Supporting Information

Ligand compatibility of salacinol-type α-glucosidase inhibitors toward the GH31 family

Fumihiro Ishikawa,[a] Aiko Hirano,[a] Yuuto Yoshimori,[a] Kana Nishida,[a] Shinya Nakamura,[b] Katsuki Takashima,[a] Shinsuke Marumoto,[c] Kiyofumi Ninomiya,[d] Isao Nakanishi,[b] Weijia Xie,[e] Toshio Morikawa,[d] Osamu Muraoka,[d] and Genzoh Tanabe[a],[d],[*]

[a] Laboratory of Pharmaceutical Organic Chemistry, Faculty of Pharmacy, Kindai University, 3-4-1 Kowakae, Higashi-Osaka, Osaka 577-8502, Japan
[b] Laboratory of Computational Drug Design and Discovery, Faculty of Pharmacy, Kindai University, 3-4-1 Kowakae, Higashi-Osaka, Osaka 577-8502, Japan
[c] Joint Research Centre, Kindai University, 3-4-1 Kowakae, Higashi-Osaka, Osaka 577-8502, Japan
[d] Pharmaceutical Research and Technology Institute, Kindai University, 3-4-1 Kowakae, Higashi-Osaka, Osaka 577-8502, Japan
[e] Department of Medicinal Chemistry, China Pharmaceutical University, 24 Tong Jia Xiang, Nanjing 210009, PR China.

*Correspondence and request for materials should be directed via email to Genzoh Tanabe (g-tanabe@phar.kindai.ac.jp).
## Contents:

| Section                                      | Page  |
|----------------------------------------------|-------|
| Supplementary Figure S1–S7                  | S3–S7 |
| Supplementary Scheme S1                     | S8    |
| Supplementary Table S1                      | S11   |
| General Methods                             | S8–S16|
| References                                  | S16   |
| Copies of $^1$H-NMR and $^{13}$C-NMR Spectra | S17–S27|
Figure S1. Inhibitory activities of rGAA (Myozyme) by salacinol (1) and neosalacinol (2). (A) Inhibition of rGAA (Myozyme) by salacinol (1). (B) Inhibition of rGAA (Myozyme) by neosalacinol (2). The reactions contained 0.5 μM rGAA (Myozyme) and 3 mM 4-nitrophenyl-α-D-glucopyranoside in 150 mM McIlvaine buffer (pH 5.2).

Figure S2. Inhibitory activities of rGAA (Myozyme) by voglibose and acarbose. (A) Inhibition of rGAA (Myozyme) by voglibose. (B) Inhibition of rGAA (Myozyme) by acarbose. The reactions contained 0.5 μM rGAA (Myozyme) and 3 mM 4-nitrophenyl-α-D-glucopyranoside in McIlvaine buffer (pH 5.2).
Figure S3. Inhibitory activities of rGAA (Myozyme) by compounds 3-7. (A) Inhibition of rGAA (Myozyme) by compound 3. (B) Inhibition of rGAA (Myozyme) by compound 4. (C) Inhibition of rGAA (Myozyme) by compound 5. (D) Inhibition of rGAA (Myozyme) by compound 6. (E) Inhibition of rGAA (Myozyme) by compound 7. The reactions contained 0.5 µM rGAA (Myozyme) and 3 mM 4-nitrophenyl-α-D-glucopyranoside in McIlvaine buffer (pH 5.2).
Figure S4. The correlation between the inhibition activities and the interaction energies of compound 1-7 (Horizontal axis : $E_{int}$(kcal/mol) , Vertical axis : $pK_i$).

