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Supplementary Information

Negishi coupling reactions as a valuable tool for [^{11}C]methyl-arene formation; first proof of principle

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1. Experimental

[^{11}C]carbon dioxide ($^{11}CO_2$) was produced by the nuclear $^{14}N[p,\alpha]^{11}C$ reaction using a Siemens Eclipse HP cyclotron by 11 MeV proton bombardment of a target containing nitrogen and 1% oxygen. $^{11}CH_3I$ was produced from $^{11}CO_2$ using the commercially-available Synthra gas phase methyl iodide module, and delivered to the reaction vessel in a stream of helium carrier gas. Glassware used for radiochemical and microwave reactions was oven dried and flushed with a stream of argon prior to use. Test radiolabelling reactions were performed in a lead-lined hotcell using a custom-built methylation apparatus consisting of an externally-controlled valve array and heater block. Complete syntheses were performed using the Eckert and Ziegler Modular-Lab apparatus, controlled externally via computer (see Supplementary Information for schematic of experimental setup). Semipreparative HPLC purifications were performed using a system consisting of a Gilson 322 binary pump, a Gilson 151 UV–vis detector and online radio-detection using Carroll and Ramsey pin diodes.

Quality control analysis of radioactive product mixtures was performed by high performance liquid chromatography (HPLC) using an Agilent 1100 system with in-line radioactivity and diode array detectors (UV) fitted with an Agilent Zorbax SB-C_{18} column (5 µm, 4.6 x 150 mm) and an eluent mixture of acetonitrile and ammonium formate at a flow of 1.5 mL/min. Radioactivity measurements were performed using a Isomed 2000 dose calibrator. ^{1}H NMR spectra were recorded using a Varian Oxford 400 MHz spectrometer. Chemical shifts are reported in parts per million and are calculated from the residual proton signal in the solvent. J values are given in Hz. Mass spectra were recorded on a Micromass LCT Premier spectrometer at the Department of Chemistry, Imperial College, London.

Reagents and anhydrous solvents were obtained from Sigma-Aldrich and used as received, with the exception of sterile water and 0.9% saline solution (Fresenius Kabi), dimethylacetamide, anhydrous ethanol and Acetonitrile (Romil), and 2-bromo-6-(2-phenylethynyl)-pyridine (synthesised according to the method described by Yu *et al.*[1]).

1.1 Synthesis of 1-[^{11}C]methylnaphthalene from commercially available 1-naphthylzinc iodide

$Pd(PPh_3)_2Cl_2$ (2.7 mg, 4 µmol) was added to a 3 mL v-bottomed glass reaction vial and a rubber septum-containing cap fitted. In a hotcell, the vial was degassed with argon for 10 min via inlet and vent needles, and dimethylacetamide (0.4 mL) added by a syringe. The vial was heated at 100^{o}C for 5 min to dissolve the $Pd(PPh_3)_2Cl_2$, forming a yellow solution, then allowed to cool to room temperature. The inlet needle was disconnected from the argon line and connected to the $^{11}CH_3I$ delivery line. The vent needle was connected to a vent line, terminating at a waste gas bag. $^{11}CH_3I$ was delivered to the vial in a helium carrier gas stream; when the radioactivity of the vial reached a maximum value, as determined using a pin-diode radioactivity detector, the flow to the vial was stopped and arylzinc halide solution (0.1 mL, 50 µmol) added via syringe. The vial was shaken to mix the reagents and allowed to stand at room temperature. After 5 min, the vial was removed from the hotcell and an aliquot of solution removed by syringe, quenched with water, and analysed by analytical HPLC (60% MeCN :40% AMF, pH 4.0, 50 mM), r.t = 5.9 min.

1.2 General procedure for preparation of arylzinc halides

Aryl halide (500 µmol) was added to a 0.5 – 2 mL microwave vial (Biotage®), either by weighing directly in to the vial (solid reagents) or by pipette (liquid reagents). A stirrer bar was added and the vial fitted with a rubber septum crimp-cap which was pierced with inlet and vent needles, and the vial purged with argon for 10 min. Zn/THF suspension (1.4 mL, 1000 µmol, 2 equivalents) was added by syringe and the needles removed from the vial. The vial was heated at 180^{o}C for 10 min in a microwave reactor (Biotage® Initiator) after which the unreacted zinc was allowed to settle out, typically over a number of hours (this process can be accelerated using a centrifuge). The resultant orange supernatant solution of arylzinc halide was then removed by needle and syringe and transferred to a separate reaction vessel for Negishi [^{11}C]methylation reactions.

1.3 General procedure for Negishi [^{11}C]methylation reaction (adding arylzinc halide before $^{11}CH_3I$)

$Pd(PPh_3)_2Cl_2$ (2.7 mg, 4 µmol) was added to a 3 mL v-bottomed glass reaction vial and a rubber septum-containing cap fitted. In a hotcell, the vial was degassed with argon for 10 min via inlet and vent needles, and dimethylacetamide (0.4 mL) added by a syringe fitted with a Na_2SO_4 drying cartridge (Biotage®). The vial was heated at 100^{o}C for 5 min to dissolve the $Pd(PPh_3)_2Cl_2$, forming a yellow solution, then allowed to cool to room temperature. Arylzinc halide solution (0.05 mL, 20 µmol) was added via syringe and the contents mixed by passing argon gas through the solution. The inlet needle was disconnected from the argon line and connected to the $^{11}CH_3I$ delivery line. The vent needle was connected to a vent line, terminating at a waste gas bag in a dose calibrator. $^{11}CH_3I$ was delivered to the vial in a helium carrier gas stream; when the radioactivity of the vial reached a maximum value, as determined using a pin-diode radioactivity detector, the gas flow to the vial was stopped and the vial allowed to stand at room temperature. After 5 min, a stream of nitrogen gas was passed through the vial to displace any

radioactive gases to the dose calibrator. When the radioactivity of the waste gases reached a steady maximum, the flow of nitrogen gas was stopped and the vial was removed from the hotcell. The radioactivity of the vial was measured and an aliquot of solution removed by syringe, quenched with water, and analysed by analytical HPLC. The eluent ratios and their respective retention times for each product are as follows: 1-[^{11}C]methylnaphthalene (60% MeCN :40% AMF, pH 4.0, 50 mM), r.t = 5.9 min; 4-[^{11}C]methyl benzonitrile (44% MeCN :56% AMF, pH 4.0, 50 mM), r.t = 5.5 min; 4-[^{11}C]methyl anisole (52% MeCN :48% AMF, pH 4.0, 50 mM), r.t = 4.6 min; 2-[^{11}C]methyl pyridine (20% MeCN :80% AMF, pH 8.0, 50 mM), r.t = 5.2 min; [^{11}C]MPEP (50% MeCN :50% AMF, pH 4.0, 50 mM), r.t = 4.7 min.

1.4 General procedure for Negishi [^{11}C]methylation reaction (adding arylzinc halide after $^{11}CH_3I$)

$Pd(PPh_3)_2Cl_2$ (2.7 mg, 4 µmol) was added to a 3 mL v-bottomed glass reaction vial and a rubber septum-containing cap fitted. In a hotcell, the vial was degassed with argon for 10 min via inlet and vent needles, and dimethylacetamide (0.4 mL) added by a syringe fitted with a Na_2SO_4 drying cartridge (Biotage®). The vial was heated at 100^{o}C for 5 min to dissolve the $Pd(PPh_3)_2Cl_2$, forming a yellow solution, then allowed to cool to room temperature. The inlet needle was disconnected from the argon line and connected to the $^{11}CH_3I$ delivery line. The vent needle was connected to a vent line, terminating at a waste gas bag in a dose calibrator. $^{11}CH_3I$ was delivered to the vial in a helium carrier gas stream; when the radioactivity of the vial reached a maximum value, as determined using a pin-diode radioactivity detector, the flow to the vial was stopped and arylzinc halide solution (0.5 mL, 140 µmol) added via syringe. The vial was shaken to mix the reagents and allowed to stand at room temperature. After 5 min, a stream of nitrogen gas was passed through the vial, displacing any radioactive gases to the dose calibrator. When the radioactivity of the waste gases reached a steady maximum, the flow of nitrogen gas was stopped and the vial was removed from the hotcell. The radioactivity of the vial was measured and an aliquot of solution removed by syringe, quenched with water, and analysed by analytical HPLC.

