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LATE-STAGE ^{11}C-CARBONYLATION OF DRUG-LIKE MOLECULES FOR PET

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Late-stage ^{11}C-carbonylation of drug-like molecules for PET

THESIS FOR DOCTORAL DEGREE (Ph.D.)

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ABSTRACT

Positron emission tomography (PET) is a non-invasive *in vivo* imaging technique used for translational biomedical research and clinical diagnosis. A fundamental pre-condition for PET is the radiolabelled tracer molecule used in the emission measurement, and there is a pressing need to accelerate the development of novel PET tracers to meet an increasing demand from the healthcare system, academia and drug industry. A cornerstone in this effort is access to methodologies for late-stage isotopic labelling of small molecules with the positron-emitting radionuclide carbon-11 (^{11}C, half-life 20.4 min).

The carbonyl group is one of the most abundant motifs in biologically important molecules and therefore a good target for isotopic labelling. The aim of this thesis was to develop ^{11}C-carbonylation reactions that made use of [^{11}C]carbon dioxide ([^{11}C]CO_2), a synthon obtained directly from medical cyclotrons. The incorporation of this radiolabelled synthon, first into model compounds and later into biologically relevant molecules, was accomplished in automated radiochemistry systems and analysed using liquid chromatography.

Papers I and **II** describe the development of two different methods for the synthesis of ^{11}C-labelled cyclic ureas. In **Paper I**, the noteworthy concept of CO_2 fixation with strong, organic bases was applied. A set of [^{11}C]benzimidazolones was thus radiolabelled with high radiochemical yields under mild conditions. In **Paper II**, the task was approached by using a click chemistry-inspired Staudinger/aza-Wittig cascade reaction. A wide selection of five and six-membered cyclic ureas was radiolabelled using this simple one-pot reaction. As a proof of concept, the β-adrenergic radioligand, (*S*)-[^{11}C]CGP12177, was eventually produced using both methods.

Paper III showcased a novel ^{12}C/^{11}C isotopic exchange reaction for labelling of phenylacetic acids. The method is based on heat-induced decarboxylation of the substrate molecule, followed by introduction of isotope-labelled [^{11}C]CO_2, without need for any additional reagents. Three small molecules, including two anti-inflammatory drugs, were labelled to prove suitability of the method for a low-concentration carbon-11 source.

In **Paper IV**, the CO_2 fixation concept was applied to the preparation of ^{11}C-labelled oxazolidinones and other cyclic urethanes, starting from corresponding anilines, benzylamines and dibromoalkanes. Five and six-membered cyclic urethanes, as well as both phenylic and benzylic compounds, were labelled with moderate to excellent radiochemical yields.

In conclusion, a set of new methodologies was developed for the ^{11}C-labelling of carbonyl groups. Each of the novel methodologies utilised [^{11}C]CO_2 obtained directly from a medical cyclotron without further chemical manipulation, and were successfully used in the preparation of cyclic ureas, benzoxazolone and benzothiazolone compounds, phenylacetic acids, *N*-aryloxazolidinones, *N*-aryloxazinanones and *N*-benzyloxazinanones. Given the useful radiochemical yields and operational simplicity of these methods, there is a reason to be optimistic about their adoption in future PET tracer development.

LIST OF SCIENTIFIC PAPERS

I. **Horkka, K.**; Dahl, K.; Bergare, J.; Elmore, C. S.; Halldin, C.; Schou, M. Rapid and efficient synthesis of ^{11}C-labeled benzimidazolones using [^{11}C]carbon dioxide. *ChemistrySelect*, **2019**, *4*, 1846-1849.

II. Del Vecchio, A.; Caillé, F.; Chevalier, A.; Loreau, O.; **Horkka**, **K.**; Halldin, C.; Schou, M.; Camus, N.; Kessler, P.; Kuhnast, B.; Taran, F.; Audisio, D. Late-stage isotopic carbon labeling of pharmaceutically relevant cyclic ureas directly from CO_2. *Angewandte Chemie*, **2018**, *130*, 9892-9896; *Angewandte Chemie International Edition*, **2018**, *57*, 9744-9748.

III. Destro, G.; **Horkka, K.**; Loreau, O.; Buisson, D.-A.; Kingston, L.; Del Vecchio, A.; Schou, M.; Elmore, C. S.; Taran, F.; Cantat, T.; Audisio, D. Transition-metal-free carbon isotope exchange of phenyl acetic acids. *Angewandte Chemie; Angewandte Chemie International Edition*. doi:10.1002/anie.202002341.

IV. **Horkka, K.**; Mannisto, J. K.; Repo, T.; Schou, M. Late-stage radiolabeling of *N*-aryl oxazolidinones and other cyclic urethanes starting from [^{11}C]carbon dioxide. Manuscript.

CONTENTS

LIST OF ABBREVIATIONS

(+)-[^{11}C]PHNO	(+)-4-[^{11}C]Propyl-3,4,4a,5,6,10b-hexahydro-2*H*-naphtho[1,2-*b*][1,4]oxazin-9-ol
[^{11}C]CURB	[^{11}C-Carbonyl]-6-hydroxy-[1,1′-biphenyl]-3-yl cyclohexylcarbamate
[^{11}C]PIB	2-(4-*N*-[^{11}C]Methylaminophenyl)-6-hydroxybenzothiazole
[^{11}C]S14506	[*O*-Methyl-^{11}C]-1-[2-(4-fluorobenzoylamino)ethyl]-4-(7-methoxynaphthyl)piperazine
[^{11}C]SL25.1188	(*S*)-5-Methoxymethyl-3-[6-(4,4,4-trifluorobutoxy) benzo[*d*]isoxazol-3-yl]oxazolidin-2-[^{11}C]one
[^{18}F]FDG	2-Deoxy-2-[^{18}F]fluoro-D-glucose
2-*t*BuTMG	2-*Tert*-butyltetramethylguanidine
$5HT_{1A}$	Serotonin receptor subtype 1A
A_m	Molar activity
BBB	Blood-brain barrier
BEMP	(2-*Tert*-butylimino-2-diethylamino-1,3-dimethylperhydro-1,3,2-diazaphosphorine)
[Carbonyl-^{11}C]WAY-100635	[Carbonyl-^{11}C]*N*-(2-(1-(4-(2-methoxyphenyl)-piperazinyl)ethyl)-*N*-pyridinyl) cyclohexanecarboxamide
CEA	French Alternative Energies and Atomic Energy Commission
CT	Computer tomography
DBAD	Di-*tert*-butyl azodicarboxylate
DBU	(1,8-Diazabicyclo[5.4.0]undec-7-ene)
DMF	*N*,*N*-Dimethylformamide
DMSO	Dimethyl sulfoxide
EOB	End of bombardment
GMP	Good manufacturing practice
HPLC	High-performance liquid chromatography
IE	Isotopic enrichment
LiHMDS	Lithium hexamethyldisilazide
LG	Leaving group
MAO-A	Monoamine oxidase A
MAO-B	Monoamine oxidase B
MeCN	Acetonitrile
MRI	Magnetic resonance imaging
OTf	Triflate, trifluoromethanesulfonate
PET	Positron emission tomography

PTC	Phase-transfer catalyst
RCP	Radiochemical purity
RCY	Radiochemical yield
RT	Room temperature
RXR	Retinoid X receptor
(*S*)-[^{11}C]CGP12177	(*S*)-4-(3-(*Tert*-butylamino)-2-hydroxypropoxy)-2*H*-benzimidazol-2[^{11}C]-one)
SAW	Staudinger/aza-Wittig reaction
$t_{1/2}$	Half-life
TBD	Triazabicyclodecene
TE	Trapping efficiency
THF	Tetrahydrofuran
TMG	Tetramethylguanidine
UV	Ultraviolet

1 INTRODUCTION

1.1 Principles of PET

1.1.1 PET methodology

Positron emission tomography (PET) is a sensitive, non-invasive biomedical imaging technique that enables localisation of radioactive molecular tracers in living human subjects and experimental animals.[1, 2] The tracers used in PET are labelled with unstable radioisotopes that emit a positron (e^+), which travels a short distance in the surrounding tissue until it collides with an electron (e^-). The energy of the positron is proportional to the travel distance of the radionuclide. The resulting annihilation event produces two 511 keV γ-rays which are emitted in nearly opposite directions. When a pair of γ-rays is coincidently detected in the scintillation crystals of the ring-shaped detector system around the subject, a signal is registered. A PET measurement consists of numbers of such coincidence events that are finally transformed into spatial information of the tracer and thus, an image. The resolution for modern pre-clinical PET is 0.8 mm^3 and for clinical PET 1.5 mm^4 full width at half maximum. Although the resolution of a PET measurement is inferior to that observed with magnetic resonance imaging (MRI), it provides an important opportunity for functional real-time imaging of metabolic or biochemical events that are very difficult to observe by other means.[5] A dual PET/MR or PET/CT (computer tomography) measurement is normally conducted to combine the biochemical information from PET with anatomical images. Importantly, time-activity curves can be generated from the PET data, which allows for quantification of tracer distribution in tissue.

1.1.2 Positron-emitting radionuclides

The most commonly used radionuclides in PET are fluorine-18, carbon-11, oxygen-15 and nitrogen-13, which share a notably short half-life (2-110 minutes).[6] Though the nuclides with short half-life are inconvenient from a radiochemistry perspective, they reduce the radiation burden for the subject and allow for multiple PET measurements in the same subject during a short time interval. PET radionuclides are commonly produced in medical cyclotrons, which are circular particle accelerators that typically have several reaction vessels (targets) in which the nuclear reactions take place. These cyclotrons are typically placed in an infrastructure together with a radiochemistry laboratory, which in turn is located at proximity of a nuclear medicine department or imaging research centre. However, not all imaging infrastructures have access to locally-produced radionuclides or PET tracers, and thus rely on external production facilities for PET tracer supply. This setup is almost exclusively adopted for ^{18}F-labelled PET tracer synthesis due to its convenient 110 minutes half-life. Furthermore, not all radionuclides require a cyclotron for their production, but can be generated locally from an unstable "mother" radionuclide that is supplied in a radionuclide generator. Gallium-68 and copper-62 are examples of generator-produced metal nuclides that have gained popularity in the past years. Table 1 shows some PET nuclides and their physical properties.[7]

Table 1. Summary of decay characteristics and common production routes of some commonly used radionuclides in PET.

