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RESEARCH ARTICLE

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Radiosynthesis of the ^{11}C-methyl derivative of LBQ657 for PET investigation of the neprilysin inhibitor sacubitril

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Neprilysin, also known as neutral endopeptidase, is a cell surface membrane metalo-endopeptidase that cleaves various peptides. Altered neprilysin expression has been correlated with various cancers and cardiovascular diseases. In this work, we present the radiosynthesis of the novel *O*-^{11}C-methylated derivative of LBQ657 (a potent neprilysin inhibitor). (2*R*,4*S*)-5-(Biphenyl-4-yl)-4-[(3-carboxypropionyl)amino]-2-methylpentanoic acid [^{11}C]methyl ester ([^{11}C]MeOLBQ) is an analog of sacubitril where the alkyl ester is a ^{11}C-methyl instead of an ethyl. [^{11}C]MeOLBQ was produced in a one-pot two-step synthesis. The *O*-^{11}C-methylation of the pentanoic acid part was done with [^{11}C]methyl triflate followed by the deprotection of the *tert*-butyl ester precursor in acidic conditions. [^{11}C]MeOLBQ ([^{11}C]**7**) was produced in 9.5 ± 2.5% RCY (25 ± 6% decay-corrected from [^{11}C]CO_2, n = 3) high molar activity 348 ± 100 GBq/μmol (9425 ± 2720 mCi/μmol) at EOS, in high chemical (>95%) and radiochemical (>99%) purities. The total synthesis time including HPLC purification and reformulation was 29 minutes. To our knowledge, this is the first PET-labeled analog of the clinically used NEP inhibitor sacubitril.

KEYWORDS
carbon-11, ^{11}C-esterification, heart failure, natriuretic peptides, radiosynthesis, sacubitril

1 | INTRODUCTION

Neprilysin (NEP), also called neutral endopeptidase, enkephalinase, or CD10, is a zinc dependent type II integral membrane peptidase that cleaves peptides such as substance P, endothelin-1, natriuretic peptides, and encephalin and thus regulates their physiological action by reducing their availability at the cell surface.[1] NEP is widely expressed in mammalian tissues, such as kidneys, brain, heart, and lung.[2] In cancer, by regulating the biological activities of peptide substrates, NEP plays an important role in maintenance of homeostasis, neoplastic transformation, and tumor progression.[3,4] Elevated NEP expression in several solid tumors and carcinomas has been reported to be a marker of cancer progression.[5,6] NEP is also a target of choice for therapeutic intervention in heart failure. NEP plays a key role into cardiorenal disorders. By breaking down natriuretic peptides, NEP

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participates in the regulation of the sodium level, water balance, and blood pressure homeostasis.[7-9] Blocking this enzyme with NEP inhibitors (NEPi) increases the concentration of the natriuretic peptides and thus has therapeutic potential.[10,11] It has been demonstrated that a combined therapy consisting of the NEPi sacubitril and the angiotensin II blocker valsartan (ie, Entresto, Novartis) improved the outcome in the treatment of heart failure and patient survival.[12] Sacubitril is metabolized into the desethylated active NEPi sacubitrilat (LBQ657).[13] Taking into account the terminal half-life of 0.7 ± 0.3 hours in humans[14] and interpatient variability of Sacubitril,[15] the novel ^{11}C-methylated derivative of the potent NEPi LBQ657 (as opposed to the ethyl ester derivative of LBQ657, sacubitril) was developed with the potential to investigate in vivo the hydrolysis and metabolism of an alkylated ester analog allowing for a better understanding of the distribution and pharmacokinetics of the prodrug. This paper describes the radiosynthesis of the ^{11}C-methylated derivative of LBQ657 ([^{11}C]MeOLBQ).

