Supporting Information

^{11}C-Carbonylation Reactions Using Gas-Liquid Segmented Microfluidics

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Supporting Information

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General experimental information. Unless otherwise noted, all chemicals and solvents were purchased from Sigma-Aldrich (Sweden) and used without further purification. High pressure liquid chromatographic analysis (HPLC) was performed using a Agilent 1200 gradient pump and a Agilent 1200 variable wavelength UV-detector in a series with a Eckert & Ziegler β^+-flow detector. Analytical HPLC analysis was performed using a reverse phase column (μBondapak, C18, 10 μm, 3.9 x 300 mm) eluted with a gradient between acetonitrile (A) and 0.1 M NH_4HCO_2 (B). The gradient was linear between 10 - 90% over 9 minutes and isocratic in between 9-10 min (MeCN: 0.1 M NH_4HCO_2, 90:10), at a flow rate of 2 ml/min. Identification of all radioactive products was confirmed by co-elution with the corresponding non-radioactive compound.

Production of [^{11}C]Carbon dioxide. No-carrier-added [^{11}C]carbon dioxide production was performed using a GEMS PETtrace cyclotron (GE, Uppsala, Sweden). The ^{14}N(p, α)^{11}C reaction was employed in a pressurized gas target (Initial pressure, 1.1 × 10^5 Pa) containing nitrogen (AGA, Nitrogen 6.0) and 0.5% oxygen (AGA, Oxygen 4.8) by bombardment with 10 μA proton beam (33 → 6 MeV) for 1 min. At the end of bombardment (EOB), the gas was delivered from the target to the Microfluidic ^{11}C-carbonylation synthesizer (Fig. 1), where the $^{11}CO_2$ was trapped on a molecular sieve column (30 μg packed in a ⅛" tube, mesh 80/100, GRACE) at room temperature. The carrier gas, nitrogen, was replaced with helium (AGA, Helium 6.0).

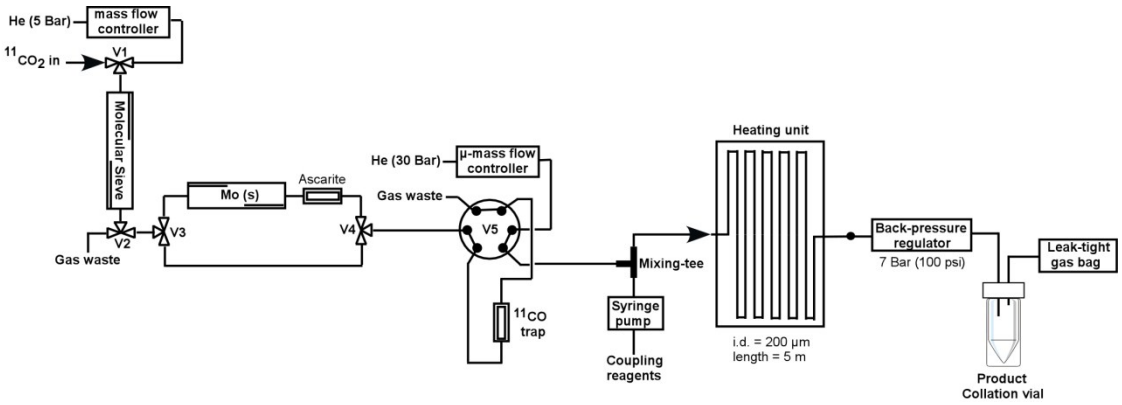


Fig. 1 Full schematic diagram of the microfluidic ^{11}C-carbonylation synthesizer.

General procedure for performing Gas-Liquid Segmented Microfluidic (MF) [^{11}C]carbonylation. The accumulated $^{11}CO_2$ was released into a controlled stream of helium (10 ml/min) using a mass-flow controller (Bronkhorst, Ruurlo, Netherlands), while heating the molecular sieve trap to 360°C. Four 3-port, two-way valve (V1-4, Parker, P/N 009-0269-900) was used to controll and direct gas flow throuhout the production procedure. Furthermore, $^{11}CO_2$ was reduced online to ^{11}CO using a pre-heated (Carbolite oven, 850°C) quartz glass column (6 x 4 x 220 mm: outer diameter x inner diameter x length) charged with Molybdenum powder (1.5 g, <150 μm, 99.99% trace metals basis, Sigma-Aldrich, Sweden). Unreacted $^{11}CO_2$ was subsequently removed by a sodium hydroxide-coated silica (0.5 g, Ascarite II, 20-30 mesh) trap and the ^{11}CO were concentrated on a silica gel (^{11}CO trap, 10 mg, 60 Å, 60 -100 mesh) trap immersed in liquid nitrogen. After completed entrapment, the trap was heated to room temperature (r.t.) while release the ^{11}CO using the μ-mass flow controller (Bronkhorst, Ruurlo, Netherlands) at 100 μl/min into a the MF reactor. A six-port, two-way valve (V5, Valco, P/N C2-2006D) was used to direct the ^{11}CO/He flow from the ^{11}CO trap to the MF reactor. At the same time the premixed coupling reagents solution (aryl halide, Pd-ligand and nucleophile in anhydrous THF) was infused into the MF reactor at a constant flow rate (30 μl/min) using a syringe pump. The micro reactor (deactivated fused-silica capillary, length = 5-m, i.d. = 200 μm, P/N 160-2205-5, Agilent technologies) was pre-heated to 100°C using an oil bath. A mixing-tee (150 μm i.d., P/N P-890-01, INEX Heath & Science) was enplayed to generate a sufficient gas and liquid contact and facilitate μ-bubble formation. The pressure inside the micro reactor was kept at 7 Bar (100 psi) using a back-pressure regulator (Supelco, P/N 5-9284). A product collection vial was connected to the reactor outlet with a leak-tight gas bag in series to receive volatile radioactive products (*e.g.* ^{11}CO). The synthesis process was controlled and monitored with in-house developed software (Labview, National Istruments).

