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Recent Developments and Trends in ^{18}F-Radiochemistry: Syntheses and Applications

Ralf Schirrmacher[1*], Carmen Wängler[2] and Esther Schirrmacher[3]

[1]*Montreal Neurological Institute, McGill University, Montreal, Canada*

[2]*Department of Radiopharmaceutical Chemistry, German Cancer Research Center, Heidelberg, Germany*

[3]*Lady Davis Institute for Medical Research, Jewish General Hospital, McGill University, Montreal, Canada*

Abstract: In this short review we describe recent methods and novel trends for the introduction of fluorine-18 into molecules which in turn are intended to serve as imaging agents for the *in vivo* imaging modality positron emission tomography (PET). These ^{18}F-labeling schemes are based on enzymatic fluorination, the use of ionic liquids, protic solvents acting as catalysts, application of "click chemistry", thiol-reactive labeling agents for peptide and protein labeling and the most recent introduction of "non-classical" radiochemistry based on organo-phosphorous, organo-boron and organo-silicon radiochemistry. The latter approach for the first time introduced an ^{18}F-chemistry characterized by high selectivity and unique efficiency making complicated work-up procedures obsolete.

Keywords: Fluorine-18, positron emission tomography (PET), labeling chemistry.

1. INTRODUCTION

Due to the high dissemination of positron emission tomography (PET) [1], an imaging modality investigating the distribution of radiolabeled biomarkers *in vivo* (humans and animals), the syntheses of radiolabeled biologically active compounds such as peptides, neuro-transmitter ligands and enzyme targets with the positron emitting radio-halide fluorine-18 has gained widespread interest in life science [2]. Fluorine-18 can be considered to be among the "ideal" positron emitters for PET because of its physical characteristics: a half-life of 110 min to conduct scans over several hours and a low positron energy allowing for images of highest resolution. The labeling methods for the introduction of ^{18}F into complex organic molecules such as peptides or proteins so far described are most often characterized by multi-step synthetic pathways, synthesizing small ^{18}F-labeled molecules (prosthetic groups) which have to be prepared in advance by complicated procedures before final conjugation to the bio-marker of interest [3]. Due to the very restricted chemistry of ^{18}F which is determined by the production method of ^{18}F (*via* irradiation of specific targets e.g. [^{18}O]water with protons or neon-20 with deuterons) [4] yielding either anionic ^{18}F$^-$ or carrier (non radioactive [^{19}F]fluorine gas) added [^{18}F]F$_2$, the general synthesis of those ^{18}F-prosthetic groups is very limited in terms of chemistry. In the case of anionic ^{18}F$^-$, the syntheses of these precursor compounds, mainly ^{18}F-labeled alkylating agents, amines, aldehydes and acid chlorides involve nucleophilic substitutions using suitable leaving groups [5]. A most recent achievement is the regioselective nucleophilic ^{18}F-fluorination of electron-rich arene compounds using heteroaromatic iodonium salts [6], a fluorination which was so far only possible by the use of activated electron-deficient aromatic systems. In the case of electrophilic [^{18}F]F$_2$, activated aromatic systems for the often unselective electrophilic substitution are used although the use of tin or mercury containing fluorination precursors result in higher regioselectivities. Besides [^{18}F]F$_2$, which is by far the most "untamed" electrophilic labeling agent, numerous attempts to introduce more selective reagents by converting [^{18}F]F$_2$ to secondary labeling synthons have been described in the literature. As all these labeling agents are based on [^{18}F]F$_2$, they all suffer from a limited specific activity[1], which of course constricts the use of electrophilic fluorinations in radiopharmaceutical chemistry [7]. However, it has been demonstrated by Solin and co-workers that relatively high specific activities of [^{18}F]F$_2$ of 4GBq/µmol are possible by a post-target conversion of [^{18}F]F$^-$ to [^{18}F]F$_2$ [8]. Furthermore, electrophilic ^{18}F-fluoronitrogen reagents have been described [9] as ^{18}F-labeling tools and the most recent study introducing N-[^{18}F]fluorobenzenesulfonimide proves that it is still a hot topic [10]. Along with their complexity, all those methods, nucleophilic as well as electrophilic approaches, yield unwanted radioactive and non-radioactive by-products which have to be separated from the product by means of High Performance Liquid Chromatography (HPLC). This is very time consuming, demands special equipment such as expensive synthesis modules, HPLC etc. and trained personnel, resulting in a restricted availability of *in vivo* imaging by PET to research centers and financially strong companies only. In the light of this situation and the high demand for ^{18}F-radiopharmaceuticals in nuclear medicine, many groups have searched for novel synthetic procedures to facilitate the introduction of ^{18}F into tracer molecules for PET. Recent synthetic procedures described e.g. the use of enzymatic chemo-selective ^{18}F-labeling reactions providing a high specificity as well as good radiochemical yields or the use of the 1,3-dipolar Huisgen cycloaddition, an example of the so-called "click chemistry", for the reaction of ^{18}F-

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*Address correspondence to this author at the Montreal Neurological Institute, McGill University, Montreal, Canada;
E-mail: ralf.schirrmacher@mcgill.ca

[1] Specific activity is expressed in GBq (amount of radioactivity)/µmol (sum of labeled- and unlabeled compound).

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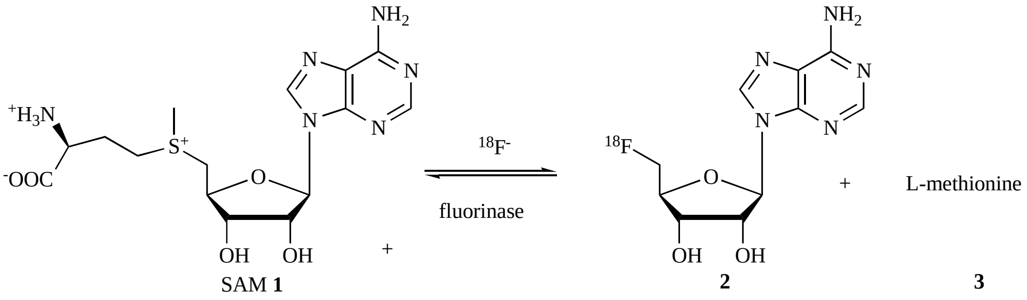
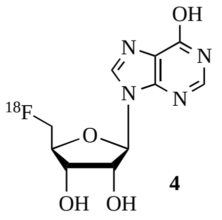
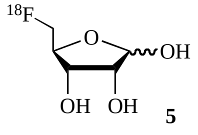
labeled azides with alkynes (or vice versa), a very promising tool in radiochemistry which deserves a special focus in this review. Thiol-reactive ^{18}F-labeled synthons emerged within the last few years as valuable tools for the ^{18}F-labeling of proteins and peptides and will therefore get particular attention. Other groups abandoned the classical carbon-fluorine chemistry and evaluated the use of phosphorous organic chemistry and the use of organo-silicon derivatives to generate novel ^{18}F-labeled molecules for their potential use in radiopharmaceutical sciences. Most of these methods introducing new radiochemical approaches are still academic and it has to be proven if they will find their way into the routine production of PET radiopharmaceuticals. One major concern, regardless of the particular labeling chemistry, is the inevitable alteration of an original biomolecule by using ^{18}F-labeling techniques. Especially the use of ^{18}F-labeled prosthetic groups for final labeling often imparts a certain level of lipophilicity to the molecule of interest and often leads to a changed *in vivo* behavior. This mini-review article thoroughly describes all recent developments and attempts in the field of ^{18}F-radiochemistry possibly leading to the introduction of new ^{18}F-based radiopharmaceuticals in life sciences. A major concern of this article is to focus on the potential strengths of the described methods but also on contingent problems and weaknesses.

2. GENERAL CONSIDERATIONS IN ^{18}F-CHEMISTRY

One general predicament in nucleophilic ^{18}F-fluorination chemistry (by far the most common labeling procedure) where a C-^{18}F bond is formed by the use of $^{18}F^-$ is the requirement for so called "naked" highly nucleophilic $^{18}F^-$ anions in dipolar aprotic solvents such as acetonitrile, DMF or DMSO [11]. In the presence of water, $^{18}F^-$ forms hydrogen bonds which decrease its nucleophilicity. The state of the art method for obtaining "naked" un-solvatized $^{18}F^-$ for further reactions involves azeotropic drying of the aqueous $(H_2[^{18}O]O)/^{18}F^-$ solution by using either a phase catalyst such as Kryptofix2.2.2® (K222) and K_2CO_3 as a base or tetrabutylammonium hydroxide or tetrabutylammonium bicarbonate with $^{18}F^-$ [12]. For removing all traces of water, the phase transfer catalyst K222 and a basic solution of K_2CO_3 (1N) are added and the mixture is dried in a stream of



enzyme system	RCY [%]	time [h]	product
wild type fluorinase	1	5	2
fluorinase (8 mg/mL)	26	4	2
fluorinase (8 mg/mL)- L-amino acid oxidase^a	70	4	2
fluorinase (24 mg/mL)- L-amino acid oxidase^b	95	2	2
fluorinase (24 mg/mL)- adenyl acid deaminase^b	75	4	 4
fluorinase(24 mg/mL) -PNP-phytase^b	45	4	 5

^{a} at 25 °C
^{b} at 35 °C

Fig. (1). Enzymatic ^{18}F-labeling using fluorinase/fluorinase enzyme combinations.

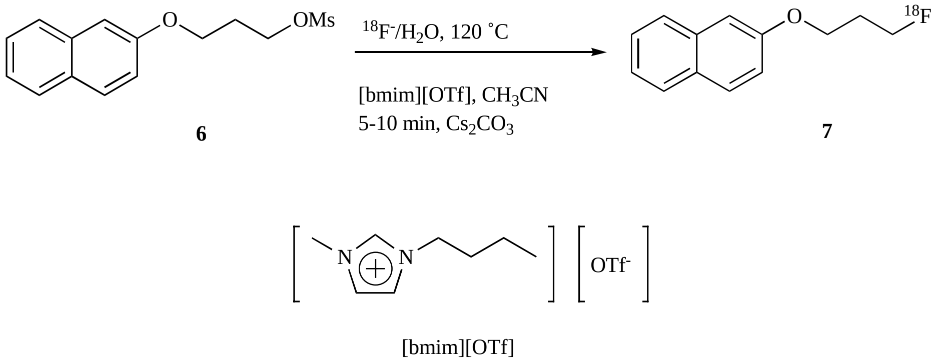
nitrogen at temperatures between 80-110ºC. The formed K^+/K222/$^{18}F^-$ is readily soluble in dipolar aprotic solvents and thus the $^{18}F^-$ is not solvatized and highly reactive. Unfortunately, the addition of K_2CO_3 or potassium oxalate (as a weaker base) is mandatory to prevent the release of H[^{18}F]F during the drying process but often leads to complications when the compound to be labeled (the precursor molecule) is base sensitive. Normally (not in every case as we will see later on), the addition of water as a co-solvent prevents the labeling reaction completely by solvatizing the $^{18}F^-$ (decreasing nucleophilicity) and generating hydroxyl ions under basic reaction conditions which are most often incompatible with precursor molecules. Therefore many research groups searched for alternative labeling reactions for the mild but selective introduction of ^{18}F into biomolecules.

