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Synthesis of empagliflozin, a novel and selective sodium-glucose co-transporter-2 inhibitor, labeled with carbon-14 and carbon-13

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Empagliflozin, (2*S*,3*R*,4*R*,5*S*,6*R*)-2-[4-chloro-3-[[4-[(3*S*)-oxolan-3-yl]oxyphenyl]methyl]phenyl]-6-(hydroxymethyl)oxane-3,4,5-triol was recently approved by the FDA for the treatment of chronic type 2 diabetes mellitus. Herein, we report the synthesis of carbon-13 and carbon-14 labeled empagliflozin. Carbon-13 labeled empagliflozin was prepared in five steps and in 34% overall chemical yield starting from the commercially available α-D-glucose-[$^{13}C_6$]. For the radiosynthesis, the carbon-14 atom was introduced in three different positions of the molecule. In the first synthesis, Carbon-14 D-(+)-gluconic acid δ-lactone was used to prepare specifically labeled empagliflozin in carbon-1 of the sugar moiety in four steps and in 19% overall radiochemical yield. Carbon-14 labeled empagliflozin with the radioactive atom in the benzylic position was obtained in eight steps and in 7% overall radiochemical yield. In the last synthesis carbon-14 uniformly labeled phenol was used to give [^{14}C]empagliflozin in eight steps and in 18% overall radiochemical yield. In all these radiosyntheses, the specific activities of the final compounds were higher than 53 mCi/mmol, and the radiochemical purities were above 98.5%.

Keywords: empagliflozin; diabetes mellitus; SGLT-2; carbon-14; carbon-13; radiosynthesis

Introduction

Diabetes is among the top ten leading causes of death in the US. More than 25 million people are inflected with this debilitating disease and the numbers continue to escalate due to obesity and an aging population.[1] Globally, 382 million people have diabetes. This number is expected to rise to 592 million or about 10% of the world's population, by 2035.[2] Type 2 diabetes is the most common form accounting for more than 90% of all causes in the developed world.[3] This disease occurs when the body does not properly produce or use insulin. Elevated blood sugar, or hyperglycemia, is a common effect of uncontrolled diabetes, which over time leads to serious damage to many of the body's organs and systems, especially the nerves, blood vessels, heart, and kidneys, resulting in blindness, low limb amputation, kidney failure, and cardiovascular disease.[4,5] The discovery of the sodium-glucose co-transporters (SGLTs), which act to ensure glucose entering the kidneys finds its way back into the blood stream,[6–12] has attracted huge interest by the pharmaceutical industry to find suitable inhibitors of these co-transporters and hence lower blood sugar by increasing the amount of sugar excreted in the urine.[13–18] Nearly all of the filtered glucose is reabsorbed in the kidneys by SGLT-1 and SGLT-2, with SGLT-2 responsible for about 90% of this re-absorption.[14] Phlorizin was the first SGLT inhibitor discovered.[19] The molecule itself was known since 1835 and is a naturally occurring compound isolated from the bark of pear, apple, cherry, and other fruit trees.[20,21] This compound was superseded by better and more selective synthetic analogs like canagliflozin,[22–24], dapagliflozin,[25,26] and empagliflozin[27,28] (Figure 1).

Empagliflozin, also known as jardiance and BI 10773, (2*S*,3*R*,4*R*,5*S*,6*R*)-2-[4-chloro-3-[[4-[(3*S*)-oxolan-3-yl]oxyphenyl]methyl]phenyl]-6-(hydroxymethyl)oxane-3,4,5-triol, was recently approved by the FDA for the treatment of diabetes mellitus-2.

Results and discussion

The synthesis of carbon-13 labeled empagliflozin was carried out starting from the commercially available α-D-glucose-[$^{13}C_6$] (**1**) (Scheme 1). Selective oxidation using Shvo's catalyst (1-hydroxytetraphenyl-cyclopentadieyl(tetraphenyl-2,4-cyclopentadien-1-one)-μ-hydrotetracarbonyldiruthenium) in the presence of cyclohexanone as a co-oxidant in dimethylformamide (DMF) provided the desired lactone (**2**) in an 90% yield.[29] Protection of the hydroxyl groups using trimethylsilyl chloride in the presence of excess *N*-methylmorpholine (NMM) in THF provided the fully protected lactone (**3**) in 89% yield.[30]

The lithiated anion of the aryl compound (**4**)[31] was prepared via halogen metal exchange at −78 °C with *n*-BuLi, and the resulting anion was reacted with the protected sugar (**3**) at −78 °C, followed by treatment with methanesulfonic acid in methanol at 45 °C to give the desired compound (**6**) according to the literature.[32,33] Reduction of (**6**) with Et_3SiH and BF_3OEt_2 gave the labeled [$^{13}C_6$]-(**7**) after re-crystallization from ethanol

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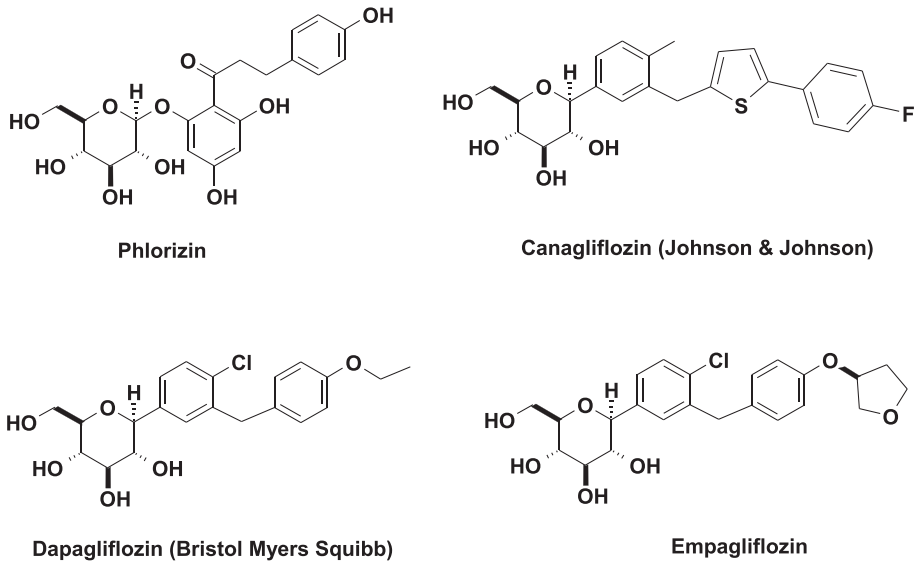
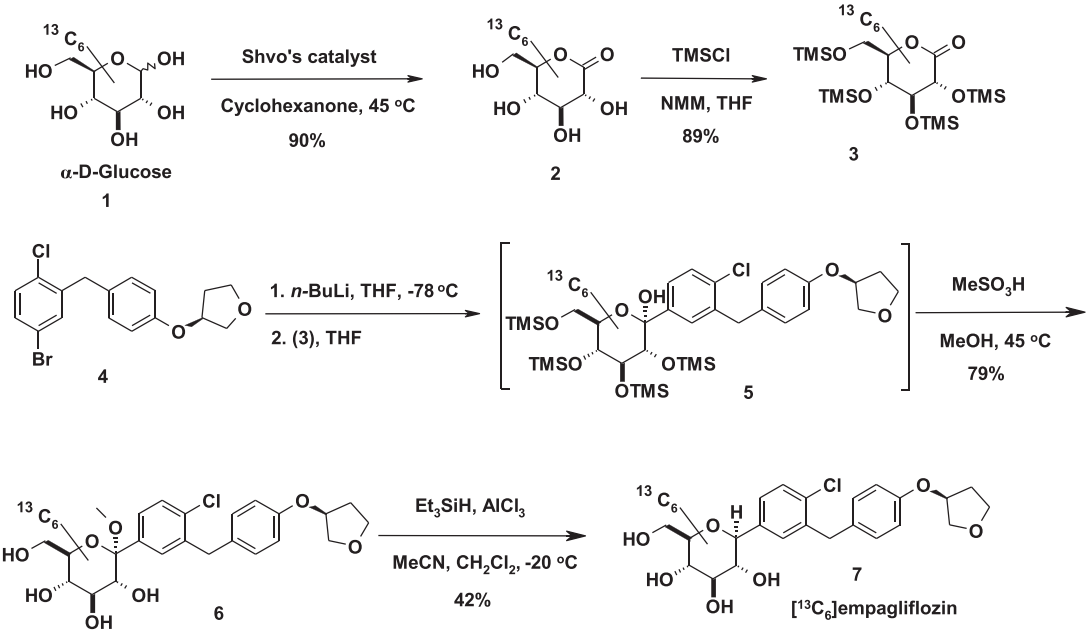


