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Quinuclidine and DABCO Enhance the Radiofluorinations of 5-Substituted 2-Halopyridines

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ABSTRACT. Positron emission tomography (PET) is an important molecular imaging technique for medical diagnosis, biomedical research and drug development. PET tracers for molecular imaging contain β^+-emitting radionuclides, such as carbon-11 ($t_{1/2}$ = 20.4 min) or fluorine-18 ($t_{1/2}$ = 109.8 min). The [^{18}F]2-fluoro-pyridyl moiety features in a few prominent PET radiotracers, not least because this moiety is usually resistant to unwanted radiodefluorination in vivo. Various methods have been developed for labeling these radiotracers from cyclotron-produced no-carrier-added [^{18}F]fluoride ion, mainly based on substitution of a leaving group, such as halide (Cl or Br), or preferably a better leaving group, such as nitro or trimethylammonium. However, precursors with a good leaving group are sometimes more challenging or lengthy to prepare. Methods for enhancing the reactivity of more readily accessible 2-halopyridyl precursors are therefore desirable, especially for early radiotracer screening programs that may require the quick labeling of several homologous radiotracer candidates. In this work, we explored a wide range of additives for beneficial effect on nucleophilic substitution by [^{18}F]fluoride ion in 5-subsituted 2-halopyridines (halo = Cl or Br). The nucleophilic cyclic tertiary amines, quinuclidine and DABCO, proved effective for increasing yields to practically useful levels (> 15%). Quinuclidine and DABCO likely promote radiofluorination through reversible formation of quaternary ammonium intermediates.

Keywords: fluorine-18; radiofluorination; organocatalysis; 2-halopyridine; reactive intermediates

Introduction

Positron emission tomography (PET) is a molecular imaging modality that has increasing importance for medical diagnosis, biomedical research[1,2] and drug development.[3,4] The scope of PET for such applications very much depends on the development of radiotracers that are either able to interact with a single specific protein to provide robust information on protein distribution, protein function, or protein interaction with existing or experimental drugs, or that report on metabolic or signal transduction processes, such as [^{18}F]FDG for glycolysis and [^{18}F]FDOPA for the presynaptic dopaminergic system. Consequently, a demand for new radiotracers is ever present. Successful PET radiotracers must satisfy a broad array of criteria with regard to physicochemical, pharmacokinetic and pharmacological properties and therefore typically many candidates must be screened to find a high performing radiotracer for a particular protein target.[5]

Usually PET radiotracers are labeled with a cyclotron-produced short-lived radionuclide, such as carbon-11 ($t_{1/2}$ = 20.4 min) or fluorine-18 ($t_{1/2}$ = 109.8 min). ^{18}F-Labeled radiotracers are especially attractive because, unlike ^{11}C-labeled radiotracers, they may be transported over considerable distances to imaging sites that do not have a cyclotron. Moreover, fluorine-18 is available as [^{18}F]fluoride ion in high activity and in high (no-carrier-added; NCA) molar activity (ratio of activity to mass) from the ^{18}O(p,n)^{18}F nuclear reaction on ^{18}O-enriched water.[6] ^{18}F-Labeled tracers should be designed to resist extensive defluorination in vivo.[5] Otherwise the generated [^{18}F]fluoride ion binds avidly to bones, causing unnecessary radiation exposure of healthy tissue as well as spillover effects.[5,7] A feature of the [^{18}F]2-fluoro-pyridyl moiety is its metabolic stability.[5,7] Therefore, there are notable examples of effective PET radiotracers that contain this moiety. These include [^{18}F]AZD4694 ([^{18}F]**1**) for imaging Aβ-amyloid,[8] [^{18}F]T807 ([^{18}F]**2**) for imaging neurofibrillary tangles,[9,10] [^{18}F]NCFHEB ([^{18}F]**3**) for imaging $\alpha_4\beta_2$-nicotinic

acetylcholine receptors,[11,12] and [^{18}F]fluoroisonicotinamide ([^{18}F]**4**) for imaging melanoma[13] (Figure 1).

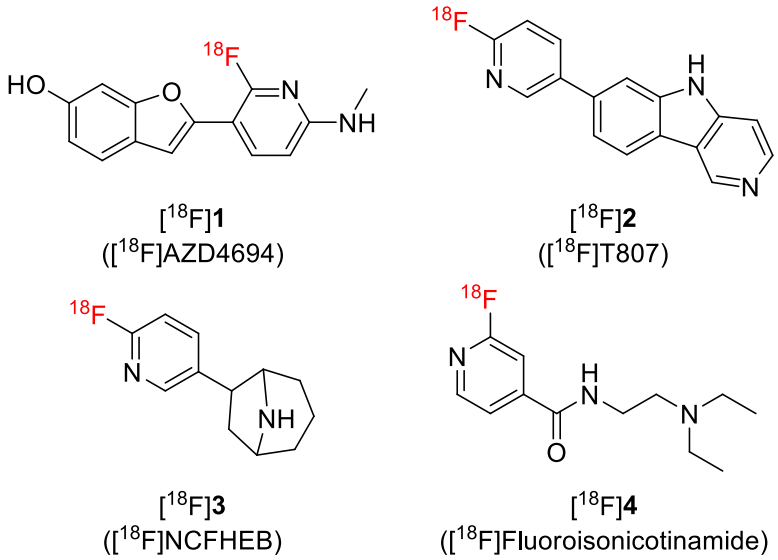

Figure 1. Examples of PET radiotracers containing an [^{18}F]2-fluoropyridyl moiety.

Reliable radiosyntheses of [^{18}F]2-fluoropyridyl compounds from NCA [^{18}F]fluoride ion are based on aromatic nucleophilic substitution of good leaving groups.[14–27] The effectiveness of the leaving group in radiofluorination follows the order: halide (Cl or Br) < nitro < trimethylammonium.[19,27] Yields from 2-chloro or 2-bromo-pyridines are not however always adequate for early candidate radiotracer evaluation experiments. Furthermore, the synthesis of precursors with 2-trimethylammonium as a leaving group on a pyridyl ring, especially when additional amino groups are present, can be challenging or lengthy. Although new methods have been introduced recently for labeling pyridines in 2-position with fluorine-18 using precursors such as diaryl sulfoxides[28] or diaryliodonium salts,[29] the preparation of these precursors usually requires halopyridines as starting materials (Figure 2). Therefore, to enable the rapid screening of homologous candidate PET radiotracers, methods for improving the radiosyntheses of [^{18}F]2-fluoropyridines directly from 2-halopyridyl precursors are desirable. Here we show that DABCO

and quinuclidine activate substituted 2-halopyridines for nucleophilic substitution with [^{18}F]fluoride ion to improve significantly radiochemical yields of [^{18}F]2-fluoropyridines.

Figure 2. Known methods for labeling pyridines in 2-position with [^{18}F]fluoride ion.

Results and discussion

Syntheses of precursors and reference compounds. 6-(6-Chloropyridin-3-yl)naphthalen-2-amine (**5**) and its 6-fluoro analog (**6**) were obtained in moderate yields by Suzuki coupling of the respective 6-halopyridin-3-ylboronic acids with 6-bromonaphthalen-2-amine (Figure 3). Treatment of amines **5** or **6** with Boc anhydride then gave the respective Boc-protected compounds **7** or **8** in high yields.

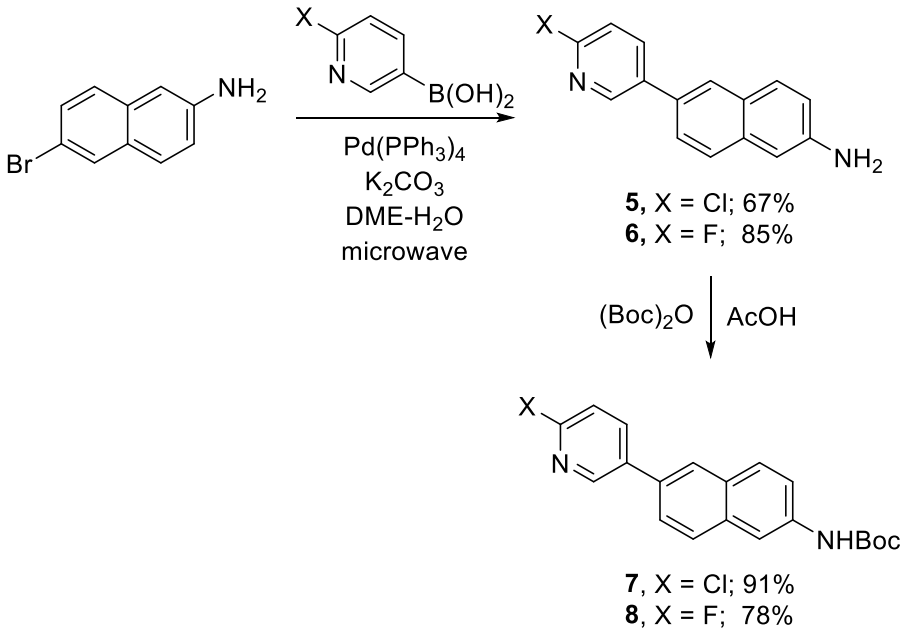

Figure 3. Syntheses of 6-(6-halopyridin-3-yl)naphthalene-2-amines and their *N*-Boc derivatives.

Other halopyridyl precursors (halo = Cl or Br) or reference 2-fluoropyridyl compounds were obtained commercially, or synthesized by well-known single-step general methods (Figure 4). Thus, 2-halo-5-alkoxy-pyridines were prepared through nucleophilic substitution in alkyl iodides with 2-halo-5-hydroxypyridines under basic conditions. 2-Halo-5-aryloxy-pyridines were prepared using copper salts as catalysts from iodoarenes. Suzuki coupling was used to prepare several 2-halo-5-aryl-pyridines from halo-pyridines.

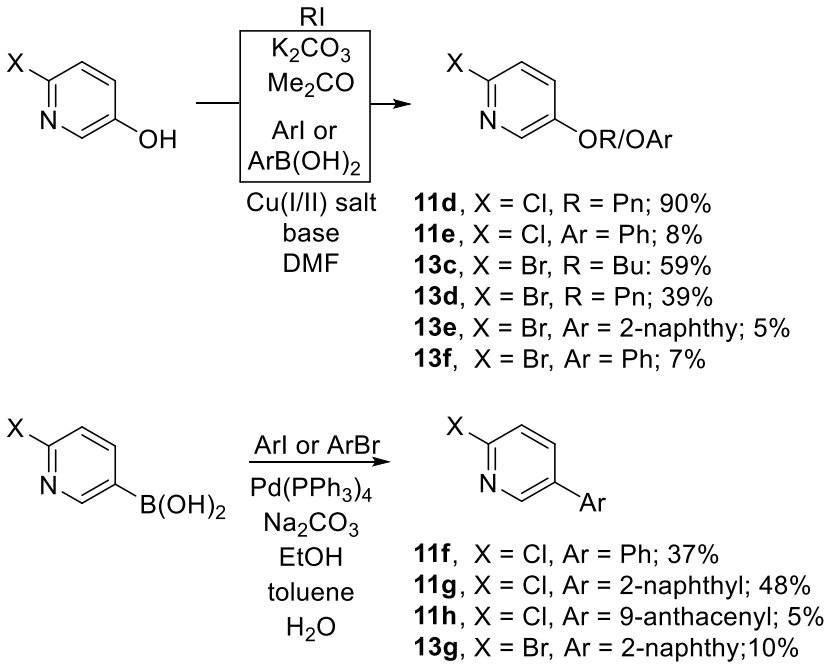

Figure 4. Methods used to prepare 5-substituted-2-halopyridines.

