

RESEARCH REPORT

Synthesis of Asymmetric Urea Scaffolds and an Organometallic Rhenium Complex for Dual-Modality Soluble Epoxide Hydrolase Radiotracer Development

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SPONSORSHIP

Canadian Nuclear Society (CNS)

Undergraduate Scholarship Awardee

October 1, 2026

Abstract

Soluble epoxide hydrolase (sEH) is a multifunctional enzyme that regulates inflammation and vascular tone, representing a promising biomarker for molecular imaging. In this study, we established the foundational chemical framework for dual-modality Positron Emission Tomography (PET) and Single-Photon Emission Computed Tomography (SPECT) sEH radiotracer development. A library of five novel asymmetric urea scaffolds (Scaffolds 1-5) was synthesized using triphosgene mediated coupling from functionalized piperidine cores, serving as benchmark precursors for future carbon-11 (^{11}C) radiolabeling with $[^{11}\text{C}]\text{CO}_2$ fixation. To address SPECT derivation, a cyclopentadienyl-rhenium tricarbonyl surrogate complex was prepared using high temperature organometallic complexation, validating metal-tricarbonyl chelation. This bench synthesis provides the necessary starting material to execute downstream NMR characterization, $[^{11}\text{C}]\text{CO}_2$ radiosynthesis, and *in vitro* binding assays, advancing these novel probes toward preclinical imaging of inflammation and carcinogenesis.

Keywords: soluble epoxide hydrolase (sEH); carbon-11 fixation; rhenium tricarbonyl; radiotracer development; positron emission tomography (PET); single-photon emission computed tomography (SPECT)

Introduction

As a multifunctional enzyme, soluble epoxide hydrolase (sEH) regulates vasoconstriction and inflammation, linking its activity to a broad spectrum of disorders and making it a highly attractive therapeutic target [1]. sEH is widely expressed in the liver, kidney, and brain, and regulates immune signaling by metabolizing anti-inflammatory epoxy fatty acids (EpFAs) into 1,2-diol forms [1]. Maintaining higher EpFA levels can prevent mitochondrial dysfunction [2], whereas the accumulation of the resultant 1,2-diols signals downstream pro-inflammatory pathways [1, 2]. Thus, increased sEH viability or activity promotes vasoconstriction and inflammation, making sEH inhibition a highly pursued therapeutic approach [1]. Currently, sEH is of significant interest in central nervous system disorders, including ischemic stroke, dementia, parkinsonism, and psychiatric conditions [3]. While most prior work has focused exclusively on sEH's role in neuroinflammatory and neurodegenerative diseases, there remains a critical need to explore its role in broader inflammatory pathways and carcinogenesis [4].

In addition to serving as a therapeutic target, radioligands selective for sEH have been used as imaging biomarkers with Positron Emission Tomography (PET) [5]. Most research to date has focused on PET imaging because of its superior imaging resolution. However, its reliance on short-lived radioisotopes through cyclotron production creates a high-cost roadblock that limits its clinical availability [6]. Developing more cost-effective and broadly available Single-Photon Emission Computed Tomography (SPECT) tracers is therefore essential to address the current accessibility gap in molecular imaging, thereby enabling clinical applications in settings where PET is not feasible [6].

To establish the foundation for dual-modality sEH radiotracer development, this study served as an initial starting point focused on the organic synthesis of novel urea-based inhibitor candidates. Specifically, our objectives were to prepare a series of core urea-based sEH inhibitor scaffolds assembled from amine precursors, establishing the chemical framework required for future carbon-11 (^{11}C) radiolabeling via $[^{11}\text{C}]\text{CO}_2$ fixation [7]. In this work, five final urea scaffolds were synthesized. This bench synthesis provides the necessary starting material for subsequent project phases, which will involve completing structural characterization across all compounds using NMR spectroscopy, validating $[^{11}\text{C}]\text{CO}_2$ radiosynthesis feasibility, evaluating *in vitro* sEH binding affinity, and utilizing the precursor amines to derive cyclopentadienyl-conjugated technetium-99m ($^{99\text{m}}\text{Tc}$) SPECT analogues.

