Synthesis of unlabelled and stable isotope labelled glucuronide metabolites of dapagliflozin and synthesis of stable isotope labelled dapagliflozin

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Summary

Two regioisomeric glucuronide metabolites of dapagliflozin (BMS-512148) were synthesized and used to elucidate the structures of dapagliflozin metabolites observed in human urine samples. The structures of the synthetic metabolites were assigned by HMBC, ROESY, and TOCSY experiments. Analogues of these metabolites containing carbon-13 as a stable-label were also prepared for use as internal standards for the analysis of urine samples obtained from patients participating in clinical studies.

Key Words: SGLT2, dapagliflozin, glucuronide metabolites, stable-isotope label

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Introduction

Approximately 25 million individuals in the US and greater than 300 million worldwide suffer from type II diabetes, also known as non-insulin dependent diabetes mellitus (NIDDM), which is characterized by hyperglycemia, insulin resistance and impaired insulin secretion.\(^1\)\(^,\)\(^2\) One method for reducing glycemic levels involves inhibition of sodium glucose co-transporter 2 (SGLT2). This transporter is located in the kidney and reabsorbs glucose from the renal filtrate.\(^3\) The inhibition of SGLT2 activity reduces plasma glucose levels through increased glucose excretion, and offsets the development of diabetic complications.\(^4\)\(^,\)\(^5\) Dapagliflozin (BMS-512148) \(^1\)\(^a\) (Figure 1) is a potent and selective SGLT2 inhibitor discovered at Bristol-Myers Squibb.\(^6\) It is currently marketed in the United States as Farxiga\(^8\) for the treatment of type 2 diabetes.

During the development of BMS-512148, quantitative metabolite identification and profiling using carbon-14 labelled BMS-512148 lead to the discovery of several metabolites present in human urine.\(^7\) Two metabolites, m10 and m15, were found to account for greater than 90% of the radioactive isolate (Figure 2). Both of these metabolites arise from glucuronidation of BMS-512148. Prior to this work, the position of glucuronidation on the parent glycoside (2-O vs 3-O) was not known. Synthetic reference standards were required to identify the molecular structures of the urine metabolites. Furthermore, stable-isotope labelled metabolite standards were required as internal standards for LC-MS analyses in support of clinical studies. Due to the need for both unlabelled and labelled synthetic standards, an efficient route for the preparation of the 2-O and 3-O glucuronide metabolites was developed. As a result of this work, the structures of the m15 \(^2\)\(^a\) and m10 \(^3\)\(^a\) metabolites were assigned as shown in Figure 3.
From $^{13}$C$_6$BMS-512148, stable isotope labeled metabolite m15 2b and m10 3b were prepared as shown in scheme 2. The work was first presented at IIS NE US chapter meeting.\textsuperscript{8} Similar syntheses for other gliflozins, for example, Empagliflozin\textsuperscript{9} and Canagliflozin\textsuperscript{10} were also reported.

**Results and discussion**

A streamlined synthetic approach was devised to access both the 2-O-glucuronide and 3-O-glucuronide metabolites from the parent BMS-512148 with and without stable-isotopic labelling. We planned to perform the glucuronidation of the partially protected glycoside of BMS-512148 in a non-regioselective fashion and then separate the regioisomers at a later stage in the synthesis. The 4- and 6-positions of the glycoside would be blocked with the di-tert-butylsilylenediyl ether group\textsuperscript{11} to allow simultaneous glucuronidation at both the 2- and 3- positions. This approach would permit the simultaneous construction of the two metabolites from a common intermediate and minimize protecting group manipulations.

