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[^{18}F]Fluoropropylsulfonyl chloride: a new reagent for radiolabeling primary and secondary amines for PET imaging

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***In vivo* molecular imaging with positron emission tomography (PET) requires the preparation of an appropriate positron-emitting radiotracer. New methods for the introduction of F-18 into biologically interesting molecules could increase the availability of specific PET radiotracers and increase the application of PET to the study of human diseases. In this work, [^{18}F]fluoropropylsulfonyl chloride was synthesized from 3-toluenesulfonyloxypropyl thiocyanate in two steps and was successfully incorporated into molecules containing a reactive amino group. Both a primary amine, L-phenylalanine ethyl ester hydrochloride, and a secondary amine, 1-(2-methoxyphenyl)-piperazine, were successfully radiolabeled by this method. The entire radiochemical synthesis required 90 min. The products were obtained in 25.7 ± 2.3% (n = 3) and 22.8 ± 9.1% (n = 6) (EOB). This method provides a useful and easy way to make new F-18 labeled radiopharmaceuticals for PET imaging.**

Keywords: fluorine-18; radiolabeling agent; [^{18}F]fluoropropylsulfonyl chloride

Introduction

Noninvasive molecular imaging using positron emission tomography (PET) is playing an increasing role in monitoring biochemical changes *in vivo* in various diseases. Despite the increasing reliance of the biomedical science on imaging, the development of new radiopharmaceuticals for PET remains a slow process. One bottleneck is the limited methods available for the introduction of radionuclide into biologically interesting molecules.[1]

Fluorine-18 ($t_{1/2}$ = 109.8 min) is a very important radionuclide in PET due to its favorable decay characteristics. Many radiopharmaceuticals containing F-18 have been found to be very useful in PET imaging.[2,3] For the preparation of fluorine-18 labeled PET radiotracer, the most widely used radiofluorination method is nucleophilic substitution (S_N) reaction employing the $K_{222}/K^{18}F$ complex in polar aprotic solvents.[4,5] The minimal structural requirement for the efficient nucleophilic substitution is the presence of a leaving group such as methanesulfonates (OMs), trifluoromethanesulfonate (OTf), toluenesulfonate (OTs) for aliphatic substitution, and nitro or aryltrimethyl anilinium for aromatic substitution. Once a biologically interesting target is selected for radiolabeling with fluorine-18, synthesis of a precursor with an appropriate leaving group is needed. It is desirable to introduce the radionuclide at the last step of the synthesis, but usually it is very difficult in more complex molecules. Not only are suitable precursor molecules with appropriate leaving group for direct nucleophilic fluorination frequently not easy to synthesize, but they are also often unstable under the direct labeling conditions. An alternative method is using a prosthetic group such as [^{18}F]fluoroalkyl moieties as labeling agent.[6–9] Amongst these agents are compounds of formula $X(CH_2)_n^{18}F$, where n may range from 1 to 4 and X may be a halogen, OMs, OTf, or OTs.[10–12] These labeling agents are often used to perform [^{18}F]ω-fluoroalkylations at nucleophilic centers such as OH, NH_2, SH.[4] Labeling with [^{18}F]fluoroalkyl moieties as prosthetic groups widely extends the spectrum of biomolecules which can be labeled.

In this report, we present a new approach using [^{18}F]fluoropropylsulfonyl chloride as a prosthetic radiolabeling agent to radiolabel target molecules with F-18 by forming sulfonamide derivatives. In some cases sulfonamides are already present in the drug molecule. For example, short chain sulfonamide moieties are present in a class of muscarinic acetylcholine antagonists[13] and in a class of glycine transportor inhibitors.[14] Compounds [^{18}F]**a** and [^{18}F]**b** were prepared as model compounds by introducing a [^{18}F]fluoropropylsulfonyl group into the corresponding primary and secondary amine (Scheme 1). The sequence was rapid and efficient; the reaction conditions were mild.

