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ortho-Substituted *C*-aryl glucosides as highly potent and selective renal sodium-dependent glucose co-transporter 2 (SGLT2) inhibitors

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ABSTRACT

A series of 2-substituted *C*-aryl glucosides have been synthesized and evaluated for inhibition of hSGLT1 and hSGLT2. Introduction of an appropriate *ortho* substituent at the proximal phenyl ring adjacent to the glycosidic bond was found to improve SGLT2 inhibitory activity and dramatically increase selectivity for hSGLT2 over hSGLT1. Selected compounds were investigated for in vivo efficacy.

1. Introduction

Inhibition of renal sodium-dependent glucose co-transporter (SGLT) proteins is an attractive strategy for the mitigation of hyperglycemia in type 2 diabetes.[1] It has been reported that 90% of renal glucose re-absorption is mediated by SGLT2, which is localized to the S1 and S2 segments of the proximal tubule.[2] The remaining renal glucose re-absorption is thought to be principally mediated by SGLT1, which is localized to the more distal S3 segment of the proximal tubule.[2] Multiple classes of metabolically instable O-glucosides[1a,3] and metabolically more robust C-glucosides, like dapagliflozin,[4] have been reported as SGLT2 inhibitors to date.

We have previously discussed the activity of *O*-spiroketal *C*-aryl glucosides (formula II, Fig. 1), as SGLT2 inhibitors.[5] Compared to dapagliflozin **1**, *O*-spiroketal glucoside **3** exhibited similar in vitro hSGLT2 inhibitory activity and slightly higher selectivity toward hSGLT2 than SGLT1, but had less in vivo activity and displayed unfavorable gastrointestinal side effects (diarrhea and cecal dilation due to gas accumulation).[5] In this study we identify ring-opened *ortho*-substituted compounds (Fig. 1), based on compounds **1**, **2** and **3**, as SGLT2 inhibitors. The synthetic strategy is centered on the introduction of a substituent at the 6-position of the proximal phenyl ring of formula I to obtain 2-substituted *C*-aryl glucoside SGLT2 inhibitors (formula III).[6]

2. Results and discussion

2.1. Chemistry

Scheme 1 describes the formation of the core aglycones, a benzyl ether series, and a phenethyl ether series, that were diversified in this study from intermediates **4a** and **4b**, the synthetic method of which was described in details in the patent WO2008122014.[6] Phenylethanols **7a** and **7b** were constructed using Brown hydroboration reaction of styrenes **6a** and **6b**, which were synthesized by the oxidation of benzylalcohols **4a** and **4b**[5] followed by Wittig olefination of **5a** and **5b**. Alkylation of benzyl alcohol **4a** and phenylethanols **7a–b** gave aglycones **8a–b** (Scheme 1, top) and **9a–b** (Scheme 1, bottom) respectively.

As illustrated in Scheme 2, the β-*C*-aryl glucosides **11a–b** and **12a–b** were synthesized via a previously reported method.[7] Lithiation of bromodiarylmethanes **8a–b** and **9a–b** followed by their

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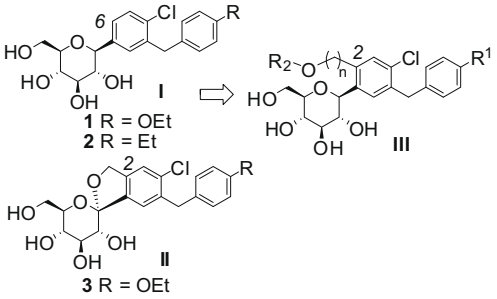
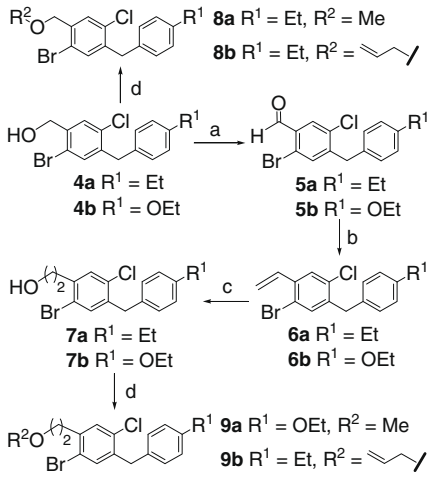


Figure 1.



Scheme 1. Construction of the aglycones. Reagents and conditions: (a) Dess–Martin periodinane, DCM, 0 °C to rt, (96% for **5a**, 89% for **5b**); (b) $Ph_3P^+MeI^-$, KHMDS, toluene, rt (81% for **6a**, 94% for **6b**); (c) 9-BBN, THF, 0 °C to rt, then MeOH, NaOH, 30% H_2O_2 (70% for **7a**, 64% for **7b**); (d) NaH, TBAI, DMF or THF, 0 °C or rt, then MeI or allylbromide (78–91%).

addition to 2,3,4,6-tetra-*O*-trimethylsilyl-D-gluconolactone **10** and finally treatment with methanesulfonic acid provided desilylated *O*-methyl lactols, which were reduced using triethylsilane and $BF_3{\cdot}OEt_2$ to give the desired *ortho*-substituted *C*-aryl glucosides.[8]

Schemes 3–7 illustrate the synthetic routes to modify the allyl moiety of glucoside **11b**. The synthesis of compounds **17**–**20** and **22** is shown in Scheme 3. Bromodiarylmethane **8b** was lithiated and added to 2,3,4,6-tetra-*O*-benzyl-D-gluconolactone **13**.[9] Subsequent reduction of the lactol gave predominantly β-linked *C*-aryl glucoside **14**[10] followed by ozonolysis to provide aldehyde **15**. Reduction of this aldehyde afforded alcohol **16** which was deprotected by hydrogenolysis to give the hydroxyl-substituted analog **17**. Methylation of alcohol **16** followed by deprotection yielded methoxy-substituted compound **18**. Fluorination of alcohol **16** and aldehyde **15** with DAST[11] followed by hydrogenolysis produced mono and difluoro-substituted analogs **19** and **20**, respectively. Trifluoroethoxy compound **22** was constructed by deallylation[12] of **14** followed by alkylation with 2,2,2-trifluoroethanol under Mitsunobu reaction conditions and finally by hydrogenolysis of the benzyl groups.

The synthesis of analogs **23**–**26** is shown in Scheme 4. A methylene extended 3-propoxy alcohol **23** was obtained directly by Brown hydroboration of alkene **11b** (path a). Wacker–Tsuji oxidation[13] of the alkene **11b** (path b) provided ketone **24**, which was subjected to reduction with sodium borohydride and to reductive amination with sodium cyanoborohydride and methylamine to provide the corresponding secondary alcohol **25** and secondary amine **26** (as a mixture of diastereomers).

Scheme 5 depicts the synthesis of fluorides **29** and **30**. Wacker–Tsuji oxidation[13] of alkene **14** generated ketone **27** which was reduced by sodium borohydride to obtain alcohol **28**. Fluorination of alcohol **28** and ketone **27** with DAST[11] followed by hydrogenolysis produced the corresponding monofluoro and difluoro-substituted analogs **29** (as a mixture of diastereomers) and **30** respectively.

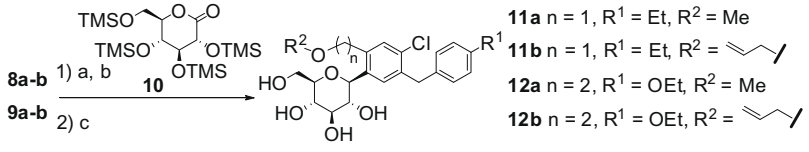
As shown in Scheme 6, peracetylation of **11b** yielded tetraacetate **31** that was subjected to deallylation[12] to give benzyl alcohol **32**, which subsequently underwent Mitsunobu reaction followed by hydrolysis to generate phenyl-substituted analog **33**.

Alkyne derivatives **34** and **35** described in Scheme 7 were obtained from the corresponding alkenes **11b** and **12b** by electrophilic addition of bromine to the double bond followed by elimination under strong alkaline conditions.

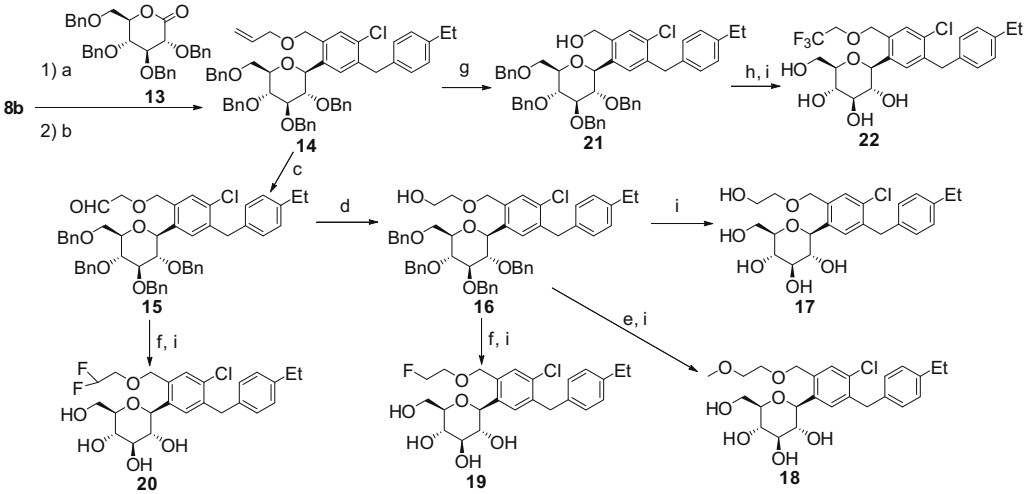
2.2. Biological activity evaluation

The target compounds were screened in cell-based SGLT functional assays, in which 293 cells expressing hSGLT2 and COS-7 cells expressing hSGLT1 were used. The results were presented in Table 1. In the benzylic ether series, 2-methoxymethyl-substituted glucoside **11a** was 7-fold more potent and 11-fold more selective for inhibition of hSGLT2 compared to dapagliflozin **1**[14] and *O*-spiroketal glucoside **3**. In general the benzylic ethers tolerated a variety of substitutions and resulted in higher selectivity for hSGLT2 compared to hSGLT1. Some notably less potent outliers were the allyl ether **11b**, which was 57-fold less active than the methyl ether, the trifluoroethyl ether **22**, which was 190-fold less active, and the hydroxypropyl ether **23**, which was 125-fold less active. The reduced activity of the trifluoroethyl ether is striking when considered in the context of the di- and monofluoroethyl ethers, both of which exhibited excellent potency and selectivity. A related steep SAR can be noted among the fluorinated propyl ethers, for example comparing the activities of the 2-fluoro and the 2,2-difluoro analogs **29** and **30**. The 1-hydroxypropyl ether **23** was significantly less active than the 2-hydroxypropyl ether **25**, and introduction of a cationic center into the side chain (compound **26**) was poorly tolerated. Similarly disruptive was the introduction of a bulky phenyl group, **33**. Surprisingly, the propargyl ether **34** displayed an exceptional combination of potency and specificity, with sub-nanomolar activity and more than 3300-fold selectivity for SGLT2.

