Supporting Information

High-yielding Automated Convergent Synthesis of No-carrier-added (n.c.a) $^{[11]}\text{C-Carbonyl}$-Labeled Amino Acids Using the Strecker Reaction

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Experimental Section

1. General considerations for chemistry

All starting materials, solvents, reagents and reference standards were purchased from commercial sources and used without further purification unless otherwise stated. NMR spectra were recorded with a Varian 400 MHz instrument at room temperature with tetramethylsilane (TMS) as an internal standard. High performance liquid chromatography (HPLC) was performed using a Shimadzu LC-2010A HT system equipped with a Bioscan B-FC-1000 radiation detector.

2. Synthesis of $^{[11}C$]sarcosine

Scheme S1: The synthesis of $^{[11}C$]Sarcosine.

$^{[11}C$]Sarcosine (1) was synthesized by our standard $^{[11}C$]methylation method using $^{[11}C$]methyl triflate, followed by saponification with sodium hydroxide at 60 °C. The reaction mixture was then diluted with water and purified using anion exchange Sep-Pak solid phase extraction. The final injectable dose was prepared by first washing the product trapped on the Sep-Pak with water, followed by elution with sodium chloride and finally passing the dose through a 0.22 μm sterile filter. Total synthesis time was approximately 25 minutes. Non-decay corrected radiochemical yield based on $^{[11}C$]CO$_2$ was 2.5-6 % (EOS, n=5) with radiochemical purity over 95%. The product was analyzed using analytical HPLC. Column: IC-Pak Cation M/D (Waters, 3.9x150 mm); mobile phase: 5 mM HCl aqueous solution; flow rate: 0.5 mL/min; Rad detector and UV 193 nm.

3. MicroPET imaging of tumor-bearing mice using $^{[11}C$]sarcosine (1)

All animal experiments were conducted in accordance with the “Guide for the care and use of laboratory animals” (8th edition, National Research Council, National Academies Press, 2011), as well as with the institutional ethic commission of the University of Michigan.

The human prostate cancer cell line PC-3 was obtained from American Type Culture Collection (ATCC; Manassas, VA, USA). Cells were cultured in RPMI in the presence of 10% of heat-inactivated fetal bovine serum, 1% of Penicillin/Streptomycin, 2 mM L-glutamine and 1 mM sodium pyruvate. At about 50% confluence, approximately 5 million, PC-3 cells were injected at the flanks of 6 to 8 week-old athymic Foxn1 nu mice (Charles River). Mice
were housed under pathogen-free conditions in micro-isolator cages, with rodent chow and water available ad libitum. Animals were examined daily and body weight and tumor sizes were determined. Tumors were allowed to grow to a diameter of 4-6 mm (short axis) before imaging.

Nude mice bearing PC-3 tumor received \([11C]\text{sarcosine} \) (500 µCi) intravenously and a 60 minute dynamic microPET scan was performed. Images were corrected for decay and photon attenuation using a \([^{68}\text{Ge}]\text{germanium rod source} \). Volumes of interest (VOIs) are drawn over tumor, muscle, liver and brain to generate time-activity curves (Figure S1). The preliminary microPET imaging data showed higher tumor-to-background ratio indicating sarcosine-based radiotracers have great potential as PET tracers for prostate cancer imaging.

![Figure S1: MicroPET image and time-activity curves](image)

4. Initial routes to \([^{11C}\text{-carbonyl}]\text{sarcosine} \) (2)

We initially designed two synthetic routes for \([^{11C}\text{-carbonyl}]\text{sarcosine} \) (Scheme S2). Intermediates \(S2\) and \(S5\) proved to be unstable. \(S2\) was easily converted to \(S4\) (Fig. S2) by reacting with \(S1\) existing in the reaction mixture, while \(S5\) can be converted back to \(S1\) during the purification process. Treatment of a mixture of \(S1\) and \(S5\) with TsCl can provide a new product monitored by thin-layer chromatography (TLC) and staining by iodine, but the new product cannot be purified by flash chromatography. Thus, we next considered using the Strecker reaction for the synthesis of \([^{11C}\text{-carbonyl}]\text{sarcosine} \).

