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Asymmetric syntheses of radioactive and stable isotope-labeled β-amino acids

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β-amino acids 1 and 2 are α2δ agonists, which were developed for the treatment of generalized anxiety disorder and insomnia. The stable and radioactive isotope-labeled β-amino acids were required to support pre-clinical and clinical studies. Asymmetric syntheses of deuterium and carbon-14 labeled β-amino acids were achieved via chiral auxiliary and diastereoselective hydrogenation methodologies, respectively. The details of these syntheses are reported.

Keywords: isotope-labeling; C-14; C-13; H-2; β-amino acids; asymmetric synthesis; anxiety disorder and insomnia

Introduction

α2δ-Ligands are biologically active compounds that selectively displace ^{3}H-gabapentin from brain membranes, indicating a high affinity interaction with the α2δ subunit of voltage-gated calcium channels.[1] α2δ-Ligands have been developed for the treatment of several conditions such as generalized anxiety disorder, insomnia, fibromyalgia, epilepsy, neuropathic pain, anxiety, depression, and attention deficient hyperactivity disorder.[2] Compounds **1** and **2** (Figure 1) were selected for detailed study of their pharmacology. Therefore, their stable-isotope labeled analogs **3** and **4** were required for use as internal standards for phase I sample assays, and radiolabeled analog **5** was needed for animal and human absorption, distribution, metabolism, and elimination (ADME) and whole body autoradiography studies.

This article describes asymmetric syntheses of radioactive and stable isotope-labeled compounds **3, 4**, and **5**. The synthesis of unlabeled β-amino acids **1** and **2** has been previously reported.[3,4] We have adapted the reported synthetic approaches to complete the syntheses of several isotope labeled β-amino acids such as **3**, **4**, and **5** through chiral auxiliary and diastereoselective hydrogenation methodologies. Both final stable isotope-labeled compounds **3** and **4** were prepared with a chemical purity of >99% and an isotopic enrichment of >98%, and carbon-14 labeled compound **5** was also synthesized with both radiochemical and chemical purity of >99%.

Results and discussion

Available methodology for asymmetric synthesis of β-amino acids with one chiral center is shown in Figure 2.[5–7] Method *I* starts with unsaturated acid **6**. Asymmetric Michael addition with chiral benzylamine **7** followed by catalytic de-benzylation gives the amino acid **13**.[8–11] Both methods *II* and *III* are based on the chiral auxiliary approach. Method *II* utilizes Evans' auxiliary to generate a chiral center through asymmetric alkylation of imide **9**. A carboxylic acid group from **9** is then converted to a β-amino group by Curtis re-arrangement.[12,13,3] Method *III* involves asymmetric Aldol reaction of chiral imines **11** and esters **10**, followed by strong acid-promoted hydrolysis of sulfonamide and ester groups.[14] Method *IV* is relatively new and uses β-ketone acid **12** as a starting material. A two-step sequence of amination and asymmetric hydrogenation offers the target **13**.[4] None of those approaches have been applied to synthesis isotope labeled β-amino acids before.

Our syntheses of stable-isotope labeled compounds **3** and **4** were accomplished in seven steps (Scheme 1) from commercially available chiral compound **14** and isotope labeled methyl iodide ($^{13}CD_3I$) and ethyl bromide (CD_3CD_2Br), respectively, based on the reported synthesis.[3] The labeled halides were first converted to Grignard reagents *in situ* then coupled with chiral bromide **14** in the presence of LiCl and CuCl to furnish the labeled chiral alkenes **15a** and **15b** in 78% and 95% yield, respectively. The labeled alkenes **15a** and **15b** were oxidized with Jones reagent to give chiral acids **16a** and **16b**. The rest of the synthesis is illustrated through the use of acid **16a** as a representative example. The purified unsaturated acid **16a** was first activated by forming a mixed anhydride with trimethylacetyl chloride *in situ* and then combined with the Evans' chiral auxiliary **17** to generate chiral oxazolidinone **18a** in 85% yield. An sodium bistrimethylsilylamide mediated alkylation of **18a** with *t*-butylbromoacetate afforded α-substituted oxazolidinone **19a** with excellent diastereoselectivity (>98% i.e., based on HPLC assay) and good yield. Compound **19a** was treated with LiOH-H_2O_2 in a mixture of THF and water resulting in the formation of sufficiently pure chiral acid **20a**. The key Curtius re-arrangement