Materials and Methods

Synthesis of Cyclopentadienyl (Cp) SPECT Precursor Scaffold

Step 1: Allylic Bromination

Ethyl 3-methyl-2-butenate (1.0 equiv), N-bromosuccinimide (NBS, 1.1 equiv), and azobisisobutyronitrile (AIBN, 0.02 equiv) were combined in a 2-5 mL microwave reaction vial. The vessel was sealed and heated to 110 °C for 15 min under low pressure. The resulting yellow reaction mixture was dissolved in dichloromethane (CH_2Cl_2 , 50 mL), washed with saturated aqueous sodium sulfite (Na_2SO_3), and dried over anhydrous sodium sulfate (Na_2SO_4). The suspension was filtered, and the filtrate was concentrated under

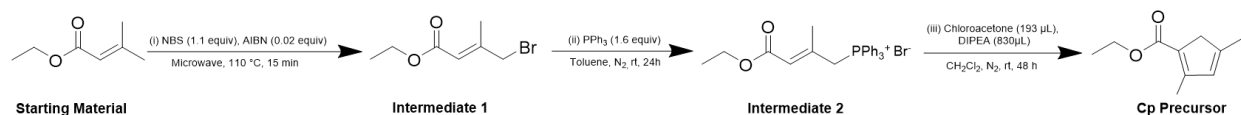
reduced pressure via rotary evaporation to yield the crude brominated intermediate (82% yield).

Step 2: Phosphonium Salt Formation

Triphenylphosphine (PPh_3 , 1.6 g) was dissolved in toluene (52 mL) in a round-bottom flask and stirred under a nitrogen (N_2) atmosphere. A solution of the brominated product from Step 1 in toluene (13 mL) was added, and the mixture was stirred at room temperature for 24 h. The resulting precipitate was collected by vacuum filtration using filter paper, and the filtrate was concentrated under reduced pressure using rotary evaporation to isolate the remaining product, affording the phosphonium salt intermediate (34.5% combined yield).

Step 3: Cyclopentadienyl Ring Annulation and Coupling

The phosphonium salt from Step 2 was dissolved in anhydrous dichloromethane (CH_2Cl_2 , 21 mL) in a round-bottom flask and stirred for 5 min under a nitrogen (N_2) atmosphere. *N,N*-Diisopropylethylamine (DIPEA, 830 μL) and chloroacetone (193 μL) were added, and the reaction mixture was stirred at room temperature for 48 h. 30 mL of water was added and the aqueous phase was washed with dichloromethane (CH_2Cl_2 , 2 x 30 mL) and dried over anhydrous sodium sulfate and concentrated using rotary evaporation. The crude mixture was purified by automated flash column chromatography using an *n*-hexane/ethyl acetate gradient (1 column volume (CV) at 2%, 12 CV from 2% to 25%, and 2 CV at 25% ethyl acetate). The target Cp intermediate was isolated as a clear oily liquid (25% yield)

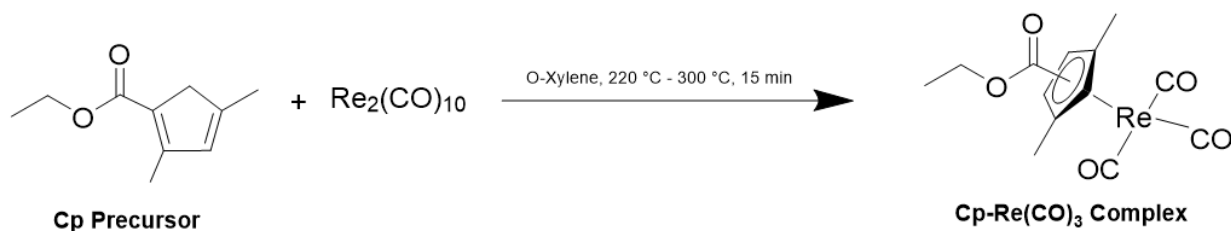


Scheme 1. Synthetic route for the cyclopentadienyl (Cp) SPECT precursor scaffold.

Reagents and reaction conditions: (i) N-bromosuccinimide (NBS, 1.1 equiv), azobisisobutyronitrile (AIBN, 0.02 equiv), microwave irradiation, 110 °C, 15 min, 82% yield; (ii) triphenylphosphine (PPh_3 , 1.6 g), toluene, N_2 , room temperature, 24h, 34.5% yield; (iii) chloroacetone (193 μL), N,N-diisopropylethylamine (DIPEA, 830 μL), anhydrous CH_2Cl_2 , N_2 , room temperature, 48 h, automated flash chromatography (n-hexane/ethyl acetate gradient) 25.0% yield.

