COMMUNICATION

Preparation of Labeled Aromatic Amino Acids via Late-Stage ^{18}F-Fluorination of Chiral Nickel and Copper Complexes

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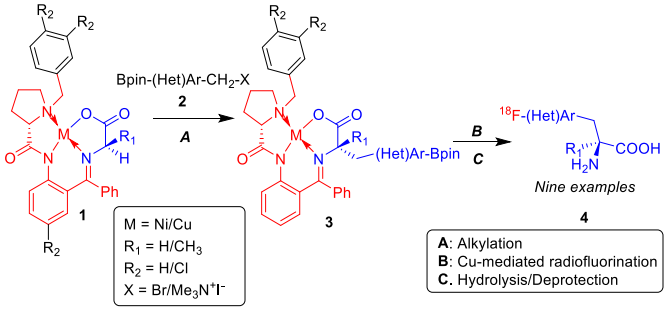
A general protocol for the preparation of ^{18}F-labeled AAAs and α-methyl-AAAs applying alcohol-enhanced Cu-mediated radiofluorination of Bpin-substituted chiral complexes using Ni/Cu-BPX templates as double protecting groups is reported. The chiral auxiliaries are easily accessible from commercially available starting materials in a few synthetic steps. The versatility of the method was demonstrated by the high-yielding preparation of a series of [^{18}F]F-AAAs and the successful implementation of the protocol into automated radiosynthesis modules.

Positron emission tomography (PET) has gained great importance as a prominent non-invasive imaging modality in clinical practice. Using tracers containing a suitable β$^+$-emitting radionuclide, PET may provide real-time visualization of complex biochemical processes at the molecular level, with high sensitivity and excellent image quality. Fluorine-18 has been established as an advantageous β$^+$-emitting radionuclide for PET, mainly due to its efficient large-scale cyclotron production in the form of [^{18}F]fluoride, relatively long half-life (109.7 min), low maximum positron energy of 0.635 MeV, and high β$^+$-decay branching intensity (97%).[1]

For decades, ^{18}F-labeled aromatic amino acids (AAA) including *O*-(2-[^{18}F]fluoroethyl)-L-tyrosine ([^{18}F]FET), 3,4-dihydroxy-6-[^{18}F]fluoro-L-phenylalanine (6-[^{18}F]FDOPA), and 2-[^{18}F]fluoro-L-tyrosine (2-[^{18}F]FTyr) have played a significant role in clinical diagnostics of cancer and neurodegenerative disorders using PET.[2-4] In combination with MRI and CT, [^{18}F]F-AAA-PET has been demonstrated to be a sensitive tool for effective tumor diagnosis and staging.[5,6] In addition, 6-[^{18}F]FDOPA, 6-[^{18}F]fluoro-meta-L-tyrosine (6-[^{18}F]FMT), and 7-[^{18}F]fluoro-L-tryptophan (7-[^{18}F]FTrp) have been used as neurotracers for the visualization of the dopaminergic and serotonergic systems.[7,8]

Accordingly, a significant amount of research has been devoted to the development of convenient and versatile protocols for the production of ^{18}F-labeled AAAs. Despite the substantial effort, the majority of the published protocols lack efficacy and are too impractical for routine applications. Consequently, the potential of ^{18}F-labeled AAAs has not been fully explored.

Selected recent preparations of ^{18}F-labeled AAAs (shown in Scheme 2) highlight challenges including multi-step precursor syntheses, low RCYs, and regioselectivity issues. The group of DiMagno reported the use diaryliodonium salts towards 6-[^{18}F]FDOPA ([^{18}F]**7**) and 4-[^{18}F]FPhe preparation, although lengthy precursor synthesis and fair RCYs limited these approaches (Scheme 2 A).[9,10] Liang *et al.* accessed ^{18}F-labeled AAAs *via* spirocyclic iodonium(III) ylides; unfortunately, demanding precursor synthesis also reduced the practicality of this method.[11] Tsushima and co-workers applied an electrophilic radiofluorination towards α-[^{18}F]FMePhes despite giving rise to multiple ^{18}F-labeled products and low specific activities (A_s).[12]



Scheme 1 Application of Ni/Cu-BPX templates for the late-stage preparation of ^{18}F-labeled AAAs.