3. ENZYMATIC ^{18}F-FLUORINATION

The idea behind using enzymes for the introduction of ^{18}F is obvious, namely the search for chemo-selectivity in ^{18}F-fluorination chemistry. In contrast to normally used often unselective conventional fluorine chemistry involving the formation of a C-F bond, an enzymatic introduction of ^{18}F would proceed bio-catalytically controlled. One obvious problem is the lack of fluorine in nature making the demand for enzymatic C-F bond formation rare. However, the recent finding that a fluorination enzyme, isolated from the bacterium *Streptomyces cattleya* is capable to form C-F bonds has given a certain prospect to the general idea of enzymatic ^{18}F-fluorination of biomolecules [13]. The first approach in this direction was done by Martarello *et al.* using the aforementioned enzyme (wild type *fluorinase*) for the chemo-specific introduction of ^{18}F into 5'-[^{18}F]fluoro-5'-deoxyadenosine ([^{18}F]-5'-FDA, **2**) as a potential tumor imaging agent [14]. As a precursor molecule for the synthesis of **2**, S-adenosyl-L-methionine (SAM, **1**) was incubated with *fluorinase* in Tris-HCl buffer (pH 7.8) of different concentrations (0.2-4 mg/ml) and $^{18}F^-$ in [^{18}O]water directly supplied from the cyclotron (the ^{18}F isotope is produced by irradiation of oxygen-18 from [^{18}O]H_2O with protons, supplied by a cyclotron *via* the nuclear reaction ^{18}O (p,n) ^{18}F) (Fig. **1**). Incubations took place at 40ºC for 5h which is, taking the ^{18}F half life of 110 min into account, far too long and the overall radiochemical yield (RCY) of the reaction was 1% only (corrected for radioactive decay) making this approach academically interesting but technically inapplicable. The reason for the low RCY was elucidated by the same group later on in 2006 pointing out that the actual ^{18}F-fluorination made by the *fluorinase* is a reversible process, impeding high RCY of ^{18}F-fluorinated product. Hence their final goal was to find a way to pull the ^{18}F-transfer towards product formation, in this particular case, [^{18}F]-5'-FDA (**2**) and derived products [15]. This was elegantly achieved using various *fluorinase (recombinant fluorinase)* coupled enzyme systems which successfully adjourned chemical equilibrium (Fig. **1**). The enzyme L-amino acid oxidase e.g. successfully converted L-methionine (**3**) (and therefore removed it from equilibrium) which is formed during the *fluorinase* reaction. By using three other enzymes coupled to the *fluorinase*, even higher amounts of labeled compounds such as [^{18}F]-5'-FDI (**4**) and [^{18}F]-5-FDR (**5**) were available. Reasonable RCY (decay corrected) between 45-75% could be achieved but the synthesis time of 1-4h is, as the authors correctly stated, relatively long to produce radiopharmaceuticals which are manufactured on a daily base for patient applications. If this method would be further improved in terms of reaction time and variety of available compounds it might have the potential to make it a standard practice.

4. ^{18}F-FLUORINATIONS IN IONIC LIQUIDS

In 2003 Kim *et al.* reported the use of ionic liquids as a reaction medium in the nucleophilic ^{18}F-labeling of an aliphatic mesylate (**6**) yielding the corresponding ^{18}F-compound (**7**), a new labeling method tolerating even the presence of water, obviating the typical time consuming drying procedure of $^{18}F^-$ [16]. As a relatively mild base, Cs_2CO_3 was added to the aqueous reaction mixture to prevent the emanation of [^{18}F]HF at reaction temperatures of 120º (Fig. **2**). The ionic liquid contains a lipophilic cation structure element based on imidazolium salts (e.g. [bmim] [OTf], Fig. **2**) and different counter ions. It has been impressively demonstrated that ionic liquids as solvents for organic reactions can be considered as valuable alternatives to the currently used volatile organic solvents such as acetonitrile [17]. Besides a successful application in the improved synthesis of [^{18}F]FDG [18], which is the most commonly applied PET-radiopharmaceutical and often called the "work horse" of nuclear medicine [19], the general value of ionic liquids as a true alternative for conventional ^{18}F-fluorination is still pending. Until now, except for the synthesis of [^{18}F]FDG, just simple ^{18}F-fluorinations of a



6 **7**

[bmim][OTf]

Fig. (2). ^{18}F-fluorination in ionic liquids.

model mesylate, namely 2-(3-methanesulfonyloxypropoxy) naphthalene (**6**) and a α-bromoacetophenone (not shown) have been successfully demonstrated. The addition of a base is mandatory and reaction temperatures of 110-140ºC are quite high and probably not tolerable for all kinds of precursor molecules. Although the reaction proceeded efficiently in the presence of small amounts of water (50 μL), the RCY dropped considerably when higher quantities (250 μL) were added. This could be a drawback in terms of generating large radioactivity amounts of radiopharmaceuticals, because the volume radioactivity of the aqueous $^{18}F^-$ has to be very high to keep the water content reasonably low.

5. PROTIC SOLVENTS AS CATALYSTS IN NUCLEOPHILIC ^{18}F-FLUORINATION

It was common knowledge for the last decades that nucleophilic ^{18}F-fluorinations do not work in aqueous media, a statement which was successfully disproved by using ionic liquids as reaction media in radioactive fluorinations. Moreover, the most recent finding illustrating that protic solvents such as tertiary alcohols can even facilitate nucleophilic reactions with alkali metal fluorides was even more unexpected because normally anion nucleophilicity is reduced as a result of the interaction with the partial positive charge present in protic solvents. It was demonstrated by Chi and co-workers that non-radioactive fluorinations of various model compounds using CsF in tert-amyl alcohol gave the corresponding fluorinated products in good to excellent yields at temperatures between 25 and 90ºC (to reflux) [20]. The use of tertiary alcohols seems to further improve selectivity towards nucleophilic reactions by concurrently reducing the amount of radioactive by-products such as alkenes, alcohols and ethers, which often occur when using conventional methods in ^{18}F-labeling chemistry. The applicability of this new method to the production of routinely produced radiotracers as molecular imaging agents was impressively proven by improving the syntheses of [^{18}F]FDG (**8**), [^{18}F]FLT (**9**), [^{18}F]FP-CIT (**10**) and [^{18}F]FMISO (**11**) (Fig. **3**). Most recently Lee *et al.* demonstrated an automated high RCY synthesis of [^{18}F]FP-CIT (**10**), a radioligand for dopamine transporter imaging, using t-BuOH as a solvent [21]. This new chemistry (radiochemistry) of $^{19/18}F^-$ in tertiary alcohols is not yet fully understood and the results are "striking" as the authors stated. One characteristic is especially interesting: the reactivity of the halide ion in these solvents seems to be reversed. The F^- is more reactive than Br^-, which is normally

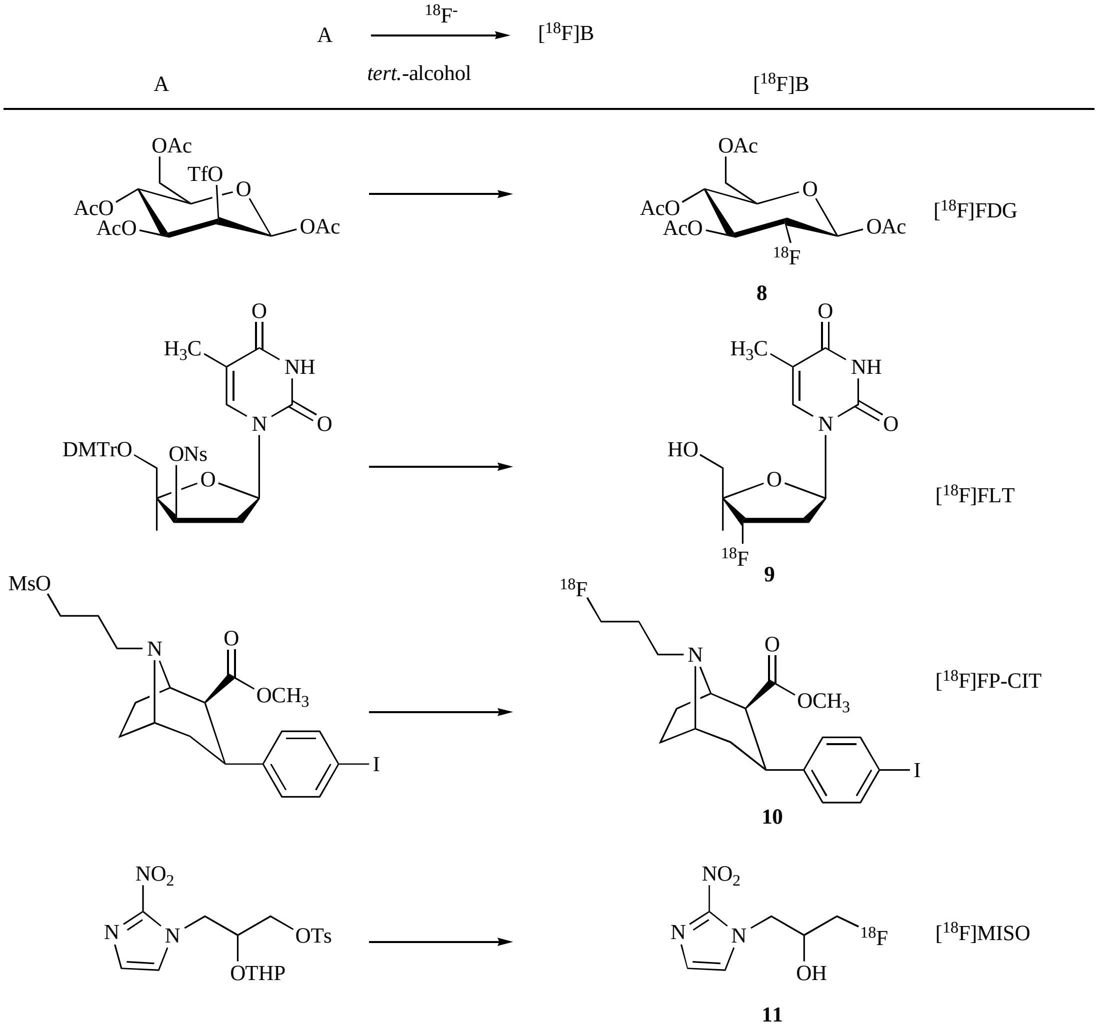


Fig. (3). ^{18}F-fluorination in tertiary alcohols.

