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Novel macrocyclic *C*-aryl glucoside SGLT2 inhibitors as potential antidiabetic agents

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This paper is dedicated to Professor Eun Lee on the occasion of his retirement from Seoul National University in August 2011.

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ABSTRACT

Novel macrocyclic *C*-aryl glucoside SGLT2 inhibitors were designed and synthesized. Two different synthetic routes of macrocyclization were adopted to prepare novel ansa SGLT2 inhibitors. Among the compounds tested, [1,7]dioxacyclopentadecine macrocycles possessing methylthiophenyl at the distal ring **40** or ethoxyphenyl at the distal ring **23** showed the best in vitro inhibitory activity in this series to date (**40**, IC_{50} = 0.778 nM and **23**, IC_{50} = 0.899 nM) against *h*SGLT2.

1. Introduction

Diabetes has become an augmenting concern to the world's population. In 2007, approximately 246 million people were influenced by the disease, with an additional 7 million people catching the disease every year.[1] The ADA states, "Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels."[19]. There are two identified form of diabetes: Type 1 diabetes is distinguished as an autoimmune disease involving pancreatic β-cells, while type 2 diabetes is associated with β-cell dysfunction[2] and is defined by a defect in glucose regulation and metabolism. Type 2 diabetes is the most dominant disorder of glucose homeostasis, accounting for nearly 90–95% of all cases of diabetes. While diabetes is often a result of impared insulin secretion or action, the actual measure of diabetes relates to glucose levels.

Sodium-dependent glucose cotransporters (SGLTs) couple the transport of glucose against a concentration gradient with the simultaneous transport of Na^+ down a concentration gradient.[3] Two important SGLT isoforms have been cloned and identified, SGLT1 and SGLT2.[4] SGLT1 is typically in the GI tract of humans and the GI tract represents a major locus of action of SGLT1 in glucose trafficking. Also, SGLT1 is a high-affinity, low-capacity transporter and therefore accounts for only a small fraction of renal glucose reabsorption.[6] In contrast, SGLT2 is a low-affinity, high-capacity transporter located exclusively at the apical domain of the epithelial cells in the early proximal convoluted tubule. It is estimated that 90% of renal glucose reabsorption is facilitated by SGLT2; the remaining 10% is likely mediated by SGLT1 in the late proximal straight tubule.[7] Since SGLT2 appears to be responsible for the majority of renal glucose reabsorption based on human mutation studies,[8] it has become a target of therapeutic interest.

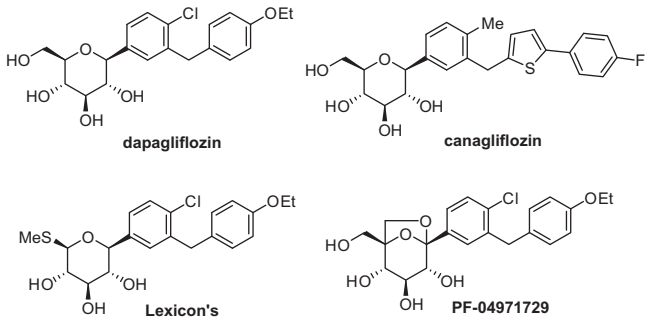

Figure 1. Structures of C-aryl glucoside SGLT2 inhibitors.

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Bristol–Myers Squibb has identified dapagliflozin (Fig. 1), a potent, selective SGLT2 inhibitor for the treatment of type 2 diabetes.[9–11] At present, dapagliflozin is the most advanced SGLT2 inhibitor in clinical trials. On the other hand, Mitsubishi Tanabe, in collaboration with Johnson & Johnson, is developing canagliflozin **2**, another novel C-glucoside-derived SGLT2 inhibitor.[12] In addition, Boehringer Ingelheim (BI 10773), Lexicon (LX4211), Astellas (ASP1941), and Pfizer (PF-04971729) are reported to be in various phase of clinical trials.[13] Our efforts on identifying inhibitors that target SGLT2 have been previously described.[14] One of our most recent efforts involved exploration of ansa-structure **A** of *C*-aryl glucoside SGLT2 inhibitors (Fig. 2). We performed synthesis of

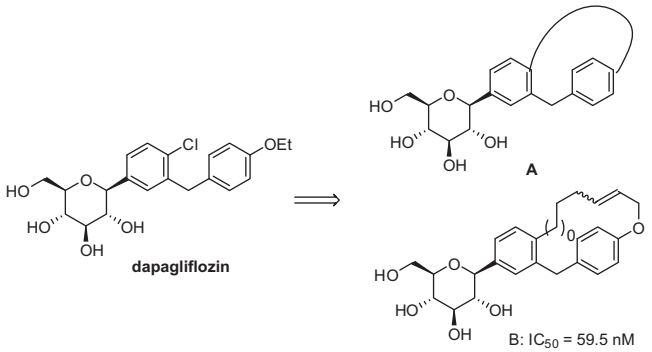


Figure 2. Glucosides with cyclic diarylpolynoid as SGLT2 inhibitor.

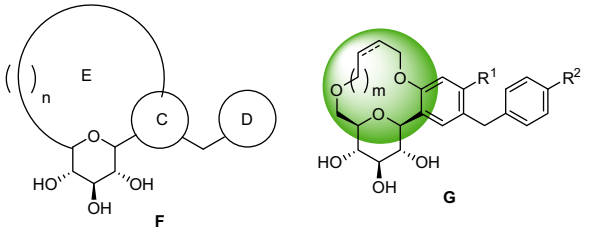


Figure 3. Exploration of C-glucoside conjoining the proximal ring with a handle of glucose ring.

C-glucosides associated with cyclic diarylpolynoid utilizing versatile organozinc chemistry and subsequent ring-closing olefin metathesis using 2nd generation Grubbs catalyst. The synthesized ansa-analogs showed the modest in vitro inhibitory activity against *h*SGLT2 (**B**, IC_{50} = 59.5 nM).[15]

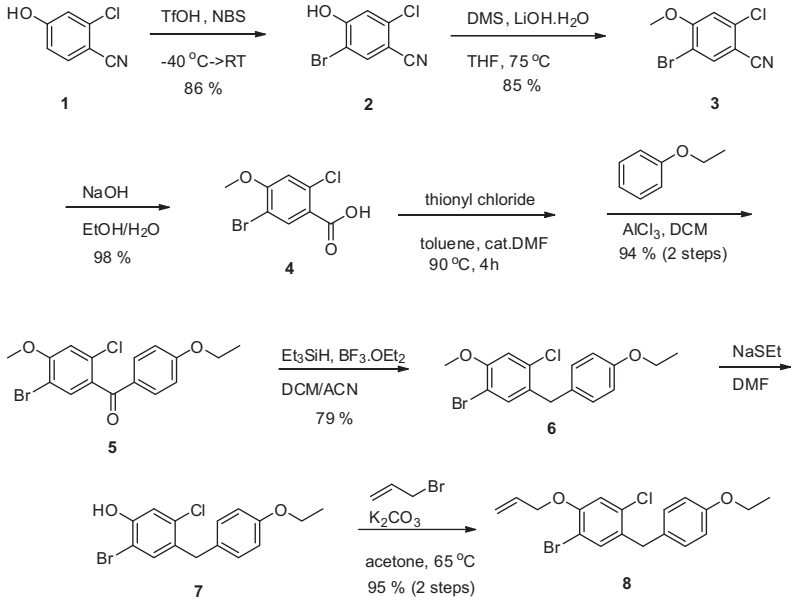
In the present study, novel macrocyclic C-glucosides connecting carbohydrate and the proximal ring were exploited in order to develop novel SGLT2 targeting antidiabetic agents. Theracos has shown that the O-spiroketal *C*-aryl glucosides and *C*-spiro *C*-aryl glucosids are potent SGLT2 inhibitors and introduction of a non-cyclized substituent at 6-position of the proximal aryl ring not only improved potency but also provided high selectivity toward SGLT2.[18] Adopting these results and ring closing strategy used in a variety of medicinal chemistry practices, we envisioned that conjoining the proximal ring of dapagliflozin with a handle of glucose ring as shown in **F** (Fig. 3) would be a valuable approach for modulating physicochemical property, and possibly improving biological activity. Thus, we decided to test this hypothesis.

To be best of our knowledge, this would be the first example of macrocyclic SGLT2 inhibitor formed by connecting glucose and proximal phenyl ring. For this purpose, structure of dapagliflozin was modified into **G** forming macrocycle between C-6 position of carbohydrate and *ortho*-position at the proximal ring as shown in Figure 3. Subsequently, we envisioned that macrocyclic ring could be obtained by ring-closing olefin metathesis using Grubbs catalyst or simple intramolecular alkylation using hydroxyl group as a nucleophile and alkyl halide as an electrophile at the other side of the molecule. Herein, we report design, synthesis and biological evaluation of novel C-glucoside macrocyclic congeners.

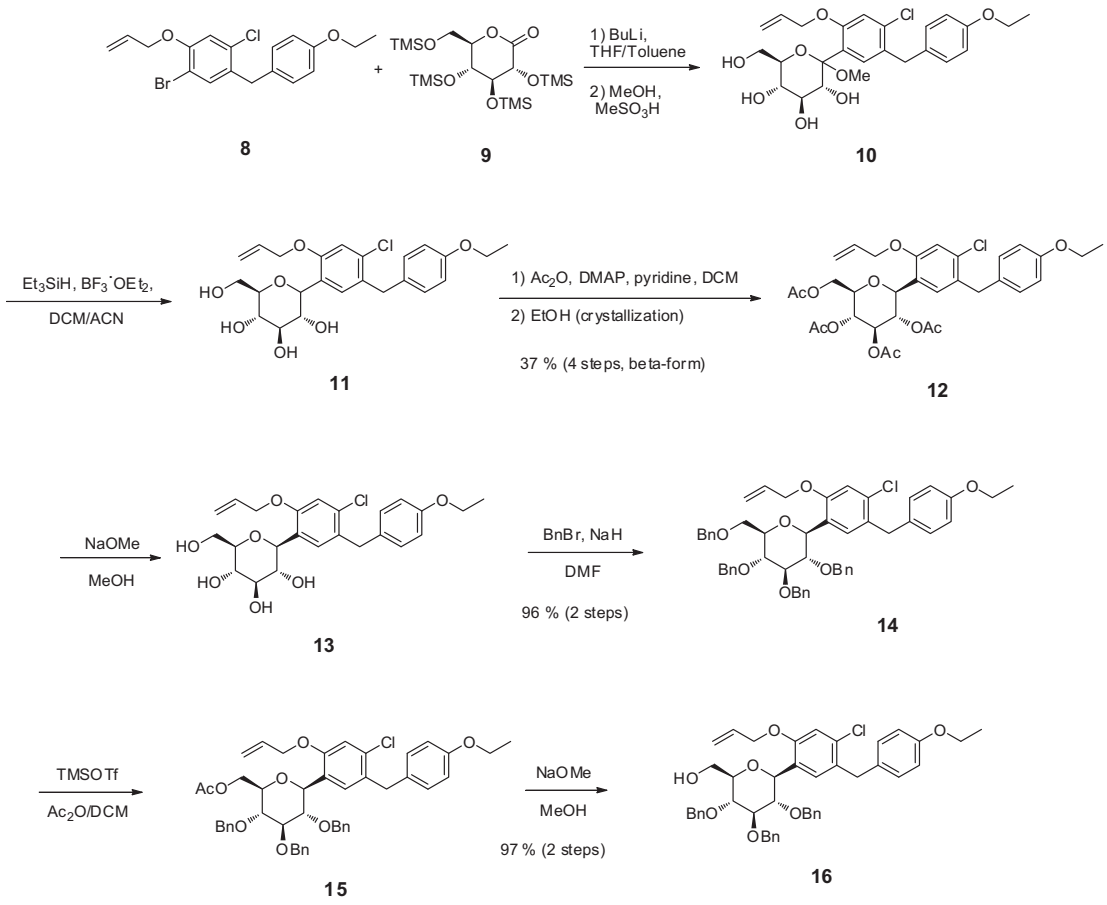
2. Results and discussion

2.1. Chemistry

As shown in Scheme 1, preparation of the target compounds commenced with preparation of aglycone **8**. Thus, 2-chloro-4-hydroxybenzonitrile (**1**) was brominated selectively to produce structure of **2** with NBS and trifluoromethanesulfonic acid. The structure of **2** was methylated with dimethylsulfate and LiOH in a suitable solvent such as THF to provide **3**. Treatment of **3** with NaOH in aqueous EtOH, followed by thionyl chloride produced



Scheme 1. Preparation of intermediate **8**. TfOH: trifluoromethanesulfonic acid; DMS: dimethylsulfate; DCM: dichloromethane; ACN:acetonitile; DMF : *N*,*N*-dimethylamide.



