^{11}C=O Bonds Made Easily for Positron Emission Tomography Radiopharmaceuticals

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Abstract

The positron-emitting radionuclide carbon-11 (^{11}C, $t_{1/2}$ = 20.3 minutes) possesses the unique potential for radiolabeling of any biological, naturally occurring, or synthetic organic molecule for *in vivo* positron emission tomography (PET) imaging. Carbon-11 is most often incorporated into small molecules by methylation of alcohol, thiol, amine or carboxylic acid precursors using [^{11}C]methyl iodide or [^{11}C]methyl triflate (generated from [^{11}C]CO_2). Consequently, small molecules that lack an easily substituted ^{11}C-methyl group are often considered to have non-obvious strategies for radiolabeling and require a more customized approach. [^{11}C]Carbon dioxide, [^{11}C]carbon monoxide, [^{11}C]cyanide, and [^{11}C]phosgene represent alternative carbon-11 reactants to enable ^{11}C-carbonylation. Methodologies developed for preparation of ^{11}C-carbonyl groups have had a tremendous impact on the development of novel PET radiopharmaceuticals and provided key tools for clinical research. ^{11}C-*Carbonyl* radiopharmaceuticals based on labeled carboxylic acids, amides, carbamates, and ureas now account for a substantial number of important imaging agents that have seen translation to higher species and clinical research of previously inaccessible targets, which is a testament to the creativity, utility, and practicality of the underlying radiochemistry.

Keywords

carbon-11, positron emission tomography, radiochemistry, carbon dioxide, phosgene, carbon monoxide, hydrogen cyanide, molecular imaging

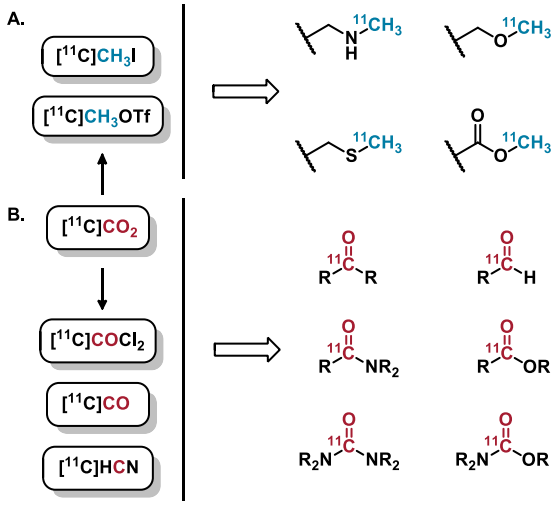
1. Introduction

1.1 Strategies for radiolabeling with carbon-11

The discovery and development of positron emission tomography (PET) radiopharmaceuticals demands an iterative approach that, even when informed by existing medicinal chemistry data and computational modeling, suffers from capricious trial-and-error based evaluation.[1–4] While advances in radiochemical methods with carbon-11 (^{11}C, $t_{1/2}$ = 20.3 min) and fluorine-18 (^{18}F, $t_{1/2}$ = 109.7 min) have enabled the preparation of a greater array of radiolabeled small molecules, relatively fewer of these methods have been applied for PET radiotracer development or led directly to the discovery of novel radiopharmaceuticals. Methods that require the synthesis of specialized precursors or which are proven only on relatively simple molecules hold limited value for radiopharmaceutical development since they require significant synthetic effort to apply them to the goals of rapidly and reliably preparing series of radiotracer candidates and eventually reproducible routine production. For these reasons, many small molecule tracer development programs still rely on a very small number of radiolabeling methods, chief among them being ^{11}C-methylation.[5,6]

The merits of ^{11}C-methylation as a choice reaction for radiotracer development have been well-recognized.[3,7,8] [^{11}C]Methyl iodide and [^{11}C]methyl triflate are nowadays readily prepared in many cyclotron-equipped radiochemistry facilities using commercial apparatus.[9] The precursors for ^{11}C-methylation are typically alcohols, amines, thiols, carboxylates, or amides, which can all be subjected to similar labeling conditions that seldom require significant optimization. The primary variables are the nature and stoichiometry of added base (if needed) and any deprotection and/or workup steps prior to purification. Contemporary methods even do away with a dedicated reaction vessel for this process, and conduct the radiolabeling inside of an

injector loop, which is subsequently flushed onto an HPLC column using mobile phase to initiate purification ("loop method").[10–12] Despite this technical simplification, ^{11}C-methylation represents only one strategy for radiolabeling, and can be applied to prepare *O*-, *N*-, *S*-, or in some cases *C*-^{11}C-methylated products,[13,14] in the presence of compatible functional groups (Scheme 1A). By relying so heavily on a single synthetic strategy, radiotracer development has necessarily been biased towards certain classes of ^{11}C-methylated products, at the expense of greater chemical diversity.



Scheme 1. A. Radiolabeling by the ^{11}C-methylation strategy. **B.** Radiolabeling by the ^{11}C-carbonylation strategy.

In this review, we will explore ^{11}C-carbonylation as an alternative set of strategies that can serve as a complementary tool to ^{11}C-methylation for radiotracer development. Carbonyl groups are found throughout bioactive molecules and are featured in a great number of functional groups utilized by medicinal chemists.[15] An analysis of drug candidates from the AstraZeneca's central nervous system portfolio in January 2012 revealed that <35% of compounds could be radiolabeled by ^{11}C-methylation alone, compared to >75% when including candidates for ^{11}C-

carbonylation.[16] Likewise, [^{11}C]carbon dioxide, [^{11}C]carbon monoxide, [^{11}C]cyanide, and [^{11}C]phosgene represent a versatile set of alternative carbon-11 reactants to enable ^{11}C-carbonylation when ^{11}C-methylation is either not applicable for preparation of ^{11}C-isotopologues or not desirable for metabolic reasons[17] (Scheme 1B). The criteria which each ^{11}C-radiolabeling methodology must satisfy to be useful for radiopharmaceutical development are outlined herein with a focus on methods for preparation of ^{11}C-carbonyl groups. We will show that the toolbox available for ^{11}C-radiotracer development includes viable methods for ^{11}C-carbonylation (using [^{11}C]CO_2, [^{11}C]$COCl_2$, [^{11}C]CO, and [^{11}C]CN), and will identify remaining challenges for widespread adoption of these for routine application in radiosynthesis.

1.2 Criteria for Practical Radiosynthesis with Carbon-11

PET is an imaging modality used to study biochemical processes by reporting on molecular interactions of *in vivo* probes.[6,18] A number of positron-emitting isotopes are available, including ^{11}C, nitrogen-13, oxygen-15,[19] ^{18}F,[5,20] copper-64, gallium-68, and zirconium-89,[21,22] and may be selected for a given imaging target based on desired half-lifes, and synthetic and biochemical compatibility. Isotopologue radiolabeling is attractive for well-characterized small molecules to take advantage of known properties, such as target affinity, selectivity, specificity, pharmacokinetics, and metabolism, which can be affected by structural modifications. The ubiquity of carbon atoms in organic compounds is complemented by highly versatile radiochemistry and the potential for multi-tracer studies in a single imaging session to establish ^{11}C as a frequently selected isotope for tracer development.

Practical methods for incorporation of ^{11}C into small molecules should possess the required qualities to ensure convenience and reliability of the production process as well as the safety and utility of the radiopharmaceutical dose for imaging protein targets *in vivo*. The following criteria

have been identified as key factors in determining whether to employ a given method or to seek alternatives for practical synthesis of radiopharmaceuticals:

- *commercial availability or facile syntheses of precursors*
- *radiochemical purity*
- *specific activity (SA)*
- *radiochemical yield (RCY)*
- *ease of automation and reproducibility*

A major strength of ^{11}C-methylation is widespread *availability of precursors* such as carboxylic acids, amines, alcohols, and thiols with which to prepare ^{11}C-methylated derivatives. On the other hand, a method such as [^{11}C]CO_2-fixation using air-sensitive and reactive Grignard reagents faces major headwinds to expanding its utility beyond relatively simple radiopharmaceuticals such as [^{11}C]acetate and [^{11}C]palmitate or labeled intermediates for acylation of more complex products such as [^{11}C]WAY-100635 and [^{11}C](+)-PHNO (*vide infra*).[23] Achieving high *radiochemical purity* of the radiotracer is essential for *in vivo* imaging studies. Radioactive impurities, even in trace amounts, can dramatically skew the interpretation of PET imaging or decay counting experiments, since the detected γ-photons do not indicate molecular identity.[24,25] Reactions that produce multiple structurally similar radioactive products can pose obstacles to efficient purification by semi-preparative HPLC and/or solid-phase extraction.[26–28]

The "tracer principle" forms the basis of diagnostic nuclear imaging, and allows for probing of biological targets *in vivo* without inducing physiological effects. The *specific activity (SA)* of a radiotracer is defined as radioactivity relative to mass and depending on the biological target,

high SA (generally considered as >1 Ci·μmol^{-1}, >37 GBq·μmol^{-1}) may or may not be required at the time-of-injection.[29] Regardless, to be applicable to a variety of radiotracers and biological targets, a labeling method should be capable of delivering high SA products. For ^{11}C, SA can be compromised by sources of non-radioactive intermediates, usually arising from atmospheric CO_2. Similarly, the chemical purity of the product should be high to maintain a low total injected mass, especially if the impurities may have affinity to the target of interest, or pose a toxicity concern.[30,31] Ideally, radiolabeling reactions proceed with high yields and selectivity, such that separation of the product radiotracer from other species by HPLC purification is trivial. Consequently, it is advantageous to deploy precursors that have substantially different chromatographic/polarity profiles from the products and at low precursor loadings (<0.5–5 mg).