![Scheme S2: Initial routes for \([^{11C}\text{-carbonyl}]\text{sarcosine} \)](image)
5. Preparation of $[^{11}\text{C}]\text{HCN}$

$[^{11}\text{C}]\text{CO}_2$ produced by the $^{14}\text{N} (p,\alpha)^{11}\text{C}$ reaction with a GE PETtrace cyclotron (40 µA for 30 minutes) and was converted to $[^{11}\text{C}]\text{HCN}$ using a GE PETtrace Carbon-11 Process Panel. Briefly, $[^{11}\text{C}]\text{CO}_2$ (~ 3,000 mCi) from the target was trapped on molecular sieves at room temperature. The $[^{11}\text{C}]\text{CO}_2$ was released from the trap at 350 °C and mixed with hydrogen gas, and then passed through a preheated nickel oven at 420 °C for conversion to $[^{11}\text{C}]\text{CH}_4$. $[^{11}\text{C}]\text{CH}_4$ gas was purified by passing it through Ascarite and Sicapent columns to remove water and unreacted $[^{11}\text{C}]\text{CO}_2$. The gas together with anhydrous ammonia gas was then sent through a high temperature (950 °C) platinum oven where the coupling of $[^{11}\text{C}]\text{CH}_4$ and $\text{NH}_3$ occurred to form $[^{11}\text{C}]\text{HCN}$. The non-decay corrected radiochemical yields of $[^{11}\text{C}]\text{HCN}$ were 700 – 1000 mCi.
6. Synthesis Module Modifications

Modifications of a commercial GE TRACERLab FX\textsubscript{M} are shown in Figure S3. Modification A: Two electronic valves (E1 and E2) were installed in the front of the chemistry module to direct [\textsuperscript{11}C]HCN from GE HCN system to FX\textsubscript{M}. V30 and V31 were removed from the HPLC pump and connected to [\textsuperscript{11}C]HCN system and reactor. A helically shaped platinum wire in a Teflon tube was inserted between V30 and V31 for purifying HCN (removal of excess NH\textsubscript{3}). V17 (N\textsubscript{2} gas line) was removed from the reactor and connected to V31 for chasing residual NH\textsubscript{3} mixed with [\textsuperscript{11}C]HCN out of solution; since residual NH\textsubscript{3} could have a negative effect on the experiments.

Modification B: Solvent reservoirs above V1 and V2 were removed and replaced with Luer lock adapters with needles, which allow the use of various size V-vials for small volumes and air sensitive reagents.

![Figure S3: Modifications to a GE TRACERLab FX\textsubscript{M} for sarcosine synthesis](image-url)
7. $^{11}$C-Carbonyl}sarcosine (2)

7.1 Synthesis of tert-butyl (cyanomethyl)(methyl)carbamate reference standard

To a solution of methylamine hydrochloride (100 mg, 1.48 mmol) in 1 mL water and 37% formaldehyde solution (112 μL, 1.48 mmol) in a 5 mL flask was added NaCN (72.59 mg, 1.48 mmol) at room temperature. After stirring 30 min at room temperature, (Boc)$_2$O (0.32 g, 1.47 mmol) in ethanol (1 mL) was added. The mixture was stirred 30 min, and extracted with dichloromethane ($2 \times 3$ mL), washed with 3 mL brine, and dried with Na$_2$SO$_4$. Evaporation of the solvent afforded a yellow oil, which was then purified by silica gel flash chromatography (1:4 hexane/ethyl acetate) affording the product as a pale oil. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 4.28 (s, 2H), 2.84 (s, 2H), 1.40 (s, 9H).

Compound S8 was difficult to purify and was used as a reference standard for $^{[11]}$C$\alpha$-aminonitrile 3 without purification. Compound S9 was prepared, purified, and identity confirmed by $^1$H NMR (Figure S4).

$^1$H NMR (400 MHz, DMSO-$d_6$)
7.2 Synthesis of $[^{11}\text{C}]{\alpha}$-aminonitrile (Table 1)

Scheme S4: The synthesis of $[^{11}\text{C}]{\alpha}$-aminonitrile (3).

Methylamine hydrochloride (1 mg, 14.8μM) in 100 μL H$_2$O and 37% formaldehyde solution (1.12 μL, 14.8μM) in 100 μL H$_2$O was added to the reactor at room temperature. After the reaction mixture was held at room temperature for 20 min, $[^{11}\text{C}]$NaCN (50 mCi) in 150 μL aqueous solution of sodium cyanide (0.72 mg, 14.8μM, Table 1, Entries 1 - 4) or 150 μL water (Table 1, Entry 5) was added. The reaction was tested at different conditions listed in Table 1 of the article. The HPLC results are shown in Figures S5 and S6, the radioactive peak around 4.5 min corresponded to $[^{11}\text{C}]{\alpha}$-aminonitrile and matched the retention time of the reference standard as observed by UV. Given the minimal effect of varying reaction time on radiochemical conversion (RCC) and the potential impurity profile at higher temperature, we selected the shortest reaction time 5 min at room temperature as the best method for production of 3 (Table 1, Entry 1).

