Electronic Supplementary Information (ESI)

Rapid 'on-column' preparation of hydrogen [11C]cyanide from [11C]methyl iodide *via* [11C]formaldehyde

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1. Experimental Procedure

1.1. General

1.1.1. Chemicals. Anhydrous-grade dimethyl sulfoxide (DMSO), *N,N*-dimethyl formamide (DMF), acetonitrile (MeCN), and tetrahydrofuran (THF) were purchased from Wako Pure Chemical Ltd. (Osaka, Japan) and used as reaction solvents. Anhydrous trimethylamine *N*-oxide (TMAO), TMAO dihydrate, and *N*-methylmorpholine *N*-oxide (NMO) were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Oxymatrine (OMT) was purchased from Cayman Chemical Company Inc. (Ann Arbor, MI, USA) and stored at $-20\,°C$. Hydroxylamine-*O*-sulfonic acid (HOSA, 99.999%) was purchased from Merck KGaA (Darmstadt, Germany) and stored at $4\,°C$. A cartridge including 2,4-dinitrophenylhydrazine (DNPH, InertSep® mini AERO DNPH) was purchased from GL Sciences Inc. (Tokyo, Japan). A standard solution of formaldehyde 2,4-dinitrophenylhydrazone was purchased from Kanto Chemical Co., Inc. (Tokyo, Japan). Water purified with a Milli-Q® system (Direct-Q® UV3, Merck KGaA) was used in this study. All other chemicals used in this study were purchased from Merck KGaA, Wako Pure Chemical Ltd., Tokyo Chemical Industry Co., Ltd., and Nacalai Tesque Inc. (Kyoto, Japan), unless otherwise noted, and used without further purification.

1.1.2. Equipment. Automated radiochemical synthesis was performed using a system built in-house. HPLC analysis was performed on an HPLC system consisting of a JASCO PU-2089 pump (JASCO Corporation, Tokyo, Japan), JASCO UV-2075 ultraviolet detector, and NaI(Tl) scintillation detector with an ACE Mate Amplifier and BIAS supply (925-SCINT, ORTEC, Oak Ridge, TN, USA) for radioactivity detection. Data acquisition and interpretation were performed with ChromNAV software (ver. 1.5.2.0, JASCO Corporation). Radioactivity was measured using dose calibrators (Curiemeter IGC-7, Hitachi Ltd., Tokyo, Japan, and CRC-55tR, Capintec Inc., Florham Park, NJ, USA). NMR spectra were obtained using an Avance Nanobay 400-MHz spectrometer (Bruker Corporation, Billerica, MA, USA). The reaction column was prepared with a small empty column made of borosilicate glass (Chromabond, 3 mL, Macherey-Nagel GmbH & Co. KG, Düren, Germany). The silicon rubber heater ($0.8\ W/cm^2$, 11 mm ID $\times$ 65 mm) was purchased from Kawai Corporation (Nagoya, Japan).

1.1.3. Radiochemical purity. The radiochemical purity of [^{11}C]methyl iodide ([^{11}C]CH$_3$I), [^{11}C]formaldehyde-2,4-dinitrophenylhydrazone ([^{11}C]CH$_2$O-DNPH), [^{11}C]hydrogen cyanide ([^{11}C]HCN), and benzyl [^{11}C]cyanide ([^{11}C]BnCN) were measured by HPLC. A portion of the sample solution (generally 20 μL) was collected in an injection syringe and the radioactivity was measured. After sample injection into the HPLC instrument, radioactivity remaining in the syringe was measured to calculate the radioactivity injected for HPLC analysis. The eluent from the HPLC system was collected in a vial on ice *via* a septum and gas evacuated from the vial was collected in a plastic syringe during the observation period. The radioactivity collected in the vial and plastic syringe was measured after HPLC analysis to calculate the recovery rate of the radioactive compounds. In most cases, the recovery was more than 95%. The decay-corrected area ratio of the corresponding peak on the

radiochromatogram was measured and the value was corrected for the recovery rate of radioactive compounds to determine the radiochemical purity.

1.1.4. Molar activity. The molar activity of [^{11}C]CH$_3$I, [^{11}C]CH$_2$O-DNPH, and [^{11}C]BnCN were measured by HPLC. The quantity of each compound in sample solutions injected into the HPLC instrument was measured using the standard curve. The radioactivity of the compounds injected for HPLC was determined using the method described above (see section 1.1.3).

