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Discovery of Tofogliflozin, a Novel *C*-Arylglucoside with an *O*-Spiroketal Ring System, as a Highly Selective Sodium Glucose Cotransporter 2 (SGLT2) Inhibitor for the Treatment of Type 2 Diabetes

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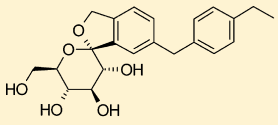
Supporting Information

ABSTRACT: Inhibition of sodium glucose cotransporter 2 (SGLT2) has been proposed as a novel therapeutic approach to treat type 2 diabetes. In our efforts to discover novel inhibitors of SGLT2, we first generated a 3D pharmacophore model based on the superposition of known inhibitors. A search of the Cambridge Structural Database using a series of pharmacophore queries led to the discovery of an *O*-spiroketal *C*-arylglucoside scaffold. Subsequent chemical examination combined with computational modeling resulted in the identification of the clinical candidate **16d** (CSG452, tofogliflozin), which is currently under phase III clinical trials.



16d

IC_{50} (hSGLT2): 2.9 nM
IC_{50} (hSGLT1): 8,444 nM
Selectivity (hSGLT1/hSGLT2)= 2,912-fold
F (%): (mice) 75%
(monkeys) 85%

INTRODUCTION

Diabetes mellitus is a progressive metabolic disease characterized by chronic hyperglycemia due to a decrease of insulin secretion from the pancreas and insulin resistance. According to the World Health Organization, more than 346 million people worldwide have diabetes, and an estimated 3.4 million people died from the consequences of high blood sugar in 2004, with this number expected to double by the year 2030.[1] Type 2 diabetes accounts for 90% of all cases of diabetes. Prevention of diabetes-related microvascular complications such as nephropathy, retinopathy, and neuropathy in these patients requires tight glycemic control.[2] Currently available hypoglycemic agents can be classified into three categories based on their mechanism of action: those promoting insulin secretion, those improving insulin sensitivity, and those delaying glucose absorption. However, sustained control of blood glucose to prevent disease progression is difficult to achieve with any single agent or with stepwise administration of these agents in combination, with the result that patients eventually require insulin therapy. In addition, currently available antidiabetic agents are known to cause undesirable side effects such as hypoglycemia, body weight gain, gastric symptoms, etc. Thus, there is a high unmet medical need for novel potent hypoglycemic agents with a good safety and tolerability profile that can be used either as a monotherapy or in combination with other antidiabetic agents.

Sodium glucose cotransporters (SGLTs) have recently attracted considerable attention as new drug targets for the treatment of diabetes.[3] Of several subtypes identified in the SGLT family, SGLT1 and 2 are expressed in the renal tubules.[4] Recent research results indicate that selective inhibition of SGLT2 could provide a well-tolerated and highly effective method of glycemic control.[5] The expression of SGLT2 appears to be kidney specific. Mutations of the SGLT2 gene cause familial renal glucosuria,[6] but daily activities are not affected. SGLT1, on the other hand, transports glucose and galactose not only in the renal tubules but also in the small intestine.[7] Mutations in human SGLT1 lead to glucose–galactose malabsorption, which is characterized by severe diarrhea and sometimes mild renal glucosuria.[8] In addition, SGLT1 is expressed in cardiac myocytes and serves as a glucose transporter in the heart.[9] Thus, inhibition of SGLT1 may cause gastrointestinal symptoms or unexpected adverse effects. Therefore, highly selective inhibition of SGLT2 versus SGLT1 is one of the important features to be achieved.

By targeting renal tubular glucose reabsorption, SGLT2-selective inhibitors exhibit a novel mechanism of action resulting in excretion of glucose into the urine. Because this effect is independent of insulin secretion or insulin sensitivity,

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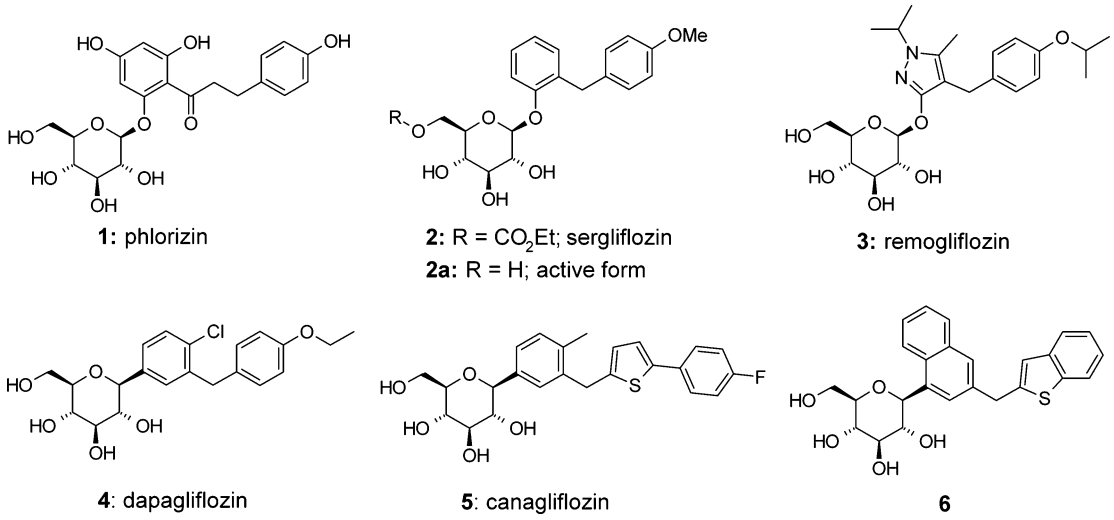

Figure 1. Selected examples of SGLT2 inhibitors.

SGLT2 inhibitors can be used in combination with various existing oral antihyperglycemia drugs to improve clinical outcomes in patients with type 2 diabetes. Moreover, SGLT2 inhibitors are also expected to reduce body weight because they excrete glucose, or "energy", leading to a lower overall caloric load. Actually, it has been reported that SGLT2 inhibitors reduced body weight in clinical trials.[10]

Phlorizin (**1**) is a natural nonselective SGLT inhibitor. This approach to SGLT inhibition was supported by the finding that chronic subcutaneous administration of **1** reduced the plasma glucose level of diabetic rodents.[11] However, **1** was not clinically developed as a drug candidate because of its poor metabolic stability to β-glucosidases.

Over nearly a decade, a lot of different compounds have been reported as SGLT2 inhibitors.[12−15] They are generally divided into two classes, *O*-glucosides and *C*-glucosides. T-1095,[16] the first structural derivative of **1** reported by the Tanabe group, was followed by sergliflozin[17] (**2**) and remogliflozin[18] (**3**), and all are representatives of the *O*-glucosides. However, there have been no reports that the *O*-glucoside class of SGLT2 inhibitors have advanced to phase II clinical trials, probably because, like phlorizin, they have inherent metabolic instability to β-glucosidases, and thus relatively large doses are required in clinic to exert the expected significant efficacy.

Meanwhile, the first of the *C*-aryl glucoside class of SGLT2 inhibitors has been reported by researchers at Bristol-Myers Squibb Co. in the previous decade. Their representative, dapagliflozin[19] (**4**), was found to have more potent hSGLT2 inhibition in vitro and stability in vivo than the *O*-glucosides and is currently being developed as an antidiabetic agent. At present, a number of companies are clinically developing the *C*-glucoside class of SGLT2 inhibitors, such as canagliflozin[20] (**5**, Mitsubishi Tanabe/Johnson & Johnson, phase III) and empagliflozin[21] (Boehringer Ingelheim, phase III), by modifying the aglycon moiety.

In the course of our efforts to explore potent, selective, and metabolically stable SGLT2 inhibitors, we successfully discovered a novel class of highly potent and SGLT2-selective inhibitors incorporating a unique spiroketal structure.[22−24] Herein we wish to report the process of producing the *O*-spiroketal *C*-arylglucosides from structure database searches based on a 3D pharmacophore model, the synthesis of the test compounds **16a−16n** and **21o−21r**, and the selection of the clinical candidate **16d** as a tofogliflozin,[25] according to the results of both pharmacological and pharmacokinetic studies.

RESULTS AND DISCUSSION

New Scaffold Exploration. Three-dimensional structures of target proteins are very informative for designing new drugs, but those of hSGLT1 and 2 have not been available so far.[26] In our efforts to identify novel SGLT2 inhibitors, we previously discovered the carba-glucopyranosides by the ligand-based approach.[27] At nearly the same time, a 3D pharmacophore-based approach for a lead discovery was applied based on the accumulated structure−activity relationship information from the carbasugar derivatives and others. At first, to construct a hypothetical SGLT2 pharmacophore model, five potent inhibitors[28] (**1**, **2a**, **3**, **4**, and **5** in Figure 1) and our potent *C*-naphthyl glucoside **6**[29] were subjected to superposition with the Flexible Alignment module in Molecular Operating Environment (MOE).[30] A conformation search by Low-ModeMD of MOE indicated that the energy difference of each compound in Figure 2a from the global minimum was within 5 kcal/mol. The reason why we utilized the additional in-house compound **6** to construct the pharmacophore model is that its naphthalene ring served as a template to superpose two kinds of central aromatic rings such as *C*-glucosides and *O*-glucosides. The superposition model in Figure 2a implies that having a properly aligned sugar ring, two aromatic moieties, and special distances between two pairs of pharmacophore points are important in order to achieve higher activity. The information obtained from a superposition model in Figure 2a is schematically summarized in Figure 2b. In Figure 2b, the two aromatic rings are depicted in blue circles and the two distances by double-headed arrows (Dist. 1, Dist. 2).

Next, we tried many searches in the Cambridge Structural Database (CSD)[31] and other available structure databases using possible combinations of the pharmacophore points shown in Figure 2b as a query. When searching databases with all three pharmacophore points and two distance constraints, no promising hit was obtained. On the other hand, the search over CSD using two pharmacophore points (the sugar moiety and the aromatic group 1 in Figure 2b) and a distance between them (Dist. 1), followed by careful visual inspection of the hit lists, provided us with three valuable structures, one of which is

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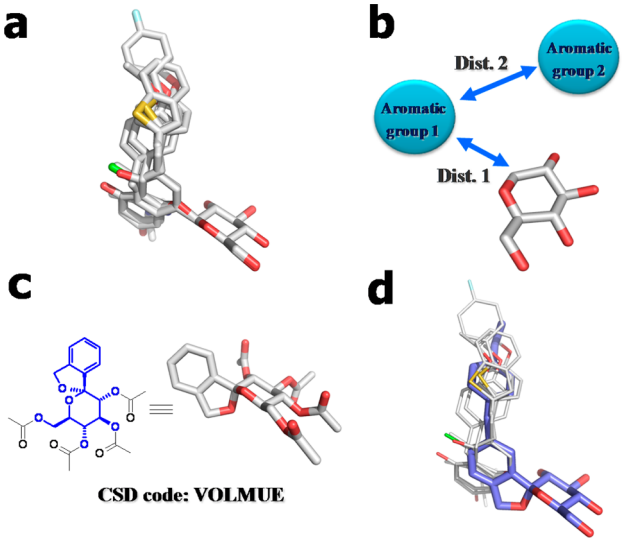


Figure 2. Generation of a new *O*-spiroketal *C*-arylglucoside scaffold. (a) Superposition model of six selected SGLT2 inhibitors, (b) a schematic representation of the pharmacophore model, (c) one of three hit compounds from CSD search, (d) superposition model of the newly designed SGLT2 inhibitor **16d** (carbon atoms in blue). Hydrogen atoms are not displayed for clarity. All the figures were prepared with PyMol (http://www.pymol.org./).

in Figure 2c (and see Supporting Information). They include a spiroketal moiety in common and are known as synthetic intermediates of antibiotic papulacandins.[32] Finally, a new *O*-spiroketal *C*-arylglucoside class of SGLT inhibitors was designed by removing presumably unnecessary groups from the CSD hits and adding *p*-substituted benzyl group to the central benzene ring, referring to the structures of sergliflozin (**2**) and dapagliflozin (**4**) (Figure 2d). The conformational energy of **16d** in Figure 2d from its global minimum is within 5 kcal/mol.