Radionuclide	Half-life	Energy of positrons (MeV)	Type of reaction	Portion of positron decay
^{15}O	2.0 min	1.72	$^{14}N(d,n)^{15}O$	100%
^{13}N	10.0 min	1.19	$^{16}O(p,\alpha)^{13}N$	100%
^{11}C	20.4 min	0.96	$^{14}N(p,\alpha)^{11}C$	100%
^{18}F	109.7 min	0.64	$^{18}O(p,n)^{18}F$	97%
^{68}Ga	68.1 min	1.90	$^{68}Ge \rightarrow {}^{68}Ga + \beta^-$	90%
^{64}Cu	12.7 h	0.65	$^{64}Ni(p,n)^{64}Cu$	18%
^{89}Zr	78.4 h	0.90	$^{89}Y(p,n)^{89}Zr$	22%

A PET tracer can be a radiolabelled drug molecule, biomolecule, receptor ligand or a simple gas. The biological half-lives vary significantly between these chemical species, it is thus important to select a radionuclide with a long enough half-life for its intended use. For example, small molecules such as pharmaceutical drugs, receptor ligands and peptides are ideally labelled using carbon-11, gallium-68 and fluorine-18. Larger biomolecules call for longer lived radionuclides, such as copper-64 and zirconium-89, whereas simple gasses like ammonia or water are labelled using nitrogen-13 and oxygen-15. To preserve the biological properties of a PET tracer, the radionuclide should ideally be introduced in a manner that does not alter its physicochemical or pharmacological properties. Nearly all biologically important molecules contain carbon and most of them contain nitrogen and/or oxygen atoms for such isotopic substitution. In contrast, fluorine, copper, gallium and zirconium atoms are scarcely observed in biomolecules and thus require structural modification to allow for their introduction. Of these, the introduction of fluorine-18 is the least perturbating whereas the labelling with metal radionuclides (^{68}Ga, ^{64}Cu and ^{89}Zr) requires a relatively large chelating moiety in the target molecule, which makes these more suitable for peptides, nucleotides, nanoparticles and antibodies.[8]

1.1.3 Applications of PET

Today, PET is an established tool in clinical oncology, where it is used for diagnosis, to distinguish malignant tumours from benign, to track metastases and to follow the efficacy of treatments. The most widely used PET radiotracer is 2-deoxy-2-[^{18}F]fluoro-D-glucose ([^{18}F]FDG), a fluorinated glucose analogue that is taken up into cells with high glucose

consumption, such as cancer cells.[9] Following cellular uptake, [^{18}F]FDG is phosphorylated but cannot be metabolised further, resulting in intracellular accumulation. In addition to [^{18}F]FDG, PET imaging in oncology also takes advantage of a variety of other type of radiotracers, both routinely and as promising new methodologies. These include targeting cell proliferation with labelled thymidine analogues,[10, 11] studying tumour blood flow with ^{15}O-labelled water,[12] targeting tumour receptors with labelled antibodies[13] and aptamers,[14] and finally, following efficacy of gene therapy.[15]

The risks associated with invasive brain research have led to the emergence of PET as an important tool in translational neuroscience research. In addition to the use of [^{18}F]FDG to identify altered states of cerebral glucose metabolism (for example in Alzheimer's disease[16]), there is an increasing arsenal of tracers available for imaging in neuroscience.[17] Radiotracers targeting the brain must efficiently penetrate the blood-brain barrier (BBB), which sets specific requirements for the size and chemical structure of the potential tracer molecule. Successful tracers enter the brain *via* passive diffusion or active transport and are not substrates for efflux transporters located at the BBB that otherwise limit their brain exposure.[18] Among the many radiotracers developed during the past decades, the most widely used tracers target the dopaminergic, serotonergic, cholinergic and glutamatergic neurotransmission systems. These tracers contribute to diagnosis and increased understanding of several neurological and psychiatric disorders such as depression, addiction, schizophrenia, Parkinson's and Alzheimer's disease. However, it is important to note that most receptors are still lacking a selective radiotracer and that there is still a great deal of work to be done in tracer development.[19] PET is also routinely used in clinical cardiology, and examples of common tracers are [^{13}N]ammonia, [^{11}C]acetate, [^{11}C]palmitate and [^{18}F]FDG.[2, 20]

PET radiotracers are often obtained at a high ratio between radioactivity and molar amount, or at a high "molar activity" (Am). A high A_m is important in order to avoid saturation of target proteins in ligand binding studies, and it is typically recommended that less than 5-10% of target sites are occupied by the tracer during the PET measurement. However, it is also important to note that not all studies require high A_m. Measurements of high capacity systems and drug biodistribution are examples of such studies.[21]

A fundamental pre-requisite for conducting imaging studies in human subjects is that the tracer production complies with good manufacturing practice (GMP).[6] To comply with GMP, a radiosynthesis should ideally be automated and carried out in an aseptic environment to exclude the introduction of microorganisms in the process. A number of quality requirements are typically imposed on GMP manufacturing procedures as well as on the end product. Among these, strict specifications are typically set to assure chemical and radiochemical purity and identity, molar activity, as well as sterility and apyrogenicity of the injection solution. However, due to short half-lives of the radionuclides, not all the quality control can be conducted before administration of the radiopharmaceutical. Strictly validated methods are thus used in radiotracer production.

1.2 PET radiochemistry

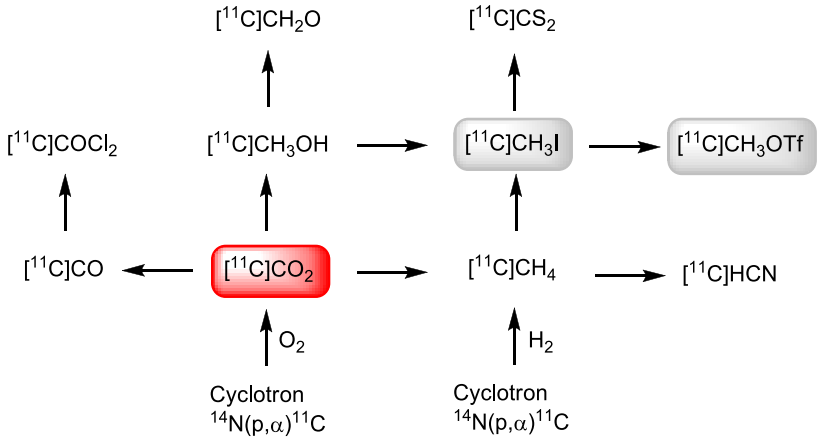
1.2.1 Fluorine-18

Fluorine-18 is the most widely used radionuclide in PET radiochemistry.[22-24] Its convenient half-life permits both centralised production and transportation to radiochemistry facilities that lack an on-site cyclotron, as well as use in multistep radiosynthesis. In a similar manner as in organofluorine chemistry, the radiochemistry of fluorine-18 can be divided into electrophilic and nucleophilic methodologies. Despite the great scope associated with electrophilic fluorinations, this methodology is far less utilised in fluorine-18 radiochemistry due to practical issues associated with [^{18}F]fluorine production, as well as the lower molar activity observed for the radiolabelled products. An exception to this is the relatively high molar activity obtained when utilising the electric discharge method, in which fluoromethane (produced from non-carrier-added [^{18}F]fluoride) is reacted with a low amount of fluorine gas[25] which, however, requires careful control over reaction conditions. Most often a nucleophilic labelling approach is sought to simplify radiofluorinations from a practical perspective. Because of its high solvation energy, nucleophilic ^{18}F-fluorinations rely on dried [^{18}F]fluoride and the use of phase-transfer catalysts (PTC) to increase its concentration in organic solvents. The dried [^{18}F]fluoride-PTC complex can subsequently be used in both aliphatic[23] and aromatic[26] radiofluorination reactions. Common leaving groups (LG) in nucleophilic aliphatic substitution include sulfonates (for example methyl and *p*-toluenesulfonate) and halides, whereas the corresponding LGs in aromatic fluorinations include nitro or trimethylammonium salts. However, not all compounds are optimally suited for nucleophilic aromatic fluorinations because they lack electron-withdrawing groups in *ortho* or *para* positions relative to the LG. Such compounds may be labelled using Cu-mediated reaction,[27] Pd-mediated electrophilic ^{18}F-fluorination[28] and aryl iodonium salts[29].

1.2.2 Carbon-11

Carbon-11 is most often produced using the ^{14}N(p,α)^{11}C nuclear reaction by bombardment of nitrogen gas with protons. By adding a small amount of oxygen or hydrogen to the target gas, the labelling precursors [^{11}C]carbon dioxide ([^{11}C]CO_2) and [^{11}C]methane ([^{11}C]CH_4) can be obtained, respectively (Scheme 1). Of these, [^{11}C]CO_2 is the more versatile precursor and, in addition to direct use, can be converted to secondary labelling precursors, like [^{11}C]methyl iodide ([^{11}C]CH_3I)[30, 31] and [^{11}C]carbon monoxide ([^{11}C]CO)[32, 33]. These can in turn be converted into other useful radioactive synthons, such as [^{11}C]phosgene ([^{11}C]$COCl_2$)[34-36], [^{11}C]methyl triflate ([^{11}C]CH_3OTf)[37, 38], [^{11}C]formaldehyde ([^{11}C]CH_2O)[39] and [^{11}C]carbon disulfide ([^{11}C]CS_2)[40]. Of these, [^{11}C]CH_3I and [^{11}C]CH_3OTf are the most frequently used in ^{11}C-radiochemistry, whereas the other radiolabelled precursors are more seldom used owing to difficulties associated with their production. In general, high effectiveness and reactivity is often counterbalanced by time-consuming preparation, maintenance of the equipment and low reproducibility.[22] [^{11}C]CH_4 does not permit direct labelling, but can be converted into [^{11}C]hydrogen cyanide ([^{11}C]HCN)[41] or [^{11}C]CH_3I. An advantage with [^{11}C]CH_3I derived from in-target produced [^{11}C]CH_4 is its higher molar activity that can be obtained due to the low

concentrations of methane in the atmosphere.[42] However, a drawback is the lower yields typically observed for in-target produced [^{11}C]CH_4 compared to [^{11}C]CO_2.



Scheme 1. Primary and secondary ^{11}C-precursors. Highlighted are [^{11}C]CO_2 (red), the most common primary ^{11}C-precursor, and [^{11}C]CH_3I and [^{11}C]CH_3OTf (grey), the most used ^{11}C-labelling synthons.

1.2.2.1 ^{11}C-Labelling using [^{11}C]carbon dioxide

Considering its 20-minute half-life, overall synthesis times with carbon-11 should be kept as short as possible. Being the most common ^{11}C-labelled precursor obtained from the cyclotron, [^{11}C]CO_2 is a compelling option to be used directly, without chemical modifications. Many of the features sought in method development for carbon-11 radiochemistry are shared with those desired for novel methods for organic chemistry. Any new method would thus be optimised to achieve the highest possible yield of the product, under the mildest possible conditions and at the highest possible chemo and/or stereoselectivity. Challenges associated with [^{11}C]CO_2 radiochemistry include low partial pressure of [^{11}C]CO_2 in inert gas, its relatively low reactivity and solubility. In addition, strict precautions need to be taken to avoid unnecessary dilution of the reaction mixture with atmospheric CO_2, since this results in poor molar activity of the desired radiolabelled product. In synthetic organic chemistry, the incorporation of unlabelled CO_2 often requires high partial pressure of the gas, high temperatures and catalysts. Since [^{11}C]CO_2 is obtained in minute quantities from the cyclotron, it is difficult to directly apply such methods to PET radiochemistry. Thus, only a handful of methods is available for direct or semi-direct incorporation of [^{11}C]CO_2.

