Bioorganic & Medicinal Chemistry 19 (2011) 5334–5341

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

5a-Carba-β-D-glucopyranose derivatives as novel sodium-dependent glucose cotransporter 2 (SGLT2) inhibitors for the treatment of type 2 diabetes

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ARTICLE INFO

Article history:
Received 16 June 2011
Revised 2 August 2011
Accepted 3 August 2011
Available online 8 August 2011

Keywords:
Type 2 diabetes
SGLT2 inhibitor
Carbasugar
5a-Carba-β-D-glucopyranose
Metabolic stabilization

ABSTRACT

5a-Carba-β-D-glucopyranose derivatives were synthesized and identified as novel SGLT2-selective inhibitors. These inhibitors exhibited potent SGLT2 inhibition with high selectivity over SGLT1. Among the tested compounds, 6**f** indicated the most potent hSGLT2 inhibition and the highest selectivity over hSGLT1. Moreover, the pharmacokinetics data also showed that **6h**, which had the same aglycon structure as sergliflozin-active (**3-active**), had a threefold longer half-life time ($T_{1/2}$) than sergliflozin (**3**) with a high distribution volume in db/db mice. Subsequently, **6h** lowered blood glucose levels as much as **3** and showed longer hypoglycemic action than **3** in db/db mice.

1. Introduction

Type 2 diabetes (T2D) mellitus is a progressive metabolic disease characterized by decreased insulin secretion and increased insulin resistance, the prevalence of which 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 co-transporter 2 (SGLT2) has been attracting attention because it is uniquely responsible for reabsorbing glucose from the renal filtrate.[1] The expression site of SGLT2 is primarily the proximal convoluted tubules, whereas those of SGLT1 are small intestine, heart, brain, and renal tubules. Furthermore, SGLT1 mutation is known to cause glucose-galactose malabsorption,[2] so SGLT1 inhibition is reported to possibly cause gastrointestinal side effects.[3] From these findings, SGLT2-selective inhibitors are considered to be safer than non-selective inhibitors.

The reported SGLT2 inhibitors are classified into *O*-aryl glucopyranosides and *C*-aryl glucopyranosides by the type of glycosidic bond (Fig. 1). The natural product phlorizin (**1**) is a non-selective *O*-glycosidic type of SGLT inhibitor, and repeated subcutaneous administration of **1** lowered the blood glucose levels of diabetic rodents.[4] T-1095 (**2**), which was reported by the Tanabe group, was the first time a structural modification of phlorizin was used to discover a selective SGLT2 inhibitor.[5] However, this inhibitor was considered to be metabolically unstable because of the cleavage of the *O*-glycosidic bond by glycosidases, so a methyl carbonate prodrug was required to avoid high dosing. Subsequently, several groups have reported potent SGLT2-selective inhibitors of *O*-glycosides, such as sergliflozin (**3**)[6] and remogliflozin etabonate,[7] but these compounds have not been launched yet. On the other hand, the Bristol–Myers Squibb group has recently disclosed the discovery of a highly potent SGLT2 inhibitor, dapagliflozin (**4**),[8] which has the benefit of long-lasting action due to being a metabolically stable *C*-aryl glycosidic type. Likewise, canagliflozin (**5**)[9] has been reported by the Mitsubishi Tanabe group. Thus, *O*-type inhibitors are generally considered to be metabolically more unstable than *C*-type inhibitors making it difficult to achieve a lower dose in vivo, however, we decided to strive for the discovery of novel *O*-type SGLT2 inhibitors which are metabolically stable and effective in diabetic animal models.

One of our strategies for discovering metabolically stable novel SGLT2 inhibitors was to convert unstable *O*-glycoside to stable glucose mimics, effectively avoiding the cleavage of the *O*-glycosidic bond by glycosidases (Fig. 2). Some investigations of the structural modification of sugar analog have been reported as

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doi:10.1016/j.bmc.2011.08.005

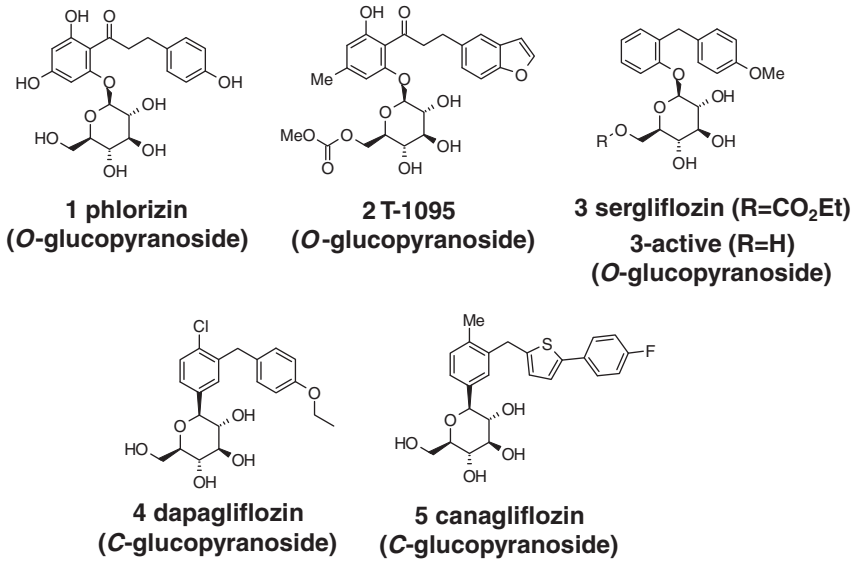


Figure 1. Structures of some known SGLT inhibitors.

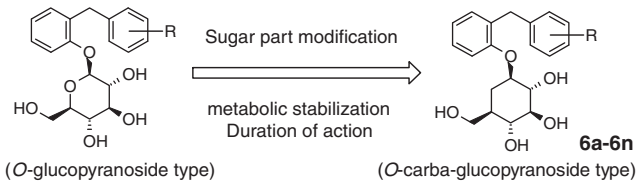


Figure 2. Our original design of carba-glucopyranoside SGLT2 inhibitors.

SGLT2-selective inhibitors,[10] but there were no reports of an SGLT2 inhibitor bearing a carbasugar.[11] Although examples of carbasugar being reported as a glucose mimetic were limited, those examples have encouraged us to prepare and evaluate carbasugar derivatives as SGLT2 inhibitors (**6a**–**6n**).[12]

2. Results and discussion

2.1. Chemistry

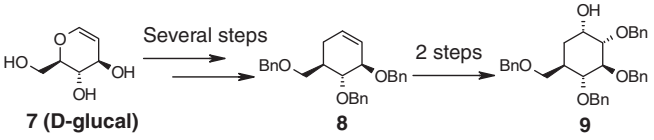
First, we prepared 2,3,4,6-tetra-*O*-benzyl-5a-carba-α-D-glucopyranose (**9**) as a key intermediate from D-glucal (**7**) by using Ogawa's method[13] and Sudha's method[14] (Scheme 1).

Next, the general synthetic methods of aglycons **11e–11f, 11i–11j, 11l–11n** are shown in Scheme 2. After lithium halogen exchange of the commercially available 2-benzyloxybromobenzene (**10**), 4-substituted benzaldehyde derivatives were added to give the corresponding alcohols in good yield. The alcohol derivatives were converted to **11** by direct route (Method A) or stepwise route (Method B), depending on their reactivity of R substituent in reduction conditions.

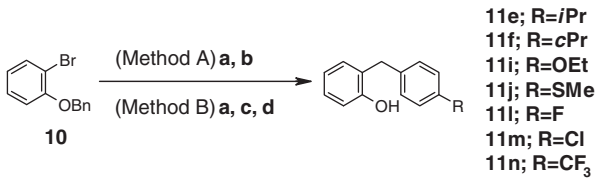
The general synthesis of the final carbasugar derivatives is shown in Scheme 3. Our key reaction was the coupling reaction between carbasugar **9** and the corresponding aglycon **11** with inversion of C-1 position on the cyclohexyl ring. In our investigation, the coupled compounds were obtained by the combination of tri-*n*butylphosphine (*n*Bu_3P) and tetramethylazodicarbox-amide (TMAD). Finally, removal of the tetra benzyl groups furnished the desired carba-glucopyranose compounds **6a–6f, 6i–6j, 6l–6n**.