The quaternary salt **17** was prepared from commercially available 6-bromonaphthalen-2-amine. First, the Boc-protected derivative (**14**) was prepared to facilitate the synthesis of the boronic ester (**15**) through Pd-catalyzed borylation. Use of $Pd(PPh_3)_4$ plus KOAc in dioxane at 98 ºC for 72 h gave **15** as a clean product in high yield (81%). $Pd(PPh_3)_4$ was also effective for coupling **15** with 3-bromopyridine oxide to give crude **16**. Finally, immediate treatment of crude **16** with $(CF_3CO)_2O$ in the presence of DABCO gave **17**, which was then purified with HPLC.

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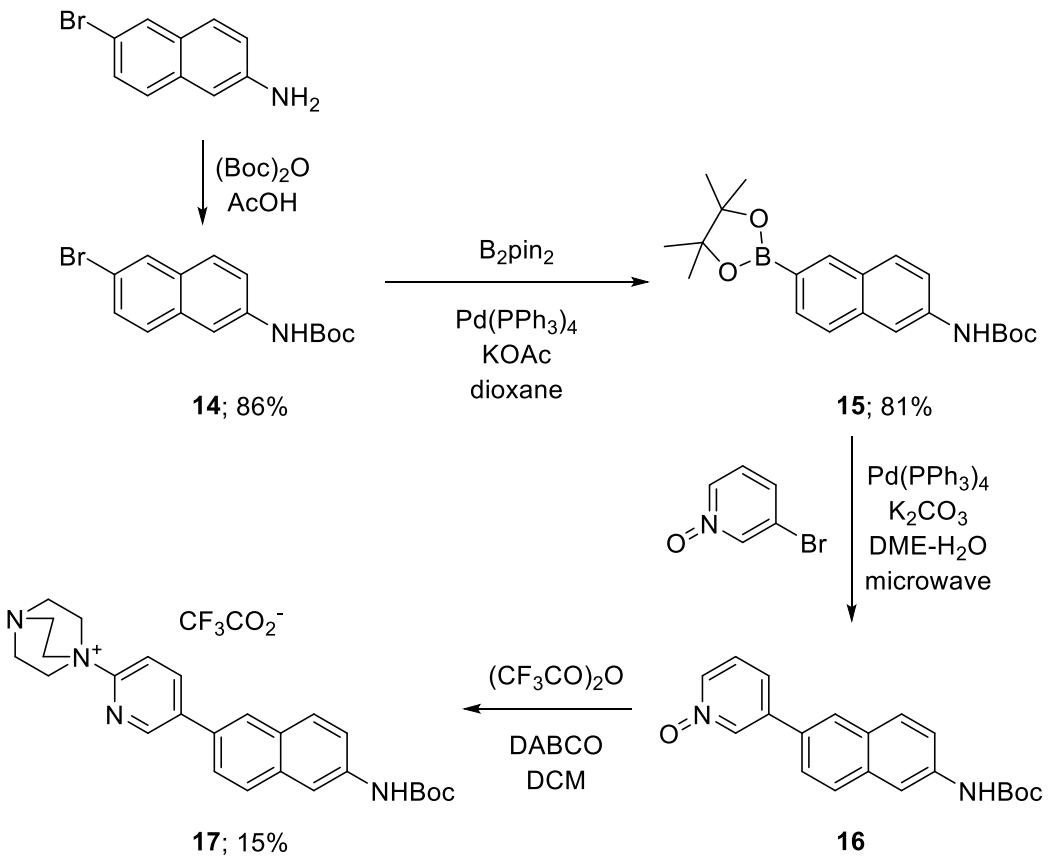


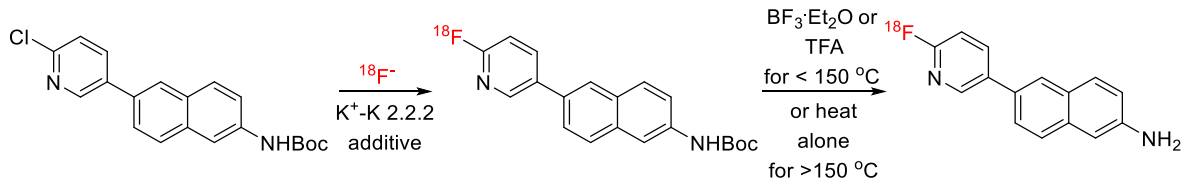
Figure 5. Synthesis of the quaternary ammonium salt, **17**.

Exploration of additives to increase radiolabeling efficiency. As part of our ongoing program to develop PET radiotracers for imaging neurofibrillary tangles,[30] we were initially interested in the radiofluorination of *tert*-butyl (6-(6-chloropyridin-3-yl)naphthalen-2-yl)carbamate (**7**) to produce [^{18}F]6-(6-fluoropyridin-3-yl)naphthalen-2-amine ([^{18}F]**6**) in two steps, namely radiofluorination with $^{18}F^-$-K^+-K 2.2.2 followed by removal of the Boc protecting group (Table 1). This labeling strategy gave no [^{18}F]**6** in DMF at 120 °C (entry 1) or in DMSO at 200 °C (entry 2) and very low yield in DMSO at 220 °C (entry 3). Consequently, we considered whether we might be able to promote the reaction with a suitable additive. We noted that DABCO (1,4-diazabicyclo[2.2.2.]octane) has been reported to catalyze the substitution of chloro leaving groups in heteroarenes by various non-halide nucleophiles as in, for example, the reaction of 2,6-

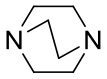
dichloronicotinate with phenols,[31] and the reactions of 2-chloropyrimidines with hydroxide or alkoxide ions.[32,33] A beneficial effect of DABCO on the preparation of ^{18}F-labeled trifluoroacetamides,[34] and the application of a DABCO-derived precursor for the preparation of 6-[^{18}F]fluoronicotinaldehyde,[35] have also been reported. We therefore decided to explore whether added DABCO might promote the radiofluorination of **7**.

No [^{18}F]**6** was obtained when DABCO was used as additive in solvents of quite similar polarity (E_t value), but somewhat lower dielectric constant (ε), such as DMF, *t*-BuOH, or NMP (Table 1, entries 4−6). DABCO altered the yield of [^{18}F]**6** in a concentration-dependent manner for reactions performed in DMSO (entries 7−10). An increase of DABCO concentration from 34 to 140 mM for reactions at 200 °C increased the yield from 9 to 43% (entries 7 and 8). However, further increases in DABCO concentration gave reduced yields (entries 9 and 10). The yield of [^{18}F]**6** was optimal when DABCO was in 8-fold molar ratio to precursor **7**. Dibasic DABCO gave better yield enhancement to monobasic quinuclidine at a higher concentration in DMSO at 180 °C for 30 min (entries 11 and 12). Because reactions were only successful in DMSO, this solvent was used throughout further experiments.

Table 1. Effect of additives on the yields of the radiofluorination of **7** [2.82 μmol (1 mg), in 0.7 mL solvent ≡ 4 mM] to give [^{18}F]**6**.



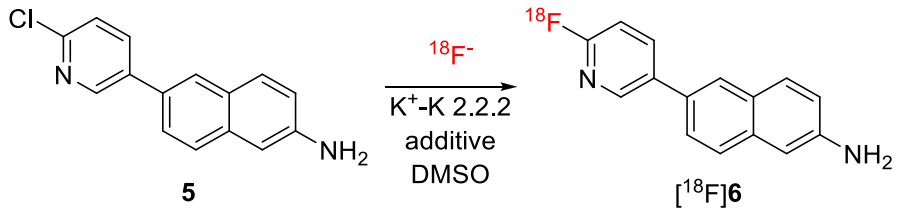
7 [^{18}F]**8** [^{18}F]**6**

Entry	Additive	Additive conc. (mM)	Solvent	Temp. (°C)	Time (min)	Yield of [^{18}F]6 (%)
1	None		DMF	120	30	0
2	None		DMSO	200	30	0
3	None		DMSO	220	30	8
4	 = DABCO	260	DMF	120	30	0
5	DABCO	140	*t*-BuOH	120	30	0

6	DABCO	260	NMP	200	15	0
7	DABCO	34	DMSO	200	15	9
8	DABCO	140	DMSO	200	15	43
9	DABCO	260	DMSO	200	15	33
10	DABCO	2500	DMSO	200	15	19
11	DABCO	130	DMSO	180	30	20
12	 = quinuclidine	300	DMSO	180	30	13

Tolerance of aryl amino groups. We noticed that the Boc protecting group was removed during the two-step radiosynthesis of [^{18}F]**6** when the reaction temperature was above 150 °C. The necessity for there to be a Boc protecting group in the precursor was therefore questionable. Consequently, we wondered if quinuclidine or DABCO would enhance radiofluorination yields even in the presence of a hydrogen bond donor such as a free aryl amino group. We tested the radiofluorination of the corresponding unprotected precursor **5** in the presence of additive (Table 2). Under similar conditions of temperature and time, dibasic DABCO gave about the same yield of [^{18}F]**6** as monobasic quinuclidine at a higher concentration (compare entries 2 and 1). The yield increased with the concentration of DABCO and with reaction temperature (entries 2 to 4), to 30% (entry 4) which was similar to that reached when precursor was Boc-protected (33%; Table 1, entry 9). Therefore, the Boc protecting group was deemed unnecessary in further experiments.

Table 2. Tolerance for hydrogen-donor group in the radiofluorination of **5** [1.0 mg (3.93 µmol) in 0.7 mL solvent] to give [^{18}F]**6**.



5 [^{18}F]**6**

Entry	Additive	Additive conc. (mM)	Temp. (°C)	Time (min)	Yield of [^{18}F]**6** (%)
1	Quinuclidine	200	120	30	14
2	DABCO	130	120	30	15

3	DABCO	160	150	30	28[a]
4	DABCO	250	200	15	30

[a] In this example, we isolated 925 MBq of [^{18}F]**6** with a molar activity of 78 GBq/μmol.

