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High-Yielding Automated Convergent Synthesis of No-Carrier-Added [^{11}C -Carbonyl]-Labeled Amino Acids Using the Strecker Reaction

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Abstract

A new variant of the Strecker synthesis using no-carrier-added [^{11}C]cyanide for the synthesis of radiolabeled amino acids is described. The protocol is fully automated using a radiochemistry synthesis module and applied to the production of a number of new PET radiotracers. [^{11}C -Carbonyl]sarcosine, [^{11}C -carbonyl]methionine, [^{11}C -carbonyl]-*N*-phenylglycine, and [^{11}C -carbonyl]glycine are all synthesized in moderate to good radiochemical yields. The synthesis of [^{11}C -carbonyl]sarcosine has been validated for production of doses for clinical use, and preliminary evaluation of the new radiotracer in PC3 tumor-bearing mice is also reported.

Graphical abstract

Keywords

PET imaging; PET radiochemistry; carbon-11

Positron emission tomography (PET) imaging is a functional imaging technique in which patients are injected with a radiotracer, a bioactive molecule tagged with a positron-emitting radionuclide such as ^{11}C or ^{18}F .¹ Increasing availability of PET instrumentation around the

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Supporting Information

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world is associated with the expanding use of PET imaging in individualized healthcare and drug discovery.² Unmet clinical challenges in the early diagnosis and/or staging of disease, as well as the pharmaceutical industry's need to radiolabel new drug scaffolds on an ongoing basis, has created an urgency for development of new PET radiotracers as well as reliable methods for their production.

Radiolabeled amino acids and related compounds are an important group of PET radiotracers used in oncologic imaging, as well as applications in neuroimaging such as neuro-oncology and Parkinson's disease.³ When radiolabeling such molecules, it is desirable to use ^{11}C so that the final product is structurally identical to the endogenous molecule. This simplifies translation into clinical use because extensive pharmacology and toxicology testing is often not required for radiolabeled versions of endogenous molecules in contrast to, for example, fluorinated analogues designed for incorporation of ^{18}F (e.g., [^{11}C]choline vs. [^{18}F]fluorocholine). Moreover, the half-life of ^{11}C (20 min) allows dual radiotracer studies to be undertaken in a patient on the same day, during a single hospital visit.

In connection with our program developing PET radiotracers based upon sarcosine for prostate cancer imaging, we initially synthesized [^{11}C]sarcosine **1**, which was readily accessible by simple N-methylation of methyl glycinate with [^{11}C]methyl triflate, followed by ester hydrolysis (see Supporting Information for details). While we were pleased to see the expected uptake of the radiotracer in preclinical models of prostate cancer (see Supporting Information),⁴ we had concerns that volatile [^{11}C]CO₂ would be exhaled during PET scans because of the metabolic demethylation of sarcosine promoted by sarcosine dehydrogenase.⁵ This enzyme converts [^{11}C]sarcosine into glycine, with release of [^{11}C]formaldehyde, with the latter being subsequently converted into [^{11}C]CO₂, via [^{11}C]formic acid, and exhaled. These concerns proved well founded, and the charcoal filters on the rodent anesthesia machines were found to have retained significant radioactivity (ca. 8–10% of the injected dose) following the pre-clinical PET scans. To overcome this limitation of the radiotracer, we next wished to evaluate [^{11}C -carbonyl]sarcosine **2** as an alternate design (Figure 1), reasoning that moving the labeled carbon would reduce or eliminate exhalation of volatile [^{11}C]CO₂.

The synthesis of [^{11}C -carbonyl]sarcosine **2** was expected to be more complicated. To label the carbonyl moiety, we initially focused on [^{11}C]CO₂ fixation strategies recently reported by our group⁶ and others,⁷ using either a Grignard reagent or organolithium, but the key halogenated intermediate required for either approach proved unstable (see Supporting Information). We next considered whether **2** could be accessed using [^{11}C]cyanide, either via a nucleophilic substitution, or using the Strecker reaction, and subsequent conversion of the cyanide into the carboxylic acid. Similar to the organometallic approaches described above, the requisite precursor for the S_N2 approach could never be synthesized (see Supporting Information), and we therefore focused our efforts on the Strecker reaction.