The compound **6g** (R = CH=CH_2) was prepared as shown in Scheme 4. Key intermediate **9** was treated with trifluoromethanesulfonic anhydride, and coupled with sodium 2-(4-bromophenylmethyl)phenoxide **13**[15] with inversion. After deprotection of four benzyl groups, vinylation was finally implemented by palladium coupling reaction with vinyl tributylstannane to afford the vinyl compound **6g**.

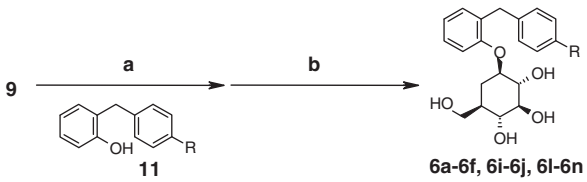
As shown in Scheme 5, **6h** (R = OMe) and **6k** (R = OH) were also synthesized. Coupling reaction of **9** and methyl salicylate was conducted under Mitsunobu condition to afford **15** with the inversion of C-1 position on carbasugar. After reducing the methyl ester, benzaldehyde **17** was obtained in good yield by using Dess–Martin reagent. It was treated with commercially available 4-methoxyphenyl Grignard reagent to afford compound **18**, followed by



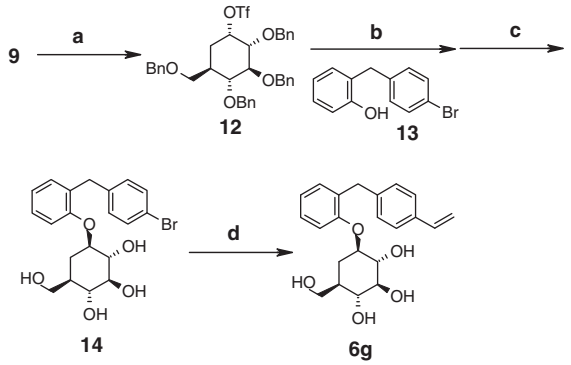
Scheme 1. Synthesis of 2,3,4,6-tetra-*O*-benzyl-5a-carba-α-D-glucopyranose (**9**)



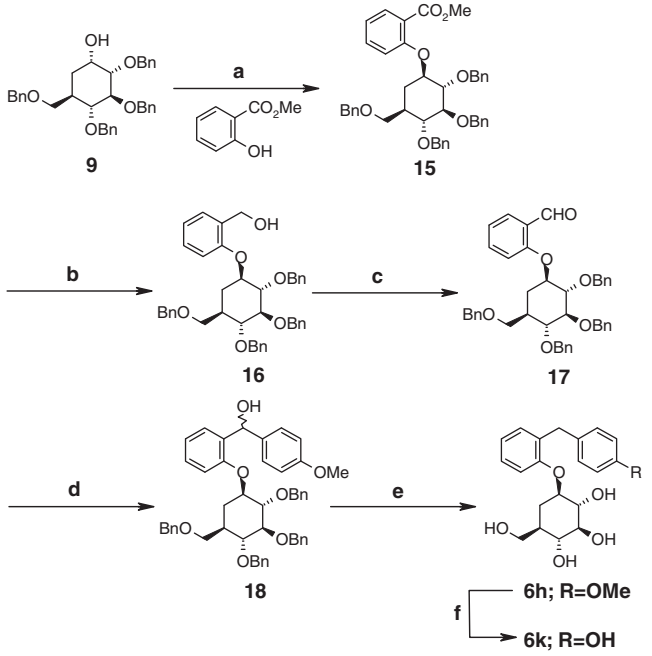
Scheme 2. Reagents and conditions: (a) *n*BuLi, THF, −78 °C then 4-substituted benzaldehyde; (b) $Pd(OH)_2$, H_2, 2 N HCl aq, MeOH/AcOEt, rt; (c) Et_3SiH, $BF_3{\cdot}OEt_2$, MeCN, −40 °C; (d) Me_2S, $BF_3{\cdot}OEt_2$, CH_2Cl_2, 0 °C to rt (**11e**; 73%, **11f**; 56%, **11i**; 38%, **11j**; 50%, **11l**; 77%, **11m**; 62%, **11n**; 71%).



Scheme 3. Reagents and conditions: (a) compound **11**, *n*Bu_3P, TMAD, toluene/THF, 0 °C to rt; (b) $Pd(OH)_2$, H_2, MeOH/THF, rt or Me_2S, $BF_3{\cdot}OEt_2$, CH_2Cl_2, 0 °C to rt (**6a**; 26%, **6b**; 6%, **6c**; 14%, **6d**; 21%, **6e**; 27%, **6f**; 25%, **6i**; 36%, **6j**; 28%, **6l**; 46%, **6m**; 35%, **6n**; 19%).



Scheme 4. Reagents and conditions: (a) Tf_2O, pyridine, CH_2Cl_2, 0 °C; (b) 60% NaH, DMF, −40 °C to 0 °C; (c) Me_2S, $BF_3{\cdot}OEt_2$, CH_2Cl_2, 0 °C to rt (43% for 3 steps); (d) nBu$_3$SnCH=CH$_2$, Pd(PPh$_3$)$_4$, 2,6-di-*tert*-Bu-4-MeC$_6$H$_3$OH, toluene, 110 °C (26%).



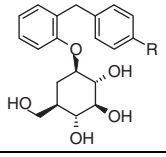
Scheme 5. Reagents and conditions: (a) PPh_3, DEAD, THF, rt (49%); (b) $LiAlH_4$, THF, 55 °C (81%); (c) Dess–Martin reagent, CH_2Cl_2, rt (81%); (d) 0.5 M 4-MeOC$_6$H$_4$MgBr in THF solution, Et_2O, rt (74%); (e) $Pd(OH)_2$, H_2, HCl–MeOH, rt (61%); (f) BBr_3, CH_2Cl_2, −78 °C to rt (47%).

dehydroxylation at 1,1-diphenylmethy position and deprotection of four benzyl groups, which were conducted simultaneously to give the methoxy compound **6h**. Finally, demethylation of the methoxy group on the distal benzene ring was conducted by use of boron tribromide (BBr_3) and gave **6k** in moderate yield.

2.2. Results and structure–activity relationship

We evaluated all the synthesized compounds **6a**–**6n** for in vitro inhibitory activity against human SGLT1 (hSGLT1) and human SGLT2 (hSGLT2) in order to investigate the effect of substituent (R) at para position on the distal benzene ring. The results are shown in Table 1. Compound **6a** (R = H) had a weak inhibitory activity for hSGLT2. Introduction of a methyl group (**6b**) resulted in the enhancement of hSGLT2 inhibition and selectivity against hSGLT1 compared with **6a**. By replacing the methyl group with an ethyl group (**6c**), its hSGLT2 inhibition and selectivity over hSGLT1 was further improved, whereas the introduction of a propyl substituent (**6d**) resulted in weak hSGLT2 inhibition and low

Table 1
In vitro data for hSGLT inhibition and selectivity



Compds	R	IC_{50} (nM)[a]		Selectivity[b]
		hSGLT2	hSGLT1	
1		16	190	12
3		260	22,000	85
3-Active		4.8	2,300	480
6a	H	690	13,000	19
6b	Me	59	6,700	110
6c	Et	28	4,800	170
6d	*n*Pr	120	7,800	65
6e	*i*Pr	150	20,000	130
6f	*c*Pr	11	5,600	510
6g	Vinyl	29	3,300	110
6h	OMe	42	9,300	220
6i	OEt	110	41,000	370
6j	SMe	19	3,500	180
6k	OH	290	19,000	66
6l	F	630	38,000	60
6m	Cl	150	22,000	150
6n	CF_3	120	57,000	480

