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C-Aryl 5a-carba-β-D-glucopyranosides as novel sodium glucose cotransporter 2 (SGLT2) inhibitors for the treatment of type 2 diabetes

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ABSTRACT

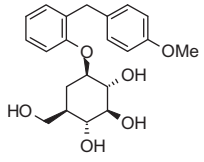
C-Aryl 5a-carba-β-D-glucopyranose derivatives were synthesized and evaluated for inhibition activity against hSGLT1 and hSGLT2. Modifications to the substituents on the two benzene rings resulted in enhanced hSGLT2 inhibition activity and extremely high hSGLT2 selectivity versus SGLT1. Using the created superimposed model, the reason for the high hSGLT2 selectivity was speculated to be that additional substituents occupied a new space, in a different way than known inhibitors. Among the tested compounds, the ethoxy compound **5h** with high hSGLT2 selectivity exhibited more potent and longer hypoglycemic action in db/db mice than our O-carbasugar compound (**1**) and sergliflozin (**2**), which could be explained by its improved PK profiles relative to those of the two compounds. These results indicated that **5h** might be a promising drug candidate for the treatment of type 2 diabetes.

1. Introduction

Type 2 diabetes (T2D) mellitus is a progressive metabolic disease characterized by decreased insulin secretion and increased insulin resistance, and the prevalence has been increasing yearly. However, it is generally accepted that current medication does not adequately control the blood glucose in patients with diabetes. In addition, hypoglycemia is reported as one of the side effects when insulin or sulfonylureas is administered. Therefore, more effective and safer new drugs for the treatment of T2D are strongly desired.

In the last decade, sodium glucose cotransporter 2 (SGLT2) has been attracting attention because it is uniquely responsible for reabsorbing glucose from the renal filtrate.[1] SGLT2 is primarily expressed at the proximal convoluted tubules, whereas SGLT1 is expressed in the small intestine, heart, brain, and renal tubules. Furthermore, SGLT1 mutation is known to cause glucose–galactose malabsorption,[2] so SGLT1 inhibition is reported to have possible gastrointestinal side effects.[3] From these findings, SGLT2-selective inhibitors are considered to be safer than non-selective inhibitors.

Recently, we reported metabolically stable *O*-aryl 5a-carba-β-D-glucopyranosides which are carbasugar analogs as novel SGLT2 inhibitors, revealing their SAR for the substituent on the distal benzene ring of the aglycon.[4] We also demonstrated that one of them (**1**, Fig. 1) showed similar levels with longer duration in vivo efficacy than sergliflozin[5] (**2**, Fig. 1). The *O*-linked carbasugar derivatives have good characteristics as selective SGLT2



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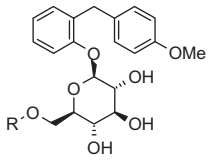
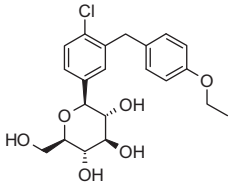
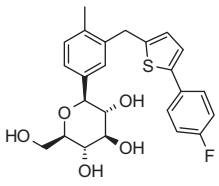


2 sergliflozin (R=CO_2Et)
2-active (2-A) (R=H)
(*O*-glucopyranoside)



3 dapagliflozin
(*C*-glucopyranoside)



4 canagliflozin
(*C*-glucopyranoside)

Figure 1. Structures of some known SGLT inhibitors.

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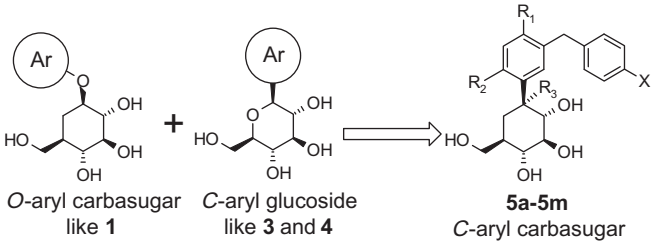


Figure 2. Design of *C*-aryl carbasugar type of SGLT2 inhibitors.

inhibitors (max. hSGLT2 IC_{50} = 11 nM and 510-fold selectivity for hSGLT2 versus hSGLT1); however, further improvement of potency and selectivity was thought to be desirable for clinically effective and safe drugs for the treatment of T2D.

Originally, many clinical trials using SGLT2 inhibitors began with the *O*-glucopyranoside type. However, the *C*-glucopyranoside type of SGLT2 inhibitors, as represented by dapagliflozin[6] (**3**) and canagliflozin[7] (**4**), have currently superseded the *O*-types due to their high potency and metabolic stability.[8] According to a report by Bristol–Myers Squibb, **3** actually appears to be 8-fold more potent and have greater hSGLT2 selectivity than **2-A** in their in vitro assay.[6a]

Taking this situation into account, we applied the carbasugar strategy into the *C*-glucopyranoside type as shown in Figure 2 in an effort to enhance hSGLT2 potency and metabolic stability. Here we wish to report a novel *C*-aryl carbasugar class with potent and selective SGLT2 inhibition, describing the SAR of compounds and providing some in vivo data.

2. Results and discussion

2.1. Chemistry

First, a key intermediate **8** was prepared from the corresponding cyclohexanol **7**[4] by oxidation using Dess–Martin periodinane (Scheme 1).

Next, aglycons **10** were synthesized according to the general method shown in Scheme 2. Addition of the corresponding Grignard reagents or lithiated reagents, generated by *n*BuLi and aryl bromides, to substituted-3-bromobenzaldehyde derivatives (**9**) followed by removal of the hydroxyl group at benzylic position gave the aglycons, **10a–10d, 10f, 10h, 10j** and **10l**.

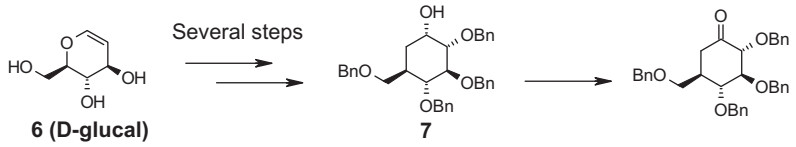
C-Aryl 5a-carba-β-D-glucopyranoside derivatives were synthesized according to the route outlined in Scheme 3. After lithiation of aglycon **10**, it was coupled with the intermediate **8** at −78 °C to give two diastereomers at position 1 on cyclohexane ring. After separation of the epimers by silicagel column chromatography, the more polar epimers were deprotected and deoxygenated to afford tetraols (**5a**, **5b**, **5f**, **5h**, and **5j**) under catalytic hydrogenation condition using $Pd(OH)_2$/C and hydrogen gas. Tetraol **5d** was obtained by stepwise conversion (method c). On the other hand, when the less polar epimers were subjected to the same hydrogenation condition, deoxygenation at the benzylic positions did not occur and pentaols (**5e**, **5g**, **5i**, **5k**, and **5m**) were obtained. The less polar compounds were successfully deoxygenated using Et_3SiH with $BF_3{\cdot}OEt_2$ at −40 °C, followed by debenzylation, to give the tetraol **5l** with the same configuration as that in the more polar compounds by method b. The configurations at position 1 on cyclohexane ring of tetraol and pentaol compounds were determined by NMR analysis.[9]

Cyclopropyl derivative **5c** was prepared by a different route due to the difficulty of the hydrogenation at the final step (Scheme 4). Pentahydroxy compound **12**, which was obtained from **8** via pentabenzyloxy **11**, was protected with two acetonide groups to give **13**. Trifluoromethanesulfonylation of **13** with *N*-(2-pyridyl)-bis(trifluoro-methanesulfonimide) ($PyNTf_2$), followed by Suzuki coupling reaction, led to **15**. Finally, acetonide deprotection under acidic condition afforded **5c** successfully.

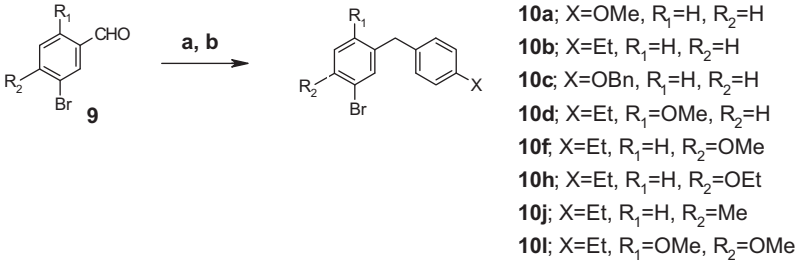
2.2. In vitro data and SAR

All the synthesized compounds **5a**–**5m** were evaluated for their in vitro inhibition activity against human SGLT1 (hSGLT1) and SGLT2 (hSGLT2). The results are shown in Table 1. Of the three compounds **5a**–**5c** with a substituent (X) at para position on the distal benzene ring of the aglycon, **5c** (X = *c*Pr) showed the most potent hSGLT2 inhibition and the highest hSGLT2 selectivity, denoting the same tendency of *O*-linked carbasugar **1** which we reported previously. The cyclopropyl derivative **5c**, however, showed high CYP3A4 time-dependent inhibition (TDI).[10] Therefore, we set the ethyl group as the *para* substituent for further examination.

