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RESEARCH ARTICLE

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"In-loop" carbonylation—A simplified method for carbon-11 labelling of drugs and radioligands

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Abstract

Transition-metal mediated carbonylation with ^{11}C-labelled carbon monoxide ([^{11}C]CO) is a versatile method for introducing ^{11}C ($t_{1/2}$ = 20.3 min) into drugs and radioligands for subsequent use in positron emission tomography (PET). The aim of the current study was to perform the ^{11}C-carbonylation reaction on the interior surface of a stainless-steel loop used for high performance liquid chromatography (HPLC). In the experimental setup, cyclotron produced ^{11}C-labelled carbon dioxide ([^{11}C]CO_2) was converted to [^{11}C]CO by reduction over heated Molybdenum and swept into an HPLC loop pre-charged with the appropriate reaction mixture. Following a 5 min reaction, the radiochemical purity (RCP) and the trapping efficiency (TE) of the reaction mixture was determined. After optimization, [^{11}C]*N*-Benzylbenzamide was obtained in quantitative radiochemical yield (RCY) following a 5 min reaction at room temperature. The methodology was further applied to label [^{11}C]benzoic acid (RCP≥99%, TE>91%), [^{11}C]methyl benzoate (RCP≥99%, TE>93%) and [^{11}C]phthalide (RCP≥99%, TE>88%). A set of pharmaceuticals was finally radiolabelled using non-optimized conditions. Excellent yields were obtained for the histamine-3 receptor radioligand [^{11}C]AZ13198083, the oncology drug [^{11}C]olaparib and the dopamine D2 receptor radioligand [^{11}C]raclopride, whereas a moderate yield was observed for the high-affinity dopamine D2 receptor radioligand [^{11}C]FLB457. The presented "in-loop" process proved efficient for diverse ^{11}C-carbonylations, providing [^{11}C]amides, [^{11}C]esters and [^{11}C]carboxylic acids in moderate to excellent RCYs. Based on the advantages associated with performing the radiolabelling step as an integrated part of the purification system, this methodology may become a valuable addition to the toolbox of methodologies used for ^{11}C-carbonylation of drugs and radioligands for PET.

KEYWORDS
carbon-11, ^{11}C-carbonylation, loop, radioligands, radiopharmaceutical chemistry

1 | INTRODUCTION

Positron emission tomography (PET) is a nuclear medicine imaging technique frequently used in translational research and drug development. After injection of a radiolabelled compound, PET produces a series of three-dimensional images of the tracer tissue distribution inside the living organism. A fundamental pre-requisite for PET is that the tracer molecule is labelled with a positron emitting radionuclide, such as ^{11}C ($t_{1/2}$ = 20.3 min). An important advantage with ^{11}C is that it can be introduced in the place of ^{12}C in bio-organic compounds without any structural modification of the target compound.

Due to the short half-life of carbon-11, its radiochemistry needs to be rapid, efficient and preferably automated to minimize radiation exposure to the radiochemist. The radionuclide is commonly produced by a medical cyclotron in the form of [^{11}C]methane or [^{11}C]carbon dioxide. Because of the convenient conversion of these species into [^{11}C]methyl iodide,[1,2] the radionuclide is most commonly introduced into tracer molecules via ^{11}C-methylation. Although methyl groups are common in bioactive molecules, the majority lack this structural motif, which makes other labelling methods necessary to avoid structural modification of the tracer molecule.