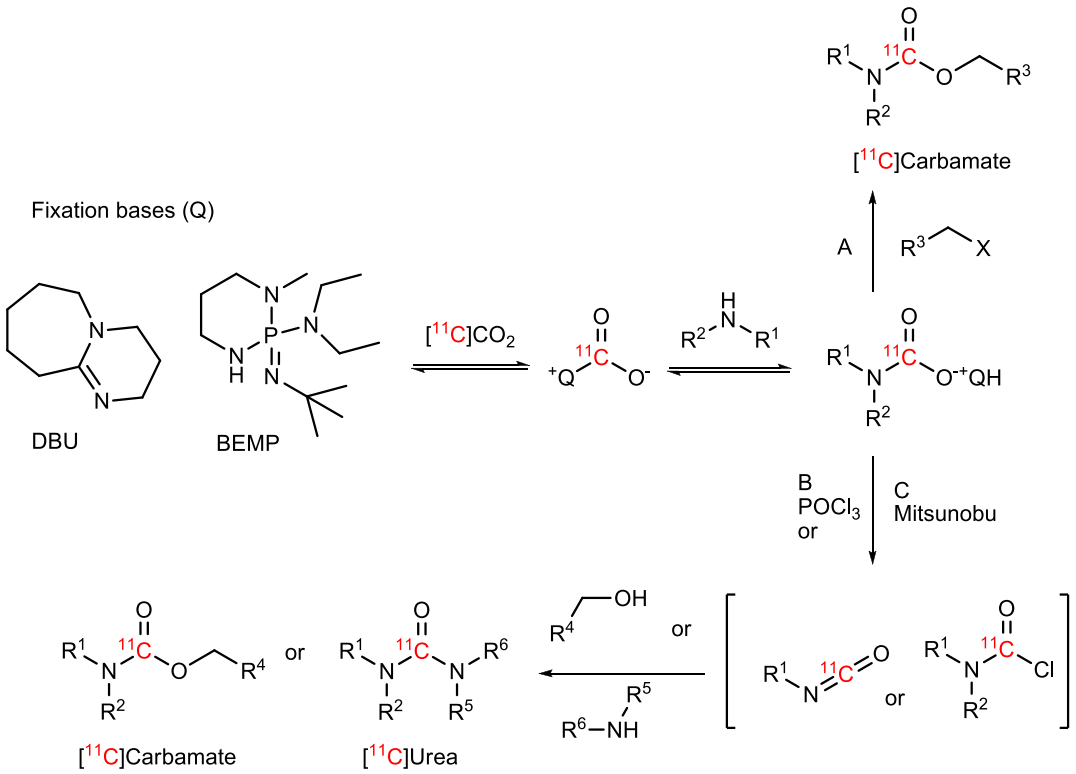
Carbamates and ureas are relatively common structural motifs in bioactive molecules and PET tracers. Although these structural motifs since long have been labelled using [^{11}C]$COCl_2$[43] the preparation of phosgene is cumbersome and hence not adopted in many PET centres. In an early [^{11}C]urea synthesis, lithium hexamethyldisilazide (LiHMDS) was reacted with [^{11}C]CO_2 and the subsequent lithium complex was hydrolysed using ammonium chloride.[44] Unfortunately, only [^{11}C]urea and [^{11}C]uracil were prepared using this method that has not found widespread use in the academic community. In another early example, [^{11}C]CO_2 was trapped at low temperature in a mixture of a substrate amine and triethylamine.[45] The

intermediate carbamic acid was dehydrated with phosphoryl chloride ($POCl_3$) to obtain ^{11}C-labelled isocyanates and ureas. However, the approach suffered from the high reactivity of the intermediate [^{11}C]isocyanate towards the precursor amine, resulting in low control over the products. There are also examples of ureas labelled using [^{11}C]carbon monoxide.[46, 47]

Considering the tedious preparation or narrow substrate scope for the previously presented *N*-^{11}C-carbonylation methods, a more straightforward and mild way of utilising [^{11}C]CO_2 was in demand. A big step forward was taken when the concept of carbon dioxide fixation for labelling was presented.[48, 49] Strong, organic bases, so-called fixating agents, enable efficient trapping of [^{11}C]CO_2 into the reaction mixture and activate it towards heteroatom-[^{11}C]carbon bond formation (Scheme 2). Although Coenen and co-workers already used triethylamine for [^{11}C]CO_2 fixation,[45] these so-called superbases, such as DBU (1,8-diazabicyclo[5.4.0]undec-7-ene) and BEMP (2-*tert*-butylimino-2-diethylamino-1,3-dimethylperhydro-1,3,2-diazaphosphorine), have proven to be excellent fixating agents even at room temperature and moderate gas flow rates. The nature of the base-CO_2 intermediate remains still unclear. A zwitterionic intermediate has generally been proposed, but at least in case of DBU, only formation of bicarbonate salt has been confirmed.[50, 51]

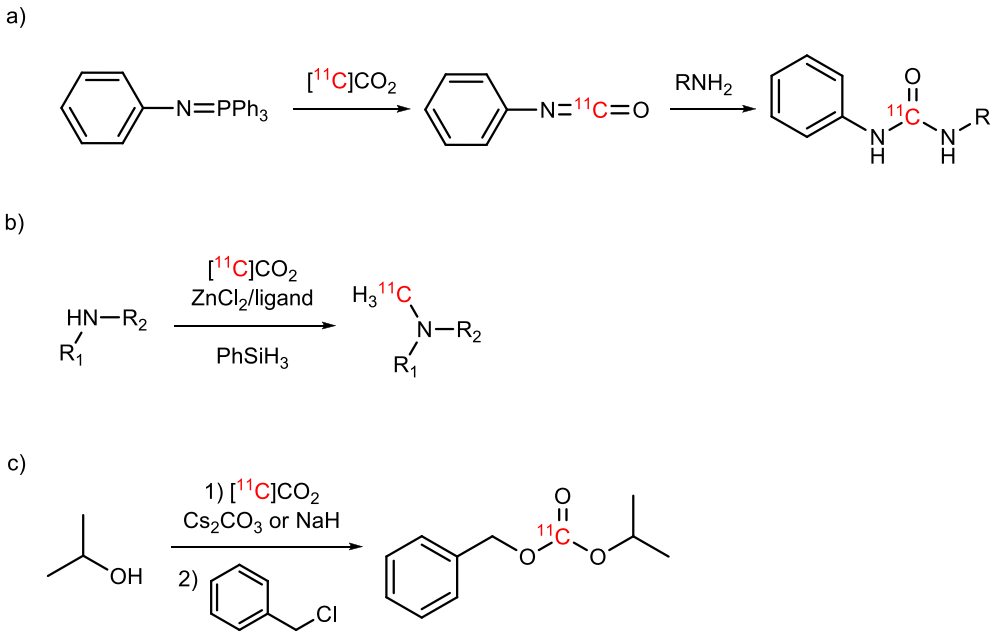
In their publications, both Hooker and Wilson used the fixation base chemistry for preparation of ^{11}C-labelled carbamates (A in Scheme 2).[48, 49] It was shown that the putative intermediate, the carbamate salt formed between the base, amine and carbon dioxide could be alkylated directly with reactive alkyl electrophiles. The method, which was operationally simple, consisted of bubbling [^{11}C]CO_2 through a solution of the fixation base (DBU or BEMP), an amine and a halide, tosylate or sulfate. It was later discovered that more structurally complex [^{11}C]carbamates could be prepared *via* an active [^{11}C]isocyanate intermediate, which could be formed by dehydrating the carbamate salt with $POCl_3$ (B in Scheme 2).[52] In the same report, Wilson and co-workers also demonstrated that the [^{11}C]isocyanate intermediate could be reacted with amines to provide the corresponding [^{11}C]ureas, although transformation was not very efficient for aromatic ureas. Other labelled heterocyclic compounds, such as [^{11}C]oxazolidinones, were also readily available when amino and hydroxy functionalities were pre-installed into the substrate, as demonstrated for [^{11}C]SL25.1188.[53] This radiotracer for monoamine oxidase-B (MAO-B), that was originally labelled using [^{11}C]$COCl_2$,[54] has also been applied to research in human subjects.[55] Furthermore, the same methodology has been applied to the production of the fatty acid amide hydrolase PET tracer [^{11}C]CURB for application in human PET studies.[56]

Mitsunobu conditions have also been applied as an efficient alternative to $POCl_3$ treatment in the synthesis of [^{11}C]isocyanates (C in Scheme 2).[57] Importantly, these conditions were mild (often carried out at room temperature) and allowed for excellent RCYs for both symmetrical and unsymmetrical [^{11}C]ureas even with aniline substrates.[57, 58] It was later demonstrated that [^{11}C]amides could be prepared when the Mitsunobu reaction was followed by alkylation with Grignard reagent.[59]



Scheme 2. Possible pathways (A-C) for [^{11}C]carbamate and [^{11}C]urea formation using [^{11}C]CO_2 fixation chemistry.

There are several other methods reported in the literature for ^{11}C-heteroatom bond formation that do not require superbases for CO_2 fixation (Scheme 3). Van Tilburg and colleagues reported an alternative for ^{11}C-labelling of ureas by using phosphinimine precursors obtained from the corresponding primary amines (Scheme 3a).[60] Though high reaction selectivity was observed for a set of linear [^{11}C]ureas, the methodology was hampered by relatively low radiochemical yield. Direct ^{11}C-methylation of amines with [^{11}C]CO_2 has also been reported, instead of using traditional [^{11}C]CH_3I or [^{11}C]CH_3OTf (Scheme 3b).[61] Although this Zn-mediated method had several drawbacks (high temperature, long reaction time and moderate substrate scope), it was reminded that direct utilisation of [^{11}C]CO_2 would often simplify set-ups and automation. The authors were able to apply the method to the radiosynthesis of [^{11}C]PIB, a β-amyloid radiotracer, with moderate RCY but low molar activity. Finally, [^{11}C]carbonates were synthesized starting from an alcohol and alkyl chloride (Scheme 3c).[62] DBU was not suitable for this method but the authors presented two possible alternative approaches. In the first one, using cesium carbonate (Cs_2CO_3) as a base, high RCYs but low molar activities were obtained. When using sodium hydride (NaH) instead, molar activities were high but RCYs only moderate. As a disadvantage, scope for the method was not presented.