The radioactivity inside the collaction vial and the leak-tight gas bag were determined using a calibrated radioisotope calibrator (Capintec INC, USA). The radioactivity inside the collaction vials was measured a second time after flushing the head-space with N_2 to remove unreacted ^{11}CO (Fig. 2). The ^{11}CO trapping efficiency (TE) was calculated by deviding the radioactivity still remaining within the collation vial after flushing with N_2, with the sum of all radioactivity exiting the micro reactor. The crude reaction mixture was diluted with mobile phase (1:1, acetonitrile:water) and the radiochemical purity (RCP) was established with radio-HPLC. The product peak area as a percentage of the sum of all radioactive peak areas, with the correction for trapping efficiency, was used to estimate the radiochemical conversion (RCC).

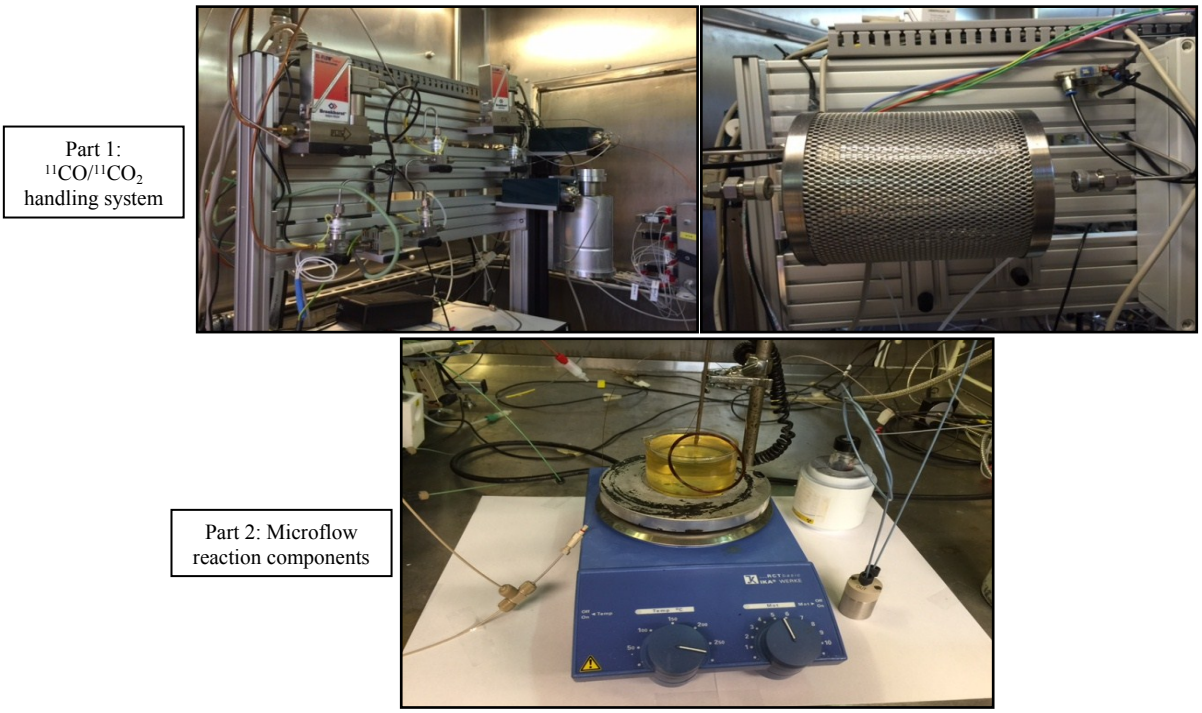


Fig. 2 The microfluidic ^{11}C-carbonylation synthesizer devided in two main parts. Part 1: the $^{11}CO/^{11}CO_2$ handling system, including a molecular sieve column, two mass-flow controllers, four 3-port valves, one 6-port valve, Mo (s) oven, acarite trap and ^{11}CO trap (silica column). Part 2: Microflow reaction components, including the capillary micro reactor, mixing-Tee, oil bath and back-pressure regulator.