Scheme 2. Preparation of advanced intermediate **16**.

the corresponding acyl chloride, which was directly reacted with ethoxybenzene and aluminum chloride in dichloromethane to generate ketone **5**. Subsequent reduction of ketone **5** with triethylsilane in the presence of boron trifluoride diethyl etherate produced diphenylmethane **6**. The requisite aglycone **8** was produced with demethylation by sodium ethanethiolate in DMF and the subsequent allylation with allyl bromide and potassium carbonate in acetone.

Preparation of the key intermediate **16** was accomplished as shown in Scheme 2. Thus, lithiation and subsequent etherification produced anomeric mixture **10** with concomitant desilylation (Scheme 2). Reduction of **10** with triethylsilane in the presence of boron trifluoride diethyl etherate, and subsequent acetylation of the resulting compound, followed by crystallization in EtOH generated beta-anomeric tetraacetate **12**. Switching its acetyl groups to benzyl groups was accomplished by hydrolysis with sodium methoxide in MeOH, and the subsequent benzylation. The requisite alcohol **16** was obtained in 97% yield overall by (i) the selective acetylation of **14** into acetate **15** with TMSOTf and acetic anhydride, and (ii) the subsequent hydrolysis.

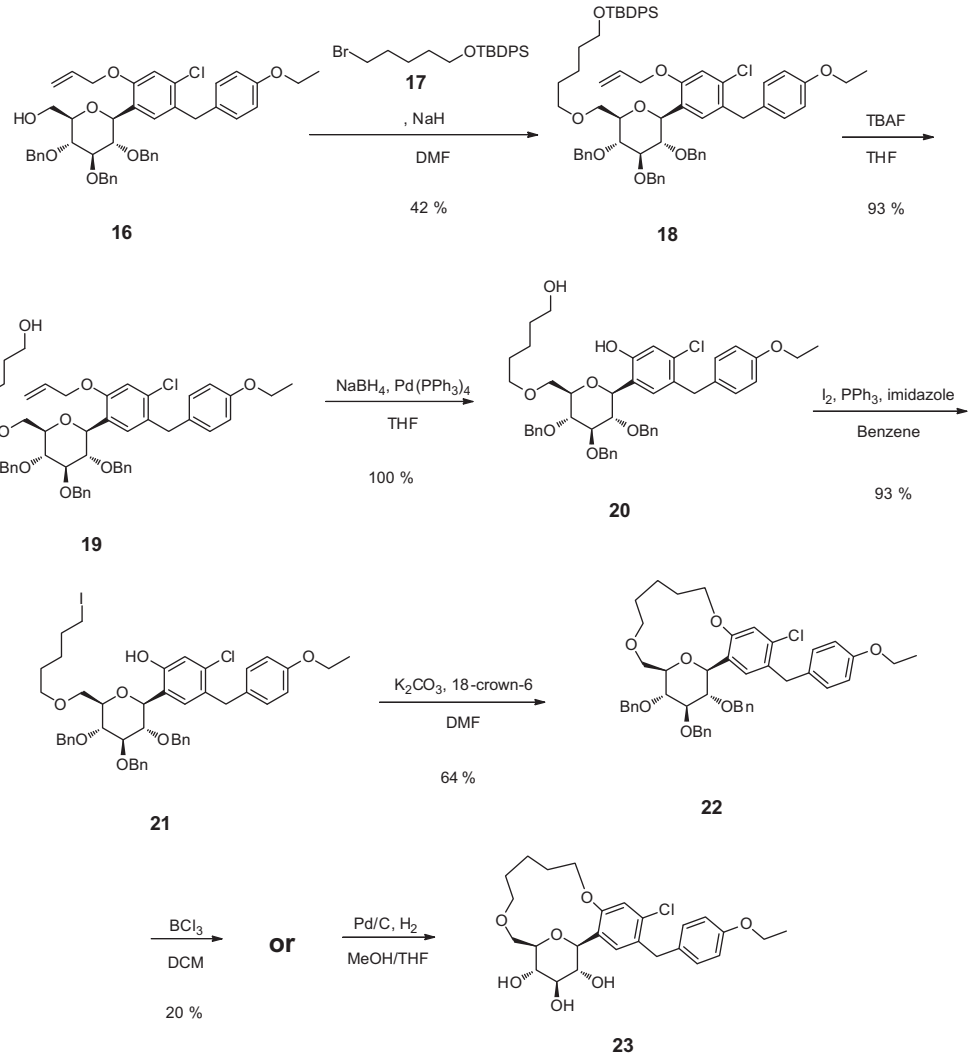
Alkylation of alcohol **16** with (5-bromopentyloxy)(*tert*-butyl)-diphenylsilane (**17**) in the presence of sodium hydride in DMF produced **18** in 42% yield. Desilylation of **18** with TBAF gave alcohol **19** in 93% yield. Removal of the allyl group was carried out smoothly using $NaBH_4$ in the presence of tetrakis[triphenylphosphine]palladium(0) to give phenol **20** in quantitative yield. The primary alcohol of **20** was transformed into the corresponding iodide **21** by action of iodine, triphenylphosphine, and imidazole in benzene. The iodide underwent macrocyclization under conditions of potassium carbonate and 18-crown-6 in DMF. Removal of the benzyl groups on the carbohydrate moiety proceeded with either BCl_3 in methylene chloride or hydrogenolysis on Pd/C in MeOH and THF to produce the target compound **23** (Scheme 3).

Another approach toward macrocyclization involves ring-closing olefin metathesis (RCM) as described in Scheme 4. Thus, compound **13** was treated with 3,3-dimethoxyprop-1-ene in the presence of CSA (10-camphorsulfonic acid) in DMF to provide the 2-vinyl-1,3-dioxane **24**. Selective and reductive ring opening using TfOH (trifluoromethanesulfonic acid) and sodium cyanoborohydride produced compound **25**. Ring-closing olefin metathesis was undergone with divinyl intermediate **26** using Grubbs 2nd generation catalyst in moderate yield. Finally, removal of acetyl groups was accomplished using sodium methoxide in methanol to generate the target compound **28**.

Another way performing RCM is described in Scheme 5. Thus, compound **16** prepared in Scheme 2, was allylated (allyl bromide, sodium hydride, DMF) to produce diene **29**. Ring-closing metathesis (RCM) using Grubbs 2nd generation catalyst afforded macrocycle **30** along with recovered **29**. Finally, subjection of **30** to hydrogen atmosphere on Pd/C in a mixture of MeOH and THF produced the target compound **31** in 22% yields.

2.2. Structure–activity relationship studies

The cell-based SGLT2 AMG (Methyl-α-D-glucopyranoside) inhibition assay was performed to evaluate the inhibitory effects of all prepared compounds on *h*SGLT2 activities.[16,17] Exploration of the SAR began by connecting *ortho*-position on the proximal phenyl ring of dapagliflozin with C-6 position of glucose moiety. These results are shown in Table 1. Synthesis of the series started from macrocyclic ring with $m = 2$ (in C_mH_n) and R_1 = OEt, since macrocyclic ring with $m = 1$ can be considered to be chemically labile.

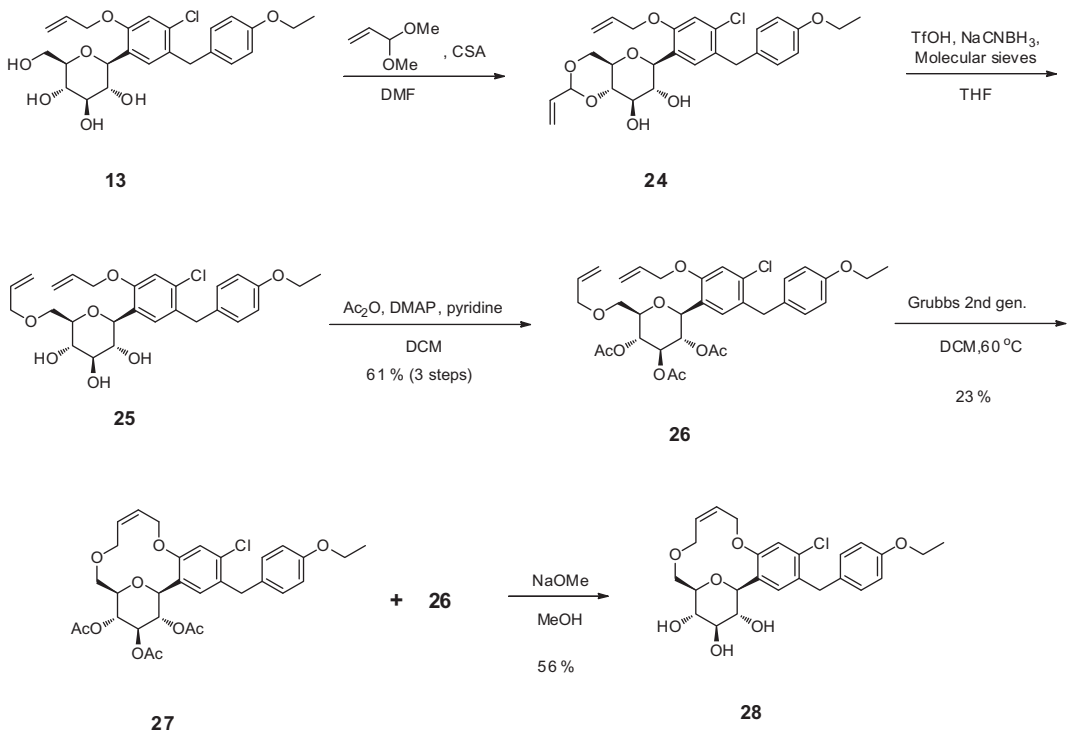


Scheme 3. Synthesis of precursor **21** and subsequent key intramolecular cyclization.