Figure 1. Sodium glucose co-transporter 2 (SGLT-2) inhibitors.

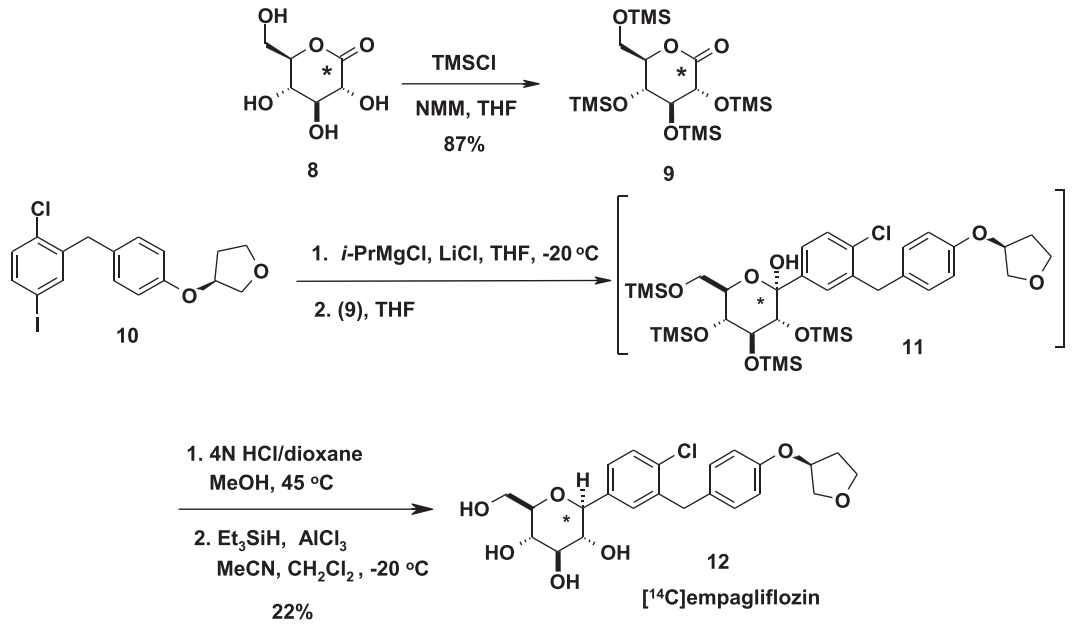


Scheme 1. Synthesis of [$^{13}C_6$]empagliflozin.

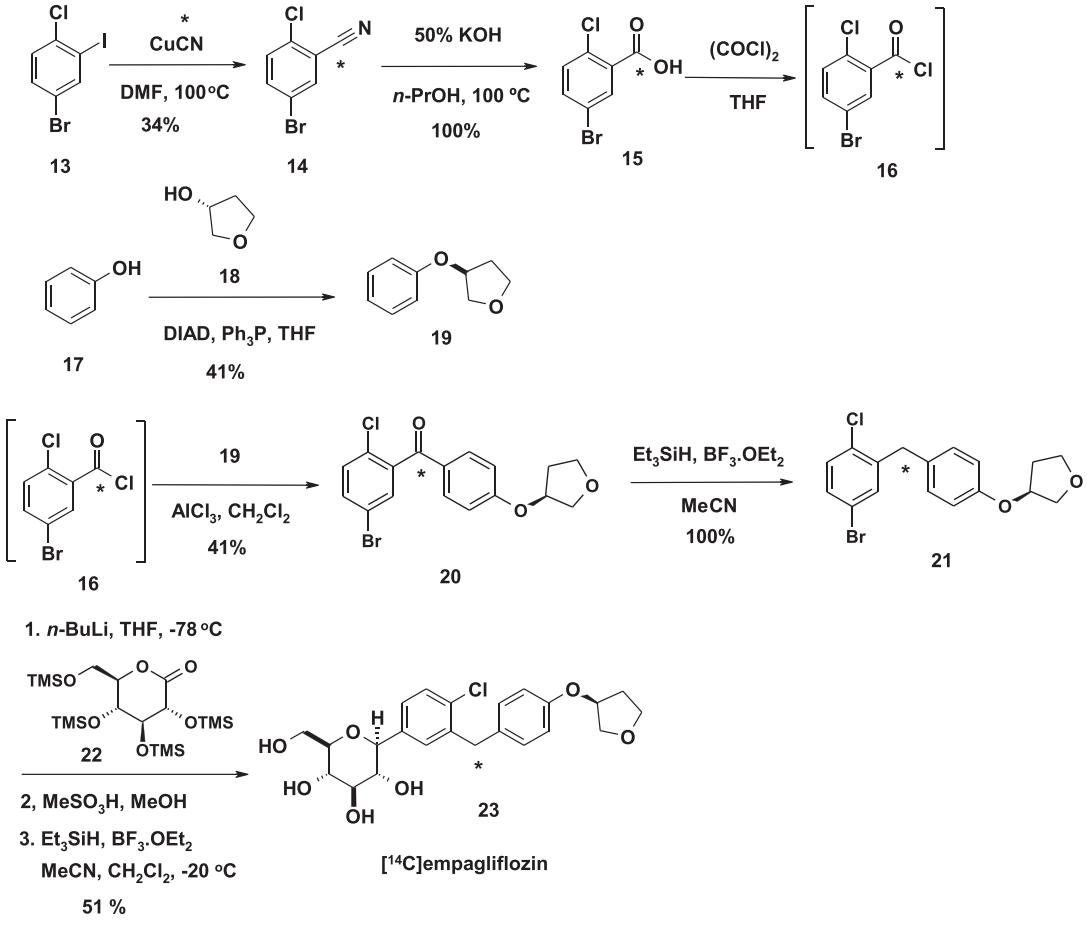
in 42% yield.[34] The crystallization removed any α-anomer formed during this final reduction of the anomeric methoxy group.[31]