Preparation of *N*-benzyl-[carbonyl-^{11}C]benzamide ([^{11}C]3) using conditions A. An oven-dried disposable 4 ml vial (chromacol) was equipped with a rubber septum, evacuated and cooled under nitrogen. All solid reagents were added by briefly removing the rubber septum: $Pd(PPh_3)_4$ (4 mg, 3.5 µmol) and iodobenzene (2 mg, 10 µmol) were dissolved in anhydrous THF (0.9 ml). The mixture was purged for 15 min with nitrogen before benzylamine (50 mg, 467 µmol) was added via syringe through the septum. The reaction mixture was loaded to syringe pump of the syntheses module 5 min prior to start of synthesis.

Preparation of *N*-benzyl-[carbonyl-^{11}C]benzamide ([^{11}C]3) using conditions B. An oven-dried disposable 4 ml vial (chromacol) was equipped with a rubber septum, evacuated and cooled under nitrogen. All solid reagents were added by briefly removing the rubber septum: $Pd_2(\pi\text{-cinnamyl})Cl_2$ (2 mg, 3.9 µmol), xantphos (4 mg, 6.9 µmol) and iodobenzene (2 mg, 10 µmol) were dissolved in anhydrous THF (0.9 ml). The mixture was purged for 15 min with nitrogen before benzylamine (50 mg, 467 µmol) was added via syringe through the septum. The reaction mixture was loaded to syringe pump of the syntheses module 5 min prior to start of synthesis.

Preparation of *N*-benzyl-[carbonyl-^{11}C]benzamide ([^{11}C]3) using conditions C. An oven-dried disposable 4 ml vial (chromacol) was equipped with a rubber septum, evacuated and cooled under nitrogen. All solid reagents were added by briefly removing the rubber septum: [$PdCl_2$-(xantphos)] (7.5 mg, 9.9 µmol) and iodobenzene (2 mg, 10 µmol) were dissolved in anhydrous toluene (1 ml). The mixture was purged for 5 min with nitrogen and heated at 100°C for an additional 5 min before benzylamine (50 mg, 467 µmol) was

added via syringe through the septum. The reaction mixture was loaded to syringe pump of the syntheses module 5 min prior to start of synthesis.

Preparation of [^{11}C]4 using conditions A. An oven-dried disposable 4 ml vial (chromacol) was equipped with a rubber septum, evacuated and cooled under nitrogen. All solid reagents were added by briefly removing the rubber septum: $Pd(PPh_3)_4$ (4 mg, 3.5 μmol) and 2-iodobenzyl alcohol (5 mg, 21 μmol) were dissolved in anhydrous THF (0.9 ml). The mixture was purged for 15 min with nitrogen. The reaction mixture was loaded to syringe pump of the syntheses module 5 min prior to start of synthesis.

Preparation of [^{11}C]5 using conditions A. An oven-dried disposable 4 ml vial (chromacol) was equipped with a rubber septum, evacuated and cooled under nitrogen. All solid reagents were added by briefly removing the rubber septum: $Pd(PPh_3)_4$ (4 mg, 3.5 μmol) and iodobenzene (2 mg, 10 μmol) were dissolved in anhydrous THF (0.8 ml). The mixture was purged for 15 min with nitrogen before 0.35 M NaOH solution (200 μl) was added via syringe through the septum. The reaction mixture was loaded to syringe pump of the syntheses module 5 min prior to start of synthesis.

Preparation of [^{11}C]6 using conditions A. An oven-dried disposable 4 ml vial (chromacol) was equipped with a rubber septum, evacuated and cooled under nitrogen. All solid reagents were added by briefly removing the rubber septum: $Pd(PPh_3)_4$ (4 mg, 3.5 μmol) and iodobenzene (2 mg, 10 μmol) were dissolved in anhydrous THF (0.4 ml). The mixture was purged for 15 min with nitrogen before methanol (400 μl) was added via syringe through the septum. The reaction mixture was loaded to syringe pump of the syntheses module 5 min prior to start of synthesis.

Preparation of [^{11}C]7 using conditions A. An oven-dried disposable 4 ml vial (chromacol) was equipped with a rubber septum, evacuated and cooled under nitrogen. All solid reagents were added by briefly removing the rubber septum: $Pd(PPh_3)_4$ (4 mg, 3.5 μmol) and iodobenzene (2 mg, 10 μmol) were dissolved in anhydrous THF (0.4 ml). The mixture was purged for 15 min with nitrogen before ethanol (400 μl) was added via syringe through the septum. The reaction mixture was loaded to syringe pump of the syntheses module 5 min prior to start of synthesis.

Preparation of [^{11}C]FLB457 ([^{11}C]8) using conditions A. An oven-dried disposable 4 ml vial (chromacol) was equipped with a rubber septum, evacuated and cooled under nitrogen. All solid reagents were added by briefly removing the rubber septum: $Pd(PPh_3)_4$ (4 mg, 3.5 μmol) and 5-bromo-1-iodo-2,3-dimethoxybenzene (4 mg, 12 μmol) were dissolved in anhydrous THF (0.9 ml). The mixture was purged for 15 min with nitrogen (S)-(-)-aminomethyl-1-ethylpyrrolidine (20 mg, 156 μmol) was added via syringe through the septum. The reaction mixture was loaded to syringe pump of the syntheses module 5 min prior to start of synthesis.

