In-Loop Flow [^{11}C]CO_2 Fixation and Radiosynthesis of *N*,*N*'-[^{11}C]Dibenzylurea

Joseph Downey,‡ Salvatore Bongarzone,‡ Stefan Hader, Antony D. Gee*

Division of Imaging Sciences and Biomedical Engineering, King's College London, 4th Floor Lambeth Wing, London SE1 7EH, UK

‡ These authors contributed equally to this work.

*Corresponding author

Abstract

Cyclotron-produced carbon-11 is a highly valuable radionuclide for the production of positron emission tomography (PET) radiotracers. It is typically produced as relatively unreactive carbon-11 carbon dioxide ([^{11}C]CO_2) which is most commonly converted into a more reactive precursor for synthesis of PET radiotracers.

The development of [^{11}C]CO_2 fixation methods have more recently enabled the direct radiolabeling of a diverse array of structures directly from [^{11}C]CO_2, and the advantages afforded by the use of a loop-based system employed in ^{11}C-methylation and ^{11}C-carboxylation reactions inspired us to apply the [^{11}C]CO_2 fixation "in-loop". In this work we developed and investigated a new ethylene tetrafluoroethylene (ETFE) loop-based [^{11}C]CO_2-fixation method, enabling the fast and efficient, direct-from-cyclotron, in-loop trapping of [^{11}C]CO_2 using mixed DBU/amine solutions. An optimised protocol was integrated into a proof-of-concept in-loop flow radiosynthesis of *N*,*N*'-[^{11}C]dibenzylurea. This reaction exhibited an average 78% trapping efficiency and a crude radiochemical purity of 83% (determined by radio-HPLC), giving an overall non-isolated radiochemical yield of 72% (decay corrected) within just 3 min from end-of-bombardment.

This proof-of-concept reaction has demonstrated that efficient [^{11}C]CO_2 fixation can be achieved in a low-volume (150 µL) ETFE loop, and that this can be easily integrated into a rapid in-loop flow radiosynthesis of carbon-11 labeled products.

This new in-loop methodology will allow fast radiolabelling reactions to be performed using cheap/disposable ETFE tubing setup (ideal for GMP production) thereby contributing to the widespread usage of [^{11}C]CO_2 trapping/fixation reactions for the production of PET radiotracers.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/jlcr.3568

Introduction

One of the most prevalent and important radionuclides used in positron emission tomography (PET) is carbon-11 (^{11}C, $t_{1/2}$ = 20.4 min), it has been incorporated into a wide variety of exogenous and endogenous ligands, used for both diagnostic and research purposes.[1–5] The short half-life potentially allows the administration and scanning of multiple PET radiotracers in the same patient on the same day, thereby combining the advantages of imaging multiple biochemical pathways; in addition, it enables the labelling of biologically relevant molecules, without changing their pharmacodynamic and pharmacokinetic properties, by virtue of its isotopology with carbon-12.

Carbon-11 is produced as $[^{11}C]CO_2$ (primary precursor), by the cyclotron proton-bombardment of nitrogen-14 *via* the $^{14}N(p,\alpha)^{11}C$ nuclear reaction. The relatively low reactivity and solubility of $[^{11}C]CO_2$ leads to its rapid conversion to more reactive secondary precursors.[1] The most prevalent of these secondary precursors is $[^{11}C]CH_3I$ for ^{11}C-methylation reactions.[1] While these reactions are used to produce the vast majority of carbon-11 radiotracers, in the time taken to convert $[^{11}C]CO_2$ to $[^{11}C]CH_3I$, significant amounts of the starting radioactivity can be lost through multistep synthesis and radioactive decay.[6] In addition, ^{11}C-methylation can somewhat limit the chemical space available for radiolabeling. As such, there have been a variety of alternative secondary and tertiary ^{11}C-precursors developed to convert the poorly reactive $[^{11}C]CO_2$ into a more versatile toolbox for the ^{11}C-radiochemist: $[^{11}C]CO$, $[^{11}C]HCN$, $[^{11}C]CS_2$, $[^{11}C]CH_3OTf$ and $[^{11}C]COCl_2$ are just a few examples.[1]

In an effort to avoid this time consuming conversion to more reactive precursors, $[^{11}C]CO_2$ has been reacted directly with Grignard reagents (alkyl magnesium bromide) to synthesise ^{11}C-carboxylic acid derivatives such as [^{11}C]acetate, [^{11}C]palmitic acid and 2-[^{11}C]octynoic acid.[7–9] In an effort to easily automate these syntheses, these reactions have been performed "in-loop" *via* a captive solvent system; whereby the walls of a small tubing loop are coated with the highly-reactive Grignard reagents, then $[^{11}C]CO_2$ is flowed through the loop, where it reacts "in-loop" to form the ^{11}C-labeled carboxylic acid product.[10,11] This "in-loop" synthetic approach has also been employed in ^{11}C-methylation reactions using gaseous $[^{11}C]CH_3I$, with the synthesis of [^{11}C]raclopride as a notable example.[12–16] This synthetic methodology lends itself particularly well to the specific demands of ^{11}C-radiochemistry, and it has a number of advantages over the standard vial-based methods. For these mixed gas-liquid phase reactions, the high surface area provided by the walls of the loop maximises gas-liquid contact, increasing the efficiency and thus decreasing the reaction times required *versus* the standard vial-based setup. In addition, since loop synthesis eliminates reaction vials, transfer losses are kept to a minimum; and since this, in effect, miniaturises these reactions, precursor quantities can be greatly reduced. Finally, these reactions are easily automated which is advantageous when considering the translational potential of a tracer.[12,13]

More recently, there has been a resurgence of interest into the direct use of $[^{11}C]CO_2$ in radiolabeling using a technique called $[^{11}C]CO_2$-fixation.[6,17] This new chemistry is a by-product of the "green-chemistry" movement and the corresponding interest in new chemical agents for carbon-capture;[6,17–19] the most commonly used compounds being the amidine base 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU),[20] and the phosphazene base, 2-tert-butylimino-2-

diethylamino-1,3-dimethylperhydro-1,3,2-diazaphosphorine (BEMP).[21] While there is still some uncertainty regarding their trapping/fixation mechanisms,[22,23] solutions of these bases with an amine substrate exhibit efficient [^{11}C]CO_2 trapping and transfer to form amine carbamate intermediates.[20] This [^{11}C]CO_2 fixation has therefore enabled many new ^{11}C-carbonylation reactions starting from [^{11}C]CO_2, and many new syntheses have been developed based on this fixation strategy (^{11}C-carbamates, ^{11}C-ureas, ^{11}C-amides and ^{11}C-oxazolidinones, among others).[20,24–29] Within our group, we have developed a Mitsunobu-based method for the synthesis of ^{11}C-ureas.[26,27] This reaction involves the fixation of [^{11}C]CO_2 by DBU and amines to form the ^{11}C-amine carbamate intermediates; Mitsunobu reagents (PBu_3/DBAD) are added to form an ^{11}C-isocyanate intermediate, which reacts with another amine molecule to form an ^{11}C-labeled urea product, in just 5 min total reaction time.[26,27]

The recent interest in [^{11}C]CO_2 fixation chemistry has the potential to revolutionise the field of carbon-11 radiochemistry, and the advantages afforded by the use of a loop-based system employed in ^{11}C-methylation and ^{11}C-carboxylation reactions inspired us to apply the [^{11}C]CO_2 fixation "in-loop".