Synthesis of Rhenium-Cyclopentadienyl (Re-Cp) Complex

The cyclopentadienyl (Cp) precursor (0.25 mmol, 2.0 equiv) and dirhenium decacarbonyl ($\text{Re}_2(\text{CO})_{10}$, 0.125 mmol, 1.0 equiv) were added to a microwave reaction vial containing o-xylene (0.5 mL). The vial was sealed, placed in an oil bath heated to 220-300 °C, and stirred for 15 min. Thin-layer chromatography (TLC) revealed an impure crude mixture, with the reaction forming a dark black oil. The crude material was purified via automated flash column chromatography using an n-hexane/ethyl acetate solvent gradient (1 CV at 2%, 12 CV from 2% - 25%, and 2 CV at 25% ethyl acetate). Two distinct chromatographic peaks were observed; the target fractions across three collection tubes were combined and concentrated under reduced pressure using rotary evaporation to yield the isolated product as white crystals (32% yield).



Scheme 2. Organometallic complexation of Cp precursor to form the cold rhenium

tricarbonyl surrogate. Cyclopentadienyl precursor (0.25 mmol, 2.0 equiv), dirhenium decarbonyl ($\text{Re}_2(\text{CO})_{10}$, 0.125 mmol, 1.0 equiv), o-xylene (0.5 mL), oil bath heating (220-300 °C), 15 min. Automated flash chromatography (2-25% ethyl acetate/n-hexane gradient), 42.0% yield (0.052 mmol, isolated as white crystals).

Synthesis of 1-(Adamantan-1-yl)-3-(piperidine-4-yl)urea Scaffold (1-Adamantyl Urea Derivative):

A solution of 1-adamantanamine (1.0 mmol) and N-N-diisopropylethylamine (DIPEA, 191 μL , 1.1 equiv) in dichloromethane (CH_2Cl_2 , 1.0 mL) was prepared in a reaction vial. In a 10 mL round-bottom flask, triphosgene (0.38 mmol, 0.38 equiv) was dissolved in anhydrous CH_2Cl_2 (2.0 mL) and cooled to 0 °C in an ice bath. The amine solution was added dropwise to the triphosgene solution with continuous stirring, and the vial was rinsed with additional CH_2Cl_2 (0.5 mL), forming a white cloudy reaction mixture. The mixture was stirred at 0 °C for 1 h to complete isocyanate formation. Subsequently, a solution of tert-butyl 4-aminopiperidine-1-carboxylate (1.0 mmol, 1.0 equiv) and DIPEA

(348 μ L, 2.0 equiv) in CH_2Cl_2 (1.0 mL) was added dropwise. The reaction was stirred for 45 min, yielding a clear-to-cloudy solution.

The reaction was quenched with water (1.5 mL) and saturated aqueous sodium chloride (brine, 1.5 mL), acidified with 3 M HCl, and extracted with CH_2Cl_2 (3 x 5 mL). The combined organic layers were dried over anhydrous sodium sulfate (Na_2SO_4), filtered, and concentrated under reducing pressure to afford a crude yellow oil. Purification was performed using automated flash column chromatography (100% CH_2Cl_2 for 2 CV, 0-7% MeOH / CH_2Cl_2 gradient over 10 CV, and 7% MeOH/ CH_2Cl_2 for 10 CV). Since the scaffold lacks a UV active chromophore, fractions were monitored and visualized using ninhydrin staining.

For Boc-deprotection, the purified intermediate (0.1 mmol scale) was dissolved in CH_2Cl_2 (1.0 mL) and treated with trifluoroacetic acid (TFA, 1.0 mL). The solution was stirred at room temperature for 1 h and concentrated using rotary evaporation to yield the target deprotected amine precursor as a dark orange oil (0.7 mmol, 70% overall yield).

Synthesis of tert-Butyl 4-(3-(4-(Trifluoromethoxy)phenyl)ureido)piperidine-1-carboxylate (4-TFM Urea Derivative)

A solution of 4-(trifluoromethoxy)aniline (1.0 mmol) and N-N-Diisopropylethylamine (DIPEA, 191 μ L, 1.1 equiv) in CH_2Cl_2 (1.0 mL) was added dropwise to a stirred solution of triphosgene (0.38 mmol) in CH_2Cl_2 (2.0 mL) at 0 $^\circ\text{C}$ in an ice bath. Active gas evolution was observed upon addition, and the mixture was stirred for 1 h. A solution of tert-butyl 4-

aminopiperidine-1-carboxylate (1.0 mmol) and DIPEA (348 μ L) in CH_2Cl_2 (1.0 mL) was added dropwise, and the resulting yellow solution was stirred for 45 min.