Figure S5. Perspective models for binding of salacinol (1) (orange) and the 3’-O-benzylated analog 6 (white) to α-glucosidase GAA (gray). Docking pose of analog 6 has been superposed to predicted complex of GAA with salacinol (1). The active-site residues of GAA with cyan mesh surface are shown and all hydrogen atoms are not shown for clarity.
Figure S6. Inhibitory activities of rGAA (Myozyme) by 3'-epi-1 (8), 3'-epi-2 (9), 2'-epi-1 (10), 2'-epi-2 (11), 2',3'-epi-1 (12), and 2',3'-epi-2 (13). (A) Inhibition of rGAA (Myozyme) by 3'-epi-1 (8). (B) Inhibition of rGAA (Myozyme) by 3'-epi-2 (9). (C) Inhibition of rGAA (Myozyme) by 2'-epi-1 (10). (D) Inhibition of rGAA (Myozyme) by 2'-epi-2 (11). (E) Inhibition of rGAA (Myozyme) by 2',3'-epi-1 (12). (F) Inhibition of rGAA (Myozyme) by 2',3'-epi-2 (13). The reactions contained 0.5 µM rGAA (Myozyme) and 3 mM 4-nitrophenyl-α-D-glucopyranoside in McIlvaine buffer (pH 5.2).
Figure S7. Inhibition profile of β-glucosidase from Aspergillus niger toward compounds 1–13. Inhibition studies were performed by pre-incubation of β-glucosidase from A. niger (0.5 U/mL) with compounds 1–13 (1 mM) for 10 min at room temperature. After 10 min at room temperature, the reaction was initiated by adding 4-nitrophenyl-β-D-glucopyranoside (10 mM) and incubated for 30 min at 37 °C. Control reactions were treated except no inhibitors were added to the reaction mixture. The activity obtained from the reaction without inhibitors is defined as 1.0.
Chemical Synthetic Procedures

Scheme S1. Synthetic routes to compounds 14, 15, and 16.

General Experimental Procedures. IR spectra were measured on a FT-IR spectrophotometer. NMR spectra were recorded on a FT-NMR spectrometers (1H, 500 or 800 MHz; 13C, 125 or 200 MHz). Chemical shifts (δ) and coupling constants (J) are given in ppm and Hz, respectively. Tetramethylsilane (TMS) was used as an internal standard for 1H NMR measurements in CDCl3, whereas 13C NMR measurements utilized the solvent signal (77.0 ppm) of CDCl3 for this purpose. When CD3OD and DMSO-d6 were used for the measurement of 1H and 13C NMR spectra, solvent signals [in CD3OD (δH 3.30 ppm and δC 49.0 ppm) and in DMSO-d6 (δH 2.49 ppm and δC 39.7 ppm)] were used as standard. Sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) was used as an external standard in the measurement of 1H and 13C NMR spectra in D2O. 1D NMR peak assignments were confirmed by COSY and HSQC spectra. High-resolution mass spectra were recorded on a double-focusing mass spectrometer (FAB). Optical rotations were determined with a digital polarimeter. All the organic extracts were dried over anhydrous Na2SO4 prior to evaporation. Column chromatography was performed over silica gel (45–106 μM).

Syntheses of cyclic sulfuric acid esters (14, 15, 16) Compound number in bold refers to the structures shown in Scheme S1.

According to the reported protocol,1 2,4-O-benzylidene-D-erythritol (B) was subjected to cyclic sulfuric acid ester formation reaction to give 2,4-O-benzylidene-D-erythritol 1,3-cyclic sulfate (16) in good yield as depicted in Scheme 1. The 2'-epimer of 16, 2,4-O-benzylidene-D-threitol 1,3-cyclic sulfate (14) was synthesized as follows. By modifying the experimental procedure reported by Haskins et al.,2 solvent free direct condensation of D-arabinitol with benzaldehyde containing anhydrous hydrogen chloride was achieved to easily give 1,3-O-benzylidene-D-arabinitol (S2), which was then oxidized by NaIO4, and subsequent NaBH4 reduction of the resulting D-erythrose derivative gave 1,3-benzylidene-D-threitol3 (S3) in 83%. Finally, S3
was converted to the desired 2,4-benzylidene-D-threitol 1,3-cyclic sulfate (14) in 72% yield in a manner similar to that used for the preparation of 16. Similarly, the antipode of 14, 2,4-benzylidene-L-threitol 1,3-cyclic sulfate (15) was prepared starting from L-arabinitol in good yield. $^1$H and $^{13}$C NMR spectroscopic properties of 14 and 15 were completely in accord with each other. In their $^1$H NMR spectra, downfield shift owing to cyclic sulfuric acid ester formation was observed with respect to the signals due to C-1 methylene ($\delta_{H1a} 4.66$ and $\delta_{H1b} 4.93$) and C-3 methine ($\delta_{H3} 4.84$) protons. Small vicinal coupling constants ($J_{2,3} = 1.4$ Hz) between H-2 and H-3 well supported the cis-fused bicyclic structure of 14 and 15, whereas the corresponding vicinal coupling constant ($J_{2,3} = 9.5$ Hz) of trans-fused 2,4-cyclic sulfate 16 was larger than that of 14 and 15. Correlation of the specific rotation value of 14 ([$\alpha$]$_D$–37.1) with that of 15 ([$\alpha$]$_D$ +37.8) well indicated that both compounds are enantiomers with each other. (Scheme 1).