2 | EXPERIMENTAL

2.1 | Materials

Commercially available chemicals were used without further purification unless otherwise noted. The syntheses of 1-*O*-*tert*-butyl 4-*O*-(2,5-dioxopyrrolidin-1-yl) butanedioate (**8**) and 1-*O*-benzyl 4-*O*-(2,-5-dioxopyrrolidin-1-yl) butanedioate (**4**) were completed as reported previously.[16,17] Flash chromatography was carried out with silica gel (40-60 μm). ^{1}H NMR and ^{13}C NMR spectra were acquired with a Bruker 300- MHz spectrometer at ambient temperature. Spectral data are reported in parts per million (ppm) using residual solvent as a reference. High resolution and accurate mass measurements were done in positive mode by flow injection analysis into a Thermo Scientific Q-Exactive Plus Orbitrap Mass Spectrometer (San Jose, CA, USA) interfaced with a heated electrospray ion source. A synthesis platform (Synthra MeIplus Research) was utilized for automated radiotracer synthesis, purification, and reformulation. Sep-Pack Vac C18 plus short (100 mg, Waters) solid-phase extraction cartridges were preconditioned by passing through ethanol (10 mL) followed by deionized water (20 Ml). Semipreparative HPLC was performed with a Phenomenex Luna C18 (2) HPLC column (250 × 10 mm, 10 μm) at a flow rate of 7 mL/min (4.5:5 acetonitrile/ammonium formate 0.1M, pH 4.8). Analytical HPLC was carried out on a Luna C18 (2) column (250 × 4.6 mm, 10 μm) at a flow rate of 3 mL/min (1:1 CH_3CN/H_2O + 0.1% TFA) with a Waters instrument equipped with a UV detector (254 nm) and Raytest Gabi Star radioactivity detector.

2.2 | Chemistry

2.2.1 | (3*R*,5*S*)-5-Biphenyl-4-ylmethyl-3-methylpyrrolidin-2-one (2)

(2*R*,4*S*)-4-Amino-5-(biphenyl-4-yl)-2-methylpentanoic acid ethyl ester (**1**) (500 mg, 1.43 mmol) was dissolved in 1:1 THF/MeOH (12 mL) and 2M NaOH (3.6 mL) was added dropwise. The reaction mixture was quenched with water and extracted with EtOAc (3 × 30 mL). The organic fractions were combined, washed with brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. Purification by silica column chromatography (30% EtOAc in hexanes) afforded compound **2** (360 mg, 1.35 mmol, 95%) as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.56 (t, *J* = 8.2 Hz, 4H), 7.44 (t, *J* = 7.4 Hz, 2H), 7.34 (t, *J* = 7.3 Hz, 1H), 7.25 (d, *J* = 7.8 Hz, 2H), 6.00 (s, 1H), 3.94-3.75 (m, 1H), 2.91-2.70 (m, 2H), 2.56-2.39 (m, 1H), 2.21-2.07 (m, 1H), 1.99-1.83 (m, 1H), 1.20 (d, *J* = 7.2 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 180.5, 140.8, 139.9, 136.8, 129.6, 128.9, 127.6, 127.4, 127.1, 53.5, 42.5, 35.2, 35.0, 16.3. HRMS (ESI): Exact mass calcd for ($C_{18}H_{19}NO$) $[M + H]^+$: 266.1539. Found: 266.1537.

2.2.2 | (2*R*,4*S*)-4-Amino-5-biphenyl-4-yl-2-methylpentanoic acid hydrochloride (3)