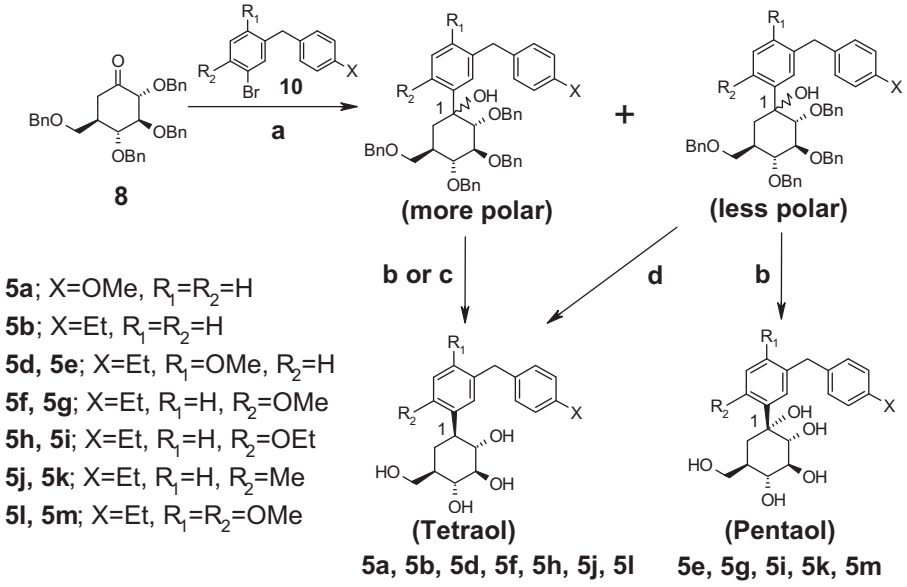
Next, we investigated the substituents (R_1 and R_2) on the central benzene ring (**5d**, **5f**, **5h**, **5j**, and **5l**). As a result, introducing the R_1



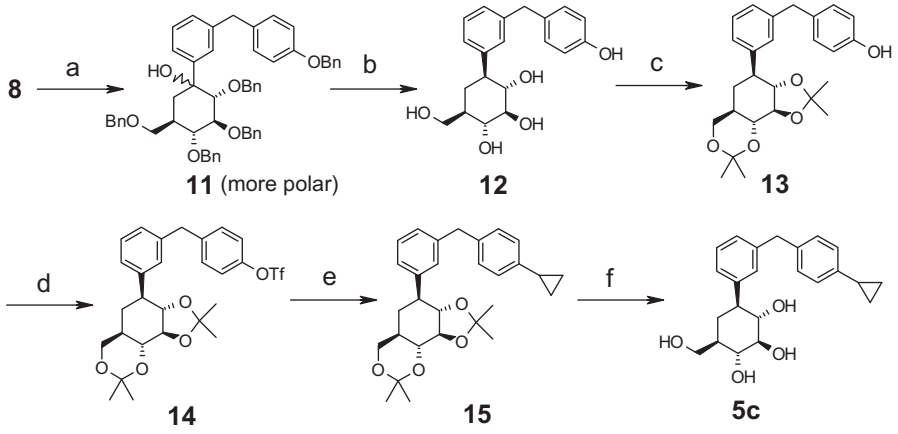
Scheme 1. Reagents and conditions: (a) Dess–Martin periodinane, CH_2Cl_2, rt, 3 h.



Scheme 2. Reagents and conditions: (a) ArMgBr or ArBr/*n*BuLi, THF, 0 °C; (b) Et_3SiH, $BF_3{\cdot}OEt_2$, CH_2Cl_2, 0 °C to rt.



Scheme 3. Reagents and conditions: (a) *n*BuLi, THF or Et_2O, −78 °C then separation by silica gel; (b) $Pd(OH)_2/C$, H_2, MeOH/THF, rt; (c) Et_3SiH, TFA, CH_2Cl_2, −10 °C to rt then $Pd(OH)_2/C$, H_2, MeOH/THF, rt; (d) Et_3SiH, $BF_3{\cdot}Et_2O$, CH_2Cl_2, −40 °C to −10 °C then $Pd(OH)_2/C$, H_2, MeOH/THF, rt.



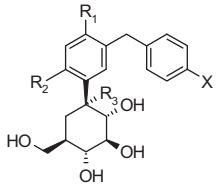
Scheme 4. Reagents and conditions: (a) **10c**, *n*BuLi, THF, −78 °C, 0.5 h (62%); (b) $Pd(OH)_2$, H_2, MeOH/THF, rt, 40 h (63%); (c) $Me_2C(OMe)_2$, DMF, *p*-TsOH, 0 °C to rt, 0.5 h (59%); (d) $PyNTf_2$, CH_2Cl_2, DMAP, 0 °C to rt, 0.5 h (83%); (e) *c*PrB$(OH)_2$, $Pd(PPh_3)_4$, NaBr, K_3PO_4, toluene–H_2O, 100 °C, 6 h (33%); (f) 2 N HCl, 1,4-dioxane, 3 h (51%).

and/or R_2 substituents showed significant effects on hSGLT2 inhibition and selectivity toward SGLT2 versus SGLT1 in most cases. Of the tested *C*-aryl carbasugar compounds, **5d** (R_1 = OMe) exhibited the most potent hSGLT2 inhibition activity (IC_{50} = 8.3 nM); however, it also inhibited hSGLT1 most strongly (IC_{50} = 510 nM), leading to a drastic decrease of the selectivity for hSGLT2 (61-fold). On the other hand, introducing alkoxy groups as the R_2 substituent turned out to be a very effective way of both enhancing hSGLT2 inhibition and lowering hSGLT1 inhibition activity. Methoxy and ethoxy compounds (**5f** and **5h**) showed improved hSGLT2 potency and very low hSGLT1 inhibition compared to non-substituted **5b**. Double methoxy introduction to R_1 and R_2 (**5l**) also supported the idea that the R_2-methoxy substitution effectively diminishes the hSGLT1 inhibition (versus **5d**). Meanwhile, methyl introduction (**5j**) had little effect on both hSGLT1 and hSGLT2 inhibition (compared with **5b**).

Another interesting finding was the effect of the hydroxyl group at benzylic position (R_3 = OH). On the whole, introducing of a hydroxyl group at R_3 position effectively reduced of hSGLT1 inhibition activity. The hSGLT1 inhibition of **5e** was 60-fold lower than that of **5d,** whereas the hSGLT2 inhibition was 10-fold lower. This result suggested that the introducing hydrogen-bonding or a hydrophilic group in R_3 would be unfavorable for hSGLT1 and hSGLT2 inhibition activity. A similar tendency was observed in **5k**. In contrast, three compounds with an alkoxy group at R_2 position (**5g**, **5i** and **5m**) showed maintained hSGLT2 inhibition and moderate to high hSGLT2 selectivity in comparison with the corresponding non-hydroxy compounds (**5f**, **5h** and **5l**). This difference implies the formation of an intramolecular hydrogen bond between the oxygen atom of the alkoxy group and the hydrogen atom of the benzylic hydroxyl group, suggesting that the hydrogen-bonding donating effect has disappeared.

Because comparing 3D structures of known inhibitors with our compounds would be very helpful for us to understand the SAR profiles, we constructed a superimposed model (Fig. 3). Several known SGLT2 inhibitors (phlorizin,[11] **2-A**, **3**, **4**, ipragliflozin,[12] luseogliflozin[13]) and our compounds (**1**, **5h** and **5i**) were subjected to superposition with the Flexible Alignment module in Molecular Operating Environment (MOE).[14] This superimposed model showed that all the inhibitors totally overlapped each other, indicating that they

Table 1
In vitro data for hSGLT inhibition activity and selectivity



5a-5m

Compounds	X	R_1	R_2	R_3	SGLT IC_{50} (nM)[a]		Selectivity[b]	IC_{50} of CYP3A4 inhibition (μM)			
					hSGLT2	hSGLT1		IC_{50}[c] (−)	IC_{50}[c] (+)	Ratio[d]	TDI[e]
Phlorizin					16	190	12	ND	ND	ND	ND
2					260	22,000	85	ND	ND	ND	ND
2-A					4.8	2300	480	ND	ND	ND	ND
3					1.3	800	620	ND	ND	ND	ND
4					6.7	1900	280	ND	ND	ND	ND
5a	OMe	H	H	H	73	48,000	660	>50	>50	n.c.	n.j.
5b	Et	H	H	H	49	33,000	670	>50	>50	n.c.	n.j.
5c	*c*Pr	H	H	H	11	27,000	2,500	76	33	2.3	(+)
5d	Et	OMe	H	H	8.3	510	61	ND	ND	ND	ND
5e	Et	OMe	H	OH	100	31,000	310	ND	ND	ND	ND
5f	Et	H	OMe	H	12	73,000	6,100	>50	>50	n.c.	n.j.
5g	Et	H	OMe	OH	14	>100,000	>7,100	ND	ND	ND	ND
5h	Et	H	OEt	H	19	>100,000	>5,300	36.6	>50	n.c.	n.j.
5i	Et	H	OEt	OH	9.9	>100,000	>10,000	ND	ND	ND	ND
5j	Et	H	Me	H	52	25000	480	ND	ND	ND	ND
5k	Et	H	Me	OH	170	>100,000	>590	ND	ND	ND	ND
5l	Et	OMe	OMe	H	8.5	4500	530	25.7	40.3	0.6	(−)
5m	Et	OMe	OMe	OH	10	8600	860	29.0	>50	n.c.	n.j.

ND = no data; n.c. = not calculated; n.j. = not judged
[a] Phlorizin was always included in the assays as reference standards.
[b] The selectivity values were calculated by IC_{50} hSGLT1/IC_{50} hSGLT2.
[c] IC_{50} (−) and IC_{50} (+) represent the IC_{50} values in which the inhibitor was added to the reaction mixture simultaneously with BFC (coincubation assay) and before the addition of BFC (preincubation assay), respectively.
[d] The ratio were calculated by IC_{50} (−)/IC_{50} (+).
[e] TDIs were judged from the literature.[10] (+) = high risk (⩾2); (−) = no risk (<1.3)

construct a common pharmacophore. Also, it is supposed that the central benzene ring of aglycon is aligned in a perpendicular direction to the sugar moiety and each central benzene ring is less overlapped, whereas each distal benzene ring is relatively well-overlapped. From this model, it was found that only the ethoxy group (R_2) of our compounds was located in a region that could not be occupied by other inhibitors. This finding suggests that some sort of negative interactions between the ethoxy moiety and the hSGLT1 protein may occur in this region. In addition, the oxygen atom of the alkoxy group (R_2) is so close to the hydrogen atom of the hydroxyl group (R_3) as to form an effective intramolecular hydrogen bond, supporting our consideration mentioned above.

2.3. In vivo and PK data

We selected paired ethoxy compounds (**5h** and **5i**) with high hSGLT2 selectivity and evaluated their blood glucose-lowering effect in db/db mice at oral administration of 30 mg/kg to compare with **2** (Fig. 4A). For 4 h after dosing, all the three compounds lowered blood glucose almost equivalently, but this changed after 4 h. That is, while the glucose-lowering effect of **2** diminished rapidly and that of **5i** gradually, that of **5h** was maintained for almost 8 h. In addition, compared with the reduction rate of blood glucose $AUC_{0-8\ h}$ of **1** at dosing of 100 mg/kg (−41.6 ± 3.5%), those of **5h** and **5i** at dosing of 30 mg/kg were almost the same (−42.6 ± 1.3% and −39.1 ± 1.3%, respectively) (Fig. 4B). These results indicate that the blood glucose-lowering effects of **5h** and **5i** were threefold stronger than that of **1**. The reason why the more potent in vitro SGLT2 inhibitor **5i** showed a little shorter and weaker in vivo activity than the less potent **5h** is not clear,[15] but may be the metabolic liability attributable to the benzylic alcohol moiety.

