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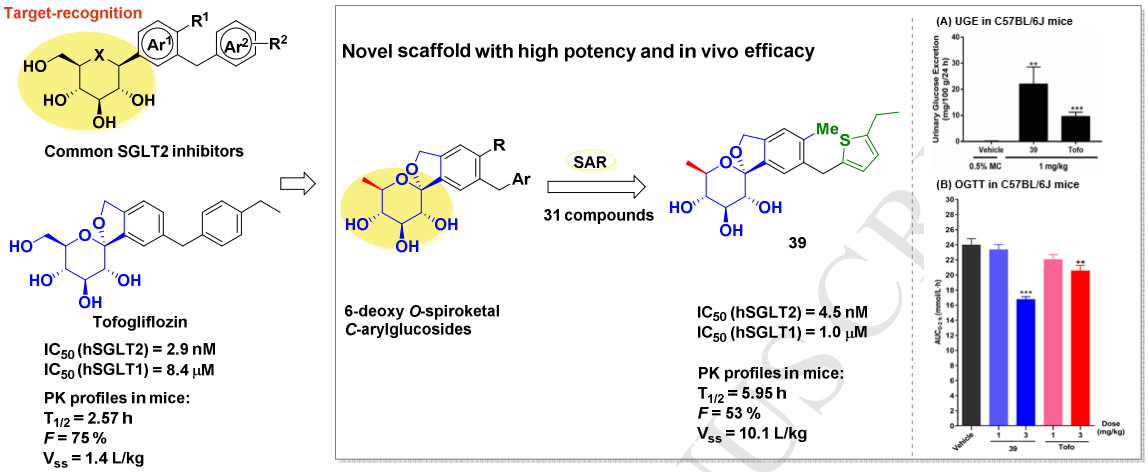
**Title:**

# Design, Synthesis and Biological Evaluation of 6-Deoxy *O*-spiroketal *C*-arylglucosides as Novel Renal Sodium-Dependent Glucose Cotransporter 2 (SGLT2) Inhibitors for the Treatment of Type 2 Diabetes

# Graphical Abstract



**Highlights:**

- Novel 6-deoxy *O*-spiroketal *C*-arylglucosides as SGLT2 inhibitors were designed, synthesized, and evaluated.
- The structure-activity relationship (SAR) of this novel series of 31 compounds was demonstrated.
- Compound **39** demonstrated excellent *in vitro* inhibitory activities against hSGLT2 ($IC_{50}$ = 4.5 nM), which was higher than positive control (dapagliflozin, $IC_{50}$ = 8.3 nM in this assay).
- Compound **39** displayed more remarkable efficacy on promoting urinary glucose excretion and improving oral glucose tolerance than marketed drug tofogliflozin did in C57BL/6J mice.
- The half-life of compound **39** in mice was longer than tofogliflozin after oral administration (5.95 h vs 2.57 h).

# Design, Synthesis and Biological Evaluation of 6-Deoxy *O*-spiroketal *C*-arylglucosides as Novel Renal Sodium-Dependent Glucose Cotransporter 2 (SGLT2) Inhibitors for the Treatment of Type 2 Diabetes

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**ABSTRACT:**

In this work, aiming at finding a novel, potent, and selective sodium-dependent glucose cotransporter 2 (SGLT2) inhibitor with good pharmacokinetic profiles for the treatment of diabetes, we focus on modifying the sugar moiety of SGLT2 inhibitors, which dominates the binding with glucose binding site of hSGLT, via removing the C-6 hydroxy group to adjust the physicochemical properties and target-recognition manners of SGLT2 inhibitors. In addition, tofogliflozin containing a special *O*-spiroketal *C*-arylglucoside scaffold, displayed good efficacy and bioavailability both in animals and in humans. Therefore, a series of 6-deoxy *O*-spiroketal *C*-arylglucosides as novel SGLT2 inhibitors were designed, synthesized, and evaluated in this work. The structure-activity relationship (SAR) research on this novel series and a comprehensive *in vitro* and *in vivo* biological evaluation afforded compound **39** with high *in vitro* hSGLT2 inhibitory activity ($IC_{50}$ = 4.5 nM), good pharmacokinetic profiles, and more remarkable efficacy in C57BL/6J mice and Sprague-Dawley rats than marketed drug tofogliflozin.

**Keywords:** SGLT2 inhibitors, Diabetes, Sugar modification, Structure-activity relationship, Urinary glucose excretion, Oral glucose tolerance

## INTRODUCTION

Diabetes Mellitus (DM), as a chronical, progressive metabolic disorder causing many devastating short and long-term effects, has represented one of the primary threats to health and human development these decades. It mainly consists of three types: type 1 diabetes (T1DM), type 2 diabetes (T2DM), and gestational diabetes (GDM), and T2DM accounts for around 90% of all cases.[1] The common medications for T2DM treatment are insulin and its analogs, as well as metformin, sulphonylureas, GLP-1 analogs, and DPP4 inhibitors.[1] For T1DM patients, current small molecular drugs usually fail in reducing blood glucose levels, and long-term injection of insulin is indispensable because the lack of basal insulin in T1DM patients.[2] However, various adverse effects of those medications such as hypoglycemia, cardiovascular risk and weight gain cannot be ignored.[3] Recently, the newly developed sodium-dependent glucose cotransporter 2 (SGLT2) inhibitors have become increasingly popular and widely accepted for T2DM therapy,[4, 5] and the clinical trials of T1DM treatment using SGLT2 inhibitors combined with insulin are well underway[6-11].

SGLT2, mostly distributed in proximal renal tubules, is a major transporter responsible for over 90% of renal glucose reabsorption that is known as one of the major pathways to maintain glucose homeostasis.[12] In diabetes, continuous hyperglycemia leads to upregulation of SGLT2, resulting in stronger absorption of renal glucose and consequently higher blood glucose levels.[13-15] Based on the revelation that mutations of the SGLT2 gene caused familial renal glycosuria without affecting daily activities, researchers have presumed that inhibiting the hyperactive SGLT2 in kidneys of diabetic patients, thus increasing glucose excretion, should be a promising approach to reduce blood glucose levels.[16, 17] Moreover, this approach causes no hypoglycemia and weight gain because the remarkable anti-diabetic effects is independent with insulin secretion or insulin action.[12, 18, 19] Most excitingly, the EMPA-REG OUTCOME trial revealed that using SGLT2 inhibitor treatment with empagliflozin displayed significant reduction of heart failure hospitalization and

cardiovascular deaths in T2DM patients[20, 21], and similar results of other gliflozins were also reported[4, 22, 23], which has never been achieved by other classes of anti-diabetic medications[3, 24-28]. Several putative mechanisms of the cardiovascular benefits have been proposed, including the amelioration of ventricular preload and afterload, improvement in cardiac metabolism, inhibition of myocardial $Na^+/H^+$ exchange, reduction of necrosis and cardiac fibrosis, and so on.[29] Besides, it has been reported that T1DM patients treated with SGLT2 inhibitors need less dosage of insulin injection to improve glycemic control, which may be associated with a lower rate of hypoglycemia events.[6-11, 30]

The first reported SGLT2 inhibitor was phlorizin (**1**, Figure 1), a natural product that was proved to cause glucosuria and hypoglycemic effect via nonselective SGLT inhibition. Nonetheless, phlorizin was not suitable for clinical use mainly because of its poor metabolic stability to *β*-glucosidases, moderate potency, and severe gastrointestinal side effects resulting from its low selectivity of hSGLT2. Therefore, large amounts of efforts have been promoted to overcome these drawbacks and plenty of promising SGLT2 inhibitors have sprung up[31, 32]. Generally, SGLT2 inhibitors are divided into three categories, *O*-glucosides, *C*-glycosides, and *O*-spiroketal *C*-arylglucosides.[33] The metabolic susceptibility to *β*-glucosidases limited the development of *O*-glucosides, while the latter two structural categories, which are proved highly stable against glucosidases, have been extensively and rapidly investigated, yielding up to seven drugs launched for the treatment of T2DM (Figure 1). Among the first few drugs on the market, dapagliflozin[34] (**2**), canagliflozin[35] (**3**), empagliflozin[36] (**4**), and ipragliflozin[37] (**5**) are *C*-glucosides; tofogliflozin[38] (**6**) is of *O*-spiroketal *C*-arylglucoside class; luseogliflozin[39] (**7**) and the latest approved ertugliflozin[40] (**8**) are special *C*-glycosides: the former one contains a *C*-phenyl 1-thio-D-glucitol scaffold, and the latter one incorporates a unique bridged ketal ring. Although up to seven gliflozins are already on the market, the hypoglycemic effects as well as cardiovascular benefits of them still need improvement, and it is worthy of efforts on developing new drugs to avert adverse effects such as ketoacidosis.

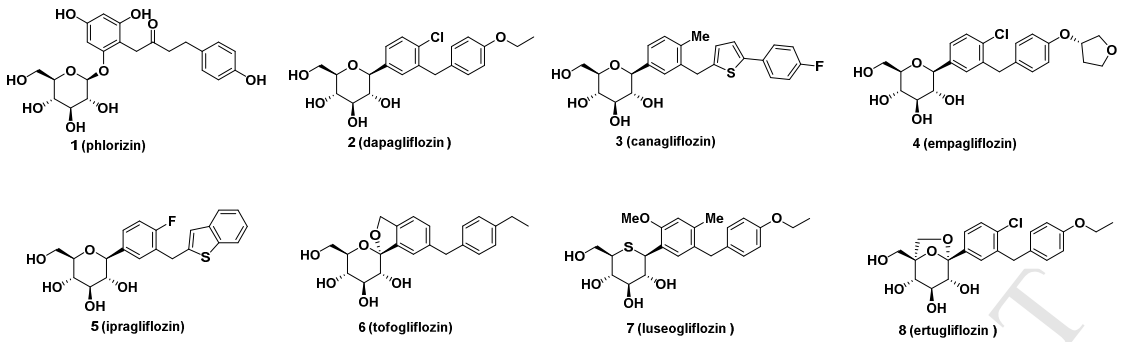



**Figure 1**. Phlorizin and the marketed SGLT2 inhibitors.

It was noticed that all the currently marketed SGLT2 inhibitors share the same pattern of four hydroxy groups in the glucose moiety. As some results of binding studies of phlorizin[41] and marketed gliflozins[36, 42, 43] with hSGLTs had been revealed, the critical role of the sugar moiety in target-recognition attracted our attention to searching for a novel and potent SGLT2 inhibitor with more satisfactory pharmacokinetics profiles via sugar modifications. Herein, we report the design, synthesis, and biological evaluation of a novel series of 6-deoxy *O*-spiroketal *C*-arylglucosides **9**-**39**, among which compound **39** was selected as a highly potent SGLT2 inhibitor.

## RESULTS AND DISCUSSION

### Design of 6-deoxy *O*-spiroketal *C*-arylglucosides

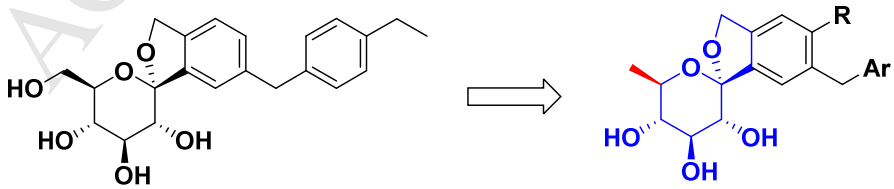
Recent decades have witnessed large amounts of global efforts on the SAR investigation of selective SGLT2 inhibitors[32, 33, 44-49]. Generally, three main elements are proved critical to the maintenance of SGLT2 inhibitory activity: a glucose moiety or its analogues with the same chair configuration, a proximal aromatic ring, and a distal ring where a lipophilic substituent is favorable.

Although the interactions between SGLT2 inhibitors and the target have not been achieved in detail because the crystal structure of SGLT2 is unavailable yet, several studies have disclosed that it was the ectodomains of SGLT that recognized the

inhibitors[17, 50-52]. The glucose-binding site of hSGLT dominates the interaction with the sugar moiety of SGLT2 inhibitors, while the binding of aglucone moiety influence the affinity of the entire inhibitor[41, 42, 52]. Furthermore, inhibition kinetics and binding studies of phlorizin[41], dapagliflozin[42], and luseogliflozin[43] suggested that modification of the sugar moiety may profoundly influence the association and dissociation manners of SGLT2 inhibitors with hSGLT2. For example, luseogliflozin, containing a distinctive 1-thio-D-glucitol sugar ring, displayed a much longer dissociation half-life (7 hours) [43] than empagliflozin (about 60 min)[36], which might interpret its long duration of action on urinary glucose excretion[53].

At present, most SGLT2 inhibitors have been designed focusing on the modification of aglucone[33], while the efforts in sugar modification were relatively rare[39, 40, 45-47]. Besides, the same pattern of four hydroxy groups in the sugar moiety was retained in all the marketed drugs (Figure 1). In the course of our efforts to the development of novel, potent and selective SGLT2 inhibitors with good pharmacokinetic profiles, we envisioned that the role of the sugar moiety in target-recognition might need more in-depth investigation, and removing the C-6 hydroxy group from the glucose moiety might not only alter the physicochemical properties of the molecule, but also influence the association and dissociation manners of SGLT2 inhibitors with the target. On the other hand, the interesting scaffold of *O*-spiroketal *C*-arylglucoside in tofogliflozin[38] attracted our attention because of its high potency and selectivity[38, 54].

Therefore, we designed and synthesized a novel series of 6-deoxy *O*-spiroketal *C*-arylglucosides **9**-**39** as SGLT2 inhibitors (Figure 2).



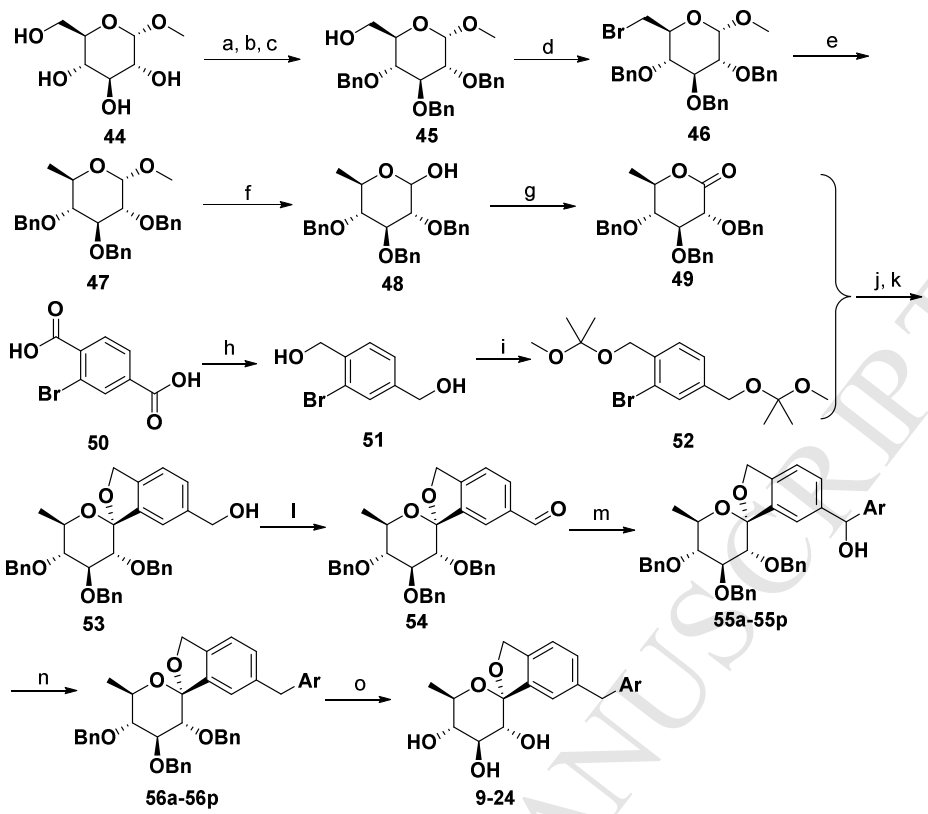

**6 (tofogliflozin)** **9-39**

**Figure 2**. Design of 6-deoxy *O*-spiroketal *C*-arylglucosides as SGLT2 inhibitors.

## Chemistry

The synthetic route of 6-deoxy *O*-spiroketal *C*-arylglucosides **9-24** is outlined in Scheme 1. Methyl glucopyranose **44** was converted to **45** in three consecutive steps to selectively leave the 6-OH group free. Direct bromination of **45** with triphenylphosphine and carbon tetrabromide, afforded quantitatively the bromide **46**. Efficient reduction of **46** with the tributyltin hydride furnished **47**. Acid hydrolysis of **47** afforded hemiacetal **48**, which was finally oxidized by DMSO to provide the key intermediate **49**. On the other hand, reduction of the two carboxyl groups of commercially available 2-bromoterephtalic acid **50**, followed by protection of the resulting hydroxy groups with 2-methoxypropene, afforded intermediate **52**. Addition of lactone **49** to intermediate **52** gave the desired *O*-spiroketal *C*-arylglucoside **53** by the treatment with *p*-toluenesulfonic acid. Oxidation of **53** provided aldehyde **54**, which was further reacted with Ar-X (X = Br or H) to afford **55a-55p**. Alcohols **55a-55p** were converted to **56a-56p** by using triethylsilane and borontrifluoride diethyletherate. Finally, debenzylation with boron trichloride or catalytic hydrogenation with palladium hydroxide under a hydrogen atmosphere afforded the target compounds **9-24**.

