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METHODOLOGICAL ADVANCEMENTS IN MICROFLUIDIC AND CARBONYLATION PET RADIOCHEMISTRY

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Methodological advancements in microfluidic and carbonylation PET radiochemistry

Thesis for doctoral degree (Ph.D.)

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To my family.

ABSTRACT

Along with the progress of PET as a powerful imaging tool in medicine, there has been increasing demand for new labeling methods. The main aim of this PhD dissertation was to develop novel labeling methodologies using the positron emitter carbon-11.

Advancements within the field of palladium-mediated carbonylation reaction are discussed in the first part of the thesis. Paper I describes the development of a Pd-ligand complex where [^{11}C]carbon monoxide (^{11}CO) is efficiently trapped and incorporated as a part of the CO-insertion procedure. The consequent carbonylation reaction proceeds smoothly in good and reproducible yields using aryl halides or triflates as substrates. As a proof of concept, the utility of the protocol was applied to the synthesis of a candidate radioligand for the histamine type-3 receptor. The same protocol was further improved using microwave heating in paper II. An improved yield was observed in the ^{11}C-aminocarbonylation of electron deficient aryl halides and even allowing for the use of an aryl chloride as substrate. Moreover, high yields for hydroxy- and alkoxycarbonylation were obtained when efficient microwave-energy absorbent nucleophiles, such as water and alcohol, were utilized as co-solvents. In paper III, an efficient and convenient carbonylative approach for the direct synthesis of ^{11}C-labeled aryl methyl ketones from aryl halides is presented, employing [^{11}C]methyl iodide ($^{11}CH_3I$) as the radioactive precursor under $Co_2(CO)_8$-mediated conditions. A total of ten model (hetero)aryl methyl ketones were obtained in a 22-63% decay-corrected radiochemical yield, based on radioactivity in the solution at the end of synthesis.

The increasing demand of rapid labeling procedures has stimulated the development of emerging technologies such as microfluidics for more flexible and efficient radioligand supply. To this end, in the second part of the thesis, microfluidic-assisted radiochemistry was evaluated for the labeling with fluorine-18 and carbon-11. Firstly, in paper IV, a commercially available microfluidic (MF) platform was evaluated using the two-step preparation of ^{18}F-fluorobenzyl amines via a reductive amination reaction. The microfluidic apparatus allowed for rapid parameter optimization and was also applied preparatively to produce adequate radioactivities for PET applications. Finally, in paper V, a novel gas-liquid segmented microfluidic platform was developed. The large gas-to-liquid interfacial area generated by the segmented approach facilitated the ^{11}CO insertion even while less reactive Pd-ligand species were applied. The Pd-mediated ^{11}C-carbonylation reaction proceeded smoothly on this platform and good to excellent radiochemical yields were observed. Twelve compounds were successfully radiolabeled in a RCY range of 41-99%, including the well estabiched D_2 receptor radioligands [^{11}C]raclopride and [^{11}C]FLB 457.

LIST OF THESIS PUBLICATIONS

I. **Kenneth Dahl**, Magnus Schou, Nahid Amini, Christer Halldin. Palladium-mediated [^{11}C]carbonylation at atmospheric pressure: A general method using xantphos as supporting ligand. *European Journal Organic Chemistry* **2013**, 1228-1231.

II. **Kenneth Dahl**, Magnus Schou, Obaidur Rahman, Christer Halldin. Improved yields for the palladium-mediated ^{11}C-carbonylation reaction using microwave technology. *European Journal Organic Chemistry* **2014**, 307-310.

III. **Kenneth Dahl**, Magnus Schou, Christer Halldin. Direct and efficient cobalt carbonyl-mediated aryl acetylation using [^{11}C]methyl iodide. *Submitted*.

IV. **Kenneth Dahl**, Magnus Schou, Christer Halldin. Radiofluorination and reductive amination using a microfluidic device. *Journal of Labelled Compounds and Radiopharmaceuticals* **2012**, 55, 455-459.

V. **Kenneth Dahl**, Magnus Schou, Johan Ulin, Carl-Olof Sjöberg, Lars Farde, Christer Halldin. ^{11}C-Carbonylation reaction using gas-liquid segmented microfluidics. *RSC Advances* **2015**, 5, 88886-88889.

LIST OF NON-THESIS PUBLICATIONS

1. **Kenneth Dahl**, Oleksiy Itsenko, Obaidur Rahman, Johan Ulin, Carl-Olof Sjöberg, Peter Sandblom, Lars-Anders Larsson, Magnus Schou, Christer Halldin. An evaluation of a high-pressure ^{11}CO apparatus. *Journal of Labelled Compounds and Radiopharmaceuticals* **2015**, 58, 220-225.

2. Obaidur Rahman, Akihiro Takano, Nahid Amini, **Kenneth Dahl**, Naoki Kanegawa, Bengt Långström, Lars Farde, Christer Halldin. Synthesis of ([^{11}C]*carbonyl*)raclopride and a comparison with ([^{11}C]*methyl*)raclopride in a monkey PET study. *Nuclear Medicine and Biology* **2015**, 11, 893-898.

CONTENTS

LIST OF ABBREVIATIONS

^{11}C	Radioactive, neutron deficient isotope of carbon
$^{11}CO_2$	[^{11}C]Carbon dioxide
$^{11}CH_4$	[^{11}C]Methane
^{11}CO	[^{11}C]Carbon monoxide
$^{11}CH_3I$	[^{11}C]Methyl iodide
$^{18}F^-$	[^{18}F]Fluoride ion
AC	Active cooling
Bq	Bequerel, SI unit of radioactivity
Carrier	Non radioactive form of radioligand
$Co_2(CO)_8$	Cobalt carbonyl
Cyclotron	Particle accelerator
dcpp	1,3-Bis(dicyclohexylphosphino)propane bis(tetrafluoroborate)
DMF	*N*,*N*-Dimethylformamide
DMSO	Dimethyl sulfoxide
DPEphos	(Oxydi-2,1-phenylene)bis(diphenylphosphine)
dppd	4-Bis(diphenylphosphino)butane
dppe	Ethylenebis(diphenylphosphine)
dppf	1,1′-Bis(diphenylphosphino)ferrocene
dppp	1,3-Bis(diphenylphosphino)propane
EtOH	Ethanol
Hot cell	Lead shielded containment box
HPLC	High performance liquid chromatography
i.d.	Inner diameter
In vivo	“in life” i.e. in the organism
Kryptofix (K_{222})	4, 7, 13, 16, 21, 24-hexaoxa-1, 10-diazabicyclo-[8.8.8]hexacosane
MeCN	Acetonitrile
MeOH	Menthanol
MeV	Mega electron volt
MF	Microfluidic
MS	Mass spectrometry
Pd	Palladium
$Pd_2(\pi\text{-cinnamyl})Cl_2$	Palladium(π-cinnamyl) chloride dimer
$Pd(PPh_3)_4$	Tetrakis(triphenylphosphine)palladium(0)
PPh_3	Triphenylphosphine
PET	Positron emission tomography
RCP	Radiochemical purity
RCY	Radiochemical yield
RT	Room temperature
Sphos	2-Dicyclohexylphosphino-2′,6′-dimethoxybiphenyl
SRA	Specific radioactivity

$t_{1/2}$	Half-life
t-Bu-Xantphos	9,9-Dimethyl-4,5-bis(di-*tert*-butylphosphino)xanthene
TE	Trapping efficiency
THF	Tetrahydrofuran
UV	Ultraviolet
Xantphos	4,5-Bis(diphenylphosphino)-9,9-dimethylxanthene

1 INTRODUCTION

The ability to image molecular events within a living organism and in real time is of great value to the natural sciences. One of the most advanced technologies for this purpose is positron emission tomography (PET), an imaging modality that offers picomolar sensitivity and can provide *in vivo* quantitative information of physiological, biochemical and pharmacological processes following the administration of a specific positron-emitting probe called radioligand[1,2]. However, the development of new radioligands for PET is far from trivial and radiochemistry, the chemistry of radioactive material, is a major limiting factor for the field of PET. The primary focus of this thesis is the development of novel synthetic strategies for the labeling of organic compounds with short-lived radionuclides, with a special emphasis on advancements within palladium-mediated ^{11}C-carbonylation reactions and microfluidic radiochemistry.

1.1 The PET technology

Imaging agents used in PET are radiolabeled with short-lived positron-emitting radionuclides and the most commonly used radionuclides for PET are shown in table 1. In PET, the radioligand is most commonly administered intravenously to the subject (patient or animal). Positron-emitting radionuclides decay by the conversion of a proton into a neutron with the simultaneous emission of a positron. The emitted positron travels a short distance in tissue and annihilates after encountering an electron. The distance traveled by the positron is known as the positron range, which generates a loss in spatial resolution. The energy of the emitted positron determines the path length before the annihilation and is different for each positron-emitting radionuclide. This annihilation gives rise to a pair of body penetrating gamma rays (511 keV) that are emitted in virtually opposite directions[3]. The coincidence events are subsequently recorded by a number of detector rings surrounding the subject. Data are collected over time and reconstructed by a computer algorithm to give a three dimensional image showing the radioactive distribution in the investigated region.

Table 1. Physical properties of ^{11}C, ^{18}F, ^{13}N and ^{15}O.

Radionuclide	Half-life (min)	Positron emission (keV)	Maximal energy (keV)	Nuclear reaction
^{11}C	20.4	99.8	0.960	^{14}N(p,α)^{11}C
^{18}F	109.7	96.7	0.635	^{18}O(p,n)^{18}F
^{13}N	10.0	99.8	1.190	^{16}O(p,α)^{13}N
^{15}O	2.1	99.9	1.720	^{14}N(d,n)^{15}O

1.2 PET radiochemistry

Along with the progress of PET as a powerful imaging tool in medicine, there has been an increasing demand for new labeling methods. However, the synthesis of these biological probes is not trivial and represents an important challenge for synthetic chemists[4]. First of all, working with short-lived radionuclides imposes strict time constraints on the overall radioligand production process. As a general rule, a PET radioligand has to be synthesized, purified, formulated and analyzed within a timescale of roughly 2 to 3 physical half-lives of the radionuclide in use; ideally, one should aim to introduce the radioactive label as late as possible in the reaction sequence. Techniques such as microwave heating[5] and microreactors[6-7] (microfluidic) have been increasingly used as a positional mean to reduce reaction times and improve selectivity.

An important aspect when working with radioactive compounds is the safety of the operator. Traditional bench-top synthetic chemistry is thus clearly not feasible. In order to avoid unnecessary radiation exposure, radiolabeling is performed in automated or robotic synthesis modules housed inside lead-shielded fume hoods (hot cells). The difficulties of working with short-lived PET radionuclides are further exacerbated by the minute chemical amounts (typically in the picomolar to nanomolar scale) of radioactive material produced from a standard medical cyclotron (particle accelerator). As a result, in the synthesis of PET radioligands, the radioactive reaction partner will always be the limiting reagent and only present in low concentration[4]. On the other hand, for some reactions, this large stoichiometric excess of non-radioactive precursor may promote enhanced reaction kinetics and lead to shorter reactions.

1.2.1 Radiolabeling with carbon-11

Carbon-11 (^{11}C) is an attractive PET radionuclide since its naturally occurring isotope, carbon-12, is present in all organic molecules. Labeling with ^{11}C can thus be achieved without altering the physicochemical or pharmacological properties of a compound[8-10]. The most frequently used method for producing ^{11}C is the $^{14}N(p, \alpha)^{11}C$ nuclear reaction. The reaction is performed by proton bombardment of target containing a gas mixture with nitrogen-14 as the main component. The two major ^{11}C-labeled products, $^{11}CO_2$ and $^{11}CH_4$, are formed, respectively, when either small amounts oxygen or hydrogen are present in the target. These simple primary precursors are often transformed via on-line chemical transformations into more reactive compounds, such as [^{11}C]carbon monoxide[11-13], hydrogen [^{11}C]cyanide, [^{11}C]phosgene[14], [^{11}C]methyl iodide[15] and [^{11}C]methyl triflate[16-17] (figure 1), before being used in ^{11}C-labeling reactions. In this thesis [^{11}C]methyl iodide (paper III) and [^{11}C]carbon monoxide (paper I-II and V) were employed as secondary ^{11}C-labeling precursors.

So far, the most widely used method for incorporation of ^{11}C is heteroatom methylation using $^{11}CH_3I$. There are two common methods to prepare $^{11}CH_3I$ (scheme 1). In the "wet" method[18], $^{11}CO_2$ is reduced to [^{11}C]methanol using $LiAlH_4$, followed by the treatment with hydriodic acid. $^{11}CH_3I$ can also be obtained using gas phase iodination of $^{11}CH_4$ with elemental iodine in a radical reaction initiated at 720^{o}C. This method is commonly referred to as the "gas-phase" method[19], and has the advantage that it delivers ^{11}C-labeled PET radioligands with higher specific radioactivity (SRA) relative the "wet" method. High specific radioactivity is often required in neuroreceptor imaging studies to avoid saturation of the receptor system by non-

radioactive carrier molecules. Furthermore, in recent years, the application of $^{11}CH_3I$ in transition-metal-mediated reactions has become more widespread for ^{11}C-labeling[16]; especially notable is the use of palladium catalyst in ^{11}C-C bond formation by Stille and Suzuki cross-coupling reactions. Despite its utility, ^{11}C-methylation reactions are limited to labeling compounds containing a methyl-group. The development of new ^{11}C-labeling routes is thus important not only in order to increase the number of compounds that can be labeled with ^{11}C but also for increasing the possibility of labeling a given compound in different positions.

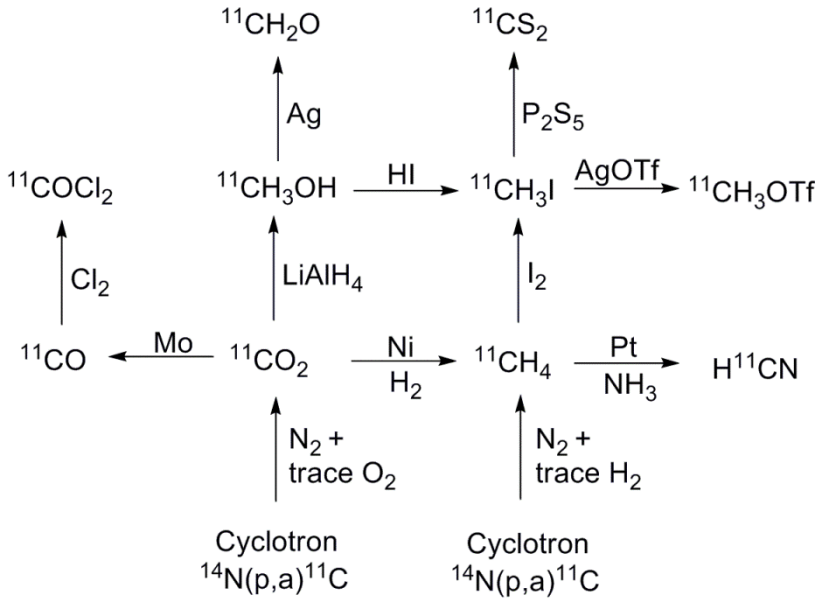


Figure 1. On-line transformations in carbon-11 radiochemistry.