Compound **2** (300 mg, 1.13 mmol) was dissolved in 1:1 acetic acid/HCl (20 mL). The reaction mixture was stirred under reflux for 20 hours and then concentrated under reduced pressure. Recrystallization from 9:1 EtOAc/acetic acid gave the product **3** (290 mg, 0.90 mmol, 80%) as a yellow powder. ^{1}H NMR (300 MHz, DMSO) δ 7.65 (t, J = 7.5 Hz, 1H), 7.46 (t, *J* = 7.5 Hz, 1H), 7.37 (d, *J* = 7.7 Hz, 1H), 3.64-3.23 (m, 1H), 3.13-2.97 (m, 1H), 2.95-2.78 (m, 1H), 2.74-2.57 (m, 1H), 1.93-1.77 (m, 1H), 1.67-1.49 (m, *J* = 13.7 Hz, 1H), 1.06 (d, *J* = 6.9 Hz, 1H). ^{13}C NMR (75 MHz, DMSO) δ 177.0, 140.2, 139.1, 136.7, 130.5, 129.4, 127.9, 127.7, 127.0, 50.8, 38.6, 36.2, 35.4, 18.0. HRMS (ESI): Exact mass calcd for ($C_{18}H_{22}NO_2^+$) $[M - HCl + H]^+$: 284.1645. Found: 284.1644.

2.2.3 | (2*R*,4*S*)-5-(Biphenyl-4-yl)-4-(4-(benzyloxy)-4-oxobutanamido)-2-methylpentanoic acid (5)

Compounds **3** (250 mg, 0.78 mmol), **4** (358 mg, 1.17 mmol, 1.5 eq.), and DMAP (955 mg, 7.8 mmol, 10 eq.) were dissolved in 1:1 DMF/PBS (20 mL), and the reaction

was stirred at room temperature for 18 hours. The mixture was quenched with water (20 mL) and extracted with EtOAc (3 × 30 mL). The organic fractions were combined and washed with 2N HCl (30 mL), water (20 mL), and brine (30 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. Compound **5** (245 mg, 0.51 mmol, 65%) was purified by silica column chromatography (40% EtOAc in hexanes). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.59-7.48 (4H, m), 7.46-7.38 (2H, m), 7.37-7.28 (6H, m), 7.21 (2H, d, J = 8.2), 6.03 (1H, d, J = 8.8), 5.09 (2H, s, J = 2.6), 4.40-4.24 (1H, m), 2.92-2.39 (7H, m), 1.99-1.87 (1H, m), 1.60-1.48 (1H, m), 1.16 (3H, d, J = 6.9). ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.7, 173.1, 172.8, 140.7, 139.7, 136.1, 135.8, 129.6, 128.8, 128.6, 128.4, 128.2, 127.3, 127.3, 127.0, 66.7, 49.0, 40.7, 39.7, 36.5, 31.0, 29.5, 17.8. HRMS (ESI): Exact mass calcd for ($C_{29}H_{31}NO_5$) $[M + H]^+$: 474.2275. Found: 474.2275.

2.2.4 | Methyl (2*R*,4*S*)-5-(biphenyl-4-yl)-4-(4-(benzyloxy)-4-oxobutanamido)-2-methylpentanoate (6)

Compound **5** (35 mg, 0.07 mmol) and Na_2CO_3 (12 mg, 0.11 mmol, 1.6 eq.) were mixed in DMF (5 mL). Methyl iodide (15 mg, 0.11 mmol, 1.6 eq.) was added, and the solution was stirred at room temperature for 4 hours. The reaction was quenched with water (20 mL) and extracted with EtOAc (3 × 15 mL). The organic fractions were combined and washed with 2N HCl (15 mL), water (20 mL), and brine (15 mL). The solution was then dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The product **6** (32 mg, 0.06 mmol, 88%) was purified by silica column chromatography (50% EtOAc in hexanes). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.58 (d, J = 7.1 Hz, 2H), 7.52 (d, J = 8.2 Hz, 2H), 7.43 (t, J = 7.4 Hz, 2H), 7.37-7.29 (m, 6H), 7.24 (d, J = 8.2 Hz, 2H), 5.62 (d, J = 8.8 Hz, 1H), 5.11 (s, 2H), 4.36-4.17 (m, 1H), 3.67 (s, J = 2.9 Hz, 3H), 2.83 (d, J = 6.4 Hz, 2H), 2.71-2.63 (m, 2H), 2.62-2.51 (m, 1H), 2.42 (t, J = 6.8 Hz, 2H), 2.00-1.88 (m, 1H), 1.59-1.45 (m, 1H), 1.16 (d, J = 7.1 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.8, 172.9, 170.9, 140.8, 139.4, 136.7, 135.8, 129.9, 128.8, 128.6, 128.3, 128.2, 127.2, 127.1, 127.0, 66.5, 51.8, 48.4, 40.5, 37.6, 36.4, 31.2, 29.6, 17.7. HRMS (ESI): Exact mass calcd for ($C_{30}H_{33}NO_5$) $[M + H]^+$: 488.2428. Found: 488.2428.