[a] The IC_{50} values are means of multiple independent experiments. Compound **1** and **3-active** as reference standards were always included in the assays.
[b] The selectivity values were calculated by IC_{50} hSGLT1/IC_{50} hSGLT2.

selectivity. Moreover, the branched and cyclic alkyl substituents were also investigated (**6e**, **6f**). The most noteworthy compound was the cyclopropyl-substituted compound **6f**, which had the most potent hSGLT2 inhibitory activity (11 nM) and the highest selectivity against hSGLT1 (510-fold). The vinyl-substituted compound (**6g**) exhibited the same potency as **6c**. The methoxy substitution (**6h**), which is the same substituent as sergliflozin (**3**), was found to exhibit potent inhibition of hSGLT2. When the methoxy group was replaced with the methylthio group (**6j**), the potency of hSGLT2 inhibition was doubled. Substitution at this position with the hydrophilic hydroxyl group (**6k**) resulted in a loss of potency. The introduction of a small sized fluoro group (**6l**) showed the same result as **6a**. The other halogen derivatives (**6m–6n**) also gave moderate inhibitory activity.

As a consequence of our investigation, we demonstrated that hSGLT1 and hSGLT2 inhibitions were affected by the substituent R. Interestingly, the order of hSGLT2 IC_{50} values in alkyl substituent series (**6a–6g**, including the vinyl group) is as follows: **6a** (R = H) >> **6b** (R = Me) > **6c** (R = Et) = **6g** (R = vinyl) > **6f** (R = *c*Pr) < **6d** (R = *n*Pr) < **6e** (R = *i*Pr). These results suggest that the substituent at para position on the distal benzene ring occupies a two- or three-carbon-sized pocket of hSGLT2. On the other hand, inhibitors bearing a relatively hydrophilic substituent such as **6h** (R = OMe), **6i** (R = OEt), and **6k** (R = OH) exhibited less potent inhibition, compared with inhibitors which have a correspondingly sized alkyl group. These results suggest that the pocket of hSGLT2 is hydrophobic. Three halogen substituted compounds (**6l–6n**), which are smaller in size than a cyclopropyl substituent, also showed relatively weak inhibition, suggesting that these inhibitors were not satisfactory either in size or hydrophobicity. Meanwhile, these compounds inhibited hSGLT1 considerably more weakly than they did hSGLT2. Importantly, all the tested compounds showed hSGLT2 selectivity over hSGLT1, extensively. From these findings, having a substituent of suitable size and hydrophobicity at para position on the distal benzene ring is considered to be critical for potent hSGLT2 inhibition and higher selectivity than to hSGLT1.

Table 2
Pharmacokinetic parameters of db/db mice for key compounds

Compds	Dose (mg/kg; po)	C_{max} (μg/mL)	CL/F (mL/h/kg)	Vz/F[a] (mL/kg)	$T_{1/2}$ (h)
3	10	3.36	2,818	3,315	0.82
6h	10	1.64	3,257	12,586	2.68

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

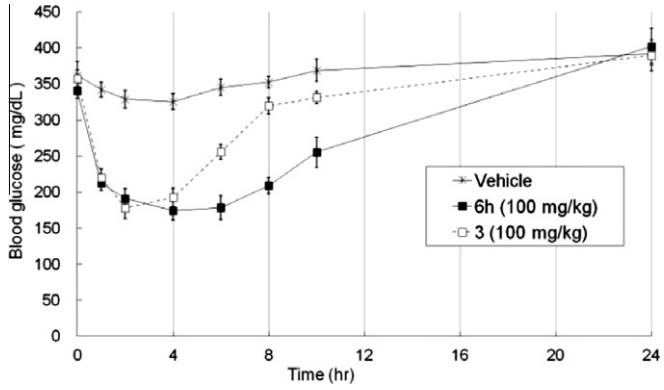


Figure 3. Blood glucose lowering effect of single oral administration of **3** and **6h** in db/db mice.

2.3. PK and in vivo studies

Among the carbasugar derivatives, **6h** was chosen for pharmacokinetic study in db/db mice to compare with **3** because **6h** had the same aglycon structure as **3-active** and the difference between compounds was only one atom structurally (Table 2). By removing the glycosidic bond, which is cleavable by β-glycosidase, we expected that the PK profile, especially the time of half-life ($T_{1/2}$), would be prolonged. In the results, **6h** showed threefold longer $T_{1/2}$ than **3,** as expected. Even though the cleavage of the glycosidic bond by β-glycosidase was successfully avoided (data not shown), the clearance (CL/F) of **6h** was not improved. The poor clearance might be attributed to metabolic factors other than cleavage of the glycosidic bond. As for the reason for the longer $T_{1/2}$, it resulted from the distribution volume (Vz/F) being greatly elevated. That is to say, the structural modification of glucose analog to carbasugar analog might increase the compound's lipophilicity and enhance distribution in tissue.

Furthermore, we studied the blood glucose lowering effect in db/db mice (Fig. 3). The resulting effects of **6h** (100 mg/kg) were as strong as those of **3** (100 mg/kg) even though each hSGLT2 inhibition value varied greatly (IC_{50}: 4.8 nM vs 42 nM). Moreover, 8 h after dosing, the blood glucose levels in the **6h**-treated mice were lower than those in the **3**-treated mice. This means that, as we expected from the PK profiles, **6h** has a longer-lasting effect than **3**.

3. Conclusion

We introduced the metabolically stable *O*-Aryl 5a-carba-β-D-glucopyranosides **6a–6n** as novel SGLT2 inhibitors with a carbasugar analog and revealed the SAR for the substituent on the distal benzene ring. Among the tested compounds, **6f** (R = *c*Pr) indicated the most potent hSGLT2 inhibition and the highest selectivity over hSGLT1. The pharmacokinetics data also showed that **6h** (R = OMe) had a three-fold longer $T_{1/2}$ than sergliflozin (**3**) with a high distribution volume in db/db mice. Subsequently, **6h** lowered blood glucose levels as much as **3** and showed longer hypoglycemic action than **3** in db/db mice. Further investigations of SGLT2 inhibitors having a carbasugar analog will be reported in the near future.

4. Experimental

4.1. Chemistry: instruments

Silica gel 60F254 precoated plates on glass from Merck KgaA were used for thin layer chromatography (TLC). Column chromatography was carried out on Merck Silica Gel 60 (230–400 mesh), if not otherwise specified. Reverse phase column chromatography was carried out on Fuji Silysia Chromatorex Pro. No. DM2035MT. ^{1}H 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 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. 2,3,4,6-Tetra-*O*-benzyl-5a-carba-α-D-glucopyranose (9)

2,3,4,6-Tetra-*O*-benzyl-5a-carba-α-D-glucopyranose (**9**) was synthesized from commercially available D-glucal (**7**) in several steps.

