(1S)-1,5-Anhydro-1-[5-(4-ethoxybenzyl)-2-methoxy-4-methylphenyl]-1-thio-D-glucitol (TS-071) is a Potent, Selective Sodium-Dependent Glucose Cotransporter 2 (SGLT2) Inhibitor for Type 2 Diabetes Treatment

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Derivatives of a novel scaffold, *C*-phenyl 1-thio-D-glucitol, were prepared and evaluated for sodium-dependent glucose cotransporter (SGLT) 2 and SGLT1 inhibition activities. Optimization of substituents on the aromatic rings afforded five compounds with potent and selective SGLT2 inhibition activities. The compounds were evaluated for in vitro human metabolic stability, human serum protein binding (SPB), and Caco-2 permeability. Of them, (1S)-1,5-anhydro-1-[5-(4-ethoxybenzyl)-2-methoxy-4-methylphenyl]-1-thio-D-glucitol (**3p**) exhibited potent SGLT2 inhibition activity (IC$_{50}$ = 2.26 nM), with 1650-fold selectivity over SGLT1. Compound **3p** showed good metabolic stability toward cryopreserved human hepatic clearance, lower SPB, and moderate Caco-2 permeability. Since **3p** should have acceptable human pharmacokinetics (PK) properties, it could be a clinical candidate for treating type 2 diabetes. We observed that compound **3p** exhibits a blood glucose lowering effect, excellent urinary glucose excretion properties, and promising PK profiles in animals. Phase II clinical trials of **3p** (TS-071) are currently ongoing.

Introduction

The number of patients diagnosed with type 2, non-insulin-dependent, diabetes mellitus (NIDDM[a]) is increasing.[1,2] The International Diabetes Federation estimates that more than 250 million people worldwide had diabetes in 2007. This total is expected to rise to 380 million by 2025.[3] NIDDM can cause diabetic complications including blindness (retinopathy), renal failure (nephropathy), the need for lower limb amputation (neuropathy), and increased risk of a cardiovascular event.[4] The treatment of NIDDM can prevent or delay the risk of complications, thus decreasing overall medical costs and helping maintain quality of life. Good control of blood glucose can be achieved by a controlled diet and physical exercise. Diabetes can also be managed by orally active drugs including sulfonylurea,[5] biganide,[6] α-glucosidase inhibitors,[7] thiazolidinediones,[8,9] and DPP4 inhibitors.[10−12] Since combination therapy is usually the most effective approach for controlling hyperglycemia, there is need for novel antidiabetic agents with unique modes of action to complement existing therapies and prevent potential side effects.

Sodium-dependent glucose transporters (SGLTs) present on the chronic membrane of the intestine and kidney play an important role in the absorption and reabsorption of glucose.[13] Approximately 180 g of plasma glucose per day is filtered in the glomerulus in the kidney, 99% of which is reabsorbed in the proximal tubule.[14,15] Sodium-dependent glucose transporter 2 (SGLT2) is mainly expressed in epithelial cells of the early S1 segment of the renal cortical tubule and is likely the major transporter for this reabsorption process.[16,17]

Phlorizin (**1**), a natural nonselective SGLT inhibitor, was used in proof of concept experiments in several diabetic animal models (Figure 1). It was shown to advance glucose excretion and lower fasting and postprandial blood glucose levels without hypoglycemic side effects.[18,19] Tanabe Seiyaku was the first to conduct a clinical trial of T-1095, which is a prodrug of an active phlorizin analogue.[20−23] This finding led the company to develop aryl and heteroaryl *O*-glucosides for treatment of type 2 diabetes and obesity in the early 2000s.[24−27] It was, however, recognized that in vivo instability of *O*-glucosides against glucosidases resulted in need of high dosage. Subsequently, Bristol-Myers Squibb[28] and Kotobuki[29] disclosed *C*-aryl glucosides in 2001, which appear to have potent inhibition and good stability in vivo. Consequently, a number of companies have pursued *C*-aryl glucosides.[30] At present, dapagliflozin (**2**) (Bristol-Myers Squibb),[31,32] ASP1941 (Kotobuki/Astellas),[33] and canagliflozin (Johnson & Johnson/Mitsubishi Tanabe)[34] with *C*-aryl glucosides are undergoing phase III clinical trials. At least three compounds including BI 10773 (Boehringer Ingelheim),[35] CSG452 (Chugai/Roche),[36]
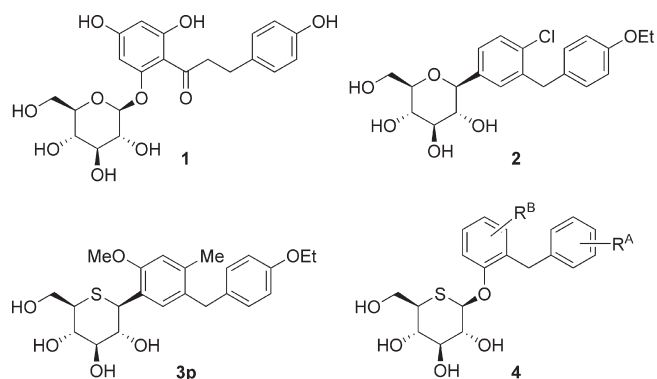
Figure 1

and LX4211 (Lexicon)[37,38] are currently in phase II clinical trials.

We have also given considerable attention to 5-thioglucose-derived SGLT2 inhibitors, which have a sulfur atom in place of the oxygen atom in the glucose ring. This glucose analogue has provided important information regarding the metabolic stability of several 5-thio-*O*-glycosides. For example, 5′-thio-*N*-acetyllactosamine is 200 times more resistant to digestion by β-galactosidase,[39] and methyl α-5′-thiomaltoside is not hydrolyzed at all by glucoamylase from *Rhizopus niveus*.[40] Thus, we had initially used *O*-aryl 5-thio-β-glucoside **4** as a SGLT inhibitor and demonstrated that it increased urinary glucose excretion and lowered blood glucose in Zucker fatty rats.[41,42] However, more metabolically robust and effective compounds were required, in order to achieve lower dose in vivo. Thus, we developed a novel synthetic strategy for preparing *C*-phenyl 1-thio-D-glucitol derivatives. These compounds produced a blood glucose lowering effect and had excellent pharmacokinetics properties. Here, we focused on the synthesis of *C*-phenyl 1-thio-D-glucitol **3a–3s** and the evaluation and selection of the clinical candidate, **3p**, as a selective SGLT2 inhibitor.
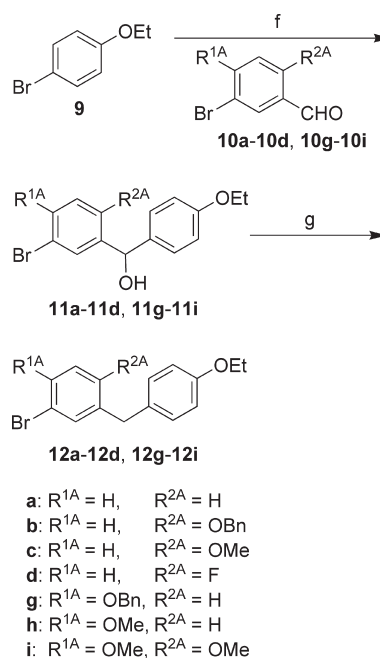
Chemistry

Bozó et al. reported that condensation of 2,3,4,6-tetra-*O*-acetyl 5-thio-D-glucopyranosyl bromide with 4-cyanoben-zenethiol in the presence of K_2CO_3 gives a mixture of α and β-*S*-5-thio-D-glucopyranoside of 4-cyanothiophenol (68%), together with a small amount of *C*-phenyl α- and β-5-thio-D-glucopyranoside derivatives.[43] This strategy for the preparation of *C*-phenyl 1-thio-D-glucitol was not suitable for our purposes, due to its lack of general versatility. Our preparation of *C*-aryl β-glucoside was inspired by the report that addition of an aryl organolithium reagent to a sugar lactone gives a mixture of lactols, which is reduced selectively to afford *C*-aryl β-glucoside.[31,44] It was expected that this approach would be applicable to the preparation of *C*-phenyl 1-thio-D-glucitol; therefore, thiolactone **8** was believed to be an important intermediate. The first synthesis of tetra-*O*-benzyl derivative **7** and thiolactone **8** was reported by Yuasa.[45] Compound **7** was prepared by acid hydrolysis of methyl tetra-*O*-benzyl-5-thio-D-glucoside in moderate yield (50%). We therefore prepared **8** in accordance with Scheme 1, due to the availability of **7**. 5-Thio-D-glucose penta-*O*-acetate **5b** can be prepared in 8 steps from commercially available D-glucur-ono-3,6-lactone (**5a**).[41,46] Anomeric acetyl group of **5b** was selectively removed with hydrazine acetate[47] or a combination of methylhydrazine and acetic acid as described previously.[48]

Scheme 1
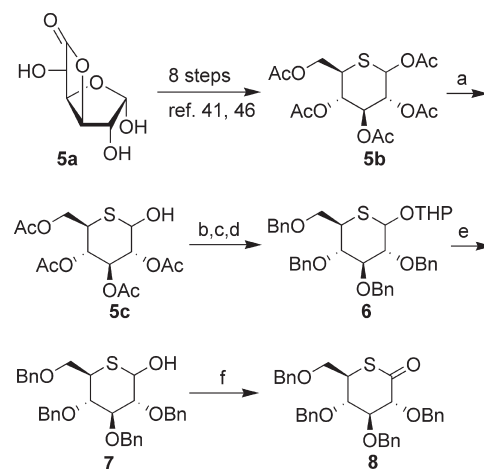
(a) MeNHNH₂, AcOH, DMF (70%); (b) 3,4-DHP, TsOH·H₂O, CHCl₃; (c) NaOMe, MeOH; (d) BnBr, NaH, DMF (96%, 3 steps); (e) PPTS, EtOH (100%); (f) DMSO, Ac₂O (82%).

Scheme 2

a: R^{1A} = H,   R^{2A} = H
b: R^{1A} = H,   R^{2A} = OBn
c: R^{1A} = H,   R^{2A} = OMe
d: R^{1A} = H,   R^{2A} = F
g: R^{1A} = OBn, R^{2A} = H
h: R^{1A} = OMe, R^{2A} = H
i: R^{1A} = OMe, R^{2A} = OMe

(f) *n*-BuLi, THF, −78 °C, then **10**; (g) Et₃SiH, BF₃OEt₂, CH₃CN/CHCl₃.

The resulting anomeric hydroxyl group of **5c** was protected with 3,4-dihydro-2*H*-pyranyl ether in the presence of *p*-tolue-nesulfonic acid monohydrate. Then, the acetyl group was removed by Zemplén deacetylation, and the resulting hydro-xyl groups were protected by a benzyl group using benzyl-bromide with sodium hydride to generate **6**. The tetra-hydropyranyl group was removed by treating **6** with PPTS, and finally DMSO oxidation of **7** provided **8** in 79% total yield.

The aglycon portions **12a–12d** and **12g–12i** were synthe-sized as shown in Scheme 2. After lithium halogen exchange of **9**, benzaldehyde derivatives **10a–10d** and **10g–10i**[49] were added to give **11a–11d** and **11g–11i** in 58–90% yield. Reduction of alcohol **11** using triethylsilane and boron trifluoride diethyletherate gave **12a–12d** and **12g–12i** in 43–99% yield.

Scheme 3
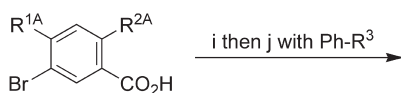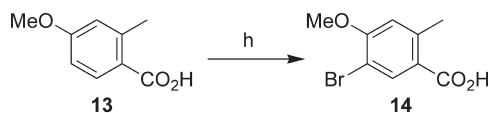
14: R^{1A} = OMe, R^{2A} = Me
15: R^{1A} = H, R^{2A} = Me
16: R^{1A} = H, R^{2A} = Cl

17e, 17f, 17j-17q

12e, 12f, 12j-12q
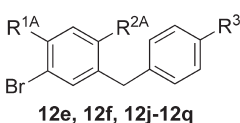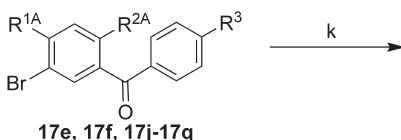
e: R^{1A} = H, R^{2A} = Me, R^3 = OEt
f: R^{1A} = H, R^{2A} = Cl, R^3 = OEt
j: R^{1A} = H, R^{2A} = Cl, R^3 = OMe
k: R^{1A} = H, R^{2A} = Cl, R^3 = Me
l: R^{1A} = H, R^{2A} = Cl, R^3 = Et
m: R^{1A} = H, R^{2A} = Cl, R^3 = iPr
n: R^{1A} = H, R^{2A} = Cl, R^3 = tBu
o: R^{1A} = H, R^{2A} = Cl, R^3 = SMe
p: R^{1A} = OMe, R^{2A} = Me, R^3 = OEt
q: R^{1A} = OMe, R^{2A} = Me, R^3 = Et

(h) Br_2, Fe (cat), $CHCl_3$; (i) $(COCl)_2$, DMF (cat), $CHCl_3$; (j) $AlCl_3$, $CHCl_3$; (k) Et_3SiH, BF_3OEt_2, $CH_3CN/CHCl_3$ for **12e−12p**; $NaBH_4$, $AlCl_3$, THF for **12q**.

The aglycon portions **12e**, **12f**[31] and **12j−12q** were synthesized as shown in Scheme 3. Commercially available 4-methoxy-2-methylbenzoic acid **13**, brominated using bromine in the presence of a catalytic amount of Fe, generated a 1:1 mixture of 3- and 5-bromo derivatives. The desired isomer **14** was isolated after two recrystallizations from MeOH. Friedel−Crafts reaction of a corresponding substituted benzene (Ph-R^3) was carried out using the acid chlorides prepared from **14** and commercially available **15** and **16** with oxalyl chloride to afford **17e**, **17f**, and **17j−17q**, respectively (43−99%). Reduction of **17e**, **17f**, and **17j−17p** using triethylsilane and boron trifluoride diethyletherate gave aglycons **12e**, **12f**, and **12j−12p** (47−96%). Compound **17q**, however, was resistant to the silane reduction conditions, and so was reduced by a combination of sodium borohydride and aluminum chloride to provide **12q** in good yield (90%).

Compound **12r** was prepared as shown in Scheme 4. Friedel−Crafts reaction of cumene using the acid chloride prepared from **13** afforded **17r**. Compound **17r** was reduced under silane reducing conditions, and the obtained diphenylmethane derivative was brominated to give **12r**.

Compound **12s** was synthesized as shown in Scheme 5. After methylation of commercially available **18** with iodomethane under basic conditions, Friedel−Crafts reaction of **19** was performed using 4-ethylbenzoyl chloride to afford **17s**. Subsequent reduction of **17s** using the same conditions described above gave the aglycon portion **12s**.

Scheme 4
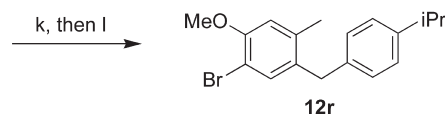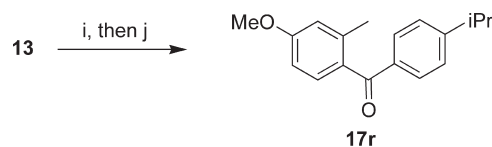
(i) $(COCl)_2$, DMF (cat), $CHCl_3$; (j) cumene, $AlCl_3$, $CHCl_3$; (k) Et_3SiH, BF_3OEt_2, $CH_3CN/CHCl_3$; (l) Br_2, AcOH.

Scheme 5
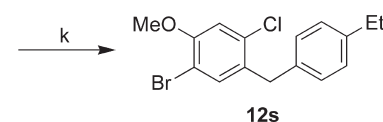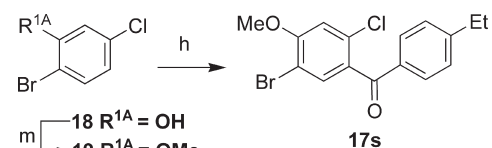
(m) MeI, K_2CO_3, *n*-Bu_4NI, DMF; (h) 4-ethylbenzoylchroride, $AlCl_3$, $CHCl_3$; (k) Et_3SiH, BF_3OEt_2, $CH_3CN/CHCl_3$.

C-Phenyl 1-thio-D-glucitols **3a−3s** were prepared as outlined in Scheme 6. Compounds **20a−20s** were obtained by adding thiolactone **8** to Grignard reagents prepared from compounds **12a−12s** and magnesium powder. Each product was found to be a single isomer, but the anomeric configuration could not be determined. The hydroxyl group of **20a−20s** was reduced β-stereoselectively to afford compounds **21a−21s**. In this reaction, the α-isomer was formed as a byproduct in a yield of several percent, but could be decreased using the following conditions. First, boron trifluoride diethyletherate was selected as the Lewis acid. Second, the most favorable solvent was chosen, and found to be acetonitrile or a mixture of acetonitrile and chloroform. For example, **21p** was obtained with a ratio of 4/96 of α/β in 77% yield. Finally, the benzyl ether of compound **21** was removed by catalytic hydrogenation with palladium hydroxide under a hydrogen atmosphere to afford **3a−3e**, **3g−3i**, and **3p−3r**. Alternatively, compounds **3f**, **3j−3o**, and **3s** were obtained by removal of the benzyl group using Lewis acid conditions to prevent reduction of the chloride.