1.2. Production of [^{11}C]carbon dioxide ([^{11}C]CO$_2$)

[^{11}C]CO$_2$ was produced by the ^{14}N(p,α)^{11}C nuclear reaction in N$_2$ gas containing 0.01% O$_2$ with 18 MeV protons using the CYPRIS HM-18 cyclotron (Sumitomo Heavy Industry, Tokyo, Japan). The yield of [^{11}C]CO$_2$ was automatically calculated and monitored in real time during irradiation. As the molar activity of [^{11}C]CO$_2$ is dependent on the irradiation conditions,[1] [^{11}C]CO$_2$ (18–20 GBq) produced under almost identical irradiation conditions (15 μA, 10 min) was used in the automated synthesis to compare the molar activity of radioactive products.

1.3. Production of [^{11}C]CH$_3$I

1.3.1. For manual synthesis. [^{11}C]CO$_2$ was transferred to a reaction vessel containing a THF solution of lithium aluminium hydride (LAH, 500 μL, 0.05 M) with the target gas flow at 500 mL/min at -10 °C. After evaporating the THF solvent, 57% hydroiodic acid (400 μL) was added to the vessel and the mixture was heated at 150 °C. The resulting [^{11}C]CH$_3$I was purged from the vessel by N$_2$ gas flow (50 mL/min), followed by passing through columns filled with P$_2$O$_5$ and Ascarite II® sequentially. [^{11}C]CH$_3$I (~500 MBq) was then collected at ambient temperature in a glass vessel containing the solvent (1 mL) used for the reaction described below (see section 1.4).

1.3.2. For automated synthesis. The target gas containing [^{11}C]CO$_2$ (18–20 GBq), which was produced using the procedure described above (see section 1.2), was introduced into a stainless-steel tube coil at -180 °C with a flow rate of 500 mL/min. The concentrated [^{11}C]CO$_2$ in the coil was released by heating and transferred to a reaction vessel containing a THF solution of LAH (100 μL, 0.05 M) under target gas flow (15 mL/min). The following procedure was similar to that of the manual synthesis, but using 300 μL of 57% hydroiodic acid and with the target gas as the carrier gas instead of N$_2$. After adding hydroiodic acid, the vessel was closed and heated at 150 °C for 1.5 min. The synthesis time from the end of bombardment to the start of [^{11}C]CH$_3$I transfer was 8.5–9 min.

1.3.3. Molar activity of [^{11}C]CH$_3$I. [^{11}C]CH$_3$I produced for the automated synthesis was collected in MeCN on ice. The molar activity of [^{11}C]CH$_3$I in the solution was measured as described above (see sections 1.1.2–1.1.4) using an HPLC column (COSMOSIL 5C$_{18}$-MS-II (4.6 mm ID $\times$ 150 mm, 5 μm) with a guard column (4.6 mm ID $\times$ 10 mm, 5 μm), Nacalai Tesque Inc.) and a mobile phase (60% MeCN in H$_2$O). The retention time of CH$_3$I on the UV detector (254 nm) at a flow rate of 1 mL/min was 3.9 min.

1.4. Conversion of [¹¹C]CH₃I to [¹¹C]CH₂O in solvents (manual synthesis)

1.4.1. Reaction. For reactions in *N*-oxide suspended solution, a solution of [¹¹C]CH₃I (111–222 MBq, 300 μL) was added to a reaction vial containing a given amount of *N*-oxide and allowed to stand for 2 min at the designated temperature on a heating block. For reactions with *N*-oxide completely dissolved in the solution, a solution of [¹¹C]CH₃I (37–74 MBq, 100 μL) was added to the *N*-oxide solution (200 μL). Generally, *N*-oxides were dissolved completely in DMSO with sonication and slight warming. The actual temperature of the heating block was monitored with a thermometer and adjusted to a given value prior to the experiment. At the end of the reaction, the reaction mixture was cooled on ice and quenched by adding 30% methanol (100 μL).

1.4.2. Dimedone precipitation assay. The yield of [¹¹C]CH₂O in the resulting mixture was measured by dimedone precipitation assay. The method was slightly modified from that reported previously.[2] An aliquot of the reaction mixture including [¹¹C]CH₂O (5 μL) and 30% HCHO aqueous solution (20 μL, 0.2 mmol) was added to 30% methanol (200 μL). The mixture (200 μL) was then added to a solution of dimedone (80 mg, 0.57 mmol) in 30% methanol (5 mL) and heated at 100 °C for 15 min. The reaction mixture was then cooled to room temperature and the radioactivity in the vial was measured. The liquid in the suspension was transferred to an empty column cartridge (Bond Elut Empty SPE Cartridge with a 20-μm pore size frit, Agilent Technologies, Inc., CA, USA), which was directly connected with a syringe filter (Millex-LH Syringe Filter, 0.45 μm, φ13 mm, Merck KGaA). The precipitate (methylene-bisdimedone) remaining in the reaction vial was rinsed with 30% methanol (1 mL) and the liquid was combined with the former liquid in the column cartridge. After the liquid was filtered off, the radioactivity in the precipitate was measured and the yield of [¹¹C]methylene-bisdimedone was determined.