Results and discussion

In this study, we used L-phenylalanine ethyl ester hydrochloride, a primary amine, and 1-(2-methoxyphenyl)piperazine, a secondary amine, as model substrates for testing the reactivity of a

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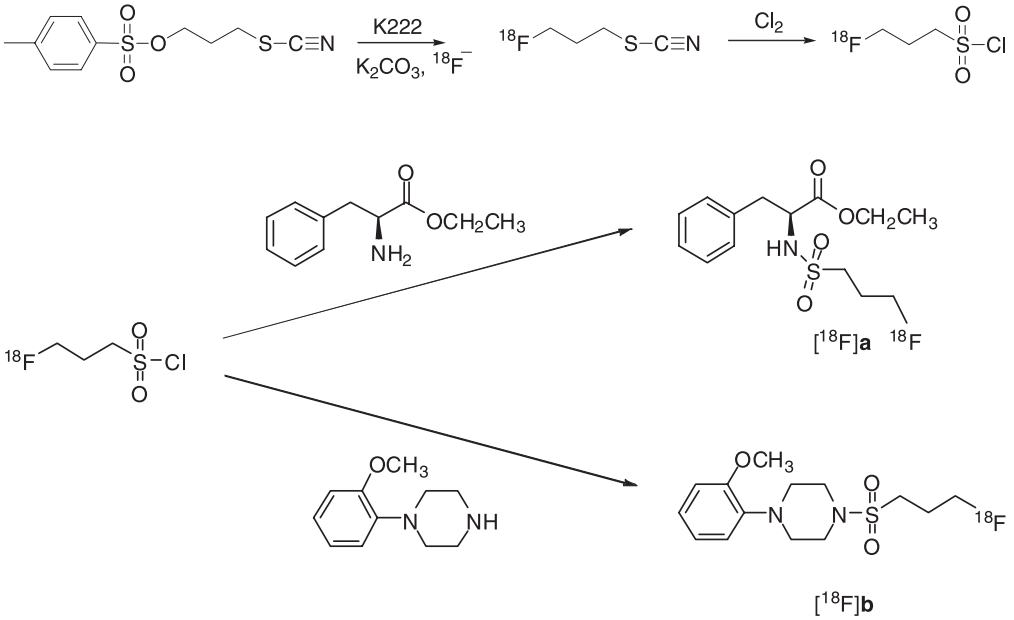
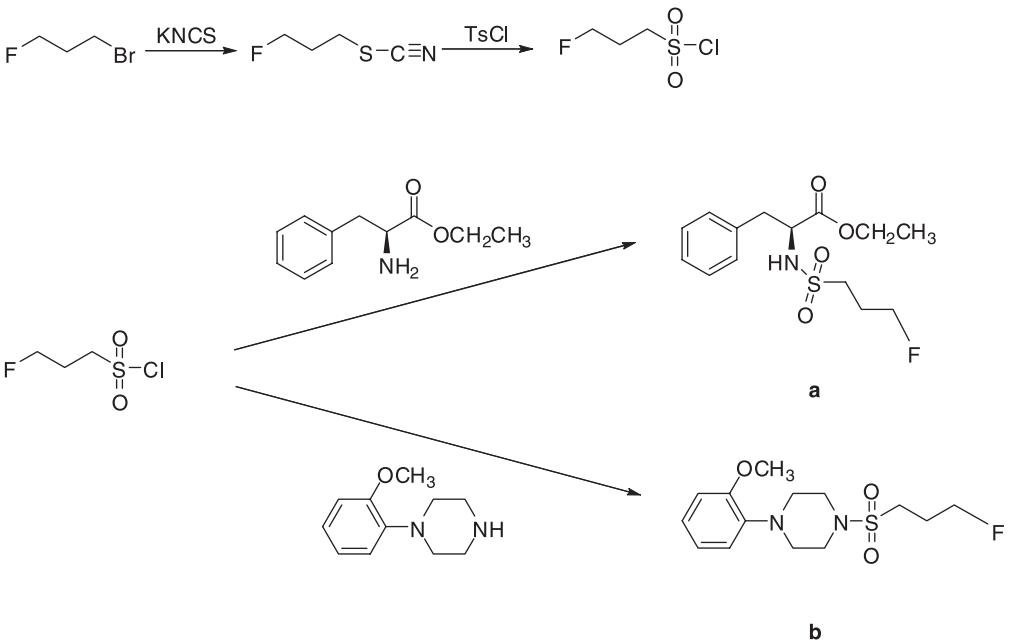


