Organic & Biomolecular Chemistry

Cite this: *Org. Biomol. Chem.*, 2011, **9**, 3313

www.rsc.org/obc

PAPER

Microfluidic reactions using [^{11}C]carbon monoxide solutions for the synthesis of a positron emission tomography radiotracer†

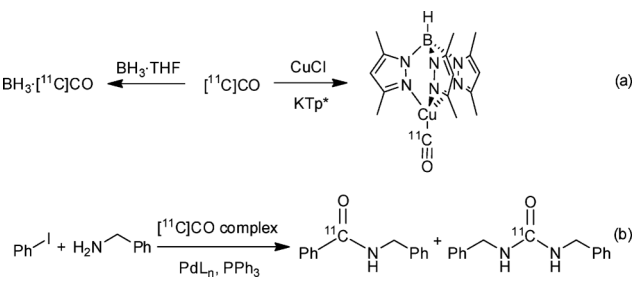
Steven Kealey,[a,b] Christophe Plisson,[a] T. Lee Collier,[c] Nicholas J. Long,[d] Stephen M. Husbands,[b] Laurent Martarello[a] and Antony D. Gee[e]

Received 26th August 2010, Accepted 6th January 2011
DOI: 10.1039/c0ob00631a

Microfluidic technology has been used to perform [^{11}C]carbonylation reactions using solutions containing [^{11}C]CO in the form of the complex, copper(I)tris(3,5-dimethylpyrazolyl)borate-[^{11}C]carbonyl (Cu(Tp*)[^{11}C]CO). The synthesis of the model compound [^{11}C]*N*-benzylbenzamide and the known tracer molecule [^{11}C]*trans*-*N*-[5-(2-flurophenyl)-2-pyrimidinyl]-3-oxospiro[5-azaisobenzofurane-1(3*H*),1′-cyclohexane]-4′-carboxamide ([^{11}C]MK-0233), a ligand for the neuropeptide Y Y5 receptor, have been performed using this technique. Following semi-preparative HPLC purification and reformulation, 1262 ± 113 MBq of [^{11}C]MK-0233 was produced at the end of the synthesis with a specific activity of 100 ± 30 GBq μmol^{-1} and a >99% radiochemical purity. This corresponds to a decay corrected radiochemical yield of 7.2 ± 0.7%. Using a 3 mL vial as the reaction vessel, and following semi-preparative HPLC purification and reformulation, 1255 ± 392 MBq of [^{11}C]MK-0233 was produced at the end of the synthesis with a specific activity of 100 ± 15 GBq μmol^{-1} and a >99% radiochemical purity. This corresponds to a decay corrected radiochemical yield of 7.1 ± 2.2%.

Introduction

Positron emission tomography (PET) is a powerful non-invasive imaging technique that uses radiolabelled compounds as molecular probes to image biological processes *in vivo*.[1] [^{11}C]Carbon monoxide is an important ^{11}C-labelling reagent for radiotracer synthesis as it can provide access to a range of ^{11}C-labelled molecules through metal-catalysed carbonylation reactions.[1,2] The low solubility of this gas and its delivery at high dilution, as well as the time restrictions imposed by the short radioactive half-life of carbon-11 (20 min), has restricted the widespread use of this radiolabelling reagent. In the past 15 years, significant advances have been made in this field through the use of micro-autoclave reactors to perform carbonylation reactions at high pressures.[3,4] We are interested in techniques that enhance the reactivity of [^{11}C]CO by increasing its solubility through chemical complexation to CO-binding molecules in solution. This was first demonstrated using a solution of BH_3·THF to form a BH_3·[^{11}C]CO adduct at low temperatures,[5] and most recently with a copper(I) tris(pyrazolyl)borate complex to form a copper [^{11}C]carbonyl complex (Cu(Tp*)[^{11}C]CO) at room temperature (Scheme 1a).[6] Both of these systems were found to trap [^{11}C]CO efficiently (>90%) and release the gas controllably, by heating in the case of BH_3·[^{11}C]CO or by the addition of triphenylphosphine in the case of Cu(Tp*)[^{11}C]CO. This led to their successful application as [^{11}C]CO-sources in low-pressure, palladium-mediated [^{11}C]carbonylation reactions to form the test compound [^{11}C]*N*-benzylbenzamide and side-product [^{11}C]dibenzylurea (Scheme 1b).



Scheme 1 (a) [^{11}C]CO trapping procedure using BH_3·THF (left) and a copper(I) complex (right). (b) Synthesis of [^{11}C]N-benzylbenzamide and side-product [^{11}C]dibenzylurea using a [^{11}C]CO solution.

