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General Last-Step Labeling of Biomolecule-Based Substrates by [^{12}C], [^{13}C], and [^{11}C] Carbon Monoxide

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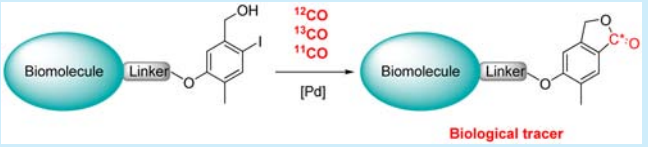
Supporting Information

ABSTRACT: Alkaloid-, steroid-, biotin-, carbohydrate-, nucleoside-, and peptide-based bioconjugates are easily labeled with CO by a last-step palladium-catalyzed carbonylation. The choice of the [^{12}C], [^{13}C], or [^{11}C] isotope opens the way to a new class of potential tracers or ligands easily available for various applications.



Specific ligands of biological targets are very important in the biologist's toolbox to reveal metabolism activity by *in vitro* and *in vivo* experiments.[1] Efficient tracers are generally obtained by combining a high-affinity biomolecule to a synthetic tag allowing precise tracking by analytical or imaging techniques. Among all the possibilities, isotopic and radioisotopic labeling is widely represented in the literature. Indeed, nuclear properties or emitted radiations can be readily detected even on a very small scale. This unique sensitivity has been used in numerous applications according to the stability and/or the nature of the emission.[2]

Carbon perfectly represents this diversity, with intensive use of the stable ^{13}C or the radioactive ^{14}C/^{11}C isotopes.[3] The first one is extremely useful for biological studies when combined with mass spectra analysis or hyperpolarized ^{13}C NMR.[4] The β^- emitting carbon 14 ($t_{1/2}$ = 5730 yrs) is particularly employed for *in vitro*/*in vivo* studies based on liquid scintillation counting or autoradiography imaging.[5] The last one is a β^+ emitter with a shorter half-life of 20.4 min which makes ^{11}C an attractive radionucleus for Positron Emission Tomography (PET). Being noninvasive, this imaging technique underwent great development in the past decade.[6] In most cases, replacing a ^{12}C in a biomolecule by its isotope counterpart permits identical biodistribution of the labeled tracer. Yet, the chemical synthesis can become very difficult for complex and highly functionalized structures, especially when dealing with radioactive material.[7]

To overcome this issue, a general and last-step incorporation of the radioisotope into the tracer is highly recommended and would limit risks, costs, and time consumption by decreasing the radio-synthesis length. For all these reasons, mild and specific ligation of the radioactive synthon to a synthetic building block linked to the biomolecule is particularly attractive. Indeed this strategy has been applied for many radioelements by covalent binding or chelation and often proved to be successful for PET imaging with ^{11}C[8] or other radionuclides[9] (Figure 1). However, carbon monoxide has never been used as an isotopic carbon source for such an approach despite numerous advantages.

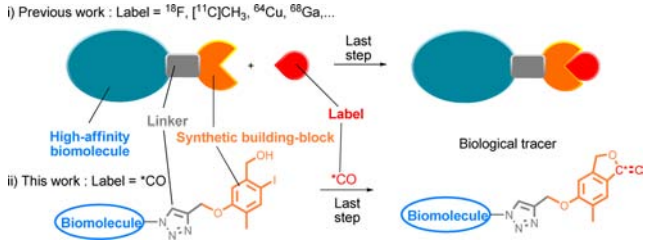


Figure 1. Strategy for the synthesis of biological tracers by a general last-step isotope labeling.

Recent methodologies allow CO[10] and carbon-labeled CO[11−13] to be produced and manipulated easily as a limiting reagent. It can be combined to efficient, selective, and highly functional-group tolerant Pd-catalyzed carbonylation reactions.[14,15] Thus, the design of new synthetic tags, the optimization for a mild carbonylation reaction, and the linking to biomolecules by Cu-catalyzed Huisgen cycloaddition is described. Then, the results of the last-step labeling of the synthesized substrates by [^{12}C], [^{13}C], and [^{11}C] carbon monoxide are disclosed, demonstrating the ease of synthesis of these potential biological tracers.

