

Late-Stage Carbon-14 Labeling and Isotope Exchange: Emerging Opportunities and Future Challenges

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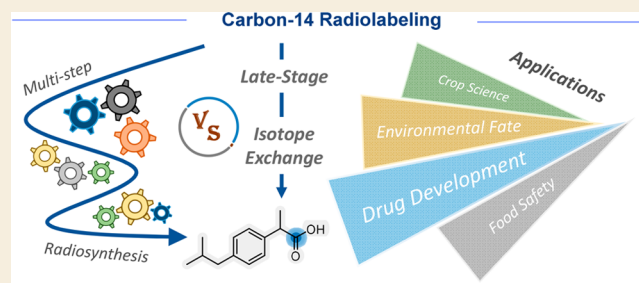
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ABSTRACT: Carbon-14 (^{14}C) is a gold standard technology routinely utilized in pharmaceutical and agrochemical industries for tracking synthetic organic molecules and providing their metabolic and safety profiles. While the state of the art has been dominated for decades by traditional multistep synthetic approaches, the recent emergence of late-stage carbon isotope labeling has provided new avenues to rapidly access carbon-14-labeled biologically relevant compounds. In particular, the development of carbon isotope exchange has represented a fundamental paradigm change, opening the way to unexplored synthetic transformations. In this Perspective, we discuss the recent developments in the field with a critical assessment of the literature. We subsequently discuss research directions and future challenges within this rapidly evolving field.

KEYWORDS: carbon-14, radiocarbon, isotope labeling, carbon isotope exchange, carbon dioxide



1. INTRODUCTION

Carbon-14 (^{14}C) is a naturally abundant radioactive isotope of carbon-12 (^{12}C), with a half-life of 5730 years. This long-lived radioisotope emits low-energy β -particle radiation (i.e., electrons with a mean energy of radiation of 56 keV) that is commonly utilized as a traceless tag for organic molecules to study their fate. This unique tool, in association with β -counting and β -imaging technologies, provides vital knowledge on the fate of synthetic organic molecules (Figure 1).¹ This information is critical to establish potential issues affecting human health and is required worldwide by regulatory agencies such as OECD, FDA, and EMA for the following:

- (1) *pharmaceutical development*: to unveil drug metabolism, disposition, and pharmacokinetics of novel pharmaceuticals^{2,3}
- (2) *animal health drug development*: to determine the metabolism, disposition, and pharmacokinetics of veterinary drugs⁴
- (3) *crop science*: to understand the plant metabolism of agrochemicals and pesticides^{5,6}
- (4) *human food safety evaluation*: to ensure that food derived from animals that have been treated by veterinary drugs is safe for human consumption⁷
- (5) *environmental fate studies*: to perform soil dissipation studies and assess potential environmental impacts associated with human and animal health product excretions that might enter the aquatic and terrestrial environment^{8,9}

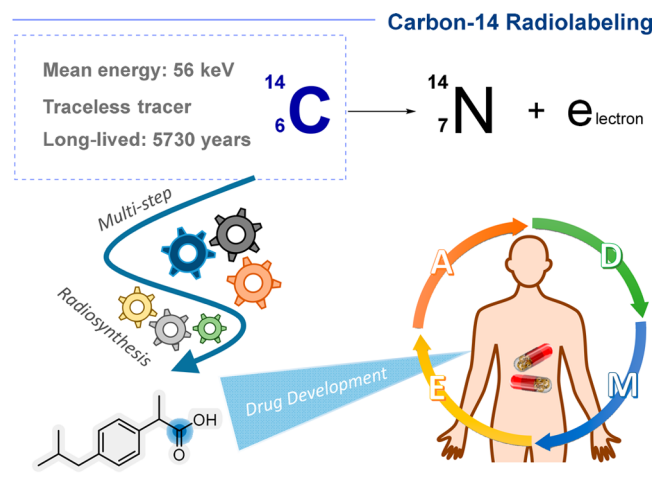


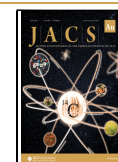
Figure 1. Carbon-14 radiolabeling: a traceless tool for tracking organic molecules, supporting drug development and the agrochemical industry.

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Rapid and straightforward access to ^{14}C -radiolabeled organic molecules is a strict requirement for accelerating research in these fields with high societal impacts. Surprisingly, carbon radiolabeling still represents a bottleneck and a largely unsolved fundamental problem.

These limitations are related to challenges associated with the fundamental source of the radioisotope, as carbon-14 is generated in nuclear reactors as $\text{Ba}[^{14}\text{C}]\text{CO}_3$.¹⁰ Consequently, all ^{14}C -labeled compounds are derived from this common carbon source. The radioactivity is incorporated into the chemical scaffold at an early stage of the synthesis and transformed, by a series of consecutive steps, into the desired molecule. This classical approach is marred by several major drawbacks, namely, the generation of massive amounts of radioactive waste (extremely difficult to dispose of) and the multistep, time-consuming nature of these synthetic approaches, which mandates the development of a specific route in line with radio-synthetic requirements and safety regulations. Last but not least, ^{14}C radiosynthesis is highly resource-demanding, as the price for 37 GBq of $\text{Ba}[^{14}\text{C}]\text{CO}_3$ (ca. 3.3 g, corresponding to ca. 800 mg of $[^{14}\text{C}]\text{CO}_2$) is 25 k€, and any additional step will skyrocket the overall cost.

While ^{14}C -labeling is constantly utilized by pharmaceutical and agrochemical companies, methodological innovation toward this isotope has been scarce until recently. In the past five years, we witnessed a sudden revival in interest toward this isotope, and new technologies have appeared, providing effective alternatives to multistep procedures. The advent of late-stage ^{14}C -radiolabeling and the conceptualization of the first practical examples of carbon isotope exchange (CIE) have provided much enthusiasm in this field with a resurgence of interest in these long-lasting challenges. In this Perspective, we aim to paint a portrait of the vibrant state of the art, focusing exclusively on the recent developments in late-stage labeling that have offered tangible evidence of its applicability to carbon-14 labeling. Consequently, all methodologies that apply uniquely to stable ^{13}C , but do not provide concrete use of the radioisotope, will not be included in this Perspective.

1.1. Brief History

Since its discovery, made on February 27, 1940, by Martin Kamen and Sam Ruben at the Lawrence Berkeley National Laboratory,¹¹ ^{14}C has been instrumental in a wide range of applications, and it particularly helped revolutionize many fields of life science (Figure 2).^{12,13} It is well-known that the utilization of $[^{14}\text{C}]\text{CO}_2$ turned out to be key in the determination of how plants utilize carbon dioxide in the process of photosynthesis.^{14,15} This was immediately recognized by Melvin Calvin who stated, in his notorious Nobel Prize Lecture in 1961, that “Ruben and Kamen provided the ideal tool for the tracing of the route along which carbon dioxide travels on its way to carbohydrate.”^{16,17}

Another early recognition of this “supremely important tracer”¹⁸ was made by Willard Libby, who conceptualized in 1946¹⁹ his groundbreaking idea on the most notorious technology related to this radionuclide: radiocarbon dating.²⁰ Libby realized that ^{14}C , formed in the atmosphere by a reaction between neutrons and nitrogen-14,^{21,22} would find its way into living organisms, which would thus be tagged with the radioisotope.²³ The “radiocarbon revolution” had tremendous effects on the fields of archeology, geology, geophysics, and other branches of science,²⁴ and Libby was recognized with the Nobel Prize in Chemistry in 1960.²⁵

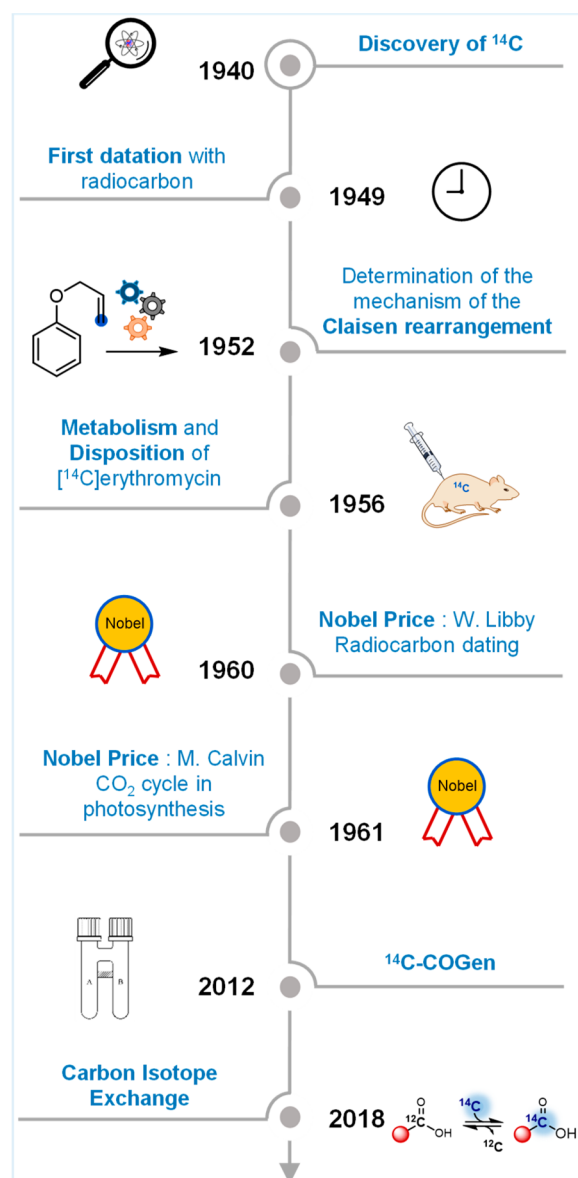


Figure 2. Historical timeline of carbon-14 uses and applications.

^{14}C has also been utilized in organic chemistry as a radioactive tracer element to elucidate and provide evidence on reaction mechanisms.²⁶ This work was prevalently performed in the 1950s, before NMR and its value in chemistry had been elucidated and commercial instruments became available.²⁷ A prominent example is the elucidation of the Claisen rearrangement by Ryan and O'Connor in 1952.²⁸ The authors managed to provide undisputable evidence of the [3,3]-sigmatropic rearrangement by inserting a ^{14}C -tag on the terminal carbon of the allyl group. After the rearrangement, the precise location of the ^{14}C -tag was unequivocally established by a series of degradative processes onto the resulting phenol, in association with *beta*-counting on the derived fractions.²⁹ Other examples of the use of ^{14}C in mechanistic investigations are the confirmation of the existence of a benzyne intermediate^{30,31} and the rearrangements of carbon atoms in *t*-butyl and *t*-amyl derivatives by J. D. Roberts;^{32–34} the series of studies on the Wagner rearrangement by Collins and co-workers;^{35–39} the reaction of diazo compounds with nitro-

olefins by O'Connor;⁴⁰ and the investigation on benzidine rearrangement by Smith.⁴¹

Today, while ^{13}C has replaced ^{14}C for routine mechanistic investigations, the role of ^{14}C is still eminent in the elucidation of biosynthetic pathways.⁴² The use of simple radiolabeled precursors, often amino acids, allows the unequivocal tracking of their evolution into final products in highly sophisticated biological environments.⁴³ A recent example is the remarkable work from Evanno, Poupon, and Thomas on the biosynthesis of the cyclic guanidine alkaloids from Crambeidae marine sponges.⁴⁴

The use of ^{14}C -labeled biologically active molecules to determine their metabolism and disposition started in the late 50s. While we cannot state with certitude the first reported study, early representative examples were the labeling of *N*-[^{14}C] methyl-erythromycin by Flynn, Murphy, and McMahon in 1955⁴⁵ and the determination of its elimination and metabolism in rats in 1956 at Eli Lilly and Company.^{46,47} The use of ^{14}C has since become common practice and a gold standard for a variety of applications in drug development such as the determination of drug absorption, distribution, metabolism, and excretion (ADME); mass balance studies;⁴⁸ and quantifying drug concentrations in target organs using whole body autoradioluminography (WBAL).⁴⁹ In this context, the capacity to introduce the radioisotope into molecules of interest in a cost-effective, timely, and efficient manner is of utmost importance.

