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Synthesis of ^{11}C-labeled Sulfonyl Carbamates through a Multicomponent Reaction Employing Sulfonyl Azides, Alcohols, and [^{11}C]CO

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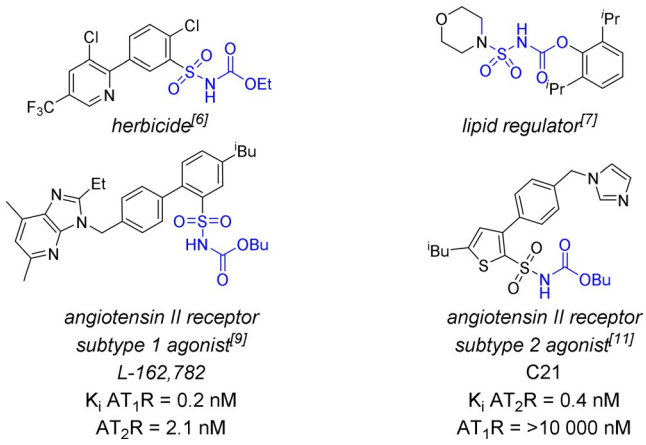
We describe the development of a new methodology focusing on ^{11}C-labeling of sulfonyl carbamates in a multicomponent reaction comprised of a sulfonyl azide, an alkyl alcohol, and [^{11}C]CO. A number of ^{11}C-labeled sulfonyl carbamates were synthesized and isolated, and the developed methodology was then applied in the preparation of a biologically active molecule. The target compound was obtained in 24±10% isolated radiochemical yield and was evaluated for binding properties in a tumor cell assay; in vivo biodistribution and imaging studies were also performed. This represents the first successful radiolabeling of a non-peptide angiotensin II receptor subtype 2 agonist, C21, currently in clinical trials for the treatment of idiopathic pulmonary fibrosis.

1. Introduction

The sulfonyl carbamate functional group is an easily accessible carboxylic acid bioisostere, and is traditionally introduced in the *N*-acylation of sulfonamides with carbonic acid derivatives such as chloroformate or anhydride.[1] Sulfonyl carbamates are also used as nitrogen nucleophiles in the Mitsonobu reaction,[2] as protecting groups for alcohols[3] and as dehydrating reagents.[4] The sulfonyl carbamate moiety can be found in molecules with anticancer,[5] herbicidal,[6] lipid-regulating,[7] and antibacterial[8] properties. Furthermore, it is present in a plethora of ligands[9] to the angiotensin II subtype 1 and 2 receptors (AT_1R and AT_2R, respectively), a subject of ongoing research interest in our laboratory.[10] Compound 21 (C21, see Scheme 1), the first non-peptide AT_2R selective agonist,[11] is currently in clinical trials for the treatment of idiopathic pulmonary fibrosis and has been the subject of intense study since its disclosure.[12] In addition to its beneficial effects in animal models of cardiovascular disorders,[13] stroke,[14] and spinal cord injury,[15] C21 represents a possible therapeutic alternative in the management of prostate cancer, the leading cause of cancer in European men.[16,17] As AT_2R expression has been shown to decrease with disease progression, we envisioned that the use of a radio-labeled selective non-peptide AT_2R agonist would provide a potential means for the monitoring and staging of pros-



Scheme 1. Representative sulfonyl carbamates, including C21—the first non-peptide angiotensin II receptor subtype 2 agonist.

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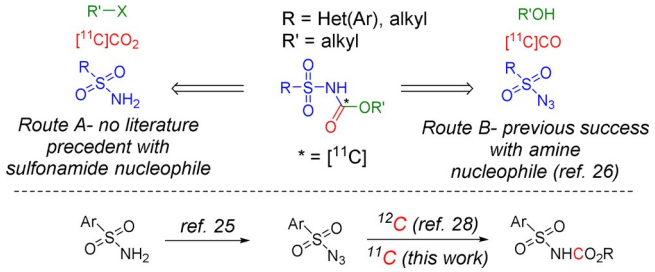
tate cancer, as well of investigating AT_2R function and the in vivo properties of non-labeled C21.

The use of positron emission tomography (PET) allows non-invasive visualization of biological processes at the molecular or cellular level.[18,19] This is achieved by the incorporation of a short-lived radionuclide such as ^{11}C ($t_{1/2}$ = 20.4 min) into an organic molecule, which can then be administered as a tracer. To this end, several classes of non-peptide AT_2R ligands have previously been reported by Hallberg and co-workers[11,20–22] and a ^{11}C-labeled benzamide ligand was evaluated for tracer properties and biodistribution.[23] Although the ligand tested did not display favorable metabolic properties, we wished to extend this work to include C21, the most potent and well-studied ligand to this receptor. To the best of our knowledge, there is no existing methodology for the preparation of ^{11}C-labeled sulfonyl carbamates. We, therefore, turned our efforts to the development of a general methodology that would enable synthesis, purification, and formulation of ^{11}C-sulfonyl carbamates within the time constraints induced by our radionuclide of choice. This methodology could then be applied to the preparation of ^{11}C-labeled C21 ($[^{11}C]$C21).

Having identified a suitable target compound, we envisioned a multicomponent[24] approach to the synthesis of $[^{11}C]$C21 containing a ^{11}C-labeled sulfonyl carbamate moiety, starting from the corresponding sulfonyl azide. A retrosynthetic analysis revealed two possible precursor molecules that could be used in the preparation of ^{11}C-sulfonyl carbamates (Scheme 2). Although elegant use has been made of $[^{11}C]CO_2$



Scheme 2. Retrosynthetic approach to ^{11}C-labeled carbamates.