Workup was conducted identically to the adamantyl derivative (quenching with 1.5 mL H_2O in 1.5 mL brine, acidification was 3 M HCl, extraction with 3 x 5 mL CH_2Cl_2 , drying over Na_2SO_4 and rotary evaporation). The crude material was purified by automated gradient flash chromatography with UV detection at 240 nm, yielding two resolved clean peaks. Concentrating the target fraction yielding the Boc-protected urea scaffold. For Boc-deprotection, the purified intermediate (0.1 mmol scale) was dissolved in CH_2Cl_2 (1.0 mL) and treated with trifluoroacetic acid (TFA, 1.0 mL). This solution was stirred at room temperature for 1 h and rotary evaporated, yielding the target deprotected urea scaffold as a white solid (69% yield).

Synthesis of Tosylated Amine Precursor (Precursor 1)

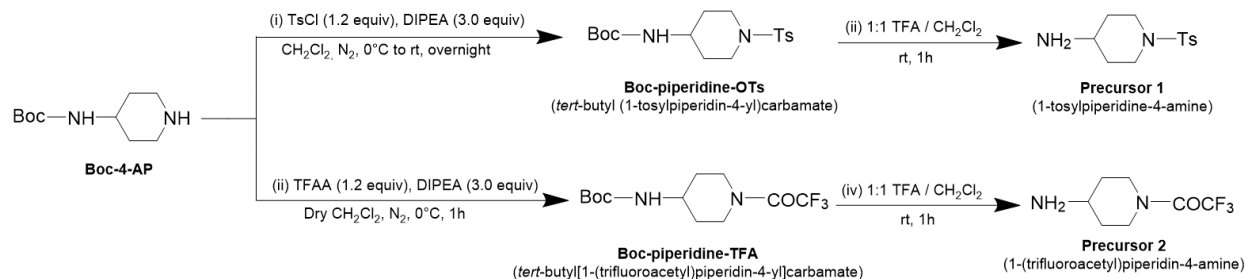
Tert- Butyl 4-aminopiperidine-1-carboxylate (1.0 mmol) was dissolved in anhydrous dichloromethane (CH_2Cl_2 , 10.0 mL) in a 25 mL round-bottom flask and stirred under a nitrogen (N_2) atmosphere at 0 $^\circ\text{C}$ in an ice bath. *N*-*N*-Diisopropylethylamine (DIPEA, 522 μ L, 3.0 equiv) was added, and the solution was stirred for 5 min. A solution of *p*-toluenesulfonyl chloride (TsCl, 1.2 mmol, 1.2 equiv) in CH_2Cl_2 (2.0 mL) was added dropwise, and the reaction was allowed to warm to room temperature and stir overnight. The reaction was quenched with water (2 x 5.0 mL), saturated aqueous sodium carbonate (Na_2CO_3), filtered through a fritted funnel, and concentrated via rotary evaporation to yield

a crude white solid. Automated flash column chromatography (100% CH₂Cl₂ for 2 CV, 0-5% MeOH/CH₂Cl₂ gradient over 10CV, and 5% MeOH/CH₂Cl₂ for 12 CV) with UV detection at $\lambda = 210$ nm and 225 nm afforded the tosylated intermediate as a white solid.

Deprotection with 1:1: TFA/CH₂Cl₂ (1.0 mL each per 0.1 mmol) for 1 h yielded the amine tosylate precursor as a TFA salt.

Synthesis of Trifluoroacetylated Amine Precursor (Precursor 2)

Tert-butyl 4-aminopiperidine-1-carboxylate (1.0 mmol) was dissolved in anhydrous CH₂Cl₂ (8.0 mL) and DIPEA (522 μ L, 3.0 equiv) under N₂ at 0 °C. Trifluoroacetic anhydride (TFA, 1.2 mmol, 1.2 equiv) in CH₂Cl₂ (1.0 mL) was added dropwise, observing intermediate white gas evolution with each drop. The mixture was stirred for 1 h and monitored by ninhydrin staining. The reaction was quenched with water (5.0 mL), washed with water (2 x 5.0 mL), saturated aqueous sodium bicarbonate (NaHCO₃, 10.0 mL), and brine (10.0 mL), dried over Na₂SO₄, and concentrated under reduced pressure. Flash column chromatography (0-5% MeOH/CH₂Cl₂) guided by ninhydrin staining isolated the intermediate. Acidolysis of the Boc group (1:1 TFA/CH₂Cl₂, 1h) yielded the trifluoroacetylated amine precursor as an oil (0.88 mmol, 88% yield).