1.5. Conversion of [¹¹C]CH₃I to [¹¹C]CH₂O in a reaction column

1.5.1. Preparation of reaction column. Quartz wool (2–6 μm, approx. 430 mg, TOSOH Corp., Tokyo, Japan) was compressed to 25 mm in a small empty column made of borosilicate glass. A silicone rubber septum (W cap, w-9, Taiyo Kogyo, Tokyo, Japan) was placed on top of the column. MeCN (750 μL) was mixed with an *N*-oxide and a sulfoxide, and the solution was then soaked up through the end of the column by drawing a syringe *via* the septum. TMAO (10 mg), NMO (10 mg), or OMT (25 mg) was used as the *N*-oxide, while DMSO (250 μL) or diphenyl sulfoxide (DPSO, 250 mg) was used as the sulfoxide. The column was wrapped with a silicone rubber heater[3] and excess solution was purged with N₂ gas flow introduced *via* the septum (100 mL/min) at 50 °C for 10 min. The video showing the method for the preparation of the reaction column is available in supplementary information. **Notes:** The glass fibre frit, which was pre-inserted in the purchased column, was removed and the column was washed well before use, because heating the frit generated a considerable amount of nonradioactive CH₂O originating from a binder used in the frit (Fig. S5). The adaptor provided as an

accessory for the column was not used because it was not firmly connected to the column. Quartz wool should be tightly compressed in the column, otherwise the amount of solution retained in the quartz wool is increased, resulting in increased radioactivity retained in the column after the reaction. Compression of the quartz wool in the column should be conducted in a fume cupboard with a face mask to avoid the inhalation of quartz wool dust.

1.5.2. Reaction. The reaction column was covered with a silicone rubber heater[3] and a thermocouple probe (type K) was placed between the column and heater. Heating the column to the designated temperature (120–150 °C) was started 1.5 min prior to starting [^{11}C]CH$_3$I transfer. [^{11}C]CH$_3$I in the carrier gas (target gas) was introduced into the heated reaction column with a flow rate of 50 mL/min for 3 min. The eluted gas containing [^{11}C]CH$_2$O was passed through a DNPH cartridge covered with aluminium foil and then collected in a sampling bag. After the reaction, radioactivity in the reaction column, DNPH cartridge, and sampling bag was measured.

1.5.3. Radiochemical yield and molar activity of [^{11}C]CH$_2$O-DNPH. Radioactive compounds in the DNPH cartridge were extracted with MeCN (6 mL) and collected in an amber vial on ice. The extraction efficiency was more than 95%. The radiochemical purity and molar activity of [^{11}C]CH$_2$O-DNPH in the MeCN solution were determined by HPLC using an XBridge C18 column (4.6 mm ID $\times$ 150 mm, 3.5 μm, Waters Corporation, Milford, MA, USA) as described above (sections 1.1.2–1.1.4). The retention time of CH$_2$O-DNPH (mobile phase of MeCN/10 mM AcONH$_4$ buffer, 5:5 (*v/v*); flow rate of 1 mL/min) on the UV detector (360 nm) was 4.7 min. The radiochemical yield of [^{11}C]CH$_2$O-DNPH was calculated as the radioactivity in the DNPH cartridge multiplied by the purity of [^{11}C]CH$_2$O-DNPH.

1.6. Conversion of [^{11}C]CH$_3$I to [^{11}C]HCN with a reaction column

1.6.1. Preparation of the reaction column with OMT solution. After the reaction column for the production of [^{11}C]CH$_2$O was prepared with the MeCN solution of OMT and DPSO, as described above (section 1.5.1), the septum was removed. A small piece of quartz wool was placed on the *N*-oxide layer, followed by a mixture of HOSA (40 mg) and quartz sand (2.3 g, 50–70 mesh, Merck KGaA). Another piece of quartz wool was then placed on the HOSA layer, and the top of the column was plugged with a silicone rubber septum. The reaction column was prepared just before use. **Note:** MeCN used to prepare the *N*-oxide layer should be free of HCN to avoid reducing the molar activity of [^{11}C]HCN.[4] The video showing the method for the preparation of the reaction column is available in supplementary information.