Scheme 1

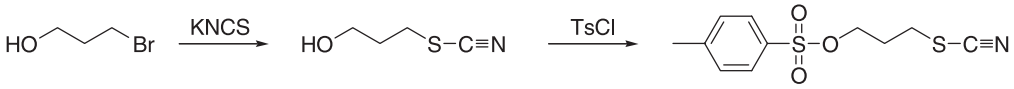
novel radiolabeled prosthetic agent, [^{18}F]fluoropropylsulfonyl chloride, and optimizing the labeling reaction conditions. The synthesis of the non-radioactive standard compounds for analytical purposes including high-performance liquid chromatography (HPLC) and gas chromatography mass spectrometry (GC-MS) was achieved according to Scheme 2. The reference amides **a** and **b** were prepared in three steps from 1-bromo-3-fluoropropane and corresponding amines, respectively. The structure of compounds **a** and **b** were confirmed by ^{1}H NMR, ^{13}C NMR, and MS. Fluoropropylsulfonyl chloride was prepared from 1-bromo-3-fluoropropane in two steps following literature methods.[15,16] The radiolabeling precursor, 3-toluenesulfonyloxypropyl thiocyanate, was prepared from 3-bromopropanol by displacement of bromide using KSCN, followed by treatment with toluenesulfonyl chloride (Scheme 3).

[^{18}F]]Fluoropropylsulfonamides were synthesized in three steps (Scheme 1) beginning by heating 3-toluenesulfonyloxypropyl thiocyanate with [^{18}F]fluoride, K_2CO_3, and Kryptofix[2.2.2.]. In our initial radiochemical synthesis, the intermediate, [^{18}F]fluoropropyl thiocyanate, was obtained with a radiochemical yield of 75.2$\pm$6.1% ($n = 7$) (decay corrected) following elution with ether through a short pipette column containing silica gel ($\sim$5 mm).

In order to investigate optimal reaction conditions for the chlorination step, small-scale reactions were conducted with non-radioactive fluoropropyl thiocyanate. Chlorine gas was



Scheme 2



Scheme 3

bubbled through the aqueous solution and the reaction was monitored by GC-MS. From the model reactions, we found that water is the only suitable solvent for the reaction; mixtures of water with CH_3CN and/or ether failed to provide the sulfonyl chloride. The GC peak at 2.85 min identified as the fluoropropylsulfonyl chloride was observed to increase within 2–3 min, but after additional time the amount of product decreased and more by-products were observed. Thus, optimal conditions for the conversion of thiocyanate to sulfonyl chloride were bubbling chlorine through an aqueous solution for 2–3 min.

For the radiochemical conversion, evaporation of the ethereal solution led to loss of the volatile intermediate [^{18}F]fluoropropyl thiocyanate. We found that the addition of water (1 mL) to the ethereal solution of [^{18}F]fluoropropyl thiocyanate, cooling to 0°C, followed by gentle evaporation of the organic solvents under an argon stream effectively reduced the loss of radioactivity to less than 5%. Residual amounts of ether or CH_3CN that may have been left in the aqueous solution did not seem to adversely affect the chlorination reaction. Chlorine gas was bubbled slowly (single bubbles observed) through the ice-cold solution for 3 min, followed by slowly bubbling argon to purge the excess chlorine. The aqueous solution was extracted with CH_2Cl_2. The radiochemical yield of [^{18}F]fluoropropylsulfonyl chloride from [^{18}F]fluoropropyl thiocyanate in water was $59.4 \pm 11.6\%$ ($n = 7$) (decay corrected).