Results and Discussion

To assess the in vitro potency of SGLT2 inhibition and its selectivity for SGLT1, the inhibitory activities of compounds on SGLTs were determined by a cell-based assay in which inhibition of the rate of glucose uptake was monitored with CHO-K1 cells expressing human SGLT1 and SGLT2.

We were interested in using thioglucose instead of glucose because the ring sulfur atom has little charge, whereas the ring oxygen atom possesses a high negative-charge density.[50] If the binding site interacts hydrophobically with the upper face of the sugar ring, the thioglucose might be a better ligand than its ring oxygen analogue. We selected 3-(4-ethoxybenzyl)phenyl

Scheme 6
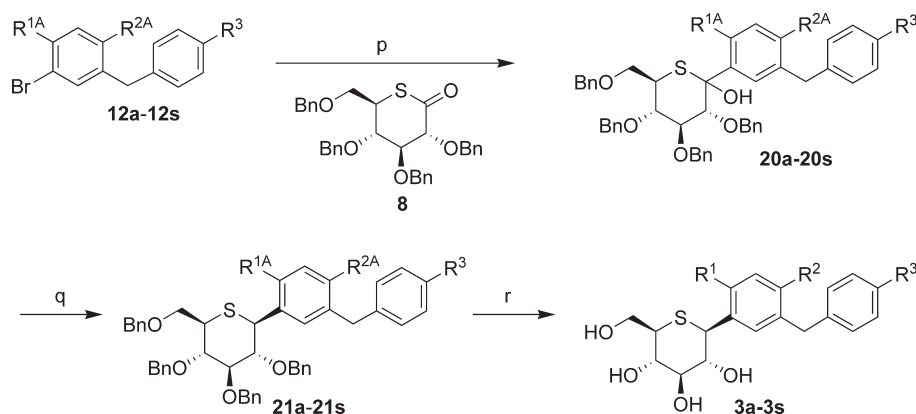
(p) Mg, THF; (q) Et$_3$SiH, BF$_3$OEt$_2$, CH$_3$CN, or CH$_3$CN/CHCl$_3$; (r) Pd(OH)$_2$/H$_2$ (for **3a**−**3e**, **3g**−**3i**, **3p**−**3r**) or AlCl$_3$ in anisole (for **3f**, **3j**−**3n**) or CF$_3$CO$_2$H, Me$_2$S, *m*-cresol, 1,2-ethanedithiol, TfOH (for **3o**, **3s**).

Table 1. hSGLT1 and hSGLT2 Inhibitory Activity[a]
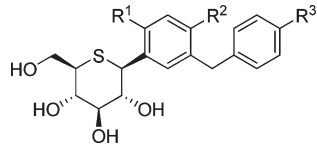

cmpds	R^1	R^2	R^3	SGLT2 (nM) mean (95% CI)	SGLT1 (nM) mean (95% CI)	T1/T2 selectivity
1				27.8 (21.8−35.3)	246 (162−374)	8.8
3a	H	H	OEt	73.6 (51.4−105)	26100 (20300−33700)	355
3b	H	OH	OEt	283 (268−298)	14600 (11500−18500)	51.6
3c	H	OMe	OEt	13.4 (11.3−15.8)	565 (510−627)	42.2
3d	H	F	OEt	9.40 (5.87−15.0)	7960 (7180−8820)	847
3e	H	Me	OEt	2.29 (1.76−2.99)	671 (230−1960)	293
3f	H	Cl	OEt	1.77 (0.95−3.30)	1210 (798−1840)	684
3g	OH	H	OEt	17.4 (15.9−19.0)	4040 (1200−13600)	232
3h	OMe	H	OEt	37.9 (26.4−54.4)	100000 (66500−151000)	2640
3i	OMe	OMe	OEt	10.8 (6.84−17.1)	4270 (1560−11600)	395
3j	H	Cl	OMe	1.68 (1.08−2.60)	260 (72.5−931)	155
3k	H	Cl	Me	1.37 (0.97−1.95)	209 (80.2−545)	153
3l	H	Cl	Et	1.78 (0.88−3.63)	602 (473−767)	338
3m	H	Cl	iPr	4.01 (1.75−9.17)	8160 (4860−13700)	2040
3n	H	Cl	tBu	18.8 (11.0−32.1)	35600 (31900−39800)	1890
3o	H	Cl	SMe	1.16 (0.73−1.85)	391 (239−641)	337
3p	OMe	Me	OEt	2.26 (1.48−3.43)	3990 (2690−5920)	1770
3q	OMe	Me	Et	1.71 (1.19−2.46)	2830 (1540−5200)	1650
3r	OMe	Me	iPr	2.68 (2.15−3.34)	17300 (14100−21100)	6400
3s	OMe	Cl	Et	1.51 (0.75−3.04)	3340 (2710−4110)	2210

[a] IC$_{50}$ values for SGLT1 and 2 activities represent the mean and the 95% confidence intervals (CI) of at least three determinations.

derivative **3a** as a simple lead compound because of its aglycon structure. According to previous reports,[31,42] the positions of substituents on aromatic rings should be important. Compound **3a** showed moderate inhibition activity (73.6 nM for SGLT2) and was 355 times more selective for SGLT2 than for SGLT1 (Table 1). First, we investigated substitution effects on the central benzene moiety. The introduction of a methoxy, fluoro, methyl, or chloro group in R^2 (**3c**−**3f**) increased SGLT2 inhibition potency. Compound **3e** with a methyl group and **3f** with a chloro group showed strong inhibition activity toward SGLT2 at 2.29 and 1.77 nM, respectively. However, the introduction of a hydroxyl group in R^2 (**3b**) decreased potency (283 nM), suggesting that the hydrogen donating property of a hydroxyl group in R^2 may be undesirable. The substitution of R^1 with a hydroxyl (**3g**) or methoxy

(**3h** and **3i**) group resulted in 2−4 times greater potency toward SGLT2 compared to **3a**. Surprisingly, **3h** had decreased SGLT1 inhibition activity compared with **3a** and resulted in a 2640-fold greater selectivity for SGLT2 than for SGLT1. However, the selectivity for SGLT2 (395-fold) of the dimethoxy analogue (**3i**) was same as that of **3a**.

We next studied the structure−activity relationships (SAR) of *para*-position derivatives of the distal benzene moiety while retaining the chloro group in the central benzene ring. From comparison of compounds **3f** and **3j**−**3o** in Table 1, except for **3n** with a tertiary butyl group, compounds with a methyl, ethyl, isopropyl, methoxy, ethoxy, or methylthio substituent strongly inhibited SGLT2 activity and showed IC$_{50}$ values of less than 5 nM. In this series, compound **3m** (with an isopropyl group) and **3n** (with a tertiary butyl group) showed high
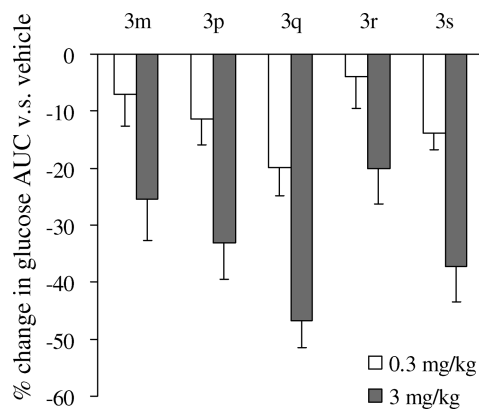
Figure 2. Suppression of glucose $AUC_{0-8\ h}$ in STZ-induced diabetic rats versus vehicle control, following an oral dose of 0.3 or 3.0 mg/kg of **3m**, **3p**, **3s** ($n = 6$) and **3q**, **3r** ($n = 5$). Data are expressed as means $\pm$ SE.

selectivity for SGLT2 over SGLT1 (2040- and 1890-fold, respectively).

Finally, we combined the substituents in R^1, R^2, and R^3 and optimized SGLT2 inhibition potency and selectivity for SGLT2. We selected the methoxy group for R^1 because of its expected high SGLT2 selectivity. A methyl and a chloro group were chosen for R^2, and an ethyl, an ethoxy, and an isopropyl group for R^3 were selected due to their expected potency and selectivity for SGLT2 inhibition activity, based on the above results. Compounds **3p**−**3s** strongly inhibited SGLT2 activity, with IC_{50} values of less than 3 nM, showing that **3p**−**3s** are 10 times more potent than phlorizin (**1**). On the other hand, their SGLT1 inhibitory activities were lower (IC_{50} values were greater than 2.8 μM), and their selectivity for SGLT2 was 1650- to 6400-fold, which is the same as or greater than the selectivity of dapagliflozin (**2**, 1200-fold)[31] and phlorizin (**1**, 8.8-fold).

The potency of **3q** (1.71 nM for SGLT2, 2830 nM for SGLT1) was the same as that of **3s** (1.51 nM for SGLT2, 3340 nM for SGLT1). Comparable potencies were also observed for **3e** and **3f**, indicating that having a methyl or chloro group in R^2 produces equivalent effects. This result indicates that a steric, rather than electronic, effect in R^2 may influence SGLT2 inhibition activity. The selectivity of **3m** and **3r** for SGLT2 was 4.6- and 3.9-fold greater than that of **3l** and **3q**, respectively. From these results, it appears that a methoxy group in R^1 and a bulky isopropyl group in R^3 contribute to a greater selectivity for SGLT2, and that a methyl and a chloro group in R^2 increase potency toward SGLT2.

We selected **3m** and **3p**−**3s**, which had strong SGLT2 inhibition potency (IC_{50} less than 5nM) and high SGLT2 selectivity (more than 1500-fold). These compounds lowered blood glucose in streptozotocin (STZ)-induced diabetic rats when orally administered at 0.3 or 3 mg/kg (Figure 2). Since these compounds showed promise for the effective treatment of type 2 diabetes, they were evaluated further.

Our intention was to identify compounds having favorable pharmacokinetics (PK) profiles in humans. Metabolic stability affects PK and plays a major role in drug clearance, with an increase in metabolic stability leading to a decrease in clearance. We therefore studied the stabilities of **3m** and **3p**−**3s** using human liver microsomes and hepatocytes. Table 2 shows that all five compounds were resistant to human microsomal degradation. However, **3q**−**3s** were largely decomposed after 240 min incubation with human cryopreserved hepatocytes, comparable to that of a control drug, verapamil,

Table 2. In Vitro Human Metabolic Stability of Verapamil and **3m**, **3p**−**3s**

	(% residual)	
cmpds	microsomes[a]	cryopreserved hepatocytes[b]
verapamil	NT[c]	3.9 ± 0.3
3m	96	22.4 ± 3.5
3p	96	69.0 ± 7.0
3q	90	4.7 ± 1.3
3r	91	6.8 ± 0.9
3s	89	3.7 ± 1.3

[a] Test compounds (5 μmol/L) were incubated with human liver microsomes (1 mg/mL protein) in the presence of an NADPH-generating system for 15 min at 37 °C. [b] Test compounds (5 μmol/L) were incubated with human cryopreserved hepatocytes (1.0×10^6 cells/mL) for 240 min at 37 °C. Each value represents the mean $\pm$ SD of triplicate samples. [c] NT, not tested.

Table 3. Serum Protein Binding of **3m**, **3p**−**3s** in Human

cmpds	serum protein binding (%)[a]
3m	99.7 ± 0.0
3p	96.2 ± 0.2
3q	98.3 ± 0.1
3r	99.1 ± 0.1
3s	99.4 ± 0.0

[a] Serum protein binding was determined by equilibrium dialysis at 1 μg/mL compounds. Each value represents the mean $\pm$ SD of triplicate samples.

which has a Cl_{human} of 11.8 ± 5.0 mL/min/kg and a F_{human} of $20 \pm 12\%$.[51] Further, we compared **3a** with **4** ($R^A = H$, $R^B = $ 4-OEt) which had the same aglycon of **3a**. The stability of **3a** was greater than that of **4** against cryopreserved hepatocytes (data not shown). Although interesting information was known that O-5-thioglucoside linkage would be resistant to bacterial glucosidases,[39,40] O-aryl 5-thio-β-glucoside **4** may be less stable in human tissues. In contrast, $22.4 \pm 3.5\%$ of **3m** and $69.0 \pm 7.0\%$ of **3p** remained after 240 min incubation with human cryopreserved hepatocytes. These results indicate that the clearance of **3m** and **3p** may be slower than that of verapamil in humans. Serum protein binding (SPB) also impacts both PK and exposure to the therapeutic target. Human SPB of **3p** was 96.2%, indicating that the free fraction of **3p** was 3.8%, which is 13 times higher than that of **3m** (free fraction of **3m** was 0.3%) (Table 3). It was expected that compound **3p** would be readily absorbed via the intestine, based on Caco-2 assay results (14.4 ± 2.2 cm/s $\times 10^6$). This result was comparable with that of a positive control, propranolol (9.1 ± 0.5 cm/s $\times 10^6$).[52] These results suggested that **3p** would have good absorption and distribution profiles and would be stable in human tissues. We therefore selected and administered **3p** orally and intravenously to rats and dogs at 1 mg/kg (Table 4). The bioavailability ($F = 35.3\%$) and total clearance (36.3 ± 2.67 mL/min/kg) of **3p** in rats were moderate. On the other hand, C_{max} was 914 ± 73.4 ng/mL at 0.67 h in dogs, with excellent bioavailability (92.7%) and a slow clearance rate of 3.18 ± 0.260 mL/min/kg. The volume of distribution at steady state (Vdss) was 0.8 ± 0.06 L/kg, similar to total body water in dog (ca. 0.6 L/kg), indicating modest extravascular distribution. Compared to the disclosed PK parameters of dapagliflozin (**2**) in dogs,[53] the bioavailability (F), Vdss, and T_{max} were similar to those of **3p**. The half-life ($T_{1/2}$) of **3p**, however, was shorter than that of **2**. This may be attributed to the considerably slower clearance rate of **2** (1.5 ± 0.2 mL/min/kg).

Table 4. Pharmacokinetic Parameters of **3p** after Oral and Intravenous Administration to Rats and Dogs

cmps (species)	**3p** (rats)[a]	**3p** (dogs)[a]	**2** (dogs)[b]
dose (mg/kg)	1	1	6.6
C_{max} (ng/mL)	35.7 ± 17.0	914 ± 73.4	10 700 ± 1600
T_{max} (h)	0.50 ± 0.00	0.67 ± 0.29	0.6 ± 0.4
$T_{1/2}$ (h)	2.93 ± 2.00	4.07 ± 0.25	7.4 ± 1.2
F (%)	35.3	92.7	83 ± 2
Vd_{ss} (L/kg)	2.63 ± 0.57	0.80 ± 0.06	0.80 ± 0.1
Cl (mL/min/kg)	36.3 ± 2.67	3.19 ± 0.26	1.5 ± 0.2

[a] Each data represents mean ± SD of three animals. [b] PK parameters of dapagliflozin (**2**) were quoted from the ref 53.

Table 5. Distribution of **3p** to the Kidney in Rats

	concentration (ng/mL or g)[a]		
time (h)	plasma	kidney	kidney/plasma ratio
1	23.1 ± 3.2	562 ± 199	23.9 ± 5.1
4	13.9 ± 3.9	488 ± 123	35.3 ± 0.8
24	NC[b]	NC	NC

[a] Concentrations of **3p** in plasma and kidney in rats were measured at 1, 4, and 24 h after oral administration at 1 mg/kg. Each value represents mean ± SD of three animals. [b] NC, (not calculated) means limit of detection.

Table 6. Enhancement of Urinary Glucose Excretion after Oral Glucose Loading in Zucker Fatty Rats and Beagle Dogs

	urinary glucose output (mg/24 h)	
	rats[a]	dogs[b]
3p (1 mg/kg)	179.4 ± 15.27	20129 ± 1870
vehicle control	6.07 ± 3.58	—
normal control	0.98 ± 0.08	7.64 ± 1.95

[a] Compound **3p** (1 mg/kg) was administered orally to Zucker fatty rats (n = 8, mean body weight, approximately 380 g) 0.5 h prior to 2 g/kg glucose loading. Urine samples were collected for 24 h using metabolic cages. [b] Compound **3p** (1 mg/kg) was administered orally to beagle dogs (n = 10, body weight, 8.1−9.6 kg) 1 h prior to 2 g/kg glucose loading. Urine samples were collected for 24 h using metabolic cages. Each value represents mean ± SD.

Furthermore, kidney/plasma ratio was 24−35-fold higher, indicating that appreciable amounts of **3p** are distributed to the target organ (kidney) (Table 5). In contrast, **3p** was excreted within 24 h in rats and was not detected in nontarget organs such as liver, heart, and brain 24 h after administration at 3 mg/kg, p.o. Most metabolites of [^{14}C] **3p** in rats were excreted to bile. (data not shown) Compound **3p** should be well-distributed to kidney and be eliminated primarily by metabolism in liver.