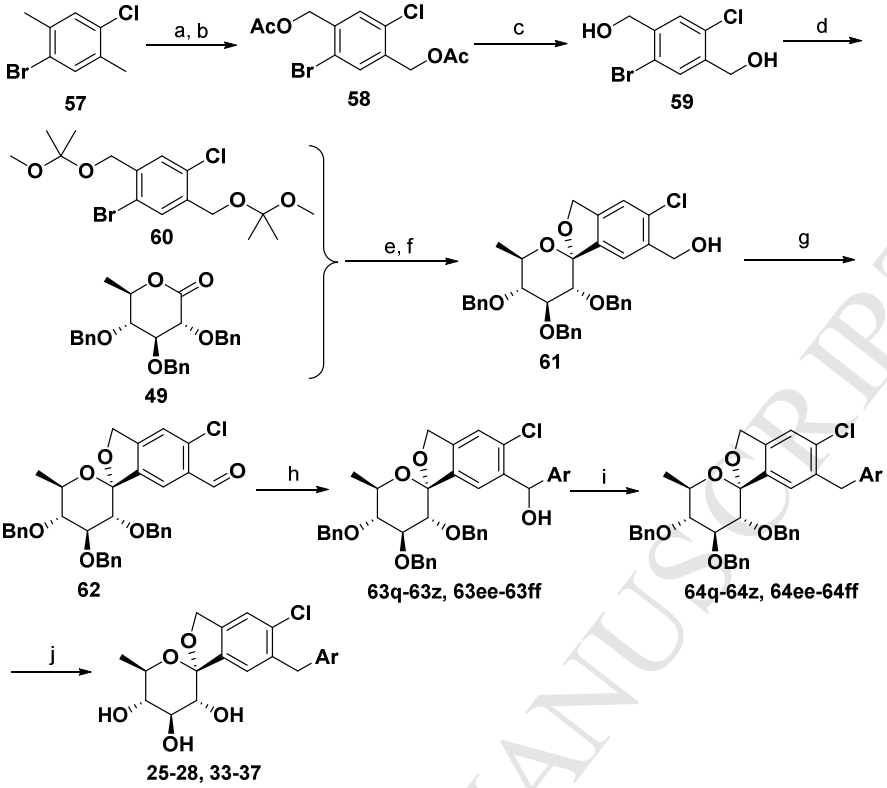
**Scheme 1. Synthetic routes of 6-deoxy *O*-spiroketal *C*-arylglucosides 9-24.**[a]




[a]Reagents and conditions: (a) Imidazole, TIPSCl, DMF, 0 °C to rt, 24 h; (b) NaH, BnBr, DMF, 0 °C to rt, 12 h; (c) TBAF, THF, rt, 12 h; (d) $Ph_3P$, $CBr_4$, THF, 0 °C, 1 h; (e) $Bu_3SnH$, AIBN, toluene, 80 °C, 4 h; (f) 3 M $H_2SO_4$ aq, AcOH, 85 °C, 2.5 h; (g) $Ac_2O$, DMSO, rt, overnight; (h) $BH_3{\cdot}SMe_2$, THF, 0 °C to 70 °C, 4 h; (i) pyridinium *p*-toluenesulfonate, 2-methoxypropene, THF, 0 °C, 2 h; (j) *n*-BuLi, THF, −78 °C, 3 h; (k) *p*-toluenesulfonic acid, THF-MeOH, rt, 15 h; (l) PCC, $CH_2Cl_2$, rt, 4 h; (m) Ar-X (X = H or Br), *n*-BuLi, THF, −78 °C, 3 h; (n) $Et_3SiH$, $BF_3{\cdot}OEt_2$, $CH_2Cl_2$, −40 °C, 1 h; (o) $H_2$, $Pd(OH)_2/C$, MeOH-AcOEt, 2 M HCl aq, rt, 5 h, or $BCl_3$, $Me_5$-benzene, $CH_2Cl_2$, −78 °C, overnight.

Synthetic routes of compounds **25-28** and **33-37** are shown in Scheme 2. Diol **59** was obtained from commercially available **57** *via* a three-step procedure consisting of dibromination, nucleophilic substitution reaction, and hydrolysis. Compound **59** was transformed into compounds **25-28** and **33-37** by a similar method to that in Scheme 1.

**Scheme 2. Synthetic routes of compounds 25-28 and 33-37.**[a]

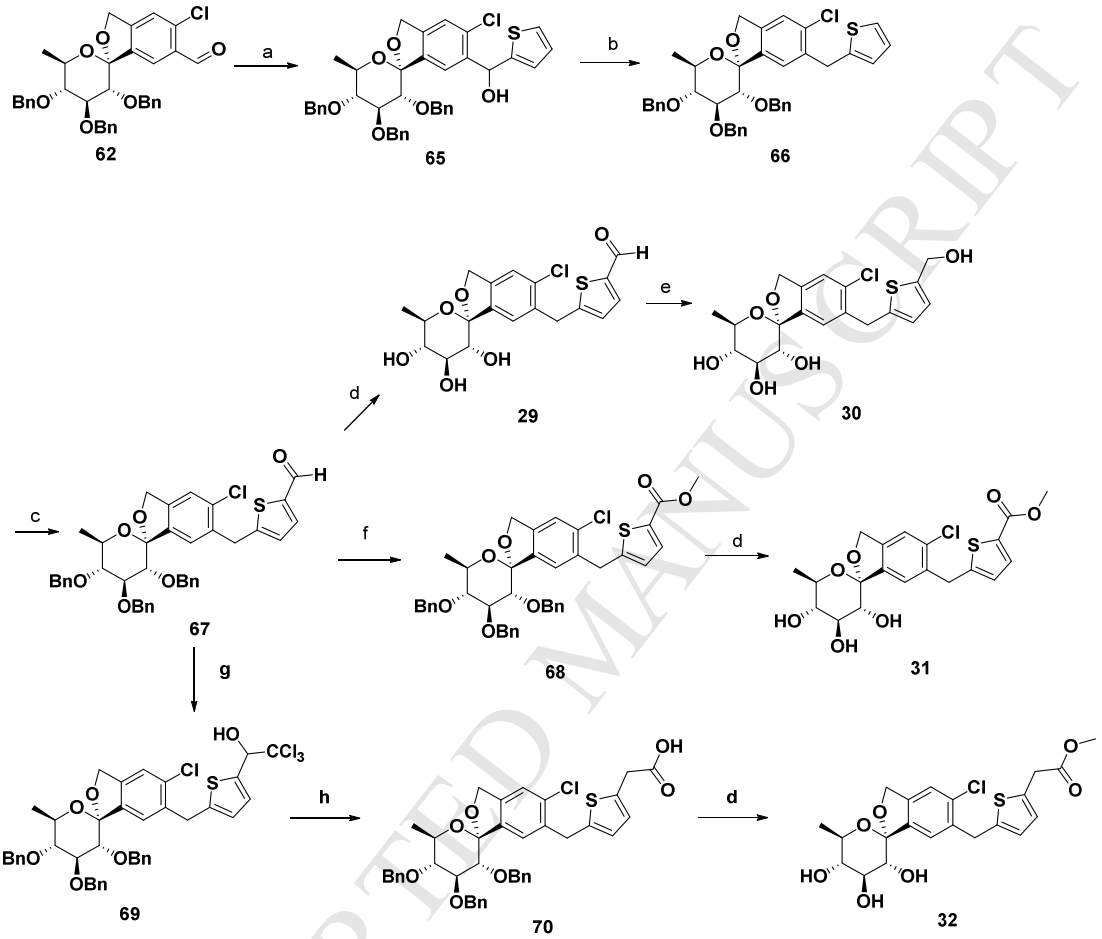



[a]Reagents and conditions: (a) NBS, AIBN, EtOAc, reflux, 0.5 h; (b) NaOAc, DMF, 80 °C, 3 h; (c) KOH, THF-EtOH-$H_2O$, 80 °C, 4.5 h; (d) pyridinium *p*-toluenesulfonate, 2-methoxypropene, THF, 0 °C, 2 h; (e) *n*-BuLi, THF, −78 °C, 3 h; (f) *p*-toluenesulfonic acid, THF-MeOH, rt, 15 h; (g) PCC, $CH_2Cl_2$, rt, 4 h; (h) Ar-X (X = H or Br), *n*-BuLi, THF, −78 °C, 3 h; (i) $Et_3SiH$, $BF_3 \cdot OEt_2$, $CH_2Cl_2$, −40 °C, 1 h; (j) $BCl_3$, $Me_5$-benzene, $CH_2Cl_2$, −78 °C, overnight.

Synthetic routes to compounds **29-32** are shown in Scheme 3. Aldehyde **62** was coupled with 2-bromothiophene followed by a reduction reaction to give intermediate **66**. A formyl group was introduced to compound **66** by the use of *N*,*N*-dimethylformamide (DMF), giving aldehyde **67**. Debenzylation of **67** afforded compound **29**, further reduction of the formyl group of **35** furnished the corresponding alcohol **30**. On the other hand, the oxidation of aldehyde **67** using iodine under basic conditions afforded ester **68**, which was further subjected to debenzylation to furnish compound **31**. Aldehyde **67** was treated with chloroform and KOH to give intermediate **69**, which was subsequently transformed[55] into aryl acetic

acid **70**. Final debenzylation of intermediate **70** followed by the after-treatment with methanol yielded compound **32**.

**Scheme 3. Synthetic routes of compounds 29-32.**[a]

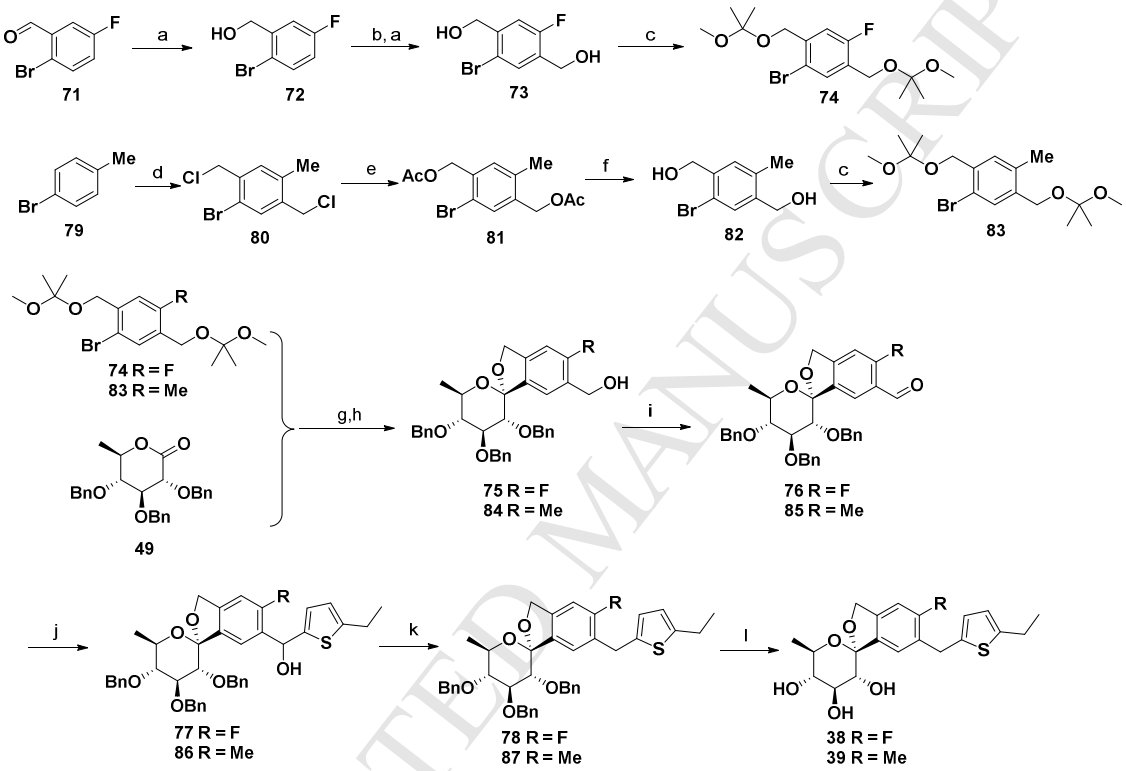



[a]Reagents and conditions: (a) 2-bromothiophene, *n*-BuLi, THF, −78 °C, 3 h; (b) $Et_3SiH$, $BF_3 \cdot OEt_2$, $CH_2Cl_2$, −40 °C, 1 h; (c) DMF, *n*-BuLi, THF, −70 °C, overnight; (d) $BCl_3$, $Me_5$-benzene, $CH_2Cl_2$, −78 °C, overnight; (e) $NaBH_4$, MeOH, rt, 2 h; (f) $I_2$, KOH, MeOH, 0 °C, 3 h; (g) $CHCl_3$, KOH, MeOH, DMF , −9 °C, 2 h; (h) NaOH (newly grinded powder), $NaBH_4$, *t*-BuOH, 35 °C, 48 h.

Compounds **38-39** were synthesized as shown in Scheme 4. Reduction of commercially available **71** under sodium borohydride condition generated (2-bromo-5-fluorophenyl)methanol **72**, which was further subjected to formylation and reduction and gave compound **73**. Diol **73** was transformed into compound **38** by

a similar synthetic method to that in Scheme 1. Compound **39** was prepared starting from commercially available **79**. Diol **82** was obtained from **79** *via* chloromethylation, substitution reaction, acetylation and hydrolysis. Compound **82** was then transformed into compound **39** by a similar synthetic route to that in Scheme 1.

**Scheme 4. Synthetic routes of compounds 38-39.**[a]




[a]Reagents and conditions: (a) $NaBH_4$, MeOH, 0 °C to rt, 2.5 h; (b) HTMP, *n*-BuLi, DMF, THF, -78 °C to rt, 3.5 h; (c) pyridinium *p*-toluenesulfonate, 2-methoxypropene, THF, 0 °C, 2 h; (d) $ZnCl_2$, paraformaldehyde, HCl (gas), 60 °C, 24 h; (e) NaOAc, DMF, 80 °C, 3 h; (f) KOH, THF-EtOH-$H_2O$, 80 °C, 4.5 h; (g) *n*-BuLi, THF, −78 °C, 3 h; (h) *p*-toluenesulfonic acid, THF-MeOH, rt, 15 h; (i) PCC, $CH_2Cl_2$, rt, 4 h; (j) 2-ethylthiophene, *n*-BuLi, THF, −78 °C, 3 h; (k) $Et_3SiH$, $BF_3 \cdot OEt_2$, $CH_2Cl_2$, −40 °C, 1 h; (l) $BCl_3$, $Me_5$-benzene, $CH_2Cl_2$, −78 °C, overnight.

## Structure−Activity Relationships of 6-deoxy *O*-spiroketal *C*-arylglucosides

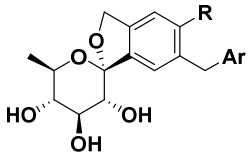
The *in vitro* cell-based SGLT2 AMG (methyl-α-*D*-glucopyranoside) assay was

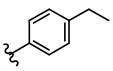
performed to evaluate the inhibitory effects of all synthesized compounds **9-39** on hSGLT2 and hSGLT1 inhibitory activities.[56] In the SAR exploration, our first attempts were focused on the series of compounds **9**-**24** with R as a hydrogen atom as in the case of tofogliflozin. The distal aromatic ring, ranging from alkylphenyl, alkoxyphenyl, alkylthiophenyl to arylthiophenyl, naphthyl and even heteroaromatic fused rings, were investigated (Table 1). Unfortunately, most of these compounds showed weak activities, and even the best one of them, namely, compound **10** with an ethylphenyl ring, just displayed an $IC_{50}$ of 63.5 nM against hSGLT2, while the $IC_{50}$ values of the others were all above 100 nM (Table 1). We assumed that the R substitution of this novel scaffold of 6-deoxy *O*-spiroketal *C*-arylglucoside might be quite different from the case of tofogliflozin, although they share similar spiroketal moieties. Then, compound **25** was designed *via* the alteration of R group to a chloride atom while the ethylphenyl group was reserved as the distal Ar moiety. To our excitement, compound **25** was about twice more potent than the hydrogen-substituted counterpart (**10**, Table 1). When the ethyl group on the distal phenyl ring was changed into a *para*-ethyoxyl group, the activity of the corresponding compound was significantly enhanced, with an $IC_{50}$ of 8.8 nM against hSGLT2 (compound **26** in Table 1). Next, several thiophenyl-substituted analogues were investigated. Compound **27**, containing a 5-methylthiophenyl substitution, was as potent as compound **26** whose Ar group was a *para*-ethyoxylphenyl ring. Compound **28** with the distal ring of 5-chlorothiophenyl group instead of alkylthiophenyl groups, showed good inhibitory activity with an hSGLT2 $IC_{50}$ of 15.9 nM. On the other hand, some electron-deficient-group substituted thiophenyl rings were not quite favored in promoting hSGLT2 inhibition, as well as in the cases of hydrophobic ester derivatives, and the corresponding compounds possessed moderate $IC_{50}$ values around 50 nM against hSGLT2 (compounds **29**, **30**, **31** and **32** in Table 1). In addition, compounds bearing hydrophobic chains that contain different alkoxy groups exhibited moderate potencies, and the extension of the length of alkoxy groups might slightly improve the potency and selectivity (compounds **33**, **34**, and **35** in Table 1). Compounds with 4-phenyl substituted thiophenyl and benzothiophenyl groups as the Ar moieties were

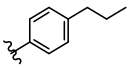
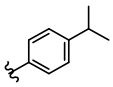
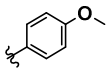
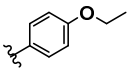
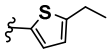
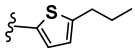
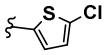
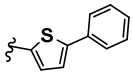
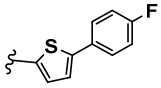
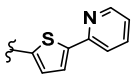
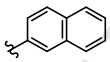
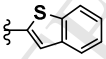
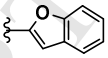
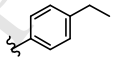
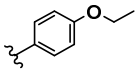
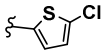
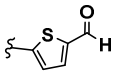
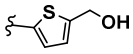
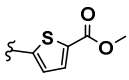
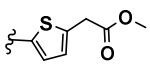
also investigated. Results showed that the compound with the latter substitution (**37**) displayed much higher inhibitory activity ($IC_{50}$ = 24.0 nM) than the compound with the former substitution (**36**, $IC_{50}$ > 100 nM). The above results indicated that some extension of the size of R substituent from the smallest hydrogen atom might be critical to the augmentation of hSGLT2 inhibitory activities. Therefore, other substituents such as fluorine atom and methyl group as R group were explored. The results showed that only the methyl-substituted compound **39** displayed satisfactory activity against hSGLT2 ($IC_{50}$ = 4.5 nM), while in the other case, the $IC_{50}$ value was around 26.2 nM (Table 1). Consequently, it was inferred that the size of R group as large as a chlorine atom or a methyl group just matched this novel scaffold; smaller R groups were not preferred.

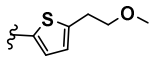
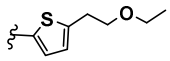
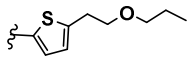
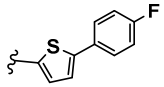
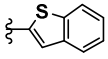
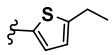
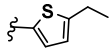
To conclude, the SAR studies showed that, i) an R group as large as a chlorine atom or a methyl group was critical to improve the hSGLT2 inhibitory activities of compounds derived from the novel scaffold of 6-deoxy *O*-spiroketal *C*-arylglucoside; ii) the electron-withdrawing group substituted aromatic rings, arylthiophenyl and aromatic fusing rings as the distal Ar moiety, were not quite favored; iii) alkyl or alkoxy substituted aromatic rings were beneficial to improving hSGLT2 activities when R was a chlorine atom or a methyl group.