The preceding labeling experiments were performed in septum-sealed glass reaction vials. We have previously used a microfluidic apparatus (Nanotek; Advion) as a convenient platform for rapidly investigating single-step radiofluorination reactions with tight control of reaction temperature, time, and concentrations of reactants.[36] Further experiments were performed with this apparatus. So as to attain 30 min reaction time, we used a "stopped flow" procedure to conduct further investigations. In this apparatus, the single-step radiosynthesis of [^{18}F]**6** at 200 °C showed optimal yield at 100 mM concentration of quinuclidine (Figure 6). This pattern of yields versus additive concentration was similar to that from radiofluorinations of **7** in a conventional reaction vial (Table 1).

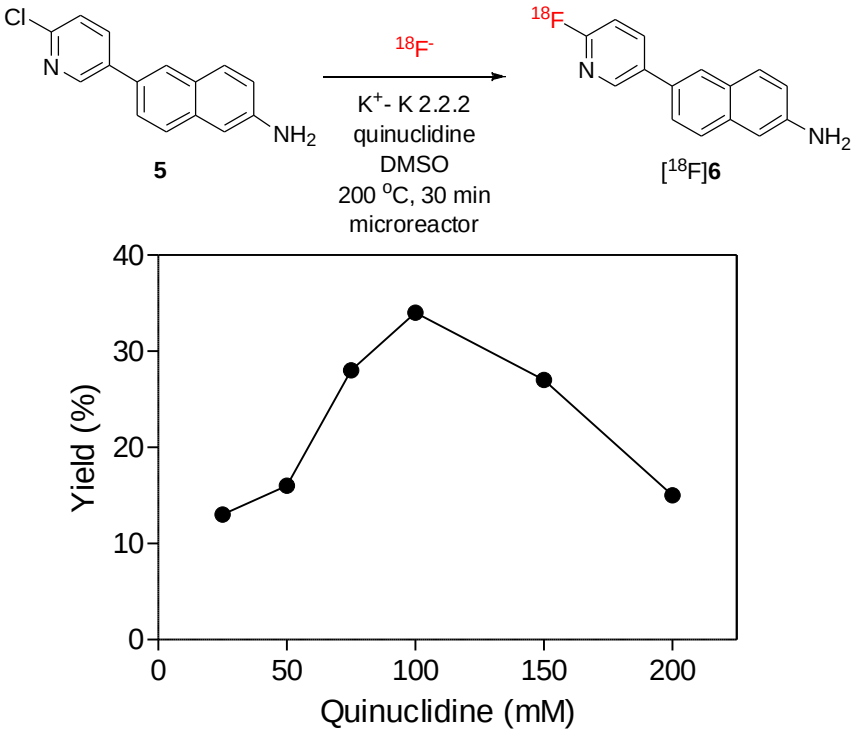


Figure 6. The effect of quinuclidine concentration on yield of [^{18}F]**6** at a precursor concentration of 8.7 mM at 200 °C in a Nanotek microfluidic apparatus.

The influences of added DABCO or quinuclidine at 100 mM concentration on yields of [^{18}F]**6** from **5** at three temperatures were further evaluated in the microfluidic apparatus (Figure 7). We were further interested in whether yield enhancement was more strongly related to additive basicity or nucleophilicity. Therefore, we examined other organic amines, namely triethylamine, a strong base but a weaker nucleophile than either DABCO or quinuclidine, and also DBU, DMAP and TMG, all weak nucleophiles but strong bases.[37] Generally, yields increased with nucleophilicity rather than the basicity of the added amine. At 200 °C, TEA slightly increased the radiofluorination yield (8% to 17%). No enhancement of yield was observed when DBU, DMAP or TMG was used. Thus, quinuclidine and DABCO were the most effective for promoting the labeling reactions.

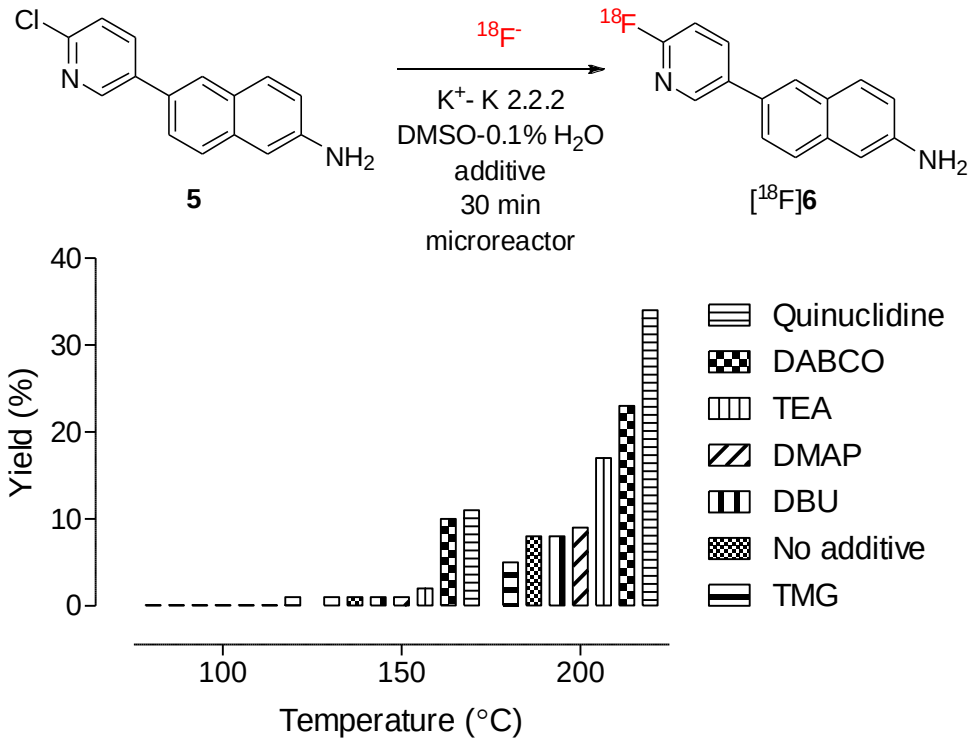


Figure 7. The effects of various organic bases at 100 mM concentration on the yield of [^{18}F]**6** at a precursor concentration of 8.7 mM at temperatures from 100 to 200 °C. (TEA = triethylamine; DMAP = *N*,*N*-dimethylpyridin-4-amine; DBU = 1,8-diazocyclo[5,4,0]undec-7-ene; TMG = 1,1,3,3-tetramethylguanidine).

As a further test of the tolerability of free aryl amino groups in the quinuclidine- or DABCO-promoted radiofluorinations of 2-chloro-heteroarenes, we performed similar experiments on 2-chloroquinolin-6-amine (**9**) in the microfluidic apparatus. The pattern of results (Figure 8), namely the beneficial effect of DABCO or quinuclidine at different temperatures, was like that obtained in the radiofluorination of **5** (Figure 7). Yields of [^{18}F]**10** were greater when either DABCO or quinuclidine was present and increased with temperature up to 200 °C, with the best yield reaching 41%. Thus, the free aryl amino group was well-tolerated in this reaction.

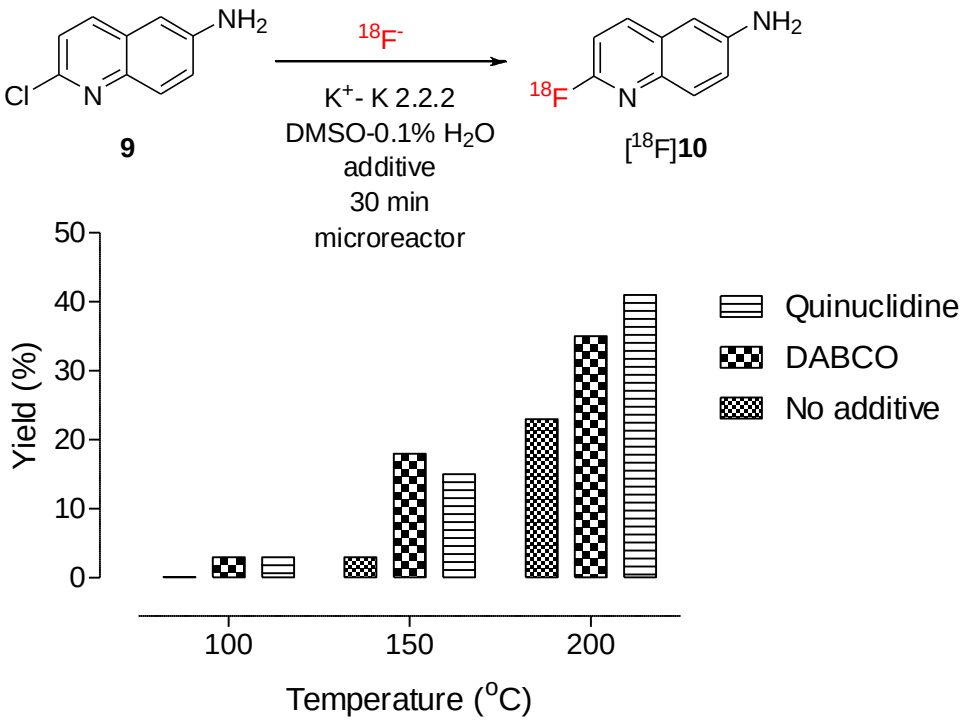


Figure 8. The effects of DABCO or quinuclidine at 100 mM concentration on the yield of [^{18}F]**10** at a precursor concentration of 8.7 mM and temperatures from 100 to 200 °C.

Radiofluorinations of other 5-substituted 2-chloropyridines. We evaluated the effects of the three nucleophilic organic bases, TEA, quinuclidine and DABCO, on the yields of reactions of [^{18}F]fluoride ion with two 5-substituted 2-chloro-pyridines, one with a 5-methyl substituent (**11a**) and another with a 5-methoxy substituent (**11b**). All three bases enhanced the yields of

[^{18}F]**12a** from **11a** at all three tested temperatures from 100 and 200 °C. Among the tested bases, quinuclidine performed best, giving strong yield enhancement, i.e. to 18% at 150 °C and 52% at 200 °C (Figure 9). Much weaker yield increase was seen with quinuclidine and DABCO for the radiofluorination of **11b** to give [^{18}F]**12b** (Figure 10). Yields were less than 10% even at 200 °C, showing that the 5-methoxy group was much more deactivating than the 5-methyl group.

