



Small Prosthetic Groups in ^{18}F -Radiochemistry: Useful Auxiliaries for the Design of ^{18}F -PET Tracers

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Prosthetic group (PG) applications in ^{18}F -radiochemistry play a pivotal role among current ^{18}F -labeling techniques for the development and availability of ^{18}F -labeled imaging probes for PET (Wahl, 2002) (¹). The introduction and popularization of PGs in the mid-80s by pioneers in ^{18}F -radiochemistry has profoundly changed the landscape of available tracers for PET and has led to a multitude of new imaging agents based on simple and efficiently synthesized PGs. Because of the chemical nature of anionic $^{18}\text{F}^-$ (apart from electrophilic low specific activity ^{18}F -fluorine), radiochemistry before the introduction of PGs was limited to simple nucleophilic substitutions of leaving group containing precursor molecules. These precursors were not always available, and some target compounds were either hard to synthesize or not obtainable at all. Even with the advent of recently introduced "late-stage fluorination" techniques for the ^{18}F -fluorination of deactivated aromatic systems, PGs will continue to play a central role in ^{18}F -radiochemistry because of their robust and almost universal usability. The importance of PGs in radiochemistry is shown by its current significance in tracer development and exemplified by an overview of selected methodologies for PG attachment to PET tracer molecules. Especially, click-chemistry approaches to PG conjugation, while furthering the historical evolution of PGs in PET tracer design, play a most influential role in modern PG utilization. All earlier and recent multifaceted approaches in PG development have significantly enriched the contingent of modern ^{18}F -radiochemistry procedures and will continue to do so.

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Introduction

Before discussing a prosthetic group (PG)'s purpose in radiochemistry,¹ its definition needs to be clarified. The use of the term "prosthetic group" has been traditionally employed in enzymology and defined as a cofactor that is "either tightly or loosely bound to the enzyme. If tightly connected, the cofactor is referred to as a prosthetic group."² How did this definition migrate into the field of radiochemistry and what is the significance of it in this particular context? It should

be recalled that for the development of a new radiotracer, usually (in almost all cases), a nonradioactive lead compound devised by medicinal chemistry is taken as a chemical template or scaffold and translated into a radiotracer for in vivo imaging by introducing a radiolabel. This general strategy holds true for tracers intended for PET, where so-called organic radionuclides such as carbon-11 and fluorine-18 (^{18}F) are commonly used, and where the size of compounds varies between small tracers for brain imaging, and peptide- and protein-based probes for cancer imaging.^{3–8} If the original lead structure does not contain, for example, fluorine, the radiochemist has to add fluorine at a position in the molecule that most likely does not interfere with its binding properties to the target. The smaller the change in the molecular structure of the lead compound, the less probable the binding behavior to the target structure will change. Therefore, radiochemists and also medicinal chemists (addition of fluorine to a molecule can have very positive effects on its bio-availability and stability in vivo⁹) often simply exchange a

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hydrogen atom in a lead with a fluorine atom based on the similar van der Waals radii of hydrogen and fluorine. Unfortunately, it is not always possible to introduce radioactive ^{18}F at the same position in a molecule where medicinal chemists are able to add stable fluorine in their multistep synthetic schemes. This ostensible contradiction can be explained easily by the different requirements for nonradioactive and radioactive fluorine in preparative chemistry. Although ^{18}F is still fluorine in chemical terms, the radioactive nature of ^{18}F introduces the problem of radioactive decay to the synthetic equation. In general, multistep syntheses up to this day are not desirable in ^{18}F radiochemistry because such laborious and lengthy procedures will never yield the desired radiotracer in high radiochemical yields (RCYs) and high specific activities, not to mention the cumbersome purification of many intermediates on the way toward the final product.

What Is a Prosthetic Group?

These ramifications are still the reasons why PGs have been invented and used in tracer development ever since. In principle, a PG overcomes the inability to radiolabel certain compounds with ^{18}F in 1-2 steps via nucleophilic $^{18}\text{F}^-$ substitution of a leaving group in a timely manner. Comparable with carbon-11 methylations using ^{11}C -methyl iodide, small reactive ^{18}F bearing labeling synthons were envisioned as analogous labeling reagents to engage nucleophilic moieties such as amino, hydroxy, carboxy, and thiol groups. This strategy made it possible to easily transfer a ^{11}C -methylation to an equivalent ^{18}F -fluoro methylation or ethylation (or alkylation in general), taking advantage of the already existing labeling precursor for ^{11}C -labeling. This concept has been recently extended to a multitude of chemical ^{18}F -bioconjugations based on click chemistry, significantly supporting the use of PG applications in radiotracer development despite the development of novel metal catalyzed late-stage ^{18}F -fluorination techniques that have substantially enriched existing ^{18}F -labeling techniques.¹⁰⁻¹⁴ Referring back to the above-introduced definition of a PG in enzymology, a PG in radiopharmaceutical chemistry is a serviceable auxiliary that can easily

be attached to a precursor molecule in analogy to existing ^{11}C -labeling techniques (alkylation, acylation, amination, etc) or click-chemistry methodologies (triazole-, dihydropyrazine-, oxime formations, etc). However, the use of PGs for tracer development carries an important limitation. Usually, a first-in-kind radiotracer (especially for small compounds of low molecular weight) is directly derived from the lead structure that has no functionalities that could be easily modified to introduce ^{18}F by a PG. As a result, in most cases, a methyl group that is usually abundantly available in most small organic compounds is substituted for a ^{11}C -methyl group. This strategy yields a radiotracer that is in every regard exactly the same as the nonradioactive lead compound. In contrast, the structural exchange of a methyl group with a higher ^{18}F -labeled homolog such as an ^{18}F -fluoroethyl group or even a closely related ^{18}F -fluoromethyl group can significantly alter the biological and chemical properties of the resulting molecule. It is the responsibility of the researcher to prove that this very minor chemical-radiochemical alteration in structure does not compromise important parameters such as toxicity, metabolism, and binding affinity to the target. This of course adds a new layer to the development process of ^{18}F -radiopharmaceuticals, a noticeable detriment in comparison with an unaltered ^{11}C -tracer that has most often already been tested for toxicity, binding affinity, and metabolism in the course of commercial drug development. This handicap, however, is offset by the advantages of ^{18}F over ^{11}C in terms of nuclide properties, and longer half-life, which allows for a prolonged duration of PET measurement and thus a higher quality of corresponding PET images.

Many of the abovementioned concepts of ^{18}F -PG introduction can be illustrated by the synthesis of 6-O-(2- ^{18}F fluoroethyl)-6-O-desmethyl diprenorphine, a PET tracer for opioid in vivo imaging, where the original methyl group in 6 position is substituted by an ^{18}F -fluoroethyl moiety (Fig. 1).

The methoxy group can be easily converted into a hydroxy group (the desmethyl precursor) that can be labeled either by [^{11}C]methyl iodide or 2- ^{18}F fluoroethyl tosylate (^{18}F FETos).¹⁵ This particular position is the most suited to make small changes to the structure of diprenorphine without compromising its binding to the opioid receptors. Another group had previously chosen the N-17 position to replace the cyclopropyl methyl group with an ^{18}F -fluoro ethyl

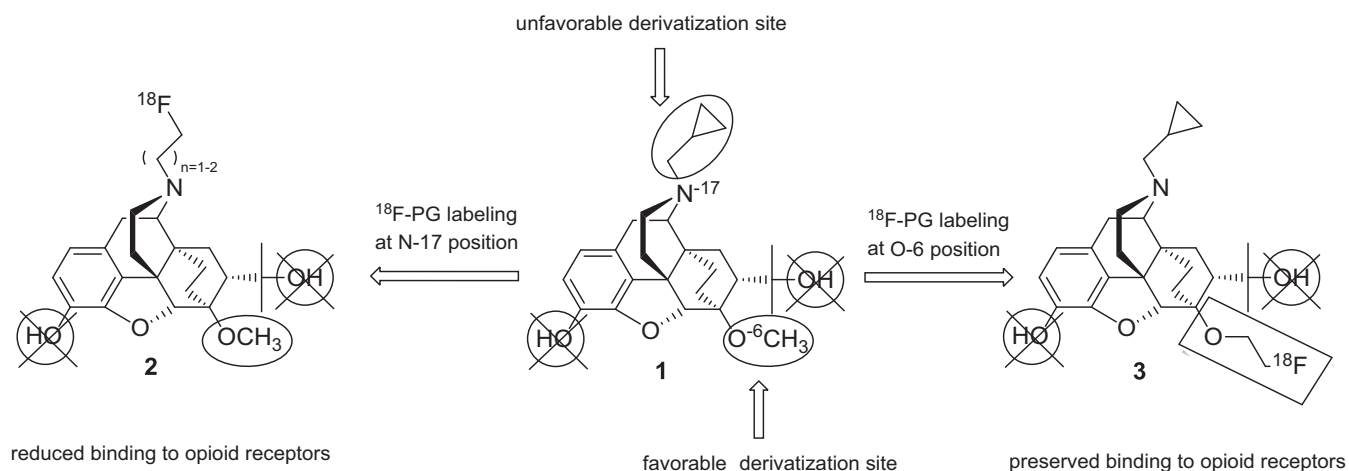


Figure 1 Derivatization sites of the opioid receptor ligand diprenorphine. Structural changes of diprenorphine at the O-6 position (right) yield preserved binding to the receptor, whereas changes at the N-17 position (left) lead to a reduced binding to the receptor.

and -propyl moiety, but the in vivo evaluation of the resulting compounds revealed reduced binding in opioid receptor-rich regions in comparison with the ^{11}C -original.¹⁶ Fortunately, the O-6 derivatized ^{18}F variant of diprenorphine overcomes the shortcoming of the original ^{11}C -labeled compound of a very limited PET scan duration and even enables shipment of the compound to PET centers without a cyclotron. This review does not claim to summarize the entire literature with regard to reported PGs over the last 30 years but will rather explain conceptual strategies behind PG utilization using demonstrative examples.