The only firm requirement for a PET labeling method with respect to *radiochemical yield (RCY)* is that *in vivo* studies are possible using convenient amounts of starting radioactivity (0.5–2 Ci, 18.5–74 GBq). For practical purposes, rapid synthetic processes are preferred with short-lived radionuclides and RCY of the entire process from isotope delivery (for ^{11}C, typically as [^{11}C]CO_2) to isolation of a sterile formulated product should be determined. Most radiopharmaceutical production is conducted within the constraints of a hot cell and automated synthesis module to ensure high levels of *reproducibility* and to take advantage of engineering controls to minimize radiation exposure to personnel. To validate a radiopharmaceutical production for human administration, it is typically repeated multiple times, according to a set protocol, to generate consistent RCY, SA, radiochemical and chemical purity, within a margin of variability, and passing several analytical tests to ensure doses are safe for injection.[31,32]

2. [^{11}C]CO_2-Fixation

2.1 Production and Handling of [^{11}C]CO_2

[^{11}C]CO_2 is a highly convenient reagent for use in radiolabeling, as it is nearly always the first carbon-11 product formed in a cyclotron target. [^{11}C]CO_2 is generated by proton bombardment of nitrogen gas (^{14}N(p,α)^{11}C) in the presence of small amounts of oxygen (typically 0.5%). [^{11}C]CO_2 can be used directly from the cyclotron, though trap-and-release purification and concentration steps are often included to remove excess oxygen and nitrous oxides that may interfere with chemical labeling reactions, and to control delivery flow rate and volume of the gaseous reagent. Two strategies exist for this process. Cryogenic purification involves condensing [^{11}C]CO_2 in a small volume vessel (often a steel tube) cooled by liquid nitrogen or argon and subsequent removal of condensable impurities by in-line chemical traps.[33] The second approach is to immobilize [^{11}C]CO_2 on a solid support such as molecular sieves,[34] with release for delivery into the reactor upon heating. The latter approach has recently been adapted for "on-cartridge" labeling and purification of fatty acids using Grignard precursors, for rapid production of [^{11}C]palmitic acid.[35] In all cases, it is prudent to equip delivery lines with terminal chemical traps for irreversible retention of [^{11}C]CO_2 (*e.g.,* soda lime, ascarite, charcoal) to prevent release of radioactivity.

[^{11}C]CO_2 is sparingly soluble in polar organic solvents, and this solubility can be dramatically improved by the presence of base. In many methodologies, organic nitrogenous bases, such as BEMP or DBU (Scheme 2) are used to trap [^{11}C]CO_2 in solution. Both appear to be highly efficient at trapping [^{11}C]CO_2 in DMF. Sequestering [^{11}C]CO_2 in this way without forming unreactive covalent bonds is appealing compared to [^{11}C]CO_2-fixation using strongly basic organometallic reagents, such as Grignard and organolithium reagents, in which trapping is

concomitant with C–^{11}C bond formation. Not only do the milder methods facilitate preparation of highly functionalized labeled compounds, but they also allow transamidation to other amines in solution.

Apparatus used for [^{11}C]CO_2-fixation should be rigorously assembled to afford a system that is leak-proof, anhydrous, free of air and constructed with efficient handling and well-controlled flow rates. Unlike other carbon-11 gaseous reagents, [^{11}C]CO_2 can easily become contaminated with atmospheric CO_2, which reduces the specific activity of the product. Fortunately, many [^{11}C]CO_2-fixation protocols are effective with relatively simple apparatus and carried out at room temperature and ambient pressure. High specific activity products are routinely achieved with good reproducibility. Radiochemical yields can also be very high with efficient incorporation levels, in part thanks to relatively rapid syntheses that do not require transformation of [^{11}C]CO_2 into other labeling intermediates. While many of the modern [^{11}C]CO_2-fixation reactions were developed using bespoke apparatus, commercially available and widely deployed synthesis apparatus (such as those for the preparation of [^{11}C]CH_3I) can be adapted for semi-automated catch-and-release of [^{11}C]CO_2.[36] Applications for [^{11}C]CO_2 will very likely continue to develop and become more commonplace in radiochemistry laboratories in the near future.

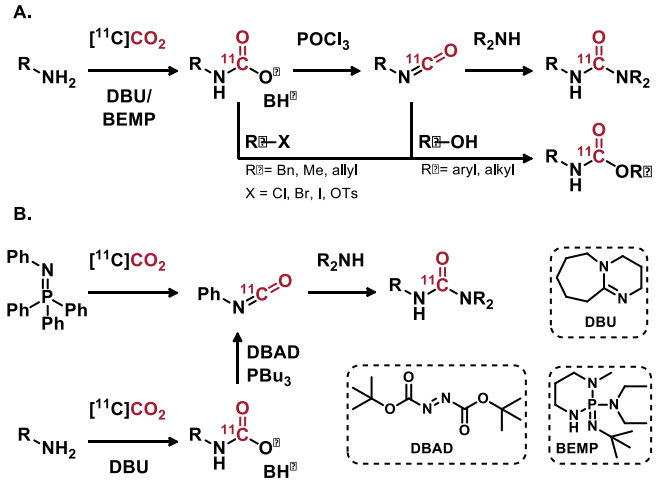
2.2 Methods for Radiotracer Synthesis

Owing to the low mass and concentration of [^{11}C]CO_2 in cyclotron target and delivery gas, efficient trapping of [^{11}C]carbon dioxide in the reaction mixture is crucial to obtaining high radiochemical yields of labeled products relative to starting radioactivity. Early examples of [^{11}C]CO_2-fixation were accomplished using highly reactive organometallic substrates (*i.e.*, Grignard and organolithium reagents) that react directly with [^{11}C]CO_2 to form C–^{11}C bonds. In terms of carbon–heteroatom bond formation, Chakraborty *et al.* reported the first synthesis of

[^{11}C]urea using lithium hexamethyldisilazide (LHMDS) to trap [^{11}C]CO_2 in a THF solution, followed by aqueous hydrolysis with ammonium chloride.[37] Unfortunately, this method is only applicable to the preparation of simple [^{11}C]urea, and derivatives such as [^{11}C-*carbonyl*]uracil by multi-step syntheses. Silanamines represent an alternative fixating agent for $^{11}CO_2$, and could be used to generate *O*-silyl carbamates, which could then be reduced to ^{11}C-methylamines using lithium aluminum hydride.[38]

In order to prepare more structurally complex ^{11}C-ureas, organic base-mediated [^{11}C]CO_2-fixation was pursued, inspired by mild, industrial-focused processes being developed contemporaneously.[23,39] In the first iteration, triethylamine in dichloromethane was used to facilitate ^{11}C-carboxylation of aniline and aliphatic amines, followed by dehydration with $POCl_3$ to generate ^{11}C-*carbonyl*-isocyanates.[40] Under these conditions, ^{11}C-isocyanates reacted immediately with the excess amine, to form symmetrical products including ^{11}C-*carbonyl*-ureas and ^{11}C-*carbonyl*-carbodiimides. While these results provided evidence for the desired reactivity using [^{11}C]CO_2, they also suggested that achieving selectivity for unsymmetrical ureas could be a practical challenge in this context. Whereas one could reasonably expect to saturate precursor amines using excess CO_2 in non-radioactive synthesis, analogous stoichiometry using no-carrier-added [^{11}C]CO_2 would demand very precise measurements and high dilution. To overcome these challenges, radiochemists then deployed organic bases such as DBU[41] and BEMP,[42] which could efficiently trap [^{11}C]CO_2 at ambient temperature and pressure, and at practical gas delivery flow rates (Scheme 2A). With BEMP, selective radiosynthesis of unsymmetrical ^{11}C-*carbonyl*-ureas was finally achieved. Formation of symmetrical products could be suppressed by using excess $POCl_3$, but this strategy also stipulated an even larger excess of the second amine nucleophile, for attack on the ^{11}C-isocyanate. Consequently, a high concentration reaction mixture would be

formed, which presented a challenge for purification. Fortunately, reducing the concentration of the initial amine (typically aliphatic primary and cyclic secondary amines) was found to be compatible with fast reaction times (≤2 min) and high radiochemical yields.[43]



Scheme 2. Synthesis of ^{11}C-ureas and -carbamates by [^{11}C]CO_2-fixation.

An alternative approach to isocyanates relied on phosphinimine precursors, prepared from azides or primary amines, for condensation with [^{11}C]carbon dioxide.[44] Phenyltriphenylphosphinimine could be used to prepare [^{11}C-*carbonyl*]phenylisocyanate, and a variety of unsymmetrical ^{11}C-ureas from aliphatic and aromatic amines (Scheme 2B).[45] In this case, [^{11}C]CO_2 was trapped in a THF solution cooled to −60 °C during gas delivery, followed by heating to 60 °C to complete the reaction. Given the high trapping efficiency in the absence of base, it is likely that [^{11}C]CO_2 forms a complex with either the phosphinimine or amine precursor. Mitsunobu chemistry could also be employed to convert ionic carbamates into isocyanates using phosphines and azo compounds (Scheme 2B).[46,47] In the presence of an additional amine, the isocyanate could be transformed *in situ* to a urea.[48] To accommodate this reaction to radiolabeling conditions, the reaction temperature was carefully controlled at 50 °C to promote the reaction and prevent

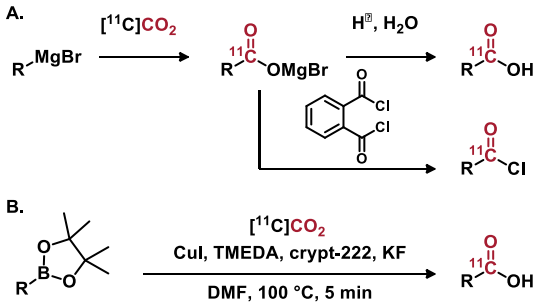
premature release of [^{11}C]CO_2.[49] Aliphatic and aromatic amines could be drawn on for ^{11}C-isocyanate formation, though secondary aliphatic amines were necessary for ^{11}C-urea formation to achieve selectivity for unsymmetrical products.[50]

Carbamates, like ureas, are attractive functional groups for drug and radiotracer design due to their stability *in vivo*, role as a linker of ligand fragments, and for drug-target interactions through the carbamate itself.[51] While ^{11}C-carbamates were previously radiolabeled using [^{11}C]phosgene or [^{11}C]carbon monoxide,[5] methods using [^{11}C]CO_2 are especially convenient for avoiding intermediate redox manipulation. Unlike ^{11}C-ureas, *O*-alkyl carbamates may be prepared without intermediate dehydration to form ^{11}C-isocyanates. Simply bubbling [^{11}C]CO_2 into a DMF solution of DBU or BEMP, an amine nucleophile, and a benzyl, allyl or methyl electrophile followed by heating produced high yields of *O*-alkyl [^{11}C]carbamates.[41,42] Excess or early addition of the methylating agent led to reduced yields of the desired compounds, suggesting the intermediacy of the carbamate ion.