1.2. Production of ^{14}C Reagents and Isotope Specificities

Carbon-14 is artificially generated by neutron bombardment of solid beryllium or aluminum nitride, a process that might range from one to three years. After treatment, this nuclear reaction leads to the isolation of barium carbonate $\text{Ba}[^{14}\text{C}]\text{CO}_3$ as the first carbon-14 building block in organic radiosynthesis. This starting material is transformed into four major derivatives from which all ^{14}C building blocks derive, namely, ^{14}C -carbon dioxide ($[^{14}\text{C}]\text{CO}_2$), metal ^{14}C -cyanides ($[^{14}\text{C}]\text{MCN}$), ^{14}C -acetylene, and ^{14}C -cyanamide ($[^{14}\text{C}]\text{BaN-CN}$).⁵⁰ These primary building blocks are further converted into more complex secondary intermediates such as $[^{14}\text{C}]\text{CH}_3\text{OH}$ or $\text{H}[^{14}\text{C}]\text{COOK}$. Of course, even more sophisticated precursors can be prepared such as mono- or multilabeled aromatic rings (Scheme 1, top).^{51,52}

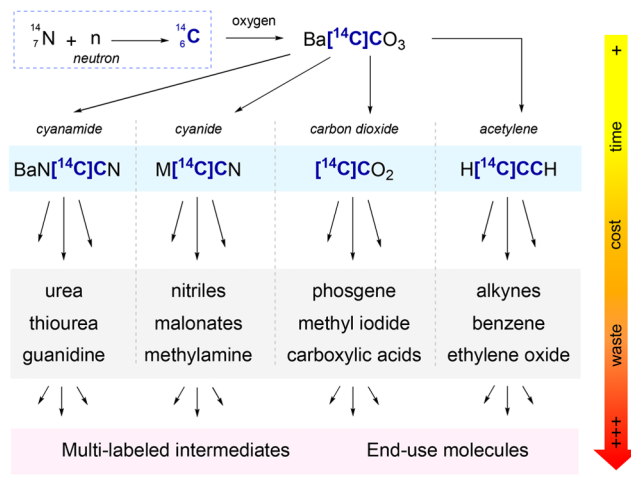
In most cases, the presence of radiocarbon in the structure of a given drug has no notable effect on its biological properties, as the size increase of the isotope is negligible compared to hydrogen–deuterium or hydrogen–tritium, and the kinetic isotope effect of carbon is minor, as well.

Nonetheless, due to the inherent electron emission of the isotope, covalent bond cleavage is possible. Indeed, molecules labeled with ^{14}C might not behave as stably labeled ^{12}C - and ^{13}C -isotopologues and might undergo decomposition, a process known as radiolysis.⁵³ For example, benzene labeled with more than three ^{14}C atoms tends to polymerize spontaneously (Scheme 1, bottom left).¹⁰ The same process has been reported for labeled monomers such as methyl methacrylate, methyl acrylate, vinyl acetate, and vinyl chloride, even at low molar activity (A_m).^{54,55} A_m is defined as the amount of radioactivity per unit mol of the element. From the theoretical point, the maximum molar activity of carbon-14 is $2.31 \text{ GBq mmol}^{-1}$.¹⁰

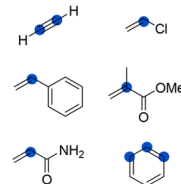
Besides radiolysis, differences in reactivity between stable ^{12}C - and ^{14}C -labeled compounds have been sporadically

Scheme 1. (Top) Arborescence of ^{14}C -Labeled Starting Materials, (Bottom Left) Monomers That Undergo Polymerization and/or Radiolysis, and (Bottom Right) Example of a Reaction Providing Different Product Outcomes between Cold and Hot Synthesis

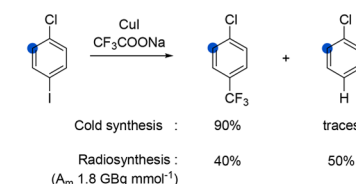
Arborescence of carbon-14 building blocks



Radiolysis



Different outcome between cold/hot reaction



reported. Parker described three examples including the trifluoromethylation of 1-chloro-4-iodobenzene (A_m $1.8 \text{ GBq mmol}^{-1}$), where product distributions differed quite remarkably between the nonlabeled and the radioactive syntheses (Scheme 1, bottom right).^{56,57} Additional examples concerned aniline bromination and a Johnson Claisen rearrangement, where differences in reactivity were noticed.⁵⁷ In 1986, Parker made the hypothesis that “if a chemical reaction can proceed by two or more pathways, whereof one involving the participation of free radicals, the latter might be favored, when a high molar activity radiolabeled species is involved.”

For the above-mentioned reasons, in the context of synthetic methodology development, we strongly encourage not considering the transfer from a stable isotope (i.e., ^{13}C) to radiocarbon as a negligible and automatic step. Additionally, as technical and stoichiometric differences are often present when using ^{14}C reagents, the case of the stoichiometry of $[^{14}\text{C}]\text{CO}_2$ being a crystal clear example, we advise against comparing results obtained with an excess of cold $[^{13}\text{C}]\text{CO}_2$ to ^{14}C radioactive molar activities.

1.3. Analysis of the Chemical Reactions and Linear Sequences in ^{14}C Radiosynthesis from the Most Common Building Blocks

In order to gain precise and quantitative information on the current state of the art in radiocarbon synthesis, we compiled an inventory of 129 publications reported on the radioisotope-specialized *Journal of Labeled Compounds and Radiopharmaceuticals*, between 2010 and 2020 (see the Supporting Information for a detailed list of publications). These articles described 154 syntheses aiming to insert ^{14}C into the molecules of interest. Of note, pharmaceutical companies

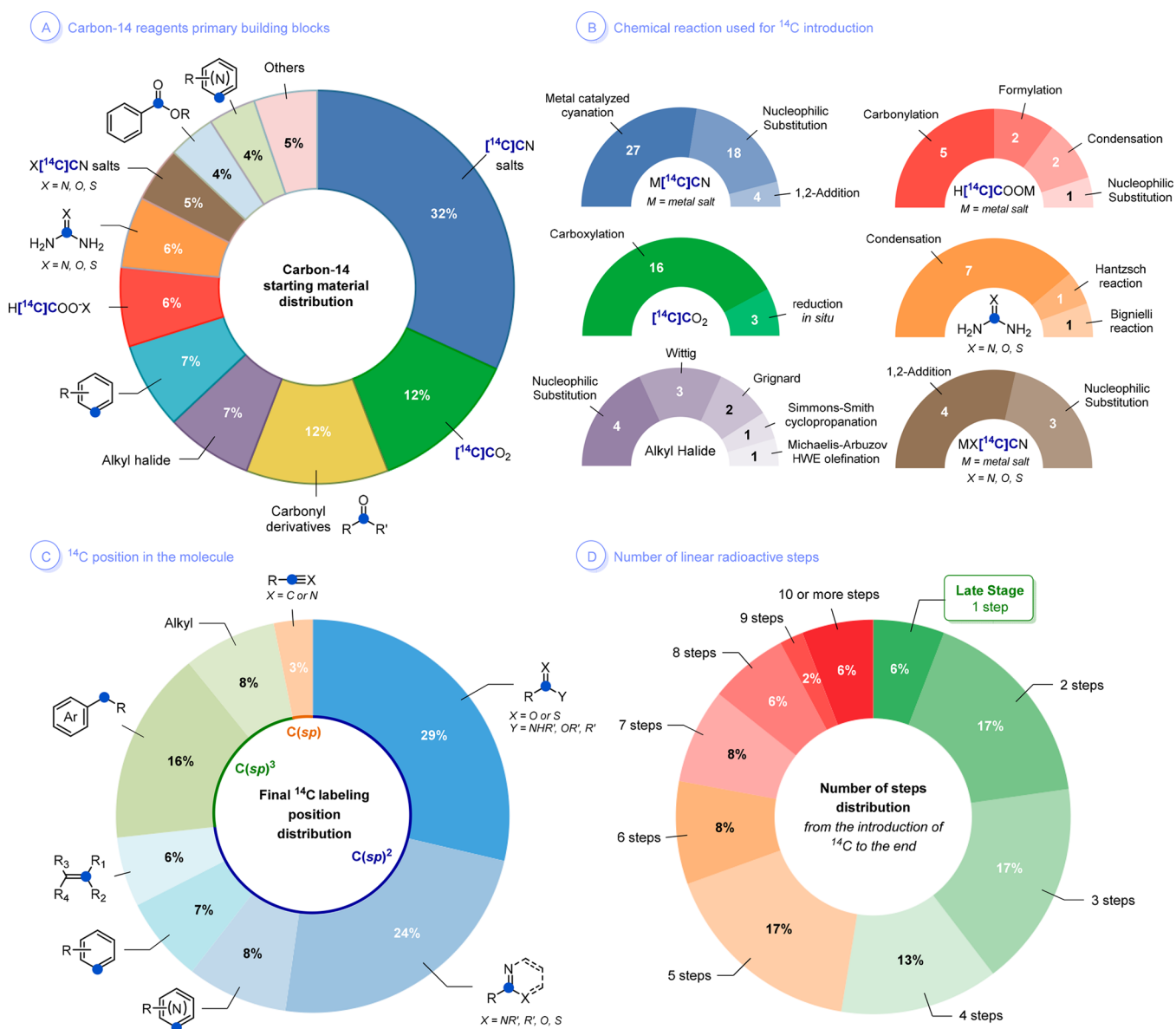


Figure 3. Analysis of 129 publications reporting ^{14}C radiosynthesis over the past decade. (A) Pie chart of building blocks utilized as a ^{14}C -source. (B) Types of reactions utilized to introduce the isotope. (C) ^{14}C -labeled positions on the final molecule of interest. (D) Number of linear steps required to complete the radiosynthesis.

published more than 75% of these articles, thus underpinning the strategic importance of this isotope in the pharmaceutical industry. An analysis of the starting material revealed that cyanide salts are by far the most used precursors, accounting for 32% of all processes (Figure 3A). The simple access to these primary sources, their high reactivity, and their simpler handling compared to gas sources might explain this observation. In comparison, $[^{14}\text{C}]\text{CO}_2$, the second most frequent source, is found in 12% of the syntheses (generated either *in situ* from solid $\text{Ba}[^{14}\text{C}]\text{CO}_3$ or directly in its gaseous form). Building blocks such as carbonyl derivatives and alkyl halides represent 12% and 7% of the isotopic sources, respectively. Technically advanced arenes account for 11% of the distribution. Finally, in a similar proportion, formate salts (6%), urea derivatives (6%), and cyanate salts (5%) are also significant precursors for radiolabeling. Chemical reactions utilized to incorporate the radionuclide strictly depend on the selected labeled building blocks (i.e., $\text{M}[^{14}\text{C}]\text{CN}$, $[^{14}\text{C}]\text{CO}_2$,

alkyl halide, etc.). For the six ^{14}C precursors selected in Figure 3B, generally one procedure prevailed over the others. For carbon dioxide, carboxylation reactions in the presence of organomagnesium or organolithium species prevail over direct reductions, while condensation reactions are most common for urea derivatives. Concerning cyanide salts, transition-metal catalysis and nucleophilic substitutions are the most representative pathways to highlight. However, for other sources such as formate salts and alkyl halides, various sequences have been reported (see the Supporting Information for a detailed list of publications).

