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The synthesis of ^{14}C-labeled, $^{13}CD_2$-labeled saxagliptin, and its $^{13}CD_2$-labeled 5-hydroxy metabolite

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^{14}C-labeled saxagliptin, $^{13}CD_2$-labeled saxagliptin, and its $^{13}CD_2$-labeled 5-hydroxy metabolite were synthesized to further support development of the compound for biological studies. This paper describes new syntheses leading to the desired compounds. A total of 3.0 mCi of ^{14}C-labeled saxagliptin was obtained with a specific activity of 53.98 μCi/mg (17.13 mCi/mmol). The radiochemical purity determined by HPLC was 99.29%, and the overall radiochemical yield was 3.0% based upon 100 mCi of $[^{14}C]CH_2I_2$ starting material. By following similar synthetic routes, 580.0 mg of $^{13}CD_2$-labeled saxagliptin and 153.1 mg of $^{13}CD_2$-labeled 5-hydroxysaxagliptin metabolite were prepared.

Keywords: ^{14}C-label; ^{13}C-label; deuterium; isotope labeling; saxagliptin; type 2 diabetes

Introduction

Dipeptidyl peptidase-4 (DPP-4) is a member of the large family of proteases and known as a novel and attractive target for potential treatment of diabetes.[1] DPP-4's role in blood glucose regulation is thought to be the degradation of incretins, such as glucagon-like peptide-1. DPP-4 inhibitors are a new class of oral hyperglycemic drugs for type 2 diabetes that glucagon-like peptide-1 and increase the release of insulin in response to food.[1] Onglyza™ (saxagliptin, Bristol-Myers Squibb and AstraZeneca) is a highly potent, competitive inhibitor of DPP-4 and is an FDA approved drug for the treatment of high blood sugar levels in patients with type 2 diabetes. ^{14}C-labeled saxagliptin (**1**), $^{13}CD_2$-labeled saxagliptin (**2**), and its $^{13}CD_2$-labeled 5-hydroxy metabolite (**3**) were synthesized to further support development of the compound for use in key biological studies. The synthesis of ^{14}C-labeled saxagliptin has previously been reported by K. Cao.[2] Cao's initial syntheses utilized [^{14}C] potassium cyanide (Scheme 1, Route 1) or ethyl[1,2-^{14}C]oxalyl chloride (Route 2) as the labeled reagents followed by several linear steps. These steps included a reductive enzymatic amination bio-conversion and elaborate purification to produce a desired enantiomeric intermediate. The radioactive enantiomeric intermediate was reacted in an amide coupling reaction and further derivatization to give the desired final compounds.

Desiring a shorter synthetic route which avoided biosynthesis and purification of labeled intermediates, our attention focused on labeling the pyrrolidine portion of the molecule. We chose to label the molecule via an organic cheletropic reaction by adapting existing methodology[3] and utilizing an often overlooked labeled precursor, diiodomethane.[4] The key step in the synthesis was the installation of C-14 via a stereospecific Simmons–Smith reaction between $[^{14}C]CH_2I_2$ and an olefin precursor to prepare the cyclopropane ring system.[5,6] This process did not require the resolution of the diastereomers. It was followed by four radiochemical steps to give the title compound (**1**). Following analogous routes, $^{13}CD_2$-labeled saxagliptin (**2**) and its $^{13}CD_2$-labeled 5-hydroxy metabolite (**3**) were prepared using $^{13}CD_2I_2$ as the label source (Scheme 2).