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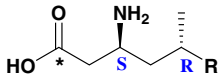


1) R=$CH_2CH_2CH_3$,* = ^{12}C
2) R=$CH_2CH_2CH_2CH_3$, * = ^{12}C
3) R=$CH_2CH_2{}^{13}CD_3$, * = ^{12}C
4) R=$CH_2CH_2CD_2CD_3$, * = ^{12}C
5) R=$CH_2CH_2CH_3$, * = ^{14}C

Figure 1. Structures of unlabeled and labeled β-amino acids **1**, **2, 3, 4, and 5**.

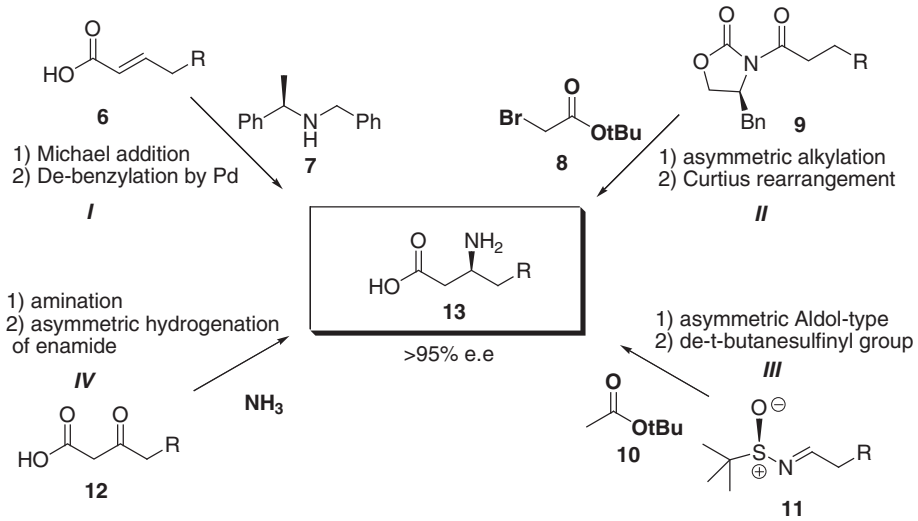
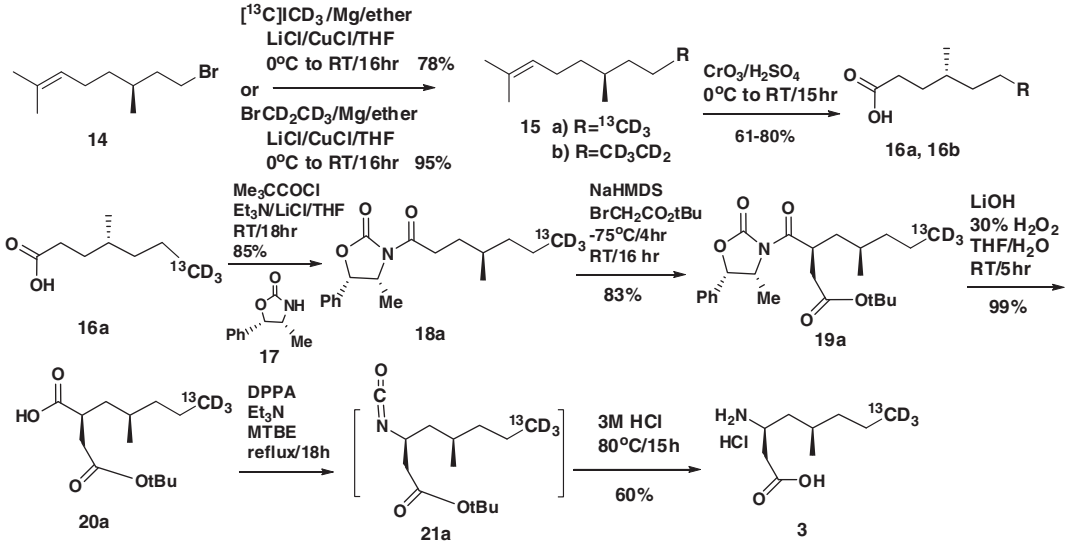


Figure 2. Available methodology for β-amino acids.