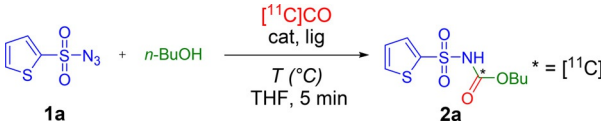
in the direct synthesis of carbamates from amines and alkyl halides,[25] to the best of our knowledge, this methodology has not been tested with sulfonamides as the nucleophilic component (route A). Rh^I-mediated carbonylation chemistry, on the other hand, is well-suited to radionuclide incorporation by using sulfonyl azides and $[^{11}C]$CO (route B).[26] We, therefore, chose to expand the scope of carbonylation chemistry, employing a sulfonyl azide and an alcohol as the nucleophilic component. Relying on recently disclosed methodology that enables confinement of $[^{11}C]$CO in small-volume reaction vessels at low pressure by using soluble xenon as the transfer gas,[27] we sought to develop a synthetic route to ^{11}C-labeled sulfonyl carbamates. The potential of multicomponent reactions in terms of robustness and operational simplicity would then be harnessed to afford rapid access to structurally diverse ^{11}C-carbamates. Herein, we describe the development of a novel method of preparing ^{11}C-sufonyl carbamates and its application in the synthesis of $[^{11}C]$C21.

2. Results and Discussion

2.1. Model Carbamates

By using chemistry developed in our group,[28,29] a number of sulfonyl azide precursors were prepared and converted to the corresponding isotopically unmodified sulfonyl carbamates, which were then used as analytical standards. With previous work on aryl azides[30] and sulfonyl azides[26] as entry points, we carried out a preliminary screening of reaction conditions (Table 1) for the synthesis of ^{11}C-sulfonyl carbamates, using

Table 1. Catalyst and ligand effect on formation of **2a** from **1a**.[a]



Entry	[Cat]	[Lig]	T [°C]	[Nu]	Conv. [%][b]	RCY [%][c]
1	A, 10	–	75	80	9	6
2	A, 2	–	100	110	68	50
3	B, 3	–	100	80	82	66
4	B, 3	–	110	80	54–61	33–35[d,e]
5	B, 3	–	120	80	18	3
6	C, 0.4	–	100	80	48	39–43[d]
7	D, 0.4	PPh_3, 1.2	75	80	95	86 (46)[e]
8	D, 0.4	PPh_3,1.2	100	80	89	75–82[d]
9	D, 0.4	PPh_3,1.2	120	80	14	11
10	**D, 0.4**	**PPh_3, 2.4**	**100**	**80**	**94**	**88 (55)[e]**
11	D, 0.4	Xantphos, 1.2	100	80	50	20[e]
12	D, 0.4	dppf, 1.2	100	80	92	56
13	D, 0.4	dppp, 1.2	100	80	52	38–48[d]

[a] Catalyst, ligand, and *n*-butanol in stock solutions. All reactions carried out in 1 mL pear-shaped vial with crimp cap under argon, final volume 0.3 mL, concentrations in mM, [**1a**] = 40 mM for all entries except for entry 2 (50 mM). [b] Conversion, percentage of non-volatile activity remaining in reaction solution after flushing with N_2, see Figure S1. [c] Non-isolated radiochemical yield, decay-corrected. [d] $n = 2$. [e] Isolated radiochemical yield. A = $PdCl_2$, B = $Pd(PPh_3)_4$, C = $Pd(OAc)_2$, D = $[Rh(COD)Cl]_2$.

thiophene-2-sulfonyl azide (**1a**) as a model substrate. Inspired by the efforts of Long and Ning,[31] we began our catalyst screen with $PdCl_2$, a readily available and inexpensive catalyst. However, it quickly became apparent that $PdCl_2$ was not amenable to the scale on which ^{11}C-labeling reactions are normally conducted. The poor solubility of $PdCl_2$ in selected solvents (DMA, DMF and THF) hindered the preparation of stock solutions, and it was observed to catalyze the formation of multiple ^{11}C-labeled side products (Table 1, entries 1 and 2). Decreasing the concentration of $PdCl_2$ and increasing reaction temperature led to an improvement in both the conversion of $[^{11}C]$CO to non-volatile components (conversion), as well as a concomitant increase in non-isolated radiochemical yield (NIRCY) (entry 2). The difficulties encountered when working with $PdCl_2$ and its propensity to oxidize CO to CO_2 in the presence of

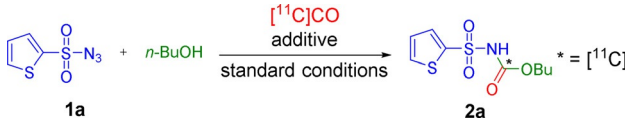
water[32] led us to select $Pd(PPh_3)_4$, a common choice for transition-metal-mediated ^{11}C-carbonylation reactions.[23,33,34] We were pleased to see a relatively large increase in conversion and NIRCY (entry 3), but increasing the temperature had a detrimental effect on both conversion and NIRCY (entries 4 and 5). $Pd(OAc)_2$ has been used[35] in the synthesis of sulfonylureas from the corresponding sulfonyl chlorides and CO, but a conversion of 48% and a non-isolated radiochemical yield of 43% prompted us to investigate Rh^I as a catalyst. Earlier work[26] in our group demonstrated the utility of Rh^I in aminocarbonylation reactions of sulfonyl azides, and $[Rh(COD)Cl]_2$ and PPh_3 at 75 °C gave a conversion of 95% and an NIRCY of 86% (entry 7). Increasing the temperature to 100 °C gave similar results, but increasing to 120 °C had a negative impact on both conversion and NIRCY (entries 8 and 9). Increasing the concentration of PPh_3 to achieve a P/Rh ratio of 3:1 in order to form the Wilkinson's catalyst[36] gave a comparable conversion and an NIRCY of 88% (entry 10), leading us to select these conditions. Moreover, we were pleased by the superior performance of the ubiquitous PPh_3 compared to bidentate ligands of varying bite angles (Xantphos, dppf, and dppp; entries 11–13).