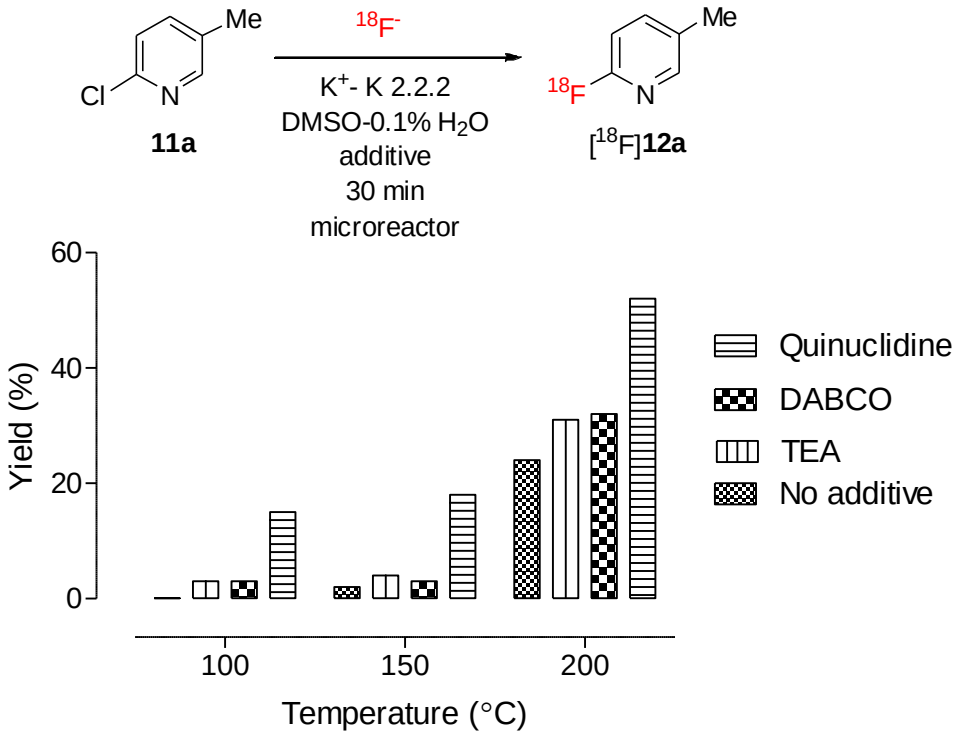


Figure 9. The effect of bases at 100 mM concentration on the yield of [^{18}F]**12a** at a precursor (**11a**) concentration of 8.7 mM and temperatures from 100 to 200 °C,

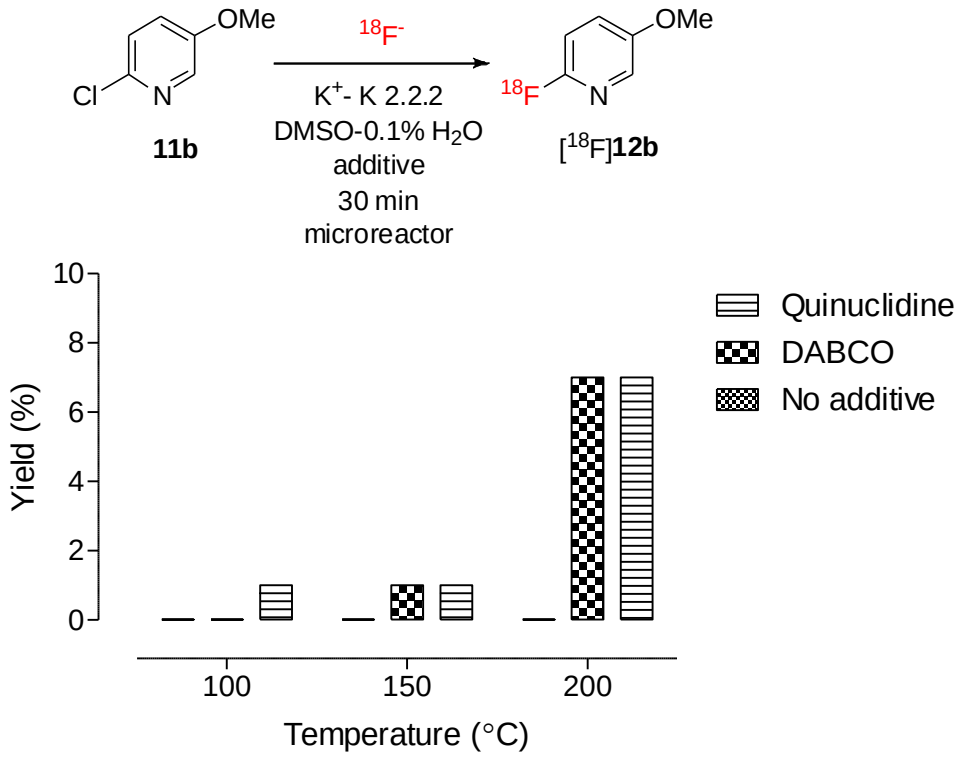


Figure 10. The effect of quinuclidine or DABCO at 100 mM concentration on the yield of [^{18}F]**12b** at a precursor (**11b**) concentration of 8.7 mM and temperatures from 100 to 200 °C.

In all preceding experiments, the effect of quinuclidine on the yield of [^{18}F]fluoropyridines was usually as good as or better than that of DABCO. Therefore, quinuclidine was used as the additive in subsequent experiments that were aimed at assessing the influence of substituents in the 5-position of the pyridyl ring. Specifically, we compared the effect of quinuclidine at 100 mM concentration on the radiofluorination yields of various 5-substituted 2-chloropyridines with and without additive at temperatures of 100, 150 and 200 °C (Figure 11). 2-Chloropyridine itself gave no yield with or without additive. In the absence of additive (Figure 11A), yields were close to zero at 100 °C for all tested substituents. Only 5-phenyl-2-chloropyridine gave a yield (54%) above 20% at 150 °C. At 200 °C, precursors that had the 5-substituent bonded through aliphatic carbon (R = Me: 24%) or aryl carbon (R = Ph: 80%, or 2-naphthyl: 54%) gave yields above 20%, except for the precursor having a 9-anthracenyl substituent (8%). Precursors that have stronger

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electron-donating substituents, such as methoxy, gave lower yields. These yields decreased in the order of the electron-donating ability of the substituent (OPh > OPn > OMe) and never exceed 20% at 200 °C. In the presence of quinuclidine (Figure 11B), yields increased at all temperatures, regardless of the nature of the substituent. For precursors bearing a 5-phenyl or 2-naphthyl substituent, the yields at 200 °C with additive were lower than control (70% vs. 80%, 50% vs. 54%), but the yields at 100 °C with additives were higher than control (67% vs 54%, 56% vs. 14%). Precursors with a 5-methyl or 9-anthracenyl substituent gave yields above 18% at both 150 (18% and 26%) and 200 °C (52% and 24%). Precursors with a 5-methoxy substituent gave enhanced yields (1% no additive vs. 7% with quinuclidine), but still below 20%, except that with a 5-phenoxy substituent at 200 °C (21% increased from 5% at 150 °C).

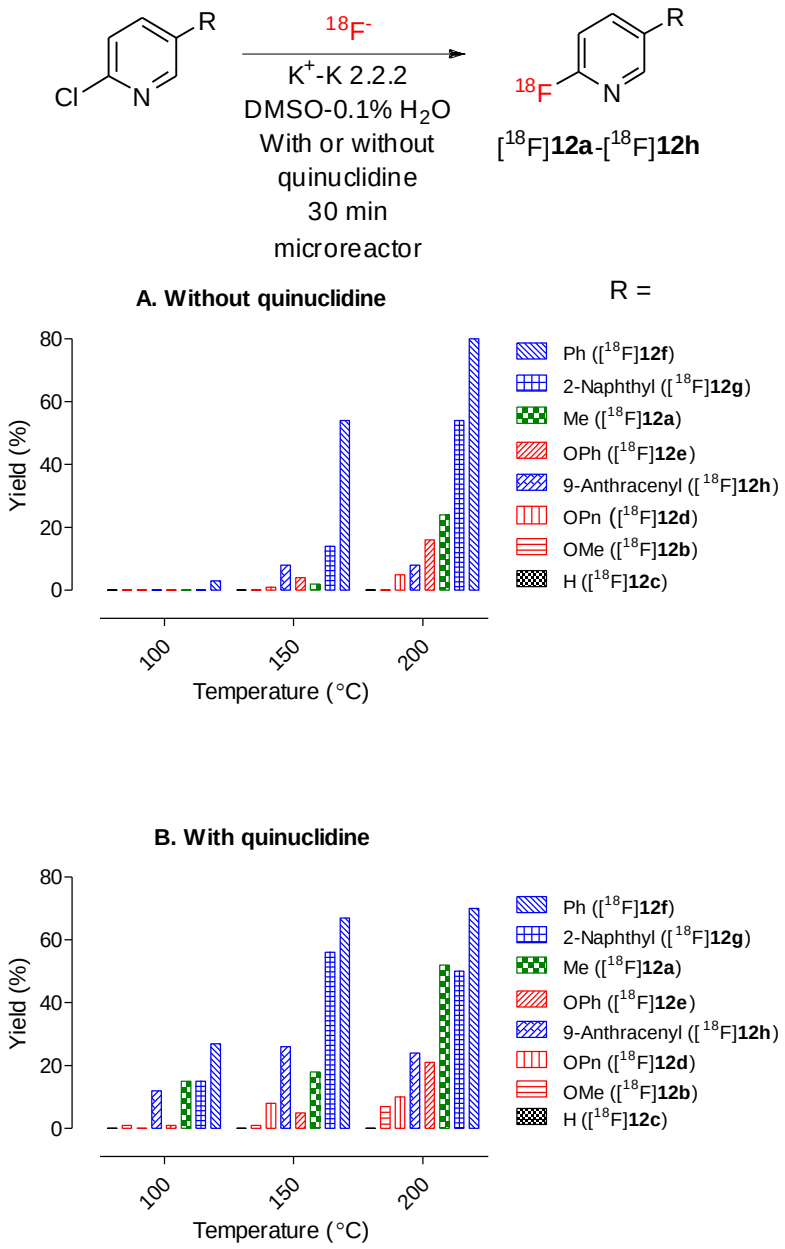


Figure 11. The effect of quinuclidine on the yields of 5-substituted [^{18}F]2-fluoropyridines from chloro precursors. Data colored green, blue and red are for precursors having the 5-subsituent bonded to the 2-chloropyridyl ring through aliphatic carbon, aryl carbon and oxygen, respectively.

Radiofluorinations of 5-substituted 2-bromopyridines. We also investigated the influence of halide leaving group on radiofluorination yields. Various 5-substituted 2-bromo-pyridines were evaluated with and without quinuclidine additive at temperatures of 100, 150 and 200 °C (Figure 12). In the absence of quinuclidine (Figure 12A), no radioactive products were

obtained at 100 °C. At 150 °C yields were slightly improved, but generally still low, except for [^{18}F]5(-(2-naphthyl))-2-fluoropyridine ([^{18}F]**12g**; 34%) which was higher than that from the corresponding chloro precursor (14%; Figure 11A). At 200 °C yields further improved. [^{18}F]2-Fluoropyridines bearing a 5-(*O*-2-naphthyl) ([^{18}F]**12i**), 5-(2-naphthyl) ([^{18}F]**12g**), 5-phenoxy ([^{18}F]**12e**) or 5-methyl ([^{18}F]**12a**) substituent gave yields of 58, 57, 44 and 19%, respectively. [^{18}F]2-Fluoropyridines bearing a 5-butoxy ([^{18}F]**12j**) or 5-pentoxy ([^{18}F]**12d**) substituent were obtained in extremely low yields. Quinuclidine greatly improved the yields (Figure 12B) for *O*-aryl and aryl substituents even at 100 and 150 °C, with the optimal temperature at 150 °C. *O*-alkyl substituted precursors gave low yields.

