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ORIGINAL ARTICLE

Investigation of S_N2 [^{11}C]cyanation for base-sensitive substrates: an improved radiosynthesis of L-[5-^{11}C]-glutamine

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Abstract Carbon-11 (β^+ emitter, $t_{1/2}$ = 20.4 min) radiolabeled L-glutamine is a potentially useful molecular imaging agent that can be utilized with positron emission tomography for both human oncological diagnosis and plant imaging research. Based upon a previously reported [^{11}C]cyanide end-capping labeling method, a systematic investigation of nucleophilic cyanation reactions and acidic hydrolysis reaction parameters, including base, metal ion source, phase transfer catalyst, solvent, reaction temperature and reaction time, was conducted. The result was a milder, more reliable, two-step method which provides L-[5-^{11}C]-glutamine with a radiochemical yield of 63.8 ± 8.7 % (range from 51 to 74 %, n = 10) with >90 % radiochemical purity and >90 % enantiomeric purity. The total synthesis time was 40–50 min from the end of bombardment. In addition, an Fmoc derivatization method was developed to measure the specific activity of this radiotracer.

Keywords L-[5-^{11}C]-glutamine · PET imaging · Nucleophilic [^{11}C]cyanation · Radiolabeling · Base sensitive · Enantiomeric purity

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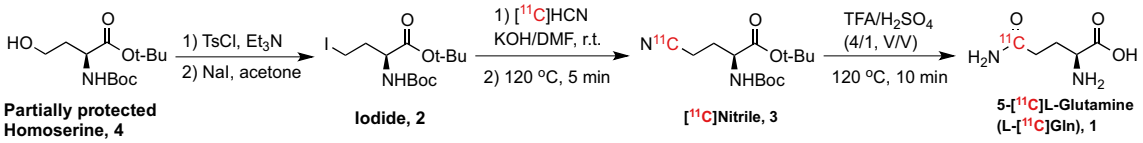
Introduction

Positron emission tomography (PET) is a powerful imaging modality that has been broadly used as a scientific and clinical research tool for medical applications (Ametamey et al. 2008; Vallabhajosula 2009), and recently in the study of plant metabolism (Buehler et al. 2011; Kiser et al. 2008). Radiotracer chemistry, especially the development of carbon-11(^{11}C, $t_{1/2}$ = 20.4 min)- and fluorine-18(^{18}F, $t_{1/2}$ = 109.7 min)-labeled PET radiotracers, provides many opportunities to image biochemical transformations non-invasively and monitor the movement of biological substrates, drugs and xenobiotics in living animals, humans and plants (Ametamey et al. 2008; Miller et al. 2008). Amino acids (AAs) are essential small organic molecules used in the synthesis of proteins and other biologically active compounds such as neurotransmitters and hormones that are present across the animal and plant kingdoms. The synthesis of positron emitter-labeled AAs has been the focus of radiotracer chemistry research for many years and a variety of ^{11}C-labeled natural and non-natural AAs have been synthesized and evaluated as potential PET imaging agents (Ermert and Coenen 2013; Miller et al. 2008).

In recent years, the need to develop renewable sources of energy that do not compete with food crop production has stimulated the genetic transformation of plants for improved biofuel and feedstock production. Fixed nitrogen, in the form of amino acids such as glutamine (Gln) and asparagine (Asn), is critical for the transport of nitrogen from the roots to the leaves in plants. Through the action of glutaminase and asparaginase, these amino acids release ammonium to distal tissues and roots (Lea et al. 2007; Sieciechowicz et al. 1988; Stitt et al. 2002). The development of molecular imaging tools, such as ^{11}C-labeled Gln and Asn, could help to image and better understand



Scheme 1 Previously reported [^{11}C]cyanide end-capping method for radiosynthesis of L-[^{11}C]Gln

the transport, storage and utilization of nitrogen in plants. Information garnered could further guide researchers to modify plants for better biological nitrogen fixation and utilization.

Previously, ^{13}N-labeled L-Gln has been synthesized enzymatically and utilized to image various spontaneous canine tumors (Gelbard et al. 1977). More recently, we reported a streamlined method for the chemical synthesis of L-[5-^{11}C]-glutamine (L-[^{11}C]Gln), using an alkyl iodide 2 precursor derivatized from partially protected homoserine 4, as well as a [^{11}C]cyanide end-capping method (Scheme 1) (Qu et al. 2012). This process included two consecutive reactions: nucleophilic [^{11}C]cyanation, as well as [^{11}C]nitrile hydrolysis and deprotection with strong acid. Solid phase extraction (SPE) and ion retardation resin chromatography methods were utilized to purify [^{11}C]nitrile, 3 and [^{11}C]Gln. To obtain an acceptable radiochemical yield and ensure the enantiomeric purity of [^{11}C]Gln, a glove box was necessary to store and prepare reaction reagents and vessels to reduce moisture, which promoted racemization. These requirements added extra precautions and complications to the [^{11}C]Gln radiosynthesis process. In addition, as we surveyed the literature we were surprised to find very little information on the reaction conditions required for nucleophilic [^{11}C]cyanation reactions using tracer levels of [^{11}C]cyanide-labeling precursor, especially when the radiosynthesis required the use of acid- or base- or moisture-sensitive substrates (Ding et al. 1989). In this research, we describe our recent systematic investigation of the effects of varying reaction parameters on the tracer level S_N2 [^{11}C]cyanation reaction, and a simplified and refined synthetic method for L-[^{11}C]Gln with a significant improvement in the reproducibility and radiochemical yield.

