

Robust and General Procedure for Carbon Isotope Labeling of Linear Urea Derivatives with Carbon Dioxide

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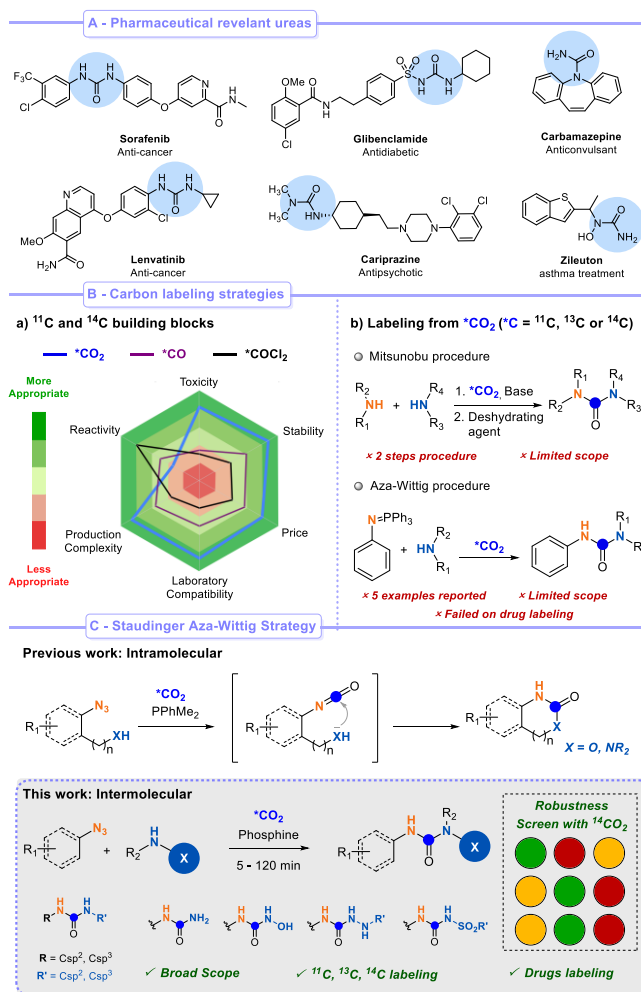
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Abstract: Carbon isotope labeling is a traceless technology, which allows tracking the fate of organic compounds either in the environment or in living organisms. Despite recent advances in the field, the development of robust and general technologies remains a significant task. This full article reports on a general approach to label urea derivatives with all carbon isotopes, including ^{14}C and ^{11}C . Based on a Staudinger aza-Wittig sequence, it provides access to all aliphatic/aromatic urea combinations as well as to semicarbazides, sulfonylureas, hydroxyl ureas, and simple terminal ureas. A de-risking approach was developed to evaluate the robustness of the reaction. This technology is based on $[^{14}\text{C}]\text{CO}_2$ screening that allowed to investigate the tolerance of the procedure with most representative heterocycles and functional groups found in FDA approved drugs.

Introduction

Radioisotope labeling is a fundamental topic in nuclear medicine, medicinal chemistry and has multiple implications in drug design and development.¹ The peculiar physical proprieties of radionuclides allow the collection of precious data for ADMET studies (Absorption, Distribution, Metabolism, Excretion and Toxicological) with long life β^- isotopes² and the diagnosis of pathological disease with non-invasive Positron Emission Tomography (PET) imaging for short life β^+ isotopes.³ In this context, carbon plays a privileged role: ubiquitously present in organic compounds, it is an ideal element for isotopic labeling of pharmaceuticals. Two radioisotopes of carbon are pharmaceutically relevant: carbon-11 (^{11}C) a positron (β^+) emitter with a remarkably short half-live ($t_{1/2} = 20.4$ minutes) and carbon-14 (^{14}C) a long-lived electron (β^-) emitter ($t_{1/2} = 5730$ years).⁴ To introduce a radioactive isotope onto a desired molecule the synthetic strategy should be compliant to specifications such as: short reaction time, high yield and generate minimal amounts of radioactive waste. In line with these recommendations, late-stage labeling appears as the most appropriate strategy.⁵

