Discovery of Dapagliflozin: A Potent, Selective Renal Sodium-Dependent Glucose Cotransporter 2 (SGLT2) Inhibitor for the Treatment of Type 2 Diabetes

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The C-aryl glucoside **6** (dapagliflozin) was identified as a potent and selective hSGLT2 inhibitor which reduced blood glucose levels in a dose-dependent manner by as much as 55% in hyperglycemic streptozotocin (STZ) rats. These findings, combined with a favorable ADME profile, have prompted clinical evaluation of dapagliflozin for the treatment of type 2 diabetes.

Introduction

The prevalence of type 2 diabetes has become an increasing concern, as it is estimated that approximately 246 million people, or 5.9%, of the world's population aged 20−79 years, will have diabetes in 2007. Moreover, the prevalence is expected to increase to some 380 million, or 7.1% of the adult population, by 2025.[2] Type 2 diabetes is characterized by hyperglycemia due to a relative deficiency in insulin secretion accompanied by insulin resistance and poses a major risk for the development of microvascular complications, including retinopathy, neuropathy, and nephropathy, as well as macrovascular changes.[3–5] Diabetes, both type 1 and type 2, poses a 2- to 6-fold risk for progressive cardiovascular disease; emerging evidence suggests that aggressive glycemic control may have some benefit in terms of modifying this risk.[6–8] Due to the progressive nature of the disease, combination therapy is usually required to achieve the target glycemic level, thereby necessitating development of alternative agents that may act by novel mechanisms to control hyperglycemia.[9,10] Indeed, new agents with novel mechanisms of action are needed at all stages of the disease, whether for monotherapy or for use in combination therapy. In a study reviewing diabetic patients for control of vascular risk factors in 2004,[11] only 37.0% of participants achieved the target goal of HbA_{1c} level of less than 7.0%. This need is also underscored by the United Kingdom Prospective Diabetes Study (UKPDS)[a] findings that typically only 25% to 50% of type 2 diabetes patients are effectively treated by current therapies.[12,13] Thus, well tolerated new agents with novel mechanisms of action are needed at all stages of the disease to control hyperglycemia whether for monotherapy or for use in combination therapy.

In healthy individuals, greater than 99% of the plasma glucose that is filtered in the kidney glomerulus is reabsorbed, resulting in less than 1% of the total filtered glucose being excreted in urine.[14,15] This reabsorption process is mediated by two sodium-dependent glucose cotransporters (SGLTs): SGLT1, a low-capacity, high-affinity transporter expressed in the gut, heart, and kidney,[16,17] and SGLT2, a high-capacity, low-affinity transporter that is expressed mainly in the kidney.[18,19] It is estimated that 90% of renal glucose reabsorption is facilitated by SGLT2 residing on the surface of the epithelial cells lining the S1 segment of the proximal tubule; the remaining 10% is likely mediated by SGLT1 localized on the more distal S3 segment of the proximal tubule.[20–25] Humans with SGLT1 gene mutations experience glucose-galactose malabsorption, resulting in frequent, watery diarrhea and dehydration when on a glucose diet, confirming that SGLT1 is the major glucose transporter in the small intestine. These individuals present with little or no glucosuria, suggesting that SGLT1 is not the major glucose transporter in the kidney.[26,27] In contrast, persistent renal glucosuria is the sole reported phenotype of humans with SGLT2 gene mutations.[28,29]

Selective inhibition of SGLT2 has been proposed to aid in the normalization of plasma glucose levels in patients with diabetes by preventing the renal glucose reabsorption process and promoting glucose excretion in urine.[30] Selective SGLT2 inhibitors would be desirable, since gastrointestinal side effects associated with SGLT1 inhibition would be minimized. This mechanism is expected to be associated with a low risk of hypoglycemia, because there is no interference with the normal counterregulatory mechanisms for glucose.