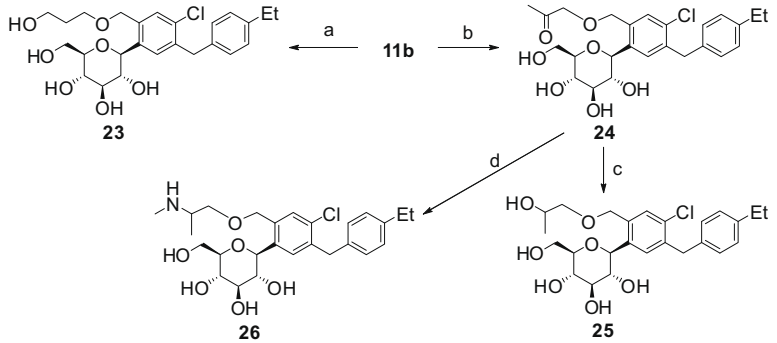
Limited comparisons between the benzyl and phenethyl ether series showed that short side chains were well tolerated in the phenethyl context, and that the allyl ether was more effective and more selective than in the benzyl context. The propargyl ether



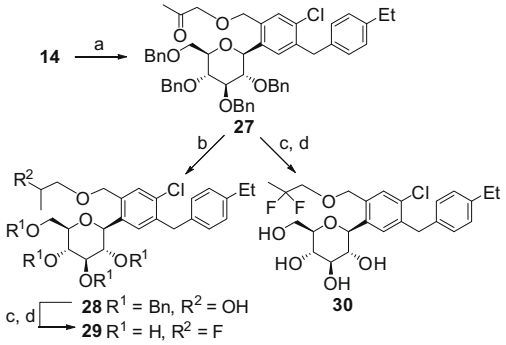
Scheme 2. General route employed in the synthesis of *C*-arylglucosides. Reagents and conditions: (a) *n*-BuLi, THF/toluene (1:2), −78 °C, then **10**; (b) $MeSO_3H$, MeOH, −78 °C to rt; (c) Et_3SiH, $BF_3{\cdot}OEt_2$, MeCN/DCM (1:1), −30 to −10 °C (22–58% three steps).



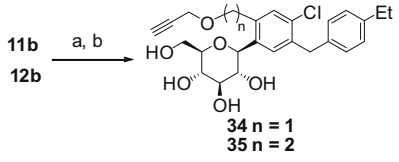
Scheme 3. Reagents and conditions: (a) *n*-BuLi, THF, −78 °C, then **13**; (b) $BF_3{\cdot}OEt_2$, Et_3SiH, MeCN, −40 °C (28% two steps); (c) O_3, −78 °C, then PPh_3 (75%); (d) $NaBH_4$, THF, 0 °C (96%); (e) NaH, THF, 0 °C, then TBAI and MeI; (f) DAST, DCM, −78 °C; (g) $PdCl_2$, AcONa, $AcOH/H_2O$ (9:1), 70 °C (73%); (h) ADDP, PBu_3, CF_3CH_2OH, toluene, rt; (i) H_2, 10% pd/C, THF/MeOH (2:1), 1,2-dichlorobenzene (71% for **17**, two steps: 45% for **18**, 48% for **19**, 39% for **20**, 10% for **22**).



Scheme 4. Reagents and conditions: (a) $BH_3{\cdot}THF$, 0 °C to rt, then 30% NaOH, 30% H_2O_2 (22%); (b) $PdCl_2$, CuCl, O_2, DMF/H_2O (10:1), rt (91%); (c) $NaBH_4$, THF, rt (67%); (d) $MeNH_2$, $NaBH_3CN$, cat. AcOH, EtOH, 0 °C to rt (9%).

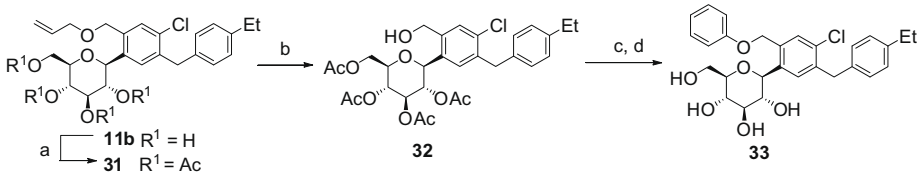


Scheme 5. Reagents and conditions: (a) $PdCl_2$, CuCl, O_2, DMF, rt (58%); (b) $NaBH_4$, THF, rt (98%); (c) DAST, DCM, −78 °C to rt; (d) H_2, 10% Pd/C, 1,2-dichlorobenzene, THF/MeOH (2:1) (two steps: 23% for **29**, 20% for **30**).



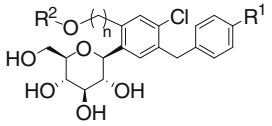
Scheme 7. Reagents and conditions: (a) Br_2, CCl_4, −5 °C; (b) KOH, EtOH, reflux (two steps: 47% for **34**, 45% for **35**).

35 had high activity and selectivity in the phenethyl context, but both attributes were diminished compared to those of the benzylic ether **34**. Both the benzyl and phenethyl ether series exhibited higher selectivity for SGLT2 over SGLT1 compared to compounds **1**, **2** and **3**.



Scheme 6. Reagents and conditions: (a) Ac_2O, pyridine, DMAP, CH_2Cl_2, rt (67%); (b) $PdCl_2$, AcONa, $AcOH/H_2O$ (9:1), 70 °C (91%); (c) phenol, DIAD, PPh_3, −5 °C to rt (30%); (d) $LiOH{\cdot}H_2O$, $THF/MeOH/H_2O$, rt (2:3:1), rt (65%).

Table 1
In vitro data for hSGLT inhibitory activity and selectivity



Compds	*n*	R_1	R_2	hSGLT2 IC_{50} (nM)	hSGLT1 IC_{50} (μM)	Selectivity versus hSGLT1
1[14]	NA	OEt	NA	6.7	0.89	130
2	NA	Et	NA	1.9	0.72	378
3	NA	OEt	NA	6.5	1.3	200
11a	1	Et	Me	0.9	>2	>2200
11b	1	Et		52	91	1700
17	1	Et	$HOCH_2CH_2$	1.2	4.2	3300
18	1	Et	$MeOCH_2CH_2$	3.6	>10	>2700
19	1	Et	FCH_2CH_2	1.6	>5	>3100
20	1	Et	F_2CHCH_2	3	>10	>3300
22	1	Et	F_3CCH_2	170	134	780
23	1	Et	$HOCH_2CH_2CH_2$	110	103	930
24	1	Et	$MeCOCH_2$	6.0	>10	>1600
25	1	Et	$MeCH(OH)CH_2$	16	60	3700
26	1	Et	$MeNHCH(Me)CH_2$	2%[a]	36%[b]	ND
29	1	Et	$MeCHFCH_2$	<10	10%[c]	ND
30	1	Et	$MeCF_2CH_2$	130	140	1000
33	1	Et		12%[a]	0%[b]	ND
34	1	Et		0.3	>1	>3300
12a	2	OEt	Me	0.9	>2	>2200
12b	2	Et		12	31	2500
35	2	Et		6.7	>20	>2900

[a] Inhibition at a screening concentration of 0.1 μM.
[b] Inhibition at a screening concentration of 100 μM.
[c] Inhibition at a screening concentration of 1 μM.

In the mouse diarrhea test (CD1 mice), no diarrhea was observed after 6 h following oral administration of a single dose of 25 mg/kg of benzyl ether analogs, such as **11a**–**b** and **20** whereas severe diarrhea was noted following oral administration of an equivalent dose of *O*-spiroketal *C*-aryl glucosides.[5b] Compounds **11b** and **12b** were evaluated for their ability to induce urinary glucose excretion in normal male Sprague-Dawley rats compared to compound **2**. When a single dose of 1.0 mg/kg of compound was administered orally, compound **11b** induced excretion of 830 mg of urinary glucose per 200 g body weight over 24 h, 34% of glucose excretion relative to reference compound **2**. However, compound **12b** induced excretion of 1170 mg of urinary glucose at the same dose, 56% of glucose excretion relative to reference compound **2**. In fact, compounds **1** and **2** had the similar in vivo activity as that the urinary glucose induced by compound **2** was 88% of that induced by compound **1** when tested in the same experiment. In the same experiment, a 31% and 38% AUC reduction in blood glucose level versus vehicle controls was observed at the 2 h time point after a single 1 mg/kg oral dose of **11b** and reference compound **2**, respectively, to normal male C57BL/6 J mice compared with a pre-dose levels of 84–115 mg/dL of blood glucose.

3. Conclusion

In summary, we have demonstrated that introduction of an appropriate *ortho* substituent adjacent to the glycosidic bond at the proximal phenyl ring resulted in improved SGLT2 inhibitory activity consistent with an analysis presented by Washburn,[3b] and also increased selectivity toward SGLT2 as compared to dapagliflozin **1**, **2** and *O*-spiroketal glucoside **3**. Further in vitro and in vivo SAR investigation of this series will be reported in due course.