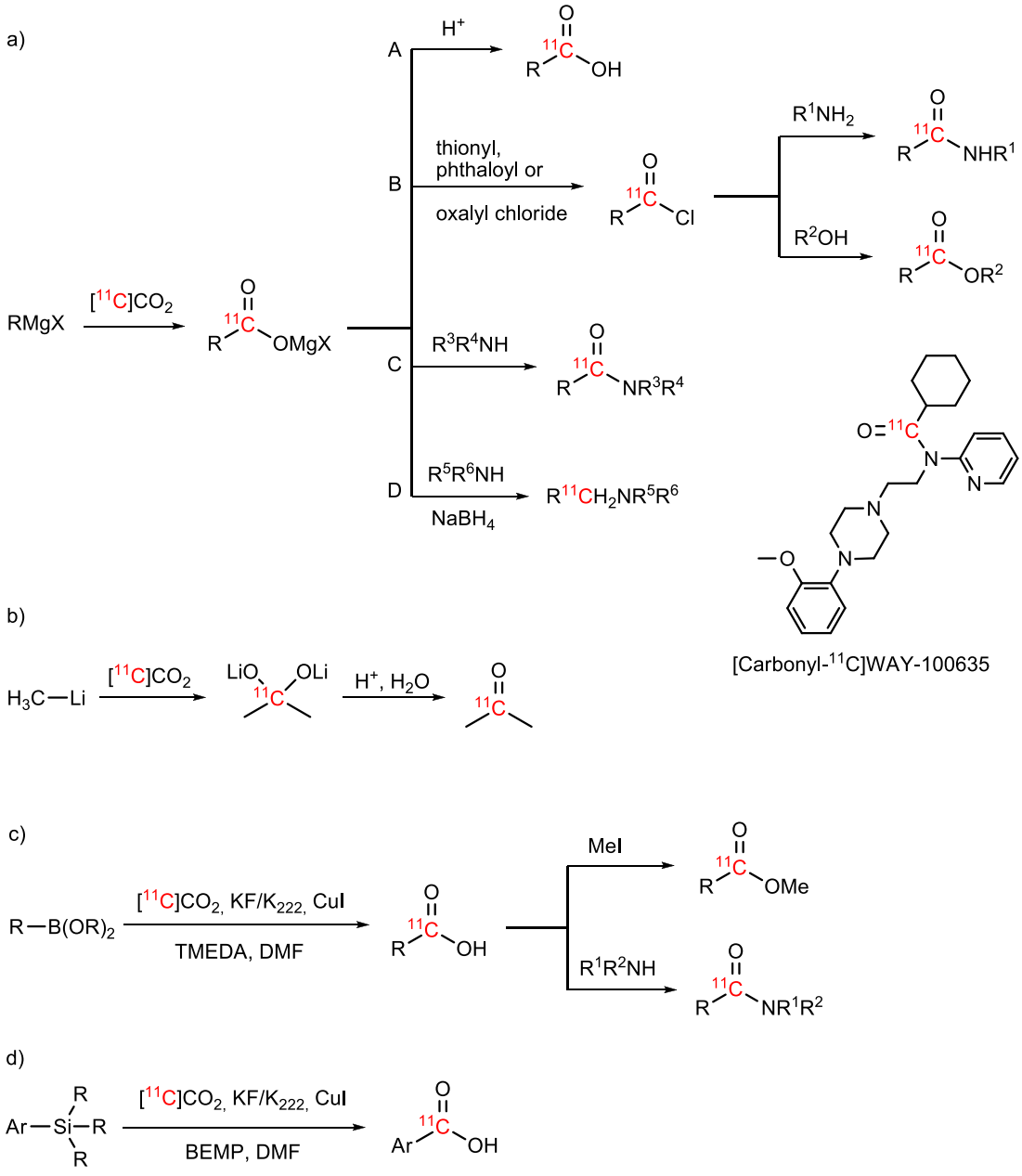


Scheme 3. Miscellaneous ^{11}C-heteroatom bond formation reactions. a) [^{11}C]Ureas from phosphinimines. b) Direct ^{11}C-methylation of amines. c) [^{11}C]Carbonates from [^{11}C]CO_2.

Strongly basic organometallic reagents have since long been used for ^{11}C-carboxylation reactions in ^{11}C-C bond formation (Scheme 4a and b). Simple [^{11}C]carboxylic acids (Route A in Scheme 4a), such as [^{11}C]acetic and [^{11}C]palmitic acid have thus been obtained by reacting the corresponding Grignard reagents with [^{11}C]CO_2.[63-65] Further functionalisation of such ^{11}C-carboxylated species into esters and amides has been facilitated by reaction of the intermediate [^{11}C]carboxymagnesium halides with thionyl chloride, phthaloyl dichloride or oxalyl chloride to form the corresponding [^{11}C]acyl halides (B in Scheme 4a).[66, 67] [Carbonyl-^{11}C]WAY-100635, prepared *via* this route, is arguably the most widely used PET tracer for imaging serotonin 1A receptors ($5HT_{1A}$) in human subjects.[68-71] Additionally, [^{11}C]amide can be reduced to [^{11}C]amine, as demonstrated in the synthesis of (+)-[^{11}C]PHNO, a dopamine D_2/D_3 radioligand.[72] Lasne and co-workers developed a direct reaction between the [^{11}C]carboxymagnesium halide and amine to obtain a set of amides (C in Scheme 4a).[73] It is noteworthy that [^{11}C]amines were accessible using this route when sodium borohydride ($NaBH_4$) was present in the reaction mixture (D in Scheme 4a).[74] Radiochemical yields (RCY) for the ^{11}C-amidation were considerably enhanced by applying microwave heating, and as a proof of concept, the $5HT_{1A}$ agonist S14506 was ^{11}C-labelled with 10-18% RCY.[75, 76]

Organolithium compounds have also found use in ^{11}C-carboxylation chemistry. The labelling synthon [^{11}C]acetone has been successfully prepared using methyllithium (Scheme 4b).[77, 78] However, due to the high reactivity and sensitivity of organometallic reagents, several problems arise. If reagents are not stored under an inert atmosphere, they are prone to react with atmospheric CO_2, leading to reduced molar activities of the ^{11}C-labelled products. The syntheses often require complicated set-ups, and functional group tolerance in the target molecule is typically poor. A recently developed copper-mediated protocol for labelling of [^{11}C]carboxylic acids and derivatives starting from the corresponding boronic acid esters

addressed some of these shortcomings (Scheme 4c).[79] Functional group tolerance was higher with this method, but only aryl, vinyl and alkynylboronates were compatible as substrates. An oxytocin receptor ligand[79] as well as the retinoid X receptor (RXR) agonist bexarotene[80] were labelled using the method. Another way to overcome the problems arising from the use of organometallic reagents was presented by Borganzone and colleagues in their work that harnessed fluorine-activated silylated aryl precursors for carboxylation (Scheme 4d).[81] Trapping efficiency for [^{11}C]CO_2 was greatly enhanced in the presence of BEMP or DBU.



Scheme 4. Synthesis of [^{11}C]carboxylic acid derivatives using a) Grignard, b) organolithium, c) boronate ester and d) silane chemistry.

AIMS OF THE THESIS

The overall aim of the thesis was to develop new ^{11}C-labelling methods using [^{11}C]carbon dioxide directly from the cyclotron, and thus facilitate development and discovery of novel radiotracers for PET. The specific aims were the following:

- To develop methods for ^{11}C-labelling of cyclic ureas and benzimidazolones, as well as to label (*S*)-[^{11}C]CGP12177, a radioligand used for imaging of β-adrenergic receptors.
- To apply dynamic isotope exchange to ^{11}C-labelling of carboxylic acids.
- To develop a method for ^{11}C-labelling of *N*-phenyl-2-oxazolidinones and other cyclic urethanes.

2 MATERIALS AND METHODS

A brief summary of materials and methods is presented below. For detailed synthesis procedures and radiochemistry methodology, the reader is referred to the full papers and manuscript.

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

All gases were purchased from AGA Gas AB (Sundbyberg, Sweden). Carbon-11 was produced with a GEMS PETtrace cyclotron (GE Uppsala, Sweden) using 16.4 MeV protons *via* the $^{14}N(p,\alpha)^{11}C$ nuclear reaction. [^{11}C]CO_2 was prepared in a 78 mL aluminium target containing nitrogen gas of scientific grade purity (99.9999%) and a small amount of oxygen (0.5 or 1%). [^{11}C]CO_2 was transferred either directly to a hot cell or *via* intermediate purification on molecular sieves inside a chemical process cabinet (GE, Uppsala, Sweden).

Inside the hot cell, [^{11}C]CO_2 was delivered to a [^{11}C]CO-synthesizer prototype from GEMS PET Systems AB (GE Healthcare, Uppsala, Sweden), where it was trapped either on a molecular sieve column (0.6 g packed in a ¼" stainless steel tube, 4 Å, mesh 80/100, GRACE) at room temperature (RT) or a stainless steel loop immersed in liquid nitrogen. The accumulated [^{11}C]CO_2 was released into a controlled stream (10 mL/min, Mass flow controller, Bronckhorst) of helium (He 6.0) upon heating. The purified [^{11}C]CO_2 was further concentrated on a silica gel (10 mg, 60 Å, 60-100 mesh) trap immersed in liquid nitrogen. After heating, [^{11}C]CO_2 was transferred *via* a phosphorus pentoxide (P_2O_5) column into a reaction vessel using a controlled stream of helium.

