Ernst Schering Research Foundation Workshop 62
PET Chemistry

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The Driving Force in Molecular Imaging

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Editors

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Preface

Personalized medicine employing patient-based tailor-made therapeutic drugs is taking over treatment paradigms in a variety of fields in oncology and the central nervous system. The success of such therapies is mainly dependent on efficacious therapeutic drugs and a selective imaging probe for identification of potential responders as well as therapy monitoring for an early benefit assessment. Molecular imaging (MI) is based on the selective and specific interaction of a molecular probe with a biological target which is visualized through nuclear, magnetic resonance, near infrared or other methods. Therefore it is the method of choice for patient selection and therapy monitoring as well as for specific end-point monitoring in modern drug development. PET (positron emitting tomography), a nuclear medical imaging modality, is ideally suited to produce three-dimensional images of various targets or processes. The rapidly increasing demand for highly selective probes for MI strongly pushes the development of new PET tracers and PET chemistry.

'PET chemistry' can be defined as the study of positron-emitting compounds regarding their synthesis, structure, composition, reactivity, nuclear properties and processes and their properties in natural and un-natural environments. In practice PET chemistry is strongly influenced by the unique properties of the radioisotopes used (e.g., half-life, chemical reactivity, etc.) and integrates scientific aspects of nuclear-, organic-, inorganic- and biochemistry.

The 62nd Ernst Schering Foundation Workshop 'PET Chemistry – The Driving Force in Molecular Imaging' is the first in the history of the Foundation devoted to special aspects of the synthesis and characterization of PET tracers. The purpose of the workshop was to gain a deeper insight into the complex applications and emerging technologies in the area of PET chemistry. New inventions in this field have a strong impact on MI, preclinical and clinical research, new therapeutic drug development, clinical routine applications and new chemical concepts.

The contributions from the invited experts that are compiled in this book demonstrate the focus and the large investment of effort over the last decade towards the advancement of PET chemistry as the cornerstone of MI today. New ideas in this exciting field translate into practical benefit very quickly. Scientific aspects covered by the workshop included reflections on a variety of PET isotopes such as ^{11}C, ^{68}Ga, ^{64}Cu, ^{86}Y, ^{76}Br, ^{77}Br, ^{124}I and their impact on the MI field. The main focus was put on ^{18}F, the 'workhorse' of PET. The contributions embraced a broad spectrum of new technical features such as the application of microwaves, microreactors and modules in the synthesis and development of new PET tracers.

The open atmosphere that prevailed during interdisciplinary discussions, and the dedication towards the goal of advancing the shared field of expertise, led to a profound contribution to PET chemistry.

We wish to express our sincere gratitude to all participants for their contributions to both the workshop and to this book. We are also grateful to the Schering Research Foundation for the generous support in making the workshop a great success.

Berlin, May 2006,
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1 Molecular Imaging with PET – Open Questions?

P.A. Schubiger

Abstract. Molecular imaging has become a very popular term in medicine and can be interpreted in many different ways. It is argued that a correct definition should be 'in vivo imaging of biological processes with appropriate molecular probes'. The real challenge in molecular imaging therefore is the search for the 'optimal' molecular imaging probes. It is discussed that nuclear, optical and magnetic probes can be used. However, only PET probes have the high sensitivity to be applied generally. To develop PET probes efficiently, methods for the in vitro and in vivo characterization are discussed and alternatives compared. Some open questions with respect to the reliability of animal imaging and evaluation of the imaging data will be elucidated.

1.1 Introduction

'PET-Chemistry: The Driving Force in Molecular Imaging' – the title of this symposium, is a strong statement about the role of PET chemistry. Consequently many eminent colleagues will discuss at this symposium efficient radiolabeling strategies and techniques for different types of biomolecules and PET radionuclides. It also means that a lot of resources are dedicated to this field, so one must be convinced that the message of this symposium as expressed in this title holds true. It may therefore be wise to ask a few questions in this context at the opening of this symposium.

The first question is about the definition of molecular imaging: What exactly is meant by the term 'molecular imaging'?

This leads us to a second question about molecular probes: Is a molecular probe or more specifically a PET molecular probe (for which we need PET chemistry) the tool to achieve molecular imaging?

Assuming that this is the case we must ask: Do we know how to develop and characterize PET ligands in the most straightforward manner or do we still lack the optimal methodology?

When we obtain molecular images with PET ligands in animal and humans, we present and publish proudly our 'pictures' as part of our results. But do we also have the proper prerequisites and means to accumulate and evaluate these images quantitatively?

1.2 Molecular Imaging?

Molecular imaging has become a very popular term in medicine. In the literature and at scientific meetings images are presented under the term 'molecular' – irrespective of the imaging method (CT, US, MRI, BLI or PET) and the information gained from the image. Thus, it appears the broad use of the label 'molecular' has probably less to do with a proper scientific definition than with clever marketing strategies and the expectations of industry and medicine to make an additional profit out of this label. This is the message of an impressive Editorial by U. Haberkorn and M. Eisenhut (Haberkorn and Eisenhut 2005).

Molecular imaging can theoretically be interpreted in two ways, namely as imaging *of* molecules or as imaging *using* molecules (i.e.

molecular probes). How is molecular imaging defined in the field of nuclear medicine? Each year at the SNM (Society of Nuclear Medicine) meeting a highlight lecture is given to address novel and relevant results, trends, and an image of the year is selected (Fig. 1). In 2005 H.H. Wagner gave this lecture for the 28th time and named one section of his speech 'Moving Toward a Molecular Focus' and asked rhetorically 'Has the time come to change the name of the SNM to the Society of Molecular Medicine' (Wagner 2005)? From a patient point of view, he defined imaging and asked what could imaging tell us about processes, state and phenotype of a disease and what could we do with this information to help a patient. This is a fair and ethical definition.

From a chemist's point of view, a molecular image is something quite different. It literally means an image which reveals information about molecules. A typical example is a structural image obtained from synchrotron radiation. Figure 2 (Lingaraju et al. 2005) shows the structure (with electron densities) of 8-oxoguanine in a novel binding mode with glycosylase from *Pyrobaculum aerophilum*. Another example is

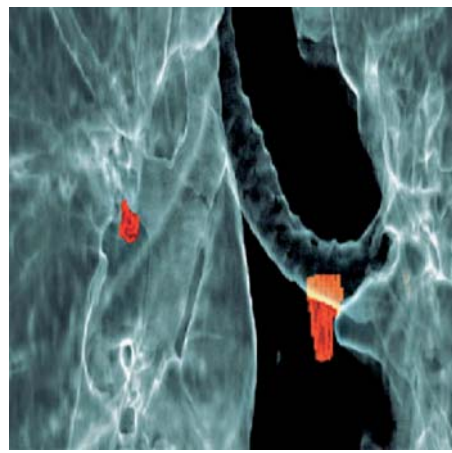

Fig. 1. 2005 SNM image of the year. Three-dimensional PET/multislice CT fusion image of virtual bronchography for presurgial evaluation (Quon et al. in Wagner 2005)

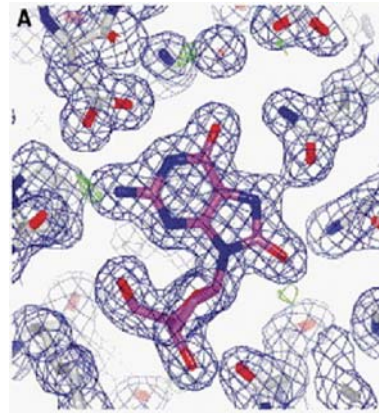

Fig. 2. A DNA glycosylase from *Pyrobaculum aerophilum* with a novel 8-oxoguanine binding mode and a noncanonical helix–hairpin–helix structure

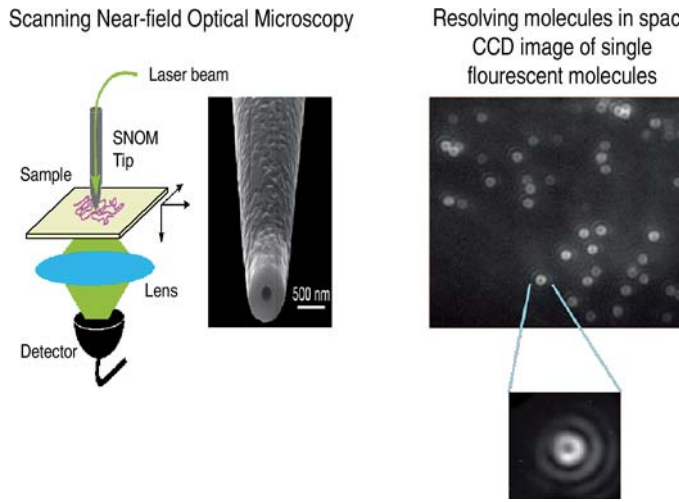

Fig. 3. Scanning near-field optical microscopy: method and example

one whereby protein molecules within the cell aggregates are visualized with a confocal microscope, or in which the resolution of molecules in space is studied with scanning near-field optical micrography (Fig. 3). With this and many more methods we can get information on various properties of molecules.

Thus it is obvious that in medicine we are not looking for images of molecules, but we are imaging biological processes by using molecular probes or in the words of S.S. Gambhir (in Marx 2005): "Molecular imaging makes molecular processes visible, quantifiable and trackable over time in a live animal or human". Conclusively, I would define molecular imaging as 'in vivo imaging of biological processes with appropriate molecular probes'.

1.3 Molecular Probes?

The real challenge in molecular imaging therefore is the search for the 'optimal' molecular imaging probes followed by the search for the appropriate (imaging) machine. Understanding biology at the molecular level needs molecules, which are part of the biological processes underlying normal or diseased states. The imaging of these molecular probes then must lead to the development of technical devices – tools to detect the signals of specific molecular probes. Imaging strategies are based on the target-specific molecular probes. Imaging methods have been established using nuclear, optical and MR imaging technology.

The functional principles of specific molecular probes are depicted in Fig. 4. The resultant image of probe localization and concentration (signal intensity) is directly related to its interaction with the target. The choice of a certain imaging modality depends primarily on the specific question to be addressed. Answering those questions requires methods with specific properties on spatial resolution, sensitivity and specificity. The strength and weakness of various methods (CT, US, MRI, BLT, FMT, FRI, PET, SPET) are discussed in many reviews (see, e.g. Rudin 2003 or Contag 2002). In Table 1 various high-resolution small animal imaging systems are compared. It is obvious that only PET has the sensitivity needed to visualize most interactions between physiological targets and ligands, such as neurotransmitter and brain receptors (imag-

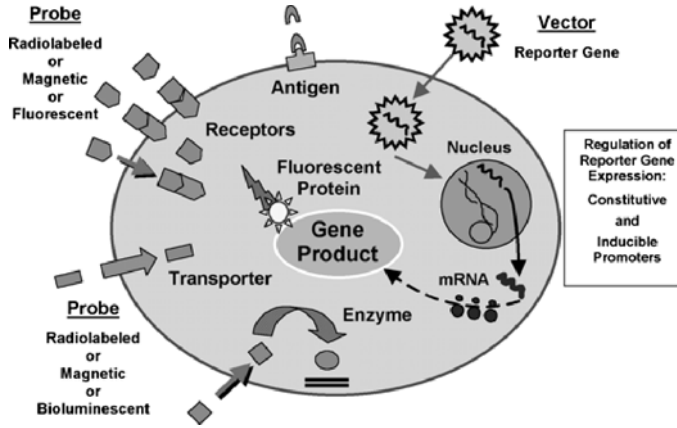

Fig. 4. Molecular probes and reporter systems. (Doubrovin 2004)

Table 1. Small animal imaging modalities (taken in part from Rudin 2003)

Imaging method	Spatial resolution	Temporal resolution	Sensitivity
Ultrasound	50 μm	> 10 ms	10^{-3} Mol
CT	50 μm	> 300 ms	10^{-3} Mol
MRI	100 μm	> 50 ms	10^{-5} Mol
MRS	1 mm	> 60 ms	10^{-5} Mol
BLI	1–3 mm (depth!)	1 ms	
PET	> 1 mm	> 15 s (6 s)	10^{-9}–10^{-12} Mol

ing quality is independent of both the localization of the organ within the body and of the distance of the organ to the body surface). Therefore, if the question concerns monitoring drug distribution, pharmacokinetics and pharmacodynamics for most organs (e.g. the brain) PET is the only choice as a nuclear imaging technique.

1.4 Development of New Molecular PET Probes – Permeation of the Blood–Brain Barrier?

Choosing the right target and defining the purpose of the molecular probes is a tedious process, but not part of this introduction. In this

context, I would like to stress one point taken from a review by Bill Eckelman (Eckelmann et al. 2005). There he discusses neurotransmitter systems for which multiple radiolabeled molecular probes have been developed. He concludes that although the rationale for the development of these radioligands often refers to a target disease, few have actually demonstrated an impact on patient care.

Once a desired PET molecular probe (PET ligand) is defined and synthesized, the in vitro and in vivo characterization should proceed in the most efficient way such that a 'go' or 'no go' decision is taken as early as possible. For the in vitro characterization, methods exist to validate binding to the target structure, to test stability in blood serum or to identify metabolites. For blood–brain barrier (BBB) permeation many methods have been proposed but the most commonly used method is the partition coefficient logP or logD which, however, is not reliable. The next example demonstrates that lipophilicity does not always predict brain permeation very reliably.

Table 2 shows structures of some potential PET-ligands for the metabotropic glutamatergic receptor subtype 5 (mGluR5) with their respective binding affinities and lipophilicity values. This is a selection of some of the ligands we synthesized and evaluated in our group.

Table 2. Structures of potential mGLUR5 PET-ligands (Ametamey et al. 2006)

Ligand	MW/ (g/mol)	tPSA	$\log P_{calc}$ ($\log D_{exp}$)	K_D (nM)	IC_{50} (nM)
MPEP 6-methyl-2-(phenylethynyl)-pyridine	193.24	11.3	3.77		34
M-MPEP 2-methyl-6-(methaxyphenyl)ethynyl))-pyridine	223.27	3.64	3.64	3.4	
M-FPEP 2-methyl-6-(3-fluorophenylethynyl))-pyridine	211.23	11.3	3.93 (2.7)	$1.4 + 1.1$	9
ABP688 3-(6-methyl-pyridin-2-ylethyl) cyclohex-2-enone *O*-methyl-axime	239.31	25.4	2.44 (2.4)	$1.7 + 0.2$	3

Ametaney et al. (2005) in collaboration with Novartis

Despite favourable in vitro properties only ABP688 (Ametamey et al. 2005) proved to be useful for in vivo studies in animals. Most of the ligands failed, due mainly to high lipophilicity, unfavourable brain uptake kinetics or rapid metabolism. So the question arises if there are more suitable BBB permeability methods on which one can rely.

The use of planar lipid bilayers such as in IAM (immobilizer artificial membrane) chromatography columns or PAMPA (parallel artificial membrane permeability assay; highly porous microfilters coated with phospholipid layers), which mimic the lipid environment found in cell membranes, give some indications about passive transport only. The lipid bilayers are known to be physically unstable and to contain residual solvent molecules. Liposomes have a better physical stability and can be prepared without solvents or other adjuvants (Krämer and Wunderli-Allenspach 2001). In the cellular model approach, in vitro techniques typically use a tissue culture model of cerebrovascular endothelial cells (MDCK or Caco2) either alone or cocultured with astroglia. These models are very time consuming and labour extensive (Löscher and Potschka 2005).

Available in vivo models mostly rely on rodents (mouse, rat, guinea pig), are well described and widely used but lead to ethical issues and overwhelming costs. Non-invasive approaches are imaging technologies that use dedicated platforms such as PET, MRI, or (immuno) histochemistry. Invasive models use, for example, the classical pharmacokinetic analysis (brain extraction, canulation), the intracerebral microdialysis, or the beta-probe method. The β-microprobe system is a local β-radioactivity counter that takes advantage of the limited range of β-particles within biologic tissues to define the detection volume in which the radioactivity is counted (Pain et al. 2004). The device has been validated for pharmacology studies, some involving coupling with microdialysis and some involving quantitative measurements of cerebral metabolism. The use of two β-microprobes allows simultaneous determination of blood and cerebral tissue-time activity curves after a single injection of a PET tracer in an animal. However, it is highly invasive and requires skilled personnel trained to place plastic catheters in small animals.

Summarizing the methods for evaluating the BBB penetration for drug development purposes, one will notice that none of the published

models has yet been adopted by a significant number of research laboratories. Research on BBB models is a relatively young field and there is not a definitive model available:

a. There is no 'golden rule' for approaching brain penetration (such as Lipinski's rule of five).
b. Among the BBB permeation or penetration models available, some are often used in parallel (in silico, in vitro and in vivo approaches).
c. Currently, the methods preferentially used in predicting drug absorption, are logP, logD data compared to liposome permeation data for membrane translation, and the use of cell-based assays (e.g. monolayers of MDCK cells).

1.5 Experimental Imaging Design and Data Evaluation: Limitations and Challenges?

In contrast to human PET imaging, PET data collection in small animals involves immobilization of the animal which is generally attained by anaesthesia during scanning. All anaesthetics have significant effects on the respiratory, cardiovascular and central nervous systems. Therefore, anaesthesia should be administered at a superficial level to reduce the effects on circulation and metabolism. Furthermore, fluctuations in the anaesthetic level among various scans may increase the interindividual variation in tracer uptake, kinetics and metabolism and should thus be kept to a minimum by standardization of anaesthetic regimens. Cross-correlation analyses using biodistribution studies with non-anaesthetized animals can help to assess the impact of the anaesthetic regimen on signal sizes and kinetics. For example, by comparing post-mortem striatal activity concentrations of the D2 receptor ligand [^{18}F]-fallypride in anaesthetized and non-anaesthetized mice a substantial influence of isoflurane anaesthesia on signal sizes was excluded (Fig. 5). Post-mortem activity concentrations in the striatum differed only by 20% at 63 min and 153 min post-injection (Honer et al. 2004), being thus in line with a marginal susceptibility of the radiotracer to metabolic breakdown as suggested by a dynamic ROI analysis (Mukherjee et al. 1999).

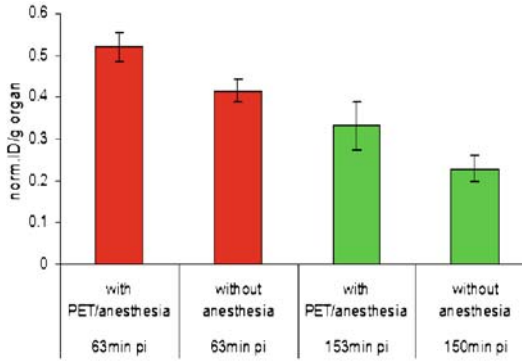

Fig. 5. [^{18}F]fallypride: effect of anaesthesia

PET studies in mice and rats without the involvement of anaesthesia remain a major challenge for small animal PET experimentation. So far, PET experiments have been performed in conscious and trained cats or monkeys (Hassoun et al. 2003; Noda et al. 2003; Tsukada et al. 2000; Zimmer et al. 2003). In a recent study, a non-anaesthetized rat was trained to accept head fixation and scanned for 60 min (Momosaki et al. 2004). Regarding animal welfare issues and scanning throughput this approach will probably not achieve general acceptance. Furthermore, the effects of the physical and mental stress imposed on the animal by active restraint are not well analysed. Alternatively, a PET system mounted to a rat's head allowed imaging of the conscious rodent brain (Vaska et al. 2004) but the practicability and utility of this experimental approach has still to be demonstrated.

Another major challenge in small animal PET imaging affects data processing and quantification. It is crucial to find ways to conduct small animal PET studies with higher efficiency and accuracy. PET imaging involves acquisition of huge data sets and timely PET data reconstruction is desired. Therefore, efficient data management, the use of high performance hardware as well as fast reconstruction algorithms are imperative for higher throughput of scanning experiments in the future. Additionally, PET imaging has the big advantage to allow the distribution of a radiotracer to be measured in quantitative units. However, only limited in-

formation is available on the feasibility of achieving absolute quantification in small animal PET imaging. Corrections for attenuation in the animal's body as well as for scattered coincidences have to be implemented in the reconstruction software. Furthermore, calibration factors should be routinely determined and applied to allow conversion between scanner units (counts/voxel/s) and radioactivity concentrations (kBq/ml). Dynamically reconstructed PET data can be further processed and quantified by applying tracer kinetic models. To perform such fully quantitative dynamic PET experiments, information on the time-course of tracer delivery to the tissue is mandatory (for a review see Laforest et al. 2005).

Another challenge to obtain precise and reliable quantitative PET data involves high intra-study stability and inter-study reproducibility of the imaging experiment. Only a detailed standardized experimental design as well as standardized evaluation techniques avoid the introduction of bias to the analysis and permit one to draw meaningful conclusions from a longitudinal study with repetitive PET scanning experiments. The significance of PET data obtained in mice and rats may be also limited if rodent data shall be translated to the human setting. Species differences in metabolism, protein binding characteristics, and target site density etc. might be considerable and complicate the prediction of whether a successfully characterized PET tracer in rodents can be effectively applied in humans. In order to facilitate the extrapolation of rodent PET data to the clinical situation, the development of meaningful assays (e.g. metabolite analyses using human hepatocytes) represents a major challenge for successful preclinical PET tracer characterization in the future.

1.6 Conclusions

Molecular imaging means in vivo imaging of biological processes with appropriate molecular probes. Today only PET has the sensitivity to visualize most physiological interactions in a volume and target-depth independent manner, and in three dimensions. Therefore the real challenge is the search for the specific PET probes, and so PET chemistry is indeed the driving force in molecular imaging. However, besides chemistry some other fields such as pharmacological characterization, animal handling or data evaluation must also be developed further.

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2 Fluorine-18 Labeling Methods: Features and Possibilities of Basic Reactions

H.H. Coenen

Dedicated to Prof. Dr. Dr. h.c. Syed M. Qaim on the occasion of this 65th birthday.

Abstract. Many experimental and established tracers make fluorine-18 the most widely used radionuclide in positron emission tomography with an increasing demand for new or simpler ^{18}F-labeling procedures. After a brief summary of the advantages of the nuclide and its major production routes, the basic features of the principal radiofluorination methods are described. These comprise direct electrophilic and nucleophilic processes, or in case of more complex molecules, the labeling of synthons and prosthetic groups for indirect built-up syntheses. While addressing the progress of no-carrier-added ^{18}F-labeling procedures, the

following chapters on more specific topics in this book are introduced. Emphasis is given to radiofluorination of arenes – especially with iodonium leaving groups. Examples of radiopharmaceutical syntheses are mentioned in order to illustrate strategic concepts of labeling with fluorine-18.

2.1 Introduction

Concomitant with the increasing variety of biochemical and pharmacological principles applied for molecular imaging and hence with the growing number of chemical compound classes used, there is a permanent demand for new or adapted methods of labeling. Fluorine-18 is the radionuclide most often used for routine diagnosis with positron emission tomography (PET). The preparation and use of ^{18}F-labeled radiopharmaceuticals and experimental compounds have been summarized in many recent reviews (Lasne et al. 2002; Wester 2003; Coenen 2003; Shiue et al. 2004; Couturier et al. 2004; Adam and Wilbur 2005). Several chapters in this book deal with the preparation of special classes of compounds, built-up and prosthetic group labeling as well as technical aspects of radiofluorination procedures. Therefore, the focus here will be on the basics of established and newer methods of the primary ^{18}F-labeling step.

The report on the first production of fluorine-18 originates from 1936 (Snell 1937) and only 4 years later it found use in health related studies on fluoride-adsorption by bone and dentine (Volker et al. 1940). Its success as routine PET nuclide in diagnosis and pharmacological research is based on its almost perfect chemical and nuclear properties. Fluorine-18 can be produced in good yields, even with low-energy cyclotrons. The half-life of 109.7 min allows time-consuming multi-step radiosyntheses as well as extended PET studies of slower biochemical processes; in addition, it makes shipment possible according to a so-called satellite concept. Thus the supply of clinics without an on-site cyclotron can be ensured. The radionuclide has a low β^+-energy of 635 keV, besides ^{64}Cu the lowest of the PET nuclides, which promises a very high resolution of down to 1 mm in PET images and guarantees minor radiation doses to the patients.

Most commonly fluorine-18 is employed in analoguous fluoroderivatives, where it sterically replaces a hydrogen atom. While the Van der Waals' radii of fluorine (1.35 Å) and hydrogen (1.20 Å) are almost the same, the differences in electronic character of the two elements, however, are very pronounced. For example, replacing a hydrogen in an aliphatic position by fluorine will decrease the lipophilicity by a factor of five while substitution in an aryl group will increase it twofold. Nevertheless, most of the ^{18}F-labeled compounds are based on the analogy in steric demands of fluorine and hydrogen. The best known example is 2-[^{18}F]fluorodeoxyglucose ([^{18}F]FDG), which is the most widely used PET radiopharmaceutical (Gallagher et al. 1978; Reivich et al. 1979). Also the analoguous amino acid L-2-[^{18}F]fluorotyrosine shows metabolic acceptance (Coenen et al. 1989; Couturier et al. 2004). This again is true for 6-[^{18}F]fluoro-3,4-dihydroxy-L-phenylalanine (6-[^{18}F]fluoro-L-DOPA), a radiodiagnostic for neurodegenerative diseases (Parkinson disease) and for oncology (Shiue et al. 2004; Couturier et al. 2004).

2.2 Nuclide Production

For fluorine-18 more than 20 nuclear reactions are known as production pathways (Qaim et al. 1993), the most common of which are listed in Table 1. The use of the ^{18}O(p,n)^{18}F ($E_p = 16 \rightarrow 3$ MeV) reaction on ^{18}O-enriched water is the most effective method and delivers [^{18}F]fluoride of high molar radioactivity. Thus, under optimized conditions high activities of [^{18}F]fluoride can easily be achieved at cyclotrons, even with low energy, within less than 1 h of irradiation time. The costly ^{3}He-irradiation of ^{16}O is of minor importance as is especially the possible production in nuclear fission reactors, where the tandem reaction ^{6}Li(n,α)^{3}H/^{16}O(^{3}H,n)^{18}F only allows activity production of < 1 GBq and causes ^{3}H-waste problems.

In case of electrophilic radiolabeling reactions [^{18}F]F_2 is required. Accordingly, ^{20}Ne and ^{18}O gas targets can be chosen. The major problem in both cases is the adsorption of the produced fluorine-18 on the target walls so that an addition of elemental fluorine to the target gas is mandatory and high molar activities cannot be attained (Hess et al.

Table 1. Production routes for fluorine-18

Reaction	$^{18}O(p,n)^{18}F$	$^{16}O(^{3}He,p)^{18}F$	$^{20}Ne(d,\alpha)^{18}F$	$^{18}O(p,n)^{18}F$
Target	$H_2^{18}O$[a]	H_2O	Ne (200 μmol F_2)[b]	$^{18}O_2$, Kr (50 μmol F_2)[c]
Particle energy [MeV]	$16 \rightarrow 3$	$36 \rightarrow 0$	$14 \rightarrow 0$	$16 \rightarrow 3$
Main product form	$^{18}F^-_{aq}$	$^{18}F^-_{aq}$	$[^{18}F]F_2$	$[^{18}F]F_2$
Exp. yield [GBq/μAh]	2.22	0.26	0.40	1.0
Specific activity [MBq/μmol]	$\approx 600 \times 10^3$	$\approx 50 \times 10^3$	≈ 100	≈ 600

[a] Ti-target with Ti-window

[b] Passivated Ni-target

[c] Two step process; Al-target

2000). Since two consecutive irradiations are necessary with the $^{18}O_2$-target for saving enriched material, the $^{20}Ne(d,\alpha)^{18}F$ reaction is most practical and common process for electrophilic fluorine-18, although production rates are lower.

A recent review on the nuclear data of the $^{18}O(p,n)^{18}F$ reaction from threshold up to 30 MeV proton energy exhibited higher theoretical production yields than the hitherto accepted data, limited to < 14 MeV. The saturation integral yield calculated from the excitation curve as depicted in Fig. 1, encourages irradiations up to about E_p 20 MeV and relativates the performance data of existing targets (Hess et al. 2001).

2.3 Direct ^{18}F-Labeling Methods

In principle, general pathways for the fluorination of organic molecules are transferable from macroscopic organic chemistry to radiochemistry. For fluorine introduction into aromatic organic compounds, preparative organic chemistry generally uses stable diazonium piperidines in the Wallach reaction (Wallach 1886) and solid diazonium tetrafluoroborates in the Balz-Schiemann reaction with better yields (Balz and Schiemann 1927). These are dediazoniation reactions, where the fluoride re-

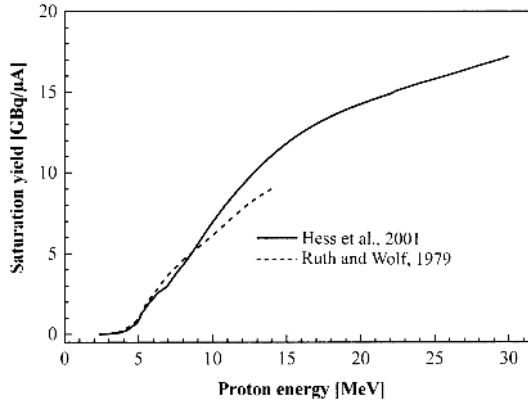


Fig. 1. Calculated saturation integral yield of fluorine-18 as a function of proton energy from 100% enriched ^{18}O. (Hess et al. 2001)

places a decomposing diazonium moiety. However, in no-carrier-added (n.c.a.) labeling syntheses these reaction types only result in low radiochemical yields (Atkins et al. 1972; Tewson and Welch 1979). For the Balz-Schiemann reaction the tetrafluoroborate counter anion leads axiomatically to a maximum radiochemical yield (RCY) of only 25% and causes isotopic carrier. Moreover, both reactions are principally of the S_N1-type, thus reactive cations evoke and interact with any nucleophilic species causing many side products. The statistical probability of the reactive intermediates to encounter a [^{18}F]fluoride ion under n.c.a. conditions is marginal and only carrier addition leads to acceptable results (Atkins et al. 1972; Tewson and Welch 1979; Coenen 1989).

Depending on the production procedure (see Table 1), fluorine-18 is obtained in a particular chemical form, i.e. as [^{18}F]F_2 or [^{18}F]fluoride, which determines the possible reactions. While both species undergo substitution reactions, elemental fluorine or derived electrophilic reagents such as acetylhypofluorite and halogenfluorides can add to olefins and form α-substituted [^{18}F]fluoroalkanes. The most prominent examples here are the original radiosyntheses for [^{18}F]FDG (Ido et al. 1978; Coenen et al. 1987a) and the halofluorination of double bonds forming, for example [^{18}F]fluoroalkyl bromides which are used for

^{18}F-fluoroalkylation (Chi et al. 1986). Stereochemically the Markovnikov product is predominantly obtained in these addition reactions and subsequent hydrolysis or elimination steps result in alkyl [^{18}F]fluorides or vinyl [^{18}F]fluorides as shown with, for example [^{18}F]FDG (Ido et al. 1978) or 5-[^{18}F]fluorouracil (Fowler et al. 1973), respectively. Today, however, electrophilic addition reactions almost do not play any role. Thus, the principal ^{18}F-labeling methods are restricted to a few and can be divided into:

– Electrophilic substitution
– Nucleophilic substitution
– ^{18}F-fluorination via built-up procedures
– ^{18}F-fluorination via prosthetic groups.

The former procedures represent direct methods and the latter indirect methods, which in turn are based on the direct methods for ^{18}F-labeling of the required synthon or prosthetic group.

2.3.1 Electrophilic Substitution

For electrophilic ^{18}F-labeling reactions carrier-added (c.a.) [^{18}F]F_2 is directly available from the target (Table 1), but only with a maximum molar activity of about 600 MBq/μmol (Hess et al. 2000). In some cases [^{18}F]F_2 is transferred into somewhat less reactive and more selective fluorination agents such as acetylhypofluorite ([^{18}F]CH_3COOF) and xenon difluoride ([^{18}F]XeF_2) (Visser et al. 1984; Chirakal et al. 1984; Constantinou et al. 2001). These offer options for the ^{18}F-fluorination of electron-rich compounds (e.g. alkenes, aromatic molecules, carbanions, etc.), which are actually unavailable with nucleophilic n.c.a. ^{18}F-labeling methods. Due to the necessary carrier addition in the [^{18}F]F_2 production and the fact that every [^{18}F]F_2 molecule carries only one ^{18}F atom, the theoretical achievable maximum RCY in electrophilic ^{18}F-substitution is limited to 50%. As a result, the electrophilic ^{18}F-labeling routes are restricted to radiopharmaceuticals where a high specific activity is not required. Due to the high reactivity of electrophilic ^{18}F-labeling agents their selectivity is rather low and undesired radical side reactions and those with solvents take place. Therefore,

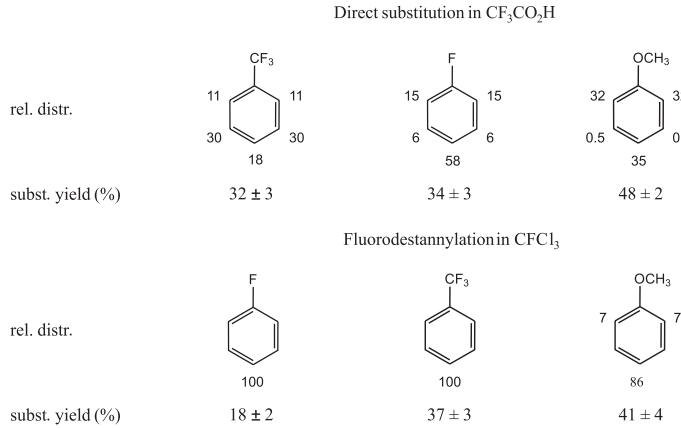
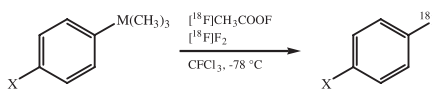


Fig. 2. Radiochemical yields and isomer patterns of electrophilic ^{18}F-substitution of hydrogen and *p*-trimethyltin groups in monosubstituted benzenes

electrophilic methods call for extensive purification procedures to meet the requirements of very high purity of radiopharmaceuticals.

The high reactivity of electrophilic radiofluorine is exemplified in Fig. 2 for three monosubstituted benzenes, where mixtures of positional fluoroisomers are indicated together with a weak dependence on the activating effect by the substituent.

In order to increase the regioselectivity in arenes, demetallation reactions of organometallic precursors can be used (see Scheme 1). Suitable organometallic precursors are aryltrimethyltin, aryltrimethylgermanium and aryltrimethylsilicon compounds, whereas the organotin moiety shows the best results (Adam et al. 1984; Coenen and Moerlein 1987). Generally a substituent (X) influences the orientation of substitution in the arene, but for the ^{18}F-fluorodemetallation reaction the effect is less



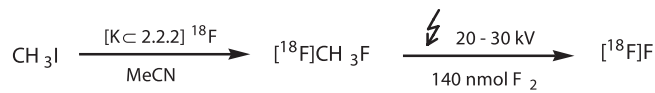
Scheme 1. Regioselective ^{18}F-labeling via electrophilic demetallation reactions (M = Sn, Ge, Si; X = OCH_3, CH_3, H, F, CF_3, NO_2) (Coenen and Moerlein 1987)

strong than in direct electrophilic ^{18}F-labeling reactions. However, in spite of the high carbanionic character of the metal-bearing carbon, side reactions to positional isomers can still be observed with strongly activating substituents due to the high reactivity of electrophilic fluorine species (see Fig. 2) (Coenen and Moerlein 1987).

Electrophilic c.a. radiofluorination can be used for preparation of analogues of endogenous compounds, such as amino acids, which exhibit a high concentration, and if the fluoroanalogues are not toxic.

However, for complex molecules including sensitive functional groups, those may have to be protected before radiofluorination and further radiosynthetic steps become necessary for the deprotection. As a first example, ^{18}F-labeling via an organotin precursor led to 25% RCY in case of 4-[^{18}F]fluoro-L-phenylalanine (Coenen et al. 1986a), while direct electrophilic labeling causes isomeric mixtures of aromatic fluoroamino acids (Coenen et al. 1988). Also 6-[^{18}F]fluoro-L-DOPA is produced for clinical routine via ^{18}F-fluorodestannylation, although the maximum RCY is only 30% and the molar activities are low (Namavari et al. 1992; De Vries et al. 1999). The lack of an efficient and simple nucleophilic alternative requires the use of the electrophilic pathway. So far, the best nucleophilic method still involves a multi-step radiosynthesis including chiral auxiliaries, and therefore complicates automation and reduces the RCY (Lemaire et al. 1994) (see below).

More recently attempts were made to reduce the amount of fluorine-19 carrier in the synthesis of electrophilic ^{18}F-species. Higher molar activities of [^{18}F]F_2 of about 30 GBq/μmol were obtained via electric gaseous discharge of [^{18}F]methylfluoride previously prepared by n.c.a. nucleophilic substitution (see Scheme 2; Bergman and Solin 1997). This, however, has the drawback of low radiochemical yields based on the radionuclide produced and a molar activity that is still lower than desired, for example, for receptor ligands.



Scheme 2. Electrophilic [^{18}F]fluorine generated by electric gaseous discharge (Bergman and Solin 1997)

2.3.2 Nucleophilic Substitution

The most important route for obtaining ^{18}F-labeled compounds is nucleophilic substitution based on n.c.a. [^{18}F]fluoride, which is directly available from the target without any carrier addition. Today, this is the only practical means of obtaining ^{18}F-radiopharmaceuticals with the highest molar activity. This is generally preferable but mandatory for investigations of low concentration binding-sites, e.g. of neuronal receptors, which must be studied without perturbation of the physiological equilibrium. N.c.a. [^{18}F]fluoride is obtained as an aqueous solution according to the production technology (see above for details). Due to the high charge density of the anion it is strongly hydrated ($\Delta H_{hydr} = 506\,kJ/mol$) and inactivated for nucleophilic reactions. In the presence of Lewis acids, the fluoride anion has a strong tendency to form complexes and will be masked by ions of heavy metals. It is very easily protonated, forming hydrogen fluoride ($E_B = 565\,kJ/mol$), which then makes it unavailable for further reactions.

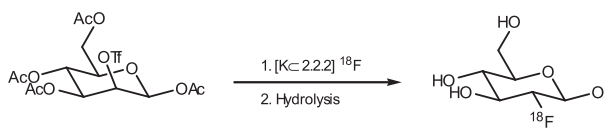
Labeling, therefore, has to take place under aprotic but polar conditions. In order to separate [^{18}F]fluoride and recover the enriched target water, anion exchange resins (Gatley and Shoughnessy 1982; Schlyer et al. 1990) are used and the water is generally subsequently removed by azeotropic distillation with acetonitrile. Recent introduction of an electrochemical cell (Hirschfelder et al. 1999; Hamacher et al. 2002a) makes thermal drying superfluous and the use of ionic liquids does not even require the absence of water (see Kim et al. 2004 and references therein) for the substitution reaction. However, routinely the nucleophilic radiofluorination with appropriate precursors is performed in dipolar aprotic solvents using fluoride salts with a soft cation (Cs^+, Rb^+) thus rendering weak – easy to separate – ion pairs and 'naked' [^{18}F]fluoride of high nucleophilicity. For further anion activation phase transfer catalysts (PTC) like tetraalkylammonium carbonates (hydrogen carbonates) (Gatley and Shoughnessy 1982; Kiesewetter et al. 1986; Kilbourn 1990) or mainly the aminopolyether Kryptofix® 2.2.2 in combination with potassium carbonate or oxalate (Coenen 1986; Coenen et al. 1986b; Coenen 1989; Hamacher and Hamkens 1995) are optimal. If the solubility product of the cryptate complex is not exceeded, the losses by wall-adsorption are negligibly small (Block et al. 1987)

and the Kryptofix system is the generally preferred one in most n.c.a. radiofluorination reactions.

In some cases tetrabutylammonium carbonate showed advantages over the Kryptofix system for activation of n.c.a. [^{18}F]fluoride (Hamacher and Coenen 2002), which was also discussed in connection with the microwave assisted preparation of [^{18}F]FDG (Taylor et al. 1996). In comparison to conventional heating, microwave technology often has a beneficial influence on labeling procedures not only with respect to saving reaction time but also to activating non-reactive precursors and avoiding destructive side reactions (Hwang et al. 1987; Stone-Elander and Elander 2002) as reviewed by Stone-Elander et al. in this volume.

The direct nucleophilic ^{18}F-fluorination of aliphatic compounds in dipolar aprotic solvents, most preferably acetonitrile (ACN), proceeds according to an S_N2-mechanism. Halogens or sulphonic acid ester groups, such as mesylate, tosylate and triflate, act as the leaving group. Generally, triflate (trifluoromethane sulphonic acid ester) precursors give the best results (for a review see Coenen 1986; Kilbourn 1990). Accordingly, the reactivity of ^{18}F-substitution decreases from primary to tertiary carbon position.

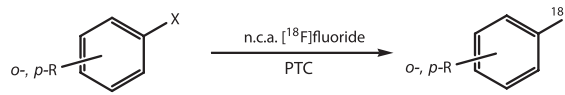
Since the replacement of the leaving group proceeds via a Walden inversion according to the stereospecific S_N2-mechanism involved, the most widely used radiopharmaceutical [^{18}F]FDG is synthesized starting from a completely acetylated mannose precursor by an ^{18}F-for-triflate exchange in ACN and a subsequent hydrolysis (see Scheme 3) (Hamacher et al. 1986). Routinely a RCY of more than 50% is attained in automated procedures. Since fluoride in dipolar aprotic solvents exhibits also a strong basic character and generally the reaction medium is basic



Scheme 3. Nucleophilic n.c.a. ^{18}F-fluorination and subsequent hydrolysis of acetylated mannose triflate for the preparation of [^{18}F]FDG. (Hamacher et al. 1986)

(CO_3^{2-}, HCO_3^-), elimination reactions can compete with nucleophilic substitution. The preparation of small bifunctional alkyl [^{18}F]fluorides (Block et al. 1987) is of special interest as they allow interesting applications in further built-up syntheses (see below). Using the methodology of fluoride activation by PTC in dipolar aprotic solvents, numerous aliphatic and aromatic n.c.a. ^{18}F-labeled tracers were prepared which are summarized in many reviews.

The nucleophilic aromatic n.c.a. ^{18}F-fluorination is of even greater importance for ^{18}F-labeled radiopharmaceuticals. The generally good metabolic stability of the resulting ^{18}F-labeled aromatic compounds is a major advantage. Nucleophilic aromatic ^{18}F-fluorination calls in the first place for activated aromatic molecules; consequently electron withdrawing substituents in *ortho*- or position to the leaving group appear to be indispensable (Scheme 4).

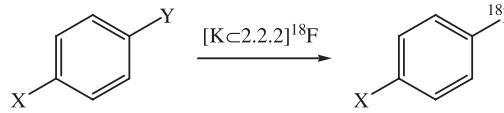


Scheme 4. Nucleophilic aromatic ^{18}F-fluorination with activated arenes, X=Br, Cl, I, NO_2, $N(CH_3)_3^+$ (counter ions: TfO^-, TsO^-, ClO_4^-, I^-); R=NO_2, CN, CHO, RCO, COOR, Cl, Br, I; PTC=$[K\subset2.2.2]_2CO_3$, $[K\subset2.2.2]_2C_2O_4/CO_3$, R_4N^+, Cs^+, Rb^+

In particular, substituents (R) with strong electron withdrawing properties such as nitro, cyano and carbonyl groups are suitable for the activation step (Angelini et al. 1984; Coenen 1989; Kilbourn 1990). Halogens, nitro and the trimethylammonium salts show increasing reactivity as the leaving group. This is clearly demonstrated in Table 2 with the strongly activating nitro- and cyano-substituent in *para*-disubstituted benzenes.

While with strongly reactive arenes (e.g. *para*-dinitrobenzene) ^{18}F-substitution succeeds with the addition of only Rb_2CO_3 or Cs_2CO_3 for activation (Shiue et al. 1984), PTCs are indispensable with less activated arenes. A problem is the basicity of the reaction solution. Thus, base labile compounds such as butyrophenone neuroleptics, for example, could only be ^{18}F-labeled directly when the $[K\subset2.2.2]_2CO_3/C_2O_4$ buffer system was used; for example, [^{18}F]*N*-methylspiperone could

Table 2. Dependence of nucleofugality of different aryl substituents activated by *p*-nitro- or cyano-groups[a]



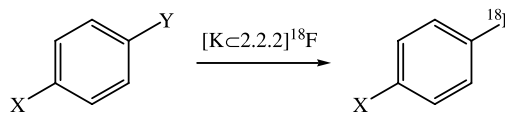
p-Substituent X	Leaving group Y					
	I	Br	Cl	F	NO_2	$NR_3^+Tfl^-$
CN	–	33	24	70	73	68
NO_2	10	35	70	47	87	–

[a]Data are percentage radiochemical yield; SD<5%; 150°C in DMSO

be obtained from the corresponding *para*-nitro precursor in good RCY (Hamacher and Hamkens 1995). Even though the nitro-moiety is a much better leaving group than halogens, substitution yields as shown in Table 3 demonstrate that with *para*-nitrophenyl halides the nitro group will not be replaced; this is confirmed also for *ortho*-bromo-nitroarenes (Van Dort et al. 1989; Karramkam et al. 2002).

With the introduction of the trimethylammonium leaving group as chloride, perchlorate or triflate salt, a major improvement was attained

Table 3. Effect of *para*-substituents on substitution of aryl nitro and trimethylammonium group[a]



Leaving group Y		*p*-Substituent X							
		NO_2	CF_3	CN	CHO	$COCH_3$	I	Br	Cl
NO_2[b]	80°C	78	33	73	75	35	–	< 0.1	
	150°C	88	67	81	77	67	–	< 0.1	
NMe_3^+[c]	140°C	88	-	87	71	79	15	12.5	-

[a]Data are percentage radiochemical yield; SD<5%

[b]In DMSO

[c]As triflate in DMAA

(Irie et al. 1982; Angelini et al. 1985; Haka et al. 1989). Of those discussed so far, this group shows the best nucleofugic quality, especially as triflate and with dimethylsulfoxide (DMSO) or even better dimetylacetamide (DMAA) as solvent. It becomes also evident from Table 3 that the superiority of the NMe_3^+ over the NO_2 leaving group is only revealed with less activating substituents such as phenone- or halogen-moieties.

In cases of less activating aryl substituents with low Hammett constants, e.g. iodine and bromine (Hansch et al. 1991), however, an aliphatic substitution occurs on the $N(CH_3)_3$ group and [^{18}F]fluoromethane formation competes with aromatic ^{18}F-labeling (Fig. 3). Thus an RCY of only about 12%–15% of 1-[^{18}F]fluoro-4-halobenzenes is attainable with these less activating substituents (Gail and Coenen 1993; Ermert et al. 1995, 2004). In this context it is interesting to note that ^{13}C-NMR was used to predict reactivity in nucleophilic ^{18}F-exchange on benzaldehydes, but only a partial linear dependence of the obtained RCY on the chemical shifts was observed (Ding et al. 1990; Rengan and Kilbourn 1993).

Here, the use of substituted diaryliodonium salts as a newer approach offers a more promising route. It is well known that diaryliodonium salts react with a wide range of nucleophiles to yield the substituted

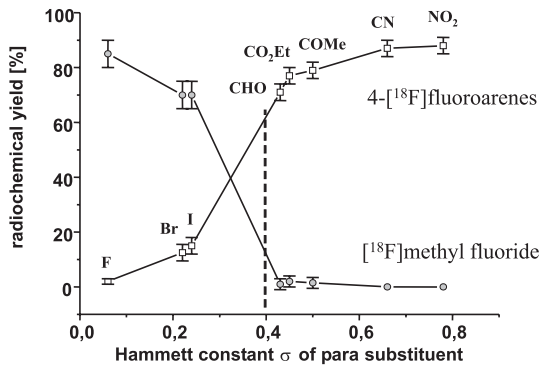
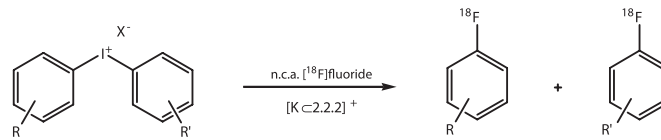


Fig. 3. Competing formation of [^{18}F]methylfluoride and of *para*-substituted [^{18}F]fluorobenzenes in nucleophilic ^{18}F-substitution on corresponding trimethylanilinium triflates

arenes and corresponding iodoarenes (Koser 1983). The first attempt was made in 1995 (Pike and Aigbirhio 1995) for nucleophilic n.c.a. ^{18}F-labeling of diaryliodonium salts with diverse substituents and counter ions in acetonitrile, activated by the Kryptofix® 2.2.2/K_2CO_3-system. Since that time a variety of simple benzene derivatives has been labeled with [^{18}F]fluoride via diaryliodonium salts.

For the latter method the resulting product distribution after the nucleophilic attack by n.c.a. [^{18}F]fluoride strongly depends on the electronic and steric character of each aryl ring, i.e. its substituents (Scheme 5). The ^{18}F-labeling reaction proceeds via a S_NAr-mechanism and in an asymmetric diaryliodonium salt the more electron deficient ring is preferably attacked by the [^{18}F]fluoride. Furthermore, steric influences, especially of *ortho*-substituents, have an impact and usually increase the RCY (Pike and Aigbirhio 1995; Gail et al. 1997). In case of *para*-substituted aryl(phenyl)iodonium salts the electronic influence of the substituent leads to decreasing product yield with the sequence bromo > chloro > methyl and 0% with methoxy; the corresponding percentage of the [^{18}F]fluorobenzene increased with the same sequence (Gail et al. 1997; Ermert et al. 2004). Surprisingly, with ortho substituents and an increasing number of methyl groups at one aromatic ring (Fig. 4), the probability for the nucleophilic attack increased in spite of an increasing steric hindrance and electronic deactivation by multi-methylation (Gail et al. 1997).



Scheme 5. General principle of ^{18}F-labeling via diaryliodonium salts (X=Cl, Br, I, TsO, ClO_4, CF_3SO_3; R, or R′=H, Cl, Br, I, NO_2, CH_3, OCH_3)

The phenomenon of the so-called '*ortho*-effect' was explained by a trigonal bipyramidal geometry, where the 'reactive' ring is occupying the equatorial position *syn* to the nucleophile. Incipiently postulated by Yamada and Okawara (1972a) this interpretation was used to explain similar results in corresponding radiofluorination reactions (Gail

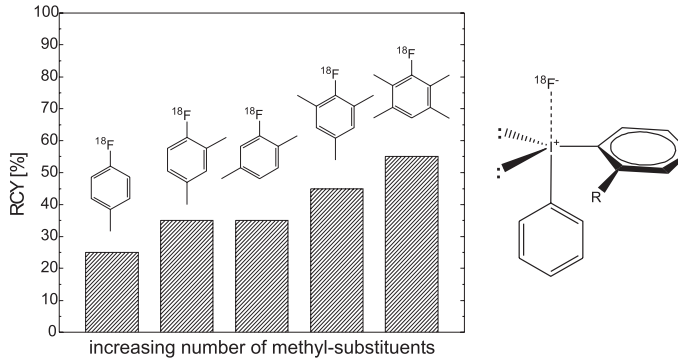


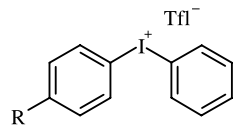
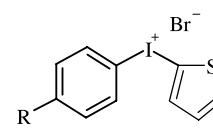
Fig. 4. Dependence of RCY on the number of methyl-substituents (Gail et al. 1997); trigonal bipyramidal geometry of the intermediate [^{18}F]fluoride complex of the nucleophilic attack

et al. 1997; Shah et al. 1998; Hostetler et al. 1999; Martin-Santamaria et al. 2000).

With regard to the positionally directing electronic influence, highly electron rich moieties such as heteroaromatic rings came into consideration. Various reactions of non-radioactive nucleophiles including fluoride as caesium or potassium fluoride had already been carried out and showed very high regioselectivity up to regiospecificity (Yamada and Okawara 1972a, 1972b; Martin-Santamaria et al. 2000). As indicated in Table 4, radiofluorination via diaryliodonium salts leads mostly to both ^{18}F-labeled arenes in different product ratios. This is avoided by using aryl(2-thienyl)iodonium precursors. Particularly for electron-rich molecules this is of major interest and in addition n.c.a. 4-halo-[^{18}F]fluoroarenes can be prepared in higher RCY by this new method and obviously without any radioactive side products (Ross et al. 2005). In contrast to the nitro and ammonium leaving groups dimethylformamide (DMF) proved to be the best solvent with iodonium salts and bromide as the optimum counter anion.

It must be mentioned, however, that in spite of the promising results with aryliodonium leaving groups, satisfactory yields were obtained only with simple substituted benzene derivatives, while RCY $< 3\%$ with complex molecules was disappointingly small (Hostetler et al. 1999).

Table 4. Comparison of n.c.a. ^{18}F-substitution on *para*-substituted aryl(2-thienyl)iodonium bromides (Ross et al. 2005) and aryl(phenyl)iodonium triflates[a]

Reference	 [^{18}F]Fluoroarene	[^{18}F]Fluorobenzene	R	 [^{18}F]Fluoroarene
Gail et al. 1997	38	13	I	60
Ermert et al. 2004	35	15	Br	70
Gail et al. 1997	30	20	Cl	62
Gail et al. 1997	23	41	CH_3	32
Pike et al. 1995	0	88	OCH_3	29

[a]Data are percentrage radiochemical yield ±5% SD

This synthesis of 2-[^{18}F]fluoroestradiol is mentioned as an instructive example, where the nucleophilic n.c.a. ^{18}F-exchange failed not only with an iodonium leaving group but also with nitro groups activated by an *ortho*-aldehyde moiety while the nucleofugality of a trimethylammonium moiety was excellent with activation by a *para*-keto-substituent (Hostetler et al. 1999) as proven also for a similar chemical system (Ermert et al. 2000).

Another approach to achieve stable n.c.a. ^{18}F-labeled aromatic compounds makes use of heteroarenes which favour nucleophilic substitution according to their electronic structure – for example, pyridines. This approach, although already verified almost 25 years ago with ^{18}F-substituted purines (Irie et al. 1982) and nicotinic acid amides (Knust et al. 1982), has attracted increasing interest only in recent years (for review see Dollé 2005), which is in part also due to the availability of newer heteroaromatic pharmaceuticals. As depicted in Fig. 5, again the use of the $[K\subset 2.2.2]_2CO_3$ system together with the suitable nucleofugic groups discussed above, provides high RCY in the activated 2- and 4-positions, while meta-substituted products could be obtained in only one case (Beer et al. 1995) with an additional nucleophilic activating group. The method was successful with various radioligands of neuronal receptors (Dollé 2005). Very recently also quinolines (Perrio et al. 2005) and 1,3-thiazoles (Simeon et al.

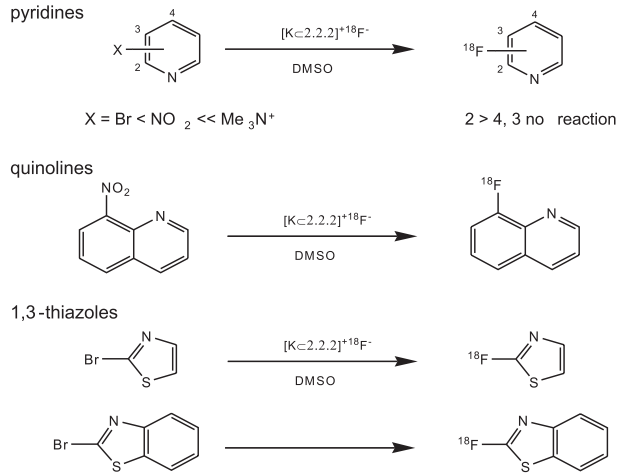


Fig. 5. [^{18}F]Fluoroheteroarenes labeled by direct n.c.a. nucleophilic substitution. (Dollé 2005; Perrio et al. 2005; Simeon et al. 2005)

2005) were introduced as activated precursors for direct nucleophilic ^{18}F-fluorination, thereby broadening this attractive pathway for radiotracer development.

2.4 Radiofluorination Via Built-Up Syntheses

2.4.1 ^{18}F-Fluorinated Synthons

For the preparation of n.c.a. radiolabeled electron rich fluoroarenes two general procedures are possible. In the first one, a precursor of a complex aromatic compound is used which is activated by an electron withdrawing group, or into which such a moiety is additionally introduced, and then the precursor is transferred to the desired electron-rich analogue by converting this group after radiofluorination. The second route makes use of simple activated arenes as listed in Scheme 4, where reactions with the activating groups are used in built-up syntheses of the final tracer. Both procedures imply multi-step radiosyntheses, where either modification or removal of the activating group is required after the ^{18}F-introduction.

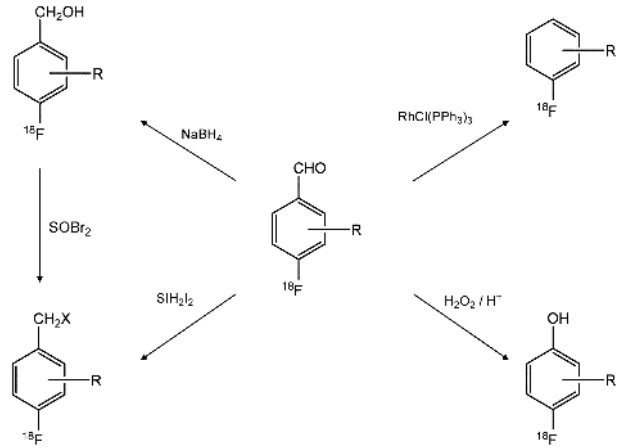


Fig. 6. Possibilities of conversion of [^{18}F]fluorobenzaldehydes as versatile synthons

For example 2- or 4-[^{18}F]fluorobenzaldehydes lend themselves as versatile synthons for this purpose (Fig. 6). Different reductive reactions lead to corresponding benzylhydroxides and halides or the complete removal of an aldehyde group, whereas oxidation provides ^{18}F-substituted phenols (Plenevaux et al. 1992; Chakraborty and Kilbourn 1991a, 1991b). For example, the above mentioned nucleophilic route to fluoroaromatic amino acids proceeds via the secondary radiosynthons of [^{18}F]fluorobenzylhalides (Lemaire et al. 1994). To a limited extent these conversions are also possible with more complex derivatives. It must, however, be pointed out that activation by an aldehyde group, ortho to a nucleofugic moiety, does not guarantee a nucleophilic substitution by n.c.a. [^{18}F]fluoride as found with homo- and heteroaromatic compounds (Hostetler et al. 1999; Perrio et al. 2005). Similarly a broad synthetic versatility was pointed out for aceto-2- and 4-[^{18}F]fluorophenone with even more reaction possibilities than [^{18}F]fluorobenzaldehydes (Banks et al. 1993).

Besides α-ketoarenes, further primary ^{18}F-labeling synthons are known and commonly used in multi-step radiosyntheses of radiopharmaceuticals. Thus, 4-cyano-1-[^{18}F]fluorobenzene has been used in the built-up ^{18}F-labeling procedure of several ^{18}F-labeled butyrophenone

neuroleptics, e.g. [^{18}F]haloperidol, [^{18}F]benperidol, etc. (Shiue et al. 1985). Also benzonitriles can be reduced to benzylamines. More versatile synthons are given by molecules such as 4-[^{18}F]fluoro-4-haloarenes or the secondary radiosynthons such as 2- or 4-[^{18}F]fluoroaniline and -phenyldiazonium salts, formed from 4-[^{18}F]fluoro-1-nitrobenzene, or [^{18}F]fluorophenol.

A further, recently developed built-up ^{18}F-labeling method is the ^{18}F-fluoroarylation. This procedure is based on metallorganic molecules such as 4-[^{18}F]fluorophenyl lithium as versatile metallorganic compound for general coupling reactions. This intermediate can be synthesized from 1-[^{18}F]fluoro-4-haloarenes. As recently compared (Fig. 7; Ermert et al. 2004) these are in turn obtainable from the *para*-halotrimethylanilinium triflates in modest radiochemical yields only (Gail et al. 1993) or more appropriately via symmetrical diaryliodonium salts

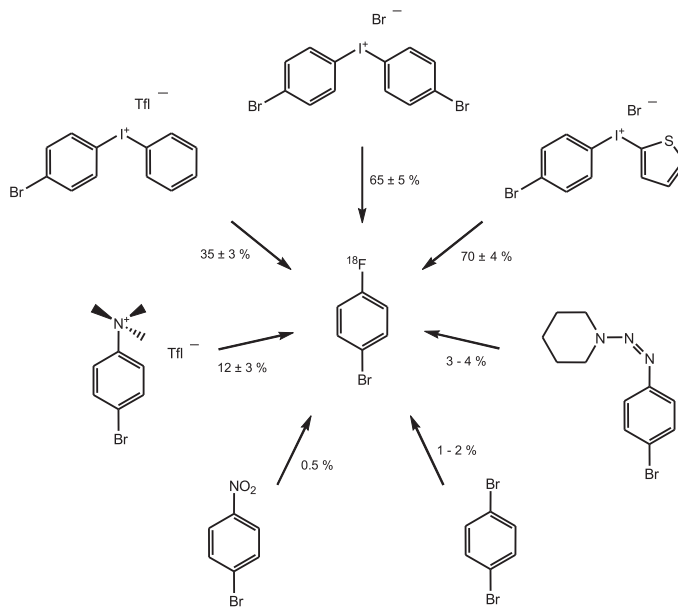


Fig. 7. One-step radiofluorination reactions to *para*-bromo-[^{18}F]fluorobenzene. (Ermert et al. 2004; Ross et al. 2005)

in good RCY of about 50%–60% (Ermert et al. 2004; Wüst and Kniess 2003). Similar RCY was obtained with halophenyl(2-thienyl)iodonium salts (Ross et al. 2005). It was demonstrated that the purity of the iodonium precursor is crucial for optimal radiofluorination yield and that the *para*-iodo-derivative is more reactive than the bromo one, as expected (Wüst and Kniess 2003).

The wide applicability of ^{18}F-fluoroarylation has been demonstrated with several different model compounds which are not accessible by direct substitution (Fig. 8; Ludwig et al. 2001a). Synthons like 1-[^{18}F]fluoro-4-haloarenes can also be used for C–C coupling via Grignard intermediates (Ludwig et al. 2001a) or as reagents for palladium-(0)-catalysed reactions such as the Stille (Allain-Barbier et al. 1998; Wüst and Kniess. 2004), the Hartwig-Buchwald (Marrière et al. 2000) and the Sonogashira reaction (Wüst and Kniess 2003) as elaborated by F. Wüst in Chap. 3 of this volume.

The use of the nitro-moiety in arenes opens another pathway to versatile intermediate [^{18}F]fluoroarenes. The nitro-moiety can not only serve as activating substituent or as nucleofugic group in substitution reactions but also lends itself to further derivatization. As depicted in Scheme 6 this is generally performed by reduction to anilines and possibly their conver-

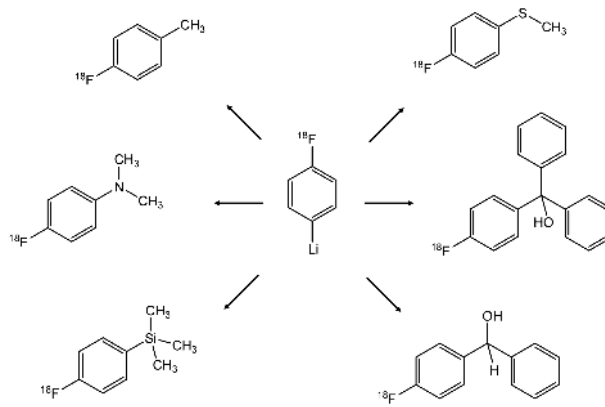
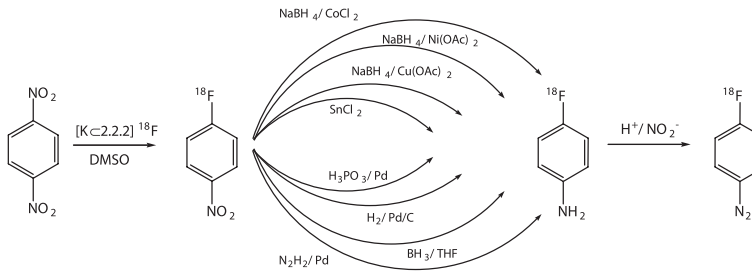


Fig. 8. ^{18}F-Fluoroarylation reactions with [^{18}F]fluorophenyl lithium. (Ludwig et al. 2001a)

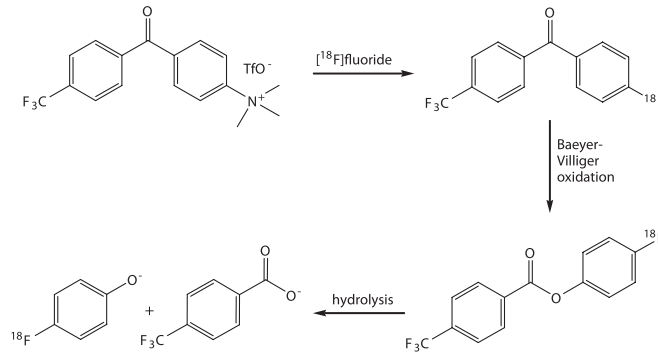
sion to diazonium salts, both of which are versatile synthons for further reactions. This was first exemplified by ^{18}F-exchange on dinitrobenzene and subsequent transformation into simple n.c.a. [^{18}F]fluorobenzenes (Shiue et al. 1984; Shiue et al. 1989).