**Table 1**. Structures and *in vitro* inhibitory activity against hSGLT2 and hSGLT1.




| Compd. | R | Ar | $IC_{50}$ ± SD | | Selectivity [a] |
|---|---|---|---|---|---|
| | | | hSGLT2 (nM) | hSGLT1 (μM) | |
| **9** | H |  | > 100 | ND[b] | ND |
| **10** | H |  | 63.5 ± 7.1 | 22.3 ± 4.3 | 351 |

| | | | | | |
|---|---|---|---|---|---|
| **11** | H |  | > 100 | ND | ND |
| **12** | H |  | > 100 | ND | ND |
| **13** | H |  | > 100 | ND | ND |
| **14** | H |  | > 100 | ND | ND |
| **15** | H |  | > 100 | ND | ND |
| **16** | H |  | > 100 | ND | ND |
| **17** | H |  | > 100 | ND | ND |
| **18** | H |  | > 100 | ND | ND |
| **19** | H |  | > 100 | ND | ND |
| **20** | H |  | > 100 | ND | ND |
| **21** | H |  | > 100 | ND | ND |
| **22** | H |  | > 100 | ND | ND |
| **23** | H |  | > 100 | ND | ND |
| **24** | H |  | > 100 | ND | ND |
| **25** | Cl |  | 29.7 ± 18.4 | 0.4 ± 0.1 | 13 |
| **26** | Cl |  | 8.8 ± 1.6 | 0.5 ± 0.1 | 60 |
| **27** | Cl |  | 8.5 ± 0.8 | 0.4 ± 0.1 | 46 |
| **28** | Cl |  | 15.9 ± 2.6 | 0.5 ± 0.1 | 30 |
| **29** | Cl |  | 51.3 ± 14.2 | 3.6 ± 0.3 | 70 |
| **30** | Cl |  | 47.2 ± 8.3 | 2.2 ± 0.2 | 47 |
| **31** | Cl |  | 51.5 ± 11.7 | 3.0 ± 0.2 | 58 |
| **32** | Cl |  | 42.4 ± 11.9 | 6.4 ± 0.8 | 151 |

| | | | | | |
|---|---|---|---|---|---|
| **33** | Cl |  | 69.4 ± 14.4 | 4.7 ± 0.3 | 68 |
| **34** | Cl |  | 67.5 ± 14.3 | 4.8 ± 0.4 | 71 |
| **35** | Cl |  | 42.3 ± 9.0 | 5.0 ± 0.5 | 117 |
| **36** | Cl |  | > 100 | ND | ND |
| **37** | Cl |  | 24.0 ± 2.4 | 2.0 ± 0.1 | 81 |
| **38** | F |  | 26.2 ± 2.0 | 25.9 ± 4.3 | 988 |
| **39** | Me |  | 4.5 ± 0.8 | 1.0 ± 0.1 | 216 |
| **Dapagliflozin** | - | - | 8.3 ± 0.7 | 12.3 ± 1.1 | 1483 |
| **Tofogliflozin**[c] | - | - | 2.9 | 8.44 | 2912 |

[a] The selectivity values were calculated by $IC_{50}$ hSGLT1/$IC_{50}$ hSGLT2;[b] ND refers to Not Determined, similarly hereinafter; [c] See ref 34.

**Urinary Glucose Excretion in C57BL/6J Mice and Sprague-Dawley Rats**

In the *in vitro* biological evaluation, compound **39** showed excellent *in vitro* inhibitory activities against hSGLT2 ($IC_{50}$ = 4.5 nM), and good selectivities against hSGLT1 (selective ratio: 216-fold), which were comparable to the marketed drugs dapagliflozin and tofogliflozin (Table 1). Therefore, compound **39** was selected for *in vivo* biological evaluation.

We explored and compared the effects of **39** and tofogliflozin on urine volume, urine glucose concentration and urinary glucose excretion (UGE) in C57BL/6J mice after single oral administration at a dose of 1 mg/kg (Figure 3A-C and Table 2). The amounts of 24-hour urinary glucose excretion caused by **39** and tofogliflozin were 110.53 mg/100 g and 48.85 mg/100 g, respectively (Figure 3C and Table 2), indicating that compound **39** displayed higher SGLT2 inhibitory effect than tofogliflozin. Similarly, **39** did not influence the urinary volume in in Sprague-Dawley (SD) rats (Figure 3D) but induced more excretion of urinary glucose (134.23 ± 46.72 mg/100 g) than tofogliflozin (73.68 ± 29.52 mg/100 g) on the same dose and duration (Figure 3F and Table 3).

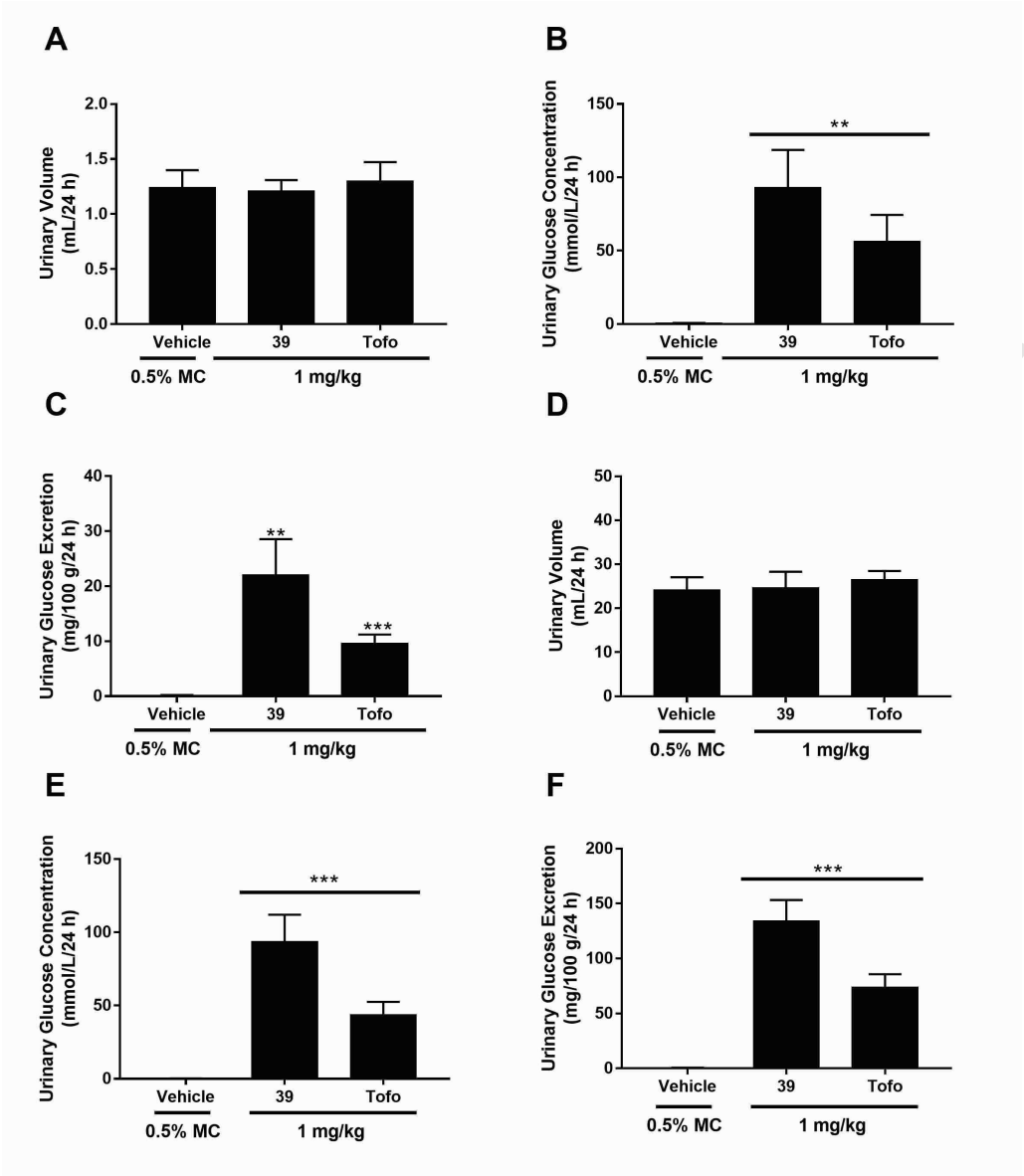



**Figure 3**. The effect of **39** on urinary glucose excretion during 24 h after oral administration. The urinary volume (A, n=9), glucose concentration (B, n=9) and glucose excretion (C, n=9) of **39** and tofogliflozin in C57BL/6J mice and the urinary volume (D, n=6), glucose concentration (E, n=6) and glucose excretion (F, n=6) of **39** and tofogliflozin in SD rats were determined by the glucose-oxidase method. All results are expressed as mean ± SEM. *, *p* value<0.05; **, *p* value<0.01; ***, *p* value < 0.001 compared with the control vehicle group by two-tails *t*-test.

**Table 2**. Effects of single oral administration of **39** and **tofogliflozin** on urinary glucose excretion in C57BL/6J mice during 24 h.

| Groups | Dose (mg/kg) | Urine glucose concentration (mmol/L) | Urine volume (mL) | Urine glucose excretion (mg/100 g) |
|---|---|---|---|---|
| **Vehicle** | 0.5% MC | 0.74±0.14 | 1.24±0.45 | 0.84±0.40 |
| **39** | 1 | 92.99±72.53** | 1.21±0.28 | 110.53±93.81** |
| **Tofogliflozin** | 1 | 56.27±51.46** | 1.30±0.49 | 48.85±24.34*** |

All results are expressed as mean ± SD (n = 9). **, *p* value<0.01, ***, *p* value < 0.001; compared with the vehicle group by two-tails *t*-test.

**Table 3**. Effects of single oral administration of **39** and **tofogliflozin** on urinary glucose excretion in SD rats during 24 h.

| Groups | Dose (mg/kg) | Urine glucose concentration (mmol/L) | Urine volume (mL) | Urine glucose excretion (mg/100 g) |
|---|---|---|---|---|
| **Vehicle** | 0.5% MC | 0.28±0.16 | 24.17±7.11 | 0.51±0.40 |
| **39** | 1 | 93.80±44.64*** | 24.67±8.91 | 134.23±46.72*** |
| **Tofogliflozin** | 1 | 43.67±21.66*** | 26.50±4.81 | 73.68±29.52*** |

All results are expressed as mean ± SD (n = 6). ***, *p* value < 0.001; compared with the vehicle group by two-tails *t*-test.

**Oral Glucose Tolerance Tests (OGTT) in C57BL/6J Mice**

Based on the excellent effect of **39** on UGE in both mice and rats, it was supposed that **39** should have good performance of improving glucose control. Further investigation of compound **39** on its hypoglycemic effect on C57BL/6J mice showed that single oral dose of compound **39** (3 mg/kg) significantly improved oral glucose tolerance in mice in a dose-dependent manner after an oral glucose overload (Figure 4A-B and Table 4). It is worth noting that compound **39** displayed more remarkable improvement on oral glucose tolerance than tofogliflozin did in C57BL/6J mice at the same dose (Figure 4A-B and Table 4), indicating its great potential of treating diabetes.

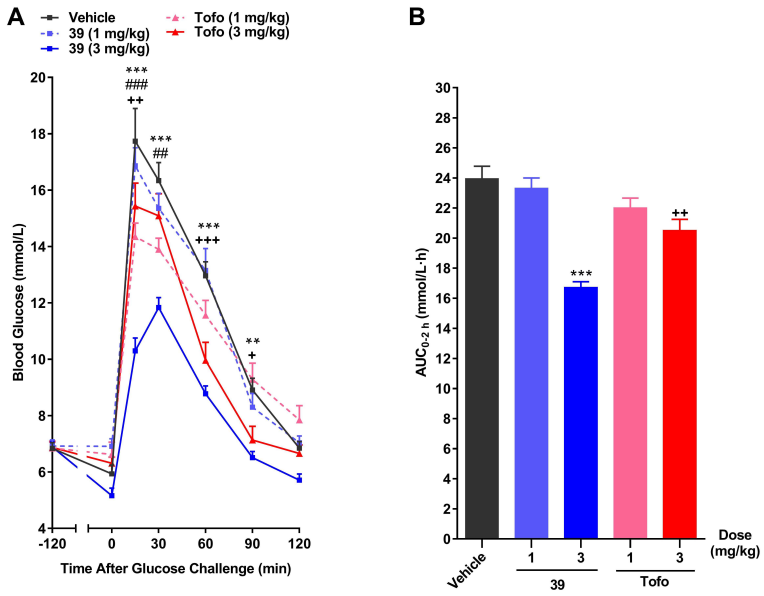



**Figure 4**. The effect of compound **39** on improving oral glucose tolerance after single-dose administration in C57BL/6J mice. (A) Blood glucose levels were determined 2 h before (-120 minutes) and at 0, 15, 30, 60, 90, and 120 minutes after oral glucose challenge in C57BL/6J mice (n=8). (B) Areas under curves ($AUC_{0\text{-}2\,h}$) in C57BL/6J mice were calculated by the trapezoidal rule. All results are expressed as mean ± SEM (n = 8). *, # and +, *p* value<0.05; **, ## and ++, *p* value<0.01; ***, ### and +++, *p* value < 0.001 compared with the control vehicle group one-way ANOVA with Dunnett's correction for OGTT or by two-tails *t*-test for $AUC_{0\text{-}2\ h}$. The significance of 3 mg/kg **39** and 1 mg/kg and 3 mg/kg Tofo compared with vehicle group are marked as *, # and + respectively. Tofo, tofogliflozin.

**Table 4**. Areas under the curve ($AUC_{0\text{-}2\ h}$) of blood glucose after single oral administration of **39** and **tofogliflozin** in C57BL/6J mice with oral glucose challenge.

| **Groups** | **Dose (mg/kg)** | **N** | **$AUC_{0\text{-}2\,h}$ (mmol/L·h)** |
|---|---|---|---|
| **Vehicle** | 0.5% MC | 8 | 23.95±2.33 |
| **39** | 1 | 8 | 27.63±4.90 |
| | 3 | 8 | $16.73±1.04^{***}$ |
| **Tofogliflozin** | 1 | 8 | 22.02±1.81 |
| | 3 | 8 | $20.52±2.04^{++}$ |

All results are expressed as mean ± SD (n = 8). ***, *p* value < 0.001 (3 mg/kg **39**); ++,

*p* value < 0.01 (3 mg/kg tofogliflozin) compared with the control vehicle group by two-tails *t*-test.

**Pharmacokinetic Profiles**

Administration of a single intravenous dose of 2 mg/kg and a single oral dose of 10 mg/kg to mice revealed that compound **39** possessed the following specialties: acceptable oral bioavailability (53.4%), longer half-life than tofogliflozin (5.95 h vs 2.57 h, p.o.), and moderate exposure in plasma but extremely large steady-state volume of distribution ($V_{ss\_obs}$ > 10 L/kg). Clearly, the $V_{ss\_obs}$ value was much greater than the total blood volume in mice[34], indicating remarkable enrichment of **39** in extravascular space[34] (e.g., the target organ kidney[39]), which may explain its advantage over tofogliflozin on promoting urinary glucose excretion and improving glucose tolerance.

**Table 5**. Pharmacokinetic parameters of **39** after i.v. and p.o. administration in mice.

| Admin. | i.v. | p.o. |
|---|---|---|
| Dose (mg/kg) | 2 | 10 |
| $T_{1/2}$ (h) | 10.8 ± 10.7 | 5.95 ± 2.32 |
| $T_{max}$ (h) | - | 0.33 ± 0.14 |
| $C_{max}$ (ng/mL) | - | 740 ± 294 |
| $AUC_{last}$ (h*ng/mL) | 1128 ± 309 | 3009 ± 408 |
| $AUC_{INF\_obs}$ (h*ng/mL) | 2322 ± 1087 | 5576 ± 1535 |
| $CL_{\_obs}$ (mL/min/kg) | 19.9 ± 11.0 | - |
| $MRT_{INF\_obs}$ (h) | 13.1 ± 13.0 | 8.9 ± 3.4 |
| $V_{ss\_obs}$ (mL/kg) | 10107 ± 3155 | - |
| *F* (%) | | 53.4 |

**CONCLUSION**

In this work, we modifed the sugar moiety of SGLT2 inhibitors via removing the C-6 hydroxy group of glucose, aiming to find a novel and potent SGLT2 inhibitor with good pharmacokinetic profiles. A novel series of 6-deoxy *O*-spiroketal

*C*-arylglucosides as SGLT2 inhibitors were designed, synthesized, and evaluated, and the structure-activity relationship (SAR) of this novel series was demonstrated. Among these newly designed and synthesized SGLT2 inhibitors, compound **39** was a promising anti-diabetic agent with high inhibitory activity against hSGLT2, favorable pharmacokinetic profiles, and excellent effects on facilitating the glucose outflow in C57BL/6J mice and SD rats and improving glucose tolerance in C57BL/6J mice, which were better than the positive control tofogliflozin.

## ■ EXPERIMENTAL SECTION

### Chemistry

All reagents (chemicals) were commercially available and used without further purification. Analytical thin-layer chromatography (TLC) was performed on HSGF 254 (0.1 mm thickness). Column chromatography was performed on silica gel 200–300 mesh to purify the compounds. NMR spectra were recorded on Varian-MERCURY Plus-400 and AVANCE III 500 in $CDCl_3$ and Methanol-$d_4$. Chemical shifts were reported in parts per million (ppm, $\delta$) downfield from tetramethylsilane. Proton coupling patterns were described as s = singlet, d = doublet, dd = doublet of doublet, t = triplet, q = quartet, m = multiplet. Low- and high-resolution mass spectra (LRMS and HRMS) were given with electrospray ionization (ESI).

All target compounds **9-39** were confirmed with over 95% purity which were determined by Agilent 1260 HPLC with binary pump, photodiode array detector (DAD), using Agilent XDB-C18 (4.6 × 250 mm, 5 μm), MeOH/$H_2O$ = 80/20 (v/v) at 1.0 mL/min, or Agilent XDB-C18 (4.6 × 250 mm, 5 μm), MeOH/$H_2O$ = 75/25 (v/v) at 1.0 mL/min, or Agilent Extend-C18 (4.6 × 250 mm, 5 μm), MeOH/$H_2O$ = 75/25 (v/v) at 1.0 mL/min and calculated the peak areas at 254 nm.

**(1*S*,3'*R*,4'*S*,5'*S*,6'*R*)-6'-methyl-6-(4-methylbenzyl)-3',4',5',6'-tetrahydro-3*H*-spiro[isobenzofuran-1,2'-pyran]-3',4',5'-triol (9).** To a solution of 1-bromo-4-methylbenzene (1.00 g, 5.85 mmol) in anhydrous THF (30 mL), 2.4 M

*n*-BuLi in hexane solution (2.44 mL, 5.85 mmol) was added dropwise at −78 °C under a nitrogen atmosphere and the resultant mixture was stirred under the same condition for 1 h. A solution of compound **54** (322.0 mg, 0.58 mmol) in anhydrous THF (5 mL) was then added dropwise to the resultant mixture. The reaction mixture was stirred for 2 h, and then water was added thereto. The resultant mixture was extracted with EtOAc. The organic layer was washed with brine, dried over $Na_2SO_4$, and then evaporated under reduced pressure to remove the solvent. The obtained residue was purified by column chromatography on silica gel (petroleum ether/EtOAc = 4/1) to give compound **55a** (338.5 mg, 90%) as a colorless oil.