The natural product O-glucoside phlorizin (**1**, Figure 1) is a well-documented, potent glucosuric agent that was subsequently shown to be a nonselective SGLT inhibitor.[31] The finding that chronic subcutaneous administration of **1** reduced plasma glucose levels of diabetic rodents supported this mechanistic approach.[32–34] However, **1** is not considered to be a suitable drug candidate because it inhibits SGLT1 and also because of its poor metabolic stability due to susceptibility to β-glucosidase-mediated cleavage, resulting in release of the aglycon phloretin.

In a series of papers, researchers at the Tanabe Seiyaku Co. disclosed the structure−activity relationships (SARs) of **1**, resulting in the identification of selective, potent SGLT2

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[a] Abbreviations: UKPDS, United Kingdom Prospective Diabetes Study; SGLT, sodium-dependent glucose cotransporter; SAR, structure–activity relationship; EC_{50}, half maximal effective concentration, STZ, streptozotocin; ADME, absorption, distribution, metabolism, and excretion.

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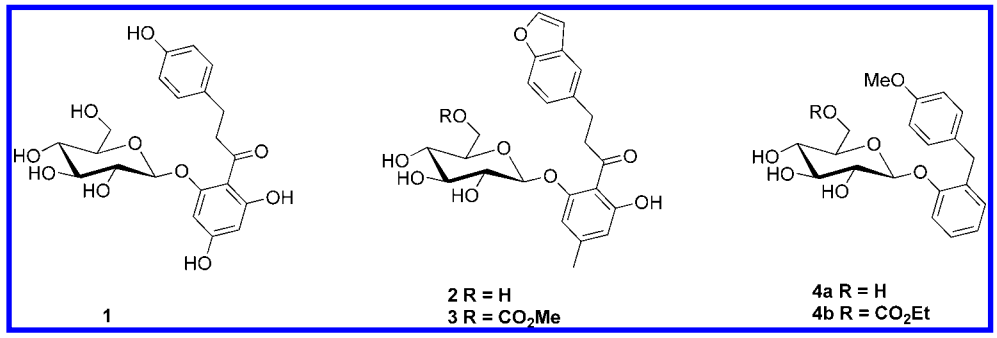


Figure 1. Structures of some known SGLT inhibitors.

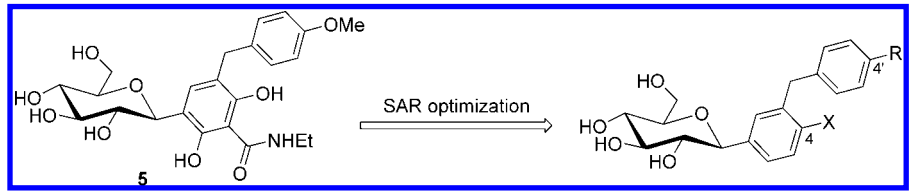


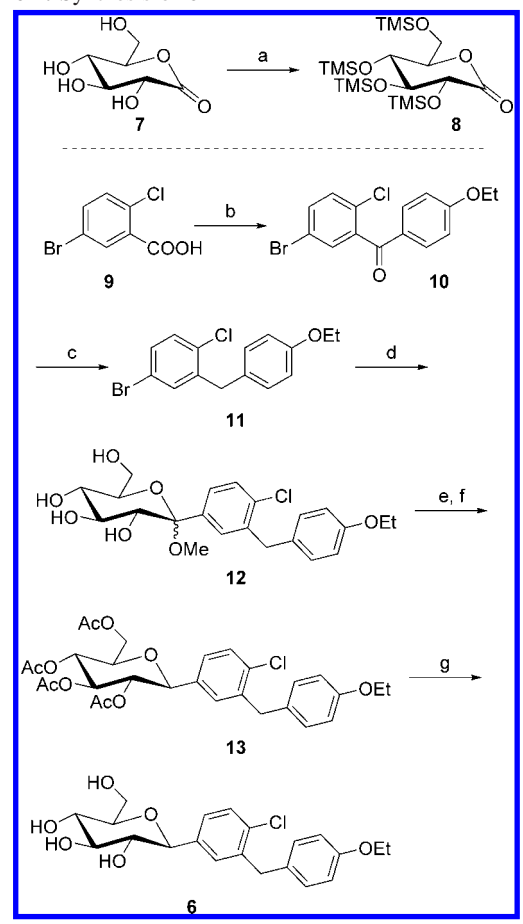
Figure 2. Origin and design of C-aryl glucoside SGLT2 inhibitors.

