nature portfolio

https://doi.org/10.1038/s41596-021-00584-4

Supplementary information

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Supplementary information

Translation of ^{11}C-Labelled Tracer Synthesis to a CGMP Environment as Exemplified by [^{11}C]ER176 for PET Imaging of Human TSPO

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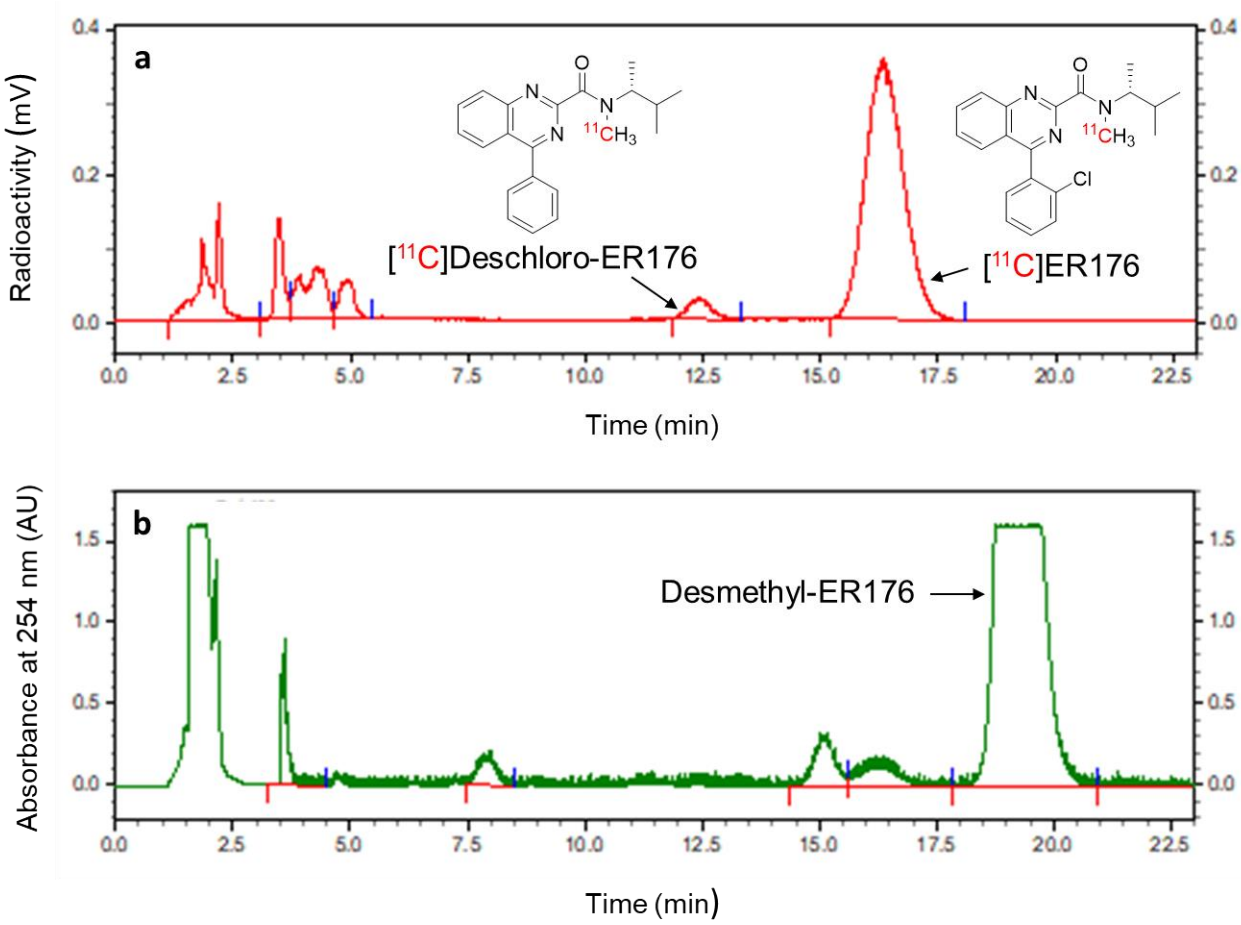
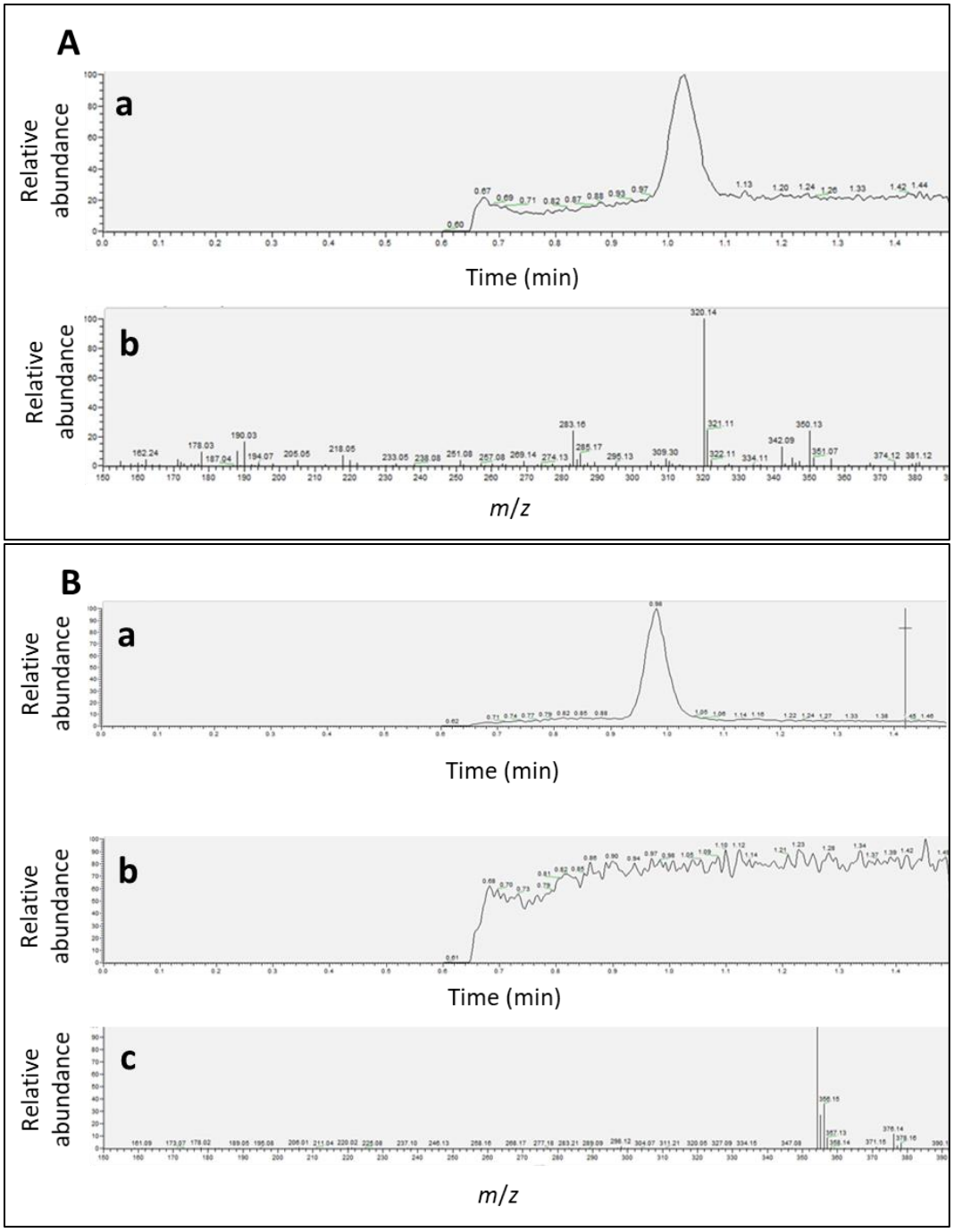
Method 1: Separation and identification of [^{11}C]deschloro-ER176

We investigated whether dechlorination of desmethyl-ER176 occurred during methylation with [^{11}C]iodomethane when using solid potassium hydroxide as base. Desmethyl-ER176 (0.62 mg) was weighed out into a conical vial and DMSO (400 μL) was added to completely dissolve the precursor. This solution was transferred to a vial containing freshly ground potassium hydroxide (1.23 mg). No-carrier-added [^{11}C]iodomethane (30 GBq) was bubbled into the solution in a stream of helium (20 mL/min) at room temperature for about 2 min until the radioactivity reached a maximum. The reaction vial was then moved to an oven using a ASPEC XL robotic arm (Gilson). The vial was heated in the oven at 80 °C for 5 min, after which water (0.5 mL) was added to the reaction mixture. Then the entire solution was pulled up into a transfer loop (1/8 in OD; 1/16 in ID) through the needle of a Gilson syringe pump 402 controlled remotely by software running on the Windows XP PC. The aliquot was injected into a 5-mL injection loop for purification on a Gold HPLC system (Beckman) using an XTerra RP18 column (7.8 mm ID × 300 mm; 10 μm; Waters) eluted with MeCN-aq.1 mM NH_4OH (37: 63 v/v) at 6 mL/min (Supplementary Fig. 1). Eluate was monitored sequentially for radioactivity and absorbance at 254 nm. The HPLC fraction eluting between 12 and 13 min (3% of the total radioactivity as estimated from the HPLC chromatogram) was collected, and a sample was analyzed with LC-MS. The $[M+H]^+$ ion (*m*/*z* 320) for carrier deschloro-ER176 was extracted from the total ion chromatogram (Supplementary Fig. 2A). Likewise, the product fraction containing [^{11}C]ER176, which eluted between 15.5 and 17.5 min (67% of the total radioactivity) was collected and analyzed with LC-MS. The $[M+H]^+$ ion (*m*/*z* 354) for carrier ER176 was extracted from the total ion chromatogram (Supplementary Fig. 2B, panel **a**). To determine whether there was any contamination of carrier ER176 with carrier deschloro-ER176, the *m*/*z* 320 ion was extracted from the total ion chromatogram (Supplementary Fig. 2B, panel b). Only baseline noise was observed, showing that the collected [^{11}C]ER176 fraction was not contaminated by [^{11}C]deschloro-ER176.