The Early Days of Prosthetic Group Chemistry

Early research was reported in the late 1970s and early 1980s, when the synthesis of halo- ^{18}F -fluoromethanes was described in the context of hot-atom recoil labeling where the precursor substrate was directly added to the neon gas target and bombarded with 14-MeV deuterons.¹⁷ The “hot” ^{18}F -formed via the $^{20}\text{Ne}(\text{d},\alpha)^{18}\text{F}$ reaction could react directly with in-target components to form the corresponding halo- ^{18}F -fluoromethanes. Unfortunately, the high energy transfer rates within a target during bombardment led to an increased formation of radioactive impurities impairing the production of higher activities. The irradiation of halo-alkanes in the presence of ^{18}F generates halo- ^{18}F -fluoro alkanes, which were characterized but not used for any kind of follow-up labeling. Another experimental study with more detailed information but still limited practical applicability for preparative ^{18}F -radiochemistry was reported by Root and Manning.¹⁸ At the same time, ^{18}F -fluorinated surface oxidized silver wool as a curious source of ^{18}F was used for the synthesis of halo- ^{18}F -fluoro alkanes. The authors did not have the sophisticated labeling utilities at their disposal, as modern radiochemists and consequently the reaction mechanism using silver wool is still not well understood.¹⁹ Another early contribution from Coenen and coworkers in 1985 fully characterized for the first time 1-bromo- ^{18}F -fluoromethane with the intent to use this synthon analogously to ^{11}C -methyl iodide.²⁰ The labeling conditions to ^{18}F -fluorinate the precursor dibromomethane via nucleophilic substitution were different from previous approaches. Almost at the same time, Dae and coworkers reported on a mechanistic study of halofluorination of olefins that paved the way towards other investigations.²¹ The full automation of the synthesis of bromo- ^{18}F -fluoromethane was achieved by automated distillation of the reaction mixture over 3 Sep-Pak Plus silica cartridges to remove the dibromomethane precursor and unreacted ^{18}F .²² The introduction of the aminopolyether Kryptofix222 and potassium carbonate system as well as the tetrabutylammonium ^{18}F -fluoride complex^{23,24} constituted important improvements enhancing the nucleophilicity of the ^{18}F -anion, via complexation of the potassium cation through Kryptofix222, leaving the ^{18}F -un-solvated and thus “naked” or through the formation of highly soluble $\text{nBu}_4\text{N}^+[\text{F}]^-$ (due to the phase transfer capabilities of nBu_4N^+), respectively. The implementation of the Kryptofix222/ $\text{K}^+[\text{F}]^-$ labeling system

marked a turning point in ^{18}F -radiochemistry. It is still an important pillar of modern ^{18}F -labeling methodology and had a catalytic effect on the further development of PGs and radiotracers in general. Previously, hard to label compounds suddenly became available through this innovation, invigorating and intensifying the research on ^{18}F -labeled compounds and making ^{18}F the most important diagnostic radionuclide today.²⁵ Besides bromo- and iodo- ^{18}F -fluoromethane, the more reactive ^{18}F -fluoromethyl triflate was introduced in 2002 via online conversion of bromo- ^{18}F -fluoromethane using silver triflate in a gas-solid phase reaction.²⁶ The year 1986 was most memorable with regard to PG development and PG application in tracer research. Several pioneering researchers in radiochemistry such as Kilbourn, Katzenellenbogen, Welch, Shiue, Wolf, Barrio, Coenen, and Stöcklin presented their early or continuing research on ^{18}F -fluorinated alkyl tosylates, -mesylates, and -halides at the Sixth International Symposium on Radiopharmaceutical Chemistry in Boston, Massachusetts, USA, initializing a new important chapter in radiochemistry that still continues to have an effect on tracer conception. It cannot be overstated that putting forward the Kryptofix222/ K_2CO_3 labeling system for anionic ^{18}F -fluorination sustainably invigorated and accelerated progress in ^{18}F -tracer development. Although some new methods have completely omitted toxic Kryptofix222 from the synthesis and also omitted the azeotropic drying procedure,²⁷ this ^{18}F -nucleophilicity-enhancing auxiliary is still in frequent use. Shiue and Wolf for example applied this labeling system in the synthesis of higher ^{18}F -fluoroalkylated analogs of spiroperidol (spiperone **4**, Fig. 2) (at the same conference there were several contributions from different groups featuring the synthesis of ^{18}F -N-alkyl labeled spiroperidols **5**, Fig. 2), an important ligand to investigate dopamine receptors in humans.²³ The motivation to use PG chemistry was clearly motivated by the difficult multistep synthesis of the original compound, making a routine application for human PET imaging unlikely. The obtained N- ^{18}F -fluoroalkyl spiroperidol derivatives **5**, despite being structurally slightly different from the lead, could be synthesized consistently from their corresponding ^{18}F -fluoroalkyl halides in radiochemical yields of up to 60% (decay corrected) (Fig. 2). Generally for convenience's sake, a 1-step fluorination is always preferable and the chosen route if a labeling precursor is available. However, if the synthesis of a direct 1-step labeling precursor is cumbersome or the direct 1-step labeling results in the formation of many side-products because of harsh labeling conditions, the PG labeling approach is preferred for reasons of more efficient synthesis and faster and immediate evaluation of the tracer. The pioneering work from all those early groups provided the necessary innovative momentum to advance radiochemistry to the next level and set the state for numerous applications, further developments, and, finally, click chemistry in PG application.²⁸⁻³¹

2- ^{18}F -fluoroethyl tosylate: The PG Workhorse

If there is one PG that almost every radiochemist has at least used once in his or her professional career, it would most probably be ^{18}F -FETos, first introduced by Block et al at the

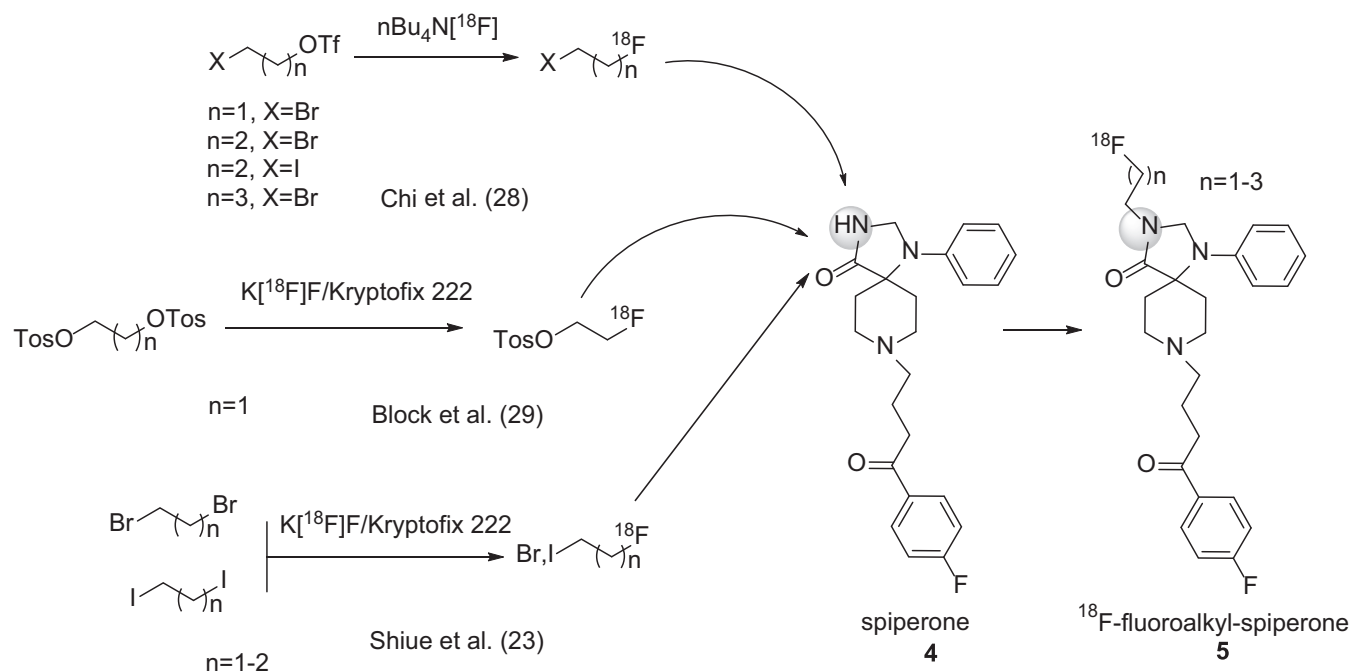


Figure 2 Synthesis of various ^{18}F -fluoro alkylation PGs for the synthesis of an ^{18}F -derivative of the D2 receptor ligand spiperone.

Forschungszentrum Juelich, Germany, in 1987.³² The easy and reliable synthesis of ^{18}F FETos, its simple and convenient purification, its perfect balance between reactivity and stability in solution, and its unprecedented number of labeling applications attesting to its usefulness make it the most stand-out PG to date (originally cited from Block et al: "This compound appears optimal as fluoroalkylation agent with respect to size, stability and ease of its preparation")³² (Fig. 2). Countless applications of ^{18}F -FETos have been published in the literature and it would be beyond the scope of a review to just count them all. Although many other similar 2- ^{18}F fluoroethyl sulfonates (and triflates cf. above) have been the subject of systematic investigations³³ (eg, for the synthesis of the dopamine reuptake ligand ^{18}F FECNT **7** from precursor **6**, Fig. 3), ^{18}F -FETos remains the most prominent labeling synthon among ^{18}F -fluoro-ethylating agents targeting amino and deprotonated hydroxy functions. ^{18}F FETos functions as an ^{18}F -surrogate PG for ^{11}C -methyl iodide³⁴ although bromo- ^{18}F fluoromethyl or ^{18}F trifluoromethyl synthons structurally more closely resemble ^{11}C methyl iodide in terms of spatial and steric demand. A drawback of these smaller PGs is their more intricate preparation (see above). Especially for less-experienced radiochemists, the synthesis of 1-bromo- ^{18}F -fluoro methane bears some preparative pitfalls, which can translate into low radiochemical yields or an impure PG. Furthermore, ^{18}F -trifluoromethyl-chemistry, despite its high synthetic potential, has only recently been introduced and has not been widely applied yet.³⁵ In stark contrast, the synthesis of ^{18}F FETos is almost foolproof. The precursor ethylene 1,2-ditosylate is commercially available, constitutes a non-volatile solid (unlike dibromo- or diiodo ethane, which are liquids but also greenhouse gases and should be used with care) and has a high molecular weight

allowing accurate, easy, and convenient weighing control. The ^{18}F -fluorination of ethylene 1,2-ditosylate reliably yields the PG between 50% and 80% RCY. The ^{18}F FETos is easily separated by High Performance Liquid Chromatography (HPLC) from its more lipophilic precursor, and final workup is equally simple via solid phase extraction. Even procedures without HPLC separation have been reported.³⁶ As for many PGs, one drawback of ^{18}F FETos should not be disregarded, namely the fact that the exchange of a ^{11}C -methyl group with a ^{18}F -fluoro ethyl group means a change in molecular structure. Albeit small, this structural dissimilarity necessitates that any resulting radiotracer has to be tested for toxicity and change in biological behavior irrespective of whether these tests have already been performed for the original ^{11}C -tracer that is most often structurally equal to a fully evaluated pharmaceutical drug. ^{18}F FETos has been challenged over time by similar PGs such as bromo- ^{18}F -fluoro ethane.^{37,38} The efficient alkali iodide promoted ^{18}F -fluoroethylation of p-anisidine **8**, a very unreactive substrate for alkylations; using both ^{18}F -FETos and bromo- ^{18}F -fluoroethane demonstrates the usefulness of both PGs for radiolabeling (Fig. 3). Several radioligands of relevance in nuclear medicine were labeled applying this methodology, for example, ^{18}F -fluoroethyl-choline **9** (Fig. 3). Although strategies have been reported to routinely prepare bromo- ^{18}F -fluoroethane by various methodologies, ^{18}F -FETos seems continuously to assert itself as the most applied PG in the history of PG applications. An interesting metabolically stable alternative to ^{18}F -FETos has been proposed in 2013 where a ^{18}F -fluorocyclobutyl PG serves as an ^{18}F -labeling agent **10** to provide an ^{18}F -labeled amino acid **11** (Fig. 3). The labeling yields using this synthon unfortunately did not compare favorably with other ^{18}F -alkylating PGs despite its higher in vivo metabolic stability.³⁹