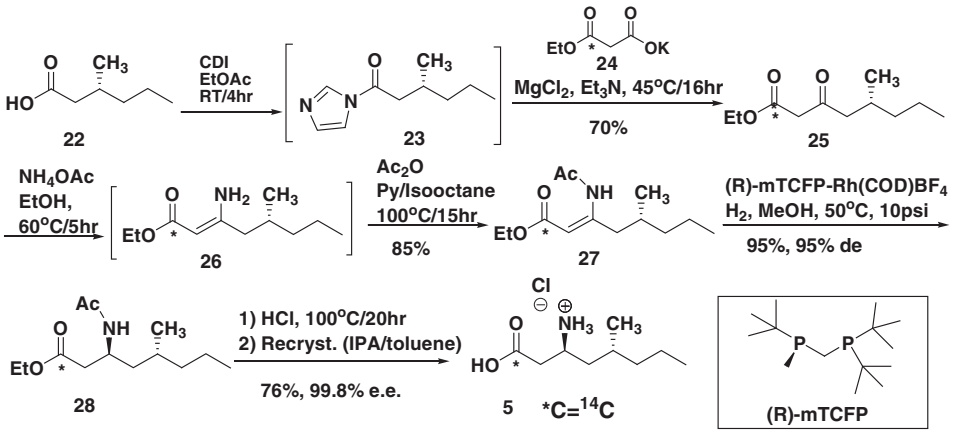


Scheme 1. Synthesis of stable-isotope labeled compounds 3 and 4.

was carried out by heating acid **20a** with diphenyl-phosphoryl azide (DPPA) in the presence of triethylamine to afford crude isocyanate **21a**. Without further purification, crude **21a** was hydrolyzed with 3M HCl to form labeled compound **3** as an HCl salt in 60% yield after purification. The overall yield of **3** from the labeled methyl iodide was 16.7% with >98% of isotope enrichment. The labeled compound **4** was prepared in a similar fashion from chiral acid **16b** (***R*** **= CD_2CD_3**).

This route proved satisfactory to provide small amounts of compounds **1** and **2** and their stable isotope-labeled analogs for preliminary studies. However, several issues were identified that did not make it amenable for scale-up[4] and current Good Manufacturing Practices (cGMP) radiosynthesis, such as the highly toxic waste produced by the Jones oxidation and the use of hazardous reagents, hydrogen peroxide, and DPPA. Therefore, a more efficient route (Scheme 2) was developed for the synthesis of compound **1** on a large scale[4] and adapted to prepare carbon-14 labeled cGMP material for supporting human ADME studies.

For our cGMP radiosynthesis, we used commercially available potassium ethyl [1-^{14}C]malonate **24** as our C-14 source and



Scheme 2. cGMP Synthesis of radioactive isotope-labeled compound 5.

diluted the labeled material with its unlabeled analog to reduce its specific activity. A 6-step cGMP radiosynthesis is shown in Scheme 2. The synthesis started with activation of (R)3-methylhexanoic acid **22** with 1,1′-carbonyldiimidazole to generate imidazolide **23** *in situ*. Imidazolide **23** was reacted with **24** (prediluted with unlabeled material) in the presence of $MgCl_2$ [15] to give the labeled β-ketoester **25** in 70% yield. Treatment of ketoester **25** with ammonium acetate in ethanol at 60°C afforded enamine **26**, which was immediately reacted with acetic anhydride in isooctane at 100°C to form enamide **27**. The key asymmetric hydrogenation of enamide **27** in methanol and isooctane was accomplished using (R)-mTCFP as a ligand and $Rh(COD)BF_4$ as a catalyst to produce the desired acetamide **28** with excellent diasteroselectivity (>95% de) in 95% yield.[16] Acidic hydrolysis of acetamide **28** with aqueous HCl solution was carried out to give the desired C-14 labeled cGMP material **5** as an HCl salt after recrystallization from IPA/toluene. The overall radiochemical yield from potassium ethyl [1-^{14}C]malonate **24** was 43% with a radiochemical purity of 99.2% and a specific activity of 8.4 mCi/mmol. This labeled cGMP material with a high specific activity was further diluted with GMP unlabeled material to furnish the final dose (105.29 μCi/mmol or 0.502 μCi/mg).