Chemistry. The synthetic route was established with reference to the methods of the synthetic intermediates of antibiotic papulacandins.[33] That is, a key synthetic intermediate **11** for **16a**−**16n** was synthesized as shown in Scheme 1. Reduction of two carboxyl groups of commercially available 2-bromoterephtalic acid **7**, followed by protection of two hydroxyl groups with triphenylmethyl, afforded **8**. Compound **10** was obtained by adding the tetra benzyl-protected gluconolactone **9** to the aryl lithium prepared in situ by treatment of **8** with *sec*-butyllithium. Deprotection followed by spirocyclization of **10** using triethylsilane and boron trifluoride-diethyl etherate ($BF_3{\cdot}OEt_2$) gave the benzyl alcohol **11**.

O-Spiroketal *C*-arylglucosides **16a**−**16n** were prepared from **11** through two pathways as outlined in Scheme 2. Compounds **13a** and **13d**−**13g** were synthesized from the aldehyde **12**, which could be obtained by oxidation of **11**, followed by addition of Grignard reagents or lithiated benzenes and reduction. Compound **16e** (R^1 = *n*-Pr) was obtained from **13g** (R^1 = *c*-Pr) by the reductive cleavage of the cyclopropane ring in the final deprotection step described below. On the other hand, **15b**, **15c**, and **15h**−**15n** were prepared utilizing the Suzuki coupling reactions. After debenzylation using boron trichloride, benzyl alcohol moiety was selectively chlorinated by treatment of chlorotrimethylsilane with dimethyl sulfoxide. Four hydroxyl groups of the resulting benzyl chloride were acetylated to afford **14**. Suzuki coupling reactions of **14** with the corresponding 4-substituted phenylboronic acids under reported conditions[34] gave **15b**, **15c**, and **15h**−**15n**. Finally, deprotections (debenzylation for **13a** and **13d**−**13g** or deacetylation for **15b**, **15c**, and **15h**−**15n**) afforded the test compounds **16a**−**16n**.

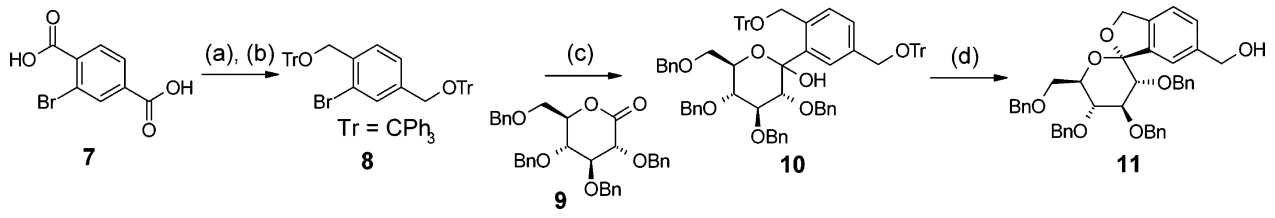
The configuration of the spiroketal was determined by single-crystal X-ray diffraction analysis. Ethyl compound **16d** was converted into monoacetate derivative **17** by treatment with acetyl chloride in 2,4,6-trimethylpyridine. Single crystal of **17** was obtained by recrystallization from acetonitrile and water as a monohydrate, and the result of X-ray analysis is shown in Figure 3 (see Supporting Information for more details).

Compounds **21o**−**21r** with a substitution at position 4 of the aglycon were synthesized as shown in Scheme 3. Diol **19** was obtained from commercially available **18** via dibromination, substitution reaction, and hydrolysis. Compound **19** was transformed into **20** by a similar method to that in Schemes 1 and 2. Palladium-catalyzed coupling reactions of the chloro-substituted **20** with boronic acids or trimetylsilylacetylene,[35,36] followed by deacetylation under basic conditions, gave **21o**−**21r**.

In Vitro Activity. The in vitro SGLT inhibitory potential of the *O*-spiroketal *C*-arylglucoside derivatives was assessed by monitoring the inhibition of methyl-α-D-[U-^{14}C]-glucopyranoside (AMG) in Chinese hamster ovary cells stably expressing human or mouse SGLT2 and SGLT1. All the inhibition activities are shown in Table 1.

Focusing initially on *para* substitution groups on the distal benzene ring, methoxy and ethoxy derivatives (**16a** and **16b**) had similarly high SGLT2 inhibition potential (IC_{50} = 9.1 and 9.6 nM, respectively). Among the three small straight-chain alkyl analogues (**16c**−**16e**), the ethyl analogue **16d** was more potent and had higher SGLT2 selectivity versus SGLT1 (approximately 3000 times the selectivity) than the two alkoxy derivatives (**16a** and **16b**). The isopropyl (**16f**), the cyclopropyl (**16g**), and the methylthio (**16n**) analogues showed similar inhibition potential to the ethyl analogue **16d**, and both **16f** and **16g** had slightly higher SGLT2 selectivity than **16d**. The

Scheme 1. Preparation of the Benzyl Alcohol 11[a]



[a]Reagents and conditions: (a) BH_3, THF, 0−40 °C, 2.5 h (90%); (b) Ph_3CCl, Et_3N, DMAP, DMF−CH_2Cl_2, 50 °C, 5.5 h (68%); (c) *sec*-BuLi, toluene, −78 °C to rt, 30 min (80%); (d) Et_3SiH, $BF_3{\cdot}OEt_2$, CH_3CN, −40 to 0 °C, 1 h (56%).

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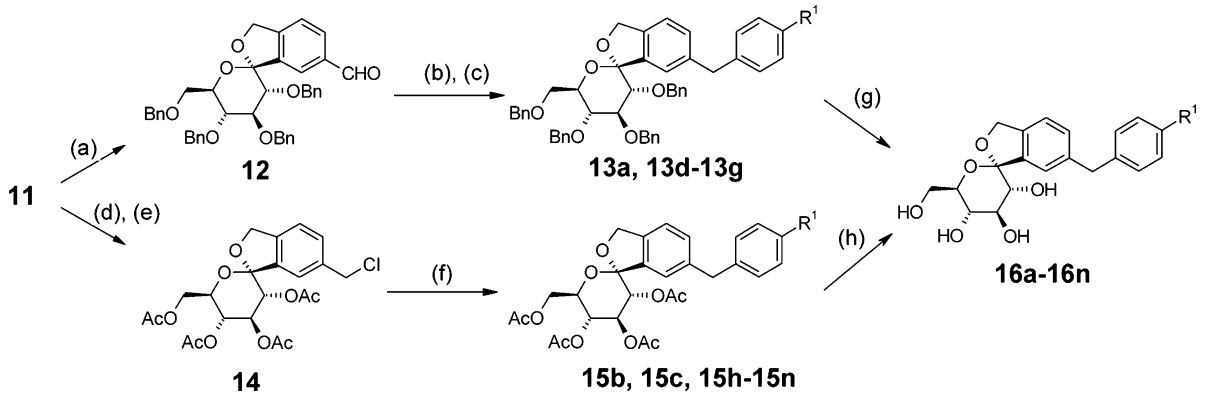
Scheme 2. Preparation of Compounds 16a−16n[a]



[a]Reagents and conditions: (a) Dess−Martin periodinane, CH_2Cl_2, rt, 30 min (33%); (b) Grignard reagents, Et_2O, 0 °C, 2.5 h (80−92%), or *p*-R^1-phenyl bromide, *n*-BuLi, THF or Et_2O, −78 °C, 1 h (86−87%); (c) Et_3SiH, $BF_3{\cdot}OEt_2$, CH_3CN or CH_2Cl_2, −40 °C, 1 h (83−90%); (d) BCl_3, Me_5-benzene, CH_2Cl_2, −78 °C, 2 h (67%); (e) TMSCl, DMSO, rt, 1.5 h; then Ac_2O, NMM, DMAP, 0 °C, 1 h (76%); (f) *p*-R^1-phenylboronic acid, $Pd(OAc)_2$, Ph_3P, K_3PO_4, toluene, 80 °C, 15 h (50−93%); (g) H_2, $Pd(OH)_2/C$, MeOH-AcOEt, 2N HCl aq, rt, 1 h (80−99%), or BCl_3, Me_5-benzene, CH_2Cl_2, −78 °C, 2 h (50%); (h) K_2CO_3, MeOH, rt, 12 h (29−61%).

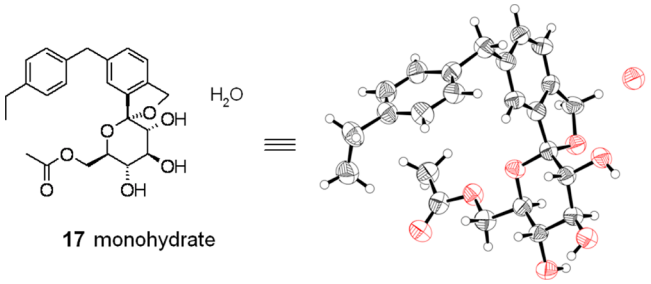


Figure 3. ORTEP representation of the X-ray crystal structure of **17** (monohydrate).

compounds with a hydrophilic group (**16h**) or electron-withdrawing groups (**16i−16m**) showed less potent inhibition potential than the alkyl or the alkoxy analogues (**16a−16g**).

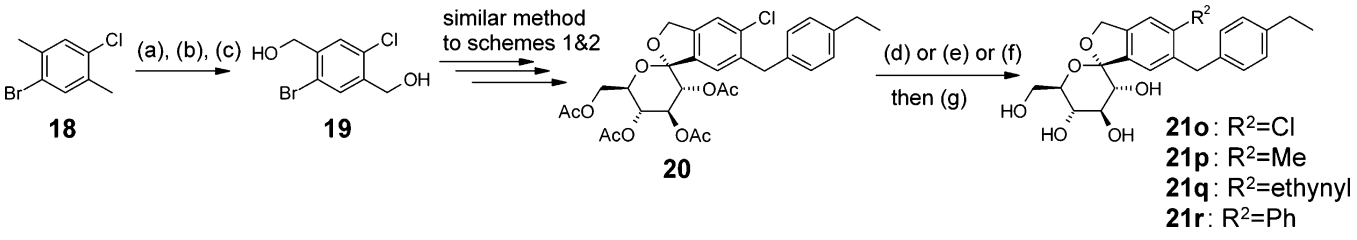
We next investigated the effects of substitution on the central benzene moiety. The introduction of a chloro, methyl, or ethynyl group in R^2 (**21o**, **21p**, and **21q**) slightly increased the SGLT2 inhibition potency compared with **16d** while it greatly increased the SGLT1 inhibition potency; that is, their SGLT2 selectivity was more than 10 times lower than that of **16d**. Interestingly, the substitution with a phenyl group (**21r**) resulted in substantially decreased SGLT2 inhibition, suggesting that the large substitution in this position (R^2) may be undesirable.

We selected **16d** and **16f**, which had strong hSGLT2 inhibition (IC_{50} < 5 nM) and high SGLT2 selectivity (around 3000-fold), for further evaluation. Cyclopropyl compound **16g** was not selected, despite having superior in vitro pharmacological profiles, because it showed high time-dependent inhibition (TDI) of CYP3A4 (see Supporting Information for more details).[37]

In Vivo Efficacy. The effects of these two compounds, **16d** and **16f**, were evaluated on renal glucose excretion and blood glucose level in db/db mice at oral administrations of 1, 3, and 10 mg/kg. Both compounds **16d** and **16f** showed a significant and similar degree of increase in renal glucose excretion in the period 0−6 h after oral dosing at 3 or 10 mg/kg (Figure 4). In addition, both compounds reduced the blood glucose level in a dose-dependent manner and to similar levels within the normal range but not below 100 mg/dL (Figure 5a,b), indicating that **16d** and **16f** could improve hyperglycemia without inducing hypoglycemia. Therefore, **16d** and **16f** were found to be promising for the treatment of type 2 diabetes.