Synthesis of the metabolites began with silylation of BMS-512148 1a selectively at the 1,3-diol (C4 and C6) to give cyclic di-tert-butylsilylenediyl ether 12a in 85% yield (Scheme 1). The glucuronide donor 13 was prepared from glucurono-6,3-lactone following a literature procedure\textsuperscript{12}. Coupling of cyclic di-tert-butylsilylenediyl ether 12a with compound 13 in the presence of TMSOTf gave a mixture of regioisomers 14a and 15a in 50% yield. The C3 isomer 14a was formed as the major isomer in a 3:1 ratio. The regioselectivity is likely due to steric hindrance from the phenyl ring at position C1. Not surprisingly, no bis-glucuronide product was observed, which presumably can be explained by steric effects. The two regio-isomers were not separated and were used directly in the following reaction. Deprotection of the cyclic di-tert-butylsilylenediyl ethers 14a and 15a was initially attempted
using tetrabutylammonium fluoride, resulting in a complicated mixture that required extensive HPLC purification. Later triethylamine trihydrofluoride was found to give a cleaner reaction wherein 16a and 17a were separated using silica gel flash chromatography (93% combined yield). Disaccharides 16a and 17a were treated separately with aqueous NaOH in MeOH to form 2a (85% yield) and 3a (81% yield).

Prior to synthesizing the stable-isotope labelled metabolites, stable-isotope labelled BMS-512148 was prepared from uniformly labelled [13C6] phenetole 4 following the published synthesis of unlabeled BMS-512148 (Scheme 2). Friedel-Crafts reaction of 2-chloro-bromobenzoyl chloride 5 with [13C6]phenetole 4 gave ketone 6. Reduction of ketone 6 by triethylsilane followed by coupling with TMS-protected glucolactone 9 (prepared by silylation of gluconolactone 8, Scheme 3) yielded compound 10. Reduction of 10 and acetylation with acetic anhydride produced the penultimate compound 11. [13C6] BMS-512148 1b was prepared by hydrolysis of compound 11 with aqueous LiOH.

Stable-isotope labelled metabolites 2b and 3b were prepared from [13C6] BMS-512148 1b by the synthetic route for the unlabelled metabolites in similar overall yield (Scheme 1). Isotopic abundances of 2b and 3b were each 96%, with 4% M+5 and no M+0 as determined by mass spectral analysis. These labelled metabolites served as internal standards for the analysis of urine samples obtained from patients participating in clinical studies.

Synthetic metabolites 2a, 2b, 3a, and 3b co-eluted with their corresponding biological standards m15 and m10 using analytical HPLC and showed the same MS-MS fragmentation patterns. A representative example of synthetic vs. human m15 2a is shown in Figure 4. The NMR spectra of 2a and 2b matched that of the m15 biological standard (Figure 5).
The TOCSY spectrum (Figure 6) of the unlabeled m15 metabolite 2a shows the proton spin system for the two sugar like moieties in the molecule. The position of the glucuronide (Gluc) in the metabolite was determined using $^1$H-$^{13}$C heteronuclear correlations. The $^1$H-$^{13}$C HMBC spectrum of the metabolite (Figure 7) shows correlations between protons 2 and 6 to carbon 4, which confirmed the position of 4. Also shown in the HMBC spectrum is the long range correlation between proton 30 (Gluc) and carbon 4 in the parent compound, identifying the attachment site of the glucuronide to carbon 4. This was further confirmed from ROESY (through space interactions). The ROESY spectrum (Figure 8) shows correlations between proton 30 and protons 3 and 4, which indicates that they are within 5Å of each other and is consistent with the proposed site of glucuronide attachment. The structure of m10 was similarly determined.

**Conclusion**

Two glucuronide metabolites of BMS-512148 were synthesized for use as reference standards, and were used to assign the structures of the m15 2a and m10 3a metabolites. The assignments were made by comparing data obtained from HPLC, LCMS, and 1D and 2D NMR spectroscopy for the synthetic reference standards and the isolated metabolites. The carbon-13 labelled metabolites 2b and 3b and carbon-13 labelled BMS-512148 1b were also prepared for use as internal standards.