Supplementary Fig. 1 | Preparative radio-HPLC chromatogram. Panel **a:** Radioactivity channel. Panel **b**: UV absorbance channel. The HPLC method is described in the text. [^{11}C]Deschloro-ER176 and [^{11}C]ER176 were well separated.



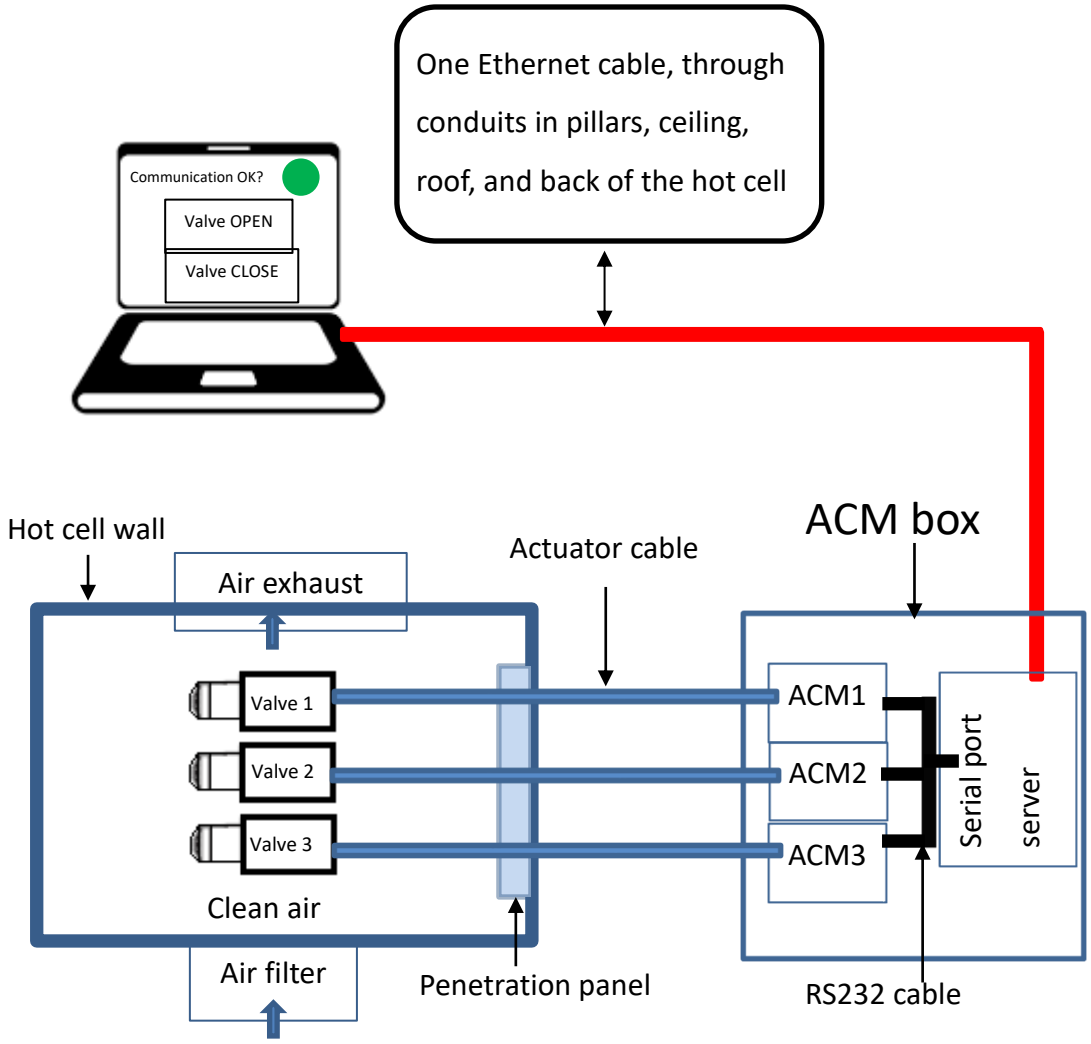
Supplementary Fig. 2 | Identification of deschloro-ER176 and ER176. **A**: LC-MS of the HPLC fraction collected between 12 and 13 min (Supplementary Fig. 1). *m/z* 320 is the $[M+H]^+$ ion from deschloro-ER176 carrier (**b**) extracted from the total ion chromatogram (**a**). **B**: LC-MS of the HPLC fraction collected between 15.5 and 17.5 min (Supplementary Fig. 1). *m/z* 354 peak is the $[M+H]^+$ ion from the carrier associated with [^{11}C]ER176 (**c**) extracted from the total ion chromatogram for the fraction collected between 15 and 17.5 min from HPLC (**a**). The middle panel (**b**) shows absence of the *m/z* 320 peak for the $[M+H]^+$ ion from deschloro-ER176 carrier.

Method 2: Detailed layout of the [^{11}C]ER176 production apparatus

As a complement to Fig. 5 in the main text, Extended Data Figure 1 details the layout of apparatus within the CGMP hot-cell, and in particular identifies the physical inter-relationships of valves, reservoirs and other equipment making up this apparatus, as referred to in the main text.

Method 3: Control of hot-cell radioactive gas distribution

In a non-CGMP laboratory, all cables can be passed into a hot-cell, through penetrations wherever needed. However, to maintain air cleanliness inside a CGMP laboratory hot-cell, all electrical cables must enter through sealed penetrations. The cable distance from the operator desktop to the actuator controller modules (ACMs) for the valves turns out to be about 30 feet. This is because cables must be routed up the walls, over the ceiling, and down the rear of the hot-cell. Use of one manual controller for each ACM would require many ribbon cables to run in conduits from the operator's desk to the hot-cell. Instead of running so many unnecessary cables and cluttering the desks with manual controllers, we installed a serial port server close to the valves themselves to handle all ACMs. With this arrangement, we only needed one Ethernet cable (CAT6a) from the user computer to a box having all the ACMs together in one instrument chassis (ACM box) for easy maintenance and inspection (see Supplementary Fig. 3). From this ACM cluster, all actuator cables to the valves ran through sealed penetration panels into the hot-cells. We placed a serial port server (see main text: Materials) inside the ACM box. Short 9 pin RS232 cables were used to interconnect individual ACMs. A graphical programming language, LabVIEW, was used to write to and read from the microcontrollers in ACMs, i.e. to send command and read the response of ACMs. Any generic high-level modern programming language is capable of handling a communication port program.



Supplementary Fig. 3 | Control of hot-cell radioactive gas distribution in CGMP environment. An actuator controller module (ACM) for each valve communicated via a serial port server from the host computer. Actuator cables pass through a sealed penetration panel as required to maintain clean air.

Method 4: Demonstration of no racemization during methylation of desmethyl-ER176

Validation of polarimeter with sucrose standard

The polarimeter (Model P-1010; Jasco) uses the wavelength of the sodium D line (589 nm). The instrument was zeroed with deionized water. Sucrose powder (0.1228 g; ultra pure, ≥ 99%, MP Biomedicals, LLC, cat. no. 802536) was weighed out with a calibrated balance (Sartorius) and dissolved in water up to the mark of a 10-mL volumetric flask (final concentration 1.228 g/100

mL). The optical rotation measured for this sucrose solution (α degrees) was converted into specific rotation according to Equation 1.

$$\alpha_D^T = \frac{100*\alpha}{l*C} \qquad \text{(Equation 1)}$$

where T = Temperature in ºC, l = length in decimeter, C = concentration in g/100 mL.

For sucrose at a concentration of 1.228 g /100 mL, with l set at 1 decimeter, α was found to be 0.8165 ± 0.0011° (n = 10) at 24.1 °C, from which the specific rotation of sucrose was calculated to be 66.45 ± 0.11° (n = 10) (lit.[1] 66.5°):

Measurement of the specific rotation of desmethyl-ER176

Desmethyl-ER176 powder (9.26 mg) was dissolved in 1.5 mL of chloroform (Sigma-Aldrich, cat no. 372978-100 ML). The polarimeter was zeroed with blank solvent ($CHCl_3$). The optical rotation of desmethyl-ER176 was measured at 1 second intervals at a concentration of 0.6173 g/100 mL with l set at 1 decimeter, giving α to be –0.0587 ± 0.00064° (n = 10; mean ± SD) at 24.1 °C, from which the specific rotation was calculated to be –9.5086 ± 0.104°. This value is very close to the specific rotation reported by our supplier (Prof. Da Settimo, Università di Pisa) of this compound (–9.74 ± 0.08°). The relative difference is 2.4%.