In order to prepare *O*-substituted ^{11}C-carbamates not readily accessible by alkylation of carbamate ions, alcohols and phenols can be used to quench ^{11}C-isocyanates, as described above (Scheme 2A).[43] A wide variety of alcohols, including hindered and electron-deficient ones such as *tert*-butanol, and hexafluoroisopropanol[52] have been successfully incorporated into ^{11}C-carbamates by this method. Using amino alcohol precursors for [^{11}C]CO_2-fixation, oxazolidinones are formed at ambient temperature after dehydration with $POCl_3$.[53]

^{11}C-Carboxylic acids have been produced from [^{11}C]CO_2, using organometallic precursors, such as methylmagnesium bromide for [^{11}C]acetate and *n*-pentadecylmagnesium bromide for [^{11}C]palmitate.[54,55] This strategy is, however, limited to ^{11}C-carboxylic acids for which a stable

organometallic precursor can be prepared. More complex ^{11}C-carboxylic acid derivatives can be prepared by multi-step syntheses wherein magnesium ^{11}C-carboxylate intermediates are converted into acid chlorides and distilled prior to being used in amide bond formation.[56] For example, [^{11}C](+)-PHNO, a D_3-preferring agonist radiotracer,[57,58] was initially prepared in this manner from ethyl magnesium bromide.[59,60] However, the synthetic apparatus needed for multi-step reactions using Grignard reagent precursors are challenging to maintain and operate, often leading to high failure rates of radiosynthesis (see, for example, the remarkable preparation of [^{11}C]lapatinib[61]), and generate an strong impetus to develop alternative methods that are simpler to operate and provide greater reliability.[62,63]



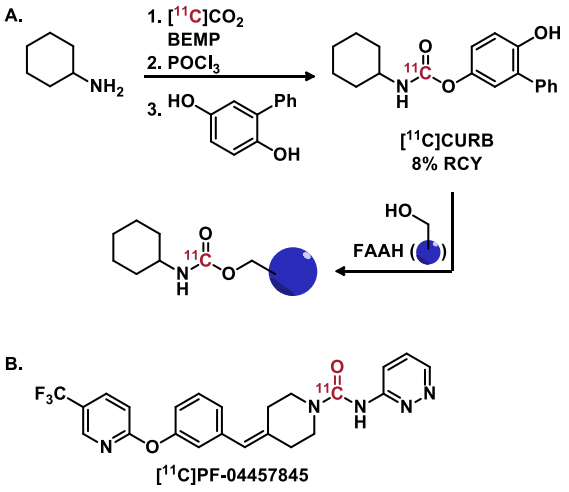
Scheme 3. [^{11}C]CO_2-fixation for the synthesis of carboxylic acids and acid chlorides using **A.** Grignard reagents and **B.** organoboron precursors and copper mediated coupling.

In the interest of expanding the utility of CO_2 as a feedstock for bulk and specialty chemicals, metal and organocatalytic approaches for CO_2-fixation to generate carboxylic acids and their derivatives have been keenly pursued.[64,65] Boronic esters have been shown to be amenable for [^{11}C]CO_2-fixation using a copper catalyst,[36] overcoming the significant challenge of much lower concentrations of [^{11}C]CO_2 available in the reaction mixture compared to CO_2 under non-radioactive conditions. As such, significant optimization was required, including shifting away

from alkoxide bases in favor of *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TMEDA), which acts both as a trapping agent for [^{11}C]CO_2 and a ligand for the copper catalyst. A variety of functional groups were tolerated under the optimized conditions and the ^{11}C-carboxylic acids could be converted to ^{11}C-*carbonyl*-esters and -amides, such as the bioconjugation reagent *N*-succinimidyl [^{11}C-*carbonyl*]4-fluorobenzoate ([^{11}C]SFB).[36]

2.3 Impact

Recently developed methods for [^{11}C]CO_2-fixation have led to a number of novel and important radiotracers for fatty acid amide hydrolase (FAAH), a serine hydrolase that regulates signaling at cannabinoid receptors CB_1 and CB_2 through metabolism of anandamide.[66] [^{11}C]CURB was designed in analogy to the selective and irreversible FAAH inhibitor and drug candidate URB597.[67] [^{11}C]CURB is an *O*-aryl carbamate prepared by [^{11}C]CO_2-fixation in 8% uncorrected radiochemical yield (RCY), with high specific activity (2.5 Ci·μmol^{-1}, 92.5 GBq·μmol^{-1}) 27 minutes after end-of-bombardment (Scheme 4A).[43] Having shown high brain penetration and target selectivity for FAAH in rats,[68] [^{11}C]CURB has now been translated for regional quantification of FAAH activity in the human brain, where it has revealed sensitivity to a functional single-nucleotide polymorphism (rs324420, C385A) that inactivates FAAH (Fig. 1).[69–71] Pfizer's urea-based irreversible FAAH inhibitor was radiolabeled as its direct isotopologue [^{11}C-*carbonyl*]PF-04457845 by [^{11}C]CO_2-fixation[72] in order to conduct biodistribution studies and confirm target engagement and irreversibility of binding to the enzyme in rats (Scheme 4B). Clinical translation of this tracer is also underway.



Scheme 4. A. Synthesis of [^{11}C]CURB, a covalent, irreversible radiotracer for fatty acid amide hydrolase (FAAH). **B.** Structure of unsymmetrical urea [^{11}C]PF-04457845, which inhibits FAAH by the same mechanism.

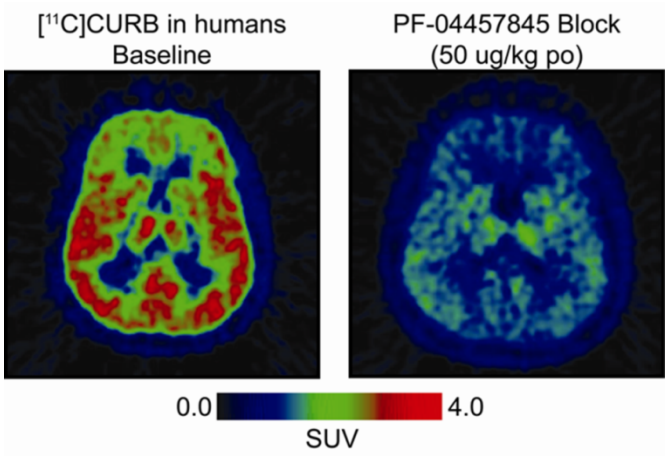


Fig. 1. Human neuroimaging of FAAH using [^{11}C]CURB. Baseline (left) and with pretreatment with a FAAH inhibitor (right). Reproduced from reference 73 with permission from the Society of Nuclear Medicine and Molecular Imaging, copyright 2014.

The potential and versatility of [^{11}C]CO_2-fixation for radiotracer discovery was demonstrated by a combinatorial approach to survey structure-activity relationships of *O*-aryl carbamates based

on the structures of URB597 and URB694 (Fig. 2). To begin, non-radioactive carbamates were prepared – from commercially available amines, bicyclic phenols, and phosgene – for *in vitro* FAAH assays and physicochemical evaluation.[74] Eight FAAH inhibitors were then radiolabeled with carbon-11 using a standard [^{11}C]CO_2-fixation protocol to generate ^{11}C-*carbonyl*-carbamate radiotracers for biodistribution studies. It was found that a linear *N*-alkyl substituent leads to increased potency over cyclic isomers and that the presence of a dihydrooxazole confers faster and greater brain uptake with lower nonspecific binding. These insights lead directly to the design of two fluorine-18 labeled FAAH radiotracers: [^{18}F]DOPP[75,76] and [^{18}F]FCHC.[77] Both show greater brain uptake *in vivo* and sensitivity to FAAH activity *in vitro* than [^{11}C]CURB. A similar combinatorial approach was implemented in the hopes of developing a monoacylglycerol lipase (MAGL) radiotracer, but unfortunately the radiolabeled compounds failed to show high brain uptake in rodents.[52] More recently, a sulfonamide-based MAGL inhibitor SAR127303 was labeled at the ^{11}C-carbamate position using [^{11}C]CO_2-fixation and demonstrated irreversible brain uptake with measurable specificity and selectivity for MAGL in rats.[78]

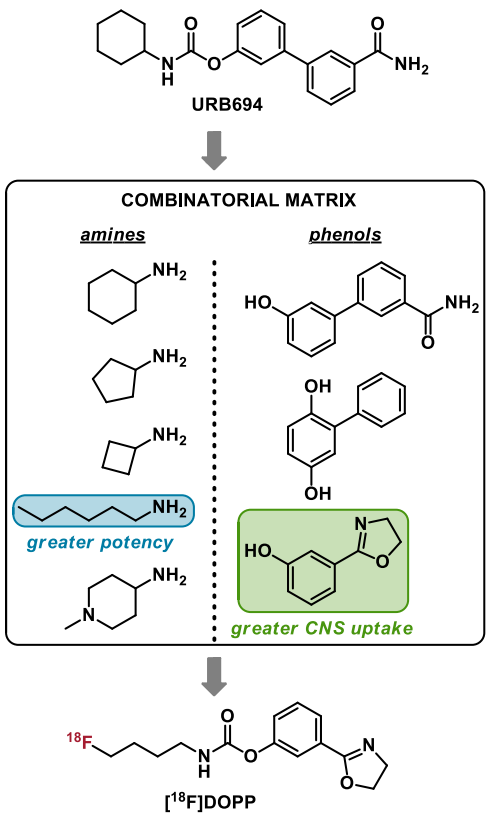


Fig. 2. Combinatorial [^{11}C]CO_2-fixation enabled the design of a fluorinated radiotracer for FAAH, [^{18}F]DOPP.