This pathway proved to be useful also with more complex compounds as shown, for example, for a ligand of the serotonoin reuptake transporter (SERT) (Kung and Shiue 2003). Similarly, receptor ligands were available through the [^{18}F]fluorophenyldiazonium intermediate from n.c.a. [^{18}F]fluoroaniline (Collins et al. 1992) and the improved generation of the latter facilitated a new route to fluorophenylureas (Olma et al. 2005). For the reduction step to [^{18}F]fluoroaniline various agents were tested and those indicated in Scheme 6 show comparably good results.

Another useful secondary synthon for ^{18}F-fluorinations is represented by para-[^{18}F]fluorophenol, which is part of several bioactive substrates (Kirk and Creveling 1984). Various attempts have been made to improve the synthesis and the availability of 4-[^{18}F]fluorophenol. The first practicable radiosynthesis of n.c.a. 4-[^{18}F]fluorophenol proceeded via hydrolysis of a 4-[^{18}F]fluorophenyldiazonium salt (Barré et al. 1993). An enhancement was achieved via the Baeyer-Villiger oxidation of ^{18}F-labeled benzaldehyde, acetophenone and benzophenone with a RCY of about 25% (Ekaeva et al. 1995). Recent detailed studies on the effects of various substituents led to (4-(trifluoromethyl)phenyl)-benzoyl-4′-*N*,*N*,*N*-trimethylammonium triflate as ideal precursor and



Scheme 6. Radiosynthesis of *p*-[^{18}F]fluoroaniline and *p*-[^{18}F]fluorophenyldiazonium



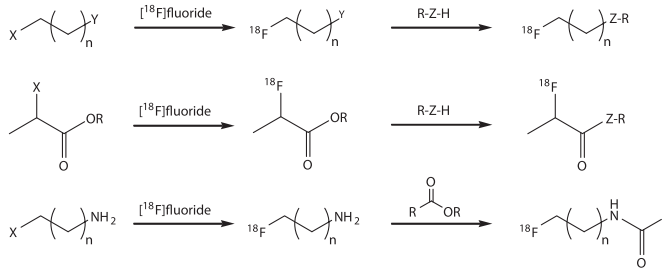
Scheme 7. Improved radiosynthesis of n.c.a. 4-[^{18}F]fluorophenol via Baeyer-Villiger oxidation. (Ludwig et al. 2002)

a 60% RCY of n.c.a. 4-[^{18}F]fluorophenol as depicted in Scheme 7 (Ludwig et al. 2002). Here again, the recent use of 4-benzyloxyphenyl(2-thienyl)iodonium bromide made 4-[^{18}F]fluorophenol available in two reaction steps only with a RCY of 32±2% (Ross et al. 2005) which is easier to automate.

Due to the improved radiosynthesis of n.c.a. 4-[^{18}F]fluorophenol, effective subsequent coupling with alkylhalides to various 4-[^{18}F]fluoroarylalkyl ethers became available; e.g. a dopamine D_4 receptor antagonist (Ludwig et al. 2001b). As examples of diarylethers n.c.a. 2-(4-[^{18}F]fluorophenoxy)-*N*-dimethylbenzylamine and n.c.a. 2-(4-[^{18}F]fluoro-phenoxy)-*N*-methylbenzylamine, structural analogues of known radioligands for SERT, were realized by Ullmann ether coupling of the corresponding 2-bromobenzoic acid amides and a subsequent reduction (Stoll et al. 2004).

2.4.2 ^{18}F-Fluorinated Prosthetic Groups

As mentioned above, another indirect method of ^{18}F-fluorination proceeds via prosthetic groups. This means that a primary ^{18}F-labeled functionalized compound is coupled with a second molecule. Important procedures via prosthetic groups are the ^{18}F-fluoroalkylation (Block et



Scheme 8. Prosthetic group via ^{18}F-fluoroalkylation, ^{18}F-fluoroacylation and ^{18}F-fluoroamidation (X, Y = Br, I, OTs, OTf; Z = N, O, S; R = alkyl, aryl)

al. 1988b), the ^{18}F-fluoroacylation (Kilbourn et al. 1987; Block et al. 1988a), and the ^{18}F-fluoroamidation (Shai et al. 1989) (see Scheme 8).

Applications of these ^{18}F-labeling pathways via prosthetic groups are widespread and can be realized with almost every molecule carrying a protic function such as a thiol, amino or hydroxyl group. Although originally defined for derivatization of peptides, the term prosthetic group was extended also to labeling of monomeric compounds. Thus, the transition from synthons to prosthetic groups is fluent, for which the ether formation with [^{18}F]fluorophenol may serve as an example. This is especially true for the fluoroalkylation of several bio-relevant molecules, e.g. receptor ligands of the dopamine (Coenen et al. 1987) and the serotonin system (Moerlein and Perlmutter 1991), benzodiazepines (Moerlein and Perlmutter 1992), analogues of cocaine (Wilson et al. 1995) and amino acids (Wester et al. 1999) may be mentioned here. Many more tracers were synthesized by this route and in particular [^{18}F]fluoroethylation was the subject of recent improvements by using alkali iodide promotion (Baumann et al. 2003) or [^{18}F]fluoroethyl aryl sulfonates of higher reactivity (Musachio et al. 2005). The ^{18}F-fluoroalkylation agents are prepared in most cases from the symmetrically 1,n-substituted precursors (Block et al. 1987; Eskola et al. 1999). While tosylates are generally preferred for ^{18}F-fluoroethylation (Block et al. 1988b), bromides appear advantageous for ^{18}F-fluoromethylation, as successfully used for labeling of a SERT-ligand (Zessin et al. 2001). This is, of course, strongly influenced by the solvent used.

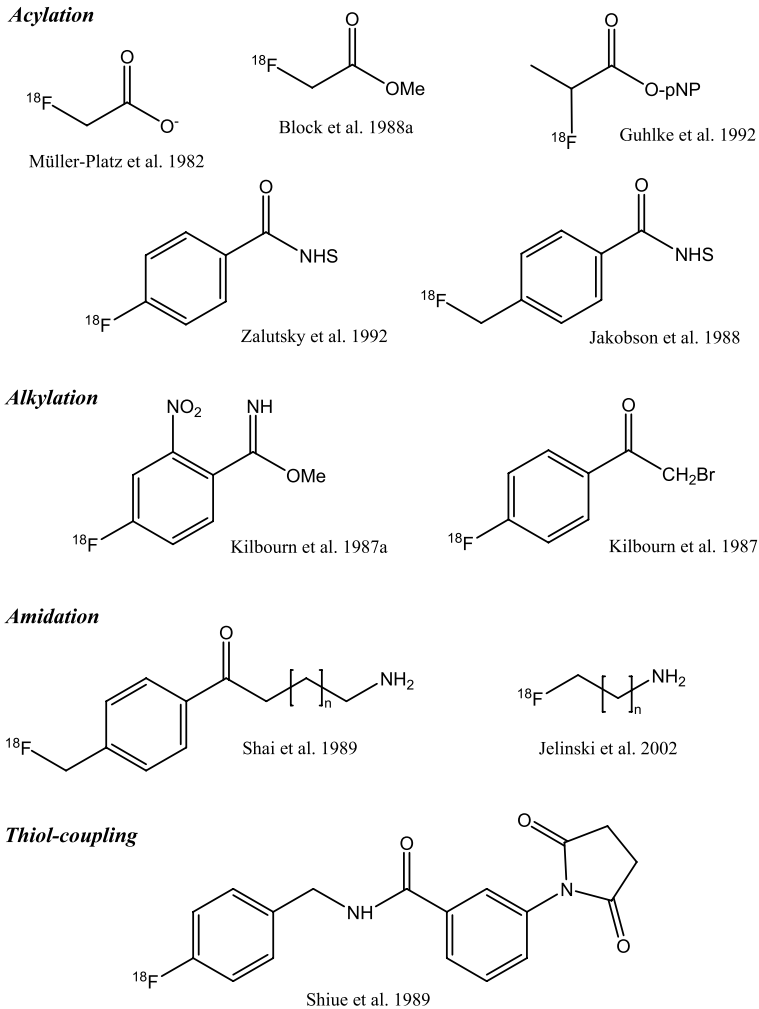


Fig. 9. Examples of prosthetic groups for radiofluorination of peptides and proteins

Unlike ^{18}F-fluoroalkylation, ^{18}F-fluoroacylation and ^{18}F-fluoroamidation (Jelinski et al. 2002) are of great interest primarily for the ^{18}F-labeling of proteins and peptides because they allow radiosyntheses by amide-bond formation in aqueous systems. As a result, ^{18}F-labeled esters (Jakobson et al. 1988; Guhlke et al. 1992) were successfully coupled with, for example, biotin (Guhlke et al. 1991) and octreotide, the first ^{18}F-labeled peptide (Guhlke et al. 1994). ^{18}F-Labeling of peptides and proteins has received much attraction during the last two decades and many more prosthetic groups, and corresponding coupling methods, have been developed, such as the less common imidation (Kilbourn et al. 1987) and photochemical conjugation (Wester et al. 1996). These are summarized exemplarily in Fig. 9 and in a detailed review by Okarvi (2001). In particular, reagents with fluorine-18 in a stable aromatic position were of primary interest (Zalutsky et al. 1992) and, as recent examples, 4-[^{18}F]fluorobenzaldehyde and 4-[^{18}F]fluorophenyldiazonium have been used in chemoselective ^{18}F-labeling of peptides (Poethko et al. 2004; Patt et al. 2002). As very new fluoroprosthetic groups for ^{18}F-labeling of heteropolymers such as peptides, glucoproteins and oligonucleotides, derivatized heteroarylfluorides (for review see Dollé 2005) and even the readily available 2-[^{18}F]fluorodeoxyglucose (Prante et al. 1999; Maschauer et al. 2005) were suggested including enzymatic methods. A dedicated chapter by H.J. Wester in this volume covers these developments.

2.5 Labeling Concepts and Prospects

For reasons of economy (decay of radioactivity), reliability (routine production in remote controlled devices) and radiation protection (of the radiochemist) it is anticipated that the nuclide will be introduced during the last step of preparation of a radiotracer. With fluorine-18 this is often possible directly, or the necessary protecting groups can be removed subsequently. In most cases, however, more complex molecules demand several radiosynthetic steps, which may be due to useful precursors, sensitive functionalities or unsuitable chemical properties of the molecules to be labeled, given the radiofluorination methodology available. This is especially true for electron-rich aromatic compounds as discussed above.

In this case, the race between establishing procedures for electrophilic fluorine species with high molar activity and for developing efficient leaving groups, such as the 2-thienyl iodonium moiety, is presently much in favour of the latter. Electrochemical procedures are presently far from useful in terms of both radiochemical yield and the obtainable molar activity (Kienzle et al. 2005 and references therein) and only marginal yields were obtained under n.c.a. conditions (Hirschfelder et al. 1999).

Impressive multi-step syntheses were elaborated for some radiopharmaceuticals, such as [^{18}F]butyrophenones (Shiue et al. 1985) and [^{18}F]fluoro-L-DOPA (Lemaire et al. 1994). However, for routine production they appear to be too tedious and too complicated. This is why direct substitution (Hamacher and Hamkens 1995) and prosthetic group labeling (Coenen et al. 1987b) were developed for butyrophenones or why an electrophlic method was established for 6-[^{18}F]fluoro-L-DOPA as a low molar activity is here acceptable (Namavari et al. 1992). Often a compromise between multi-step synthesis with a radiosynthon and prosthetic group labeling is decided upon. Examples with limited success so far are the n.c.a. syntheses of 6-[^{18}F]fluoro-L-DOPA (Tierling et al. 2001) and of 4-[^{18}F]fluorometaraminol (Ermert et al. 2000). The latter uses fewer radiosynthetic steps than a previous route (Langer et al. 2001), but it requires more purification steps.

While prosthetic group labeling appears to be necessary for polymeric substances there is an alternative of using a bifunctional [^{18}F]fluoroalkane for the preparation of fluoroalkylated monomeric compounds by final substitution on the already alkylated compound bearing a leaving group. In the case of O-([^{18}F]fluoroethyl)-L-tyrosine the latter proved to be the more effective procedure (Wester et al 1999; Hamacher and Coenen 2002).

Thus, in spite of the many ^{18}F-fluorination methods available, a rational design of a labeling concept cannot be set up for each new molecule. This is in part due to missing procedures, but also due to a lack of predictability of the reaction possibilities of a more complex molecule; this includes missing knowledge on the detailed reaction mechanisms involved. In conclusion, there is still a wide field for further improvement or development of n.c.a. radiofluorination methods. It seems advisable to test and compare various available methods and concepts for the ^{18}F-labeling of new tracer molecules.

Acknowledgements. The author gratefully acknowledges dicussion and support given by Drs. Johannes Ermert and Tobias Ross.

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3 Fluorine-18 Labeling of Small Molecules: The Use of ^{18}F-Labeled Aryl Fluorides Derived from No-Carrier-Added [^{18}F]Fluoride as Labeling Precursors

F. Wuest

Abstract. The favourable long-half life, the ease of production and the low energy of the emitted positron make ^{18}F an ideal radionuclide for PET imaging. Radiochemistry of ^{18}F basically relies on two distinctive types of reactions: nucleophilic and electrophilic reactions. All syntheses of ^{18}F-labeled radiotracers are based on either [^{18}F]fluoride ion or [^{18}F]fluorine gas as simple primary labeling precursors which are obtained directly from the cyclotron. They can be applied either directly to the radiosynthesis or they can be transformed into more complex labeling precursors enabling the multi-step build-up of organic tracer molecules. The topic of this review is a survey on the application of several ^{18}F-labeled aryl fluorides as building blocks derived from no-carrier-added (n.c.a.) [^{18}F] fluoride to build up small monomeric PET radiotracers at high specific radioactivity by multi-step synthesis procedures.

3.1 Basic Considerations of Organic PET Radiochemistry with ^{18}F

Today's PET chemistry has evolved into a complex chemical science, and special attention is focused on radiosyntheses with the most prominent short-lived positron emitters ^{11}C ($t_{1/2}$ = 20.4 min) and ^{18}F ($t_{1/2}$ = 109.8 min). Despite the low abundance of fluorine-containing natural products (O'Hagan and Harper 1999), unlike ^{11}C, the use of ^{18}F offers several advantages. The relatively long half-life, the low positron range and the ease of cyclotron production make ^{18}F an extremely useful PET radioisotope which allows extended multi-step radiosynthesis procedures and monitoring of moderately slow biological processes over scanning periods exceeding 2 h with high spatial resolution. Moreover, in the last decade many important fluorinated small monomeric drugs have been developed which may allow isotopic substitution with ^{18}F (Ismail 2002; Park et al. 2001). In addition to isotopic substitution with ^{18}F, the directed bioisosteric substitution for hydrogen or a hydroxyl group by fluorine is an important alternative strategy for the design of novel ^{18}F-labeled PET radiotracers.

The basic chemical reactions used for the introduction of ^{18}F into a given molecule can generally be subdivided into nucleophilic and electrophilic reactions. Thus, all syntheses of ^{18}F-labeled radiotracers basically commence either with no-carrier-added (n.c.a; high specific radioactivity) nucleophilic [^{18}F]fluoride ion or carrier-added (c.a.; low

specific radioactivity) electrophilic [^{18}F]fluorine gas as the primary labeling precursors. Excellent and very comprehensive reviews on ^{18}F radiochemistry have been published (Coenen 1986; Lasne et al. 2002).

Initially, many ^{18}F-labeled radiotracers were synthesized using electrophilic reactions. The classical example is the original electrophilic radiosynthesis of 2-[^{18}F]fluoro-deoxy-D-glucose ([^{18}F]FDG) which is now almost exclusively conducted via the nucleophilic reaction pathway. However, the ongoing demand for novel specific-binding ^{18}F radiotracers at high specific radioactivity has led to a shift in favour of nucleophilic radiofluorination reactions.

Today's growing list of clinically established and novel potential ^{18}F-labeled radiopharmaceuticals comprises small monomeric compounds which are mainly prepared either by aliphatic or aromatic nucleophilic substitution reactions. In order to obtain PET radiotracers in high radiochemical yields and at high specific radioactivity the radiolabel should be incorporated as late as possible within the synthesis sequence. However, such preferred single-step nucleophilic radiofluorination reactions with n.c.a. [^{18}F]fluoride on suitable labeling precursor molecules are not always applicable. This is especially the case when electron-rich aromatic compounds should be labeled with n.c.a. [^{18}F]fluoride. Moreover, reactions involving a [^{18}F]fluoride ion as the labeling precursor usually require harsh reaction conditions (high temperatures, strongly basic) which often are not compatible with functional groups and may even lead to decomposition of sensitive compounds. As a consequence and to overcome the aforementioned problems, several small ^{18}F bifunctional labeling agents, also referred to as prosthetic groups, have been developed for mild and site-specific incorporation of ^{18}F into a given molecule. The incorporation of the ^{18}F-labeled prosthetic group can be accomplished via alkylation, acylation or photochemical reactions (Kilbourn et al. 1987; Okarvi 2001; Wester et al. 1996; Wuest 2005). The concept of using prosthetic groups was applied especially successfully to the ^{18}F labeling of biomacromolecules such as peptides, proteins and antibodies.

This review is a survey on the application of several ^{18}F-labeled aryl fluorides derived from n.c.a. [^{18}F]fluoride as labeling precursors to build up small monomeric PET radiotracers by multi-step synthesis procedures.

3.2 Application of ^{18}F-Labeled Aryl Fluorides Derived from n.c.a. [^{18}F]Fluoride as Labeling Precursors to the Synthesis of Small Monomeric PET Radiotracers

3.2.1 [^{18}F]Fluorobenzaldehydes in Reductive Amination Reactions

The convenient and high yield synthesis of [^{18}F]fluorobenzaldehydes via nucleophilic aromatic substitutions with readily available [^{18}F]fluoride make these compounds very versatile labeling precursors for a broad range of different chemical reactions involving electrophilic carbon atoms as found in aldehydes.

Several ^{18}F-labeled benzaldehydes have been used in the synthesis of *N*-[^{18}F]fluorobenzylamines via reductive amination reaction. Reductive amination of ketones and aldehydes in the presence of reducing agents are commonly applied reactions in organic synthesis for the formation of amines (Abdel-Magid et al. 1996). The reductive alkylation of amines with [^{18}F]fluorobenzaldehydes was first reported by Wilson and coworkers (Wilson et al. 1990). The reactions are usually performed as one-pot two-step synthesis procedures using dimethylsulfoxide (DMSO) or dimethylfluoride (DMF) as the preferred solvents, acidic acid as a weak acid to adjust pH appropriately to use $NaBH_3CN$ as the reducing agent. The application of one-pot reaction sequences is highly desirable for many reasons, including reducing synthesis time and ease of automation. The general outline of the reductive amination reaction with ^{18}F-labeled benzaldehydes is given in Fig. 1.

The reaction proceeds particularly well and in good radiochemical yields when strong basic secondary amines such as piperidines or piperazines are subjected to reductive amination reaction conditions. Figure 2 shows a selection of various *N*-[^{18}F]fluorobenzylamines which have been synthesised starting from ^{18}F-labeled benzaldehydes and several primary and secondary amines in one-pot two-step procedures according to a reductive amination reaction protocol.

The obtained good radiochemical yields (20%–40%, decay-corrected, based upon [^{18}F]fluoride) and significantly reduced synthesis time make reductive amination a very attractive alternative synthesis method com-

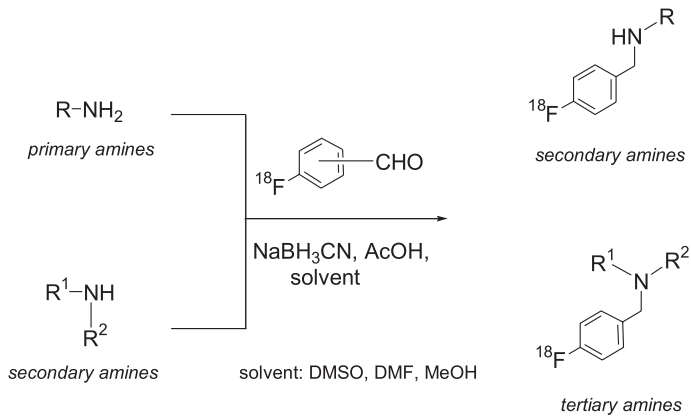


Fig. 1. Reductive aminations with ^{18}F-labeled benzaldehydes

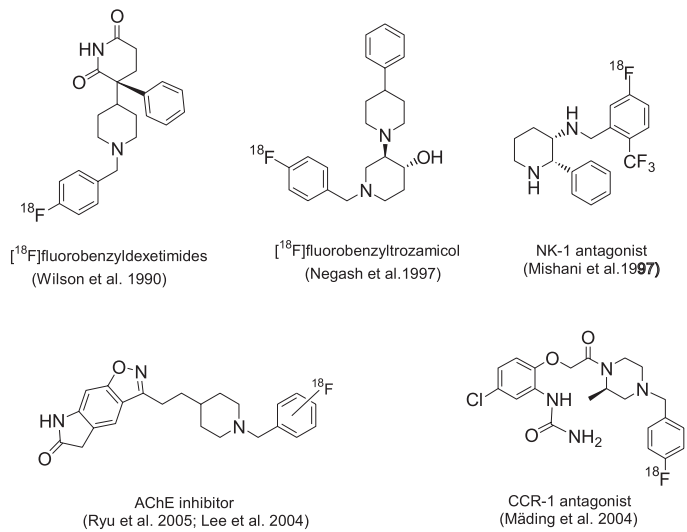


Fig. 2. *N*-[^{18}F]fluorobenzylamines prepared via reductive amination with ^{18}F-labeled benzaldehydes

pared to alkylation reactions with [^{18}F]fluorobenzyl halides (see Sect. 3.2.3) to give the corresponding *N*-[^{18}F]fluorobenzylamines.

3.2.2 [^{18}F]Fluorobenzaldehydes in Carbonyl-Olefination Reactions

The highly electrophilic character of ^{18}F-labeled benzaldehydes make these compounds useful reagents in several carbonyl-olefination reactions involving ylides prepared from phosphoranes (Wittig reaction) or phosphonates (Horner-Wadsworth-Emmons reaction), and C–H acidic compounds lacking an α hydrogen (Knoevenagel reaction).

All these base-mediated condensation reactions of active-hydrogen compounds were performed with 4-[^{18}F]fluorobenzaldehyde as the carbonyl component.

The radiosynthesis of L-4-[^{18}F]fluorophenylalanine using a Knoevenagel reaction of 4-[^{18}F]fluorobenzaldehyde with 2-phenyl-5-oxazolone as the key step was reported by Lemaire and co-workers (Lemaire et al. 1987). Acidic hydrolysis of the intermediate benzylidene compound resulted in the formation of racemic [4-^{18}F]fluorophenylalanine. Enantiomeric pure L-4-[^{18}F]fluorophenylalanine was obtained in 5% radiochemical yield after chiral HPLC purification. The entire synthesis sequence of L-[4-^{18}F]fluorophenylalanine is depicted in Fig. 3.

Wittig reactions of 4-[^{18}F]fluorobenzaldehyde with several ylides derived from phosphonium salts have been reported in the literature (Piarraud 1993). However, despite the obtained reasonable radiochemical yields (RCY) selectivity problems regarding to the formation of *E*- and *Z*-isomers in course of the reaction have been observed (Fig. 4).

Thus, stable ylides preferentially lead to the formation of the thermodynamically stable *E*−isomer, whereas less stable ylids lead to the corresponding *Z*−isomer. Ylides containing aryl substituents are considered to be semi-stable and, therefore, forming mixtures of both isomers. In order to circumvent the *E*-/*Z*- selectivity problems the Horner-Wadsworth-Emmons reaction was used as an alternative carbonyl-olefination reaction capable of creating a carbon–carbon double bond, which exclusively displays the desired *E*-isomer configuration of the resulting olefin.

Thus, coupling of phosphonic acid diester with readily available 4-[^{18}F]fluoro-benzaldehyde and subsequent cleavage of the MOM-pro-

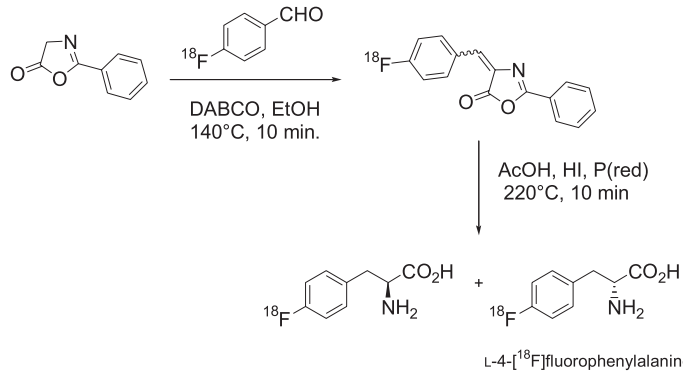


Fig. 3. Radiosynthesis of L-[4-^{18}F]fluorophenylalanine using a Knoevenagel reaction as the key step

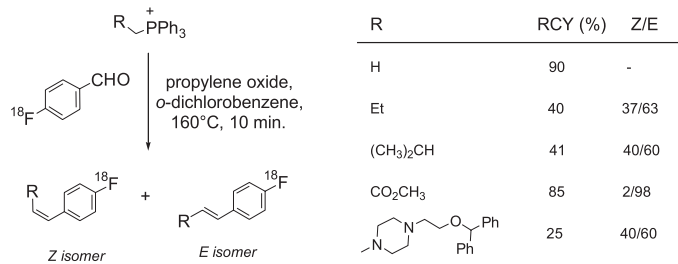


Fig. 4. Wittig reactions with 4-[^{18}F]fluorobenzaldehyde

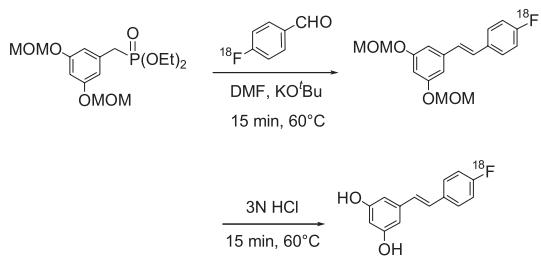


Fig. 5. Horner-Wadsworth-Emmons reaction with 4-[^{18}F]fluorobenzaldehyde

tecting groups led to a stilbene derivative as the desired *E*−isomer exclusively (Gester et al. 2005). The reaction proceeded in 9% RCY (decay-corrected, based upon [^{18}F]fluoride). DMF proved to be the solvent of choice for the three-step one-pot reaction sequence (Fig. 5).

3.2.3 Nucleophilic Additions to [^{18}F]Fluorobenzaldehydes

Several nucleophilic addition reactions of various carbon nucleophiles to [^{18}F]fluorobenzaldehydes have been used for the synthesis of ^{18}F-labeled radiotracers.

The preparation of the catecholamine 6-[^{18}F]fluoronorepinephrine has been performed in a multi-step synthesis sequence by the addition of trimethylsilylcyanide to 3,4-*O*-isopropylidene-6-[^{18}F]fluorobenzaldehyde as the key step. 3,4-*O*-Isopropylidene-6-[^{18}F]fluorobenzaldehyde as labeling precursor was synthesised via aromatic nucleophilic substitution with [^{18}F]fluoride starting from the corresponding nitro compound. Subsequent reduction of the formed cyanohydrin trimethylsilyl ether and cleavage of the 1,2-diol protecting group afforded racemic 6-[^{18}F]fluoronorepinephrine in 20% RCY at the end-of-bombardment (Ding et al. 1991). The synthesis scheme is summarized in Fig. 6.

The synthesis of 4- and 6-[^{18}F]fluorometaraminol at high specific radioactivity employing the nucleophilic addition of nitroethane as a carbon nucleophile to a *O*-benzyl protected ^{18}F-labeled benzaldehyde is another example for this class of reaction (Langer et al. 2001). Cleavage of the benzyl ether protecting group by means of Pd/C-ammonium formate and simultaneous reduction of the nitro group gave 4- and 6-[^{18}F]fluorometaraminol as a mixture of diastereomers (Fig. 7). The desired stereoisomers could be obtained by means of two subsequent HPLC purifications.

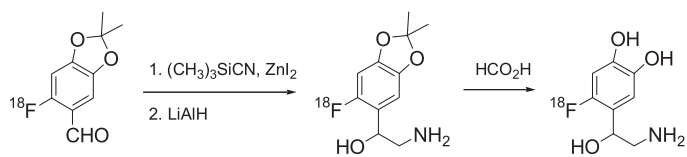


Fig. 6. Synthesis of 6-[^{18}F]fluoronorepinephrine

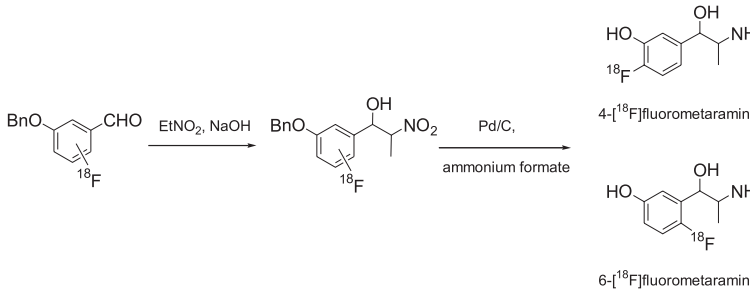


Fig. 7. Radiosynthesis of diastereomeric 4- and 6-[^{18}F]fluorometaraminol

3.2.4 [^{18}F]Fluorobenzyl Halides in Heteroatom Alkylation Reactions

[^{18}F]Fluorobenzaldehydes can easily be transformed into various [^{18}F]fluoro-benzyl halides as useful reagents for heteroatom alkylation reactions (Dence et al. 1997; Hatano et al. 1991; Iwata et al. 2000a; Lemaire et al. 2004; Mach et al. 1993). The most prominent [^{18}F]fluorobenzyl halides for alkylation reactions are [^{18}F]fluorobenzyl iodide and [^{18}F]fluorobenzyl bromide.

Principally [^{18}F]fluorobenzylations can be performed on amino, thiol and hydroxyl groups. However, most reactions were carried out using compounds bearing primary or secondary amines. As typical S_N2 reactions the use of aprotic polar solvents (DMF, DMSO, CH_3CN) is preferred. The appropriate reaction conditions (temperature, auxiliary base) mainly depend on the nucleophilicity of the heteroatom used.

The first example of using [^{18}F]fluorobenzyl halides in alkylation reactions was demonstrated by the synthesis of neuroleptics via *N*-alkylation with [^{18}F]fluoro-benzyl bromide (Hatano et al. 1991). When 4-[^{18}F]fluorobenzyl bromide is used the reaction is performed in CH_3CN as the solvent and using $NaHCO_3$ as the base. However, these reaction conditions fail in the case of 2-[^{18}F]fluorobenzyl bromide, and DMSO as the solvent and tetrabutylammonium hydroxide (TBAOH) as the base give the best yield. The synthesis scheme is given in Fig. 8.

In a series of alkylation reactions the reactivity of 4-[^{18}F]fluorobenzyl iodide as the most prominent 4-[^{18}F]fluorobenzyl halide in alkylation re-

actions towards several sulphur- and nitrogen-containing substrates with different nucleophilicity was investigated (Mach et al. 1993). Reactions of 4-[^{18}F]fluorobenzyl iodide with secondary amines such as piperidine and piperazine derivatives gave the corresponding *N*-4-[^{18}F]fluorobenzyl amines in high yield under mild reaction conditions (ambient temperature, 5 min) (Fig. 9).

Similar results were found in the case of thiol group-containing compounds but utilization of a base is necessary. Thus, reaction of 4-[^{18}F]fluorobenzyl iodide with the thiol group of 4′-mercapto SCH 23390 in the presence of triethylamine as the base occurred very rapidly at room temperature to form D_1 receptor ligand [^{18}F]BT-SCH in 40%–60% decay-corrected yield (Fig. 10).

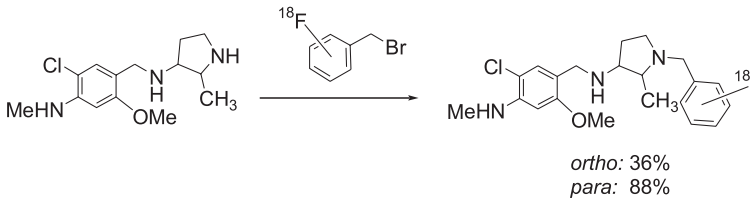


Fig. 8. *N*-Alkylation reactions with 2- and 4-[^{18}F]fluorobenzyl bromide

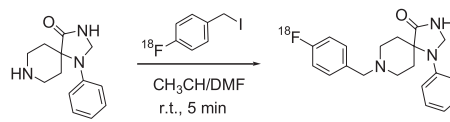


Fig. 9. Alkylation of piperidine nitrogen with 4-[^{18}F]fluorobenzyl iodide

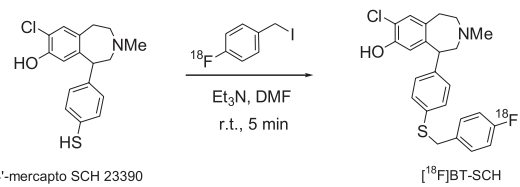


Fig. 10. Alkylation of thiol 4′-mercapto SCH 23390 with 4-[^{18}F]fluorobenzyl iodide

Aniline nitrogens as lower-reactive nitrogen nucleophiles require more vigorous reaction conditions (higher temperature) and a mixture of DMF and CH_3CN as the solvent. Amide nitrogen can also be alkylated with 4-[^{18}F]fluorobenzyl iodide when CH_3CN as the solvent and tetraethylammonium hydroxide (TEOH) as the base are used (Fig. 11). The use of tetrabutylammonium hydroxide instead of TEOH gives lower radiochemical yields. A drawback of this reaction is the formation of substantial amounts of 4-[^{18}F]fluorobenzyl alcohol due to hydrolysis of 4-[^{18}F]fluorobenzyl iodide under these conditions.

The alkylation of a hydroxyl group with 4-[^{18}F]fluorobenzyl halides was exemplified by the synthesis of histamine H_3 receptor ligand [^{18}F]fluoroproxyfan (Iwata et al. 2000b). The synthesis involved *O*-alkylation of trityl-protected (1*H*-imidazol-4-yl)propanol with 4-[^{18}F]fluorobenzyl bromide in the presence of silver triflate (AgOTf) and 2,6-di-*tert*-butylpyridine as a non-nucleophilic amine base. Subsequent cleavage of the trityl protecting group gave [^{18}F]fluoroproxyfan in 10% decay-corrected RCY (based upon [^{18}F]fluoride). The synthesis scheme is shown in Fig. 12.

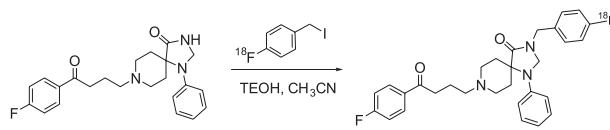


Fig. 11. Alkylation of amides with 4-[^{18}F]fluorobenzyl iodide

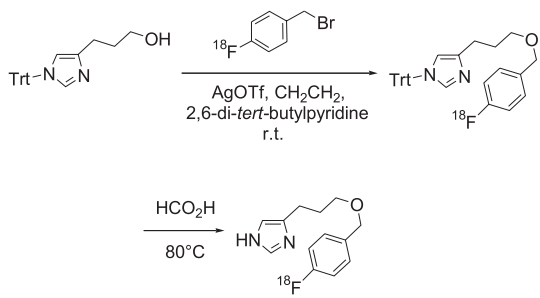


Fig. 12. Alkylation of alcohols with 4-[^{18}F]fluorobenzyl bromide

The easy access to ^{18}F-labeled benzyl halides and the possibility to alkylate various nitrogen, sulphur and oxygen nucleophiles make [^{18}F]fluorobenzylation reactions a valuable approach for the synthesis of a broad variety of PET radiotracers. However, [^{18}F]fluorobenzylation reactions usually consist of a time-consuming multi-pot/multi-step synthesis sequence involving: (1) preparation of [^{18}F]benzaldehyde from cyclotron-produced n.c.a. [^{18}F]fluoride; (2) transformation of [^{18}F]benzaldehyde into the corresponding [^{18}F]benzyl halide; and (3) subsequent heteroatom benzylation reaction which make automation of the radiosynthesis process a special challenge.

3.2.5 4-[^{18}F]Fluorohalobenzenes in Palladium-Mediated C–C and C–N Bond Formations

In addition to significant progress in radionuclide production and automation, recent advances in modern preparative organic chemistry have stimulated novel developments in radiochemistry with the short-lived positron emitters ^{11}C and ^{18}F. In the last decade especially the use of palladium-mediated reactions has proved to be an exceptionally valuable approach for rapid and efficient syntheses of a wide variety of ^{11}C-labeled radiotracers via ^{11}C–C bond forming reactions (Langström et al. 1999). However, only a few attempts have been made to adapt the recent advances in palladium-mediated reactions to the synthesis of ^{18}F-labeled radiotracers. The palladium-catalysed cross-coupling reactions of organometallic compounds such as Grignard reagents, organotin, or organoboron derivatives with organic halides suggests the use of ^{18}F-labeled aryl halides as the electrophilic coupling partners. This approach can be regarded as a general method for the mild and efficient introduction of a 4-[^{18}F]fluorophenyl group into a wide variety of complex functionalized target molecules. In recent years several synthetic pathways for the preparation of ^{18}F-labeled aryl halides have been developed (Allain-Barbier et al. 1998; Ermert et al. 2004; Gail and Coenen 1994; Gail et al. 1997; Ross et al. 2005; Shah et al. 1998; Wuest and Kniess 2003, 2004) making these versatile ^{18}F-labeling precursors now readily available. These developments have stimulated many efforts to prepare ^{18}F-labeled radiotracers via palladium-mediated cross-coupling with 4-[^{18}F]bromofluorobenzene and 4-[^{18}F]fluoroiodo-benzene.

One of the first palladium-mediated cross-coupling reactions applied to ^{18}F chemistry was the Stille reaction (Stille 1986).

The Stille reaction enjoys much success in synthetic organic synthesis because it proceeds in high yields under mild reaction conditions while tolerating a broad range of functional groups (e.g. CO_2R, CN, OH) on either coupling partner. The organostannanes as coupling partner possess some beneficial properties that similar organometallics do not. Organostannanes can easily be synthesized and isolated without excessive precautions. Moreover, many organostannanes are also commercially available. They are typically air- and moisture-stable compounds allowing their trouble-free storage. The coupling between an organic electrophile and an organostannane can be achieved through an array of different palladium complexes such as $Pd(PPh_3)_4$, $Pd_2(dba)_3$ or $Pd(PPh_3)_2Cl_2$. The development of novel ligands and the use of additives have further expanded the scope of the Stille reaction. The Stille cross-coupling process follows the general catalytic cycle for palladium-mediated cross-coupling reactions involving oxidative addition-transmetalation-reductive elimination as depicted in Fig. 13.

The beneficial features of the Stille reaction with ^{18}F-labeled aryl halides for the synthesis of several 4-[^{18}F]fluorophenyl-containing radiotracers was exemplified by the radiosynthesis of nicotinic acetylcholine receptor ligand 9-(4′-[^{18}F]fluorophenyl)cytisine (Marriére et al. 1999), [^{18}F]fluvastatin (Forngren et al. 1998), several 5-(4′-[^{18}F]fluorophenyl)-substituted nucleosides (Wuest and Kniess. 2004) and 4-[^{18}F]fluorophenyl-substituted COX-2 inhibitors (Wuest et al. 2005). Careful optimization of the reaction conditions by screening several Pd-complexes,

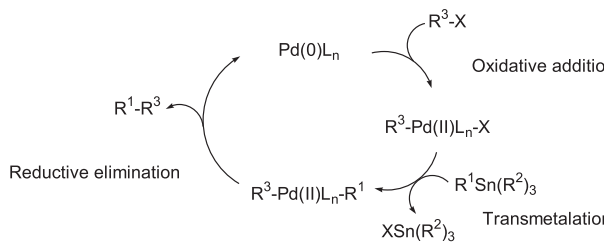


Fig. 13. Catalytic cycle for the Stille reaction

solvents, co-ligands and additives gave cross-coupling yields of up to 98% (based upon 4-[^{18}F]fluorohalobenzene). A selection of ^{18}F-labeled compounds prepared according to a Stille cross-coupling of organostannanes with 4-[^{18}F]fluorohalobenzene is given in Fig. 14.

The palladium(0)/copper(I)-catalysed coupling of terminal alkynes with aromatic and vinyl halides, also known as the Sonogashira reaction, is an other example of an effective and widely used method to form C–C bonds (Sonogashira et al. 1975). The Sonogashira reaction follows the normal oxidative addition-reductive elimination process common to palladium-catalysed carbon–carbon bond forming reactions. It can be performed under mild reaction conditions and tolerates a broad range of functional groups. The presumed catalytic cycle for the Sonogashira reaction is given in Fig. 15.

The cross-coupling of 4-[^{18}F]fluorohalobenzenes with terminal alkynes under classical Sonogashira reaction conditions leads to the formation of 4-[^{18}F]fluorophenylethynyl-substituted compounds. The general reaction conditions for the cross-coupling of terminal alkynes with 4-[^{18}F]fluoroiodobenzene were optimized by using the 17α-ethynyl substituted steroid mestranol as a model compound to give the corre-

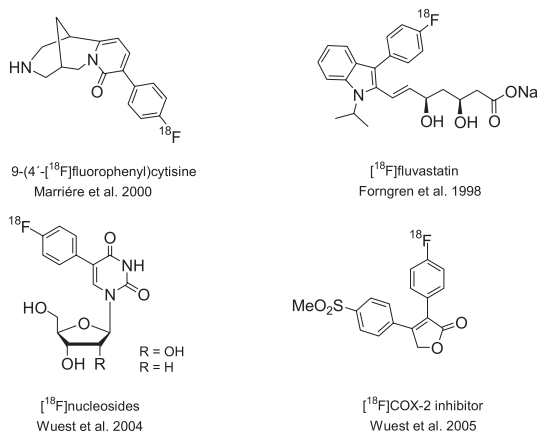


Fig. 14. Selection of ^{18}F-labeled compounds prepared according to a Stille reaction

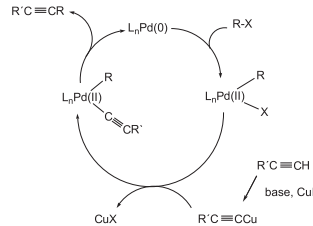


Fig. 15. Presumed catalytic cycle for the Sonogashira reaction

sponding 4-[^{18}F]fluorophenyl-substituted mestranol derivative (Wuest and Kniess 2003) (Fig. 16).

Optimized reaction conditions ($Pd(PPh_3)_4$/CuI in THF/triethylamine, 20 min at 120 °C) gave the desired cross-coupled product in yields of up to 64% (based upon 4-[^{18}F]fluoroiodobenzene). Optimized reaction conditions were applied to the synthesis of other 17α-ethynyl substituted steroids to give the corresponding 17α-(4-[^{18}F]fluorophenylethynyl)-substituted compounds as shown in Table 1.

The short reaction time, the high radiochemical yield and the tolerance toward several functional groups make the Sonogashira reaction and Stille reaction with 4-[^{18}F]fluorohalobenzenes a valuable approach for the synthesis of 4-[^{18}F]fluoro-phenyl group containing PET radiotracers via directed C–C bond formations.

Moreover, 4-[^{18}F]fluorohalobenzenes can also be used in cross-coupling reactions involving heteroatoms. In recent years several research groups have demonstrated very efficient synthetic methods for aromatic carbon-nitrogen bond formation (Wolfe et al. 1998). The first application of a aromatic carbon–nitrogen bond formation, also referred to as *N*-ary-

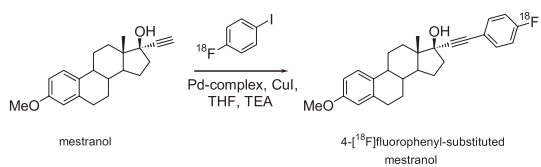
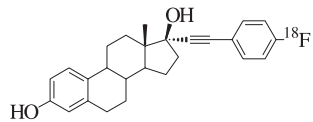
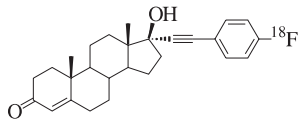
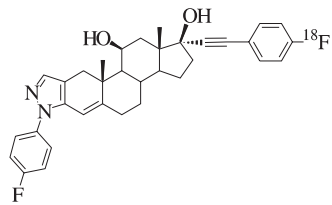


Fig. 16. Sonogashira cross-coupling reaction of mestranol with 4-[^{18}F]fluoro-iodobenzene

Table 1. Radiochemical yields for the cross-coupling of terminal alkynes with 4-[^{18}F]fluoroiodo-benzene

Product	Radiochemical yield (based upon 4-[^{18}F]fluoroiodobenzene)[a]
	65%–88%
	90%
	81%

[a] Reactions were carried out employing optimized reaction conditions (3 mg alkyne, 3 mg CuI, 3 mg $Pd(PPh_3)_4$, THF/TEA, 120 °C, 20 min)

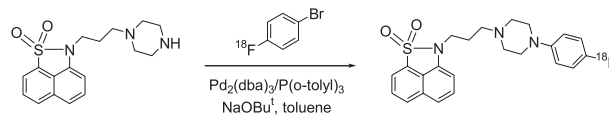


Fig. 17. Synthesis of [^{18}F]RP 62203 via *N*-arylation with 4-[^{18}F]bromofluorobenzene

lation reaction, in ^{18}F chemistry was exemplified by the synthesis of the selective 5-HT_{2A} receptor ligand [^{18}F]RP 62203 via palladium-assisted *N*-arylation of the piperazine moiety with 4-[^{18}F]bromofluorobenzene as shown in Fig. 17 (Marriére et al. 1999).

An extension of the *N*-arylation reaction with 4-[^{18}F]fluorohalobenzenes to indoles was achieved by the synthesis of two ^{18}F-labeled σ_2 receptor ligands (Wuest and Kiess 2005) (Fig. 18).

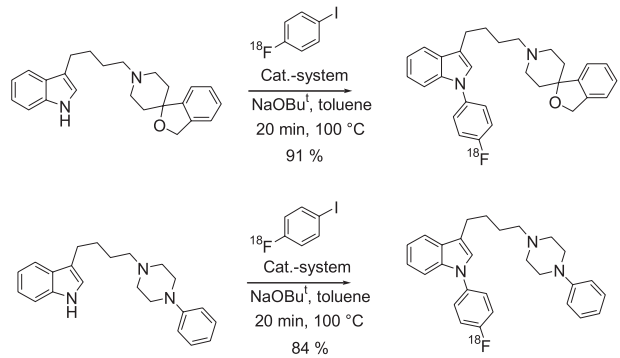


Fig. 18. Radiolabeling of σ_2 receptor ligands via *N*-arylation with 4-[^{18}F]fluoroiodobenzene

A catalyst system consisting of $Pd_2(dba)_3$ as the palladium complex and 2-(dicyclohexyl-phosphino)-2′-(*N*, *N*-dimethylamino)-biphenyl as an electron-rich phosphine ligand proved to provide the highest radiochemical yields of 91% and 84%, respectively (based upon 4-[^{18}F]fluoroiodobenzene).

3.2.6 4-[^{18}F]Fluorohalobenzenes in ^{18}F-Fluoroarylation Reactions via ^{18}F-Labeled Organometallic Reagents

The conversion of 4-[^{18}F]fluorohalobenzenes into organometallic 4-[^{18}F]fluoro-phenyl compounds and their application to coupling reactions represents another interesting approach for ^{18}F-fluoroarylation reactions. The preparation of ^{18}F-labeled organometallic reagents such as 4-[^{18}F]fluorophenylmagnesium bromide and 4-[^{18}F]fluorophenyllithium has been reported recently (Ludwig et al. 1998, 2001a).

The synthesis of 4-[^{18}F]fluorophenyllithium was carried out starting from 4-[^{18}F]bromofluorobenzene using lithium metal and bromobenzene as a co-reacting compound under ultrasound activation. Likewise, a comparable procedure was used for the synthesis of 4-[^{18}F]fluorophenylmagnesium bromide by the reaction of 4-[^{18}F]bromofluorobenzene in the presence of magnesium metal and 1,2-dibromoethane as activator.

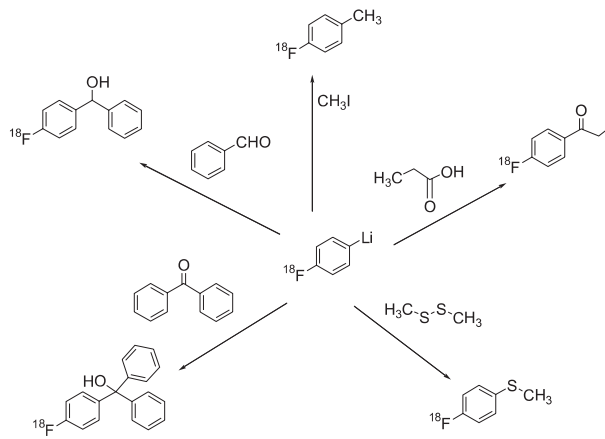


Fig. 19. Radiosyntheses using 4-[^{18}F]fluorophenyllithium via ^{18}F-fluoroarylation reactions

The feasibility of organometallic-mediated ^{18}F-fluoroarylation reactions was demonstrated with several model compounds, such as alkylhalides, carboxylic acids, disulfides, ketones and aldehydes, to give the corresponding [^{18}F]fluoroalkylarenes, ^{18}F-fluorophenyl ketones, ^{18}F-fluorophenyl thioethers, and 3° or 2° aromatic ^{18}F-fluorophenyl alcohols (Fig. 19).

The results demonstrate the usefulness of ^{18}F-fluoroarylation reaction via ^{18}F-labeled organometallic reagents for the synthesis of a broad variety of compounds exhibiting different substitution pattern.

3.2.7 4-[^{18}F]Fluorobenzylamines, [^{18}F]Fluoroanilines, 4-[^{18}F]Fluorophenol and 4-[^{18}F]Fluoroacetophenone in the Synthesis of Small Monomeric PET Radiotracers

Compared to the dominant role of [^{18}F]fluorobenzaldehydes, [^{18}F]fluorobenzyl halides and [^{18}F]fluorohalobenzenes in the synthesis of small monomeric PET radiotracers other ^{18}F-labeled aryl fluorides (4-[^{18}F]fluorobenzyl amine, [^{18}F]fluoroanilines, 4-[^{18}F]fluorophenol and 4-[^{18}F]fluoroacetophenone) were utilized to a much lower extent. Unlike its frequent application in oligonucleotide labelings with the

4-[^{18}F]fluorobenzyl amine-derived prosthetic group *N*-4-[^{18}F]fluorobenzyl-α-bromoacetamide (Dollè et al. 1996; Kuhnast et al. 2000) there are only few examples using 4-[^{18}F]fluorobenzyl amine itself as a labeling precursor for the synthesis of small monomeric PET radiotracers. 4-[^{18}F]Fluorobenzylamine can be prepared in a one-pot synthesis using 4-*N*,*N*,*N*-trimethylammoniumbenzonitrile triflate as the starting material. After reaction with [^{18}F]fluoride the mixture containing 4-[^{18}F]fluorobenzonitrile as intermediate was treated with a reducing reagent (e.g. $LiAlH_4$, $NaBH_4$-$CoCl_2$, BH_3-Me_2S) to give the desired 4-[^{18}F]fluorobenzylamine (Haradahira et al. 1998; Shiue et al. 1989). Moreover, 4-[^{18}F]fluorobenzonitrile itself was shown to be an interesting labeling precursor for the synthesis of ^{18}F-labeled butyrophenone neuroleptics benperidol, haloperidol, spiroperidol and pipamperone (Shiue et al. 1985).

An application of 4-[^{18}F]fluorobenzylamine as labeling precursor involves the synthesis of the ^{18}F-labeled analogue of an anti-tumour prostaglandin Δ^7-PGA_1 methyl ester. Treatment of 4-[^{18}F]fluorobenzyl amine with the *N*-succinimidyl active ester of 15-deoxy-13,14-dihydro-Δ^7-PGA_1 in acetonitrile at room temperature for 15 min gave the desired compound in 58% decay-corrected RCY (based upon 4-[^{18}F]fluorobenzylamine) (Haradahira et al. 1998). The synthesis scheme is given in Fig. 20.

Several [^{18}F]fluoroanilines can conveniently be prepared by the reduction of the corresponding [^{18}F]fluoronitrobenzens (VanBrocklin et al. 2001). The common 4-anilino substitution pattern in a broad range of quinazoline-based epidermal growth factor receptor (EGFR) ligands

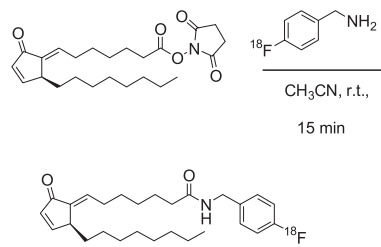


Fig. 20. Synthesis of 15-deoxy-13,14-dihydro-Δ^7-PGA_1 4-[^{18}F]fluorobenzylamide

have stimulated many efforts to make use of various [^{18}F]anilines as key labeling precursors in the synthesis of ^{18}F-labeled EGFR ligands. However, the use of 4-[^{18}F]fluoroaniline in the synthesis of ^{18}F-labeled EGFR ligands seems to be not optimal as the corresponding 4′-[^{18}F]fluoroanilinoquinazolines were shown to metabolically radiodefluorinate (Snyder et al. 2000; VanBrocklin et al. 2003).

The most prominent synthesis approach consists of the condensation of [^{18}F]anilines with 4-chloroquinazoline intermediates at elevated temperatures in 2-propanol or DMF as the solvent to afford the corresponding [^{18}F]aniline-quinazolines (VanBrocklin et al. 2001; Seimbille et al. 2005). The synthesis of three ^{18}F-labeled PET radiotracers based on the therapeutic agents Tarceva, Iressa and ZD6474 is depicted in Fig. 21.

An alternative approach to [^{18}F]anilinoquinazolines was recently reported (Vasdev et al. 2005). This approach involves a ring closure reaction of *N*′-(2-cyano-4-nitrophenyl)-*N*,*N*-dimethylimidoformamide with 2-[^{18}F]aniline as the key step to form a 6-nitro-4-anilino-quinazoline which was further converted into 6-acryl-amido-4-(2-[^{18}F]fluoroanilino)quinazoline (Fig. 22).

The improved availability of n.c.a. 4-[^{18}F]fluorophenol (Barrè et al. 1993; Ludwig et al. 2001b, 2002; Stoll et al. 2004) offers the possibility to prepare various PET radiotracers containing a 4-[^{18}F]fluorophenoxy moiety. 4-[^{18}F]fluorophenol was applied to the synthesis of a highly

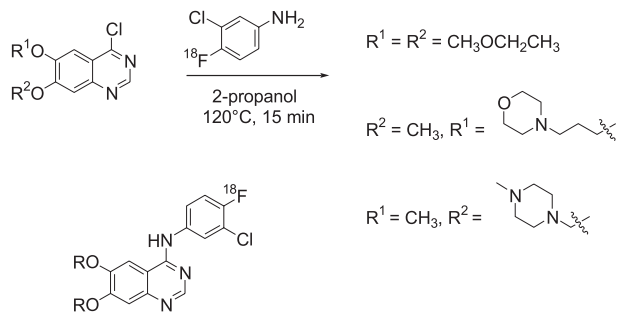


Fig. 21. Synthesis ^{18}F-labeled PET radiotracers based on the therapeutic agents Tarceva, Iressa and ZD6474

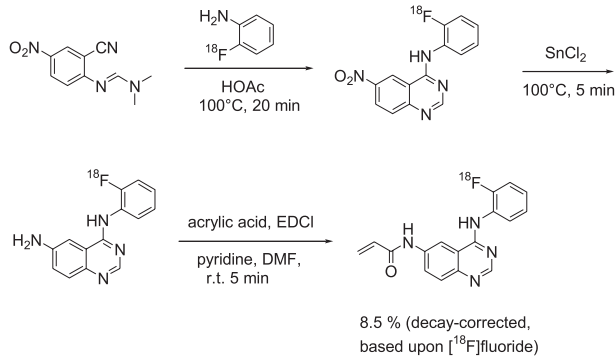


Fig. 22. Radiosynthesis 6-acryl-amido-4-(2-[^{18}F]fluoroanilino)quinazoline

selective dopamine D_4 receptor ligand (Ludwig et al. 2001b). In a one-pot react ion 4-[^{18}F]fluorophenol was used in the alkylation of 1,3-dibromopropane followed by a second alkylation reaction of 4-[^{18}F]fluorophenoxypropyl bromide with 3-(4-tolyloxy)-ethylamine to give the D_4 selective radioligand in 50% yield (based upon 4-[^{18}F]fluorophenol). The radiosynthesis of the D_4 selective ligand is summarized in Fig. 23.

Another example of using 4-[^{18}F]fluorophenol as a labeling precursor was reported by the synthesis of 2-(4-[^{18}F]fluorophenoxy)-benzylamines employing an Ullmann ether synthesis as the key step in the reaction sequence (Stoll et al. 2004) (Fig. 24).

The easy access to 4-[^{18}F]fluoroacetophenone via nucleophilic aromatic substitution with [^{18}F]fluoride starting from 4-nitroacetophenone makes this compound an attractive labeling precursor. Transformation of 4-[^{18}F]fluoro-acetophenone onto a solid phase (A-26, Br_3^-) gave

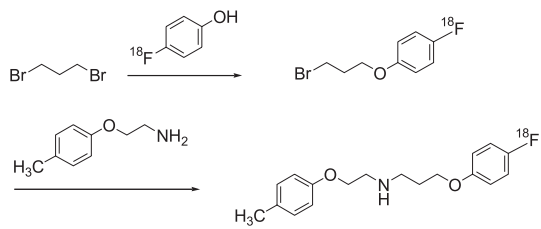


Fig. 23. Radiosynthesis of a D_4 selective ligand using 4-[^{18}F]fluorophenol

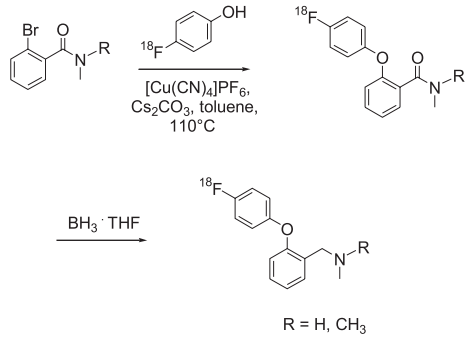


Fig. 24. Synthesis of diarylethers via Ullman coupling with 4-[^{18}F]fluorophenol

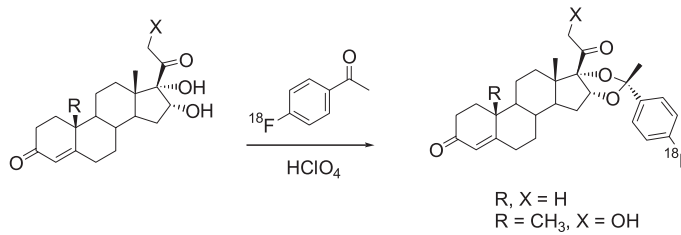


Fig. 25. Ketalization reactions with 4-[^{18}F]fluoroacetophenone

4-[^{18}F]fluorophenacylbromide, a compound which was shown to be a promising reagent for conjugation to peptides containing thiol groups (Downer et al. 1997). 4-[^{18}F]Fluoroacetophenone as a labeling precursor was used for the synthesis of two ^{18}F-labeled steroid hormones via acid-promoted ketalization reactions (Kochanny et al. 1993). However, considerable adjustment of reaction conditions was required to effect ketalization using n.c.a. 4-[^{18}F]fluoroacetophenone. The reaction scheme is given in Fig. 25.

3.3 Conclusion

Today's arsenal of ^{18}F-labeled PET radiotracers comprise several hundred compounds, most of them representing small monomeric substances. The increasing number of compounds with more and more complex structures often demands labeling strategies based upon multi-step syntheses involving suitable ^{18}F labeling precursors. In this connection much successful work has been done by the radiochemists over the last two decades. Moreover, there is still fertile ground for further improvements and novel developments of n.c.a. radio-fluorination strategies. This fact is, for example, demonstrated by the recent utilization of [^{18}F]fluorohalobenzenes in various palladium-mediated cross-coupling reactions and the use of [^{18}F]fluorophenol in the synthesis of [^{18}F]fluorophenyl ethers.

However, all this basic radiochemistry work now has to be transformed into synthesis protocols enabling the large-scale production of ^{18}F-labeled PET with pharmaceutical quality. This challenge implies special consideration of good manufacturing practice and good laboratory practice. Therefore, further work especially should be focused on multi-step radiosyntheses via ^{18}F labeling precursors which can be performed in remotely-controlled synthesis apparatus. The currently commercially available synthesis apparatus are not well suited for complex multi-pot/multi-step radiosyntheses. Here, special synthesis apparatus are needed which allow, on the one hand, the convenient preparation of the n.c.a. ^{18}F labeling precursor and, on the other hand, the application of the ^{18}F labeling precursor in complex built-up radiosyntheses including solid-phase-extraction procedures and HPLC purification in the same apparatus.

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4 Fluorine-18 Labeling of Peptides and Proteins

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Abstract. The pool of promising peptides worthy of investigation and evaluation for clinical use is continuously filled from different sources. Driven by the promising results obtained with peptides addressing somatostatin-2 receptor positive (sst_2+) neuroendocrine tumours, other peptides targeting further receptor systems are being studied and evaluated. Progress in profiling the density and incidence of peptide hormone receptors in human cancer has initiated and will further promote research on the corresponding peptidic binders. In addition, industrial pharmaceutical research will be another significant source of peptides in the future. A recent prognosis revealed that about 50% of the drugs entering clinical trials in the next years will be peptides. The extensive research activities in genomics and proteomics will point out and quantify new and already known target structures upregulated in specific diseases. Based on the knowledge of their endogenous ligands or via selection of suitable candidates by phage display, suitable peptide ligands for e.g. membrane associated receptors can be identified and thus allow targeting of such binding sites. Thus, bioactive peptides specifically addressing relevant molecular targets are expected to become an important class of tracers, also due to the possibility of bridging imaging with therapeutic approaches. In this brief overview a summary of methods and strategies for the ^{18}F-labeling of peptides and proteins is given.

4.1 Introduction

After more than 15 years of engaged research on peptide radiopharmaceuticals, we impartially note that the current impact of peptides on clinical PET is comparatively low. The investigations on radiolabeled peptides so far is limited to some examples, i.e. analogues of octreotide, gastrin, neurotensin or RGD-peptides. These peptides were predominantly developed for SPECT imaging and/or labeled with positron emitters for dosimetry of the corresponding analogues that were assessed for peptide receptor radiotherapy (PRRT) approaches. In a majority of studies, peptides have been labeled by radiometals, whereas only a few peptides were radiohalogenated. Studies on ^{18}F-labeling of peptides are rare and to the best of our knowledge, only two preliminary studies with ^{18}F-labeled peptides have been carried out in humans so far (Wester et al. 2003a; Haubner et al. 2005b; Beer et al. 2005).