1.2.2 Radiolabeling with fluorine-18

Fluorine-18 (^{18}F) is the most frequently employed radionuclide in PET. Its physical properties enables for multi-step synthesis, longer *in vivo* investigations, relatively high resolution imaging and distribution to other clinics without an on-site cyclotron. On the other hand, fluorine does not generally occur in biological important molecules, and the introduction of an ^{18}F atom alters a compounds physicochemical property. There are two main strategies for the preparation of ^{18}F-labeled molecules, namely electrophilic[20-21] and nucleophilic ^{18}F-fluorination[22-23]. One key difference between these two approaches is the specific radioactivity. Nucleophilic [^{18}F]fluoride ion is produced by the efficient^{18}O(*p*,*n*)^{18}F nuclear reaction under aqueous conditions and is normally obtained in high specific radioactivity.

Although the [^{18}F]fluoride ion is a strong nucleophile, in aqueous solution it forms strong hydrogen bonds with surrounding water molecules and becomes unreactive for nucleophilic substitution. The addition of a cationic counter ion and phase-transfer-catalyst (PTC), followed by the removal of water has proven to be crucial in improving the reactivity of the [^{18}F]fluoride

ion. Once the process of drying is completed, fluoride can be introduced by S_N2 mechanism into aliphatic positions or via nucleophilic aromatic substitution (S_NAr) into aromatic molecules. An aromatic tertiary amine was ^{18}F-labeled using a two-step microfluidic approach in paper IV.

Traditionally, aromatic no-carrier-added ^{18}F nucleophilic substitutions are only feasible on activated aromatic systems. Strong electron-withdrawing groups such as nitro-, cyano- and carbonyl-, position in *ortho* or *para* position to the leaving group are suitable for activation. There are several possible alternatives for leaving groups but mostly nitro or trimethylammonium salts are employed. However, recent advances have expanded the scope of nucleophilic aromatic ^{18}F-fluorinations to also include labeling of unactivated arenes. Most noteworthy is the development of the activated leaving group iodonium ylide[24] and transition-metal-mediated[25] approaches to ^{18}F-labeled arenes[26].

1.3 ^{11}C-Carbonylation reactions

Palladium-catalyzed carbonylation reaction is the coupling of a substrate (aryl, vinyl, benyl) functionalized with a leaving-group (halide, triflate, etc.), carbon monoxide and an appropriate nucleophile to give carboxylic acid derivatives, amides, aldehydes and ketones[27]. The basics of the transformation were first established in the mid-1970s by Heck and co-workers[28-29], ever since the scope and number of reactions have been expanded drastically. The general catalytic cycle for this palladium-catalyzed carbonylation reaction is illustrated in figure 2, and the fundamental processes involved in this transformation are described in the following section[30].

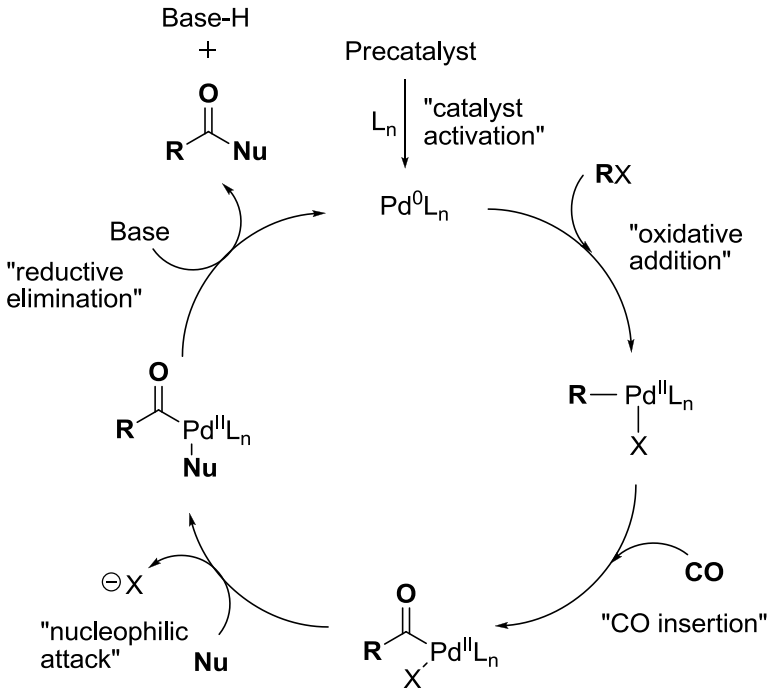


Figure 2. Catalytic cycle for the Pd-catalyzed carbonylation reaction
R = aryl, alkene; X = leaving group; L = Ligand.

Catalyst activation is the first reaction in palladium-catalyzed reactions where the precatalyst is converted *in situ* to the active palladium(0)-complex (Pd^0L_n). This process often requires the loss or exchange of ligands. Most Pd-based precatalysts applied in cross-coupling chemistry are found in oxidation states Pd^0 or Pd^{II}. Pd^{II} complexes are known to be more air stable and therefore easier to handle than Pd^0 complexes.

Oxidative addition is the first fundamental reaction of the catalytic cycle. During the oxidative addition, the Pd^0L_n complex is oxidized to Pd^{II}, and a carbon-(pseudo)halide bond of the aryl halide substrate is cleaved and two new σ-bonds to palladium are formed. The common monodentate phosphines ligand PPh_3 and the bidentate phosphine ligands, xantphos and dppp (figure 3), tend to form bi-ligated complexes.

CO insertion and coordination is defined as the insertion of carbon monoxide into a ligand-metal bond. For the catalytic process to produce the desired carbonylated product, coordination and insertion of carbon monoxide to generate a Pd^{II} acyl complex has to be faster than the competing nucleophilic attack, which would afford an undesired cross-couplings bi-product.

Nucleophilic attack (or transmetallation) is the transfer of nucleophile to the Pd centre, subsequently followed by the loss of a Pd coordinated (pseudo)halide, thus keeping the coordination and oxidation state intact. Amines and alcohols are examples of common nuclephiles applied in Pd-catalyzed carbonylation reactions.

Reductive elimination is the final step in the catalytic cycle, and is usually considered to be rate limiting step. During the course of a reductive elimination, a new carbon-carbon bond is formed between two groups positioned *cis* to each other in the Pd^{II} acyl complex. The reaction is essentially the reverse of oxidative addition, and thus the oxidation state and coordination number of the metal is decreased by two, and the original Pd^0L_n complex is regenerated by deprotonation using a base.

Ligand selection is an important factor to consider[31]. A ligand is defined as anything capable of coordinating to the palladium center. A ligand, however, has the ability to fine-tune the catalytic properties of the Pd-complex especially the bulkiness and electron density of these ligands are two parameters of particular importance for the reactivity of the Pd catalyst.

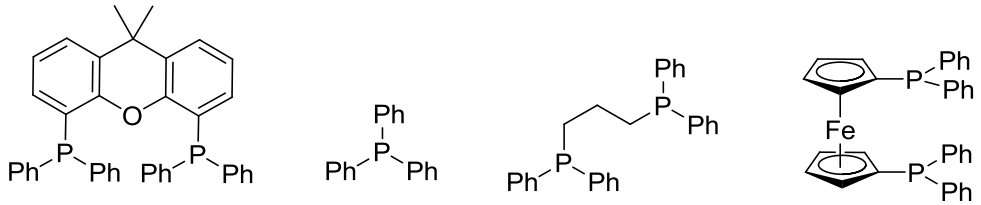


Figure 3. Ligands employed in the work presented in this thesis.

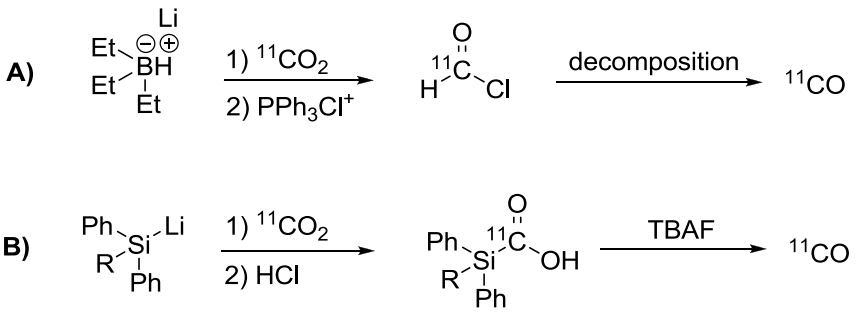
Advances in traditional synthetic carbonylation chemistry have, to some extent, been reflected in [^{11}C]carbon monoxide radiochemistry. Traditionally, carbonylations are performed using high CO pressure at elevated temperatures, owing to the poor solubility of CO in common organic solvents. However, as mentioned in section 1.2 of this thesis, cyclotron produced radionuclides is only present in minute amounts and low concentration, which precludes the use of high ^{11}CO pressures. Furthermore, owing from the low ^{11}CO concentration and the fact that the coupling reagents are present in large excess, the term "mediated" is used, rather than

catalyzed. In section 1.3.2, a selection of the most promising methodologies used to overcome the challenges associated with ^{11}CO chemistry is highlighted.

1.3.1 [^{11}C]Carbon monoxide production

The synthesis of ^{11}CO is rather straightforward, using one of the two following methods. The first method is a simple online gas-phase reduction of cyclotron produced $^{11}CO_2$ over zinc (400°C)[32] or molybdenum (850°C)[33]. The process is fast and provides considerably high radiochemical yields (RCY). Although, zinc reduction provides higher RCY (>99%), the molybdenum reduction method (RCY = 70 %) is generally preferred owing to its greater reproducibility over time.

The second method described in the literature is a one-pot two-step homogeneous conversion of $^{11}CO_2$ to ^{11}CO at room temperature. The first report using this approach was presented in 2004 by Roeda and co-workers[34], where lithium [^{11}C]formate was formed upon $^{11}CO_2$ capture in $LiEt_3BH$-THF solution. Lithium [^{11}C]formate was subsequently dehydrated to form [^{11}C]formyl chloride, which is instantly decomposed to ^{11}CO (scheme 1, entry A). More recently, a similar approach was reported using a silyllithium regent as $^{11}CO_2$ trapping regent to form [^{11}C]silacarboxylic acid after HCl treatment. Decarbonylation of [^{11}C]silacarboxylic acid is subsequently effectuated with a fluoride source (scheme 1, entry B)[25-36]. Both these methodologies provide near quantitative RCY and represent an interesting alternative to the standard gas-phase reduction methods.



Scheme 1. Methods for homogenous chemical synthesis of [^{11}C]carbon monoxide.

1.3.2 ^{11}C-Carbonylation methodologies

[^{11}C]Carbon monoxide has many attractive features as a synthon in PET radiochemistry, including its facile production and high versatility in transition-metal-mediated carbonylation reactions. Although ^{11}CO was one of the first ^{11}C-labeled radiotracers used in medicine, the widespread use of ^{11}CO as a labeling precursor has been restricted, mainly due to its poor reactivity. This poor reactivity is a consequence of low solubility of carbon monoxide in organic solvents, relatively high inertness, and the high dilution of ^{11}CO in inert carrier gas[11]. Over the years, several solutions have been introduced to overcome the above shortcomings, both from technical and chemical points of view. The first reports on ^{11}C-carbonylation in the literature

described a single pass procedure carried out in conventional glass reactors at near to atmospheric pressures[37-38]. The reactions were generally selective, but approximately 90% of the ^{11}CO remained unreacted. In a first attempt to enhance the transfer of ^{11}CO from the gas phase into the solution, a recirculation technique was applied, in which unreacted ^{11}CO was pumped back into the solution, resulting in an increased RCY in the range of 35 – 62%[39]. However, a breakthrough came in 1999, where Kihlberg and co-workers introduced a method where ^{11}CO was allowed to react in a small autoclave under high solvent pressure (>350 Bar)[40]. The high-pressure reactor methodology exhibited nearly quantitative ^{11}CO trapping efficiency (TE) and high radiochemical yield. Even though this method has exemplified the importance of ^{11}CO as a labeling precursor it has not gained broad adoption in the PET radiochemistry community. This can partly be attributed to the overall complexity of the autoclave system and the relatively high level of service needed to maintain the system operational. Moreover, the repeated use of an integrated stainless steel reactor may infer issues related to transition metal build up over time, which is problematic in reaction development and system validation.

Increasing the interfacial contact area between two phases is another way to enhance mass transfer. This has been achieved using packed tube reactors and microfluidic systems. The microtube reactor system relies on use of silica-supported Pd catalyst packed into a standard teflon tube[41]. The high surface area of the support material improves the gas-liquid interaction between the gaseous ^{11}CO (in N_2) and precursor solution. The system was used to label four simple amides in moderate to good RCY. In a more recent study, Miller *et al.* presented a MF approach to ^{11}C-labeled products[42]. The reaction was performed by generating an annular flow of ^{11}CO (in N_2) inside a liquid stream. Five different amides and one lactone were successfully obtained in good to excellent radiochemical yields. Later, a commercially available MF device was used to perform ^{11}C-carbonylation reactions, in which a liquid solution of Cu(Tp*)^{11}CO was applied as a CO donor[43].

An alternative strategy to improve ^{11}CO reactivity is through chemical complexation, where a trapping reagent that selectively binds ^{11}CO and subsequently being used as an ^{11}CO-donor for the Pd-mediated ^{11}C-carbonylation reaction. To date, two such methods have been presented. In 2004, Audrain *et al.* reported the use of a BH_3THF solution to trap ^{11}CO at room temperature in the form of an ^{11}CO-borane adduct[44]. ^{11}CO was subsequently released by heating. Two model compounds were synthesized in reasonable yields using this methodology. More recently, Kealey and co-workers developed a copper complex which coordinates and retains ^{11}CO efficiently (>95%)[45]. Upon treatment with PPh_3 ^{11}CO is released and thus accessible for synthesis of radiolabeled compounds. Although this approach is very appealing due to its apparent simplicity, current complexation methods suffer from some drawbacks, which may limit their wide application in radiosynthetic chemistry. Such drawbacks include tedious reagent preparation, limitations in the choice of solvent for the carbonylation reaction and an unavoidable contamination of the subsequent carbonylation reaction mixture with the complexation reagents.

Most recently, a novel protocol was recently reported by Eriksson and co-workers in which ^{11}C-carbonylation reactions were achieved without the need for high-pressure equipment or chemical complexation reagents[46-47]. The high solubility of xenon gas in organic solvents was exploited as an effective way of transferring ^{11}CO into a sealed reaction vessel (1 ml). Using

this setup, three model compounds were successfully radiolabeled in acceptable to good radiochemical yields.