4.1.2. Aglycons (11a, 11b, 11c)

Aglycons **11a** and **11b** were commercially available. These were used without further purification. **11c** was prepared by the procedure of the patent.[16]

4.1.3. 2-(4-Isopropylbenzyl)phenol (11e) (direct route, method A)

In a nitrogen stream, 2.44 M *n*BuLi in hexane (5.14 mL, 12.5 mmol) was added dropwise to a solution of 1-benzyloxy-2-bromobenzene (3.0 g, 11.4 mmol) in THF (114 mL) at −78 °C and the mixture was stirred at the same temperature for 20 min. To this solution, a solution of 4-isopropylbenzaldehyde (1.41 g, 9.49 mmol) in THF (38 mL) was added dropwise at −78 °C. The mixture was stirred at the same temperature for 2 h, and then saturated aq NH_4Cl was added thereto and the mixture was extracted with AcOEt. The organic layer was washed with brine and dried with $MgSO_4$, and then the solvent was removed under reduced pressure. The obtained residue was purified by silica gel column chromatography (AcOEt/hexane = 1:5) to obtain 2-benzyloxyphenyl-4-isopropylphenylmethanol (2.63 g, 84%). To a solution of the obtained product in MeOH (50 mL), 20% $Pd(OH)_2$ (263 mg) was added and furthermore 2 N HCl (400 μL) was added thereto. The mixture was stirred under a hydrogen atmosphere for 15 h, and then the catalyst was filtered. The solvent was removed under reduced pressure and the obtained residue was purified by silica gel column chromatography (AcOEt/hexane = 1:4) to obtain the title compound (1.31 g, 73%). ^{1}H NMR ($CDCl_3$) δ: 1.24 (6H, d, J = 7.8 Hz), 2.82–2.92 (1H, m), 3.96 (2H, s), 4.67 (1H, s), 6.79 (1H, d, J = 9.0 Hz), 6.88 (1H, t, J = 8.4 Hz), 7.10–7.15 (6H, m).

4.1.4. 2-(4-Cyclopropylbenzyl)phenol (11f) (stepwise route, method B)

4.1.4.1. 2-Benzyloxyphenyl-(4-cyclopropylphenyl)methanol. In a nitrogen stream, 2.6 M *n*BuLi in hexane (3.5 mL, 9.1 mmol) was added dropwise to a solution of 1-benzyloxy-2-bromobenzene (2.2 g, 8.3 mmol) in THF (83 mL) at −78 °C and the mixture was stirred at the same temperature for 30 min. To this solution, a solution of 4-cyclopropylbenzaldehyde (1.1 g, 6.9 mmol) in THF (280 mL) was added dropwise at −78 °C. The reaction mixture was stirred at the same temperature for 1 h. Water was added thereto and the mixture was extracted with AcOEt. The organic

layer was washed with brine and dried with $MgSO_4$, and then the solvent was removed under reduced pressure. The obtained residue was purified by silica gel column chromatography (AcOEt/hexane = 1:5) to obtain the title compound (1.7 g, 75%). ^{1}H NMR ($CDCl_3$) δ: 0.65–0.69 (2H, m), 0.92–0.97 (2H, m), 1.86–1.90 (1H, m), 2.88 (1H, d, J = 6.0 Hz), 5.03 (2H, s), 6.03 (1H, d, J = 6.0 Hz), 7.17–7.26 (5H, m), 7.32–7.35 (4H, m); MS (ESI) m/z: 315 ($[M+Na]^+$).

4.1.4.2. 1-Benzyloxy-2-(4-cyclopropylbenzyl)benzene. In a nitrogen stream, Et_3SiH (0.73 mL, 4.6 mmol) and $BF_3{\cdot}Et_2O$ (0.5 mL, 4.0 mmol) were added to a solution of 2-benzyloxyphenyl-4-cyclopropylphenyl-methanol (1.3 g, 4.0 mmol) in MeCN (7 mL) at −40 °C and the mixture solution was stirred at the same temperature for 1.5 h and furthermore at 0 °C for 30 min. Water was added thereto and the mixture was extracted with CH_2Cl_2. The organic layer was washed with brine and dried with $MgSO_4$, and then the solvent was removed under reduced pressure. The obtained residue was purified by silica gel column chromatography (AcOEt/hexane = 1:7) to obtain the title compound (1.2 g, 95%). ^{1}H NMR ($CDCl_3$) δ: 0.63–0.67 (2H, m), 0.89–0.94 (2H, m), 1.84–1.87 (1H, m), 3.98 (2H, s), 5.06 (2H, s), 6.87–6.97 (4H, m), 7.08–7.26 (4H, m), 7.31–7.37 (5H, m); MS (ESI) m/z: 332 ($[M+H_2O]^+$).

4.1.4.3. 2-(4-cyclopropylbenzyl)phenol. In a nitrogen stream, Me_2S (2.2 mL, 51.7 mmol) and $BF_3{\cdot}Et_2O$ (1.1 mL, 8.8 mmol) were added to a solution of 1-benzyloxy-2-(4-cyclopropyl-benzyl)benzene (1.1 g, 3.5 mmol) in CH_2Cl_2 (24 mL) under cooling with ice, and the reaction mixture was stirred for 23 h while gradually raising its temperature to room temperature. Water was added thereto under cooling with ice and the mixture was extracted with CH_2Cl_2. The organic layer was washed with brine and dried with $MgSO_4$, and then the solvent was removed under reduced pressure. The obtained residue was purified by silica gel column chromatography (AcOEt/hexane = 1:4) to obtain the title compound (621 mg, 79%). ^{1}H NMR ($CDCl_3$) δ: 0.65–0.67 (2H, m), 0.90–0.94 (2H, m), 1.85–1.86 (1H, m), 3.95 (2H, s), 4.90 (1H, s), 6.79 (1H, d, J = 8.1 Hz), 6.88 (1H, t, J = 7.7 Hz), 7.11 (2H, d, J = 8.1 Hz), 7.20 (4H, d, J = 7.7 Hz); MS (ESI) m/z: 247 ($[M+Na]^+$).

4.1.5. 2-(4-Ethyoxybenzyl)phenol (11i)

This compound was prepared from 1-benzyloxy-2-bromobenzene and 4-ethoxybenzaldehyde in the same manner as described in Section 4.1.3. Yield 38%. ^{1}H NMR ($CDCl_3$) δ: 1.39 (3H, t, J = 7.0 Hz), 3.93 (2H, s), 3.99 (2H, q, J = 7.0 Hz), 6.77–6.90 (4H, m), 7.09–7.14 (4H, m).

4.1.6. 2-(4-Methylsulfanylbenzyl)phenol (11j)

This compound was prepared from 1-benzyloxy-2-bromobenzene and 4-methylsulfanylbenzaldehyde in the same manner as described in Section 4.1.4. Yield 50%. ^{1}H NMR ($CDCl_3$) δ: 2.46 (3H, s), 3.95 (2H, s), 4.65–4.75 (1H, br s), 6.78 (1H, d, J = 7.6 Hz), 6.89 (1H, t, J = 7.6 Hz), 7.09–7.21 (6H, m).

4.1.7. 2-(4-Fluorobenzyl)phenol (11l)

This compound was prepared from 1-benzyloxy-2-bromobenzene and 4-fluorobenzaldehyde in the same manner as described in Section 4.1.3. Yield 77%. ^{1}H NMR ($CDCl_3$) δ: 3.95 (2H, s), 4.72 (1H, s), 7.77 (1H, dd, J = 1.1, 7.9 Hz), 6.88 (1H, dt, J = 1.1, 7.5 Hz), 6.96 (1H, dd, J = 8.7, 8.7 Hz), 7.06–7.20 (4H, m).

4.1.8. 2-(4-Chlorobenzyl)phenol (11m)

This compound was prepared from 1-benzyloxy-2-bromobenzene and 4-chlorobenzaldehyde in the same manner as described in Section 4.1.4. Yield 62%. ^{1}H NMR ($CDCl_3$) δ: 3.95 (2H, s), 4.60 (1H, s), 6.75–6.78 (1H, m), 6.86–6.92 (1H, m), 7.07–7.27 (6H, m).

4.1.9. 2-(4-Trifluoromethylbenzyl)phenol (11n)

This compound was prepared from 1-benzyloxy-2-bromobenzene and 4-trifluoromethylbenzaldehyde in the same manner as described in Section 4.1.3. Yield 71%. ^{1}H NMR ($CDCl_3$) δ: 4.03 (2H, s), 4.72 (1H, s), 6.77 (1H, d, J = 6.0 Hz), 6.89 (1H, t, J = 4.8 Hz), 7.08–7.15 (2H, m), 7.33 (2H, d, J = 6.0 Hz), 7.52 (2H, d, J = 6.0 Hz); MS (ESI) m/z: 275 ($[M+Na]^+$).