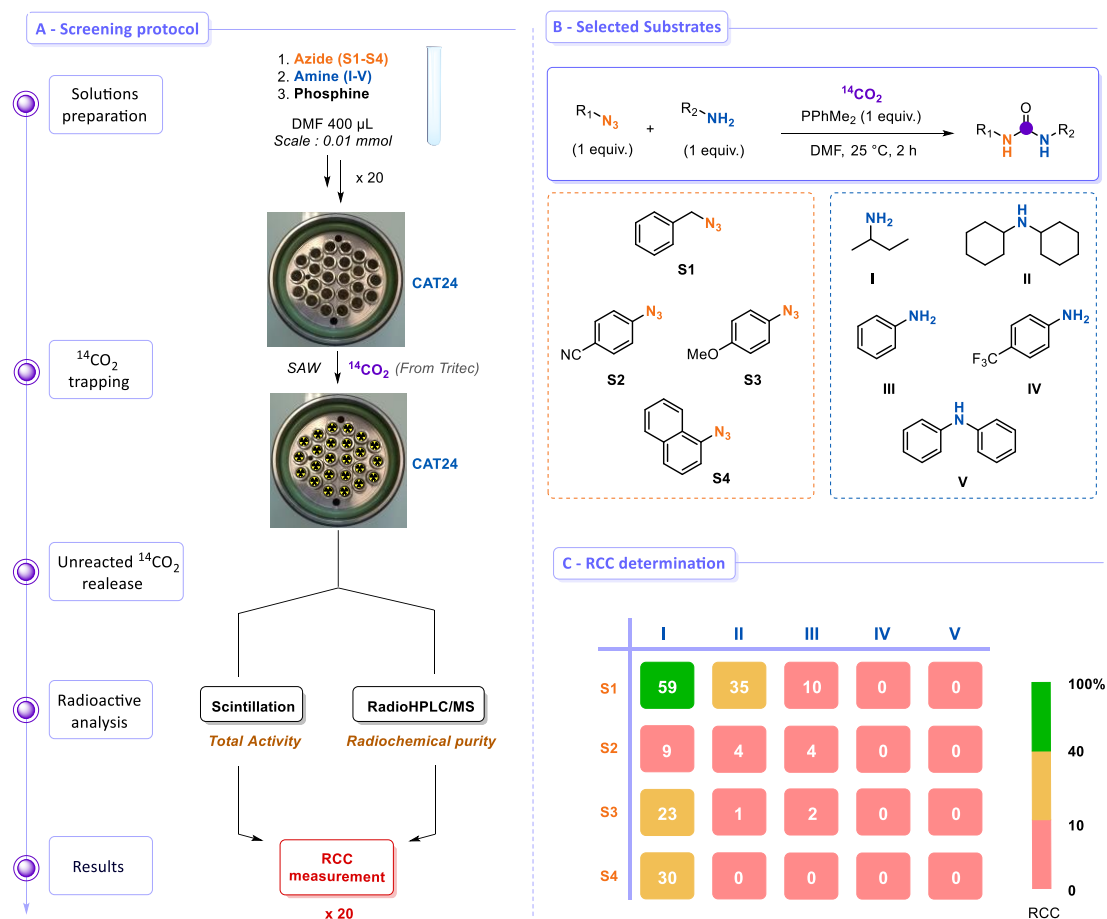


Scheme 1. A - Relevant examples of ureas substructures; B – a) Carbon isotope building blocks used for labeling; b) Synthetic strategies to access radiolabeled ureas; C - Staudinger Aza-Wittig procedure.

The urea substructure is a fundamental building block in medicinal chemistry. It is found in numerous FDA approved drugs for a variety of human diseases and countless number of pharmaceutically relevant compounds (Scheme 1A).⁶ According to a recent study by Ertl *et al.*, it is the 14th most frequent functional group occurring in bioactive molecules described in medicinal chemistry literature.⁷ This functional group represents an attractive target for the labeling of drugs at the carbonyl group. Three main reagents are known for the radiocarbonylation of ureas (Scheme 1B,a).⁸ [$^{11/14}\text{C}$]Phosgene was frequently used due to its high reactivity, but its complex chemical production requiring hazardous reactants, such as C-labeled CO and Cl_2 , and the need for specialized equipments drastically reduced its utilization.^{9,10} [$^{11/14}\text{C}$]Carbon monoxide is a building block suitable for installing the carbonyl group of ureas into organic compounds, through metal-catalyzed procedures or selenium mediated synthesis.¹¹ Nevertheless, its utilization is not trivial due to the inherent toxicity and the time-consuming production, which might require harsh conditions.¹² In addition, [^{14}C]CO was described to undergo radiolysis and its limited stability prevents storage.¹³ [$^{11/14}\text{C}$]Carbon dioxide¹⁴ fulfills the handiest criteria as precursor: it is the primary radioactive carbon source from which derive all ^{11}C - and ^{14}C -labeled compounds, has a remarkable stability to radiolysis and has a suitable safety profile compared to CO and COCl_2 . Two main procedures have been established for the labeling of linear ureas directly from [$^{11/14}\text{C}$]CO₂. On one hand, a Mitsunobu approach using a base (BEMP or DBU) to trap CO₂ and a dehydrating agent (POCl_3) to afford unsymmetrical ureas.¹⁵ This two-step procedure has been

utilized both for ^{11}C and ^{14}C labeling, but it is limited by a moderate functional group compatibility. In 2006, van Tilburg published an Aza-Wittig approach starting from commercially available triphenylphosphine phenylimide and amines affording unsymmetrical ureas with moderate yields.¹⁶ To date, this methodology was applied only to four amine substrates. Importantly, it was unsuccessful on drug radiolabeling despite different attempts to modify and reverse the nature of the triphenylphosphinimine/amine partners.¹⁷ It is worth of note that all previous procedures are limited to ureas bearing carbon-substituents on positions 1 and 3. Scaffolds broadly found in pharmaceuticals such as sulfonylureas, hydroxyl ureas, semicarbazides or simple terminal ureas still constitute a formidable challenge for radiolabeling.¹⁸

The current state of the art highlights the lack of a robust and general method to efficiently label unsymmetrical ureas and their derivatives. We recently reported an intramolecular Staudinger Aza-Wittig (SAW) approach to label cyclic ureas and carbamates from stoichiometric $^{11/14}\text{CO}_2$ (Scheme 1C).¹⁹ The close proximity of the nucleophile to the azide was fundamental for a facile intramolecular attack onto the isocyanate generated *in situ*.



Scheme 2. $^{14}\text{CO}_2/\text{Screen}$. A) Screening procedure based on the use of ^{14}C CO₂. For additional details please see the SI. B) Substrates selected for the screening. C) Results of the screening expressed as radiochemical conversion (RCC).

We now look to develop an intermolecular SAW procedure to offer a straightforward access to unsymmetrical carbon labeled ureas including hydroxyl ureas, terminal ureas, semicarbazides, S-thiocarbamates and sulfonylureas. At the outset, a number of challenges were anticipated, such as: (1) the parasite formation of symmetrical ureas, (2) difference in reactivity based on the electronic nature

of both the azide and amine partners, (3) a lack of generality of the methodology and (4) an undesired reaction, such as amine carbonation, competing with the minute amounts of CO₂ available. To anticipate and manage these issues, a de-risking strategy based on an unprecedented carbon-14 parallel screening was elaborated and applied to the SAW procedure in the perspective of determining at a glance the radiolabeling practicability of complex polyfunctionalized molecules.

Results and Discussion

Assessment of the reaction applicability and development of a parallel ¹⁴C-screening technology

To rapidly assess the generality and potential applicability of an intermolecular SAW with CO₂, we developed a unique ¹⁴C-screening technology (¹⁴CO₂/Screen, Scheme 2A).

Four azides (**S1 to S4**) and five amines (**I to V**) were selected based on their different electronic and steric nature (Scheme 2B), thus providing a representative overview of structures found in pharmaceutically relevant ureas (Scheme 1A). Specifically, we aimed to consider the following combinations: (a) alkyl azide with alkyl amine (b) alkyl azide with aryl amine (c) aryl azide with alkyl amine and (d) aryl azide with aryl amine.