1.4 Microfluidic-assisted radiochemistry

Microfluidic technology is a rapidly growing field in science[48-51]. MF reactors are by definition devices consisting of a network of micron-sized channels (typically 10-500 µl) embedded in solid material. Glass is often the choice for chemists owing to its mechanical and chemical stability to a wide range of chemicals and solvents. However, polymeric materials such as silicon give a greater flexibility in fabrication. In solution-based MF synthesis, the channel networks are connected to a series of reservoirs containing chemical reagents to form the complete device. The reagents is mixed in a specific sequence and allowed to react for a specified time. Liquid flow is generally generated using hydrodynamic pumping (e.g syringe or diaphragm pumps)[52]. A laminar flow is generally achieved in most microchannels, however, the flow profile is influenced by different factors, such as media viscosity, channel geometry and flow rate[52]. MF is particularly well suited to conducting multi-phase gas-liquid or gas-liquid-solid because of their capability to sustain high-pressure[53]. The pressurization allows reactions to be performed at elevated temperature and also permits the dissociation of reactant gas into the flow stream. There are two distinctly different flow patterns for multi-phase MF reactions[53]. The first condition is annular flow and is characterized as a rapid gas flow inside the centre of a liquid film coated on the internal surface of the MF reactor. The second is called segmented flow and relies on the continuous formation of bubbles within a moving liquid[54-55].

1.4.1 Microfluidics for PET radioligand synthesis

MF reactors for PET radiosynthesis have generated considerable interest primarily because miniaturisation reaction systems have the potential to address many of the current issues associated with PET radioligand production. The technical advantages of MF, in particular the high surface-to-volume ratio, can increase the speed of labeling reaction and improve the overall efficiency of the radiolabeling reaction process[6-7]. In addition, the potential for automation of MF processes and the obvious capability of easily shielding such small devices make MF, in many regards, the ideal platform for performing the rapid types of radiolabeling reactions required for PET. To date, a majority of the applications of MF for PET have been focusing on device design and proof-of-principle studies. The first account of reporting the application of MF to radiochemistry was published by Lu *et al.* in 2004[56]. A glass fabricated, T-shaped micro reactor was used to study the reaction of carboxylic acids with different ^{11}C and ^{18}F-alkylating agents. After this pioneering work, the progress in this research field has continued both using home-made devices and commercially available systems. The full on-chip radiosynthesis of the well established PET radioligand, [^{18}F]2-fluoro-2-deoxy-D-glucose ([^{18}F]FDG), has been widely adapted as a benchmark in multiple MF studies[57]. It is noteworthy that although the on-chip synthesis of [^{18}F]FDG was reported nearly a decade ago, no commercial apparatus is yet available that relies on this technology. Nevertheless, the benefits of this technology for PET radiochemistry have been demonstrated, and the advancement in this very hot area of research has been summarized in a series of review articles[6-7, 58-61].

2 AIMS OF THE THESIS

The overall aim of the present thesis was to develop or improve novel methodologies for labeling molecules with the short-lived positron emitting radionuclides ^{11}C and ^{18}F. The specific aims were the following:

- To develop a simple method where the ^{11}C-carbonylation reactions is performed at atmospheric pressure in disposable glass vessels.
- To develop alternative methodologies to produce ^{11}C-carbonyl labeled compounds using [^{11}C]methyl iodide as a radiolabeled precursor.
- To evaluate a commercial microfluidic platform in one- and two-step radiosynthesis procedure.
- To develop and evaluate a novel microfluidic platform for performing multiphase gas-liquid ^{11}C-labeling reactions.

3 MATERIALS AND METHODS

The present chapter briefly summarizes the methods used during this thesis work. For complete experimental description the reader is referred to the full papers and manuscript that follow.

3.1 Positron emitting isotope production

All irradiations were performed on a GEMS PETtrace cyclotron (GE, Uppsala, Sweden) using 16.4 MeV protons.
The $^{14}N(p, \alpha)^{11}C$ nuclear reaction was employed to produce carbon-11. A pressurized 78 ml aluminum gas target containing nitrogen gas of scientific grade purity (99.9999%) and small amounts either oxygen (0.5%) or hydrogen (10%) yielding $^{11}CO_2$ and $^{11}CH_4$, respectively. The gas mixtures were purified using gas purifier (All pure^{TM}, Alltech) to remove any traces of carbon before entering the target. The produced $^{11}CO_2$ and $^{11}CH_4$ were either directly transferred to the dedicated hot cell in a stream of the target gas or via intermediate purification on molecular sieves inside a chemical process cabinet (GE, Uppsala, Sweden).
Fluorine-18 labeled fluoride (^{18}F-) was obtained from the $^{18}O(p, \alpha)^{18}F$ nuclear reaction by irradiating a liquid target prefilled with ^{18}O-enriched water ($[^{18}O]H_2O$). The target content was subsequently transferred to the dedicated hot cell by helium pressure. All gases were purchased from AGA gas AB (Sundbyberg, Sweden).

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

^{11}CO was produced using the previously published gas-phase conversion method[32]. In short, in-target produced $^{11}CO_2$ was trapped on a molecular sieve column at room temperature. The accumulated $^{11}CO_2$ was released into a controlled steam of helium (10 ml/min) while heating the molecular sieve column at 360°C. $^{11}CO_2$ was reduced online to ^{11}CO using a pre-heated (850°C) quarts glass column charged with molybdenum powder (in papers I and II the purified $^{11}CO_2$ was pre-concentrated before the gas-phase reduction using a silica gel trap immersed in liquid nitrogen (-196°C)). Unreacted $^{11}CO_2$ was subsequently removed by a sodium hydroxide-coated silica trap (Ascarite), and the ^{11}CO was concentrated using a silica gel trap immersed in liquid nitrogen. After complete entrapment, the produced ^{11}CO was released and transferred to the reactor using a controlled steam of helium while heating the silica gel trap to RT. A representative synthesis scheme is shown in figure 4.

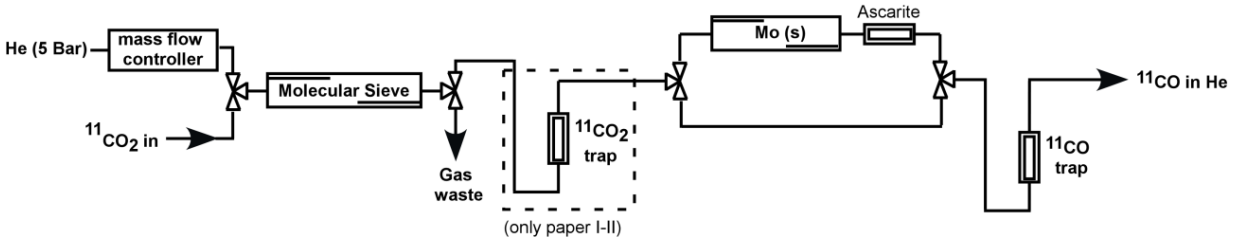


Figure 4. A representative flowchart for the preparation of [^{11}C]carbon monoxide.

3.3 Production of [^{11}C]methyl iodide

Starting from in-target produced $^{11}CH_4$, $^{11}CH_3I$ was produced using an automated radiochemical synthesis module (Scansys, Copenhagen, Denmark) according to previously published methods [62]. In short, $^{11}CH_4$ was released from the target (500 ml/min) and passed through a phosphorous pentoxide trap to remove traces of ammonia and water produced during the nuclear reaction. The $^{11}CH_4$ was subsequently trapped on HayeSep Q trap cooled to -180°C. The trap was purged with helium (200 ml/min) at a slightly higher temperature (-130°C) for one minute to remove target gas (10% H_2 in N_2). The accumulated $^{11}CH_4$ was then released into a recirculation system by gentle heating to -10°C. The recirculation consists of three ovens, a HaySep Q trap, a quartz tube containing iodine, a glass tube charged with ascarite and a micro diaphragm gas pump (NMP830KVDC, KNF Neuberger, Freiburg, Germany) to generate a stable flow (900 ml/min). The $^{11}CH_4$ thus first enters the quartz tube where it is mixed with a vapor of iodine crystals at 60°C and the radical formation of $^{11}CH_3I$ occurs at 720°C. After the reaction the iodine and HI are removed ascarite while the $^{11}CH_3I$ was collected in the HaySep Q trap at -10°C and the unreacted $^{11}CH_4$ recirculated for three minutes. The accumulated $^{11}CH_3I$ was released and transferred to the reaction vessel using a controlled stream of helium (70 ml/min) while heating the HaySep Q trap at 200°C.

3.4 [^{18}F]Fluoride ion handling and microfluidic radiofluorination

A commercially available microfluidic device (NanoTek, Advion, USA) was used as a synthesis platform for the preparation of fluorine-18 labeled tertiary amines in a two-step procedure (paper IV). The microfluidic system consists of a concentrator module for $^{18}F^-$ drying, a reactor module capable of performing 4 constituent reactions, a syringe pump module, and 3 loading loops for reagents. The automatic microfluidic apparatus was either setup for one- or two-step radiosynthesis. A complete schematic of the NanoTek platform is shown in figure 5.

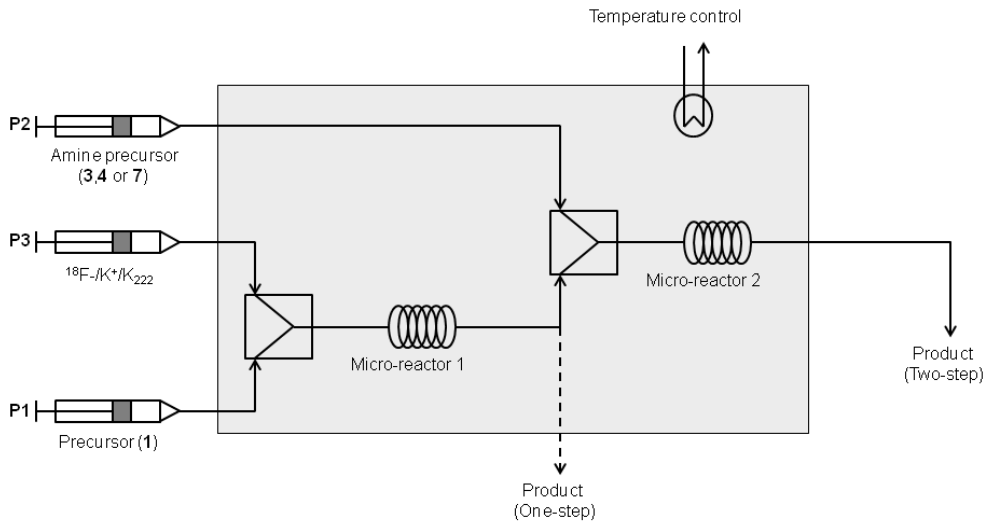


Figure 5. Schematic of the NanoTek platform used in this thesis

Dry $^{18}F^-/K^+$/Kryrofix-2.2.2 (K_{222}) was generated according to previously reported methods [63-64]. In short, in-target produced $^{18}F^-$ was first isolated onto an anion resin (MP-1) cartridge, and subsequently eluted with a solution of K_2CO_3, and K_{222} in water (18 MΩ) and acetonitrile (MeCN) into a 2 ml vial. The solvents were evaporated at 105°C under continuous nitrogen flow. Five portions (100 µl) of MeCN were added to the vessel over a 15 min drying time to form the activated $^{18}F^-/K^+/K_{222}$ complex.
In a typical microfluidic reaction, dry $^{18}F^-/K^+/K_{222}$ in anhydrous MeCN and the precursor solutions were subsequently loaded onto their respective storage loops prior to start of reaction, whereby the reagents were infused into a pre-heated (100 - 200°C) capillary-reactor (inner diameter (i.d.) = 100 µm) using a syringe pump. A back-pressure regulator was also applied to keep the pressure constant in the system. The synthesis process was controlled and monitored using a Labview-based software.

3.5 Gas-liquid segmented microfluidic system

The microfluidic system (figure 6) consists of a precision syringe pump, a µ-mass flow controller, a mixing-tee (i.d. = 150 µm) to permit gas-to-liquid contact, and a 5 m fused-silica capillary reactor (i.d. = 200 µm) located within a pre-heated oil bath, as well as a back-pressure regulator (100 psi). In a typical reaction, the produced ^{11}CO was trapped and concentrated on a silica column at -196°C, after which the accumulated ^{11}CO was transferred into the micro-reactor using a micro flow controller (helium). At the same time the premixed coupling reagents in anhydrous THF was infused into the micro-reactor using a syringe pump. The product was finally collected in a vial connected to the micro-reactor outlet with a leak-tight gas bag in series to receive volatile radioactive products (*e.g.* ^{11}CO). The synthesis process was controlled and monitored with in-house developed software.

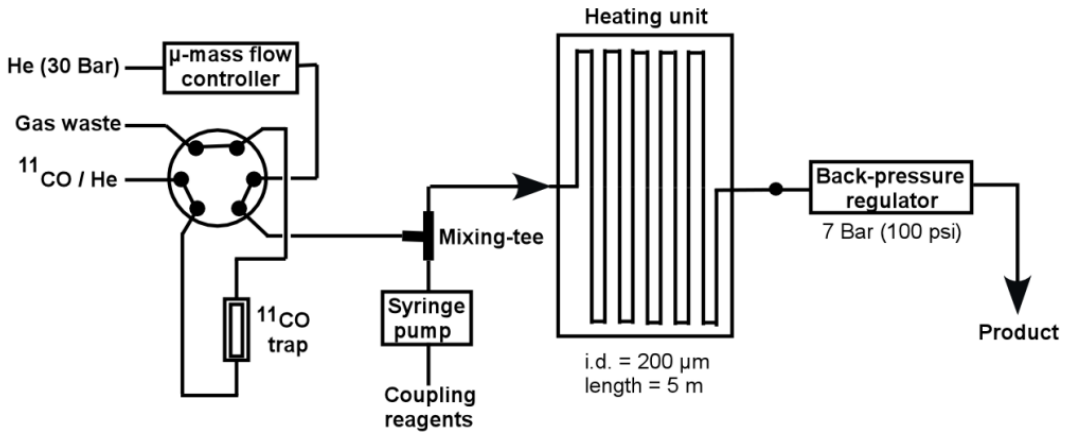


Figure 6. Schematic of the ^{11}CO microfluidic platform used in this thesis.

3.6 Microwave reactor

The microwave-assisted reactions conducted in this thesis were performed using a single-mode microwave cavity (Scancys, Copenhagen, Denmark) equipped with air pressure cooling and IR-sensor for temperature control. The custom made system utilizes a Samsung magnetron with an output effect of 380 W. Microwave reactions were performed for a pre-set period of time, temperature and power in a standard borosilicate glass vial (Chromacol 3.5-HRSV, Scantec Lab, Sweden) which was sealed using a dedicated cap equipped with a PTFE-coated septum (½" Microsep, GRACE). The system was controlled and monitored using Labview-based software.