Carbonyl groups are present in most biologically active molecules and are featured in a large number of drug molecules. Significant efforts have thus been put into the development of methods that use [^{11}C]carbon monoxide ([^{11}C]CO), available in one step from [^{11}C]CO_2, to introduce the ^{11}C-carbonyl motif into tracer molecules. Radiochemistry with [^{11}C]CO is far from trivial, however, because of its low reactivity, poor solubility in organic solvents, high dilution in inert gas and the low concentration of isotopically labelled [^{11}C]CO.[3] Many of these challenges have been successfully addressed via recirculating [^{11}C]CO through the reaction media,[4] by performing the ^{11}C-carbonylation in a micro-autoclave (at 350 bar)[3,5] or inside a microtube reactor.[6] Other fruitful methods for improving the yield for these reactions include chemical complexation of [^{11}C]CO with diborane (BH_3-[^{11}C]CO),[7] copper scorpionates (Cu-Sc-[^{11}C]CO),[8] or introducing [^{11}C]CO into the reaction media in a stream of Xenon carrier gas.[9] These developments have resulted in numerous PET studies with tracer molecules labelled using palladium mediated ^{11}C-carbonylation chemistry.[10-15]

In an effort to simplify ^{11}C-carbonylation reactions from a technical perspective, we were inspired by previous work that employed captive solvents and reagents confined within plastic or stainless-steel tubes for the radiochemical synthesis.[16,17] It was considered that such an approach, which maximizes the surface area between the gas and liquid phase, may be ideally suited for [^{11}C]CO radiochemistry because of the increased opportunity for the radioactive gas to equilibrate with solution. In addition, an approach in which the HPLC loop is the reaction vessel for the ^{11}C-carbonylation was considered ideal, since transfer losses in such case are minimized and process automation simplified according to Wilson et al.[17] Thus, the aim of this study was to develop a novel "in-loop" method for PET tracer synthesis via ^{11}C-carbonylation using [^{11}C]CO (Figure 1).

2 | RESULTS AND DISCUSSION

An initial requirement for most "in-loop" ^{11}C-labelling processes is that the product must be formed in a one-pot, one-step procedure. We thus started our investigation on the "in-loop" ^{11}C-aminocarbonylation of iodobenzene with benzylamine, forming the product [^{11}C]*N*-benzylbenzamide, a reaction that has been well characterized previously.[18] DMF was first tested as a solvent for the reaction because of its captive properties and compatibility with "in-loop" ^{11}C-methylation.[17] Likewise, the Pd-xantphos catalytic system was used as a starting point for the optimization, since it has been shown to

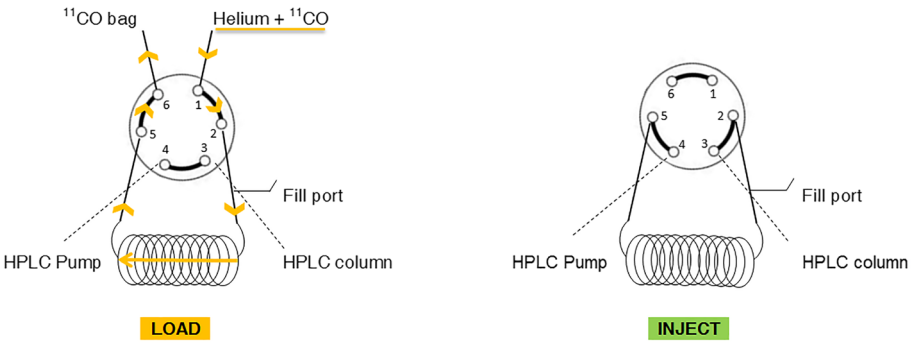


FIGURE 1 Schematic representation of the HPLC loop applied in the ^{11}C-carbonylation procedure. The yellow arrows indicate the flow direction for [^{11}C]CO

be efficient in promoting ^{11}C-aminocarbonylation reactions.[18,19] To our delight, a 5 min reaction at room temperature with Pd-xantphos in DMF furnished [^{11}C]*N*-benzylbenzamide at high trapping efficiency (TE), albeit at a moderate radiochemical purity (RCP) (Table 1, entry 1). Other solvents, such as DMSO and toluene, also provided good TEs in the "in-loop" ^{11}C-carbonylation (Table 1, entries 2 and 3), but the best yields were obtained in 1,4-dioxane and THF with near quantitative radiochemical yields (RCYs) (Table 1, entries 4 and 5). It is worth noting that an autoradiography experiment revealed that the majority of the radioactivity was trapped in the first quarter (~0.5 mL) of the stainless steel loop (Figure 2). In comparison, a control reaction with Pd$(PPh_3)_4$ provided [^{11}C]*N*-benzylbenzamide in 70% radiochemical purity, but with a poor TE (0.2%) and thus low RCY (0.14%).

