Development of Customized $[^{18}\text{F}]$ Fluoride Elution Techniques for the Enhancement of Copper-Mediated Late-Stage Radiofluorination

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1. Materials and Methods

Reagents and solvents were purchased and used without further purification unless otherwise noted. HPLC grade acetonitrile, HPLC grade methanol, anhydrous sodium sulfate, potassium chloride, potassium bromide, potassium iodide, potassium dihydrogen phosphate, potassium hydrogen phosphate, potassium acetate, potassium hydroxide, sodium bicarbonate, and potassium carbonate were purchased from Fisher Scientific. Anhydrous N,N-dimethylformamide (DMF) and 4-acetylphenylboronic acid, pinacol ester were purchased from Acros Organics. Potassium trifluoromethanesulfonate was purchased from Oakwood Scientific. Copper(II) trifluoromethanesulfonate (Cu(OTf)$_2$) and 4-acetylphenylboronic acid, along with all other nitrogenous weak bases and preconditioning salts were purchased from Sigma-Aldrich. Sterile product vials were purchased from Hollister-Stier. QMA-light Sep-Paks were purchased from Waters Corporation. QMA-light Sep-Paks were conditioned with sequential flushes with 10 mL of ethanol, followed by 10 mL of 0.5 M preconditioning solution (*vide infra*), and 10 mL of sterile water prior to use.

2. Evaluation of Eluents and Preconditioning Reagents

2.1 Preparation of aqueous [$^{18}$F]Fluoride. All loading operations were conducted under an ambient atmosphere. Argon was used as a pressurizing gas during automated sample transfers. Fluorine-18 was produced via the $^{18}$O(p,n)$^{18}$F nuclear reaction using a GE PETTrace cyclotron (40 μA beam for 2 min generated ca. 150 mCi of fluorine-18). Fluorine-18 was delivered to a TRACERLab FX$_{FN}$ automated radiochemistry synthesis module in a 1.5 mL bolus of [$^{18}$O]water and collected in a sterile vial. Aqueous fluorine-18 produced in this way was further diluted with Milli-Q water to c.a. 1-3 mCi/mL prior to use in fluorine-18 elution studies.

2.2 [$^{18}$F]Fluoride elution studies method. Waters QMA-light Sep-Paks were washed sequentially with 10 mL ethanol, 10 mL 0.5 M preconditioning agent in water, and 10 mL Milli-Q water. 0.5 mL of aqueous [$^{18}$F]fluoride was passed through a QMA cartridge followed by 2 mL air, and the activity of the QMA cartridge was determined with a Capintec® dose calibrator. [$^{18}$F]fluoride was then eluted from the QMA cartridge into a 4mL vial with 0.5 mL eluent solution, followed by 2 mL of air. Activity of the 4 mL vial (eluate) and QMA cartridge (residual
[¹⁸F]fluoride) were determined with a Capintec dose calibrator. Activity data was used to calculate % fluoride recovery.

\[
\%[^{18}F]\text{Fluoride Recovery} = \frac{\text{Eluate Activity}}{\text{Eluate Activity} + \text{Final QMA Activity}}
\]

2.3 Evaluation of Eluents and Preconditioning Reagents: Raw Data

2.3.1 [¹⁸F]Fluoride elution studies data: Copper Triflate as eluent

| CuOTf₂ concentration (M) | QMA initial | QMA final | eluate | ¹⁸F recovery (%) |
|--------------------------|-------------|-----------|--------|-----------------|
| 0.01                     | 409         | 311       | 90     | 22%             |
| 0.025                    | 460         | 66        | 373    | 85%             |
| 0.05                     | 447         | 20        | 415    | 95%             |
| 0.075                    | 432         | 20        | 388    | 95%             |
| 0.1                      | 334         | 10        | 317    | 97%             |
| 0.1 (MeCN)               | 430         | 317       | 107    | 25%             |
| 0.1 (MeOH)               | 421         | 220       | 203    | 48%             |

Table 2.3.1A: Elution of [¹⁸F]Fluoride with Cu(OTf)₂ after NaHCO₃ Preconditioning

| CuOTf₂ concentration (M) | QMA initial | QMA final | eluate | ¹⁸F recovery (%) |
|--------------------------|-------------|-----------|--------|-----------------|
| 0.01                     | 416         | 188       | 217    | 54%             |
| 0.025                    | 459         | 40        | 406    | 91%             |
| 0.05                     | 441         | 20        | 407    | 95%             |
| 0.075                    | 420         | 30        | 397    | 93%             |
| 0.1                      | 326         | 40        | 284    | 88%             |
| 0.1 (MeCN)               | 408         | 286       | 114    | 29%             |
| 0.1 (MeOH)               | 446         | 160       | 272    | 63%             |

Table 2.3.1B: Elution of [¹⁸F]Fluoride with Cu(OTf)₂ after KOTf Preconditioning
### Table 2.3.1C: Elution of $[^{18}\text{F}]$Fluoride with Cu(OTf)$_2$ after Na$_2$SO$_4$ Preconditioning

| CuOTf$_2$ concentration (M) | QMA initial | QMA final | eluate | $^{18}$F recovery (%) |
|-----------------------------|-------------|-----------|--------|-----------------------|
| 0.01                        | 447         | 99        | 330    | 77%                   |
| 0.025                       | 481         | 40        | 416    | 91%                   |
| 0.05                        | 436         | 30        | 396    | 93%                   |
| 0.075                       | 469         | 40        | 404    | 91%                   |
| 0.1                         | 353         | 20        | 315    | 94%                   |
| 0.1 (MeCN)                  | 419         | 311       | 110    | 26%                   |
| 0.1 (MeOH)                  | 440         | 209       | 229    | 52%                   |

### Table 2.3.2: Elution of $[^{18}\text{F}]$Fluoride with KOH after NaHCO$_3$, KOTf or Na$_2$SO$_4$ Preconditioning

#### 2.3.2 $[^{18}\text{F}]$Fluoride elution studies data: KOH as eluent

| KOH concentration (M) | QMA initial | QMA final | eluate | $^{18}$F recovery (%) |
|-----------------------|-------------|-----------|--------|-----------------------|
| Preconditioning Agent= 0.5M NaHCO$_3$ |
| 0.02                  | 534         | 75        | 451    | 84%                   |
| 0.01                  | 579         | 123       | 433    | 75%                   |
| 0.0075                | 556         | 349       | 190    | 34%                   |
| 0.005                 | 547         | 423       | 116    | 21%                   |

| KOH concentration (M) | QMA initial | QMA final | eluate | $^{18}$F recovery (%) |
|-----------------------|-------------|-----------|--------|-----------------------|
| Preconditioning Agent= 0.5M Na$_2$SO$_4$ |
| 0.02                  | 830         | 67        | 739    | 89%                   |
| 0.01                  | 824         | 163       | 640    | 78%                   |
| 0.0075                | 780         | 162       | 612    | 78%                   |
| 0.005                 | 780         | 300       | 484    | 62%                   |

| KOH concentration (M) | QMA initial | QMA final | eluate | $^{18}$F recovery (%) |
|-----------------------|-------------|-----------|--------|-----------------------|
| Preconditioning Agent= 0.5M KOTf |
| 0.02                  | 546         | 375       | 169    | 31%                   |
| 0.01                  | 600         | 418       | 171    | 29%                   |
| 0.0075                | 625         | 605       | 22     | 4%                    |
| 0.005                 | 591         | 592       | 0      | 0%                    |
2.3.3 \[^{18}\text{F}]\)Fluoride elution studies data: Preconditioning studies with KOH as Eluent

| Preconditioning Agent (as 0.5M aqueous solution) | Activity in microcuries (µCi) | Eluent = 0.02M KOH |
|--------------------------------------------------|-------------------------------|-------------------|
|                                                  | QMA initial | QMA final | eluate | $^{18}\text{F}$ recovery (%) |
| NH$_4$HCO$_3$                                    | 1089        | 250       | 820    | 77%                       |
| NEt$_4$HCO$_3$                                   | 1160        | 282       | 830    | 75%                       |
| Na$_2$CO$_3$                                     | 1120        | 85        | 1035   | 92%                       |
| K$_2$CO$_3$                                      | 1138        | 70        | 1030   | 94%                       |
| KOH                                             | 1290        | 343       | 900    | 72%                       |
| KCl                                             | 1185        | 522       | 629    | 55%                       |
| KBr                                             | 1260        | 395       | 821    | 68%                       |
| KI                                              | 1160        | 249       | 895    | 78%                       |
| KH$_2$PO$_4$                                     | 2340        | 730       | 1498   | 67%                       |
| K$_2$HPO$_4$                                     | 1170        | 70        | 1085   | 94%                       |
| NaHCO$_2$                                       | 1175        | 822       | 340    | 29%                       |
| KOAc                                            | 1086        | 900       | 170    | 16%                       |
| PyridiniumOTs                                    | 1347        | 448       | 925    | 67%                       |

| Preconditioning Agent (as 0.5M aqueous solution) | Activity in microcuries (µCi) | Eluent = 0.0075M KOH |
|--------------------------------------------------|-------------------------------|-------------------|
|                                                  | QMA initial | QMA final | eluate | $^{18}\text{F}$ recovery (%) |
| NH$_4$HCO$_3$                                    | 1040        | 885       | 160    | 15%                       |
| NEt$_4$HCO$_3$                                   | 1200        | 1100      | 85     | 7%                        |
| Na$_2$CO$_3$                                     | 1070        | 190       | 875    | 82%                       |
| K$_2$CO$_3$                                      | 1135        | 235       | 840    | 78%                       |
| KOH                                             | 960         | 298       | 635    | 68%                       |
| KCl                                             | 1110        | 1000      | 70     | 7%                        |
| KBr                                             | 1020        | 950       | 55     | 5%                        |
| KI                                              | 1100        | 890       | 209    | 19%                       |
| KH$_2$PO$_4$                                     | 1100        | 1030      | 69     | 6%                        |
| K$_2$HPO$_4$                                     | 1142        | 365       | 760    | 68%                       |
| NaHCO$_2$                                       | 1157        | 1115      | 17     | 2%                        |
| KOAc                                            | 1096        | 1076      | 30     | 3%                        |
| PyridiniumOTs                                    | 1090        | 766       | 300    | 28%                       |