Experimental

General: Radioactivity was measured with a PerkinElmer Liquid Scintillation Analyzer, Tri-Carb Model 2900TR. Mass spectra were obtained with a Finnigan LXQ mass spectrometer. Proton and ^{13}C NMR spectra were recorded on a Bruker Avance Ultrashield NMR operating at 400 or 500 MHz and 100 MHz, respectively. Chemical and radiochemical purities were determined by HPLC (Shimadzu SPD 10A UV detector and LC-10AT pumps or Agilent Technologies 1100 Series HPLC System and IN/US System β-Ram radiometric flow detector with a 0.5 mL flow cell). Analytical HPLC methods. Method 1: Phenomenex C18 Luna, 5 μm, 4.6 × 150 mm, detected at 205 nm. Mobile phase A, 0.05% trifluoroacetic acid (TFA) in water; mobile phase B, 0.05% TFA in acetonitrile. Gradient: 0 min 5% B, 6 min 5% B, 11 min 95% B, 13 min 95% B, 15 min 5% B. Flowrate = 1.0 mL/min. Method 2: Same column, mobile phases, and wavelength as method 1 but with a longer gradient: 0 min 5% B, 8 min 50% B, 15 min 95% B, 20 min 95% B, 23 min 5% B. Method 3: Same column, mobile phases, and gradient as method 2 but detected at 220 nm. Method 4: Zorbax Eclipse XDB-C18, 5 μm, 4.6 × 150 mm, detected at 215 nm. Mobile phase A, MeOH: Water: TFA (10:90:0.1); mobile phase B, MeOH: Water: TFA (90:10:0.1). Gradient: 0 min 0% B, 20 min 20% B, 40 min 100% B, 45 min 100% B, 46 min 0%

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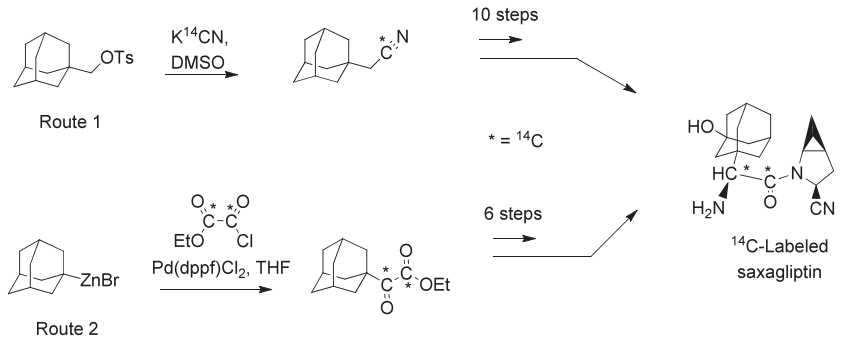
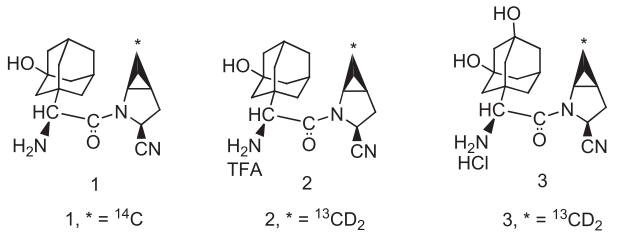


Scheme 1. Two initial syntheses of ^{14}C-labeled saxagliptin.[2]



Scheme 2. ^{14}C-labeled saxagliptin (**1**), $^{13}CD_2$-labeled saxagliptin (**2**), and $^{13}CD_2$-labeled 5-hydroxysaxagliptin metabolite (**3**).

B, 55 min 0% B. Flowrate = 0.6 mL/min. Method 5: Same column, mobile phases, and gradient as method 2 but detected at 215 nm. Method 6: Phenomenex Synergi Polar-RP 80A, 4 μm, 4.6 × 150 mm. A = 0.15% TFA in water, B = 0.15% TFA in acetonitrile. Flowrate = 1 mL/min, detected at 215 nm. 5 min 5% B, 15 min 70% B, 20 min 95% B, 23 min 95% B, 25 min 5% B. Semi-preparative HPLC Conditions. Method 1: Phenomenex Luna C18, 5 μm, Axia, 250 × 21.2 mm. UV detection: 215 nm, flowrate = 11 mL/min. Mobile phase A, water; mobile phase B, acetonitrile. Gradient: 0 min 30% B, 6 min 40% B, 11 min 95% B, 13 min 95% B, 18 min 30% B. Method 2: Phenomenex Synergi C18, 4 μm, 21.2 × 250 mm, mobile phase A = 0.1% TFA in 900 Water: 100 Acetonitrile, mobile phase B = 0.1% TFA in 900 Acetonitrile: 100 water, flowrate = 15 mL/min, 0.0 min 0% B, 6.0 min 0% B, 11 min 95% B, 13 min 95% B, 15 min 5% B, 15.01 min 0% B, detected at 220 nm. TLC was performed on 60 F_{254} silica gel plates (Merck) with UV or iodine detection. Flash chromatography was conducted using Teledyne Isco RediSep Rf prepacked columns using an AnaLogix BSR pump. Radiolabeled products were compared with authentic standards when possible. All reagents and solvents were ACS grade or better. [^{14}C]CH_2I_2 was purchased from ViTrax, (Placentia CA, >98%) and stabilized with copper. The specific activities of [^{14}C] saxagliptin and other intermediates were determined gravimetrically.