Pharmacokinetics. To select a clinical candidate, we compared the pharmacokinetic profiles of the two compounds (**16d** and **16f**) in normal ICR mice and cynomolgus monkeys. As shown in Table 2, oral bioavailability (*F*) of **16d** was higher (75% in mice and 85% in monkeys) than that of **16f** (46% in mice and 21% in monkeys). The large difference in the oral bioavailability in monkeys is most probably attributable to the

Scheme 3. Preparation of Compounds 21o−21r[a]



[a]Reagents and conditions: (a) NBS, AIBN, EtOAc, reflux, 20 min; (b) NaOAc, DMF, 80 °C, 3 h (39% from **17**); (c) KOH, THF−EtOH−H_2O, 80 °C, 3 h (97%); (d) methylboronic acid, $Pd(dba)_2$, *t*-$Bu_3P{\cdot}BF_4$, K_3PO_4, xylene, 145 °C, 15 h (28%); (e) TMS−acetylene, $PdCl_2(CH_3CN)_2$, Xphos, Cs_2CO_3, CH_3CN, 90 °C, 2 h (81%); (f) phenylboronic acid, $Pd(dba)_2$, CTC-Q-PHOS, THF, 100 °C, 30 min, microwave (quant); (g) K_2CO_3, MeOH, rt, 1.5 h (36−54%).

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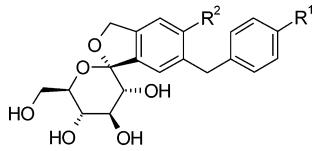
Table 1. In Vitro Data for hSGLT Inhibition



compd	R^1	R^2	IC_{50} ± SD (nM)[a] hSGLT2	IC_{50} ± SD (nM)[a] hSGLT1	selectivity[b]
1			16.4 ± 5.2	185 ± 70	11
4			1.3 ± 0.2	800 ± 210	615
16a	OMe	H	9.1 ± 0.5	8482 ± 2228	932
16b	OEt	H	9.6 ± 1.6	18745 ± 3791	1953
16c	Me	H	4.8 ± 1.1	3086 ± 202	643
16d	Et	H	2.9 ± 0.7	8444 ± 2310	2912
16e	*n*-Pr	H	7.5 ± 3.8	13137 ± 3523	1752
16f	*i*-Pr	H	3.2 ± 1.2	12335 ± 3235	3855
16g	*c*-Pr	H	1.6 ± 0.4	5314 ± 724	3321
16h	OH	H	29.9 ± 1.1	4987 ± 1255	167
16i	F	H	122.7 ± 42.4	18697 ± 449	152
16j	Cl	H	16.8 ± 6.1	6281 ± 625	374
16k	CN	H	117.9 ± 35.3	81487 ± 9225	691
16l	CF_3	H	14.2 ± 3.4	24146 ± 5207	1700
16m	OCF_3	H	14.5 ± 1.2	28918 ± 8099	1994
16n	SMe	H	3.6 ± 0.8	5902 ± 533	1639
21o	Et	Cl	1.3 ± 0.2	158 ± 30	122
21p	Et	Me	1.4 ± 0.3	126 ± 11	90
21q	Et	C≡CH	1.4 ± 0.3	388 ± 24	277
21r	Et	Ph	240.8 ± 56.1	5132 ± 249	21

[a]In vitro human SGLT1 or SGLT2 inhibition activities of compounds were determined by evaluating sodium-dependent uptake of methyl-α-D-[U-^{14}C]glucopyranoside in Chinese hamster ovary (CHO) cells stably expressing SGLT1 or SGLT2. All the values represent the mean of three determinations except for **1** and **16d** (n = 22) and **4** (n = 15). [b]The selectivity values were calculated by IC_{50} hSGLT1/IC_{50} hSGLT2.

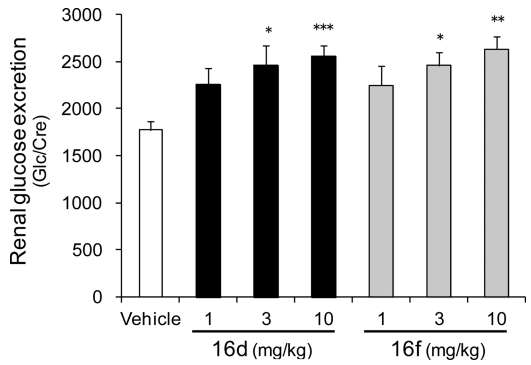


Figure 4. Effects of single oral administration of **16d** and **16f** on renal glucose excretion (0–6 h) in db/db mice. Compounds **16d** and **16f** (1, 3, and 10 mg/kg) or vehicle were administered to db/db mice by oral gavage. The renal glucose excretion (urine glucose concentration/urine creatinine concentration) was calculated from the urine collected between 0 and 6 h after drug administration under nonfasting conditions. Data are expressed as mean ± SEM (n = 6). *P < 0.05, **P < 0.01, ***P < 0.001 versus vehicle-treatment group by Dunnett's multiple comparison test.

difference in absorption because CL_{total} of both compounds is almost the same and not as high as the hepatic blood flow of monkeys, indicating that the hepatic first pass effects of both the compounds can be considered low. In addition, because the target organ of SGLT2 inhibitors is the kidney tubule and they may act from the apical side, a greater amount of inhibitor is expected to be excreted into the urine. In this regard, urinary excretion amounts of the two compounds in mice and monkeys were also compared. We measured the value of the renal clearance (CL_{renal}) as a measure of the renal excretion ability and calculated its ratio to total clearance (CL_{renal}/CL_{total}). When 1.0 mg/kg of **16d** or **16f** were administered intravenously to monkeys, the urinary excretion ratios (CL_{renal}/CL_{total}) were 21.4% for **16d** and 8.82% for **16f**, respectively, indicating that the ratio of the hepatic metabolism of **16d** is lower than that of **16f**, and consequently the risk of drug–drug interaction (DDI) is expected to be low. On the basis of the results described above, compound **16d** (tofogliflozin, CSG452) was selected as a clinical candidate.[38]

CONCLUSION

We discovered a novel class of SGLT2 inhibitors characterized by the *O*-spiroketal *C*-arylglucoside scaffold through a structural database search using a 3D pharmacophore model that was derived from known inhibitors. We revealed the SAR for substitution of various groups on the aromatic rings. Regarding the para-substitution on the distal benzene ring, small alkyl groups were most favorable for achieving both SGLT2 inhibition activity and SGLT2 selectivity versus SGLT1. As for the 4-substitution on the central benzene ring, small groups, such as a chloro, enhanced the SGLT2 inhibition activity but lowered the SGLT2 selectivity versus SGLT1. Compounds **16d** and **16f**, with a well-balanced profile for potency and selective SGLT2 inhibition, were nominated for further evaluations. These two compounds almost equally enhanced the renal glucose excretion and lowered the blood glucose in diabetic db/db mice. As a result of the pharmacokinetic studies, it was

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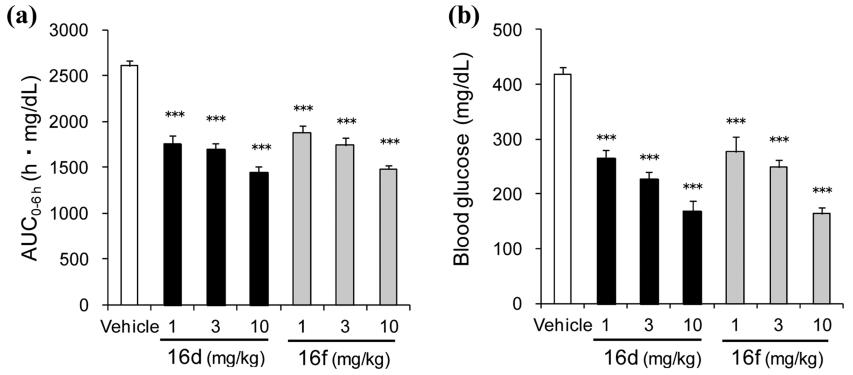

Figure 5. Effects of single oral administration of **16d** and **16f** on blood glucose level in db/db mice. (a) blood glucose AUC for 6 h period ($AUC_{0-6\ h}$), (b) blood glucose level at 6 h after administration. Compounds **16d** and **16f** (1, 3, and 10 mg/kg) or vehicle were administered to db/db mice by oral gavage. Blood glucose levels were measured before (0 h) and at 1, 2, 4, and 6 h after drug administration under nonfasting condition. Data are expressed as mean ± SEM (n = 6). ***P < 0.001 versus vehicle-treatment group by Dunnett's multiple comparison test.

Table 2. Pharmacokinetic Parameters of 16d and 16f after Oral and Intravenous Administration to ICR Mice and Cynomolgus Monkeys[a]

	mice				monkeys			
	16d		**16f**		**16d**		**16f**	
	po	iv	po	iv	po	iv	po	iv
dose (mg/kg)	2.8	2.9	2.6	3.1	1.7	1.0	2.8	1.0
C_{max} (μg/mL)	1.35		0.47		0.93		0.53	
T_{max} (h)	0.63		0.44		2.00		1.75	
$T_{1/2}$ (h)	2.57	2.66	2.90	2.48	3.72	3.54	3.75	4.30
AUC_{inf} (μg·h/mL)	3.98	4.75	1.85	4.21	4.03	2.74	1.99	3.37
F (%)[b]	75		46		85		21	
V_{ss} (mL/kg)		1416		2046		1409		1364
CL_{total} (mL/h/kg)		634		748		356		307
CL_{renal}[c]/CL_{total} (%)		7.92		4.06		21.4		8.82

[a]Plasma concentrations were measured by LC-MS/MS. PK parameters were calculated by noncompartmental analysis using WinNonlin 5.0. Data are expressed as mean (n = 4). [b]Oral bioavailability. [c]CL_{renal} = {cumulative drug amount excreted into urine (0−8 h for mice, 8−24 h for monkeys)}/AUC ($AUC_{0-8\ h}$ for mice, $AUC_{8-24\ h}$ for monkeys).

found that **16d** had more desirable profiles in oral bioavailability and renal excretion than **16f**. Therefore, **16d** was chosen as a clinical candidate. Compound **16d** is currently undergoing phase III clinical trials for the treatment of type 2 diabetes.

EXPERIMENTAL SECTION

All reactions were carried out under an argon or nitrogen atmosphere. All solvents and reagents were purchased from commercial sources without further drying. Silica gel 60F254 precoated plates on glass from Merck KgaA were used for thin layer chromatography (TLC) and preparative TLC (PTLC). Column chromatography was carried out on Merck Silica Gel 60 (230−400 mesh) if not otherwise specified. Preparative HPLC was carried out using a Rainin HPLC employing a 41.4 mm × 300 mm, 8 μm, Dynamax C-18 column at a flow of 49 mL/min and employing a gradient of acetonitrile/water (each containing 0.75% TFA) typically from 5 to 95% acetonitrile over 35−40 min. Melting points (mp) were determined with a Yanagimoto micro melting point apparatus. ^{1}H NMR spectra were recorded on JEOL EX-270, Varian Mercury 300, or JEOL JNM-ECP-400 or Varian 400-MR. Chemical shifts were reported in parts per million (δ) downfield from tetramethylsilane (δH 0.00) as an internal standard. Data are presented as follows: chemical shift (δ, ppm), integration, multiplicity (br = broad, s = singlet, d = doublet, t = triplet, q = quartet, and m = multiplet), and coupling constant. ^{13}C NMR spectra were recorded on a Varian 400-MR (100 MHz) spectrometer. The following internal reference was used (CD_3OD, δ 49.0; $CDCl_3$, δ 77.0). Mass spectra (MS) were measured by a Thermo Electron LCQ Classic or Micromass ZQ from Waters (ESI). High resonance mass spectra (HRMS) were recorded by a Micromass Q-Tof Ultima API mass spectrometer. Analytical HPLC was measured by a 2690/2996 from Waters or a LC2010A from Shimadzu. The measurement conditions for HPLC were as follows: (condition A) Column: YMC-Pack ODS-A 6.0 mm × 150 mm, 5 μm. Mobile phase: elution by applying a gradient of from 0.1% TFA/MeCN (5%) plus 0.1% TFA/H_2O (95%) to 0.1% TFA/MeCN (100%) for 20 min, and thereafter under the same condition [0.1% TFA/MeCN (100%)] for 5 min. Flow Rate: 1.5 mL/min. Column temperature: room temperature. Detection condition: total plot of the entire wavelength of 230−400 nm. (condition B) Column: YMC-Pack ODS-A 6.0 mm × 150 mm, 5 μm. Mobile phase: elution by applying a gradient of from 0.1% TFA/MeCN (5%) plus 0.1% TFA/H_2O (95%) to 0.1% TFA/MeCN (100%) for 13 min, and thereafter under the same condition [0.1% TFA/MeCN (100%)] for 7 min. Flow rate: 1.0 mL/min. Column temperature: 25 °C. Detection condition: wavelength of 254 nm. All the test compounds were determined to be >95% pure by HPLC.