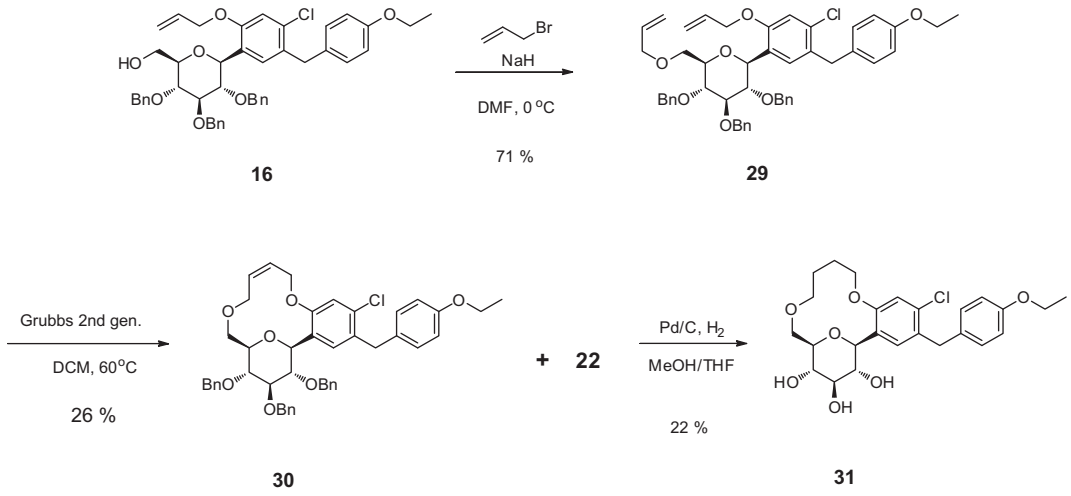
To our delight, the first compound we prepared in this series turned out to be as good as dapagliflozin in terms of inhibitory activity against *h*SGLT2 (**32**, IC_{50} = 1.34 nM vs dapagliflozin, IC_{50} = 1.35 nM), suggesting that C-6 OH on glucose is not necessarily critical for inhibitory activity against *h*SGLT2 as long as the molecule is poised to adopt favorable conformation for optimal inhibitory activity. As we tested macrocycle homologated by one more carbon (m = 3) in this series, the compound proved to be twofold less active (**33**, IC_{50} = 2.95 nM). One more homologation **31** slightly improved in vitro inhibitory activity, but failed to reach the same level of activity as that of compound **32** (**31**, IC_{50} = 2.27 nM). However, one more homologation into [1,7]dioxacyclopentadecine **23** appears to provide better inhibitory activity against *h*SGLT2 (IC_{50} = 0.899 nM). The higher homolog (m = 6) becomes less potent, the compound become less inhibitory against *h*SGLT2. The observations are more clearly described in Figure 4. This is a kind of combination of bell-shaped curve and zig–zag curve depending on the number of the bridging carbon atoms. Interestingly, the olefinic macrocycle **28** demonstrated subnanomolar inhibitory activity against *h*SGLT2, showing the molecule can adopt appropriate conformation with the olefinic character in the macrocycle. Next, the compounds with R_1 = Et on the distal ring were tested to examine if the particular modification would influence the inhibitory activity against *h*SGLT2. As shown in Table 1, regardless of macrocycle ring size (m = 3–6), the compounds in the series show consistently good activity, displaying IC_{50} = 1.38–1.59 nM (**35**–**38**). Further investigation was carried out to other substituents such as R_1 = SMe or R_1 and R_2 connected by 1,4-dioxane bridge. For both cases, the similar observations were made as described previously in 4-OEt or 4-Et series. Thus, [1,7]dioxacyclopentadecine compounds with m = 5 showed the excellent activity, exhibiting IC_{50} = 0.778 nM (**40**) and IC_{50} = 1.38 nM (**42**), respectively. On the basis of the excellent in vitro inhibitory data against *h*SGLT2, compound **23** and **37** were evaluated briefly for its potential to induce UGE (urine glucose excretion) in vivo. UGE values of dapagliflozin, **23**, and **37** in normal SD rats were 1648 ± 228 mg/200 g body weight, 226 ± 97 mg/200 g body weight, and 116 ± 50 mg/200 g body weight at a single oral dose of 1 mg/kg, respectively. Thus, it is concluded that the decreased in vivo efficacy of the current macrocycle SGLT2 inhibitors **23** and **37** compared with dapagliflozin, should be attributed to the poor pharmacokinetic properties, since there appears to be no significant difference in inherent in vitro potencies.

3. Conclusion

In the present study, novel macrocyclic *C*-aryl glucoside SGLT2 inhibitors were designed and synthesized. We envisioned that conjoining the proximal ring of dapagliflozin with a handle of



Scheme 4. Preparation of diene precursor **26** and Grubbs catalyst-mediated macrocyclization.



Scheme 5. Preparation of diene precursor **29** and Grubbs catalyst-mediated macrocyclization.

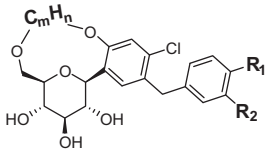
glucose ring would be a valuable approach for modulating physicochemical property, and possibly improving biological activity. We trust that this would be the first example of macrocyclic SGLT2 inhibitor formed by connecting glucose and proximal phenyl ring. For this purpose, structure of dapagliflozin was modified into macrocycles between C-6 position of carbohydrate and *ortho*-position at the proximal ring. Two different synthetic routes of macrocyclization such as ring-closing olefin metathesis using Grubbs catalyst and simple intramolecular alkylation using hydroxyl group as a nucleophile and alkyl halide as an electrophile at the other side of the molecule, were adopted to prepare novel macrocyclic SGLT2 inhibitors. Among the compounds tested, [1,7]dioxacyclopentadecine macrocycles possessing ethylphenyl at the distal ring **40** or ethoxyphenyl at the distal ring **23** showed the best in vitro inhibitory activity in this series to date (**40**, IC_{50} = 0.778 nM and **23**, IC_{50} = 0.899 nM) against *h*SGLT2. The promising profile of **23** prompted evaluation of its potential to induce UGE in vivo, but **23** turned out to show only moderate in vivo UGE value.

4. Experimental

4.1. General methods

All reactions are conducted under an inert atmosphere at room temperature, unless otherwise noted. All reagents were purchased at the highest commercial quality and used without further purification, unless otherwise indicated. Microwave reaction was conducted with a Biotage Initiator microwave reactor. NMR spectra were obtained on a Varian 400-MR (400 MHz ^{1}H, 100 MHz ^{13}C) spectrometer. NMR spectra were recorded in ppm (δ) relative to

Table 1
In vitro inhibitory activity against *h*SGLT2. Ref. Dapagliflozin (1.35 ± 0.15)[a]



Compd	R_1, R_2	C_mH_n	*h*SGLT2 IC_{50}[b] (nM)	Compd	R_1, R_2	C_mH_n	*h*SGLT2 IC_{50}[b] (nM)
32[c]	OEt, H	C_2H_4	1.34	**36**[c]	Et, H	C_4H_8	1.40
33[c]	OEt, H	C_3H_6	2.95	**37**[c]	Et, H	C_5H_{10}	1.38
28	OEt, H	C_4H_6	0.974	**38**[c]	Et, H	C_6H_{12}	1.40
31	OEt, H	C_4H_8	2.27	**39**[c]	SMe, H	C_4H_8	1.35
23[c]	OEt, H	C_5H_{10}	0.899	**40**[c]	SMe, H	C_5H_{10}	0.778
34[c]	OEt, H	C_6H_{12}	3.63	**41**[c]	1,4-Dioxane	C_4H_8	3.65
35[c]	Et, H	C_3H_6	1.59	**42**[c]	1,4-Dioxane	C_5H_{10}	1.38

[a] This data was obtained by multiple determinations.
[b] These data were obtained by single determinations.
[c] These compounds were synthesized by the same procedure shown in Scheme 3.

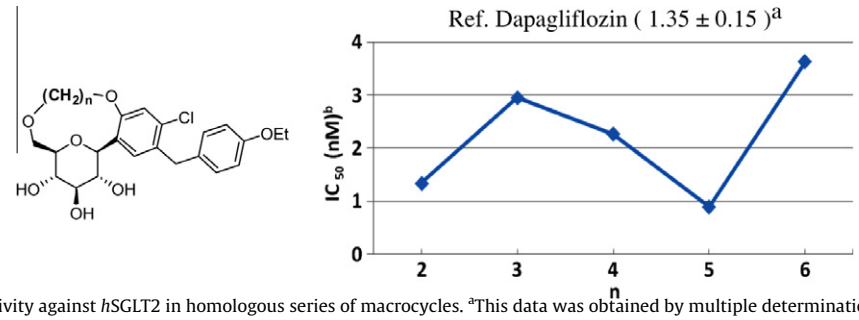


Figure 4. In vitro inhibitory activity against *h*SGLT2 in homologous series of macrocycles. [a]This data was obtained by multiple determinations. [b]These data were obtained by single determinations.

tetramethylsilane (δ = 0.00) as an internal standard unless stated otherwise and are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, quint = quintet, sext = sextet, m = multiplet, and br = broad), coupling constant, and integration. ^{13}C NMR spectra were referenced to the residual chloroform-d_1 (δ = 77.0) or DMSO-d_6 (δ = 39.7). Mass spectra were obtained with an Agilent 6110 quadruple LC-MSD (ESI^+). High resolution mass spectra were obtained on a Jeol JMS-700 Mstation (10 kV, HFAB). Optical rotations were obtained on a Rudolph Autopol III digital polarimeter. Preparative HPLC purifications were performed on a Gilson purification system. For preparative HPLC, ca. 100 mg of a product was injected in 1 mL of methanol onto a SunFire Prep C18 OBD 5 μm 30 × 100 mm Column with a 30 min gradient from 5% to 90% acetonitrile in water and a 45 mL/min flow rate. Biotage SP1 and Isolera purification systems were used for normal phase column chromatography with ethyl acetate and hexane. Flash chromatography was performed using E. Merck 230–400 mesh silica gel according to the procedure of Still et al. Reactions were monitored by either thin-layer chromatography (TLC) on 0.25 mm E. Merck silica gel plates (60F-254) using UV light and *p*-anisaldehyde solution as visualizing agents or HPLC analysis on an Agilent 1200 series system.

4.2. Chemistry

4.2.1. Preparation of intermediate 8

4.2.1.1. 5-Bromo-2-chloro-4-hydroxybenzonitrile (2). To a solution of 2-chloro-4-hydroxybenzonitrile (**1**, 10 g, 65 mmol) in acetonitrile (200 mL) at −30 °C was added dropwise trifluoromethanesulfonic acid (10 mL, 71 mmol). The solution is stirred for 10 min at −30 °C, before adding of *N*-bromosuccinimide (16.2 g, 91 mmol). After 18 h stirring at ambient temperature, the solution was quenched with aqueous saturated sodium hydrogen carbonate. The organic layers was extracted with ethyl acetate two times, washed with brine, dried over magnesium sulfate, filtered and concentrated in vacuo. The resulting crude residue was purified on a Biotage® purification apparatus (silica gel, 5–40% ethyl acetate in hexanes gradient) to yield the title compound (13 g, 56 mmol, 86%) as a white solid. ^1H NMR (400 MHz, CD_3OD) δ 7.84 (s, 1H), 6.99 (s, 1H); MH^+ 232.

4.2.1.2. 5-Bromo-2-chloro-4-methoxybenzonitrile (3). To a solution 5-bromo-2-chloro-4-hydroxybenzonitrile (**2**, 29 g, 124 mmol) in tetrahydrofuran (500 mL) was added lithium hydroxide monohydrate (6.7 g, 161 mmol) and dimethyl sulfite (15.2 mL, 161 mmol). The resulting mixture was heated at 75 °C for 5 h, re-cooled to room temperature, and quenched with water. The mixture was extracted with ethyl acetate and the organic layer was washed with brine, dried over magnesium sulfate, filtered and evaporated in vacuo. The crude residue was purified on Biotage® purification apparatus (silica gel, 3–10% ethyl acetate in hexanes) to yield the title compound (26 g, 104 mmol, 85%) as a white solid. ^1H NMR (400 MHz, $CDCl_3$) δ 7.82 (s, 1H), 7.00 (s, 1H), 3.98 (s, 3H).