[a]*GlaxoSmithKline Clinical Imaging Centre, Hammersmith Hospital, London, W12 0NN, UK*
[b]*Department of Pharmacy and Pharmacology, University of Bath, Bath, BA2 7AY, UK*
[c]*Advion BioSystems, Inc., Division of Imaging Sciences, Ithaca, NY, 14850, USA*
[d]*Department of Chemistry, Imperial College London, London, SW7 2AZ, UK*
[e]*King's College London, Rayne Institute, St. Thomas' Hospital, London, SE1 7EH, UK. E-mail: antony.gee@kcl.ac.uk*
† Electronic supplementary information (ESI) available. See DOI: 10.1039/c0ob00631a

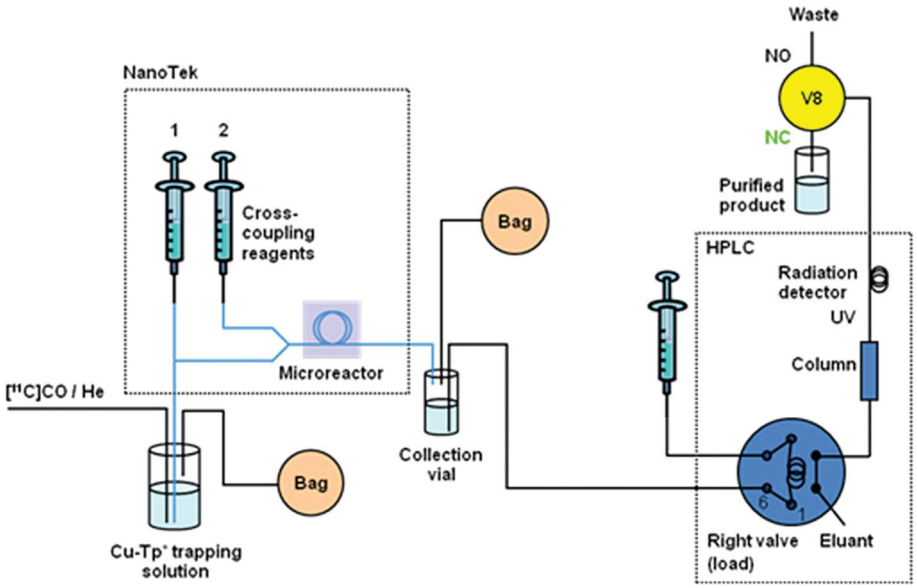


Fig. 1 Schematic of the microfluidic apparatus (HPLC purification apparatus used for the synthesis of [^{11}C]MK-0233).

The ability to handle this useful reagent in solution opens up a new radiolabelling technique in which solutions of [^{11}C]CO may be used in continuous flow microfluidic reactions. The use of microfluidics for radiotracer synthesis has begun to attract considerable attention in recent years,[7–16] as this technology is well-placed to overcome some of the constraints associated with PET radiochemistry, including the use of sub-micromolar quantities of radiolabelling reagents, the requirement for short reaction times and the need for an automated, reproducible process.

While a macroscopic tube reactor has previously been reported for performing continuous flow [^{11}C]carbonylation reactions,[17] microfluidics has not made a significant impact on [^{11}C]CO chemistry, despite its enormous potential. This may be a result of the restrictions caused by the low concentration and solubility of this gas when using conventional techniques, as well as the mechanistic difficulties of controlling the [^{11}C]CO/carrier gas flow. The recent emergence of efficient [^{11}C]CO trapping systems therefore provides an excellent opportunity to open up the field of [^{11}C]CO chemistry to microfluidics. It was postulated that microfluidics would be especially suited to performing [^{11}C]carbonylation reactions, since the [^{11}C]CO gas that is released from the complex will be constrained within the channels of the microreactor, thus keeping it in contact with the reagents. This could be advantageous over conventional vial-based reactions, where [^{11}C]CO gas is likely to escape to the headspace of the vessel, thus reducing its interactions with the substrate in solution.

Results and discussion

In order to test the suitability of the microfluidic approach to solution-based [^{11}C]carbonylation chemistry, the synthesis of the test compound [^{11}C]*N*-benzylbenzamide (and side-product [^{11}C]dibenzylurea) was initially explored using Cu(Tp*)[^{11}C]CO solutions. The ease of formation of Cu(Tp*)[^{11}C]CO (trapping occurs at room temperature in a one-pot process with a high trapping efficiency) made this reagent the obvious candidate for performing these reactions. A commercially-available continuous flow microfluidic device, the Advion NanoTek®,[18] was chosen for these experiments. This integrated device contains syringe pumps that are connected to a microreactor—a coiled silica capillary—which is housed in a metal casing and seated in a heater block. The device was placed inside the hot-cell and connected to a robotic valve setup (Fig. 1).

Microfluidic reactions for the synthesis of test compound [^{11}C]*N*-benzylbenzamide

A significant feature of microfluidics is the ability to perform reactions using small quantities of reagents. In radiolabelling chemistry, this could be exploited to allow multiple reactions to be performed from a single production of radioisotope. In order to examine the feasibility of this process for microfluidic [^{11}C]carbonylation reactions, the palladium-mediated coupling of iodobenzene and benzylamine (Scheme 1b) was chosen as a model reaction. Having been performed previously in glass vials,[6] this reaction has been shown to yield [^{11}C]*N*-benzylbenzamide in good yield in the presence of a palladium(0) catalyst, while the use of a palladium(II) catalyst results in significant formation of a by-product, [^{11}C]dibenzylurea. By performing multiple reactions with microfluidics, it was decided to examine the effect of microreactor temperature on the [^{11}C]*N*-benzylbenzamide/[^{11}C]dibenzylurea product distribution formed in the presence of the palladium(II) species Pd(dppp)Cl_2.