inhibitors. However, in order to achieve a significant reduction of hyperglycemia with a concurrent increase of glucosuria, the metabolic instability of the O-glucoside linkage necessitated oral administration of their lead compound, T-1095A (**2**, Figure 1), to *KK-A*^{y} mice as the methyl carbonate pro-drug T-1095 (**3**).[30,35–41] Subsequently, Kissei disclosed two other series of O-glucosides containing SGLT2 inhibitors as potential treatments for type 2 diabetes.[42–45] Again, concern regarding gut glucosidase-mediated degradation resulted in the lead compound, **4a**, being administered as the ethyl carbonate prodrug sergliflozin (**4b**).

Although we had initially also pursued O-glucoside-derived SGLT2 inhibitors,[46–48] that focus shifted to metabolically more robust C-aryl glucosides once compound **5** (Figure 2) was found to be modestly active (SGLT2 50% inhibitory concentration [EC_{50}] = 1300 nM). SAR exploration of the C-aryl glucosides revealed meta-substituted diarylmethanes to be superior SGLT2 ligands to their biphenyl and 1,2-diarylethane counterparts.[49] A number of compounds with preferred C-4′ and C-4 substitutions were synthesized and evaluated.[50] This SAR culminated in the discovery of **6**, a potent, selective SGLT2 inhibitor that exhibited properties warranting further progression as a clinical candidate for the treatment of type 2 diabetes.

Chemistry. The synthesis of **6** is shown in Scheme 1. Persilylated gluconolactone **8** was prepared in 99% yield by a slow addition of trimethylsilyl chloride to commercially available gluconolactone **7** in *N*-methylmorpholine and tetrahydrofuran.[51,52] Friedel–Crafts acylation of phenetole with 5-bromo-2-chlorobenzoyl chloride, formed from commercially available 5-bromo-2-chlorobenzoic acid **9** with oxalyl chloride, generated a 7:1 mixture of regioisomers in favor of the desired *p*-benzophenone **10**, which was subsequently isolated pure in 64% yield following two recrystallizations from ethanol. Reduction of **10** by triethylsilane and $BF_3 \cdot OEt_2$ provided aglycon **11** in 62% yield. Lithium halogen exchange, followed by addition of the nascent lithiated aromatic to **8**, gave a mixture of lactols, which were converted in situ to the desilylated O-methyl lactols **12** by treatment with methanesulfonic acid in methanol.[53] Reduction of the anomeric methoxy group of **12** using triethylsilane and $BF_3 \cdot OEt_2$, followed by peracetylation, yielded tetraacetate **13** in 55% for the two steps after recrystallization from ethanol to remove the small amount of the α-anomer formed during the reduction.[54] Hydrolysis of **13** with lithium hydroxide generated **6** in quantitative yield.

Scheme 1. Synthesis of **6**[a]



[a] (a) TMSCl, NMM, THF, 35 °C, 99%; (b) $(COCl)_2$, CH_2Cl_2, DMF, then phenetole, $AlCl_3$, 0 °C, 64%; (c) Et_3SiH, $BF_3 \cdot OEt_2$, $ClCH_2CH_2Cl$, CH_3CN, 10–50 °C, 62%; (d) *n*-BuLi, THF, $PhCH_3$, −78 °C, then **8** followed by MeOH, CH_3SO_3H, 85%; (e) Et_3SiH, $BF_3 \cdot OEt_2$, CH_2Cl_2, CH_3CN, −10 °C; (f) Ac_2O, pyr, CH_2Cl_2, DMAP, 55%; (g) $LiOH \cdot H_2O$, THF, H_2O, MeOH, 100%.

