

One-Pot, Direct Incorporation of [^{11}C] CO_2 into Carbamates**

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Positron emission tomography (PET) is a prevailing non-invasive research tool for the investigation of biochemical processes and the elucidation of molecular interactions relevant to human health.^[1] The enabling technology that underlies PET and other radiotracer imaging methods is radiotracer chemistry. Thus, the continued success of PET and the expansion of its potential relies on the development of methods to incorporate positron-emitting isotopes into compounds intended, for example, as biomarkers for human disease. Arguably, one of the most important isotopes for PET research is carbon-11 ($t_{1/2} = 20.4$ min) as a result of its ubiquity in pharmacologically active compounds and its favorable physical properties.^[2] However, only a limited number of reactions that meet the special demands and constraints of carbon-11 chemistry have been developed.^[3] Even fewer are routinely employed owing to process complexity and/or the requirement for special equipment. Recently, we have placed a focus on the development of chemical methods for carbon-11 labeling that can be easily and immediately implemented,^[4] and herein we describe a new method that addresses the carbamate functional group.^[5]

Synthesis with carbon-11 begins with the use of a cyclotron that produces $^{11}\text{CO}_2$ or $^{11}\text{CH}_4$ via a nuclear reaction (typically, [$^{14}\text{N}(\text{p}, \alpha)^{11}\text{C}$]).^[6] In most cases, this “starting material” must be converted rapidly into a more useful reagent, for example, $^{11}\text{CH}_3\text{I}$,^[7] which is then used to label a precursor compound. The process of reagent synthesis alone can consume more than half of the radioactivity (if one considers decay during reaction time \times yield of each

conversion step \times trapping efficiency).^[8] In this respect, chemical reactions in which $^{11}\text{CO}_2$ or $^{11}\text{CH}_4$ is used directly have a clear advantage in terms of radiochemical yield. If properly designed, direct incorporation strategies can eliminate or reduce the need for special equipment.

We have now developed a one-pot, operationally simple method for the direct incorporation of $^{11}\text{CO}_2$ into carbamate-containing compounds. This functional group is an attractive target for radiochemical incorporation in light of its versatility for the modular construction of organic compounds and its chemical and metabolic stability. Other methods to label the carbamate carbon atom have utilized [^{11}C]phosgene^[9] or [^{11}C]carbon monoxide,^[10] both of which present technical difficulties and equipment needs that currently make their routine use somewhat impractical.

The development of our method was guided by previous studies in which excess, typically high-pressure, carbon dioxide was used in a variety of chemical-fixation reactions,^[11] including carbamate synthesis.^[12] However, at the outset we anticipated significant differences in reactions with trace carbon dioxide ($^{11}\text{CO}_2$) at atmospheric pressure and recognized process differences that would dictate the general applicability and use of a new radiochemical method. Previous studies^[13] demonstrated the feasibility of using no-carrier-added $^{11}\text{CO}_2$ in the conversion of amines into isocyanates and ureas. Encouraged by these studies, we examined a variety of reaction conditions by using substoichiometric $^{12}\text{CO}_2$ in model reactions. Furthermore, we optimized the reaction in terms of $^{11}\text{CO}_2$ -trapping efficiency at room temperature, capture flow rate, the solvent, and the catalyst/base through screening.^[14]

From these preliminary experiments, we quickly identified DBU (1,8-diazabicyclo[5.4.0]undec-7-ene) as both a superior trapping reagent and a catalyst for the reaction. Solutions of DBU (100 mM) in MeCN, *N,N*-dimethylformamide (DMF), and dimethyl sulfoxide (DMSO) trapped more than 95 % of the $^{11}\text{CO}_2$ introduced in a constant flow of helium (50 mL min⁻¹), whereas DMAP (4-(dimethylamino)-pyridine) or DABCO (1,4-diazabicyclo[2.2.2]octane) in the same solvents (these were also prepared at 100 mM in MeCN, DMF, and DMSO) trapped less than 10 % of the $^{11}\text{CO}_2$.