The process began with [^{11}C]CO trapping to form the Cu(Tp*)[^{11}C]CO solution. This solution was then aspirated into syringe 1 of the NanoTek®, while a separate solution containing the cross-coupling reagents (triphenylphosphine, palladium catalyst, benzylamine, iodobenzene) was aspirated into syringe 2. To perform a reaction, aliquots of both solutions were dispensed into the microreactor and the crude product that flowed out was collected in a vial for analysis. The radiolabelled product distribution was measured by radioanalytical HPLC to give the radiochemical purity of each component in the mixture.[19] 100 μL aliquots of the Cu(Tp*)[^{11}C]CO solution were used for each

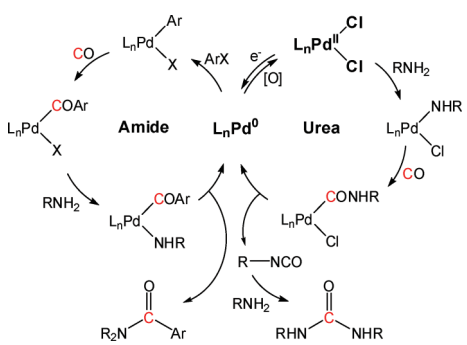
Table 1 Results of microfluidic [^{11}C]carbonylation reactions for the synthesis of [^{11}C]*N*-benzylbenzamide and [^{11}C]dibenzylurea

Temp./°C	RCP [^{11}C]urea[a]	RCP [^{11}C]amide[a]
100	73 ± 2	12 ± 6
125	73 ± 6	19 ± 4
150	52 ± 3	44 ± 3
175	32 ± 6	61 ± 5
200	22 ± 1	69 ± 1

[a] Average of 2 runs (± standard deviation. Flow rate = 20 μL min^{-1} per syringe.

experiment, allowing up to four reactions to be performed from a single 400 μL batch of Cu(Tp*)[^{11}C]CO. Three productions of Cu(Tp*)[^{11}C]CO enabled a range of temperatures between 100 and 200 °C to be probed with a repeat run at each temperature, the results of which are summarised in Table 1.

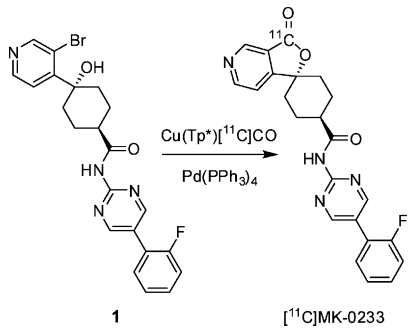
Microfluidics was indeed found to be suitable for performing [^{11}C]carbonylation reactions, producing the expected ^{11}C-labelled products in a distribution that appeared to be temperature dependent. High microreactor temperatures (>150 °C) were found to favour [^{11}C]*N*-benzylbenzamide formation, while lower temperatures (<150 °C) produced [^{11}C]dibenzylurea as the major product. This phenomenon is believed to arise from the two distinct catalytic cycles that drive these processes (Scheme 2) and the effect that the palladium oxidation state has on which path is favoured. Amide formation is known to occur *via* a palladium(0)-mediated process,[20] while urea formation is thought, in this case, to occur *via* a palladium(II)-mediated oxidative carbonylation process.[21–31] As the starting material used is a palladium(II) species, at lower temperatures, it is expected that this species will remain largely unreduced and therefore act as the starting point for the oxidative carbonylation reaction to yield the ^{11}C-labelled urea (Scheme 2, RHS). At higher temperatures, reduction of the palladium(II) by excess phosphine and/or amine may be accelerated, thus yielding a palladium(0) species that can catalyse the aminocarbonylation reaction to form the ^{11}C-labelled amide (Scheme 2, LHS). Since these experiments involve nanomolar quantities of [^{11}C]CO, any palladium species will be in relative excess to [^{11}C]CO and, as such, regeneration of the palladium would not be required in order to convert all of the [^{11}C]CO to ^{11}C-labelled products.



Scheme 2 Competing catalytic cycles in the formation of ^{11}C-labelled amide and ^{11}C-labelled urea using palladium catalysts in the presence of amine and aryl halide. L_n = 1,3-bis(diphenylphosphino)propane.

Microfluidic reactions the for synthesis of [^{11}C]MK-0233

Since the ultimate goal is to apply this technology to the clinical production of PET radiotracers, it was decided to examine the suitability of the microfluidic system towards the synthesis of a biologically-active tracer molecule. The neuropeptide Y Y5 receptor antagonist [^{11}C]MK-0233, previously synthesised by Burns *et al.*[32] and used to examine the role of this receptor in weight loss,[33] was chosen for these studies. This labelling reaction proceeds *via* a palladium(0)-mediated ring-closing [^{11}C]carbonylation reaction between pyridyl-bromide and hydroxyl functional groups of the starting material, **1**, to form a ^{11}C-labelled lactone, [^{11}C]MK-0233 (Scheme 3).



Scheme 3 Synthesis of [^{11}C]MK-0233 *via* a palladium-mediated [^{11}C]carbonylation reaction following a copper-mediated [^{11}C]CO trapping process.

Before performing a complete synthesis and purification, the optimum reaction conditions were first sought by performing small-scale reactions using a single batch of Cu(Tp*)[^{11}C]CO. As for the test reaction, this process began with [^{11}C]CO trapping to form the Cu(Tp*)[^{11}C]CO solution, which was aspirated into syringe 1. This solution was then dispensed into the microreactor along with the cross-coupling reagents (triphenylphosphine, palladium catalyst, **1**) from syringe 2 (Fig. 1).