1,3-$O$-Benzylidene-D-threitol [2,4-$O$-benzylidene-D-threitol] (S3). A hydrogen chloride solution in benzaldehyde (20.9 g) was pre-prepared by bubbling of a slow stream of hydrogen chloride (2.9 g) into freshly distilled benzaldehyde (18.0 g, 170 mmol) under ice-water cooling. To the solution (4.9 g), D-arabinitol (5.0 g, 32.9 mmol) was added at room temperature and D-arabinitol dissolved in 20 min. The resulting mixture solidified during stirring at room temperature for another 20 min and was allowed to stand at room temperature for further 18 h. The solid mass was finely crushed and triturated with a mixture of the solution of sodium hydroxide (2.0 g, 50 mmol) in water (30 mL) and methanol (10 mL). The deposited solid was collected by filtration, and then successively washed with water and diethyl ether to give 1,3-benzylidene-D-arabinitol 2 ($S_2$, 5.2 g) as a colorless solid. The combined filtrate and washings were washed with diethyl ether and was condensed to give $S_3$ (2.1 g). Compound $S_2$ was pure enough for further reaction.

To a mixture of the crude $S_2$ (3.71 g), saturated aqueous sodium hydrogen carbonate (15 mL), and dichloromethane (45 mL) was added portionwise sodium metaperiodate (6.6 g, 30.8 mmol) at room temperature. The heterogeneous mixture was stirred for 1 h at room temperature, and the insoluble solid was filtered off, and washed with ethyl acetate. The combined filtrate and washings were condensed, and the residue was dissolved in methanol (300 mL). To the mixture was added portionwise sodium borohydride (3.51 g, 92.8 mmol) at 0 °C. After being stirring for 1 h at room temperature, the reaction was quenched by addition of saturated aqueous ammonium chloride. Methanol was evaporated at reduced pressure, and the residue was extracted with ethyl acetate (3×50 mL). The extract was successively washed with aqueous sodium thiosulfate-sodium hydrogen carbonate and brine, and condensed to give the title compound $S_3$ (2.90 g, 83% from D-arabinitol as a colorless microcrystalline solid, which was pure enough for further reaction. For analytical purpose a small portion of $S_3$ was recrystallized from a mixture of ethyl acetate and n-hexane to give colorless needles, mp 133–135 °C, lit. 3a mp. 123 °C, lit. 3b mp 130–131 °C. [$\alpha$]$^D_{25}$ –6.3 (c = 1.13, CHCl$_3$), lit. 3a [$\alpha$]$^D_{23}$ –6 (c = 1.0, MeOH), lit. 3b [$\alpha$]$^D_{25}$ –3.1 (c = 1.01, MeOH). IR (nujol): 3263, 1087, 1060, 1002 cm$^{-1}$. $^1$H NMR (800 MHz, DMSO-$d_6$) $\delta$: 3.49 (1H, dtd, $J$ = 6.6, 1.6, 1.5, H-2), 3.50 (1H, ddd, $J$ = 11.6, 6.6, 5.5, H-4a), 3.56 (1H, ddd, $J$ = 11.6, 5.9, 5.9, H-4b), 3.86 (1H, ddd, $J$ = 6.6, 5.9, 1.5, H-3), 4.10 (2H, d, $J$ = 1.6, H-1), 4.63 (1H, ddd, $J$ = 5.9, 5.5, $\text{OH}$), 4.73 (1H, d, $J$ = 6.6, $\text{OH}$), 5.54 (1H, s, $\text{CHPh}$), 7.32–7.37 (3H, m, arom.), 7.46–7.48 (2H, m, arom.). $^{13}$C NMR (200 MHz, CDCl$_3$) $\delta$: 61.2 (C-4), 62.6 (C-2), 72.4 (C-1), 80.2 (C-3), 100.5 (CHPh), 126.6/128.0/128.7 (d, arom.), 139.0 (s, arom.).