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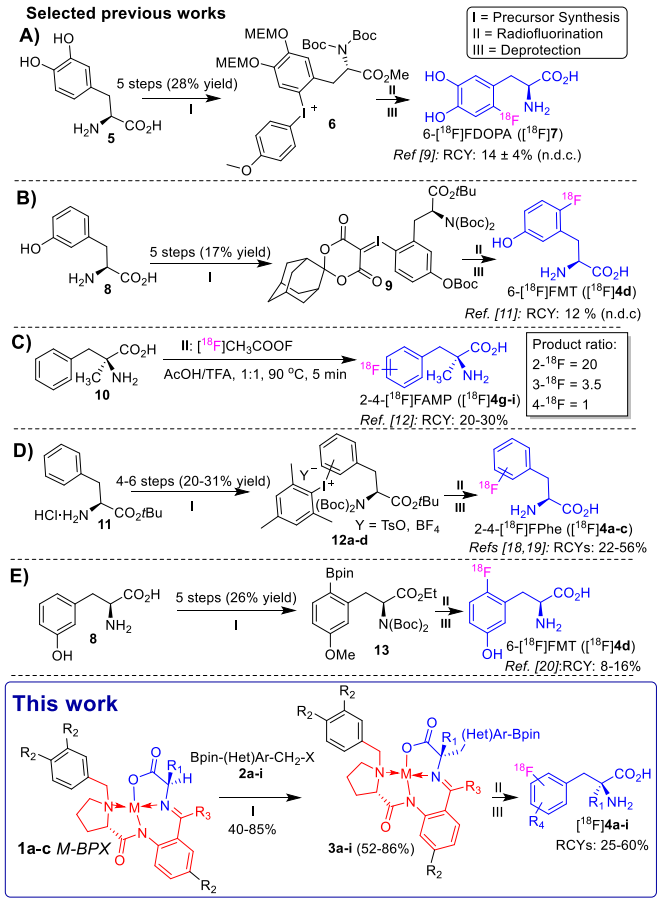
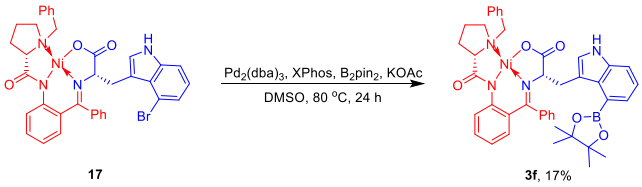


Scheme 2. Selected examples of ^{18}F-labeled AAA preparations. Conditions for radiolabeling steps (**II**): A): 1) azeotropic drying of [^{18}F]KF/K2.2.2 (×3), 2) diglyme, 140 °C, 5 min; B) 1) azeotropic drying of [^{18}F]Et_4NF (×3), 2) DMF, 120 °C, 20 min; C): [^{18}F]AcOF, AcOH/TFA 1:1, 90 °C, 5 min; D: [^{18}F]Et_4NF, $Cu(MeCN)_4OTf$, DMF or MeOH/DMF, 85–95 °C, 20 min, air or Ar; E): 1) azeotropic drying of [^{18}F]KF/K2.2.2 (×5), 2) $Cu(py)_4(OTf)_2$, DMF, 120 °C, 20 min, air; this work: [^{18}F]Et_4NF, $Cu(py)_4(OTf)_2$, nBuOH/DMA, 110 °C, 15 min, air. Procedures D and the novel procedure (this work) avoid any azeotropic drying steps.

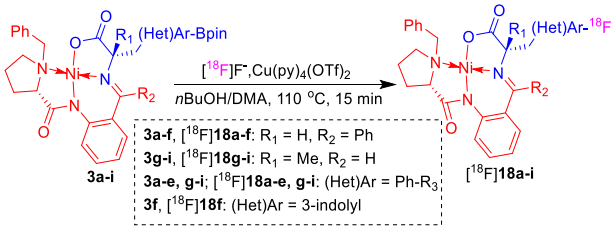
In recent years, Cu-mediated radiofluorination strategies have received much attention, owing to high RCYs, the wide substrate scope and their facile implementations.[13-15] More recently, additives including alcoholic co-solvents and pyridinium salts have been demonstrated to further enhance the efficiency of Cu-mediated radiofluorination reactions.[16,17] Cu-mediated radiolabeling of (aryl)(mesityl) iodonium salts proved a promising method, although possible epimerization and multistep precursor synthesis remained significant drawbacks (Scheme 2 D).[18,19] Gouverneur and coworkers prepared 6-[^{18}F]FMT ([^{18}F]**4d**) *via* Cu-mediated radiofluorination of the corresponding pinacol arylboronate (Bpin) in 15±1% RCY (Scheme 2 E); however, the five-step precursor synthesis was low-yielding and required HPLC purification.[20] In our previous work, we have successfully employed a chiral Schöllkopf auxiliary towards 6-[^{18}F]fluoro-L-tryptophan (6-[^{18}F]FTrp, [^{18}F]**16**); however, we encountered several challenges.[21] The laborious six-step precursor synthesis (overall yield 37%) was expensive and time-consuming, the