Obtaining a positive result in in vivo pharmacological study, we conducted a pharmacokinetic (PK) study of **5h** in db/db mice at oral single dosage of 10 mg/kg and compared the result with those of **1** and **2** (Table 2). Outstandingly, the *C*-aryl carbasugar compound **5h** showed an apparent lower clearance (CL/F) and higher AUC_{inf} than **1** and **2.** These results suggested that **5h** may become metabolically more stable than **1** and **2** by being converted from the *O*-linked type to the *C*-linked type, being interpreted as meaning that **5h** has more potent and longer in vivo efficacy action than **1** and **2**.

3. Conclusion

In the course of our research on the carbasugar class of SGLT2 inhibitors, we successfully synthesized *C*-aryl derivatives. Several *C*-linked compounds showed more potent in vitro hSGLT2 inhibition and higher hSGLT2 selectivity than those of *O*-linked compound **1**. Using the created superimposition model of some known SGLT2 inhibitors, the reason for the high hSGLT2 selectivity, especially the diminished hSGLT1 inhibition, was speculated to be that additional substituents occupied a new space, in a different way than known inhibitors. Of the tested compounds, highly SGLT2-selective **5h** was found to have a more potent and longer lasting blood glucose-lowering effect in db/db mice than **1** and **2**, which might be attributed to its stronger SGLT2 inhibition activity and the better

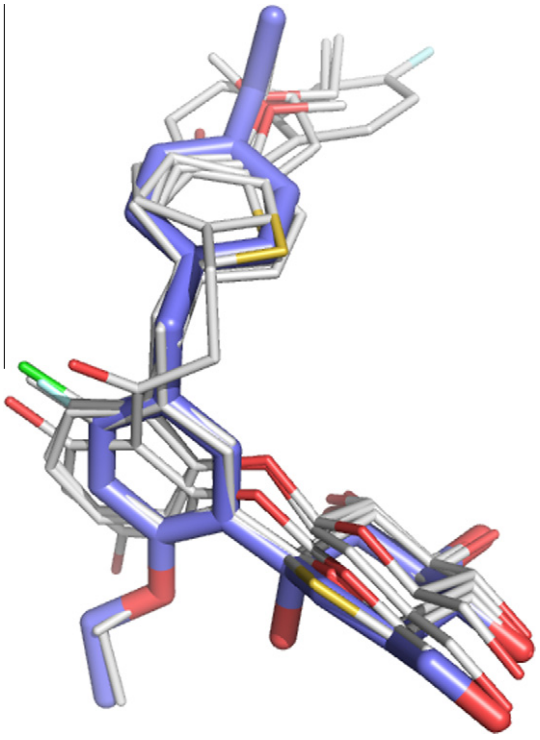


Figure 3. Superimposed model of SGLT2 inhibitors. Compound **5i** is depicted in a thicker stick model (carbon atoms in blue). Hydrogen atoms are not displayed for clarity. See Experimental section for further explanation.

PK profiles than those of **1** and **2**. These results demonstrate that **5h** might be a promising drug candidate for the treatment of T2D.

4. Experimental

4.1. Chemistry: instruments

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) or Shoko Scientific Purif-Pack SI (60 μm), if not otherwise specified. Melting points (mp) were determined with a Yanagimoto micro melting point apparatus. ^{1}H and ^{13}C NMR spectra were recorded on JEOL EX-270 (270 MHz), Varian Mercury300 (300 MHz) or JEOL JNM-ECP-400 (400 MHz), and chemical shifts were reported in parts per million (δ) downfield from tetramethylsilane as an internal standard. The peak patterns are shown as the following abbreviations: br, broad; s, singlet; d, doublet; t, triplet; q, quartet; and m, multiplet. Mass spectra (MS) were measured by a Thermo Electron LCQ Classic or a Micromass ZQ of Waters (ESI). High resolution mass spectra (HRMS) were recorded by a Micromass Q-Tof Ultima API mass spectrometer. All reagents and the solvent were commercially available unless otherwise indicated.

4.1.1. (2*R*,3*S*,4*R*,5*R*)-2,3,4-Tris(benzyloxy)-5-[(benzyloxy) methyl]cyclohexanone (8)

To a solution of (1*S*,2*S*,3*S*,4*R*,5*R*)-2,3,4-tris(benzyloxy)-5-[(benzyloxy)methyl]cyclohexanol **7** (236 mg, 0.44 mmol) in CH_2Cl_2 (1.2 mL) was added Dess–Martin periodinane (280 mg, 0.66 mmol) portionwise at room temperature (rt) and the mixture solution was stirred for 3 h. The solvent was concentrated and the obtained residue was purified by silica gel chromatography (AcOEt/hexane = 1:4) to obtain the title compound (201 mg, 85%) as a white solid. mp 89–91 °C; ^{1}H NMR ($CDCl_3$) δ: 1.84–1.94 (1H, m), 2.41 (1H, dd, J = 14.3, 3.9 Hz), 2.64 (1H, dd, J = 14.3, 13.7 Hz), 3.39 (1H, dd, J = 9.0, 2.5 Hz), 3.71 (1H, dd, J = 9.6, 9.3 Hz), 3.77 (1H, dd, J = 9.2, 3.5 Hz), 3.90 (1H, dd, J = 10.8, 9.0 Hz), 4.13 (1H, d, J = 9.3 Hz), 4.43 (2H, s), 4.55 (2H, dd, J = 11.7, 2.9 Hz), 4.78 (1H, d, J = 10.8 Hz), 4.91–4.97 (3H, m), 7.18–7.21 (2H, m), 7.26–7.40 (18H, m).

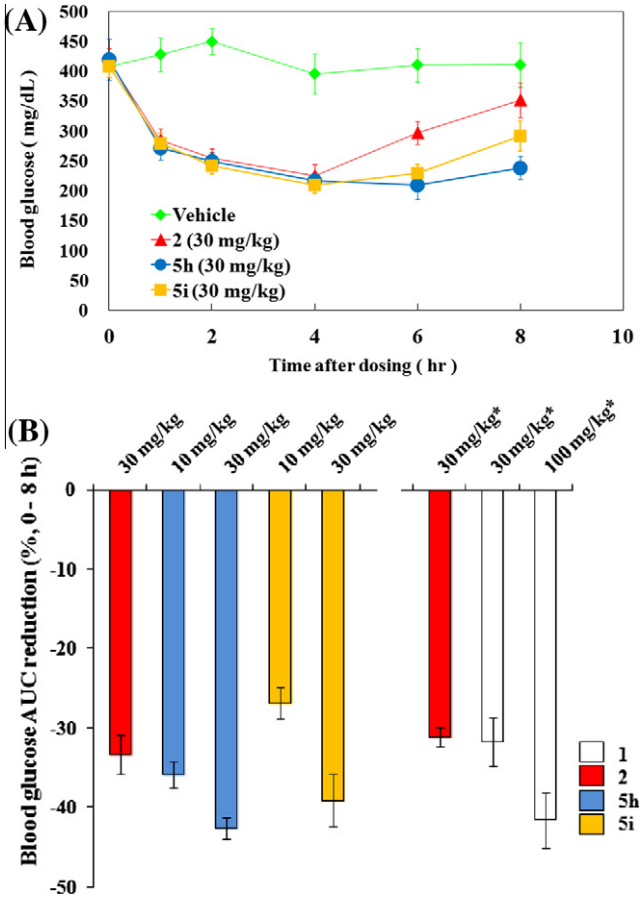


Figure 4. Blood glucose-lowering effect of single oral administration in db/db mice; (A) change in blood glucose levels of **2**, **5h**, and **5i**; (B) Reduction rate of blood glucose $AUC_{0-8\ h}$ in db/db mice (**1**, **2**, **5h**, and **5i**) versus vehicle control. Data are expressed as means ± SE (n = 6). ∗Based on our previous data.[4b]

Table 2
Pharmacokinetic parameters of **1**, **2**, and **5h** in db/db mice

Compounds	1	2	5h
Dose (mg/kg; po)	10	10	10
$T_{1/2}$ (h)	2.68	0.82	1.57
T_{max} (h)	0.25	0.25	0.25
C_{max} (μg/mL)	1.64	3.36	3.68
AUC_{inf} (μg·h/mL)	3.07	2.97	6.24
Vz/F[a] (mL/kg)	12,586	3315	3629
CL/F (mL/hr/kg)	3257	2818	1602

[a] The apparent volume of distribution during terminal phase at oral administration.

4.1.2. 1-Bromo-3-(4-methoxylbenzyl)benzene (10a)

4.1.2.1. (3-Bromophenyl)(4-methoxyphenyl)methanol. In a nitrogen stream, to a solution of 3-bromo-benzaldehyde (423 mg, 2.29 mmol) in THF (10 mL) was added 0.5 M methoxylphenylmagnesium bromide in THF solution (6.86 mL, 3.43 mmol) dropwise at 0 °C. The reaction mixture was stirred at the same temperature for 10 min and warmed up to rt. After being stirred for 30 min at rt, the mixture was stopped by addition of satd. NH_4Cl aq solution. The obtained mixture was extracted with AcOEt, and the extracts were

washed with water and then dried over $MgSO_4$. The organic solvent was concentrated, and the residue was purified by silica gel column chromatography (AcOEt/hexane = 1:9 to 1:3) to obtain the title compound (905 mg, quant) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ: 2.75 (1H, br s), 3.80 (3H, s), 5.76 (1H, s), 6.86–6.90 (2H, m), 7.17–7.21 (1H, m), 7.24–7.29 (3H, m), 7.37–7.40 (1H, m), 7.55–7.56 (1H, m).

4.1.2.2. 1-Bromo-3-(4-methoxylbenzyl)benzene. To a solution of obtained (3-bromophenyl)(4-methoxyphenyl)methanol (670 mg, 2.29 mmol) in CH_2Cl_2 (10 mL) were added Et_3SiH (0.73 mL, 4.57 mmol) and $BF_3{\cdot}OEt_2$ (0.32 mL, 2.51 mmol) at 0 °C and the mixture was stirred for 30 min. Water (4 mL) was added to the reaction mixture and the resulting mixture was extracted with AcOEt. The extracted organic layer was washed with brine, and then dried over $MgSO_4$. The organic solvent was concentrated, and the residue was purified by silica gel column chromatography (AcOEt/hexane = 1:9) to obtain the title compound (586 mg, 92%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ: 3.78 (3H, s), 3.88 (2H, s), 6.83–6.86 (2H, m), 7.07–7.16 (4H, m), 7.31–7.33 (2H, m).

4.1.3. 1-Bromo-3-(4-ethylbenzyl)benzene (10b)

This compound was prepared from 3-bromobenzaldehyde and 0.5 M 4-ethylphenylmagnesium bromide in THF solution in the same manner as described in Section 4.1.2. The total yield was 63%. Colorless oil. ^{1}H NMR ($CDCl_3$) δ: 1.22 (3H, t, J = 7.6 Hz), 2.64 (2H, q, J = 7.6 Hz), 3.90 (2H, s), 7.07–7.16 (6H, m), 7.31–7.33 (2H, m).