In keeping with the conditions reported by Burns *et al.*,[32] the palladium(0) catalyst $Pd(PPh_3)_4$ was used for these studies. Optimisation reactions were carried out using 30 μL aliquots of the Cu(Tp*)[^{11}C]CO and cross-coupling reagent solutions to probe the effects of temperature and flow rate on the radiochemical purity of the crude (unpurified) product, as measured by analytical HPLC. These reactions were performed at a range of temperatures at a fixed arbitrary flow rate of 30 μL min^{-1} delivered from each syringe (Table 2, entries 1–6). This showed that negligible product formation was observed below 130 °C, leaving most of the Cu(Tp*)[^{11}C]CO complex unreacted, while a significant increase in RCP was observed upon increasing the temperature to 150 °C. Further raising the temperature at 10 °C intervals revealed that the yield reached a maximum value of 81% at 160 °C, which was not improved by further raising the temperature. The combined flow rate of 60 μL min^{-1} used in these experiments corresponds to a residence time of a quarter of a minute for the reagents within the microreactor. Next, the impact of flow rate (and therefore residence time of the reagents within the microreactor) on yield was examined. At a constant temperature of 180 °C, experiments were performed at flow rates of 50, 100 and 150 μL min^{-1} (Table 2, entries 7–9). This showed that the greater the flow rate, the lower

Table 2 Effect of temperature and flow rate on the radiochemical purity of [^{11}C]MK-0233

Entry	Temp./°C	Flow rate/μL min^{-1}	Residence time/s[a]	RCP (%)
1	120	30	31	0
2	130	30	31	9
3	150	30	31	67
4	160	30	31	81
5	170	30	31	79
6	180	30	31	79
7	180	50	19	76
8	180	100	9	73
9	180	150	6	72

[a] 100 μm × 2 m microreactor, volume = 16 μL.

the yield, although the deterioration was only slight, suggesting that, under these microfluidic conditions, the reaction proceeds so quickly that reducing the residence time to the order of seconds does not prevent the reaction from occurring.

For the subsequent full-scale synthesis, aiming to produce [^{11}C]MK-0233 for potential use in PET experiments, the cyclotron bombardment time was increased to a 'full bombardment', giving approximately 37 GBq of [^{11}C]CO after the reduction of [^{11}C]CO_2. Following the optimisation process, 400 μL of the Cu(Tp*)[^{11}C]CO solution was used and, as a compromise between maximising yields and minimising synthesis time, reactions were performed at 180 °C with a flow rate of 100 μL min^{-1}. Following the microfluidic reaction, the solution was purified by semi-preparative HPLC (Fig. 1, RHS) and then reformulated to give the product in a potentially injectable ethanol–saline solution. The radiochemical purity and specific activity of the product was then measured by analytical HPLC. Using this technique, over three consecutive runs, 1262 ± 113 MBq of [^{11}C]MK-0233 was produced at end of the synthesis with a specific activity of 100 ± 30 GBq μmol^{-1} and a >99% radiochemical purity. The total preparation time from the end of cyclotron bombardment to receiving the reformulated dose was 27 min. This corresponds to a decay-corrected radiochemical yield of 7.2 ± 0.7%, calculated from the total amount of [^{11}C]CO delivered to the copper(I) trapping solution.

Vial reactions for the synthesis of [^{11}C]MK-0233

While low pressure [^{11}C]carbonylation reactions using Cu(Tp*)[^{11}C]CO solutions have previously been employed for the synthesis of the test compound, this method has not been demonstrated for the synthesis of biologically-active tracer molecules—for potential use in PET imaging studies. In order to examine the feasibility of this process and for comparison with the microfluidic method, the synthesis of [^{11}C]MK-0233 was performed using this low pressure technique.

The reactions were performed using a 3 mL glass vial fitted with a rubber septum cap and pierced with needles to allow the transfer of reagents into and out of the vessel. An array of three-way valves connected by PTFE tubing were used to control the flow of gaseous and liquid reagents in this process (Fig. 2). As before, the apparatus was placed in a lead-shielded fume hood (hot-cell) and controlled externally by a computer. In these experiments the [^{11}C]CO trapping process was performed initially to give a solution of Cu(Tp*)[^{11}C]CO, to which the cross-coupling reagent solution (PPh_3, $Pd(PPh_3)_4$, **1**) was added using an argon gas sweep. Following this, the vial was sealed and heated to 150 °C for 5 min.