In Zucker fatty rats after oral glucose loading, a single oral dose of **3p** (1 mg/kg) increased urinary glucose excretion to 30 times that of the vehicle control and 180 times that of normal control Zucker lean rats over 24 h. Experiments in dogs showed over 2600-fold enhancement of the urinary glucose level after 1 mg/kg oral administration of **3p** (Table 6). This may be attributed to higher bioavailability of **3p** in dogs (Table 4).

Conclusion

We described the synthesis of *C*-phenyl 1-thio-D-glucitols **3a**−**3s** and revealed the SAR for the substitution of various groups on the aromatic rings. A methoxy group adjacent to the sugar moiety was important for expression of SGLT2 selectivity, whereas a chloro or methy group *para* to the sugar moiety strongly increased SGLT2 potency. Optimization of

the substituents on the aromatic rings generated potent SGLT2 selective inhibitors (**3m**, **3p**−**3s**) which lowered blood glucose in STZ-induced diabetic rats. In order to estimate the pharmacological effects in humans, we conducted further in vitro evaluations and found that compound **3p** was 1650-fold more selective for SGLT2 with an IC_{50} of 2.26 nM than for SGLT1 and the highest metabolic stability toward degradation by human cryopreserved hepatocytes. Compound **3p** also had the lowest human serum protein binding of the five compounds tested. Further, the Caco-2 permeability of **3p** was comparable with that of propranolol. For these reasons, it was anticipated that compound **3p** would have good PK profiles in humans. Compound **3p** ((1*S*)-1,5-anhydro-1-[5-(4-ethoxybenzyl)-2-methoxy-4-methylphenyl]-1-thio-D-glucitol; **TS-071**) was therefore chosen as a clinical candidate. In order to confirm the pharmacology of **3p**, PK profiles and urinary glucose excretion effects were evaluated in animals. Compound **3p** showed excellent urinary glucose excretion and PK profiles in dogs. Provided that compound **3p** has desirable PK profiles in humans, **3p** will be useful for treating type 2 diabetes by means of SGLT2 inhibition. These results demonstrate the utility of thioglucose as a SGLT2 inhibitor, and indicate that thioglucose works as a glucose analogue. Compound **3p** is currently undergoing phase II clinical trials.

Experimental Section

Chemistry. Melting points were determined on a Yanaco MP-500D melting point apparatus and are uncorrected. All reactions were monitored using TLC plates Silicagel gel 60F$_{254}$ (Merck). 200, 300, and 600 MHz NMR spectra were obtained using a Varian Gemini 200, Varian Unity Inova 300, or JEOL JNM-ECA600, respectively. Chemical shifts are reported in parts per million relative to tetramethylsilane (TMS) used as an internal standard. Electron impact (EI) mass spectra were taken on a Perkin-Elmer Sciex API-300 mass spectrometer. Electrospray ionization (ESI) mass spectra were taken on a Micromass Platform LC mass spectrometer. Elemental analyses were performed on a Perkin-Elmer 2400 elemental analyzers. Silica gel column chromatography was performed on Silica gel 60 (Kanto Chemical) or NH-silica gel chromatorex 1020 (Fuji Silicia), using the solvent systems (volume ratios) indicated below. Purity of the test compounds was determined to be >95% by HPLC (shiseido capcell pak UG120 4.6 × 150 mm, acetonitrile/water = 4/6, 1 mL/min of flow rate at 40 °C, and detection at 220 nm) or elemental analyses.

General Methods for Removal of the Benzyl Group (Method A). (1*S*)-1,5-Anhydro-1-[3-(4-ethoxybenzyl)phenyl]-1-thio-D-glucitol (3a). A mixture of (1*S*)-1,5-anhydro-2,3,4,6-tetra-*O*-benzyl-1-[3-(4-ethoxybenzyl)phenyl]-1-thio-D-glucitol (**21a**) (550 mg, 0.732 mmol), 20% palladium hydroxide on carbon of Degussa type (550 mg) in 15 mL of ethyl acetate and 15 mL of ethanol was stirred under a hydrogen atmosphere at room temperature for 48 h. The insoluble components were filtered off using a Celite pad and the filtrate was concentrated. The residue was purified by silica gel column chromatography (CHCl$_3$/MeOH = 10/1) to give 0.5 hydrate of **3a** as a colorless powder (60 mg, 21%). mp 110.0−113.0 °C. ^{1}H NMR (300 MHz, MeOH-d_4) δ 1.35 (t, *J* = 7.0 Hz, 3 H), 2.92−3.03 (m, 1 H), 3.19−3.28 (m, 1 H), 3.59 (dd, *J* = 9.1, 10.2 Hz, 1 H), 3.69−3.78 (m, 3 H), 3.88 (s, 2 H), 3.90−4.04 (m, 3 H), 6.80 (d, *J* = 8.7 Hz, 2 H), 7.04−7.11 (m, 3 H), 7.14−7.25 (m, 3 H). MS (ESI) *m/z* 413 (M+Na), 389 (M−H). Anal. Calcd for (C$_{21}$H$_{26}$O$_5$S·0.5H$_2$O) C, 63.14; H, 6.81. Found C, 63.38; H, 6.73.

(1*S*)-1,5-Anhydro-1-[3-(4-ethoxybenzyl)-4-hydroxyphenyl]-1-thio-D-glucitol (3b). Compound **3b** (0.117 g, 66%) was prepared

as a colorless powder from **21b** (0.566 g, 0.660 mmol) according to the method described for the synthesis of **3a**. (Method A) mp 95.0−100.0 °C. ^{1}H NMR (300 MHz, MeOH-d_4) δ 1.35 (t, J=6.9 Hz, 3 H), 2.89−2.98 (m, 1 H), 3.16−3.24 (m, 1 H), 3.56 (dd, J = 9.0, 10.2 Hz, 1 H), 3.60−3.76 (m, 3 H), 3.83 (s, 2 H), 3.88−3.95 (m, 1 H), 3.98 (q, J=6.9 Hz, 2 H), 6.69−6.80 (m, 3 H), 6.96−7.04 (m, 2 H), 7.06−7.15 (m, 2 H). MS (ESI) m/z 424 (M+Na), 405 (M−H). Anal. Calcd for ($C_{21}H_{26}O_6S \cdot 1.1H_2O$) C, 59.17; H, 6.67. Found C, 59.14; H, 6.32.

(1*S*)-1,5-Anhydro-1-[3-(4-ethoxybenzyl)-4-methoxyphenyl]-1-thio-D-glucitol (3c). Compound **3c** (0.385 g, 66%) was prepared as a colorless powder from **21c** (0.880 g, 1.13 mmol) according to the method described for the synthesis of **3a**. (Method A) mp 89.0−95.0 °C. ^{1}H NMR (300 MHz, MeOH-d_4) δ 1.35 (t, J=7.0 Hz, 3 H), 2.91−3.01 (m, 1 H), 3.18−3.25 (m, 1 H), 3.57 (dd, J=9.0, 10.3 Hz, 1 H), 3.68−3.76 (m, 3 H), 3.79 (s, 3 H), 3.84 (s, 2 H), 3.89−4.02 (m, 3 H), 6.76 (d, J=8.7 Hz, 2 H), 6.88 (d, J=8.7 Hz, 1 H), 7.03−7.11 (m, 3 H), 7.17 (dd, J=2.3, 8.6 Hz, 1 H). MS (ESI) m/z 443 (M+Na), 419 (M−H). Anal. Calcd for ($C_{22}H_{28}O_6S \cdot H_2O$) C, 60.25; H, 6.90. Found C, 60.49; H, 6.75.

(1*S*)-1,5-Anhydro-1-[3-(4-ethoxybenzyl)-4-fluorophenyl]-1-thio-D-glucitol (3d). Compound **3d** (0.305 g, 75%) was prepared as a colorless powder from **21d** (0.767 g, 0.997 mmol) according to the method described for the synthesis of **3a**. (Method A) mp 60.0−65.0 °C. ^{1}H NMR (300 MHz, MeOH-d_4) δ 1.35 (t, J=7.0 Hz, 3 H), 2.91−3.03 (m, 1 H), 3.19−3.25 (m, 1 H), 3.58 (dd, J = 9.0, 10.3 Hz, 1 H), 3.68−3.79 (m, 3 H), 3.86−4.04 (m, 5 H), 6.77−6.82 (m, 2 H), 6.95−7.04 (m, 1 H), 7.07−7.12 (m, 2 H), 7.15−7.24 (m, 2 H). MS (ESI) m/z 431 (M+Na). Anal. Calcd for ($C_{21}H_{25}FO_5S \cdot 0.5H_2O$) C, 60.41; H, 6.28. Found C, 60.22; H, 6.09.

(1*S*)-1,5-Anhydro-1-[3-(4-ethoxybenzyl)-4-methylphenyl]-1-thio-D-glucitol (3e). Compound **3e** (0.181 g, 33%) was prepared as a colorless powder from **21e** (1.04 g, 1.36 mmol) according to the method described for the synthesis of **3a**. (Method A) mp 105.0−107.0 °C. ^{1}H NMR (300 MHz, MeOH-d_4) δ 1.35 (t, J = 6.9 Hz, 3 H), 2.17 (s, 3 H), 2.93−3.03 (m, 1 H), 3.19−3.28 (m, 1 H), 3.59 (dd, J=9.0, 10.2 Hz, 1 H), 3.68−3.79 (m, 3 H), 3.89 (s, 2 H), 3.93 (dd, J = 3.7, 7.9 Hz, 1 H), 3.97 (q, J = 6.9 Hz, 2 H), 6.74−6.82 (m, 2 H), 6.96−7.04 (m, 2 H), 7.05−7.15 (m, 3 H). MS (ESI) m/z 422 (M+NH4), 403 (M−H). Anal. Calcd for ($C_{22}H_{28}$-$O_5S \cdot 0.4H_2O$) C, 64.18; H, 7.05. Found C, 64.13; H, 6.94.

General Methods for Removal of the Benzyl Group (Method B). (1*S*)-1,5-Anhydro-1-[4-chloro-3-(4-ethoxybenzyl)phenyl]-1-thio-D-glucitol (3f). To a solution of **21f** (1.58 g, 2.01 mmol) in anisole (15 mL) at 4 °C was added AlCl3 (2.68 g, 21.1 mmol). The reaction mixture was allowed to warm to room temperature, stirred for 0.5 h, then poured into ice. The resulting mixture was extracted with ethyl acetate twice, and the combined organic layers were washed with 1 M hydrochloric acid, saturated aqueous NaHCO3, and brine, and then dried over MgSO4 and concentrated. The residue was purified by silica gel column chromatography (CHCl3/MeOH = 10/1) to give **3f** (0.16 g, 20%) as a colorless powder. mp 79.0−83.0 °C. ^{1}H NMR (300 MHz, MeOH-d_4) δ 1.36 (t, J = 7.0 Hz, 3 H), 2.94−3.03 (m, 1 H), 3.22 (t, J = 8.2 Hz, 1 H), 3.57 (dd, J = 9.0, 10.3 Hz, 1 H), 3.65−3.78 (m, 3 H), 3.89−4.05 (m, 5 H), 6.80 (d, J=8.7 Hz, 2 H), 7.08 (d, J=8.7 Hz, 2 H), 7.16−7.23 (m, 2 H), 7.32 (d, J=8.3 Hz, 1 H). MS (ESI) m/z 447 (M+Na), 449 (M+2+Na). Anal. Calcd for ($C_{21}H_{25}ClO_5S \cdot 0.7H_2O$) C, 57.65; H, 6.08. Found C, 57.56; H, 6.00.

(1*S*)-1,5-Anhydro-1-[5-(4-ethoxybenzyl)-2-hydroxyphenyl]-1-thio-D-glucitol (3g). Compound **3g** (0.142 g, 37%) was prepared as a colorless powder from **21g** (0.810 g, 0.945 mmol) according to the method described for the synthesis of **3a**. (Method A) mp 145.0−150.0 °C. ^{1}H NMR (600 MHz, MeOH-d_4) δ 1.37 (t, J=7.0 Hz, 3 H), 2.94−3.05 (m, 1 H), 3.22−3.29 (m, 1 H), 3.58−3.62 (m, 1 H), 3.69−3.88 (m, 4 H), 3.90−4.04 (m, 3 H), 4.33 (d, J=10.6 Hz, 1 H), 6.71 (d, J=8.2 Hz, 1 H), 6.76−6.90 (m, 3 H), 7.03−7.15 (m, 3 H). MS (ESI) m/z 429 (M+Na), 405 (M−H).

Anal. Calcd for ($C_{21}H_{26}O_6S \cdot 1.2H_2O$) C, 58.92; H, 6.69. Found C, 58.87; H, 6.40.

(1*S*)-1,5-Anhydro-1-[5-(4-ethoxybenzyl)-2-methoxyphenyl]-1-thio-D-glucitol (3h). Compound **3h** (0.037 g, 7.2%) was prepared as a colorless powder from **21h** (0.87 g, 1.11 mmol) according to the method described for the synthesis of **3a**. (Method A) mp 130.0−130.5 °C. ^{1}H NMR (600 MHz, MeOH-d_4) δ 1.33 (t, J = 7.1 Hz, 3 H), 2.90−2.98 (m, 1 H), 3.22 (t, J = 8.9 Hz, 1 H), 3.51−3.61 (m, 1 H), 3.65−3.74 (dd, J = 6.4, 11.5 Hz, 1 H), 3.76−3.84 (m, 6 H), 3.91 (dd, J = 3.7, 11.5 Hz, 1 H), 3.96 (q, J=7.1 Hz, 2 H), 4.31 (brs 1 H), 6.77 (d, J=8.7 Hz, 2 H), 6.85 (d, J=8.7 Hz, 1 H), 7.00 (dd, J=2.3, 8.7, Hz, 1 H), 7.04 (d, J=8.7 Hz, 2 H), 7.16 (brs, 1 H). MS (ESI) m/z 443 (M+Na). Anal. Calcd for ($C_{22}H_{28}O_6S \cdot H_2O$) C, 60.25; H, 6.90. Found C, 60.00; H, 6.76.

(1*S*)-1,5-Anhydro-1-[2,4-dimethoxy-5-(4-ethoxybenzyl)phenyl]-1-thio-D-glucitol (3i). Compound **3i** (0.156 g, 66%) was prepared as a colorless powder from **21i** (0.428 g, 0.527 mmol) according to the method described for the synthesis of **3a**. (Method A) mp 158.0−160.0 °C. ^{1}H NMR (600 MHz, MeOH-d_4) δ 1.34 (t, J = 7.0 Hz, 3 H), 2.88−2.99 (m, 1 H), 3.22 (t, J=8.8 Hz, 1 H), 3.51−3.59 (m, 1 H), 3.66−3.79 (m, 4 H), 3.81 (s, 3 H), 3.84 (s, 3 H), 3.88−4.01 (m, 3 H), 4.21−4.32 (m, 1 H), 6.57 (s, 1 H), 6.75 (d, J = 8.7 Hz, 2 H), 7.03 (s, 1 H), 7.04 (d, J=8.7 Hz, 2 H). MS (ESI) m/z 468 (M+NH4), 449 (M−H). Anal. Calcd for ($C_{23}H_{30}$-$O_7S \cdot 0.5H_2O$) C, 60.11; H, 6.80. Found C, 60.22; H, 6.61.

(1*S*)-1,5-Anhydro-1-[4-chloro-3-(4-methoxybenzyl)phenyl]-1-thio-D-glucitol (3j). Compound **3j** (0.100 g, 19%) was prepared as a colorless powder from **21j** (1.01 g, 1.31 mmol) according to the method described for the synthesis of **3f**. (Method B) mp 71.0−74.0 °C. ^{1}H NMR (600 MHz, MeOH-d_4) δ 2.96−3.01 (m, 1 H), 3.21−3.25 (m, 1 H), 3.57 (dd, J = 8.9, 10.3 Hz, 1 H), 3.66−3.74 (m, 3 H), 3.75 (s, 3 H), 3.93 (dd, J=3.7, 11.5 Hz, 1 H), 3.98−4.05 (m, 2 H), 6.82 (d, J=8.7 Hz, 2 H), 7.09 (d, J=8.7 Hz, 2 H), 7.18−7.22 (m, 2 H), 7.32 (d, J=8.3 Hz, 1 H). MS (ESI) m/z 428 (M+NH4), 430 (M+2+NH4). Anal. Calcd for ($C_{20}H_{23}O_4$-ClS) C, 58.46; H, 5.46. Found C, 58.36; H, 5.55.