Preparation of [^{11}C]9 using conditions A. An oven-dried disposable 4 ml vial (chromacol) was equipped with a rubber septum, evacuated and cooled under nitrogen. All solid reagents were added by briefly removing the rubber septum: $Pd(PPh_3)_4$ (4 mg, 3.5 μmol) and methyl iodide (2 mg, 14 μmol) were dissolved in anhydrous THF (0.9 ml). The mixture was purged for 15 min with nitrogen before amine-precursor (10 mg, 23 μmol) was added by briefly removing the rubber septum. The reaction mixture was loaded to syringe pump of the syntheses module 5 min prior to start of synthesis.

Preparation of [^{11}C]10 using conditions A. An oven-dried disposable 4 ml vial (chromacol) was equipped with a rubber septum, evacuated and cooled under nitrogen. All solid reagents were added by briefly removing the rubber septum: $Pd(PPh_3)_4$ (4 mg, 3.5 μmol) and iodo-precursor (5 mg, 10 μmol) were dissolved in anhydrous THF (0.6 ml). The mixture was purged for 15 min with nitrogen before 2 M dimethylamine in THF (400 μl) was added via syringe through the septum. The reaction mixture was loaded to syringe pump of the syntheses module 5 min prior to start of synthesis.

Preparation of [^{11}C]11 using conditions A. An oven-dried disposable 4 ml vial (chromacol) was equipped with a rubber septum, evacuated and cooled under nitrogen. All solid reagents were added by briefly removing the rubber septum: $Pd(PPh_3)_4$ (4 mg, 3.5 μmol) and iodo-precursor (5 mg, 11.5 μmol) were dissolved in anhydrous THF (0.5 ml). The mixture was purged for 15 min with nitrogen before 2 M

dimethylamine in THF (500 µl) was added via syringe through the septum. The reaction mixture was loaded to syringe pump of the syntheses module 5 min prior to start of synthesis.

Preparation of [^{11}C]12 using conditions A. An oven-dried disposable 4 ml vial (chromacol) was equipped with a rubber septum, evacuated and cooled under nitrogen. All solid reagents were added by briefly removing the rubber septum: $Pd(PPh_3)_4$ (4 mg, 3.5 µmol) and iodo-precursor (5 mg, 10 µmol) were dissolved in anhydrous THF (0.9 ml). The mixture was purged for 15 min with nitrogen before piperidine (43 mg, 506 µmol) was added via syringe through the septum. The reaction mixture was loaded to syringe pump of the syntheses module 5 min prior to start of synthesis.

Preparation of [^{11}C]13 using conditions B. An oven-dried disposable 4 ml vial (chromacol) was equipped with a rubber septum, evacuated and cooled under nitrogen. All solid reagents were added by briefly removing the rubber septum: $Pd_2(\pi\text{-cinnamyl})Cl_2$ (2 mg, 3.9 µmol), xantphos (4 mg, 6.9 µmol) and bromo-precursor (3 mg, 8.3 µmol) were dissolved in anhydrous THF (0.5 ml). The mixture was purged for 15 min with nitrogen before 2 M ethylamine in THF (500 µl) was added via syringe through the septum. The reaction mixture was loaded to syringe pump of the syntheses module 5 min prior to start of synthesis.

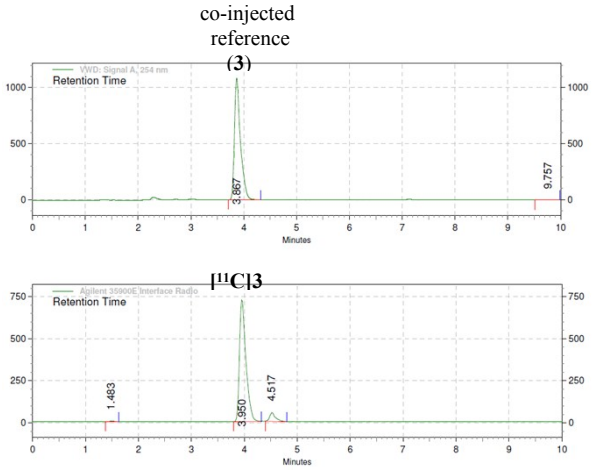
Preparation of [^{11}C]Raclopride ([^{11}C]14) using conditions B. An oven-dried disposable 4 ml vial (chromacol) was equipped with a rubber septum, evacuated and cooled under nitrogen. All solid reagents were added by briefly removing the rubber septum: $Pd_2(\pi\text{-cinnamyl})Cl_2$ (2 mg, 3.9 µmol), xantphos (4 mg, 6.9 µmol) and 4,6-dichloro-2-iodo-3-methoxyphenol (3 mg, 9.4 µmol) were dissolved in anhydrous THF (0.9 ml). The mixture was purged for 15 min with nitrogen before (S)-(-)-aminomethyl-1-ethylpyrrolidine (20 mg, 156 µmol) was added via syringe through the septum. The reaction mixture was loaded to syringe pump of the syntheses module 5 min prior to start of synthesis.