Figure S5: HPLC trace of compound 3
Figure S6: HPLC trace for the synthesis of compound 3 at different conditions (see Table 1)
7.3 Hydrolysis of $[^{11}\text{C}]\alpha$-aminonitrile to yield $[^{11}\text{C}-\text{carbonyl}]$sarcosine (Table 2)

Hydrolysis of $^3$ to generate $[^{11}\text{C}-\text{carbonyl}]$sarcosine ($^2$) was next investigated. Literature reports typically employ 10 M NaOH at $\geq$100 °C to convert nitriles to carboxylic acids. Employing these standard conditions for 5 min resulted in 59% conversion to $^2$ (Fig. S7). Attempts to reduce the concentration of NaOH were unsuccessful, as using 5M NaOH resulted in reduced conversion of 15%. The hydrolysis of $^3$ was also sensitive to temperature, and reducing the hydrolysis temperature to 60 °C resulted in a significantly improved conversion of 97%.

Figure S7: HPLC trace for the hydrolysis of $^3$ (60 °C for 5 min).
7.4 Preparative HPLC conditions:

Four different preparative HPLC columns were tested (Table S1). An ion exchange chromatography column was the first choice and the retention time for the standard was acceptable. However, the high concentration of salts in the reaction mixture caused a loss of retention, resulting in everything eluting from the column within 3 min without any separation. Reverse phase chromatography was next investigated with both Hydro-RP or ODS column. We had tried purification by ion exchange first as low organic modifies in mobile phase led us to hypothesize that reverse phase would not provide adequate separation for purification of the product. As expected, $[^{11}\text{C-carbonyl}]$sarcosine did not retain on the column long enough to separate from impurities under these reverse phase conditions. Finally, a Luna-NH$_2$ amino-capped column was tested. In theory, one should avoid injecting an aldehyde onto an amino-capped column as imine formation can occur, damaging the stationary phase. However, in our case the amount of aldehyde was minimal (0.56 μL, 7.41μM). After reaction and hydrolysis, residue of aldehyde in the reaction mixture was very limited. A guard column was used to protect the column. The best retention and purification have been achieved using a Luna-NH$_2$ column with 60% acetonitrile, 10 mM NaH$_2$PO$_4$, pH 5.6 buffer (Table S1).

<table>
<thead>
<tr>
<th>Column</th>
<th>Mobile phase</th>
<th>RT (min)</th>
<th>Flow Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luna 10u SCX</td>
<td>100%H$_2$O, 10mM NaH$_2$PO$_4$</td>
<td>6.93</td>
<td>4 mL/min</td>
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<td>150 x 10 mm</td>
<td>100%H$_2$O, 20mM NaH$_2$PO$_4$</td>
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<td>Hydro-RP80</td>
<td>5%MeCN, 20mM NH$_4$OAC</td>
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<td>4 mL/min</td>
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<td>5%MeCN, 20mM NH$_4$OAC, pH=4.5</td>
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</tr>
<tr>
<td>Prodigy ODS</td>
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<td>60%MeCN, 10mM NaH$_2$PO$_4$</td>
<td>10.45</td>
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</table>

Table S1: HPLC conditions tested for $[^{11}\text{C-carbonyl}]$sarcosine (2)
7.5 Fully-automated n.c.a synthesis of [11C-carbonyl]sarcosine for clinical use

7.5.1 Reagents preparation

Scheme S6: The synthesis of n.c.a [11C-carbonyl]sarcosine (2)

Methylamine hydrochloride (0.5 mg, 7.41 μM) in 50 μL water and 37% formaldehyde solution (0.56 μL, 7.41μM) in 50 μL H2O was placed into the reactor of GE TRACERLab FXM at room temperature 20 min prior to end of beam. The platinum wire was preconditioned with sodium hydroxide (200 μL 1M NaOH and 2 mL air run through the platinum channel successively) and installed between V30 and V31. Two 1 mL V-vials containing water (150 μL) and 10 M NaOH (250 μL) were connected above V1 and V2, respectively. HPLC buffer (60% MeCN, 10 mM NaH2PO4, pH 5.6, 0.6 mL) was placed in the solvent reservoir above V3. The preparative HPLC column (Luna NH2, 250 x 10 mm) was installed onto the FXM module and mobile phase buffer solution was placed into the Eluent 1 bottle. The flow rate was set to 4 mL/min and the wavelength of UV detector was set at 220 nm. 60 mL of water containing 0.2 mL of 1M NaOH was placed in round bottom dilution flask. Sterile water for injection (5 mL each) was added into the reservoirs above V5 and V7. A solution of 0.5 mL 2 M NaCl was added into solvent reservoir above V6. Sterile water for injection (4.3 mL) and USP phosphate buffer (0.2 mL) were added into the product vial between V12 and V13. A pre-conditioned SAX extraction cartridge (Grace Alltech SAX Maxi-Clean Cartridge, 600 mg) was installed on the FXM module between V11 and V12. A sterile dose vial with a 0.22 μm filter was attached to the product line from V13.

