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Alpha selective epoxide opening with $^{18}F^-$: synthesis of 4-(3-[^{18}F]fluoro-2-hydroxypropoxy)benzaldehyde ([^{18}F]FPB) for peptide labeling

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ABSTRACT

Strained tricyclic ring systems such as epoxides are rarely used as precursors for the introduction of anionic fluorine-18 into organic compounds intended for positron emission tomography (PET). Here we report the alpha selective ring opening of epoxides for the introduction of fluorine-18 into small as well as larger biomolecules via 1- and 2-step protocols. [^{18}F]fluoromisonidazole ([^{18}F]MISO), a tracer for hypoxia imaging, and the tumor targeting peptide Tyr^3-octreotate (TATE) were radiolabeled using epoxide opening reactions. In the latter case, the new prosthetic labeling synthon 4-(3-[^{18}F]fluoro-2-hydroxypropoxy)benzaldehyde ([^{18}F]FPB) has been used for ^{18}F-introduction.

Fluorine-18 (^{18}F) is one of the most important radioisotopes used for the labeling of imaging agents utilized in Positron Emission Tomography as non-invasive imaging tools to detect and quantify biological processes in vivo. Over the last years, radiochemistry has devised numerous methods to introduce ^{18}F into small as well as large biomolecules by direct fluorination as well as multistep procedures.[1,2] Strained small cyclic ring systems such as aziridines and epoxides appear to be well-suited precursors for the introduction of $^{18}F^-$ into even complex biomolecules.[3,4] One possible application of this ring opening reaction could be the synthesis of the hypoxia imaging agent [^{18}F]MISO. However, only one example of a successful application of an epoxide precursor in the synthesis of [^{18}F]MISO has been described by Welch and co-workers in 1985 so far, yielding the labeled compound in low radiochemical yields (RCYs).[5] A high yield procedure for [^{18}F]MISO utilizing an epoxide precursor has been reported in a recent patent application where the epoxide opening was carried out in a mixture of acetonitrile and 2-methyl-2-butanol (*t*-amyl-OH) at 100 °C,[6] making use of the accelerating effect of tertiary alcohols in ^{18}F-fluorinations.[7,8] Encouraged by these findings, we systematically investigated the suitability of various epoxide precursors for the one-step introduction of nucleophilic $^{18}F^-$ into model compounds in various solvent and base systems. Furthermore, we explored the labeling of an epoxide-bearing TATE peptide with $^{18}F^-$ in one step and introduced the labeling synthon [^{18}F]FPB derived from its epoxide precursor for the ^{18}F-labeling of aminooxy-derivatized TATE.

The opening of epoxides with fluoride, anhydrous hydrogen fluoride and various other reagents, such as HF-amine complexes, potassium hydrogen fluoride, and silicon tetrafluoride to name just a few is an often used procedure in the preparation of fluorohydrines.[9,10] In ^{18}F-radiochemistry, however, there are only two ^{18}F-species available, namely $^{18}F^-$ and H[^{18}F]F as a result of the production process. Since H[^{18}F]F is difficult to handle and unselective, we focused our investigation on $^{18}F^-$ only. We initially studied the reaction of simple racemic epoxides such as 2-benzyloxirane (**1a**) and 2-(phenoxymethyl)-oxirane (**1b**) in dipolar aprotic solvents, such as acetonitrile, DMF, and DMSO as those are usually used in nucleophilic fluorinations. Using azeotropically 'dried' $^{18}F^-$ in the form of its K^+/Kryptofix2.2.2®/$^{18}F^-$ complex in the presence of either K_2CO_3 or potassium oxalate, RCYs between <1% and 30% were obtained for the corresponding ^{18}F-fluorohydrines at temperatures of 100–160 °C (Table 1). Acetonitrile consistently yielded the highest RCYs followed by DMF and DMSO (<1%). Knowing that tertiary alcohols greatly facilitate nucleophilic reactions between sulfonic acid esters (e.g., tosylates and mesylates) and $^{18}F^-$,[7] we also performed the labeling reactions in *t*-amyl-OH as solvent at elevated temperatures using potassium oxalate as the base. RCYs between 77% and 83% were obtained for both labeled compounds **2a** and **2b** after 30 min at 110 °C (Fig. 1). In the radiosyntheses of **2a–c** we found 7–15% higher RCYs using potassium oxalate as the base compared with K_2CO_3 and thus used the *t*-amyl-OH/oxalate system in all following experiments.