TABLE 1 Investigation of solvent effects on the radiochemical yield of [^{11}C]*N*-benzylbenzamide

Entry	Solvent[a]	TE (%)	RCP (%)	RCY (%)
1	DMF	92	52	48
2	DMSO	95	88	84
3	Toluene	84	96	81
4	1,4-Dioxane	98	99±0.5[b]	97
5	THF	97	95±5[b]	92

Reaction conditions: $Pd_2(\pi\text{-cinnamyl})Cl_2$ (1 eq), Xantphos (2eq), Iodobenzene(1,4 μL), THF(700 μL) in appropriate solvent (150 μL) and Benzylamine (10 μL)

[a]Oxidative addition takes place during evaporation of THF (700μL)

[b]$n = 3$ (triplicate)

With the improved conditions in hand, the methodology was next applied to radiolabel functional groups other than amides. Thus, a lactone, an ester and a carboxylic acid, were each radiolabelled with only minor modifications to those previously used in the radiosynthesis of [^{11}C]*N*-benzylbenzamide (Scheme 1). Modifications to the original procedure include an increase in aryl halide concentration to improve trapping efficiency for [^{11}C]phthalide, and an increased temperature to improve the RCP of [^{11}C]*methyl b*enzoate. The observed yields from these reactions are on par with those observed for reactions performed in either vial or in micro-reactor setups, thus demonstrating that the utility of the developed "in-loop" ^{11}C-carbonylation method extends beyond aminocarbonylation.[18,20]

The "in-loop" carbonylation was finally tested on a set of drug-like molecules (Scheme 2). Thus, carbon-11 radiolabelling of the histamine type-3 receptor radioligand AZ13198083, the oncology drug olaparib and the dopamine D_2 receptor radioligands raclopride[14]

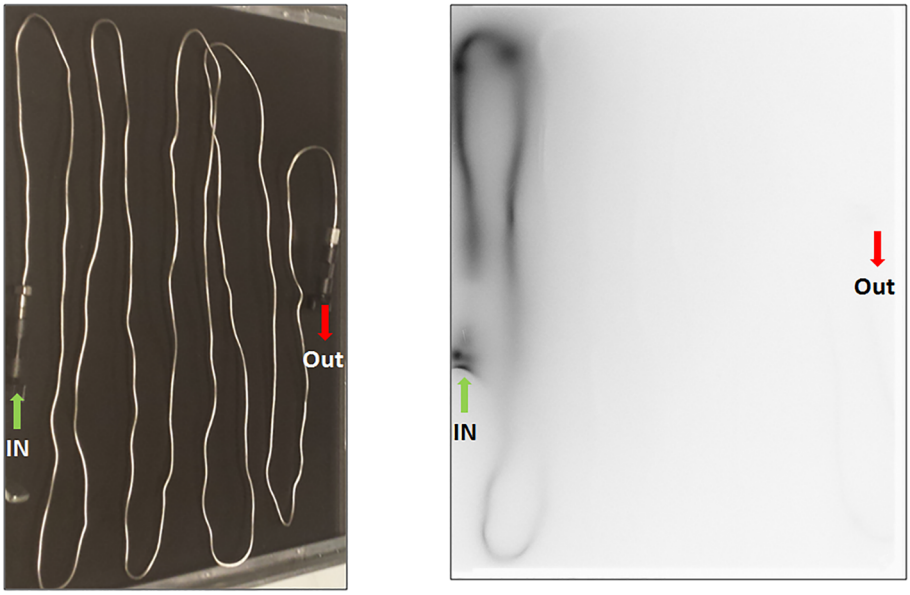
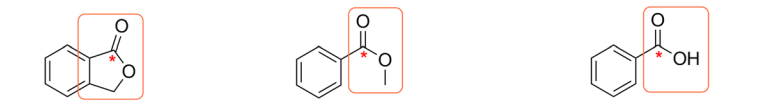