Sulfonyl chloride coupling reactions were also explored on a small scale using the product from chlorination of non-radioactive fluoropropyl thiocyanate to guide the radiochemical procedure. Initially reactions between fluoropropylsulfonyl chloride and L-phenylalanine ethyl ester hydrochloride using two equivalents of triethylamine in methylene chloride failed. GC-MS analysis showed that the fluoropropylsulfonyl chloride peak at 2.85 min disappeared, but no new peak was observed. In retrospect, we may have predicted the deleterious effects of residual water. At pH > 6.7 sulfonyl chlorides are known to hydrolyze by direct nucleophilic attack by water.[17,18] Removal of water from methylene chloride extract with a small Na_2SO_4 column and using one equivalent of triethylamine permitted the reaction to proceed. We dissolved the amine and 1 equivalent of triethylamine in CH_2Cl_2 and then added the Na_2SO_4 dried solution of fluoropropylsulfonyl chloride. The mixture was heated at 85°C for 10 min and then at 105°C for an additional 5 min. From GC-MS, the sulfonyl chloride peak at 2.85 min disappeared and a new peak at 7.43 min formed, which was identified as the expected product based on comparison of the mass spectral fragment ions at 208, 226, and 176 with an authentic product.

We subsequently carried out the radiochemical reaction between [^{18}F]fluoropropylsulfonyl chloride and L-phenylalanine ethyl ester hydrochloride utilizing the conditions described above to produce compound **a**. High radiochemical yield was achieved ($80.5 \pm 6.5\%$; $n = 7$) for this coupling step (decay corrected).

The conversion of thiocyanate into sulfonyl chloride during this three-step radiosynthesis still contained procedures, including tedious evaporation of solvents and a liquid–liquid extraction, that cause loss of the desired radioactive product. In order to simplify these procedures, we investigated chlorination of the thiocyanate on a C-18 solid phase extraction column (100 mg). After the fluoride incorporation reaction, water was added to the solution and the [^{18}F]fluoropropyl thiocyanate was trapped on a C-18 column. Chlorine gas was passed through the column to convert thiocyanate into sulfonyl chloride. After purging with argon to remove excess chlorine, [^{18}F]fluoropropylsulfonyl chloride was eluted with CH_2Cl_2 through a Na_2SO_4 column and into a solution of 1-phenylalanine ethyl ester hydrochloride containing 1 equivalent triethylamine. This improved procedure increased the yield of sulfonyl chloride from thiocyanate to $71.2 \pm 4.2\%$ ($n = 3$) (decay corrected), the overall radiochemical yield from fluoride to $25.7 \pm 2.3\%$ ($n = 3$) (decay corrected), and shortened the overall reaction time from delivery of aqueous [^{18}F]fluoride until isolation of the product from HPLC to 90 min. The radiochemical purity was > 99%.

The secondary amine, 1-(2-methoxyphenyl)-piperazine, was radiolabeled using a similar procedure. Because the substrate is a free base, no triethylamine was required. The radiochemical yield for the reaction between 1-(2-methoxyphenyl)-piperazine and [^{18}F]fluoropropylsulfonyl chloride was $69.4 \pm 8.7\%$ ($n = 6$) and the overall yield was $22.8 \pm 9.1\%$ ($n = 6$) (decay corrected). The radiochemical purity was > 99%.

Experimental

General

Kryptofix [2.2.2] was purchased from EM Reagents and chlorine gas was obtained from Air Products and Chemicals Inc. L-Phenylalanine ethyl ester hydrochloride (99%), 1-(2-methoxyphenyl)-piperazine (98%), 1-bromo-3-fluoropropane (98%), 3-bromo-1-propanol (97%), and other common reagents were purchased from Aldrich Chemical Company and used as received. Solvents were obtained from commercial sources and were used without further purification. ^{1}H NMR and ^{13}C NMR were obtained on a Bruker Avance 300 spectrometer at 300 and 75 MHz, respectively, using tetramethylsilane as internal standard. HPLC-MS was obtained on a ThermoFinnigan LCQ spectrum coupled to a HP1100 HPLC system using electrospray as the ionization method with HPLC employing a Phenomenex Luna C-18 column (150 mm × 4.6 mm) and isocratic elution (50% CH_3CN: 50% NH_4OAc) unless otherwise noted. Analytical HPLC employed a Phenomenex C-18 column (150 mm × 4.6 mm) eluted with 40% CH_3CN and 60% NH_4OAc (50 mM) at 1 ml/min. Gas chromatography utilized a Restek RTX5-MS column with oven temperature programming (50°C for 2 min, programmed at 30°C/min to 270°C).