2.2.5 | (2*R*,4*S*)-5-(Biphenyl-4-yl)-4-[(3-carboxypropionyl)amino]-2-methylpentanoic (7), MeOLBQ standard

Compound **6** (25 mg, 0.05 mmol) was dissolved in methanol (6 mL); 10 wt% Pd/C (3 mg, 0.002 mmol, 0.04 eq.) was added, and the reaction was stirred at room temperature for 5 minutes under nitrogen atmosphere. The reaction was then placed under an atmosphere of H_2 (balloon) which was bubbled through the mixture for 20 minutes. The reaction was stirred for an additional 20 minutes, filtered over celite, and concentrated under reduced pressure. The product **7** (18 mg, 0.04 mmol, 88%) was purified by silica column chromatography (50% EtOAc in hexanes + 0.1% acetic acid). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.56 (d, J = 7.1 Hz, 2H), 7.51 (d, J = 8.1 Hz, 2H), 7.41 (t, J = 7.4 Hz, 2H), 7.32 (t, J = 7.3 Hz, 1H), 7.22 (d, J = 8.1 Hz, 2H), 6.02 (d, J = 8.8 Hz, 1H), 4.32-4.15 (m, 1H), 3.65 (s, 3H), 2.90-2.76 (m, 2H), 2.66-2.51 (m, 3H), 2.41 (t, J = 6.5 Hz, 2H), 2.00-1.85 (m, 1H), 1.63-1.47 (m, 1H), 1.15 (d, J = 7.1 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.9, 176.6, 172.0, 140.8, 139.4, 136.6, 129.8, 128.8, 127.2, 127.1, 127.0, 51.9, 48.8, 40.5, 37.4, 36.4, 30.8, 29.7, 17.6. HRMS (ESI): Exact mass calcd for $C_{23}H_{28}NO_5$ $[M + H]^+$: 398.1962. Found: 398.1961.

2.2.6 | (2*R*,4*S*)-5-(Biphenyl-4-yl)-4-(4-(*tert*-butoxy)-4-oxobutanamido)-2-methylpentanoic acid (9)

Compounds **3** (50 mg, 0.15 mmol), **8** (85 mg, 0.31 mmol), and DMAP (191 mg, 1.5 mmol) were dissolved in 1:1 DMF/PBS (20 mL), and the reaction was stirred at room temperature for 18 hours. The mixture was quenched with 10% citric acid (20 mL) and extracted with EtOAc (3 × 35 mL). The organic fractions were combined and washed with 10% citric acid (2 × 50 mL), water (50 mL), and brine (30 mL). The solution was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. Compound **9** (23 mg, 0.05 mmol, 34%) was purified by silica column chromatography (40% EtOAc in hexanes + 0.1% acetic acid). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.57 (d, J = 7.1 Hz, 2H), 7.52 (d, J = 8.2 Hz, 2H), 7.43 (t, J = 7.4 Hz, 2H), 7.33 (t, J = 7.3 Hz, 1H), 7.23 (d, J = 8.2 Hz, 2H), 6.16 (d, J = 8.8 Hz, 1H), 4.40-4.22 (m, 1H), 2.96-2.75 (m, 2H), 2.69-2.32 (m, 5H), 1.99-1.86 (m, 1H), 1.60-1.47 (m, 1H), 1.42 (s, 9H), 1.17 (d, J = 6.8 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.8, 172.9, 170.9, 140.8, 139.4, 136.7, 135.8, 129.9, 128.8, 128.6, 128.3, 128.2, 127.2, 127.1, 127.0, 66.5, 51.8, 48.4, 40.5, 37.6, 36.4, 31.2, 29.6, 17.7. HRMS (ESI): Exact mass calcd for ($C_{26}H_{33}NO_5$) $[M + H]^+$: 440.2431. Found: 440.2431.