The reaction vessel pre-charged with the reaction mixture, was equipped with an ascarite (sodium hydroxide-coated silica, 8-20 or 20-30 mesh) trap to measure untrapped [^{11}C]CO_2. Following completed entrapment, the needles were removed, and the vessel was kept at the desired temperature for the specified reaction time. By comparing decay-corrected activities (A) in the vial and the ascarite trap, trapping efficiency 1 (TE_1) could be determined using the following Equation 1. TE_1 gives an estimation about the ability of the reaction mixture to retain [^{11}C]CO_2, and is subject to improvement by using CO_2 trapping agents or optimising the conditions, such as temperature or gas flow rate. Nevertheless, part of the radioactive gas is naturally in the headspace of the reaction vial, thus creating uncertainty to the TE_1 values.

$$\text{Equation 1: } TE_1 = \left(\frac{A_{1,\,\text{vial}}}{A_{1,\,\text{vial}} + A_{1,\,\text{ascarite}}}\right) \times 100\%$$

When the reaction was complete and quenched, the vial was flushed with N_2 to release the [^{11}C]CO_2 that still remained unreacted, into a second ascarite trap to estimate trapping efficiency 2 (TE_2) according to Equation 2. TE_2 can be seen as a measurement of the efficacy of the reaction in terms of [^{11}C]CO_2 usage.

$$\text{Equation 2: } TE_2 = \left(\frac{A_{2,\,\text{vial}}}{A_{2,\,\text{vial}} + A_{2,\,\text{ascarite}}}\right) \times 100\%$$

2.2 Product identification and yield determination

Analytical high-performance liquid chromatography (HPLC) system equipped with both radioactivity and UV detectors, was used for product identification. By co-injecting a non-radioactive reference compound, the radioactive peak could be associated with the corresponding UV peak co-eluting with the desired product. Radiochemical purity (RCP) of the desired product in the crude mixture was evaluated by comparing the radioactivity peak areas in the chromatogram. Total radiochemical yield (RCY) was then calculated using the Equation 3:

Equation 3: $\mathrm{RCY} = \mathrm{TE}_1 \times \mathrm{TE}_2 \times \mathrm{RCP}$

2.3 Molar activity

Molar activity (A_m, GBq/µmol) was calculated by dividing the activity (GBq) of the product sample with the chemical amount of the tracer molecule (µmol). Analytical HPLC was used for determination of the concentration of carrier in the product solution.

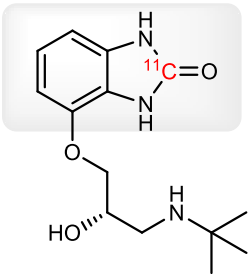
3 RESULTS AND DISCUSSION

3.1 ^{11}C-labelling of cyclic ureas (Papers I and II)

Five and six-membered cyclic ureas, including benzimidazolones, are commonly found in biologically and pharmaceutically relevant molecules. These structural motifs are traditionally labelled using [^{11}C]$COCl_2$.[43] To circumvent the difficulties associated with preparation and use of [^{11}C]$COCl_2$, we directed our efforts to developing methods for direct ^{11}C-carbonylation using cyclotron-produced [^{11}C]CO_2. This first subproject thus introduces two complementary ways to label such motifs.

3.1.1 ^{11}C-labelling of benzimidazolones using CO_2 fixation chemistry

The aim of the first study presented in **Paper I** was to develop an efficient method for preparation of ^{11}C-labelled benzimidazolones, including the PET tracer (*S*)-[^{11}C]CGP12177 ((*S*)-4-(3-(*tert*-butylamino)-2-hydroxypropoxy)-2*H*-benzimidazol-2[^{11}C]-one) (Figure 1) that has been used for β-adrenergic receptor imaging.[82-84] The method was inspired by the fruitful concept of CO_2 fixation that allows for utilising the minute amounts of ^{11}C-labelled CO_2 obtained from the cyclotron target.



(*S*)-[^{11}C]CGP12177

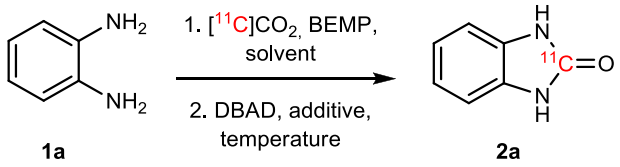
Figure 1. Structure of (*S*)-[^{11}C]CGP12177. The [^{11}C]benzimidazolone moiety is highlighted.

For optimisation, ^{11}C-carbonylation of *o*-phenylenediamine (**1a**) with [^{11}C]CO_2 was selected as a model reaction (Table 2). DBU and BEMP were selected as fixating agents, as it has been shown that [^{11}C]CO_2 can be efficiently trapped in a reaction mixture by using these superbases.[48, 49, 85] The radiolabelling procedure was started by trapping [^{11}C]CO_2 at room temperature in the presence of the fixating agent and *o*-phenylenediamine dissolved in a solvent of choice. Subsequently, a dehydrating agent was added and the reaction vessel was kept at a designated temperature for 4 min.

Following the conditions outlined in previous methodologies,[45, 52] $POCl_3$ was first tested as a dehydrating agent for the intermediate [^{11}C]isocyanate formation. Although trapping efficiency in the presence of BEMP was high, [^{11}C]benzimidazolone (**2a**) was afforded with low RCP (11%, Entry 1). Alternatively, aromatic ^{11}C-labelled ureas have previously been prepared *via* a Mitsunobu reaction approach.[57, 58, 86] More specifically, a complex of a tertiary phosphine and dialkyl azodicarboxylate is used to activate carbamic acid formed by amine and [^{11}C]CO_2. Gratifyingly, by adding a mixture of di-*tert*-butyl azodicarboxylate (DBAD) and *n*-

tributylphosphine (PBu_3) after [^{11}C]CO_2 trapping, the target molecule was afforded in high RCP (98%, Entry 2). DBU used in Entry 2 was less efficient as a trapping agent, and later comparison between DBU and BEMP verified this observation. Solvent screening revealed that MeCN, DMF and DMSO afforded both high TEs and RCPs (Entries 4-6), while THF was less suitable for CO_2 fixation (Entry 3). Next, a comparison of three different phosphines (PPh_3, $PMePh_2$ and PBu_3) was conducted (Entries 7-9). Although RCYs were similar for all the phosphines, PBu_3 was selected due to the combination of high reactivity in the solution and stability on the bench. It was also noticed that the reaction proceeded well at room temperature. Finally, in the absence of a fixating base, only moderate TE was achieved (Entry 10).

Table 2. Optimisation of reaction conditions for the synthesis of [^{11}C]benzimidazolone.



1a 2a

Entry[a]	Solvent	Additive	T [°C]	TE [%][b]	RCP [%][c]	RCY [%][d]
1[e]	MeCN	$POCl_3$	RT	93	11	10
2[f]	MeCN	PBu_3	50	38	98	37
3	THF	PPh_3	RT	48	80	38
4	DMSO	PBu_3	50	80	96	77
5	DMF	PBu_3	50	92	85	78
6	MeCN	PBu_3	50	96	99	95
7	MeCN	PPh_3	RT	85	95	81
8	MeCN	$PMePh_2$	RT	99	95	94
9[g]	MeCN	PBu_3	RT	92 ± 4	99 ± 1	91 ± 1
10[h]	MeCN	PBu_3	RT	51	99	50

[a] Reaction conditions: [^{11}C]CO_2 was bubbled into a solution of *o*-phenyleneamine (22.9 μmol, 2.5 mg) and BEMP (1.4 equiv) dissolved in the designated solvent (0.5 mL) at room temperature. The solution was reacted for 1 minute, after which a solution of DBAD (2.0 equiv) and additive (2.0 equiv) in 0.1 mL of the same solvent was added. The reaction mixture was kept at the designated temperature for additional 4 min. [b] Trapping efficiency, $TE = TE_1 \times TE_2$. [c] Radiochemical purity, decay-corrected, estimated by radio-HPLC. [d] Radiochemical yield, RCY = TE x RCP. [e] *o*-Phenyleneamine (27.5 μmol), solvent 0.6 mL, BEMP 0.2 equiv, no DBAD, additive 0.08 equiv. [f] DBU (0.044 equiv) instead of BEMP. According to Haji Dheere et al.[57] [g] The mean value ± SD, n=3. [h] No fixating agent eg. BEMP.

With optimised conditions in hand, we studied the suitability of the reaction for labelling of benzimidazolones with different functional groups (Figure 2). High RCYs, varying from 83% to 99%, were achieved from *o*-phenylenediamines bearing both electron-withdrawing and donating groups (**2b**-**f**). Also, other heterocycles, namely oxazolidinone **2g** and thiazolone **2h**, were labelled with 83% and 95% RCY, respectively. *N*-Methylated substrate **1i** was readily transformed to the desired product, but *N*,*N*´-dimethylated **1j** required heating to afford 18% RCY. The lower reactivity could be resulting from steric hindrance caused by the methyl groups, or from the requirement for the reaction to proceed *via* a different mechanism. Isocyanate formation after dehydration of the carbamate salt can occur only for primary amines (Scheme 5, Route A). Hence, in the case of *N*,*N*´-dimethyl-*o*-phenylenediamine, formation of isocyanate is ruled out and a direct attack of the amino group to the activated carbamoyloxyphoshonium would occur instead (Route B).

