

Enantioselective Radiosynthesis of Positron Emission Tomography (PET) Tracers Containing [¹⁸F]Fluorohydrins

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Supporting Information

ABSTRACT: Herein, we describe an operationally straightforward radiosynthesis of a chiral transition metal fluoride catalyst, [¹⁸F](salen)CoF, and its use for late-stage enantioselective aliphatic radiofluorination. We demonstrate the utility of the method by preparing single enantiomer experimental and clinically validated PET tracers that contain base-sensitive functional groups, epimerizable stereocenters, and nitrogen-rich motifs. Unlike the conventional radiosyntheses of these targets with [¹⁸F]KF, labeling with (salen)CoF is possible in the last step and under exceptionally mild conditions. These results constitute a rare example of a nucleophilic radiofluorination using a transition metal fluoride and highlight the potential of such reagents to enhance traditional methods for labeling aliphatic hydrocarbons.

The use of [¹⁸F]-labeled small molecules for positron emission tomography (PET) represents one of the most promising approaches to detect disease progression and evaluate therapeutic effectiveness *in vivo*.^{1–3} However, the radiochemical methods available to introduce [¹⁸F]fluoride into bioactive probes severely limit the potential scope of the imaging modality.^{4,5} The short half-life (110 min) and low available concentrations of ¹⁸F (ranging from nM to μM), compounded with the general difficulties posed by C–¹⁹F bond formation, make the identification of broadly applicable radiofluorinations of complex molecules incredibly challenging.^{6,7} Nevertheless, the past five years have witnessed the discovery of new methods that begin to address the limited scope of radiolabeling with [¹⁸F]fluoride. The majority of these solutions have focused on the challenge of [¹⁸F]aryl fluoride synthesis.^{8–10} In contrast, methods for improving the scope of aliphatic radiofluorination remain significantly underdeveloped.^{11,12} Although numerous modern synthetic methods have been reported that achieve mild and selective aliphatic carbon–fluorine bond formation, these methods utilize electrophilic ¹⁹F sources.¹³ Nucleophilic fluoride is currently the only practical and generally available source of ¹⁸F to prepare PET tracers in high specific activity.¹³ As such, these electrophilic methods have proven less useful for applications in PET.

PET tracers containing aliphatic C–¹⁸F labels are typically prepared using a substitution reaction with activated alcohol derivatives (i.e., tosylate, mesylate) and [¹⁸F]KF in the presence of cryptands such as Kryptofix 2.2.2. (K₂₂₂). Substrates

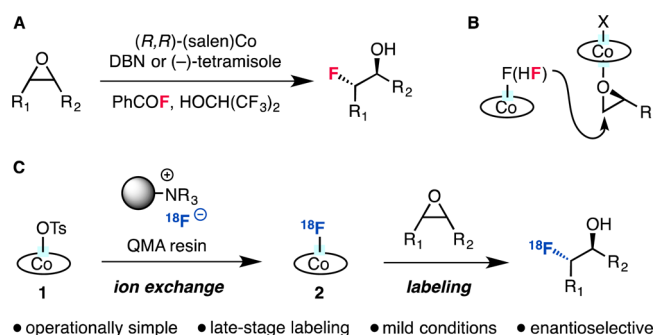


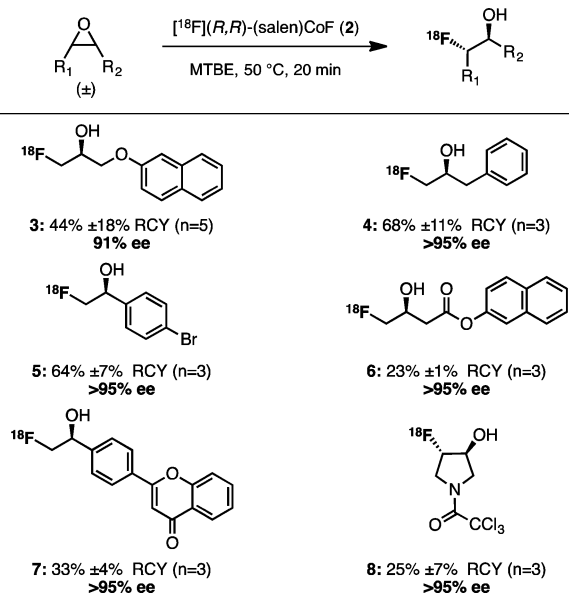
Figure 1. (A) Asymmetric fluoride ring opening of epoxides catalyzed by (salen)Co; DBN: 1,5-diazabicyclo(4.3.0)non-5-ene. (B) Proposed homobimetallic mechanism. (C) Strategy for direct radiosynthesis of [¹⁸F]fluorohydrins.