4.1.4. 1-Bromo-3-(4-benzyloxybenzyl)benzene (10c)

In a nitrogen stream, to a solution of 1-benzyloxy-4-bromobenzene (2.0 g, 7.6 mmol) in THF (70 mL) was added 2.7 M *n*BuLi in hexane solution (3.1 mL, 8.36 mmol) dropwise at −78 °C and the mixture solution was stirred for 30 min. Then, a solution of 3-bromobenzaldehyde (1.17 g, 6.31 mmol) in THF (30 mL) was added dropwise thereto and the reaction mixture was stirred at −78 °C for 1 h. The mixture was stopped by addition of satd. NH_4Cl aq solution. The mixture was extracted with AcOEt, and the organic layer was washed with brine, and then dried over anhydrous $MgSO_4$. The solvent was concentrated, and the residue was purified by silica gel chromatography (AcOEt/hexane = 1:8) to obtain (3-bromophenyl)(4-benzyloxyphenyl)methanol (2.26 g, 97%) as a colorless oil.

To a solution of obtained (3-bromophenyl)(4-benzyloxyphenyl)methanol (1.0 g, 2.70 mmol) in CH_2Cl_2 (3 mL) and MeCN (3 mL) were added Et_3SiH (0.52 mL, 3.25 mmol) and $BF_3{\cdot}OEt_2$ (0.36 mL, 2.83 mmol) at −40 °C and the mixture was stirred for 30 min. Water (4 mL) was added to the reaction mixture and the resulting mixture was extracted with AcOEt. The extracted organic layer was washed with brine, and then dried over anhydrous $MgSO_4$. The organic solvent was concentrated, and the residue was purified by silica gel column chromatography (AcOEt/ hexane = 1:20) to obtain the title compound (863 mg, 90%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ: 3.87 (2H, s), 5.03 (2H, s), 6.88–6.92 (2H, m), 7.05–7.15 (5H, m), 7.29–7.43 (6H, m).

4.1.5. 1-Bromo-4-methoxy-3-(4-ethylbenzyl)benzene (10d)

This compound was prepared from 5-bromo-2-methoxybenzaldehyde and 0.5 M 4-ethylphenylmagnesium bromide in THF solution in the same manner as described in Section 4.1.2. The total yield was 99%. Colorless oil. ^{1}H NMR ($CDCl_3$) δ: 1.22 (3H, t, J = 7.8 Hz), 2.61 (2H, q, J = 7.8 Hz), 3.79 (3H, s), 3.88 (2H, s), 6.71 (1H, d, J = 8.7 Hz), 7.10–7.15 (5H, m), 7.26 (1H, dd, J = 8.7, 2.3 Hz).

4.1.6. 2-Bromo-1-methoxy-4-(4-ethylbenzyl)benzene (10f)

This compound was prepared from 3-bromo-4-methoxybenzaldehyde and 0.5 M 4-ethylphenylmagnesium bromide in THF solution in the same manner as described in Section 4.1.2. The total yield was 86%. Colorless oil. ^{1}H NMR ($CDCl_3$) δ: 1.22 (3H, t, J = 7.5 Hz), 2.61 (2H, q, J = 7.5 Hz), 3.85 (5H, s), 6.79 (1H, d, J = 8.4 Hz), 7.05–7.24 (5H, m), 7.35–7.37 (1H, m).

4.1.7. 2-Bromo-1-ethoxy-4-(4-ethylbenzyl)benzene (10h)

This compound was prepared from 3-bromo-4-ethoxybenzaldehyde and 0.5 M 4-ethylphenylmagnesium bromide in THF solution in the same manner as described in Section 4.1.2. The total yield was 88%. Colorless oil. ^{1}H NMR ($CDCl_3$) δ: 1.22 (3H, t, J = 7.6 Hz), 1.45 (3H, t, J = 7.0 Hz), 2.62 (2H, q, J = 7.6 Hz), 3.85 (2H, s), 4.06 (2H, q, J = 7.0 Hz), 6.79 (1H, d, J = 8.4 Hz), 7.00–7.15 (5H, m), 7.36 (1H, d, J = 2.1 Hz).

4.1.8. 2-Bromo-1-methyl-4-(4-ethylbenzyl)benzene (10j)

This compound was prepared from 5-bromo-2,4-dimethoxybenzaldehyde and 0.5 M 4-ethylphenyl-magnesium bromide in THF solution in the same manner as described in Section 4.1.2. The total yield was 54%. Colorless oil. ^{1}H NMR ($CDCl_3$) δ: 1.22 (3H, t, J = 7.6 Hz), 2.35 (3H, s), 2.61 (2H, q, J = 7.6 Hz), 3.87 (2H, s), 7.01 (1H, dd, J = 8.0, 1.7 Hz), 7.06–7.14 (5H, m), 7.36 (1H, d, J = 1.7 Hz).

4.1.9. 1-Bromo-5-(4-ethylbenzyl)-2,4-dimethoxybenzene (10l)

This compound was prepared from 5-bromo-2,4-dimethoxybenzaldehyde and 0.5 M 4-ethylphenyl-magnesium bromide in THF solution in the same manner as described in Section 4.1.2. The total yield was 68%. White solid. mp 48–49 °C; ^{1}H NMR ($CDCl_3$) δ: 1.22 (3H, t, J = 7.6 Hz), 2.61 (2H, q, J = 7.6 Hz), 3.82 (3H, s), 3.83 (2H, s), 3.89 (3H, s), 6.47 (1H, s), 7.10 (4H, s), 7.20 (1H, s).

4.1.10. [1*S*,2*R*,3*R*,4*R*,6*S*]-4-Hydroxymethyl-6-[3-(4-methoxybenzyl)phenyl]cyclohexane-1,2,3-triol (5a)

4.1.10.1. [1*R*,2*R*,3*S*,4*R*,5*R*]- and [1*S*,2*R*,3*S*,4*R*,5*R*]-2,3,4-trisbenzyloxy-5-benzyloxymethyl-1-[3-(4-methoxybenzyl)phenyl] cyclohexanol. To a solution of 3-(4-methoxybenzyl)-1-bromobenzene **10a** (77 mg, 0.28 mmol) in THF (0.5 mL) was added 2.5 M *n*BuLi in hexane solution (0.10 mL, 0.27 mmol) dropwise at −78 °C and the mixture solution was stirred for 15 min. Then, a solution of **8** (100 mg, 0.186 mmol) in THF (0.50 mL) was added dropwise thereto and the reaction mixture was stirred for 1 h. To the reaction mixture was added satd. NH_4Cl aq solution and the mixture was extracted with AcOEt, and the organic layer was washed with brine, and then dried over anhydrous $MgSO_4$. The solvent was concentrated, and the obtained residue was purified by silica gel chromatography (AcOEt/hexane = 2:5) to obtain a less polar compound (53 mg, 39%) and a more polar compound (68 mg, 50%).

(Less polar compound): R_f 0.35 (AcOEt/hexane = 1:4); ^{1}H NMR ($CDCl_3$) δ: 1.88 (1H, dd, J = 14.5, 3.9 Hz), 1.97–2.02 (1H, m), 2.26–2.34 (1H, m), 2.98 (1H, br s), 3.39–3.43 (1H, m), 3.70–3.96 (10H, m), 4.42–4.46 (3H, m), 4.62 (1H, d, J = 10.4 Hz), 4.83 (1H, d, J = 10.8 Hz), 4.91 (1H, d, J = 10.7 Hz), 4.93 (1H, d, J = 10.7 Hz), 6.74–6.77 (3H, m), 7.04–7.21 (7H, m), 7.21–7.37 (18H, m).

(More polar compound): R_f 0.30 (AcOEt/hexane = 1:4); ^{1}H NMR ($CDCl_3$) δ: 1.40–1.54 (2H, m), 1.84–1.91 (1H, m), 2.42 (1H, dd, J = 14.1, 3.7 Hz), 2.54 (1H, s), 3.37 (1H, dd, J = 9.0, 2.5 Hz), 3.58 (1H, dd, J = 9.0, 4.7 Hz), 3.64–3.69 (4H, m), 3.74 (1H, d, J = 10.4 Hz), 3.90–3.97 (3H, m), 4.42 (2H, s), 4.51 (1H, d, J = 10.8 Hz), 4.73 (2H, d, J = 11.0 Hz), 4.81 (1H, d, J = 11.0 Hz), 4.86 (1H, d, J = 10.8 Hz), 5.02 (1H, d, J = 11.7 Hz), 6.73–6.77 (2H, m), 7.06–7.09 (3H, m), 7.16–7.20 (2H, m), 7.22–7.35 (18H, m), 7.55–7.58 (1H, m), 7.65–7.67 (1H, m).

4.1.10.2. [1*S*,2*R*,3*R*,4*R*,6*S*]-4-Hydroxymethyl-6-[3-(4-methoxybenzyl)phenyl]cyclohexane-1,2,3-triol. To a solution of the obtained more polar compound (50 mg, 0.068 mmol) in MeOH (5.0 mL)-THF (1.0 mL) was added 20% $Pd(OH)_2$ (27 mg) and the

mixture solution was stirred under hydrogen atmosphere for 3 h. The reaction mixture was filtered and the filtrate was concentrated. The obtained residue was purified by PTLC (MeOH/CH_2Cl_2 = 1:9) to obtain the title compound (11 mg, 45%) as a colorless amorphous. ^{1}H NMR (CD_3OD) δ: 1.44–1.50 (1H, m), 1.65–1.75 (1H, m), 1.85 (1H, dt, J = 3.7, 13.7 Hz), 2.58–2.64 (1H, m), 3.34–3.40 (2H, m), 3.51–3.57 (1H, m), 3.62 (1H, dd, J = 10.6, 6.5 Hz), 3.79 (3H, s), 3.80 (1H, dd, J = 10.4, 4.1 Hz), 3.92 (2H, s), 6.83–6.87 (2H, m), 7.04–7.06 (1H, m), 7.10–7.16 (4H, m), 7.22–7.27 (1H, m); ^{13}C NMR (CD_3OD) δ: 159.4, 144.5, 143.2, 134.8, 130.8, 129.5, 129.4, 128.0, 126.5, 114.8, 81.2, 77.4, 75.1, 64.4, 55.6, 50.0, 45.4, 42.0, 34.5; MS (ESI) m/z: 376 ([M+H_2O]$^+$); HRMS calcd for $C_{21}H_{25}O_5$ ([M−H]$^-$) 357.1697. Found 357.1699.