7.5.2 Radiosynthesis

[11C]HCN released from GE HCN system was trapped as [11C]NaCN on the platinum wire. After residual NH3 was removed by stream of nitrogen gas via V17, water above V1 was transferred through Pt channel to deliver the [11C]NaCN (~900 mCi) into the reactor. The reaction mixture was then stirred at room temperature for 5 min. 10 M NaOH was added via V2 to the reaction mixture. The reactor was heated at 60 °C for 5 min. Afterwards, the mixture was cooled to 35 °C with liquid nitrogen, HPLC solvent was added via V3. The reaction mixture was injected onto the preparative HPLC column. The product peak between 10 and 11.5 minutes (see Figure S8 for a typical semi-preparative HPLC trace) was collected and diluted into 60 mL of water. The solution was loaded onto the SAX cartridge and washed with water via V5. The final dose was eluted with 0.5 mL 2M NaCl into the product vial containing 0.2 mL of sodium phosphate buffer and 4.3 mL of sterile followed by a rinse with 5 mL of sterile water to bring the dose to its final volume and composition. Total synthesis time was 40 min from end of beam. The dose was passed though a sterile 0.22 μm filter into a sterile dose vial and submitted for QC testing (see section 7.5.3 below). Non-decay corrected radiochemical yields (RCY) based on [11C]HCN were 30±12 mCi (1%, n=3). Radiochemical
purities (RCP) were >90%. Specific activities (SA) were greater than 1500 Ci/mmol based on limit of detection for sarcosine (0.3μg/mL).

Figure S8: Semi-preparative HPLC trace for the synthesis of [11C-carbonyl]sarcosine (2)

7.5.3 Quality control

Quality control of radiopharmaceuticals prepared for clinical use at the University of Michigan PET Center is carried out using guidelines outlined in Chapter 823 of USP and as detailed in the next paragraphs. The key data is summarized in Table S2.

7.5.3.1 Visual inspection

Doses were visually examined and required to be clear, colorless and free of particulate matter.

7.5.3.2 Dose pH

The pH of the doses was determined by applying a small amount of the dose to pH-indicator strips and determined by visual comparison to the scale provided. Acceptable doses were between 4.5 and 7.5.

7.5.3.3 Radiochemical purity and radiochemical identity

Radiochemical purity and identity are analyzed using an HPLC equipped with a radioactivity detector and a UV detector. Column: IP-Pak cation M/D 150 × 3.9 mm; mobile
phase: 5 mM HCl; flow rate: 0.5 mL/min. The product $t_R$: ~ 4.7 min ([Fig. S9](#)) Radiochemical purity for doses must be > 90%. Sarcosine (Aldrich, 99%) was used as the non-radio-active carbon-12 reference standard. Radiochemical identity was confirmed and quantified by calculating the relative retention time ($\text{RRT} = \frac{[\text{retention time of } ^{11}\text{C-sarcosine}]}{[\text{retention time of } ^{12}\text{C-sarcosine}]}$), and was required to be 0.9–1.10.

### 7.5.3.4 Radionuclidic identity

Radionuclidic identity is confirmed by measuring the half-life of $^{[11]}\text{C}$sarcosine doses and comparing it to the known half-life of carbon-11 (20.8 min). Activities are measured using a Capintec CRC-15 Radioisotope Dose Calibrator and half-life ($t_{1/2}$) was calculated according to equation. Calculated half-life must be 18.4-22.4 min.

$$t_{1/2} = \ln(2)\frac{\text{Time difference}}{\ln(\text{ending activity/stating activity})}$$

### 7.5.3.5 Sterile filter integrity (Bubble Point) test

Sterile filter from dose (with needle still attached) was connected to a nitrogen supply via a regulator. The needle was then submerged in water and the nitrogen pressure gradually increased. If the pressure was raised above the filter acceptance pressure (typically 40 psi) without seeing a stream of bubbles, the filter is considered intact.