The reaction with carbon-11 was optimized with operational simplicity in mind. Conceptually, the reaction occurs as detailed in Scheme 1; however, it is most likely that many equilibria exist in solution and that (bi)carbonate salts of DBU are important reactive intermediates.^[14,15] Solutions of benzylamine (**1**), benzyl chloride (**2**), and DBU were combined in a standard cone-bottomed glass vial with a septum and a screw cap, and the resulting mixture was used to trap $^{11}\text{CO}_2$ (10–40 mCi) from a constant stream of helium (within 2 min). The inlet and outlet lines were then removed or closed, and the reaction solution was heated. At the end of a

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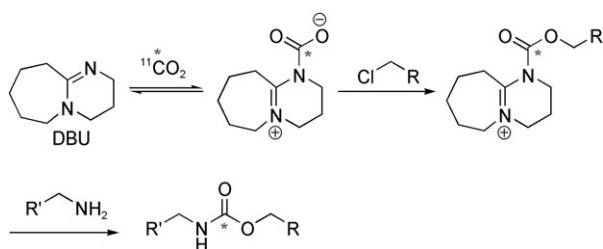
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[**] This research was carried out at Brookhaven National Laboratory (contract DE-AC02-98CH10886 with the US Department of Energy and supported by its Office of Biological and Environmental Research). J.M.H. was supported by the NIH (1F32EB008320-01) and a Goldhaber Fellowship at BNL. A.T.R. was supported by Deutscher Akademischer Austauschdienst (DAAD) and BNL.

Supporting information for this article is available on the WWW under <http://dx.doi.org/10.1002/ange.200900112>.



Scheme 1. Conceptual mechanism for the DBU-catalyzed incorporation of [^{11}C]carbon dioxide.

given reaction time, the reaction was quenched by the addition of excess acid, and the amount of volatile radioactivity (i.e. unreacted $^{11}\text{CO}_2$) was determined. A sample was then analyzed by radio-TLC or radio-HPLC for chemical identity and radiochemical purity. From these data, we calculated the radiochemical yield under various reaction conditions (Table 1).

Table 1: Preliminary reaction optimization.^[a]

Entry	[1] [mM]	[2] [mM]	[DBU] [mM]	<i>t</i> [min]	<i>T</i> [°C]	Yield [%] ^[b]
1	100	100	100	5.0	75	60
2	1	100	100	5.0	100	69
3	1	100	100	7.5	75	81
4	1	100	100	7.5	100	83
5	1	100	100	10	25	17
6	1	100	100	10	75	85
7	1	100	100	10	100	75
8	1	100	100	12	75	83
9	1	100	100	12	100	81
10	10	100	100	10	75	73
11	100	10	100	10	75	51
12	100	100	10	10	75	10
13	100	100	0	10	75	7

[a] Reactions were carried out in DMF (300 μL) in a sealed vessel under a helium atmosphere. No-carrier-added $^{11}\text{CO}_2$ was trapped from a stream of helium gas. [b] See the Supporting Information for details related to the calculation of radiochemical yields.

The desired [^{11}C]carbamate was formed in high radiochemical yield in less than 10 min at a slightly elevated temperature (Table 1, entry 6). The yield was lower when the reaction was carried out at room temperature, but perhaps sufficient for the labeling of temperature-sensitive compounds. When comparing this method to other carbon-11 labeling methods, one must bear in mind that the entire process, including trapping and the desired reaction, can be accomplished in less than 10 minutes. Therefore, yields for this direct method with $^{11}\text{CO}_2$ are much higher than yields (without a correction for radioactive decay) for other, more time intensive, labeling strategies.^[16]

From this series of experiments we determined that the radiochemical yield was more sensitive to the concentrations of DBU and benzyl chloride than to the benzylamine

concentration. In the absence of DBU, a small amount of labeling was observed; however, the trapping efficiency was poor. In the absence of benzylamine, [^{11}C]dibenzyl carbonate (**3**) was formed in less than 1 % yield. This result may indicate that the soluble [^{11}C]carbonate salt of DBU does not participate or is alkylated reversibly with the release of $^{11}\text{CO}_2$ upon acidification. The only productive (irreversible) and high-yielding pathway involved the amine. In fact, the use of a mixture of benzyl alcohol, benzyl chloride, and DBU under the optimized reaction conditions provided [^{11}C]dibenzyl carbonate in a radiochemical yield of less than 3 %.