Chemistry

Synthesis of 3-Toluenesulfonyloxypropyl Thiocyanate

3-Bromopropanol (2 g, 14.4 mmol) and potassium thiocyanate (1.4 g, 14.4 mmol) were dissolved in MeOH (30 mL). The reaction mixture was stirred at 60°C for 5 h.

Solvent was removed under reduced pressure. The residue was taken up in water (20 mL) and extracted with $CHCl_3$ (2×50 mL) and the combined organic layers were dried (Na_2SO_4) and evaporated. The 3-hydroxypropyl thiocyanate was used for the next reaction without further purification.

3-Hydroxypropyl thiocyanate (1.5 g, 12.8 mmol) and triethylamine (1.78 mL, 12.8 mmol) were dissolved in $CHCl_3$ (25 mL). Toluenesulfonyl chloride (2.5 g, 12.8 mmol) in $CHCl_3$ (5 mL) was added dropwise to this solution at 0°C. The reaction mixture was stirred overnight at room temperature. The solvent was evaporated and the residue taken up in $CHCl_3$ (25 mL). The solution was washed with water (10 mL × 2), dried (Na_2SO_4), and evaporated. ^{1}H NMR ($CDCl_3$) δ: 2.19 (tt, 2H, $J = 5.7$, 6.6 Hz), 2.47 (s, 3H), 3.02 (t, 2H, $J = 6.9$ Hz), 4.19 (t, 2H, $J = 5.7$ Hz), 7.38 (d, 2H, $J = 8.1$ Hz), 7.79 (dd, 2H, $J = 1.5$, 8.6 Hz); ^{13}C NMR ($CDCl_3$) δ: 21.7, 29.1, 29.9, 66.8, 111.4, 127.9 (2C), 130.1 (2C), 132.5, 145.3.

Synthesis of (S)-ethyl 2-(3-fluoropropylsulfonamido)-3-phenylpropanoate **(a)**

L-Phenylalanine ethyl ester hydrochloride (1.15 g, 5 mmol) and triethyl amine (1.50 mL, 10 mmol) were dissolved in CH_2Cl_2 (20 mL). Fluoropropylsulfonyl chloride (0.80 g, 5 mmol) was added. The solution was heated at reflux for 2 h. NaOH (1 N, 20 mL) was added and the organic layer was separated. The aqueous layer was extracted with CH_2Cl_2 (20 mL × 2) and the combined organic layers were dried (Na_2SO_4) and evaporated. The residue was subjected to flash chromatography eluting with Hexane:EtOAc [80:20] to yield the desired product (1.38 g, 92%). ^{1}H NMR ($CDCl_3$) δ: 1.29 (t, 3H, $J = 7.2$ Hz), 2.02 (m, 2H), 2.86 (t, 2H, $J = 7.5$ Hz), 3.01 (dd, 1H, $J = 7.5$, 13.8 Hz), 3.16 (dd, 1H, $J = 5.1$, 13.8 Hz), 4.23 (q, 2H, $J = 7.2$ Hz), 4.35 (m, 2H), 4.49 (dd, 1H, $J = 5.7$ Hz), 4.79 (d, NH, $J = 9.3$ Hz), 7.18–7.36 (m, 5H); ^{13}C NMR ($CDCl_3$) δ: 14.1, 24.9 ($J = 20$), 39.6, 49.9 ($J = 4.5$), 57.2, 62.1, 81.54 ($J = 166$), 127.5, 128.8 (2C), 129.5 (2C), 135.4, 171.4. GC-MS 7.49 min, $m/z = 244.30$ $[M+H-C_3H_5O_2]^+$, 226.21 $[M+H-C_7H_7]^+$, and 176.35 $[M+H-C_3H_7SO_2FN]^+$.