(1*S*)-1,5-Anhydro-1-[4-chloro-3-(4-methylbenzyl)phenyl]-1-thio-D-glucitol (3k). Compound **3k** (0.183 g, 23%) was prepared as a colorless powder from **21k** (1.54 g, 2.04 mmol) according to the method described for the synthesis of **3f**. (Method B) mp 151.0−153.0 °C. ^{1}H NMR (300 MHz, MeOH-d_4) δ 2.28 (s, 3 H), 2.94−3.02 (m, 1 H), 3.18−3.26 (m, 1 H), 3.57 (dd, J = 8.9, 10.2 Hz, 1 H), 3.65−3.77 (m, 3 H), 3.93 (dd, J = 3.7, 11.4 Hz, 1 H), 4.02 (s, 2 H), 7.02−7.10 (m, 4 H), 7.16−7.24 (m, 2 H), 7.29−7.35 (m, 1 H). MS (ESI) m/z 412 (M+Na), 414 (M+2+Na). Anal. Calcd for ($C_{20}H_{23}ClO_4S \cdot 0.5H_2O$) C, 59.74; H, 5.97. Found C, 59.66; H, 5.90.

(1*S*)-1,5-Anhydro-1-[4-chloro-3-(4-ethylbenzyl)phenyl]-1-thio-D-glucitol (3l). Compound **3l** (0.299 g, 26%) was prepared as a colorless powder from **21l** (2.13 g, 2.77 mmol) according to the method described for the synthesis of **3f**. (Method B) mp 142.0−144.0 °C. ^{1}H NMR (300 MHz, MeOH-d_4) δ 1.20 (t, J = 7.6 Hz, 3 H), 2.59 (q, J = 7.6 Hz, 2 H), 2.94−3.03 (m, 1 H), 3.18−3.27 (m, 1 H), 3.57 (dd, J=9.1, 10.3 Hz, 1 H), 3.66−3.78 (m, 3 H), 3.93 (dd, J=3.6, 11.5 Hz, 1 H), 4.03 (s, 2 H), 7.06−7.11 (m, 4 H), 7.17−7.25 (m, 2 H), 7.33 (d, J=8.1 Hz, 1 H). MS (ESI) m/z 431 (M+Na), 433 (M+2+Na). Anal. Calcd for ($C_{21}H_{25}$-$ClO_4S \cdot 0.4H_2O$) C, 60.61; H, 6.25. Found C, 60.55; H, 6.13.

(1*S*)-1,5-Anhydro-1-[4-chloro-3-[4-(propan-2-yl)benzyl]phenyl]-1-thio-D-glucitol (3m). Compound **3m** (0.280 g, 26%) was prepared as a colorless powder from **21m** (2.01 g, 2.57 mmol) according to the method described for the synthesis of **3f**. (Method B) mp 72.0−83.0 °C. ^{1}H NMR (300 MHz, MeOH-d_4) δ 1.21 (d, J=6.8 Hz, 3 H), 2.76−2.92 (m, 1 H), 2.94−3.03 (m, 1 H), 3.19−3.27 (m, 1 H), 3.58 (dd, J = 9.2, 10.1 Hz, 1 H), 3.66−3.79 (m, 3 H), 3.94 (dd, J=3.6, 11.4 Hz, 1 H), 4.03 (s, 2 H), 7.06−7.15 (m, 4 H), 7.17−7.26 (m, 2 H), 7.30−7.35 (m, 1 H). MS (ESI) m/z 445 (M+Na), 447 (M+2+Na), 421 (M−H). Anal.

Calcd for (C$_{22}$H$_{27}$ClO$_4$S·0.5H$_2$O) C, 61.17; H, 6.53. Found C, 61.06; H, 6.44.

(1*S*)-1,5-Anhydro-1-[3-(4-*tert*-butylbenzyl)-4-chlorophenyl]-1-thio-D-glucitol (3n). Compound **3n** (0.101 g, 18%) was prepared as a colorless powder from **21n** (1.04 g, 1.30 mmol) according to the method described for the synthesis of **3f**. (Method B) mp 94.0−100.0 °C. ^{1}H NMR (300 MHz, MeOH-d_4) δ 1.29 (s, 9 H), 2.90−3.05 (m, 1 H), 3.23 (t, *J* = 8.7 Hz, 1 H), 3.58 (dd, *J* = 8.7, 10.1 Hz, 1 H), 3.64−3.80 (m, 3 H), 3.94 (dd, *J* = 3.5, 11.4 Hz, 1 H), 4.04 (s, 2 H), 7.10 (d, *J* = 8.2 Hz, 2 H), 7.16−7.37 (m, 5 H). MS (ESI) *m/z* 454 (M+NH$_4$), 456 (M+2+NH$_4$). Anal. Calcd for (C$_{23}$H$_{29}$ClO$_4$S) C, 63.22; H, 6.69. Found C, 62.83; H, 6.64.

General Methods for Removal of the Benzyl Group (Method C). (1*S*)-1,5-Anhydro-1-[4-chloro-3-[4-(methylsulfanyl)benzyl]phenyl]-1-thio-D-glucitol (3o). To a stirred mixture of **21o** (3.00 g, 3.81 mmol) in trifluoroacetic acid (1.5 mL), dimethylsulfide (9.0 mL), *m*-cresol (2.4 mL), and 1,2-ethanedithiol (0.6 mL) was added trifluoromethane sulfonic acid (3.0 mL) at −15 °C. After stirring for 20 min, the mixture was poured into a mixture of ice and saturated aqueous NaHCO$_3$. The resulting mixture was extracted with ethyl acetate, and the organic layer was washed with saturated aqueous NaHCO$_3$ twice, brine, and then dried over MgSO$_4$ and concentrated. The residue was purified by silica gel column chromatography (CHCl$_3$/MeOH = from 100/1 to 10/1) to give a residue, which was recrystallized from MeOH/water = 2/1 to afford **3o** (0.865 g, 53%) as a colorless powder. ^{1}H NMR (600 MHz, MeOH-d_4) δ 2.43 (s, 3 H), 2.95−3.03 (m, 1 H), 3.23 (t, *J* = 8.7 Hz, 1 H), 3.58 (dd, *J* = 8.9, 10.3 Hz, 1 H), 3.68−3.77 (m, 3 H), 3.93 (dd, *J* = 3.2, 11.5 Hz, 1 H), 4.00−4.09 (m, 2 H), 7.09−7.13 (m, 2 H), 7.15−7.19 (m, 2 H), 7.21 (dd, *J* = 2.3, 8.3 Hz, 1 H), 7.23 (d, *J* = 2.3 Hz, 1 H), 7.33 (d, *J* = 8.3 Hz, 1 H). MS (ESI) *m/z* 449 (M+Na), 451 (M+2+Na), 425 (M−H) 427 (M+2−H). Anal. Calcd for (C$_{20}$H$_{23}$ClO$_4$S$_2$·0.5H$_2$O) C, 55.09; H, 5.56. Found C, 54.90; H, 5.55.

(1*S*)-1,5-Anhydro-1-[5-(4-ethoxybenzyl)-2-methoxy-4-methylphenyl]-1-thio-D-glucitol (3p). Compound **3p** (0.281 g, 81%) was prepared as a colorless powder from **21p** (0.630 g, 0.792 mmol) according to the method described for the synthesis of **3a**. (Method A) mp 155.0−157.0 °C. ^{1}H NMR (600 MHz, MeOH-d_4) δ 1.35 (t, *J* = 6.9 Hz, 3 H), 2.17 (s, 3 H), 2.92−3.01 (m, 1 H), 3.24 (t, *J* = 8.7 Hz, 1 H), 3.54−3.60 (m, 1 H), 3.72 (dd, *J* = 6.4, 11.5, Hz, 1 H), 3.81 (s, 3 H), 3.83 (s, 2 H), 3.94 (dd, *J* = 3.7, 11.5 Hz, 1 H), 3.97 (q, *J* = 6.9 Hz, 2 H), 4.33 (brs, 1 H), 6.77 (d, *J* = 8.3 Hz, 2 H), 6.76 (s, 1 H), 6.99 (d, *J* = 8.3 Hz, 2 H), 7.10 (s, 1 H). MS (ESI) *m/z* 452 (M+NH$_4$). Anal. Calcd for (C$_{23}$H$_{30}$O$_6$S·0.5H$_2$O) C, 62.28; H, 7.06. Found C, 62.39; H, 7.10.

(1*S*)-1,5-Anhydro-1-[5-(4-ethylbenzyl)-2-methoxy-4-methylphenyl]-1-thio-D-glucitol (3q). Compound **3q** (0.61 g, 68%) was prepared as a colorless powder from **21q** (1.68 g, 2.16 mmol) according to the method described for the synthesis of **3a**. (Method A) ^{1}H NMR (300 MHz, MeOH-d_4) δ 1.19 (t, *J* = 7.6 Hz, 3 H), 2.17 (s, 3 H), 2.58 (q, *J* = 7.6 Hz, 2 H), 2.91−3.02 (m, 1 H), 3.19−3.28 (m, 1 H), 3.58 (dd, *J* = 9.2, 10.3 Hz, 1 H), 3.67−3.89 (m, 7 H), 3.94 (dd, *J* = 3.7, 11.5 Hz, 1 H), 4.25−4.39 (m, 1 H), 6.76 (s, 1 H), 6.96−7.09 (m, 4 H), 7.12 (s, 1 H). MS (ESI) *m/z* 441(M+Na), 417 (M−H). Anal. Calcd for (C$_{23}$H$_{30}$O$_5$S·0.3H$_2$O) C, 65.16; H, 7.27. Found C, 65.18; H, 7.32.

(1*S*)-1,5-Anhydro-1-[2-methoxy-4-methyl-5-[4-(propan-2-yl)benzyl]phenyl]-1-thio-D-glucitol (3r). Compound **3r** (0.31 g, 83%) was prepared as a colorless powder from **17r** (0.69 g, 0.87 mmol) according to the method described for the synthesis of **3a**. (Method A) ^{1}H NMR (600 MHz, MeOH-d_4) δ 1.21 (d, *J* = 6.9 Hz, 6 H), 2.17 (s, 3 H), 2.80−2.86 (m, 1 H), 2.94−2.99 (m, 1 H), 3.25 (m, 1 H), 3.58 (dd, *J* = 9.2, 10.1 Hz, 1 H), 3.72 (dd, *J* = 6.6, 11.5 Hz, 1 H), 3.77−3.91 (m, 6 H), 3.94 (dd, *J* = 3.7, 11.5 Hz, 1 H), 4.28−4.38 (m, 1 H), 6.76 (s, 1 H), 7.00 (d, *J* = 8.3 Hz, 2 H,) 7.08 (d, *J* = 8.3 Hz, 2 H), 7.13 (s, 1 H). MS (ESI) *m/z* 455 (M+Na), 431 (M−H). Anal. Calcd for (C$_{24}$H$_{32}$O$_5$S·0.6H$_2$O) C, 65.01; H, 7.55. Found C, 65.07; H, 7.49.

(1*S*)-1,5-Anhydro-1-[4-chloro-5-(4-ethylbenzyl)-2-methoxyphenyl]-1-thio-D-glucitol (3s). Compound **3s** (0.35 g, 24%) was prepared as a colorless powder from **21s** (2.65 g, 3.31 mmol) according to the method described for the synthesis of **3o**. (Method C) ^{1}H NMR (600 MHz, MeOH-d_4) δ 1.19 (t, *J* = 7.6 Hz, 3 H), 2.59 (q, *J* = 7.6 Hz, 2 H), 2.93−3.00 (m, 1 H), 3.23 (t, *J* = 8.9 Hz, 1 H), 3.56 (dd, *J* = 8.9, 10.3 Hz, 1 H), 3.67−3.87 (m, 5 H), 3.90−4.02 (m, 3 H), 4.22−4.37 (m, 1 H), 6.99 (s, 1 H), 7.03−7.11 (m, 4 H), 7.23 (s, 1 H). MS (ESI) *m/z* 461 (M+Na). Anal. Calcd for (C$_{22}$H$_{27}$ClO$_5$S·H$_2$O) C, 56.70; H, 6.50. Found C, 56.40; H, 6.45.

2,3,4,6-Tetra-*O*-acetyl-5-thio-D-glucopyranose (5c). To a solution of 1,2,3,4,6-penta-*O*-acetyl-5-thio-D-glucopyranose (**5b**)[41,46] (42.0 g, 103 mmol) in DMF (300 mL) was added a mixture of methylhydrazine (5.76 g, 125 mmol), AcOH (7.50 g, 125 mmol), and DMF (125 mL), and the resulting mixture was stirred at room temperature for 2 h, followed by adding an additional mixture of 0.967 g methylhydrazine, 1.26 g AcOH, and 21 mL DMF and stirring for additional 1 h. The reaction mixture was diluted with 400 mL of ethyl acetate and poured into a mixture of brine (1.0 L) and ethyl acetate (1.0 L), followed by separation. The organic layer was washed with 0.5 M hydrochroric acid (400 mL), brine (400 mL), then dried over MgSO$_4$ and concentrated. The residue was purified by silica gel column chromatography (hexane/ethyl acetate = 65/35) to give 26.5 g of **5c** for α/β anomeric mixture (91.4/8.6) as a colorless crystal. ^{1}H NMR of α anomer (300 MHz, CDCl$_3$) δ 2.02, 2.04, 2.08, 2.08 (each s, each 3H), 3.70 (ddd, *J* = 3.3, 5.0, 8.3 Hz, 1 H), 4.08 (dd, *J* = 3.3, 12.0 Hz, 1 H), 4.38 (dd, *J* = 5.0, 12.0 Hz, 1 H), 5.15−5.19 (m, 2 H), 5.31 (dd, *J* = 9.6, 10.9 Hz, 1 H), 5.55 (t, *J* = 9.6 Hz, 1 H).

2,3,4,6-Tetra-*O*-benzyl-5-thio-D-glucono-1,5-lactone (8). To a stirred solution of 2,3,4,6-tetra-*O*-acetyl-5-thio-D-glucopyranose (**5c**) (2.0 g, 5.49 mmol) in 40 mL of CHCl$_3$ were added 3,4-dihydro-2*H*-pyran (1.5 mL, 16.5 mmol) and *p*-toluenesulfonic acid monohydrate (104 mg, 0.549 mmol) at room temperature, then the mixture was stirred at room temperature for 1 h. The reaction mixture was poured into saturated aqueous NaHCO$_3$, and the layers were separated. The aqueous layer was extracted with CHCl$_3$. The combined organic layers were washed with brine, dried over MgSO$_4$, and concentrated. The residue was purified by silica gel column chromatography (hexane/ethyl acetate = 1/1) to give 2.56 g of tetrahydro-2*H*-pyran-2-yl 2,3,4,6-tetra-*O*-acetyl-5-thio-D-glucopyranose as a pale yellow amorphous, which was taken up into 40 mL of anhydrous methanol and treated with a 25 wt % solution of sodium methoxide in methanol (0.11 mL, 0.55 mmol). The resultant mixture was stirred at room temperature for 3 h. After neutralization by the addition of dry ice, the mixture was concentrated. The residue was taken up into DMF (20 mL) and added to a suspension of sodium hydride (1.3 g, 32.9 mmol; 60% oil) in DMF (4 mL) dropwise at 0 °C. After stirring at room temperature for 20 min, the mixture was cooled to 4 °C and treated with benzyl bromide (5.6 g, 32.9 mmol). The resulting mixture was stirred at room temperature for 12 h and quenched by the addition of methanol (5 mL). After an additional 30 min stirring at room temperature, the reaction mixture was poured into ice water and extracted with ethyl acetate. The organic layer was washed with brine, dried over MgSO$_4$, and concentrated. The residue was purified by silica gel column chromatography (hexane/ethyl acetate = 6/1) to give compound **6** (3.36 g, 96% for three steps). A mixture of **6** (3.30 g, 5.15 mmol) and pyridinium *p*-toluenesulfonate (518 mg, 2.06 mmol) in ethanol (58 mL) was stirred at 80 °C for 2 h. The resulting mixture was cooled to room temperature and concentrated. The residue was dissolved in ethyl acetate and washed with saturated aqueous NaHCO$_3$ and brine, dried over MgSO$_4$, and concentrated. The residue was purified by silica gel column chromatography (hexane/ethyl acetate = 3/1) to give 2,3,4,6-tetra-*O*-benzyl-5-thio-D-glucopyranose (**7**) (2.89 g, quant) as a colorless crystal. ^{13}C NMR (125 MHz, CDCl$_3$) δ 41.3, 67.8, 71.6, 73.0, 73.2, 75.6, 76.2, 81.9, 82.9,

84.4, 127.5, 127.7, 127.8, 127.9, 128.0, 128.3, 128.4, 128.5, 137.8, 138.3, 138.8.

A mixture of **7** (2.82 g, 5.07 mmol) and acetic anhydride (39 mL) in DMSO (47 mL) was stirred at room temperature for 12 h. The reaction mixture was poured into ice water and extracted with ethyl acetate. The organic layer was washed with water, saturated aqueous NaHCO$_3$, and brine, then dried over MgSO$_4$ and concentrated. The residue was purified by silica gel column chromatography (hexane/ethyl acetate = 6/1) to give **8** (2.3 g, 82%) as a colorless oil. ^{1}H NMR (200 MHz, CDCl$_3$) δ 3.70 (d, J = 4.8 Hz, 2 H), 3.86−4.02 (m, 2 H), 4.09−4.22 (m, 2 H), 4.40−4.68 (m, 7 H), 4.83 (d, J = 11.4 Hz, 1 H), 7.12−7.41 (m, 20 H).