In summary, we have utilized a chiral auxiliary methodology to synthesize two stable isotope-labeled β-amino acids from a readily available stable isotope sources. Also, we have demonstrated that cGMP radioactive materials were prepared in four steps using a more efficient approach with a key asymmetric reduction step.

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Experimental section

General methods: All reactions were carried out under an atmosphere of nitrogen unless otherwise stated. Liquid chromatography-mass spectrometry (LC-MS) data were obtained on a Waters Micromass LCT mass spectrometer with flow injection analysis and electrospray ionization. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Gemini 300 MHz instrument. Chemical purity of all compounds was determined by HPLC and LC-MS. Purifications were performed using flash column chromatography on Biotage Flash 40 system. Quantitation of radioactivity of C-14 labeled compounds was performed using a Packard 2200CA liquid scintillation analyzer, with Scintiverse BD cocktail used throughout. Commercial reagents and solvents were purchased from Aldrich and used as received unless otherwise noted. Potassium ethyl [1-$^{14}C_2$]malonate **24** (50 mCi, 54.7 mCi/mmol) was purchased from PerkinElmer. [^{13}C,D_3]methyl iodide and [D_5]ethylbromide were purchased from CDN Isotopes, Inc. Intermediate **22** and ligand (R)-mTCFP were provided by Chemical R&D, Sandwich Lab, Pfizer Inc. All known compounds were identified by comparison of NMR spectra to those reported in the literature or authentic samples.

9-([^{13}C]-Trideuteriomethyl)-(R)-2,6-dimethylnon-2-ene (15a)

A flame-dried three-necked flask was charged with magnesium (4.5 g, 186 mmol) and diethylether (60 mL). A solution of $^{13}CD_3I$ (Aldrich, 15.0 g, 104 mmol) in diethylether (15 mL) was added slowly. Upon addition of all the iodide, the mixture was heated at 30°C for 1 hour and then cooled at room temperature. A second flame-dried three-necked flask was charged with lithium chloride (0.9 g, 21 mmol), cuprous chloride (1.04 g, 10.5 mmol) and tetrahydrofuran (distilled, 75 mL). (*S*)-(+)-citronellylbromide **14 (** 11.5 g, 52 mmol) was added slowly at room temperature. The resulting mixture was stirred at 25°C for 10 minutes and at 0°C for an additional 30 minutes. The solution of the Grignard reagent was slowly added to the solution of the cuprate mixture at 0°C. The resulting mixture was stirred for 16 hours at room temperature. After the mixture was quenched with ammonium chloride (25 mL, saturated), it was poured into a mixture of diethyl ether (500 mL) and ammonium chloride (500 mL, 2N). The organic phase was separated, washed with brine, dried (Na_2SO_4), and concentrated under vacuum to give **15a** (6.4 g, 78%) as a colorless oil. ^{1}HNMR ($CDCl_3$): δ 5.03 (t sept, ^3J = 7.1 Hz, ^4J = 1.5 Hz, 1H), 1.89 (m, 2H), 1.61 (d, ^4J = 1.2 Hz, 3H), 1.53 (s, 3H), 1.41–1.12 (m, 5H), 1.11–0.95 (m, 2H), 0.79 (d, J = 6.2 Hz, 3H).