1.6.2. Preparation of the reaction column with OMT powder. DPSO and OMT were finely powdered with an alumina mortar and pestle under ambient conditions. From the SiO$_2$ granules purchased (99.99%, 200–700 μm, Umicore Thin Film Products AG, Balzers, Liechtenstein), granules with a particle size of 200–300 μm were manually selected with sieves. The powdered DPSO (75 mg) and OMT (25 mg) were mixed sequentially with SiO$_2$ granules (1.6 g) in a vial under ambient conditions.

The mixture was poured into the column, and a piece of quartz wool was placed on the mixture. The following procedure was the same as described above (see section 1.6.1). The quartz sand used for the HOSA layer was also applied to preparation of the N-oxide layer instead of SiO_2 granules. However, this tended to increase the breakthrough of $[^{11}C]CH_3I$.

1.6.3. Reaction. For the method using a reaction column prepared with OMT solution, the procedure for $[^{11}C]HCN$ production was similar to that of $[^{11}C]CH_2O$ production with a reaction column (see section 1.5.2). The reaction temperature was set at 150 °C. Gas eluted from the reaction column was bubbled into DMF (1 mL) in a vial on ice to collect $[^{11}C]HCN$ and radioactive compounds that escaped from DMF were collected in a sampling bag. $[^{11}C]CH_3I$ transfer was continued until the radioactivity in the collection vial reached a plateau. For the method using a reaction column prepared with OMT powder, the procedure for $[^{11}C]HCN$ production was slightly modified from the method using a reaction column prepared with OMT solution. The flow rate for $[^{11}C]CH_3I$ transfer was reduced from 50 to 40 mL/min. $[^{11}C]CH_3I$ was introduced into the reaction column at 150–160 °C and the temperature was further increased to 170 °C. In both procedures, radioactivity in the reaction column, the collection vial containing DMF, and the sampling bag was measured after the reaction. The synthesis time from the end of bombardment to the end of $[^{11}C]HCN$ collection was 9.8–10.4 min. The schematic diagram for the $[^{11}C]HCN$ production system for the current method is shown in Fig. S6.

1.6.4. Radiochemical yield and purity of $[^{11}C]HCN$. The radiochemical purity of $[^{11}C]HCN$ collected in DMF was determined using an ion exchange column (Shim-pack Amino-Na, 6 mm ID × 100 mm, 5 μm, Shimazu GLC Ltd., Kyoto, Japan) with a guard column (Shim-pack IC-CN(G), 6 mm ID × 10 mm, 5 μm, Shimazu GLC) and tartrate buffer (10 mM, pH 4.2) as the mobile phase, as described above (see sections 1.1.2 and 1.1.3). 1 M NaOH solution (1 mL) was added to the vial used to collect the eluent from the HPLC system. The retention time of $[^{11}C]HCN$ (5.2 min on a radioactivity detector) at a flow rate of 0.6 mL/min was confirmed using $[^{11}C]HCN$ produced with the traditional method (see section 1.7.1). The radiochemical yield of $[^{11}C]HCN$ was calculated as the radioactivity in the DMF solution multiplied by the purity of $[^{11}C]HCN$.

1.7. Preparation of $[^{11}C]HCN$ using the traditional method

1.7.1. Reaction. The preparation of $[^{11}C]HCN$ using the traditional method was performed as previously reported with modifications.[5] The target gas containing $[^{11}C]CO_2$, which was produced with the procedure described above (see section 1.2), was introduced into a stainless-steel tube coil at −180 °C with a flow rate of 500 mL/min. Concentrated $[^{11}C]CO_2$ in the coil was released by heating under target gas flow (10 mL/min) and mixed with H_2 gas. The gas mixture was introduced into a methanizer (211MT, GL Science Inc., Tokyo, Japan) and $[^{11}C]CO_2$ was converted into $[^{11}C]CH_4$ over a Ni-filled column heated at 400 °C. To remove water and unreacted $[^{11}C]CO_2$, the gas containing

[^{11}C]CH$_4$ was passed through columns filled with P$_2$O$_5$ and Ascarite II® sequentially. The generated [^{11}C]CH$_4$ was trapped in a stainless-steel tube coil containing Porapak Q (80–100 mesh) at −180 °C and H$_2$ contained in the gas was purged. [^{11}C]CH$_4$ was released by heating under N$_2$ gas flow (40 mL/min) and fed to the NH$_3$ gas stream (5% NH$_3$ in N$_2$, 500 mL/min), followed by passing through a Pt-filled column heated at 950 °C, in which [^{11}C]CH$_4$ was converted into [^{11}C]HCN. [^{11}C]HCN in the NH$_3$ gas was collected in solution at −20 °C for further experiments. The total synthesis time from the end of bombardment to the end of [^{11}C]HCN collection was around 7 min.

