

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

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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 $[\text{}^{18}\text{F}]\text{fluoride ion}$, a reagent available in much higher specific activity than electrophilic sources of $[\text{}^{18}\text{F}]\text{fluorine}$. 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).

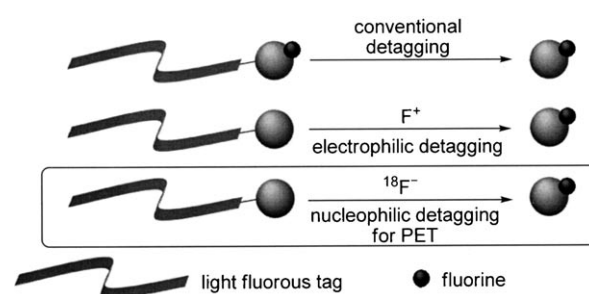


Figure 1. Fluorous tag displaceable upon nucleophilic fluorination.

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 $[\text{}^{18}\text{F}]\text{fluoride ion}$ 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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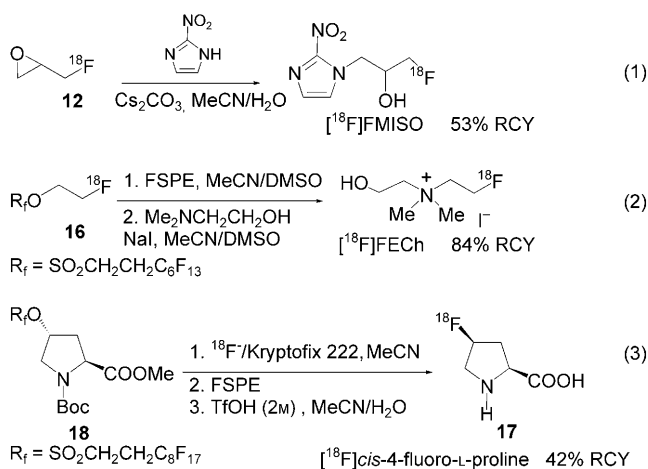
the click reaction in a subsequent step (see the Supporting Information). [^{18}F]-Epifluorohydrin **12**, a prosthetic group known to couple successfully with various nucleophiles, was synthesized in higher RCY than previously reported (70–75 %) and successfully purified by FSPE (Table 1, entry 3).^[19] 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} fluororous tag **2b**. Importantly, the product **16** was separated from the heavy fluororous tagged precursor ($\text{C}_{12}\text{F}_{26}$) **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 fluororous 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 fluororous 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 theoret-

ical maximum value of $6.3 \times 10^4 \text{ GBq } \mu\text{mol}^{-1}$ 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 fluororous analogue **5**. Starting from both the fluororous and the non-fluorous precursors, [^{18}F]-4-phenylbutyl fluoride **6** was obtained in 91 % and 90 % RCY, with specific activities of 1–10 $\text{GBq } \mu\text{mol}^{-1}$ ($m=3$) and greater than 100 $\text{GBq } \mu\text{mol}^{-1}$ ($m=2$), starting from approximately 200 MBq [^{18}F] $^-$ ions. It therefore appears that, under the radiolabeling conditions, the fluororous 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 fluororous-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 fluororous 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 fluororous 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 fluororous 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. Fluororous synthesis of known ^{18}F radiotracers.

Experimental Section

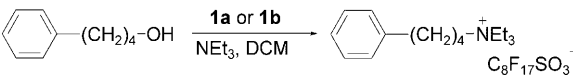
Typical procedure: FSPE cartridges were prepared in-house using a Sep-Pak Light tube (Waters, Milford, MA) and fluororous silica (180 mg; Fluorous Technology, Pittsburgh, PA). ^{18}F Fluorination was typically performed in acetonitrile (0.3–0.5 mL) using dry [^{18}F] $^-/\text{K}^+$ -Kryptofix 222 and 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.