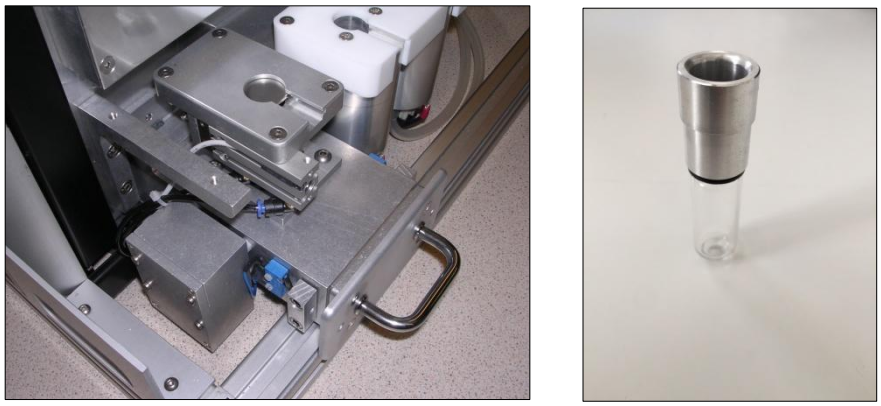


Figure 7. The single-mode microwave heater and a picture illustrating the reaction vessel used in this thesis

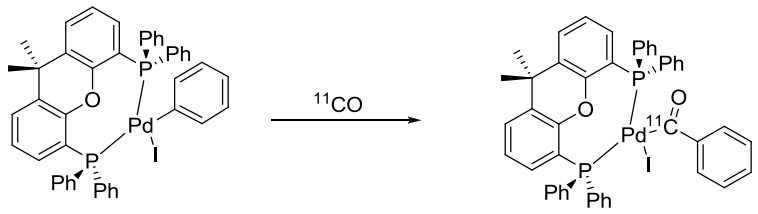
3.7 Product identification and yield calculation

Product identification was performed using analytical high performance liquid chromatography (HPLC) with co-injection of non-radioactive reference compound. The HPLC system was equipped with both radioactivity and UV detectors. The compounds are co-eluted and the retention time for the UV peak and the radioactive peak are compared for identification. The identity of compounds produced on a preparative scale in paper I ([^{11}C]**7**) and IV([^{19}F]**16**) was also identified using LC-MS/MS. The effectiveness of a reaction was determined using the parameters radiochemical purity (RCP) and analytical radiochemical yield (RCY). The RCP is defined as the fraction of the product in relation the other labeled entities and is determined using Radio-HPLC, while the RCY is the amount of product in relation to the starting radioactivity. However, if the radioactive precursor is volatile, e.g. in the case of [^{11}C]carbon monoxide, the parameter trapping efficiency (TE) must be taken into consideration. The TE is defined as the total amount of ^{11}CO that is converted to nonvolatile products at the end of reaction. The RCY was calculated using the following equation, RCY = RCP ∗ TE.

4 RESULTS AND COMMENTS

4.1 Pd-xantphos mediated ^{11}C-carbonylation (paper I)

[^{11}C]Carbon monoxide is a useful ^{11}C-reagent in PET radiochemistry, as it can be incorporated into a wide range of biological-active molecules through metal-mediated ^{11}C-carbonylation reactions. Despite the potential of ^{11}CO as labeling synthon for PET, its widespread use has been hindered by its poor solubility and high dilution in inert carrier gas. The aim with this study was to develop a palladium-ligand complex where ^{11}CO is trapped and incorporated as a part of the CO insertion procedure at close to atmospheric pressure, its simplicity would make it extremely useful in radiochemistry (scheme 2).



Scheme 2. The entrapment of ^{11}CO during the insertion/migration procedure exemplified with the bidentate phosphine ligand, xantphos.

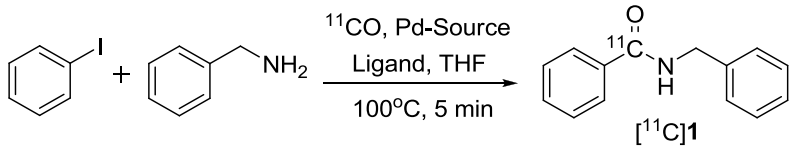
4.1.1 Otimization of reaction conditions

The focus was initially to discover a palladium complex that allows for an efficient trapping (TE) of ^{11}CO in solution. Arguably the most important aspect of tuning the reactivity of Pd catalyst is ligand selection. Inspired by the work of Buchwald[65] et al., a series of bidentate phosphine ligands with neutral bite angles from 78 to 110° was tested in the ^{11}C-aminocarbonylation of iodobenzene with benzylamine using $Pd(OAc)_2$ as catalyst (table 2). In addition, the commonly used ligands PPh_3 and Sphos were included for comparative purposes. In a typical experiment, ^{11}CO was first concentrated and subsequently transferred into a sealed glass vial (4 ml) pre-charged with coupling reagent using a controlled stream of He (20 ml/min). The reactions were standardized and performed at 100°C for 5 min.

The standard ligand for Pd-mediated ^{11}C-carbonylation reactions, PPh_3, showed poor TE (21%) and gave a low radiochemical yield (RCY) of the desired [^{11}C]benzamide (7%). Furthermore, a number of bidentate ligands gave low to moderate yields (Table 2, entries 1-6). The best results were obtained with xantphos, a bidentate ligand developed by van Leeuwen in the 1995[66], for which we obtained a quantitative TE (>99%), and [^{11}C]*N*-benzylbenzamide ([^{11}C]**1**) in a reproducible RCY of 54±1%. Remarkably, when changing the Pd-scource to $Pd_2(\pi$-cinnamyl$)Cl_2$, an almost quantitative conversion to the desired product was observed, with an

average RCY of 99±1% (table 2, entry 16). The flexible backbone structure of xantphos (bite angle, 97 – 133°) may partly account for this phenomenon[65-66].
A small solvent screening concluded the methodology also showed good compatibility with a range of commonly used organic solvents, such as DMF, DMSO, MeCN, Toluene and 1,4-Dioxane. RCYs above 78% were observed for all examined solvents. In addition, we also investigated the reaction at lower temperatures using THF as solvent. A 10% decrease in product formation was observed at room temperature compared to 100°C.

Table 2. Effect of the ligand and Pd-source on the ^{11}CO trapping efficiency and the radiochemical conversion.



Entry	Ligand	Bite angle[b]	Pd-Source	Trapped ^{11}CO[c] [%]	RCP[d] [%]	RCY[e] [%]
1	dppe	78	$Pd(OAc)_2$	31	67	21
2	S-BINAP	92	$Pd(OAc)_2$	18	17	3
3	dppp	95	$Pd(OAc)_2$	63	49	31
4	dppb	99	$Pd(OAc)_2$	40	24	10
5	dppf	106	$Pd(OAc)_2$	85	35	30
6	DPEphos	108	$Pd(OAc)_2$	55	34	19
7	Xantphos	110	$Pd(OAc)_2$	>99	54±1[f]	54
8	dcpp		$Pd(OAc)_2$	11	0	0
9	PPh_3		$Pd(OAc)_2$	21	35	7
10	Sphos		$Pd(OAc)_2$	9	42	4
11	t-Bu-Xantphos	140	$Pd(OAc)_2$	12	18	2
12	EA-Xantphos		$Pd(OAc)_2$	34	35	12
13	Xantphos	110	$PdCl_2$	82	96	79
14	Xantphos	110	$Pd_2(dba)_3$	>99	93	92
15	Xantphos	110	$Pd(PPh_3)_4$	96	85	82
16	Xantphos	110	$Pd_2[\pi\text{-cinnamyl}]Cl_2$	>99	99±1[f]	98

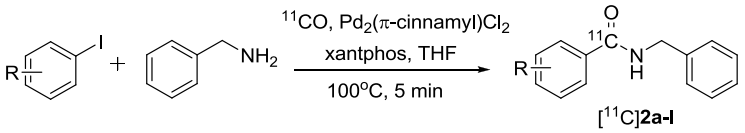
[a] Conditions: iodobenzene (20 µmol), benzylamine (100 µl), Ligand (14-48 µmol), Pd-source (14 µmol), THF (0.7 ml), 100°C, 5 min. [b] See ref 65. [c] Decay-corrected, the fraction of radioactivity left in the crude product after. purging with nitrogen. [d] Radiochemical purity determined by radio-analytical HPLC. [e] Radio-chemical yields based on the total radioactivity delivered to the reaction vial. [f] Average of 3 runs.

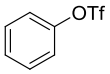
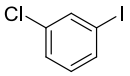
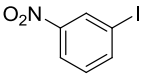
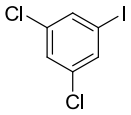
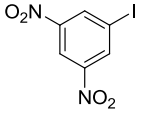
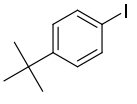
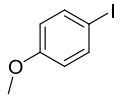
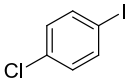
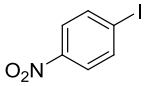
4.1.2 Scope of the methodology

The scope and limitations of the method was evaluated by including substrates with different reactivity and electron density. A series of functionalized aryl halides was successfully converted to their corresponding ^{11}C-labeled amides in a RCY range of 13-98% (table 3). In general, aryl iodides with electron-donating substituents gave excellent RCY (table 3, entries 9-10), whereas electron-withdrawing groups could be more or less well tolerated (table 3, entries

5-8 and 11-12). However, chlorobenzene (table 3, entry 3) was found to be ineffective as a substrate under these conditions. The radiochemical yield was strongly dependent on the nature of the substrate, and its tendency to engage in oxidative addition with the Pd^0-complex.

Table 3. Effect of nature of the substrate on the radiochemical conversion.



Entry	Substrate	Trapped ^{11}CO [%]	RCP[b] [%]	RCY [%]
1		>99	99±1	98
2		>99	97±1	96
3		98	<1	<1
4		>99	78±3	77
5		98	84±4	82
6		73	64±3	46
7		66	70±5	43
8		23	57±4	13
9		>99	97±2	96
10		>99	98±3	97
11		>99	91±5	91
12		66	72±3	48

[a] Conditions: Substrate (20 µmol), benzylamine (100 µl), Xantphos (14 µmol), Pd_2[π-cinnamyl]Cl_2 (14 µmol), THF (0.7 ml), 100°C, 5 min. [b] Average of 3 runs.

The conditions were also employed in the labeling of an ester, a carboxylic acid, an aldehyde and a ketone (figure 8). Finally, a candidate radioligand ([^{11}C]**7**) for the histamine type-3 receptor was synthesized using this novel methodology (scheme 3). In a preparative run, 4170 MBq of [^{11}C]**7** was obtained in an 88% (decay corrected) RCY from ^{11}CO. The radiochemical purity was 97% and the SRA was 121 GBq/µmol (3280 Ci/mmol).

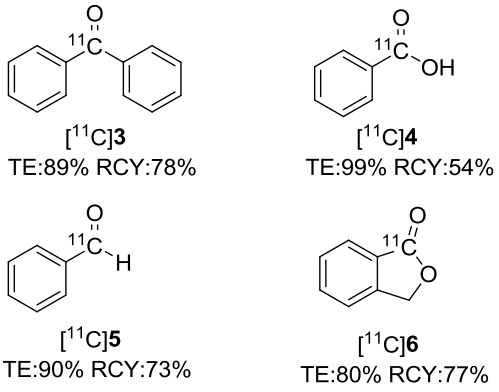
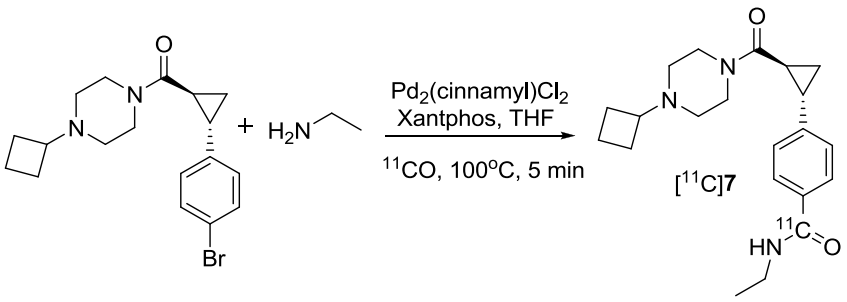


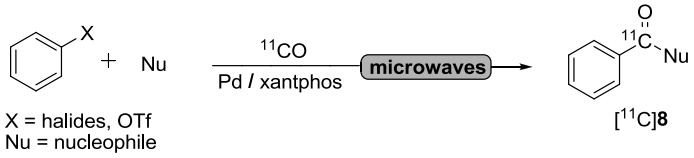
Figure 8. Structures, TEs and RCYs of the prepared [^{11}C]ketone, [^{11}C]carboxylic acid, [^{11}C]aldehyde and [^{11}C]ester.



Scheme 3. Radiosynthesis of a histamine type-3 receptor radioligand.

4.2 Microwave-assisted ^{11}C-carbonylation (paper II)

The application of microwaves as an efficient heating source for organic transformations was first recognized in mid-1980s[67]. Shortly thereafter microwaves were also applied in the synthesis of radiopharmaceuticals for PET[68]. In an effort to widen the scope of our newly developed methodology (paper I), we thus decided to examine microwave-assisted heating as a potential means to reduce reaction time and improve selectivity for the ^{11}C-carbonylation reaction (scheme 4).

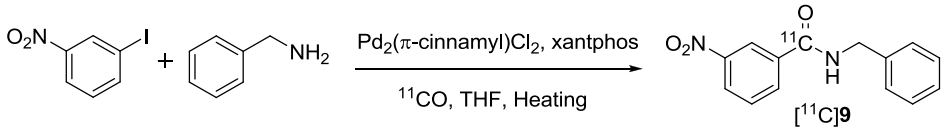


Scheme 4. The microwave-enhanced Pd-mediated ^{11}C-carbonylation route to form the [^{11}C]carbonyl containing product.

4.2.1 Otimization of reaction conditions

To establish preferred microwave conditions for the ^{11}C-aminocarbonylation reaction, [^{11}C]3-nitro-benzylbenzamide ([^{11}C]**9**) was selected as a target compound for the investigation using the established conditions from paper I. A series of experiments was conducted in which the TE and RCY of [^{11}C]**9** were investigated as a function of time and temperature (table 4).

Table 4. Microwave condition optimization in regards to temperature and time for the [^{11}C]**9** formation.