Scheme 3. Synthetic routes for tosylated (Precursor 1) and trifluoroacetylated

(Precursor 2) piperidine cores. (i) *tert*-Butyl 4-aminopiperidine-1-carboxylate (1.0 mmol) *p*-toluenesulfonyl chloride (TsCl, 1.2 equiv), DIPEA (3.0 equiv), anhydrous CH_2Cl_2 , N_2 , 0°C to room temperature, overnight, 37% yield. (ii) 1:1 TFA/ CH_2Cl_2 , room temperature, 1 h, quantitative yield (Precursor 1, isolated as TFA salt). (iii) *tert*-Butyl 4-aminopiperidine-1-carboxylate (1.0 mmol), trifluoroacetic anhydride (TFAA, 1.2 equiv), DIPEA (3.0 equiv), anhydrous CH_2Cl_2 , N_2 , 0°C , 1 h. (iv) 1:1 TFA/ CH_2Cl_2 , room temperature, 1 h, 88% overall yield (Precursor 2).

General Procedure for Triphosgene-Mediated Urea Coupling

A primary amine stock (1-adamantanamine or 4-trifluoromethoxy)aniline, 1.0-1.2 equiv) and DIPEA (1.5-2.0 equiv) in anhydrous CH_2Cl_2 (1.0 mL) were added dropwise to a stirred solution of triphosgene (0.30-0.35 equiv) in CH_2Cl_2 (2.0 mL) at 0°C in an ice bath. The reaction was stirred for 30 min to complete *in-situ* iso-cyanate formation.

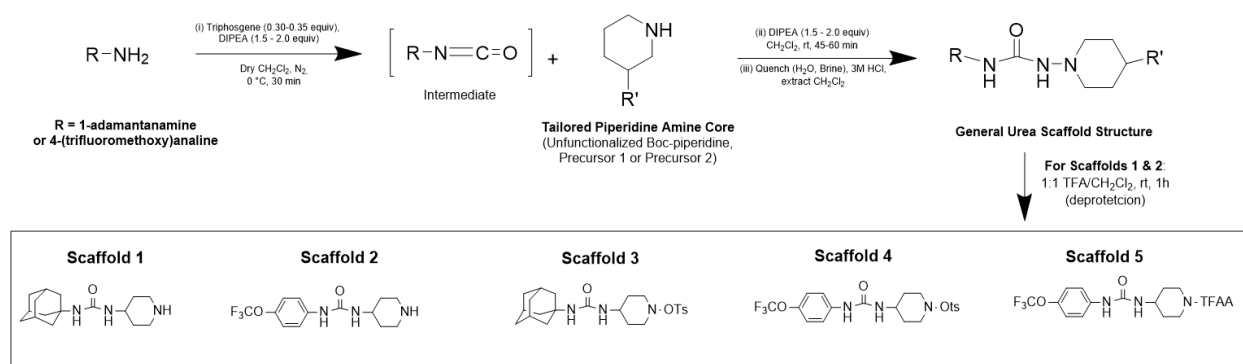
Subsequently, a solution of the functionalized piperidine precursor (Precursor 1 or Precursor 2, 1.0 equiv) and DIPEA (1.5-2.0 equiv) in CH_2Cl_2 (1.0 mL) was added dropwise. The reaction was stirred at room temperature for 1 h, quenched with water and brine

solution (1.5 mL each), acidified with 3 M HCl, extracted with CH₂Cl₂ (3 x 5.0 mL), dried over Na₂SO₄, and purified by automated flash column chromatography (n-hexane/ethyl acetate or MeOH/CH₂Cl₂ gradient).

Urea Scaffold 3 (Adamantyl-Piperidine-OTs): Prepared from 0.70 mmol 1-adamantanamine, 0.20 mmol triphosgene, and Precursor 1. Isolated as an off-white solid (0.23 mmol, 62% yield).

Urea Scaffold 4 (4-TFM-Piperidine-OTs): Prepared from 0.70 mmol 4-(trifluoromethoxy)aniline, 0.20 mmol triphosgene, and Precursor 1. Purified at $\lambda = 210$ nm and 242 nm to yield white crystalline solids (0.25 mmol, 68% yield).

Urea Scaffold 5 (4-TFM-Piperidine-TFAA): Prepared from 0.151 mmol 4-(trifluoromethoxy)aniline, 0.04 mmol triphosgene, and 0.126 mmol Precursor 2. Isolated as a white solid (0.086 mmol, 68% yield).



Scheme 4. Triphosgene-mediated asymmetric coupling for urea-scaffolds 1-5. (i)

Primary amine stock 1-adamantanamine or 4-(trifluoromethoxy)aniline, 1.0 equiv), DIPEA (1.5- 2.0 equiv), triphosgene (0.30 - 0.35 equiv), anhydrous CH₂Cl₂, N₂, 0 °C, 30-60 min. (ii) Unfunctionalized or tailored piperidine precursor (Precursor 1 or Precursor 2, 1.0 equiv),

DIPEA (1.5 - 2.0 equiv), CH₂Cl₂), room temperature, 45-60 min. (iii) Quench (H₂O/brine), acidification (3 M HCl), extraction (CH₂Cl₂). Automated gradient flash chromatography (62-70% yields).