The Strecker reaction, first reported in 1850,⁸ is a valuable multicomponent reaction for synthesizing amino acids from aldehydes or ketones (Scheme 1).⁹ Traditionally, an aldehyde is condensed with ammonium chloride in the presence of a source of cyanide (usually potassium cyanide) to form an α -aminonitrile, which is subsequently hydrolyzed to yield the

corresponding amino acid. Radiochemical versions,¹⁰ employing [¹¹C]cyanide^{10a-h,10j-n} or [¹¹C]aldehydes,¹⁰ⁱ have also been developed for the convergent radiosynthesis of amino acid based PET tracers. We noted several limitations with these early examples, however: i) most reported examples require the need for addition of carrier cyanide or intermediate purifications, likely due to residual components from production of [¹¹C]cyanide (e.g., ammonia) poisoning the reaction(s); ii) carrier-added syntheses by definition provide radiotracers with lower specific activities; and iii) early reactions were often conducted in sealed tubes, because of the high temperatures and pressures required, making them incompatible with modern automated radiochemistry synthesis modules. To address this, we have modified the Strecker reaction and herein report a new no-carrier-added (n.c.a.) version that is fully automated using a TRACERLab synthesis module and apply it to the synthesis of [¹¹C-*carbonyl*]sarcosine as well as several other labeled amino acids of interest to our clinical and preclinical imaging colleagues.

The proposed Strecker synthesis of [¹¹C-*carbonyl*]sarcosine required condensation of formaldehyde with methylamine and [¹¹C]NaCN to generate [¹¹C]α-aminonitrile **3** (Table 1). In preliminary work, we tested the reaction with carrier added NaCN. Cyclotron-produced [¹¹C]CO₂ was converted into [¹¹C]HCN using published methods.¹¹ [¹¹C]HCN was trapped on a column composed of platinum wire coated with NaOH to simultaneously purify it (removing excess NH₃) and convert into [¹¹C]NaCN.^{10c} [¹¹C]NaCN was then eluted from the column with aqueous NaCN into an aqueous solution of methylamine hydrochloride and formaldehyde. The reaction was stirred at room temperature for five minutes and resulted in 63% radiochemical conversion (RCC¹²) to α-aminonitrile **3** (Table 1, entry 1). Extending the reaction time to 12 minutes resulted in a negligible increase in RCC to 65% (Table 1, entry 2). We next investigated the effect of temperature on the reaction. Heating at 50 °C for five minutes also had little impact, and **3** was obtained in 68% RCC (Table 1, entry 3). Increasing the reaction temperature to 80 °C gave 81% RCC to **3** (Table 1, entry 4) but, notably, we observed the appearance of several other radiolabeled impurities in the crude reaction mixture that we anticipated could be difficult to separate from **3**. Given the minimal effect of varying reaction time on RCC and potential impurity profile at higher temperatures, we elected to move forward with the original conditions (r.t. for 5 min, Table 1, entry 1). We next attempted the reaction using water to elute n.c.a. [¹¹C]NaCN off the platinum wire, and were gratified to observe 49% RCC to [¹¹C]α-aminonitrile **3** (Table 1, entry 5).

Hydrolysis of **3** to generate [¹¹C-*carbonyl*]sarcosine **2** was next investigated. Literature reports typically employ 10 M NaOH at 100 °C to convert nitriles into carboxylic acids,¹⁰ and employing these standard conditions for five minutes resulted in 59% RCC to **2** (Table 2, entry 1). Attempts to reduce the concentration of NaOH were unsuccessful, as using 5 M NaOH resulted in reduced RCC of 15% (Table 2, entry 2). The hydrolysis of **3** was also sensitive to temperature, and reducing the hydrolysis temperature to 60 °C resulted in a significantly improved RCC of 97% (Table 2, entry 3).