1,3-$O$-Benzylidene-L-threitol [2,4-$O$-benzylidene-L-threitol] (S5). In a similar manner used for the
preparation of S3. L-arabinitol (5.0 g, 32.9 mmol) was treated with a solution of hydrogen chloride in benzaldehyde (4.9 g). A similar work-up gave a practically pure 1,3-benzylidene-L-arabinitol (S4, 7.5 g) as colorless solid, a part (2.47 g) of which was oxidized with sodium metaperiodate (4.4 g, 20.5 mmol) in a mixture of saturated aqueous sodium hydrogen carbonate (10 mL), and dichloromethane (30 mL). Work-up gave an aldehyde intermediate, which was then reduced with sodium borohydride (2.34 g, 61.9 mmol) in methanol (200 mL) to give the title compound S5 (1.92 g, 85% from L-S3) as a colorless microcrystalline solid, which was pure enough for further reaction. \(^1\)H and \(^{13}\)C NMR spectroscopic properties of S5 were completely in accord with those of S3. For analytical purpose a small portion of S5 was recrystallized from a mixture of ethyl acetate and \(n\)-hexane to give colorless needles, mp 133–135 °C, lit.\(^4\) Mp 133–134 °C. \([\alpha]_D^24^4\) + 7.44 (c = 1.05, CHCl\(_3\)), lit.\(^4\) \([\alpha]_D^24^4\) +8.0 (c = 1.2, pyridine). \(^1\)H and \(^{13}\)C NMR spectroscopic properties of S5 were completely in accord with those of S3.

2,4-O-Benzylidene-D-threitol 1,3-Cyclic Sulfate (14). A solution of freshly distilled thionyl chloride (0.39 mL, 5.4 mmol) in dry dichloromethane (10 mL) was added dropwise to a stirred mixture of diol S3 (0.8 g, 3.8 mmol), triethylamine (1.42 mL, 10.3 mmol) and dry dichloromethane (15 mL) at 0 °C. After being stirred at 0 °C for 15 min, the mixture was poured into ice-cooled and vigorously stirred saturated aqueous sodium hydrogen carbonate (50 mL), and extracted with dichloromethane (1×30 mL, 2×10 mL). The extract was washed with brine, and condensed to give the corresponding sulfite (1.07 g) as a pale brown solid, which was immediately used in the next step without purification.