1.5 Synthesis, purification and formulation of 1-[^{11}C]methylnaphthalene

$Pd(PPh_3)_2Cl_2$ (2.7 mg, 4 µmol) was added to a 3 mL v-bottomed glass reaction vial and a rubber septum-containing cap fitted. In a hotcell, the vial was purged with argon gas for 10 min via inlet and vent needles, and dimethylacetamide (0.4 mL) added by a syringe fitted with a Na_2SO_4 drying cartridge (Biotage®). The vial was heated at 100^{o}C for 5 min to dissolve the $Pd(PPh_3)_2Cl_2$, forming a yellow solution, then allowed to cool to room temperature. 1-naphthylzinc iodide (0.05 mL, 20 µmol) was added via syringe and the contents mixed by passing argon gas through the solution. The vial was connected to the Eckert and Ziegler Modular-Lab apparatus via inlet and vent needles and cooled to -5^{o}C. $^{11}CH_3I$ was delivered to the vial and when the radioactivity reached a maximum value the flow was stopped. The solution was quenched with HPLC eluent (1.0 mL) and passed through a Saulen cartridge (Chromabond) containing a filter frit and subsequently purified by semi-preparative HPLC (Agilent Zorbax SB-C_{18} column, 250 x 9.4 mm, 5 µm) using a mobile phase of 60% MeCN : 40% ammonium formate pH 4, 50 mM at a flow of 8 mL/min. The desired fraction was collected (retention time = 6.5–

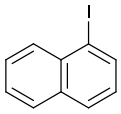
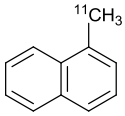
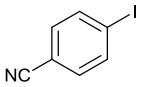
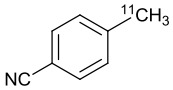
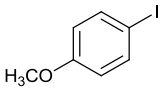
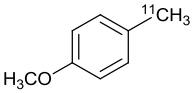
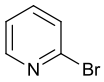
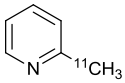
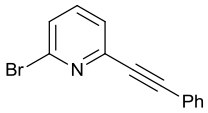
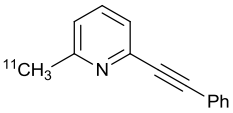
7.0 min) in a vessel containing 25 mL sterile water. The resultant solution was passed through C_{18} Sep-Pak Classic cartridge (Waters) which was then washed with sterile water (10 mL). 1-[^{11}C]methylnaphthalene was eluted from the cartridge using anhydrous ethanol (1 mL) followed by 0.9% saline solution (9 mL) and the resultant solution delivered to a sterile glass vial. Radioactivity at end of synthesis = 2.82 ± 0.47 GBq (n = 2); radiochemical purity >99%; total synthesis time = 26 min; decay corrected radiochemical yield = 62% based on an average delivery of 9 GBq of $^{11}CH_3I$ under the cyclotron irradiation parameters employed (55 µA, 21 min bombardment).

1.6 Synthesis, purification and formulation of 2-[^{11}C]methyl-6-(phenylethynyl)-pyridine ([^{11}C]MPEP)

$Pd(PPh_3)_2Cl_2$ (2.7 mg, 4 µmol) was added to a 3 mL v-bottomed glass reaction vial and a rubber septum-containing cap fitted. In a hotcell, the vial was purged with argon gas for 10 min via inlet and vent needles, after which dimethylacetamide (0.4 mL) was added by a syringe fitted with a Na_2SO_4 drying cartridge (Biotage®). The vial was heated at 100°C for 5 min to dissolve the $Pd(PPh_3)_2Cl_2$, forming a yellow solution, then allowed to cool to room temperature. 2-bromozinc-6-(2-phenylethynyl)-pyridine (0.4 mL, 25 µmol) was added via syringe and the contents mixed by passing argon gas through the solution. The vial was heated at 100°C for 2 min, allowed to cool to room temperature, connected to the Eckert and Ziegler Modular-Lab apparatus via inlet and vent needles and cooled to -5°C. $^{11}CH_3I$ was delivered to the vial and when the radioactivity reached a maximum value the flow was stopped and the vial heated at 120°C for 5 min. The solution was quenched with HPLC eluent (1.0 mL) and passed through a Saulen cartridge (Chromabond) containing a filter frit and subsequently purified by semi-preparative HPLC (Agilent Zorbax SB-C_{18} column, 250 x 9.4 mm, 5 µm) using a mobile phase of 50% MeCN : 50% ammonium formate pH 4, 50 mM at a flow of 8 mL/min. The desired fraction was collected (retention time = 9.0– 10.0 min) in a vessel containing 25 mL sterile water. The resultant solution was passed through C_{18} Sep-Pak Classic cartridge (Waters) which was then washed with sterile water (10 mL). 1-[^{11}C]methylnaphthalene was eluted from the cartridge using anhydrous ethanol (1 mL) followed by 0.9% saline solution (9 mL) and the resultant solution delivered to a sterile glass vial. Radioactivity at end of synthesis = 1.56 ± 0.85 GBq (n = 4); radiochemical purity >99%; total synthesis time = 32 ± 1 min; decay corrected radiochemical yield = 29% based on an average delivery of 13 GBq of $^{11}CH_3I$ under the cyclotron irradiation parameters employed (55 µA, 30 min bombardment).

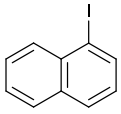
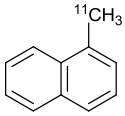
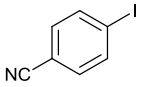
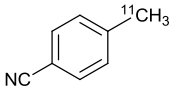
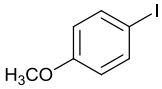
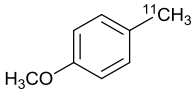
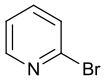
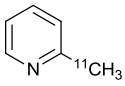
2. Results

Table S1. [^{11}C]methyl arene formation via palladium-mediated Negishi reaction of organozinc reagents (adding ArZnX before $^{11}CH_3I$).

Substrate	Product	Incorporation[a]	RCP[a]	RCY[a]
		99 ± 1%	100 ± 0%	99 ± 1%
		76 ± 8%	91 ± 2%	69 ± 5%
		94 ± 2%	98 ± 4%	91 ± 1%
		40 ± 29% 28 ± 1%[b] 51 ± 4%[c]	0 ± 0% 99 ± 1 % 100 ± 0 %	0 ± 0% 28 ± 1% 51 ± 1%
		74 ± 2%[d]	93 ± 8%	69 ± 4%

(a) Average of two runs. (b) Negishi Reaction performed at 120°C. (c) $P(PPh_3)Cl_2$ and organozinc solution preheated at 100°C. Negishi reaction performed at 120°C (d) $Pd(PPh_3)Cl_2$ and organozinc solution preheated at 100°C. Negishi reaction performed at 110°C.

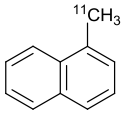
Table S2. [^{11}C]methyl arene formation via palladium-mediated Negishi reaction of organozinc reagents (adding $^{11}CH_3I$ before ArZnX).