### 7.5.3.6 Bacterial endotoxins

Endotoxin content in radiopharmaceutical doses is analyzed by a Charles River Laboratories EndoSafe® Portable Testing System and according to the US Pharmacopeia. Doses must contain <175 Endotoxin Units (EU).

### 7.5.3.7 Sterility

Culture tubes of fluid thioglycolate media (FTM) and tryptic soy broth (TSB) are inoculated with samples of the radiolabeled product and incubated (along with positive and negative controls) for 14 days. FTM is used to test for anaerobes, aerobes and microaerophiles whilst TSB is used to test for non-fastidious and fastidious microorganisms. Culture tubes are visually inspected on the 3rd, 7th and 14th days of the test period and compared to the positive and negative standards. Positive standards must show growth (turbidity) in the tubes, and dose/negative controls must have no culture growth after 14 days to be indicative of sterility.
Figure S9: Analytical HPLC trace of formulated $[^{11}C$-carbonyl]sarcosine (2).

| Table S2. Quality control data for $[^{11}C$-carbonyl]sarcosine (2) |
|-----------------------------|-------------|-----------|-----------|-----------|
|                             | Release criteria | Run 1     | Run 2     | Run 3     |
| Visual inspection           | Clear, no ppt  | Pass      | Pass      | Pass      |
| pH                          | 4.5-7.5       | 7.5       | 6.5       | 6.5       |
| Radiochemical purity        | > 90%         | 90.4%     | 96.2%     | 97%       |
| Radiochemical identity      | 0.9-1.10      | 1.02      | 1.00      | 1.00      |
| Radionuclidic identity      | T<sub>1/2</sub>=18.2-22.4 | 22.2 | 22.0 | 22.2 |
| Filter integrity            | > 40 psi      | 45        | 46        | 45        |
| Endotoxin                   | < 17.5EU/mL   | < 2       | < 2       | < 2       |
| Sterility                   | Sterile       | Sterile   | Sterile   | Sterile   |
8. MicroPET imaging of tumor-bearing mice using \[^{11}C\text{-carbonyl}]sarcosine\ (2)

All animal experiments were conducted in accordance with the “Guide for the care and use of laboratory animals” (8th edition, National Research Council, National Academies Press, 2011), as well as with the institutional ethic commission of the University of Michigan.

The human prostate cancer cell line PC-3 was obtained from American Type Culture Collection (ATCC; Manassas, VA, USA). Cells were cultured in RPMI in the presence of 10% of heat-inactivated fetal bovine serum and 2 mM L-glutamine. At about 50% confluence, approximately 5 million, PC-3 cells were injected at the flanks of 6 to 8 week-old athymic nude – Fox n1\(^{nu}\) mice (Charles River). Mice were housed under pathogen-free conditions in micro-isolator cages, with rodent chow and water available \textit{ad libitum}. Animals were examined daily and body weight and tumor sizes were determined. Tumors were allowed to grow to a diameter of 4-6 mm (short axis) before imaging.

Nude mice bearing PC-3 tumor received \[^{11}C\text{-carbonyl}]sarcosine\ (250 \mu\text{Ci}) intravenously and a 60 minute dynamic microPET scan was performed. Images were corrected for decay and photon attenuation using a \[^{68}\text{Ge}]\text{germanium}\ rod source. Volumes of interest (VOIs) are drawn over tumor, muscle, liver and brain to generate time-activity curves (Figure 3). The preliminary microPET imaging data showed higher tumor-to-background ratio indicating sarcosine-based radiotracers have great potential as PET tracers for prostate cancer imaging.
9. $[^{11}\text{C-}\text{Carbonyl}]$methionine (Table 3, Entry 1)

3-(Methylthio)propanal (1.0 mg, 9.6 μmol) in 50 μL ethanol, NH$_4$Cl (1.54 mg, 28.80 μmol) in 50 μL H$_2$O and 37% ammonium hydroxide (50 μL) was added into reactor of GE Tracerlab FX$_M$ at room temperature. $[^{11}\text{C}]$NaCN (725 mCi) in 200 μL was added and the reaction was stirred at room temperature for 5 min. 350 μL of 10 M NaOH was added to the reaction mixture which was held at 60 °C for 5 min. Then the reaction was cooled to 35 °C. 600 μL of HPLC buffer (see below) was added and used to transfer the reaction mixture to the HPLC loop. The product was purified by RP-HPLC (column: Prodigy 5μ ODS3 100R, 250×10.00 mm; mobile phase: 2% EtOH, 20 mM NaH$_2$PO$_4$; flow rate: 4 mL/min, room temperature). Total synthesis time was 33 min from end of beam and gave 43 mCi of isolated and formulated product. Non-decay corrected radiochemical yield (RCY) based on $[^{11}\text{C}]$HCN was 5%. Radiochemical purity (RCP) was >90% with a specific activity (SA) of 1256 Ci/mmol.