Measurement of the specific rotation of desmethyl-ER176 after treatment with base

The radiochemistry proposed for producing [^{11}C]ER176 in the CGMP laboratory was methylation of desmethyl-ER176 with [^{11}C]iodomethane at room temperature in 80 µL of DMSO within a 2-mL stainless steel HPLC injection loop in the presence of one equivalent of potassium tertiary butoxide. Therefore, it was investigated whether any racemization of precursor occurred during this procedure.

Desmethyl-ER176 powder (20.04 mg; 58.8 µmol) was dissolved in DMSO (800 µL). Potassium tertiary butoxide in THF (1 M; 59 µL) was added to this solution which was left to stand at room temperature for 14 minutes. The reaction was quenched with deionized water (20 mL) and then extracted twice with ethyl acetate (20 mL). The organic layers were combined and dried ($MgSO_4$). After removal of solvent and drying under high vacuum overnight, 16.32 mg of compound was recovered. The material was dissolved in chloroform (1 mL) and the optical rotation was measured again to calculate the specific rotation. The measured optical rotation for a concentration of 1.6320 g/100 mL with l set to 0.5 decimeter was –0.0777 ± 0.0035° (n = 10) at

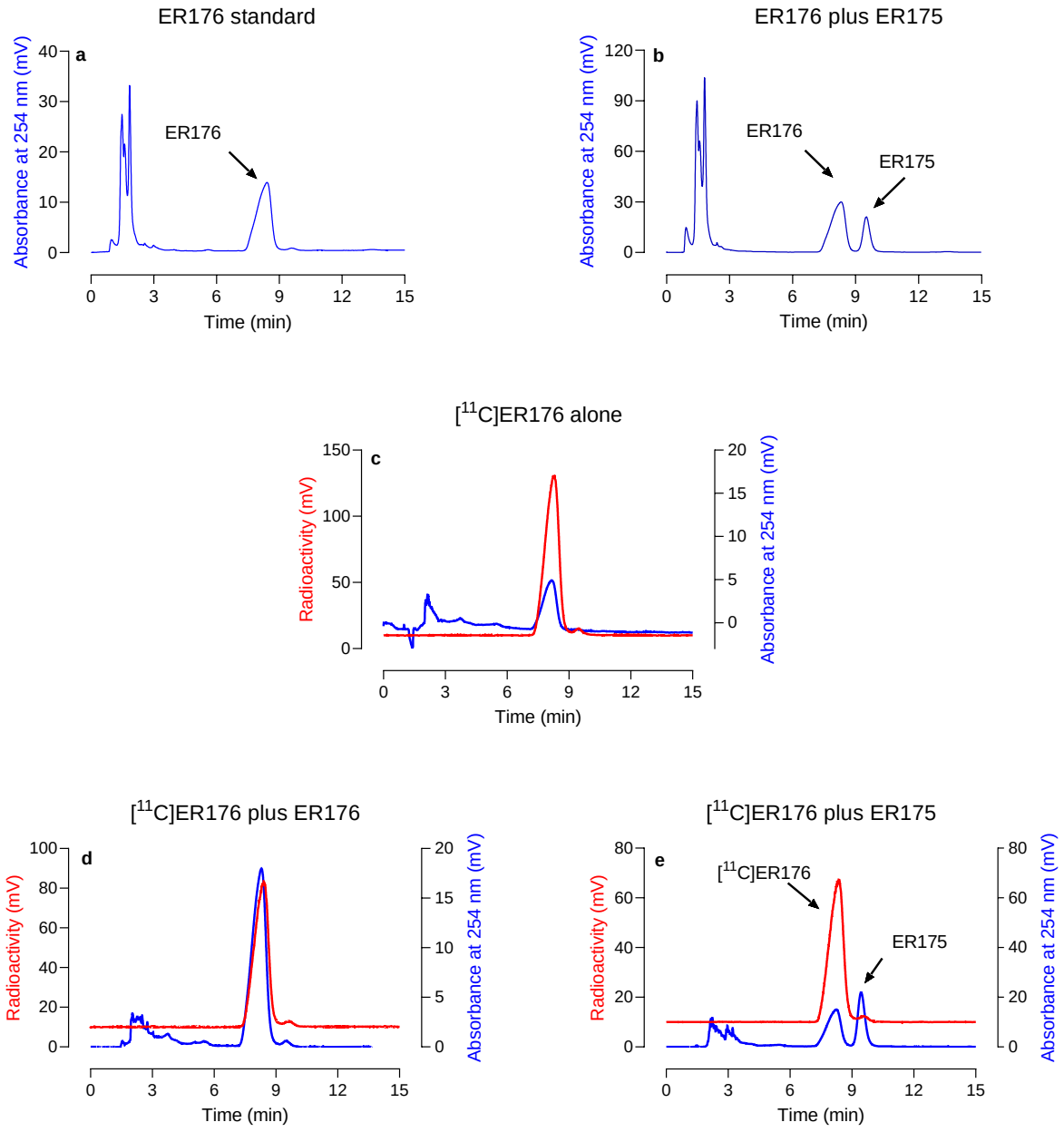
23.5 °C, corresponding to a specific rotation of –9.52 ± 0.21°. This value is almost identical to that of the starting material (–9.51 ± 0.10°). Therefore, desmethyl-ER176 does not undergo racemization under the basic methylation conditions selected for producing [^{11}C]ER176 in the CGMP laboratory.

Reference

1. Handbook of Chemistry and Physics, 42nd ed., 1960–1961, p 1784, The Chemical Rubber Co., Ohio, U.S.A.).

Method 5: Chiral HPLC of [^{11}C]ER176, ER176, and its enantiomer (ER175)

Samples of ER176 (~ 0.09 mg) and its enantiomer ER175 (~ 0.34 mg) were each dissolved in ethanol-hexane (35: 75 v/v; 5 mL). A sample (30 μL) of each solution was analyzed by injection onto an (*S*,*S*)-Whelk-O1 column (4.6 mm × 250 mm, 5 μm; Regis Technologies Inc.) eluted with ethanol-hexane (35: 75, v/v) at 2 mL/min, with eluate monitored for absorbance at 254 nm (Supplementary Figs. 4a). An aliquot (10 μL) of ER175 solution was added to an aliquot (40 μL) of ER176 solution and the sample (50 μL) was injected onto the chiral column to show separation of enantiomers (Supplementary Fig. 4b). A sample of [^{11}C]ER176 solution in HPLC mobile phase, when injected onto the same HPLC system having a radioactivity detector immediately after the absorbance detector, showed the same retention time as ER176 and evidence of only 3% contamination by enantiomer (Supplementary Fig. 4c). This result was confirmed by analysis of a same sample of the [^{11}C]ER176 spiked with reference ER176 (Supplementary Fig. 4d) and by analysis of the [^{11}C]ER176 spiked with reference ER175 (Supplementary Fig. 4e). The small amount of enantiomer impurity in the [^{11}C]ER176 almost certainly derives from enantiomer impurity in the precursor, desmethyl-ER176. This impurity may itself derive from enantiomer jmpurity in the (*R*)-*sec*-butylamine used in its synthesis. The enantiomers of desmethyl-ER176 could not be separated with baseline resolution in this chiral chromatography method.



Supplementary Fig. 4 | Chiral HPLC of [^{11}C]ER176, ER176, and its enantiomer (ER175). Panel **a**: reference ER176. Panel **b**: mixture of reference ER176 and ER175 (enantiomer of ER176). Panel **c**: [^{11}C]ER176 alone. Panel **d**: [^{11}C]ER176 spiked with ER176. Panel **e**: [^{11}C]ER176 spiked with ER175.

Method 6: Calibration of GC response for ethanol and acetonitrile

Gas chromatography (GC) calibration curves are used to calculate the amounts of ethanol and acetonitrile in the final formulated [^{11}C]ER176. Construction of the calibration curves requires reference solutions of acetonitrile and ethanol as well as a solution of internal standard (propionitrile) for accurate quantification.

An analytical balance is checked for accuracy with 10 mg and 1.00000 g certified standards. The accuracy of pipettes (1-mL and 50-µL) and of a Hamilton syringe (200-µL) must be verified before use. For example, for the 1-mL pipette, dispense 1 mL of HPLC from the pipette into a weighed vial. Weigh the amount of water dispensed into the vial. Perform the operation three times to see if all the measured weights fall within specification (assuming water density of 1.00 g/mL).

Serial dilutions are made to make accurate internal standard solutions of propionitrile, ethanol and acetonitrile. An aliquot (200 µL) of propionitrile is accurately weighed into a sealed vial and then diluted with water in a 10-mL volumetric flask to make a stock solution (~ 17,500 ppm). An aliquot of this propionitrile stock solution (200 µL) is diluted with water in a 10-mL volumetric flask to give an internal standard of about 350 ppm. Initial measurement of the material on the analytical balance ensures the accuracy of the measurement. (Measurement with a pipette has a much larger error than with an analytical balance). Acetonitrile standard and ethanol standard solutions are prepared similarly, as follows.

An aliquot of acetonitrile (~ 127 µL) is weighed and transferred to a 100-mL volumetric flask. Water is added to the mark. The concentration of acetonitrile in this stock solution is about 1 mg/mL Using five 10-mL volumetric flasks the stock solution is diluted as shown in Supplementary Table 1. Stock and diluted solutions of ethanol are prepared likewise (Supplementary Table 1). When stock solutions are transferred to the 10 mL volumetric flask, the 1.5-mL vials are thoroughly rinsed for quantitative transfer. Additionally, a sixth solution is prepared for system suitability testing. The system suitability standard is injected onto the gas chromatograph to check for proper function before injecting the sample from the final product for human subject. System suitability injections are done using the same method as for quality control.