For the reactions reported in Table 1, we used dry solvents that had been stored over molecular sieves. However, we found that dry solvents were not necessary, and even in the presence of 10 mg of water, the reaction yields were comparably high. We have not eliminated the possibility that water is important in the reaction and are working to perform the reaction under rigorously anhydrous conditions to determine whether water plays an essential role in the mechanism. We have found that the use of aqueous NaOH in DMF under the same conditions is not effective, but mechanisms involving water with DBU cannot be excluded. Clearly, the role of DBU in sequestering CO_2 either as a zwitterion or a (bi)carbonate salt is important, but the precise nature of this interaction and related reactive intermediate(s) cannot yet be discerned, especially with the use of tracer (nanomolar) quantities of $^{11}\text{CO}_2$.^[14]

To probe the scope of this direct $^{11}\text{CO}_2$ -incorporation method, we examined the reaction of a series of amines and alkyl halides (Table 2). Secondary and α -disubstituted amines were converted into the corresponding [^{11}C]carbamates in good yields (Table 2, entries 1 and 2). Less nucleophilic amines, such as anilines, participated in the reaction but with lower levels of conversion under these conditions (Table 2, entries 3–5). Given the simplicity of the reaction, the direct use of $^{11}\text{CO}_2$, and the short reaction times, we anticipate that even with these lower radiochemical yields, this method will be competitive with alternative methods for carbamate labeling. We are currently investigating reaction conditions and cocatalysts for the conversion of alcohols and less nucleophilic amines in increased radiochemical yields.^[17]

We examined the reaction of benzylamine with a series of alkyl halides (Table 2, entries 6–10). For the desired $^{11}\text{CO}_2$ -incorporation reaction to occur in an acceptable radiochemical yield, the reactivity of the electrophile had to be modulated so that competitive alkylation of the amine or DBU and elimination of the alkyl halide were minimal. In general, alkyl chlorides were more suitable than the corresponding bromides. However, in some cases, alkyl bromides were more effective. In these cases, alkylation of the amine was not competitive (Table 2, entry 8). Thus, the reactivity can be “tuned” by the choice of an appropriate nucleophile/leaving-group pair. We are now investigating the extent to which we can develop rules to guide the selection of such pairs and thus expand the scope and predictability of these reactions.

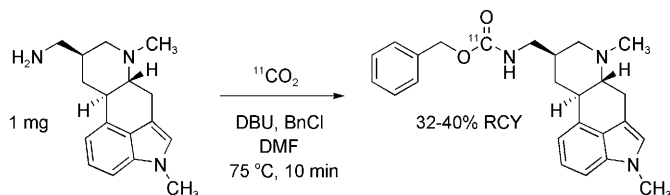
Finally, to test the reaction in the context of a pharmacologically active compound with potential as a PET imaging

Table 2: Scope of the DBU-mediated incorporation of $^{11}\text{CO}_2$.^[a]

$\text{R-NHR}' + \text{Cl-CH}_2\text{-R}'' \xrightarrow[75^\circ\text{C, 10 min}]{^{11}\text{CO}_2, \text{DBU, DMF}} \text{R-N}(\text{R}')\text{-C}(=\text{O})\text{-O-CH}_2\text{-R}''$					
Entry	Product	Yield [%] ^[b]	Entry	Product	Yield [%] ^[b]
1		60	6		77 ± 8 ^[d] 33 ^[c]
2		48	7		33 11 ^[c]
3		16	8		12 69 ^[c]
4		7 16 ^[c]	9		6
5		4	10		< 1

[a] Reactions were carried out with the amine, the alkyl chloride, and DBU, each at a concentration of 100 mM, in DMF (300 μL). [b] Average radiochemical yield for two or more reactions. [c] Radiochemical yield for the reaction with the corresponding alkyl bromide. [d] Mean value \pm standard deviation ($n = 10$).

tracer, we labeled metergoline,^[18] an antagonist of the serotonin (5HT) receptor (Scheme 2). Benzyl carbamate containing molecules, such as metergoline, are attractive targets for this methodology, because the labeling precursor

**Scheme 2.** Synthesis of $[^{11}\text{C}]$ metergoline. Bn = benzyl, RCY = radiochemical yield.

(an amine) can be produced by deprotection with H_2 and Pd/C. (We anticipate similar strategies for other common carbamates that are used as protecting groups.) The reaction setup was extremely simple; a single vessel was required. $^{11}\text{CO}_2$ was trapped directly from the cyclotron target gases in the reaction solution (300 μL), which contained DBU (100 mM), the amine precursor (1 mg), and benzyl chloride (100 mM). Given the simplicity of the method and the short overall process, including the short reaction time from the end of bombardment (EOB), the radiochemical yield was excellent (32% calculated to EOB). We have now scaled this reaction in terms of radioactivity to the use of up to 400 mCi of $^{11}\text{CO}_2$ and can produce $[^{11}\text{C}]$ metergoline at a high specific activity (up to 5 Ci μmol^{-1}) from less than 1 mg of the precursor. (The evaluation of $[^{11}\text{C}]$ metergoline as a radiotracer for the 5HT receptor system in the brain will be reported elsewhere.)