Having demonstrated the viability of this strategy, we then applied it to the synthesis of a variety of functionalized ^{11}C-labeled sulfonyl carbamates (Table 2). We began by investigating the scope of the alcohol component, using *p*-toluene sulfonyl azide (tosyl azide, TsN_3, **1b**) as the azide scaffold. Gratifyingly, *n*-butanol performed well with TsN_3, forming ^{11}C-sulfonyl carbamate **2b** in a slightly lower isolated yield as compared to thiophene-2-sulfonyl azide. Secondary alcohols gave comparable yields (**2c** and **2d**), but the use of benzyl alcohol resulted in a lower isolated radiochemical yield, despite good conversion of gaseous [^{11}C]CO (**2e**). 2-Thiophenemethanol gave an isolated radiochemical yield of 23% (**2f**), whereas hindered alcohols gave low, albeit isolable yields (**2g**, **2h**), and in the case of **2g**, a number of unidentified labeled side products were observed.

Having explored the scope of alcohols that could be used in this reaction, we then investigated a number of sulfonyl azides (Table 3). We were pleased to observe the compatibility of structurally diverse sulfonyl azides with our reaction system. Electron-rich and electron-poor arylsulfonyl azides gave comparable isolated radiochemical yields (**3a** and **3b**) with slightly lower conversion of gaseous [^{11}C]CO as compared to TsN_3. Notably, the use of a sulfonyl azide on an sp^3 carbon gave good conversion and an isolated radiochemical yield of 51% (**3c**). This observation is in agreement with our results when using isotopically unmodified CO and $PdCl_2$.[28] The use of a heteroaryl sulfonyl azide gave a reduced, albeit isolable yield (**3d**), and in the case of **3e**, we were pleased to see a conversion of 85% and a non-isolated radiochemical yield of 71%.

Inspired by the work of Collins and Glorius,[37] we then tested the susceptibility of our reaction manifold to several potential contaminants and other detrimental factors (Table 3). To our surprise, the presence of both a free carboxylic acid and a basic nitrogen center that could potentially interfere with metal catalysts was tolerated (albeit with the formation of several ^{11}C-labeled side products; Table 3, entry 1). Similarly, a non-isolated radiochemical yield of 50% was observed when using phenylacetylene as an additive (entry 2). This indicated that the sulfonyl azide starting material was available for reaction, despite its ability to undergo azide–alkyne cycloaddition.[38] Conversely, the addition of excess H_2O proved to be detrimental, as did the omission of catalyst (entries 3 and 4). Finally, the use of 24 h old stock solutions exposed to light resulted in a non-isolated radiochemical yield of 6%, most probably owing to catalyst decomposition (entry 5).

Table 3. Robustness screen of the radiosynthesis of **2a** from **1a**[a]

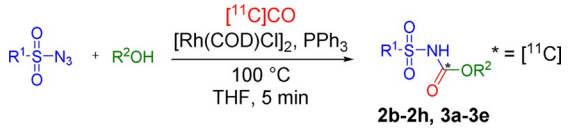


1a **2a**

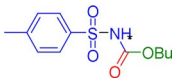
Entry	Additive/change	Conv. [%]	RCY[b] [%]
1	picolinic acid, 120 mM	77	50
2	phenylacetylene, 120 mM	52	50
3	H_2O, 440 mM	7	4
4	no catalyst	5	4
5	old stock solutions[c]	14	6

[a] Reaction conditions: see Table 1, entry 10. [b] Determined by radio-HPLC. [c] 24 h old previously-used stock solutions stored in 1 mL flat-bottomed vials in anhydrous THF under N_2 atmosphere.

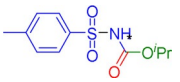
Table 2. Azide and alcohol scope in multicomponent assembly of ^{11}C-labeled sulfonyl carbamates[a]



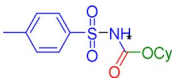
2b-2h, 3a-3e



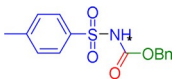
2b, 55% (88%)



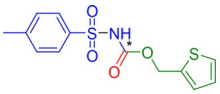
2c, 57% (77%)



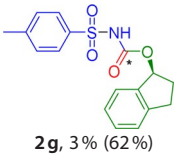
2d, 45% (87%)



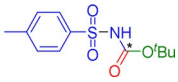
2e, 23% (75%)



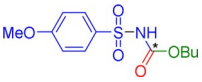
2f, 23% (60%)



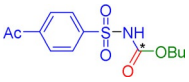
2g, 3% (62%)



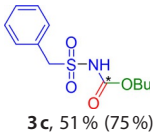
2h, 2% (33%)



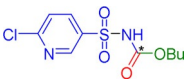
3a, 40% (68%)