Materials and methods

General

All reagents and solvents used for the radiosynthesis process were purchased from Sigma-Aldrich (MO, USA) with a minimum of ACS reagent grade and used without further purification. Solid phase extraction (SPE) cartridges (Sep-Pak® C18 Plus) were obtained from Waters (Waters® Association, MA, USA). Ag®11-A8 ion retardation resin was purchased from BIO-RAD, USA. High- and low-level radioactivity measurements were performed using a Capintec CRC-712MV and a Capintec CRC-ultra radioisotope dose calibrator (Capintec Inc., NJ, USA) respectively. The analytical radio-HPLC analysis was performed using a Knauer HPLC system (Sonntek Inc., NJ, USA) equipped with a Knauer pump (model K-501 or model K-1001) and using a model 87 variable wavelength monitor for detecting UV signal. The radioactivity signal was measured with an NaI detector or a Geiger Muller ionization detector. The HPLC chromatograph data were collected using an SRI PeakSimple data acquisition system. The radiochemical purity and enantiomeric purity (percentages of L- and - isomers) of the final product L-[^{11}C]Gln were determined by analytical radio-HPLC with an Phenomenex® Chirex 3126 (D)-penicillamine column (250 × 4.60 mm), mobile phase: isopropanol/2 mM $CuSO_4$ aqueous solution 2/100 (V/V), flow rate 1.0 mL/min, $\lambda = 254$ nm, Rt of L-[^{11}C]Gln = 7–8 min.

[^{11}C]HCN production

Using an EBCO TR-19 cyclotron, carbon-11 was generated as [^{11}C]CO_2 by bombarding an N_2 gas target (400 psi 99.9999 % pure N_2 doped with 400–500 ppm O_2) with 17.4 MeV protons to induce the ^{14}N(p, α)^{11}C nuclear reaction. Typical irradiation current used for the bombardment was 33 μA. Following the irradiation, the target gas was released and delivered to an in-house-built, fully automated [^{11}C]HCN production system (Kim et al. 2013) for converting [^{11}C]CO_2 to [^{11}C]HCN. Briefly, the [^{11}C]CO_2 delivered from the cyclotron target is trapped at room temperature on a nickel (Ni) catalyst mixed with molecular sieves (MS). After pressurizing to 15 psi with hydrogen gas, the Ni/MS furnace was sealed and heated to 420 °C for 3 min to reduce [^{11}C]CO_2 to [^{11}C]CH_4. Next, helium gas was allowed to flowed through the Ni/MS furnace to carry [^{11}C]CH_4 and mix it with ammonia gas (NH_3). This mixed gas was passed over a pre-heated platinum (Pt)-furnace (950 °C) and the desired [^{11}C]HCN was synthesized and delivered to a pre-installed reaction vessel for radioactivity collection. Usually, 1.11–1.85 GBq (30–50 mCi) of H^{11}CN was produced and trapped in a reaction vessel following a 12–14 min production cycle with 1 min cyclotron beam time (generating 3.7 GBq (100 mCi) $^{11}CO_2$).

Chemistry and radiochemistry

Preparation of weak base stock solution

A stock solution was prepared by mixing an 18-crown-6 (18-C-6) CH_3CN solution (18-C-6, 160 mg, 0.61 mmol dissolved 17 mL of CH_3CN) with a weak base aqueous solution (for example, $CsHCO_3$ aqueous solution: weigh $CsHCO_3$, 60 mg, 0.31 mmol and dissolve in 3 mL of H_2O). The mixture was well shaken to form a homogenous phase that is ready for use. The pH value of this stock solution was determined by measuring the weak base aqueous solution with a pH meter.

[^{11}C]HCN trapping solution preparation

The weak basic stock solution (1 mL) was added to a conical glass vial (RV01) and the solvent was evaporated by heating at 120 °C under a mild argon stream. After most solvent was evaporated, RV01 was further dried azeotropically with CH_3CN (2 × 1 mL) to remove all moisture. Once the drying process was complete, RV01 was cooled to room temperature, 0.3 mL DMF was added and RV01 was ready for trapping radioactivity [^{11}C]HCN.