General Methods for Synthesis of Aglycon 12a−12s. 1-Bromo-3-(4-ethoxybenzyl)benzene (12a). To a stirred solution of 4-bromophenetole (**9**) (2.87 g, 0.0143 mol) in THF (30 mL) at −78 °C was added dropwise a 2.6 M hexane solution of *n*-BuLi (5.8 mL, 0.0150 mol). After stirring for 30 min, a THF (15 mL) solution of 3-bromobenzaldehyde (**10a**) (2.65 g, 0.0143 mol) was added dropwise at −78 °C. After stirring for 15 min, the reaction mixture was allowed to warm to room temperature, and then, saturated aqueous NH$_4$Cl was added. The resulting mixture was extracted with ethyl acetate twice, and the combined organic layers were washed with brine, dried over MgSO$_4$, and concentrated. The residue was purified by silica gel column chromatography (hexane/ethyl acetate = from 7/1 to 5/1) to give 3.94 g of a colorless oil. This was taken up into 11 mL of CHCl$_3$ and 11 mL of CH$_3$CN. The mixture was cooled to −60 °C and Et$_3$SiH (4.09 mL, 0.0256 mol) and then BF$_3$·Et$_2$O (1.47 mL, 0.0116 mol) were added sequentially. After stirring for 1 h, the reaction mixture was allowed to warm to room temperature, and then, saturated aqueous Na$_2$CO$_3$ was added. The resulting mixture was extracted with CHCl$_3$, and the organic layer was washed with brine, dried over MgSO$_4$, and concentrated. The residue was purified by silica gel column chromatography (hexane/ethyl acetate = 10/1) to give **12a** (2.84 g, 68%, 2 steps) as a colorless oil. ^{1}H NMR (300 MHz, CDCl$_3$) δ 1.40 (t, J = 7.0 Hz, 3 H), 3.88 (s, 2 H), 4.01 (q, J = 7.0 Hz, 2 H), 6.83 (d, J = 8.9 Hz, 2 H), 7.07 (d, J = 8.9 Hz, 2 H), 7.09−7.18 (m, 2 H), 7.29−7.34 (m, 2 H).

1-(Benzyloxy)-4-bromo-2-(4-ethoxybenzyl)benzene (12b). Compound **12b** (5.88 g, 61%, 2 steps) was prepared according to the method described for the synthesis of **12a** using **9** (5.12 g, 0.0255 mol) and **10b** (10.0 g, 0.0345 mol) as a colorless oil. ^{1}H NMR (200 MHz, CDCl$_3$) δ 1.40 (t, J = 6.8 Hz, 3 H), 3.90 (s, 2 H), 4.01 (q, J = 6.8 Hz, 2 H), 5.03 (s, 2 H), 6.72−6.85 (m, 3 H), 7.02−7.13 (m, 2 H), 7.15−7.43 (m, 7 H).

4-Bromo-2-(4-ethoxybenzyl)-1-methoxybenzene (12c). Compound **12c** (3.12 g, 41%, 2 steps) was prepared according to the method described for the synthesis of **12a** using **9** (4.99 g, 0.0248 mol) and **10c** (5.08 g, 0.0236 mol) as a colorless oil. ^{1}H NMR (300 MHz, CDCl$_3$) δ 1.40 (t, J = 7.0 Hz, 3 H), 3.79 (s, 3 H), 3.85 (s, 2 H), 4.01 (q, J = 7.0 Hz, 2 H), 6.72 (d, J = 8.6 Hz, 1 H), 6.81 (d, J = 8.7 Hz, 2 H), 7.09 (d, J = 8.7 Hz, 1 H), 7.13 (d, J = 2.5 Hz, 1 H), 7.27 (dd, J = 2.5, 8.6 Hz, 1 H).

4-Bromo-2-(4-ethoxybenzyl)-1-fluorobenzene (12d). Compound **12d** (2.46 g, 33%, 2 steps) was prepared according to the method described for the synthesis of **12a** using **9** (10.4 g, 0.0517 mol) and **10d** (10.0 g, 0.0493 mol) as a colorless amorphous substance. ^{1}H NMR (200 MHz, CDCl$_3$) δ 1.40 (t, J = 7.0 Hz, 3 H), 3.88 (s, 2 H), 4.01 (q, J = 7.0 Hz, 2 H), 6.79−6.96 (m, 3 H), 7.05−7.16 (m, 2 H), 7.19−7.32 (m, 2 H). MS (EI) m/z 309 (M$^+$), 311 (M+2).

4-Bromo-2-(4-ethoxybenzyl)-1-methylbenzene (12e). Compound **12e** (2.34 g, 96%) was prepared according to the method described for the synthesis of **12p** using **15** (4.29 g, 0.0199 mol) and phenetole (2.52 g, 0.0209 mol) as a colorless oil. ^{1}H NMR (300 MHz, CDCl$_3$) δ 1.40 (t, J = 7.0 Hz, 3 H), 2.18 (s, 3 H), 3.86 (s, 2 H), 4.00 (q, J = 7.0 Hz, 2 H), 6.76−6.87 (m, 2 H), 6.94−7.07 (m, 3 H), 7.17−7.30 (m, 2 H). MS (EI) m/z 304 (M$^+$), 306 (M+2).

1-(Benzyloxy)-2-bromo-4-(4-ethoxybenzyl)benzene (12g). Compound **12g** (8.80 g, 86%, 2 steps) was prepared according to the method described for the synthesis of **12a** using **9** (7.30 g, 0.0366

mol) and **10g** (7.0 g, 0.0244 mol) as a colorless oil. ^{1}H NMR (300 MHz, CDCl$_3$) δ 1.40 (t, J = 7.0 Hz, 3 H), 3.82 (s, 2 H), 4.00 (q, J = 7.0 Hz, 2 H), 5.12 (s, 2 H), 6.78−6.87 (m, 3 H), 6.98−7.10 (m, 3 H), 7.27−7.50 (m, 6 H).

2-Bromo-4-(4-ethoxybenzyl)-1-methoxybenzene (12h). Compound **12h** (12.2 g, 82%, 2 steps) was prepared according to the method described for the synthesis of **12a** using **9** (8.81 g, 0.0488 mol) and **10h** (10.0 g, 0.0465 mol) as a colorless powder. ^{1}H NMR (300 MHz, CDCl$_3$) δ 1.40 (t, J = 7.0 Hz, 3 H), 3.83 (s, 2 H), 3.86 (s, 3 H), 4.01 (q, J = 7.0 Hz, 2 H), 6.78−6.85 (m, 3 H), 7.03−7.10 (m, 3 H), 7.35 (d, J = 2.2 Hz, 1 H). MS (EI) m/z 320 (M$^+$), 322 (M+2).

1-Bromo-2,4-dimethoxy-5-(4-ethoxybenzyl)benzene (12i). Compound **12i** (1.80 g, 51%, 2 steps) was prepared according to the method described for the synthesis of **12a** using **9** (2.34 g, 0.0117 mol) and **10i** (3.11 g, 0.0127 mol) as a colorless powder. ^{1}H NMR (200 MHz, CDCl$_3$) δ 1.39 (t, J = 7.0 Hz, 3 H), 3.80 (s, 2 H), 3.82 (s, 3 H), 3.88 (s, 3 H), 4.00 (q, J = 7.0 Hz, 2 H), 6.47 (s, 1 H), 6.75−6.85 (m, 2 H), 7.02−7.12 (m, 2 H), 7.17 (s, 1 H). MS (EI) m/z 350 (M$^+$), 352 (M+2).

4-Bromo-1-chloro-2-(4-methoxybenzyl)benzene (12j). Compound **12j** (3.88 g, 74%) was prepared according to the method described for the synthesis of **12p** using 5-bromo-2-chlorobenzoic acid (**16**) (4.00 g, 0.0170 mol) and anisole (1.88 mL, 0.0173 mol) as a colorless gum. ^{1}H NMR (300 MHz, CDCl$_3$) δ 3.80 (s, 3 H), 3.99 (s, 2 H), 6.82−6.89 (m, 2 H), 7.06−7.13 (m, 2 H), 7.19−7.30 (m, 3 H).

4-Bromo-1-chloro-2-(4-methylbenzyl)benzene (12k). Compound **12k** (2.86 g, 57%) was prepared according to the method described for the synthesis of **12p** using **16** (5.00 g, 0.0212 mol) and toluene (2.48 mL, 0.0234 mol) as a colorless oil. ^{1}H NMR (200 MHz, CDCl$_3$) δ 2.33 (s, 3 H), 4.02 (s, 2 H), 7.03−7.16 (m, 4 H), 7.18−7.32 (m, 3 H). MS (EI) m/z 294 (M$^+$), 296 (M+2).

4-Bromo-1-chloro-2-(4-ethylbenzyl)benzene (12l). Compound **12l** (2.98 g, 47%) was prepared according to the method described for the synthesis of **12p** using **16** (5.00 g, 0.0212 mol) and ethylbenzene (2.86 mL, 0.0234 mol) as a colorless oil. ^{1}H NMR (200 MHz, CDCl$_3$) δ 1.23 (t, J = 7.7 Hz, 3 H), 2.63 (q, J = 7.7 Hz, 2 H), 4.02 (s, 2 H), 7.04−7.18 (m, 4 H), 7.18−7.32 (m, 3 H). MS (EI) m/z 308 (M$^+$), 310 (M+2).

4-Bromo-1-chloro-2-[4-(propan-2-yl)benzyl]benzene (12m). Compound **12m** (3.28 g, 48%) was prepared according to the method described for the synthesis of **12p** using **16** (5.00 g, 0.0212 mol) and cumene (3.24 mL, 0.0234 mmol). ^{1}H NMR (200 MHz, CDCl$_3$) δ 1.24 (d, J = 7.0 Hz, 6 H), 2.80−2.97 (m, 1 H), 4.02 (s, 2 H), 7.06−7.32 (m, 7 H).

4-Bromo-2-(4-*tert*-butylbenzyl)-1-chlorobenzene (12n). Compound **12n** (4.44 g, 62%) was prepared according to the method described for the synthesis of **12p** using **16** (5.00 g, 0.0212 mol) and *t*-butylbenzene (2.90 g, 0.0216 mol) as a colorless oil. ^{1}H NMR (200 MHz, CDCl$_3$) δ 1.31 (s, 9 H), 4.03 (s, 2 H), 7.11 (d, J = 7.9 Hz, 2 H), 7.22−7.37 (m, 5 H).

4-Bromo-1-chloro-2-[4-(methylsulfanyl)benzyl]benzene (12o). Compound **12o** (2.83 g, 41%) was prepared according to the method described for the synthesis of **12p** using **16** (5.00 g, 0.0212 mol) and thioanisole (2.60 mL, 0.0220 mol). ^{1}H NMR (300 MHz, CDCl$_3$) δ 2.47 (s, 3 H), 4.01 (s, 2 H), 7.06−7.14 (m, 2 H), 7.17−7.32 (m, 5 H).

1-Bromo-5-(4-ethoxybenzyl)-2-methoxy-4-methylbenzene (12p). To a stirred suspension of 5-bromo-4-methoxy-2-methylbenzoic acid (**14**) (4.93 g, 0.0201 mol) in CHCl$_3$ (15 mL) was added oxalyl chloride (1.79 mL, 0.0209 mol) and DMF (0.02 mL). The resulting mixture was stirred for 2 h. An additional amount of oxalyl chloride (0.5 mL, 5.83 mmol) was added, and the mixture was stirred for 15 h. The mixture was concentrated, and the residual colorless solid was dissolved in CHCl$_3$ (30 mL). To this solution were added phenetole (2.50 g, 0.0205 mol) and then AlCl$_3$ (2.71 g, 0.0203 mol) portionwise so that the temperature did not exceed −4 °C. After being stirred at 5 °C for 3 h, the mixture was poured into ice water and extracted with CHCl$_3$

three times. The combined organic layers were washed with 1 M hydrochloric acid, water, and brine, then dried over MgSO$_4$ and concentrated. The residual solid was crystallized from a 4:1 mixture of hexane and ethyl acetate to give 5.75 g (82%) of (5-bromo-4-methoxy-2-methylphenyl)(4-ethoxyphenyl)methanone (**17p**) as a colorless crystal. ^{1}H NMR (200 MHz, CDCl$_3$) δ 1.45 (t, J = 7.0 Hz, 3 H), 2.33 (s, 3 H), 3.95 (s, 3 H), 4.11 (q, J = 7.0 Hz, 2 H), 6.79 (s, 1 H), 6.87−6.97 (m, 2 H), 7.51 (s, 1 H), 7.69−7.80 (m, 2 H).

To a stirred solution of **17p** (5.75 g, 0.0165 mol) in 30 mL of CHCl$_3$ and 30 mL of CH$_3$CN at 4 °C were added Et$_3$SiH (6.57 mL, 0.0411 mol), then BF$_3$·Et$_2$O (3.14 mL, 0.0248 mol), and the resulting mixture was warmed to room temperature and stirred for 12 h. The mixture was poured into saturated aqueous NaHCO$_3$ and extracted with CHCl$_3$ twice. The combined organic layers were washed with brine, dried over MgSO$_4$, and concentrated. The residue was purified by silica gel column chromatography (hexane/ethyl acetate = 15/1) to give a colorless oily compound **12p** (5.50 g, 99%). ^{1}H NMR (300 MHz, CDCl$_3$) δ 1.40 (t, J = 7.0 Hz, 3 H), 2.19 (s, 3 H), 3.82 (s, 2 H), 3.87 (s, 3 H), 4.00 (q, J = 7.0 Hz, 2 H), 6.71 (s, 1 H), 6.77−6.83 (m, 2 H), 6.95−7.04 (m, 2 H), 7.24 (s, 1 H). MS (EI) m/z 335 (M$^+$), 337 (M+2).

1-Bromo-5-(4-ethylbenzyl)-2-methoxy-4-methylbenzene (12q). Compound **17q** (90.43 g, 0.27 mol), which was prepared according to the method described for the synthesis of **17p** using **14** and ethylbenzene, was dissolved in THF (450 mL) and cooled to 4 °C. To the solution was added NaBH$_4$ (32.41 g, 0.82 mol), then AlCl$_3$ (36.17 g, 0.271 mmol) was added portionwise under a nitrogen atmosphere. The reaction mixture was stirred for 3 h at 50 °C, cooled to 4 °C, then poured into ice water cautiously to avoid a rise in temperature. The resulting mixture was extracted with ethyl acetate. The organic layer was washed with 1 M hydrochloric acid, water, saturated aqueous NaHCO$_3$, and finally brine, and then dried over MgSO$_4$ and concentrated. The residue was purified by silica gel column chromatography (hexane/ethyl acetate = 1/1) to give **12q** (77.0 g, 90%). ^{1}H NMR (300 MHz, CDCl$_3$) δ 1.22 (t, J = 7.6 Hz, 3 H), 2.20 (s, 3 H), 2.61 (q, J = 7.6 Hz, 2 H), 3.85 (s, 2 H), 3.87 (s, 3 H), 6.71 (s, 1 H), 6.97−7.14 (m, 4 H), 7.27 (s, 1 H). MS (EI) 318 (M$^+$).

1-Bromo-2-methoxy-4-methyl-5-[4-(propan-2-yl)benzyl]benzene (12r). Compound **17r** (4.53 g, 56%) was prepared according to the method described for the synthesis of **17p** using 4-methoxy-2-methylbenzoyl chloride prepared from **13** (5.00 g, 30.08 mmol) and cumene (4.60 mL, 33.08 mmol). Compound **17r** (4.53 g, 16.9 mmol) was reduced with Et$_3$SiH (8.10 mL, 0.0506 mol) and BF$_3$·Et$_2$O (3.14 mL, 0.0338 mol) according to the reduction method described for the synthesis of **12p** to give 1-(4-iso-propylbenzyl)-4-methoxy-2-methylbenzene (3.79 g) as a pale yellow oil. To an AcOH (30 mL) solution of 1-(4-isopropyl-benzyl)-4-methoxy-2-methylbenzene (3.79 g, 14.89 mmol) at 4 °C was added bromine (0.76 mL, 14.89 mmol). The mixture was stirred at 100 °C for 2 h and allowed to cool to room temperature, then was diluted with ethyl acetate. The resulting solution was washed with saturated aqueous NaHCO$_3$, brine, and then dried over MgSO$_4$ and concentrated. The residue was purified by silica gel column chromatography (hexane/ethyl acetate = 15/1) to give **12r** (4.21 g, 80%) as a yellow oil. ^{1}H NMR (300 MHz, CDCl$_3$) δ 1.23 (d, J = 7.0 Hz, 6 H), 2.21 (s, 3 H), 2.81−2.92 (m, 1 H), 3.85 (brs, 2 H), 3.87 (s, 3 H), 6.71 (s, 1 H), 6.98−7.06 (m, 2 H), 7.10−7.16 (m, 2 H), 7.28 (s, 1 H). MS (EI) 322 (M$^+$), 334 (M+2).