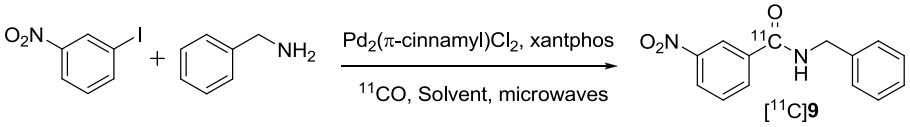


Entry	Heating technique	Temperature [%]	Active cooling	Time [min]	Trapped ^{11}CO [%]	RCY [%]
1	Thermal	100	-	5	73	46
2	Microwave	100	no	5	82	62
3	Microwave	100	yes	5	96	76
4	Microwave	120	yes	5	>99	81
5	Microwave	140	yes	5	98	69
6	Microwave	120	yes	3	85	61
7	Microwave	140	yes	3	98	67
8	Microwave	160	yes	2	97	56

[a] Conditions: Substrate (20 µmol), benzylamine (100 µl), Xantphos (14 µmol), Pd_2[π-cinnamyl]Cl_2 (14 µmol), THF (0.7 ml), Microwaves or theramal heating for 2-5 min.

The best results, quantitative TE and a RCY of 81% (table 4, entry 4), were obtained at 120°C for 5 min using active cooling (AC), which is a 78% increase in RCY compared with conventional heating. Next, a series of solvents with different microwave properties was investigated. Large variation in RCY (6-83%) was observed for the different solvents, interestingly, the highly protic solvent MeOH exhibits similar or better RCY compared with the investigated aprotic solvents under microwave conditions (table 5, entry 9). One would expect that the corresponding methyl [^{11}C]benzoate would be produced as the main product.

Table 5. Microwave condition optimization in regards to solvent for the [^{11}C]**9** formation.



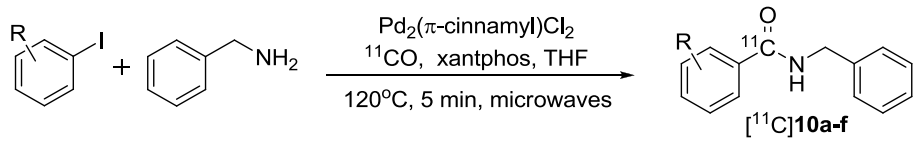
Entry	Solvent	Trapped ^{11}CO [%]	RCY [%]
1	THF	>99	81
2	MeCN	98	75
3	2-methyl-2-BuOH	98	36
4	1,4-Dioxane	70	33
5	DMSO	73	29
6	DMF	98	38
7	Water	>99	8
8	Toluene	98	53
9	MeOH	>99	83
10	EtOH	>99	6

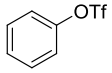
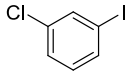
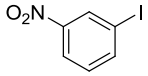
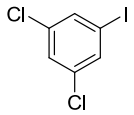
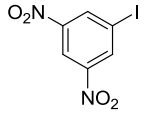
[a] Conditions: Substrate (20 µmol), benzylamine (100 µl), Xantphos (14 µmol), Pd_2[π-cinnamyl]Cl_2 (14 µmol), Solvent (0.7 ml), 120°C, 5 min, microwaves (AC).

4.2.2 Microwave vs thermal heating

A direct comparison between thermal and microwave heating was conducted using the ^{11}C-aminocarbonylation reaction using a set of electron-deficient aryl iodides, an aryl triflate and an aryl chloride with benzylamine as a model amine nucleophile (table 6). An improved selectivity and higher yield were observed using microwave heating in four of the six investigated reactions. The approach even allowed the use of an aryl chloride as substrate (table 6, entry 1), which is, to the best of our knowledge, the first example of a successful ^{11}C-carbonylation of an aryl chloride. These results provide evidence that microwave-assisted heating indeed can have a significant effect on the outcome for ^{11}C-carbonylation reactions.

Table 6. Difference in ^{11}CO trapping efficiency and radiochemical conversion was examined for 6 different aminocarbonylation reactions between microwave and thermal heating.



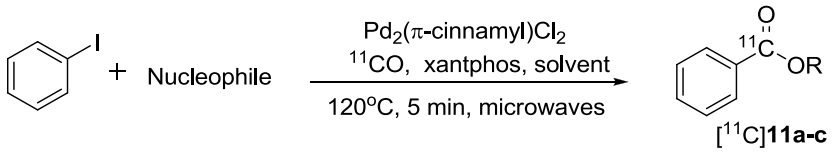
Entry	Substrate	Thermal Heating		Microwave Heating	
		TE [%]	RCC [%]	TE [%]	RCY [%]
1		>99	<1	88±3	58±1
2		>99	77	>99	91±1
3		98	82	>99	92±1
4		73	46	>99	81±3
5		61	42	94±3	62±3
6		23	13	44±1	13±2

[a] Conditions: Substrate (20 µmol), benzylamine (100 µl), Xantphos (14 µmol), Pd_2[π-cinnamyl]Cl_2 (14 µmol), THF (0.7 ml), 120°C, 5 min, microwaves (AC). [b] Average of 2 runs.

4.2.3 Hydroxy- and alkoxycarbonylation

Microwave-enhanced heating relies on the ability of a material (solvent or reagent) to absorb microwave energy and convert it into heat[69]. Polar protic solvents such as water and alcohols are high absorbing materials under microwave irradiation. To this end, two esters and a carboxylic acid were produced using the nucleophiles (water, methanol and ethanol) as co-solvent. When ^{11}C-hydroxycarbonylation was conducted in an equal mixture of aqueous sodium hydroxide and THF, the corresponding carboxylic acid was produced in 97% RCY (table 7, entry 1). Furthermore, two alkyl [^{11}C]benzoates were produced in excellent and reproducible RCYs using a THF and alcohol mixture (Table 7, entries 2-3). These encouraging results imply that microwave-enhanced ^{11}C-carbonylation may present a facile route to ^{11}C-carboxylic acid and ^{11}C-esters labeled in the carbonyl position.

Table 7. Results for the Pd-mediated ^{11}C-carbonylation using *O*-centered nucleophiles.



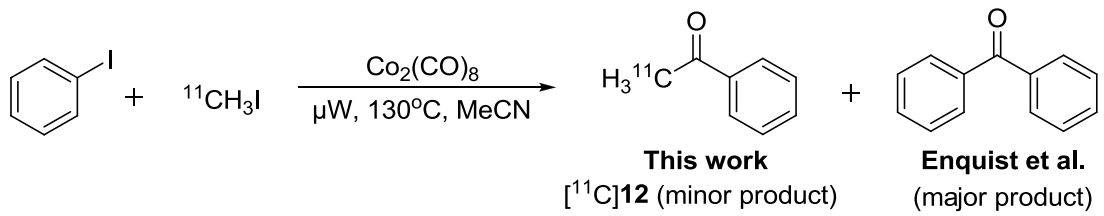
[^{11}C]**11a-c**

Entry	Nucleophile	Trapped ^{11}CO [%]	RCY [%]
1	H2O	>99	97±1
2	MeOH	>99	93±1
3	EtOH	>99	82±1
4	LiOEt	>99	73±6

[a] Conditions: Substrate (20 µmol),Nucleophile (400 µl), Xantphos (14 µmol), Pd_2[π-cinnamyl]Cl_2 (14 µmol), THF (400 µl), 120°C, 5 min, microwaves (AC). [b] 70 µmol NaOH as base. [c] Reaction was performed with 100 µmol of LiOEt in THF. [d] Average of 2 runs.

4.3 Cobalt carbonyl-mediated ^{11}C-acetylation (paper III)

Aryl methyl ketones are a valuable class of carbonyl derivates, which can serve as synthetic building block to bioactive molecules[70]. ^{11}C-labeled aryl methyl ketones have, so far, been prepared by palladium-mediated ^{11}C-carbonylation reactions using either [^{11}C]carbon monoxide[71-72] or [^{11}C]acetyl chloride[73-74]. A drawback with these methodologies is that they are based on cyclotron produced [^{11}C]carbon dioxide, which is often produced in rather low specific radioactivity due to isotopic dilution of $^{12}CO_2$.
Inspired by the work of Enquist et al., a new method was developed allowing for the direct ^{11}C-acetylation of aryl halides (scheme 5) using the standard radioactive precursor [^{11}C]methyl iodide. A compound that is prepared on a routinely basis with high SRA in our laboratory from in-target [^{11}C]methane[62].

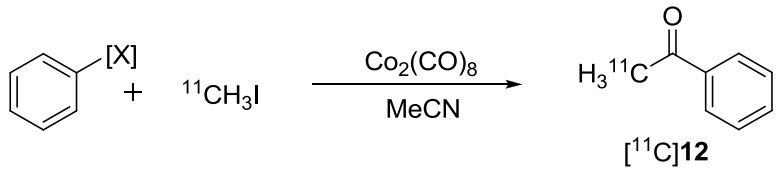


Scheme 5. Routes to ^{11}C-labelled aryl methyl ketone and the competing diaryl ketone formation reaction.

4.3.1 Otimization of reaction conditions

The [^{11}C]acetophenone ([^{11}C]**12**) formation was selected as a target reaction, a compound prepared via direct coupling of aryl halides and $^{11}CH_3I$ using a cobalt carbonyl-mediated protocol using microwave irradiation. A series of experiments was thus conducted in which the RCY of [^{11}C]**12** was investigated in relation to substrate type and reaction temperature (table 8). Initial experiments were conducted using iodobenzene as substrate (table 8, entries 1-3). Interestingly, a better RCY was obtained at a lower temperature (100°C). This may partly be explained by rapid substrate consumption, owing from the highly rapid competing diaryl formation reaction[75]. In support of this hypothesis, the less reactive aryl halides, bromobenzene and chlorobenzene, generated the desired product in good and reproducible yields, 62% and 63±2%, respectively (table 8, entries 4-5).

Table 8. Optimization of the direct ^{11}C-acetylation of aryl halides with $^{11}CH_3I$.



Entry[a]	Substrate (**1a-e**)	T (^{o}C)	RCY (%)[f]
1	Iodobenzene	130	47
2	Iodobenzene	100	54
3	Iodobenzene	100	40
4	Bromobenzene	130	62
5	Chlorobenzene	130	63±2[g]
6	Phenyl triflate	130	16
7	Fluorobenzene	130	0
8	Chlorobenzene	130	50
9	Chlorobenzene	130	54
10	Chlorobenzene	130	63
11	Chlorobenzene	140	61
12	Chlorobenzene	120	58

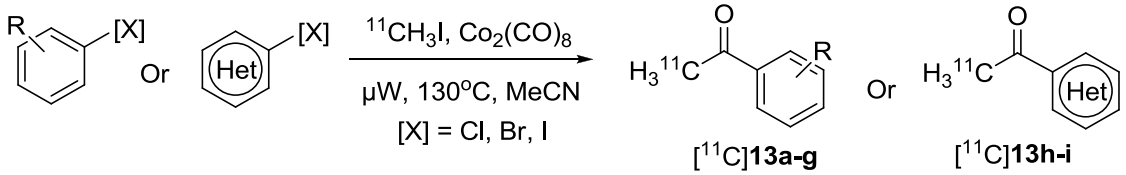
[a] Reaction conditions: Substrate (20 µmol), $Co_2(CO)_8$ (100 µmol), MeCN (1 ml), microwave heating. [b] Thermal heating. [c] Substrate (10 µmol). [d] $Co_2(CO)_8$ (50 µmol). [e] $Co_2(CO)_8$ (200 µmol). [f] Determined by radio-HPLC. [g] Average of two runs.

4.3.2 Scope of the methodology

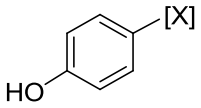
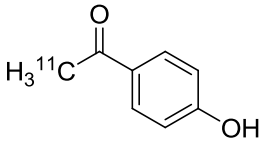
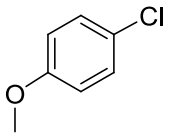
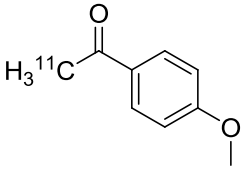
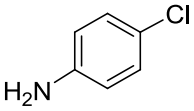
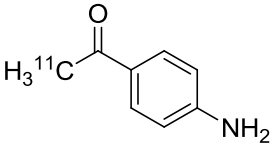
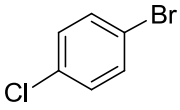
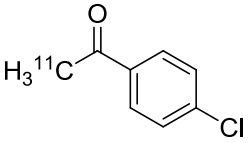
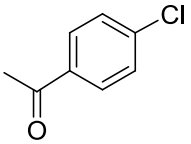
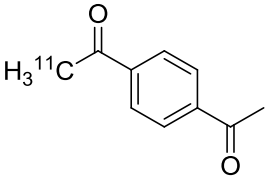
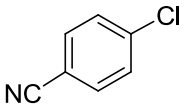
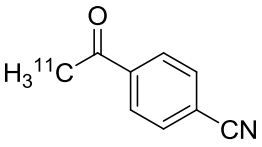
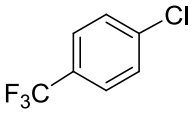
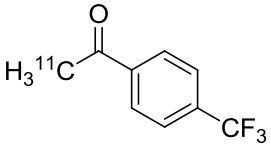
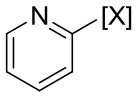
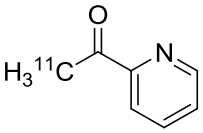
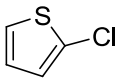
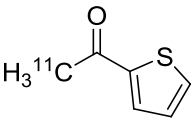
Nine functionalized aryl methyl ketones were successfully synthesized. The radiochemical yield varied from 22-59% (table 9). Aryl chlorides with electron-withdrawing groups in *para* position gave good RCYs (table 9, entries 4-7), whereas electron-donating groups were more or less tolerated using these conditions (table 9, entries 1-3). Finally, two heteroarenes, 2-bromopyridine and 2-chlorothiophene, were also converted to the corresponding aryl methyl ketone, RCYs was 22% and 31%, respectively.

In a final experiment, [^{11}C]**12** was prepared on a preparative scale. 1500 MBq (40.3 mCi) isolated product ([^{11}C]**12**) was obtained in a 43% decay-corrected RCY calculated from $^{11}CH_3I$ delivered to the reaction vessel. The RCP was greater than 99% and the specific radioactivity was 230 GBq µmol^{-1} (6212 Ci mmol^{-1}).

Table 9. Direct ^{11}C-acetylation of aryl halides with $^{11}CH_3I$.



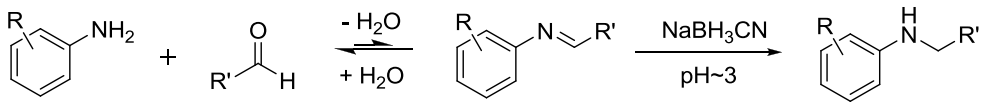
[^{11}C]**13a-g** [^{11}C]**13h-i**

Entry	Substrate	Trapped ^{11}CO [%]	RCP [%]
1			<1[a] 23±1[c]
2			47±2
3			46±1
4			41±1
5			49±2
6			59±1
7			58±1
8			16±2[a] 22±1[b] 17±1[c]
9			31±2

[a] Reaction conditions: aryl chloride substrate (20 μmol), $Co_2(CO)_8$ (100 μmol), MeCN (1 ml), microwave heating. [b] Aryl bromide substrate (20 μmol). [c] Aryl iodide substrate (20 μmol). [d] Determined by radio-HPLC. [e] Average of two runs.