Radiosynthesis of L-[^{11}C]Gln

Upon completion of the radioactivity trapping process, the radiolabeling precursor, tert-butyl (*2S*)-2-[(tert-butoxycarbonyl)amino]-4-iodobutanoate, 2 (2 mg, predissolved in 0.2 mL DMF), was quickly added to RV01 and heated to 90 °C for 8 min. The reaction mixture was diluted with 10 mL H_2O. The diluted mixture was passed through a C18Plus cartridge. Following washes with H_2O (2 × 10 mL), the radioactivity ([^{11}C]nitrile, 3) trapped on the cartridge was eluted into a second conical vial (RV02) with CH_3CN (1.5 mL). RV02 was heated in a 120 °C oil bath under a mild argon stream to remove all solvent. Once most liquid had evaporated, the radioactivity was further dried azeotropically with CH_3CN (2 × 1 mL) to remove all moisture. After drying, TFA/H_2SO_4 (0.2 mL, 4/1, V/V) was added and the mixture was heated at 90 °C for 5 min. Following dilution with H_2O (2 mL), the radioactive mixture was loaded onto an Ag11-A8 resin column (3.0 g of resin, preloaded in a 0.8 cm I.D. Econo-Column®, washed with 20 mL of 0.5 M NaCl and 100 mL of D.I. H_2O, consecutively). Once the column had drained completely, a second portion of H_2O (4 mL) was used to elute the majority of radioactivity as the final product L-[^{11}C]Gln (normally 185–370 MBq (5–10 mCi) activity). Total synthesis time from end of bombardment (EOB) to delivery of radiotracer for a plant study was around 40–50 min.

Fmoc derivatization of L-[^{11}C]Gln for specific activity (SA) determination

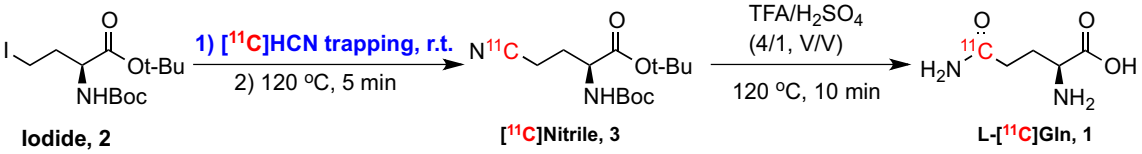
Product SA was determined by HPLC analysis using the fluorenylmethyloxycarbonyl (Fmoc)-derivitization method: 100 µL of L-[^{11}C]Gln product sample was mixed with 200 µL of freshly prepared Fmoc-Cl (1.5 mg/mL CH_3CN) and 20 µL of aqueous saturated sodium borate. After maintaining the reaction mixture at room temperature for 15 min with periodic vortexing, the sample was subjected to HPLC analysis with a Gemini C18 column (250 × 4.60 mm, 5 µ), mobile phase: 0.05 M sodium acetate (pH 4.2, pH adjusted with glacial acetic acid)/CH_3CN 70/30 (V/V), flow rate: 1.0 mL/min, $\lambda = 263$ nm. The radioactivity signals were measured with a Biodex Model 3 radiodetector (Rt of Fmoc-[^{11}C]Gln: 11 min). A 10 µL aliquot was injected on the HPLC and triplicate 30 µL aliquots were measured in an NaI well counter (Picker International, Cleveland, Ohio), decay-corrected back to EOB and normalized to the derivitization yield (determined from the analytic radio-HPLC trace). If necessary, the counting aliquots were allowed to decay until the count range was within that appropriate for avoiding dead-time issues with the well counter.

Results and discussion

Investigation of the nucleophilic [^{11}C]cyanation reaction

The investigation of reaction parameters started with the nucleophilic [^{11}C]cyanation reaction (Table 1). With the incentive to minimize stringent processes (i.e., handling all reaction agents inside a glove box for preparing the reaction kit), we first tested the use of 0.1 M KOH (in MeOH) solution instead of solid KOH powder for trapping [^{11}C]HCN and handling the DMF solvent and iodide precursor with simple inert gas balloons instead of a glove box (Qu et al. 2012). After pre-evaporation of MeOH at 120 °C, DMF was added for trapping [^{11}C]HCN. Once the radioactivity was trapped, the iodide precursor (dissolved in DMF) was added and the reaction mixture was heated at 120 °C for 5 min. Surprisingly, there was no detectable reaction under these conditions (Table 1, Entry 1). Liquid chromatography–mass spectrometry (LC–MS) analysis revealed that the lactone and the dimerized compounds were formed (Fig. 1). The unexpected cyclization could be induced by the trace amount of MeOH trapped in KOH. Instead of nucleophilic cyanation, the iodide precursor went through a cyclized reaction that was triggered by a strong base methoxide ion and was consumed rapidly (Scheme 2). Similar phenomena have been observed previously in reports of the synthesis of (2S,4S)- and (2S,4R)-5-fluoroleucine, as well as optically pure 4-fluoroglutamines (Charrier et al. 2004; Qu et al. 2011). Upon the