To a solution of compound **55a** (338.0 mg, 0.53 mmol) in $CH_2Cl_2$ (30 mL), triethylsilane (419.9 μL, 2.63 mmol) and $BF_3{\cdot}OEt_2$ (71.4 μL, 0.58 mmol) were added at −40 °C and the mixture was stirred at the same temperature for 1 h. After addition of water, the reaction mixture was extracted with $CH_2Cl_2$. The organic layer was washed with brine, dried over $Na_2SO_4$, and then evaporated under reduced pressure to remove the solvent. The obtained residue was purified by column chromatography on silica gel (petroleum ether/EtOAc = 10/1) to give compound **56a** (310.0 mg, 94%) as a colorless oil.

To a solution of compound **56a** (310.0 mg, 0.49 mmol) and pentamethylbenzene (733.2 mg, 4.95 mmol) in $CH_2Cl_2$ (30 mL) was added 1.0 M $BCl_3$ in toluene solution (4.95 mL, 4.95 mmol) at −78 °C under a nitrogen atmosphere, and the mixture was stirred at the same temperature overnight. After addition of MeOH (10 mL), the reaction mixture was warmed to rt and evaporated under reduced pressure to remove the solvent. The obtained residue was purified by column chromatography on silica gel ($CH_2Cl_2$/MeOH = 20/1) to give compound **9** (100 mg, 57%) as a white solid. HPLC purity: 95.84%. m.p. 145–146 °C; $^1$H NMR (400 MHz, Methanol-$d_4$) $\delta$ 7.24 – 7.18 (m, 2H), 7.16 – 7.12 (m, 1H), 7.12 – 7.05 (m, 4H), 5.15 – 5.04 (m, 2H), 3.96 (s, 2H), 3.91 – 3.81 (m, 1H), 3.77 – 3.66 (m, 2H), 3.18 – 3.10 (m, 1H), 2.29 (s, 3H), 1.20 (d, $J$ = 6.3 Hz, 3H). $^{13}$C NMR (125 MHz, Methanol-$d_4$) $\delta$ 142.7, 140.3, 139.7, 139.3, 136.7, 131.1, 130.1, 129.9, 123.4, 121.8, 111.5, 77.4, 76.1, 75.2, 73.4, 71.4, 42.2, 21.1, 18.2. LRMS (ESI) m/z: 357 $[M+H]^+$; HRMS (ESI) cacld for $C_{21}H_{24}O_5Na$ $[M+Na]^+$:

379.1516; found: 379.1510.

**((2*R*,3*R*,4*S*,5*R*,6*S*)-3,4,5-tris(benzyloxy)-6-methoxytetrahydro-2*H*-pyran-2-yl)methanol (45).** To a cooled solution (0 °C) of α-*D*-Methylglucoside **44** (20.00 g, 103.00 mmol) and imidazole (21.04 g, 308.99 mmol) in DMF (180 mL), TIPSCl (24.27 mL, 113.30 mmol) was added dropwise over a period of one hour. After 24 h at rt, the reaction was diluted with water and extracted with $CH_2Cl_2$. The combined organic layer was washed with brine, dried over $Na_2SO_4$, concentrated in vacuo. A solution of this crude product and BnBr (61.17 mL, 514.98 mmol) in DMF (350 mL) was cooled to 0 °C, and NaH (60% in mineral oil, 20.60 g, 514.98 mmol) was added. The reaction mixture was allowed to warm to r.t. After 12 h, the reaction mixture was carefully quenched by water. The reaction mixture was extracted with EtOAc. The combined organic layer was washed with brine, dried over $Na_2SO_4$, and concentrated in vacuo. The crude product was combined with TBAF (53.86 g, 205.99 mmol) and THF (350 mL) was added. The reaction solution was stirred at rt for 12 h, diluted with $H_2O$, and extracted with EtOAc. The combined organic layer was washed with brine, dried over $Na_2SO_4$, concentrated in vacuo, and purified by column chromatography on silica gel (petroleum ether/EtOAc = 2/1) to give compound **45** (37.50 g, 78%) (3 steps) as a colorless syrup. $^1$H NMR (400 MHz, Chloroform-*d*) $\delta$ 7.40 – 7.27 (m, 15H), 4.99 (d, $J$ = 10.9 Hz, 1H), 4.92 – 4.77 (m, 3H), 4.70 – 4.61 (m, 2H), 4.56 (d, $J$ = 3.6 Hz, 1H), 4.01 (t, $J$ = 9.3 Hz, 1H), 3.81 – 3.61 (m, 3H), 3.58 – 3.45 (m, 2H), 3.37 (s, 3H). LRMS (ESI, *m/z*): 487 $[M+Na]^+$.

**(2*S*,3*R*,4*S*,5*R*,6*R*)-3,4,5-tris(benzyloxy)-2-methoxy-6-methyltetrahydro-2*H*-pyran (47).** At 0 °C, $Ph_3P$ (25.41 g, 96.87 mmol) and $CBr_4$ (32.12 g, 96.87 mmol) were added successively to a solution of **45** (30.00 g, 64.58 mmol) in THF (300ml). The mixture was stirred for 1 h and then filtered. The filtrate was concentrated in vacuo and purified by column chromatography on silica gel (petroleum ether/EtOAc = 10/1) to give compound **46** (33.70 g, 99%) as a colorless syrup. LRMS (ESI, *m/z*): 549 $[M+Na]^+$.

To a solution of compound **46** (31.89 g, 60.46 mmol) in dry toluene (250 mL) at 20 $^{o}$C was added $Bu_3SnH$ (19.45 mL, 72.55 mmol) and AIBN (992.82 mg, 6.05 mmol) successively. The mixture was stirred for 4 h at 80 °C. The solution was evaporated to syrup which was purified by column chromatography on silica gel (petroleum ether/EtOAc = 10/1) to give compound **47** (23.59 g, 87%) as a colorless syrup. $^1$H NMR (400 MHz, Chloroform-*d*) $\delta$ 7.42 – 7.22 (m, 15H), 4.98 (d, *J* = 10.9 Hz, 1H), 4.90 (d, *J* = 10.9 Hz, 1H), 4.86 – 4.76 (m, 2H), 4.72 – 4.60 (m, 2H), 4.53 (d, *J* = 3.6 Hz, 1H), 3.95 (t, *J* = 9.3 Hz, 1H), 3.79 – 3.66 (m, 1H), 3.52 (dd, *J* = 9.7, 3.6 Hz, 1H), 3.37 (s, 3H), 3.13 (t, *J* = 9.3 Hz, 1H), 1.24 (d, *J* = 6.3 Hz, 3H). LRMS (ESI, *m/z*): 471 $[M+Na]^+$.

**(3*R*,4*S*,5*R*,6*R*)-3,4,5-tris(benzyloxy)-6-methyltetrahydro-2*H*-pyran-2-one (49).** To a solution of compound **47** (15.10 g, 33.66 mmol) in glacial acetic acid (300 mL) was added 3.0 M sulfuric acid (33.66 mL, 100.99 mmol). After stirring at 85 $^{o}$C for 2.5 h, the reaction mixture was cooled to room temperature. $CH_2Cl_2$ was added to the system and the mixture was added sat. aq. $NaHCO_3$ until no gas generated. The organic layer was separated, washed with brine, dried over $Na_2SO_4$. Concentrated in vacuo, and purified by column chromatography on silica gel (petroleum ether/EtOAc = 2/1) to give compound **48** (13.01 g, 89%) as a white solid. LRMS (ESI, *m/z*): 457 $[M+Na]^+$.

To a solution of compound **48** (25.20 g, 57.99 mmol) in DMSO (200 mL) was added acetic acid anhydride (50 mL). The mixture was stirred overnight before diluted with water, then sat. aq. $NaHCO_3$ was added into the mixture until no gas generated. The mixture was extracted with EtOAc. The combined organic layer was washed with brine, dried over $Na_2SO_4$, concentrated in vacuo, and purified by column chromatography on silica gel (petroleum ether/EtOAc = 10/1) to give compound **49** (24.70 g, 98%) as a white solid. $^1$H NMR (400 MHz, Chloroform-*d*) $\delta$ 7.48 – 7.19 (m, 15H), 4.95 (d, *J* = 11.5 Hz, 1H), 4.74 – 4.61 (m, 3H), 4.61 – 4.48 (m, 3H), 4.12 (d, *J* = 5.0 Hz, 1H), 3.97 – 3.87 (m, 1H), 3.46 (dd, *J* = 8.8, 5.7 Hz, 1H), 1.41 (d, *J* = 6.4 Hz, 3H). LRMS (ESI, *m/z*): 433 $[M+H]^+$.

**2-bromo-1,4-bis(((2-methoxypropan-2-yl)oxy)methyl)benzene (52).** To a solution of 2-bromo-terephthalic acid **50** (15.00 g, 61.22 mmol) in anhydrous THF (200 mL) was added 2.0 M $BH_3{\cdot}SMe_2$ in THF (91.83 mL, 183.65 mmol) at 0 °C. The mixture was stirred for 2 h at 70 °C. After the exothermic reaction was over, the reaction was cooled by ice-water bath. The reaction was quenched by dropwise addition of water. The resulting mixture was extracted with EtOAc. The organic layer was washed with brine and concentrated under reduced pressure to give compound **51** (11.90 g, 89%) as an off-white solid.

To a solution of **51** (11.90 g, 54.82 mmol) in anhydrous THF (200 mL) was added 2-methoxypropene (51.61 mL, 548.24 mmol) and pyridinium *p*-toluenesulfonate (275.55 mg, 1.10 mmol) at 0 °C, and the resultant mixture was stirred at 0 $^{o}$C for 2 h. Sat. aq. $NaHCO_3$ was added thereto, and then the resultant mixture was extracted with EtOAc (500 mL) containing $Et_3N$ (1.5 mL). The organic layer was washed with brine, dried over $Na_2SO_4$, concentrated in vacuo, and purified by column chromatography on silica gel (petroleum ether / EtOAc = 20/1) to give compound **52** (13.62 g, 69%) as a colorless oil. $^1$H NMR (400 MHz, Chloroform-*d*) $\delta$ 7.54 (d, $J$ = 1.3 Hz, 1H), 7.50 (d, $J$ = 7.9 Hz, 1H), 7.28 (dd, $J$ = 7.9, 1.6 Hz, 1H), 4.53 (s, 2H), 4.44 (s, 2H), 3.24 (s, 3H), 3.23 (s, 3H), 1.45 (s, 6H), 1.42 (s, 6H).

**(1*S*,3'*R*,4'*S*,5'*R*,6'*R*)-3',4',5'-tris(benzyloxy)-6'-methyl-3',4',5',6'-tetrahydro-3*H*-spiro[isobenzofuran-1,2'-pyran]-6-carbaldehyde (54).** To a solution of compound **52** (10.00 g, 27.68 mmol) in anhydrous THF (100 mL), 2.4 M *n*-BuLi in hexane solution (12.69 mL, 30.45 mmol) was added dropwise at −78 °C under a nitrogen atmosphere and the resultant mixture was stirred under the same condition for 1 h. A solution of compound **49** (10.54 g, 24.36 mmol) in anhydrous THF (50 mL) was then added dropwise to the resultant mixture. The reaction mixture was stirred for 2 h, and then water was added thereto. The resultant mixture was extracted with EtOAc. The resultant organic layer was washed with water and brine and then dried over $Na_2SO_4$. The solvent was then removed by distillation under reduced pressure. The obtained

residue was dissolved in a mixed solvent of THF (100 mL) and MeOH (50 mL), and *p*-toluenesulfonic acid (5.24 g, 30.45 mmol) was added thereto. The mixture was stirred at rt for 15 h. After most of the MeOH was evaporated, the solution was extracted with EtOAc. The combined organic layer was washed with sat. aq. $NaHCO_3$, brine, dried over $Na_2SO_4$, concentrated in vacuo, and purified by column chromatography on silica gel (petroleum ether/EtOAc = 4/1) to give compound **53** (7.67 g, 57%) as a colorless oil. LRMS (ESI, *m/z*): 553 $[M+H]^+$.

To a solution of compound **53** (11.20 g, 20.27 mmol) in $CH_2Cl_2$ (120 mL), pyridinium chlorochromate (PCC) (6.55 g, 30.40 mmol) and silica gel (200-300 mesh, 15 g) were added at rt and the mixture was stirred for 4 h. After completion, the reaction mixture was evaporated under reduced pressure to remove the solvent. The obtained residue was purified by column chromatography on silica gel (petroleum ether/EtOAc = 10/1) to give compound **54** (9.00 g, 81%) as a white solid. $^1$H NMR (400 MHz, Chloroform-*d*) $\delta$ 9.86 (s, 1H), 7.88 (d, *J* = 7.7 Hz, 1H), 7.50 (s, 1H), 7.41 (d, *J* = 7.8 Hz, 1H), 7.38 – 7.27 (m, 10H), 7.13 (t, *J* = 7.2 Hz, 1H), 7.07 (t, *J* = 7.3 Hz, 2H), 6.77 (d, *J* = 7.3 Hz, 2H), 5.24 (s, 2H), 4.97 (d, *J* = 11.1 Hz, 3H), 4.73 (d, *J* = 10.9 Hz, 1H), 4.65 (d, *J* = 11.4 Hz, 1H), 4.25 (d, *J* = 11.2 Hz, 1H), 4.15 (t, *J* = 9.3 Hz, 1H), 4.11 – 4.01 (m, 1H), 3.92 (d, *J* = 9.5 Hz, 1H), 3.36 (t, *J* = 9.3 Hz, 1H), 1.26 (d, *J* = 6.2 Hz, 3H). LRMS (ESI, *m/z*): 573 $[M+Na]^+$.

**(1*S*,3'*R*,4'*S*,5'*S*,6'*R*)-6-(4-ethylbenzyl)-6'-methyl-3',4',5',6'-tetrahydro-3*H*-spiro[isobenzofuran-1,2'-pyran]-3',4',5'-triol (10).** Compound **10** was prepared from **54** and 1-bromo-4-ethylbenzene according to the procedure described for compound **9** in 38% yield (3 steps) as a white solid. HPLC purity: 96.43%. m.p. 136–138 °C; $^1$H NMR (400 MHz, Methanol-$d_4$) $\delta$ 7.24 – 7.17 (m, 2H), 7.16 (s, 1H), 7.11 (d, *J* = 1.8 Hz, 4H), 5.16 – 5.03 (m, 2H), 3.97 (s, 2H), 3.91 – 3.81 (m, 1H), 3.77 – 3.66 (m, 2H), 3.18 – 3.10 (m, 1H), 2.59 (q, *J* = 7.6 Hz, 2H), 1.24 – 1.16 (m, 6H). $^{13}$C NMR (125 MHz, Methanol-$d_4$) $\delta$ 143.2, 142.7, 140.3, 139.7, 139.6, 131.2, 129.9, 128.9, 123.4, 121.8, 111.5, 77.4, 76.1, 75.2, 73.5, 71.4, 42.2, 29.4, 18.2, 16.3. LRMS

(ESI) m/z: 371 $[M+H]^+$; HRMS (ESI) cacld for $C_{22}H_{26}O_5Na$ $[M+Na]^+$: 393.1672; found: 393.1668.

**(1*S*,3'*R*,4'*S*,5'*S*,6'*R*)-6'-methyl-6-(4-propylbenzyl)-3',4',5',6'-tetrahydro-3*H*-spiro[isobenzofuran-1,2'-pyran]-3',4',5'-triol (11).** Compound **11** was prepared from **54** and 1-bromo-4-propylbenzene according to the procedure described for compound **9** in 50% yield (3 steps) as a white solid. HPLC purity: 97.18%. mp 135–138 °C; $^1$H NMR (400 MHz, Methanol-$d_4$) $\delta$ 7.25 – 7.18 (m, 2H), 7.17 (s, 1H), 7.10 (q, $J$ = 8.2 Hz, 4H), 5.14 – 5.05 (m, 2H), 3.97 (s, 2H), 3.92 – 3.80 (m, 1H), 3.77 – 3.67 (m, 2H), 3.19 – 3.10 (m, 1H), 2.60 – 2.48 (m, 2H), 1.69 – 1.54 (m, 2H), 1.20 (d, $J$ = 6.3 Hz, 3H), 0.92 (t, $J$ = 7.4 Hz, 3H). $^{13}$C NMR (100 MHz, Methanol-$d_4$) $\delta$ 142.7, 141.5, 140.3, 139.7, 139.7, 131.2 129.8, 129.6, 123.4, 121.8, 111.5, 77.4, 76.1, 75.2, 73.5, 71.4, 42.2, 38.7, 25.8, 18.2, 14.1. LRMS (ESI) m/z: 385 $[M+H]^+$; HRMS (ESI) cacld for $C_{23}H_{28}O_5Na$ $[M+Na]^+$: 407.1829; found: 407.1829.

**(1*S*,3'*R*,4'*S*,5'*S*,6'*R*)-6-(4-isopropylbenzyl)-6'-methyl-3',4',5',6'-tetrahydro-3*H*-spiro[isobenzofuran-1,2'-pyran]-3',4',5'-triol (12).** Compound **12** was prepared from **54** and 1-bromo-4-isopropylbenzene according to the procedure described for compound **9** in 56% yield (3 steps) as a white solid. HPLC purity: 95.26%. mp 134–136 °C; $^1$H NMR (400 MHz, Methanol-$d_4$) $\delta$ 7.25 – 7.18 (m, 2H), 7.17 (s, 1H), 7.13 (s, 4H), 5.14 – 5.05 (m, 2H), 3.97 (s, 2H), 3.91 – 3.82 (m, 1H), 3.78 – 3.67 (m, 2H), 3.18 – 3.10 (m, 1H), 2.91 – 2.80 (m, 1H), 1.24 – 1.18 (m, 9H). $^{13}$C NMR (100 MHz, Methanol-$d_4$) $\delta$ 147.8, 142.7, 140.3, 139.8, 139.7, 131.2, 129.9, 127.4, 123.4, 121.8, 111.5, 77.4, 76.1, 75.2, 73.5, 71.4, 42.2, 35.0, 24.5, 18.2. LRMS (ESI) m/z: 385 $[M+H]^+$; HRMS (ESI) cacld for $C_{23}H_{28}O_5Na$ $[M+Na]^+$: 407.1829; found: 407.1825.