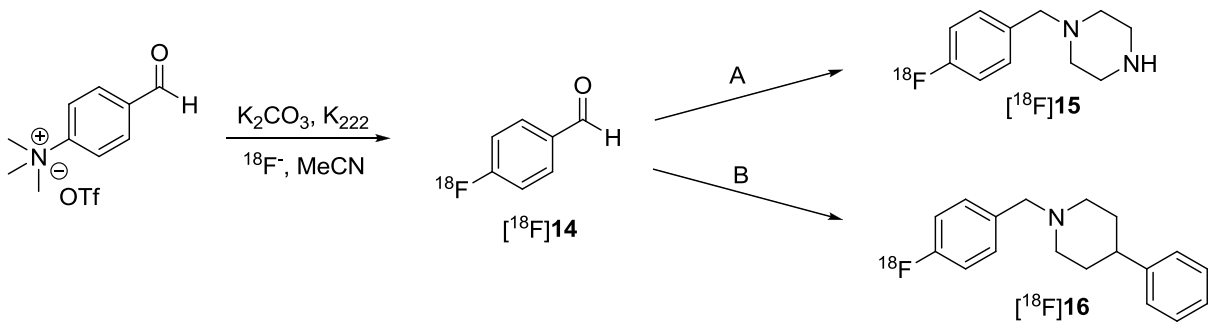
4.4 Two-Step radiofluorination using microfluidics (paper IV)

Amines are one of the most abundant functional groups in biologically important compounds and druglike molecules, thus, an easy access to radiolabeled amines would be highly desirable in PET. Reductive amination is one of the most powerful methods for the synthesis of substituted amines[76]. The direct reductive amination involves the initial formation of an intermediate hemiaminal which dehydrates to form an imine. Under acidic condition the imine is protonated to form an iminium ion and is subsequently reduced to produce the corresponding alkylated amine (scheme 6).



Scheme 6. Reductive amination mechanism

The application of microfluidic reactors to perform radiosynthesis is currently attracting a great deal of interest because of their potential to deliver many advantages over conventional labeling systems. The aim of this study was to apply a commercially available microfluidic radiochemistry system (NanoTek, Advion) as a synthetic platform for the two-step preparation of fluorine-18 labeled fluorobenzyl amine, via the initial aromatic nucleophilic ^{18}F-fluorination, followed by the reductive amination starting from a mono substituted benzaldehyde compound (scheme 7)[77].



Scheme 7. The two-step synthesis route for two fluorine-18 labelled fluorobenzyl amines. (A) piperazine, sodium cyanoborohydride, acidic acid in 1:1 mixture between MeCN/MeOH; (B) 4-phenyl-piperidine, sodium cyanoborohydride, acidic acid in 1:1 mixture between MeCN/MeOH.

4.4.1 ^{18}F-Fluorination reaction optimization

The aromatic aldehyde, [^{18}F]4-fluorobenzaldehyde ([^{18}F]**14**), was selected as the model substrate for the following reductive amination reaction. The conditions for the initial radiofluorination step were first examined. Thus, a series of experiments was conducted in which the RCY of [^{18}F]**14** was investigated as a function of precursor concentrations and temperature (figure 9). All reactions were conducted in a two meter micro-reactor (i.d. = 100 µm) using MeCN as solvent. The highest RCY based on [^{18}F]fluoride, 93%, was obtained at 200°C with a precursor concentration of 31.9 mM. However, since a lower concentration of precursor would be beneficial for the subsequent reductive amination, we chose to use 4.8 mM at a reaction temperature of 160°C for the continued study.

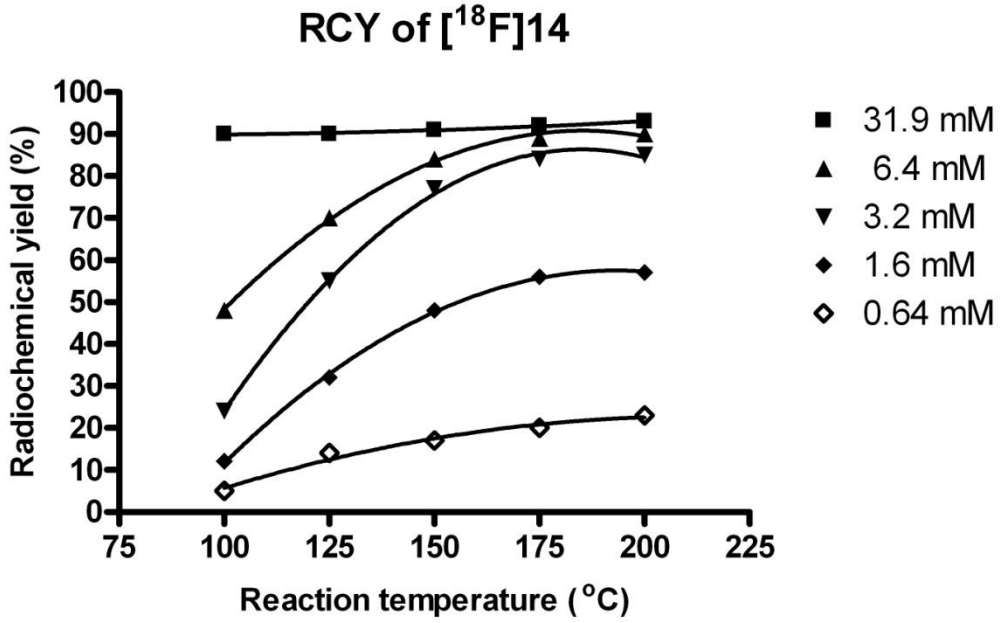


Figure 9. RCY based on [^{18}F]fluoride is presented as a function of temperature and precursor concentration for the radiofluorination step, establish by analytical radio-TLC.

4.4.2 Reductive amination reaction optimization

We proceeded to study [^{18}F]**14** reactivity in the subsequent reductive amination reaction. Using a similar approach as for the first step, the RCY of [^{18}F]**15** and [^{18}F]**16** was investigated as a function of amine concentrations and temperature (figure 10). The crude reaction mixture from the first step was mixed online with an amine solution (piperazine or 4-phenyl-piperidine, sodium cyanoborohydride, acetic acid and menthanol) at the inlet of the second micro-reactor (4 m long, i.d. = 100 µm). The overall labeling occurred as a single process without intermediate purification step. The highest RCY of [^{18}F]**15** was 70% and the corresponding value for [^{18}F]**16**

was 75%. The reproducibility in the preparation of [^{18}F]**15** and [^{18}F]**16** was also studied by three consecutive production cycles at an amine concentration of 70 mM.
Finally, [^{18}F]**16** was prepared on a preparative scale. 1050 MBq (28.4 mCi) isolated product was obtained in a 37.5% (decay corrected) RCY calculated from [^{18}F]fluoride. The radiochemical purity was greater than 99% and the specific radioactivity was 298 GBq/µmol (8052 Ci/mmol).

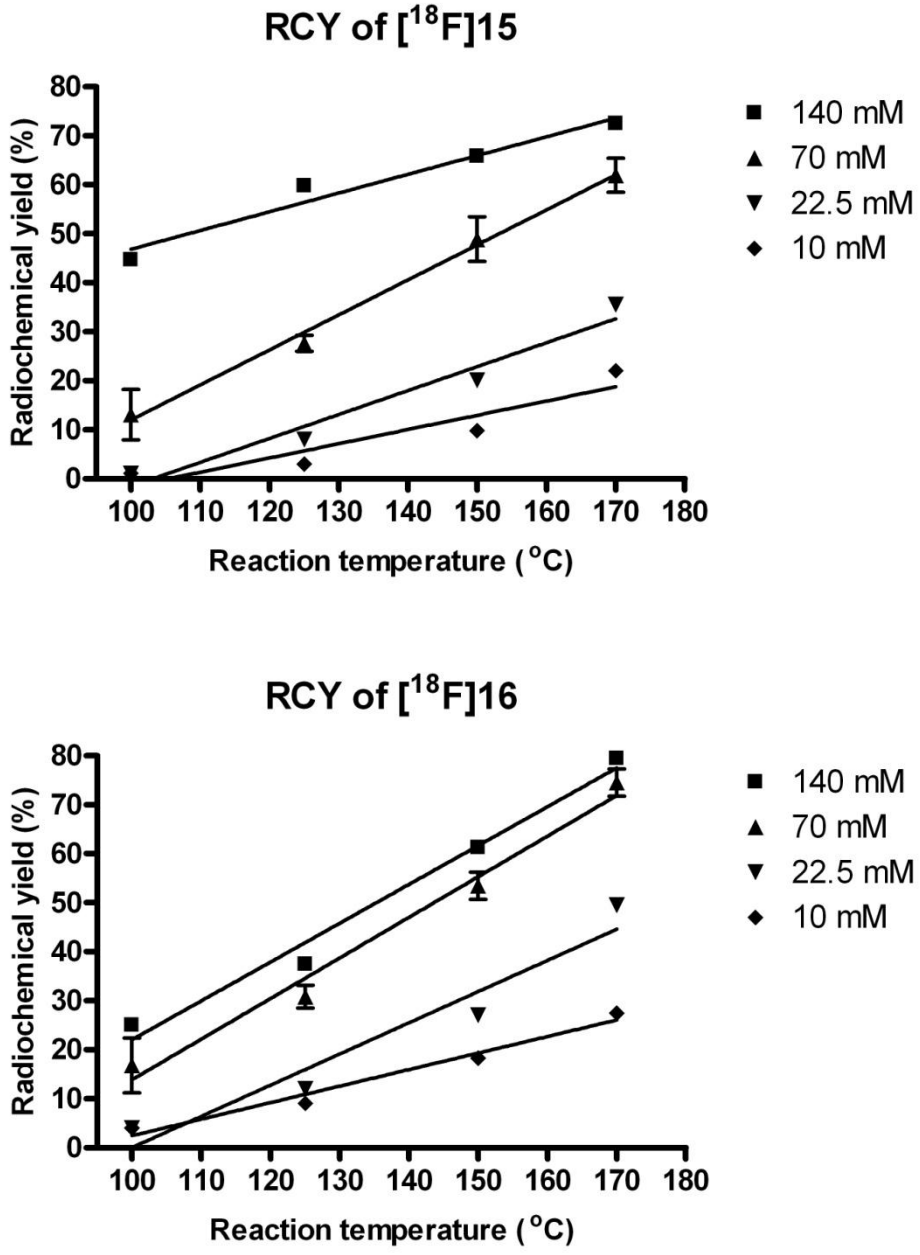
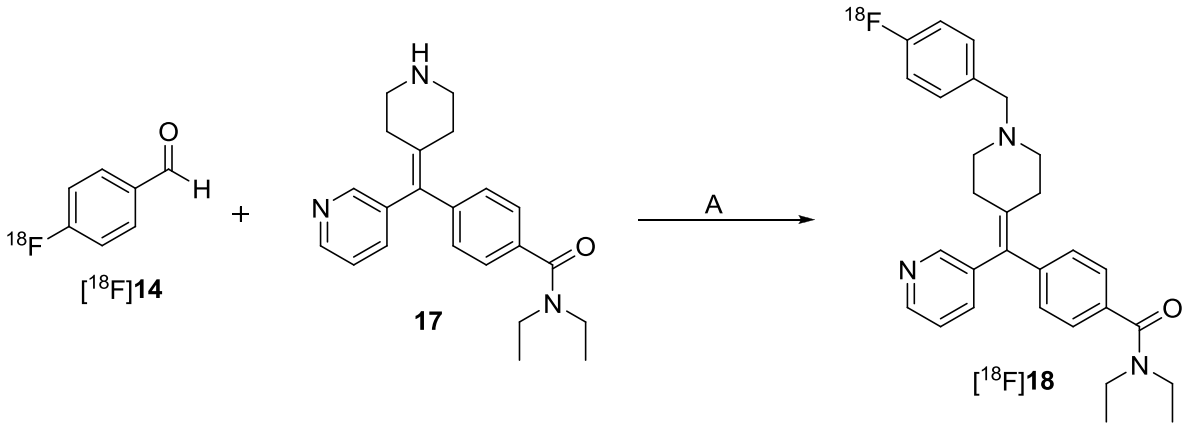


Figure 10. RCY of [^{18}F]**15** and [^{18}F]**16** based on [^{18}F]**14** are presented as a function of temperature and precursor concentration for the reductive amination step, established by analytical radio-HPLC. RCY values at 70 mM is a mean from three consecutive runs, n = 3.

4.4.3 Radiosynthesis of [^{18}F]AZ12439516

To further test the utility of this methodology, we also radiolabeled a delta opioid agonist, [^{18}F]**18** (scheme 8), using the optimal conditions established for compounds [^{18}F]**15** and [^{18}F]**16**. The analytical RCY of the crude product was 29%.



Scheme 8. The two-step synthesis of AZ12439516 ([^{18}F]**18**) via the reductive amination of [^{18}F]**14**. (A) amine precursor (**17**), $NaBH_3CN$, acidic acid in 1:1 mixture of between MeCN/MeOH.

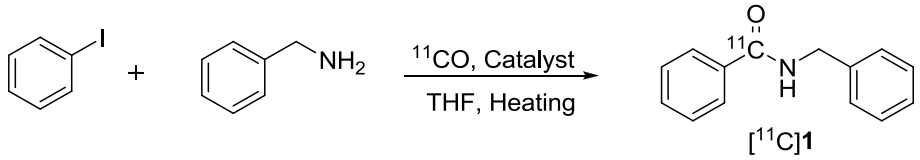
4.5 Gas-liquid segmented microfluidics (paper IV)

Microflow reactors are a powerful tool to accelerate gas-liquid segmented heterogeneous reaction, such as hydrogenations[78], fluorinations[79], and carbonylations[53, 80]. The significant gain in interfacial gas-liquid contact area can lead to enhanced reactivity. This is particular appealing for ^{11}C-carbonylation because of the low solubility and resultant poor reactivity of [^{11}C]carbon monoxide. The aim with this study was to develop a general platform where ^{11}C-carbonylation reactions are performed in a gas-liquid segmented microfluidic system.

4.5.1 Otimization of reaction conditions

Experiments were initially performed at different temperatures and gas flow rates using a micro mixing-tee (i.d. = 50 µm) in order to identify conditions with sufficient gas-to-liquid interfacial area. A series of experiments was performed using the synthesis of well-characterized [^{11}C]*N*-benzylbenzamide ([^{11}C]**1**) as a model reaction (Table 10). To our delight, at 100°C and gas flow rate of 100 µl/min using the standard Pd-ligand catalyst, Pd(PPh$_3$)$_4$, [^{11}C]**1** was obtained in a reproducible RCY of 95±2% (Table 10, entry 2). Attempts to perform the reactions at lower temperatures (80°C) or at higher gas flows (200 µl/min) resulted in a decreased TE and thereby a lower RCY. On the other hand, with Pd$_2$(cinnamyl)Cl$_2$-xantphos as catalyst a RCY of 98% was obtained already at room temperature (table 10, entry 6).