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Table 1
Optimized conditions for the opening of epoxides with $^{18}F^-$ in CH_3CN and *t*-amyl-OH

Epoxide/labeled compound	Solvent/temperature (°C)/time (min)[a]	Base system	RCY% ± (%) *n* = 10
1a/**2a**	CH_3CN/100/30	K_2CO_3	24 ± 2
1a/**2a**	CH_3CN/100/30	$K_2(COO)_2$	30 ± 3
1a/**2a**	*t*-Amyl-OH/110/30	$K_2(COO)_2$	80 ± 3
1b/**2b**	CH_3CN/100/30	K_2CO_3	22 ± 4
1b/**2b**	CH_3CN/100/30	$K_2(COO)_2$	28 ± 2
1b/**2b**	*t*-Amyl-OH/110	$K_2(COO)_2$	86 ± 3
1c/**2c**	CH_3CN/100/30	K_2CO_3	7 ± 3
1c/**2c**	CH_3CN/100/30	$K_2(COO)_2$	10 ± 4
1c/**2c**	*t*-Amyl-OH/100/30	$K_2(COO)_2$	90 ± 5
1d/**2d**[b]	*t*-Amyl-OH/100/30	$K_2(COO)_2$	87 ± 7

[a] The maximum RCY for all reactions was reached at approx. 30 min.
[b] The reaction of **1d** with $^{18}F^-$ was only performed in *t*-amyl-OH.

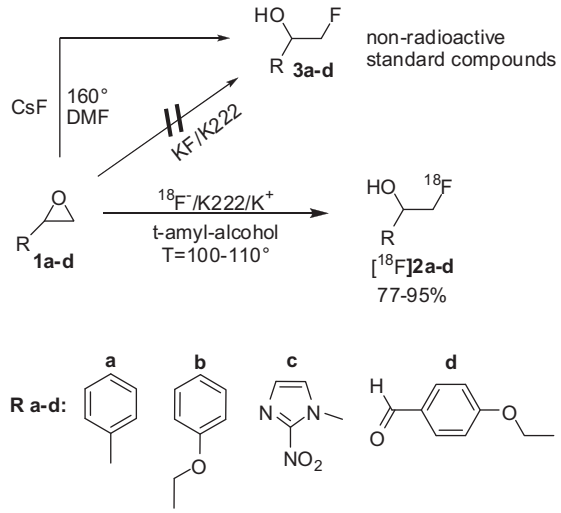

Figure 1. α-Selective epoxide opening of model compounds **1a** and **1b**, the labeling precursor **1c** for the synthesis of [^{18}F]FMISO and **1d** yielding [^{18}F]**2d** for peptide labeling via oxime formation. Non-radioactive standard compounds **3a**–**d** were synthesized reacting **1a**–**d** with CsF in DMF at 160 °C.

Interestingly and in contrast to the reactions performed in dipolar aprotic solvents, in *t*-amyl-OH no radioactive side products were detected either by radio-TLC or radio-HPLC. The same reaction conditions were applied to the synthesis of [^{18}F]MISO (**2c**) from its epoxide precursor 2-nitro-1-(oxiran-2-ylmethyl)-1*H*-imidazole (**1c**). [^{18}F]MISO was obtained in RCYs of 80–95% in pure *t*-amyl-alcohol after 30 min reaction time at 100 °C using $K_2(COO)_2$ as base. With this labeling reaction, the [^{18}F]MISO formation reached a plateau of about 90(±5)% RCY after 30 min. After 5, 10, and 20 min, the RCYs were about 45(±5)%, 61(±5)%, and 78%(±5), respectively. The corresponding reaction in CH_3CN gave 7–10% RCY only (Table 1). The preparative RCYs of [^{18}F]MISO after HPLC purification were 55% after a total synthesis time of 60 min.

In order to validate if those conditions are applicable to the ^{18}F-labeling of more complex biomolecules such as peptides, Tyr^3-octreotate was N-terminally modified with aminooxy-acetic acid[11,12] (**6**, Fig. 2) and conjugated to racemic 4-(oxiran-2-ylmethoxy)benzaldehyde (**1d**) by chemoselective oxime formation yielding peptide **4** (Fig. 2).[12] The direct ^{18}F-fluorination of **4** under various conditions did not yield the desired labeled peptide [^{18}F]**5**. Neither dipolar aprotic solvents nor *t*-amyl-OH resulted in the formation of radiolabeled [^{18}F]**5**. Besides unreacted $^{18}F^-$, different non-radioactive side products could be detected by HPLC (UV channel) which were not further analyzed. This is in contrast to the results reported for the one-step aziridine opening of complex biomolecules by $^{18}F^-$.[4] It is likely that the N-terminal epoxide moiety reacts unselectively with side chain functionalities of the peptide.