4. Experimental

4.1. Chemistry

4.1.1. General procedures

All reagents and solvents were commercially available. Commercial grade anhydrous solvents were purchased from SK Chemical. Silica gel (Qingdao Haiyang) was used for analytical TLC (F254 plates) and flash chromatography (200–300 mesh). ^1H, ^{13}C and ^{19}F NMR spectra were recorded on a Varian Mercury Plus 300 MHz or Bruker 400 MHz using $CDCl_3$, CD_3OD or DMSO-d_6 as solvent and TMS as internal reference. Chemical shifts values are expressed in δ ppm. The data were processed using the program NUTS 4.9.9.9. Abbreviations used for signal patterns are as follows: s, singlet; bs, broad singlet; d, doublet; t, triplet; q, quadruplet; m, multiplet. LC–MS system was a Waters ZQ2000 mass spectrometer, interfaced with a Waters series 2695 liquid chromatography and a Waters 2996 photodiode array detector. Electrospray (ESI^+) was used as the ionization technique. Ion polarity was positive and negative. Chromatographic separation was achieved using a Waters XTerra C18 column (5 μm, 50 × 2.1 mm column). The mobile phases A and B were 0.045% ammonium formate in acetonitrile and 0.1% ammonium formate or formic acid in Milli-Q water, respectively. A gradient elution was performed from 10% to 95% A in 6 min, followed by a 2 min isocratic step at 95% A, at a flow rate of 1.0 mL/min. The photodiode array detector used wavelengths between 190 and 400 nm. Preparative LC–MS system was a Waters ZQ2000 mass spectrometer, interfaced with a Waters series 2525 liquid chromatography and a Waters 2487 UV absorbance detector. The mobile phases A and B were 100% acetonitrile and 0.1% ammonium formate or formic acid in Milli-Q water, respectively. Chromatographic separation was achieved using a Waters

XTerra Prep MS C18 OBD column (5 μm, 19 × 100 mm column). A gradient elution was performed from 33% to 95% A in 15 min, followed by a 5 min isocratic step at 95% A, at a flow rate of 17 mL/min. UV absorbance detector at wavelength 225 nm was used to monitor the chromatography.

4.1.2. Synthesis of 2-bromo-5-chloro-4-(4-ethylbenzyl)benzaldehyde (5a)

To a stirred, cold (−5 °C) solution of **4a** (9.7 g, 29.4 mmol) in anhydrous DCM (100 mL) under argon was added a solution of Dess–Martin's reagent (18.7 g, 44 mmol) in DCM (100 mL) dropwise. After stirring for 2 h, the reaction was then quenched with 1 M aqueous sodium hydroxide (10 mL). After separation, the organic layer was washed with saturated aqueous sodium bisulfite (1×), saturated aqueous sodium bicarbonate (2×) and brine (3×), and dried over anhydrous sodium sulfate. After filtration and removal of the volatiles under reduced pressure, **5a** was obtained as a white solid (9.3 g, 96%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.24 (s, 1H), 7.91 (s, 1H), 7.40 (s, 1H), 7.17 (d, J = 8.1 Hz, 2H), 7.10 (d, J = 8.1 Hz, 2H), 4.08 (s, 2H), 2.64 (q, J = 7.8 Hz, 2H), 1.23 (t, J = 7.8 Hz, 3H).

4.1.3. Synthesis of 2-bromo-5-chloro-4-(4-ethoxybenzyl)benzaldehyde (5b)

Compound **5b** was prepared from **4b** in 89% yield using a similar method as used for the preparation of **5a**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.23 (s, 1H), 7.90 (s, 1H), 7.36 (s, 1H), 7.09 (d, J = 8.7 Hz, 2H), 6.86 (d, J = 8.7 Hz, 2H), 4.08 (s, 2H), 4.03 (q, J = 7.2 Hz, 2H), 1.41 (t, J = 7.2 Hz, 3H).

4.1.4. Synthesis of 1-bromo-4-chloro-5-(4-ethylbenzyl)-2-vinylbenzene (6a)

To a stirred solution of $Ph_3P^+CH_3I^-$ (14.4 g, 35.7 mmol) in anhydrous toluene (100 mL) under argon was added potassium hexamethyldisilazane (15% in toluene, 47.5 g, 35.7 mmol) dropwise. After stirring for 15 min, a solution of **5a** (10 g, 29.8 mmol) in toluene (100 mL) was added dropwise to the reaction mixture. After stirring for 2.5 h at room temperature, the reaction was quenched with saturated aqueous sodium bicarbonate (15 mL). After separation, the organic layer was washed with water (2×) and brine (2×), and dried over anhydrous sodium sulfate. After filtration and removal of the volatiles under reduced pressure, the residue was purified by flash column using petroleum ether (PE) as eluent to give **6a** as a colorless oil (8.1 g, 81%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.54 (s, 1H), 7.33 (s, 1H), 7.15 (d, J = 8.1 Hz, 2H), 7.10 (d, J = 8.1 Hz, 2H), 6.94 (dd, J = 17.4, 10.8 Hz, 1H), 5.69 (d, J = 17.4 Hz, 1H), 5.37 (d, J = 10.8 Hz, 1H), 4.02 (s, 2H), 2.63 (q, J = 7.5 Hz, 2H), 1.23 (t, J = 7.5 Hz, 3H).

4.1.5. Synthesis of 1-bromo-4-chloro-5-(4-ethoxybenzyl)-2-vinylbenzene (6b)

Compound **6b** was prepared from **5b** in 94% yield as a yellow solid using a similar method as used for the preparation of **6a**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.53 (s, 1H), 7.29 (s, 1H), 7.09 (d, J = 8.7 Hz, 2H), 6.93 (dd, J = 17.7, 11.1 Hz, 1H), 6.83 (d, J = 8.7 Hz, 2H), 5.68 (d, J = 17.7 Hz, 1H), 5.37 (d, J = 11.1 Hz, 1H), 4.01 (q, J = 7.5 Hz, 2H), 3.97 (s, 2H), 1.40 (t, J = 6.9 Hz, 3H).

4.1.6. Synthesis of 2-(2-bromo-5-chloro-4-(4-ethylbenzyl)-phenyl)ethanol (7a)

To a stirred 0 °C solution of **6a** (453 mg, 1.3 mmol) in THF (5 mL) was added 9-BBN (0.5 M in THF, 3.2 mL, 1.6 mmol) dropwise over 30 min. After the addition was complete, the ice bath was removed and the reaction mixture was stirred at room temperature overnight. After cooling to 0 °C, methanol (2.2 mL), 2 M aqueous sodium hydroxide (5.6 mL) and 30% hydrogen peroxide (1.2 mL) were added to the reaction mixture successively. After stirring for 3 h at room temperature, the mixture was extracted 3× with ethyl acetate. The combined organic extracts were washed with brine and dried over sodium sulfate. After filtration and removal of the volatiles under reduced pressure, the residue was purified by flash column using 100% PE to 5:1 PE/ethyl acetate as eluent to give **7a** (336 mg, 70%) as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.34 (s, 1H), 7.30 (s, 1H), 7.13 (q, 4H), 4.00 (s, 2H), 3.87 (t, J = 6.6 Hz, 2H), 2.96 (t, J = 6.6 Hz, 2H), 2.63 (q, J = 7.6 Hz, 2H), 1.23 (t, J = 7.6 Hz, 3H).

4.1.7. Synthesis of 2-(2-bromo-5-chloro-4-(4-ethoxybenzyl)-phenyl)ethanol (7b)

Compound **7b** was prepared from **6b** in 64% yield using a similar method as used for the preparation of **6a**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.30 (s, 1H), 7.29 (s, 1H), 7.09 (d, J = 8.0 Hz, 2H), 6.83 (d, J = 8.0 Hz, 2H), 4.01 (t, J = 6.9 Hz, 2H), 3.96 (s, 2H), 3.87 (t, J = 6.6 Hz, 2H), 2.95 (t, J = 6.6 Hz, 2H), 1.40 (t, J = 6.9 Hz, 3H).

4.1.8. Synthesis of 1-bromo-4-chloro-5-(4-ethylbenzyl)-2-(methoxymethyl)benzene (8a)

To a stirred solution of **4a** (0.5 g, 1.5 mmol) and tetrabutylammonium iodide (42 mg) in anhydrous THF (5 mL) under argon was added sodium hydride (60% suspension in mineral oil, 0.11 g, 2.8 mmol) in portions. After stirring for 20 min, iodomethane (0.43 g, 3 mmol) was added dropwise to the reaction mixture. After stirring overnight at room temperature, the reaction was quenched with ice water and extracted 3× with ethyl acetate. The combined organic extracts were washed with brine and dried over anhydrous sodium sulfate. After filtration and removal of the volatiles under reduced pressure, the residue was purified by flash column using 10:1 PE/ethyl acetate as eluent to give **8a** (404 mg, 78%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 (s, 1H), 7.35 (s, 1H), 7.18 (d, J = 8.4 Hz, 2H), 7.13 (d, J = 8.4 Hz, 2H), 4.55 (s, 2H), 4.02 (s, 2H), 3.43 (s, 3H), 2.65 (q, J = 7.8 Hz, 2H), 1.25 (t, J = 7.8 Hz, 3H).

4.1.9. Synthesis of 1-(allyloxymethyl)-2-bromo-5-chloro-4-(4-ethylbenzyl)benzene (8b)

To a stirred, cold (−5 °C) solution of **4a** (3.4 g, 10 mmol) in anhydrous DMF (50 mL) under argon was added sodium hydride (60% suspension in mineral oil, 0.8 g, 20 mmol) in portions. After stirring for 1 h, tetrabutylammonium iodide (0.37 g, 1 mmol) was added followed by addition of a solution of allyl bromide (1.45 g, 12 mmol) in DMF (10 mL). After stirring for 1 h at the same temperature, the reaction was quenched with ice water and extracted 3× with ethyl acetate. The combined organic extracts were washed with brine and dried over anhydrous sodium sulfate. After filtration and removal of the volatiles under reduced pressure, the residue was purified by flash column using 10:1 PE/ethyl acetate as eluent to give **8b** (3.22 g, 85%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.56 (s, 1H), 7.35 (s, 1H), 7.18 (d, J = 8.4 Hz, 2H), 7.13 (d, J = 8.4 Hz, 2H), 6.07–5.96 (m, 1H), 5.42–5.36 (m, 1H), 5.29 (dd, J = 17.0, 10.2 Hz, 1H), 4.54 (s, 2H), 4.16–4.14 (m, 2H), 4.06 (s, 2H), 2.67 (q, J = 7.6 Hz, 2H), 1.27 (t, J = 7.8 Hz, 3H).

4.1.10. Synthesis of 1-bromo-4-chloro-5-(4-ethoxybenzyl)-2-(2-methoxyethyl)benzene (9a)

Compound **9a** was prepared from **7a** in 82% yield using a similar method as used for the preparation of **8a**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.32 (s, 1H), 7.0 (s, 1H), 7.08 (d, J = 8.4 Hz, 2H), 6.78 (d, J = 8.4 Hz, 2H), 4.01 (q, J = 7.2 Hz, 2H), 3.35 (s, 3H), 3.66 (t, J = 7.0 Hz, 2H), 2.98 (t, J = 7.0 Hz, 2H), 1.35 (t, J = 7.2 Hz, 3H).