4.1.11. (1*R*,2*R*,3*S*,4*S*,6*R*)-4-[3-(4-Ethylbenzyl)-phenyl]-6-(hydroxymethyl)cyclohexane-1,2,3-triol (5b)

This compound was prepared from **8** and **10b** in the same manner as described in Section 4.1.10**.** The total yield was 44%. Colorless amorphous. ^{1}H NMR (CD_3OD) δ: 1.24 (3H, t, J = 7.5 Hz), 1.43–1.53 (1H, m), 1.61–1.78 (1H, m), 1.85 (1H, dt, J = 3.7, 13.4 Hz), 2.56–2.68 (3H, m), 3.29–3.40 (2H, m), 3.50–3.66 (2H, m), 3.80 (1H, dd, J = 10.9, 3.9 Hz), 3.94 (2H, s), 7.04–7.27 (8H, m); ^{13}C NMR (CD_3OD) δ: 144.5, 143.0, 142.9, 139.9, 129.9, 129.6, 129.4, 128.8, 128.0, 126.5, 81.2, 77.4, 75.1, 64.4, 50.0, 45.4, 42.5, 34.5, 29.4, 16.3; MS (ESI) m/z: 357 ([M+H]$^+$); HRMS calcd for $C_{22}H_{27}O_4$ ([M−H]$^-$) 355.1904. Found 355.1908.

4.1.12. (1*R*,2*R*,3*S*,4*S*,6*R*)-4-[3-(4-Cyclopropyl-benzyl)phenyl-6-(hydroxymethyl)cyclohexane-1,2,3-triol (5c)

4.1.12.1. (1*R*,2*R*,3*S*,4*R*,5*R*)- and (1*S*,2*R*,3*S*,4*R*,5*R*)-2,3,4-Tris(benzyloxy)-1-[3-(4-(benzyloxy)benzyl)phenyl-5-(benzyloxy)methyl] cyclohexanol (11). In a nitrogen stream, to a solution of 1-bromo-3-(4-benzyloxybenzyl)benzene (**10c**) (753 mg, 2.1 mmol) in THF (10 mL) was added 2.5 M *n*BuLi in hexane solution (0.8 mL, 2.0 mmol) dropwise at −78 °C and the reaction mixture was stirred at the same temperature for 10 min. To this solution was added a solution of **8** (715 mg, 1.33 mmol) in THF (2 mL) dropwise at −78 °C and the resulting solution was stirred for 30 min. The reaction was stopped by addition of satd. NH_4Cl aq solution. The obtained solution was extracted with AcOEt, and the organic layer was washed with water and then dried over anhydrous $MgSO_4$. The solvent was concentrated, and the obtained residue was purified by silica gel column chromatography (AcOEt/hexane = 1:5 to 1:1) to obtain a less polar compound (295 mg, 27%) and a more polar compound (670 mg, 62%).

(More polar): R_f 0.28 (AcOEt/hexane = 1:4); ^{1}H NMR (CDCl3) δ: 1.40–1.60 (2H, m), 1.82–1.92 (1H, m), 2.39 (1H, dd, J = 13.8, 3.6 Hz), 2.56 (1H, s), 3.36 (1H, dd, J = 8.9, 2.5 Hz), 3.54–3.76 (3H, m), 3.90 (2H, s), 3.92–3.98 (1H, m), 4.41 (2H, s), 4.49 (1H, d, J = 13.4 Hz), 4.70–4.90 (6H, m), 5.02 (1H, d, J = 11.7 Hz), 6.81–6.86 (2H, m), 7.06 (2H, d, J = 8.6 Hz), 7.15–7.38 (27H, m), 7.54–7.66 (2H, m).

4.1.12.2. (1*R*,2*R*,3*S*,4*S*,6*R*)-4-[3-(4-Hydroxybenzyl)phenyl-6-(hydroxymethyl)cyclohexane-1,2,3-triol (12). To a solution of the obtained more polar compound (659 mg, 0.81 mmol) in THF (2 mL) and MeOH (10 mL) was added 20% $Pd(OH)_2$ (200 mg) and the mixture was stirred under hydrogen atmosphere for 64 h. After filtering the catalyst, the filtrate was concentrated under reduced pressure. The obtained residue was purified by silica gel column chromatography (MeOH/CH_2Cl_2 = 1:10) to obtain the title compound (176 mg, 63%) as a colorless oil. ^{1}H NMR (CD_3OD) δ: 1.47 (1H, t, J = 13.2 Hz), 1.61–1.78 (1H, m), 1.85 (1H, dt, J = 13.5, 3.7 Hz), 2.52–2.68 (1H, m), 3.32–3.40 (2H, m), 3.50–3.66 (2H, m), 3.76–3.84 (1H, m), 3.88 (2H, s), 6.68–6.74 (2H, m), 7.01- 7.32 (6H, m).

4.1.12.3. 4-{3-[(3a*S*,4*S*,5a*R*,9a*R*,9b*R*)-2,2,8,8-Tetramethyl-hexahydro[1,3]dioxolo[4',5':3,4]benzo-[1,2-*d*][1,3]dioxin-4-yl]benzyl}phenol (13). To a solution of the above obtained compound (47 mg, 0.136 mmol) in DMF (1 mL) were added 2,2-dimethoxypropane (142 mg, 1.36 mmol) and *p*-toluenesulfonic acid hydrate (2 mg) at 0 °C. The mixture was stirred at rt for 30 min, and the reaction was stopped by addition of satd. $NaHCO_3$ aq solution. The mixture was extracted with AcOEt, and the organic layer was washed with water and dried over anhydrous $MgSO_4$. The solvent was concentrated, and the obtained residue was purified by PTLC (AcOEt/hexane = 1:4) to obtain the title compound (34 mg, 59%). ^{1}H NMR ($CDCl_3$) δ: 1.11–1.24 (1H, m), 1.42 (6H, s), 1.46 (3H, s), 1.53 (3H, s), 1.73 (1H, dt, J = 13.7, 3.9 Hz), 1.80–1.93 (1H, m), 2.94 (1H, dt, J = 11.0, 4.0 Hz), 3.59–3.92 (7H, m), 4.85 (1H, s), 6.71–6.76 (2H, m), 7.00–7.08 (5H, m), 7.19–7.23 (1H, m) .

4.1.12.4. 4-{3-[(3a*S*,4*S*,5a*R*,9a*R*,9b*R*)-2,2,8,8-Tetramethylhexahydro-3a*H*-[1,3]dioxolo[4',5':5,6]benzo [1,2-*d*][1,3]dioxin-4-yl]benzyl}phenyl trifluoromethane sulfonate (14). To a solution of the obtained compound (34 mg, 0.08 mmol) in CH_2Cl_2 (0.8 mL) were added pyridine (15 mg, 0.19 mmol), 2-[*N*,*N*-bis(trifluoromethane-sulfonyl) amino]pyridine (34 mg, 0.096 mmol), and DMAP (1 mg) at 0 °C. The reaction mixture was stirred at rt for 30 min, and the reaction was stopped by addition of water. The mixture was extracted with CH_2Cl_2 and the organic layer was washed with water and dried over anhydrous $MgSO_4$. The solvent was concentrated, and the obtained residue was purified by PTLC (AcOEt/hexane = 1:4) to obtain the title compound (37 mg, 83%). ^{1}H NMR ($CDCl_3$) δ: 1.11–1.24 (1H, m), 1.42 (6H, s), 1.43 (3H, s), 1.53 (3H, s), 1.74 (1H, dt, J = 13.7, 3.9 Hz), 1.80–1.93 (1H, m), 2.96 (1H, dt, J = 11.1, 3.6 Hz), 3.59–3.92 (5H, m), 3.98 (2H, s), 7.00–7.03 (2H, m), 7.10–7.35 (6H, m).

4.1.12.5. (3a*S*,4*S*,5a*R*,9a*R*,9b*R*)-4-[3-(4-Cyclopropylbenzyl)phenyl]-2,2,8,8-tetramethylhexahydro-3a*H*-[1,3]dioxolo[4',5':5,6] benzo [1,2-*d*][1,3]dioxine (15). In a nitrogen stream, to a solution of the obtained compound (38 mg, 0.068 mmol) in toluene (0.5 mL)-water (0.017 mL) were added K_3PO_4 (65 mg, 0.31 mmol), NaBr (7 mg, 0.068 mmol), cyclopropylboronic acid (9 mg, 0.10 mmol) and $Pd(PPh_3)_4$ (8.0 mg, 0.007 mmol). The reaction mixture was heated at 100 °C for 6 h. The reaction mixture was cooled to 0 °C and water was added thereto. The resulting solution was extracted with AcOEt, and the organic layer was washed with brine and dried over anhydrous $MgSO_4$. The solvent was concentrated, and the obtained residue was purified by PTLC (AcOEt/hexane = 1:4) to obtain the title compound (10 mg, 33%). ^{1}H NMR ($CDCl_3$) δ: 0.62–0.68 (2H, m), 0.89–0.96 (2H, m), 1.10–1.24 (1H, m), 1.42 (3H, s), 1.43 (3H, s), 1.46 (3H, s), 1.54 (3H, s), 1.74 (1H, dt, J = 13.7, 3.9 Hz), 1.80–1.93 (2H, m), 2.94 (1H, dt, J = 10.9, 3.6 Hz), 3.59–3.96 (8H, m), 6.96–7.07 (7H, m), 7.16–7.25 (1H, m).

4.1.12.6. (1*R*,2*R*,3*S*,4*S*,6*R*)-4-[3-(4-Cyclopropylbenzyl) phenyl]-6-(hydroxymethyl) -cyclohexane-1,2,3-triol (5c). To a solution of the obtained compound (10 mg, 0.022 mmol) in 1,4-dioxane (0.2 mL) was added 2 N HCl aq solution (0.2 mL) at 0 °C dropwise. The reaction mixture was stirred for 3 h, and then sat. $NaHCO_3$ aq solution was added thereto. The resulting solution was extracted with CH_2Cl_2 and the organic layer was washed with brine and dried over anhydrous $MgSO_4$. The solvent was concentrated, and the obtained residue was purified by PTLC (MeOH/CH_2Cl_2 = 1:10) to obtain the title compound (4.5 mg, 51%) as a colorless amorphous. ^{1}H NMR (CD_3OD) δ: 0.63–0.68 (2H, m), 0.91–0.98 (2H, m), 1.42–1.52 (1H, m), 1.62–1.78 (1H, m), 1.80–1.94 (2H, m), 2.54–2.66 (1H, m), 3.33–3.40 (2H, m), 3.50–3.68 (2H, m), 3.79 (1H, dd, J = 10.9, 3.9 Hz), 3.93 (2H, s), 6.85–7.16 (7H, m), 7.21–7.26 (1H,

m); MS (ESI) *m*/*z*: 386 ([M+H_2O]$^+$); HRMS calcd for $C_{23}H_{27}O_4$ ([M−H]$^-$) 367.1904. Found 367.1907.

