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Rapid Carbon-11 Radiolabelling for PET Using Microfluidics

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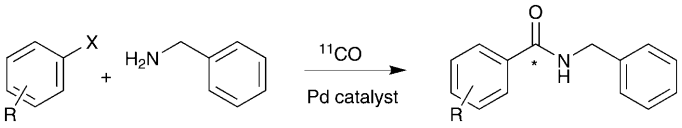
Positron emission tomography (PET) is an imaging technique routinely used for screening, diagnosing and staging chronic conditions such as cancer and neurodegenerative diseases.[1] In addition to clinical applications, PET is also widely used to gain a fundamental understanding of the underlying biology of these diseases and to discover new treatments.[2] All PET scans require a positron emitting radioisotope to enter the body, usually in the form of an injected radiopharmaceutical. The synthesis, preparation and purification of radiopharmaceuticals for PET imaging are not easy processes. The short half-lives of the common cyclotron-generated PET radioisotopes (^{11}C: $t_{1/2}=20.4$ min, ^{18}F: $t_{1/2}=110$ min, ^{13}N: $t_{1/2}=9.96$ min, ^{15}O: $t_{1/2}=2.04$ min), coupled with extremely low radioisotopic concentrations (pM–nM) represent the main challenges in the synthesis of positron emitting labelled compounds.[3] Microfluidics has recently emerged as an important technology for the rapid synthesis of short-lived radiopharmaceuticals for PET.[4] The advantages of using microfluidic reactors for organic chemistry are well documented and include the benefits associated with miniaturisation: smaller reaction volumes (nL–μL) and lower reagent quantities (nmol–μmol), controlled and predicable mixing, efficient heat transfer and enhanced processing capabilities.[5] Microfluidic reactors for PET radiosynthesis have generated considerable interest primarily because miniaturised reaction systems have the potential to address the challenges of increasing the speed of labelling reactions, reducing their scale and improving the overall efficiency of radiolabelling reaction processes.

Our group[6] and others[7] have recently reported the use of microfluidic reactors for rapid and high-yielding carbonylation reactions. These Pd-catalysed carbonylation reactions present an efficient synthetic route to a range of carbonyl-containing organic compounds with biological relevance.[8] For this reason Pd-mediated ^{11}C carbonylation reactions (Scheme 1) have attracted considerable interest for the preparation of ^{11}C-carbonyl-labelled compounds for PET.[9] Radiochemical synthesis with ^{11}CO is challenging and presents two key difficulties: firstly, carbon monoxide is sparingly soluble in common organic solvents, and secondly, ^{11}CO, produced through the reduction of $^{11}CO_2$, is delivered at extremely low concentrations (<nM) in an inert carrier gas. ^{11}CO is always accompanied by a much larger quantity of stable [$^{12}C/^{13}C$]CO, typically in >1000-fold excess. Several technologies have emerged in recent years to improve the processing and the reactivity of ^{11}CO; these include high-pressure micro-autoclave systems,[10] supported catalysts[11] and inorganic ^{11}CO trapping reagents.[12] Here, we report the first example of a ^{11}CO-labelling procedure using a microfluidic reactor that exploits enhanced gas–liquid contact.



Scheme 1. The three-component Pd-mediated aminocarbonylation reaction to form an amide. The asterisk indicates the ^{11}C-labelling position.

We designed a glass-fabricated microfluidic reactor (Figure 1) with two inlet channels for the gaseous and liquid reagents, a mixing-tee motif to permit gas and liquid contact, a long residence channel to enhance reactivity and an outlet port for product collection. The etched microchannels have a semicircular cross-sectional profile and are 220 μm

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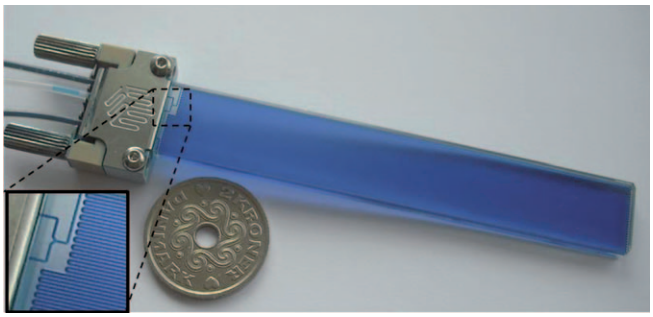


Figure 1. Microfluidic device used for ^{11}CO carbonylation reactions. The device is filled with a blue dye to emphasise the channel structure. Bottom left hand quadrant shows a close-up of the inlet channels. See the Supporting Information for details of the microfluidic reactor design and substrate solution preparation.

wide and 100 μm deep. The residence channel is 5 m in length and occupies most of the device's footprint area (90× 15 mm). In a previous study we found that a long residence channel was necessary to give reasonably high chemical yields for Pd-catalysed carbonylations under annular type flow regimes.[6b] A schematic of our microfluidic radiolabelling reaction setup is shown in Figure 2 and consists of a