7-([^{13}C-Trideuteriomethyl)-(R)-4-methylheptanoic acid (16a)

A round-bottomed flask was charged with chromium trioxide (21.2 g, 212 mmol), H_2O (60 mL) and cooled to 0°C. Sulfuric acid (18.0 mL, 335 mmol) was slowly added to this cold solution. The resulting mixture was stirred at 0°C for 15 minutes. Another flask was charged with **15a** (6.4 g, 40.5 mmol) and acetone (50 mL) and cooled to 0°C. The solution of the oxidant via a dropping funnel at 5°C over a period of 4 hours was slowly added to this cold solution. After the addition, the mixture was stirred for 16 hours at room temperature, quenched with brine (20 mL), concentrated under vacuum, and further diluted with brine

(20 mL). The aqueous layer was extracted with *tert*-butyl methyl ether (4 × 60 mL). The combined organic layers were washed with brine (200 mL), dried (Na_2SO_4), and concentrated under vacuum to give a viscous brown tar. The crude material was purified by flash column chromatography (heptane/ethyl acetate, gradient 6:1 to 2:1) to afford **16a** (4.4 g, 73.3%) as a colorless oil. ^{1}HNMR ($CDCl_3$): δ 2.30 (m, 2H), 1.60 (m, 1H), 1.46–1.32 (m, 2H), 1.30–1.14 (m, 3H), 1.10–0.99 (m, 1H), 0.81 (d, J = 6.7 Hz, 3H).

7-(^{13}C-Trideuteriomethyl)-(4R,5S)-4-methyl-3-[(R)-4-methylheptanoyl]-5-phenyloxazolidin-2-one (18a)

Triethylamine (4.4 mL, 60 mmol) and trimethylacetyl chloride (4.34 g, 36.0 mmol) at 0°C with stirring was added to a solution of **16a** (4.4 g, 29.7 mmol) in THF (200 mL). The resulting mixture was stirred at 0°C for 1 hour. A mixture of (*4R,5S*)-(+)-4-methyl-5-phenyl-2-oxazolidinone (**17**, 6.0 g, 34 mmol) and LiCl (1.27 g, 30 mmol) were added. The resulting mixture was stirred for 18 hours at room temperature. The precipitate formed in the reaction mixture was removed by filtration, washed with diethyl ether (2 × 10 mL). The filtrate was washed with brine (2 × 30 mL), dried over $MgSO_4$, and concentrated under vacuum. The crude product (11.2 g) was purified by flash column chromatography (heptane-ethyl acetate, 10:1) to afford **18a** (7.5 g, 82%) as a colorless oil. ^{1}HNMR ($CDCl_3$): δ 7.48–7.30 (m, 5H), 5.68 (d, J = 5.8 Hz, 1H), 4.78 (qn, J = 7.3 Hz, 1H), 3.08–2.85 (m, 2H), 1.78–1.63 (m, 1H), 1.61–1.45 (m, 2H), 1.41–1.25 (m, 3H), 1.23–1.10 (m, 1H), 0.93 (d, J = 6.3 Hz, 3H), 0.91 (d, J = 6.7 Hz, 3H).

8-(^{13}C-Trideuteriomethyl)-(3S,5R)-ter-butyl-5-methyl-3-((4R,5S)-4-methyl-2-oxo-5-phenyloxazolidine-3-carbonyl)octanoate (19a)

A flame-dried three-necked flask was charged with **18a** (7.5 g, 24.3 mmol) and anhydrous THF (100 mL). Sodium bistrimethylsilylamide (1M in THF, 30 mL) was added dropwise at −70°C over 10 minutes. The mixture was stirred at −70°C for 1 hour. A solution of *tert*-butyl bromoacetate (15.0 g, 77 mmol) in anhydrous THF (10 mL) was added via syringe over 30 minutes. The mixture was stirred for 4 hours at −70°C and then was warmed at room temperature and stirred for 16 hours. The mixture was quenched by addition of H_2O (10 mL) and then ammonium chloride (2N, 100 mL). The mixture was extracted with *tert*-butyl methyl ether (3 × 100 mL). The combined organic phases were washed with brine (100 mL), dried over Na_2SO_4, and concentrated under vacuum to give the crude product as a colorless oil, which was further purified by flash column chromatography (heptane-ethyl acetate, 9:1) to give **19a** (8.5 g, 83%) as a white solid. ^{1}HNMR ($CDCl_3$): δ 7.40–7.22 (m, 5H), 5.58 (d, J = 7.4 Hz, 1H), 4.69 (qn, J = 5.9 Hz, 1H), 4.25 (m, 1H), 2.50 (ddd, ^2J = 83.1 Hz, ^3J = 9.7 Hz, 5.1 Hz, 2H), 1.65 (qn, J = 6.8 Hz, 1H), 1.45–0.98 (m, 7H), 1.34 (s, 9H), 0.85 (d, J = 6.3 Hz, 3H), 0.82 (d, J = 6.7 Hz, 3H).