Unlike the microfluidic method, repeated small-scale reactions using one production of [^{11}C]CO could not easily be performed for the optimisation process. Instead, a series of [^{11}C]CO productions were used to perform test reactions, and initially these suffered difficulties due to radioactive gaseous releases from the apparatus,

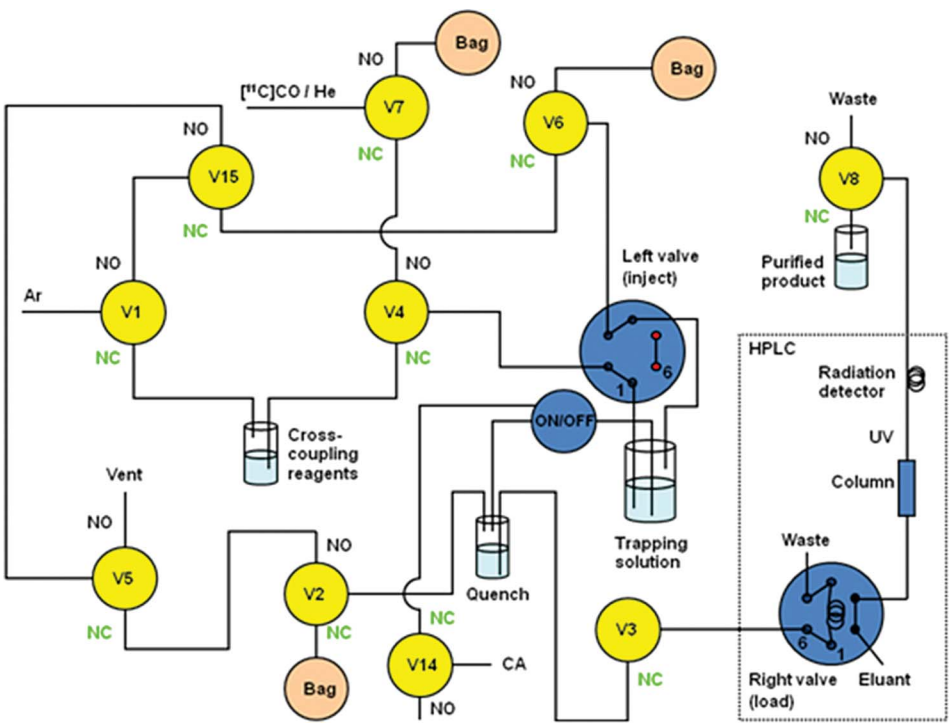


Fig. 2 Valve setup for the reaction and purification of [^{11}C]MK-0233 using the vial technique.

especially when heating at temperatures in excess of 150 °C due to pressurisation of the solvents. These releases were attributed to leaks from the three-way valves that were initially used to seal the reaction vial during heating. To avoid this problem, a Rheodyne® HPLC valve and a compressed air-activated two-way valve were used to seal the vial, and these proved to be leak-tight. To further minimise the release of [^{11}C]CO from the reaction vial, two rubber septa were placed together in the cap to seal the vial, and repeated puncturing of the septa with needles was avoided. At 150 °C, we found that [^{11}C]CO release was minimised, while the reaction proceeded cleanly to give crude radiochemical purities of >90%. The overall radiochemical yields were not measured at this stage due to variations between runs in the amount of [^{11}C]CO_2 produced.

As for the microfluidic process, after optimisation was complete, the synthesis of [^{11}C]MK-0233 was performed on a larger scale using full cyclotron bombardment and purification by semi-preparative HPLC, and reformulation to give the product as a potentially injectable ethanol–saline solution. The radiochemical purity and specific activity was then measured by analytical HPLC. Using this technique, over three consecutive runs, 1255 ± 392 MBq of [^{11}C]MK-0233 was obtained at end of synthesis with a specific activity of 100 ± 15 GBq μmol^{-1} and a radiochemical purity >99%. The total preparation time from the end of cyclotron bombardment to receiving the reformulated dose was 27 min. This corresponds to a decay-corrected radiochemical yield of 7.1 ± 2.2%, calculated from the total amount of [^{11}C]CO delivered to the copper(I) trapping solution.

Comparison of methods

The radiochemical yields obtained through the microfluidic method and the vial method were found to be almost identical, with both processes taking the same amount of time (from the delivery of [^{11}C]CO_2 to receiving the dose of purified [^{11}C]MK-0233). Despite our predictions, a greater yield was not achieved through microfluidics, which could result from a number of factors. Firstly, in the microfluidic experiments, in order to avoid aspirating air into the system, 700 μL of trapping solution was used, while only 400 μL of this was used for radiolabelling. We believe this wastage could be all but eliminated by further reducing the trapping volume, which would be expected to significantly improve radiochemical yields. A second factor that is likely to contribute to the lowering of yields is the use of high flow rates in the microfluidic process in order to allow the full bolus of reactants to pass through the reactor in a timeframe similar to the heating step in corresponding batch reactions. Microfluidic flow rates of 100 μL per syringe correspond to a residence time of the reagents within the microreactor of under 10 s, and this is likely to result in incomplete conversion of [^{11}C]CO to radiolabelled product. It is thought that increasing the reactor length would improve yields by providing an increase in reagent residence time without sacrificing reaction time (as would occur if the flow rate was lowered instead). This will be investigated in future studies.