Previously designed radiotracers have also been prepared by [^{11}C]CO_2-fixation to improve their syntheses or *in vivo* imaging properties. The reversible monoamine oxidase B (MAO-B) PET radiotracer SL25.1188 was developed using [^{11}C]$COCl_2$, but this method presents technical challenges for widespread use.[79] In a bid to improve the accessibility and the isolated yield of [^{11}C]SL25.1188, the amino alcohol precursor was deployed in [^{11}C]CO_2-fixation and enabled translation for human PET imaging in clinical research studies (Fig. 3).[53,80] [^{11}C]GR103545 is a methyl carbamate PET radiotracer for κ-opioid receptors that was originally radiolabeled using [^{11}C-*methyl*]methyl chloroformate,[81] and later using [^{11}C]CH_3I[82] or [^{11}C]CH_3OTf[83] for methylation of the precursor carbamate anion. [^{11}C]CO_2-fixation presented an operationally

simple alternative to prepare [^{11}C-*carbonyl*]GR103545 at room-temperature in high yield (13% RCY) in 23 minutes including synthesis and purification.[42] To probe glycogen synthase kinase 3β (GSK-3β), a labeled urea [^{11}C-*carbonyl*]AR-A014418 was prepared by [^{11}C]CO_2-fixation[84] as an alternative to the original ^{11}C-methylation procedure.[85] Though AR-A014418 was found to have low brain uptake, [^{11}C]CO_2-fixation presents an opportunity for testing analogues of the same scaffold without mandating methyl ethers.

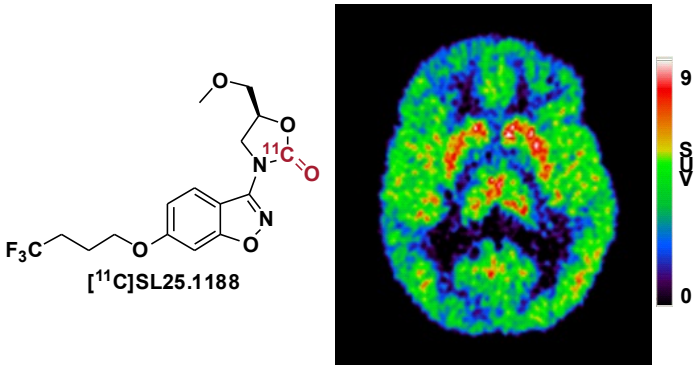


Fig. 3. Structure and human PET neuroimaging of [^{11}C]SL25.1188 for MAO-B. Reproduced from reference 73 with permission from the Society of Nuclear Medicine and Molecular Imaging, copyright 2014.

With hopes of imaging 5-HT_{1A} receptors in the brain, the ^{11}C-*methyl* isotopologue of WAY-100635 was prepared and evaluated *in vivo* (Fig. 4). However the major metabolic pathway, amide hydrolysis in the periphery, releases an amine (M1) that crosses the blood-brain barrier and engages in specific and nonspecific interactions. Quantitative imaging using [^{11}C-*methyl*]WAY-100635 is therefore confounded by [^{11}C]M1.[86] Using a multi-step synthesis involving [^{11}C]CO_2-fixation with a Grignard reagent precursor, imaging with [^{11}C-*carbonyl*]WAY-100635 leads to lower observed nonspecific uptake in the tissue of interest and superior kinetics for target quantification, as the major radiolabeled metabolite [^{11}C]M2 is

ionized and does not enter the brain (Fig. 4).[87,88] In this case, the ^{11}C-carbonyl radiopharmaceutical was made necessary by troublesome radiometabolites, and ^{11}C-carbonyl labeling[89,90] meant WAY-100635 could be rescued for clinical research imaging studies.

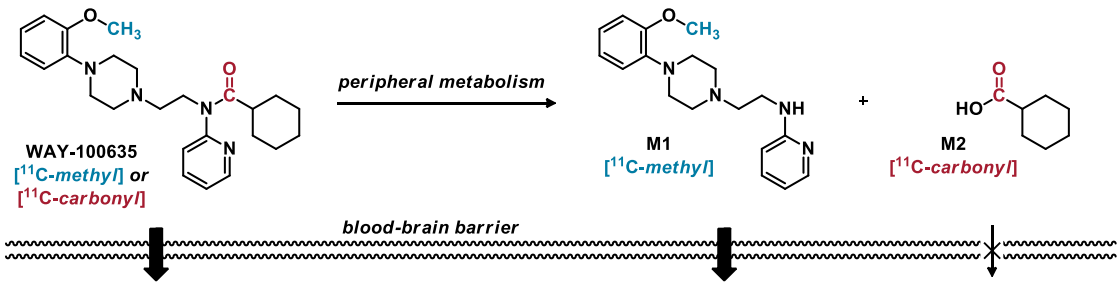


Fig. 4. Peripheral metabolism of the 5-HT$_{1A}$ receptor antagonist WAY-100635 produces a nonpolar (M1) and a polar (M2) metabolite. If the tracer is labeled at the methyl position (blue), M1 will be radioactive and confound imaging as it enters the brain and engages in specific and nonspecific interactions. If the tracer is labeled at the carbonyl position (red), the parent compound accounts for most of the PET signal in the brain, since the radioactive metabolite M2 is ionized at physiological pH and does not cross the blood-brain barrier.[88]

Numerous other drugs and pharmaceutical candidates have been radiolabeled by [^{11}C]CO_2-fixation for biodistribution and pharmacokinetic evaluation. These include a serotonin receptor antagonist, metergoline;[41] the histone deacetylase (HDAC) inhibitor MS-275 (entinostat);[91] and an anti-cancer therapeutic and carbamate analogue of camptothecin, irinotecan.[92] In order to radiolabel irinotecan, both [^{11}C]CO_2-fixation and [^{11}C]$COCl_2$ were evaluated. Specific activities and time-of-synthesis were comparable, but the yield of [^{11}C]irinotecan using [^{11}C]CO_2-fixation was nearly twice that of the ^{11}C-phosgenation method.

Bexarotene, a synthetic retinoid X receptor (RXR) agonist, is currently in clinical trials for treatment of Alzheimer's disease, and a brain-penetrating, RXR-selective radiotracer could assist in ApoE-mediated management of brain amyloidosis.[93] The ^{11}C-carboxylic acid isotopologue has been synthesized by copper-mediated [^{11}C]CO_2-fixation and used for PET-MR imaging in non-human primate to demonstrate brain uptake.[94] Further imaging studies with [^{11}C]bexarotene are underway in our laboratories to assess specific binding and occupancy.

3. Radiochemistry with [^{11}C]$COCl_2$

[^{11}C]Phosgene has played a relatively modest but continuous and manifest role in the history of PET radiochemistry. First reported in the 1970s, [^{11}C]$COCl_2$ was proposed as a useful complement to the already existing one-carbon precursors [^{11}C]CO_2, [^{11}C]CH_3I, [^{11}C]CH_3OTf, H[^{11}C]CHO, and H[^{11}C]CN. While these latter carbon-11-labeled reagents were mainly used for the introduction of ^{11}C-*methyl* or ^{11}C-*carboxylate* groups, [^{11}C]$COCl_2$ allowed the insertion of a labeled carbonyl between two heteroatoms without the need for dehydration, redox operations, or transition metal catalysis. ^{11}C-Carbonyl heterocycles are directly accessible using [^{11}C]$COCl_2$ and represent several key radiotracers including ^{11}C-imidazolones (NMDA inhibitor and β-adrenoreceptor radioligands), ^{11}C-oxazolidinones for each of MAO-A and MAO-B, and an ^{11}C-oxazolidindione for *in vivo* intracellular pH sensing.[95]

3.1 Production and Handling of [^{11}C]$COCl_2$

Despite its advantageous reactivity, [^{11}C]$COCl_2$ has never become as generally available as [^{11}C]CO_2, [^{11}C]CH_3I, or, later, [^{11}C]CH_3OTf, because of its rather complicated production that requires intensive upkeep of specialized apparatus, including replacement of key components prior to each use. Still, improvements in methods for preparation of this reagent have maintained

a constant presence in the PET chemistry literature throughout the years. Table 1 compiles the principle methods used up to now for producing [^{11}C]$COCl_2$. These methods are all two-step-based processes, starting from cyclotron-produced [^{11}C]CO_2 or [^{11}C]CH_4, the latter being also available by reduction of [^{11}C]CO_2 with hydrogen over a nickel catalyst.