(1*S*,3′*R*,4′*S*,5′*R*,6′*R*)-3′,4′,5′-Tris(benzyloxy)-6′-((benzyloxy)-methyl)-3′,4′,5′,6′-tetrahydro-3*H*-spiro[2-benzofuran-1,2′-pyran]-6-carbaldehyde (Intermediate 12). To a solution of 2-bromo-terephthalic acid (7) (185.0 g, 755 mmol) in THF (1.85 L), a THF solution of BH_3 (1.1 M, 2.0 L) was added at 0 °C dropwise for 1.5 h and the mixture was stirred for 1 h at 40 °C. After the exothermic reaction was over, the reaction was cooled by ice−water bath. The reaction was quenched by dropwise addition of water. The resulting mixture was extracted with AcOEt. The organic layer was washed with brine and concentrated under reduced pressure to give (2-bromo-4-hydroxymethylphenyl)methanol (148 g, 90%) as an off-white solid. ^{1}H

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NMR (270 MHz, DMSO-d_6) δ: 4.47 (2H, d, J = 5.1 Hz), 4.49 (2H, d, J = 5.1 Hz), 5.27 (1H, t, J = 6.0 Hz), 5.37 (1H, t, J = 5.7 Hz), 7.31 (1H, d, J = 7.8 Hz), 7.45−7.49 (2H, m). MS (ESI) m/z: 240 $[M + Na]^+$.

To a solution of the above obtained diol (148 g, 682 mmol) and triphenylmethyl chloride (TrCl) (411 g, 1.47 mol) in DMF (300 mL) and CH_2Cl_2 (1.0 L), triethylamine (335 mL, 2.40 mol) and DMAP (20 g, 163.7 mmol) were added and the mixture was stirred for 1.5 h at 50 °C. Triethylamine (38 mL, 273 mmol) and TrCl (76 g, 273 mmol) were added, and the mixture was stirred for 4 h at 50 °C. The resulting mixture was cooled, and then white solid was crystallized out. The solid was filtered off and washed with Et_2O−water (2:7) and Et_2O and dried in vacuo to give **8** (327.0 g, 68%) as a off-white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 4.14 (2H, s), 4.20 (2H, s), 7.22−7.34 (21H, m), 7.47−7.53 (12H, m).

To a solution of **8** (255.3 mg, 0.36 mmol) in toluene (1.5 mL), 0.99 M *sec*-BuLi in cyclohexane solution (367 mL, 0.36 mmol) was added dropwise at rt, and the solution was stirred for 30 min. This solution was added dropwise at −78 °C to a solution of commercially available **9** (140 mg, 0.26 mmol) in toluene (1.5 mL), and the mixture was stirred at the same temperature for 30 min. After addition of water, the reaction mixture was extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$, and then evaporated under reduced pressure to remove the solvent. The obtained residue was purified by silica gel column chromatography (AcOEt/hexane = 1:5) to give **10** (242 mg, 80%) as a mixture of diastereomers. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.34 (1H, t, J = 9.3 Hz), 3.46−3.51 (3H, m), 3.76 (1H, d, J = 10.8 Hz), 3.92 (1H, t, J = 9.3 Hz), 4.00−4.05 (1H, m), 4.08−4.16 (3H, m), 4.26−4.31 (3H, m), 4.41 (1H, d, J = 12.3 Hz), 4.49−4.58 (2H, m), 4.77−4.84 (3H, m), 6.71−6.76 (2H, m), 6.93−6.97 (2H, m), 7.02−7.07 (1H, m), 7.11−7.32 (35H, m), 7.47−7.59 (12H, m), 7.69 (1H, d, J = 7.5 Hz). MS (ESI) m/z: 1184 $[M + Na]^+$.

To a solution of the above intermediate **10** (242 mg, 0.21 mmol) in MeCN (3 mL), triethylsilane (36 mL, 0.23 mmol) and $BF_3{\cdot}OEt_2$ (29 mL, 0.23 mmol) were added at −40 °C and the mixture was stirred at the same temperature for 1 h. After stirring at 0 °C for an additional 1 h, water was added and the reaction mixture was extracted with CH_2Cl_2. The organic layer was washed with brine, dried over $MgSO_4$, and then evaporated under reduced pressure to remove the solvent. The obtained residue was purified by silica gel column chromatography (AcOEt/hexane = 1:4) to give **11** (77.5 mg, 56%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.66 (1H, d, J = 11.1 Hz), 3.77−3.93 (4H, m), 4.07−4.18 (4H, m), 4.40−4.63 (5H, m), 4.83−4.95 (3H, m), 5.17 (2H, s), 6.72−6.76 (2H, m), 7.05−7.35 (20H, m), 7.40−7.55 (1H, m). MS (ESI) m/z: 681 $[M + Na]^+$.

To a solution of the above alcohol **11** (77.5 mg, 0.12 mmol) in CH_2Cl_2 (1.5 mL), Dess−Martin periodinane (74.8 mg, 0.18 mmol) was added at rt and the mixture was stirred for 30 min. After addition of water, the reaction mixture was extracted with CH_2Cl_2. The organic layer was washed with brine, dried over $MgSO_4$, and then evaporated under reduced pressure to remove the solvent. The obtained residue was purified by silica gel column chromatography (AcOEt/hexane = 1:4) to give **12** as a colorless amorphous compound (25.2 mg, 33%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.61−3.66 (1H, m), 3.76−3.95 (3H, m), 4.08−4.11 (1H, m), 4.15−4.27 (2H, m), 4.47 (1H, d, J = 12.0 Hz), 4.56 (1H, d, J = 12.0 Hz), 4.63 (2H, d, J = 10.8 Hz), 4.89 (1H, d, J = 10.8 Hz), 4.95 (2H, s), 5.24 (2H, s), 6.75 (2H, d, J = 6.9 Hz), 7.03−7.15 (3H, m), 7.19−7.41 (16H, m), 7.53 (1H, s), 7.88 (1H, d, J = 7.8 Hz), 9.85 (1H, s). MS (ESI) m/z: 679 $[M + Na]^+$.

(1*S*,3′*R*,4′*S*,5′*R*,6′*R*)-6′-(Acetoxymethyl)-6-(chloromethyl)-3′,4′,5′,6′-tetrahydro-3*H*-spiro[2-benzofuran-1,2′-pyran]-3′,4′,5′-triyl Triacetate (Intermediate 14). To a solution of the prepared **11** (0.59 g, 0.90 mmol) and pentamethylbenzene (1.33 g, 8.95 mmol) in CH_2Cl_2 (48 mL) was added 1.0 M BCl_3 in CH_2Cl_2 solution (8.95 mL, 8.95 mmol) at −78 °C under a nitrogen stream, and the mixture was stirred at the same temperature for 2 h. After addition of MeOH (48 mL), the reaction mixture was warmed to rt and evaporated under reduced pressure to remove the solvent. The obtained residue was purified by silica gel column chromatography (MeOH/CH_2Cl_2 = 1:6) to give the pentaol compound (0.18 g, 67%).

To a solution of the above pentaol (100 mg, 0.34 mmol) in DMSO (0.19 mL, 2.68 mmol), chlorotrimethylsilane (114 mL, 0.91 mmol) was added dropwise at rt and the mixture was stirred at the same temperature for 1.5 h. To the crude product obtained by distilling off volatile components, *N*-methylmorpholine (0.74 mL, 6.70 mmol), DMAP (41 mg, 0.34 mmol), and acetic anhydride (0.32 mL, 3.35 mmol) were added sequentially and the mixture was stirred at 0 °C for 1 h. After addition of brine (1 mL) and water (1 mL), the reaction mixture was extracted with AcOEt (10 mL). The organic layer was washed with water (1.5 mL) and brine (1 mL), dried over Na_2SO_4, and then concentrated under reduced pressure. The obtained residue was purified by silica gel column chromatography (AcOEt/hexane = 2:5) to give **14** as a colorless amorphous (123 mg, 76%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.74 (3H, s), 2.01 (3H, s), 2.05 (1H, s), 2.08 (3H, s), 3.99−4.08 (1H, m), 4.24−4.37 (2H, m), 4.61 (2H, s), 5.15−5.32 (3H, m), 5.57−5.65 (2H, m), 7.22−7.28 (1H, m), 7.38−7.47 (2H, m). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 20.6, 20.6, 20.6, 20.7, 45.6, 61.9, 68.4, 70.0, 70.8, 71.7, 72.9, 108.6, 121.2, 122.9, 130.6, 136.3, 137.6, 140.3, 169.3, 169.5, 170.1, 170.7. MS (ESI) m/z: 485 $[M + H]^+$.

General Procedures for Synthesis of Test Compounds via Aldehyde (Method A). *(1S,3′R,4′S,5′S,6′R)-6′-(Hydroxymethyl)-6-[(4-methoxyphenyl)methyl]-3′,4′,5′,6′-tetrahydro-3H-spiro[2-benzofuran-1,2′-pyran]-3′,4′,5′-triol (**16a**).* To a solution of aldehyde **12** (2.01 g, 3.06 mmol) in Et_2O (24 mL), 0.5 M 4-methoxyphenylmagnesium bromide in THF solution (12.24 mL, 6.12 mmol) was added at 0 °C and the mixture was stirred for 2.5 h at rt. After addition of water, the reaction mixture was extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$, and then evaporated under reduced pressure to remove the solvent. The obtained residue was purified by silica gel column chromatography (AcOEt/hexane = 1:4) to give the intermediate (2.15 g, 92%).

To a solution of the above intermediate (270 mg, 0.353 mmol) in CH_2Cl_2 (2.7 mL), triethylsilane (281 μL, 1.764 mmol) and $BF_3{\cdot}OEt_2$ (47 μL, 0.37 mmol) were added at −40 °C and the mixture was stirred at the same temperature for 15 min. After addition of water, the reaction mixture was extracted with CH_2Cl_2. The organic layer was washed with brine, dried over $MgSO_4$, and then evaporated under reduced pressure to remove the solvent. The obtained residue was purified by silica gel column chromatography (AcOEt/hexane = 1:10) to give the intermediate compound (260 mg, 90%).

To a solution of the above intermediate (280 mg, 0.381 mmol) in MeOH (1 mL) and AcOEt (1 mL), 10% $Pd(OH)_2$ (28.7 mg) was added and 2 N HCl (15.2 μL) was further added. Under a hydrogen atmosphere, the reaction mixture was stirred for 45 min at rt and then filtered to remove the catalyst. After distilling off the solvent under reduced pressure, the obtained residue was purified by silica gel column chromatography (MeOH/CH_2Cl_2 = 1:10) to give **16a** (114 mg, 98%) as a colorless amorphous compound; t_R = 9.6 min (condition A). ^{1}H NMR (400 MHz, CD_3OD) δ: 3.42−3.49 (1H, m), 3.63−3.67 (1H, m), 3.74 (3H, s), 3.74−3.87 (4H, m), 3.93 (2H, s), 5.07 (1H, d, J = 12.3 Hz), 5.13 (1H, d, J = 12.4 Hz), 6.80−6.83 (2H, m), 7.12−7.20 (2H, m), 7.21−7.22 (3H, m). ^{13}C NMR (100 MHz, CD_3OD) δ: 41.8, 55.6, 62.8, 71.9, 73.4, 74.9, 76.2, 76.4, 111.6, 114.9, 121.8, 123.5, 130.9, 131.1, 134.5, 139.8, 140.2, 142.8, 159.6. MS (ESI): 389 $[M + H]^+$. HRMS (ESI), m/z calcd for $C_{21}H_{25}O_7$ $[M + H]^+$ 389.1595, found 389.1594.