Synthesis of 1-(3-fluoropropylsulfonyl)-4-(2-methoxyphenyl)piperazine **(b)**

1-(2-Methoxyphenyl)-piperazine (0.96 g, 5 mmol), triethyl amine (0.75 mL, 5 mmol), and fluoropropylsulfonyl chloride (0.80 g, 5 mmol) were dissolved in CH_2Cl_2 (20 mL). The solution was heated at reflux for 2 h. NaOH (1 N, 20 mL) was added and the organic layer was separated. The aqueous layer was extracted with CH_2Cl_2 (20 mL × 2) and the combined organic layers were dried (Na_2SO_4) and evaporated. The residue was subjected to flash chromatography on silica gel eluting with 25% ethyl acetate in hexane to yield the desired product (1.40 g, 89%). ^{1}H NMR ($CDCl_3$) δ: 2.31 (m, 2H), 3.08–3.18 (m, 6H), 3.49–3.52 (m, 4H), 3.90 (s, 3H), 4.43 (t, 1H, $J = 5.7$ Hz), 4.73 (t, 1H, $J = 5.7$ Hz), 6.89–6.96 (m, 3H), 7.06 (m, 1H); ^{13}C NMR ($CDCl_3$) δ: 24.6 ($J = 21$), 44.9 ($J = 4.5$), 46.1 (2C), 50.5 (2C), 55.5, 81.8 ($J = 166$), 111.3, 118.6, 121.1, 123.8, 140.4, 152.2.

Radiochemistry

Radionuclide Production

[^{18}F]Fluoride aqueous solutions were prepared by a ^{18}O(p, n)^{18}F reaction in a GE PETrace cyclotron using a 1.8 ml target of 95% ^{18}O-enriched water irradiated by a 14.1 MeV beam at 20–25 μA for 60–90 min. Generally, this method produces [^{18}F]fluoride ion with a specific radioactivity exceeding 400 GBq/μmol. Aliquots (20–200 μl) of the irradiated water were used for individual experiments in this study.

Synthesis of [^{18}F]Fluoropropylsulfonyl Amides

To a 1 mL V-vial containing 4 μmol of potassium carbonate in 20 μL of water and 8 μmol of Kryptofix 2.2.2. in 40 μL of acetonitrile, aqueous F-18 fluoride activity (10–15 mCi) was added. The water was evaporated with an argon stream while heating to 105°C in a heating block. In order to encourage further removal of residual water, three portions of anhydrous acetonitrile (100 μL) were added and each, in turn, evaporated under the argon stream while heating in the hot block. To the above vial containing the anhydrous [^{18}F]fluoride ion, 3-toluenesulfonyloxypropyl thiocyanate (4 mg) in 100 μL acetonitrile was added. The vial was sealed and heated on the heating block at 105°C for 10 min. The reaction mixture was cooled to room temperature and diluted with water (1 mL). The solution was passed through a 1 mL (100 mg) C-18 BondElut column, excess liquid blown out with argon, and chlorine gas was passed through the C-18 column for 2 min. The column was purged with argon for 15 s and [^{18}F]fluoropropylsulfonyl chloride, which had formed on the column, was eluted with dichloromethane (1 mL). The dichloromethane solution was passed through a sodium sulfate column into a 10 mL test tube containing 5 mg amine (for 1-phenylalanine ethyl ester hydrochloride, 1.5 mL triethylamine was added) in 1 mL dichloromethane. The test tube was heated at 80°C for 10 min and 105°C for 5 min until the solvent was evaporated. Acetonitrile (250 μL) was added and the entire solution was injected onto a semipreparative HPLC column (Phenomenex Luna C-18(2) 250 × 10 mm) and eluted with 50% CH_3CN, 50% 50 mM NH_4OAc at 5 mL/min. The eluate was monitored with online radioactivity and UV detectors (220 nm). The fraction containing the product was collected. Radioactivity assay of the product was corrected for decay to the start of the reaction and radiochemical yield calculated. An aliquot of the product was reinjected onto an analytical HPLC column (Phenomenex Luna C-18(2) 150 × 4.6 mm eluted with 40% CH_3CN, 60% 50 mM NH_4OAc at 1 mL/min) to verify radiochemical purity and identity based on retention time. For each of the two radiochemical products, the radioactivity peak co-eluted with the peak of the authentic product. In addition we were able to obtain mass spectral data on the co-eluting mass peak from these no-carrier-added syntheses. [^{18}F](S)-ethyl 2-(3-fluoropropylsulfonamido)-3-phenylpropanoate: HPLC-ESI-MS: m/z $[M+1] = 318$: calculated ($C_{14}H_{20}FNO_4S$) m/z 317.11. [^{18}F]1-(3-fluoropropylsulfonyl)-4-(2-methoxyphenyl)piperazine]: HPLC-ESI-MS: m/z $[M+1] = 317$; calculated ($C_{14}H_{21}FN_2O_3S$) 316.13.