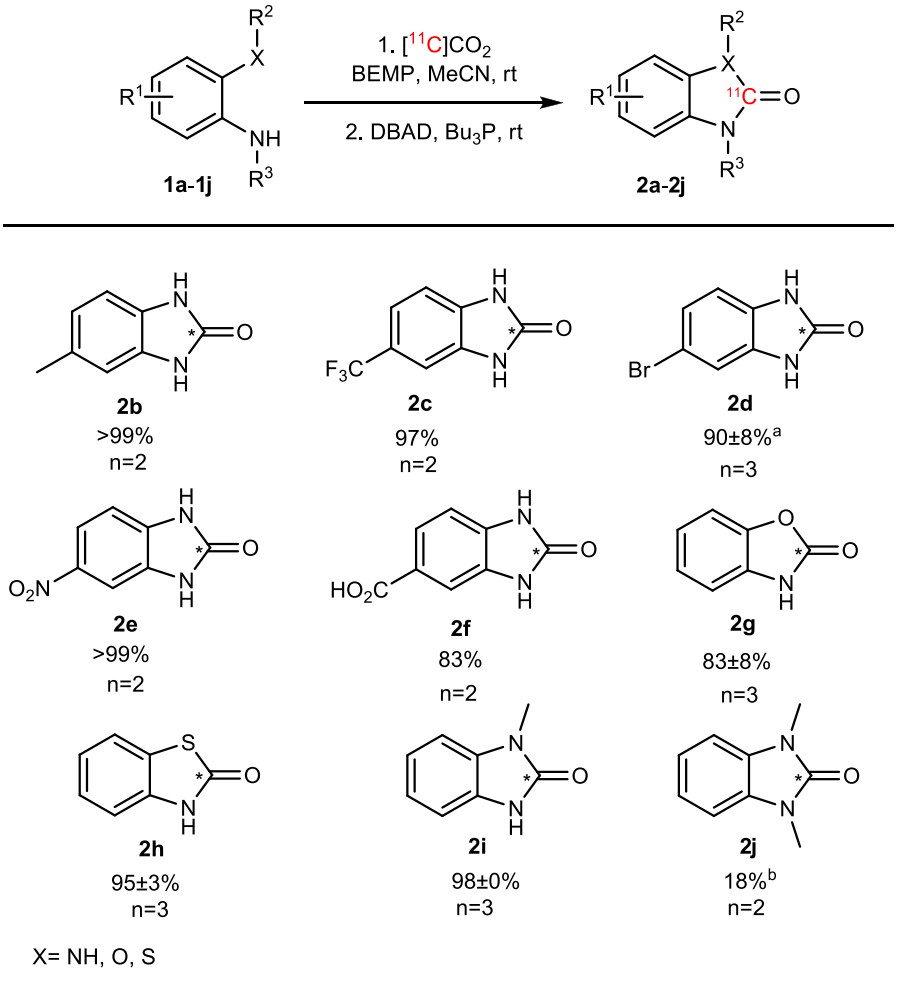
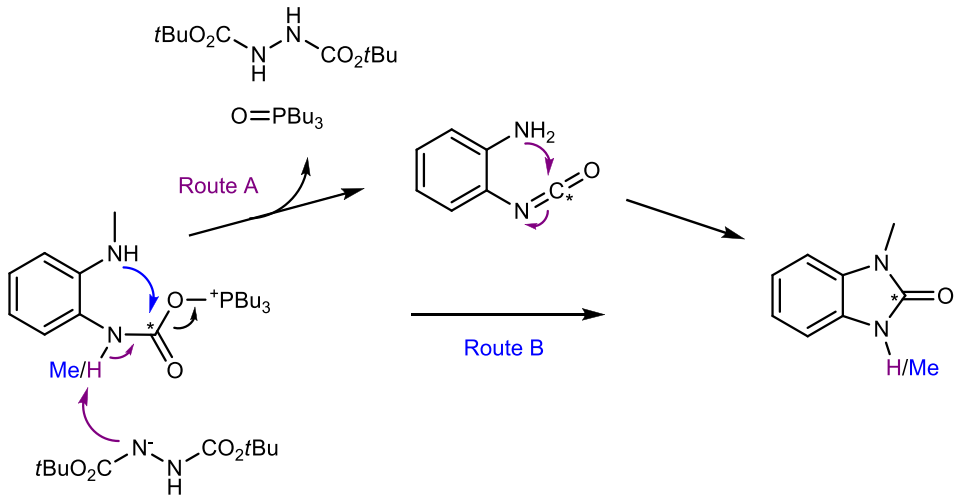
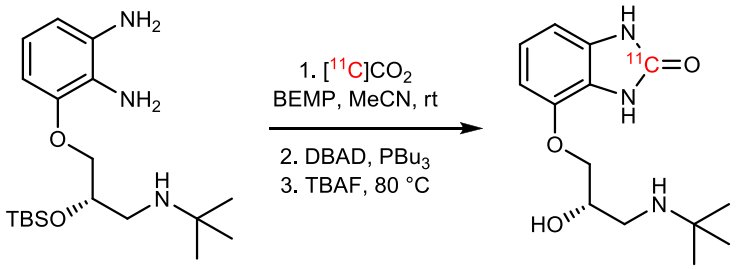


Figure 2. Substrate scope for the ^{11}C-labelling of benzimidazolones. The isotope-labelled carbon-11 is marked with an asterisk. Percentages represent RCYs. [a] Solvent MeCN/DMF 1:1. [b] Reaction at 60 °C.



Scheme 5. Two possible reaction routes for the formation of benzimidazolone under Mitsunobu conditions. Route A (purple): Isocyanate formation followed by ring closure. Route B (blue): Direct attack to the carbamoyloxyphosphonium group. The isotope-labelled carbon is marked with an asterisk.

As mentioned, the method was developed in attempt to facilitate ^{11}C-labelling of (*S*)-CGP12177. In the first labelling attempt with an unprotected hydroxy-diamine precursor, only 8% RCP was achieved. Nevertheless, after protecting the reactive hydroxyl group, radiolabelling was achieved with >99% conversion (Scheme 6). In a preparative run, (*S*)-[^{11}C]CGP12177 was afforded with 23% RCY after deprotection and semi-preparative HPLC purification. Radiochemical purity was >99% and molar activity 14 GBq/µmol.

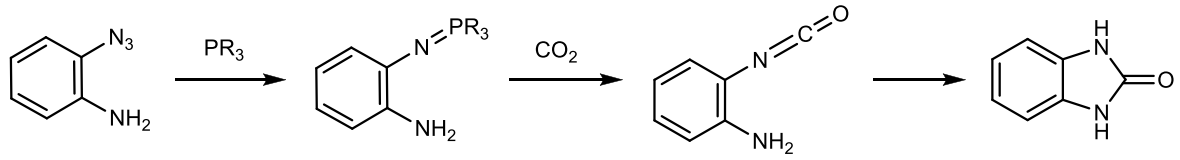


Scheme 6. Labelling and subsequent deprotection of (*S*)-[^{11}C]CGP12177.

Labelling of [^{11}C]CGP12177 with [^{11}C]$COCl_2$ has previously afforded molar activities of 4.8 GBq/µmol for the racemic mixture[83] and 252-518 GBq/µmol for the *S*-enantiomer[87] in 25 minutes from end of bombardment (EOB). [^{11}C]$COCl_2$ has been prepared from [^{11}C]CO and later, from [^{11}C]CH_4.[36, 88] Despite the advantage obtained in terms of higher molar activity, production of [^{11}C]$COCl_2$ is troublesome. All preparation methods are time-consuming multistep syntheses, and a characteristic problem for production of [^{11}C]$COCl_2$ is low reproducibility. So far, there is no widespread production system for [^{11}C]$COCl_2$ and only few laboratories produce it routinely. In this sense, production of [^{11}C]CGP12177 using [^{11}C]CO_2 directly from the cyclotron using the new method presented herein, is clearly advantageous. As a downside, due to reactivity of the hydroxyl group, an additional protection-deprotection procedure is required.

3.1.2 ^{11}C-Labelling of cyclic ureas using click chemistry

Another method for ^{11}C-labelling of cyclic ureas directly from [^{11}C]CO_2 was developed in close collaboration with colleagues at the French Alternative Energies and Atomic Energy Commission (CEA) in Saclay, Paris and is presented in **Paper II**. This novel method relied on the Staudinger/aza-Wittig reaction, a "click"-type reaction that uses organic azides as substrates in the formation of isocyanate intermediates. An azide reacts with a phosphine (PR_3) to form an iminophosphorane which then reacts with CO_2 (Scheme 7). The resulting isocyanate reacts readily with the nearby amino group to close the cyclic structure.



Scheme 7. Staudinger/aza-Wittig cascade reaction to form cyclic urea from an aza-amino precursor.

The method was first developed for carbon-14 labelling at the CEA. Initial reaction optimisation was conducted with the cheaper alternative, [^{13}C]CO_2. *o*-Azidoaniline was used as a model substrate to select adequate phosphine, solvent, temperature and time. Using highly reactive $PPhMe_2$ in DMF, the target molecule was afforded with 95% yield in only 5 minutes at 25 °C for both carbon-13 and carbon-14. Encouraged by the short reaction time, the conditions were tested for carbon-11 labelling. Gratifyingly, 79% RCP was obtained when trapping of [^{11}C]CO_2 at -50 °C was followed by the reaction at 25 °C.

The optimised conditions were next applied to labelling of several cyclic ureas, of which seven (Figure 3), including (*S*)-[^{11}C]CGP12177 were labelled at the Karolinska Institutet PET centre and the rest at the CEA. Excellent RCPs (85-99%) were obtained for the compounds **4b**-**g**, including five and six-membered ureas. A preparative run for (*S*)-[^{11}C]CGP12177 afforded the target molecule **4h** in 34% isolated yield after HPLC purification and in a molar activity of 32.5 GBq/µmol. Though somewhat better than that obtained with the Mitsunobu method presented earlier (14 GBq/µmol), the obtained A_m is still not high enough for receptor occupancy studies. Yet, both of these molar activities are useful for tracking distribution of the tracer in the body. Compared to the laborious synthesis of [^{11}C]CGP12177 using [^{11}C]$COCl_2$, for this Staudinger/aza-Wittig method, the same important advances apply: Use of [^{11}C]CO_2 directly from cyclotron in a rapid and mild one-pot reaction affords the labelled molecule in one step. As a disadvantage, an azide is needed as starting material, which could possess special safety restrictions, and requires some synthetic efforts. The method has excellent chemoselectivity, as relatively complex molecules, including a peptide and (*S*)-[^{11}C]CGP12177 were labelled without any pre-protection.

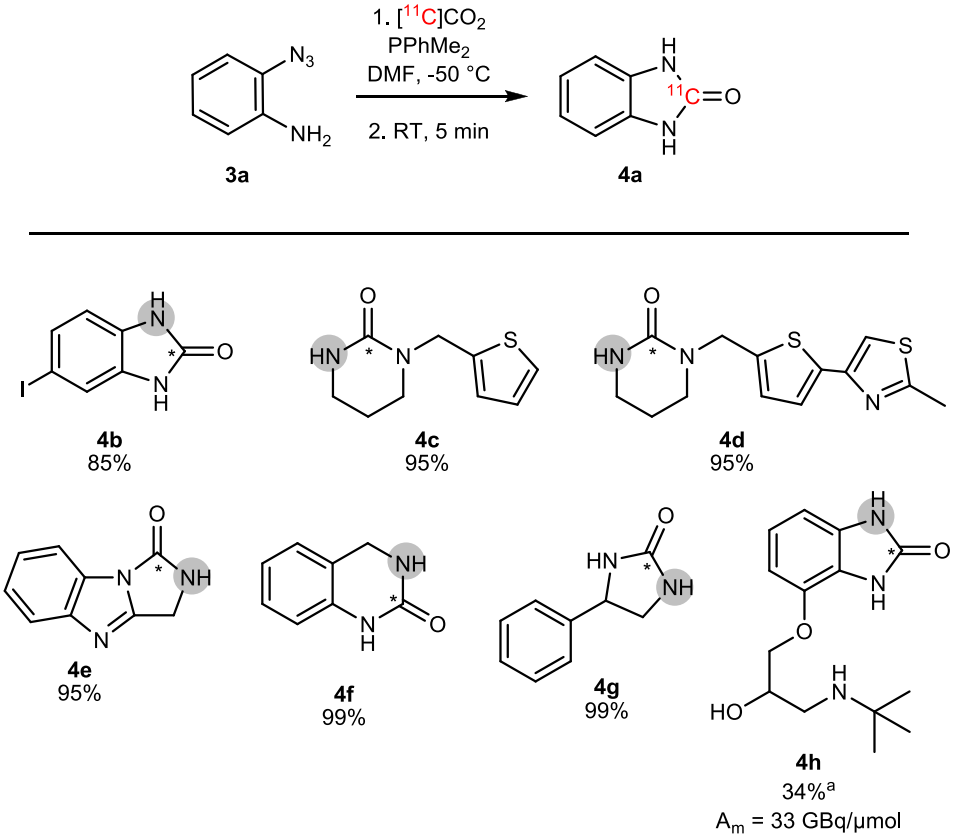
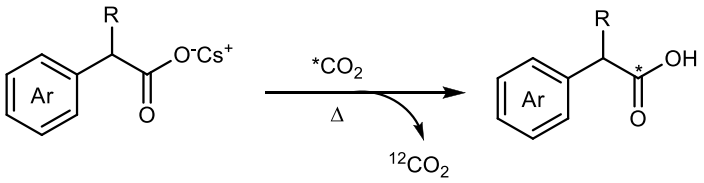


Figure 3. Scope for ^{11}C-labelling of cyclic ureas using Staudinger/aza-Wittig approach. Presented are the compounds labelled at the KI PET centre. The isotope-labelled carbon is marked with an asterisk. The nitrogen originating from the azido group is marked with a grey circle. The percentages represent RCPs estimated from radio-HPLC. [a] Isolated RCY.