Table 1. hSGLT2 and hSGLT1 Inhibitory Activity for **1**, **5**, and **6**[a]

no.	hSGLT2 EC_{50} (nM)	hSGLT1 EC_{50} (nM)	selectivity vs hSGLT1 (fold)
1	35.6 ± 4.2 (n = 11)	330 ± 50 (n = 10)	10
2	6.6 ± 0.7 (n = 3)	211 ± 29 (n = 3)	30
4a	9.2 ± 0.8 (n = 3)	>8000 (n = 2)	>90
5	1300 ± 600 (n = 3)	>8000 (n = 2)	>10
6	1.1 ± 0.06 (n = 18)	1390 ± 7 (n = 16)	1200

[a] Assays were performed in protein-free buffer as described in the Supporting Information.

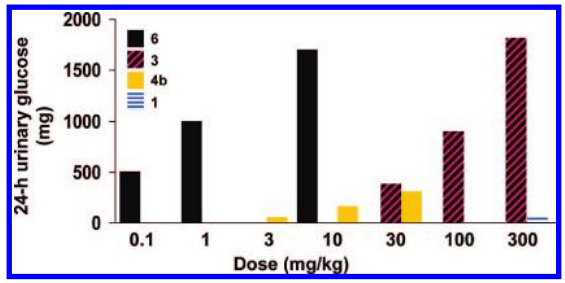


Figure 3. Dose-dependent glucosuric response of selected SGLT2 inhibitors over 24 h per 200 g body weight following oral administration to normal rats.

Results and Discussion

The in vitro SGLT inhibitory potential (EC_{50}) of **6** and analogues was assessed by monitoring inhibition of accumulation of radiolabeled α-methyl-*D*-glucopyranoside (AMG) by Chinese hamster ovary cells stably expressing human or rat SGLT2 and SGLT1. As shown in Table 1, EC_{50} values of 1.1 nM for human SGLT2 (hSGLT2) and 1.4 μM for hSGLT1 determined for **6** corresponded to 1200-fold selectivity for SGLT2 as compared with phlorizin's 10-fold selectivity. The inhibitory potencies of **6** against rat SGLT (rSGLT)2 and hSGLT2 were comparable (EC_{50} of 3 vs 1.1nM), but the selectivity of **6** for rSGLT2 versus rSGLT1 decreased to 200-fold. At 20 μM, **6** was also found to weakly inhibit (8%) 2-deoxyglucose uptake in human adipocytes mediated by GLUT 1 or GLUT 4 facilitative glucose transporters.[55] In our in vitro assay, **6** appears to be 6- and 8-fold more potent than **2** and **4a**, the respective active agents of **3** and **4b**. In addition, selectivity versus SGLT1 appears to be greater than **2** or **4a**.

Statistically significant dose-dependent glucosuria occurred over a 24-h period following oral administration of doses from 0.01 to 10 mg/kg of **6** to normal Sprague–Dawley rats, resulting in a 1000- to 10000-fold elevation in glucose disposal relative to vehicle controls. In this study, the oral administration of a single oral dose of 0.1, 1.0, and 10 mg/kg of **6** to rats induced respective losses of 550, 1100, and 1900 mg of glucose per 200 g of body weight over 24 h.[56] Figure 3 compares the dose-dependent glucosuric response with previously reported studies for **1**, **3**, and **4b**.[30,57] Since the difference in glucosuric potency of **6** vs the three O-glucosides markedly exceeds the difference in inherent in vitro potencies, we attribute the enhanced glucosuric response of **6** in part to the metabolic stability conferred by the C-glucoside linkage, thereby rendering **6** impervious to the intestinal, hepatic, and renal glucosidases that can rapidly hydrolyze labile O-glucoside bonds.