**Table 2.3.3:** Elution of \[^{18}\text{F}]\)fluoride with KOH after Different QMA Preconditioning Agents
2.3.4 [¹⁸F]Fluoride elution studies data: Preconditioning studies with copper elution

We tested the impact of preconditioning agent on [¹⁸F]fluoride recovery with aqueous Cu(OTf)₂, to see if this effect was unique for basic eluents. The results were less conclusive, as Cu(OTf)₂ led to very high % [¹⁸F]fluoride recovery at most concentrations tested (within 0.025-0.1 M), but it was again observed that Na₂SO₄ led to greater % [¹⁸F]fluoride recovery at the lowest concentration in water (0.01 M). Interestingly, a different preconditioning trend was observed with the poorer-performing methanolic and acetonitrile elution, where KOTf preconditioning promoted greater % [¹⁸F]fluoride recovery than either NaHCO₃ or Na₂SO₄. This may be due to its less ionic character in an organic medium and/or lower solubility of these salts in organic solvents.

| Entry | salt      | eluent solvent | concentration (M) | ¹⁸F recovery (%) |
|-------|-----------|----------------|-------------------|------------------|
|       |           |                |                   | KOTf | NaHCO₃ | Na₂SO₄ |
| 1     | Cu(OTf)₂  | water          | 0.01              | 54   | 22    | 77    |
| 2     | Cu(OTf)₂  | water          | 0.025             | 91   | 85    | 91    |
| 3     | Cu(OTf)₂  | water          | 0.05              | 95   | 95    | 93    |
| 4     | Cu(OTf)₂  | water          | 0.075             | 93   | 95    | 91    |
| 5     | Cu(OTf)₂  | water          | 0.1               | 88   | 97    | 94    |
| 6     | Cu(OTf)₂  | methanol       | 0.1               | 63   | 48    | 52    |
| 7     | Cu(OTf)₂  | acetonitrile   | 0.1               | 29   | 25    | 26    |

Table 2.3.4: Elution of [¹⁸F]fluoride with Cu(OTf)₂ after Different QMA Preconditioning Agents
2.3.5 \[^{18}\text{F}]\text{Fluoride elution studies data: Weak bases as eluents}

| Eluent       | Activity in microcuries (µCi) | Preconditioning Agent= 0.5M NaHCO\(_3\) |
|--------------|-------------------------------|------------------------------------------|
|              | QMA initial       | QMA final       | eluate | \(^{18}\text{F}\) recovery (%) |
| pyridine     | 519              | 526             | 0       | 0%                             |
| aniline      | 870              | 890             | 0       | 0%                             |
| imidazole    | 820              | 810             | 0       | 0%                             |
| morpholine   | 830              | 493             | 291     | 35%                            |
| DABCO        | 820              | 366             | 430     | 52%                            |
| kryptofix    | 748              | 108             | 630     | 84%                            |
| ammonia      | 719              | 210             | 493     | 69%                            |
| ethanolamine | 749              | 197             | 521     | 70%                            |
| methylamine  | 720              | 89              | 610     | 85%                            |
| diethylamine | 740              | 65              | 654     | 88%                            |
| triethylamine| 660              | 80              | 563     | 85%                            |
| DIPEA        | 663              | 71              | 584     | 88%                            |
| DBU          | 634              | 15              | 592     | 93%                            |
| 4OMe pyridine| 615              | 595             | 12      | 2%                             |
| lutidine     | 584              | 580             | 7       | 1%                             |
| collidine    | 577              | 534             | 28      | 5%                             |
| DMAP         | 621              | 149             | 453     | 73%                            |

**Table 2.3.5A:** Elution of \[^{18}\text{F}]\text{Fluoride with Weak Bases after NaHCO\(_3\) Preconditioning}

| Eluent       | Activity in microcuries (µCi) | Preconditioning Agent= 0.5M KOTf |
|--------------|-------------------------------|----------------------------------|
|              | QMA initial       | QMA final       | eluate | \(^{18}\text{F}\) recovery (%) |
| pyridine     | 518              | 516             | 0       | 0%                             |
| aniline      | 830              | 810             | 0       | 0%                             |
| imidazole    | 820              | 840             | 0       | 0%                             |
| morpholine   | 798              | 738             | 50      | 6%                             |
| DABCO        | 810              | 725             | 70      | 9%                             |
| kryptofix    | 752              | 461             | 290     | 39%                            |
| ammonia      | 690              | 475             | 203     | 29%                            |
| ethanolamine | 690              | 490             | 205     | 30%                            |
| methylamine  | 735              | 254             | 440     | 60%                            |
| diethylamine | 691              | 123             | 523     | 76%                            |
| triethylamine| 663              | 208             | 458     | 69%                            |
| DIPEA        | 654              | 211             | 436     | 67%                            |
| DBU          | 662              | 27              | 622     | 94%                            |
| 4OMe pyridine| 582              | 579             | 2       | 0%                             |
| lutidine     | 670              | 684             | 0       | 0%                             |
| collidine    | 601              | 593             | 8       | 1%                             |
| DMAP         | 592              | 390             | 205     | 35%                            |

**Table 2.3.5B:** Elution of \[^{18}\text{F}]\text{Fluoride with Weak Bases after KOTf Preconditioning}
| Eluent                 | Activity in microcuries (μCi) | Preconditioning Agent= 0.5M Na₂SO₄ | ¹⁸F recovery (%) |
|-----------------------|-------------------------------|-------------------------------------|-----------------|
|                       | QMA initial | QMA final | eluate |                  |
| pyridine              | 868          | 820       | 30     | 3%               |
| aniline               | 960          | 940       | 20     | 2%               |
| imidazole             | 970          | 497       | 438    | 45%              |
| morpholine            | 910          | 280       | 599    | 66%              |
| DABCO                 | 900          | 265       | 633    | 70%              |
| kryptofix             | 970          | 132       | 810    | 84%              |
| ammonia               | 880          | 168       | 678    | 77%              |
| ethanolamine          | 890          | 104       | 768    | 86%              |
| methylamine           | 850          | 98        | 730    | 86%              |
| diethylamine          | 820          | 73        | 741    | 90%              |
| triethylamine         | 880          | 87        | 792    | 90%              |
| DIPEA                 | 842          | 69        | 758    | 90%              |
| DBU                   | 840          | 30        | 803    | 96%              |
| 4OMe pyridine         | 960          | 713       | 210    | 22%              |
| lutidine              | 900          | 719       | 182    | 20%              |
| collidine             | 940          | 245       | 687    | 73%              |
| DMAP                  | 791          | 134       | 637    | 81%              |

