JOC The Journal of Organic Chemistry

Article

pubs.acs.org/joc

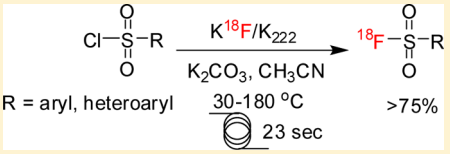
Ascertaining the Suitability of Aryl Sulfonyl Fluorides for [^{18}F]Radiochemistry Applications: A Systematic Investigation using Microfluidics

Lidia Matesic, Naomi A. Wyatt, Benjamin H. Fraser,* Maxine P. Roberts, Tien Q. Pham, and Ivan Greguric

LifeSciences Division, Australian Nuclear Science and Technology Organisation (ANSTO), Locked Bag 2001, Kirrawee DC, NSW 2232, Australia

Supporting Information

ABSTRACT: Optimization of [^{18}F]radiolabeling conditions and subsequent stability analysis in mobile phase, PBS buffer, and rat serum of 12 aryl sulfonyl chloride precursors with various substituents (electron-withdrawing groups, electron-donating groups, increased steric bulk, heterocyclic) were performed using an Advion NanoTek Microfluidic Synthesis System. A comparison of radiochemical yields and reaction times for a microfluidics device versus a conventional reaction vessel is reported. [^{18}F]Radiolabeling of sulfonyl chlorides in the presence of competing nucleophiles, H-bond donors, and water was also assessed and demonstrated the versatility and potential utility of [^{18}F]sulfonyl fluorides as synthons for indirect radiolabeling.



INTRODUCTION

Positron emission tomography (PET) is a molecular imaging technique that gives detailed three-dimensional information on functional processes in the body. PET has established diagnostic applications in oncology,[1,2] neurology[2,3] and cardiology[4] but is also proving extremely useful in drug discovery[5] and for understanding disease pathology.[6] Although many PET radioisotopes (gallium-68, zirconium-89, iodine-124) are becoming routinely available for research purposes, fluorine-18 ([^{18}F]) remains very popular due to favorable physical and chemical properties. These include low energy positron emission (0.202 MeV), excellent decay profile (97% β^+ emission), advantageous half-life (110 min) and similar steric and electronic properties to the hydroxyl group.[7,8] Although the use of the diagnostic radiopharmaceutical [^{18}F]fluorodeoxyglucose ([^{18}F]FDG) is very widespread, single-photon emission computed tomography (SPECT) agents containing technetium-99m remain the most widely prescribed diagnostic radiopharmaceuticals. This is in large part due to their simple preparation. The radiometal and ligand components are, often, simply mixed together and then injected into a patient. The development of similar, straightforward methods for [^{18}F] will be essential for ensuring it remains an important class of radiopharmaceuticals into the future. The majority of [^{18}F]radiopharmaceuticals are prepared using no-carrier-added (n.c.a.) [^{18}F]fluoride, which is incorporated into radiotracers either directly or indirectly. The direct route usually involves [^{18}F]fluoride displacement of a suitable leaving group (halogen, sulfonate ester, nitro group, ammonium cation) from an aliphatic (S_N2) or aromatic (S_NAr) precursor.[8] Direct routes are typically used for small molecules that can tolerate high reaction temperatures or when sensitive functional groups can be protected and deprotected after [^{18}F]fluoride incorporation. In contrast, indirect routes involve incorporation of [^{18}F]fluoride into a radiolabeling "synthon", which is then attached to the biological vector. Synthons are used for radiolabeling macromolecules such as peptides, proteins or antibodies that would decompose at higher temperatures or that contain hydrogen bond donor groups (−NH_2, −OH, −COOH) that would interfer with [^{18}F]fluoride incorporation. Currently there are numerous indirect radiolabeling synthons available including, but not limited to, 1-bromo-2-[^{18}F]-fluoroethane ([^{18}F]BFE; alkylation),[9] 1-[^{18}F]fluoro-4-iodobenzene (Pd catalyzed cross-coupling),[10] *N*-succinimidyl 4-[^{18}F]-fluorobenzoate ([^{18}F]SFB; acylation)[11] and 4-[^{18}F]-fluorobenzaldehyde ([^{18}F]FBA; oxime formation)[12] (Figure 1).

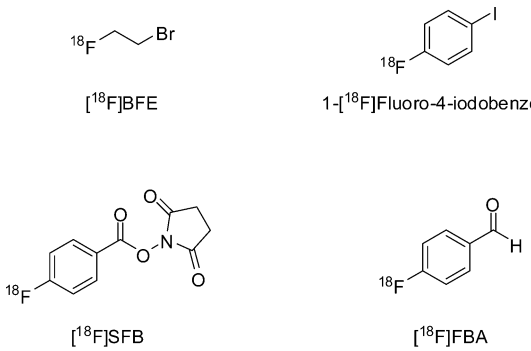


Figure 1. Radiosynthons in [^{18}F]radiolabeling.

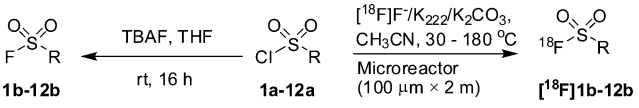
Received: August 12, 2013
Published: October 18, 2013

All the above synthons contain the carbon-[^{18}F]fluorine bond and require multistep syntheses, are water incompatible during [^{18}F]fluoride incorporation and are usually isolated in low radiochemical yields. Over the past decade there have been limited reports[13−18] using phosphorus, silicon, boron, aluminum and sulfur containing functional groups as alternatives for indirect [^{18}F]radiolabeling. The advantages of noncarbon based functional groups are typically lower [^{18}F]fluoride incorporation temperatures, shorter reaction times, higher radiochemical yields and increased water compatibility/tolerance.[19] The disadvantage is generally a decrease in stability of the fluorine to heteroatom bond although it has been shown that [^{18}F]trifluoroborates have excellent stability *in vivo*.[20] During the course of our current investigations the use of substituted benzenesulfonyl fluorides were reported by the group of Inkster.[18] In this work an aldehyde functionalized [^{18}F]-benzenesulfonyl fluoride synthon was coupled to the peptide bombesin in very good radiochemical yield (64%). The radiotracer was completely stable in 10% DMSO/water at physiological pH and temperature but was only 55% intact after 15 min in mouse serum. Given these encouraging radiochemical and stability results, which also demonstrate the clear need for improvement, we have systematically explored the effects of temperature, precursor amount, reaction time, precursor sterics, precursor electronics, presence of water and other nucleophiles upon the radiochemical yield and subsequent stability of various substituted [^{18}F]arylsulfonyl fluorides. The findings from this study increases the very limited fundamental knowledge of [^{18}F]sulfonyl fluoride radiochemistry and provides insight for the future design of [^{18}F]sulfonyl fluoride based radiotracers. This work is especially relevant given the increasing amount of literature describing biologically active molecules containing the sulfonyl fluoride functional group.[21−23]

RESULTS AND DISCUSSION

Twelve aryl sulfonyl chloride precursors (**1a−12a**) were selected in order to assess the effect of precursor electronics and sterics upon radiochemical yield (Scheme 1). Analogues

Scheme 1. Synthesis of Sulfonyl Fluorides 1b−12b and [^{18}F] 1b−12b



1 R = C_6H_5
2 R = 4-$NHCOCH_3$-C_6H_4
3 R = 4-CH_3-C_6H_4
4 R = 4-OCH_3-C_6H_4
5 R = 4-NO_2-C_6H_4
6 R = 4-F-C_6H_4
7 R = 4-Cl-C_6H_4
8 R = 4-Br-C_6H_4
9 R = 4-I-C_6H_4
10 R = 2,4,6-CH_3-C_6H_2
11 R = 2,4,6-$CH(CH_3)_2$-C_6H_2
12 R = 2-thiophene

bearing neutral (**1a**, based on Hammett constants),[24] electron-donating (**2a−4a**) and electron-withdrawing (**5a−9a**) functional groups, together with sulfonyl chlorides containing varying degrees of steric bulk (**10a**, **11a**) and one heterocyclic sulfonyl chloride (**12a**), were examined.