In summary, the direct incorporation of $^{11}\text{CO}_2$ into the carbamate functional group has been demonstrated. We are

currently optimizing additional parameters to improve the radiochemical yield and reaction scope. However, owing to its simplicity and efficiency, this reaction should be immediately useful for the incorporation of carbon-11 and expand the number and types of molecules that can be used in PET and other radiotracer applications. We are currently working to gain a better understanding of the mechanism of the reaction and the role played by DBU under tracer-scale conditions.

Experimental Section

Separate solutions (each 300 mM) of the amine, DBU, and the alkyl chloride were prepared in DMF (which had been sparged previously with helium gas to remove any CO_2 from the atmosphere). Aliquots (100 μL) of each solution were combined in a reaction vessel, which was then sealed with a septum and screw cap.

The resulting solution (300 μL ; 0.1M in each reagent) was sparged with helium gas for 2–3 min. $[^{11}\text{C}]$ Carbon dioxide was released in a stream of helium (ca. 50 mL min^{-1}) from an automated trap-and-release system and introduced into the reaction solution under constant flow at room temperature. The amount of $^{11}\text{CO}_2$ trapped was monitored and recorded, and the capture step was continued until the radioactivity curve reached a maximum (typically < 2 min). After removing the inlet and outlet needles, the sealed vessel was transferred to a heating bath. Most reactions were carried out for 10 min at 75 °C. Other conditions are noted in Table 1. To determine the radiochemical yield, reaction solutions were placed in contact with a miniature radiation detector; inlet and outlet lines were introduced by using needles inserted through the septa. The solution was acidified with 1.0M HCl (100 μL ; an excess amount relative to that of DBU and the amine). The residual $^{11}\text{CO}_2$ trapped in solution was then removed from the solution by sparging with a stream of helium gas (ca. 50 mL min^{-1}). When the radioactivity in the solution became constant (ignoring decay), an aliquot of the solution was analyzed by radio-TLC and/or radio-HPLC. The percentage of radioactivity coincident with that of a reference compound was multiplied by the decay-corrected percentage of radioactivity that remained in the solution following the removal of excess $^{11}\text{CO}_2$.

Received: January 8, 2009

Revised: March 4, 2009

Published online: April 6, 2009

Keywords: carbamates · carbon-11 · isotopic labeling · positron emission tomography · radiochemistry

- [1] R. L. Wahl, *Principles and Practice of PET and PET/CT*, 2nd ed., Lippincott Williams&Wilkins, Philadelphia, PA, 2009.
- [2] G. Antoni, T. Kihlberg, B. Långström, "Aspects on the Synthesis of ^{11}C -Labeled Compounds in *Handbook of Radiopharmaceuticals: Radiochemistry and Applications* (Eds.: M. J. Welch, C. S. Redvanly), West Sussex, England, 2003, p. 141.
- [3] a) P. W. Miller, N. J. Long, R. Vilar, A. D. Gee, *Angew. Chem.* 2008, 120, 9136; *Angew. Chem. Int. Ed.* 2008, 47, 8998; b) M.