**Table 2.3.5C:** Elution of [¹⁸F]Fluoride with Weak Bases after Na₂SO₄ Preconditioning
2.3.6 [¹⁸F]fluoride elution studies data: 4-OMe Pyridine and Pyridine elution optimization studies

| Preconditioning Agent (as 0.5M aqueous solution) | Activity in microcuries (µCi) | Eluent = 0.1M 4-OMe Pyridine | QMA initial | QMA final | eluate | ¹⁸F recovery (%) |
|------------------------------------------------|------------------------------|------------------------------|-------------|----------|--------|-----------------|
| NaHCO₃                                         |                              |                              | 534         | 521      | 0      | 0%              |
| KOTf                                           |                              |                              | 518         | 517      | 0      | 0%              |
| Na₂CO₃                                         |                              |                              | 495         | 487      | 0      | 0%              |
| K₂CO₃                                         |                              |                              | 491         | 498      | 0      | 0%              |
| K₂HPO₄                                         |                              |                              | 465         | 351      | 108    | 24%             |
| Na₂SO₄                                         |                              |                              | 493         | 253      | 238    | 48%             |

| Preconditioning Agent (as 0.5M aqueous solution) | Activity in microcuries (µCi) | Eluent = 0.1M Pyridine | QMA initial | QMA final | eluate | ¹⁸F recovery (%) |
|------------------------------------------------|------------------------------|------------------------|-------------|----------|--------|-----------------|
| NaHCO₃                                         |                              |                         | 519         | 526      | 0      | 0%              |
| KOTf                                           |                              |                         | 518         | 516      | 0      | 0%              |
| Na₂CO₃                                         |                              |                         | 504         | 500      | 0      | 0%              |
| K₂CO₃                                         |                              |                         | 514         | 495      | 0      | 0%              |
| K₂HPO₄                                         |                              |                         | 502         | 480      | 20     | 4%              |
| Na₂SO₄                                         |                              |                         | 498         | 407      | 70     | 15%             |

| 4-OMe pyridine conc. (M) | Activity in microcuries (µCi) | Preconditioning Agent: 0.5M Na₂SO₄ | QMA initial | QMA final | eluate | ¹⁸F recovery (%) |
|--------------------------|------------------------------|---------------------------------|-------------|----------|--------|-----------------|
| 0.1                      |                              |                                 | 493         | 253      | 238    | 48%             |
| 0.25                     |                              |                                 | 466         | 204      | 245    | 55%             |
| 0.5                      |                              |                                 | 445         | 205      | 232    | 53%             |
| 0.75                     |                              |                                 | 425         | 172      | 245    | 59%             |
| 1                        |                              |                                 | 429         | 139      | 270    | 66%             |

| pyridine conc. (M) | Activity in microcuries (µCi) | Preconditioning Agent: 0.5M Na₂SO₄ | QMA initial | QMA final | eluate | ¹⁸F recovery (%) |
|-------------------|------------------------------|---------------------------------|-------------|----------|--------|-----------------|
| 0.1               |                              |                                 | 498         | 407      | 70     | 15%             |
| 0.25              |                              |                                 | 417         | 323      | 99     | 23%             |
| 0.5               |                              |                                 | 445         | 355      | 88     | 20%             |
| 0.75              |                              |                                 | 449         | 343      | 91     | 21%             |
| 1                 |                              |                                 | 434         | 228      | 195    | 46%             |

Table 2.3.6: Elution of [¹⁸F]Fluoride with Pyridine or 4-OMe-Pyridine
3. Synthesis of $[^{18}\text{F}]-4$-fluoroacetophenone via manual reactions

3.1 Preparation of $[^{18}\text{F}]$fluoride solution for manual syntheses. All loading operations were conducted under an ambient atmosphere. Argon was used as a pressurizing gas during automated sample transfers. Potassium $[^{18}\text{F}]$fluoride ($[^{18}\text{F}]$KF), was prepared using a TRACERLab FX$_{FN}$ automated radiochemistry synthesis module (General Electric, GE). Fluorine-18 was produced via the $^{18}\text{O}(p,n)^{18}\text{F}$ nuclear reaction using a GE PETTrace cyclotron (40 μA beam for 2 min generated ca. 150 mCi of fluorine-18). Fluorine-18 was delivered to the synthesis module in a 1.5 mL bolus of $[^{18}\text{O}]$water and trapped on a QMA-light Sep-Pak (as $[^{18}\text{F}]$fluoride) to remove $[^{18}\text{O}]$water and other impurities. This was followed by elution of $[^{18}\text{F}]$fluoride into the reactor, followed by the addition of acetonitrile with/without phase transfer reagents (see below). Azeotrophic drying was achieved by heating the reaction vessel to 100 °C and drawing vacuum for 6 min. The reaction vessel was then subjected to a stream of Ar and simultaneous vacuum draw for an additional 6 min at 100 °C. Following azeotrophic drying, 6 mL of DMF was added to the dried reagent, and heated at 100 °C with stirring for 5 min. The resulting stock solution was cooled to 50 °C and transferred to a sterile vial for subsequent use in reactions. A 2 min bombardment typically led to a final stock activity of ~36-72 (6-12 mCi/mL) for the preparations below corresponding to 24-48% transfer efficiency based on starting activity (~150 mCi), with losses likely related to the efficiency of dissolving all of the $[^{18}\text{F}]$fluoride and transferring it out of the synthesis module. The amounts of $[^{18}\text{F}]$fluoride obtained were adequate for conducting the reactions described herein, although there is still scope for further optimization.

$[^{18}\text{F}]$KF/K$_2$CO$_3$/K$_{2.2.2}$: $[^{18}\text{F}]$Fluoride was eluted into the reaction vessel using 500 μL of 7mg/mL aqueous K$_2$CO$_3$. K$_{2.2.2}$ (15 mg) in MeCN (1 mL) was added to the reaction vessel, and the resulting solution was dried by azeotropic distillation to provide dry $[^{18}\text{F}]$KF/K$_2$CO$_3$/K$_{2.2.2}$.

$[^{18}\text{F}]$KF/KOTf: $[^{18}\text{F}]$Fluoride was eluted into the reaction vessel using 500 μL of 10mg/mL aqueous KOTf. MeCN (1 mL) was added to the reaction vessel, and the resulting solution was dried by azeotropic distillation to provide dry $[^{18}\text{F}]$KF/KOTf.

$[^{18}\text{F}]$DMAPH$^+$/F$^-$: $[^{18}\text{F}]$Fluoride was eluted into the reaction vessel using 500 μL of 0.1 M aqueous dimethylaminopyridine (DMAP). MeCN (1 mL) was added to the reaction vessel, and the resulting solution was dried by azeotropic distillation to provide dry $[^{18}\text{F}]$DMAPH$^+$/F$^-$. 
3.2 Manual Synthesis Method

Stock solutions of acetylphenylboronic acid, pinacol ester (APBpin) or other Bpin-appended precursor, copper (II) trifluoromethanesulfonate (Cu(OTf)$_2$), dimethylaminopyridine (DMAP), and/or other additive(s) in DMF were prepared immediately prior to the start of the reaction. Aliquots of these solutions were used to carry out subsequent [$^{18}$F]fluorination reactions. In a typical reaction, 660 µL anhydrous DMF was mixed with 40 µL Cu(OTf)$_2$ solution (8 µmol, 0.16 equiv, 200 mM), 100 µL Bpin solution (50 µmol, 1 equiv, 500mM) and 100 µL DMAP solution (50 µmol, 1 equiv, 500 mM) in a colorless borosilicate 4 mL scintillation vial. The reaction vial was sealed under ambient atmosphere with a PTFE/Silicone septum cap, and 100 µL of [$^{18}$F]fluoride stock (150-500 µCi) was added to the reaction vial through the septum via syringe. Additional anhydrous DMF was added (as required) to bring the total solution volume to 1000 µL. The vial was then heated in an aluminum block (Chemglass Part# CG-1991-04) without stirring at 110 ºC for 20 min. After 20 min, the reaction was allowed to cool to room temperature.

3.3 Radiolabeling Optimization Screens

4-acetylphenylboronic acid, pinacol ester (APBpin) was used as the [$^{18}$F]-fluorination substrate for all chemistry optimization screens. The reaction scheme, as well as accompanying tables in each subsection describes the reaction conditions employed; with bold typeface in the reaction scheme denoting the variable tested in each case. All reactant values are expressed in µmol quantities for brevity and simplicity. Red typeface denotes the [$^{18}$F]fluoride source used.
S3.3.1 Reagent Loading Screen

Stock solutions of 4-acetylphenylboronic acid, pinacol ester (APBpin, 100 µmol/mL), Cu(OTf)$_2$ (200 µmol/mL) and DMAP (500 µmol/mL) in anhydrous DMF were prepared 15-30 min prior to the start of the reaction(s). Appropriate volumes of the reagent solutions were added to colorless borosilicate 4 mL scintillation vials via disposable pipette to obtain the desired reagent quantity/ratios (see Table 3.3.1 below for exact reagent quantities), and additional anhydrous DMF was added to bring the total volume in the vials to 900 µL. The reaction vials were sealed under ambient atmosphere with a PTFE/Silicone septum cap, and 100 µL of [$^{18}$F]KF/KOTf DMF stock (approx. 500 µCi) was added to the reaction vial through the septum via syringe. The vial was then heated in an aluminum block (Chemglass Part# CG-1991-04) without stirring at 110 ºC for 20 min. The reaction was then allowed to cool, and once the reaction mixture was sufficiently cool to handle, Radio-TLC analysis was conducted to determine radiochemical conversion (RCC in %). Crude reaction mixture was spotted onto standard silica coated glass plates and developed with 1:1 hexane/ethyl acetate in a glass TLC chamber. The RCC was determined by dividing the integrated area under the fluorinated product spot by the total integrated area of all peaks on the TLC plate.
Reagent loading screen