In contrast to the current disappointing status of peptides in clinical PET imaging, the pool of promising compounds that are worthy of investigation and evaluation is continuously filled from different sources.

The display of $>10^9$ variants of peptides on the surface of phages by the use of libraries and selection of candidates with high target affinity is one source. The isolated peptides can be regarded as 'raw material', and need to be optimized, e.g. with respect to proteolytic stability, pharmacokinetics, and receptor selectivity. Driven by the promising results obtained with peptides addressing somatostatin-2 receptor positive (sst_2+) neuroendocrine tumours, other peptides targeting further receptor systems are currently being studied and evaluated. Progress in profiling the density and incidence of peptide hormone receptors in human cancer has initiated and further promoted the research on the corresponding peptidic binders. Peptides addressing structure proteins or enzymes represent another source for peptides, e.g. peptide tracers to quantify angiogenic (MMP-2, MMP-9, $\alpha v\beta 3$) or apoptotic processes (caspase substrates or inhibitors). In addition, industrial pharmaceutical research will be another significant source of peptides in the future. A recent prognosis revealed that about 50% of the drugs entering clinical trials in the next years will be peptides. Furthermore, the extensive research activities in genomics and proteomics will point out and quantify new and already known target structures upregulated in specific diseases. Based on the knowledge of their endogenous ligands or via selection of suitable candidates by phage display, suitable peptide ligands for e.g. membrane associated receptors can be identified and thus allow targeting of such binding sites. Thus, bioactive peptides specifically addressing relevant molecular targets are expected to become an important class of tracers, also due to the possibility of bridging imaging with therapeutic approaches.

A variety of methods for radiolabeling these complex biomolecules with positron emitting radionuclides has been developed (Stöcklin and Wester 1998; Okarvi 2001).

None of the available radiometals, however, has optimal radionuclide characteristics for routine PET imaging purposes. They suffer from less than optimal half-lives (^{86}Y, 14.7 h; ^{64}Cu, 12.7 h; ^{66}Ga, 9.49 h), low β^+-percentage branching (^{86}Y, 33%; ^{64}Cu, 18%; ^{66}Ga, 57%), high β^+-energy (^{86}Y, 1.3 MeV; ^{66}Ga, 1.7 MeV; ^{68}Ga, 1.9 MeV) as well as co-emission of a substantial amount of γ-radiation (^{64}Cu, also β^- 37%), all of which results in increased radiation doses for the patient. In contrast, ^{18}F with its half-life of 109.8 min and low β^+-energy (0.64 MeV) represents the ideal radionuclide for routine PET imaging. Due to its

low positron energy it has a short positron linear range in tissue, leading to particularly high resolution in PET imaging. Furthermore, compared to other short lived radionuclides, such as ^{11}C,its half life is long enough to allow syntheses and imaging procedures to be extended over hours, thus facilitating kinetic studies and high-quality metabolite and plasma analysis.

Unfortunately, direct no-carrier-added (n.c.a.) ^{18}F-labeling of complex biomolecules such as peptides or antibodies is not possible. For the n.c.a nucleophilic introduction of $^{18}F^-$,basic conditions are necessary which lead to proton abstraction from the precursor molecules and the formation of [^{18}F]HF as well as denaturing of sensitive organic substrates. Therefore, other ^{18}F-labeling methods, most of which include nucleophilic ^{18}F-labeling of small organic prosthetic groups, their activation and subsequent coupling to specific functional groups within the peptide or protein/antibody (such as amino or sulfhydryl groups), have been developed. They differ vastly with respect to the number of reaction steps involved, overall reaction time, labeling yield of the prosthetic group used, efficiency of the subsequent conjugation to the biomolecule, chemoselectivity of the conjugation step and also the influence of the prosthetic group on ligand pharmacokinetics (see Table 1 and Fig. 1).

As previously stated, strategies to prepare ^{18}F-labeled peptides have to address two major limitations: (1) the presence of acidic functionalities, such as hydroxyls, amides, alcohols, amines or thiols, does not allow for a direct nucleophilic introduction of n.c.a ^{18}F-fluoride; and (2) electrophilc fluorination can only be achieved in the presence of carrier and thus does not represent a strategy generally suited for the production of peptide receptor ligands.

4.2 Direct Electrophilic [^{18}F]Fluorination

Direct electrophilic fluorination was initially proposed by Hebel et al. (1990) as a method for introducing ^{18}F regiospecifically (ortho to OH) into the phenolic ring of tyrosine-containing bioactive peptides via [^{18}F]AcOF ([^{18}F]acetyl hypofluorite). However, synthesis of [^{18}F]F_2 for electrophilic fluorination agents is possible only in the presence of carrier, which ultimately leads to ^{18}F-labeled peptides with very low spe-

cific activity. Furthermore, reaction conditions lead to rapid oxidation of Met-residues, limiting the applicability of the method (Labroo et al. 1991). Recently, Ogawa et al. (2003) reported the direct electrophilic fluorination of cyclo(RGDfMeV) for potential use in $\alpha_v\beta_3$-integrin imaging in vivo. This labeling procedure, however, has the disadvantages of very low regioselectivity of the ^{18}F-labeling position in the Phe-residue, leading to a multitude of isomers, as well as very low specific activity (32.8 GBq/mmol). Moreover, the ^{18}F-labeled RGD-analogues presented in this study showed high accumulation in the excretion organs as well as very low tumour-to-organ ratios.

Generally, the low specific activity of tracers obtained via electrophilic ^{18}F-fluorination makes them unsuitable for clinical application, especially when used in receptor studies where ligand affinities are in the subnano- or nanomolar ranges. To overcome these limitations, ^{18}F-labeled prosthetic groups have been developed that allow mild and site-specific introduction of ^{18}F into bioactive molecules (for an overview see Okarvi 2001; Wilbur 1992).

4.3 ^{18}F-Acylation

4.3.1 Aliphatic Acylation Agents

Among the approaches investigated for the labeling of peptides, ^{18}F-acylation is the most prominent route. Very early attempts exploited less activated ^{18}F-fluorinated esters, e.g. methyl [^{18}F]fluoropropionate (Block et al. 1988). In contrast to the fluoropropionyl residue, which exhibits high stability towards defluorination in vivo, the corresponding [^{18}F]fluoroacetate derivative was found to be less stable. Higher conjugation yields were achieved using activated esters. 4-nitrophenyl 2-[^{18}F]fluoropropionate (Guhlke et al. 1994), synthesized via a three-step ^{18}F-fluorination of 9-anthrylmethyl 2-bromopropionate, separation, hydrolysis and subsequent activation with bis-4-nitrophenyl carbonate, has been successfully applied for the labeling of octreotide analogues (Guhlke et al. 1994) and RGD-peptides (Haubner et al. 2001a, 2001b, 2003, 2004, 2005; Beer et al. 2005). In an alternative strategy, 4-dimethylamino pyridine was added after fluorination to convert the

excess bromo precursor to a cationic species, thus allowing separation of the excess bromo precursor via a cartridge separation process.

KOBt, the potassium salt of hydroxybenzotriazole, was found to catalyse acylations with 4-nitrophenyl 2-[^{18}F]fluoropropionate in aqueous buffered solutions. In addition, side-chain specific labeling of unprotected model peptides (e.g. Phe–Lys) was successfully carried out by pH adjustment and KOBt catalysed acylation with nitrophenyl 2-[^{18}F]fluoropropionate in aqueous solutions (Wester et al. 1996).

The somatostatin receptor ligand octreotide was the first clinically relevant peptide labeled with 4-nitrophenyl 2-[^{18}F]fluoropropionate (Guhlke et al. 1994). Acylation of ε-Lys(Boc)^5-octreotide was carried out in CH_2Cl_2 in the presence of HOBt and 2,6-di-*t*-butyl-4-methyl pyridine in acetonitrile for 5 min at 70 °C. Subsequent acidic deprotection for 3 min and HPLC purification led to the product in 65% radiochemical yield (RCY) based on the fluoroacylation agent. Although the receptor affinity was in the low nanomolar range, the high lipophilicity prevented further evaluation of the compound in humans.

It has been recently shown that sugar conjugation is a powerful means of improving radioligand pharmacokinetics. Carbohydration leads to reduced lipophilicity of small radiolabeled peptides and thus to a dramatic reduction of hepatobiliary excretion in favour of renal excretion (Leisner et al. 1999; Haubner et al. 2001a; Schottelius et al. 2002, 2005a and b; Wester et al. 2002, 2004). Furthermore, it has been demonstrated that depending on the carbohydrate used, tumour uptake of radioiodinated Tyr^3-octreotide (TOC) and Tyr^3-octreotate (TOCA) may be substantially increased by glycosylation (Wester et al. 2002, 2004; Vaidyanathan et al. 2003; Schottelius et al. 2002, 2004). Based on these findings, an ^{18}F-labeled sugar analogue of TOCA, N$^{\alpha}$-(1-deoxy-d-fructosyl)-*N*$^{\varepsilon}$-(2-[^{18}F]fluoropropionyl)-Lys^0-Tyr^3-octreotate (Gluc-Lys([^{18}F]FP)-TOCA) (Wester et al. 2003) has been developed. To allow both glycosylation and prosthetic group labeling, both of which require a free amino functionality in the peptide, Lys^0 was introduced as a trifunctional linker. In AR42 J tumour bearing nude mice, Gluc-Lys([^{18}F]FP)-TOCA showed very low hepatic and intestinal uptake, renal excretion and high tumour accumulation. PET studies in patients with neuroendocrine tumour performed with this radioligand demonstrated excellent tumour to background ratios even at time points $\ll$ 1 h (Wester et al. 2003).

Table 1. Methods for the ^{18}F-labeling of peptides and proteins; structures of numbered prosthetic groups are depicted in Fig. 1

N°	Prosthetic group	Preparation			Conjugation			Overall		Comments	References
		Steps	RCY (%)	Time (min)	Steps	RCY (%)	Time (min)	RCY (%)	Time (min)		
	Amine reactive										
1	*N*-Succinimidyl 4-([^{18}F]fluoromethyl)benzoate	1	16–21	40–50	1	20–40	10	–	45–75	Low yield, moderate stability	Lang and Eckelman 1994, 1997
	methyl2-[^{18}F]fluoropropionate	1	15	90	–	–	–	–	–	Low reactivity	Block et al. 1988
2	4-[^{18}F]Fluorobenzoicacid (coupling via DCC/HOAt)	2	50–60	20	–	–	–	–	–		Hostetler et al. 1999
	4-[^{18}F]Fluorobenzoic acid (coupling via HATU/DIPEA)	2	–	10	1	89–90	20			Solid phase labeling	Sutcliffe-Goulden et al. 2002
3	4-Nitrophenyl 2-[^{18}F]fluoropropionate/KOBt	3	60	90	2	60–70	5	25–30	120–130	High in vivo stability	Guhlke et al. 1994a, 1994b, Westeret al. 1997
4	*N*-Succinimidyl-8-[4′-[^{18}F]fluorobenzylamino]-suberate	3	25–40	60	1	30–45	15	–	–	Bulky group	Garg et al. 1991

Table 1. (continued)

N°	Prosthetic group	Preparation			Conjugation			Overall		Comments	References
		Steps	RCY (%)	Time (min)	Steps	RCY (%)	Time (min)	RCY (%)	Time (min)		
5	*N*-Succinimidyl 4-[^{18}F]fluorobenzoate (DCC activated)	3	25	100	1	40–60	15–20	–	–		Vaidyanathan et al. 1992
	N-succinimidyl 4-[^{18}F]fluorobenzoate (DSC activated)	3	35	55	1	–	–	–	–	High in vivo stability	Vaidyanathan and Zalutsky 1994
	N-Succinimidyl 4-[^{18}F]fluorobenzoate (TSTU activated)	3	50–60	35	1	90	8	–	–		Wester et al. 1996, Mäding et al 2005
6	4-[^{18}F]Fluorophenacyl bromide	3	28–40	75	1	7–70	60	–	–	Low reactivity (higher reactivity towards thiols)	Kilbourn et al. 1987, Downer et al. 1993
7	3-[^{18}F]Fluoro-5-nitrobenzimidate	3	20–33	45	1	up to 95	5–60	–	–	Low reactivity	Kilbourn et al. 1987

Table 1. (continued)

N°	Prosthetic group	Preparation			Conjugation			Overall		Comments	References
		Steps	RCY (%)	Time (min)	Steps	RCY (%)	Time (min)	RCY (%)	Time (min)		
	Carboxylate reactive										
8	1-[4-([^{18}F]Fluoromethyl)benzoyl]-aminobutane-4-amine	2	52	80	–	–	–	–	–	Requires protected peptides or peptides without amines	Shai et al. 1989
9	2-[^{18}F]Fluoroethylamine	2	46	30–45	2	75–90	1–2	–	–		Jelinski et al. 2002
	Thiol reactive										
10	*N*-(*p*-[^{18}F]fluorophenyl)maleimide	4	15	100	–	–	–	–	–		Shiue et al. 1989
11	*m*-Maleimido-*N*-(*p*-[^{18}F]fluorobenzyl)-benzamide	3	10	70	1	50	–	–	–		Shiue et al. 1989
12	*N*-{4-[4-[^{18}F]Fluorobenzylidene(aminooxy)-butyl}-maleimide	2	35	60	1	5/70	10	–	–		Toyokuni et al. 2003
13	[^{18}F]FpyME	3	2 8–37	110	1	60–70*	10	–	130–140		de Bruin et al. 2005
14	[^{18}F]*N*-(4-fluorobenzyl)-2-bromoacetamide	3	20–30	85–95	1	45–55	10	7–13	180		Tavitian et al. 1998, Kuhnast et al. 2000

Table 1. (continued)

N°	Prosthetic group	Preparation			Conjugation			Overall		Comments	References
		Steps	RCY (%)	Time (min)	Steps	RCY (%)	Time (min)	RCY (%)	Time (min)		
15	3-[^{18}F]Fluoro-1-mercaptopropane	2	70	30	1	93	30	10	90	Unstable precursor	Glaser et al. 2004
16	2-{2-[2-(2-[^{18}F]Fluoro-ethoxy)ethoxy]-ethoxy}mercapto-ethane	2	73	30	1	41	30	32	90		Glaser et al. 2004
17	3-[^{18}F]Fluoro-methyl-*N*-(2-mercaptoethyl)-benzamide	2	16	40	1	86	30	1	100		Glaser et al. 2004
	Chemoselective										
18	4-[^{18}F]Fluoro-benzaldehyde (oxime formation)	1	50–55	30[a]/55[b]	1	60–90	15	25–40	65–90		Poethko et al. 2004a
	4-[^{18}F]Fluoro-benzaldehyde (hydrazone formation)	1	50–55	25[a]/50[b]	1	85	10	–	–		Bruus-Jensen et al. 2005
	4-[^{18}F]Fluoro-benzaldehyde (hydrazone formation)	1	52–82	25	1	25–90	30	–	65	No data given on chemical purity of 4-[^{18}F]FBA	Chang et al. 2005

Table 1. (continued)

N°	Prosthetic group	Preparation			Conjugation			Overall		Comments	References
		Steps	RCY (%)	Time (min)	Steps	RCY (%)	Time (min)	RCY (%)	Time (min)		
	Non-selective photochemical conjugation										
19	4-Azidophenacyl [^{18}F]fluoride	1	71	15	1	11–25	5	–	–		Wester et al. 1996
20	[^{18}F]-3,5-Difluorophenyl azide (and 5 derivatives)	1	1–12	55	1	7	120	–	175	Carrier added	Hashizume et al. 1995
21	3-Azido-5-nitrobenzyl-[^{18}F]fluoride	1	30–50	45	1	20	10	1–7	135–150		Lange et al. 2002

[a]Non decay corrected isolated yield [b]Efficiency of ^{18}F-incorporation [c]Cartridge purification [d]HPLC-purification

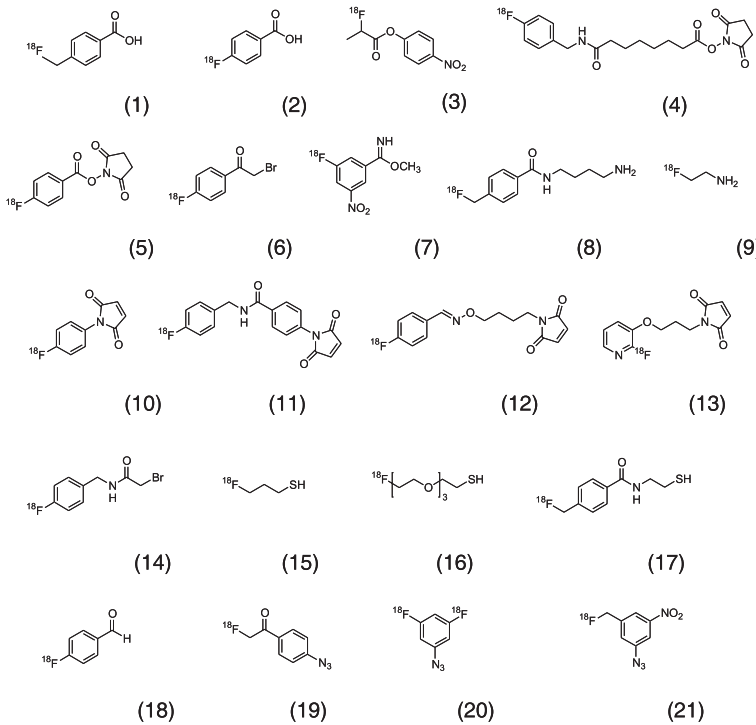

Fig. 1. Prosthetic groups for the ^{18}F-labeling of peptides and proteins; numbers refer to the numbering of the prosthetic groups in Tab. 1

Cyclic pentapeptides containing the Arg-Gly-Asp sequence (RGD peptides), which binds with high affinity to the αvβ3 integrin, have been proposed as tracers for the determination of the integrin status during antiangiogenic therapies and for the pretherapeutic recognition of those patients most amenable to αvβ3-directed therapies. To overcome problems arising from a high lipophilicity (Haubner et al. 1999), a sugar amino acid synthesized by catalysed oxidation of the primary hydroxyl group of *N*-(fluorenylmethoxycarbonyl)-β-D-galactopyranosylmethylamine was conjugated to the lysine side chain of c(RGDfK) (Galakto-RGD), and labeled at the methylamine group with 4-nitro-

phenyl 2-[^{18}F]fluoropropionate (Haubner et al. 2001a, 2001b). In vitro binding assays showed that the reference compound was able to fully suppress the binding of the endogenous ligands. The IC_{50}-values for [^{18}F]Galakto(RGD)-binding to different integrin subtypes were 5 nM ($\alpha v\beta 3$), 1,000 nM ($\alpha v\beta 5$) and 6,000 nM ($\alpha IIb\beta 3$), respectively. Evaluation in M21-$\alpha v\beta 3^{+}$ and M21-$\alpha v\beta 3^{-}$ melanoma bearing animals demonstrated receptor specific uptake and high target/non-target ratios, e.g. tumour/blood 27.5 and tumour/muscle 10.2 at 120 min p.i. (Haubner et al. 2001, 2005).

[^{18}F]Galakto(RGD) showed a highly favourable biodistribution in humans and allows the visualization of $\alpha v\beta 3$ expression with high contrast (Beer et al 2005; Haubner et al. 2005).

4.3.2 Labeling Via 4-[^{18}F]Fluorobenzoates

Although resulting in products with a somewhat higher lipophilicity, the 4-[^{18}F]fluorobenzoyl moiety has been extensively used for peptide labeling. Activation agents, such as 1,3-dicyclohexylcarbodiimide/1-hydroxy-7-azabenzotriazole (DCC/HOAt) or *N*-[(dimethylamino)-1H-1,2,3-triazolyl[4,5]pyridine-1-yl-methylene]-N-methyl-methanaminium hexafluorophosphate *N*-oxide (HATU/DIPEA) have been used for the in situ activation of 4-[^{18}F]benzoic acid in solution and solid phase synthesis, respectively (Hostetler et al. 1999; Sutcliffe-Goulden et al. 2000, 2002; Vaidyanathan and Zalutsky 1992, 1994). To overcome problems during HPLC purification after DCC activation, n.c.a. *N*-succinimidyl 4-[^{18}F]fluorobenzoate was isolated to allow for clean and optimized peptide (and protein or antibody) conjugation in aqueous and organic solvents. Activations were carried out with *N*,*N*′-disuccinimidyl carbonate or O-(*N*-succinimidyl)-*N*-*N*-*N*′-*N*′-tetramethyluronium tetrafluoroborate (TSTU) (Wester et al. 1996). The charged nature of the latter compound has been exploited for easy elimination of the excess of activation reagent from the reaction mixture after ester formation by a solid phase extraction process on a polystyrene resin.

A modified approach for the convenient synthesis of [^{18}F]SFB has been recently described (Wüst et al. 2003). [^{18}F]SFB could be obtained in decay-corrected RCY of 44%–53% (n=20) and radiochemical purity >95% within 40 min after EOB. These authors used a microwave-

assisted one-pot preparation of [^{18}F]fluorobenzoic acid via nucleophilic fluorination, acidic saponification of *tert*-butyl 4-trimethylammonium-benzoate triflate, subsequent separation on a polystyrene cartridge, and elution with acetonitrile. Activation was carried out after addition of a few microlitres of tetramethylammonium hydroxide and drying with TSTU. An automated strategy allowing the three-step production of [^{18}F]SFB in a remote controlled module in 34%–38% RCY, within a synthesis time of 68 min, was also recently published (Mäding et al. 2005).

The F(ab′)$_2$ fragment of Mel-14, an antibody reactive with gliomas and other tumours, was labeled by reaction with [^{18}F]SFB. In athymic mice with subcutaneous D-54 MG human glioma xenografts, uptake in tumour was rapid with levels as high as 18.7±1.1% ID/g 4 h p.i. (Vaidynathan et al. 1992; Vaidyanathan and Zalutsky 1992). This antibody fragment has also been labeled with *N*-succinimidyl 8-[(4′-[^{18}F]fluorobenzyl)amino]suberate (SFBS). The immunoreactivity for labeled Mel-14 F(ab′)$_2$ using the two methods was similar. Tissue distribution and pharmacokinetics were also comparable for the labeled fragments, as demonstrated in foxhounds (Page et al. 1994).

Recently, Annexin-V was labeled with [^{18}F]SFB by several groups (Zijlstra et al. 2003; Toretsky et al. 2004; Murakami Y et al. 2004; Yagle et al. 2005). Annexin V was radiolabeled within 2.5–3 h in an overall yield of 17%–40% RCY. Highest yields were observed with 125–500 μg annexin-V at a concentration of 5 μg/μl. In vitro, cancer cells treated with the chemotherapeutic agent, etopsoside, showed a 88% increase in binding of annexin-V (Toretsky et al. 2005). In ischaemic animals, accumulation of [^{18}F]annexin V and [^{99m}Tc]annexin V in the infarct area was about threefold higher than in the non-infarct area (Toretsky et al. 2005). Furthermore, the ratio of accumulation in the normal heart to the blood radioactivity was not significantly different between the tracers (Murakami et al. 2004). Pretreatment of rats with cycloheximide resulted in a three- to ninefold increase in uptake of ^{18}F-annexin V in the liver of treated animals at 2 h, compared with controls (Yagle et al. 2005). Together these studies indicate that ^{18}F-annexin V binds specifically to apoptotic tissues in this experimental model.

The anti-granulocyte antibody BW 250/183 (anti-CD66) was labeled with [^{18}F]SFB and antibody biodistribution was measured by PET (Neu-

maier et al. 2002). In patients with high risk leukaemia [^{18}F]anti-CD66 showed high and preferential uptake in the bone marrow and spleen and only low activity retention in residual body tissues.

Cyclic RGD-peptides have also been labeled with [^{18}F]SFB. (RGDyK([^{18}F]FB) has been investigated in a glioblastoma model using nude mice for imaging of αvβ3 integrins (Chen et al. 2004c, 2004d). As previously stated, the high lipophilicity of these RGD-peptides result in high hepatic extraction and biliary excretion. Thus, a benzoylation strategy, without further peptide modifications to reduce the lipophilicity, is unsuitable for the development of integrin receptor ligands. Consequently, a polyethylene glycol (PEG)-linker was conjugated to c(RGDyK) allowing [^{18}F]SFB labeling at the terminal aminogroup of the heterobifunctional PEG-linker. Improved tumour retention and in vivo kinetics were noted with [^{18}F]FB-PEG-RGD compared to [^{18}F]FB-RGD (Chen et al. 2004b).

Further examples for [^{18}F]fluroacylations of peptides via [^{18}F]SFB are: proinsulin connecting peptide (C-peptide) (Fredriksson et al. 2001), endothelin-1 (Johnström et al. 2005), α-MSH (Vaidyanathan and Zalutsky 1997), (Arg^{15}, Arg^{21})VIP (Moody et al. 1998), neurotensin (8–13) (Bergmann et al. 2002) and substance P.

4.3.3 Other ^{18}F-Acylation Agents

Several other ^{18}F-fluoroacylation reagents have been investigated. *N*-succinimidyl 8-[4′-([^{18}F]fluorobenzyl)amino]suberate ([^{18}F]SFBS) has been prepared in a three-step procedure starting with the fluorination of 4-nitrobenzonitrile, reduction to 4-[^{18}F]fluorobenylamine and coupling with disuccinimidyl suberate (DSS; 30% RCY, 60 min; Garg et al. 1991). Conjugation of an anti-myosin MAb and corresponding antibody fragments with [^{18}F]SFBS (0.2–1.0 mg, pH 8.5, 100–200 μl) led to 30%–45% RCY. With the exception of two studies using this antibody in canine models, no further studies were performed with this acylation reagent (Page et al. 1994; Zalutsky et al. 1992).

Few studies have investigated the direct fluorination of an activated ester. As previously demonstrated, [^{18}F]fluorination of free acids as well as active esters results in decomposition or unwanted side reactions (Jacobson et al. 1988). However, Lang and Eckelmann (1994) success-

fully prepared *N*-succinimidyl 4-([^{18}F]fluoromethyl)benzoate from the corresponding 4-nitrobenzenesulfonyl-oxy precursor. They obtained the best yields using Kryptofix, carbonate as counter ion and acetone as the solvent at room temperature (18%, 30–35 min). Due to the low in vivo stability of [^{18}F]fluoromethylbenzoates and other similar substituted aromatic systems (see, for example, Magata et al. 2000), this activated ester was found to have limited applicability for peptide and/or protein labeling (Lang and Eckelman 1997; Aloj et al. 1996).

4.3.4 Solid Phase Conjugation Strategy

To further optimize the coupling yields and offer alternative strategies for purification of the labeled peptides, methods of labeling on solid supports have been investigated. [^{18}F]SFB, prepared via the conventional route described above, was added to the immobilized peptides on a modified PEG-PS resin in the presence of the aminium activation agent HATU/DIPEA (3 min, > 95% RCY). Optimum cleavage was achieved within 7 min using trifluoroacetic acid/phenol/water/triisopropylsilane (Sutcliffe-Goulden et al. 2000). This method was also applied for the synthesis of RGD peptides (Sutcliffe-Goulden et al. 2002). Whether solid phase strategies will offer significant advantages over conventional homogeneous reactions has yet to be investigated in comparative studies.

4.4 ^{18}F-Amidation

Amide formation for peptide labeling using a radiolabeled amine and an activated carboxylic group on the peptide offers significant advantages when compared to the reverse strategy, i.e. the use of a radiolabeled and activated ester for coupling with an amine on the peptide. Whereas hydrolysis of the activated ester as limiting side reaction will dramatically reduce the yields when n.c.a. ^{18}F-acylation agents are used, the yields will not be significantly affected when some hydrolysis will occur on the high excess (compared to n.c.a. radiolabeled amine counterpart) of activated peptide when coupled with ^{18}F-amines. Unfortunately, only a few peptides seem to be useful for this strategy. The peptides should

have only one carboxylate for activation, and should not have free amine functionalities in order to avoid inter- and intramolecular cross-linking. The latter can be circumvented by suitable protection chemistry, which will make an additional final deprotection step necessary.

The first labeling of insulin was carried out via an amidation strategy (Shai et al. 1989). In the first step, the A1 and B29 amino residues of insulin were Boc-protected. The product was treated with a large excess of disuccinimidyl suberate resulting in a B1 activated, A1 and B29-Boc protected insulin precursor. Conjugation was carried out in a complicated multi-step procedure by the addition of *N*-[4-[^{18}F]fluoromethyl)benzoyl]butane-1,4-diamine, to the activated insulin. After subsequent deprotection, [^{18}F]insulin was isolated in an overall RCY of 52% (80 min) (Shai et al. 1989). As demonstrated by in vitro binding assays, [^{18}F]insulin completely retained the biological properties of native insulin and competed for the binding to insulin receptors on human lymphoblastoid cells in a manner indistinguishable from that of insulin. Although the selection of the ^{18}F-amine component was suboptimal, this early example demonstrates the high potential of the method.

Recently, oxytocin has also been labeled via the amidation strategy (Jelinski et al. 2002). [^{18}F]fluoroethylamine, prepared from TsO-CH_2-CH_2-NHBoc and subsequent deprotection, was added to fully side chain protected Gly^9-oxytocin in the presence of TBTU and triethylamine in DMF. Acidic deprotection led to the formation of the product within 75 min with a RCY of 30%.

4.5 Thiol-Reactive Prosthetic Groups

Free sulfhydryl (-SH) groups are not abundant in most peptides and proteins. Therefore, the use of thiol-reactive prosthetic groups allows modification of peptides and proteins at specific sites and thus provides substantially increased chemoselectivity compared to the amine- and carboxylate reactive reagents.

In 1989, Shiue et al. (1989) were the first to report the synthesis of two ^{18}F-labeled *N*-substituted maleimides, *N*-(*p*-[^{18}F]fluorophenyl)-maleimide and *m*-maleimido-*N*-*p*-[^{18}F]fluorobenzyl)-benzamide, for

protein labeling. They labeled a Fab′ fragment from rabbit IgG with a yield of 50%.

Recently, however, several new approaches towards thiol-selective ^{18}F-labeling of complex biomolecules have been reported. They are based on two different strategies: firstly, the use of the chemoselective Michael addition of thiols to maleimides, and secondly, thiol-selective alkylation reactions.

One method, developed by Toyokuni et al. (2003) is based on the first strategy and uses the chemoselective oxime formation between 4-[^{18}F]fluorobenzaldehyde and *N*-[4-(aminooxy)butyl]maleimide to create a thiol-reactive conjugate. This compound, *N*-{4-[4-[^{18}F]fluorobenzylidene(aminooxy)-butyl}maleimide, was synthesized in $\sim$ 35% yield (decay corrected) in an overall reaction time of 60 min from EOB (specific activity: $\sim$ 3,000 Ci/mmol). The thiol-containing tripeptide glutathione and a 5′-SH-functionalized oligodesoxynucleotide were conjugated with this prosthetic group in $\sim$ 70% and $\sim$ 5% RCY, respectively, within 10 min at room temperature. These conjugation yields, however, are not optimized and are preliminary in nature.

The second approach based on Michael addition chemistry utilizes [^{18}F]FpyME, a [^{18}F]fluoropyrimidine-based maleimide reagent, for highly effective (60%–70% isolated and non-decay-corrected yield) ^{18}F-labeling of a thiol- and amine-containing model peptide and of two 8-kDa proteins (de Bruin et al. 2005). The prosthetic group is synthesized in a three-step procedure including: (1) high yield nucleophilic heteroaromatic ortho-radiofluorination of [3-(3-Boc-aminopropoxy)pyridin-2-yl]trimethyl-ammonium trifluoromethanesulfonate; (2) rapid and quantitative Boc-deprotection; and (3) maleimide formation using *N*-methoxycarbonylmaleimide. [^{18}F]FpyME was obtained in RCY of 28%–37% within 110 min overall reaction time. Given the relatively high RCY both for the synthesis of [^{18}F]FpyME and for the conjugation step, as well as the high chemoselectivity of the latter, this methodology certainly represents a promising alternative to conventional non-selective prosthetic group labeling strategies.

In the case of alkylation strategies, two different approaches are possible: the synthesis of an ^{18}F-labeled prosthetic group, which is then used to selectively alkylate the thiol-functionalized biomolecule (Tavitian et al. 1998, 2003; Kuhnast et al. 2000), or the inverse strategy using

^{18}F-labeled fluorothiols, which are then 'alkylated' by, e.g. a haloacetylated peptide precursor (Glaser et al. 2004).

In the first approach [^{18}F]*N*-(4-fluorobenzyl)-2-bromoacetamide was synthesized from its 4-cyano-*N*,*N*,*N*-trimethylanilinium trifluoromethanesulfonate precursor in 20%–30% RCY. This involved a three-step procedure with nucleophilic $^{18}F^{-}$-incorporation, reduction of the cyano group and bromoacetylation of the resulting benzylamine (Kuhnast et al. 2000). Thiol-selective coupling of [^{18}F]*N*-(4-fluorobenzyl)-2-bromoacetamide to a model oligonucleotide bearing a phosphorothioate group at its 3'end was performed at high temperature (120 °C, 10 min) and afforded the ^{18}F-labeled oligonucleotide in 45%–55% yield (based on starting ^{18}F-labeled prosthetic group).

In the second 'inverse' ^{18}F-alkylation strategy, ^{18}F-labeled fluorothiols that are selectively alkylated by chloroacetylated model peptides are used (Glaser et al. 2004). Three different [^{18}F]fluorothiols were evaluated: 3-[^{18}F]Fluoro-1-mercaptopropane ([^{18}F]-**1**), 2-{2-[2-(2-[^{18}F]Fluoroethoxy)ethoxy]-ethoxy}mercaptoethane ([^{18}F]-**2**) and 3-[^{18}F]Fluoromethyl-*N*-(2-mercaptoethyl)-benzamide ([^{18}F]-**3**). With respect to efficiency of the ^{18}F-incorporation into the trityl-protected precursor (80 °C, 5 min), yields were comparably high for the first two compounds (~80%), but low for the third (20%). The conjugation to the chloroacetylated model peptide (80 °C, 30 min) was most efficient for [^{18}F]-**1** (93%), followed by [^{18}F]-**3** (86%) and [^{18}F]-**2** (41%). Overall RCY of isolated ^{18}F-labeled peptide were highest for [^{18}F]-**2** (32% decay corrected). To date, no data are available on the in vitro and in vivo behaviour of these conjugates. However, this methodology certainly holds promise for ^{18}F-labeling of peptides.

4.6 Photochemical Conjugation

Photoconjugation techniques, such as photoaffinity labeling, are well established methods in biochemistry. These techniques exploit compounds, such as aryl azides, which upon photolysis generate reactive species such as nitrenes. According to the general proposed reaction pathways, phenylnitrenes mainly react by nucleophilic addition to form substituted ^{3}H-azepines, whereas polyfluorinated nitrenes mainly re-

act by insertion. To investigate the suitability of photochemical conjugation for ^{18}F-labeling of peptides and proteins, the UV sensitive ^{18}F-fluorination agent, 4-azidophenacyl-[^{18}F]fluoride ([^{18}F]APF) was synthesized from the corresponding bromo precursor in about 70% RCY within 15 min (Wester et al. 1996). Irradiation of [^{18}F]APF with UV light of 365 nm, in the presence of proteins, such as human serum albumin (HSA), transferrin, avidin or a model IgG, resulted in up to 30% radiochemical conjugation yield within 5 min (complete decomposition of the azide). However, compared to the conventional acylation procedures, significantly higher protein concentrations are required (e.g. HSA, 1 mg/100μl, pH 8.5; [^{18}F]SFB, 90% RCY; 4-nitrophenyl 2-[^{18}F]fluoropropionate, 20%; 4-nitrophenyl 2-[^{18}F]fluoropropionate in the presence of 0.1 MHOBt, 43%; [^{18}F]APF, 25%). To overcome this disadvantage and increase the amount of nitrene which reacts by insertion, perfluorinated 4-azidophenacyl analogues have also been investigated. However, the higher conjugation yields were overcompensated by high unspecific protein binding, caused by the high lipophilicity of these analogues (Wester et al. unpublished results). In a second study, Lange et al. (2002) introduced 3-azido-5-nitrobenzoyl [^{18}F]fluoride ([^{18}F]ANBF) for the labeling of oligonucleotides (30% RCY from the corresponding tosylate). Labeling yields of up to 20% were obtained at aptamer optimal concentrations of approximately 25 μg/μl. Hashizume et al. (1995) described the synthesis of polyfluorinated azides via ^{18}F-for-^{19}F-substitution.

4.7 Chemoselective [^{18}F]Fluorination Methods

The thiol-selective prosthetic groups discussed above allow site-specific ^{18}F-labeling of high molecular mass biomolecules and offer a substantial improvement in chemoselectivity over amine-reactive prosthetic groups. This allows the use of unprotected peptide- and oligonucleotide-precursors and thus reduces the number of steps needed for the conjugation step to one. This in turn leads to a greater ease of synthesis and a reduction of overall reaction time. However, all methods based on sulfhydryl-reactive prosthetic groups involve multistep (two to four) preparations of the prosthetic group. For example, although [^{18}F]FpyME

has been synthesized in amounts allowing peptide labeling on a scale suitable for clinical application (130–180 mCi) (de Bruin et al. 2005), its preparation is tedious and time-consuming for routine syntheses in a clinical setting.

4.7.1 Formation of Oximes

In view of the disadvantages with fluorination discussed above, there was a need for a labeling strategy suitable for faster and straightforward large-scale production of ^{18}F-labeled peptidic tracers. The optimal approach with respect to the amount of reaction steps involved and thus overall reaction time would be a '1+1'-step labeling method. The first step is the one-step high-yield synthesis of a ^{18}F-labeled prosthetic group, which is stable against in vivo defluorination. The second step is its one-step chemoselective conjugation to a specifically functionalized, unprotected peptide precursor. To be generally applicable to sensitive substrates, the latter step should be feasible in aqueous media under mild conditions.

These prerequisites are met by the chemoselective formation of an oxime bond between 4-[^{18}F]fluorobenzaldehyde ([^{18}F]FB-CHO) or a radiohalogenated ketone or aldehyde and a peptide functionalized with an aminooxy-group. This methodology had already been applied in the radioiodination of antibodies (Kurth et al. 1993) and has been proposed for the radioiodination of small peptides (Thumshirn et al. 2003). Recently, chemoselective oxime ligation has been successfully applied in our lab for high-yield ^{18}F-labeling of a variety of peptides(Poethko et al. 2004a, 2004b).

[^{18}F]FB-CHO was obtained in one step from the trimethylanilinium precursor in 50% non-optimized RCY within 30 min. The efficiency of the oxime bond formation with a variety of aminooxy-functionalized peptides such as minigastrin, RGD or octreotide analogues was found to be strongly dependent on pH, peptide concentration, reaction time and temperature (Poethko et al. 2004a). Optimal results at low peptide concentrations (0.5 mM) were obtained within 15 min at 60 °C and pH 2–3, independent of the peptide used. Under these conditions, the *N*-(4-[^{18}F]fluorobenzylidene)oxime ([^{18}F]FBOA)-peptides were obtained in 60%–80% RCY.

This highly suitable strategy for the production of ^{18}F-labeled peptide receptor ligands, with excellent pharmacokinetic properties, has been demonstrated in the case of [^{18}F]FBOA-labeled RGD-multimers as well as for a glycosylated, [^{18}F]FBOA-labeled Tyr^3-octreotate analogue, Cel-*S*-Dpr([^{18}F]FBOA)TOCA (Schottelius et al. 2004). The latter was specifically optimized with respect to its excretion profile and tumour accumulation. Introduction of the aromatic [^{18}F]FBOA-moiety into the peptide invariably leads to an increased lipophilicity of the peptide, which leads to a shift from renal towards hepatobiliary clearance. This has been demonstrated for Gluc-*S*-Dpr([^{18}F]FBOA)TOCA as compared to its [^{18}F]fluoropropionylated analogue, N$^{\alpha}$-(1-deoxy-d-fructosyl)-N$^{\varepsilon}$-(2-[^{18}F]fluoropropionyl)-Lys^0-Tyr^3-octreotate (Gluc-Lys([^{18}F]FP)-TOCA) (Wester et al. 2003; Schottelius et al. 2004). Thus, to compensate for the increased lipophilicity introduced by the [^{18}F]FBOA-group, the monosaccharide glucose (Gluc) was replaced by the disaccharide cellobiose (Cel), leading to significantly reduced intestinal and hepatic tracer uptake, increased tumour accumulation and thus improved tumour-to-background ratios.

4.7.2 Labeling of Peptide Multimers

Multimers were developed to address the question of multimeric integrin binding by 'poly-potent' ligands, and to initiate and target integrin clusters with n.c.a. radiopharmaceuticals (Bock et al 1999; Wester and Kessler 2005). The above mentioned αvβ3-selective-peptides cyclo(-RGDfK-) or cyclo(-RGDfE-) were linked to polyethyleneglycol (PEG)-amino acid and other spacers (Thumshirn et al. 2003; Poethko et al. 2004b). Monomeric units were bridged by lysine or a lysine tree to form dimeric, tetrameric and octameric RGD-oligomers in a well-controlled, defined and characterized manner. Labeling was carried out by oxime ligation, e.g. using 4-[^{18}F]fluorobenzaldehyde. Comparison of the IC_{50} of cyclo(-RGDfK-) and cyclo(-RGDfE-) containing monomers, dimers, tetramers and octamers for vitronection binding to αvβ3 revealed significantly increasing affinity in the series monomer < dimer < tetramer < octamer. In contrast, the affinity of reference and control peptides carrying only one cyclo(-RGDfK-)- (or cyclo(-RGDfE-)-peptide, but otherwise cyclo(-RADfK-)- or cyclo(-RADfE-)-sequences,

respectively, was lower or similar to that of the corresponding monomers. Together, these experiments clearly demonstrate the 'multimer effect' in vitro with similar molecular structures, which is independent of differences in charge, size or shape. These data were also confirmed in vivo in M21 melanoma tumour-bearing mice (Poethko et al. 2004b). Both tumour uptake and tumour-to-organ ratios increased in the series monomer < dimer < tetramer, leading to a significant improved imaging with the ^{18}F-labeled RGD-tetramer.

4.7.3 Formation of Hydrazones

Another chemoselective reaction that is highly efficient and suitable for [^{18}F]fluorination of peptides and proteins is hydrazone formation between [^{18}F]FB-CHO and a hydrazino-group in the biomolecule. Hydrazinonicotinic acid (HYNIC) derivatized peptides are widely used as precursors for ^{99m}Tc-labeling. Therefore, this group is not only ideally suited to introduce a hydrazino group into peptides or proteins for chemoselective ^{18}F-labeling, but also offers the unique opportunity to use identical labeling precursors both for SPECT- and for PET-imaging applications.

Recently, different HYNIC analogues of Tyr^3-octreotide labeled with ^{18}F using [^{18}F]FB-CHO have been synthesized in our laboratory (Bruus-Jensen et al. 2005). With respect to conjugation yields, this method is as efficient as oxime formation (85% RCY within 15 min). Further, certain peptide optimizations, with respect to lipophilicity via, e.g. carbohydration, might offer access to sst-binding peptides that can be labeled both with ^{18}F and ^{99m}Tc for scintigraphic detection of sst-expressing tumours using SPECT or PET. The only potential advantage of oxime- versus hydrazone-based ^{18}F-labeling might lie in the greater stability of the oxime linkage in aqueous media. The oxime bond is stable in the pH range of 3–7 over 24 h and is only slightly degraded at pH 9 within that time frame (21% of compound degraded; Shao and Tam 1995). [^{18}F]FB-CH=N-HYNIC-TOCam showed rapid release of [^{18}F]FB-CHO: at pH 4 only 65%, and at neutral pH only 87% of the intact peptide was detected after 5 h. This lower stability at acidic pH will not have a significant impact on ligand pharmacokinetics, but can be used to in increase the intercellular trapping of residualizing prosthetic

groups after internalization and cleavage under the acidic endosomal conditions. The only study so far, where a protein was labeled with ^{18}F via hydrazone bond formation was by Chang et al. (2005). HSA was functionalized with HYNIC, ranging from 5.2 to 23.2 HYNIC moieties per HSA molecule, and then reacted with [^{18}F]FB-CHO. The conjugation yield ranged from 25% to 90%, with the latter being reached only with high protein concentrations (2 mg/ml), high reaction temperature (50–60 °C) and elongated reaction times (20–30 min). It is noteworthy that non-modified HSA also showed 10% conjugation yield with [^{18}F]FB-CHO, challenging the applicability of this method to large proteins.

4.8 Miscellaneous

4.8.1 Enzymatic ^{18}F-Glycosylation

In order to overcome the problem of poor regioselectivity noted with the common strategy to label proteins via ^{18}F-labeled prosthetic groups, the use of enzyme systems along with the corresponding ^{18}F-labeled coenzymes could provide a reliable, regioselective and mild labeling method. The substrate flexibility of β-(1,4)-galactosyltransferase and its frequent use in organic carbohydrate synthesis has been described in a number of studies. Nevertheless, its applicability for radiopharmaceutical syntheses of oligosaccharides or glycoconjugates has not yet been systematically investigated.

Prante et al. (1999) developed a chemo-enzymatic synthesis of the transferase cofactor uridine diphospho-2-deoxy-2-[^{18}F]fluoro-α-D-glucose (UDP-[^{18}F]FGlc) starting from 1,3,4,6-tetra-*O*-acetyl-2-[^{18}F]fluoro-2-deoxy-D-glucose, the intermediate product of [^{18}F]FDG synthesis. The chemical phosphorylation via MacDonald reaction and subsequent deprotection led to a RCY of 55% of [^{18}F]FDG-1-phosphate. UDP-[^{18}F]FGlc was synthesized enzymatically by condensation of [^{18}F]FDG-1-phosphate with UTP in presence of UDP-Glc PPase. In order to overcome a decrease in enzyme activity, the reaction was performed in a minimized reaction volume with optimized UTP-concentration of 0.5 mM leading to an overall radiochemical yield of 20% of UDP-[^{18}F]FGlc within 110 min. The ^{18}F-labeled coenzyme UDP-[^{18}F]FGlc was used

as a tool for ^{18}F-glycosylation of *N*-acetylglucosamine mediated by β-(1,4)-galactosyltransferase. The ^{18}F-glycosylated product was obtained with a RCY of 56% and was easily isolated by solid phase extraction. However, the chemical nature of this ^{18}F-glycosylated product remains to be elucidated. In addition to the general availability of [^{18}F]FDG worldwide, this strategy for enzymatic transfer of 'activated [^{18}F]FDG' has demonstrated its potential as a highly selective and mild ^{18}F-labeling method for glycosylated biopolymers.

4.8.2 Thiol-Selective Glycosylation

As a new approach to exploit [^{18}F]FDG as a prosthetic group and combine the conjugation technique with the carbohydration strategy, a radiochemical method for the ^{18}F-glycosylation of amino acid side chains was developed starting from peracetylated 2-deoxy-2-[18F]fluoroglucopyranoside (TA-[^{18}F]FDG). O-(2-deoxy-2-[^{18}F]fluoro-D-glucopyranosyl)-L-serine and the corresponding threonyl compound were obtained in a RCY of 25% and 12% (related to [^{18}F]fluoride), respectively, after deprotection within a total reaction time of 90 min. The anomeric configuration of the corresponding ^{19}F-substituted compounds revealed preferential α-stereoselectivity (Maschauer et al. 2005). However, this approach may be problematic when applied to the labeling of peptides: the use of BF_3 as a Lewis acid promoter could cause side-reactions, such as cleavage of protecting groups and thereby limits the choice of the reaction solvent. In order to circumvent these drawbacks, the development of an alternative ^{18}F-glycosylation method that prevents the need for peptide protecting groups is useful. This strategy makes use of an ^{18}F-labeled glycophenylthiosulfonate reagent reacting specifically and quantitatively with thiols.

Starting from 1,3,4,6-tetra-*O*-acetyl-2-[^{18}F]fluoro-2-deoxy-D-glucose, the intermediate of [^{18}F]FDG synthesis, the corresponding 1-phenylthiosulfonate was obtained by a two-step procedure. First, the peractetylated ^{18}F-labeled glucosyl intermediate was converted into the glycosyl 1-bromide by HBr/AcOH. Next, sodium phenylthiosulfonate in DMF/CH_3CN was used to obtain the thiol-reactive ^{18}F-labeled prosthetic group 3,4,6-tri-*O*-acetyl-2-deoxy-2-[^{18}F]fluoroglucopyranosyl-1-phenylthiosulfonate (Ac_3-[^{18}F]FDG-PTS). Without further optimiza-

tion, the non-decay-corrected RCY of Ac_3-[^{18}F]FDG-PTS was 13%–17% within 90 min, including two HPLC purification steps. The chemical ligation of Ac_3-[^{18}F]FDG-PTS with a model peptide (CAKAY) and a cyclic RGD-peptide [c(RGDfC)] revealed a radiochemical yield of $>$ 95% under mild reaction conditions (Tris-buffer, pH 8–9, RT, $t = 15$ min). As a proof of principle, the S-S′-glycosylated cyclic RGD-peptide (c(RGDfC(S,S′-Ac_3-[^{18}F]FGlc), (1) was compared to the non-glycosylated analogues c(RGDfC) (2) and c(RGDfS) (3) with respect to $\alpha_v\beta_3$ binding affinities using a solid phase receptor assay and human endothelial cells with the disintegrin ^{125}I-echistatin. Binding studies confirmed a high $\alpha_v\beta_3$ binding affinity as compared to 2 and 3. This strategy of ^{18}F-glycosyl ligation for thiol-containing peptides could be adopted to alternative bioactive peptides. Further, it suggests the application of ^{18}F-synthesis of glycopeptide derivatives, using the ^{18}F-labeled prosthetic group Ac_3-[^{18}F]FDG-PTS, for the development of novel glycoconjugates as PET radiopharmaceuticals.

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5 [^{18}F]Fluoropyridines: From Conventional Radiotracers to the Labeling of Macromolecules Such as Proteins and Oligonucleotides

F. Dollé

Abstract. Molecular in vivo imaging with the high-resolution and sensitive positron emission tomography (PET) technique requires the preparation of a positron-emitting radiolabeled probe or radiotracer. For this purpose, fluorine-

18 is becoming increasingly the radionuclide of choice due to its adequate physical and nuclear characteristics, and also because of the successful use in clinical oncology of 2-[^{18}F]fluoro-2-deoxy-D-glucose ([^{18}F]FDG), which is currently the most widely used PET-radiopharmaceutical and probably the driving force behind the growing availability and interest for this positron-emitter in radiopharmaceutical chemistry. With a few exceptions, radiofluorinations involving fluorine-18 of high specific radioactivity (e.g. $>$ 185 GBq/μmole) had, until recently, been limited to nucleophilic substitutions in *homo*aromatic and aliphatic series with [^{18}F]fluoride. Considering chemical structures showing a fluoropyridinyl moiety, nucleophilic *hetero*aromatic substitution at the *ortho*-position with no-carrier-added [^{18}F]fluoride, as its K[^{18}F]F-K_{222} complex, appears today as a highly efficient method for the radiosynthesis of radiotracers and radiopharmaceuticals. This chapter summarizes the recent applications of this methodology and highlights its potential in the design and preparation of, often drug-based, fluorine-18-labeled probes of high specific radioactivity for PET imaging, including macromolecules of biological interest such as peptides, proteins and oligonucleotides.

5.1 Introduction

Positron emission tomography (PET) is a high-resolution, sensitive, functional-imaging technique, that permits repeated, non-invasive assessment and quantification of specific biological and pharmacological processes in humans. It is probably the most advanced technology currently available for studying in vivo molecular interactions and represents the method of choice to assess the distribution, pharmacokinetics and pharmacodynamics of a molecule in vivo (Valk et al. 2003). Molecular imaging with PET, however, requires the preparation of a positron-emitting radiolabeled probe or radiotracer (Stöcklin and Pike 1993; Welch and Redvanly 2003). For this purpose, fluorine-18 is becoming increasingly the radionuclide of choice due to its adequate physical and nuclear characteristics, and also due to the successful use in clinical oncology of 2-[^{18}F]fluoro-2-deoxy-D-glucose ([^{18}F]FDG), the currently most widely used PET-radiopharmaceutical and probably the driving force behind the growing availability and interest for this positron emitter in radiopharmaceutical chemistry.

5.1.1 Fluorine-18 as the Positron Emitter

Compared with other positron-emitting radiohalogens used in PET such as bromine-76 (half-life: 16.1 h) or iodine-124 (half-life: 4.18 days), fluorine-18 displays simpler decay- and emission properties with a high positron abundance (97%) (Browne et al. 1978). Because of its shorter half-life (109.8 min) and its lower positron energy (maximum 635 keV), administration of fluorine-18-labeled radiopharmaceuticals also gives a lower radiation dose to patients. Compared with the other conventional short-lived positron-emitting radionuclides carbon-11, nitrogen-13 and oxygen-15 with equally simple decay schemes, fluorine-18 has once more a relatively low positron energy and the shortest positron linear range in tissue (maximum 2.3 mm), resulting in the highest resolution in PET imaging. Its half-life is long enough to give access to relatively extended imaging protocols compared to what is possible with carbon-11. Therefore, this facilitates kinetic studies and high-quality metabolite- and plasma analysis simply because of higher count rates and better statistics. On the other hand, the half-life is too long for repeated imaging with the same or different radiotracers, things that are possible with carbon-11, nitrogen-13 and oxygen-15. From a chemical point of view, the half-life of fluorine-18 allows multi-step synthetic approaches that can be extended over hours and fluorine-18 has therefore been effectively used for the labeling of numerous relatively simple or complex bioactive chemical structures (Kilbourn and Welch 1986; Kilbourn 1990; Lasne et al. 2002; Welch and Redvanly 2003), including high molecular weight macromolecules such as peptides and proteins (Kilbourn et al. 1987; Wilbur 1992; Okarvi 2001; de Bruin et al. 2005 and references therein) and oligonucleotides (Dollé et al. 1997; Hedberg and Långström 1997, 1998; Tavitian et al. 1998, 2003, 2004; Lange et al. 2002; Younes et al. 2002; Kühnast et al. 2004 and references therein). Fluorine-18 can be reliably and routinely produced at the multi-Curie level (Qaim and Stöcklin 1983), using the well-characterized (p,n) nuclear reaction on an oxygen-18-enriched water target on widely implemented biomedical cyclotrons of relatively low-energy proton beam (e.g. 18 MeV). This distinctive advantage, combined with its favourable half-life, permits the transport and the use of fluorine-18-labeled radiopharmaceuticals (such

as the archetype [^{18}F]FDG) at 'satellite' PET units that do not have an on-site cyclotron facility (Stöcklin 1998; Varagnolo et al. 2000).

5.1.2 Fluorine-18 Radiochemistry: Basic Principles

Even though fluorine-18 appears today as the most attractive positron-emitting radioisotope for radiopharmaceutical chemistry and PET imaging, its use also presents some drawbacks. First of all, isotopic or 'true' labeling is limited to chemical structures already containing a fluorine atom: most of the molecules of interest for biological applications do not originally contain fluorine, and compounds containing C–F bonds are rare in living nature. Another disadvantage of fluorine-18 is the restricted versatility of the possible labeling strategies, especially when compared with carbon-11, for the preparation of radiotracers and radiopharmaceuticals of high specific radioactivity (e.g. $>$ 185 GBq/μmole). With a few exceptions, radiofluorinations had been, until recently, limited to nucleophilic substitutions in *homo*aromatic and aliphatic series (Kilbourn 1990; Lasne et al. 2002; Welch and Redvanly 2003). *Homo*aromatic nucleophilic substitutions with [^{18}F]fluoride usually require activated aromatic rings, bearing both a good leaving group (e.g. a halogen, a nitro- or a trimethylammonium group) and a strong electron-withdrawing substituent (e.g. a nitro-, cyano- or acyl group) preferably placed *para* to the leaving group, whereas aliphatic nucleophilic substitutions require only a good leaving group (usually a halogen or a sulphonic acid derivative such as mesylate, tosylate, or triflate). Labeling procedures involve pre-activation of cyclotron-produced, no-carrier-added, aqueous [^{18}F]fluoride by evaporation to dryness from an added base (typically K_2CO_3) and, for example, the added kryptand Kryptofix-222, in order to form the so-called naked fluoride anion as its K[^{18}F]F-K_{222} complex (Coenen et al. 1986; Hamacher et al. 1986). Nucleophilic substitutions are then performed in an aprotic polar solvent under alkaline conditions, leading either directly to the target molecule (one-step procedure) or to a molecule needing further chemical modification, often deprotection, before yielding the target molecule (a two- or multistep procedure).

5.2 *Hetero*aromatic Nucleophilic Fluorination in the Pyridine Series

During the last decade, the scope of nucleophilic radiofluorinations has been open to *hetero*aromatic substitutions, particularly in the pyridine series (Dollé 2005 and references therein), promoted mainly by the fact that some important nicotinic receptor drug families, containing a fluoropyridine moiety, have appeared. Only a small number of references exist in the literature describing these nucleophilic substitutions in stable-fluorine-19 chemistry of *hetero*aromatic derivatives such as substituted fluoropyridines and relatives (fluoroquinolines, -isoquinolines, -naphthyridines and pyridoquinolines) (Fig. 1). This is simply due to the fact that these derivatives are usually prepared in good yields from the corresponding amino-substituted *hetero*aromatic compounds by treatment with sodium nitrite and hydrofluoric acid or fluoboric acid for example (March 1992 and references therein). The latter methods, however, are unsuitable for high-specific-radioactivity fluorine-18 labeling.

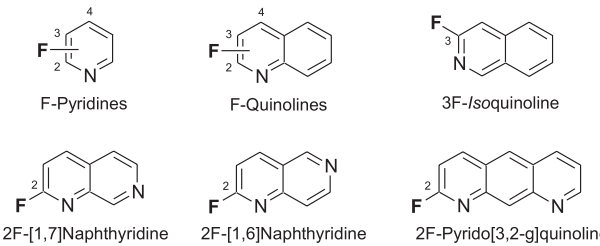


Fig. 1. Fluoropyridine structures prepared by nucleophilic aromatic substitution with fluoride

5.2.1 State-of-the-Art in Stable-Fluorine-19 Chemistry

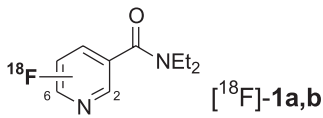
Taking the synthesis of 2-fluoropyridine as a model reaction, nucleophilic substitutions at the 2-position with [19F]fluoride usually involve chloride or bromide ions as the leaving group. 2-Fluoropyridine was obtained at a yield of 49% by heating 2-chloropyridine and [19F]fluoride in dimethylsulfone at 210 °C for 21 days (Finger et al. 1963). It was also obtained at a yield of 42% by heating 2-bromopyridine and [19F]fluoride

in dimethylsulfone at 200 °C for 7 days (Finger et al. 1963). No example of 2-fluorodeiodination can be found in the literature. 2-Fluoropyridine was also prepared from 2-nitropyridine at a yield of 60% by fluorodenitration using [^{19}F]fluoride ion in hexamethylphosphoric triamide (HMPT) at 160 °C for 24 h (Bartoli et al. 1972). Taking the synthesis of 3- or 4-fluoropyridine as a model reaction, no example of nucleophilic substitution at the 3- or 4-position with [^{19}F]fluoride can be found in the literature (Bibliographic search using MDL CrossFire Commander, Version 7.0, copyright 1995–2004, MDL Information Systems GmbH). Fluorination using nucleophilic substitution with [^{19}F]fluoride at the 2-position of pyridines bearing another function (such as nitro at the 3- or 5- position, halogen at the 3-, 5- or 6- position, cyano at the 6-position, pyrazolyl at the 3-position) have been described but exclusively involved fluorodechlorination (Peet and Sunder 1986; Miyamoto et al. 1987; Clark and Macquarrie 1987; Differding et al. 1990; Radl and Hradil 1991; Rewcastle et al. 1996; Matthews 1998; Sledeski et al. 2000; Carroll et al. 2002; Hoyte et al. 1992). One example of nucleophilic fluorodechlorination at the 3-position of a substituted pyridine (trifluoromethyl at the 5- position and an amide at the 2- position) can also be found (Sakamoto et al. 2000). Fluorinations using nucleophilic substitution with [^{19}F]fluoride at the 4-position of substituted pyridines (pyrazolyl at the 3-position or perchloropyridines) have been described and exclusively involved fluorodechlorination (Sledeski et al. 2000; Hoyte et al. 1992; Aksenov et al. 1985; Hitzke 1980). Concerning 2-fluoroquinolines, references can be found describing nucleophilic substitution with [^{19}F]fluoride at the 2-position. For example, 2-fluoroquinoline was prepared from 2-chloroquinoline and [^{19}F]fluoride to a yield of 60% after 5 days in boiling dimethylsulfone (Hamer et al. 1962). Fluorination at the 2-position of the pyridine ring of quinolines bearing another function (such as methyl at the 4-, 6-, 7-, or 8- position or formyl at the 3-position), again involving a chloride ion as the leaving group, have also been described (Kidwai et al. 1998, 1999; Kato et al. 2000). Fluorination at the *ortho*-position of the pyridine ring of substituted isoquinolines, pyrido[3,2-g]quinoline or [1,6]- and [1,7]naphthyridine have been reported and exclusively involved fluorodechlorination (Matthews and Matthews 2000; Kong et al. 2000; Chan et al. 2000; Gueret et al. 2001; Bagot-Gueret et al. 2003). One example of nucle-

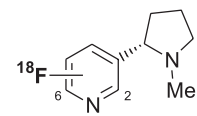
ophilic fluorodechlorinations at the 4-position of substituted quinolines can also be found (Outt et al. 1998).

5.2.2 Early Examples in Fluorine-18 Chemistry

The earliest examples of fluorine-18-labeling of fluoropyridines using nucleophilic aromatic substitution with [18F]fluoride are the preparation of 2-[18F]fluoronicotinic acid diethylamide ([18F]-**1a**), 6-[18F]fluoronicotinic acid diethylamide ([18F]-**1b**), 2-[18F]fluoronicotine ([18F]-**2a**) and 6-[18F]fluoronicotine ([18F]-**2b**) (Fig. 2). [18F]-**1a** and [18F]-**1b** were obtained in up to 40%–50% radiochemical yield (decay-corrected) from the corresponding 2- and 6-chloropyridine derivatives and [18F]fluoride as its caesium salt, in acetamide at 200 °C (Knust et al. 1982a, 1982b, 1983). [18F]-**2a** and [18F]-**2b** too were obtained using caesium[18F]fluoride. The radiochemical yield (decay-corrected) was 30%–40% in dimethylsulfoxide (DMSO) at 210 °C for 30 min from the corresponding 2- and 6-bromopyridine derivatives (Ballinger et al. 1984).



[18F]-**1a,b**



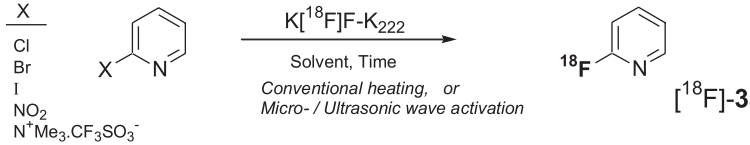
[18F]-**2a,b**

Fig. 2. Chemical structures of 2- and 6-[18F]fluoronicotinic acid diethylamide ([18F]-**1a**,**b**) and 2- and 6-[18F]fluoronicotine ([18F]-**2a**,**b**)

5.2.3 The Radiosynthesis of 2-, 3- and 4-[18F]Fluoropyridines: Scope and Limitations of These Model Reactions

Recently, the scope and limitations of these nucleophilic aromatic fluorinations of the pyridine ring with no-carrier-added [18F]fluoride as its activated K[18F]F-K_{222} complex have been evaluated. In a first study (Dolci et al. 1999a), the synthesis of 2-[18F]fluoropyridine ([18F]-**3a**) was chosen as a model reaction (Scheme 1). The parameters studied included the nature of the leaving group in the 2 position of the pyridine ring (-Cl, -Br, -I, $-NO_2$ or $-N^+Me_3.CF_3SO_3^-$), the quantity of the

precursor used, the type of activation (conventional heating, micro- and ultrasonic wave irradiation), the solvent, the temperature and the duration of the reaction.



