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Synthesis of ^{11}C-Labelled Symmetrical Ureas via the Rapid Incorporation of [^{11}C]CO_2 into Aliphatic and Aromatic Amines

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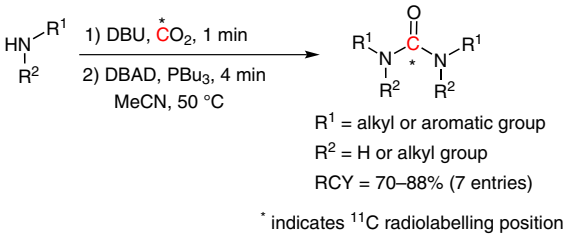

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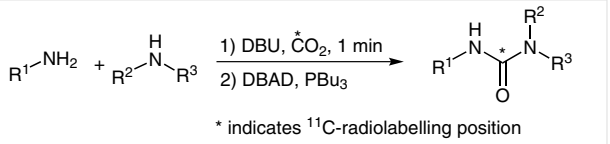
Abstract An efficient method to radiolabel symmetrical [^{11}C]ureas using 1,8-diazabicycloundec-7-ene (DBU) and cyclotron-produced [^{11}C]CO_2 has been developed. [^{11}C]urea derivatives were obtained when aliphatic and aromatic amines were used with excellent radiochemical yields (RCYs) of over 70%. The mechanism of the reaction is proposed on the basis of control experiments. This simple and robust methodology provides a powerful tool to prepare ^{11}C-labelled ureas previously inaccessible by existing methods and enable their utilisation for in vivo molecular imaging applications.

Key words carbon-11, carbon dioxide, Mitsunobu reaction, symmetrical urea, DBU

Positron emission tomography (PET) is a molecular imaging technique that is increasingly being used for diagnosis, biological research and drug development studies.[1] Short-lived carbon-11 ($t_{1/2}$ = 20.4 min) is one of the most commonly used radioisotopes for PET imaging. Carbon-11 (^{11}C) is produced by a cyclotron in the form of [^{11}C]CH_4 or [^{11}C]CO_2 by the ^{14}N(p,α)^{11}C nuclear reaction.[1] From a synthetic perspective, both products are converted into more reactive secondary synthons such as [^{11}C]methyl iodide, [^{11}C]methyl tosylate for radiolabelling.[2] Although these secondary synthons are undoubtedly useful they require time-consuming processing. Therefore, the development of methods to radiolabel compounds directly and efficiently with [^{11}C]CO_2 is of significant interest.[1–3] Due to low CO_2 reactivity, there are only a handful of radiosynthetic methods available to incorporate [^{11}C]CO_2 directly into target molecules of interest.[4] A challenge that needs to be addressed when developing synthetic methods with ^{11}C is that reactions are typically performed with reactants and supporting reagents in large excess compared to the ^{11}C synthons (typically [^{11}C]CO_2 is used on a nanomolar scale).

We have recently developed a method to radiolabel asymmetric ureas with ^{11}C using [^{11}C]CO_2 directly from the cyclotron (Scheme 1).[5] However, when the method was applied to the synthesis of symmetrical ureas, low radiochemical yields (RCYs) were observed.



Scheme 1 Synthesis of asymmetric [^{11}C]ureas using [^{11}C]CO_2

We hereby report a new development of the previous methodology that allows the radiolabelling of symmetrical ureas in moderate to excellent RCYs using a Mitsunobu reaction.

Initially, ^{11}C-radiolabelling of bis(4-methylpiperazin-1-yl)methanone ([^{11}C]**1**) was performed in two steps. Cyclotron-produced [^{11}C]CO_2 was bubbled in a solution containing *N*-methyl piperazine (18.3 µmol), 1,8-diazabicycloundec-7-ene (DBU, 0.8 µmol) in acetonitrile (MeCN, 300 µL) at room temperature (r.t.). The solution was heated at 50 °C and Mitsunobu reagents, di-*tert*-butyl azodicarboxylate (DBAD, 36.6 µmol) and tributylphosphine (PBu_3, 36.6 µmol), dissolved in MeCN (100 µL) were added. The mixture was stirred for 4 minutes at 50 °C.[5] Following this procedure, [^{11}C]**1** was obtained with a RCY of 31% (Table 1, entry 1). RCY was determined by radio-HPLC and defined as the amount of labelled [^{11}C]urea as a percentage of the cyclotron-produced [^{11}C]CO_2 trapped in solution.

To optimise the RCY of [^{11}C]**1** further, the effects of varying reaction time, solvent, temperature and reagent concentrations were studied.