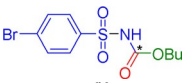
3b, 37% (65%)



3c, 51% (75%)



3d, 12% (42%)

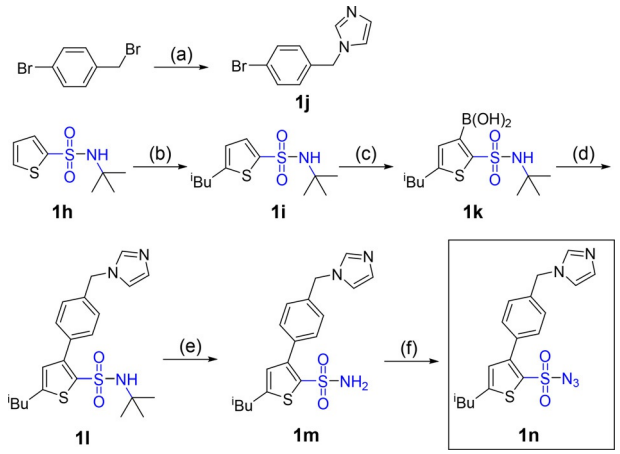


3e, 71%[b] (85%)

[a] Isolated yields, decay-corrected, based on activity remaining after N_2 purge (conversion, percentage of non-volatile products formed from [^{11}C]CO). [b] Non-isolated radiochemical yield. See Table 1, entry 9 for reactant concentrations.

2.2. Synthesis of Key Sulfonyl Azide Precursor

Following route B, the key sulfonyl azide precursor **1n** was prepared in five steps, starting from the commercially available *tert*-butyl sulfonamide **1h** (Scheme 3). Treatment of **1h** with

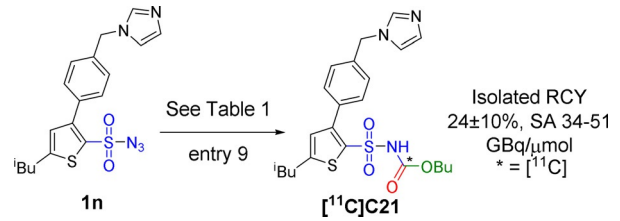


Scheme 3. Precursor synthesis. Reagents and conditions: a) KOH, imidazole, DMF, reflux, 16 h; b) *n*BuLi, THF, −78 °C (i), 1-bromo-2-methylpropane, r.t. 16 h (ii); c) *n*BuLi, THF, −78 °C (i), $B(OiPr)_3$, r.t. 16 h (ii); d) **1j**, $Pd(PPh_3)_4$, NaOH (aq), toluene, reflux, 3 h; e) TFA, 16 h; f) imidazole-1-sulfonyl azide hydrogen sulphate, *i*PrOH/H_2O, K_2CO_3, r.t., 20 h.

excess *n*BuLi followed by 1-bromo-2-methylpropane afforded the 5-alkylated product **1l**, and subsequent lithiation and borylation led to boronic acid **1i**. This was then subjected to a Suzuki–Miyaura[39] cross-coupling reaction with imidazole derivative **1j**, synthesized from 4-bromobenzyl bromide, to give **1l**. After deprotection with neat TFA, the primary sulfonamide **1m** was converted into key sulfonyl azide precursor **1n** through a diazotransfer from an imidazole-1-sulfonyl azide salt, demonstrating the utility of this methodology for late-stage azidation.[29,40]

2.3. Preparation of [^{11}C]C21

With precursor **1n** in hand, we then turned our attention to the preparation of [^{11}C]C21 (see Scheme 4). Following our newly-developed protocol, [^{11}C]C21 was isolated in 24±10% radiochemical yield (decay-corrected, $n=9$) and the specific activity after [^{11}C]CO introduction was 34–51 GBq μmol^{-1} ($n=3$). Gratifyingly, the synthesis of [^{11}C]C21 was robust and multiple batches could be prepared for biological evaluation and imaging purposes.



Scheme 4. Preparation of [^{11}C]C21 from **1n**.

2.4. In Vitro Binding Specificity

The DU145 cell line (a brain metastasis of human prostate carcinoma[41]) was chosen for evaluation of the in vitro binding characteristics of [^{11}C]C21. Measured activity was significantly lower in samples that were pre-incubated with non-labeled C21, indicating specific binding (see Figure 1). As demonstrated by Guimond et al.,[16] the presence of AT_2R in non-tumoral prostate tissue and early-stage prostate tumors suggests limited proliferation. A radiolabeled imaging agent could, therefore, be used to stage disease progression and aid in the provision of individualized therapy.

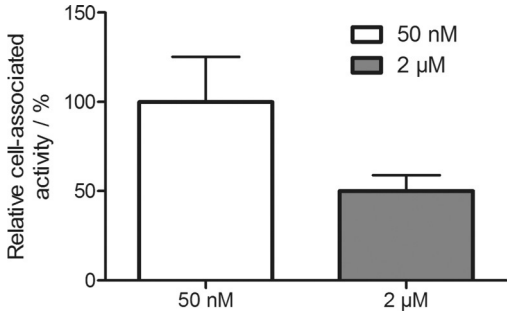


Figure 1. Specific binding of [^{11}C]C21 to DU145 cells. For the pre-saturation of receptors, an excess of non-radioactive C21 was added. Data are presented as mean values from three samples ± SD.