**Materials and Methods**

$^1$H and proton decoupled $^{13}$C NMR were recorded on a Bruker DRX-400 MHz (Billerica, MA) or a Bruker Avance II 300 MHz spectrometer. NMR structure elucidation of m15 2a and m10 3a were performed on a Bruker Avance II 600 MHz spectrometer using $^1$H,
proton decoupled $^{13}$C, double-quantum filtered correlation spectroscopy (DQCOSY), total correlation spectroscopy (TOCSY), $^{1}$H-$^{13}$C heteronuclear multiple-quantum correlation ($^{1}$H-$^{13}$C HMQC), $^{1}$H-$^{13}$C Heteronuclear Multiple-Bond Correlation ($^{1}$H-$^{13}$C HMBC), rotating-frame NOE spectroscopy (ROESY) experiments. Mass spectra were taken on either a Thermo LCQ or a LXQ spectrometer (Waltham, MA). HPLC purification was performed on a Varian ProStar HPLC system consisting of two SD-218 pumps, a PrepStar detector, HPLC analysis was conducted on an Agilent 1100 (Santa Clara, CA). The mobile phase for HPLC consisted of solvent A: 5 mM NH$_4$OAc, 0.1% formic acid, B: acetonitrile. Analytical HPLC was conducted on an Agilent Eclipse XDB C18, 4.6 mm x 150 mm, flow rate 0.8 mL/min, wavelength 220 nm, gradient from 10% B to 45% B in 35 min. Preparative HPLC was conducted on a Phenomenex Hydro RP C18 (21.2 mm x 250 mm) column (Torrance, CA) at 220 nm, 15 mL/min, gradient from 35% B to 45% B in 28 min. TLC was carried out on silica gel 60 F254 plates (Merck) with Hanessian dip visualization. All other reagents were obtained from Aldrich Chemical Company (Milwaukee, WI) and were either American Chemical Society grade or the highest quality material commercially available. Identities of labelled compounds were established by co-injection with fully characterized unlabelled compounds via HPLC along with NMR and Mass spectrometry when possible.

(2S,3S,4S,5R,6R)-2-(methoxycarbonyl)-6-(2,2,2-trichloro-1-iminoethoxy)tetrahydro-2H-pyran-3,4,5-triy1 tris(2-methylpropanoate) 13 was prepared according to literature procedure.\textsuperscript{7}

**Experimental**

(4aR,6S,7R,8R,8aS)-2,2-di-tert-Butyl-6-(4-chloro-3-(4-ethoxybenzyl)phenyl)hexahydro[3,2-d][1,3,2]dioxasilane-7,8-diol (12a): BMS-512148 (1a, 2.04g, 5 mmol) in dichloromethane (100 mL) was added 2, 6-lutidine (3 mL,
25.7 mmol) at 0 °C under Ar. Di-tert-butylsilyl-bis(trifluoromethanesulfonate) (3.74 mL, 11.5 mmol) was then added and stirred at 0 °C. After 30 min, solvent was evaporated under reduced pressure. Flash chromatography of the crude (10% EtOAc in hexanes to 35% in 25 min) gave 2.35 g 12a, yield 85%. \(^1\)H-NMR (300 MHz, CDCl\(_3\)) δ (ppm): 1.00 (s, 9H), 1.07 (s, 9H), 1.39 (t, J = 7 Hz, 3H), 3.55 (m, 2H), 3.63 (t, J = 8.7 Hz, 1H), 3.77 (t, J = 9.0 Hz, 1H), 3.87 (t, J = 10.2 Hz, 1H), 4.0 (m, 4H), 4.17 (m, 2H), 6.81 (d, J = 8.7 Hz, 2H), 7.08 (d, J = 8.7 Hz, 2H), 7.17 (m, 2H), 7.36 (d, J = 7.9 Hz, 1H); \(^1\)C-NMR (75.48 MHz, CDCl\(_3\)) δ (ppm): 157.46, 139.22, 136.77, 134.37, 131.15, 130.20, 129.84, 129.72, 126.32, 114.51, 81.41, 78.19, 74.74, 74.61, 66.34, 63.39, 38.38, 27.46, 26.99, 22.67, 19.98, 14.86. MS (ESI) m/z: [M+H\(^+\)] calculated 549, 551, observed 549, 551.

