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A general method for the synthesis of aryl [^{11}C]methylsulfones: Potential PET probes for imaging cyclooxygenase-2 expression

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Abstract—A general one-pot method has been developed for the conversion of an aryl thiol moiety masked as the butyrate ester to the corresponding ^{11}C-labeled methylsulfone group. The potential of this methodology has been demonstrated by the successful radiosynthesis of carbon-11 analogues of several highly selective cyclooxygenase-2 (COX-2) inhibitors such as Rofecoxib, Etoricoxib, and 3-(4-methylsulfonylphenyl)-4-phenyl-5-trifluoromethyl isoxazole in high yield. The chemical and radiochemical purities obtained for the ^{11}C-labeled COX-2 inhibitors are >99% with a specific activity >1000 Ci/mmol.

Cyclooxygenase-2 (COX-2) enzyme is up-regulated as part of the inflammatory pathogenesis in conditions such as cancers, arthritis, ischemic heart disease, stroke, organ rejection, and neurodegenerative diseases like Alzheimer's and Parkinson's disease.[1–6] The involvement of COX-2 in these diseases or disease processes raise the possibility that quantification of COX-2 expression might be a biological marker for early diagnosis, monitoring of disease progression, and an indicator of effective treatment. One approach to non-invasive monitoring of COX-2 in vivo is PET or SPECT. PET and SPECT tracers specifically targeting COX-2 enzyme are needed to implement this approach.

In spite of considerable efforts from several research groups, currently no specific COX-2 PET imaging agents are available for in vivo monitoring of COX-2 expression.[7] McCarthy et al. reported the radiosynthesis of [^{18}F]SC58125 as a COX-2 PET tracer.[8] However, specific binding could not be demonstrated by in vivo blocking of tracer uptake by carrier SC58125. [^{18}F]-Desbromo-DuP-697, another COX-2 inhibitor radiotracer exhibited substantial non-specific uptake in fat and intestine.[9] Isakson et al.[10] Toyokuni et al.[11] and Marnett et al.[12] have reported the synthesis of several labeled COX-2 inhibitors without demonstrating specific binding in vivo. More recently, Stille coupling of 4-[^{18}F]fluoroiodobenzene has been utilized for the synthesis of ^{18}F-labeled PET probes for monitoring COX-2 expression.[13] However, in vivo studies have not been published with these compounds.

In the course of our studies to develop a specific PET tracer for imaging COX-2,[14] we now report development of a general method for rapid one-pot conversion of masked aryl thiols to ^{11}C-labeled methylsulfone group. We propose that such a methodology could be conveniently adopted for the facile synthesis of [^{11}C] analogues of several highly selective COX-2 inhibitors like 3-(4-methylsulfonyl-phenyl)-4-phenyl-5-trifluoromethyl isoxazole (TMI), Rofecoxib, and Etoricoxib. The presence of a methylsulfone or sulfonamide group attached to an aryl ring is a characteristic feature of a large number of COX-2 selective inhibitors (COXIBs) (e.g., Fig. 1). In this communication, we describe the successful implementation of our strategy to synthesize aryl-[^{11}C]methylsulfones in good yield and specific activity.

TMI is considered as a potential imaging agent for COX-2 due to its high affinity to COX-2 (<1 nM) and excellent selectivity over COX-1 (COX-1/COX-2 = >100,000) and

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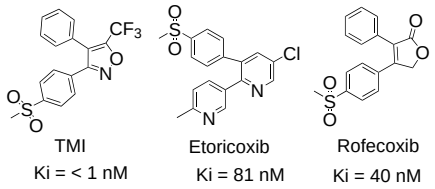
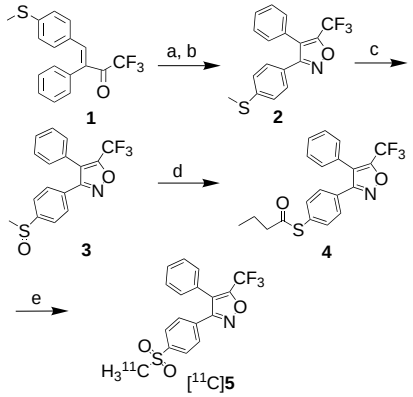


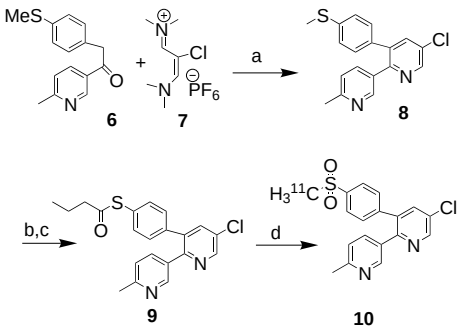
Figure 1. Examples of highly selective COX-2 inhibitors having methylsulfone group.