Synthesis

(*1S,3S,5S*)-*tert*-butyl 3-carbamoyl-6-[^{14}C]-2-azabicyclo[3.1.0] hexane-2-carboxylate (**5**)

[^{14}C]Diiodomethane (1.75 mL, 50 mCi = 0.925 mmol, ViTrax, >98% radiochemical purity, specific activity = 54 mCi/mmol, packaged at 28.6 mCi/mL in DCM:Pentane, 1:1, over copper) was charged into a 10-mL flask containing a stir bar under nitrogen. DCM (0.4 mL) was used to rinse the [^{14}C]CH_2I_2 shipping vial and was added to the reaction. Unlabeled diiodomethane (75.3 uL, 249 mg, 0.93 mmol) was added. (*S*)-*tert*-butyl 2-carbamoyl-2,3-dihydro-1*H*-pyrrole-1-carboxylate (**4**) (0.2 g, 0.94 mmol) and water (3.40 μL, 0.19 mmol) were added to the reaction flask. The solution became a clear light-pink color. The reaction flask was cooled to −10°C using an ice and MeOH bath. Diethyl zinc (1.70 mL, 1.89 mmol, 1.1 M solution in toluene) was syringed in by a syringe pump slowly over 3 h at −10°C to 0°C. The reaction mixture was stirred at 0°C for an additional 1 h as a white zinc precipitate dropped out of the solution. The reaction was warmed to room temperature for 17 h. Progress of the reaction was followed by HPLC using method 1. The reaction was cooled to 0°C, and EtOAc (8 mL) was added. The suspension slowly dissolved in EtOAc. The mixture was transferred to a separatory funnel containing 3.5 mL of a solution of ethylenediaminetetraacetic acid disodium dihydrate (4 g, 13.69 mmol) and sodium hydroxide (1.10 g, 27.4 mmol) in water (28 mL). The aqueous layer was further extracted with EtOAc (2 × 6 mL). The combined organic extracts were washed with 20% brine, dried over Na_2SO_4, filtered, and then concentrated by rotovap. The crude product was purified using a 12-g silicagel RediSep Rf column and eluted with 0–50% EtOAc in Hexane. The pure fractions were pooled, solvents removed by rotovap, and dried under vacuum to afford 59.2 mg of product (**5**) as a white solid (5.12 mCi, 10.2% radiochemical yield, 97.6% radiochemically pure with a specific activity of 19.58 mCi/mmol or 86.54 μCi/mg). HPLC (method 1, product R_t = 7.12 min). MS ESI$^+$ [^{12}C, ^{14}C M + H]$^+$ = 226.83, 228.83. This step was repeated using a second 50 mCi quantity of [^{14}C] diiodomethane to produce 38.30 mg of (**5**) as a white solid (2.69 mCi, 5.4% radiochemical yield, 98.65% radiochemical pure with a specific activity of 14.52 mCi/mmol or 64.17 μCi/mg). The average percent yield for the two reactions is equal to 7.8%.