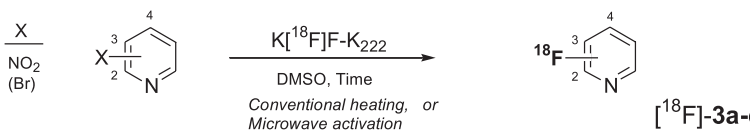
Scheme 1. Radiosynthesis of 2-[^{18}F]fluoropyridine ([^{18}F]-**3a**) from the corresponding 2-Cl, 2-Br, 2-I, 2-NO_2 and 2-$N^+Me_3.CF_3SO_3^-$ precursor

As shown in that study, yields of formation of 2-[^{18}F]fluoropyridine ([^{18}F]-**3a**), using conventional heating in DMSO (selected as a good solvent), were higher when -NO_2 or -N^+Me_3 were used as the leaving group and this was independent of the reaction time (5, 10 or 20 min) and temperature used (120 °C, 150 °C or 180 °C). Very high incorporation yields were observed with the N^+Me_3-precursor from 5 min of reaction on (81% and 88% radiochemical yield at 120 °C and 150 °C, respectively). Comparable yields were only obtained for the NO_2-precursor when longer reaction times (10 or 20 min) or higher temperatures were applied. The iodo-derivative was the less reactive precursor in all of the conditions used (maximum radiochemical yield of 2-[^{18}F]fluoropyridine: 19%, for 180 °C/20 min), although the iodo substituent is usually considered an excellent leaving group. For the other halo-precursors, incorporation yields increase with the reaction time and the temperature used, the Br-precursor giving higher radiochemical yield than the Cl-precursor (2-[^{18}F]fluoropyridine: 3% and 28% after 10 min for the Cl-precursor at 150 °C and 180 °C, respectively; 16% and 60% after 20 min for the Br-precursor at 150 °C and 180 °C, respectively) and both precursors are unreactive at 120 °C (20 min). Using microwave irradiation (MicroWell 10–2.45 GHz, Labwell, Sweden), excellent incorporation yields (96%) were observed for the N^+Me_3-precursor from a 1-min of reaction at 100 W in DMSO. Concerning the Cl-, Br- and NO_2-precursor, the use of 100-W microwave irradiation for 2 min gave radiochemical yields comparable to those obtained for 10 min of conventional heating at 180 °C (22%, 71% and 88% respectively). No incorporation of radioac-

tivity could be detected when ultrasonic waves were applied, even with a long reaction time and high power.

In a second study (Karramkam et al. 2003a), the syntheses of 3-[^{18}F]fluoropyridine ([^{18}F]-**3b**) and 4-[^{18}F]fluoropyridine ([^{18}F]-**3c**) were also evaluated and the radiochemical yields obtained for these *meta*- and *para*-nucleophilic aromatic fluorinations were compared to those obtained for the synthesis of 2-[^{18}F]fluoropyridine ([^{18}F]-**3a**) (Scheme 2). The parameters studied included the influence of the position of the leaving group at the pyridine ring (2-, 3- and 4-position), as well as the type of activation (conventional heating to various temperatures or microwave irradiation) and the duration of the reaction. Using the corresponding NO_2-precursor, high radiochemical yields (94%) for the formation of 2-[^{18}F]fluoropyridine ([^{18}F]-**3a**) were confirmed using microwaves (100 W) for 2 min in DMSO. Good yields (up to 72%) were observed at the 4-position for the formation of 4-[^{18}F]fluoropyridine ([^{18}F]-**3c**) using similar conditions. Using conventional heating and the corresponding NO_2-precursor at 145 °C for 10 min, 2- and 4-[^{18}F]fluoropyridine ([^{18}F]-**3a** and [^{18}F]-**3c**) could be obtained in 66% and 60% radiochemical yield, respectively. Whatever the conditions used, practically no 3-[^{18}F]fluoropyridine ([^{18}F]-**3b**) could be obtained from either from the corresponding NO_2-precursor or from the Br-precursor.

These model studies validated the feasibility and the potential of nucleophilic *hetero*aromatic fluorination for the design of new fluorine-18-labeled radiotracers. One can say today that, in chemical structures showing a fluoropyridinyl moiety, nucleophilic *hetero*aromatic substitution at the *ortho*-position with no-carrier-added [^{18}F]fluoride as its activated K[^{18}F]F-K_{222} complex, is the most efficient method for the radiosynthesis of radiopharmaceuticals of high specific radioactivity when compared to both *homo*aromaticand aliphatic nucleophilic radiofluorination.



Scheme 2. Radiosynthesis of 2-, 3- and 4-[^{18}F]fluoropyridine ([^{18}F]-**3a-c**) from the corresponding -Br and -NO_2 precursor

5.2.4 Mechanistic Aspects of Formation of [^{18}F]Fluoropyridines: The Addition-Elimination Sequence

Nucleophilic substitution of aromatic compounds usually proceeds via an addition (of a nucleophile) then elimination (of a good leaving group, often a negatively charged entity such as a halogen) two-step sequence of which the first step is normally the rate determining one. It is the stabilization of the negatively charged intermediates (also called Meisenheimer complexes) that is the key to such processes (Joule and Mills 2000). This mechanism remains applicable to nucleophilic substitution of *hetero*cyclic aromatic compounds, and especially to 6-membered electron-poor systems such as the pyridines. Figure 3 illustrates this aspect using the [^{18}F]fluoride anion and a halogen as the nucleophilic species and the leaving group, respectively.

Position α and γ to the imine nitrogen are activated for the initial addition of a nucleophile by the following two factors: (a) inductive and mesomeric withdrawal of electrons by the nitrogen (leading to electron-deficiency at all carbons, but especially at the α- and γ-positions); and (b) inductive withdrawal of electrons by the halogen (further increasing the electron-deficiency notably at its adjacent carbon). The σ-adduct intermediate is therefore especially stabilized when the nucleophile attacks at these α- and γ-positions as in these intermediates the negative charge resides largely on the nitrogen. Nucle-

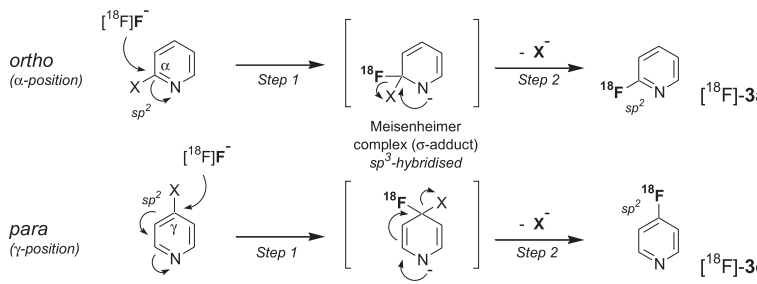


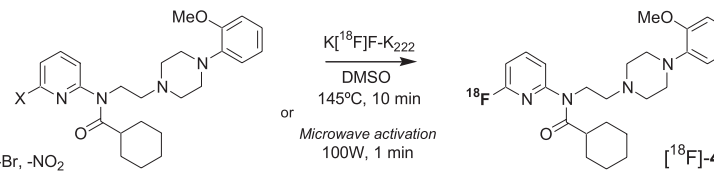
Fig. 3. Addition–elimination process involved in nucleophilic *hetero*aromatic fluorination with [^{18}F]fluoride

ophilic displacements at the β-position are therefore expected to be much slower, and for practical purposes, they can be considered not to occur.

5.3 Radiosynthesis of [Pyridinyl-^{18}F]Radiotracers

A recent example of application of these nucleophilic *hetero*aromatic substitutions can be found in the field of the design and synthesis of radiofluorinated derivatives of the 5-HT_{1A} receptor antagonist WAY-100635.

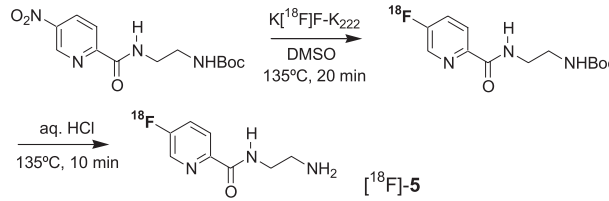
The 6-fluoropyridinyl analogue of WAY-100635, 6-[^{18}F]fluoro-WAY-100635 ([^{18}F]-**4**, *N*-(2-(1-(4-(2-methoxyphenyl)piperazinyl)ethyl))-*N*-(2-(6-[^{18}F]fluoro)pyridinyl)cyclohexane carboxamide) was efficiently synthesized in one radiochemical step and was obtained in 15%–25% non-decay-corrected overall radiochemical yield in 50–70 min total synthesis time (Scheme 3). This one-step reaction was performed using K[^{18}F]F-K_{222}, either from the corresponding bromo-precursor and conventional heating at 145 °C for 10 min (60%–65% radiochemical yield) or from the corresponding nitro-precursor and microwave activation (MicroWell 10–2.45 GHz, Labwell, Sweden) at 100 W for 1 min (up to 93% radiochemical yield) (Karramkam et al. 2003b). Whereas [^{18}F]-**4** was shown to be only moderately effective for imaging brain 5-HT_{1A} receptors in vivo, it was clearly demonstrated from a metabolic point of view that the fluorine substituent at the *ortho*-position of the pyridinyl ring is resistant to defluorination (Sandell et al. 2001; McCarron et al. 2004).



Scheme 3. Radiosynthesis of [6-pyridinyl-^{18}F]fluoro-WAY-100635 ([^{18}F]-**4**,*N*-(2-(1-(4-(2-methoxyphenyl)piperazinyl)ethyl))-*N*-(2-(6-[^{18}F]fluoro)pyridinyl)-cyclohexane carboxamide)

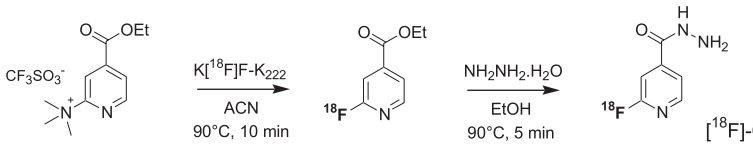
The fluorine-18-labeled 5-fluoropyridinyl analogue of WAY-100635 (5-[^{18}F]fluoro-WAY-100635, *N*-(2-(1-(4-(2-methoxyphenyl)piperazinyl)ethyl))-*N*-(2-(5-[^{18}F]fluoro)pyridinyl)cyclohexane carboxamide, structure not shown) could not be synthesized from the corresponding 5-bromo- and 5-nitro-derivatives as precursors (whatever the conditions used, conventional heating or microwave activation), clearly confirming that in the pyridine series, *meta*-[^{18}F]fluorination is normally not of interest for the preparation of a fluorine-18-labeled radiotracer by nucleophilic *hetero*aromatic substitution (Karramkam et al. 2003b).

In fact, there is only one example of a *meta*-[^{18}F]fluoropyridine derivative known to date: *N*-(2-aminoethyl)-5-[^{18}F]fluoropyridine-2-carboxamide ([^{18}F]-**5**), a potential MAO-B imaging radiotracer (Scheme 4) (Beer et al. 1995). [^{18}F]-**5** was prepared in 37% overall radiochemical yield (decay-corrected) from the corresponding nitro-precursor (using K[^{18}F]F-K$_{222}$ and conventional heating in DMSO at 135 °C for 20 min). Thus [^{18}F]fluorination at the *meta*-position is possible, but only in structures bearing an activating group (strongly electron-withdrawing), in this case *para* to the leaving group, as in conventional nucleophilic *homo*aromatic substitutions.



Scheme 4. Radiosynthesis of *N*-(2-aminoethyl)-5-[^{18}F]fluoropyridine-2-carboxamide ([^{18}F]-**5**)

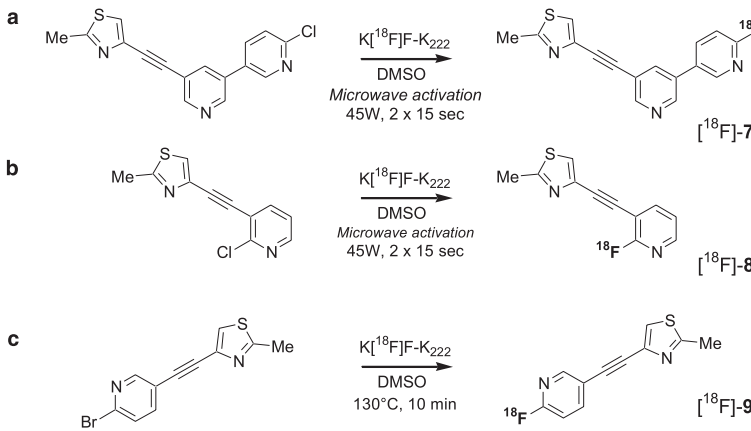
Another recent example is the preparation of 2-[^{18}F]fluoroisonicotinic acid hydrazide ([^{18}F]-**6**, a fluoro analogue of the highly effective antituberculosis agent Isoniazid®) (Scheme 5) (Amartey et al. 2002), designed as a PET-radiotracer for the detection of central nervous system located tuberculoma. [^{18}F]-**6** was synthesized in two radiochemical steps, starting from the corresponding trimethylammonium-precursor and was obtained in 70% overall radiochemical yield (decay-corrected) in 60 min



Scheme 5. Radiosynthesis of 2-[^{18}F]fluoroisonicotinic acid hydrazide ([^{18}F]-**6**)

total synthesis time. The nucleophilic *hetero*aromatic substitution with fluorine-18 was performed using K[^{18}F]F-K_{222} and conventional heating in acetonitrile at 90 °C for 10 min. Note that the reaction conditions are milder than those described above for the corresponding model reaction (Dolci et al. 1999a) (Scheme 1), whilst the radiochemical yields are at least comparable, possibly due to the activating group (electron-withdrawing) in the *meta*-position.

Three fluorine-18-labeled fluoropyridine derivatives for imaging the metabotropic glutamate receptors (subtype 5, mGluR5) in the brain were recently described (Scheme 6).

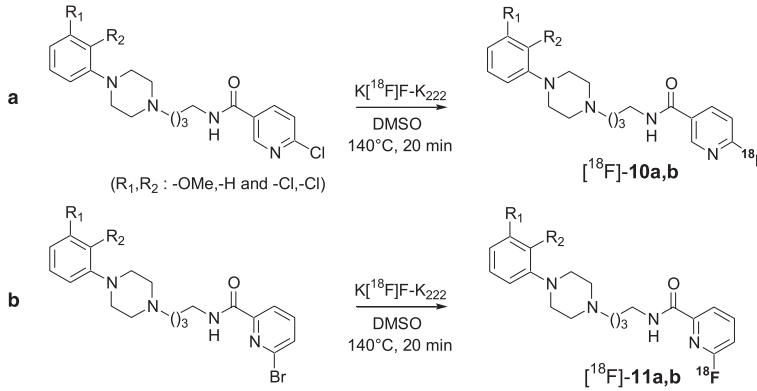


Scheme 6. Radiosyntheses of 6′-[^{18}F]fluoro-5-[(2-methyl-1,3-thiazol-4-yl)ethynyl]-3,3′-bipyridine ([^{18}F]-**7**), 2-[^{18}F]fluoro-3-[(2-methyl-1,3-thiazol-4-yl)ethynyl]pyridine ([^{18}F]-**8**) and 2-[^{18}F]fluoro-5-[(2-methyl-1,3-thiazol-4-yl)ethynyl]-pyridine ([^{18}F]-**9**)

Firstly, 6′-[^{18}F]fluoro-5-[(2-methyl-1,3-thiazol-4-yl)ethynyl]-3,3′-bipyridine ([^{18}F]-**7**) (Scheme 6a) and 2-[^{18}F]fluoro-3-[(2-methyl-1,3-thiazol-4-yl)ethynyl]pyridine ([^{18}F]-**8**) (Scheme 6b) were synthesized in one radiochemical step from the appropriate chloro-precursors and were obtained in > 40% radiochemical yield (decay-corrected) within 30 min total synthesis time (Cosford et al. 2004). The nucleophilic *hetero*aromatic substitutions with fluorine-18 were performed using K[^{18}F]F-K_{222} and microwave activation (Resonance Instruments 521, USA) at 45 W for about 30 s (up to 75% radiochemical yield). Finally, 2-[^{18}F]fluoro-5-[(2-methyl-1,3-thiazol-4-yl)ethynyl]pyridine ([^{18}F]-**9**) (Scheme 6c) was synthesized in one radiochemical step from the appropriate bromo-precursor (Yu et al. 2005). The nucleophilic *hetero*aromatic substitution with fluorine-18 was performed using K[^{18}F]F-K_{222} and conventional heating in DMSO at 130 °C for 10 min (the radiochemical yields and synthesis time were not reported).

Four fluorine-18-labeled fluoropyridine derivatives for imaging the dopaminergic D3 receptors in the brain have just recently been described (Scheme 7).

6-[^{18}F]fluoro-*N*-{4-[4-(2-methoxy-phenyl)-piperazin-1-yl]-butyl}-nicotinamide ([^{18}F]-**10a**) and 6-[^{18}F]fluoro-*N*-{4-[4-(2,3-dichloro-phenyl)-piperazin-1-yl]-butyl}-nicotinamide ([^{18}F]-**10b**) were synthesized in one radiochemical step from the appropriate chloro-precursors (Scheme 7a, total synthesis time and final radiochemical yield were not indicated) (Hocke et al. 2005). Similarly, 6-[^{18}F]fluoro-pyridine-2-carboxylic acid {4-[4-(2-methoxy-phenyl)-piperazin-1-yl]-butyl}-amide ([^{18}F]-**11a**) and 6-[^{18}F]fluoro-pyridine-2-carboxylic acid {4-[4-(2,3-dichloro-phenyl)-piperazin-1-yl]-butyl}-amide ([^{18}F]-**11b**) were synthesized in one radiochemical step from the appropriate bromo-precursors (Scheme 7b, total synthesis time and final radiochemical yield not-indicated) (Hocke et al. 2005). The nucleophilic *hetero*aromatic substitutions with fluorine-18 were performed using K[^{18}F]F-K_{222} and conventional heating in DMSO at 140 °C for 20 min with decay-corrected radiochemical yields of 81±5%, 82±4%, 76±6% and 78±5% for [^{18}F]-**10a,** [^{18}F]-**10b**, [^{18}F]-**11a** and [^{18}F]-**11b**, respectively. The radiochemical yields obtained for these fluorodehalogenations at the 2-position are higher than those described above for the corresponding model reaction

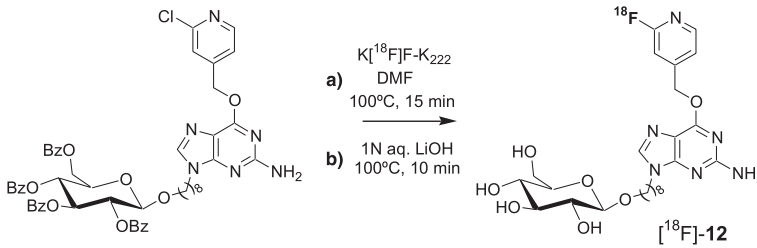


Scheme 7. Radiosyntheses of 6-[^{18}F]fluoro-*N*-{4-[4-(2-methoxy-phenyl)-piperazin-1-yl]-butyl}-nicotinamide ([^{18}F]-**10a**), 6-[^{18}F]fluoro-*N*-{4-[4-(2,3-dichloro-phenyl)-piperazin-1-yl]-butyl}-nicotinamide ([^{18}F]-**10b**), 6-[^{18}F]fluoro-pyridine-2-carboxylic acid {4-[4-(2-methoxy-phenyl)-piperazin-1-yl]-butyl}-amide ([^{18}F]-**11a**) and 6-[^{18}F]fluoro-pyridine-2-carboxylic acid {4-[4-(2,3-dichloro-phenyl)-piperazin-1-yl]-butyl}-amide ([^{18}F]-**11b**)

(Dolci et al. 1999a, Scheme 1), probably due to the presence of the electron-withdrawing amide group attached to the pyridine moiety.

In a completely different field of imaging, oncology, 2-amino-6-(2-[^{18}F]fluoropyridin-4-ylmethoxy)-9-(gβ-D-glucosyl-octyl)-purine ([^{18}F]-**12**) has been designed as a novel fluorine-18-labeled radioligand for the study of the N^6-methylguanine-DNA methyltransferase (MGMT) status of tumour tissue (Scheme 8) (Schirrmacher et al. 2002a, 2002b). [^{18}F]-**12** was synthesized in two radiochemical steps from the *O*-benzoyl-protected chloro-precursor and was obtained in 5% overall radiochemical yield (decay-corrected) in about 60 min total synthesis time. The nucleophilic *hetero*aromatic substitution with fluorine-18 was performed using K[^{18}F]F-K$_{222}$ and conventional heating in dimethylfluoride (DMF) at 100 °C for 15 min (15% radiochemical yield), followed by alkaline-removal of the *O*-benzoyl protective groups.

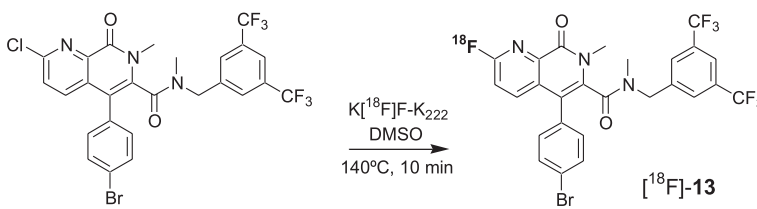
One example can also be found in the naphthyridine series, where *N*-[3,5-bis(trifluoromethyl)benzyl]-*N*-methyl-5-(4-bromophenyl)-2-[^{18}F]fluoro-7-methyl-8-oxo-7,8-dihydro-[1,7]naphthyridine-6-carboxa-



Scheme 8. Radiosynthesis of 2-amino-6-(2-[^{18}F]fluoropyridin-4-ylmethoxy)-9-(β-D-glucosyl-octyl)-purine ([^{18}F]-**12**)

mide ([^{18}F]-**13**) has been described as a potent antagonist of the NK1 (Substance P-preferring) receptors (Scheme 9) (Gueret et al. 2001; Bagot-Gueret et al. 2003). [^{18}F]-**13** was synthesized in one radiochemical step from the Cl-precursor and was routinely obtained in 25% overall radiochemical yield (decay-corrected) in about 110 min total synthesis time (Scheme 9). The nucleophilic *hetero*aromatic substitution with fluorine-18 was performed using K[^{18}F]F-K$_{222}$ and conventional heating in DMSO at 140 °C for 10 min (occasionally up to 88% radiochemical yield). This exceptionally high yield for this fluorodechlorination at the 2-position is probably due to the presence of the electron-withdrawing carbonyl group of the 8-oxo-7,8-dihydro-[1,7]naphthyridine moiety.

The nucleophilic *hetero*aromatic radiofluorination has been applied mostly to the design of new fluorine-18-labeled radiotracers for the in

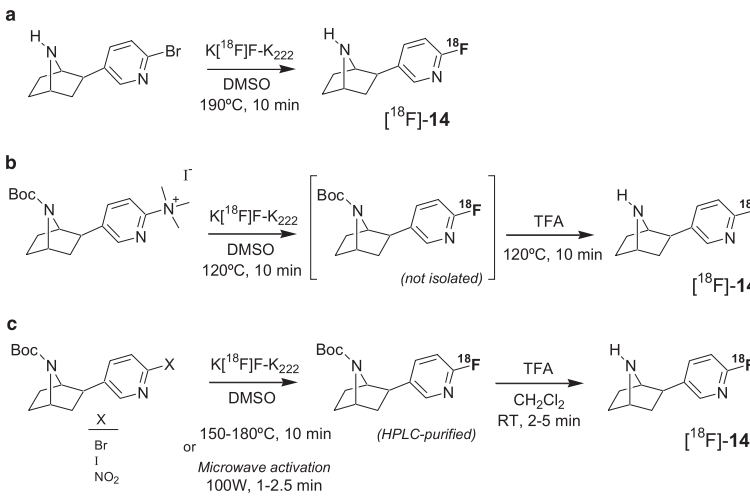


Scheme 9. Radiosynthesis of *N*-[3,5-bis(trifluoromethyl)benzyl]-*N*-methyl-5-(4-bromophenyl)-2-[^{18}F]fluoro-7-methyl-8-oxo-7,8-dihydro-[1,7]naphthyridine-6-carboxamide ([^{18}F]-**13**)

vivo imaging and study of the central nicotinic cholinergic $\alpha_4\beta_2$ system in human brain with PET, starting either from the natural products epibatidine and (–)-cytisine or from a well-characterized drug from Abott Laboratories, coded A-85380.

The earliest example is the preparation of the potent nicotinic acetylcholine receptor ligand norchloro[^{18}F]fluoroepibatidine ([^{18}F]-**14**, (±)-*exo*-2-(6-[^{18}F]fluoro-3-pyridyl)-7-azabicyclo [2.2.1]heptane) (Scheme 10). This compound is a fluorine-18-labeled fluoro analogue of epibatidine, a natural compound, isolated from the skin of the Ecuadoran poisonous frog *Epipedobates tricolor*, characterized as a very potent high-affinity nicotinic acetylcholine receptor (nAChR) agonist also showing antinociceptive activity.

Norchloro[^{18}F]fluoroepibatidine ([^{18}F]-**14**) was first synthesized in one radiochemical step implying a nucleophilic aromatic bromo-to-fluoro substitution (Horti et al. 1996, 1997; Villemagne et al. 1997) (Scheme 10a) from the corresponding non-protected bromo-precursor (using K[^{18}F]F-K_{222} and conventional heating in DMSO at 190 °C



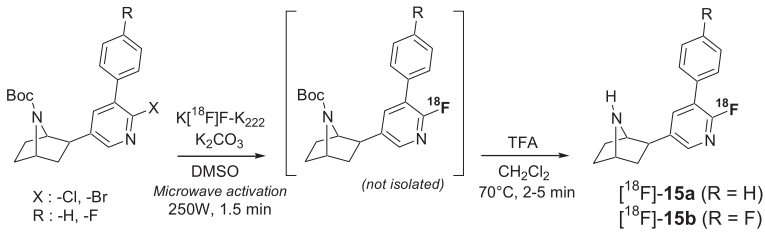
Scheme 10. Radiosyntheses of norchloro[^{18}F]fluoroepibatidine ([^{18}F]-**14**, (±)-*exo*-2-(6-[^{18}F]fluoro-3-pyridyl)-7-azabicyclo [2.2.1]heptane)

for 10 min) and was obtained in 10% radiochemical yield (decay-corrected) in 50 min total synthesis time. [^{18}F]-**14** was also synthesized in two radiochemical steps from the corresponding *N*-Boc-protected trimethylammonium-precursor and was obtained in 55%–65% overall radiochemical yield (decay-corrected) in 65 min total synthesis time (Ding et al. 1996, 1997; Liang et al. 1997) (Scheme 10b). The nucleophilic *hetero*aromatic substitution with fluorine-18 was performed using K[^{18}F]F-K_{222} and conventional heating in DMSO at 120 °C for 10 min (in up to 70% radiochemical yield), followed by quantitative trifluoroacetic acid (TFA)-removal of the *N*-Boc protective group. Using a similar radiochemical two-step approach, [^{18}F]-**14** was also synthesized from the corresponding *N*-Boc-protected bromo- and nitro-precursor and was obtained in 20%–28% overall radiochemical yield (decay-corrected) in 110 min total synthesis time (Dolci et al. 1999b) (Scheme 10c). The nucleophilic *hetero*aromatic substitution with fluorine-18 was performed using K[^{18}F]F-K_{222} and conventional heating in DMSO at 180 °C for 10 min (in up to 51% radiochemical yield with the bromo-precursor) or microwave activation (MicroWell 10–2.45 GHz, Labwell, Sweden) at 100 W, for 1–2.5 minutes (20% and 49% radiochemical yield at 1 min for the bromo- and nitro-precursor, respectively, and up to 72% yield for the bromo-precursor at 2.5 min). In this study, the corresponding *N*-Boc-protected iodo-precursor was also evaluated and was again (see above) found poorly reactive (only 14% radiochemical yield in DMSO using conventional heating at 180 °C for 10 min and 9% radiochemical yield using microwave activation at 100 W for 1 min). Note that in general, protection of the free amine function is highly advisable although not mandatory (compare Scheme 10c/10a). Norchloro[^{18}F]fluoroepibatidine ([^{18}F]-**14**) showed an exceptionally promising brain distribution and in vivo pharmacological characteristics in non-human primates (Villemagne et al. 1997; Gatley et al. 1998; Ding et al. 1999, 2000a). However, although the mass of this fluoro-analogue of epibatidine needed to image nAChRs in humans with PET was estimated to be far below pharmacologically active doses, its toxicity prohibited its use in humans (Molina et al. 1997).

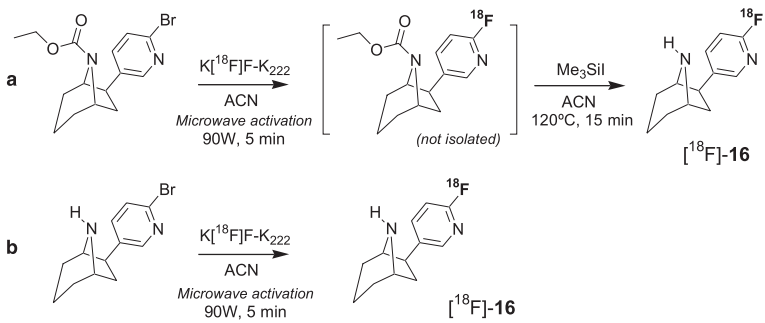
Recently, two new epibatidine-based antagonists, showing less toxicity (Carroll et al. 2001, 2004; Huang et al. 2005) as well as one non-toxic

homo-epibatidine analogue have also been reported and labeled with fluorine-18 (Schemes 11 and 12).

A first compound, coded [^{18}F]FPhEP ([^{18}F]-**15a**, 2-*exo*-(2′-[^{18}F]fluoro-3′-phenyl-pyridin-5′-yl)-7-azabicyclo[2.2.1]heptane) was synthesized in two radiochemical steps from the corresponding *N*-Boc-protected chloro- and bromo-precursor and was obtained in 10%–20% non-decay-corrected overall radiochemical yield in 75–85 min total synthesis time (Roger et al. 2005; Valette et al. 2005b) (Scheme 11). A second compound, coded [^{18}F]F$_2$PhEP ([^{18}F]-**15b**, 2-*exo*-(2′-[^{18}F]fluoro-3′-(4-fluorophenyl)-pyridin-5′-yl)-7-azabicyclo[2.2.1]heptane) was also synthe-



Scheme 11. Radiosynthesis of [^{18}F]FPhEP ([^{18}F]-**15a**, 2-*exo*-(2′-[^{18}F]fluoro-3′-phenyl-pyridin-5′-yl)-7-azabicyclo[2.2.1]heptane) and [^{18}F]F$_2$PhEP ([^{18}F]-**15b**, 2-*exo*-(2′-[^{18}F]fluoro-3′-(4-fluorophenyl)-pyridin-5′-yl)-7-azabicyclo[2.2.1]-heptane)



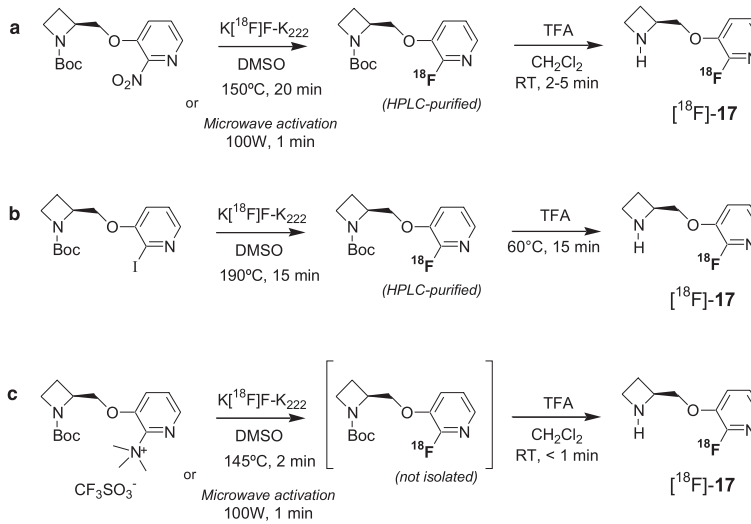
Scheme 12. Radiosyntheses of [^{18}F]NCFHEP ([^{18}F]-**16**, *exo*-6-(2-[^{18}F]fluoro-5-pyridyl)-8-azabicyclo[3.2.1]octane)

sized using similar conditions and yields (Roger et al. 2005; Valette et al. 2005b). In both cases, the nucleophilic *hetero*aromatic substitutions with fluorine-18 were performed using K[^{18}F]F-K_{222} and microwave activation (MicroWell 10–2.45 GHz, Labwell, Sweden) at 250 W for 90 s (25%–40% radiochemical yield), followed by quantitative TFA-removal of the *N*-Boc protective group.

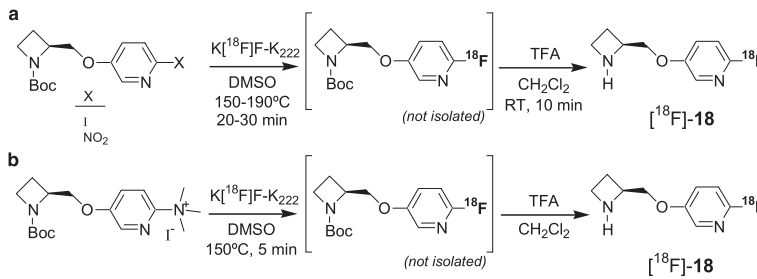
The *homo*-epibatidine analogue, coded [^{18}F]NCFHEP ([^{18}F]-**16**, *exo*-6-(2-[^{18}F]fluoro-5-pyridyl)-8-azabicyclo[3.2.1]octane) was synthesized in two radiochemical steps from the corresponding *N*-ethoxycarbonyl-protected bromo-precursor and was obtained in only 2% overall radiochemical yield (decay-corrected) (total synthesis time not indicated) (Patt et al. 2003; Deuther-Conrad et al. 2004) (Scheme 12a). [^{18}F]-**16** was also synthesized from the unprotected bromo-precursor in only one radiochemical step and 1.5%–3.5% overall radiochemical yield (decay-corrected) (Patt et al. 2005) (Scheme 12b). In both cases, the nucleophilic *hetero*aromatic substitutions with fluorine-18 were performed using K[^{18}F]F-K_{222} and microwave activation (CEM Discover, USA) at 90 W for 5 min. The *N*-ethoxycarbonyl (Scheme 12a) was removed with trimethylsilyl iodide.

The two following examples concern the preparation of other potent nAChR ligands, 2-[^{18}F]fluoro-A-85380 ([^{18}F]-**17**, 2-[^{18}F]fluoro-3-[2(S)-2-azetidinylmethoxy]pyridine) and 6-[^{18}F]fluoro-A-85380 ([^{18}F]-**18**, 6-[^{18}F]fluoro-3-[2(S)-2-azetidinylmethoxy]pyridine) (Schemes 13 and 14). Both compounds are fluorine-18-labeled fluoro analogues of A-85380 (Abott laboratories), the lead compound of a series of 3-pyridyl ethers (Abreo et al. 1996; Sullivan et al. 1996; Holladay et al. 1998a), that are potent and selective ligands for the human $\alpha_4\beta_2$ nAChR subtype. This series not only possesses subnanomolar affinity for brain nAChRs and differentially acts on subtypes of neuronal nAChR but also shows a satisfactory safety profile (Holladay et al. 1998a, 1998b). The novel structural features of these ligands retain the high potency of epibatidine and impart a subtype selectivity not observed with the latter.

2-[^{18}F]fluoro-A-85380 ([^{18}F]-**17**) was first synthesized in two radiochemical steps from the *N*-Boc-protected NO_2-precursor and was obtained in 49%–64% overall radiochemical yield (decay-corrected) in 105–110 min total synthesis time (Scheme 13a) (Dollé et al. 1998). The nucleophilic *hetero*aromatic substitution with fluorine-18 was per-



Scheme 13. Radiosyntheses of 2-[18F]fluoro-A-85380 ([18F]-**17**, 2-[18F]fluoro-3-[2(S)-2-azetidinylmethoxy]pyridine)



Scheme 14. Radiosyntheses of 6-[18F]fluoro-A-85380 ([18F]-**18**, 6-[18F]fluoro-3-[2(S)-2-azetidinylmethoxy]pyridine)

formed using K[18F]F-K222 and conventional heating in DMSO at 150 °C for 20 min or microwave activation (MicroWell 10–2.45 GHz, Labwell, Sweden) at 100 W for 1 min (70%, and occasionally up to 90% radiochemical yield), followed by quantitative TFA-removal of the

N-Boc protective group. This radiochemical two-step process was recently simplified and [^{18}F]-**17** can be obtained in 45%–55% overall radiochemical yield (decay-corrected) in only 55–60 min total synthesis time (Liu et al. 2002). The nucleophilic *hetero*aromatic substitution with fluorine-18 was performed using the same *N*-Boc-protected NO_2-precursor and K[^{18}F]F-K_{222} and conventional heating in DMSO at 120 °C for 10 min (50%–65% radiochemical yield). [^{18}F]-**17** was also synthesized in two radiochemical steps from the *N*-Boc-protected iodo-precursor and was obtained in 21% overall radiochemical (decay-corrected) yield in 120 min total synthesis time (Scheme 13b) (Horti et al. 1998a). The nucleophilic *hetero*aromatic substitution with fluorine-18 was performed using K[^{18}F]F-K_{222} and conventional heating in DMSO at 190 °C for 15 min (40% radiochemical yield). Attempts to perform a one-step radiosynthesis from the corresponding non-protected iodo-precursor failed, due to thermal instability of [^{18}F]-**17** in the rather severe conditions used (Horti et al. 1998a). [^{18}F]-**17** was finally also synthesized in two radiochemical steps from the *N*-Boc-protected N^+Me_3-precursor and was obtained in 68%–72% overall radiochemical yield (decay-corrected) in 50–53 min total synthesis time (Scheme 13c). The nucleophilic *hetero*aromatic substitution with fluorine-18 was performed using K[^{18}F]F-K_{222} and conventional heating in DMSO at 145 °C for 2 min or microwave activation (MicroWell 10–2.45 GHz, Labwell, Sweden) at 100 W for 1 min (85%–90% radiochemical yield) (Dollé et al. 1999). This radiochemical two-step process was recently simplified (avoiding final HPLC purification and only using Sep-Pak cartridges) and [^{18}F]-**17** can be obtained in only 35 min total synthesis time in up to 55% overall radiochemical yield (decay-corrected) (Schmaljohann et al. 2004, 2005).

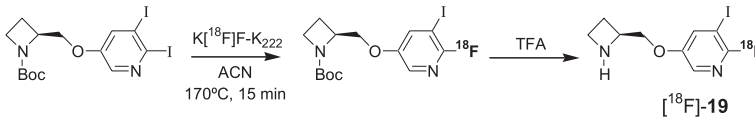
6-[^{18}F]fluoro-A-85380 ([^{18}F]-**18**), the second closely-related fluorine-18-labeled fluoro analogue of A-85380, was first synthesized in two radiochemical steps from the *N*-Boc-protected iodo-precursor and was obtained in 28% overall radiochemical yield (decay-corrected) in 85–95 min total synthesis time (Scheme 14a) (Horti et al. 2000; Scheffel et al. 2000). The nucleophilic *hetero*aromatic substitution with fluorine-18 was performed using K[^{18}F]F-K_{222} and conventional heating in DMSO at 190 °C for 30 min (up to 33% radiochemical yield), followed by TFA-removal of the *N*-Boc protective group. Note that in

these alkoxy-substituted pyridines, the radiochemical yields observed for these fluorodeiodinations (40% and 28% for the preparation of *N*-Boc-protected [^{18}F]-**17** and [^{18}F]-**18**, respectively) are considerably higher than those described above for the corresponding model reaction [maximum 19% for the preparation [^{18}F]-**3a** (Dolci et al. 1999a) in Scheme 1]. [^{18}F]-**18** was also synthesized in two radiochemical steps from the *N*-Boc-protected NO_2-precursor (Scheme 14a) and was obtained in non-optimized 16%–24% overall radiochemical yield (decay-corrected) in 110 min total synthesis time (Koren et al. 2000). The nucleophilic *hetero*aromatic substitution with fluorine-18 was performed using K[^{18}F]F-K_{222} and conventional heating in DMSO at 150 °C for 20 min (up to 25% radiochemical yield). Finally, [^{18}F]-**18** was also synthesized in two radiochemical steps from the *N*-Boc-protected N^+Me_3-precursor and was obtained in 38%–48% overall radiochemical yield (decay-corrected) in 50–55 min total synthesis time (Scheme 14b) (Ding et al. 2000b). The nucleophilic *hetero*aromatic substitution with fluorine-18 was performed using K[^{18}F]F-K_{222} and conventional heating in DMSO at 150 °C for 5 min (45%–60% radiochemical yield).

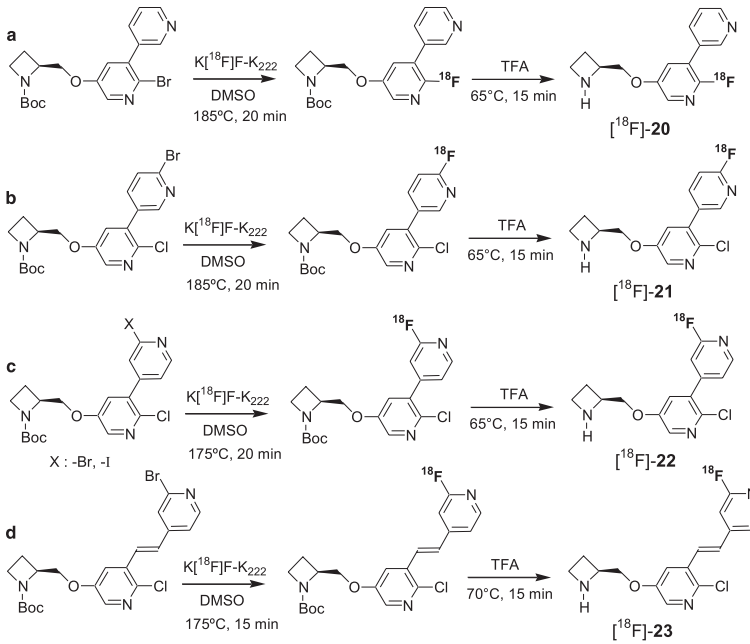
2-[^{18}F]fluoro-A-85380 ([^{18}F]-**17**) shows high affinity and selectivity for the $\alpha_4\beta_2$ nAChR subtype (Koren et al. 1998; Horti et al. 1998b; Valette et al. 1999a; Vaupel et al. 2005) and has already been used extensively in non-human primate studies (Valette et al. 1999b, 2003, 2005a; Chefer et al. 1999, 2003). It also displays a safe profile for its use as a PET probe in humans (Mitkovski et al. 2005; Gallezot et al. 2005) : low toxicity (Valette et al. 2002; Vaupel et al. 2005), lack of mutagenicity (Valette et al. 2002; Vaupel et al. 2005) and acceptable effective dose equivalent to the patient in dosimetric studies (Bottlaender et al. 2003; Kimes et al. 2003; Vaupel et al. 2005). So far, 6-[^{18}F]fluoro-A-85380 ([^{18}F]-**18**) has been studied less but seems to show similar pharmacological, toxicological and imaging properties as 2-[^{18}F]fluoro-A-85380 ([^{18}F]-**17**) (Ding et al. 2004; Gündisch et al. 2005).

More lipophilic fluorine-18-labeled fluoro-analogues of A-85380 have also been prepared recently (Schemes 15 and 16).

First of all, 6-[^{18}F]fluoro-5-iodo-A-85380 ([^{18}F]-**19**, 6-[^{18}F]fluoro-5-iodo-3-[2(S)-2-azetidinylmethoxy]pyridine) was synthesized in two radiochemical steps from the *N*-Boc-protected iodo-precursor and was obtained in 1% overall radiochemical yield (decay-corrected) in 100 min



Scheme 15. Radiosynthesis of 6-[^{18}F]fluoro-5-iodo-A-85380 ([^{18}F]-**19**, 6-[^{18}F]-fluoro-5-iodo-3-[2(S)-2-azetidinylmethoxy]pyridine)



Scheme 16. Radiosyntheses of [^{18}F]NIDA52158 ([^{18}F]-**20**, 5-(azetidin-2-ylmethoxy)-2-[^{18}F]fluoro-[3,3′]bipyridinyl), [^{18}F]NIDA52189([^{18}F]-**21**, 5-(azetidin-2-ylmethoxy)-2-chloro-6′-[^{18}F]fluoro-[3,3′]bipyridinyl), [^{18}F]NIDA522131 ([^{18}F]-**22**, 5-(azetidin-2-ylmethoxy)-2-chloro-2′-[^{18}F]fluoro-[3,4′]bipyridinyl) and [^{18}F]NIDA52289 ([^{18}F]-**23**, 5-(azetidin-2-ylmethoxy)-2-chloro-3-[2-(2-[^{18}F]fluoro-pyridin-4-yl)-vinyl]-pyridine)

total synthesis time (Scheme 15) (Koren et al. 2001). The nucleophilic *hetero*aromatic substitution with fluorine-18 was performed using

K[^{18}F]F-K_{222} and conventional heating in acetonitrile at 170 °C for 15 min (the corresponding radiochemical yield was not indicated), followed by TFA-removal of the *N*-Boc protective group.

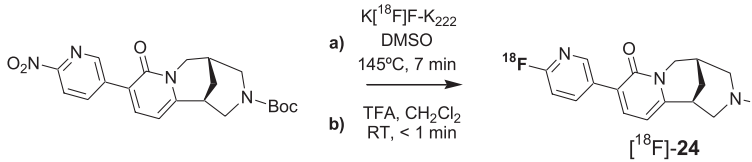
Also, four azetidine-based, 6-[^{18}F]fluoro-5-substituted-pyridinyl derivatives, namely [^{18}F]NIDA52158 ([^{18}F]-**20,** 5-(azetidin-2-ylmethoxy)-2-[^{18}F]fluoro-[3,3′]bipyridinyl), [^{18}F]NIDA52189 ([^{18}F]-**21**, 5-(azetidin-2-ylmethoxy)-2-chloro-6′-[^{18}F]fluoro-[3,3′]bipyridinyl), [^{18}F]NIDA522131 ([^{18}F]-**22**, 5-(azetidin-2-ylmethoxy)-2-chloro-2′-[^{18}F]fluoro-[3,4′]bipyridinyl) and [^{18}F]NIDA52289 ([^{18}F]-**23**, 5-(azetidin-2-ylmethoxy)-2-chloro-3-[2-(2-[^{18}F]fluoro-pyridin-4-yl)-vinyl]-pyridine) were recently described (Scheme 16) (Zhang and Horti 2004; Zhang et al. 2004a, 2004b).

[^{18}F]-**20** was synthesized in two radiochemical steps from the *N*-Boc-protected bromo-precursor and was obtained in non-optimized 30% non-decay-corrected overall radiochemical yield in 120 min total synthesis time (Scheme 16a) (Zhang et al. 2004b). The nucleophilic *hetero*aromatic substitution with fluorine-18 was performed using K[^{18}F]F-K_{222} and conventional heating in DMSO at 185 °C for 20 min (the corresponding radiochemical yield was not indicated), followed by TFA-removal of the *N*-Boc protective group. [^{18}F]-**21** was synthesized in two radiochemical steps from the *N*-Boc-protected bromo-precursor and was obtained in only 5%–10% (non-optimized) non-decay-corrected overall radiochemical yield in 120 min total synthesis time (Scheme 16b) (Zhang et al. 2004b). The nucleophilic *hetero*aromatic substitution with fluorine-18 was performed using K[^{18}F]F-K_{222} and conventional heating in DMSO at 185 °C for 20 min (30%–40% non-optimized radiochemical yield), followed by TFA-removal of the *N*-Boc protective group. [^{18}F]-**22** was first synthesized in two radiochemical steps from the *N*-Boc-protected bromo-precursor and was obtained in low radiochemical yield (Scheme 16c) (Zhang and Horti 2004): The nucleophilic *hetero*aromatic substitution with fluorine-18 was performed using K[^{18}F]F-K_{222} and conventional heating in DMSO at 185 °C for 20 min in only 2%–7% radiochemical yield, followed by TFA-removal of the *N*-Boc-protective group. HPLC purification of the *N*-Boc-protected fluorine-18-labeled intermediate was required in order to obtain > 99% pure [^{18}F]-**22**. It was finally synthesized from the *N*-Boc-protected iodo-precursor

and was obtained in 4%–8% non-decay-corrected overall radiochemical yield in 140 min total synthesis time (Scheme 16c) (Zhang and Horti 2004). The nucleophilic *hetero*aromatic substitution with fluorine-18 was performed using K[^{18}F]F-K_{222} and conventional heating in DMSO at 185 °C for 20 min in 8%–15% radiochemical yield. Note that in this example, the radiochemical yields observed for these fluorodeiodinations (8%–15%) are higher than those obtained for the corresponding fluorodebrominations (2%–7%). These data differ from that obtained for the corresponding model reaction (Scheme 1), where the iodo-derivative was found less reactive than the bromo (Dolci et al. 1999a). [^{18}F]-**23** was synthesized in two radiochemical steps from the *N*-Boc-protected bromo-precursor and was obtained in 10% non-decay-corrected overall radiochemical yield in 120 min total synthesis time (Scheme 16d) (Zhang et al. 2004a). The nucleophilic *hetero*aromatic substitution with fluorine-18 was performed using K[^{18}F]F-K_{222} and conventional heating in DMSO at 175 °C for 15 min (15%–18% radiochemical yield), followed by TFA-removal of the *N*-Boc protective group. HPLC purification of the *N*-Boc-protected fluorine-18-labeled intermediate was also required in order to obtain > 99% pure ([^{18}F]-**23**). In this case, a fluorine-18-labeled side-product was isolated and was identified as the chloro for fluoro substituted product (2-[^{18}F]fluoro-5-((1-tert-butoxycarbonyl)-2-(*S*)-azetidinyl)methoxy)-3-[((*E*)-2-(2-bromo-pyridin-4-yl)vinyl]pyridine, structure not shown).

In another series, a derivative of (–)-cytisine, (–)-9-(2-[^{18}F]fluoropyridinyl)cytisine ([^{18}F]-**24**, ((–)-(1R,5S)-9-(2-[^{18}F]fluoro-5-pyridyl)-1,2,3,4,5,6-hexahydro-1,5-methano-pyrido[1,2-a][1,5]diazocin-8-one) (Scheme 17) was recently described (Roger et al. 2003).

[^{18}F]-**24** was synthesized in two radiochemical steps from the *N*-Boc-protected NO_2-precursor and was obtained in 47% overall radiochemical yield (decay-corrected) (occasionally up to 71%) in about 70–75 min total synthesis time (Scheme 17) (Roger et al. 2003). The nucleophilic *hetero*aromatic substitution with fluorine-18 was performed using K[^{18}F]F-K_{222} and conventional heating in DMSO at 145 °C for 7 min (up to 85% radiochemical yield), followed by quantitative TFA-removal of the *N*-Boc protective group. Like derivatives [^{18}F]-**20–23** described above (Scheme 16), [^{18}F]-**24** is an example where attachment of a fluoropyridinyl to a pharmacologically active molecule is tolerated

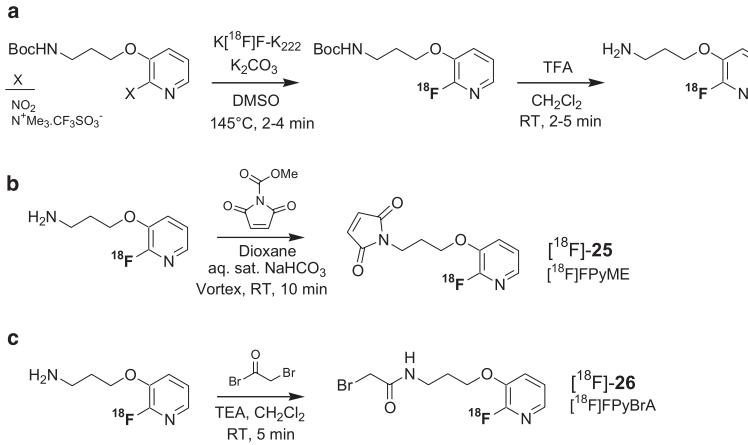


Scheme 17. Radiosynthesis of (–)-9-(2-[^{18}F]fluoropyridinyl)cytisine ([^{18}F]-**24**, ((–)-(1R,5S)-9-(2-[^{18}F]fluoro-5-pyridyl)-1,2,3,4,5,6-hexahydro-1,5-methanopyrido[1,2-a][1,5]diazocin-8-one)

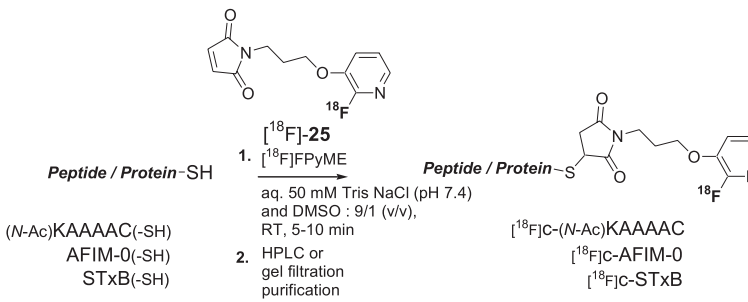
in terms of biological activity. However, in spite of its in vitro nanomolar affinity and selectivity, [^{18}F]-**24** does not have the required properties for imaging nAChRs using PET.

5.4 Applications to the Fluorine-18-Labeling of Macromolecules

In the field of macromolecule fluorine-18-labeling, two distinctive 2-[^{18}F]fluoropyridinyl-based reagents were recently designed for the prosthetic labeling of peptides, proteins and oligonucleotides *via* selective conjugation of sulfur-containing functions (Schemes 18–20). First of all, a maleimido [^{18}F]reagent, coded [^{18}F]FPyME ([^{18}F]-**25**, 1-[3-(2-[^{18}F]fluoro-pyridin-3-yloxy)-propyl]-pyrrole-2,5-dione), dedicated to the alkylation of thiol (sulfhydryl) functions (Cys-SH), was developed for the fluorine-18-labeling of peptides and proteins (Dollé 2004; de Bruin et al. 2005). Secondly, a new 2-bromoacetamide [^{18}F]reagent, coded [^{18}F]FPyBrA ([^{18}F]-**26**, *N*-[3-(2-[^{18}F]fluoropyridin-3-yloxy)-propyl]-2-bromoacetamide), dedicated to the alkylation of phosphorothioate monoester functions (-O-P(O)(-OH)-SH), was developed for the fluorine-18-labeling of oligonucleotides and derivatives (Kühnast et al. 2004). In both these reagents, the pyridinyl moiety carries the radioactive fluorine which can be efficiently incorporated *via* a nucleophilic *hetero*aromatic substitution, and the reactive entities, the maleimido group and the 2-bromoacetamide function, assure the efficient alkylation of free sulfur-containing functions: thiols as borne by



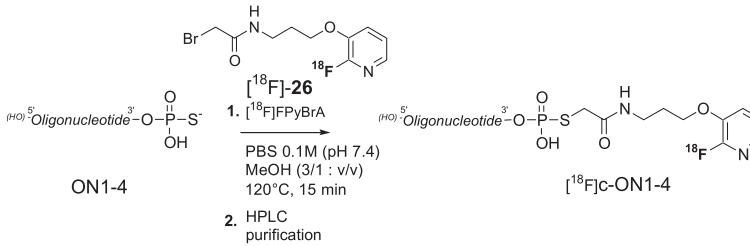
Scheme 18. Radiosyntheses of 1-[3-(2-[^{18}F]fluoro-pyridin-3-yloxy)-propyl]-pyrrole-2,5-dione ([^{18}F]-**25**, [^{18}F]FPyME) and *N*-[3-(2-[^{18}F]fluoropyridin-3-yloxy)-propyl]-2-bromoacetamide ([^{18}F]-**26**, [^{18}F]FPyBrA)



Scheme 19. Peptide and protein conjugations with 1-[3-(2-[^{18}F]fluoro-pyridin-3-yloxy)-propyl]-pyrrole-2,5-dione ([^{18}F]-**25**, [^{18}F]FPyME).

cysteine residues and phosphorothioate monoester groups borne at the 3′- or 5′-end of single-stranded oligonucleotides, respectively.

[^{18}F]FPyME ([^{18}F]-**25**, HPLC-purified) was prepared in 17%–20% non decay-corrected yield using the three-step radiochemical pathway outlined in Scheme 18. The developed procedure involves: (1) a high-



Scheme 20. Oligonucleotide conjugation with *N*-[3-(2-[^{18}F]fluoropyridin-3-yloxy)-propyl]-2-bromoacetamide ([^{18}F]-**26**, [^{18}F]FPyBrA).
Sequences: ON1: ACCGATCCG;
ON2: AGAATACAGGGTCCAAAT;
ON3: AGAAUACAGGGUCCAAAU;
ON4: A*UUU*GGA*CCCU*G*U*A*UUC*A
(C, T, A, G, C, U, A, G, *U* and *C* for cytidine, thymidine, adenosine, guanosine, 2′methoxycytidine, 2′methoxythymidine, 2′methoxyadenosine, 2′methoxyguanosine, 2′fluorouridine and 2′fluorocytidine, respectively

yield nucleophilic *hetero*aromatic *ortho*-radiofluorination on the appropriate trimethylammonium precursor as the fluorine-18 incorporation-step (performed using K[^{18}F]F-K_{222} and conventional heating in DMSO at 145 °C for 2 min in 70%–85% radiochemical yield); followed bybreak (2) rapid and quantitative TFA-induced removal of the *N*-Boc-protective group (Scheme 18a); and (3) optimized maleimide formation using *N*-methoxycarbonylmaleimide (performed at room temperature in a mixture of dioxane and aqueous saturated $NaHCO_3$ in 45%–65% radiochemical yield) (Scheme 18b). Typically, 4.8–6.7 GBq (130–180 mCi) of radiochemically pure [^{18}F]-**25** could be obtained after semi-preparative HPLC in 110 min starting from a cyclotron production batch of 33.3 GBq (900 mCi) of [^{18}F]fluoride (overall radiochemical yield (decay-corrected) based on starting [^{18}F]fluoride: 28%–37%) (Dollé 2004; de Bruin et al. 2005). [^{18}F]FPyBrA ([^{18}F]-**26**, HPLC-purified) was prepared in 18%–20% non decay-corrected yield using a similar three-step radiochemical pathway. As illustrated in Scheme 18a, the two first steps are the same as those described for the preparation of [^{18}F]-**25**. The third and last step is the condensation with 2-bromoacetyl bro-

mide (performed at room temperature in a mixture of dichloromethane and triethylamine in 64%–77% radiochemical yield) (Scheme 18c). Typically, 3.3–3.7 GBq (90–100 mCi) of radiochemically pure [^{18}F]-**26** could be obtained after semi-preparative HPLC in 80–85 min from a cyclotron production batch of 18.5 GBq (500 mCi) of [^{18}F]fluoride (overall radiochemical yield (decay-corrected) based on starting [^{18}F]fluoride: 30%–34%). In preliminary attempts, [^{18}F]-**25** and [^{18}F]-**26** had first been synthesized using a similar three-step approach, but from the NO_2-precursor. The nucleophilic *hetero*aromatic substitution with fluorine-18 was performed using K[^{18}F]F-K_{222} and conventional heating in DMSO at 145 °C for 4 min (45%–70% yield, and occasionally up to 85% radiochemical yield) (Kühnast et al. 2004).

[^{18}F]FPyME ([^{18}F]-**25**) was conjugated (Scheme 19) with a small model hexapeptide ((*N*-Ac)KAAAAC), confirming the excellent chemoselectivity of the coupling reaction (CH_2-SH versus -CH_2-NH_2). It was then conjugated with two 8-kD proteins of interest, currently being developed as tumour imaging agents (c-AFIM-0 and c-STxB). Conjugation was achieved in high yields (60%–70%, isolated and non decay-corrected) and used optimized, short-time reaction conditions [a 1/9 (v/v) mixture of DMSO and 0.05 M aqueous Tris/NaCl buffer (pH 7.4) or 0.1 M phosphate buffer system (pH 8, PBS), at room temperature for 10 min) and purification conditions (a gel filtration using a Sephadex® NAP-10 cartridge or a SuperDex™ Peptide HR 10/30 column), both compatible with the chemical stability of the proteins and the relatively short half-life of the radioisotope concerned. The whole radiosynthetic procedure, including the preparation of the fluorine-18-labeled reagent, the conjugation with the protein and the final purification took 130–140 min (de Bruin et al. 2005).

[^{18}F]FPyME ([^{18}F]-**25**) represents a new, valuable, thiol selective, fluorine-18-labeled reagent for the prosthetic labeling with fluorine-18 of peptides and proteins. Because of its excellent chemoselectivity, [^{18}F]-**25** offers an interesting alternative to the use of the non-selective carboxylate and amine-reactive [^{18}F]reagents (Okarvi 2001 and references therein) and can therefore advantageously be used for the design and development of new peptide- and protein-based radiopharmaceuticals for PET.

[^{18}F]FPyBrA ([^{18}F]-**26**) was regioselectively conjugated (Scheme 20) with 9-mer and 18-mer single-stranded oligonucleotides, provided with a phosphorothioate monoester group at their 3′-end. Both natural phosphodiester DNAs and in vivo-stable, 2-methoxy and -fluoro-modified RNAs were used. Conjugation uses optimized, short time reaction conditions (MeOH/0.1 M PBS pH 7.4, 15 min, 120 °C) and purification conditions (RP-HPLC followed by Sephadex NAP-10 cartridge desalting), both compatible with the chemical stability of the oligonucleotides and the half-life of fluorine-18. The whole radiosynthetic procedure, including the preparation of the fluorine-18-labeled reagent, the conjugation with the oligonucleotide and the final HPLC-purification and formulation lasted 140–160 min (Kühnast et al. 2004).

[^{18}F]FPyBrA ([^{18}F]-**26**) represents a valuable alternative to the already reported *N*-(4-[^{18}F]fluorobenzyl)-2-bromoacetamide (structure not shown, Dollé et al. 1997) for the design and development of oligonucleotide-based radiopharmaceuticals for PET. Of note, is that the latter reagent and the associated original strategy (the efficient and regioselective conjugation with an oligonucleotide provided with a phosphorothioate monoester group at its 3′- or 5′-end) have been reliably and routinely applied to the fluorine-18-labeling of natural phosphodiester DNA oligodeoxyribonucleotides. The methodology has also been applied to all the popular chemical modifications of oligonucleotides, such as full-length phosphorothioate diester internucleosidic-bond deoxyribonucleotides, hybrid methylphosphonate/phosphodiester internucleosidic-bond deoxyribonucleotides and 2′O-methyl-modified oligoribonucleotides (Dollé et al. 1997; Tavitian et al. 1998, 2002; Marzabal et al. 1999; Kühnast et al. 2000a, 2000b, 2003a). Furthermore, it has been successfully applied to the labeling of Spiegelmers (l-RNA or l-DNA, Nolte et al. 1996; Wlotzka et al. 2002; Kühnast et al. 2003b; Boisgard et al. 2005) and peptide nucleic acids (PNA) (Kühnast et al. 2001, 2005; Hamzavi et al. 2003), another unique class of synthetic macromolecules (the deoxyribose phosphate backbone of DNA is replaced by a pseudo-peptide *N*-(2-aminoethyl)glycyl backbone, while retaining the nucleobases of DNA (Nielsen et al. 1991; Nielsen and Egholm 1999), originally designed as ligands for the recognition of double-stranded DNA.

5.5 Conclusions

Considering chemical structures showing a 2-fluoropyridinyl moiety, nucleophilic *hetero*aromatic substitution at the *ortho*-position with no-carrier-added [^{18}F]fluoride appears today as the most efficient method for the radiosynthesis of radiotracers and radiopharmaceuticals of high specific radioactivity when compared to *homo*aromatic-, but also aliphatic, nucleophilic radiofluorination (Dollé 2005). As for the aliphatic nucleophilic radiofluorinations, only a good leaving group is required (a halogen, or better a nitro- or a trimethylammonium group). There is no need for an additional strong electron-withdrawing substituent for activation of the aromatic ring such as in the *homo*aromatic nucleophilic radiofluorinations, except if one considers *meta*-fluorination. It is essential that the labeling procedures involve pre-activation of cyclotron produced, no-carrier-added, aqueous [^{18}F]fluoride as for example, its K[^{18}F]F-K_{222} complex. Nucleophilic *hetero*aromatic substitution and consequent fluorine-18 incorporation are generally performed in DMSO using conventional heating at a moderately high temperature (120–150 °C) or microwave irradiation (100 W) for a short period of time (1–2 min) and often lead to high radiochemical yields. These significant advantages clearly highlight the potential of these nucleophilic *hetero*aromatic substitutions in the pyridine series, especially at the *ortho*-position, in the design and preparation, of often drug-based, fluorine-18-labeled radiotracers and radiopharmaceuticals of high specific radioactivity for PET imaging. Although *para*-fluorinations are feasible, there are no applications so far.