- Allard, E. Fouquet, D. James, M. Szlosek-Pinaud, *Curr. Med. Chem.* **2008**, *15*, 235.
- [4] J. M. Hooker, M. Schönberger, H. Schieferstein, J. S. Fowler, *Angew. Chem.* **2008**, *120*, 6078; *Angew. Chem. Int. Ed.* **2008**, *47*, 5989.
- [5] For a new, practical method for the synthesis of methyl carbamates from $^{11}\text{CH}_3\text{I}$, see: B. W. Schoultz, E. Årstad, J. Marton, F. Willoch, A. Drzezga, H.-J. Wester, G. Henriksen, *Open Med. Chem. J.* **2008**, *2*, 72.
- [6] D. R. Christman, R. D. Finn, K. I. Karlstrom, A. P. Wolf, *Int. J. Appl. Radiat. Isot.* **1975**, *26*, 435.
- [7] a) B. Långström, H. Lundqvist, *Int. J. Appl. Radiat. Isot.* **1976**, *27*, 357; b) J. M. Link, K. A. Krohn, J. C. Clark, *Nucl. Med. Biol.* **1997**, *24*, 93.
- [8] a) J. S. Fowler, A. P. Wolf, *Acc. Chem. Res.* **1997**, *30*, 181; b) B. Långström, T. Kihlberg, M. Bergstrom, G. Antoni, M. Bjorkman, B. H. Forngren, T. Forngren, P. Hartvig, K. Markides, U. Yngve, M. Ogren, *Acta Chem. Scand.* **1999**, *53*, 651.
- [9] L. Lemoucheux, J. Rouden, M. Ibazizene, F. Sobrio, M. C. Lasne, *J. Org. Chem.* **2003**, *68*, 7289.
- [10] a) T. Kihlberg, F. Karimi, B. Långström, *J. Org. Chem.* **2002**, *67*, 3687; b) H. Doi, J. Barletta, M. Suzuki, R. Noyori, Y. Watanabe, B. Långström, *Org. Biomol. Chem.* **2004**, *2*, 3063.
- [11] a) M. Costa, G. P. Chiusoli, M. Rizzardi, *Chem. Commun.* **1996**, 1699; b) D. Chaturvedi, A. K. Chaturvedi, N. Mishra, V. Mishra, *Synth. Commun.* **2008**, *38*, 4013.
- [12] a) M. Shi, Y. M. Shen, *Helv. Chim. Acta* **2001**, *84*, 3357; b) D. B. Dell'Amico, F. Calderazzo, L. Labella, F. Marchetti, G. Pampaloni, *Chem. Rev.* **2003**, *103*, 3857; c) D. Chaturvedi, S. Ray, *Monatsh. Chem.* **2006**, *137*, 127; d) P. Tascetta, E. Dunach, *Chem. Commun.* **2000**, 449; e) M. Aresta, E. Quaranta, *Tetrahedron* **1992**, *48*, 1515; f) E. R. Pérez, M. O. da Silva, V. C. Costa, U. P. Rodrigues-Filho, D. W. Franco, *Tetrahedron Lett.* **2002**, *43*, 4091.
- [13] a) A. Schirbel, M. H. Holschbach, H. H. Coenen, *J. Labelled Compd. Radiopharm.* **1999**, *42*, 537; b) E. W. Van Tilburg, A. D. Windhorst, M. Van der Mey, J. D. M. Herscheid, *J. Labelled Compd. Radiopharm.* **2006**, *49*, 321.
- [14] See the Supporting Information for model reactions with CO_2 , $^{11}\text{CO}_2$ -trapping experiments, and additional mechanistic discussion.
- [15] Mechanistic details related to the role of DBU and alkyl amines in similar reactions have been discussed extensively; see, for example: a) D. J. Heldebrant, P. G. Jessop, C. A. Thomas, C. A. Eckert, C. L. Liotta, *J. Org. Chem.* **2005**, *70*, 5335; b) W. Mcghee, D. Riley, K. Christ, Y. Pan, B. Parnas, *J. Org. Chem.* **1995**, *60*, 2820; c) E. R. Pérez, R. H. A. Santos, M. T. P. Gambardella, L. G. M. de Macedo, U. P. Rodrigues-Filho, J. C. Launay, D. W. Franco, *J. Org. Chem.* **2004**, *69*, 8005; d) K. Masuda, Y. Ito, M. Horiguchi, H. Fujita, *Tetrahedron* **2005**, *61*, 213.
- [16] For further discussion, see: F. R. Wuest, *Trends Org. Chem.* **2003**, *10*, 61.
- [17] Cocatalysts have been employed successfully in CO_2 -incorporation reactions involving epoxides; see, for example: Y. M. Shen, W. L. Duan, M. Shi, *Adv. Synth. Catal.* **2003**, *345*, 337.
- [18] C. Beretta, R. Ferrini, A. H. Glasser, *Nature* **1965**, *207*, 421.