7-(^{13}C-Trideuteriomethyl)-(2S,4R)-2-(2-tert-butoxy-2-oxoethyl)-4-methylheptanoic acid (20a)

A solution of lithium hydroxide hydrate (1.68 g, 40 mmol) in hydrogen peroxide (30%, 14.4 mL) and water (32 mL) at 0°C was slowly added to a solution of **19a** (8.4 g, 19.9 mmol) in THF (180 mL) and water (60 mL). Then, the resulting mixture was warmed at room temperature and stirred for 5 hours. A solution of sodium sulfite (6.0 g) and sodium bisulfite (6.0 g) in water (60 mL) was added. The mixture was extracted with a mixture of *tert*-butyl methyl ether and heptane (50/50 v/v, 250 mL). The organic phase was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was dissolved in pentane (50 mL), and a brief agitation caused the chiral auxiliary to precipitate. The mixture was left to stand for 16 hours at room temperature. The solids were removed by filtration, and the filtrate was concentrated under vacuum to afford **20a** (5.2 g, 99%) as a colorless oil. ^{1}HNMR ($CDCl_3$): δ 2.81 (m, 1H), 2.41 (ddd, ^2J = 77.6 Hz, ^3J = 9.7 Hz, 5.1 Hz, 2H), 1.62 (m, 1H), 1.49–1.32 (m, 2H), 1.36 (s, 9H), 1.29–0.98 (m, 5H), 0.85 (d, J = 6.4 Hz, 3H).

8-(^{13}C-Trideuteriomethyl)-(3S, 5R)-3-amino-5-methyloctanoic acid (3)

Triethylamine (3.5 mL, 25 mmol) and diphenylphosphoryl azide (DPPA, 4.7 mL, 22.6 mmol) was added to a mixture of **20a** (5.2 g, 20 mmol) in anhydrous toluene (120 mL). The resulting mixture was stirred for 30 minutes at room temperature, then for 18 hours at reflux. After the mixture was cooled at room temperature, hexane (7 mL) and water (8 mL) were added. The organic layer was separated and washed with water (4 mL). The solvent was evaporated under vacuum to give crude **21a**. The crude **21a** was then dissolved in 3M HCl (10 mL) and heated at 80°C for 15 hours. After cooling at room temperature, the reaction mixture was diluted with diethyl ether (8 mL). The aqueous layer was separated and extracted with diethyl ether (5 mL) and treated with charcoal at 60°C for 30 minutes. After removal of charcoal, the aqueous layer was evaporated under vacuum to dryness. The crude product was triturated in acetonitrile at 23°C for 1 hour to afford **3** as an HCl salt (2.56 g, 60%). Mp.133–134°C. ^{1}HNMR (CD_3OD): δ 3.63(m, 1H), 2.68 (ddd, ^2J = 38.9 Hz, ^3J = 7.2 Hz, 4.9 Hz, 2H), 1.73–1.53 (m, 2H), 1.52–1.12 (m, 5H), 0.98 (d, J = 5.9 Hz, 3H). ^{13}CNMR (CD_3OD): δ 172.4, 46.7, 40.1, 39.1, 36.6, 28.7, 19.7, 18.4, 13.5 (septet, intense). MS(APCI$^+$): *m/z* 178 (M + H).

Analytical data for (*3S,5R*)-3-amino-5-methyl[D_5]nonanoic acid (**4**): ^{1}HNMR (CD_3OD); δ 5.13 (bs, 3H), 3.38–3.58(m, 1H), 2.42–2.57 (m, 1H), 2.23–2.42 (m, 1H), 1.56–1.72 (m, 2H), 1.17–1.46 (m, 5H); 0.92 (d, 3H).^{13}CNMR (CD_3OD): δ 176.4, 47.5, 40.5, 38.9, 38.6, 36.4, 29.0, 28.9, 22.8 (m), 18.4, 13.2 (m). MS (APCI$^+$): *m/z* 193 (M + H).