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6 Production of Non-standard PET Radionuclides and the Application of Radiopharmaceuticals Labeled with these Nuclides

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Abstract. The field of positron emission tomography (PET) has expanded dramatically over recent years. In spite of this expansion the large majority of clinical studies are carried out utilizing one radiopharmaceutical–2-fluoro-2-deoxyglucose. Many research groups are developing novel radiopharmaceuticals. A major emphasis is on other agents labeled with ^{18}F. Several other positron emitting radionuclides can be prepared in high yields in small biomedical cyclotrons. Some of these have half-lives that make delivery significantly easier

than the delivery of ^{18}F compounds. These radionuclides include: ^{64}Cu (half life 12.7 h), ^{76}Br (half life 16.2 h), ^{86}Y (half life 14.74 h) and ^{124}I (half life 4.2 days). The method of production of these and other 'non-standard' PET radionuclides will be discussed and the method of labeling radiopharmaceuticals with these radionuclides described. Several of these radiopharmaceuticals have been studied in animal models as well and a limited number translated to the human situation.

6.1 Introduction

The majority of centers carrying out positron emission tomography (PET) use the short half-lived radionuclides ^{15}O (half-life 2.04 min), ^{13}N (half-life 9.96 min), ^{11}C (half-life 20.4 min) and ^{18}F (half-life 1.83 h). These radiopharmaceuticals are produced on compact biomedical cyclotrons by nuclear reactions such as (p,n), (d,n) and (p,α). As has been discussed (Nickles 1991) low energy biomedical cyclotrons can be used to produce a whole series of other radionuclides besides these four widely used short-lived radionuclides. The nuclides produced at Washington University are listed in Table 1 together with their decay characteristics as well as the target used to produce these nuclides by the (p,n) nuclear reaction. The production of longer-lived nuclides expand the applications of PET, for example, these nuclides include ^{45}Ti. Titanium compounds have been used as anti-cancer drugs (Koepf-Maier et al. 1986; Guo et al. 2000) and ^{45}Ti with a 3-h half-life allows the pharmacokinetics of these agents to be studied. Copper-60, ^{61}Cu and ^{64}Cu have half-lives varying from 23 min to 12.7 h allowing the imaging of physiological processes with differing biological half-lives.

The positron emitting halogens ^{76}Br and ^{124}I can be used to label a whole series of compounds that have previously been labeled with other heavy halogen radionuclides. Bromine-77 is a nuclide that decays partially by Auger emission and has been shown (Kassis et al. 1982) to be highly lethal when incorporated into the DNA of mammalian cells. Bromine-77, therefore, has potential as a therapeutic agent.

Several therapeutic radiopharmaceuticals, including the FDA approved agent Zevalin® labeled with the β^-emitting nuclide ^{90}Y, are being used as therapeutic radiopharmaceuticals. In the majority of clinical applications it is essential to determine the biodistribution of the agent prior to administering the therapeutic dose. In general, this is

Table 1. Decay characteristics of the nuclides

Isotope	$t_{1/2}$	Decay mode (% branching ratio)	βmax (MeV)	βmean (MeV)	Main γ (% abundance)	Target material for production by the (p,n) nuclear reaction
^{45}Ti	3.08 h	β + (84.83) EC (15.17)	1.0404	0.4391	0.511 (169.66)	^{45}Sc
^{60}Cu	23.7 min	β + (93.00) EC (7.00)	3.7723	0.9733	0.511 (185.19) 0.826 (21.7) 1.333 (88.0) 1.791 (45.4)	^{60}Ni
^{61}Cu	3.333 h	β + 61.00) EC (39.00)	1.2152	0.4991	0.283 (12.2) 0.511 (122.87) 0.656 (10.8)	^{61}Ni
^{64}Cu	12.7 h	β + (17.40) β$^-$ EC (43.60)	0.6531 0.5787	0.2782 0.1902	0.511 (34.79) 1.346 (0.473)	^{64}Ni
^{76}Br	16.2 h	β + (54.7) EC (45.3)	3.941	1.180	0.511 (109.46) 0.559 (74.0) 0.657 (15.9) 1.854 (14.7)	^{76}Se
^{77}Br	57.036 h	β + (0.74) EC (99.26)	0.343	0.152	0.239 (23.1) 0.297 (4.16) 0.511 (1.48) 0.521 (22.4)	^{77}Se
^{86}Y	14.74 h	β + (31.9)	3.141	0.664	0.443 (16.9) 0.511 (63.89) 0.627 (32.6) 0.703 (15.4) 0.777 (22.4) 1.077 (82.5) 1.115 (30.5) 1.921 (20.8) 1.854 (17.2)	^{86}Sr
^{124}I	4.176 days	β + (23.0) EC (77.0)	2.138	0.819	0.511 (45.96) 0.603 (62.9) 0.723 (10.35)	^{124}Te

carried out by using the ^{111}In labeled radiopharmaceutical. Indium and yttrium do, however, have different stabilities and therefore different *in vivo* behavior to the chelates used as bifunctional agents. Yttrium-86, therefore, has the potential to provide quantitative biodistribution prior to application of a ^{90}Y radiopharmaceutical.

6.2 Targetry and Processing Systems

We initially developed targetry for use on the Washington University CS15 cyclotron for the production of ^{64}Cu and other copper radionuclides (McCarthy et al. 1997, 1999). This target has been in continuous use for almost a decade and has produced the copper radionuclides listed in Table 1 on a regular basis during that time. The target has been adapted to irradiate foils, powders and compounds such as copper selenide and telluride to produce the bromine radionuclides. This technology has been used to produce the other radionuclides listed in Table 1 (Vavere et al. 2005; Yoo et al. 2005b; Gaehle et al. 2003). The target designed for the production of copper radionuclides (McCarthy et al. 1997, 1999) has been redesigned to allow a series of nuclides to be produced without reloading the target (Gaehle et al. 2003) (Fig. 1). Figure 1 shows this target that was designed for the Washington University Japan Steel Works 16/8 cyclotron prior to being mounted on the cyclotron. The upper panel shows the target, the bottom panel the target cassette with three targets loaded, one being an electroplated target, the second being a powder target and the third a high current slanted target for the production of the bromine and iodine radionuclides.

The Washington University group has spent a significant amount of effort on the development of remote systems for the processing of the radionuclides. A prototype system for the processing of ^{64}Cu has been described (McCarthy et al. 1998). This system is shown in Fig. 2. This system used commercially available pre-sterilized disposable components mounted on a reusable backplate. Although this system produced ^{64}Cu, its reliability was insufficient for our applications, so an improved system (Tang et al. 2003a) has been developed. Automated systems for the production of the halogen radionuclides (Tang et al. 2003b) and ^{86}Y (Yoo et al. 2005a) are under development.

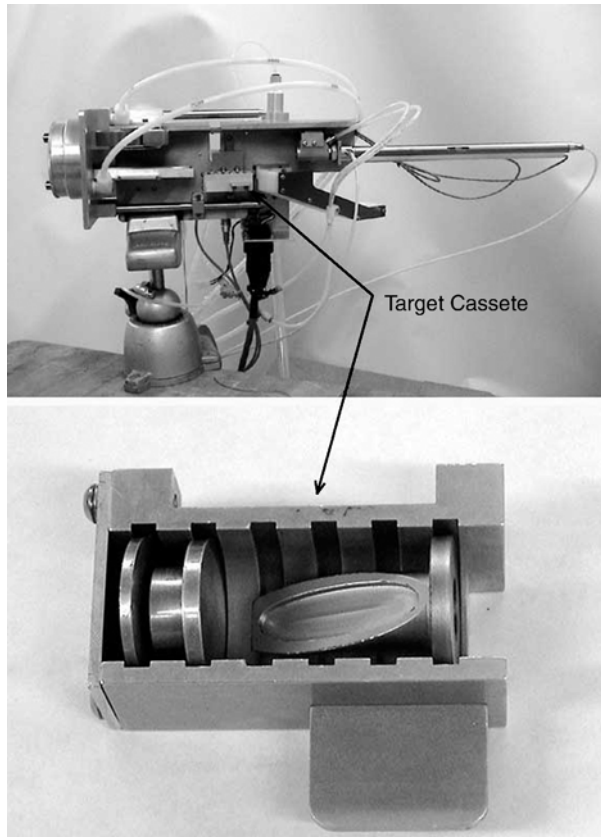


Fig. 1. Target and target cassette holder designed for use on the Japan Steel Works (JSW) 16/8 cyclotron. The cassette holder holds up to six targets to be irradiated. In the example given, three targets are mounted in the cassette holder, a target on which the target material has been electroplated, a target where the material is in a powder form and a high powered slanted target designed for the production of ^{76}Br, ^{77}Br and ^{124}I. The target is fitted on to the cyclotron (the beam window is at the left of the target) and is separated by a foil from helium cooling installed on the JSW cyclotron. This target can be adapted for use on the majority of small biomedical cyclotrons

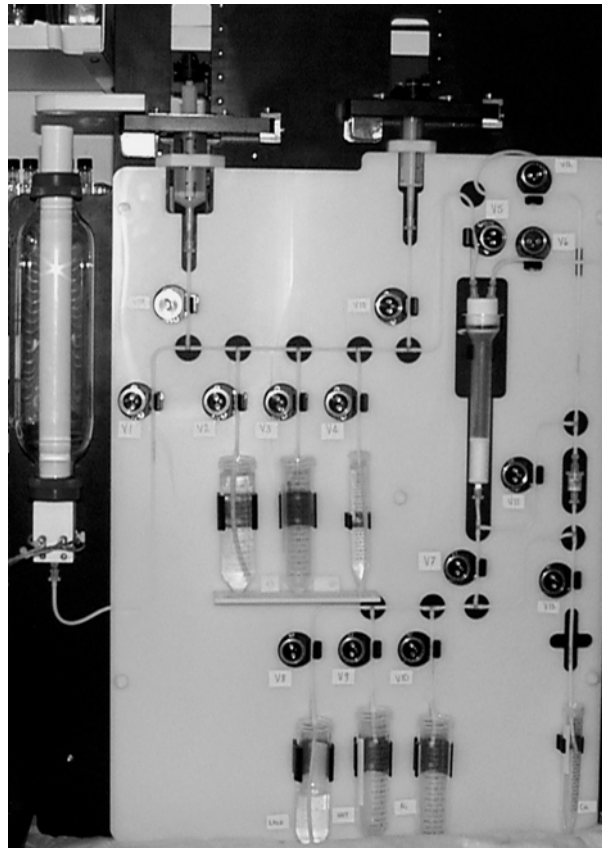


Fig. 2. Photograph of the initial prototype separation system for ^{64}Cu. In this system the heating vessel is on the *left* of the photograph and the chromatographic separation column on the *middle right*. The liquid flow is controlled by the specially designed valves. An improved system is currently being tested

6.3 Distribution

Funded by the United States National Cancer Institute, we are distributing the radionuclides discussed above to research centers throughout the USA. The researchers at these centers carry out various scientific projects largely related to radiopharmaceutical development. The number of shipments and the amount of activity shipped during the year December 1 2004 to November 30 2005 is given in Table 2. This table shows the number of customers to whom each nuclide was shipped, the number of 'large shipments' (shipments over limited quantity of radionuclides for which a contract with Washington University is required) as well as the shipments of limited quantities of radionuclides. It also shows the number of total shipments and the amount of activity shipped. As can be seen from this table, a significant number of researchers are using these radionuclides and, in fact, at least two United States companies (Eastern Isotopes and Nordion) are beginning to ship the radionuclides listed in Table 2 commercially.

Table 2. Activity shipped to other researchers from Washington University

Isotope	Customers	Large shipments	Limited quantity	Total shipments	Total mCi shipped
Bromine-76	4	9	4	13	44.069
Yttrium-86	2	4	0	4	16.8
Iodine-124	3	1	2	3	1.713
Copper-64	33	196	161	357	5467.5

6.4 PET Imaging with Non-standard Radionuclides

As can be seen from Table 1, some of the radionuclides listed possess complex decay schemes leading to the emission of both high energy positrons and several prompt gamma rays. The positron range has been recognized as a fundamental factor limiting the ultimate spatial resolution in PET. By using human PET scanners with imaging phantoms (Dehdashti et al. 2003a) it has been shown that positron emitting nuclides with complex decay schemes give a very similar imaging quality than that compared to phantoms filled with fluorine-18. This is not the

case when using high resolution small animal PET scanners such as the microPET-Focus (Tai et al. 2005). It is worth noting that the volumetric resolution in a typical human PET scanner is approximately 100 mm^3, whereas the volumetric resolution of the center of a microPET Focus scanner is approximately 5 mm^3. This significant increase in absolute resolution makes the effect of positron range much more significant in microPET scanners compared to human scanners. Images of several of these nuclides in a phantom using a microPET Focus are shown in Fig. 3. Laforest et al. (2002) evaluated the effect of positron range and gamma rays on imaging quality in phantoms. Dramatic differences were observed in many phantoms between nuclides such as ^{13}N and ^{66}Ga.

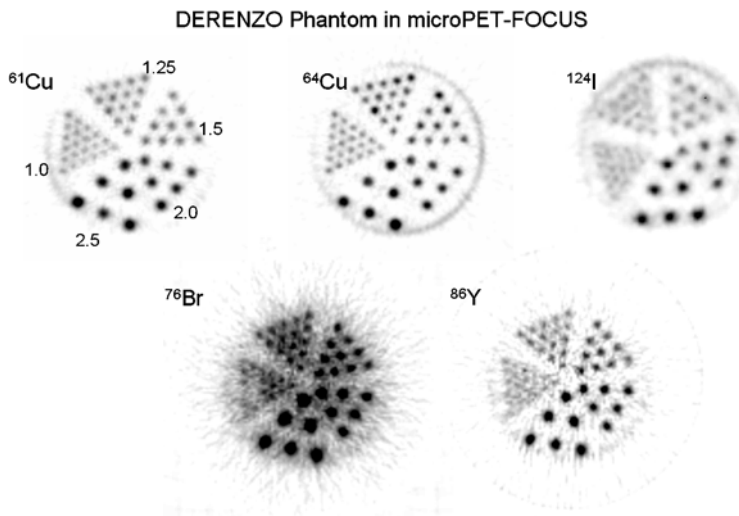
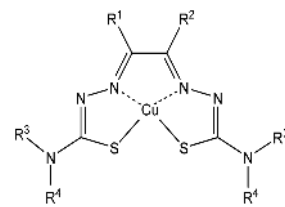


Fig. 3. A mini Derenzo phantom filled with various radionuclide imaged on the microPET Focus scanner. This phantom consists of radioactive rods of specified diameter separated by four times the diameter. These images were reconstructed utilizing the filtered back projection. It is seen that the nuclides with higher energy positrons and prompt gamma rays produce the images that are degraded compared to those with a single low energy positron (for example, ^{64}Cu)

Mathematical algorithms (maximum a posteriori) have been developed to take both the system geometry (Mumcuoglu et al. 1996; Chatzioannou et al. 2000) and the positron range into consideration in order to improve resolution with high energy positron emitters and nuclides that also emit prompt gamma rays. Ruangma et al. (2006) have imaged phantoms with the three positron emitting nuclides of copper (^{60}Cu, ^{61}Cu, ^{64}Cu) and imaged mice and rats using two radiopharmaceuticals labeled with these three radionuclides. These data have shown that inclusion of a positron range model in the maximum a posteriori algorithm results in significant resolution recovery for the radionuclides with large positron ranges. It is possible that by using these algorithms the deficiencies in image quality can be significantly improved.

6.5 Applications of Non-standard Positron Emitting Copper Radionuclides

Copper-64 as elemental copper has been used to study various diseases involving copper metabolism. These diseases include Menkes' disease (Herd et al. 1987) and Wilson's disease (Gunther et al. 1988). Copper-64 has been used to study copper metabolism and excretion in sheep (Mason et al. 1988). Radiopharmaceuticals labeled with a variety of copper radionuclides have been developed for several applications. These



R^1, R^2, R^3 and R^4 independently represents hydrogen, alkyl or alkoxy
H_2ATSM: R^1, R^2 = CH_3, R^3 = CH_3, R^4 = H
H_2PTSM: R^1 = H, R^2 = CH_3, R^3 = CH_3, R^4 = H
Cu represents any nuclide of copper (e.g., Cu-60, Cu-61, Cu-64 or Cu-67)

Fig. 4. Structures of Cu-thiosemicarbazones, for PTSM R_3=H_1R_4=CH_3 and R_1=CH_3. For PTSM R_2=H_1. For ATSM R_2=CH_3

radiopharmaceuticals can be grouped into three general categories, these being small molecules, radiolabeled peptides and radiolabeled antibodies. Copper-labeled PTSM (Green et al. 1988) (pyruvaldehyde bis(N^4-dimethylthiosemicarbazone) (Fig. 4) has been shown to be an agent that measures blood flow in the heart (Shelton et al. 1989, 1990) and brain (Mathias et al. 1990; Green et al. 1990). The mechanism of uptake has been proposed (Barnhart-Bott and Green 1991) to result from the reductive decomposition of the copper-II complex by intracellular sulfhydryls (such as glutathione). It was shown that only low levels of intracellular sulfhydryls were needed to trap the copper from Cu-PTSM. A thiosemicarbazone with a structure similar to Cu-PTSM, copper-ATSM Cu(II)-diacetyl-bis(N^4-methylthiosemicarbazone) has been shown to be trapped only in hypoxic cells (Fujibayashi et al. 1997). The initial study was carried out in isolated hearts. Several studies have been carried out to understand the mechanism of uptake of Cu-ATSM in hypoxic tissue (Fujibayashi et al. 1999; Lewis et al. 2001a; Takahashi et al. 2001; Dearling et al. 2002; Lewis et al. 2002b; Obata et al. 2003). Both ^{64}Cu-ATSM and ^{64}Cu-PTSM have been used as agents for therapeutic applications (Obata et al. 2005; Lewis et al. 2001a, 2001b; Lewis et al. 2002a; Aft et al. 2003). These studies all show that when administered in large quantities, ^{64}Cu-ATSM and ^{64}Cu-PTSM can be effective radiotherapy agents. Copper-60 ATSM has been evaluated in several human tumors to assess tumor hypoxia. In both cervical cancer (Dehdashti et al. 2003b) and lung cancer Cu-ATSM (Dehdashti et al. 2003a) uptake could differentiate patients who responded to conventional radiation therapy and those who did not. It appears as if the tumor uptake of Cu-ATSM reveals clinically unique information about tumor oxygenation that is predictive of tumor response to conventional therapy.

In these studies, ^{60}Cu-ATSM was used due to the low radiation dose which was approved by the Washington University Radioactive Drug Research Committee. In order to widen the application of Cu-ATSM a full toxicology study was carried out on Cu-ATSM funded by the National Cancer Institute DCIDE Program. The mutagenicity assays, cardiovascular and pulmonary safety testing in Beagle dogs, neurological safety assessment in rats as well as both acute and subacute toxicity studies in rats and rabbits were carried out under this program. An Inves-

tigative New Drug application has recently been approved by the United States Food and Drug Administration and it is anticipated that human trials using ^{64}Cu-ATSM commenced in January 2006.

Several other small molecules have been labeled with copper-radionuclides. Amongst these are a series of methanephosphonate tetrase (macrocyclic ligands) (Sun et al. 2003). One of these compounds showed very high bone accumulation. This compound, when administered in larger quantities, has been shown to have a therapeutic effect on bone tumors. Another small molecule that has been studied is the copper complex of 1,4,7-tris(carboxymethyl)-10-(tetradecyl)-1,4,7,10-tetraazadodecane (Yoo et al. 2005b); this has been shown to be an agent that can diagnose Dubin-Johnson Syndrome (DJS). This syndrome is caused by a deficiency of the human canalicular multispecific organic anion transporter (cMOAT). Studies in transgenic animals show totally different biodistributions in normal and cMOAT-deficient rats.

A second major area of research that uses copper-radionuclides is the labeling of peptides. Copper-64 octreotide type conjugates have been studied in an preliminary human study (Anderson et al. 1995a) where high target to non-target ratios were observed. Improved peptides have been studied (Lewis et al. 1999) and by using a cross-bridged chelate, CBTE2A, (Sprague et al. 2004) significantly higher target to non-target ratios were observed. It was hypothesized that by using conventional macrocyclic ligands TETA (1,4,8,11-tetraazacyclotetradecane-N,N',N'',N'''-tetraacetic acid) and DOTA (1,4,7,10-tetraazacyclododecan-N,N',N'',N'''-tetraacetic acid)] some of the copper was released, whereas with the cross-bridge ligand a more stable chelate was formed. Other peptides have been labeled with copper, including a gastrin releasing peptide (Rogers et al. 2003) a labeled RDG peptide targeting $\alpha_V\beta_3$integrin expression (Chen et al. 2004a, 2004b) as well as vasoactive intestinal peptide (Thakur et al. 2004).

These studies using copper radiolabeled peptide show the breadth of studies possible with labeled peptides.

Copper-64 labeled monoclonal antibodies have also been studied both in animal models and in humans. In animal models (Anderson et al. 1995b) biodistribution and dosimetry prior to human studies were carried out. The radiolabeled 1A3 antibody has been evaluated in humans both to detect tumor metastases (Philpott et al. 1995) and to carry out

human dosimetry (Cutler et al. 1995). In this study 36 patients who were suspected of having advanced primary or metastatic colorectal cancer were studied by using the ^{64}Cu antibody. All patients were imaged twice, and were also studied with fluorodeoxyglucose. Positive predictive value of the monoclonal antibody PET was excellent ranging from 89% to 96% depending on the ultimate classification of three imaged positive, but as yet unconfirmed, tumor sites.

These studies show that copper-labeled antibodies have potential for application in PET imaging.

A final application of ^{64}Cu is to label separated cellular fractions in order to image cell trafficking. Adonai and co-workers (Adonai et al. 2002) labeled C-6 rat glioma cells with ^{64}Cu-PTSM and used microPET imaging to study cell trafficking.

Copper-64 has also been used to label nanoparticles (Sun et al. 2005a). Amphiphilic core-shell nanoparticles have drawn considerable interest in biomedical applications. The precise control over their physicochemical parameters and the ability to attach various ligands within specific domains suggest that these shell cross-linked (SCK) nanoparticles nanoparticles can be used as scaffolds for drug delivery. We have functionalized nanoparticles with a series of groups (Fig. 5) and studied their in vivo biodistribution by microPET imaging with ^{64}Cu. In initial studies the effects of SCK nanoparticle size, core composition and surface PEGylation on in vivo biodistribution was examined. It was shown that both core composition and the hydrodynamic diameter of the nanoparticles play an important role in the respective biodistibution (Sun et al. 2005a). Smaller rigid particles showed the longest blood retention and the lowest liver uptake. We have also labeled these particles with folate as a targeting agent (Rossin et al. 2005a). In this study we showed that SCK folate particles were competitively blocked by excess folate in small size tumors. However, in large tumors it appears that the uptake occurs mainly through extravasation from blood vessels into the vascular tumors. We have also studied tumor uptake of upstream of *N*-ras (UNR) mRNA targeted peptide in nucleic acids (Sun et al. 2005b; Rossin et al. 2005b), both with the agent as a small molecule (Sun et al. 2005b) and attached to nanoparticles (Rossin et al. 2005b); in both cases specific peptide nucleic acid (PNA) group enhances tumor uptake. Similar tumor-to-blood and tumor-to-muscle ratios were obtained when

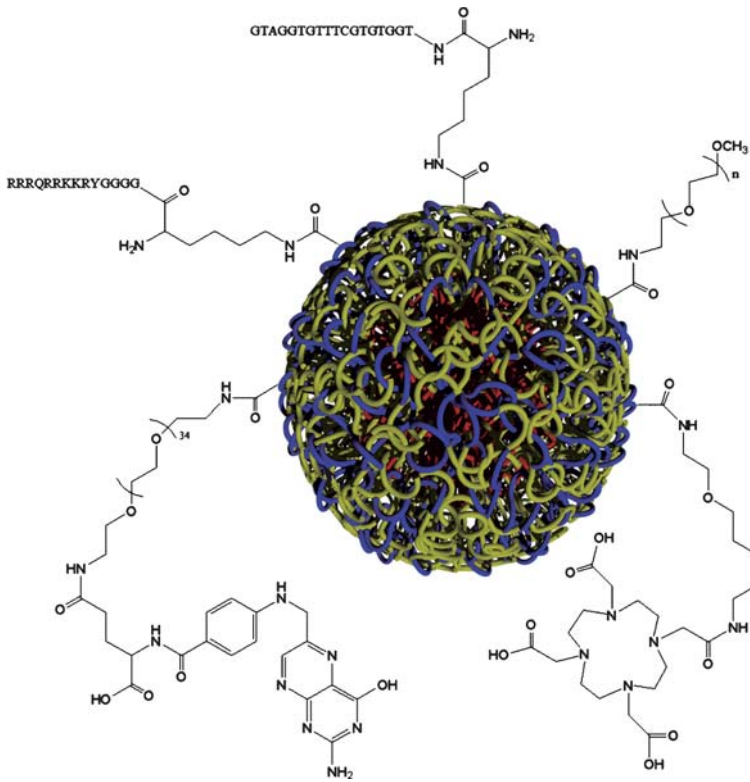


Fig. 5. Schematic of functionalized nanoparticles, functionalized with the groups discussed in the text

administering mice bearing MCF-7 xenografts with a PNA conjugate SCK or the nonconjugated PNA. The SCK conjugation, however, improves the PNA biodistribution so it produces lower non-specific uptake in the non-target tissues. These studies show the potential of utilizing non-standard PET radionuclides specifically ^{64}Cu and understanding the behavior of nanoparticles.

6.6 Yttrium-86

As discussed in the Introduction, one of the potential uses for ^{86}Y is to determine accurately the radiation dose prior to administration of ^{90}Y compounds for radiopharmaceutical therapy by using the ^{86}Y complex with PET. Applications of ^{86}Y to determine the radiation dose of ^{90}Y citrate and ^{90}Y EDTMP (ethylene diamine tetramethylene phosphonate) (Rosch et al. 1996) as well as a somatostatin analogue had been discussed. Again as discussed in the Introduction, the stability of yttrium and indium chelates to the same chelate are different. The biodistribution of ^{86}Y and ^{111}In labeled monoclonal antibodies were compared in a nude mouse model (Lovqvist et al. 2001). In this case a non-macrocyclic chelate was used and it was observed that 4 days after administration, the ^{86}Y activity was significantly higher in several tissues, including tumor and bone. The authors reached the conclusion that quantitative information offered by PET combined with the identical biodistribution of ^{86}Y and ^{90}Y should enable more accurate radiation dose estimates for ^{90}Y radioimmunotherapy.

Labeled peptides targeting the somatostatin receptor have been compared with ^{111}In labeled peptides (Forster et al. 2001). Another application of ^{80}Y involves the study of a radiolabeled peptide targeting α-melanocyte stimulating hormone, a receptor expressed in melanoma tissue (McQuade et al. 2005). In this publication DOTA labeled peptide was radiolabeled with ^{64}Cu and ^{86}Y and the biodistribution was compared both by small animal PET imaging and biodistribution studies. Significantly higher target-to-non-target ratios were obtained utilizing ^{90}Y compared to ^{64}Cu presumably due to the greater stability of the chelate with yttrium. It appears, therefore, that utilizing DOTA yttrium peptides are likely to have lower non-target uptake.

6.7 Bromine-76, Bromine-77, Iodine-124

Bromine-77 labeled estrogens (Katzenellenbogen et al. 1981, 1982) were developed and studied in humans (McElvany et al. 1982) prior to the development of ^{18}F-labeled steroids. The technology developed in the early 1980s for the production of ^{77}Br-labeled steroids can be applied to the development of ^{76}Br estrogens for imaging, and to ^{77}Br estrogens

for therapy. The therapeutic potential of ^{77}Br has been discussed previously. A ^{124}I radiolabeled androgen has been described (Downer et al. 2001). A similar compound in which the iodine is replaced with bromine could also be prepared by a similar reaction mechanism. These agents would allow the delivery of long-lived steroid hormones both for diagnosis and therapy. The therapeutic potential of bromine-labeled steroid hormone ligands has been shown by using bromine-80m-labeled estrogens as well as ^{77}Br-labeled compounds (Downer, et al. 2001; DeSombre et al. 1988, 1990a, 1990b). Bromine-76 bromodeoxyuridine (Bergstrom et al. 1998; Ryser et al. 1999) has been evaluated as potential tracer for the measurement of cell proliferation. One concern in the application of this compound is the amount of free bromide produced (Lu et al. 1999). It has been suggested that administration of diuretics to eliminate the bromide may facilitate the use of ^{76}Br-bromodeoxyuridine as a tracer for the measurement of DNA synthesis using PET.

A series of ligands for neuroreceptors has been labeled with ^{76}Br and studied in animal models or humans. Targets include the central muscarinic receptor (Loc'h et al. 1996a) the dopamine D2 receptor (Loc'h et al. 1996b) the dopamine D1 receptor (Foged et al. 1996) and the benzodiazepine receptor (Foged et al. 1997).

Antisense ligand nucleotides have been labeled with ^{76}Br (Wu et al. 2000, 2004) and it has been confirmed that labeling with bromine does not change the hybridization ability of antisense oligonucleotides.

Techniques to label proteins with bromine were described in the early 1980s (McElvany et al. 1980; McElvany and Welch 1980). Several brominated antibodies have been prepared and their biodistribution compared with that of other halogenated antibodies (Orlova et al. 2002; Winberg et al. 2004; Lovqvist et al. 1997a, 1997b).

6.8 Conclusions

A series of 'non-standard' positron emitting radionuclides can be prepared routinely and in high yield utilizing small biomedical cyclotrons. The research at Washington University has concentrated on the radionuclides of copper as well as ^{86}Y, ^{76}Br, ^{77}Br and ^{124}I. The whole range of small molecules, peptides, antibodies and nanoparticles can be labeled

with these nuclides. The widespread availability of medium half-life (3 h to 4 d) positron emitting radionuclides widens the potential clinical useful positron emitting radiopharmaceuticals.

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7 Carbon-11 Labeling Chemistry Based upon [^{11}C]Methyl Iodide

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This article is dedicated to Prof. Dr. B. Johannsen on occasion of his 67th birthday.

Abstract. Radiochemistry with the short-lived positron emitter ^{11}C (half-life 20.38 min) represents special challenges in terms of synthesis time and labeling techniques. The recent developments in ^{11}C radiochemistry have steadily expanded the number of ^{11}C labeled compounds. This chapter addresses selected chemical and technical aspects of ^{11}C chemistry based on the readily available labeling precursors [^{11}C]methyl iodide and, to a lesser extent, [^{11}C]methyl triflate. Special emphasis is placed on heteroatom methylation reactions and ^{11}C–C bond formations.

7.1 Radionuclide Production and Carbon-11 Labeling Precursors

The progress of positron emission tomography (PET) as a powerful imaging technique in nuclear medicine and drug research and development is accompanied by an increasing demand for new radiolabeling methods especially for the short-lived positron emitters ^{11}C (half-life 20.4 min) and fluorine-18 (half-life 109.8 min). Compared to the almost 2 h half-life of fluorine-18 the shorter half-life of ^{11}C provides the advantage to perform repeated PET studies while still allowing, to some extent, multi-step radiosynthesis sequences. Moreover, isotopic labeling through substitution of a stable carbon atom with ^{11}C makes the corresponding ^{11}C labeled radiotracers indistinguishable from their stable counterparts within the biological system.

Several nuclear reactions can be used to produce ^{11}C (for reviews see Wolf and Redvantly 1977; Ferrieri and Wolf 1983). Among these processes the $^{14}N(p,\alpha)^{11}C$ nuclear reaction on a nitrogen target gas is by far the most convenient and most commonly used method of producing ^{11}C. The radionuclide can be produced from nitrogen as a non carbon-containing target material, thus providing ^{11}C at high specific radioactivity. Sufficient amounts of radioactivity can be produced within reasonable irradiation times as the $^{14}N(p,\alpha)^{11}C$ nuclear reaction has cross-section of 250 mbarns using a relatively low threshold energy of 3.1 MeV which allows production of ^{11}C on a small biomedical cyclotron. With the addition of oxygen (up to 2%) or hydrogen (5%–10%) to the nitrogen target gas, ^{11}C is obtained in the target either as [^{11}C]carbon dioxide or [^{11}C]methane, respectively, as primary labeling precursor. However, [^{11}C]carbon dioxide is the most important and most versatile primary labeling precursor. Cyclotron-produced [^{11}C]carbon dioxide can directly be used for the ^{11}C labeling of organic molecules. This includes the reaction of [^{11}C]carbon dioxide with primary amines to give [^{11}C]ureas and [^{11}C]isocyanates (Schirbel et al. 1999) and the reaction of [^{11}C]carbon dioxide with organolithium and organomagnesium compounds. In this line, the most important application is the preparation of [1-^{11}C]acetate via carboxylation of Grignard reagents (MeMgCl or MeMgBr) with [^{11}C]carbon dioxide (Kruijer et al. 1995).

The short 20.38 min half-life of ^{11}C imposes major constraints on the synthesis time of ^{11}C-labeled compounds. Hence, methods for the incorporation of this isotope tend to be limited to those based on a few readily available labeling precursors, and the ^{11}C label should be introduced at the latest time possible within the synthesis sequence. The availability of a wide array of different ^{11}C labeling precursors is an important prerequisite for the flexible and position specific radiolabeling of substances.

Starting from [^{11}C]carbon dioxide as the most important and versatile primary labeling precursor a broad spectrum of different ^{11}C-labeled synthetic intermediates as useful secondary labeling precursors can be prepared.

Figure 1 shows a selection of ^{11}C-labeled secondary labeling precursors derived from [^{11}C]carbon dioxide (Langström et al. 1999).

Among the known secondary ^{11}C labeling precursors, [^{11}C]methyl iodide is the most important and most frequently used. [^{11}C]Methyl iodide has been used extensively as an alkylating agent for carbanions and heteroatom nucleophiles. More recently, [^{11}C]methyl iodide was used as electrophile in several palladium-mediated cross-coupling reactions to form distinct ^{11}C–C bonds. Finally, [^{11}C]methyl iodide is needed for the preparation of other labeling precursors such as [^{11}C]methyl lithium, [^{11}C]nitromethane [^{11}C]methyltriphenyl-phosphorane and triphenylarsonium [^{11}C]methylide.

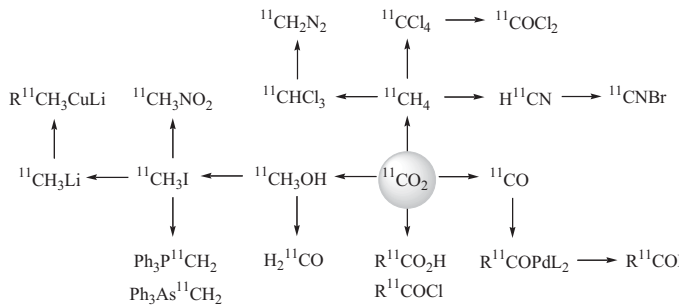


Fig. 1. Selection of ^{11}C-labeled precursors derived from [^{11}C]CO_2

Other important labeling precursors are hydrogen [^{11}C]cyanide and [^{11}C]carbon monoxide. The latter is used for the synthesis of a broad range of carbonyl compounds via palladium- or selenium-mediated reactions (Antoni et al. 2003 and references therein). Recent technical improvements for the handling of [^{11}C]carbon monoxide in carbonylative coupling reactions made this labeling precursor almost equal in importance to [^{11}C]methyl iodide in the synthesis of ^{11}C labeled radiotracers.

In this chapter we wish to give an overview of ^{11}C chemistry based on the readily available labeling precursors [^{11}C]methyl iodide and [^{11}C]methyl triflate. Especial attention is given to heteroatom carbon-11 methylation reactions including recent important technical developments and aspects. Furthermore, recent developments in palladium-mediated ^{11}C–C bond forming reactions with [^{11}C]methyl iodide as an important approach to the position-specific labeling of substances with [^{11}C]methyl groups are discussed.

7.2 Preparation of [^{11}C]Methyl Iodide and [^{11}C]Methyl Triflate

[^{11}C]Methyl iodide as the most versatile ^{11}C labeling precursor can be synthesized via two distinct methods. The so-called ‘wet’ method is based on reduction of cyclotron-produced [^{11}C]carbon dioxide with $LiAlH_4$ in tetrahydrofuran or diethylether (0.05–0.1 M). After evaporation of the solvent hydriodic acid is added and the [^{11}C]methyl iodide formed is distilled off at elevated temperatures in a stream of nitrogen or helium through a $NaOH/P_2O_5$ trap into a reaction vial where the methylation reaction is carried out. This method was developed in the 1970s (Comar et al. 1973; Langström and Lundquvist 1976). Alternatively to hydriodic acid, diphosphorous tetraiodide (Oberdorfer et al. 1985) or triphenylphosphine diiodide (Holschbach et al. 1993) were used to convert [^{11}C]methanol into [^{11}C]methyl iodide. The general reaction sequence to prepare [^{11}C]methyl iodide according to the ‘wet’ chemistry route is outlined in Fig. 2.

The ‘wet’ method is very reliable in terms of radiochemical yields, however, the use of $LiAlH_4$ as reducing agent also has a major draw-

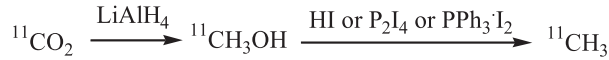


Fig. 2. Synthesis of [^{11}C]methyl iodide via the 'wet' chemistry route

back. $LiAlH_4$ represents the major source of cold carbon dioxide as the most crucial contamination which may cause drastic decrease of specific radioactivity of [^{11}C]methyl iodide and, hence, result in low specific radioactivity of the final ^{11}C labeled radiotracer. Thus, reduction of the amount of $LiAlH_4$ leads to a higher specific radioactivity of the end product (Matarrese et al. 2003). An amount of 5–7 μmol $LiAlH_4$ was shown to be adequate for sufficient trapping of [^{11}C]carbon dioxide whilst providing satisfactory specific radioactivities (2–10 Ci/μmol at the end of synthesis) of the final product (Matarrese et al. 2003).

An alternative method, also referred to as the 'gas phase' method, was developed in the 1990s. This method exploits the conversion of [^{11}C]methane into [^{11}C]methyl iodide by free radical iodination with iodine vapour at elevated temperatures (700–750 °C) in the gas phase (Larsen et al. 1997; Link et al. 1997) (Fig. 3).

[^{11}C]Methane can either be produced directly in the target chamber via the $^{14}N(p,\alpha)^{11}C$ nuclear reaction using a 5% or 10% H_2/N_2 target mix (Buckley et al. 2000, 2004) or by hydrogen reduction of cyclotron-produced [^{11}C]carbon dioxide on a nickel catalyst (Larsen et al. 1997, Link et al. 1997).

In order to enable sufficient conversion of [^{11}C]methane into [^{11}C]methyl iodide the gas-phase iodination is performed as a circulation process. The formed [^{11}C]methyl iodide is continuously removed from the circulation process by using a Porapak trap, which is heated afterwards to release [^{11}C]methyl iodide. An alternative gas phase bromination of [^{11}C]methane with bromine at 550 °C to give [^{11}C]methyl bromide in 75% yield was reported by Mock (Mock et al. 1999).

The preparation of [^{11}C]methyl iodide via the 'gas phase' method offers several advantages over the 'wet' chemistry route. Firstly, it cir-

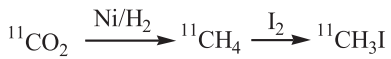


Fig. 3. Synthesis of [^{11}C]methyl iodide via the 'gas phase' method

cumvents the problem associated with the use of $LiAlH_4$, hence leading to higher specific radioactivity. In this respect, the highest specific radioactivities of up to 4,700 GBq/μmol are obtained when [^{11}C]methane is produced in situ in the target chamber and [^{11}C]methyl iodide is synthesised by a single pass iodination procedure in a heated quartz tube (Zhang and Suzuki 2005).

Secondly, avoidance of hydriodic acid prevents tubing and valves from deterioration. Moreover, the 'wet' method using $LiAlH_4$ and hydriodic acid requires intense and time-consuming cleaning and drying procedures of reaction vials and tubings of the ^{11}C methylation apparatus which may limit the number of [^{11}C]methyl iodide preparations possible per day.

Some PET radiotracers were shown to give only moderate or even low radiochemical yields in heteroatom methylation reactions when [^{11}C]methyl iodide is used. Thus, the reactivity of [^{11}C]methyl iodide can be increased by conversion into more reactive [^{11}C]methyl triflate (Jewett 1992).

The use of [^{11}C]methyl triflate as a more reactive methylation agent compared to [^{11}C]methyl iodide provides several advantages. [^{11}C]Methyl triflate is less volatile and thus more easily trapped in small volumes of solvent. Usually heteroatom methylation reactions with [^{11}C]methyl triflate proceed in higher radiochemical yields with shorter reaction times and lower reaction temperatures compared to reactions using [^{11}C]methyl iodide (Nagren et al. 1995a, b; Nagren and Halldin 1998; Lundkvist et al. 1998). Moreover, smaller amounts of desmethyl precursor (<1 mg) are needed which is important for facile final product purification and cost of the precursor.

The synthesis of [^{11}C]methyl triflate is performed as an on-line process by passing [^{11}C]methyl iodide or [^{11}C]methyl bromide (Mock et al. 1999) in a gentle stream of helium or nitrogen through a small column containing silver triflate or silver triflate on graphitised carbon spheres which is preheated at 200–300 °C (Fig. 4).

Typical reaction conditions for heteroatom methylation reactions using [^{11}C]methyl iodide and [^{11}C]methyl triflate are opposed in Table 1 (Elsinga 2002).

Other ^{11}C labeling precursors such as [^{11}C]ethyl iodide (Erikson et al. 2004; Bergström et al. 1998; Schmitz et al. 1995), [^{11}C]propyl iodide

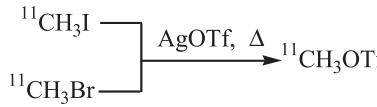


Fig. 4. Synthesis of [^{11}C]methyl triflate

Table 1. Comparison of reaction conditions using [^{11}C]methyl iodide and [^{11}C]methyl triflate (Elsinga 2002)

Reaction condition	[^{11}C]methyl triflate	[^{11}C]methyl iodide
Temperature (°C)	20–60	80–120
Reaction time (min)	1	2–10
Desmethyl precursor (mg)	< 1	1–10

(Ishiwata et al. 1999; Antoni and Langström 1987a) and [^{11}C]formaldehyde (Langer et al. 2005) have also been used in heteroatom alkylation reactions although by far not as frequently as [^{11}C]methyl iodide and [^{11}C]methyl triflate.

Table 2 shows some selected radiotracers and their specific radioactivities reached through heteroatom methylation reactions with [^{11}C]methyl iodide or [^{11}C]methyl triflate. [^{11}C]Methyl iodide was prepared either via the 'wet' method or the 'gas phase' method.

Based on the data summarised in Table 2 it is clear that the 'gas phase' method generally provides ^{11}C-labeled compounds at higher specific radioactivity. Thus, the 'gas phase' method is the method of choice when radiotracers with high specific radioactivity such as receptor ligands or enzyme inhibitors are needed.

Table 2. Examples of specific radioactivities at end-of-synthesis reached in methylation reactions with [^{11}C]methyl iodide and [^{11}C]methyl triflate

Method[a]	Labeling precursor	Radiotracer	SA[b] (GBq/μmol)	Reference
A	[^{11}C]CH_3OTf	[^{11}C]PE2I	29–4	Dolle et al. 2000
A	[^{11}C]CH_3OTf	[^{11}C]HED, [^{11}C]HPED	47–60	van Dort et al. 2000
A	[^{11}C]CH_3I	[^{11}C]DMT, [^{11}C]MPTP, [^{11}C]HED, Dilthiazem, [^{11}C]YM-09151–2	11–74	Iwata et al. 1988
A	[^{11}C]CH_3OTf	[^{11}C]FLB457, [^{11}C]MDL100907, [^{11}C]β-CIT-FE	74–93	Lundkvist et al. 1998
A	[^{11}C]CH_3OTf	[^{11}C]FLB457	78	Sandell et al. 2000
A	[^{11}C]CH_3I	[^{11}C]YM-50001	47–99	Zhang and Suzuki 2002
A	[^{11}C]CH_3I	[^{11}C]MeI	74–175	Crouzel et al. 1987
A	[^{11}C]CH_3OTf	[^{11}C](*R*)MDL-100907	37–370	Matarrese et al. 2003
B	[^{11}C]CH_3I	[^{11}C]MeI	125	Oberdorfer et al. 1985
C	[^{11}C]CH_3I	[^{11}C]β-CPPIT	74–100	Schönbächler et al. 1999
C	[^{11}C]CH_3OTf	[^{11}C]FLB457	126	Sandell et al. 2000
C	[^{11}C]CH_3I	[^{11}C]MeI	370	Larsen et al. 1995
C	[^{11}C]CH_3I	[^{11}C]MHED	444	Link et al. 1997
C	[^{11}C]CH_3I	[^{11}C]MHED	451	Link et al. 1995
C	[^{11}C]CH_3I	[^{11}C]MeI	550	Larsen et al. 1997
C	[^{11}C]CH_3I	[^{11}C] [^{11}C]YM-50001	1810	Zhang et al. 2002
C	[^{11}C]CH_3I	[^{11}C]Flumazenil	2440	Zhang and Suzuki 2005
C	[^{11}C]CH_3I	[^{11}C]Ro15–4513	4700	Noguchi and Suzuki 2003
D	[^{11}C]CH_3OTf	Different compounds[c]	92	Mock et al. 1999

[a] A, "wet" method ($LiAlH_4$/HI); B, "wet method" ($LiAlH_4/P_2I_4$); C, "gas phase" method (iodination); D, "gas phase" method (bromination)

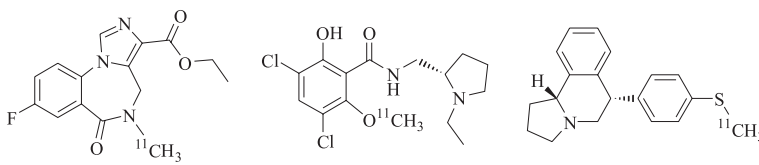
[b] SA=specific radioactivity at end-of-synthesis

[c] Compounds are not explicit specified

7.3 Heteroatom Alkylation Reactions with [^{11}C]Methyl Iodide and [^{11}C]Methyl Triflate

The great majority of carbon-11 labeled compounds has been prepared via *N*-, *O*- and *S*-methylation reactions by using [^{11}C]methyl iodide and [^{11}C]methyl triflate as labeling reagents. Well known examples comprise the synthesis of the PET radiopharmaceuticals [*N*-methyl-^{11}C]flumazenil, [*O*-methyl-^{11}C]raclopride and L[*S*-methyl-^{11}C]McN-5652 (Fig. 5).

The general feature of *N*-, *O*- or *S*-heteroatom ^{11}C methylation reactions is characterized by an extraordinary stoichiometic relationship between the desmethyl precursor and [^{11}C]methyl iodide or [^{11}C]methyl triflate as typically found for radiosyntheses using radionuclides at high specific radioactivity. The corresponding desmethyl precursor is present in an excess of a few orders of magnitude compared to [^{11}C]methyl iodide or [^{11}C]methyl triflate. The resulting stoichiometrical relation can reach a factor of 10^4 : 1. This extraordinary stoichiometry results in a pseudo-first order kinetics of heteroatom methylation reactions with [^{11}C]methyl iodide or [^{11}C]methyl triflate. As a consequence, the conversion rate is highly increased and the radioactive labeling reagent is consumed very rapidly to give satisfactory radiochemical yields within a short reaction time of 5–10 min. Moreover, no problems with polyalkylation occur as otherwise observed when alkylation reactions are performed in stoichiometric amounts of the amine and methyl iodide. The use of trace amounts of compound also simplifies technical handlings such as transfer and purification steps. It also offers the opportunity to



[*N*-methyl-^{11}C]flumazenil [*O*-methyl-^{11}C]raclopride [*S*-methyl-^{11}C]McN-5652

Fig. 5. ^{11}C-labeled radiopharmaceuticals prepared via *N*-, *O*- and *S*-methylation reactions

perform the radiolabeling reaction in small-scale apparatus thus facilitating automation.

Heteroatom alkylation reactions with [^{11}C]methyl iodide and [^{11}C]methyl triflate can either be carried out in solution, on solid-phase support or, more recently, in micro reactors.

The most prominent and most frequently used ^{11}C methylation technique is the execution of the reaction in solution. Commonly used solvents are dimethylsulfoxide (DMSO), dimethylfloride (DMF), acetone and acetonitrile. [^{11}C]Methyl iodide or [^{11}C]methyl triflate is distilled into a vial containing a small amount (0.5–10 mg) of the corresponding desmethyl precursor dissolved in a small volume (<1 ml) of the appropriate solvent. In many cases the use of a base such as K_2CO_3, TBAOH or NaOH is required. The trapping of [^{11}C]methyl iodide in the solution should occur at low temperature (e.g. 0 °C) to enable sufficient trapping. Since trapping of [^{11}C]methyl iodide in solution is not quantitative, insertion of an active charcoal trap attached to the end of waste line is recommended. Trapping of [^{11}C]methyl triflate in solution is usually complete. The vial containing trapped [^{11}C]methyl iodide or [^{11}C]methyl triflate is sealed and heated for a few minutes (1–10 min) for completion of the reaction. The reaction mixture containing the ^{11}C-labeled compound must be subjected onto a semi-preparative HPLC as the almost universal procedure to remove residues of the desmethyl precursor and any other by-products to give a chemically and radiochemically pure compound.

Carbon-11 methylation reactions on a solid support have gained increasing attention over the last years. Reactions on a solid support comprise the use of solid-phase-extraction (SPE) cartridges or a thin tubing loop mounted to the HPLC injector system. Both approaches are characterized by its simple and time-saving operation without significant losses of radioactivity. The simple experimental set-up is easy to automate.

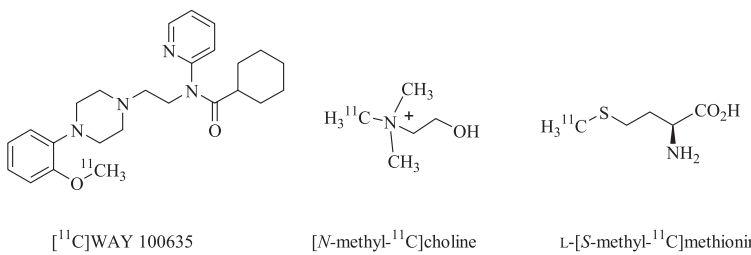
Reactions on SPE cartridges usually make use of disposable C_{18} reverse-phase cartridges which are loaded with a solution (100–200 µl) containing the desired desmethyl precursor and an auxiliary base such as TBAOH or NaOH. [^{11}C]Methyl iodide or [^{11}C]methyl triflate is swept by a gentle stream of nitrogen into the cartridge where the reaction occurs rapidly at ambient temperature. After completion of the reaction the ^{11}C methylated product is removed from the cartridge by an appropriate

solvent. The eluate containing the product can be subjected onto a HPLC injection loop for subsequent conventional HPLC purification.

According to this method, the selective 5-HT_{1A} antagonist [*O*−methyl-^{11}C]-*N*-[2-[4-(-methoxyphenyl)-1-piperazinyl]ethyl-*N*-(2-pyridinyl)cyclohexane carboxamide ([^{11}C]WAY 100635) (Wilson et al. 1995), the amino acid L[*S*-methyl-^{11}C]methionine (Pascali et al. 1999) and [*N*-methyl-^{11}C]choline (Pascali et al. 2000) have been prepared (Fig. 6).

An alternative on-line ^{11}C methylation approach on a solid support was reported by Iwata (Iwata et al. 1992). The key idea of the on-line ^{11}C methylation approach is the incorporation of a short column, which is used as an adsorber, reaction vessel and injection loop, in a HPLC injector. [^{11}C]Methyl iodide is first trapped in the short column containing an appropriate adsorber (e.g. silica gel or Porapak Q) and the coated desmethyl labeling precursor. It was shown that [^{11}C]methyl iodide is almost quantitatively adsorbed by only 30 µl of silica gel. The ^{11}C methylation reaction occurs after DMF as the solvent was introduced into the column. Likewise, a DMF solution of the desmethyl labeling precursor can be added to the short silica gel column after the trapping of [^{11}C]methyl iodide. After heating the column the reaction mixture can easily injected into a HPLC column to purify and isolate the desired ^{11}C-labeled compound.

An advancement of ^{11}C methylation reactions on solid-phase support was achieved by the introduction of the loop method for the automated preparation of ^{11}C-labeled compounds (Watkins et al. 1988; Wilson et al. 2000; Iwata et al. 2001, 2002; Studenov et al. 2004). A stainless steel



[^{11}C]WAY 100635 [*N*-methyl-^{11}C]choline L-[*S*-methyl-^{11}C]methionine

Fig. 6. Carbon-11-labeled radiopharmaceuticals prepared on solid support using a C_{18} SPE cartridge

standard HPLC injection loop or a Teflon loop is coated internally with a thin film of a solution (80–100 μl) containing the desmethyl labeling precursor. [^{11}C]Methyl iodide or [^{11}C]methyl triflate in a gentle stream of nitrogen or helium (flow rate 8–15 ml/min) is passed through the loop for 1–5 min. The content of the loop is injected onto a HPLC column for purification. As no vials, transfer lines, cooling, heating, or sealing valves are required, no transfer losses occur, and the clean-up is minimal. These advantages make the loop method an ideal technique for the convenient preparation of broad range of ^{11}C-labeled radiopharmaceuticals such as [*O*-methyl-^{11}C]raclopride (Wilson et al. 2000; Iwata et al. 2001), [^{11}C]carfentanil (Studenov et al. 2004), [^{11}C]SCH23390 (Wilson et al. 2000; Studenov et al. 2004), [^{11}C]RO5–4864 (Watkins et al. 1988) and [^{11}C]DASB (Wilson et al. 2000) (Fig. 7).

Recently, mircofluidic-based devises have been reported to be capable of performing a wide range of single and multistep synthesis (Ratner et al. 2004). Such continuous-flow microreactors have been used to perform chemical reactions on nanolitre to microlitre scales. This technique proved to be a valuable tool for optimizing synthetic efficiency, particularly when sensitive compounds are used. The distinct advantages of

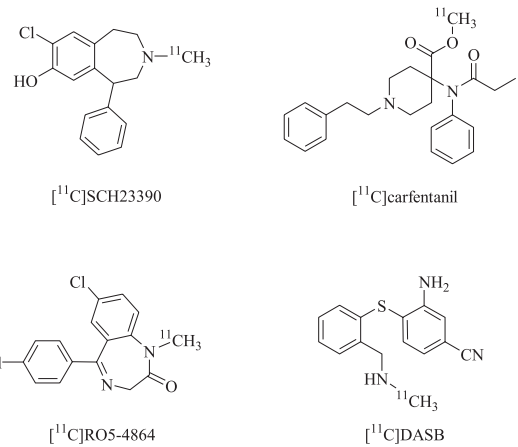


Fig. 7. Carbon-11-labeled radiopharmaceuticals prepared via in-loop radiosynthesis

microfluid technology has also entered the field of PET radiochemistry (Gillies et al. 2006a, 2006b; Lee et al. 2005; Lu et al. 2004). In a proof-of-principle study this technology was applied to the radiosynthesis of 2-deoxy-2-[^{18}F]fluoro-D-glucose ([^{18}F]FDG) (Lee et al. 2005). An extention of microreactor technology to ^{11}C chemistry was achieved by the synthesis of ^{11}C-labeled carboxylic esters as depicted in Fig. 8 (Lu et al. 2004).

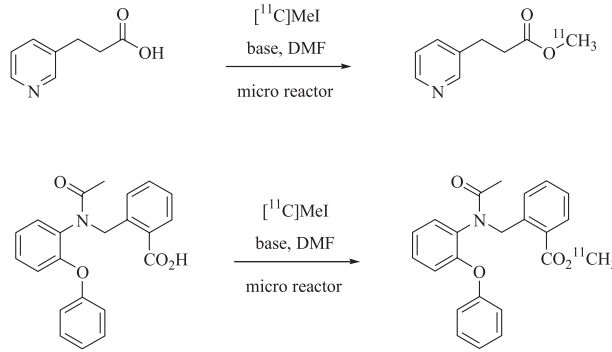


Fig. 8. Carbon-11-labeled esters prepared using microreactor technology

Carbon-11-labeled esters were obtained in radiochemical yields of 56% and 45%, respectively, at an infusion rate of 10 μl/min. Reduction of the infusion rate to 1 μl/min gave increased radiochemical yields of 88% and 65%, respectively.

These promising initial results make mircofluidic-based radiosyntheses a very attractive approach for producing radiotracers for PET. The technology bears many potential advantages (e.g. work with small amounts of compounds, enhanced reactions, rapid reaction optimisation, easy product purification), and it certainly will have an important impact on radiopharmaceutical chemistry in the future.

7.4 Palladium-Mediated ^{11}C–C Bond Formations with [^{11}C]Methyl Iodide

To further expand the number of ^{11}C-labeled compounds as molecular probes for PET the development of novel ^{11}C–C bond forming reactions gains more and more attention. The interest in these reactions stems from the possibility to place the ^{11}C label at a distinct position of a given molecule.

The readily availability of [^{11}C]methyl iodide makes this ^{11}C labeling precursor an ideal reagent for distinct ^{11}C–C bond forming reactions. Several routes for ^{11}C–C bond formations involving [^{11}C]methyl iodide have been developed. Thus, alkylation reactions of stabilized carbanions with [^{11}C]methyl iodide were used to synthesise ^{11}C-labeled amino acids (Antoni and Langström 1987b; Bjurling et al. 1989; Fasth et al. 1988, 1995; Fasth and Langström 1990; Gee and Langström 1991a,1991b; Goethals et al. 1996; Harada et al. 2000; Ikemoto et al. 1999; Mosevich et al. 1996, 1997, 1999; Sasaki et al. 2000; Studenov et al. 2003), nucleoside analogues (Conti et al. 1995; de Vries et al. 2000; Lu et al. 2002), chloride-ion channel blockers (Snyder et al. 1995), methylated thiophenes (Karramkam et al. 2003), dihydroxyvitamin D_3 (Bonasera et al. 2001), oestrogens (Dence et al. 1996) and fatty acids (Hostetler et al. 1998).

Wittig reactions employing [^{11}C]methylenetriphenylphosphorane or triphenylarsonium [^{11}C]methylide afforded [beta-^{11}C]styrene (Kihlberg et al. 1990), 6-[^{11}C]-D-glucose (Grierson et al. 1993) and [2-^{11}C]indole (Zessin et al. 1999). Wittig reaction followed by a Heck coupling led to functionalized olefins (Bjorkman and 2000).

The synthesis of ^{11}C-labeled fatty acids was achieved by conversion of various organocopper compounds with [^{11}C]methyl iodide (Kihlberg and Langström 1994; Neu et al. 1997a, 1997b; Wuest et al. 2000).

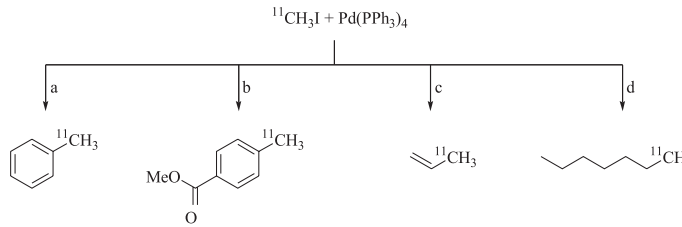
However, the aforementioned methods often require difficult synthetic sequences and they are not compatible with many functional groups. In order to overcome these obstacles, novel technically simple, high-yielding and functional group-tolerating synthetic methods for ^{11}C–C bond formations are of particular interest.

In the last decade, several palladium-mediated cross-coupling reactions have been shown to be effective and very innovative approaches for distinct ^{11}C–C bond formations.

The first application of [^{11}C]methyl iodide in palladium-mediated cross-coupling reactions was reported in 1995 (Andersson et al. 1995). The feasibility of incorporating [^{11}C]methyl groups into arenes, alkenes as well as alkanes was demonstrated by the reaction with the corresponding organostannanes and boranes in Stille and Suzuki cross-coupling reactions (Fig. 9).

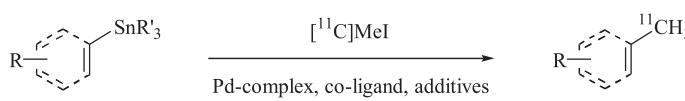
Especially the Stille coupling reaction with [^{11}C]methyl iodide was extensively used in the synthesis of several ^{11}C-labeled compounds (Fig. 10).

The selected palladium complex, the co-ligand and additives have a strong influence on the cross-coupling reaction (Samuelsson and Langström 2003; Suzuki et al. 1997). The generation of the reactive palladium species in situ from $Pd_2(dba)_3$ and a co-ligand is convenient because the type and the amount of co-ligand can be varied. Reaction



a) $PhSnMe_3$, DMSO, 75-85 %; b) Methyl 4-($SnBu_3$)benzoate, DMF or NMP, 30-40 %;
c) Vinyl-$SnBu_3$, DMSO, 75-85 %; d) 9-Hexyl-9-BBN, benzene or dioxane or THF, 60-70 %

Fig. 9. Stille and Suzuki couplings using [^{11}C]methyl iodide



R' = Me, n-B

Fig. 10. Stille cross-coupling reaction

between $Pd_2(dba)_3$ and tri-*o*-tolylphosphine ($P(o\text{-Tol})_3$) as a co-ligand generates the reactive Pd(0) species [$(o\text{-Tol})_3$P-Pd-$P(o\text{-Tol})_3$]. $P(o\text{-Tol})_3$ is superior to other co-ligands because of the large cone angle (194°) which results in the release of steric strain in the transmetalation step. The transmetalation step is considered to be rate determining within the catalytic cycle of the Stille reaction. In many cases addition of CuCl or CuI also results in better yields. After oxidative addition of [^{11}C]methyl iodide into the reactive Pd(0) complex ($Pd[P(o\text{-Tol})_3]_2$) the formed Pd^{II}-complex readily reacts with an organocopper compound which was generated in situ by transmetalation of the stannane with CuCl or CuI.

In a typical reaction [^{11}C]methyl iodide is trapped in a solution containing the Pd-complex (e.g. $Pd_2(dba)_3$) and the co-ligand (e.g. $P(o\text{-Tol})_3$) in DMF. The resulting mixture is transferred into a vial containing the stannane (and CuCl or CuI). After heating the reaction mixture for a couple of minutes, the desired product is subsequently purified by semi-preparative HPLC.