FIGURE 2 Picture (left) and autoradiographical image (right) of the HPLC loop following entrapment of [^{11}C]CO in the synthesis of [^{11}C]*N*-benzylbenzamide. The arrows indicate the entry and exit points of the He carrier gas through the HPLC loop

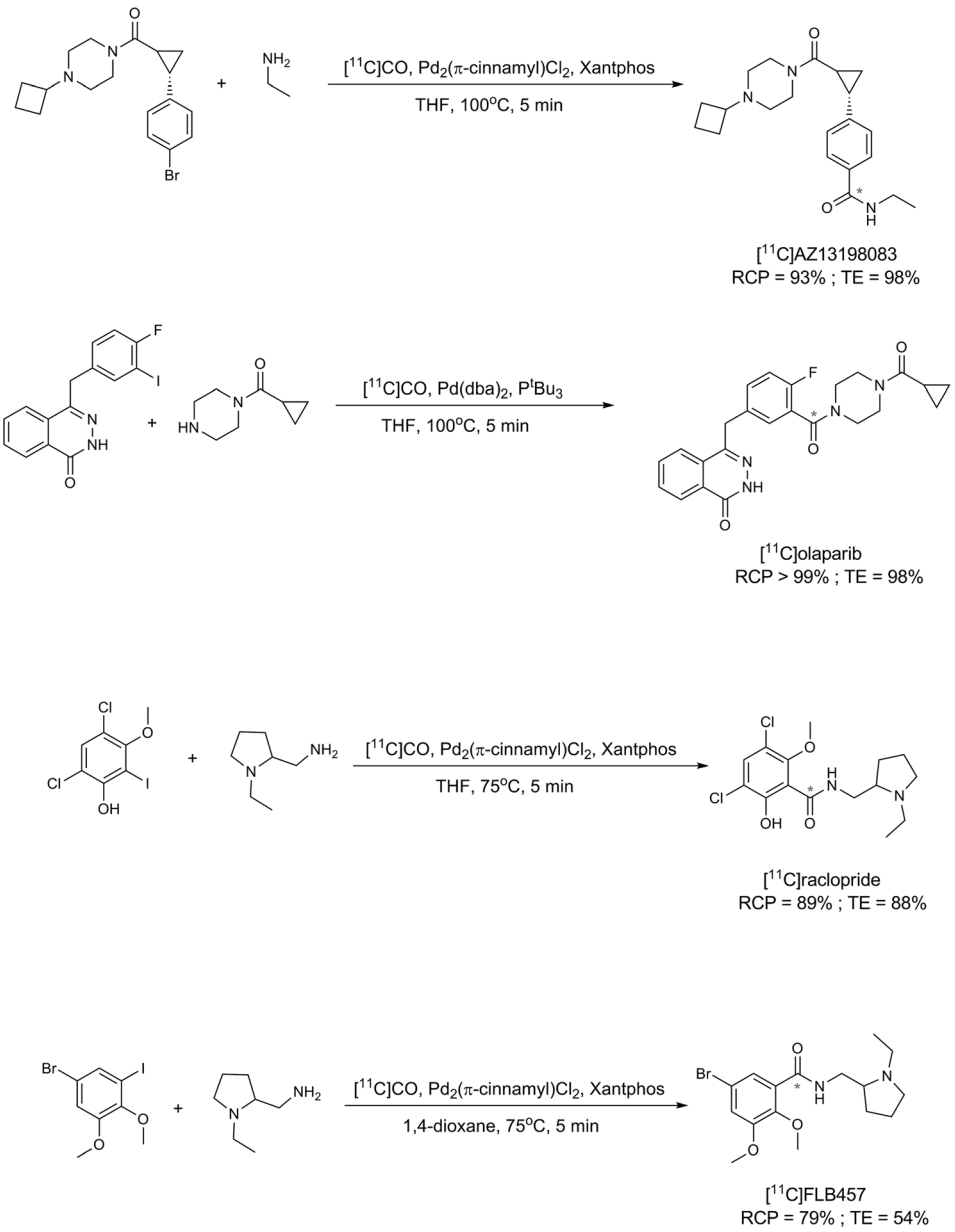


[^{11}C]phtalide
RCC ≥ 99% ; TE = 88%

[^{11}C]methylbenzoate
RCC ≥ 99% ; TE = 93%

[^{11}C]benzoic acid
RCC ≥ 99% ; TE = 91%

SCHEME 1 Synthesis of [^{11}C]phthalide, [^{11}C]methyl benzoate and [^{11}C]benzoic acid, via "in-loop" ^{11}C-carbonylation.



SCHEME 2 "In-loop" ^{11}C-carbonylation applied to four drug-like molecules.

and FLB457 were performed. Again, some modifications to the developed procedure were important to achieve good RCYs for these compounds. Most importantly, heating was required for all substrates to obtain the target tracers at high RCP. In addition, as was previously demonstrated by Skrydstrup et al., [^{11}C]olaparib cannot be produced with the Pd-xantphos system because of a phenyl scrambling from xantphos onto the aryl palladium complex.[21] Instead, the Pd-^{t}Bu$_3$ system was used to produce the target compound, thus demonstrating that other catalytic systems than the Pd-xantphos system are also compatible with the "in-loop" ^{11}C-carbonylation methodology. It is noteworthy that [^{11}C]FLB457 could only be produced at a moderate TE under the employed conditions, but this may well be improved following further optimization. Finally, after integrating the loop system into a commercially available radiochemistry system (GE Tracerlab FX-C, Uppsala, Sweden), [^{11}C]AZ13198083 was obtained in a 38% decay-corrected RCY. The isolated radiochemical yield was lower than expected because of losses during HPLC isolation, formulation and sterile filtration. However, the RCP was >99% and the molar activity was 110 GBq/μmol.