4.1.11. Synthesis of 1-(2-(allyloxy)ethyl)-2-bromo-5-chloro-4-(4-ethylbenzyl)benzene (9b)

Compound **9b** was prepared from **7b** in 91% yield using a similar method as used for the preparation of **8b**. ^{1}H NMR (300 MHz,

$CDCl_3$) δ 7.31 (s, 1H), 7.30 (s, 1H), 7.14 (d, J = 8.4 Hz, 2H), 7.10 (d, J = 8.4 Hz, 2H), 5.97–5.84 (m, 1H), 5.31–5.23 (m, 1H), 5.21–5.15 (m, 1H), 4.02–3.98 (m, 4H), 3.64 (t, J = 7.0 Hz, 2H), 2.98 (t, J = 7.0 Hz, 2H), 2.63 (q, J = 7.8 Hz, 2H), 1.24 (t, J = 7.8 Hz, 3H).

4.1.12. Synthesis of (2*S*,3*R*,4*R*,5*S*,6*R*)-2-(4-chloro-5-(4-ethylbenzyl)-2-(methoxymethyl)phenyl)-6-(hydroxymethyl)tetrahydro-2*H*-pyran-3,4,5-triol (11a)

To a stirred −78 °C solution of **8a** (54 mg, 0.15 mmol) in 2:1 anhydrous toluene/THF (0.8 mL) was added *n*-BuLi (2.5 M in hexane, 0.07 mL, 0.18 mmol) dropwise, keeping the internal temperature below −60 °C. After stirring for 40 min, a solution of **10** (86 mg, 0.18 mmol) in toluene (0.8 mL) was added to the above reaction solution. After stirring for another 1.5 h, the reaction was quenched with saturated aqueous ammonium chloride (5 mL). The mixture was extracted 3× with ethyl acetate. The combined organic extracts were washed with brine and dried over anhydrous sodium sulfate. After filtration and removal of the volatiles, the resulting residue was redissolved in 1:1 DCM/acetonitrile (0.8 mL) and cooled to −15 to −10 °C. Et_3SiH (48 μL, 0.3 mmol) was added followed by addition of $BF_3{\cdot}OEt_2$ (29 μL, 0.23 mmol) while keeping the internal temperature below −25 °C. After the addition was complete, the reaction mixture was allowed to warm to −15 °C and stirred for 5 h. Saturated aqueous sodium bicarbonate was then added to quench the reaction. After concentration, the resulting residue was extracted 3× with ethyl acetate. The combined organic extracts were washed 1× with water and brine respectively, and dried over anhydrous sodium sulfate prior to filtration and concentration under reduced pressure. The resulting residue was purified by preparative LC–MS to give **11a** (15 mg, 22%) as a white solid. ^{1}H NMR (400 MHz, CD_3OD) δ 7.47 (s, 1H), 7.40 (s, 1H), 7.09 (s, 4H), 4.69 (d, J = 12.4 Hz, 1H), 4.49 (d, J = 12.4 Hz, 1H), 4.42 (d, J = 8.8 Hz, 1H), 4.08 (d, J = 15.0 Hz, 1H), 4.03 (d, J = 15.0 Hz, 1H), 3.86 (d, J = 12.4 Hz, 1H), 3.70–3.63 (m, 1H), 3.50–3.37 (m, 7H), 2.58 (q, J = 7.8 Hz, 2H), 1.19 (t, J = 7.8 Hz, 3H); ESI MS (m/z): 437 $[M+H]^+$, 481 $[M+HCO_2]^-$, calcd 436.

4.1.13. Synthesis of (2*S*,3*R*,4*R*,5*S*,6*R*)-2-(2-(allyloxymethyl)-4-chloro-5-(4-ethylbenzyl)phenyl)-6-(hydroxymethyl)tetrahydro-2*H*-pyran-3,4,5-triol (11b)

Compound **11b** was prepared from **8b** in 35% yield as a white solid using a similar method as used for the preparation of **11a**. ^{1}H NMR (400 MHz, CD_3OD) δ 7.48 (s, 1H), 7.43 (s, 1H), 7.11 (s, 4H), 6.04–5.98 (m, 1H), 5.35 (dd, J = 16.4, 1.6 Hz, 1H), 5.23 (dd, J = 10.0, 1.6 Hz, 1H), 4.76 (d, J = 12.2 Hz, 1H), 4.68 (d, J = 12.2 Hz, 1H), 4.47 (d, J = 9.2 Hz, 1H), 4.13–4.02 (m, 4H), 3.87 (dd, J = 12.4, 2.4 Hz, 1H), 3.71–3.66 (m, 1H), 3.50–2.74 (m, 4H), 2.60 (q, J = 7.2 Hz, 2H), 1.13 (d, J = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 140.3, 136.4, 135.5, 135.2, 135.1, 132.9, 131.5, 128.8, 127.2, 126.8 (2C), 125.9 (2C), 114.8, 79.5, 77.0, 75.9, 73.5, 69.5, 68.9, 66.9, 60.1, 36.5, 26.5, 13.4; ESI MS (m/z): 463 $[M+H]^+$, 480 $[M+NH_4]^+$, 507 $[M+HCO_2]^-$, calcd 462.

4.1.14. Synthesis of (2*S*,3*R*,4*R*,5*S*,6*R*)-2-(4-chloro-5-(4-ethoxybenzyl)-2-(2-methoxyethyl)phenyl)-6-(hydroxymethyl)tetrahydro-2*H*-pyran-3,4,5-triol (12a)

Compound **12a** was prepared from **9a** in 40% yield as a white solid using a similar method as used for the preparation of **11a**. ^{1}H NMR (300 MHz, CD_3OD) δ 7.37 (s, 1H), 7.24 (s, 1H), 7.08 (d, J = 8.7 Hz, 2H), 6.78 (d, J = 8.7 Hz, 2H), 4.42 (d, J = 9.0 Hz, 1H), 4.02–3.94 (m, 4H), 3.85 (d, J = 12.3 Hz, 1H), 3.67–3.58 (m, 3H), 3.49–3.43 (m, 2H), 3.40–3.36 (m, 2H), 3.34 (s, 3H), 3.10–2.99 (m, 1H), 2.95–2.85 (m, 1H), 1.35 (t, J = 7.2 Hz, 3H); ESI MS (m/z): 467 $[M+H]^+$, 511 $[M+HCO_2]^-$, calcd 466.

4.1.15. Synthesis of (2*S*,3*R*,4*R*,5*S*,6*R*)-2-(2-(2-(allyloxy)ethyl)-4-chloro-5-(4-ethylbenzyl)phenyl)-6-(hydroxymethyl)tetrahydro-2*H*-pyran-3,4,5-triol (12b)

Compound **12b** was prepared from **9b** in 58% yield as a white solid using a similar method as used for the preparation of **11a**. ^{1}H NMR (300 MHz, CD_3OD) δ 7.39 (s, 1H), 7.26 (s, 1H), 7.08 (s, 4H), 5.97–5.84 (m, 1H)?5.29–5.12 (m, 2H)?4.44 (d, J = 9.0 Hz, 1H), 4.03–3.97 (m, 4H), 3.85 (d, J = 11.1 Hz, 1H), 3.70–3.62 (m, 3H), 3.48–3.37 (m, 4H), 3.12–3.03 (m, 1H), 2.95–2.86 (m, 1H), 2.58 (q, J = 7.6 Hz, 2H), 1.19 (t, J = 7.6 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 140.2, 136.5, 135.4, 135.1, 135.0, 133.1, 131.3, 128.8, 128.7, 126.8 (2C), 125.8 (2C), 144.3, 79.5, 77.0, 76.1, 73.1, 69.9, 68.9 (2C), 60.1, 36.5, 30.5, 26.5, 13.3; ESI MS (m/z): 477 $[M+H]^+$, 521 $[M+HCO_2]^-$, calcd 476.

4.1.16. Synthesis of (2*S*,3*S*,4*R*,5*R*,6*R*)-2-(2-(allyloxymethyl)-4-chloro-5-(4-ethylbenzyl)phenyl)-3,4,5-tris(benzyloxy)-6-(benzyloxymethyl)tetrahydro-2*H*-pyran (14)

To a stirred −78 °C solution of **8b** (3.2 g, 8.5 mmol) in anhydrous THF (30 mL) was added *n*-BuLi (2.5 M in hexane, 3.7 mL, 9.4 mmol) dropwise, keeping the internal temperature below −60 °C. After stirring for 2 h, a solution of **13** (6.9 g, 12.8 mmol) in THF (20 mL) was added to the above reaction solution. After stirring for another 2 h, the reaction was quenched with saturated aqueous ammonium chloride (10 mL). The mixture was extracted 3× with ethyl acetate. The combined organic extracts were washed with brine and dried over anhydrous sodium sulfate. After filtration and removal of the volatiles, the resulting residue was redissolved in acetonitrile (30 mL) and cooled to −40 °C. Et_3SiH (1.5 g, 13 mmol) was added followed by addition of $BF_3{\cdot}OEt_2$ (1.8 g, 13 mmol) while keeping the internal temperature below −25 °C. After the addition was complete, the reaction mixture was allowed to warm to −15 °C and stirred for 2 h. Saturated aqueous sodium bicarbonate was then added to quench the reaction. After concentration, the resulting residue was extracted 3× with ethyl acetate. The combined organic extracts were washed 1× with water and brine, respectively, and dried over anhydrous sodium sulfate prior to filtration and concentration under reduced pressure. The resulting residue was purified by flash column using 40:1 PE/ethyl acetate as eluent to give **14** (2.0 g, 28%). ESI MS (m/z): 840 $[M+NH_4]^+$, calcd 822.

4.1.17. Synthesis of 2-(5-chloro-4-(4-ethylbenzyl)-2-((2*S*,3*S*,4*R*,5*R*,6*R*)-3,4,5-tris(benzyloxy)-6-(benzyloxymethyl)tetrahydro-2*H*-pyran-2-yl)benzyloxy)acetaldehyde (15)

Ozone was bubbled through a stirred −78 °C solution of **14** (0.2 g, 0.24 mmol) in DCM (100 mL) until the color of solution turned to blue. At this point, argon was bubbled through the reaction solution until blue disappeared. A solution of triphenylphosphine (0.19 g, 0.72 mmol) in DCM (2 mL) was then added. The reaction solution was allowed to warm to room temperature and stirred for 30 min. After removal of the volatiles under reduced pressure, the residue was purified by flash column using 5:1 PE/ethyl acetate as eluent to give **15** (152 mg, 75%). ^{1}H NMR (300 MHz, CD_3OD) δ 9.57 (s, 1H), 7.29 (m, 13 H), 7.22 (m, 6H), 7.04 (m, 4H), 6.84 (m, 2H), 4.87 (m, 4H), 4.52 (m, 1H), 4.43 (m, 5H), 4.05 (dd, J = 18.0, 15.0 Hz, 2H), 3.96 (m, 5H), 3.77 (m, 5H), 3.71 (m, 2H), 2.56 (q, J = 7.5 Hz, 2H), 1.76 (t, J = 7.5 Hz, 3H); ESI MS (m/z): 842 $[M+NH_4]^+$, calcd 824.