Substrate	Product	Incorporation[a]	RCP[a]	RCY[a]
		78 ± 1%	100 ± 0 %	78 ± 1%
		66 ± 1 %	97 ± 5 %	63 ± 3 %
		86 %	93 %	80 %
		15 ± 2%[b]	98 ± 4%[b]	15 ± 3%[b]

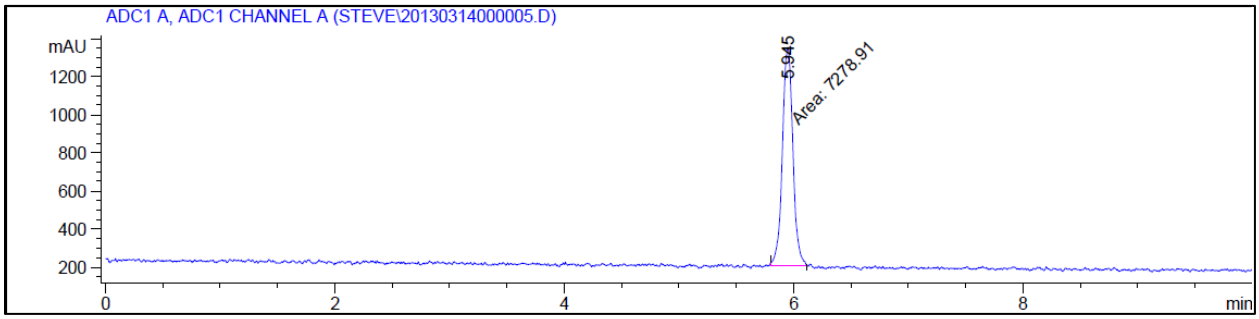
(a) Average of two runs. (b) Negishi Reaction performed at 100°C.

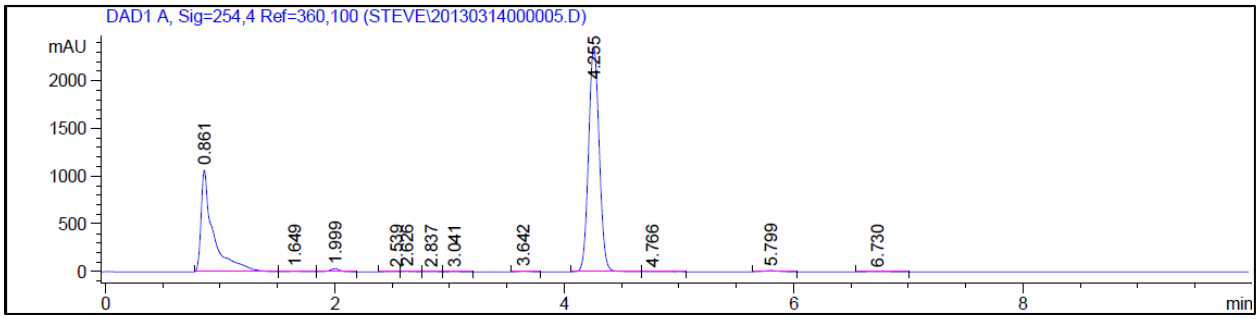
2.1 Analytical HPLC chromatograms of crude reaction mixtures

2.1.1 1-[^{11}C]methyl naphthalene



Crude





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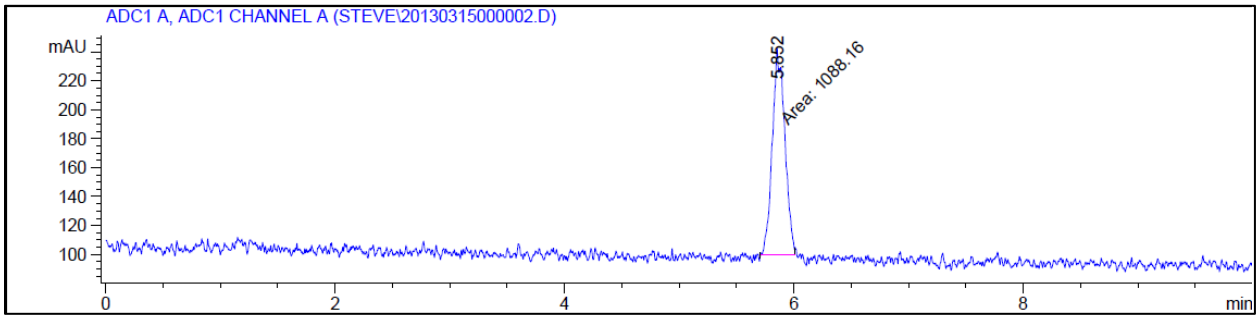
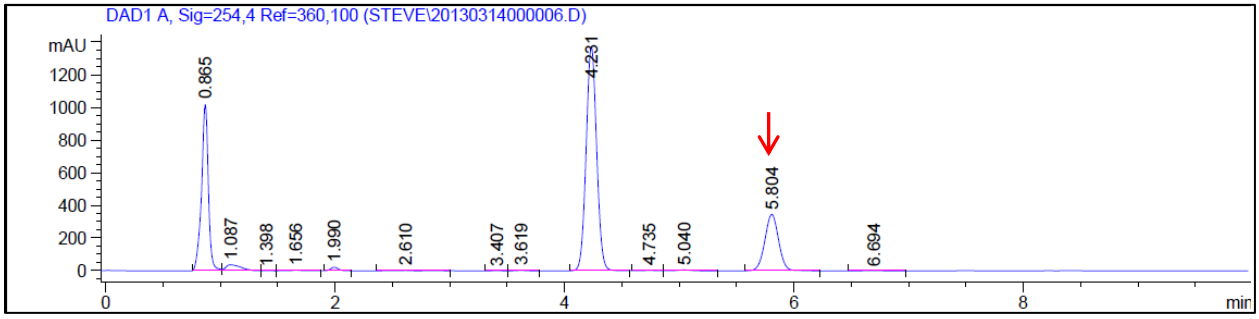
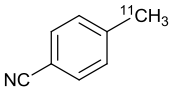




2.1.2 4-[^{11}C]methyl benzonitrile 

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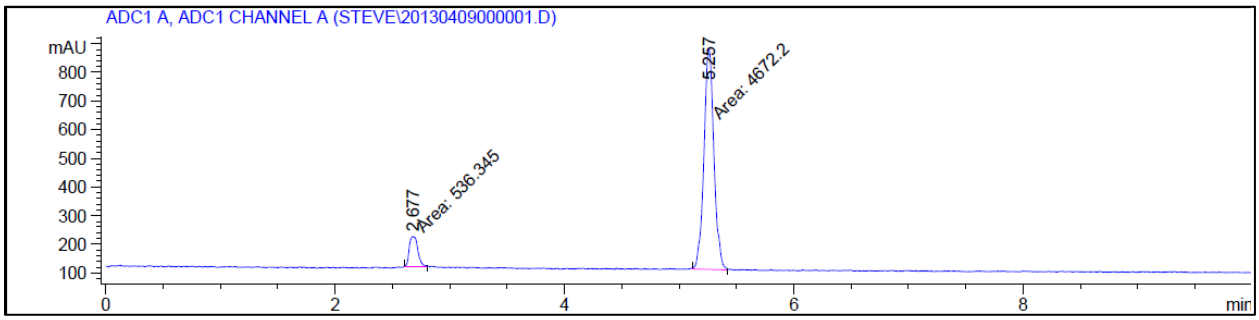