The first two methods were developed in the late 1970s and rely on the intermediate preparation of [^{11}C]CO, which was most often generated by reduction of [^{11}C]CO_2 on zinc at 400 °C. In the first case, [^{11}C]CO was passed through a quartz tube coiled around a strong UV lamp while chlorine gas was mixed in to concentrations of 15–50%.[96,97] [^{11}C]$COCl_2$ was produced in up to 90% radiochemical yields (decay-corrected) and easily separated from Cl_2 by passing the mixture through antimony or amalgamated copper. The second method was a non-photochemical one, producing [^{11}C]$COCl_2$ in 30–50% RCY.[98–102] For this, cryogenically concentrated [^{11}C]CO was swept by a carrier gas through $PtCl_4$ that was heated up to 430 °C at the last moment. Technical problems and a certain propensity to failure were often reported together with relatively low measured specific radioactivities.[103,104]

Beginning in the 1980s, [^{11}C]CO was abandoned for producing [^{11}C]$COCl_2$ and four alternative methods were described using [^{11}C]CCl_4 as the common intermediate.[105–112] Invariably, the first step consisted of mixing [^{11}C]CH_4 with a certain amount of Cl_2 and passing the mixture through a heated glass or quartz tube filled with cupric chloride on pumice stone,[105,106] empty[109,110,112] or filled with glass beads.[107] Using the latter, >90% RCY for the production [^{11}C]CCl_4 been reported,[111] higher than the 60–65% with the catalyst.[105] The second step consisted of on-line transformation of [^{11}C]CCl_4 into [^{11}C]$COCl_2$. For this, [^{11}C]CCl_4 was swept through a second heated tube normally containing iron filings[105,106] or an iron oxide/iron mixture,[109,110] but also an empty quartz tube at 750 °C is effective, as trace oxygen in the carrier gas or available at the hot

glass or quartz surface were proven to be sufficient to produce [^{11}C]$COCl_2$.[112] Finally, a commercially available tube originally designed for the measurement of trace amounts of CCl_4 in air and containing an immobilized mixture of I_2O_5 and fuming H_2SO_4 (serving here as the oxygen source) effects the transformation at room temperature in the highest yield reported so far (80%).[111] All methods use an antimony guard placed at the end of the flow system for trapping excess Cl_2.

[^{11}C]$COCl_2$ is well soluble at trace levels in various organic solvents (CH_3CN, toluene, CH_2Cl_2, $CHCl_3$, Et_2O), and is usually directly trapped at room temperature or below in a solution containing the labeling precursors. As it is the case for the use of other carbon-11-labeled gases (such as [^{11}C]CO_2), apparatus designed for the use of [^{11}C]$COCl_2$ needs to be rigorously leak-proof, and constructed with efficient handling and well-controlled flow rates. However, if properly produced, the use of [^{11}C]$COCl_2$ remains of interest for the reactivity and high specific radioactivity that can be achieved.

Table 1. [^{11}C]$COCl_2$: a compilation of selected practical methods for its production.

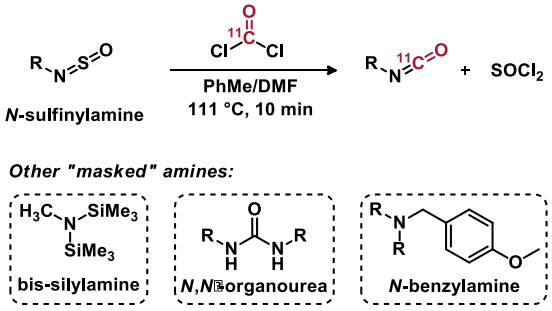
Primary precursor	Conditions (Step 1)	Intermediate product	Conditions (Step 2)	[^{11}C]$COCl_2$ Yield[a] (time)	Refs
[^{11}C]CO_2	Zn 400 °C	**[^{11}C]CO**	Cl_2, hν	80–90% (25 min)	96,97
			$PtCl_4$ 430 °C	30–50% (15–22 min)	98–101
[^{11}C]CH_4	Cl_2, $CuCl_2$ 380 °C *or* Cl_2, empty tube or glass beads 510–560 °C	**[^{11}C]CCl_4**	Fe 360 °C	35–65% (15–20 min)	105–107
			Fe/Fe_2O_3 320 °C		109,110
			Empty tube 750 °C	35% (12–13 min)	112
			I_2O_5/H_2SO_4 fum. rt	80% (10 min)	111

[a] decay-corrected

3.2 Methods for Radiotracer Synthesis

The reactivity of [^{11}C]$COCl_2$ is well-characterized as an activated ^{11}C-carbonylating reagent. Among the earliest applications for [^{11}C]$COCl_2$ included treatment with aqueous ammonia to generate [^{11}C]urea,[113] which itself served as a precursor to labeled barbituric acids[114] and nucleosides such as thymidine.[107] Similarly, symmetric ^{11}C-carbonates and ^{11}C-ureas are readily prepared from [^{11}C]$COCl_2$,[96] as well as the heterocycles hydantoins[114] and imidazolones.[99] Unsymmetrical acyclic carbamates or ureas present a greater challenge using [^{11}C]$COCl_2$ due to the highly imbalanced stoichiometry of the reactions; ^{11}C-isocyanates are readily prepared by treatment of [^{11}C]$COCl_2$ with an alkyl amine, however these are highly susceptible to excess amine present to generate symmetrical products.[115] This is particularly true with low molecular weight and gaseous amines, as well as amine salts which are difficult to accurately dispense at

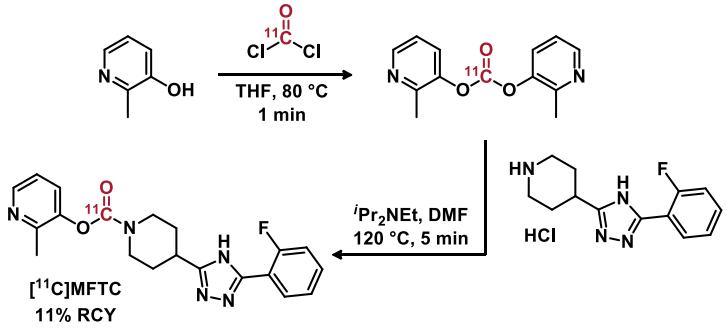
microscale levels.[108] Overcoming this obstacle has been a major emphasis for researchers and led to the development of "masked" amines as precursors to ^{11}C-isocyanates or ^{11}C-carbamyl chlorides, which can then be used for acylation of other nucleophiles. One such precursor class is *N*-sulfinyl amines, which are converted to ^{11}C-isocyanates by heating with [^{11}C]$COCl_2$ in anhydrous toluene (50–70% RCC, >1 Ci·μmol^{-1}, 37 GBq·μmol^{-1}) (Scheme 5).[108] Symmetrical ureas could be used in a similar manner, albeit in lower yields, and surprisingly without diminished specific activity products.[108] *N*,*N*-bis(trimethylsilyl)methylamine has been demonstrated to be an effective precursor to methyl [^{11}C-*carbonyl*]isocyanate, proceeding in 55–60% RCC upon warming the cooled mixture to room temperature.[115,116] Secondary amine precursors can be effectively "masked" as tertiary benzylamines, using a phosgene-promoted debenzylation protocol.[117] In the presence of [^{11}C]$COCl_2$, ^{11}C-carbamyl chlorides were generated, which could then be treated with amines, alkoxides, Grignard reagents or arylcuprates to produce ^{11}C-ureas, -carbamates, or -amides, respectively.



Scheme 5. Preparation of isocyanates from [^{11}C]$COCl_2$ using "masked" amines.

Finally, the high reactivity of [^{11}C]$COCl_2$ can be tempered by using it for the formation of less reactive ^{11}C-carbonate intermediates. To complete the synthesis of an *O*-aryl-^{11}C-carbamate, [^{11}C]MFTC, Zhang and co-workers first prepared the symmetrical diaryl ^{11}C-carbonate, which

could undergo transamidation directly into the desired labeled product by treatment with the requisite amine (Scheme 6).[118] This represents a strategy for preparing ^{11}C-carbamates from [^{11}C]$COCl_2$ using commonly available precursors, such as phenols and secondary amines.



Scheme 6. Synthesis of [^{11}C]MFTC by way of a symmetrical ^{11}C-carbamate intermediate.

3.3 Impact

In order to study the mechanism of action of the anticancer drug temozolomide, two isotopologues were prepared for human PET imaging studies from [^{11}C]methylisocyanate ([^{11}C]MIC). [^{11}C-*carbonyl*]MIC was prepared from [^{11}C]$COCl_2$ using the "masked" amine approach and used in the synthesis of [^{11}C-*carbonyl*]temozolomide (Fig. 5). *In vivo*, this isotopologue was converted to [^{11}C]CO_2 to a greater degree than the ^{11}C-*methyl* labeled drug.[116,119] These studies confirmed that the temozolomide undergoes decarboxylation *in vivo* to release a highly reactive methyldiazonium ion for DNA alkylation. Labeled hydantoins, such as [^{11}C]phenytoin can be readily prepared from [^{11}C]$COCl_2$ by condensation with *α*-aminoamides.[114] This straightforward synthesis is readily amenable to preparation of a wide variety of derivatives, and the biodistribution of [^{11}C]phenytoin was evaluated in epilepsy patients.[120]

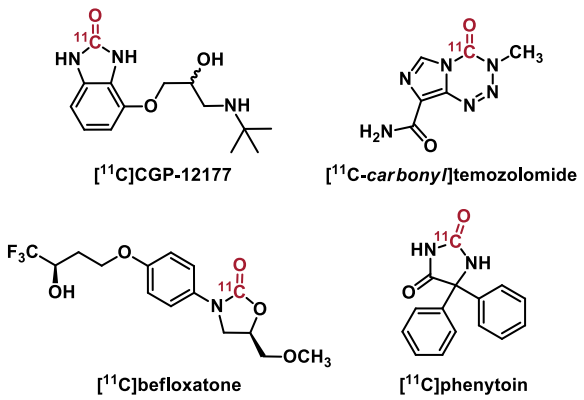


Fig. 5. A selection of radiotracers prepared from [^{11}C]$COCl_2$ that have advanced for human PET imaging.