Chart S3.3.1: Reagent loading screen

| Sample #     | 1      | 2   | 3    | 4    | 5    | 6    |
|--------------|--------|-----|------|------|------|------|
| DMAP µmol    | 50     | 50  | 50   | 50   | 50   | 50   |
| APBpin µmol  | 2.5    | 5   | 10   | 15   | 25   | 50   |
| Cu(OTf)$_2$ 4 µmol | 8.2%  | 14.4% | 26.0% | 34.0% | 43.1% | 45.6% |
| Cu(OTf)$_2$ 8 µmol | 4.8%  | 8.9%  | 16.5% | 25.8% | 39.1% | 57.9% |
| Cu(OTf)$_2$ 20 µmol | 4.0%  | 7.6%  | 12.8% | 22.1% | 42.9% | 52.0% |
| Cu(OTf)$_2$ 50 µmol | 3.0%  | 6.1%  | 13.8% | 19.5% | 23.0% | 37.6% |

Table 3.3.1: Reagent loading screen
S3.3.2 Order of Addition Screen (Manual)

Stock solutions of 4-acetylphenylboronic acid, pinacol ester (APBpin, 100 μmol/mL), Cu(OTf)$_2$ (200 μmol/mL) and DMAP (500 μmol/mL) in anhydrous DMF were prepared 60 min prior to the start of the reaction(s). Sixteen colorless borosilicate 4 mL scintillation vials were charged with 260 μL anhydrous DMF via pipette. Aliquots of APBpin (50 μmol, 500 μL), Cu(OTf)$_2$ (8 μmol, 40 μL), DMAP (50 μmol, 100 μL), and/or $[^{18}\text{F}]\text{KF}/\text{KOTf}$ DMF stock (approx. 500 μCi, 100 μL) were then added via pipette, with each vial containing a different mixture of reagents (i.e., specific reagents were added to some vials, but not others; there are coincidentally sixteen different reagent mixture variations ($2^4$) that can be made from four different reagents). Table 3.3.2 shows which reagents were added at this stage of the reaction and which were not ($\text{X} = \text{added}; \text{O} = \text{not added}$). Following reagent addition, the reaction vials were sealed under ambient atmosphere with a PTFE/Silicone septum cap and heated in an aluminum block (Chemglass Part# CG-1991-04) without stirring at 110 °C for 20 min. The reaction was allowed to cool to room temperature and the reagents not added previously were added to the cooled reaction solution (i.e., all vials now contained equivalent aliquots of all four reagents). The vials were re-sealed under ambient atmosphere with a PTFE/Silicone septum cap and heated again in an aluminum block (Chemglass Part# CG-1991-04) without stirring at 110 °C for 20 min. After this second round of heating, the reaction was allowed to cool, and once the reaction mixture was sufficiently cool to handle, Radio-TLC analysis was conducted to determine radiochemical conversion (RCC in %). Crude reaction mixture was spotted onto standard silica coated glass plates and developed with 1:1 hexane/ethyl acetate in a glass TLC chamber. The RCC was determined by dividing the integrated area under the fluorinated product spot by the total integrated area of all peaks on the TLC plate.
**Order of Addition screen (Manual)**

8 μmol Cu(OTf)$_2$

50 μmol DMAP

KOTf/K$^{18}$F

1 mL DMF total

step 1) X reagents added

20min, 110 °C

step 2) O reagents added

20min, 110 °C

(see table below for X and O)

---

**Procedure:**

step 1) Added X; stirred @ 110C for 20 min; cooled;

step 2) added O; stirred @ 110C for 20 min; cooled; radio TLC

|                  | DMAP | Cu(OTf)$_2$ | APBpin | [F$^{18}$]KF/KOTf | RCC (%) |
|------------------|------|-------------|---------|-------------------|---------|
|                  | X    | X           | X       | O                 | 59.0%   |
|                  | O    | X           | O       | O                 | 4.2%    |
|                  | X    | X           | O       | O                 | 65.5%   |
|                  | X    | O           | O       | O                 | 40.4%   |
|                  | O    | O           | O       | O                 | 11.9%   |
|                  | X    | X           | X       | X                 | 63.1%   |
|                  | O    | X           | X       | X                 | 54.3%   |
|                  | X    | X           | X       | X                 | 63.7%   |

|                  | DMAP | Cu(OTf)$_2$ | APBpin | [F$^{18}$]KF/KOTf | RCC (%) |
|------------------|------|-------------|---------|-------------------|---------|
|                  | X    | X           | X       | O                 | 4.2%    |
|                  | O    | X           | O       | O                 | 4.0%    |
|                  | X    | X           | O       | O                 | 67.2%   |
|                  | X    | O           | O       | O                 | 45.2%   |
|                  | O    | O           | O       | O                 | 23.8%   |
|                  | X    | X           | X       | X                 | 62.6%   |
|                  | O    | O           | O       | O                 | 60.4%   |
|                  | X    | X           | X       | X                 | 57.8%   |

**Table 3.3.2:** Order of Addition screen (Manual)
S3.3.3 Order of Addition Screen (Automated)

We conducted automated optimization and order of addition studies using a TRACERLab FX<sub>FN</sub> synthesis module. As the radiochemical yield of a radiolabeling reaction is dependent on both recovery during [<sup>18</sup>F]fluoride elution and the reaction RCC, three preconditioning strategies were tested separately due to their different [<sup>18</sup>F]fluoride recovery yields, but unknown effects on RCC (Table 3.3.3, entries 1-3). The highest RCC (5%) was observed with NaHCO<sub>3</sub> preconditioning (entry 1). In addition to low RCC, KOTf preconditioning (entry 2) also led to unacceptably low [<sup>18</sup>F]fluoride recovery and hence was not explored further. Given the potential improvements offered by QMA preconditioning with Na<sub>2</sub>SO<sub>4</sub>, we also tested this possibility (entry 3). Unfortunately, in this case, there was no product formation, likely due to deactivation of the catalyst via the coordination of SO<sub>4</sub><sup>2-</sup> to Cu<sup>2+</sup>. This result was not entirely unexpected, as it is in line with our previous findings that CuSO<sub>4</sub> is an inadequate catalyst for this chemistry (see main manuscript). This finding demonstrates the need to carefully consider fluoride processing when designing and/or optimizing late-stage fluorination approaches.

The order of reagent addition was then re-evaluated to examine if it impacts the reactivity. Order of addition was examined for both the manual reactions (see Section 3.3.2 above) and automated reactions performed in the TRACERLab FX<sub>FN</sub> synthesis module, using NaHCO<sub>3</sub> as the preconditioning agent. Anhydrous [<sup>18</sup>F]fluoride was pre-dissolved in either Cu(OTf)<sub>2</sub> or arylBpin DMF solutions (110 °C, 5 min) prior to addition of the other reagents and radiolabeling (140 °C, 20 min). Pre-dissolving [<sup>18</sup>F]fluoride with the Cu(OTf)<sub>2</sub> solution again led to low RCCs of 2-4% (Table 3.3.3, entries 4 and 5), but pre-dissolving [<sup>18</sup>F]fluoride with arylBpin 1 led to an RCC of 19% (Table 3.3.3, entry 6). Conducting this reaction using the optimized reagent ratio (see Figure 2b of the main manuscript) further increased the RCC to 38% and 45%, when the reaction was carried out at 140 °C and 110 °C, respectively (Table 3.3.3, entries 7 and 8). Interestingly when conducting manual reactions, pre-mixing and heating Cu(OTf)<sub>2</sub>, substrate, and DMAP prior to the addition of [<sup>18</sup>F]fluoride also led to significant yield suppression, while simultaneous mixing of all the reactants with [18F]fluoride stock solution did not (see Section 3.3.2 above), suggesting that dissolution of [18F]fluoride is a critical step in this synthesis, and must occur prior to the addition of [18F]fluoride or substrate to Cu(OTf)<sub>2</sub>. Mechanistic studies into these effects are currently underway.
| Entry | Reaction conditions | Precond. agent | Reaction temp (°C) | Target Water Activity (mCi) | Final Dose Activity (mCi) | RCC (%) |
|-------|---------------------|----------------|-------------------|-----------------------------|---------------------------|---------|
| 1     | $^{18}$F mixed with all reactants | NaHCO$_3$ | 110 | 120 | 12 | 5 |
| 2     | $^{18}$F mixed with all reactants | KOTf | 140 | 150 | 12 | 2 |
| 3     | $^{18}$F mixed with all reactants | Na$_2$SO$_4$ | 140 | 150 | 37 | 0 |
| 4     | $^{18}$F mixed with CuOTf$_2$  | NaHCO$_3$ | 1: 110 | 2: 140 | 160 | 41 | 2 |
|       | 2: Precursor, DMAP added    |               |                   |                             |                           |         |
| 5     | $^{18}$F mixed with CuOTf$_2$  | NaHCO$_3$ | 1: 110 | 2: 140 | 150 | 38 | 4 |
|       | 2: Precursor added          |               |                   |                             |                           |         |
| 6     | $^{18}$F mixed with Precursor | NaHCO$_3$ | 1: 110 | 2: 140 | 150 | 39 | 19 |
|       | 2: CuOTf$_2$, DMAP added    |               |                   |                             |                           |         |