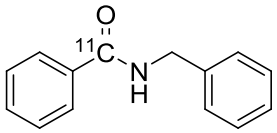
Table 10. Condition screening using [^{11}C]*N*-benzylbenzamide as a model reaction.



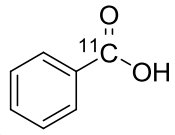
[^{11}C]**1**

Entry[a]	*T* (°C)	Gas flow (µl/min)	Liquid flow (µl/min)	Mixing tee (i.d. , µm)	Catalyst	Trapped ^{11}CO (%)[b]	RCP (%)[c]	RCY (%)[d]
1	100	200	20	50	Pd(PPh$_3$)$_4$	53	71	37
2	100	100	20	50	Pd(PPh$_3$)$_4$	>99	96	95±2[e]
3	80	100	20	50	Pd(PPh$_3$)$_4$	89	67	59
4	120	100	20	50	Pd(PPh$_3$)$_4$	>99	94	93
5	100	100	20	50	Pd$_2$(cinnamyl)Cl$_2$-xantphos	>99	99	99
6	r.t.	100	20	50	Pd$_2$(cinnamyl)Cl$_2$-xantphos	>99	98	98
7	100	100	20	150	Pd(PPh$_3$)$_4$	95	91	86
8	100	100	30	150	Pd(PPh$_3$)$_4$	>99	96	95±1[e]
9	100	200	30	150	Pd(PPh$_3$)$_4$	91	90	82

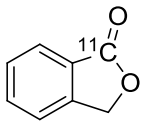
[a] Reaction conditions: iodobenzene (20 µmol), benzylamine (50 µl), Pd-source (14 µmol), Ligand (14 µmol), THF (1 ml), 100°C. [b] Decay corrected; the fraction of radioactivity left in the crude product after purging with nitrogen. [c] Radiochemical purity determined by radioanalytical HPLC. [d] Radiochemical conversion based on the total radioactivity delivered to the collection vial. [e] Average of two runs



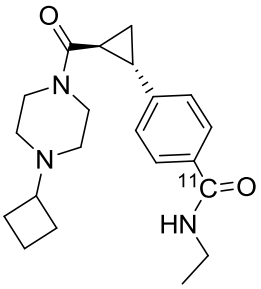
[^{11}C]**1**. **A**: 95±1,
B: 99±1, **C:** 99±1



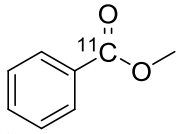
[^{11}C]**4**. **A**: 95±1 .



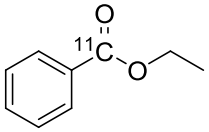
[^{11}C]**6**. **A**: 99±1



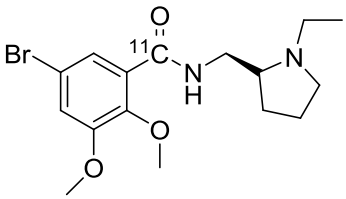
[^{11}C]**7**. **A**: n.d.
B: 79±1



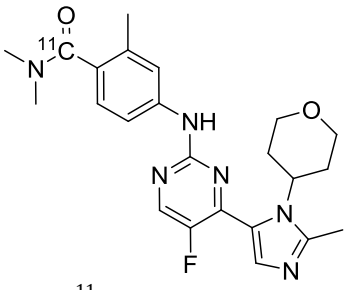
[^{11}C]**19**. **A**: 97±1



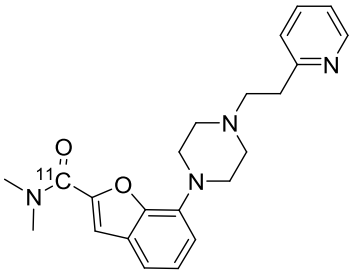
[^{11}C]**20**. **A**: 79±3



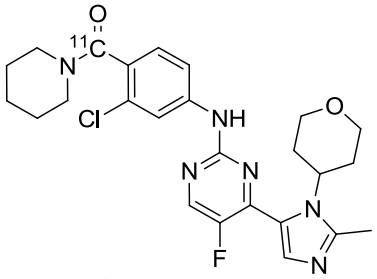
[^{11}C]**21**. **A**: 61±4



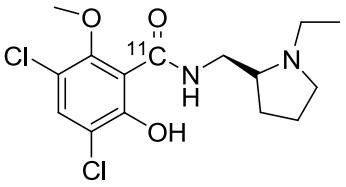
[^{11}C]**22**. **A**: 65±1



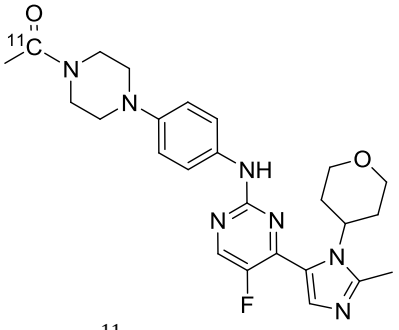
[^{11}C]**23**. **A**: 87±4



[^{11}C]**24**. **A**: 67±7



[^{11}C]**25**. **A**: n.d.
B: 41±1



[^{11}C]**26**. **A**: 38±8

Figure 11. Compounds produced using the gas-liquid segment microfluidic approach. Conditions **A**: aryl-halide, nucleophile, $Pd(PPh_3)_4$, THF, 100°C. Conditions **B**: aryl-halide, nucleophile, $Pd_2(cinnamyl)Cl_2$, xantphos, THF, 100°C. Conditions **C**: iodobenzene, benzylamine, [$PdCl_2$-(xantphos)], Toluene, 100°C. Average of two runs.

Unfortunately, at this stage we experienced issues related to reagent accumulations in the micro mixing-tee. As a precaution and potential means to improve the reliability of the present method, we decided to test a mixing-tee with a larger inner diameter (i.d. = 150 µm). Further alterations to the conditions were thus conducted (table 10, entries 7 - 9). Noteworthy, simply increasing the liquid flow rate from 20 to 30 µl/min resulted in a near quantitative conversion (95±1%) to the desired product (table 10, entry 8).

4.5.2 Scope of the methodology

With a suitable protocol for the microfluidic-assisted ^{11}C-carbonylation reactions in hand, the applicability of the newly developed method was exemplified in the synthesis of a set of ^{11}C-labeled test compounds and drug-like molecules (figure 11).
In general, good to excellent RCYs were observed when using $Pd(PPh_3)_4$ as catalyst, as exemplified by the D_2 receptor radioligand, [^{11}C]FLB 457 ([^{11}C]**21**)[81], which was produced in a RCC of 61±4%. However, for [^{11}C]**7** and [^{11}C]raclopride ([^{11}C]**25**)[82] $Pd(PPh_3)_4$ was found ineffective as a catalyst. For these molecules, the more active $Pd_2(cinnamyl)Cl_2$-xantphos catalyst provided RCYs of 79±1%and 41±1%, respectively.
Furthermore, when comparing the synthesis of [^{11}C]**1** in the current work with the previously reported gas-liquid annular microfluidic approach,[42] published by Miller *et al.*, we observed a 12% increase in RCY with our setup. We attribute this finding to the larger gas-liquid interface generated using the gas-liquid segmented approach. An enlarged photo of the fused-silica capillary is shown in figure 12, in which this flow profile is confirmed.
To further test the utility of this methodology, two compounds ([^{11}C]**7** and [^{11}C]**24)** were produced on a preparative scale (table 11). Both compounds were isolated using preparative HPLC and produced in sufficient radioactivity for PET studies. The radiochemical purity was more than 99%, while the specific radioactivity was acceptable (SRA, 40 and 54 GBq/µmol).

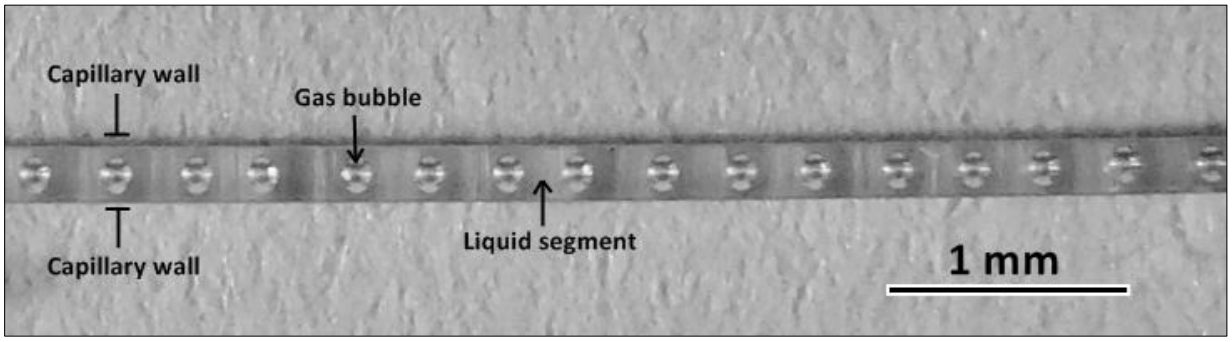
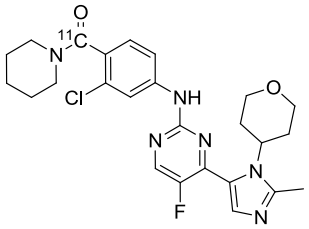
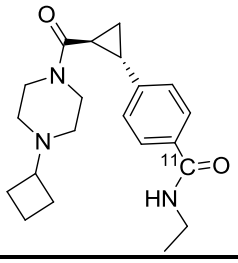


Figure 12. Photographic image of the flow profile inside the fused-silica capillary.

Table 11. Isolated yields of compounds synthesized using the gas-liquid segmented ^{11}C-carbonylation reaction.

Product	Isolated yield (MBq)	SRA (GBq/μmol)	RCP (%)	Synthesis time (min)
	1200	40	>99	49
	2800	54	>99	52

5 CONCLUDING REMARKS

The work presented in this thesis describes the methodological advancements in ^{11}C-carbonylation and microfluidic radiochemistry. The specific results and conclusions are summarized below.

- A Pd-mediated carbonylation protocol employing the bidentate phosphine ligand, xantphos, as the supporting ligand was developed for the preparation of a set of ^{11}C-carbonyl labeled functional groups. The reaction proceeds at ambient pressure with aryl halides and aryl triflates as substrates. The scope and limitation of the method was examined by ^{11}C-aminocarbonylation of a variety of functionalized aryl halides, indicating that the radiochemical yield is strongly dependent on the nature of the substrate. In addition, improved yields were observed for microwave-assisted ^{11}C-carbonylation reaction, even allowing the use of an aryl chloride as substrate. The method is general, simple, and has the potential to bring wide access to the attractive synthon [^{11}C]carbon monoxide.

- A fast and simplified route ^{11}C-labeled aryl methyl ketones was developed, employing [^{11}C]methyl iodide as the labeling agent. Cobalt carbonyl, $Co_2(CO)_8$, was used as a combined aryl halide activator and carbon monoxide source for the ^{11}C-acetylation reaction. Ten different functionalized (hetero)aryl methyl ketones were successfully radiolabeled in moderate to good radiochemical yields using the novel methodology.

- A commercially avalible microfluidic device was evaluated using the two-step preparation of ^{18}F-fluorobenzyl amines via the reductive amination reaction. The microfluidic apparatus allowed for a rapid parameter optimization and were scalable to produce adequate radioactivities for PET applications.

- A novel gas-liquid segmented microfluidic platform was developed for the preparation of drug-like molecules ^{11}C-labeled in the carbonyl position. The technology was used in the synthesis of twelve different ^{11}C-labeled compounds, including the well established D_2 receptor radioligands [^{11}C]raclopride and [^{11}C]FLB 457. This represents the first application of gas-liquid segmented microfluidics in the field of radiochemistry.

6 FUTURE PERSPECTIVES

- Considerable advances have been made in recent years within the field of ^{11}C-carbonylation radiochemistry, partly presented in this thesis and by other research groups. There are now a number of technically simplified low-pressure protocols that can be used for rapid and high yield ^{11}CO labeling. However, the need for tailor made and GMP compliant, commercially available synthesis modules is the main limitation, in order to streamline these existing methodologies and apply them to PET radioligand development in a clinical setting.

- The main efforts in microfluidic development for radiolabeling, to date, has been focused on proof-of-principles and illastrating the advantages associated with the technology. So also the results presented in this thesis. In general, microfluidic techniques have demonstrated tremendous potential for allowing rapid and cost-effective reaction optimization of new radioligands. However, in my view, the future of microfluidic-assisted chemistry for PET, is in the lab-on-a-chip technology using disposable kits, which would translate into cost advantage during fabrication, better reproducibility and simplify the time consuming validation procedure. However, significant further developments are needed if the technology is to reach its full potential.

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8 REFERENCES

1. Phelps M. E. PET: a biological imaging technique. *Neurochem Res* **1991**, 16, 929-940.
2. Halldin C., Gulyas B., Langer O. and Farde L. Brain radioligands – state of the art and new trends. *J Nucl Med* **2001**, 45, 139-152.
3. Ametamey S. M., Honer H. and Schubiger P. A. Molecular imaging with PET. *Chem Rev* **2008**, 108, 1501-1516.
4. Miller P. W., Long N. J., Vilar R. And Gee A. D. Synthesis of ^{11}C, ^{18}F, ^{15}O, and ^{13}N radiolabels for positron emission tomography. *Angew Chem Int Ed* **2008**, 47, 8998-9033.
5. Elander N., Jones J. R., Lu S. and Stone-Elander S. Microwave-enhanced radiochemistry. *Chem Soc Rev* **2000**, 29, 239-249.
6. Miller P. W. Radiolabelling with short-lived PET (positron emission tomography) isotopes using microfluidic reactors. *J Chem Technol Biotechnol* **2009**, 84, 309-315.
7. Pascali G., Watts P. and Salvadori P. A. Microfluidics in radiopharmaceutical chemistry. *Nucl Med Biol* **2013**, 40, 776-787.
8. Antoni G. Development of carbon-11 labelled PET tracers – radiochemical and technological challenges in a historic perspective. *J Labelled Compd Radiopharm* **2015**, 58, 65-72.
9. Scott P. Methods for the incorporation of carbon-11 to generate radiopharmaceuticals for PET imaging. *Angew Chem Int Ed* **2009**, 48, 6001-6004.
10. Pretze M., Große-Gehling P. and Mamat C. Cross-coupling as a versatile tool for the preparation of PET radiotracers. *Molecules* **2011**, 16, 1129-1165.
11. Långström B., Itsenko O. and Rahman O. [^{11}C]carbon monoxide, a versitile and useful precursor in labelling chemistry for PET-ligand development. *J Labelled Compd Radiopharm* **2007**, 50, 794-810.
12. 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.
13. 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.
14. Roeda D. and Dollé F. [^{11}C]Phosgene: A versatile reagent for radioactive carbonyl insertion into medicinal radiotracers for positron emission tomography. *Current topics in Med Chem* **2010**, 10, 1680-1700.
15. Halldin C. Gulyas B. and Farde L. Studies with carbon-11 radioligands in neuropsychopharmacological drug development. *Current Pharmaceutical design* **2001**, 7, 1907-1929.
16. Jewett D. M. A simple synthesis of [^{11}C]methyl triflate. *Int Appl Radiat Isot* **1992**, 43, 1383-1385.
17. 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.
18. Långström B. and Lundqvist H. The preperation of ^{11}C-methyl iodide and its use in the synthesis of ^{11}C-methyl-L-methionine. *Int Appl Radiat Isot* **1976**, 27, 357-363.