Changing the reaction time from 5 to 15 minutes had no effect on the RCY (Table 1, entry 2). Next, screening of different solvents was carried out. [^{11}C]**1** was not obtained when toluene, THF, and DMF were used as solvent (Table 1, entries 3–5) whereas DMSO (Table 1, entry 6) afforded the desired product with low RCY (24%). MeCN (Table 1, entry 1) produced the highest RCY (31%). Therefore, it was selected as the solvent of choice for further investigations.

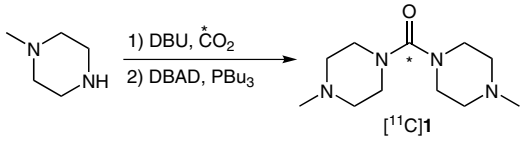
We have previously reported that a reaction temperature of 50 °C is optimal for the formation of asymmetrical [^{11}C]ureas.[5] A similar trend was observed for the synthesis of symmetrical [^{11}C]ureas whereby the RCY decreased from 31% at 50 °C to 14% at 25 °C (Table 1, entries 1 and 7).

Increasing the concentration of the secondary amine did not improve the RCY noticeably (Table 1, entry 8). However, increasing the concentration of DBU from 0.8 µmol to 3.4 µmol (Table 1, entry 9) significantly increased the RCY from 35% to 84% while trapping the [^{11}C]CO_2 efficiently (95%). The increase in RCY can be explained by the influence of DBU on the reaction mixture as it promotes the formation of the carbamate anion (intermediate **I**, Scheme 2). A further increase in DBU concentration from 0.8 to 6.8 µmol caused a drop of the RCY to 15% (Table 1, entry 10). The latter may be due to high concentrations of DBU sequestering protons needed for the protonation of Mitsunobu intermediate **I** (see Supporting Information, Figure SI2) resulting in the low RCY.

In attempts to increase the RCY further, DBU was replaced with another trapping reagent, 2-*tert*-butylimino-2-diethylamino-1,3-dimethylperhydro-1,3,2-diazaphosphorine (BEMP).[4] High [^{11}C]CO_2 trapping was obtained when BEMP was used however, no [^{11}C]**1** was observed (see Supporting Information, Table SI1 and Table 1, entry 11).

To explore the applicability of the developed method, the optimised conditions were applied to the radiolabelling of various symmetrical ureas using a range of aliphatic and aromatic amines (Table 2). High RCYs (>80%) of the desired [^{11}C]ureas were observed for primary and secondary amines such as 1-tetrahydroisoquinoline, benzylamine and cyclohexylamine (**2**–**4**). The less reactive aromatic amine aniline was also tested and interestingly, **5** was obtained with a high RCY of 79%. The effect of functional groups on aniline was studied. Aniline derivatives bearing electron-

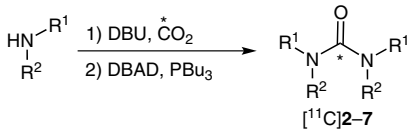
Table 1 Reaction Optimisation to Radiolabel [^{11}C]**1**[6]

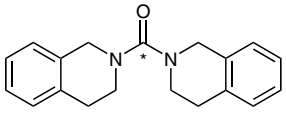
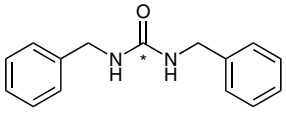
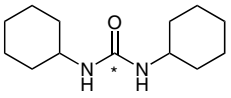
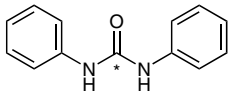
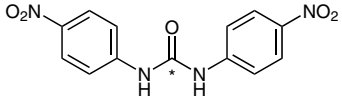
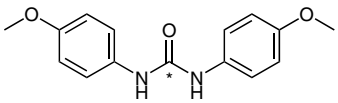


Entry[a]	Time (min)	Trapping reagent	Solvent	RCY (%)[b]
1	5	DBU	MeCN	31 ± 8
2	15	DBU	MeCN	33 ± 2
3	5	DBU	toluene	0
4	5	DBU	THF	0
5	5	DBU	DMF	0
6	5	DBU	DMSO	24 ± 12
7[c]	5	DBU	MeCN	14 ± 1
8[d]	5	DBU	MeCN	35 ± 3
9[e]	5	DBU	MeCN	84 ± 4
10[f]	5	DBU	MeCN	15 ± 7
11	5	BEMP	MeCN	0
12	5	–	MeCN	0

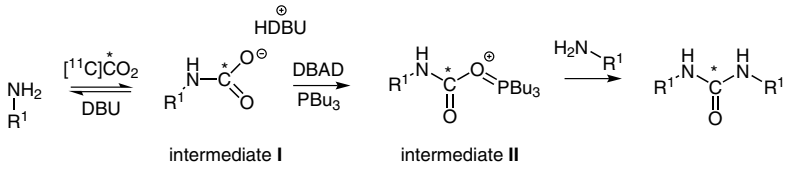
[a] Reaction conditions: [^{11}C]CO_2, amine (18.3 µmol), DBU (0.8 µmol), Mitsunobu reagents (36.6 µmol) in MeCN (400 µL) at 50 °C for 5 min.
[b] RCY was determined by radio-HPLC (n = 3).
[c] Reaction was performed at 25 °C.
[d] Further 18.3 µmol amine was added after trapping.
[e] Amount of DBU used was 3.4 µmol.
[f] Amount of DBU used was 6.8 µmol.