The previous report describing the synthesis of [^{11}C]MK-0233,[32] performed at high pressure using a micro-autoclave reactor, does not state the yield or specific activity of the product, so no comparison with our methods can be made. For the purposes of our investigation, to examine the viability of microfluidics for solution-based carbonylations, we have found the technique to be suitable for tracer production, with the potential to outperform the vial method. Microfluidics was found to be advantageous when using a volatile, radioactive reagent such as [^{11}C]CO as unwanted radioactive emissions were not observed, most likely because during the heating process, the gases are held within the confines of the reactor. Vial reactions, however, are prone to occasional and unpredictable releases of [^{11}C]CO into the hot cell as a consequence of elevated pressures within the vial during heating, potentially lowering the radiochemical yield. As such, the upper temperature for vial reactions is limited to that of the boiling point of the solvent, while the increased pressure tolerance of the microfluidic setup allowed temperatures above the normal boiling point of the solvent to be used. The automation that can be achieved with microfluidic devices means that the apparatus can be easily cleaned between runs using a pre-programmed macro. Microfluidic technology carries the significant advantage of allowing reaction optimisation to be performed rapidly using sub-milligram quantities of reagents and often using only one production of [^{11}C]CO. The optimisation process for vial reactions, on the other hand, were more time consuming, requiring multiple productions of [^{11}C]CO to test a range of temperatures and alterations in the valve setup.

Conclusion

A copper(I)[^{11}C]carbonyl complex has been successfully used to perform [^{11}C]carbonylation reactions using microfluidic technology or conventional glass reaction vials. Both approaches gave yields on a scale suitable for performing *in vivo* PET studies, despite being unoptimised in terms of catalyst/ligand choice. Despite the longer optimisation process associated with the vial technique, reasonable radiochemical yields were obtained for the tracer compound, indicating that this low-cost approach provides a viable method for performing [^{11}C]carbonylation reactions for clinical tracer production.

Microfluidic technology was found to be well-suited to performing solution-[^{11}C]CO chemistry and enabled reaction optimisation to be performed rapidly using small quantities of reagents. As well as performing these test reactions, the process was successfully scaled-up, as a proof-of-concept, to produce the tracer molecule on a scale suitable for PET studies. We believe that the combination of [^{11}C]CO solutions with microfluidics can play an important role in the future radiolabelling of PET tracers. One such role is the development of more potent and selective tracer molecules, where pseudo-combinatorial syntheses could be performed to label a series of different precursors using a single batch of Cu(Tp*)[^{11}C]CO. A mini-library of related radiotracers could be synthesised in this manner and studied in parallel, for example by tissue section autoradiography on adjacent tissue sections, thus enabling rapid screening of radioligands to probe structure–activity relationships. These studies are currently being performed in our laboratories and will be reported in due course.

Experimental section

General procedures

All chemicals and solvents were purchased from commercial sources, except for *trans*-4-(3-bromopyridin-4-yl)-4-hydroxy-*N*-[5-(2-fluorophenyl)-2-pyrimidinyl]-cyclohexanecarboxamide (**1**), which was synthesised using the procedure described by Burns

et al.[32] Semi-preparative HPLC purification was performed using a reversed-phase column (Agilent Zorbax SB-C18, 5 μm, 9.4 × 250 mm) using a mobile phase of MeCN + 0.1% trifluoroacetic acid and water using an initial gradient (0–2 min from 5–40% MeCN) followed by an isocratic 40% MeCN at a flow rate of 8 mL min^{-1}. UV (254 nm) and radioactivity (pin diode detector) signals were recorded using Chromeleon software. An unlabelled reference sample of MK-0233 was used to determine the retention time of this compound (9.3 min). QC of the final reformulated dose was performed by analytical HPLC using a reversed phase column (Agilent Zorbax SB-Phenyl, 3.5 μm, 4.6 × 150 mm) and a 60 : 40 ammonium formate (50 mM, pH 4) : MeCN mobile phase at a flow rate of 1.5 mL min^{-1} (retention time = 5.6 min).

[^{11}C]Carbon dioxide was produced using a Siemens Eclipse HP cyclotron by 11 MeV proton bombardment (45 min at 55 μA) of a target containing nitrogen and 1% oxygen. [^{11}C]CO was produced using an Eckert and Ziegler reduction module using the following procedure: [^{11}C]CO_2 was delivered from the cyclotron and trapped at room temperature in a stainless steel loop containing molecular sieves. The [^{11}C]CO_2 was then released by passing a helium flow through the loop while heating to 400 °C. The resultant [^{11}C]CO_2/He gas stream was passed through a glass tube packed with molybdenum powder at 850 °C, converting the [^{11}C]CO_2 to [^{11}C]CO, with any unreduced [^{11}C]CO_2 being trapped using Ascarite. The resultant [^{11}C]CO/He gas stream was delivered to the trapping solution at a flow rate of 20 mL min^{-1}. The time taken from the end of cyclotron bombardment to complete delivery of [^{11}C]CO to the trapping vial was 5 to 6 min.

Microfluidic reactions for the synthesis of test compound [^{11}C]*N*-benzylbenzamide