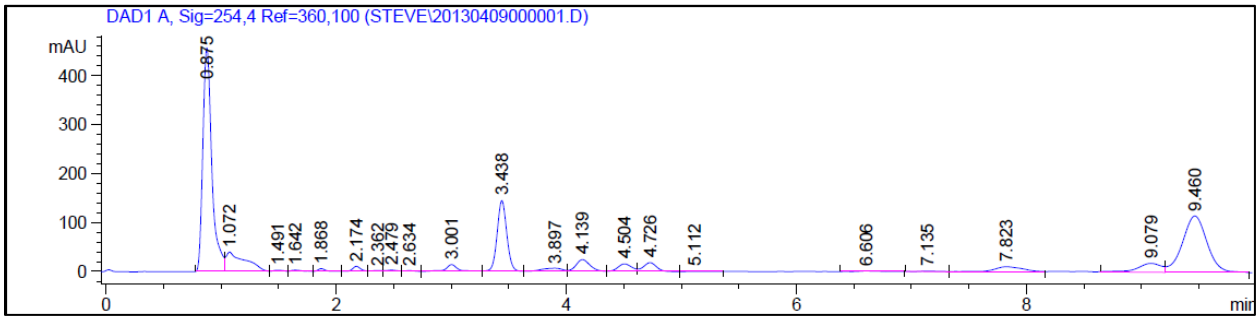

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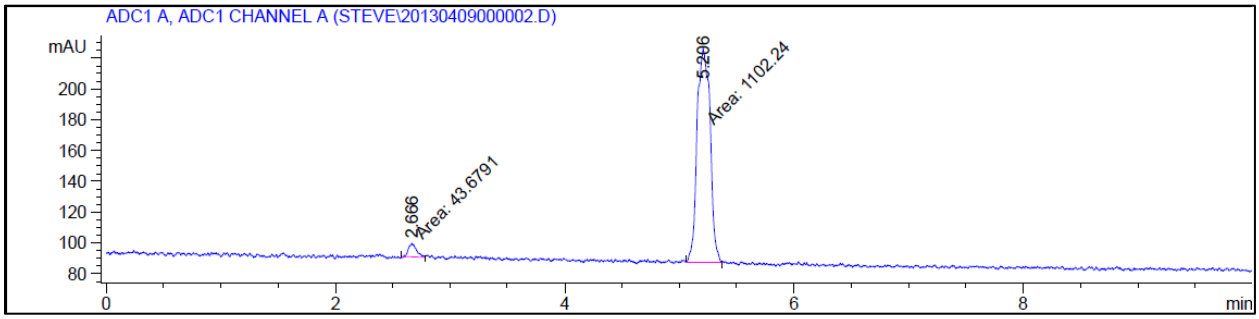
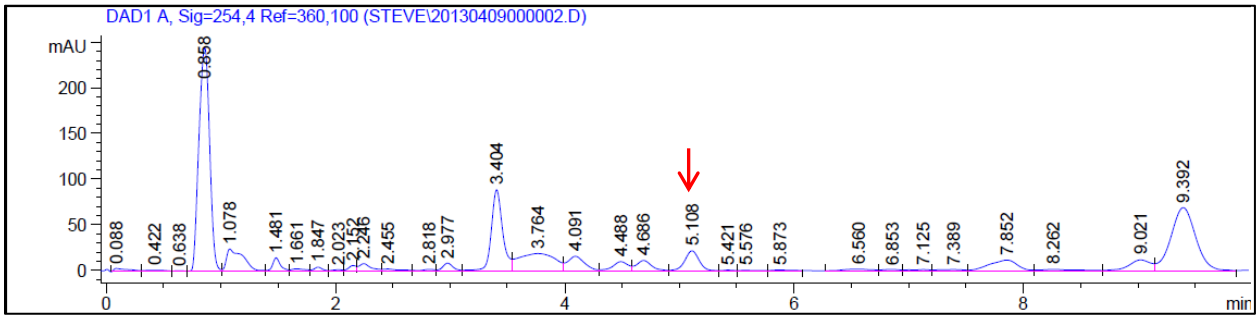
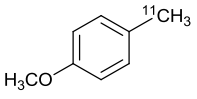




2.1.3 4-[^{11}C]methyl anisole 

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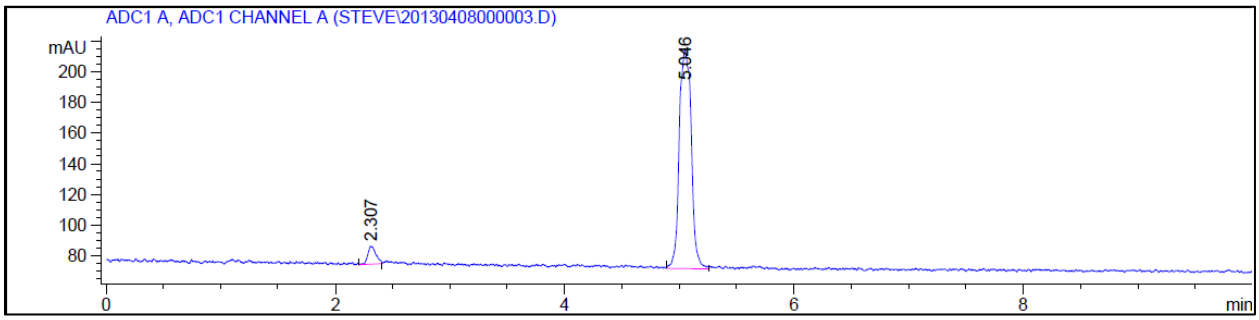
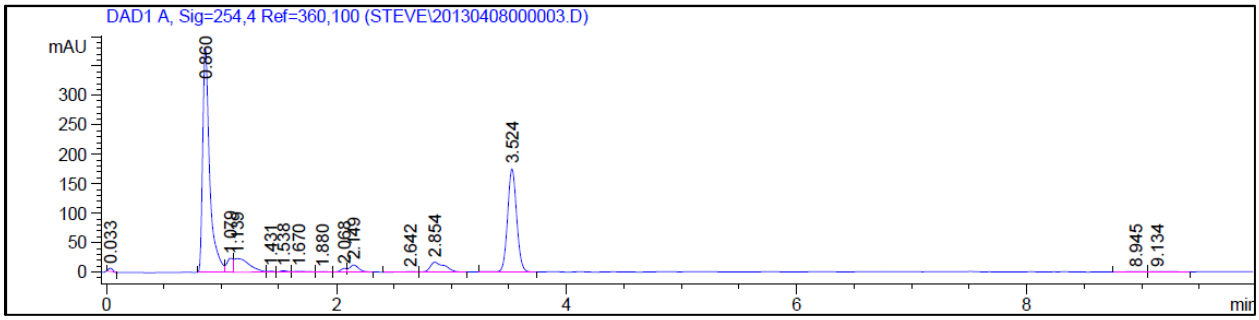
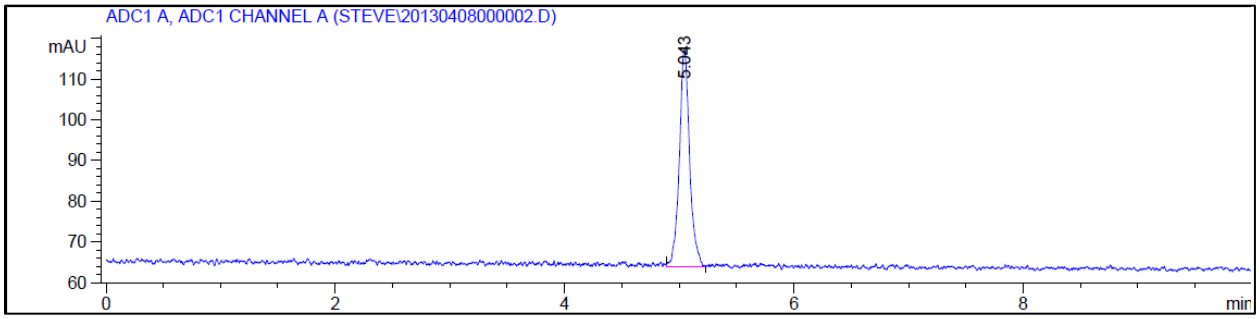
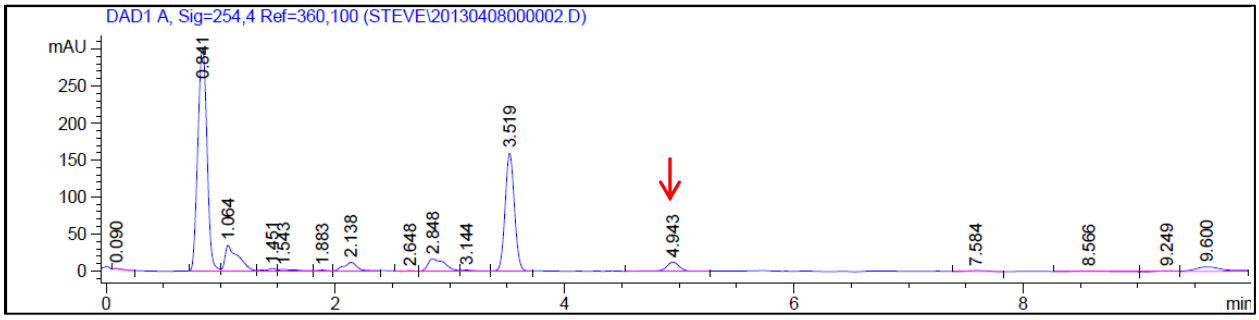
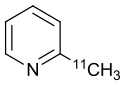