Table 2 ^{11}C-Radiolabelling of Different Symmetrical Ureas[7]



Compound[a]	[^{11}C]Urea	RCY (%)[b]
2		88 ± 6
3		85 ± 5
4		83 ± 2
5		79 ± 14
6		70 ± 2
7		72 ± 6

[a] Reaction conditions: [^{11}C]CO_2, amine (18.3 µmol), DBU (0.8 µmol), Mitsunobu reagents (36.6 µmol) in MeCN (400 µL) at 50 °C for 5 min.
[b] RCY determined by radio-HPLC (n = 3).



Scheme 2 Proposed route for the synthesis of symmetrical [^{11}C]urea

donating (OMe, **6**) or electron-withdrawing (NO_2, **7**) groups in *para* position were radiolabelled in over 70% RCYs. Interestingly, similar RCYs were observed for the synthesis of [^{11}C]**3** when the Mitsunobu reaction time was reduced to one minute.

The reaction for the synthesis of symmetrical [^{11}C]ureas is proposed to occur in three steps (Scheme 2): (i) formation of intermediate **I** from [^{11}C]CO_2 and amine in the presence of DBU; (ii) reaction of **I** with the Mitsunobu reagents giving intermediate **II**; (iii) formation of the urea by nucleophilic attack of an another amine on **II**. The formation of intermediate **I** requires the concomitant presence of an amine and the trapping reagent DBU (see Supporting Information, Table SI1 and Table 1, entry 12). These results are in agreement with the previously proposed trapping mechanism whereby the amine traps the [^{11}C]CO_2 forming a zwitterion which is deprotonated by DBU giving a carbamate anion (intermediate **I**).[8] When Mitsunobu reagents are added to the reaction mixture, a phosphine oxonium ion (intermediate **II**) is formed (see Supporting Information, Figure SI2). In the last step, intermediate **II** reacts with another molecule of amine producing the [^{11}C]urea.

In conclusion, a rapid method to radiolabel symmetrical ureas with ^{11}C has been developed, significantly improving previously reported routes.[5] The method incorporates [^{11}C]CO_2 directly from the cyclotron into primary and secondary amines to form the corresponding [^{11}C]ureas. Aliphatic, benzylic and aromatic molecules were radiolabelled with high RCYs greater than 70%. The developed method avoids the need for converting short-lived [^{11}C]CO_2 into other reactive radiolabelling species and enables the opportunity to study the fate of urea-containing molecules (e.g. Suramin,[9] 1,3-dibenzylurea[10] and 1,3-dicyclohexylurea[11]) *in vivo* using PET.

Acknowledgment

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Supporting Information

Supporting information for this article is available online at http://dx.doi.org/10.1055/s-0034-1381055.

References and Notes

(1) (a) Miller, P.; Long, N.; Vilar, R.; Gee, A. *Angew. Chem. Int. Ed.* **2008**, *47*, 8998. (b) Gee, A. *Br. Med. Bull.* **2003**, *65*, 169. (c) Cunningham, V.; Parker, C.; Rabiner, E.; Gee, A.; Gunn, R. *Drug Discovery Today* **2005**, *2*, 311. (d) Kealey, S.; Turner, E.; Husbands, S.; Salinas, C.; Jakobsen, S.; Tyacke, R.; Nutt, D.; Parker, C.; Gee, A. *J. Nucl. Med.* **2013**, *54*, 139. (e) Ametamey, S.; Horner, M.; Schubiger, P. *Chem. Rev.* **2008**, *108*, 1501. (f) Antoni, G. *J. Label. Compd. Radiopharm.* **2015**, *58*, 65. (g) Kealey, S.; Gee, A.; Miller, P. *J. Label. Compd. Radiopharm.* **2014**, *57*, 195.