(*1S,3S,5S*)-*tert*-butyl 3-cyano-6-[^{14}C]-2-azabicyclo[3.1.0]hexane-2-carboxylate (**7**)

To a 10-mL flask was added compound (**5**) (97.50 mg, 0.43 mmol, 7.81 mCi) dissolved in DMF (0.65 mL). The solution was cooled to 0°C, and cyanuric chloride (80 mg, 0.43 mmol) was added. The reaction was stirred for 1.2 h. Progress of the reaction was monitored by TLC (stained with I_2, 30% EtOAc in Hexane, starting material R_f = 0.2, product R_f = 0.7) and HPLC (method 2, product R_t = 11.55 min). At 0°C, water (11.5 μL, 0.64 mmol) was added to the reaction, and it was stirred for 30 min until all solids dissolved. To the reaction at 0°C was added cold saturated $NaHCO_3$ (3.0 mL). The pH of the solution was 8.5. The aqueous layer was extracted with EtOAc (4 × 5 mL). The combined organic extracts were washed with brine, dried over Na_2SO_4, filtered, and rotovapped to afford the desired crude product. The crude product was purified by silica gel flash chromatography eluting with 0–50% EtOAc in Hexane to give 75.9 mg of a white solid, 85% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ 4.92 (d, J = 9.9 Hz, 1H), 3.58–3.44 (m, 1H), 2.18 (d, J = 13.7 Hz, 1H), 1.68 (br. s., 1H), 1.44 (s, 10H), 0.91–0.77 (m, 1H), 0.68 (br. s., 1H). MS ESI$^+$ [^{12}C, ^{14}C M + H]$^+$ = 209.17, 211.08.

(*1S,3S,5S*)-6-[^{14}C]-2-azabicyclo[3.1.0]hexane-3-carbonitrile hydrochloride (9)

To a 10-mL flask under nitrogen was added compound (**7**) (75.9 mg, 0.36 mmol) and 1,4-dioxane (0.2 mL). The flask was charged with 4 M hydrogen chloride in 1,4-dioxane (0.72 mL, 2.8 mmol). The reaction was heated to 47°C for 1.2 h while being monitored by TLC (stained with I_2, 5% MeOH in DCM, product $R_f = 0.3$) and HPLC (method 2). HPLC showed complete disappearance of the starting material and formation of the product ($R_t = 2.21$ min). The solvent was removed by rotovap, and the crude product was azeotropped with acetonitrile (2 × 1 mL). The crude product was dried under vacuum for 18 h and used as the HCl salt (59.07 mg) in the next step without further purification.

[^{14}C]saxagliptin (1)

The amide coupling reaction to form compound (**12**) followed by deprotection to form compound (**14**) was performed in a similar way as described by K. Cao.[2] To a 10-mL vial equipped with a stir bar containing crude 14 (74.3 mg, 0.21 mmol) dissolved in 2-propanol (0.076 mL) was added 1:1 dichloromethane:water (4 mL). The reaction vial was cooled to 10°C, and 10 N sodium hydroxide (0.023 mL, 0.23 mmol) and potassium carbonate (79 mg, 0.14 mmol) were added slowly. The two phases were stirred for 5 min at room temperature. The reaction mixture was transferred to a separatory funnel, and the layers were separated. The aqueous layer was extracted with CH_2Cl_2 (3 × 4 mL). The combined organic extracts were washed with brine, dried over Na_2SO_4, and filtered. The solvent was removed by rotovap. The crude product was dissolved in 1:1 water:acetonitrile (1.2 mL) and purified by semi-preparative HPLC using method 1 (product $R_t = 9.12$ min). The pure fractions were pooled, solvent removed by rotovap, and the product was dried under vacuum to give 55.44 mg of a white foam (HPLC method 4, 99.29% radiochemically pure, specific activity = 53.98 μCi/mg or 17.13 mCi/mmol). Mass spectroscopy data were consistent with that previously published.[2] ^{1}H NMR (300 MHz, DMSO-d_6) δ 5.09 (d, J = 10.6 Hz, 1H), 4.35 (s, 1H), 3.88 (br. s., 1H), 3.45 (s, 1H), 2.18 (d, J = 14.5 Hz, 1H), 2.13–2.04 (m, 3H), 1.84 (br. s., 1H), 1.75–1.60 (m, 3H), 1.57–1.40 (m, 6H), 1.32 (d, J = 10.7 Hz, 3H), 1.02–0.91 (m, 1H), 0.70 (br. s., 1H).