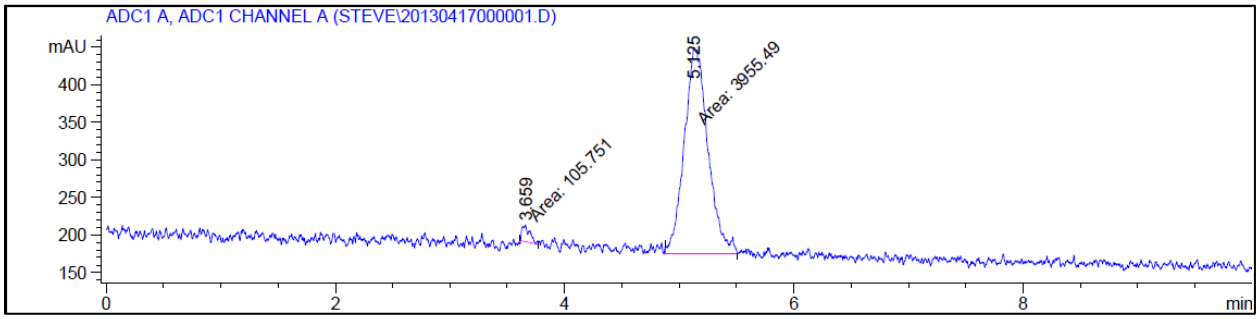
Coinjection

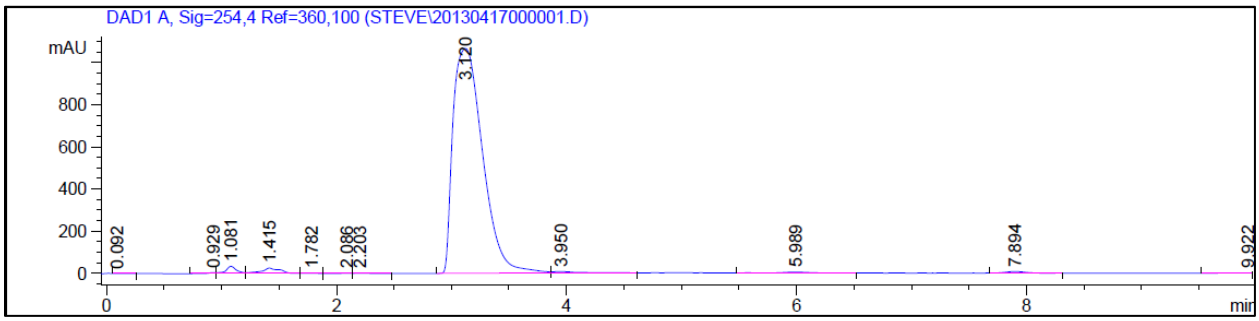




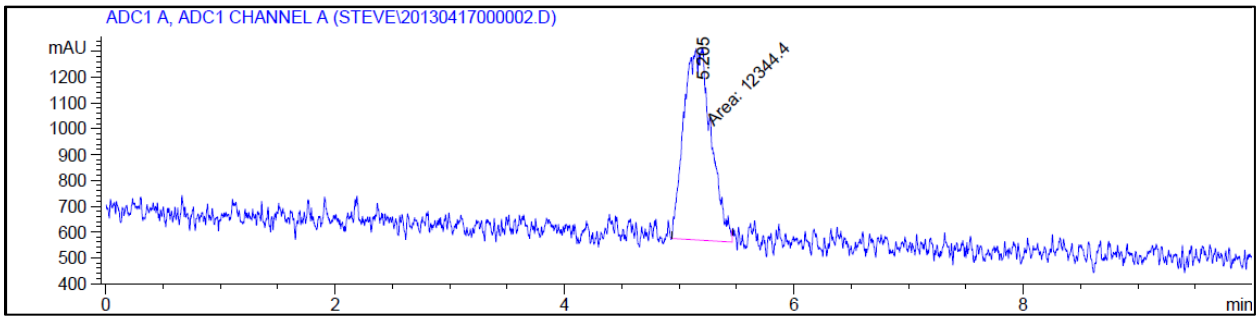
2.1.4 2-[^{11}C]methyl pyridine 

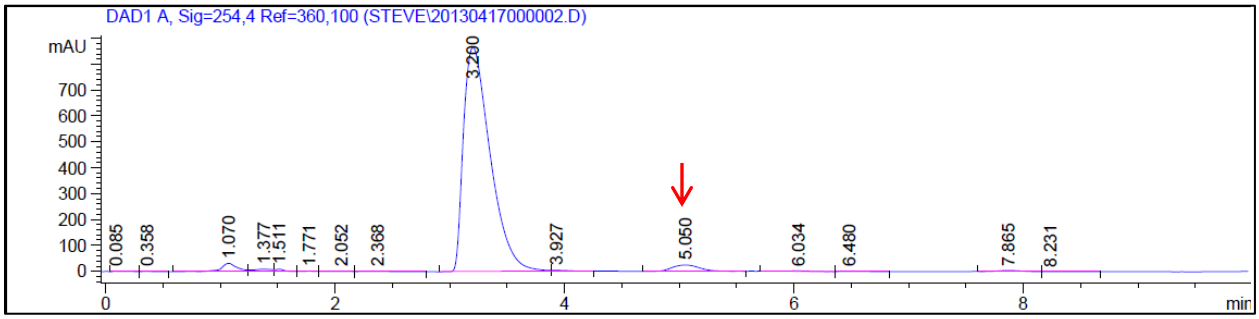
Crude



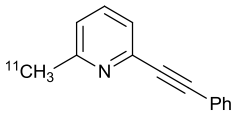


Coninjection

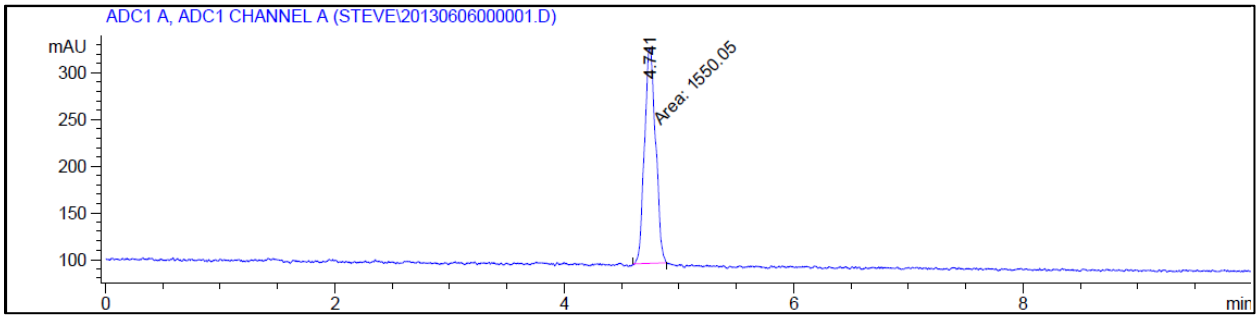


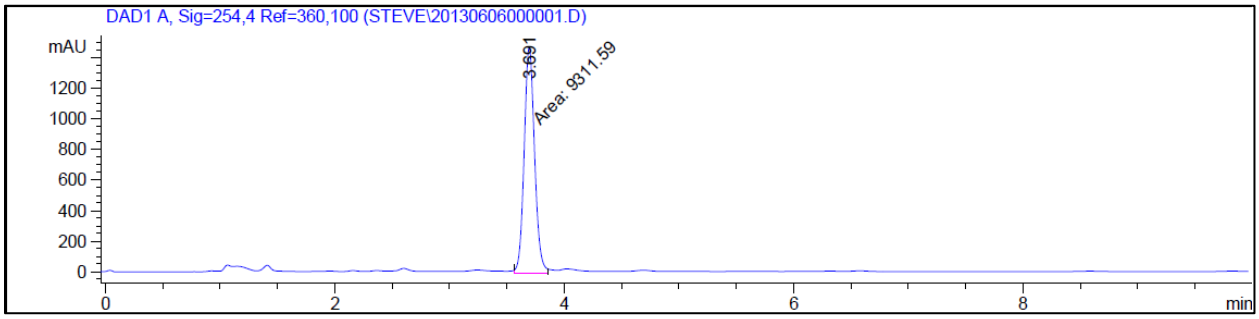


2.1.5 2-[^{11}C]methyl-6-(phenylethynyl)-pyridine ([^{11}C]MPEP)