(2R,3R,4S,5S,6S)-2-(((4aR,6S,7R,8R,8aS)-2,2-di-tert-Butyl-6-(4-chloro-3-(4-ethoxybenzyl)phenyl)-8-hydroxyhexahydropyran[3,2-d][1,3,2]dioxasilin-7-yl)oxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl tris(2-methylpropanoate) (14a) and (2R,3R,4S,5S,6S)-2-(((4aR,6S,7S,8R,8aR)-2,2-di-tert-Butyl-6-(4-chloro-3-(4-ethoxybenzyl)phenyl)-7-hydroxyhexahydropyran[3,2-d][1,3,2]dioxasilin-8-yl)oxy)-6-(ethoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl tris(2-methylpropanoate) (15a): A mixture of silyl protected BMS-512148 12a (848 mg, 1.54 mmol), (2S,3S,4S,5R,6R)-2-(methoxycarbonyl)-6-(2,2,2-trichloro-1-iminoethoxy)tetrahydro-2H-pyran-3,4,5-triyl tris(2-methylpropanoate) 13 (1.08 g, 1.9 mmol), and 4A powdered molecular sieves (1.0 g) in anhydrous dichloromethane was stirred at room temperature for 1 h under Ar. Me\(_3\)SiOTf (100 uL, 0.46 mmol) was added and stirred at room temperature for 1 h. More Me\(_3\)SiOTf (100 uL, 0.46 mmol) was added and stirred at room temperature for 1 h. Solvent was removed under reduced pressure. Flash chromatography (100% hexanes to 20% ethyl acetate in 40 min, then changed to 70% ethyl acetate in 25 min) gave a mixture of regioisomers (14a+15a) 675 mg, yield 46%. The mixture was used directly for the following step. LC-MS (ESI) m/z:
[M+NH₄]⁺ calculated 966, 968, observed 966, 968; [M+Na]⁺ calculated 971, 973, observed 971, 973.

(2R,3R,4S,5S,6S)-2-(((2S,3S,4R,5R,6R)-2-(4-chloro-3-(4-ethoxybenzyl)phenyl)-3,5-dihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-4-yl)oxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triy1 tris(2-methylpropanoate) (16a) and (2R,3R,4S,5S,6S)-2-(((2S,3R,4S,5S,6R)-2-(4-chloro-3-(4-ethoxybenzyl)phenyl)-4,5-dihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-3-yl)oxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triy1 tris(2-methylpropanoate) (17a): To a cooled (0 °C) solution of 14a and 15a (mixture of unresolved regioisomers) (650mg, 0.68 mmol) in anhydrous THF (20 mL) was added Et₃N·3HF (0.34 mL) and stirred at 0 °C for 5 h. Solvent was evaporated under reduced pressure. Flash chromatography (5% methanol in dichloromethane) gave two major products: 16a 366 mg and 17a 150 mg, yield 93%. 16a ¹H-NMR (300 MHz, CDCl₃) δ (ppm): 1.06 (m, 18H), 1.39 (t, J = 7 Hz 3 H), 2.47 (m, 3H), 3.48 (m, 2H), 3.55 (t, J = 8.5 Hz , 1H), 3.63 (t, J = 9.0 Hz, 1H), 3.74 (s, 3H), 3.76 (m, 1H), 3.92 (m, 4H), 4.11 (m, 2H), 4.80 (d, J = 7.8 Hz, 1H), 5.08 (dd, J = 9.3, 7.8 Hz, 1H), 5.25 (t, J = 9.6 Hz, 1H), 5.35 (t, J = 9.4 Hz, 1H), 6.80 (d, J = 8.6 Hz, 2H), 7.08 (d, J = 8.5 Hz, 2H), 7.13 (d, J = 1.8 Hz, 1H), 7.16 (dd, J = 8.3, 2.0 Hz, 1H), 7.37 (d, J = 8.0 Hz, 1H); ¹³C-NMR (100.62 MHz, CDCl₃) δ (ppm): 175.76, 175.32, 166.89, 157.47, 139.42, 136.51, 134.47, 130.98, 130.10, 129.81, 126.32, 114.47, 101.56, 89.48, 81.07, 79.74, 73.88, 71.78, 71.38, 71.18, 69.74, 68.46, 63.36, 63.11, 53.12, 38.37, 33.82, 18.83, 18.79, 18.74, 18.71, 18.70, 18.67, 14.87. MS (ESI) m/z: [M+NH₄]⁺ calculated 826, 828, observed 826, 828; [M+Na]⁺ calculated 831, 833, observed 831, 833. 17a ¹H-NMR (300 MHz, CDCl₃) δ (ppm): 1.08 (m, 18H), 1.38 (t, J = 7 Hz 3 H), 2.43 (m, 3H), 3.35 (d, J = 9.8 Hz, 1H), 3.43 (m, 1H), 3.60 (m, 3H), 3.64 (s, 3H), 3.76 (m, 1H), 3.85 (m, 1H), 3.92-4.12 (m, 4H), 4.18 (dd, J = 9.2 Hz, 1H), 4.41 (dd, J = 7.9 Hz, 1H), 4.82 (t, J = 8.6 Hz, 1H), 4.98 (t, J = 9.7 Hz, 1H), 5.11(t,
$J = 9.5 \text{ Hz, } 1\text{H}$, 6.87 (d, $J = 8.7 \text{ Hz, } 2\text{H}$), 7.08-7.19 (m, 3H), 7.27 (m, 1H); $^{13}$C-NMR (100.62 MHz, CDCl$_3$) δ (ppm): 175.79, 175.73, 175.18, 166.64, 157.57, 138.82, 136.29, 134.05, 131.27, 130.67, 129.80, 129.11, 127.19, 114.54, 100.22, 82.40, 79.83, 78.94, 77.66, 77.21, 72.26, 71.37, 70.74, 68.89, 63.34, 62.66, 52.78, 38.25, 33.93, 33.76, 33.69, 18.86, 18.77, 18.67, 14.87. MS (ESI) m/z: [M+NH$_4$]$^+$ calculated 826, 828, observed 826, 828; [M+Na]$^+$ calculated 831, 833, observed 831, 833.