To finally obtain [^{18}F]**5**, the new prosthetic labeling precursor [^{18}F]FPB ([^{18}F]**2d**) was synthesized from **1d** in high RCYs between 80% and 95% (based on HPLC) and conjugated to **6** in aqueous solution at pH 4 to form [^{18}F]**5** via chemoselective oxime formation in reliable RCYs of 60–70% as determined by analytical HPLC. The ^{18}F-labeled product could be isolated in preparative RCYs of 28–32% after 65–70 min total preparation time (Figs. 1 and 2). The preparative HPLC purification of [^{18}F]**5** was particularly easy since no radioactive side products were formed during the conjugation reaction (Fig. 3).

The preparation of the secondary labeling precursor [^{18}F]FPB as well as its conjugation efficiency to aminooxy-derivatized peptides are thus comparable to the currently, mainly used aldehyde-comprising secondary labeling precursor ^{18}F-fluorobenzaldehyde[13,14] and identify [^{18}F]FPB as a well-suited secondary labeling precursor for the radiolabeling of complex aminooxy-derivatized molecules.

In all investigated reactions, the opening was 100% α-selective with the $^{18}F^-$ reacting at the sterically less hindered position which is in accordance to the findings of Park et al.[15] During the epoxide ring opening reactions, two different enantiomers are formed which should in general be of no concern for an in vivo application of labeled biomolecules obtained by this reaction type. For [^{18}F]MISO, the formation of enantiomers was postulated not to negatively influence the pharmacologic properties of the radiotracer as well.[16] Nevertheless, the formation of enantiomers produced during the epoxide opening may have negative effects on the pharmacologic properties of small molecules and thus has to be kept in mind.

Interestingly, the non-radioactive ^{19}F-standard compounds **3a**–**d** synthesized for analytical purposes could not be obtained using the corresponding ^{18}F-labeling conditions employing macroscopic amounts of KF/K222 in *t*-amyl-OH.

The ^{19}F-fluorinated compounds **3a** and **3b** could only be synthesized from the epoxide precursors using CsF in DMF at 160 °C for 24 h and analytical data (^{1}H, ^{13}C NMR, mass) were compared with literature data.[17,18] Compound **3c**, which was used as non-radioactive standard compound, was purchased from ABX (Radeberg, Germany). The non-radioactive standard compound **3d** was obtained as follows: The racemic epoxide **1d** (0.35 g, 1.96 mmol) and CsF (0.89 g, 5.88 mmol) were dissolved in DMF (7 mL) and reacted at 160 °C for 24 h. Compound **3d** was purified via column chromatography. *Compound* **3d**: ^{1}H NMR (400 MHz, $CDCl_3$) δ (9.84 (s, 1H), 7.8 (d, 2H, J = 8.85 Hz), 6.98 (d, 2H, J = 8.74 Hz), 4.57 (ddd, 2H, J = 46.97 Hz, 4.63 Hz, 2.31 Hz); ^{13}C NMR (400 MHz, $CDCl_3$) δ 190.80, 132.15, 115.08, 76.80, 69.19, 50.00, 44.72, 29.87, ESI-MS: m/z 199 ([M+H]$^+$).

The synthesis of aminooxy-derivatized TATE (**6**) was performed as previously described.[19] The non-radioactive peptide **5** and the