4.1.10. (2-Benzylphenyl)-5a-carba-β-D-glucopyranoside (6a)

In a nitrogen stream, 2,3,4,6-tetra-*O*-benzyl-5a-carba-α-D-glucopyranose (**9**) (500 mg, 0.93 mmol) and nBu$_3$P (0.35 mL, 1.39 mmol) were added to a solution of commercially available 2-benzylphenol (257 mg, 1.39 mmol) in toluene (2 mL) under cooling with ice, and then tetramethylazodicarboxamide (TMAD, 239 mg, 1.39 mmol) was added thereto at the same temperature. The reaction mixture was stirred for 21 h while gradually raising its temperature to room temperature. The reaction mixture was concentrated under reduced pressure, and the obtained residue was treated by silica gel column chromatography (AcOEt/hexane = 1:10). The obtained crude product was dissolved in a MeOH/THF (1:1) mixed solution (14 mL) and 20% $Pd(OH)_2$ (119 mg) was added thereto and the mixture was stirred under a hydrogen atmosphere for 15 h, and then the catalyst was filtered off. The solvent was removed under reduced pressure and the obtained residue was purified by silica gel column chromatography ($MeOH/CH_2Cl_2$ = 1:10) to obtain the title compound (84 mg, 26%) as a colorless amorphous solid. ^{1}H NMR (CD_3OD) δ: 0.95 (1H, dd, J = 11.7, 12.0 Hz), 1.49–1.59 (1H, m), 2.03–2.09 (1H, m), 3.20 (1H, d, J = 9.0 Hz), 3.25 (1H, d, J = 5.7 Hz), 3.44–3.50 (2H, m), 3.68 (1H, dd, J = 3.9, 4.2 Hz), 3.94 (1H, d, J = 15.0 Hz), 4.00 (1H, d, J = 15.0 Hz), 4.13–4.22 (1H, m), 6.84 (1H, t, J = 6.3 Hz), 7.03 (1H, d, J = 6.3 Hz), 7.06–7.24 (7H, m); MS (ESI) m/z: 345 ($[M+H]^+$); HRMS calcd for $C_{20}H_{23}O_5$ ($[M-H]^-$) 343.154. Found 343.1539.

4.1.11. [2-(4-Methylbenzyl)phenyl]-5a-carba-β-D-glucopyranoside (6b)

This compound was prepared from **9** and 2-(4-methylbenzyl)phenol (**11b**) in the same manner as described in Section 4.1.10. Yield 6%. ^{1}H NMR (CD_3OD) δ: 0.84–0.97 (1H, m), 1.44–2.03 (1H, m), 1.96–2.03 (1H, m), 2.22 (3H, s), 3.13–3.30 (2H, m), 3.39–3.46 (2H, m), 3.64 (1H, dd, J = 4.5, 10.8 Hz), 3.95 (2H, m), 4.12 (1H, m), 6.76–6.81 (1H, m), 6.92–7.03 (5H, m), 7.06–7.11 (2H, m); MS (ESI) m/z: 381 ($[M+Na]^+$); HRMS calcd for $C_{21}H_{25}O_5$ ($[M-H]^-$) 357.1697. Found 357.1694.

4.1.12. [2-(4-Ethylbenzyl)phenyl]-5a-carba-β-D-glucopyranoside (6c)

This compound was prepared from **9** and 2-(4-ethylbenzyl)phenol (**11c**) in the same manner as described in Section 4.1.10. Yield 14%. ^{1}H NMR (CD_3OD) δ: 0.86–1.00 (1H, m), 1.19 (3H, t, J = 7.6 Hz), 1.45–1.62 (1H, m), 2.00–2.11 (1H, m), 2.57 (2H, q, J = 7.6 Hz), 3.16–3.30 (2H, m), 3.43–3.49 (2H, m), 3.70 (1H, dd, J = 4.1, 10.6 Hz), 3.84–4.02 (2H, m), 4.12–4.24 (1H, m), 6.83 (1H, t, J = 7.4 Hz), 6.99–7.16 (7H, m); MS (ESI): 395 ($[M+Na]^+$); HRMS calcd for $C_{22}H_{27}O_5$ ($[M-H]^-$) 371.1853. Found 371.1850.

4.1.13. [2-(4-*n*Propylbenzyl)phenyl]-5a-carba-β-D-glucopyranoside (6d)

1-Benzyloxy-2-(4-cyclopropylbenzyl)benzene (641 mg, 2.0 mmol) as synthesized in Section 4.1.4. was dissolved in 2,2-dimethylpropanol (12 mL). 20% $Pd(OH)_2$ (64 mg) was added thereto and the mixture was stirred for 2 h under a hydrogen atmosphere, and then the catalyst was filtered off. The solvent was removed under reduced pressure, and the obtained residue was treated by silica gel column chromatography (AcOEt/hexane = 1:4). To a solution of the obtained 2-(4-*n*Propylbenzyl)phenol (348 mg)

in toluene (3.5 mL), **9** (557 mg, 1.03 mmol) and nBu$_3$P (0.39 mL, 1.55 mmol) were added under cooling with ice, and then TMAD (267 mg, 1.55 mmol) was added thereto at the same temperature. The reaction mixture was stirred for 20 h while gradually raising its temperature to room temperature. The reaction mixture was concentrated under reduced pressure, and the obtained residue was treated with silica gel column chromatography (AcOEt/hexane = 1:5). The obtained product was dissolved in 2,2-dimethylpropanol (15 mL). 20% Pd(OH)$_2$ (159 mg) was added thereto and the mixture was stirred under a hydrogen atmosphere for 17 h, and the catalyst was filtered off. The solvent was removed under reduced pressure, and the obtained residue was purified by silica gel column chromatography (MeOH/CH_2Cl_2 = 1:9) to obtain the title compound (165 mg, 21%) as a colorless amorphous solid. ^{1}H NMR (CD_3OD) δ: 0.88–0.94 (4H, m), 1.48–1.63 (3H, m), 2.05–2.09 (1H, m), 2.52 (2H, t, J = 7.7 Hz), 3.13–3.30 (2H, m), 3.46 (2H, m), 3.70 (1H, m), 3.87 (1H, d, J = 15.0 Hz), 3.98 (1H, d, J = 15.0 Hz), 4.60–4.88 (1H, m), 6.84 (1H, t, J = 7.3 Hz), 7.00–7.16 (7H, m); MS (ESI) m/z: 387 ([M+H]$^+$); HRMS calcd for $C_{23}H_{29}O_5$ ([M−H]$^-$) 385.2010. Found 385.2011.

4.1.14. [2-(4-Isopropylbenzyl)phenyl]-5a-carba-β-D-glucopyranoside (6e)

This compound was prepared from **9** and 2-(4-isopropylbenzyl)phenol (**11e**) in the same manner as described in Section 4.1.10. Yield 27%. ^{1}H NMR (CD_3OD) δ: 0.94 (1H, dd, J = 12.0, 12.9 Hz), 1.22 (6H, d, J = 6.9 Hz), 1.48–1.58 (1H, m), 2.02–2.09 (1H, dt, J = 3.9, 4.2 Hz), 2.78–2.87 (1H, m), 3.12–3.24 (2H, m), 3.43–3.49 (2H, m), 3.68 (1H, dd, J = 4.2, 6.6 Hz), 3.89 (1H, d, J = 14.4 Hz), 4.01 (1H, d, J = 14.7 Hz), 4.13–4.21 (1H, m), 6.84 (1H, t, J = 6.3 Hz), 7.02 (1H, d, J = 6.3 Hz), 7.06–7.15 (6H, m); MS (ESI) m/z: 387 ([M+H]$^+$); HRMS calcd for $C_{23}H_{29}O_5$ ([M−H]$^-$) 385.2010. Found 385.2006.