**(1*S*,3'*R*,4'*S*,5'*S*,6'*R*)-6-(4-methoxybenzyl)-6'-methyl-3',4',5',6'-tetrahydro-3*H*-spiro[isobenzofuran-1,2'-pyran]-3',4',5'-triol (13).** Compound **56e** was prepared from **54** and 1-bromo-4-methoxybenzene according to the procedure described for **56a** in

87% yield (2 steps) as colorless oil. Then, to a solution of compound **56e** (297.00 mg, 0.46 mmol) in MeOH (1.5 mL) and EtOAc (1.5 mL), 20% $Pd(OH)_2$ (16.22 mg, 0.023mmol) was added and 2 *N* HCl (27.72 μL, 0.055mmol) was further added. Under a hydrogen atmosphere, the reaction mixture was stirred for 5 h and then filtered to remove the catalyst. After distilling off the solvent under reduced pressure, the obtained residue was purified by silica gel column chromatography ($CH_2Cl_2$ / MeOH = 20:1) to give compound **13** (125.00 mg, 73%) as a white solid. HPLC purity: 96.62%. m.p. 139–140 °C; $^1$H NMR (400 MHz, Methanol-$d_4$) $\delta$ 7.24 – 7.18 (m, 2H), 7.16 – 7.09 (m, 3H), 6.86 – 6.79 (m, 2H), 5.14 – 5.05 (m, 2H), 3.95 (s, 2H), 3.91 – 3.82 (m, 1H), 3.75 (s, 3H), 3.74 – 3.68 (m, 2H), 3.18 – 3.10 (m, 1H), 1.20 (d, $J$ = 6.3 Hz, 3H). $^{13}$C NMR (125 MHz, Methanol-$d_4$) $\delta$ 159.6, 142.9, 140.3, 139.7, 134.5, 131.1 130.9, 123.3, 121.8, 114.9, 111.5, 77.4, 76.1, 75.2, 73.4, 71.4, 55.6, 41.7, 18.2. LRMS (ESI) m/z: 373 $[M+H]^+$; HRMS (ESI) cacld for $C_{21}H_{24}O_6Na$ $[M+Na]^+$: 395.1465; found: 395.1457.

**(1*S*,3'*R*,4'*S*,5'*S*,6'*R*)-6-(4-ethoxybenzyl)-6'-methyl-3',4',5',6'-tetrahydro-3*H*-spiro[isobenzofuran-1,2'-pyran]-3',4',5'-triol (14).** Compound **14** was prepared from **54** and 1-bromo-4-ethoxybenzene according to the procedure described for **9** in 49% yield (3 steps) as a white solid. HPLC purity: 96.39%. m.p. 134–136 °C; $^1$H NMR (400 MHz, Methanol-$d_4$) $\delta$ 7.25 – 7.17 (m, 2H), 7.17 – 7.07 (m, 3H), 6.85 – 6.78 (m, 2H), 5.16 – 5.04 (m, 2H), 3.99 (q, $J$ = 7.0 Hz, 2H), 3.94 (s, 2H), 3.91 – 3.81 (m, 1H), 3.77 – 3.66 (m, 2H), 3.14 (t, $J$ = 8.9 Hz, 1H), 1.36 (t, $J$ = 7.0 Hz, 3H), 1.20 (d, $J$ = 6.3 Hz, 3H). $^{13}$C NMR (125 MHz, Methanol-$d_4$) $\delta$ 158.8, 142.9, 140.3, 139.7, 134.4, 131.1, 130.9, 123.3, 121.8, 115.5, 111.5, 77.4, 76.1, 75.2, 73.5, 71.4, 64.4, 41.7, 18.2, 15.2. LRMS (ESI) m/z: 387 $[M+H]^+$; HRMS (ESI) cacld for $C_{22}H_{26}O_6Na$ $[M+Na]^+$: 409.1622; found: 409.1622.

**(1*S*,3'*R*,4'*S*,5'*S*,6'*R*)-6'-methyl-6-((5-methylthiophen-2-yl)methyl)-3',4',5',6'-tetrahydro-3*H*-spiro[isobenzofuran-1,2'-pyran]-3',4',5'-triol (15).** Compound **15** was prepared from **54** and 2-methylthiophene according to the procedure described for **9**

in 49% yield (3 steps) as a white solid. HPLC purity: 96.76%. m.p. 153–154 °C; $^{1}$H NMR (400 MHz, Methanol-$d_4$) $\delta$ 7.27 (dd, $J$ = 7.8, 1.3 Hz, 1H), 7.24 – 7.18 (m, 2H), 6.60 (d, $J$ = 3.3 Hz, 1H), 6.57 – 6.54 (m, 1H), 5.16 – 5.05 (m, 2H), 4.10 (s, 2H), 3.92 – 3.82 (m, 1H), 3.78 – 3.67 (m, 2H), 3.19 – 3.11 (m, 1H), 2.43 – 2.35 (m, 3H), 1.21 (d, $J$ = 6.3 Hz, 3H). $^{13}$C NMR (125 MHz, Methanol-$d_4$) $\delta$ 142.7, 141.9, 140.4, 140.1, 139.5, 130.8, 126.2, 125.8, 123.1, 121.9, 111.5, 77.4, 76.1, 75.3, 73.5, 71.5, 36.8, 18.2, 15.2. LRMS (ESI) m/z: 363 $[M+H]^+$; HRMS (ESI) cacld for $C_{19}H_{23}O_5S$ $[M+H]^+$: 363.1261; found: 363.1261.

**(1*S*,3'*R*,4'*S*,5'*S*,6'*R*)-6-((5-ethylthiophen-2-yl)methyl)-6'-methyl-3',4',5',6'-tetrahydro-3*H*-spiro[isobenzofuran-1,2'-pyran]-3',4',5'-triol (16).** Compound **16** was prepared from **54** and 2-ethylthiophene according to the procedure described for **9** in 57% yield (3 steps) as a white solid. HPLC purity: 99.41%. m.p. 142–144 °C; $^{1}$H NMR (500 MHz, Methanol-$d_4$) $\delta$ 7.29 – 7.25 (m, 1H), 7.22 (d, $J$ = 8.5 Hz, 2H), 6.62 (d, $J$ = 3.3 Hz, 1H), 6.58 (d, $J$ = 3.4 Hz, 1H), 5.17 – 5.04 (m, 2H), 4.11 (s, 2H), 3.95 – 3.82 (m, 1H), 3.81 – 3.63 (m, 2H), 3.26 – 3.08 (m, 1H), 2.75 (q, $J$ = 7.5 Hz, 2H), 1.32 – 1.15 (m, 6H). $^{13}$C NMR (125 MHz, Methanol-$d_4$) $\delta$ 147.2, 142.4, 141.9, 140.3, 140.1, 130.8, 125.9, 124.0, 123.2, 121.9, 111.5, 77.4, 76.1, 75.2, 73.5, 71.5, 36.8, 24.3, 18.2, 16.5. LRMS (ESI) m/z: 377 $[M+H]^+$; HRMS (ESI) cacld for $C_{20}H_{24}O_5NaS$ $[M+Na]^+$: 399.1237; found: 399.1231.

**(1*S*,3'*R*,4'*S*,5'*S*,6'*R*)-6'-methyl-6-((5-propylthiophen-2-yl)methyl)-3',4',5',6'-tetrahydro-3*H*-spiro[isobenzofuran-1,2'-pyran]-3',4',5'-triol (17).** Compound **17** was prepared from **54** and 2-propylthiophene according to the procedure described for **9** in 59% yield (3 steps) as a light yellow solid. HPLC purity: 99.23%. m.p. 156–157 °C; $^{1}$H NMR (400 MHz, Methanol-$d_4$) $\delta$ 7.25 (dd, $J$ = 20.4, 9.3 Hz, 3H), 6.62 (d, $J$ = 3.2 Hz, 1H), 6.58 (d, $J$ = 3.1 Hz, 1H), 5.21 – 5.03 (m, 2H), 4.12 (s, 2H), 3.96 – 3.82 (m, 1H), 3.81 – 3.65 (m, 2H), 3.15 (t, $J$ = 9.0 Hz, 1H), 2.70 (t, $J$ = 7.4 Hz, 2H), 1.70 – 1.56 (m, 2H), 1.21 (d, $J$ = 6.3 Hz, 3H), 0.94 (t, $J$ = 7.3 Hz, 3H). $^{13}$C NMR (125 MHz, Methanol-$d_4$) $\delta$ 145.3, 142.5, 141.8, 140.3, 140.1, 130.8, 125.9, 124.8, 123.2, 121.9,

111.5, 77.4, 76.1, 75.3, 73.5, 71.5, 36.8, 33.1, 26.1, 18.2, 13.9. LRMS (ESI) m/z: 391 $[M+H]^+$; HRMS (ESI) cacld for $C_{21}H_{26}O_5NaS$ $[M+Na]^+$: 413.1393; found: 413.1384.

**(1*S*,3'*R*,4'*S*,5'*S*,6'*R*)-6-((5-chlorothiophen-2-yl)methyl)-6'-methyl-3',4',5',6'-tetrahydro-3*H*-spiro[isobenzofuran-1,2'-pyran]-3',4',5'-triol (18).** Compound **18** was prepared from **54** and 2-chlorothiophene according to the procedure described for **9** in 53% yield (3 steps) as a light yellow solid. HPLC purity: 98.59%. m.p. 150–152 °C; $^1$H NMR (400 MHz, Methanol-$d_4$) $\delta$ 7.33 – 7.18 (m, 3H), 6.77 (d, $J$ = 3.7 Hz, 1H), 6.68 (d, $J$ = 3.7 Hz, 1H), 5.18 – 5.05 (m, 2H), 4.13 (s, 2H), 3.93 – 3.82 (m, 1H), 3.80 – 3.66 (m, 2H), 3.21 – 3.10 (m, 1H), 1.21 (d, $J$ = 6.3 Hz, 3H). $^{13}$C NMR (125 MHz, Methanol-$d_4$) $\delta$ 144.6, 140.9, 140.6, 140.5, 130.8, 128.7, 127.1, 125.9, 123.3, 122.1, 111.5, 77.4, 76.1, 75.3, 73.5, 71.5, 36.8, 18.2. LRMS (ESI) m/z: 383 $[M+H]^+$; HRMS (ESI) cacld for $C_{18}H_{19}O_5ClNaS$ $[M+Na]^+$: 405.0534; found: 405.0527.

**(1*S*,3'*R*,4'*S*,5'*S*,6'*R*)-6'-methyl-6-((5-phenylthiophen-2-yl)methyl)-3',4',5',6'-tetrahydro-3*H*-spiro[isobenzofuran-1,2'-pyran]-3',4',5'-triol (19).** Compound **19** was prepared from **54** and 2-phenylthiophene according to the procedure described for **9** in 55% yield (3 steps) as a white solid. HPLC purity: 98.11%. m.p. 149–151 °C; $^1$H NMR (500 MHz, Methanol-$d_4$) $\delta$ 7.53 (d, $J$ = 7.5 Hz, 2H), 7.36 – 7.28 (m, 3H), 7.28 – 7.19 (m, 3H), 7.18 (d, $J$ = 3.6 Hz, 1H), 6.82 (d, $J$ = 3.5 Hz, 1H), 5.16 – 5.07 (m, 2H), 4.19 (s, 2H), 3.95 – 3.84 (m, 1H), 3.78 (d, $J$ = 9.6 Hz, 1H), 3.72 (t, $J$ = 9.2 Hz, 1H), 3.17 (t, $J$ = 9.2 Hz, 1H), 1.22 (d, $J$ = 6.2 Hz, 3H). $^{13}$C NMR (125 MHz, Methanol-$d_4$) $\delta$ 144.9, 144.1, 141.5, 140.5, 140.3, 135.9, 130.9, 129.9, 128.2, 127.6, 126.3, 123.9, 123.3, 122.1, 111.5, 77.4, 76.1, 75.3, 73.5, 71.5, 36.8, 18.2. LRMS (ESI) m/z: 425 $[M+H]^+$; HRMS (ESI) cacld for $C_{24}H_{24}O_5NaS$ $[M+Na]^+$: 447.1237; found: 447.1224.

**(1*S*,3'*R*,4'*S*,5'*S*,6'*R*)-6-((5-(4-fluorophenyl)thiophen-2-yl)methyl)-6'-methyl-3',4',5',6'-tetrahydro-3*H*-spiro[isobenzofuran-1,2'-pyran]-3',4',5'-triol (20).** Compound **20** was prepared from **54** and 2-(4-fluorophenyl)thiophene according to the procedure described for **9** in 49% yield (3 steps) as a white solid. HPLC purity: 96.06%. m.p.

174–176 °C; $^{1}$H NMR (400 MHz, Methanol-$d_4$) $\delta$ 7.54 (dd, $J$ = 8.7, 5.3 Hz, 2H), 7.32 (d, $J$ = 8.1 Hz, 1H), 7.25 (d, $J$ = 7.8 Hz, 2H), 7.13 (d, $J$ = 3.5 Hz, 1H), 7.07 (t, $J$ = 8.7 Hz, 2H), 6.82 (d, $J$ = 3.5 Hz, 1H), 5.17 – 5.06 (m, 2H), 4.19 (s, 2H), 3.96 – 3.83 (m, 1H), 3.82 – 3.65 (m, 2H), 3.16 (t, $J$ = 9.1 Hz, 1H), 1.21 (d, $J$ = 6.2 Hz, 3H). $^{13}$C NMR (125 MHz, Methanol-$d_4$) $\delta$ 163.5 (d, $J$ = 243.6 Hz), 145.0, 143.0, 141.5, 140.5, 140.3, 132.4, 132.4, 130.9, 128.2 (d, $J$ = 8.0 Hz), 127.6, 124.0, 123.3, 122.1, 116.6 (d, $J$ = 21.9 Hz), 111.5, 77.4, 76.1, 75.3, 73.5, 71.5, 36.8, 18.2. LRMS (ESI) m/z: 443 $[M+H]^+$; HRMS (ESI) cacld for $C_{24}H_{24}O_5FS$ $[M+H]^+$: 443.1323; found: 443.1328.

**(1*S*,3'*R*,4'*S*,5'*S*,6'*R*)-6'-methyl-6-((5-(pyridin-2-yl)thiophen-2-yl)methyl)-3',4',5',6'-tetrahydro-3*H*-spiro[isobenzofuran-1,2'-pyran]-3',4',5'-triol (21).** Compound **21** was prepared from **54** and 2-(thiophen-2-yl)pyridine according to the procedure described for **9** in 27% yield (3 steps) as a white solid. HPLC purity: 99.00%. m.p. 146–147 °C; $^{1}$H NMR (400 MHz, Methanol-$d_4$) $\delta$ 8.65 – 8.58 (m, 1H), 8.45 (td, $J$ = 8.3, 1.5 Hz, 1H), 8.19 (d, $J$ = 8.3 Hz, 1H), 7.89 (d, $J$ = 3.9 Hz, 1H), 7.82 – 7.74 (m, 1H), 7.41 – 7.34 (m, 1H), 7.30 (d, $J$ = 7.5 Hz, 2H), 7.16 (d, $J$ = 3.9 Hz, 1H), 5.13 (d, $J$ = 2.7 Hz, 2H), 4.35 (s, 2H), 3.93 – 3.83 (m, 1H), 3.79 – 3.68 (m, 2H), 3.19 – 3.11 (m, 1H), 1.21 (d, $J$ = 6.3 Hz, 3H). $^{13}$C NMR (125 MHz, Methanol-$d_4$) $\delta$ 155.0, 148.3, 147.3, 142.8, 140.9, 140.9, 140.3, 132.5, 131.0, 129.3, 125.1, 125.1, 123.5, 122.4, 111.5, 77.4, 76.1, 75.3, 73.4, 71.5, 36.9, 18.2. LRMS (ESI) m/z: 426 $[M+H]^+$; HRMS (ESI) cacld for $C_{23}H_{24}O_5NS$ $[M+H]^+$: 426.1370; found: 426.1366.

**(1*S*,3'*R*,4'*S*,5'*S*,6'*R*)-6'-methyl-6-(naphthalen-2-ylmethyl)-3',4',5',6'-tetrahydro-3*H*-spiro[isobenzofuran-1,2'-pyran]-3',4',5'-triol (22).** Compound **22** was prepared from **54** and 2-Bromonaphthalene according to the procedure described for **9** in 36% yield (3 steps) as a white solid. HPLC purity: 96.87%. m.p. 148–149 °C. $^{1}$H NMR (500 MHz, Methanol-$d_4$) $\delta$ 7.82 – 7.72 (m, 3H), 7.67 (s, 1H), 7.46 – 7.37 (m, 2H), 7.34 (dd, $J$ = 8.4, 1.6 Hz, 1H), 7.31 – 7.26 (m, 1H), 7.22 (d, $J$ = 8.6 Hz, 2H), 5.20 – 5.02 (m, 2H), 4.17 (s, 2H), 3.94 – 3.82 (m, 1H), 3.79 – 3.64 (m, 2H), 3.20 – 3.07 (m, 1H), 1.20 (d, $J$ = 6.2 Hz, 3H). $^{13}$C NMR (125 MHz, Methanol-$d_4$) $\delta$ 227.3, 142.3,

140.4, 140.0, 139.9, 135.1, 133.6, 131.3, 129.1, 128.6, 128.6, 128.1, 127.0, 126.4, 123.6, 122.0, 111.5, 77.4, 76.1, 75.2, 73.5, 71.5, 42.7, 18.2. LRMS (ESI, *m/z*): 393 $[M+H]^+$; HRMS (ESI) cacld for $C_{24}H_{24}O_5Na$ $[M+Na]^+$: 415.1516; found: 415.1509.

**(1*S*,3'*R*,4'*S*,5'*S*,6'*R*)-6-(benzo[*b*]thiophen-2-ylmethyl)-6'-methyl-3',4',5',6'-tetrahydro-3*H*-spiro[isobenzofuran-1,2'-pyran]-3',4',5'-triol (23).** Compound **23** was prepared from **54** and benzo[b]thiophene according to the procedure described for **9** in 51% yield (3 steps) as a white solid. HPLC purity: 96.75%. m.p. 178–180 °C; $^1$H NMR (400 MHz, Methanol-$d_4$) $\delta$ 7.73 (d, $J$ = 7.8 Hz, 1H), 7.67 (d, $J$ = 7.5 Hz, 1H), 7.36 (dd, $J$ = 7.7, 1.5 Hz, 1H), 7.32 – 7.19 (m, 4H), 7.09 (s, 1H), 5.19 – 5.06 (m, 2H), 4.29 (s, 2H), 3.87 (dd, $J$ = 9.6, 6.3 Hz, 1H), 3.80 – 3.64 (m, 2H), 3.20 – 3.09 (m, 1H), 1.21 (d, $J$ = 6.3 Hz, 3H). $^{13}$C NMR (125 MHz, Methanol-$d_4$) $\delta$ 146.2, 141.5, 141.2, 140.8, 140.5, 140.5, 131.0, 125.2, 124.8, 124.0, 123.4, 123.0, 123.0, 122.1, 111.5, 77.4, 76.1, 75.2, 73.5, 71.5, 37.4, 18.2. LRMS (ESI) m/z: 399 $[M+H]^+$; HRMS (ESI) cacld for $C_{22}H_{22}O_5NaS$ $[M+Na]^+$: 421.1080; found: 421.1071.