Preperative production of compound [^{11}C]12 using conditions A. An oven-dried disposable 4 ml vial (chromacol) was equipped with a rubber septum, evacuated and cooled under nitrogen. All solid reagents were added by briefly removing the rubber septum: $Pd(PPh_3)_4$ (4 mg, 3.5 µmol) and iodo-precursor (5 mg, 10 µmol) were dissolved in anhydrous THF (0.9 ml). The mixture was purged for 15 min with nitrogen before piperidine (43 mg, 506 µmol) was added via syringe through the septum. The reaction mixture was loaded to syringe pump of the syntheses module 5 min prior to start of synthesis. The reaction was carried out in accordence with the general procidure, after which the crude reaction mixture quenched with 4 ml of a 1:1 ratio between mobile phase and 0.1 M NH_4HCO_2. Semi-preparative HPLC purification was performed on a reversed-phase column (µBondapak – C18, 10 µm, 10 x 300 mm) eluted with an isocratic mobile phase consisting of acetonitrile and 0.1 M NH_4HCO_2 (40:60, v/v) at a flow rate of 6 ml/min to afford [^{11}C]**12** (t_r = 18 min) in a isolated yield of 1200 MBq. HPLC analysis t_r = 6.100 min; >99% radiochemical purity and a Specific Radioactivity (SRA) of 49 GBq/µmol (1070 Ci/mmol).

Preperative production of compound [^{11}C]13 using conditions B. An oven-dried disposable 4 ml vial (chromacol) was equipped with a rubber septum, evacuated and cooled under nitrogen. All solid reagents were added by briefly removing the rubber septum: $Pd_2(\pi\text{-cinnamyl})Cl_2$ (2 mg, 3.9 µmol), xantphos (4 mg, 6.9 µmol) and bromo-precursor (3 mg, 8.3 µmol) were dissolved in anhydrous THF (0.5 ml). The mixture was purged for 15 min with nitrogen before 2 M ethylamine in THF (500 µl) was added via syringe through the septum. The reaction mixture was loaded to syringe pump of the syntheses module 5 min prior to start of synthesis. The reaction was carried out in accordence with the general procidure, after which the crude reaction mixture quenched with 4 ml of a 1:1 ratio between mobile phase and 0.1 M NH_4HCO_2. Semi-preparative HPLC purification was performed on a reversed-phase column (µBondapak – C18, 10 µm, 10 x 300 mm) eluted with an isocratic mobile phase consisting of acetonitrile and 0.1 M NH_4HCO_2 (25:75, v/v) at a flow rate of 6 ml/min to afford [^{11}C]**12** (t_r = 15 min) in a isolated yield of 2800 MBq. HPLC analysis t_r = 5.110 min; >99% radiochemical purity and a Specific Radioactivity (SRA) of 52 GBq/µmol (1470 Ci/mmol).

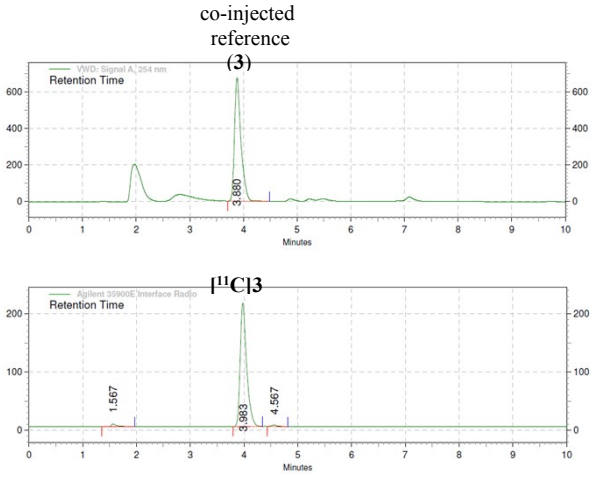
Product determination by Radio-HPLC of compounds [^{11}C]3 – 14.

1. Radio-HPLC determination for compound **3** synthesized using conditions A.



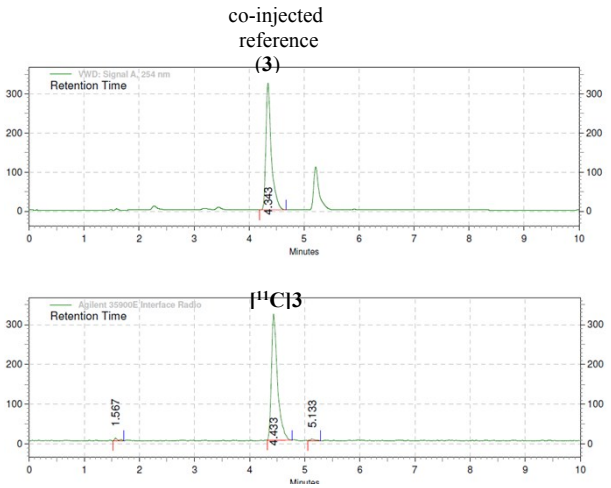
Reaction data: TE = >99%, RCC = TE • RCP / 100 = 99 • 0.96 = 95%. **Chromatogarphy data:** Retention time (3) = 3.867 min, Retention time ([^{11}C]3) = 3.950 min, Retention time difference = 0.083 min, RCP ([^{11}C]3) = 96%.

