From THE DEPARTMENT OF CLINICAL NEUROSCIENCE
Karolinska Institutet, Stockholm, Sweden

METHOD DEVELOPMENT FOR [^{11}C]CARBON MONOXIDE RADIOCHEMISTRY

Mélodie Ferrat

Karolinska Institutet

Stockholm 2021

All previously published papers were reproduced with permission from the publisher.
Published by Karolinska Institutet.
Printed by Universitetsservice US-AB, 2021
© Mélodie Ferrat, 2021
ISBN 978-91-8016-132-9
Cover illustration: Palladium-mediated ^{11}CO-carbonylation reactions illustration for the insertion of ^{11}CO into molecules

Method development for [^{11}C]carbon monoxide radiochemistry
THESIS FOR DOCTORAL DEGREE (Ph.D)

By

Mélodie Ferrat

The thesis will be defended in public at Karolinska Institutet in Solna, Stockholm, March 19th 2021

Principal Supervisor:
Associate Professor Magnus Schou
AstraZeneca PET Science Centre
Karolinska Institutet
Department of Clinical Neuroscience

Co-supervisor(s):
Professor Christer Halldin
Karolinska Institutet
Department of Clinical Neuroscience

Dr. Kenneth Dahl
Karolinska University Hospital
Department of Radiopharmacy

Dr. Sangram Nag
Karolinska Institutet
Department of Clinical Neuroscience

Opponent:
Assistant Professor Johnny Vercouillie
Tours University
Department of Biophysics and Mathematics

Examination Board:
Associate Professor Daniëlle Vugts
VU Amsterdam
Department of Radiology and Nuclear Medicine

Associate Professor Thuy Tran
Karolinska Institutet
Department of Oncology and Pathology

Professor Jaak Järv
University of Tartu
Institute of Chemistry

To my family

Séparation Hivernale

Toi qui es loin de nous, mon âme est près de toi
Parce que tu es l'oiseau qui remplit jour et nuit ma mémoire
Chaque fois que je suis seul avec le clair de lune
Tu regardes les portes et le toit de mon cœur depuis les horizons

Parce qu'ouvrir ta lettre dégouline de la tristesse dans mon cœur
Tes mots racontent l'histoire de tes solitudes
Tel est le cas avec le roseau isolé
Chaque partie de ton corps exprime la tristesse et la séparation

Je m'assois seul la nuit en pensant à toi
Chaque mélodie que j'entends me rappelle ta voix…
Les jours où je traverse des ruelles familières
Partout où je vais, je dis que ce sont tes traces!

À chaque nuit lumineuse, la lune enchanteresse me sourit,
Oh merveille! Je te vois face à la lune
Mon sommeil agité est le miroir de ton image
Je me retourne partout et rêve que tu es là

En hiver, je me dis la séparation du jour et de la nuit
Souviens-toi des jours où nous avions un nouveau printemps ensemble
Le destin nous a enlevé nos nuits
Oh la bonne journée que nous avons eue ensemble!

Ô bienheureux ange de la chance, vole vers moi
Sans toi, mes larmes, mon ombre, ma pierre, je ne sais pas ce que je suis…
La feuille de mon âme tremble de la tempête de la mort
J'ai peur du jour où tu reviendras mais je ne serai plus là... !

Mehdi Soheili

ABSTRACT

Positron emission tomography (PET) is a non-invasive molecular imaging technique that has found extensive utility in biomedical research and in drug development. A fundamental pre-requisite for PET is the tracer, which is a biologically relevant molecule, labeled with a short-lived radionuclide. One of the most attractive radionuclides for PET is carbon-11 (^{11}C) that has a half-life of only 20 minutes (^{11}C, $t_{1/2}$=20.3 min). This radionuclide can be introduced via transition-metal mediated carbonylation with [^{11}C]carbon monoxide ([^{11}C]CO), a reaction that has found utility in the production of a wide range of drug-like molecules and radioligands. Transition-metal mediated ^{11}C-carbonylation is typically performed at high pressure and high temperature due to the poor solubility of [^{11}C]CO in organic solvents and its high dilution in inert gas. Because of its radioactive nature, chemical processes with ^{11}C not only need to be fast, but also need to be automated inside a lead-shielded fume cupboard to ensure operator safety.

The current PhD thesis aimed to develop novel and simplified methods for the introduction of ^{11}C into one of the most abundant functional groups in bioactive molecules, namely the carbonyl group. **Paper I** describes the development of a new stainless-steel loop method for ^{11}C-carbonylation reactions, in which a thin film of reagents is created on the interior surface of the loop. This operation creates a large surface area, which facilitates exchange between the liquid and gas phase and thus enhances trapping and incorporation of [^{11}C]CO into target compounds via reactive palladium complexes. The method was applied to a set of test compounds and proved to be useful to provide ^{11}C-labeled amides, esters and carboxylic acids with good to excellent yields. As a proof of concept, the histamine-3 receptor radioligand [^{11}C]AZ13198083, the oncology drug [^{11}C]olaparib, the dopamine D2 receptor radioligands [^{11}C]raclopride and [^{11}C]FLB457 were produced using the same method.

To allow tracers labeled by ^{11}C-carbonylation to be used in studies of human physiology and pathophysiology, **Paper II** described the development of a new automated system for [^{11}C]CO radiochemistry that complies with all regulations associated with such studies (e.g. good manufacturing practice (GMP)). The aim was to develop the first commercially available [^{11}C]CO system with the purpose of making [^{11}C]CO radiochemistry accessible to the wider PET community. Following development and optimization of each part of the GMP system, the [^{11}C]CO synthesizer was successfully used to produce the histamine type-3 radioligand, [^{11}C]AZ13198083.

In **Paper III**, a novel and simple method for the synthesis of ^{11}C-labeled primary amides was developed. This process consists on the Pd-mediated ^{11}C-aminocarbonylation of aryl halides via intermediate electrophilic aroyl-DMAP-salts (DMAP – 4-dimethylaminopyridine). The method provided a range of substrates with good to excellent yields and was finally successfully applied to the radiolabeling of the two cancer drugs [^{11}C]niraparib and [^{11}C]veliparib for preclinical studies.

To conclude, a variety of new methodologies have been described for ^{11}C-labeling carbonyl groups that have the potential to be widely implemented in the development of new tracer molecules for PET imaging.

LIST OF SCIENTIFIC PAPERS

I. **Mélodie Ferrat**, Kenneth Dahl, Christer Halldin, Magnus Schou. "In-loop" carbonylation - A simplified method for carbon-11 labelling of drugs and radioligands. *Journal of labelled compounds and radiopharmaceuticals*, **2020**, 1-8.

II. **Mélodie Ferrat**, Youssef El Khoury, Peter Larsen, Kenneth Dahl, Christer Halldin, Magnus Schou. Development of a fully automated low-pressure [^{11}C]CO carbonylation apparatus. *Journal of labelled compounds and radiopharmaceuticals*, **2020**, 63, 517-522.

III. **Mélodie Ferrat**, Kenneth Dahl, Magnus Schou. One-pot synthesis of ^{11}C-labelled benzamides via intermediate [^{11}C]aroyl dimethylaminopyridinium salts. *SUBMITTED*

CONTENTS

LIST OF ABBREVIATIONS

^{11}B	Boron-11
^{11}C	Carbon-11, radioactive carbon
$^{11}CO_2$	[^{11}C]Carbon dioxide
$^{11}CH_4$	[^{11}C]Methane
^{11}CO	[^{11}C]Carbon monoxide
^{13}N	Nitrogen-13
$^{14}N(p,\alpha)^{11}C$	Nitrogen-14 + 1 proton (p) $\rightarrow$ ^{4}He (α particle) + carbon-11
^{18}F	Fluorine-18
[^{18}F]FDG	2-Deoxy-2-[^{18}F]fluoro-D-glucose
[^{18}F]-L-DOPA	[^{18}F]L-dihydroxyphenylalanine
^{15}O	Oxygen-15
Å	Ångström
A_m	Molar activity
$A_{(t=0)}$	Activity at time 0
Bar	Unit of pressure, 1 bar = 10^5 Pa
Boc_2O	Di-*tert*-butyl dicarbonate
Carrier	Natural isotope ^{12}C
D1	Dee 1, D-shaped metallic plates in a cyclotron
D2	Dee 2, D-shaped metallic plates in a cyclotron
DMAP	4-Dimethylaminopyridine
DMF	*N*,*N*-Dimethylformamide
DMSO	Dimethyl sulfoxide
e	Electron
EOB	End of bombardment
F-DOPA	Fluorodopa, fluorinated form of L- DOPA
GBq	Gigabecquerel, SI unit of radioactivity, 1GBq=10^3MBq
GMP	Good manufacturing practice
He	Helium
HPLC	High-performance liquid chromatography

$K_{(decay)}$	Rate constant of decay, each radioactive isotope has different $K_{(decay)}$
KeV	Kilo-electron volt, unit of energy
HMDS	Hexamethyldisilazane
MBq	Megabecquerel, SI unit of measurement of radioactivity
MeCN	Acetonitrile
mg	Milligrams
mm	Millimeters
Mo	Molybdenum
N	Number of neutrons
Nu^-	Nucleophile
OTf	Triflate, trifluoromethanesulfonate
PARP	Poly- ADP ribose polymerase, enzyme helps damaged cells to repair
PET	Positron emission tomography
Pd	Palladium
Pd-L_2	Palladium-ligands
PPh_3	Triphenylphosphine
PTC	Phase-transfer catalyst
RCP	Radiochemical purity
RCY	Radiochemical yield
RT	Room temperature
R_t	Retention time
SPE	Solid phase extraction
$t_{1/2}$	Half-life
TE	Trapping efficiency
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
UV	Ultraviolet
Z	Atomic number, number of protons
Zn	Zinc
α	Particle alpha
β	Beta-decay, type of radioactive decay

β^+	Positron emission, once a proton is converted to neutron it creates a positron
γ	Gamma
µmol	Micromole, 1 mol = 10^6µmol
µA	Microampere, a unit of electric current

1 INTRODUCTION

1.1 THE HISTORY OF RADIOACTIVITY

Radioactivity is everywhere, and we are constantly in contact with ionizing radiation due to the natural radioactivity. Henri Becquerel discovered natural radioactivity in 1896 during an experiment in which uranium salts were put in contact with a photographic plate. Unexpectedly, the plate was exposed even though the uranium salts had not been exposed to light and it was concluded that uranium emits its own radiation.[1] Becquerel shared the Nobel Prize in 1903 with his student Marie Curie and her husband Pierre following their discovery of the radioactive metals Polonium and Radium. Since then, radioactivity has had a tremendous impact on medicine, industry and science, sometimes with serious consequences on the user's health, but when properly managed the positive aspects can outweigh the risks. Today, the use of radioactivity is strictly regulated to ensure the protection of humans and the environment.

1.2 PRINCIPLES OF PET

Positron Emission Tomography (PET) is a non-invasive nuclear imaging technique used in clinical diagnosis, drug development and biomedical research. PET enables the visualization of radioactive tracers, specially-designed radioactive molecules that reveal pharmacological and biological processes in living subjects. These radiotracers are bioactive molecules labeled with positron emitters (e.g., ^{11}C, ^{15}O, ^{18}F, ^{13}N). Once the radioactive molecule is injected intravenously into the subject (patient or experimental animal) it is transported by the blood stream to the target of interest. The nuclei of positron emitting radionuclides are rich in protons, so during their decay a proton is converted into a neutron and a positron is emitted. This positron travels a short distance before annihilating with an electron from the matter. Different isotopes emit positrons at different energies so the positron emitted by ^{15}O travels ≈ 2.5 mm, the one from ^{18}F ≈ 0.6 mm and the one emitted by ^{11}C ≈ 1.1 mm.[2] The annihilation event generates two gamma (γ) rays at nearly opposite directions (~180°), each with an energy of 511 keV (Figure 1). When two gamma rays are detected simultaneously by the detector ring that surrounds the research subject, they are registered as a coincidence event. Only coincidence events are used to generate the final PET image.

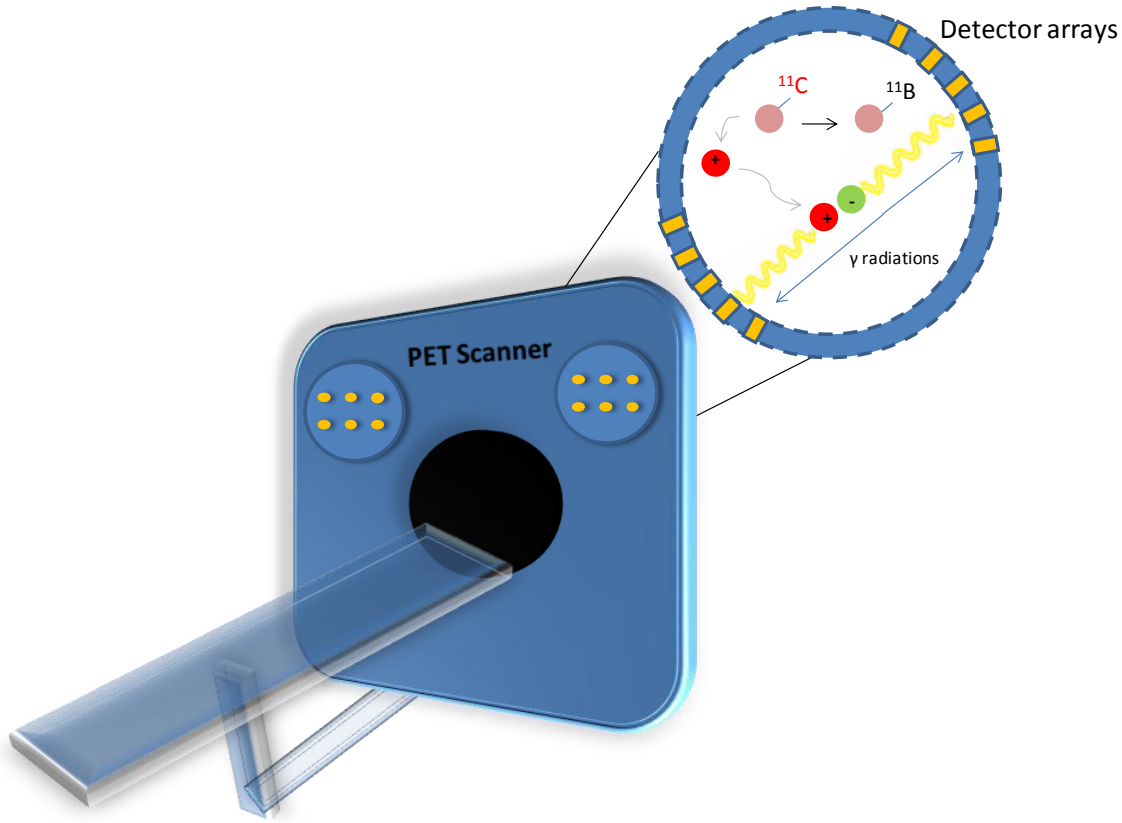


Figure 1: Graphical representation of the PET system and the mechanism of detecting the short-lived radionuclide carbon-11

1.2.1 Applications of PET in clinical diagnosis, biomedical research and drug development

Nowadays, PET has an important role in clinical oncology where it is useful as a diagnosis tool; to determine whether a tumor is malignant or benign, track the spread of cancer and provide information on treatment effect. The most widely used tracer in PET is [^{18}F]2-fluorodeoxyglucose (FDG), an analogue of glucose, which accumulates in cells with high glucose consumption (e.g. tumors) and because its metabolism is obstructed by a fluorine in the 2-position, [^{18}F]FDG accumulation is irreversible. In addition to being commonplace in clinical oncology, [^{18}F]FDG PET has also been applied to study regional cerebral and cardiac metabolism, respectively. Other tracers that are integrated in routine clinical imaging include [^{13}N]ammonia (cardiology), [^{11}C]acetate (oncology), [^{18}F]fluorodopa (neurology, oncology) and a number of labeled amino acid derivatives, including [^{11}C]methionine and [^{18}F]fluorocholine (both used in oncology).[3]

In addition to the emerging role of PET in clinical diagnosis, it is increasingly used in translational biomedical research. PET was early adopted by neuroscientists to study the pathophysiology of neurological and neuropsychiatric disorders without the need for risky biopsies from brain. Other scientific disciplines followed shortly after and today there are numerous examples of translational research on disease biology facilitated by PET across all

therapeutic areas.[4,5] Although a plethora of tracers have been developed to aid in such research, suitable tracer molecules are still a major bottleneck in PET imaging.