Results

Multi-Step Synthesis of Urea-Based sEH Inhibitor Scaffolds

To construct a benchmark library for future ¹¹C-CO₂ fixation and PET imaging, five novel urea-based soluble epoxide hydrolase (sEH) inhibitors were synthesized from their corresponding amine precursors. The multi-step organic synthesis was carried out using temperature-controlled reaction conditions, including cryogenic (ice-bath) and reflux heating setups. Following crude reaction workups via liquid-liquid extraction and vacuum filtration, the urea intermediates were isolated using rotary evaporation and purified by automated flash column chromatography. Structural confirmation and chemical purity of the initial urea scaffolds were verified using ¹H and ¹³C Nuclear Magnetic Resonance (NMR) spectroscopy.

Preparation of Cyclopentadienyl (Cp) Precursor for SPECT Derivation

To establish the chemical foundation for parallel Single-Photon Emission Computed Tomography (SPECT) tracer development, a cyclopentadienyl (Cp)-

functionalized precursor was synthesized in a three-step sequence. Allylic bromination of ethyl 3-methyl-2-butenate afforded the brominated intermediate in 82% yield. Subsequent nucleophilic substitution with triphenylphosphine yielded the phosphonium salt intermediate (34.5% yield). Final annulation with chloroacetone in the presence of DIPEA yielded the target Cp-functionalized precursor as a clear oil in 25% yield after automated gradient flash chromatography. This Cp scaffold provides the necessary chelating framework for future technetium-99m (^{99m}Tc) radiolabeling via tricarbonyl coordination.

Organometallic Complexation and Isolation of Re-Cp Surrogate

To validate the chelation capability of the cyclopentadienyl scaffold as a surrogate for future technetium-99m (^{99m}Tc) SPECT radiolabeling, a cold complexation reaction was performed using dirhenium decacarbonyl ($\text{Re}_2(\text{CO})_{10}$). High-temperature reaction in o-xylene (220–300 °C) for 15 min converted the starting materials into a dark oil. While initial TLC monitoring indicated an impure crude mixture, automated flash column chromatography successfully resolved two distinct elution peaks. Combining and concentrating the pure product fractions yielded white crystals, confirming successful formation and isolation of the cyclopentadienyl rhenium tricarbonyl complex.

Triphosgene-Mediated Synthesis of Asymmetric Urea Scaffolds

To construct core urea-based soluble epoxide hydrolase (sEH) inhibitors, asymmetric coupling reactions were conducted using triphosgene as a carbonyl

equivalency reagent. Intermediate *in-situ* isocyanate formation from primary aromatic and aliphatic amines proceeded smoothly at 0 °C, followed by nucleophilic attack by *tert*-butyl 4-aminopiperidine-1-carboxylate to form the central urea linkages.

Adamantyl Scaffold

Coupling 1-adamantanamine with the piperidine core yielded a crude yellow oil. Since aliphatic adamantyl scaffolds lack strong UV absorbance, fraction collection during flash chromatography was guided by ninhydrin staining. Subsequent acidolysis of the *N*-Boc protecting group using 1:1 TFA/CH₂Cl₂ afforded the deprotected amine precursor as a dark orange oil (0.7 mmol, 70% yield).

4-(Trifluoromethoxy)phenyl Scaffold

Reaction of 4-(trifluoromethoxy)aniline generated visible gas evolution during isocyanate formation. Following nucleophilic addition, purification monitored at $\lambda = 240$ nm resolved two distinct peaks, isolating the intact Boc-protected urea scaffold. Deprotection using 1:1 TFA/CH₂Cl₂ isolated deprotected urea scaffold as a crystalline white solid (69% yield).

These two synthesized urea candidates represent key benchmark scaffolds for subsequent [¹¹C]CO₂ fixation testing and amine-precursor coupling for dual-modality PET/SPECT sEH imaging.

Multi-Step Synthesis of Urea-Based sEH Inhibitor Scaffolds

To construct a benchmark library for future ^{11}C -CO₂ fixation and PET imaging, five novel urea-based soluble epoxide hydrolase (sEH) inhibitors were synthesized from their corresponding primary amine starting materials and functionalized piperidine cores. Triphosgene-mediated asymmetric couplings proceeded via *in situ* isocyanate formation at 0 °C, followed by nucleophilic attack by the respective piperidine derivative:

1. Unfunctionalized Piperidine Scaffolds (Scaffolds 1 and 2)

Coupling 1-adamantanamine with tert-butyl 4-aminopiperidine-1-carboxylate followed by TFA acidolysis yielded Scaffold 1 (1-adamantyl derivative) as a dark orange oil (0.7 mmol, 70% yield). The corresponding reaction with 4-(trifluoromethoxy)aniline created Scaffold 2 (4-TFM derivative) as a white crystalline solid (0.69 mmol, 69% yield).