¹⁴CO₂/Screen was implemented by synergically merging the RC Tritec technology, for precise delivery of [¹⁴C]CO₂ and the multi-sample high throughput screening reactor CAT24. The 20 azide-amine couples (ratio 1:1) were screened in DMF according to standard SAW labeling conditions.¹⁹ One equivalent of PPhMe₂ phosphine was added in each vial and a precise amount of labeled [¹⁴C]CO₂ was delivered in the reactor (Scheme 2A, see SI for the detailed experimental procedure). After two hours, unreacted [¹⁴C]CO₂ was removed and the crude reaction mixtures analyzed by scintillation counting, to quantify the residual ¹⁴C radioactivity inside each reaction vial, and subsequently coupled to a RadioHPLC/MS to provide a quantitative assessment of the radiochemical conversion (RCC) of each vial.

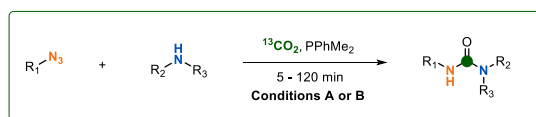
When the aliphatic benzyl azide **S1** was used, the corresponding linear urea was detected with aliphatic amine **I** and **II** in 59% and 35% RCC, respectively (Scheme 2C). In presence of aniline **III**, the unsymmetrical linear urea **S1-III** was obtained in only 10% RCC, while another radioactive by-product was detected in the same amount (11% RCC). The by-product was identified as the symmetrical benzyl urea generated from the addition of benzyl amine, formed by the Staudinger reduction of **S1** under the reaction conditions, to the C-labeled isocyanate. When electron deficient aniline **IV** or secondary aniline **V** were screened, the desired products **S1-IV** and **S1-V** were not formed and only the labeled symmetrical benzyl urea was observed. When aromatic azides were utilized, a dramatic decrease of the reactivity was witnessed. Limited RCC was detected with *sec*-butylamine **I** (9 to 30%). Unfortunately, negligible RCC occurred with the remaining combinations (Scheme 2C). Traces of symmetrical aromatic ureas was also detected in presence of azide **S3** and **S4** with aniline derivatives **III**, **IV** and **V**.

These results clearly show the limitations of the current intermolecular SAW protocol. Specifically, the presence of aromatic amines and azides seriously prevents the reactivity and undermine its generality. To increase its compatibility with various combinations of azides and amines, specific optimizations were necessary.

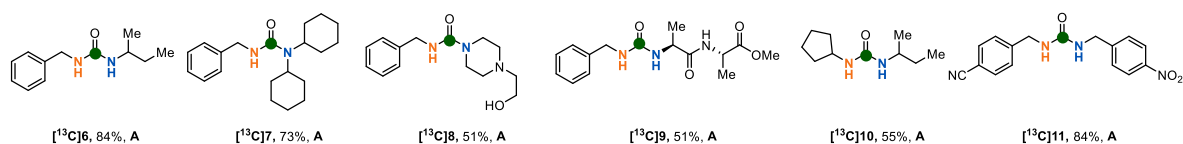
Optimization of the reaction and investigation of the scope

At first, we explored the SAW procedure with a combination of aliphatic azides and aliphatic amines (Scheme 3A). The reaction was performed on model substrates benzylazide and *s*-butylamine in acetonitrile, in presence of one equivalent of PPhMe₂. [¹³C]CO₂ was utilized as a cheaper ¹⁴C-surrogate, and its precise addition was monitored utilizing a RC Tritec manifold, under identical conditions as for ¹⁴C. Introduction of [¹³C]CO₂ resulted in rapid formation of expected urea [¹³C]**6** at room temperature in less than 5 minutes in 84% isolated yield. The procedure is tolerant towards sterically bulky dicyclohexylamine and chemoselectivity for aliphatic amine in respect to alcohols: ureas [¹³C]**7** and [¹³C]**8** were isolated in 73 and 51% yield. The use of a dipeptide was suitable and the corresponding ¹³C urea [¹³C]**9** synthesized with 51% isolated yield. Varying the nature of the azide did not affect the reaction. Secondary cycloalkyl azide provided [¹³C]**10** in 55% yield. It is worth to indicate that in all the reactions performed, no parasite formation of symmetrical urea was observed in ¹³C-NMR of the crude mixture.

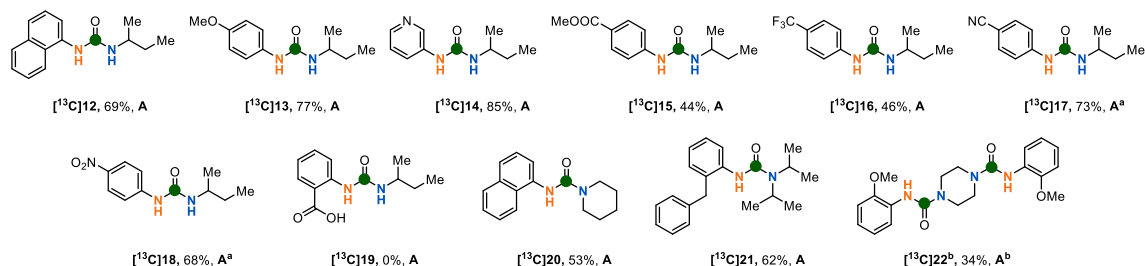
In light with these results, we subsequently explored the SAW procedure in presence of aromatic azides (Scheme 3B). The use of 1-naphtylazide afforded the unsymmetrical urea [¹³C]**12** with an isolated yield of 69%. The presence of electron donating groups like methoxy [¹³C]**13**, or benzyl [¹³C]**21**, are tolerated and the corresponding ureas were isolated in 77% and 62% yield. 3-azidopyridine provided the corresponding product [¹³C]**14** in 85% yield. On the other hand, the presence of electron withdrawing groups on the aromatic azide negatively impacted the outcome of the reaction. When 4-trifluoromethyl and 4-methylbenzoate azides were utilized, the corresponding ureas were isolated only in moderate yields of 46% and 44%, respectively. The anilines corresponding to the hydrolysis of the iminophosphorane (IP) intermediates were observed as major by-products of the transformation. In presence of stronger electron withdrawing groups (EWG) such as cyano or nitro, only the aniline by-products were recovered and no traces of the desired ureas were observed. To prevent the hydrolysis of the IP, the reactions were performed under strict anhydrous conditions in the glovebox using deuterated solvent. Under such conditions, we could observe the formation of the expected urea by ¹³C-NMR, but the major product detected in the reaction was the IP intermediate (³¹P-NMR δ = 11.9 ppm). These results highlight the poor nucleophilic nature of IPs bearing EWGs, which are more prone to hydrolysis than nucleophilic attack to CO₂.