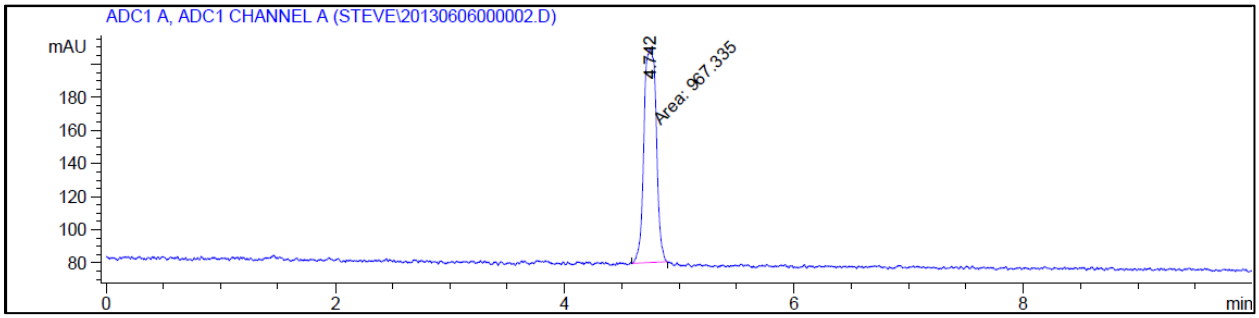


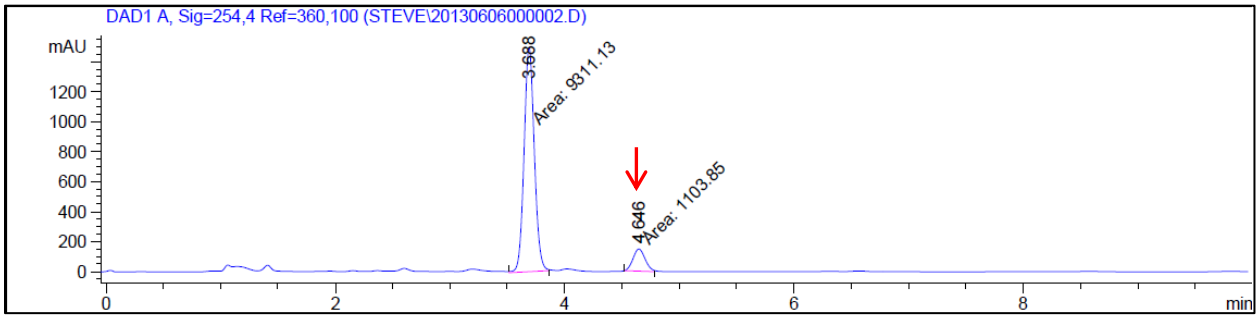
Crude





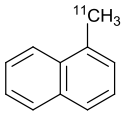
Coinjection



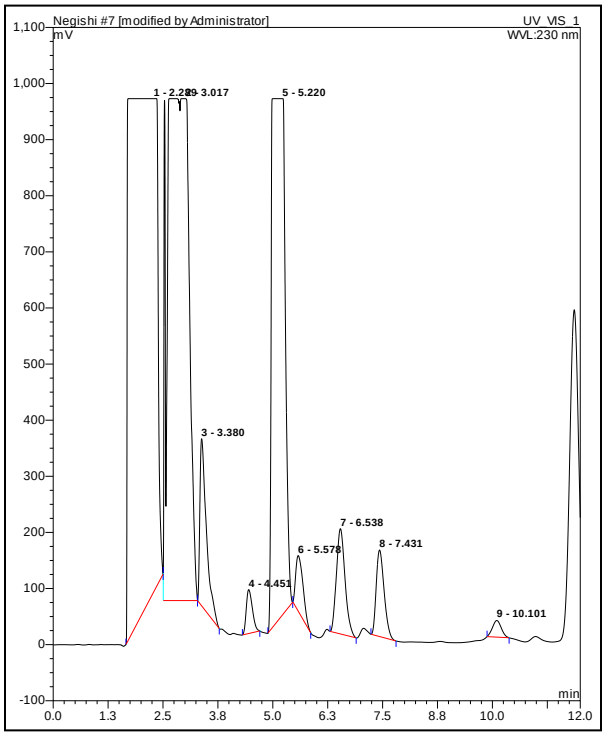


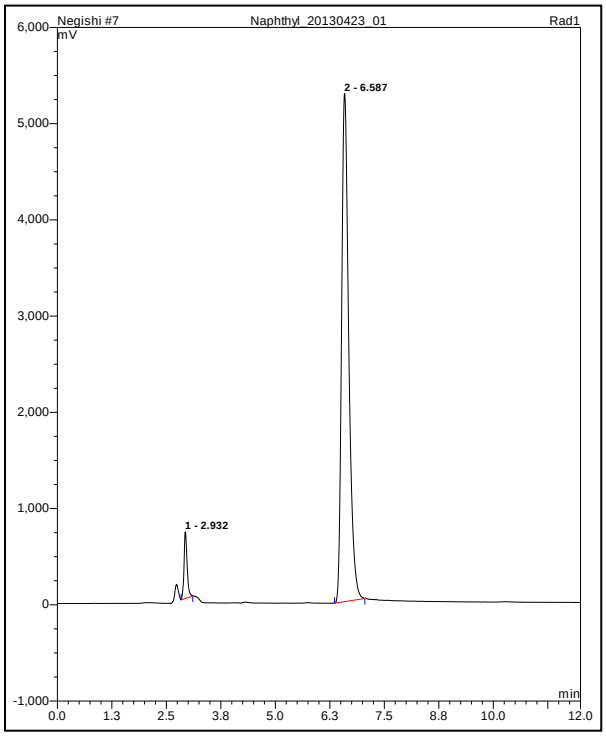
2.2 Full syntheses: Semi-preparative HPLC chromatograms from purification step and analytical HPLC chromatograms from QC analysis of isolated dose.