3 | CONCLUSION

The presented "in-loop" process proved to be useful for diverse ^{11}C-carbonylation, providing both simple reference

compounds (*e.g.* [^{11}C]amides, [^{11}C]esters and [^{11}C]carboxylic acids) as well as PET tracer molecules (*e.g.* [^{11}C] raclopride, [^{11}C]olaparib) in moderate to excellent RCYs. Given its simplicity and efficiency, the methodology has the potential to be widely implemented for carbonylations in PET tracer synthesis.

4 | MATERIALS AND METHODS

General experimental information

All chemical and solvents were obtained from Sigma-Aldrich (Sweden) and used without further purification, unless specified otherwise.

High performance liquid chromatography analysis (HPLC) was performed using a Hitachi L-6200 gradient pump and a Hitachi L-4000 variable wavelength UV-detector in a series with a Bioscan β+-flow detector. Analytical HPLC analysis was performed using a reverse phase column (XBridge, C18, 5 μm, 4.6 x 150 mm):

a-. [^{11}C]*N*-benzylbenzamide was eluted with a gradient between acetonitrile (A) and 0.1% TFA (B). The gradient was linear between 10 and 90% over 5 minutes and isocratic in between 5 and 8 min (ACN: 0.1% TFA, 90:10), at a flow rate of 3 ml/min.

b-. [^{11}C]phthalide was eluted with a gradient between acetonitrile (A) and 0.1 M NH_4HCO_2 (B). The gradient was linear between 10 and 90% over 9 minutes and isocratic in between 9 and 10 min (ACN: 0.1 M NH_4HCO_2, 90:10), at a flow rate of 2 ml/min.

c-. [^{11}C]methyl *b*enzoate was eluted with a gradient between acetonitrile (A) and 0.1% TFA (B). The gradient was linear between 10 and 90% over 5 minutes and isocratic in between 5 and 0 min (ACN: 0.1% TFA, 90:10), at a flow rate of 3 ml/min.