(*1S,3S,5S*)-*tert*-butyl 3-carbamoyl-6-[$^{13}CD_2$]-2-azabicyclo[3.1.0]hexane-2-carboxylate (6)

To a flask under nitrogen was weighed (*S*)-*tert*-butyl 2-carbamoyl-2,3-dihydro-1*H*-pyrrole-1-carboxylate (**4**) (0.4 g, 1.89 mmol). The solid was dissolved in CH_2Cl_2 (3.6 mL) and toluene (1.5 mL). The solution was cooled to 0°C. Diethylzinc (15% by weight in toluene, 3.39 mL, 3.77 mmol) was added over 30 min, and then $^{13}CD_2I_2$ (1.02 g, 3.77 mmol) was added dropwise. The reaction was stirred at 0°C for 1 h and at room temperature for 16 h. The reaction was cooled to 0°C, diluted with CH_2Cl_2 (10 mL), and saturated $NaHCO_3$ (15 mL) was added. The layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (3 × 20 mL). The combined organic extracts were washed with brine, dried over Na_2SO_4, filtered, and concentrated to approximately 3 mL. The crude product was purified by silica gel flash chromatography using 0–80% EtOAc in Hexane to afford 172 mg of a white solid (40% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.33–7.20 (m, 1H), 6.95–6.83 (m, 1H), 4.40–4.26 (m, 1H), 3.39–3.27 (m, 2H), 2.85 (m, 1H), 1.88–1.72 (m, 1H), 1.42 (s, 9H). ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 11.62 (s). HPLC (method 2, product $R_t = 7.58$ min). MS ESI$^+$ [$^{13}CD_2$ M-Boc + H]$^+$ = 130.08.

(*1S,3S,5S*)-*tert*-butyl 3-cyano-6-[$^{13}CD_2$]-2-azabicyclo[3.1.0]hexane-2-carboxylate (8)

Method of preparation is the same as (**7**), 60% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 4.87 (dd, J = 10.3, 1.8 Hz, 1H), 3.50 (d, J = 6.5 Hz, 1H), 2.64–2.52 (m, 1H), 2.25–2.13 (m, 1H), 1.68 (t, J = 5.9 Hz, 1H), 1.47 (s, 9H).

(*1S,3S,5S*)-6-[$^{13}CD_2$]-2-azabicyclo[3.1.0]hexane-3-carbonitrile hydrochloride (10)

Method of preparation is the same as (**9**), 92% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.20 (br. s., 1H), 8.64 (br. s., 1H), 8.01–7.58 (m, 2H), 4.39 (dd, J = 10.8, 2.8 Hz, 1H), 3.25 (d, J = 5.8 Hz, 1H), 2.10 (dt, J = 13.8, 3.5 Hz, 1H), 1.71 (t, J = 5.0 Hz, 1H).

***tert*-Butyl ((*S*)-2-((*1S,3S,5S*)-3-cyano-6-[$^{13}CD_2$]-2-azabicyclo[3.1.0]hexan-2-yl)-1-(3-hydroxyadamantan-1-yl)-2-oxoethyl) carbamate (13)**

Prepared in the same way as that described by K. Cao,[2] 70% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.88 (d, J = 8.5 Hz, 1H), 5.13 (d, J = 10.3 Hz, 1H), 4.42 (s, 1H), 4.33 (d, J = 9.0 Hz, 1H), 3.84 (d, J = 6.5 Hz, 1H), 2.18 (d, J = 13.8 Hz, 1H), 2.13–2.06 (m, 3H), 1.87 (t, J = 5.9 Hz, 1H), 1.66–1.30 (m, 21H).