Microfluidic Radiosynthesis. The use of microfluidic systems to accelerate reactions in conventional synthetic chemistry is well established; however, this technology remains significantly underutilized in radiochemistry,[25−27] although the first account of a PET radiotracer for human use, produced in batch mode on a microfluidic system, has just been reported.[28] Microfluidic devices contain microreactors with internal diameters of 10−300 μm and have much larger surface to volume ratio compared to conventional vessel-type reactors. As a result, microfluidic reactors mix fluids with high speed and precision, facilitate rapid and uniform heat transfer, give shorter reaction times, reduce consumption of reagents[29] and decrease user exposure to ionizing radiation due to the ease of shielding.[25]

Radiolabeling under microfluidic conditions was performed using the Advion NanoTek Microfluidic Synthesis System, which consists of a base module containing two syringe pumps each connected to a multifunctional valve, a reactor module encompassing a syringe pump connected to a multifunctional valve plus a heating area for the independent heating of up to four microreactors, and a concentrator/evaporator module containing a syringe pump linked to a multiport valve plus a vessel heater which is connected to an auxiliary diaphragm pump (Figure 2). Detailed descriptions of the NanoTek system have been reported previously.[30]

Within the microfluidic apparatus, the concentrator/evaporator module prepared the anhydrous potassium [^{18}F]fluoride-Kryptofix [2.2.2] ($K^{18}F/K_{222}$) complex, before being dissolved in acetonitrile and transferred to loop 3 via pump 3, ready to be dispensed as required. Approximately 20 radiosynthetic reactions could be performed from one batch of (n.c.a.) [^{18}F]fluoride (500−2300 MBq) loaded onto the loop inside the microfluidic system. Likewise, the appropriate sulfonyl chloride precursor (**1a−12a**) was dissolved in acetonitrile and loaded into loop 1 via pump 1. The remaining pump module (in our case, pump 2) is typically used for two-step reactions, whereby another precursor is required and, hence, was not utilized for our reactions. The precursor and the activated $K^{18}F/K_{222}$ complex in solution were transferred from their corresponding modules into the microreactor where the reaction took place. Parameters such as precursor mass (0.5−10 mg/mL), temperature (30−180 °C) and precursor volume (10−30 μL) were controlled in the Discovery Mode of the NanoTek software. The microreactors were made of tightly coiled silica tubing (100 μm × 2 m) with an internal volume of 15.7 μL. Hence, at a flow rate of 20 μL/min for each, the precursor and $K^{18}F/K_{222}$ complex, the residence (or reaction) time inside the microreactor was 23.6 s per reaction. In conjunction with the final sweeping of the product through the system, each reaction was completed within 2 min on the microfluidic system. The end product from the microfluidic reaction was diluted with water before being injected onto an analytical HPLC system for analysis and purification. Isolated radiochemical yields (RCY) of [^{18}F]**1b−12b** were calculated based on the initial radioactivity injected. Nonradioactive reference standards (**1b−12b**) used to confirm the radiosynthesis of [^{18}F]**1b−12b**, were synthesized by reacting **1a−12a** with TBAF overnight (Scheme 1).

Precursor **4a** (R = 4-OCH_3−C_6H_4) was chosen for initial precursor amount optimization experiments. At concentrations of 2 mg/mL and 10 mg/mL, [^{18}F]**4b** was able to be prepared in >75% RCY (n = 3) at all temperatures between 30 and 180 °C (Figure 3). When the precursor amount was reduced to 0.5 mg/mL, the yield remained >75% at 30 °C but steadily decreased to <50% with increasing temperatures up to 180 °C. This is most likely due to precursor decomposition at higher temperatures but is not pertinent as excellent radiochemical

dx.doi.org/10.1021/jo401759z | J. Org. Chem. 2013, 78, 11262−11270

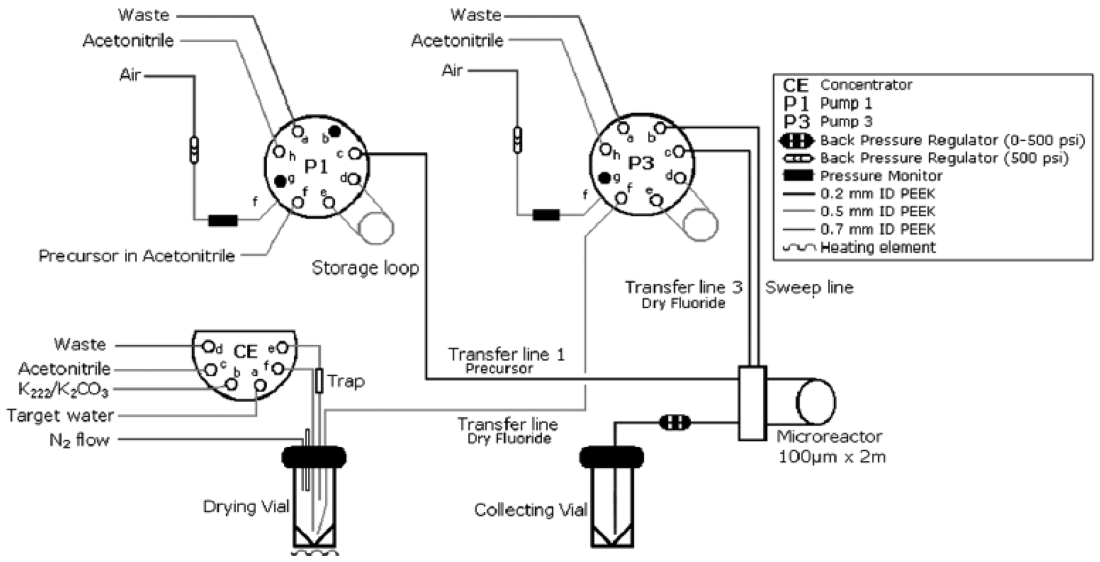


Figure 2. Schematic representation of the NanoTek microfluidic system setup. This figure originally appeared in Ungersboeck et al.[31] reproduced with permission.

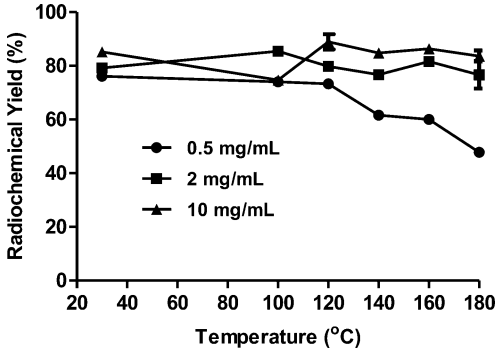


Figure 3. Effects of varying precursor mass on the radiochemical yield of [^{18}F]**4b**. Each data point represents the mean of at least three independent experiments ± SD. (Some error bars are smaller than the data points).

yields were obtained at 30 °C. An example of a typical HPLC radio-chromatogram is displayed in Figure 4.

The remaining sulfonyl chlorides **1a**−**12a** were then radiolabeled (1−40 MBq) in acetonitrile using 0.5 mg/mL of precursor (pump 1), a precursor volume of 10 μL, a 450 μL solution containing [^{18}F]fluoride with 5 mg/mL of K_2CO_3 and

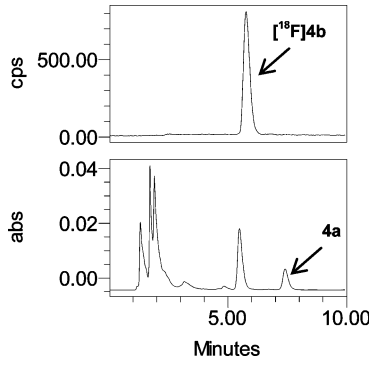


Figure 4. Crude radio-HPLC chromatogram of [^{18}F]**4b** after reaction was performed at 140 °C on the microfluidic system; (top) radioactivity (cps); (bottom) UV at 254 nm (abs).

20 mg/mL of K_{222} (pump 3), pump 1 to pump 3 ratio of 1, flow rate of 20 μL/min and temperatures of 30−180 °C on the microfluidic system (Figure 5). Although microfluidic systems are able to operate under supercritical conditions, whereby solvents can be heated well-above their boiling points, temperatures greater than 180 °C were not used to minimize the risk of damage to the microreactors.