**(1*S*,3'*R*,4'*S*,5'*S*,6'*R*)-6-(benzofuran-2-ylmethyl)-6'-methyl-3',4',5',6'-tetrahydro-3*H*-spiro[isobenzofuran-1,2'-pyran]-3',4',5'-triol (24).** Compound **24** was prepared from **54** and benzofuran according to the procedure described for **9** in 48% yield (3 steps) as a white solid. HPLC purity: 97.12%. m.p. 162–163 °C; $^1$H NMR (400 MHz, Methanol-$d_4$) $\delta$ 7.51 – 7.44 (m, 1H), 7.36 (d, $J$ = 7.4 Hz, 2H), 7.33 – 7.24 (m, 2H), 7.23 – 7.11 (m, 2H), 6.54 – 6.44 (m, 1H), 5.18 – 5.07 (m, 2H), 4.17 (s, 2H), 3.93 – 3.82 (m, 1H), 3.80 – 3.67 (m, 2H), 3.20 – 3.10 (m, 1H), 1.21 (d, $J$ = 6.3 Hz, 3H). $^{13}$C NMR (125 MHz, Methanol-$d_4$) $\delta$ 159.1, 156.4, 140.5, 140.5, 138.6, 131.2, 130.2, 124.5, 123.6, 123.6, 122.1, 121.5, 111.6, 111.5, 104.3, 77.4, 76.1, 75.3, 73.5, 71.5, 35.4, 18.2. LRMS (ESI) m/z: 383 $[M+H]^+$; HRMS (ESI) cacld for $C_{22}H_{23}O_6$ $[M+H]^+$: 383.1489; found: 383.1486.

**(2-bromo-5-chloro-1,4-phenylene)dimethanol (59).** To a solution (150 mL) of 1-bromo-4-chloro-2,5-dimethylbenzene **57** (25.00 g, 113.89 mmol) in EtOAc was

added *N*-bromosuccinimide (NBS) (50.68 g, 284.73 mmol) and 2,2′-azobis(isobutyronitrile) (AIBN) (935.1 mg, 5.69 mmol), and the resultant mixture was stirred for 0.5 h at reflux temperature. The reaction mixture was cooled to rt, and then EtOAc was added thereto. The resultant mixture was washed with water and brine. The organic layer was dried over $Na_2SO_4$, and the solvent was then removed by distillation under reduced pressure. The obtained crude product was dissolved in DMF (200 mL), and AcONa (28.03 g, 341.68 mmol) was added thereto. The resultant mixture was stirred for 3 h at 80 °C. The reaction mixture was cooled to rt, and then $CH_2Cl_2$ was added thereto. The mixture was washed with water and brine. The organic layer was dried over $Na_2SO_4$, and the solvent was then removed by distillation under reduced pressure. To the obtained residue was added EtOAc (200 mL), and this solution was stirred for 15 h at rt. Undissolved material was collected by filtration to obtain the compound **58** (10.32 g, 27%) as a white solid.

To a solution of compound **58** (10.32 g, 30.75 mmol) in a mixture of THF (80 mL), EtOH (80 mL) and water (40 mL), potassium hydroxide (5.18 g, 92.26 mmol) was added, and the resultant mixture was stirred for 4.5 h at 80 °C. The reaction mixture was cooled to rt, and then solvent was removed by distillation under reduced pressure. To the resulting residue were added water (70 mL) and AcOEt (35 mL), and this mixture was stirred for 1 h at rt. Undissolved material was collected by filtration and dried to give compound **59** (7.10 g, 92%) as a white solid. $^1$H NMR (400 MHz, Methanol-$d_4$) $\delta$ 7.71 (s, 1H), 7.52 (s, 1H), 4.66 (s, 2H), 4.61 (s, 2H).

**1-bromo-4-chloro-2,5-bis(((2-methoxypropan-2-yl)oxy)methyl)benzene (60).** Compound **60** was prepared from **59** according to the procedure described for **52** in 91% yield as a white solid. $^1$H NMR (400 MHz, Chloroform-*d*) $\delta$ 7.68 (s, 1H), 7.51 (s, 1H), 4.53 (s, 2H), 4.48 (s, 2H), 3.24 – 3.21 (m, 6H), 1.47 – 1.43 (m, 12H).

**(1*S*,3'*R*,4'*S*,5'*R*,6'*R*)-3',4',5'-tris(benzyloxy)-5-chloro-6'-methyl-3',4',5',6'-tetrahydro-3*H*-spiro[isobenzofuran-1,2'-pyran]-6-carbaldehyde (62).** Compound **62** was prepared from **49** and **60** according to the procedure described for **54** in 38% yield (3

steps) as a colorless oil. $^{1}$H NMR (400 MHz, Chloroform-*d*) $\delta$ 10.35 (s, 1H), 7.57 (s, 1H), 7.40 – 7.27 (m, 11H), 7.16 – 7.04 (m, 3H), 6.83 – 6.76 (m, 2H), 5.19 (s, 2H), 4.99 – 4.88 (m, 3H), 4.69 (dd, *J* = 18.4, 11.2 Hz, 2H), 4.23 (d, *J* = 11.6 Hz, 1H), 4.11 – 3.96 (m, 2H), 3.85 (d, *J* = 9.5 Hz, 1H), 3.33 (t, *J* = 9.4 Hz, 1H), 1.24 (d, *J* = 6.2 Hz, 3H). LRMS (ESI, *m/z*): 607 $[M+Na]^+$.

**(1*S*,3'*R*,4'*S*,5'*S*,6'*R*)-5-chloro-6-(4-ethylbenzyl)-6'-methyl-3',4',5',6'-tetrahydro-3*H*-spiro[isobenzofuran-1,2'-pyran]-3',4',5'-triol (25).** Compound **25** was prepared from **62** and 1-bromo-4-ethylbenzene according to the procedure described for **9** in 52% yield (3 steps) as a white solid. HPLC purity: 95.48%. m.p. 130–132 °C. $^{1}$H NMR (500 MHz, Methanol-$d_4$) $\delta$ 7.35 (s, 1H), 7.18 (s, 1H), 7.10 (s, 4H), 5.12 – 5.04 (m, 2H), 4.08 (s, 2H), 3.89 – 3.81 (m, 1H), 3.73 – 3.66 (m, 2H), 3.16 – 3.09 (m, 1H), 2.60 (q, *J* = 7.6 Hz, 2H), 1.24 – 1.16 (m, 6H). $^{13}$C NMR (125 MHz, Methanol-$d_4$) $\delta$ 143.4, 141.9, 139.7, 139.3, 137.9, 136.2, 129.9, 128.9, 125.6, 123.1, 111.3, 77.3, 76.0, 75.2, 72.9, 71.6, 39.8, 29.4, 18.2, 16.2. LRMS (ESI, *m/z*): 405 $[M+H]^+$; HRMS (ESI) cacld for $C_{22}H_{25}O_5ClNa$ $[M+Na]^+$: 427.1283; found: 427.1273.

**(1*S*,3'*R*,4'*S*,5'*S*,6'*R*)-5-chloro-6-(4-ethoxybenzyl)-6'-methyl-3',4',5',6'-tetrahydro-3*H*-spiro[isobenzofuran-1,2'-pyran]-3',4',5'-triol (26).** Compound **26** was prepared from **62** and 1-bromo-4-ethoxybenzene according to the procedure described for **9** in 48% yield (3 steps) as a white solid. HPLC purity: 97.30%. m.p. 109–112 °C. $^{1}$H NMR (500 MHz, Methanol-$d_4$) $\delta$ 7.35 (s, 1H), 7.16 (s, 1H), 7.12 – 7.07 (m, 2H), 6.84 – 6.79 (m, 2H), 5.12 – 5.04 (m, 2H), 4.05 (s, 2H), 3.99 (q, *J* = 7.0 Hz, 2H), 3.89 – 3.82 (m, 1H), 3.72 – 3.65 (m, 2H), 3.17 – 3.09 (m, 1H), 1.36 (t, *J* = 7.0 Hz, 3H), 1.20 (d, *J* = 6.3 Hz, 3H). $^{13}$C NMR (125 MHz, Methanol-$d_4$) $\delta$ 158.9, 141.8, 140.0, 139.3, 136.1, 132.5, 130.9, 125.5, 123.1, 115.5, 111.3, 77.3, 76.0, 75.2, 72.9, 71.6, 64.4, 39.3, 18.2, 15.2. LRMS (ESI, *m/z*): 421 $[M+H]^+$; HRMS (ESI) cacld for $C_{22}H_{25}O_6ClNa$ $[M+Na]^+$: 443.1232; found: 443.1221.

**(1*S*,3'*R*,4'*S*,5'*S*,6'*R*)-5-chloro-6'-methyl-6-((5-methylthiophen-2-yl)methyl)-3',4',5**

**',6'-tetrahydro-3*H*-spiro[isobenzofuran-1,2'-pyran]-3',4',5'-triol (27).** Compound **27** was prepared from **62** and 2-bromo-5-methylthiophene according to the procedure described for **9** in 36% yield (3 steps) as a white solid. HPLC purity: 97.24%. $^{1}$H NMR (400 MHz, Methanol-$d_4$) δ 7.33 (s, 1H), 7.24 (s, 1H), 6.63 – 6.50 (m, 2H), 5.07 (t, *J* = 1.4 Hz, 2H), 4.23 – 4.13 (m, 2H), 3.85 (dq, *J* = 9.7, 6.2 Hz, 1H), 3.74 – 3.65 (m, 2H), 3.14 (ddd, *J* = 9.3, 5.8, 3.1 Hz, 1H), 2.37 (s, 3H), 1.20 (d, *J* = 6.3 Hz, 3H). $^{13}$C NMR (125 MHz, Methanol-$d_4$) δ 140.82, 139.35, 138.18, 138.01, 137.71, 134.40, 125.17, 124.46, 123.90, 121.79, 109.99, 109.93, 75.89, 74.65, 73.84, 71.59, 70.20, 33.16, 16.84, 13.84. LRMS (ESI, *m/z*): 396.9 $[M+H]^+$; HRMS (ESI) cacld for $C_{19}H_{22}ClO_5S[M+H]^+$: 397.0871; found: 397.0861.

**(1*S*,3'*R*,4'*S*,5'*S*,6'*R*)-5-chloro-6-((5-chlorothiophen-2-yl)methyl)-6'-methyl-3',4',5',6'-tetrahydro-3*H*-spiro[isobenzofuran-1,2'-pyran]-3',4',5'-triol (28).** Compound **28** was prepared from **62** and 2-bromo-5-chlorothiophene according to the procedure described for **9** in 56% yield (3 steps) as a white solid. HPLC purity: 98.09%. $^{1}$H NMR (400 MHz, Methanol-$d_4$) δ 7.38 (d, *J* = 1.1 Hz, 1H), 7.30 (s, 1H), 6.76 (dd, *J* = 3.8, 0.9 Hz, 1H), 6.65 (dd, *J* = 3.7, 1.0 Hz, 1H), 5.09 (d, *J* = 2.5 Hz, 2H), 4.22 (s, 2H), 3.90 – 3.80 (m, 1H), 3.75 – 3.65 (m, 2H), 3.30 (p, *J* = 1.6 Hz, 1H), 3.17 – 3.11 (m, 1H), 1.20 (d, *J* = 6.3 Hz, 3H). $^{13}$C NMR (125 MHz, Methanol-$d_4$) δ 141.05, 141.03, 138.00, 136.48, 134.19, 127.09, 125.43, 124.62, 123.82, 121.72, 109.67, 75.66, 74.38, 73.60, 71.32, 69.98, 33.09, 16.55. LRMS (ESI, *m/z*): 417.1 $[M+H]^+$; HRMS (ESI) cacld for $C_{18}H_{19}Cl_2O_5S$ $[M+H]^+$: 417.0325; found: 417.0325.

**(1*S*,3'*R*,4'*S*,5'*S*,6'*R*)-5-chloro-6-((5-(2-methoxyethyl)thiophen-2-yl)methyl)-6'-methyl-3',4',5',6'-tetrahydro-3*H*-spiro[isobenzofuran-1,2'-pyran]-3',4',5'-triol (33).** Compound **33** was prepared from **62** and 2-(2-methoxyethyl)thiophene according to the procedure described for **9** in 49% yield (3 steps) as a white solid. HPLC purity: 98.43%. $^{1}$H NMR (400 MHz, Methanol-$d_4$) δ 7.35 (s, 1H), 7.26 (s, 1H), 6.68 – 6.60 (m, 2H), 5.08 (d, *J* = 2.2 Hz, 2H), 4.21 (d, *J* = 3.7 Hz, 2H), 3.85 (dq, *J* = 9.7, 6.2 Hz, 1H), 3.73 – 3.65 (m, 2H), 3.56 (t, *J* = 6.6 Hz, 2H), 3.32 (s, 3H), 3.14 (ddd, *J* = 9.3, 6.7, 2.3 Hz, 1H), 2.96 (t, *J* = 6.6 Hz, 2H), 1.20 (d, *J* = 6.3 Hz, 3H). $^{13}$C NMR (100 MHz, Methanol-$d_4$) δ 142.77, 141.93, 141.90, 139.94, 139.52, 136.29, 126.84, 126.42,

125.88, 123.69, 111.82, 77.79, 76.52, 75.72, 74.85, 73.47, 72.09, 59.31, 35.08, 31.85, 18.73. LRMS (ESI, *m/z*): 462.9 $[M+Na]^+$; HRMS (ESI) cacld for $C_{21}H_{26}ClO_6S$ $[M+H]^+$: 441.1133; found: 441.1131.

**(1*S*,3'*R*,4'*S*,5'*S*,6'*R*)-5-chloro-6-((5-(2-ethoxyethyl)thiophen-2-yl)methyl)-6'-methyl-3',4',5',6'-tetrahydro-3*H*-spiro[isobenzofuran-1,2'-pyran]-3',4',5'-triol (34).** Compound **34** was prepared from **62** and 2-(2-ethoxyethyl)thiophene according to the procedure described for **9** in 44% yield (3 steps) as a white solid. HPLC purity: 98.18%. $^1$H NMR (400 MHz, Methanol-$d_4$) δ 7.38 (s, 1H), 7.29 (s, 1H), 6.70 – 6.63 (m, 2H), 5.11 (d, *J* = 2.6 Hz, 2H), 4.25 (d, *J* = 5.2 Hz, 2H), 3.87 (dd, *J* = 9.6, 6.3 Hz, 1H), 3.75 – 3.67 (m, 2H), 3.63 (t, *J* = 6.7 Hz, 2H), 3.52 (q, *J* = 7.0 Hz, 2H), 3.16 (ddd, *J* = 9.2, 6.4, 2.5 Hz, 1H), 3.03 – 2.95 (m, 2H), 1.23 (d, *J* = 6.2 Hz, 3H), 1.19 (t, *J* = 7.0 Hz, 3H). $^{13}$C NMR (125 MHz, Methanol-$d_4$) δ 140.63, 139.85, 139.75, 137.81, 137.39, 134.16, 124.65, 124.23, 123.71, 121.52, 109.68, 75.66, 74.39, 73.60, 71.31, 70.63, 69.94, 65.62, 32.92, 29.90, 16.55, 13.80. LRMS (ESI, *m/z*): 476.9 $[M+Na]^+$; HRMS (ESI) cacld for $C_{22}H_{28}ClO_6S$ $[M+H]^+$: 455.1290; found: 455.1292.

**(1*S*,3'*R*,4'*S*,5'*S*,6'*R*)-5-chloro-6'-methyl-6-((5-(2-propoxyethyl)thiophen-2-yl)methyl)-3',4',5',6'-tetrahydro-3*H*-spiro[isobenzofuran-1,2'-pyran]-3',4',5'-triol (35).** Compound **35** was prepared from **62** and 2-(2-propoxyethyl)thiophene according to the procedure described for **9** in 40% yield (3 steps) as a white solid. HPLC purity: 97.36%. $^1$H NMR (400 MHz, Methanol-$d_4$) δ 7.38 (s, 1H), 7.29 (s, 1H), 6.66 (d, *J* = 4.4 Hz, 2H), 5.11 (d, *J* = 2.8 Hz, 2H), 4.25 (d, *J* = 6.2 Hz, 2H), 3.94 – 3.83 (m, 1H), 3.76 – 3.67 (m, 2H), 3.62 (t, *J* = 6.6 Hz, 2H), 3.43 (t, *J* = 6.6 Hz, 2H), 3.16 (ddd, *J* = 9.3, 6.5, 2.4 Hz, 1H), 3.00 (t, *J* = 6.6 Hz, 2H), 1.59 (h, *J* = 7.1 Hz, 2H), 1.23 (d, *J* = 6.2 Hz, 3H), 0.93 (t, *J* = 7.4 Hz, 3H). $^{13}$C NMR (125 MHz, Methanol-$d_4$) δ 140.63, 139.93, 139.75, 137.80, 137.41, 134.17, 124.60, 124.22, 123.71, 121.52, 109.68, 75.67, 74.39, 73.60, 72.05, 71.31, 70.82, 69.94, 32.93, 29.90, 22.25, 16.54, 9.34. LRMS (ESI, *m/z*): 490.9 $[M+Na]^+$; HRMS (ESI) cacld for $C_{23}H_{30}ClO_6S$ $[M+H]^+$: 469.1446; found: 469.1457.

**(1*S*,3'*R*,4'*S*,5'*S*,6'*R*)-5-chloro-6-((5-(4-fluorophenyl)thiophen-2-yl)methyl)-6'-methyl-3',4',5',6'-tetrahydro-3*H*-spiro[isobenzofuran-1,2'-pyran]-3',4',5'-triol (36).** Compound **36** was prepared from **62** and 2-(4-fluorophenyl)thiophene according to the procedure described for **9** in 51% yield (3 steps) as a light yellow solid. HPLC purity: 97.24%. mp 115–117 °C. $^{1}$H NMR (500 MHz, Methanol-$d_4$) $\delta$ 7.58 – 7.51 (m, 2H), 7.38 (s, 1H), 7.33 (s, 1H), 7.12 (d, $J$ = 3.6 Hz, 1H), 7.11 – 7.03 (m, 2H), 6.80 (d, $J$ = 3.6 Hz, 1H), 5.14 – 5.06 (m, 2H), 4.30 (s, 2H), 3.92 – 3.81 (m, 1H), 3.77 – 3.67 (m, 2H), 3.20 – 3.10 (m, 1H), 1.21 (d, $J$ = 6.3 Hz, 3H). $^{13}$C NMR (125 MHz, Methanol-$d_4$) $\delta$ 163.51 (d, $J$ = 243.8 Hz), 143.1, 143.0, 142.5, 139.6, 138.7, 135.9, 132.4, 132.3, 128.2 (d, $J$ = 8.0 Hz), 127.9, 125.4, 124.0, 123.3, 116.6 (d, $J$ = 21.9 Hz), 111.3, 77.3, 76.1, 75.3, 73.0, 71.6, 34.7, 18.2. LRMS (ESI, *m/z*): 477 $[M+H]^+$; HRMS (ESI) cacld for $C_{24}H_{22}O_5ClFNaS$ $[M+Na]^+$: 499.0753; found: 499.0739.