4.2.1.3. 5-Bromo-2-chloro-4-methoxybenzoic acid (4). To a solution of 5-bromo-2-chloro-4-methoxybenzonitrile (**3**, 30.6 g, 124 mmol) in ethanol (450 mL)/H_2O (225 mL) was added sodium hydroxide (124 g, 3.1 mol). The solution was refluxed at 100 °C overnight, cooled to room temperature and evaporated ethanol.

The aqueous layer was cooled to 0 °C, acidified with concentrated hydrogen chloride (190 mL). The generated white solid was filtered, washed with water and dried in vacuo to yield the title compound (32 g, 122 mmol, 98%) as a white solid.

4.2.1.4. (5-Bromo-2-chloro-4-methoxyphenyl)(4-ethoxyphenyl)-methanone (5). To a solution of 5-bromo-2-chloro-4-methoxybenzoic acid (**4**, 15 g, 56.5 mmol) in toluene (72 mL) was added thionyl chloride (8.24 mL, 113 mmol) and *N,N*-dimethylformamide (0.1 mL). The solution was refluxed at 90 °C for 4 h, cooled to room temperature and evaporated toluene and residual reagent. The obtained acyl chloride was diluted with dichloromethane (240 mL) and added portionwise aluminum chloride (8.3 g, 62.2 mmol) and phenetole (7.2 mL, 56.5 mmol) at 0 °C. The reaction mixture was stirred at room temperature overnight, quenched with 1 N HCl (15 mL) and H_2O (15 mL). The organic layer was extracted with dichloromethane two times, washed with 1 N HCl and brine, dried over magnesium sulfate, filtered and concentrated in vacuo to yield the title compound as a yellowish solid which was used without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (d, J = 9.2 Hz, 2H), 7.57 (s, 1H), 6.94 (s, 1H), 6.92 (d, J = 9.6 Hz, 2H), 4.11 (q, J = 7.2 Hz, 2H), 3.96 (s, 3H), 1.43 (t, J = 6.4 Hz, 3H); MH^+ 368.

4.2.1.5. 1-Bromo-4-chloro-5-(4-ethoxybenzyl)-2-methoxybenzene (6). To a stirred −10 °C solution of (5-bromo-2-chloro-4-methoxyphenyl)(4-ethoxyphenyl)methanone (54.3 mmol) from Step 4 in dichloromethane (150 mL)/acetonitile (150 mL) was added triethylsilane (20 mL, 109 mmol) followed by boron trifluoride diethyl etherate (16 mL, 109 mmol) at −10 °C. The solution was allowed to warm to 0 °C over 2 h prior to quenching with saturated sodium carbonate solution. After removal of organic volatiles under reduced pressure, the residue was partitioned between ethyl acetate and water. Following extraction of the aqueous layer with ethyl acetate, the combined organic layers were washed with water prior to drying over magnesium sulfate. Filtration and concentration under reduced pressure yield the title compound as a yellowish solid which was used without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29 (s, 1H), 7.07 (d, J = 8.8 Hz, 2H), 6.90 (s, 1H), 6.83 (d, J = 8.8 Hz, 2H), 4.00 (q, J = 6.8 Hz, 2H), 3.93 (s, 2H), 3.87 (s, 3H), 1.40 (t, J = 6.8, 3H).

4.2.1.6. 2-Bromo-5-chloro-4-(4-ethoxybenzyl)phenol (7). To a solution of 1-bromo-4-chloro-5-(4-ethoxybenzyl)-2-methoxybenzene (**6**, 11.3 mmol) in *N,N*-dimethylformamide (50 mL) was added sodium ethane thiolate (3.17 g, 34 mmol) and heated at 90 °C for 3 h. The reaction mixture was cooled to 0 °C, neutralized with 1 N HCl, and the organic layer was separated and the aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over magnesium sulfate, filtered and concentrated in vacuo to yield the title compound as a yellow oil which was used without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26 (s, 1H), 7.07 (d, J = 8.4 Hz, 2H), 7.06 (s, 1H), 6.83 (d, J = 8.4 Hz, 2H), 4.01 (q, J = 6.8 Hz, 2H), 3.93 (s, 2H), 1.40 (t, J = 6.8 Hz, 3H).

4.2.1.7. 1-(Allyloxy)-2-bromo-5-chloro-4-(4-ethoxybenzyl)benzene (8). To a solution of 2-bromo-5-chloro-4-(4-ethoxybenzyl)phenol (**7**, 55 mmol) in acetone (180 mL) was added potassium carbonate (15.2 g, 110 mmol) and allyl bromide (7 mL, 83 mmol) and heated at 65 °C for 3 h. The insoluble material was removed by filter and the filtrate was evaporated under reduced pressure. The crude residue was purified on Biotage® purification apparatus (silica gel, 3–10% ethyl acetate in hexanes) to yield the title compound (21 g, 52 mmol, 95%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29 (s, 1H), 7.07 (d, J = 8.4 Hz, 2H), 6.90 (s, 1H), 6.83 (d, J = 8.0 Hz, 2H), 6.09–5.99 (m, 1H), 5.47 (d, J = 17.2 Hz, 1H), 5.33 (d, J = 10.4 Hz, 2H), 4.57 (d, J = 4.8 Hz, 2H), 4.01 (q, J = 6.8 Hz, 2H), 3.93 (s, 2H), 1.40 (t, J = 6.8, 3H).

4.2.2. Preparation of intermediate 21

4.2.2.1. (2*R*,3*R*,4*R*,5*S*,6*S*)-2-(Acetoxymethyl)-6-(2-(allyloxy)-4-chloro-5-(4-ethoxybenzyl)phenyl)tetrahydro-2*H*-pyran-3,4,5-triyl triacetate (12). To a solution of (**8**, 10.2 g, 27 mmol) in tetrahydrofuran (25 mL)/toluene (50 mL) at −78 °C under an atmosphere of nitrogen was added dropwise *n*-butyllithium (2.5 M in hexanes, 12 mL, 30 mmol), and the mixture was stirred for 1 h at the same temperature. Then a solution of 2,3,4,6-tetra-*O*-trimetylsilyl-β-D-glucolatone (**9**, 13.8 g, 30 mmol) in tetrahydrofuran (30 mL) was added dropwise, and the mixture was stirred for 1.5 h at the same temperature. The reaction mixture was quenched by addition of saturated ammonium chloride solution. After complete addition, the solution was gradually raised to room temperature. The organic layer was separated and the aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over magnesium sulfate, filtered and concentrated in vacuo to yield the title compound (**10**) as a yellowish solid which was used without further purification.

To a stirred −25 °C solution of *O*-methylglucoside (**10**) in dichloromethane (50 mL)/acetonitile (50 mL) was added triethylsilane (8.7 mL, 54 mmol) followed by boron trifluoride diethyl etherate (5.2 mL, 41 mmol) at −25 °C. The solution was allowed to warm to 0 °C over 3 h prior to quenching with saturated sodium carbonate solution. After removal of organic volatiles under reduced pressure, the residue was partitioned between ethyl acetate and water. Following extraction of the aqueous layer with ethyl acetate, the combined organic layers were washed with water prior to drying over magnesium sulfate. Filtration and removal of volatiles under reduced pressure yield desired tetraol (**11**) as a yellowish solid.

The obtained tetraol (**11**) was diluted dichloromethane (60 mL) and added acetic anhydride (22.2 mL, 235 mmol), DMAP (165 mg, 1.35 mmol) and pyridine (19 mL, 235 mmol). After 18 h, the reaction was quenched by addition of H_2O, whereupon the resulting mixture was extracted with dichloromethane (2×). The combined organic layers were washed with 1 N HCl (2×) and brine (2×) prior to drying over magnesium sulfate. After filtration and concentration under reduced pressure, residue was slurried in ethanol (80 mL) and heated to reflux with stirring. The reaction mixture was held at reflux for 1 h to ensure that all of solution had homogenized; it was then cooled evenly at 15 °C/h to ambient temperature and stirred overnight at this temperature. The resulting solid was isolated by filtration and dried in vacuo to yield the title compound (**12**, 7.0 g, 11 mmol, 41%, ca. 60% separation yield) as a white solid. MNa^+ 655.

4.2.2.2. (2*S*,3*R*,4*R*,5*S*,6*R*)-2-(2-(Allyloxy)-4-chloro-5-(4-ethoxybenzyl)phenyl)-6-(hydroxyl-methyl)tetrahydro-2*H*-pyran-3,4,5-triol (13). To a solution (2*R*,3*R*,4*R*,5*S*,6*S*)-2-(acetoxymethyl)-6-(2-(allyloxy)-4-chloro-5-(4-ethoxybenzyl)phenyl)tetrahydro-2*H*-pyran-3,4,5-triyl triacetate (**12**, 4 g, 6.32 mmol) from Step 1 in methanol (90 mL) was added sodium methoxide (25% in methanol, 9 mL) and the reaction mixture stirred at ambient temperature for 5 h. The solution was cooled to 0 °C prior to neutralizing with acetic acid (4.5 mL). After removal of organic volatiles under reduced pressure, the residue was diluted with ethyl acetate and water. The organic layer was separated and the aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with sodium hydrogen carbonate, brine, dried over magnesium sulfate, filtered and concentrated in vacuo to yield the title compound (**13**) as a white solid which was used without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.19 (s, 1H), 7.05 (d, J = 8.4 Hz, 2H),

6.92 (s, 1H), 6.79 (d, J = 8.8 Hz, 2H), 6.06–5.96 (m, 1H), 5.40 (dd, J = 1.6, 17.6 Hz, 1H), 5.30 (dd, J = 1.6, 10.4 Hz, 1H), 4.66 (d, J = 9.6 Hz, 2H), 4.58–4.49 (m, 2H), 4.01–3.96 (m, 4H), 3.89–3.86 (m, 1H), 3.80–3.75 (m, 1H), 3.66–3.62 (m, 2H), 3.55–3.47 (m,2H), 3.22 (m, 1H), 3.05 (m, 1H), 2.42 (m, 1H), 2.13–2.11 (m, 1H), 1.38 (t, J = 6.8 Hz, 3H), MNa^+ 487.

4.2.2.3. (2*S*,3*S*,4*R*,5*R*,6*R*)-2-(2-(Allyloxy)-4-chloro-5-(4-ethoxybenzyl)phenyl)-3,4,5-tris(benzyloxy)-6-(benzyloxymethyl)tetrahydro-2*H*-pyran (14). To a solution of tetraol (**13**, 8.4 g, 18 mmol) from Step 2 in *N*,*N*-dimethylformamide (100 mL) at 0 °C under an atmosphere of nitrogen was added sodium hydride (60% dispersion in mineral oil, 10.1 g, 252 mmol), and the mixture was stirred for 30 min at the same temperature. Then benzyl bromide (19.5 mL, 162 mmol) was added dropwise, and the mixture was stirred with gradual warming to ambient temperature over 5 h. After re-cooling to 0 °C, the reaction mixture was quenched by addition of water (100 mL). The mixture was diluted with water and extracted with ethyl acetate. The organic layer was washed with brine, dried over magnesium sulfate, filtered and evaporated in vacuo. The resulting crude residue was purified on a Biotage® purification apparatus (silica gel, 5–20% tetrahydrofuran in hexanes gradient) to yield the title compound (**14**, 14.8 g, 18 mmol, 100%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.43 (br, 2H), 7.33–7.25 (m, 12H), 7.21–7.13 (m, 5H), 7.06–7.04 (m, 3H), 6.83 (d, J = 6.4 Hz, 1H), 6.71 (d, J = 8.8 Hz, 1H), 6.00–5.93 (m, 1H), 5.38 (d, J = 17.2 Hz, 1H), 5.18 (dd, J = 1.6, 10.8 Hz, 1H), 4.80 (s, 2H), 4.76 (d, J = 11.2 Hz, 1H), 4.59–4.40 (m, 6H), 3.94–3.86 (m, 5H), 3.75 (m, 2H), 3.63–3.58 (m, 4H), 1.26 (t, J = 6.8 Hz, 3H); MNa^+ 847.