(2) (a) Iwata, R.; Pascali, C.; Bogni, A.; Miyake, Y.; Yanai, K.; Ido, T. *Appl. Radiat. Isot.* **2001**, *55*, 17. (b) Cleij, M.; Clark, J.; Baron, J.; Aigbirhio, F. *J. Label. Compd. Radiopharm.* **2007**, *50*, 19. (c) Kealey, S.; Husbands, S.; Bennacef, I.; Gee, A.; Passchier, J. *J. Label. Compd. Radiopharm.* **2014**, 57, 202. (d) Child, C.; Kealey, S.; Jones, H.; Miller, P.; White, A.; Gee, A.; Long, N. *J. Chem. Soc., Dalton Trans.* **2011**, *40*, 6210. (e) Hooker, J. M.; Reibel, A. T.; Hill, S. M.; Schueller, M. J.; Fowler, J. S. *Angew. Chem. Int. Ed.* **2009**, *48*, 6001.

(3) (a) van Tilburg, E.; Windhorst, A.; van der Mey, M.; Herscheid, J. *J. Labelled Compd. Radiopharm.* **2006**, *49*, 321. (b) Hooker, J. M.; Reibel, A. T.; Hill, S. M.; Schueller, M. J.; Fowler, J. S. *Angew. Chem. Int. Ed.* **2009**, *48*, 3482. (c) Wilson, A. A.; Garcia, A.; Houle, S.; Vasdev, N. *Org. Biomol. Chem.* **2010**, *8*, 428. (d) Riss, P.; Lu, S.; Telu, S.; Aigbirhio, F.; Pike, V. *Angew. Chem. Int. Ed.* **2012**, *51*, 2698. (e) Rotstein, B. H.; Hooker, J. M.; Woo, J.; Collier, T. L.; Brady, T. J.; Liang, S. H.; Vasdev, N. *Med. Chem. Lett.* **2014**, *5*, 668.

(4) Rotstein, B. H.; Liang, S. H.; Holland, J. P.; Collier, T. L.; Hooker, J. M.; Wilson, A. A.; Vasdev, N. *Chem. Commun.* **2013**, *49*, 5621.

(5) Haji Dheere, A.; Yusuf, N.; Gee, A. *Chem. Commun.* **2013**, *49*, 8193.

(6) See Supporting Information for full experimental details and compound characterization.
Synthesis of Compound 1: Diisopropylethylamine (194 mg, 1.50 mmol) was added to mixture of 1-methylpiperazine (50 mg, 0.50 mmol), and 4-methylpiperazin-l-ylcarbonyl chloride (122 mg, 0.75 mmol) in CH_2Cl_2 (20 mL). The resulting mixture was stirred at r.t. for 1 h. Then, the mixture was concentrated and recrystallised from EtOH to give **1** (30 mg, 27%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.59 (m, 8 H), 2.56 (m, 8 H), 2.90 (s, 6 H). ^{13}C NMR (100 MHz, CD_3OD): δ = 163.65, 54.03, 45.04, 43.79. HRMS: *m*/*z* [M + H]$^+$ calcd for $C_{11}H_{22}N_4O$: 227.1794; found: 227.2003.

(7) **General Radiolabelling Procedure of [^{11}C]Ureas**: [^{11}C]CO_2 from the cyclotron target was bubbled in a stream of helium gas at a flow rate of 1.4 mL/min post target depressurisation directly into a reaction vial containing an amine (18.3 µmol) and DBU (0.13 µL, 0.9 µmol) in MeCN (300 µL). The resulting solution was stirred and heated at 50 °C for 1 min. In a separate vial, PBu_3 (9 µL, 36.6 µmol) was added to a solution containing DBAD (8 mg, 36.6 µmol) in MeCN (100 µL) under argon at r.t. The resulting solution was transferred into the reaction mixture and stirred for 4 min at 50 °C. The reaction was quenched with H_2O and the crude product was analysed by radio-HPLC.

(8) (a) Peterson, S.; Stucka, S.; Dinsmore, C. *Org. Lett.* **2010**, *12*, 1340. (b) Dinsmore, C.; Mercer, S. *Org. Lett.* **2004**, *6*, 2885. (c) Swamy, K.; Kumar, N.; Balaraman, E.; Kumar, K. *Chem. Rev.* **2009**, *109*, 2551. (d) Ma, C.; Pietrucci, F.; Andreoni, W. *J. Chem. Theory Comput.* **2015**, *11*, 3189.

(9) Nunziante, M.; Kehler, C.; Maas, E.; Kassack, M.; Groschup, M.; Schatzl, H. *J. Cell Sci.* **2005**, *118*, 4959.

(10) Sashidhara, K.; Rosaiah, J.; Tyagi, E.; Shukla, R.; Raghubir, R.; Rajendran, S. *Eur. J. Med. Chem.* **2009**, *44*, 432.

(11) Hwang, S.; Tsai, H.; Liu, J.; Morisseau, C.; Hammock, B. *J. Med. Chem.* **2007**, *16*, 3825.