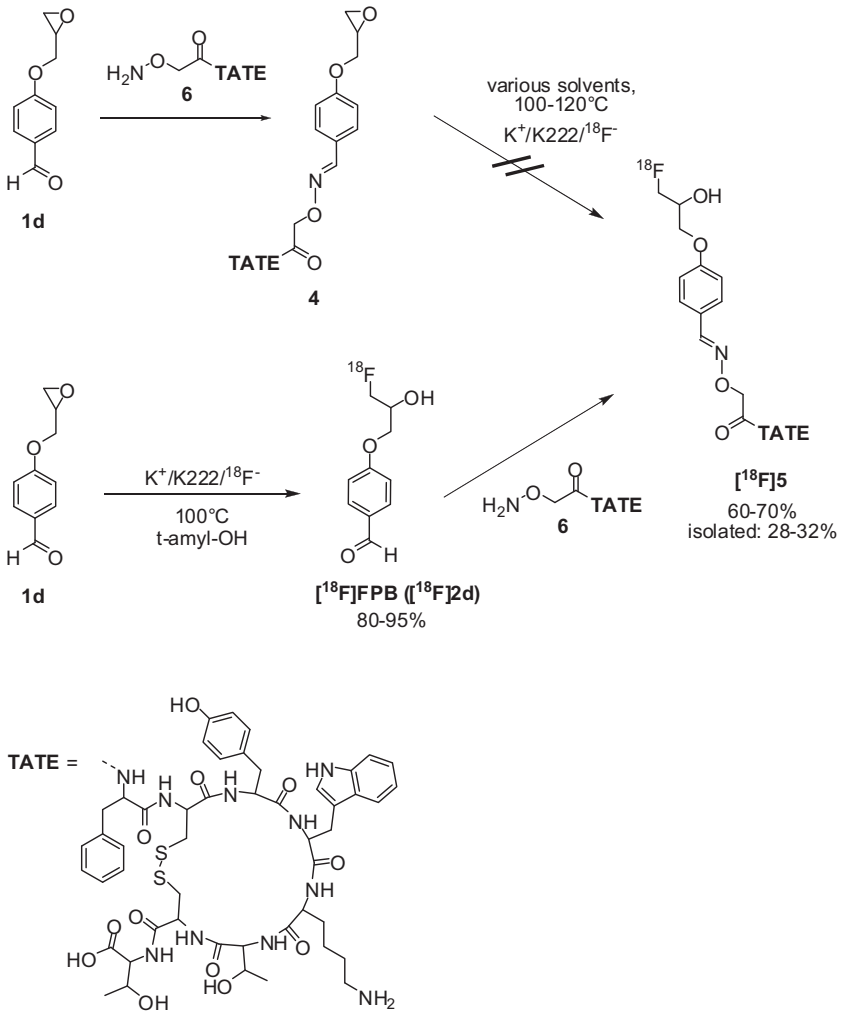

Figure 2. Synthesis of [^{18}F]**5** via oxime formation between [^{18}F]FPB ([^{18}F]**2d**) and **6** at pH 4. The reaction between **4** and $^{18}F^-$ did not yield [^{18}F]**5**.

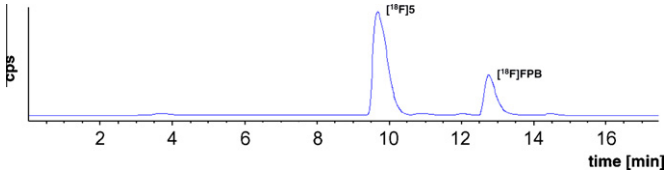

Figure 3. Radio-HPLC chromatogram of the crude reaction mixture of **6** and [^{18}F]FPB ([^{18}F]**2d**) yielding [^{18}F]**5** (column: Chromolith® Performance RP-18 100∗4.6 mm, acetonitrile/H_2O gradient 100% H_2O at t = 0–100% acetonitrile at t = 30 min, flow: 1.5 mL/min).

labeling precursor **4** for the one-step reaction with $^{18}F^-$ were synthesized following standard procedures.[20,13] *Compound* **4**: MALDI-TOF-MS: m/z 1281.5 ([M+H]$^+$). *Compound* **5**: MALDI-TOF-MS: m/z 1301.6 ([M+H]$^+$).

*Synthesis of [^{18}F]FPB ([^{18}F]**2d**) and [^{18}F]**5***: To azeotropically dried $^{18}F^-$/Kryptofix2.2.2®/K^+/$K_2(COO)_2$ (50–60 mCi) in *t*-amyl-OH (0.5 mL) was added **1d** (3 mg, 16.8 μmol) and the mixture was heated to 100 °C in a sealed Wheaton Vial (5 mL) for 30 min. The solution was diluted with CH_3CN (1 mL) and [^{18}F]**2d** was purified by semi-preparative HPLC. Water (5 mL) was added to the collected [^{18}F]**2d** fraction and the mixture was passed through a C18 SepPak cartridge (Waters). The cartridge was eluted with DMSO (0.7 mL) quantitatively eluting [^{18}F]**2d** (25–42 mCi, preparative yield). Subsequently, [^{18}F]**2d** (5–10 mCi) was reacted with **6** (0.2 mg, 0.17 μmol) following a published procedure[19] and purified by HPLC.

In conclusion, we have demonstrated that nucleophilic α- selective opening of epoxides with $^{18}F^-$ is well-suited to prepare secondary labeling precursors such as [^{18}F]FPB which in turn can be used for efficient peptide labeling. The ^{18}F-labeling of epoxides is strongly facilitated by the use of the tertiary alcohol *t*-amyl-OH.

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