The synthetic route described above was applied to the first synthesis of [^{14}C]empagliflozin, (Scheme 2). Carbon-14 D-(+)-gluconic acid δ-lactone (**8**) labeled specifically at carbon-1 of the sugar was custom made. Reaction of the protected tetrahydro-2*H*-pyran-2-one (**9**) with the anion of the aryl (**10**),[31] prepared via halogen metal exchange at −20 °C, followed by treatment with 4 N HCl (solution in 1,4-dioxane) in MeOH at 45 °C, and finally reduction with Et_3SiH and $AlCl_3$ gave pure [^{14}C]-(**12**) after crystallization from ethanol with radiochemical purity of 99.2%, a specific activity of 57.5 mCi/mmol, and in 19% overall radiochemical yield.

However, the synthesis of the lactone (**8**) is somewhat problematic as the oxidation of carbon-14 labeled glucose can be sluggish and yields were inconsistent. Hence in the second synthesis of [^{14}C]empagliflozin, we considered the preparation of 5-bromo-2-chlorobenzoic acid (**15**) with the label on the carboxyl carbon. We envisioned that starting from 5-bromo-2-chloro-iodobenzene (**13**) we could obtain the desired benzoic acid (**15**) in two steps via the nitrile (**14**). Carbon-14 cuprous cyanide ($Cu^{14}CN$) was reacted with (**13**) in DMF at 100 °C, (Scheme 3). Column chromatography afforded the desired product (**15**) and 4-chloro-5-iodobenzonitrile up to 89:11 ratios. Hydrolysis using 50% KOH gave the desired 5-bromo-2-chlorobenzoic acid (**15**) which can be crystallized from methanol and water. Friedel Crafts reaction was performed using freshly prepared acid chloride and the phenyl-3-(*S*)-hydroxytetrahydrofuran (**19**), prepared from phenol (**17**) and (*R*)-3-hydroxytetrahydrofuran (**18**)[35] to give the desired benzophenone (**20**) in a 41% yield. Reduction of the



Scheme 2. First synthesis of [^{14}C]empagliflozin (**12**), asterisk indicates position of carbon-14.



Scheme 3. Second synthesis of [^{14}C]empagloflizin (23).

carbonyl was accomplished in quantitative yield using Et_3SiH in the presence of BF_3OEt_2, and carbon-14 labeled empagliflozin was obtained as described before after re-crystallization from ethanol in 51% yield. The final product (**23**) was obtained in 7.1% overall radiochemical yield (4.57 mCi) with a specific activity of 54.27 mCi/mmol and a radiochemical purity of 99%.

The third synthesis of [^{14}C]empagliflozin was completed in five synthetic steps, starting from [U-^{14}C]phenol (**24**) as the radioactive starting material (Scheme 4). Mitsunobu's reaction with (*R*)-3-hydroxy-tetrahydrofuran (**18**) proceeded smoothly to give the desired ether (**25**) in a 76% yield with the desired *S*-configuration as described above. Friedel Crafts acylation with freshly prepared 5-bromo-2-chlorobenzoyl chloride (**26**) gave the desired benzophenone (**27**) in quantitative yield. Reduction to compound (**28**) as reported above was accomplished in a 93% yield. Then, reaction of the protected sugar (**22**) with the lithiated anion of (**28**), followed by treatment with methanesulfonic acid in methanol and reaction with triethyl silane as described above furnished the final product (**29**) after crystallization from ethanol in the amount of 36.0 mCi with a specific activity of 53.4 mCi/mmol. The radio-purity was found to be 99.4%, with an 18% overall radio-chemical yield.

Chiral HPLC showed that carbon-14 labeled empagliflozin did not contain any of the (*R*)-diastereomer of the hydroxyl-tetrahydrofuran moiety (**30**) (Figure 2). The same finding was observed from the synthesis described in Scheme 3. See Figure 3.

