Radiochemistry

DOI: 10.1002/ange.200803897

Fluorous Synthesis of ^{18}F Radiotracers with the [^{18}F]Fluoride Ion: Nucleophilic Fluorination as the Detagging Process**

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Positron emission tomography (PET) is a molecular imaging method of ever-increasing importance for diagnostic medicine and clinical pharmaceutical research.[1] The availability of structurally assorted radiotracers is crucial for breakthrough progress and is currently limited by the speed and efficiency of synthetic methods. This is particularly relevant for radiolabeled molecular probes derived from short-lived isotopes (for example, ^{11}C or ^{18}F). In radiosynthesis, the nonradioactive precursor is often used in large excess (μmol–mmol) relative to the amount of radiolabeling agent (pmol–nmol). The radiolabeled compound must be separated from the excess precursor prior to clinical use or for further chemical transformations. This separation is critical when the precursor can compete with the radiolabeled probe for the in vivo target or cause unwanted biological effects. Purification of radiolabeled compounds is conventionally performed by using one of four techniques. Whilst HPLC is a very powerful technique for separation, it is time-consuming and is not an attractive option for short-lived radionuclides as decay leads to a significant loss of radioactivity. Based on the same principle as HPLC, chromatography by means of solid phase extraction is fairly narrow in scope, as the technique requires the precursor and the radiotracer to have significantly different affinities for the stationary phase. Distillation is a useful technique for volatile radiolabeled compounds but this process is time consuming, difficult to implement, and may suffer from poor reproducibility. In addition, heating of the reaction mixtures may result in decomposition.[2,3] In recognition of these limitations, the strategy of solid-phase labeling has emerged as an attractive alternative technology. The labeling reaction allows the radiotracer to be released from the insoluble solid-supported substrate, which itself is removed by simple filtration.[4] Although conceptually elegant, the radiolabeling reaction itself typically requires extensive optimization and could display an unfavorable kinetic profile because of the heterogeneity of the reaction mixture.

In contrast to solid-phase methods, fluorous chemistry is best considered as solution phase synthesis with the benefit of separation tagging. Light-fluorous technology is particularly attractive since the unique separation properties of molecules containing a highly perfluorinated domain allows for the rapid purification of crude reaction mixtures by using fluorous solid phase extraction (FSPE).[5] Radiolabeling strategies that rely on the use of fluorous soluble supports are extremely scarce, notable exceptions being the preparation of radiolabeled compounds ^{125}I and ^{35}S that have relatively long half-lives.[6] Since ^{18}F is one of the most attractive radionuclides because of its routine accessibility by cyclotron production, the high resolution of images obtained and its intermediate half-life of 109.7 min, a key application of fluorous synthesis is in the preparation of ^{18}F-labeled molecular probes. As part of our continuing investigations into novel fluorination methodology, we developed a fluorous strategy featuring an electrophilic fluorination as the detagging process.[7] ^{18}F Radiochemistry is currently dominated by synthetic routes based on nucleophilic fluorination with the [^{18}F]fluoride ion, a reagent available in much higher specific activity than electrophilic sources of [^{18}F]fluorine. A platform technology based on the detagging of a fluorous-tagged precursor upon nucleophilic fluorination is therefore a compelling application of fluorous chemistry (Figure 1).

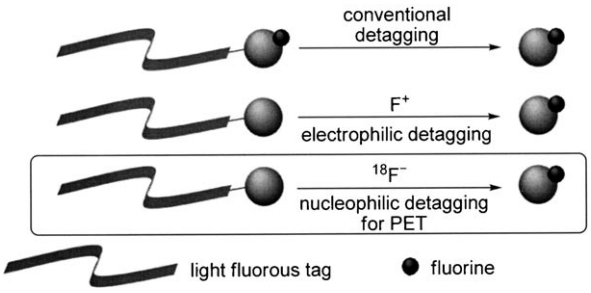

Figure 1. Fluorous tag displaceable upon nucleophilic fluorination.