General Procedures for Synthesis of Test Compounds via Benzyl Chloride (Method B). *(1S,3′R,4′S,5′S,6′R)-6-[(4-Ethoxyphenyl)methyl]-6′-(hydroxymethyl)-3′,4′,5′,6′-tetrahydro-3H-spiro[2-benzofuran-1,2′-pyran]-3′,4′,5′-triol (**16b**).* To a solution of benzyl chloride **14** (200 mg, 0.413 mmol) in toluene (2.0 mL), triphenylphosphine (5.41 mg, 0.021 mmol), palladium acetate (2.36 mg, 0.010 mmol), 4-ethoxyphenylboronic acid (0.103 g, 0.619 mmol), and potassium phosphate (0.181 g, 0.825 mmol) were added and the mixture was heated at 80 °C. After the mixture was stirred for 15 h, water and AcOEt were added, and the mixture was washed with brine. The organic layer was dried over $MgSO_4$, filtered, and then evaporated under reduced pressure. The obtained residue was purified by silica gel column chromatography (AcOEt/hexane = 1:2) to give the titled compound (0.117 g, 50%) as a colorless amorphous compound. ^{1}H

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NMR ($CDCl_3$) δ 1.39 (3H, t, J = 7.1 Hz), 1.71 (3H, s), 2.00 (3H, s), 2.05 (3H, s), 2.07 (3H, s), 3.88−4.09 (5H, m), 4.21−4.36 (2H, m), 5.07−5.24 (2H, m), 5.25−5.33 (1H, m), 5.55−5.65 (2H, m), 6.79−6.85 (2H, m), 7.01−7.09 (2H, m), 7.11−7.19 (2H, m), 7.23 (1H, s).

To a solution of the above compound (0.210 g, 0.368 mmol) in MeOH (5 mL), K_2CO_3 (100 mg, 0.709 mmol) was added and the mixture was stirred for 12 h at rt. After addition of water, the reaction mixture was extracted with AcOEt. The organic layer was washed with brine and dried over $MgSO_4$. After filtration, the solvent was concentrated under reduced pressure. The obtained residue was purified by silica gel column chromatography ($MeOH/CH_2Cl_2$ = 1:15) to give the titled compound (42.5 mg, 29%) as a colorless amorphous compound; t_R = 8.1 min (condition B). ^1H NMR (400 MHz, CD_3OD) δ: 1.35 (3H, t, J = 6.9 Hz), 3.42−3.48 (1H, m), 3.63−3.67 (1H, m), 3.74−3.83 (4H, m), 3.92 (2H, s), 3.98 (2H, q, J = 6.9 Hz), 5.07 (1H, d, J = 12.3 Hz), 5.13 (1H, d, J = 12.3 Hz), 6.77−6.83 (2H, m), 7.07−7.14 (2H,m), 7.17−7.22 (3H, m). ^{13}C NMR (100 MHz, CD_3OD) δ: 15.2, 41.8, 62.8, 64.4, 71.8, 73.4, 74.9, 76.2, 76.4, 111.6, 115.5, 121.8, 123.5, 130.9, 131.1, 134.5, 139.8, 140.2, 142.8, 158.8. MS (ESI): 403 $[M + H]^+$. HRMS (ESI), m/z calcd for $C_{22}H_{27}O_7$ $[M + H]^+$ 403.1751, found 403.1748.

*(1S,3′R,4′S,5′S,6′R)-6′-(Hydroxymethyl)-6-[(4-methylphenyl)-methyl]-3′,4′,5′,6′-tetrahydro-3H-spiro[2-benzofuran-1,2′-pyran]-3′,4′,5′-triol (**16c**).* Compound **16c** was prepared from **14** and 4-methylphenylboronic acid following a similar procedure to that described in Method B (yield 32%, colorless amorphous); t_R = 8.1 min (condition B). ^1H NMR (400 MHz, CD_3OD) δ: 2.27 (3H, s), 3.42−3.47 (1H, m), 3.63−3.67 (1H, m), 3.73−3.83 (4H, m), 3.94 (2H, s), 5.07 (1H, d, J = 12.3 Hz), 5.13 (1H, d, J = 12.5 Hz), 7.04−7.10 (4H, m), 7.17−7.22 (3H, m). ^{13}C NMR (100 MHz, CD_3OD) δ: 21.0, 42.2, 62.8, 71.8, 73.4, 74.9, 76.2, 76.4, 111.6, 121.8, 123.6, 129.9, 130.1, 131.1, 136.6, 139.4, 139.9, 140.2, 142.6. MS (ESI): 395 $[M + Na]^+$. HRMS (ESI), m/z calcd for $C_{21}H_{25}O_6$ $[M + H]^+$ 373.1646, found 373.1647.

*(1S,3′R,4′S,5′S,6′R)-6-[(4-Ethylphenyl)methyl]-6′-(hydroxymethyl)-3′,4′,5′,6′-tetrahydro-3H-spiro[2-benzofuran-1,2′-pyran]-3′,4′,5′-triol (**16d**, CSG452, Tofogliflozin).* Compound **16d** was prepared from **12** and 0.5 M 4-ethylphenylmagnesium bromide in THF solution following a similar procedure to that described in Method A (yield 66%, colorless amorphous); t_R = 11.4 min (condition A). ^1H NMR (400 MHz, CD_3OD) δ: 1.20 (3H, t, J = 7.6 Hz), 2.58 (2H, q, J = 7.6 Hz), 3.42−3.47 (1H, m), 3.63−3.67 (1H, m), 3.75−3.88 (4H, m), 3.95 (2H, s), 5.06 (1H, d, J = 12.3 Hz), 5.12 (1H, d, J = 12.5 Hz), 7.07−7.14 (4H, m), 7.17−7.23 (3H, m). ^{13}C NMR (100 MHz, CD_3OD) δ: 16.3, 29.4, 42.3, 62.8, 71.9, 73.4, 74.9, 76.2, 76.4, 111.6, 121.8, 123.6, 128.9, 129.9, 131.1, 139.7, 139.9, 140.2, 142.6, 143.2. MS (ESI): 387 $[M + H]^+$. HRMS (ESI), m/z calcd for $C_{22}H_{27}O_6$ $[M + H]^+$ 387.1802, found 387.1801.

*(1S,3′R,4′S,5′S,6′R)-6′-(Hydroxymethyl)-6-[(4-propylphenyl)-methyl]-3′,4′,5′,6′-tetrahydro-3H-spiro[2-benzofuran-1,2′-pyran]-3′,4′,5′-triol (**16e**).* Intermediate **13g** (1.68 g, 2.21 mmol) prepared from **12** and 4-cyclopropylphenylmagnesium bromide generated by treatment of magnesium with 1-bromo-4-cyclopropylbenznene was dissolved in MeOH (10 mL) and AcOEt (10 mL). To this solution, 10% $Pd(OH)_2$ (0.21 g) was added. Under a hydrogen atmosphere, the reaction mixture was stirred for 1.5 h at rt and then filtered to remove the catalyst. After distilling off the solvent under reduced pressure, the obtained residue was purified by silica gel column chromatography ($MeOH/CH_2Cl_2$ = 1:10) to give **16e** (0.65 g, 73%) as a colorless amorphous compound; t_R = 12.3 min (condition A). ^1H NMR (400 MHz, CD_3OD) δ: 0.92 (3H, t, J = 7.4 Hz), 1.56−1.65 (2H, m), 2.51−2.55 (2H, m), 3.42−3.47 (1H, m), 3.63−3.67 (1H, m), 3.74−3.83 (4H, m), 3.96 (2H, s), 5.07 (1H, d, J = 12.3 Hz), 5.13 (1H, d, J = 12.5 Hz), 7.06−7.14 (4H, m), 7.18−7.23 (3H, m). ^{13}C NMR (100 MHz, CD_3OD) δ: 14.1, 25.8, 38.7, 42.3, 62.8, 71.9, 73.4, 74.9, 76.2, 76.4, 111.6, 121.8, 123.6, 129.6, 129.8, 131.2, 139.7, 139.9, 140.2, 141.5, 142.6. MS (ESI): 401 $[M + H]^+$. HRMS (ESI), m/z calcd for $C_{23}H_{29}O_6$ $[M + H]^+$ 401.1959, found 401.1956.

*(1S,3′R,4′S,5′S,6′R)-6′-(Hydroxymethyl)-6-[(4-isopropylphenyl)-methyl]-3′,4′,5′,6′-tetrahydro-3H-spiro[2-benzofuran-1,2′-pyran]-3′,4′,5′-triol (**16f**).* Compound **16f** was prepared from **12** and 4-isopropylphenyl lithium generated by treatment of *n*-BuLi with 1-bromo-4-isopropylbenzene following a similar procedure to that described in Method A (yield 59%, colorless amorphous); t_R = 12.1 min (condition A). ^1H NMR (400 MHz, CD_3OD) δ: 1.21 (6H, d, J = 6.9 Hz), 2.82−2.86 (1H, m), 3.43−3.47 (1H, m), 3.64 (1H, dd, J = 12.1, 5.8 Hz), 3.74−3.81 (4H, m), 3.95 (2H, s), 5.07 (1H, d, J = 12.3 Hz), 5.14 (1H, d, J = 12.3 Hz), 7.09−7.13 (4H, m), 7.17−7.24 (3H, m). ^{13}C NMR (100 MHz, CD_3OD) δ: 24.5, 24.5, 35.0, 42.2, 62.8, 71.8, 73.4, 74.9, 76.2, 76.4, 111.6, 121.8, 123.6, 127.5, 129.9, 131.2, 139.9, 140.2, 142.6, 147.8. MS (ESI): 401 $[M + H]^+$. HRMS (ESI), m/z calcd for $C_{23}H_{29}O_6$ $[M + H]^+$ 401.1959, found 401.1956.

*(1S,3′R,4′S,5′S,6′R)-6-[(4-Cyclopropylphenyl)methyl]-6′-(hydroxymethyl)-3′,4′,5′,6′-tetrahydro-3H-spiro[2-benzofuran-1,2′-pyran]-3′,4′,5′-triol (**16g**).* Compound **13g** was prepared from **12** and 4-cyclopropylphenyl magnesium bromide generated by treatment of magnesium with 1-bromo-4-cyclopropylbenznene following a similar procedure to that described in Method A (yield 73%). Then, to a solution of **13g** (2.55 g, 3.36 mmol) and pentamethylbenzene (4.99 g, 33.7 mmol) in CH_2Cl_2 (185 mL) was added 1.0 M BCl_3 in CH_2Cl_2 solution (33.3 mL, 33.3 mmol) at −78 °C under a nitrogen stream, and the mixture was stirred at the same temperature for 2 h. After addition of MeOH (185 mL), the reaction mixture was warmed to rt and evaporated under reduced pressure to remove the solvent. The obtained residue was purified by silica gel column chromatography ($MeOH/CH_2Cl_2$ = 1:10) to give **16g** (0.67 g, 50%) as a colorless amorphous compound; t_R = 11.4 min (condition A). ^1H NMR (400 MHz, CD_3OD) δ: 0.59−0.63 (2H, m), 0.88−0.92 (2H, m), 1.81−1.87 (1H, m), 3.42−3.47 (1H, m), 3.61 (1H, dd, J = 11.8, 5.6 Hz), 3.74−3.83 (4H, m), 3.94 (2H, s), 5.06 (1H, d, J = 12.3 Hz), 5.13 (1H, d, J = 12.3 Hz), 6.94−6.97 (2H, m), 7.05−7.08 (2H, m), 7.17−7.22 (3H, m). ^{13}C NMR (100 MHz, CD_3OD) δ: 9.4, 9.4, 15.8, 42.2, 62.8, 71.8, 73.4, 74.9, 76.2, 76.4, 111.6, 121.8, 123.6, 126.7, 129.8, 131.1, 139.4, 139.8, 140.2, 142.6, 143.0. MS (ESI): 399 $[M + H]^+$. HRMS (ESI), m/z calcd for $C_{23}H_{27}O_6$ $[M + H]^+$ 399.1802, found 399.1800.