As shown in Figure 5, at a reaction temperature of 30 °C all sulfonyl chlorides (**1a**−**12a**), with the exception of nitro precursor **5a** (R = 4-NO_2-C_6H_4) were radiolabeled in >60% RCY ($n \geq 3$). At 100 °C the RCY of [^{18}F]**8b** (R = 4-Br-C_6H_4) decreased dramatically (Figure 5C) while all remaining [^{18}F]sulfonyl fluorides were prepared in 48−78% RCY ($n \geq 3$). At 120 °C, the RCY of [^{18}F]**7b** (R = 4-Cl-C_6H_4) also decreased (Figure 5C) but all other [^{18}F]sulfonyl fluorides could still be produced in 39−76% RCY ($n \geq 3$). The results indicate that the RCY at higher reaction temperatures is lowest for precursors bearing electron-withdrawing groups in the 4-position (e.g., **5a**, **7a**, **8a**) (Figure 5C) and that the magnitude of this decrease is proportional to the Hammett constants of **5a** (σ_p = 0.78), **7a** (σ_p = 0.23) and **8a** (σ_p = 0.23) (see Supporting Information for full table of Hammett constants). The RCY for precursors encompassing electron-donating substituents also followed this trend with **2a** (σ_p = −0.15), **3a** (σ_p = −0.15) and **4a** (σ_p = −0.27) all obtained in >70% RCY ($n \geq 3$) at temperatures of 120 °C, with [^{18}F]**4b** (R = 4-OCH_3-C_6H_4) being formed in >50% RCY ($n \geq 3$) at 180 °C (Figure 5B). At 180 °C the *N*-acetyl product, [^{18}F]**2b** was synthesized in the greatest RCY of all 12 sulfonyl fluorides (70 ± 9%, n = 3, Figure 5B). This trend suggests that decreases in RCY either through decomposition of the [^{18}F]labeled compound or precursor occurs through a mechanism in which the rate limiting step is nucleophilic attack at the sulfonyl group. Sulfonyl fluorides with increased steric bulk [^{18}F]**10b** (R = 2,4,6-CH_3-C_6H_2) and [^{18}F]**11b** (R = 2,4,6-$CH(CH_3)_2$-C_6H_2) or a heterocyclic moiety [^{18}F]**12b** (R = 2-thiophene) were produced in good RCY at temperatures up to 120 °C after which significant decreases in yield were observed (Figure 5D). However, the sulfonyl fluoride containing the greatest amount of steric bulk, the triisopropyl-substituted [^{18}F]**11b** still managed to exhibit a

dx.doi.org/10.1021/jo401759z | J. Org. Chem. 2013, 78, 11262−11270

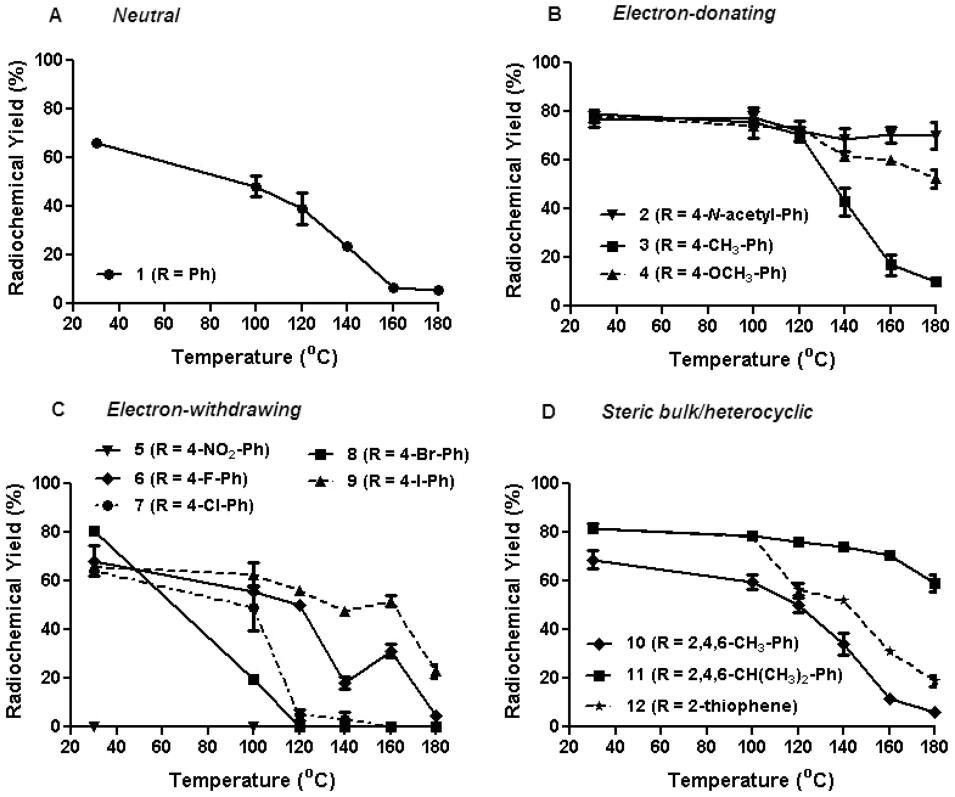


Figure 5. Effects of varying temperature on the incorporated radiochemical yields of [^{18}F]**1b**−**12b**. (A) [^{18}F]Sulfonyl fluorides containing a neutral substituent (σ_p = 0.00); (B) [^{18}F]sulfonyl fluorides containing an electron-donating substituent (σ_p = −0.15 − −0.27); (C) [^{18}F]sulfonyl fluorides containing an electron-withdrawing substituent (σ_p = 0.06 − 0.78); (D) [^{18}F]sulfonyl fluorides containing sterically bulky (σ_p = −0.15 − −0.17) or heterocyclic (σ_p = N/A) substituents. Each data point represents the mean of at least three independent experiments ± SD. (Some error bars are smaller than the data points).

RCY of 59 ± 6% (n = 3) at 180 °C. This shows steric bulk does not inhibit radiolabeling but subsequently protects the compound from decomposition. The precursor containing the most electron-withdrawing group **5a** (R = 4-NO_2-C_6H_4, σ_p = 0.78), gave no detectable radiolabeled product at temperatures of 30−180 °C. However, by altering the precursor mass to 10 mg/mL, it was possible to produce [^{18}F]sulfonyl fluorides containing electron-withdrawing groups such as [^{18}F]**5b** in high RCY (>75%), at a range of temperatures (30−180 °C) and with excellent radiochemical purity (>97%).

Conventional Vessel-based Radiosynthesis. Sulfonyl chlorides **2a**, **4a** and **5a** with Hammett constants −0.15, −0.27 and 0.78 respectively, were also radiolabeled (200−300 MBq) in conventional vessel-type reactions in order to compare the RCY to the microfluidic reactions. To mimic the microfluidic conditions as closely as possible, precursors **2a**, **4a** and **5a** (at 0.5 mg/mL) were heated to 100 °C in acetonitrile (higher temperatures were not used due to the low boiling point of acetonitrile and the pressure limitations of the reaction vessel) for 2 min with 5 mg/mL of K_2CO_3 and 20 mg/mL of K_{222}. Under these conditions the nitro precursor **5a** (R = 4-NO_2-C_6H_4) did not yield a detectable radiolabeled product, (consistent with the microfluidic system) while the methoxy precursor **4a** (R = 4-OCH_3-C_6H_4) produced the radiolabeled product [^{18}F]**4b** in 1% RCY (*c.f.* 74% at 100 °C on the microfluidic system). The *N*-acetyl precursor **2a** (R = 4-$NHCOCH_3$-C_6H_4) was radiolabeled to give [^{18}F]**2b** in the greatest RCY at 30%, however, this yield is still much lower than the 78% RCY produced at 100 °C on the microfluidic system. These results are not surprising, as the use of excess equivalents of K_{222} and K_2CO_3 are known to reduce [^{18}F]fluoride incorporation and can lead to decomposition of the precursor.[32,33] On the contrary, short residence times and the superior mixing and heating capabilities of the microfluidics system may circumvent some of the decomposition problems often observed with conventional or automated synthesis conditions. Higher RCYs using conventional heating could be achieved by using 0.5 mg/mL of precursor, in acetonitrile at 100 °C for 2 min with a lower ratio of K_{222} (1 mol equiv) and K_2CO_3 (2 mol equiv). This resulted in [^{18}F]**4b**, [^{18}F]**2b** and [^{18}F]**5b** being synthesized in 88 ± 2%, 87 ± 3% and 74 ± 6% RCY, respectively ($n \geq 3$), with no significant difference in RCY when the reactions were performed at 30 °C. [^{18}F]**4b** was also synthesized in high yield (72% RCY, n = 3) at 100 °C after a reaction time of only 30 s, verifying that [^{18}F]sulfonyl fluorides are produced extremely rapidly. The radiolabeling efficiency of [^{18}F]**4b** was further explored by not drying the $K^{18}F/K_{222}$ complex and adding water prior to adding the precursor **4a**. Reaction mixtures containing 3% and 10% water led to the production of [^{18}F]**4b** in 53% and 19% RCY respectively at 30 °C using 0.5 mg/mL of **4a**. This result is consistent with Inkster et al. who reported that [^{18}F]-benzenesulfonyl fluorides may be successfully prepared in a 1:1 mixture of aqueous/organic solvent at rt.[18]