In this work, our aim was therefore to: *i)* establish a novel in-loop trapping/fixation of [^{11}C]CO_2 for the investigation and optimisation of different trapping solutions; and *ii)* implement the setup in an in-loop flow-radiosynthesis of ^{11}C-labeled urea functional groups from [^{11}C]CO_2.

Results and Discussion

Three-component [^{11}C]CO_2 loop trapping

To assess the potential for in-loop trapping/fixation of [^{11}C]CO_2 we designed a novel prototype three-component loop trapping apparatus (**Figure 1**). In designing the system, good manufacturing practice (GMP) requirements led us to opt for a single-use disposable ethylene tetrafluoroethylene (ETFE) based loop system since it obviates the need for rigorous cleaning etc. The system consists of three easily-separable components: a trapping loop (1/16" O.D. ETFE tubing, 150 µL volume), the walls of which will be coated with a trapping solution; a crimped glass waste vial; and a trapping cartridge able to fix all unreacted [^{11}C]CO_2 (ascarite trap, **Figure 1**). On passing [^{11}C]CO_2 through this system, the [^{11}C]CO_2 is totally trapped within these three components. The components are then simply separated and the radioactivity levels of the loop, waste vial and ascarite trap (R_{loop}, R_{waste} and R_{trap} respectively) are measured. Comparison of these values allows the calculation of trapping efficiencies for a given solution.

To pre-coat the walls of the loop, it is half filled with 75 µL trapping solution, then it is connected to the cyclotron delivery line and flushed with helium gas through to the waste vial. The waste vial contains the trapping solution that was not retained by the loop. Therefore when [^{11}C]CO_2 is passed through the three-component system, it is trapped within the loop and any untrapped [^{11}C]CO_2 will be fixed in the solution within the waste vial and the trapping cartridge.

To assess a solution's overall trapping efficiency as well as its suitability for our applications, we extracted two values from the acquired data: these are termed total-solution trapping (T_{sol}) and loop-trapping (T_{loop}).

T_{sol} ($T_{sol} = (R_{loop} + R_{waste})/(R_{loop} + R_{waste} + R_{trap})$) gives an insight into a solution's ability to trap $[^{11}C]CO_2$ in bulk and is a proxy measure for the chemical trapping efficiency of a given solution. While a low T_{sol} indicates that a solvent mixture is unsuitable for application within our in-loop $[^{11}C]CO_2$ trapping/fixation method, a high T_{sol} does not conversely guarantee success for our work. Since a highly efficient trapping solution may still have poor loop-retention – the degree to which the trapping solution is retained on the walls of the loop – and thus $[^{11}C]CO_2$ trapping/fixation will not occur in-loop but in the waste vial.

Instead T_{loop} ($T_{loop} = R_{loop}/(R_{loop} + R_{waste} + R_{trap})$) accounts both for the chemical trapping ability of a solution, but also the degree to which it is retained within our 150 µL ETFE loop. High values of T_{sol} and T_{loop} should guarantee success in developing an in-loop $[^{11}C]CO_2$ fixation methodology.

Our model trapping solutions contained varying concentrations of benzylamine and DBU dissolved in acetonitrile (MeCN). DBU was chosen instead of BEMP, since in previous work using these Mitsunobu reagents, both compounds gave good $[^{11}C]CO_2$ trapping but only DBU led to ^{11}C-urea formation.[27]

To ensure MeCN does not exhibit any $[^{11}C]CO_2$ trapping itself, pure MeCN trapping experiments were attempted and – as expected considering the low solubility of $[^{11}C]CO_2$ in MeCN – negligible trapping was seen for this experiment (T_{sol} = 6.5 ± 0.1%, T_{loop} = 0.2 %, n = 2).

Firstly, we explored the effect of varying benzylamine concentration (1%, 5% and 10%) in the presence of a fixed content of DBU (10% v/v) (**Figure 2**). A solution of 10% DBU in MeCN without adding benzylamine (**Figure 2**) showed good T_{sol} (62.7 ± 2.3%), but very low T_{loop} (2.1 ± 0.1%). While the solution can trap $[^{11}C]CO_2$ reasonably well, it is poorly retained in the loop. Any solutions to be used within this loop trapping/fixation methodology must therefore exhibit high T_{loop}. Increasing benzylamine content up to 10%, both T_{sol} and T_{loop} increased. Notably, adding 1%, 5% and 10% benzylamine, T_{sol} showed near-quantitative trapping of total $[^{11}C]CO_2$ (93.8 ± 2.5%, 97.6 ± 0.8% and 97.5 ± 0.4%, respectively). However despite the comparable T_{sol} seen for these solutions, there was a marked difference in T_{loop} values (**Figure 2**). Using 1% and 5% benzylamine content showed a two-fold increase of T_{loop} (10.9 ± 4.4% and 24.4 ± 8.0% respectively), however increasing further the content of benzylamine up to 10% T_{loop} did not significantly improve (27.0 ± 3.2%).

These results show that – chemically – these mixed benzylamine/DBU solutions, are exquisitely fine $[^{11}C]CO_2$ trapping/fixation solutions, even at relatively low concentrations of benzylamine (1%, T_{sol} > 90%). However for their usage in loop trapping/fixation, only the solutions with high T_{loop} values are suitable (10% benzylamine, T_{sol} = 97%, T_{loop} = 27%). We suspected that the increased benzylamine content of the solutions with the highest T_{loop} values resulted in the increased viscosity of these solutions, replacing less-viscous MeCN (0.343 mPa.s)[30] with more-viscous benzylamine (1.492 mPa.s),[31] which therefore gave an increased retention of the solution on the walls of the loop.