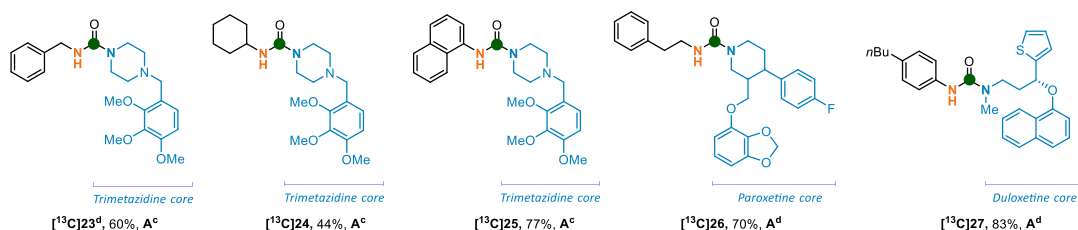
A - Alkyl azide / Alkyl amine



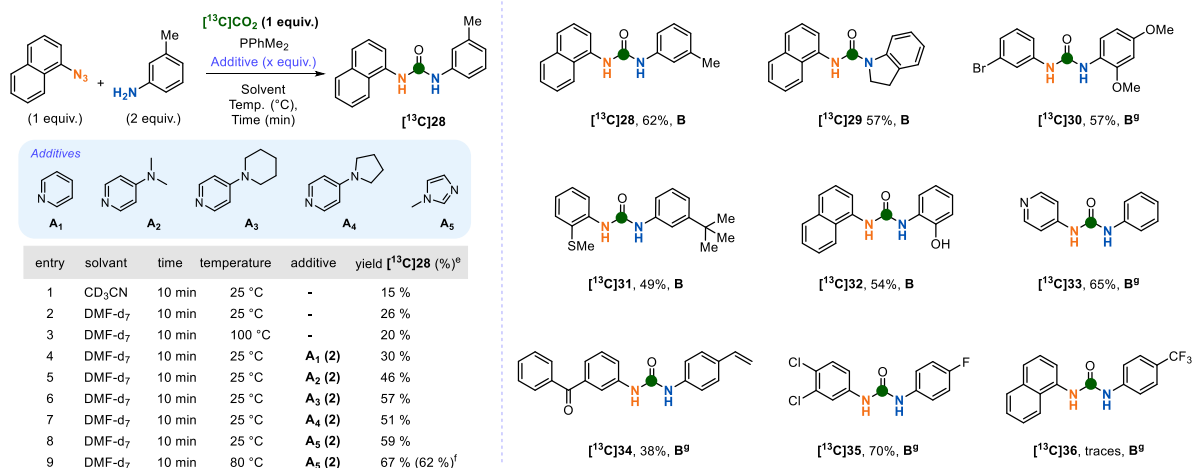
B - Aryl azide / Alkyl amine



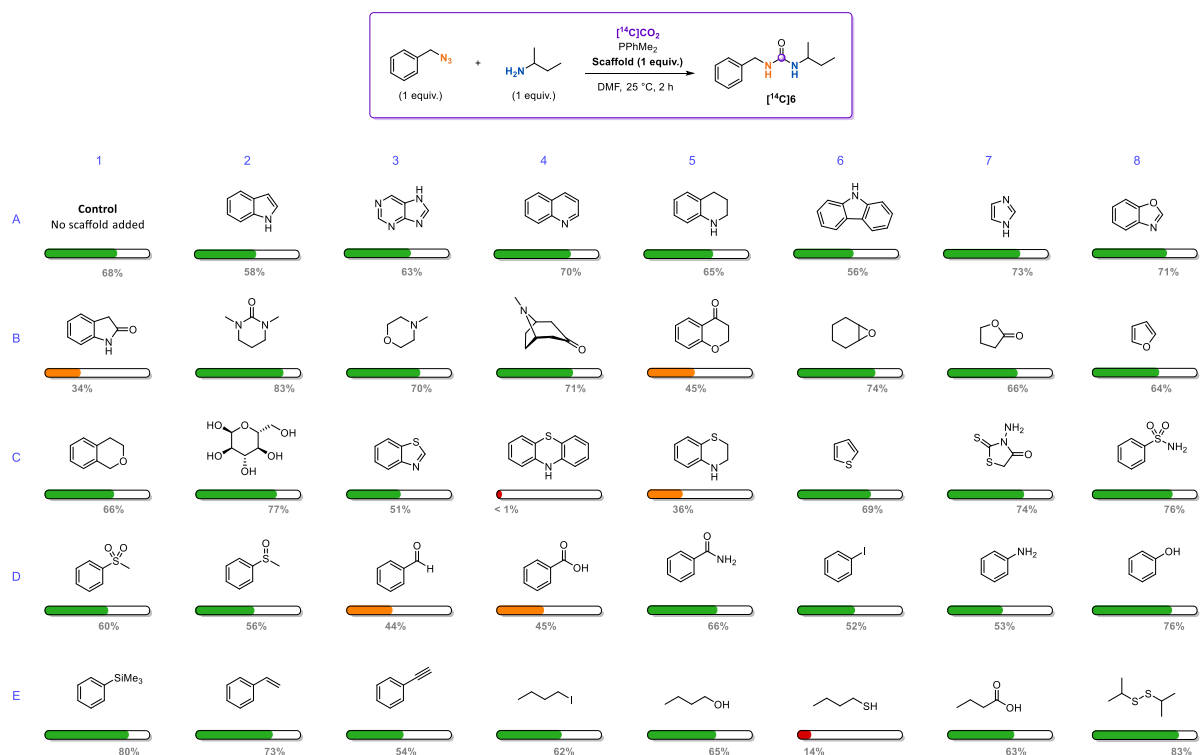
C - Late-stage drugs diversification



D - Aryl azide / Aryl amine



Scheme 3. Scope of the reaction. Green colored circles denotes the position of the carbon atoms labeled. Reaction conditions: A / azide (0.1 mmol, 1 equiv.), amine (0.1 mmol, 1 equiv.), PPhMe₂ (0.1 mmol, 1 equiv.), [¹³C]CO₂ (1.1 equiv.), CH₃CN (0.6 mL), 5 min, 25 °C. B / azide (0.1 mmol, 1 equiv.), amine (0.2 mmol, 2 equiv.), N-methylimidazole (0.2 mmol, 2 equiv.), PPhMe₂ (0.1 mmol, 1 equiv.), [¹³C]CO₂ (1.1 equiv.), DMF (0.6 mL), 10 min, 80 °C. [a] 70 °C instead of 25 °C, [b] azide (0.2 mmol, 2 equiv.), amine (0.1 mmol, 1 equiv.), PPhMe₂ (0.2 mmol, 2 equiv.), [¹³C]CO₂ (2.2 equiv.) [c] DIPEA (2 equiv.) was added. [d] DIPEA (1 equiv.) was added. [e] NMR yield, MTBE was used as internal standard. [f] Isolated yield. [g] 2 hours instead of 10 min.



Scheme 4. ^{14}C -Robustness screening. Effect exerted by various additives on the SAW procedure. The compatibility of each additive was calculated using the $^{14}\text{CO}_2$ /Screen procedure developing. Colored bars (red $\text{RCC} < 20\%$, orange $20\% < \text{RCC} < 50\%$, green $\text{RCC} > 50\%$).

In order to favor the aza-Wittig step, the procedure was reproduced in the glovebox and the reaction heated at 70°C for 10 minutes. By simply adapting the protocol, it was possible to isolate the corresponding linear ureas $[^{13}\text{C}]\mathbf{17}$ and $[^{13}\text{C}]\mathbf{18}$ in 73% and 68% yield, respectively. On the other hand, *ortho*-azidobenzoic acid was not suitable for this procedure. After addition of the phosphine, the carboxylate stabilized the IP forming a 6-members ring that prevented the aza-Wittig reaction with CO_2 ($[^{13}\text{C}]\mathbf{19}$ not observed). In presence of a diamine such as piperazine, a double SAW took place in presence of two equivalents of phosphine, azide and $[^{13}\text{C}]\text{CO}_2$ and bis-urea $[^{13}\text{C}]\mathbf{22}$ was isolated in 34%. Though this result was not optimized further, it paves the way for the development of high molar activity carbon-14 labeling strategies. Finally, the direct diversification of drugs using various aliphatic and aromatic azides was performed. The three commercially available pharmaceuticals Trimetazidine, Paroxetine and Duloxetine were easily functionalized, in presence of one single equivalent of carbon dioxide in 44% to 83% yield (Scheme 3C).

With these results in hand, we next explored the SAW in the presence of less nucleophilic aniline derivatives (Scheme 3D). When standard conditions were utilized with *m*-toluidine, the expected urea $[^{13}\text{C}]\mathbf{28}$ was obtained in poor yield, in agreement with what highlighted by the $^{14}\text{CO}_2$ /Screen. The use of DMF as solvent and higher temperatures slightly improved the yield (Scheme 3D, entries 2 and 3).