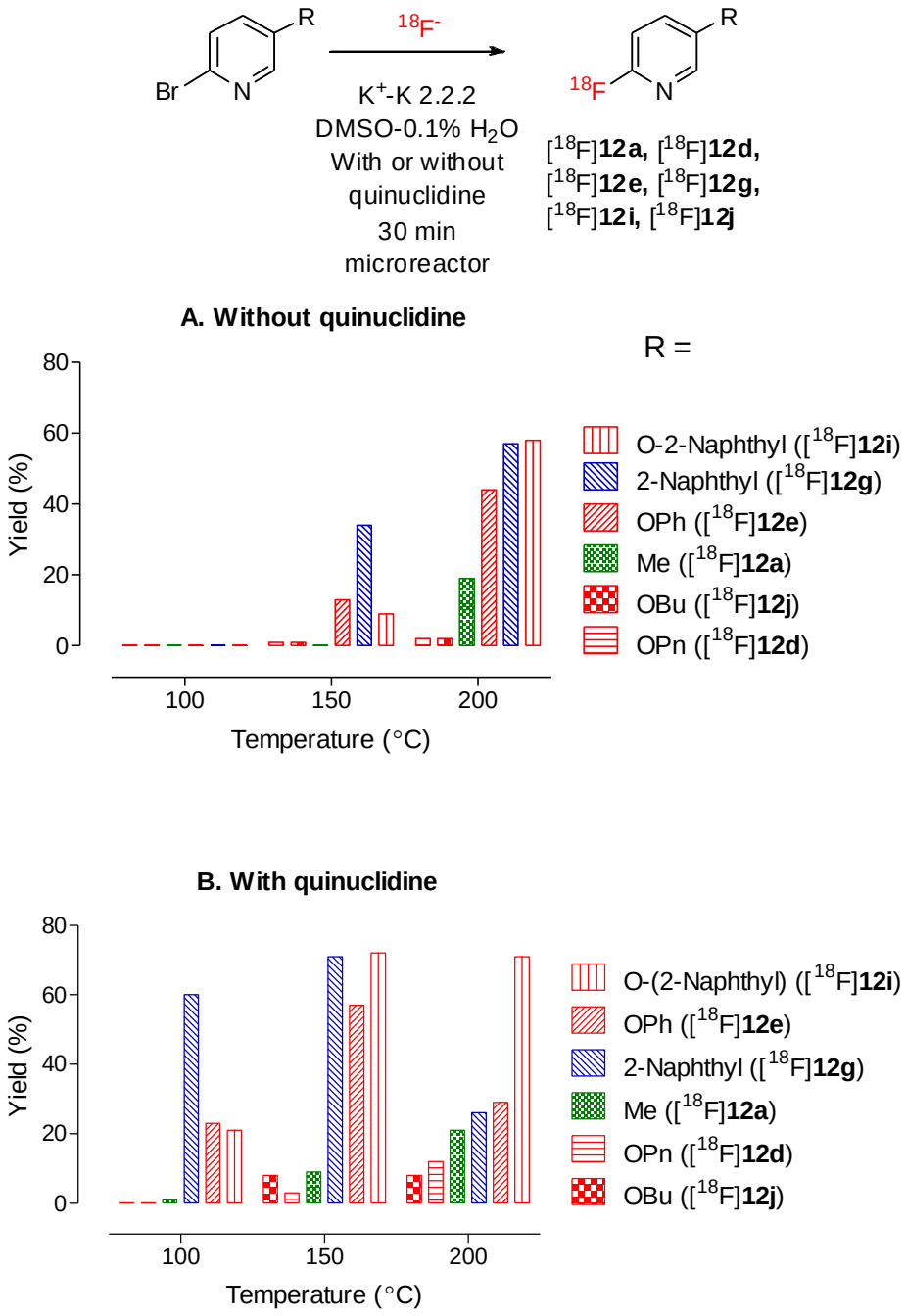


Figure 12. The effect of quinuclidine on the yields of 5-substituted [^{18}F]2-fluoro-pyridines from bromo-precursors. Data colored green, blue and red are for precursors having the 5-subsituent bonded to the 2-bromopyridyl ring through aliphatic carbon, aryl carbon and oxygen, respectively.

Mechanistic interpretation of quinuclidine- or DABCO-enhanced radiofluorinations of halo-pyridines. In this study we observed that: 1) the highly nucleophilic tertiary amine bases

quinuclidine and DABCO were the most effective for enhancing the radiofluorinations of 2-halopyridines; 2) triethylamine, a tertiary amine base with weaker nucleophilicity, was less effective; 3) weakly nucleophilic but very strong organic bases, such as DBU, were ineffective; 4) bromide was a better leaving group than chloride in the presence or absence of quinuclidine; 5) only a polar solvent with relatively high dielectric constant, such as DMSO, was suitable for the reaction in the presence of tertiary amine additive; 6) the enhancement of yield by DABCO or quinuclidine was concentration-dependent, first increasing with increasing concentration and then declining with further increase; and 7) in the presence of DABCO or quinuclidine the radiofluorination reactions tolerate free aryl amino groups in the precursor. These observations lead us to suggest that the tertiary amines, DABCO or quinuclidine, form a more reactive quaternary ammonium intermediate with a 2-halopyridine. A 2-fluoro substituent has been shown to be an effective leaving group in S_NAr reactions on pyridines.[38] Therefore, we also speculate that the radiofluorinated product may form the same quaternary ammonium intermediate reversibly. Such an intermediate must be formed in relatively low concentration, as we were unable to observe formation in situ by either NMR or HPLC, when precursor and DABCO were heated together in DMSO at temperatures up to 150 °C. Finally, the tolerance of the reactions to a free aryl amino group can perhaps be rationalized as being due to a strong charge interaction between the free [^{18}F]fluoride ion and the positively charged quaternary nitrogen at the expense of any weak interaction of the [^{18}F]fluoride ion with a free amino group.

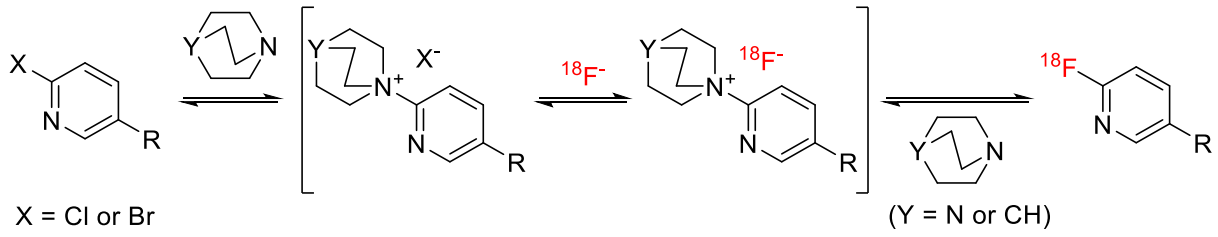
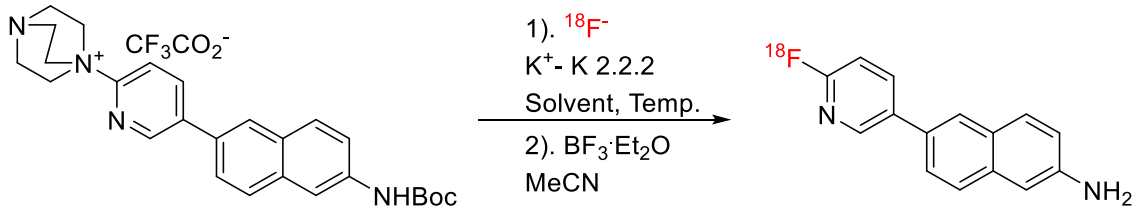


Figure 13. Proposed pathway for the DABCO or quinuclidine mediated radiofluorinations of 2-halopyridines.

To further probe our mechanistic interpretation, we made the quaternary ammonium salt, **17**, and explored its reactivity towards [^{18}F]fluoride ion. Radiofluorination of this salt in DMSO and Boc-deprotection gave a high yield of [^{18}F]**6** without any additional additive (Table 3, entry 1), supporting the possible importance of such quaternary intermediates in the quinuclidine- or DABCO-assisted radiofluorination reactions. Radiofluorination of **17** became possible in DMF (entry 2) but not in less polar acetonitrile (entry 3).

Table 3. Conversion of the quaternary ammonium salt **17** into [^{18}F]**6**.



17 [^{18}F]**6**

Entry	Solvent	Temp (°C)	Time (min)	Yield of [^{18}F]6 (%)
1	DMSO	120	10	36-58
2	DMF	120	10	25-45
3	MeCN	110	20	0

Conclusions

Various tertiary amines were evaluated as additives to enhance the radiofluorination of 5-substituted 2-halopyridines. DABCO and quinuclidine emerged as the most effective among those tested. These additives either increased the radiolabeling yields to more useful levels or lowered the temperatures needed for the radiolabeling reactions. Based on our observations, we suggest that the tertiary amine-enhanced reactions occur through a reversibly formed quaternary ammonium intermediate. A further useful feature of these reactions is their tolerance for free aryl amino groups.

Experimental

General

All solvents, bases, and reagents were purchased from Sigma-Aldrich (St. Louis, MO) or Combi-Blocks (San Diego, CA) and were used without further purification. Non-substituted and substituted 2-halopyridines (halo = F, Cl, or Br) were obtained commercially, except for those with synthesis described below. All reactions were performed under inert atmosphere in oven-dried glassware and monitored with TLC on silica layers (0.2 mm; Polygram® Sil G/UV254; Grace; Deerfield, IL) visualized under UV light (254 nm). Column chromatography was performed with a Combi*Flash*®R_f+ apparatus (Teledyne Isco; Lincoln, NE) on silica Redi*Sep*®R_f cartridges (Teledyne Isco) with gradient elution (EtOAc/hexanes, 2: 98 v/v → EtOAc/hexanes, 10: 90 v/v). ^{1}H- (400 MHz) and ^{13}C- (100 MHz) NMR spectra were recorded in deuterated solvents on an Avance 400 instrument (Bruker; Billerica, MA). Chemical shifts are given in parts per million (ppm) (δ) downfield from the signal for tetramethylsilane. All new compounds were analyzed with high resolution mass spectrometry (HRMS) at the Mass Spectrometry Laboratory, School of Chemical Sciences, University of Illinois (Urbana-Champaign, IL) with a Micromass Q-T of Ultima instrument for ESI (Waters Corp; Columbia, MD). Compound purities were determined with HPLC on a LC-20AD instrument (Shimadzu; Columbia, MD) equipped with a diode array detector (254 nm), and a Luna C18 column (5 µm; 100 Å; 4.6 × 250 mm; Phenomenex; Torrance, CA) eluted with MeCN: H_2O (9: 1 v/v) at 1 mL/min. The purity of all final compounds was ≥ 95%.