The major applications of PET in support of drug development include i) microdosing, in which the in vivo distribution of a labeled drug is studied. ii) target occupancy studies, in which the in vivo drug-target interaction is studied. iii) biomarker studies, in which an imaging biomarker of pathophysiology is used for diagnosis and/or monitoring disease progression.[6] The most sophisticated radiochemical methods are typically required for PET microdosing studies, where radiochemists are faced with labeling drug molecules that were not developed with the intent of being labeled.

1.2.2 Radionuclide production

The radionuclides used in PET are typically produced in circular particle accelerators also known as cyclotrons. The fundament of a cyclotron is the electromagnet that generates a vertical magnetic field and the two D-shaped metallic plates called dees. These dees are connected to an alternating electric source in which the polarity of the electric field changes periodically. Charged particles, often hydride ions, are generated at the center of the cyclotron and are subsequently attracted by the first positive dee (D1). Once the hydride leaves the D1 plate and enters a gap between the dees, the polarity of the two plates change and the other plate (D2) becomes positively charged. The hydride will now be repelled by the D1 plate and attracted toward the D2 plate, resulting in an increase of its energy. By alternating the charge of the two dees, hydride ions will be accelerated in a cyclic fashion with increasing energy resulting from each orbit. When the desired energy has been attained, the electrons are stripped off the hydride ion and the now positively charged particle will be repelled from the orbit and shot into a reaction vessel for the nuclear reaction. This reaction vessel, commonly referred to as a "target", may be filled with a liquid, solid or a gas, depending on the desired radionuclide (table 1).

Radionuclide	Target	Nuclear reaction	$T_{1/2}$ (min)	Product
^{11}C	N_2(g) + 0.1% O_2 (g)	$^{14}N(p,\alpha)^{11}C$	20.3	$[^{11}C]CO_2$
	N_2(g) + 10% H_2 (g)	$^{14}N(p,\alpha)^{11}C$		$[^{11}C]CH_4$
^{13}N	H_2O (l) + EtOH(l)	$^{16}O(p,\alpha)^{13}N$	10.0	$[^{13}N]NH_3$
^{15}O	N_2(g) + 0.2% O_2 (g)	$^{14}N(d,n)^{15}O$	2.0	$[^{15}O]O_2$
^{18}F	$H_2{}^{18}O$ (l)	$^{18}O(p,n)^{18}F$	109.7	$[^{18}F](H_2O)_n$

Table 1: Characteristics of commonly used radionuclides.

1.3 PET RADIOCHEMISTRY

The chemical elements used in PET have diverse properties and thus require fundamentally different radiolabeling methods for their introduction (table 1). Whereas covalent bonding is the preferred method for labeling with carbon and heteroatoms, chelation is the preferred method for labeling with the larger metal radionuclides. Radiochemical methods are also limited by the physical half-lives of the radionuclides. As a rule of thumb, not more than three half-lives should be used for synthesis, purification and formulation of a PET tracer to avoid excessive radioactive decay. Thus, the short half-lives of oxygen-15 and nitrogen-13 restrict their use to single step labeling of simple molecules such as water and ammonia. Although many PET radionuclides can be used in direct radiolabeling of tracer molecules in a similar fashion, it is not uncommon that cyclotron-produced radionuclides require further chemical manipulation before they can be used in radiolabeling. A wide range of ^{18}F and ^{11}C radiolabeling agents have been prepared and are currently used as synthons in PET chemistry.

1.3.1 Molar activity

PET is a sensitive imaging technique that allows for the measurement of radiolabeled tracers at picomolar concentrations. This exquisite sensitivity stems from the high ratio between radioactivity and molar amount, or the high "molar activity" (A_m), that often can be obtained for PET radionuclides. A high A_m is important to avoid saturation of target proteins in binding studies, and it is typically recommended that less than 5-10% of these proteins are occupied by the tracer to ensure that the biological system of interest remains unperturbed during the PET measurement. However, it is also important to note that not all studies require high A_m. For instance, studies of high-capacity systems (e.g., with F-DOPA and FDG) and drug biodistribution do not require a high A_m.[6]

1.3.2 Radiolabeling with Carbon-11

Carbon-11 (^{11}C) has an unstable nucleus (Z = 6, N = 5) and decays almost exclusively via positron emission > 99.7 % to boron-11 (Z = 5, N = 6): $^{11}C \rightarrow {}^{11}B + \beta^+$ with a 20.3 min half-life. Only negligible amounts decay via electron capture

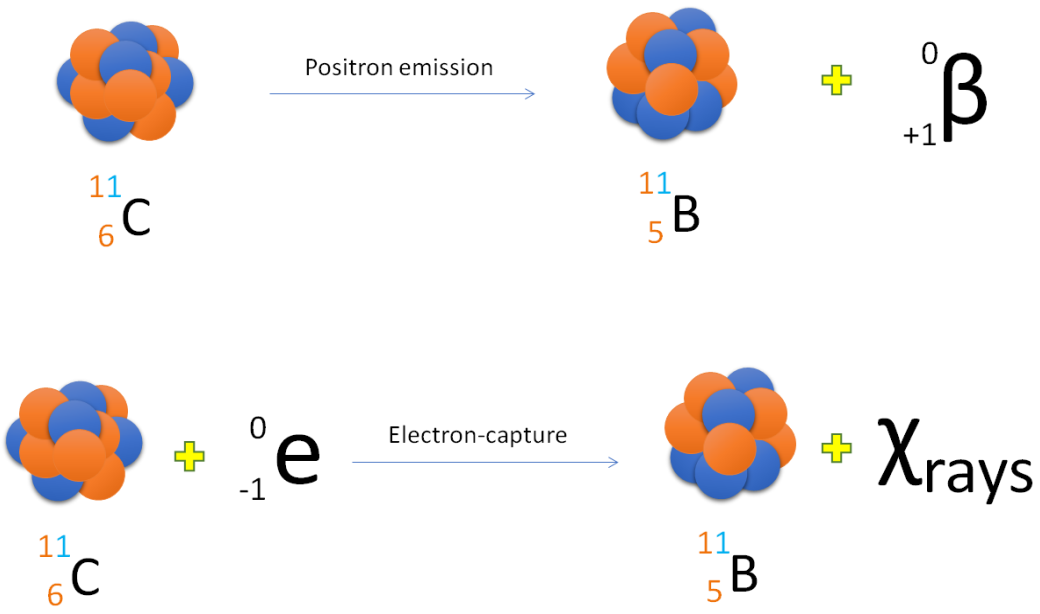


Figure 2: The two decay pathways for carbon-11.

The short half-life of ^{11}C can be perceived as both strength and weakness. While it leads to low radiation doses to subjects and allows for multiple studies to be performed on the same day, it also introduces a significant challenge to the radiochemist. Despite its short half-life, ^{11}C is still considered an attractive PET radionuclide because it enables substitution of ^{12}C in bio-organic compounds without structural modifications. ^{11}C is produced with a cyclotron via the $^{14}N(p,\alpha)^{11}C$ nuclear reaction by proton bombardment of nitrogen gas containing oxygen (0.1-1%) or hydrogen (5-10%) in order to produce [^{11}C]CO_2 and [^{11}C]CH_4, respectively. These radiolabeled precursors are efficiently purified by passing the target gas through columns that either selectively trap them or absorb chemical impurities. Thus, [^{11}C]CO_2 is typically purified from oxygen and nitrous oxides by selective trapping on molecular sieves or in a cryogenic loop. Ammonia, which is the main impurity observed following [^{11}C]CH_4 production, can be efficiently removed by a phosphorous pentoxide trap in-series with the cryogenic trap for [^{11}C]CH_4. [^{11}C]CH_4 and [^{11}C]CO_2 are typically transformed into secondary labeling precursors such as [^{11}C]carbon monoxide ([^{11}C]CO),[7-9] [^{11}C]hydrogen cyanide ([^{11}C]HCN),[10] [^{11}C]methyl iodide ([^{11}C]CH_3I),[11] [^{11}C]methyl triflate ([^{11}C]CH_3OTf),[12,13] or [^{11}C]phosgene ([^{11}C]$COCl_2$)[14-16] prior to the radiolabeling reaction (Figure 3).

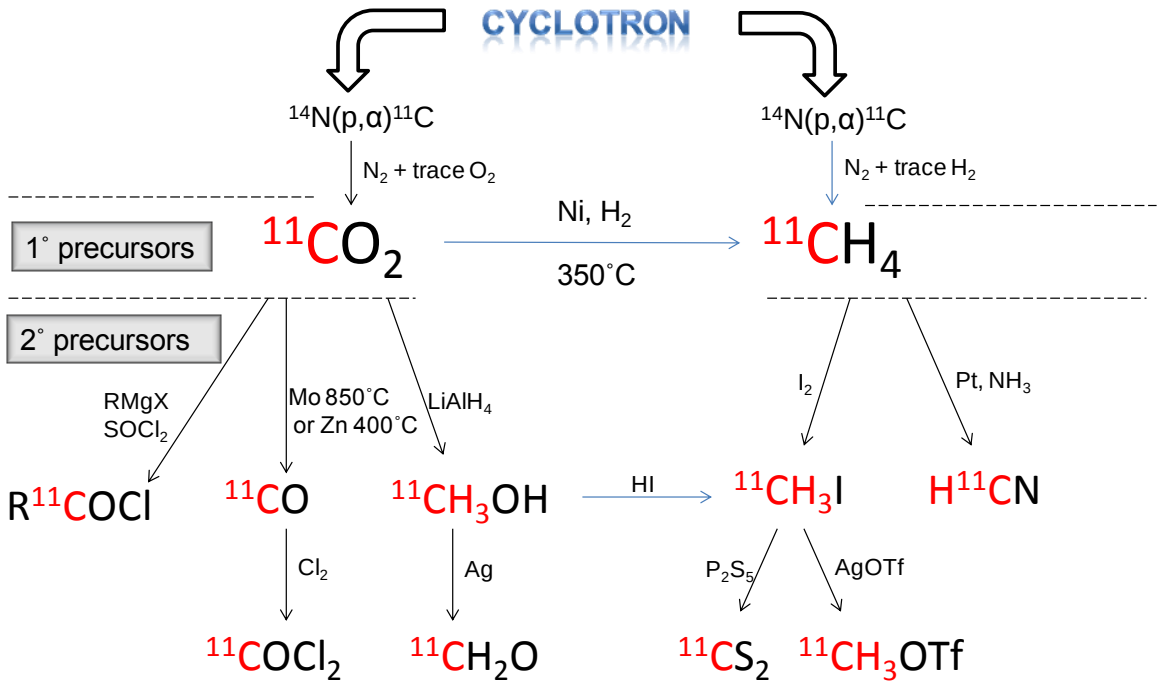


Figure 3: ^{11}C-labeled synthons for use in PET chemistry.

1.3.3 Radiolabeling with Fluorine-18

Fluorine-18 is the premier radionuclide for PET[17-20] due to its physical and nuclear properties. Its relatively long half-life ($t_{1/2}$ = 110 min) enables both multi-step synthesis and transportation to other clinics without an on-site cyclotron. It decays almost exclusively via positron emission > 96.9 % with low energy which leads to PET-images of high resolution. From a chemical point of view, fluorine-18 can be introduced via electrophilic[20,21] or nucleophilic[17,18] ^{18}F-fluorination methods. The main difference between these two approaches is associated to the molar activity, which is crucial while working with low capacity systems (eg. ligand-receptor binding). Electrophilic ^{18}F-fluorination agent is produced by irradiation of neon gas rich in F_2 following the nuclear reaction $^{20}Ne(d,\alpha)^{18}F$ to form $[^{18}F]F_2$ gas. The produced $[^{18}F]F_2$ can be used as is ($[^{18}F]F_2$) or transformed into less reactive reagents (e.g., acetyl [^{18}F]hypofluorite[22] or [^{18}F]Selectfluor)[20] prior to the labelling reactions. Common substrates for electrophilic fluorination include aryl, vinyl or alkyl organoelement substrates (eg. tin reagents) [23].

Nucleophilic fluorine-18 is generally produced via irradiation of oxygen-18 enriched water with protons in the $^{18}O(n,p)^{18}F$ nuclear reaction. This nuclear reaction produces aqueous [^{18}F]fluoride ions ($[^{18}F]F^-$), but due to its capability of forming strong hydrogen bonds, $[^{18}F]F^-$ is generally unreactive for nucleophilic substitution in this native form. It is thus commonplace to eliminate water, add a cationic counter ion (e.g. Potassium carbonate) and use a phase transfer catalyst (PTC) to increase its reactivity. [^{18}F]Fluoride can be introduced via nucleophilic substitution reactions into aliphatic and aromatic substrates.[18,24]

Electrophilic $[^{18}F]$-F_2 has a lower molar activity (100-600 MBq/µmol) compared to nucleophilic ^{18}F-fluoride with A_m in the range of $1x10^2$ GBq/µmol. Therefore, the importance

of high molar activity for PET imaging leads to the use of nucleophilic fluorine-18 for further fluorine-18 reactions.