d-. [^{11}C]benzoic acid was eluted with a gradient between acetonitrile (A) and 0.1% TFA (B). The gradient was linear between 10 and 90% over 7 minutes and isocratic in between 7 and 10 min (ACN: 0.1% TFA, 90:10), at a flow rate of 3 ml/min.

e-. [^{11}C]AZ13198083 was eluted with a gradient between acetonitrile (A) and 0.1 M NH_4HCO_2 (B). The gradient was linear between 10 and 90% over 9 minutes and isocratic in between 9 and 10 min (ACN: 0.1 M NH_4HCO_2, 90:10), at a flow rate of 2 ml/min.

f-. [^{11}C]olaparib was eluted with a gradient between acetonitrile (A) and 0.01 M NH_4HCO_2 (B). The gradient was linear between 30 and 70% over 5 minutes and isocratic in between 5 and 6 min (ACN: 0.1 M NH_4HCO_2, 90:10), at a flow rate of 3 ml/min.

g-. [^{11}C]raclopride was eluted with a gradient between acetonitrile (A) and 0.1% TFA (B). The gradient was linear between 10 and 90% over 9 minutes and isocratic in between 9 and 10 min (ACN: 0.1% TFA, 90:10), at a flow rate of 2 ml/min.

h-. [^{11}C]FLB457 was eluted with a gradient between acetonitrile (A) and 0.1 M NH_4HCO_2 (B). The gradient was linear between 30 and 70% over 9 minutes and isocratic in between 9 and 10 min (ACN: 0.1 M NH_4HCO_2, 90:10), at a flow rate of 2 ml/min.

The identity of all radioactive products was confirmed by co-elution with the corresponding non-radioactive compound.

4.1 | Radiochemistry

All experiments were performed in accordance with local and national rules and laws that govern the work with open sources of radiation. The study was carried out at the PET Centre, Karolinska Institutet (Stockholm, Sweden).

[^{11}C]CO_2 was produced by a GEMS PETtrace cyclotron (General Electrics Medical Systems, Uppsala, Sweden). The $^{14}N(p,\alpha)^{11}C$ nuclear reaction, performed by proton bombardment of a pressurized gas target containing nitrogen (AGA, Nitrogen 6.0) and 1% oxygen (AGA, Oxygen 4.8), was used to produce [^{11}C]CO_2. At the end of bombardment (EOB), [^{11}C]CO_2 was transferred from the target to a [^{11}C]CO-synthesizer prototype (General Electrics Medical Systems, Uppsala, Sweden). [^{11}C]CO_2 was trapped on a molecular sieve column (0.6 mg packed in a ¼" tube, mesh 80/100, GRACE, USA) at room temperature. The accumulated [^{11}C]CO_2 was released into a controlled stream of helium (10 ml/min) while heating the molecular sieve trap to 360°C. The purified [^{11}C]CO_2 was pre-concentrated by a stainless steel loop trap immersed in liquid nitrogen (-196°C) prior to on-line reduction over a heated Molybdenum column (850°C). The effluent gas was purified through an Ascarite® (sodium hydroxide-coated silica, 20–30 mesh, Sigma-Aldrich, Sweden) column prior to concentrating the [^{11}C]CO on a silica gel trap immersed in liquid nitrogen. Following completed entrapment, [^{11}C]CO was released in a stream of Helium into a stainless steel HPLC loop for the ^{11}C-carbonylation reaction. Untrapped [^{11}C]CO was collected in a gas-tight bag (Tedlar® gas sampling bag push lock valve 1L, Sigma-Aldrich, Sweden).