**Unoptimized conditions**$^{(a)}$

**Optimized conditions**$^{(b)}$

| Entry | Reaction conditions | Precond. agent | Reaction temp (°C) | Target Water Activity (mCi) | Final Dose Activity (mCi) | RCC (%) |
|-------|---------------------|----------------|-------------------|-----------------------------|---------------------------|---------|
| 7     | $^{18}$F mixed with Precursor | NaHCO$_3$ | 1: 110 | 2: 140 | 150 | 42 | 38 |
|       | 2: CuOTf$_2$, DMAP added    |               |                   |                             |                           |         |
| 8     | $^{18}$F mixed with Precursor | NaHCO$_3$ | 1: 110 | 2: 110 | 150 | 47 | 45 |

### Table 3.3.3: Automated synthesis results using aqueous dimethylaminopyridine as the eluent and order of addition as a variable. (a) **Unoptimized conditions**: 4-dimethylaminopyridine (Eluent + 50 µmol, 2.5 equiv, 100 mM) copper (II) trifluoromethanesulfonate (20 µmol, 2 equiv, 20 mM), and 4-acetylphenylboronic acid, pinacol ester (40 µmol, 2 equiv, 40 mM) in a total volume of 1 mL anhydrous dimethylformamide; (b) **Optimized conditions**: 4-dimethylaminopyridine (Eluent + 50 µmol, 1 equiv, 100 mM), copper (II) trifluoromethanesulfonate (8 µmol, 0.16 equiv, 16 mM), 4-acetylphenylboronic acid, pinacol ester (50 µmol, 1 equiv, 100 mM) in a total volume of 1 mL anhydrous dimethylformamide. (Step 1 = 5 min; Step 2 = 20 min).
3.4 Substrate Scope

Radiofluorination of several Bpin substrates was conducted as described in Section 3.2, however overall reagent loading was halved (see figure below). RCC was determined using radioTLC (in 1:1 hexanes: ethyl acetate eluent) and identity was determined using HPLC (see Section 6 for raw data).

![Reaction Scheme]

**Chart S3.4: Substrate Screen**

| Substrate (R =) | 4-acetyl (2) | 2-methyl ester (4) | 4-cyano (5) | 4-nitro (6) | 3-F,4-amino (7) | 5-Indole (8) |
|-----------------|--------------|--------------------|-------------|-------------|----------------|--------------|
| 52.3            | 51.8         | 42.4               | 19.9        | 1.3         | 31.0           |              |
| 41.1            | 70.1         | 66.2               | 48.9        | 2.1         | 28.5           |              |
| 34.6            | 34.3         | 30.2               | 14.7        | 1.2         | 24.7           |              |
| Mean            | 43           | 52                 | 46          | 28          | 1.5            | 28           |
| SD              | 9            | 18                 | 18          | 18          | 0.5            | 3           |

**Table 3.4: Substrate Screen**
4. Synthesis of $[^{18}\text{F}]$fluoroacetophenone in a TRACERlab FX$_{FN}$ Synthesis Module.

4.1 General Considerations: Fluorine-18 was produced via the $^{18}\text{O}(p,\text{n})^{18}\text{F}$ nuclear reaction using a GE PETTrace cyclotron (40 μA beam for 2 min generated ca. 150 mCi of fluorine-18 as measured by synthesis module detector). The $[^{18}\text{F}]$fluoride was delivered to a TRACERLab FX$_{FN}$ automated radiochemistry synthesis module in a 1.5 mL bolus of $[^{18}\text{O}]$water and collected in a sterile vial. Fluorine-18 containing target water was then reprocessed and used for radiofluorination reactions according to methods described in sections 4.4-4.6. All synthesis module loading operations were conducted under an ambient atmosphere. Argon was used as a pressurizing gas during automated sample transfers. Total recovered activity at the end of synthesis was measured with a Capintec® dose calibrator.

4.2 Thin Layer Chromatography (TLC) Analysis: Radio-TLC analysis was conducted to determine % RCC using a Bioscan AR-2000 TLC scanner. Crude reaction mixture (undiluted) was spotted onto standard silica coated glass plates and developed with 1:1 hexane/ethyl acetate in a glass TLC chamber. The RCC was determined by dividing the integrated area under the fluorinated product spot by the total integrated area of the TLC plate.
4.3 HPLC analysis
Radio-HPLC analyses were conducted using a Shimadzu LC-2010A HT system equipped with a Bioscan B-FC-1000 radiation detector. To prepare samples for HPLC analysis, approx. 100 µL crude reaction mixture was added directly into HPLC vials without further dilution. To confirm identity, crude reaction mixture was spiked with 1 mg/mL 4-fluoroacetophenone in acetonitrile (typically 50 µL standard solution was added to 100 µL crude reaction mixture and briefly agitated). Eluent systems and columns used for HPLC analysis are described below.

**HPLC Condition A.**

**Condition:** 40 % MeCN in H₂O, 10mM NH₄OAc pH: 6.1  
**Flow Rate:** 1 mL/min  
**Column:** Phenomenex® Luna C-8 Column 150 x 4.6 mm. 3 µm.

**HPLC Condition B.**

**Condition:** 40 % MeCN in H₂O, 10mM NH₄OAc pH: 6.1  
**Flow Rate:** 1 mL/min  
**Column:** Waters® Spherisorb C-8 Column 150 x 4.6 mm. 5 µm.

**HPLC Condition C.**

**Condition:** 40 % MeCN in H₂O, 10mM NH₄OAc pH: 6.1  
**Flow Rate:** 4 mL/min  
**Column:** Phenomenex® Luna C-18 Column 250 x 10 mm. 5 µm.

**HPLC Condition D.**

**Condition:** 40 % MeCN in H₂O, 10mM NH₄OAc pH: 6.1  
**Flow Rate:** 2 mL/min  
**Column:** Waters® Spherisorb C-8 Column 150 x 4.6 mm. 5 µm.

**HPLC Condition E.**

**Condition:** 5-95 % MeCN gradient (time = 0-20 min), then held at 5% MeCN (time = 20-25 min). Water contained 0.1% trifluoroacetic acid, MeCN contained no additives.  
**Flow Rate:** 2 mL/min,  
**Column:** Waters® Spherisorb C-8 Column 150 x 4.6 mm. 5 µm.
4.4 General synthetic procedures in a TRACERLab FX\textsubscript{FN} synthesis module

4.4.1 Synthesis of \([^{18}\text{F}]\)-4-fluoroacetophenone (\([^{18}\text{F}]\text{FAP}\)) with DMAP elution

Waters QMA-light cartridges was sequentially rinsed with 10 mL ethanol, 10 mL 0.5M preconditioning salt, and 10 mL water prior to use. Dry \([^{18}\text{F}]\text{DMAP}\)●HF was produced by trapping \(^{18}\text{F}\) from target water on the QMA cartridge followed by elution with 0.5 mL aqueous DMAP (50 \(\mu\)mol, 1 equiv, 100 mM) (vial 1) and azeotropic drying with 1 mL acetonitrile (vial 2) at 100 °C. The reactor housing the dry \([^{18}\text{F}]\)fluoride was cooled to 50 °C with compressed air and the reactants (vial 3 and/or 4) in a total volume of 1 mL of anhydrous DMF were added to the reactor by applying Argon push gas through the valve containing the reagent solution. All open valves leading out of the reactor were then closed and the mixture was stirred at either 110 or 140 °C for 20 min. Under optimized conditions, the reactant ratios used were: Cu(OTf)_2 (8 \(\mu\)mol, 0.16 equiv), 4-acetylphenylboronic acid, pinacol ester (50 \(\mu\)mol, 1 equiv), DMAP (50 \(\mu\)mol, 1 equiv). In several instances, one or two of the reactants (vial 3) in 0.5 mL anhydrous DMF were added to the dry \([^{18}\text{F}]\)fluoride first, and "pre-stirred" with the \([^{18}\text{F}]\)fluoride for 5 min at 110 °C, cooled to 50 °C, and finally mixed with the remaining reactant(s) (vial 4) in 0.5 mL DMF. The (complete) reactant mixture was then stirred at either 110 or 140 °C for 20 min. The reactor was then cooled to 50 °C and 5 mL of DMF was added to the reactor. The mixture was stirred ~2 min and was transferred to an 8 mL sterile product vial with Argon push gas. The product vial was transferred out of the synthesis module in a lead pig. Total activity of recovered material was measured using a Capintec dose calibrator. Radiochemical conversion (RCC) was determined with radio-TLC (Eluent = 1:1 hexanes: ethyl acetate). Identity of the product was confirmed with HPLC. The preconditioning salts and eluents used, as well as the order of addition and amounts of reactants, and radiochemical conversions are detailed in Table 5A. Activities at various timepoints during synthesis as well as radiochemical conversions are detailed in Table 5A. Spectroscopic data (radio-TLC and HPLC spectra) and HPLC conditions (column, flow rate, and eluent used) are located in section 5.1.1-5.1.10.
4.4.2 Synthesis of [\(^{18}\)F]-4-Fluorophenacyl Bromide ([\(^{18}\)F]FPB) with DMAP Elution