4.1.13. (1*R*,2*R*,3*S*,4*S*,6*R*)-4-[3-(4-Ethylbenzyl)-4-methoxyphenyl]-6-(hydroxymethyl)cyclohexane-1,2,3-triol (5d)

4.1.13.1. (1*R*,2*R*,3*S*,4*R*,5*R*)- and (1*S*,2*R*,3*S*,4*R*,5*R*)-2,3,4-Tris(benzyloxy)-5-[(benzyloxy)methyl]-1-[3-(4-ethylbenzyl)-4-methoxyphenyl]cyclohexanol. In a nitrogen stream, to a solution of 4-bromo-2-(4-ethylbenzyl)-1-methoxybenzene (**10d**) (908 mg, 2.98 mmol) in THF (10 mL) was added 2.70 M *n*BuLi in hexane solution (1.03 mL) dropwise at -78 °C and the reaction mixture was stirred for 10 min. To this solution was added a solution of **8** (1.00 g, 1.86 mmol) in THF (2 mL) at -78 °C dropwise and the resulting solution was stirred for 30 min. The reaction was stopped by addition of satd. NH_4Cl aq solution. The obtained solution was extracted with AcOEt, and the organic layer was washed with water and then dried over anhydrous $MgSO_4$. The solvent was concentrated, and the obtained residue was purified by silica gel column chromatography (AcOEt/hexane = 1:5 to 1:1) to obtain a less polar compound (320 mg, 23%) and a more polar compound (890 mg, 64%).

(Less polar): R_f 0.38 (AcOEt/hexane = 1:4); ^{1}H NMR (CDCl3) δ: 1.17 (3H, t, *J* = 7.6 Hz), 1.80–2.00 (2H, m), 2.20–2.36 (1H, m), 2.55 (2H, d, *J* = 7.6 Hz), 2.91 (1H, d, *J* = 2.0 Hz), 3.35–3.42 (1H, m), 3.65–4.05 (10H, m), 4.41–4.45 (3H, m), 4.59 (1H, d, *J* = 10.7 Hz), 4.81 (1H, d, *J* = 10.7 Hz), 4.87 (1H, d, *J* = 10.7 Hz), 4.90 (1H, d, *J* = 10.7 Hz), 6.78–6.84 (3H, m), 6.99–7.20 (6H, m), 7.20–7.40 (18H, m).

(More polar): R_f 0.31 (AcOEt/hexane = 1:4); ^{1}H NMR (CDCl3) δ: 1.12 (3H, t, *J* = 7.6 Hz), 1.40–1.54 (2H, m), 1.78–1.88 (1H, m), 2.35 (1H, dd, *J* = 13.7, 3.3 Hz), 2.43 (1H, s), 2.47 (2H, q, *J* = 7.6 Hz), 3.34 (1H, dd, *J* = 8.9, 0.9 Hz), 3.53–3.72 (4H, m), 3.80 (3H, s), 3.86–3.93 (2H, m), 4.41 (2H, s), 4.49 (1H, d, *J*=10.7 Hz), 4.67–4.79 (2H, m), 4.83 (1H, d, *J* = 10.7 Hz), 5.02 (1H, d, *J* = 11.7 Hz), 6.78 (1H, d, *J* = 8.4 Hz), 7.00 (2H, d, *J* = 7.9 Hz), 7.10 (2H, d, *J* = 7.9 Hz), 7.14–7.20 (2H, m), 7.21–7.40 (18H, m), 7.56–7.63 (2H, m).

4.1.13.2. ((((1*S*,2*R*,3*R*,4*R*,6*S*)-4-[(Benzyloxy)methyl]-6-[3-(4-ethylbenzyl)-4-methoxyphenyl]cyclohexane-1,2,3-triyl)tris(oxy))tris(methylene))tribenzene. To a solution of the above more polar compound (124 mg, 0.16 mmol) in CH_2Cl_2 (2 mL) were added Et_3SiH (0.39 mL, 2.44 mmol) and trifluoroacetic acid (0.12 mL, 1.6 mmol) at −5 °C. The reaction mixture was stirred for 1 h, and then satd. $NaHCO_3$ aq solution was added thereto. The resulting mixture was extracted with CH_2Cl_2 and the organic layer was washed with brine and then dried over anhydrous $MgSO_4$. The solvent was concentrated, and the obtained residue was purified by silica gel column chromatography (AcOEt/hexane = 1:9) to obtain the title compound (more polar, 10 mg, 8%) and a diastereoisomer (less polar, 93 mg, 77%). R_f 0.68 (AcOEt/hexane = 1:4); ^{1}H NMR ($CDCl_3$) δ: 1.17 (3H, t, *J* = 7.6 Hz), 1.58–1.96 (3H, m), 2.56 (2H, q, *J* = 7.6 Hz), 2.55–2.70 (1H, m), 3.43–3.62 (5H, m), 3.78–4.00 (6H, m), 4.43 (2H, s), 4.44 (1H, d, *J* = 9.9 Hz), 4.56 (1H, d, *J* = 10.8 Hz), 4.82–4.93 (3H, m), 6.75–6.82 (3H, m), 6.99–7.18 (9H, m), 7.20–7.38 (15H, m).

4.1.13.3. (1*R*,2*R*,3*S*,4*S*,6*R*)-4-[3-(4-Ethylbenzyl)-4-methoxyphenyl]-6-(hydroxymethyl)cyclohexane-1,2,3-triol. To a solution of the obtained compound (10 mg, 0.013 mmol) in THF (0.2 mL) and MeOH (1 mL) was added 20% $Pd(OH)_2$ (10 mg) and the mixture was stirred under hydrogen atmosphere at rt for 13 h. After filtering the catalyst, the filtrate was concentrated under reduced pressure. The obtained residue was purified by PTLC ($MeOH/CH_2Cl_2$ = 1:10) to obtain the title compound (3.9 mg, 75%) as a colorless amorphous. ^{1}H NMR (CD_3OD) δ: 1.23 (3H, t, *J* = 7.4 Hz), 1.34–1.48 (1H, m), 1.60–1.76 (1H, m), 1.83 (1H, dt, *J* = 13.5, 3.5 Hz), 2.48–2.66 (3H, m), 3.30–3.38 (2H, m), 3.42–3.52 (1H, m), 3.58–3.65 (1H, m), 3.77 (1H, d, *J* = 4.0 Hz), 3.82 (3H, s), 3.92 (2H, s), 6.91 (1H, d, *J* = 8.2 Hz), 7.02–7.14 (6H, m); MS (ESI) *m*/*z*: 404 ([M+H_2O]$^+$); HRMS calcd for $C_{23}H_{29}O_5$ ([M−H]$^-$) 385.2010. Found 385.2011.

4.1.13.4. (1*R*,2*R*,3*S*,4*R*,5*R*)-1-[3-(4-Ethylbenzyl)-4-methoxyphenyl]-5-(hydroxymethyl)cyclohexane-1,2,3,4-tetraol (5e). To a solution of the less polar compound which was obtained at 1st step as described in Section 4.1.13 (50 mg, 0.067 mmol) in THF (0.5 mL) and MeOH (2 mL) was added 20% $Pd(OH)_2$ (10 mg) and the mixture was stirred under hydrogen atmosphere at room temperature for 15 h. After filtering the catalyst, the filtrate was concentrated under reduced pressure. The obtained residue was purified by PTLC ($MeOH/CH_2Cl_2$ = 1:10) to obtain the title compound (20 mg, 74%) as a colorless amorphous. ^{1}H NMR (CD_3OD) δ: 1.22 (3H, t, *J* = 7.6 Hz), 1.65–1.84 (2H, m), 2.01–2.14 (1H, m), 2.60 (2H, q, *J* = 7.6 Hz), 3.39–3.46 (1H, m), 3.62–3.75 (4H, m), 3.82 (3H, s), 3.94 (2H, s), 6.94 (1H, d, *J* = 8.4 Hz), 7.06 (2H, d, *J* = 8.2 Hz), 7.13 (2H, d, *J* = 8.2 Hz), 7.30–7.38 (2H, m); ^{13}C NMR (CD_3OD) δ: 157.5, 142.7, 139.9, 139.5, 130.5, 129.8, 128.6, 128.6, 125.4, 111.2, 78.5, 77.8, 76.9, 75.0, 64.2, 55.9, 41.0, 40.2, 36.7, 29.4, 16.3; MS (ESI+): 420 [M+H_2O]$^+$; HRMS calcd for $C_{23}H_{29}O_6$ ([M-H]$^-$) 401.1959. Found 401.1956.

4.1.14. (1*R*,2*R*,3*S*,4*S*,6*R*)-4-[5-(4-Ethylbenzyl)-2-methoxyphenyl]-6-(hydroxymethyl)cyclohexane-1,2,3-triol (5f)

This compound was prepared from **8** and **10f** in the same manner as described in Section 4.1.13. The total yield was 3%. Colorless amorphous. ^{1}H NMR (CD_3OD) δ: 1.18 (3H, t, *J* = 7.6 Hz), 1.24–1.50 (1H, m), 1.54–1.85 (2H, m), 2.57 (2H, q, *J* = 7.6 Hz), 2.90–3.18 (1H, m), 3.24–3.34 (2H, m), 3.54 (1H, dd, *J* = 10.7, 5.9 Hz), 3.60–3.78 (2H, m), 3.76 (3H, s), 3.83 (2H, s), 6.82 (1H, d, *J* = 8.4 Hz), 6.96 (1H, dd, *J* = 8.4, 2.0 Hz), 7.01–7.11 (5H, m); ^{13}C NMR (CD_3OD) δ: 157.4, 142.9, 140.4, 134.9, 131.9, 129.8, 128.8, 128.8, 128.5, 111.9, 81.4, 76.0, 75.2, 64.5, 56.0, 45.5, 41.8, 41.8, 33.2, 29.4, 16.3; MS (ESI) *m*/*z*: 404 ([M+H_2O]$^+$); HRMS calcd for $C_{23}H_{29}O_5$ ([M−H]$^-$) 385.2010. Found 385.2011.