**Figure S16:** Semi-preparative HPLC trace of $[^{11}\text{C-}\text{carbonyl}]$methionine (4)
<table>
<thead>
<tr>
<th>Column</th>
<th>Mobile phase</th>
<th>R.T.(min)</th>
<th>Fluent rate</th>
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<td>Hydro-RP80, 250×10.00 mm</td>
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</tr>
<tr>
<td>Luna, 10u, NH2,100R</td>
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<td>4.12</td>
<td>4 mL/min</td>
</tr>
<tr>
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<td>50% MeCN, 20 mM NH₄OAc</td>
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</tr>
</tbody>
</table>

**Table S4:** HPLC conditions tested for $[^{11}C]$carbonyl]methionine (4)

$[^{11}C]$Carbonyl]methionine quality control

Column: Luna 10μ NH2 100R 250 × 4.60 mm; mobile phase: 60% MeCN, 10 mM NaH₂PO₄, UV = 210 nM; flow rate: 1 mL/min. product t<sub>R</sub>: ~5.68 min (Figure S17)

**Figure S17:** Analytical HPLC trace of $[^{11}C]$carbonyl]methionine (4)
10. $^{[11]}$C-"Carbonyl"glycine (Table 3, Entries 2 and 3)

We first applied the exact same synthetic procedure for $^{[11]}$C-carbonylsarcosine to appropriate precursor for the generation of $^{[11]}$C-carbonylglycine, but no product was obtained. Thus, modified Strecker conditions (Bucherer-Strecker) were used instead.

Scheme S7: The synthesis of $^{[11]}$C-carbonylglycine (5).

(NH$_4$)$_2$CO$_3$ (4.0 mg, 41.64 μmol) and 37% formaldehyde solution (2 μL, 26.86μmol) in 2 μL water was added to reactor of GE Tracerlab FXM at room temperature. After the reaction mixture was kept at room temperature for 20 min, $^{[11]}$CNaCN (800 mCi) in 250 μL water was added, and the mixture was heated with stirring for 7 min at 100 °C. The intermediate hydantoin was then hydrolyzed by treatment with 10 M NaOH (250μL) for 8 min at 100 °C. Then the mixture was cooled to 35 °C with liquid nitrogen. The reaction was quenched with 600μL of 10 mM NaH$_2$PO$_4$ aqueous solution, and the reaction mixture was injected onto the HPLC column. The product was purified by HPLC (column: Luna 10μ NH$_2$ 100R, 250 × 10 mm; mobile phase: 60% MeCN, 10 mM NaH$_2$PO$_4$, pH 5.6; flow rate: 4 mL/min, room temperature). The radioactive product peak between 7 and 9 min was collected. Total synthesis time was 38 min from end of beam and yielded 108 mCi of isolated product (non-decay corrected radiochemical yield base on $^{[11]}$CHCN) was 14%. Radiochemical purity was greater than 95% and specific activity was >1500 Ci/mmol based on limit of detection for glycine (5 μg/mL).

Fig S10: Semi-preparative HPLC trace for the synthesis of $^{[11]}$C-"carbonyl"glycine (5)
[11C-Carbonyl]glycine Quality Control
Column: IV-Pak cation M/D 150 × 3.9 mm; mobile phase: 5 mM HCl; flow rate: 0.5 mL/min
wavelength: 193 nm; room temperature; product \( t_R \): ~4.9 min (Fig. S11)

**Figure S11:** Analytical HPLC trace of [11C-carbonyl]glycine (5).

11. [11C-Carbonyl]-N-phenylglycine (Table 3, Entry 4)

11.1 Synthesis of 2-(phenylamino)acetonitrile reference standard (S12)

\[
\begin{align*}
\text{NH}_2 + \text{HCl}, \text{NaCN} & \rightarrow \text{NH} - \text{CN} \\
\text{HCHO, 80 °C, 8 h} & \rightarrow \text{S12}
\end{align*}
\]

Scheme S8: The synthesis of reference standard S12.