The analysis of the labeled position on the final molecule clearly shows that carbon $\text{C}(\text{sp}^2)$ hybridization is preferred, as observed in three-quarters of the labeled molecules (74%, Figure 3C). Carbonyl (29%), imine derivatives (24%), and labeled arenes (15%) are the most recurrent labeled positions encountered. With regard to the other hybridization states of carbon, the $\text{C}(\text{sp}^3)$ distribution nearly reaches one-quarter of

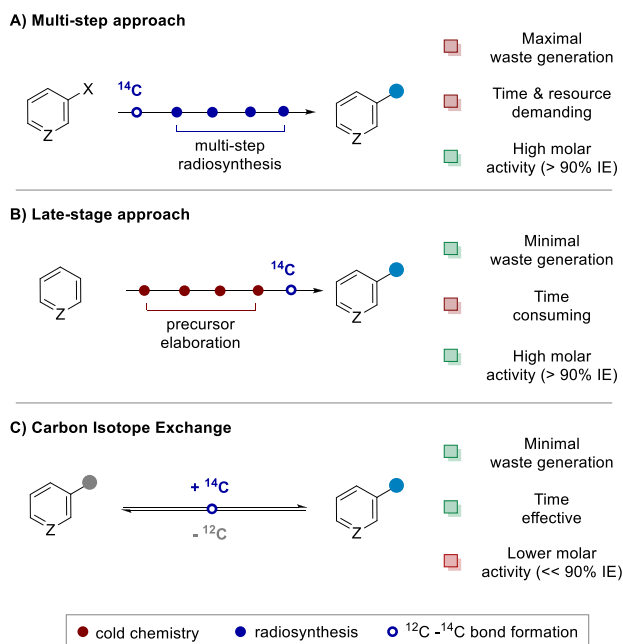
the substrate inventory (24%), while C(sp¹) labeling is rarely targeted (3%).

Lastly, we looked to the number of linear steps required for the incorporation of the radionuclide onto the final labeled molecule (Figure 3D). Most commonly, two, three, or five steps are necessary to obtain the final substrate with a median value that was determined to be 4 steps. However, in 30% of reports, 6 steps or more are required to provide the labeled compounds, including 6% of processes with a nightmarish 10 step radiosynthesis. In this context, it is somehow shocking to notice that late-stage procedures (i.e., the use of one single radioactive reaction) account only for 6% of the total number of radiosyntheses analyzed.

2. RADIOCARBON LABELING

Compared to long multistep radiosynthesis (Scheme 2A), a tardive insertion of the isotope has the advantage to reduce the

Scheme 2. Comparison between (A) Classical Multi-Step Radiosynthesis, (B) a Late-Stage Approach, and (C) Carbon Isotope Exchange



overall cost of the approach, the time required to obtain the molecule, and the amount of radioactive waste generated, with a beneficial impact on the overall efficiency of the process.

In this area, we can distinguish two possible approaches. On one hand, the late-stage incorporation of ¹⁴C, ideally at the last step of the process (Scheme 2B), has the benefit of inserting the ¹⁴C without isotopic dilution (i.e., high *A_m*). The main drawback is the necessity to prepare a suitably decorated precursor, elaborated specifically to adapt to the methodology. Though this process does not require handling of radioactive material, it might be time-demanding and require the design of an alternative retrosynthesis compared to the molecule of interest.

Recently, an alternative strategy has been reported: carbon isotopic exchange (CIE, Scheme 2C). This approach aims to replace a ¹²C functional group (–COOH, –CN) with its

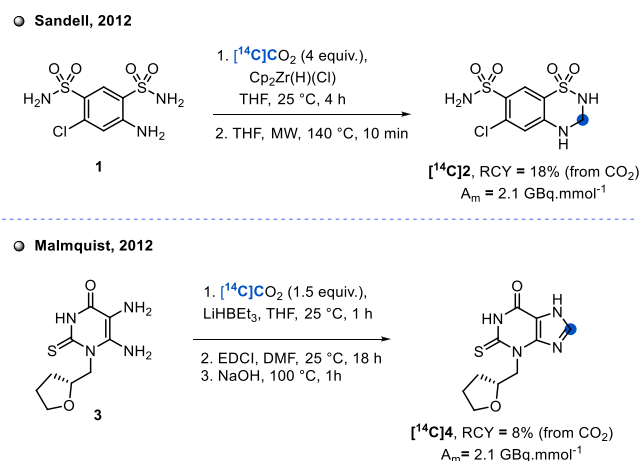
radiolabeled counterpart in one single operation, directly on the final compound of interest. CIE is appealing as it avoids the requirement for the elaboration of a precursor and is the most straightforward way to access ¹⁴C-labeled molecules. As these transformations are based on equilibria, ¹⁴C is inserted with some degree of isotope dilution, and the *A_m* will be lower compared to the other strategies.

2.1. Late-Stage ¹⁴C-Labeling with High Molar Activity

2.1.1. Late-Stage Labeling from [¹⁴C]CO₂. Carbon dioxide is the primary radiocarbon source and one of the most attractive building block to incorporate the radionuclide. Nevertheless, its high thermodynamic stability represents a severe challenge and a limitation for effective utilization.⁵⁸ In addition, compared to cold unlabeled chemistry, the use of a large excess and high pressures of [¹⁴C]CO₂ is simply inconceivable from both financial and safety standpoints.

In 2012, radiochemists at AstraZeneca published two strategies based on the partial reduction of CO₂ and its subsequent utilization for ring closure reactions (Scheme 3).

Scheme 3. One-Pot CO₂ Reduction Strategies: (Top) Reduction to Formaldehyde to Label Hydrochlorothiazide and (Bottom) Reduction to Formate and Subsequent Cyclization



Using an excess of [¹⁴C]CO₂ in the presence of the Schwartz reagent,⁵⁹ ¹⁴C-formaldehyde was generated *in situ* and immediately reacted with the sulfonamide precursor 1 to obtain the [¹⁴C]hydrochlorothiazide 2, a diuretic drug (Scheme 3, top).⁶⁰ Though the radiochemical yield (RCY) from labeled CO₂ was modest (18%), the advantage of this one-pot strategy is not negligible.

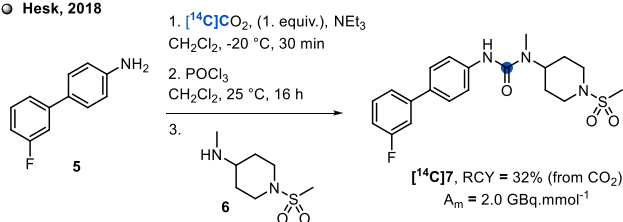
Xanthine derivative 4, a myeloperoxidase inhibitor, was labeled on the imidazole backbone via a 2-step process from CO₂ (Scheme 3, bottom). Lithium ¹⁴C-formate was prepared *in situ* after a reduction of [¹⁴C]CO₂ with superhydride LiEt₃BH. After the solution was concentrated, the precursor 3 was added in the presence of EDCI to form the corresponding formylated intermediate, which was further converted into the desired product 4 under basic conditions.⁶¹ The overall radiochemical yield from CO₂ was low (8%), but sufficient compound was delivered to be administered to rats for quantitative whole body autoradiography (QWBA) experiments.

Urea derivatives are common functional groups in medicinal chemistry and are often found in pharmaceuticals.^{62,63} In 2018,

Hesk and co-workers at Merck applied a strategy, published a few years earlier,⁶⁴ for the labeling of **7**, a potent inhibitor of the NPY₅ receptor.⁶⁵ A trapping of radioactive carbon dioxide by the aniline **5** under basic conditions, followed by an addition of POCl₃ as a dehydrating agent, led to the generation of an isocyanate intermediate. The subsequent addition of aliphatic amine **6** afforded the expected labeled urea **7** with a radiochemical yield of 32% and high *A_m* (Scheme 4). While

Scheme 4. Labeling of Urea from [¹⁴C]CO₂ Using a Dehydration Protocol in the Presence of POCl₃

© Hesk, 2018



effective, the disadvantage of this reaction, which is commonly used in ¹¹C-labeling, is the requirement for a dehydrating agent that is poorly tolerant with elaborated functional groups.

The same year, our group reported a last-step Staudinger aza-Wittig procedure (SAW) for the labeling of cyclic ureas.⁶⁶ Inspired by the biorthogonal Staudinger ligation,⁶⁷ we aimed to develop a broadly tolerant methodology suitable to a large variety of substructures (Scheme 5). A treatment of an azido-amine derivative with dimethylphenylphosphine resulted in the instantaneous formation of diatomic nitrogen and the corresponding iminophosphorane. To this frozen solution, a stoichiometric amount of [¹⁴C]CO₂ was precisely delivered to the reaction using an RC Tritic carboxylation manifold, generating an intermediate isocyanate, and subsequent intra-

molecular nucleophilic addition delivered the cyclized urea products (Scheme 5, left).

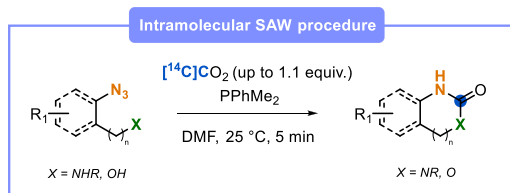
This procedure was shown to be extremely mild and effective: [¹⁴C]CO₂ was converted into the desired cyclic ureas within 5 min at room temperature. This protocol was applied with success to the labeling of four pharmaceutically relevant cyclic urea derivatives, including flibanserin (**8**) and oxatomide (**9**) in suitable radiochemical yields. Functional group compatibility was showcased by the labeling of an unprotected heptapeptide bearing multiple functional groups, including alcohol, carboxylic acid, amine, and indole. Finally, it was shown that the methodology could be successfully applied to the short-lived carbon-11 isotope.

In 2020, this procedure was applied to the labeling of 5- and 6-membered cyclic carbamates (Scheme 5, left).⁶⁸ Using the same approach with more challenging hydroxyl nucleophiles (both alcohols and phenols), nine carbamates were radiolabeled, including five pharmaceuticals in 40–59% radiochemical isolated yields. Once more, the applicability of the procedure to ¹¹C radiolabeling was identified on 24 substrates, including a linear carbamate. To avoid precursor elaboration, we validated a 3-step disconnection/reconnection strategy, involving ring opening/isotopic closure, which allowed the labeling of ¹⁴C-zolmitriptan starting from the commercially available unlabeled pharmaceutical.