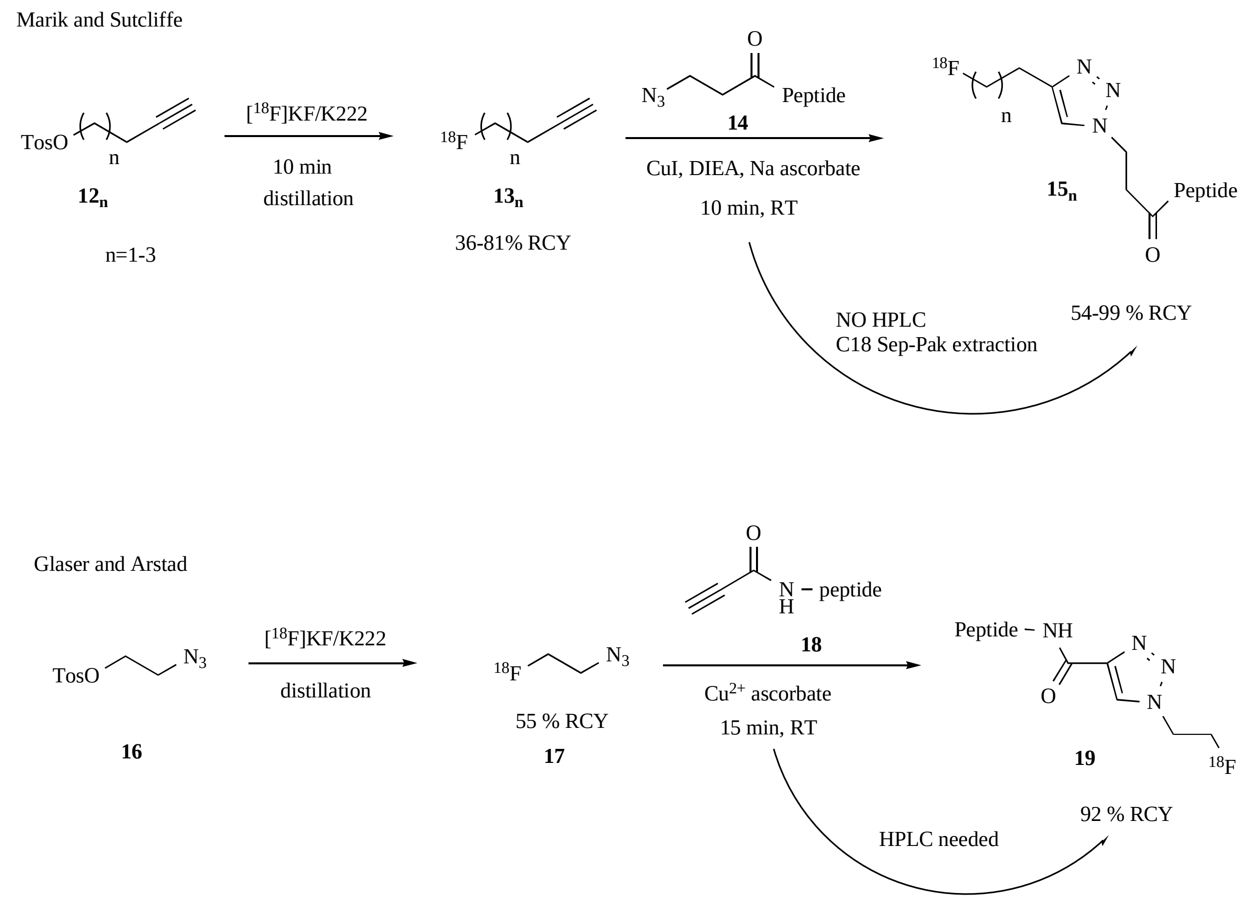


Fig. (4). ^{18}F-labeling using "click-chemistry".

the other way around in protic solvents. Although these first results are strongly encouraging, the future will tell whether this ^{18}F-labeling chemistry finds its way into the routine production of ^{18}F-radiopharmaceuticals.

6. ^{18}F-CLICK-CHEMISTRY

Recently, the usefulness of the 1,3-dipolar Huisgen cycloaddition, also called "click-chemistry" for the preparation of PET radiopharmaceuticals has been demonstrated. The term was coined by K. Barry Sharpless in an article published in Angew. Chem. Int. Ed. and denotes chemical reactions selectively providing high yields of products from a variety of easily accessible building blocks [22]. The most commonly used click-reaction is the Cu(I) catalyzed Huisgen reaction, a 1,3-dipolar cycloaddition of terminal alkynes with azides, yielding 1,4-disubstituted 1,2,3-triazoles under mild conditions. This reaction owns its usefulness to the relative ease with which both necessary functional moieties, azide and alkyne, can be introduced into various molecules. Both groups are relatively stable to the majority of common reaction conditions in organic synthesis so that they can be introduced into target molecules whenever convenient [23]. The uncatalyzed Huisgen cycloaddition usually yields a mixture of 1,4- and 1,5-disubstituted triazoles and proceeds rather slowly, so that its applicability for radiochemistry with short-lived isotopes had not been recognized [24]. It was only the recent discovery by Huisgen and Meldal that Cu(I) catalysis leads to 1,4-regioisomers only while drastically enhancing reaction rates that led to the application in radiochemistry [25].

The first paper using click-chemistry for radiolabeling was published by Marik and Sutcliffe in 2006, describing a procedure for obtaining ^{18}F-fluoropeptides [26]. They reacted ω-[^{18}F]fluoroalkynes (**13**$_{\mathbf{n=1-3}}$) with peptides bearing *N*-(3-azidopropionyl)-groups (**14**) (Fig. **4**). The syntheses of the three different ^{18}F-fluoroalkynes, the butyne, pentyne and hexyne were accomplished by reacting the corresponding tosylalkynes (**12**$_{\mathbf{n=1-3}}$) with dried ^{18}F$^-$/K222/K$^+$ complex for 10 minutes, followed by a co-distillation with acetonitrile. While the reported radiochemical purities were high (>98%) in all cases, the reaction yields varied significantly. The 4-[^{18}F]fluoro-1-butyne (**13**$_{\mathbf{n=1}}$) was obtained in 31% yield only, while 5-[^{18}F]fluoro-1-pentyne (**13**$_{\mathbf{n=2}}$) and 6-[^{18}F]fluoro-1-hexyne (**13**$_{\mathbf{n=3}}$) were obtained with a yield of 81% and 61% respectively. The authors did not state the reason for the differences, but it seems likely that the co-distillation with acetonitrile could be responsible for varying RCY. In the case of **13**$_{\mathbf{n=1}}$, the boiling point of 45°C probably leads to trapping difficulties, whereas the boiling point of **13**$_{\mathbf{n=3}}$ (106°C) prevents a complete distillation into the product vial (distillation temperature given as 100°C). Nonetheless, the speed and ease of the experimental set-up more than make up for the loss in radiochemical yield.

The subsequent reaction of the [^{18}F]fluoroalkynes (**13**$_{\mathbf{n=1-3}}$) with the azide-derivatized peptides (**14**) proceeded with radiochemical yields of 10% within 30 min of the ^{18}F-labeled peptides (**15**$_{n=1-3}$), using Cu(II)sulfate and sodium ascorbate as catalyst. This in situ reduction of Cu(II) to obtain the Cu(I) catalyst was originally published by Sharpless [27]. Marik and Sutcliffe found that a Cu(I) iodide together with a nitrogen base resulted in drastically improved radiochemical yields of nearly 100% after 10 min reaction time only. Sodium ascorbate was added to prevent the oxidation of Cu(I) by atmospheric oxygen. Several papers support the vital role of the catalyst systems on obtainable yields. Fazio and co-workers studied reactions using Cu(I) iodide with triethyl amine and diisopropyl ethyl amine (DIPEA) or without base in organic solvents [28]. They found that in water-free environments, the absence of any base led to very slow reaction rates, probably due to the absence of the copper acetylide. However, not only the presence of a base, but also its nature affects the reaction yields: they report that the use of triethylamine resulted in no product formation, while the use of DIPEA led to reaction yields of 38%. Marik and Sutcliffe tested DIPEA, pyridine and piperidine and reported that although the reaction rate increased with piperidine, this led to the formation of unspecified by-products. They obtained the best results with DIPEA, and in the case of one peptide found that the addition of pyridine improved the purity of the product. The nitrogen base was present in 10fold excess relative to the Cu(I) iodide, or 400fold excess relative to the azide-derivatized peptide. The two radioactive reaction steps, the synthesis of the ω-[^{18}F]fluoroalkyne (**13**$_{n=1-3}$) and the reaction with the azide component, proceed rapidly and in good to excellent yields, with final specific activities of > 35GBq/μmol of the ^{18}F-labeled peptides. The strength of Marik and Sutcliffe's approach is undoubtedly the decision to use ω-[^{18}F]fluoroalkynes (**13**$_{n=1-3}$) instead of ^{18}F-fluoroalkylazides (cf. Glaser and Arstad, Fig. **4**) as secondary labeling synthons since this allows the purification of both the ω-[^{18}F]fluoroalkyne (**13**$_{n=1-3}$) and the final ^{18}F-labeled peptide by distillation (in the latter case by removing unreacted ω-[^{18}F]fluoroalkyne) rather than time-consuming and cumbersome HPLC purification. They thus achieved the ^{18}F-labeling of peptides in reaction times of 30 min in sufficient yields.

Some months after Marik and Sutcliffe's publication, Glaser and Årstadt published a similar approach [29]. They also reported a click-labeling approach with the secondary labeling precursor 2-[^{18}F]fluoroethylazide (**17**). They decided on the ^{18}F-azide because alkynes are more readily available and less hazardous than organic azides. Thus, their approach to click-labeling used 2-azidoethyl 4-methylbenzenesulfonate (**16**), which was converted to **17** by reacting with the dried ^{18}F$^-$/K222/K$^+$ complex in acetonitrile (Fig. **4**). After 15 min the ^{18}F-azide was purified by distillation providing decay-corrected RCYs of 54%. Glaser and Årstadt reported the use of this labeling synthon to obtain different 1,4-disubstituted triazoles in the presence of amine and carboxylic groups, among others. They tested different catalysts, Cu(II)-sulfate with sodium ascorbate and copper powder. The reaction was allowed to proceed for 15 min at room temperature, and yields varied considerably, depending not only on the catalyst, but also on the alkyne substrate used. After heating the reaction mixture to 80°C, the reaction was allowed to proceed for another 15 min, which then resulted in moderate to excellent yields (15-99%) of the triazoles. At 80°C, the Cu(II)sulfate proved to be the better catalyst for all published alkynes.