1.4 [^{11}C]CARBON MONOXIDE IN PET CHEMISTRY

Carbonyl groups are present in most biologically active molecules and drug molecules. As such, these groups are obvious targets for isotopic labeling via transition metal mediated carbonylation with labeled CO. Although the synthesis of [^{11}C]CO was reported already in 1976, [25] the use of this radiolabeled precursor has been challenging and hence restricted to a few PET centers in the world. Some of the challenges of using [^{11}C]CO in radiochemistry include its low reactivity, its poor solubility in organic solvents, its high dilution in inert gas[6] and the low concentration of isotopically labeled [^{11}C]CO.[3]

1.4.1 [^{11}C]Carbon monoxide production

The production of [^{11}C]CO is typically performed by two different methods. The first is a gas-phase method in which [^{11}C]CO_2 is reduced by passing through a heated column filled with zinc (400˚C) or molybdenum (850˚C).[26] The difference between zinc (Zn) and molybdenum (Mo) is that Zn requires more column maintenance, gives high and irreproducibility yield and its melting point is close to the temperature needed for reducing [^{11}C]CO_2 (400˚C). On the contrary, Mo provides reproducible yields (70%) with less maintenance.

The second method is a "wet method", which relies on the decomposition of [^{11}C]formyl chloride[27] or [^{11}C]silacarboxylic acids[28] to form [^{11}C]CO (Figure 4). The gas-phase method with Mo as reductant is often preferred because of higher reproducibility[25] although it has lower yield than the other methods. In the current project, the reduction of [^{11}C]CO_2 to [^{11}C]CO was exclusively performed via the gas-phase method with Mo as reductant.

1.4.2 Transition metal mediated ^{11}C-carbonylation

The first transition metal mediated ^{11}C-carbonylation reaction was carried out in a conventional glass vial at atmospheric pressure, but because of the abovementioned reasons only 10% of the utilized [^{11}C]CO reacted and remained trapped in the solution.[29,30] Although the poor trapping was improved by introducing a recirculating technique that allowed unreacted [^{11}C]CO to be recycled through the reaction media,[31] the major breakthrough came after the development of a micro-autoclave system by Kihlberg et al., in which [^{11}C]CO was introduced at high solvent pressure.[32] The high pressure (350 bar) allowed for the [^{11}C]CO to be efficiently trapped and provided excellent radiochemical yields (RCYs) for a wide range of substrates (figure 4).[33-35]

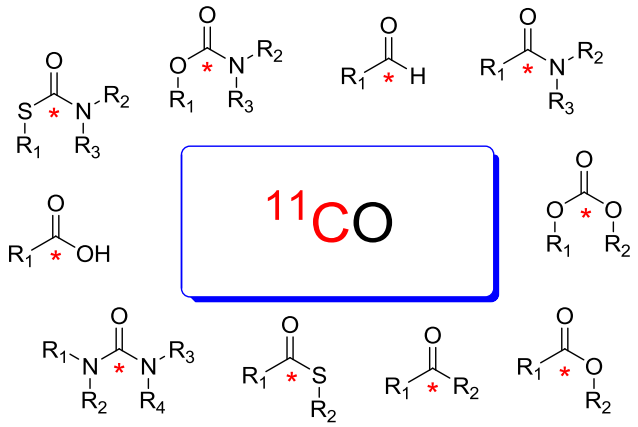


Figure 4: Wide range of target molecules obtained from labeling with [^{11}C]CO.

Even though the micro-autoclave method is still in use, it has not gained wide adoption in the PET chemistry community, possibly because of the lack of fit-for-purpose commercially available synthesis modules. Other technical developments with the potential of simplifying ^{11}C-carbonylations are microfluidic devices, where annular and segmented ^{11}CO flows have provided good trapping and RCYs.[29,33] Additional fruitful methods for improving the yield for these reactions include the use of the highly efficient Pd-mediated ^{11}C-carbonylations with Xantphos as a supporting ligand,[34] chemical complexation of [^{11}C]CO with diborane (BH_3-[^{11}C]CO)[37] or copper scorpionates (Cu[Tp*]^{11}CO), [38] or introducing [^{11}C]CO into the reaction media in a stream of xenon carrier gas.[39]

1.4.3 The catalytic cycle in Pd-mediated ^{11}C-carbonylation reactions

Five principal steps have been postulated that describe the Pd-mediated ^{11}C-carbonylative coupling reaction. Although these steps are commonly presented in the form a catalytic cycle, the stoichiometry of a radiolabeling reaction with [^{11}C]CO is fundamentally different from that used in traditional organic chemistry and it is thus unlikely that a cycle is necessary to generate the final radiolabeled product (Scheme 1). Nevertheless, the mechanism below is important in relation to understanding and potentially improving the yields of transition metal mediated ^{11}C-carbonylative coupling reactions.

1- **Catalyst activation:** A fundament behind the many successful applications of Pd in cross coupling reactions is its ability to easily transition between oxidation states. The activation of many Pd(II) pre-catalysts is important for *in situ* generation of Pd(0).

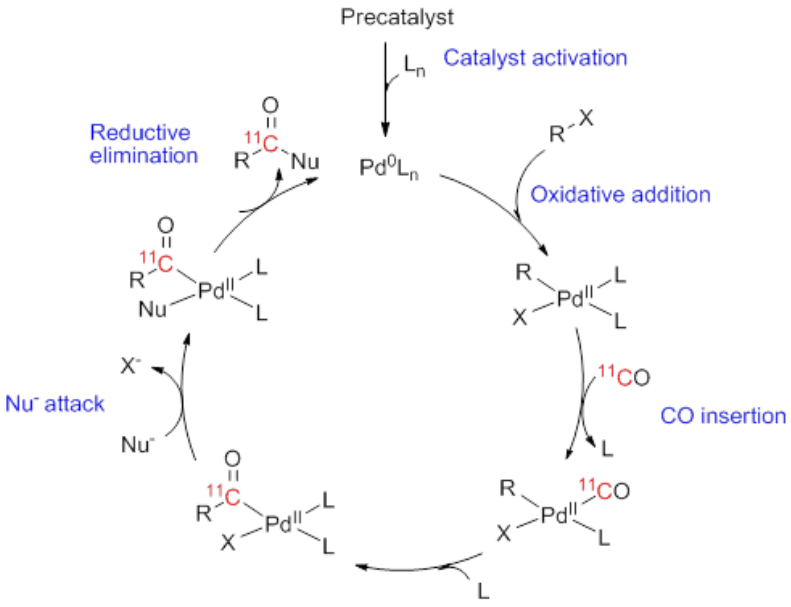
2- **Oxidative addition:** Oxidative addition occurs at low coordinated Pd(0) (eg. PdL_2, PdL). In this step, Pd(0) adds to the aryl-halide bond (R-Pd-X) and is oxidized in the process to Pd(II) aryl halo complexes, $[PdL_n]$ + R-X → $[Pd^{II}L_n(R)(X)]$.

3- **CO coordination and insertion:** In carbonylation reactions, the coordination of CO to the palladium center occurs first, followed by the insertion of CO into ligand-metal bond. The CO and the electrophile are in c*is*-position in order to allow the organo-

group to migrate to the CO carbon and form an acyl-palladium complex. In PET chemistry, the amount of non-volatile radioactive matter in solution after the reaction with [^{11}C]CO is referred to as "trapped". The term "trapping efficiency" (TE) relates the radiation intensity of non-volatile compounds to the overall radioactivity in the reaction vessel and is given in percent.

4- **Nucleophilic attack:** This step delivers the desired nucleophile to the palladium center. The nucleophilic attack is followed by the elimination of a Pd coordinated pseudo-halide.

5- **Reductive elimination:** The last step in this catalytic cycle is the formation of a new carbon-carbon bond between the electrophilic acyl group and the nucleophile. The non-bonded electron pair of Pd is now back and the active Pd^0L_2 species is regenerated.



Scheme 1: Catalytic cycle of Pd-catalyzed carbonylation reactions[40-44]

The yield of a Pd-mediated carbonylative coupling reaction depends on several factors, including the nature of the substrate, the Pd-ligand complex, the nucleophile and the solvent. Aryl, vinyl or allyl halides are common substrates for this reaction, since these chemical motifs are unsaturated and cannot undergo beta-hydrideelimination. In addition, electron-poor aryl substrates favor the oxidative addition of Pd and thus increase both the rate and yield of the overall reaction. Second, there is a vast difference in the reactivity of Pd-complexes in carbonylative coupling. More reactive Pd-complexes, such as Pd-$(PtBu_3)_2$ and Pd-Xantphos, have been shown to allow for CO insertion at lower pressure.[34,45] Finally, the ligand will promote solubility of the transition metal in the solvent used for the reaction, which is typically a moderately polar aprotic solvent such as THF or dioxane, although DMF, DMSO, toluene and acetonitrile have also been used in the reaction.

1.4.4 ^{11}C-carbonylation of alkyl substrates

Carbon-11 labeled aliphatic acids and amides are typically prepared from [^{11}C]CO_2 using organometallic reagents. Though very efficient for the preparation of simple substrates, such as [^{11}C]acetate, these methods suffer from poor functional group tolerance and are less suitable for late-stage labeling of drug-like molecules.

Because alkyl acids and amides are common chemical motifs in biologically active molecules, there is a great demand for method development in this area. A few efforts of applying [^{11}C]CO in the ^{11}C-carbonylation of alkyl substrates have been reported in the literature. Itsenko et al. first reported on the ^{11}C-carbonylation of aliphatic substrates in a high-pressure autoclave equipped with a sapphire window for ultraviolet light irradiation of the reaction mixture.[46,47] Next, Rahman et al. reported a nickel-mediated protocol for the carbonylative coupling in ordinary glass vials.[48] More recently, Roslin et al. reported on the use of a radical initiator for producing the desired ^{11}C-labeled aliphatic acids and amides.[49] Some challenges with the previously reported methods include the need for sophisticated equipment, the use of air sensitive reagents and moderate trapping efficiency.

2 AIMS OF THE THESIS

The general aim of the thesis was to develop and implement novel methodologies for rapid and efficient introduction of the short-lived radionuclide carbon-11 into carbonyl groups via [^{11}C]carbon monoxide, and to enable tracers labeled by ^{11}C-carbonylation to be used in studies of human physiology and pathophysiology. Some specific aims of the thesis work are described in below:

a) To develop a novel and simple "in-loop" methodology where ^{11}C-carbonylation reactions are performed on the interior surface of a stainless-steel injection loop used for high performance liquid chromatography.

b) To develop an automated low-pressure system for [^{11}C]CO carbonylation radiochemistry that complies with good manufacturing practice (GMP) to enable imaging studies in human subjects.

c) To develop a convenient and efficient method for the synthesis of ^{11}C-labeled benzamides via [^{11}C]aroyl dimethylaminopyridinium salts.

3 MATERIALS AND METHODS

The material and methods section in the present thesis is briefly presented in the below sections. For more detailed descriptions of experimental procedures, the reader is referred to the full papers and manuscript.

3.1 Preparation and handling of [^{11}C]carbon dioxide

Carbon-11 was produced by a GEMS PETtrace cyclotron (GE Uppsala, Sweden) using 16.4 MeV protons via the nuclear reaction $^{14}N(p,\alpha)^{11}C$. [^{11}C]CO_2 was prepared in a pressurized 78 mL aluminum gas target containing nitrogen gas of scientific grade purity (99.9999%) and a small amount of oxygen (0.5% or 1%). All gases used in the thesis were purchased from AGA gas AB (Sundbyberg, Sweden). A gas purifier (All pureTM, Alltech) was placed in series with the gas target to remove all traces of carbon before entering the target. The produced [^{11}C]CO_2, was transferred to the desired hot-cell by applying a stream of target gas pressure through the target. Importantly, oxygen and other impurities can be present after the production of [^{11}C]CO_2, therefore, they can be removed either by trapping [^{11}C]CO_2 on a molecular sieve column (0.6 g packed in a ¼" stainless-steel tube, 4 Å, mesh 80/100, GRACE) at room temperature (RT) or on a stainless-steel loop immersed in liquid nitrogen (T = -196˚C). The accumulated [^{11}C]CO_2 was released by applying a controlled flow of helium (10 mL/min, Mass flow controller, Bronckhorst) and by heating either the molecular sieve column at 360˚C or by applying air flow to thaw the stainless-steel loop (In paper I, [^{11}C]CO_2 was trapped and released from molecular sieves whereas in papers II and III, stainless-steel loop immersed in liquid nitrogen was used).

3.2 Preparation and handling of [^{11}C]CO

The production of [^{11}C]CO was performed according to the gas method where [^{11}C]CO_2 was reduced to [^{11}C]CO through a heated quartz glass column (T = 850˚C) containing molybdenum powder. The produced [^{11}C]CO was passed through a sodium hydroxide-coated silica trap (Ascarite) to remove any trace of unreacted [^{11}C]CO_2. The purified [^{11}C]CO was concentrated on a silica gel trap (60 Å, 60-100 mesh) immersed in liquid nitrogen. After complete accumulation of [^{11}C]CO, it was transferred either into a stainless-steel loop (Figure 6, pathway A) or into a sealed vial (Figure 6, pathway B) for further synthesis.

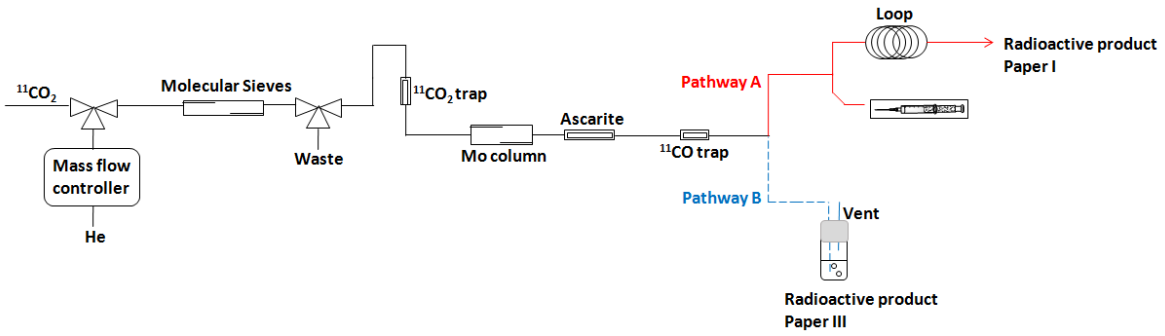


Figure 6: A representative flowchart for the production of [^{11}C]CO and its use in pathway A: "In-loop" ^{11}C-carbonylation (project 1) and pathway B: ^{11}C-labeling of primary amides (project 2).