1.7.2. Radiochemical yield and purity of [^{11}C]HCN. The radiochemical yield and purity of [^{11}C]HCN collected in DMF was determined in the same manner as described above (see section 1.6.4). The DMF solution of [^{11}C]HCN was diluted 10-fold or more with ice-cold tartrate buffer, which was used for HPLC analysis, prior to analysis to sufficiently acidify the solution.

1.7.3. Removal of NH$_3$ from the product gas. NH$_3$ in the product gas containing [^{11}C]HCN was removed by passing through a column filled with KHSO$_4$. As KHSO$_4$ is highly hygroscopic, excess water in the KHSO$_4$ column was removed before use by passing through N$_2$ gas at a flow rate of 100 mL/min for 10 min at 50 °C. The reduction in NH$_3$ content achieved by the KHSO$_4$ column was preliminarily confirmed as follows. N$_2$ gas containing 5% NH$_3$ was passed through the KHSO$_4$ column at a flow rate of 500 mL/min for 30 s and the eluted gas was collected in a sampling bag (Tedlar® bag, n=3). The concentration of NH$_3$ in the eluted gas was measured using a detector tube (105SD, Komyo Rikagaku Kogyo K.K., Kanagawa, Japan) and found to be less than the quantitation limit (0.2 ppm). Indeed, the odour threshold for ammonia was reported to be 2.6 ppm,[6] but the collected gas was odourless.

1.8. Preparation of [^{11}C]BnCN

1.8.1. Reaction. [^{11}C]HCN was transferred to a reaction vessel containing a DMF solution (300 µL) of benzyl bromide (1 µL) and 1,8-diazabicyclo[5.4.0]undec-7-ene (1 µL) at −20 °C until the radioactivity in the vessel reached a plateau. The reaction mixture was heated at 120 °C for 5 min, and then cooled and diluted with the mobile phase (MeCN/10 mM AcONH$_4$, 4:6 (*v/v*)) used for HPLC separation. [^{11}C]BnCN was purified by HPLC (XBridge Prep C18, 10 mm ID × 250 mm, 5 µm). The radioactive fraction corresponding to [^{11}C]BnCN (retention time of 8.2 min at a flow rate of 5 mL/min) was collected in a vial and the activity of the solution in the vial was measured. Although the synthesis time of [^{11}C]HCN using the traditional method was 3 min shorter than that using the current method, the total synthesis time of [^{11}C]BnCN from the end of bombardment to the end of collection was comparable between these methods (around 26 min). This was due to the difference in synthesis modules used for [^{11}C]BnCN preparation.

1.8.2. Radiochemical yield and purity of [^{11}C]BnCN. The radiochemical purity and molar activity of [^{11}C]BnCN were measured by HPLC (J'sphere ODS-H80 column (4.6 mm ID × 150 mm, 5 µm, YMC Co. Ltd., Kyoto, Japan); mobile phase of MeCN/10 mM AcONH$_4$ = 3/7 (*v/v*); flow rate of 1 mL/min) as

described above (see sections 1.1.2–1.1.4). The retention time of [^{11}C]BnCN on the UV detector (254 nm) was 13.5 min. The radiochemical purity was always more than 99% (Fig. S7). The radiochemical yield of [^{11}C]BnCN was calculated as the radioactivity of the collected solution in the vial multiplied by the purity of [^{11}C]BnCN. **Note:** When the XBridge C18 column (4.6 mm ID × 150 mm, 3.5 μm) was used to measure the molar activity of [^{11}C]BnCN, an impurity peak overlapped with that of [^{11}C]BnCN, resulting in underestimation of the molar activity.