2.2.1 1-[^{11}C]methyl naphthalene

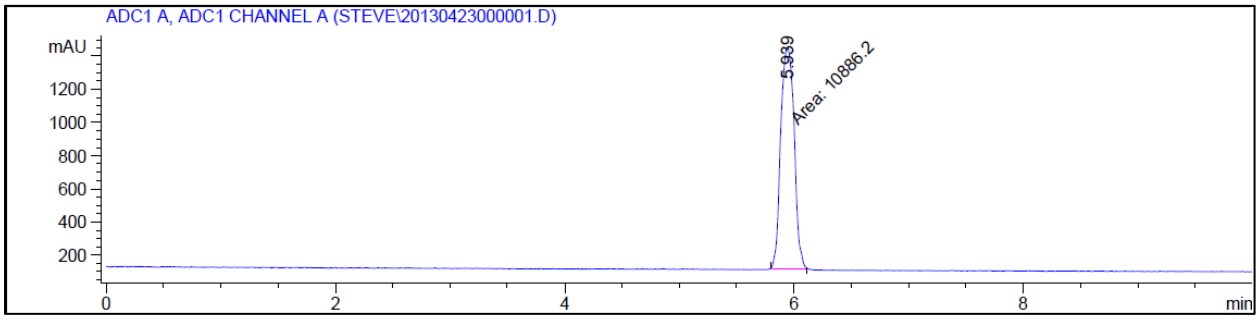


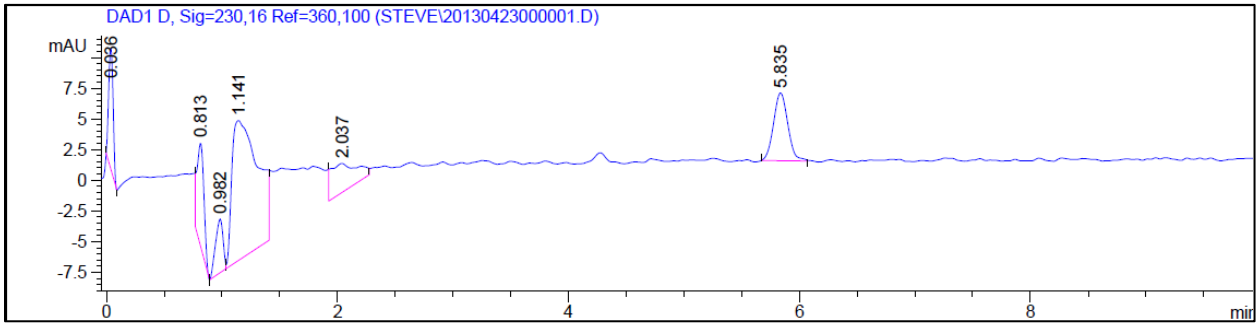
Semi-preparative HPLC



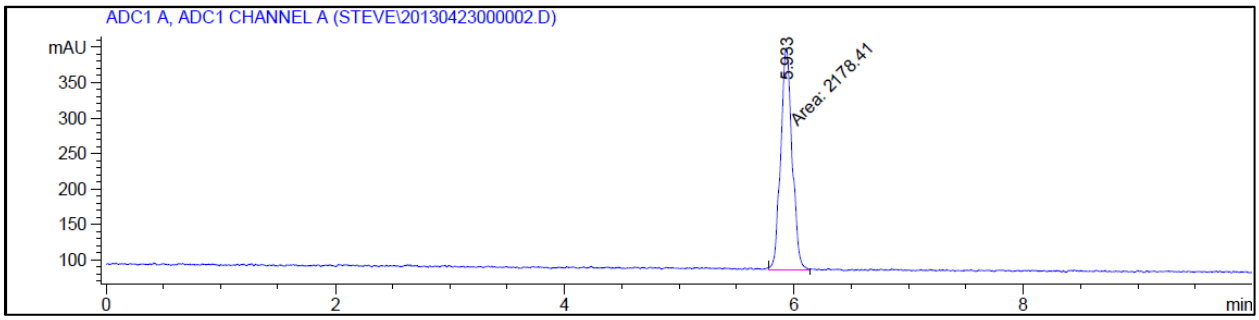


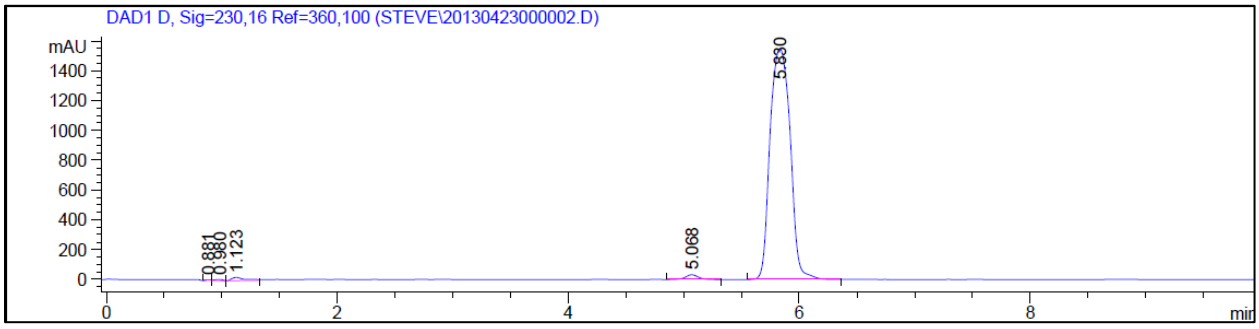
QC: Analytical HPLC of dose

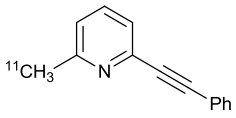




QC: Analytical HPLC of dose (coinjection)