4.2.2.4. ((2*R*,3*R*,4*R*,5*S*,6*S*)-6-(2-(Allyloxy)-4-chloro-5-(4-ethoxybenzyl)phenyl)-3,4,5-tris(benzyloxy)tetrahydro-2*H*-pyran-2-yl)-methyl acetate (15). To a stirred −55 °C solution of (2*S*,3*S*,4*R*,5*R*,6*R*)-2-(2-(allyloxy)-4-chloro-5-(4-ethoxybenzyl)phenyl)-3,4,5-tris(benzyloxy)-6-(benzyloxymethyl)tetrahydro-2*H*-pyran (**14,** 8.54 g, 10 mmol) from Step 3 in acetic anhydride (50 mL)/dichloromethane (50 mL) was dropwise added trimethylsilyl trifluoromethanesulfonate (4.7 mL, 26 mmol) at a rate such that the reaction temperature was maintained between −50 and −55 °C. The solution was allowed to warm to −45 °C over 1.5 h prior to quenching with saturated sodium hydrogen carbonate solution. The reaction mixture was extracted with dichloromethane and the combined organic layers were washed with saturated sodium hydrogen carbonate solution prior to drying over magnesium sulfate. Filtration and concentration under reduced pressure and the crude residue was purified on a Biotage® purification apparatus (silica gel, 5–20% tetrahydrofuran in hexanes gradient) to yield the title compound (**15**, 7.8 g, 10 mmol, 100%) as a white solid. MNa^+ 799.

4.2.2.5. ((2*R*,3*R*,4*R*,5*S*,6*S*)-6-(2-(Allyloxy)-4-chloro-5-(4-ethoxybenzyl)phenyl)-3,4,5-tris(benzyloxy)tetrahydro-2*H*-pyran-2-yl)-methanol (16). To a solution ((2*R*,3*R*,4*R*,5*S*,6*S*)-6-(2-(allyloxy)-4-chloro-5-(4-ethoxybenzyl)phenyl)-3,4,5-tris(benzyloxy)tetrahydro-2*H*-pyran-2-yl)methyl acetate (**15**, 7.8 g, 10 mmol) from Step 4 in methanol (130 mL) was added sodium methoxide (25% in methanol, 13 mL) and the reaction mixture vigorously stirred at ambient temperature for 5 h. The solution was cooled to 0 °C prior to neutralizing with acetic acid (6.5 mL). After removal of organic volatiles under reduced pressure, the residue was diluted ethyl acetate and water. The organic layer was separated and the aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with sodium hydrogen carbonate, brine, dried over magnesium sulfate, filtered and concentrated in vacuo. The crude residue was purified on Biotage® purification apparatus (silica gel, 5–30% tetrahydrofuran in hexanes) to yield the title compound (**16**, 7.2 g, 9.8 mmol, 98%) as a white solid. MNa^+ 757.

4.2.3. Synthesis of precursor 31 and subsequent key intramolecular cyclization

4.2.3.1. (5-Bromopentyloxy)(*tert*-butyl)diphenylsilane (17). To a solution of 5-bromopentan-1-ol (0.43 mL, 3.00 mmol) in tetrahydrofuran (15 mL) at 0 °C under an atmosphere of nitrogen was added imidazole (210 mg, 3.00 mmol) and *t*-butylchlorodiphenylsilane (0.78 mL, 3.00 mmol) at 0 °C. After 5 h at room temperature, the reaction mixture was quenched by addition of water (5 mL) and the mixture was extracted with diethyl ether. The organic layer was washed with brine, dried over magnesium sulfate, filtered and evaporated in vacuo. The resulting crude residue was purified on a Biotage® purification apparatus (silica gel, 0–10% ethyl acetate in hexanes gradient) to yield the title compound (**17**, 980 mg, 2.42 mmol, 81%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72–7.69 (m, 4H), 7.48–7.39 (m, 6H), 3.70 (t, J = 6.4 Hz, 1H), 3.42 (t, J = 6.8 Hz, 1H), 1.91–1.84 (m, 2H), 1.62–1.58 (m, 2H), 1.47–1.42 (m, 4H), 1.09 (s, 9H).

4.2.3.2. (5-(((2*R*,3*R*,4*R*,5*S*,6*S*)-6-(2-(Allyloxy)-4-chloro-5-(4-ethoxybenzyl)phenyl)-3,4,5-tris-(benzyloxy)tetrahydro-2*H* -pyran-2-yl)methoxy)pentyloxy)(*tert*-butyl)diphenylsilane (18). To a solution of ((2*R*,3*R*,4*R*,5*S*,6*S*)-6-(2-(allyloxy)-4-chloro-5-(4-ethoxybenzyl)phenyl)-3,4,5-tris(benzyloxy)tetrahydro-2*H*-pyran-2-yl)methanol (**26**, 1.1 g, 1.51 mmol) in *N*,*N*-dimethylformamide (25 mL) was added sodium hydride (60% dispersion in mineral oil, 120.4 mg, 3.01 mmol) at 0 °C, and the reaction mixture stirred at ambient temperature for 0.5 h. After 1 h at room temperature, the reaction mixture was re-cooled to 0 °C and (5-bromopentyloxy)(*tert*-butyl)diphenylsilane (**17**, 980 mg, 2.42 mmol) was added dropwise, and the mixture was stirred with gradual warming to ambient temperature over 5 h. After re-cooling to 0 °C, the reaction mixture was quenched by addition of water (25 mL). The mixture was diluted with water and extracted with ethyl acetate. The organic layer was washed with brine, dried over magnesium sulfate, filtered and evaporated in vacuo. The resulting crude residue was purified on a Biotage® purification apparatus (silica gel, 5–20% tetrahydrofuran in hexanes gradient) to yield the title compound (**18**, 664 mg, 0.63 mmol, 42%) as a yellowish oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.69–7.67 (m, 4H), 7.45–7.40 (m, 6H), 7.35–7.32 (m, 10H), 7.26–7.14 (m, 6H), 7.06–7.02 (m, 1H), 6.92–6.86 (m, 3H), 6.77–6.75 (m, 1H), 6.04–5.94 (m,1H), 5.43–5.39 (m, 1H), 5.27–5.24 (m, 1H), 4.96–4.89 (m, 3H), 4.72 (d, J = 11.2 Hz, 1H), 4.49–4.43 (m, 3H), 4.05–3.89 (m, 5H), 3.85–3.77 (m, 2H), 3.75–3.68 (m, 2H), 3.67–3.64 (m, 3H), 3.56–3.47 (m, 2H), 3.42–3.34 (m, 1H), 1.63–1.53 (m, 2H), 1.40 (t, J = 6.8 Hz, 3H), 1.06 (s, 9H).

4.2.3.3. 5-(((2*R*,3*R*,4*R*,5*S*,6*S*)-6-(2-(Allyloxy)-4-chloro-5-(4-ethoxybenzyl)phenyl)-3,4,5-tris(benzyloxy)tetrahydro-2*H*-pyran-2-yl)methoxy)pentan-1-ol (19). To a solution of (5-(((2*R*,3*R*,4*R*,5*S*,6*S*)-6-(2-(allyloxy)-4-chloro-5-(4-ethoxybenzyl)phenyl)-3,4,5-tris(benzyloxy)tetrahydro-2*H*-pyran-2-yl)methoxy)pentyloxy)(*tert*-butyl)diphenylsilane (**18**, 664 mg, 0.626 mmol) from Step 1 in tetrahydrofuran (8 mL) was added tetrabutylammonium fluoride (1.0 M in tetrahydrofuran, 1.9 mL, 1.88 mmol) and the reaction mixture stirred at ambient temperature for 2 h. After removal of organic volatiles under reduced pressure, the residue was partitioned between ethyl acetate and saturated ammonium chloride solution. The organic layer was separated and the aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over magnesium sulfate, filtered and concentrated in vacuo. The crude residue was purified on Biotage® purification apparatus (silica gel, 10–60% tetrahydrofuran

in hexanes) to yield the title compound (**19**, 479 mg, 0.583 mmol, 93%) as a white. MNa^+ 843.

4.2.3.4. 5-Chloro-4-(4-ethoxybenzyl)-2-((2*S*,3*S*,4*R*,5*R*,6*R*)-3,4,5-tris(benzyl-oxy)-6-((5-hydr-oxypentyloxy)methyl)tetrahydro-2*H*-pyran-2-yl)phenol (20). To a solution of 5-(((2*R*,3*R*,4*R*,5*S*,6*S*)-6-(2-(allyloxy)-4-chloro-5-(4-ethoxybenzyl)phenyl)-3,4,5-tris-(benzyloxy)tetrahydro-2*H*-pyran-2-yl)methoxy)pentan-1-ol (**19**, 479 mg, 0.584 mmol) from Step 2 in tetrahydrofuran (6 mL) was added sodium borohydride (177 mg, 4.668 mmol) and tetrakis(triphenylphosphine)palladium (67.5 mg, 0.058 mmol). The reaction mixture was stirred at room temperature overnight. The mixture was diluted with ethyl acetate and quenched with saturated ammonium chloride, extracted with ethyl acetate. The organic layer was washed with brine, dried over magnesium sulfate, filtered and evaporated in vacuo. Flash chromatography on a Biotage® apparatus (silica gel, 10–50% tetrahydrofuran in hexanes gradient) gave the title compound (**20**, 459 mg, 0.58 mmol, 100%) as a colorless oil. . ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.84 (s, 1H), 7.38–7.27 (m, 10H), 7.23–7.10 (m, 5H), 7.07–7.02 (m, 2H), 6.93–6.86 (m, 3H), 6.76–6.74 (m, 1H), 4.81–4.78 (m, 3H), 4.65–4.60 (m, 2H), 4.42–4.37 (m, 1H), 4.00–3.85 (m, 4H), 3.76–3.67 (m, 2H), 3.65–3.51 (m, 4H), 3.47–3.40 (m, 1H), 3.35–3.28 (m, 2H), 3.25–3.20 (m, 2H), 1.53–1.33 (m, 4H), 1.36–1.21 (m, 2H), 1.29 (t, J = 6.8 Hz, 3H); MNa^+ 803.

4.2.3.5. 5-Chloro-4-(4-ethoxybenzyl)-2-((2*S*,3*S*,4*R*,5*R*,6*R*)-3,4,5-tris(benzyloxy)-6-((5-iodo-pentyloxy)methyl)tetrahydro-2*H*-pyran-2-yl)phenol (21). To a solution of 5-chloro-4-(4-ethoxybenzyl)-2-((2*S*,3*S*,4*R*,5*R*,6*R*)-3,4,5-tris(benzyloxy)-6-((5-hydroxypentyloxy)methyl)tetrahydro-2*H*-pyran-2-yl)phenol (**20**, 459 mg, 0.58 mmol) in benzene (3 mL) under an atmosphere of nitrogen was added imidazole (448 mg, 1.76 mmol) and triphenylphosphine (467 mg,1.76 mmol). After 5 min, iodine (448 mg, 1.76 mmol) in benzene (2 mL) was added dropwide to the reaction mixture at room temperature. The reaction solution was stirred for 1 h, diluted with diethyl ether, quenched with saturated sodium hydrogen carbonate, extracted with diethyl ether (2×). The organic layer was washed with brine, dried over magnesium sulfate, filtered and evaporated in vacuo. The resulting crude residue was purified on a Biotage® purification apparatus (silica gel, 5–25% tetrahydrofuran in hexanes gradient) to yield the title compound (**21**, 485 mg, 0.54 mmol, 93%) as a yellow foam. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.83 (s, 1H), 7.38–7.28 (m, 10H), 7.24–7.10 (m, 5H), 7.06–7.04 (m, 2H), 6.92–6.86 (m, 3H), 6.76–6.74 (m, 1H), 4.82–4.79 (m, 3H), 4.66–4.60 (m, 2H), 4.42–4.35 (m, 1H), 4.00–3.85 (m, 4H), 3.76–3.67 (m, 2H), 3.65–3.51 (m, 4H), 3.47–3.40 (m, 1H), 3.35–3.28 (m, 2H), 3.25–3.20 (m, 2H), 1.80–1.70 (m, 2H), 1.53–1.43 (m, 2H), 1.41–1.32 (m, 2H), 1.28 (t, J = 6.8 Hz, 3H); MNa^+ 913.