4.1.18. Synthesis of 2-(5-chloro-4-(4-ethylbenzyl)-2-((2*S*,3*S*,4*R*,5*R*,6*R*)-3,4,5-tris(benzyloxy)-6-(benzyloxymethyl)tetrahydro-2*H*-pyran-2-yl)benzyloxy)ethanol (16)

To a stirred 0 °C solution of **15** (0.14 g, 0.17 mmol) in THF (10 mL) was added solid sodium borohydride (13 mg, 0.34 mmol). After stirring for 1.5 h at room temperature, the reaction was

quenched with methanol (2 mL). After removal of the volatiles under reduced pressure, the residue was purified by flash column using 5:1 PE/ethyl acetate as eluent to give **16** (135 mg, 96%). ESI MS (*m*/*z*): 844 $[M+NH_4]^+$, calcd 826.

4.1.19. Synthesis of (2*S*,3*R*,4*R*,5*S*,6*R*)-2-(4-chloro-5-(4-ethylbenzyl)-2-((2-hydroxyethoxy)methyl)phenyl)-6-(hydroxymethyl)-tetrahydro-2*H*-pyran-3,4,5-triol (17)

The mixture of **16** (50 mg, 0.06 mmol), 10% Pd/C (50 mg), 1,2-dichlorobenzene (45 mg, 0.3 mmol) and 2:1 THF/methanol (3 mL) was stirred for 3 h under hydrogen atmosphere (1 atm.). After filtration of the solid and removal of the volatiles under reduced pressure, the residue was purified by preparative LC–MS to give **17** (20 mg, 71%) as a white solid. ^{1}H NMR (300 MHz, CD_3OD) δ 7.46 (s, 1H), 7.45 (s, 1H), 7.07 (s, 4H), 4.74 (d, J = 12.0 Hz, 1H), 4.60 (d, J = 12.0 Hz, 1H), 4.47 (d, J = 9.3 Hz, 1H), 4.08 (d, J = 14.7 Hz, 1H), 4.02 (d, J = 15.0 Hz, 1H), 3.86 (d, J = 11.8 Hz, 1H), 3.72–3.58 (m, 5H), 3.51–3.37 (m, 4H), 2.57 (q, J = 7.5 Hz, 2H), 1.18 (t, J = 7.5 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 141.9, 138.0, 137.1, 136.8 (2C), 133.0, 130.4, 129.0, 128.3 (2C), 127.4 (2C), 81.0, 78.5, 77.3, 75.1, 71.6, 70.5, 69.6, 61.7, 60.8, 38.1, 28.1, 14.9; ESI MS (*m*/*z*): 467 $[M+H]^+$, 511 $[M+HCO_2]^-$, calcd 466.

4.1.20. Synthesis of (2*S*,3*R*,4*R*,5*S*,6*R*)-2-(4-chloro-5-(4-ethylbenzyl)-2-((2-methoxyethoxy)methyl)phenyl)-6-(hydroxymethyl)-tetrahydro-2*H*-pyran-3,4,5-triol (18)

To a stirred 0 °C solution of **16** (60 mg, 0.072 mmol) in anhydrous THF (2 mL) under argon was added 60% NaH (suspended in mineral oil, 35 mg, 0.86 mmol). After stirring for 1 h at the same temperature, tetrabutylammonium iodide (5 mg) and iodomethane (15 mg, 0.11 mmol) was added to the reaction mixture. After stirring overnight at room temperature, the reaction was quenched with ice water and extracted 3× with ethyl acetate. The combined organic extracts were washed with brine and dried over anhydrous sodium sulfate. Removal of the volatiles under reduced pressure gave benzyl-protected product (60 mg). Debenzylation of the above intermediate using a similar method as used for the preparation of **17** gave **18** (16 mg, 45% two steps) as a white solid. ^{1}H NMR (300 MHz, CD_3OD) δ 7.46 (s, 1H), 7.42 (s, 1H), 7.07 (s, 4H), 4.74 (d, J = 12.0 Hz, 1H), 4.58 (d, J = 12.0 Hz, 1H), 4.46 (d, J = 9.3 Hz, 1H), 4.08 (d, J = 15.0 Hz, 1H), 4.02 (d, J = 15.0 Hz, 1H), 3.85 (d, J = 12.0 Hz, 1H), 3.68–3.62 (m, 3H), 3.60–3.56 (m, 2H), 3.47–3.33 (m, 7H), 2.58 (q, J = 7.5 Hz, 2H), 1.19 (t, J = 7.5 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 140.3, 136.5, 135.4, 135.3, 135.2, 131.4, 128.8, 127.3, 126.8 (2C), 125.9 (2C), 79.5, 77.0, 75.8, 73.6, 69.9, 68.9, 68.0, 67.7, 60.2, 56.2, 36.5, 26.5, 13.3; ESI MS (*m*/*z*) 481 $[M+H]^+$, 498 $[M+NH_4]^+$, 525 $[M+HCO_2]^-$, calcd 480.

4.1.21. Synthesis of (2*S*,3*R*,4*R*,5*S*,6*R*)-2-(4-chloro-5-(4-ethylbenzyl)-2-((2-fluoroethoxy)methyl)phenyl)-6-(hydroxymethyl)tetrahydro-2*H*-pyran-3,4,5-triol (19)

To a stirred −78 °C solution of **16** (150 mg, 0.18 mmol) in anhydrous DCM (2 mL) under argon was added DAST (36 μL, 0.27 mmol). The reaction solution was allowed to slowly warm to room temperature and stirred for 3 h. The reaction was then quenched with methanol and the mixture was evaporated in vacuum. The residue was taken up with water and ethyl acetate. After separation of the layers, the organic layer was washed with brine and dried over anhydrous sodium sulfate. After filtration and removal of the volatiles under reduced pressure, the residue was purified by preparative TLC using 5:1 PE/ethyl acetate as eluent to give benzyl-protected product (100 mg). Debenzylation of the above intermediate using a similar method as used for the preparation of **17** gave **19** (41 mg, 48% two steps) as a white solid. ^{1}H NMR (300 MHz, CD_3OD) δ 7.47 (s, 1H), 7.42 (s, 1H), 7.07 (s, 4H), 4.80 (d, J = 12.3 Hz, 1H), 4.66–4.59 (m, 2H), 4.50–4.44 (m, 2H), 4.08 (d, J = 15.0 Hz, 1H), 4.02 (d, J = 15.0 Hz, 1H), 3.84–3.78 (m, 2H), 3.71–3.61 (m, 2H), 3.45–3.31 (m, 4H), 2.58 (q, J = 7.8 Hz, 2H), 1.18 (t, J = 7.8 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 140.3, 136.5, 135.3, 135.2, 135.1, 131.5, 128.8, 127.2, 126.8 (2C), 125.9 (2C), 81.1 (d, J = 167 Hz), 79.4, 77.0, 75.8, 73.5, 68.9, 67.9, 67.8, 60.1, 36.6, 26.5, 13.4; ^{19}F NMR (376 MHz, CD_3OD) δ −224.2 to −224.5 (m, 1F); ESI MS (*m*/*z*): 469 $[M+H]^+$, 486 $[M+NH_4]^+$, 513 $[M+HCO_2]^-$, calcd 468.

4.1.22. Synthesis of (2*S*,3*R*,4*R*,5*S*,6*R*)-2-(4-chloro-2-((2,2-difluoroethoxy)methyl)-5-(4-ethylbenzyl)phenyl)-6-(hydroxymethyl)-tetrahydro-2*H*-pyran-3,4,5-triol (20)

Compound **20** was prepared from **15** in 39% yield as a white solid using a similar method as used for the preparation of **19**. ^{1}H NMR (300 MHz, CD_3OD) δ 7.46 (s,1H) 7.41 (s, 1H), 7.07 (s, 4H), 5.99 (tt, J = 55.2, 3.9 Hz, 1H), 4.86 (d, J = 12.6 Hz, 1H), 4.65 (d, J = 12.6 Hz, 1H), 4.44 (d, J = 9.0 Hz, 1H), 4.08 (d, J = 15.0 Hz, 1H), 4.02 (d, J = 15.0 Hz, 1H), 3.85 (d, J = 11.4 Hz, 1H), 3.78–3.62 (m, 3H), 3.50–3.35 (m, 4H), 2.58 (q, J = 7.5 Hz, 2H), 1.19 (t, J = 7.5 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 140.3, 136.7, 135.2, 135.1, 134.8, 131.5, 128.9, 127.1, 126.8 (2C), 125.9 (2C), 113.1 (t, J = 238 Hz), 79.4, 76.9, 75.8, 73.4, 68.8, 68.5, 67.5 (t, J = 27 Hz), 36.5, 26.5, 13.3; ^{19}F NMR (376 MHz, CD_3OD) δ -126.9 (t, J = 14.3 Hz, 1F), -127.1 (t, J = 14.3 Hz, 1F); ESI MS (*m*/*z*): 504 $[M+NH_4]^+$, 531 $[M+HCO_2]^-$, calcd 486.

4.1.23. Synthesis of (5-chloro-4-(4-ethylbenzyl)-2-((2*S*,3*S*,4*R*,5*R*,6*R*)-3,4,5-tris(benzyloxy)-6-(benzyloxymethyl)tetrahydro-2*H*-pyran-2-yl)phenyl)methanol (21)

The mixture of **14** (50 mg, 0.061 mmol), $PdCl_2$ (24 mg, 0.13 mmol), sodium acetate (76 mg, 0.29 mmol) and 9:1 acetic acid/H_2O (1 mL) was stirred at 70 °C for 1 h. Upon cooling to room temperature, water and ethyl acetate was added. After separation of the layers, the aqueous layer was extracted 2× with ethyl acetate. The combined extracts were washed 1× with water and brine, respectively, and dried over sodium sulfate. After filtration and removal of the volatiles under reduced pressure, the residue was purified by preparative TLC using 3:1 PE/ethyl acetate as eluent to give **21** (30 mg, 63%). ESI MS (*m*/*z*): 800 $[M+NH_4]^+$, calcd 782.