Since we observed no significant improvement of T_{loop} using 5% or 10% of benzylamine we decided to fix the content of benzylamine to 10% in the next experiments. We then explored the effect of varying DBU concentration in our solutions (**Figure 3**). 10% benzylamine in MeCN (no DBU added) showed a reasonable T_{sol} (44.1 ± 15.4%), but very low T_{loop} (1.6 ± 1.1%). These results are very similar to those seen for a solution of 10% DBU in MeCN (**Figure 2**), indeed while both solutions (10% DBU in MeCN or 10% benzylamine in MeCN) can trap [^{11}C]CO_2 fairly well, their poor loop-retention hampers the T_{loop}. Adding DBU at 10%, 50% and 90% led to near-quantitative T_{sol} (97.5 ± 0.4%, 96.5 ±1.8%, and 89.9 ± 4.0%, respectively), again demonstrating that these are highly powerful [^{11}C]CO_2 trapping/fixation solutions (**Figure 3**). However T_{loop} increased by adding 10%, 50%, and 90% DBU (27.0 ± 3.2%, 26.8 ± 15.6%, and 41.8 ± 7.1, respectively). The highest T_{loop} value (42%) for this three-component trapping setup was obtained using a MeCN-free system containing 90% DBU and 10% benzylamine (v/v) mixture. This again supports the suggestion that solution viscosity dictates loop-retention and therefore affects T_{loop}, since we are increasing the content of more-viscous DBU (11.76 mPa.s)[32] and decreasing the content of less-viscous MeCN (0.343 mPa.s).[31]

This section of work demonstrated the successful trapping and fixation of [^{11}C]CO_2 using a mixture of benzylamine/DBU solutions coating the walls of a 150 µL ETFE loop. The modular three-component experimental setup was easy to set-up as well as to disconnect for radioactivity measurements, and so allows the rapid screening of a number of different trapping solutions. We next wanted to explore the potential integration of this in-loop [^{11}C]CO_2 trapping into more complex flow syntheses of ^{11}C-labeled compounds. To investigate this, we performed a proof-of-concept flow synthesis of *N*,*N*'-[^{11}C]dibenzylurea using a more complex setup, and using the optimal trapping solution developed above: 90% DBU and 10% benzylamine (v/v) mixture.

In-loop flow radiosynthesis of *N*,*N*'-[^{11}C]dibenzylurea

The apparatus setup for the flow-synthesis section of this work was based on the simple trapping apparatus described above. The [^{11}C]CO_2 trapping/fixation in-loop was initially performed in a similar manner, before passing a solution of Mitsunobu reagents (PBu_3 and DBAD in MeCN) through the trapping loop, through a second 150 µL reaction loop (to ensure adequate mixing) and into a product vial (**Figure 4**). The [^{11}C]CO_2 is trapped initially as an *N*-[^{11}C]benzyl carbamate intermediate, which is converted by the Mitsunobu reagents to a highly reactive ^{11}C-isocyanate intermediate. This then undergoes attack from another molecule of benzylamine, to form the desired *N*,*N*'-[^{11}C]dibenzylurea product (**Figure 4**).

The setup (**Figure 4**) includes two switching valves (V1 and V2) on an E&Z Modular Lab automated synthesis system. The trapping loop is connected to these two valves, the inlet is connected to V1, and the outlet is connected to V2. V1 can be switched to either the cyclotron outlet (for helium flush and [^{11}C]CO_2 delivery), or to a syringe containing the Mitsunobu reagents. V2 can divert the flow towards either the waste vial or through the reaction loop and into the product vial (see materials and methods section for step-by-step procedure). To ascertain the quantity of [^{11}C]CO_2 delivered through the system per-experiment, the system was washed with MeCN and the radioactivity of all the washings and components (lines, fittings and needles) was measured.

An additional feature of this setup is the 1 mL H_2O added as a quenching solution in the final product vial. This ensures that any ^{11}C-urea derivatives detected in the crude HPLC are products formed exclusively in the loop. Without this simple addition, the reaction could feasibly be simply occurring in the product vial that receives the reaction mixture, during the time taken to measure product radioactivity and prepare the sample for HPLC analysis. The active Mitsunobu intermediate formed is a Morrison-Brunn-Huisgen betaine, which will react with protic substrates: alcohols, amines and carboxylic acids.[33–37] Our own non-radioactive experiments have confirmed that this betaine reacts with water forming the tri-n-butylphosphine oxide (PBu_3O) and di-*tert*-butyl hydrazodiformate (DBAD-H_2). Therefore in the ^{11}C-urea synthesis, any remaining unreacted urea-forming betaine will react with the excess water (in the product vial) upon leaving the reaction loop, ensuring that the crude-HPLC is representative of the ^{11}C-labeled species formed in-loop, not in-vial. In addition this serves to pre-dilute the crude product ready for HPLC injection, which helps to streamline the synthetic process.

One notable observation during these ^{11}C-urea syntheses was that we saw a significantly increased T_{loop} (78.3 ± 3.6%) compared to that seen within the trapping experiments (41.8 ± 7.1%), indicating an increase in loop-retention of our optimised trapping solution. This variance in T_{loop} value might be due to the difference between the two setups; in the flow synthesis of *N*,*N*'-[^{11}C]dibenzylurea the loops are routed *via* the E&Z switching valves (V1 and V2). We speculate that the use of these valves increases the backpressure in the system, and correspondingly increases the loop-retention of the trapping solution.

Due to the demanding time-constraints placed upon carbon-11 radiochemistry in the development of this method we attempted to minimise all process-times, avoiding any product losses due to radioactive decay. Since we avoided pre-trapping or concentration of [^{11}C]CO_2 (instead delivered diluted in the helium carrier gas), and the rate of delivery was not slowed from the cyclotron's 70 mL/min, the [^{11}C]CO_2 is delivered through the system and trapped in the loop within 105 sec of the end-of-bombardment (EOB). V1 and V2 are instantly switched and the trapping loop is filled with Mitsunobu reagents within 30 sec (**Figure 4**). V1 is then switched back to a helium flush and the reagents are pushed through the reaction loop and into the product vial at 70 mL/min. Therefore, the process is complete within just 3 min from EOB, and 1 min from end-of-delivery (EOD).

In the synthesis of our model substrate, *N*,*N*'-[^{11}C]dibenzylurea, we had to consider the concentration and stoichiometry of our Mitsunobu reagents added to the 150 µL trapping loop. Based on the assumption of 7-8 µL trapping solution retention (based on preliminary non-radioactive flushing experiments filling the loop with 75 µL of solution) and considering the optimal conditions found in our previous radiosynthesis of ^{11}C-symmetrical ureas,[27] the reagent concentration was selected to ensure a 2:1 stoichiometric ratio of Mitsunobu reagents (PBu_3/DBAD) to benzylamine.

Using the in-loop flow radiosynthesis setup: the radio-HPLC of the crude solution showed a radiochemical purity (RCP) of 82.6 ± 3.3%. This coupled with the overall T_{loop} of 78.3 ± 3.6%, led to a decay-corrected non-isolated radiochemical yield (RCY) of 72.3 ± 5.1% (n=3) for the synthesis of *N*,*N*'-[^{11}C]dibenzylurea, in 3 min from EOB, with a molar radioactivity of 0.72 ± 0.17 GBq/µmol (for 300-350 MBq initial [^{11}C]CO_2; other work in the group has demonstrated that this molar radioactivity would be expected to increase towards

60-70 GBq/μmol for an initial 30 GBq [^{11}C]CO_2 production,[28] which is consistent with the molar radioactivities obtained for other clinical ^{11}C-labelled radiotracers within our institution).[38] This therefore presents a reproducible, rapid, and high-yielding synthesis of a carbon-11 labeled urea product directly from [^{11}C]CO_2.

The radiochemical yields for the synthesis of *N*,*N*'-[^{11}C]dibenzylurea using this in-loop method (72% decay-corrected) are slightly lower than those for the traditional in-vial method (82% decay-corrected), due to a lower [^{11}C]CO_2 trapping efficiency (78% *versus* 96%), but with a comparable RCP (83% vs 85%, by crude radio-HPLC).[27] However this in-loop method: uses smaller quantities of reagents compared to the in-vial method (75 μL *versus* 400 μL), which should simplify purification; will minimise most of the transfer losses associated with in-vial synthesis; and the use of cheap, disposable, ETFE loops means that this method is particularly well suited to GMP production. These factors combined therefore mean that this in-loop method presents an appealing alternative to in-vial [^{11}C]CO_2 fixation reactions.

Conclusions

In this work we demonstrated that cyclotron-produced [^{11}C]CO_2 can be fixed in a low-volume (150 μL) ETFE loop. We showed that while amine/DBU solutions are chemically efficient [^{11}C]CO_2 fixation agents in-vial, both the chemical and physical properties (primarily viscosity) of these solutions determine their degree of loop trapping efficiency (T_{loop}). This optimised direct-from-cyclotron [^{11}C]CO_2 fixation methodology avoids the need for cryogenic pre-concentration commonly used in carbon-11 procedures. This setup was implemented in a proof-of-concept in-loop flow radiosynthesis of *N*,*N*'-[^{11}C]dibenzylurea by passing Mitsunobu reagents through the loop containing a trapped *N*-[^{11}C]benzyl carbamate intermediate and benzylamine. *N*,*N*'-[^{11}C]dibenzylurea was obtained with high non-isolated radiochemical yields (~ 72%), comparable to those previously reported in-vial (~ 82%), within 3 min from EOB (recently presented in abstract form at the International Symposium on Radiopharmaceutical Sciences, ISRS, Dresden, 14-19 May 2017).[39] This novel methodology has demonstrated the potential for direct-from-cyclotron in-loop [^{11}C]CO_2 fixation and has demonstrated that this can be employed as part of a more complex synthesis (eg. amides, carbamates).[28] The speed of the reaction (3 min from EOB) and the cheap/disposable ETFE tubing setup (ideal for GMP production) mean that this method should be suitable for further applications in the direct trapping/fixation reactions of [^{11}C]CO_2. We anticipate that this new methodology will facilitate a more widespread uptake and application of these powerful reactions, for the radiolabeling of a diverse array of structures directly from [^{11}C]CO_2.

Experimental

Materials and general methods

Anhydrous acetonitrile (MeCN, 99.8%), ascarite, benzylamine (99%), di-*tert*-butyl-azodicarboxylate (DBAD, 98%), triethylamine (Et_3N, ≥ 99.5%) and tri-n-butyl phosphine (PBu_3, 99%) were purchased from Sigma-Aldrich. Ethyl acetate (EtOAc, ≥ 99.5%) was purchased from Fisher Scientific. Anhydrous magnesium sulfate ($MgSO_4$, 98%) was

purchased from Fluka. 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU, 99%) and benzyl isocyanate (98%) were purchased from Alfa Aesar. Carbon dioxide (CO_2) was purchased from BOC Gases.

ETFE tubing (1/16" O.D. x 0.75 mm I.D., 25 m/pkg) was obtained from VICI Jours. The ascarite traps were constructed from empty SPE-ED cartridges, obtained from Biosys Solutions Ltd: Fritted Empty MiniSPE-ED Cartridges, part # 2447. All fluidic connections were obtained from Upchurch Scientific, the product codes are as follows: fingertight flangeless fitting short, PEEK, XP-235X; female to male quick-connect Luer adapter, P-675-01.

$[^{11}C]CO_2$ was produced using a Siemens RDS112 cyclotron in a $^{14}N(p,\alpha)^{11}C$ reaction, by the 11 MeV proton bombardment of nitrogen (+ 1% O_2) gas. The cyclotron produced $[^{11}C]CO_2$ was transferred in a stream of helium gas at 70 mL/min directly into a switching valve of an E&Z Modular Lab automated synthesis unit. Unless otherwise specified, all radioactive experiments used a 5µA bombardment for 1 min, giving on average 300-350 MBq $[^{11}C]CO_2$ at EOB. RCYs reported are calculated as a percentage of the total radioactivity delivered from the cyclotron. Unless otherwise stated, all experiments were repeated three times (n = 3).

^{1}H and $^{13}C\{^{1}H\}$ NMR spectra were recorded on a Bruker Avance DRX 400 MHz spectrometer at 294 K. Chemical shifts are reported in parts per million (ppm) relative to the residual solvent proton impurities (^{1}H) or the residual solvent carbon impurities (^{13}C), as internal standards.

HPLC analysis was performed on an Agilent 1200 system, with a variable wavelength UV detector and a LabLogic Flow-RAM β^+ detector equipped in series. Analytical reverse-phase column: Agilent XDB-C18, 5 µm, 4.6 x 150 mm. Gradient used: 95% H_2O, 5% MeCN; to 5% H_2O, 95% MeCN; over 9 min. Identity of radioactive products was confirmed by co-elution with the non-radioactive standard compounds. HPLC was used to determine molar radioactivities, by reference to a variable dilution calibration curve. Previous experiments within our lab have shown that scaling reactions to higher starting radioactivities, with all other factors kept constant, leads to correspondingly higher molar radioactivities. From these results we assume that a 100-fold increase from approx. 300 MBq (preliminary experiments) to 30 GBq initial $[^{11}C]CO_2$ (clinical production) will give a roughly 100-fold increase in molar radioactivity.

Mitsunobu solutions were prepared by dissolving DBAD (21.1 mg, 91.6 µmol, 3 eq.) in 1 mL MeCN (anhydrous). PBu_3 (22.9 µL, 91.6 µmol, 3 eq.) was added and the mixture was briefly shaken. A colour change from pale yellow-to-colourless was observed on successful formation of the active Mitsunobu intermediate. Trapping solutions were prepared as 1 mL solutions by diluting the corresponding percentages (v/v) of DBU and benzylamine in MeCN. All trapping (benzylamine/DBU/MeCN) and Mitsunobu (PBu_3/DBAD/MeCN) solutions were prepared under an inert argon atmosphere, using anhydrous MeCN.

Three-component $[^{11}C]CO_2$ trapping apparatus: design and setup

The trapping apparatus setup is shown in **Figure 1**. One end of a 35 cm length of ETFE tubing (**trapping loop**, 1/16" O.D., 0.75mm I.D., 150 µL volume) was fitted with a fingertight screw fitting, and the other end was cleanly cut at a 45º taper. The loop was tightly

coiled and placed inside a 10 mL glass vial for ease of handling. The tapered end was inserted through the rubber septum of a sealed crimped 10 mL **waste vial**. This vial was vented *via* a needle through an **ascarite trap** into a gas waste bag. The loop was half-filled with 75 µL trapping solution, using a 100 µL syringe, and connected *via* a luer slip fitting to the $[^{11}C]CO_2$ outlet line from the E&Z Modular Lab. Providing a simple, modular and easy to disconnect setup which traps all $[^{11}C]CO_2$ passed through.

^{11}C-Urea derivative flow synthesis apparatus: design and setup

The ^{11}C-urea synthesis apparatus builds upon the trapping apparatus proof-of-concept, and is shown in **Figure 4**. The cyclotron $[^{11}C]CO_2$ outlet line was connected to switching valve 1 (**V1**) as was a syringe containing Mitsunobu reagents (PBu_3 and DBAD in MeCN). Both ends of a 35 cm length of ETFE tubing (**trapping loop**, 1/16" O.D., 0.75mm I.D., 150 µL volume) were fitted with fingertight screw fittings, and the loop was half filled with 75 µL trapping solution. One end was attached to the outlet of **V1** and the other to the inlet of switching valve 2 (**V2**). To one outlet of **V2,** a short length of ETFE tubing was connected to a **waste vial**, vented *via* an **ascarite trap**. To the other outlet is connected a second 35 cm length of coiled ETFE tubing (**reaction loop**, 1/16" O.D., 0.75mm I.D., 150 µL) running into a sealed crimped product vial (containing 1 mL water), vented *via* an ascarite trap.

Generic procedure: $[^{11}C]CO_2$ trapping

The preloaded loop was connected to the $[^{11}C]CO_2$ delivery line from the cyclotron and helium was flushed through the system for 3 min at 70 mL/min, leaving a just a small, residual amount of trapping solution coating the walls of the trapping loop, and flushing the bulk of the trapping solution into the waste vial. The cyclotron produced $[^{11}C]CO_2$ was then directly delivered diluted in carrier helium gas (without prior trapping and concentration) at 70 mL/min into the E&Z Modular Lab and subsequently through the three-component trapping apparatus. Since all $[^{11}C]CO_2$ is trapped within either the trapping loop, the waste vial, or the ascarite trap, these three components are quickly separated and their radioactivities measured within a Capintec® dose calibrator. Comparison of the distribution of radioactivity within this apparatus allowed calculation of different solvent trapping efficiencies.

Generic procedure: ^{11}C-urea derivative synthesis

1) Helium was flushed through the preloaded trapping loop to the waste vial for 3 min at 70 mL/min. 2) Cyclotron produced $[^{11}C]CO_2$ was then directly delivered, diluted in carrier helium gas (without prior trapping and concentration) at 70 mL/min, through the trapping loop, waste vial and ascarite trap. 3) **V1** and **V2** are switched and the trapping loop was filled with 150 µL Mitsunobu solution (PBu_3/DBAD in MeCN). 4) **V1** was switched and a 70 mL/min helium flush from the cyclotron transferred the contents of the trapping loop through the reaction loop and into the product vial (containing 1 mL water as a quench). 5) The crude products were analysed by radio-HPLC and the system was washed with MeCN and all washings and component radioactivities were measured to determine the total $[^{11}C]CO_2$ radioactivity delivered from the cyclotron.

Synthesis of *N*,*N*'-Dibenzylurea

Benzylamine (1.2 mmol, 131 µL, 3 eq.) was dissolved in 2 mL EtOAc with stirring at room temperature. To this was added Et_3N (1.2 mmol, 167 µL, 3 eq.) and benzyl isocyanate (400 µmol, 49.4 µL, 1 eq.). Solution was stirred for 2 hours under ambient conditions. 1M HCl was added and the solution was extracted three times with EtOAc. The combined organic extracts were washed with brine and dried over $MgSO_4$. The solvent was removed under reduced pressure to yield a white solid (83.5 mg, 87% yield); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25-7.1 (m, 10H, CH), 4.75 (br s, 2H, NH), 4.26 (d, 4H, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.1 (CO), 139.0 (C), 128.6 (CH), 127.4 (CH), 127.3 (CH), 44.5 (CH_2).

Acknowledgments

This work was supported by the Medical Research Council (MRC, MR/K022733/1) and the King's College London and Imperial College London EPSRC Centre for Doctoral Training in Medical Imaging (EP/L015226/1). The authors acknowledge financial support from the Department of Health via the National Institute for Health Research (NIHR) comprehensive Biomedical Research Centre award to Guy's & St Thomas' NHS Foundation Trust in partnership with King's College London and King's College Hospital NHS Foundation Trust and the Centre of Excellence in Medical Engineering funded by the Wellcome Trust and EPSRC under grant number WT088641/Z/09/Z.

References

1. Miller PW, Long NJ, Vilar R, Gee AD. Synthesis of ^{11}C, ^{18}F, ^{15}O, and ^{13}N Radiolabels for Positron Emission Tomography. *Angew Chemie Int Ed*. 2008;47(47):8998-9033. doi:10.1002/anie.200800222.
2. Antoni G. Development of carbon-11 labelled PET tracers-radiochemical and technological challenges in a historic perspective. *J Labelled Comp Radiopharm*. 2015;58(3):65-72. doi:10.1002/jlcr.3258.
3. Tu Z, Mach RH. C-11 radiochemistry in cancer imaging applications. *Curr Top Med Chem (Sharjah, United Arab Emirates)*. 2010;10(11):1060-1095. doi:10.2174/156802610791384261.
4. Ametamey SM, Honer M, Schubiger PA. Molecular imaging with PET. *Chem Rev*. 2008;108(5):1501-1516. doi:10.1021/cr0782426.
5. Piel M, Vernaleken I, Rösch F. Positron emission tomography in CNS drug discovery and drug monitoring. *J Med Chem*. 2014;57(22):9232-9258. doi:10.1021/jm5001858.
6. Rotstein BH, Liang SH, Holland JP, et al. $^{11}CO_2$ fixation: a renaissance in PET radiochemistry. *Chem Commun*. 2013;49(50):5621. doi:10.1039/c3cc42236d.
7. Pike VW, Eakins MN, Allan RM, Selwyn AP. Preparation of carbon-11 labelled acetate and palmitic acid for the study of myocardial metabolism by emission-computerised axial tomography. *J Radioanal Chem*. 1981;64(1-2):291-297. doi:10.1007/BF02518360.
8. Kawashima H, Yajima K, Kuge Y, Hashimoto N, Miyake Y. Synthesis of [1-^{11}C]-2-octynoic acid, [1-^{11}C]-2-decynoic acid and [1-^{11}C]-3-(R,S)-methyloctanoic acid as potential markers for PET studies of fatty acid metabolism. *J Label Compd Radiopharm*. 1997;39(3):181-193. doi:10.1002/(SICI)1099-1344(199703)39:3<181::AID-JLCR962>3.0.CO;2-W.
9. Runkle AC, Shao X, Tluczek LJM, Henderson BD, Hockley BG, Scott PJH. Automated production of [^{11}C]acetate and [^{11}C]palmitate using a modified GE Tracerlab FXC-Pro. *Appl Radiat Isot*. 2011;69(4):691-698. doi:10.1016/j.apradiso.2011.01.002.
10. Davenport RJ, Dowsett K, Pike VW. A simple technique for the automated production of no-carrier-added [1-^{11}C]acetate. *Appl Radiat Isot*. 1997;48(8):1117-1120. doi:10.1016/S0969-8043(97)00118-8.
11. Rami-Mark C, Ungersboeck J, Haeusler D, et al. Reliable set-up for in-loop 11C-carboxylations using Grignard

reactions for the preparation of [carbonyl-^{11}C]WAY-100635 and [^{11}C]-(+)-PHNO. *Appl Radiat Isot*. 2013;82:75-80. doi:10.1016/j.apradiso.2013.07.023.

12. Wilson AA, Garcia A, Jin L, Houle S. Radiotracer synthesis from [^{11}C]-iodomethane: A remarkably simple captive solvent method. *Nucl Med Biol*. 2000;27(6):529-532. doi:10.1016/S0969-8051(00)00132-3.

13. Wilson AA, Garcia A, Houle S, Vasdev N. Utility of commercial radiosynthetic modules in captive solvent [^{11}C]-methylation reactions. *J Label Compd Radiopharm*. 2009;52(11):490-492. doi:10.1002/jlcr.1618.

14. Iwata R, Pascali C, Bogni A, Miyake Y, Yanai K, Ido T. A simple loop method for the automated preparation of [^{11}C]raclopride from [^{11}C]methyl triflate. *Appl Radiat Isot*. 2001;55(1):17-22. doi:10.1016/S0969-8043(00)00368-7.

15. Shao X, Kilbourn MR. A simple modification of GE tracerlab FX C Pro for rapid sequential preparation of [^{11}C]carfentanil and [^{11}C]raclopride. *Appl Radiat Isot*. 2009;67(4):602-605. doi:10.1016/j.apradiso.2008.12.013.

16. Shao X, Schnau PL, Fawaz M V., Scott PJH. Enhanced radiosyntheses of [^{11}C]raclopride and [^{11}C]DASB using ethanolic loop chemistry. *Nucl Med Biol*. 2013;40(1):109-116. doi:10.1016/j.nucmedbio.2012.09.008.

17. Rotstein BH, Liang SH, Placzek MS, et al. ^{11}C=O bonds made easily for positron emission tomography radiopharmaceuticals. *Chem Soc Rev*. 2016;45(17):4708-4726. doi:10.1039/C6CS00310A.

18. Jessop PG, Heldebrant DJ, Li X, Eckert CA, Liotta CL. Green chemistry: Reversible nonpolar-to-polar solvent. *Nature*. 2005;436(7054):1102-1102. doi:10.1038/4361102a.

19. Heldebrant DJ, Yonker CR, Jessop PG, Phan L. Organic liquid CO_2 capture agents with high gravimetric CO_2 capacity. *Energy Environ Sci*. 2008;124(July):487-493. doi:10.1039/b809533g.

20. Hooker JM, Reibel AT, Hill SM, Schueller MJ, Fowler JS. One-Pot, Direct Incorporation of [^{11}C]CO_2 into Carbamates. *Angew Chemie Int Ed*. 2009;48(19):3482-3485. doi:10.1002/anie.200900112.

21. Wilson AA, Garcia A, Houle S, Vasdev N. Direct fixation of [^{11}C]-CO_2 by amines: formation of [^{11}C-carbonyl]-methylcarbamates. *Org Biomol Chem*. 2010;8(2):428-432. doi:10.1039/B916419G.

22. Heldebrant DJ, Jessop PG, Thomas CA, Eckert CA, Liotta CL. The reaction of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) with carbon dioxide. *J Org Chem*. 2005;70(13):5335-5338. doi:10.1021/jo0503759.

23. Nicholls R, Kaufhold S, Nguyen BN, et al. Observation of guanidine–carbon dioxide complexation in solution and its role in the reaction of carbon dioxide and propargylamines. *Catal Sci Technol*. 2014;4(10):3458-3462. doi:10.1039/C4CY00480A.

24. Vasdev N, Sadovski O, Garcia A, et al. Radiosynthesis of [^{11}C]SL25.1188 via [^{11}C]CO_2 fixation for imaging monoamine oxidase B. *J Label Compd Radiopharm*. 2011;54(10):678-680. doi:10.1002/jlcr.1908.

25. Wilson AA, Garcia A, Houle S, Sadovski O, Vasdev N. Synthesis and Application of Isocyanates Radiolabeled with Carbon-11. *Chem - A Eur J*. 2011;17(1):259-264. doi:10.1002/chem.201002345.

26. Haji Dheere AK, Yusuf N, Gee A. Rapid and efficient synthesis of [^{11}C]ureas via the incorporation of [^{11}C]CO_2 into aliphatic and aromatic amines. *Chem Commun (Camb)*. 2013;49(74):8193-8195. doi:10.1039/c3cc44046j.

27. Dheere A, Bongarzone S, Taddei C, Yan R, Gee A. Synthesis of ^{11}C-Labelled Symmetrical Ureas via the Rapid Incorporation of [^{11}C]CO_2 into Aliphatic and Aromatic Amines. *Synlett*. 2015;26(16):2257-2260. doi:10.1055/s-0034-1381055.

28. Bongarzone S, Runser A, Taddei C, et al. From [^{11}C]CO_2 to [^{11}C]amides: a rapid one-pot synthesis via the Mitsunobu reaction. *Chem Commun*. 2017;53(38):5334-5337. doi:10.1039/C7CC01407D.

29. Mossine A V., Brooks AF, Jackson IM, et al. Synthesis of Diverse ^{11}C-Labeled PET Radiotracers via Direct Incorporation of [^{11}C]CO_2. *Bioconjug Chem*. 2016;27(5):1382-1389. doi:10.1021/acs.bioconjchem.6b00163.

30. CHERIC. CHERIC: Chemical Engineering and Materials Research Information Center, Pure Component Properties, Acetonitrile. https://www.cheric.org/research/kdb/hcprop/showcoef.php?cmpid=1386&prop=VSL. Accessed June 14, 2017.

31. Weng WL, Chang LT, Shiah IM. Viscosities and densities for binary mixtures of benzylamine with 1-pentanol, 2-pentanol, 2-methyl-1-butanol, 2-methyl-2-butanol, 3-methyl-1-butanol, and 3-methyl-2-butanol. *J Chem Eng Data*. 1999;44(5):994-997. doi:10.1021/je990031d.

32. Fisher Scientific. DBU MSDS, Fisher Scientific. https://www.fishersci.com/shop/msdsproxy?productName=AC160610025&productDescription=1+8-DIAZABICYCLO+5.4.0+U+2.5KG&catNo=AC160610025&vendorId=VN00032119&storeId=10652. Published 2014. Accessed June 14, 2017.

33. Brunn E, Huisgen R. Structure and Reactivity of the Betaine Derived from Triphenylphosphine and Dimethyl Azodicarboxylate. *Angew Chemie Int Ed English*. 1969;8(7):513-515. doi:10.1002/anie.196905131.

34. Satish Kumar N, Praveen Kumar K, Pavan Kumar KVP, Kommana P, Vittal JJ, Kumara Swamy KC. Diverse Modes of Reactivity of Dialkyl Azodicarboxylates with P(III) Compounds: Synthesis, Structure, and Reactivity of Products Other than the Morrison-Brunn-Huisgen Intermediate in A Mitsunobu-Type Reaction. *J Org Chem*. 2004;69(6):1880-1889. doi:10.1021/jo035634d.

35. But TYS, Toy PH. The Mitsunobu reaction: Origin, mechanism, improvements, and applications. *Chem - An Asian J*. 2007;2(11):1340-1355. doi:10.1002/asia.200700182.

36. Swamy KCK, Kumar NNB, Balaraman E, Kumar KVPP. Mitsunobu and Related Reactions: Advances and Applications. *Chem Rev*. 2009;109(6):2551-2651. doi:10.1021/cr800278z.

37. Fletcher S. The Mitsunobu reaction in the 21 st century. *Org Chem Front*. 2015;2(6):739-752. doi:10.1039/C5QO00016E.

38. One example of a clinical radiotracer produced at our institution is [^{11}C]methionine, which is obtained by ^{11}C-methylation using [^{11}C]CH_3I produced *via* the gas-phase method.

39. Downey J, Bongarzone S, Hader S, Gee AD. Oral Presentations (O 066). In: *Journal of Labelled Compounds and Radiopharmaceuticals*. Vol 60. ; 2017:S88-S89. doi:10.1002/jlcr.3507.

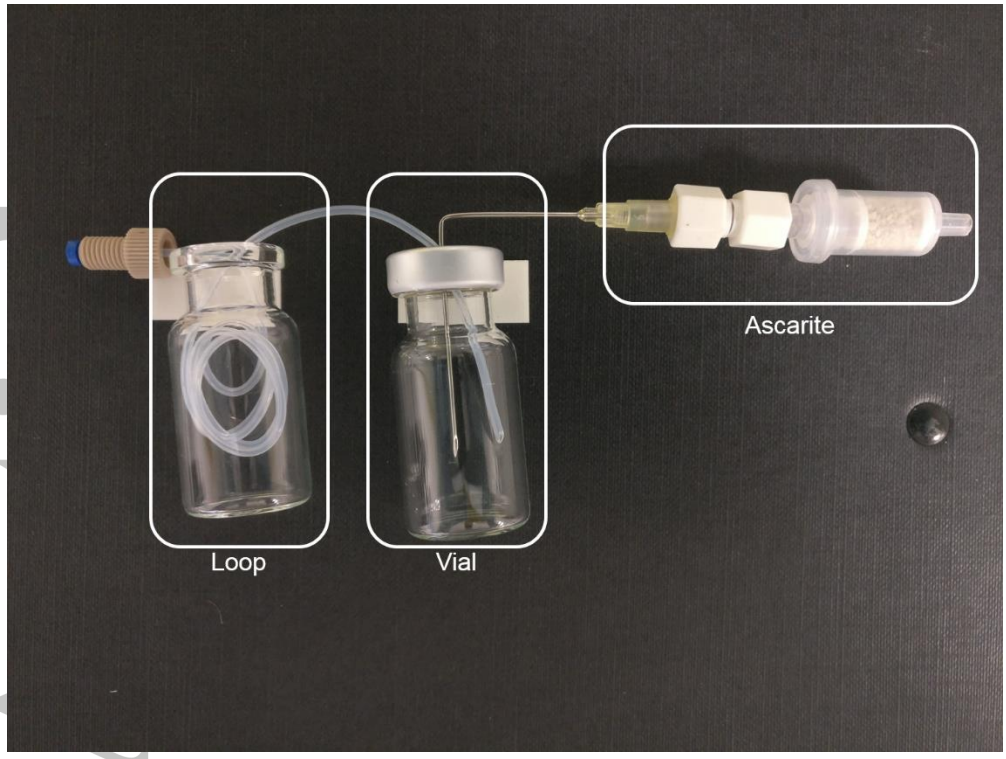

Figure 1. Representative three-component [^{11}C]CO_2 trapping: trapping loop, vial and [^{11}C]CO_2 trapping cartridge (ascarite).