1-Bromo-4-chloro-5-(4-ethylbenzyl)-2-methoxybenzene (12s). Compound **12s** (4.46 g, 27%) was prepared according to the method described for the synthesis of **12p** using 4-ethylbenzoic acid (6.78 g, 45.1 mmol) and **19** (10.0 g, 45.1 mmol). ^{1}H NMR (300 MHz, CDCl$_3$) δ 1.22 (t, J = 7.6 Hz, 3 H), 2.62 (q, J = 7.6 Hz, 2 H), 3.87 (s, 3 H), 3.97 (s, 2 H), 6.91 (s, 1 H), 7.04−7.18 (m, 4 H), 7.32 (s, 1 H). MS (EI) 338 (M$^+$), 340 (M+2), 342 (M+4).

5-Bromo-4-methoxy-2-methylbenzoic acid (14). To a stirred suspension of 4-methoxy-2-methylbenzoic acid (**13**) (10 g, 0.060

mol) and Fe (0.20 g, 3.61 mmol) in CHCl$_3$ (10 mL) was added bromine (3.87 mL, 0.076 mol) dropwise at 5 °C, and the resulting mixture was allowed to warm to room temperature and stirred overnight. The mixture was diluted with CHCl$_3$ (600 mL) and washed with 200 mL of 10% sodium hydrogen sulfate twice, then with brine, dried over MgSO$_4$, and concentrated to give a pale yellow solid which was recrystallized from methanol twice to give **14** (4.96 g, 34%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.53 (s, 3 H), 3.90 (s, 3 H), 7.05 (s, 1 H), 7.99 (s, 1 H), 12.82 (brs, 1 H). MS (ESI) m/z 243, 245 (M−H).

1-Bromo-4-chloro-2-methoxybenzene (19). To a suspension of 2-bromo-5-chlorophenol (**18**) (34.3 g, 0.165 mol), K$_2$CO$_3$ (22.8 g, 0.165 mol), and n-Bu$_4$NI (0.60 g, 1.65 mmol) in DMF (95 mL) was added iodomethane (12.9 mL, 0.206 mol). After stirring the mixture for 3 h, ice water was added, and the resulting mixture was extracted with ethyl acetate twice. The combined organic layers were washed with brine, dried over MgSO$_4$, and concentrated. The residue was purified by silica gel column chromatography (hexane/ethyl acetate = 95/5) to give **19** (33.3 g, 91%) as a colorless oil. ^{1}H NMR (300 MHz, CDCl$_3$) δ 3.89 (s, 3H), 6.71−6.94 (m, 2H), 7.45 (d, J = 8.4 Hz, 1H).

General Methods for Coupling Reaction with Grignard Reagents. 2,3,4,6-Tetra-O-benzyl-1-C-[3-(4-ethoxybenzyl)phenyl]-5-thio-D-glucopyranose (20a). A stirred suspension of magnesium (137 mg, 5.63 mmol) and 1-bromo-3-(4-ethoxybenzyl)benzene (**12a**) (1.38 g, 4.73 mmol) in THF (5 mL) was treated with 1,2-dibromoethane (0.05 mL) and heated to reflux for 2 h. After cooling to room temperature, the mixture was stirred for 4 h. To the resulting Grignard reagent at 0 °C was added a solution of **8** (1.25 g, 2.25 mmol) in 5.0 mL of THF. The reaction mixture was stirred at room temperature for 1 h and poured into saturated aqueous NH$_4$Cl. The resulting mixture was extracted with ethyl acetate twice, and the combined organic layers were washed with brine, dried over MgSO$_4$, and concentrated. The residue was purified by silica gel column chromatography (hexane/ethyl acetate = 4/1) to give **20a** (0.580 g, 34%) as a colorless oil. ^{1}H NMR (300 MHz, CDCl$_3$) δ 1.38 (t, J = 7.0 Hz, 3 H), 3.04 (s, 1 H), 3.48−3.57 (m, 1 H), 3.64 (dd, J = 2.6, 9.8 Hz, 1 H), 3.74 (d, J = 10.1 Hz, 1 H), 3.88−4.16 (m, 8 H), 4.41 (d, J = 10.1 Hz, 1 H), 4.52 (s, 2 H), 4.65 (d, J = 10.7 Hz, 1 H), 4.79−4.96 (m, 3 H), 6.67−6.79 (m, 4 H), 7.00−7.35 (m, 22 H), 7.47−7.55 (m, 2 H). MS (ESI) m/z 789 (M+NH$_4$).

2,3,4,6-Tetra-O-benzyl-1-C-[4-(benzyloxy)-3-(4-ethoxybenzyl)-phenyl]-5-thio-D-glucopyranose (20b). Compound **20b** (1.00 g, 42%) was prepared according to the method described for the synthesis of **20a** using **8** (1.50 g, 2.70 mmol) and **12b** (2.15 g, 5.41 mmol) as a yellow oil. ^{1}H NMR (300 MHz, CDCl$_3$) δ 1.38 (t, J = 7.0 Hz, 3 H), 2.97 (s, 1 H), 3.46−3.55 (m, 1 H), 3.63 (dd, J = 2.7, 9.6 Hz, 1 H), 3.80 (d, J = 10.3 Hz, 1 H), 3.86−4.11 (m, 8 H), 4.42 (d, J = 10.3 Hz, 1 H), 4.52 (s, 2 H), 4.64 (d, J = 10.4 Hz, 1 H), 4.79−4.95 (m, 3 H), 5.07 (s, 2 H), 6.68−6.77 (m, 4 H), 6.90 (d, J = 9.0 Hz, 1 H), 7.02−7.35 (m, 25 H), 7.42−7.48 (m, 2 H). MS (ESI) m/z 895 (M+Na).

2,3,4,6-Tetra-O-benzyl-1-C-[3-(4-ethoxybenzyl)-4-methoxy-phenyl]-5-thio-D-glucopyranose (20c). Compound **20c** (1.59 g, 74%) was prepared according to the method described for the synthesis of **20a** using **8** (1.50 g, 2.70 mmol) and **12c** (1.73 g, 5.40 mmol) as a colorless amorphous. ^{1}H NMR (600 MHz, CDCl$_3$) δ 1.37 (t, J = 6.9 Hz, 3 H), 2.97 (s, 1 H), 3.50 (s, 1 H), 3.63 (dd, J = 2.8, 10.1 Hz, 1 H), 3.78−3.88 (m, 5 H), 3.89−3.98 (m, 5 H), 4.01−4.10 (m, 2 H), 4.41 (d, J = 10.1 Hz, 1 H), 4.52 (s, 2 H), 4.64 (d, J = 10.6 Hz, 1 H), 4.80−4.94 (m, 3 H), 6.71−6.76 (m, 4 H), 6.84 (d, J = 8.7 Hz, 1 H), 7.03−7.20 (m, 7 H), 7.23−7.34 (m, 13 H), 7.41 (d, J = 2.3 Hz, 1 H), 7.45−7.49 (m, 1 H). MS (ESI) m/z 819 (M+Na).

2,3,4,6-Tetra-O-benzyl-1-C-[3-(4-ethoxybenzyl)-4-fluorophenyl]-5-thio-D-glucopyranose (20d). Compound **20d** (1.83 g, 86%) was prepared according to the method described for the synthesis of **20a** using **8** (1.50 g, 2.70 mmol) and **12d** (1.67 g, 5.41 mmol) as a colorless amorphous substance. ^{1}H NMR (300 MHz, CDCl$_3$)

δ 1.37 (t, *J* = 7.0 Hz, 3 H), 3.00 (s, 1 H), 3.46−3.53 (m, 1 H), 3.62 (dd, *J* = 2.8, 9.8 Hz, 1 H), 3.79 (d, *J* = 10.3 Hz, 1 H), 3.83−4.09 (m, 8 H), 4.45 (d, *J* = 10.0 Hz, 1 H), 4.51 (s, 2 H), 4.64 (d, *J* = 10.7 Hz, 1 H), 4.79−4.94 (m, 3 H), 6.68−6.78 (m, 4 H), 6.97−7.20 (m, 8 H), 7.25−7.34 (m, 13 H), 7.43−7.50 (m, 2 H). MS (ESI) *m/z* 807 (M+Na).

2,3,4,6-Tetra-*O*-benzyl-1-*C*-[3-(4-ethoxybenzyl)-4-methyl-phenyl]-5-thio-D-glucopyranose (20e). Compound **20e** (1.53 g, 50%) was prepared according to the method described for the synthesis of **20a** using **8** (2.20 g, 3.93 mmol) and **12e** (1.20 g, 3.93 mmol) as a colorless amorphous. ^{1}H NMR (300 MHz, CDCl$_3$) δ 1.37 (t, *J* = 7.0 Hz, 3 H), 2.23 (s, 3 H), 3.02 (s, 1 H), 3.48−3.57 (m, 1 H), 3.64 (dd, *J* = 2.7, 9.9 Hz, 1 H), 3.81−4.17 (m, 9 H), 4.44 (d, *J* = 10.1 Hz, 1 H), 4.52 (s, 2 H), 4.62−4.72 (m, 1 H), 4.78−4.96 (m, 3 H), 6.66−6.82 (m, 4 H), 6.95 (d, *J* = 8.7 Hz, 2 H), 7.08−7.38 (m, 19 H), 7.41−7.47 (m, 2 H). MS (ESI) *m/z* 798 (M+NH$_4$), 779 (M−H).

2,3,4,6-Tetra-*O*-benzyl-1-*C*-[4-chloro-3-(4-ethoxybenzyl)-phenyl]-5-thio-D-glucopyranose (20f). Compound **20f** (3.80 g, 89%) was prepared according to the method described for the synthesis of **20a** using **8** (3.00 g, 5.40 mmol) and **12f** (3.52 g, 10.8 mmol) as a colorless oil. ^{1}H NMR (300 MHz, CDCl$_3$) δ 1.38 (t, *J* = 7.0 Hz, 3 H), 3.01 (s, 1 H), 3.45−3.54 (m, 1 H), 3.62 (dd, *J* = 2.8, 10.0 Hz, 1 H), 3.77−4.13 (m, 9 H), 4.46 (d, *J* = 10.3 Hz, 1 H), 4.51 (s, 2 H), 4.64 (d, *J* = 10.7 Hz, 1 H), 4.78−4.94 (m, 3 H), 6.68−6.79 (m, 4 H), 7.00−7.52 (m, 23 H). MS (ESI) *m/z* 823 (M+Na).

2,3,4,6-Tetra-*O*-benzyl-1-*C*-[2-(benzyloxy)-5-(4-ethoxybenzyl)-phenyl]-5-thio-D-glucopyranose (20g). Compound **20g** (1.48 g, 59%) was prepared according to the method described for the synthesis of **20a** using **8** (1.60 g, 2.88 mmol) and **12g** (2.29 g, 5.76 mmol) as a pale yellow powder. ^{1}H NMR (300 MHz, CDCl$_3$) δ 1.38 (t, *J* = 6.9 Hz, 3 H), 3.52−3.70 (m, 2 H), 3.79−4.08 (m, 9 H), 4.51 (s, 2 H), 4.59−4.73 (m, 3 H), 4.80−4.93 (m, 3 H), 5.09 (s, 2 H), 6.64−7.40 (m, 32 H). MS (ESI) *m/z* 895 (M+Na).

2,3,4,6-Tetra-*O*-benzyl-1-*C*-[5-(4-ethoxybenzyl)-2-methoxy-phenyl]-5-thio-D-glucopyranose (20h). Compound **20h** (1.71 g, 79%) was prepared according to the method described for the synthesis of **20a** using **8** (1.50 g, 2.70 mmol) and **12h** (1.74 g, 5.41 mmol) as a pale yellow solid. ^{1}H NMR (300 MHz, CDCl$_3$) δ 1.37 (t, *J* = 7.1 Hz, 3 H), 3.54−3.70 (m, 2 H), 3.78−4.09 (m, 12 H), 4.51 (s, 2 H), 4.58−4.72 (m, 2 H), 4.81−4.98 (m, 3 H), 6.67−6.90 (m, 5 H), 6.95−7.35 (m, 22 H). MS (ESI) *m/z* 819 (M+Na).

2,3,4,6-Tetra-*O*-benzyl-1-*C*-[5-(4-ethoxybenzyl)-2,4-dimethoxy-phenyl]-5-thio-D-glucopyranose (20i). Compound **20i** (1.76 g, 79%) was prepared according to the method described for the synthesis of **20a** using **8** (1.50 g, 2.70 mmol) and **12i** (1.80 g, 5.12 mmol) as a colorless amorphous. ^{1}H NMR (300 MHz, CDCl$_3$) δ 1.36 (t, *J* = 7.0 Hz, 3 H), 3.50−3.70 (m, 2 H), 3.74−4.11 (m, 15 H), 4.51 (s, 2 H), 4.57−4.68 (m, 2 H), 4.77−4.97 (m, 3 H), 6.44 (s, 1 H), 6.64−6.80 (m, 4 H), 6.98−7.36 (m, 21 H). MS (ESI) *m/z* 849 (M+Na).

2,3,4,6-Tetra-*O*-benzyl-1-*C*-[4-chloro-3-(4-methoxybenzyl)-phenyl]-5-thio-D-glucopyranose (20j). Compound **20j** (1.93 g, 91%) was prepared according to the method described for the synthesis of **20a** using **8** (1.50 g, 2.70 mmol) and **12j** (1.68 g, 5.40 mmol) as a colorless oil. ^{1}H NMR (300 MHz, CDCl$_3$) δ 3.00 (s, 1 H), 3.45−3.54 (m, 1 H), 3.62 (dd, *J* = 2.9, 10.0 Hz, 1 H), 3.74 (s, 3 H), 3.80−4.12 (m, 7 H), 4.42−4.54 (m, 3 H), 4.64 (d, *J* = 10.9 Hz, 1 H), 4.77−4.96 (m, 3 H), 6.68−6.80 (m, 4 H), 7.03−7.37 (m, 21 H), 7.39−7.51 (m, 2 H). MS (ESI) *m/z* 809 (M+Na).

2,3,4,6-Tetra-*O*-benzyl-1-*C*-[4-chloro-3-(4-methylbenzyl)-phenyl]-5-thio-D-glucopyranose (20k). Compound **20k** (2.50 g, 67%) was prepared according to the method described for the synthesis of **20a** using **8** (2.68 g, 4.84 mmol) and **12k** (2.86 g, 9.68 mmol) as a colorless oil. ^{1}H NMR (300 MHz, CDCl$_3$) δ 2.28 (s, 3 H), 3.00 (s, 1 H), 3.45−3.54 (m, 1 H), 3.62 (dd, *J* = 2.6, 9.9 Hz, 1 H), 3.79−4.15 (m, 7 H), 4.43−4.53 (m, 3 H), 4.64 (d, *J* = 10.7 Hz, 1 H), 4.76−4.96 (m, 3 H), 6.69−6.77 (m, 2 H), 6.98−7.54 (m, 25 H). MS (ESI) *m/z* 793 (M+Na).

2,3,4,6-Tetra-*O*-benzyl-1-*C*-[4-chloro-3-(4-ethylbenzyl)phenyl]-5-thio-D-glucopyranose (20l). Compound **20l** (3.03 g, 80%) was prepared according to the method described for the synthesis of **20a** using **8** (2.67 g, 4.81 mmol) and **12l** (2.98 g, 9.62 mmol) as a colorless amorphous. ^{1}H NMR (300 MHz, CDCl$_3$) δ 1.19 (t, *J* = 7.7 Hz, 3 H), 2.58 (q, *J* = 7.7 Hz, 2 H), 3.00 (s, 1 H), 3.45−3.53 (m, 1 H), 3.62 (dd, *J* = 2.9, 9.9 Hz, 1 H), 3.79−4.17 (m, 7 H), 4.43−4.53 (m, 3 H), 4.64 (d, *J* = 10.6 Hz, 1 H), 4.78−4.95 (m, 3 H), 6.68−6.77 (m, 2 H), 7.01−7.54 (m, 25 H). MS (ESI) *m/z* 807 (M+Na).

2,3,4,6-Tetra-*O*-benzyl-1-*C*-[4-chloro-3-[4-(propan-2-yl)benzyl]-phenyl]-5-thio-D-glucopyranose (20m). Compound **20m** (2.65 g, 65%) was prepared according to the method described for the synthesis of **20a** using **8** (2.80 g, 5.07 mmol) and **12m** (3.28 g, 10.13 mmol) as a yellow powder. ^{1}H NMR (300 MHz, CDCl$_3$) δ 1.19 (d, *J* = 6.8 Hz, 6 H), 2.77−2.89 (m, 1 H), 3.01 (s, 1 H), 3.45−3.54 (m, 1 H), 3.62 (dd, *J* = 2.7, 9.9 Hz, 1 H), 3.79−4.20 (m, 7 H), 4.46 (d, *J* = 10.3 Hz, 1 H), 4.51 (s, 2 H), 4.64 (d, *J* = 10.7 Hz, 1 H), 4.79−4.95 (m, 3 H), 6.69−6.76 (m, 2 H), 7.05−7.21 (m, 8 H), 7.24−7.37 (m, 15 H), 7.39−7.46 (m, 1 H), 7.52 (d, *J* = 2.3 Hz, 1 H). MS (ESI) *m/z* 821 (M+Na).