3.3 Product identification and calculation of radiochemical yields

All radioactive reaction mixtures and products were analyzed using analytical high-performance liquid chromatographic (HPLC) with ultraviolet and radiation detectors on the column effluent. Each product was identified by co-elution with the corresponding non-radioactive authentic reference standard. Radiochemical purity (RCP) was determined by integrating the product peak and expressing its area counts as a percentage of the total area counts in the chromatogram. Trapping efficiency (TE) was defined as the fraction of radioactivity retained in solution after sparging a reaction mixture with He to remove volatile radioactive byproducts and unreacted [^{11}C]CO). For paper I, the total TE was a product of TE in the vial and TE in the stainless steel loop, which was assessed by measuring radioactivity in a gas-tight bag placed in series directly after the loop, following equation 1.

Equation 1: $$A_{(t=0)} = \frac{A_t^*}{e^{-\left(K_{(decay)} x\, t\right)}} \quad \text{and} \quad t_{1/2} = \frac{\ln 2}{K_{(decay)}}$$

$$A_{(t=0)} = \frac{A_t^*}{e^{-\left(\frac{\ln 2}{t_{1/2}} x\, t\right)}}$$

The radiochemical yield (RCY) was calculated following equation 2.

Equation 2: $$RCY = TE * RCP$$

3.4 Molar activity

Molar activity (A_m) is the ratio between the radioactivity (GBq) and the chemical amount of the carrier molecule (µmol) and is calculated according to equation 3. The molar amount of carrier was determined using analytical HPLC.

Equation 3: $$A_m = \frac{A_{(radioligand)}}{n_{(carrier)}}$$

4 RESULTS AND DISCUSSION

4.1 Development of an "in-loop" method for transition-metal mediated ^{11}C-carbonylation using [^{11}C]CO (Paper I)

Despite the broad utility of ^{11}C-carbonylation reactions for producing diverse chemotypes for PET imaging, its widespread use has so far been hampered by the need of complex technical solutions for its application. New simplified technologies for ^{11}C-carbonylation radiochemistry are thus needed, although such development represents a considerable challenge due to the poor solubility of [^{11}C]CO in organic solvents and its high dilution in inert carrier gas under PET chemistry conditions. In the first project, based on its previous successful application in radiochemical synthesis with [^{11}C]methyl iodide and [^{11}C]CO_2,[50,51] it was hypothesized that an ordinary stainless-steel loop used for HPLC injections could serve as the reactor for the transition-metal mediated ^{11}C-carbonylation. The smaller inner diameter of such a loop was considered advantageous for this radiochemistry, since it maximizes the surface area between the gas (i.e. He/[^{11}C]CO mix) and liquid phases (i.e. reaction mixture) and thus facilitates equilibration between them. Further advantages of such a method would be that transfer losses are minimized and process automation can be simplified (Figure 7).[50]

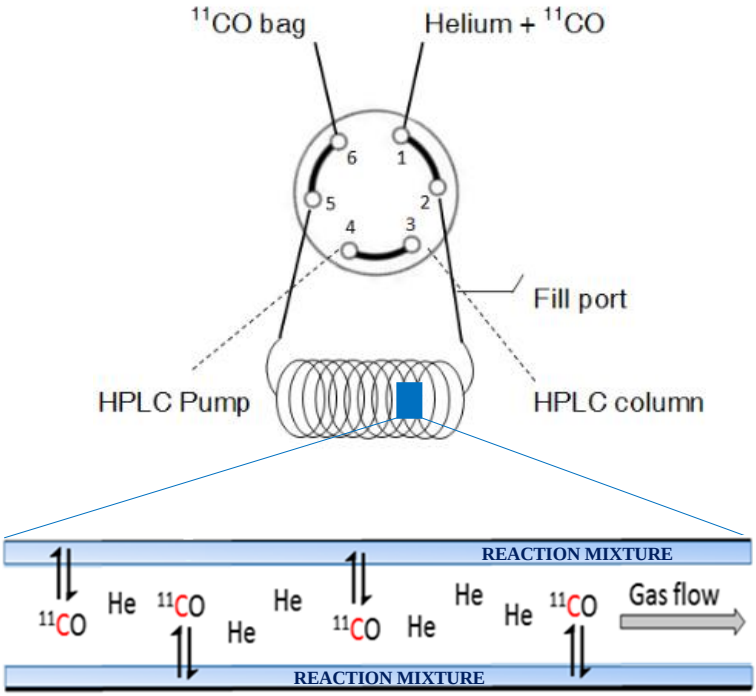


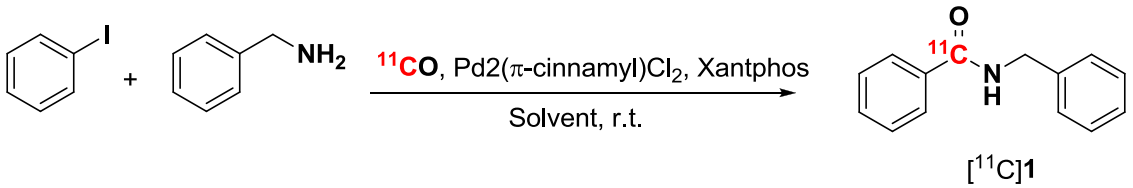
Figure 7: Schematic of the "in-loop" ^{11}C-carbonylation with [^{11}C]CO.

4.1.1 Optimization of the ”in-loop” methodology

The “in-loop” methodology was optimized on a well characterized model reaction, namely the ^{11}C-aminocarbonylation of iodobenzene with [^{11}C]CO and benzylamine, forming [^{11}C]*N*-benzylbenzamide ([^{11}C]**1**).[34] In our experimental setting, [^{11}C]CO was swept into a stainless-steel loop, which had been pre-charged with coupling reagents (amine, aryl halide, Pd and ligand dissolved in THF). A gas-tight bag was connected in series with the stainless-steel loop to permit determination of the breakthrough of [^{11}C]CO during its transfer to the loop. Most of the reactions were performed at room temperature for 5 minutes, after which the reaction mixture was transferred into a sealed tube, where trapping efficiency could be determined.

Although the palladium-Xantphos system has been shown to be more efficient in enabling the trapping and insertion of [^{11}C]CO at near-atmospheric pressure, the most reported ligand in Pd-mediated ^{11}C-aminocarbonylation is triphenylphosphine (PPh_3). We therefore started to test the “in-loop” ^{11}C-aminocarbonylation with palladium-tetrakis(triphenylphosphine) ($Pd(PPh_3)_4$) at room temperature in THF and with a reaction time of 5 minutes. Under these conditions, compound [^{11}C]**1** was obtained in good RCP (70%), but at low TE (0.2%), leading to low overall RCY (0.14%). When instead using Pd-Xantphos in DMF, the most well-used solvent for in-loop ^{11}C-methylation[50], high TE (92%) and moderate RCP (52%) was observed (Table 2, entry 1). Other solvents such as DMSO and toluene also showed high TE (Table 2, entry 2 and 3) while reactions in THF and 1,4-dioxane provided the best results in terms of yields and also proved to be reproducible (Table 2, entries 4 and 5).

Table 2: Solvent screening of [^{11}C]N-benzylbenzamide



[^{11}C]**1**

Entry	Solvent[a]	[^{11}C]**1** TE (%)	[^{11}C]**1** RCP (%)	[^{11}C]**1** 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 the corresponding solvent (150 µL) and benzylamine (10 µL)
[a] Oxidative addition takes place during evaporation of THF (700µL)
[b] n = 3 (triplicate)

4.1.2 Autoradiographic study of radioactivity distribution in the loop

In effort to increase our understanding of the trapping efficiency inside the loop, an autoradiographic study was conducted in which the loop was disconnected and sealed after [^{11}C]CO had been swept through and mixed with the reaction mixture (entry 4 conditions). After leaving the radioactivity in the loop to decay to allow for transportation out of the hot-cell, an experiment was conducted where the loop was placed on a phosphorimaging plate to analyze the distribution of the radioactivity in the loop. It has revealed that most of the radioactivity was trapped at the beginning of the loop (≈ 0.5 mL). This allows us to speculate that a smaller loop volume and/or higher transfer flow through the loop could be tolerated without compromising the RCY (Figure 8).

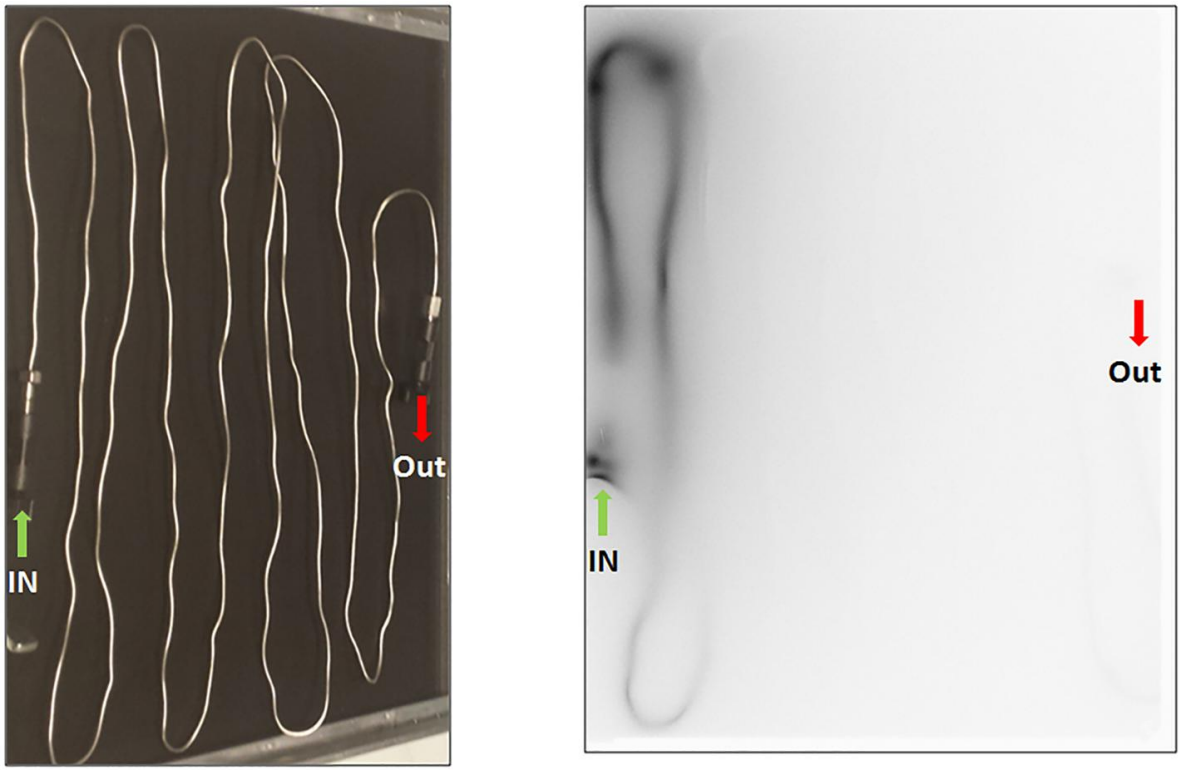


Figure 8: Synthesis of [^{11}C]*N*-benzylbenzamide in a stainless-steel loop (left) where the entrapment of [^{11}C]CO was shown in an autoradiographic image (right).

4.1.3 Scope of the methodology

With optimized conditions in hand, the scope and limitations of the method were studied by its application to molecules containing other functional groups and finally also drug-like molecules. As could be expected, a lactone, a carboxylic acid and esters were synthesized following the method used for radiolabeling of [^{11}C]N-benzylbenzamide with some minor modifications. For [^{11}C]**3,** an increase of the amount of the corresponding precursor was found necessary to improve TE (≥99%) whereas for [^{11}C]**4** and [^{11}C]**5**, RCP was improved by conducting the reaction at a higher temperature (Figure 9).

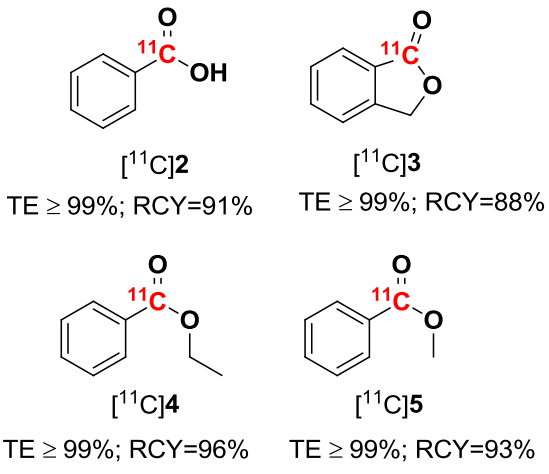
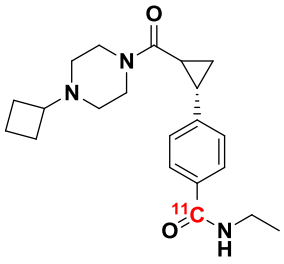


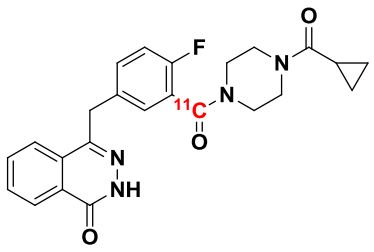
Figure 9: In-loop ^{11}C-carbonylation of [^{11}C]**2,** [^{11}C]**3,** [^{11}C]**4** and [^{11}C]**5** via [^{11}C]CO.

Next, a set of pharmaceutical druglike molecules such as the histamine type-3 receptor radioligand[52] AZ13198083 ([^{11}C]**6**), the oncology drug olaparib ([^{11}C]**7**) and the dopamine D2 receptor radioligands raclopride[53] ([^{11}C]**8**) and FLB457[54] ([^{11}C]**9**) were synthesized using the new in-loop methodology. Though each of these compounds required elevated temperatures (75-100˚C) to obtain products at high RCP, the most extensive deviation from the general protocol, changing the Pd-source to $Pd(dba)_2$ and the supporting ligand to P^tBu_3, was required to allow for the synthesis of [^{11}C]**7** at high RCY (≈97%). In agreement with the observation by Skrydstrup et al., a near quantitative conversion into an undesired by-product (aryl scrambling of the phenyl group from Xantphos into the aryl-Pd complex) was observed when using Pd-Xantphos in this reaction, thus restricting its use for the preparation of [^{11}C]**7**.[55] Importantly, however, this example highlights the application and compatibility of multiple Pd-complexes with the devised "in-loop" technology. Finally, a more modest yield was obtained in the preparation of [^{11}C]**9** (RCY=43%), albeit one could foresee that this yield could be improved following additional optimization, which was outside the scope of this study.