Experimental

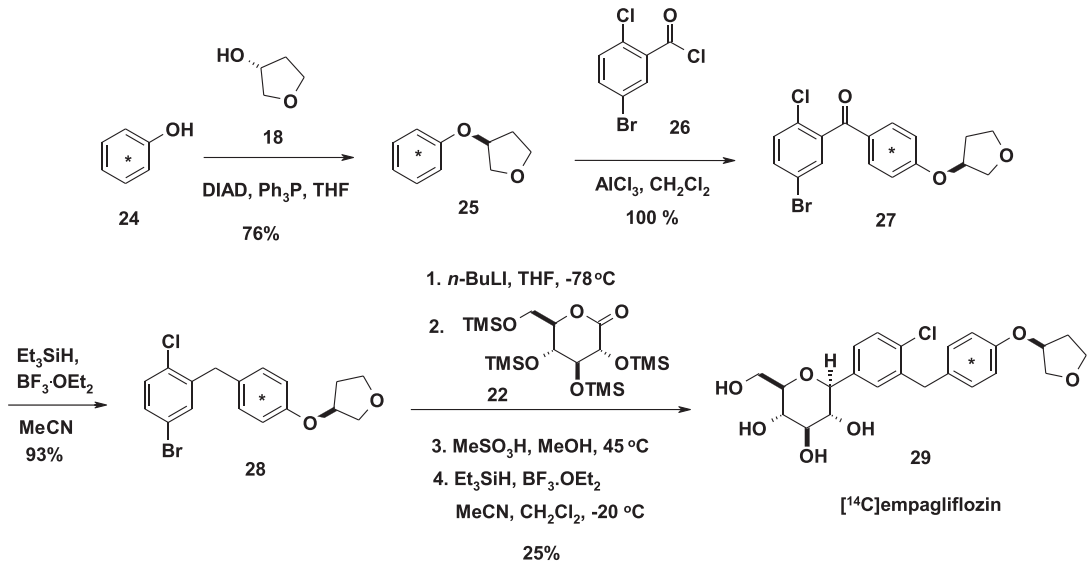
Materials and methods

NMR spectra of radioactive compounds were recorded on a Bruker 500-MHz spectrometer using double encapsulated NMR tubes in deuterated dimethyl sulfoxide. Liquid scintillation counting was accomplished using a Beckman LS6500TA and UltimaGold™ cocktail (PerkinElmer, Boston, MA). Pre-coated TLC sheets (silica gel 60 F_{254}) were obtained from EM Science (Gibbstown, NJ). HPLC analysis was performed on an Agilent 1200 instrument. HPLC Conditions: (a) Mobile Phase gradient 20 to 100% (MeCN/H_2O both 10 mM TFA) over 30 min, column: Zorbax SB C8 4.5 × 150 mm; (b) Mobile Phase gradient 20 to 100% (MeCN/H_2O both 10 mM TFA) over 20 min; (c) 70% A to 60% A in 7 min, then to 5% A in 8 min. A = water (0.1% TFA), B = MeCN. Flow rate: 1.2 mL/min, UV detection at 224 nm, column: Zorbax Eclipse XDB C8 (3.5 μm, 4.6 mm × 150 mm). The chemical purity of prepared compounds was greater than 98%. The radiochemical purity was measured using a radio-HPLC detector β-Ram model 4 or model 3 (LabLogic systems, Inc. Brandon, FL) connected to the Agilent instrument using IN-FLOW™ 2:1 liquid scintillation (LabLogic systems, Inc. Brandon, FL). The radiochemical purity of the final compounds was greater than 98%. For enantiomeric purity, the mobile phase consisted of isocratic water with 0.1% AcOH, pH 7.0 with NH_4OH and MeCN (99/1). Flow rate at 2.0 mL/min for 20 min and UV detection at 230 nm and using ChromTech Chiral AGP column (5 μm, 4.0 × 150 mm, ID 4.6 mm). [U-^{14}C]Phenol was purchased PerkinElmer (Boston, MA). Cuprous[^{14}C]cyanide was purchased from Quotient Bioresearch (Cardiff, UK). D-(+)-Gluconic acid δ-lactone [2-^{14}C] was custom made by Moravek Biochemicals (Brea, CA). (*R*)-3-Hydroxytetrahydrofuran was obtained from CODEXIS® (Redwood City, CA). The rest of the reagents were purchased from Sigma-Aldrich Company.

Synthesis of [$^{13}C_6$]-empagliflozine

[$^{13}C_6$]-(3R,4S,5S,6R)-3,4,5-Trihydroxy-6-hydroxymethyl-tetrahydro-2H-pyran-2-one (2)

Cyclohexanone (20 mL) was added to [$^{13}C_6$]-α-D-glucose (540 mg, 2.90 mmol) and Shvo's catalyst (40 mg) at room temperature. The mixture was stirred at 45 °C for 16 h. The mixture was transferred to a centrifuge tube and centrifuged at 3000 rpm for 5 min. The solvent was decanted off, and the solid was dried under high vacuum giving 482 mg of (**2**) in 90% yield as an off white solid. LCMS *m/z*: 185 (MH^+). ^{1}H NMR (500 MHz, D_3COD) δ: 5.81 (1H, m), 5.41 (2H, m), 4.88 (1H, m), 4.10 (1H, bd, *J* = 97 Hz), 3.83 (1H, m). ^{13}C NMR (100 MHz, D_3COD) δ:



Scheme 4. Third synthesis of [^{14}C]empagliflozin (**29**).

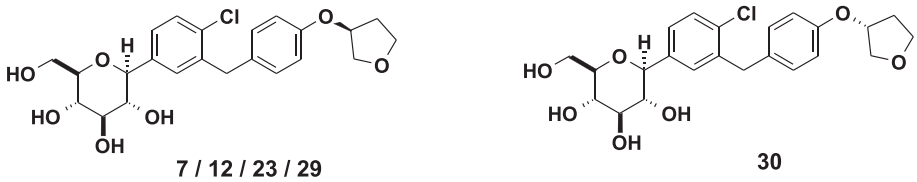


Figure 2. Empagliflozin and its (*R*)-3-phenoxy-tetrahydrofuran diastereomer.

171.9 (d, $J = 54$ Hz), 81.3 (t, $J = 41$ Hz), 73.9 (t, $J = 41$ Hz), 71.5 (ddd, $J = 3$, 41, 54 Hz), 67.9 (dt, $J = 3$, 41 Hz), 60.2 (d, $J = 41$ Hz).

[$^{13}C_6$]-(3R,4S,5R,6R)-3,4,5-Tris-trimethylsilyloxy-6-trimethylsilyloxymethyl-tetrahydro-2H-pyran-2-one (3)

TMSCl (1.7 g, 15.6 mmol) was added slowly to a solution of the above lactone (480 mg, 2.60 mmol) and NMM (2.1 g, 20.8 mmol) in THF (10 mL) at 0 °C. The mixture was warmed to room temperature and stirred for 20 h. The reaction mixture was diluted with toluene and H_2O. The organic layer was washed sequentially with aq. Na_2HPO_4 (1 g/20 mL), brine, dried over Na_2SO_4, and concentrated. The residue was diluted with heptane and concentrated. The residue was purified by flash chromatography on Combi-Flash® Companion (40 g, 0–10% EtOAc/Hexane) to give 1.1 g of (**3**) as a colorless oil in 89% yield. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 4.30 (1H, bd, $J = 67$ Hz), 3.94 (3H, m), 3.61 (2H, m), 0.15 (9H, s), 0.14 (9H, s), 0.13 (9H, s), 0.09 (9H, s).

[$^{13}C_6$]-(2S,3R,4S,5S,6R)-2-{4-Chloro-3-[4-(tetrahydro-furan-(3S)-yloxy)-benzyl]phenyl}-6-hydroxymethyl-2-methoxytetrahydro-2H-pyran-3,4,5-triol (6)

A solution of 3(*S*)-[3-(5-bromo-2-chloro-benzyl)-phenoxy]-tetrahydrofuran (**4**) (685 mg, 1.9 mmol) in THF (2 mL) was added to a solution of *n*-BuLi (1.0 mL, 2.3 mmol) in THF (5 mL) at −78 °C. After stirring for 1 h, the lactone (**3**) (1.1 g, 2.3 mmol) in THF (3 mL) was added dropwise (5 min). The mixture was stirred for 1.5 h at −78 °C. The reaction was quenched by addition of AcOH/H_2O (300 μL/10 mL), diluted with EtOAc (20 mL). The organic layer was washed with brine, dried over Na_2SO_4, and concentrated giving (**5**). The residue was diluted with MeOH (10 mL), then $MeSO_3H$ (125 μL) was added and the mixture was stirred at 45 °C for 2.5 h. The reaction mixture was cooled to room temperature and quenched by addition of $NaHCO_3$ (10 mL). The mixture was extracted with EtOAc (10 mL × 3). The combined extracts were washed with brine and dried over Na_2SO_4 and concentrated giving 720 mg of (**6**) in 79% yield as an off white foam. LCMS *m/z*: 509 ($M + Na^+$), which was used as it is in the next step.