2.3 | Radiochemistry

2.3.1 | Production of [^{11}C]CH_3I and [^{11}C]CH_3OTf

[^{11}C]CO_2 was produced using our IBA Cyclone 18/9 cyclotron *via* the $^{14}N(p,\alpha)^{11}C$ nuclear reaction by

irradiating a gas target of 0.2% O_2 in N_2 with a 40 to 45 μA beam at 15-16 MeV for 20 to 25 minutes. Following transfer with the target gas, [^{11}C]CO_2 was trapped at −180°C and reduced to [^{11}C]CH_4 using a nickel catalyst in the presence of H_2 gas at 425°C. The [^{11}C]CH_4 was trapped on a Carbosphere column at −120°C. Recirculation using high purity He (grade 6) following release of [^{11}C]CH_4 by temperature increase allowed reaction with elemental iodine at 750°C to produce [^{11}C]CH_3I that was trapped on a Porapak column at room temperature. When maximum activity level was reached, the Porapak column was heated at 200°C to permit the transfer with He (grade 6) of [^{11}C]CH_3I through an online silver triflate column heated at 200°C, allowing the generation of [^{11}C]CH_3OTf which was then bubbled into the reactor. Alternatively, [^{11}C]CH_3I was transferred directly from the Porapak column into the reactor for ^{11}C-methylation.

2.3.2 | Automated radiosynthesis of [^{11}C](2*R*,4*S*)-5-(biphenyl-4-yl)-4-[(3-carboxypropionyl)amino]-2-methylpentanoic acid methyl ester ([^{11}C]MeOLBQ, [^{11}C]7)

Radiolabeling optimization reactions were performed with approximately 2 Ci (74 GBq) of [^{11}C]CO_2 at end of beam. ^{11}C-Methylation was carried out with either [^{11}C]methyl iodide or [^{11}C]methyl triflate with a mixture of the *tert*-butyl-protected precursor **9** (0.5 mg, 1.13 μmol), potassium carbonate (4 mg, 28.9 μmol, 26 eq.), and Kryptofix 222 (5.1 mg, 13.5 μmol, 12 eq.) dissolved in DMF (200 μL) in various conditions (see Table 1). [^{11}C]CH_3I or [^{11}C]CH_3OTf was bubbled (flow 35 mL/min) and trapped at −25°C in the reaction mixture before heating the vial at 50°C or 120°C for 1 or 3 minutes to conduct the alkylation reaction. The deprotection reaction was then completed at 50°C to 90°C for 0.5 to 3 minutes using 200 μL of 18% HCl, 6% HCl or TFA. The reaction mixture was cooled to 20°C, quenched with the HPLC buffer (0.8 mL), and then injected onto semipreparative HPLC.

2.3.3 | Semipreparative HPLC purification and reformulation

The semipreparative HPLC fraction containing [^{11}C]**7** (t_R = 5.8 minutes, Figure 1) was collected into a vessel containing Milli-Q water (40 mL). A flow of He was used to transfer the solution through a preconditioned Sep-Pack Vac C18 1 cc cartridge. The cartridge was washed with sterile water (10 mL) to remove residual HPLC solvent. [^{11}C]**7** was eluted with USP ethanol (0.6 mL) followed by saline (5.4 mL) into a sterile vial to provide the final product. (Figure 1A).