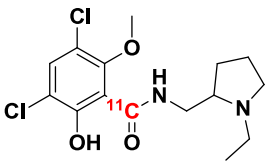
In a preparative run, the loop system was integrated into a commercially available synthesis module for carbon-11 chemistry (GE Tracerlab FX-C, Uppsala, Sweden). Following isolation, formulation and sterile filtration, [^{11}C]**6** was produced with a 38% RCY (decay-corrected, based on the amount of [^{11}C]CO delivered to the loop), which enables production of substantial amounts of the target radioligand, and thus demonstrated the proof of concept for applying the novel "in-loop" ^{11}C-carbonylation technology for radioligand synthesis. The discrepancy between the preparative yield and that obtained during method development (crude yield) was likely a result of losses during HPLC and SPE purification of the radioligand. The radiochemical purity of [^{11}C]**6** was high (>99%) and the molar activity was 110 GBq/µmol, both sufficiently high to allow for preclinical and clinical PET studies.[56]



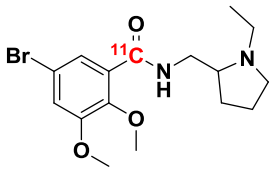
[^{11}C]**6**
RCP = 93%; TE = 98%



[^{11}C]**7**
RCP > 99%; TE = 98%



[^{11}C]**8**
RCP= 89%; TE = 88%



[^{11}C]**9**
RCP= 79%; TE = 54%

Figure 10: Radiolabeling of several druglike molecules using in-loop ^{11}C-carbonylation**.**

4.2 Development of a new and automated GMP system for ^{11}C-carbonylation reactions with [^{11}C]CO (Paper II)

Since most PET centers do not have the combination of research radiochemists and engineers in their staff, and the associated capability to construct sophisticated radiochemistry hardware, we hypothesized that widespread adoption of [^{11}C]CO radiochemistry likely would need to be accelerated by a fit-for-purpose commercially available radiochemistry apparatus for this chemistry. Whilst automation would be a strict necessity for its utility, compatibility with good manufacturing practice (GMP) was also desired, since this would pave the way for PET studies in human subjects and thus increase utility and usage of the system. In this project, a new GMP-complying radiochemistry prototype for [^{11}C]CO chemistry was designed, constructed and developed through the collaboration with a Danish PET chemistry hardware company (Scansys A/S). This novel equipment is, to the best of our knowledge, the first commercially available apparatus for this purpose.

4.2.1 System development

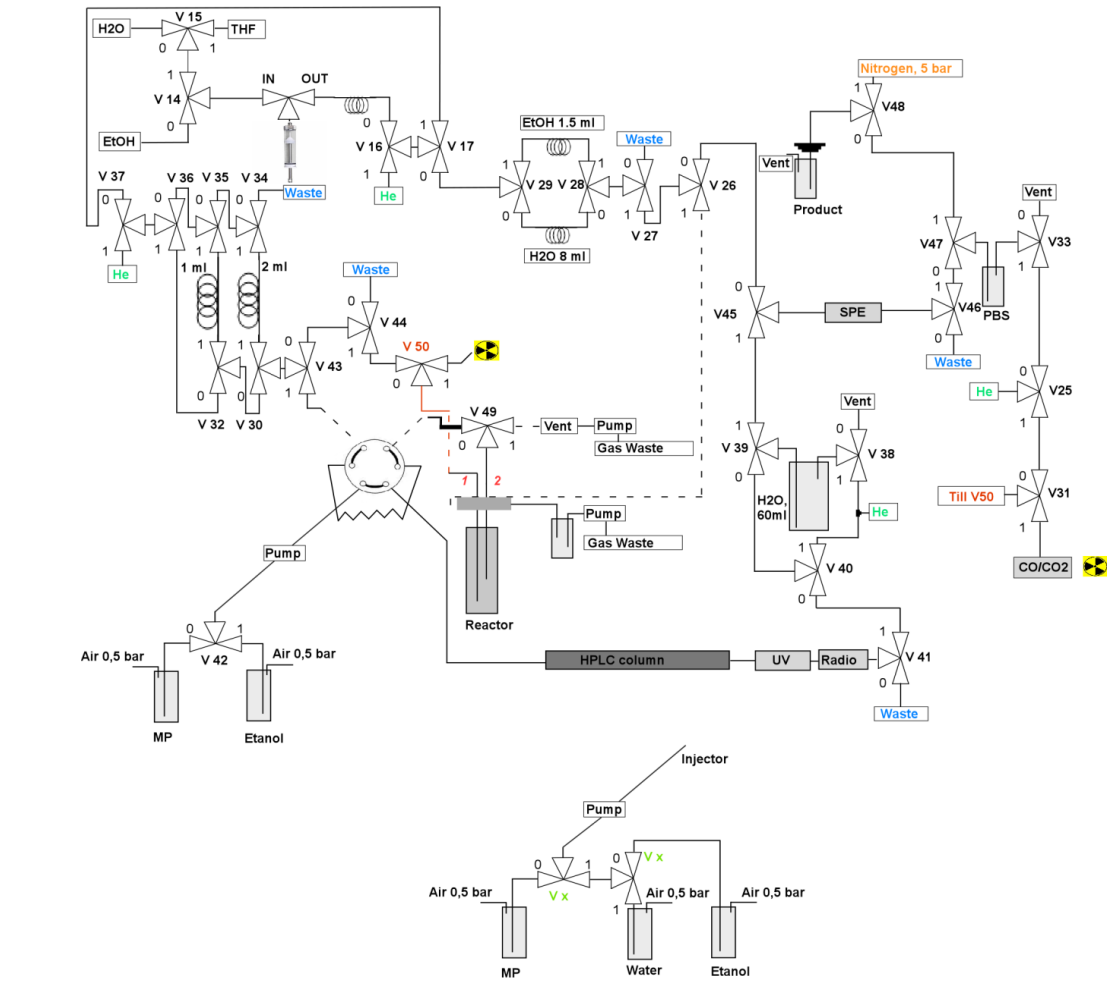
Following installation at the Karolinska Institutet (KI) PET Centre, five distinct subsystems were developed and integrated into the GMP radiochemistry synthesizer. The subsystems consist of multiple valves, ovens, reactors, pumps and flow controllers that are controlled using a single software (Labview). This software also enables data monitoring and collection during production. The five subsystems are shortly described in the following (Figure 11, A):

a) **Gas handling system:** Traps and purifies cyclotron produced [^{11}C]CO_2 and subsequently converts it into [^{11}C]CO. Also purifies the [^{11}C]CO to enable its use in the next step.

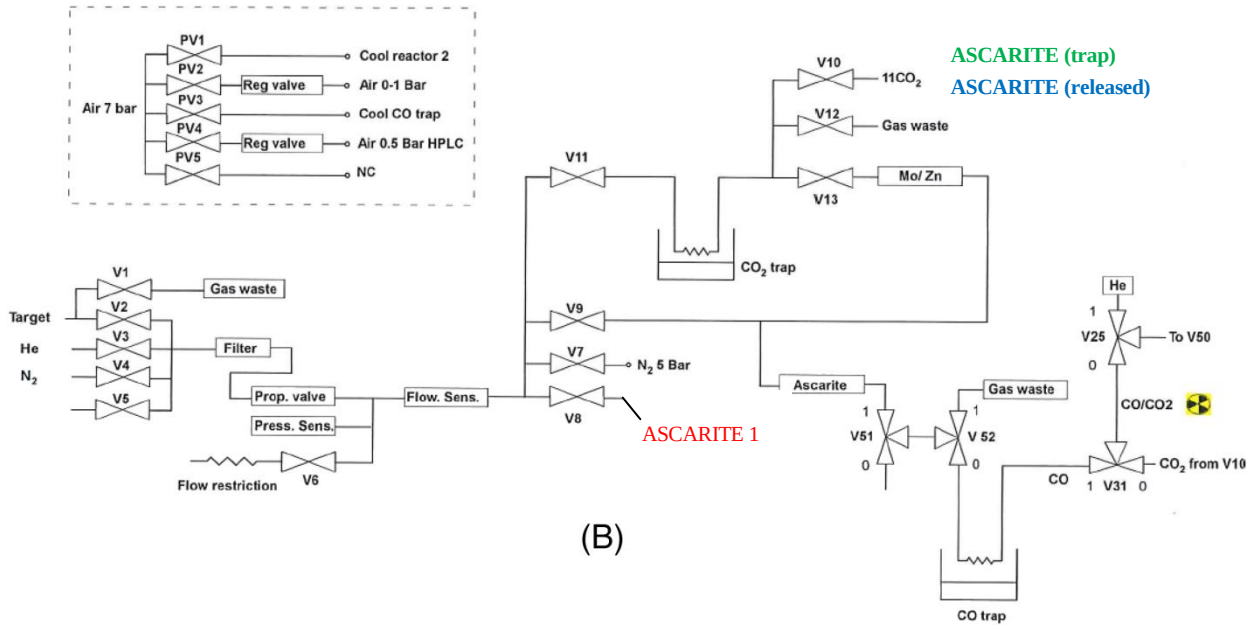
b) **Reactor system:** Concentrates purified [^{11}C]CO on a small silica trap to allow for its rapid transfer into the sealed reaction vessel where the ^{11}C-carbonylation reaction takes place. Has option to perform secondary reaction (e.g., deprotection), evaporating off solvents and enables dilution of reaction mixture with a suitable matrix for the ensuing purification.

c) **Purification system:** Purifies the crude ^{11}C-labeled tracer from other components in the reaction mixture using HPLC and subsequently enables its isolation from mobile phase constituents using solid phase extraction (SPE).

d) **Formulation and sterilization system:** Formulates the ^{11}C-labeled tracer in a vehicle suitable for intravenous injection in human subjects and subsequently sterilizes the solution via membrane filtration.



(A)



(B)

Figure 11: Flowchart of the low-pressure ^{11}C-carbonylation prototype

4.2.2 Process optimization

Each subsystem was optimized using a short, fixed irradiation time (1 min) and current (35 μA) that reproducibly yielded a reasonably low starting radioactivity for the investigation (3.1 GBq, Table 3, entry 1). [^{11}C]CO_2, produced in a gas target containing a mixture of oxygen (1%) and nitrogen, was quantitatively trapped in a stainless steel tube filled with porapak Q immersed in liquid nitrogen. This setting, although with the drawback of requiring liquid nitrogen, has the advantages of providing a longer lifetime and higher A_m than molecular sieves, which tend to perform worse over time and also need to be conditioned prior to each synthesis since they continuously adsorb CO_2 from the atmosphere. It was found that no additional traps were required to purify [^{11}C]CO_2 before its use in the conversion to [^{11}C]CO over heated molybdenum. However it could not be excluded that a purification step could increase the lifetime for the Mo column from the observed 50 runs under the adopted experimental setup. Finally, [^{11}C]CO was purified from residual [^{11}C]CO_2 over an Ascarite trap, concentrated in a stainless-steel tube pre-filled with silica before being released into the reactor. After the delivery of [^{11}C]CO, the reactor containing the ^{11}C-aminocarbonylation reaction contained 1.6 GBq of radioactivity, which equates to 71% RCY based on the starting activity (Table 3, entry 2).

Table 3: Measurement of [^{11}C]CO_2 and [^{11}C]CO with low-pressure system

Entry	Information	Measured activity[a] (MBq)	Time[b] (min)	Yield uncorrected[c] (%)	Yield corrected[d] (%)
1	Starting activity	3108 ± 250	6.5	100	100
2	Reactor	1618 ± 160	9	50	71

[a] Average of 3 runs
[b] Time after EOB until measurement
[c] Yield calculated without taking the half-life into account
[d] Yield calculated by taking the half-life into account

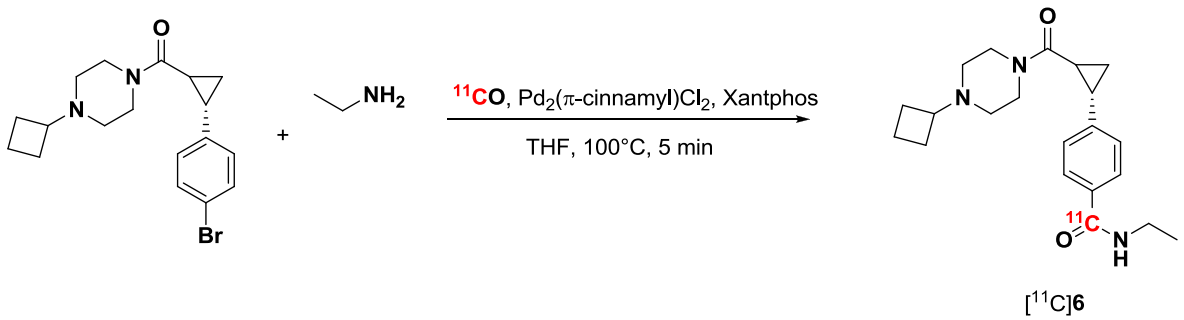
The conventional method for purifying PET radioligands is via semi-preparative HPLC with the effluent monitored for ultraviolet absorbance and radiation. A particularly useful feature of the developed system is the HPLC column scanner, which allows the operator to view movement of activity inside the column during the separation and can provide information on yield prior to collecting the product fraction. Standard methods for SPE were used to recover the radiopharmaceutical product from the mobile phase and to provide it in a sterile solution

containing less than 10% ethanol. After each synthesis, the system is automatically cleaned using a validated cleaning procedure.

4.2.3 Process evaluation

Three consecutive batches of [^{11}C]**6** were produced to test the overall performance of the synthesis prototype, with each produced batch measured for radioactivity and tested against a set of typical quality control (QC) specifications for GMP produced radiopharmaceuticals. Following a full cyclotron production (55 µA, 30 min), 8.54 ± 1.4 GBq of [^{11}C]**6** was obtained in a formulation suitable for intravenous injection. Each batch met the specifications for all conducted QC tests, including radiochemical purity, pH, presence of residual solvents, sterility and bacterial endotoxins (Table 4).

Table 4: Batch results from the production of [^{11}C]**6**.