[$^{13}C_6$]-(2S,3R,4R,5S,6R)-2-{4-Chloro-3-[4-(tetrahydro-furan-3(S)-yloxy)-benzyl]phenyl}-6-hydroxymethyl-tetrahydro-2H-pyran-3,4,5-triol (7)

BF_3OEt_2 (162 mg, 1.13 mmol) was added dropwise to a solution of (**6**) (250 mg, 0.51 mmol) and Et_3SiH (177 mg, 1.53 mmol) in CH_2Cl_2/MeCN (0.5 mL/2 mL) at −25 °C. The mixture was warmed slowly to 10 °C in 2 h period. The mixture was cooled to −10 °C, and quenched by addition of a saturated aqueous solution of $NaHCO_3$ (10 mL), extracted with EtOAc (10 mL × 3). The combined EtOAc extracts were concentrated, then diluted with EtOH and concentrated giving off white foam. The foam was diluted with EtOH (2.5 mL) warmed, covered, and stirred overnight. The solid was collected by vacuum filtration giving 98 mg of (**7**) in 42% yield as a white solid. HPLC (retention time): 5.9 min.[b] LCMS *m/z*: 457 (MH^+). ^{1}H NMR (500 MHz, DMSO-d_6) δ: 7.37 (1H, d, $J = 8.5$ Hz), 7.33 (1H, bs), 7.23 (1H, bd, $J = 8.0$ Hz), 7.11 (2H, d, $J = 8.0$ Hz), 6.82 (2H, d, $J = 8.0$ Hz), 4.93 (3H, m), 4.80 (1H, bs), 4.42 (1H,bs), 4.00 (1H, d, $J = 15$ Hz), 3.96 (1H, d, $J = 15$ Hz), 3.83 (3H, m), 3.74 (2H, m), 3.56 (1H, m), 3.10 (1H,m), 3.00 (1H,m), 2.18 (1H, m), 1.93 (1H,m). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 155.5, 139.7 (d, $J = 52$ Hz), 137.8 (d, $J = 3$ Hz), 131.6, 130.9, 129.7, 128.7 (d, $J = 3$ Hz), 127.5, 115.2, 81.2 (d, $J = 42$ Hz), 80.4 (d, $J = 40$ Hz), 78.4 (t, $J = 40$ Hz), 77.0, 74.7 (t, $J = 40$ Hz), 72.4, 70.3 (t, $J = 40$ Hz), 66.5, 61.5 (d, $J = 44$ Hz), 37.7, 32.5.

Synthesis of [^{14}C]empagliflozin

[^{14}C]Empagliflozin labeled in the sugar moiety

(3R,4S,5R,6R)-3,4,5-Tris-trimethylsilyloxy-6-trimethylsilyloxymethyl-tetrahydro-2H-pyran-[2-^{14}C]-2-one (9). Trimethylsilyl chloride (1.98 g, 18.0 mmol) was added slowly to a solution of [^{14}C]-D-(+)-gluconic acid δ-lactone (**8**), (150 mCi, ≈ 3.0 mmol) and NMM (2.44 g, 24.0 mmol) in THF (15 mL) at 0 °C. The mixture was warmed to room temperature and stirred for 20 h. The mixture was diluted with toluene (20 mL) and H_2O (15 mL). The organic layer was washed sequentially with aq. Na_2HPO_4 (1 g/20 mL water) and brine (15 mL), dried over Na_2SO_4, and concentrated. The residue was diluted with heptane (20 mL) again and concentrated. The residue was purified by flash chromatography (0–10% EtOAc/Hexane) to give 130 mCi or 1.06 g of (**9**) as a colorless oil in 87% yield.

(2S,3R,4R,5S,6R)-2-{4-Chloro-3-[4-(tetrahydro-furan-3-yloxy)-benzyl]phenyl}-6-hydroxymethyl-tetrahydro-2H-pyran-[2-^{14}C]-3,4,5-triol (12). A solution of (**10**) (0.75 g, 1.8 mmol) was dissolved in THF (10 mL) in a round bottom flask under N_2 and cooled to −20 °C. Then, *i*-PrMgCl/LiCl (1.3 M in THF, 1.45 mL, 1.89 mmol) was slowly added, keeping internal temperature below −10 °C. The mixture was stirred at −7 °C for 40 min. The reaction was cooled to −20 °C and a solution of the protected lactone (**9**) (75 mCi, 1.5 mmol) in THF (3 mL) was added, keeping temperature below −10 °C. The reaction was stirred at −10 °C for 1.5 h then, warmed to 0 °C over 30 min and stirred at 0 °C for 20 min. The reaction was cooled to −10 °C, and MeOH (15 mL) was added slowly, keeping internal temperature below 5 °C. A solution of 4 N HCl in dioxane (1 mL, 4 mmol) was added and the solution was concentrated. Methanol (10 mL) was added followed with a solution of 4 N HCl in dioxane (1.0 mL, 1.0 mmol) until pH ≈ 2.5. The mixture was stirred at 45 °C for 2 h and slowly cooled to 22 °C and stirred for 6 h at 22 °C. The mixture was quenched by addition of 5% Na_2CO_3 (20 mL) and extracted with EtOAc (20 mL × 3). The combined extracts were washed with brine and dried over Na_2SO_4 and concentrated to a residue. This residue (40 mCi, 0.77 mmol), MeCN (2 mL), and CH_2Cl_2 (2 mL) were added. In a separate flask, $AlCl_3$ (205 mg, 1.54 mmol) was added followed by CH_2Cl_2 (3 mL) and cooled to 0 °C. MeCN (3 mL) was added slowly, keeping internal temperature below 20 °C. The slurry turned into solution after the addition was completed. Et_3SiH (242 mg, 2.08 mmol) was then added. A solution of the starting material was slowly added keeping internal temperature below 15 °C. The solution was warmed to 20 °C and stirred for 30 min. The mixture was cooled to 0 °C, and water (10 mL) was slowly added with rapid stirring, keeping internal temperature below 15 °C. The mixture was stirred for 15 min and extracted with EtOAc (20 mL × 3). The combined EtOAc extracts were dried over Na_2SO_4 and concentrated giving crude [^{14}C]-(**12**). The crude material was purified by silica gel chromatography (0–15%MeOH/CH_2Cl_2) to give 19 mCi of product as a white foam. Further crystallization from ethanol gave 16.5 mCi of material as a white solid after drying under high vacuum with a specific activity of 57.5 mCi/mmol and a radiochemical purity of 99.2%. ^{1}HNMR was identical to unlabeled standard.