In order to increase the electrophilicity of the transient isocyanate, we decided to investigate the use of various additives. Addition of DMAP (\mathbf{A}_2) to the reaction mixture provided $[^{13}\text{C}]\mathbf{28}$ in 46% yield (entry 5). Other pyridine derivatives significantly increase the yield of $[^{13}\text{C}]\mathbf{28}$ (entries 4 to 7). Finally, *N*-methylimidazole (\mathbf{A}_5) was identified as most suitable additive, affording the expected compound in ten minutes at 80°C in 62% isolated yield (entry 9).

We next investigated the scope of this new procedure for bis-aromatic ureas preparation (Scheme 3D). As expected, electron-donating anilines reacted smoothly affording the labeled unsymmetrical urea

[¹³C]28-31 in presence of both primary and secondary amines. Interestingly, the SAW exhibited high chemoselectivity toward aniline in respect of phenol: **[¹³C]32** was isolated in 54% yield and no traces of carbamate was detected in the reaction crude. Linear urea labeling was also possible with poor electron withdrawing aniline leading to aromatic ureas **[¹³C]34** and **[¹³C]35**. However, in presence of strong electron-withdrawing anilines, the reaction did not occur and the expected urea **[¹³C]36** was not observed.

It is noteworthy to highlight that the current protocol is of interest not only in the field of carbon isotope labeling, but also compared to other state of the art methodologies for the synthesis of ureas directly from CO₂. For example, the group of Stephan described in 2019 the synthesis of urea derivatives from CO₂ and silylamines.^{14b} While very effective, this procedure is applicable only to symmetrical ureas, is performed at 120-150 °C and utilized undetermined excess of CO₂ (2 to 5 atm).²⁰

Robustness screening: de-risking the SAW

Understanding the practical limitations of a chemical transformation is a milestone for efficiently planning the synthesis of a desired compound of interest. This is even more critical in radioisotope labeling where costs, related to the isotope source and waste disposal, severely impact the choice of retrosynthetic strategies.² Nonetheless, published reports of the scope of novel transformations are often biased towards the more successful results and lack of information on the compatibility with functional groups and tolerance to heterocycles, thus severely restraining the capacity to foretell reaction outcomes for undescribed substrates.

With the aim of de-risking the SAW and applying it to relevant pharmaceutical molecules, we decided to evaluate its robustness. This valuable approach, introduced by the work of Glorius in 2013,²¹ and not frequently encountered since,²² was applied once in radiochemistry by the group of Gouverneur with fluorine-18.^{23,24} The advantage of such a strategy is that it only takes a glance to predict the success of the labeling on a complex molecule, thus significantly reducing the time invested in the assembly of reaction precursors. To realize this de-risking approach, we took advantage of the **¹⁴CO₂/Screen** previously implemented and we selected the formation of urea **[¹⁴C]6** as model reaction.