[^{11}C]**6**

Test	Specification	Batch 1	Batch 2	Batch 3
Radioactivity	N.A.	7050 MBq	8730 MBq	9830 MBq
pH	4.5-8.0	7.0	7.5	7.5
Product identification	R_t Radiopeak-R_t UV = 0.3-0.5 min	0.35 min	0.33 min	0.38 min
Radiochemical purity	Not less than 95%	>99%	>99%	>99%
Molar activity	N.A.	38,1 GBq/µmol	54,6 GBq/µmol	108,7 GBq/µmol
Filter integrity	Not less than 3.5 bar	3.6 bar	3.7 bar	4.1 bar
Residual acetone	Not more than 5000 ppm	2652 ppm	2097 ppm	2978 ppm
Residual acetonitrile	Not more than 410 ppm	8.9 ppm	Not detected	Not detected
Ethanol content	Not more than 10%	3.6%, 15 mL	3.4 %, 15 mL	3.7%, 15 mL

Furthermore, the automated [^{11}C]CO synthesizer allowed for the production of additional tracers with radiochemical yields sufficiently high for preclinical studies (unpublished

results). Three such examples are the poly(ADP-ribose) polymerase (PARP) inhibitors depicted in figure 12 below. The produced amount of activity of these radiotracers studied was 5.15 GBq for [^{11}C]**7**, 1.05 GBq for [^{11}C]**10** and [^{11}C]600 MBq for [^{11}C]**11** with the corresponding molar activities, 140 GBq/µmol, 15.9 GBq/µmol and 9.9 GBq/µmol. Radiochemical purity was >99% for [^{11}C]**7**, [^{11}C]**10** and [^{11}C]**11** at the time of administration (Figure 12).[57]

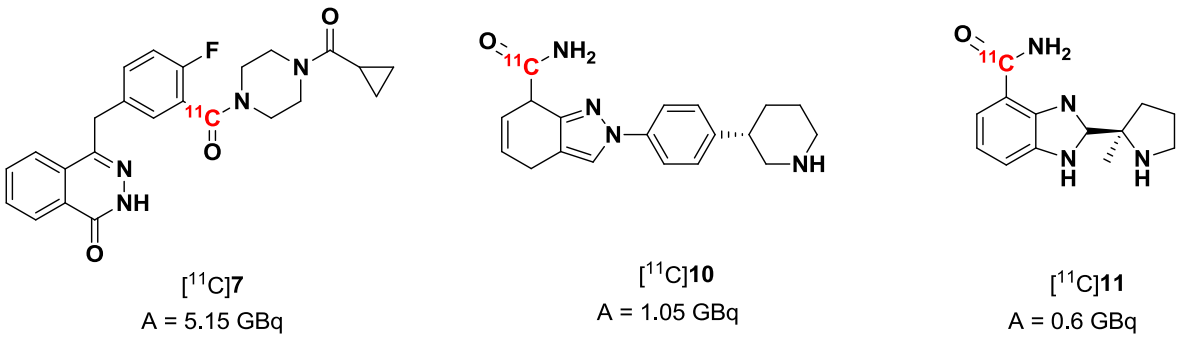


Figure 12: Three PARP inhibitors produced with the GMP radiochemistry prototype. N.B: Methodology for labeling compounds [^{11}C]**10** and [^{11}C]**11** were developed in Paper III.

4.3 One-pot synthesis of ^{11}C-labelled benzamides via intermediate [^{11}C]aroyl dimethylaminopyridinium salts (Paper III)

The preparation of ^{11}C-labeled primary amides has received far less attention in the literature than that of secondary and tertiary [^{11}C]amides, despite of the fact that benzamides are present in many biologically important molecules, including cancer and CNS drugs (figure 13). For PET imaging purposes, the motif has previously been labeled using either Pd-mediated [^{11}C]cyanation, followed by hydrolysis of the intermediate nitrile, or via ^{11}C-aminocarbonylation with ammonia.[51,58,59] Although both methods have provided useful radiochemical yields (RCYs), they suffer from a few drawbacks, including the inconvenient use of toxic ammonia gas and the use of a high-pressure micro-autoclave equipment.

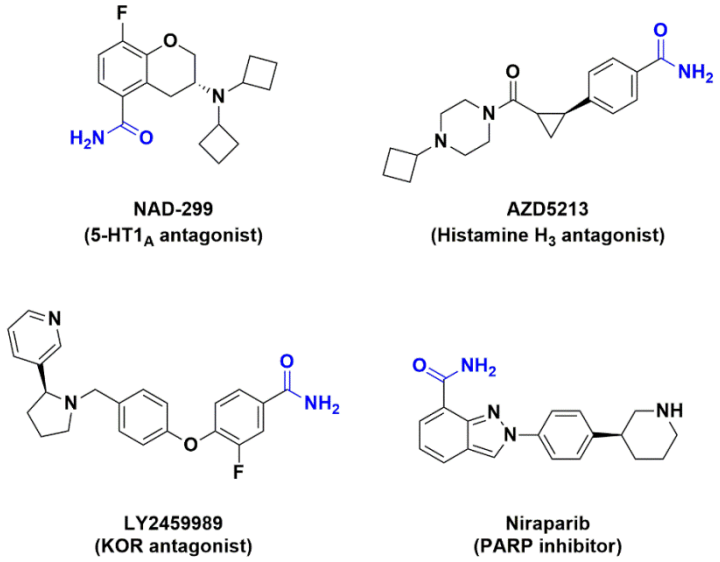


Figure 13: Structures of drug molecules containing the benzamide structural motif.

Recently, Arndtsen et al. reported the preparation of aromatic amides and esters via formation of aroyl dimethylaminopyridinium (aroyl-DMAP) salts. These salts are potent electrophiles that are often used without isolation as intermediates in acylation reactions. [60,61] We herein set out to develop an alternative and more convenient method for the preparation of ^{11}C-labeled benzamides via intermediate aroyl-DMAP salts. It was hypothesized that the recent progress in the development of low-pressure ^{11}C-carbonylation protocols would be compatible with such an approach.[34,36-38,44]

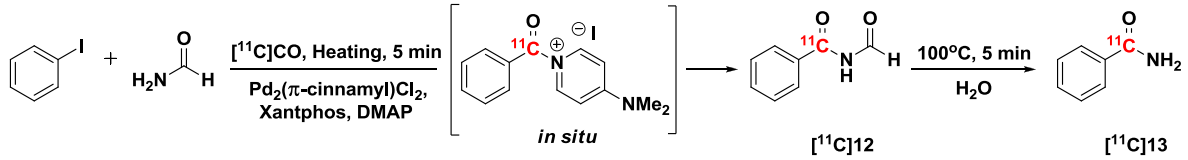
4.3.1 Optimization of the reaction conditions

[^{11}C]Benzamide ([^{11}C]**13**) was selected as the target molecule for investigating the palladium-mediated ^{11}C-aminocarbonylation of iodobenzene, with DMAP as an organic additive and employing ammonia surrogates as safer alternatives to toxic ammonia gas. Several ammonia surrogates were tested, of which hexamethyldisilazane (HMDS) and ammonium carbamate were inefficient, whereas formamide provided [^{11}C]**13** in 48% RCY after hydrolysis of the intermediate formimide [^{11}C]**12** (Table 1, entry 1). The reaction was performed in a sealed vial at 100˚C with an overall synthesis time of 10 min, of which 5 minutes was dedicated to the hydrolysis of the formimide.

The ensuing optimization was conducted using formamide as ammonia surrogate and focused on investigating the effect of typical parameters on the reaction yield (e.g., solvent, temperature, concentration). First, the effect of the DMAP concentration was investigated. Without the use of DMAP, only 9% RCY of [^{11}C]**13** was obtained (Table 1, entry 4) whereas 91% RCY was observed in a reaction with a large excess of DMAP (Table 1, entry 3). It was finally found that a 50% reduction in DMAP concentration (from entry 3 conditions) was tolerated without having a critical impact on the RCY (Table 1, entry 2).

Next, the effect of temperature was investigated. As expected, there was a relationship between the RCY of [^{11}C]**13** and the temperature, with a 30% lower yield observed at 60˚C (57% RCY, table 5, entry 5) compared to that observed at 150˚C RCY (90%, table 5, entry 6). Since heating at 100˚C is more compatible with THF as solvent, the marginal reduction in yield from the higher temperature (5%) was deemed acceptable (Table 1, entry 2 and 6).

Table 5: Optimization of [^{11}C]**13**.



Entry	Solvent	Temp (˚C)	DMAP (eq)	TE (%)[a]	RCP (%)[b]	RCY (%)[c]
1	THF	100	19	100	48	48
2	THF	100	52	95	90	85 ±2[d]
3	THF	100	104	100	91	91
4	THF	100	0	97	9	9
5	THF	60	52	100	57	57
6	THF	150	52	97	93	90
7	THF[e]	100	52	100	70	70
8	ACN	100	52	98	77	75
9	Formamide	100	52	92	91	83
10	Formamide	150	52	95	94	89

Reaction conditions: iodobenzene (12.6 µmol), Pd_2(µ-cinnamyl)Cl_2 (2.2 mg), Xantphos (5.0 mg), DMAP (25 mg), formamide (100 µL) solvent (1.3 mL).

[a] Trapping efficiency (TE); the fraction of radioactivity left the crude product after purging with nitrogen.
[b] The radiochemical purity (RCP) is determined by radioanalytical HPLC.
[c] The radiochemical yield (RCY) is based on the total radioactivity delivered to the reaction vessel.
[d] Average of four experiments.
[e] Formamide 60µL

Moreover, it was observed that a reduction in the volume of formamide, from 100 µL to 60 µL, produced a 15% reduction in the RCY of [^{11}C]**13** (Table 1, entries 2 and 7). It was also found that the reaction was compatible with both MeCN and formamide as solvents, although the latter provided higher yields (Table 1, entries 8-10). However, since THF can be widely applied in transition metal mediated chemistry, and its lower boiling point enables evaporation of the reaction solvent prior to HPLC, entry 2 conditions were selected for further investigations of the scope of the novel methodology.

4.3.2 Scope of the methodology

From a general perspective, the methodology was compatible with most of the studied substituents and also tolerated an aryl bromide as substrate, albeit at the expense of a slightly reduced yield (Figure 14). A limitation with the methodology was the low yields obtained with unprotected amine substituents present on the aromatic ring ([^{11}C]**11**-**12**). However, when the amine was *N*-Boc-protected, a drastic improvement in RCY was observed ([^{11}C]**12**[b]). Electron withdrawing groups were well tolerated in the reaction, as showcased by the high RCYs of nitro-, trifluoromethyl, cyano-, or even di-trifluoromethyl substituted benzamides ([^{11}C]**6**-**10**). However, *o*-substituted trifluoromethyl groups only provided the desired benzamide product in low yield ([^{11}C]**10**), which may be explained by the increased steric hindrance observed for *o*-substituted substrates (also see [^{11}C]**3** vs. [^{11}C]**4**). Heterocycles and heteroatoms were also well tolerated, as shown by the high RCYs observed for the unprotected phenol and the *p*-bromine-, thiophene- and pyridine derivatives ([^{11}C]**14**-**17**). Finally, excellent RCYs were obtained with neutral or electron rich arenes, as illustrated by the naphtyl-, anisolyl and tolyl substituted ^{11}C-labelled benzamides ([^{11}C]**2**-**5**).

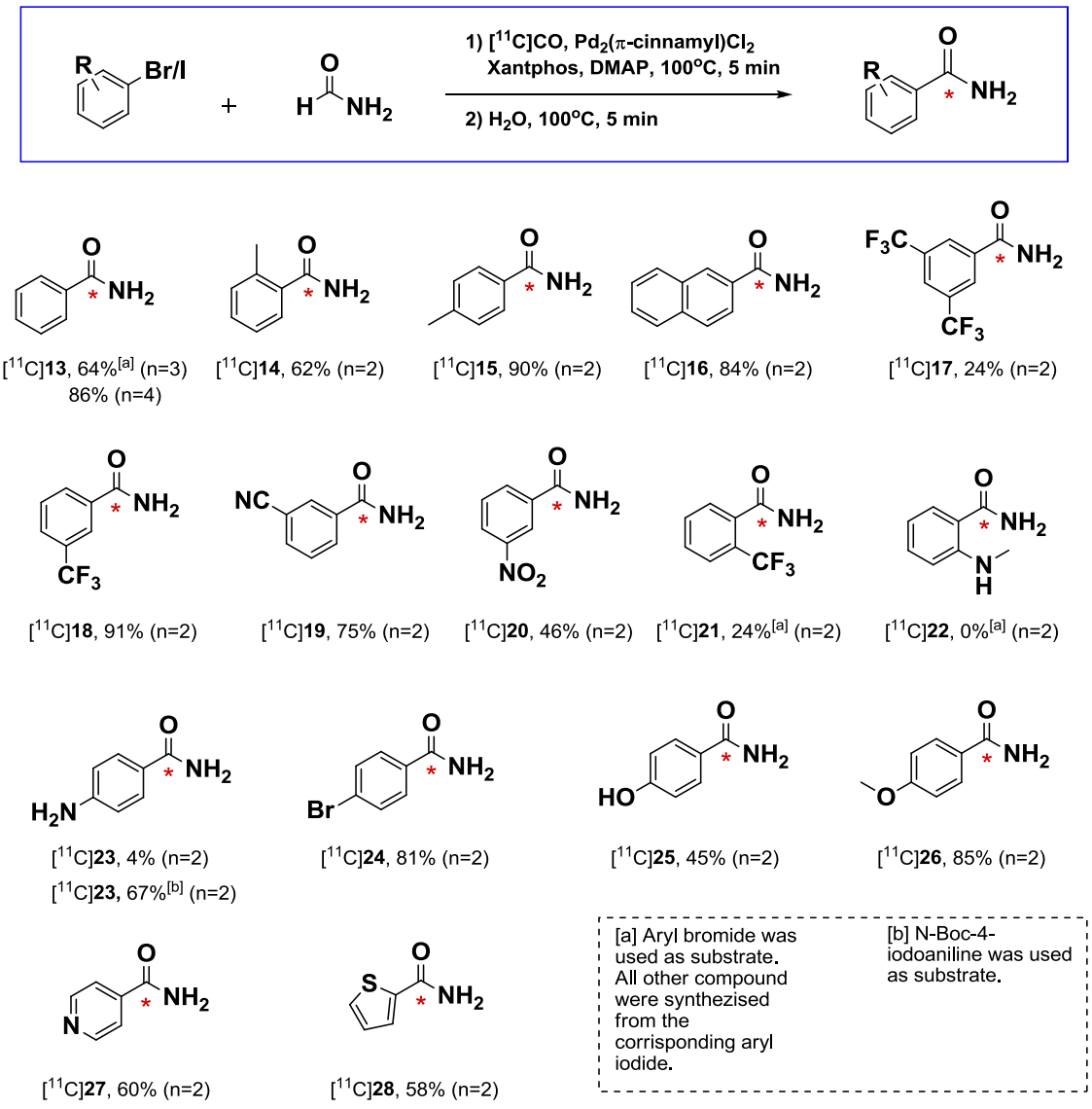
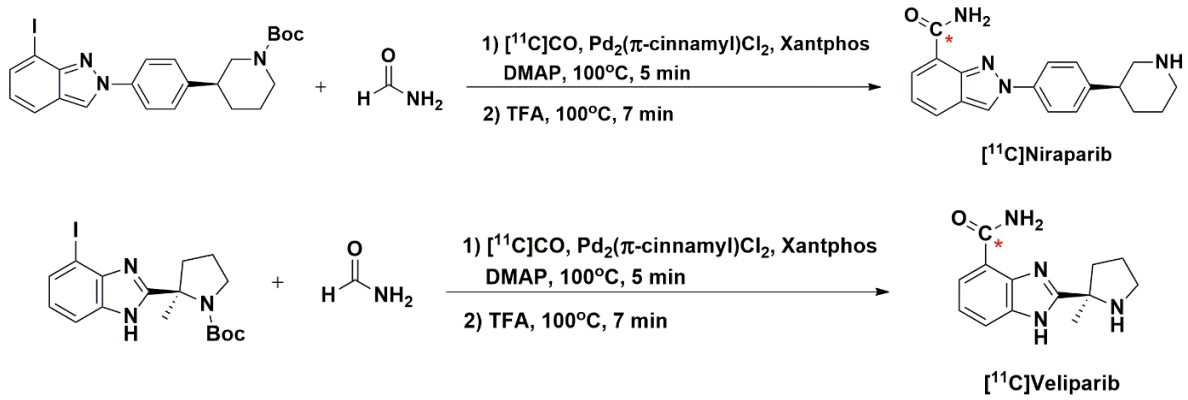


Figure 14: ^{11}C-labeled benzamides formed via intermediate [^{11}C]aroyl-DAMP salts.