Various PET radiotracers were synthesised by the palladium-mediated reaction of stannanes with [^{11}C]methyl iodide employing Stille reaction conditions as shown in Fig. 11, e.g. serotonin transporter ligands: [^{11}C]citalopram analogue (Madsen et al. 2003), 5-[^{11}C]methyl-6-nitroquipazine (Sandell et al. 2002a, 2002b), [*p*-^{11}C-methyl]-MADAM (Tarkiainen et al. 2001); ligands for metabotropic glutamate subtype 5 receptor: [^{11}C]MPEP (Yu et al. 2005), [^{11}C]M-MTEB (Hamill et al. 2005) [alternatively, [^{11}C]M-MTEB was synthesised using a Suzuki reaction; the Suzuki coupling route gave higher radiochemical yields compared to the Stille reaction (see Fig. 14)]; and glutamate 1 receptor: [^{11}C]JNJ-16567083 (Huang et al. 2005); prostaglandins: [^{11}C]methyl $PGF_{y\alpha}$-analogue (Bjorkman et al. 2000), [^{11}C]TIC methyl esters (Bjorkman et al. 1998; Suzuki et al. 2000, 2004); COX-2 inhibitors: [^{11}C]celecoxib (Prabhakaran et al. 2005), [^{11}C]FMAU (Samuelsson and Langström 2003); tracer for assessment of myocardial sympathetic innervation: 4-[^{11}C]methylmetaraminol (Langer et al. 2003); and ligands for the nicotinic acetyl choline receptor: 5-[^{11}C]methyl-A-85380 (Iida et al. 2004; Karimi and Langström 2002) (Fig. 11). A general protocol for the synthesis of labeled methylalkenes was

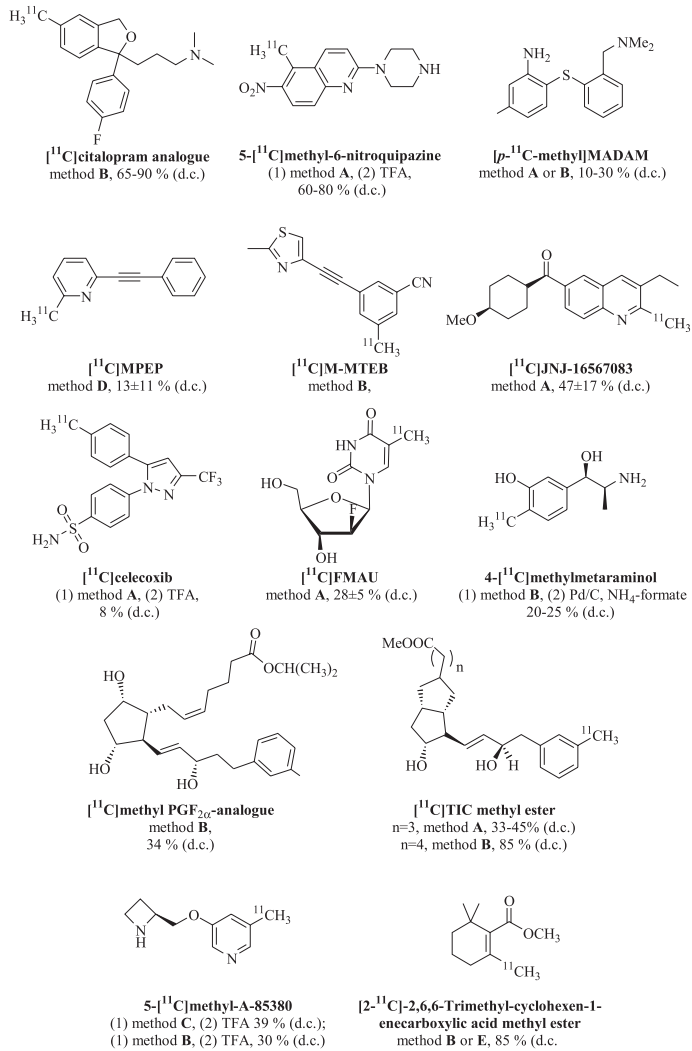


Fig. 11. Carbon-11-labeled radiotracers prepared by Stille reaction (d.c. = decay-corrected). (**A**) $Pd_2(dba)_3$, P(o-Tol)$_3$, DMF. (**B**) $Pd_2(dba)_3$, P(o-Tol)$_3$, CuCl, K_2CO_3, DMF. (**C**) $Pd_2(dba)_3$, P(o-Tol)$_3$, CuCl, K_2CO_3, DMSO. (**D**) $Pd(PPh_3)_4$, toluene. (**E**) $Pd_2(dba)_3$, P(o-Tol)$_3$, CuBr, CsF, DMF

demonstrated by the synthesis of [2-^{11}C]-2,6,6-trimethyl-cyclohexen-1-enecarboxylic acid methyl ester (Hosoya et al. 2006).

Beside the conversion of organostannanes with [^{11}C]methyl iodide the preparation of [^{11}C]monomethyltin reagents (Fig. 12) and their subsequent palladium-mediated reaction with organohalides have been described (Forngren et al. 2004; Huiban et al. 2006). 5-[^{11}C]Methyl-1-aza-5-stanna-bicyclo[3.3.3]undecane was synthesized from the corresponding chloro compound and [^{11}C]methyl lithium (Forngren et al. 2004). The use of the ^{11}C-labeled stannane was demonstrated in palladium-mediated Stille reactions with aromatic, heteroaromatic and vinylic halides to build up [^{11}C]methyl-substituted compounds. An alternative ^{11}C-labeled monomethylstannate was obtained by the conversion of Lappert's stannylene ($Sn[N(TMS)_2]_2$) with [^{11}C]methyl iodide and subsequent activation of the intermediate with tetra-*n*-butyl-ammonium

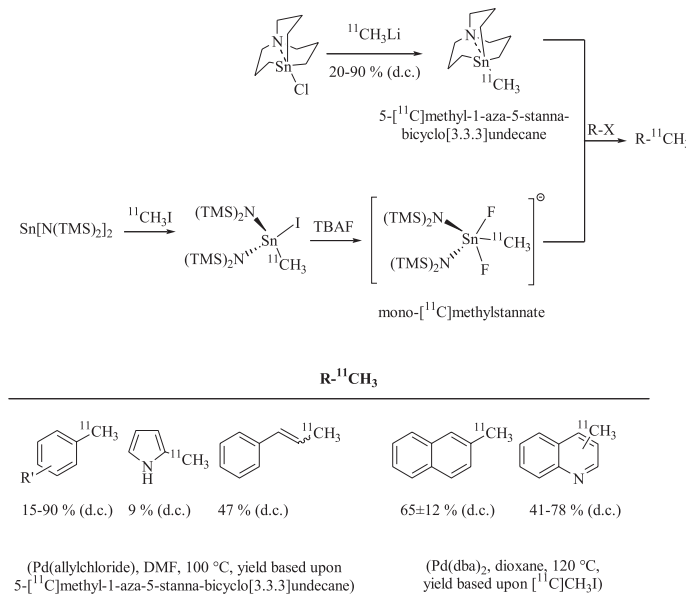


Fig. 12. Carbon-11-labeled monomethyltin reagents in Stille cross-coupling reactions (*d.c.*, decay-corrected)

fluoride (TBAF) (Huiban et al. 2006). Rapid Stille cross-coupling reaction under ligand-free conditions afforded various ^{11}C methylated naphthalenes and quinolines in high radiochemical yields.

However, sometimes toxic tin-containing contaminants are difficult to remove completely from the reaction mixture obtained in Stille cross-coupling reactions. This may limit the application of the Stille reaction especially when pharmaceuticals are synthesized. An alternative to the Stille reaction is the palladium-mediated cross-coupling of boronic acids or boronic esters with electrophiles, also referred to as the Suzuki reaction. Both electron-rich and electron-poor aryl boronic esters and aryl boronic acids bearing a wide range of functional groups were coupled with [^{11}C]methyl iodide in good yields (Hostetler et al. 2005). Thus, several [^{11}C]toluenes could be synthesised via Suzuki cross-coupling reactions (Fig. 13).

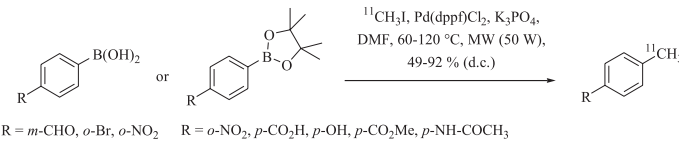


Fig. 13. Preparation of carbon-11-labeled toluenes (*d.c.*, decay-corrected)

First, [^{11}C]methyl iodide was distilled into a solution containing Pd(dppf)Cl_2 in DMF. This mixture was transferred to a vial containing a solution of the aryl boronic acid or ester and K_3PO_4 in DMF. After heating the reaction mixture by microwave (MW) activation (100 °C, 90 s at 50 W) the reaction was quenched by the addition of water. The ^{11}C-labeled toluenes were purified by HPLC.

The glutamate receptor subtype 5 PET radiotracer [^{11}C]M-MTEB could be synthesized effectively starting from a aryl boronic acid and [^{11}C]methyl iodide according to a Suzuki coupling reaction (Hamill et al. 2005; Madsen et al. 2003) (Fig. 14). This synthetic approach was more effective than the Stille reaction using the corresponding trimethylstannane (see Fig. 12).

The coupling of alkyl boranes with [^{11}C]methyl iodide provides a useful excess to ^{11}C-labeled fatty acids (Hostetler et al. 1998). The carboxyl moiety was protected as *tert*-butyl ester or masked as furane (Fig. 15).

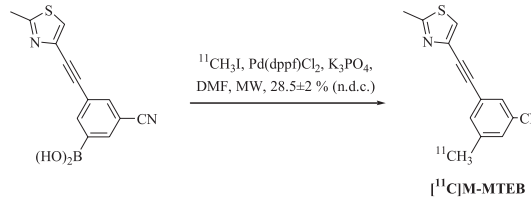


Fig. 14. Synthesis of [^{11}C]M-MTEB employing Suzuki cross-coupling reaction (*n.d.c.*, not decay-corrected)

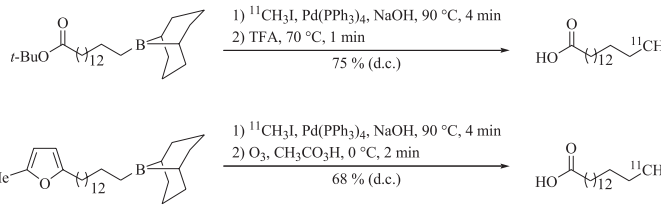


Fig. 15. Carbon-11-labeled fatty acids through Suzuki coupling (*d.c.*, decay-corrected)

Another approach for palladium-mediated ^{11}C–C bond formation with [^{11}C]methyl iodide is the conversion of terminal alkynes according to a Sonogashira cross-coupling reaction. This reaction of a terminal alkynes with [^{11}C]methyl iodide results in the formation of 3′-[^{11}C]prop-1-ynyl-substituted compounds. However, application of the classical Sonogashira reaction conditions is not feasible when [^{11}C]methyl iodide is used as the electrophile. [^{11}C]Methyl iodide will immediately be consumed by the commonly used strong amine base (e.g. triethylamine) used in the classical Sonogashira reaction to form the corresponding quaternary ammonium salt. Consequently, one has to modify the reaction conditions to be compatible with the use of [^{11}C]methyl iodide as the electrophile in the Sonogashira cross-coupling reaction. A combination of $Pd_2(dba)_3$ as palladium complex, $AsPh_3$ as co-ligand and TBAF as activator was shown to give sufficient radiochemical yields of up to 64% (based upon [^{11}C]methyl iodide) of the desired compound (Wuest et al. 2003). After transferring of [^{11}C]methyl iodide into a solution

of $Pd_2(dba)_3$ and $AsPh_3$ in THF, the mixture was heated at 60 °C for 2–3 min. TBAF and the terminal alkyne in THF were added, and the mixture was heated at 60 °C for 3 min. The final product was separated by semi-preparative HPLC (Fig. 16).

A strategy to form ^{11}C-labeled α,α′-dimethyl substituted alkenes comprises the formation of alkenylzirconocenes by the *syn*-insertion of a C–C triple bond into the Zr-H bond of Schwartz reagent [$Cp_2Zr(H)Cl$] followed by palladium-mediated ^{11}C–C bond formation with [^{11}C]methyl iodide under retention of the configuration of the C–C double bond (Wuest and Berndt 2006) (Fig. 17).

The palladium complex $Pd(PPh_3)_4$ proved to be superior to $Pt(PPh_3)_4$ or $Ni(PPh_3)_4$ as a transition metal complex. First, a solution of the alkyne and Schwartz's reagent in THF was stirred for 3 h at ambient temperature, $Pd(PPh_3)_4$ was added, and the mixture was stirred for 5 min. An aliquot of the resulting orange-coloured palladium complex/alkenylzirconocene solution was used for cross-coupling with [^{11}C]methyl iodide which took place on heating the reaction mixture at 60 °C for 5 min. The scope and limitations of the palladium-mediated cross-coupling reaction of alkenyl-zirconocenes with [^{11}C]methyl iodide were tested with various internal alkynes. Radiochemical yields of up to 75% (based upon [^{11}C]methyl iodide) could be achieved.

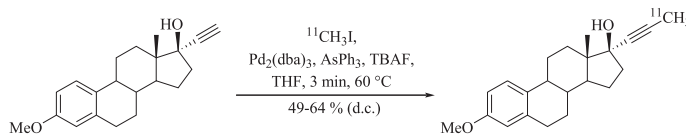


Fig. 16. ^{11}C-C bond formation via a modified Sonogashira cross-coupling (*d.c.*, decay-corrected)

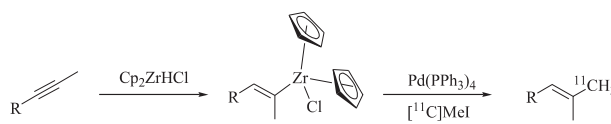


Fig. 17. Synthesis of carbon-11-labeled α,α′-dimethyl alkenes by palladium-mediated cross-coupling alkenylzirconocenes with [^{11}C]methyl iodide

7.5 Conclusion

Among the plethora of ^{11}C-labeling precursors, [^{11}C]methyl iodide is one of the most versatile ^{11}C building blocks for the synthesis of a wide variety of PET radiotracers. [^{11}C]Methyl iodide can easily produced in automated synthesis apparatus in high radiochemical yields and high specific radioactivity. Recent technical improvements and developments have made heteroatom methylation reactions with [^{11}C]methyl iodide a powerful and convenient synthesis route for the preparation of ^{11}C-labeled PET radiotracers for clinical routine and research purposes. Moreover, the scope of [^{11}C]methyl iodide as useful labeling precursor was significantly expanded through the application of transition metal-mediated reactions for distinct ^{11}C–C bond formations. In this line, especially palladium-mediated ^{11}C–C bond formations have proved to be exceptionally valuable to further expand the arsenal of ^{11}C-labeled compounds. Thus, recent developments in ^{11}C radiochemistry are an important prerequisite to further stimulate the progress of PET as a powerful imaging technique in clinical routine and research, and drug research and development.

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8 ^{68}Ga-PET Radiopharmacy: A Generator-Based Alternative to ^{18}F-Radiopharmacy

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Abstract. Positron emission tomography (PET) is becoming a dominating method in the field of molecular imaging. Most commonly used radionuclides are accelerator produced ^{11}C and ^{18}F. An alternative method to label biomolecules

is the use of metallic positron emitters; among them ^{68}Ga is the most promising as it can be produced from a generator system consisting of an inorganic or organic matrix immobilizing the parent radionuclide ^{68}Ge. Germanium-68 has a long half-life of 271 days which allows the production of long-lived, potentially very cost-effective generator systems. A commercial generator from Obninsk, Russia, is available which uses TiO_2 as an inorganic matrix to immobilize ^{68}Ge in the oxidation state IV+. ^{68}Ge(IV) is chemically sufficiently different to allow efficient separation from ^{68}Ga(III). Ga^{3+} is redox-inert; its coordination chemistry is dominated by its hard acid character. A variety of mono- and bifunctional chelators were developed which allow immobilization of $^{68}Ga^{3+}$ and convenient coupling to biomolecules. Especially peptides targeting G-protein coupled receptors overexpressed on human tumour cells have been studied preclinically and in patient studies showing high and specific tumour uptake and specific localization. ^{68}Ga-radiopharmacy may indeed be an alternative to ^{18}F-based radiopharmacy. Freeze-dried, kit-formulated precursors along with the generator may be provided, similar to the ^{99}Mo/^{99m}Tc-based radiopharmacy, still the mainstay of nuclear medicine.

Abbreviations

DOTA	1,4,7,10-Tetraazacyclododecane-*N*,*N*′,*N*″,*N*‴-tetraacetic acid
DTPA	Diethylenetriaminepentaacetic acid
HBED	*N*,*N*′-di(2-hydroxybenzyl)ethylenediamine-*N*,*N*′-diacetic acid
NODAGA	1,4,7-Triazacyclononane-*N*-glutaric acid-*N*′,*N*″-diacetic acid
NODASA	1,4,7-Triazacyclononane-*N*-succinic acid-*N*′,*N*″-diacetic acid
NOTA	1,4,7-Triazacyclononane-*N*,*N*′,*N*″-triacetic acid
NOTP	1,4,7-Triazacyclononane-*N*,*N*′,*N*″-tris(methylenephosphonic) acid
NOTPME	1,4,7-Triazacyclononane-*N*,*N*′,*N*″-tris(methylenephosphonate-monoethylester)
TAME	1,1,1-tris(aminomethyl)ethane

8.1 Introduction

Positron emission tomography is becoming the dominating imaging method in nuclear medicine. It combines the potential to quantify the tracer uptake within lesions with a relatively high resolution. In addition, biomolecules are frequently labeled with 'biological' positron-emitting isotopes such as ^{11}C, ^{13}O, ^{15}N which render any molecule chemically unchanged compared to the original molecule. The most commonly used PET-radionuclide is ^{18}F which often replaces a hydrogen atom as it has a similar van der Waals radius as ^{1}H. These radioisotopes are produced in a cyclotron. Another strategy to produce positron-emitting radionuclides is via a generator; examples are the ^{68}Ge/^{68}Ga, ^{82}Sr/^{82}Rb and the ^{62}Zn/^{62}Cu generators. Generators have the advantage that they allow clinical studies without an on-site cyclotron or if cyclotron beam time may not be available.

8.1.1 Why ^{68}Ga?

Gallium-68 as a positron emitter has some important advantages besides being available from a generator. It has a physical half-life of 68 min which is compatible with the pharmacokinetics of most radiopharmaceuticals of low molecular weight such as antibody fragments, peptides, aptamers, oligonucleotides and others.

Gallium-68 decays to 89% by positron emission and to 11% via electron capture. The maximum positron energy is 1,899 keV (the average energy per disintegration is 740 keV). The parent isotope ^{68}Ge has a very long half-life of 271 days which allows routine manufacture and shipment while the chemical properties of Ge(IV) and Ga(III) are sufficiently different to allow several different methods of efficient separation.

The use of a generator allows the development of a range of cold, freeze-dried kits which can be reconstituted and labeled, and requested at any time.

There is a well-established coordination chemistry of Ga^{3+} which allows developing agents resistant to in vivo transchelation of $^{68}Ga^{3+}$.

8.2 The ^{68}Ge/^{68}Ga-Generators

Radionuclide generators have the advantage of providing radionuclides on demand. This is potentially an inexpensive and convenient alternative to the on-site cyclotron production of short-lived radionuclides.

Radionuclide generators usually contain a long-lived parent radionuclide which decays to a short-lived daughter. There needs to be a sufficient chemical difference to allow efficient chemical separation of the stationary parent from the soluble daughter.

The development of the ^{68}Ge/^{68}Ga generator has been reviewed in several articles (Lambrecht and Sajjad 1988; Mirzadeh and Lambrecht 1996). The long half-life ($t_{1/2}$ =270.8 days) of the parent ^{68}Ge combined with the half-life of ^{68}Ga ($t_{1/2}$ =68 min), suitable for radiopharmaceutical synthesis, makes this pair ideal for a generator strategy. The preferred production route of ^{68}Ge is via the (p, 2n) reaction on gallium targets (Fig. 1). This reaction provides a significant cross section but experimental yields amount to 0.74 MBq/μ A/h only; therefore high current accelerators are needed for sufficient batch yields (Rösch and Knapp 2003).

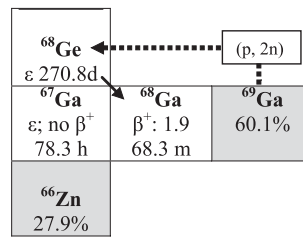

Fig. 1. Production of ^{68}Ge by the (p, 2n) reaction of ^{69}Ga

Due to the long half-life of ^{68}Ge a good separation system of mother and daughter to avoid breakthrough is mandatory. Two different strategies have been employed to afford this separation of $^{68}Ge^{IV}$ from $^{68}Ga^{III}$.

8.2.1 Organic Matrices

Ge^{IV} forms very stable complexes with phenolic groups. This property was used by Schuhmacher et al. (Schuhmacher and Maier-Borst 1981) to prepare pyrogallol-formaldehyde polymer resins which allow elution of $^{68}Ga^{3+}$ as $^{68}GaCl_4^-$ with 5.5 M HCl, followed by purification from low levels of breakthrough ^{68}Ge and concentration by adsorption on a small Dowex anion-exchange column as $^{68}GaCl_4^-$ which then was eluted with water to afford a concentrated solution of $^{68}Ga^{3+}$ in 0.5 M HCl. This home-made generator shows a very low breakthrough and is still successfully being used at the German Cancer Centre. Recently a macroporous organic polymer based on *N*-methylglucamine was also shown to be suitable as an organic matrix. Elution is performed with 0.1 M trisodium citrate and a high elution efficiency of 90% and a ^{68}Ge-leakage of $< 0.0004\%$ (Nakayama et al. 2002).

8.2.2 Inorganic Oxides As Matrices Such As Al_2O_3, SnO_2, Sb_2O_5, ZrO_2, and TiO_2

Inorganic matrices were the first to be used for efficient separation. Al_2O_3 or ZrO_2 (Greene and Tucker 1961) was used and $^{68}Ga^{3+}$ was eluted as $^{68}Ga(EDTA)^-$ complex which made every synthesis of a radiopharmaceutical time-consuming and tedious. Therefore, generators providing $^{68}Ga^{3+}$ were developed. In 1980, Loc'h et al. (Loc'h et al. 1980) reported a generator with very low ^{68}Ge breakthrough (10^{-6}–$10^{-5}\%$) and a high elution yield of 70%–80% in 1 M HCl.

Recently a TiO_2-based generator has become commercially available (Cyclotron Co, Obninsk, Russia). This generator provides $^{68}Ga^{3+}$ in 0.1 M HCl. In Europe, approximately 25 centres use this generator currently. A few groups developed automated modules to purify the eluate from Fe, Zn, and Ti impurities and to minimize the labeling volume. Meyer et al. used a microchromatography column carrying a strongly basic anion-exchange resin to concentrate the eluate and to reduce the acidity (Meyer et al. 2004). The anion exchanger adsorbs $GaCl_4^-$ present in aqueous HCl solution.

Velikyan et al. used a similar approach and in addition employed microwave heating to achieve high labeling yields ($> 98\%$) and specific

activities of >1 GBq/μ mol at very short times (1–2 min) (Velikyan et al. 2004). This group has fully automated the system (B. Langstrom, personal communication). Breeman et al. report also very high labeling yields and specific activities without the need for purification of the eluate (Breeman et al. 2004). A similar approach is chosen by Decristoforo et al. (Decristoforo et al. 2005), but they use SepPak purification of the DOTA-peptides which they employ in (pre)clinical studies. This procedure reduces the low ^{68}Ge breakthrough by a factor of 200. Rösch et al (Rösch et al. 2005) use a cation exchange resin to purify the eluate from $^{68}Ge^{IV}$, Zn^{II}, Ti^{IV}, and Fe^{III}. They reduce the low ^{68}Ga breakthrough by an additional factor of 1000. With their procedure they provide an injectable (^{68}Ga-DOTA,Tyr^3]octreotide labeling solution within 25 min.

8.3 Chemistry of Gallium

8.3.1 Aqueous and Coordination Chemistry

Gallium is the third element of Group 13 of the Periodic Table. The +3 oxidation state of this metal is the most stable in aqueous solution. Ga^{2+} has been shown to exist in the presence of high concentration of Cl^- ions but it is thermodynamically unstable to further oxidation to Ga^{3+}. In aqueous solution the free hydrated Ga^{3+} ion is stable only under acidic conditions. In the pH range of 3–7 it can hydrolyse to insoluble trihydroxide if its concentration exceeds nanomolar level. Nevertheless this precipitation can be avoided in the presence of stabilizing agents. At physiological pH the solubility of gallium is high due to the almost exclusive formation of $[Ga(OH)_4]^-$ ions (Green and Welch 1989; Moerlein and Welch 1981).

The coordination chemistry of $Ga^{3+}$is quite similar to that of the high spin Fe^{3+}ion. The two ions have the same charge, similar ionic radii (62 pm for Ga^{3+} and 65 pm for Fe^{3+}) and the same major coordination number of six (Ga^{3+} chelates sometimes are four- and five-coordinated). In order to be suitable as a radiopharmaceutical, a Ga^{3+} chelate has to be thermodynamically stable towards hydrolysis at physiological pH or to be kinetically stable during the period of clinical use. Another requirement is that the Ga^{3+} chelate does not undergo exchange with the abundant blood serum protein transferrin, which displays two iron-

binding sites with high affinity for this metal ion. At serum bicarbonate concentrations the human transferrin has a binding affinity for Ga^{3+} given by log K_{ST}=20.3 (Harris and Pecoraro 1983).

The Ga^{3+} ion is classified as a hard Lewis acid, forming thermodynamically stable complexes with ligands that are hard Lewis bases. Thus ligands with oxygen and/or nitrogen donor atoms (such as carboxylate, phosphonate, phenolate, hydroxamate and amine groups) constitute good chelating agents for Ga^{3+}. Thiols also have been shown to be good coordinating groups. Several suitable chelators have been proposed, developed and coupled to biomolecules for gallium labeling. Often ^{67}Ga is used as a replacement for ^{68}Ga in radiopharmaceutical development.

8.3.2 Mono- and Bifunctional Chelators

Gallium(III) is suitable for complexation with polydentate ligands, both cyclic and open chain structures. The majority of ligands designed for Ga^{3+} are hexadentate although several chelates have been reported which are stable in vivo and have coordination numbers of four and five. If the ligand, besides binding the metal cation, also presents a functionality (often $–NH_2$ or –COOH groups) that allows covalent coupling to a targeting vector, it is called a bifunctional chelator. A bifunctional chelator should meet the following criteria: (1) when linked to a macromolecule it should chelate the radiometal rapidly and sufficiently; (2) the obtained chelate should be kinetically stable to demetallation over a pH range of 4–8 and in the presence of other serum cations (Ca^{2+}, Zn^{2+}, Mg^{2+}).

Desferrioxamine-B (DFO) (Fig. 2) is a ligand that complexes Ga^{3+}rapidly and with high affinity. It has three hydroxamate groups as metal coordinating sites.

DFO has been used as a bifunctional chelator for high radiochemical yield labeling with $^{67/68}Ga^{3+}$ (Caraco et al. 1998; Furukawa et al. 1991; Mathias et al. 1996; Smith-Jones et al. 1994; Yokoyama et al. 1982). Caraco et al. studied the effect of low DFO concentrations and the different incubation conditions on the stability of the Ga^{3+}-DFO chelate, concluding that at the nanomolar level DFO does not act as a good chelating agent for gallium (Caraco et al. 1998). This chelator displays a $–NH_2$ group available for coupling to biomolecules. Another approach

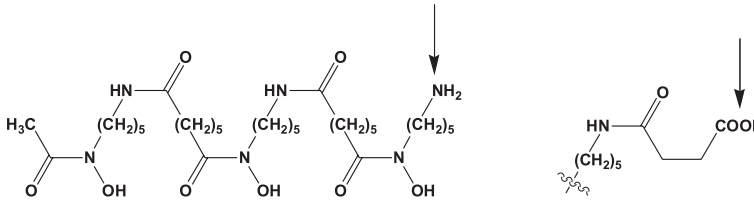

Fig. 2. Desferrioxamine-B (DFO). Coupling sites to biomolecules: NH_2 or a succinyl spacer

is the coupling via a succinyl spacer (Maecke et al. 1993; Smith-Jones et al. 1994; Stolz et al. 1994).

Sun et al. (Sun et al. 1996) measured the stability constants (Table 1) and the in vitro and in vivo stabilities of a series of Ga^{3+} and In^{3+} chelates of mercapto amino ligands with different denticities (ligands 1–8 in Fig. 3).

The liver clearance of the metal complexes indicates that the more stable complexes are cleared rapidly, whereas the less stable complexes are retained in the liver, probably exchanging with transferrin. This study allowed to conclude that tetra- and pentadentate ligands can form chelates sufficiently stable so that their biodistribution pattern is determined by the Ga(III)-complex itself and not by the exchange of gallium to pro-

Table 1. Equilibrium constants for In^{3+} and Ga^{3+} complexes of various chelators (Anderson et al. 1995; Clarke and Martell 1991; Harris and Martell 1976; Ma et al. 1995; Sun et al. 1996) (25 °C, 0.10 M KNO_3 or KCl)

Equilibrium quotient	4SS	5SS	6SS	EDDASS	EC	TACN-TM	NOTA	NS3	HBED
					In^{3+}				
$\frac{[ML]}{[M][L]}$	27.34	30.9	39.8	37.0	33.0	36.1	26.2	21.2	
pM	21.7	23.7	30.9	30.4	26.2	23.9		-	
					Ga^{3+}				
$\frac{[ML]}{[M][L]}$	24.73	27.37	41.0	35.6	31.5	34.2	30.98	20.5	38.51
pM	22.6	22.1	31.6	29.0	24.7	23.6	26.4	-	28.6

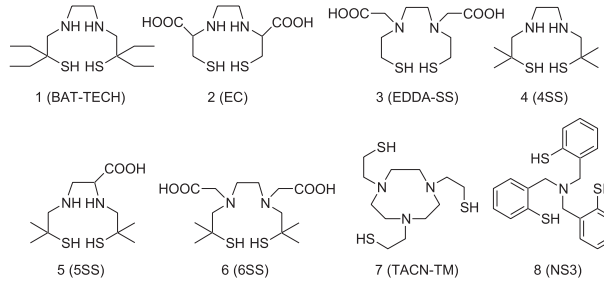

Fig. 3. Mercapto amino ligands with different denticities

teins. Nevertheless, hexadentate ligands are the best candidates for forming bifunctional chelators through covalent linkage to biomolecules. In particular, the ligands 6SS and EDDASS were shown to be the best candidates.

Cutler et al. (Cutler et al. 1997) have shown that the Ga^{3+} complex of the tetradentate tripodal ligand NS_3 (Govindasvamy et al. 1995) is a neutral lipophilic species which passes the blood–brain barrier and exhibits a high heart-to-blood ratio. This is a chelate that even in the presence of water retains its tetrahedral geometry. The conjugation of the NS_3 chelator to phenylalanine demonstrated the feasibility of this system in the preparation of stable $^{67/68}$Ga-labeled peptides (Fig. 4) (Luyt and Katzenellenbogen 2002).

Recently other tripodal polycarboxylic acid chelators have been reported which are based on the TAME structure. Their Ga^{3+} chelates were shown to be stable against *trans*-chelation by a 1000-fold excess of DTPA (Arslantas et al. 2004).

Macrocyclic chelators, in particular triaza ligands, display high conformational and size selectivity towards metal ions. The high thermodynamic stability of the Ga^{3+}chelates of triazamacrocyclic ligands is

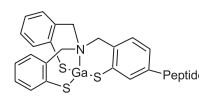

Fig. 4. A trithiolate tripodal gallium(III) bioconjugate

due to the good fit of the relatively small cation in the cyclic cavity. This category of chelators can encapsulate the metal ion with high efficiency, keeping it away from competitor species like blood transferrin. TACN-TM, a triaza ligand with three mercaptoethyl pendant arms (Ma et al. 1995); NOTA, a triazatricarboxilic ligand (Fig. 5) (Broan et al. 1991; Clarke and Martell 1992; Craig et al. 1989), and NOTP and NOTPME, two phosphonate-bearing triaza ligands (Prata et al. 1999, 2000), are appropriate hexadentate chelators for gallium. Neutral complexes are less sensitive to acid/cation-promoted dissociation compared to the anionic complexes.

This is well illustrated by the neutral Ga(NOTA), which only undergoes acid-catalysed dissociation at non-physiological low pH values. Ga(NODASA) (Fig. 6) equally is a very stable gallium chelate which additionally has potential for biomolecule coupling using the prelabeling approach.

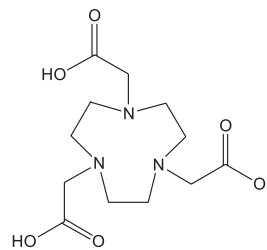

Fig. 5. NOTA

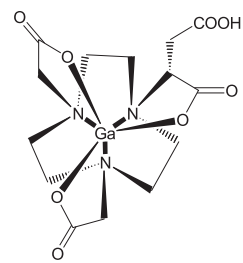

Fig. 6. Structural formula of Ga(NODASA)

As demonstrated by its X-ray structure (Fig. 7) it displays a free carboxylate group available for coupling to biomolecules whereas the other three carboxylates are protected by the metal ion.

This chelate has been rapidly coupled to D-phenylalanineamide as a model peptide which renders the coupling of ^{68}Ga(NODASA) to peptides promising for PET applications (Andre et al. 1998). The same type of chelator has been derivatized (NODAGATOC) in order to make it also available for the post-labeling approach (Fig. 8) (Eisenwiener et al. 2002).

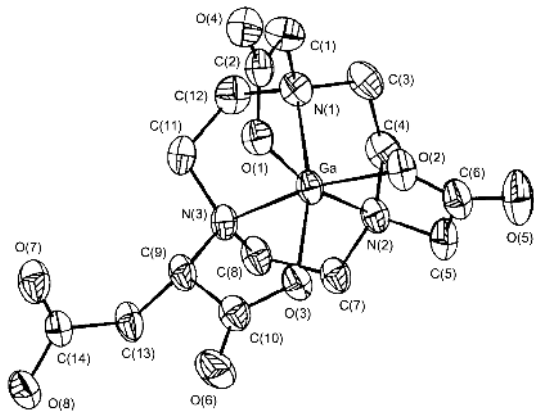

Fig. 7. ORTEP plot of structure Ga(NODASA) (André et al. 1998) (reproduced by permission of the Royal Society of Chemistry)

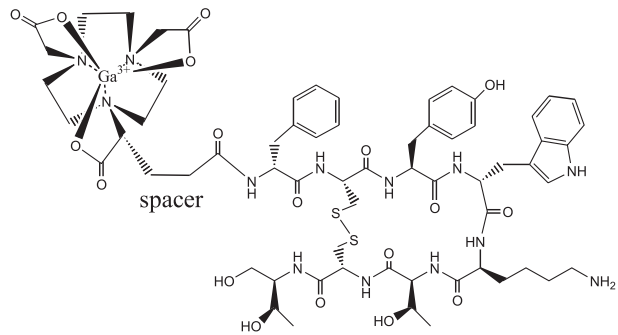

Fig. 8. Structural formula of NODAGA-Tyr^3-Octreotide (NODAGATOC)

The thermodynamic stability constant of the Ga^{3+} complex of the tetraaza tetraacetic acid chelator DOTA (Fig. 9) is much lower ($\log K = 21.33$) (Clarke and Martell 1992) than that of Ga(NOTA) ($\log K = 30.98$) (Clarke and Martell 1991) due to the larger dimensions of its cavity.

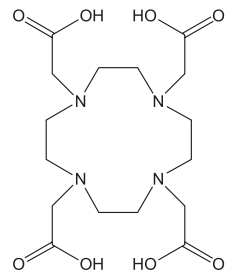

Fig. 9. Structural formula of 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA)

Despite the lower value of its stability constant, Ga(DOTA) is sufficiently stable to be used in clinical practice. The DOTA monoamide conjugated peptide [Tyr^3]-octreotide (DOTATOC) showed very promising in vivo and in vitro properties when labeled with ^{67}Ga, including higher binding affinity for the somatostatin-receptor subtype 2 and approx. 2.5 times higher tumour uptake in a mouse model and lower kidney uptake than $^{111}In/^{90}Y$-DOTATOC (Heppeler et al. 1999a). The X-ray crystal structure of the model peptide Ga^{3+}-DOTA-D-PheNH_2 showed that DOTA adopts a *cis*-pseudo-octahedral geometry with a folded macrocyclic unit (2424 conformation) (Fig. 10).

The equatorial plane is formed by two transannular nitrogens of the cyclen ring and two oxygens of the corresponding carboxylate groups. The two axial positions are occupied by the remaining two ring nitrogens. Two potential coordination sites of the chelator are free, namely one carboxylate and the amide carboxy oxygen. This contrasts with the quite different structure of Y^{3+}(In^{3+})-DOTA-D-PheNH_2 (octacoordinated) and might account for the improved radiopharmaceutical properties of the gallium conjugate (Heppeler et al. 1999b). ^1H and ^{13}C nuclear

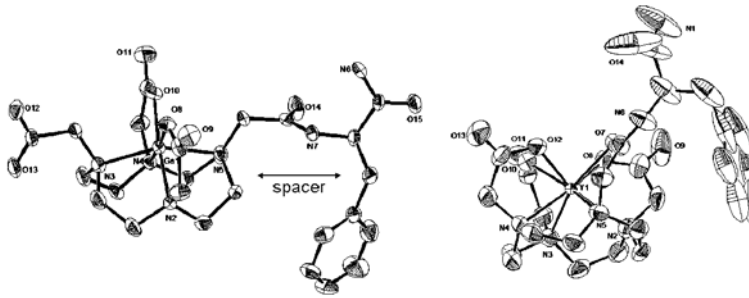

Fig. 10. [Ga^{III}-DOTA-D-PheNH_2] 6-coordinate (left) and [Y^{III}-DOTA-D-PheNH_2] 8-coordinate (right) (reproduced by permission of John Wiley & Sons)

magnetic resonance (NMR) studies in solution confirmed the structural differences between the Ga^{3+} and Y^{3+} complexes of DOTATOC, namely on the D-Phe[1] residue (Fig. 11).

The yttrium conjugate leads to two conformers exchanging slowly on the NMR time scale – cis/trans isomers at the amide bond between the DOTA chelator and the first amino acid residue of the peptide (Deshmukh et al. 2005).

8.3.3 Prochelators and Coupling Chemistry

The compatibility with peptide synthesis requires that bifunctional chelators, often displaying carboxylic acid groups, have the possibility of orthogonal protection. Additionally the monoreactive prochelator has to be soluble in the solvents commonly used in peptide synthesis.

Different syntheses of bifunctional NOTA derivatives have been reported previously by several groups (Cox et al. 1990; McMurry et al. 1993; Studer and Meares 1992). Their approaches included intra- or intermolecular cyclization strategies as key steps, affording NOTAs functionalized on a carbon of the macrocyclic ring. These compounds were not designed for coupling to peptides, and harsh conditions for deprotection and reduction were required.

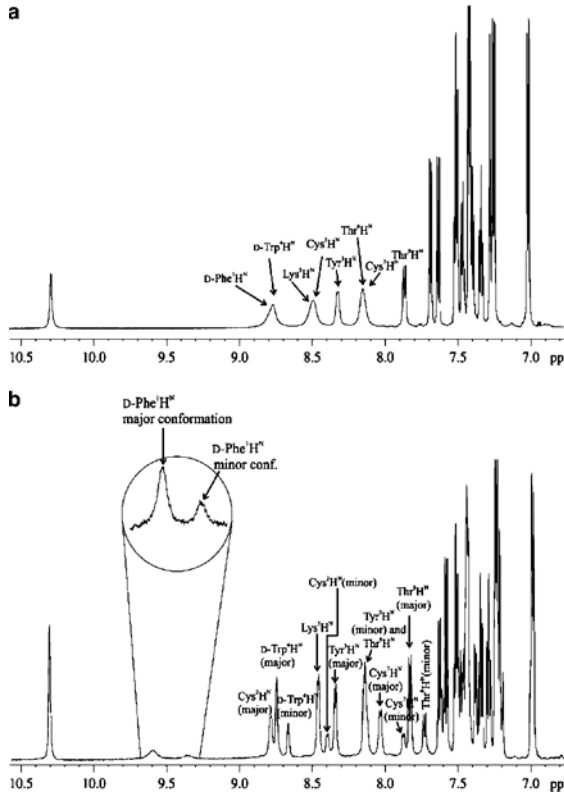

Fig. 11. One-dimensional proton NMR spectra of Ga^{III}-DOTA-TOC (**a**) and Y^{III}-DOTA-TOC (**b**) at 290 K. (Reproduced by permission of the American Chemical Society)

The attachment of a prochelator to a vector molecule can be achieved via the carboxylate group or the functionalized carbon skeleton. Often the former strategy is used. A bifunctional NOTA for antibody modification has been described (Brechbiel et al. 1993). Several functionalizations of DOTA have been reported (Chappell et al. 1999; Kruper et al. 1993; Lewis et al. 1994). The monoreactive prochelator DOTA-tris(tBu) (Fig. 12) prepared in a three step-synthesis constitutes a syn-

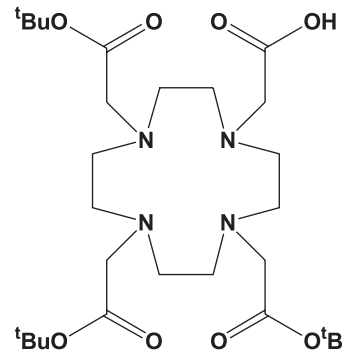

Fig. 12. Monoreactive prochelator DOTA-tris(tBu)

thon that can be coupled, both in solution and in solid phase, to the *N*-terminus of peptides. It has been coupled to the somatostatin analogue Tyr^3-Lys^5(BOC)-octreotide in dimethylfluoride, followed by deprotection in trifluoroacetic acid (TFA)/H_2O (Heppeler et al. 1999a).

More recently NOTA has been coupled to peptides using an alternative strategy. Figure 13 shows the five-step synthesis of the monoreactive prochelator [1-(1-carboxy-3-carbo-*tert*-butoxypropyl)-4,7-(carbo-*tert*-butoxy-methyl)-1,4,7-triazacyclononane (NODAGA(t-Bu_3)] (Eisenwiener et al. 2000, 2002). The resulting conjugate is deprotected by hydrogenation in a suspension of Pd/C in propanol/H_2O. For the coupling of the deprotected compound to the linear [Tyr^3]octreotide (appropriately protected) the latter is assembled on a trityl chloride resin. The cleavage is followed by oxidative cyclization using I_2 and deprotection in a mixture of TFA/phenol/thioanisole/water.

The bifunctional version of the tetradentate tripodal NS_3 has been synthesized in 13 steps and subsequently linked to model peptides through an aromatic amine using carbodiimide chemistry (Fig. 4) (Luyt and Katzenellenbogen 2002).

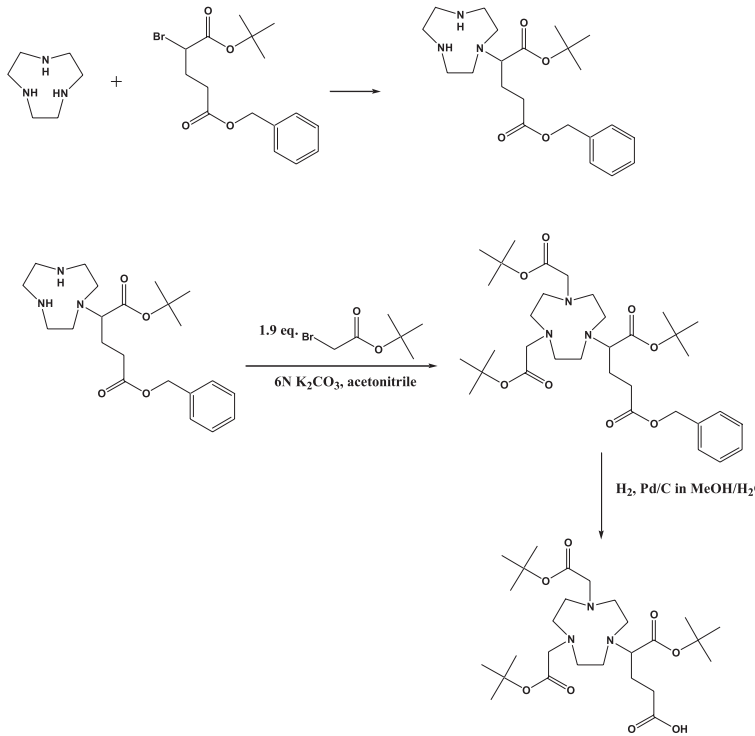

Fig. 13. Five-step synthesis of the monoreactive prochelator (1-(1-carboxy-3-carbo-*tert*-butoxypropyl)-4,7-(carbo-*tert*-butoxy-methyl)-1,4,7-triazacyclononane (NODAGA(t-Bu_3))

8.4 Radiopharmaceuticals

8.4.1 Preclinical Studies

In a review on 'the continuing role of radionuclide generator systems for nuclear medicine' in the European Journal of Nuclear Medicine in 1994 (Knapp and Mirzadeh 1994) the authors state that "despite the availability of the ^{68}Ge/^{68}Ga generator application of ^{68}Ga radiopharmaceuticals may suffer from the complex ligand chemistry required for Ga^{3+} com-

Table 2. Gallium-68 radiopharmaceuticals (see Green 1993)

^{68}Ga-transferrin	Plasma protein volume
^{68}Ga-DTPA-albumin	
^{68}Ga-albumin microspheres	Pulmonary
^{68}Ga-macroaggregated albumin	Myocardial and cerebral perfusion
^{68}Ga-Fe(OH)$_3$colloid	Liver/spleen RES function
^{68}Ga-alizarin	
^{68}Ga-[(5-MeOsal)$_3$tame]	Myocardial blood flow
^{68}Ga-BAT-TECH	
^{68}Ga-EDTA	Detection of blood-brain barrier defects
^{68}Ga-PLED	Renal function
^{68}Ga-EDTA	
^{68}Ga-polymetaphosphate	
^{68}Ga-*t*-butyl-HBED	Hepatobiliary function
^{68}Ga-BP-IDA	
^{68}Ga-(3,4DiP-LICAM)	
^{68}Ga-Br-EHPG	
^{68}Ga-Br-HBED	
^{68}Ga-DTPA-antimyosin	Detection of myocardial infarction/necrosis
^{68}Ga-DTPA-low density lipoprotein	Lipoprotein metabolism
^{68}Ga-galactosyl-neoglycoalbumin	Hepatocyte function
^{68}Ga-EDTMP	Bone scanning (metastatic tumour detection)
^{68}Ga-red blood cells	Thrombosis localization
^{68}Ga-platelets	

plexation to useful tissue-specific radiopharmaceuticals". Indeed, at that time no ^{68}Ga-based radiopharmaceuticals were in clinical studies and no EMEA or FDA approved ^{68}Ga radiopharmaceuticals are available currently. However, a variety of ^{68}Ga radiopharmaceuticals for different organ functions were synthesized and studied preclinically (Table 2) and only few of these have found their way into the clinic.

A renaissance of ^{68}Ga radiopharmacy came with the development of small peptides with tumour affinity, most notably those targeting somatostatin receptors. A first conjugate was developed using the high affinity Ga^{3+} and Fe^{3+} chelator desferrioxamine-B (Fig. 2) which was coupled via a succinyl spacer to octreotide. Preclinical data showed high binding affinity to rat brain cortex membrane, comparable to octreotide, and high and specific tumour uptake in a rat bearing a pancreatic islet cell tumour. Clinical studies in eight patients were somewhat disappointing

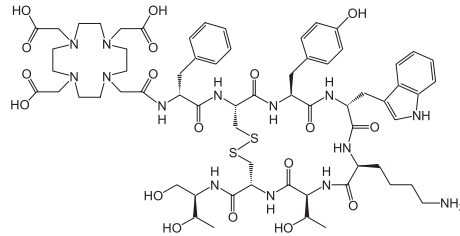

Fig. 14. Structural formula of DOTA-TOC

because of a slow blood clearance which was due to strong protein binding in human plasma (Maecke et al. 1999).

A more promising compound was [^{67}Ga/^{68}Ga-DOTA,Tyr^3]octreotide (^{67}Ga/^{68}Ga-DOTA-TOC, Fig. 14). It showed higher somatostatin receptor subtype 2 affinity than ^{111}In/^{90}Y-DOTA-TOC but also a 2.5-fold higher tumour uptake in a mouse model bearing the sst2-positive AR4–2J tumour.

Other small molecules were labeled with ^{67}Ga/^{68}Ga, e.g. different somatostatin-based peptides such as DOTA-lanreotide (Traub et al. 2005) and [DOTA,1-Nal^3]octreotide (DOTA-NOC) (Baum et al. 2005; Hofmann et al. 2005).

Another peptide receptor is being targeted with ^{68}Ga-labeled vectors, the MC1R which is expressed by cutaneous melanocytes and is overexpressed on both melanotic and amelanotic melanomas. Preclinical studies using ^{68}Ga DOTA-conjugated α-melanocyte stimulating hormone (α-MSH) derivatives showed high tumour and low kidney uptake. PET studies using [^{68}Ga-DOTA, Nle^4-Asp^5, D-Phe^7] α-MSH (4–11) revealed high contrast images 1 h after tracer administration (Froidevaux et al. 2004).

The bombesin receptor family has also been targeted with the peptide [^{68}Ga-DOTA-PEG2, D-Tyr^6, βAla^{11}, Thi^{13}, Nle^{14}]bombesin (6–14) which showed a high internalization rate into the bombesin receptor-positive AR4–2J cell line. The tumour uptake in the AR4–2J animal model was also high and specific (Schumacher et al. 2005). One hour after tracer injection PET-imaging showed specific uptake in the tumour and the bombesin receptor-positive organs such as the bowel and

the pancreas. Relatively high uptake in the kidney was also demonstrated.

Additional non-peptide based small molecules coupled to DOTA have been prepared for ^{68}Ga labeling. Velikyan et al. (Velikyan et al. 2005) prepared and evaluated ^{68}Ga-DOTA-hEGF for the visualization of epidermal growth factor receptor (EGFR) expression in tumours. They labeled the small protein with ^{68}Ga using microwave heating and reached a labeling yield of 77 $\pm$ 4% within 1 min. The radiogallium-labeled protein was rapidly internalized into EGFR-positive tumour cells and biodistribution studies showed specific uptake in a tumour xenograft and in EGFR-expressing organs. A microPET imaging study enabled visualization of tumours within a few minutes.

Roivainen et al. (Roivainen et al. 2004) synthesized DOTA-coupled 17-mer oligonucleotides as PET-imaging agents for tumours containing the K-ras point mutations in codon 12. The modified and labeled oligonucleotides showed unaltered hybridization properties. High quality PET images allowed visualization of the tumour and quantification of the pharmacokinetics in the tumour and major organs. The authors conclude that "^{68}Ga labeling of oligonucleotides is a convenient approach for *in vivo* PET imaging of the biodistribution and quantification of oligonucleotide biokinetics in living animals".

For use in a two-step pretargeting approach with a bispecific antibody against carcino-embryionic antigen (CEA), Griffiths et al. (Griffiths et al. 2004) synthesized and studied a bivalent peptidic hapten coupled to DOTA for ^{68}Ga-labeling. They concluded from the preclinical study that the approach deserves further development for improved cancer imaging.

8.4.2 Patient Studies

As indicated above, ^{68}Ga-DOTA-TOC was one if not the breakthrough vector molecule and seminal to the development of present ^{68}Ga radiopharmacy and the ^{68}Ge/^{68}Ga generator.

To our knowledge, currently five different peptides labeled by ^{68}Ga are in clinical trials:

1. [^{68}Ga-DOTA, Tyr^3]octreotide (DOTA-TOC); [^{68}Ga-DOTA, 1-Nal^3]-octreotide (DOTA-NOC); [^{68}Ga-DOTA-2-Nal, Tyr^3, ThrNH$_2^8$]octreo-

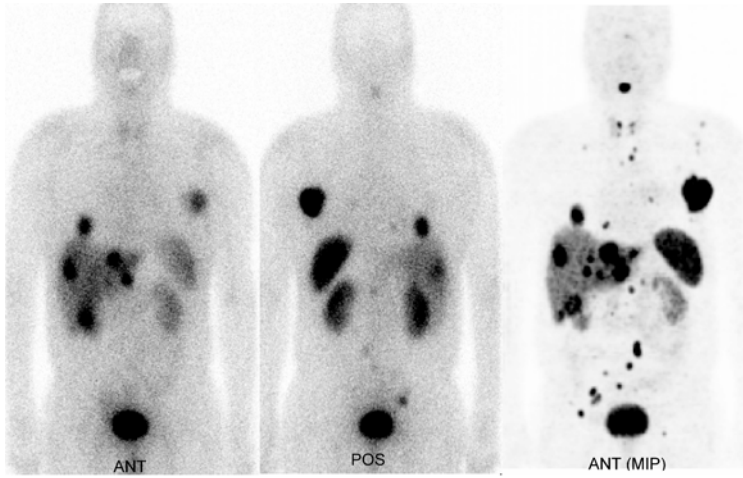

Fig. 15. Comparison of ^{111}In-DTPAOC with ^{68}Ga-DOTA-TOC in the same tumour patient

tide (DOTA-lanreotide) which target several subtypes of somatostatin receptors.
2. [^{68}Ga-DOTA]bombesin, a peptide which binds to the three bombesin receptor subtypes and is being studied in gastrointestinal tumours and in prostate cancer.
3. [^{68}Ga-DOTA-D-Glu]gastrin which is studied in medullary thyroid cancer patients.

^{68}Ga-DOTA-TOC was studied in different somatostatin receptor-positive tumour entities and published in different papers and abstracts (Henze et al. 2001, 2004, 2005; Hofmann et al. 2001; Kowalsky et al. 2003).

In two recent abstracts Aschoff et al. (Aschoff et al. 2005a, 2005b)) concluded that the sensitivity of ^{68}Ga-DOTA-TOC in neuroendocrine tumours exceeded that of OctreoScan; in 24 of 36 patients additional lesions were found (Fig. 15). In addition, in 94% of patients the final diagnosis and the visualization of all lesions was possible 10 min

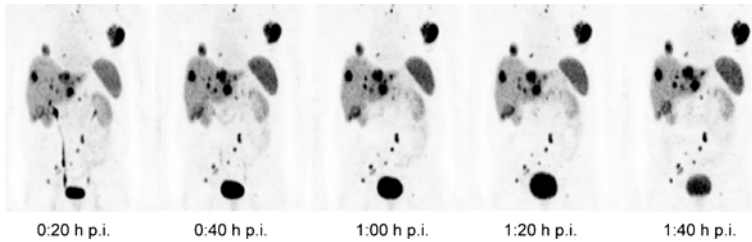

Fig. 16. Pharmacokinetics using ^{68}Ga-DOTA-TOC in a patient with a neuroendocrine lung tumour

post-injection of the tracer. The authors conclude that for daily practice, because of the short duration of the scan and the optimal pharmacokinetics (Fig. 16), the final diagnosis is available within 1 h. An important additional aspect is the much lower radiation dose to the patient.

A second paper by the same authors deals with the sensitivity of ^{68}Ga-DOTA-TOC to image bone metastases in neuroendocrine tumours, and the question if a bone scan using ^{99m}Tc-labeled phosphonates provides additional information. Based on 10 patients they conclude that PET/CT using ^{68}Ga-DOTA-TOC is superior to bone scans in the visualization of bone metastases in neuroendocrine tumours and makes the latter unnecessary. This, they state, "is due to the superiority of the new tracer but also to the high sensitivity of PET compared to SPECT".

Gabriel et al. (Gabriel et al. 2005) arrive at a similar result with regard to the sensitivity of ^{68}Ga-DOTA-TOC PET vs. ^{99m}Tc-HYNIC-TOC and ^{111}In-DOTA-TOC SPECT.

^{68}Ga-DOTA-NOC may have an advantage over ^{68}Ga-DOTA-TOC as it shows a broader somatostatin receptor subtype profile than the TOC-derivative. Hofmann et al. compared the two PET tracers in 40 patients and concluded that ^{68}Ga-DOTA-NOC displays a not only 2.5-fold higher uptake in tumours but also a higher uptake in some normal organs (Hofmann et al. 2005).

In a similar comparative study, Baum et al. conclude that "^{68}Ga-DOTA-NOC advances to the new gold standard for the imaging of neuroendocrine tumours" (Baum et al. 2005).

8.5 Conclusion and Prospects

The commercial availability of the ^{68}Ge/^{68}Ga generator and the development of several peptides with high affinity to different G-protein coupled receptors overexpressed on major human tumours has led to much activity in the field of ^{68}Ga-based radiopharmacy. Also, due to cooperations and networking within the COST action B12 'Radiotracers for in vivo assessment of biological functions', about 25 centres in Europe use this generator, develop it further and most of them use it in clinical studies. The breakthrough came with the somatostatin receptor targeting radiopeptide [^{68}Ga-DOTA, Tyr^3]octreotide (^{68}Ga-DOTA-TOC). Other peptides followed and new ^{68}Ga-based radiopharmaceuticals are being developed in different laboratories.

These developments and the success stories in clinical studies argue for the development of a pharmaceutical grade generator. If this is the case, a new radiopharmacy may develop, providing PET radiopharmaceuticals as freeze-dried, kit-formulated, good manufacturing-produced versions, similar to the ^{99}Mo/^{99m}Tc-generator-based ^{99m}Tc-radiopharmaceuticals. This may indeed be an alternative to ^{18}F-based radiopharmaceuticals if the appending of ^{68}Ga-chelates does not alter the pharmacokinetics of the biomolecules studied.

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9 Microwaving in F-18 Chemistry: Quirks and Tweaks

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Abstract. Since the late 1980s, microwave dielectric heating has been used to speed up chemical transformations, also in radiolabeling tracers for positron emission tomography. In addition to shorter reaction times, higher yields, cleaner product mixtures and improved reproducibility have also been obtained for reactions involving polar components that require heating at elevated temperatures. The conditions used in microwave chemistry can differ considerably from those in conventional heating. Understanding the factors that influence the interaction of the electromagnetic field with the sample is critical for the successful implementation of microwave heating. These parameters are discussed here and exemplified with radiolabelings with fluorine-18.

9.1 Introduction

Microwaves are electromagnetic waves with vacuum wavelengths between 0.1 and 100 cm (frequencies between 0.3 and 300 GHz). Since the mid-1980s it has been convincingly demonstrated that the way in which microwaves interact with electric dipoles and charges can be used to rapidly heat reaction samples to a degree and at a speed that can be difficult to achieve with conventional heating.

It is generally agreed that it is the electric component of the electromagnetic wave that exerts the principal effect. Charged species are dragged by the reversing field. Molecules with permanent dipoles are polarized and attempt to rotate with the direction of the field change. In liquids and solids, however, the motion is restricted and rotations are turned into hindered rotations, which have frequencies in the low GHz range. A recent study has suggested that microwave absorption of liquids and solids is the result of the excitation of these hindered rotations (Kalhori et al. 2002). The electromagnetic energy is transferred at a faster rate than the molecules can relax. The micromotion in response to the oscillating field will therefore lead to a very rapid heating of the sample. In contrast to conventional, diffusive heating applied from the surface of the vessel, all the elements that respond to dipole polarization or ionic conduction will be affected simultaneously. Samples can reach a very high temperature nearly instantaneously – temperatures that are considerably higher than their normal boiling points, so-called superheating (Baghurst and Mingos 1992). In accordance with the Arrhenius law, the rates of reactions will increase as the temperature is raised. Therefore, all

reactions that involve polar components that can absorb microwaves and that typically require heating at elevated temperatures may potentially be accelerated through the use of microwave dielectric heating.

Microwaves have proven to be a highly effective heating source for a wide range of chemical conversions (reviewed, for example, in Abramovitch 1991; Mingos and Baghurst 1991; Caddick 1995; Lidström et al. 2001; Kappe 2004). Not only have the reactions been accelerated, but also higher yields, cleaner product mixtures and improved reproducibility have been reported. The special aspects of using microwave techniques in PET radiochemistry, with emphasis on observations from fluorine-18 applications, are discussed here.

9.2 Microwave Equipment in PET Radiochemistry

Microwave devices that might leak are required to operate at the ISM (Industrial, Scientific, Medical) bands, 915 MHz or 2.45 GHz, to avoid interference with radar or telecommunications. The most widely used band is 2.45 GHz, since this frequency has a penetration depth that is appropriate for most reaction samples.

For reproducible results in microwave-induced chemical transformations, the field to which the sample is exposed should be controllable and well defined. However, not only does the geometry of the field differ for each individual microwave device but it will also be affected by the load (the reaction vessel and the components in the sample) and its temperature. Although many radiolabelings have been performed using domestic multi-mode microwave ovens, the development of and the commercial availability of dedicated single-mode devices have been the most important reasons for the increasing use of microwave techniques in PET as well as in many other areas of synthetic organic chemistry. In single-mode cavities it is possible to generate focused and optimized standing waves with well-defined positions of maximum and minimum intensity. Holders guide the vessels to an antinode of the field, and the shift of the field by the load can be compensated in a predictable fashion. These devices give better reproducibility and shorter reaction times than multi-mode ovens, simply due to the fact that the interaction between the microwave field and the sample is controlled and can thereby be optimized.

Fig. 1. Commercially supplied single-mode microwave cavities being used in PET. (**a**) The Microwell 10 (Labwell AB, Uppsala, Sweden) with a footprint of 18×34×30 cm (w×d×h). (**b**) The Model 521 (Resonance Instruments Inc., Skokie, Ill., USA, diameter=15 cm) shown here in a hot cell at NIMH (provided by Shui-Yu Lu). (**c**) The Discover (CEM, Matthews, N.C., USA; 36 cm×44 cm×22 (w×d×h) is shown here integrated in a hot cell at NIMH (provided by Shui-Yu Lu). (**d**) The SmithCreator (Biotage, Uppsala, Sweden) shown here in a laboratory environment (provided by Hans Ehrsson). (**e**) The custom-designed 600-W microwave cavity (Scansys Laboratorieteknik, Værløse, Denmark) integrated into a modular PET radiosynthesis system (provided by Peter Larsen)

The commercially supplied single-mode devices that are, to our knowledge, currently being used for PET radiochemistry are shown in Fig. 1. Most of these consist of a microwave power generator, an interconnecting cable and a remote cavity, which is ideal for radiolabeling set-ups since the equipment that needs to be inside the hot cell is kept at a minimum. The Microwell 10 (Labwell AB, Fig. 1a) is no longer commercially available, but has/is being used by around ten radiolabeling groups. Resonance Instruments Inc. (Skokie, Ill., USA) supplies several compact systems (Models 510, 520A and 521). These instruments, originally custom-designed for PET radiochemistry, are the most commonly used today, with more than 15 systems sold to PET facilities (W. Openlander, personal communication). The Model 521 cavity integrated into the radiolabeling set-up at NIMH is shown in Fig. 1b (courtesy of Shui-Yu Lu). Two larger single-mode cavities, marketed for general synthetic applications, have also been used in PET. The CEM Discover (Matthews, N.C., USA), here integrated into the hot cell at NIMH (Fig. 1c, courtesy of Shui-Yu Lu), has been used, for example, to accelerate the syntheses of [^{18}F]FDG (Nickles et al. 2003) and [carbonyl-^{11}C]amides (Lu et al. 2003). Radiolabeling of peptides with ^{68}Ga (Velikyan et al. 2004) has been improved by using the Biotage SmithCreator (Uppsala, Sweden, Fig. 1d). Though the power supply and cavity are not separated on this apparatus, control from outside the hot cell can be achieved by attaching a remote laptop or mouse. A more compact device, the Initiator, has recently succeeded the SmithCreator. A new custom-designed single-mode cavity for radiolabeling applications (Fig. 1e) was recently presented (Gillings and Larsen 2005). The cavity is currently integrated as one of several modular units in a system for performing typical operations in carbon-11 and fluorine-18 radiosyntheses, but will soon also be available as a stand-alone system (P. Larsen, personal communication). All of these cavities except the first Microwell 10 have either standard or optional devices for monitoring and optimizing conditions in the sample (see Sect. 9.3).

As mentioned previously, the geometry of the field differs in each individual microwave device. Inter-laboratory implementations of a reported procedure will therefore be simplified by either calibrating for possible differences between the equipments used or by measuring conditions in the sample so they can be recreated in an apparatus-independent

fashion. To our knowledge, there has only been one direct comparison of the performances of different monomodal microwave cavities in PET radiolabelings: that of the RI Model 520A, the CEM Discover and Labwell's Microwell 10 in a seven-step procedure for synthesizing [^{18}F]FDG (Nickles et al. 2003). The total production times were found to be comparable using the three instruments (52±2 min) and the radiolabeling results obtained were generally the same, regardless of which of four operators performed the procedures (nine syntheses each). However, 20% more [^{18}F]FDG was obtained when using the RI instrument. This difference was interpreted as being due primarily to two features of that cavity: (1) it was fitted with an up/down stage that allowed variation of the depth of the vessel in the cavity, which can be important when the volume of a sample varies during, for example, evaporation and (2) it is so compact that the operator could easily see what was happening in the vessel so the microwave treatment could be adjusted if, for instance, bumping occurred during the heating. The large improvement in yields with the Model 520A motivated subsequent in-house modifications of the Discover and the Microwell 10 to improve visibility in the cavity during the synthesis and to remotely readjust the positioning of the vessels in the field maximum.