Waters QMA-light cartridge was sequentially rinsed with 10 mL ethanol, 10 mL 0.5M preconditioning salt, and 10 mL water prior to use. Dry [\(^{18}\)F]DMAP•HF was produced by trapping \(^{18}\)F from target water on the QMA cartridge followed by elution with 0.5 mL aqueous DMAP (50 µmol, 1 equiv, 100 mM) (vial 1) and azeotropic drying with 1 mL acetonitrile (vial 2) at 100 °C. The reactor housing the dry [\(^{18}\)F]fluoride was cooled to 50 °C with compressed air, and then a solution (vial 3) containing 4-acetylphenylboronic acid, pinacol ester (50 µmol, 1 equiv) in 500 µL anhydrous DMF was added to the reactor using Ar push gas. The reactor was heated to 110 °C for 5 min and subsequently cooled to 50 °C with compressed air. A second solution (vial 4) containing Cu(OTf)$_2$ (8 µmol, 0.16 equiv) and DMAP (50 µmol, 1 equiv) in 500 µL anhydrous DMF was added to the reactor using Ar push gas, heated to 110 °C for 20 min and subsequently cooled to 50 °C with compressed air. Finally, a third solution (vial 5) containing methanesulfonic acid (1000 µmol, 20 equiv) and N-bromosuccinimide (150 µmol) in 1000 µL anhydrous acetonitrile was added to the reactor using Ar push gas, heated to 110 °C for 20 min and subsequently cooled to 50 °C with compressed air. 5 mL of DMF was added to the reactor, the mixture was stirred for ~2 min and was then transferred to an 8 mL sterile product vial with Argon push gas. Total activity of recovered material was measured using a Capintec dose calibrator. Radiochemical conversion (RCC) to [\(^{18}\)F]fluorinated organics was determined with radio-TLC (Eluent = 1:1 hexanes: ethyl acetate). The identity and ratio of organic [\(^{18}\)F]fluorinated products in the reaction mixture, which included [\(^{18}\)F]-4-fluoroacetophenone ([\(^{18}\)F]FAP), [\(^{18}\)F]-4-fluorophenacyl bromide ([\(^{18}\)F]FPB), and [\(^{18}\)F]-4-fluorophenacyl dibromide ([\(^{18}\)F]FPDB), was determined using HPLC. Activities at various timepoints during synthesis as well as radiochemical conversions are detailed in Table 5B. The relative percentages of [\(^{18}\)F]FAP, [\(^{18}\)F]FPB, and [\(^{18}\)F]FPDB are detailed in Table 5C. Spectroscopic data (radio-TLC and HPLC spectra) and HPLC conditions (column, flow rate, and eluent used) are located in section 5.2.1.
4.4.3 Synthesis of \([^{18}F]\)-4-Fluorophenacyl Bromide (\([^{18}F]\)FPB) with DMAP Elution: in-box purification and reformulation.

Waters QMA-light cartridge was sequentially rinsed with 10 mL ethanol, 10 mL 0.5M preconditioning salt, and 10 mL water prior to use. Dry \([^{18}F]\)DMAP●HF was produced by trapping \(^{18}\)F from target water on the QMA cartridge followed by elution with 0.5 mL aqueous DMAP (50 µmol, 1 equiv, 100 mM) (vial 1) and azeotropic drying with 1 mL acetonitrile (vial 2) at 100 °C. The reactor housing the dry \([^{18}F]\)fluoride was cooled to 50 °C with compressed air, and then a solution (vial 3) containing 4-acetylphenylboronic acid, pinacol ester BPin-1 (50 µmol, 1 equiv) in 500 µL anhydrous DMF was added to the reactor using Ar push gas. The reactor was heated to 110 °C for 5 min and subsequently cooled to 50 °C with compressed air. A second solution (vial 4) containing Cu(OTf)$_2$ (8 µmol, 0.16 equiv) and DMAP (50 µmol, 1 equiv) in 500 µL anhydrous DMF was added to the reactor using Ar push gas, heated to 110 °C for 20 min and subsequently cooled to 50 °C with compressed air. Finally, a third solution (vial 5) containing methanesulfonic acid (1000 µmol, 20 equiv) and N-bromosuccinimide (150 µmol, 3 equiv) in 1000 µL anhydrous acetonitrile was added to the reactor using Ar push gas, heated to 110 °C for 20 min and subsequently cooled to 50 °C with compressed air. A solution (vial 6) containing KOH (750 µmol, 15 equiv) in 2 mL of water was added to the reactor to neutralize excess acid, and the mixture was loaded onto a semi-preparative column (HPLC condition C). The \([^{18}F]\)FPB product peak was collected (22.1-24.0 min) into a dilution flask containing 50 mL of water. The diluted product was then passed through a Waters C18 1cc vac cartridge, and the cartridge was rinsed with 10 mL of water (vial 9). Product trapped on the cartridge was eluted with 0.5 mL ethanol (vial 8), followed by 4.5 mL sterile saline buffer into a collection flask. The formulated product was then transferred to an 8 mL sterile product vial with Argon push gas. Total activity of recovered material was 13 mCi as measured using a Capintec dose calibrator. The identity (matched with co-injected FPB standard), specific activity (8,097 Ci/mmol) and radiochemical purity (99%) were determined using HPLC (condition D). HPLC data and HPLC conditions (column, flow rate, and eluent used) are located in section 5.2.2.

4.4.4 Synthesis of \([^{18}F]\)-4-Fluoroacetophenone (\([^{18}F]\)FAP) with Cu(OTf)$_2$ Elution

Waters QMA-light cartridge was sequentially rinsed with 10 mL ethanol, 10 mL 0.5M potassium trifluoromethanesulfonate, and 10 mL water. Dry \([^{18}F]\)Cu(OTf)$_2$●F$^-$ was produced by trapping \(^{18}\)F from target water on the QMA cartridge followed by elution with 500 µL of aqueous
(5.3.1) or methanolic (5.3.2) Cu(OTf)$_2$ (50 µmol, 6.25 equiv, 100 mM, vial 1), and azeotropic drying with 1 mL acetonitrile (vial 2) at 100 °C. The reactor housing the dry [$^{18}$F]fluoride was cooled to 50 °C with compressed air and a solution containing 4-acetylphenylboronic acid (8 µmol, 1 equiv, 4 mM), and pyridine (1000 µmol, 125 equiv, 500 mM) in 2 mL anhydrous DMF (vial 3) was added to the reactor by applying Ar push gas through the vial containing the reagent solution. All open valves leading out of the reactor were then closed and the mixture was stirred for 20 min at 140 °C. The mixture was cooled to 50 °C with compressed air cooling and 5 mL of DMF was added to the reactor. The mixture was stirred for ~2 min and was transferred to an 8 mL sterile product vial with Ar push gas. The product vial was transferred out of the synthesis module in a lead pig. Total activity of recovered material was measured using a Capintec dose calibrator. Radiochemical conversion (RCC) was determined with radio-TLC (Eluent = 1:1 hexanes: ethyl acetate). Identity of the product was confirmed with HPLC. The activities at various timepoints during synthesis and radiochemical conversions are detailed in Table 5B. Spectroscopic data (radio-TLC and HPLC spectra) and HPLC conditions (column, flow rate, and eluent used) are located in section 5.3.1 and 5.3.2.
5. Spectroscopic data and accompanying data tables

| Entry | QMA preparation | μmol reagents used | Order of reagent addition | reaction temperature (°C) | RCC (%) |
|-------|----------------|--------------------|---------------------------|--------------------------|---------|
|       | Precondition Salt (0.5 M aq. soln) | CuOTf | AP-Bpin | DMAP\(^1\) | "pre-stirring" (5 min, 110 °C) | reaction (20 min, X °C) |
| 5.1.1 | NaHCO\(_3\) | 40 | 20 | n/a | n/a | 140 | 5 |
| 5.1.2 | Na\(_2\)SO\(_4\) | 40 | 20 | n/a | n/a | 140 | 0 |
| 5.1.3 | KOTf | 40 | 20 | n/a | n/a | 140 | 2 |
| 5.1.4 | NaHCO\(_3\) | 40 | 20 | n/a | X | 140 | 2 |
| 5.1.5 | NaHCO\(_3\) | 40 | 20 | 20 | X, O | 140 | 4 |
| 5.1.6 | NaHCO\(_3\) | 8 | 50 | 50 | O | 140 | 19 |
| 5.1.7 | NaHCO\(_3\) | 8 | 50 | 50 | O, # | 140 | 38 |
| 5.1.8 | Na\(_2\)SO\(_4\) | 8 | 50 | 50 | O, # | 140 | 2 |
| 5.1.9 | Na\(_2\)SO\(_4\) | 8 | 50 | 50 | O, # | 110 | 45 |
| 5.1.10 | Na\(_2\)SO\(_4\) | 8 | 50 | 50 | O, # | 110 | 0 |