To a well stirred mixture of the crude sulfite (1.02 g), sodium hydrogen carbonate (800 mg, 9.5 mmol), carbon tetrachloride (20 mL), acetonitrile (20 mL), and water (10 mL) was added dropwise a brown mixture of sodium metaperiodate (1.96 g, 9.2 mmol), ruthenium chloride \(n\)-hydrate (30 mg), and water (15 mL) at 0 °C. After being stirred at 0 °C for 30 min, the reaction was quenched by the addition of aqueous sodium thiosulfate–sodium hydrogen carbonate. The resulting purple suspension was filtered by suction, and the filter cake was washed with ethyl acetate. The combined filtrate and washings was extracted with ethyl acetate (3×30 mL). The extract was washed with brine, and condensed to give a colorless solid (930 mg), which on column chromatography (\(n\)-hexane/CH\(_2\)Cl\(_2\), 2/1→1/1→1/2) gave the title compound 14 (745 mg, 72 % from S3) as a colorless microcrystalline solid, mp. 125–127 °C. \([\alpha]_D^{25}\) –37.1 (c = 1.00, CHCl\(_3\)). IR (nujol): 1404, 1199, 1026 cm\(^{-1}\). \(^1\)H NMR (500 MHz, CDCl\(_3\)) \(\delta\): 4.08 (1H, ddd, \(J = 2.0, 1.4, 1.2\), H-2), 4.20 (1H, dd, \(J = 13.5, 1.8\), H-4a), 4.40 (1H, dd, \(J = 13.5, 1.4\), H-4b), 4.66 (1H, dd, \(J = 12.6, 1.2\), H-1a), 4.84 (1H, ddd, \(J = 1.8, 1.4, 1.4\), H-3), 4.93 (1H, dd, \(J = 12.6, 2.0\), H-1b), 5.62 (1H, s, CHPh), 7.38–7.55 (5H, m, arom.). \(^{13}\)C NMR (125 MHz, CDCl\(_3\)) \(\delta\): 67.2 (C-2), 68.0 (C-4), 74.9 (C-1), 77.1 (C-3), 101.3 (CHPh), 126.2/128.4/129.7 (d, arom.), 136.4 (s, arom.). HRMS (FAB) \(m/z\): [M+H]\(^+\) Calcd for C\(_{11}\)H\(_{13}\)O\(_6\)S 273.0433; Found 273.0445.

2,4-O-Benzylidene-L-threitol 1,3-Cyclic Sulfate (15). In a similar mannar used for the preparation of 14, diol S5 (0.8 g, 3.81 mmol) was treated with a solution of thionyl chloride (0.39 mL, 5.4 mmol) in dichloromethane (25 mL) in the presence of triethylamine (1.42 mL, 10.3 mmol). A similar work-up gave the corresponding sulfite (1.02 g) as a pale brown solid, which was immediately used in the next step without purification. The crude sulfite (1.00 g) was oxidized with ruthenium tetroxide, which was generated from mixing sodium metaperiodate (1.92 g, 9.0 mmol) and ruthenium chloride \(n\)-hydrate (30 mg) in water (15 mL), in a mixture of sodium hydrogen carbonate (800 mg, 9.5 mmol), carbon tetrachloride (20 mL), acetonitrile (20 mL), and water
(10 mL) to give a colorless solid (934 mg). Column chromatography (n-hexane/CH₂Cl₂, 2/1→1/1→1/2) gave the title compound 15 (777 mg, 75% from S5) as a colorless microcrystalline solid, mp. 124–126 °C. [α]$_{D}^{24}$ + 37.8 (c = 1.07, CHCl₃). $^1$H and $^{13}$C NMR spectroscopic properties of 15 were completely in accord with those of 14.

**Syntheses of sulfonium sulfate inner salts (2', 3'-epi-Salacinol (12), 3'-epi-Salacinol (8), 2'-epi-Salacinol (10)) and sulfonium chlorides (2', 3'-epi-Neosalacinol (13), 3'-epi-Neosalacinol (9), 2'-epi-Neosalacinol (11)).** Compound number in bold refers to the structures shown in Scheme 1.