4.2.3.6. (9*R*,10*S*,11*R*,12*R*,13*S*)-10,11,12-Tris(benzyloxy)-16-chloro-15-(4-ethoxybenz-yl)-3,4-,5,6,8,9,10,11,12,13-decahydro-2*H*-9,13-epoxybenzo[*h*]-[1,7]dioxacyclopentadecine (32). To a solution of 5-chloro-4-(4-ethoxybenzyl)-2-((2*S*,3*S*,4*R*,5*R*,6*R*)-3,4,5-tris(benzyloxy)-6-((5-iodopentyloxy)methyl)tetrahydro-2*H*-pyran-2-yl)phenol (**21**, 480 mg, 0.54 mmol) from Step 4 in toluene (54 mL) was added potassium carbonate (15 mg, 1.08 mmol) and 18-crown-6 (288 mg, 1.08 mmol). The resulting mixture was stirred at room temperature overnight and quenched with H_2O. After dilution with ethyl acetate, the organic layer was washed with water and brine prior to drying over magnesium sulfate. Filtration and removal of volatiles under reduced pressure, then flash chromatography on a Biotage® apparatus (silica gel, 10–50% tetrahydrofuran in hexanes gradient) gave the macrocyclic compound (**22**, 261 mg, 0.34 mmol, 64%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.36–7.27 (m, 10H), 7.26–7.12 (m, 7H), 7.10–7.07 (m, 1H), 6.89–6.82 (m, 2H), 6.78–6.71 (m, 1H), 4.81–4.77 (m, 3H), 4.67 (d, J = 10.8 Hz, 1H), 4.48–4.43 (m, 1H), 4.26–4.23 (m, 2H), 4.10–4.01 (m, 2H), 3.98–3.88 (m, 6H), 3.80–3.60 (m, 4H), 3.48–3.41 (m, 3H), 1.95–1.86 (m, 2H), 1.64–1.61 (m, 2H), 1.31 (t, J = 6.8 Hz, 3H); MNa^+ 785.

4.2.3.7. (9*R*,10*S*,11*R*,12*R*,13*S*)-16-Chloro-15-(4-ethoxybenzyl)-3,4,5,6,8,9,10,11,12,13-decah-ydro-2*H*-9,13-epoxybenzo[*h*][1,7]-dioxacyclopentadecine-10,11,12-triol (23). To a solution of (2*S*,3*R*,4*R*,5*S*,6*R*)-2-(4-chloro-3-((5-(pyrazin-2-yl)-1,3,4-thiadiazol-2-yl)methyl)phenyl)-6-(hydroxymethyl)-tetrahydro-2*H*-pyran-3,4,5-triol (**22**, 261 mg, 0.34 mmol) in dichloromethane (12 mL) at 0 °C was added boron trichloride (1.0 M in dichloromethane, 1.8 mL, 5.3 mmol). The reaction mixture was stirred at 0 °C for 0.5 h and quenched with methanol (3 mL), then the solution was concentrated in vacuo. Purification by reverse phase preparative HPLC (Waters®, SunFire™ Prep, 5–50% acetonitrile in water gradient) provided the title compound (**23**, 33 mg, 0.067 mmol, 20%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.26 (s, 1H), 7.11 (d, J = 8.4 Hz, 2H), 7.08 (s, 1H), 6.84 (d, J = 8.8 Hz, 2H), 5.08 (m, 1H), 5.01 (m, 1H), 4.83–4.82 (m, 1H), 4.21 (d, J = 10.0 Hz, 1H), 4.04 (m, 2H), 3.98 (q, J = 7.2 Hz, 2H), 3.91 (d, J = 12.4 Hz, 2H), 3.71–3.67 (m, 1H), 3.59 (d, J = 11.6 Hz, 1H), 3.48–3.39 (m, 4H), 3.31–3.26 (m, 2H), 3.16–3.3.14 (m, 1H), 1.70–1.68 (m, 3H), 1.54–1.42 (m, 3H), 1.31 (t, J = 6.8 Hz, 3H); M^+–H_2O 475, M^+–NH_4^+ 457.

*4.2.3.7.1. (6R,7S,8R,9R,10S)-13-Chloro-12-(4-ethoxybenzyl)-2,3,5,6,7,8,9,10-octahydro-6,10-epoxybenzo[e][1,4]dioxacyclododecine-7,8,9-triol (**32**).* ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.12 (s, 1H), 7.05 (d, J = 8.4 Hz, 2H), 6.80 (d, J = 8.4 Hz, 2H), 6.52 (s, 1H), 4.97 (br, 2H), 4.41 (d, J = 9.2 Hz, 1H), 3.97 (q, J = 6.8 Hz, 2H), 3.81 (d, J = 3.6 Hz, 2H), 3.69 (d, J = 10.8 Hz, 1H), 3.48–3.25 (m, 8H), 3.18–3.12 (m, 3H), 1.31 (t, J = 7.2 Hz, 3H); MH^+–$4H^+$ 447.

*4.2.3.7.2. (7R,8S,9R,10R,11S)-14-Chloro-13-(4-ethoxybenzyl)-3,4,6,7,8,9,10,11-octahydro-2H-7,11-epoxybenzo[f][1,5]dioxacyclotridecine-8,9,10-triol (**33**).* ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.47 (s, 1H), 7.27 (s, 1H), 7.11 (d, J = 8.8 Hz, 1H), 6.84 (d, J = 8.8 Hz, 1H), 5.33–4.79 (br, 2H), 4.25 (d, J = 10.0 Hz, 1H), 4.18–4.14 (m, 1H), 4.01–3.95 (m, 3H), 3.91–3.80 (m, 3H), 3.74–3.67 (m, 2H), 3.61 (t, J = 9.6 Hz, 1H), 3.46–3.40 (m, 2H), 3.38–3.34 (m, 3H), 3.15–3.11 (m, 1H), 1.93–1.84 (m, 1H), 1.79–1.73 (m, 1H), 1.31 (t, J = 6.8 Hz, 3H); MH+–H_2O 447.

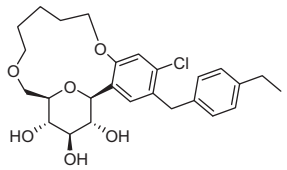
*4.2.3.7.3. (10R,11S,12R,13R,14S)-17-Chloro-16-(4-ethoxybenzyl)-2,3,4,5,6,7,9,10,11,12,13,14 -undecahydro-10,14-epoxybenzo[h][1,8]-dioxacyclohexadecine-11,12,13-triol (**34**).* ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.29 (s, 1H), 7.10 (d, J = 8.8 Hz, 2H), 7.03 (s, 1H), 6.83 (d, J = 8.8 Hz, 2H), 5.11 (br, 1H), 5.02 (br, 1H), 4.83 (br, 1H), 4.39 (d, J = 10.0 Hz, 1H), 4.15–4.13 (m, 1H), 4.01–3.96 (m, 3H), 3.89–3.86 (m, 2H), 3.66 (t, J = 9.2 Hz, 1H), 3.53 (d, J = 12.0 Hz, 1H), 3.46 (t, J = 8.0 Hz, 2H), 3.38–3.30 (m, 2H), 2.99–2.97 (m, 1H), 1.82 (m, 1H), 1.68–1.66 (m, 2H), 1.54–1.39 (m, 5H), 1.31 (t, J = 6.8 Hz, 3H); MNa^+ 529, MH^+–H_2O 489, MH^+–$NH4^+$ 471, IR (neat, cm^{-1}) 3429, 2917, 1608, 1509, 1242, 1077, 1040, 989, 833.

*4.2.3.7.4. (7R,8S,9R,10R,11S)-14-Chloro-13-(4-ethylbenzyl)-3,4,6,7,8,9,10,11-octahydro-2H-7,11-epoxybenzo[f][1,5]dioxacyclotridecine-8,9,10-triol (**35**).* ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.50 (s, 1H), 7.29 (s, 1H), 7.12 (s, 4H), 5.17–5.14 (m, 2H), 5.03 (d, J = 5.6 Hz, 1H), 4.25 (d, J = 10.0 Hz, 1H), 4.17–4.15 (m, 1H), 4.01 (d, J = 14.8 Hz, 1H), 3.92 (d, J = 14.4 Hz, 1H), 3.90–3.80 (m, 2H), 3.74–3.68 (m, 2H), 3.64–3.58 (m, 1H), 3.46–3.38 (m, 2H), 3.15–3.09 (m, 1H), 2.55 (q, J = 7.6 Hz, 2H), 1.90–1.84 (m, 1H), 1.78–1.72 (m, 1H), 1.16 (t, J = 7.6 Hz, 1H),3H); MH^+–H_2O 431, IR (neat, cm^{-1}) 3374, 2962, 2927, 2872, 1607, 1488, 1381, 1251, 1087, 1039, 987, 846.

*4.2.3.7.5. (8R,9S,10R,11R,12S)-15-Chloro-14-(4-ethylbenzyl)-2,3,4,5,7,8,9,10,11,12-decahyd-ro-8,12-epoxybenzo[g][1,6]dioxacyclotetradecine-9,10,11-triol (**36**).* ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.32 (s, 1H), 7.13 (s, 4H), 7.07 (s, 1H), 5.05 (d, J = 4.8 Hz, 1H), 5.00 (d,

J = 5.2 Hz, 1H), 4.92 (d, J = 6.4 Hz, 1H), 4.17 (d, J = 10.0 Hz, 1H), 3.98 (d, J = 14.8 Hz, 1H), 3.91 (d, J = 14.8 Hz, 1H), 3.71–3.61 (m, 3H), 3.56–3.52 (m, 1H), 3.40–3.37 (m, 4H), 3.31–3.25 (m, 1H), 3.19–3.15 (m, 1H), 2.56 (q, J = 7.6 Hz, 2H), 1.79 (m, 2H), 1.73–1.69 (m, 1H), 1.61–1.60 (m, 1H), 1.16 (t, J = 7.6 Hz, 1H); MNa^+ 485, MH^+–H_2O 445, MH^+–NH_4^+ 427, IR (neat, cm^{-1}) 3375, 2964, 2860, 1616, 1495, 1329, 1253, 1086, 1048, 983, 832.