2.3.4 | Analytical HPLC analyses

An aliquot of the final product was removed for analytical HPLC to determine the chemical and radiochemical

TABLE 1 Optimization of reaction conditions

Entry	Reagent MeI/MeOTf	Reaction Time of Alkylation, s	Temperature of Alkylation, °C	Reaction Time of Deprotection, s	Temperature of Deprotection, °C	Acid for Deprotection	RCY,[a] %
1	MeI	180	120	60	60	HCl 18%	12
2	MeI	180	120	60	60	HCl 18%	10
3	MeOTf	180	50	60	60	HCl 18%	22
4	MeOTf	60	50	60	60	HCl 18%	9
5	MeOTf	180	50	90	90	HCl 18%	5
6	MeOTf	180	50	120	60	HCl 18%	10
7	MeOTf	180	50	180	60	HCl 18%	8
8	MeOTf	180	50	30	60	HCl 18%	19
9	MeOTf	180	50	60	60	HCl 6%	10
10	MeOTf	180	50	30	60	HCl 6%	6
11	MeOTf	180	50	30	90	HCl 6%	2
12	MeOTf	180	50	60	60	TFA	0

[a]RCY (decay-corrected from CO_2) corresponds to the radioactivity values of [^{11}C]**7** from the recovered semipreparative HPLC peak without reformulation.

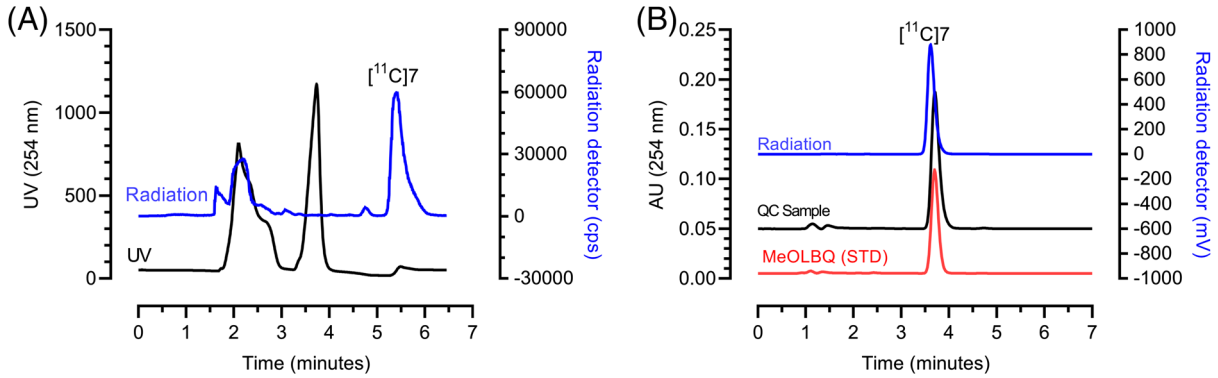


FIGURE 1 A, Representative semipreparative HPLC chromatogram of [^{11}C]**7**, radioactivity in blue ([^{11}C]**7** t_R = 5.8 min) and UV in black (λ = 254 nm). B, Representative analytical HPLC chromatogram of [^{11}C]**7**, radioactivity in blue ([^{11}C]**7** t_R = 3.7 min), UV in black (λ = 254 nm, t_R = 3.8 minutes) and the nonradioactive standard **7** in red (UV, λ = 254 nm, t_R = 3.8 min). The difference in the retention times between radiation and UV detection is due to detectors being in series