possessing protic functional groups (e.g., alcohols) and functionality prone to elimination are generally not tolerated under these reaction conditions due to the high temperatures (>100 °C) necessary for labeling and the basicity of [¹⁸F]KF/K₂₂₂.^{6,7} Furthermore, despite the importance of stereochemistry with regard to biological activity, the preparation of single stereoisomer PET probes is often challenging owing to the propensity of [¹⁸F]fluoride reagents to induce epimerization. As such, PET tracers are often evaluated as racemic mixtures or they are subjected to time-consuming chiral HPLC separation.^{14,15} To the best of our knowledge, methods capable of late-stage enantioselective labeling with [¹⁸F]fluoride are completely unknown. Herein, we report an asymmetric, no-carrier-added radiosynthesis of [¹⁸F]fluorohydrins by ring opening of epoxides with chiral cobalt catalysts. In addition to offering direct access to single enantiomer tracers in the last synthetic step, the method also addresses many of the noted deficiencies associated with aliphatic labeling using [¹⁸F]KF.

[¹⁸F]Fluorohydrins represent a useful motif in probe design and are featured in several experimental and clinically validated PET tracers.^{14,16–18} They are typically prepared through selective displacement of differentially protected diols followed by deprotection of the remaining protecting group (*vide infra*). As such, preparation of a single enantiomer PET probe containing an [¹⁸F]fluorohydrin requires that stereochemistry be set within an organic molecule prior to labeling. Asymmetric

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Table 1. Scope of Radiofluorination with **2**^a

^aRadiochemical yields (RCYs) are the average of *n* runs and are based on activity added to each reaction. RCY was determined by radio-TLC. The identity and ee of the product were determined by radio-HPLC. See SI for details; MTBE: methyl *tert*-butyl ether.

ring-opening of epoxides is an attractive alternative approach to [¹⁸F]fluorohydrins because it obviates the need for multistep preparation of differentially protected enantiopure 1,2-diols and it introduces the radioisotope in the last step. However, ring-opening reactions of epoxides with [¹⁸F]fluoride generally cannot be achieved due to poor rates and regioselectivity.^{19,20}

We recently demonstrated that a Lewis acidic chiral (salen)Co and an isothiourea or amidine cocatalyst promote enantioselective, nucleophilic fluoride ring opening of epoxides (Figure 1A).²¹ This approach represents a rare example of a catalyst-controlled enantioselective nucleophilic fluorination and features remarkably mild and functional group tolerant conditions. Key to its success is the use of benzoyl fluoride (PhCOF) and an alcohol additive as a latent source of HF, which avoids an unselective background reaction and catalyst poisoning. Based on these promising attributes, we sought to translate the methodology to a radiofluorination protocol. However, a number of issues inhibited direct application, not the least of which was that use of [¹⁸F]PhCOF would require its radiosynthesis and purification prior to labeling. Instead, we relied on insight gathered from our mechanistic analysis of the catalytic reaction, which revealed that a (salen)CoF(HF) species generated *in situ* from PhCOF serves as the active fluorinating agent and that fluorination occurs through a homobimetallic pathway in which one (salen)Co center activates the epoxide while the second delivers fluoride (Figure 1B).²² As such, we envisioned that an alternative synthesis of (salen)CoF by counterion metathesis between [¹⁸F]fluoride and a suitable (salen)Co(III)X (X = anionic counterion) species could lead to the development of a successful [¹⁸F]fluoride ring opening (Figure 1C).²³ In the event, we found that, by using (*R,R*)-(salen)CoOTs (**1**) as a precursor, a [¹⁸F](salen)CoF (**2**) species suitable for the radiofluorination of epoxides was generated by elution of [¹⁸F]fluoride from an ion-exchange cartridge containing a quaternary ammonium cation (QMA). Importantly, the preparation of **2** is directly analogous to the preparation of [¹⁸F]KF and can be carried out under air without the use of rigorously dried solvents or glassware.^{6,7}

We next investigated the scope and generality of the epoxide radiofluorination with **2** (Table 1). Representative epoxides were