To prove that their approach also works satisfactorily for peptide labeling, Glaser and Årstadt labeled a model peptide derivatized with propargylic acid (**18**) using **17** at room temperature in 15 min (Fig. **4**). The reaction yields reported were excellent (92%, decay corrected), but unfortunately HPLC purification was required to obtain the ^{18}F-labeled peptide. The overall preparative yields for this model peptide were 50%, the synthesis time was not explicitly stated, but with 2 steps at 15 min each and a distillation step followed by HPLC purification, it is safe to assume that the overall synthesis time was in the range of 45 min at least. Although no *in vivo* data have yet been published, click-chemistry for fluorine-18 labeling has the potential to develop into a versatile labeling tool, provided that toxicity and *in vivo* stability prove to be satisfactory. The current interest in click chemistry for ^{18}F-labeling has been substantiated by recent reports from the 17th International Symposium on Radiopharmaceutical Science of several new ^{18}F-radiopharmaceuticals based on 1,3-dipolar cycloaddition [30].

7. ^{18}F-LABELING OF PEPTIDES WITH A FOCUS ON THIOL LABELING AGENTS

Most F-18 bearing labeling synthons for peptides and proteins such as carboxylic acids or esters target primary amino functions, either at the N-terminus of peptides (α-NH$_2$) or at internal lysine residues (ε-NH$_2$). The disadvantage of this approach is the unselectivity of the radioactive label introduction, due to the relative abundance of lysine in proteins. Many different strategies to label larger multifunctional molecules such as peptides and proteins have been described over the years. Acylation and photochemical conjugation [31] as well as the use of alkylating agents [32], N-succinimidyl 4-[^{18}F]fluorobenzoate [33], 2-[^{18}F]fluoropropionic acid [34], p-[^{18}F]fluorophenacyl bromide [35], 4-[^{18}F]fluorobenzyl halides [36], ^{18}F-labeled thiols [37], solid phase ^{18}F-fluorinations [38] and a hydrazone-formation by coupling 4-[^{18}F]fluorobenzaldehyde to a hydrazinonicotinic acid (HYNIC) derivatized human serum albumine [39] have been described. A free thiol function, on the other hand, is not very common in most proteins, and is only present in cysteine residues. When using radiolabeling synthons targeting thiol groups, a more site-specific modification of peptides and proteins becomes feasible. Furthermore, under physiological conditions, the thiol moiety is more nucleophilic than amines. Most strategies for developing thiol reactive secondary labeling precursors are based on a maleimide group for thiol specific Michael addition reactions. Apart from maleimides, Kuhnast *et al.* have described the use of N-(4-[^{18}F]fluorobenzyl)-2-bromoacetamide as a thiol-reactive synthon in the synthesis of ^{18}F-labeled peptide nucleic acids (PNAs) [40]. It was later demonstrated by Kuhnast *et al.* that the same synthon can be used for the ^{18}F-labeling of N-terminus-modified PNAs [41].

The first instance of a thiol-reactive labeling reagent was published by Shiue *et al.* in 1998 [42]. They used 1-(4-[^{18}F]fluorophenyl)pyrrole-2,5-dione ([^{18}F]FPPD, not shown) and *N*-[3-(2,5-dioxo-2,5-dihydropyrrol-1-yl)phenyl]-4-[^{18}F]

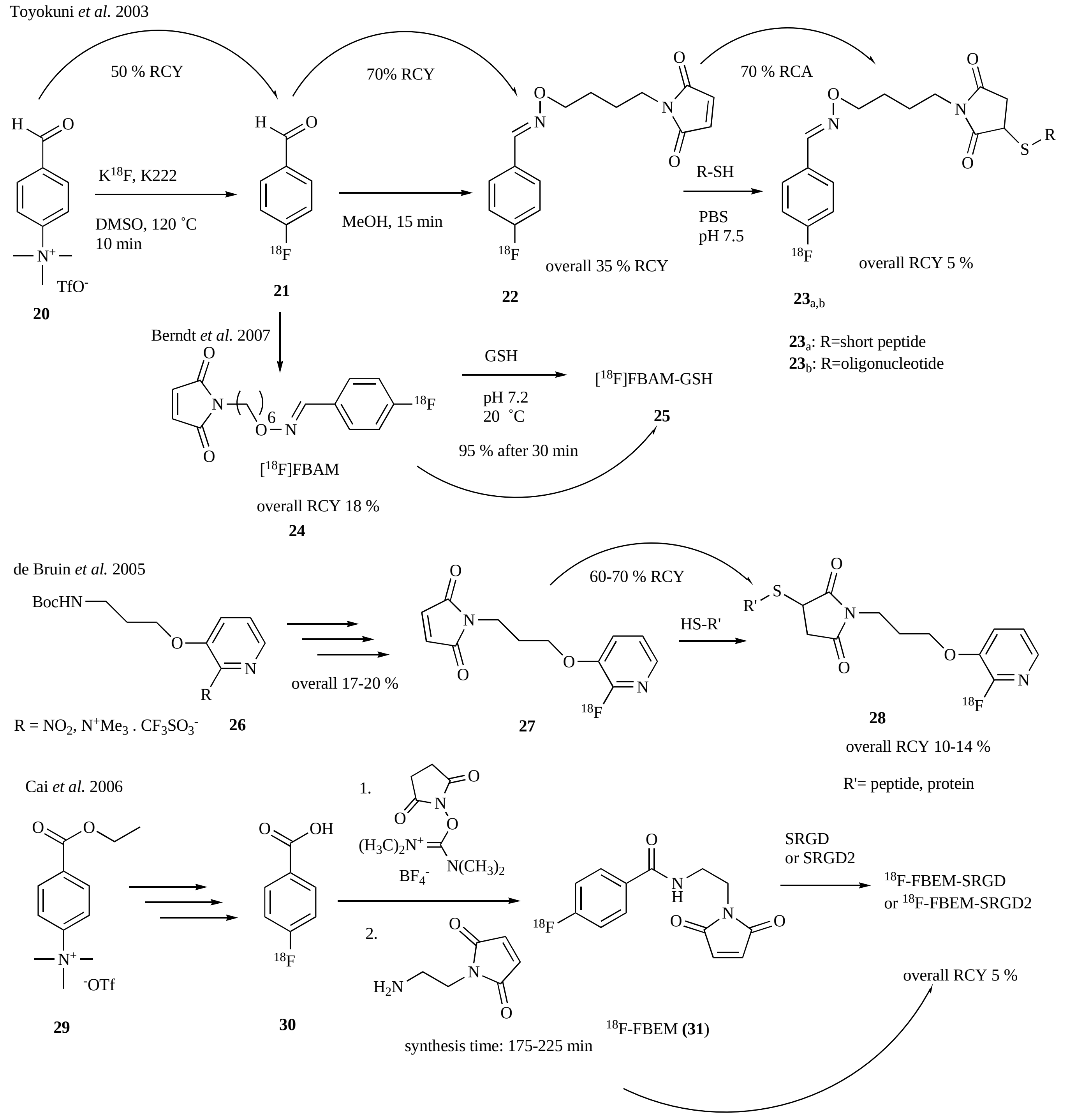


Fig. (5). Thiol reactive ^{18}F-labeling agents.

fluorobenzamide ([^{18}F]DDPFB, not shown) for radiolabeling monoclonal antibodies in a multistep preparation. Since then, little efforts had been made on the development of thiol-reactive labeling precursors until the last 4 years. In 2003, Toyokuni *et al.* reported the synthesis of *N*-{4-[(4-[^{18}F]fluorobenzylidene)aminooxy]butyl}malei-mide (**22**) (Fig. **5**) [43]. Their first reaction step was the formation of 4-[^{18}F]fluorobenzaldehyde (**21**) from the trimethyl ammonium labeling precursor (**20**), followed by reaction with an aminooxy derivatized butyl maleimide derivative to yield **22**. While the first reaction step yielding **21** only required a rapid SepPak solid phase extraction, the final labeling synthon (**22**) needed an HPLC purification. All in all, the radiosynthesis took 60 min and yielded the secondary thiol reactive labeling precursor in 35% RCY (decay corrected). They also report the reaction of **22** with a model peptide and a thiol-functionalized oligodeoxy-nucleotide in phosphate buffered saline at room temperature to yield the corresponding ^{18}F-labeled compounds (**23$_{a,b}$**). While the model peptide could be labeled in yields of 70% in 30 min, the oligodeoxynucleotide only gave yields of 5% after gel filtration purification in 60 min (Fig. **5**).

In 2007, Berndt *et al.* published another thiol reactive reagent, *N*-[6-(4-[^{18}F]fluorobenzylidene)aminooxyhexyl]maleimide ([^{18}F]FBAM, **24**). Their synthesis also used **20**, affording **21**, which in turn was reacted with *N*-(6-aminohexyl)maleimide to obtain **24** with radiochemical yields of 29% within 70 min reaction time, including a final HPLC purification (Fig. **5**) [44]. This approach is quite similar to the one chosen by Toyokuni *et al.* [40], which also used **21** as the radioactive intermediate to obtain the homologous, two carbons shorter synthon. Although no purification seemed to be necessary, [^{18}F]FBAM (**24**) does offer only slight advantages in terms of chemistry, since Toyokuni's method resulted in 60 min reaction time and a radiochemical yield of 35%. Specific activities of their respective thiol reactive labeling precursors were similar with 76 GBq/μmol for [^{18}F]FBAM (**24**) and 125 GBq/μmol for Tokoyuni's secondary labeling precursor. The reactivity of **24** was tested with the tripeptide glutathione and different apolipoproteins of human low-density lipoproteins (LDL). Reaction yields of the ^{18}F-labeled compounds (**25**) were in the range of 20% within 45 min for LDL (not shown), the short peptide (GSH) reacted within 30 min with radiochemical yields of 95%. It was further mentioned by the authors that the lipophilicity of **24**, when introduced into biomolecules, might be a concern regarding its *in vivo* behavior.

A different approach to thiol reactive labeling precursors was published by deBruin *et al.* in 2005. Instead of using homoaromatic nucleophilic substitutions to introduce the fluorine-18 label into the labeling synthon, they chose a heteroaromatic nucleophilic substitution to improve radiochemical yields (Fig. **5**) [45]. They introduced ^{18}F$^-$ into a Boc-protected aminopropoxy-pyridine (**26**) with either a nitro or trimethylamino leaving group. After cleavage of the Boc group with trifluoroacetic acid, the amino function was reacted with *N*-methoxycarbonylmaleimide to obtain the final secondary labeling precursor (**27**). After this 3 step radiosynthesis, the thiol-reactive labeling precursor [^{18}F]FPyMe (**27**) was obtained in 28-37% RCY (decay corrected) after 110 min reaction time including an HPLC purification. They report the use of this labeling synthon to obtain a fluorine-18 labeled model peptide and two 8 kDa proteins. The conjugation step of **27** to the biomolecules proceeded in buffer at extremely mild reaction conditions yielding the ^{18}F-labeled compounds (**28$_{a,b}$**) with good yields (60-70% isolated) within 10 min. So the overall reaction time to obtain fluorine-18 labeled proteins with **27** was 130-140 min.