Scheme 3. Suzuki-Miyuara borylation upon Ni-BPB-AAA.

enantiomeric purity of 6-[^{18}F]FTrp was suboptimal (89% *ee*). Moreover, the harsh deprotection conditions (50% H_2SO_4, 130 °C, 15 min) led to the partial decomposition of the tracer. In our revised strategy, we were attracted by the possibility of using Ni-BPX complexes to overcome these limitations.

Developed by Belokon *et al*. as early as the 1980s, the chiral Ni-BPB-Gly (**1a**) and Ni-BPA-(*RS*)-Ala complexes (**1b**) were designed to enable the stereoselective synthesis of α-amino and α-amino-α-methyl acids.[22] In particular, the commercial availability/simplicity of their multi-gram scale preparations, high crystallinity, moisture stability, facile purification and rapid decomposition (to yield the free AAA) under rather mild acidic conditions is advantageous for synthetic as well as radiosynthetic purposes. Whilst multi-step AAA radiosyntheses using Belokon complexes have been reported,[4,23,24] the application of late-stage Cu-mediated radiofluorination upon Ni/Cu-BPB complexes has never been previously disclosed. The Bpin-substituted precursors (**3a–i**) were accessed via alkylation upon (S)-M-BPX (**1a–c**) (Scheme 1 A) using NaH in DMF/MeCN, furnishing the desired (S)-M-BPX-AAA complexes **3a–i** in good yields. Three of the requisite alkylating agents used for the synthesis of six tracer precursors were commercially available



Entry	Precursor	Product (RCY %)
1	***3a,*** *R_3=H, 2-Bpin*	*[^{18}F]**18a** (92±5)*
2	***3b,*** *R_3=H, 3-Bpin*	*[^{18}F]**18b** (90±2)*
3	***3c,*** *R_3=H, 4-Bpin*	*[^{18}F]**18c** (86±4)*
4	***3d,*** *R_3= 5-OMOM, 2-Bpin*	*[^{18}F]**18d** (90±3)*
5	***3e,*** *R_3= 4-OMOM, 2-Bpin*	*[^{18}F]**18e** (90±6)*
6	***3f,*** *R_3 = H, 4-Bpin*	*[^{18}F]**18f** (94±2)*
7	***3g,*** *R_3 = H, 2-Bpin*	*[^{18}F]**18g** (84±6)*
8	***3h,*** *R_3 = H, 3-Bpin*	*[^{18}F]**18h** (89±7)*
9	***3i,*** *R_3 = H, 4-Bpin*	*[^{18}F]**18i** (92±4)*

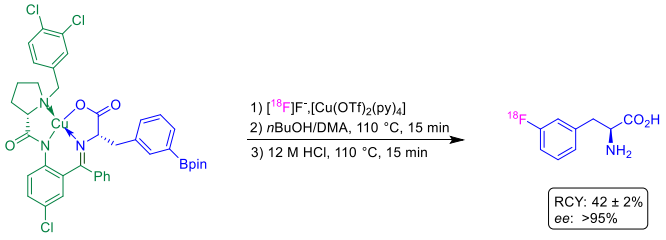
Table 1. ^{18}F-Labeling: Ni-BPX-AAA (**3a–i**, 10 µmol), $Cu(py)_4(OTf)_2$ (20 µmol), [^{18}F]F^-/Et_4NHCO_3 (50–1500 MBq), 250 µL nBuOH/750 µL DMA, air, 110 °C, 15 min. All experiments were carried out at least six times. Radio-HPLC was used to determine RCYs, given in the form of mean ± standard deviation.

(Scheme 1 A & B, **2a–c**). The synthetic routes towards 6-[^{18}F]FMT ([^{18}F]**4d**), 2-[^{18}F]FTyr ([^{18}F]**4e**), 4-[^{18}F]FTrp ([^{18}F]**4f**) precursors using alkylating agents (**2d–f**) are outlined in the Supporting Information. We also investigated the use of the bromomethyl substituted arylboronic acids as alkylating agents; however, problems with the purification of the corresponding Ni-BPX complexes prevented us from pursuing this pathway.