Befloxatone is an oxazolidinone-based selective and reversible inhibitor of monoamine oxidase A (MAO-A), and was first radiolabeled for PET using [^{11}C]$COCl_2$ in high yield and specific activity in a single step from the corresponding amino alcohol precursor.[121,122] In preclinical imaging studies, [^{11}C]befloxatone has been used for cardiac imaging after tobacco smoke inhalation,[123] and also demonstrated highly specific uptake in the brain, reversibility and high selectivity for MAO-A over MAO-B, all properties that indicated a high chance of success for human imaging studies.[124] Indeed, [^{11}C-*methyl*]befloxatone is currently used in human imaging research.[125] As discussed earlier, reversible oxazolidinone MAO-B radioligands [^{11}C]SBox-13 and [^{11}C]SL25.1188 were originally developed using [^{11}C]$COCl_2$,[79,126,127] but improvements in the synthesis of the latter compound using [^{11}C]CO_2-fixation have enabled human translation (Fig. 3).[53,80] An imidazolone β-adrenergic receptor antagonist (CGP-12177) was radiolabeled using [^{11}C]$COCl_2$ from the diamine precursor,[106] and has shown utility for imaging this target in myocardium and pulmonary tissue.[128] The synthesis was considered a major challenge to widespread use of this radiotracer, to the point where attempts were made to design simpler

derivatives for radiolabeling.[129] More recently, improvements in handling of [^{11}C]$COCl_2$ appear to have made this radiopharmaceutical available at some sites.[110,130]

Efforts to develop a selective radiotracer for NR2B-containing NMDA receptors have heavily relied on [^{11}C]$COCl_2$ for radiolabeling of antagonists, which typically contain a benzoxazolone or benzimidazolone pharmacophore.[131–133] This approach has allowed for rapid and direct radiosynthesis of candidate ligands to be evaluated *in vivo*, although to date a successful radiotracer for this target has proven elusive. [^{11}C]Phosgene continues to play a significant role in development of next-generation FAAH radiotracers. Iterative structural refinements of carbamate and urea-based ^{11}C-ligands have produced [^{11}C]MFTC, which shows unfavourable nonspecific uptake, and more recently [^{11}C]DPFC, which suffers from slow brain uptake kinetics.[118,134] A comprehensive survey of the [^{11}C]$COCl_2$ literature would identify several more applications of its use for synthesis of radiolabeled molecules, including urea, thymidine,[107] and heterocyclic ligands for numerous targets. Ultimately, however, obstacles to the widespread routine use of [^{11}C]$COCl_2$ have curtailed its use, and instead directed efforts to more routine labeling strategies and alternative radiotracers, such as [^{18}F]fluorothymidine ([^{18}F]FLT).

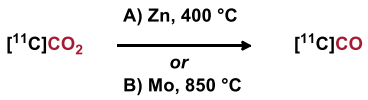
4. [^{11}C]CO Carbonylation

[^{11}C]Carbon monoxide has been used for carbonylation and preparation of myriad ^{11}C-ketones, -amides, -ureas, -carboxylic acids, and -esters.[135] This versatile reactivity is enabled by transition metal catalysts adept at coordination of CO and facilitating insertion and reductive elimination of C–C, C–N, and C–O bonds. The major obstacle to radiosynthesis with [^{11}C]CO is its low solubility in organic solvents, which is exasperated by the trace amounts available. Similar to

[^{11}C]$COCl_2$, many of the major advances in [^{11}C]CO radiochemistry have addressed the technical challenges of making the reagent available for chemical transformations in solution.

4.1 Production and Handling of [^{11}C]CO

Carbon monoxide is most commonly prepared in good yields with minimal processing time by passing cyclotron-produced [^{11}C]CO_2 in a stream of helium or nitrogen carrier gas through a heated quartz tube containing zinc (400 °C) or molybdenum (850 °C) (equation 1). Despite its ease of preparation, its utility in labeling reactions has only become more widespread over recent years after the development of methods to overcome the poor solubility of [^{11}C]CO in organic solvents: repeated recirculation of carbon monoxide through the reaction solution; the use of high pressure autoclaves; and the development of carbon monoxide trap-and-release reagents.



(*1*)

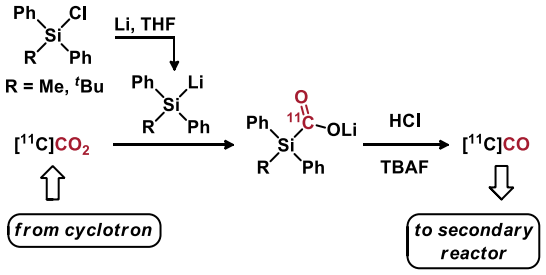
Recirculation of [^{11}C]CO through the reaction solvent results in a 4–10-fold increase in trapped radioactivity compared to single-pass trapping to achieve efficiencies in excess of 60%.[136] A micro-autoclave system was designed with quantitative [^{11}C]CO trapping efficiency and to facilitate radiolabeling at high pressure (35 MPa) and temperature (up to 200 °C).[137] Using this approach [^{11}C]CO was released into a small volume reactor (250 μL) containing reactants and catalyst that was pressurized with an HPLC pump and could be heated to carry out labeling reactions. A technically simpler system based on a commercially available HPLC injector has also been developed.[138] Flow-through systems for aminocarbonylation have also been developed, including disposable microreactors loaded with immobilized silica-supported palladium catalysts as well as gas-liquid segmented microfluidic reactors.[139,140] However, with the desire to achieve

wide dissemination within the field, much subsequent activity has focused on the development of low pressure molecule-mediated CO trap-and-release agents for ^{11}C-labeling applications.

The conversion of [^{11}C]CO into solvent-soluble adducts was first reported using a solution of THF·BH_3 leading to the 'on-line' formation of BH_3·^{11}CO (b.p. -64 °C), which could be trapped in organic solvents at ambient temperature and pressure with >95% efficiency for subsequent use in palladium-mediated reactions.[141] Commercially available copper(I) tris(pyrazolyl)borate ligands (so-called 'scorpionate' ligands) were found to be effective trap-and-release agents, relying on the coordination of [^{11}C]CO with the central copper(I) ion.[142] These complexes are technically simple to produce, enable quantitative CO trapping, and are compatible with suitable catalysts and substrates for ^{11}C-carbonylation in conventional batch reactions and in microfluidic syntheses.[143] The high solubility of $Xe_{(g)}$ in organic solvents can be exploited to process [^{11}C]CO with a technically simple procedure.[144] Using $Xe_{(g)}$ to transfer pre-concentrated [^{11}C]CO to a glass reaction vial containing a solution of an appropriate catalyst and substrates resulted in high trapping efficiency and radiochemical yields of labeled products upon heating. Pd complexes possessing high CO trapping efficiency, (*e.g.*, Pd-xantphos) have been shown to trap [^{11}C]CO from helium of nitrogen gas streams at ambient pressure producing ^{11}C-*carbonyl* labeled products in excellent yield and without any need for high pressure infrastructure.[145] Given the simplicity of accommodating these solutions into existing infrastructure for preparing and handling [^{11}C]CO, $Xe_{(g)}$ as well as Pd-xantphos complexes and selenium-TBAF mixtures[146] (*vide infra*) appear to be practical and effective solutions to the solubility problem, and are likely to see widespread future application.

Another recent advance that should enable easier access to [^{11}C]CO in radiochemistry labs is its facile production from [^{11}C]CO_2 through [^{11}C]silacarboxylic acid intermediates, which obviates

the need for specialized equipment to achieve this reduction.[147,148] [^{11}C]CO_2 is trapped in solution by lithium silane chloride complexes and [^{11}C]CO is then rapidly released in a small volume and at practical flow rates upon addition of TBAF (Scheme 7). In comparison to metal catalyzed reduction methods, [^{11}C]CO can be produced for ^{11}C-aminocarbonylation with improvements in speed and efficiency of the process, without the need for specialized apparatus and infrastructure.

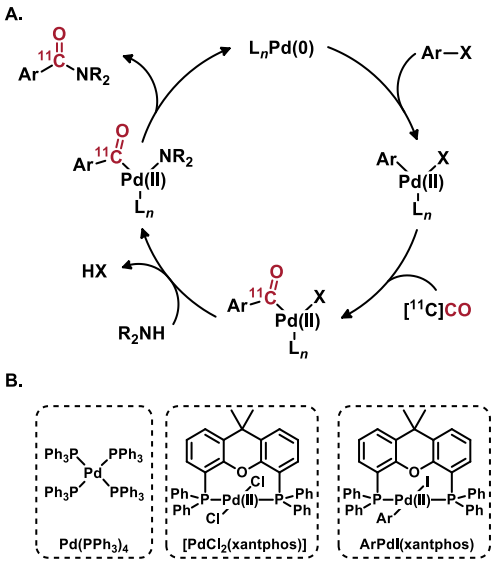


Scheme 7. Preparation of [^{11}C]CO from [^{11}C]CO_2 using lithium silane reagents.

4.2 Methods for Radiotracer Synthesis

The chemistry of carbon monoxide gives access to a plethora of carbonyl compounds of biological interest. Initial carbonylation reactions using organoborane or organolithium reagents and yielding labeled aliphatic ketones,[149] alcohols,[150] or carboxylic amides[151] demonstrated the potential for radiolabeling, but were not widely used in the preparation of PET radiopharmaceuticals due to poor trapping efficiency of [^{11}C]CO in organic solvents, even at low temperatures. Over the last two decades, the versatility of transition metal-mediated carbonylation reactions has continued to be translated to use in ^{11}C-radiochemistry. Though a variety of transition metals have been utilized, palladium catalysts have played a dominant role and of these, $Pd(PPh_3)_4$ has been by far the most commonly utilized catalyst for CO insertion reactions. Oxidative addition of aryl halides or their equivalents to Pd(0) is followed by CO-

insertion, coordination of a suitable nucleophile to the Pd(II) centre and finally reductive elimination to release catalyst and product (Scheme 8). Of Pd(0) catalysts, Pd-xantphos was surfaced from a screening array for its superior catalytic activity at relatively lower temperatures giving excellent radiochemical yields in aminocarbonylation.[152] Recently, Pd(II)-aryl precursors have been developed as isolable stoichiometric reagents for aminocarbonylation to directly prepare labeled amides rapidly and with high radiochemical purity from [^{11}C]CO.[153] In this case as well, Pd-xantphos complexes appeared to be among the most reactive precursors, though electron-deficient aryl precursors demanded Pd-P(*t*-Bu)$_3$ to prevent arene scrambling with the with phosphine ligand. This approach appears to be very promising, though the need to balance each of [^{11}C]CO trapping efficiency, Pd-aryl complex reactivity, and amine nucleophilicity, all of which are highly dependent on the precursor, suggests that significant optimization may be important for each substrate.