Cai *et al.* published a p-fluorobenzamidoethyl maleimide ([^{18}F]FBEM, **31**) as a thiol-reactive secondary labeling precursor (Fig. **5**) [46]. They report the preparation consisting of 3 steps, with 5% non-corrected radiochemical yields and a reaction time of 150 min. The starting material was the trimethyl ammonium salt (**29**), which was ^{18}F-fluorinated and subsequently hydrolyzed to obtain 4-[^{18}F]fluorobenzoic acid (**30**). After conversion into **30** [47], obtained and purified *via* solid phase extraction, the final reaction step with N-(2-aminoethyl) maleimide yielded their secondary labeling precursor [^{18}F]FBEM (**31**), which was purified by HPLC. Specific activity was determined to be in the range of 150-200 GBq/μmol. Cai *et al.* also reported the successful use of this thiol reactive labeling precursor for the labeling of monomeric and dimeric sulfhydryl-RGD peptides yielding the ^{18}F-labeled peptides in 85% (non-decay corrected, based on **31** starting activity) after 20 min reaction time under mild conditions (PBS buffer, pH 7-7.5). *In vivo* experiments proved the metabolic stability of the ^{18}F-C bond by the absence of radioactivity uptake into bone as a result of non bound ^{18}F$^-$ even after 4 h.

The most recent development in thiol reactive labeling precursors was published by Prante *et al.* in 2007 [48]. This study described the synthesis of an ^{18}F-labeled glycosyl synthon Ac$_3$-[^{18}F]FGlc-PTS (**35**), an acetyl protected 2-deoxy-2-[^{18}F]fluoroglucopyranosyl phenylthiosulfonate (Fig. **6**). Their approach aims at combining the radiolabeling step with a glycosylation for improving the biokinetics of prospective radiotracers and to enhance bioavailability and *in vivo* clearance [49]. **35** was synthesized in 3 steps with an overall RCY of 33% within 90 min (Fig. **6**). The first reaction step is based on the FDG synthesis [50], where an acetylated manose triflate labeling precursor (**32**) is reacted with ^{18}F$^-$. Reaction with hydrogen bromide in acetic acid converted the tetra acetylated 2-deoxy-2-[^{18}F]fluoroglucose (**33**) into the corresponding α-bromide (**34**), which was

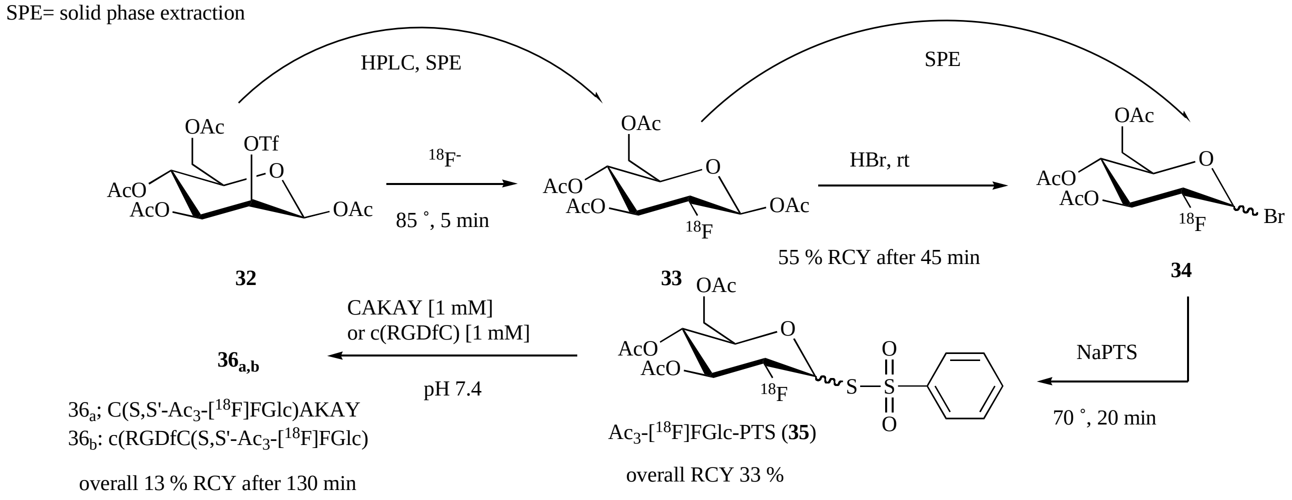
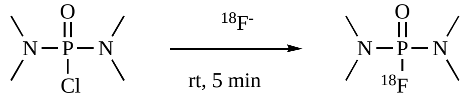


Fig. (6). A thiol ^{18}F-labeling agent based on glucose.

purified by solid phase extraction. The subsequent reaction step for conversion into the phenylthiosulfonate (**35**) yielded the secondary labeling precursor after 20 min. In order to assess the ^{18}F-labeling ability of **35** for the labeling of peptides, the labeling of a model pentapeptide (CAKAY) and a cyclo-RGD derivative was performed. This conjugation step was highly efficient yielding the ^{18}F-labeled peptides (**36$_{a,b}$**) in 90-95% within 15 min under mild conditions (Fig. **6**). Incubation experiments of **36$_{a,b}$** with human serum confirmed metabolic stability of the ^{18}F-fluoroglycosylated RGD (**36$_a$**) derivative for the investigated 90 min. In comparison to the other published thiol reactive labeling synthons, Ac$_3$-[^{18}F]FGlcy-PTS (**35**) yields approximately the same RCYs for the ^{18}F-peptides. Notably, the concept of using ^{18}F-labeled sugars as prosthetic groups in radiochemistry has been continued by introducing UDP-2-deoxy-2-[^{18}F]fluoro-α-D-glucopyranose (not shown), a derivative of 2-[^{18}F]FDG, as a potential substrate for glycosyltransferase [51]. Whether or not the simultaneous glycosylation has a beneficial impact on lipophilicity of prospective radiotracers, and whether or not the other thiol reactive secondary labeling precursors add lipophilicity to their respective target molecules remains to be elucidated and would be an interesting study for the future.

8. INTRODUCTION OF P-^{18}F, B-^{18}F AND SI-^{18}F CHEMISTRY: A POSSIBLE ALTERNATIVE?

All the above mentioned methods using the conventional formation of a C-^{18}F bond, although great improvements by all means, still suffer from various shortcomings such as multistep synthetic pathways, time consuming procedures and most notably the need for specially trained personnel to cope with all the complicated aspects of ^{18}F-radiochemistry. This is the main reason why PET radiopharmacy has not gained the same impact as its direct competitor Single-Photon-Emission-Tomography (SPECT) [52] in terms of widespread application in nuclear medicine. Although SPECT is inferior regarding spatial resolution and sensitivity, it is still the dominating methodology in nuclear medicine [53]. The most important reason for this is that the synthesis of SPECT radiopharmaceuticals, based e.g. on the radioisotope ^{99m}Tc, is characterized by easy labeling procedures which can be handled by technicians rather than radiochemists [54]. These labeling procedures are most often just one-pot labeling reactions where the ^{99m}Tc (as pertechnetate) is added to a prepared and sterile mixture of labeling precursors and additives. No final purification with HPLC or solid phase extraction is needed before the ^{99m}Tc-radiopharmaceutical can be injected into humans. This feature characterizes this kind of labeling as a true “Kit Formulation”, something still missing in ^{18}F-radiochemistry. Although the benefit of having Kit Formulations for the synthesis of ^{18}F-radiopharmaceuticals is quite obvious, only a few research groups have hitherto searched for new chemical pathways to introduce ^{18}F into biomolecules by abandoning the conventional methods of C-^{18}F bond formation. The introduction of new radiolabeling chemistry utilizing the formation of a phosphorous-^{18}F bond has been described recently by Studenov and co-workers [55]. As proof of principle, they demonstrated the synthesis of the ^{18}F-labeled cholinesterase inhibitor Dimefox (*N,N,N’,N’*-tetramethylphosphorodiamidic acid [^{18}F]fluoride, **37**) in high RCYs of 96% reacting the corresponding chloro-precursor Dimefox (**36**) with azeotropically dried ^{18}F$^-$ at room temperature for 5 min (Fig. **7**). The stability of [^{18}F]Dimefox (**37**) against hydrolysis was assessed by mixing an aliquot of the reaction mixture with water. Approximately 25% of the P-^{18}F bond was hydrolyzed within 30 min at room temperature. Unfortunately the authors did not investigate the stability of the compound under physiological conditions (pH 7.4) but it was mentioned that a higher stability of P-^{18}F compounds might be achieved by introducing phosphorofluoridate monoester moieties having higher hydrolytic stability.



Dimefox (**36**)

[^{18}F]Dimefox (**37**)
96% RCY

Fig. (7). ^{18}F-labeling of Dimefox *via* formation of a P-^{18}F bond.

In contrast to P-^{18}F chemistry, substantially more data are available regarding the formation of a silicon-^{18}F bond, serving as a new tool in ^{18}F-radiochemistry. Interestingly the first Si-^{18}F bond formation dates back to 1985 when Rosenthal *et al.* reported the reaction of chlorotrimethylsilane (**38**) with ^{18}F$^-$ in aqueous acetonitrile yielding the corresponding Si-^{18}F compound (**39**) in 65% RCY (Fig. **8**) [56]. This compound was subjected to a preliminary *in vivo* experiment to elucidate the stability of the Si-^{18}F bond. It was found that the Si-^{18}F bond in this particular compound was hydrolyzed very fast yielding the corresponding silanol (**40**), thus resulting in the impression that Si-^{18}F comprising molecules are unsuitable for the development of PET imaging agents in general. When **39** was inhaled by rats, most of the radioactivity was found in the bone structure as a result of fast decomposition of the Si-^{18}F bond (^{18}F$^-$ is readily incorporated into bones). Notably the authors suggested the use of more sterical hindered Si-^{18}F compounds to avoid hydrolytic loss of ^{18}F$^-$ which eventually turned out to be the right strategy. Except for the proposed reaction between H^{18}F and organosilanoles by Walsh and co-workers in a symposium abstract from 1999 [57], these preliminary findings are probably the reason why until 2005 no one tried to apply Si-based ^{18}F-fluorination chemistry. In 2005 Ting *et al.* described the high-yielding aqueous biomolecular ^{18}F-labeling of arylfluoroborates and alkylfluorosilicates as novel PET imaging agents [58]. They introduced biotinylated *p*-aminophenylboronylpinacolate (**41**) and biotinylated (aminopropyl)triethoxysilane (**42**) for protein targeting of avidin in order to have an analytical system to determine ^{18}F-fluoride incorporation into these compounds by trapping the ^{18}F-labeled compounds on an avidin matrix. After treatment of these compounds with fluoride, they observed the expected formation of the corresponding trifluoroborate and tetrafluorosilicate which they named “ate” salts. To transfer these findings to a radioactive labeling approach with ^{18}F$^-$ they added ^{18}O-target water containing ^{18}F$^-$ plus KHF$_2$ to solutions of compound **41** and **42** (Fig. **8**). The added carrier ^{19}F$^-$ ensured the targeted B-F and Si-F ratio of 1:3 or 1:4 respectively which was also confirmed by NMR and low-resolution ESI. The successful incorporation of the radioactive ^{18}F$^-$ yielding compound **43**