[^{14}C]Empagliflozin labeled in the benzylic position

5-Bromo-2-chlorobenzo-[^{14}C]nitrile (14). A suspension of Cu^{14}CN (200 mCi, 340 mg, 3.7 mmol) in DMF (5 mL) was added to 5-bromo-2-chloroiodobenzene (**13**) (1.2 g, 3.7 mmol) in DMF (5 mL) at room temperature; the mixture was heated to 100 °C overnight. The reaction mixture was cooled to room temperature, diluted with EtOAc (20 mL), filtered through Celite®, washed sequentially with water (5 mL × 4), brine, filtered through a membrane filter, and concentrated giving a light brown solid. The solid was purified by silica gel chromatography (5% EtOAc/Hexane) to give a 90:10 mixture of 5-bromo-2-chlorobenzo-[^{14}C] nitrile **(14)** and the undesired 2-chloro-5-iodobenzo-[^{14}C]nitrile (280 mg, 68 mCi) in 34% yield as a white solid.

5-Bromo-2-chlorobenzoic acid-[carboxyl-^{14}C] (15). The above mixture (280 mg, 1.29 mmol) was diluted with 50% KOH (2.0 mL) and *n*-propanol (2.0 mL). The mixture was heated at 100 °C overnight. Then 4 N HCl (6 mL) was added, and the mixture was allowed to cool to room temperature. The solid was collected by vacuum filtration to give a light brown solid. The solid was diluted with MeOH (2.5 mL) warmed to 70 °C, then filtered, the liquid was heated to 70 °C, then water (8 mL) was added, and the mixture was stirred at 70 °C for 0.5 h. The solution was allowed to cool to room temperature, and the solid was collected by vacuum filtration to give 305 mg of (**15**) in quantitative yield as an off white solid. HPLC: (retention time) 7.6 min, co-eluted with an unlabeled commercial material.

(S)-3-Phenoxy-tetrahydrofuran (19). Mitsunobu coupling of phenol (**17**) (753 mg, 8.0 mmol) and (*R*)-3-hydroxytetrahydrofuran (**18**) (775 mg, 8.8 mmol) according to the general procedure gave (*S*)-3-phenoxy-tetrahydrofuran (540 mg, 41%) as a colorless oil. LCMS *m/z*: 165 (MH^+). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.28 (2H, m), 6.96 (1H, m), 6.87 (2H, m), 4.93 (1H, ddd, $J = 2$, 4.5, 8 Hz), 4.00 (2H, m), 3.90 (1H, ddd, $J = 4.5$, 8, 8 Hz), 2.18 (2H, m). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 157.4, 129.6, 121.0, 115.4, 73.2, 67.2, 33.0.

(S)-(5-Bromo-2-chlorophenyl)-(4-tetrahydrofuran-3-yloxyphenyl)-[^{14}C]methanone (20). Oxalyl chloride (160 μL, 1.89 mmol) was added dropwise to a mixture of (**15**) (300 mg 1.27 mmol) and DMF (50 μL) in THF (10 mL) at room temperature. The mixture was stirred for 1.5 h then, concentrated. The residue was diluted with heptane (15 mL) and concentrated to give a light yellow oil. The oil was diluted with CH_2Cl_2 (5 mL) and cooled to 0 °C, then $AlCl_3$ (173.3 mg, 1.30 mmol) was added followed by (**19**) (213.5 mg, 1.30 mmol) in CH_2Cl_2 (3 mL). A second portion of $AlCl_3$ (173.3 mg, 1.30 mmol) was added, and the mixture was stirred overnight. HPLC revealed incomplete conversion; therefore, an additional portion of $AlCl_3$ (173.3 mg, 1.30 mmol) was added, and the mixture was stirred for an additional 4 h. The reaction was quenched by the addition of water (15 mL) and extracted with CH_2Cl_2 (20 mL × 3). The combined extracts were dried over Na_2SO_4, filtered through a membrane filter and concentrated to give a brown oil. The crude oil was purified by silica gel chromatography (40 g, 10–30% EtOAc/hexanes) to give (**20**) (201 mg, 28 mCi) in 41% yield as colorless oil. HPLC (retention time): 10.9 min.[(c)]

(S)-3-[4-[(5-Bromo-2-chlorophenyl)-[^{14}C]methyl]phenoxy]tetrahydrofuran (21). BF_3OEt_2 (93.0 μL, 0.79 mmol) was added to a mixture of the above material (201 mg, 0.52 mmol) and Et_3SiH (435.2 μL, 2.62 mmol) in MeCN (8.0 mL) MeCN (8.0 mL) at room temperature. The mixture was stirred overnight. The reaction was quenched by addition of 1 N NaOH (5 mL) and stirred for 20 min. The mixture was diluted with water and extracted with CH_2Cl_2 (20 mL × 3). The combined extracts were dried over Na_2SO_4, filtered through a membrane filter, and concentrated to give 240 mg (28 mCi) of (**21**) as a colorless oil. HPLC (retention time): 12.5 min.[(c)]