4.2 | In-loop ^{11}C-carbonylation setup

A commercial HPLC injection valve (VICI, #C52-12061, Switzerland) was equipped with a stainless-steel loop (2 mL, VICI, #SL2KCUW, Switzerland) and an injection port (for 1/16" fill port, VICI #VISF-2, Switzerland). Prior to

start of synthesis, the injection valve was set to LOAD position (Figure 1) and a solution of aryl halide, palladium-source, supporting ligand and nucleophile in anhydrous solvent, was injected using a 1 mL syringe capped with a filter (Acrodisc 13 mm minispike with 0.45 μm GHP Membrane, Sigma-Aldrich, Sweden) through the injection port of the loop. The isolated and concentrated [^{11}C]CO was then swept into the loop at 5 mL/min, and after a 5 min reaction, the contents of the loop were transferred to a sealed test tube by purging the loop with THF (~2 mL). After transfer to the test tube, the radioactivity in the test tube was measured before and after it was purged with N_2 (to assess TE), followed by a measurement of the gas waste bag that was connected on the outlet of the HPLC loop. The radioactivity in the test tube was also analyzed using radio-HPLC to assess the RCP that gives information about reaction selectivity. Moreover, as [^{11}C]CO is a radioactive gas, the parameter TE needs to be taken into account. TE is the total amount of [^{11}C]CO trapped in the reaction solution that is converted to non-volatile entities at the end of the reaction. RCY was calculated from the RCP and TE (*i.e.* RCY = RCP x TE).

4.3 | Application to GE Tracerlab FX-C

A Tracerlab FX-C system (GE PETtrace, Uppsala, Sweden) was used for semi-preparative HPLC purification, in parallel with the [^{11}C]CO-synthesizer prototype (General Electrics Medical Systems, Uppsala, Sweden) to produce [^{11}C]**7**. In short, ^{11}C-carbon monoxide was transferred from the [^{11}C]CO-system to the stainless-steel loop in the Tracerlab FX-C system where the reaction was taking place. The crude product was obtained in the HPLC loop and then purified using a semi-preparative HPLC column (ACE C-18, 5μm, 10 × 250 mm; Advanced Chromatography Technologies Ltd; Aberdeen, UK) eluted with an isocratic mobile phase consisting of 30% MeCN in HCO_2NH_4 (0.1M). The eluate was monitored for absorbance (λ = 254 nm) and for radioactivity. [^{11}C]**7** (t_R = 8–10 min) was collected and diluted with water (~50 mL) and then concentrated on a C-18 SepPak (Waters, Sweden). The SepPak was then washed with water (~8 mL) prior to elution with ethanol (99%, 1.5 mL) into saline (10 mL). The formulation was finally filtered through a yellow sterile filter (0.22 μm, Millipore, Millex®GV) to yield the final product in a sterile injection vial.

4.4 | Synthesis of [^{11}C]*N,N*-benzylbenzamide

An oven-dried disposable 4 mL vial was equipped with a screw thread cap, a rubber septum and cooled under Nitrogen (N_2) atmosphere. Pd_2[π-cinnamyl]Cl_2 (2.3 mg; 1eq), xantphos (5.0 mg; 2 eq), iodobenzene (1.4 μL, 1.42 eq) were dissolved in anhydrous THF (700 μL) and the resulting mixture was flushed with N_2 until the THF was fully evaporated (20 min). 1,4-Dioxane (150 μL) and Benzylamine (10 μL) were added and the reaction mixture was introduced into the HPLC loop approximately 2 min prior to start of synthesis. The resulting mixture was allowed to react for 5 min at r.t.

4.5 | Synthesis of [^{11}C]phthalide

Pd_2[π-cinnamyl]Cl_2 (6.7 mg; 3 eq), xantphos (7.5 mg; 3 eq) and iodobenzyl alcohol (3.0 μL, 3 eq) were dissolved in anhydrous THF (700 μL) kept at r.t. for 15 min and then heated at 75°C during 5 min. THF (150 μL) was added and the resulting mixture was introduced into the HPLC loop approximately 2 min prior to start of synthesis. The resulting mixture was allowed to react for 5 min at 75°C.