*(1S,3′R,4′S,5′S,6′R)-6-[(4-Hydroxyphenyl)methyl]-6′-(hydroxymethyl)-3′,4′,5′,6′-tetrahydro-3H-spiro[2-benzofuran-1,2′-pyran]-3′,4′,5′-triol (**16h**).* Compound **16h** was prepared from **14** and 4-benzyloxyphenylboronic acid following a similar procedure to that described in Method B (yield 12%, colorless amorphous); t_R = 6.3 min (condition B). ^1H NMR (400 MHz, CD_3OD) δ: 3.42−3.52 (1H, m), 3.63−3.67 (1H, m), 3.74−3.83 (4H, m), 3.90 (2H, s), 5.07 (1H, d, J = 12.5 Hz), 5.13 (1H, d, J = 12.5 Hz), 6.66−6.70 (2H, m), 6.99−7.02 (2H, m), 7.17−7.22 (3H, m). ^{13}C NMR (100 MHz, CD_3OD) δ: 41.8, 62.8, 71.9, 73.4, 74.9, 76.2, 76.4, 111.6, 116.2, 121.8, 123.5, 130.9, 131.1, 133.3, 139.8, 140.1, 143.1, 156.7. MS (ESI): 397 $[M + Na]^+$. HRMS (ESI), m/z calcd for $C_{20}H_{23}O_7$ $[M + H]^+$ 375.1438, found 375.1439.

*(1S,3′R,4′S,5′S,6′R)-6-[(4-Fluorophenyl)methyl]-6′-(hydroxymethyl)-3′,4′,5′,6′-tetrahydro-3H-spiro[2-benzofuran-1,2′-pyran]-3′,4′,5′-triol (**16i**).* Compound **16i** was prepared from **14** and 4-fluorophenylboronic acid following a similar procedure to that described in Method B (yield 26%, colorless amorphous); t_R = 7.7 min (condition B). ^1H NMR (400 MHz, CD_3OD) δ: 3.42−3.47 (1H, m), 3.63−3.67(1H, m), 3.74−3.83 (4H, m), 3.98 (2H, s), 5.07 (1H, d, J = 12.5 Hz), 5.13 (1H, d, J = 12.5 Hz), 6.95−7.00 (2H, m), 7.19−7.23 (5H, m). ^{13}C NMR (100 MHz, CD_3OD) δ: 41.7, 62.8, 71.8, 73.4, 74.9, 76.2, 76.4, 111.6, 116.0 (d, J = 21.7 Hz), 122.0, 123.6, 131.1, 131.5 (d, J = 8.2 Hz), 138.5 (d, J = 3.0 Hz), 140.1, 140.3, 142.2, 162.9 (d, J = 242.3 Hz). MS (ESI): 399 $[M + Na]^+$. HRMS (ESI), m/z calcd for $C_{20}H_{22}FO_6$ $[M + H]^+$ 377.1395, found 377.1396.

*(1S,3′R,4′S,5′S,6′R)-6-[(4-Chlorophenyl)methyl]-6′-(hydroxymethyl)-3′,4′,5′,6′-tetrahydro-3H-spiro[2-benzofuran-1,2′-pyran]-3′,4′,5′-triol (**16j**).* Compound **16j** was prepared from **14** and 4-chlorophenylboronic acid following a similar procedure to that described in Method B (yield 25%, colorless amorphous); t_R = 8.3 min (condition B). ^1H NMR (400 MHz, CD_3OD) δ: 3.43−3.47 (1H, m), 3.63−3.68 (1H, m), 3.75−3.84 (4H, m), 3.99 (2H,s), 5.07 (1H, d, J = 12.5 Hz), 5.13 (1H, d, J = 12.5 Hz), 7.17−7.26 (7H, m). ^{13}C NMR (100 MHz, CD_3OD) δ: 41.8, 62.8, 71.8, 73.4, 74.9, 76.2, 76.4, 111.6, 122.0, 123.7, 129.5, 131.1, 131.5, 132.9, 140.2, 140.4, 141.4, 141.8. MS

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(ESI): 393 $[M + H]^+$. HRMS (ESI), m/z calcd for $C_{20}H_{22}ClO_6$ $[M + H]^+$ 393.1099, found 393.1102.

*4-(((1S,3′R,4′S,5′S,6′R)-3′,4′,5′-Trihydroxy-6′-(hydroxymethyl)-3′,4′,5′,6′-tetrahydro-3H-spiro[2-benzofuran-1,2′-pyran]-6-yl)-methyl)benzonitrile (**16k**).* Compound **16k** was prepared from **14** and 4-cyanophenylboronic acid following a similar procedure to that described in Method B (yield 43%, colorless amorphous); t_R = 7.2 min (condition B). ^{1}H NMR (400 MHz, CD_3OD) δ: 3.43−3.47 (1H, m), 3.63−3.68(1H, m), 3.75−3.84 (4H, m), 4.09 (2H, s), 5.08 (1H, d, J = 12.5 Hz), 5.14 (1H, d, J = 12.5 Hz), 7.24−7.25 (3H, m), 7.40 (2H, d, J = 8.4 Hz), 7.61−7.63 (2H, m). ^{13}C NMR (100 MHz, CD_3OD) δ: 42.5, 62.7, 71.8, 73.4, 74.9, 76.2, 76.4, 111.0, 111.6, 119.8, 122.2, 123.9, 131.0, 131.3, 133.4, 140.5, 140.6, 140.8, 148.6. MS (ESI): 406 $[M + Na]^+$. HRMS (ESI), m/z calcd for $C_{21}H_{22}NO_6$ $[M + H]^+$ 384.1442, found 384.1446.

*(1S,3′R,4′S,5′S,6′R)-6′-(Hydroxymethyl)-6-[(4-(trifluoromethyl)-phenyl)methyl]-3′,4′,5′,6′-tetrahydro-3H-spiro[2-benzofuran-1,2′-pyran]-3′,4′,5′-triol (**16l**).* Compound **16l** was prepared from **14** and 4-trifluoromethylphenylboronic acid following a similar procedure to that described in Method B (yield 57%, colorless amorphous); t_R = 8.7 min (condition B). ^{1}H NMR (400 MHz, CD_3OD) δ: 3.43−3.47 (1H, m), 3.63−3.68 (1H, m), 3.75−3.83 (4H, m), 4.10 (2H,s), 5.08 (1H, d, J = 12.5 Hz), 5.14 (1H, d, J = 12.5 Hz), 7.22−7.26 (3H, m), 7.39−7.42 (2H, m), 7.55−7.57 (2H, m). ^{13}C NMR (100 MHz, CD_3OD) δ: 42.3, 62.8, 71.8, 73.4, 74.9, 76.2, 76.4, 111.6, 122.1, 123.8, 125.8 (q, J = 271.5 Hz), 126.3 (q, J = 3.8 Hz), 129.4 (q, J = 31.5 Hz), 130.5, 131.2, 140.4, 140.5, 141.2, 147.3. MS (ESI): 427 $[M + H]^+$. HRMS (ESI), m/z calcd for $C_{21}H_{22}F_3O_6$ $[M + H]^+$ 427.1363, found 427.1365.

*(1S,3′R,4′S,5′S,6′R)-6′-(Hydroxymethyl)-6-[(4-(trifluoromethoxy)-phenyl)methyl]-3″,4′,5′,6′-tetrahydro-3H-spiro[2-benzofuran-1,2′-pyran]-3′,4′,5′-triol (**16m**).* Compound **16m** was prepared from **14** and 4-trifluoromethoxyphenylboronic acid following a similar procedure to that described in Method B (yield 32%, colorless amorphous); t_R = 8.9 min (condition B).; ^{1}H NMR (400 MHz, CD_3OD) δ: 3.43−3.48 (1H, m), 3.64−3.68 (1H, m), 3.75−3.84 (4H, m), 4.03 (2H, s), 5.08 (1H, d, J = 12.5 Hz), 5.14 (1H, d, J = 12.5 Hz), 7.14−7.32 (7H, m). ^{13}C NMR (100 MHz, CD_3OD) δ: 41.8, 62.8, 71.8, 73.4, 74.9, 76.2, 76.4, 111.6, 122.0 (q, J = 256.5 Hz), 122.1, 122.1, 123.7, 131.2, 131.4, 140.2, 140.4, 141.7, 142.0, 148.9 (q, J = 2.2 Hz). MS (ESI): 443 $[M + H]^+$. HRMS (ESI), m/z calcd for $C_{21}H_{22}F_3O_7$ $[M + H]^+$ 443.1312, found 443.1314.

*(1S,3′R,4′S,5′S,6′R)-6′-(Hydroxymethyl)-6-[(4-(methylthio)-phenyl)methyl]-3′,4′,5′,6′-tetrahydro-3H-spiro[2-benzofuran-1,2′-pyran]-3′,4′,5′-triol (**16n**).* Compound **16n** was prepared from **14** and 4-(methylthio)phenylboronic acid following a similar procedure to that described in Method B (yield 30%, colorless amorphous); t_R = 8.3 min (condition B). ^{1}H NMR (400 MHz, CD_3OD) δ: 2.42 (3H, s), 3.42−3.47 (1H, m), 3.63−3.67 (1H, m), 3.74−3.83 (4H, m), 3.96 (2H, s), 5.07 (1H, d, J = 12.5 Hz), 5.13 (1H, d, J = 12.5 Hz), 7.12−7.23 (7H, m). ^{13}C NMR (100 MHz, CD_3OD) δ: 16.0, 42.0, 62.8, 71.8, 73.4, 74.9, 76.2, 76.4, 111.6, 121.9, 123.6, 128.1, 130.5, 131.1, 137.4, 139.5, 140.0, 140.3, 142.2. MS (ESI): 405 $[M + H]^+$. HRMS (ESI), m/z calcd for $C_{21}H_{25}O_6S$ $[M + H]^+$ 405.1366, found 405.1366.

*(1S,3′R,4′S,5′S,6′R)-3′,4′,5′-Tri(acetoxy)-6′-acetoxymethyl-5-chloro-6-[(4-ethylphenyl)methyl]-3′,4′,5′,6′-tetrahydrospiro-[isobenzofuran-1(3H),2′-[2H]pyran] (**20**).* To a solution (45 mL) of 1-bromo-4-chloro-2,5-dimethylbenzene **18** (10.0 g, 45.5 mmol) in AcOEt was added *N*-bromosuccinimide (NBS) (21.0 g, 118.4 mmol) and 2,2′-azobis(isobutyronitrile) (AIBN) (300 mg), and the resultant mixture was stirred for 20 min at reflux temperature. The reaction mixture was cooled to rt, and then AcOEt was added thereto. The resultant mixture was washed with water and brine. The organic layer was dried over $MgSO_4$, and the solvent was then removed by distillation under reduced pressure. The obtained crude product (20.8 g) was dissolved in DMF (100 mL), and AcONa (11.2 g, 136.5 mmol) was added thereto. The resultant mixture was stirred for 3 h at 80 °C. The reaction mixture was cooled to rt, and then CH_2Cl_2 was added thereto. The mixture was washed with water and brine. The organic layer was dried over $MgSO_4$, and the solvent was then removed by distillation under reduced pressure. To the obtained residue was added AcOEt (100 mL), and this solution was stirred for 15 h at rt. Undissolved material was collected by filtration to obtain (4-acetoxymethyl-2-bromo-5-chloro)benzyl acetate (5.9 g, 39%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.15 (3H, s), 2.16 (3H, s), 5.14 (2H, s), 5.16 (2H, s), 7.42 (1H, s), 7.61 (1H, s).