The cross-coupling reagent solution was prepared by the addition of Pd(dppp)Cl_2 (1.3 mg, 2 μmol), PPh_3 (5.8 mg, 22 μmol), iodobenzene (2.3 mg, 11 μmol) and benzylamine (0.1 mL) into a 1 mL vial, flushing with nitrogen for 10 min, followed by the addition of anhydrous dimethylformamide (0.6 mL). This solution was transferred to a 400 μL loop by aspirating syringe 1 of the NanoTek®. The trapping solution was prepared by the addition of CuCl (1.1 mg, 11 μmol) and K[Tp*] (3.7 mg, 11 μmol) to a 3 mL V-bottomed glass vial, flushing with nitrogen for 10 min, followed by the addition of anhydrous tetrahydrofuran (1.0 mL). The solution was then filtered into another 3 mL V-bottomed glass vial under a nitrogen atmosphere. [^{11}C]CO/He was bubbled through the trapping solution and the radioactivity of the vial monitored using a non-calibrated pin diode detector. Following complete delivery of the radioactivity to the vial, corresponding to the formation of Cu(Tp*)[^{11}C]CO, the solution was transferred into a 400 μL loop by aspirating syringe 2 of the NanoTek®. The cross-coupling reagent solution (100 μL) and the [^{11}C]CO solution (100 μL) were then dispensed simultaneously through a 100 μm × 4 m microreactor situated in a heater block. The mixture leaving the microreactor was collected in a 1 mL vial containing water (0.1 mL) and the product mixture analysed by analytical HPLC using a reversed-phase column (Agilent XDC C_{18}, 5 μm, 4.6 × 150 mm) and a 60 : 40 water–MeCN mobile phase at a flow rate of 1.5 mL min^{-1} (amide retention time = 4.5 min, urea retention time = 4.1 min).

Microfluidic reactions the for synthesis of [^{11}C]MK-0233

The cross-coupling reagent solution was prepared by the addition of Pd(PPh_3)$_4$ (3.0 mg, 3 μmol), PPh_3 (4.2 mg, 16 μmol) and **1** (1.0 mg, 2 μmol) into a 1 mL vial, flushing with nitrogen for 10 min, followed by the addition of anhydrous dimethylformamide (0.5 mL). This solution was transferred to a 400 μL loop by aspirating syringe 1 of the NanoTek®. The trapping solution was prepared by the addition of CuCl (1.1 mg, 11 μmol) and K[Tp*] (3.7 mg, 11 μmol) to a 3 mL V-bottomed glass vial, flushing with nitrogen for 10 min, followed by the addition of anhydrous MeCN (0.7 mL). The solution was then passed through a 0.45 μm filter into another 3 mL V-bottomed glass vial under a nitrogen atmosphere. [^{11}C]CO/He was bubbled through the trapping solution and the radioactivity of the vial monitored using a non-calibrated pin diode detector. Following complete delivery of the radioactivity to the vial, corresponding to the formation of Cu(Tp*)[^{11}C]CO, the solution was transferred into a 400 μL loop by aspirating syringe 2 of the NanoTek®. The cross-coupling reagent solution (400 μL) and the [^{11}C]CO solution (400 μL) were then dispensed simultaneously at a flow rate of 100 μL min^{-1} per syringe into a 100 μm × 2 m microreactor situated in a heater block at 180 °C. The mixture leaving the microreactor was collected in a vial and injected into the HPLC for semi-preparative purification. Upon observation of the radioactivity peak at the expected retention time, the flow was diverted into a vial containing a solution of K_2CO_3 (100 mg) in water (30 mL). This solution was then passed through a C_{18} Sep-Pak cartridge to trap the product, [^{11}C]MK-0233, and washed with water (6 mL). The product was eluted from the Sep-Pak cartridge into a 10 mL dose vial using EtOH (2 mL) an then saline solution (8 mL). The radioactivity of the dose vial was measured in a dose calibrator, and the radiochemical purity and specific activity of the product were measured by analytical HPLC.

Vial reaction for the synthesis of [^{11}C]MK-0233

The trapping solution was prepared by the addition of CuCl (1.1 mg, 11 μmol) and K[Tp*] (3.7 mg, 11 μmol) into a 3 mL V-bottomed glass vial, flushing with nitrogen for 10 min, followed by the addition of anhydrous THF (1.1 mL). The carbonylation reagents were prepared by the addition of Pd(PPh_3)$_4$ (2.4 mg, 2 μmol), PPh_3 (5.8 mg, 22 μmol) and **1** (1.0 mg, 2 μmol) to a second vial, flushing it with nitrogen for 10 min, followed by the addition of anhydrous dimethylformamide (0.5 mL). [^{11}C]CO/He was bubbled through the trapping solution and the radioactivity of the vial monitored using a non-calibrated pin diode detector. Following complete delivery of the radioactivity to the vial, the carbonylation reagents were added using an argon gas push. The vial was subsequently sealed and heated at 150 °C in a heating block for 6 min. The reaction mixture was then transferred to the quench vial using a positive pressure of argon gas and injected into an HPLC for semi-preparative purification. Upon observation of the radioactivity peak at the expected retention time, the flow was diverted into a vial containing a solution of K_2CO_3 (100 mg) in water (30 mL). This solution was then passed through a C_{18} Sep-Pak cartridge to trap the product, [^{11}C]MK-0233, and washed with water (6 mL). The product was eluted from the Sep-Pak cartridge into a 10 mL dose vial using EtOH (2 mL) and then saline solution

(8 mL). The radioactivity of the dose vial was measured in a dose calibrator, and the radiochemical purity and specific activity of the product were measured by analytical HPLC.

Acknowledgements

We thank Valeria Rampazzo for the synthesis of the radiolabelling precursor and the reference sample of MK-0233. N. J. L. would like to thank the Leverhulme Trust for the award of a Research Fellowship.