2. Radio-HPLC determination for compound **3** synthesized using conditions B.



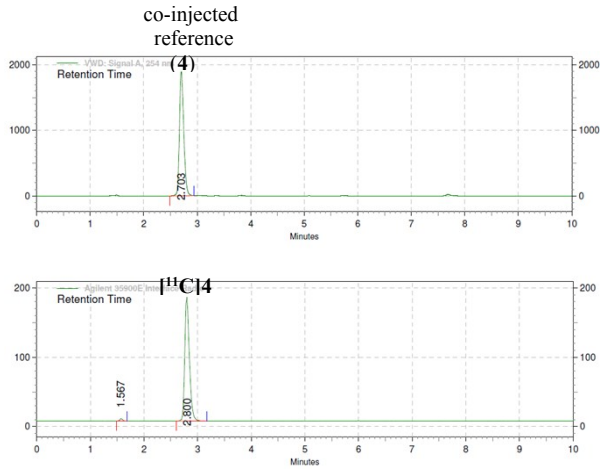
Reaction data: TE = >99%, RCC = TE • RCP / 100 = 99 • 0.99 = 98%. **Chromatogarphy data:** Retention time (3) = 3.880 min, Retention time ([^{11}C]3) = 3.983 min, Retention time difference = 0.103 min, RCP ([^{11}C]3) = 98%.

3. Radio-HPLC determination for compound 3 synthesized using conditions C.



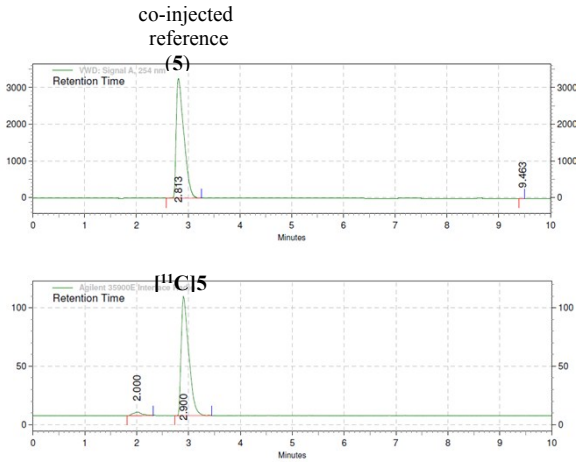
Reaction data: TE = >99%, RCC = TE • RCP / 100 = 99 • 0.99 = 98%. **Chromatogarphy data:** Retention time (3) = 4.343 min, Retention time ([^{11}C]3) = 4.433 min, Retention time difference = 0.09 min, RCP ([^{11}C]3) = 98%.

4. Radio-HPLC determination for compound **4** synthesized using conditions A.



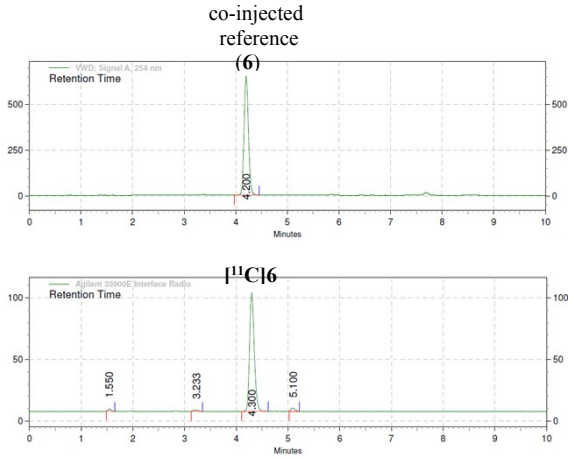
Reaction data: TE = >99%, RCC = TE • RCP / 100 = 99 • 0.99 = 98%. **Chromatogarphy data:** Retention time (4) = 2.703 min, Retention time ([^{11}C]4) = 2.800 min, Retention time difference = 0.097 min, RCP ([^{11}C]4) = 98%.

5. Radio-HPLC determination for compound **5** synthesized using conditions A.



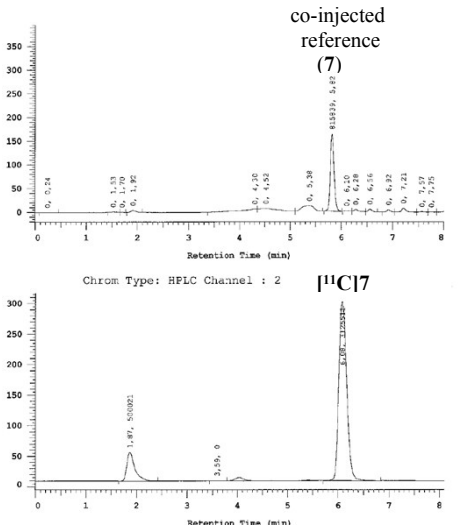
Reaction data: TE = >99%, RCC = TE • RCP / 100 = 99 • 0.97 = 96%. **Chromatogarphy data:** Retention time (5) = 2.813 min, Retention time ([^{11}C]5) = 2.900 min, Retention time difference = 0.087 min, RCP ([^{11}C]5) = 97%.