Table 1 Optimization of the [^{11}C]cyanation reaction



Entry	[^{11}C]HCN trapping conditions	[^{11}C]nitrile **3** yield (%)[a]	Overall yield (%)[b]	Radiochemical and optical purities (%)[c]
1	KOH in MeOH, pre-drying of MeOH at 120 °C	N/A	N/A	N/A
2[d]	KOH in MeOH, pre-drying of MeOH at 120 °C and at 140 °C azeotropically with DMF	60–70	45–52	79–90/5–10
3[e]	KH_2PO_4 (pH = 4.5)	6.0	N/A	N/A
4	K_2CO_3 (pH = 11.8)	5.4	N/A	N/A
5	$KHCO_3$ (pH = 9.8)	22	18	76/n/a
6	$KHCO_3$/K222, pre-drying of solvent at 120 °C azeotropically with CH_3CN	30	15	35/36
7[f]	$KHCO_3$/18-C-6, pre-drying of solvent at 120 °C azeotropically with CH_3CN	**78**	**59**	**96/0.9**
8	$K_2C_2O_4$ (pH = 6)/18-C-6	15	N/A	N/A
9	$K_2C_2O_4$/K_2CO_3 (95/5) (pH = 10.8)/18-C-6	12	N/A	N/A
10	KI (pH = 7.6)/18-C-6	8.3	N/A	N/A
11	K_2CO_3 (pH = 11.8)/18-C-6	66	47	70/26
12	$CsHCO_3$ (pH = 9.6)/18-C-6	**85**	**59**	**90/4.0**
13	$TBAHCO_3$ (pH = 9.5)/18-C-6	28	16	24/17

n/a no detectable D-[^{11}C]Gln

N/A The acidic hydrolysis and deprotection reactions were not carried out

All reactions started with 1.11–1.85 GBq (30–50 mCi) [^{11} C]HCN that was produced from our in-house, fully automated [^{11}C]HCN production system

Bold values indicate the significance of the combination of $KHCO_3$ and 18-C-6 as well as the combination of $CsHCO_3$ and 18-C-6

[a] Isolated yields and decay-corrected yield (DCY) from total collected [^{11}C]HCN activity

[b] Isolated yields and DCY from total collected [^{11}C]HCN activity

[c] Radiochemical and optical purity data are shown as percentages of both L- and D-[^{11}C]Gln in the final products

[d] Results summarized from four experiments

[e] All pH values listed in this table were obtained by checking the aqueous solutions of various salts

[f] Pre-prepared $KHCO_3$/18-C-6 1 mL was dried by heating in a 120 °C oil bath under a stream of argon to remove most of the solvent. Then, 2 × 1 mL CH_3CN was added and evaporated in the same manner for the removal of H_2O

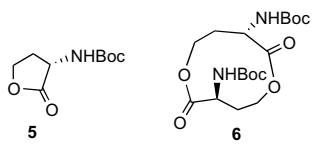
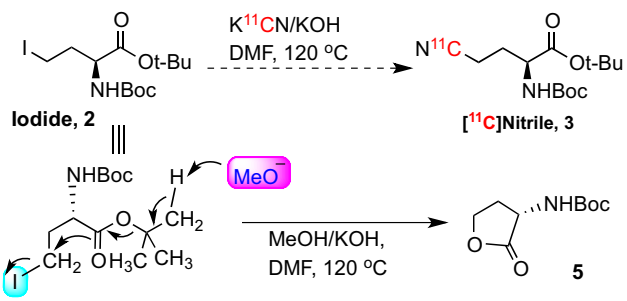


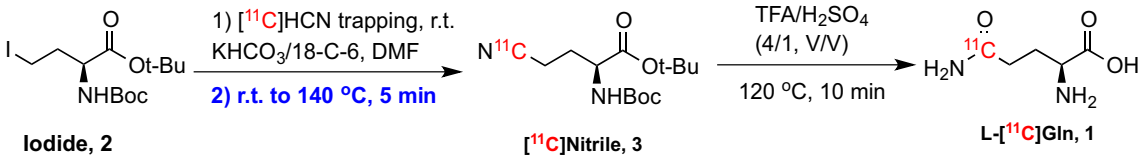
Fig. 1 Structures of two by-products in Table 1, Entry 1

identification of this problem, we modified the evaporation to ensure removal of MeOH from KOH. After evaporation of MeOH at 120 °C under argon flow, DMF (0.2 mL) was added and the reaction vial was heated to 140 °C with an