To a solution of the above intermediate (28.6 g, 85.2 mmol) in a mixture of THF (250 mL), EtOH (250 mL), and water (125 mL) was added potassium hydroxide (14.3 g, 256 mmol), and the resultant mixture was stirred for 3 h at 80 °C. The reaction mixture was cooled to rt, and then solvent was removed by distillation under reduced pressure. To the resulting residue were added water (200 mL) and AcOEt (100 mL), and this mixture was stirred for 1 h at rt. Undissolved material was collected by filtration and dried to obtain (2-bromo-5-chloro-4-hydroxymethylphenyl)methanol (**19**) (20.7 g, 97%). ^{1}H NMR (300 MHz, CD_3OD) δ: 4.61 (2H, s), 4.66 (2H, s), 7.52 (1H, s), 7.70 (1H, s).

To a solution (500 mL) of **19** (20.7 g, 82.3 mmol) in anhydrous THF were added 2-methoxypropene (78.8 mL, 823.1 mmol) and pyridinium *p*-toluenesulfonate (207 mg, 0.823 mmol) at 0 °C, and the resultant mixture was stirred for 2 h. Aqueous potassium carbonate solution (1 M, 200 mL) was added thereto, and then the resultant mixture was extracted with AcOEt (800 mL) containing Et_3N (2.5 mL). The organic layer was washed with water (500 mL) and brine (500 mL) and then dried over $MgSO_4$. The solvent was then removed by distillation under reduced pressure to obtain 1-bromo-4-chloro-2,5-bis[(1-methoxy-1-methyl)ethoxymethyl]benzene (33.2 g, 100%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.45 (12H, s), 3.22 (6H, s), 4.48 (2H, s), 4.53 (2H, s), 7.51 (1H, s), 7.68 (1H, s).

To a solution (500 mL) of the above intermediate (24.7 g, 62.53 mmol) in anhydrous THF, 1.6 M *n*-BuLi in hexane solution (39.1 mL, 62.53 mmol) was added dropwise over 5 min at −78 °C under a nitrogen atmosphere and the resultant mixture was stirred under the same condition for 20 min. A solution of prepared 2,3,4,6-tetrakis-*O*-trimethylsilyl-D-glucono-1,5-lactone[39] (26.54 g, 56.85 mmol) in THF (40 mL) was then added dropwise over 5 min to the resultant mixture. The reaction mixture was stirred for 1 h, and then water was added thereto. The resultant mixture was extracted with Et_2O. The resultant organic layer was washed with water and brine and then dried over $MgSO_4$. The solvent was then removed by distillation under reduced pressure. The obtained residue (46.97 g) was dissolved in a mixed solvent of THF (94 mL) and MeOH (47 mL), and *p*-toluenesulfonic acid (2.16 g) was added thereto. The mixture was stirred at rt for 15 h and then cooled with ice. *tert*-Butyl methyl ether (188 mL) was added thereto, and the precipitate was collected by filtration. The obtained solid was dried under reduced pressure to obtain (1*S*,3′*R*,4′*S*,5′*S*,6′*R*)-5-chloro-3′,4′,5′,6′-tetrahydro-6,6′-bis(hydroxymethyl)-spiro[2-benzofuran-1(3*H*),2′-[2*H*]pyran]-3′,4′,5′-triol (12.7 g, 67%). ^{1}H NMR (300 MHz, CD_3OD) δ: 3.44−3.50 (1H, m), 3.63−3.84 (5H, m), 4.71 (2H, s), 5.07 (1H, d, J = 12.3 Hz), 5.13 (1H, d, J = 12.3 Hz), 7.33 (1H, s), 7.55 (1H, s).

To a solution of the above pentaol (1.0 g, 3.0 mmol) in DMSO (1.7 mL, 24.0 mmol) was added chlorotrimethylsilane (1.1 mL, 8.4 mmol) at rt, and the resultant mixture was stirred under this condition for 4 h. The reaction mixture was concentrated under reduced pressure, and the obtained residue was dried under reduced pressure for 15 h. To a solution (20 mL) of the obtained residue in THF were added *N*-methylmorpholine (3.7 mL, 30 mmol) and DMAP (385 mg, 3.15 mmol) at 0 °C, and Ac_2O (1.7 mL, 18 mmol) was then added dropwise to the resultant mixture. The reaction mixture was stirred under this condition for 30 min and then stirred for another 30 min at rt. Excess aqueous phosphoric acid was added thereto, and the resultant mixture was extracted with AcOEt. The organic layer was washed with water and brine, and then dried over $MgSO_4$. The solvent was then removed by distillation under reduced pressure. The resulting residue was purified by silica gel column chromatography (AcOEt/hexane = 1:1) to obtain (1*S*,3′*R*,4′*S*,5′*R*,6′*R*)-6′-(acetoxymethyl)-6-(chloromethyl)-3′,4′,5′,6′-tetrahydro-3*H*-spiro[2-benzofuran-1,2′-pyran]-3′,4′,5′-triyl triacetate (840 mg, 54%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.77 (3H, s), 2.01 (3H, s), 2.05 (3H, s), 2.08 (3H, s), 4.01−4.06 (1H m), 4.25−4.33 (2H, m), 4.70 (2H, d, J = 2.3

Hz), 5.13 (1H, d, J = 13.4 Hz), 5.20 (1H, d, J = 13.4 Hz), 5.25−5.31 (1H, m), 5.55−5.64 (2H, m), 7.32 (1H, s), 7.51 (1H, s).

To a solution of the above compound (300 mg, 0.58 mmol) in DMF (2.85 mL) and water (0.15 mL) were added 4-ethylphenylboronic acid (174 mg, 1.16 mmol), tetrakis(triphenylphosphine)palladium (34 mg, 0.029 mmol), sodium carbonate (184 mg, 1.74 mmol), and tetrabutylammonium bromide (39 mg, 0.116 mmol), and the resultant mixture was stirred for 15 h at 85 °C. The reaction mixture was cooled to rt, and then water (10 mL) was added thereto. The resultant mixture was extracted with AcOEt (100 mL). The organic layer was washed with water and brine and then dried over $MgSO_4$. The solvent was then removed by distillation under reduced pressure. The obtained residue was purified by silica gel column chromatography (AcOEt/hexane = 1:3) to obtain **20** (236 mg, 69%) as a colorless amorphous compound. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.22 (3H, t, J = 7.6 Hz), 1.75 (3H, s), 2.00 (3H, s), 2.04 (3H, s), 2.06 (3H, s), 2.62 (2H, q, J = 7.6 Hz), 4.01−4.13 (3H, m), 4.22−4.33 (2H, m), 5.09 (1H, d, J = 12.6 Hz), 5.17 (1H, d, J = 13.1 Hz), 5.23−5.30 (1H, m), 5.52−5.63 (2H, m), 7.07−7.14 (4H, m), 7.26 (2H, s).

*(1S,3′R,4′S,5′S,6′R)-5-Chloro-6-[(4-ethylphenyl)methyl]-6′-(hydroxymethyl)-3′,4′,5′,6′-tetrahydro-3H-spiro[2-benzofuran-1,2′-pyran]-3′,4′,5′-triol (**21o**).* To a solution of **20** (236 mg, 0.40 mmol) in MeOH (16 mL) was added potassium carbonate (166 mg, 1.20 mmol), and the resultant mixture was stirred for 1 h at rt. Water (5 mL) was added thereto, and the resultant mixture was extracted with AcOEt (50 mL). The organic layer was washed with brine and then dried over $MgSO_4$. The solvent was then removed by distillation under reduced pressure. The resulting residue was purified by silica gel column chromatography (MeOH/CH_2Cl_2 = 1:15) to obtain **21o** (110 mg, 65%) as a colorless amorphous compound; t_R = 11.2 min (condition B). ^{1}H NMR (400 MHz, CD_3OD) δ: 1.20 (3H, t, J = 7.6 Hz), 2.59 (2H, q, J = 7.6 Hz), 3.38−3.46 (1H, m), 3.63−3.81 (5H, m), 4.04−4.13 (2H, m), 5.07 (1H, d, J = 12.7 Hz), 5.12 (1H, d, J = 12.7 Hz), 7.10 (4H, s), 7.25 (1H, s), 7.36 (1H, s). ^{13}C NMR (100 MHz, CD_3OD) δ: 16.2, 29.5, 39.8, 62.7, 71.7, 72.9, 74.9, 76.3, 76.4, 111.4, 123.1, 125.9, 128.9, 129.9, 136.2, 137.9, 139.2, 139.6, 142.1, 143.4. MS (ESI): 443 $[M + Na]^+$, 863 $[2M + Na]^+$. HRMS (ESI), m/z calcd for $C_{22}H_{26}ClO_6$ $[M + H]^+$ 421.1412, found 421.1415.

*(1S,3′R,4′S,5′S,6′R)-6-[(4-Ethylphenyl)methyl]-6′-(hydroxymethyl)-5-methyl-3′,4′,5′,6′-tetrahydro-3H-spiro[2-benzofuran-1,2′-pyran]-3′,4′,5′-triol (**21p**).* A solution of **20** (138 mg, 0.23 mmol), bis(dibenzylideneacetone)palladium(0) (Pd(dba)$_2$) (132 mg, 0.23 mmol), tri-*tert*-butylphosphonium tetrafluoroborate (*t*-$Bu_3P{\cdot}BF_4$) (67 mg, 0.23 mmol), methylboronic acid (28 mg, 0.46 mmol), and K_3PO_4 (146 mg, 0.69 mmol) in xylene (2.8 mL) was stirred for 15 h at 145 °C. The reaction mixture was concentrated under reduced pressure, and the obtained residue was purified by silica gel column chromatography (AcOEt/hexane = 2:5) and preparative HPLC to obtain intermediate (37 mg, 28%). To a solution of the above compound (37 mg, 0.065 mmol) in MeOH (1 mL) was added K_2CO_3 (9 mg, 0.065), and the resultant mixture was stirred for 1 h at rt. The solvent was then removed by distillation under reduced pressure. The obtained residue was purified by silica gel column chromatography (MeOH/CH_2Cl_2 = 15:85) and preparative HPLC to obtain **21p** (14 mg, 54%) as a colorless amorphous compound; t_R = 11.9 min (condition A). ^{1}H NMR (400 MHz, CD_3OD) δ: 1.19 (3H, t, J = 7.6 Hz), 2.22 (3H, s), 2.58 (2H, q, J = 7.6 Hz), 3.41−3.47 (1H, m), 3.64−3.68 (1H, m), 3.73−3.84 (4H, m), 3.97 (2H, s), 5.03 (1H, d, J = 12.5 Hz), 5.13 (1H, d, J = 12.4 Hz), 7.01−7.14 (6H, m). ^{13}C NMR (100 MHz, CD_3OD) δ: 16.2, 20.1, 29.4, 40.1, 62.8, 71.8, 73.4, 74.9, 76.2, 76.5, 111.7, 123.4, 124.6, 128.8, 129.7, 137.8, 138.7, 139.3, 140.1, 140.4, 143.1. MS (ESI): 401 $[M + H]^+$. HRMS (ESI), m/z calcd for $C_{23}H_{29}O_6$ $[M + H]^+$ 401.1959, found 401.1956.