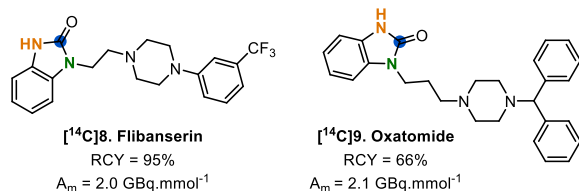
In 2021, we reported a linear version of the SAW procedure for the labeling of urea substructures (Scheme 5, right).⁶⁹ By using all combinations of aliphatic/aromatic azides and amines, access to dissymmetric linear ureas was granted, including derivatives such as hydroxyureas, semicarbazides, or sulfonylureas. This procedure was applied on 43 derivatives using [¹³C]CO₂ under identical stoichiometric conditions compared to ¹⁴C. It was shown that, in the presence of less nucleophilic anilines, the use of *N*-methylimidazole additive (2 equiv) was

Scheme 5. Last-Step Radiolabeling of Ureas and Carbamates Using a Staudinger Aza-Wittig Approach with [¹⁴C]CO₂

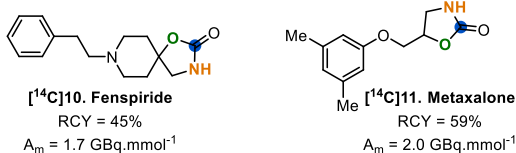
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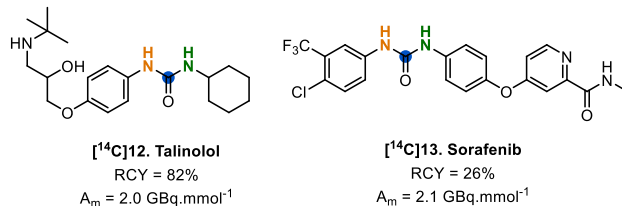
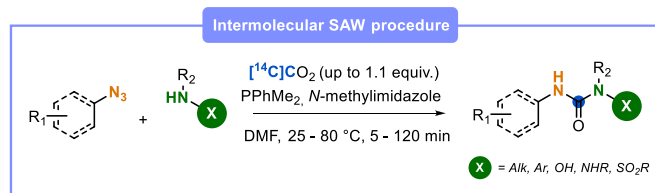
Cyclic ureas examples



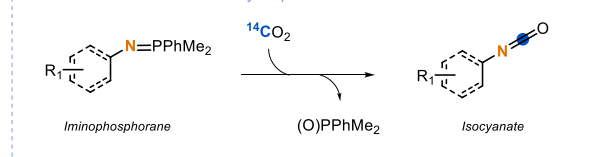
Cyclic carbamates examples



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Key step of mechanism



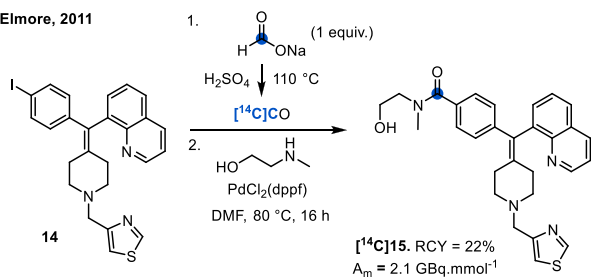
required to obtain satisfactory yields. This sequence enabled the ^{14}C -labeling of four drugs from 26% to 82% isolated yields.

2.1.2. Late-Stage Labeling from ^{14}C CO. Carbonylation reactions are very frequently encountered in late-stage labeling, in particular for stable ^{13}C and short-lived ^{11}C .⁷⁰ On the contrary, this is not the case for ^{14}C CO, which has only rarely been reported as a building block. Besides the inherent toxicity of ^{14}C CO that requires obvious safety precautions, its use has been underexploited due, in particular, to its poor stability. Hargraves and co-workers investigated the cryogenic isotopic distillation of ^{14}C CO as a technology for isotope concentration and found that ^{14}C CO undergoes significant radiolysis even when stored at low temperatures.⁷¹ Similar observations were reported by Hardy and co-workers.⁷² Consequently, this gas is generated *in situ* and utilized directly for further functionalization.

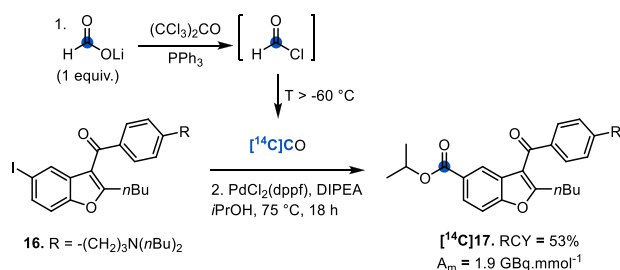
^{14}C Formate salts have been successfully reported to generate ^{14}C CO in the presence of concentrated sulfuric acid.⁷³ In 2011, Elmore and co-workers at AstraZeneca published the synthesis of a *delta* opioid agonist (**15**) based on a palladium-catalyzed amino-carbonylation as the final step (Scheme 6, top). In this procedure, ^{14}C CO was produced by

Scheme 6. Carbonylative Cross-Coupling Utilizing ^{14}C CO Generated from ^{14}C Formate Sources

© Elmore, 2011



© Whitehead, 2013



^{14}C sodium formate dehydration after the addition of sulfuric acid at 110 °C.⁷⁴ The same strategy was used for a series of other carbonylations from aryl halides, such as hydroxycarbonylations,^{75,76} alkoxycarbonylations,⁷⁷ and intramolecular aminocarbonylations.⁷⁸

In 2013, Whitehead et al. developed an alternative procedure that enables ^{14}C CO generation under milder conditions and applied it to the radiosynthesis of celivarone **17** (Scheme 6, bottom).⁷⁹ Carbon monoxide was liberated after an addition of lithium ^{14}C formate to a chlorophosphonium cation, previously generated from triphenylphosphine and perchloroacetone.⁸⁰ Under these conditions, the formate salt is immediately transformed into ^{14}C formyl chloride that readily dissociate at temperatures exceeding -60 °C to give ^{14}C CO. ^{14}C Celivarone was labeled in 53% RCY by a subsequent palladium-catalyzed alkoxycarbonylation reaction.

Skrydstrup's group pioneered the development of solid carbon monoxide sources, which allows the release of the poisonous gas under safer and simple conditions.⁸¹ In collaboration with Elmore at AstraZeneca, they reported in 2012 a method to generate ^{14}C CO based on a solid and bench stable ^{14}C CO precursor, COgen (**20**).⁸² Utilizing a two-chamber glassware reactor, the release of ^{14}C CO takes place in the first chamber through a palladium-catalyzed decarbonylation of **20**, and gas diffusion in the second chamber allows the desired carbonylation reaction (Scheme 7B). $^{14}\text{COgen}$ is synthesized in two steps from ^{14}C CO₂ by lithiation of 9-methyl-9H-fluorene **19** and subsequent carboxylation, which is followed by activation of the carboxylic acid with oxalyl chloride with an overall radiochemical yield of 91% (Scheme 7A). Using this method, a variety of combinations of aryl halides and amines or boronic acids led to the labeling of relevant amides or ketones, respectively. In the presence of isotopically diluted ^{14}C COgen, three drugs were labeled with success (Scheme 7). Olaparib **21** and thalidomide **23** were obtained by aminocarbonylation in 37% and 70% RCY, respectively. Fenofibrate **22** was labeled in 72% RCY starting from the corresponding boronic acid.

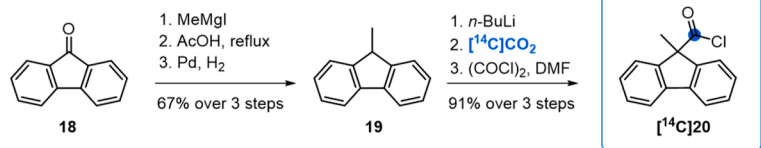
In 2019, Sardana et al. developed a visible-light-mediated late-stage aminocarbonylation methodology, starting from alkyl iodides and secondary amines in the presence of stoichiometric amounts of labeled CO (Scheme 8).⁸³ After extensive optimization with ^{13}C , palladium catalysis afforded the labeled amide at ambient temperature using COgen as a limiting reagent. Using isotopically diluted ^{14}C COgen with unlabeled material (at 5% $^{12}\text{C}/^{14}\text{C}$, A_m 0.11 GBq mmol⁻¹), this procedure was successfully applied to the radiocarbonylation of two amides **24** and **25** with the expected molar activities (Scheme 8).

Ketones are common functional groups in organic chemistry and are found in fine chemicals and particularly in pharmaceuticals. While (hetero)aryl ketones can be prepared and labeled by carbonylative cross-couplings using palladium catalysis (see fenofibrate **22**, Scheme 7), the propensity for palladium-alkyl intermediates to undergo competing *beta*-hydride elimination has limited the development of effective carbonylative solutions to aliphatic ketones.⁸⁴ In 2020, Skrydstrup's group reported a stoichiometric nickel-mediated carbonylative coupling of alkyl zinc reagents with alkyl iodide to access labeled alkyl ketones (Scheme 9).⁸⁵ Starting from NN₂ pincer nickel(II) complex (A), an addition of alkyl zinc reagent produced a nickel(II) alkyl complex (B). *Ex situ* generation of CO in a double-chamber COware reactor was followed by insertion into the Ni^{II}-alkyl complex (B), thus generating the corresponding Ni^{II}-acyl species (C). A subsequent addition of alkyl iodide to a second nickel(I) complex, reduced by an addition of pincer nickel species (A) and manganese Mn, led to the generation of an alkyl radical that provided the Ni^{III}-(alkyl)acyl complex (D). Finally, reductive elimination afforded the expected labeled alkyl ketone (E). Based on this procedure and using a stoichiometric amount of nickel(II) and nickel(I) complexes, ^{14}C -labeled nabumetone **27**, a nonsteroidal anti-inflammatory drug (NSAID), was isolated with a radiochemical yield of 36% and an A_m of 0.5 GBq mmol⁻¹.

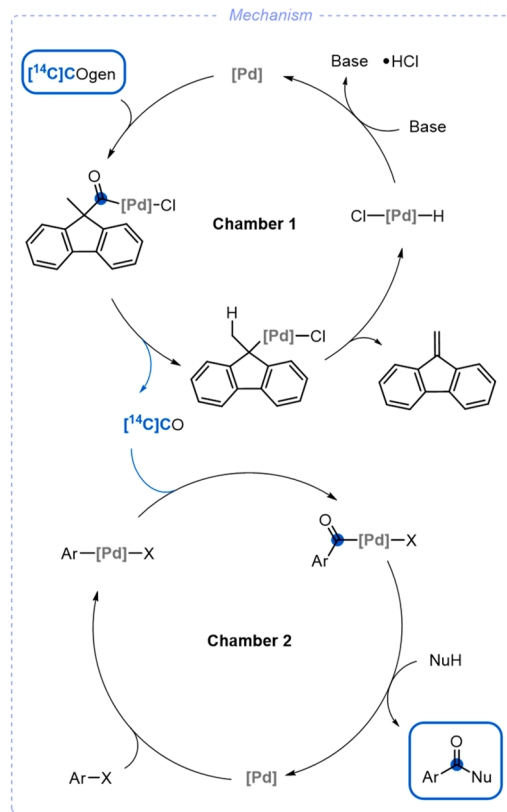
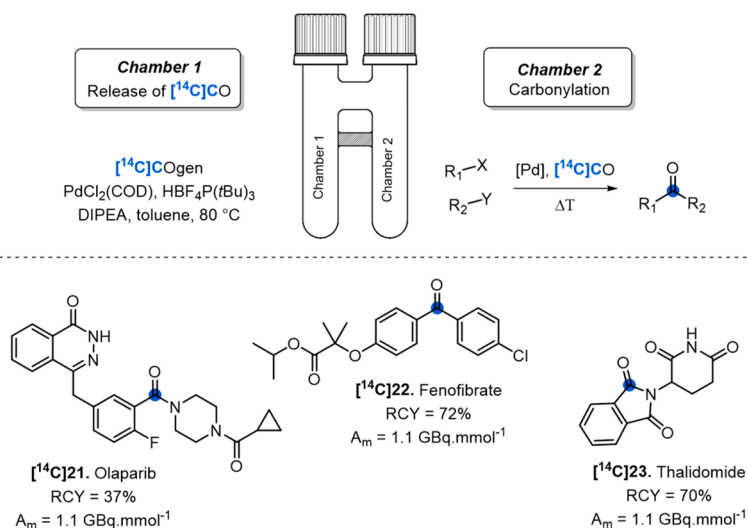
2.1.3. Late-Stage Labeling from ^{14}C CH₃I. ^{14}C -labeled methyl iodide represents a tempting secondary building block because it provides a wide range of opportunities to introduce the radionuclide: in particular, access to labeled O- and N-