Competition Studies. Sulfonyl fluorides are known to react with various nucleophiles (−NH_2, −OH) which displace the fluoride group.[34] Additionally, hydrogen bond donor groups (−NH_2, −OH, −COOH) interfere with [^{18}F]-radiolabeling and generally require a protecting group strategy. Therefore, to examine the utility of sulfonyl fluoride radio-

dx.doi.org/10.1021/jo401759z | J. Org. Chem. 2013, 78, 11262−11270

labeling, competition studies with nucleophiles and H-bond donors were performed. Radiolabeling of precursor **4a** (R = 4-OCH_3-C_6H_4) at 100 °C in the presence of an equimolar amount of D-tyrosine did not diminish the RCY of [^{18}F]**4b** (86% after 2 min). Additionally, radiolabeling of **4a** in the presence of an equimolar amount of benzylamine gave [^{18}F]**4b** in excellent RCY (73%). The byproduct *N*-benzyl-4-methoxybenzenesulfonamide (**13**, Figure 6) was observed but the high

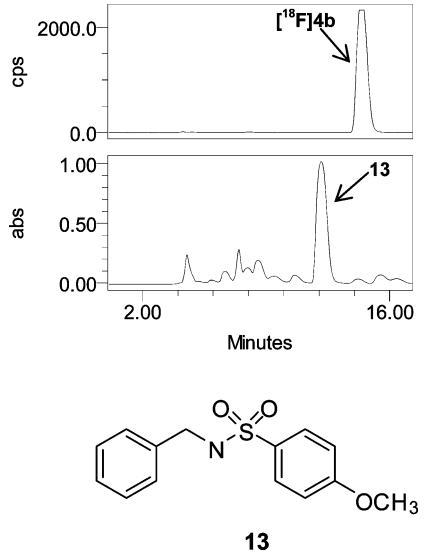


Figure 6. Crude radio-HPLC chromatogram of [^{18}F]**4b** after reaction was performed at 100 °C in the presence of benzylamine; top panel: radioactivity (cps); presence of [^{18}F]**4b**; bottom panel: UV at 254 nm (abs); presence of sulfonamide **13**.

RCY shows the amine reacts with the excess sulfonyl chloride precursor and not the [^{18}F]sulfonyl fluoride product. This shows sulfonyl fluorides can be produced in excellent yields in the presence of H-bond donors which usually render the [^{18}F]fluoride non-nucleophilic. It also demonstrates that [^{18}F]sulfonyl fluorides are relatively stable and not prone to significant degradation by competing nucleophiles such as amines or hydroxyl groups.

Stability Studies. Due to the high radiochemical yields, tolerance for H-bond donor groups and resistance to competing nucleophiles during radiolabeling, time-course stability studies for [^{18}F]**2b** (R = 4-$NHCOCH_3$-C_6H_4), [^{18}F]**4b** (R = 4-OCH_3-C_6H_4) and [^{18}F]**5b** (R = 4-NO_2-C_6H_4) were performed. The three products were initially found to be >97% stable after 3 h at rt in a solution of mobile phase (55% CH_3CN/45% H_2O/0.1% TFA) and in a 1:1 mixture of EtOH/saline as determined by radio-HPLC. Stability of the three [^{18}F]sulfonyl fluorides was also assessed in buffer (~20% EtOH in 0.1 M PBS pH 7.4) at rt (Figure 7). Sulfonyl fluorides containing electron-donating groups [^{18}F]**2b** (R = 4-$NHCOCH_3$-C_6H_4) and [^{18}F]**4b** (R = 4-OCH_3-C_6H_4) displayed 98 ± 1% stability after 3 h (n = 3) as determined by HPLC, however, the stability of the electron-withdrawing sulfonyl fluoride [^{18}F]**5b** (R = 4-NO_2-C_6H_4), decreased dramatically, with only 32 ± 3% of the product intact after 1 h and 8 ± 2% intact after 3 h (n = 5).

As a comparison, the known [^{18}F]sulfonyl fluoride, [^{18}F]4-formylbenzenesulfonyl fluoride[18] ([^{18}F]**14b**, Scheme 2), was synthesized and its stability assessed in 0.1 M PBS pH 7.4. The

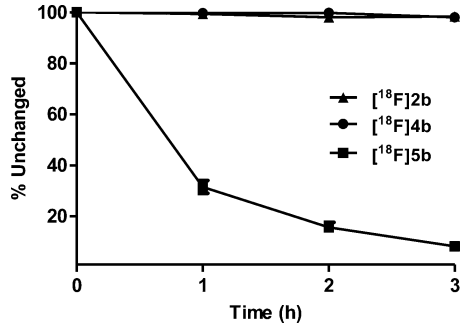


Figure 7. Stability of [^{18}F]sulfonyl fluorides [^{18}F]**2b**, [^{18}F]**4b** and [^{18}F]**5b** in 0.1 M PBS pH 7.4 (containing ~20% EtOH) at rt over 3 h. Each data point represents the mean of at three independent experiments ± SD. (Some error bars are smaller than the data points).

nonradioactive reference standard **14b** was also prepared to confirm the identity of [^{18}F]**14b**.

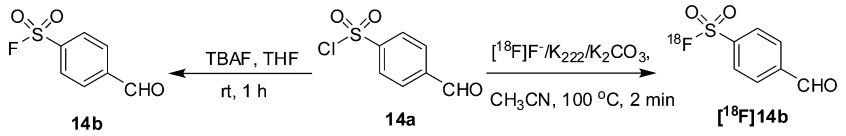
Stability of [^{18}F]**14b** in 0.1 M PBS pH 7.4 buffer containing ~20% EtOH (as above) was evaluated. After 3 h at rt only 19% of [^{18}F]**14b** remained as determined by radio-HPLC. This is in agreement with Inkster et al. who demonstrated that the stability of [^{18}F]**14b** is only 1% after incubation at the elevated temperature of 37 °C for 2.5 h in 10% DMSO in 0.15 M PBS pH 7.4.[18] The formyl substituent has a Hammett (σ_p) value of 0.42 and the observed instability is consistent with our determined stability trends. Specifically, the stability of [^{18}F]**14b** (19%) lies between the values obtained for [^{18}F]**2b** (σ_p = −0.15) and [^{18}F]**5b** (σ_p = 0.78). This demonstrates, again, that [^{18}F]sulfonyl fluoride stability is inversely proportional to the magnitude of the substituent Hammett constant (σ_p).