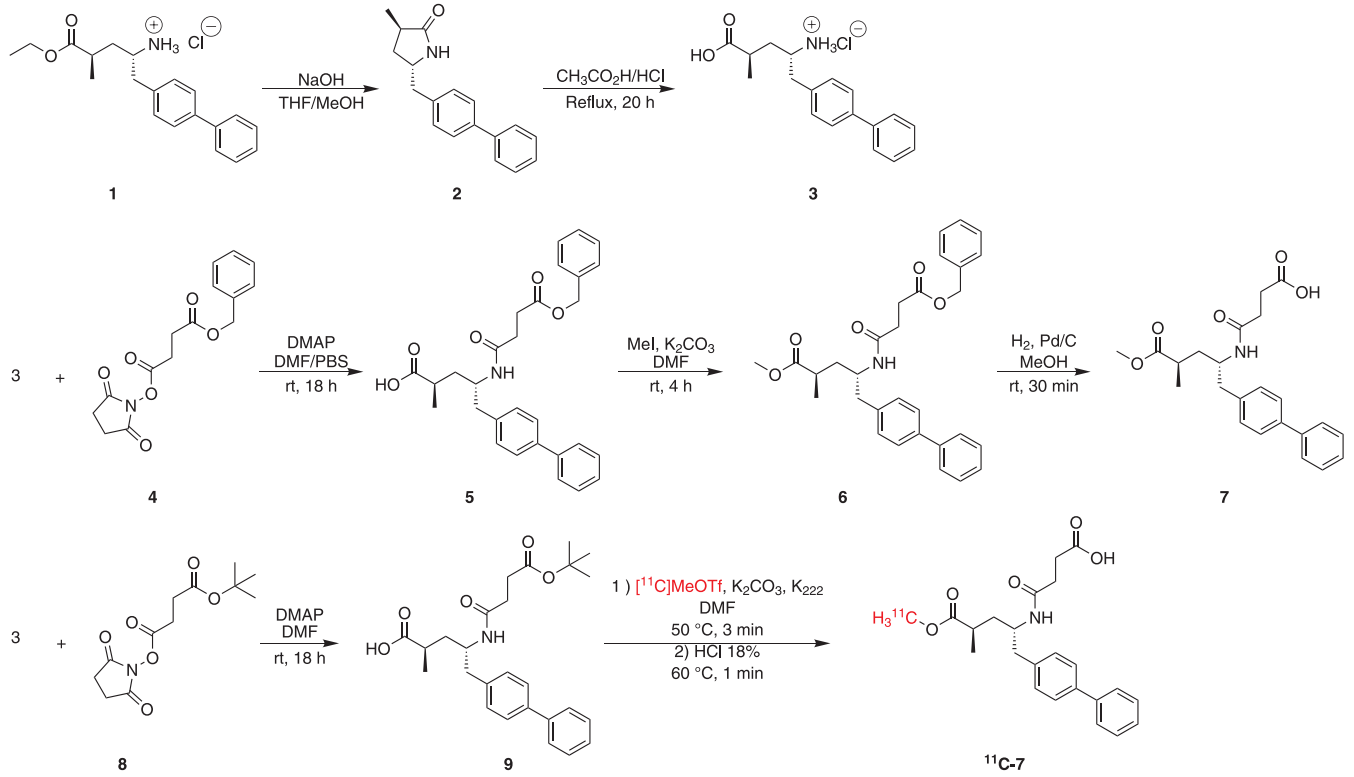
purities. The chemical identity of [^{11}C]MeOLBQ was confirmed by a coelution with the nonradioactive standard **7**. The molar activity was measured by comparing the UV mass of [^{11}C]MeOLBQ sample at 254 nm (t_R = 3.7 minutes) with the UV response of a single injection of the nonradioactive standard **7** (Figure 1B).

3 | RESULTS AND DISCUSSION

The production of [^{11}C]MeOLBQ by a simple one-step ^{11}C-methylation of LBQ657 cannot be realized due to the presence of two carboxylic acids which would require the synthesis of both methyl ester derivatives as standards and precise analytical HPLC process in order to confirm selective methylation. Direct methylation of the free carboxylic acid precursor with the protected oxobutanoic acid moiety has thus been developed (Scheme 1), which allows the selective methylation of the 2-methylbutanoic acid moiety. The benzyl ester precursor **6** was synthesized to produce the standard compound **7**. However, the use of Pd/C and H_2 for deprotection of the [^{11}C]MeOLBQ requires a filtration of the catalyst before semipreparative HPLC. The filtration reduced significantly the amount of the radiolabeled product in the final formulation and was not optimal. Therefore, we decided to use another approach to produce [^{11}C]**C-7** via ^{11}C-methylation of the *tert*-butyl ester precursor **9** (instead of **6**) allowing rapid deprotection in acidic conditions without the need of solid catalyst before HPLC purification.