2.5. Biodistribution

Having demonstrated the in vitro utility of [^{11}C]C21, we then wished to evaluate its in vivo biodistribution in rats and potential as a PET tracer. To evaluate the kinetics of [^{11}C]C21 in vivo, biodistribution studies were carried out in healthy female rats. Results from ex vivo organ distribution are shown as standardized uptake values (*SUV*s) in Figure 2. The radioactivity rapidly disappeared from blood, as evidenced by *SUV* values of approximately 0.3 within 10 min post-injection. The highest uptake of radioactivity was observed in the liver, kidneys, and

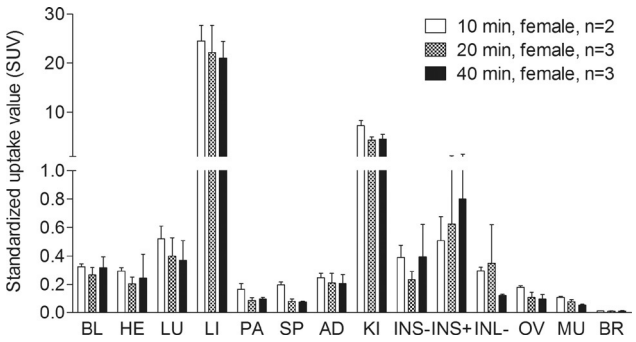


Figure 2. Organ uptake of [^{11}C]C21 in healthy female rats. BL–blood; HE–heart; LU–lungs; LI–liver; PA–pancreas; SP–spleen; AD–adrenals; KI–kidneys; INS—small intestine without contents; INS+–small intestine with contents; INL—large intestine without contents; OV–ovaries; MU–muscle; BR–brain. Data are presented as mean values and error bars represent the SD.

intestine with contents that, in conjunction with rapid clearance from blood, indicate fast elimination kinetics. Very low levels of activity were detected in all other organs. Although the AT_2R has been previously detected in the pancreas and sexual organs,[42] only low activity was detected in these organs.

2.6. MicroPET-CT

The fused PET/CT images over the abdomen showed the same distribution pattern for the anaesthetized male rats (Figure 3) as that determined by ex vivo biodistribution studies when using un-sedated female rats. The only organ that was clearly distinguishable was the liver. However, by using early frames, regions of interest (ROIs) could be drawn also for the aorta and the left kidney, and time activity curves were generated. The kinetics of [^{11}C]C21 in these organs are shown in Figure S4 in the Supporting Information.

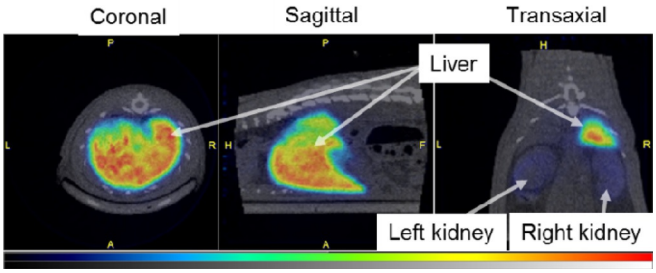


Figure 3. PET/CT image, showing in vivo distribution of [^{11}C]C21 in a healthy male rat. Grey scale (CT), 200–1500 Hounsfield units, colour scale (PET) SUV = 0 to 20 (black to red). White arrows show liver in 3 projections and kidneys in transaxial projection.

3. Conclusions

A general methodology for the ^{11}C-labeling of sulfonyl carbamates in a Rh^{I}-mediated multicomponent reaction has been developed and demonstrated by the preparation of 12 ^{11}C-sulfonyl carbamates. The radiolabeling of a potent, non-peptide AT_2R agonist (C21) was then successfully performed and the isolated compound was found to bind specifically in prostate tumor cells expressing AT_2R. The biological evaluation of [^{11}C]C21 indicates rapid metabolism and excretion, thus precluding its use as a PET tracer. However, given the potential utility of an AT_2R-selective imaging agent in individualized management of prostate cancer therapy, these results provide an entry point into the development of future tracers to meet this need.

Experimental Section

Chemistry

General

All reagents were purchased at the highest commercial quality and used without further purification. Solvents used for extraction and silica gel chromatography (EtOAc, hexanes, *n*-pentane, dichloromethane and methanol) were used without purification or desiccation. Yields are for isolated and spectroscopically pure material, unless otherwise stated. Reaction progress was monitored by using thin-layer chromatography (0.25 mm E. Merck silica plates, 60F-254), visualized by fluorescence quenching with UV light at 254 nm or by iodine staining. Silica gel chromatography was carried out by using E. Merck silica gel (60 Å pore size, particle size 40–63 nm). ^{1}H NMR spectra were recorded at 400 MHz and ^{13}C NMR spectra at 100 MHz by using a Bruker Avance III NMR spectrometer. The chemical shifts for ^{1}H NMR and ^{13}C NMR were referenced to TMS through residual solvent signals (^{1}H, $CDCl_3$ at 7.26 ppm; ^{13}C, $CDCl_3$ at 77.16 ppm; ^{1}H, [D_6]DMSO at 2.45 ppm; ^{13}C, [D_6]DMSO at 39.43 ppm). LC–MS was performed on an instrument equipped with a CP-Sil 8 CB capillary column (50×3.0 mm, particle size 2.6 μm, pore size 100 Å), operating at an ionization potential of 70 eV, using a 2 min 5–100% CH_3CN/H_2O gradient (0.05% HCOOH in both CH_3CN & H_2O). Accurate mass values were determined by using an electrospray ionization source with a 7-T hybrid ion trap and a TOF detector or through chemical ionization by using ammonia as carrier gas. Sulfonyl azides **1a**–**1g**,[29] precursors **1h**–**1l**,[11] and the non-labeled standards for compounds **2a**–**2h** and **3a**–**3d**[28] were synthesized following the literature procedures and spectral data were in agreement with published values.