(2S,3S,4S,5R,6R)-6-(((2S,3S,4R,5R,6R)-2-(4-Chloro-3-(4-ethoxybenzyl)phenyl)-3,5-dihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-4-yl)oxy)-3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylic acid (2a): To a solution of protected glucuronide 16a (346 mg, 0.42 mmol) in methanol (17 mL) was added aq. NaOH (1N, 3.5 mL) and stirred at ambient temperature for 1.5 h. The solvent was removed under reduced pressure and the crude was purified by preparative HPLC. Pooled fractions were combined, evaporated under reduced pressure and lyophilized to yield 2a 205 mg as a white lyophile, yield 85%. $^1$H-NMR (400 MHz, DMSO-$d_6$) δ (ppm): 1.28 (t, $J = 7 \text{ Hz, } 3\text{H}$), 3.0-3.5 (m, 13H), 3.71 (d, $J = 11.3 \text{ Hz, } 1\text{H}$), 3.90-4.00 (m, 4H), 4.09 (d, $J = 9.1 \text{ Hz, } 1\text{H}$), 4.23 (d, $J = 7.6 \text{ Hz, } 1\text{H}$), 6.82 (d, $J = 8.8 \text{ Hz, } 2\text{H}$), 7.08 (d, $J = 8.8 \text{ Hz, } 2\text{H}$), 7.25 (dd, $J = 8.4, 2.0 \text{ Hz, } 1\text{H}$), 7.34 (d, $J = 2.0 \text{ Hz, } 1\text{H}$), 7.37 (d, $J = 8.3 \text{ Hz, } 1\text{H}$); $^{13}$C-NMR (100.62 MHz, DMSO-$d_6$) δ (ppm): 172.46, 165.55, 165.88, 139.23, 137.91, 132.05, 131.19, 130.78, 129.52, 128.71, 127.32, 114.29, 103.58, 89.64, 80.97, 79.92, 76.02, 73.70, 73.04, 72.96, 72.03, 68.77, 62.87, 61.21, 37.61, 14.68. MS (ESI) m/z: [M+NH$_4$]$^+$ calculated 602, 604, observed 602, 604; [M+Na]$^+$ calculated 607, 609, observed 607, 609.