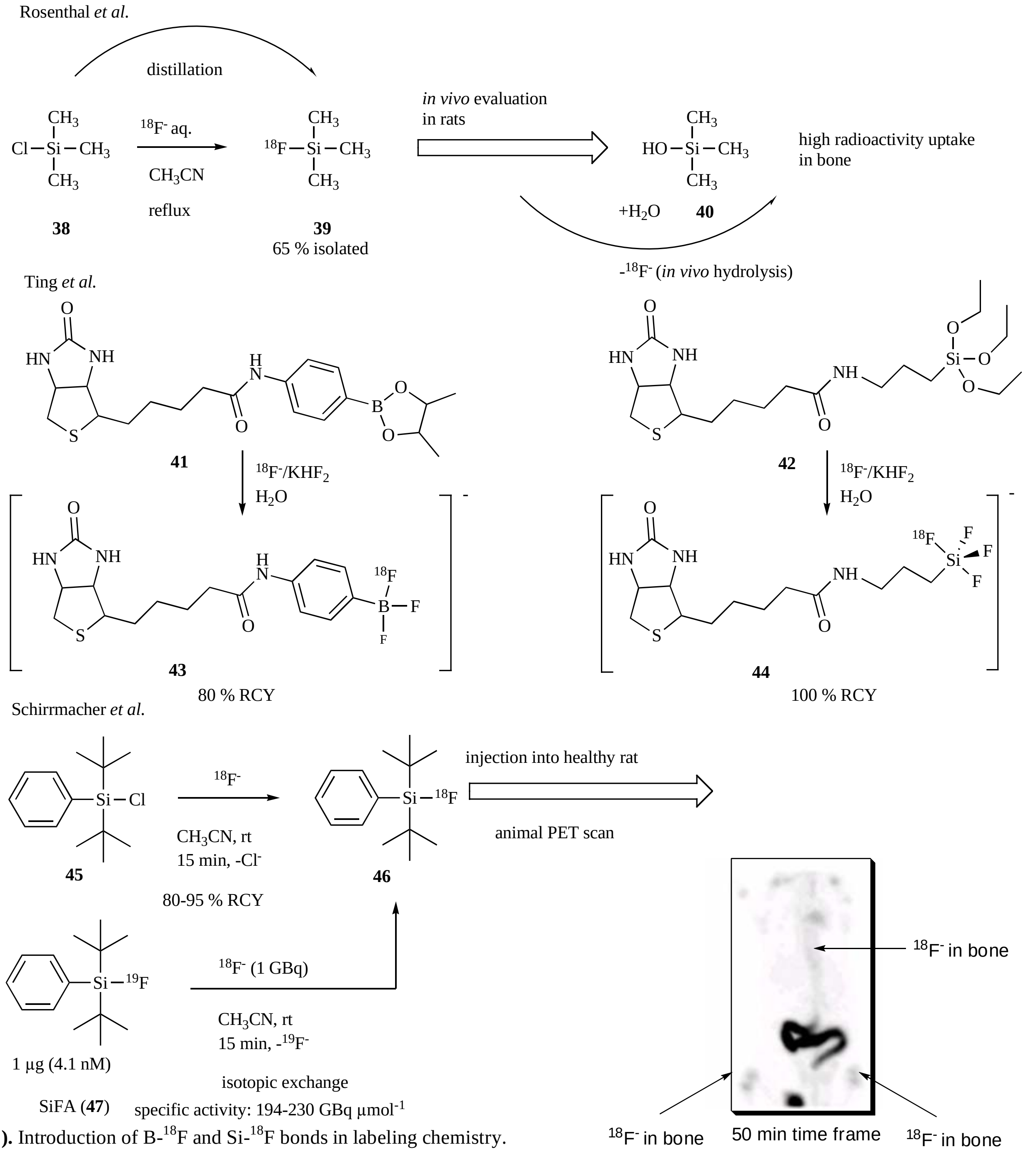
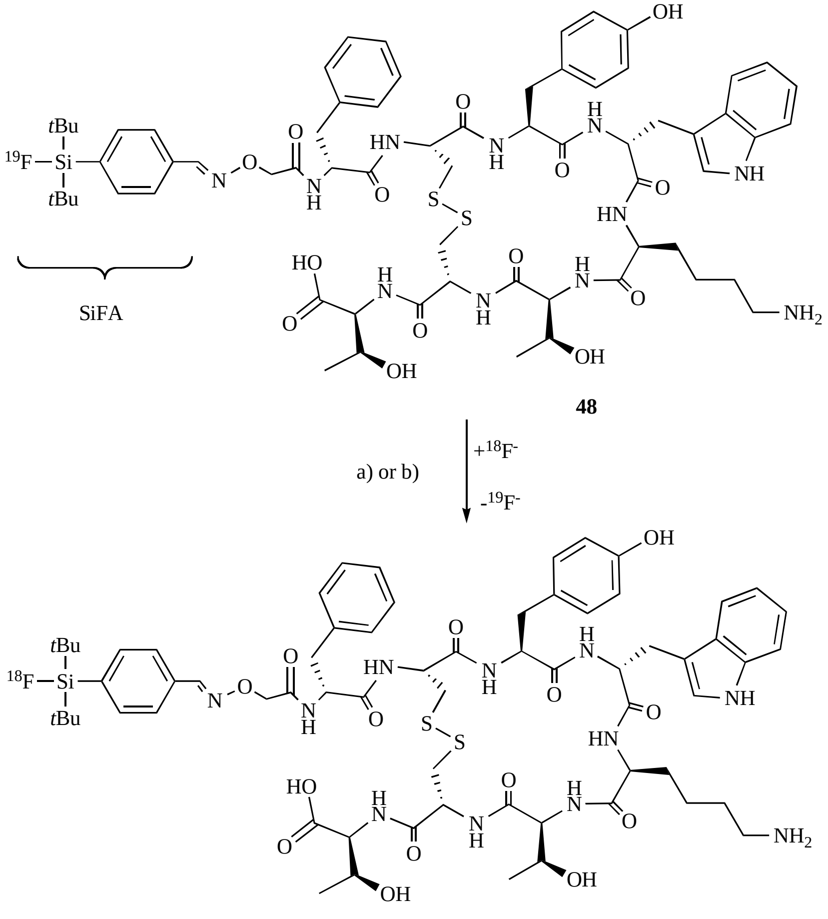


Fig. (8). Introduction of B-^{18}F and Si-^{18}F bonds in labeling chemistry.

and **44** respectively was confirmed by trapping the labeled biotinylated compounds to polydisperse avidin magnetic particles (AMPs) with a binding capacity for biotin of 525 pmol and subsequent autoradiography of the affixed AMPs. Labeling efficiency for both compounds was found to be exceptionally high (80-100 %). The hydrolytic stability of **43** and **44** was assessed by dilution with carbonate buffer and with $KH^{19}F_2$ solution. The latter was added to ensure that no back reaction of dissociated $^{18}F^-$ would occur. The ^{18}F-tetrafluorosilicate **44** was found to be moderately stable under these conditions (rate constant of hydrolysis: 0.01 min^{-1}) in contrast to the ^{18}F-trifluoroborate **43** which displayed no decomposition at all. An additional experiment was done under physiological conditions by incubating the compounds in either serum or whole blood where also no decomposition of the internalized ^{18}F-radioactivity could be observed. Unfortunately the authors did not apply larger amounts of radioactivity (several GBq) to their labeling protocol due to radiation safety concerns. To finally prove this method applicable to the synthesis of routine ^{18}F-radiopharmaceuticals, it must be demonstrated that large amounts of $^{18}F^-$ can be incorporated into these new "ate" compounds. Besides these minor concerns this new labeling method shows great potential for the labeling of small "ate" bearing prosthetic groups which in turn might serve as secondary labeling precursors for efficient labeling of proteins and peptides. An outstanding feature is that the described ^{18}F-chemistry works well under aqueous conditions.



48

49

A: 95-97 %

B: 70-90 %

Fig. (9). First example of using SiFA in the synthesis of the ^{18}F-labeled peptide Tyr^3-octreotate a) using "dried" ^{18}F$^-$ b) using ^{18}F$^-$ in ^{18}O-water.

A similar approach, entirely build on the concept of isotopic ^{19}F-^{18}F exchange at a silicon-core was introduced by Schirrmacher *et al.* in 2006 [59]. The focus was laid on finding silicon-^{18}F compounds displaying high stability under physiological conditions (pH 7.4 in blood serum) but also on easily applicable radiochemistry to form the silicon-^{18}F bond. Progress was made insofar that the stability of the silicon-^{18}F bond against hydrolysis could be substantially enhanced by connecting bulky substituents such as *tert*-butyl groups to the ^{18}F bearing silicon atom. One compound in particular, di-*tert*-butyl-phenyl[^{18}F]fluorosilane (**46**), synthesized by reacting the corresponding chloro-silane (**45**) with ^{18}F$^-$ in acetonitrile was found to be extremely stable under physiological conditions (60 min in human serum, 37.4°C, pH 7.4-7.6) and even when injected into rats (Fig. **8**). However, chloro-silanes are highly unstable and readily hydrolyzed when subjected to aqueous conditions proving them unsuitable to be coupled to peptides or smaller molecules (where aqueous workup can not be avoided). During the process of purification, the Si-Cl bond would get hydrolyzed and the resulting Si-OH moiety could not easily be used for ^{18}F-labeling. To circumvent this shortcoming an isotopic exchange reaction using a Si-^{19}F bond and ^{18}F$^-$ was considered by the authors as a possible alternative although isotopic exchange reactions in general have rarely been acknowledged as valuable alternatives in ^{18}F-labeling chemistry [60]. The achievable specific activity of labeled compounds *via* isotopic exchange reactions is normally very low because a huge amount of ^{19}F-compound is needed to achieve high yields of the isotopic exchange. Such a method is therefore unsuitable for most PET applications involving the detection of receptor densities in the human brain where the amount of binding sites for the ^{18}F-labeled radioligand could be very low. A decreased specific activity of the ^{18}F-compound by "dilution" with non-radioactive ^{19}F generally results in a low image quality of the PET scan because the signal-to-noise ratio deteriorates too much.