[$^{13}CD_2$]saxagliptin (2)

To a flask containing compound (**13**) (832 mg, 1.99 mmol) was added 2-propanol (10 mL). To this solution was added 2 M hydrogen chloride in dioxane (13.92 mL, 27.80 mmol). The solution was heated to 70°C, and reaction progress was monitored by HPLC using method 3. After 1.5 h, the reaction was complete. The solution was rotovapped to dryness. The crude HCl salt product was dried under vacuum for 18 h at rt, dissolved in 7:3 water: acetonitrile (1.5 mL) and purified by semi-preparative HPLC using method 2. Pure fractions determined by analytical HPLC using method 3 were pooled, and solvent was removed by rotovap. The purified product was dried under vacuum to give 580 mg of a white solid as the TFA salt (67.0% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.22 (br. s., 3H), 5.24 (dd, J = 10.5, 1.8 Hz, 1H), 4.25 (d, J = 4.5 Hz, 1H), 4.09 (d, J = 6.3 Hz, 1H), 2.26 (d, J = 13.8 Hz, 1H), 2.16 (br. s., 2H), 1.95 (t, J = 5.4 Hz, 1H), 1.65 (d, J = 11.8 Hz, 3H), 1.61–1.54 (m, 2H), 1.53–1.39 (m, 7H). ^{13}C NMR (100 MHz, DMSO-d_6) 12.34. HPLC 99.3% (method 3, product $R_t = 6.37$ min). MS ESI$^+$ [$^{13}CD_2$ M + H]$^+$ = 319.33, mass isotopic distribution *m/z* 319.33 = 97.5%, 318.42 = 2.5%.

(*S*)-2-((*tert*-butoxycarbonyl)amino)-2-(3,5-dihydroxyadamantan-1-yl)acetic acid (15)

Potassium permanganate (1.34 g, 8.45 mmol) was dissolved in 2% aqueous KOH (25 mL). The solution was heated to 60°C, and (*S*)-2-((*tert*-butoxycarbonyl)amino)-2-(3-hydroxyadamantan-1-yl)acetic acid (**11**) (2.5 g, 7.68 mmol) was added in small portions. The mixture was heated to 85°C for 1.5 h. HPLC analysis using method 2 showed the formation of a more polar product $R_t = 7.12$ min versus the starting material $R_t = 9.60$ min. The black solution was cooled to 0°C, acidified with 1 N HCl (20 mL) to pH 3, and extracted with EtOAc (3 × 10 mL). The combined organic

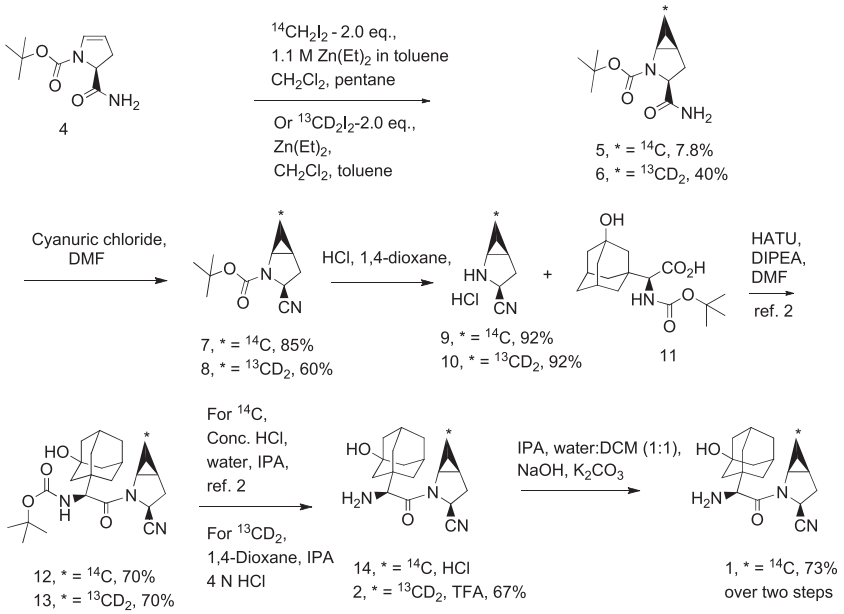
extracts were washed with brine, dried over Na_2SO_4, filtered, and the solvent was removed by rotovap. The crude product was purified by silica gel flash chromatography using 0–10% MeOH in CH_2Cl_2 and then with AcOH:MeOH:CH_2Cl_2 (0.05:0.95:9) to afford 0.67 g of a white solid (25% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.82 (d, J = 8.8 Hz, 1H), 4.46 (s, 2H), 3.73 (d, J = 9.0 Hz, 1H), 2.14 (br. s., 1H), 1.46–1.18 (m, 22H). MS ESI$^+$ [M-Boc + H]$^+$ = 242.25.