Supplementary Table 1 | Volumes used for preparation of the acetonitrile and ethanol standard solutions and for suitability testing.

Solution	Acetonitrile stock solution (mL)	Ethanol stock solution (mL)
Level 5	5.0	1.4
Level 4	2.5	1.0
Level 3	1.25	0.8
Level 2	0.50	0.6
Level 1	0.25	0.4
System suitability	5.0	1.0

When the gas chromatograph is not in use, a default method is downloaded to maintain the column at 150 ºC. For analysis of the sample, the column temperature is held at 50˚C for 1 min, and then increased to 150 ˚C at a rate of 20 ˚C/min. The temperature is held at this temperature for 0.5 min, and then increased to 220 ˚C at a rate of 50 ˚C/min. After 3 min, the column temperature is returned to the starting temperature of 50 ˚C.

The flame ionization detector (FID) is supplied with hydrogen at a flow rate of 40 mL/min and air at a flow rate of 450 mL/min. Make up flow of helium is set to 45 mL/min and the detector is operated at 250 ˚C. The needle and syringe are rinsed with deionized water four times before direct injection of a sample and two times after injection. The equipment we use for determining residual solvents content in the final dose vial is listed in the main manuscript. The instrument parameters are summarized in Supplementary Table 2.

Supplementary Table 2 | GC method parameters.

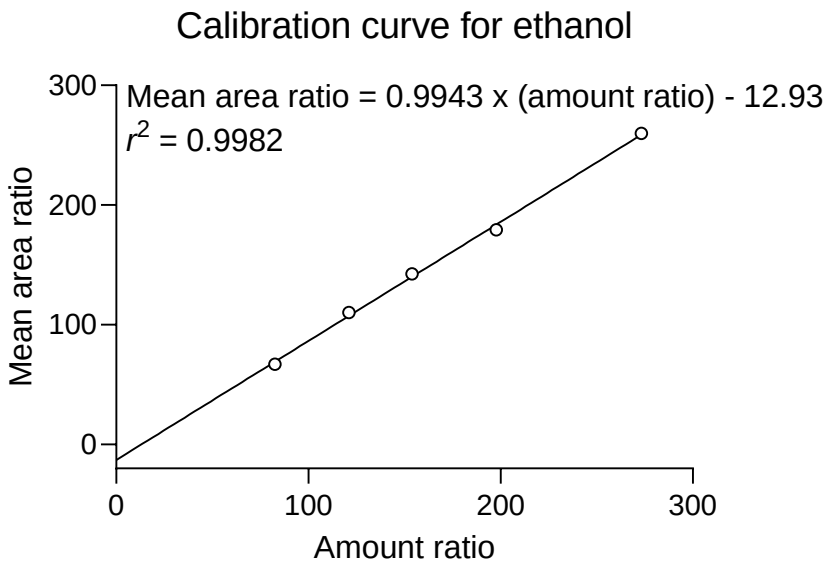
Injection port temperature	250 °C
Split ratio	20:1
Helium carrier gas flow rate	2 mL/min
Air at a flow rate	350 mL/min
H_2 flow rate	40 mL/min
Make up flow rate of He	45 mL/min
FID detector temperature	250 °C

For construction of the calibration curve, 50 µL of each calibration level standard is added to 50 µL of internal standard and vortexed to mix thoroughly. Five replicate direct injections of 1 µL from each level are made using an autosampler. The peak area ratios of acetonitrile to propionitrile (the internal standard) and that of ethanol to internal standard are calculated and

plotted with the instrument software (Agilent Chemstation). For a calibration curve to be valid, %RSD values for each level must be ≤ 5%, the tailing factor of the propionitrile peak ≤ 2, and the resolution between acetonitrile and propionitrile ≥ 3.5. Periodic validation using triplicate direct injections of the system suitability standard is recommended every six months, and a new curve using new standard solutions should be generated every two years. Supplementary Table 3 shows a sample data set for ethanol. In this example, the internal standard (ITSD) concentration was 392.32 ppm. By linear regression of the calibration curve not forced to go through the origin (Supplementary Fig. 5), we obtain the equation Area ratio = (0.9943 × amount ratio) –12.93 with r^2 = 0.9982.

Supplementary Table 3 | Calibration data for ethanol.

Ethanol (ppm)	Peak area	Amount ratio	Mean area ratio	%RSD of area ratio
32431.00	10177.78	82.66	67.07	1.09
47472.00	16139.15	121.00	110.20	1.85
60397.00	20287.87	153.95	142.65	1.51
77580.00	25763.76	197.74	179.40	1.89
107205.00	36625.80	273.26	259.80	0.37

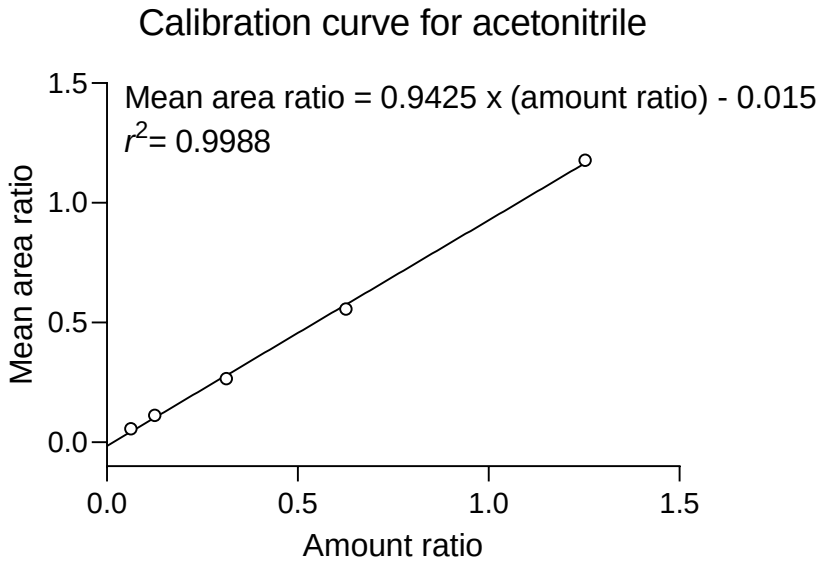


Supplementary Fig. 5| Constructed calibration curve for ethanol. Area ratios of ethanol peak to internal standard (propionitrile) peak in each level were plotted against the amount ratios of ethanol to propionitrile. Both X and Y axes are unitless.

A calibration table for acetonitrile is prepared identically from 25 direct injections of standard solutions. An example of acquired data is given below (Supplementary Table 4). In all five levels, %RSDs of the ratio of the area of acetonitrile to ITSD were less than 2%, which confirmed the precision of this method. Supplementary Fig. 6 shows the corresponding calibration curve. For the line not going through the origin, $r^2 = 0.9988$. The calculated detection limit of this method is 16.41 ppm using the equation specified by the International Council Harmonization guideline: detection limit = (3.3 × standard deviation of Y intercept)/slope.

Supplementary Table 4 | Calibration data for acetonitrile

Acetonitrile (ppm)	Peak area	Amount ratio	Mean area ratio	%RSD of area ratio
24.59	8.52	0.06266	0.05623	1.12
49.17	16.53	0.12533	0.11284	1.62
122.93	37.80	0.31332	0.26574	0.82
245.85	79.86	0.62665	0.55602	1.31
491.70	165.91	1.25330	1.17790	0.20



Supplementary Fig. 6 | Constructed calibration curve for acetonitrile. Area ratios of acetonitrile peak to internal standard (propionitrile) peak in each level were plotted against the amount ratios of acetonitrile to propionitrile.. Both X and Y axes are unitless.

The amounts of acetonitrile and ethanol present in each batch of formulated [^{11}C]ER176 are

calculated using the currently valid calibration curve. To the 50 µL sample from the final product vial, 50 µL of the internal standard is added using a pipette, and 1 µL of sample injected directly. The peak area ratios of solvents (MeCN, EtOH) to the internal standard are calculated. These ratios are converted into the concentrations using the established calibration curves and the concentrations are reported as ng/µL.

Method 7: SOP for operation and maintenance of TRACERlab FX C Pro

1. PURPOSE

To describe the setup and basic operation of the TRACERlab FX C Pro synthesis module and software.

2. RESPONSIBILITY

Production personnel.

3. EQUIPMENT & MATERIALS

3.1. TRACERlab FX C Pro synthesis module.

3.2. Computer with TRACERlab software.

3.3. TRACERlab FX C Pro Service Manual (DIRECTION 23685618-100), REV 3.

4. PROCEDURE

4.1. Operation

4.1.1. System is operated from a computer running the TRACERlab software.

4.1.2. Software has two modes of operation; manual mode and programmed methods/time-lists.

4.1.3. Manual mode – allows manual operation of valves, ovens and other instrument components. Typically used for routine system checks, method development and diagnostics/maintenance. Should not be used for production of PET drug products.

4.1.3.1. Enter manual mode by clicking the icon with the image of a yellow hand. Software instrument diagram will display current status of all sensors and valves.

4.1.3.2. Valves are colored red in the closed position and green in the open position. Valves and other system components are operated by clicking on the component icon in the instrument diagram.