6. Radio-HPLC determination for compound **6** synthesized using conditions A.



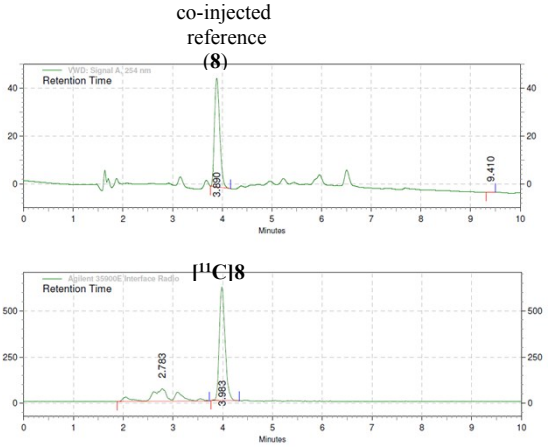
Reaction data: TE = >99%, RCC = TE • RCP / 100 = 99 • 0.98 = 97%. **Chromatogarphy data:** Retention time (6) = 4.200 min, Retention time ([^{11}C]6) = 4.300 min, Retention time difference = 0.100 min, RCP ([^{11}C]6) = 97%.

7. Radio-HPLC determination for compound **7** synthesized using conditions A.



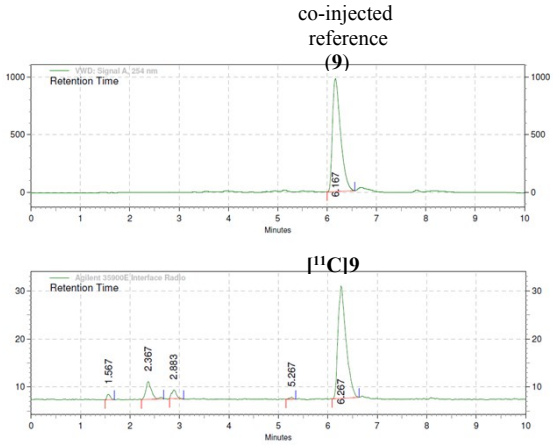
Reaction data: TE = >99%, RCC = TE • RCP / 100 = 99 • 0.80 = 79%. **Chromatogarphy data:** Retention time (7) = 5.82 min, Retention time ([^{11}C]7) = 6.08 min, Retention time difference = 0.26 min, RCP ([^{11}C]7) = 80%. The analysis was performed with a different Radio-HPLC system. (Hitatchi 6000 Model)

8. Radio-HPLC determination for compound **8** synthesized using conditions A.



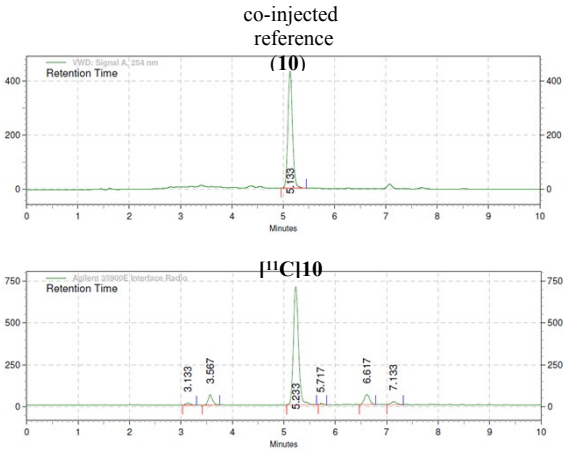
Reaction data: TE = 82%, RCC = TE • RCP / 100 = 82 • 0.74 = 61%. **Chromatogarphy data:** Retention time (8) = 3.890 min, Retention time ([^{11}C]8) = 3.983 min, Retention time difference = 0.093 min, RCP ([^{11}C]8) = 74%.

9. Radio-HPLC determination for compound **9** synthesized using conditions A.



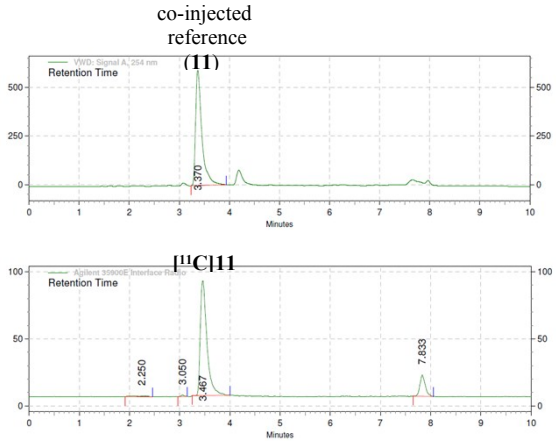
Reaction data: TE = 45%, RCC = TE • RCP / 100 = 45 • 0.86 = 38%. **Chromatogarphy data:** Retention time (9) = 6.167 min, Retention time ([^{11}C]9) = 6.267 min, Retention time difference = 0.100 min, RCP ([^{11}C]9) = 86%.