In a separate experiment, a 55% reduction in blood glucose level versus controls was observed at 5 h after a single 0.1 mg/kg oral dose of **6** was administered to streptozotocin (STZ)-induced diabetic rats with starting blood glucose levels of 480–530 mg/dL, which were then food-restricted for 5 h postdose (Figure 4). In two separate but identical studies to that described above, administration of a single 0.01- and 0.03-mg/kg oral dose of **6** produced 17% and 45% reductions, respectively, in blood glucose level versus controls at 5 h postdose. The above correlation of SGLT2 inhibition, glucosuria, and dose-dependent blood glucose-lowering effects suggest that selective SGLT2 inhibition holds promise as a viable approach to treat type 2 diabetes.

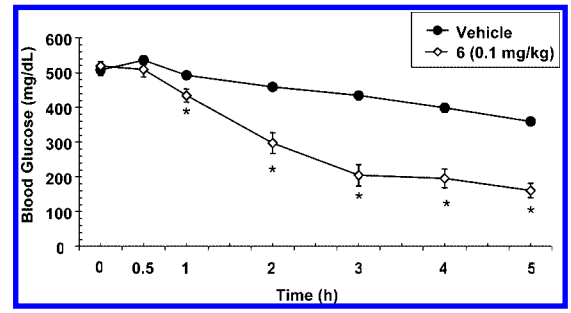


Figure 4. Mean blood glucose values in STZ-induced diabetic Sprague–Dawley rats following a single oral dose of 0.1 mg/kg of **6**. n = 6. *P < 0.05 vs control group using a paired Student t-test.

Compound **6** displayed a favorable absorption, distribution, metabolism, and excretion (ADME) profile conducive to further development. At 10 μM in serum from rats and humans, the free fraction of **6** was 3% and 4%, respectively. Compound **6** is anticipated to be orally bioavailable in humans based on a high (>150 nm/s) permeability value for the Caco-2 cell monolayer and 84% oral bioavailability in rats. The steady-state volume of distribution value (1.6 L/kg) was greater than the total blood volume in rats, indicating that **6** distributed into the extravascular space. Low to intermediate in vitro metabolic rates were observed upon incubation of **6** with liver microsomes and hepatocytes from rats and humans. After oral administration of a 1-mg/kg dose of **6** to rats, a C_{max} of 0.6 μg/mL was obtained at 1.7 h with a low systemic clearance rate of 4.8 mL/min/kg. The elimination half-life for **6** following intraarterial administration was 4.6, 7.4, and 3.0 h in rats, dogs, and monkeys, respectively.

In summary, **6** is a potent, metabolically robust, selective SGLT2 inhibitor that is not subject to O-glucosidase degradation. As a consequence, **6** is a much more potent stimulator of glucosuria in normal rats than other SGLT2 inhibitors hitherto disclosed. The promising significant reduction of blood glucose levels in diabetic STZ rats, combined with a favorable ADME profile, prompted further evaluation of **6** (dapagliflozin, **BMS-512148**) in the clinic for the treatment of type 2 diabetes.

Experimental Section

***O*-Methoxyglucoside (12).** To a stirred −78 °C solution of 5-bromo-2-chloro-4′-ethoxydiphenylmethane **11** (150 g, 0.5 mol) in 1:2 THF/toluene (1.2 L) under Ar was added *n*-BuLi (2.5 M in hexane, 184 mL, 0.5 mol) dropwise while keeping the temperature below −70 °C. After 30 min, this solution was transferred by cannula to a stirred −78 °C solution of 2,3,4,6-tetra-*O*-trimethylsilyl-β-D-glucolactone **8** (236 g, 0.5 mol) in toluene (1.1 L) at a rate that maintained the reaction temperature below −70 °C. After 30 min, methanesulfonic acid (0.6 N in MeOH, 1 L) was added; whereupon, the reaction was allowed to slowly warm to room temperature over 16 h. The reaction was then quenched with saturated aqueous $NaHCO_3$ (~200 mL). After extraction with EtOAc (3×), the combined organic layers were washed with brine and dried over Na_2SO_4 prior to filtration and concentration under reduced pressure. The resulting residue, upon dissolution in hot toluene (150 mL), was poured into hexanes (1 L) to precipitate **12**. *O*-Methylglucoside **12** (171 g, 85%) was isolated as a white solid comprised of a ~85:15 mixture of anomers after vacuum filtration, washing the filter cake with hexanes (2 × 500 mL) and air drying.