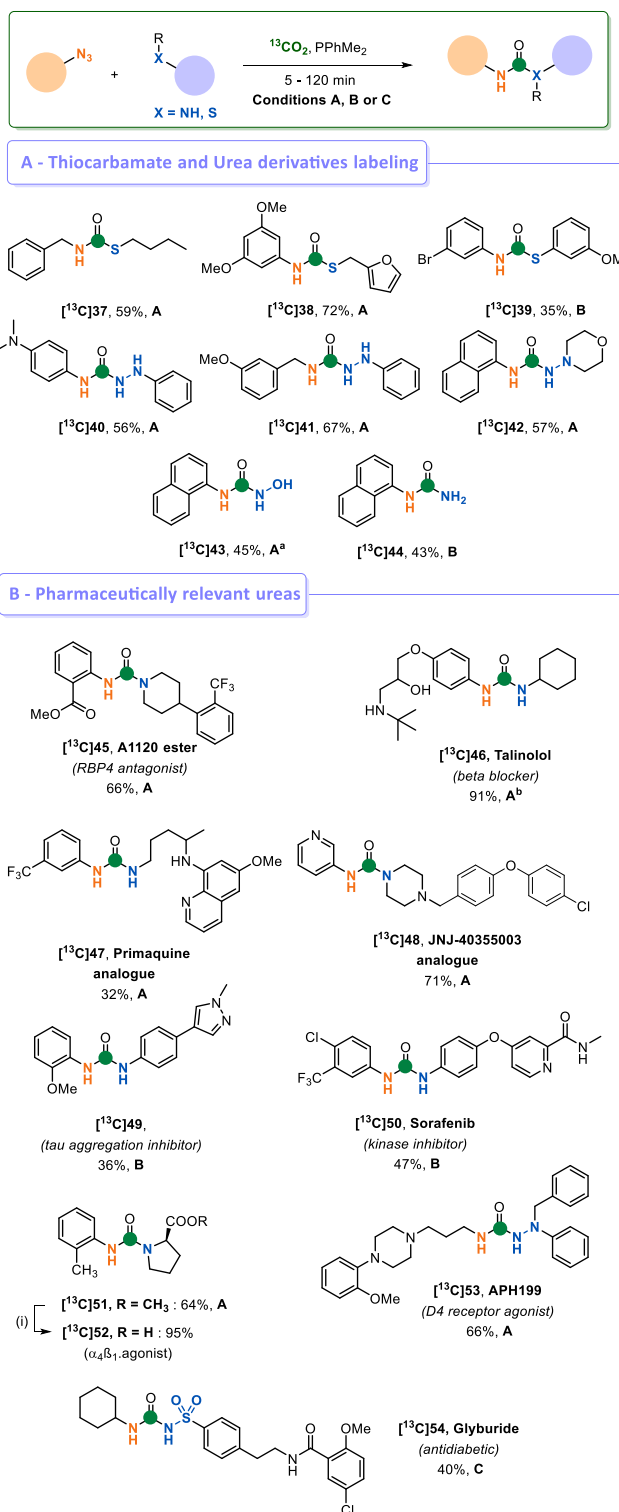
Equimolar amounts of 1-naphtylazide, *sec*-butylamine and phosphine were added in the mixture, in presence of one molar equivalent of a molecular fragment, likely to interfere. The reaction was performed using [¹⁴C]CO₂ and coupled to scintillation counting and radio HPLC, providing the RCC of each reaction. Additives were selected based on the work of Njardarson, where the most frequent nitrogen heterocycles, oxygen heterocycles and sulfur-containing groups in FDA approved drugs are tallied.²⁵

As shown in Scheme 4, most of the nitrogen-containing heterocycles (**A2-A8**, **B2-B4**, **C3**) screened were compatible with the reaction and no impact on the radiolabeling was observed.

Oxygen containing heterocycle (**B3**, **B5-C2**) were also well tolerated and even the challenging unprotected D-(+)-glucose **C2**, one of the major components found in pharmaceuticals, was suitable. However, in presence of some carbonyl-containing functional groups lower conversions were observed. Imine formation with benzaldehyde **D3** or chromanone **B5** and acido-basic reaction with carboxylic acid **D4** could parasite the reaction.

The presence of sulfur derivatives was also investigated. Chemical functions such as sulfone **D1** sulfoxide **D2** and sulfonamide **C8** had no impact on the labeling of the linear urea, affording **[¹⁴C]6** with

similar RCC to the control experiment. However, the RCC was dramatically affected when phenothiazine **C4** was present as additive and no radioactive compound was detected by radioHPLC.



Scheme 5. Application of the SAW to thiocarbamate, urea derivatives and pharmaceuticals. Green colored circles denotes the position of the carbon atoms labeled. Reaction conditions: A / azide (0.1 mmol, 1 equiv.), amine (0.1 mmol, 1 equiv.), PPhMe₂ (0.1 mmol, 1 equiv.), [¹³C]CO₂ (1.1 equiv.), CH₃CN (0.6 mL), 5 min, 25 °C. B / azide (0.1 mmol, 1 equiv.), amine (0.2 mmol, 2 equiv.), *N*-methylimidazole (0.2 mmol, 2 equiv.), PPhMe₂ (0.1 mmol, 1 equiv.), [¹³C]CO₂ (1.1 equiv.), DMF (0.6 mL), 120 min, 80 °C. C / azide (0.1 mmol, 1 equiv.), amine (0.1 mmol, 1 equiv.), NaH (0.2 mmol, 2 equiv.) PPhMe₂ (0.1 mmol, 1 equiv.), [¹³C]CO₂ (1.1 equiv.), DMF (0.6 mL), 5 min, 80 °C. [a] 3 equivalents of DIPEA was added, [b] 5 equivalents of cyclohexylamine was added instead of one. (i) LiOH, THF/H₂O (2/1), 25 °C, 18 h, 95%.

It is worth to note, that while disulfide **E8** did not affect the transformation, the use of butanethiol **E6** showed low radio chemical conversion indicating the presence of a competitive reaction. Radio-HPLC/MS allowed to identify the thiocarbamate as major product of the reaction (radio-HPLC ratio: 4/1). Overall, this robustness screening chemical showed a good tolerance and chemoselectivity toward a whole range of chemical functions investigated, thus providing guidance for future applications.