To demonstrate the utility of this new methodology, the PARP inhibitors veliparib and niraparib were labeled using a two-step one-pot procedure starting from their corresponding *N*-Boc-protected iodo-substrates. For this synthesis, the aqueous hydrolysis step of the formimide could be omitted, since non-anhydrous TFA was used for the deprotection. The synthesis of these radiolabeled drugs was carried out in a fully automated fashion on the radiochemistry system that was described in the previous section (4.2). Preparative runs furnished both compounds in suitable yields for preclinical PET studies. Thus, following a full cyclotron production (55 µA for 30 min), 541 MBq of [^{11}C]veliparib was obtained at a A_m of 9.9 GBq/µmol and a radiochemical purity exceeding 99%. The isolated radiochemical yield of [^{11}C]niraparib was higher than [^{11}C]veliparib – 1422 MBq, at a A_m of 10.0 GBq/µmol and a radiochemical purity exceeding 99% (Scheme 3).



Scheme 3: Radiolabeling of [^{11}C]niraparib and [^{11}C]veliparib for preclinical studies.

5 CONCLUDING REMARKS

The present thesis has contributed to the advancement of radiopharmaceutical science by describing the development of novel methodologies that provide access to structurally diverse radiotracers via late-stage palladium-mediated ^{11}C-carbonylation using [^{11}C]CO (Figure 15). The described methodologies have the potential to accelerate the development of new PET tracers, which in turn plays an important role in improving our understanding of human pathophysiology. Some of the key findings are highlighted in the below:

First, a new, simple and automated "in-loop" methodology for ^{11}C-carbonylation using [^{11}C]CO was developed. The method does not only provide high yields of simple model substrates, but also highly functionalized druglike molecules and PET tracers. Apart from its simplicity, the method has the advantage of reducing transfer losses between the reactor and the preparative HPLC system since the HPLC loop is the reaction vessel.

Second, a novel automated GMP-compliant radiochemistry system was developed, allowing for production at high yield and in suitable quality for enabling PET studies in human subjects. Since its development, this radiochemistry system has enabled the preparation of several PET tracers for preclinical studies and is validated for use in a clinical study. This radiochemistry system represents the first commercially-available system for ^{11}C-carbonylation using [^{11}C]CO, and it is our hope that access to this system will spark more wide use of [^{11}C]CO in the radiopharmaceutical community.

Finally, a new convenient route to ^{11}C-labeled benzamides was developed. The method relied on a combination of formamide as an ammonia surrogate and the *in situ* generation of electrophilic ^{11}C-aroyl-DMAP salts. The method was rapid, efficient and provided a wide range of substituted [^{11}C]benzamides in good to excellent radiochemical yields. The utility of the methodology was showcased in the labeling of PET tracers [^{11}C]niraparib ([^{11}C]**10**) and [^{11}C]veliparib ([^{11}C]**11**). Furthermore, this is, to the best of our knowledge, the first report of electrophilic ^{11}C-aroyl-DMAP salts, a new class ^{11}C-labeled carbonylation intermediates for PET radiopharmaceutical synthesis.

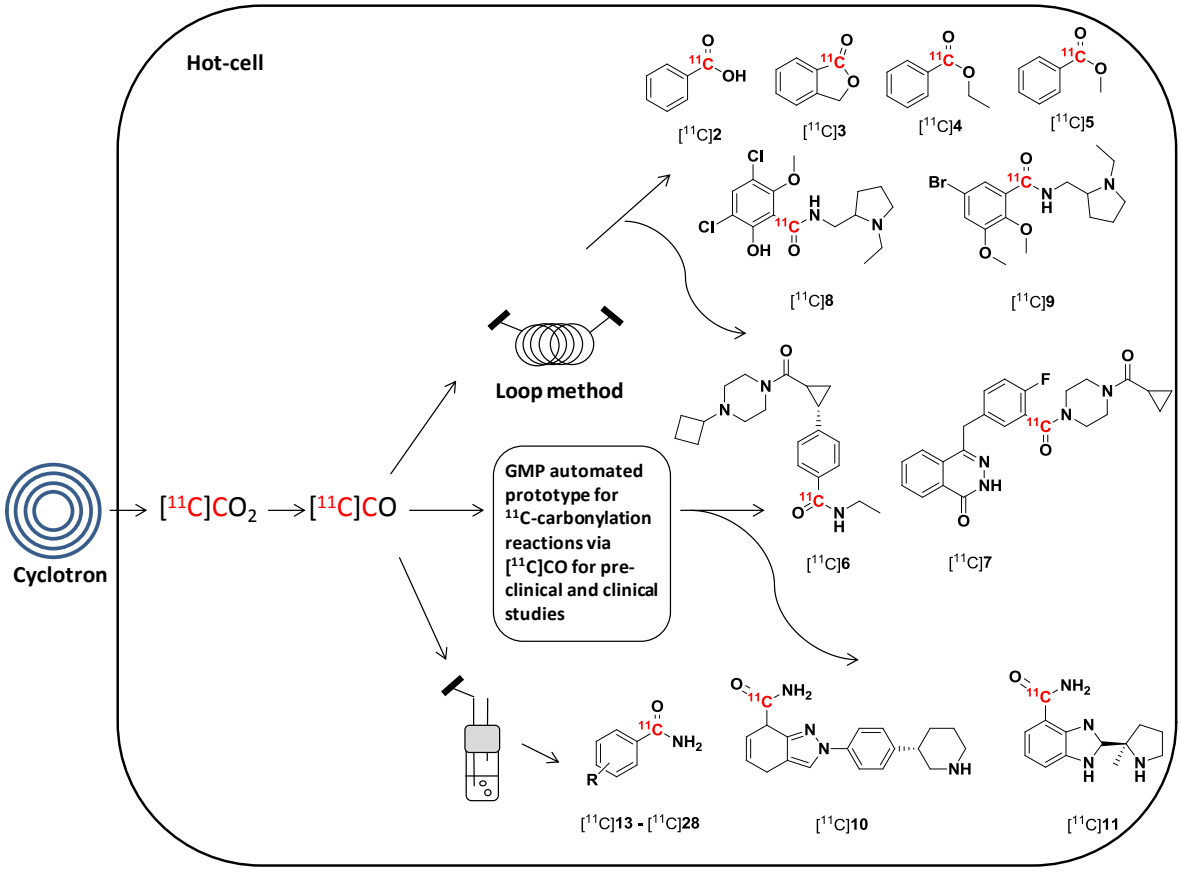


Figure 15: New methodologies developed during this thesis work.

6 FUTURE PERSPECTIVES

Vast improvements in ^{11}C-carbonylation chemistry have been reported during the past decades. The development of radiochemistry methods, partly described in this thesis, is needed to meet the increasing demand for suitable PET radioligands from academia, healthcare and drug industry.

There are several natural extensions of the research reported in this thesis; the "in-loop" ^{11}C-carbonylation methodology (paper 1) could be integrated in the commercially available automated radiochemistry system (paper 2) and there are good reasons to believe that the electrophilic ^{11}C-aroyl-DMAP salts could be successfully applied as intermediates in the synthesis of ^{11}C-carbonyl species originating from weak nucleophiles. Finally, a major limitation with transition-metal mediated ^{11}C-carbonylation is that it is restricted to producing aromatic ^{11}C-carbonyl species due to extensive β–elimination in alkyl substrates. Recent progress in photoredox chemistry may have an important part to play in overcoming this limitation, although this represents a significant challenge.

7 ACKNOWLEDGEMENTS

The present thesis was part of the European Union's Horizon 2020 programme within ISOTOPICS project. Therefore, I would like to express my sincere acknowledgement to everyone who makes this thesis possible.

My main supervisor Magnus Schou, even though I still don't know how to pronounce your last name, I still appreciate all the progress you have done to finally said mine, it meant so much to me. Many thanks for introducing me to the world of PET radiochemistry; your useful comments and guidance showed how to be an independent researcher in the long term.

Professor Christer Halldin, my co-supervisor for giving me the opportunity to do my doctoral training at Karolinska Institutet. It was a real pleasure to be part of your team.

Kenneth Dahl and Sangram Nag, my co-supervisors, for their valuable support and suggestion. Peter Johnström, Kenneth Dahl, Mikhail Kondrashov and Miguel Cortes Gonzalez for spending time to revise this thesis.

All the persons with who I had the chance to work with, at our magnificent U1 basement: Ana Vazquez Romero, Patricia Miranda Azpiazu, Youssef El Khoury, Tian Qiu, Kaisa Horkka, Maria Johansson, Vladimir Stepanov, Mikhail Kondrashov, Andrea Varrone, Raisa Krasikova, Magnus Schou, Peter Johnström, Arsalan Amir, Guennadi Jogolev, Kenneth Dahl, Zhisheng Jia, Prodip Datta, Yaser Khani, Ali Ekhtiari, Reza Mohammadi, Mohammad Moein, Johan Ulin, Dinahlee Saturnino Guarino, Mathangi Palanivel, Zsolt Sarnyai, Åsa Södergren, Siv Eriksson, Anton Lindberg, Urban Hansson, Jessica Bridoux, Kenneth Stålmo, Miguel Cortés Gonzalez, Arindam Das, Petra Agirman, Mahabuba Jahan, Sangram Nag, Hemantha Mallapura.

A special thanks to: **Youssef**, for his unlimited help in the lab; he reminds me a cartoon called "Bob le bricoleur" translated as Bob the builder. I hope that Youssef AB Company coming from our imagination will became true. The person who is reading my thesis, for further collaboration, please make a call to Youssef the engineer, available 24H; he will send someone to help you asap (N.B: Tools included). **Patricia**, for being my wonderful friend, I will always remember your "caliente" Spanish voice in my mind calling me "bébé". KI most famous Casanova, **Zsolt** for his ability to flirt with most of the girls at the same time. **Mikey**, for all the Rock'N'Roll music's in the lab and his positive attitude at the office (especially the "guitar guitar" song). **Kaisa** for her daily word "sure" that brings happiness at work. The most "True love" person ever met, **Prodip** for his kindness. **Miguel**, for your "Krikri". **Yaser**, for our "Miaou" conversations. **Ali** and **Reza,** for being fun. **Vlad** and his unconditional "Coffee?" followed by his intellectual anecdotes. My crazy friend **Tian,** for being the funniest person ever met and making me discover Chinese food such as chicken feet, yummy!

I enjoyed every moment spent with all of you either at or outside work.

To all the member of KI PET centre group for making a successful work environment.

Many thank to my previous supervisors from France, Françoise Dumas and Franck Le Bideau, with who I developed a passion for chemistry and their advices for applying to this thesis position.

All the members of the ISOTOPICS consortium and all PhDs part of this network: Kaisa, Laura, Agostinho, Mégane, Francesco, Mateusz, Viktor, Alexandra, Anna Chiara, Donia, Gianluca, Alberto, Malvika and Antonio with who I had the chance to go through this journey.

Importantly, a huge thanks to SFI for teaching me all the useful Swedish sentences needed for having a real conversation such as “Tjena min kompis”, “Jag heter Mélodie och jag älskar fransk vin med ost”, “Vad Kul!” and “Hejdå”, It did help to make Swedish friends (I was ironic here).

I would like to thank the most important persons in my life, my mother Elodie and my brother Maxime for their love and support.

Warm acknowledgement to my friends in France: Steffie, Stephane, Safaa, Deepak, Baptiste, Laura, Fanny, Jess, Sandra, Diana and many others.
Other friends that I met in Stockholm: Doris, Noëmie, Marie, Flavia, Marialena, Nancy, Charlotte, Salim (Monsieur briquet), Floriane, Niranjan, Romain, Elo, Eva, Tamara, Linnsi, Lilly, Demetria, Émile, Amélie, Arjhun, Anton, Phill, Jessica (mama Jess), Margot and many others (Not enough space for everyone).

Special thanks to my dearest Joakim for always being there.

8 REFERENCES

1. Blaufox, M.D.; Becquerel and the Discovery of Radioactivity: Early Concepts, Seminars in Nuclear Medicine. **1996**, Vol XXVI, No 3, 145-154.

2. L. Jødal1, C. Le Loirec and C. Champion ; Positron range in PET imaging: an alternative approach for assessing and correcting the blurring, *Phys. Med. Biol*. **2012**, 57, 3931-3943.

3. Phelps, M.E.; Hoffman, E. J.; Mullani, N.A.; Ter-Pogossian,M.M. Application of annhilation coincidence detection to transaxial reconstruction tomography. *J.Nucl.Med*. **1975**,16(3), 210-224.

4. Bergström, M.; Langström, B.; Pharmacokinetics studies with PET. In progress in drug research: Imaging in drug discovery and early clinical trials; Rudin, M.; Ed.; Birkhäuser Verlag: Basel, **2005**; 279-318.