*4.2.3.7.6. (9R,10S,11R,12R,13S)-16-Chloro-15-(4-ethylbenzyl)-3,4,5,6,8,9,10,11,12,13-decah-ydro-2H-9,13-epoxybenzo[h][1,7]dioxacyclopentadecine-10,11,12-triol (**37**).*



^1H NMR (400 MHz, DMSO-d_6) δ 7.29 (s, 1H), 7.13 (s, 4H), 7.09 (s, 1H), 5.06 (br, 1H), 5.00 (br, 1H), 4.84 (br, 1H), 4.21 (d, J = 10.0 Hz, 1H), 4.04 (m, 2H), 4.99 (d, J = 14.8 Hz, 1H), 3.91 (d, J = 14.8 Hz, 1H), 3.72–3.68 (m, 1H), 3.60 (d, J = 12.0 Hz, 1H), 3.46–3.39 (m, 4H), 3.30–3.26 (m, 1H), 3.16–3.14 (m, 1H), 2.56 (q, J = 7.6 Hz, 1H), 1.68 (m, 3H), 1.50–1.42 (m, 3H), 1.16 (t, J = 7.6 Hz, 1H); MNa^+ 499, MH^+–H_2O 459, MH^+–NH_4^+ 441, IR (neat, cm^{-1}) 3358, 2918, 1608, 1568, 1493, 1466, 1257, 1212, 1062, 991, 915, 829.

*4.2.3.7.7. (10R,11S,12R,13R,14S)-17-Chloro-16-(4-ethylbenzyl)-2,3,4,5,6,7,9,10,11,12,13,14-undecahydro-10,14-epoxybenzo[h][1,8]-dioxacyclohexadecine-11,12,13-triol (**38**).* ^1H NMR (400 MHz, DMSO-d_6) δ 7.31 (s, 1H), 7.12 (s, 4H), 7.03 (s, 1H), 5.08 (d, J = 4.4 Hz, 1H), 5.01 (d, J = 4.4 Hz, 1H), 4.82 (d, J = 5.2 Hz, 1H), 4.39 (d, J = 10.0 Hz, 1H), 4.16–4.13 (m, 1H), 4.01 (d, J = 14.8 Hz, 1H), 3.93–3.86 (m, 2H), 3.70–3.65 (m, 1H), 3.53 (d, J = 12.0 Hz, 1H), 3.48–3.44 (m, 2H), 3.38–3.29 (m, 2H), 2.97 (m, 1H), 2.56 (q, J = 7.2 Hz, 1H), 1.82 (m, 1H), 1.68–1.66 (m, 2H), 1.54–1.49 (m, 1H), 1.46–1.39 (m, 4H), 1.16 (t, J = 7.6 Hz, 1H); MNa^+ 513, MH^+–H_2O 473, MH–NH_4^+ 455; IR (neat, cm^{-1}) 3396, 2917, 1610, 1497, 1323, 1255, 1134, 1059, 1003, 944, 827.

*4.2.3.7.8. (8R,9S,10R,11R,12S)-15-Chloro-14-(4-(methylthio)-benzyl)-2,3,4,5,7,8,9,10,11,12-d ecahydro-8,12-epoxybenzo[g][1,6]-dioxacyclotetradecine-9,10,11-triol (**39**).* ^1H NMR (400 MHz, DMSO-d_6) δ 7.30 (s, 1H), 7.23–7.09 (m, 4H), 7.06 (s, 1H), 5.07–4.89 (m, 3H), 4.15 (d, J = 9.8 Hz, 1H), 4.05–3.93 (m, 4H), 3.74–3.58 (m, 3H), 3.56–3.61 (m, 1H), 3.41–3.31 (m, 2H), 3.29–3.21 (m, 1H), 3.19–3.09 (m, 1H), 2.42 (s, 3H), 1.85–1.55 (m, 4H); MNa^+ 503; IR (neat, cm^{-1}) 3387, 2917, 1492, 1254, 1081, 1052, 1002, 914, 834.

*4.2.3.7.9. (9R,10S,11R,12R,13S)-16-Chloro-15-(4-(methylthio)-benzyl)-3,4,5,6,8,9,10,11,12,13-decahydro-2H-9,13-epoxybenzo[h][1,7]-dioxacyclopentadecine-10,11,12-triol (**40**).* ^1H NMR (400 MHz, DMSO-d_6) δ 7.27 (s, 1H), 7.21–7.10 (m, 4H), 7.07 (s, 1H), 5.05 (d, J = 4.4 Hz, 1H), 4.99 (d, J = 5.3 Hz, 1H), 4.81 (d, J = 5.8 Hz, 1H), 4.19 (d, J = 10.0 Hz, 1H), 4.06–3.88 (m, 4H), 3.71–3.62 (m, 1H), 3.58 (d, J = 11.6 Hz, 1H), 3.52–3.37 (m, 4H), 3.27–3.21 (m, 1H), 3.18–3.08 (m, 1H), 1.77–1.58 (m, 3H), 1.56–1.39 (m, 3H); MNa^+ 517; IR (neat, cm^{-1}) 3378, 2918, 1609, 1492, 1255, 1034, 997, 833, 774.

*4.2.3.7.10. (8R,9S,10R,11R,12S)-15-Chloro-14-((2,3-dihydro-benzo[b][1,4]dioxin-6-yl)-methyl)-2,3,4,5,7,8,9,10,11,12-decahydro-8,12-epoxybenzo[g][1,6]dioxacyc-lotetradecine-9,10,11-triol (**41**).* ^1H NMR (400 MHz, DMSO-d_6) δ 7.28 (s, 1H), 7.05 (s, 1H), 6.77–6.71 (m, 1H), 6.69–6.62 (m, 2H), 5.04 (d, J = 4.0 Hz, 1H), 4.99 (d, J = 4.4 Hz, 1H), 4.91 (d, J = 5.4 Hz, 1H), 4.22–4.11 (m, 5H), 4.09–3.96 (m, 2H), 3.84 (quartet, J = 14.8 Hz, 2H), 3.70–3.58 (m, 3H), 3.57–3.44 (m, 1H), 3.39–3.32 (m, 2H), 3.29–3.22 (m, 1H), 3.17–3.09 (m, 1H), 1.85–1.52 (m, 4H); MNa^+ 515; IR (neat, cm^{-1}) 3380, 2917, 1506, 1463, 1284, 1255, 1122, 1087, 1067, 1045, 1000, 982, 952, 919, 885.

*4.2.3.7.11. (9R,10S,11R,12R,13S)-16-Chloro-15-((2,3-dihydro-benzo[b][1,4]dioxin-6-yl)-methyl)-3,4,5,6,8,9,10,11,12,13-decahydro-2H-9,13-epoxybenzo[h][1,7]diox-acyclopentadecine-10,11,12-triol (**42**).* ^1H NMR (400 MHz, DMSO-d_6) δ 7.25 (s, 1H), 7.06 (s, 1H), 6.77–6.71 (m, 1H), 6.68–6.63 (m, 2H), 5.05 (d, J = 4.4 Hz, 1H), 5.00 (d, J = 5.5 Hz, 1H), 4.82 (d, J = 6.1 Hz, 1H), 4.23–4.16 (m, 5H), 4.08–3.97 (m, 2H), 3.84 (quartet, J = 14.8 Hz, 2H), 3.71–3.63 (m, 1H), 3.58 (d, J = 11.9 Hz, 1H), 3.51–3.37 (m, 4H), 3.32–3.23 (m, 1H), 3.18–3.09 (m, 1H), 1.78–1.59 (m, 3H), 1.57–1.38 (m, 3H); MNa^+ 529; IR (neat, cm^{-1}) 3375, 2912, 1591, 1507, 1495, 1285, 1256, 1126, 1069, 1034, 1003, 920, 882.

4.2.4. Preparation of diene precursor and macrocyclization

4.2.4.1. (4a*R*,6*S*,7*R*,8*R*,8a*S*)-6-(2-(Allyloxy)-4-chloro-5-(4-ethoxybenzyl)phenyl)-2-vinylhexahydropyrano[3,2-*d*][1,3]dioxine-7,8-diol (24). To a solution of intermediate tetraol (**13**, 500 mg, 1.08 mmol) in *N*,*N*-dimethylformamide (3 mL) under an atmosphere of nitrogen was added acrolein diethyl acetal (0.51 mL, 3.23 mmol) and camphorsulfonic acid (64 mg, 0.26 mmol), and the mixture was stirred at the same temperature overnight. The reaction mixture was quenched by addition of triehylamine (0.045 mL, 0.32 mmol) and the removal of volatiles under reduced pressure yielded desired acetal compound (**24**) as a white-off solid which was used without further purification. MNa^+ 525.

4.2.4.2. (2*S*,3*R*,4*R*,5*S*,6*R*)-2-(2-(Allyloxy)-4-chloro-5-(4-ethoxybenzyl)phenyl)-6-(allyloxyme thyl)tetrahydro-2*H*-pyran-3,4,5-triol (25). To a solution (4^a*R*,6*S*,7*R*,8*R*,8^a*S*)-6-(2-(allyloxy)-4-chloro-5-(4-ethoxybenzyl)phenyl)-2-vinylhexahydropyrano[3,2-*d*]-[1,3]dioxine-7,8-diol (**24**, 669 mg, 1.33 mmol) from Step 1 in tetrahydrofuran (13 mL) was added sodium cyanoborohydride(642 mg, 9.71 mmol), molecular sieves (407 mg) and was added cautiously trifluoromethanesulfonic acid. After addition, the mixture was stirred for another 0.5 h, before being poured into H_2O. The aqueous phase was extracted with dichloromethane and the combined organic fractions were washed with brine. The organic layer was dried over magnesium sulfate and filtered through a pad of silica gel. The filtrate was evaporated and went to next step. MNa^+ 527.

4.2.4.3. (2*S*,3*S*,4*R*,5*R*,6*R*)-2-(2-(Allyloxy)-4-chloro-5-(4-ethoxybenzyl)phenyl)-6-(allyloxyme thyl)tetrahydro-2*H*-pyran-3,4,5-triyl triacetate (26). The obtained triol (**25**) was diluted dichloromethane (15 mL) and added acetic anhydride (1.1 mL, 12.1 mmol), DMAP (9.2 mg, 0.076 mmol) and pyridine (1.0 mL, 12.11 mmol). After 18 h, the reaction was quenched by addition of H_2O, whereupon the resulting mixture was extracted with dichloromethane (2×). The combined organic layers were washed with 1 N HCl (2×) and brine (2×) prior to drying over magnesium sulfate. After filtration and concentration under reduced pressure , the resulting oil was purified on Biotage® purification apparatus (silica gel, 5–30% tetrahydrofuran in hexanes) to yield the title compound (**26**, 580 mg, 0.92 mmol, 61%; three steps) as a white solid. ^1H NMR (400 MHz, $CDCl_3$) δ 7.20 (s, 1H), 7.04 (d, J = 8.8 Hz, 2H), 6.84 (s, 1H), 6.79 (d, J = 8.4 Hz, 2H), 6.07–5.99 (m, 1H), 5.85–5.78 (m, 1H), 5.44 (dd, J = 1.6, 17.6 Hz, 1H), 5.34–5.23 (m, 3H), 5.19–50.13 (m, 2H), 4.82 (d, J = 9.2 Hz, 1H), 4.50 (d, J = 4.0 Hz, 1H), 4.02–3.90 (m, 6H), 3.79–3.74 (m, 1H), 3.53 (d, J = 4.4 Hz, 1H), 2.04 (s, 3H), 2.00 (s, 3H), 1.74 (s, 3H), 1.39 (t, J = 7.2 Hz, 3H); MNa^+ 653.