(R)-Ethyl 5-methyl-3-[1-^{14}C]oxooctanoate (25)

A 100 mL flask was charged with 1,1′-carbonyldiimidazole (0.7293 g, 4.50 mmol) and ethyl acetate (4.20 mL) to form a suspension. A solution of compound **22** (0.5855 g, 4.5 mmol) in ethyl acetate (4.2 mL) at room temperature was added slowly to the suspension. The resulting mixture was stirred at room temperature for 4 hours and then evaporated under vacuum to remove CO_2 to give a solution ***A***. Another flask was charged with [1-^{14}C]potassium ethyl malonate **24** (50 mCi, 0.914 mmol, 54.7 mCi/mmol), potassium ethyl malonate (0.603 g, 3.54 mmol), $MgCl_2$ (0.381 g, 4.00 mmol) and ethyl acetate (5.0 mL). The resulting mixture was stirred at room temperature for 10 minutes. Triethylamine (0.4624 g, 4.57 mmol) dropwise was added to this mixture at such a rate as to maintain the internal temperature below 30°C to form a suspension. The suspension was stirred at room temperature for 1 hour. The solution ***A*** was added to the suspension at room temperature. The resulting mixture was stirred at 45°C for 16 hours and then cooled to room temperature. 4M HCl (5.7 mL) was added to this mixture at such a rate to keep the internal temperature below 25°C. The mixture was allowed to stand and to form a clear bi-phase mixture. The organic layer was separated and washed with water (2 × 3.0 mL), 6% $NaHCO_3$ (4.6 mL) and water (2 × 3.0 mL) and dried over Na_2SO_4 (5.0 g). The solvent was evaporated under vacuum to give compound **25** (834.5 mg, 70%) with a total activity of 35.0 mCi and a specific activity of 8.40 mCi/mmol. ^{1}HNMR ($CDCl_3$): δ 4.20 (q, 2H), 3.42 (s, 2H), 2.52 (dd, 1H), 2.33 (dd, 1H), 2.03 (m, 1H), 1.30–1.10 (m, 6H), 1.28 (t, 3H), 0.90 (m, 6H).

(R,Z)-Ethyl 3-acetamido-5-methyl[1-^{14}C]oct-2-enoate (27)

A 50 ml flask was charged with $AcONH_4$ (0.6985 g, 9.06 mmol), **25** (0.7943 g, 3.96 mmol, 33.3 mCi) and anhydrous ethanol (6.0 mL) to form a suspension. The suspension was stirred at 60°C for a minimum of 5 hours and then cooled to 35°C and diluted with

isooctane (4.0 mL). The mixture was concentrated *in vacuo* to form a semi-solid. Isooctane (4 mL) was added to the semi-solid, and the resulting mixture was stirred for 5 minutes. The solid was removed by filtration and washed with isooctane (3 × 2.0 mL). The filtrate was concentrated to the volume of about 1 mL. Isooctane (4.5 mL), Ac_2O (0.90 mL), and pyridine (0.90 mL) were added to the residue. The resulting mixture was heated at 100°C for 15 hours with stirring and then cooled to 5°C. Water (3.0 mL) was added, and the resulting mixture was stirred for 2.5 hours at room temperature. The organic layer was separated, washed with water (2 × 2.0 mL), H_2SO_4 solution (2 × 2.0 mL, 1.0 mL conc. H_2SO_4, and 10.0 mL water), then water (2 × 2.0 mL) and dried over anhydrous Na_2SO_4. The Na_2SO_4 was filtered off and washed with isooctane (3 × 2.0 mL). The filtrate was concentrated under vacuum to give **27** (0.8165 g, 8.4 mCi/mmol, 28.4 mCi, 85.3%). ^{1}HNMR ($CDCl_3$): δ 11.20 (s, 1H), 4.90 (s, 1H), 4.17 (q, 2H), 2.88 (dd, 1H), 2.34 (dd, 1H), 2.14 (s, 3H), 1.79 (m, 1H), 1.30 (m, 7H), 0.89 (m, 6H); ^{13}CNMR ($CDCl_3$): δ 169.1 (1C), 168.3 (1C), 157.8 (1C), 97.2 (1C), 59.8 (1C), 42.7 (1C), 36.3 (1C), 31.4 (1C), 29.0 (1C), 28.8 (1C), 22.9 (1C), 19.0 (1C), 14.2 (1C).