Chemistry

6-(6-Chloropyridin-3-yl)naphthalen-2-amine (5). 6-Bromonaphthalen-2-amine (1.1 g, 4.95 mmol), (6-chloropyridin-3-yl)boronic acid (1.0 g, 6.35 mmol), K_2CO_3 (1.2 g, 8.68 mmol), and $Pd(PPh_3)_4$ (0.54 g, 0.467 mmol), were dissolved in DME-H_2O (1: 1, v/v; 20 mL). The mixture was subjected to microwave irradiation (90°C20min150W250psi). HPLC analysis of an aliquot showed 90% conversion. Another portion of $Pd(PPh_3)_4$ (0.15 g, 0.130 mmol) was added, and the mixture was irradiated under the same conditions. HPLC analysis of an aliquot showed 95% conversion. The mixture was partitioned between $CHCl_3$ and H_2O (100 mL each), and the aqueous phase was extracted with $CHCl_3$ (100 mL × 2). The combined organic phase was dried ($MgSO_4$). After filtration, the solvent was removed. The residue was washed with EtOAc and the solid was dried under vacuum to give **5** (0.86 g; 67%). ^{1}H-NMR in DMSO-d_6: δ 8.80 (d, $^4J_{HH}$ = 2.5 Hz, 1H, Ar-H), 8.21 (dd, $^3J_{HH}$ = 8.4 Hz, $^4J_{HH}$ = 2.6 Hz, 1H, Ar-H), 8.05 (s, 1H, Ar-H), 7.69−7.58 (m, 4H, Ar-H), 6.98 (dd, $^3J_{HH}$ = 8.8 Hz, $^4J_{HH}$ = 2.2 Hz, 1H, Ar-H), 6.84 (d, $^4J_{HH}$ = 2.0 Hz, 1H, Ar-H), 5.56 (s, 2H, NH_2). HRMS calculated for $C_{15}H_{12}ClN_2$ $[M + H]^+$: m/z = 255.0689, found 255.0690. Error (ppm): 0.4.

6-(6-Fluoropyridin-3-yl)naphthalen-2-amine (6). 6-Bromonaphthalen-2-amine (100 mg, 0.450 mmol), (6-fluoropyridin-3-yl)boronic acid (98 mg, 0.695 mmol), K_2CO_3 (124 mg, 0.897 mmol), and $Pd(PPh_3)_4$ (31.2 mg, 0.0270 mmol) were dissolved in DME-H_2O (1: 1, v/v; 10 mL). The mixture was subjected to microwave irradiation (80°C40min80W250psi). The mixture was partitioned between $CHCl_3$ and H_2O (100 mL each), and the aqueous phase was extracted with $CHCl_3$ (100 mL × 2). The combined organic phase was dried ($MgSO_4$). After filtration, the solvent was removed. The residue was washed with EtOAc, and dried under vacuum to give **6** (91 mg; 85%). ^{1}H-NMR in DMSO-d_6: δ 8.59 (d, $^4J_{HH}$ = 2.5 Hz, 1H, Ar-H), 8.87 (dt, $^3J_{HF}$ = $^3J_{HH}$ = 8.3 Hz, $^4J_{HH}$ = 2.6 Hz, 1H, Ar-H), 8.01 (s, 1H, Ar-H), 7.67 (d, $^3J_{HH}$ = 8.8 Hz, 1H, Ar-H), 7.64 (ABd, $^3J_{HH}$

= 8.6 Hz, $^4J_{HH}$ = 1.8 Hz, 1H, Ar-H), 7.61 (AB, $^3J_{HH}$ = 8.6 Hz, 1H, Ar-H), 7.27 (dd, $^3J_{HH}$ = 8.5 Hz, $^4J_{HH}$ = 2.9 Hz, 1H, Ar-H), 6.98 (dd, $^3J_{HH}$ = 8.7 Hz, $^4J_{HH}$ = 2.2 Hz, 1H, Ar-H), 6.84 (d, $^4J_{HH}$ = 2.0 Hz, 1H, Ar-H), 5.53 (s, 2H, NH_2). $^{13}C\{^1H\}$-NMR in DMSO-d_6: δ 162.3 (d, $^1J_{CF}$ = 233.9 Hz, 1C, 2-Py), 147.5, 145.0 (d, $^3J_{CF}$ = 14.8 Hz, 1C, 6-Py), 140.0 (d, $^3J_{CF}$ = 7.9 Hz, 1C, 4-Py), 134.7, 129.3, 128.2, 126.5, 126.2, 125.7, 124.8, 119.1, 109.7 (d, $^2J_{CF}$ = 37.6 Hz, 1C, 3-Py), 105.6. HRMS calculated for $C_{15}H_{12}FN_2$ $[M + H]^+$: *m*/*z* = 239.0985, found 239.0984. Error (ppm): −0.4.

***tert*-Butyl (6-(6-chloropyridin-3-yl)naphthalen-2-yl)carbamate (7)**. Compound **5** (1.0 g; 3.93 mmol) and Boc_2O, (1.0 mL; *d* = 0.95 g/mL, 4.35 mmol) were dissolved in AcOH (3.0 mL). The mixture was stirred overnight. The mixture was partitioned between EtOAc and H_2O (100 mL each), and the aqueous phase was extracted with EtOAc (2 × 100 mL). The combined organic phase was dried ($MgSO_4$). After filtration, the solvent was removed. The residue was washed with EtOAc, and the solid was dried under vacuum, to give **7** (1.27 g; 91%). ^{1}H-NMR in DMSO-d_6: δ 9.66 (brs, 1H, NH), 8.87 (d, $^4J_{HH}$ = 2.1 Hz, 1H, Ar-H), 8.28 (dd, $^3J_{HH}$ = 8.6 Hz, $^4J_{HH}$ = 2.6 Hz, 1H, Ar-H), 8.22 (s, 1H, Ar-H), 8.16 (s, 1H, Ar-H), 7.91 (d, $^3J_{HH}$ = 8.6 Hz, 1H, Ar-H), 7.89 (d, $^3J_{HH}$ = 8.7 Hz, 1H, Ar-H), 7.82 (dd, $^3J_{HH}$ = 8.5 Hz, $^4J_{HH}$ = 1.5 Hz, 1H, Ar-H), 7.63 (d, $^3J_{HH}$ = 8.3 Hz, 1H, Ar-H), 7.55 (dd, $^3J_{HH}$ = 8.9 Hz, $^4J_{HH}$ = 1.8 Hz, 1H, Ar-H), 1.52 (s, 9H, CH_3). LC-MS, 355.0 ($[M+H]^+$, 100%), 357.1 ($[M+H]^+$, 32%), 299.2 ($[M-CH_2=C(CH_3)_2+H]^+$, 26%), 301.2 ($[M-CH_2=C(CH_3)_2+H]^+$, 9%), 255.3 ($[M-CH_2=C(CH_3)_2-CO_2+H]^+$, 22%), 257.3 ($[M-CH_2=C(CH_3)_2-CO_2+H]^+$, 6%); HRMS calculated for $C_{20}H_{20}ClN_2O_2$ $[M + H]^+$: *m*/*z* = 355.1213, found 355.1217. Error (ppm): 1.1.

***tert*-Butyl (6-(6-fluoropyridin-3-yl)naphthalen-2-yl)carbamate (8)**. Compound **6** (1.0 g; 3.93 mmol) and Boc_2O (1.0 mL; *d* = 0.95 g/mL, 4.35 mmol) were dissolved in AcOH (3.0 mL). The mixture was stirred overnight. The mixture was partitioned between EtOAc and H_2O (100

mL each), and the aqueous phase extracted with EtOAc (2 × 100 mL). The combined organic phase was dried ($MgSO_4$). After filtration, the solvent was removed. The residue was washed with EtOAc and dried under vacuum to give **8** (1.12 g; 78%). ^{1}H-NMR in DMSO-d_6: δ 9.67 (brs, 1H, NH), 8.67 (d, $^4J_{HH}$ = 2.1 Hz, 1H, Ar-H), 8.28 (dd, $^3J_{HH}$ = 8.2 Hz, $^4J_{HH}$ = 2.5 Hz, 1H, Ar-H), 8.18 (s, 1H, Ar-H), 8.16 (s, 1H, Ar-H), 7.89, 7.88 (AB, $^3J_{HH}$ = 8.5 Hz, 2H, Ar-H), 7.80 (dd, $^3J_{HH}$ = 8.6 Hz, $^4J_{HH}$ = 1.7 Hz, 1H, Ar-H), 7.54 (dd, $^3J_{HH}$ = 8.8 Hz, $^4J_{HH}$ = 2.0 Hz, 1H, Ar-H), 7.31 (dd, $^3J_{HH}$ = 8.5 Hz, $^4J_{HH}$ = 2.8 Hz, 1H, Ar-H), 1.52 (s, 9H, CH_3). ^{13}C{^{1}H}-NMR in DMSO-d_6: δ 162.6 (d, $^1J_{CF}$ = 234.3 Hz, 1C, 2-Py), 153.0, 145.5 (d, $^3J_{CF}$ = 15.0 Hz, 1C, 6-Py), 140.5 (d, $^3J_{CF}$ = 8.1 Hz, 1C, 4-Py), 137.9, 134.3, 133.2, 131.5, 129.4, 129.0, 128.2, 125.6, 125.3, 120.2, 113.2, 109.8 (d, $^2J_{CF}$ = 37.5 Hz, 1C, 3-Py), 79.6 (s, 1C, C-O), 28.3 (s, 3C, CH_3). HRMS calculated for $C_{20}H_{20}FN_2O_2$ $[M + H]^+$: m/z = 339.1509, found 339.1506. Error (ppm): −0.9.

2-Chloro-5-(pentyloxy)pyridine (11d). 2-Chloro-5-hydroxypyridine (500 mg, 3.9 mmol), 1-iodopentane (852 mg, 4.3 mmol), and K_2CO_3 (539 mg, 3.9 mmol) were refluxed in acetone (7 mL). After 16 h, the reaction mixture was cooled to rt, filtered, and the filtrate concentrated. The crude residue was diluted in DCM, evaporated onto silica gel, and purified with column chromatography to afford **11d** (703 mg; 90%). ^{1}H-NMR: δ 8.02–8.01 (m, 1H), 7.20–7.15 (m, 2H), 3.96 (t, J = 6.4 Hz, 2H), 1.78 (pentet, J = 6.4 Hz, 2H), 1.46–1.34 (m, 4H), 0.94 (t, J = 7.2 Hz, 3H); HRMS calculated for $C_{10}H_{14}ClNO$ $[M + H]^+$: m/z = 200.0837, found 200.0834. Error (ppm): 1.35.