Importantly, the choice of the carbonylative reaction was crucial due to the potential applications. The pallado-catalyzed intramolecular alkoxycarbonylation of 2-iodobenzylalcohol derivatives was quickly identified as a promising candidate. Indeed, this reaction is known to be selective and highly efficient under mild conditions,[16] and 2-halogenobenzylalcohols have already proven to be good substrates for specific ^{11}CO carbonylation.[17] Thus, the synthetic building-block **2** was easily obtained from commercial compound **1** in a three-step procedure in an 84% overall yield (Scheme 1). This phenol **2** was then functionalized by propargyl and 3-azidopropyl groups to lead respectively to synthetic tags **3** and **4** with an identical

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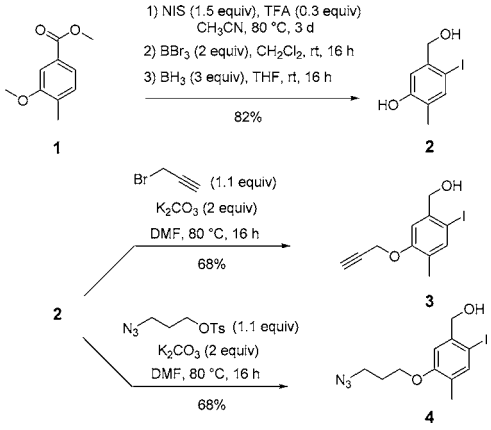
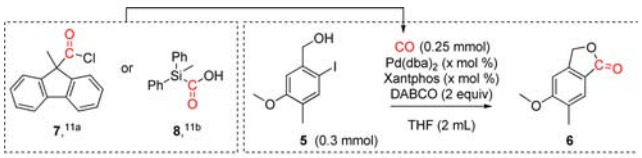
Scheme 1. Synthesis of Propargylated and Azido Tags for Specific CO Capture



yield of 68%. Preliminary studies were first done on the model substrate 2-iodo-4-methyl-5-methoxybenzyl alcohol **5** to tune optimal carbonylation conditions (Scheme 2).

Scheme 2. Optimized Conditions of the Intramolecular Pd-Catalyzed Alkoxycarbonylation



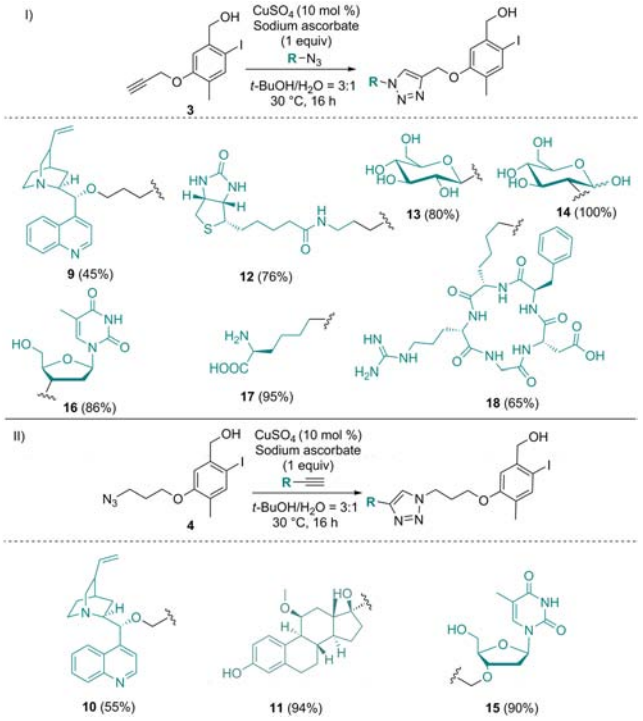
conditions	6, yield
8, [Pd] (5 mol %), 40 °C, 16 h	**6**, 100%
[^{13}C]-**8**, [Pd] (10 mol %), 70 °C, 1 h	**6***, 93%