To further assess the stability of [^{18}F]sulfonyl fluorides, selected compounds were evaluated in rat serum. The most stable [^{18}F]sulfonyl fluorides in PBS buffer, [^{18}F]**2b** and [^{18}F]**4b**, were chosen to be assessed in normal rat plasma, in conjunction with [^{18}F]**11b** (R = 2,4,6-$CH(CH_3)_2$-C_6H_2). Despite [^{18}F]**11b** not being initially evaluated in PBS buffer, there are suggestions in the literature that increased steric bulk aids in the stability of non C−F bonds such as Si−F under physiological conditions by shielding the Si−F bond from hydrolysis.[15] It was postulated that the steric bulk of the triisopropyl groups in [^{18}F]**11b** could contribute to the compound's stability in plasma. The serum stability of compounds [^{18}F]**2b**, [^{18}F]**4b** and [^{18}F]**11b** were analyzed by incubation in normal rat plasma with 3% EtOH at 37 °C for 120 min (n = 3). An aliquot was removed after 1, 15, 30, 60, and 120 min, diluted with water and injected onto an analytical HPLC system. Radio-HPLC analysis was performed following the method of Hilton.[35] For comparative purposes, [^{18}F]**2b**, [^{18}F]**4b** and [^{18}F]**11b** were also incubated in 0.1 M PBS buffer pH 7.4 containing 3% EtOH at 37 °C for 120 min. All three compounds demonstrated ≥95% stability after 120 min.

Compound [^{18}F]**4b** (σ_p value of −0.27) exhibited moderate stability in rat plasma with some degradation in the first 60 min, however, only 44 ± 8% of the compound remained after 120 min (Figure 8). [^{18}F]Sulfonyl fluoride [^{18}F]**2b** (σ_p value of −0.15) displayed signs of degradation after 15 min, with 84 ± 9% remaining (Figure 8). Decomposition of this compound increased rapidly in the first 60 min and only 3 ± 3% remained after 120 min. The remainder of the radioactivity at 120 min was detected within the first minute in the radio-HPLC and can be attributed to degradation products. Free fluoride was

dx.doi.org/10.1021/jo401759z | J. Org. Chem. 2013, 78, 11262−11270

Scheme 2. Synthesis of Sulfonyl Fluorides 14b and [^{18}F]14b



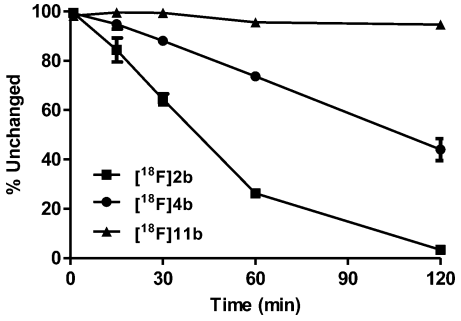


Figure 8. Stability of [^{18}F]sulfonyl fluorides [^{18}F]**2b**, [^{18}F]**4b** and [^{18}F]**11b** in normal rat plasma (containing 3% EtOH) at 37 °C over 120 min. Each data point represents the mean of at least three independent experiments ± SD. (Some error bars are smaller than the data points).

detected "bleeding" off the HPLC column, making the baseline not uniform, an observation that has been reported previously.[18] (See Supporting Information for a typical radio-HPLC chromatogram). Surprisingly, the 2,4,6-triisopropylbenzenesulfonyl fluoride [^{18}F]**11b**, with a Hammett constant of −0.15 exhibited excellent stability in rat plasma at 37 °C. No degradation of the compound was observed in the initial 30 min, followed by 96 ± 1% and 95 ± 1% of the compound remaining in serum after 60 and 120 min, respectively (Figure 8). This suggests that steric hindrance is the more important factor than substituent electronics in affecting radiotracer stability. The specific activity of the two most stable compounds, [^{18}F]**11b** and [^{18}F]**4b**, were calculated to be 397 ± 49 GBq/μmol (10.7 ± 1.3 Ci/μmol) and 335 ± 117 GBq/μmol (9.0 ± 3.2 Ci/μmol) (EOS), respectively.

CONCLUSION

A fundamental study into the radiolabeling and subsequent stability of 12 aryl [^{18}F]sulfonyl fluorides was achieved using an Advion NanoTek microfluidic synthesis system. The microfluidic device allowed rapid optimization of RCY by allowing fast screening of reaction conditions including temperature, precursor amount, reaction time, precursor sterics and electronics. Most [^{18}F]sulfonyl fluorides were radiolabeled at concentrations as low as 0.5 mg/mL and reaction times of less than 1 min. Radiochemical yields were typically higher, at higher reaction temperatures (100−120 °C) for aryl sulfonyl fluorides bearing electron-donating groups (*e.g.*, [^{18}F]**4b**) compared to electron-withdrawing groups (*e.g.*, [^{18}F]**5b**). Almost all aryl [^{18}F]sulfonyl fluorides, however, were obtained in very good RCY (>75%) at temperatures between 30 and 100 °C and with excellent radiochemical purity (>97%). Investigations into the radiolabeling of [^{18}F]sulfonyl fluorides under conventional heating conditions showed that [^{18}F]-sulfonyl fluorides are reasonably stable and can be prepared in good yields in the presence of water, other nucleophiles and unprotected chemical groups i.e. amines, acids and phenols. Barely any radiosynthons reported in the literature can tolerate these conditions and thus, our results give precedence to radiochemists to produce simple, faster, higher yielding, robust reaction pathways.

Stability studies in mobile phase (55% CH_3CN/45% H_2O/0.1% TFA) showed [^{18}F]sulfonyl fluorides bearing electron-donating and electron-withdrawing groups were >97% intact after 3 h at rt. Further stability studies in buffer (~20% EtOH in 0.1 M PBS pH 7.4) showed that [^{18}F]sulfonyl fluorides bearing electron-donating groups were >98% intact after 3 h at rt but those with strongly electron-withdrawing groups were only 8% intact after 3 h at rt. Stability studies in rat serum at 37 °C after 120 min showed that [^{18}F]sulfonyl fluorides with electron donating groups [^{18}F]**2b** (44%) and [^{18}F]**4b** (3%) were significantly diminished compared to PBS buffer. The sterically hindered [^{18}F]**11b**, however, was >95% intact in serum after 120 min at 37 °C. This result suggests that steric hindrance is more important than substrate electronics for increasing radiotracer stability. From this work it is predicted that [^{18}F]sulfonyl fluoride functional groups will be most stable *in vivo* when they have a combination of electron-donating groups and increased steric bulk near the sulfonyl fluoride group. This work not only makes an important contribution to our fundamental understanding of [^{18}F]sulfonyl fluoride radiochemistry but is also the first step toward our longer term goal of developing novel [^{18}F]radiolabeling methodologies. With the insights gained during this study, we are now investigating and developing a high yielding, water compatible, one-step radiolabeling procedure for a stabile [^{18}F]sulfonyl fluoride synthon with bioconjugation functionality. Our results from this work will be reported in due course.

EXPERIMENTAL SECTION

General Methods. Unless otherwise stated, all reactions were performed under an atmosphere of nitrogen and all solvents used were HPLC grade. Automated flash chromatography was performed using normal phase silica gel cartridges. ^{1}H, ^{13}C and ^{19}F nuclear magnetic resonance (NMR) spectra were acquired on a 400 MHz spectrometer; ^{19}F samples were calibrated externally to trifluorotoluene. IR spectra were acquired using a FT-IR spectrometer. Mass spectrometry (MS) was performed on a quadrupole spectrometer, while high-resolution MS was performed on a Q-TOF spectrometer.

General Procedure for the Synthesis of 1b, 2b, 4b−12b, 14b. The appropriate sulfonyl chloride (**1a**, **2a**, **4a−12a**, **14a**; 1−5 mmol, 1 equiv) and TBAF (1 M in THF; 2 equiv) were stirred at rt for 1−16 h. The reaction mixture was then purified using an automated flash chromatography system (silica, petroleum ether/ethyl acetate gradient) to yield the sulfonyl fluorides **1b**, **2b**, **4b−12b**, **14b**.

Benzenesulfonyl Fluoride (1b). Yield (154 mg, 57%); yellow oil. ^{1}H NMR (400 MHz, acetone-d_6): δ 8.11 (t, J = 2.0 Hz, 2H), 7.98 (t, J = 7.2 Hz, 1H), 7.85−7.81 (m, 2H). ^{13}C NMR (100 MHz, acetone-d_6): δ 137.2, 133.6 (J = 24 Hz), 131.1, 129.2. ^{19}F NMR (376 Hz, acetone-d_6): δ 64.1. IR (cm^{-1}): 1451, 1405, 1210, 750.