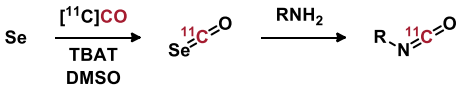


Scheme 8. A. General mechanism for Pd-mediated aminocarbonylation with [^{11}C]CO. **B.** Commonly used Pd-complexes for ^{11}C-aminocarbonylation, including isolated arylpalladium precursors.

There is considerable potential in the use of non-palladium based catalysts, however the scope of these has only been explored to a limited extent.[154] Rh(I) catalysts, for example, have been deployed to produce small collections of ^{11}C-ureas by aminocarbonylation in the presence of aryl or sulfonyl azides, including VEGFR-2 ligands.[155,156]

Selenium mediated carbonylation has positioned [^{11}C]CO as a possible replacement for [^{11}C]COCl$_2$ for synthesis of cyclic or acyclic ^{11}C-ureas or carbamates from similar precursors.[146] Indeed, the initial report developing this chemistry featured a labeled oxadiazolone, [^{11}C]SBox-13, previously only prepared from [^{11}C]COCl$_2$. These reactions are suggested to proceed through [^{11}C]carbonyl selenide that undergoes substitution with an amine to produce ^{11}C-isocyanate intermediates that are susceptible to a second incoming nucleophile (Scheme 9). In order to improve the solubility of Se, tetrabutylammonium fluoride (TBAF) in DMSO was used as the

reaction medium in a microautoclave. Under these conditions trapping efficiency often exceeded 90% with substrate dependent and typically good radiochemical conversions.



Scheme 9. Selenium-mediated preparation of ^{11}C-isocyanates from [^{11}C]CO

Alkyl radicals generated using UV light and photosensitizers have been used to perform carbonylation with [^{11}C]CO to obtain ^{11}C-labeled amides, esters and carboxylic acids using aliphatic iodides and nucleophiles.[157–159] Detailed mechanistic studies suggest key roles for solvent in initiating radical sequences and facilitating formation of acyl iodide intermediates. Despite the apparently limited functional group tolerance, this method represents a novel and appealing approach for ^{11}C-carbonyl labeling.

4.3 Impact

Despite the flexibility of its synthetic applications, the use of [^{11}C]CO for PET radiopharmaceutical development has to date been quite limited. Very few radiotracers prepared from [^{11}C]CO have advanced for human imaging studies, perhaps due to the low availability of this reagent worldwide and the bespoke apparatus that have typically been required for practical handling. One notable exception is [^{11}C]zolmitriptan, an oxazolidinone 5-HT$_{1B/1D}$ receptor agonist prepared by Se-mediated ^{11}C-carbonylation in an autoclave (Fig. 6).[160,161] Zolmitriptan is indicated for acute treatment of migraines, and the ^{11}C-isotopologue was used to study uptake kinetics and distribution in healthy volunteers.

A selective NPY Y5 receptor antagonist, [^{11}C]MK-0233 is a lactone radiolabeled by palladium mediated carbonylation. This compound has been utilized for measuring drug occupancy of the

target in the non-human primate brain, but does not appear to have seen further adoption in academic or clinical research. [^{11}C]Benzyl acetate was proposed as a superior probe for glial acetate metabolism than [^{11}C]acetate, due to its higher potential for brain uptake. Indeed, [^{11}C]BnOAc, prepared from iodomethane, [^{11}C]CO, and benzyl alcohol by palladium-catalyzed carbonylation, displays relatively high peak brain uptake in non-human primate and could find utility in imaging.[162]

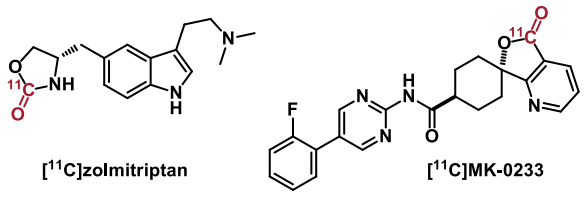


Fig. 6. Relatively few radiotracers developed using [^{11}C]CO have undergone translational imaging.

A number of other labeled compounds have been prepared using [^{11}C]CO, including [^{11}C-*carbonyl*]raclopride (which shows similar imaging properties to the more common ^{11}C-*methyl* isomer[163]), the RXR agonist [^{11}C]Am80,[164] and an H3 receptor antagonist.[148] Several radiotracer development efforts have attempted to leverage the combinatorial potential of metal-catalyzed ^{11}C-carbonylation for targets including TSPO, EGFR, VEGFR-2, VAChT, and TG2.[155,165–168] It is hoped that recent advances in production and handling of [^{11}C]CO will unlock this highly versatile reagent for greater use in novel radiotracer development efforts in the near future.

5. [^{11}C]HCN Cyanation

Carbon-11 carbonyl groups have long been prepared by substitution with [^{11}C]HCN, followed by hydrolysis to give ^{11}C-amides or -carboxylic acids. This strategy has most notably been applied

for the radiosynthesis of ^{11}C-carbonyl amino acids and derivatives. Recent examples include the preparation of [^{11}C]glutamine for metabolic imaging of tumors, [^{11}C]leucine for amino acid transport, and [^{11}C]auxins for plant imaging. This route is also ideal for preparation of ^{11}C-benzamide derivatives, whose applications include non-human primate imaging with tuberculosis therapeutics isoniazid and pyrazinamide and human imaging with the CNS penetrant κ-opioid receptor antagonist radiotracer [^{11}C]LY2795050.[169,170]

5.1 Production and Handling of [^{11}C]HCN

The first practical method for producing carrier-free [^{11}C]HCN was reported in 1973,[171] and continues to form the basis of similar production processes in use today. [^{11}C]CH_4, produced in the cyclotron target or prepared by passing [^{11}C]CO_2 with hydrogen gas over a nickel catalyst at ca. 400 °C is converted in-line to [^{11}C]HCN (or more accurately [^{11}C]NH_4CN) by introduction of ammonia gas and passing the gas mixture over a platinum catalyst at high temperature (ca. 900 °C) (equation 2).

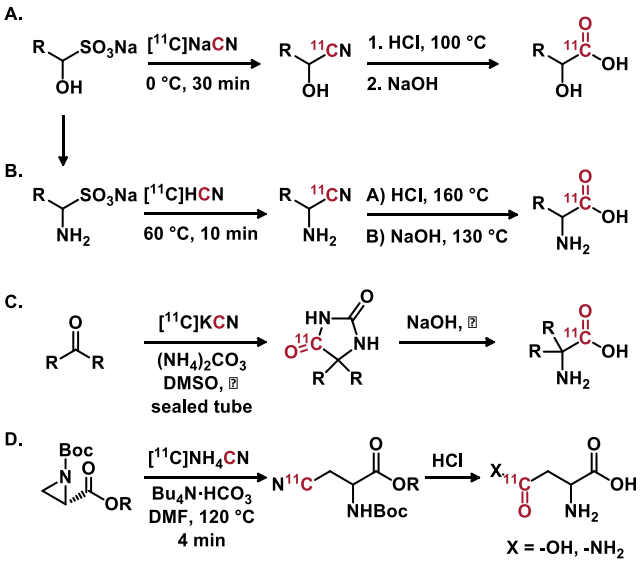
(*2*)

Compared to other ^{11}C labeling reagents discussed above, [^{11}C]HCN is relatively easy to capture in a small volume of organic solvent (*e.g.*, DMSO) at atmospheric pressure. A principal challenge is the exclusion of excess NH_3 that may be incompatible with the precursor or other reaction components, but this can be partially improved upon using in-line P_2O_5 traps.

5.2 Methods for Radiotracer Synthesis

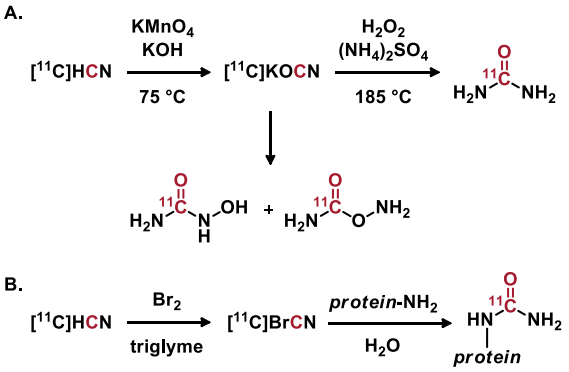
Cyanide is a highly polarized soft base that readily participates in nucleophilic substitution reactions with aliphatic halides or quaternary ammonium salts to furnish organic nitriles, which

themselves can be transformed under mild conditions to amides, aldehydes, amines, or carboxylic acids.[172] This versatility allows for facile functionalization and derivatization for development of ^{11}C-*carbonyl* radiotracers. Among the earliest radiochemical applications of [^{11}C]HCN was substitution of aldehyde–bisulfite adducts to prepare ^{11}C-cyanohydrin intermediates en route to ^{11}C-methyleneamines[173] or ^{11}C-hydroxycarboxylic acids (Scheme 10A).[174] ^{11}C-*Carbonyl*-amino acids could be prepared by the same strategy using modified Bücherer-Strecker chemistry,[175,176] (Scheme 10B) or by enzymatic methods.[177–179] Multicomponent reactions using [^{11}C]cyanide salts have proven fruitful for radiolabeling and radiotracer development, particularly in the case of hydantoins, accessed directly from [^{11}C]cyanide salts using the Bücherer-Bergs reaction (Scheme 10C).[180–182] Aziridines represent an alternative electrophile for radiolabeling with [^{11}C]HCN. In a single report, ring-opening of aziridine-2-carboxylates was employed to prepare ^{11}C-*β*-cyano-amino acids, which were transformed into [^{11}C-*carbonyl*]asparagine and aspartic acid (Scheme 10D).[183]



Scheme 10. Conversion of [^{11}C]cyanide to amino acids and hydroxyacids.