Carbon monoxide was produced *ex situ* by using a two-chamber system in combination with a CO precursor whose ^{13}C-isotopic version was easily available.[11] Both 9-methyl-9*H*-fluorene-9-carbonyl chloride **7**[11a] and silacarboxylic acid **8**[11b] were investigated, but the latter was chosen for its ability to decarbonylate within minutes at rt in the presence of fluoride. After optimization of the conditions (see Supporting Information (SI) for details), a quantitative CO capture under mild conditions (16 h at 40 °C) was obtained using the $Pd(dba)_2$/Xantphos/1,4-diazabicyclo[2.2.2]octane (DABCO) system. Interestingly, increasing the temperature to 70 °C and catalyst loading to 10 mol % allowed the formation of the desired product in 93% yield within 1 h. In this case, starting from [^{13}C]-**8** led directly to the [^{13}C]-labeled compound **6***.

Next investigations were focused on the synthesis of the biomolecules-based substrates. Easy and efficient linking to a corresponding partner was achieved for both **3** and **4** by using standard conditions for Cu-catalyzed Huisgen cycloadditions[18] (Scheme 3). For example, various molecules bearing an azido residue were reacted with the propargylated tag **3** in the presence of copper sulfate (10 mol %) and sodium ascorbate (1 equiv) at 30 °C for 16 h. The desired bioconjugates were obtained in good yields ranging from 45% to quantitative yields (Scheme 3, I). The same conditions were also applied successfully to the azido tag **4** with alkyne-substituted substrates, giving products in 55% to 94% yields (Scheme 3, II).

All these biomolecules-based substrates were submitted to the carbonylation conditions previously optimized, i.e. 16 h at 40 °C

Scheme 3. Preparation of Biomolecules-Based Substrates by Copper-Catalyzed Huisgen Cycloaddition with 3 or 4



with **8** as the CO precursor (Scheme 4, Procedure A) or 1 h at 70 °C using [^{13}C]-**8** (Scheme 4, Procedure B).[19]

The two O-functionalized cinchonidine derivatives **9** and **10** were used as model compounds to investigate a possible linkage effect. The first one issued from tag **3** allowed isolation of pure **19** in quantitative yield when using procedure A, while **19*** was obtained in 74% yield with procedure B. These results were in good accordance with the experiments from the optimization part (Scheme 2). The second substrate coming from the coupling with tag **4** was carbonylated to synthesize **20** and **20***. Both compounds were obtained with yields above 80%, indicating a limited influence of the linking moiety. Then, more biologically relevant molecules were examined. The bioconjugate **11** derived from moxestrol (a specific ligand to estrogen receptor) was submitted to conditions A and B to give products **21** and **21*** with very good yields of 96% and 94%. As a potential estrogen receptor tracer, the radioactive **21** could be useful for instance to detect hormone responsive cancers.[20] An excellent result was also obtained for the preparation of the biotin-conjugate **22** (Conditions A, 100% yield) or the labeled version **22*** (Conditions B, 79% yield). This demonstrated the compatibility of the methodology with urea and sulfur-containing functionalities. Further possibilities for *in vitro* application could be envisaged through streptavidin/avidin–biotin technology.[21] Starting from commercially available 1- or 2-azidodeoxyglucose-tag structures with conditions A lead respectively to compound **23** (quantitative yield) and **24** (90% yield). Labeled **23*** and **24*** were also produced in good yield (87% and 80%) in 1 h at 70 °C (Conditions B). Compound **23*** was even obtained in 80% yield in 20 min by dividing by three the amount of CO precursor. These glucose analogs could then be well-suited for glucose-uptake measurement and imaging when a radioactive source of CO is used in the carbonylative step.