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- [1] a) *Handbook of Radiopharmaceuticals* (Eds.: M. J. Welch, C. S. Redvanly), Wiley, New York, **2003**; b) S. M. Ametamey, M. Honer, P. A. Schubiger, *Chem. Rev.* **2008**, *108*, 1501; c) D. W. Townsend, *J. Nucl. Med.* **2008**, *49*, 938.
- [2] T. J. Tewson, *Nucl. Med. Biol.* **1997**, *24*, 755.
- [3] M. Glaser, E. Årstad, *Bioconjugate Chem.* **2007**, *18*, 989.
- [4] a) K. Kowai, H. Ohta, M. A. Channing, A. Kubodera, W. C. Eckelman, *Appl. Radiat. Isot.* **1996**, *47*, 37; b) D. H. Hunter, X. Zhu, *J. Labelled Compd. Radiopharm.* **1999**, *42*, 653; c) R. Dunn-Dufault, A. Pollak, J. Fitzgerald, J. R. Thornback, J. R. Ballinger, *Nucl. Med. Biol.* **2000**, *27*, 803; d) A. Pollak, D. G. Roe, C. M. Pollock, L. F. L. Lu, J. R. Thornback, *J. Am. Chem. Soc.* **1999**, *121*, 11593; e) S. Mundwiler, L. Candrea, P. Hafliger, K. Ortner, R. Alberto, *Bioconjugate Chem.* **2004**, *15*, 195; f) L. J. Brown, D. R. Bouvet, S. Champion, A. M. Gibson, Y. Hu, A. Jackson, I. Khan, N. Ma, N. Millot, H. Wadsworth, R. C. D. Brown, *Angew. Chem.* **2007**, *119*, 959; *Angew. Chem. Int. Ed.* **2007**, *46*, 941.
- [5] a) *Handbook of Fluorous Chemistry* (Eds.: J. A. Gladysz, D. P. Curran, I. T. Horváth), Wiley-VCH, Weinheim, **2004**; b) D. P. Curran, *Synlett* **2001**, 1488; c) W. Zhang, D. P. Curran, *Tetrahedron* **2006**, *62*, 11837.
- [6] a) A. Donovan, J. Forbes, P. Dorff, P. Schaffer, J. Babich, J. F. Valliant, *J. Am. Chem. Soc.* **2006**, *128*, 3536; b) A. S. Zhang, C. S. Elmore, M. A. Egan, D. G. Melillo, D. C. Dean, *J. Labelled Compd. Radiopharm.* **2005**, *48*, 203.
- [7] S. Boldon, J. E. Moore, V. Gouverneur, *Chem. Commun.* **2008**, 3622.
- [8] a) F. Dollé, D. Roeda, B. Kuhnast, M.-C. Lasne in *Fluorine and Health* (Eds.: A. Tressaud, G. Haufe), Elsevier, Dordrecht, **2008**; b) L. Cai, S. Lu, V. W. Pike, *Eur. J. Org. Chem.* **2008**, 2853.
- [9] a) X. Hao, A. Yoshida, J. Nishikido, *J. Fluorine Chem.* **2006**, *127*, 193; b) L. Zu, J. Wang, H. Li, W. Wang, *Org. Lett.* **2006**, *8*, 3077.
- [10] P. M. Savu, US Patent 08/341448, **1994**.
- [11] 
- [12] a) Y. Li, Z.-Q. Chen, J. Tian, Y.-B. Zhou, Z.-X. Chen, *J. Fluorine Chem.* **2004**, *125*, 1077; b) Y. Li, Z.-Q. Chen, J. Tian, Y.-B. Zhou, Z.-X. Chen, Z.-J. Liu, *J. Fluorine Chem.* **2005**, *126*, 888.
- [13] a) F. Szonyi, A. Cambon, *J. Fluorine Chem.* **1989**, *42*, 59; b) L. Foulletier, Deutsches Patent P1667117, **1969**; c) H. Stach, Deutsches Patent P2059257, **1970**.