However, when ^{18}F$^-$ in acetonitrile was added to nanomolar quantities of di-*tert*-butyl-phenyl-fluorosilane (**47**) the isotopic exchange reaction took place most efficiently within 15 min at room temperature yielding **46** in high RCY. This unexpected finding laid the foundation for the so called SiFA labeling approach, where SiFA is the abbreviation of **Si**licon-**F**luoride-**A**cceptor and refers to a compound characterized by 1) a silicon core, 2) two *tert*-

butyl groups and 3) a phenyl system which is amenable to modifications for chemoligation. Following the SiFA approach, RCYs of 80-95% were possible exceeding the RCYs normally obtained by nucleophilic ^{18}F-substitutions of activated aromatic compounds requiring high temperatures and long reaction times [61]. The labeling of peptides described in the literature is characterized by multistep labeling procedures which are time consuming and laborious, finally providing the ^{18}F-labeled peptide in unsatisfying RCYs [62]. A one-step ^{18}F-labeling of peptides, bearing a variety of different functional groups had not been described so far and has been recently rated as one of the most important tasks in radiochemistry [63]. To apply the SiFA strategy to the ^{18}F-labeling of peptides, the model SiFA compound **47** was derivatized with an aldehyde moiety at the para position of the phenyl group for chemoselective conjugation to aminooxy derivatized peptides, a valuable method used in peptide derivatisation which has already been applied in ^{18}F-radiochemistry by Wester and co-workers [64]. The resulting oxime is stable and obtainable in high yields. As proof of principle the *N*-aminooxy derivatized peptide Tyr^3-octreotate was coupled to *p*-(di-*tert*-butylfluorosilyl) benzaldehyde and the resulting purified peptide **48** could be labeled with ^{18}F$^-$ in acetonitrile yielding the ^{18}F-labeled peptide **49** in RCYs of 95-97 % after 10-15 min reaction time at room temperature (Fig. **9**). The labeling also worked well under aqueous conditions, where the ^{18}F$^-$ in ^{18}O-water is used directly for labeling, but higher temperatures, longer reaction times and a very good quality of $H_2{}^{18}O$ was crucial, making the labeling in acetonitrile far more applicable. No formation of radioactive side products were observed by HPLC. The workup of the labeled peptide was easily achieved by solid phase extraction. The authors report that no HPLC was needed at any time of the synthesis allowing this new labeling approach to be adapted for a "Kit Labeling" procedure. The advantages of this approach are its applicability to the labeling of complex molecules without the need for protecting groups and its simplicity, so that the labeling could even be carried out by untrained personnel in 4 steps (1. add radioactivity 2. dilute with water 3. fix on cartridge 4. elute and do sterile filtering). However, the reported specific activities for **49** were in the range of 3-5 GBq μmol^{-1} which is probably too low for receptor imaging and should be further improved. A second concern is the high lipophilicity introduced by the SiFA compound. First preliminary *in vivo* experiments of compound **49** in tumor bearing rats proved a high radioactivity uptake in liver [65]. It would be worthwhile to positively or negatively charge the SiFA compound either by means of direct derivatization or by connecting the SiFA group to small charged linkers suitable of bioconjugation to peptides.

CONCLUSIONS

Novel labeling methods in ^{18}F-radiochemistry are highly desired to reduce the scale of effort necessary to obtain ^{18}F-labeled compounds for their application as imaging agents in nuclear medicine and life science. Great endeavors are currently made by many groups to find novel routes to introduce the ^{18}F-isotope into molecules for *in vivo* imaging using PET. A special focus has been laid clearly on the labeling of larger biomolecules such as peptides which have not been amenable to simple labeling procedures for a long time. From an economical point of view, to strengthen the role of radiochemistry in medicine and life science, it is necessary to find reliable methods for ^{18}F-labeling which can be applied by technicians on a daily basis to produce PET radiopharmaceuticals for various applications. The refinement and/or combination of the above described methods could be a crucial step into this direction.

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REFERENCES

[1] a) Wahl, R. L.; Buchanan, J. W. *Principles and Practice of Positron Emission Tomography*, Lippincott Williams & Wilkins, **2002**; b) Phelps, M. E. *Proc. Natl. Acad. Sci. USA*, **2000**, *97*, 9226; c) Marx, V. *Chem. Eng. News*, **2005**, *83*, 25.

[2] Bolton, R. *J. Label. Compd. Radiopharm*., **2002**, *45*, 485.

[3] a) Okarvi, S. M. *Eur. J. Nucl. Med*., **2001**, *28*, 929; b) Wester, H. J.; Hamacher, K.; Stoecklin, G. *Nucl. Med. Biol*., **1996**, *23*, 365; c) Wilbur, D. S. *Bioconjug. Chem*., **1992**, *3*, 433; d) Lundqvist, H.; Tolmachev, V. *Biopolymeres (Pept. Sci.)*, **2002**, *66*, 381; e) Heppeler, A.; Froidevaux, S.; Eberle, A. N.; Maecke, H. R. *Curr. Med. Chem*., **2000**, *7*, 971; f) Knight, L. C. *Radiolabeled Peptides for Tumor Imaging*. Handbook of Radiopharmaceuticals, Radiochemistry and Applications, Wiley, Chichester, **2003**.

[4] Schleyer, D. J. *Production of Radionuclides in Accelerators*. Handbook of Radiopharmaceuticals, Radiochemistry and Applications, Wiley, Chichester, **2003**.

[5] a) Ferrieri, R. A. *Production and Application of Synthetic Precursors Labeled with Carbon-11 and Fluorine-18*. Handbook of Radiopharmaceuticals, Radiochemistry and Applications, Wiley, Chichester, **2003**.

[6] Ross, T. L.; Ermert, J.; Hocke, C.; Coenen, H. H. *J. Am. Chem. Soc*., **2007**, *129*, 8025.

[7] Snyder, S. E.; Kilbourn, M. R. *Chemistry of Flourine-18 Radiopharmaceuticals*. Handbook of Radiopharmaceuticals, Radiochemistry and Applications, Wiley, Chichester, **2003**.

[8] a) Bergman, J.; Solin, O. *Nucl. Med. Biol*., **1997**, *24*, 677; b) Vähätalo, J. K.; Eskola, O.; Bergman, J.; Forsback, S.; Lehikoinen, P.; Jääskeläinen, J.; Solin, O. *J. Label. Compds. Radiopharm*., **2002**, *45*, 697.

[9] a) Oberdorfer, F.; Hofmann, E.; Maier-Borst, W. *J. Label. Compds. Radiopharm*., **1988**, *25*, 999; b) Oberdorfer, F.; Hofmann, E.; Maier-Borst, W. *Appl. Radiat. Isot*., **1988**, *39*, 685; c) Satyamurthy, N.; Bida, G. T.; Phelps, M. E.; Barrio, J. R. *Appl. Radiat. Isot*., **1990**, *41*, 733; d) Oberdorfer, F.; Dietzel, G. *J. Label. Compd. Radiopharm*., **2003**, *46*, S216.

[10] Teare, H.; Robins, E. G.; Arstad, E.; Luthra, S. K.; Gouverneur, V. *Chem. Commun*., **2007**, 2330.

[11] Reichardt, C. *Solvents and Solvent Effects in Organic Chemistry*, Whiley-VCH, **2002**.

[12] a) Chi, D. Y.; Kilbourn, J. A.; Katzenellenbogen. M. J.; Welch, A. *J. Org. Chem*., **1987**, *52*, 658; b) Choe, Y. S.; Song, D. H.; Lee, K.-J.; Kim, S. E.; Choi, Y.; Lee, K. H.; Kim, B.-T.; Oh, S. J.; Chi, D. Y. *Appl. Radiat. Isot*., **1998**, *49*, 73.

[13] a) Sanada, M.; Miyana, T.; Iwadare, S.; Williamson, J. M.; Arison, B. H.; Smith, J. L.; Douglas, A. W.; Liesch, J. M.; Inamine, E. *J. Antibiot*., **1984**, *39*, 259; b) O'Hagan, D.; Schaffrath, C.; Cobb, S. L.; Hamilton, J. T. G.; Murphy, C. D. *Nature*, **2002**, *416*, 279; c) Schaffrath, C.; Deng, H.; O'Hagan, D. *FEBS Lett*., **2003**, *547*, 111.

[14] Martarello, L.; Schaffrath, C.; Deng, H.; Gee, A. D.; Lockhart, A.; O'Hagan, D. *J. Label. Compd. Radiopharm*., **2003**, *46*, 1181.

[15] Deng, H.; Cobb, S. L.; Gee, A. D.; Lockhart, A.; Martarello, L.; McGlinchey, R. P.; O'Hagan, D.; Onega, M. *Chem. Commun*., **2006**, 652.

[16] Kim, D. W.; Choe, Y. S.; Chi, D. Y. *Nucl. Med. Biol*., **2003**, *30*, 345.

[17] a) Kim, D. W.; Song, C. E.; Chi, D. Y. *J. Am. Chem. Soc*., **2002**, *124*, 10278; b) Kim, D. W.; Song, C. E.; Chi, D. Y. *J. Org. Chem*., **2003**, *68*, 4281; c) Kim, D. W.; Chi, D. Y. *Angew. Chem. Int. Ed*., **2004**, *43*, 483.

[18] Kim, H. W.; Jeong, J. M.; Lee, Y.-S.; Chi, D. Y.; Chung, K.-H.; Lee, D. S.; Chung, J.-K.; Lee, M. C. *Appl. Radiat. Isot.*, **2004**, *61*, 1241.

[19] Stöcklin, G. H. Eur. *J. Nucl. Med*., **1998**, *25*, 1612.

[20] Kim, D. W.; Ahn, D.-S.; Oh, Y.-H.; Lee, S.; Kil, H. S.; Oh, S. J.; Lee, S. J.; Kim, J. S.; Ryu, J. S.; Moon, D. H.; Chi, D. Y. *J. Am. Chem. Soc*., **2006**, *128*, 16394.

[21] Lee, S. J.; Oh, A. J.; Chi, D. Y.; Kang, S. H.; Kil, H. S.; Kim, J. S.; Moon, D. H. *Nucl. Med. Biol*., **2007**, *34*, 345.

[22] Kolb, H. C.; Finn, M. G.; Sharpless, K. B. *Angew. Chem. Int. Ed*., **2001**, *40*, 2004.

[23] Bock, V.; Hiemstra, H.; von Maarseveen, J. H. *Eur. J. Org. Chem*., **2006**, 51.

[24] Gothelf, K.V.; Jorgensen, K.A. *Chem. Rev*., **1998**, *98*, 863.

[25] Appukkuttan, P.; Dehaen, W.; Fokin, V.V.; van der Eycken, E. *Org. Lett*., **2004**, *6*, 4223.

[26] Marik, J.; Sutcliffe, J. L. *Tetrahedron Lett*., **2006**, *47*, 6681.