To a mixture of aniline (100 mg, 1.07 mmol) in 1 mL ethanol and 37% HCl (97.6 μL, 1.07 mmol) was added 37% formaldehyde solution (79.94μL, 1.07 mmol) and NaCN (52.62 mg) in 1 mL water. The reaction mixture was kept at 80 °C for 8 h, and then cooled to room temperature, quenched with 2 mL, extracted with dichloromethane (2 × 3 mL), washed with 3 mL brine, and dried over Na₂SO₄. Evaporation of the solvent afforded a yellow solid, which was then purified by silica gel column chromatography (1:1 hexane/ethyl acetate) affording 91 mg (yield: 64.12%) of the product as a white solid. \(^1\H NMR (400 MHz, DMSO- d_6) \delta 7.29 (m, 2H), 6.90(m, 2H), 6.72(m, 2H), 4.07 (s, 2H), 4.00 (br. s, 1H).

When the reaction was repeated in the automatic synthesis module GE Tracerlab FXm mimicking the carbon-11 procedures with small amount of reagents (aniline, 1 mg), no product was obtained. This was most likely caused by the change of the solution pH due to the NaOH coated on the platinum wire. Additional amount of HCl (aniline : HCl = 1 : 2) gave
the product in 25% yield. Figure S13 showed the HPLC trace of crude product (S14). Column: Prodigy 5μ ODS3 100R, 250×4.6 mm; mobile phase: 10%MeCN, 20 mM NH₄OAc; flow rate: 1.0 mL/min wavelength: 254 nm; room temperature; product tᵣ: ~7.68 min.

Figure S12: $^1$H NMR of reference standard S12

Figure S13: Analytical HPLC trace of reference standard S12
11.2 Synthesis of $[^{11}\text{C-carbonyl}]$-N-phenylglycine (6)

As aniline was not as reactive as methylamine, the first step required heating to 80°C. Reverse-phase HPLC was chosen for purification (Table S3).

Scheme S9: The synthesis of $[^{11}\text{C-carbonyl}]$-N-phenylglycine (6).

To a mixture of aniline (0.5 mg, 5.37 μmol) in 50 μL ethanol and 37% HCl (0.97 μL, 10.74μmol) in 50 μL H2O was added 37% formaldehyde solution (0.4 μL) in 50 μL H2O. $[^{11}\text{NaCN}]$ (725 mCi) in 200 μL was added and the reaction was stirred at 80°C for 5 min. 350 μL of 10 N NaOH was added to the reaction mixture which was held at 80°C for 5 min. Then the reaction was cooled to 35 °C with liquid nitrogen. 600 μL of HPLC buffer (see below) was added and used to transfer the reaction mixture to the HPLC loop. The product was purified by RP-HPLC (column: Prodigy 5μ ODS3 100R, 250×10.00 mm; mobile phase: 10%MeCN, 20 mM NH4OAc; flow rate: 4 mL/min, room temperature). Total synthesis time was 33 min from end of beam and gave 14 mCi of isolated and formulated product. Non-decay corrected radiochemical yield (RCY) based $[^{11}\text{C}]$HCN was 2%. Radiochemical purities (RCP) was > 99%. Specific activity was 15,453 Ci/mmol.

Figure S14: Semi-preparative HPLC trace of $[^{11}\text{C-carbonyl}]$-N-phenylglycine (6)
<table>
<thead>
<tr>
<th>Column</th>
<th>Mobile phase</th>
<th>R.T.(min)</th>
<th>Fluent rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydro-RP80, 250×10.00 mm</td>
<td>1 % MeCN, 20 mM NH$_4$OAc, pH=4.18</td>
<td>14.3</td>
<td>4 mL/min</td>
</tr>
<tr>
<td></td>
<td>5 % MeCN, 20 mM NH$_4$OAc, pH=4.18</td>
<td>7.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10 % MeCN, 20 mM NH$_4$OAc, pH=4.18</td>
<td>4.1</td>
<td></td>
</tr>
<tr>
<td>Prodigy 5μ ODS3 100R, 250×10.00 mm</td>
<td>5 % MeCN, 20 mM NH$_4$OAc</td>
<td>10.8</td>
<td>4 mL/min</td>
</tr>
<tr>
<td></td>
<td>10 % MeCN, 20 mM NH$_4$OAc</td>
<td>7.4</td>
<td></td>
</tr>
<tr>
<td>Luna, 10u, NH2,100R, 250×10.00 mm</td>
<td>50 % MeCN, 20 mM NH$_4$OAc, pH=4.20</td>
<td>4.4</td>
<td>4 mL/min</td>
</tr>
<tr>
<td></td>
<td>60 % MeCN, 20 mM NH$_4$OAc, pH=4.20</td>
<td>6.9</td>
<td></td>
</tr>
</tbody>
</table>