4.1.24. Synthesis of (2*S*,3*R*,4*R*,5*S*,6*R*)-2-(4-chloro-5-(4-ethylbenzyl)-2-((2,2,2-trifluoroethoxy)methyl)phenyl)-6-(hydroxymethyl)tetrahydro-2*H*-pyran-3,4,5-triol (22)

To a stirred solution of **21** (0.78 g, 1 mmol) in toluene (2 mL) was added ADDP (0.5 g, 2 mmol) at room temperature. The reaction mixture was then stirred for 10 min and tributylphosphine (0.5 mL, 2 mmol) was added. After stirring for another 1 h, 2,2,2-trifluoroethanol (0.72 mL, 10 mmol) was added. The reaction mixture was stirred overnight. After removal of the volatiles, the residue was purified by flash column using 10:1 to 3:1 PE/ethyl acetate as eluent to give benzyl-protected product. Debenzylation of the above intermediate using a similar method as used for the preparation of **17** gave **22** (50 mg, 10% two steps) as a white solid. ^{1}H NMR (300 MHz, CD_3OD) δ 7.47(s, 1H), 7.41 (s, 1H), 7.08 (s, 4H), 4.94 (d, J = 12.0 Hz, 1H), 4.71 (d, J = 12.0 Hz, 1H), 4.42 (d, J = 9.3 Hz, 1H), 4.06 (d, J = 6.6 Hz, 2H), 3.98 (t, J = 8.7 Hz, 2H), 3.85 (d, J = 1.2 Hz, 1H), 3.69–3.63 (m, 1H), 3.46–3.36 (m, 4H), 2.58 (q, J = 7.5 Hz, 2H), 1.18 (t, J = 7.8 Hz, 3H); ESI MS (*m*/*z*): 505 $[M+H]^+$, 549 $[M+HCO_2]^-$, calcd 504.

4.1.25. Synthesis of (2*S*,3*R*,4*R*,5*S*,6*R*)-2-(4-chloro-5-(4-ethylbenzyl)-2-((3-hydroxypropoxy)methyl)phenyl)-6-(hydroxymethyl)-tetrahydro-2*H*-pyran-3,4,5-triol (23)

To a stirred 0 °C solution of **11b** (22 mg, 0.048 mmol) in THF (0.6 mL) was added BH_3·THF (1 M in THF, 0.5 mL, 0.5 mmol) dropwise. After stirring for 2 h at room temperature, 30%

aqueous sodium hydroxide (1 mL) was added followed by addition of 30% aqueous hydrogen peroxide (1 mL). After stirring for another 1.5 h, the mixture was extracted 3× with ethyl acetate. The combined extracts were washed 1× with water and brine respectively, and dried over sodium sulfate. After filtration and removal of the volatiles under reduced pressure, the residue was purified by preparative TLC using 10:1 ethyl acetate/methanol as eluent to give desired product **23** (5 mg, 22%) and isomer **25** (4 mg, 17%) as white solid. ^{1}H NMR (300 MHz, CD_3OD) δ 7.46 (s, 1H), 7.40 (s, 1H), 7.08 (s, 4H), 4.73 (d, J = 12.2 Hz, 1H), 4.53 (d, J = 12.2 Hz, 1H), 4.45 (d, J = 9.0 Hz, 1H), 4.08 (d, J = 15.0 Hz, 1H), 4.02 (d, J = 15.0 Hz, 1H), 3.86 (d, J = 12.0 Hz, 1H), 3.68–3.60 (m, 4H), 3.44–3.29 (m, 5H), 2.58 (q, J = 7.6 Hz, 2H), 1.86–1.79 (m, 2H), 1.19 (t, J = 7.6 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 140.3, 136.4, 135.6, 135.2, 135.1, 131.4, 128.8, 127.3, 126.8 (2C), 125.8 (2C), 79.5, 77.0, 75.9, 73.5, 68.9, 67.8, 65.6, 60.1, 57.0, 36.5, 30.7, 26.5, 13.3; ESI MS (m/z): 481 $[M+H]^+$, 525 $[M+HCO_2]^-$, calcd 480.

4.1.26. Synthesis of 1-(5-chloro-4-(4-ethylbenzyl)-2-((2*S*,3*R*,4*R*,5*S*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-2-yl)benzyloxy)propan-2-one (24)

The mixture of $PdCl_2$ (5.3 mg, 0.03 mmol), CuCl (15 mg, 0.15 mmol) and 10:1 DMF/H_2O (2 mL) was stirred for 2.5 h under oxygen atmosphere (1 atm.). Compound **11b** (0.13 g, 0.28 mmol) was then added. After stirring overnight at room temperature, water was added and the mixture was extracted 3× with ethyl acetate. The combined extracts were washed 1× with water and brine respectively, and dried over sodium sulfate. After filtration and removal of the volatiles under reduced pressure, the residue was purified by preparative TLC using 10:1 ethyl acetate/methanol as eluent to give **24** (122 mg, 91%) as gel. ^{1}H NMR (300 MHz, CD_3OD) δ 7.47 (s, 1H), 7.44 (s, 1H), 7.08 (s, 4H), 4.79 (d, J = 12.0 Hz, 1H), 4.60 (d, J = 12.0 Hz, 1H), 4.55 (d, J = 9.6 Hz, 1H), 4.22 (s, 2H), 4.10 (d, J = 14.8 Hz, 1H), 4.05 (d, J = 14.8 Hz, 1H), 3.85 (dd, J = 11.7, 2.1 Hz, 1H), 3.68–3.63 (m, 1H), 3.50–3.36 (m, 4H), 2.58 (q, J = 7.8 Hz, 2H), 2.12 (s, 3H), 1.19 (t, J = 7.8 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 205.8, 140.3, 136.8, 135.5, 135.2, 134.7, 131.5, 128.9, 127.6, 126.8 (2C), 125.9 (2C), 79.4, 76.9, 75.7, 73.6, 73.2, 68.9, 68.1, 60.1, 36.5, 26.5, 23.3, 13.3; ESI MS (m/z): 479 $[M+H]^+$, 523 $[M+HCO_2]^-$, calcd 478.

4.1.27. Synthesis of (2*S*,3*R*,4*R*,5*S*,6*R*)-2-(4-chloro-5-(4-ethylbenzyl)-2-((2-hydroxypropoxy)methyl)phenyl)-6-(hydroxymethyl)-tetrahydro-2*H*-pyran-3,4,5-triol (25)

To a stirred solution of **24** (3.7 mg, 0.008 mmol) in anhydrous THF (0.5 mL) under argon was added sodium borohydride (1.8 mg, 0.046 mmol) at room temperature. After stirring for 2 h, the reaction was quenched with saturated aqueous ammonium chloride. The mixture was extracted 2× with ethyl acetate. The combined organic extracts were washed with water and brine, dried over sodium sulfate. After filtration and removal of the volatiles under reduced pressure, the residue was purified by preparative TLC using 10:1 ethyl acetate/methanol as eluent to give **25** (2.5 mg, 67%) as white solid. ^{1}H NMR (300 MHz, CD_3OD) δ 7.45 (s, 1H), 7.43 (s, 1H), 7.08 (s, 4H), 4.85–4.71 (m, 2H), 4.58 (dd, J = 12.0, 3.9 Hz, 1H), 4.47 (dd, J = 9.3, 3.3 Hz, 1H), 4.10 (d, J = 14.8 Hz, 1H), 4.05 (d, J = 14.8 Hz, 1H), 3.95–3.83 (m, 1H), 3.85 (dd, J = 11.7, 1.5 Hz, 1H), 3.68–3.62 (m, 1H), 3.47–3.33 (m, 5H), 2.60 (q, J = 7.8 Hz, 2H), 1.19 (t, J = 7.8 Hz, 3H), 1.15 (dd, J = 6.6, 1.8 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 140.3, 136.5, 135.5, 135.3, 135.2, 131.4, 128.8, 127.4, 126.8 (2C), 125.9 (2C), 79.5, 77.0, 75.7, 74.2, 73.5, 68.9, 68.2, 64.5, 60.1, 36.5, 26.5, 16.8, 13.3; ESI MS (m/z): 481 $[M+H]^+$, 525 $[M+HCO_2]^-$, calcd 480.

4.1.28. Synthesis of (2*S*,3*R*,4*R*,5*S*,6*R*)-2-(4-chloro-5-(4-ethylbenzyl)-2-((2-(methylamino)propoxy)methyl)phenyl)-6-(hydroxymethyl)tetrahydro-2*H*-pyran-3,4,5-triol (26)

To a stirred 0 °C solution of **24** (0.48 g, 1 mmol) in ethanol (5 mL) was added a solution of methylamine in ethanol (10 mmol) and followed by addition of catalytic amount of acetic acid. After stirring for 1 h, sodium cyanoborohydride (0.19 g, 3 mmol) was added. The reaction was monitored by LC–MS. Once the reaction was complete, the reaction mixture was evaporated prior to adding water and ethyl acetate. The organic layer was separated, washed with brine, and dried over sodium sulfate. After filtration and removal of the volatiles under reduced pressure, the residue was purified by preparative LC–MS to give **26** (44 mg, 9%) as white solid. ^{1}H NMR (300 MHz, CD_3OD) δ 7.45 (s, 1H), 7.41 (s, 1H), 7.06 (s, 4H), 4.90–4.77 (m, 2H), 4.62–4.57 (m, 1H), 4.49 (d, J = 9.0 Hz, 1H), 4.04 (s, 2H), 3.88 (d, J = 11.7 Hz, 1H), 3.75–3.59 (m, 2H), 3.55–3.38 (m, 5H), 2.65–2.53 (m, 6H), 1.27 (d, J = 6.9 Hz, 3H), 1.19 (t, J = 7.5 Hz, 3H); ESI MS (m/z): 494 $[M+H]^+$, 538 $[M+HCO_2]^-$, calcd 493.

4.1.29. Synthesis of 1-(5-chloro-4-(4-ethylbenzyl)-2-((2*S*,3*S*,4*R*,5*R*,6*R*)-3,4,5-tris(benzyloxy)-6-(benzyloxymethyl)tetrahydro-2*H*-pyran-2-yl)benzyloxy)propan-2-one (27)

Compound **27** was prepared from **14** in 58% yield using a similar method as used for the preparation of **24**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.46 (s, 2H), 7.32–7.27 (m, 13H), 7.20–7.10 (m, 5H), 7.04 (d, J = 8.1 Hz, 2H), 6.99 (d, J = 8.1 Hz, 2H), 6.79 (dd, J = 7.8, 1.8 Hz, 2H), 4.79 (s, 2H), 4.76–4.71 (m, 2H), 4.64–4.32 (m, 6H), 4.13 (s, 2H), 4.00 (s, 2H), 3.82–3.76 (m, 2H), 3.63–3.54 (m, 5H), 2.45 (q, J = 8.1 Hz, 2H), 1.99 (s, 3H), 1.06 (t, J = 8.1 Hz, 3H); ESI MS (m/z): 839 $[M+H]^+$, calcd 838.