Nucleoside analogs that are often used for monitoring cellular proliferation, thymidine and azidothymidine were combined to

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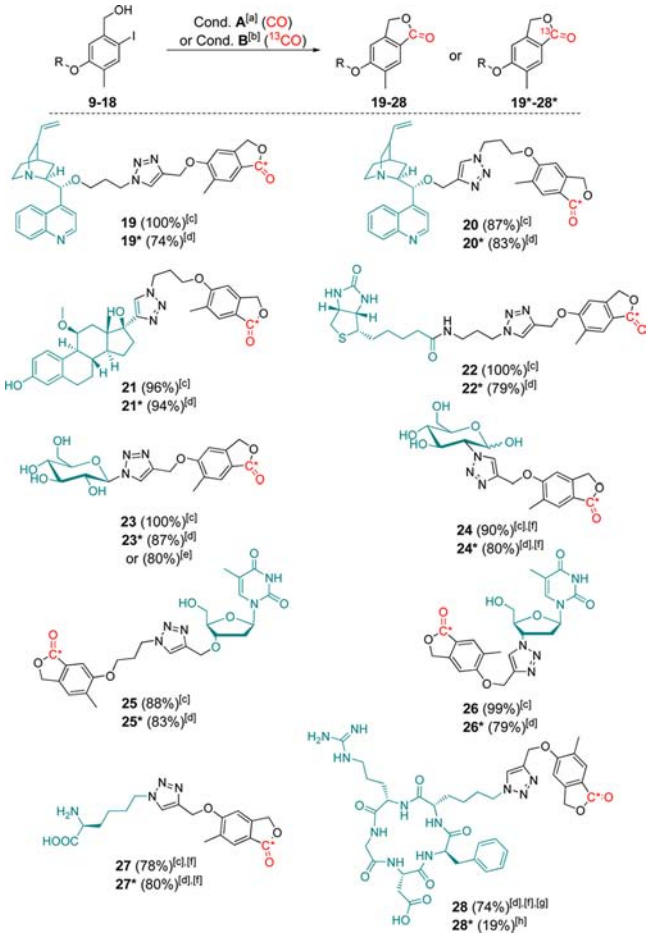
Scheme 4. [^{12}C]-/[^{13}C]-Carbonylations of Substrates 9−18[a]

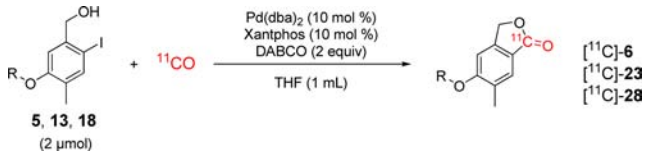


[a]Aryl iodide (0.2 mmol), $Pd(dba)_2$ (5 mol %), Xantphos (5 mol %), DABCO (2 equiv), THF (2 mL) in chamber A and **8** (0.25 mmol), TBAF (10 mol %) in chamber B at 40 °C for 16 h. [b] Aryl iodide (0.2 mmol), $Pd(dba)_2$ (10 mol %), Xantphos (10 mol %), DABCO (2 equiv), THF (2 mL) in chamber A and **8*** (0.15 mmol), TBAF (10 mol %) in chamber B at 70 °C for 1 h. [c] Isolated yield. [d] Yield after purification by column chromatography, isolated in mixture with the starting material. [e] Conditions B with [^{13}C]-**8** (0.05 mmol), **13** (0.2 mmol), 70 °C, 20 min, ^{1}H NMR yield. [f] THF/H_2O mixture (7:1) used as solvent. [g] Aryl iodide (0.05 mmol), $Pd(dba)_2$ (10 mol %), Xantphos (10 mol %), DABCO (2 equiv), THF/H_2O mixture (7:1) (2 mL) in chamber A and **8** (0.1 mmol), TBAF (10 mol %) in chamber B at 40 °C for 16 h. [h] Based on the isolated **28*** after semipreparative HPLC purification of a portion of the crude material, corresponding to a corrected yield of 73% (see SI for details).