(2S,3S,4S,5R,6R)-6-(((2S,3R,4S,5S,6R)-2-(4-Chloro-3-(4-ethoxybenzyl)phenyl)-4,5-dihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-3-yl)oxy)-3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylic acid (3a): Compound 17a (150 mg, 0.185 mmol) was hydrolyzed as described for 16a. Preparative HPLC yielded 3a 87 mg as a white
lyophile, yield 81%. $^1$H-NMR (400 MHz, DMSO-$d_6$) δ (ppm): 1.28 (t, $J = 6.9$ Hz, 3H), 2.82 (t, $J = 8.1$ Hz, 1H), 2.90-3.05 (m, 2H), 3.15-3.30 (m, 2H), 3.41 (dd, $J = 5.8, 11.9$ Hz, 1H), 3.51-3.70 (m, 3H), 3.92-4.00 (m, 4H), 4.18 (d, $J = 9.3$ Hz, 1H), 4.30 (d, $J = 7.8$ Hz, 1H), 6.79 (d, $J = 8.8$ Hz, 2H), 7.10 (d, $J = 8.6$ Hz, 2H), 7.27 (d, $J = 8.4$ Hz, 1H), 7.36 (dd, $J = 8.4, 2.0$ Hz, 1H), 7.50 (d, $J = 1.8$ Hz, 1H); $^{13}$C-NMR (100.62 MHz, DMSO-$d_6$) δ (ppm): 172.48, 165.70, 156.74, 138.08, 137.83, 132.02, 131.71, 131.50, 129.59, 128.51, 128.24, 114.15, 101.47, 80.94, 80.33, 78.44, 77.10, 76.45, 74.26, 73.75, 72.12, 70.15, 62.83, 61.19, 37.36, 14.70. MS (ESI) $m/z$: [M+NH$_4$]$^+$ calculated 602, 604, observed 602, 604; [M+Na]$^+$ calculated 607, 609.

(4aR,6S,7R,8R,8aS)-2,2-di-tert-Butyl-6-(4-chloro-3-((4-ethoxyphenyl)-1,2,3,4,5,6-$^{13}$C$_6$)(methyl)phenyl)hexahydropyrano[3,2-d][1,3,2]dioxasilin-7,8-diyl (12b): Compound 12b (1.08 g, 88%) was prepared from compound 1b following the procedure described for unlabeled 12a. MS (ESI) $m/z$: [M+H$^+$] calculated 555, 557, observed 555, 557.

(2R,3R,4S,5S,6S)-2-(((4aR,6S,7S,8R,8aR)-2,2-di-tert-Butyl-6-(4-chloro-3-((4-ethoxyphenyl)-1,2,3,4,5,6-$^{13}$C$_6$)(methyl)phenyl)-7-hydroxyhexahydropyrano[3,2-d][1,3,2]dioxasilin-8-yl)oxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl tris(2-methylpropanoate) (14b) and (2R,3R,4S,5S,6S)-2-(((4aR,6S,7R,8R,8aS)-2,2-di-tert-Butyl-6-(4-chloro-3-((4-ethoxyphenyl)-1,2,3,4,5,6-$^{13}$C$_6$)(methyl)phenyl)-8-hydroxyhexahydropyrano[3,2-d][1,3,2]dioxasilin-7-yl)oxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl tris(2-methylpropanoate) (15b): Compounds 14b and 15b were prepared as a mixture of regioisomers (669 mg, 36% yield) from compound 12b following the procedure described for unlabeled 14a and 15a. LC-MS (ESI) $m/z$: [M+NH$_4$]$^+$ calculated 972, 974, observed 972, 974; [M+Na]$^+$ calculated 977, 978, observed 977, 979.
(2R,3R,4S,5S,6S)-2-(((2S,3S,4R,5R,6R)-2-(4-chloro-3-((4-ethoxyphenyl-1,2,3,4,5,6-13C6)methyl)phenyl)-3,5-dihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-4-yl)oxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyltriis(2-methylpropanoate) (16b) and (2R,3R,4S,5S,6S)-2-(((2S,3R,4S,5S,6R)-2-(4-chloro-3-((4-ethoxyphenyl-1,2,3,4,5,6-13C6)methyl)phenyl)-4,5-dihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-3-yl)oxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyltriis(2-methylpropanoate) (17b):