4-Acetamidobenzene-1-sulfonyl Fluoride (2b). Yield (580 mg, 61%); white/beige crystals; mp 175−176 °C (lit.[36] 175.2 °C). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.69 (bs, 1H, NH), 7.87 (s, 4H, CH × 4), 2.21 (s, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.6, 145.3, 129.9, 122.7, 119.6, 24.8. ^{19}F NMR (376 Hz, acetone-d_6): δ 65.3. IR (cm^{-1}): 3339, 1690, 1589, 1531, 1490, 1399, 1207, 1178, 763, 617. MS

dx.doi.org/10.1021/jo401759z | J. Org. Chem. 2013, 78, 11262−11270

(ESI+) m/z: 218 $[M+H]^+$. HRMS (ESI+) m/z: calcd for $C_8H_9FNO_3S$: 218.0287 $[M+H]^+$; found 218.0297 $[M+H]^+$.

4-Methoxybenzene-1-sulfonyl Fluoride (4b). Yield (570 mg, 62%); clear oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.93 (dt, J = 9.2 Hz, 3.2 Hz, 2H, CH × 2), 7.05 (dt, J = 8.8 Hz, 2.8 Hz, 2H, CH × 2), 3.91 (s, 3H, OCH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.4, 130.9, 124.1 (J = 24 Hz), 56.0. ^{19}F NMR (376 Hz, acetone-d_6): δ 65.7. IR (cm^{-1}): 1595, 1501, 1398, 1267, 1206, 1172, 807, 750, 670.

4-Nitrobenzene-1-sulfonyl Fluoride (5b). Yield (400 mg, 42%); beige crystals; mp 75−77 °C (lit.[37] 77.5−78.5 °C). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.48 (dt, J = 8.8 Hz, 2.4 Hz, 2H, CH × 2), 8.24 (dt, J = 8.8 Hz, 2.4 Hz, 2H, CH × 2). ^{13}C NMR (100 MHz, $CDCl_3$): δ 152.0, 138.5 (J = 27 Hz), 130.2, 125.0. ^{19}F NMR (376 Hz, acetone-d_6): δ 64.0. IR (cm^{-1}): 1609, 1532, 1417, 1348, 1214, 784, 615.

4-Fluorobenzene-1-sulfonyl Fluoride (6b). Yield (152 mg, 52%); yellow oil. ^{1}H NMR (400 MHz, acetone-d_6): δ 8.23 (q, J = 5.2 Hz, 2H), 7.60 (t, J = 8.4 Hz, 2H). ^{13}C NMR (100 MHz, acetone-d_6): δ 166.7, 132.8 (J = 11 Hz), 129.6 (J = 25 Hz), 118.4 (J = 23 Hz). ^{19}F NMR (376 Hz, acetone-d_6): δ −101.9, 64.9. IR (cm^{-1}): 1592, 1407, 1209, 837, 754, 672.

4-Chlorobenzene-1-sulfonyl Fluoride (7b). Yield (163 mg, 62%); white powder; mp 47−48 °C (lit.[38,39] 38.4−39.8 °C; 48−49 °C). ^{1}H NMR (400 MHz, acetone-d_6): δ 8.14 (dt, J = 8.4 Hz, 1.6 Hz, 2H), 7.86 (dt, J = 8.4 Hz, 2.4 Hz, 2H). ^{13}C NMR (100 MHz, acetone-d_6): δ 143.3, 132.1 (J = 24 Hz), 131.4, 131.2. ^{19}F NMR (376 Hz, acetone-d_6): δ 64.5. IR (cm^{-1}): 1572, 1473, 1405, 1209, 1088, 775, 740, 648.

4-Bromobenzene-1-sulfonyl Fluoride (8b). Yield (194 mg, 63%); beige solid; mp 52−54 °C (lit.[38] 58.1−59.8 °C). ^{1}H NMR (400 MHz, acetone-d_6): δ 8.04 (q, J = 8.8 Hz, 4H). ^{13}C NMR (100 MHz, acetone-d_6): δ 134.4, 132.6 (J = 26 Hz), 132.0, 131.0. ^{19}F NMR (376 Hz, acetone-d_6): δ 64.5. IR (cm^{-1}): 1573, 1468, 1403, 1207, 1065, 770, 731, 640.

4-Iodobenzene-1-sulfonyl Fluoride (9b). Yield (176 mg, 57%); beige powder; mp 70 °C. ^{1}H NMR (400 MHz, acetone-d_6): δ 8.24 (dd, J = 1.6 Hz, 8.8 Hz, 2H), 7.88 (dd, J = 2.0 Hz, 8.8 Hz, 2H). ^{13}C NMR (100 MHz, acetone-d_6): 140.5, 133.1 (J = 25 Hz), 130.5, 105.2. ^{19}F NMR (376 Hz, acetone-d_6): δ 64.3. IR (cm^{-1}): 1567, 1469, 1403, 1207, 767, 724, 602.

2,4,6-Trimethylbenzene-1-sulfonyl Fluoride (10b). Yield (550 mg, 59%); white powder; mp 71−73 °C (lit.[40] 73−73.5 °C). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.03 (s, 2H, CH × 2), 2.64 (d, J = 1.6 Hz, 6H, CH_3 × 2), 2.34 (s, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 145.2, 140.2, 132.0 (J = 1 Hz), 129.3 (J = 20 Hz), 22.5 (J = 2 Hz), 21.3. ^{19}F NMR (376 Hz, CD_3CN-d_3): δ 72.3. IR (cm^{-1}): 1604, 1440, 1392, 1204, 748, 663. MS (EI) m/z: 202 [M+]. HRMS (ESI+) m/z: calcd for $C_9H_{12}FO_2S$: 203.0542 $[M+H]^+$; found 203.0543 $[M+H]^+$.

2,4,6-Triisopropylbenzene-1-sulfonyl Fluoride (11b). Yield (310 mg, 65%); white crystals; mp 68−69 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.28 (s, 2H, CH × 2), 4.02−3.94 (m, 2H, CH × 2), 3.00−2.93 (m, 1H, CH), 1.33−1.29 (m, 18H, CH_3 × 6). ^{13}C NMR (100 MHz, $CDCl_3$): δ 155.5, 150.9, 128.1 (J = 19 Hz), 124.1, 34.5, 30.3 (J = 2 Hz), 24.6. ^{19}F NMR (376 Hz, CD_3CN-d_3): δ 67.1. IR (cm^{-1}): 2960, 1599, 1431, 1399, 1363, 1205, 846, 764, 667.

Thiophene-2-sulfonyl Fluoride (12b). Yield (181 mg, 60%); yellow oil. ^{1}H NMR (400 MHz, acetone-d_6): δ 8.35 (ddd, J = 5.2 Hz, 1.6 Hz, 0.4 Hz, 1H), 8.14 (dt, J = 4.0 Hz, 1.6 Hz, 1H), 7.45 (ddd, J = 4.8 Hz, 4.0 Hz, 0.8 Hz, 1H). ^{13}C NMR (100 MHz, acetone-d_6): δ 139.4 (J = 3 Hz), 138.8 (J = 2 Hz), 131.3 (J = 30 Hz), 129.8. ^{19}F NMR (376 Hz, acetone-d_6): δ 70.2. IR (cm^{-1}): 1400, 1204, 1024, 859, 767, 672.

4-Formylbenzene-1-sulfonyl Fluoride (14b). Yield (62 mg, 29%); white powder; mp 59−60 °C (lit.[18] 60 °C). ^{1}H NMR (400 MHz, acetone-d_6): δ 10.26 (s, 1H), 8.35 (d, J = 8.4 Hz, 2H), 8.32 (d, J = 8.8 Hz, 2H). ^{13}C NMR (100 MHz, acetone-d_6): δ 192.1, 142.5, 137.7 (J = 25 Hz), 131.5, 130.2. ^{19}F NMR (376 Hz, acetone-d_6): δ 63.9. IR (cm^{-1}): 2866, 1702, 1407, 1205, 773, 697, 605. MS (ESI+) m/z: 188 $[M+H]^+$.