A fluorous variant of the commonly used nucleophilic fluorination of alkyl sulfonates was chosen as a proof-of-principle study,[8] and a light fluorous tag that could be substituted with the [^{18}F]fluoride ion was identified and prepared. Though some perfluorinated sulfonates (triflate and nonaflate) are widely used to carry out aliphatic nucleophilic substitution, none of them have the character-

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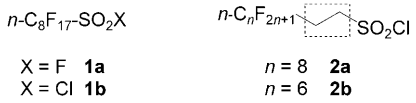
[**] This work was generously supported by the DIUS and Siemens Molecular Imaging (Project No TP/16636)

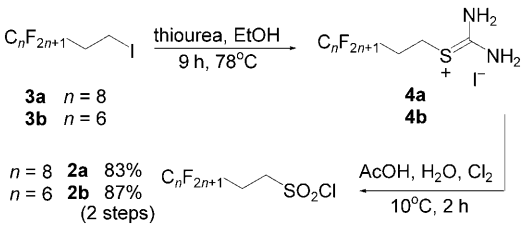
Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.200803897.

istic feature of a fluorous tag. A modification of the sulfonate with a polyfluorinated alkyl chain (typically with 9–17 fluorine atoms) should provide a leaving group with such properties. The first attempts performed with the commercially available perfluorooctanesulfonyl halides **1**, typically used to obtain sulfonamides,[9] were unsuccessful. Although the synthesis of alkyl perfluorooctane sulfonates has been reported,[10] an ammonium sulfonate salt was the only product formed in this case, because of the high reactivity of the alkyl perfluorosulfonate that was presumably formed.[11,12] The alternative tags **2a**–**b** with a spacer of two carbon atoms between the fluorous chain and the sulfonyl chloride group were therefore prepared as the distancing of the sulfonyl chloride functionality from the fluorous domain should modulate reactivity (Scheme 1).



Scheme 1. First- and second-generation tagging reagents.

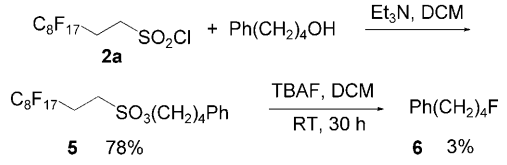
The second-generation fluorous tags **2a**–**b** were synthesized following the two-step procedure outlined in Scheme 2.[13] Compound **2a** was further reacted with 4-



Scheme 2. Synthesis of the fluorous tagging reagents **2a**–**b**.

phenyl-butan-1-ol, which resulted in the production of 4-phenyl-butyl-1*H*,1*H*,2*H*,2*H*-perfluorodecane-1-sulfonate **5** in 78% yield. The nucleophilic fluorination of **5** was carried out with tetrabutylammonium fluoride (TBAF), a reagent used as a surrogate for "hot" [^{18}F]fluoride ions (Scheme 3). Although only traces of the desired fluorinated product were seen, this encouraging result prompted us to validate the radiosynthesis of both ^{18}F-labeled prosthetic groups and molecular probes commonly used for PET.

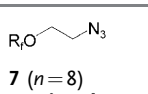
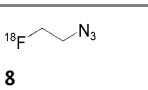
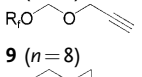
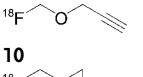
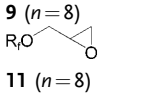
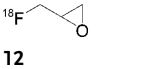
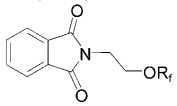
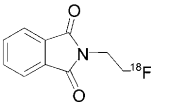
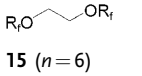
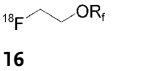
Although it is preferable to carry out the radiolabeling as the last step of the synthetic sequence, this is not always possible since the conditions required for the fluorination may not be compatible with the radiotracer of interest.



Scheme 3. Validation of nucleophilic fluoro-detagging.

Consequently, transformations that are referred to as "click reactions", for example, the Cu^{I}-catalyzed Huisgen 1,3-cycloaddition or the nucleophilic opening of an epoxide,[14] have proven highly valuable for the coupling of ^{18}F-radiolabeled prosthetic groups with sensitive substrates under mild or aqueous conditions.[3,15] To test the practical utility of the fluoro-detagging methodology, various fluorous precursors of ^{18}F-radiolabeled prosthetic groups were synthesized and subjected to fluorination (Table 1). ^{18}F Fluorination was

Table 1: Fluorous synthesis of ^{18}F prosthetic groups.