Compound 16b (298 mg, 52% yield) and compound 17b (120 mg, 21% yield) were prepared from regioisomeric mixture of 14b and 15b following the procedure described for unlabeled 16a and 17a. Compound 16b 1H-NMR(400 MHz, CDCl3) δ (ppm): 1.02-1.14 (m, 18H), 1.39 (t, J = 7 Hz, 3H), 2.47 (m, 3H), 3.44-3.51 (m, 2H), 3.56 (t, J = 8.5 Hz , 1H), 3.63 (t, J = 9.0 Hz, 1H), 3.74 (s, 3H), 3.77 (m, 1H), 3.90-4.05 (m, 4H), 3.99 (dd, J = 2.2, 6.9 Hz, 2H), 4.08 (d, J = 9.3 Hz, 1H), 4.13(d, J = 9.8 Hz, 1H), 4.80 (d, J = 7.8 Hz, 1H), 5.08 (dd, J = 9.3, 7.8 Hz, 1H), 5.24 (t, J = 9.6 Hz, 1H), 5.34 (t, J = 9.4 Hz, 1H), 5.55-6.65 (m, 1H), 6.80-6.95 (m, 1H), 6.96-7.04 (m, 1H), 7.13 (d, J = 2 Hz, 1H), 7.16 (dd, J = 8.3, 2.1 Hz, 1H), 7.26-7.33 (m, 1H), 7.37 (d, J = 8.2 Hz, 1H). 13C-NMR (75.48 MHz, CDCl3) δ (ppm): 157.46 (ddd, J = 7.9, 67.3, 67.3 Hz), 128.9-131.8 (m), 113.6-115.2 (m). MS (ESI) m/z: [M+NH4]+ calculated 832, 834, observed 832, 834; [M+Na]+ calculated 837, 839, observed 837, 839. Compound 17b. 1H-NMR(400 MHz, CDCl3) δ (ppm): 1.01-1.14 (m, 18H), 1.37 (t, J = 6.9 Hz, 3H), 2.43 (m, 3H), 3.32-3.40 (m, 2H), 3.41 (d, J = 10.1 Hz , 1H), 3.47-3.69 (m, 4H), 3.62 (s, 3H), 3.71-3.87 (m, 2H), 3.92-4.10 (m, 2H), 4.01 (dd, J = 2.1, 6.9 Hz, 2H), 4.16 (d, J = 9.3 Hz, 1H), 4.48(d, J = 8.1 Hz, 1H), 4.82 (dd, J = 7.9, 9.4 Hz, 1H), 4.98 (t, J = 9.7 Hz, 1H), 5.12 (t, J = 9.6 Hz, 1H), 6.62-6.72 (m, br, 1H), 6.86-6.97 (m, br, 1H), 7.02-7.10 (m, 1H), 7.13-7.20 (m, 2H), 7.26 (d, J = 8.1 Hz, 1H), 7.25-7.35 (m, 1H). 13C-NMR (75.48 MHz, CDCl3) δ (ppm): 157.46 (ddd, J = 7.9, 67.3, 67.3 Hz), 129.0-132.0 (m), 113.7-115.1(m). MS (ESI) m/z:
(2S,3S,4S,5R,6R)-6-(((2S,3S,4R,5R,6R)-2-(4-Chloro-3-((4-ethoxyphenyl-1,2,3,4,5,6-
$^{13}$C$_8$-methyl)phenyl)-3,5-dihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-4-yl)oxy)-
3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylic acid (2b): Compound 2b (102 mg, 49% yield) was prepared from compound 16b following the procedure described for unlabeled 2a. $^1$H-NMR (400 MHz, DMSO-$d_6$) δ (ppm): 1.28 (t, $J = 6.9$ Hz, 3H), 3.01-3.52 (m, 11H), 3.71 (d, $J = 11.3$ Hz, 1H), 3.91-4.04 (m, 4H), 4.09 (d, $J = 9.3$ Hz, 1H), 4.25 (d, $J = 7.9$ Hz, 1H), 6.40-6.55 (m, br, 1H), 6.70-6.85 (m, br, 1H), 6.90-7.05 (m, br, 1H), 7.15-7.21 (m, 1H), 7.23-7.36 (m, 1H), 7.26-7.33 (m, 3H). $^{13}$C-NMR (100.62 MHz, DMSO-$d_6$) δ (ppm): 157.85 (ddd, $J = 8.1$, 66.7, 66.7 Hz), 128.6-132.0 (m), 113.4-115.1 (m). MS (ESI) $m/z$: [M+NH$_4$]$^+$ calculated 591, 593, observed 591, 593; [M+Na]$^+$ calculated 613, 615, observed 613, 615.