Table 2. Pharmacokinetic Profile of **6** in Rats

dose (mg/kg)	1
C_{max} (PO dose, μg/mL)	0.6
T_{max} (PO dose, h)	1.7
$T_{1/2}$ (h)	4.6
F (%)	84
V_{ss} (L/kg)	1.6
Cl (mL/min/kg)	4.8

For the major anomer: HPLC $t_R = 3.45$ min, purity 100%; ^{1}H NMR (400 MHz, CD_3OD) δ 7.54 (d, $J = 2.2$, 1H), 7.45 (dd, $J = 2.2$, 8.4, 1H), 7.35 (d, $J = 8.4$, 1H), 7.08 (d, $J = 8.8$, 2H), 6.79 (d, $J = 8.8$, 2H), 4.08 (d, $J = 15.0$, 1H), 3.99 (d, $J = 15.0$, 1H), 3.98 (q, $J = 7.0$, 2H), 3.92 (dd, $J = 2.2$, 11.8, 1H), 3.80 (dd, $J = 5.3$, 11.9, 1H), 3.74 (t, $J = 9.2$, 1H), 3.57 (m, 1H), 3.41 (d, $J = 8.8$, 1H), 3.08 (d, $J = 9.7$, 1H), 3.06 (s, 3H), 1.35 (t, $J = 7.0$, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.2, 139.0, 138.5, 134.2, 132.4, 131.2, 130.1, 129.2, 127.6, 114.8, 101.8, 78.0, 75.3, 74.5, 71.1, 63.8, 62.1, 49.0, 38.7, 14.5. Anal. Calcd for $C_{22}H_{27}ClO_7$: C, 60.20; H, 6.20; Cl, 8.07. Found: C, 60.05; H, 6.21; Cl, 8.01.

***tetra*-Acetylated β-C-glucoside (13).** To a stirred −10 °C solution of *O*-methylglucoside **12** (123 g, 0.3 mol) in 1:1 CH_2Cl_2/MeCN (1.1 L) was added Et_3SiH (65 g, 0.6 mol) followed by $BF_3 \cdot OEt_2$ (60 g, 0.4 mol) at a rate such that the reaction temperature was maintained between −5 and −10 °C. The solution was allowed to warm to 0 °C over 5 h prior to quenching with saturated aqueous $NaHCO_3$ (310 mL). After removal of organic volatiles under reduced pressure, the residue was partitioned between 2 L each of EtOAc and H_2O. Following extraction of the aqueous layer with EtOAc (2 × 2 L), the combined organic layers were washed with H_2O (2 L) and brine (2 L) prior to drying over $MgSO_4$. Filtration and concentration under reduced pressure yielded a yellow foam (105 g). Peracetylation was achieved by addition of Ac_2O (261 g, 2.6 mol) and DMAP (1.6 g, 13.1 mmol) to a solution of this residue in CH_2Cl_2 (750 mL) and pyridine (200 g, 2.5 mol). After 1.5 h, the reaction was quenched by addition of H_2O (1.8 L), whereupon the resulting mixture was extracted with CH_2Cl_2 (2×). The combined organic layers were washed with 1 N HCl (2 × 1.8 L) and brine (2 × 1.8 L) prior to drying over $MgSO_4$. After filtration and concentration under reduced pressure, the residue was recrystallized from absolute EtOH to yield the desired tetra-acetylated β-C-glucoside **13** (90 g, 55% for two steps) as a white solid (the stereochemistry for the anomeric position of **13** was established according to ref 51). The mother liquors contained the corresponding α-C-glucoside as well as a more polar furanose isomer: HPLC $t_R = 3.98$ min, purity 100%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 (d, $J = 8.4$, 1H), 7.19 (dd, $J = 1.8$, 8.4, 1H), 7.07 (d, $J = 1.8$, 1H), 7.05 (d, $J = 8.8$, 2H), 6.82 (d, $J = 8.8$, 2H), 5.28 (t, $J = 9.2$, 1H), 5.20 (t, $J = 9.2$, 1H), 5.05 (t, $J = 9.2$, 1H), 4.31 (d, $J = 9.7$, 1H), 4.26 (dd, $J = 4.8$, 12.8, 1H), 4.14 (dd, $J = 2.2$, 12.4, 1H), 3.95–4.07 (m, 4H), 3.80 (m, 1H), 2.08 (s, 3H), 2.04 (s, 3H), 1.99 (s, 3H), 1.71 (s, 3H), 1.40 (t, $J = 7.0$, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.7, 170.3, 169.45, 168.7, 157.5, 139.1, 135.1, 134.6, 131.0, 129.79, 126.0, 114.5, 79.5, 76.1, 74.1, 72.5, 68.5, 63.4, 62.3, 38.2, 20.7, 20.6, 20.3, 14.8; mp 120 °C (uncorrected); HRMS calcd for $C_{29}H_{33}ClNaO_{10}$ $(M + Na)^+$ 599.1660, found 599.1649. Anal. Calcd for $C_{29}H_{33}ClO_{10}$: C, 60.36; H, 5.76. Found: C, 60.43; H, 5.56.