Scheme 2 Plausible explanation for the formation of cyclized by-product **5**

Table 2 Temperature effect on [^{11}C]cyanation



Entry	[^{11}C]cyanation temperature	[^{11}C]nitrile 3 yield (%)[a]	Overall yield (%)[b]	Radiochemical and optical purities (%)[c]
1[d]	Room temperature	17	12	95/n/a
2	60 °C	47	41	96/1.4
3	90 °C	57	44	94/1.6
4	120 °C	78	59	96/0.9
5	140 °C	84	60	89/7.2
6[e]	120 °C	64	56	87/8.7
7[f]	120 °C	66	55	89/6.0

[a] Isolated yields and decay-corrected yield (DCY) based on total collected [^{11}C]HCN activity

[b] Isolated yields and DCY based on total collected [^{11}C]HCN activity

[c] Radiochemical and optical purity data expressed as percentages of both L- and D-[^{11}C]Gln in the final products

[d] $CsHCO_3$ was used; n/a: no detectable amount of D-[^{11}C]Gln

[e] During the pre-preparation, the [^{11}C]cyanide production system was required to be flushed with helium flow for 1 h. In this experiment, the line-flushing process was done for only 10 min

[f] Water (2 μL) was intentionally added to the reaction vial

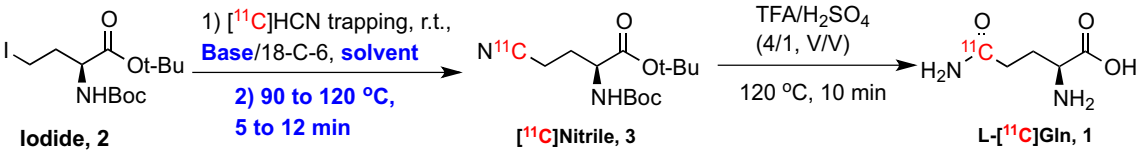
argon flow to remove DMF and trace amounts of MeOH. This azeotropic evaporation allowed the complete removal of MeOH. The subsequent [^{11}C]cyanation gave [^{11}C]Gln with comparable yields to our previous report (Qu et al. 2012). However, the radiochemical and enantiomeric purities of the product were not reproducible under the above conditions (Entry 2). Next, less basic conditions were tested for the trapping of [^{11}C]HCN and the cyanation reaction. Both acidic KH_2PO_4 and basic K_2CO_3 could trap [^{11}C]HCN radioactivity efficiently, but only a negligible amount of [^{11}C] nitrile **3** was formed (Entry 3 and 4). When slightly basic $KHCO_3$ was tested, [^{11}C]nitrile 3 was clearly formed (Entry 5). More importantly, the analytical radio-HPLC analysis results showed that 76 % of radioactivity in the final product was L-[^{11}C]Gln, and no D-[^{11}C]Gln was detected. By introducing the phase transfer catalyst (PTC) Krypotfix 222 (4,7,13,16,21,24-Hexaoxa-1,10-diazabicyclo[8.8.8]-hexacosane, K222) to the reaction, the yield improved significantly (Entry 6). However, unexpected isomerization occurred, and equal amounts of L- and D- forms of [^{11}C]Gln were detected in the final product. The basicity of reagent K222 may be responsible for this observed epimerization at α-position of [^{11}C]Gln. When neutral form PTC, 18-C-6, was tested, the cyanation reaction yield improved dramatically to 78 % and the overall radiochemical yield reached 59 % (Entry 7). More delightfully, HPLC analysis revealed that the product had the desired radiochemical and enantiomeric purities (96 % L-[^{11}C]Gln/0.9 % D-[^{11}C]Gln).

Given the encouraging results obtained from the addition of 18-C-6, this neutral PTC was used in all subsequent [^{11}C]cyanation reactions listed in this research. Both slightly acidic $K_2C_2O_4$ and basic $K_2C_2O_4/K_2CO_3$ (95/5, mol/mol) salts (Entry 8 and 9), which had been used in radio-fluorination for synthesizing ^{18}F-labeled homocysteine (Bourdier et al. 2011), provided very low [^{11}C]cyanation yields. A similar result was obtained when KI was tested, despite the fact that the pH value of its aqueous solution was close to neutral (Entry 10). Relatively basic K_2CO_3 led to the formation of more undesired D-[^{11}C]Gln, although the overall reaction yield was acceptable (Entry 11). When $CsHCO_3$, another slightly basic salt (comparable with $KHCO_3$), was tested, the results were more encouraging (Entry 12). Both the [^{11}C]cyanation and the overall radiochemical yields were improved, and the quality of the final product was also excellent. Tetrabutylammonium bicarbonate ($TBAHCO_3$), which has a pH value similar to $KHCO_3$, proved to be inferior for [^{11}C]cyanation (Entry 13). Similar results were reported for the synthesis of optically pure 4-fluoro-glutamines (Qu et al. 2012).