Notes and references

1 P. W. Miller, N. J. Long, R. Vilar and A. D. Gee, *Angew. Chem., Int. Ed.*, 2008, **47**, 8998.
2 B. Långström, O. Itsenko and O. Rahman, *J. Labelled Compd. Radiopharm.*, 2007, **50**, 794.
3 E. D. Hostetler and H. D. Burns, *Nucl. Med. Biol.*, 2002, **29**, 845.
4 T. Kihlberg and B. Langstrom, *PCT Int. Appl.* WO 2002102711 A1, 2002.
5 H. Audrain, L. Martarello, A. Gee and D. Bender, *Chem. Commun.*, 2004, 558.
6 S. Kealey, P. W. Miller, N. J. Long, C. Plisson, L. Martarello and A. D. Gee, *Chem. Commun.*, 2009, 3696.
7 A. Elizarov, R. M. van Dam, Y. S. Shin, H. C. Kolb, H. C. Padgett, D. Stout, J. Shu, J. Huang, A. Daridon and J. R. Heath, *J. Nucl. Med.*, 2010, **51**, 282.
8 S. Y. Lu, P. Watts, F. T. Chin, J. Hong, M. J. L. E. Briard and V. W. Pike, *Lab Chip*, 2004, **4**, 523.
9 C. C. Lee, G. Sui, A. Elizarov, C. J. Shu, Y. S. Shin, A. N. Dooley, J. Huang, A. Daridon, P. Wyatt, D. Stout, H. C. Kolb, O. N. Witte, N. Satyamurthy, J. R. Heath, M. E. Phelps, S. R. Quake and H. R. Tseng, *Science*, 2005, **310**, 1793.
10 J. M. Gillies, C. Prenant, G. N. Chimon, G. J. Smethurst, W. Perrie, I. Hamblett, B. Dekker and J. Zweit, *Appl. Radiat. Isot.*, 2006, **64**, 325.
11 H. M. Wu, G. Sui, C. C. Lee, M. L. Prins, W. Ladno, H. D. Lin, A. S. Yu, M. E. Phelps and S. C. Huang, *J. Nucl. Med.*, 2007, **48**, 837.
12 C. J. Steel, A. T. O'Brien, S. K. Luthra and F. Brady, *J. Labelled Compd. Radiopharm.*, 2007, **50**, 308.
13 H. Audrain, *Angew. Chem., Int. Ed.*, 2007, **46**, 1772.
14 P. W. Miller, *J. Chem. Technol. Biotechnol.*, 2009, **84**, 309.
15 A. M. Elizarov, *Lab Chip*, 2009, **9**, 1326.
16 J. H. Chun, S. Lu, Y. S. Lee and V. W. Pike, *J. Org. Chem.*, 2010, **75**, 3332.
17 P. W. Miller, N. J. Long, A. J. De Mello, R. Vilar, H. Audrain, C. Bender, J. Passchier and A. Gee, *Angew. Chem., Int. Ed.*, 2007, **46**, 2875.
18 http://www.advion.com/biosystems/nanotek/nanotek-positron-emission-tomography.php.
19 Radiochemical purity (RCP) refers to the radioactivity of a particular component in a solution as a proportion of the total radioactivity of the solution, as judged by analytical HPLC. This does not account for unreacted [^{11}C]CO due to its low solubility.
20 C. F. J. Barnard, *Organometallics*, 2008, **27**, 5402.
21 K. Orito, M. Miyazawa, T. Nakamura, A. Horibata, H. Ushito, H. Nagasaki, M. Yuguchi, S. Yamashita, T. Yamazaki and M. Tokuda, *J. Org. Chem.*, 2006, **71**, 5951.
22 B. Gabriele, G. Salerno, R. Mancuso and M. Costa, *J. Org. Chem.*, 2004, **69**, 4741.
23 I. Chiarotto and M. Feroci, *J. Org. Chem.*, 2003, **68**, 7137.
24 F. Shi, Y. Deng, T. SiMa and H. Yang, *Tetrahedron Lett.*, 2001, **42**, 2161.
25 H. Yang, Y. Deng and F. Shi, *J. Mol. Catal. A: Chem.*, 2001, **176**, 73.
26 G. M. Dahlen and A. Sen, *Macromolecules*, 1993, **26**, 1784.
27 B. M. Choudary, R. K. Koteswara, S. D. Pirozhkov and A. L. Lapidus, *Synth. Commun.*, 1991, **21**, 1923.
28 I. Pri-Bar and H. Alper, *Can. J. Chem.*, 1990, **68**, 1544.
29 S. P. Gupte and R. V. Chaudhari, *J. Catal.*, 1988, **114**, 246.
30 P. Giannoccaro, *J. Organomet. Chem.*, 1987, **336**, 271.
31 J. Tsuji and N. Iwamoto, *J. Chem. Soc. D*, 1966, 380.
32 H. D. Burns, R. E. Gibson, T. G. Hamill and T. Fukami, *PCT Int. Appl.* WO 03/010175 A2, 2003.
33 N. Erondu, I. Gantz, B. Musser, S. Suryawanshi, M. Mallick, C. Addy, J. Cote, G. Bray, K. Fujioka, H. Bays, P. Hollander, S. M. Sanabria-Bohorquez, W. Eng, B. Langstrom, R. J. Hargreaves, H. D. Burns, A. Kanatani, T. Fukami, D. J. MacNeil, K. M. Gottesdiener, J. M. Amatruda, K. D. Kaufman and S. B. Heymsfield, *Cell Metab.*, 2006, **4**, 275.