The size of the microwave equipment continues to be a determining factor for how feasibly the technique can be integrated into PET radiolabeling procedures. For example, a rapid and high-yield microwave-assisted preparation of 4-[^{18}F]fluorobenzoic acid was developed to improve the synthesis of *N*-succinimidyl-4-[^{18}F]fluorobenzoate (Wüst et al. 2003). In a subsequent study, however, it was reported that, although the microwave-based procedure was better, it had been discontinued due to difficulties in fitting the microwave equipment into the automated synthesis equipment used for the entire radiosynthesis (Mäding et al. 2005). The compactness of the RI cavities is not only an advantage considering current trends toward using smaller hot cells, but also for its compatibility with other radiosynthesis devices (Fig. 1b). The fact that two recently reported module-based radiosynthesis systems (Gillings and Larsen 2005; Bonasera et al. 2005) have been dimensioned for including microwave cavities is a recognition of the increasingly important role that microwave dielectric heating is playing in PET radiolabeling.

9.3 Monitoring While Microwaving

It has been observed that radiolabelings using microwave heating can be more reproducible than the corresponding procedures performed with conventional heating (e.g., Taylor et al. 1996; Ponde et al. 2004). A major reason for this is that the reaction components are rapidly and uniformly heated throughout – *as soon as the field is turned on.* Likewise, when the field is turned off, the reaction components are no longer excited and the sample can be rapidly cooled, sometimes with the help of pressurized gas. Such fine control over the exact amount and duration of heating can be an important advantage when trying to force very activated substrates to react in ultra-short times, as is generally desired in PET radiolabeling.

Identifying the optimal microwave conditions requires that the chemist understands how to best take advantage of the characteristics of a particular single-mode cavity and which parameters to iteratively change when optimizing a given reaction. Achieving reproducibility requires that you can furthermore recreate those conditions in experiment after experiment. Both optimizing yields and standardizing conditions *in* the sample can be facilitated by the monitoring devices, which are now standard features of newer, commercially available microwave devices.

The temperature inside a vessel in a microwave field is most easily monitored by calibrating, and thereafter using an infrared measurement of the outside temperature of the reaction vessel. This is the technique most commonly used in the commercially available cavities today. To accurately measure the temperature inside the vessel during treatment, a microwave-transparent fiberoptic probe can be placed directly in the sample (Brown et al. 1996). For approximate measurements of relative, total changes in conditions, a thermocouple can be inserted into the sample as soon as the microwave is turned off (Taylor et al. 1996).

Measuring the pressure in closed vessels can not only be useful for maximizing the heating, but may also be necessary to avoid exceeding the pressure tolerances of the vessel when heating highly microwave-susceptible liquids. The measuring devices that have been used vary from pressure gauges simply connected via Teflon or peek tubing through septum-capped Pyrex vessels (Thorell et al. 1995) to those connected to acid digestion bombs capable of withstanding many atmospheres overpressure (Zjilstra et al. 1993) It is, however, important to realize

that if lines are left in the vessel during the microwave heating, there is a risk that the sample (and microwaves) may leak through the septum and tubing unless there is an appreciable head space above the microwave field for cooling. Sophisticated equipment such as that in Fig. 1c and d monitor the distension of the closed septum to estimate the pressure generated inside the vessel.

There is an added advantage if the monitoring read-out can be used to on-line adjust the input power so as to fine-tune for variations in the sample's response to the treatment during and between experiments. For example, as the amount of sample decreases during evaporation, it may be necessary to reduce the input power to avoid overheating. Also, if repositioning cannot be performed to keep the decreasing sample in the same relative field intensity, the input power may instead be varied to maintain the desired temperature. In some cases, the heating profile may completely change as the reaction progresses if the microwave susceptibility of the starting materials and products differ greatly and these are the major components in the sample. By continuously monitoring, e.g., the temperature in the sample, these types of phenomena that are special for microwave heating can be more readily detected and managed.

9.4 The Experimental Parameters

The conditions used in microwave chemistry can differ considerably from what is customary in conventional heating. An awareness of these differences and how to use them to your advantage is important for successfully implementing microwave dielectric heating.

9.4.1 Vessels

The commercial vendors of single-mode devices commonly supply their own custom-designed vessels that are optimized for the geometry of their cavity or they specify which types of geometries are recommended for the sample sizes and types to be heated. The reason is that the results obtained are directly dependent on how well the sample is positioned in the maximum field intensity. Aside from the dimensions that are accepted, the other basic requirement is that the vessel is essentially microwave-transparent. Borosilicate glass is the most commonly used

material and it is customary to fit the vessels with some kind of means of releasing pressure, in case the pressure tolerances are exceeded. The walls may be quite thick to accommodate higher pressures and the closures can vary from screw caps with septa to crimp-seals. Although Teflon does not absorb microwaves, it is not entirely inert to many of the solvents/reagents used at the high temperatures attainable with microwave heating and should therefore be avoided. At least one study has indicated that using the different vessels permitted or required with different cavities can negatively influence the radiochemistry performed (Nickles et al. 2003).

9.4.2 Atmospheric or Overpressure

In liquid-phase microwave reactions, solvents are often heated many degrees higher than their normal boiling points. At atmospheric pressure, i.e., in open vessels, higher boiling and less volatile organic solvents such as DMSO, *N*-methyl-2-pyrrolidone, 1,2-dichlorobenzene, ethylene glycol and DMF may be used as long as the other components are equally non-volatile. Samples that are only slightly microwave susceptible may also be heated in open vessels. Using open vessels in radiolabeling can be convenient since the problems of adding or removing reagents and opening or closing the vessel while maintaining good radiation protection are minimized. Open vessels have, for example, been used successfully in the microwave-induced nucleophilic fluoridations of substituted pyridines in DMSO (e.g., Dolci et al. 1999; Karramkam et al. 2003b).

In closed vessels that can tolerate higher pressures, the boiling point elevation would be much higher than for a sample heated at atmospheric pressure – sometimes as much as 50–100 °C higher. A radiolabeling, which under conventional heating requires 30 min at 90 °C, would then require less than 1 min at 150 °C. Thus lower boiling (more readily removed) solvents such as acetonitrile can be used, and still achieve the very short reaction times possible when running the reactions at very high temperatures. If the pressure build-up is too high with these solvents, a higher-boiling but otherwise similar solvent can be used to reduce the risks for vessel rupture (for example, use $ClCH_2CH_2Cl$ instead of CH_2Cl_2 and $CH_3CH_2CH_2OH$ instead of CH_3CH_2OH or CH_3OH).

9.4.3 Input Power and Time

The time and degree of heating are mostly limited by how well the vessel can contain the sample at the conditions of temperature and pressure generated and what the components (starting materials as well as products) can withstand without degrading. Since the components are heated extremely rapidly in microwave cavities, reactions can be started immediately at higher temperatures than is feasible with conventional bulk heating. The heating stops as soon the microwave energy is turned off and the temperature may be rapidly decreased by forced cooling. The ability to perform reactions in these sometimes short time windows between production and destruction is one of the main reasons for the remarkable results often obtained with microwave heating. Not only can higher yields be obtained but also cleaner reaction mixtures, which will facilitate purification procedures. Moreover, recent studies have indicated that it can also be advantageous to simultaneously cool the bulk temperature of the sample *during* the microwave treatment (e.g., Chen and Deshpande 2003). This would allow a higher total input of microwave energy, which can be needed when performing reactions with high activation energies.

9.4.4 Stirring

If microwave-absorbing solids are absorbed on or sedimented near the vessel walls, a highly localized heating may occur and can cause the vessel to rupture. Some of the more sophisticated microwave systems are equipped with magnetic stirring features. Often, though, it may be sufficient to vortex or otherwise swirl the sample immediately prior to placing the vessel in the cavity and then to quickly heat to reflux to keep the heterogeneous mixture stirred. Boiling chips (Taylor et al. 1996) and glass beads (Ponde et al. 2004) have also been added to the vessels to minimize bumping and to aid in the heat distribution during microwave treatment.

9.4.5 Sample Components

Both the ability of the media to be polarized by the field and the efficiency with which the electromagnetic energy can be converted into heat will

determine the degree to which the sample will be heated by microwave treatment. Since almost all non-radioactive components are in excess of the labeling agent in PET radiochemistry, the microwaving conditions will therefore most often be fine-tuned using the properties of sample components other than the one whose conversions are to be optimized.

9.4.5.1 Solvents

Properties determining the extent to which solvents interact with the electromagnetic field have been discussed in depth elsewhere (Gabriel et al. 1998). Briefly, polar solvents such as DMSO, DMF, alcohols, amines, and H_2O are microwave-susceptible and heated more or less rapidly, while solvents such as ethers, chlorinated hydrocarbons, and hydrocarbons are slightly or not at all heated. However, the latter can also be heated to high temperatures if other more microwave-susceptible materials are added (see, e.g., Sects. 9.4.5.3 and 9.5.4). If the temperature achieved with a given solvent is not high enough, it might be better to use a higher-boiling homolog.

Ionic liquids, which have high dielectric constants, low vapor pressures and good thermal stability, are interesting alternatives to dipolar aprotic solvents in microwave syntheses. As extremely microwave-susceptible media, they can be instantaneously heated to very high temperatures. Alternatively, small amounts can be added to less polar solvents to dramatically increase their heating temperatures. For example, in one report the microwave heating in sealed vessels of hexane was increased from 46 °C to 217 °C and of toluene from 109 °C to 195 °C (Leadbeater and Torenius 2002). However, concerns about possible side reactions that might label the ionic liquids and their documented decomposition at high microwaving temperatures should be considered before using them in radiolabeling procedures.

9.4.5.2 Reagents

The microwave susceptibility of the reagents used may be just as important as their chemical reactivity. For example, the use of metal agents may be problematic, ionic reagents may absorb too much energy too rapidly and apolar reagents must rely on other components for the heat-

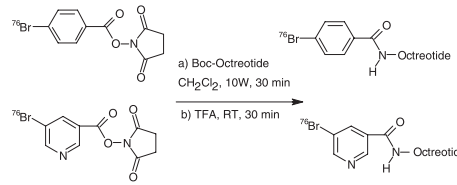
ing process. Therefore, the contribution of the reagents' concentrations and volatility to the sample's bulk properties must be optimized (see, e.g., Sect. 9.5.5).

9.4.5.3 Salts

Non-reacting salts may be purposefully added in order to change the dielectric properties of the sample. The coupling with the electromagnetic field is increased and consequently the heating will be more rapid and more effective. Salt additions have been utilized for that purpose to improve the yields in ^{11}C-cyano-de-chlorinations (Thorell et al. 1992), *N*-alkylations with ^{18}F-labeled alkyl halides (Zjilstra et al. 1993) and *N*- and *O*-alkylations with [^{18}F]β-fluoroethyl tosylate (Lu et al. 2004) – see Sect. 9.5.4. The increases in the ionic strength increased the absorption of the microwave energy, which led to higher reaction temperatures at the higher pressures contained in the vessels, even though the solvent in the ^{18}F-labelings was the relatively low-boiling acetonitrile.

9.4.5.4 Substrates

The polarizability of substrates and therefore their disposition for certain reactions may be enhanced by the oscillating electromagnetic field. In PET the substrates are also often present in relatively low concentrations. It might therefore also be feasible to use a less common but interesting special microwave heating approach – to selectively heat the microwave-susceptible substrates while using a solvent or medium that is poorly or not at all heated. This type of selective substrate heating may have contributed to the results in the ^{76}Br-prosthetic group labeling of the *N*-terminal of octreotide (Scheme 1) (Yngve et al. 2001). The solvent used (CH_2Cl_2) has a low microwave susceptibility, while the reactants are polar molecules and would be expected to couple more readily with the electromagnetic field. The authors used a very low input power for a relatively long exposure (30 min) to achieve radiolabeling yields of nearly 50%. All other conventional and microwave conditions attempted were unsuccessful. Selective heating may thus offer a means to avoid high bulk temperatures while still heating the substrates, which

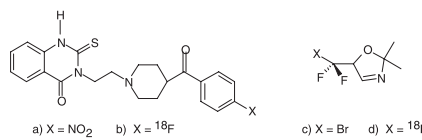


Scheme 1.

can be important when dealing with thermally labile substrates like peptides and proteins.

9.4.6 Concentrations of Starting Materials

Using a large excess of non-radioactive starting materials compared to the fluorinating reagent is typically used to help drive radiolabeling reactions toward completion. However, in the $^{18}F^-$ for NO_2^- substitution yielding [^{18}F]altanserin (Scheme 2a, b), consistently higher yields could be obtained with lower amounts of starting materials when using microwave treatment instead of conventional heating (Lemaire et al. 1991). Precious starting materials were saved and difficulties in isolating the product from the structurally similar starting materials and side-products that arose during prolonged heating were reduced. Similarly, in a fluorodebromination performed on a polyfluorinated oxazoline (Scheme 2c, d), it was observed that less starting material and shorter reaction times could be used during microwave heating (Johnström and Stone-Elander 1996). Fluorine-containing substrates can decompose in polar media and release $^{19}F^-$, which can compete with $^{18}F^-$. Thus the microwave conditions improved not only the time for the reaction, but also the radiolabeling yields and the specific activity of the product. The possibility



Scheme 2.

of being able to vary the amount of substrate over a wider range without appreciable decreases in yields can therefore have a number of practical implications for PET radiolabelings.

9.5 Microwaving in Fluorine-18 Radiochemistry: Selected examples

The use of microwave dielectric heating in radiolabeling applications has been thoroughly reviewed previously (Elander et al. 2000; Jones and Lu 2002; Stone-Elander and Elander 2002). The examples discussed below have been chosen because they illustrate certain aspects of typical procedures in radiofluorinations. When possible, an effort has been made to highlight observations in newer papers that have been published since 2002. For a more detailed examination of the literature, the reader is referred to the previously published reviews.

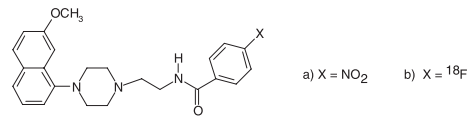
9.5.1 Evaporation of Target Water/Solvents

The particular considerations associated with decreasing the sample volume and adjusting the microwave conditions as the load changes have been alluded to in the discussions above. Heating with microwaves is dependent on having something in the sample that will absorb the microwaves and that something is most often the solvent. It will therefore be necessary to adjust the input power as the solvent decreases. This is more easily achieved using the cavities equipped with the on-line monitoring discussed previously. Alternatively, a combination of scintillators collimated to the bottom reaction zone of the vessel, an open construction or cameras that allow the operator to follow the progress of the evaporation, along with mechanisms for adjusting the position of the vessel in the microwave field have been reported to be important features needed for achieving controllable heating (Nickles et al. 2003). In several studies, microwave heating has been used to evaporate the target water and azeotropically dry the $^{18}F^{-}$ residue (for example, Taylor et al. 1996; Monclus et al. 1998; Wüst et al. 2003; Nickles et al. 2003; Hamill et al. 2005), The large majority of procedures, however, still use conventional heating.

9.5.2 Nucleophilic Aromatic Substitutions

Probably the most common route for introducing fluorine is through nucleophilic aromatic substitutions using [^{18}F]F^{-}, crown ether or phase transfer catalysts and polar, aprotic solvents. Depending on the degree of activation of the substrate, conventional heating can require 10–30 min at temperatures up to 180 °C. Microwave dielectric heating considerably reduces the reaction times for nucleophilic aromatic fluorinations to 15 s up to 5 min, depending on the equipment and substrate.

The leaving groups most optimal for microwave procedures are often, but not necessarily, the same as in conventional heating. [^{18}F]Fluoro-for-nitro substitutions are commonly used (see examples reviewed in Stone-Elander and Elander 2002) and reaction times can be considerably reduced with microwave treatment, even when the sample temperature is the same as that generated with conventional heating (Scheme 3a, b) (Lu et al. 2005).

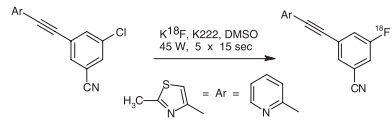


Scheme 3.

The [^{18}F]fluoro-for-$^{+}$NMe$_3$ exchange can also work well (e.g., Stone-Elander and Elander 1993; Hostetler et al. 1999; Dolci et al. 1999; Wüst et al. 2003), although a competing reaction with the alkyl side chain producing labeled gases has been reported (Banks et al. 1994). It is also important not to overheat these substrates, since they, similar to the behavior of ionic liquids, are strongly affected by the electromagnetic field and can decompose.

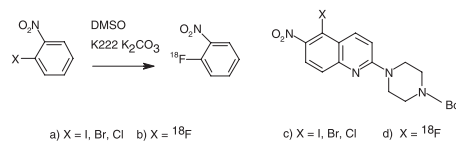
[^{18}F]Fluoro-to-halogen substitutions are becoming increasingly popular. For example, in a recent paper describing several strategies for labeling ligands for metabotropic glutamate receptors (Hamill et al. 2005), the argument was made that being able to use aryl chlorides as precursors is attractive since they are generally more readily available than the corresponding nitro substrates. Sufficient yields of the ^{18}F-labeled compounds (Scheme 4) for the planned animal validation studies were

obtained from the chlorides by using short microwave pulses with 15 s pauses in between. If the in vivo results call for scaling up to larger amounts of a particular ligand, then synthetic efforts can be more rationally used to synthesize that compound with a better leaving group for the nucleophilic substitution.



Scheme 4.

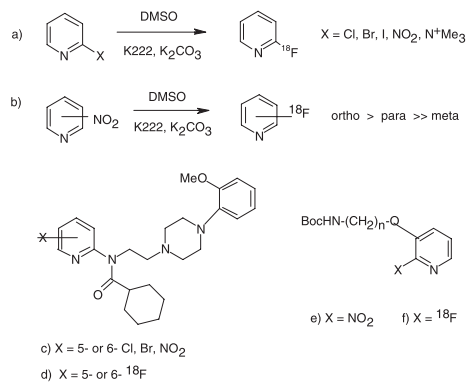
Achieving selectivity when several potential leaving groups are present in the starting material can be a concern in substitution reactions (Stone-Elander and Elander 1991). In a recent model reaction of [^{18}F]fluoro-for-halo substitutions in nitro-containing benzenes (Scheme 5a, b), only the halide was displaced and only the chloride was found to be reactive, with up to 70% yields obtained with microwaves (100 W, 150 s) or 48% with conventional heating (145 °C, 300 s) (Karramkam et al. 2002b). These reactions were subsequently extended to more complicated substrates in the synthesis of 5-[^{18}F]fluoro-6-nitroquipazine (Scheme 5c, d) (Karramkam et al. 2002a). In this case 20% (50 W, 30 s) to 30% (145 °C, 3 min) incorporation of [^{18}F]F$^-$ was obtained with both the chloro- and bromo-derivatives. Higher temperatures or higher input caused decomposition of both substrates. Selectivity of [^{18}F]fluoro-for-halogen vs. [^{18}F]fluoro-for-nitro substitution was observed for both substrates and both modes of heating.



Scheme 5.

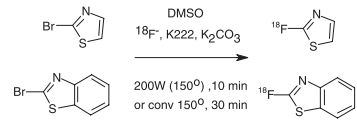
The [^{18}F]fluorinations of substituted heteroaromatics (pyridines) with microwave and conventional heating have been investigated (Dolci et al. 1999; Karramkam et al. 2003b). Comparable yields were obtained faster

with microwave (100 W, 1–2 min) than with conventional (145–180 °C, 10 min) heating for the *ortho*-bromo, the *ortho*-trimethylammonium and the *ortho*- and *para*-nitropyridines (Scheme 6a, b). These studies were then successfully applied in the preparation of 6-[^{18}F]fluoro-WAY-100635 from the corresponding 6-bromo precursor (Scheme 6c, d) (conventional heating 145 °C, 10 min) or from the corresponding 6-nitro precursor (microwave 100 W, 1 min) (Karramkam et al. 2003a). Short reaction times were important since prolonged heating resulted in degradation of the product 6-[^{18}F]fluoropyridine. Substitution at the *meta* position was not successful in any of these syntheses. In a further *ortho*-fluorination of a pyridine substrate, [(2-[^{18}F]fluoropyridin-3-yloxy)alkyl]carbamic acid tert-butyl ester (Scheme 6e, f) required conventional heating at 145 °C for up to 30 min or microwave heating with 100 W for up to 3 min (de Bruin et al. 2005). These examples also illustrate that model reactions of simple molecules can be useful for identifying the labeling conditions for single-step radiolabelings of complex substrates.



Scheme 6.

In a recent preliminary communication (Simeon and Pike 2005), 1,3-thiazoles were radiofluorinated in a one-step [^{18}F]fluoro-for-bromo substitution (Scheme 7). Microwave heating gave higher yields in one third the time required with conventional heating, even though the same temperature (150 °C) was maintained. These [2-^{18}F]1,3-thiazoles may



Scheme 7.

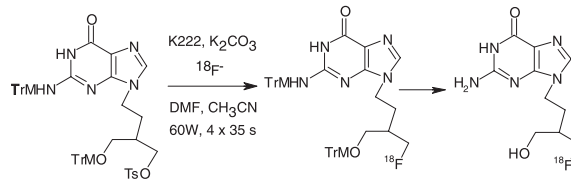
provide a labeled correlate for the more difficultly labeled pyridinyl rings of many drug candidates.

Nucleophilic aromatic substitutions can require 25%–50% of the total time for many tracer productions using conventional heating. Reducing time in this one step can therefore be important not only for the yields and specific activities of the products but also for scheduling and turn-around time, which explains why this application of dielectric heating has so frequently been the focus of PET microwavers.

9.5.3 Nucleophilic Aliphatic Fluorinations

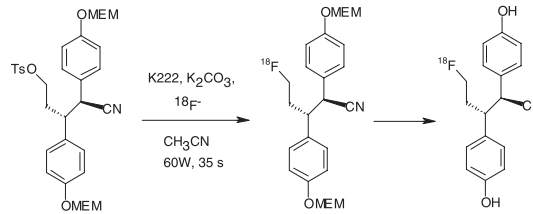
Nucleophilic aliphatic fluorinations, as in aromatic substitutions, may be used in one-step radiolabeling procedures with appropriate substrates containing typically a sulfonate ester or halide leaving group. Competing eliminations or other degradations due to the basicity of the media or the high temperatures and long reaction times may limit the yields obtained. In many cases microwave heating can reduce the heating times while achieving comparable or better yields and often in cleaner product mixtures. Several new one-step aliphatic radiofluorinations that illustrate these advantages have recently been reported.

In the first step of the synthesis of 9-(4-[^{18}F]fluoro-3-hydroxymethyl-butyl)guanine ([^{18}F]FHBG), the microwave-assisted [^{18}F]fluoro-for-tosyl substitution (Scheme 8) in either DMSO or DMF:CH_3CN (1:1) was found to give superior yields (30%–40% vs. 4%–5%) and considerably better reproducibility than the corresponding procedure with conventional heating (Ponde et al. 2004). Using the DMF:CH_3CN mixture allowed the subsequent hydrolysis step to be performed in the same pot, without the intermediate solvent removal that had been required for DMSO.



Scheme 8.

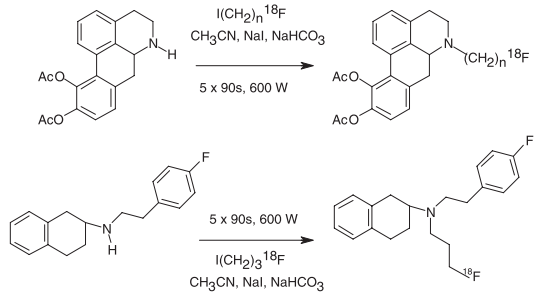
Another [^{18}F]fluoro-for-tosyl substitution, in the synthesis of an estrogen receptor β-selective ligand (Scheme 9), was successfully achieved with microwave heating (Yoo et al. 2005). The very short heating times that could be used in microwave heating were a deciding factor since the tosyl precursor was very sensitive to base and degraded quickly with longer or repeated cycles of heating.



Scheme 9.

9.5.4 Alkylations with Labeled Precursors

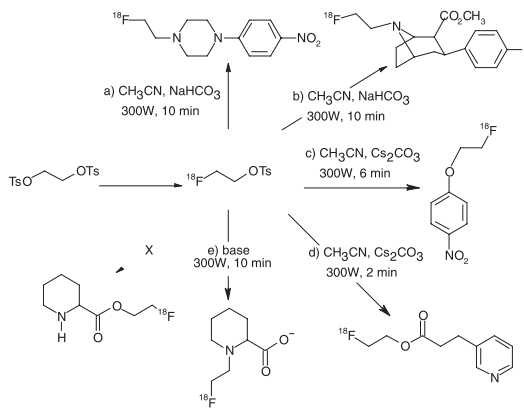
An alternative method of introducing fluorine into side chains of target molecules involves first synthesizing the precursors, fluoroethyl or fluoropropyl halides or sulfonates, for subsequent alkylations of amines, alcohols or thiols. However, these alkylations can be difficult. Advantages of microwave techniques for performing this type of reactions have been convincingly demonstrated in the *N*-alkylations of apomorphine and tetralin derivatives with [^{18}F]ethyl iodide and [^{18}F]propyl iodide (Scheme 10) (Zijlstra et al. 1993). Sodium salts were added to the samples dissolved in acetonitrile in digestion vessels to improve the microwave coupling. The pressure and the temperature increased



Scheme 10.

concomitantly to higher levels than had been achieved with conventional heating. The syntheses of these fluoroalkyl compounds in useful quantities and in reasonable reaction times by conventional heating were seriously hampered by their slow reaction kinetics and competing degradation.

Recently non-reacting Cs_2CO_3 and $NaHCO_3$ were added to fluoroethylations of amines (Scheme 11a, b), phenol (Scheme 11c) and carboxylate (Scheme 11d) performed with [^{18}F]β-fluoroethyl tosylate (Scheme 11) (Lu et al. 2004). Reaction temperatures of 150 °C were

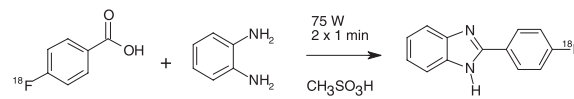


Scheme 11.

obtained even though the solvent used here was again the lower boiling and lower microwave-absorbing acetonitrile. The yields obtained were higher and the reaction times shorter than with the comparable, conventionally heated procedures: (a) 51% at 10 min vs. 5% at 25 min; (b) 78% at 10 min vs. 60% at 45 min; (c) 77% at 6 min vs. 59% at 10 min and (d) 89% at 2 min vs. 66% at 10 min. Furthermore, in labeling of pipecolinic acid, the [^{18}F]β-fluoroethyl tosylate reacted exclusively with the amino group (Scheme 11e) in > 90% yields after 10 min of microwave treatment.

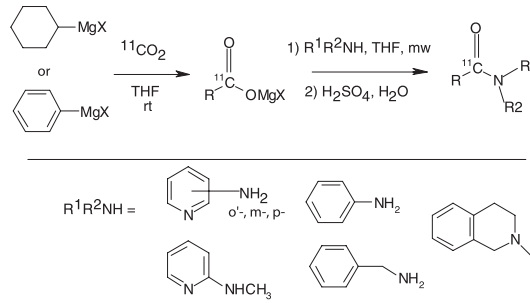
9.5.5 Condensations with Labeled Precursors

Condensations forming rings generally require high temperatures and can be difficult to accomplish in the time available in PET radiolabeling procedures. Scheme 12 shows the use of microwave dielectric heating to accelerate the formation of a heteroaromatic ring by the cyclocondensation of 1,2-diaminobenzene with [4-^{18}F]fluorobenzoic acid (Getvoldsen et al. 2004). The mineral acids HCl and H_2SO_4 are typically used when performing these syntheses with conventional heating. Due to the danger of their runaway heating by microwaves and explosions (Kingston and Haswell 1997; Thorell et al. 1995) the higher boiling methanesulfonic and polyphosphoric acids were instead used. High temperatures (> 200 °C) could be rapidly achieved without containment problems and the 2-([4^{18}F]fluorophenyl)benzimidazole was obtained in 50% conversions after only 2 min.



Scheme 12.

Amide formations by the condensation of primary and secondary amines with in-situ generated carbon-11-labeled carboxymagnesium halides (Scheme 13) have been accelerated with microwave heating (Lu et al. 2003). Comparisons made at two temperatures (70 and 100°C) showed that microwave heating gave nearly twice as much product as the corresponding conventionally heated synthesis. Furthermore, even

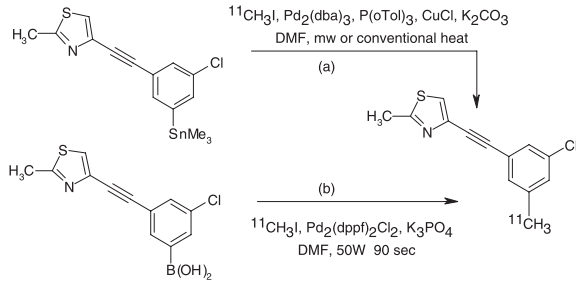


Scheme 13.

higher reaction temperatures (up to 130 °C) could be achieved with microwaves, even though the boiling point of the solvent, THF, is only 67 °C. Both aromatic and aliphatic amides were successfully synthesized. The method was more successful with primary than secondary amines. Without the microwave reactor, control of the reaction at the desired temperature was difficult to achieve. This procedure gives useful amounts of products in times that are practical for carbon-11 chemistry and can of course potentially be extended to procedures using fluorine-18-labeled acids or amines.

9.5.6 Carbon–Carbon Coupling Reactions with Potential for Fluorine-18 Labeling Applications

Transition-metal-catalyzed carbon–carbon coupling reactions offer attractive routes for building drug libraries. Though these reactions are widely used in medicinal chemistry, there are only a few reports of their uses in PET radiolabeling. Recently a comparison of the microwave-induced Stille and Suzuki couplings of the appropriate aromatic substrates with [^{11}C]CH_3I was reported (Hamill et al. 2005). Both routes gave the desired product (Scheme 14), but the Suzuki coupling (b) gave higher yields and had the additional advantage of using a commercially available stable catalyst. The yields obtained were approximately 30% (uncorrected at EOS) and the total synthesis time for the procedure was only around 20 min. The extension of these findings to, e.g., ^{18}F-labeled halides could facilitate new radiolabeling strategies (Wüst et al. 2005).



Scheme 14.

9.6 In Summary

Microwave heating allows us to sometimes do what cannot be achieved by other methods in the time frame we have available in PET radiochemistry. The large amounts of energy that can be applied can force difficult reactions to go more quickly. The rapidity with which the energy can be added and removed can further favor desired over undesired reactions. The advantages of saving time in reactions with short-lived radionuclides, which are disappearing the whole time you are working with them, are unique in microwaving chemistry. The availability of process-conforming equipment and the growing understanding of the mechanisms behind microwave dielectric heating will continue to contribute its increasing use to facilitate and diversify PET radiochemistry.

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10 Micro-reactors for PET Tracer Labeling

S.Y. Lu, V.W. Pike

Abstract. Miniaturization of PET radiosynthesis devices (micro-reactors or microfluidic systems) is an emerging area that has the potential to deliver many advantages, such as more efficient use of hot-cell space for production of multiple radiotracers; use of less non-radioactive precursor for saving precious material and a reduced separation challenge; highly controlled, reproducible and reliable radiotracer production; and cheap, interchangeable, disposable and quality-assured radiochemistry processors. Several 'proof of principle' examples along with basics of micro-reactor flow control, mixing principle and design, and device fabrication are discussed in this chapter.

10.1 Introduction

Positron-emission tomography (PET) is a radiotracer imaging modality that is used to provide quantitative information on physiological and biochemical phenomena in animals and human subjects in vivo (Phelps and Mazziota 1985; Phelps 1991, 2000). As such, PET is highly valuable for clinical research (Wagner 1991) and drug development (Comar 1995; Pike 1997; Burns et al. 1999). The biochemical scope and specificity of PET is determined by the available array of positron-emitting radiotracers (Iwata 2004), which are generally labeled with positron-emitters such as carbon-11 ($t_{1/2}$ =20.4 min) or fluorine-18 ($t_{1/2}$ =109.8 min) at high (no-carrier-added; NCA) specific radioactivity.

Because of their short half-lives, these radiotracers must be produced as needed from cyclotron sources of carbon-11 and fluorine-18, which are usually [^{11}C]carbon dioxide, [^{11}C]methane and [^{18}F]fluoride ion (Fowler and Wolf 1982, 1997, Stöcklin and Pike 1993; Elsinga 2002; Welch and Redvanly 2003). [^{11}C]Carbon dioxide and [^{18}F]fluoride ion may be used directly in labeling reactions. Otherwise, primary cyclotron products are converted into useful secondary labeling agents, such as [^{11}C]iodomethane (Crouzel et al. 1987; Larsen et al. 1997; Link et al. 1997) or [^{18}F]2-fluoroethyl tosylate (Block et al. 1987; Studenov and Berridge 2001). In a typical labeling procedure, a very large (10^2 to 10^4-fold) excess of the non-radioactive reactant (precursor) is used to promote rapid and efficient incorporation of the radioisotope into the target radiotracer. The reaction volume is typically 0.3–1.0 ml with the vessel sealed and heated. These conditions necessitate rapid separation of a low quantity of radioactive product ($\sim$ μg) from a large excess of unreacted precursor ($\sim$ mg) that is usually achieved with single-pass high-performance liquid chromatography (HPLC) on a semi-preparative size column. Moreover, in practice, efficient transfers of radioactive product to HPLC are not always possible, since they may require intervening concentration of the reaction mixture by evaporation or solid-phase extraction. In addition, the reliable and regular production of PET radiotracers must be performed in a lead-shielded hot-cell with remotely controlled and preferably automated equipment that is capable of (a) synthesis of the radiotracer from high initial levels of radioactivity (up to 5 Ci; 1 Ci=37 GBq), (b) purification of the radiotracer and

(c) formulation of the radiotracer for intravenous injection, all within only two to three half-lives of the radioisotope.

Micro-reactor devices (Ehrfeld et al. 2000; Fletcher et al. 2002; Jähnisch et al. 2004), consisting of a network of micron-sized channels (typical dimensions in the range 10–300 μm), filters, separation columns, electrodes and reaction loops/chambers etched onto a solid substrate, are now emerging as an extremely useful technology for the intensification and miniaturization of chemical processes. The ability to manipulate, process and analyze reagent concentrations and reaction interfaces in both space and time within the channel network of a micro-reactor provides the fine level of reaction control that is desirable in PET radiochemistry practice. In this chapter, we will show through our research, as well as contributions from elsewhere, that miniaturization of radiosyntheses with carbon-11 or fluorine-18 could lead to several benefits such as the use of less material (especially precursor, which may be precious or difficult to obtain) and easier and faster purification with greater conservation of radioactive product and its specific radioactivity. Basic principles and design of the micro-reactors are also discussed.

10.2 Micro-reactors for Synthetic Chemistry

Many widely used organic reactions have been successfully performed in micro-reactors and the benefits of micro-reactors to synthetic chemistry convincingly demonstrated (DeWittt 1999; Fletcher et al. 2002; Watts and Haswell 2003; Jähnisch et al. 2004; Feng et al. 2004; Cullen et al. 2004; Yoshida 2005). Purer products, higher yields, shorter reaction times than for corresponding batch reactions, and more benign reaction conditions are some of the major advantages of this technology. In some cases, enantioselectivity and regioselectivity are also enhanced. The reactions are carried out on nano- to micro-molar scale, but in sufficient quantities to perform full characterization, also with the capability to expand the production capacity by adopting parallel (intensification) procedures. There is no doubt that micro-reactor chemistry has great promise as a foundation for new chemical technology and processes.

Micro-reactors can be fabricated from polymers, metals, quartz, silicon or glass. A wide variety of fabrication methods are available so that an informed choice can be made based on considerations, such as the

nature of reaction to be performed, materials choice, process cost, reliability, accessibility etc. (Harrison et al. 1993; Jakeway et al. 2000; Becker and Lacascio 2002; Reyes et al. 2002; Huikko et al. 2003; Stroock and Whitesides 2003; Vilkner et al. 2004; Fiorini and Chiu 2005). For example, glass is often the first choice of chemists because it is mechanically strong, compatible with a huge range of chemicals and solvents, optically transparent and supports different operation modes. Alternatively, polymers, especially poly(dimethylsiloxane) (PDMS) (McDonald and Whitesides 2002), give great flexibility in fabrication and valve control ability, but applications in such devices are restricted to those not involving organic solvents that cause swelling or damage.

Micro-reactors can be coupled to several separation and detection methods for 'on-chip' or 'post-chip' separation and analysis of products. Among these, the 'on-chip' capillary electrophoresis (CE) separation and 'post-chip' HPLC separation are most useful (Auroux et al. 2002).

The advantages of micro-reactors derive from their physical and geometrical properties. Each property and its benefit are summarized in Table 1. Some important aspects of micro-reactor operation, such as (a) operation control methods and (b) mixing principles and design, will be discussed in detail in the following sections.

10.2.1 Hydrodynamic vs. Electrokinetic Flow Control

For solution-based chemistry, the channel networks are connected to a series of reservoirs containing chemical reagents to form the complete device with overall dimensions of a few centimeters. In the micro-reactor, reagents can be brought together in a specific sequence, mixed and allowed to react for a specified time in a controlled region of the channel network using hydrodynamic or electrokinetic pumping (Fig. 1). Hydrodynamic pumping exploits conventional or micro-scale pumps, notably syringe pumps, diaphragm pumps or an inert gas manifold, to maneuver solutions around the channel network. Other micro-pump concepts and systems are being developed and one day may be useful in micro-reactors for chemical synthesis (Nguyen et al. 2002; Laser and Santiago 2004; Woias 2005). Electrokinetic [electroosmotic (EOF) and electrophoretic] systems (Gawron et al. 2001; Ghosal 2004) use the sequential application of voltages through electrodes that are located

Table 1. Key micro-reactor properties and their benefits

	Properties	Characteristics and benefits
1	Compact linear dimension	Typical channel cross-section widths range between 50 and 500 μm. Total dimension is less than several cm^2. Heat transfer, mass transport, diffusion rate are improved. More control at reaction temperature, concentration, density and pressure are attained.
2	Small reactor volume	The internal volume ranges from 1 to 10s of μl. Minimal amounts of material are used, hold-up is small, and process safety is increased. Short residence time leads to improved selectivity, purity and higher yields.
3	High surface to volume ratio	Specific surface of a microchannel is in the range of 10,000 to 50,000 m^2m^{-3}, compared to 100 to 1,000 m^2m^{-3} in a conventional reaction vessel. This results in more efficient heat transfer and better catalytic properties.
4	High specific phase interface	Specific phase interfaces are between 5,000 and 50,000 m^2m^{-3} for liquid mixer, and up to 2,000 m^2m^{-3} for gas-liquid mixer, which promotes better mixing.
5	Short mixing time	Diffusion-based mixing time is in the ms to s range. Residence time is also reduced to the range of s to min.
6	Rapid heat transfer	Heat-transfer coefficient up to 25,000 $Wm^{-2}K^{-1}$, is useful in exothermic or endothermic reactions, with improved safety.
7	Mode of flow	Laminar flow can be regulated with hydrodynamic or electrokinetic controls. Continuous flow improves processibility
8	Scope for parallel process and intensification	The reaction conditions can be quickly transferred to 'cloned' devices. Subunits can be quickly stacked up, and therefore adapted for high throughput or combinatorial synthesis.

in the appropriate reservoirs. It offers a simple but effective method of moving and separating reactants and products within a micro-reactor, without the need for moving parts.

When an electric field is applied across the channel length, solvent and any uncharged solutes move in an EOF having a velocity v_{eof} defined as in Eq. 1:

$$v_{eof} = \frac{E\varepsilon\varepsilon_0\zeta}{\eta} \tag{1}$$

where E is the electric field (voltage), ε is the dielectric constant of the liquid, ε_0 is the permittivity of free space and ζ is the zeta potential of

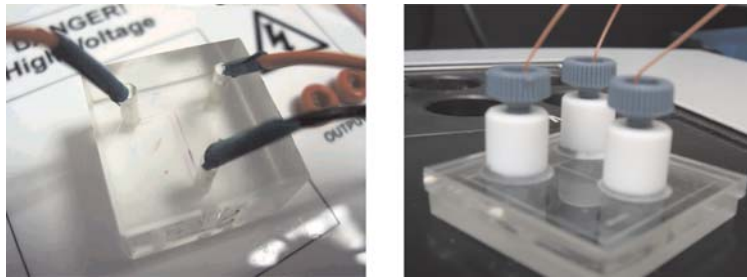


Fig. 1. Examples of an EOF-controlled (*left*) and a syringe pump-controlled (*right*) micro-reactor for organic syntheses in their simplest form

the channel wall–solution interface and η is the liquid viscosity. EOF is not obtained with semiconductor materials such as silicon and does not occur with low-polarity solvents such as alkane.

Within the electric field, charged solutes have an additional electrophoretic velocity v_{ph} which is given as in Eq. 2:

$$v_{ph} = \frac{zeED}{kT} \tag{2}$$

where z is the charge number on the species, e is the electronic charge, D is the diffusion coefficient of the species, k is the Boltzmann constant and T is the absolute temperature. The magnitude of v_{ph} for a typical micro-reactor operating voltage is commonly comparable with v_{eof}.

A comparison of characteristics and the advantages and disadvantages for the two operating modes are summarized in Table 2.

10.2.2 Mixing Principles and Design

Thorough and rapid mixing is prerequisite for reactions involving more than two reactants. Miniaturized mixing devices or micro-mixers can be generally divided into two major categories: passive micro-mixers and active micro-mixers (Nguyen and Wu 2005). The former do not require external energy and the mixing process relies on diffusion or chaotic advection only. The latter uses the disturbance generated by external forces which could be, for example, pressure, temperature, electrohydrody-

Table 2. A comparison of characteristics, advantages and disadvantages for hydrodynamic and EOF operating modes

		Hydrodynamic control	EOF control
1	Control method	Pressure through syringe pump etc.	Voltage
2	Materials restriction	Applicable to all liquids and micro-reactors	Only to polar and charged molecules. Micro-reactors need to be fabricated from materials with ionizable surface, such as glass. EOF does not work with semiconductor materials, such as silicon
3	Flow profile	High velocity in the channel center, slow velocity near the wall	EOF velocity is constant across the channel. Channel dimension must be sufficiently small to limit hydrodynamic flow
4	Flow rate	Wide range of flow rate from 0.1 to 100 $\mu l\,min^{-1}$	Usually with low flow rate such as $< 10\ \mu l\ h^{-1}$
5	Back pressure	High back pressure, especially with viscous liquids	Virtually no back pressure
6	Side reactions	None	Excessive voltage may cause electrochemical reactions
7	Automation	Involving more mechanical or moving parts, requires more space	Computerized voltage control; no other feeding tubes
8	In-chip separation of product	Impossible	Possible

namics, dielectrophoretics, electrokinetics, magnetohydrodynamics or acoustics.

There are two modes of mixing, having very different characteristics, that are used commonly in EOF micro-reactors for synthesis. The first is 'diffusive mixing' – two laminar flows of reagent streams inter-diffuse along the channel. Slow flow, which increases the contact time, promotes complete mixing. Alternatively, faster flows can be used with a smaller channel cross-section within extended channels to achieve the same level of mixing. The second is 'slug injection' – a slug of one reagent is generated within a flowing stream of a second reagent in a 'flow–inject–flow' sequence. Obviously a series of many narrow slugs is more

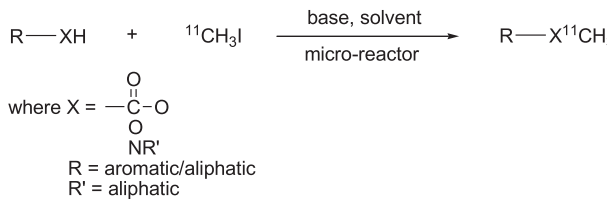
effective than a single long slug to achieve thorough mixing. Both modes are neatly illustrated with visually recognizable dye solution (Fletcher et al. 2002).

With hydrodynamically controlled micro-reactors for synthesis, again, the majority of mixing can be achieved through diffusion without any assistance of turbulence because the small channel dimensions form a laminar flow profile. Large contact surfaces and small diffusional paths, that can be obtained simply by dividing a main stream into many small sub-streams or reducing the channel width along the flow axis, are useful to promote rapid mixing (Ehrfeld et al. 2000). A number of other mechanisms [e.g., geometrical (Johnson et al. 2002; Ratner et al. 2005), mechanical (Seong and Crooks 2002; Nagasawa et al. 2005), thermal (Schwalbe et al. 2004), vibrational (Liu et al. 2003), or electrical] are used for designing new micro-reactor systems that are of enhanced performance.

10.3 Micro-reactors for PET Radiochemistry

The application of micro-reactors in radiochemistry is still in its infancy (Lucignani 2006). The limited number of published examples are mainly on reaction investigation or device design as proof-of-principle studies. Nevertheless, the applicability and benefits of this new technology to PET radiosynthesis have been convincingly demonstrated in some examples such as in the synthesis of NCA ^{11}C-labeled amines (Brady et al. 2003; Jeffery et al. 2004), NCA ^{11}C and ^{18}F-labeled carboxylic esters (Lu et al. 2004a, b) and [^{18}F]FDG (Brady et al. 2003; Gillies et al. 2005, 2006; Jeffery et al. 2004; Liow et al. 2005; Lee et al. 2005).

10.3.1 [^{11}C]Methylation in Micro-reactors

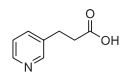
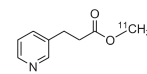
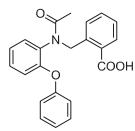
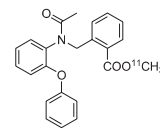
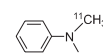
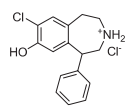
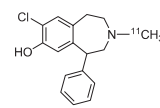
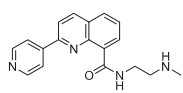
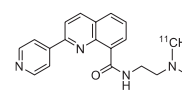


[^{11}C]Methylation is one of the most widely used labeling methods in PET radiochemistry (Bolton 2001) and was selected as a model reaction for investigation in micro-reactors.

[^{11}C]*O*-Methylation in a micro-reactor has been demonstrated in the synthesis (with iodomethane) and radiolabeling (with [^{11}C]iodomethane) of 3-(3-pyridinyl)propionic acid methyl ester (Table 3, entry 1) and a peripheral benzodiazepine receptor (PBR, Okubo et al. 2004) ligand (Table 3, entry 2) within a simple T-shaped micro-reactor (Lu et al. 2004a).

Reaction behavior in the micro-reactor was first investigated with non-radioactive labeling agent. For the reaction of 3-(3-pyridinyl)propionic acid (**1**) with iodomethane**,** the methyl ester was obtained at room tem-

Table 3. Examples of [^{11}C]methylation within micro-reactors

Entry	Precursor	Reaction conditions	Product	RCY (%)	Reference
1		Bu_4NOH, DMF, RT		≤ 88	Lu et al. 2004
2		Bu_4NOH, DMF, RT		≤ 65	Ibid
3		NaOH, acetone, RT		Not reported	Brady et al. 2003
4		Et_3N, acetone, RT		5–19	Ibid Jeffrey et al. 2004
5		Acetone, RT		10	Brady et al. 2003

perature (RT), and its yield increased with higher reagent concentration and with reduced infusion rate (Fig. 2). Reactions carried out with radioactive labeling agent followed the same trend. For example, at an infusion rate of 10 μl min^{-1} the [^{11}C]methyl ester was obtained in 56% decay-corrected radiochemical yield (RCY). When the infusion rate was reduced to 1 μl min^{-1}, the RCY increased to 88%. The lower infusion rate allows more time in the micro-reactor (residence time) for reactant mixing by diffusion, and for reaction. At 1 μl min^{-1} the residence is about 12 s and total processing time 10 min, the latter is comparable to reaction times currently used in PET radiotracer synthesis. With new micro-reactor designs it should be possible to achieve thorough mixing of reagents at higher infusion rates, further accelerating the process.

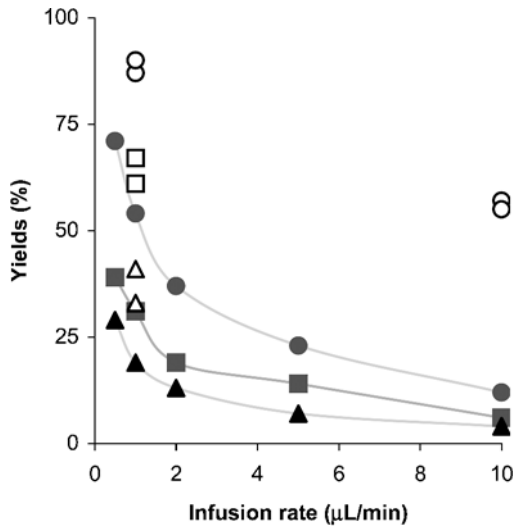
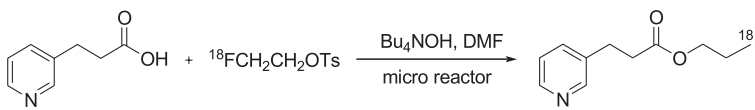


Fig. 2. Effect of infusion rate and reagent concentration on the yield of 3-(3-pyridinyl)propionic methyl ester from the reactions of **1** with MeI at 10 mM (●), 5 mM (■), 2 mM (▲) and RCYs of [^{11}C]3-(3-pyridinyl)propionic methyl ester from the reaction of acid **1** with [^{11}C]MeI at 10 mM (○), 5 mM (□) and 2 mM (△). All reactions were performed at RT. (*Lab Chip*, 2004, **4**, 523–525. Reproduced by permission of *The Royal Society of Chemistry*)

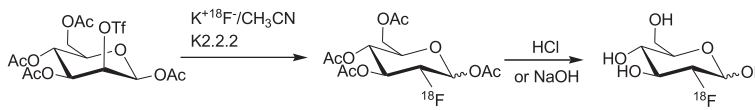
[^{11}C]*N*-methylations (Table 3, entries 3, 5) have been performed within a micro-reactor fabricated from glass (Brady et al. 2003). The methylation of *N*-methylaniline was the most difficult, requiring heating of the micro-reactor to obtain an amount of *N*,*N*-[^{11}C]dimethylaniline detectable with HPLC. A radiochemical yield for this reaction was not reported. Reaction of [^{11}C]iodomethane with the other two precursors resulted in 5–19 % RCYs at infusion rates between 10 and 100 μl min^{-1}. In the case of (*R*)-(+)-7-chloro-8-hydroxy-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine HCl salt, a brain dopamine D_1 receptor antagonist (Halldin et al. 1986) (Table 3, entry 4), excess triethylamine was needed for the methylation to take place, whilst in the case of 2-pyridine-4-yl-quinoline-8-carboxylic acid (2-methylamino-ethyl) amide, a potential tumor imaging agent (Osman et al. 2001) (Table 3, entry 5), no additional amine was required.

10.3.2 [^{18}F]Fluoroethylation in Micro-reactors



The synthesis of ^{18}F-labeled ester using 2-[^{18}F]fluoroethyl tosylate has also been investigated (Lu et al. 2004a). Initially the fluoroethylation of 3-(3-pyridinyl)propionic acid did not proceed at RT. Raising the temperature of the micro-reactor on a heating block to 80 °C achieved moderate RCYs (14–17 %). The reaction can be performed with as low as 0.75 μg (5 nmol) of **1** in 10 μl of solution. At an infusion rate of 1 μL min^{-1}, reaction of **1** with [^{18}F]fluoroethyl tosylate at 80 °C gave the ^{18}F-labeled ester in 10% RCY.

10.3.3 [^{18}F]FDG Synthesis in Micro-reactors



2-[^{18}F]Fluoro-2-deoxy-D-glucose ([^{18}F]FDG) is the most widely used radiotracer for PET imaging in oncology, cardiology and neurology (Fowler and Ido 2002). Rapid expansion in clinical applications demands convenient, reliable and economical production of [^{18}F]FDG in high quality accessible to clinical and research centers. It is not surprising, therefore, that the miniaturization of [^{18}F]FDG production has became an important test case for micro-reactor technology.

The first example of [^{18}F]FDG synthesis in a glass-based micro-reactor started with the conventional azeotropic method for drying aqueous [^{18}F]fluoride ion (Brady et al. 2003). The dried K^{18}F with Kryptofix 222 (K2.2.2) in acetonitrile was infused into the micro-reactor through one of two inlets while the precursor, 1,3,5,6-*tetra*-acetyl-D-mannose triflate in acetonitrile, was infused through the other inlet at a rate said to be 5–100 μl min^{-1}. The single-step RCY was 20–49 % by radio-HPLC when the micro-reactor was conditioned at 75 °C. The reaction product, 2-[^{18}F]fluoro-1,3,5,6-*tetra*-acetyl-D-deoxyglucose, was fed into one port of another identical micro-reactor whilst sodium hydroxide (NaOH) solution was infused through another inlet at RT. The hydrolysis reaction in the micro-reactor gave [^{18}F]FDG in 24% RCY. The combined residence time within the two micro-reactors was less than 1 min.

A full production run starting with a large quantity of NCA [^{18}F]fluoride ion (1 Ci) was reported as an update of the above-mentioned work (Liow et al. 2005). Again, the drying of [^{18}F]fluoride ion was carried out using the conventional method. The two-stage synthesis was accomplished on a single micro-reactor with three inlets from which the precursor, dried [^{18}F]fluoride ion with K2.2.2 and NaOH solution, can be infused at a rate of 50 μl min^{-1}. The overall RCY was 15–20 %. Optimization and automation are in progress.

The multi-step synthesis of [^{18}F]FDG, including concentration of [^{18}F]fluoride ion, water evaporation, radiofluorination, solvent exchange and hydrolytic deprotection in acidic medium, has also been accomplished with a PDMS-based, automated integrated micro-fluidic chemical reaction circuit (CRC) (Lee et al. 2005). The authors took the view that radiotracer in a small dose that is still enough for small animal imaging can be prepared within the CRC. The total synthesis time was 14 min with a reported RCY of 26% and radiochemical purity of 98%.

This CRC has achieved a high degree of automation and integration. The concentration of [^{18}F]fluoride ion was realized through the use of a miniaturized anion exchange resin column. The radioactivity was washed off the column with a potassium carbonate (K_2CO_3) solution that was dried in a rectangular loop heated with a hot-plate. Anhydrous acetonitrile was introduced to mimic the azeotropic distillation for complete drying. A solution of the precursor with K2.2.2 in anhydrous acetonitrile was introduced into the reaction loop where the dried K^{18}F was deposited and the mixture heated (100 °C for 30 s then 120 °C for 50 s). At the end of the reaction acetonitrile was removed, hydrochloric acid (HCl, 3 N) was injected and the mixture was heated at 60 °C to obtain the final product.

Although moisture and acetonitrile vapor could be observed to penetrate and escape the gas-permeable PDMS matrix, it did not seem to affect the radiosynthesis. When starting with 720 μCi of [^{18}F]fluoride ion, 500 μCi of [^{18}F]fluoride ion was retained on the anion resin column, which in turn produced 190 μCi product in 14 min. Scale-up based on the design saw the authors producing [^{18}F]FDG in larger quantity (1.74 mCi). No specific radioactivity was reported. The final product was used for mouse tumor imaging with satisfactory results.

10.4 Conclusions

The preparation of PET radiotracers in micro-reactors has been demonstrated through the synthesis of NCA ^{11}C and ^{18}F-labeled carboxylic esters, ^{11}C-labeled amines and [^{18}F]FDG. Miniaturized reactions are rapid and efficient in hydrodynamically driven micro-reactors or integrated microfluidic circuits. These results exemplify some of the potential advantages of this methodology for radiotracer synthesis, which should be amenable to greater sophistication to encompass entire radiosyntheses in a versatile high-throughput manner.

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11 Synthesis Modules and Automation in F-18 Labeling

R. Krasikova

Abstract. Fast implementation of PET into clinical studies and research has resulted in high demands in the automated modules for the preparation of PET radiopharmaceuticals in a safe and reproducible manner. ^{18}F-labeled radiotracers are of considerable interest due to longer half-life of fluorine-18 allowing remote site application, as demonstrated by [^{18}F]FDG. In this chapter, the state of the art of commercially available modules for [^{18}F]FDG is reviewed with

the emphasis on multibatch production of this important radiotracer. Examples are given on the syntheses of other clinically relevant ^{18}F-labeled radiotracers by using existing [^{18}F]FDG synthesizers or with the help of general-purpose [^{18}F]nucleophilic fluorination modules. On-going research and progress in the automation of complex radio labeling procedures followed by development of flexible multipurpose automated apparatus are discussed. The contribution of radiochemists in facilitating automation via introduction of new ^{18}F-labeling techniques and labeling synthons, *on-line* reactions and purifications etc. is outlined.

11.1 Introduction

In terms of perspective application of positron emission tomography (PET), the use of ^{18}F offers a number of advantages because of its longer half-life (110 min), availability in large amounts from modern PET dedicated cyclotrons and high specific activity of the radiotracers. As demonstrated by the clinical success of 2-[^{18}F]fluoro-deoxy-D-glucose ([^{18}F]FDG), ^{18}F-labeled radiopharmaceuticals (RPs) can be synthesized in quantities sufficient for the formulation of multiple doses from a single production run and for up to 2-h shipment away from the production/cyclotron site. The introduction of PET with [^{18}F]FDG (Ido et al. 1977) has revolutionized the field of oncology. Development of efficient synthetic methods and excellent fully automated modules for multiscale productions of the [^{18}F]FDG has contributed to a great extent to the widespread use and commercialization of this radiotracer.

Although [^{18}F]FDG is an effective tumor-imaging agent, its utilization by various types of cells has resulted in several well-known limitations. Thus, the introduction of new PET radiopharmaceuticals to complement [^{18}F]FDG and provide more accurate characterization of tumors is a “hot-spot” in PET-radiochemistry (Couturier et al. 2004). In neurology, ^{18}F-labeled analogues of well-established ^{11}C-labeled PET radioligands are of particular interest due to the possibility of obtaining high specific radioactivity tracers and higher contrast images. For the pharmaceutical industry, specific molecular probes are in high demand for the imaging and quantification of drug binding sites in the human body.

Depending on the structure and location of the ^{18}F-label, some of these new radiotracers can be prepared in a similar manner to [^{18}F]FDG, via nucleophilic radiofluorination of suitable precursors and subsequent hydrolysis/deprotection step. Their synthesis can be adapted to conventional [^{18}F]FDG apparatus after some modifications in the programs and hardware. However, the preparation of many important classes of ^{18}F-radiotracers involves a wide range of chemical transformations resulting in a complex multistep labeling procedure. Here the automation task becomes much more complicated: an automated module must be developed on the individual base to allow reliable production of a particular radiotracer.

In general, the design and construction of automated modules for the synthesis of PET radiotracers have been an important focus of radiochemistry research since the earliest times of PET development. The historical background and evolution of automated syntheses apparatus are given in an excellent review (Alexoff 2003). Due to continuous progress in automation, modern [^{18}F]FDG synthesizers have been designed representing the best example of the automated routine production process. Nowadays a number of companies provide small PET dedicated cyclotrons with various [^{18}F]FDG modules (for review, see Satyamurthy et al. 1999).

In this chapter the state of the art of commercially available modules for [^{18}F]FDG is reviewed with emphasis on multibatch production of this radiotracer. Some examples are given on the syntheses of other clinically relevant ^{18}F-labeled radiotracers by using existing [^{18}F]FDG synthesizers or with the help of general-purpose [^{18}F]nucleophilic fluorination modules. On-going research in the automation of complex radio labeling procedures followed by development of flexible multipurpose automated apparatus is discussed. The contribution of radiochemists in facilitating automation via introduction of new ^{18}F-labeling techniques and labeling synthons, *on-line* reactions and purifications etc. is outlined. Automated dispensing systems, hot cells and other equipment related to automated production are out of the scope of this paper. Due to limited size of the chapter, robotic production methods will not be discussed.

11.2 Automation in Routine Production of [^{18}F]FDG

There can be no doubts that the use of automatic synthesizers minimizes the risk of radiation burden to personnel. Radiation protection becomes increasingly important with the introduction of a new generation of PET dedicated cyclotrons equipped with high-pressure/high-beam targets for generating [^{18}F]fluoride via ^{18}O(p,n)^{18}F nuclear reaction. For example, using IBA C-18/9cyclotron, an average operational radioactivity of [^{18}F]fluoride from 2-h bombardment is about 8.5 Ci, which gives about 5.2 Ci of [^{18}F]FDG (Kiselev et al. 2004). An improved ^{18}F-target system from IBA (XXL target) will allow production of over 10 Ci of radionuclide after single target irradiation. With PETtrace cyclotron using a dual-beam option, the amount of [^{18}F] fluoride reaches 12 Ci.

As for the choice of the automation approach or particular automated apparatus, it is dependent on the type of facility: clinical PET center, large-scale production of [^{18}F]FDG for centralized distribution, research and development projects or combined facilities. General requirements for automated modules for routine production of [^{18}F]FDG or other ^{18}F-labeled radiopharmaceuticals (RPs) are presented in Table 1.

All these features are realized in the design of modern [^{18}F]FDG synthesizers which can be subdivided into two main categories. The first ones are the stationary systems where all connections of the tubes (plumbing) and valves are permanent and are not changed in day-to-day operation. Rinsing all vessels and connecting tubing with solvents without removing them from the apparatus accomplishes cleaning. It is usually referred to as a “clean in place” (CIP) procedure. The CIP requires extensive validation to ensure the safe levels of cleaning solvents and the absence of cross-contamination between batches. In practice, for radiation safety reasons such systems are normally used only once per day because the levels of radiation after cleaning are still high to allow reload of the chemicals, cartridges etc. in a safe manner. Examples of such systems include: TRACERlab Fx_{FDG} (GE Medical Systems, Münster, Germany: former Nuclear Interface module); CPCU, Chemistry Process Control Unit (CTI, Knoxville, TN, USA); EBCO/Jaltech FDG synthesis module (EBCO, Canada); Synchrom (raytest, Isotopenmeesgerate GmbH, Germany). To increase production capability, the TRACERlab Fx_{FDG} apparatus was upgraded allowing operation of two

Table 1. General requirements for automated apparatus for routine production of PET RPs

Production	Process operation, control and validation	Preparation and cleaning	Other demands
High reproducibility	PC or PCU (Process Control Unit) operation	Easy set-up system	Compact size
Reliability	Minimized number of errors	Quick re-load of the chemicals and exchangeable components between runs	Safe access to final product vial for dispensing or transportation
High radiochemical yield	Monitoring of radioactivity transfer during run		GMP and GLP compliance
Product quality in correspondence to the QC demands	IQ, OQ, PQ validations[a]	Automated cleaning and sterilization between runs	Cost-efficiency
Possibility to prepare multiple batches within 1 day		Automated removal of disposable kit after run	The use of materials with high chemical and radiation resistance

[a] Installation Qualification, Operational Qualification, Performance Qualification: EU Guide to GMP, Annex 15, "Working Party on Control of Medicines and Inspections", Qualification and Validation

consecutive runs of [^{18}F]FDG without reloading of chemicals and opening of the hot-cell door (Dual FDG Synthesizer).

With alternate types of automated systems, all the chemical steps for [^{18}F]FDG production take place within a disposable process cassette (disposable kit). The cassette has to be replaced after each batch with "no-cleaning" procedure. Examples of "cassette-type" apparatus (also referred to as "black boxes") are: TRACERlab Mx FDG synthesis module (GE Healthcare Technologies: former FDG synthesizer from Coincidence); PETtrace FDG II Microlab (GE Medical Systems, Uppsala, Sweden). A fully disposable cassette contains reagent vials, tubing, syringes and valves assembled on the stationary platform. The cassette is connected to the syringes and radioactivity inlet. In the above-mentioned

modules, the cassette could not be remotely removed after the synthesis, which caused limitations due to radiation precautions. To overcome these difficulties, a post-run cleaning sequence was introduced in the recently developed FDG-Plus synthesizer (BIOSCAN Inc., Washington DC, USA). After cleaning, less than 0.1% of the total activity is left on the hardware kit, so that even with a starting activity of 10 Ci, the radioactivity levels are acceptable for the preparation for the next run.

A recent technical innovation in the automation is the implementation of a pneumatically actuated kit holder. This provides a new option for the automatic removal of radioactive hardware into a special shielded box without opening the hot-cell door. A new apparatus, Synthera (IBA, Louvain la Neuve, Belgium), is purposely developed for multirun productions of [^{18}F]FDG and other radiotracers. This synthesizer is very compact (16 cm wide) due to specially designed valves and connections. All the materials in the kit construction are carefully selected to provide the best chemical and radiation resistance.