4.1.30. Synthesis of 1-(5-chloro-4-(4-ethylbenzyl)-2-((2*S*,3*S*,4*R*,5*R*,6*R*)-3,4,5-tris(benzyloxy)-6-(benzyloxymethyl)tetrahydro-2*H*-pyran-2-yl)benzyloxy)propan-2-ol (28)

Compound **28** was prepared from **27** in 98% yield using a similar method as used for the preparation of **16**. ESI MS (m/z): 860 $[M+NH_4]^+$, calcd 842.

4.1.31. Synthesis of (2*S*,3*R*,4*R*,5*S*,6*R*)-2-(4-chloro-5-(4-ethylbenzyl)-2-((2-fluoropropoxy)methyl)phenyl)-6-(hydroxymethyl)-tetrahydro-2*H*-pyran-3,4,5-triol (29)

Compound **29** was prepared from **28** in 23% yield as a white solid using a similar method as used for the preparation of **19**. ^{1}H NMR (300 MHz, CD_3OD) δ 7.46 (s, 1H), 7.41 (s, 1H), 7.08 (s, 4H), 4.80 (d, J = 12.6 Hz, 1H), 4.59 (d, J = 12.3 Hz, 1H), 4.46 (dd, J = 9.3, 2.4 Hz, 1H), 4.08 (d, J = 15.0 Hz, 1H), 4.02 (d, J = 15.0 Hz, 1H), 3.85 (d, J = 12.0 Hz, 1H), 3.68–3.61 (m, 2H), 3.55 (d, J = 4.8 Hz, 1H), 3.46–3.35 (m, 4H), 2.58 (q, J = 7.5 Hz, 2H), 1.31 (ddd, J = 23.4, 6.6, 0.6 Hz, 3H), 1.19 (t, J = 7.5 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 140.3, 136.5, 135.3, 135.2, 135.1, 131.4, 128.8, 127.2, 126.8 (2C), 125.9 (2C), 87.5 (d, J = 167 Hz), 79.4, 77.0, 75.8, 73.5, 71.6 (d, J = 22 Hz), 70.7, 68.6, 59.7, 36.5, 26.5, 14.7 (d, J = 22 Hz), 13.3; ^{19}F NMR (376 MHz, CD_3OD) δ −180.2 to −180.7 (m, 1F); ESI MS (m/z): 483 $[M+H]^+$, 500 $[M+NH_4]^+$, 527 $[M+HCO_2]^-$, calcd 482.

4.1.32. Synthesis of (2*S*,3*R*,4*R*,5*S*,6*R*)-2-(4-chloro-2-((2,2-difluoropropoxy)methyl)-5-(4-ethylbenzyl)phenyl)-6-(hydroxymethyl)tetrahydro-2*H*-pyran-3,4,5-triol (30)

Compound **30** was prepared from **27** in 20% yield as a white solid using a similar method as used for the preparation of **19**. ^{1}H NMR (300 MHz, CD_3OD) δ 7.47 (s, 1H), 7.41 (s, 1H), 7.08 (s, 4H), 4.87 (d, J = 12.0 Hz, 1H), 4.64 (d, J = 12.0 Hz, 1H), 4.45 (d, J = 9.0 Hz, 1H), 4.08 (d, J = 15.0 Hz, 1H), 4.03 (d, J = 15.0 Hz, 1H), 3.85 (d, J = 12.0 Hz, 1H), 3.72–3.63 (m, 3H), 3.50–3.35 (m, 4H), 2.58 (q, J = 7.8 Hz, 2H), 1.64 (t, J = 18.6 Hz, 3H), 1.18 (t, J = 7.8 Hz,

3H); ^{13}C NMR (100 MHz, CD_3OD) δ 140.3, 136.6, 135.2 (2C), 134.9, 131.5, 128.8, 127.1, 126.8 (2C), 125.9 (2C), 120.6 (t, J = 237 Hz), 79.4, 76.9, 75.8, 73.5, 70.0 (t, J = 32 Hz), 68.8, 68.6, 60.1, 36.5, 26.5, 18.2 (t, J = 25 Hz), 13.4; ^{19}F NMR (376 MHz, CD_3OD) δ −99.3 to −99.5 (m, 2F); ESI MS (m/z): 518 $[M+NH_4]^+$, 545 $[M+HCO_2]^-$, calcd 500.

4.1.33. Synthesis of (2*R*,3*R*,4*R*,5*S*,6*S*)-2-(acetoxymethyl)-6-(2-(allyloxymethyl)-4-chloro-5-(4-ethylbenzyl)phenyl)tetrahydro-2*H*-pyran-3,4,5-triyl triacetate (31)

To a stirred solution of **11b** (1.2 g, 2.6 mmol) in DCM (18 mL) was added pyridine (2.1 mL, 26 mmol), acetic anhydride (2.7 mL, 29 mmol) and DMAP (32 mg) successively at room temperature. After stirring overnight, the reaction was quenched with water (10 mL). The mixture was extracted 2× with DCM. The combined organic extracts were washed 2× with 1 N HCl and brine, and dried over sodium sulfate. After filtration and removal of the volatiles under reduced pressure, the residue was purified by flash column using 3:1 PE/ethyl acetate as eluent to give **31** (1.1 g, 67%) as a white solid. ^{1}H NMR (300 MHz, CD_3OD) δ 7.61 (s, 1H), 7.26 (s, 1H), 7.10 (d, J = 8.4 Hz, 2H), 7.05 (d, J = 8.4 Hz, 2H), 6.04–5.93 (m, 1H), 5.52 (t, J = 9.6 Hz, 1H), 5.43–5.36 (m, 1H), 5.27–5.20 (m, 3H), 4.85 (d, J = 13.8 Hz, 1H), 4.58 (d, J = 13.5 Hz, 1H), 4.36 (dd, J = 12.3, 4.8 Hz, 1H), 4.16–4.10 (m, 2H), 4.08–3.95 (m, 5H), 2.59 (q, J = 7.8 Hz, 2H), 2.10 (s, 3H), 2.07–2.04 (m, 6H), 1.94 (s, 3H), 1.19 (t, J = 7.8 Hz, 3H); ESI MS (m/z): 648 $[M+NH_4]^+$, 675 $[M+HCO_2]^-$, calcd 630.

4.1.34. Synthesis of (2*R*,3*R*,4*R*,5*S*,6*S*)-2-(acetoxymethyl)-6-(4-chloro-5-(4-ethylbenzyl)-2-(hydroxymethyl)phenyl)tetrahydro-2*H*-pyran-3,4,5-triyl triacetate (32)

Compound **32** was prepared from **31** in 91% yield as a white solid using a similar method as used for the preparation of **21**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 (s, 1H), 7.12–7.05 (m, 5H), 5.36–5.27 (m, 2H), 5.20 (t, J = 9.6 Hz, 1H), 4.69 (s, 2H), 4.57 (d, J = 10.0 Hz, 1H), 4.23–4.12 (m, 2H), 4.06 (d, J = 15.2 Hz, 1H), 3.99 (d, J = 15.2 Hz, 1H), 3.85–3.81 (m, 1H), 2.60 (q, J = 7.6 Hz, 2H), 2.07 (s, 3H), 2.05 (s, 3H), 1.99 (s, 3H), 1.63 (s, 3H), 1.19 (t, J = 9.6 Hz, 3H); ESI MS (m/z): 608 $[M+NH_4]^+$, 635 $[M+HCO_2]^-$, calcd 590.

4.1.35. Synthesis of (2*S*,3*R*,4*R*,5*S*,6*R*)-2-(4-chloro-5-(4-ethylbenzyl)-2-(phenoxymethyl)phenyl)-6-(hydroxymethyl)tetrahydro-2*H*-pyran-3,4,5-triol (33)

A solution of phenol (22 mg, 0.23 mmol) and triphenylphosphine (60 mg, 0.23 mmol) in anhydrous THF (0.5 mL) under argon was stirred for 1 h at −5 °C. Then DIAD (45 μL, 0.23 mmol) was added dropwise to the above solution. After stirring for 1 h below 10 °C, a solution of **32** (68 mg, 0.12 mmol) in THF (0.5 mL) was added dropwise. The reaction mixture was stirred for another 5 h prior to concentration in vaccum. The resulting residue was purified by preparative TLC using 4:1 PE/ethyl acetate as eluent to give 23 mg of product as a white solid, which was redissolved in 2:3:1 THF/methanol/H_2O (0.6 mL). Solid $LiOH{\cdot}H_2O$ (1.8 mg, 0.043 mmol) was added to the above solution. After stirring overnight at room temperature, the reaction mixture was evaporated and the resulting residue was taken up with ethyl acetate and water. After separation, the organic layer was washed with brine, dried over sodium sulfate. After filtration and removal of the volatiles under reduced pressure, the residue was purified by preparative TLC using 8:80:1 PE/ethyl acetate/methanol as eluent to give **33** (11 mg, 65%) as a colorless gel. ^{1}H NMR (300 MHz, CD_3OD) δ 7.49 (s, 1H), 7.48 (m, 1H), 7.30–7.23 (m, 2H), 7.09 (s, 4H), 7.04–6.98 (m, 2H), 6.93 (t, J = 7.5 Hz, 1H), 5.31 (d, J = 12.6 Hz, 1H), 5.16 (d, J = 12.9 Hz, 1H), 4.44 (d, J = 9.3 Hz, 1H), 4.09 (d, J = 15.0 Hz, 1H), 4.03 (d, J = 15.0 Hz, 1H), 3.86 (d, J = 11.7 Hz, 1H), 3.70–3.62 (m, 1H), 3.47–3.35 (m, 4H), 2.58 (q, J = 7.5 Hz, 2H), 1.19 (t, J = 7.5 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 157.0, 140.3, 136.4, 135.2, 134.7, 134.6, 131.6, 128.9, 127.6 (2C), 126.8 (2C), 126.6, 125.9 (2C), 119.1, 113.0 (2C), 79.5, 76.9, 76.2, 73.4, 68.8, 64.9, 60.1, 36.5, 26.5, 13.3; ESI MS (m/z): 516 $[M+NH_4]^+$, 543 $[M+HCO_2]^-$, calcd 498.