4.1.15. [2-(4-Cyclopropylbenzyl)phenyl]-5a-carba-β-D-glucopyranoside (6f)

In a nitrogen stream, **9** (557 mg, 1.03 mmol) and nBu$_3$P (0.37 mL, 1.55 mmol) were added to a solution of 2-(4-cyclopropylbenzyl)phenol (**11f**) (348 mg, 1.55 mmol) in toluene (3.5 mL) under cooling with ice, and then TMAD (267 mg, 1.55 mmol) was added thereto at the same temperature. The reaction mixture was stirred for 15 h while gradually raising its temperature to room temperature. The reaction mixture was concentrated under reduced pressure, and the obtained residue was treated with silica gel column chromatography (AcOEt/hexane = 1:10). The obtained product was dissolved in CH_2Cl_2 (3.5 mL). Me_2S (1.3 mL, 30.3 mmol) and $BF_3{\cdot}Et_2O$ (0.65 mL, 5.1 mmol) were added thereto under cooling with ice. The reaction mixture was stirred for 14 h while gradually raising its temperature to room temperature, and then water was added thereto under cooling with ice and the mixture was extracted with CH_2Cl_2. The organic layer was washed with brine and dried with $MgSO_4$, and then the solvent was removed under reduced pressure. The obtained residue was purified by silica gel column chromatography (MeOH/CH_2Cl_2 = 1:9) to obtain the title compound (98 mg, 25%) as a colorless amorphous solid. ^{1}H NMR (CD_3OD) δ: 0.57–0.61 (2H, m), 0.81–0.95 (3H, m), 1.50 (1H, s), 1.79–1.85 (1H, m), 1.99–2.03 (1H, m), 3.15–3.33 (2H, m), 3.42–3.48 (2H, m), 3.66–3.72 (1H, m), 3.81–3.99 (2H, q, J = 14.7 Hz), 4.41–4.85 (1H, m), 6.80–7.15 (8H, m); MS (ESI) m/z: 407 ([M+Na]$^+$); HRMS calcd for $C_{23}H_{27}O_5$ ([M−H]$^-$) 383.1853. Found 383.1849.

4.1.16. [2-(4-Vinylbenzyl)phenyl]-5a-carba-β-D-glucopyranoside (6g)

4.1.16.1. (1*S*,2*R*,3*S*,4*R*,5*R*)-2,3,4-Trisbenzyloxy-(5-benzyloxymethyl)cyclohexyl trifluoromethanesulfonate. (12). Pyridine (205 μL, 2.53 mmol) was added to a solution of **9** (300 mg, 0.557 mmol) in CH_2Cl_2 (5.5 mL) and the mixture was cooled to 0 °C, and then Tf_2O (210 μL, 1.25 mmol) was added thereto. The reaction mixture was stirred at the same temperature for 1 h, and then saturated aq $NaHCO_3$ was added thereto and the mixture was extracted with CH_2Cl_2. The combined organic layer was washed with saturated aq $KHSO_4$ and brine, and dried over $MgSO_4$. The solvent was removed to obtain the crude titled product (380 mg). ^{1}H NMR ($CDCl_3$) δ: 1.83 (1H, dd, J = 14.0, 15.9 Hz), 2.00–2.14 (2H, m), 3.39 (1H, dd, J = 2.3, 9.2 Hz), 3.50 (1H, dd, J = 2.6, 9.6 Hz), 3.56 (1H, dd, J = 9.3, 10.2 Hz), 3.75 (1H, dd, J = 3.4, 9.2 Hz), 3.86 (1H, dd, J = 9.3, 9.5 Hz), 4.40 (2H, s), 4.50 (1H, d, J = 10.8 Hz), 4.62 (1H, d, J = 11.4 Hz), 4.79 (1H, d, J = 10.7 Hz), 4.82 (1H, d, J = 11.5 Hz), 4.89 (1H, d, J = 10.7 Hz), 4.92 (1H, d, J = 10.7 Hz), 5.33 (1H, br), 7.15–7.30 (20H, m).

4.1.16.2. 2-(4-Bromobenzyl)phenol (13). In a nitrogen stream, Me_2S (1.83 mL, 42.46 mmol) and $BF_3{\cdot}Et_2O$ (0.9 mL, 7.07 mmol) were added to a solution of 1-benzlyoxy-2-(4-bromobenzyl)benzene (1.0 g, 2.83 mmol) in CH_2Cl_2 (30.0 mL) under ice-cooling in the same manner as described in Section 4.1.4.. The reaction mixture was stirred for 19 h while gradually raising its temperature to room temperature, and then water was added thereto under cooling with ice and the mixture was extracted with CH_2Cl_2. The organic layer was washed with brine and dried with $MgSO_4$, and then the solvent was removed under reduced pressure. The obtained residue was purified by silica gel column chromatography (AcOEt/hexane = 1:5) to obtain the title compound (527 mg, 71%). ^{1}H NMR ($CDCl_3$) δ: 3.92 (2H, s), 4.62 (1H, s), 6.73–6.76 (1H, m), 6.85–6.99 (1H, m), 7.06–7.14 (4H, m), 7.35–7.40 (2H, m).

4.1.16.3. [2-(4-Bromobenzyl)phenyl]-5a-carba-β-D-glucopyranoside (14). In a nitrogen stream, a solution of **13** (386 mg, 1.46 mmol) in DMF (1 mL) was cooled in an ice bath, and NaH (60%, 52 mg) was added thereto. The reaction mixture was stirred at the same temperature for 10 min, and then this reaction mixture was added dropwise to a suspension of **12** (655 mg, 0.97 mmol) in DMF (2.5 mL) at −40 °C. The reaction mixture was stirred at the same temperature for 2 min, at −40 °C for 30 min and then at 0 °C for 1 h. To the reaction mixture was added brine and water. The resulting solution was extracted with Et_2O, and the organic layer was washed with water and brine and dried over Na_2SO_4. The residue obtained by removing the solvent was roughly purified by silica gel column chromatography (AcOEt/hexane = 1:5) to obtain a crude product (200 mg). The obtained crude product was dissolved in CH_2Cl_2 (2.5 mL) in a nitrogen stream. Me_2S (0.6 mL, 13.48 mmol) and $BF_3{\cdot}Et_2O$ (284 μL, 2.2 mmol) were added thereto under cooling with ice. The reaction mixture was stirred for 25 h while gradually raising its temperature to room temperature, then water was added thereto under cooling with ice and the mixture was extracted with CH_2Cl_2. The organic layer was washed with brine and dried with $MgSO_4$, and then the solvent was removed under reduced pressure. The obtained residue was purified by silica gel column chromatography (MeOH/CH_2Cl_2 = 1:10) to obtain the title product (40.0 mg, 43%). ^{1}H NMR (CD_3OD) δ: 0.85 (1H, dd, J = 11.1, 13.2 Hz), 1.44–1.59 (1H, m), 2.03–2.09 (1H, m), 3.13 (1H, d, J = 8.7 Hz), 3.19 (1H, d, J = 5.1 Hz), 3.37–3.45 (2H, m), 3.63–3.68 (1H, dd, J = 3.9, 10.6 Hz), 3.81 (1H, d, J = 14.4 Hz), 4.10 (1H, d, J = 14.4 Hz), 4.09–4.18 (1H, m), 6.79 (1H, t, J = 7.5 Hz), 6.96 (1H, d, J = 9.0 Hz), 7.03–7.13 (4H, m), 7.27–7.32 (2H, m).