Access to other derivatives

At first, we looked into aliphatic thiols and thiophenols. Pleasingly, under the same conditions, three thiocarbamates [**¹³C37-39] were isolated in 35 to 72% yield. We next investigated the labeling of semicarbazides: one of the building blocks to conduct to semicarbazones, an important family known for its anti-viral properties, anti-cancer activity and its ability to chelate metals.²⁶ To the best of our knowledge, no radiochemical procedure has been described for radio-carbonylation of this family of derivatives. To access to non-isotopically labeled semicarbazides, two main chemical pathways are described. Most commonly, they are obtained by nucleophilic addition of hydrazide derivative onto isocyanates, commercially available or generated *in situ* from phosgene, Curtius rearrangement or hydrogen carbonate.²⁷**

As shown in Scheme 5A, the use of both aromatic and aliphatic hydrazines enabled accessing to ¹³C-labeled semicarbazides [**¹³C40-42] in 56 to 67% yield, using the standard conditions. To access to 1-hydroxy-3-(naphthalen-1-yl)urea [**¹³C43], hydroxylamine hydrochloride was utilized as nitrogen source in presence of DIPEA. Pleasingly, enrichment was observed in ¹³C-NMR corresponding to the expected hydroxyurea, which was next isolated in 45% yield. Applying the same conditions in presence of ammonium chloride was not successful to obtain terminal urea [**¹³C44]. The utilization of a commercially available solution of ammonia in methanol as nitrogen source assisted by *N*-methylimidazole at 80 °C allowed to isolate the desired terminal urea in 43% yield. This moderate yield can be explained by the hydrolysis of the starting azide and the recovery of the corresponding aniline. Nonetheless, this procedure allows to access to terminal ureas in one single operation from CO₂ with no need of protecting groups.******

Carbon labeling of pharmaceutically relevant urea derivatives

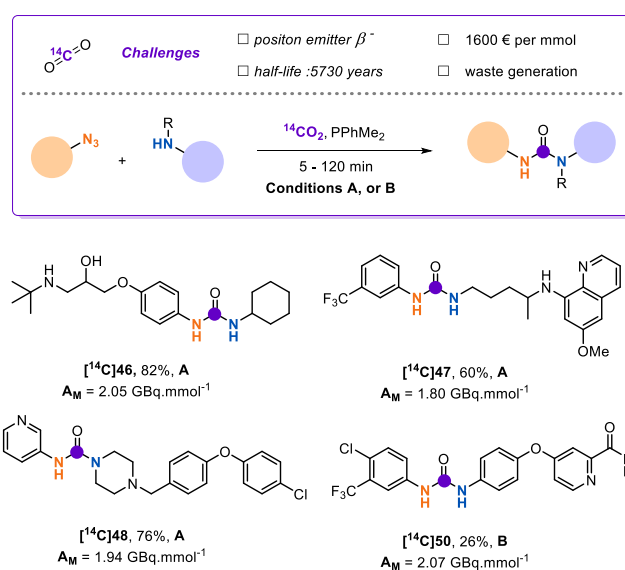
Driven by these results, a series of representative pharmaceutical ureas were labeled starting from appropriate combinations of azides and amines (Scheme 5B). A1120 is an inhibitor of Retinol Binding Protein 4 (RBP4) reported by Amgen in 2009.²⁸ Taking into consideration the previous limitation observed on substrate **19**, we started from the *ortho*-carboxylic acid protected azide and, under SAW procedure, [**¹³C45] was isolated with 66% yield. Next, we focused on Talinolol **46**. The structure of this beta blocker was particularly interesting as its labeling would provide a severe challenge using phosgene or the Mitsunobu/BEMP strategies, given the presence of the *beta*-hydroxyl amine side chain. On the other hand, the SAW enabled to obtain [**¹³C46] in a fully chemo-selective and high yielding manner. [**¹³C47], a primaquine analogue,²⁹ and [**¹³C48], a positional isomer of JNJ-40355003, an inhibitor of the Fatty Acid Amide Hydrolase (FAAH)³⁰ were synthesized in 32% to 91% yield, respectively. Aromatic urea [**¹³C49], patented by Merck as potential tau aggregation inhibitor in Alzheimer disease,³¹ was obtained in 36% yield, while kinase inhibitor Sorafenib [**¹³C50] in 47% yield in presence of *N*-methylimidazole. Urea [**¹³C52], an $\alpha_1\beta_1$ integrin agonist reported by Spampinato and**************

Giacomini in 2019,³² was obtained from L-proline methyl ester and 2-azidotoluene following by saponification in 61% yield over two steps.

Next, semicarbazide [¹³C]46, recently described as a full agonist of the D₄ receptor,³³ was labeled with 66% yield. This example illustrate the use of the SAW as a convergent alternative for the synthesis of such analogues. Finally, antidiabetic sulfonylurea Glyburide [¹³C]48 was labeled for the first time on the carbonyl position. After deprotonation of the corresponding sulfonamide with sodium hydride, the sequence successfully provided the labeled urea with 40% yield using cyclohexylazide as the other partner.

Carbon-14 Radioisotope Labeling

To evaluate the potential of this methodology and to assess its compliance to the recommendations for late-stage labeling, commercially available pharmaceutical drugs were labeled using high molar activity [¹⁴C]CO₂ (A_m: 2.17 GBq.mmol⁻¹). In agreement with the results obtained with carbon 13 isotope, four drugs and analogues were labeled with carbon 14 in high molar activities, negligible isotope dilution and high radiochemical purities (Scheme 6). Talinolol [¹⁴C]47 was labeled with a significant molar activity of 2.05 GBq.mmol⁻¹ and 82% yield.