10. Radio-HPLC determination for compound **10** synthesized using conditions A.



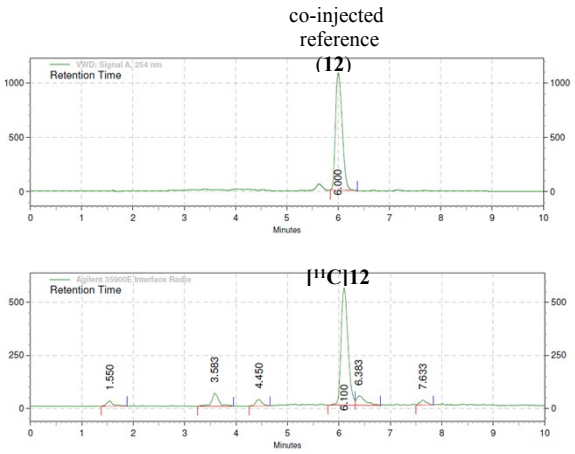
Reaction data: TE = 79%, RCC = TE • RCP / 100 = 79 • 0.82 = 65%. **Chromatogarphy data:** Retention time (10) = 5.133 min, Retention time ([^{11}C]10) = 5.233 min, Retention time difference = 0.100 min, RCP ([^{11}C]10) = 82%.

11. Radio-HPLC determination for compound **11** synthesized using conditions A.



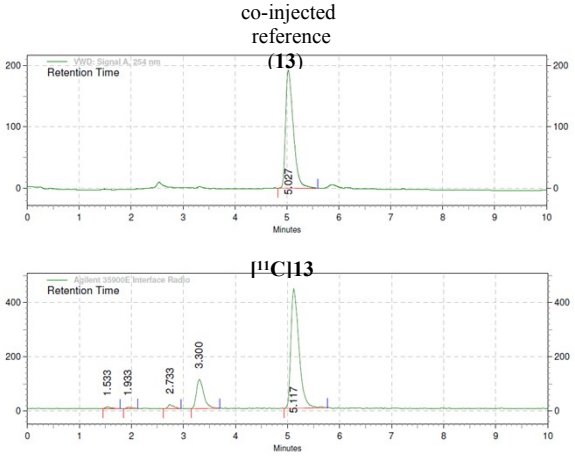
Reaction data: TE = 97%, RCC = TE • RCP / 100 = 97 • 0.86 = 83%. **Chromatogarphy data:** Retention time (11) = 3.370 min, Retention time ([^{11}C]11) = 3.467 min, Retention time difference = 0.097 min, RCP ([^{11}C]11) = 86%.

12. Radio-HPLC determination for compound **12** synthesized using conditions A.



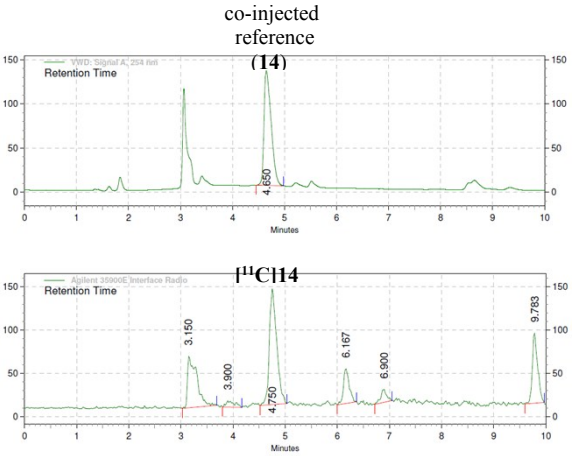
Reaction data: TE = 89%, RCC = TE • RCP / 100 = 89 • 0.76 = 68%. **Chromatogarphy data:** Retention time (12) = 6.000 min, Retention time ([^{11}C]12) = 6.100 min, Retention time difference = 0.100 min, RCP ([^{11}C]12) = 76%.

13. Radio-HPLC determination for compound **13** synthesized using conditions B.



Reaction data: TE = >99%, RCC = TE • RCP / 100 = 99 • 0.80 = 79%. **Chromatogarphy data:** Retention time (13) = 5.027 min, Retention time ([^{11}C]13) = 5.117 min, Retention time difference = 0.090 min, RCP ([^{11}C]13) = 80%.

14. Radio-HPLC determination for compound **14** synthesized using conditions B.



Reaction data: TE = >99%, RCC = TE • RCP / 100 = 99 • 0.42 = 41%. **Chromatogarphy data:** Retention time (14) = 4.650 min, Retention time ([^{11}C]14) = 5.750 min, Retention time difference = 0.100 min, RCP ([^{11}C]13) = 42%.