Scheme 7. Formation of $[^{14}\text{C}]\text{CO}$ from $[^{14}\text{C}]\text{COgen}$ and Subsequent Use for Pd-Catalyzed Carbonylation Reactions^a

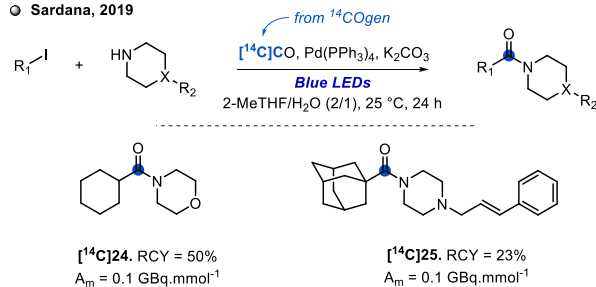
© Skrydstrup, 2012

A Synthesis of $[^{14}\text{C}]\text{COgen}$ 

B Decarbonylation and Carbonylation in COWare

^aProf. T. Skrydstrup is kindly acknowledged for providing the image of the two-chamber glassware reactor.Scheme 8. Visible-Light-Mediated Aminocarbonylation of Unactivated Alkyl Iodides with Stoichiometric $[^{14}\text{C}]\text{CO}$

© Sardana, 2019

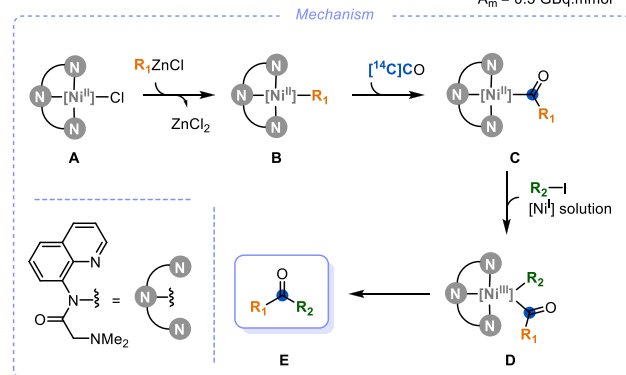
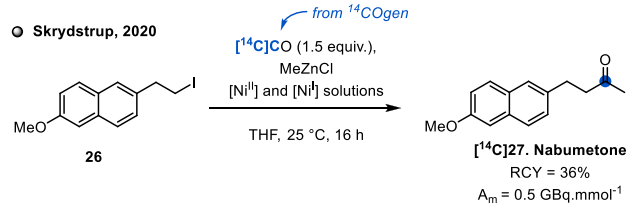


$[^{14}\text{C}]$ methylated substrates granted by electrophilic methylation. $[^{14}\text{C}]\text{CH}_3\text{I}$ is prepared by LiAlH_4 reduction of $[^{14}\text{C}]\text{CO}_2$ to $[^{14}\text{C}]\text{CH}_3\text{OH}$, followed by thermal treatment with HI, and is subsequently employed for alkylation.¹⁰

In 2012, Tanga and co-workers published the radiosynthesis of the pimaradiene diterpene acanthoic acid $[^{14}\text{C}]\text{28}$, using a 3-step deconstruction/reconstruction strategy (Scheme 10, top). Acanthoic acid was dihydroxylated with osmium tetroxide to give vicinal diol **29**; subsequent oxidative cleavage with sodium periodate provided the aldehyde precursor **30**. A late-stage Wittig reaction was utilized for the introduction of the carbon-14 radioisotope.⁸⁶ The labeled phosphonium ylide was prepared from $[^{14}\text{C}]\text{CH}_3\text{I}$ and triphenylphosphine under basic conditions. An excess of the ^{14}C -methylene Wittig reagent was added to the solution containing aldehyde **30**, affording the labeled compound with a modest radiochemical yield of 9% and high molar activity ($1.9 \text{ GBq mmol}^{-1}$).

Scheme 9. Nickel Complexes Mediated Access to ^{14}C -Labeled Aliphatic Ketones

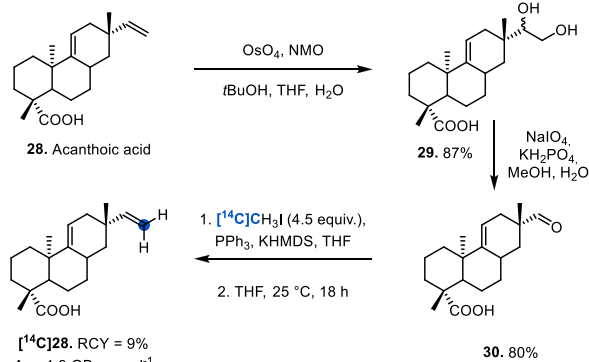
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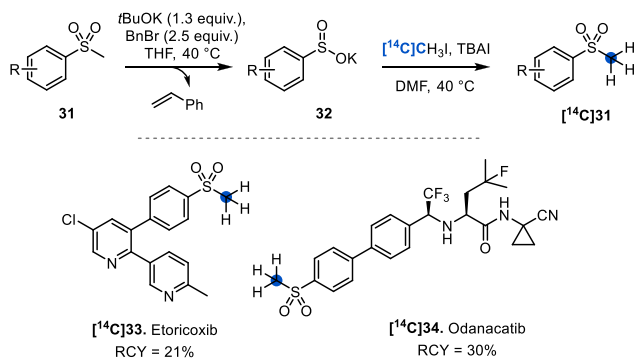
In 2016, Gauthier Jr. and co-workers at Merck presented a deconstruction/reconstruction strategy based on the demethylation/ $[^{14}\text{C}]$ methylation of methylsulfones (Scheme 10, bottom).⁸⁷ Under optimal conditions, methyl phenyl sulfones **31** were treated with benzyl bromide in the presence of excess *t*BuOK followed by *in situ* elimination of the resulting styrene, thus leading to the formation of sulfinate salt **32**. A final

Scheme 10. Radiosynthesis of Acanthoic Acid and Methylsulfones by Deconstruction/Reconstruction Strategies

© Tanga, 2012



© Gauthier, 2016



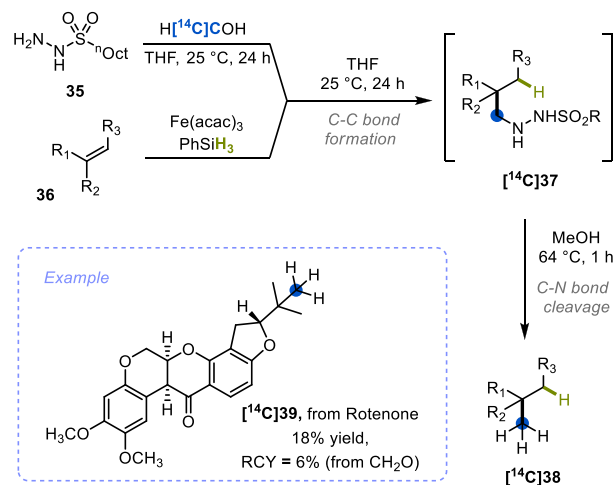
alkylation with $[^{14}\text{C}]\text{CH}_3\text{I}$ promoted the formation of the labeled methylsulfones $[^{14}\text{C}]\text{31}$. This strategy was applied to the synthesis of two pharmaceutically active molecules, etoricoxib **33** and odanacatib **34**. Using high-molar-activity $[^{14}\text{C}]\text{CH}_3\text{I}$ ($A_m = 2.1 \text{ GBq mmol}^{-1}$), **33** was isolated in 21% RCY. According to the authors, the reaction with stably labeled CH_3I proceeded in >90% yield, and the low RCY is due to the incomplete transfer of the radioactive gas alkylating agent. This is another example where technical issues due to the manipulation of the radioisotope might deeply affect the transposition of the cold synthesis. ^{14}C -labeling of odanacatib **34** (30% RCY), a selective inhibitor of cathepsin K, was required for environmental risk assessment studies.

2.1.4. Late-Stage Labeling from $[^{14}\text{C}]\text{CH}_2\text{O}$. In 2015, Baran and co-workers reported a methodology for the hydromethylation of unactivated alkenes promoted by $\text{Fe}(\text{acac})_3$ (Scheme 11).⁸⁸ In the first step, a sulfonylhydrazone was generated by condensation of formaldehyde on the corresponding sulfonylhydrazide **35**. An excess of this reagent was added to a solution of alkene, in the presence of phenylsilane and an iron complex carrying out the formation of alkylhydrazine **37**. Finally, a solvent switch to methanol and gentle heating of the reaction at 60 °C yielded the hydromethylated adduct **38**, by reductive C–N bond cleavage, and a release of sulfinic acid.

This procedure was successfully applied to a variety of substrates using unlabeled formaldehyde (6 equiv) and even on rotenone, an olefin bearing insecticide, in 50% yield on a gram scale. Using $[^{14}\text{C}]\text{HCOH}$ ($A_m = 2.1 \text{ GBq mmol}^{-1}$), iron-mediated hydromethylation of rotenone was successfully

Scheme 11. Hydromethylation of Unactivated Alkenes with Formaldehyde

© Baran, 2015



realized in collaboration with the Maxwell group at Bristol Myers Squibb. The ^{14}C -labeled compound **39** was isolated in 18% yield from rotenone (6% RCY from $[^{14}\text{C}]\text{HCOH}$). We might speculate that, to reduce costs associated with the labeled reagent for the ^{14}C reaction, the number of equivalents of ^{14}C -formaldehyde was limited to 3.2, which can explain the lower yield compared to the unlabeled experiment.