4.1.16.4. [2-(4-Vinylbenzyl)phenyl]-5a-carba-β-D-glucopyranoside (6g). In a nitrogen stream, **14** (40 mg, 0.094 mmol) was dissolved in toluene (2 mL). Tributylvinyltin (36 mg, 0.11 mmol), Pd(PPh$_3$)$_4$ (2.7 mg) and 2,6-di-*tert*-butyl-4-methylphenol were added thereto, and the mixture was refluxed at 110 °C for 15 h.

The reaction mixture was cooled to room temperature and the solvent was removed under reduced pressure. The obtained residue was purified by preparative thin-layer chromatography (PTLC) ($MeOH/CH_2Cl_2$ = 1:10) to obtain the title product (9 mg, 26%) as a colorless amorphous solid. ^1H NMR (CD_3OD) δ: 0.86 (1H, dd, J = 11.7, 12.0 Hz), 1.42–1.55 (1H, m), 1.96–2.03 (1H, m), 3.10–3.19 (2H, m), 3.36–3.44 (2H, m), 3.63 (1H, dd, J = 4.1, 10.6 Hz), 3.81–4.08 (2H, m), 4.09–4.16 (1H, m), 5.07 (1H, dd, J = 1.2, 11.0 Hz), 5.63 (1H, dd, J = 1.2, 18.0 Hz), 6.61 (1H, dd, J = 11.0, 18.0 Hz), 6.78 (1H, t, J = 7.3 Hz), 7.03 (1H, d, J = 6.3 Hz), 7.02–7.24 (6H, m); MS (ESI) m/z: 370 ($[M]^+$); HRMS calcd for $C_{22}H_{25}O_5$ ($[M-H]^-$) 369.1697. Found 369.1698.

4.1.17. [2-(4-Methoxybenzyl)phenyl]-5a-carba-β-D-glucopyranoside (6h)

4.1.17.1. 2-(2,3,4,6-Tetra-O-benzyl-5a-carba-β-D-gluco-pyranosyl)benzoic acid methyl ester (15). In a nitrogen stream, **9** (200 mg, 0.371 mmol) and Ph_3P (146 mg, 0.557 mmol) were added to a solution of methyl salicylate (72 μL, 0.557 mmol) in THF (400 μL), and then diethyl azodicarboxylate (DEAD, 88 μL, 0.557 mmol) was added dropwise thereto at room temperature. The mixture solution was stirred at room temperature for 10 h. The reaction mixture was concentrated under reduced pressure, and the obtained residue was purified by PTLC (AcOEt/hexane = 1:3) to obtain the title compound (123 mg, 49%). ^1H NMR ($CDCl_3$) δ: 1.60–1.80 (2H, m), 2.15–2.24 (1H, m), 3.48–3.64 (5H, m), 3.83 (3H, s), 4.43 (3H, s), 4.53 (1H, d, J = 10.7 Hz), 4.51–4.98 (5H, m), 6.95–7.02 (1H, m), 7.10–7.50 (22H, m), 7.78 (1H, dd, J = 1.7, 7.8 Hz); MS (ESI) m/z: 695 ($[M+Na]^+$).

4.1.17.2. 2-(2,3,4,6-Tetra-O-benzyl-5a-carba-β-D-glucopyranosyl)benzyl alcohol (16). Lithium aluminum hydride (10.4 mg, 0.274 mmol) was added to a solution of **15** (123 mg, 0.183 mmol) in THF (360 μL) in small portions and the reaction mixture was stirred at 55 °C for 3 h. The reaction mixture was cooled to room temperature, and then water was added thereto and extracted with AcOEt. The organic layer was washed with brine and dried with $MgSO_4$, and then the solvent was removed under reduced pressure. The obtained residue was purified by PTLC (AcOEt/hexane = 1:3) to obtain the title compound (95 mg, 81%). ^1H NMR ($CDCl_3$) δ: 1.60–1.80 (2H, m), 2.16–2.22 (1H, m), 3.46–3.74 (5H, m), 4.43 (3H, s), 4.54 (1H, d, J = 10.7 Hz), 4.66 (2H, br s), 4.81–4.96 (6H, m), 6.94–7.31 (24H, m); MS (ESI) m/z: 667 ($[M+Na]^+$).

4.1.17.3. 2-(2,3,4,6-Tetra-O-benzyl-5a-carba-β-D-glucopyranosyl)benzaldehyde (17). To a solution of **16** (95 mg, 0.147 mmol) in CH_2Cl_2 (1.5 mL) was added Dess–Martin reagent (94 mg, 0.221 mmol), and the mixture solution was stirred at room temperature for 45 min. Insolubles were removed from the reaction mixture by filtration and the filtrate was concentrated under reduced pressure. The obtained residue was purified by PTLC (AcOEt/hexane = 1:3) to obtain the title compound (77 mg, 81%). ^1H NMR ($CDCl_3$) δ: 1.60–1.80 (2H, m), 2.17–2.23 (1H, m), 3.48–3.78 (5H, m), 4.44 (3H, s), 4.54 (1H, d, J = 10.7 Hz), 4.73–4.97 (5H, m), 7.00–7.32 (22H, m), 7.50 (1H, dd, J = 1.5, 7.8 Hz), 7.83 (1H, dd, J = 1.5, 7.6 Hz), 10.4 (1H, s).

4.1.17.4. [2-(2,3,4,6-Tetra-O-benzyl-5a-carba-β-D-gluco-pyranosyl)phenyl]-4-methoxyphenylmethanol (18). To a solution of **17** (77 mg, 0.119 mmol) in Et_2O (120 μL) was added 0.5 M 4-methoxyphenyl-magnesiumbromide in THF solution (480 μL, 0.238 mmol), and the mixture solution was stirred at room temperature for 13 h. A saturated aq NH_4Cl was added thereto and the mixture was extracted with AcOEt. The organic layer was dried with $MgSO_4$ and the solvent was concentrated under reduced pressure. The obtained residue was purified by PTLC (AcOEt/hexane = 1:3) to obtain the title compound (66 mg, 74%). ^1H NMR ($CDCl_3$) δ: 1.60–1.80 (2H, m), 1.95–2.09 (1H, m), 2.66 (1H, d, J = 4. 8 Hz), 3.38–3.79 (5H, m), 3.65 (1H, s), 3.69 (2H, s), 4.33–4.93 (9H, m), 5.96–6.16 (1H, m), 6.73–7.42 (28H, m); MS (ESI) m/z: 773 ($[M+Na]^+$).

4.1.17.5. [2-(4-Methoxybenzyl)phenyl]-5a-carba-β-D-glucopyranoside (6h). To a solution of **18** (66 mg, 0.0879 mmol) in 0.1 M HCl–MeOH (2 mL) was added 20% $Pd(OH)_2$ (10 mg), and the reaction mixture was stirred under a hydrogen atmosphere for 3 h. After the reaction, the reaction mixture was filtered and the filtrate was concentrated under reduced pressure, and the obtained residue was purified by PTLC ($MeOH/CH_2Cl_2$ = 1:10) to obtain the title compound (20 mg, 61%) as a colorless amorphous solid. ^1H NMR (CD_3OD) δ: 0.89–1.03 (1H, m), 1.40–1.60 (1H, m), 2.02–2.10 (1H, m), 3.18–3.34 (2H, m), 3.45–3.51 (2H, m), 3.67–3.71 (1H, m), 3.73 (3H, s), 3.82–3.99 (2H, m), 4.13–4.22 (1H, m), 6.78 (2H, d, J = 8.6 Hz), 6.80–6.86 (1H, m), 6.99–7.16 (5H, m); MS (ESI) m/z 397 ($[M+Na]^+$); HRMS calcd for $C_{21}H_{25}O_6$ ($[M-H]^-$) 373.1646. Found 373.1651.