1.9. Synthesis of quinuclidine *N*-oxide

Quinuclidine *N*-oxide was prepared as previously described with slight modifications.[7] To an ice-cold solution of quinuclidine (500 mg, 4.5 mmol) in EtOH (5 mL) was slowly added 30% H_2O_2 (3 mL) under Ar. The reaction mixture was allowed to stand for 18 h at ambient temperature. After the disappearance of quinuclidine was confirmed using an NH silica TLC plate (Chromatorex, Fuji Silysia Chemical LTD., Kasugai, Japan) with AcOEt/2-PrOH (1:1, *v/v*) as solvent, excess H_2O_2 was destroyed by adding MnO_2 (2 mg) under ice-cold conditions and the reaction mixture was stirred on ice for an additional 1 h. The mixture was slowly warmed to ambient temperature and the disappearance of H_2O_2 was confirmed using potassium iodide starch test paper. After the removal of MnO_2 by filtration with Celite®, MeCN (50 mL) was added to the filtrate and the solvent was evaporated in vacuo. The resulting residue was redissolved in MeCN (50 mL) and the solvent was evaporated. This was repeated twice to remove water. The resulting solidified residue was dried under reduced pressure overnight, and then washed with AcOEt by decantation. Finally, the product was purified by column chromatography over NH silica gel (Chromatorex, Fuji Silysia Chemical LTD.) eluting with AcOEt/2-PrOH (1:1, *v/v*) to yield quinuclidine *N*-oxide (577 mg, 4.5 mmol, 101%), which contained some water as previously reported.[8] The NMR data agreed with literature values.[9] ^{1}H-NMR (400 MHz, DMSO-d$_6$) δ(ppm): 1.82–1.86 (m, 7H), 3.08–3.12 (m, 6H). ^{13}C NMR (100 MHz, DMSO-d$_6$) δ(ppm): 20.19, 26.46, 63.50.
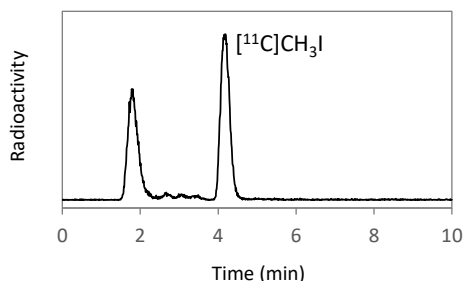
2. Supplemental Figures