4.1.3.3. Reactor temperatures and [^{11}C]methane trap temperature are set by clicking the set point box and entering the required temperature.

4.1.4. Methods and time lists – runs a pre-programmed list of commands. Should be used for routine cleaning processes and for production of PET drug products.

4.1.4.1. Methods are composed of up to four individual time lists. Time lists may be used in multiple methods.

4.1.4.2. Selecting "Methods >> Methods and Time Lists" will bring up a window with five tabs:

4.1.4.2.1. Methods: creation of new methods, selection of component time lists.

4.1.4.2.2. Time List Editor: editing of individual time lists.

4.1.4.2.3. Preparation: editing of preparation checklist to be displayed prior to running of method.

4.1.4.2.4. Recording and Regions: selection of data channels to be monitored (e.g., reactor temperatures and pressures, HPLC signals).

4.1.4.2.5. Special Methods: definition of methods for general procedures (not typically utilized).

4.1.4.3. Methods are run by selecting the method name from the drop-down box in the upper left corner of the software screen and selecting "Synthesis >> Start". Data specified in the method setup will be recorded and accessible from the Synthesis Log.

4.1.4.4. Individual time lists may be run by selecting the time list name from the drop-down box in the upper right corner of the screen and clicking "Run Time List". Run data will not be saved in the Synthesis Log.

4.1.4.5. Before running any method or time list, check pressures of all gases. Refer to SOP Table 1 for operating pressures.

SOP Table 1. Operating pressures of all gases

Gas	Air	Helium for liquid nitrogen (He1)	Helium for transferring $^{11}CO_2$ (He2)	Hydrogen
Pressure, (bar)	6.5	0.5	1.0	2.0

4.1.4.6. Before running any method or time list utilizing the vacuum pump, ensure the

vacuum trap is sufficiently empty and the Dewar flask is sufficiently filled with a dry ice/isopropanol slurry.

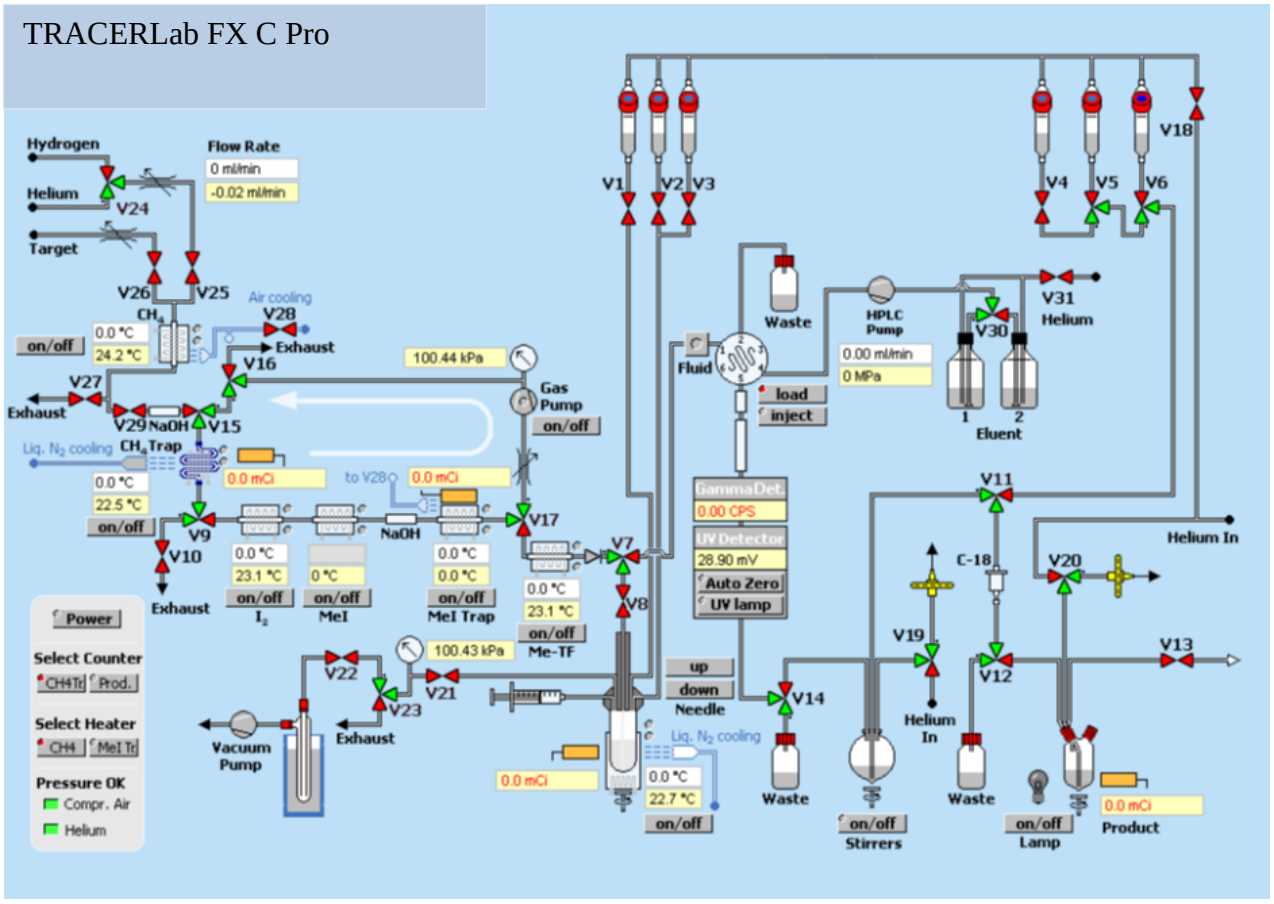
4.2. Cleaning

4.2.1. Cleaning process between syntheses of a single PET drug product consists of running the “Cleaning” method to rinse the formulation system.

4.2.1.1. Add 200 proof ethanol to V4 (10-mL), V5 (2-mL), V6 (10-mL), and the round bottom flask (40-mL).

4.2.1.2. Remove any used cartridges and connect all waste lines and the product line to waste bottles.

4.2.1.3. Program will rinse the formulation system and dry the product outlet line.



SOP Figure 1. Graphical User Interface of TRACERlab FX C Pro.

4.3. Setup and System Checks

4.3.1. Leak test

4.3.1.1. Running "Leak Test ver 3" will test the leak in the FXC Pro box sequentially from V25, V26, V15, V9, V10, V17, V16. The leak rate in the recirculation loop shall be measured by observing the pressure drop over a short period of time (3 min) when the recirculation loop is pressurized and isolated with the valves (V15, V 9, V 17, and V16) from the rest of the system. The pressure should not decrease significantly and only a small decrease (typically, 1.2 kPa) will occur. If a pressure drop of more than 5 kPa occurs, maintenance service shall be performed.

4.3.2. Cleaning and purging the [^{11}C]iodomethane production system.

4.3.2.1. From the drop down list under the main menu bar, select a method called "Preparation for NIMH MeI" and enter the file name to be saved for recorded data. This will remove any residual [^{11}C]carbon dioxide and organic substance in the system to improve molar activity (specific activity).

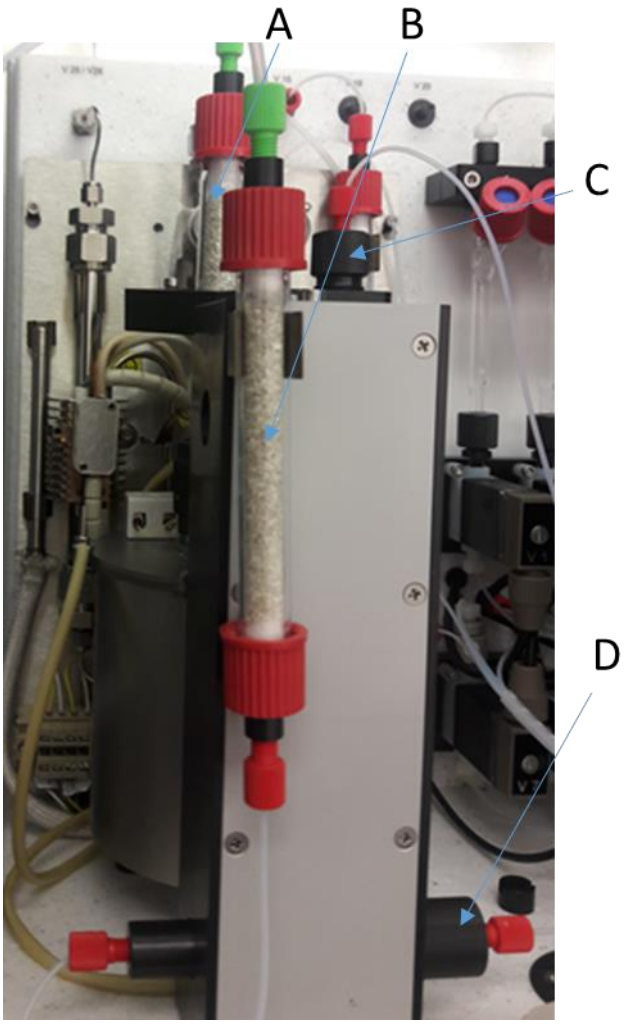
4.3.2.2. The program will heat the molecular sieve at 350 °C for 10 minutes while purging the [^{11}C]carbon dioxide trap with hydrogen and then heat [^{11}C]iodomethane trap at 190 °C while purging with helium through the recirculation path using the gas pump, and finally heat the [^{11}C]methane trap at 120 °C for 10 min.