4.1.15. (1*R*,2*R*,3*S*,4*R*,5*R*)-1-[5-(4-Ethylbenzyl)-2-methoxyphenyl]-5-(hydroxymethyl)cyclohexane-1,2,3,4-tetraol (5g)

This compound was prepared from **8** and **10f** in the same manner as described in Section 4.1.14. The total yield was 47%. Colorless amorphous. ^{1}H NMR (CD_3OD) δ: 1.13 (3H, t, *J* = 7.6 Hz), 1.56 (1H, dd, *J* = 14.1, 3.9 Hz), 1.94–2.04 (1H, m), 2.24 (1H, dd, *J* = 13.9, 13.2 Hz), 2.51 (2H, q, *J* = 7.5 Hz), 3.24–3.37 (1H, m), 3.57–3.63 (3H, m), 3.74 (3H, s), 3.80–3.90 (2H, m), 4.22 (1H, d, *J* = 9.1 Hz), 6.80 (1H, d, *J* = 8.4 Hz), 6.95–7.01 (5H, m), 7.41 (1H, d, *J* = 1.2 Hz); ^{13}C NMR (CD_3OD) δ: 156.0, 142.9, 140.5, 134.9, 134.3, 129.8, 129.5, 129.3, 128.8, 112.5, 78.0, 77.5, 75.7, 75.3, 64.5, 55.7, 42.0, 40.9, 36.2, 29.5, 16.3; MS (ESI) *m*/*z*: 425 ([M+Na]$^+$); HRMS calcd for $C_{23}H_{29}O_6$ ([M−H]$^-$) 401.1959. Found 401.1958.

4.1.16. (1*R*,2*R*,3*S*,4*S*,6*R*)-4-[2-Ethoxy-5-(4-ethylbenzyl)phenyl]-6-(hydroxymethyl)cyclohexane-1,2,3-triol (5h)

4.1.16.1. (1*R*,2*R*,3*S*,4*R*,5*R*)- and (1*S*,2*R*,3*S*,4*R*,5*R*)-2,3,4-Trisbenzyloxy-5-benzyloxy-methyl-1-[2-ethoxy-5-(4-ethylbenzyl)phenyl]-cyclohexanol. In a nitrogen stream, to a solution of 2-bromo-1-ethoxy-4-(4-ethylbenzyl) benzene (**10h**) (1.18 g, 3.70 mmol) in THF (9 mL) was added 1.59 M *n*BuLi in hexane solution (2.30 mL, 3.66 mmol) dropwise at -78 °C and the reaction mixture was stirred for 1 h. Then, to this solution was added a solution of **8** (1.50 g, 2.80 mmol) in THF (4.5 mL) dropwise at -78 °C. After being stirred for 10 min, the reaction was stopped by addition of satd. NH_4Cl aq solution. The resulting mixture was extracted with AcOEt, and the organic layer was washed with brine and then dried over anhydrous $MgSO_4$. The solvent was concentrated, and the obtained residue was purified by silica gel column chromatography

(AcOEt/hexane = 1:4) to obtain a less polar compound (884 mg, 41%) and a more polar compound (740 mg, 34%).

(Less polar): R_f 0.39 (AcOEt/hexane = 1:4); ^{1}H NMR ($CDCl_3$) δ: 1.23 (3H, t, J = 7.6 Hz), 1.37 (3H, t, J = 7.0 Hz), 1.70 (1H, dd, J = 14.3, 4.1 Hz), 2.20–2.35 (1H, m), 2.62 (2H, q, J = 7.6 Hz), 2.67–2.82 (1H, m), 3.00–3.30 (1H, br), 3.43 (1H, dd, J = 8.8, 2.0 Hz), 3.71 (1H, dd, J = 9.8, 9.8 Hz), 3.83 (1H, dd, J = 8.9, 4.0 Hz), 3.86–4.10 (4H, m), 3.91 (2H, s), 4.44 (2H, s), 4.48 (1H, d, J = 10.3 Hz), 4.58 (1H, br), 4.63 (1H, d, J = 10.3 Hz), 4.85 (1H, d, J = 11.0 Hz), 4.88 (1H, d, J = 11.0 Hz), 4.95 (1H, d, J = 10.8 Hz), 6.75 (1H, d, J = 8.4 Hz), 6.79 (2H, d, J = 7.0 Hz), 7.00–7.40 (23H, m), 7.53 (1H, d, J = 2.0 Hz).

(More polar): R_f 0.32 (AcOEt/hexane = 1:4); ^{1}H NMR ($CDCl_3$) δ: 1.12 (3H, t, J = 7.6 Hz), 1.30 (3H, t, J = 7.2 Hz), 1.40–1.55 (1H, m), 1.76 (1H, dd, J = 13.3, 13.3 Hz), 2.50 (2H, q, J = 7.6 Hz), 2.74 (1H, dd, J = 13.7, 3.2 Hz), 3.38 (1H, dd, J = 9.0, 2.8 Hz), 3.54 (1H, dd, J = 9.0, 5.3 Hz), 3.64 (1H, dd, J = 10.8, 8.7 Hz), 3.86 (1H, dd, J = 9.5, 9.5 Hz), 3.89 (2H, s), 4.00–4.10 (2H, m), 4.39 (2H, s), 4.53 (1H, d, J = 11.0 Hz), 4.64–4.72 (3H, m), 4.77 (1H, d, J = 11.7 Hz), 4.79 (1H, d, J = 10.5 Hz), 4.87 (1H, d, J = 10.8 Hz), 4.98 (1H, d, J = 11.7 Hz), 6.98–7.42 (26H, m), 7.67 (1H, d, J = 1.8 Hz).

4.1.16.2. (1*R*,2*R*,3*S*,4*S*,6*R*)-4-[2-Ethoxy-5-(4-ethylbenzyl)phenyl]-6-(hydroxymethyl)cyclohexane-1,2,3-triol. To a solution of the obtained more polar compound (402 mg, 0.517 mmol) in THF (4 ml) and methanol (2 mL) was added 20% $Pd(OH)_2$ (78 mg) and the mixture solution was stirred at rt under hydrogen atmosphere for 19 h. After filtering the catalyst, the filtrate was concentrated under reduced pressure. The obtained residue was purified by silica gel column chromatography ($MeOH/CH_2Cl_2$ = 1:10) to obtain the title compound (42 mg, 20%) as a colorless amorphous. ^{1}H NMR (CD_3OD) δ: 1.18 (3H, t, J = 7.6 Hz), 1.25–1.50 (1H, m), 1.38 (3H, t, J = 7.0 Hz), 1.62 (1H, m), 1.78 (1H, m), 2.57 (2H, q, J = 7.6 Hz), 2.95–3.15 (1H, br), 3.25–3.33 (2H, m), 3.54 (1H, dd, J = 10.8, 6.1 Hz), 3.73 (1H, dd, J = 10.8, 3.8 Hz), 3.65–3.85 (1H, m), 3.83 (2H, s), 4.00 (2H, q, J = 7.0 Hz), 6.81 (1H, d, J = 8.3 Hz), 6.94 (1H, dd, J = 8.3, 2.1 Hz), 7.04 (1H, d, J = 2.1 Hz), 7.06 (4H, m); ^{13}C NMR (CD_3OD) δ: 156.7, 142.9, 140.4, 134.8, 132.0, 129.8, 129.8, 128.8, 128.5, 113.1, 81.4, 75.8, 75.3, 64.9, 64.6, 49.2, 45.5, 41.8, 33.0, 29.4, 16.3, 15.3; MS (ESI) m/z: 401 [M+H]; HRMS calcd for $C_{24}H_{31}O_5$ ([M−H]$^-$) 399.2166. Found 399.2163.

4.1.17. (1*R*,2*R*,3*S*,4*R*,5*R*)-1-[2-Ethoxyl-5-(4-ethylbenzyl)phenyl]-5-(hydroxymethyl)cyclohexane-1,2,3,4-tetraol (5i)

To a solution of the less polar compound (600 mg, 0.772 mmol) in THF (6 mL) and MeOH (3 mL) was added 20% $Pd(OH)_2$ (60 mg) and the mixture was stirred under hydrogen atmosphere for 2.5 h. After filtering the catalyst, the filtrate was concentrated under reduced pressure. The obtained residue was purified by silica gel column chromatography ($MeOH/CH_2Cl_2$ = 1:10) to obtain the title compound (197 mg, 61%) as a colorless amorphous. ^{1}H NMR (CD_3OD) δ: 1.18 (3H, t, J = 7.6 Hz), 1.43 (3H, t, J = 7.0 Hz), 1.60 (1H, dd, J = 14.3, 4.1 Hz), 2.01 (1H, m), 2.43 (1H, dd, J = 14.0, 13.0 Hz), 2.57 (2H, q, J = 7.5 Hz), 3.39 (1H, dd, J = 10.5, 9.3 Hz), 3.60–3.68 (3H, m), 3.85 (2H, s), 4.03 (2H, m), 4.34 (1H, d, J = 9.2 Hz), 6.82 (1H, d, J = 8.2 Hz), 6.99 (1H, dd, J = 8.4, 2.3 Hz), 7.04–7.14 (4H, m), 7.45 (1H, d, J = 2.3 Hz); ^{13}C NMR (CD_3OD) δ: 155.2, 142.9, 140.5, 134.7, 134.3, 129.8, 129.4, 129.2, 128.8, 113.2, 77.9, 77.5, 75.7, 75.2, 64.9, 64.5, 42.0, 41.0, 36.2, 29.4, 16.3, 15.3; MS (ESI) m/z: 434 ([M+H_2O]$^+$); HRMS calcd for $C_{24}H_{31}O_6$ ([M−H]$^-$) 415.2115. Found 415.2113.

4.1.18. (1*R*,2*R*,3*S*,4*S*,6*R*)-4-[5-(4-Ethylbenzyl)-2-methylphenyl]-6-(hydroxymethyl)cyclohexane-1,2,3-triol (5j)

This compound was prepared from **8** and **10j** in the same manner as described in Section 4.1.13. The total yield was 25%. Colorless amorphous. ^{1}H NMR (CD_3OD) δ: 1.19 (3H, t, J = 7.6 Hz), 1.24–1.37 (1H, m), 1.59–1.81 (2H, m), 2.30 (3H, s), 2.57 (2H, q, J = 7.6 Hz), 2.93 (1H, m), 3.34 (2H, m), 3.50–3.63 (2H, m), 3.75 (1H, dd, J = 10.7, 4.2 Hz), 3.86 (2H, s), 6.87 (1H, d, J = 7.6 Hz), 7.03 (1H, dd, J = 7.6, 2.1 Hz), 7.07 (4H, m), 7.11 (1H, d, J = 2.1 Hz); ^{13}C NMR (CD_3OD) δ: 142.9, 142.3, 140.5, 140.2, 135.3, 131.4, 129.8, 128.8, 127.5, 127.2, 81.3, 77.3, 75.1, 64.4, 45.6, 44.4, 42.3, 34.0, 29.4, 19.5, 16.3; MS (ESI) m/z: 369 ([M−H]$^-$); HRMS calcd for $C_{23}H_{29}O_4$ ([M−H]$^-$) 369.2060. Found 369.2058.