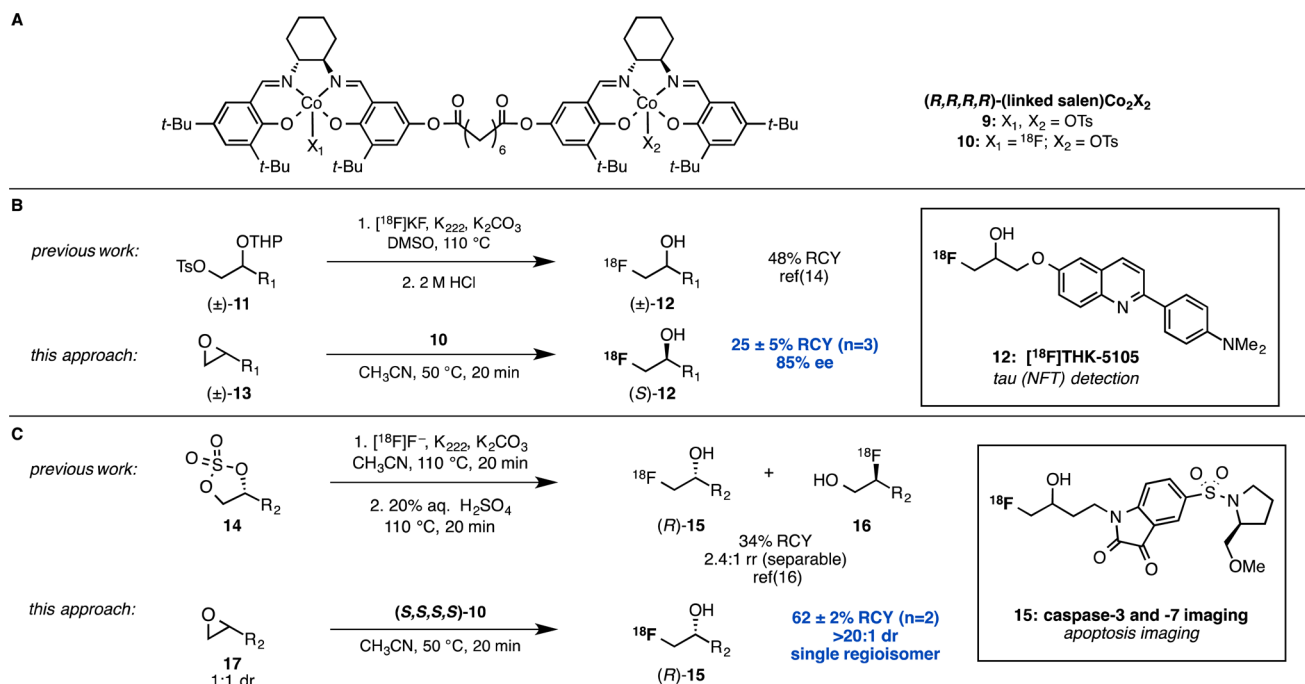


Figure 2. (A) Linked-catalyst employed in asymmetric radiofluorination of complex epoxides. (B) Comparative radiosyntheses of [¹⁸F]THK-5105. (C) Comparative radiosyntheses of [¹⁸F]isatin sulfonamides. Radiochemical yields (RCYs) are the average of *n* runs and are based on activity added to each reaction. RCY was determined by radio-TLC. The identity and ee of the product were determined by radio-HPLC. The dr of **15** was determined used Mosher ester analysis on [¹⁹F]**15**. See SI for details.

added to the MTBE solution of **2** at 50 °C for 20 min, and the radiofluorination reactions were analyzed by radio-TLC for radiochemical yield and by chiral HPLC for product identity and ee determination. Using this procedure, a variety of racemic terminal epoxides could be radiolabeled in 23–68% RCY and >90% ee. Ether, aliphatic, and styrenyl epoxides were all well tolerated (**3**, **4**, **5**, **7**). Additionally an epoxide adjacent to relatively acidic C–H bonds, which undergo undesired elimination under standard conditions with KF, provided **6** in 23% RCY and >95% ee (see Supporting Information (SI) for details). Furthermore, a meso epoxide underwent desymmetrization under the same conditions in 25% RCY and >95% ee, allowing the generation of **8**, bearing a stereogenic C–¹⁸F center.²⁴ Disappointingly, labeling of epoxides possessing Lewis basic nitrogens or α -branching with **2** provided poor radioisotope incorporation (<1% RCY). This presented a significant limitation due to the prevalence of such functionality among bioactive molecules.