4.3.3. Changing ascarite (sodium hydroxide) traps.

4.3.3.1. Approximately after every ten [^{11}C]iodomethane productions, radiochemists must change out the ascarite columns. Check the color of ascarite. If the band of yellow colored zone covers the entire ascarite column, ascarite must be changed out.

4.3.3.2. SOP Figure 2 shows two ascarite columns that must be changed out regularly. Remove them by hand by unscrewing them from the system. Remove the old ascarite material carefully with a stainless spatula and brush the inside surface with 10% Liquinox soap solution. Using copious amount of D.I. water, rinse out the Liquinox and rinse with acetone. Place the column in the oven at 100 °C and dry it completely. After cooling down the columns, fill each

column with ascarite. For the first (bigger) column (A in SOP Figure 2), leave the lower 50% of the column empty. In this empty space, sublimed iodine vapor from the iodine oven will be deposited during the run. Fill only the top 50% of the column with ascarite. Install quartz wool on both side of column to prevent any material from being displaced from the column. For the second column, fill column with ascarite and insert quartz wools (0.3 cm in height) on both ends of the column.



SOP Figure 2. Locations of the components in the TRACERlab FX C Pro.

A: First ascarite trap; B: Second ascarite trap; C: Inlet to the iodine reservoir; D: [^{11}C]iodomethane trap.

4.3.4. Checking the iodine level in the iodine reservoir.

4.3.4.1. Check the iodine level using the tool.

4.3.4.2. Open the inlet to the iodine reservoir by unscrewing the black nuts, C in SOP Figure 2. Insert the measuring tool to measure the iodine level. The measuring tool has markers for full and empty level of the iodine reservoir. Refill iodine using the funnel when necessary. Do not exceed the full level. While adding small iodine crystals, frequently measure the level of iodine such that iodine is not added excessively. Make sure that [^{11}C]iodomethane trap is inserted into the heated zone as shown in the picture. When the [^{11}C]iodomethane trap is displaced out of the oven, it cannot release [^{11}C]iodomethane from the trap.

4.3.5. Production of [^{11}C]iodomethane.

4.3.5.1. From the drop down list under the main menu bar, select a method called "MeI batch" which uses a time list, MeI batch NIH & GE V3. A tracer specific method also contains a time list for formulating the product. The time list for formulation is an instruction on how to concentrate the product on C-18 Sep-Pak cartridge and how to wash and elute the product with water, ethanol and saline through the sterile filter into a sterile vial.

4.4. Maintenance

4.4.1. The TRACERlab FX C Pro has several types of user-serviceable parts:

4.4.1.1. All valves, lines and fittings may be replaced on an as-needed basis. Valves should be replaced with identical parts and may be obtained from GE Healthcare or directly from the manufacturers, Burkert and Parker Hannifin. Lines and fittings should be replaced with identical or equivalent parts with the same level of chemical resistance.

4.4.1.2. Interface module cards may be replaced with identical cards obtained from GE Healthcare.

4.4.1.3. Computer may be replaced with an equivalent system, provided that the software may be successfully installed and operated.

4.4.1.4. The following critical chemicals in TRACERlab FX C Pro shall be changed out at least once every two years.

4.4.1.4.1. Molecular sieve and nickel in [^{11}C]methane oven

4.4.1.4.2. Porapak N in [^{11}C]iodomethane oven.

4.4.1.4.3. Carbosphere or Carboxen in methane cryogenic trap.

4.4.1.4.4. Iodine in iodine oven. After the complete removal of old iodine, clean the quartz tube thoroughly with acetone and dried in the 100 ºC oven.

4.4.2. The hardware components shall be serviced regularly.

4.4.2.1. Change out the Teflon tubes (1/16" O.D. and 0.02" I.D. or greater than 0.02" I.D.) and Burkert valves on the recirculation path annually.

4.4.2.2. Change out the O-ring (¼" O.D.; Duran, part no. 7624-42, GL18 for 5.5-6.5 mm tubing or equivalent) every six months on the outlet side of the spiral shaped quartz tube in the furnace. Make sure the Teflon side of the O-ring face the glassware containing ascarite. Change out the Teflon faced O-ring (5/8") on the other side of the this ascarite trap when damage is observed.

4.4.3. In the event of a major operational issue which cannot be resolved by the above steps, detecting leaks or clogs in the lines or valves, or restarting the equipment, contact the GE Healthcare for service.

5. FREQUENCY

5.1. Cleaning and system checks should be done in advance of each PET drug preparation as described.

5.2. Maintenance should be performed as needed.

6. ASSOCIATED FORMS

6.1. FE109: Equipment Maintenance Record.

Method 8: Time lists for TRACERlab FX C Pro program

1. Method ER176

1.1: Time list for step 1: [^{11}C]methane – oven conditioning

Time List: Methane prep 7/15/2017

Time		Device		Value	Dur.	Comme
0	Set	Power	=	On		2.0.7 2007-06-18
2	Set	V25	=	Open		
3	Set	V24	=	b (up)		H2 flow
3.2	Set	V27	=	Open		
5	Set	Flow Rate Set Point	=	50		
8	Set	Temp. Set Point CH4	=	350		
10	Wait	Temp. Reg. Status CH4	=	Temp. OK		
t1+0	Set	Process Control	=	Show message and wait		Stop Conditioning of Methane Oven with hydrogen?
t2+1	Set	V27	=	Close		
t2+2	Set	Temp. Reg. Switch CH4	=	Off		
t2+3	Set	V28	=	Open		
t2+4	Set	Process Control	=	STOP		

1. Time list for step 2: [^{11}C]iodomethane batch & GE V8 with He conditioning

Time List: MeI batch NIH & GE v8 with He Conditioning 12/10/2018

Time		Device		Value	Dur.	Comme
0	Set	Power	=	On		17 AUG 2018 JH
0.4	Set	Temp. Reg. Switch MeI	=	On		
0.6	Set	Flow Rate Set Point	=	50		
1	Set	V25	=	Open		
1.5	Set	HPLC UV Detetor Lamp	=	On		Turn on UV detector
2	Set	Select Counter	=	3A on, 3B off		methane trap counter
5	Set	V28	=	Open		
6	Set	V24	=	b (up)		H2 flow
6.7	Set	V24	=	a (down)		Select Helium
7.1	Set	Flow Rate Set Point	=	8		
7.2	Set	V27	=	Open		
38	Wait	Temp. CH4	<=	45		Waiting for CH4 trap to cool prior to activity
t1+1	Set	Process Control	=	Show message and wait		Inject the precursor and press 'Okay' to condition t
t2+1	Set	Flow Rate Set Point	=	0		
t2+2	Set	V27	=	Close		
t2+3	Set	V29	=	Open		
t2+3.1	Set	V15 CH4 Trap Input	=	b (left)		
t2+3.2	Set	V17	=	b (down)		
t2+3.3	Set	V09	=	b (right)		flip to the right
t2+3.4	Set	V08	=	Open		
t2+8	Set	Flow Rate Set Point	=	8		
t2+1'4	Set	Process Control	=	Show message and wait		Click Okay to finish the conditioning the loop.

t3+2	Set	V08	=	Close	
t3+3.1	Set	V24	=	b (up)	
t3+3.2	Set	V29	=	Close	
t3+3.3	Set	V15 CH4 Trap Input	=	a (right)	
t3+3.4	Set	V17	=	a (up)	
t3+4.5	Set	Flow Rate Set Point	=	50	
t3+10	Set	Temp. Set Point CH4 Trap	=	-75	
t3+12	Wait	Temp. Reg. Status CH4 Trap	=	Temp. OK	
t4+1	Set	Process Control	=	Show message and wait	Press 'Okay' when delivery of activity begins. Open
t5+1	Set	Temp. Set Point I2	=	100	
t5+2	Set	V25	=	Close	
t5+3	Set	V26	=	Open	C11 activity release
t5+5	Set	V27	=	Open	
t5+6	Set	Process Control	=	Show message and wait	Press 'Okay' when delivery of activity is complete.
t6+1	Set	V26	=	Close	C11activity stop
t6+2	Set	Flow Rate Set Point	=	100	
t6+5	Set	V25	=	Open	
t6+1'50	Set	V27	=	Close	
t6+1'55	Set	V28	=	Close	
t6+2'0	Set	V25	=	Close	
t6+2'5	Set	V24	=	a (down)	Switch to He
t6+2'6	Set	Temp. Set Point CH4	=	350	
t6+2'7	Wait	Temp. Reg. Status CH4	=	Temp. OK	
t7+2'20	Set	V10 Exhaust	=	Open	
t7+2'21	Set	V15 CH4 Trap Input	=	b (left)	
t7+2'22	Set	Temp. Set Point CH4 Trap	=	-75	
t7+2'23	Wait	Temp. Reg. Status CH4 Trap	=	Temp. OK	
t8+1	Set	Process Control	=	Start Region	CH4
t8+3	Set	V29	=	Open	
t8+4	Set	Flow Rate Set Point	=	100	
t8+5	Set	V25	=	Open	Purge and transport with He
t8+6	Set	V15 CH4 Trap Input	=	b (left)	
t8+7	Set	V09	=	a (left)	
t8+8	Set	V10 Exhaust	=	Open	