The nonradioactive standard **7** was synthesized in a five-step route. The first step of the synthesis involved the hydrolysis of the amino pentanoate **1** with sodium hydroxide to generate product **2** in 95 % yield. The ring of the formed lactam **2** was then opened after treatment with acetic acid and hydrochloric acid under reflux to give the ammonium salt **3** in high yield (80%). The ammonium salt **3** was then coupled with the NHS-activated acid **4** in DMF/PBS with DMAP to form the oxobutanamido benzylic protected ester **5** which was then methylated in DMF in the presence of K_2CO_3 (88%). The benzyl group was cleaved using H_2 with Pd/C in methanol at room temperature to afford the standard, MeOLBQ (**7**), in an overall yield of 38%. The *tert*-butyl protected carboxylic acid **9** was synthesized by coupling the ammonium salt **3** with the NHS-activated acid **8** in DMF with DMAP to give the precursor **9** in 34 % yield. An overall yield of 26% was obtained for the three-step synthesis of the precursor **9** as shown in Scheme 1.

[^{11}C]MeOLBQ was synthesized by ^{11}C-methylation of the *tert*-butyl ester precursor **9** followed by deprotection under acid conditions. ^{11}C-Methylation was carried out in the presence of Kryptofix 222 and potassium carbonate. Utilization of this cryptant was previously reported to enhance the reactivity of the base increasing the formation of the carboxylate ion for methylation.[18,19] In order to improve the RCY of the reaction, both methylating agents were tested, [^{11}C]methyl iodide and [^{11}C]methyl triflate. As expected, [^{11}C]methyl triflate resulted in a higher RCY (Table 1, entries 1-3). Higher RCYs were obtained at lower temperature and less time. Reducing the time of the methylation from 3 to 1 minute was not beneficial (Table 1, entry 4), indicating that a longer reaction time is needed to complete the alkylation reaction in high RCY. The duration of the deprotection reaction was then studied. Prolonged deprotection reaction times (Table 1, entries 5-7) led to diminished RCYs. Reducing the time of deprotection from 60 to 30 seconds did not offer a significant improvement in RCY (Table 1, entries 3 and 8). It was thus concluded that 1 minute was sufficient for the deprotection reaction. Given that longer



SCHEME 1 Synthetic route of [^{11}C]MeOLBQ

deprotection reaction times resulted in lower RCY, various concentrations of HCl were tested for the deprotection reaction, while minimizing the potential degradation of the ^{11}C-methyl ester derivative. Reducing the HCl concentration from 18% to 6% did not increase the final yield (Table 1, entries 3 and 9-11). Deprotection with TFA was not successful (Table 1, entry 12). The best results were therefore obtained with 3 minutes of [^{11}C]MeOTf alkylation at 50°C, followed by deprotection with 18% HCl for 1 minute at 60°C. These conditions were tested in validation runs including reformulation to obtain [^{11}C]MeOLBQ ([^{11}C]**7**) in 9.5 ± 2.5% RCY (25 ± 6% decay-corrected from [^{11}C]CO_2, n = 3) and high molar activity 348 ± 100 GBq/μmol (9425 ± 2720 mCi/μmol) at EOS, in high chemical (>95%) and radiochemical (>99%) purities. The total synthesis time including HPLC purification and reformulation was 29 minutes.

4 | CONCLUSION

The radiosynthesis of the novel [^{11}C]MeOLBQ has been successfully completed using [^{11}C]MeOTf in high radiochemical yield and molar activity. The fast and reproducible one-pot two-step process has been fully automated. This strategy includes selective methylation of the carboxylic acid followed by deprotection of the *tert*-butyl ester precursor with acidic condition. To our knowledge [^{11}C]MeOLBQ is the first PET-labeled analog of the clinically used NEPi sacubitril.

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