The effect of temperature on [^{11}C]cyanation

Next, we investigated the effect of reaction temperature on the [^{11}C]cyanation reaction. The reaction yields were clearly improved by increasing the reaction temperature (Table 2, Entry 1–5). When the temperature was above

Table 3 Further optimization of ^{11}C-cyanation reaction



Entry	[^{11}C]cyanation reaction conditions	[^{11}C]nitrile 3 yield (%)[a]	Overall yield (%)[b]	Radiochemical and optical purities (%)[c]
1	$KHCO_3$/DMF, 120 °C for 5 min	78	59	96/0.9
2	$KHCO_3$/DMSO, 120 °C for 5 min	52	36	92/n/a
3	$KHCO_3$/tetraethylene glycol, 120 °C for 5 min	3.5	N/A	N/A
4	$KHCO_3$/2-methyl-2-butanol, 120 °C for 5 min	60	35	76/18
5	$KHCO_3$/CH_3CN, 90 °C for 5 min	65	59	93/1.1
6	$KHCO_3$/CH_3CN, 90 °C for 8 min	79	49	95/n/a
7	$KHCO_3$/CH_3CN, 90 °C for 12 min	87	62	93/2.2
8	$CsHCO_3$/CH_3CN, 90 °C for 8 min	85	46	96/1.0
9	$CsHCO_3$/DMF, 90 °C for 8 min	**94**	**46**	**95/1.6**

n/a no detectable D-[^{11}C]Gln

N/A The acidic hydrolysis and deprotection reactions were not carried out

Bold values indicate the significance of the best [^{11}C]cyanation reaction conditions

[a] Isolated yields and decay-corrected yield (DCY) based on total collected [^{11}C]HCN activity

[b] Isolated yields and DCY based on total collected H^{11}CN activity

[c] Radiochemical and optical purities data are reported as percentages of both L- and D-[^{11}C]Gln in the final product

120 °C, there was more D-[^{11}C]Gln in the final product (Entry 5, 140 °C). For all [^{11}C]cyanation tests, to eliminate the perturbation of varying amounts of trace moisture in the transfer line, the entire [^{11}C]HCN production system was flushed with helium flow for 1 h prior to transfer of the [^{11}C]CO_2 from the cyclotron target. In one test, this helium flushing process was accidentally reduced to 10 min and the [^{11}C]cyanation yield dropped by more than 10 % and a higher percentage of D-[^{11}C]Gln was formed (Entry 6). It was speculated that moisture left in the [^{11}C]HCN production system was responsible for the decrease in both reaction yield and enantiomeric purity of the final product. To verify our hypothesis, slightly wet DMF solvent (500 μL mixed with 2 μL H_2O) was used for the [^{11}C]cyanation reaction and the results clearly showed that water was detrimental to the synthesis of L-[^{11}C]Gln (Entry 7). Any precautions that eliminate moisture from the [^{11}C]cyanation reaction will presumably improve the synthesis of this radiotracer.

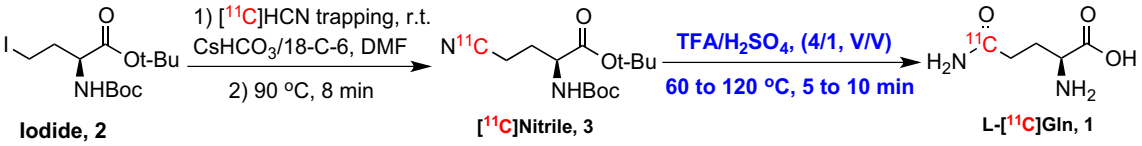
Further optimization of [^{11}C]cyanation: solvent and reaction time

After the investigation of the effect of temperature on the [^{11}C]cyanation reaction was complete, our attention turned to the reaction solvents and time (Table 3). Neither the polar aprotic solvent DMSO (Entry 2) nor alcoholic solvents tetraethylene glycol (Lee et al. 2009) and 2-methyl-2-butanol (Entries 3 and 4), which had been reported to be beneficial for S_N2 nucleophilic substitution reaction (Kim et al. 2006), improved the results. Another polar aprotic solvent CH_3CN provided comparable results to DMF (Entry 5–8). Considering the potential of generating [^{12}C]cyanide ion during reactions under basic conditions and high reaction temperatures (Ding et al. 1989), CH_3CN was excluded from subsequent experiments. Increasing reaction time (Entry 6 and 7) and changing the [^{11}C]HCN trapping salt from $KHCO_3$ to $CsHCO_3$ (Entry 8) both helped to improve the overall yields of L-[^{11}C]Gln. After combining the optimized reaction conditions (Entry 9), we found that the yield of [^{11}C]cyanation reaction yield increased to 94 %.