various competitive brain receptors, transporters, and proteins (affinity >10 μM).[15,16] Our initial strategy was to synthesize 4-(4-phenyl-5-trifluoromethylisoxazol-3-yl)benzenethiol, the required free thiol, as the radiolabeling precursor for [^{11}C]TMI. Toward this end, we pursued a palladium catalyzed coupling of 3-(4-bromophenyl)-4-phenyl-5-trifluoro-methylisoxazole with potassium tri(isopropyl)silane thiol[17] followed by preparative thin-layer chromatography generating free thiol in 40% and corresponding dimer in considerable yield. However, the lack of reproducibility of the reaction in acceptable yield as well as the instability of the free thiol prompted us to follow an alternative strategy for the synthesis of stable thiobutyrate ester, as shown in Scheme 1. The thiomethyl ether **2**, the intermediate required for the synthesis of precursor for radiosynthesis of TMI, was prepared by another modified procedure.[16] Accordingly, an isoxazole ring was established from the prop-2-ene-1-one **1** by treatment with hydroxylamine followed by the oxidative cyclization of the adduct in 88% yield. The synthesis of thiol precursor for radiosynthesis by deprotection of methyl sulfide **2** using several alkyl thiolates[18] was unsuccessful. We then pursued an alternative strategy for the synthesis of the stable thiobutyrate ester **4** from methylsulfide **2** via oxidation to sulfoxide **3** followed by Pummerer rearrangement[19] and quenching of the intermediate free thiol with butyryl chloride, as shown in Scheme 1. The desired precursor **4** was formed in 59% yield along with traces of disulfide.[20] Radiolabeling conditions for the synthesis of [^{11}C]TMI (**5**) from precursor **4** were initially optimized by mimicking the radiosynthesis with non-radioactive methyl iodide. Optimum yields were obtained by unraveling the free thiol from thiobutyrate ester **4** using tetrabutylammonium hydroxide in tetrahydrofuran (THF) followed by addition of methyl iodide (MeI), stirring for 5 min at rt, and then heating the reaction mixture for 2 min at 70 °C in presence of excess Oxone® in methanol/water (1:1 v/v). Under identical conditions, labeling with [^{11}C]MeI provided [^{11}C]TMI (**5**) in 30 min with 37% yield (n = 3, SD = ±3) at end of bombardment (EOB) based on [^{11}C]MeI and with >99% chemical and radiochemical purity (Scheme 1). The product was purified by reverse phase-high performance liquid chromatography (RP-HPLC, Phenomenex C18, 10 × 250 mm, 10 μm, mobile phase: acetonitrile/0.1 M ammonium formate solution 60:40, flow rate: 10 mL/min) followed by C-18 Sep-Pak® purification. The formation of [^{11}C]TMI was confirmed by co-injecting the [^{11}C]-product with non-radioactive compound and comparing the HPLC retention times of the two compounds. Specific activity of [^{11}C]TMI obtained was 2×10^3 Ci/mmol (n = 3, SD = ±250) based on a standard mass curve.

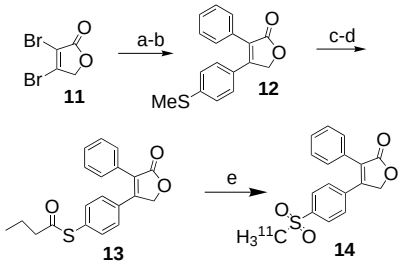
We then adopted a similar strategy for the synthesis of [^{11}C]Etoricoxib (**10**) (Scheme 2). The central pyridine ring of **8** (74%) was introduced by ring annulation of α-aryl ketone **6** with vinamidinium hexafluorophosphate salt **7**.[21] The attempted deprotection of methylthio group in **8** by sodium thiomethoxide resulted in the displacement of chloro substituent in the central pyridine ring by thiomethyl substituent. However, oxidation of thiomethyl group to the corresponding sulfoxide[22] followed by Pummerer rearrangement and protection of the free thiol group as the thiobutyrate ester proceeded uneventfully with an overall yield of 48% in three steps to give compound **9**.[23] The radiosynthesis of [^{11}C]Etoricoxib was achieved in 28% yield [(n = 3, SD = ±4), EOB based on $^{11}CH_3I$] by an analogous procedure adopted for the synthesis of [^{11}C]TMI.[24]



Scheme 1. Radiosynthesis of [^{11}C]TMI. Reagents and conditions: (a) $NH_2OH{\cdot}HCl$, NaOAc, EtOH–H_2O, reflux, 90 °C, 65%; (b) KI, I_2, $NaHCO_3$, Water–THF, reflux, 7 h, rt, 12 h, 88%; (c) m-CPBA, CH_2Cl_2, −20 °C, 1 h, 84%; (d) (i) $(CF_3CO)_2O$, 2,6-lutidine, CH_3CN, −20 °C, 1 h; (ii) butyryl chloride, pyridine, CH_2Cl_2, 0 °C, 30 min, 59% (two steps); (e) (i) [^{11}C]CH_3I, tetrabutylammonium hydroxide, THF, rt, 5 min; (ii) Oxone®, MeOH/H_2O (1:1), 70 °C, 2 min, 37% (EOB).