3-[4-[(1*H*-Imidazol-1-yl)methyl]phenyl]-5-isobutylthiophene-2-sulfonamide (1m)

1l (0.29 g, 0.68 mmol) was dissolved in 2 mL TFA and stirred overnight at ambient temperature. Upon full consumption of starting material (as confirmed by LC–MS), the volatiles were evaporated. The crude product (colorless oil) was used in the next step without further purification (0.25 g, 89%). R_f (SiO_2) = 0.24 (1:9 MeOH/DCM); ^{1}H NMR (400 MHz, [D_6]DMSO): δ = 9.14 (d, J = 1.3 Hz, 1H), 7.82–7.74 (m, 1H), 7.65–7.62 (m, 4H), 7.62–7.60 (m, 1H), 7.48–7.38 (m, 2H), 6.91 (t, J = 0.8 Hz, 1H), 5.45 (s, 2H), 2.68 (dd, J = 7.0, 0.8 Hz, 2H), 1.99–1.80 (m, 1H), 0.94 ppm (d, J = 6.6 Hz, 6H); ^{13}C NMR (100 MHz, [D_6]DMSO): δ = 146.6, 141.8, 137.9, 136.2, 135.4, 135.1, 130.13, 130.08, 128.2, 122.3, 121.9, 51.5, 38.5, 30.4, 22.4 ppm; MS (ESI): *m/z* calcd for $C_{18}H_{23}N_3O_2S_2$: 376.1153 [M + H$^+$]; found: 376.1163.

3-[4-[(1*H*-Imidazol-1-yl)methyl]phenyl]-5-isobutylthiophene-2-sulfonyl azide (1n)

To a stirred mixture of **1m** (55.1 mg, 0.14 mmol) and K_2CO_3 (86.1 mg, 0.62 mmol) in 1:1 *i*PrOH/H_2O (10 mL) was added imidazole-1-sulfonyl azide hydrogen sulfate (56.8 mg, 0.21 mmol). The reaction mixture was stirred for 16 h and then quenched by the addition of 10 mL $NaHCO_3$. EtOAc (20 mL) was added and the resulting aqueous phase was extracted with 20 mL EtOAc. The combined organics were washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The crude yellow liquid obtained directly after workup was of sufficient purity (ca. 2% starting material, as confirmed by ^{1}H NMR) to be used directly in Rh^{I}-mediated carbonylation chemistry. Purification by using silica gel chromatography (0–10% MeOH in $CHCl_3$) significantly reduced the isolated yield (colorless liquid, 11 mg, 20%), possibly owing to stability issues with the sulfonyl azide moiety. The purified compound was stable for > 1 month at −21 °C. R_f (SiO_2) = 0.32 (1:9 MeOH/DCM; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.49 (s, 1H), 7.33–7.22 (m, 2H), 7.03–6.98 (m, 2H), 6.91 (t, J = 0.8 Hz, 1H), 6.72 (s, 1H), 6.63 (s, 1H), 4.98 (s, 2H), 2.51 (dd, J = 7.1, 0.8 Hz, 2H), 1.82–1.58 (m, 1H), 0.78 ppm (d, J = 6.6 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 153.8, 147.2, 136.1, 135.2, 134.1, 131.0, 130.9, 130.6, 128.5, 122.5, 121.1, 39.8, 31.1,

22.7 ppm; IR (KBr) 2170 cm^{-1}; MS (ESI): *m/z* calcd for $C_{18}H_{20}N_5O_2S_2$: 402.1058 $[M+H^+]$; found: 402.1048.

Radiochemistry

General

All radiochemical work was carried out at Uppsala University Hospital PET Centre. $[^{11}C]CO_2$ was produced by using a Scanditronix MC-17 cyclotron through the $^{14}N(p,\alpha)^{11}C$ nuclear reaction, whereby the target gas (N_2, containing 50 ppm O_2) was bombarded with 17 MeV protons. Thereafter, the $[^{11}C]CO_2$ was transferred to the low-pressure xenon system in a stream of helium gas. $[^{11}C]CO_2$ and $[^{11}C]CO$ were concentrated on anhydrous silica gel at −196 °C. The radioactive gases were released from both traps by removal of liquid nitrogen containers and heating with coiled cable heaters. Heated zinc (400 °C) was used to obtain $[^{11}C]CO$ from $[^{11}C]CO_2$. When releasing the $[^{11}C]CO$ from the CO trap, the transfer gas was changed from helium to xenon (≥99.995%, 1.5 mL min^{-1}) and the transfer needle was placed into the solvent in a sealed reaction vial (Grace 11 mm silicon/PTFE septum, disposable). Analytical radio-HPLC was carried out by using a VWR LaChrom ELITE system (L-2130, L-2200, L-2400) equipped with a Merck Chromolith Performance RP-18e column (4.6×100 mm). Method: 5–95% MeCN in H_2O (0.05% TFA), 10 min, 4 mL min^{-1}. The UV signal was monitored at 254 nm and radioactivity was monitored by using a Bioscan Flow-Count PMT radioactivity detector. The ^{11}C-labeled carbamates were isolated through semi-preparative HPLC (VWR LaPrep HPLC system P110, P311, equipped with a Reprosil Basic C_{18}-HL column, 10×250 mm). Method: 5–95% MeCN in $(NH_4)_2CO_3$ (pH 8.9), 8 mL min^{-1}. UV purity was monitored at 254 nm and radioactivity was monitored by using a Bioscan Flow-Count PMT radioactivity detector. All products were identified by co-elution with the corresponding isotopically unmodified compound (prepared following the literature procedure[28]) and were >95% radiochemically pure, as confirmed by analytical radio-HPLC (see above). The identity of $[^{11}C]$C21 was further confirmed by radio-LC–MS (Waters Quattro Premier Genesis C_{18} column, 4μ particle size, 150 mm×4.6 mm, MS running in selective ion recording ES^+ mode) and radio-TLC carried out on batches of $[^{11}C]$C21 prepared for imaging. For radio-TLC, a co-spot of isotopically unmodified C21 was run with the reaction mixture and its retention was visualized by fluorescence quenching (UV light, 254 nm). This value (in mm) was identical to that of the labeled compound.