In order to address this limitation we explored the use of dimeric cobalt catalyst **9** (Figure 2A), reasoning that previously observed rate enhancements induced by **9** for the fluorination of epoxides may enable increased scope.²² The radiosynthesis of dimeric catalyst **10** was achieved in a manner directly analogous to **2** (see SI for details). In an effort to evaluate the reactivity of dimeric catalyst **10**, we selected an array of clinically validated and recently disclosed preclinical PET tracers as case studies.²⁵

To examine the compatibility of **10** with N-containing substrates, [¹⁸F]THK-5105 (**12**) was selected (Figure 2B). [¹⁸F]THK-5105 (**12**) is an experimental PET tracer for imaging of tau pathology, which is an important biomarker for Alzheimer's disease.¹⁴ The reported preparation of **12** relies on the commonly employed strategy of differential protection of a 1,2-diol as a tosylate and THP-ether (Figure 2B). Radiosynthesis is achieved by nucleophilic displacement of the tosylate **11**, followed by acidic cleavage of THP-protected alcohol to provide racemic **12**. In contrast, we found that dimeric cobalt catalyst **10** labeled racemic epoxide **13** in 25% RCY and 85% ee to directly deliver enantioenriched [¹⁸F]THK-5105 (Figure 2B, (S)-**12**). Although the radiochemical yield is diminished compared to that in the reported preparation of [¹⁸F]THK-5105, this result demonstrates the utility of the method with respect to tracer candidates containing Lewis basic functionality.

[¹⁸F]Isatin sulfonamide **15** was selected in order to probe the regioselectivity of [¹⁸F]fluoride delivery with catalyst **10**. [¹⁸F]Isatin sulfonamide **15** is an experimental tracer for imaging of caspases-3 and -7, which are implicated in the process of apoptosis.¹⁶ It was reported that the stereochemistry of the fluorohydrin is integral to the selectivity of caspase inhibition. The reported strategy to prepare **15** utilizes a two-step protocol employing ring opening of diastereomerically pure cyclic sulfonate **14** followed by deprotection to gain access to [¹⁸F]fluorohydrin **15** (Figure 2C). Unfortunately, under the described labeling conditions, cyclic sulfonate **14** undergoes unselective ring opening and results in the formation of a separable mixture of both possible regioisomers, (*R*)-**15** and **16**. In contrast, we observed that dimeric catalyst (*S,S,S,S*)-**10** was capable of labeling a 1:1 mixture of diastereomers (**17**), producing (*R*)-**15** as a single regioisomer in 62% RCY with high levels of diastereocontrol (>20:1), demonstrating the unique selectivity of **10** relative to traditional [¹⁸F]fluoride sources.

Given the mildness of these radiofluorinations (50 °C) in comparison to the fluorohydrin syntheses with [¹⁸F]KF (>100

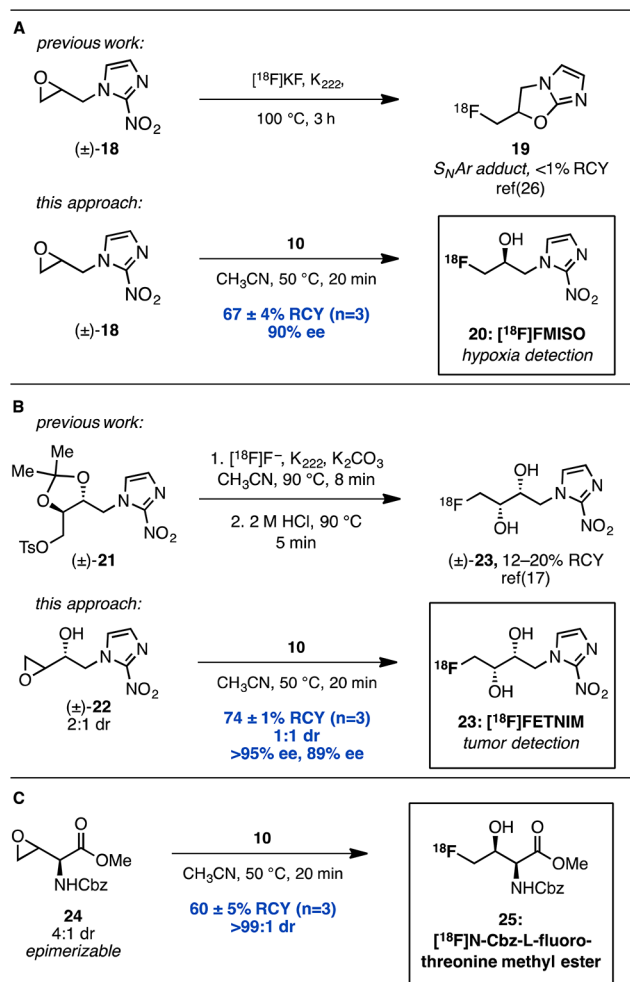


Figure 3. (A) Comparative radiosyntheses of [¹⁸F]FMISO. (B) Comparative radiosyntheses of [¹⁸F]FETNIM. (C) Direct radiosynthesis of [¹⁸F]threonine precursor. Radiochemical yields (RCYs) are the average of *n* runs and are based on activity added to each reaction. RCY was determined by radioTLC. The identity and ee of the product were determined by radio-HPLC. See SI for details.