Entry	Precursors[a]	^{18}F-Radiolabeled products[b]	RCY [%][c]	FSPE ratio MeCN/H_2O
1	 **7** ($n=8$)	 **8**	> 76[d] ($m=2$)	7:3
2	 **9** ($n=8$)	 **10**	> 79[d]	7:3
3	 **11** ($n=8$)	 **12**	> 89[d]	7:3
4	 **13** ($n=8$)	 **14**	50 ($m=3$)	7:3
5	 **15** ($n=6$)	 **16**	82 ($m=3$)	9:1[e]

[a] $n=8$ $R_f=C_8F_{17}(CH_2)_2$-SO_2-; $n=6$ $R_f=C_6F_{13}(CH_2)_2$-SO_2-. [b] Precursor (10 mg), MeCN (0.3 mL), 15 min, 120°C. [c] crude RCY: decay-corrected radiochemical yield or mean RCY based on *m* experiments. [d] Volatile product; RCY was determined after subsequent coupling. [e] FSPE was also successful with MeCN/DMSO 9:1.

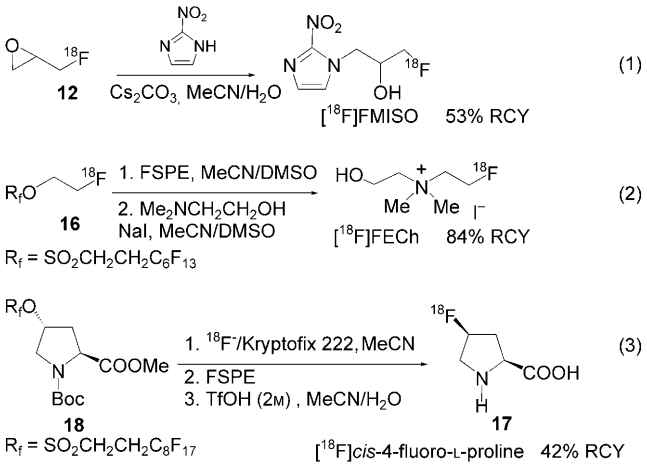
performed in acetonitrile using [^{18}F]$^-$/K$^+$-Kryptofix 222 and FSPE implemented to separate the radiolabeled product from the fluorous precursor, which was typically used in large excess. After dilution with a fluorophobic solvent, the crude reaction mixture was eluted through a FSPE cartridge and washed with a fluorophobic eluent. Pleasingly, less than 5% of radiolabeled material remained trapped on the cartridge, while most of the unreacted fluoride was not eluted with organic solvent. The fluorous starting material was recovered in 20–40% yield by eluting the FSPE cartridge with acetonitrile; another fraction of the precursor usually precipitated in the reaction vessel while diluting with the fluorophobic solvent. None or only a trace amount of fluorous precursors could be detected in the eluted fraction by HPLC. The click reagent [^{18}F]-2-fluoroethylazide **8**, an ^{18}F building block useful for "click coupling" was prepared upon nucleophilic fluoro-detagging of **7** and was subsequently purified by FSPE. This protocol was superior to previous radiosyntheses that rely on purification by distillation (Table 1, entry 1).[3,16,17] [^{18}F]-(2-Fluoroethoxy)prop-1-yne **10**, another compound belonging to the class of "click partners" was obtained with good radiochemical yield (RCY) after FSPE without the need to have recourse to distillation (Table 1, entry 2).[15,18] Both the ^{18}F-labeled dipole **8** and dipolarophile **10** successfully underwent

the click reaction in a subsequent step (see the Supporting Information). [^{18}F]-Epifluorohydrin **12**, a prosthetic group known to couple successfully with various nucleophiles, was synthesized in higher RCY than previously reported (70–75%) and successfully purified by FSPE (Table 1, entry 3).[19] Similarly, the detagging of **13** afforded [^{18}F]-*N*-(2-fluoroethyl)phthalimide **14** in good RCY (Table 1, entry 4). FSPE purification of **14** followed by deprotection with hydrazine hydrate led to unmasked [^{18}F]-2-fluoroethylamine.[2,20] The doubly tagged diol **15**, derived from ethylene glycol, was found to be amenable to monofluorination and afforded the monotagged substrate **16** in 82% RCY. For this reaction, it was essential to use the lighter C_6F_{13} fluorous tag **2b**. Importantly, the product **16** was separated from the heavy fluorous tagged precursor ($C_{12}F_{26}$) **15** by FSPE, which suggests that it is no longer necessary to use HPLC for the purification of the non-fluorous ^{18}F-fluoroalkylating agent [^{18}F]-2-fluoroethyltosylate (Table 1, entry 5).[21]