Scheme 2. Radiosynthesis of [^{11}C]Etoricoxib. Reagents and conditions: (a) (i) KOt-Bu, THF, rt, (ii) AcOH, TFA, rt, (iii) concd NH_3, reflux, 6 h, 74% (three steps); (b) (i) m-CPBA, CH_2Cl_2, −20 °C, 1 h, 84%; (c) (i) $(CF_3CO)_2O$, 2,6-lutidine, CH_3CN, −20 °C, 1 h; (ii) butyryl chloride, pyridine, CH_2Cl_2, 0 °C, 30 min, 57% (two steps); (d) (i) [^{11}C]CH_3I, tetrabutylammonium hydroxide, THF, rt, 5 min; (ii) Oxone®, MeOH/H_2O (1:1), 75 °C, 3 min, 28% (EOB).



Scheme 3. Radiosynthesis of [^{11}C]Rofecoxib. Reagents and conditions: (a) 4-thiomethylphenylboronic acid, $PdCl_2(PPh_3)_2$, CsF, $BnEt_3N^+Cl^-$, 3 d, 74%; (b) phenylboronic acid, $PdCl_2(PPh_3)_2$, CsF, $BnEt_3N^+Cl^-$, 3 d, 71%; (c) *m*-CPBA, CH_2Cl_2, −30 °C, 1 h, 93%; (d) (i) $(CF_3CO)_2O$, 2,6-lutidine, CH_3CN, −20 °C, 1 h; (ii) butyryl chloride, pyridine, CH_2Cl_2, 0 °C, 30 min, 54% (two steps); (e) (i) [^{11}C]CH_3I, pyrrolidine, DMF, rt, 5 min; (ii) Oxone®, MeOH/H_2O (1:1), 70 °C, 2 min, 20% (EOB).

The α,β-unsaturated γ-butyrolactone ring in Rofecoxib provided an unexpected challenge for its radiosynthesis. The precursor for radiosynthesis was prepared by initially establishing the diaryl lactone ring in **12** by two consecutive Suzuki couplings of mucobromic acid **11**, as shown in Scheme 3.[25] Selective mono-oxidation of **12**, Pummerer rearrangement, and protection of the thiol group proceeded with an overall yield of 50% to give the thiobutyrate ester **13**. However, attempted deprotection of the free thiol group by adding tetrabutylammonium hydroxide resulted in the cleavage of γ-butyrolactone ring. A host of other mild bases also failed to release the thiol group without destroying the unsaturated lactone. The in situ deprotection and methylation of the thiobutyrate ester was finally achieved successfully by carrying out [^{11}C]methylation in the presence of excess of pyrrolidine in DMF at room temperature.[26] Oxidation with Oxone® proceeded as expected to furnish [^{11}C]Rofecoxib (**14**) in 20% yield [(n = 3, SD = ±4), based on ^{11}C-CH_3I at EOB].

In conclusion, we have designed a one-pot method for the synthesis of ^{11}C-labeled methylsulfone group from the corresponding aryl thiol protected as the butyrate ester. The methodology was successfully exploited for the radiosynthesis of carbon-11 analogues of three highly selective COX-2 inhibitors Rofecoxib, Etoricoxib, and TMI in high radiochemical purity. In vivo studies with these potential PET probes for imaging COX-2 expression are currently in progress.

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22. 5-Chloro-3-(4-methanesulfinylphenyl)-6′-methyl-[2,3′]bipyridine. A solution of thioether **6** (147 mg, 0.45 mmol) in CH_2Cl_2 (2 mL) was cooled to −40 °C and stirred vigorously. Then a solution of *m*-CPBA (106 mg of 77 % water suspension, 0.47 mmol) in CH_2Cl_2 (2 mL) was added dropwise. The mixture was stirred at −20 °C for 40 min. Then $Ca(OH)_2$ (60 mg, 0.81 mmol) and $MgSO_4$ (200 mg) were added, and stirring was continued for 30 min. After filtration and evaporation, the resultant colorless oil was column chromatographed (4% MeOH in CH_2Cl_2) to yield the desired sulfoxide **8** as a colorless puffy solid (130 mg, 84%). Compound **8**: mp: 123 °C; ^{1}H NMR: δ 2.54 (s, 3H), 2.75 (s, 3H), 7.07 (d, J = 8.0 Hz, 1H), 7.35–7.37 (m, 2H), 7.56 (dd, J = 8.0, 2.3 Hz, 1H), 7.61–7.63 (m, 2H), 7.74 (d, J = 2.4 Hz, 1H), 8.42 (d, J = 2.1 Hz, 1H), 8.70 (d,

J = 2.4 Hz, 1H); HRMS Calcd for $C_{18}H_{16}ClN_2OS$ (MH^+): 343.0672; Found: 343.0679.