4.2.4.4. (8*R*,9*S*,10*R*,11*R*,12*S*)-9,10,11-Tris(acetyloxy)-15-chloro-14-(4-ethoxybenzyl)-5,7,8,9, 10,11,12-hexahydro-2*H*-8,12-epoxybenzo[*g*][1,6]dioxacyclotetradecine (27). To a solution of (2*S*,3*S*,4*R*,5*R*,6*R*)-2-(2-(allyloxy)-4-chloro-5-(4-ethoxybenzyl)phenyl)-

6-(allyloxymethyl)tetrahydro-2*H*-pyran-3,4,5-triyl triacetate (**26**, 580 mg, 0.92 mmol) from Step 3 in dichloromethane (184 mL, 0.005 M) under an atmosphere of nitrogen was added Grubbs 2nd generation catalyst (156 mg, 0.184 mmol), and the mixture was heated at 60 °C for three days. After re-cooling to room temperature, the reaction mixture was filtered through a pad of Celite®, washed with ethyl acetate (20 mL) and the filtrated was evaporated under reduced pressure. The resulting crude residue was purified on a Biotage® purification apparatus (silica gel, 5–20% tetrahydrofuran in hexanes gradient) to yield the title compound (**27**, (*Z*)-form, 125 mg, 0.208 mmol, 23%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.22 (s, 1H), 7.18 (s, 1H), 7.11 (d, *J* = 8.4 Hz, 2H), 6.82 (d, *J* = 8.8 Hz, 2H), 5.95–5.87 (m, 1H), 5.72 (dt, *J* = 4.0, 15.2 Hz, 1H), 5.56 (t, *J* = 9.2 Hz, 1H), 5.35–5.28 (m, 1H), 4.92 (t, *J* = 10.0 Hz, 1H), 4.77–4.67 (m, 2H), 4.41–4.36 (m, 1H), 4.14–4.11 (m, 1H), 4.05–3.94 (m, 5H), 3.72 (t, *J* = 8.8 Hz, 1H), 3.65 (d, *J* = 13.2 Hz, 1H), 3.54–3.48 (m, 1H), 2.07 (s, 3H), 2.02 (s, 3H), 1.75 (s, 3H), 1.41 (t, *J* = 7.2 Hz, 3H); MNa^+ 625.

4.2.4.5. (8*R*,9*S*,10*R*,11*R*,12*S*)-15-Chloro-14-(4-ethoxybenzyl)-5,7,8,9,10,11,12-hexahydro-2*H*-8,12-epoxybenzo[*g*][1,6]dioxacyclotetradecine-9,10,11-triol (28). To a solution (2*S*,3*S*,4*R*,5*R*,6*R*)-2-(2-(allyloxy)-4-chloro-5-(4-ethoxybenzyl)phenyl)-6-(allyloxymethyl)tetrahydro-2*H*-pyran-3,4,5-triyl triacetate (**27**, 125 mg, 0.208 mmol) from Step 4 in methanol (4 mL) was added sodium methoxide (25% in methanol, 0.6 mL) and the reaction mixture stirred at ambient temperature for 1 h. The solution was cooled to 0 °C prior to neutralizing with acetic acid (0.3 mL). After removal of organic volatiles under reduced pressure, the residue was diluted with methanol. Purification by reverse phase preparative HPLC (Waters®, SunFire™ Prep, 5–50% acetonitrile in water gradient) provided the title compound (**28**, 56 mg, 0.117 mmol, 56%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.33 (s, 1H), 7.30 (s, 1H), 7.29 (d, *J* = 8.4 Hz, 2H), 6.82 (d, *J* = 8.8 Hz, 2H), 5.86–5.80 (m, 1H), 5.72 (dt, *J* = 7.6, 15.2 Hz, 1H), 5.07 (d, *J* = 4.4 Hz, 1H), 5.01 (d, *J* = 5.6 Hz, 1H), 4.79 (d, *J* = 6.0 Hz, 1H), 4.62 (dd, *J* = 8.8, 12.0 Hz, 1H), 4.38 (dd, *J* = 5.6, 11.6 Hz, 1H), 4.23 (d, *J* = 10.0 Hz, 1H), 4.00–3.88 (m, 6H), 3.83 (d, *J* = 11.6 Hz, 1H), 3.66–3.60 (m, 1H), 3.28–3.24 (m, 3H), 2.98–2.93 (m, 1H), 1.30 (t, *J* = 7.2 Hz, 3H); MH^+ 499, IR (neat, cm^{-1}) 3425, 2922, 1607, 1511, 1485, 1245, 1079, 1028, 974, 826.

4.2.4.6. (2*S*,3*S*,4*R*,5*R*,6*R*)-2-(2-(Allyloxy)-4-chloro-5-(4-ethoxybenzyl)phenyl)-6-(allyloxyme thyl)-3,4,5-tris(benzyloxy)tetrahydro-2*H*-pyran (29). To a solution of primary alcohol (**16**, 1.5 g, 2.04 mmol) from Step 1 in *N*,*N*-dimethylformamide (10 mL) at 0 °C under an atmosphere of nitrogen was added sodium hydride (60% dispersion in mineral oil, 123 mg, 3.06 mmol), and the mixture was stirred for 30 min at the same temperature. Then allyl bromide (0.26 mL, 3.06 mmol) was added dropwise, and the mixture was stirred with gradual warming to ambient temperature over 5 h. After re-cooling to 0 °C, the reaction mixture was quenched by addition of water (10 mL). The mixture was diluted with saturated ammonium chloride and extracted with ethyl acetate. The organic layer was washed with brine, dried over magnesium sulfate, filtered and evaporated in vacuo. The resulting crude residue was purified on a Biotage® purification apparatus (silica gel, 5–20% tetrahydrofuran in hexanes gradient) to yield the title compound (**29**, 1.12 g, 1.44 mmol, 71%) as a white solid. MNa^+ 797.

4.2.4.7. (*Z*)-((8*R*,9*S*,10*R*,11*R*,12*S*)-9,10,11-Tris(benzyloxy)-15-chloro-14-(4-ethoxybenzyl)-5, 7,8,9,10,11,12-hexahydro-2*H*-8,12-epoxybenzo[*g*][1,6]dioxacyclotetradecine (30). To a solution of (2*S*,3*S*,4*R*,5*R*,6*R*)-2-(2-(allyloxy)-4-chloro-5-(4-ethoxybenzyl)phenyl)-6-(allyloxymethyl)-3,4,5-tris(benzyloxy)tetrahydro-2*H*-pyran (**29**, 1.12 g, 1.44 mmol) from Step 3 in dichloromethane (150 mL, 0.01 M) under an atmosphere of nitrogen was added Grubbs 2nd generation catalyst (100 mg, 0.16 mmol), and the mixture was heated at 60 °C overnight. After re-cooling to room temperature, the reaction mixture was filtered through a pad of Celite®, washed with ethyl acetate (100 mL) and the filtrated was evaporated under reduced pressure. The resulting crude residue was purified on a Biotage® purification apparatus (silica gel, 5–20% tetrahydrofuran in hexanes gradient) to yield the title compound (**30**, (*Z*)-form, 279 mg, 0.374 mmol, 26%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.29–7.25 (m, 10H), 7.22–7.13 (m, 5H), 7.12–7.02 (m, 3H), 6.89 (s, 1H), 6.86–6.82 (m, 2H), 6.79–6.69 (m, 2H), 5.96–5.92 (m, 2H), 4.79–4 (d, *J* = 5.6 Hz, 1H), 4.65 (d, *J* = 6.0 Hz, 1H), 4.15 (d, *J* = 10.0 Hz, 1H), 4.02.77 (m, 3H), 4.68–4.65 (m, 1H), 4.56–4.53 (m, 1H), 4.49–4.43 (m, 1H), 4.41–4.34 (m, 2H), 4.22–4.13 (m, 1H), 4.12–4.02 (m, 2H), 3.99–3.81 (m, 5H), 3.76–3.71 (m, 1H), 3.68–3.63 (m, 2H), 3.47–3.45 (m, 1H), 1.32 (t, *J* = 6.8 Hz, 3H); MNa^+ 769.

4.2.4.8. (8*R*,9*S*,10*R*,11*R*,12*S*)-15-Chloro-14-(4-ethoxybenzyl)-2,3,4,5,7,8,9,10,11,12-decahyd ro-2*H*-8,12-epoxybenzo[*g*][1,6]-dioxacyclotetradecine-9,10,11-triol (31). To a solution of (2*S*,3*R*,4*R*,5*S*,6*R*)-2-(4-chloro-3-((5-(pyrazin-2-yl)-1,3,4-thiadiazol-2-yl)methyl)phenyl)-6-(hydroxymethyl)-tetrahydro-2*H*-pyran-3,4,5-triol (**30**, 279 mg, 0.374 mmol) in methanol (3 mL)/tetrahydrofuran (3 mL) was added 10% palladium on charcoal (22 mg). The reaction mixture was stirred under hydrogen gas overnight. The reaction solution was filtered through syringe filter and the filtrate was evaporated under reduced pressure. The crude compound was diluted with methanol and purified by reverse phase preparative HPLC (Waters®, SunFire™ Prep, 5–50% acetonitrile in water gradient) provided the title compound (**31**, 38 mg, 0.08 mmol, 22%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.27 (s, 1H), 7.09 (d, *J* = 8.8 Hz, 1H), 7.05 (s, 1H), 6.82 (d, *J* = 8.4 Hz, 1H), 5.01 (d, *J* = 4.4 Hz, 1H), 4.96 (d, *J* = 5.6 Hz, 1H), 4.65 (d, *J* = 6.0 Hz, 1H), 4.15 (d, *J* = 10.0 Hz, 1H), 4.02–3.94 (m, 2H), 3.96 (q, *J* = 7.2 Hz, 2H), 3.89 (d, *J* = 9.2 Hz, 1H), 3.67–3.59 (m, 2H), 3.54–3.50 (m, 1H), 3.38–3.36 (m, 1H), 3.29–3.23 (m, 1H), 3.15–3.14 (m, 1H), 1.77–1.58 (m, 4H), 1.29 (t, *J* = 7.2 Hz, 3H); MH^+–H_2O 461, MH^+–$NH4^+$ 443, IR (neat, cm^{-1}) 3444, 3288, 2977, 2931, 1608, 1512, 1476, 1392, 1349, 1238, 1176, 1012, 959, 842.

4.3. In vitro assay

4.3.1. Cloning and cell line construction for human SGLT2

Human SGLT2 (*h*SGLT2) gene was amplified by PCR from cDNA-Human Adult Normal Tissue Kidney (Invitrogen, Carlsbad, CA). The hSGLT2 sequence was cloned into pcDNA3.1(+) for mammalian expression and were stably transfected into Chinese hamster ovary (CHO) cells. SGLT2-expressing clones were selected based on resistance to G418 antibiotic (Geneticin®, Invitrogen, Carlsbad, CA) and activity in the ^{14}C-α-methyl-D-glucopyranoside (^{14}C-AMG) uptake assay.

4.3.2. Inhibitory effects on human SGLT2 activities

For sodium-dependent glucose transport assay, cells expressing *h*SGLT2 were seeded into a 96-well culture plate at a density of 5×10^4 cells/well in RPMI medium 1640 containing 10% fetal bovine serum. The cells were used one day after plating. They were incubated in pretreatment buffer (10 mM HEPES, 5 mM Tris, 140 mM choline chloride, 2 mM KCl, 1 mM $CaCl_2$, and 1 mM $MgCl_2$, pH 7.4) at 37 °C for 10 min. They were then incubated in uptake buffer (10 mM HEPES, 5 mM Tris, 140 mM NaCl, 2 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, and 1 mM ^{14}C-nonlabeled AMG pH 7.4) containing ^{14}C-labeled (8 μM) and inhibitor or dimethyl sulfoxide (DMSO) vehicle at 37 °C for 2 h. Cells were washed twice with washing buffer (pretreatment buffer containing 10 mM AMG at room temperature) and then the radioactivity was measured using

a liquid scintillation counter. IC_{50} was determined by nonlinear regression analysis using GraphPad PRISM.[16,17]

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Further reading

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