2,3,4,6-Tetra-*O*-benzyl-1-*C*-[3-(4-*tert*-butylbenzyl)-4-chloro-phenyl]-5-thio-D-glucopyranose (20n). Compound **20n** (1.73 g, 79%) was prepared according to the method described for the synthesis of **20a** using **8** (1.50 g, 2.70 mmol) and **12n** (1.82 g, 5.40 mmol) as a colorless gum. ^{1}H NMR (300 MHz, CDCl$_3$) δ 1.26 (s, 9 H), 3.01 (s, 1 H), 3.45−3.55 (m, 1 H), 3.62 (dd, *J* = 2.7, 9.7 Hz, 1 H), 3.80−4.18 (m, 7 H), 4.42−4.54 (m, 3 H), 4.64 (d, *J* = 10.7 Hz, 1 H), 4.78−4.96 (m, 3 H), 6.68−6.76 (m, 2 H), 7.04−7.38 (m, 23 H), 7.40−7.56 (m, 2 H). MS (ESI) *m/z* 835 (M+Na), 811 (M−H).

2,3,4,6-Tetra-*O*-benzyl-1-*C*-[4-chloro-3-[4-(methylsulfanyl)-benzyl]phenyl-5-thio-D-glucopyranose (20o). Compound **20o** (2.00 g, 92%) was prepared according to the method described for the synthesis of **20a** using **8** (1.50 g, 2.70 mmol) and **12o** (1.77 g, 5.40 mmol) as a colorless amorphous. ^{1}H NMR (300 MHz, CDCl$_3$) δ 2.42 (s, 3 H), 3.01 (s, 1 H), 3.45−3.55 (m, 1 H), 3.62 (dd, *J* = 2.7, 9.9 Hz, 1 H), 3.79−4.14 (m, 7 H), 4.43−4.55 (m, 3 H), 4.64 (d, *J* = 10.7 Hz, 1 H), 4.77−4.96 (m, 3 H), 6.69−6.76 (m, 2 H), 7.01−7.38 (m, 23 H), 7.40−7.53 (m, 2 H). MS (ESI) *m/z* 825 (M+Na), 801 (M−H).

2,3,4,6-Tetra-*O*-benzyl-1-*C*-[5-(4-ethoxybenzyl)-2-methoxy-4-methylphenyl]-5-thio-D-glucopyranose (20p). Compound **20p** (2.20 g, 75%) was prepared according to the method described for the synthesis of **20a** using **8** (2.00 g, 3.61 mmol) and **12p** (2.71 g, 8.08 mmol) as a yellow oil. ^{1}H NMR (300 MHz, CDCl$_3$) δ 1.37 (t, *J* = 6.9 Hz, 3 H), 2.21 (s, 3 H), 3.51−4.20 (m, 14 H), 4.51 (s, 2 H), 4.60−4.75 (m, 2 H), 4.78−4.99 (m, 3 H), 6.59−7.43 (m, 26 H). MS (ESI) *m/z* 812 (M+NH$_4$).

2,3,4,6-Tetra-*O*-benzyl-1-*C*-[5-(4-ethylbenzyl)-2-methoxy-4-methylphenyl]-5-thio-D-glucopyranose (20q). Compound **20q** (2.25 g, 48%) was prepared according to the method described for the synthesis of **20a** using **8** (3.25 g, 5.87 mmol) and **12q** (3.75 g, 11.74 mmol) as a yellow solid. ^{1}H NMR (300 MHz, CDCl$_3$) δ 1.17 (t, *J* = 7.7 Hz, 3 H), 2.22 (s, 3 H), 2.49−2.62 (m, 2 H), 3.53−4.16 (m, 12 H), 4.51 (s, 2 H), 4.58−4.74 (m, 2 H), 4.80−4.98 (m, 3 H), 6.71−6.86 (m, 2 H), 6.89−7.03 (m, 3 H), 7.06−7.41 (m, 21 H). MS (ESI) *m/z* 817 (M+Na), 793 (M−H).

2,3,4,6-Tetra-*O*-benzyl-1-*C*-[2-methoxy-4-methyl-5-[4-(propan-2-yl)benzyl]phenyl]-5-thio-D-glucopyranose (20r). Compound **20r** (1.14 g, 23%) was prepared according to the method described for the synthesis of **20a** using **8** (3.30 g, 5.94 mmol) and **12r** (4.00 g, 12.0 mmol) as a yellow amorphous substance. ^{1}H NMR (300 MHz, CDCl$_3$) δ 1.18 (d, *J* = 6.7 Hz, 6 H), 2.23 (s, 3 H), 2.76−2.88 (m, 1 H), 3.52−4.16 (m, 12 H), 4.51 (s, 2 H), 4.57−4.69 (m, 2 H), 4.81−4.96 (m, 3 H), 6.71−6.84 (m, 2 H), 6.90−7.38 (m, 24 H). MS (ESI) *m/z* 831 (M+Na), 807 (M−H).

2,3,4,6-Tetra-*O*-benzyl-1-*C*-[4-chloro-5-(4-ethylbenzyl)-2-methoxyphenyl]-5-thio-D-glucopyranose (20s). Compound **20s** (3.12 g, 74%) was prepared according to the method described

for the synthesis of **20a** using **8** (2.86 g, 5.15 mmol) and **12s** (3.50 g, 10.30 mmol) as a pale yellow crystal. ^{1}H NMR (300 MHz, CDCl$_3$) δ 1.18 (t, J=7.5 Hz, 3 H), 2.57 (q, J=7.5 Hz, 2 H), 3.51−3.71 (m, 2 H), 3.83 (s, 3 H), 3.88−4.09 (m, 6 H), 4.50 (s, 2 H), 4.58−4.70 (m, 2 H), 4.79−4.96 (m, 4 H), 6.75 (d, J=7.0 Hz, 2 H), 6.85−7.39 (m, 24 H).

General Methods for Reduction of Anomeric Hydroxyl Group. (1S)-1,5-Anhydro-2,3,4,6-tetra-O-benzyl-1-[3-(4-ethoxy-benzyl)phenyl]-1-thio-D-glucitol (21a). To an CH$_3$CN (6 mL) solution of 2,3,4,6-tetra-O-benzyl-1-C-[3-(4-ethoxybenzyl)phenyl]-5-thio-D-glucopyranose (**20a**) (570 mg, 0.743 mmol) was added Et$_3$SiH (0.71 mL, 4.46 mmol) and then BF$_3$·Et$_2$O (0.38 mL, 2.97 mmol) at −15 °C, and the mixture was stirred for 1 h. The reaction was quenched by addition of saturated aqueous NaHCO$_3$ and extracted with ethyl acetate twice. The combined organic layers were washed with brine, dried with MgSO$_4$, and concentrated. The residue was purified by silica gel column chromatography (hexane/ethyl acetate = 9/1) to give **21a** (330 mg, 59%). ^{1}H NMR (600 MHz, CDCl$_3$) δ 1.38 (t, J=7.0 Hz, 3 H), 3.04 (s, 1 H), 3.48−3.57 (m, 1 H), 3.64 (dd, J=2.6, 9.8 Hz, 1 H), 3.74 (d, J=10.1 Hz, 1 H), 3.88−4.16 (m, 8 H), 4.41 (d, J=10.1 Hz, 1 H), 4.52 (s, 2 H), 4.65 (d, J=10.7 Hz, 1 H), 4.79−4.96 (m, 3 H), 6.67−6.79 (m, 4 H), 7.00−7.35 (m, 22 H), 7.47−7.55 (m, 2 H). MS (ESI) m/z 773 (M+Na).

(1S)-1,5-Anhydro-2,3,4,6-tetra-O-benzyl-1-[4-(benzyloxy)-3-(4-ethoxybenzyl)phenyl]-1-thio-D-glucitol (21b). Compound **21b** (566 mg, 58%) was prepared as a colorless powder from **20b** (1.00 g, 1.15 mmol) according to the method described for the synthesis of **21a** following recrystallization from hexane/ethyl acetate. ^{1}H NMR (300 MHz, CDCl$_3$) δ 1.38 (t, J=6.9 Hz, 3 H), 3.02−3.14 (m, 1 H), 3.51 (t, J=8.7 Hz, 1 H), 3.66−4.05 (m, 10 H), 4.47 (d, J=10.3 Hz, 1 H), 4.52 (s, 2 H), 4.59 (d, J=10.4 Hz, 1 H), 4.83−4.94 (m, 3 H), 5.06 (s, 2 H), 6.64−6.74 (m, 4 H), 6.84−6.91 (m, 1 H), 6.96−7.38 (m, 27 H). MS (ESI) m/z 874 (M+NH$_4$).

(1S)-1,5-Anhydro-2,3,4,6-tetra-O-benzyl-1-[3-(4-ethoxybenzyl)-4-methoxyphenyl]-1-thio-D-glucitol (21c). Compound **21c** (930 mg, 60%) was prepared as a colorless amorphous from **20c** (1.58 g, 1.98 mmol) according to the method described for the synthesis of **21a**. ^{1}H NMR (600 MHz, CDCl$_3$) δ 1.37 (t, J=6.9 Hz, 3 H), 3.05−3.11 (m, 1 H), 3.50 (t, J=8.9 Hz, 1 H), 3.70 (dd, J=2.8, 9.6 Hz, 1 H), 3.76−3.84 (m, 6 H), 3.84−3.92 (m, 3 H), 3.92−3.99 (m, 3 H), 4.45 (d, J=10.1 Hz, 1 H), 4.52 (s, 2 H), 4.59 (d, J=10.6 Hz, 1 H), 4.85 (s, 2 H), 4.89 (d, J=11.0 Hz, 1 H), 6.67−6.75 (m, 4 H), 6.83 (d, J=8.3 Hz, 1 H), 7.02−7.18 (m, 8 H), 7.22−7.35 (m, 14 H). MS (ESI) m/z 803 (M+Na).

(1S)-1,5-Anhydro-2,3,4,6-tetra-O-benzyl-1-[3-(4-ethoxybenzyl)-4-fluorophenyl]-1-thio-D-glucitol (21d). Compound **21d** (767 mg, 49%) was prepared as a colorless powder from **20d** (1.60 g, 2.04 mmol) according to the method described for the synthesis of **21a** following recrystallization from hexane/ethyl acetate. ^{1}H NMR (300 MHz, CDCl$_3$) δ 1.38 (t, J=7.0 Hz, 3 H), 3.03−3.13 (m, 1 H), 3.46−3.54 (m, 1 H), 3.66−4.00 (m, 10 H), 4.45−4.53 (m, 3 H), 4.59 (d, J=10.7 Hz, 1 H), 4.84−4.93 (m, 3 H), 6.65−6.77 (m, 4 H), 6.96−7.34 (m, 23 H). MS (ESI) m/z 791 (M+Na).

(1S)-1,5-Anhydro-2,3,4,6-tetra-O-benzyl-1-[3-(4-ethoxybenzyl)-4-methylphenyl]-1-thio-D-glucitol (21e). Compound **21e** (1.04 g, 69%) was prepared as a colorless powder from **20e** (1.53 g, 1.96 mmol) according to the method described for the synthesis of **21a**. ^{1}H NMR (300 MHz, CDCl$_3$) δ 1.38 (t, J=7.0 Hz, 3 H), 2.22 (s, 3 H), 3.06−3.14 (m, 1 H), 3.52 (t, J=8.9 Hz, 1 H), 3.68−4.00 (m, 10 H), 4.46−4.54 (m, 3 H), 4.60 (d, J=10.7 Hz, 1 H), 4.84−4.93 (m, 3 H), 6.67−6.76 (m, 4 H), 6.92−6.98 (m, 2 H), 7.08−7.35 (m, 21 H). MS (ESI) m/z 782 (M+Na).

(1S)-1,5-Anhydro-2,3,4,6-tetra-O-benzyl-1-[4-chloro-3-(4-ethoxybenzyl)phenyl]-1-thio-D-glucitol (21f). Compound **21f** (2.62 g, 71%) was prepared as a colorless powder from **20f** (3.79 g, 4.73 mmol) according to the method described for the synthesis of **21a** following recrystallization from hexane/ethyl

acetate. ^{1}H NMR (300 MHz, CDCl$_3$) δ 1.38 (t, J=6.9 Hz, 3 H), 3.00−3.15 (m, 1 H), 3.50 (t, J=8.9 Hz, 1 H), 3.70 (dd, J=3.0, 9.9 Hz, 1 H), 3.75−3.80 (m, 2 H), 3.82−3.99 (m, 4 H), 4.06 (d, J=15.6 Hz, 1 H) 4.47−4.53 (m, 3 H), 4.59 (d, J=10.6 Hz, 1 H), 4.82−4.88 (m, 2 H), 4.89 (d, J=10.6 Hz, 1 H), 6.70 (d, J=6.9 Hz, 2 H), 6.74 (d, J=8.7 Hz, 2 H), 7.03 (d, J=8.7 Hz, 2 H), 7.09−7.37 (m, 21 H). MS (ESI) m/z 807 (M+Na).

(1S)-1,5-Anhydro-2,3,4,6-tetra-O-benzyl-1-[2-(benzyloxy)-5-(4-ethoxybenzyl)phenyl]-1-thio-D-glucitol (21g). Compound **21g** (0.210 g, 99%) was prepared from **20g** (0.216 g, 0.247 mmol) according to the method described for the synthesis of **21a**. ^{1}H NMR (300 MHz, CDCl$_3$) δ 1.36 (t, J=6.9 Hz, 3 H), 2.97−3.17 (m, 1 H), 3.47−3.62 (m, 1 H), 3.62−3.75 (m, 1 H), 3.76−4.03 (m, 8 H), 4.40−4.67 (m, 5 H), 4.82−5.12 (m, 5 H), 6.62−7.42 (m, 32 H). MS (ESI) m/z 879 (M+Na).

(1S)-1,5-Anhydro-2,3,4,6-tetra-O-benzyl-1-[5-(4-ethoxybenzyl)-2-methoxyphenyl]-1-thio-D-glucitol (21h). Compound **21h** (0.87 g) was prepared as a pale yellow oil from **20h** (1.71 g, 2.15 mmol) according to the method described for the synthesis of **21a**. ^{1}H NMR (600 MHz, CDCl$_3$) δ 1.37 (t, J=7.1 Hz, 3 H), 3.07−3.15 (m, 1 H), 3.54 (t, J = 9.2 Hz, 1 H), 3.62−3.99 (m, 11 H), 4.47−4.62 (m, 6 H), 4.84−4.93 (m, 3 H), 6.61−7.41 (m, 27 H). MS (ESI) m/z 803 (M+Na).

(1S)-1,5-Anhydro-2,3,4,6-tetra-O-benzyl-1-[5-(4-ethoxybenzyl)-2,4-dimethoxyphenyl]-1-thio-D-glucitol (21i). Compound **21i** (0.358 g, 30%) was prepared as a yellow oil from **20i** (1.22 g, 1.48 mmol) according to the method described for the synthesis of **20a**. ^{1}H NMR (300 MHz, CDCl$_3$) δ 1.35 (t, J=6.9 Hz, 3 H), 3.06−3.15 (m, 1 H), 3.52 (t, J=8.9 Hz, 1 H), 3.64−3.98 (m, 14 H), 4.45−4.62 (m, 4 H), 4.71 (s, 2 H), 4.84−4.93 (m, 3 H), 6.45 (s, 1 H), 6.63−6.72 (m, 4 H), 6.99−7.38 (m, 21 H). MS (ESI) m/z 828 (M+NH$_4$).

(1S)-1,5-Anhydro-2,3,4,6-tetra-O-benzyl-1-[4-chloro-3-(4-methoxybenzyl)phenyl]-1-thio-D-glucitol (21j). Compound **21j** (1.01 g, 55%) was prepared as a colorless powder from **20j** (1.87 g, 2.37 mmol) according to the method described for the synthesis of **21a** following recrystallization from ethyl acetate. ^{1}H NMR (300 MHz, CDCl$_3$) δ 3.01−3.16 (m, 1 H), 3.50 (t, J=8.9 Hz, 1 H), 3.66−3.72 (m, 1 H), 3.76 (s, 3 H), 3.76−3.99 (m, 6 H), 4.02−4.14 (m, 1 H), 4.46−4.53 (m, 3 H), 4.59 (d, J=10.7 Hz, 1 H), 4.82−4.95 (m, 3 H), 6.63−6.82 (m, 4 H), 7.01−7.36 (m, 23 H). MS (ESI) m/z 788 (M+NH$_4$).

(1S)-1,5-Anhydro-2,3,4,6-tetra-O-benzyl-1-[4-chloro-3-(4-methylbenzyl)phenyl]-1-thio-D-glucitol (21k). Compound **21k** (1.54 g, 63%) was prepared as a colorless powder from **20k** (2.50 g, 3.24 mmol) according to the method described for the synthesis of **21a** following recrystallization from hexane/ethyl acetate. ^{1}H NMR (300 MHz, CDCl$_3$) δ 2.30 (s, 3 H), 3.04−3.13 (m, 1 H), 3.46−3.54 (m, 1 H), 3.66−4.13 (m, 8 H), 4.47−4.53 (m, 3 H), 4.59 (d, J=11.0 Hz, 1 H), 4.84−4.92 (m, 3 H), 6.67−6.72 (m, 2 H), 7.02 (s, 4 H), 7.08−7.35 (m, 21 H). MS (ESI) m/z 772 (M+NH$_4$), 774 (M+2+NH$_4$).