Time		Device		Value	Dur.	Comme
t8+49	Set	V10 Exhaust	=	Close		
t8+50	Set	V09	=	b (right)		
t8+55	Set	V16 Exhaust	=	b (up)		
t8+1'24	Set	V16 Exhaust	=	a (down)		
t8+1'30	Set	V15 CH4 Trap Input	=	a (right)		
t8+1'31	Set	Gas Pump	=	On		start recirculation
t8+1'32	Set	Temp. Set Point CH4 Trap	=	80		
t8+1'33	Set	V25	=	Close		
t8+1'34	Set	V29	=	Close		
t8+1'35	Set	Select Heater	=	3B on, 3A off		
t8+5'14	Set	Process Control	=	End Region		CH4
t8+6'15	Set	Gas Pump	=	Off		end RECirculation
t8+6'16	Set	Temp. Reg. Switch I2	=	Off		
t8+6'16	Set	Temp. Reg. Switch MeI	=	Off		
t8+6'16	Set	Temp. Reg. Switch CH4 Trap	=	Off		
t8+6'17	Set	V15 CH4 Trap Input	=	b (left)		
t8+6'18	Set	V29	=	Open		
t8+6'19	Set	V25	=	Open		
t8+6'20	Set	V16 Exhaust	=	b (up)		
t8+6'26	Set	Flow Rate Set Point	=	50		
t8+6'30	Set	Temp. Set Point MeI Trap	=	190		
t8+6'31	Set	Process Control	=	Start Region		MeI
t8+6'50	Set	Process Control	=	End Region		MeI
t8+6'57	Set	V16 Exhaust	=	b (up)		
t8+6'58	Set	V17	=	a (up)		
t8+6'59	Set	Process Control	=	Show message and wait		Press 'Okay' to release activity into the loop. Clic
t9+0	Set	V08	=	Open		
t9+0.2	Set	V16 Exhaust	=	a (down)		
t9+0.5	Set	V17	=	b (down)		
t9+1	Set	V21	=	Open		
t9+2	Set	V23	=	b (down)		
t9+3	Set	Flow Rate Set Point	=	20		
t9+10	Set	Process Control	=	Show message and wait		Press 'Okay' when activity trapped in the loop has m
t10+1	Set	V08	=	Close		
t10+2	Set	V17	=	a (up)		
t10+2.1	Set	V16 Exhaust	=	b (up)		
t10+3	Set	Process Control	=	Show message and wait		Prep 'Okay' after you start prep HPLC data acquisiti

1.3. Time list for step 3: ER176 formulation

Time List: ER176 formulation 12/10/2018

Time		Device		Value	Dur.	Comme
0.1	Set	Select Counter	=	3B on, 3A off		
0.5	Set	Power	=	On		
0.7	Set	Select Counter	=	3B on, 3A off		
1	Set	HPLC UV Detetor Lamp	=	On		
1.2	Set	Stirrer	=	On		start stirring
1.5	Set	V16 Exhaust	=	b (up)		
2	Set	HPLC UV Detector Auto Zero	=	On		
5	Wait	Chromatography Peak Detector	=	Start of Peak		Click the rising edge icon
t1+5	Wait	Chromatography Peak Detector	=	End of Peak		Click the falling edge icon.
t2+0.5	Set	V19	=	b (down)		Load product on C-18 Sepak.
t2+4'19	Set	Process Control	=	Show message and wait		Click Okay after the round bottom flask is emptied.
t3+1	Set	V11	=	b (right)		stop loading product on C-18 Sepak
t3+1.4	Set	V18	=	Open		
t3+1.8	Set	V04 Vial 4	=	Open		wash C-18 with water
t3+2	Set	V19	=	a (up)		He push gas off
t3+51.1	Set	V12	=	b (right)		product goes to the mixing vial.
t3+51.2	Set	Lamp FDG1	=	On		turn on the lamp
t3+52	Set	V04 Vial 4	=	Close		finished with washing with water
t3+53	Set	V05 Vial 5	=	b (up)		elute product from C-18 with EtOH
t3+1'6	Set	V05 Vial 5	=	a (down)		
t3+1'7	Set	V06 Vial 6	=	b (up)		Elute with Saline
t3+1'11	Set	Process Control	=	Start Region		PROD
t3+1'41	Set	V06 Vial 6	=	a (down)		stop eluting with Saline
t3+1'42	Set	V11	=	a (left)		
t3+1'42	Set	V12	=	a (left)		
t3+1'42	Set	V18	=	Close		
t3+1'42	Set	V20	=	b (left)		He push gas on
t3+1'42	Set	Process Control	=	End Region		PROD
t3+1'42	Set	Stirrer	=	Off		
t3+1'43	Set	V13	=	Open		valve to sterile vial open
t3+2'29	Set	Load/Inject Valve	=	Load		
t3+2'30	Set	HPLC UV Detetor Lamp	=	Off		
t3+2'31	Set	Process Control	=	Show message and wait		Click Okay when transfer is complete.
t4+0	Set	Process Control	=	Reset		

2. Time list for cleaning

Time List: Cleaning 7/15/2017

Time		Device		Value	Dur.	Comme
0	Set	Process Control	=	Reset		
0.1	Set	V19	=	b (down)		cleaning reservoir bottle.
1	Set	Stirrer	=	On		Two stirrers are on
5'0	Set	V19	=	a (up)		
5'1	Set	V18	=	Open		
5'2	Set	V04 Vial 4	=	Open		Clean V4
5'2	Set	V11	=	b (right)		
6'30	Set	V04 Vial 4	=	Close		
6'30	Set	V05 Vial 5	=	b (up)		Clean V5
6'30	Set	V12	=	b (right)		
6'40	Set	V05 Vial 5	=	a (down)		
6'41	Set	V06 Vial 6	=	b (up)		Clean V6
7'40	Set	V06 Vial 6	=	a (down)		
7'40	Set	V20	=	b (left)		
7'40	Set	V13	=	Open		
12'40	Set	Process Control	=	Reset		reset

3. Time lists for the preparation of [^{11}C]iodomethane

3.1 Time List for Step 1: [^{11}C]carbon dioxide trap cleaning

Time List: CO2 trap cleaning v2 7/15/2017

Time		Device		Value	Dur.	Comme
0	Set	Power	=	On		2.0.7 2007-06-18
2	Set	V25	=	Open		
3	Set	V24	=	b (up)		H2 flow
3.2	Set	V27	=	Open		
5	Set	Flow Rate Set Point	=	50		
8	Set	Temp. Set Point CH4	=	350		
10	Wait	Temp. Reg. Status CH4	=	Temp. OK		
t1+2'0	Set	Temp. Set Point CH4	=	0		Stop Conditioning of Methane Oven with hydrogen?
t1+2'2	Set	V28	=	Open		air cooling
t1+2'3	Set	Power	=	Off		
t1+2'4	Set	Temp. Reg. Switch CH4	=	Off		
t1+2'5	Set	V27	=	Close		
t1+2'6	Set	V24	=	a (down)		selecting helium

3.2. Time list for Step 2: [^{11}C]methane and [^{11}C]iodomethane preparation

Time List: CH4 and MeI preparation NIMH 7/15/2017

Time		Device		Value	Dur.	Comme
0	Set	Power	=	On		2.0.7 2007-06-18
0.1	Set	Select Heater	=	3B on, 3A off		
0.2	Set	Temp. Reg. Switch MeI	=	On		Heating up MeI oven
1.2	Set	Temp. Set Point MeI Trap	=	190		Conditioning of MeI trap
1.3	Set	V09	=	b (right)		
1.4	Set	V15 CH4 Trap Input	=	b (left)		
1.5	Set	V25	=	Open		
1.6	Set	V29	=	Open		
1.7	Set	V16 Exhaust	=	b (up)		
1.8	Set	Flow Rate Set Point	=	30		
5'4	Set	Process Control	=	REPEAT LOOP	3	flush loop with He, especial tube connecting V16 and□
t1+0	Set	V16 Exhaust	=	a (down)		
t1+0.1	Set	V15 CH4 Trap Input	=	a (right)		
t1+0.2	Set	Gas Pump	=	On		
t1+10	Set	Gas Pump	=	Off		
t1+10.1	Set	V15 CH4 Trap Input	=	b (left)		
t1+10.2	Set	V16 Exhaust	=	b (up)		
t1+1'0	Set	Process Control	=	END LOOP		after 3 loops, t2=4'00
t2+0	Set	Temp. Reg. Switch MeI Trap	=	Off		Cooldown with He purge
t2+0	Set	V16 Exhaust	=	a (down)		Closing MeI circ.loop
t2+1	Set	Select Heater	=	3A on, 3B off		
t2+20	Set	V09	=	a (left)		
t2+20.5	Set	V10 Exhaust	=	Open		
t2+21	Set	Temp. Set Point CH4 Trap	=	120		Conditioning CH4 trap
t2+22	Wait	Temp. Reg. Status CH4 Trap	=	Temp. OK		
t3+10'22	Set	V10 Exhaust	=	Close		
t3+10'43	Set	Process Control	=	Reset		