4.1.18. [2-(4-Ethoxybenzyl)phenyl]-5a-carba-β-D-glucopyranoside (6i)

This compound was prepared from **9** and 2-(4-ethoxybenzyl)phenol (**11i**) in the same manner as described in Section 4.1.10. Yield 36%. ^1H NMR (CD_3OD) δ: 0.89–1.03 (1H, m), 1.35 (3H, t, J = 7.1 Hz), 1.48–1.62 (1H, m), 2.02–2.11 (1H, m), 3.18–3.33 (3H, m), 3.44–3.52 (2H, m), 3.68–3.74 (1H, m), 3.81–4.02 (3H, m), 4.13–4.23 (1H, m), 6.74–6.87 (4H, m), 6.99–7.17 (4H, m); MS (ESI) m/z: 411 ($[M+Na]^+$); HRMS calcd for $C_{22}H_{27}O_6$ ($[M-H]^-$) 387.1802. Found 387.1797.

4.1.19. [2-(4-Methylsulfanylbenzyl)phenyl]-5a-carba-β-D-glucopyranoside (6j)

This compound was prepared from **9** and 2-(4-methylsulfanylbenzyl)phenol (**11j**) in the same manner as described in Section 4.1.15. Yield 28%. ^1H NMR (CD_3OD) δ: 0.80–0.95 (1H, m), 1.45–1.60 (1H, m), 2.00–2.10 (1H, m), 2.42 (3H, s), 3.12–3.33 (2H, m), 3.40–3.50 (2H, m), 3.63–3.70 (1H, m), 3.86 (1H, d, J = 15.0 Hz), 4.00 (1H, d, J = 15.0 Hz), 4.15–4.23 (1H, m), 6.84 (1H, t, J = 7.0 Hz), 7.00–7.18 (7H, m); MS (ESI) m/z: 391 ($[M+H]^+$); HRMS calcd for $C_{21}H_{25}O_5S$ ($[M-H]^-$) 389.1417. Found 389.1415.

4.1.20. [2-(4-Hydroxybenzyl)phenyl]-5a-carba-β-D-glucopyranoside (6k)

In a nitrogen stream, 1.0 M BBr_3 in CH_2Cl_2 (0.80 mL, 0.80 mmol) was added to a solution of **6h** (100 mg, 0.27 mmol) in CH_2Cl_2 (1.3 mL) at −78 °C. The cooling bath was removed and the reaction mixture was stirred at room temperature for 1 h. The reaction mixture was concentrated under reduced pressure and the obtained residue was purified by reverse phase column chromatography (MeOH/20 mM $AcONH_4$ aqueous solution = 5:95–50:50) to obtain the title compound (45 mg, 47%) as a colorless amorphous solid. ^1H NMR (CD_3OD) δ: 0.92–1.06 (1H, m), 1.47–1.67 (1H, m), 2.03–2.11 (1H, m), 3.18–3.34 (2H, m), 3.45–3.52 (2H, m), 3.71 (1H, dd, J = 4.1, 10.7 Hz), 3.79–3.95 (2H, m), 4.12–4.22 (1H, m), 6.65 (2H, d, J = 8.4 Hz), 6.83 (1H, t, J = 7.3 Hz), 6.99–7.16 (5H, m); MS (ESI) m/z: 361 ($[M+H]^+$); HRMS calcd for $C_{20}H_{23}O_6$ ($[M-H]^-$) 359.1489. Found 359.1481.

4.1.21. [2-(4-Fluorobenzyl)phenyl]-5a-carba-β-D-glucopyranoside (6l)

This compound was prepared from **9** and 2-(4-fluorobenzyl)phenol (**11l**) in the same manner as described in Section 4.1.10. Yield 46%. ^1H NMR (CD_3OD) δ: 0.99 (1H, m), 1.55 (1H, m), 2.08 (1H, m), 3.22 (1H, m), 3.30 (1H, m), 3.48 (1H, m), 3.51 (1H, dd, J = 6.0, 10.8 Hz), 3.70 (1H, dd, J = 4.2, 10.8 Hz), 3.90 (1H, d,

J = 14.7 Hz), 4.02 (1H, d, J = 14.7 Hz), 4.20 (1H, ddd, J = 4.8, 9.0, 11.4 Hz), 6.85 (1H, t, J = 7.5 Hz), 6.93 (1H, m), 7.02 (1H, d, J = 8.1 Hz), 7.06–7.23 (5H, m); MS (ESI) m/z: 363 ($[M+H]^+$); HRMS calcd for $C_{20}H_{22}FO_5$ ($[M-H]^-$) 361.1446. Found 361.1441.

4.1.22. [2-(4-Chlorobenzyl)phenyl]-5a-carba-β-D-glucopyranoside (6m)

This compound was prepared from **9** and 2-(4-chlorobenzyl)phenol (**11m**) in the same manner as described in Section 4.1.15. Yield 35%. ^{1}H NMR (CD_3OD) δ: 0.95 (1H, dd, J = 11.1, 13.2 Hz), 1.53 (1H, m), 2.00–2.09 (1H, m), 3.20 (1H, d, J = 8.7 Hz), 3.25 (1H, d, J = 5.1 Hz), 3.43–3.51 (2H, m), 3.68–3.71 (1H, m), 3.91 (1H, d, J = 14.7 Hz), 3.99 (1H, d, J = 15.0 Hz), 4.15–4.23 (1H, m), 6.85 (1H, t, J = 7.5 Hz), 7.03 (1H, d, J = 9.0 Hz), 7.08–7.23 (6H, m); MS (ESI) m/z: 379 ($[M+H]^+$); HRMS calcd for $C_{20}H_{22}ClO_5$ ($[M-H]^-$) 377.1150. Found 377.1152.

4.1.23. [2-(4-Trifluoromethylbenzyl)phenyl]-5a-carba-β-D-glucopyranoside (6n)

This compound was prepared from **9** and 2-(4-trifluoromethylbenzyl)phenol (**11n**) in the same manner as described in Section 4.1.10. Yield 19%. ^{1}H NMR (CD_3OD) δ: 0.89 (1H, dd, J = 11.7, 12.9 Hz), 1.48–1.61 (1H, m), 2.05 (1H, dt, J = 13.2, 4.2 Hz), 3.15–3.34 (2H, m), 3.42–3.49 (2H, m), 3.69 (1H, dd, J = 3.9, 10.5 Hz), 3.96–4.15 (2H, m), 4.17–4.24 (1H, m), 6.87 (1H, dt, J = 1.2, 7.5 Hz), 7.02–7.20 (3H, m), 7.38 (2H, d, J = 8.1 Hz), 7.51 (2H, d, J = 8.4 Hz); MS (ESI) m/z: 435 ($[M+Na]^+$); HRMS calcd for $C_{21}H_{22}F_3O_5$ ($[M-H]^-$) 411.1414. Found 411.1410.

5. Biology

5.1. In vitro SGLT inhibition assay

Chinese hamster ovary-K1(CHO) cells stably expressing human SGLT2 (NM_003041) and human SGLT1 (NM_000343) 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 activities were 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.).

5.2. 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 **3** and **6h** (100 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, 8, 10, and 24 h after dosing. Blood glucose levels were measured by the hexokinase method (Autosera S GLU, Daiichi Pure Chemicals, Japan).

5.3. 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 **3** and **6h** at a dose of 10 mg/kg (n = 6). Blood samples were collected at 0.25, 0.5, 1, 2, 4, 6, 8, 10 (for **6h**), and 24 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/minute. The injection cycle of each sample was set at 10 min. The transitions for multiple reaction monitoring were 394–180 (**3-active**), 392–267 (**6h**). Non-compartmental pharmacokinetic parameters were calculated based on the averaged plasma concentration-time data using WinNonlin Professional 5.0 (Pharsight, Mountain View, CA).

Acknowledgements

We are very thankful to Ms. Hitomi Suda of Chugai Pharmaceutical Co., Ltd for HRMS measurements of the compounds, and Editing Services at Chugai Pharmaceutical Co., Ltd for assistance with English usage.

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