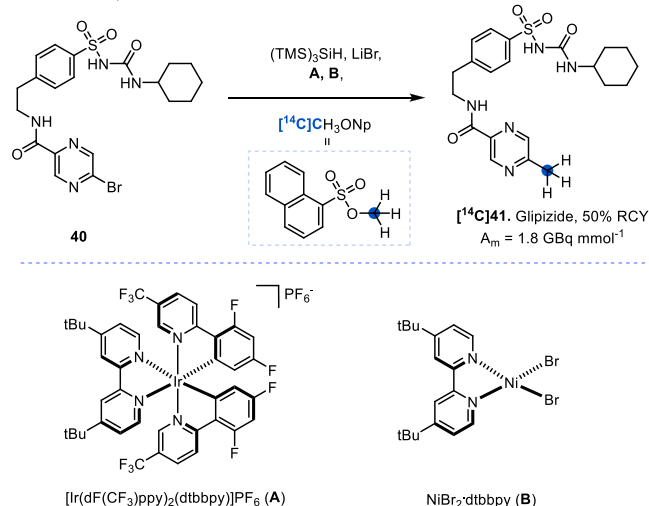
Based on their previous findings on the metallaphotoredox cross-electrophile coupling strategy,^{89,90} in 2021, MacMillan and co-workers reported a radiomethylation procedure suitable to multiple isotopes, including tritium, ^{11}C , and ^{14}C (Scheme 12).⁹¹

Starting from the alkyl or aryl bromide derivatives and a corresponding radiolabeled methyl source ($[^{14}\text{C}]\text{CH}_3\text{ONp}$), the desired molecules were synthesized based on a dual photoredox and metal catalysis mechanism, mediated by silyl radical activation of alkyl halides. Aiming for practical applicability, the authors selected isotopically labeled methyl naphthalene-2-sulfonate as a limiting reagent, owing to its nonvolatility compared to MeI . In the presence of the additive LiBr , $[^{14}\text{C}]\text{CH}_3\text{ONp}$ undergoes an *in situ* Finkelstein-like reaction to form $[^{14}\text{C}]\text{CH}_3\text{Br}$.

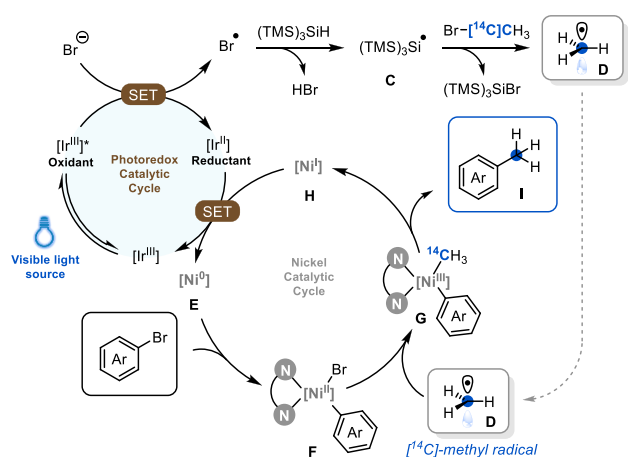
Under blue light irradiation, the photoredox catalyst (**A**) is excited to generate, after single-electron oxidation of bromide (a dissociable ligand of nickel **B**), a bromine radical and the reduced photocatalyst. Bromine radical-promoted hydrogen-atom abstraction from tris(trimethylsilyl)silane ($(\text{TMS})_3\text{SiH}$) generates the stabilized silyl radical intermediate (**C**), which in turns affords the radiolabeled methyl radical (**D**) group through halogen-atom abstraction. On the other hand, zerovalent nickel catalyst undergoes oxidative addition with the corresponding aryl bromide to furnish a Ni^{II} intermediate (**F**). Oxidative capture of a labeled methyl radical leads to the formation of Ni^{III} (**G**), and subsequent reductive elimination provides the expected product (**I**) and the corresponding Ni^{I} complex (**H**). Lastly, single-electron transfer from the reduced photocatalyst to the Ni^{I} (**H**) simultaneously regenerates both catalysts. This procedure was applied to the labeling of several ^3H and ^{11}C radiotracers. On the other hand, only a single example using ^{14}C was reported: glipizide **41** was labeled with

Scheme 12. Metallaphotoredox Cross-Electrophile Coupling Strategy for the Radiomethylation of Aryl and Alkyl Bromides

© MacMillan, 2021



Mechanism

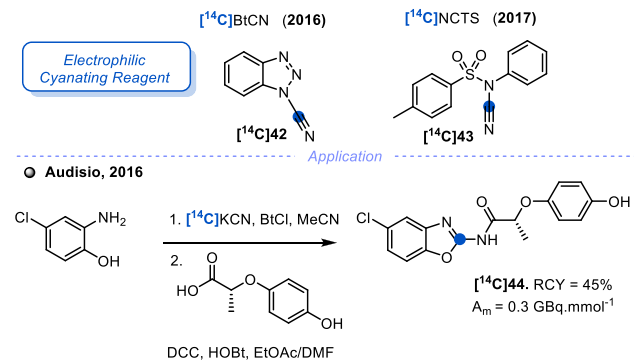


50% isolated radiochemical yield and high molar activity (Scheme 12).

2.1.5. Late-Stage Labeling from [14C]CN. Cyanide salts are the most used reagents for ¹⁴C incorporation due to their suitable handling, facile accessibility, and versatility of the corresponding nitrile functional group (Figure 3A). While the synthesis and reactivity of electrophilic cyanating sources have been largely investigated,^{92–94} most are unfortunately unsuited to carbon radioisotope labeling. In 2016, our group developed an access to 2-aminobenzothiazole and 2-aminobenzoxazole derivatives via a KCN polarity inversion strategy using 1-chlorobenzotriazole (BtCl, Scheme 13).⁹⁵ By mixing BtCl and [14C]KCN, electrophilic cyanating agent [14C]BtCN 42 was generated *in situ*. Subsequent addition of the aminophenol or aminothiophenol led to the formation of the labeled heterocycles. This method allowed the labeling of an advanced herbicide synthetic intermediate 44 in a one-pot approach. Starting from isotopically diluted [14C]KCN (A_m = 0.3 GBq mmol⁻¹), compound 44 was labeled with 45% RCY based on this procedure coupled with sequential peptide bond formation with the corresponding carboxylic acid.

In 2017, Song's group at Johnson & Johnson described the synthesis of the electrophilic cyanating reagent [14C]NCTS

Scheme 13. ¹⁴C-Labeled Electrophilic Cyanating Reagents

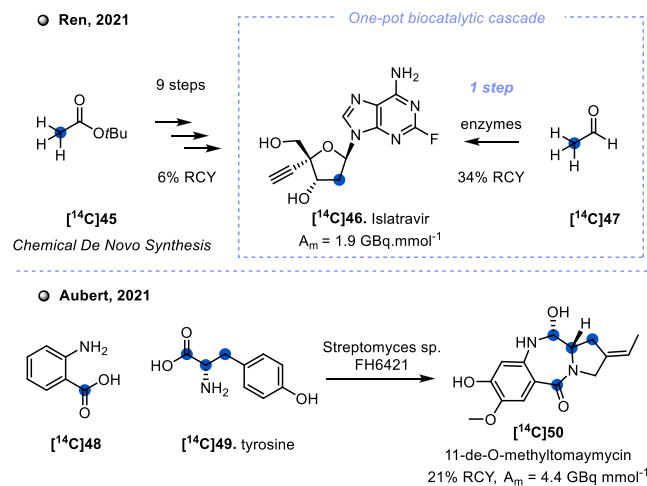


43.⁹⁶ A treatment of low-molar-activity [14C]KCN (A_m = 0.1 GBq mmol⁻¹) with bromine formed *in situ* cyanogen bromide, which was reacted directly with *N*-phenyl-*p*-toluenesulfonamide to provide the desired reagent in 68% RCY. In the paper, while the authors reported a method for decarboxylative cyanation catalyzed by palladium using stable [13C]NCTS, no application with ¹⁴C was provided.

2.1.6. Labeling Using Biocatalytic Enzymatic Reactions. Biocatalytic cascade reactions are a cost-effective alternative to multistep synthesis for carbon-14 incorporation. Using well-known biological mechanisms, this approach might provide access to complex labeled molecules in a one-pot procedure based on successive enzymatic catalysis. In 2000, Bacher's group reported the biosynthetic labeling of an isoprenoid biosynthesis intermediate, 4-diphosphocytidyl-2C-methyl-D-erythritol, through a 4-step enzyme cascade reaction system that provided the expected ¹⁴C-labeled compound in 47% isolated yield from [2-¹⁴C]pyruvate.⁹⁷ In 2007, Roy's group reported the use of nitrile hydrolyzing enzymes (nitrilases) as an attractive alternative to selectively hydrolyze nitrile derivatives under mild conditions.⁹⁸

In 2021, Ren and co-workers from Merck published a biocatalytic synthesis of [14C]islatravir 46 (Scheme 14, top). This labeled adenosine-based nucleoside reverse transcriptase

Scheme 14. (Top) Comparison of Synthetic versus Biocatalytic Approaches to [14C]Islatravir Radiosynthesis and (Bottom) Biocatalytic Route to [14C]50 from [14C]Tyrosine



translocation inhibitor, under investigation for the treatment of HIV infection, was required to support drug metabolism and pharmacokinetics studies.⁹⁹

Their chemical radiosynthesis started from [¹⁴C]*t*-butyl acetate **45**, providing **46** in an overall 6% RCY, in 9 linear steps. The low yield and the high cost, time, and waste generated by this approach necessitated the development of a “greener” alternative. Based on previous developments at Merck on the manufacturing of unlabeled islatravir by means of biocatalytic cascades,¹⁰⁰ the authors aimed to adapt it to ¹⁴C. Using [¹⁴C]acetaldehyde **47**, a delicate starting material that undergoes slow radiolysis (10% per month), the biocatalytic approach provided **46** in a much improved 37% RCY and high *A_m* in just one single reaction step. This work is a beautiful example of how biocatalysis might support the development of ¹⁴C tracers.

11-De-O-methyltomaymycin **50** is a highly toxic antitumor antibiotic utilized as a payload for antibody drug conjugate (ADC) development. To support *in vivo* pharmacokinetic and metabolism studies at Sanofi, Aubert and co-workers required a ¹⁴C-labeled version of the molecule.¹⁰¹ While a multistep approach appeared to be too ambitious, the authors turned their attention to a fermentation process starting from labeled precursors.

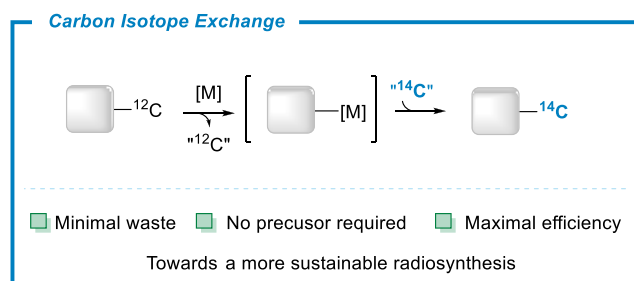
After precise optimization, the authors were able to achieve their goal using the *Streptomyces* sp. FH6421 strain (Scheme 14, bottom). In the presence of ¹⁴C-labeled anthranilic acid **48** (2.1 GBq mmol⁻¹) and tyrosine **49** (6.3 GBq mmol⁻¹) as radiolabeled precursors, ¹⁴C-labeled 11-de-O-methyltomaymycin **50** could be isolated in 21% RCY and high *A_m* (4.4 GBq mmol⁻¹) and up to 97% radiochemical and chemical purity.

While biocatalytic cascade reactions have not been extensively utilized for ¹⁴C-labeling, the stunning achievements presented herein show that when a biocatalytic approach is possible, efforts should be directed toward its implementation.

2.2. Carbon Isotope Exchange

Carbon isotope exchange (CIE, Schemes 2C and 15) aims to selectively replace a ¹²C functional group (i.e., –COOH, –CN) with its radiolabeled counterpart directly on the final compound of interest, in one single operation.