*(1S,3′R,4′S,5′S,6′R)-6-[(4-Ethylphenyl)methyl]-5-ethynyl-6′-(hydroxymethyl)-3′,4′,5′,6′-tetrahydro-3H-spiro[2-benzofuran-1,2′-pyran]-3′,4′,5′-triol (**21q**).* A solution of **20** (97 mg, 0.165 mmol), dichlorobis(acetonitrile)palladium(II) (9 mg, 0.035 mmol), 2-dicyclohexylphosphino-2′,4′,6′-triisopropylbiphenyl (53 mg, 0.111 mmol), and cesium carbonate (141 mg, 0.433 mmol) in MeCN (3.35 mL) was stirred for 0.5 h at rt. To the mixture was then added trimethylsilylacetylene (0.26 mL, 1.84 mmol), and the resultant mixture was stirred for 2 h at 90 °C. The reaction mixture was concentrated under reduced pressure, and the resulting residue was purified by silica gel flash column chromatography (AcOEt/hexane = 1:2) to obtain the intermediate (96 mg, 81%). To a solution of the above compound (101 mg, 0.155 mmol) in MeOH (1 mL) was added K_2CO_3 (14 mg), and the resultant mixture was stirred for 1.5 h at rt. The solvent was then removed by distillation under reduced pressure. The obtained residue was purified by silica gel column chromatography (MeOH/CH_2Cl_2 = 15:85) to obtain **21q** (23 mg, 36%) as a colorless amorphous compund; t_R = 11.6 min (condition A). ^{1}H NMR (400 MHz, CD_3OD) δ: 1.19 (3H, t, J = 7.6 Hz), 2.58 (2H, q, J = 7.6 Hz), 3.40−3.45 (1H, m), 3.62−3.82 (6H, m), 4.12 (1H, d, J = 14.8 Hz), 4.19 (1H, d, J = 14.8 Hz), 5.06 (1H, d, J = 12.7 Hz), 5.12 (1H, d, J = 12.7 Hz), 7.07 (2H, d, J = 8.0 Hz), 7.14 (2H, d, J = 8.0 Hz), 7.21 (1H, s), 7.42 (1H, s). ^{13}C NMR (100 MHz, CD_3OD) δ: 16.2, 29.4, 40.4, 62.7, 71.7, 73.1, 74.8, 76.2, 76.3 76.3, 82.9, 111.5, 124.3, 124.4, 126.3, 128.8, 129.9, 138.9, 140.2, 140.8, 143.2, 144.9. MS (ESI): 411 $[M + H]^+$. HRMS (ESI), m/z calcd for $C_{24}H_{27}O_6$ $[M + H]^+$ 411.1802, found 411.1802.

*(1S,3′R,4′S,5′S,6′R)-6-[(4-Ethylphenyl)methyl]-6′-(hydroxymethyl)-5-phenyl-3′,4′,5′,6′-tetrahydro-3H-spiro[2-benzofuran-1,2′-pyran]-3′,4′,5′-triol (**21r**).* A solution of **20** (67 mg, 0.114 mmol), bis(dibenzylideneacetone)palladium(0) (0.7 mg, 0.001 mmol), 1,2,3,4,5-pentaphenyl-1′-(di-*t*-butylphosphino)ferrocene (CTC-Q-PHOS) (1.7 mg, 0.002 mmol), phenylboronic acid (21 mg, 0.172 mmol), and KF (20 mg, 0.344 mmol) in THF (0.22 mL) was stirred for 0.5 h at 100 °C under microwave irradiation. The reaction mixture was concentrated under reduced pressure, and the obtained residue was purified by silica gel column chromatography (AcOEt/hexane = 1:2), to obtain the intermediate (77 mg, quant). To a solution of the above compound (77 mg, 0.114 mmol) in MeOH (2 mL) was added K_2CO_3 (34 mg, 0.246 mmol), and the resultant mixture was stirred for 1.5 h at rt. The solvent was then removed by distillation under reduced pressure. The obtained residue was purified by silica gel column chromatography (MeOH/CH_2Cl_2 = 1:9) and preparative HPLC to obtain **21r** (24 mg, 45%) as a colorless amorphous compound; t_R = 13.5 min (condition A). ^{1}H NMR (400 MHz, CD_3OD) δ: 1.17 (3H, t, J = 7.6 Hz), 2.55 (2H, q, J = 7.6 Hz), 3.43−3.47 (1H, m), 3.65−3.69 (1H, m), 3.76−3.86 (4H, m), 3.90 (2H, d, J = 2.4 Hz), 5.12 (1H, d, J = 12.7 Hz), 5.19 (1H, d, J = 12.7 Hz), 6.82 (2H, d, J = 8.0 Hz), 6.98 (2H, d, J = 8.0 Hz), 7.13 (1H, s), 7.16−7.18 (2H, m), 7.26 (1H, s), 7.32−7.36 (3H, m). ^{13}C NMR (100 MHz, CD_3OD) δ: 16.2, 29.4, 39.7, 62.9, 71.9, 73.4, 75.0, 76.3, 76.5, 111.7, 123.4, 125.0, 128.2, 128.7, 129.1, 129.8, 130.3, 139.3, 139.6, 139.7, 140.2, 142.9, 143.0, 145.0. MS (ESI): 463 $[M + H]^+$. HRMS (ESI), m/z calcd for $C_{28}H_{31}O_6$ $[M + H]^+$ 463.2115, found 463.2114.

*((1S,3′R,4′S,5′S,6′R)-6-[(4-Ethylphenyl)methyl]-3′,4′,5′-trihydroxy-3′,4′,5′,6′-tetrahydro-3H-spiro[2-benzofuran-1,2′-pyran]-6′-yl)methyl Acetate (**17**) for Single Crystal Analysis.* To a solution of **16d** (CSG452) (100 mg, 0.26 mmol) in 2,4,6-trimethylpyridine (0.68 mL) was added acetyl chloride (74 μL, 1.04 mmol) at −10 °C, and the resulting mixture was stirred for 2 h at rt. Water was added thereto, and the mixture was extracted with AcOEt. The organic layer was washed with brine and then dried over $MgSO_4$. The solvent was then removed by distillation under reduced pressure. The obtained residue was purified by silica gel column chromatography (MeOH/CH_2Cl_2 = 1:10) to obtain monoacetylated compound **17** (81 mg, 73%) as a colorless solid. Single crystals of monohydrate **17** were obtained by recrystallization from MeCN−H_2O as the solvent; mp 168−169 °C. ^{1}H NMR (400 MHz, CD_3OD) δ: 1.19 (3H, t, J = 7.6 Hz), 1.99 (3H, s), 2.58 (2H, q, J = 7.6 Hz), 3.42−3.47 (1H, m), 3.73−3.76 (2H, m), 3.95−4.00 (3H, m), 4.15 (1H, dd, J = 5.6, 12.0 Hz), 4.31 (1H, dd, J = 2.0, 11.9 Hz), 5.07 (1H, d, J = 12.5 Hz), 5.12 (1H, d, J = 12.5 Hz), 7.08−7.22 (6H, m). ^{13}C NMR (100 MHz, CD_3OD) δ: 16.2, 20.7, 29.4, 42.1, 65.1, 71.8, 73.5, 73.5, 74.8, 76.2, 111.6, 121.8, 123.4, 128.9, 129.9, 131.2, 139.5, 139.6, 139.8, 142.7, 143.1, 172.9. MS (ESI): 429 $[M + H]^+$.

Molecular Modeling. All molecular modeling procedures were performed using Molecular Operating Environment (MOE) version 2010.10.[30] Six known SGLT2 inhibitors (**1**, **2a**, **3**, **4**, **5**, **6** in Figure 1) were constructed and minimized by using a MMFF94 force field in default condition.[40] Superposed models of inhibitors were derived by the FlexibleAlignment module of MOE in which the H-bond donor and the aromaticity were assigned for similarity terms. Six atoms in the pyranose ring moiety of each inhibitor were fixed during the Flexible Alignment. Although several energetically equivalent models were generated because of the higher flexibility of inhibitors, the differences among these models are due to the relative configurations of sugar and distal aromatic moieties bound to the central aromatic ring. One of them, which has a similar configuration to the crystal structure of phlorizin (Cambridge Structural Database ID: CEWWAC01), was chosen for further discussion. The global minimum energy of each inhibitor was estimated by the LowModeMD search method.

In Vitro SGLT Inhibition Assay. Chinese hamster ovary-K1(CHO) cells stably expressing human SGLT2 (NM003041) and human SGLT1 (NM000343) were used for the sodium-dependent methyl-α-D-glucopyranoside (AMG) uptake inhibition assay. The cells were incubated in reaction buffer with the compound and 1 mM AMG containing [^{14}C]AMG for 45 min. AMG uptake activity was determined by counting the radioactivity of the cell lysates. IC_{50} values were calculated by curve fitting using a four-parameter logistic model (XLfit, ID Business Solutions Ltd.).

In Vivo Efficacy in db/db Mice. Male db/db mice were purchased from CLEA Japan (Tokyo, Japan) and maintained on a regular diet (CE2, CLEA Japan). All animal care and experiments were performed in accordance with the guidelines for the care and use of laboratory animals at Chugai Pharmaceutical Co., Ltd. Compounds **16d**, **16f** (1, 3, or 10 mg/kg), or a vehicle (0.5% CMC) was orally administered to nonfasted db/db mice. Blood samples were collected from the tail vein immediately before administration (0 h) and at 1, 2, 4, and 6 h after administration. Urine samples were collected from mice metabolic cages for 6 h from drug administration, and the urinary glucose and creatinine concentrations were measured. Blood and urine glucose concentrations were measured by the hexokinase method (Autosera S GLU, Daiichi Pure Chemicals, Japan). Urinary creatinine concentrations were measured by the enzymatic method (Sikaliquid-N Cre, Kanto Chemicals, Japan).

Pharmacokinetics Studies. Male Crlj:CD1 (ICR) mice (8-weeks old; Charles River Laboratories Japan, Inc., Japan) and male cynomolgus monkeys (body weight: 3.1−3.8 kg) were used for the pharmacokinetics study. Animals were given a single intravenous or oral administration of **16d** and **16f** under fasted conditions. Dosing vehicles used were 10% (v/v) Cremophor EL aqueous solution (Sigma Chemical Co., MO, USA) for intravenous and 0.5% (w/v) carboxymethyl-cellulose sodium salt for oral dosing. Blood samples were collected at 5 min, 0.25, 0.5, 1, 2, 4, and 8 h after administration to mice and at 0.25, 0.5, 1, 2, 4, 8, and 24 h after administration to monkeys. The urine samples were collected during the 8 h just after administration to mice and collected from 8 to 24 h after administration to monkeys. The drug concentrations in samples were determined using an LC-MS/MS system after deproteinization. LC-MS/MS analysis was performed using a Shimadzu LC-10AD pump (Shimadzu Co., Japan) and an Applied Biosystems/MDS Sciex API-300 mass spectrometer (Toronto, Canada) with a TurboIonSpray source. Chromatographic separation was achieved using a CAPCELL PAK C18 column (2.0 mm × 150 mm, 5 μm) (Shiseido Co., Japan). The mobile phase consisted of acetonitrile/10 mM ammonium acetate solution (4:6, v/v), and the flow rate was set at 0.2 mL/min. The injection cycle of each sample was set at 10 min. The transitions for multiple reaction monitoring were 387−267 (**16d**) and 401−281 (**16f**). Noncompartmental pharmacokinetic parameters were calculated based on the plasma concentration−time data using WinNonlin Professional 5.0 (Pharsight, Mountain View, CA). The cumulative drug amount excreted into urine was divided by the corresponding AUC to estimate renal clearance (CL_{renal}).

■ ASSOCIATED CONTENT

Supporting Information

The structures of three hit compounds from a CSD search and CYP3A4 inhibition assay data, X-ray crystallographic data of **17**, and in vitro chemical and metabolic stability data of **16d**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

AcOEt, ethyl acetate; AIBN, 2,2′-azobis(2-methylpropionitrile); AMG, methyl-α-D-glucopyranoside; AUC, area under the plasma concentration time curve; CL, clearance; CSD, Cambridge Structural Database; CYP3A4, cytochrome P450 3A4; 3D, three-dimensional; dba, dibenzylideneacetone; DDI, drug−drug interaction; DMAP, *N*,*N*-dimethylaminopyridine; DMF, *N*,*N*-dimethylformamide; DMSO, dimethylsulfoxide; *F*, oral bioavailability; IC_{50}, half-maximal inhibitory concentration; MOE, Molecular Operating Environment; mp, melting point; NBS, *N*-bromosuccinimide; PK, pharmacokinetics; SD, standard deviation; SGLT, sodium glucose cotransporter; TDI, time-dependent inhibition; TFA, trifluoroacetic acid; THF, tetrahydrofuran; TMS, trimethylsilyl; Tr, triphenylmethyl; t_R, retention time; V_{ss}, steady-state volume of distribution

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