Investigation of acidic deprotection and hydrolysis conditions

Once all conditions for the [^{11}C]cyanation reaction had been optimized, our attention turned to the second step of the [^{11}C]Gln synthesis: one-pot acidic deprotection and hydrolysis reactions. The previous reaction conditions were based upon a highly selective hydrolysis protocol for organonitrile compounds (Moorthy and Singhal 2005). This method proved to be efficient in this series of research experiments (Table 4, Entry 1). Given the fact that the synthesis and application of carbon-11

Table 4 Optimization of acidic deprotection and hydrolysis conditions



Entry	Acidic hydrolysis conditions	[^{11}C]nitrile **3** yield (%)[a]	Overall yield (%)[b]	Radiochemical and optical purities (%)[c]
1	120 °C for 10 min TFA/H_2SO_4 (4/1, V/V)	94	46	95/1.6
2[d]	110 °C for 8 min, 0.1 M HCl for acidic hydrolysis	80	64	76/13
3[e]	90 °C for 10 min TFA/H_2SO_4 (4/1, V/V)	83	59	97/0.5
4[f]	90 °C for 7 min TFA/H_2SO_4 (4/1, V/V)	76	55	94/0.3
5	90 °C for 5 min TFA/H_2SO_4 (4/1, V/V)	**90**	**60**	**96/0.1**
6	60 °C for 5 min TFA/H_2SO_4 (4/1, V/V)	86	60	95/1.6

Bold values indicate the significance of the best acidic hydrolysis/deprotection reaction conditions

[a] Isolated yields and decay-corrected yield (DCY) based on total collected [^{11}C]HCN activity

[b] Isolated yields and DCY based on total collected [^{11}C]HCN activity

[c] Radiochemical and optical purity data are reported as percentages of both L- and D-[^{11}C]Gln in final products

[d] Radiosynthesis started with 0.87 GBq (23.6 mCi) [^{11}C]CsCN radioactivity

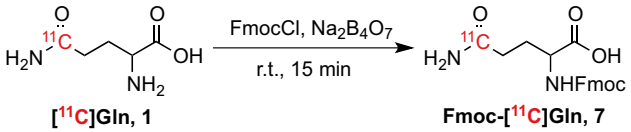
[e] Radiosynthesis started with 0.65 GBq (17.5 mCi) [^{11}C]CsCN radioactivity

[f] Radiosynthesis started with 0.73 GBq (19.8 mCi) [^{11}C]CsCN radioactivity

($t_{1/2}$ = 20.4 min)-labeled radiotracers must be "working against time" (Fowler and Wolf 1997), it is essential to optimize the rate of reaction, which will not only help to increase the actual amount of radiotracer available for biological applications, but also possibly decrease the radiation exposure to the radiochemist. In this investigation, a method (0.1 M HCl, 110 °C) that had been used to synthesize [^{11}C]asparagine was first tested for the hydrolysis of [^{11}C]nitrile 3 and the results were unsatisfactory (Gillings and Gee 2001). An increased amount of D-[^{11}C]Gln was detected in the final product (Entry 2). Next, a mixed, strong acid system (TFA/H_2SO_4, 4/1, V/V) was tested with lower reaction temperatures and shorter reaction times (Entry 3–6) (Moorthy and Singhal 2005; Qu et al. 2012). The results were satisfactory: the intermediate [^{11}C]nitrile 3 could be successfully converted to the desired L-[^{11}C]Gln in 5 min with heating in the temperature range of 60–120 °C. Eventually, the conditions listed in Entry 5 were chosen as the standard conditions for the second step of synthesis of L-[^{11}C]Gln.

Specific activity (SA) measurement, method development and finalization of the complete radiosynthesis process

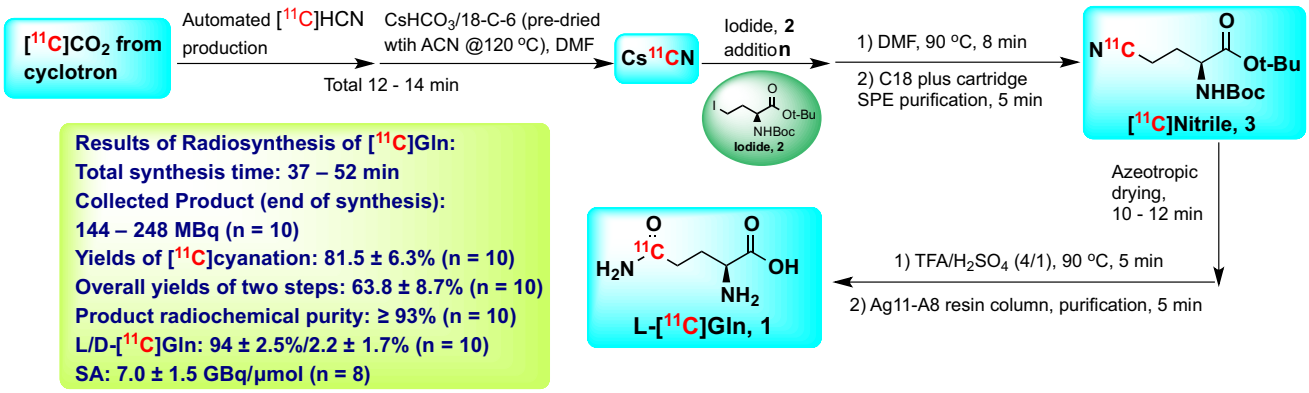
Specific activity (SA) is an important property for most PET radiotracers (Lapi and Welch 2013). The SA of [^{11}C]Gln in the original report (Qu et al. 2012) was based on an analytical radio-HPLC method. Since there are only micrograms of carbon-12-labeled glutamine ([^{12}C]Gln)