Scheme 15. Carbon Isotope Exchange



Isotope exchange technologies have been extensively developed over the past decades for hydrogen, providing a vast arsenal of opportunities to introduce deuterium and tritium on pharmaceutical compounds.¹⁰² On the other hand, this concept lay largely unexplored for the carbon isotope until recently.¹⁰³ Despite early examples on decarboxylative carboxylation of aliphatic carboxylates,^{104–108} the synthetic applicability of such processes, requiring harsh thermal

conditions ($T > 300\text{ }^{\circ}\text{C}$), often pyrolytic,^{109,110} did not find practical applications. One report on a biochemical isotope exchange strictly limited to 4-hydroxybenzoate, and catalyzed by phenol carboxylase, was reported by groups Aresta and Fuchs.¹¹¹

2.2.1. CIE Using [¹⁴C]CO₂. In early 2019, three independent reports based on transition-metal-assisted decarboxylative carboxylation sequences appeared. First, carboxylic acids are activated allowing the release of CO₂ molecules and the creation of a transient C-metal bond. Then, in the presence of labeled CO₂, competition between [¹²C]CO₂ and [¹⁴C]CO₂ for carboxylation occurred, providing the desired labeled carboxylic acid products as an inseparable mixture of ¹²C and ¹⁴C isotopomers.

As CIE is oftentimes based on reversible transformations and equilibria, ¹⁴C is incorporated in the desired products with some degree of isotope dilution with natural ¹²C. In order to evaluate the efficiency of isotope exchange and compare different procedures, a major parameter of interest is the isotope enrichment (% IE, indicating the ratio of ¹²C over ¹⁴C), which is directly proportional to the *A_m* of the molecule (Figure 4).

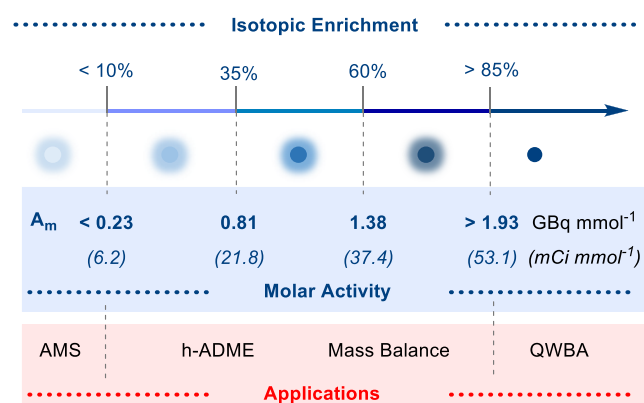


Figure 4. Correlation between isotopic enrichment (% IE) and molar activity (*A_m*) for carbon-14. Color code assignment is arbitrarily selected by the authors, with the aim to present in a scholarly manner the CIE section of the review. Note: correlations between *A_m* and their applications provide general indications. They might vary according to national laws, the internal company rules, and the inherent molecule specifications and study.

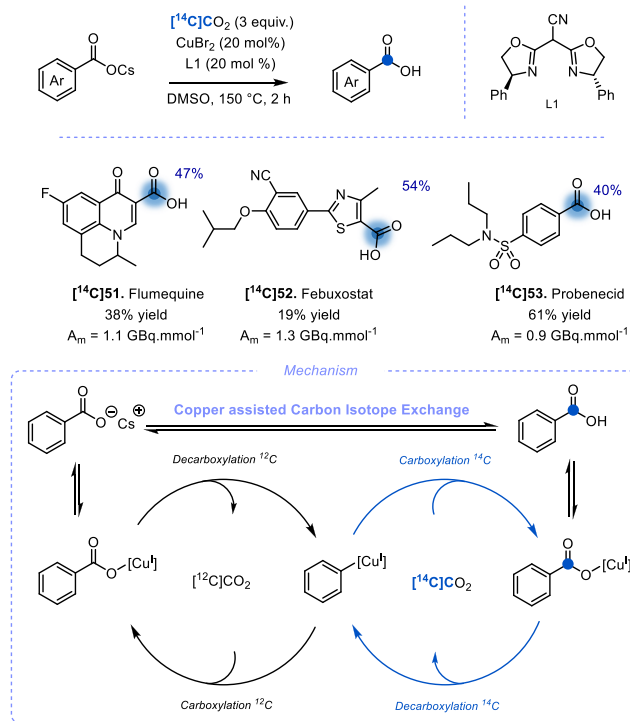
For applications in drug development, the *A_m* is a parameter of utmost importance. For example, low-*A_m* compounds (i.e., low IE < 10%) will only be useful for accelerator mass spectrometry (AMS) studies.¹¹² On the contrary, CIE methods allowing for an IE higher than 40% might be of interest for broader practical applications (human-ADME, mass balance).^{113,114} On the other hand, for animal mass balance¹¹⁵ and QWBA¹¹⁶ studies as well as for agrochemical studies, a high IE (>1.9 GBq mmol⁻¹) is normally used. From the theoretical point of view, the maximum molar activity (i.e., 100% IE) of carbon-14 is 2.31 GBq mmol⁻¹ (or 62.4 mCi mmol⁻¹).

Our group in collaboration with Thibault Cantat patented in 2018¹¹⁷ a copper-catalyzed CIE starting from cesium carboxylate salts.¹¹⁸ Using the RC Tritec carboxylation manifold to precisely deliver 3 equiv of labeled carbon dioxide, the mixture was heated at 150 °C leading to [¹²C]CO₂

extrusion of aromatic carboxylates in the presence of copper(I) catalyst. The organometallic intermediate subsequently reacted with ^{14}C -labeled CO_2 affording the expected carboxylic acids (Scheme 16). This procedure was applied with success to

Scheme 16. Copper-Catalyzed Carbon Isotope Exchange

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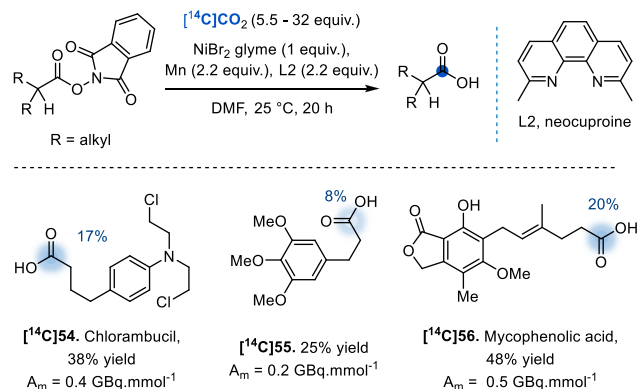
various aryl and heteroaryl carboxylates allowing carbon isotope incorporation in 2 h. Finally, ^{14}C -labeling on several biological acids with good molar activities was performed. Interestingly, only 3 equiv of labeled CO_2 was required (with a maximal theoretical IE of 75%) to obtain an IE in the range 40–54% (Scheme 16). Limitations of the current method are the sensitivity to labile protons that favors the formation of the proto-decarboxylation byproduct, and the scope which tolerates aromatic carboxylated and is preferable with electron-withdrawing substituents. On the other hand, the 1-step radiolabeling of flumequine **51** showcases the effectiveness of this procedure over multistep approaches. Previously, ^{14}C **51** was labeled in 10 linear steps from ^{14}C CO_2 for rat and dog disposition studies.¹¹⁹

In 2019, Baran's group reported a procedure based on a stoichiometric nickel(II) transformation utilizing activated *N*-hydroxyphthalimide (NHP) redox-active esters from alkyl carboxylate derivatives (Scheme 17).¹²⁰ The introduction of labeled CO_2 allows the CIE at room temperature, and the reaction exhibited a wide tolerance toward primary and secondary alkyl carboxylates. In collaboration with the department of radiochemistry at Bristol Myers Squibb, the sequence was implemented to biologically relevant compounds using a large excess of ^{14}C CO_2 (5.5–32 equiv). In particular, chlorambucil **54** and mycophenolic acid **56** were isolated with 17% and 20% IE, respectively.

Concomitantly to this work, Martin's group independently published an analogous CIE procedure suitable for aliphatic NHP redox active esters.¹²¹ The direct exchange was

Scheme 17. Stoichiometric Nickel-Assisted CIE

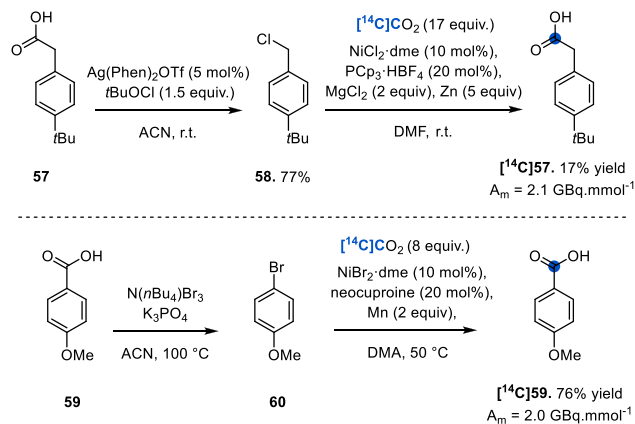
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performed using a substituted 2,2'-bipyridine ligand, which allowed a reduction in the catalyst loading to 10 mol %. The procedure was only performed with ^{13}C CO_2 , and no data on ^{14}C were provided. In addition, the authors reported a 2-step full isotope replacement of alkyl and aryl carboxylic acids by merging decarboxylative halogenations with catalytic carboxylation of organic halides (Scheme 18). Silver-catalyzed

Scheme 18. Full Isotope Replacement of Carboxylic Acids

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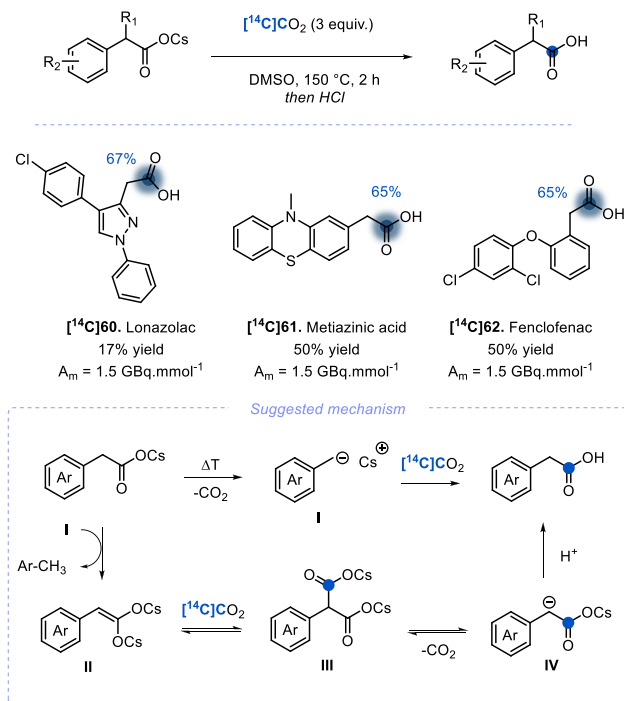


decarboxylation halogenation of aliphatic carboxylic acids¹²² followed by nickel-catalyzed carboxylation enables isolating the corresponding labeled acids with high carbon isotope incorporation for a broad scope of substrates using ^{13}C , including phenyl acetic acid ^{14}C **57**. This method is also suitable for aryl acids. Compound **59** underwent Hunsdiecker-type decarboxylative bromination, according to the procedure reported by Larrosa.¹²³ Further Ni-catalyzed carboxylation delivered ^{14}C **59** with excellent molar activity.^{124,125}

In 2020, our group described a transition-metal-free decarboxylation carboxylation sequence on phenylacetic acids (PAA, Scheme 19).¹²⁶ The importance of PAA in medicinal chemistry is unique, as it is a main substructure representative of NSAIDs. Under thermal conditions, phenyl-acetate cesium salts were decarboxylated to generate benzylic anion intermediates (I). From this anionic species, two possible

Scheme 19. Transition-Metal-Free Carbon Isotope Exchange of Phenyl Acetic Acids

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pathways are suggested: On one hand, the benzylic anion can directly trap a labeled molecule of CO_2 leading to the expected compound. On the other hand, a dienolate moiety (II) might be generated first. This species can then undergo nucleophilic attack on labeled CO_2 leading to the malonate derivative (III). Subsequent decarboxylation followed by protonation affords the expected labeled carboxylic acid. This procedure was applied with success to various drugs such as lonazoloc **60**, metiazinic acid **61** and fenclofenac **62** with ^{14}C , and the molar activities in line with ADMET studies were obtained. In addition, this CIE was also applied for the first time to short-lived ^{11}C .¹²⁷ The same year, Lundgren and co-workers published a very similar methodology starting from potassium salts. The procedure was only applied to ^{13}C .¹²⁸ While the effectiveness of the thermal CIE of PAA is evident, the thermal conditions required for exchange are not ideal for radioactive gas handling.