Technical details of the specific automated systems, flowchart diagrams and related information can be found on the corresponding websites of the major commercial suppliers mentioned above.

11.3 The Role of Radiochemistry in the Automation of Routine [^{18}FF]FDG Production

The chemistry process in most of the automated [^{18}F]FDG synthesizers is based on the nucleophilic synthetic method suggested in 1986 (Hamacher et al. 1986) with some modifications (Fig. 1). The major steps in this radiochemical synthesis include delivery of [^{18}F]fluoride

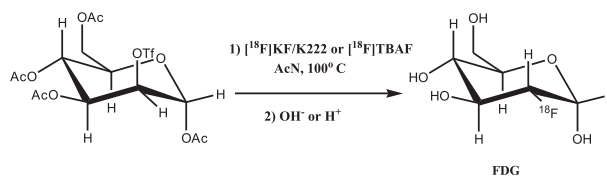

Fig. 1. Stereospecific synthesis of [^{18}F]FDG using nucleophilic displacement reaction in the presence of phase transfer catalyst

from the target, recovery of [^{18}O]enriched water, preparation of a reactive complex of [^{18}F]fluoride with phase transfer catalyst (kryptofix 2.2.2 or tetrabutylammonium carbonate), its resolubilization in an aprotic dipolar solvent (acetonitrile), nucleophilic displacement reaction with 1,3,4,6-tetra-*O*-acetyl-2-*O*-trifluoromethanesulfonyl-D-mannopyranose (mannose triflate), hydrolysis and on-line purification using a solid phase extraction (SPE) technique.

An alternative approach (Toorongian et al. 1990) was used in the FDG Microlab (GE Medical Systems, Uppsala, Sweden). [^{18}F]fluoride was collected on a quaternary 4-(*N*,*N*-dialkylamino)-pyridinium functionalized polystyrene anion exchange resin packed in a small column, dried by passing acetonitrile through the column and then allowed to react with mannose triflate. Labeled intermediate was eluted to the hydrolysis vessel to remove acetyl protecting groups by heating with an acid.

Correspondingly, the operational program represents a set of sequences reflecting these major steps. While in the first automated apparatus (Hamacher et al. 1990) the radiochemical yield of the [^{18}F]FDG was relatively low, it was greatly improved after further optimization of the synthesis. First, the separation of [^{18}F]fluoride from [^{18}O]H_2O (allowing its recycling use) on the anion exchange resins was introduced (Schlyer et al. 1990). In modern automated apparatus, ^{18}F-recovery is accomplished using standard SepPak QMA light cartridges. [^{18}F]fluoride retained on the QMA resin can be eluted with a solution of potassium carbonate and kryptofix in the water–acetonitrile mixture which contained only 4% of water (Gomzina et al. 2001, 2002). After evaporation of the solvents, the dry [K/K2.2.2]$^{+}$$^{18}F^{-}$ complex obtained can be used directly in nucleophilic fluorination reactions, avoiding a commonly performed azeotropic drying procedure. This results in simplified automation and shorter synthesis time. For example, by implementing this procedure into the Synchrom automated system (raytest, Isotopenmeesgerate GmbH, Germany) the [^{18}F]FDG synthesis was shortened to 18 min.

Alkali hydrolysis of tetra acetylated [^{18}F]FDG ([^{18}F]TAG) suggested in 1996 (Fuchetner et al. 1996) was immediately used by radiochemists in automated routine productions. In contrast to high-temperature acid hydrolysis (Hamacher et al. 1986), the cleavage of acetyl groups under

alkali conditions is performed very fast at room temperature. An excellent example of this improved technology is the TRACERlab Mx FDG apparatus from GE Healthcare Technologies (formerly FDG synthesizer from Coincidence). In this cassette-type apparatus the alkali hydrolysis is performed *on-line* using the tC18 SepPak cartridge which greatly facilitates automation (Mosdzianowski et al. 1999; Lemaire et al. 2002). The alkali hydrolysis is also implemented into an upgraded version of the PETtrace FDG II Microlab. It is used in another cassette-type apparatus, the FDG-Plus synthesizer (BIOSCAN Inc.).

At present alkali hydrolysis is taken up into [^{18}F]FDG synthesis procedures by most of the users of TRACERlab Fx_{FDG} (GE Medical Systems, Münster, Germany: former Nuclear Interface module). To provide reliable multiscale productions all the plumbing in the Nuclear Interface FDG module was modified followed by substantial changes in the synthetic procedure, so as to make this module more suitable for large-scale production of [^{18}F]FDG. For example, to prevent degradation in radiochemical purity due to radiolysis (a problem arising in multiscale [^{18}F]FDG productions), alkali hydrolysis was accomplished by adding a controlled amount of ethanol (Kiselev et al. 2004).

Despite the differences in automation approaches and design, the radiochemical yields of [^{18}F]FDG announced by commercial suppliers of the modules are quite similar, they are in the range of 55%–60% at the end of the synthesis (EOS, not corrected for radionuclide decay). The selected data from several PET centers and production sites are shown in Table 2.

For large clinical PET centers or centralized distribution units, the necessity to deliver large amounts of [^{18}F]FDG under a tight schedule put extremely high demands on the reproducibility and reliability of the procedure. Figure 2 gives an excellent illustration of multiscale [^{18}F]FDG productions using a home-made automated apparatus based on the nuclear interface components. The number of failures has been evaluated to be as low as 1%.

Table 2. Radiochemical yields of [^{18}F]FDG using various automated synthetic procedures

PET center/company	Cyclotron	Automated module	Type of hydrolysis	Synthesis time (min)	[^{18}F]FDG yield, EOS	Number of runs
Hopitaux Universitaires du Geneva	IBA 18/9	Nuclear Interface	Acidic	16	48.2	200
Eastern Isotopes (USA)	IBA 18/9	Home-made	Alkali plus ethanol	23	62.2	23123
Eastern Isotopes (USA)	IBA 18/9	Coincidence	Alkali	27	55.6	134
ARGOS Zyklotron GesmbH, Linz, Austria	GE PETtrace	Coincidence	Alkali	27	60.8	45
PET center AZG/RUG[a]	MC17, SCX	Coincidence	Alkali	27	58.9	218
Karolinska Pharmacy, Karolinska Hospital, Stockholm	GE PETtrace	FDG II Microlab	Alkali	35	44.0	133
Institute of Human Brain, St. Petersburg	MC17, SCX	Anatech RB 86 Robot	Alkali	48	63.3	40

[a] Data from Annual Report 2004, PET Center AZG/RUG, Groningen University Hospital, Groningen, The Netherlands

11.4 Automated Production of ^{18}F-Labeled Radiotracers Using Commercial Modules for [^{18}F]FDG

The possibility to produce another ^{18}F-labeled radiotracer using commercial [^{18}F]FDG synthesizers allowing economical use of expensive equipment is very attractive for radiochemists. Many radiosyntheses of ^{18}F-labeled compounds comprise sequential steps of direct nucleophilic substitutions of the leaving group in desired molecule and hydrolysis/deprotection. Here the synthetic procedure is similar to that for [^{18}F]FDG. Well-known examples are: (a) hypoxic agents: [^{18}F]fluoromi-

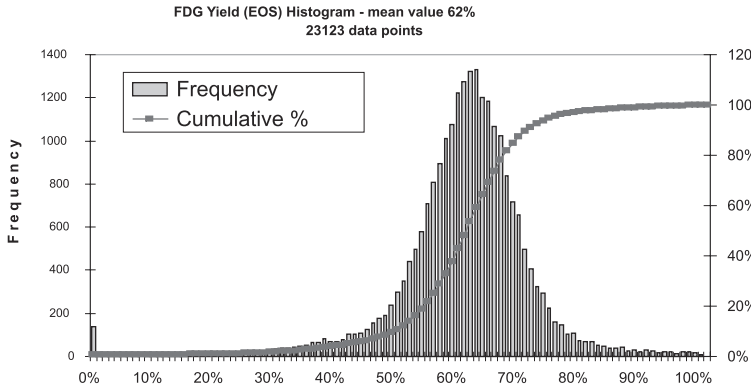

Fig. 2. [^{18}F]FDG production yields obtained at the Eastern Isotopes (Kiselev et al. 2004)

sonidazole ([^{18}F]FMISO) (Lim and Berridge 1993) and ^{18}F-labeled fluoroazomycin arabinoside ([^{18}F]FAZA) (Kumar et al. 2002); (b) tumor-seeking agents: 3′-deoxy-3′-[^{18}F]fluorothymidine ([^{18}F]FLT) (Shields et al. 1998), *O*-[2-^{18}Ffluoroethyl]-L-tyrosine ([^{18}F]FET) (Hamacher and Coenen 2002); (c) receptor radioligands: [^{18}F]flumazenil (central BZ) (Ryzhikov et al. 2005), *p*-[^{18}F]MPPF ($5HT_{1A}$) (Schiue et al. 1997), [^{18}F]altanserine ($5HT_{2A}$) (Lemaire et al.1991) and others. As an example, the synthesis scheme for [^{18}F]flumazenil is shown in Fig. 3.

Despite the general features in the radiolabeling approach, the synthetic procedure regarding solvents and reaction conditions for a particular compound may be different from [^{18}F]FDG. An additional SPE step may be necessary to purify the ^{18}F-labeled intermediates. Thus, the standard module has to be substantially modified and adapted to the

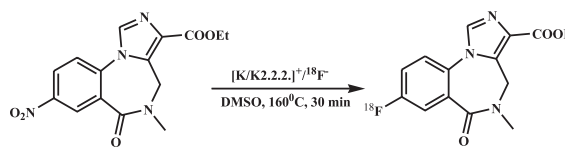

Fig. 3. Synthesis of [^{18}F]flumazenil via nucleophilic displacement reaction

production of new compounds. For the stationary systems (TRACERlab Fx_{FDG}, former Nuclear Interface) with an open access to radiochemistry hardware, the reassembly of the valves and plumbing is easily achieved to allow new operational sequences.

As for the most popular cassette-type apparatus, TRACERlab Mx FDG (former Coincidence FDG synthesizer), made of standard molded stopcock manifolds, it is almost impossible to modify the plumbing pattern, as the manifolds come in one configuration only. Another limitation is that the cassette material is not resistant to certain solvents, for example, to methylene chloride or DMSO. Even so, this synthesizer was adapted for routine productions of several useful tracers: [^{18}F]fluroerythro nitroimidazole (FETNIM), [^{18}F]FMISO, [^{18}F]FET and [^{18}F]FLT (ARGOS Zyklotron GesmbH, Linz, Austria). A detailed description of the modifications made in the TRACERlab Mx FDG (both in the stopcock manifold and cassette) to accomplish the syntheses of [^{18}F]FLT and [^{18}F]FMISO can be found in recent publications (Oh et al. 2004, 2005). The syntheses of [^{18}F]FMISO (Patt et al. 1999; Tang et al. 2005) and [^{18}F]FAZA (Reischl et al. 2005) using TRACERlab FX_{F-N} synthesizer have been reported.

In contrast to [^{18}F]FDG, for most radiotracers a semi-preparative HPLC purification is required to ensure the separation of labeled product from the synthetic precursor. Therefore, the commercial FDG module has to be extended to provide an HPLC purification option. The automated injection of the reaction mixture into an HPLC injection loop is not a trivial task. This step is accomplished with losses of the product due to either incomplete delivery of solution into the loop or unavoidable introduction of air, which results in overpressure and plugging of the HPLC column. To solve this problem, the radioactivity detector is usually installed in combination with a photo- or optical sensor. The solution to be purified is sent to the sample loop of the HPLC apparatus through the photo sensor and a rotary six-way valve. When the photo sensor detects the end of solution, the rotary six-way valve rotates the position automatically, and the mixture in the sample loop is then loaded to the HPLC column by an HPLC pump.

In many laboratories this step is performed manually with the use of an external syringe connected via tubing to the HPLC injection loop equipped with a radioactivity detector. A commercially available

module, TRACERlab FX_{F-N} (GE Medical Systems), is equipped with a built-in preparative radio/UV HPLC system. The HPLC injection option is also part of the recently developed module for nucleophilic fluorinations, Synchrom (raytest, Isotopenmeesgerate GmbH, Germany).

After collecting the product peak, the HPLC mobile phase has to be removed allowing the radiotracer solution to be obtained in a ready-to-use form for PET study (after an appropriate quality control procedure). The automation of this step is also associated with difficulties. In general, the solvents can be removed by three different ways:

- Conventional evaporation with the use of a rotary evaporator under visual control of the dryness. Radiolabeled product is removed from the rotary evaporator flask by buffer solution via external tubing connected to the syringe.
- Extraction of the product on the SPE cartridge. To complete the extraction, the product peak (fraction) is collected in the separated flask and diluted by a large volume of water. The diluted mixture is passed via pre-conditioned SPE cartridge (normally, C18 SepPak light). Finally, labeled product is eluted by 1 ml of ethanol or suitable buffer solution.
- On-line evaporation of the solvents by means of carburetor-type apparatus. The HPLC mobile phase at the exit from the column is continuously carbureted by a controlled flow of helium over the heated spiral where the labeled product is accumulated. Formulation of the final product is accomplished by rinsing the spiral with sterile buffer solution.

The traditional rotary-evaporator procedure is time consuming and requires manual intervention to remove the product from the rotary flask. SPE purification is advantageous for automation (Lemaire et al. 1999) and widely accepted by many PET-chemistry labs. However, the necessity to install a bulky vessel for dilution of product fraction results in enlargement of the size of the apparatus. Technically, the simplest evaporator technique is a carburetor-type apparatus. It has been in use in the Karolinska Institutet since 1997 (Larsen et al. 1997) as part of an [^{11}C]methyl iodide/triflate methylation system and has proved to be very reliable. An important factor is that no radiolysis was observed using the carburetor, which is often the case for conventional rotary

evaporation. A solvent-removal carburetor is an integral part of a new highly flexible modular radiochemistry system (ScanSys, Vaerloeser, Denmark) (Gillings and Larsen 2005).

11.5 Automation of Multistage Synthesis Based on Nucleophilic Displacement Reactions with ^{18}F-Fluoride

Introduction of ^{18}F-label via direct nucleophilic substitutions of the leaving group in a desired position of the targeted molecule is not always possible. Therefore, a complex multistage chemical process for ^{18}F-labeling is often required. This process usually includes the preparation of highly reactive ^{18}F-intermediate (^{18}F-labeling synthone) by nucleophilic substitution reaction, followed by a second reaction, for example, alkylation. Depending on the leading structure, more steps and more purifications in between may be involved. The final step is usually deprotection reaction and semi-preparative HPLC purification of the labeled product. To operate this multistage synthesis, home-made semi-automated apparatus is usually constructed in almost every PET laboratory. Numerous examples are regularly presented at the Workshop on Targetry and Target Chemistry (Automation section). In the context of this short chapter, several automated systems are described with examples on the preparation of clinically relevant radiotracers via ^{18}F-alkylation reactions.

11.6 Semi-automated and Automated Synthetic Modules for ^{18}F-Fluoroalkylations

^{18}F-fluoroalkylation is an effective way to introduce ^{18}F-fluorine into various molecules with hydroxy-, amino- or amido moieties. In carbon-11 chemistry, ^{11}C-alkylations reactions (mainly, ^{11}C-methylations) are fully automated. The automated ^{11}C-methyl iodide synthesis module (GEMS, Uppsala, Sweden) is an excellent example. However, ^{18}F-alkylation reactions are not so common in routine syntheses, mainly because of difficulties in the automation. Due to recent progress in the synthesis and purification of ^{18}F-alkylating agents (Iwata et al. 2000,

2002), automation has become easier allowing wider applications of this type of reaction in semi- and fully automated systems.

For example, synthesis of 4-^{18}F-labeled benzyl bromide, a useful synthone for introducing ^{18}F into the aromatic part of various molecules, has been performed using a remotely controlled module (Krasikova et al. 2002). The synthesis involves three major steps: (1) nucleophilic fluorination of 4-nitro benzaldehyde in DMSO at 150 °C for 7 min; (2) on-line reduction of resulting 4-^{18}F-fluorobenzaldehyde with an aqueous $NaBH_4$ on the C18 SepPak followed by on-line purification on a combined drying column; (3) room temperature bromination of the purified 4-^{18}F-fluorobenzyl alcohol with Ph_3PBr_2(3 min stirring in CH_2Cl_2). The automation of the last step becomes possible only due to the use of Ph_3PBr_2, a stable commercially available brominating agent introduced for the first time in radioactive synthesis by Iwata and co-workers (Iwata et al. 2000). The automation of the bromination procedure using previously reported agents ($SOBr_2$, gaseous HBr, HBr in ether) has been associated with difficulties due to the aggressive nature of these chemicals.

To carry out this process, a remote-controlled apparatus was designed in the Karolinska Institutet, Department of Clinical Neuroscience, Psychiatry section, in co-operation with Laboratech, Sweden. It consists of nine three-way valves (Burkert Compromatic), a heating block with a cooling gun, SepPak and drying column holders and Vortex (Fig. 4). All the system components were placed in a chemical-proof stainless steel box with a front door. Electronic parts, cables, motors etc. were placed inside this box to avoid the damage of expensive electronics by chemicals. The valves, tubing, heating block and others were connected to the front door allowing easy access and replacement of chemicals. The synthesis operation was performed using bottoms-board. A separate module was designed for trapping of [^{18}F]fluoride on the QMA SepPak light cartridge from the irradiated [^{18}O]H_2O. This module was attached to the inner surface of the hot-cell wall and connected to the "main" module by Teflon tubing.

An upgraded version of this apparatus, which includes 19 valves, two heating blocks adapted for different sizes of reactive vessels (from 1 to 10 ml volume), two column ovens, a remotely controlled injection into the HPLC loop, radioactivity monitoring via six scintillation-type

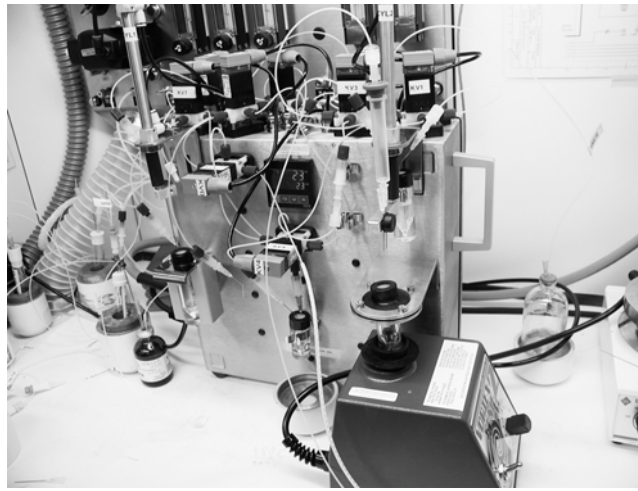

Fig. 4. Front view of remotely controlled apparatus for the synthesis of 4-[^{18}F]benzyl bromide

detectors (Carroll–Ramsey Associates, USA), was designed to operate more complex synthetic procedures. This apparatus was used in a regular production of a new norepinephrine transporter (NETs) radioligand, (*S*,*S*)-[^{18}F]FMeNER-D_2 (Schou et al. 2004). The module was PLC controlled via an external touch screen, where the information on the temperature settings/readings as well as radioactivity detector readings was displayed. The synthesis of this compound was performed via alkylation reaction of desfluoromethoxy-(*S*,*S*)-FMeNER-D_2 with [^{18}F]bromofluoromethane. The latter was synthesized by nucleophilic substitution of CH_2Br_2 with no-carrier-added [^{18}F]fluoride and purified by four Sep-Pak Plus silica cartridges connected in a series. This purification technique (Iwata et al. 2002) is very simple and much easier for automation, compared with earlier published methods based on gas chromatography separation of [^{18}F]CH_2BrF and volatile precursor, CH_2Br_2 (DeGrado et al. 2000).

A more reactive [^{18}F]fluoromethylating agent, [^{18}F]fluoromethyl triflate ([^{18}F]CH_2FOTf), was prepared on-line from [^{18}F]CH_2BrF (Iwata et al. 2002) and applied in a remotely controlled synthesis of [^{18}F]fluorome-

thyl choline, a well-known radiotracer for PET imaging of prostate cancer. A fully automated version of this synthesis has been recently developed in ARGOS Zyklotron GesmbH (Fig. 5).

The apparatus consists of a reactive vessel for nucleophilic fluorination of CH_2Br_2, a purification line (four silica cartridges in series, shown by arrow), and a triflate-production oven with the outlet connected to the C18 cartridge preloaded with *N*,*N*-dimethylaminoethanol (starting material in methylation reaction) (Fig. 6). The purification was achieved by SPE technique using an Accell plus cation exchange cartridge. PC-controlled synthesis operated by LabView software was accomplished within 40 min and provided radiochemically pure [^{18}F]fluoromethyl choline in 20% yield (EOS). The cleaning procedure was fully automated.

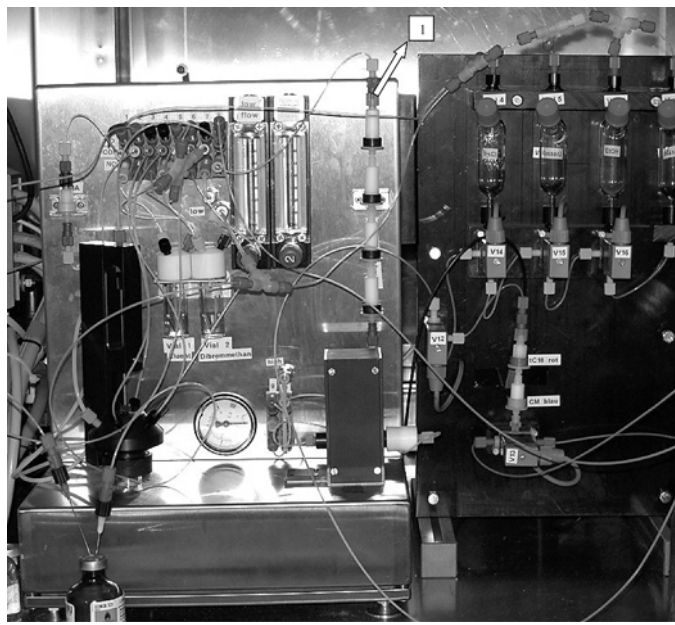

Fig. 5. A general view of fully automated apparatus for synthesis of [^{18}F]fluoromethyl choline (ARGOS Zyklotron GesmbH, Linz, Austria)

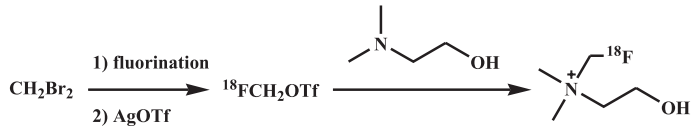

Fig. 6. Synthesis of [^{18}F]fluoromethyl choline via [^{18}F]fluoromethylation of *N*,*N*-dimethyl amino ethanol with [^{18}F]CH_2OTf

For ^{18}F-fluoroethylations reactions, [^{18}F]fluoroethyltosylate ([^{18}F]-FETos) is the most common labeling agent due to its ease of preparation, good stability and wide applicability. The reaction of [^{18}F]FETos with dipotassium salt of L-tyrosine was suggested for the preparation of *O*-(2′-[^{18}F]fluoroethyl)-L-tyrosine ([^{18}F]FET), which very quickly became the most popular amino acid tracer for tumor imaging with PET (Westera et al. 1999). However, the automation of this two-step procedure is associated with difficulties due to the necessity to apply semi-preparative HPLC purification of the reactive intermediate [^{18}F]FETos.

Even so, the synthesis of [^{18}F]FET including two consequent HPLC purification steps was fully automated at the Centre for Radiopharmaceutical Science, ETH, PSI and USZ, Zurich, and implemented into routine productions of this radiotracer (Kaim et al. 2002). In the automated synthesis a unique versatile synthesis apparatus for ^{18}F-fluorinations was employed. This apparatus with controlling software programmed on LabView is very flexible and makes it possible to prepare various ^{18}F-labeled radiopharmaceuticals via nucleophilic or electrophilic approaches. The major system components and operational principles are briefly described elsewhere (Westera et al. 1999). A flowchart diagram (LabView operational Window on the PC) for the synthesis of [^{18}F]FET and [^{18}F]fluoromethylcholine is shown in Fig. 7.

Automation of two-step synthesis of [^{18}F]FET using commercially available PETtrace FDG Microlab synthesizer has been reported (Tang et al. 2003). In the reported procedure, the HPLC purification was replaced by SPE on the standard SepPak cartridges. However, there were no data on the chemical and enantiomeric purity of the final product, while purification by SPE technique requires a very careful validation of the synthetic procedure.

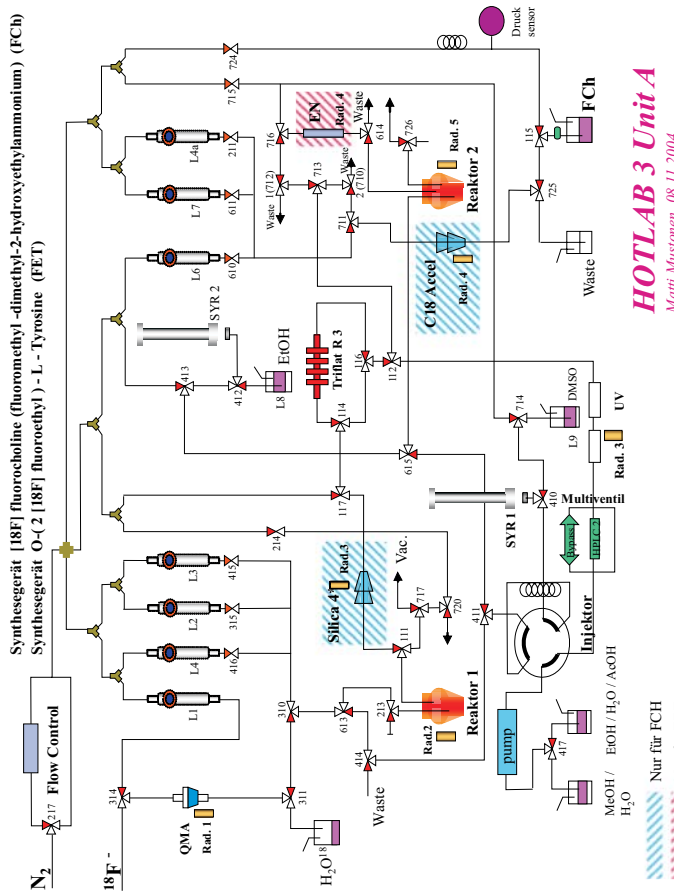

Fig. 7. Flowchart (LabView operational Window) for the synthesis of [^{18}F]FET and [^{18}F]fluoromethylcholine

At present, due to the development of a direct nucleophilic radiofluorination method for [^{18}F]FET (Hamacher and Coenen 2002), an automated production of this radiotracer is well established with the use of commercially available [^{18}F]FDG synthesizers. As a result, there is a remarkable growth in clinical applications of this useful tumor-seeking agent.

11.7 Automated Synthesis of [^{18}F]Fluorinated Compounds via Electrophilic Fluorination

An alternate approach for introducing ^{18}F-label into organic molecules is electrophilic fluorination reaction using gaseous [^{18}F]F_2 or [^{18}F]acetyl fluoride. This method is currently employed in routine production of aromatic [^{18}F]fluorinated α-amino acids which contain ^{18}F-label in the aromatic ring. Most of the interest is focused on 6-[^{18}F]fluoro-L-3,4-dihydroxyphenylalanine (6-[^{18}F]FDOPA), a well-established tracer for Parkinson's disease (Barrio et al. 1997). Recently, 6-[^{18}F]FDOPA has been extensively evaluated as an agent for PET imaging of tumors. 2-[^{18}F]fluoro-L-tyrosine is a second important amino acid tracer suggested as a tumor-seeking agent in 1986. Both compounds can be produced via electrophilic regiospecific fluoro-de-stannylation reactions (Fig. 8) using commercially available stannyl precursors.

In general, the electrophilic approach is facile and easy to automate. The major obstacle in wide application of the electrophilic method is production of the radionuclide itself. [^{18}F]F_2 can be obtained either via ^{20}Ne(d,α)^{18}F nuclear reaction or ^{18}O(p,n)^{18}F nuclear reaction (Nickles et al. 1984) performed in the gas target. The target gas should con-

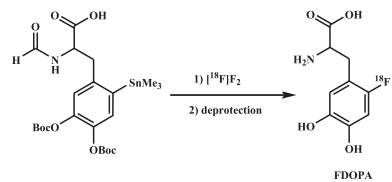

Fig. 8. Synthesis of 6-[^{18}F]FDOPA via electrophilic regiospecific fluoro-de-stannylation reaction

tain a controlled amount of elemental fluorine (< 1%) for recovery of [^{18}F]F_2, which results in radiotracers with low specific radioactivity (SRA). When radionuclide is generated via ^{18}O(p,n)^{18}F nuclear reaction, a double-shut irradiation protocol is required for complete recovery.

The most popular commercially available apparatus for fully automated production of 6-[^{18}F]FDOPA is TRACERlab FXFDOPA (GE Medical Systems, former Nuclear Interface). It is equipped with a semi-preparative HPLC column with UV/radio detectors allowing complete preparation of injectable solution. The mobile phase for HPLC purification of 6-[^{18}F]FDOPA does not contain organic solvents or additives (0.01 M CH_3COOH aqueous solution, pH 4), therefore a solvent evaporation step is not necessary. Automated synthesis of 6-[^{18}F]FDOPA with the use of Nuclear Interface apparatus (first prototype) is described in detail elsewhere (de Vries et al. 1999). This apparatus was also applied in electrophilic synthesis of 2-[^{18}F]fluoro-L-tyrosine (Hess et al. 2002). The electrophilic 6-FDOPA module is provided by other commercial suppliers, for example by raytest, Isotopenmeesgerate GmbH, Germany. However, there are no data available on its practical application. In Table 3 the selected data on the production yield of 6-[^{18}F]FDOPA are presented.

In general, the electrophilic method for synthesis of 6-[^{18}F]FDOPA is capable of generating clinically useful doses of radiotracer. With an efficient production method for [^{18}F]F_2, sufficient amounts can be produced for delivery to remote sites. However, the application of the nucleophilic approach allowing one to start with much higher activity of [^{18}F]fluoride is still a great challenge. Asymmetric synthesis of 6-[^{18}F]FDOPA allowing one to get a reasonable yield of radiotracer (10%–12%, EOS) can be easily operated by the Anatech RB86 laboratory robot (Krasikova et al. 2004). However, despite considerable efforts, this complex multistage synthesis procedure has been found to be difficult for implementation into automated modules (Fedorova et al. 2003).

Table 3. Productions yields of 6-[^{18}F]FDOPA using various automated synthetic procedures

PET center/company	Irradiation	Target	Target gas	Automated Module	[^{18}F]F_2, GBq	6-FDOPA, GBq
ARGOS Zyklotron GesmbH, Linz, Austria	GE PETtrace 40/25 μA 120±15 min	Home-made	$^{18}O_2$/Ar-F_2mixture	TRACERlab FXFDOPA	50	7–14
PET center AZG/RUG[a]	MC17, SCX 25 μA 60 min	SCX nickel plated 150 ml	0.44% F_2 in neon, 75 psi	Nuclear Interface	2,4–6,0	0.8
USZ, Zurich[b]	GE PETtrace 40 μA 60 min	GE	0.40% F_2 in neon, 140 KPa	Home-made	8–9.2	0.7

[a] Data from Annual Report 2004, PET Center AZG/RUG, Groningen University Hospital, Groningen, The Netherlands; the apparatus used is described in de Vries et al. 1999

[b] Preliminary data for 6-FDOPA productions using versatile synthesis apparatus at the Center for Radiopharmaceutical Science ETH, PSI and USZ, Zurich

11.8 Modular Concept as a New Philosophy in Automation

All the automated synthesis types of apparatus described above are based on the principle "to perform all the necessary chemical operations with radionuclide in one unit." This concept works well for one-step labeling synthetic strategies. However, in complex multistage synthesis it is frequently necessary to use two or more different reactors to conduct the separated stages of the process, to eliminate carry-over of solvents and impurities from stage to stage. As a result, relatively large synthesis modules have to be constructed. These highly complex systems typically require significant effort to maintain, since automated cleaning of such a big assembly of plumbing is hardly possible.

An alternative approach to provide an economical and compact solution to multistage synthesis is the modular concept. Within this concept very compact identical modules are placed within the same hot-cell and connected in a way that allows use of each module for one step with an automatic transfer of resulting radiolabeled intermediates from one

stage to another (i.e., from the first module to the second one etc.). The entire process is operated by one software program. The first prototype of a module-type apparatus "Synthera" was recently developed at the IBA, Louvain la Neuve, Belgium (patent is under application) (Fig. 9).

In this apparatus each module is equipped with a kit containing a sufficient number of reagent vials, reactors and filter holding positions, especially designed for a particular stage of the multistage process. Because each module is only 16 cm wide, it allows for placement of at least three or more of these modules within one hot-cell. This method will allow for virtually unlimited flexibility in use of the systems and will enable production of a great variety of radiotracers using standardized single-use kits.

This concept seems to be really challenging for application to such a complex multistage synthesis, such as nucleophilic production of 6-[^{18}F]FDOPA. A possible limitation of this modular approach is that the use of pre-packed kits of vials and reagents is expected to be very expensive.

Another principle has been applied in a modular system that is under development in ScanSys, Vaerloese, Denmark (Gillings and Larsen

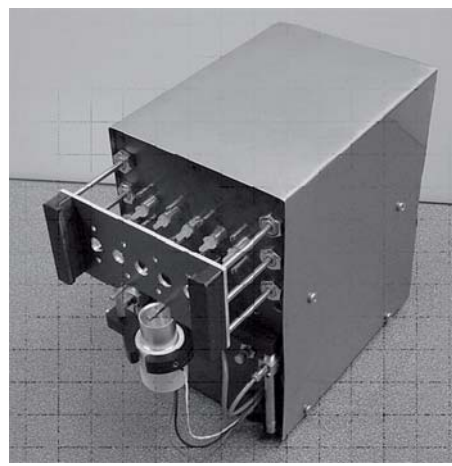

Fig. 9. General view of a single-module unit in Synthera apparatus

2005). A series of modules were designed that can quickly be combined to each other within one rack made from alumina. In Fig. 10 an assembly of modules in an aluminum rack is shown which includes: eight pneumatically operated three-way valves, Rheodyne; eight-channel radioactivity detectors unit; eight valves for compressed air supply; two HPLC columns unit; an HPLC columns selector; module with three reactors and a moving panel for addition of regents.

In fact, the modular concept was successfully realized in an automated system for ^{11}C-methylation (Larsen et al. 1997). [^{11}C]methyl

Fig. 10. Modular system components combined in aluminum rack

iodide produced in a GEMS methylation apparatus was transferred online into [^{11}C]methyl triflate. The modular design is very well suited to ^{11}C-methylations chemistry, which is quite simple and well known. All the methylation reactions using [^{11}C]methyl triflate were performed at room temperature in disposable glass mini-vials placed in an HPLC autosampler (Gilson). One may expect that the application of the modular principle to complex multistage synthesis with [^{18}F]fluoride will require much more efforts.

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12 Pharmacological Prerequisites for PET Ligands and Practical Issues in Preclinical PET Research

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Abstract. The development of PET radiopharmaceuticals for the non-invasive imaging of cancerous lesions, brain receptors, transporters and enzymes started more than 25 years ago. But till today no established algorithms exist to predict the success of a PET radiopharmaceutical. PET radioligand development is a challenging endeavor and predicting the success of PET ligand can be an elusive undertaking. A large number of PET radiopharmaceuticals have been developed for imaging, but so far only a few have found application as imaging agents in vivo in humans. Typically, the potential compound selected for development usually has the desired in vitro characteristics but unknown in vivo properties. The purpose of this chapter is to highlight some of the pharmacological constraints and prerequisites. Interspecies difference in metabolism and mass effects are discussed with examples. Finally, some of the practical issues related to laboratory animal imaging using anesthetic agents are also presented.

12.1 Introduction

Positron emission tomography (PET) is an imaging modality that allows study of physiological, biochemical and pharmacological functions at the molecular level. The development of PET radiopharmaceuticals for the non-invasive imaging of cancerous lesions, brain receptors, transporters and enzymes started more than 25 years ago, but till today no established algorithms exist to predict the success of a PET radiopharmaceutical. Driven by new post-genomic techniques such as proteomics, genomics and protein–protein interactions, pharmaceutical companies have developed and continue to develop a large number of drugs for various targets. The general assumption is for the most part that drugs which are fetching money for the pharmaceutical companies would also be excellent PET radiopharmaceuticals if amenable to labeling with PET isotopes. This assumption is not necessarily true. For example, the antidepressant fluoxetine (Prozac), although performing well on the drug market as a pharmaceutical, is due to high non-specific binding in vivo unsuitable as a PET ligand for imaging the serotonin transporter (Shiue et al. 1995). PET radioligand development is a challenging endeavor and predicting the success of PET ligand can sometimes be an elusive undertaking. A large number of PET ligands have been developed for imaging, but so far only a few have found application as imaging agents in vivo in humans. As with drug development, there are various stages involved in the PET radiopharmaceutical development process starting from candidate compound selection through to human application. Typically, the potential compound selected for development usually has the desired in vitro characteristics such as high affinity, selectivity and other appropriate pharmacologic properties for the target under investigation. But factors such as in vivo affinity, metabolic stability and pharmacokinetics are for the most part unknown and contribute to a great extent to the failure of PET radiopharmaceuticals. The question that arises is which in vivo parameters, criteria or factors should one be aware of or carefully consider when developing PET radiopharmaceuticals in order to reduce attrition in the PET radiopharmaceutical development process. Ideally, PET radiopharmaceuticals should fulfill several key requirements. The ligand should have high affinity and selectivity to provide an adequate target-to-non-target ratio. The labeled compound should be produced in

high specific radioactivity and for PET radiotracers of the central nervous system (CNS) metabolism should not yield radioactive metabolites that cross the blood–brain barrier. The compound should be non-toxic and not have affinity for efflux pumps.

This chapter describes with illustrative examples some major pharmacological properties of PET radiotracers as well as practical issues which should be considered during the development and validation phase of a new PET radiopharmaceutical.

12.2 Pharmacological Constraints

PET imaging studies generally use tracer doses containing only very small amounts of the radiopharmaceutical (in the range of picograms to micrograms) which conform to true tracer kinetics and have only marginal or negligible pharmacological effects and toxicity. The limited sensitivity of small animal PET imaging systems compared with clinical tomographs requires a higher number of coincidence events detected from a rodent imaging voxel to achieve a comparable count statistics as in human PET studies. Therefore, significantly higher doses of radioactivity have to be administered to a rodent. Such an approach, however, may be associated with the injection of a considerable mass of stable compound.

The impact of administrating high doses to mice and rats is closely related to the target protein density or capacity and the affinity of the tracer. Depending on the mechanism of uptake and retention, PET radiopharmaceuticals can be classified in three main classes (Jagoda et al. 2004): (1) non-saturable systems (e.g., [^{18}F]-fluoride uptake in bone), (2) intermediate saturable systems (e.g., [^{18}F]-FDG measuring GLUT-1 capacity and hexokinase activity), and (3) saturable binding sites, typically receptor binding sites with low density (e.g., [^{18}F]-fallypride binding to D2 receptors).

Tracers belonging to class 1 pose the least challenges; their uptake in the target region will not be affected even at very high injected doses. For class 2 tracers such as [^{18}F]-FDG, the situation may already become more problematic when glucose levels in the blood are high and the saturation level of the hexokinase is potentially reached (Jagoda

et al. 2004). The hypoxia tracer [^{18}F]-FMISO represents a comparable case to [^{18}F]-FDG. [^{18}F]-FMISO undergoes reduction in hypoxic tissue requiring intact nitroreductase enzymes and it is still a matter of debate whether the nitroreductase enzymes become saturated depending on the injected mass of unlabeled FMISO and therefore cause a non-oxygenation-related reduction in radioactivity uptake in hypoxic tissue even though a 'no-carrier-added' method is used for the preparation of the radiotracer. The quantification of [^{18}F]-FMISO uptake in B16 melanoma-bearing mice using injectants with escalating amounts of unlabeled FMISO demonstrated that even co-injection of 'no-carrier-added' [^{18}F]-FMISO with up to a thousand-fold excess of cold FMISO did not show any significant correlation between [^{18}F]-FMISO uptake and injected mass of FMISO (Fig. 1).

For class 3 radiotracers, the target saturation can be easily assessed by in vivo small animal imaging studies or ex vivo dissection techniques. Using escalating carrier-added doses, the ligand dose which is blocking 50% of specific binding (ED_{50}) can be determined. The ED_{50} value was

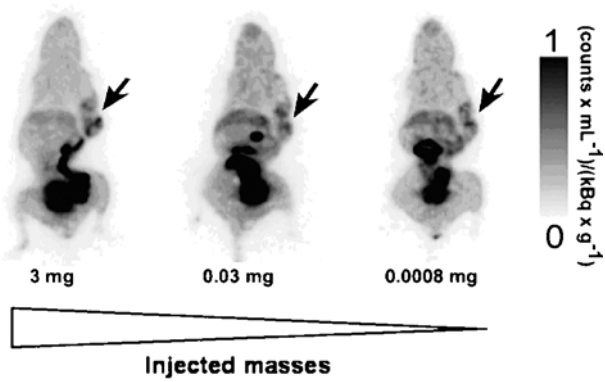

Fig. 1. The same B16 melanoma-bearing mouse scanned on three different days after [^{18}F]-FMISO injection with a decreasing injected mass of unlabeled FMISO. Upon visual inspection, no difference in radiotracer uptake or image quality is apparent. The tumor is marked by an arrow. (Modified from Wyss et al. 2005)

found to be unpredictable from the binding potential ($BP = B_{max}/K_d$) as measured by in vitro binding assays (Hume et al. 1995). The discrepancy of in vitro and in vivo values may be explained by factors such as non-specific binding, soft tissue retention, metabolism, blood–brain barrier penetration, and micro-environment of the target site. Therefore, the term 'apparent' K_d for values determined in vivo is often used to reflect these differences between both types of analyses (Kung et al. 2005). Especially for high-affinity PET tracers (K_d <0.1 nM) involved in easily saturated processes (e.g., ligand–receptor interactions) the specific activity of the radiotracer plays an important role and can limit its usefulness as an imaging agent. Even small amounts of injected tracer may lead to a significant receptor occupancy which is associated with a deterioration of signal-to-noise ratios, the violation of kinetic modeling assumptions (postulating less than 5% receptor occupancy), and – depending on the target and its effector system – with pharmacological or toxic effects. Even for PET tracers with intermediate affinity such as [^{11}C]-raclopride (K_d=1 nM) or with low affinity (e.g., [^{11}C]-CFT; K_d=10 nM) a considerable degree of receptor occupancy may be reached (Hume et al. 1995). For future studies, increasing the specific activity of the radiotracer and the sensitivity of the small animal PET system may be crucial to avoid conflicts with this pharmacological constraint.

12.3 Radiotracer Metabolism

In general, the difficulty in predicting metabolism of a new candidate ligand stems from the fact that numerous factors such as enzyme expression, interspecies differences, inter-animal or inter-individual variability, age and hormonal status all play a role in determining the metabolic fate of a drug (Delaforge 1998). For CNS PET radiotracers, as mentioned above, radiolabeled metabolites should not enter the brain and confound the PET signal. 6-[^{18}F]-Fluoro-L-DOPA ([^{18}F]-FDOPA) is one of the most widely used PET tracers for studying presynaptic dopaminergic function in Parkinson's disease (PD). In the brain, [^{18}F]-FDOPA is decarboxylated to [^{18}F]-Fluoro-L-dopamine and like dopamine accumulates in dopaminergic terminals. Metabolic studies showed that besides [^{18}F]-Fluoro-L-dopamine, other radiolabeled metabolites are

also formed which cross the blood–brain barrier and contribute to non-specific binding in the brain. As such for an accurate analysis of [^{18}F]-FDOPA uptake the determination of [^{18}F]-FDOPA and its radioactive metabolites in blood plasma is essential. Despite this shortcoming, [^{18}F]-FDOPA has proven its utility in the clinical diagnosis of PD. We evaluated the possibility of using [^{18}F]-FDOPA in a mouse brain and found that unlike in humans and primates [^{18}F]-FDOPA was only uniformly distributed in mouse brain with no specific accumulation in the striatum (Fig. 2) despite the concomitant use of aromatic amino acid decarboxylase (AADC) and catechol-*O*-methyltransferase (COMT) inhibitors (Honer et al. 2005). Hume and colleagues reported similar findings in rat brain using [^{18}F]-FDOPA (Hume et al. 1996). The use of [^{18}F]-FMT (6-fluoro-meta-tyrosine), a structural analogue that is not a substrate of COMT, also did not provide a useful image contrast (Fig. 2).

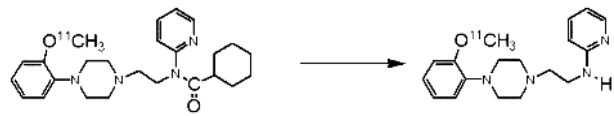

Fig. 2. Metabolism of [*O*-methyl-^{11}C]-WAY100635 in primates

Another illustrative example is the serotonin 5-HT$_{1A}$ antagonist [*O*-methyl-^{11}C]-WAY100635 which was identified as the first useful radioligand for visualizing the 5-HT$_{1A}$receptors in rodents. In rats, the radioactive metabolites formed were more polar than the parent compound and did not enter rat brain (Pike et al. 1995). However, PET and plasma metabolic studies in humans and primates indicated that a different radiolabeled metabolite, the des-cyclohexanecarbonyl derivative [*O*-methyl-^{11}C]-WAY100634 (Fig. 3) was formed that passed into the brain and contributed to specific and non-specific binding (Osman et al. 1996). Further optimizations eventually led to the development of more metabolic stable compounds which are currently being used to study the 5-HT$_{1A}$ receptors in humans. The lessons from ^{18}F-FDOPA and [*O*-methyl-^{11}C]-WAY100635 metabolism remind us that interspecies differences in radioligand metabolism should always be taken into account and wrong decisions could be taken when the radioligand is re-

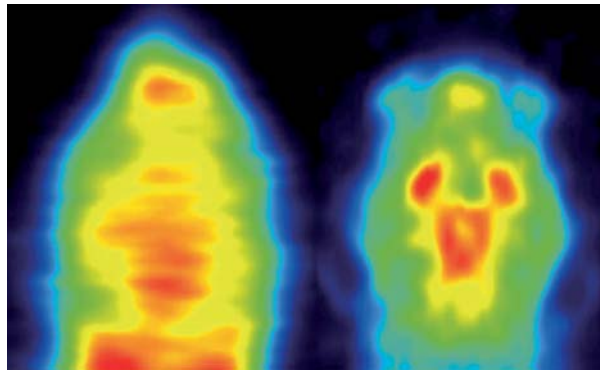

Fig. 3. Comparison of representative coronal PET images through the brains of two different C57/BL6 mice injected with [^{18}F]-FDOPA (reconstructed data from 15–45 min p.i.) and [^{18}F]-FMT (reconstructed data from 88–128 min p.i.). The plane shown passes through the level of the striatum

jected too early or too late when no early information on potential metabolism problems in humans is available. In vitro metabolism studies using rat or human hepatocytes in combination with high-pressure liquid chromatography and mass spectrometry can very well predict in vivo metabolism as demonstrated recently by Ma and colleagues (Ma et al. 2003).

12.4 Practical Constraints: Anesthesia

Small animal PET imaging involves the necessity to immobilize the animal during the scanning procedure. The immobilization is generally accomplished using anesthetics which, however, affect physiological and hemodynamic parameters of the animal. Therefore, tracer uptake, kinetics and metabolism may be influenced in vivo by the use of anesthesia, thus complicating data interpretation of animals under anesthetized conditions. Particularly, PET imaging using radiotracers that are rapidly metabolized in vivo will be flawed by anesthesia.

Several issues have to be considered to reduce the confounding influence of anesthesia on the function of interest. In order to minimize

the effects on animal physiology, anesthesia should be administered in a controllable manner and at a superficial level. For such an anesthesia, the most consistent and convenient regimen in small animal PET imaging involves inhalation anesthetics and spontaneously breathing animals. Isoflurane is the most commonly used inhalation anesthetic in small animal imaging, but is also known to have some effects on regional cerebral blood flow and brain glucose metabolism (Fig. 4). Depending on the PET tracer, scanning protocol and function of interest, the ideal anesthetic regime should be selected for each experimental protocol. In addition, for each tracer employed in small animal PET scanning it should be considered whether the anesthetics may directly or indirectly interact with the target site of the tracer (Votaw et al. 2003; Opacka-Juffry et al. 1991; Tsukada et al. 1999). Apart from isoflurane inhalation anesthesia, other commonly used anesthetics in small animal imaging are pentobarbital, α-chloralose, propofol and ketamine/xylazine.

An optimal anesthetic protocol also involves reliable monitoring of the depth of anesthesia using the respiratory frequency as the best monitoring parameter. Homeostasis of fluid and electrolyte balance, acid/base balance and blood glucose levels by fluid supplementation during anesthesia may also become very important, especially for long-term scanning (>30 min). In addition, body temperature should be controlled by a rectal probe and an automated feedback temperature control system

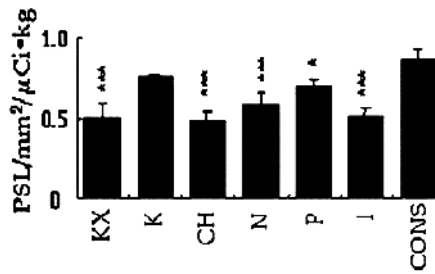

Fig. 4. Average normalized activity uptake in whole brain of rats injected with [^{18}F]-FDG and anesthetized with various regimen (*KX*, ketamine/xylazine; *K*, ketamine; *CH*, chloral hydrate; *N*, pentobarbital; *P*, propofol; *I*, isoflurane; *CONS*, controls). (Adapted from Matsumura et al. 2003)

connected to an appropriate heating device. Precise monitoring of anesthesia is also crucial for the maintenance of physiological and hemodynamic stability within and across PET studies, thus increasing the intrastudy and interstudy reproducibility of PET data.

PET studies in rodents without anesthesia remain challenging for small animal PET experimentation. In a recent study, a non-anesthetized rat was trained to accept head fixation and scanned for 1 h (Momosaki et al. 2004). However, the effects of physical and mental stress imposed on the animal by active restraint have to be analyzed. Furthermore, the compromising effects of anesthesia can be largely avoided for tracers that are efficiently trapped in their target compartment. The animal is then allowed to remain awake during uptake and accumulation of the radioactive probe and is anesthetized only at a later time point when tracer uptake is complete and a steady state has been reached. [^{18}F]-FDG is a good example of a tracer that may be used in such a manner (Matsumura et al. 2003).

12.5 Conclusions

In conclusion, PET radiopharmaceutical development is an iterative process, requires a bit of luck and it can sometimes be a trial and error exercise. One may follow all the guidelines and criteria needed for a successful ligand but there is no guarantee that a successful tracer for human application will be the end product and obviously no successful radiopharmaceutical can fulfill all the requirements.

Suitable animal models may help to facilitate PET tracer development. For example, animals with a targeted disruption of a specific gene can be elegantly used to demonstrate in vivo specificity of tracer binding to the target gene product (Ametamey et al., 2006). However, the validity of each animal model (e.g., murine models for Alzheimer's or Parkinsons's disease) for tracer development has to be carefully evaluated in advance.

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13 Positron Emission Tomography Imaging as a Key Enabling Technology in Drug Development

T.J. McCarthy

Abstract. The use of positron emission tomography (PET) in drug development has become more common in the pharmaceutical industry in recent years. One of the biggest challenges to gaining acceptance of this technology is for project teams to understand when to use PET. This chapter reviews the usage of PET in drug development in the context of target, mechanism and efficacy biomarkers. Examples are drawn from a number of therapeutic areas, but we also show that the relative penetration of this technology beyond CNS and oncology applications has been relatively small. However, with the increasing availability of PET

and development of novel radiotracers it is expected that the utilization will be much broader in future years, with the additional expectation that the use of PET as an efficacy biomarker will also become more evident.

13.1 Introduction

The goal of this manuscript is to describe how noninvasive imaging using positron emission tomography (PET) is positioned to significantly impact drug development. In order to best illustrate this opportunity, examples will be given in the context of a biomarker framework (Littman and Williams 2005) that deals with target, mechanism and efficacy applications. It will be clear from this paper that PET (as well as other noninvasive imaging modalities) can be applied at all stages of the pharmaceutical life-cycle.

One of the key challenges to achieving the optimal use of this technique is to ensure that project teams have clearly identified the questions that are faced at a particular juncture in the discovery and development paradigm. With this information in hand, technology groups can identify the range of potential solutions and find the optimal solution, while avoiding the problem of using technology for technology's sake.

13.2 Biomarker Definitions

There are three definitions of biomarker levels that are commonly used to describe the particular application of a biomarker:

1. Proof of Target (POT)
   In this category, techniques are used to confirm that the pharmaceutical candidate is reaching the desired target in order to confirm the pharmacological mechanism of action. A target biomarker will ideally be able to measure the level of pharmacological inhibition and the duration of action at the target site. In some cases it may be more limited in nature and simply confirm that the drug has reached the target site (for example, brain penetration or tumor uptake). Typically these types of studies are acute in nature and involve looking at changes within a subject from a baseline condition to one following dosing with the candidate pharmaceutical (either single dose or multi-dose).

Demonstration of POT is something that is ideally demonstrated as a part of a phase 1 clinical trial, and so typically examples of this technique are conducted in healthy volunteers.

2. Proof of Mechanism (POM)
   In this category we are looking to confirm that the candidate pharmaceutical is affecting a downstream component of the biochemical pathway related to target inhibition. This might be related to metabolism, flow, proliferation or one of many outcomes. Some mechanistic biomarkers can be evaluated in normal subjects, while others are restricted to patient populations (e.g., tumor effects, etc).
3. Proof of Efficacy (POE)
   Biomarkers of this category are of the highest value to a development program. Changes associated with these biomarkers will have been demonstrated to be directly linked to clinical outcome, and these changes will manifest ahead of the standard clinical response. It is conceivable that a POM biomarker could become an efficacy biomarker following rigorous qualification; examples of biomarkers in this situation will be discussed later. By default, efficacy biomarkers are restricted to a patient population and may require the use of large patient populations in multi-center studies, which raises the need for standardization in the technique and central data analysis.

As one progresses across this biomarker paradigm, it is clear that the rigor in, and cost of, validation of the biomarker increases dramatically for efficacy biomarkers. Target and mechanism biomarkers are likely to be used for internal go/no-go decision making, while *qualified* efficacy biomarkers will form a pivotal component of a regulatory submission package. However, it is likely that regulatory agencies will be interested in supplemental biomarker data to demonstrate that a novel therapeutic agent is able to elicit specific target- and mechanism-related changes.

13.3 PET as a Tool for Proof of Target

Historically the application of PET to drug development has its origins in the CNS therapeutic area. In the 1980s significant advances were made in the development of radiopharmaceuticals to target specific receptor

subtypes. Of those the dopamine D_2 receptor drew significant interest from the community and a number of candidate radiotracers were developed (Volkow et al. 1996). One of the earliest examples reported for receptor occupancy measurements using PET of a novel therapeutic candidate was for ziprasidone (CP-88,059–01); dose-dependent blockade of [^{11}C]raclopride was demonstrated in healthy male volunteers following oral administration of this pharmaceutical and 85% blockade was confirmed with the highest doses administered (Bench et al. 1993).

The typical paradigm used to measure receptor occupancy involves obtaining a baseline PET scan and magnetic resonance (MR) image (as an anatomic reference), the subject is then dosed with the experimental pharmaceutical (typically oral dosing) in either a single- or multi-dose regimen. A subsequent PET scan is obtained along with plasma pharmacokinetic (PK) samples to determine exposure levels of the experimental compound. From these data, receptor occupancy can be derived and a dose- or exposure-to-occupancy relationship obtained from a group of subjects. By addition of the third imaging session after discontinuation of dosing, one is also able to look at the central washout of the drug and compare this with the peripheral PK.

In the case of ziprasidone, temporal binding to striatal D_2 receptors was investigated and the measured binding potential was found to correlate significantly with the serum levels of ziprasidone (Bench et al. 1996). Since ziprasidone also had activity at the $5HT_2$ receptor, additional studies were conducted to investigate the time course of $5HT_2$ receptor occupancy following a single oral dose of ziprasidone using [^{18}F]septoperone, a $5HT_2$ selective radiopharmaceutical (Fischman et al. 1996).

Another more recent example using [^{11}C]raclopride to measure central receptor occupancy described studies of the antipsychotic drug aripiprazole (OPC 14597). In these studies, occupancy was measured (Yokoi et al. 2002) after 14 days of oral dosing and dose-dependent receptor occupancy ranged between 40% and 95%. This study highlights another key facet of target biomarkers; it is extremely important to link receptor occupancy with a clinical endpoint in order to fully comprehend the data. In this case the highest receptor occupancies were not accompanied by extrapyramidal side effects (common issue for dopamine-D_2 antagonism at high levels of occupancy). These data helped to build

confidence that aripiprazole was acting as a partial dopamine receptor agonist (Gründer and Wong 2003).

One of the most valuable contributions of PET-based target biomarkers is to provide sufficient data to confidently stop a research program early. Confirming that a molecule does not reach its target in sufficient concentrations to likely have a therapeutic effect can lead to significant savings by not advancing to a phase 2 "proof of concept" (POC) study; instead alternative chemical matter can be identified and brought forward to the clinic. On the other hand, if acceptable receptor occupancy levels are obtained and one has a negative POC outcome in phase 2, then a clear decision can be made that the underlying hypothesis for that therapeutic approach is invalid. A recent example of this has been highlighted by Merck in the development of NK1 antagonists (Hargreaves 2002), which have been successful in treating emesis, but have failed in other indications. By using PET ([^{18}F]FSPA-RQ) to demonstrate that adequate levels of target inhibition had been achieved, confidence was increased in terminating development for those indications.

13.4 PET as a Tool for Proof of Mechanism

[^{18}F]Fluorodeoxyglucose (FDG) is currently the most widely used diagnostic PET radiopharmaceutical; utilization of this radiotracer to stage and re-stage various cancers has grown enormously in recent years (Juweid and Cheson 2006). Technological advances have also helped to seed this growth; for example, the introduction of the combined PET/CT camera has revolutionized the ability to delineate metastatic disease. In recent years, a growing body of data has suggested that FDG-PET has the potential to monitor cancer therapy (Weber 2005). This has been driven by the need to identify biomarkers of response that occur sooner than reduction in tumor burden.

While FDG-related changes have been investigated in standard chemo- and radio-therapy regimens, a growing number of examples are emerging for novel targeted therapies, especially antiangiogenic agents. One of the most widely cited examples has been the treatment of patients with gastrointestinal tumors (GIST) using a receptor tyrosine kinase in-

hibitor, imatinib mesylate (Gleevec). Profound changes in FDG uptake were noted after a single cycle of therapy (Joensuu et al. 201); this effect has also been observed in other kinase inhibitors, such as sunitinib (SU011248, or Sutent) (Demetri et al. 2003). In both cases the manifestation of an early metabolic response ahead of clinical benefit helped to confirm that the drug was having an effect at the target site.

A key mechanistic feature of antiangiogenic or antivascular therapies is the direct effect on the tumor vasculature, which is unlikely to elicit immediate morphological changes within the tumor. Therefore, the ability to noninvasively monitor changes in *tumor* blood flow would clearly be of great value in exploratory development. Using PET, changes in tumor perfusion and specific measurements of tumor blood flow can be achieved using [^{15}O]water. The first reported use of this approach in a clinical trial was for the novel tubulin-binding inhibitor, combrestatin A4 phosphate (CA4P). In this phase 1 dose-escalation trial, subjects treated with CA4P demonstrated rapid changes in tissue perfusion demonstrating the immediate mechanistic interaction of this agent upon the tumor vasculature.

It is worthwhile digressing for a moment to mention another nonnuclear-based approach to study the effects of these types of interventions. Using diffusion contrast-enhanced MRI (dceMRI) one is able to monitor changes of two key parameters, K_{trans} and IAUC, which are hallmarks of perfusion and vascular permeability. In a recent phase 1 trail of AG-013736, a novel receptor tyrosine kinase inhibitor, this approach was used to show antivascular effects in subjects within 2 days of treatment (Liu et al. 2005).

Returning to FDG, it should be mentioned that as a mechanistic biomarker, a positive signal of target modulation is not limited to *decreases* in uptake of FDG. An example of increased uptake following therapeutic intervention has been shown in the treatment of metastatic breast cancer with tamoxifen (Moritmer et al. 2001). In this study increases in FDG uptake shortly after treatment with tamoxifen were associated with a good response to therapy and indicative of the mechanism-related hormonal flare associated with this therapy.

The examples highlighted in this section emphasize the value of mechanistic PET biomarkers across programs, which contrasts with target biomarkers whose utility is typically restricted to individual projects

or programs, due to the specific nature of the biomarker. Strategically, investment in the development of novel mechanistic biomarkers that have broad utility and can therefore be used for various therapeutic strategies is extremely useful from both economic and operational perspectives. There are a number of other PET radiotracers that are in development and likely to have a significant impact on cancer drug development in the future (Jager et al. 2005); these include markers of proliferation, hypoxia and apoptosis. In the meantime FDG continues to be evaluated in other therapeutic applications and has been demonstrated to be of great value in many areas including diabetes (Virtanen et al. 2003) and neurodegenerative diseases (Reiman et al. 2004).

13.5 PET as a Tool for Proof of Efficacy

The use of PET as a biomarker of efficacy has not fully been exploited, but it is quite likely that this will change in the near future. Since the qualification of an efficacy biomarker involves relatively large clinical trails, the costs of acquiring the PET data have been a barrier to progress. As an example we can return to the application of FDG in oncology, where data are needed to link the response in serial FDG images with an accepted clinical endpoint such as overall survival, or response as defined by RECIST. A number of small single-center trails have demonstrated that FDG-PET changes are correlated with clinical benefit in a number of cancers (Goerres et al. 2005), but the overall numbers of subjects studied have been too small to provide the statistical significance for qualification.

The issue of efficacy biomarker qualification is currently a major focus of the Food and Drug Administration (FDA) and forms a central component of the "Critical Path Initiative" (FDA 2005). This is occurring in collaboration with the National Cancer Institute (NCI) and their efforts (NCI 2006) in the Oncology Biomarker Qualification Initiative (OBQI). At the present time consortia are being assembled in order to conduct the first large-scale demonstration projects of FDG PET as a biomarker of efficacy. These initial projects will lay the groundwork for qualification of PET and other molecular imaging methods as efficacy biomarkers, by identifying and solving many of the questions related to multi-center

image acquisition and analysis. A central issue to this whole program is the need for standardization of the entire process.

13.6 PET as a Tool to Monitor Pharmacokinetics

One application of PET in drug development that is not adequately identified in this biomarker paradigm is the information obtained from simply radiolabeling the drug candidate itself. By administering a radiolabeled candidate, one is able to collect direct pharmacokinetic information about the compound itself and this is a fundamental premise of the microdosing concept (Lappin and Garner 2003). A few examples have been published which demonstrate the spectrum of questions that can be answered with this technique, ranging from monoclonal antibody distribution (Jayson et al. 2002) to the inhaled distribution of antiinflammatory steroids (Berridge et al. 2000).

13.7 The Role of Preclinical PET Imaging in Drug Discovery and Development

This document has focused on the clinical applications of PET in drug development. A significant effort is currently underway within the pharmaceutical industry to evaluate the value of preclinical PET imaging to address the biomarker questions outlined here. A number of companies have made significant internal investments into the technology, while others have entered into strategic alliances with diagnostic imaging companies or academic partners. The challenges of incorporating this technology into such a time-sensitive environment are enormous; however, some early indications of success have been reported (Hamill et al. 2005).

13.8 Conclusion

In this paper the application of PET imaging as a key enabling tool for drug development has been outlined. As can be seen, there are many opportunities for PET in this arena. It is anticipated that the utilization of

this and other noninvasive imaging technologies technology will grow rapidly in drug development in the years to come.

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