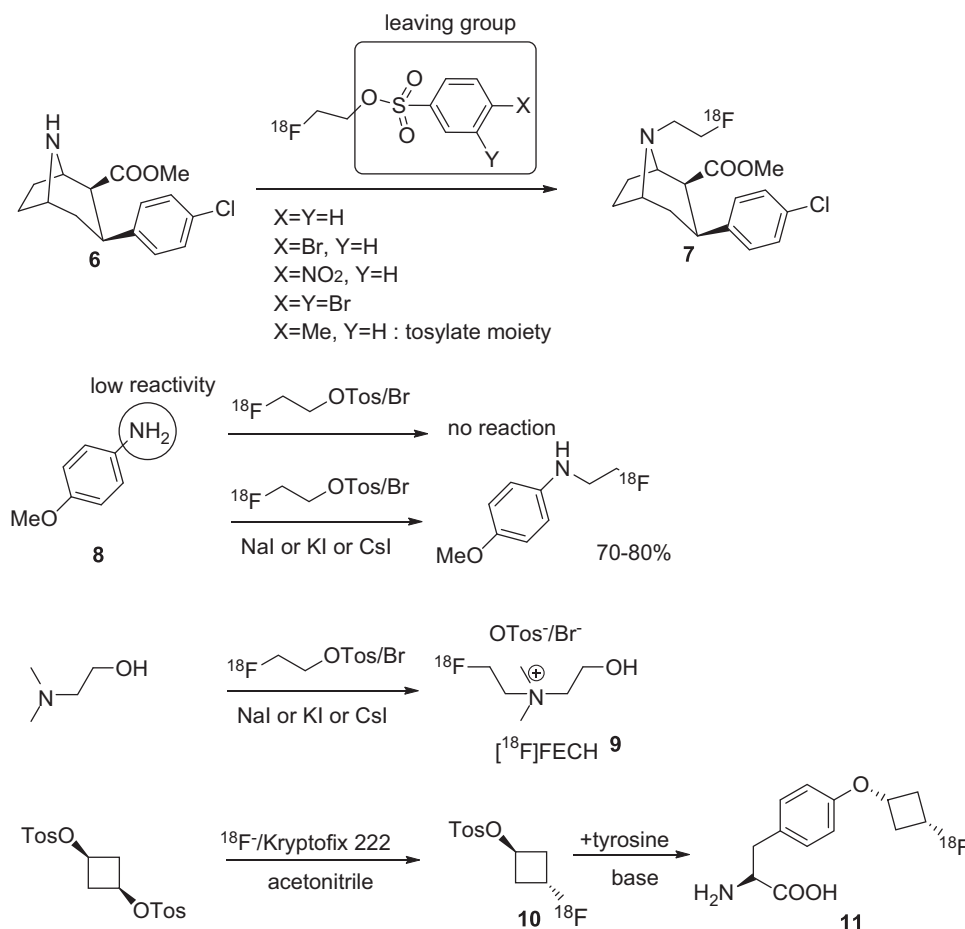


Figure 3 Application of several different ^{18}F -fluoroalkylating PGs for the ^{18}F -fluoroalkylation of different precursor molecules such as the dopamine re-uptake radioligand ^{18}F -FECNT **7**, ^{18}F -FECH **9**, and the tyrosine derivative **11**.

^{18}F -Active Esters: Unspecific but Highly Reactive Labeling Agents

In spite of ^{18}F FETos's usefulness in labeling organic molecules of low to medium molecular weight (<1000 u), it is less suited to react with complex molecules such as peptides and proteins for diverse reasons. First of all, ^{18}F FETos is not "super" reactive (OTos is a nucleofuge of medium reactivity) and requires some energy (eg, higher reaction temperatures) to react efficiently and in high radiochemical yields. Proteins in particular do not tolerate high temperatures and denature quickly. Peptides, if not fully side-chain protected, can easily yield more than just 1 ^{18}F -fluoroalkylation product, demanding laborious purification via HPLC. ^{18}F FETos has never found widespread application for peptide (or other larger compounds such as oligonucleotides) labeling, and conditions for labeling have not been optimized because there are far better PGs for that purpose. Highly reactive chemically activated esters, radiolabeled with ^{18}F , have early on been envisioned as reactive secondary labeling precursors for peptide and protein labeling under preferably aqueous conditions because water perfectly dissolves both. Direct ^{18}F -labeling approaches in water were not available at that time and were only introduced later in 2005-2006 using unconventional radiochemistries based on B- ^{18}F and Si- ^{18}F

formation.⁴⁰ The most prominent example of an activated ester-based PG is *N*-succinimidyl-4- ^{18}F fluorobenzoate (^{18}F SFB) introduced by Vaidyanathan and Zalutsky in the 1990s.^{41,42} Its publication has set a field-wide search in motion for new and even more efficient active ester-based PGs despite the report of significant radioactive side-product formation. The initial synthetic procedure (Fig. 4) where 4-formyl-*N,N,N*-trimethyl anilinium trifluoromethane sulfonate **12** is radiolabeled with ^{18}F to yield 4- ^{18}F fluorobenzaldehyde **13**, which is oxidized into 4- ^{18}F fluorobenzoic acid **14** and finally converted in situ into ^{18}F SFB **15**, has been improved, modified, and automatized to make it more convenient and guarantee high RCYs for routine applications. Depending on the setup and method used, the overall synthesis can take approximately 2 hours, which is decidedly at the higher end of the preferable time line for PG syntheses. Automation of this usually 3-step preparation includes the initial labeling of the triflate salt of ethyl 4-(*N,N,N*-trimethylammonium)benzoate, its ester hydrolysis, and final in situ active ester formation. It was always a challenge for radiochemists to automatize the ^{18}F -SFB synthesis to make its use more attractive for routine radiochemistry. The introduction of other ester moieties such as *tert*-butyl ester into the labeling precursor **16** that is easily cleavable after ^{18}F -fluorination via trifluoroacetic acid yielding ^{18}F -fluorobenzoic acid **14**, has improved the original procedure for ^{18}F SFB synthesis

Vaidyanathan et al. (41, 42)

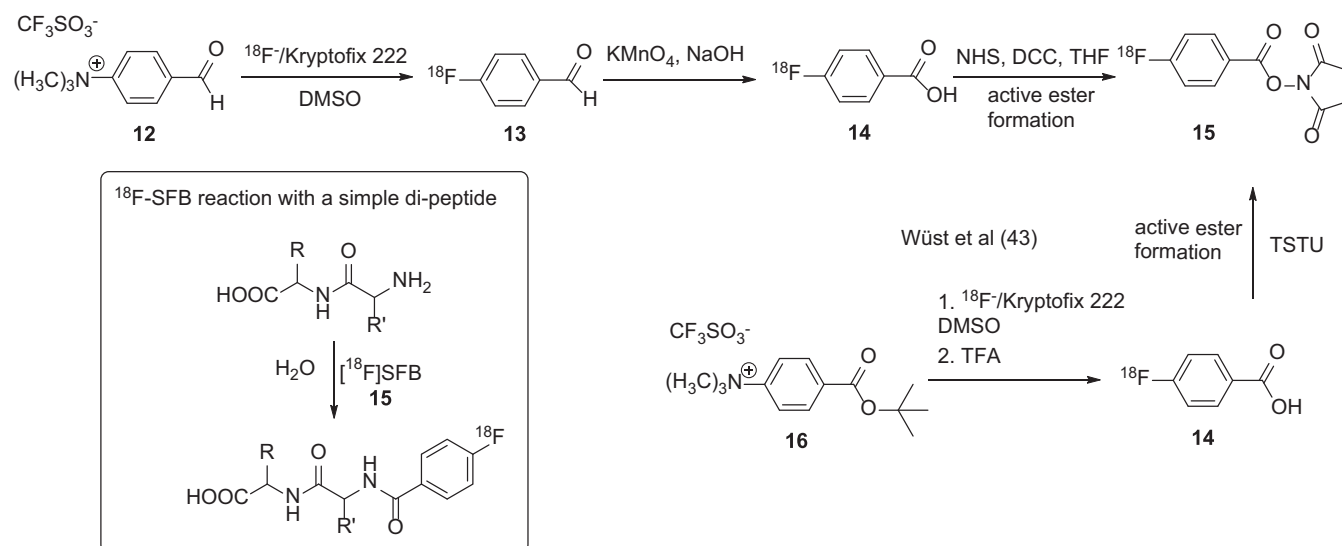


Figure 4 Synthesis of ^{18}F -SFB **15** and its application in radiolabeling of a dipeptide (box).

(Fig. 4).⁴³ Besides technical advancements in ^{18}F -SFB synthesis such as a recently reported 3-step 1-pot synthesis for automation,⁴⁴ other active esters were devised to challenge ^{18}F -SFB with regard to ease of preparation, synthesis time, and labeling efficiency. The most desirable synthetic route toward an ^{18}F -active ester would be a single-step fluorination. One complication on the way toward a 1-step labeling procedure is caused by the inherent lability of the active ester moiety. This strategy was tried very early in 1988 and proved to be unsuccessful at that time.⁴⁵ Although desirable for the intended labeling of proteins, this chemical reactivity hinders the 1-step ^{18}F -fluorination of an active ester precursor. Even if possible, the reactivity of the active ester moiety leads to side products that have to be removed before the subsequent labeling with this PG. The synthesis of N-succinimidyl 4-(^{18}F -fluoromethyl)benzoate (^{18}F -SFMB) **18** by Lang and Eckelman was the first example of a 1-step active ester labeling that yielded the reactive PG in 18% RCY at the end of a 30- to 35-minute synthesis demonstrating impressively the reduction in synthesis time in comparison with the originally published ^{18}F -SFB synthesis.⁴⁶ The relatively low yields of the ^{18}F -fluorination of precursor **17** were explained through experimentally confirmed formation of radioactive side products **19** and products of active ester hydrolysis **20-21** (Fig. 5). ^{18}F -Fluoromethyl benzoate synthesis was further improved by changing the leaving group of the precursor molecule from Tos to Nos (4-nitrobenzene sulfonate) as demonstrated in a 1-step labeling reaction of peptides.^{47,48} In 2010, Olberg et al and Malik et al reported on the 1-step synthesis of the ^{18}F -labeled nicotinic acid derived active ester ^{18}F -Fpy-TFP **23**, where the 2,3,5,6-tetrafluorophenyl active ester group survived the labeling of the trimethylammonium moiety at the 6-position of nicotinic acid **22**.^{49,50} The ^{18}F -fluorination surprisingly takes place at room temperature (Fig. 5). This PG **23** has been used in the synthesis of a PSMA targeting ligand. The purification of the PG was conveniently achieved by simple Sep-Pak cartridge separation, omitting the need for an HPLC system. The yields of the labeling reaction were surprisingly high with 60%-70% at just 40°C, and the con-

secutive reaction with several peptides (eg, arginylglycylaspartic acid (RGD)) was almost quantitative at relatively low peptide concentrations. The recently introduced non-canonical labeling approaches based on B- ^{18}F ^{51,52} and Si- ^{18}F ^{40,53} that are characterized by an easy 1-step introduction of ^{18}F into small as well as complex organic compounds at very mild temperatures, led to the introduction of exotic PGs such as ^{18}F -SiFB **24**,^{54,55} which is structurally very similar to ^{18}F -SFB. One caveat, however, is the higher lipophilicity of ^{18}F -SiFB in comparison with ^{18}F -SFB, limiting its use to protein labeling only, which can sufficiently compensate for the higher lipophilic character of the introduced silicon fluoride acceptor (SiFA) group.⁵⁵ However, the synthesis of ^{18}F **24** is outstandingly convenient, relying on the concept of isotopic exchange and one simple cartridge purification step only as a result of the chemical identity of precursor and labeled product (Fig. 5). Another PG bearing an active ester group has been published in 2013, tackling the high lipophilicity of the transferred ^{18}F -fluoro benzyl group that can sometimes cause trouble when attached to small peptides.⁵⁶ Although not based on a convenient 1-step reaction, this sulfonated hetero-bifunctional ^{18}F -crosslinking PG **28** is based on the ring opening of a 1,3-propane sultone **25** with ^{18}F -yielding **26**, its deprotection to compound **27**, and its final conversion into an N-hydroxysuccinimidyl ester **28** (Fig. 6). The ^{18}F -ring opening yielded only low amounts of product under conventional ^{18}F -labeling conditions (acetonitrile, Kryptofix222, base) but works exceptionally well in tertiary alcohols (up to 90% radiochemical yield) at 90°C, a new labeling paradigm introduced by Kim et al.⁵⁷ The sultone ring opening leads to the liberation of a sulfonic acid group, which imparts hydrophilicity to the PG. The PG **28** has only been used so far in the synthesis of an ^{18}F -labeled cyanine dye, and its usefulness for peptide and protein labeling has still to be proven. Shortly after, the same group applied a sultone-based labeling approach featuring a bis-sultone cross-linker to the synthesis of a peptide without implication of an active ester moiety.⁵⁸ Another well-tolerated and synthetically proven ^{18}F -active ester was introduced by Gohlke et al in 1994,⁵⁹ and

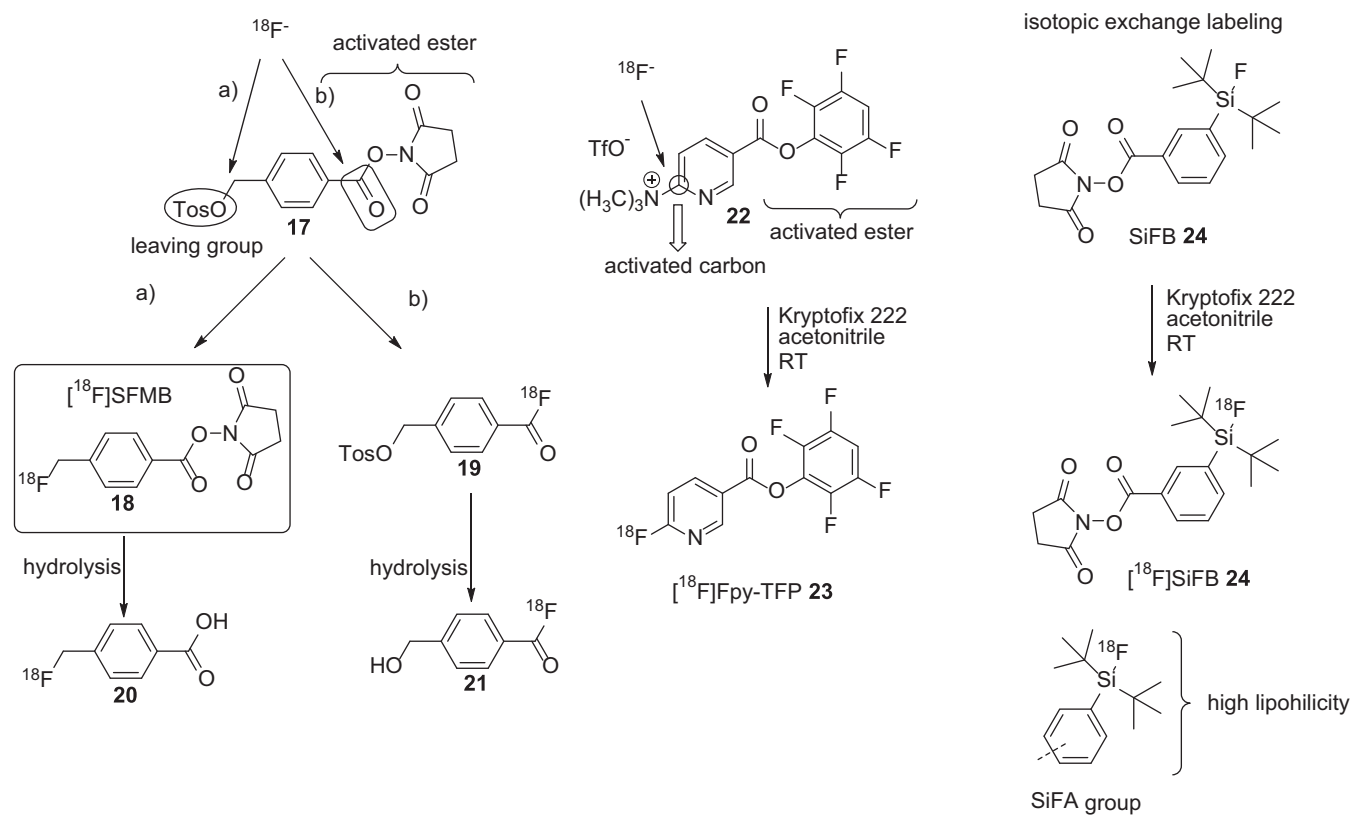


Figure 5 Synthesis of different active ester PGs (18, 22, and 24) and possible side reactions (hydrolysis of 18 to 20 and 19 to 21).

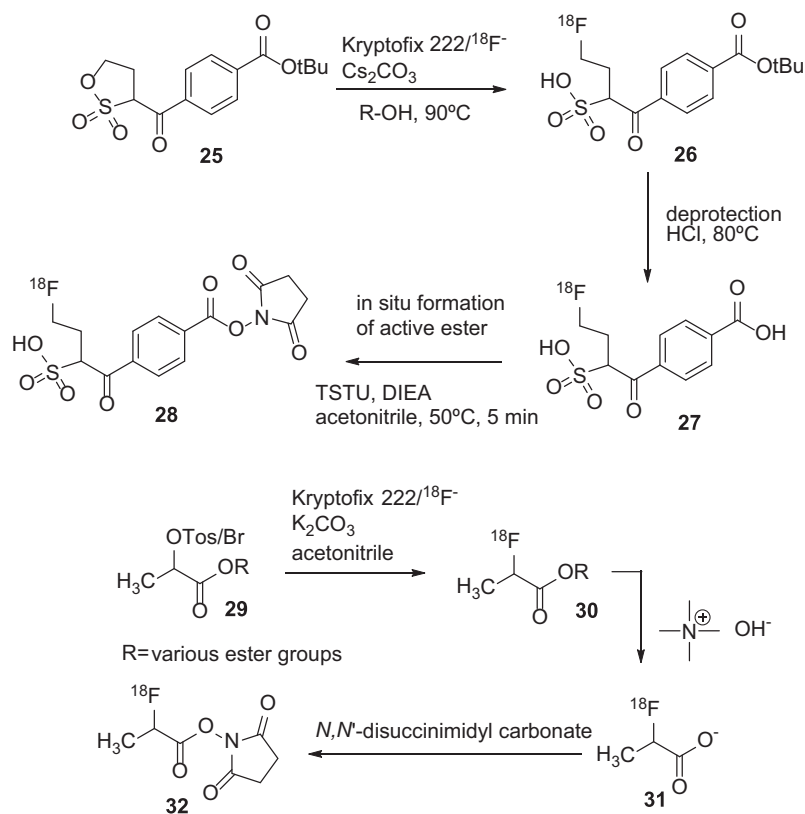


Figure 6 Synthesis of active esters PGs 28 (sultone based) and N -succinimidyl-2- $[^{18}\text{F}]$ fluoropropionate 32.

used in the synthesis of ^{18}F -peptides.⁶⁰⁻⁶² The small PG N-succinimidyl-2- ^{18}F fluoropropionate **32** was used in the labeling of RGD dimer peptides, confirming its usefulness for peptide-based PET tracer development.⁶³ The synthesis of **32** starts from either the bromo or the tosyl precursor **29**, which is ^{18}F -labeled under standard conditions, yielding ester compound **30**. The ester moiety is cleaved by a base, and the resulting ^{18}F -acid **31** is converted into the final active ester PG **32** (Fig. 6). A simplified 1-step synthesis for **32** has been added recently to the portfolio of active ester PGs.⁶⁴

Click Chemistry: Modern Tools for PG Development

Besides recent metal-mediated late-stage ^{18}F -fluorination (see above), the facilitation and perfecting of click chemistry for ^{18}F -radiolabeling (and other radioactive tags) has been seriously changing radiochemistry over the last 2 decades after K. Barry Sharpless introduced the terminology to organic chemistry in 2001.⁶⁵⁻⁶⁷ Click chemistry applications in all fields of radiochemistry have extensively been reviewed previously.⁶⁸⁻⁷³ Especially, ^{18}F -PG development has tremendously benefited from the concept of click chemistry. The chemical efficiency of ^{18}F -PG bioconjugation has been maximized through the utilization of a wide contingent of click-chemistry reactions such as oxime formation, Michael addition (eg, thiol-maleimide conjugation), 1,3-dipolar Huisgen cycloaddition (eg, (Cu(I) assisted as well as catalyst-free triazole formation), and more recently Diels-Alder-like reactions (eg, tetrazine-dienophile reactions). One outstanding feature of click chemistry is its orthogonality, which adds an unprecedented chemoselectivity to PG conjugation to organic compounds that contain a large number of different functional chemical groups. A click chemistry reaction should be simple, fast, and modular. High chemical (and radiochemical) yields of stable products (such as radiotracers) and easy removable side products (no chromatographic removal) characterize a successful click reaction. If applied to radiolabeling, another important feature has to be added to click chemistry's list of requirements, namely a fast kinetic profile that is compatible to the physical half-life of the applied radionuclide and applicability to low nanomolar concentrations of the ^{18}F -PG. Some of the most frequently used click chemistry reactions in radiochemistry are discussed below.

1,3-Dipolar Huisgen Cycloadditions: Catalyzed and Non-catalyzed [2 + 3]Cycloadditions

Triazoles, besides being very stable and biocompatible, bear topological and electronical similarities to an amide bond. Their introduction into the context of ^{18}F -labeling chemistry in 2006-2007 by Marik and Sutcliffe,⁷⁴ and Glaser and Årstad⁷⁵ (almost

simultaneously) set off extraordinary research efforts toward ^{18}F -radiotracer development using click chemistry (Fig. 7). ^{18}F -Labeled ^{18}F -fluoroalkynes **33** (Marik and Sutcliffe's approach) as well as ^{18}F -azides **34** (Glaser and Årstad's strategy) quickly proved their value as PGs for biomolecular labeling where a compatible alkyne or azide moiety was conjugated to the N-terminal end of a peptide or a derivatized compound of lower molecular weight.⁷⁶⁻⁸⁰ The Cu-catalyzed formation of the triazole proceeds via a [2 + 3]cycloaddition (1,3-dipolar Huisgen cycloaddition) and is usually fast and proceeds without the formation of major side products. However, the catalytic system has to be individually adjusted for each click situation. Some spin-off labeling synthons using different ^{18}F -fluoroalkynes and ^{18}F -fluoroazide-bearing PGs (eg, various carbohydrate-based azides)⁸¹ have been described in recent years⁸²⁻⁹³ (Fig. 7, bottom). For example, 2-deoxy-2- ^{18}F fluoroglucopyranosyl azide **37**, introduced by Maschauer and Prante in 2009-2010, was proven to be a valuable tool for the labeling of biomolecules adding a biocompatible glucose moiety to the tracer compound counteracting the lipophilicity other PGs impart on the resulting molecule.^{94,95} The synthesis starts from a fully protected trifluoromethansulfonyl-beta-D-mannopyranosyl azide **35**, which can be ^{18}F -radiolabeled in high radiochemical yields to obtain the PG intermediate **36** (Fig. 7). The acetyl protecting groups are removed under basic conditions to yield PG **37** for further click reactions. In every individual case and for each application, the resulting compound from click labeling has to be evaluated again toward its binding affinity to the molecular target. Silicon- as well as boron-based ^{18}F -radiochemistry has been used to devise new alkyne and azide containing PGs for click radiolabeling⁹⁶⁻⁹⁸ (Fig. 7). Triazole formation has evolved over time,⁹⁹ omitting the metal catalyst from the reaction mixture exploiting conformationally optimized cyclooctyne systems that react immediately with azides as a result of a reduced activation energy for the cycloaddition.¹⁰⁰⁻¹⁰⁴ This so called strain-promoted copper-free click chemistry has been introduced in 2011 by several groups. Campbell-Verduyn et al introduced ^{18}F -PEGglyated azide **38** and performed the reaction with aza-benzocyclooctyne derivatives in plasma-dimethylsulfoxide (DMSO) mixtures to prove biocompatibility of the click reaction for future in vivo labeling (Fig. 8).¹⁰¹ The resulting strain-promoted conjugation product **39** was obtained in 42% radiochemical yield. Other groups explored the ^{18}F -labeling of the aza-dibenzocyclooctyne building block and its reaction with azide-modified peptides. The cyclooctyne can be labeled in sufficient radiochemical yields (cf. compounds **40** and **41**) either directly with ^{18}F -¹⁰⁰ or by using the PG [^{18}F]SFB,¹⁰² and it reacts smoothly with azide-modified peptides (Fig. 8). A general concern regarding the use of ^{18}F -labeled dibenzocyclooctynes is their high lipophilicity that negatively impacts in vivo PET imaging via unfavorable bio-distribution patterns (high liver uptake). Kettenbach and Ross therefore introduced a hydrophilic, ^{18}F -labeled PEG linker to the dibenzocyclooctyne structure, reducing the overall lipophilicity of PG **42** (Fig. 8).¹⁰⁴

Strictly speaking the [3 + 2]cycloadditions comply only with the specifications of click reactions if the reaction is performed

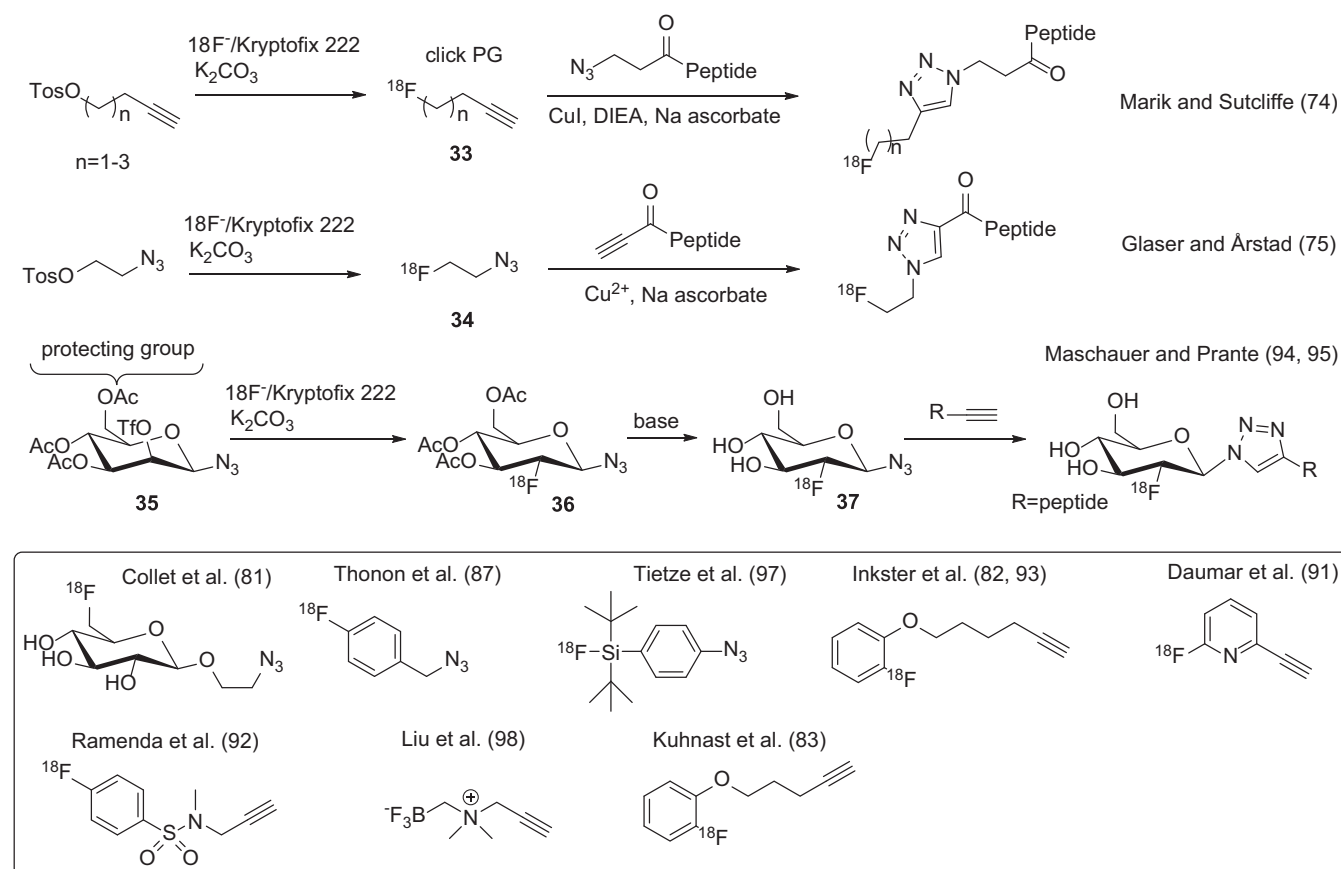


Figure 7 Copper-I catalyzed 1,3-Dipolar Huisgen cycloadditions introducing ^{18}F -labeled alkynes (eg, **33**) and azides (eg, **34** and **37**) as efficient PGs for peptide labeling. BOX: different ^{18}F -alkynes and azides employed as PGs.

in an organic solvent. As demonstrated by Campbell-Verduyn et al, the radiochemical yields in biological plasma samples are not approaching quantitative conversion. However, it is remarkable that the reaction proceeds at all in a complex reaction environment like this, further attesting to its bio-orthogonality.

^{18}F -Aldehydes: A Clickable PG for Peptide Labeling

Aldehydes are extensively being used for the ^{18}F -labeling of peptides via bio-orthogonal click chemistry. Several functional groups such as aminoxy-, amino-, and hydrazine groups mildly and selectively react with radiolabeled aldehydes to oximes, Schiff bases, and hydrazones. Schiff base formation, however, is less suitable for bioconjugations because of the sheer abundance of amino functions in proteins and its lower stability under physiological conditions. Hydrazines react slower with aldehydes¹⁰⁵ than aminoxy derivatives, making them less common click partners for the ^{18}F -labeling of peptides. Even though not the most reactive of aldehydes, 4- ^{18}F fluorobenzaldehyde **44**¹⁰⁶ is the most commonly applied ^{18}F -aldehyde in radiochemistry and has been used numerous times to introduce ^{18}F into peptides, multimeres, and proteins¹⁰⁶⁻¹¹¹ (Fig. 9). Its synthesis is straightforward starting from 4-trimethylammonium benzaldehyde triflate **43** and performed

under standard ^{18}F -labeling conditions. It has also been converted elegantly into ^{18}F -4-fluorobenzyl iodide for the labeling of dopamine receptor ligands.¹¹² Data from the literature demonstrate that a PEG linker between the ^{18}F and the aldehyde moiety (cf compound **45**) led to a more favorable binding conservation of derivatized RGD peptides for integrin $\alpha_v\beta_3$ imaging.¹⁰⁷ Another ^{18}F fluoro-PEG-benzaldehyde **46** was reported for peptide labeling via oxime formation that can be purified by simple solid-phase extraction.¹¹³ A heteroaromatic ^{18}F -aldehyde, 2- ^{18}F fluoro-pyridinecarboxaldehyde **47**, has been applied in the fully automated synthesis of A β peptides (Fig. 9).¹¹¹ Even no-carrier-added 2- ^{18}F FDG **48**, in its open, non-cyclic form **49**, can be used directly for oxime formation as a result of its naturally occurring aldehyde group. A direct site-specific radiolabeling approach has been reported for cyclo RGD peptides from two groups^{114,115} and further used to ^{18}F -label folic acid derivatives for oncological imaging.¹¹⁶ An ^{18}F -labeled aldehyde based on the SiFA building block has been introduced by Schirmacher et al using isotopic exchange labeling in high radiochemical yields and specific activity. The SiFA PG **50** could be prepared without HPLC purification and conveniently used for aminoxy-derivatized peptide conjugation.¹¹⁷ Isotopic exchange has also been used by Herman et al for the synthesis of ^{18}F -pentafluoro benzaldehyde **51**. The specific activity of **51**, despite being low, was sufficient to radiolabel HSA protein via reductive amination.¹¹⁸ The smallest ^{18}F -aldehyde reported is ^{18}F fluoroacet aldehyde **52**, which was elegantly obtained via dimethyl sulfoxide oxidation of

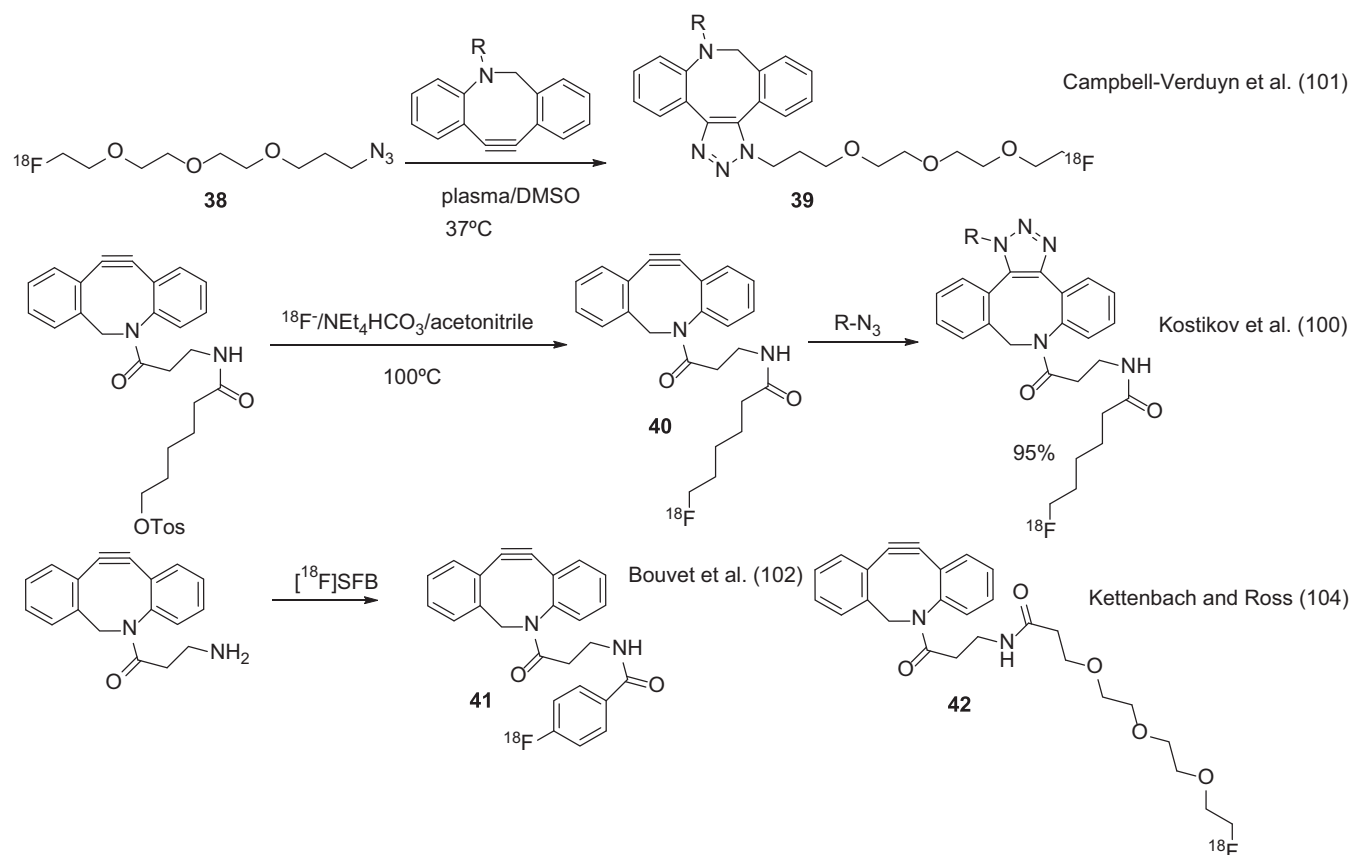


Figure 8 Strain promoted, catalyst-free 1,3-dipolar cycloadditions using both ^{18}F -azides (eg, **38**) and ^{18}F -aza-dibenzocyclooctynes (eg, **40**, **41**, and **42**) for peptide labeling.

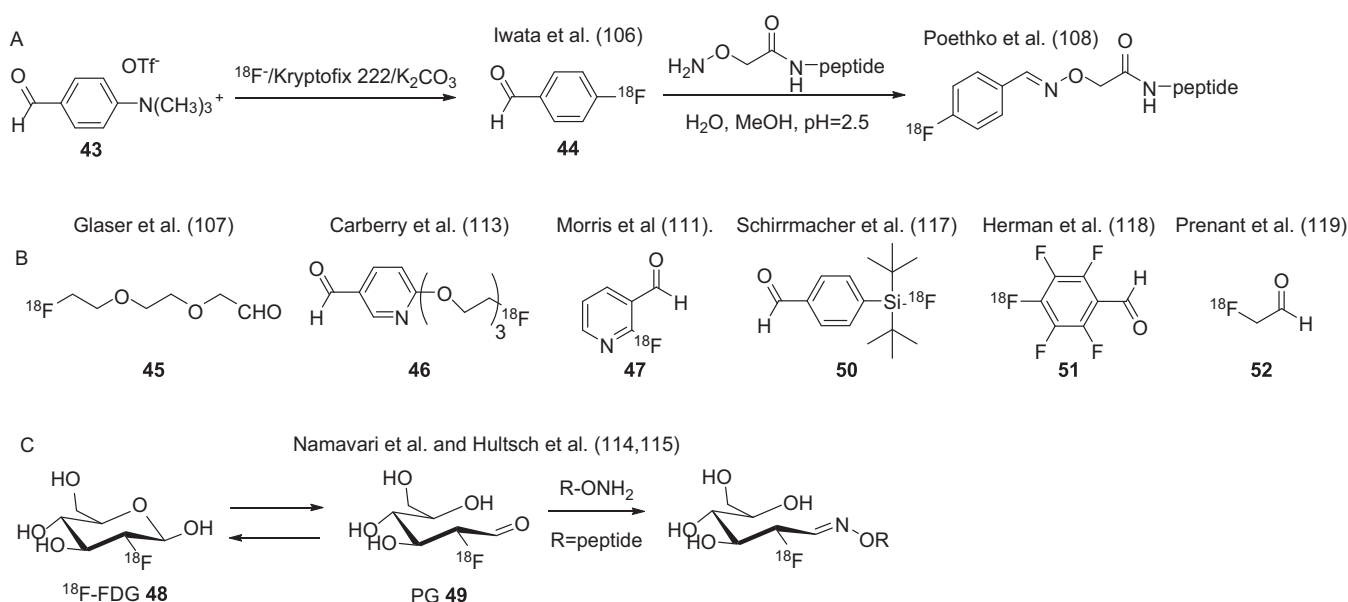


Figure 9 (A) Synthesis of 4-[^{18}F]fluorobenzaldehyde **44** and its reaction with aminooxy derivatized peptides. (B) Structures of various ^{18}F -labeled aldehydes. (C) Equilibrium between [^{18}F]FDG **48** and its open chain form **49** (the actual PG) bearing an aldehyde group for conjugation to aminooxy-derivatized peptides.

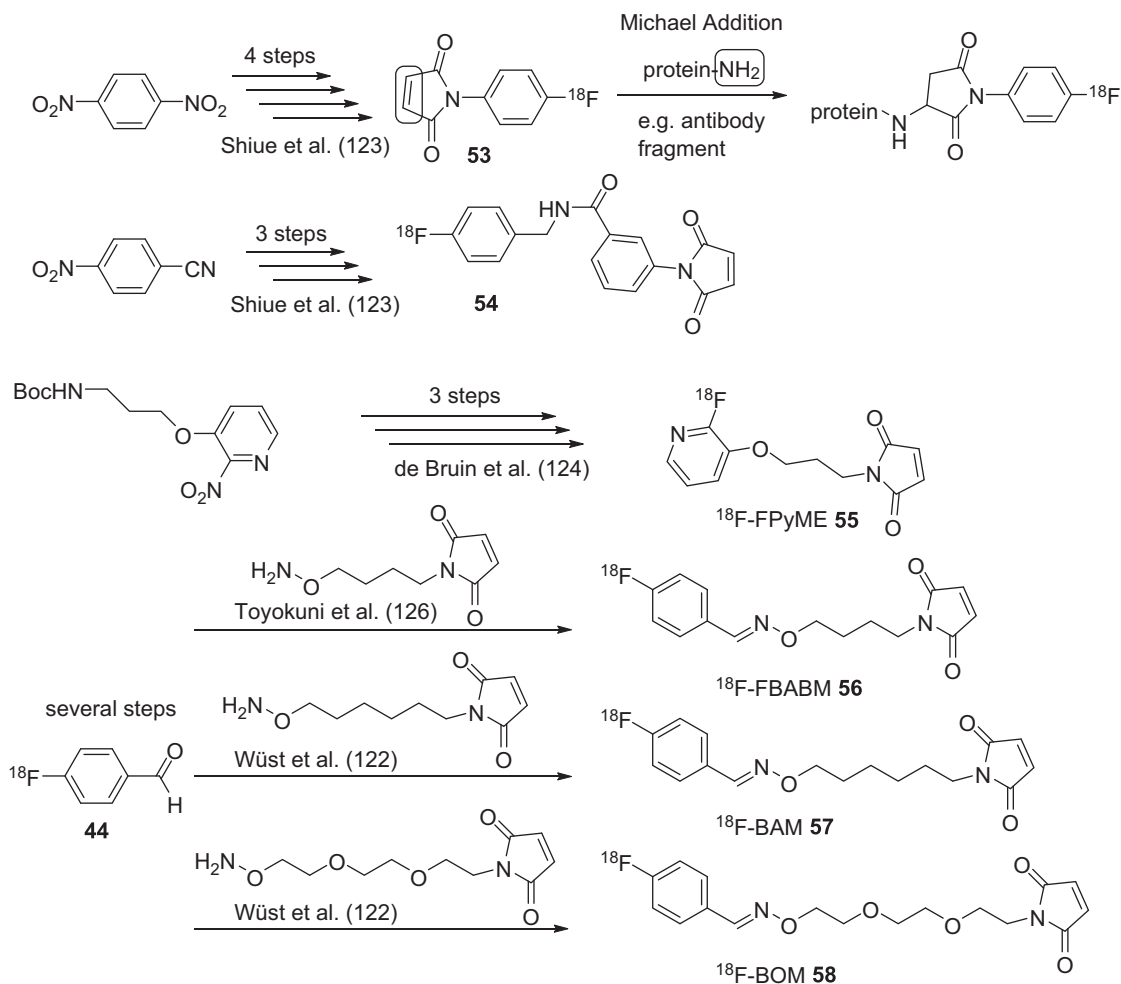


Figure 10 Syntheses of various maleimide-based PGs (53-58) for radiolabeling of biomolecules through targeting of amino and thiol groups.

^{18}F FETos and advertised as a possible substitute for 4- ^{18}F fluorobenzaldehyde¹¹⁹ (Fig. 9).

Maleimide- and Thiol-based PGs for Fast Michael Additions

Amines or thiols and maleimides quickly and chemoselectively react to form amino- or thiopyrrolidine-2,5-diones via Michael addition (Fig. 10). This labeling strategy has been used both for peptide and for protein labeling in high radiochemical yields. Proteins for example contain amino groups as well as thiol-bearing cysteine side chains, which react easily with maleimide-bearing PGs. Even if proteins do not contain sufficient thiol functional groups (thiols react much better with maleimides than amino groups), it is possible to introduce them artificially, determine their number analytically (eg, Ellman assay), and use them for a following radiolabeling. If carefully performed, the addition of those extra thiols or maleimides does not negatively impact the biological properties of the protein.¹²⁰⁻¹²² ^{18}F -labeled maleimides have received special attention for the development of PGs and were first introduced in 1989 by Shiue et al for

the labeling of an antibody fragment (presumably at an NH₂ group).¹²³ The two ^{18}F -maleimide PGs 53 and 54 were laboriously synthesized in 4 and 3 steps, respectively. Although a demonstration of radiochemical preparative skill, these PGs did not find any future applications as a result of their far too complicated syntheses for radiotracer development. Today, the ^{18}F is almost exclusively introduced into an activated aromatic system such as benzaldehyde comprising a leaving group in 4-position or an activated pyridine system.¹²⁴ For example, in a preliminary reaction 4- ^{18}F fluorobenzaldehyde, which is a popular PG itself (cf. ^{18}F -aldehydes) or even ^{18}F FDG, the most popular ^{18}F -radiotracer today can be reacted with an aminoxy-derivatized maleimide (Fig. 10). This strategy has proven valuable in the syntheses of N-[4- ^{18}F fluorobenzylidene]aminoxy]hexyl]maleimide 56,^{125,126} N-[4-(aminoxy)butyl]maleimide 57,¹²² and 4- ^{18}F fluorobenzaldehyde-O-(2-[2-(pyrrol-2,5-dione-1-yl)ethoxy]-ethoxy)-ethyl]oxime 58.^{122,125} De Bruin et al introduced 1-[3-(2-[^{18}F fluoropyridin-3-yloxy]propyl)pyrrole-2,5-dione 55¹²⁴ for peptide and protein labeling following a different multistep synthesis strategy. Unfortunately, conventional labeling methodologies for ^{18}F -introduction do not permit a 1-step labeling of maleimide-bearing PG precursors