Scheme 3 Fmoc derivatization for SA measurement of [^{11}C]Gln

in the final product, the UV absorption signal of [^{12}C]Gln from the analytical sample was quite small and this SA analytical method can only provide a crude estimation of the SA of this important radiotracer. For a more accurate measurement, an Fmoc-derivatization method (Zhou et al. 2011) was adapted to convert [^{11}C]Gln to Fmoc-[^{11}C]Gln (Scheme 3). By reacting a sub-nanomole amount of [^{11}C/^{12}C]Gln sample with 9-fluorenylmethoxycarbonyl chloride (Fmoc-Cl), the majority of [^{11}C/^{12}C]Gln was converted to Fmoc-[^{11}C/^{12}C]Gln product and the very strong UV absorption of Fmoc chromophore at 263 nm and the high hydrophobicity of this functional group assured an accurate HPLC measurement of the SA.

Once the method for SA measurement was developed, the refinement for synthesis of the biologically interesting PET radiotracer L-[^{11}C]Gln was complete (Scheme 4). By replacing the strong base (KOH) with a weak base ($CsHCO_3$) and utilizing a neutral PTC (18-C-6), the tedious and time-consuming reagent and solvent preparation requiring a glove box was eliminated. This change obviously simplified the synthesis process. In addition, the



Scheme 4 Optimized process for synthesis of L-[^{11}C]Gln

investigation of other [^{11}C]cyanation reaction parameters, such as solvent, reaction temperature, reaction time, etc., helped to further improve the [^{11}C]cyanation reaction. So far, the highest radiochemical yield of [^{11}C]cyanation we obtained was 94 % (DCY, based upon starting [^{11}C]HCN radioactivity), which is exceptionally high for general ^{11}C-radiosynthesis reactions. Subsequent exploration to improve the acidic deprotection and hydrolysis reactions helped to shorten the reaction time even under the milder reaction conditions (60 °C vs 120 °C). Given the short half-life of carbon-11 ($t_{1/2}$ = 20.4 min), the shorter overall synthesis time will greatly facilitate the application of L-[^{11}C]Gln in biology and medicine. To further confirm the robustness of our current synthetic method, we repeated the synthetic process of L-[^{11}C]Gln ten times under the optimized reaction conditions (Scheme 4). The results show the high reliability of this newly established synthetic process. With 1 min cyclotron beam time, which generates ~3.7 GBq (100 mCi) [^{11}C]CO_2 radioactivity, 144–248 MBq (3.9–6.7 mCi) [^{11}C]Gln product was obtained at the end of synthesis (EOS) with a total synthesis time ranging from 37 to 52 min. The overall yield (DCY) of the complete synthesis was 63.8 ± 8.7 %. The radiochemical purity of the final product was ≥93 %, with L-[^{11}C]Gln > 90 % and D-[^{11}C]Gln < 4 %. The SA of the final product [^{11}C]Gln, based upon our Fmoc-derivatization method, was 7.0 ± 1.5 GBq/μmol.

Conclusion

In summary, we have developed an improved method for synthesizing the biologically interesting PET radiotracer L-[^{11}C]Gln, which features mild reaction conditions and increased yields. The synthesis process was repeated ten times under optimized reaction conditions and statistical data proved the reproducibilty of this method. Additionally, the conditions that we report here are amenable to full-scale automation and would also be adaptable to a commercially available radiosynthesizer. Moreover, by systematic investigation of reaction parameters which included varied [^{11}C]cyanide trapping conditions, PTC, solvents, temperatures and reaction times, as well as investigating the impact of a trace amount of H_2O, etc., we now have a better understanding of the S_N2 [^{11}C]cyanation of a base- and moisture-sensitive substrate with non-carrier-added conditions. The results of this systematic investigation should be very beneficial to future [^{11}C]cyanide-based radiotracer development.

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Conflict of interest The authors declare that they have no conflict of interest.

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