Conclusion

A new and efficient radiolabeling agent, [^{18}F]fluoropropylsulfonyl chloride, was developed and successfully reacted with both primary and secondary amines. Optimal reaction conditions were determined for each step and all the reactions had very good reproducibility. The optimized three-step procedure required 90 min from delivery of aqueous [^{18}F]fluoride until isolation of the product. The overall yields for the reaction with phenylalanine ethylester and 1-(2-methoxyphenyl)-piperazine were 25.7 ± 2.3% ($n = 3$) and 22.8 ± 9.1% ($n = 6$) (EOB), respectively. Since many

interesting biological compounds contain an amino group, this new method may prove useful in the preparation of new F-18-labeled radiopharmaceuticals for PET imaging.

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References

[1] J. S. Fowler, A. P. Wolf, *Acc. Chem. Res.* **1997**, *30*, 181–188.
[2] M. E. Phelps, J. C. Mazziotta, *Science* **1985**, *228*, 799–809.
[3] M. E. Phelps, *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 9226–9233.
[4] K. Hamacher, H. H. Coenen, G. Stockling, *J. Nucl. Med.* **1986**, *27*, 235–238.
[5] M. C. Lasne, C. Perrio, J. Rouden, L. Barre, D. Roeda, F. Dolle, C. Crouzel, *Contrast Agents II: Top. Curr. Chem.* **2002**, *222*, 201–258.
[6] R. A. Ferrieri, in *Handbook of Radiopharmaceuticals, Chapter 7* (Eds.: M. J. Welch, C. S. Redvanly), Wiley, Chichester, UK, **2003**, pp. 229–282.
[7] D. Block, H. H. Coenen, G. Stöcklin, *J. Label. Compd. Radiopharm.* **1987**, *24*, 1029–1042.
[8] D. Block, H. H. Coenen, G. Stöcklin, *J. Label. Compd. Radiopharm.* **1988**, *25*, 201–215.
[9] D. Y. Chi, M. R. Kilbourn, J. A. Katzenellenbogen, M. J. Welch, *J. Org. Chem.* **1987**, *52*, 658–664.
[10] K. C. Lee, D. Y. Chi, *J. Org. Chem.* **1999**, *64*, 8576–8581.
[11] S. Comagic, M. Piel, R. Schirrmacher, S. Höhnemann, F. Rösch, *Appl. Radiat. Isot.* **2002**, *56*, 847–851.
[12] A. A. Wilson, J. N. Da Silva, S. Houle, *Appl. Radiat. Isot.* **1995**, *51*, 765–770.
[13] Y. Wang, S. Chackalamannil, W. Chang, W. Greenlee, V. Ruperto, R. A. Duffy, R. McQuade, J. E. Lachowicz, *Biorg. Med. Chem. Lett.* **2001**, *11*, 891–894.
[14] C. W. Lindsley, Z. Zhao, W. H. Leister, J. O'Brien, W. Lemaire, D. L. Williams Jr., T.-B. Chen, R. S. L. Chang, M. Burno, M. A. Jacobson, C. Sur, G. G. Kinney, D. J. Pettibone, P. R. Tiller, S. Smith, N. N. Tsou, M. E. Duggan, P. J. Conn, G. D. Hartman, *J. Med. Chem.* **2006**, *1*, 807–811.
[15] W. C. Howell, J. E. Millington, F. L. M. Pattison, *J. Am. Chem. Soc.* **1956**, *78*, 3843–3845.
[16] J. E. Millington, G. M. Brown, F. L. M. Pattison, *J. Am. Chem. Soc.* **1956**, *78*, 3846–3847.
[17] H. K. Hall, *J. Am. Chem. Soc.* **1956**, *78*, 1450–1454.
[18] J. F. King, J. Y. L. Lam, S. Skonieczny, *J. Am. Chem. Soc.* **1992**; *114*, 1743–1749.