Table S3: HPLC conditions tested for [C-carbonyl]-N-phenylglycine (6)

It should be noted that the final radioactive dose was not stable based on QC HPLC. The first injected sample showed 97% purity (Figure S15), but samples injected at later times showed degradation to 85% and 50% after 15 and 30 min, respectively. Since N-phenylglycine standard was stable in HPLC buffer, the degradation was most likely caused by radiolysis, but the details have not yet been fully investigated.

[11C-Carbonyl]-N-phenylglycine Quality Control
Column: Prodigy 5μ ODS3 100R, 250×4.6 mm; mobile phase: 10%MeCN, 20 mM NH$_4$OAc; flow rate: 1.2 mL/min wavelength: 254 nm; room temperature; product t<sub>R</sub>: ~6.15 min (Figure S15)

![Figure S15: Analytical HPLC trace of [11C-carbonyl]-N-phenylglycine (6)](image-url)
12. \([^{11}C]-Carbonyl\) 2-amino-2-methyl-3-phenylpropanoic acid (Table 3, Entries 5 and 6)

12.1 Synthesis of 2-amino-2-methyl-3-phenylpropanenitrile reference standard (S13)

![Scheme S11: The synthesis of reference standard S13.](image)

To ethanol (2 mL) was added 1-phenylpropan-2-one (100 mg, 0.75 mmol), NH4Cl (79.73 mg, 1.50 mmol), ammonium hydroxide (0.5 mL) and NaCN (36.52 mg, 0.75 mmol). The mixture was heated at 80 °C for 8 h, cooled, quenched with water (2 mL), extracted with dichloromethane (2 × 3 mL), washed with brine (5 mL), and dried over Na2SO4. Evaporation of the solvent afforded a yellow oil, which was then purified by silica gel flash chromatography (1:1 hexane/ethyl acetate), affording 80 mg (yield: 67%) of the product as a yellow oil. \(^1\)H NMR (400 MHz, CDCl3) \(\delta 7.34-7.38 \text{ (m, 5H)}\), \(3.0 \text{ (d, 1H, } J = 3.0 \text{ Hz)}\), \(2.83 \text{ (d, 1H, } J = 2.8 \text{ Hz)}\), \(1.56 \text{ (s, 3H)}\).

![Figure S18: \(^1\)H NMR of reference standard S13](image)
12.2 Attempts Synthesis of n.c.a (±)[11C]-2-amino-2-methyl-3-phenylpropanenitrile (7, Table 3, Entry 5)

The reaction without carrier was also tested. Increasing the reaction temperature to 100 °C resulted in no desired product. Using DMSO as solvent, the reaction temperature was tested at both 100 °C and 150 °C, but no product was observed in either case (Table S5). In addition, switching the amine to ammonium carbonate had no effect on the reaction outcome.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>T(°C)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H₂O/EtOH</td>
<td>80</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>H₂O/DMSO</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td></td>
</tr>
</tbody>
</table>

Table S5: Attempts to synthesize n.c.a (±)[11C]-2-amino-2-methyl-3-phenylpropanenitrile 7

12.3 Synthesis of carrier-added (±)[11C]-2-amino-2-methyl-3-phenylpropanenitrile (7, Table 3, Entry 6)

![Scheme S12: The synthesis of [11C]α-aminonitrile 7.](attachment:image)

1-phenylpropan-2-one (2 mg, 14.91 µmol), NH₄Cl (1.59 mg, 29.82 µmol), ethanol (50 µL) and ammonium hydroxide (50 µL) was added into 5 ml reactor at room temperature. After the reaction mixture was kept at room temperature for 20 min, [11C]NaCN (175 mCi) in 200 µl water that contained NaCN (0.73 mg, 14.91 µmol) was added, and the mixture was heated with stirring for 15 min at 80 °C. Then the mixture was cooled to 35 °C. The identity and purity of the product was checked by RP-HPLC (Prodigy 5µ ODS3 100R, 250 × 4.600 mm; mobile phase: 40% MeCN, 20 mM NH₄OAc, room temperature) (Figure S19)
Figure S19: Analytical HPLC trace of compound [11C]α-aminonitrile 7