Radiochemistry. Aqueous H[^{18}F] was produced on a GE PETtrace cyclotron (Cyclotek, Bundoora, Australia) or an IBA Cyclone 18 Twin cyclotron (ANSTO, Camperdown, Australia) using the ^{18}O(p, n)^{18}F nuclear reaction. Microfluidic radiosyntheses were performed in Discovery Mode using a NanoTek LF Microfluidic Synthesis System (Advion, Ithaca, NY) connected to a standard laptop using the NanoTek software, V1.4.0 GMP Lite. Microreactors were made of fused silica tubing (100 μm × 2 m), coiled tightly into a brass ring containing a thermoresistant polymer to hold the tubing in place. [^{18}F]**1b−12b** and [^{18}F]**14b** were purified by HPLC using a Waters 515 pump, a Linear UVis 200 detector (λ = 254 nm) together with a Carroll and Ramsey model 105S gamma detector. [^{18}F]**1b−12b** samples from the microfluidic reactor were purified using a Phenomenex Luna C18 column (150 × 4.6 mm, 5 μm) at 1.2 mL/min with CH_3CN/H_2O/TFA (55:45:0.1, v/v) as the mobile phase, with the following exceptions: [^{18}F]**2b** (52.5% CH_3CN); [^{18}F]**7b** (65% CH_3CN); [^{18}F]**8b** (65% CH_3CN); [^{18}F]**9b** (70% CH_3CN); [^{18}F]**10b** (90% CH_3CN); [^{18}F]**11b** (70% CH_3CN). [^{18}F]**2b**, [^{18}F]**4b**, [^{18}F]**5b**, [^{18}F]**11b** and [^{18}F]**14b** samples from the conventional radiosynthetic methods were purified using Phenomenex Luna C18 columns (250 × 10 mm, 5 or 10 μm) at 2 mL/min with CH_3CN/H_2O/TFA (70:30:0.1, v/v) as the mobile phase (90:10:0.1, v/v for [^{18}F]**11b**). Specific activity values for [^{18}F]**4b** and [^{18}F]**11b** were obtained by measuring the radioactivity injected and the UV absorbance associated with the radioactive peak by analytical HPLC. The concentration of the sample was found by comparison of the UV area under the curve to the concentration calibration curve of the analogous reference standard. Radioactivity was measured using a Capintec R15C dose calibrator. Isolated radiochemical yields of [^{18}F]**1b−12b**, **14b** were collected HPLC yields calculated as a percentage of the initial radioactivity injected. Solid phase extraction Sep-pak C18 Light cartridges were purchased from Waters Corporation (Milford, MA) and activated with 5 mL of EtOH and 20 mL of H_2O.

Microfluidic Procedure. No-carrier-added (n.c.a.) aqueous [^{18}F]-fluoride (500−2300 MBq) was trapped onto an anion-exchange resin (MP1) and eluted with a solution of K_2CO_3 (5 mg/mL) and Kryptofix [2.2.2] (20 mg/mL) in CH_3CN/H_2O (90:10 v/v, 450 μL). Subsequent azeotropic drying (2 × 500 μL L) was performed at 95 °C, followed by the addition of 500 μL of CH_3CN. The loop of pump 1 was filled with the precursor solution (0.5−10 mg/mL in CH_3CN), while the loop of pump 3 was filled with the dried $K^{18}F/K_{222}$ complex. Equal volumes of $K^{18}F/K_{222}$ and the precursor (10 μL) were released from each pump and pushed through the microreactor at a flow rate of 20 μL/min, at temperatures between 30 and 180 °C. The crude product was released from the microreactor with 200 μL of CH_3CN into an Eppendorf tube, before 300 μL of H_2O was added and the whole sample was injected onto an analytical HPLC system for analysis and purification.

Conventional Procedure. An aqueous H[^{18}F] solution (100−500 MBq) was added to a 2.5 mL vial containing a solution of Kryptofix [2.2.2] (1.24−1.83 mg, 12.4−18.3 μL in CH_3CN, 2 equiv) and K_2CO_3 (0.23−0.34 mg, 2.3−3.4 μL in H_2O, 1 equiv). The solvent was evaporated under a stream of N_2 at 100 °C under vacuum and the residue was azeotropically dried with 3 × 1 mL anhydrous CH_3CN. The precursor **2a**, **4a**, **5a**, **11a** or **14a** (0.5 mg, 1 equiv) was dissolved in anhydrous CH_3CN (1 mL) and added to the dried $K^{18}F/K_{222}$ complex before being heated at 100 °C for 2 min. (For reactions at 30 °C, the reaction vial was cooled in H_2O for 5 min before the addition of the precursor). A 200 μL aliquot was added to 500 μL of mobile phase and purified using HPLC to yield [^{18}F]**2b** (30 °C: 86 ± 3% RCY, n = 3; 100 °C: 87 ± 3% RCY, n = 4), [^{18}F]**4b** (30 °C: 84 ± 2% RCY, n = 3; 100 °C: 88 ± 1% RCY, n = 3), [^{18}F]**5b** (30 °C: 77 ± 7% RCY, n = 4; 100 °C: 74 ± 6% RCY, n = 4), [^{18}F]**11b** (100 °C: 64 ± 1% RCY, n = 3) and [^{18}F]**14b** (100 °C: 58 ± 11% RCY, n = 3). [^{18}F]**4b** was also produced in 72 ± 11% RCY (n = 3) after being heated at 100 °C for 30 s.

Competition Studies. *Addition of an Unprotected Amino Acid.* The radiosynthesis was performed using the conventional method. The precursor **4a** (0.5 mg, 2.42 μmol) and D-tyrosine (0.44 mg, 2.42 μmol) were dissolved in anhydrous CH_3CN (1 mL) and added to the dried $K^{18}F/K_{222}$ complex before being heated at 100 °C for 2 min. A

200 μL aliquot was added to 500 μL of mobile phase and purified using HPLC to yield [^{18}F]**4b** in 86% RCY.

Addition of an Amine. The radiosynthesis was performed using the conventional method. The precursor **4a** (0.5 mg, 2.42 μmol) and benzylamine (0.26 mg, 2.42 μmol) were dissolved in anhydrous CH_3CN (1 mL) and added to the dried $K^{18}F/K_{222}$ complex before being heated at 100 °C for 2 min. A 200 μL aliquot was added to 500 μL of mobile phase and purified using HPLC to yield [^{18}F]**3b** in 73 ± 22% RCY, n = 3.

Stability Studies. *Buffer.* A HPLC purified sample of [^{18}F]**2b**, [^{18}F]**4b**, [^{18}F]**5b** or [^{18}F]**14b** (2−3 mL) was diluted with H_2O (∼15 mL) and loaded onto a preactivated Sep-pak C18 Light cartridge. The cartridge was eluted with EtOH (300 μL), 0.1 M PBS pH 7.4 (1 mL) was added and the sample was incubated at rt for 3 h, with an aliquot (400 μL) injected onto the HPLC system after 1, 2, and 3 h to assess the percentage of the [^{18}F]sulfonyl fluoride still intact. Briefly, a precolumn and a reverse phase HPLC column were used in series, with a switching valve between columns. First, the precolumn was washed with 1% CH_3CN in water for 3 min to elute polar impurities and then the solvent direction was switched to include the HPLC column. Both columns in the series were then eluted. The radioactivity peak corresponding to the appropriate [^{18}F]sulfonyl fluoride was compared to the total activity in the radiochromatogram to give the percentage of intact [^{18}F]sulfonyl fluoride.

Serum. A HPLC purified sample of [^{18}F]**2b**, [^{18}F]**4b** or [^{18}F]**11b** (2−3 mL) was diluted with H_2O (∼15 mL) and loaded onto a preactivated Sep-pak C18 Light cartridge. The cartridge was eluted with EtOH (300 μL). An aliquot (3 μL) was removed, added to normal rat plasma (97 μL) and heated at 37 °C. Aliquots (1 μL) were removed after 1, 15, 30, 60, and 120 min and quenched with H_2O (99 μL). 50 μL of this sample was then injected onto the HPLC system (<20 cps) to assess the percentage of the [^{18}F]sulfonyl fluoride still intact. For comparison, a 3 μL aliquot of the [^{18}F]sulfonyl fluoride sample was also added to 0.1 M PBS pH 7.4 (97 μL) and heated at 37 °C. Aliquots (1 μL) were removed after 1, 60, and 120 min and quenched with H_2O (99 μL). 50 μL of this sample was then injected onto the HPLC system to assess the percentage of the [^{18}F]sulfonyl fluoride still intact.