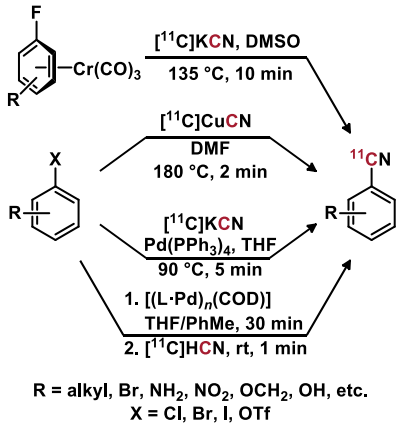
Several useful labeled intermediates are available from [^{11}C]cyanide. Conversion of [^{11}C]HCN to [^{11}C]urea[184,185] provides a labeled precursor for heterocycles including hydantoins[186] and nucleosides.[187] Oxidation of K^{11}CN with potassium permanganate produces the ^{11}C-cyanate salt, which can be converted into hydroxyurea or isohydroxyurea (Scheme 11A).[188] [^{11}C]HCN can be converted into an electrophilic ^{11}C-radiolabeling reagent, [^{11}C]cyanogen bromide by treatment with bromine (Scheme 11B).[189] This intermediate could be used to label a range of small molecules, including guanidines,[190] and biomolecules such as polysaccharides[191] and proteins.[192]



Scheme 11. Reactions of [^{11}C]cyanate and [^{11}C]cyanogen bromide.

Cyanide coordinates extremely well with soft metals like Cu^{+}, Pd^{2+}, Pt^{2+}, a property that assists metal catalyzed cyanation of aryl and vinyl halides (Scheme 12). The first report of ^{11}C-cyanation of arenes using transition metals utilized aryl fluoride–chromium tricarbonyl complexes as precursors for nucleophilic aromatic substitution with [^{11}C]HCN.[193] These complexes are extremely air sensitive and the relatively harsh reaction conditions were tolerated by only a narrow substrate scope. Copper(I) [^{11}C]cyanide was also developed as a radiolabeling reagent to prepare aryl [^{11}C]nitriles from aryl halides by the Rosenmund-von Braun reaction.[194] Following reports of Pd-catalyzed cyanation of aryl triflates,[195] the extension of this methodology to PET radiochemistry to prepare aryl [^{11}C]nitriles using [^{11}C]HCN and $Pd(PPh_3)_4$

was demonstrated with a wide substrate scope including heterocycles, electronically diverse arenes, and protic functional groups using iodoarene precursors (72–99% RCY).[196] Recently, a method was reported describing near instantaneous, room temperature coupling of [^{11}C]HCN to arylpalladium precursor complexes formed *in situ* from aryl halides and triflates.[197] A variety of arenes, heteroarenes, and drug molecules were radiolabeled using this method, which takes advantage of an optimized sterically hindered Pd(biarylphosphine) precursor complex and shows high tolerance for the presence of protic and basic additives. It may be anticipated that selective and mild direct ^{11}C-cyanation of complex molecules will have a major impact on radiotracer synthesis and design going forward.



Scheme 12. Synthesis of ^{11}C-cyanoarenes.

5.3 Impact

PET imaging with [^{11}C]urea and substituted ^{11}C-ureas were among the earliest applications of radiochemistry with [^{11}C]HCN. Substituted ureas, including hydantoins such as the anticonvulsant [^{11}C]phenytoin, underwent extensive preclinical evaluation to determine biodistribution and to evaluate their potential as radiotracers.[180,181] It is now known that

[^{11}C]phenytoin is a weak P-glycoprotein substrate, and may have utility in reporting on the activity of this efflux pump.[198]

The greatest impact [^{11}C]HCN radiolabeling has had on clinical research may be the development of numerous ^{11}C-*carbonyl*-amino acids, primarily used for imaging amino acid transport in tumors.[199] Both unnatural amino acids[182] and natural ones have been prepared from [^{11}C]HCN, including [^{11}C-*carbonyl*]tyrosine[200] and [^{11}C-*carbonyl*]leucine (Fig. 7),[177] both of which are useful tools for imaging L-type amino acid transporter 1 (LAT1), protein synthesis in the brain, and gliomas.[201,202] L-[5-^{11}C]Glutamine represents the endogenous parent isotopologue of a series of radiotracers designed to measure glutaminolysis through selective imaging of the amino acid transporters SNAT and ASCT2. This tracer was prepared and characterized in cells and rodent tumor models by PET, and indicated the need for a radiotracer with a longer half-life for *in vivo* imaging, resulting in a number of ^{18}F-labeled analogues that have now been designed.[203–205]

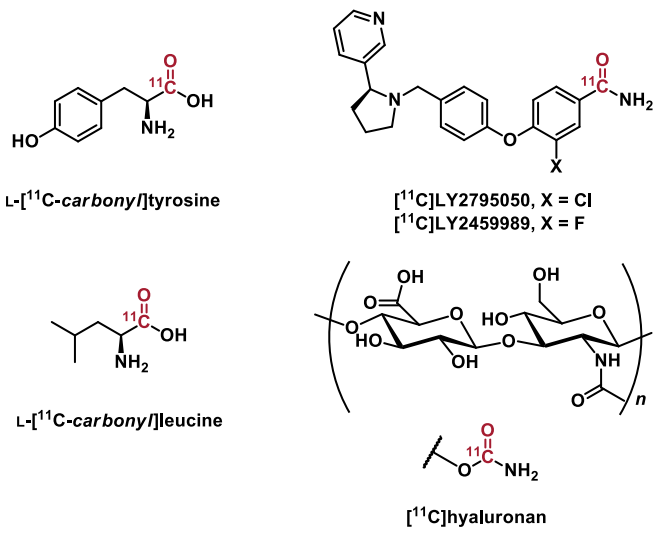


Fig. 7. Several radiotracers based on biogenic compounds and synthetic drugs have been developed using ^{11}C-cyanide. [^{11}C]Hyaluronan labeling likely occurs at one of several hydroxyl groups and specific activity measurements suggest ~1:1 stoichiometry.

Conversion of aryl ^{11}C-nitriles to ^{11}C-benzamides can be accomplished in a single step following radiolabeling and gives access to [^{11}C]nicotinamide and similar compounds that were identified as inhibitors of and potential radiotracers for poly(ADP-ribose) synthetase.[206] Unfortunately, these compounds showed low brain uptake and were not further explored. Other radiopharmaceutical development efforts have used Pd-mediated radiolabeling with [^{11}C]HCN to install [^{11}C-*carbonyl*]benzamides. The κ-opioid receptor (KOR) antagonist [^{11}C]LY2795050 was evaluated in non-human primates to determine metabolism, distribution, target selectivity and specificity of radiotracer uptake.[207,208] [^{11}C]LY2795050 has since been translated to human KOR neuroimaging studies in normal[169,170] and diseased states.[209] Furthermore, this strategy and method for radiolabeling has proven useful in development of a second-generation KOR antagonist PET radiotracer with improved KOR selectivity and binding, [^{11}C]LY2459989.[210]

A series of tuberculosis chemotherapeutics was radiolabeled with carbon-11, including using [^{11}C]HCN, and studied in non-human primates.[211] In addition to the rates and routes of metabolism, the rank order of brain penetration of the drugs was determined, which could in turn inform treatment of disseminated TB in the brain. [^{11}C]Hyaluronan is a labeled polysaccharide prepared using [^{11}C]cyanogen bromide (Scheme 11B) designed to report on hyaluronan kinetics and metabolism in different organs.[191] Hyaluronan is found in high concentration in connective tissue and in joints, and is extracted from the bloodstream by the liver and kidneys. PET studies using [^{11}C]hyaluronan were conducted in patients with liver diseases and showed significantly lower uptake kinetics than in healthy subjects.[212] It is possible that this test could further be useful in identifying regional differences in liver function.

6. Conclusions and Outlook

Radiolabeling of an organic molecule as its direct isotopologue with carbon-11 for PET imaging represents an appealing strategy for radiotracer development. Complementary to ^{11}C-methylation, new methodologies for ^{11}C-carbonylation using [^{11}C]carbon dioxide, [^{11}C]phosgene, [^{11}C]carbon monoxide, and [^{11}C]cyanide reagents have begun to satisfy the demand for practical and efficient PET radiolabeling of a wider variety of functional groups, including ureas, carbamates, carboxylic acids, esters, amides, and related heterocycles. Robust and convenient methods for radiolabeling these functional groups with carbon-11 have had a transformative impact on PET radiochemistry and contributed to the development of several novel ^{11}C-*carbonyl* based radiopharmaceuticals for clinical research, including targets such as FAAH, LAT1, MAO-A and -B, as well as κ-opioid, D3, and 5-HT$_{1A}$ receptors.

Further advances and refinements in this space are needed to overcome remaining obstacles for widespread ^{11}C-*carbonyl* radiopharmaceutical research. In addition to the challenges of selective and rapid isotope incorporation into small molecules, new basic chemistry and applied radiochemistry for preparation and use of these reagents suggests wider dissemination of these techniques for development of both labeling methodologies and novel ^{11}C-*carbonyl* radiotracers. Indeed, novel reagents for ^{11}C-carbonylation, ^{11}C-cyanation, and radiolabeling of alternative functional groups may too be on the horizon, as suggested by recent forays using [^{11}C]methyl azide,[213] [^{11}C]formaldehyde,[214] and [^{11}C]carbon disulfide.[215] Continued advances to these and other methods will streamline *in vivo* evaluation of labeled compounds, facilitate access to compounds with greater structural diversity, and expand the library of radiopharmaceuticals for PET imaging.

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