(3S,5R)-Ethyl 3-acetamido-5-methyl[1-^{14}C]octanoate (28)

A pressure reactor was charged with compound **27** (0.8165, 8.4 mCi/mmol, 28.4 mCi) and isooctane (3.0 mL, pre-treated with dry N_2 gas for 10 minutes). A solution of (*R*)-mTCFPRhCODBF$_4$ (11.94 mg, 0.0213 mmol) in MeOH (3.0 mL, pre-treated with dry N_2 gas for 10 min) under N_2 gas was added to the reaction mixture. The resulting mixture was purged with N_2 (three times) and then with H_2 gas (four times). A H_2 gas pressure of 10 psi was applied to the pressure reactor. The reactor was heated at 50°C for 15 hours. After completion of the reaction determinate by GC-MS, the mixture was evaporated under vacuum to give the crude product. The crude material was dissolved in isooctane (15.0 mL) and washed with water (2.0 mL × 2). The organic layer was filtered and concentrated under vacuum to give the desired compound **28** (0.7796 g, 94.7%, 26.88 mCi, 8.4 mCi/mmol). ^{1}HNMR ($CDCl_3$): δ 5.98 (d, 1H), 4.35 (m, 1H), 4.15 (q, 2H0, 2.47 (m, 2H), 1.97 (s, 3H), 1.57 (m 1H), 1.00–1.40 (m, 8H), 0.90 (m, 6H).

(3S,5R)-3-Amino-5-methyl[1-^{14}C]octanoic acid hydrochloride (5)

Compound **28** (0.7796 g, 3.20 mmol, 26.88 mCi, 8.40 mCi/mmol) was stirred in MeOH (1.2 mL) and water (2.0 mL) at 35°C. A total of 37% HCl (2.2 mL) in one portion was added to this mixture. The resulting mixture was heated at 100°C for not less than 20 hours and then cooled to 55°C. Toluene (3.0 mL) was added, and the resulting mixture was heated at 55°C for 10 minutes with stirring. After standing for 10 minutes, the top toluene layer was removed, and the aqueous layer was extracted with toluene (3.0 mL × 2) at 60°C. Toluene (2.0 mL) was added to the aqueous layer at 60°C. The final mixture was cooled slowly from 60°C to −10°C over a period of 3 hours and kept at −10°C for 5 hours to facilitate precipitation. The solid was collected by filtration with Buchner funnel fitted with a filter paper while it was cold, and washed with cold toluene pre-cooled at 0°C (4.0 mL × 4). The resultant solid was dried under high vacuum overnight and further recrystallized from a mixture of 2-propanol and toluene (1/2, 4.5 mL) to give **5** (0.592, 76%, 8.4 mCi/mmol). After radio-dilution, the final cGMP material (6.0284 g) was obtained with a specific activity of 105.29 μCi/mmol (0.502 μCi/mg) and radiochemical and chemical purity of >99.0%. ^{1}HNMR (DMSO-d_6): δ 12.7 (bs, 1H), 8.17 (bs, 1H), 3.38 (m, 1H), 2.69 (dd, *J* = 6, 17 Hz, 1H), 2.53 (dd, *J* = 7, 17 Hz, 1H), 1.61 (m, 2H), 1.2 (m, 4H), 1.10 (m, 1H), 0.80 (m, 6h); ^{13}CNMR (DMSO-d_6): δ 172.3 (1C), 46.5(1C), 40.2(1C), 39.2(1C), 36.5(1C), 28.8(1C), 19.5(1C), 18.6(1C), 13.4 (1C); HPLC method: Column Phenomenex, Synergi Polar-RP, 4 μm, 150x4.6; Mobile phase A = Buffer (25 mM potassium phosphate pH6.3):MeOH 90:10, B = Buffer:MeOH 10:90; from 0%B to 100% in 60 minutes; Flow rate = 1 mL/minute, Temperature = 30°C; UV @210 nm.

Conflict of Interest

The authors did not report any conflict of interest.

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