[27] Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem. Int. Ed*., **2002**, *41*, 2596.

[28] Fazio, F.; Bryan, M.C.; Blixt, O.; Paulson, J.C.; Wong, C.-H. *J. Am. Chem. Soc*., **2002**, *124*, 14397.

[29] Glaser, M.; Arstad, E. *Bioconjug. Chem*., **2007**, *18*, 989.

[30] a) Mindt, T. L.; Ross, T.; Schibli, R. *J. Label. Compd. Radiopharm*., **2007**, *50*, S34; b) Ross, T. L.; Mindt, T.; Baumann, S.; Weber, A.; Grohn, V.; Honer, M.; Schibli, R.; Schubiger, P. A.; Ametamey, S. M. *J. Label. Compd. Radiopharm*., **2007**, *50*, S35; c) Glaser, M.; Arstad, E. *J. Label. Compd. Radiopharm*., **2007**, *50*, S36; d) Sirion, U.; Kim, H. J.; Lee, B. S.; OH, S. J.; Chi, D. Y. *J. Label. Compd. Radiopharm*., **2007**, *50*, S37; e) Wust, F.; Ramenda, T.; Bergmann, R. *J. Label. Compd. Radiopharm*., **2007**, *50*, S38; f) Marik, J.; Hausner, S. H.; Gagnon, M. K. J.; Sutcliffe, J. L. *J. Label. Compd. Radiopharm*., **2007**, *50*, S39; g) Kim, D. H.; Choe, Y. S.; Choi, J. Y.; Choi, Y.; Lee, K. H.; Kim, B. T. *J. Label. Compd. Radiopharm*., **2007**, *50*, S40.

[31] Wester, H. J.; Hamacher, K.; Stoecklin, G. *Nucl. Med. Biol*., **1996**, *23*, 365.

[32] Vries, E. F. J.; Vroegh, J.; Elsinga, P. H.; Vaalburg, W. *Appl. Radiat. Isot*., **2003**, *58*, 469.

[33] Vaidyanathan, G.; Zalutsky, M. R. *Nucl. Med. Biol*., **1992**, *19*, 275.

[34] a) Guhlke, S.; Wester, H. J.; Bruns, C.; Stoecklin, G. *Nucl. Med. Biol*., **1994**, *21*, 819; b) Marik, J.; Hausner, S. H.; Fix, L. A.; Gagnon, K. J.; Sutcliffe, J. L. *Bioconjug. Chem*., **2006**, *17*, 1017.

[35] Downer, J. B.; McCarthy, T. J.; Edwards, W. B.; Anderson, C. J.; Welch, M. *J. Appl. Radiat. Isot*., **1997**, *48*, 907.

[36] Iwata, R.; Pascali, C.; Bogni, A.; Horvath, G.; Kovacs, Z.; Yanai, K.; Ido, T. *Appl. Radiat. Isot*., **2000**, *52*, 87.

[37] Glaser, M.; Karlsen, H.; Solbakken, M.; Arukwe, J.; Brady, F.; Luthra, S. K.; Cuthbertson, A. *Bioconjug. Chem*., **2004**, *15*, 1447.

[38] Sutcliffe-Goulden, J. L.; O'Doherty, M. J.; Bansal, S. *Bioorg. Med. Chem. Lett*., **2000**, *10*, 1502; b) Sutcliffe-Goulden, J. L.; O'Doherty, M. J.; Marsden, P. K.; Hart, I. R.; Marshall, J. F.; Bansal, S. S. *Eur. J. Nucl. Med*., **2002**, *29*, 754.

[39] Chang, Y. S.; Jeong, J. M.; Lee, Y.-S.; Kim, H. W.; Rai, G. B.; Lee, S. J.; Lee, D. S.; Chung, J.-K.; Lee, M. C. *Bioconjug. Chem*., **2005**, *16*, 1329.

[40] Kuhnast, B.; Dolle, F.; Tavitian, B. *J. Label. Compd. Radiopharm*., **2002**, *45*, 1.

[41] Kuhnast, B.; Hinnen, F.; Hamzavi, R.; Boisgard, R.; Tavitian, B.; Nielsen, P. E.; Dollé, F. *J. Label. Compd. Radiopharm.*, **2005**, *48*, 51.

[42] Shiue, C.-Y.; Wolf, A. P.; Hainfeld, J. F. *J. Label. Compd. Radiopharm*., **1998**, *26*, 287.

[43] Toyokuni, T.; Walsh, J. S.; Dominguez, A.; Phelps, M. E.; Barrio, J. R.; Gambhir, S. S.; Satyamurthy, N. *Bioconjug. Chem*., **2003**, *14*, 1253.

[44] Berndt, M.; Pietzsch, J.; Wuest, F. *Nucl. Med. Biol*., **2007**, *34*, 5.

[45] De Bruin, B.; Kuhnast, B.; Hinnen, F.; Yaouancq, L.; Amessou, M.; Johannes, L.; Samson, A.; Boisgard, R.; Tavitian, B.; Dolle, F. *Bioconjug. Chem*., **2005**, *15*, 406.

[46] Cai, W.; Zhang, X.; Wu, Y.; Chen, X. *J. Nucl. Med*., **2006**, *47*, 1172.

[47] Zhang, X.; Xiong, Z.; Wu, Y.; Cai, W.; Tseng, J. R.; Gambhir, S. S.; Chen, X. *J. Nucl. Med*., **2006**, *47*, 113.

[48] Prante, O.; Einsiedel, J.; Haubner, R.; Gmeiner, P.; Wester, H. J.; Kuwert, T.; Maschauer, S. *Bioconjug. Chem*., **2007**, *18*, 254.

[49] a) Haubner, R.; Wester, H. J.; Burkhart, F.; Senekowitsch-Schmidtke, R.; Weber, W.; Goodmann, S. L.; Kessler, H.; Schwaiger, M. *J. Nucl. Med*., **2001**, *42*, 326; b) Schottelius, M.; Rau, F.; Reubi, J. C.; Schwaiger, M.; Wester, H.-J. *Bioconjug. Chem*., **2005**, *16*, 429.

[50] Hamacher, K.; Coenen, H. H.; Stöcklin, G. *J. Nucl. Med*., **1986**, *27*, 235.

[51] Prante, O.; Hamacher, K.; Coenen, H. H. *J. Label. Compd. Radiopharm*., **2007**, *50*, 55 .

[52] a) Bybel, B.; Brunken, R. C.; Sha, S. N.; Turbiner, E.; Neumann, D. R. *Cleve. Clin. J. Med*., **2007**, *74*, 71; b) Kjaer, A. *Adv. Exp. Med. Biol*., **2006**, *587*, 277; c) Moresco, R. M.; Matarrese, M.; Fazio, F. *Curr. Top. Med. Chem*., **2006**, *6*, 2027; d) Tatsch, K.; Ell, P. *J. Clin. Med*., **2006**, *6*, 259.

[53] O'Connor, M. K.; Kemp, B. J. *Semin. Nucl. Med*., **2006**, *36*, 258.

[54] Docic, D. D. *Med. Pregl*., **2005**, *58*, 180.

[55] Studenov, A. R.; Adam, M. J.; Wilson, J. S.; Ruth, T. J. *J. Label. Compd. Radiopharm.*, **2005**, *48*, 497.

[56] Rosenthal, M. S.; Bosch, A. L.; Nickles, R. J.; Gateley, S. J. *Int. J. Appl. Radiat. Isot*., **1985**, *36*, 318.

[57] Walsh, J. C.; Akhoon, K. M.; Satyamurthy, N.; Barrio, J. R.; Phelps, M. E.; Gambhir, S. S.; Toyokuni, T. *J. Label. Compd. Radiopharm*., **1999**, *42*, S1.

[58] Ting, R.; Adam, M. J.; Ruth, T. J.; Perrin, D. M. *J. Am. Chem. Soc*., **2005**, *127*, 13094.

[59] Schirrmacher, R.; Bradtmoeller, G.; Schirrmacher, E.; Thews, O.; Tillmanns, J.; Siessmeier, T.; Buchholz, H. G.; Bartenstein, P.; Waengler, B.; Niemeyer, C. M.; Jurkschat, K. *Angew. Chem. Int. Ed*., **2006**, *45*, 6047.

[60] a) Kilbourn, M. R.; Welch, M. J.; Dence, C. S.; Tewson, T. J.; Saji, H.; Maeda, M. *Appl. Radiat. Isot*., **1984**, *35*, 592; b) Kilbourn, M. R.; Sabramanian, R. *J. Label. Compd. Radiopharm*., **1990**, *28*, 1355; c) Hashizume, K.; Hashimoto, N.; Miyake, Y. *J. Org. Chem*., **1995**, *60*, 6680; d) Costantinou, M.; Aigbirhio, F. I.; Smith, R. G.; Ramsden, C. A.; Pike, V. W. *J. Am. Chem. Soc*., **2001**, *123*, 1780; e) Ryzhikov, N. N.; Gomzina, N. A.; Fedorova, O. S.; Vasilev, D. A.; Kostikov, A. P.; Krasikova, R. N. *Radiochemistry*, **2004**, *46*, 290.

[61] Haka, M. S.; Kilbourn, M. R.; Watkins, G. L.; Toorongian, S. A. *J. Label. Compd. Radiopharm*., **1989**, *27*, 823.

[62] For reviews: a) Kilbourn, M. R.; Dence, C. S.; Welch, M. J.; Mathias, C. J. *J. Nucl. Med*., **1987**, *28*, 462; b) Glaser, M.; Luthra, S. K.; Brady, F. *Int. J. Oncol*., **2003**, *22*, 253; c) Okarvi, S. M. *Eur. J. Nucl. Med*., **2001**, *28*, 929; d) Okarvi, S. M. *Med. Res. Rev*., **2004**, *24*, 357; e) Wilbur, D. S. *Bioconjug. Chem*., **1992**, *3*, 433; f) Giblin, M. F.; Veerendra, B.; Smith, C. J. *In Vivo*, **2005**, *19*, 9; g) Lundquist, H.; Tolmachev, V. *Biopolymers (Pept. Sci)*, **2002**, *66*, 381; h) Heppeler, A.; Froidevaux, S.; Eberle, A. N.; Maecke, H. *Curr. Med. Chem*., **2000**, *7*, 971.

[63] Blower, P. *Dalton Trans*., **2006**, 1705.

[64] a) Tumshirn, G.; Hersel, U.; Goodman, S. L.; Kessler, H. *Chem. Eur. J*., **2003**, *9*, 2717; b) Poethko, T.; Schottelius, M.; Thumshirn, G.; Hersel, U.; Herz, M.; Henriksen, G.; Kessler, H.; Schwaiger, M.; Wester, H. J. *J. Nucl. Med*., **2004**, *45*, 892.

[65] Schirrmacher, R. **2006**, unpublished results.

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