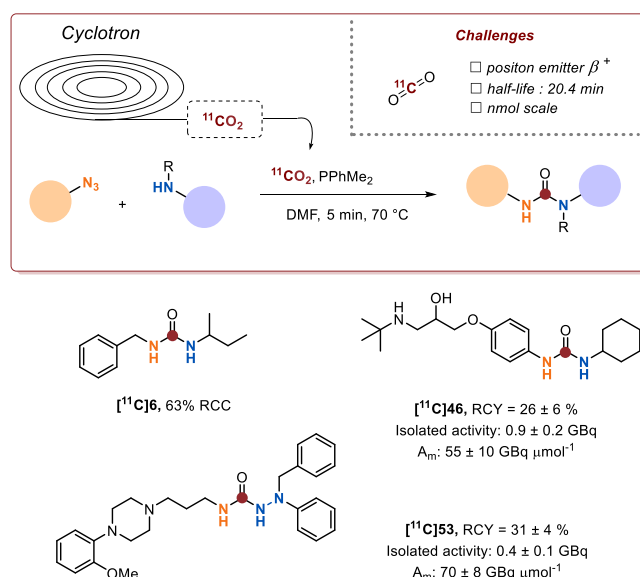


Scheme 6: ¹⁴C Late-stage carbon isotope labeling of pharmaceutically relevant linear ureas. Purple colored circles denotes the position of the carbon atoms labeled. Reaction conditions: A / azide (0.1 mmol, 1 equiv.), amine (0.1 mmol, 1 equiv.), PPhMe₂ (0.1 mmol, 1 equiv.), [¹⁴C]CO₂ (1.1 equiv.), CH₃CN (0.6 mL), 5 min, 25 °C. B / azide (0.1 mmol, 1 equiv.), amine (0.2 mmol, 2 equiv.), N-methylimidazole (0.2 mmol, 2 equiv.), PPhMe₂ (0.1 mmol, 1 equiv.), [¹³C]CO₂ (1.1 equiv.), DMF (0.6 mL), 120 min, 80 °C.

Primaquine analogue [¹⁴C]47 and FAAH inhibitor [¹⁴C]48 were respectively labeled with molar activities of 1.80 GBq.mmol⁻¹ and 1.94 GBq.mmol⁻¹ in 60% and 76% yields. Sorafenib used in chemotherapy to treat kidney, liver, and thyroid cancer has been previously labeled using carbon 11 using phosgene.³⁴ Here, we show that this methodology open access to other radioactive carbon isotope such as carbon-14 for the labeling of drugs such as Sorafenib [¹⁴C]50 obtained with a molar activity of 2.07 GBq.mmol⁻¹, opening a gateway to study their metabolism.

Carbon-11 Radioisotope Labeling

Based on the short reaction time associated to the SAW procedure, application to ^{11}C -labeling seemed promising. Nonetheless, a series of challenges have to be faced: the high energy β^+ emission that require the use of specialized automated systems, the minute concentrations of $[^{11}\text{C}]\text{CO}_2$ produced in the cyclotron and its short half-life (20.4 min).³⁵ Despite the diametrically different technical environment compared to carbon-14 and the CO_2 stoichiometry, model substrate **[^{11}C]**6** could be effectively labeled in 63% RCC, by simply adapting the procedure (Scheme 7). With the SAW approach, the non-purified **[^{11}C]**6** could be synthesized in only 10 minutes. Considering the robustness of the method even when the challenging carbon-11 isotope is concerned, we next decided to label Talinolol. As its structure lack of methyl moieties, radiomethylation of this beta blocker would not be feasible for ^{11}C labeling. Under standard conditions, **[^{11}C]**46** was isolated in $26 \pm 6\%$ RCY and high molar activity (A_m , $55 \pm 10 \text{ GBq } \mu\text{mol}^{-1}$). To the best of our knowledge, carbonyl ^{11}C -labeling of semicarbazides is unprecedented, thus compound **53** looked as an ideal example to explore this SAW application. Pleasingly, **[^{11}C]**53** could be labeled in $31 \pm 4\%$ RCY with a A_m of $70 \pm 8 \text{ GBq } \mu\text{mol}^{-1}$. These preliminary examples of linear urea derivatives showcase that the SAW approach will broaden the scope of original ^{11}C -labeled PET tracers available for imaging applications.********



Scheme 7. Late-stage ^{11}C -radiolabeling of pharmaceutically relevant linear ureas. RCY: decay-corrected radiochemical yield; A_m : decay-corrected molar activity. See SI for experimental details.

Conclusion

Carbon isotope labeling is an effective technology to track the fate of organic compounds either in the environment or in living organisms. Given the ubiquitous presence of this element in nature, such traceless modification is virtually suitable to any organic compounds. Linear ureas and derivatives such as semicarbazides, sulfonylureas, hydroxyl ureas, or simple terminal ureas are pharmacophore moieties frequently present in biologically relevant molecules, including pharmaceuticals and agrochemicals. In this article, we reported a general Staudinger aza-Wittig (SAW) approach which enabled accessing all urea derivatives in a convergent and straightforward manner. This strategy was carefully developed utilizing CO_2 as limiting reagent and has proven to be effective and compatible with all isotopes of carbon: ^{13}C , ^{14}C and ^{11}C . As radiosynthesis with these isotopes is mostly performed when there is a need in preclinical or clinical developments, we have here provided general guidelines

on scope and limitation of these reactions. All four possible combinations of aliphatic/aromatic azide/amines partners were studied in details so to generate a predictability for immediate application. In addition, a parallel screening was implemented to provide further information on the tolerance of the SAW with most representative heterocycles and functional groups found in FDA approved drugs. This empirical approach consists of evaluating the fluctuation in the outcome (RCC) of a reaction model in presence of such additives. This required the development of a technology utilizing [^{14}C] CO_2 as radioactive screening agent, for the first time. Based on the knowledge developed herein, it was possible to label in one single and unique step nine pharmaceutically relevant molecules, including commercial drugs with carbon-13. The technology was validated on radiocarbon and four pharmaceuticals were labeled with ^{14}C in high molar activities, suitable for all possible applications in pharmaceutical industry. Finally, proof of concept was also obtained using short-lived ^{11}C : even the unprecedented labeling of a bioactive semicarbazide compound could be easily performed using this same method. The current work provides a general method for the labeling of urea derivatives which will fill a gap in the literature and provide robust alternative for diagnostic and drug development programs. Furthermore, the robustness screening based on [^{14}C] CO_2 herein developed could be applied to de-risking other radiolabeling approaches, beyond this SAW reaction, thus providing rapid assessment of applicability of future radiolabeling technologies.

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