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

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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 Nevertheless, the last 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 electrophilic19F sources.13 Nucleophilic fluoride is currently the only practical and generally available source of 19F 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 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
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). 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). 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). 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.

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

Table 1. Scope of Radiofluorination with 2

<table>
<thead>
<tr>
<th>Entry</th>
<th>R1</th>
<th>R2</th>
<th>Radiochemical Yield (RCY)</th>
<th>Enantiomeric Excess (ee)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>OH</td>
<td>OH</td>
<td>44 ± 18% (n=5)</td>
<td>91% ee</td>
</tr>
<tr>
<td>4</td>
<td>OH</td>
<td>Br</td>
<td>68 ± 11% (n=3)</td>
<td>&gt;95% ee</td>
</tr>
<tr>
<td>5</td>
<td>OH</td>
<td>OH</td>
<td>64 ± 27% (n=3)</td>
<td>&gt;95% ee</td>
</tr>
<tr>
<td>6</td>
<td>OH</td>
<td>OH</td>
<td>23 ± 21% (n=3)</td>
<td>&gt;95% ee</td>
</tr>
<tr>
<td>7</td>
<td>OH</td>
<td>OH</td>
<td>33 ± 4% (n=3)</td>
<td>&gt;95% ee</td>
</tr>
<tr>
<td>8</td>
<td>OH</td>
<td>OH</td>
<td>25 ± 7% (n=3)</td>
<td>&gt;95% ee</td>
</tr>
</tbody>
</table>

“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; MTBE: methyl tert-butyl ether.

Table 2. Comparative Radiosyntheses

<table>
<thead>
<tr>
<th>Entry</th>
<th>Previous Work</th>
<th>This Approach</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>OTHP</td>
<td>(R,R,R)-linked salen)Co2X3</td>
</tr>
<tr>
<td>(a)-11</td>
<td>(a)-13</td>
<td>25 ± 5% (n=3) 85% ee</td>
</tr>
<tr>
<td>10</td>
<td>CH2CN, 90 °C, 20 min</td>
<td></td>
</tr>
</tbody>
</table>

Figure 2. (A) Linked-catalyst employed in asymmetric radiofluorination of complex epoxides. (B) Comparative radiosyntheses of [18F]THK-5105. (C) Comparative radiosyntheses of [18F]satin sulfonamides. 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. 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-S105 (12) was selected (Figure 2B). [18F]THK-S105 (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-S105 (Figure 2B, (S)-12). Although the radiochemical yield is diminished compared to that in the reported preparation of [18F]THK-S105, 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 diastereoccontrol (>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 °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$_2$Ar 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 \(^{18}\text{F}\)KF, substrates prone to base induced epimerization frequently undergo loss of steremochemical information.\(^5\) 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 \(^{18}\text{F}\)FMISO. Utilizing a remote-controlled microwave cavity integrated into an automated liquid handler, 12.3 mCi \(^{18}\text{F}\)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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**REFERENCES**

(23) Under radiosynthese with low concentrations of \(^{18}\text{F}\) fluoride, it is unlikely that a \(^{18}\text{F}\)Co(III)F(HF) species is the active nucleophile as was proposed in our cold catalytic system. We therefore describe this species as a \(^{18}\text{F}\)Co(III)F; however, the exact structure is unknown at this time. Furthermore, it is unclear if the OTs counterions undergo additional exchange (e.g., with MoOH 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.