(2S,3S,4S,5R,6R)-6-(((2S,3R,4S,5S,6R)-2-(4-Chloro-3-((4-ethoxyphenyl-1,2,3,4,5,6-
$^{13}$C$_8$-methyl)phenyl)-4,5-dihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-3-yl)oxy)-
3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylic acid (3b): Compound 3b (35 mg, 50% yield) was prepared from compound 17b following the procedure described for unlabeled 3a. $^1$H-NMR (400 MHz, DMSO-$d_6$) δ (ppm): 1.28 (t, $J = 6.9$ Hz, 3H), 2.81-2.85 (m, 1H), 2.95-3.10 (m, 3H), 3.10-3.30 (m, 3H), 3.41 (dd, $J = 5.7$, 11.7 Hz, 1H), 3.51-3.71 (m, 3H), 3.92-4.03 (m, 2H), 3.95 (dd, $J = 2.0$, 7.1 Hz, 2H), 4.17 (d, $J = 9.3$ Hz, 1H), 4.30 (d, $J = 7.5$ Hz, 1H), 6.54-6.64 (m, br, 1H), 6.84-6.94 (m, br, 1H), 6.95-7.04 (m, 1H), 7.27 (d, $J = 8.4$ Hz, 1H), 7.22-7.33 (m, br, 1H), 7.35 (dd, $J = 2.0$, 8.3 Hz, 1H), 7.46 (dd, $J = 1.7$ Hz). $^{13}$C-NMR (100.62 MHz, DMSO-$d_6$) δ (ppm): 156.71 (ddd, $J = 7.9$, 66.7, 66.7 Hz), 128.2-132.4 (m), 113.4-114.8 (m). MS (ESI) $m/z$: [M+NH$_4$]$^+$ calculated 591, 593, observed 591, 593; [M+Na]$^+$ calculated 613, 615, observed 613, 615.
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References


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Figure 1. Structure of dapagliflozin (BMS-512148) 1a.
Figure 2. Radio-chromatographic profile of human urine samples (0-12 h pooled).
Figure 3. Assigned structures of m10 and m15 metabolites.
Figure 4. MS chromatogram of m15 metabolite 2a synthetic standard versus human urine isolate.
Figure 5. $^1$H-NMR spectra of m15 biological standard (top) isolated from human urine, synthetic standard 2a (middle) and [$^{13}$C$_6$] labelled standard 2b (bottom). The coupling and peak broadening observed for 2b arise from $^1$H-$^{13}$C coupling.
Figure 6. TOCSY spectrum of the synthetic metabolite m15, 2a (highlighting the two sugars spin systems: pink A and blue B).
Figure 7. $^1$H-$^{13}$C HMBC spectrum of the synthetic metabolite m15, 2a (cross peaks between protons 30, 6, 2 and carbon 4).
Figure 8. ROESY spectrum of the synthetic metabolite m15, 2a (cross peaks between proton 30 and protons 3 and 4).
Scheme 1. Synthesis of glucuronide metabolites m15 (2a) and m10 (3a).
Scheme 2. Synthesis of stable isotope labeled m15 (2b) and m10 (3b).