**(1*S*,3'*R*,4'*S*,5'*S*,6'*R*)-6-(benzo[b]thiophen-2-ylmethyl)-5-chloro-6'-methyl-3',4',5',6'-tetrahydro-3*H*-spiro[isobenzofuran-1,2'-pyran]-3',4',5'-triol (37).** Compound **37** was prepared from **62** and benzo[b]thiophene according to the procedure described for **9** in 49% yield (3 steps) as a white solid. HPLC purity: 99.14%. Mp 119–121 °C. $^{1}$H NMR (500 MHz, Methanol-$d_4$) $\delta$ 7.73 (d, $J$ = 8.0 Hz, 1H), 7.65 (s, 1H), 7.40 (s, 1H), 7.36 (s, 1H), 7.31 – 7.20 (m, 2H), 7.03 (s, 1H), 5.15 – 5.07 (m, 2H), 4.38 (s, 2H), 3.91 – 3.81 (m, 1H), 3.76 – 3.65 (m, 2H), 3.18 – 3.09 (m, 1H), 1.21 (d, $J$ = 6.3 Hz, 3H). $^{13}$C NMR (125 MHz, Methanol-$d_4$) $\delta$ 144.4, 142.6, 141.4, 141.1, 139.6, 138.2, 136.0, 125.6, 125.2, 124.8, 124.0, 123.3, 123.3, 123.0, 111.3, 77.3, 76.0, 75.2, 73.0, 71.6, 35.3, 18.2. LRMS (ESI, *m/z*): 433 $[M+H]^+$; HRMS (ESI) cacld for $C_{22}H_{21}O_5ClNaS$ $[M+Na]^+$: 455.0690; found: 455.0681.

**5-(((1*S*,3'*R*,4'*S*,5'*R*,6'*R*)-3',4',5'-tris(benzyloxy)-5-chloro-6'-methyl-3',4',5',6'-tetrahydro-3*H*-spiro[isobenzofuran-1,2'-pyran]-6-yl)methyl)thiophene-2-carbaldehy**

**de (67)**. To a solution of 2-bromothiophene (1.00 g, 6.15 mmol) in anhydrous THF (30 mL), 2.5 M *n*-BuLi in hexane solution (2.71 mL, 6.77 mmol) was added dropwise at −78 °C under a nitrogen atmosphere and the resultant mixture was stirred under the same condition for 1 h. A solution of compound **62** (720.0 mg, 1.23 mmol) in anhydrous THF (5 mL) was then added dropwise to the resultant mixture. The reaction mixture was stirred for 2 h, and then water was added thereto. The resultant mixture was extracted with EtOAc. The organic layer was washed with brine, dried over $Na_2SO_4$, and then evaporated under reduced pressure to remove the solvent. The obtained residue was purified by column chromatography on silica gel (petroleum ether/EtOAc = 4/1) to give compound **65** (576.5 mg, 70%) as a colorless oil.

To a solution of compound **65** (550.0 mg, 0.82 mmol) in $CH_2Cl_2$ (50 mL), triethylsilane (656.3 μL, 4.11 mmol) and $BF_3{\cdot}OEt_2$ (121.7 μL, 0.99 mmol) were added at −40 °C and the mixture was stirred at the same temperature for 1 h. After addition of water, the reaction mixture was extracted with $CH_2Cl_2$. The organic layer was washed with brine, dried over $Na_2SO_4$, and then evaporated under reduced pressure to remove the solvent. The obtained residue was purified by column chromatography on silica gel (petroleum ether/EtOAc = 10/1) to give compound **66** (397.3 mg, 74%) as a colorless oil. $^1$H NMR (400 MHz, Chloroform-*d*) $\delta$ 7.34 (dd, $J$ = 7.8, 3.7 Hz, 11H), 7.21 – 7.11 (m, 5H), 6.90 (dd, $J$ = 5.1, 3.4 Hz, 1H), 6.83 – 6.77 (m, 3H), 5.22 – 5.12 (m, 2H), 4.99 – 4.88 (m, 3H), 4.72 (d, $J$ = 11.0 Hz, 1H), 4.57 (d, $J$ = 10.9 Hz, 1H), 4.35 – 4.19 (m, 2H), 4.13 – 4.03 (m, 3H), 3.82 (d, $J$ = 9.5 Hz, 1H), 3.33 (t, $J$ = 9.4 Hz, 1H), 1.28 (d, $J$ = 6.2 Hz, 3H).

To a solution of compound **66** (392.0 mg, 0.60 mmol) in anhydrous THF (20 mL) under argon atmosphere, 2.5 M *n*-BuLi in hexane solution (360.1 μL, 0.90 mmol) was added dropwise at −78 °C and the resultant mixture was stirred under 0 °C for 1 h. Then, the reaction mixture was cooled to -78 °C, and was added with DMF (92.4 μL, 1.20 mmol). The resulting mixture was stirred overnight under -70 °C. After completion, water was added to the reaction mixture. The resultant solution was extracted with EtOAc. The organic layer was washed with brine, dried over $Na_2SO_4$, and then evaporated under reduced pressure to remove the solvent. The obtained

residue was purified by column chromatography on silica gel (petroleum ether/EtOAc = 8/1) to give compound **67** (305.4 mg, 75%) as a light-yellow solid. $^1$H NMR (400 MHz, Chloroform-*d*) $\delta$ 9.71 (s, 1H), 7.58 (s, 1H), 7.53 – 7.47 (m, 2H), 7.37 – 7.21 (m, 15H), 7.10 (d, *J* = 7.5 Hz, 1H), 4.75 – 4.55 (m, 6H), 4.46 (dd, *J* = 14.3, 12.3 Hz, 2H), 4.37 (d, *J* = 7.0 Hz, 1H), 4.31 (d, *J* = 12.4 Hz, 1H), 4.21 (td, *J* = 13.1, 12.5, 6.3 Hz, 2H), 3.88 (t, *J* = 7.0 Hz, 1H), 3.47 (t, *J* = 7.0 Hz, 1H), 1.34 (d, *J* = 6.8 Hz, 3H).

**5-(((1*S*,3'*R*,4'*S*,5'*S*,6'*R*)-5-chloro-3',4',5'-trihydroxy-6'-methyl-3',4',5',6'-tetrahydro-3*H*-spiro[isobenzofuran-1,2'-pyran]-6-yl)methyl)thiophene-2-carbaldehyde (29).** To a solution of compound **67** (120.0 mg, 0.176 mmol) and pentamethylbenzene (268.2 mg, 1.76 mmol) in $CH_2Cl_2$ (30 mL) was added 1.0 M $BCl_3$ in toluene solution (1.06 mL, 1.06 mmol) at −78 °C under a nitrogen atmosphere, and the mixture was stirred at the same temperature overnight. After addition of MeOH (10 mL), the reaction mixture was warmed to rt and evaporated under reduced pressure to remove the solvent. The obtained residue was purified by column chromatography on silica gel ($CH_2Cl_2$/MeOH = 15/1) to give compound **29** (45.3 mg, 62%) as a white solid. HPLC purity: 97.21%. $^1$H NMR (400 MHz, Methanol-$d_4$) $\delta$ 9.79 (s, 1H), 7.77 (d, *J* = 3.8 Hz, 1H), 7.41 (d, *J* = 16.2 Hz, 2H), 7.05 (d, *J* = 3.8 Hz, 1H), 5.13 (d, *J* = 2.7 Hz, 2H), 4.42 (s, 2H), 3.94 – 3.83 (m, 1H), 3.80 – 3.66 (m, 2H), 3.17 (t, *J* = 8.9 Hz, 1H), 1.23 (d, *J* = 6.3 Hz, 3H). $^{13}$C NMR (150 MHz, Methanol-$d_4$) $\delta$ 183.58, 154.15, 142.38, 141.66, 138.41, 137.72, 136.15, 134.60, 127.15, 124.37, 122.17, 109.93, 75.90, 74.61, 73.83, 71.61, 70.26, 33.90, 16.85. LRMS (ESI, *m/z*): 432.7 $[M+Na]^+$ and 445.0 $[M+Cl]^-$; HRMS (ESI) cacld for $C_{19}H_{20}ClO_6S$ $[M+H]^+$: 411.0664; found: 411.0663.

**(1*S*,3'*R*,4'*S*,5'*S*,6'*R*)-5-chloro-6-((5-(hydroxymethyl)thiophen-2-yl)methyl)-6'-methyl-3',4',5',6'-tetrahydro-3*H*-spiro[isobenzofuran-1,2'-pyran]-3',4',5'-triol (30).** To a solution of compound **29** (33.0 mg, 0.08 mmol) in methanol (4 mL) was added $NaBH_4$ (6.1 mg, 0.16 mmol) at 0 °C, and the reaction mixture was stirred at room temperature for 1 h. After completion, the reaction mixture was evaporated under reduced pressure to remove the solvent. The obtained residue was purified by column

chromatography on silica gel ($CH_2Cl_2$/MeOH = 10/1) to give compound **30** (33.1 mg, 99%) as a white solid. HPLC purity: 95.11%. $^1$H NMR (400 MHz, Methanol-$d_4$) $\delta$ 7.35 (s, 1H), 7.27 (s, 1H), 6.78 (d, $J$ = 3.4 Hz, 1H), 6.68 (d, $J$ = 3.4 Hz, 1H), 5.08 (d, $J$ = 2.4 Hz, 2H), 4.63 (s, 2H), 4.31 – 4.19 (m, 2H), 3.85 (dq, $J$ = 9.7, 6.2 Hz, 1H), 3.73 – 3.65 (m, 2H), 3.13 (ddd, $J$ = 9.3, 6.1, 2.8 Hz, 1H), 1.20 (d, $J$ = 6.3 Hz, 3H). $^{13}$C NMR (125 MHz, Methanol-$d_4$) $\delta$ 143.10, 141.65, 140.72, 137.86, 137.25, 134.17, 124.68, 124.37, 123.72, 121.56, 109.68, 75.67, 74.39, 73.59, 71.30, 69.94, 58.45, 33.02, 16.55. LRMS (ESI, *m/z*): 434.7 $[M+Na]^+$ and 446.9 $[M+Cl]^-$; HRMS (ESI) cacld for $C_{19}H_{21}Cl_2O_6S$ $[M+Cl]^-$: 447.0441; found: 447.0445.

**methyl 5-(((1*S*,3'*R*,4'*S*,5'*R*,6'*R*)-3',4',5'-tris(benzyloxy)-5-chloro-6'-methyl-3',4',5',6'-tetrahydro-3*H*-spiro[isobenzofuran-1,2'-pyran]-6-yl)methyl)thiophene-2-carboxylate (68)**. To a solution of compound **67** (200.0 mg, 0.29 mmol) in methanol (4 mL) under 0 $^o$C, a methanol solution (2 mL) of KOH (42.8 mg, 0.76 mmol) and $I_2$ (96.9 mg, 0.38 mmol) was added and the resulting mixture was stirred at 0 $^o$C for 3 h. After completion, saturated $Na_2SO_3$ aq. was added dropwise into the reaction mixture until the solution did not change color any more, then remove the methanol via rotary evaporation. The residue was extracted with dichloromethane, and the organic layer was washed with brine, dried over $Na_2SO_4$, and then concentrated under reduced pressure. The obtained residue was purified by column chromatography on silica gel (petroleum ether/EtOAc = 12/1) to give compound **68** (104.0 mg, 50%) as a white solid. $^1$H NMR (400 MHz, Methanol-$d_4$) $\delta$ 7.59 (d, $J$ = 3.7 Hz, 1H), 7.35 (dd, $J$ = 9.0, 2.8 Hz, 11H), 7.23 – 7.12 (m, 4H), 6.82 (d, $J$ = 7.3 Hz, 2H), 6.77 (d, $J$ = 3.8 Hz, 1H), 5.23 – 5.13 (m, 2H), 4.99 – 4.89 (m, 3H), 4.73 (d, $J$ = 11.0 Hz, 1H), 4.60 (d, $J$ = 11.1 Hz, 1H), 4.33 – 4.18 (m, 2H), 4.15 – 4.03 (m, 3H), 3.82 (d, $J$ = 9.2 Hz, 1H), 3.81 (s, 3H), 3.34 (t, $J$ = 9.4 Hz, 1H), 1.28 (d, $J$ = 6.1 Hz, 3H).

**methyl 5-(((1*S*,3'*R*,4'*S*,5'*S*,6'*R*)-5-chloro-3',4',5'-trihydroxy-6'-methyl-3',4',5',6'-tetrahyd**

**ro-3*H*-spiro[isobenzofuran-1,2'-pyran]-6-yl)methyl)thiophene-2-carboxylate (31).** Compound **31** was prepared from intermediate **68** according to the procedure described for **29** in 62% yield as a white solid. HPLC purity: 97.94%. $^{1}$H NMR (400 MHz, Methanol-$d_4$) $\delta$ 7.62 (d, $J$ = 3.8 Hz, 1H), 7.40 (s, 1H), 7.36 (s, 1H), 6.90 (d, $J$ = 3.8 Hz, 1H), 5.20 – 5.06 (m, 2H), 4.33 (s, 2H), 3.90 (dt, $J$ = 9.6, 6.3 Hz, 1H), 3.83 (s, 3H), 3.80 – 3.70 (m, 2H), 3.18 (t, $J$ = 8.9 Hz, 1H), 1.23 (d, $J$ = 6.2 Hz, 3H). $^{13}$C NMR (150 MHz, Methanol-$d_4$) $\delta$ 162.75, 150.74, 141.49, 138.33, 136.48, 134.55, 133.49, 131.33, 126.42, 124.26, 122.11, 109.93, 75.89, 74.63, 73.83, 71.61, 70.26, 51.22, 33.53, 16.87. LRMS (ESI, *m/z*): 462.9 $[M+Na]^+$ and 475.0 $[M+Cl]^-$; HRMS (ESI) cacld for $C_{20}H_{22}ClO_7S$ $[M+H]^+$: 441.0769; found: 441.0770.

**methyl 2-(5-(((1*S*,3'*R*,4'*S*,5'*S*,6'*R*)-5-chloro-3',4',5'-trihydroxy-6'-methyl-3',4',5',6'-tetrahydro-3*H*-spiro[isobenzofuran-1,2'-pyran]-6-yl)methyl)thiophen-2-yl)acetate (32).** The DMF solution of compound **67** (300.0 mg, 0.44 mmol) was protected with argon and cooled to -9 $^{o}$C. The cooled solution was added with chloroform (87.6 μL, 1.10 mmol), then was added dropwise with a methanol solution (300 μL) of KOH (19.8 mg, 0.35 mmol) at the same temperature. The reaction was stirred for 2 h under -9 $^{o}$C and then quenched with 1 *N* HCl aq. The resulting mixture was extracted with EtOAc. The organic layer was washed with saturated $NaHCO_3$ aq., and brine, dried over $Na_2SO_4$, and then evaporated under reduced pressure to remove the solvent. The obtained residue was purified by column chromatography on silica gel (petroleum ether/EtOAc = 10/1) to give compound **69** (286.0 mg, 81%) as a white solid. $^{1}$H NMR (400 MHz, Chloroform-*d*) $\delta$ 7.34 (dd, $J$ = 5.4, 3.1 Hz, 11H), 7.21 – 7.11 (m, 4H), 7.07 – 7.04 (m, 1H), 6.78 (dt, $J$ = 8.2, 1.9 Hz, 2H), 6.72 (dd, $J$ = 3.7, 1.1 Hz, 1H), 5.26 (s, 1H), 5.23 – 5.12 (m, 2H), 4.99 – 4.88 (m, 3H), 4.76 – 4.70 (m, 1H), 4.58 (dd, $J$ = 10.8, 3.5 Hz, 1H), 4.37 – 4.02 (m, 5H), 3.82 (dd, $J$ = 9.5, 3.6 Hz, 1H), 3.37 – 3.30 (m, 1H), 1.27 (d, $J$ = 6.2 Hz, 3H).

To a solution of compound **69** (630.0 mg, 0.79 mmol) in *t*-BuOH (10 mL) was added with NaOH (powder, 103.9 mg, 2.60 mmol) at 30 $^{o}$C, then the mixture was stirred

vigorously for 10 min, followed by the addition of $NaBH_4$ (59.5 mg, 1.57 mmol). The heterogeneous mixture was warmed to 35 $^oC$ and stirred vigorously at for 48 h. After completion, the solvent *t*-BuOH was removed by rotary evaporation and the residue was dissolved in diethyl ether and water (12 mL+12 mL). The resulting solution was cooled to 0 $^oC$ and was adjusted to pH 1 with 1 *N* HCl. The product was extracted with diethyl ether, dried over $Na_2SO_4$, and then evaporated under reduced pressure to remove the solvent. The obtained residue was purified by column chromatography on silica gel (petroleum ether/EtOAc = 4/1 +0.5%HOAc) to give compound **70** (235.10 mg, 42%) as a yellow oil. LRMS (ESI, *m/z*): 709 $[M-H]^-$.

Compound **32** was prepared from intermediate **70** according to the procedure described for **29** in 24% yield as a white solid. HPLC purity: 95.52%. $^1H$ NMR (400 MHz, Methanol-$d_4$) $\delta$ $^1H$ NMR (400 MHz, Methanol-$d_4$) δ 7.38 (s, 1H), 7.31 (s, 1H), 6.75 (dt, *J* = 3.4, 1.0 Hz, 1H), 6.72 – 6.66 (m, 1H), 5.16 – 5.06 (m, 2H), 4.26 (d, *J* = 2.4 Hz, 2H), 3.92 – 3.83 (m, 1H), 3.79 (d, *J* = 0.9 Hz, 2H), 3.75 – 3.71 (m, 2H), 3.70 (s, 3H), 3.17 (ddd, *J* = 9.7, 7.3, 1.6 Hz, 1H), 1.23 (d, *J* = 6.3 Hz, 3H). $^{13}C$ NMR (125 MHz, Methanol-$d_4$) $\delta$ 171.48, 141.65, 140.99, 138.09, 137.41, 134.43, 133.99, 126.31, 125.01, 124.03, 121.84, 109.99, 109.92, 75.90, 74.63, 73.82, 71.57, 70.20, 51.27, 34.56, 33.17, 16.81. LRMS (ESI, *m/z*): 476.9 $[M+Na]^+$ and 489.0 $[M+Cl]^-$; HRMS (ESI) cacld for $C_{21}H_{23}Cl_2O_7S$ $[M+Cl]^-$: 489.0547; found: 489.0547.