°C), we sought to demonstrate the utility of the new process for the preparation of nitroimidazole based tracer [¹⁸F]FMISO (**20**), which was developed to image hypoxia within tumors (Figure 3A).¹⁸ Reported attempts at direct radiochemical synthesis of [¹⁸F]FMISO (**20**) from epoxide precursor **18** with [¹⁸F]KF require 3 h at 100 °C and lead primarily to S_NAr chemistry between the nitroimidazole and the pendant alcohol formed as a result of ring opening with fluoride (Figure 3A, **19**).²⁶ In contrast, we found that **10** facilitates the direct radiochemical synthesis of enantioenriched [¹⁸F]FMISO (**20**) in 67% RCY and 90% ee at 50 °C. Given these results, we anticipated that the related tracer [¹⁸F]FETNIM (**23**), which bears an additional hydroxyl group, might be amenable to labeling without the need for protection of epoxy alcohol **22** (Figure 3B).¹⁷ Gratifyingly, formation of [¹⁸F]FETNIM (**23**) was accomplished in 74% RCY directly from racemic epoxy alcohol **22** (2:1 *syn/anti* mixture of diastereomers), and the desired *syn* diastereomer was generated in >95% ee. These examples demonstrate that the low temperature and attenuated basicity of this new radiofluorination protocol enable efficient product formation in the presence of reactive functionality.

Under the conditions utilized for labeling with [^{18}F]KF, substrates prone to base induced epimerization frequently undergo loss of stereochemical information.¹⁵ Given the reduced basicity of **10**, we expected that the integrity of epimerizable stereocenters within substrates could be maintained during labeling. We selected 4-fluorothreonine, a rare example of a fluorine-containing natural product, as a substrate bearing a potentially epimerizable stereocenter. Prepared as a mixture of enantiomerically pure diastereomers (4:1 *syn:anti*), epoxide **24** was subjected to **10**, affording the desired product in 60% RCY (Figure 3C, **25**). It was also observed that the matched catalyst preferentially reacted with the desired *syn*-diastereomer producing **25** as a single diastereomer (>99:1).

Having shown the utility of this method for the manual preparation of known tracers, we next sought to demonstrate the feasibility of a remote, semiautomated radiosynthesis of [^{18}F]FMISO. Utilizing a remote-controlled microwave cavity integrated into an automated liquid handler, 12.3 mCi [^{18}F]FMISO was isolated in 10.6% nondecay corrected RCY (from activity delivered, 37 min total synthesis time) following semipreparative-HPLC. The isolated tracer possessed a Co-content of 5 ppb (ICP-MS) and specific activity of 3.7 Ci/ μmol (EOB), indicating that this method is capable of providing a PET tracer useful for *in vivo* studies.¹⁶

In conclusion, we have demonstrated that commercially available catalyst **2** and dimeric catalyst **10** are capable of radiolabeling a diverse array of complex epoxides with high levels of stereocontrol and functional group compatibility under mild conditions. The protocol makes use of an air-stable catalyst and is operationally simple to carry out. We anticipate that it will facilitate the synthesis of novel PET tracers and also allow investigators to better understand the relationship between stereochemistry and radiotracer imaging properties. Furthermore, the utility of **10** and other transition metal fluorides for mild and selective radiofluorination is a topic of ongoing research in our laboratory.

■ ASSOCIATED CONTENT

● Supporting Information

Experimental procedures, additional reaction optimization and spectroscopic data for all new compounds. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

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- (23) Under radiolabeling conditions with low concentrations of [^{18}F] fluoride, it is unlikely that a [^{18}F]Co(III)F(HF) species is the active nucleophile as was proposed in our cold catalytic system. We therefore describe this species as a [^{18}F]Co(III)F; however, the exact structure is unknown at this time. Furthermore, it is unclear if the OT's counterions undergo additional exchange (e.g., with MeOH or H₂O) during the preparation of **10**.
- (24) To date, attempts at employing nonsymmetrical 1,2-disubstituted epoxides have met with failure with both the mono- and dimeric catalyst systems. See SI for further details.
- (25) Our radiochemical yields are decay-corrected and based on activity added to each substrate. In contrast, the published radiochemical yields for **12**, **15**, **20**, and **23** are based on initial activity delivered from a cyclotron and total activity isolated following purification and dose formulation.
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