2-Chloro-5-phenoxypyridine (11e). A mixture of iodobenzene (204 mg, 1.0 mmol), 2-chloro-5-hydroxypyridine (155 mg, 1.2 mmol), K_2CO_3 (166 mg, 1.2 mmol), and CuI (9.5 mg, 0.050 mmol) in DMF (1 mL) was stirred at rt for 30 min. The mixture was then transferred to a hot oil bath (110 °C). After 24 h, the reaction mixture was cooled to rt, diluted with EtOAc (3

mL), and filtered over celite. The reaction mixture was then concentrated under reduced pressure, diluted in DCM, evaporated onto silica gel, and purified with column chromatography to afford **11e** (16 mg; 8%). ^{1}H-NMR: δ 8.16 (t, J = 2.0 Hz, 1H), 7.40–7.36 (m, 2H), 7.27 (d, J = 2.0 Hz, 2H), 7.20–7.16 (m, 1H), 7.04–7.01 (m, 2H); HRMS calculated for $C_{11}H_9ClNO$ $[M + H]^+$: m/z = 206.0367, found 206.0363. Error (ppm): 2.39.

2-Chloro-5-phenylpyridine (11f). A solution of 6-chloro-3-pyridylboronic acid (448 mg, 2.85 mmol) in EtOH (1.5 mL) was added to a mixture of $Pd(PPh_3)_4$ (150 mg, 0.13 mmol), iodobenzene (526 mg, 2.58 mmol), aq. Na_2CO_3 (2 M; 2.85 mL), and toluene (5 mL). The reaction mixture was heated to 80 °C, stirred overnight, and cooled to rt. The reaction mixture was then partitioned between EtOAc (20 mL) and H_2O (10 mL), and the organic layer was collected, dried ($MgSO_4$), filtered, and evaporated to dryness under reduced pressure. The residue was diluted in DCM, evaporated onto silica gel, and purified with column chromatography to afford **11f** (180 mg; 37%). All characterization data agrees with the literature.[39]

2-Chloro-5-(naphthalen-2-yl)pyridine (11g). The reaction of 6-chloro-3-pyridylboronic acid (448 mg, 2.85 mmol) with 2-iodonaphthalene (656 mg, 2.58 mmol) gave **11g** (294 mg, 48%). ^{1}H-NMR: δ 8.73 (dd, J = 2.4, 0.4 Hz, 1H), 8.01 (d, J = 1.6 Hz, 1H), 7.98–7.94 (m, 2H), 7.93–7.87 (m, 2H), 7.66 (dd, J = 8.4, 2.0 Hz, 1H), 7.57–7.51 (m, 2H), 7.44 (dd, J = 8.4, 0.4 Hz, 1H); HRMS calculated for $C_{15}H_{10}ClN$ $[M + H]^+$: m/z = 240.0580, found 240.0582. Error (ppm): 0.8.

5-(Anthracen-9-yl)-2-chloropyridine (11h). The reaction of 6-chloro-3-pyridylboronic acid (448 mg, 2.85 mmol) with 9-bromoanthracene (663 mg, 2.58 mmol) gave **11h** (41 mg; 5%). ^{1}H-NMR: δ 8.53 (s, 1H), 8.46 (dd, J = 2.4, 0.4 Hz, 1H), 8.04 (d, J = 8.4 Hz, 2H), 7.71 (dd, J = 8.0, 2.4 Hz, 1H), 7.57–7.54 (m, 3H), 7.49–7.45 (m, 2H), 7.41–7.37 (m, 2H); HRMS calculated for $C_{19}H_{13}ClN$ $[M + H]^+$: m/z = 290.0731, found 290.0729. Error (ppm): 0.7.

2-Bromo-5-butoxypyridine (13c). Application of the conditions used for the synthesis of **11d** to 2-bromo-5-hydroxypyridine (679 mg, 3.9 mmol) and 1-iodobutane (791 mg, 4.3 mmol) gave **13c** (529 mg; 59%). ^{1}H-NMR: δ 8.03 (d, J = 3.2 Hz, 1H), 7.33 (d, J = 8.8 Hz, 1H), 7.08 (dd, J = 8.8, 3.2 Hz, 1H), 3.97 (t, J = 6.4 Hz, 2H), 1.76 (pentet, J = 6.4 Hz, 2H), 1.48 (sextet, J = 7.6 Hz, 2H), 0.99 (t, J = 7.2 Hz, 3H); HRMS calculated for $C_9H_{13}BrNO$ $[M + H]^+$: m/z = 230.0175, found 230.0172. Error (ppm): 1.32.

2-Bromo-5-(pentyloxy)pyridine (13d). Application of the conditions used for the synthesis of **11d** to 2-bromo-5-hydroxypyridine (679 mg, 3.9 mmol) and 1-iodopentane (852 mg, 4.3 mmol) gave **13d** (371 mg; 39%). ^{1}H-NMR: δ 8.02 (d, J = 3.2 Hz, 1H), 7.32 (d, J = 8.8 Hz, 1H), 7.08 (dd, J = 8.8, 3.2 Hz, 1H), 3.95 (t, J = 6.8 Hz, 2H), 1.78 (pentet, J = 6.4 Hz, 2H), 1.45–1.34 (m, 4H), 0.92 (t, J = 7.2 Hz, 3H); HRMS calculated for $C_{10}H_{15}BrNO$ $[M + H]^+$: m/z = 244.0332, found 244.0330. Error (ppm): 0.63.

2-Bromo-5-(naphthalen-2-yloxy)pyridine (13e). Application of the conditions used for the synthesis of **13d** to 2-iodonaphthalene (508 mg, 2.0 mmol), 2-bromo-5-hydroxypyridine (418 mg, 2.4 mmol), and CuI (20 mol%) gave **13e** (26 mg; 5%). ^{1}H-NMR: δ 8.39 (dd, J = 4.8, 1.6 Hz, 1H), 8.10–8.07 (m, 3H), 7.79–7.75 (m, 3H), 7.58–7.54 (m, 1H), 7.51–7.49 (m, 1H), 7.29–7.25 (m, 1H).

2-Bromo-5-phenoxypyridine (13f). Activated 4 Å molecular sieves (1.0 g), $Cu(OAc)_2$ (363 mg, 2.0 mmol), and trimethylamine (1.0 g, 10 mmol) were added sequentially to a solution of 2-bromo-5-hydroxypyridine (348 mg, 2.0 mmol), and phenylboronic acid (488 mg, 4.0 mmol) in DMF (10 mL). The reaction mixture was cooled to 0 °C (ice-bath) and stirred under air for 6 h. The reaction mixture was filtered over celite, diluted in EtOAc (30 mL), and washed with HCl (1 M; 10 mL × 2), water (10 mL), and brine (15 mL × 2). The organic layer was then dried ($MgSO_4$)

and concentrated under reduced pressure. The crude residue was diluted in DCM, evaporated onto silica gel, and purified with column chromatography to afford **13f** (29 mg; 7%). All characterization data agrees with the literature.[40]

2-Bromo-5-(naphthalen-2-yl)pyridine (13g). The reaction of 6-bromo-3-pyridylboronic acid (575 mg, 2.85 mmol) and 2-iodonaphthalene (656 mg, 2.58 mmol) gave **13g** (70 mg; 10%). All characterization data agree with the literature.[41]

***tert*-Butyl (6-bromonaphthalen-2-yl)carbamate (14)**. 6-Bromonaphthalen-2-amine (2.0 g, 9.01 mmol), $(Boc)_2O$ (2.7 mL, 11.8 mmol), and AcOH (10 mL) were mixed and stirred for 6 d. The mixture was partitioned between EtOAc and saline. The combined EtOAc phase was dried ($MgSO_4$). After removing solvent, the solid was washed with hexane (10 mL× 3). The red hexane solution was decanted, and the white solid was dried in a vacuum-oven to give **14** (2.5 g; 86%). ^{1}H-NMR in DMSO-d6: δ 9.65 (s, 1H, NH), 8.12 (s, 1H, Ar-H), 8.07 (d, $^4J_{HH}$ = 1.8 Hz, 1H, Ar-H), 7.79 (d, $^3J_{HH}$ = 8.9 Hz, 1H, Ar-H), 7.75 (d, $^3J_{HH}$ = 8.8 Hz, 1H, Ar-H), 7.53 (d, $^3J_{HH}$ = 8.6 Hz, $^3J_{HH}$ = 1.6 Hz, 2H, Ar-H), 1.50 (s, 9H, CH_3). HRMS calculated for $C_{15}H_{16}BrNO_2Na$ $[M + Na]^+$: m/z = 344.0262, found 344.0266. Error (ppm): 1.2.

***tert*-Butyl (6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)naphthalen-2-yl)carbamate (15)**. Compound **14**, 0.704 g (2.18 mmol), B_2pin_2, 0.872 g (3.43 mmol), $Pd(PPh_3)_4$, 0.101 g (0.0874 mmol), and KOAc, 2.34 g (23.8 mmol) were suspended in 1,4-dioxane (12 mL). The mixture was heated at 98 °C for 3 d. The mixture was partitioned between EtOAc and brine (100 mL each), and the aqueous phase was extracted with EtOAc (100 mL× 2). The combined organic phase was dried ($MgSO_4$). After filtration, the solvent was removed. The resulting oily product was dissolved in DCM (50 mL), and silica gel (20 mL) was added. After the solvent was removed, the product was purified by Combi-Flash chromatography using EtOAc increased from

0 to 100%, balanced by *n*-hexane. After the solvent was removed, the product was transferred into a vial and dried by Centrifan to give **15** (0.65 g; 81%). ^{1}H-NMR in $CDCl_3$: δ 8.25 (s, 1H, NH), 7.96 (s, 1H, Ar-H), 7.77 (d, $^3J_{HH}$ = 8.4 Hz, 1H, Ar-H), 7.71 (d, $^3J_{HH}$ = 8.2 Hz, 1H, Ar-H), 7.30 (dd, $^3J_{HH}$ = 8.8 Hz, $^4J_{HH}$ = 2.1 Hz, 1H, Ar-H), 6.64 (s, 1H, Ar-H), 1.53 (s, 9H, CH_3), 1.36 (s, 12H, CH_3). LC-MS, 531.4 (30%), 369.2 ([M + H]$^+$, 50%), 287.2 (30%), 277.2 (80%), 255.2 (100%), 187.1 (25%); HRMS calculated for $C_{21}H_{28}BNO_4Na$ [M + Na]$^+$: *m*/*z* = 391.2045, found 391.2050. Error (ppm): 1.3.