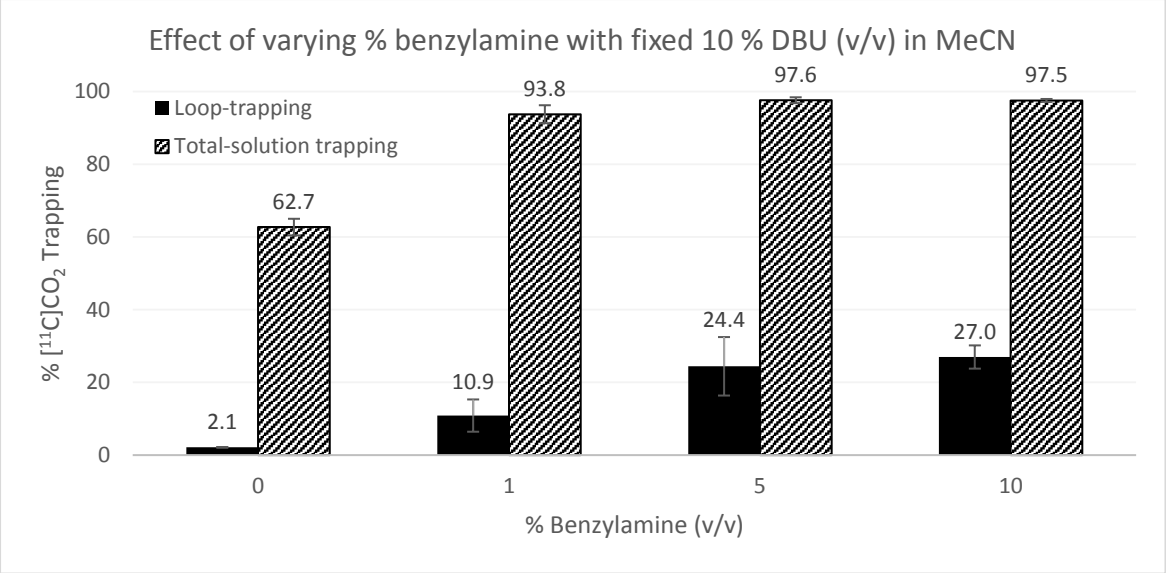

Figure 2. Effect of varying % benzylamine with fixed 10 % DBU (v/v) in MeCN.

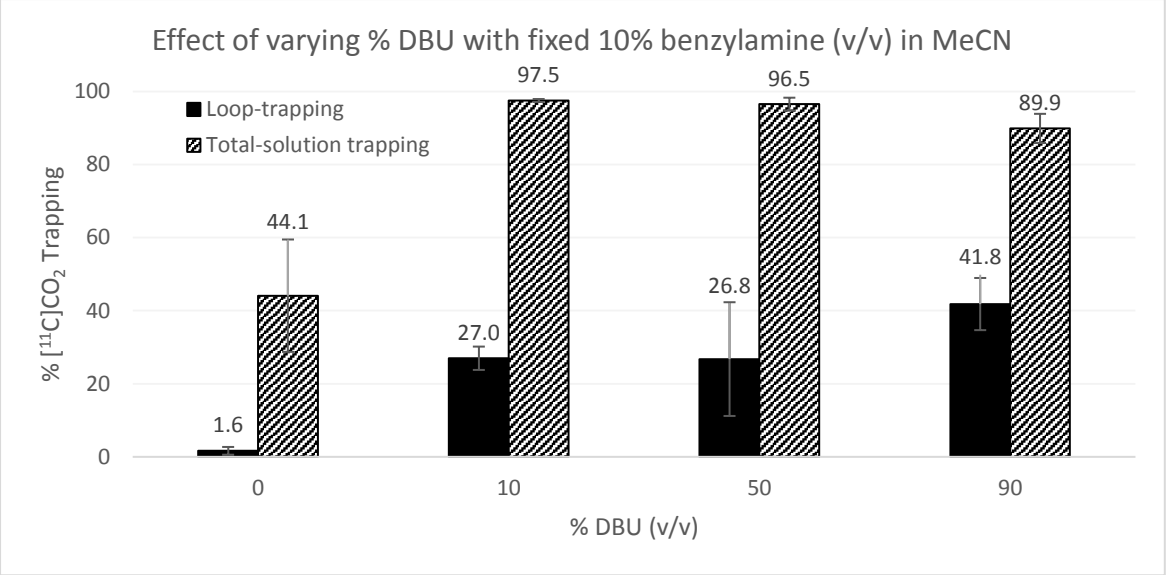

Figure 3. Effect of varying % DBU with fixed 10% benzylamine (v/v) in MeCN.

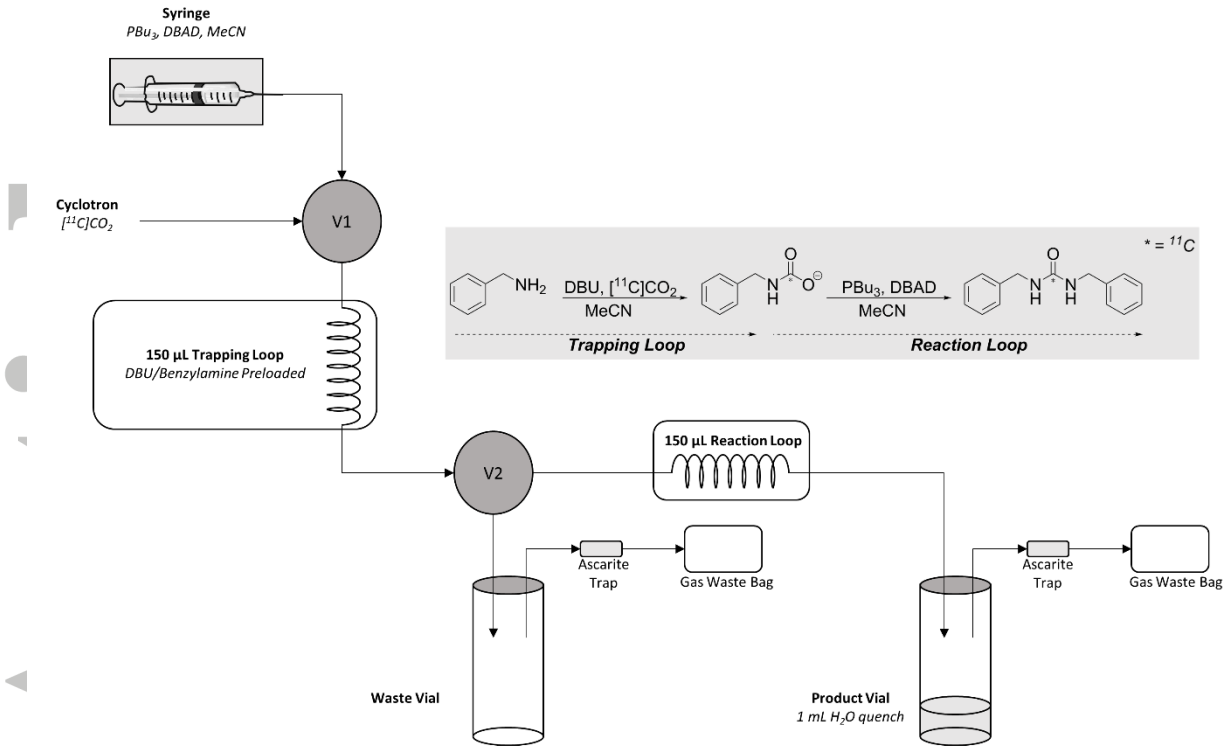

Figure 4. Schematic of in-loop flow radiosynthetic setup showing the $[^{11}C]CO_2$ trapping loop as well as the additional reaction loop to allow Mitsunobu reaction to form *N*,*N*'-[^{11}C]dibenzylurea.