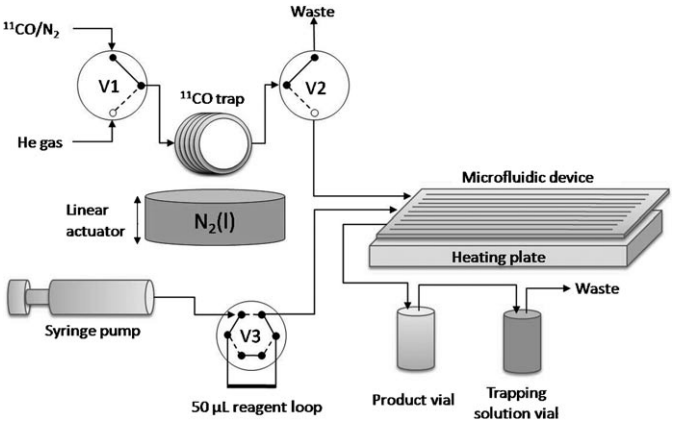


Figure 2. Schematic of the microfluidic ^{11}CO carbonylation setup.

series of valves to control and direct gas flow, a stainless steel loop packed with molecular sieves, liquid reagent injector port, microfluidic device, heating plate, solvent pump, collection vial and ^{11}CO trapping vial. In a typical ^{11}CO-labelling experiment the coupling reagents (aryl halide, Pd catalyst and amine) were premixed and loaded into a 50 μL loop on a Rheodyne valve connected to an external syringe pump charged with toluene. The ^{11}CO was trapped and concentrated into a smaller volume at −196 °C by using a molecular sieve stainless steel loop. This trapping stage is necessary to increase the speed of the overall labelling process by reducing the total volume of gas that has to be processed through the chip device. The coupling reagents were infused into the microfluidic device while, simultaneously, the trapped ^{11}CO was controllably released from the molecular sieve trap into a stream of helium gas at room temperature. A sample vial attached to the device exit port was used to collect the labelled product after flushing the chip device with toluene. A second sample vial containing a solution of copper tris(3,5-dimethylpyrazolyl)-borate ([Cu(Tp*)], see the Supporting Information) was used to trap and measure any unreacted ^{11}CO. This [Cu(Tp*)] trapping solution has recently been developed within our group[12a] and has proven to be a highly efficient reagent for trapping ^{11}CO. Here, it is used as a convenient way of trapping unreacted ^{11}CO in order to calculate the radioactivity trapping efficiencies (RTE) of these reactions.

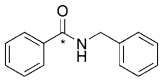
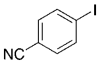
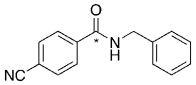
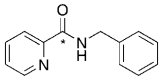
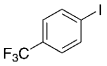
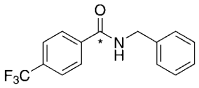
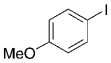
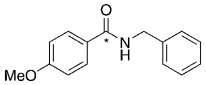
An annular type flow regime was imposed on our reaction system as it provides a large gas–liquid contact area[13] and importantly can be generated much faster and more reliably than segmented flow within the short timeframes required for ^{11}C-labelling reactions. The entire labelling process was typically complete within 15 min from the end of radionuclide production. The ^{11}CO trapping, pre-concentration and release process takes approximately 5–6 min whereas the microfluidic chip reaction and subsequent chip flushing step take a further 7–8 min to complete.

A series of [carbonyl-^{11}C]amides and a lactone were synthesised from aryl iodide substrates (Table 1) through the Pd-mediated [^{11}C]carbonylation reaction by using our setup. The catalyst used in all labelling reactions was [$PdCl_2$-(xantphos)] (xantphos = 4,5-bis(diphenylphosphino)-9,9-dimethylxanthene), which we previously found gave exceptionally high yields within short reaction times for carbonylation reactions.[6a] The model Pd-mediated ^{11}C carbonylation reaction of iodobenzene and benzylamine was used as a benchmark to test the efficiency of ^{11}CO labelling reactions. Under our microfluidic reaction conditions the ^{11}C-carbonylative coupling reaction of iodobenzene with benzylamine (Table 1, entry 1) gave encouragingly high RTEs averaging 88 %. Analysis of this radiolabelled product by HPLC showed the exclusive formation of the [carbonyl-^{11}C]*N*-benzylbenzamide with exceptionally high radiochemical purities (RCP > 99 %). The intramolecular Pd-mediated ^{11}CO coupling reaction of 2-iodobenzyl alcohol (Table 1, entry 2) to form the lactone, [carbonyl-^{11}C]phthalide, also gave excellent RTE (87 %) and RCP values (>99 %). A range of other aryl iodide substrates with the activated electron withdrawing groups *p*-nitrile, *o*-pyridyl and *p*-trifluoromethyl, in addition to the deactivated *p*-anisole group, was investigated (Table 1). Good to excellent RTEs were obtained in all cases, however, RCPs were decreased owing to the formation of unknown radioactive byproducts.