2. Tosylated Piperidine Scaffolds (Scaffolds 3 and 4)

Reaction of the tosylated piperidine precursor (Precursor 1) with 1-adamantanamine and 4-(trifluoromethoxy)aniline afforded Scaffold 3 (off-white solid, 0.23 mmol, 62% yield) and Scaffold 4 (white crystalline solid, 0.25 mmol, 68% yield), respectively.

3. Trifluoroacetylated Piperidine Scaffold (Scaffold 5)

Coupling 4-(trifluoromethoxy)aniline with the trifluoroacetylated piperidine precursor (Precursor 2) yielded Scaffold 5 as a white solid (0.086 mmol, 68% yield). (Note:

The attempted coupling of the adamantyl analogue with Precursor 2 was unviable and omitted from the library).

Table 1. Isolated yields, physical appearances, and purification parameters for synthesized sEH inhibitor scaffolds and precursors.

Scaffold	Target Modality / Chemical Pharmacophore	Isolated Yield (mmol / %)	Physical Appearance
Scaffold 1	PET / 1-Adamantyl-3-(piperidine-4-yl)urea	0.70 mmol (70%)	Dark orange oil
Scaffold 2	PET / 4-TFM-phenyl-3-(piperidin-4-yl)urea	0.69 mmol (69%)	Crystalline white solid
Scaffold 3	PET / 1-Adamantyl-3-(piperidin-4-yl-OTs)urea	0.23 mmol (62%)	Off white solid
Scaffold 4	PET / 4-TFM-phenyl-3-(piperidin-4-yl-OTs)urea	0.25 mmol (68%)	White crystalline solid
Scaffold 5	PET / 4-TFM-phenyl-3-(piperidin-4-yl-TFA)urea	0.086 mmol (68%)	White solid
Cp Precursor	SPECT / Cyclopentadienyl-Rhenium complex	0.052 mmol (42%)	White solid

Yields represent purified products following automated flash column chromatography or recrystallization. Abbreviation definitions: TFM, trifluoromethoxy; OTs, tosylate; TFA, trifluoroacetyl; Cp, cyclopentadienyl; PET, positron emission tomography; SPECT, single-photon emission computed tomography.

Discussion

In this study, we established the foundational chemical framework for dual-modality soluble epoxide hydrolase (sEH) radiotracer development by successfully synthesizing a library of five novel asymmetric urea scaffolds (Scaffolds 1-5) and an

organometallic cyclopentadienyl-rhenium tricarbonyl surrogate complex. Soluble epoxide hydrolase is a multifunctional enzyme that regulates inflammation and vascular tone by metabolizing anti-inflammatory epoxy fatty acids into pro-inflammatory diols [1]. While sEH has been extensively investigated as a therapeutic target in neurodegenerative disorders [3, 8], its role as a master regulator in peripheral inflammatory pathways and carcinogenesis remains an important area of exploration [4]. By physically preparing this benchmark chemical library, we established the requisite starting materials to evaluate both high-fidelity carbon-11 (^{11}C) PET probes and accessible technetium-99m ($^{99\text{m}}\text{Tc}$) SPECT analogues.

The synthetic strategy for the PET candidate library relied on triphosgene-mediated asymmetric coupling, proceeded using *in situ* carbonyl activation at 0 °C followed by nucleophilic addition of functionalized piperidine cores [1]. Scaffolds 1 and 2 established the baseline unfunctionalized urea scaffolds, isolating 1-adamantyl derivative (Scaffold 1) as a dark orange oil in 70% yield and the 4-(trifluoromethoxy)phenyl derivative (Scaffold 2) as white crystalline solid in 69% yield. The presence of the aromatic 4-(trifluoromethoxy)phenyl moiety in Scaffold 2 provided strong ultraviolet chromophores for automated flash chromatographic resolution at $\lambda = 240\text{ nm}$. Conversely, the aliphatic 1-adamantyl scaffold lacked UV activity, necessitating alternative reaction monitoring with ninhydrin staining to track primary amine conversion.