3-(6-((*t*-Butoxycarbonyl)amino)naphthalen-2-yl)pyridine 1-oxide (16). The boronic ester **15** (139.6 mg, 0.378 mmol), 3-bromopyridine 1-oxide (289.1 mg, 1.66 mmol), $Pd(PPh_3)_4$ (110.8 mg, 0.0959 mmol), K_2CO_3 (277.1 mg, 2.01 mmol) were suspended in DME-H_2O (4: 1; 5.0 mL). The reaction was subjected to microwave irradiation (100°C60min80W250psi). After the reaction was complete, the mixture became homogeneous. This was then partitioned between EtOAc and brine. The aqueous phase was extracted with EtOAc (100 mL× 2). The combined organic phase was dried ($MgSO_4$). After filtration, the solvent was removed. The residue was purified on Combi-Flash with MeOH (0 to 20%) balanced with EtOAc to give **16** as a white powder (183.1 mg), which was used without further purification.

1-(5-(6-((*t*-Butoxycarbonyl)amino)naphthalen-2-yl)pyridin-2-yl)-1,4-diazabicyclo[2.2.2]octan-1-ium trifluoroacetate (17). The crude pyridine oxide **16** (183.1 mg; 0.544 mmol) was dissolved in DCM (10 mL), and DABCO (404.4 mg, 3.60 mmol) was added. The mixture was stirred until it became homogeneous. $(CF_3CO)_2O$ (0.40 mL, *d* = 1.49 g/mL, 2.84 mmol) was added dropwise. The solution changed from colorless to pale yellow. The solution was stirred overnight. When all **16** had been consumed, as shown by HPLC, K_2CO_3 (1.1 g, 7.96 mmol) was added. The mixture was stirred for 0.5 h, and solid was filtered off. The solution was

added to diethyl ether (250 mL) with stirring. A white precipitate formed. The suspension stood overnight to form a clear solution with a transparent solid on the surface of the glassware. The solid was dissolved in the minimal amount of DMF and purified with HPLC, to give **17** (45.6 mg; 15%) after removing solvent on a rotary evaporator with a bath at < 40 °C. ^{1}H-NMR in CD_3OD: δ 9.00 (d, $^{4}J_{HH}$ = 2.2 Hz, 1H, Ar-H), 8.46 (dd, $^{3}J_{HH}$ = 8.7 Hz, $^{4}J_{HH}$ = 2.4 Hz, 1H, Ar-H), 8.16 (s, 1H, Ar-H), 8.09 (s, 1H, Ar-H), 7.91 (d, $^{3}J_{HH}$ = 8.6 Hz, 1H, Ar-H), 7.91 (s, 1H, Ar-H), 7.89 (s, 1H, Ar-H), 7.77 (dd, $^{3}J_{HH}$ = 8.5 Hz, $^{4}J_{HH}$ = 1.6 Hz, 1H, Ar-H), 7.55 (dd, $^{3}J_{HH}$ = 8.8 Hz, $^{4}J_{HH}$ = 2.0 Hz, 1H, Ar-H), 3.98 (t, $^{3}J_{HH}$ = 7.4 Hz, 6H, CH_2), 3.40 (t, $^{3}J_{HH}$ = 7.4 Hz, 6H, CH_2), 1.58 (s, 9H, CH_3). ^{13}C{^{1}H}-NMR in CD_3OD: δ 161.6 ($CF_3\underline{C}O_2^-$), 156.0, 155.4, 148.5, 140.7, 140.0, 139.7, 135.8, 132.2, 131.4, 130.4, 129.8, 127.8, 126.1, 121.7, 116.9, 115.2, 81.4 ($O\underline{C}(CH_3)_3$), 55.7 ($N\underline{C}H_2$), 46.7 ($N\underline{C}H_2$), 28.9 ($\underline{C}H_3$). HRMS calculated for $C_{26}H_{31}N_4O_2$ [ammonium cation]$^+$: *m*/*z* = 431.2447, found 431.2452. Error (ppm): 1.2.

Radiochemistry

General

All radiochemistry was performed in a lead-shielded hot-cell for personnel protection. Radioactivity was measured with a calibrated Atomlab 300 ionization calibrator (Biodex Medical Systems; Shirley, NY) and was corrected for background and decay. Radio-HPLC was performed with a Model 166 UV absorbance detector (Beckman Coulter; CA) coupled with a PMT flow count radioactivity detector (Bioscan, Washington DC). The radioactive compounds were eluted with a gradient of a binary solvent mixture (H_2O-MeCN) on a Luna C18 column (5 µm; 100 Å; 4.6 × 250 mm; Phenomenex; Torrance, CA). The mobile phase was initially composed of 35% MeCN, which was linearly increased to 95% MeCN over 5 min, and then maintained at this composition for the remainder of the run.

Production of [^{18}F]fluoride ion-K 2.2.2 reagent. No-carrier-added [^{18}F]fluoride ion was produced by proton irradiation of [^{18}O]water (95 atom%) with a beam (40 μA) of 16.5 MeV protons from a biomedical cyclotron (Petrace; GE). At the end of the irradiation (90 min), the [^{18}F]fluoride ion solution from the cyclotron target (~ 7.4 GBq; 300–450 μL) was added to a 5-mL V-vial containing K_2CO_3 (0.53 mg, 3.3 μmol) and K 2.2.2 (4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane; 3.0 mg, 7.3 μmol) in MeCN-H_2O (9: 1 v/v; 100 μL). The solution was then dried by four cycles of azeotropic evaporation with acetonitrile (0.45 mL) at 120 °C.

"Stopped flow" radiofluorinations within a microfluidic apparatus. A NanoTek microfluidic apparatus (Advion, Louisville, TN) was used to conduct experimental radiofluorinations. This apparatus and its use for such a purpose have been described previously.[36] The apparatus was cleaned by flushing the tubing and microreactor with DMSO before and after each experiment. A stock vial was prepared containing a solution (0.4 mL) of anhydrous [^{18}F]fluoride ion (1.9–5.6 GBq), K_2CO_3, and K 2.2.2 in DMSO (with 0.1% H_2O v/v), and another stock vial was prepared containing a solution (17.4 mM) of 2-halopyridine precursor plus any desired additive in DMSO. Each of these solutions was loaded into its respective reservoir loop on the microfluidic apparatus. Each radiofluorination reaction was performed by infusion of an equal volume of solution (15 μL) from each loop into the microreactor (31.7 μL internal volume). The flow from the apparatus was then stopped and the reaction mixture was then heated in the microreactor at a pre-set temperature for 30 min. DMSO was then used to sweep the reaction mixture from the microreactor into a collection vessel containing MeCN-H_2O (1: 1 v/v; 0.5 mL) at rt. The identity of the radiofluorinated product was verified with LC-MS of the carrier associated with the radioactive product after its HPLC separation. Decay-corrected yields were calculated as follows. The amount of radioactivity introduced into the Advion microreactor (A_1)

was calculated from the measured radioactivity concentration of the $^{18}F^{-}$-K^{+}-K 2.2.2 stock solution and the volume infused (15 μL). The total radioactivity swept out from the microreactor was measured (A_2). The peak areas of the radioactive product, unreacted [^{18}F]fluoride ion and other species in the radio-HPLC chromatograms were decay-corrected to give the percentage of product (A_3%). Both A_1 and A_2 were decay-corrected to the time of infusion. The yield was defined as ($A_2 \times A_3$%)/A_1.

Batch mode radiosyntheses of [^{18}F]6. *Method 1*. The protected precursor **7** (1.0 mg, 2.82 μmol) was used. NCA [^{18}F]fluoride ion (3.7–9.3 GBq in 0.25 mL of [^{18}O]H_2O) was added with K 2.2.2 (5 mg, 13.3 μmol) and K_2CO_3 (0.5 mg; 3.6 μmol) in MeCN-water (95: 5 v/v; 0.1 mL), and dried azeotropically with four addition-evaporation cycles of MeCN (2 mL) within a Tracerlab FX_{FN} automated synthesis unit (GE Healthcare Bio-Sciences, Pittsburgh, PA). The precursor and the additive(s) were loaded into a closed glass reaction vessel, and the anhydrous [^{18}F]fluoride ion-K^{+}-K 2.2.2 in solvent (0.7 mL) was added. The mixture was heated at a set temperature for a specific time (see Table 1 for solvent, temperature and time used). The solvent was removed with a stream of nitrogen gas, first at 90 °C, and then at 120 °C. If the reaction temperature was greater than 150 °C, no de-protection step was needed because the Boc group was removed during the reaction. For reactions below 150 °C, $BF_3 \cdot Et_2O$ (14 μL) in MeCN (0.7 mL) was added. After incubating the solution at 60 °C for 5 min, the solvent was removed by a stream of nitrogen gas at 60 °C for 5 min, until the solution was reduced to less than 0.1 mL. Subsequent work-up was as follows. Water (1.0 mL) was added to the residual solvent, and the radioactive product was purified with HPLC on an X-Bridge C-18 column (10 × 250 mm, 10 μm; Waters) eluted at 6.2 mL/min with a binary gradient of A (H_2O + 0.1% TFA) and B (MeOH + 0.1% TFA). The gradient was consecutively, B at 10% for 10 min, 10−20% for 5−10 min, 30−40% for 10−50 min, 40−90%

for 50−55 min, 90% for 55−75 min. Eluate was monitored for absorbance at 258 nm and for radioactivity with a γ-ray detector (Bioscan Flow Count fitted with a NaI(Tl) detector). The radioactive fraction with the same retention time as the respective reference ligand (t_R = 33.6 min) was collected.

Method 2. The unprotected precursor **5** (1.0 mg, 3.93 μmol) was used. The anhydrous [^{18}F]fluoride ion-K^+-K 2.2.2 reagent was prepared as described above. The precursor and the additive(s) were loaded into a closed glass reaction vessel, and the dried [^{18}F]fluoride ion in 0.7 mL solvent was added. The mixture was heated at a set temperature for a specific time (see Table 2 for solvent, temperature and time used). The solvent was removed by a stream of nitrogen gas, first at 90 °C, and then at 120 °C, until the volume was less than 0.1 mL. Subsequent work-up was as for Method 1.

Method 3. The quaternary ammonium salt precursor **17** (1.0 mg, 2.74 μmol) was used. The anhydrous [^{18}F]fluoride ion-K^+-K 2.2.2 reagent was prepared as described above. The precursor and the additive were loaded into a closed glass reaction vessel, and the anhydrous [^{18}F]fluoride ion reagent in 0.7 mL solvent was added. The mixture was heated at a set temperature for a specific time (see Table 3 for solvent, temperature and time used). The solvent was removed with a stream of nitrogen gas first at 90 °C then at 120 °C, until the volume was less than 0.1 mL. The Boc group was removed with added $BF_3 \cdot Et_2O$ (14 μL) in MeCN (0.7 mL). Subsequent work-up was the same as that for Method 1.

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Graphical abstract: