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

Ralf Schirrmacher, PhD,* Björn Wängler, PhD,† Justin Bailey, PhD,* Vadim Bernard-Gauthier, PhD,* Esther Schirrmacher, PhD,* and Carmen Wängler, PhD‡

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}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 bioavailability and stability in vivo[9]) often simply exchange a

*Medical Isotope and Cyclotron Facility, Cross Cancer Institute, University of Alberta, Alberta, Canada.
†Molecular Imaging and Radiochemistry, Department of Clinical Radiology and Nuclear Medicine, Medical Faculty Mannheim of Heidelberg University, Germany.
‡Biomedical Chemistry, Department of Clinical Radiology and Nuclear Medicine, Medical Faculty Mannheim of Heidelberg University, Germany.
Address reprint requests to Ralf Schirrmacher, PhD, Medical Isotope and Cyclotron Facility, Cross Cancer Institute, University of Alberta, AB, Canada. E-mail: schirrma@ualberta.ca

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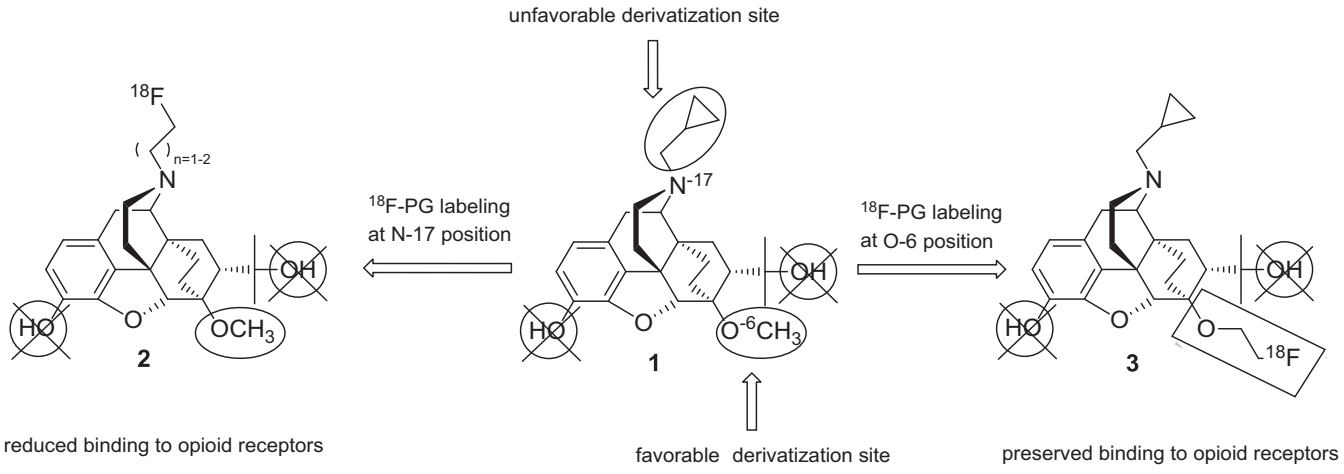
hydrogen atom in a lead with a fluorine atom based on the similar van der Waals radii of hydrogen and fluorine. Unfortunately, it is not always possible to introduce radioactive ^{18}F at the same position in a molecule where medicinal chemists are able to add stable fluorine in their multistep synthetic 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}F$^-$ substitution of a leaving group in a timely manner. Comparable with carbon-11 methylations using ^{11}C-methyliodide, 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



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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}Ne(d,α)^{18}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-solvatized and thus "naked" or through the formation of highly soluble nBu_4N[^{18}F]F (due 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

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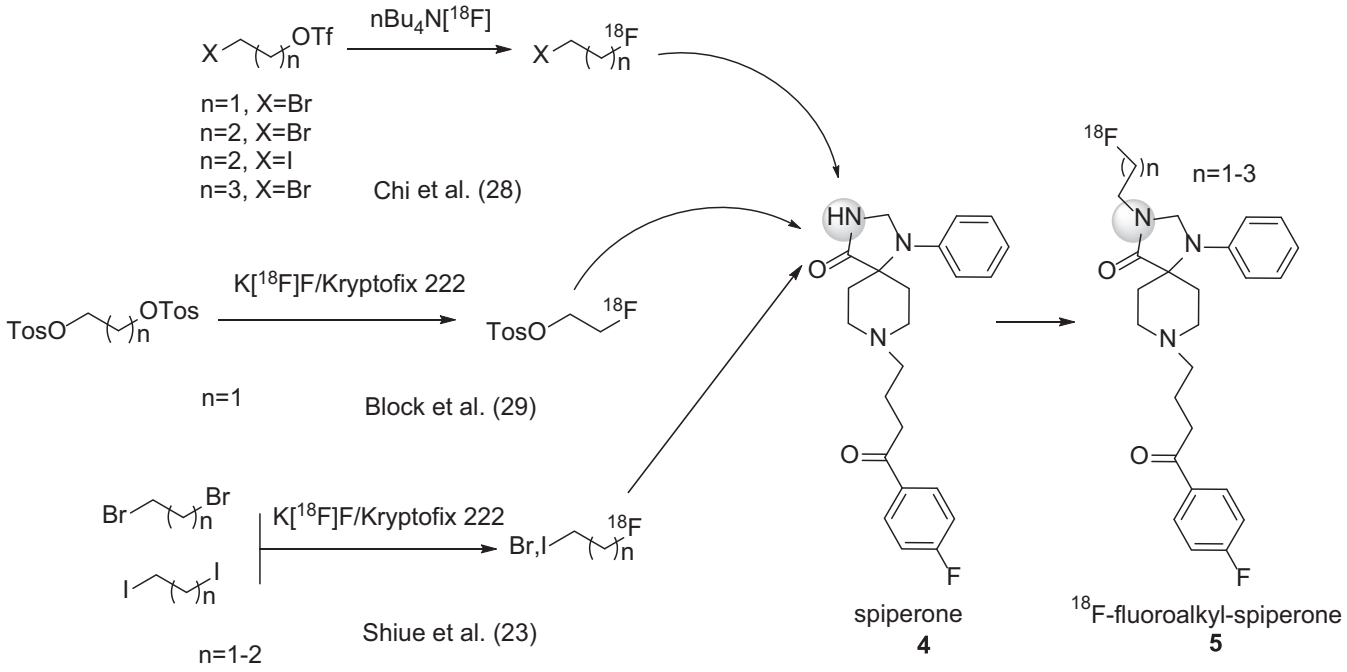

Figure 2 Synthesis of various ^{18}F-fluoro alkylation PGs for the synthesis of an ^{18}F-derivative of the D2 receptor ligand spiperone.

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 investigations[33] (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-surogate PG for [^{11}C]-methyl iodide[34] 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 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 unfortu-

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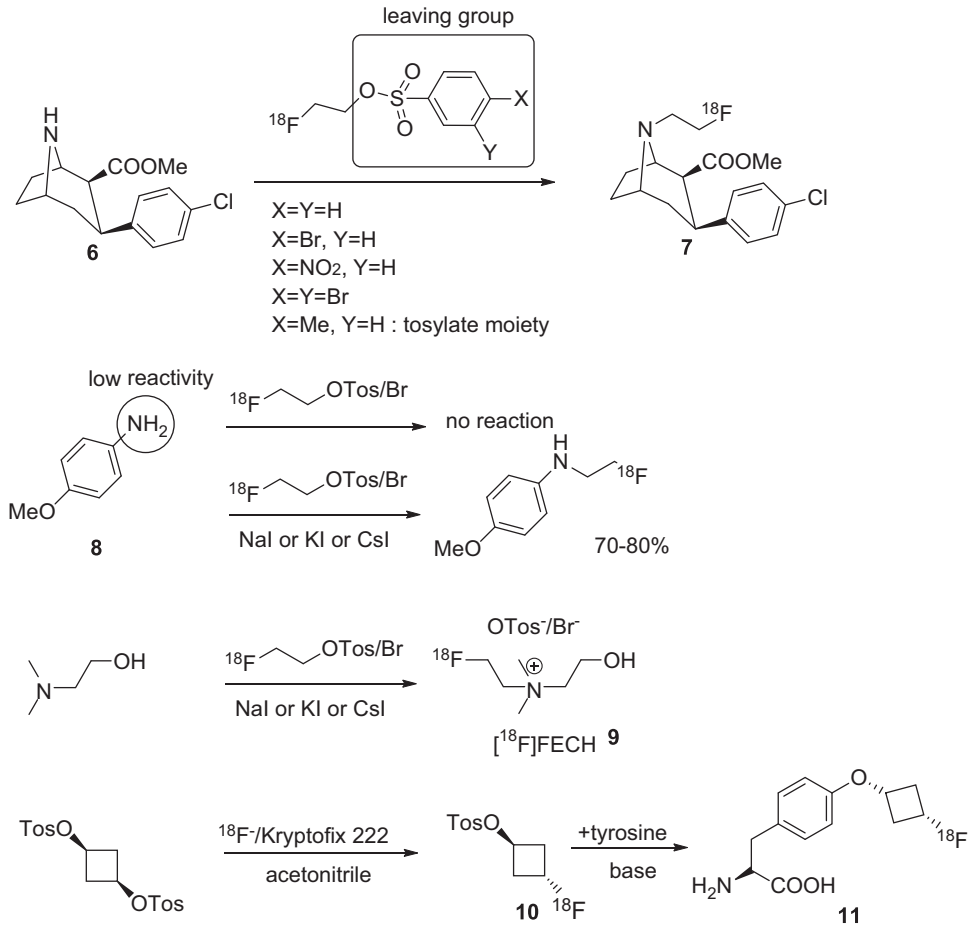

Figure 3 Application of several different ^{18}F-fluoroalkylating PGs for the ^{18}F-fluoroalkylation of different precursor molecules such as the dopamine re-uptake radioligand ^{18}F-FECNT **7**, ^{18}F-FECH **9**, and the tyrosine derivative **11**.

^{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 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]fluoro benzoic 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

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