General Procedure for the Preparation of Stock Solutions of Catalyst, Ligand, and Alcohol

Three oven-dried flat-bottomed vials (1 mL) were charged with catalyst (2 μmol), ligand (14 μmol), or alcohol (48 μmol), respectively, capped, and flushed with argon gas for 1 min. THF (1 mL, freshly distilled over sodium metal, benzophenone indicator) was added by using a Hamilton syringe, and argon gas was bubbled through the resulting solution for 30 s. The vials were then vortexed for 5 min, after which all stock solutions were homogeneous. Prepared stock solutions of the catalyst could be used for up to 12 h, after which their color gradually changed from light yellow to yellow-brown. Although all reactant vials were sealed and kept filled with argon, atmospheric poisoning by water or oxygen cannot be ruled out (see above for stability screen).

General Method for the Preparation of ^{11}C-Sulfonyl Carbamates 2a–2h and 3a–3d

An oven-dried conical vial (1 mL, PTFE/silicate crimp cap) was charged with azide (12 μmol), capped, and flushed with argon gas for 30 s. Catalyst and ligand (0.12 and 0.72 μmol, respectively) in THF were added by using a Hamilton syringe and the reaction mixture was diluted to a total volume of 300 μL with THF. Alcohol (24 μmol in 50 μL THF) was added just prior to $[^{11}C]CO$ introduction (typically 10–15 min after catalyst and ligand) to avoid potential side reactions. $[^{11}C]CO$ was introduced into the reaction mixture under a stream of xenon gas (see above) until measured activity peaked. Thereafter, the reaction vial was placed in a heating block for 5 min, after which activity (A_0) was measured in a well-counter. In a test reaction ($n=3$), the activity in the vial was checked directly after CO introduction and then after heating to see whether any leakage had occurred. We were pleased to observe that all activity remained in the vial. The reaction vial was then equipped with a vent needle and flushed with a stream of nitrogen gas (10 mL min^{-1}) for 30–60 s, and the remaining activity was measured (see the Supporting Information for a discussion on the calculation of the radiochemical yield). An aliquot (typically 1–5 μL) was taken for crude HPLC analysis and comparison with an analytical standard.[28] In parallel, the reaction mixture was diluted with 100 μL 1:1 MeCN/H_2O and purified by semi-preparative HPLC to give 2.2 GBq of sulfonyl carbamate **2a** 52 min post EOB (decay-corrected isolated RCY 55%), starting from 18 GBq $[^{11}C]CO$. To ensure that the recovery of activity was high and comparable throughout all experiments, HPLC columns, injection ports, and reaction vials were checked for residual activity after all preparative and analytical HPLC runs.

Butyl tosyl-[1-^{11}C]carbamate (2b)

Prepared following the general procedure, starting from 24 GBq $[^{11}C]CO$, 6.4 GBq isolated 31 min post-EOB (decay-corrected IRCY 49%).

Isopropyl tosyl-[1-^{11}C]carbamate (2c)

Prepared following the general procedure, starting from 12 GBq $[^{11}C]CO$, 2.5 GBq isolated 34 min post-EOB (decay-corrected IRCY 57%).

Cyclohexyl tosyl-[1-^{11}C]carbamate (2d)

Prepared following the general procedure, starting from 13 GBq $[^{11}C]CO$, 2.7 GBq isolated 33 min post-EOB (decay-corrected IRCY 45%).

Benzyl tosyl-[1-^{11}C]carbamate (2e)

Prepared following the general procedure, starting from 13 GBq $[^{11}C]CO$, 1.0 GBq isolated 40 min post-EOB (decay-corrected IRCY 23%).

Thiophen-2-ylmethyl tosyl-[1-^{11}C]carbamate (2f)

Prepared following the general procedure, starting from 19 GBq $[^{11}C]CO$, 2.0 GBq isolated 30 min post-EOB (decay-corrected IRCY 23%).

(*S*)-2,3-Dihydro-1*H*-inden-1-yl tosyl-[1-^{11}C]carbamate (2 g)

Prepared following the general procedure, starting from 12 GBq [^{11}C]CO, 0.1 GBq isolated 38 min post-EOB (decay-corrected IRCY 3%).

tert-Butyl tosyl-[1-^{11}C]carbamate (2 h)

Prepared following the general procedure, starting from 16 GBq [^{11}C]CO, 0.1 GBq isolated 39 min post-EOB (decay-corrected IRCY 2%).