tert-Butyl((*S*)-2-((*1S,3S,5S*)-3-(6-[$^{13}CD_2$])-cyano-2-azabicyclo [3.1.0]hexan-2-yl)-1-3,5-dihydroxyadamantan-1-yl)-2-oxoethyl) carbamate (16)

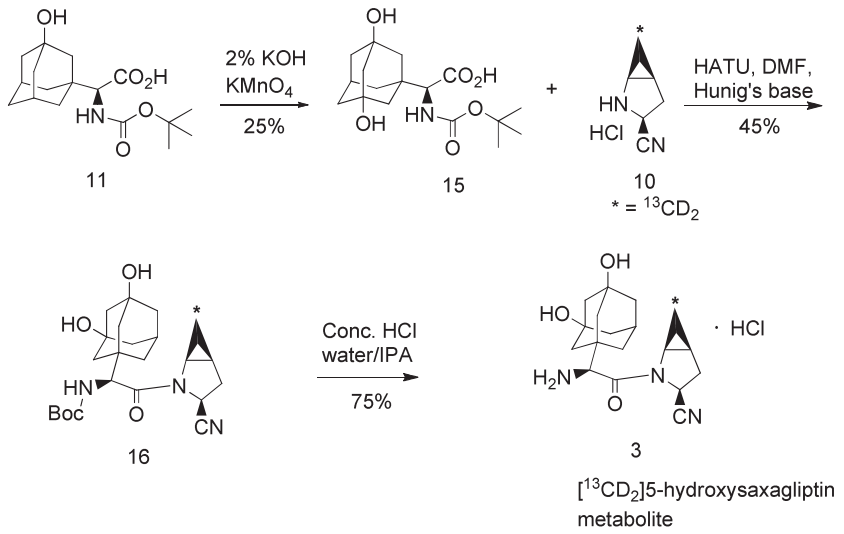
The $^{13}CD_2$ isotopologue was prepared from 118 mg (0.80 mmol) of (**10**) in the same way as that described by K. Cao[2] to produce 157.2 mg of (**16**) as a white solid, 45% yield. HPLC method 5, product R_t = 12.56 min. ^{1}H NMR (500 MHz, DMSO-d_6) δ 6.17 (br. s., 1H), 5.09 (d, J = 11.1 Hz, 1H), 4.38 (d, J = 8.7 Hz, 1H), 3.81 (d, J = 6.6 Hz, 1H), 2.65–2.55 (m, 1H), 2.20 (dt, J = 6.4, 3.2 Hz, 2H), 1.88 (br. s., 1H), 1.63–1.44 (m, 13H)1.45 (s, 9H).

$^{13}CD_2$-labeled 5-hydroxysaxagliptin metabolite (3)

The $^{13}CD_2$ isotopologue was prepared from 265 mg (0.61 mmol) of (**16**) in the same way as that described by K. Cao.[2] After the reaction, the resulted solution was evaporated to dryness, and the crude solid was dried under high vacuum for 18 h. The crude product was purified by trituration under nitrogen in diethyl ether (3 × 0.5 mL) to produce 153.1 mg of (**3**) as a white solid (75% yield). HPLC method 6, product R_t = 9.16 min, 97.3% pure. ^{1}H NMR (400 MHz, MeOH-d_4) δ 5.12 (dd, J = 10.8, 2.2 Hz, 1H), 3.91–3.83 (m, 2H), 2.72–2.55 (m, 1H), 2.39–2.28 (m, 2H), 1.95 (t, J = 5.8 Hz, 1H), 1.81–1.38 (m, 12H). ^{13}C NMR (100 MHz, DMSO-d_6) 13.94. MS ESI$^+$ [^{13}C, D_2 M + H]$^+$ = 335.24, mass isotopic distribution *m/z* 335.24 (97.87%), 334.28 (2.13%).