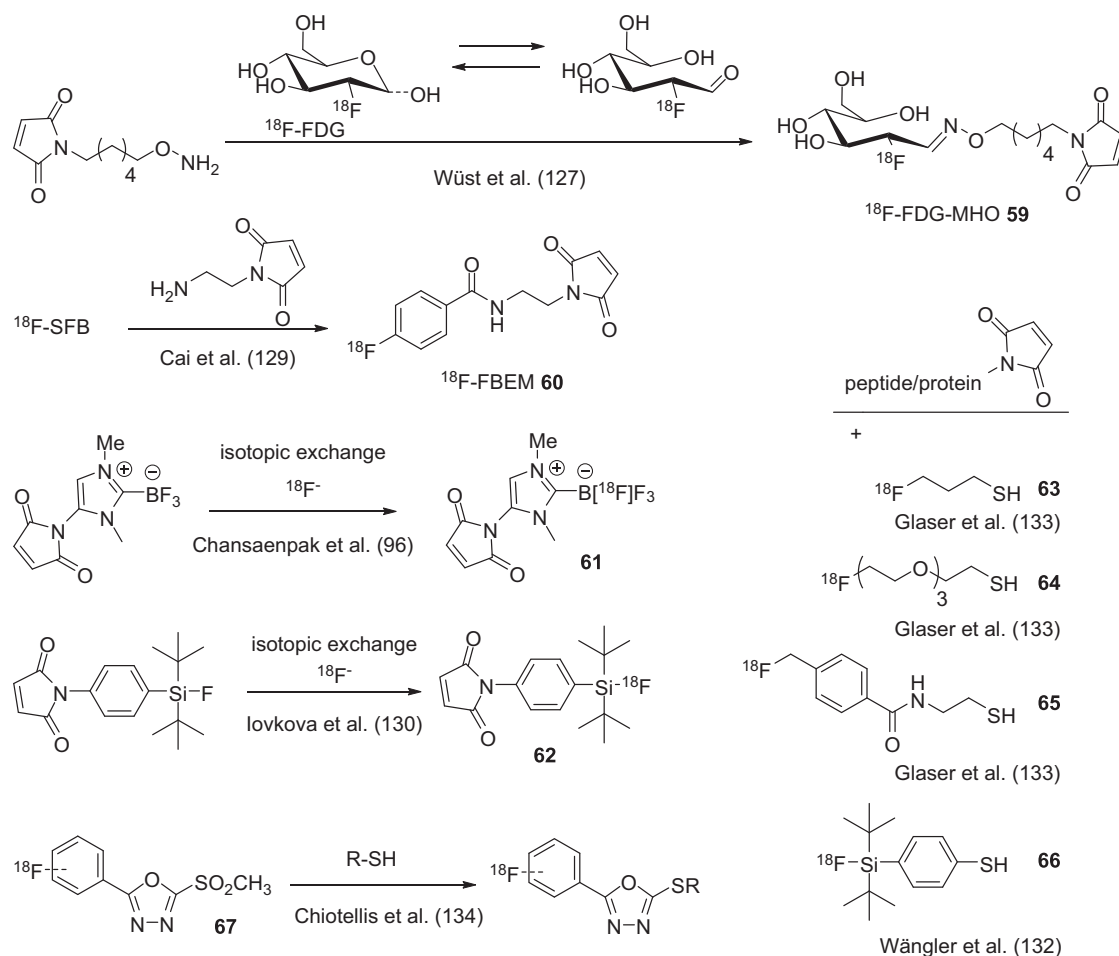


Figure 11 ^{18}F -Maleimides (eg, **59–62**) and ^{18}F -labeled thiols (compounds **63–66**) for peptide and protein labeling via Michael addition and [^{18}F]fluorophenylloxadiazole methyl sulfone **67** as an alternative thiol reactive PG.

because of the instability of the maleimide group under basic labeling conditions. In an elegant work, ^{18}F -FDG was used directly as a PG for the synthesis of a maleimide PG, [^{18}F]FDG-MHO **59**, for peptide labeling¹²⁷ (Fig. 11). *N*-[2-(4-[^{18}F]fluorobenzamido)ethyl]maleimide **60**,^{128,129} another maleimide PG, used [^{18}F]SFB in a coupling reaction with a maleimide for the synthesis of ^{18}F -labeled RGD peptides.¹²⁹ Generally, the use of a multi-chain PG application is time-consuming, intricate, and inconvenient. If more than just one PG is used to label a compound of interest, the resulting complexity of the labeling reaction quickly gets out of hand and makes these approaches less attractive for routine production of clinically used radiotracers. Both B- ^{18}F ⁹⁶ and Si- ^{18}F ^{130,131} as non-canonical chemistries contributed to the vast contingent of maleimide PGs introducing easy to synthesize synthons **61** and **62** for ^{18}F -labeling (Fig. 11). Both methodologies are characterized by efficient 1-step labeling procedures, significantly cutting the time required for synthesis and are generally eligible for peptide and protein labeling. The maleimide SiFA PG **62** was used for the labeling of rat serum albumin, which was artificially modified with additional thiol groups (Fig. 11). Naturally, the labeling of a biomolecule will also work the other way around by labeling a thiol-containing PG such as **63–66** with ^{18}F and react it with a maleimide group present

in the precursor molecule (peptide or protein). PGs bearing an SH group have been synthesized for this purpose and used in biomolecular labeling settings.^{132,133} A most recent addition to thiol-homing PGs is the introduction of a heterocyclic methylsulfone derivative by Chiotellis et al for the ^{18}F -labeling of biomolecules under mild aqueous conditions¹³⁴ (Fig. 11). The methyl sulfonyl group of compound **67** acts as a leaving group and is replaced by the thiol-bearing biomolecule. The thiol group is one of the strongest nucleophiles in chemistry and therefore reacts not only with maleimides but also with bromo acetamides, which constitutes another possible bioconjugation venue for SH-containing PGs. Bromo acetamides themselves are valuable labeling synthons for a variety of labeling applications and have been used frequently.⁷⁰

^{18}F -Amines as PGs for ^{18}F -radiolabeling

^{18}F -Labeled active esters introduced earlier are primarily targeting the N-terminal ending (or lysine amino acid side chain functionalities if present) of a peptide. The labeling of the C-terminus has been achieved via ^{18}F -fluoroalkylamidation after in situ activation of the carboxylic group. In the style of

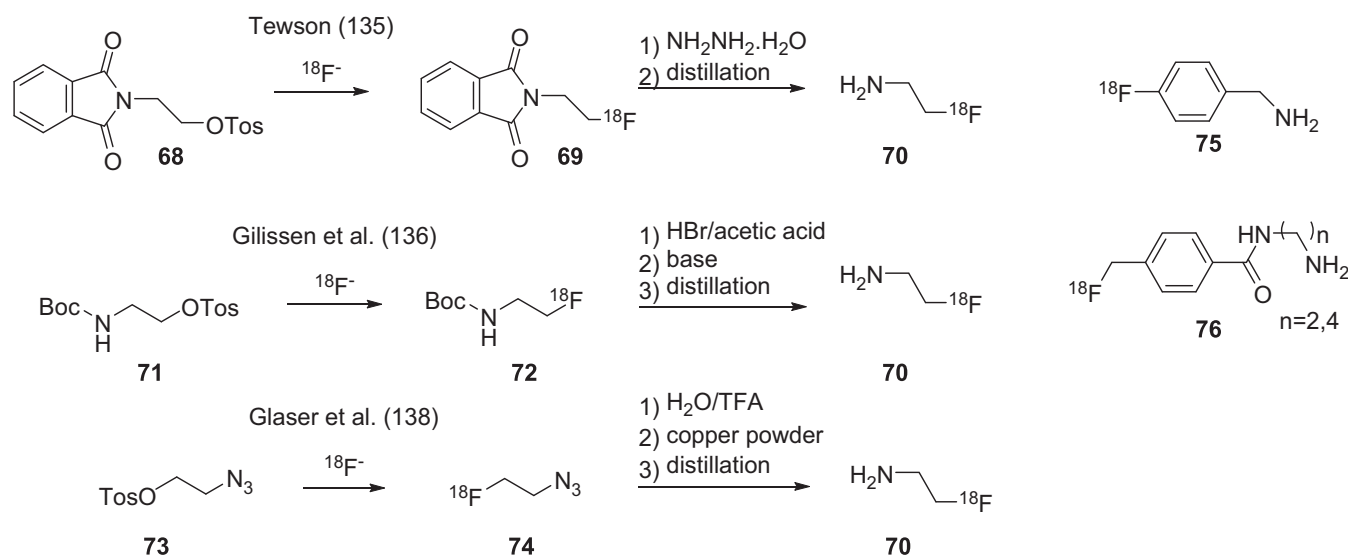


Figure 12 Different synthetic strategies to obtain 2-¹⁸F-fluoroethyl amine **70** as a highly nucleophilic PG and structures of other amine bearing PGs (**75** and **76**) for ¹⁸F-radiolabeling of biomolecules.

¹⁸F-fluoroethyl transfer reminiscent of ¹⁸F-FETos or ¹⁸F-bromo-fluoro-ethane, the introduction of a short ¹⁸F-fluoroethyl group was believed to minimize the structural alteration of the peptide to be labeled. The synthesis of ¹⁸F-fluoroethyl amine was first described by Tewson who reacted N-[2-(toluene-4-sulfonyloxy)ethyl]-phthalimide **68** with ¹⁸F⁻ yielding the protected intermediate **69**, which was deprotected to 2-¹⁸F-fluoroethyl amine **70**¹³⁵ (Fig. 12) and further optimized by Gilissen et al.¹³⁶ ¹⁸F-Fluorination of 2 different precursors, N-[2-(p-toluenesulfonyloxy)-ethyl]phthalimide **68** and N-t-Boc-[2-(p-toluenesulfonyloxy)ethylamine] **71**,¹³⁶ yielded 2-[¹⁸F]fluoroethylamine **70** after deprotection of either **69** or **72** and final distillation for further radiolabeling of activated carboxylic acids. The latter route was further improved by Jelinski et al by simply changing the reaction solvent from acetonitrile to DMSO and introducing a more sophisticated purification procedure based on solid-phase extraction omitting the cumbersome distillation step.¹³⁷ In 2012, a new radiochemistry was described that completely dismissed the necessity for an amine-protecting group. Previously, this was needed to prevent the amine group from intra-molecularly reacting with the carbon atom carrying the tosyl leaving group. 2-¹⁸F-fluoroethyl amine **70** was obtained by copper-mediated reduction of easy to synthesize 2-¹⁸F-fluoroethylazide **74** from the tosyl precursor **73**. The azide group does not need to be protected as a result of its inertness to the ¹⁸F-labeling conditions. However, purification entailed a final distillation, which is generally less suited for automation.^{75,138} 2-¹⁸F-fluoroethyl amine is one of those PGs that would find far more applications in tracer preparation if the synthesis would be less finicky. 4-[¹⁸F]fluorobenzylamine **75**, a more lipophilic amino PG, has been proven valuable for the preparation of maleimide-containing PGs and might also find applications in amidation chemistry.¹³⁹ Its synthesis was improved over previously described procedures¹⁴⁰ and can be fully automated.¹⁴¹ One of the very first ¹⁸F-labeled amines described was

1-[4-([¹⁸F]-fluoromethyl)benzoyl]aminobutane-4-amine **76**, an early PG employed for the labeling of insulin.¹⁴² This PG, however, is sterically more demanding than 2-[¹⁸F]fluoroethyl amine and did not find further application. The availability of far more convenient PGs for peptides and proteins made its use less appealing.