Butyl [(4-methoxyphenyl)sulfonyl]-[1-^{11}C]carbamate (3 a)

Prepared following the general procedure, starting from 12 GBq [^{11}C]CO, 1.7 GBq isolated 47 min post-EOB (decay-corrected IRCY 40%).

Butyl [(4-acetylphenylphenyl)sulfonyl)-[1-^{11}C]carbamate (3 b)

Prepared following the general procedure, starting from 12 GBq [^{11}C]CO, 1.8 GBq isolated 35 min post-EOB (decay-corrected IRCY 37%).

Butyl(benzylsulfonyl)-[1-^{11}C]carbamate (3 c)

Prepared following the general procedure, starting from 10 GBq [^{11}C]CO, 1.8 GBq isolated 43 min post-EOB (decay-corrected IRCY 51%).

Butyl [(6-chloropyridin-3-yl)sulfonyl)-[1-^{11}C]carbamate (3 d)

Prepared following the general procedure, starting from 10 GBq [^{11}C]CO, 0.2 GBq isolated 42 min post-EOB (decay-corrected IRCY 12%).

Butyl [(4-bromophenyl)sulfonyl)-[1-^{11}C]carbamate (3 e)

Prepared following the general procedure, selectivity 86%, conversion 82% (decay-corrected NIRCY 71%).

Butyl [(3-(4-((1*H*-imidazol-1-yl)methyl)phenyl)-5-isobutylthiophen-2-yl)sulfonyl)-[1-^{11}C]carbamate ([^{11}C]C21)

Prepared following the general procedure (see Table 1, entry 9). A mixture of **1 n**, *n*BuOH, [^{11}C]CO, $[Rh(COD)Cl]_2$, and PPh_3 in anhydrous THF was heated to 100 °C for 5 min. Purification of this mixture with semi-preparative reversed-phase HPLC gave the title compound. After reformulation in phosphate-buffered saline (pH 7.4), the radiochemical purity was >95% with no detectable amounts of reagents and starting materials present. The product was radiochemically stable after 60 min at ambient temperature in phosphate buffer.

Biological Evaluation

Animal Experiments

The animals used in this study were handled in accordance with the guidelines set up by the Swedish Animal Welfare Agency and were approved by the local animal ethics committee at Uppsala (permit number: C11/15). For the biodistribution experiments, healthy female Sprague Dawley rats weighing 300±80 g (mean± SD) were used. The animals were housed in a ventilated and thermo-regulated environment with a 12 h light/dark cycle and were allowed free access to food and water.

Cell Lines

Cells were purchased from the American Type Tissue Culture Collection (ATCC via LGC Promochem, Borås, Sweden). The cells were cultured in RPMI media supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (PEST) and grown in an incubator at 37 °C and 5% CO_2. Trypsin–EDTA (0.25% trypsin, 0.02% EDTA in buffer, Biochrom AG, Berlin Germany) was used to detach the cells. A solution of [^{11}C]C21 (50 nM) was added to plated cells. For blocking, 2 μM of non-labeled C21 was added 45 min before addition of the radiolabeled substance to saturate the receptors. The cells were incubated for 30 min at 4 °C to prevent internalization. Thereafter, the media was collected, the cells were detached by using trypsin–EDTA solution (0.25% trypsin, 0.02% EDTA in buffer, Biochrom AG, Berlin, Germany), re-suspended, and the radioactivity in the cells and media was measured to enable calculation of the fraction of cell-bound radioactivity. Experiments were performed in triplicate. Data were assessed by using an unpaired, two-sided *t*-test with GraphPad Prism (version 4.00 for Windows GraphPad Software) to determine significant differences ($p < 0.05$).

Biodistribution

The tissue distribution and kinetics of [^{11}C]C21 were studied by using healthy female Sprague–Dawley rats. Unsedated rats were injected with formulated (phosphate buffer pH 7.4) [^{11}C]C21 (11–13 MBq) through a lateral tail vein as a bolus. The rats were euthanized at defined time points by CO_2–O_2 inhalation. Selected time points were 10 ($n=2$), 20, and 40 min post-injection ($n=1$ for 20 and 40 min). In total, blood and 13 organs were collected; blood, heart, lungs, liver, pancreas, spleen, adrenals, kidneys, intestines (with and without contents), ovaries, muscle, and brain. The radioactivity and weight of all of the collected organs were measured and the injection site was checked for residual activity. The organ uptake of [^{11}C]C21 was expressed as SUVs, according to Equation (1). *RA* (Bq/g) is the concentration of radioactivity in the organ, *InjRA* (Bq) is the amount of radioactivity injected, and *TBW* (g) is the total body weight of the animal. All radioactivity measurements were corrected for decay.

$$SUV = \frac{RA\,(\mathrm{Bq/g})}{InjRA\,(\mathrm{Bq})/TBW\,(g)} \quad (1)$$

Small-Animal PET

Two male Sprague–Dawley rats were anesthetized with isoflurane (2–3%) in a 50/50% mixture of oxygen and air and placed on the temperature-controlled bed of a small-animal PET/CT scanner (Triumph trimodality, GE Healthcare). They were positioned with the abdomen in the field of view. Thereafter, [^{11}C]C21 (ca. 10 MBq) was administered as a bolus injection in the tail vein and a dynamic

PET scan was conducted for 40 min, followed by a CT scan. PET data were reconstructed by using maximum likelihood estimation maximization (10 iterations). Volumes of interest (VOIs) were drawn manually in the averaged images, for the liver, and in early frames, for the kidney and aorta. VOIs were then transferred to all frames to generate time–activity curves.

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