5. Langström, B,; Kihlberg, T.; Bergström, M. ; Antoni, G.; Björkman, M.; Forngren, B.H.; Forngren, T.; Hartvig, P.; Markides, K.; Ynhve, U.; Ögren, M.; Compounds labelledwith short-lived beta-emitting radionuclides and some applications in life sciences. The importance of a time as a parameter. *Acta Chem. Scand*. **1999**,53,651-669.

6. Chi-Ming Lee and Lars Farde; Using positron emission tomography to facilitate CNS drug development; TRENDS in pharmacological sciences, **2006**, Vol 27, 310-316.

7. B. Långström, O. Itsenko, O. Rahman; [^{11}C]Carbon monoxide, a versatile and useful precursor in labelling chemistry for PET-ligand development; *J Label Compd Radiopharm*. **2007**; 50: 794–810.

8. Kealey S., Gee A. and Miller P. W. Transition metal mediated [^{11}C]carbonylation reactions: recent advances and applications. *J Labelled Compd Radiopharm*. **2014**, 57, 195-201.

9. Rahman O. [^{11}C]carbon monoxide in labeling chemistry and positron emission tomography tracer development: scope and limitations. *J Labelled Compd Radiopharm.* **2015**, 58, 86-98.

10. Iwata, R.; Ido, T.; Takahashi, T.; Nakanishi, H.; Iida, S., Optimization of [11C]HCN production and no-carrier-added [1-11C]amino acid synthesis. International Journal of Radiation Applications and Instrumentation. Part A. Applied Radiation and Isotopes 1987, 38 (2), 97-102.

11. Halldin C. Gulyas B. and Farde L. Studies with carbon-11 radioligands in neuropsychopharmacological drug development. *Current Pharmaceutical design*. **2001**, 7, 1907-1929.

12. Jewett D. M. A simple synthesis of [^{11}C]methyl triflate. *Int Appl Radiat Isot*. **1992**, 43, 1383-1385.

13. Någren K., Müller L., Halldin C., Swahn C. G. and Lehikoinen P. Improved synthesis of some commonly used PET radioligands by the use of [^{11}C]methyl triflate. *Nucl Med Biol*. **1995**, 22, 235-239.

14. Landais, P.; Crouzel, C., A new synthesis of carbon-11 labelled phosgene. International Journal of Radiation Applications and Instrumentation. Part A. Applied Radiation and Isotopes 1987, 38 (4), 297-300.

15. Roeda, D.; Dolle, F., [11C]Phosgene: A Versatile Reagent for Radioactive Carbonyl Insertion Into Medicinal Radiotracers for Positron Emission Tomography. Current Topics in Medicinal Chemistry 2010, 10 (16), 1680-1700.

16. Nishijima, K.-i.; Kuge, Y.; Seki, K.-i.; Ohkura, K.; Motoki, N.; Nagatsu, K.; Tanaka, A.; Tsukamoto, E.; Tamaki, N., A simplified and improved synthesis of [11C]phosgene with iron and iron (III) oxide. Nuclear Medicine and Biology 2002, 29 (3), 345-350.

17. Miller, P. W.; Long, N. J.; Vilar, R.; Gee, A. D., Synthesis of ^{11}C, ^{18}F, ^{15}O, and ^{13}N Radiolabels for Positron Emission Tomography. Weinheim, **2008**; Vol. 47, pp 8998-9033.

18. Cai, L.; Lu, S.; Pike, V. W., Chemistry with [^{18}F]Fluoride Ion. *European Journal of Organic Chemistry 2008*, **2008** (17), 2853-2873.

19. Littich, R.; Scott, P. J. H., Novel Strategies for Fluorine-18 Radiochemistry. *Angew Chem Int Ed*. **2012**, 51 (5), 1106-1109.

20. Bergman, J.; Solin, O., Fluorine-18-labeled fluorine gas for synthesis of tracer molecules. *Nuclear Medicine and Biology*. **1997**, 24 (7), 677-683.

21. Teare H., Robins E. G., Kirjavainen A., Forsback S., Sandford G., Solin O., Luthra S. K.and Gouverneur V. Radiosynthesis and evaluation of [^{18}F]selectfluor bis (triflate). *Angew Chem Int Ed*. **2010**, 49, 6821-6824.

22. Lasne M-C et al, chemistry of beta emitting compounds based on fluorine-18, Topics in current chemistry, W. Krauss (ed.), **2002**, 22, p-201.

23. Dollé. F, Demphel S., Hinnen F., Fournier D., Vaufrey F., Crouzel. C., 6-[18F]Fluoro-L-DOPA by radiofluoro destannylation : a short and simple synthesis of a new labelling precursor, *J Labelled Compd Radiopharm*, **1998**, 41, 105.

24. Preshlock, S.; Tredwell, M.; Gouverneur, V., ^{18}F-labeling of arenes and heteroarenes for applications in positron emission tomography, *Chemical Reviews*. **2016**, 116 (2), 719-766.

25. Clark JC, Buckingham PD. The preparation and storage of carbon-11 labelled gases for clinical use. *Int J Appl Radiat Isot*. **1971**; 22 (11), 639–46.

26. S. K. Zeisler, M. Nader, A. Theobald and F. Oberdorfer., *Appl. Radiat. lsot*. **1997**, Vol. 48, No. 8,1091-1095.

27. Roeda D, Crouzel C, Dollé F. A rapid, almost quantitative conversion of [^{11}C]carbon dioxide into [^{11}C]carbon monoxide via [^{11}C]formate and [^{11}C]formyl chloride. *Radiochimica Acta*. **2004**; 92, 329–332.

28. P. Nordeman, S. D. Friis, T. L. Andersen, H. Audrain, M. Larhed, T. Skrydstrup and G. Antoni., Rapid and efficient conversion of $^{11}CO_2$ to ^{11}CO through silacarboxylic acids: applications in Pd-Mediated carbonylations, *Chem. Eur. J*. **2015**, 21, 17601 – 17604.

29. Y. Andersson and B. Långström, Synthesis of ^{11}C-labelled ketones via carbonylative coupling reactions using [^{11}C]carbon monoxide, *J. Chem. Soc. Perkin Trans. I*, **1995**, 287-289.

30. M. Al-Qahtani and V. Pike, *J Labelled Compd Radiopharm*, **2000**, 43, 825-835.

31. P. Lidström, T Kihlberg and B Långström, [^{11}C]Carbon monoxide in the palladium-mediated synthesis of ^{11}C-labelled ketones, *J. Chem. Soc., Perkin Trans. 1*, **1997**, 2701-2706.

32. Tor Kihlberg and Bengt Långström, Biologically active ^{11}C-labeled amides using palladium-mediated reactions with aryl halides and [^{11}C]carbon monoxide, *J. Org. Chem*. **1999**, 64, 9201-9205.

33. S. Roslin, P. Brandt, P. Nordeman, M. Larhed, L. R. Odell , J. Eriksson; Synthesis of ^{11}C-labelled ureas by palladium(II)-mediated xxidative carbonylation; *Molecules* **2017**, 22, 1688.

34. P. W. Miller, N. J. Long, A. J. de Mello, R. Vilar, H. Audrain, D. Bender, J. Passchier, A. Gee; Rapid multiphase carbonylation reactions by using a microtube reactor: Applications in positron emission tomography ^{11}C-Radiolabeling; *Angew. Chem. Int. Ed*. **2007**, 46, 2875 –2878.

35. K. Dahl, M. Schou, N. Amini, C. Halldin; Palladium-mediated [^{11}C]carbonylation at atmospheric pressure: A general method using xantphos as supporting ligand; *Eur. J. Org. Chem*. **2013**, 1228–1231.

36. Philip W. Miller, H. Audrain, D. Bender, A J. deMello, A. D. Gee, N. J. Long, R. Vilar; Rapid carbon-11 radiolabelling for PET using microfluidics; *Chem. Eur. J*. **2011**, 17, 460 – 463.

37. H. Audrain, L. Martarello, A. Gee, D. Bender; Utilisation of [^{11}C]-labelled boron carbonyl complexes in palladium carbonylation reaction; *Chem.Commun*. **2004**, 558-559.

38. S. Kealey, P. W. Miller, N. J. Long, C. Plisson, L. Martarello, A. D. Gee, Copper (I) scorpionate complexes and their application in palladium-mediated [^{11}C]carbonylation reactions, *Chem.Commun*. **2009**, 3696-3698.

39. Eriksson. J., Van den Hoek. J., Windhorst. A. D., Transition metal mediated synthesis using [^{11}C]CO at low pressure - A simplified method for ^{11}C-carbonylation, *J Labelled Compd Radiopharm*, **2012**, 55 (6), 223-228.

40. Brennfhrer A., Neumann H. and Beller M. Palladium-catalyzed carbonylation reactions of aryl halides and related compounds. *Angew. Chem. Int. Ed*. **2009**, 48, 4114 – 4133.

41. J. F. Hartwig, S. Richards, David Barañano, and F. Paul., Influences on the relative rates for C-N bond-forming reductive elimination and β-hydrogen elimination of amides. A case study on the origins of competing reduction in the palladium-catalyzed amination of aryl halides, *J. Am. Chem. Soc*., **1996**, Vol. 118 (15), 3626-3633.

42. M. S. Driver and J. F. Hartwig., Carbon-nitrogen-bond-forming reductive elimination of arylamines from palladium (II) phosphine complexes, *J. Am. Chem. Soc*. **1997**, 119, 8232-8245.

43. M. S. Driver and J. F. Hartwig., A rare, low-valent alkylamido complex, a diphenylamido complex, and their reductive elimination of amines by three-coordinate intermediates, *J. Am. Chem. Soc*. **1995**, 117, 4708-4709.

44. R. A. Widenhoefer and S. L. Buchwald., Halide and amine influence in the equilibrium formation of palladium Tris(o-tolyl)phosphine mono(amine) complexes from palladium aryl halide dimers, *Organometallics*. **1996**, 15, 2755-2763.

45. T.L. Andersen, S. D. Friis, H. Audrain, P. Nordeman, G. Antoni and T. Skrydstrup., Efficient ^{11}C-carbonylation of isolated aryl palladium complexes for PET: Application to challenging radiopharmaceutical synthesis, *J. Am. Chem. Soc*. **2015**, 137, 1548−1555.

46. O. Itsenko and B. Långström., Radical-mediated carboxylation of alkyl iodides with [^{11}C]carbon monoxide in solvent mixtures, *J. Org. Chem*. **2005**, 70, 2244-2249.

47. O. Itsenko, T. Kihlberg, B. Långström., Synthesis of aliphatic [carbonyl-^{11}C]esters using [^{11}C]carbon monoxide, *Eur. J. Org. Chem*. **2005**, 3830–3834.

48. O. Rahman, B. Långström and C. Halldin., Alkyl iodides and [^{11}C]CO in nickel-mediated cross-coupling reactions: Successful use of alkyl electrophiles containing α, β-hydrogen atom in metal-mediated [^{11}C]carbonylation, *ChemistrySelect*. **2016**, 1, 2498 – 2501.

49. Chow, S.Y., Odell, L.R. and Eriksson, J., Low-Pressure Radical ^{11}C-Aminocarbonylation of Alkyl Iodides through Thermal Initiation. Eur. J. Org. Chem., **2016**: 5980-5989.

50. McCarron JA, Turton DR, Pike VW, Poole K.G. Remotely-controlled production of the 5-HT1A receptor radioligand, [carbonyl-^{11}C]WAY-100635, via ^{11}C-carboxylation of an immobilized Grignard reagent. J Label Compd Radiopharm **1996**, 38(10), 941-953.

51. Wilson AA, Garcia A, Jin L, Houle S. Radiotracer synthesis from [C-11]-iodomethane: A remarkably simple captive solvent method. *Nucl Med Biol* **2000**, 27(6), 529-532.

52. Dahl. K., Nakao. R., Amini. N.,, Mahdi Moein. M., Finnema. S., Malmquist. J., Varnäs. K and Schou. M. Development of [carbonyl-^{11}C]AZ13198083, a novel

histamine type-3 receptor radioligand with favorable kinetics. *ACS Chem. Neurosci*. **2018**, 9, 5, 906–911.

53. Rahman. O., Takano. A., Amini. N., Dahl. K., Kanegawa. N., Långström. B., Farde. L. Halldin. C., Synthesis of ([^{11}C]carbonyl)raclopride and a comparison with ([^{11}C]methyl)raclopride in a monkey PET study. *Nuclear Medicine and Biology* 42 **2015**, 893–898.

54. Airaksinen. A. J., Nag. S., Finnema. S. J., Mukherjee. J., Chattopadhyay. S., Gulyás. B., Farde. L. Halldin. C., [^{11}C]Cyclopropyl-FLB 457: A PET radioligand for low densities of dopamine D2 receptors. *Bioorganic & Medicinal Chemistry* 16, **2008** 6467–6473.

55. Andersen. T. L., Friis. S.D., Audrain. H., Nordeman. P., Antoni. G. and Skrydstrup. T. Efficient 11C‑Carbonylation of Isolated Aryl Palladium Complexes for PET: Application to Challenging Radiopharmaceutical Synthesis. *J. Am. Chem. Soc*. **2015**, 137, 1548−1555.

56. Ferrat. M., Dahl. K., Halldin. C and Schou. M. "In-loop" carbonylation—A simplified method for carbon-11 labelling of drugs and radioligands. *J Label Compd Radiopharm*. **2020**; 1–8.

57. Ferrat. M., El Khoury. Y., Larsen. P., Dahl.K., Halldin. C and Schou. M., Development of a fully automated low-pressure [^{11}C]CO carbonylation apparatus. *J Label Compd Radiopharm*. **2020**; 63, 517–522.

58. Y. Andersson, B. Långström, Transition metal-mediated reactions using [^{11}C]cyanide in synthesis of ^{11}C-labelled aromatic compounds. *J. Chem. Soc*., Perkin Trans. 1 **1994**, 1395-1400.

59. F. Karimi, B. Långström, Palladium-mediated synthesis of [carbonyl-11C]amides and hydrazides using [^{11}C]carbon monoxide *J. Chem. Soc., Perkin Trans. 1* **2002**, 2111-2116.

60. P. Lagueux-Tremblay, A. Fabrikant, B. A. Arndtsen, Palladium-catalyzed carbonylation of aryl chlorides to electrophilic Aroyl-DMAP salts. *ACS Catal*. **2018**, 8, 6, 5350–5354.

61. J. Quesnel, A. Fabrikant, B. A. Arndtsen, A flexible approach to Pd-catalyzed carbonylations via aroyl dimethylaminopyridinium salts. *Chem. Sci*. **2016**, 7, 295-300.