(1S)-1,5-Anhydro-2,3,4,6-tetra-O-benzyl-1-[4-chloro-3-(4-ethylbenzyl)phenyl]-1-thio-D-glucitol (21l). Compound **21l** (2.13 g, 72%) was prepared as a pale yellow powder from **20l** (3.03 g, 3.86 mmol) according to the method described for the synthesis of **21a** following recrystallization from hexane/ethyl acetate. ^{1}H NMR (300 MHz, CDCl$_3$) δ 1.19 (t, J = 7.8 Hz, 3 H), 2.58 (q, J = 7.8 Hz, 2 H), 3.04−3.13 (m, 1 H), 3.50 (t, J=8.7 Hz, 1 H), 3.66−4.14 (m, 8 H), 4.46−4.53 (m, 3 H), 4.59 (d, J=10.7 Hz, 1 H), 4.84−4.92 (m, 3 H), 6.66−6.72 (m, 2 H), 7.00−7.36 (m, 25 H). MS (ESI) m/z 791 (M+Na).

(1S)-1,5-Anhydro-2,3,4,6-tetra-O-benzyl-1-[4-chloro-3-[4-(propan-2-yl)benzyl]phenyl]-1-thio-D-glucitol (21m). Compound **21m** (2.01 g, 77%) was prepared as a colorless powder from **20m** (2.65 g, 3.31 mmol) according to the method described for the synthesis of **21a** following recrystallization from hexane/ethyl acetate. ^{1}H NMR (300 MHz, CDCl$_3$) δ 1.20 (d, J = 7.0 Hz, 6 H), 2.77−2.89 (m, 1 H), 3.05−3.13 (m, 1 H), 3.51 (t, J=8.9 Hz, 1 H), 3.66−4.15 (m, 8 H), 4.46−4.54 (m, 3 H), 4.59 (d, J=10.7 Hz, 1 H), 4.83−4.92 (m, 3 H),

6.65−6.72 (m, 2 H), 7.04−7.37 (m, 25 H). MS (ESI) *m/z* 805 (M+Na).

(1*S*)-1,5-Anhydro-2,3,4,6-tetra-*O*-benzyl-1-[3-(4-*tert*-butyl-benzyl)-4-chlorophenyl]-1-thio-D-glucitol (21n). Compound **21n** (1.05 g, 64%) was prepared as a colorless powder from **20n** (1.68 g, 2.07 mmol) according to the method described for the synthesis of **21a** following recrystallization from hexane/ethyl acetate. ^{1}H NMR (300 MHz, CDCl$_3$) δ 1.26 (s, 9 H), 3.05−3.20 (m, 1 H), 3.51 (t, *J* = 8.9 Hz, 1 H), 3.63−3.93 (m, 6 H), 3.93−4.03 (m, 1 H), 4.06−4.17 (m, 1 H), 4.45−4.54 (m, 3 H), 4.59 (d, *J* = 10.6 Hz, 1 H), 4.84−4.93 (m, 3 H), 6.69 (dd, *J* = 1.5, 8.0 Hz, 2 H), 7.04−7.38 (m, 25 H). MS (ESI) *m/z* 819 (M+Na).

(1*S*)-1,5-Anhydro-2,3,4,6-tetra-*O*-benzyl-1-[4-chloro-3-[4-(methyl-sulfanyl)benzyl]phenyl]-1-thio-D-glucitol (21o). Compound **21o** (1.20 g, 62%) was prepared as a colorless powder from **20o** (1.98 g, 2.46 mmol) according to the method described for the synthesis of **21a** following recrystallization from hexane/ethyl acetate. ^{1}H NMR (600 MHz, CDCl$_3$) δ 2.42 (s, 3 H), 3.05−3.12 (m, 1 H), 3.51 (t, *J* = 8.9 Hz, 1 H), 3.70 (dd, *J* = 3.0, 9.9 Hz, 1 H), 3.74−3.82 (m, 2 H), 3.82−3.92 (m, 3 H), 3.93−4.01 (m, 1 H), 4.07 (d, *J* = 15.1 Hz, 1 H), 4.48−4.54 (m, 3 H), 4.59 (d, *J* = 10.6 Hz, 1 H), 4.82−4.87 (m, 2 H), 4.89 (d, *J* = 10.6 Hz, 1 H), 6.70 (d, *J* = 7.3 Hz, 2 H), 7.00−7.38 (m, 25 H). MS (ESI) *m/z* 804 (M+NH$_4$).

(1*S*)-1,5-Anhydro-2,3,4,6-tetra-*O*-benzyl-1-[5-(4-ethoxybenzyl)-2-methoxy-4-methylphenyl]-1-thio-D-glucitol (21p). Compound **21p** (0.640 g, 77%) was prepared as a colorless syrup from **20p** (0.840 g, 1.0 mmol) according to the method described for the synthesis of **21a**. ^{1}H NMR (600 MHz, CDCl$_3$) δ 1.35 (t, *J* = 6.9 Hz, 3 H), 2.21 (s, 3 H), 3.02−3.21 (m, 1 H), 3.55 (t, *J* = 9.4 Hz, 1 H), 3.69−3.72 (m, 1 H), 3.74−3.97 (m, 10 H), 4.00−4.05 (m, 1 H), 4.45−4.56 (m, 3 H), 4.60 (d, *J* = 10.6 Hz, 2 H), 4.86 (s, 2 H), 4.90 (d, *J* = 10.6 Hz, 1 H), 6.58−6.76 (m, 4 H), 6.90 (d, *J* = 7.3 Hz, 1 H), 7.09−7.19 (m, 5 H), 7.23−7.35 (m, 16 H). MS (ESI) *m/z* 812 (M+NH$_4$).

(1*S*)-1,5-Anhydro-2,3,4,6-tetra-*O*-benzyl-1-[5-(4-ethylbenzyl)-2-methoxy-4-methylphenyl]-1-thio-D-glucitol (21q). Compound **21q** (1.68 g, 76%) was prepared as a colorless oil from **20q** (2.25 g, 2.83 mmol) according to the method described for the synthesis of **21a**. ^{1}H NMR (300 MHz, CDCl$_3$) δ 1.15 (t, *J* = 7.5 Hz, 3 H), 2.22 (s, 3 H), 2.54 (q, *J* = 7.5 Hz, 2 H), 3.06−3.16 (m, 1 H), 3.49−4.07 (m, 11 H), 4.45−4.65 (m, 5 H), 4.84−4.94 (m, 3 H), 6.69−6.76 (m, 3 H), 6.89−6.99 (m, 4 H), 7.07−7.19 (m, 5 H), 7.22−7.35 (m, 14 H). MS (ESI) *m/z* 801 (M+Na).

(1*S*)-1,5-Anhydro-2,3,4,6-tetra-*O*-benzyl-1-[2-methoxy-4-methyl-5-[4-(propane-2-yl)benzyl)phenyl]-1-thio-D-glucitol (21r). Compound **21r** (0.69 g, 62%) was prepared from **20r** (1.14 g, 1.41 mmol) according to the method described for the synthesis of **21a**. ^{1}H NMR (300 MHz, CDCl$_3$) δ 1.16 (d, *J* = 6.8 Hz, 3 H), 2.23 (s, 3 H), 2.70−2.86 (m, 1 H), 3.03−3.20 (m, 1 H), 3.55 (t, *J* = 8.9 Hz, 1 H), 3.64−4.08 (m, 10 H), 4.43−4.66 (m, 5 H), 4.80−4.95 (m, 3 H), 6.67−6.78 (m, 3 H), 6.89−7.00 (m, 4 H), 7.05−7.19 (m, 5 H), 7.21−7.37 (m, 14 H). MS (ESI) *m/z* 815 (M+Na).

(1*S*)-1,5-Anhydro-2,3,4,6-tetra-*O*-benzyl-1-[4-chloro-5-(4-ethyl-benzyl)-2-methoxyphenyl]-1-thio-D-glucitol (21s). Compound **21s** (2.65 g, 87%) was prepared as a pale yellow crystal from **20s** (3.12 g, 3.83 mmol) according to the method described for the synthesis of **21a**. ^{1}H NMR (300 MHz, CDCl$_3$) δ 1.14 (t, *J* = 7.8 Hz, 3 H), 2.54 (q, *J* = 7.8 Hz, 2 H), 3.06−3.16 (m, 1 H), 3.53 (t, *J* = 8.8 Hz, 1 H), 3.64−4.13 (m, 10 H), 4.46−4.65 (m, 5 H), 4.83−4.95 (m, 3 H), 6.64−6.72 (m, 2 H), 6.87−7.35 (m, 24 H). MS (ESI) *m/z* 821 (M+Na).

Glucose Uptake Inhibition Assay. Chinese hamster ovary - K1 cells stably expressing human SGLT2 (NM_003041) and human SGLT1 (NM_000343) were used for the sodium dependent glucose transport inhibition test. Cells were incubated with 20 μL of pretreatment buffer solution (140 mM choline chloride, 2 mM KCl, 1 mM CaCl$_2$, 1 mM MgCl$_2$, 10 mM HEPES/5 mM Tris, pH 7.4) for 20 min. The pretreatment buffer was removed

and 75 μL of uptake buffer containing test compounds (methyl-α-D-glucopyranoside containing [^{14}C] methyl-α-D-glucopyranoside (0.1 mM for SGLT1 inhibition, 1 mM for SGLT2 inhibition), 140 mM NaCl, 2 mM KCl, 1 mM CaCl$_2$, 1 mM MgCl$_2$, 10 mM HEPES/5 mM Tris, pH 7.4) were added, and the uptake reaction was performed at 37 °C for 30 min (SGLT1) or for 1 h (SGLT2). After the reaction, cells were washed with 200 μL of washing buffer (10 mM methyl-α-D-glucopyranoside, 140 mM choline chloride, 2 mM KCl, 1 mM CaCl$_2$, 1 mM MgCl$_2$, 10 mM HEPES/5 mM Tris, pH 7.4) twice and dissolved in 75 μL of 0.25 M NaOH. Opti phase Super Mix (Perkin-Elmer Corporation) was added and mixed well with the sample, then radio-activity was measured with a Micro Beta Trilux (Perkin-Elmer Corporation). Uptake buffer containing no test compound was used as a control. Another uptake buffer containing choline chloride in place of NaCl was also prepared for background.

In order to determine IC$_{50}$ values, each test compound was tested at six suitable concentrations. The concentration at which glucose uptake was inhibited by 50% (IC$_{50}$ value) compared to glucose uptake in the control (100%) was calculated.

Hypoglycemic Effect in STZ-induced Diabetic Rats. Male Sprague−Dawley rats (7-weeks old, Charles River, Japan) were intravenously injected with STZ (50 mg/kg, Sigma) to induce hyperglycemia. One week later, a group of 5 or 6 nonfasted STZ rats were given an oral dose (0.3 or 3 mg/kg) of one of test compounds **3m** and **3p−3s**. Blood was collected from the orbital venous sinus under ether anesthesia at serial time points, and plasma glucose levels were measured using a Glucose C2 Test Wako (Wako pure Chemical Industries, Japan). Male SD rats (8 weeks old) not treated with STZ were used as the normal control.

Urinary Glucose Excretion in Zucker Fatty Rats. After fasting for 17 h, Zucker fatty rats (10 weeks old, mean body weight, approximately 380 g, Charles River Japan) were given a single oral dose (1 mg/kg) of **3p** 30 min prior to a 2 g/kg glucose load. Urine was collected for 24 h using metabolic cages, and glucose content was measured using Glucose C2 Test Wako. Male ZF-lean rats (10 weeks old) were used as the normal control.

Urinary Glucose Excretion in Beagle Dogs. After fasting for 21 h, beagle dogs (7 to 12 months old, body weight, 8.1−9.6 kg, Charles River, Japan) were given a single oral dose (1 mg/kg) of **3p** 1 h prior to a 2 g/kg glucose load. Urine was collected for 24 h using metabolic cages, and glucose content was measured using Glucose C2 Test Wako.

Metabolic Stability. Human Liver Microsomal Stability. Test compounds **3m** and **3p−3s** (5 μM) were incubated with 1.0 mg protein/mL of human liver microsomes (Xenotech LLC, USA) in 0.255 M phosphate buffer containing 0.5% (w/v) KCl (pH 7.4) and NADPH-generating system (2.4 mM MgCl$_2$, 1.5 mM glucose-6-phosphate, 0.18 U/mL glucose-6-phosphate dehydrogenase, 0.16 mM NADP$^+$) at 37 °C for 15 min. Reactions were quenched by the addition of DMSO, and supernatants were analyzed by LC/MS/MS.

Human Cryopreserved Hepatocyte Stability. Test compounds **3m** and **3p−3s** (5 μM) were incubated with 1 × 10^6 human cryo-preserved hepatocytes (In Vitro Technologies, USA) in pre-pared Hank's Balanced Salt Solution (KAC, Japan) at 37 °C for 240 min. Reactions were quenched by the addition of DMSO, and supernatants were analyzed by LC/MS/MS.

Serum Protein Binding. Human blood was obtained from healthy male and female volunteers by venous puncture without anticoagulants, as approved by the institutional ethics committee. The serum was obtained by centrifugation. Compounds **3m** and **3p−3s** (1 μg/mL) were added to serum and dialyzed against 0.05 M sodium phosphate buffer (pH 7.4) containing 0.07 M sodium chloride. Dialysis was performed at 37 °C for 6 h using a 96-well equilibrium dialysis equipment. The concentrations of the test compounds in serum and buffer were analyzed by LC/MS/MS.

Caco-2 Monolayer Permeability. Caco-2 cells were obtained from the American Type Culture Collection (Rockville, MD). Cells were grown in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum, 1% L-glutamine, 1% nonessential amino acids, and a 1% antibiotic−antimycotic mixture (10 000 U/mL penicillin G, 10 000 μg/mL streptomycin sulfate, and 25 μg/mL amphotericin B in 0.85% saline) at 37 °C in culture flasks in a humidified 5% CO_2 atmosphere. For cell permeability assessment, cells (passage number 50 to 60) were seeded on 24-well cell culture inserts (Transwell, 0.3 μm pores, 6.5 mm id) and cultured for 21 days. The integrity of the cell monolayer was evaluated by measuring transepithelial electric resistance (TEER) and ^{14}C-mannitol permeability before the transport experiment. Hank's balanced salt solution (HBSS) was used in all experiments after adjusting the pH to 6.5 for the apical side and the pH 7.4 for the basolateral side. Compound **3p** or propranolol was dissolved in HBSS (pH 6.5) at a final concentration of 10 μM. After 20 min incubation of both sides of the monolayer with drug-free HBSS, **3p** or propranolol contained HBSS (pH 6.5) was added to the apical side of the monolayers. Samples (50 μL) were taken from the basolateral side at 30, 60, 90, and 120 min after dose administration. The volume of the basolateral side was maintained by adding HBSS (pH 7.4). All experiments were performed in triplicate at 37 °C. Concentrations of compound **3p** and propranolol were determined by LC-MS/MS.

The apparent permeability coefficients (P_{app}) for each well were calculated using the following equation:

$$P_{app}(cm/s) = (\Delta cVR/\Delta tA)co$$

Where $\Delta cVR/\Delta t$ is the cumulative amount transported as a function of time (seconds), VR is the volume of the receiver chamber (mL), A is the surface area of the cell monolayer (cm^2), and co is the concentration of the compound at $t = 0$.

Pharmacokinetics. Plasma Concentrations. After administration of compound **3p**, 0.3 (rats) or 2 (dogs) mL blood samples were taken from a vein and drawn into a tube, and the plasma was separated by centrifugation. To a 50 μL aliquot of plasma sample, 25 μL of I.S. methanol solution (100 ng/mL) and 500 μL of 10 mM ammonium acetate solution were added, and the samples were vortexed. The samples were loaded into SPE cartridges (OASIS HLB, Waters USA), washed, then eluted with acetic acid/methanol (0.1/100). The eluates were evaporated to dryness, reconstituted with 100 μL of CH$_3$CN/10 mM ammonium acetate (20/80), and a 10 μL aliquot of the sample was analyzed by LC/ESI-MS/MS. The lower limit of quantification (LLOQ) of plasma was 0.3 ng/mL.

Kidney Concentrations. After oral administration of compound **3p**, rats were decapitated following blood sampling at 1, 4, and 24 h post dose. The kidneys were immediately excised, weighed, and 4-fold saline was added, followed by homogenization. To a 50 μL aliquot of plasma or kidney homogenate sample, 200 μL of I.S. CH$_3$CN/MeOH (9/1) solution (250 ng/mL) was added, and the sample was vortexed. A 10 μL aliquot of the supernatant was analyzed by LC-MS/MS system. LLOQs of plasma and kidney were 3 ng/mL and 50 ng/g, respectively.

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