Fig. S1   Conversion of [^{11}C]CH$_3$I into [^{11}C]CH$_2$O using a reaction column prepared with a MeCN solution of TMAO. The reaction column was prepared in the same manner as described in section 1.5.1. [^{11}C]CH$_3$I was introduced into the column at 120 °C for 3 min. The product gas was collected in 80% DMSO and radioactive compounds in the solution were analysed by HPLC under the same conditions used for the analysis of [^{11}C]CH$_2$O-DNPH (see section 1.5.3).
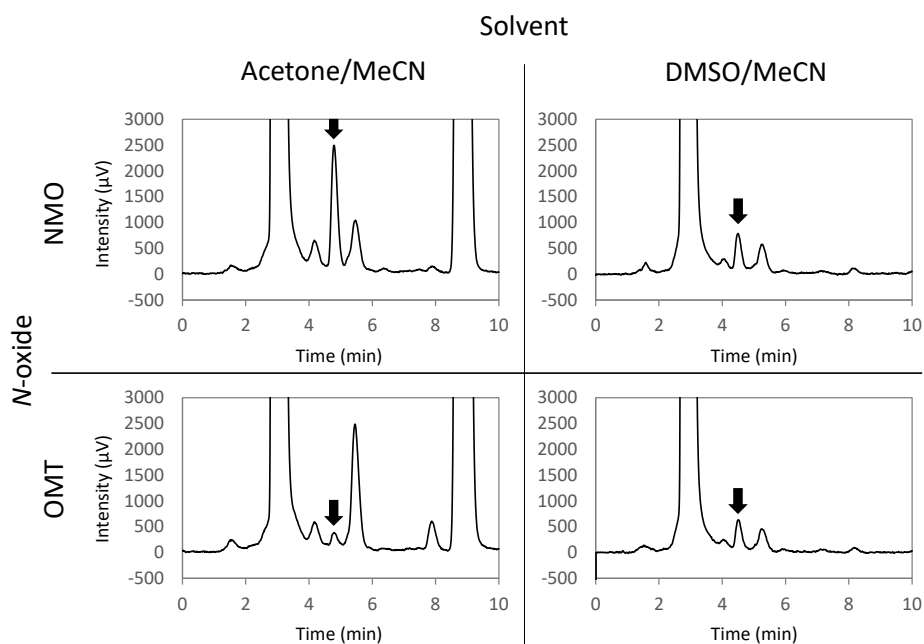
Fig. S2   Generation of CH$_2$O by heating NMO and OMT. Nitrogen gas (50 mL/min) was passed through the reaction columns, which were prepared in the same manner as described in sections 1.5.1 and 1.5.2, for 3 min. A mixture of acetone and MeCN (1:1, *v/v*) and a mixture of DMSO and MeCN (1:3, *v/v*) (left and right panels, respectively) were used as solvents for the NMO and OMT solutions (upper and lower panels, respectively) to prepare the reaction columns. The columns containing NMO and OMT were heated at 120 and 150 °C, respectively. CH$_2$O in the gas eluted from the reaction column was derivatized to CH$_2$O-DNPH using a DNPH cartridge and detected by HPLC, as described in section 1.5.2. Acetone (left panels) was used to reduce the amount of solvent remaining in the reaction column as much as possible after purging excess reagents. Although further investigation is required, the generation of CH$_2$O from NMO seemed to be suppressed in DMSO (upper panels). Arrows indicate the CH$_2$O-DNPH peak.
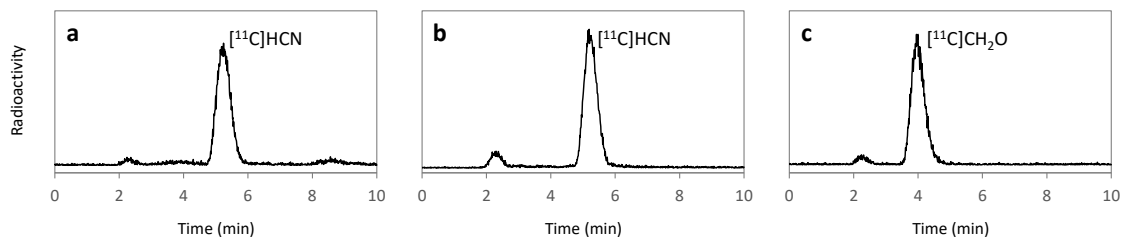
Fig. S3 Conversion of $[^{11}C]CH_2O$ into $[^{11}C]HCN$ in DMSO and water. $[^{11}C]CH_2O$ was produced by passing $[^{11}C]CH_3I$, which was prepared for manual synthesis (see section 1.3.1), through a reaction column prepared with TMAO and DMSO at 120 °C, as described in section 1.5.1. The produced $[^{11}C]CH_2O$ was collected in a vial containing DMSO or water (1 mL). The $[^{11}C]CH_2O$ solution (200 μL) was added to a solution of HOSA (12 mg in DMSO, 6 mg in water) in DMSO or water (100 μL) and reacted for 3 min at 120 °C or 100 °C, respectively. After the reaction vial was cooled on ice, the formed $[^{11}C]HCN$ was analysed by HPLC as described above in section 1.6.4. (a) $[^{11}C]HCN$ formed in DMSO; (b) $[^{11}C]HCN$ formed in water; (c) $[^{11}C]CH_2O$ collected in DMSO.

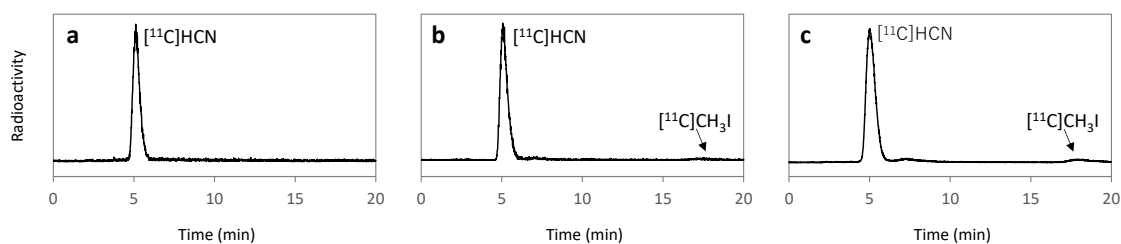**Fig. S4** Representative radiochromatograms for $[^{11}C]HCN$ produced by (a) the traditional method, and by reaction columns prepared with (b) OMT solution and (c) OMT powder.
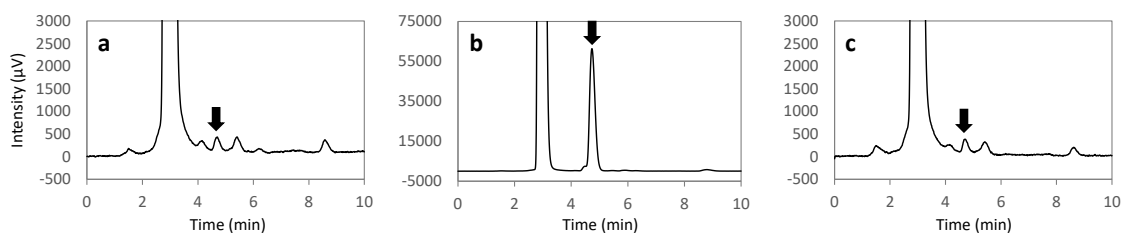