The long-term goal of this work is clinical evaluation of [¹¹C-*carbonyl*]sarcosine in our prostate cancer patient population. It was therefore necessary to adapt this optimized radiosynthesis for use in a radiochemistry synthesis module to enable a fully automated

synthesis compliant with current Good Manufacturing Practice (cGMP) regulations. To accomplish this, a GE TRACERLab FX_M synthesis module was adapted to enable automated Strecker reactions. Briefly, two sets of electronic valves were installed on the module that allowed delivery of [¹¹C]HCN from the cyclotron and automated purification using the platinum wire described above (see Supporting Information for full details of module modifications). The optimized conditions worked out above were then programmed into the module. Following synthesis, the crude reaction mixture was purified by semipreparative HPLC. A range of HPLC conditions were tested (see Supporting Information), and an acetonitrile/sodium dihydrogen phosphate mobile phase in combination with a Luna-NH₂ amino-capped silica reverse-phase column proved optimal. The fraction corresponding to [¹¹C-*carbonyl*]sarcosine **2** was collected and reformulated into an injectable buffer suitable for intravenous injection. Three qualification runs were completed using this automated method, qualifying the site for production of [¹¹C-*carbonyl*]sarcosine for use in clinical PET studies.¹³ Preliminary imaging in PC-3 human prostate cancer bearing nude mice revealed excellent uptake and better retention of the tracer in the tumor (Figure 2) as well as slower metabolism. Clinical trials in prostate cancer patients have commenced and results will be communicated in due course.

The general applicability of this automated Strecker reaction was next investigated by applying it to the synthesis of a number of radiolabeled amino acids using different amines and carbonyl compounds (Table 3).¹⁴ For example, [¹¹C-*carbonyl*]methionine **4** was prepared from n.c.a. [¹¹C]NaCN with 3-(methylthio)propanal and ammonium hydroxide (Table 3, entry 1). Analogous conditions using formaldehyde were attempted to synthesize [¹¹C-*carbonyl*]glycine **5** (Table 3, entry 2), but no product was obtained in this case. Switching the amine to ammonium carbonate, per a previous literature report,^{10g,j} generated [¹¹C-*carbonyl*]glycine **5** (Table 3, entry 3). We also explored variation of the amine and carbonyl sources used in the reaction. Thus, the synthesis of [¹¹C-*carbonyl*]-*N*-phenylglycine **6** demonstrated that anilines were tolerated, although the initial Strecker reaction required heating at 80 °C (Table 3, entry 4). Changing the carbonyl source from an aldehyde to a ketone proved more challenging, however. For example, we wished to synthesize 2-amino-2-methyl-3-phenylpropanoic-1-¹¹C acid from 1-phenylpropan-2-one and ammonia using n.c.a. [¹¹C]NaCN. However, the Strecker reaction resulted in none of the desired [¹¹C]α-aminonitrile **7**, even if the reaction was heated (Table 3, entry 5). Since ketones are known to react more slowly than aldehydes in the Strecker reaction,¹⁵ this was not entirely unexpected. To address this issue, we repeated the reaction in the presence of one equivalent of carrier NaCN and were pleased to see 4% RCC to the desired [¹¹C]α-aminonitrile **7** (Table 3, entry 6). While the need to add carrier NaCN when using ketones is not ideal, it does offer a means for accessing initial doses of new radiotracers for preliminary evaluation, recognizing that a n.c.a. synthesis would [likely] be needed prior to translation into any clinical imaging trials.

In summary, a no-carrier-added Strecker amino acid synthesis has been developed, which enables the fully automated production of carbon-11-labeled amino acids using modern radiochemistry synthesis platforms. The reaction is tolerant of a range of amines and carbonyl sources, although the use of ketones does require the addition of cyanide carrier.