Scheme 3. Synthesis of ^{14}C and $^{13}CD_2$-labeled saxagliptin.



Scheme 4. Synthesis of $^{13}CD_2$-labeled 5-hydroxysaxagliptin metabolite.

Results and discussion

The ^{14}C-label of ^{14}C-labeled saxagliptin was introduced using a stereospecific Simmons–Smith cyclopropanation reaction.[5] The cyclopropanation was directed using chelation control between zinc and the amide functionality to exclusively provide the cis product. This avoided a costly diastereomeric separation. The average radioactive yield for this step, formation of compound (**5**) from two different campaigns was 7.8%. In unlabeled pilot reactions, the yields for the Simmons–Smith step were typically between 39% and 60%, when two equivalents of CH_2I_2 were used. The lower yield obtained in the radiochemical synthesis was possibly due to degradation of the precursor. It is worth noting that [^{14}C]CH_2I_2 was utilized within 2 weeks of its delivery. The reagent was easy to handle and did not suffer from extensive decomposition. On the larger scale of the stable-labeled isotope synthesis to form (**6**), the cyclopropanation reaction stalled without going to completion. In this case, the reaction was reinitiated by transferring the contents to a new reaction flask, and an additional 1.5 equivalents of $^{13}CD_2I_2$ was added. The dehydration reaction of primary amides (**5**) or (**6**) was carried out in the presence of cyanuric chloride in DMF to form (**7**) or (**8**), respectively. ^{14}C-labeled compound (**12**) was prepared[2] in an amide coupling reaction between (**9**) and adamantyl acid (**11**) to yield 4.1 mCi (70% radiochemical yield) of product (**12**) at 99.40% radiochemical purity. The Boc group of (**12**) was removed using concentrated HCl in water and 2-propanol. The HCl salt was converted to the free base form using NaOH and K_2CO_3. The crude product was purified by semi-preparative HPLC using no modifiers to ensure structure stability and only the formation of the free base form. A total of 3.0 mCi of ^{14}C-labeled saxagliptin monohydrate was obtained with a specific activity of 53.98 μCi/mg (17.13 mCi/mmol). The radiochemical purity was determined by HPLC to be 99.29%, and the overall radiochemical yield was 3.0%.

Following an analogous route, 580.0 mg of [$^{13}CD_2$]saxagliptin TFA salt, (**2**) was synthesized. To prepare (**3**), the synthesis began with hydroxylation of (**11**) with basic potassium permanganate to form (**15**) in 25% yield. Subsequent amide coupling reaction followed by deprotection with concentrated HCl in aqueous IPA afforded 153.1 mg of [$^{13}CD_2$]5-hydroxy metabolite HCl salt, (**3**). Both stable-isotope labeled products had undetectable levels of unlabeled products by LC-MS and were suitable for use as internal standards to support bioanalytical LC-MS analyses of biological samples (Schemes 3 and 4).

Conclusion

The hallmark of our synthesis is to utilize the method of stereospecific Simmons–Smith cyclopropanation reaction. In the presence of diethyl zinc, either $^{14}CH_2I_2$ or $^{13}CD_2I_2$ was used as labeling reagent, which was added to a chiral alkene to exclusively provide the cis cyclopropyl product. ^{14}C-labeled saxagliptin, $^{13}CD_2$-labeled saxagliptin, and its $^{13}CD_2$-labeled 5-hydroxy metabolite were prepared to further support development of the compound for biological studies. A total of 3.0 mCi of ^{14}C-labeled saxagliptin was obtained with a specific activity of 53.98 μCi/mg (17.13 mCi/mmol). The radiochemical purity determined by HPLC was 99.29%, and the overall radiochemical yield was 3.0% based upon 100 mCi of [^{14}C]CH_2I_2 starting material. By following similar synthetic routes, 580.0 mg of $^{13}CD_2$-labeled saxagliptin and 153.1 mg of $^{13}CD_2$-labeled 5-hydroxysaxagliptin metabolite were synthesized. The strategy used in this synthesis efficiently avoided a difficult diastereomeric separation.

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Conflict of Interest

The authors did not report any conflict of interest.

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