- [14] H. C. Kolb, M. G. Finn, K. B. Sharpless, *Angew. Chem.* **2001**, *113*, 2056; *Angew. Chem. Int. Ed.* **2001**, *40*, 2005.
- [15] J. Marik, J. L. Sutcliffe, *Tetrahedron Lett.* **2006**, *47*, 6681.
- [16] a) L. Carroll, R. Bejot, R. Huting, S. Bayly, J. Dilworth, A. Gee, J. Declerck, V. Gouverneur, *J. Nucl. Med.* **2008**, *49*, 98P; b) M. Glaser, M. Solbakken, J. Arukwe, H. Karlsen, A. Cuthbertson, S. Luthra, E. Årstad, *J. Nucl. Med.* **2008**, *49*, 96P.
- [17] The RCY and distillation efficiency of [¹⁸F]-2-fluoroethylazide were reported as 54 % and 63 %, respectively.^[3]
- [18] U. Sirion, H. J. Kim, J. H. Lee, J. W. Seo, B. S. Lee, S. J. Lee, S. J. Oh, D. Y. Chi, *Tetrahedron Lett.* **2007**, *48*, 3953.
- [19] a) J. R. Grierson, J. M. Link, C. A. Mathis, J. S. Rasey, K. A. Krohn, *J. Nucl. Med.* **1989**, *30*, 343; b) E. L. Kämäräinen, T. Kyllönen, O. Nihtila, H. Björk, O. Solin, *J. Labelled Compd. Radiopharm.* **2004**, *47*, 37.
- [20] a) C. Gilissen, G. Bormans, T. de Groot, A. Verbruggen, *J. Labelled Compd. Radiopharm.* **1998**, *41*, 491; b) S. Lehel, G. Horváth, I. Boros, P. Mikecz, T. Márián, A. J. Szentmiklósi, L. Trón, *J. Labelled Compd. Radiopharm.* **2000**, *43*, 807; c) J. S. Rasey, P. D. Hofstrand, L. K. Chin, T. J. Tewson, *J. Nucl. Med.* **1999**, *40*, 1072.
- [21] a) D. Block, H. H. Coenen, G. Stocklin, *J. Labelled Compd. Radiopharm.* **1988**, *25*, 201; b) A. Bauman, M. Piel, R. Schirmacher, F. Rösch, *Tetrahedron Lett.* **2003**, *44*, 9165.
- [22] a) M. Piel, A. Bauman, R. P. Baum, S. Höhnemann, I. Klette, R. Wortmann, F. Rösch, *Bioorg. Med. Chem.* **2007**, *15*, 3171; b) T. Hara, N. Kosaka, H. Kishi, *J. Nucl. Med.* **2002**, *43*, 187; c) T. R. DeGrado, S. W. Baldwin, S. Wang, M. D. Orr, R. P. Liao, H. S. Friedman, R. Reiman, D. T. Price, R. E. Coleman, *J. Nucl. Med.* **2001**, *42*, 1805.
- [23] a) K. J. Langen, K. Hamacher, D. Pauleit, F. W. Floeth, G. Stoffels, D. Bauer, G. Reifemberger, K. Zilles, H. H. Coenen, *Anat. Embryol.* **2005**, *210*, 455; b) K.-J. Langen, K. Hamacher, D. Bauer, S. Broer, D. Pauleit, H. Herzog, F. Floeth, K. Zilles, H. H. Coenen, *J. Cereb. Blood Flow Metab.* **2005**, *25*, 607.
- [24] K. Hamacher, *J. Labelled Compd. Radiopharm.* **1999**, *42*, 1135.
- [25] a) S. M. Mazza, *J. Labelled Compd. Radiopharm.* **2000**, *43*, 1047; b) H.-J. Wester, M. Herz, R. Senekowitsch-Schmidtke, M. Schwaiger, G. Stocklin, K. Hamacher, *Nucl. Med. Biol.* **1999**, *26*, 259.
- [26] 50 µL triflic acid (2 M) was sufficient to deprotect **18** after FSPE, compared to the reported amount of 0.5 mL.
- [27] a) M.-C. Lasne, C. Perrio, J. Rouden, L. Barré, D. Roeda, F. Dolle, C. Crouzel, in *Contrast Agents II, Vol. 222* (Ed.: W. Krause), Springer, Berlin, **2002**, pp. 201; b) W. C. Eckelman, M. Bonardi, W. A. Volkert, *Nucl. Med. Biol.* **2008**, *35*, 523.
- [28] P. S. Johnström, S. Stone-Elander, *Appl. Radiat. Isot.* **1996**, *47*, 401.
- [29] C. Chen, W. Zhang, *Mol. Diversity* **2005**, *9*, 353.