■ ASSOCIATED CONTENT

Supporting Information

Table of Hammett constants, HPLC purity data, radio-HPLC chromatograms, ^{1}H, ^{13}C and ^{19}F NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: benjamin.fraser@ansto.gov.au. Tel: +61 2 9717 3887. Fax: +61 2 9717 9262.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors gratefully acknowledge Dr. Nigel Lengkeek for assistance with ^{19}F NMR, Ms. Cathy Jiang for technical assistance, Dr. Vu Nguyen for the supply of rat plasma and Dr. Giancarlo Pascali for useful discussions.

■ REFERENCES

(1) Kumar, R.; Halanaik, D.; Dahiya, S. PET in Anti-Cancer Drug Development and Therapy. In *Frontiers in Anti-Cancer Drug Discovery*; Rahman, A. U., Choudhary, M. I., Eds.; Bentham Science Publishers: Sharjah, 2010; Vol. *1*, p 541.
(2) Coenen, H. H.; Elsinga, P. H.; Iwata, R.; Kilbourn, M. R.; Pillai, M. R. A.; Rajan, M. G. R.; Wagner, H. N., Jr.; Zaknun, J. J. *Nucl. Med. Biol.* **2010**, *37*, 727.
(3) Luthra, S. K.; Robins, E. G. ^{18}F-Labelled Tracers for PET Oncology and Neurology Applications. In *Fluorine in Pharmaceutical and Medicinal Chemistry*; Gouverneur, V., Müller, K., Eds.; Imperial College Press: London, 2012; Vol. *6*, p 383.
(4) Kitson, S. L. *Curr. Radiopharm.* **2009**, *2*, 224.
(5) Fernandes, E. *Curr. Radiopharm.* **2012**, *5*, 90.
(6) Hendrikse, N. *Curr. Opin. Drug Discovery Dev.* **2008**, *11*, 717.
(7) Le Bars, D. *J. Fluor. Chem.* **2006**, *127*, 1488.
(8) Miller, P. W.; Long, N. J.; Vilar, R.; Gee, A. D. *Angew. Chem., Int. Ed.* **2008**, *47*, 8998.
(9) Comagic, S.; Piel, M.; Schirrmacher, R.; Höhnemann, S.; Rösch, F. *Appl. Radiat. Isot.* **2002**, *56*, 847.
(10) Wüst, F. R.; Kniess, T. *J. Label. Compd. Radiopharm.* **2003**, *46*, 699.
(11) Mäding, P.; Füchtner, F.; Wüst, F. *Appl. Radiat. Isot.* **2005**, *63*, 329.
(12) Speranza, A.; Ortosecco, G.; Castaldi, E.; Nardelli, A.; Pace, L.; Salvatore, M. *Appl. Radiat. Isot.* **2009**, *67*, 1664.
(13) Studenov, A. R.; Adam, M. J.; Wilson, J. S.; Ruth, T. J. *J. Label. Compd. Radiopharm.* **2005**, *48*, 497.
(14) Ting, R.; Adam, M. J.; Ruth, T. J.; Perrin, D. M. *J. Am. Chem. Soc.* **2005**, *127*, 13094.
(15) Wängler, C.; Kostikov, A.; Zhu, J.; Chin, J.; Wängler, B.; Schirrmacher, R. *Appl. Sci.* **2012**, *2*, 277.
(16) Mu, L. *Curr. Radiopharm.* **2010**, *3*, 224.
(17) McBride, W. J.; D'Souza, C. A.; Sharkey, R. M.; Karacay, H.; Rossi, E. A.; Chang, C.-H.; Goldenberg, D. M. *Bioconjugate Chem.* **2010**, *21*, 1331.
(18) Inkster, J. A. H.; Liu, K.; Ait-Mohand, S.; Schaffer, P.; Guérin, B.; Ruth, T. J.; Storr, T. *Chem.—Eur. J.* **2012**, *18*, 11079.
(19) Smith, T. A. D. *J. Label. Compd. Radiopharm.* **2012**, *55*, 281.
(20) Ting, R.; Harwig, C.; auf dem Keller, U.; McCormick, S.; Austin, P.; Overall, C. M.; Adam, M. J.; Ruth, T. J.; Perrin, D. M. *J. Am. Chem. Soc.* **2008**, *130*, 12045.
(21) Gu, C. *Chem. Biol.* **2013**, *20*, 541.
(22) Grimster, N. P.; Connelly, S.; Baranczak, A.; Dong, J.; Krasnova, L. B.; Sharpless, K. B.; Powers, E. T.; Wilson, I. A.; Kelly, J. W. *J. Am. Chem. Soc.* **2013**, *135*, 5656.
(23) Brouwer, A. J.; Jonker, A.; Werkhoven, P.; Kuo, E.; Li, N.; Gallastegui, N.; Kemmink, J.; Florea, B. I.; Groll, M.; Overkleeft, H. S.; Liskamp, R. M. J. *J. Med. Chem.* **2012**, *55*, 10995.
(24) Hansch, C.; Leo, A.; Taft, R. W. *Chem. Rev.* **1991**, *91*, 165.
(25) Gillies, J. M.; Prenant, C.; Chimon, G. N.; Smethurst, G. J.; Perrie, W.; Hambletta, I.; Dekker, B.; Zweit, J. *Appl. Radiat. Isot.* **2006**, *64*, 325.
(26) Miller, P. W. *J. Chem.Technol. Biot.* **2009**, *84*, 309.
(27) Elizarov, A. M. *Lab Chip* **2009**, *9*, 1326.
(28) Lebedev, A.; Miraghaie, R.; Kotta, K.; Ball, C. E.; Zhang, J. Z.; Buchsbaum, M. S.; Kolb, H. C.; Elizarov, A. *Lab Chip* **2013**, *13*, 136.
(29) Steel, C. J.; O'Brien, A. T.; Luthra, S. K.; Brady, F. *J. Label. Compd. Radiopharm.* **2007**, *50*, 308.
(30) Pascali, G.; Mazzone, G.; Saccomanni, G.; Manera, C.; Salvadori, P. A. *Nucl. Med. Biol.* **2010**, *37*, 547.
(31) Ungersboeck, J.; Philippe, C.; Mien, L. K.; Haeusler, D.; Shanab, K.; Lanzenberger, R.; Spreitzer, H.; Keppler, B. K.; Dudczak, R.; Kletter, K.; Mitterhauser, M.; Wadsak, W. *Nucl. Med. Biol.* **2011**, *38*, 427.
(32) Suehiro, M.; Vallabhajosula, S.; Goldsmith, S. J.; Ballon, D. J. *Appl. Radiat. Isot.* **2007**, *65*, 1350.
(33) Hayashi, K.; Furutsuka, K.; Ito, T.; Muto, M.; Aki, H.; Fukumura, T.; Suzuki, K. *J. Label. Compd. Radiopharm.* **2012**, *55*, 120.
(34) Kamoshenkova, O. M.; Boiko, V. N. *J. Fluor. Chem.* **2010**, *131*, 248.
(35) Hilton, J.; Yokoi, F.; Dannals, R. F.; Rauert, H. T.; Szabo, Z.; Wong, D. F. *Nucl. Med. Biol.* **2000**, *27*, 627.
(36) Qu, Q.; Tang, X.; Wang, C.; Yang, G.; Hu, X.; Lu, X.; Liu, Y.; Li, S.; Yan, C. *Anal. Chim. Acta* **2006**, *572*, 212.
(37) Jenkins, F. E.; Hambly, A. N. *Aust. J. Chem.* **1953**, *6*, 318.

(38) Chen, G.; Chen, F.; Zhang, Y.; Yang, X.; Yuan, X.; Wu, F.; Yang, X. *J. Fluor. Chem.* **2012**, *133*, 155.
(39) Kulka, M. *J. Am. Chem. Soc.* **1950**, *72*, 1215.
(40) Steinkopf, W.; Buchheim, K.; Beythien, K.; Dudek, H.; Eisold, J.; Gall, J.; Jaeger, P.; Reumuth, H.; Semenoff, A.; Wemme, A. *J. Prakt. Chem.* **1927**, Chem. Abstr. CAN21:29985.