4.1.19. (1*R*,2*R*,3*S*,4*R*,5*R*)-1-[5-(4-Ethylbenzyl)-2-methylphenyl]-5-(hydroxymethyl)cyclohexane-1,2,3,4-tetraol (5k)

This compound was prepared from **8** and **10j** in the same manner as described in Section 4.1.14. The total yield was 20%. Colorless amorphous. ^{1}H NMR (CD_3OD) δ: 1.19 (3H, t, J = 7.6 Hz), 1.70 (1H, m), 1.94 (1H, m), 2.05 (1H, m), 2.53 (3H, s), 2.58 (2H, q, J = 7.6 Hz), 3.40 (1H, d, J = 9.2 Hz), 3.67 (2H, d, J = 4.6 Hz), 3.73 (1H, d, J = 9.2 Hz), 3.87 (2H, s), 4.01 (1H, d, J = 9.2 Hz), 6.91 (1H, dd, J = 7.6, 1.5 Hz), 7.01 (1H, d, J = 7.6 Hz), 7.07 (4H, m), 7.42 (1H, d, J = 1.5 Hz); ^{13}C NMR (CD_3OD) δ: 144.4, 142.9, 140.2, 140.0, 134.3, 134.0, 129.8, 128.8, 128.5, 128.3, 78.5, 78.0, 76.7, 74.8, 64.1, 42.3, 40.9, 37.8, 29.4, 22.5, 16.3; MS (ESI) m/z: 409 ([M+Na]$^+$); HRMS calcd for $C_{23}H_{29}O_5$ ([M−H]$^-$) 385.2010. Found 385.2009.

4.1.20. (1*R*,2*R*,3*S*,4*S*,6*R*)-4-[5-(4-Ethylbenzyl)-2,4-dimethoxyphenyl]-6-(hydroxymethyl)cyclohexane-1,2,3-triol (5l)

To a solution of **10l** (840 mg, 2.51 mmol) in Et_2O (1 mL) was added 1.58 M *n*BuLi in hexane solution (1.60 mL, 2.53 mmol) dropwise at −78 °C and the mixture solution was stirred for 15 min. Then, to this solution was added a solution of **8** (1.03 g, 1.93 mmol) in THF (3 mL) dropwise at −78 °C. The reaction mixture was stirred at −78 °C for 2.5 h and then at −40 °C for 0.5 h. To the reaction mixture was added satd. NH_4Cl aq solution and the mixture was extracted with AcOEt. The organic layer was washed with brine, and then dried over anhydrous $MgSO_4$. The solvent was concentrated and the obtained residue was purified by silica gel chromatography (AcOEt/hexane = 1:5 to 1:2) to obtain a less polar compound (1100 mg, 72%) and a more polar compound (108 mg, 7%).

To a solution of the above less polar compound (747 mg, 0.943 mmol) in CH_2Cl_2 (9.5 mL) were added Et_3SiH (1.51 mL, 9.43 mmol) and $BF_3{\cdot}OEt_2$ (1.19 mL, 9.43 mmol) at −40 °C. The reaction mixture was stirred at −40 °C for 11 h and warmed up to −10 °C for 1 h, and then satd. $NaHCO_3$ aq solution was added thereto. The resulting mixture was extracted with CH_2Cl_2 and the organic layer was washed with brine and then dried over anhydrous $MgSO_4$. The solvent was concentrated and the obtained residue was purified by silica gel column chromatography (AcOEt/hexane = 1:4) to obtain a more polar compound (164 mg, 22%) and a less polar compound (475 mg, 65%) as a diastereoisomer.

To a solution of the above more polar compound (164 mg, 0.211 mmol) in THF (4 mL) and MeOH (2 mL) was added 20% $Pd(OH)_2$ (55 mg) and the mixture was stirred under hydrogen atmosphere at rt for 40 min. After filtering the catalyst, the filtrate was concentrated under reduced pressure. The obtained residue was purified by PTLC ($MeOH/CH_2Cl_2$ = 1:10) to obtain the title compound (81 mg, 92%) as a colorless amorphous. ^{1}H NMR (CD_3OD) δ: 1.17 (3H, t, J = 7.6 Hz), 1.24–1.48 (1H, m), 1.52–1.82 (2H, m), 2.56 (2H, d, J = 7.6 Hz), 2.86–3.08 (1H, m), 3.24–3.29 (1H, m), 3.48–3.67 (2H, m), 3.68–3.84 (2H, m), 3.79 (3H, s), 3.81 (3H, s), 6.56 (1H, s), 6.93 (1H, s), 7.01 (2H, d, J = 8.6 Hz), 7.05 (2H, d, J = 8.6 Hz); ^{13}C NMR (CD_3OD) δ: 158.5, 157.9, 142.5, 140.4, 130.6, 129.6, 128.5, 123.5, 122.7, 96.9, 81.5, 76.2, 75.3, 64.6, 56.2, 56.0, 45.5, 35.9, 34.9, 33.2, 29.4, 16.3; MS (ESI) m/z: 416 ([M]$^+$); HRMS calcd for $C_{24}H_{31}O_6$ ([M−H]$^-$) 415.2115. Found 415.2118.

4.1.21. (1*R*,2*R*,3*S*,4*R*,5*R*)-1-[5-(4-Ethylbenzyl)-2,4-dimethoxyphenyl]-5-(hydroxymethyl)cyclohexane-1,2,3,4-tetraol (5m)

This compound was prepared from **8** and **10l** in the same manner as described in Section 4.1.14. The total yield was 52%. Colorless amorphous. ^{1}H NMR (CD_3OD) δ: 1.17 (3H, t, J = 7.6 Hz), 1.61 (1H, dd, J = 13.9, 3.8 Hz), 1.90–2.10 (1H, m), 2.22 (1H, dd, J = 13.9, 13.2 Hz), 2.55 (2H, q, J = 7.6 Hz), 3.32–3.42 (1H, m), 3.58–3.70 (3H, m), 3.76–3.88 (2H, m), 3.80 (3H, s), 3.84 (3H, s), 4.19 (1H, d, J = 9.1 Hz), 6.58 (1H, s), 7.00 (2H, d, J = 8.2 Hz), 7.06 (2H, d, J = 8.2 Hz), 7.35 (1H, s); ^{13}C NMR (CD_3OD) δ: 158.6, 156.9, 142.5, 140.4, 130.6, 129.7, 128.5, 126.0, 122.4, 97.3, 77.9, 77.2, 76.0, 75.3, 64.6, 56.1, 55.9, 40.8, 36.6, 36.0, 29.4, 16.3; MS (ESI) m/z: 455 ([M+Na]$^+$); HRMS calcd for $C_{24}H_{31}O_7$ ([M−H]$^-$) 431.2064. Found 431.2059.

4.2. Molecular modeling

All molecular modeling procedures were performed using Molecular Operating Environment (MOE) ver.2010.10.[14] Six known SGLT2 inhibitors and our three compounds were constructed and minimized with use of MMFF94 force field.[16] Superposed models of selected inhibitors were derived by the FlexibleAlignment module of MOE in which H-bond donor and acceptor, hydrophobe, and aromaticity were assigned for similarity terms. Although several energetically equivalent models were generated because of 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 Structure Database ID: CEWWAC01), was chosen for further discussion. Global minimum energy of each inhibitor was estimated by the LowModeMD search method. The energy difference of each compound aligned was within 5 kcal/mol from the global minimum.

4.3. Biology

4.3.1. 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 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.).

4.3.2. CYP3A4 inhibition assay[10]

The evaluation of CYP3A inhibition was carried out on 96-well microtiter plates using MultiPROBE™ II_{EX} Liquid Handling Robotics (PerkinElmerJapan, Yokohama, Japan). Fluorescence detection of the metabolite of 7-benzyloxy–4-(trifluoromethyl)-coumarin (BFC) was performed using a Spectra Max Gemini microplate reader (Molecular Devices, Sunnyvale, CA, USA). Incubations and calculations of IC_{50} were conducted based on the method provided on the BD Biosciences website.[17] IC_{50} (−) and IC_{50} (+) represent the IC_{50} values in which the inhibitor was added to the reaction mixture simultaneously with BFC (coincubation assay) and before the addition of BFC (preincubation assay), respectively. The criteria to determine positive TDI were based on a twofold value of the coefficient of the variation (CV) of the IC_{50} (−) of ketoconazole.

4.3.3. In vivo blood glucose-lowering test in db/db mice

Male db/db mice were purchased from CLEA Japan (Tokyo, Japan) and maintained on a regular diet (CE2, CLEA Japan). The animals received a single oral dose of **2** (30 mg/kg), **5h** and **5i** (10 and 30 mg/kg) formulated as homogenous suspensions in 0.5% carboxymethyl-cellulose sodium salt via oral gavage. Blood samples were collected just before and at 1, 2, 4, 6, and 8 h after dosing. Blood glucose levels were measured by the hexokinase method (Autosera S GLU, Daiichi Pure Chemicals, Japan).

4.3.4. Pharmacokinetics studies

The db/db mice were administered a drug, and subjected to blood sampling under the same conditions as the animals used for the in vivo blood glucose- lowering test. Animals received a single oral administration of **2** and **5h** at a dose of 10 mg/kg (n = 6). Blood samples were collected at 0.25, 0.5, 1, 2, 4, 6, and 8 h after administration. The drug concentration in plasma sample was measured using an LC-MS/MS system after deproteinization. LC-MS/MS analysis was performed using a Shimadzu LC-10AD pump and an Applied Biosystems/MDS Sciex API-300 mass spectrometer with a TurboIonSpray source. Chromatographic separation was achieved using a Shiseido CAPCELL PAK C18 column (2.0 × 150 mm, 5 μm). 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 394 to 180 (**2-active**), 418 to 259 (**5h**). Non-compartmental pharmacokinetic parameters were calculated based on the averaged plasma concentration-time data using WinNonlin Professional 5.0 (Pharsight, Mountain View, CA).

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tertiary alcohol **5k** by ROESY in DMSO-d_6 shows the correlation between C(1)-OH and C(3)-H_{ax}, C(5)-H_{ax}. It means that the configuration of C(1)-OH of **5k** is also axial as described in our Figures.

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