To conclude, both of these methods presented in the **Papers I** and **II**, introduce a mild and fast way to label cyclic ureas with [^{11}C]CO_2. Suitability of these methods for labelling of PET tracers was highlighted by successful labelling of (*S*)-[^{11}C]CGP12177.

3.2 ^{12}C/^{11}C Isotopic exchange in phenylacetic acids (Paper III)

Today, isotopic exchange is a well-established method in labelling of molecules, especially with deuterium and tritium.[89-91] Unfortunately, labelling with carbon isotopes has traditionally required multistep syntheses. The journey towards carbon isotope exchange started by discovery of copper-assisted decarboxylation of aryl carboxylic acids.[92] Inspired by this pioneering work, in 2019 Destro and co-workers at the CEA used this as the starting point for ^{12}C/^{13}C and ^{12}C/^{14}C isotope exchange.[93] The same year, Kingston and co-workers published a nickel-mediated decarboxylative isotopic exchange for alkyl carboxylic acids.[94] Moon et al. discovered that phenyl carboxylic acids undergo heat-induced decarboxylation,[95] and inspired by this, our collaborators at the CEA developed a transition metal-free procedure for carbon isotope exchange (Scheme 8). In collaboration with the CEA, we applied this method to ^{11}C-labelling, as there are no current examples of ^{12}C/^{11}C isotope exchange.



Scheme 8. Dynamic isotopic exchange for aryl carboxylic acids. Asterisk refers to $^{11}C/^{13}C/^{14}C$.

In this method presented by the collaborators at the CEA, a cesium salt of phenylacetic acid is simply heated in presence of $[^{13}C/^{14}C]CO_2$. It was hypothesized that after decarboxylation, the benzylic anion would react directly with labelled CO_2 or it would generate a dienolate species. This intermediate would attack to CO_2 and form a malonate that would finally lose the excessive carboxyl group. Nevertheless, in a recent article by Kong and co-workers,[96] the authors ^{13}C-labelled α-substituted carboxylates that cannot form an enolate, thus arguing that the route including the dienolate intermediate is not necessary for the reaction to proceed.

The reaction conditions for the isotopic exchange were optimised by the collaborators at the CEA. For the model compound, cesium salt of 4-methoxyphenylacetate, in presence of 3 equiv. of $[^{13}C]CO_2$, a reaction in anhydrous DMSO at 150 °C afforded the highest isotopic enrichment (IE) (43%). Nevertheless, during the substrate scope studies, it was noticed that many substrates required a specific temperature and time for the optimal IE, ranging between 80 and 190 °C and from 5 minutes to 6 hours. 2-(4-Nitrophenyl)acetic acid, flurbiprofen and tolmetin precursors were amongst the swiftly reacting compounds, and they were selected for further ^{11}C-labelling tests at the KI PET centre.

For ^{11}C-labelling of 2-(4-nitrophenyl)acetic acid (**6a**), some further optimisation was conducted. The optimal results were achieved when $[^{11}C]CO_2$ was trapped at room temperature in the presence of 50 μmol of the cesium salt precursor **5a** in 0.5 mL DMSO, followed by heating at 80 °C for 5 min, affording 61% TE_1, 82% TE_2, >99% RCP and 50% total RCP (Figure 4). A preparative run for **5a** was conducted using the optimised conditions. After HPLC purification, the ^{11}C-labelled target molecule was achieved with >99% RCP and 11% overall RCY. Molar activity was 0.16 GBq/μmol.

After trials and errors, ^{11}C-labelling of flurbiprofen (**6b** in Figure 4) and tolmetin (**6c**) were conducted in 10 minutes at 150 °C, affording RCYs of 7% and 3%, respectively.

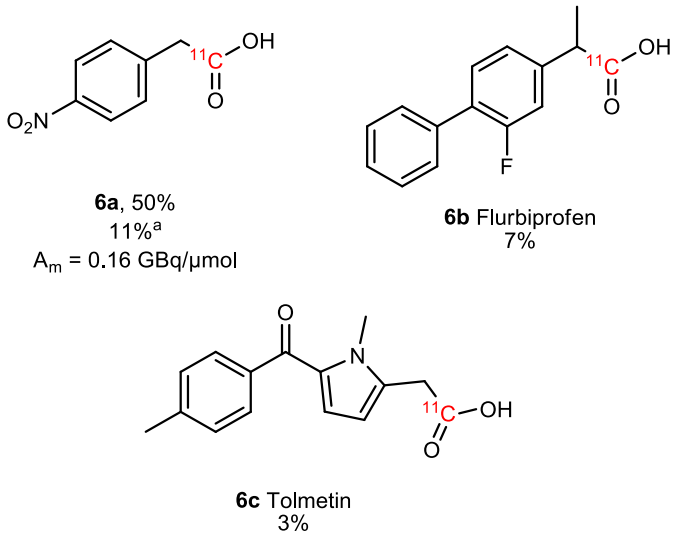


Figure 4. Isotopic exchange with [^{11}C]CO_2. Yields refer to RCY = TE_1 x TE_2 x RCP. [a] Preparative run, isolated RCY.

In this pioneering study for ^{12}C/^{11}C exchange, three substrates were radiolabelled with carbon-11 although higher number of suitable substrates can be expected to be found by further screening. High reaction temperatures could be an issue affecting to general suitability of the method. The same concern arises with the loss of stereochemistry, which is a built-in feature of the method. Lastly, RCYs and molar activities for this CO_2 exchange were low, though potentially useful. It is important to emphasise the simplicity of this method; Labelling was completed in one step using [^{11}C]CO_2 directly, and without need for special precursors or reagents.

3.3 ^{11}C-Labelling of oxazolidinones and other cyclic urethanes (Paper IV)

Encouraged by CO_2 fixation and ^{11}C-labelling of benzimidazolones in the 1st project, our next goal was to label other heterocycles, namely cyclic urethanes. Owing to their superior features *in vivo*, such as higher stability, compared to peptides, urethanes have become particularly important within medicinal chemistry.[97] To obtain ^{11}C-labelled oxazolidinones, one possible approach is to construct a β-amino alcohol precursor that undergoes intramolecular cyclisation in presence of either [^{11}C]$COCl_2$ or [^{11}C]CO_2. Two PET tracers for monoamine oxidase receptor imaging have been prepared using either of these methodologies: [^{11}C]befloxatone for MAO-A[98] and [^{11}C]SL25.1188 for MAO-B[53, 54]. Additionally, selenium-mediated synthesis of five and six-membered cyclic urethanes with [^{11}C]CO has been presented.[47]

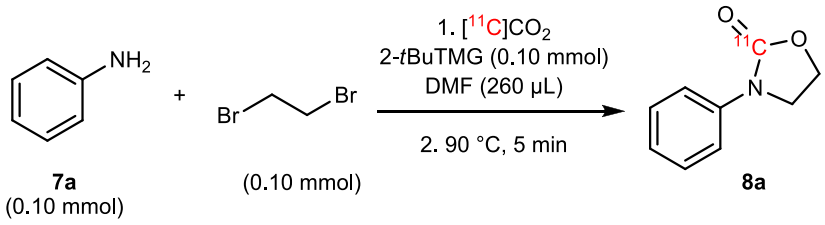
In synthetic chemistry with unlabelled CO_2, there are various ways to construct oxazolidinones but few of them are suitable for late-stage carbon-11 chemistry due to requirements in starting material functionalisation or reaction conditions, as most often high CO_2 pressure is applied. The Repo group at the University of Helsinki developed a simple method to construct *N*-aryl and *N*-benzyl oxazolidinones using moderate CO_2 pressure and simple precursors.[99] The oxazolidinone ring is formed between an aniline precursor, CO_2 and 1,2-dibromoethane, and bigger rings are achieved using dibromoalkanes with longer alkyl chain. In collaboration with Repo and co-workers, our aim was to adapt the method to ^{11}C-labelling.

The method development was initiated using aniline dissolved in MeCN as precursor, 1,2-dibromoethane as alkylating reagent, [^{11}C]CO_2 as labelling agent, and with 2-*tert*-butyltetramethylguanidine (2-*t*BuTMG) and cesium carbonate (Cs_2CO_3) as additives. The reaction was conducted in one pot by trapping [^{11}C]CO_2 into the reaction mixture at room temperature and keeping the vial at 60 °C for 15 minutes. Delightfully, the first reaction furnished the target compound with 64% TE_1, 19% TE_2, 95% RCP and total RCY of 11%.

After initial screening for solvent, additives, substrate concentrations, time and temperature, we found out that 0.34 M of aniline, 1,2-dibromoethane and 2-*t*BuTMG afforded high TE_1 (82%) and RCY (66%) in 5 minutes at 90 °C (Table 2, Entry 1). Chemically, high [^{11}C]CO_2 trapping was obtained in concert with the fixating agent and aniline but physical features arising from the reaction set up and vial dimensions also affected the TE_1.

Other fixating agents at the same basicity area were tested and of these, BEMP performed as well as 2-*t*BuTMG, while tetramethylguanidine (TMG) and DBU were less successful. Surprisingly, triazabicyclodecene (TBD) was unable to yield any product despite the similar pK_a value. More reactive electrophiles ethylene di(*p*-toluenesulfonate) and 2-bromoethyl *p*-toluenesulfonate were not compatible. Reducing the amount of 1,2-dibromoethane further to 0.03 M affected TE_2 negatively (Entry 2). A reaction conducted at 50 °C afforded also lower TE_2 (Entry 3). Prolongation of the reaction time up to 10 minutes enhanced RCY (Entry 4) but the improvement was cancelled by decay of carbon-11. Finally, the necessity of a fixating base was highlighted as the absence of 2-*t*BuTMG affected negatively to TE_1 and prevented formation of the target compound (Entry 5).

Table 2. Optimisation of reaction conditions for ^{11}C-labelling of oxazolidinones.