Fig. S5 Generation of CH_2O by heating a glass fibre frit. Nitrogen gas (50 mL/min) was passed through a reaction column, which was prepared with OMT and DPSO as described in section 1.5.1, at 150 °C for 3 min. CH_2O in the gas eluted from the reaction column was derivatized to CH_2O-DNPH using a DNPH cartridge and detected by HPLC, as described in sections 1.5.2 and 1.5.3. (a–c) CH_2O generated in reaction columns prepared (a) without the glass fibre frit, (b) with the frit, and (c) without the frit and reagents. The frit was placed on the OMT layer after reaction column preparation. Arrows indicate the CH_2O-DNPH peak.
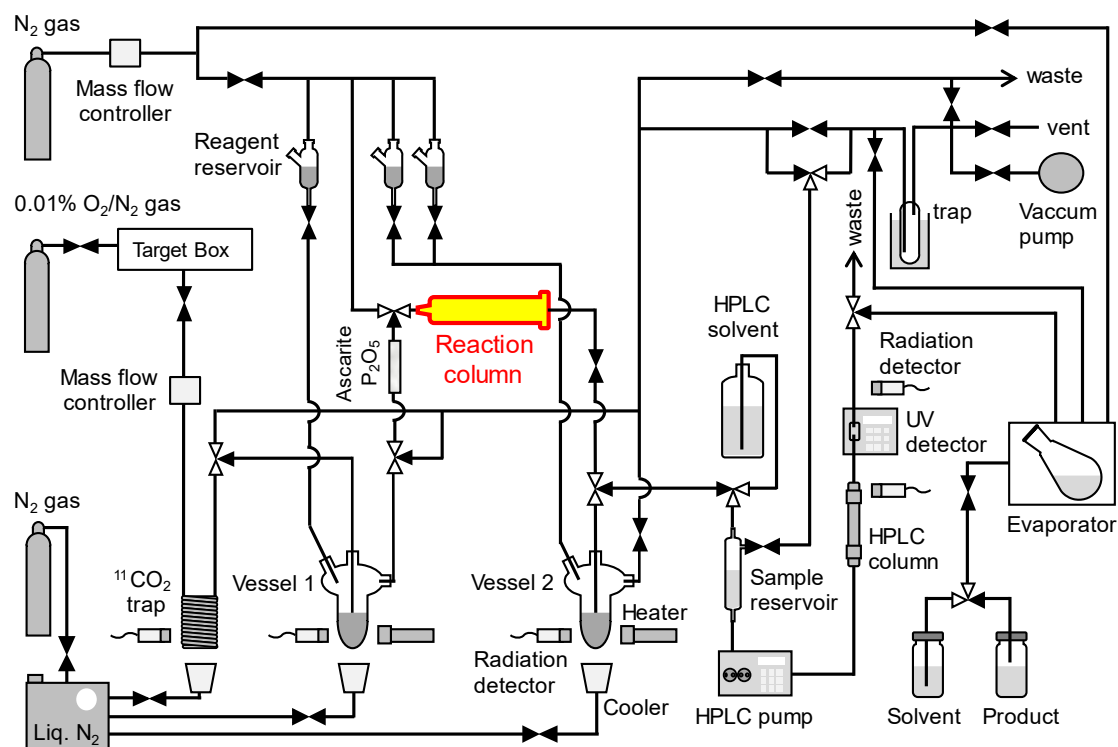
Fig. S6 Schematic diagram for the [^{11}C]HCN production system for the current method. In an in-house built synthesis system used for ^{11}C-labelling with [^{11}C]CH$_3$I, the reaction column was placed in-line between the Ascarite/P$_2$O$_5$ column and the Vessel 2 using luer fittings. [^{11}C]CH$_3$I and [^{11}C]BnCN was prepared in the Vessel 1 and the Vessel 2, respectively.

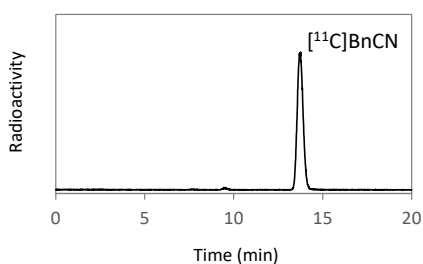**Fig. S7** A representative radiochromatogram for [^{11}C]BnCN.

References

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