4.6 | Synthesis of [^{11}C]methyl benzoate

Pd_2[π-cinnamyl]Cl_2 (2.3 mg; 1eq), xantphos (5.0 mg; 2eq) and iodobenzene (2.2 μL, 2.9 eq) were dissolved in anhydrous THF (700 μL) and the resulting mixture was flushed with N_2 until the THF was fully evaporated (20 min). THF (150 μL) and a solution of lithium methoxide in MeOH (1M, 40 μL) were added and the reaction mixture was introduced into the HPLC loop approximately 2 min prior to start of synthesis. The resulting mixture was allowed to react for 5 min at 120°C.

4.7 | Synthesis of [^{11}C]benzoic acid

Pd_2[π-cinnamyl]Cl_2 (2.2 mg), xantphos (5.0 mg) and iodobenzene (1.4 μL) were dissolved in anhydrous THF (700 μL) and the resulting mixture was flushed with N_2 until the THF was fully evaporated (20 min). 1,4-Dioxane (150 μL) and a solution of NaOH (0.35 M, 3.5 μL) were added and the reaction mixture was introduced into the HPLC loop approximately 2 min prior to start of synthesis. [^{11}C]CO was swept at r.t to the loop in which the coupling reagents were already added. The resulting mixture was allowed to react for 5 min at r.t.

4.8 | Synthesis of [^{11}C]AZ13198083

Pd_2[π-cinnamyl]Cl_2 (2.2 mg; 1 eq), xantphos (5.0 mg; 2 eq), ((2S)-2-(4-bromophenyl)cyclopropyl)(4-cyclobutylpiperazin-1-yl)methanone (3.3 μL) were dissolved in

anhydrous THF (700 μL) and the resulting mixture was flushed with N_2 until the THF was fully evaporated (20 min). A solution of ethylamine in THF (2M, 600 μL) was added and the reaction mixture was introduced into the HPLC loop approximately 2 min prior to start of synthesis. The resulting mixture was allowed to react for 5 min at 100°C.

4.9 | Synthesis of [^{11}C]olaparib

Pd (dba)$_2$ (11.5 mg), P^{t}Bu$_3$ (4.9 μL) and 4-(4-fluoro-3-iodobenzyl)phthalazin-1(2H)-one (7.6 mg) were dissolved in anhydrous THF (700 μL) and the resulting mixture was flushed with N_2 until the THF was fully evaporated (20 min). THF (150 μL) and cyclopropyl (piperazin-1-yl)methanone (10.1 μL) were added and the reaction mixture was introduced into the HPLC loop approximately 2 min prior to start of synthesis. The resulting mixture was allowed to react for 5 min at 100°C.

4.10 | Synthesis of [^{11}C]raclopride

Pd_2[π-cinnamyl]Cl_2 (6.4 mg; 1 eq), xantphos (7.1 mg; 1eq) and 4,6-dichloro-2-iodo-3-methoxyphenol (5.9 mg, 1.5 eq) were dissolved in anhydrous THF (1 mL) and the resulting mixture was flushed with N_2 until the THF was fully evaporated (20 min). THF (150 μL) and (S)-(-)-2-Aminomethyl-1-ethylpyrrolidine (30 μL) were added and the reaction mixture was introduced into the HPLC loop approximately 2 min prior to start of synthesis. The resulting mixture was allowed to react for 5 min at 75°C.

4.11 | Synthesis of [^{11}C]FLB457

Pd_2[π-cinnamyl]Cl_2 (2.2 mg; 1 eq), Xantphos (5.0 mg; 2 eq), 5-bromo-1-iodo-2,3-dimethoxybenzene (5.1 mg) were dissolved in anhydrous THF (700 μL) and the resulting mixture was flushed with N_2 until the THF was fully evaporated (20 min). 1,4-Dioxane (150 μL) and (S)-(-)-2-Aminomethyl-1-ethylpyrrolidine (27 μL) were added and the reaction mixture was introduced into the HPLC loop approximately 2 min prior to start of synthesis. The resulting mixture was allowed to react for 5 min at 75°C.

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