Entry	Deviation from standard conditions	TE_1 [%]	TE_2 [%]	RCP [%]	RCY [%][a]
1	none[b]	82 ± 6	91 ± 2	88 ± 1	66 ± 7
2	0.01 mmol 1,2-dibromoethane[c]	84	77	85	55
3	50 °C[c]	76	64	85	41
4	10 min[c]	87	94	91	74
5	no fixating base[c]	40	11	0	0

[a] RCY = TE_1 x TE_2 x RCP. [b] Conditions: **7a** (0.10 mmol, 0.34 M), 1,2-dibromoethane (0.10 mmol, 0.34 M), 2-*t*BuTMG (0.10 mmol, 0.34 M) and [^{11}C]CO_2 in DMF (250 µL) at 90 °C for 5 min. n=3. [c] n=1.

The optimised reaction conditions (Entry 1 in Table 2) were applied to labelling of different substrates. First, oxazolidinones with different functional groups were studied (Figure 5). Methoxy-substituted oxazolidinone was labelled with high RCY (**8b**, 79%), as well as 3-(3-fluoro-4-morpholinophenyl)oxazolidinone (**8e**, 78%), a motif found in antibiotic Linezolid. Electron-withdrawing groups lowered the yields for **8c** and **8d**. Ring expansion to oxazinanones was made simply by using 1,3-dibromopropane as an electrophile, allowing for labelling of **8f**-**h**. Another possible application arised from labelling of benzylic oxazinanones by using benzylamine as a precursor, as shown with **8i**-**k**. By lowering the amount of fixating base to 0.1 equiv. the higher reactivity of benzylamine was controlled.

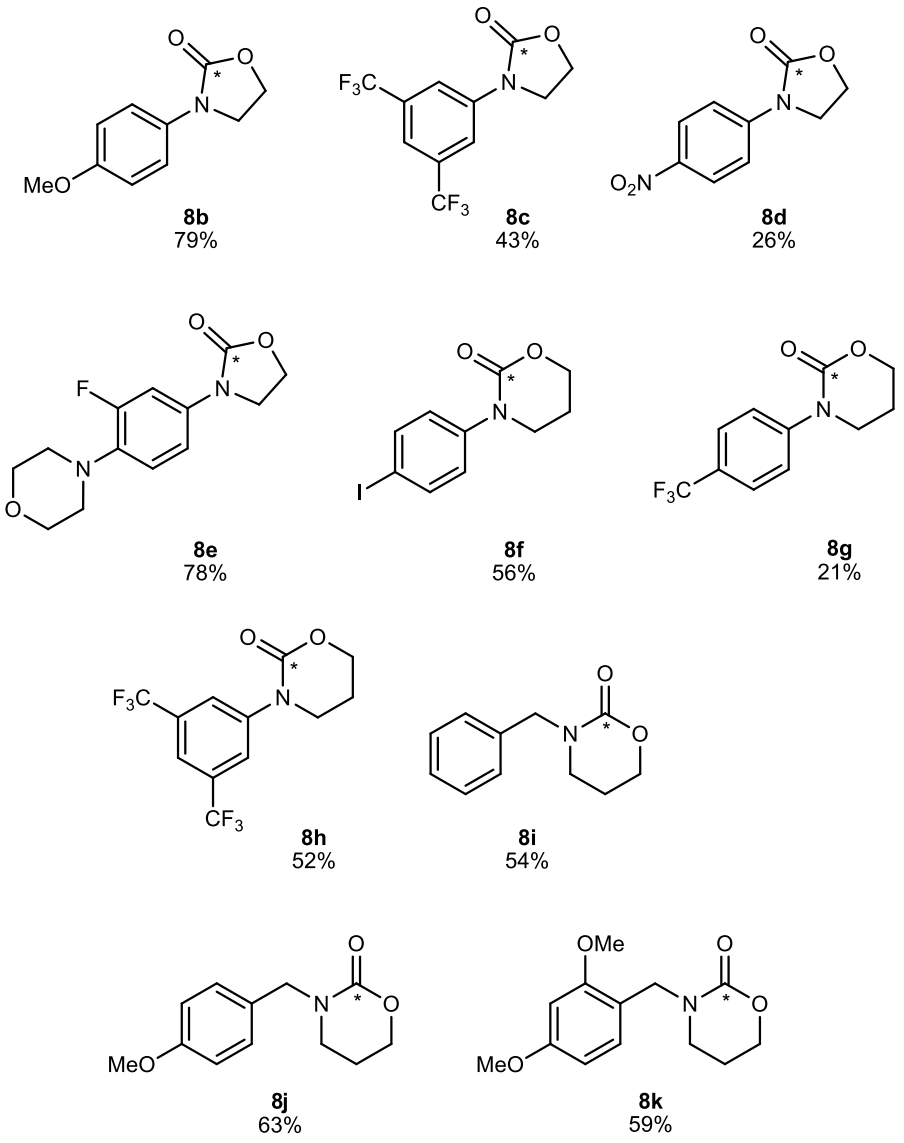


Figure 5. RCYs for the labelled [^{11}C]phenyleneoxazolidinones **8a-e**, [^{11}C]phenyleneoxazinanones **8f-h** and [^{11}C]benzyloxazinanone derivatives **8i-k** using the optimised conditions, n = 2. The carbon-11 isotope is marked with an asterisk.

Finally, a preparative run for **8e** was conducted. After HPLC purification, the radiolabelled product was isolated in 63% RCY, while RCP was over 99%. The determined molar activity was 4.7 GBq/µmol, which would be suitable for drug distribution studies. The radiotracers [^{11}C]SL25.1188 and [^{11}C]befloxatone are the only examples of ^{11}C-labelled oxazolidinones,

and any general method for labelling this structural motif starting from corresponding anilines and dihaloalkanes has not been published so far. The method presented herein approaches from a direction that utilises simple, convertible building blocks, allowing for easy construction of different cyclic urethanes.

In general, several reasons can be recognised for the molar activities that do not reach high values during these projects. In addition to site-specific restrictions in target efficacy, dilution of radiolabelled [^{11}C]CO_2 by atmospheric CO_2 is a feature that is difficult to eliminate. Use of CO_2 trapping agents (**Papers I** and **IV**), such as BEMP and 2-*t*BuTMG, is a potential reason for lower A_m. Additionally, the reaction mechanism of isotope exchange reaction (**Paper III**) lowers A_m inevitably.

4 CONCLUDING REMARKS

In this thesis, novel methodologies were developed that allowed access to ^{11}C-labelled cyclic ureas, benzoxazolones and benzothiazolones, phenylacetic acids, *N*-aryloxazolidinones, *N*-aryl and *N*-benzyl oxazinanones directly from cyclotron-produced [^{11}C]CO_2 (Figure 6). The methods shared some important features, namely operational simplicity (one-pot procedures), short reaction times, and generally high radiochemical yield and selectivity of the target compounds. This work has contributed to availability of ^{11}C-labelling methods for small molecules, which has an impact on PET tracer development. The studies presented herein have a possibility to add knowledge about labelling methods with other carbon isotopes, as well as to contribute to CO_2 chemistry in general.

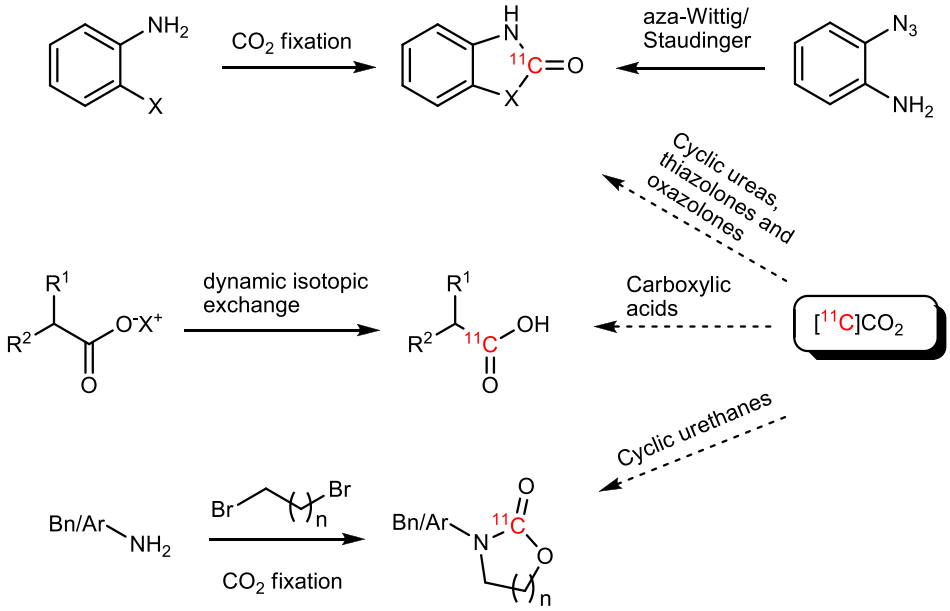


Figure 6. Summary of the ^{11}C-carbonylations and carboxylations presented in this thesis.

The key conclusions from **Papers I-IV** are the following:

- The work in **Paper I** presented a new simple method to label benzimidazolones using [^{11}C]CO_2 fixation and Mitsunobu reaction. In **Paper II**, a complementary way to label cyclic ureas using a Staudinger/aza-Wittig reaction was presented. The yields of both reactions were high, and the utility was showcased in each case by labelling of the PET tracer (*S*)-[^{11}C]CGP12177.
- In **Paper III**, a novel dynamic isotopic exchange reaction was presented. To the best of our knowledge, this is the first time that isotope exchange was used in carbon-11 chemistry. The utility of the methodology was demonstrated in the labelling of three phenylacetic acid compounds, including two anti-inflammatory drugs in useful radiochemical yields for imaging studies.

- In **Paper IV**, a novel method for ^{11}C-labelling of aryl oxazolidinones and other cyclic urethanes was presented. In this general method, aniline and dihaloalkane building blocks were used to construct ^{11}C-labelled phenylic and benzyl oxazolidinones as well as oxazinanones. The reaction provided the target products in moderate to excellent radiochemical yields.

5 FUTURE CONSIDERATIONS

PET imaging is a well-established tool in diagnosis, research and development. Nevertheless, the number of radiotracers available is still limited compared to possible imaging targets. On one hand, development of radiochemistry methods for late-stage labelling is important in order to achieve goals in radioligand discovery. Despite the recent advancements, there is still plenty of space and demand for new ^{11}C-labelling methods that are general and preferably compatible for clinical use.

Ease of automation is an important feature of a good labelling method, and this would be a plausible next step for some of the methods presented in this thesis. The exciting isotope exchange reaction that was debuting with carbon-11, will require more attention in terms of generality, substrate scope and molar activity. Molar activity has been a challenge for all of the presented methods, and solutions could be assessed when possible.

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