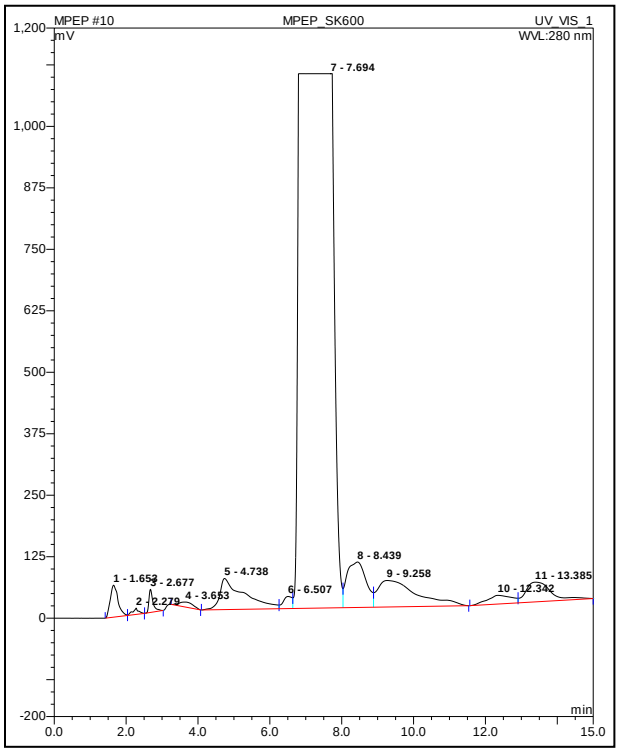


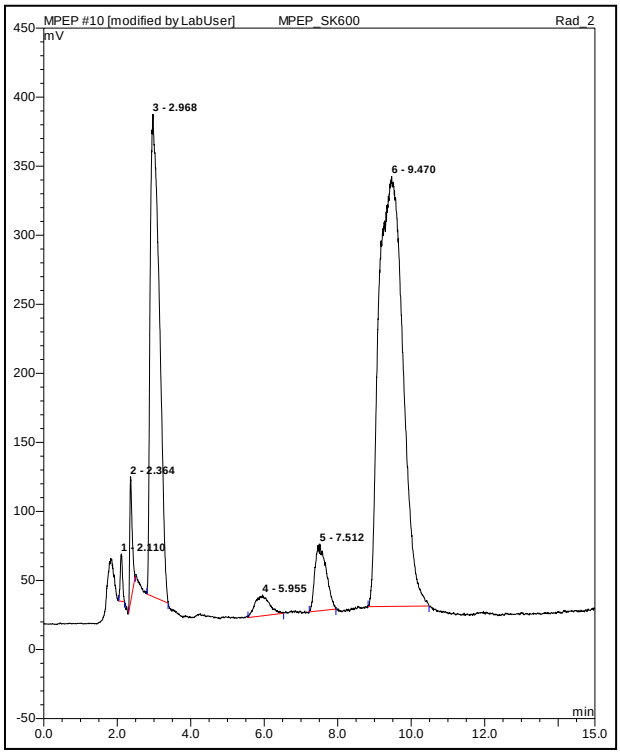




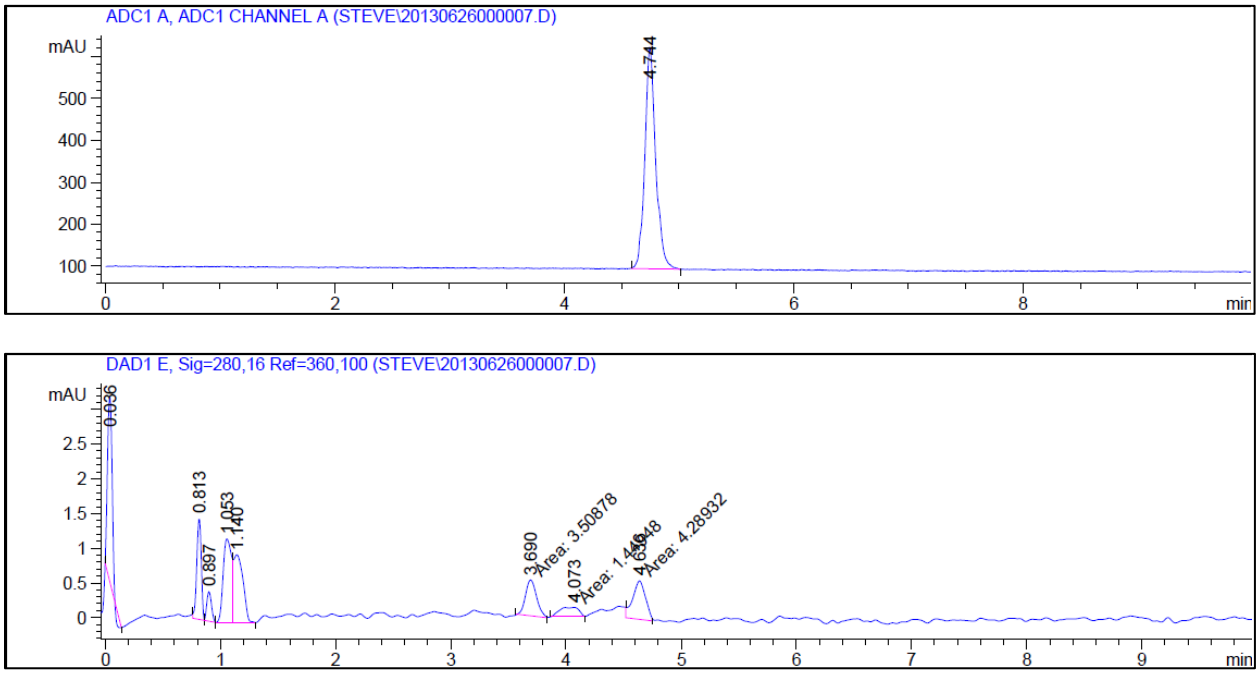
2.2.2 2-[^{11}C]methyl-6-(phenylethynyl)-pyridine ([^{11}C]MPEP)

Semi-preparative HPLC

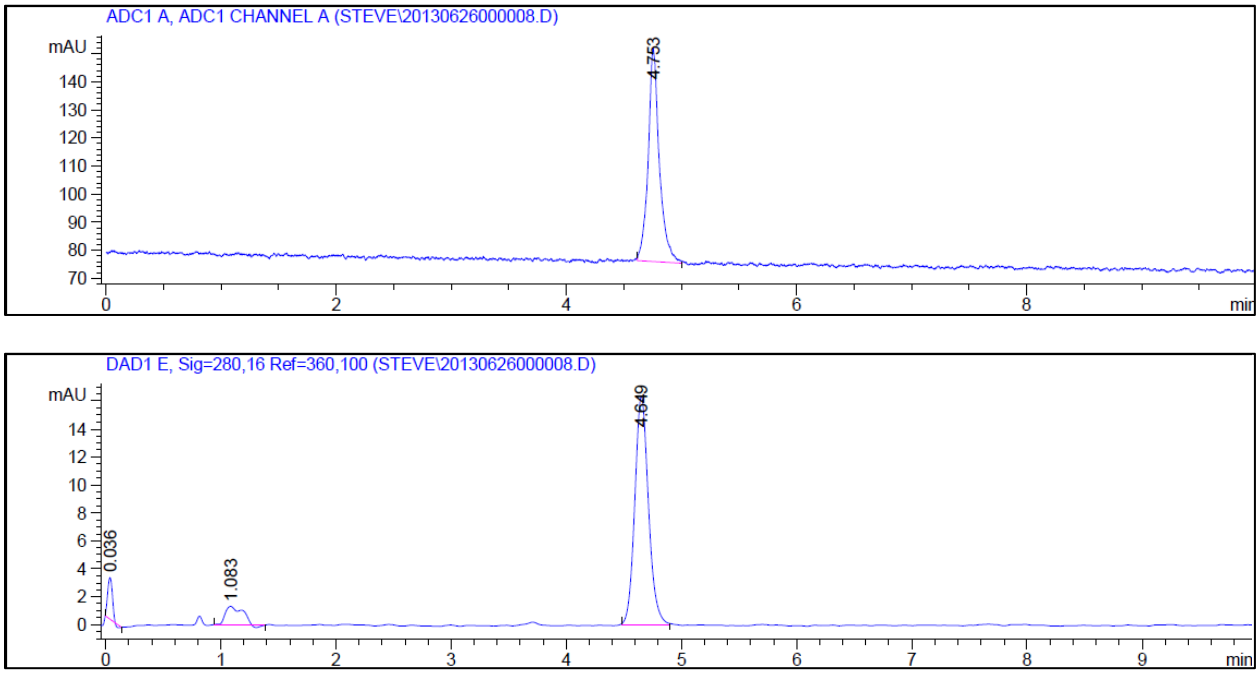




QC: Analytical HPLC of dose



QC: Analytical HPLC of dose (coinjection)



References

[1] M. Yu, W. Tueckmantel, X. Wang, A. Zhu, A. P. Kozikowski, A.-L. Brownell, *Nucl. Med. Biol.* 2005, *32*, 631-640.