Suitability for production of clinical radiotracer doses has been demonstrated through validation of a method for the synthesis of [^{11}C -carbonyl]sarcosine. This production method is being used in our facility to prepare doses of this new radiotracer for use in ongoing clinical trials imaging prostate cancer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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 12. Radiochemical conversion was determined by HPLC; %RCC = (area of product radioactivity peak/total area of all radioactive peaks) × 100.
 13. **Automated Synthesis of [¹¹C-Carbonyl]sarcosine 2** Methylamine hydrochloride (0.5 mg in 50 μL H₂O) and 37% formaldehyde (0.56 μL in 50 μL H₂O) were added to the reaction vessel of a TRACERLab FXm synthesis module. [¹¹C]NaCN (ca. 900 mCi) was delivered to the reaction vessel from the PETTrace cyclotron, and the reaction was stirred for 5 min at r.t. 10 M NaOH (250 μL) was then added to the reaction vessel and was heated to 60 °C for 5 min to perform the hydrolysis. The crude reaction mixture was cooled, diluted with HPLC mobile phase (0.6 mL), and purified by semipreparative HPLC (column: Phenomenex Luna NH₂ 250 × 10 mm; mobile phase: 10 mM NaH₂PO₄ in 60% MeCN, pH 5.6; flow rate: 4 mL/min; *t_R* = 10 min). The fraction corresponding to [¹¹C-carbonyl]sarcosine was collected into 60 mL of Milli-Q water containing 1 M NaOH (0.2 mL), and the resulting solution was passed through a SAX cartridge to trap the radiotracer. The cartridge was washed with sterile water for injection, USP (SWI) and then [¹¹C-carbonyl]sarcosine and eluted with 2 M NaCl (0.5 mL) into a product collection vial precharged with sterile water (4.3 mL) and sodium phosphates, USP (0.2 mL). The SAX cartridge was rinsed with additional SWI (5 mL) to give a final dose of 10 mL. This dose was passed through a 0.22 μm sterile filter into a sterile dose vial and submitted for QC testing (see Supporting Information for details of QC methods). The yield of [¹¹C-carbonyl]sarcosine **2** was 30±12 mCi (n = 3), corresponding to 1% isolated and formulated end-of-synthesis radiochemical yield (RCY) based on [¹¹C]CO₂, or 3% based on [¹¹C]HCN). The product was also obtained in high radiochemical purity (RCP >90%) and specific activity (SA >1500 Ci/mmol using the 0.3 μg/mL HPLC limit-of-detection for sarcosine).
 14. **General Procedure (Table 3, Entries 1–4)** ■■■details differ from table 3■■■The carbonyl compound (1 equiv) and amine (1–3 equiv) in water and/or EtOH were added to the reaction vessel of a TRACERLab FXm synthesis module. [¹¹C]NaCN (ca. 100–800 mCi) in H₂O (200–250 μL) was added, and reaction was stirred for 5–7 ■■■5?■■■ min at r.t. to 100■■■80?■■■ °C. After this time, 10 M NaOH (250–350 μL) was added, and the reaction mixture was heated at 60–100■■■80?■■■ °C for 5–7■■■5?■■■ min. The reaction was cooled, diluted, and purified by semipreparative HPLC to yield [¹¹C-carbonyl] methionine **4** (RCY = 5%; RCP >90%; SA = 1256 Ci/mmol), [¹¹C-carbonyl]glycine **5** (RCY = 14%; RCP >95%; SA >1500 Ci/mmol), or [¹¹C-carbonyl]-*N*-phenylglycine **6** (RCY = 2%; RCP >99%; SA = 15453 Ci/mmol).
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Figure 1.
Radiolabeled versions of sarcosine under evaluation

Figure 2. PET images of nude mouse showing uptake of [^{11}C -carbonyl]sarcosine **2** in implanted PC-3 tumor (a) and associated tumor and muscle time-radioactivity curves (b)

Scheme 1.
The Strecker reaction

Table 1Synthesis of [^{11}C]- α -Aminonitrile **3** via the Strecker Reaction

Entry	$\text{MeNH}_2\cdot\text{HCl}/\text{HCHO}/\text{NaCN}$	Time (min)	Temp ($^{\circ}\text{C}$)	RCC (%) ^a
1	1:1:1	5	r.t.	63
2	1:1:1	12	r.t.	65
3	1:1:1	5	50	68
4	1:1:1	5	80	81
5	1:1:0 (n.c.a.)	5	r.t.	49

^aRCC: radiochemical conversion.

Table 2Hydrolysis of **3** to Generate [^{11}C -Carbonyl]sarcosine **2**

Entry	[NaOH]	Time (min)	Temp ($^{\circ}\text{C}$)	RCC (%) ^a
1	10 M	5	100	59
2	5 M	5	100	15
3	10 M	5	60	97

^aRCC: radiochemical conversion.

Table 3Application of the Method to Other ^{11}C -Labeled Amino Acids

Entry	Amine	Carbonyl	Product	RCY (%) ^a
1	NH ₄ OH			5
2	NH ₄ OH			0
3	(NH ₄) ₂ CO ₃			14
4	PhNH ₂			2
5	NH ₃ ·H ₂ O			0
6	NH ₃ ·H ₂ O			4 ^b

^a Isolated and formulated noncorrected radiochemical yields (RCY) based upon [^{11}C]HCN.^b Radiochemical conversion.