To expand the structural diversity and tune the lipophilicity and pharmacokinetics of the library, functionalized piperidine precursors containing tosyl (-OTs) and trifluoroacetyl (-TFA) tail groups were incorporated into Scaffolds 3-5 [9]. Tosylated

derivatives yielded Scaffold 3 (adamantyl-OTs, 62% yield) and Scaffold 4 (4-TFM-OTs, 68%) as stable off-white and white crystalline solids. The trifluoroacetylated derivative, Scaffold 5 (4-TFM-TFAA), was isolated in 68% yield as a white solid. Notably, the parallel attempt to couple the 1-adamantanamine starting materials with the trifluoroacetylated piperidine precursor was unviable under these reaction conditions. This synthetic limitation was likely caused by steric hinderance surrounding the bulky, rigid adamantyl cage combined with the reduced nucleophilicity of the secondary amine precursor, leading to unreacted starting materials or competitive side reactions. Modulating piperidine tail groups is known to significantly impact molecular size, charge distribution, and plasma protein binding, which are key determinants for enzyme active-site binding affinity and *in-vivo* tissue accumulation [1, 9].

To address the SPECT imaging stream, we executed a cold organometallic complexation reaction using dirhenium decarbonyl ($\text{Re}_2(\text{CO})_{10}$) and the cyclopentadienyl (Cp) precursor, isolating the target tricarbonyl complex as white crystals in 42% yield (0.052 mmol). Due to technetium-99m ($^{99\text{m}}\text{Tc}$) is a radioactive isotope, non-radioactive rhenium (Re) serves as an ideal chemical and structural surrogate due to the identical coordination geometry and ionic radii shared by Group 7 transition metals [10, 11]. High-temperature complexation (200-300 °C in o-xylene) successfully driven to completion confirms that the cyclopentadienyl ligands effectively coordinates the $[\text{M}(\text{CO})_3]^+$ metal tricarbonyl core ($\text{M} = \text{Re}/^{99\text{m}}\text{Tc}$) [10]. This establishes the chemical feasibility of utilizing these precursor amines to prepare stable amide-based SPECT radiotracers.

For dual-modality design evaluated in this project addresses a major diagnostic trade-off in current nuclear medicine [6]. PET imaging utilizing ^{11}C - CO_2 fixation enables the synthesis of native urea structures identical to potent cold inhibitors, providing a high-fidelity benchmark for quantitative imaging [7]. However, the short physical half-life of Carbon-11 ($t_{1/2} = 20.4$ min) mandates on-site cyclotron production, creating a high-cost burden that restricts PET availability to specialized medical centers [12]. In contrast, SPECT radiopharmaceuticals labeled with single-photon gamma emitters like Technetium-99m ($t_{1/2} = 6.0$ h) are substantially cheaper and easily distributed using commercial generator kits, making SPECT a more accessible modality for routine clinical use [6, 11].

Several limitations of the present study should be noted. The experimental scope was restricted to organic synthesis and precursor isolation. Full structural characterization through ^1H and ^{12}C Nuclear Magnetic Resonance (NMR) spectroscopy remains pending for the final three urea scaffolds. Furthermore, downstream evaluation, including automated $[^{11}\text{C}]\text{CO}_2$ radiosynthesis, commercial fluorometric *in vitro* sEH binding affinity (IC_{50}) assays, and cancer cell line uptake studies were not completed during this initial project phase.

Future research will focus on completing NMR characterization for all five urea scaffolds and executing $[^{11}\text{C}]\text{CO}_2$ fixation radiosynthesis to confirm radiochemical purity and yield. Subsequently, fluorometric inhibition assays should be conducted to verify that the introduced piperidine modifications retain nanomolar binding affinity for sEH active site. Validated candidates will then be evaluated in cell-based cancer models to assess tracer uptake and specificity, advancing these novel PET and SPECT radiotracers towards *in vivo* preclinical imaging of inflammation and carcinogenesis.

Acknowledgments

I would like to express my sincere gratitude to everyone who supported me throughout the completion of this research project. I am thankful to my primary supervisor, Dr. Justin Hicks, for giving me the opportunity to work in his lab, providing insightful guidance and mentorship, and investing in my growth; work was never a dread in his lab. I am also extremely grateful to Dr. Ghazaleh Takalloobanafshi, for her invaluable hands-on teaching, endless patience, and friendship. I would like to extend my gratitude to the Canadian Nuclear Society (CNS) for providing generous financial support through the Canadian Nuclear Society Undergraduate Scholarship, which made this research opportunity possible. Lastly, thank you to the Hicks Lab and the Lawson Cyclotron Facility team for providing access to incredible infrastructure, a welcoming collaborative environment, and technical assistance over the past few months.

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