4. Time list for leak check

Time List: Leak Test ver. 3 12/12/2017

Time		Device		Value	Dur.	Comme
0	Set	Power	=	On		
0.1	Set	V24	=	a (down)		Selecting Helium gas.
1	Set	V29	=	Open		
2	Set	V15 CH4 Trap Input	=	b (left)		
3	Set	V09	=	b (right)		
4	Set	V16 Exhaust	=	b (up)		
22	Set	V16 Exhaust	=	a (down)		
23	Set	V09	=	a (left)		
24	Set	V15 CH4 Trap Input	=	a (right)		
25	Set	V29	=	Close		
26	Set	Flow Rate Set Point	=	100		
33	Wait	Flow Rate	<=	1		
t1+0	Set	V25	=	Open		
t1+5	Wait	Flow Rate	<=	1		
t2+0	Set	V29	=	Open		
t2+5.2	Wait	Flow Rate	<=	1		
t3+0	Set	V15 CH4 Trap Input	=	b (left)		
t3+5.4	Wait	Flow Rate	<=	1		
t4+0	Set	V09	=	b (right)		
t4+5.1	Wait	Flow Rate	<=	1		
t5+20	Set	V25	=	Close		
t5+21	Set	Process Control	=	Show message and wait		Record loop pressure
t6+3'0	Set	Process Control	=	Show message and wait		Subtract from initial loop pressure reading and devi
t7+1	Set	V17	=	b (down)		
t7+1	Set	V25	=	Open		
t7+8	Wait	Flow Rate	<=	0.9		
t8+1	Set	V17	=	a (up)		
t8+2	Set	V09	=	a (left)		
t8+3	Set	V29	=	Close		
t8+4	Set	V25	=	Close		
t8+5	Set	V18	=	Open		
t8+6	Set	V01 Vial 1	=	Open		
t8+7	Set	V21	=	Open		
t8+7.5	Set	V08	=	Open		
t8+8	Set	V01 Vial 1	=	Close		
t8+19	Set	Process Control	=	Show message and wait		Record Beginning Reactor Pressure.
t9+3'0	Set	Process Control	=	Show message and wait		Record Ending Reactor Pressure and devide by 3 minut
t10+0.4	Set	V08	=	Close		
t10+4	Set	Process Control	=	Stop Synthesis		
t10+5	Set	Process Control	=	Reset		
t10+6	Set	V15 CH4 Trap Input	=	a (right)		
t10+9	Set	V18	=	Close		
t10+9.1	Set	Process Control	=	Show message and wait		Open V2 on the Autoloop. Measure the flow rate throg
t11+1.1	Set	Flow Rate Set Point	=	20		
t11+9.2	Set	V25	=	Open		
t11+9.3	Set	V29	=	Open		
t11+9.4	Set	V15 CH4 Trap Input	=	b (left)		
t11+9.5	Set	V09	=	b (right)		
t11+9.6	Set	V17	=	b (down)		
t11+9.7	Set	V07	=	a (left)		
t11+9.8	Set	V08	=	Open		
t11+5'10	Set	Process Control	=	Show message and wait		Record the flow rate. The flow should be within +/-
t12+0	Set	Process Control	=	Reset		

Method 9: Calibration of HPLC absorbance response for ER176

An analytical HPLC calibration curve for absorbance response versus mass of ER176 injectate is used to calculate molar activity and mass dose for each batch of [^{11}C]ER176. To construct a calibration curve, a stock solution of reference ER176 is prepared. The reference compound (2 to 3 mg) is weighed accurately into a 1.5-mL V-vial on a validated analytical balance. The compound is dissolved with anhydrous acetonitrile, and the solution is transferred into a 10-mL volumetric flask and diluted to the mark. This solution is mixed thoroughly and used to prepare a diluted (1: 100) reference solution in acetonitrile-water (1: 1, v/v) followed by three further serial dilutions (1: 1) to prepare calibration standards for injection onto HPLC. In all cases, dilution is made with acetonitrile-water (1: 1, v/v). A detailed example of solutions prepared for constructing a calibration curve is shown in Supplementary Table 5.

Supplementary Table 5| Preparation of the standards from the stock solution.

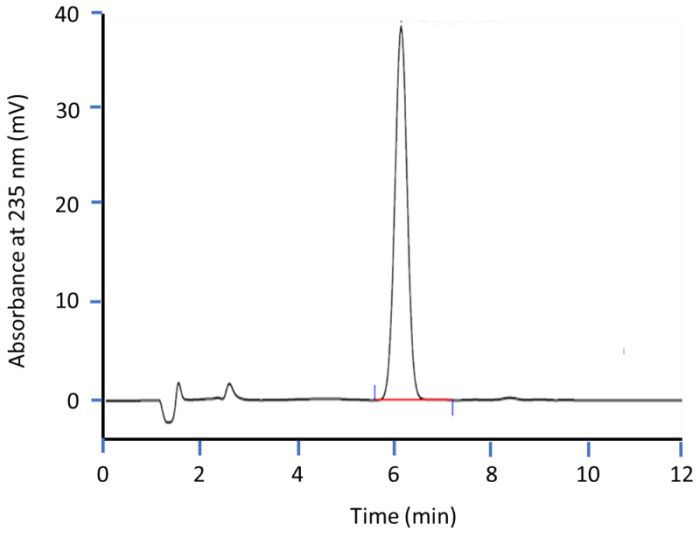
Solution	Preparation	Concentration (ng/µL)
ER176 stock	2.52 mg ER176 in 10-mL volumetric flask	252
ER176 for HPLC (Level 4)	1: 100 dilution of ER176 stock in 10-mL volumetric flask	2.52
Level 3	1: 1 dilution of 1 mL Level 4 using a calibrated pipette	1.26
Level 2	1: 1 dilution of 1 mL Level 3 using a calibrated pipette	0.63
Level 1	1: 1 dilution of 1 mL Level 2 using a calibrated pipette	0.315

The HPLC method used for quality control of the final product is used for constructing the calibration curve, namely use of a Luna C18(2) column (4.6 × 250 mm, 10 µm; Phenomenex), eluted with methanol-water (7: 3, v/v) at 2 mL/min. Eluate is monitored for absorbance at 235 nm (ER176, t_R = 6.3 min) (Supplementary Fig. 7). Five replicate injections of 100-µL samples at each dilution level of standard ER176 solution ware performed using an autosampler. The UV absorbance chromatograms are integrated to determine peak areas, and these values are plotted against injectate masses. The Y intercept of the fitted linear curve is set to zero, and the slope of the linear curve is obtained by linear regression. For the calibration curve to be valid, relative

standard deviations (RSDs) for peak areas for each level of sample dilution have to be ≤ 3%, and the coefficient of determination (r^2) greater than or equal to 0.98. A new curve is generated every six months using new diluted standard solutions. These are prepared from the original stock solution of ER176 if still adequately pure (i.e., > 95% by HPLC). The periodic curves are analyzed with only three replicate injections at each level, provided that RSD and r^2 met the acceptance criteria, as in Supplementary Table 6.

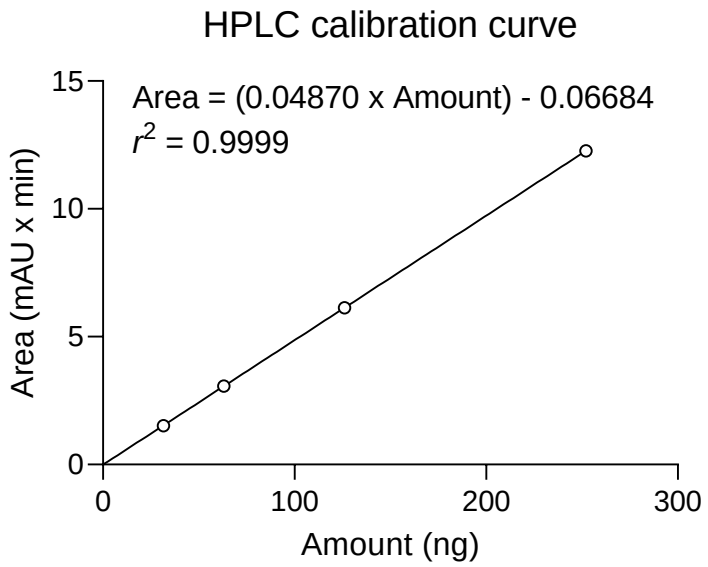
Supplementary Table 6| Example of calibration data for ER176.

Levels	Amount (ng)	Area (mAU x min)
1	31.5	1.5215
1	31.5	1.5176
1	31.5	1.5136
2	63	3.0927
2	63	3.0703
2	63	3.0582
3	126	6.1752
3	126	6.102
3	126	6.1011
4	252	12.3302
4	252	12.2171
4	252	12.249



Supplementary Fig. 7 | HPLC chromatogram for ER176 level 4 standard.

Mass concentration and molar activity are calculated for each batch of [^{11}C]ER176 using the currently valid calibration curve (e.g. Supplementary Fig. 8). The radioactivity injected onto the HPLC is calculated by subtracting the residual activity in the syringe from the initial measurement for the 100-µL sample and both values are decay-corrected to the end of synthesis (EOS). Radioactivity measurements are made in a calibrated ionization chamber. This value is divided by the mass in the sample derived from the calibration curve (converted into µmol) to obtain molar activity (A_m) in units of GBq/µmol (referred to EOS). This measurement is made only once for each production of [^{11}C]ER176.



Supplementary Fig. 8 | Constructed calibration curve for ER176. The unit of amount on the X-axis is ng and the unit of area on the Y-axis is (mAU × min). The equation of the linear curve is Area = (0.04870 × Amount) – 0.06684. The amount of ER176 is calculated using this equation from the measured area arising from the injected sample.