(2S,3R,4R,5S,6R)-2-{4-Chloro-3-[4-(tetrahydro-furan-(3S)-yloxy)-[^{14}C]benzyl]phenyl}-6-hydroxymethyl-tetrahydro-2H-pyran-3,4,5-triol (23). *n*-BuLi 2.5 M in hexanes (0.3 mL, 0.65 mmol) was added to a solution of (**21**) (240.0 mg, 0.65 mmol) in THF (3 mL) at −78 °C. After stirring for 1 h, (**22**) (303.5 mg, 0.65 mmol) in THF (1 mL) was added dropwise. The mixture was stirred at −78 °C for 1.5 h and then quenched by addition of AcOH/water (0.1 mL/3 mL). The mixture was warmed to room temperature then diluted with EtOAc (10 mL) and brine (5 mL). The organic material was dried over Na_2SO_4 and concentrated. The residue was diluted with MeOH (4 mL), and then $MeSO_3H$ (0.1 mL, 1.16 mmol) was added, and the mixture was heated at 45 °C for 2 h. The mixture was cooled to room temperature then quenched by addition of a saturated solution of $NaHCO_3$ (10 mL). The mixture was extracted with EtOAc (10 mL × 3); the combined extracts were dried over Na_2SO_4 and concentrated to give a light yellow oil. The oil was diluted with CH_2Cl_2 (1 mL), then heptane (4 mL) was added to precipitate the product out of solution. The precipitate was washed with heptane then diluted with CH_2Cl_2 (20 mL) and transferred to a tarred flask. The solution was concentrated to give (3*R*,4*S*,5*S*,6*R*)-2-{4-chloro-3-[4-(tetrahydro-furan-(3*S*)-yloxy)-[^{14}C]benzyl]-phenyl}-6-hydroxymethyl-2-methoxy-tetrahydro-2*H*-pyran-3,4,5-triol (161 mg, 51%) as a white foam. HPLC (retention time) 6.5 min.[(c)] A solution of BF_3OEt_2 (142 mg, 1.0 mmol) was added dropwise to a solution of the above material (161 mg, 0.33 mmol) and Et_3SiH (116.3 mg, 1.0 mmol) in CH_2Cl_2 (1.0 mL)/MeCN (4.0 mL) at −25 °C. The mixture was allowed to warm to −10 °C over 1 h, then held at −10 °C for 0.5 h. The reaction was quenched by addition of saturated solution of $NaHCO_3$ (10 mL), extracted with EtOAc (20 mL × 3), and the combined extracts were dried over Na_2SO_4 and concentrated. The crude residue was diluted with EtOH (2.0 mL) warmed until all the material was dissolved. The solution was allowed to cool to room temperature with stirring. Then unlabeled empagliflozin seeds (ca. 1 mg) were added, and the mixture was stirred overnight. The solid was collected by vacuum filtration to give [^{14}C]empagliflozin (35 mg, 23%) as white solid. The mother liquor was concentrated and purified by silica gel chromatography (12 g Cartridge, 0-10% MeOH/CH_2Cl_2) to give 84 mg, or 56% as a colorless glass. A total of 119 mg or 14 mCi were obtained in 50% overall yield. The specific activity was 54.27 mCi/mmol; radiochemical purity of 98.87%, and chemical purity of 99.16%. HPLC (retention time): 4.9 min.[(c)]

[U-^{14}C]Empagliflozin labeled in the phenyl group

(S)-3-[U-^{14}C]Phenoxy-tetrahydrofuran (25). DIAD (890 mg, 4.4 mmol) was added dropwise to a solution of [U-^{14}C]phenol (**24**) (200 mCi, ≈ 4.0 mmol), Ph_3P (1.2 g, 4.4 mmol), and (*R*)-3-hydroxytetrahydrofuran (**18**) (387 mg, 4.4 mmol) in THF (15 mL). The solution was stirred overnight at room temperature (HPLC[b] showed product:phenol ratio of 70:30). A second portion of Ph_3P (600 mg, 2.2 mmol), (*R*)-3-hydroxytetrahydrofuran (193 mg, 2.2 mmol) in THF (2 mL), and DIAD (445 mg, 2.2 mmol) was added. The reaction mixture was stirred for 2 days. The reaction mixture was concentrated and purified by Combi-Flash® Companion (40 g, 5–50% EtOAc/Hexane) to give 500 mg (151 mCi) of (**25**) in 76% yield as a colorless oil. HPLC (retention time): 8.9[(b)]

(S)-(5-Bromo-2-chlorophenyl)-[3(S)-(tetrahydro-furan-3-yloxy)-[U-^{14}C]phenyl]-methanone (27). Oxalyl chloride (460 mg, 3.6 mmol) was added dropwise to a solution of 5-bromo-2-chlorobenzoic acid (730 mg, 3.1 mmol) and DMF (100 μL) in THF (15 mL) at room temperature. After stirring for 2 h, the reaction mixture was concentrated. The residue was diluted with heptane (25 mL) and concentrated and dried under high vacuum. The residue was diluted with CH_2Cl_2 (10 mL) cooled to 0 °C, then $AlCl_3$ (400 mg, 3.0 mmol) was added. The mixture was warmed to room temperature and (**25**) (500 mg, 3.05 mmol) in CH_2Cl_2 (10 mL) was added followed by a second portion of $AlCl_3$ (400 mg, 3.0 mmol). The reaction mixture was stirred overnight. The reaction was quenched by addition of H_2O (10 mL), filtered through Celite®, and extracted with CH_2Cl_2 (20 mL × 3). The combined extracts were dried over Na_2SO_4 and concentrated giving 1.18 g (150 mCi) of (**27**) as a colorless oil in quantitative yield. HPLC (retention time): 14.3 min.[(b)]

3(S)-[3-(5-Bromo-2-chlorobenzyl)-[U-^{14}C]phenoxy]-tetrahydrofuran (28). BF_3OEt_2 (567 mg, 4.0 mmol) was added dropwise to a solution of (**27**) (1.18 g, 3.6 mmol) and Et_3SiH (1.26 g, 10.9 mmol) in CH_2Cl_2:MeCN (5 mL:20 mL) at room temperature. The mixture was stirred overnight. The mixture was filtered and quenched by addition of 1 M NaOH (5 mL). The mixture was diluted with CH_2Cl_2 (10 mL) and H_2O (10 mL) and stirred for 20 min. The organic material was removed by syringe, and the aqueous layer was extracted with CH_2Cl_2 (10 mL × 2). The combined extracts were dried over Na_2SO_4 and concentrated giving 1.02 g (139.5 mCi) of (**28**) in 93% yield as a colorless oil. HPLC (retention time): 16.6 min.[(b)]

(2S,3R,4R,5S,6R)-2-{4-Chloro-3-[4-(tetrahydrofuran-3(S)-yloxy)-benzyl]-[U-^{14}C]phenyl}-6-hydroxymethyl-tetrahydro-2H-pyran-3,4,5-triol (29). *n*-BuLi (1.3 mL, 3.34 mmol) was added to a solution of (**28**) (1.02 g, 2.78 mmol) in THF (10 mL) at −78 °C. After stirring for 1 h, (**22**) (1.56 g, 3.34 mmol) in THF (5 mL) was added dropwise (5 min). The mixture was stirred for 1.5 h at −78 °C. The reaction was quenched by addition of AcOH:H_2O (0.6 mL:20 mL), diluted with EtOAc (30 mL). The organic layer was washed with brine, dried over Na_2SO_4, and concentrated. The residue was diluted with MeOH (10 mL), then $MeSO_3H$ (200 μL) was added, and the mixture was stirred at 45 °C for 2.5 h. The reaction mixture was cooled to room temperature and quenched by addition of a saturated solution of $NaHCO_3$ (10 mL). The mixture was extracted with EtOAc (10 mL × 3). The combined extracts were washed with brine and dried over Na_2SO_4 and concentrated giving (3*R*,4*S*,5*S*,6*R*)-2-{4-chloro-3-