23. Thiobutyric acid *S*-[4-(5-chloro-6′-methyl-[2,3′]bipyridinyl-3-yl)-phenyl] ester. To a solution of sulfoxide **8** (50 mg, 0.15 mmol) in acetonitrile (0.75 mL), 2,6-lutidine (60 μL, 0.52 mmol) was added, and the mixture was cooled to −20 °C. To the resulting suspension was added TFAA (60 μL, 0.46 mmol) dropwise to give a clear yellow solution. The reaction mixture was stirred at −10 °C for 1 h and then cooled to 0 °C. All volatile materials were evaporated under inert atmosphere and the residue was dissolved in a precooled (0 °C) mixture of triethylamine (0.3 mL) and methanol (0.3 mL). After 30 min at 0 °C, all volatile materials were evaporated at low temperature. The residual yellow oil was dissolved in dichloromethane (1 mL), treated with pyridine (0.29 mmol, 25 μL) and cooled to 0 °C. Butyryl chloride (0.22 mmol, 23 μL) was added to the reaction mixture and allowed to warm to room temperature over 30 min. The solution was then poured into cold water and extracted with dichloromethane. The combined organic phases were dried over $MgSO_4$ and concentrated under reduced pressure and column chromatographed (70:30 hexane/EtOAc) to yield the thiobutyric ester **9** as a viscous liquid (32 mg, 57%). Compound **9**: ^{1}H NMR: δ 1.00 (t, J = 7.4 Hz, 3H), 1.75 (sextet, J = 7.4 Hz, 2H), 2.53 (s, 3H), 2.65 (t, J = 7.4 Hz, 2H), 7.05 (d, J = 7.9 Hz, 1H), 7.20–7.22 (m, 2H), 7.36–7.38 (m, 2H), 7.53 (dd, J = 8.0, 2.3 Hz, 1H), 7.75 (d, J = 2.4 Hz, 1H), 8.46 (d, J = 2.0 Hz, 1H), 8.67 (d, J = 2.4 Hz, 1H), HRMS Calcd for $C_{21}H_{20}ClN_2OS$ (MH^+): 383.0985; Found: 383.0992.
24. Radiosynthesis of [^{11}C]Etoricoxib (**10**): The precursor thiobutyrate ester **9** (1.0 mg) was dissolved in 400 μL of freshly distilled anhydrous THF in a capped 5 mL V-vial. Tetrabutylammonium hydroxide (10 μL, 1 M in MeOH) was added and the resultant pale yellow solution allowed to stand for 2 min. High specific active [^{11}C]CO_2 was produced from RDS112 Cyclotron (~37 GBq) and subsequently converted into [^{11}C]CH_3I. [^{11}C]Methyl iodide was transported by a stream of argon (20–30 mL/min) into the vial over approximately 5 min at room temperature. At the end of the trapping, a suspension of Oxone® (5 mg) in MeOH/H_2O (1:1 v/v; 200 μL) was introduced into the reaction mixture and was heated on a waterbath at 75 °C for 3 min. The suspension was filtered through a Nylon filter and the dark yellow solution then directly injected into a semi-preparative RP-HPLC (Phenomenex C18, 10 × 250 mm, 10 μm) and eluted with acetonitrile/0.1 M ammonium formate solution (35:65) at a flow rate of 10 mL/min. The precursor appeared after 5–6 min retention time during the HPLC analysis. The product fraction with a retention time of 9–10 min based on γ-detector was collected, diluted with 100 mL of deionized water, and passed through a classic C-18 Sep-Pak® cartridge. Reconstitution of the product in 1 mL of absolute ethanol afforded [^{11}C]**10** (28% yield, based on $^{11}CH_3I$ at EOB). A portion of the ethanol solution was analyzed by analytical RP-HPLC (Phenomenex, Prodigy ODS(3) 4.6 × 250 mm, 5 μm; mobile phase: acetonitrile/0.1 M ammonium formate solution 40:60, flow rate: 2 mL/min, retention time: 6.9 min, wavelength: 254 nm) to determine the specific activity and radiochemical purity. Then the final solution of the [^{11}C]**10** in 10% ethanol-saline was analyzed to confirm the chemical and radiochemical purities.
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