19. Larsen P., Ulin J., Dahlström K. and Jensen M. Synthesis of [^{11}C]iodomethane by iodination of [^{11}C]methane. *Appl Radiat Isot* **1997**, 48, 153-157.
20. Bergman J. and Solin O. Fluorine-18-labeled fluorine gas for the synthesis of tracer molecules. *Nucl Med Biol* **1997**, 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 [18F]selectfluor bis (triflate). *Angew Chem Int Ed* **2010**, 49, 6821-6824.
22. Cai L., Lu S. and Pike V. W. Chemistry with [^{18}F]Fluoride ion. *Eur. J. Org. Chem.*, **2008**, 2853-2873.
23. Littich R. and Scott P. Novel strategies for fluorine-18 radiochemistry. *Angew Chem Int Ed* **2012**, 51, 1106-1109.
24. Rotstein B. H., Stephenson N. A., Vasdev N. And Liang S. H. Spirocyclic hypervalent iodine(III)-medieted radiofluorination of non-activated and hindered aromatics. *Nature Communications* **2014**, 4, 4365-4372.
25. Tredwall M., Preshlock S. M., Taylor N. J., Gruber S., Huiban., Passchier J., Mercier J., Génicot C. and Gouverneur V. *Angew Chem Int Ed* **2014**, 126, 7885-7889.
26. Preshlock S., Tredwall M. and Gouverneur V. ^{18}F-labeling of arenes and heteroarenes for applications in positron emission tomography. *Chem Rew* **2016**, 116, 719-766.
27. Brennführer A., Neumann H. and Beller M. Palladium-catalyzed carbonylation reactions of aryl halides and related compounds. *Angew Chem Int Ed* **2009**, 48, 4114-4133.
28. Schoenberg A., Bartoletti I. and Heck R. F. Palladium-catalyzed carboalkoxylation of aryl, benzyl and vinylic halides. *J. Org. Chem.*, **1974**, 39, 3318-3326.
29. Schoenberg A. and Heck R. F. Palladium-catalyzed amidation of aryl, heterocyclic and vinylic halides. *J. Org. Chem.*, **1974**, 39, 3327-3331.
30. Colquhoun H. M., Thompson D. J. and Twigg M. V. Carbonylation, direct synthesis of carbonyl compounds. *Plenum*, **1991**, New York.
31. van Leeuwen P., Kamer P., Reek J. and Dierkes P. Ligand bite angle effects in metal-catalyzed c-c bond formation. *Chem Rew* **2000**, 100, 2741-2769.
32. Dahl K., Itsenko O., Rahman O., Ulin J., Sjöberg C., Sandblom P., Larsson L., Schou M. and Halldin C. An evaluation of a high-pressure ^{11}CO carbonylation apparatus. *J Labelled Compd Radiopharm* **2015**, 58, 220-225.
33. Zeisler S. K., Nader M. and Oberdorfer F. Conversion of no-carrier-added [^{11}C]carbon dioxide to [^{11}C]carbon monoxide on molybdenum for the synthesis of ^{11}C-labelled aromatic ketones. *Appl Radiat Isot* **1997**, 48, 1091-1095.
34. Roeda D., Crouzel C. and 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. *Radiochim Acta* **2004**, 92, 329-331.
35. Taddei C., Bongarzone S., Dheere A. and Gee A. [^{11}C]CO2 to [^{11}C]CO conversion mediated by [^{11}C]silanes: a novel route for [^{11}C]carbonylation reactions. *Chem Comm* **2015**, 51, 11795-11797.
36. Nordeman P., Friis S. D., Andersen T. L., Audrain H., Larhed M., Skrydstrup T. And Antoni G. Rapid and efficient conversion of $^{11}CO_2$ to ^{11}CO through silacarboxylic acids: application in Pd-mediated carbonylations. *Chem Eur J* **2015**, 21, 17601-17604.

37. Andersson Y. and Långström B. Synthesis of ^{11}C-labelled ketones via carbonylative couplings reaction using [^{11}C]carbon monoxide. *J Chem Soc Perkin Trans 1* **1995**, 287-289.
38. Al-Qahtani M. and Pike V. W. Palladium(II)-mediated ^{11}C-carbonylative coupling of diaryliodonium salts with organostannanes – a new, mild and rapid synthesis of aryl [^{11}C]ketones. *J Chem Soc Perkin Trans 1* **2000**, 1033-1036.
39. Lidström P., Kihlberg T. and Långström B. [^{11}C]carbon monoxide in the palladium-mediated synthesis of ^{11}C-labelled ketones. *J Chem Soc Perkin Trans 1* **1997**, 2701-2706.
40. Kihlberg T. and Långström B. Biologically active amides using palladium-mediated reactions with aryl halide and [^{11}C]carbon monoxide. *J. Org. Chem.*, **1999**, 64, 9201-9205.
41. Miller P. W., Long N., de Mello A., Vilar R., Audrain H., Bender D., Passchier J. and Gee A. Rapid multiphase carbonylation reactions using a microtube reactor: applications in positron emission tomography ^{11}C-radiolabeling. *Angew Chem Int Ed* **2007**, 46, 2875-2878.
42. Miller P. W., Audrain H., Bender D., de Mello A., Gee A. D., Long N. and Vilar R. Rapid carbon-11 radiolabelling for PET using microfluidics. *Chem Eur J* **2011**, 17, 460-463.
43. Kealey S., Plisson C., Collier T. L., Long N., Husbands S. M., Martarello L. and Gee A. D. Microfluidic reactions using [^{11}C]carbon monoxide solutions for the synthesis of a positron emission tomography radiotracer. *Org Biomol Chem* **2011**, 9, 3313-3319.
44. Audrain H., Martarello L., Gee A. and Bender D. Utilization of [^{11}C]-labelled boron carbonyl complexes in palladium carbonylation reaction. *Chem Comm* **2004**, 558-559.
45. Kealey S., Miller P. W., Long N., Plisson C., Martarello L. and Gee A. D. Copper(I) scorpionate complexes and their application in palladium-mediated [11C]carbonylation reactions. *Chem Comm* **2009**, 3696-3698.
46. Eriksson J., van den Hoek J. and 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, 223-228.
47. van der Wildt B., Wilhelmus M., Bijkerk J., Haveman L., Kooijman E., Schuit R., Bol J., Jongenelen C., Lammertsma A., Drukarch B. and Windhorst A. D. *Nucl Med Biol* **2016**, 43, 232-242.
48. Ahmed-Omer B., Brandt J. and Wirth T. Advanced organic synthesis using microreactor technology. *Org Biomol Chem* **2007**, 5, 733-740.
49. Watts P. and Haswell S. J. The application of micro reactors for organic synthesis. *Chem Soc Rev* **2005**, 34, 235-246
50. Abou-Hassan A., Sandre O. and Cabuil V. Microfluidics in inorganic chemistry. *Angew Chem Int Ed* **2010**, 49, 6268-6286.
51. Song H., Chen D. L. and Ismagilov R. F. Reactions in droplets in microfluidic channels. *Angew Chem Int Ed* **2006**, 45, 7336-7356.
52. Atencia J. and Beebe D. J. Controlled microfluidic interfaces. *Nature* **2005**, 437, 648-655.
53. Gunther A. and Jensen K. F. Multiphase microfluidics: from flow characteristics to chemical and material synthesis. *Lab Chip* **2006**, 6, 1487-1503.

54. Gong X., Miller P. W., Gee A. D., Long N., de Mello A. and Vilar R. Gas-liquid segmented microfluidics for screening Pd-catalyzed carbonylation reactions. *Chem Eur J* **2012**, 18, 2768-2772.
55. Günther A., Khan S. A., Thalmann M., Trachsel F. and Jensen K. F. Transport and reaction in microscale segmented gas-liquid flow. *Lab Chip* **2004**, 4, 278-286.
56. Lu S., Watts P., Chin F., Hong J., Musachio J. L., Briard E. and Pike V. W. Synthesis of 11C- and 18F-labeled carboxylic esters within a hydrodynamically-driven micro-reactor. *Lab Chip* **2004**, 4, 523-525.
57. Gillies J. M., Prenant C., Chimon G. N., Smethurst G. J., Dekker B. A. and Zweit J. Microfluidic technology for PET radiochemistry. *Appl Radiat Isot* **2006**, 64, 333-336.
58. Elizarov A. M. Microreactors for radiopharmaceutical synthesis. *Lab Chip* **2009**, 9, 1326-1333.
59. Rensch, C., Jackson A., Lindner S., Salvamoser R., Samper V., Riese S., Bartenstein P., Wängler C. and Wängler B. Microfluidics: A groundbreaking technology for PET tracer production?. *Molecules* **2013**, 18, 7930-7956.
60. Wang M., Lin W., Liu K., Masterman-Smith M. and Shen C. Microfluidics for positron emission tomography (PET) imaging probe development. *Mol. Imaging* **2010**, 9,175-191.
61. Keng P. Y., van Dam M. Digital microfluidics: a new paradigm for radiochemistry. *Mol. Imaging* **2015**, 14,579-594.
62. Andersson J., Truong P. and Halldin C. In-target produced [^{11}C]methane: Increased specific radioactivity. *Appl Radiat Isot* **2009**, 67, 106-110.
63. Liang S. H., Yokell D., Normandin M., Rice P., Jackson P., Shoup T., Brady T., El Fakhri G., Collier T. L. and Vasdev N. First human use of radiopharmaceutical prepared by continuous-flow microfluidic radiofluorination: proof of concept with the tau imaging agent [^{18}F]T807. *Mol. Imaging* **2014**, 13, 1-5.
64. Lu S., Giamis A. and Pike V. W. Synthesis of [^{18}F]fallypride in a micro-reactor. *Current Radiopharm* **2009**, 2, 49-53.
65. Martinelli J. R., Freckmann D. and Buchwald S. L. Convienient method for the preperation of weinreb amides via Pd-catalyzed aminocarbonylation of aryl bromides at atmospheric pressure. *Org Lett* **2006**, 8, 4843-4846.
66. Kamer P., van Leeuwen P. and Reek J. Wide bite angle diphosphines: xantphos ligands in transition metal complexes catalysis. *Acc Chem Res* **2001**, 34, 895-904.
67. Gedye R., Smith K., Westaway K., Ali H., Baldisera L., Laberge L. and Rousell J. The use of microwave ovens for rapid organic synthesis. *Tetrahedron Lett* 1986, 27, 279-282.
68. Hwang D. R., Moerlein S. M., Lang L., Welch M. J., Application of microwave technology to the synthesis of short-lived radiopharmaceuticals. *J Chem Soc Chem Comm* 1987, 1799-1801.
69. Kappe O. Controlled microwave heating in modern organic synthesis. *Angew Chem Int Ed* **2004**, 43, 6250-6284.
70. Ramgren S. and Garg N. Palladium-catalyzed acetylation of arenes. *Org Lett* **2014**, 16, 824-827.
71. Rahman O., Kihlberg T. and Långström B. Synthesis of ^{11}C/^{13}C-ketones by suzuki coupling. *Eur J Org Chem* **2004**, 474-478.

72. Karimi F., Barletta J. and Långström B. Palladium-mediated 11C-carbonylative cross-coupling of alkyl/aryl iodides with organostannanes: a afficient synthesis of unsymmetrical alkyl/aryl [^{11}C-carbonyl]ketones. *Eur J Org Chem.*, **2005**, 2374-2378.
73. Arai T., Zhang M., Ogawa M., Fukumura T., Kato K. and Suzuki K. Efficient and reproducible synthesis of [1-^{11}C]acetyl chloride using the loop method. *Appl Radiat Isot* **2009**, 67, 296-300.
74. Arai T., Kato K. and Zhang M. Synthesis of [carbonyl-^{11}C]acetophenone via the stille cross-couplings reaction of [1-^{11}C]acetyl chloride with tributylphenylstannane mediated by $Pd_2(dba)_3$/P($MeNCH_2CH_2$). *Tetrahedron Lett* **2009**, 50, 4788-4791.
75. Enqvist P., Nilsson P. and Larhed M. Ultra-fast chemistry: cobalt carbonyl-mediated synthesis of diaryl ketones under microwave irradiation. *Org Lett* **2003**, 5, 4875-4878.
76. Abdel-Magid A. F., Carson K. G., Harris B. D., Maryanoff C. A. and Shah R. D. Reductive amination of aldehydes and ketones with sodium triacetoxyborohydride: studies on direct and indirect reductive amination procedures. *J. Org. Chem.*, **1996**, 61, 3849-3862.
77. Wilson A. A., Dannals R. F., Ravert H. T. and Wagner H. N. Reductive amination of [^{18}F]fluorobenzaldehydes: Radiosyntheses of [2-^{18}F]- and [4-^{18}F]fluorodexetimides. . *J Labelled Compd Radiopharm* **1990**, 28, 1189-1199.
78. Kobayashi J., Mori Y., Okamoto K., Akiyama R., Ueno M., Kitamori T. and Kobayashi S. A microfluidic device for conducting gas-liquid-solid hydrogenation reactions. *Science* **2004**, 304,1305-1308.
79. Chambers R., Fox M., Holling D., Nakano T., Okazoe T. and Sandford G. Elemental fluorine part 16. Versatile thin-film gas-liquid multi-channel microreactors for effective scale-up. *Lab Chip* **2005**, 5, 191-198.
80. Fukuyama T., Totoki T. and Ryu I. Carbonylation in microflow: close encounters of CO and reactive species. *Green Chem* **2014**, 16, 2042-2050.
81. Halldin C., Farde L., Högberg H., Mohell N., Hall H., Suhara T., Karlsson P., Nakashima Y. and Swahn C. Carbon-11-FLB 457: a radioligand for extrastriatal D_2 dopamine receptors. *J Nucl Med* **1995**, 36, 1275-1281.
82. Ehrin E., Farde L., de Paulis T., Eriksson L., Greitz T., Johnström P., Nillson J., Sedvall G., Stone-Elander S. and Ögren S. Preperation of ^{11}C-labelled raclopride, a new potent dopamine receptor antagonist: preliminary PET studies of cerebral dopamine receptor in monkey. *Int Appl Radiat Isot* **1985**, 36, 269-273.