Our attention then turned towards the synthesis of ^{18}F radiotracers frequently used for PET (Scheme 4). [^{18}F]FMISO, a biomarker for tissue hypoxia, was successfully prepared in moderate radiochemical yield from epifluorohydrin **12** (Scheme 4, reaction (1)). [^{18}F]FECh, a potential tracer for metabolic cancer imaging,[22] was obtained in 84% RCY from **16**. For this transformation, a mixture of acetonitrile/DMSO was used to elute the FSPE cartridge in order to avoid the presence of water that can compete with the amine for the substitution of the sulfonate group (Scheme 4, reaction (2)). The fluorous protocol was also applied to the synthesis of [^{18}F]-*cis*-4-fluoro-L-proline **17**, a biomarker used for the imaging of brain tumors.[23] A variant on the reported radiosynthesis method,[24,25] was developed using the fluorous precursor **18**. [^{18}F]-*cis*-*N*-(*tert*-Butoxycarbonyl)-4-fluoroproline methyl ester was prepared in a moderate radiochemical yield of 42% ($m = 3$) and the nucleophilic fluorination occurred with clean inversion.[24] FSPE purification and quantitative deprotection with triflic acid afforded **17** (Scheme 4, reaction (3)).[26]

An important factor that requires consideration in PET is the specific activity, defined as the level of radioactivity per amount of tracer.[27] For ^{18}F-labeled radiotracers, the theoretical maximum value of 6.3×10^4 GBq μmol^{-1} is never reached because of contamination with the stable isotope originating from the radionuclide production, solvents, chemicals, and impurities. It is thus essential to verify to what extent the polyfluorinated tag may induce unwanted contamination with "cold" ^{19}F. The specific activity was evaluated for the fluorination of both 4-phenylbutyl tosylate and its fluorous analogue **5**. Starting from both the fluorous and the non-fluorous precursors, [^{18}F]-4-phenylbutyl fluoride **6** was obtained in 91% and 90% RCY, with specific activities of 1–10 GBq μmol^{-1} ($m = 3$) and greater than 100 GBq μmol^{-1} ($m = 2$), starting from approximately 200 MBq [^{18}F]$^-$ ions. It therefore appears that, under the radiolabeling conditions, the fluorous tag induced a decrease of the specific activity possibly from leaching of "cold" fluoride ion from the tag,[28] a limitation which has to be taken into consideration if the tracer is toxic or able to saturate the receptor sites.

We have demonstrated that ^{18}F-radiolabeled material can be prepared by nucleophilic fluorination using fluorous-tagged precursors and purified by FSPE regardless of the affinity of the untagged substrate for the stationary phase. FSPE-purified labeled compounds can then be used in subsequent reactions or more easily purified by HPLC before administration. FSPE is an easily implemented separation technique, which can be run by automated systems. One key feature of fluorous radiochemistry is the possibility to perform reactions in homogeneous phases. This allows for more favorable reaction kinetics than solid-phase synthesis.[29] Furthermore, the light fluorous approach requires minimal optimization, as the reaction conditions are comparable to those reported in the literature using conventional sulfonates. Further extensions are underway to tune the reactivity of the precursors by using various linkers between the fluorous chain and the sulfonyl moiety. Indeed, the optimum choice of the leaving group is usually strongly dependent on the substrate (e.g., for FDG production, a triflate group is used as the leaving group in preference to a tosylate group). The above technology offers an appealing versatility for the synthesis of radiotracers used in PET, but can be applied to the preparation of biomarkers required for alternative imaging modalities, for example, single photon emission computed tomography (SPECT).



Scheme 4. Fluorous synthesis of known ^{18}F radiotracers.

Experimental Section

Typical procedure: FSPE cartridges were prepared in-house using a Sep-Pak Light tube (Waters, Milford, MA) and fluorous silica (180 mg; Fluorous Technology, Pittsburgh, PA). ^{18}F Fluorination was typically performed in acetonitrile (0.3–0.5 mL) using dry [^{18}F]$^-$/K$^+$-Kryptofix 222 and precursor (10 mg). After heating for 15 min at 100–120 °C, the crude reaction mixture was cooled to room temperature and diluted with a fluorophobic solvent such as water or DMSO to obtain a fluorophilic/fluorophobic ratio of typically 7:3, prior to elution through a FSPE cartridge. The cartridge was subsequently washed with the fluorophilic/fluorophobic eluent (0.5 mL) to collect the ^{18}F-radiolabeled compound.

Received: August 7, 2008
Revised: October 10, 2008
Published online: December 3, 2008

Keywords: fluorine · positron emission tomography · radiopharmaceuticals · synthetic methods

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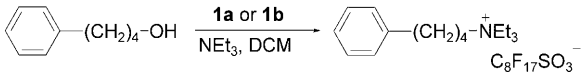
[11] 

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