PGs for the Inverse Electron Demand Diels-Alder Reactions

Most recently, the inverse electron demand Diels-Alder reaction (IEDDA) has captured the attention of radiochemists for the use in tracer development.¹⁴³⁻¹⁴⁷ The highly efficient reaction between 1,2,4,5-tetrazines and strained dienophiles proved to be a fast and clean bioorthogonal conjugation method (Fig. 13). The reaction is furthermore catalyst free, which favorably translates into easier purification of the final radiotracer. The kinetics of the IEDDA are a lot faster than those of other bioconjugation click chemistries such the 1,3-dipolar azide-alkyne formation. The ¹⁸F-label can be either introduced into the tetrazine building block or the dienophile. ¹⁸F-Labeled trans-cyclooctenes (eg, compound **77**) have been jointly investigated as a PG for tetrazine-derivatized biomolecules (eg, peptides) by the Conti and Fox groups.¹⁴⁸⁻¹⁵⁰ ¹⁸F-PG **77** reacts readily with tetrazine-derivatized peptide **78** to the IEDDA product **79** (Fig. 13). A difficulty, however, is the delicate stability of tetrazines in general, which can pose a problem for the conjugation of tetrazines to biomolecules. Another ¹⁸F-building block for IEDDA has been described by Li et al where a complex bicyclo[6.1.0]nonyne-based ¹⁸F-PG **80** was employed to label tetrazine derivatized anti-microRNA-21 **81**.¹⁵¹ Targeting the tetrazine moiety for ¹⁸F-labeling has its advantages because the bioconjugation of a complementary dienophile to a biomolecule of interest suffers less from stability issues. The downside of tetrazine ¹⁸F-labeling is,

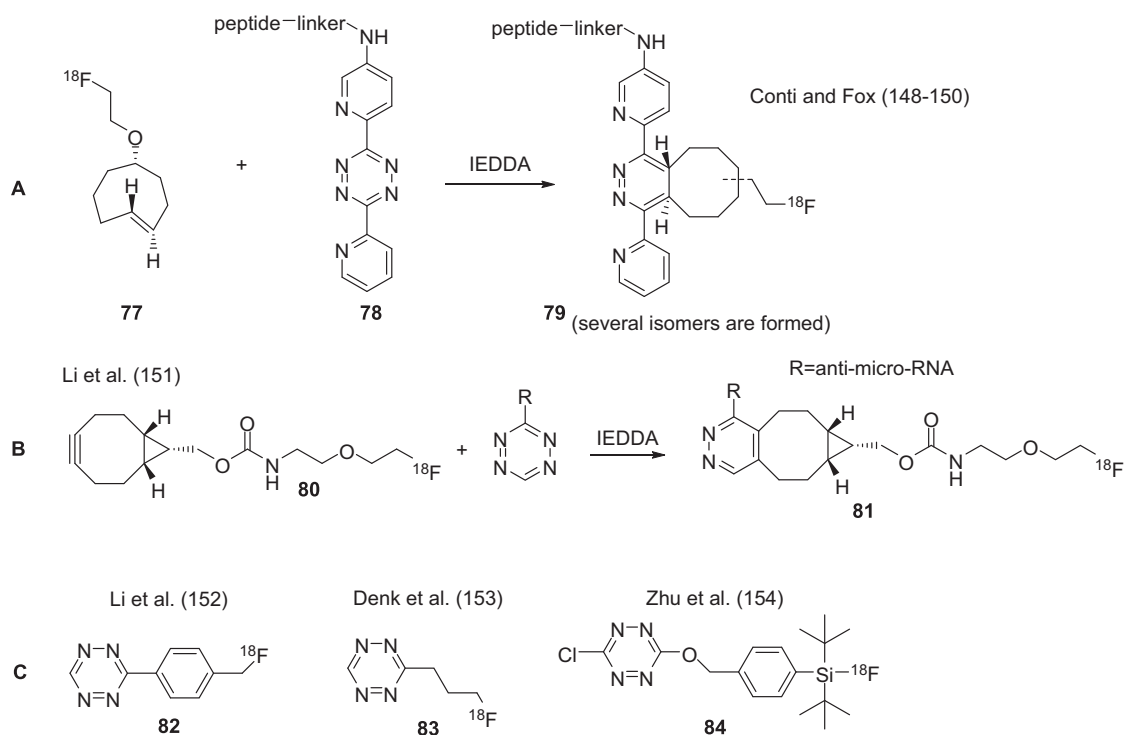


Figure 13 PGs for the inverse electron demand Diels-Alder reaction (IEDDA): (A) ^{18}F -Labeled trans-cyclooctene PG 77 for the labeling of tetrazine-derivatized peptides. (B) The bicyclononyne-derived PG 80 was used in the synthesis of ^{18}F -labeled anti-micro RNA. (C) Structures of several recently described ^{18}F -tetrazine PGs (82-84) for targeting strained unsaturated cyclic systems.

however, to ensure that the tetrazine itself survives the labeling procedure and yields sufficient ^{18}F -tetrazine for the final bio-labeling. Several groups labeled tetrazine derivatives with ^{18}F with radiochemical yields ranging from less than 1% to 80% (Fig. 13). Key to a successful introduction of anionic $^{18}\text{F}^-$ into a molecule comprising a tetrazine moiety are the labeling conditions applied. Heating under very basic conditions to do a simple nucleophilic exchange of a common leaving group with $^{18}\text{F}^-$ seems to have a detrimental effect on radiochemical yields as reported by Li et al for compound 82.¹⁵² Improved radiochemical yields of ^{18}F -labeled tetrazine 83 and 84 were reported by Denk et al¹⁵³ and by Zhu et al for a SiFA-PG.¹⁵⁴ Especially the use of the mild SiFA labeling chemistry by Zhu and coworkers at room temperature avoided the formation of any radioactive side products and made the overall purification of the tetrazine PG easy and reliable. With regard to general biocompatibility of the resulting ^{18}F -Diels-Alder addition products, the IEDDA labeling approach still has to be optimized to reduce the overall lipophilicity of the participating educts and solve the problem of different isomers that are formed by the IEDDA (cf. compound 79) reaction pathway. In terms of radiochemistry, this click methodology bears tremendous potential and prospect for new developments.

Miscellaneous PGs for ^{18}F -labeling

Peptides have generally more functional groups than compounds of low molecular weight. Introducing new labeling

techniques to selectively target one amino acid within a peptide (or perhaps even a protein) would add some desirable control to the labeling reaction and final compound characterization. Recently, new PGs for the specific labeling of tyrosine have been reported, introducing 2 novel ^{18}F -labeled 1,2,4-triazole-3,5-dione synthons [^{18}F]PTAD 86 (5-step reaction starting from 85) and [^{18}F]SF-PTAD 88 (2-step reaction starting from 87),^{155,156} exploiting an ene-type (tyrosine-click) reaction introduced first by Ban et al as a general click chemistry tool for small as well as larger organic compounds such as peptides and proteins¹⁵⁷ (Fig. 14). So far, this exciting chemistry has only been used for the ^{18}F -labeling of small molecules of low complexity, and its value as a peptide or protein labeling methodology still has to be verified. As a result of the instability of the 1,2,4-triazole-3,5-dione structure, it is doubtful whether a 1-step labeling is possible to circumvent multistep procedures. ^{18}F -Isocyanates and isothiocyanates have been of limited value to ^{18}F -radiochemistry so far despite their immense employment in organic preparative chemistry.⁷⁰ One reason is their moderate reactivity and rather long reaction times needed for their introduction into peptides and proteins at lower temperatures. Only a few ^{18}F -labeled isothiocyanates and isocyanates have been applied for radiolabeling with isothiocyanates being more reactive than isocyanates. The mild SiFA chemistry enabled a smooth introduction of $^{18}\text{F}^-$ into the PGs (both isocyanate and isothiocyanate functionalized), resulting in labeling synthons applicable to protein or peptide labeling.^{130,158} The [^{18}F]SiFA-PGs reactivity at room temperature was surprisingly higher than that of ^{18}F -fluoromethyl phenyl isothiocyanate.¹⁵⁹

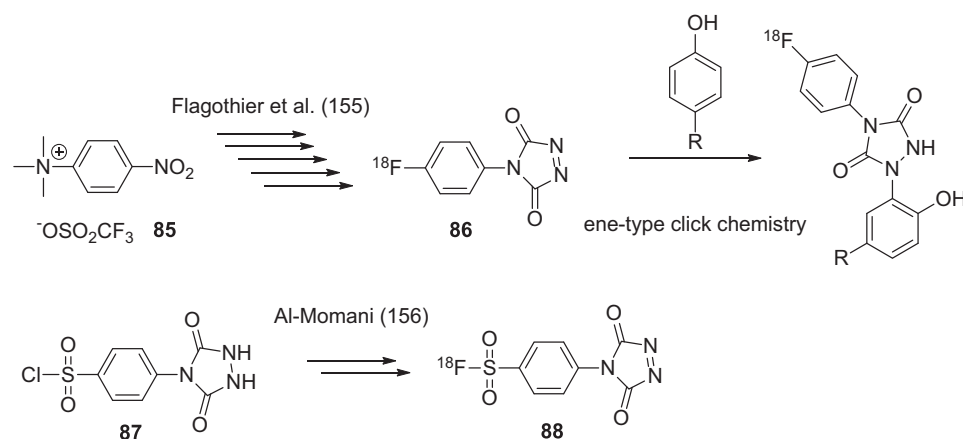


Figure 14 Syntheses of ¹⁸F-labeled 1,2,4-triazole-3,5-dione PGs **86** and **88** for tyrosine-specific labeling of biomolecules.

[¹⁸F]SiFA-isothiocyanate proved to be eligible for protein labeling, yielding almost quantitative radiochemical yields after 10- to 30-minute reaction time with proteins like rat serum albumin, apotransferrin, and bovine IgG. Extensive in vivo PET data, however, are still lacking for all ¹⁸F-isothiocyanates. Like cyanates and isothiocyanates, epoxides are known to be extremely useful building blocks in organic chemistry but did not find many applications as ¹⁸F-labeled PGs so far. Only [¹⁸F]-epi-fluorhydrin has been used for the labeling of [¹⁸F]FMISO, a tracer to image hypoxia in tumors.¹⁶⁰ Yields of epoxide openings are so far low to moderate and might benefit from the application of catalysts weakening the carbon-oxygen bond. A very early approach to use ¹⁸F-labeled azides (predating many other PGs for protein labeling) before the introduction of triazole click chemistry was its photochemical conjugation to biomolecules. Hashizume et al.¹⁶¹ and Wester et al.¹⁶² independently demonstrated in 1995-1996 that photochemical activation of ¹⁸F-phenyl azides such as 4-azidophenyl-¹⁸F-fluoride and ¹⁸F-3,5-difluorophenyl azide undergo a rearrangement to form an azepin intermediate that reacts predominantly with lysine side chains. Later 3-azido-5-nitrobenzyl-[¹⁸F]fluoride has been introduced to ¹⁸F-label an oligonucleotide aptamer, but like the other 2 reported azido-PGs, the reaction product was not further investigated in vivo.¹⁶³

Summary

PGs in radiochemistry for the purpose of radiotracer development have consolidated their value over decades. PET and especially ¹⁸F-based tracer probes have benefitted from a multitude of different PGs devised to target a great variety of functional groups in biomolecules. Some of these groups are endogenous to the molecule (eg, NH₂, COOH, OH, and SH groups in peptides and proteins) and others have artificially been introduced to accommodate novel developments in click chemistry and enhance bio-orthogonality of the PG molecular reactions. The toolbox of available PGs for ¹⁸F-radiolabeling is huge and sometimes overwhelming. The right choice of a

PG for a particular labeling situation is not easy and requires knowledge about the compound to be labeled, the biological target structure of the resulting radiotracer, and of course the preparative skills of the radiochemistry group dealing with the tracer synthesis. Some PGs are easier to prepare than others, and compromises have to be made. The overall trend in ¹⁸F-labeling chemistry moves toward synthetic comfort. The reduction of synthetic effort as well as purification has been in the focus of the radiochemical community for years to make ¹⁸F-labeling simpler. In combination with non-canonical labeling procedures and modern late-stage fluorination methodologies, PGs in ¹⁸F-radiochemistry will continue to play an integral part in PET tracer development.

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