The microfluidic reactions gave exceptionally good radiochemical yields considering the short residence times of both the gas (2 s) and liquid (2 min) reagents.[1] The high efficiency of radiolabelling is attributed to the improved gas/liquid contact and heat transfer within the microchannels of

[1] The liquid residence times were determined experimentally to be ≈2 min whereas the gas residence time was calculated to be 2.1 s at a gas flow rate of 2.5 $cm^3 min^{-1}$ (device volume/flow rate = 0.087/2.5× 60 = 2.1 s).

Table 1. Results from Pd-mediated microfluidic [^{11}C]carbonylation reactions.

Entry	Substrate	Product	RTE [%][a]	RCY [%][b]
1			88 ($n=4$)	88
2			87 ($n=2$)	87
3			78 ($n=2$)	76
4			65 ($n=2$)	44
5			81 ($n=2$)	56
6			90 ($n=2$)	58

[a] Radioactivity trapping efficiency, based on the radioactivity trapped in the collection vial expressed as a fraction of total of radioactivity (i.e., combination of collection and trapping vials). [b] Decay corrected RCY (radiochemical yield), based on the total radioactivity delivered to vial corrected for radiochemical purity; n = number of runs.

the device, which we believe enhances the ^{11}CO insertion step even at these low isotopic dilutions. One factor that limits the overall speed of this labelling procedure is the rate of ^{11}CO gas processing through the device, currently this process takes 7–8 min. Significant reductions in time may be achieved by either increasing the gas flow rate or by adding extra devices to run in parallel, thus, there is the potential to reduce reaction times from minutes to seconds.

In summary, a microfluidic reactor has been used to rapidly and efficiently radiolabel simple amide molecules through the highly versatile Pd-mediated ^{11}CO carbonylation reaction. RCYs are on a par with, or exceed that, of currently used methods.[9,11,12] This method is technically straightforward and further demonstrates the utility and potential of ^{11}CO-labelling reactions. We are presently applying this method to more challenging coupling reactions for the ultra rapid synthesis of potential ^{11}C PET tracer molecules.

Experimental Section

General microfluidic radiolabelling procedure: [^{11}C]carbon dioxide was produced by using a GE PETtrace cyclotron by 16 MeV proton bombardment of a target containing nitrogen and 1% oxygen. ^{11}CO was produced by using the GE process cabinet reduction module by passing $^{11}CO_2$ over a molybdenum wire packed into a quartz tube at 850 °C. In a typical procedure, $^{11}CO/N_2$ gas stream was passed through the molecular sieve trap at −196 °C at a flow rate of 50 cm^3 min^{-1}. A radioactivity detector was placed adjacent to the molecular sieve loop to monitor the activity trapping. Following the peak in activity on the ^{11}CO trap, valves V1 and V2 (Figure 2) were switched to direct helium flow through the microfluidic device at a pressure of 3 bar and flow rate of 2.5 cm^3 min^{-1}. The trapped ^{11}CO was controllably released by allowing the molecular sieve loop to slowly warm to room temperature by removing the liquid nitrogen Dewar over a period of two minutes by using a remote-controlled linear actuated platform that was built in-house. The radiodetector indicated the controlled loss of activity from the trapping loop. At the same time as ^{11}CO release from the trap, the premixed carbonylation reagents were infused into the microfluidic device at a flow rate of 5 μL min^{-1} from a pre-loaded 50 μL loop on a Rheodyne injector 7725i valve (V3) by using a Harvard pump 11 syringe pump charged with anhydrous toluene. The microfluidic device was pre-heated to 150 °C for the synthesis of [carbonyl-^{11}C]amides and 110 °C for [carbonyl-^{11}C]phthalide. A collection vial was connected to the chip outlet vial PTFE tubing. A second vial containing the ^{11}CO trapping complex [Cu(Tp*)] in anhydrous toluene solution (1 mL) was connected to the product collection vial to receive the vented gases and to trap any unreacted ^{11}CO. Following a 10 min reaction period, the microfluidic device was rapidly flushed with 100 μL of toluene. After this time the collection and trapping vials were removed from the hot cell and their radioactivity measured in a dose calibrator. In a typical labelling reaction 400–700 MBq of radioactivity was recorded in the collection vial after 20 min from EOB (typical bombardment time was 10 min at 40 μA). The identities of the ^{11}C-labelled compounds were confirmed by using HPLC by co-injection of authentic reference samples. The contents of the collection vial were diluted with acetonitrile (250 μL) and a 20 μL aliquot removed for UV/Vis and radio-HPLC analysis (solvent: 60:40 acetonitrile/sodium phosphate buffer solution (70 mM), flow: 1.5 mL min^{-1}, column: phenomenex sphereclone ODS, 250 × 4.6 mm). The radio-HPLC system consisted of a Dionex Summit system (P680 pump and UVD 170U detector) connected in series with a NaI radiodetector of in-house design. Dionex Chromeleon 6.8 software was used for data acquisition and analysis.

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Keywords: carbon · microfluidic reactors · positron emission tomography · radiochemistry · radiopharmaceuticals

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