4.1.36. Synthesis of (2*S*,3*R*,4*R*,5*S*,6*R*)-2-(4-chloro-5-(4-ethylbenzyl)-2-((prop-2-ynyloxy)methyl)phenyl)-6-(hydroxymethyl)-tetrahydro-2*H*-pyran-3,4,5-triol (34)

To a stirred, cold (−5 °C) solution of **11b** (306 mg, 0.66 mmol) in chloroform (5.0 mL) was added a solution of bromine (0.1 g, 0.66 mmol) in chloroform (1 mL) dropwise until the color of reaction solution turned from colorless to yellow. After stirring for 30 min, water (10 mL) was added to the reaction solution. The resulting mixture was extracted 2× with ethyl acetate. The combined organic layer was washed 2× with brine, dried over sodium sulfate, and finally evaporated to give 368 mg of dibromide as a yellow solid. A mixture of above obtained dibromide (53 mg, 0.086 mmol), solid potassium hydroxide (82%, 11 mg) and ethanol (1 mL) was refluxed for 6 h. After concentration of the solution, the residue was taken up with water and ethyl acetate. After separation of the layers, the organic layer was washed with 1 N HCl (2×) and brine (3×), dried over sodium sulfate. After filtration and removal of the volatiles under reduced pressure, the residue was purified by preparative LC–MS to give **34** (21 mg, 47% two steps) as a white solid. ^{1}H NMR (400 MHz, CD_3OD) δ 7.48 (s, 1H), 7.42 (s, 1H), 7.11 (s, 4H), 4.85 (d, J = 12.0 Hz, 1H), 4.62 (d, J = 12.0 Hz, 1H), 4.51 (d, J = 9.2 Hz, 1H), 4.23 (s, 2H), 4.10 (d, J = 14.8 Hz, 1H), 4.05 (d, J = 14.8 Hz, 1H), 3.88 (d, J = 12.4 Hz, 1H), 3.71–3.67 (m, 1H), 3.52–3.41 (m, 4H), 2.94 (s, 1H), 2.60 (q, J = 7.6 Hz, 2H), 1.21 (t, J = 7.6 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 141.9, 138.3, 136.9, 136.7, 136.3, 133.0, 130.4, 129.4, 128.4 (2C), 127.5 (2C), 81.0, 79.2, 78.5, 77.4, 75.2, 74.9, 70.4, 67.9, 61.7, 56.9, 38.2, 28.1, 15.0; ESI MS (m/z): 461 $[M+H]^+$, 478 $[M+NH_4]^+$, 505 $[M+HCO_2]^-$, calcd 460.

4.1.37. Synthesis of (2*S*,3*R*,4*R*,5*S*,6*R*)-2-(4-chloro-5-(4-ethylbenzyl)-2-(2-(prop-2-ynyloxy)ethyl)phenyl)-6-(hydroxymethyl)-tetrahydro-2*H*-pyran-3,4,5-triol (35)

Compound **35** was prepared from **11b** in 45% yield as a white solid using a similar method as used for the preparation of **34**. ^{1}H NMR (300 MHz, CD_3OD) δ 7.38 (s, 1H), 7.26 (s, 1H), 7.10 (s, 4H), 4.45–4.42 (m, 1H), 4.15 (d, J = 2.4 Hz, 2H), 4.05 (d, J = 15.6 Hz, 1H), 3.99 (d, J = 15.6 Hz, 1H), 3.85 (dd, J = 12.3, 1.5 Hz, 1H), 3.74 (t, J = 7.1 Hz, 2H), 3.67–3.62 (m, 1H), 3.48–3.45 (m, 2H), 3.40–3.37 (m, 2H), 3.13–3.04 (m, 1H), 2.96–2.89 (m, 1H), 2.83 (t, J = 2.4 Hz, 1H), 2.57 (q, J = 7.6 Hz, 2H), 1.18 (t, J = 7.6 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 140.3, 136.4, 135.4, 135.2, 135.1, 131.5, 128.9, 128.7, 126.8 (2C), 125.9 (2C), 79.5, 77.0, 76.2, 73.1, 73.0, 69.0, 68.4, 60.2, 55.9 (2C), 36.5, 30.3, 26.5, 13.3; ESI MS (m/z): 475 $[M+H]^+$, 492 $[M+NH_4]^+$, 519 $[M+HCO_2]^-$, calcd 474.

4.2. In vitro test

4.2.1. Preparation of human SGLT2 expression vector

A full-length cDNA clone expressing human SGLT2 (GenScript Corporation) was subcloned into *Hind III* and *Not I* sites of the pEAK15 expression vector. Clones harboring the cDNA inserts were identified by restriction analysis.

4.2.2. Preparation of a cell line stably expressing human SGLT2

A plasmid containing human SGLT2 was linearized with *Nsi I* and purified by agarose gel electrophoresis. Using Lipofectamine 2000 Transfection Reagent (Invitrogen Corporation), DNA was transfected into HEK293.ETN cells and cultured in Dulbecco's

Modified Eagle Medium (DMEM) containing 10% fetal bovine serum (FBS) at 37 °C under 5% carbon dioxide for 24 h. Transfectants were selected in the same growth medium supplemented with puromycin (Invitrogen Corporation) for two weeks. Puromycin-resistant cells were recovered and seeded on a fresh 96-well plate (single cell per well) and cultured in the presence of puromycin until cells became confluent. Puromycin-resistant clones were evaluated for SGLT2 activity in the methyl-α-D-[U-14C]glucopyranoside uptake assay described below. The clone that exhibited the highest signal-to-background ratio was used for the methyl-α-D-[U-14C]glucopyranoside uptake assay.

4.2.3. Preparation of human SGLT1 expressing cells

Full-length human SGLT1 cDNA in the pDream 2.1 expression vector was obtained from GenScript Corporation and propagated in *Escherichia coli* strain DH5 using Luria–Bertani (LB) medium containing ampicillin. Plasmid DNA was isolated using the QIAGEN Plasmid Midi Kit (QIAGEN Inc.). Human SGLT1 expression plasmid DNA was transfected into COS-7 cells (American Type Culture Collection) using Lipofectamine 2000 Transfection Reagent according to a manufacturer suggested protocol. Transfected cells were stored in DMEM containing 10% dimethyl sulfoxide (DMSO) at −80 °C.

4.2.4. Methyl-α-D-[U-14C]glucopyranoside uptake assay

Cells expressing SGLT1 or SGLT2 were seeded on 96-well ScintiPlates (PerkinElmer, Inc.) in DMEM containing 10% FBS (1×10^5 cells per well in 100 μL medium) incubated at 37 °C under 5% carbon dioxide for 48 h prior to the assay. Cells were washed twice with 150 μL of either sodium buffer (137 mM NaCl, 5.4 mM KCl, 2.8 mM $CaCl_2$, 1.2 mM $MgCl_2$, 10 mM tris(hydroxymethyl)aminomethane/*N*-2-hydroxyethylpiperazine-*N*′-ethanesulfonic acid [Tris/Hepes], pH 7.2) or sodium-free buffer (137 mM *N*-methyl-glucamine, 5.4 mM KCl, 2.8 mM $CaCl_2$, 1.2 mM $MgCl_2$, 10 mM Tris/Hepes, pH 7.2). Test compound in 50 μL each of sodium or sodium-free buffer containing 40 μCi/mL methyl-α-D-[U-14C]glucopyranoside (Amersham Biosciences/GE Healthcare) was added per well of a 96-well plate and incubated at 37 °C with shaking for either 2 h (SGLT1 assay) or 1.5 h (SGLT2 assay). Cells were washed twice with 150 μL of wash buffer (137 mM *N*-methylglucamine, 10 mM Tris/Hepes, pH 7.2) and methyl-α-D-[U-14C]glucopyranoside uptake was quantitated using a TopCount scintillation counter (PerkinElmer, Inc.). Inhibitors were assayed at 8 concentrations in triplicates. Sodium-dependent glucopyranoside uptake was calculated by subtracting the values obtained with sodium-free buffer from those obtained using sodium buffer. In general, ratios of sodium-dependent to sodium-independent AMG uptake in SGLT1 and SGLT2 expressing cells were 10–15 and 15–20, respectively. Results of AMG uptake were analyzed using GraphPad Prism (Intuitive Software for Science). IC_{50} calculations were performed using nonlinear regression with variable slope. As a reference standard, compound **2** was routinely included in the assays. In 26 independent evaluations, the reference compound inhibited SGLT2 activity by 69.7 ± 9.6% and SGLT1 by 72.7 ± 6.7% at 10 nM and 10 μM respectively.

4.3. In vivo test

4.3.1. Diarrhea test

Male CD-1 mice (18–22 g) was orally administered compounds or 10% NMP (vehicle control) at the dose of 25 mg/kg. After single dose, all animals were observed hourly within 8 h post-dose. The status of diarrhea was recorded and evaluated as the criteria as following: (−) normal feces (black color, shaped, hard), (+) soft feces (light color, shaped, soft), (++) soft diarrhea (light color, no shaped, soft), (+++) water-like diarrhea.

4.3.2. Urinary glucose excretion in SD rats

Reference compound **2** was used as a positive control in every test. Each test compound was dissolved in 30% PEG400 and administered orally to overnight-fasted normal male SD rats by gavage at the dose level of 1 mg/kg. Control rats were given 30% PEG400 only. One hour post dosing, glucose solution (2 g/kg, 10 mL/kg) was administered by oral gavage. Urine was collected within metabolic cages from 0 to 24 h post-dosing for urine volume and glucose measurement. Food was removed 16 h before dosing and then provided 4 h after dosing. Water was supplied ad libitum. The concentration of urinary glucose was determined at 24 h post-dose on the Integra 400 Plus Automatic Biochemistry Analyzer (Roche).

4.3.3. AUC reduction in blood glucose level test

Reference compound **2** was used as a positive control in the test. Compounds are dissolved in 30% PEG400 and administered orally to overnight fasted C57BL/6 J mice by gavage at different doses. Control mice are given vehicle only. After 1 h the administration of the drugs or vehicle, glucose solution (2 g/kg) is given orally. Blood samples are collected from tail tips just before and 5, 15, 30, 60, and 120 min after glucose loading for determination of glucose. The blood glucose was determined by a glucose meter (Johnson & Johnson). The AUC was calculated using trapezoidal rule.

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14. The published in vitro data for dapagliflozin **1** show an IC_{50} value of 1.1 nM for SGLT2 and a selectivity (hSGLT1/hSGLT2) of 1200-fold (see Ref. 4a). In these assays, an IC_{50} value of 6.7 nM and 130-fold selectivity for hSGLT2 were observed. See Section 4.2 for further details on the in vitro experimental methods used in this paper.