**Table 5A: Reaction conditions used in syntheses 5.1.1 – 5.1.10**

| Entry | Target water\(^2\) | After elution\(^2\) | After Azeotropic Drying\(^2\) | Final activity in dose vial\(^3\) | RCC (%) |
|-------|------------------|-----------------|-----------------|--------------------------|---------|
|       | (2 min beam) | After elution\(^2\) | After Azeotropic Drying\(^2\) | Final activity in dose vial\(^3\) | RCC (%) |
| 5.1.1 | 120 | 50 | 25 | 12 | 5 |
| 5.1.2 | 150 | 140 | 90 | 37 | 0 |
| 5.1.3 | 150 | 45 | 30 | 12 | 2 |
| 5.1.4 | 160 | 140 | 90 | 41 | 2 |
| 5.1.5 | 150 | 120 | 100 | 38 | 4 |
| 5.1.6 | 150 | 90 | 50 | 39 | 19 |
| 5.1.7 | 150 | 80 | 65 | 42 | 38 |
| 5.1.8 | 165 | 145 | 80 | 34 | 2 |
| 5.1.9 | 150 | 100 | 70 | 47 | 45 |
| 5.1.10 | 160 | 155 | 110 | 42 | 0 |
| 5.2.1 | 150 | 90 | 70 | 30 | 26 |
| 5.3.1 | 150 | 140 | 65 | 19\(^4\) | 10 |
| 5.3.2 | 150 | 50 | 25 | 16 | 12 |

**Table 5B: Activity (mCi) during various stages of synthesis**

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\(^1\) This is DMAP that was added in addition to the DMAP present from the eluent in all syntheses 5.1. It should be noted, however, that an unknown fraction of the DMAP present from the eluent could be lost during \[^{18}\text{F}\]fluoride reprocessing and/or azeotropic evaporation (thus necessitating this "extra" DMAP).

\(^2\) Readouts are from uncalibrated TRACERLab detectors and should be used as a rough guide only.

\(^3\) Final activities are measured in a Capintec dose calibrator.

\(^4\) In 5.3.1, a significant amount of product was lost on its way to the product vial due to a leaking line.
| Entry | $[^{18}F]$FAP | $[^{18}F]$FPB | $[^{18}F]$FPDB | RCC (to all organics) |
|-------|----------------|----------------|----------------|----------------------|
|       | Det. Resp. $^5$ | %              | Det. Resp. %   | Det. Resp. %         | (%)                  |
| 5.2.1 | 11897          | 7              | 114432         | 69                   | 40260 24            |

Table 5C: Ratio of $[^{18}F]$fluorinated organics (%) in synthesis 5.2.1

$^5$ Det. Resp. = HPLC RAD detector response (in mA). Ratio of $[^{18}F]$fluorinated products (expressed as %) was determined by comparing HPLC detector response values of FAP, FPB, and FPDB. Overall RCC (of all three products vs. "inorganic" fluoride) was determined using radio TLC and is shown in the rightmost column.
5.1 Synthesis of $[^{18}\text{F}]-4$-fluoroacetophenone with DMAP elution

5.1.1

![Chemical structure and reaction](image)

40 μmol Cu(OTf)$_2$
50 μmol DMAP (eluent)

DMAP$^{+^{18}\text{F}^-}$
NaHCO$_3$ QMA precond.

1 mL DMF
20min, 140 °C

$^{18}\text{F}$

5% RCC

**Figure 5.1.1A:** HPLC spectra of crude reaction mixture from 5.1.1. RAD trace (black, bottom) and UV trace (256 nm, red, top). HPLC conditions A.
Figure 5.1.1B: HPLC spectra of crude reaction mixture from 5.1.1 spiked with 4-fluoroacetophenone reference standard. RAD trace (black, bottom) and UV trace (256 nm, red, top). HPLC conditions A.

Figure 5.1.1C: Radio-TLC spectrum of the crude reaction mixture from 5.1.1
Figure 5.1.2A: HPLC spectra of crude reaction mixture from 5.1.2. RAD trace (black, bottom) and UV trace (256 nm, red, top). HPLC conditions A.

Figure 5.1.2B: Radio-TLC spectrum of the crude reaction mixture from 5.1.2
5.1.3

Figure 5.1.3A: HPLC spectra of crude reaction mixture from 5.1.3. RAD trace (black, bottom) and UV trace (256 nm, red, top). HPLC conditions A.

Figure 5.1.3B: Radio-TLC spectrum of the crude reaction mixture from 5.1.3.
Figure 5.1.4A: HPLC spectra of crude reaction mixture from 5.1.4. RAD trace (black, bottom) and UV trace (256 nm, red, top). HPLC conditions A.

Figure 5.1.4B: Radio-TLC spectrum of the crude reaction mixture from 5.1.4.
5.1.5

40 μmol Cu(OTf)$_2$
50 μmol DMAP (eluent)
+20 μmol DMAP (extra)
DMAPH$^{+18}$F$^{-}$
NaHCO$_3$ QMA precond.
1 mL DMF
5 min, 110$^\circ$C prestir (Cu)
20 min, 140$^\circ$C rxn (Bpin, DMAP)

$^{18}$F$^{-}$
4% RCC

**Figure 5.1.5A:** HPLC spectra of crude reaction mixture from 5.1.5. RAD trace (black, bottom) and UV trace (256 nm, red, top). HPLC conditions A.

**Figure 5.1.5B:** Radio-TLC spectrum of the crude reaction mixture from 5.1.5.
5.1.6

![Reaction Scheme]

- **40 µmol Cu(OTf)$_2$**
- **50 µmol DMAP (eluent)**
- **+20 µmol DMAP (extra)**
- **DMAPH$^{18}$F$^-$**
- **NaHCO$_3$ QMA precond.**
- **1 mL DMF**
- **5 min, 110°C prestr (Bpin)**
- **20 min, 140°C rxn (Cu, DMAP)**

19% RCC

**Figure 5.1.6A:** HPLC spectra of crude reaction mixture from 5.1.6. RAD trace (black, bottom) and UV trace (256 nm, red, top). HPLC conditions A.

**Figure 5.1.6B:** HPLC spectra of crude reaction mixture from 5.1.6. spiked with 4-fluoroacetophenone reference standard. RAD trace (black, bottom) and UV trace (256 nm, red, top). HPLC conditions A.
Figure 5.1.6C: Radio-TLC spectrum of the crude reaction mixture from 5.1.6.
5.1.7

8 μmol Cu(OTf)_2
50 μmol DMAP (eluent)
+50 μmol DMAP (extra)
DMAPH⁺^{18}\text{F}⁻
NaHCO₃ QMA precond.
1 mL DMF
5min, 110°C prestir (Bpin)
20min, 140 °C rxn (Cu, DMAP)

Figure 5.1.7A: HPLC spectra of crude reaction mixture from 5.1.7. RAD trace (black, bottom) and UV trace (256 nm, red, top). HPLC conditions A.

Figure 5.1.7B: HPLC spectra of crude reaction mixture from 5.1.7. spiked with 4-fluoroacetophenone reference standard. RAD trace (black, bottom) and UV trace (256 nm, red, top). HPLC conditions A.
**Figure 5.1.7C:** Radio-TLC spectrum of the crude reaction mixture from 5.1.7.
5.1.8

8 μmol Cu(OTf)$_2$
50 μmol DMAP (eluent)
+50 μmol DMAP (extra)
DMAPH$^{+18}$F$^-$
Na$_2$SO$_4$ QMA precond.
1 mL DMF
5 min, 110°C pre stir (Bpin)
20 min, 140°C rxn (Cu, DMAP)

$^{18}$F

2% RCC

Figure 5.1.8A: HPLC spectra of crude reaction mixture from 5.1.8. RAD trace (black, bottom) and UV trace (256 nm, red, top). HPLC conditions A.

Figure 5.1.8B: HPLC spectra of crude reaction mixture from 5.1.8. spiked with 4-fluoroacetophenone reference standard. RAD trace (black, bottom) and UV trace (256 nm, red, top). HPLC conditions A.
Figure 5.1.8C: Radio-TLC spectrum of the crude reaction mixture from 5.1.8.
5.1.9

8 μmol Cu(OTf)$_2$
50 μmol DMAP (eluent)
+50 μmol DMAP (extra)

DMAPH$^{+18F}$
NaHCO$_3$ QMA precond.
1 mL DMF
5min, 110°C prestir (Bpin)
20min, 110 °C rxn (Cu, DMAP)

50 μmol

45% RCC

Figure 5.1.9A: HPLC spectra of crude reaction mixture from 5.1.9. RAD trace (black, bottom) and UV trace (256 nm, red, top). HPLC conditions B.

Figure 5.1.9B: HPLC spectra of crude reaction mixture from 5.1.9. spiked with 4-fluoroacetophenone reference standard. RAD trace (black, bottom) and UV trace (256 nm, red, top). HPLC conditions B.
Figure 5.1.9C: Radio-TLC spectrum of the crude reaction mixture from 5.1.9.
5.1.10

8 μmol Cu(OTf)$_2$

50 μmol DMAP (eluent)

+50 μmol DMAP (extra)

DMAPH$^{+18F^-}$

Na$_2$SO$_4$ QMA precond.

1 mL DMF

5 min, 110°C prestrir (Bpin)

20 min, 110°C rxn (Cu, DMAP)

0% RCC

Figure 5.1.10A: HPLC spectra of crude reaction mixture from 5.1.10. RAD trace (black, bottom) and UV trace (256 nm, red, top). HPLC conditions B.