(2*S*,3*R*,4*R*,5*S*,6*R*)-2-(3-(4-Ethoxybenzyl)-4-chlorophenyl)-6-hydroxymethyltetrahydro-2*H*-pyran-3,4,5-triol (6). To a stirred solution of tetra-acetylated β-C-glucoside **13** (27 g, 47 mmol) in 2:3:1 THF/MeOH/H_2O (480 mL) was added $LiOH \cdot H_2O$ (2.3 g, 55 mmol). After the mixture was stirred overnight, the volatiles were removed under reduced pressure. The residue, after dissolution in EtOAc (300 mL), was subsequently washed with brine (150 mL), brine containing 10 mL of 5% aq $KHSO_4$ (50 mL) and brine (50 mL) prior to drying over Na_2SO_4. Filtration and removal of the volatiles under reduced pressure yielded desired (2*S*,3*R*,4*R*,5*S*,6*R*)-2-(3-(4-ethoxybenzyl)-4-chlorophenyl)-6-hydroxymethyl-tetrahydro-2*H*-pyran-3,4,5-triol **6** (20.4 g, 100%) as a glassy off-white amorphous solid: HPLC $t_R = 3.26$ min, purity 99%; ^{1}H NMR (500 MHz, CD_3OD) δ 7.33 (d, $J = 6.0$, 1H), 7.31 (d, $J = 2.2$, 1H), 7.31 (dd, $J = 2.2$, 6.0, 1H), 7.07 (d, $J = 8.8$, 2H), 6.78 (d, $J = 8.8$, 2H), 4.07–3.90 (m, 7H), 3.85 (d, $J = 10.6$, 1H), 3.69 (dd, $J = 5.3$, 10.6, 1H), 3.42–3.25 (m, 4H), 1.34 (t, $J = 7.0$, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ 158.8, 140.0, 139.9, 134.4, 132.9, 131.9, 130.8, 130.1, 128.2, 115.5, 82.9, 82.2, 79.7, 76.4, 71.9, 64.5, 63.1, 39.2, 15.2; HRMS calcd for $C_{21}H_{25}ClNaO_6$ $(M + Na)^+$ 431.1237, found 431.1234. Anal. Calcd for $C_{21}H_{25}ClO_6$: C, 61.68; H, 6.16. Found: C, 61.16; H, 6.58.

Supporting Information Available: Descriptions of hSGLT1 and hSGLT2 binding assays, in vivo pharmacology including glucosuria and blood glucose-lowering experiments. Detailed experimental procedures, physical state, and characterization for compounds **5**, **8**, **10**, and **11**.

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