In order to provide a milder access to CIE of PAA, in 2021, our group reported a photochemical approach with ^{14}C - CO_2 .¹²⁹ Lundgren's group reported at the same time an analogous transformation for ^{13}C -labeling, but no proof of concept for ^{14}C was reported.¹³⁰ Utilizing 4-CzIPN as an organic photocatalyst, carbon isotopes were inserted onto various phenyl acetic acids without any prefunctionalization. Under basic conditions and blue LED light irradiation, the *in situ* formed carboxylate underwent photocatalytic oxidation followed by a rapid decarboxylation to generate a benzyl radical (Scheme 20). The latter was further reduced to a benzyl carbanion through a second single-electron transfer step. Final carboxylation with a suitable source of CO_2 (3 equiv) provided the expected labeled material. This procedure was applied to ^{14}C -labeling of model substrate **63**, which was isolated in 47% yield and 1.4 GBq mmol^{-1} activity (62% IE). This mild protocol was utilized in the labeling of ibuprofen, the ultimate NSAID. ^{14}C **64** was isolated in 29% yield and 53% IE, which is suitable for a large panel of biological applications.

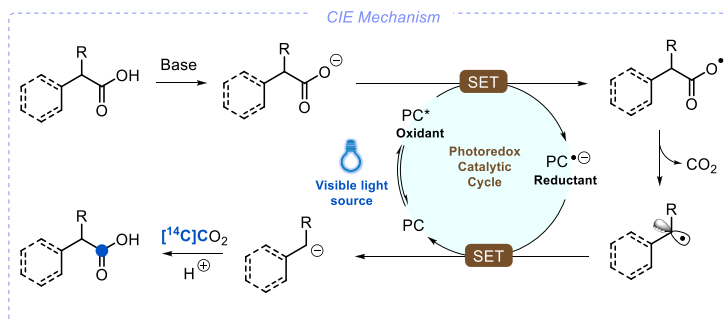
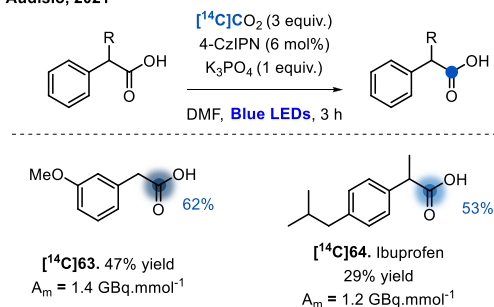
2.2.2. CIE Using ^{14}C CO. In 2018, Gauthier and co-workers from Merck developed a palladium-catalyzed CIE of aliphatic and aromatic carboxylic acids starting from acyl chlorides using ^{14}C CO as an isotopic source.¹³¹ The authors made the hypothesis that, after an oxidative addition of zerovalent palladium onto the acid chloride, an equilibration between the nonlabeled/labeled CO ligand might take place under optimized conditions. Final reductive elimination and hydrolysis of the solution would thus afford the expected labeled carboxylic acid (Scheme 21).

Using this protocol, various exchanges on biologically active substrates were performed with carbon-13, and five examples with carbon-14 were reported. Radioactive compounds were isolated in 16–41% yield and with A_m ranging from 0.6 to 1.0 GBq mmol^{-1} . Representative examples are compounds **65** and **66**. The advantage of this CIE procedure is its adaptability to both aliphatic and aromatic acids. The authors even showed that, under a specific optimization of selected substrates, the chirality in the α position to the carbonyl could be maintained. The downside is the necessity to use a secondary more expensive ^{14}C source and the activation of the carboxylic acid to a hydrolysis-sensitive acid chloride intermediate.

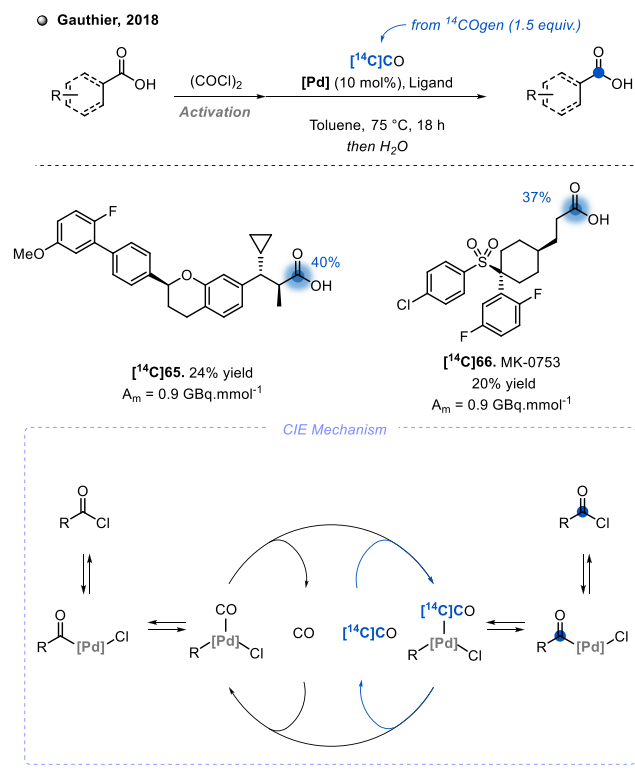
2.2.3. CIE Using ^{14}C CN Salts. Until 2021, CIE procedures could only tackle carboxylic acids. While this functional group is representative of a large variety of biologically active pharmaceuticals and agrochemicals, it is far from ubiquitous. In order to expand the chemical space of CIE, our group and independently Reilly and Strotman

Scheme 20. Photochemical Strategy for Carbon Isotope Exchange with CO_2

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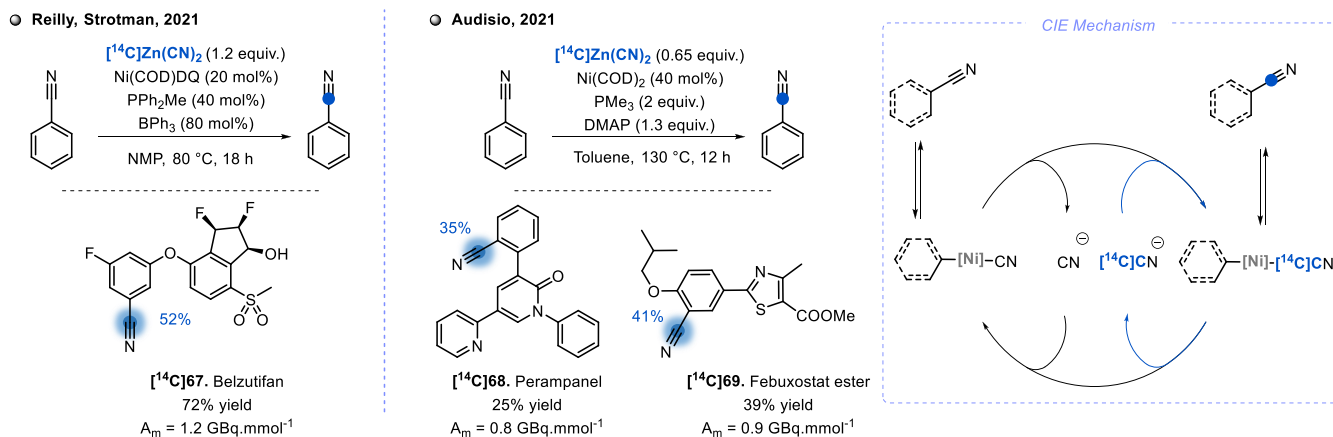


Scheme 21. Palladium-Catalyzed CIE of Acid Chlorides with $[^{14}\text{C}]\text{CO}$



simultaneously published in 2021 two similar procedures allowing CIE of aryl nitriles (Scheme 22).^{132,133} In the presence of nickel(0) catalyst, an $\eta^2\text{-CN}$ complex was formed prior to oxidative addition and metal insertion into the C–CN bond. Thus, a nitrile isotope ligand exchange occurred, and subsequent reductive elimination delivers the expected labeled aromatic nitrile. The procedure exhibited a broad scope including heteroaryl nitriles. Using $\text{Zn}(\text{CN})_2$ as a nitrile source, three pharmaceuticals were successfully labeled with enough isotope incorporation of carbon-14 radionuclide for potential applications in ADMET studies.

Scheme 22. Late-Stage CIE of Aryl Nitriles through Ni-Catalyzed C–CN Bond Activation



3. CONCLUSION

Carbon-14 has a long history of successful use in life science and particularly in the field of human health. Today, its use as a tracer remains a necessary step in the long process of bringing drugs to market. However, despite recent significant improvements, carbon-14 labeling remains difficult for a large number of compounds. Among recent advances, the strategy of isotopic exchange of carbon is certainly the most promising for future developments of carbon radiosynthesis. However, it is still limited to some specific functional groups, such as carboxylic acids and nitriles. Although these functions are common and present in pharmaceuticals, there is a need to extend this strategy to a wider range of chemical functions.

Besides CIE, efforts should be addressed to envision novel late-stage ^{14}C -labeling methodologies for high-molar-activity tracers. For example, direct access to simple building blocks as ^{14}C -labeled carbon monoxide, directly from $[^{14}\text{C}]\text{CO}_2$, under mild reaction conditions is still a challenge. Though this concept has been largely explored in electro- and photochemistry, the attention has thus far been essentially focused on the efficiency of the catalyst, while the effectiveness of the conversion of CO_2 is not investigated, and a large excess of CO_2 is most often utilized. Consequently, no methods such as these have been used in ^{14}C radiosynthesis.

Long-lasting ^{14}C -radioactive waste is extremely difficult to deal with and dispose of. We believe that new efforts should be focused on creative ways to recycle radioactive waste. In our opinion, it is past due to adapt the recently coined concept of green radiochemistry to carbon-14. While Scott's group has conceptualized it for ^{11}C ,^{134,135} ^{11}C waste does not represent a problem due to the short half-life. This is definitely not the case for ^{14}C , and to meet today's societal and environmental needs, there is a compelling urgency for the development of a green and more sustainable radiochemistry (GMSR).

Although these goals represent major challenges, they might provide a source of inspiration and motivation to develop new fundamental chemistry.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/jacsau.2c00030>.

Detailed list of publication for the analyses and statistics exposed in Figure 3 (PDF)

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Author Contributions

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Notes

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