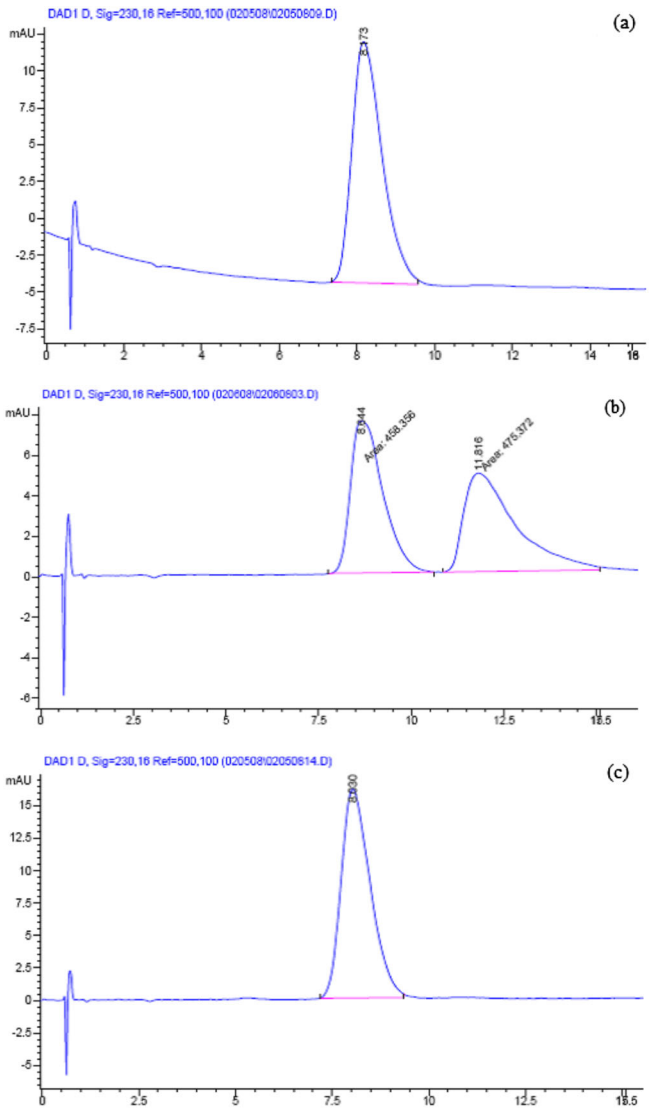


Figure 3. (a) Chiral HPLC of empagliflozin, (b) mixture of empagliflozin and (30), and (c) [^{14}C]empagliflozin.

[4-(tetrahydro-furan-(3*S*)-yloxy)-benzyl]-[U-^{14}C]phenyl}-6-hydroxymethyl-2-methoxy-tetrahydro-2*H*-pyran-3,4,5-triol (1.07 g) as an off white foam. A solution of BF_3OEt_2 (930 mg, 6.6 mmol) was added dropwise to a solution of the above material (1.07 g, 2.2 mmol) and Et_3SiH (765 mg, 6.6 mmol) in CH_2Cl_2/MeCN (2.5 mL/10 mL) at −25 °C. The mixture was warmed slowly to −10 °C over a period of 2 h. The reaction was quenched by addition of saturated $NaHCO_3$ (10 mL) and extracted with EtOAc (20 mL × 3). The combined EtOAc extracts were dried over Na_2SO_4 and concentrated, then diluted with EtOH and concentrated (2 mL × 2) giving an off white foam. The crude material was purified by Combi-Flash Companion (40 g, 0–15% MeOH/CH_2Cl_2) to give the product as white foam. The foam was diluted with EtOH (5 mL) warmed, covered, and stirred overnight. The solid was collected by vacuum filtration giving [^{14}C]empagliflozin (304 mg, 35.78 mCi) as a white solid after drying under high vacuum with a specific activity of 53.4 mCi/mmol and radiochemical purity of 98.6%. ^{1}H NMR (500 MHz, CD_3OD) δ: 7.33 (2H, m), 7.27 (1H, d, *J* = 8 Hz), 7.11 (2H, d, *J* = 8.5 Hz), 6.79 (2H, d, *J* = 8.5 Hz), 4.95 (1H, m), 4.08 (1H, d, *J* = 10 Hz), 4.03 (2H,d, *J* = 10 Hz), 3.93 (2H, m), 3.87 (3H, m), 3.69(1H, bd, *J* = 11 Hz), 3.44(1H, m), 3.39(2H, m), 3.31 (1H, m), 2.20 (1H, m), 2.08 (1H, m). HPLC (retention time): 8.5 [(a)]

Chiral purity determination of [^{14}C] empagliflozin

To determine if [^{14}C]empagliflozin (Figure 3) contained any of the diastereomer shown in Figure 2, especially the product produced from Scheme 3 and Scheme 4. We are not concerned about the small amounts of α-anomer formed at the sugar moiety which were usually removed during crystallization. Unlabeled empagliflozin (2 mg) was dissolved in methanol (10 mL). The diastereomer (**30**) (2 mg) was dissolved in methanol (10 mL). A mixture of 0.5 mL of each of the solutions was mixed. [^{14}C]-Empagliflozin, 1.0 mM solution in EtOH, 200 μL was diluted with 300 μL MeOH.

HPLC conditions: System: Agilent 1100 (Quaternary Pump); Column: ChromTech Chiral AGP 4.0 mm × 150 mm ID: 4.6 mm particle size 5 μm; column temperature: 15 °C; mobile phase: A, HPLC water with 0.1% AcOH, pH 7.0 with NH_4OH; B, HPLC MeCN; elution mode: isocratic 99/1 A/B; flow rate: 2.0 mL/min. Run time: 20 min; detection: UV at 230 nm; injection: 5 μL. HPLC showed no detected amounts of diastereomer (**30**).

Conclusion

Carbon-13 labeled empagliflozin was prepared in five steps and in 34% overall chemical yield starting from the commercially available α-D-glucose-[$^{13}C_6$]. For the radiosynthesis, the carbon-14 atom was introduced in three different positions of empagliflozin. In the first synthesis, carbon-14 D-(+)-gluconic acid δ-lactone was used according to the literature to prepare carbon-14 specifically labeled empagliflozin in carbon-1 of the sugar moiety. This four step-synthesis gave the desired material in 19% overall radiochemical yield and with a specific activity of 57.5 mCi/mmol and a radiochemical purity of 99.2%. Carbon-14 labeled empagliflozin with the radioactive atom in the benzylic position was obtained in eight steps and in 7% overall radiochemical yield with a specific activity of 54.3 mCi/mmol and a radiochemical purity of 98.9%. In the last synthesis carbon-14 uniformly labeled phenol was used to give [^{14}C]empagliflozin in eight steps and in 18% overall radiochemical yield with a specific activity of 53.4 mCi/mmol and a radiochemical purity of 98.6%. Labeled empagliflozin was indispensable in DMPK and other studies.

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Conflict of Interest

The authors did not report any conflict of interest.

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