azido-tag **4** and propargylated-tag **3**. Resulting precursors **15** and **16** produced modified nucleosides **25**/**25*** and **26**/**26*** after the pallado-catalyzed carbonylation. Very good yields of 79%−99% were obtained depending on the precursor (**15** or **16**) and the conditions (A or B). Amino acids based substrates were also investigated for their high importance in the biological process. Carbonylation conditions proved to be compatible with the free lysine-based conjugate **17**, affording **27** and **27*** in 78% and 80% yield, respectively. The starting material complexity was also increased by testing the labeling of the more challenging and useful cyclo-RGD (**18**). This cyclopeptide is particularly interesting for its high specificity for integrin $\alpha_v\beta_3$ receptors implied in cancer and cardiovascular diseases.[9d] As expected, the carbonylation of **18** at 40 °C for 16 h afforded product **28** with a good yield of 74%. This set of conditions was well-suited for labeling highly functionalized material with stable/long half-life carbon isotopes, but procedure B was used to decrease the reaction time. The resulting mixture was a bit more complex. However, HPLC analysis showed that compound **28*** was the main product and ^{13}C NMR confirmed the presence of only one [^{13}C]-carbonylated product (see SI).

Conditions B were considered as a model setup for the more challenging [^{11}C]-carbonylation, and substrates **5**, **13**, and **18** were selected for further [^{11}C]-labeling experiments in triplicates (Table 1).

Table 1. [^{11}C]-Carbonylations of Substrates 5, 13, and 18



entry	starting material	product	conditions	RCY[a] (%)
1	**5**	[11C]-**6**	5 min, 70 °C	93 ± 2
2	**13**	[11C]-**23**	5 min, 90 °C	75 ± 19
3	**18**	[11C]-**28**	5 min, 90 °C[b]	48 ± 17

[a]Mean ± standard deviation of three experiments. RCY were determined by multiplying the trapping of ^{11}CO (fraction of the total radioactivity recovered in the reaction mixture) by the radiochemical purity (determined by radio-HPLC). See Supporting Information for details [b]THF/H_2O mixture (7:1) used as solvent.

Model substrate **5** was first submitted to [^{11}C]-carbonylation for 5 min at 70 °C. Product [^{11}C]-**6** was obtained with a good radiochemical yield of 93% (Table 1, entry 1) and an excellent standard deviation of 2%. Increasing the temperature to 90 °C for the more complex substrate **13** allowed [^{11}C]-**23** to be synthesized with a good RCY despite a higher standard deviation (entry 2, 75 ± 19% RCY). Reaction of the cyclo-RGD precursor **18** with [^{11}C]-CO led to the labeled product [^{11}C]-**28** (entry 3) with a slightly lower radiochemical yield (48 ± 17%).[22] Nonetheless, this yield still remains correct for providing, without any optimization of the conditions, such a highly functionalized structure. Improvement could also be achieved employing an advanced [^{11}C]-carbonylation setup such as a microautoclave,[23] Xenon transfer,[13a] or microwave heating.[24]

In summary, we described the synthesis of new tags for a carbonylation reaction under mild conditions. After their linking to biomolecules by click chemistry, the precursors were labeled in a final step by [^{12}C], [^{13}C], and [^{11}C] carbon monoxide in good yields or radiochemical yields. The ease of synthesis for a wide scope of substrates demonstrates the high potential of this new methodology, allowing various application possibilities depending on the chosen carbon isotope. Currently under investigation, the biological evaluation for PET imaging of [^{11}C]-**23** and [^{11}C]-**28** will be published in due time.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures and spectroscopic data (^{1}H, ^{13}C NMR) for [^{12}C]- and [^{13}C]-products, experimental procedures and radio-HPLC traces for [^{11}C]-products. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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