**(2-bromo-5-fluorophenyl)methanol (72).** To a solution of 2-bromo-5-fluorobenzaldehyde **71** (25.00 g, 123.15 mmol) in MeOH (200 mL) was added $NaBH_4$ (4.66 g, 123.15 mmol) portionwise at 0 °C, and the mixture was stirred at room temperature for 2.5 h. Water was added, and the solvent was removed under reduced pressure to about a half volume. The mixture was poured into EtOAc and water. The organic layer was washed with brine and dried over anhydrous $Na_2SO_4$. The solvent was removed under reduced pressure to give compound **72** (21.00 g, 83%) as a white solid. $^1H$ NMR (400 MHz, Methanol-$d_4$) $\delta$ 7.53 (dd, *J* = 8.7, 5.2 Hz, 1H), 7.30 (dd, *J* = 9.7, 3.1 Hz, 1H), 6.95 (td, *J* = 8.4, 3.4 Hz, 1H), 4.61 (s, 2H).

**(2-bromo-5-fluoro-1,4-phenylene)dimethanol (73).** Tetramethylpiperidine (34.57 mL, 204.85 mmol) was dissolved in THF (250 mL). To the resultant solution was added 2.4 M n-BuLi in hexane solution (85.36 mL, 204.85 mmol) at 0°C., and this solution was stirred for 15 minutes. The resultant mixture was cooled to -78 °C. and a solution of compound **72** (20 g, 97.55 mmol) in THF (100 mL) was added dropwise thereto. The temperature of the solution was raised over 2 h to -40 °C. The solution was again cooled to -78 °C., and then DMF (18.80 mL, 243.87 mmol) was added thereto. The temperature of the solution was raised to room temperature, and the solution was stirred for 1 h. Saturated aqueous ammonium chloride was then added thereto, and the resultant mixture was extracted with ethyl acetate. The organic layer was dried over anhydrous $Na_2SO_4$, and then concentrated under reduced pressure. The obtained residue was dissolved in MeOH (200 mL), and $NaBH_4$ (3.69 g, 97.55 mmol) was added thereto portionwise at 0 °C. The mixture was stirred at room temperature for 2.5 h. Water was added, and the solvent was removed under reduced pressure to about a half volume. The mixture was poured into EtOAc and water. The organic layer was washed with brine and dried over anhydrous $Na_2SO_4$, and then evaporated under reduced pressure to remove the solvent. The obtained residue was purified by column chromatography on silica gel (petroleum ether/EtOAc = 1/1) to give compound **73** (7.62 g, 33%) as a white solid (2 steps). $^1$H NMR (400 MHz, Methanol-$d_4$) $\delta$ 7.62 (d, $J$ = 6.8 Hz, 1H), 7.26 (d, $J$ = 10.8 Hz, 1H), 4.63 (s, 2H), 4.60 (s, 2H).

**1-bromo-4-fluoro-2,5-bis(((2-methoxypropan-2-yl)oxy)methyl)benzene (74).** Compound **74** was prepared from **73** according to the procedure described for **52** in 96% yield as a colorless oil. $^1$H NMR (400 MHz, Chloroform-*d*) $\delta$ 7.59 (d, $J$ = 6.7 Hz, 1H), 7.26 (d, $J$ = 10.8 Hz, 1H), 4.50 (s, 2H), 4.48 (s, 2H), 3.24 (s, 3H), 3.22 (s, 3H), 1.45 (s, 6H), 1.43 (s, 6H).

**(1*S*,3'*R*,4'*S*,5'*R*,6'*R*)-3',4',5'-tris(benzyloxy)-5-fluoro-6'-methyl-3',4',5',6'-tetrahydro-3*H*-spiro[isobenzofuran-1,2'-pyran]-6-carbaldehyde (76).** Compound **76** was

prepared from **49** and **74** according to the procedure described for **54** in 25% yield (2 steps) as a white solid. $^1$H NMR (400 MHz, Chloroform-*d*) $\delta$ 10.20 (s, 1H), 7.52 (d, *J* = 6.0 Hz, 1H), 7.38 – 7.27 (m, 10H), 7.17 – 7.06 (m, 3H), 7.03 (d, *J* = 9.5 Hz, 1H), 6.84 – 6.77 (m, 2H), 5.20 (s, 2H), 4.95 (d, *J* = 12.7 Hz, 3H), 4.70 (dd, *J* = 17.8, 11.2 Hz, 2H), 4.24 (d, *J* = 11.5 Hz, 1H), 4.10 (t, *J* = 9.3 Hz, 1H), 4.07 – 3.97 (m, 1H), 3.85 (d, *J* = 9.5 Hz, 1H), 3.33 (t, *J* = 9.4 Hz, 1H), 1.24 (d, *J* = 6.3 Hz, 3H). LRMS (ESI, *m/z*): 567 $[M-H]^-$.

**(1*S*,3'*R*,4'*S*,5'*S*,6'*R*)-6-((5-ethylthiophen-2-yl)methyl)-5-fluoro-6'-methyl-3',4',5',6'-tetrahydro-3*H*-spiro[isobenzofuran-1,2'-pyran]-3',4',5'-triol (38).** Compound **38** was prepared from **76** and 2-ethylthiophene according to the procedure described for **9** in 36% yield (3 steps) as a white solid. HPLC purity: 97.31%. mp 127–128 °C. $^1$H NMR (500 MHz, Methanol-$d_4$) $\delta$ 7.22 (d, *J* = 6.7 Hz, 1H), 7.03 (d, *J* = 9.4 Hz, 1H), 6.62 (d, *J* = 3.4 Hz, 1H), 6.58 (d, *J* = 3.4 Hz, 1H), 5.13 – 5.04 (m, 2H), 4.10 (d, *J* = 3.5 Hz, 2H), 3.91 – 3.79 (m, 1H), 3.75 – 3.65 (m, 2H), 3.18 – 3.10 (m, 1H), 2.75 (q, *J* = 7.5 Hz, 2H), 1.27 – 1.17 (m, 6H). $^{13}$C NMR (125 MHz, Methanol-$d_4$) $\delta$ 162.9 (d, *J* = 244.0 Hz), 147.3, 142.6 (d, *J* = 9.3 Hz), 140.7, 135.9, 128.8 (d, *J* = 17.5 Hz), 126.0, 125.4 (d, *J* = 5.3 Hz), 124.0, 111.3, 109.0 (d, *J* = 25.1 Hz), 77.3, 76.1, 75.3, 73.1 (d, *J* = 2.0 Hz), 71.5, 30.1 (d, *J* = 3.8 Hz), 24.3, 18.2, 16.5. LRMS (ESI, *m/z*): 395 $[M+H]^+$; HRMS (ESI) cacld for $C_{20}H_{24}O_5FS$ $[M+H]^+$: 395.1323; found: 395.1313.

**1-bromo-2,5-bis(chloromethyl)-4-methylbenzene (80)**. A 100 g (584.68 mmol) sample of 1-bromo-4-methylbenzene **79** was melted in the bottom of a flask held at 60 $^o$C in an oil bath, and then powdered zinc chloride (63.75 g, 467.74 mmol) and paraformaldehyde (52.67 g) were added. Hydrogen chloride gas was blown over the surface of the reaction mixture. After the mixture was stirred at 60 $^o$C for 6 h, zinc chloride (4 g) and paraformaldehyde (4 g) were added every hour for the next 3 h. After stirring at 60 °C for an additional overnight, the resulting mixture was cooled, water was added, and the reaction mixture was extracted with $CH_2Cl_2$. The organic layer was dried over $Na_2SO_4$, and the solvent was then removed by distillation under

reduced pressure. The obtained residue was recrystallized from toluene to give compound **80** (33.05 g, 21%) as a white solid. $^1$H NMR (400 MHz, Chloroform-*d*) $\delta$ 7.54 (s, 1H), 7.31 (s, 1H), 4.65 (s, 2H), 4.53 (s, 2H), 2.38 (s, 3H).

**(2-bromo-5-methyl-1,4-phenylene)bis(methylene)diacetate (81).** To a solution of compound **80** (33.00 g, 123.14 mmol) in DMF (180 mL), and AcONa (30.31 g, 369.43 mmol) was added thereto. The resultant mixture was stirred for 3 h at 80 °C. The reaction mixture was cooled to rt, and then $CH_2Cl_2$ was added thereto. The mixture was washed with water and brine. The organic layer was dried over $Na_2SO_4$, and the solvent was then removed by distillation under reduced pressure. To the obtained residue was added EtOAc (70 mL) and n-hexane (100 mL), and this solution was stirred for 15 h at rt. Undissolved material was collected by filtration to obtain the compound **81** (32.15 g, 83%) as a white solid. $^1$H NMR (400 MHz, Chloroform-*d*) $\delta$ 7.54 (s, 1H), 7.22 (s, 1H), 5.15 (s, 2H), 5.06 (s, 2H), 2.30 (s, 3H), 2.14 (s, 3H), 2.12 (s, 3H).

**1-bromo-2,5-bis(((2-methoxypropan-2-yl)oxy)methyl)-4-methylbenzene (83).** Compound **83** was prepared from **81** according to the procedure described for **60** in 63% yield as a colorless oil (two steps). $^1$H NMR (400 MHz, Chloroform-*d*) $\delta$ 7.57 (s, 1H), 7.29 (s, 1H), 4.50 (s, 2H), 4.41 (s, 2H), 3.25 (s, 3H), 3.23 (s, 3H), 2.27 (s, 3H), 1.45 (s, 6H), 1.44 (s, 6H).

**(1*S*,3'*R*,4'*S*,5'*R*,6'*R*)-3',4',5'-tris(benzyloxy)-5,6'-dimethyl-3',4',5',6'-tetrahydro-3*H*-spiro[isobenzofuran-1,2'-pyran]-6-carbaldehyde (85).** Compound **85** was prepared from **49** and **83** according to the procedure described for **54** in 42% yield as a white solid (3 steps). $^1$H NMR (400 MHz, Chloroform-*d*) $\delta$ 10.05 (s, 1H), 7.43 (s, 1H), 7.38 – 7.28 (m, 10H), 7.17 – 7.03 (m, 4H), 6.84 – 6.76 (m, 2H), 5.20 (s, 2H), 5.00 – 4.86 (m, 3H), 4.73 (d, *J* = 10.9 Hz, 1H), 4.65 (d, *J* = 11.4 Hz, 1H), 4.26 (d, *J* = 11.4 Hz, 1H), 4.13 (t, *J* = 9.3 Hz, 1H), 4.09 – 4.00 (m, 1H), 3.91 (d, *J* = 9.5 Hz, 1H),

3.35 (t, *J* = 9.4 Hz, 1H), 2.69 (s, 3H), 1.25 (d, *J* = 6.3 Hz, 3H). LRMS (ESI, *m/z*): 565 $[M+H]^+$

**(1*S*,3'*R*,4'*S*,5'*S*,6'*R*)-6-((5-ethylthiophen-2-yl)methyl)-5,6'-dimethyl-3',4',5',6'-tetrahydro-3*H*-spiro[isobenzofuran-1,2'-pyran]-3',4',5'-triol (39).**

Compound **39** was prepared from **85** and 2-ethylthiophene according to the procedure described for **9** in 36% yield (3 steps) as a white solid. HPLC purity: 95.51%. mp 139–141 °C. $^1$H NMR (500 MHz, Methanol-$d_4$) $\delta$ 7.14 (s, 1H), 7.10 (s, 1H), 6.56 (d, *J* = 3.4 Hz, 1H), 6.50 (d, *J* = 3.4 Hz, 1H), 5.13 – 5.04 (m, 2H), 4.10 (s, 2H), 3.92 – 3.83 (m, 1H), 3.78 – 3.65 (m, 2H), 3.20 – 3.11 (m, 1H), 2.75 (q, *J* = 7.5 Hz, 2H), 2.31 (s, 3H), 1.27 – 1.16 (m, 6H). $^{13}$C NMR (125 MHz, Methanol-$d_4$) $\delta$ 146.9, 141.9, 140.6, 139.7, 139.1, 138.0, 125.7, 124.0, 123.9, 123.5, 111.6, 77.4, 76.2, 75.3, 73.4, 71.4, 34.9, 24.3, 19.9, 18.2, 16.5. LRMS (ESI, *m/z*): 391 $[M+H]^+$; HRMS (ESI) cacld for $C_{21}H_{27}O_5S$ $[M+H]^+$: 391.1574; found: 391.1573.

**Pharmacology**

***In Vitro* SGLT Inhibition Assay**

**Cell Line Culture and Generation of hSGLT1 or hSGLT2-Expressing Cell Lines.**

NIH3T3 cells were cultured at 37 $^o$C in DMEM supplemented with 25 mmol/L glucose and 10% FBS (DMEM-HG-10% FBS). Human SGLT1 and SGLT2 cDNAs were amplified by PCR and subcloned as EcoR I-Sal I fragments into the pBABE-puro expression vector and sequences were confirmed by DNA sequencing. Then, NIH3T3 cells were infected with retroviral containing full-length hSGLT1 or hSGLT2. For infection, cells were plated with the viral supernatant supplemented with 8 µg/mL polybrene and incubated in 5% $CO_2$ at 37 $^o$C for 24 h. After infection, transduced cells were selected using puromycin (2 µg/mL) for 2 weeks to generate stable cell lines.

**Radioactive Flux Measurements**

NIH3T3 cells stably overexpressing human SGLT1 or SGLT2 were plated into

96-well IsoPlate (PerkinElmer) at $3.0\times10^5$ cell /well for 2 days prior to the assay. Then the medium was changed to DMEM-HG-10% FBS containing 2 mmol/L sodium butyrate on the second day. For uptake assay, cells were washed three times with 100 μL uptake buffer (137 mmol/L NaCl, 5.4 mmol/L KCl, 2.8 mmol/L $CaCl_2$, 1.2 mmol/L $MgCl_2$, 10 mmol/L tris(hydroxymeth-yl)aminomethane/ *N*-2-hydroxyethylpiperazine-*N*-ethane sulfonic acid, pH 7.2)[44], and incubated with 100 μL sodium buffer for 30 min. Cells were moved to 60 μL uptake buffer containing tested compounds and 1.67 mmol/L (0.05 μCi per well) methyl-a-D-[U-$^{14}$C]glucopyranoside (PerkinElmer) and incubated at 37 $^{o}$C for 1 h. Cells were washed three times with 100 μL cold PBS (137 mmol/L NaCl, 2.7 mmol/L KCl, 10 mmol/L $Na_2HPO_4{\cdot}12H_2O$, 2 mmol/L $KH_2PO_4$) and dissolved with 50 μL 1‰ Triton-X 100 for 10 min. 150 μL scintillation solution was added to measure their radioactivity by scintillation counter (PerkinElmer 1450-023). Uptake buffer without tested compounds and sodium-free uptake buffer (137 mmol/L *N*-methyl-glucamine, 5.4 mmol/L KCl, 2.8 mmol/L $CaCl_2$, 1.2 mmol/L $MgCl_2$, 10 mmol/L Tris/Hepes, pH 7.2)[44] instead of sodium buffer were used as negative control and blank control.

### *In Vivo* Study

#### Animals and Groups

Male C57BL/6J mice and male SD rats were bought from Shanghai Slac Laboratory Animal Co., Ltd.. All were bred at a SPF animal room (temperature: 22-24□, humidity: 45-80%, lighting: 150-300 lx with 12-hour day and night alternate) in Shanghai Insitute of Materia Medica and maintained with free access to water and food. Animal experiments were approved by the Animal Care and Use Committee, Shanghai Institute of Materia Medica. C57BL/6J mice and SD rats were randomized to various treatment groups by body weight and postprandial blood glucose (PBG) levels at about 9 weeks of age.

#### Urinary Glucose Excretion Assay

At about 9 weeks of age, the animals were randomized by body weight to receive

a single dose of vehicle (0.9% NaCl solution containing 0.5% methyl cellulose, n = 9 for mice and n = 6 for rats) or indicated dosage of compounds (1 mg/kg, n = 9 for mice and n = 6 for rats) by gavage and housed alone in metabolic cages (Tecniplast) for 24 h. After the urine of each mouse or rat was collected and its volume measured, an aliquot of each urine sample was collected and stored at -20□ until use. The urinary glucose concentration was determined by a kit based on the glucose-oxidase method (Shanghai Mind Biological Engineering Co., Ltd.) in a SpectraMax M5 microplate reader (Molecular Devices) and the urinary glucose excretion were calculated as follow:

UGE (mg/100 g) = 18.016 × the urine volume (mL) × the glucose concentration (mM)/body weight (g).

**Oral Glucose Tolerance Test**

After overnight fasting (14 h), SD rats or C57BL/6J mice were randomized to receive 0.9% NaCl solution containing 0.5% methyl cellulose as vehicle control group or indicated dosage of **39** or tofogliflozin by gavage, followed by glucose overloading at 3 g/kg by gavage after 2 hours. Blood glucose was measured before grouping and at 0, 15, 30, 60, 90, and 120 minute after glucose overload.

**Statistical Analysis**

All results are showed as mean ± SEM. Differences between two groups were analyzed by two-tails *t*-test. Differences among various groups were compared by one-way ANOVA with Dunnett's correction. Only comparisons among various groups in fasting blood glucose and postprandial blood glucose during 5-week administration were performed by two-way ANOVA with Dunnett's correction. $P < 0.05$ was considered to be statistically significant.

## ■ ASSOCIATED CONTENT

**Supporting Information**

Copies of $^{1}H$ and $^{13}C$ NMR spectra and HPLC experiments. (PDF)

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**Notes**

The authors declare no competing financial interest.

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## ■ ABBREVIATIONS USED

SGLT, sodium-dependent glucose cotransporter; T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus; GDM, gestational diabetes mellitus; SAR, structure-activity relationship; UGE, urinary glucose excretion; SD, Sprague–Dawley; PK, pharmacokinetics; $IC_{50}$, half-maximal inhibitory concentration; THF, tetrahydrofuran; DMF, *N*,*N*-dimethylformamide; DMSO, dimethyl sulfoxide; TBAF, tetrabutyl ammonium fluoride; AIBN, 2,2-azobisisobutyronitrile; DMAP,

dimethylaminopyridine; IDF, International Diabetes Federation; DM, diabetes mellitus .

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