We considered various protecting groups, bearing in mind that rapid and high yielding deprotection would be most practical for radiosynthesis and selected *O*-methoxymethyl (MOM) groups, which could be introduced in near quantitative yields and rapidly cleaved. Suzuki-Miyuara borylation was used to incorporate the Bpin leaving groups at the desired aryl positions.

An alternative synthetic approach *via* the direct Suzuki-Miyuara borylation upon Ni-BPB-AAA (**18**) also proved to be feasible, affording the desired precursor **3f** in an unoptimized yield of 17% (Scheme 3). Radiosynthesis commenced with the loading of [^{18}F]fluoride onto an anion exchange resin followed by its subsequent elution with Et_4NHCO_3 in MeOH. The amount of Et_4NHCO_3 required for successful elution was minimized considering the base-sensitivity of $Cu(py)_4(OTf)_2$. The low boiling point of MeOH (65 °C) facilitated its subsequent rapid removal within 2–3 min, which was followed by the addition of the corresponding Ni-BPX-AAA precursor and $Cu(py)_4(OTf)_2$ in *n*BuOH/DMA (1:2). The elution of $^{18}F^-$ using Et_4NHCO_3 in *n*BuOH/DMA avoiding any evaporation steps was also evaluated. However, in this case, $^{18}F^-$ recovery was slightly lower (90–95%) and higher amounts of precursor (30 instead of 10 µmol) were necessary for the subsequent radiolabeling step. The precursor/Cu-complex ratio of 1:2 provided the highest ^{18}F-incorporation rates (Scheme 4).[25] The removal of volatiles after radiolabeling required to achieve efficient decomposition of the radiolabeled complexes and simultaneous cleavage of protecting groups. The latter was carried out using 37% HCl at 110 °C for 15 min.[26] Complete hydrolysis was pleasantly indicated by a sharp color change from deep red to pale yellow.

It was also found that higher temperatures caused a partial decomposition of 6-[^{18}F]FMT ([^{18}F]**4d**), 2-[^{18}F]FTyr ([^{18}F]**4e**) and 4-[^{18}F]FTrp ([^{18}F]**4f**). Purification of the resulting AAA tracers ([^{18}F]**4a–i**) was achieved by semi-preparative HPLC applying aqueous EtOH as a mobile phase yielded AAA tracers ([^{18}F]**4a–i**) in decay-corrected radiochemical yields (RCYs) of 25–60% within approximately 90 min with a radiochemical purity (RCP) and enantiomeric excess (*ee*) of > 95% as ready-to-use solutions.



Scheme 4. Preparation of [^{18}F]**4b** via alcohol-enhanced Cu-mediated radiofluorination of (S)-Cu-BPB-AAA complex **19**.

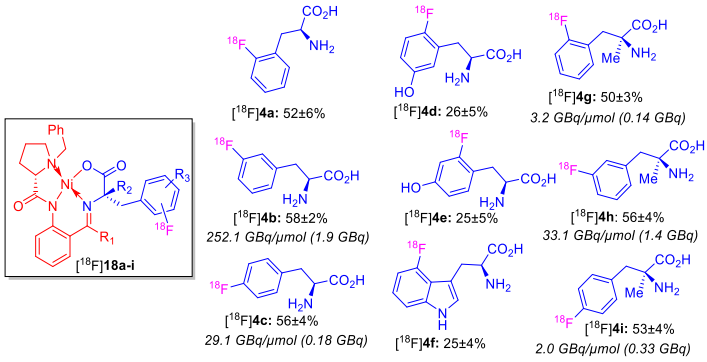


Figure 1. ^{18}F-Labeled AAAs ([^{18}F]**4a-i**) prepared using the novel method. All RCYs are isolated yields corrected for decay and determined at least in triplicate. All tracers were enantiomerically pure (ee > 95%). Furthermore, for [^{18}F]**4b,c** and [^{18}F]**4g–i** molar activities (A_m) are provided. For [^{18}F]**4b** and [^{18}F]**4h** A_m values at different activity amounts were additionally determined: 4.6 GBq/µmol (0.13 GBq), 76.3 GBq/µmol (1.1 GBq) ([^{18}F]**4b**) and 61.2 GBq/µmol (0.5 GBq) ([^{18}F]**4h**), respectively.