Figure 5.1.10B: HPLC spectra of crude reaction mixture from 5.1.10. spiked with 4-fluoroacetophenone reference standard. RAD trace (black, bottom) and UV trace (256 nm, red, top). HPLC conditions B.
Figure 5.1.10C: Radio-TLC spectrum of the crude reaction mixture from 5.1.10.
5.2 Synthesis of $[^{18}\text{F}]4$-fluorophenacylbromide with DMAP elution

5.2.1

Figure 5.2.1A: HPLC spectra of crude reaction mixture from 5.2.1. RAD trace (black, bottom) and UV trace (256 nm, red, top). HPLC conditions B.
Figure 5.2.1B: HPLC spectra of crude reaction mixture from 5.2.1 spiked with reference standards. RAD trace (black, bottom) and UV trace (256 nm) with FAP (red), FPB (blue), FPDB (green). HPLC conditions B.

Figure 5.2.1C: Radio-TLC spectrum of the crude reaction mixture from 5.2.1.
5.2.2

Figure 5.2.2A: HPLC spectra of formulated $[^{18}\text{F}]$FPB from 5.2.2. RAD trace (black, bottom) and UV trace (256 nm, red, top). HPLC conditions D.

Figure 5.2.2B: HPLC spectra of crude reaction mixture from 5.2.2 spiked with FPB reference standard. RAD trace (black, bottom) and UV trace (256 nm). HPLC conditions D.
Figure 5.2.2C: Semipreparative HPLC spectrum during purification of the crude reaction mixture from 5.2.2.
5.3 Synthesis of $[^{18}F]-4$-fluoroacetophenone with Cu(OTf)$_2$ elution

5.3.1

50 µmol Cu(OTf)$_2$
(aqueous eluent)
1000 µmol pyridine
CuOTf$_2^{18}$F$^-$
KOTf QMA precond.
2 mL DMF
20min, 140 °C

18% RCC

Figure 5.3.1A: HPLC spectra of crude reaction mixture from 5.3.1 RAD trace (black, bottom) and UV trace (256 nm, red, top). HPLC conditions A.
Figure 5.3.1B: HPLC spectra of crude reaction mixture from 5.3.1 spiked with 4-fluoroacetophenone reference standard (note: standard 254nm signal is unusually small). RAD trace (black, bottom) and UV trace (256 nm, red, top). HPLC conditions A.

Figure 5.3.1C: Radio-TLC spectrum of the crude reaction mixture from 5.3.1
Figure 5.3.2A: HPLC spectra of crude reaction mixture from 5.3.2 RAD trace (black, bottom) and UV trace (256 nm, red, top). HPLC conditions A.
**Figure 5.3.2B**: HPLC spectra of crude reaction mixture from 5.3.2 spiked with 4-fluoroacetophenone reference standard. RAD trace (black, bottom) and UV trace (256 nm, red, top). HPLC conditions A.

**Figure 5.3.2C**: Radio-TLC spectrum of the crude reaction mixture from 5.3.2
6. Substrate Scope Spectroscopic Data

6.1 4-[\textsuperscript{18}F]Fluoroacetophenone (2)

Figure 6.1A: 4-[\textsuperscript{18}F]Fluoroacetophenone (2) Radio-TLC spectrum

Figure 6.1B: 4-[\textsuperscript{18}F]Fluoroacetophenone (2) RÅD trace overlaid with UV trace (256 nm), HPLC Conditions E
Figure 6.1C: $^{18}$FFluoroacetophenone (2) RAD trace overlaid with UV trace (256 nm) spiked with 4-fluoroacetophenone reference standard, HPLC Conditions E
6.2 2-[^18F]Fluoromethylbenzoate (4)

Figure 6.2A: 2-[^18F]fluoromethylbenzoate (4) Radio-TLC spectrum

Figure 6.2B: 2-[^18F]Fluoromethylbenzoate (4) RAD trace overlaid with UV trace (256 nm), HPLC Conditions E
Figure 6.2C: 2-[^{18}F]Fluoromethylbenzoate (4) RAD trace overlaid with UV trace (256 nm) spiked with 2-fluoromethylbenzoate reference standard, HPLC Conditions E.

[Note: this compound was very poorly visible in the 200-300 nm range normally utilized for HPLC UV detection and can be seen as an increase in the size of the UV peak immediately prior to the RAD product peak].
6.3. **4-[^18]F]Fluorobenzonitrile (5)**

![Chemical Structure](image)

**Figure 6.3A**: 4-[^18]F]Fluorobenzonitrile (5) Radio-TLC spectrum

**Figure 6.3B**: 4-[^18]F]Fluorobenzonitrile (5) RAD trace overlaid with UV trace (256 nm), HPLC Conditions E
Figure 6.3C: 4-[¹⁸F]Fluorobenzonitrile (5) RAD trace overlaid with UV trace (256 nm) spiked with 4-fluorobenzonitrile reference standard, HPLC Conditions E.
6.4 4-$[^{18}F]$Fluoronitrobenzene (6)

Figure 6.4A: 4-$[^{18}F]$Fluoronitrobenzene (6) Radio-TLC spectrum

Figure 6.4B: 4-$[^{18}F]$Fluoronitrobenzene (6) RAD trace overlaid with UV trace (256 nm), HPLC Conditions E
Figure 6.4C: 4-$^{18}$FFluoronitrobenzene (6) RAD trace overlaid with UV trace (256 nm) spiked with 4-fluoronitrobenzene reference standard, HPLC Conditions E.
6.5 4-[\textsuperscript{18}F]-2,4-Difluoroaniline (7)

![Diagram of 4-[\textsuperscript{18}F]-2,4-Difluoroaniline (7)]

**Figure 6.5A:** 4-[\textsuperscript{18}F]-2,4-Difluoroaniline (7) Radio-TLC spectrum

![Radio-TLC spectrum graph]

**Figure 6.5B:** 4-[\textsuperscript{18}F]-2,4-difluoroaniline (7) RAD trace overlaid with UV trace (256 nm), HPLC Conditions E

![RAD trace overlaid with UV trace graph]
Figure 6.5C: 4-[\(^{18}\)F]-2,4-difluoroaniline (7) RAD trace overlaid with UV trace (256 nm) spiked with 2,4-difluoroaniline reference standard, HPLC Conditions E.
6.6: 5-[^{18}F]Fluoroindole (8)

Figure 6.6A: 5-[^{18}F]fluoroindole (8) Radio-TLC spectrum

Figure 6.6B: 5-[^{18}F]fluoroindole (8) RAD trace overlaid with UV trace (256 nm), HPLC Conditions E
Figure 6.6C: 5-[^18]Ffluoroindole (8) RAD trace overlaid with UV trace (256 nm) spiked with 5-fluoroindole reference standard, HPLC Conditions E.
7. Regression Analysis

To develop a predictive model for $[^{18}\text{F}]$fluoride recovery vs. pKa, regression analysis was conducted on the data collected in this experiment using GraphPad software (90% confidence level, Table 7). Regression equations were obtained with high correlation ($R^2>0.9$) for each preconditioning agent and serve as a crude predictor of $[^{18}\text{F}]$fluoride recovery when similar non-ionic eluents are use. The equations predict that bases with a conjugate acid pKa of ≥10.4 are optimal for recovering >50% $[^{18}\text{F}]$fluoride from ion exchange cartridges preconditioned with KOTf, whereas pKa values ≥8.9 and ≥7.7 are required when preconditioning with NaHCO$_3$ and Na$_2$SO$_4$, respectively. A similar relationship can be expressed in terms of pH instead of pKa, and the regression equations corresponding to $[^{18}\text{F}]$fluoride recovery versus pH (predicted from solution concentration and pKa) are also shown in Table S7. These equations are not bound by concentration and can thus predict $[^{18}\text{F}]$fluoride recovery from any non-ionic aqueous solution (both sets of equations approach zero at the lower conjugate pKa values and are only suitable for basic eluents. It can be envisioned that strong acid solutions that dissociate into H$_3$O$^+$ and A$^-$ ion pairs would also be suitable eluents, likely following the same trend with pKb).

\[
\% \text{Fluoride Recovery} = 100 \div \left(1 + 10^{(A + B \cdot pKa)}\right)
\]

| cartridge precondition. Agent | A  | B    | $R^2$ |
|-------------------------------|----|------|-------|
| KOTf                          | 0.508 | 10.38 | 0.976 |
| NaHCO$_3$                     | 0.553 | 8.94  | 0.963 |
| Na$_2$SO$_4$                  | 0.356 | 7.68  | 0.921 |

| cartridge precondition. Agent | A  | B    | $R^2$ |
|-------------------------------|----|------|-------|
| KOTf                          | 1.016 | 11.69 | 0.976 |
| NaHCO$_3$                     | 1.105 | 10.97 | 0.963 |
| Na$_2$SO$_4$                  | 0.712 | 10.34 | 0.921 |

**Table 7.** Regression equation for $[^{18}\text{F}]$fluoride recovery vs pKa/pH of aq. base solution