Molar activities (A_m) of [^{18}F]AAAs prepared using the novel method were comparable to or better than those for [^{18}F]AAAs produced using published protocols (Figure 1).

The implementation of the protocol into automated modules was straightforward owing to the simplicity of the radiosynthesis. The procedure allowed the rapid preparation of a series of ^{18}F-labeled AAAs shown in Scheme 1 C under general conditions without the need for further optimization.

Additionally, the applicability of a Cu complex **19** as an alternative to Ni-BPX radiolabelling precursors was investigated (Scheme 4).[27] The use of Cu- instead of Ni-containing precursors would mean that only a single trace metal determination required for quality control in *c*GMP production. The test radiosynthesis furnished 3-[^{18}F]FPhe in a good RCY of 42% and confirmed the applicability of the appropriate Cu-complexes for radiolabeling. The Ni & Cu content amounted to 0.001–0.37 mg/preparation (determined by ICP-MS) and was significantly below any level of concern according to the ICH Guideline of Elemental Impurities (Q3D).[28]

The AAA PET-tracers were compared to clinically established [^{18}F]FET, which provides information on the LAT1 transporter expression. As the majority of malignant tumor cells overexpress such transporters, [^{18}F]FET can afford invaluable information on the location and size of tumors.[2,29] The uptake of 3-[^{18}F]FPhe ([^{18}F]**4b**) in PC3 and MCF7 cells was significantly higher than of [^{18}F]FET (Chart S1; Supp. Information). Uptake of 3-[^{18}F]FPhe and [^{18}F]FET in MDA-MB-231 cells was comparable.

One of the most important applications of radiolabeled AAAs is the detection of cerebral tumors. Consequently, high tracer uptake in the brain is a necessary property of a successful candidate probe. Brain accumulation of 3-[^{18}F]FPhe ([^{18}F]**4b**) in healthy rats was higher than that of [^{18}F]FET with a mean value of 57.7±2.9 SUVbw during the first 30 min p. i. A distinct pattern was visible with high uptake in the olfactory bulb, frontal cortex, occipital cortex, thalamus, inferior colliculus, cerebellum, and in the areas around the 4th ventricle (Figure 2). This brain distribution pattern was similar to that of [^{18}F]FET.

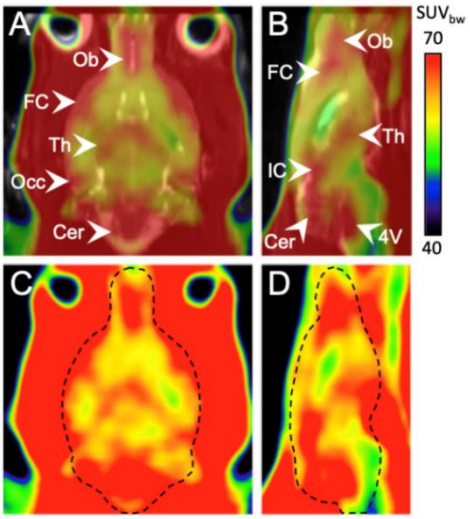


Figure 2. PET image of 3-[^{18}F]FPhe uptake in rat brain; average of n=3 healthy rats, 0-30 min p.i. Upper row: Projection of the PET image onto an MRI template in horizontal (A) and sagittal view (B). Lower row: The same PET image in horizontal (C) and sagittal view (D) with brain contour (dashed line). Abbreviations: 4V: areas around the 4th ventricle, Cer: cerebellum, FC: frontal cortex, IC: inferior colliculus, Ob: olfactory bulb, Occ: occipital cortex, Th: thalamus.

In summary, alcohol-enhanced Cu-mediated radiofluorination of Bpin-substituted Ni-BPX-AAA complexes is a simple, yet powerful method for the fast production of structurally diverse radiolabeled AAAs. The attractiveness of the procedure is highlighted by an efficient precursor synthesis, high RCYs, and amenability to automation. The utilization of M-BPX complexes facilitates precursor synthesis and allows a series of AAA tracers to be accessed in a short time and practical manner. In addition, the use of chiral Cu(II) complexes towards radiofluorinated AAAs has been described for the first time. The proposed protocol could be easily implemented also for the preparation of radiofluorinated D-AAAs owing to accessibility of the corresponding BPX-AA complexes. Notably, 3-[^{18}F]FPhe showed higher or similar uptake in tumor cells, and higher brain uptake in healthy rats compared to the clinically-established PET-tracer [^{18}F]FET.

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Conflicts of interest

There are no conflicts to declare.

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