By applying Ghavami’s conditions for the synthesis of salacinol (1), coupling reactions of thiosugar (17) with cyclic sulfates (14, 15, 16) were carried out in 1,1,3,3,3-hexafluoropropanol (HFIP), where an α-facial attack of 14, 15, 16 to the sulfur atom of 17 preferentially took place to give coupled products 18, 19 and 20 in 76%, 87% and 89% yield, respectively. Subsequently, compounds (18, 19 and 20) was treated with aqueous TFA, where p-methoxybenzyl (PMB) group and benzylidene acetal moiety were simultaneously removed to afford desired sulfonium salts (8, 10 and 12) in good yield. As shown Table 1, the $^{13}$C NMR spectral properties of 8, 10 and 12 were similar to that of salacinol (1), well supporting the formation of salacinol-type sulfonium inner salt structure. To remove sulfo group at the C-3’ oxygen atom, 8, 10 and 12 were subjected to acidic methanolysis to give corresponding sulfonium salts (9, 11 and 13, X = CH₂OSO₃), the anion of which was then exchanged with resin IRA400I (Cl⁻ form) to give the corresponding chlorides (9, 11 and 13) in good yield. The $^{13}$C NMR spectroscopic properties of 9, 11 and 13 were similar with each other, except for the signal due to C-3’ methine carbon (δ$_{C}$ 74.2–75.1), which is significantly shifted upfield in comparison with that of 8, 10 and 12 (δ$_{C}$ 82.0–82.8), supporting their de-O-sulfonated structure.

**Table S1.** $^{13}$C NMR data for salacinol (1) and its analogs 3'-epi-1 (8), 2'-epi-1 (10) and 2', 3'-epi-1 (12) in D$_2$O and neosalacinol (2) and its analogs 3'-epi-2 (9), 2'-epi-2 (11) and 2', 3'-epi-2 (13) in CD$_3$OD (125 MHz, δ in ppm)

|        | 1$^{(1)}$ | 12$^{(2)}$ | 3'-epi-1 (8)$^{(3)}$ | 2'-epi-1 (10)$^{(3)}$ | 2$^{(4)}$ | 3'-epi-7 (9)$^{(3)}$ | 2'-epi-2 (11)$^{(3)}$ | 13$^{(3)}$ |
|--------|----------|----------|----------------------|----------------------|---------|----------------------|----------------------|---------|
| C1     | 50.5     | 49.8     | 50.4                 | 49.4                 | 51.8    | 52.3                 | 50.3                 | 50.8    |
| C2     | 79.5     | 79.5     | 79.5                 | 79.4                 | 79.4    | 79.4                 | 79.4                 | 79.4    |
| C3     | 80.3     | 80.5     | 80.1                 | 80.4                 | 79.5    | 79.6                 | 79.8                 | 79.7    |
| C4     | 72.7     | 72.3     | 72.3                 | 72.6                 | 73.7    | 73.5                 | 73.5                 | 73.5    |
| C5     | 61.7     | 61.7     | 61.7                 | 61.7                 | 61.0    | 61.1                 | 61.1                 | 61.1    |
| C1’    | 52.4     | 52.1     | 52.0                 | 51.5                 | 52.1    | 52.2                 | 51.3                 | 51.4    |
| C2’    | 68.3     | 68.6     | 68.4                 | 68.7                 | 69.6    | 69.2                 | 69.1                 | 69.5    |
| C3’    | 82.6     | 82.8     | 82.1                 | 82.0                 | 75.3    | 74.2                 | 74.3                 | 75.1    |
| C4’    | 62.2     | 62.2     | 62.2                 | 62.2                 | 64.0    | 63.6                 | 63.6                 | 64.0    |

a) BMCL2009 19 2195-2198; b) BMC2007(15) 3926-3973; c) 125 MHz; d) 200 MHz

**Coupling reaction between cyclic sulfates (14, 15, 16) and thiosugar (17).** According to the literature, cyclic sulfates (14, 15, 16) were treated with thiosugar (17) in 1,1,3,3,3-hexafluoropropanol (HFIP).

**With cyclic sulfates (16).** In a sealed tube, a mixture of 16$^6$ (234 mg, 0.86 mmol), potassium carbonate (20 mg, 0.14 mmol), and HFIP (1.2 ml) was stirred at 70 °C for 120 h. After removal of the solvent, the residue was purified on column chromatography (CH₂Cl₂→CH₂Cl₂/MeOH, 20/1) to give 1,4-dideoxy-2,3,5-tri-O-(p-methoxybenzyl)-1-O-[(S)-2,4-O-benzylidene-1-deoxy-3-O-sulfo-D-erythritol-1-yl]episulfoniumylidene]-D-arabinitol inner salt (20, 516 mg, 89%) as a colorless amorphous, [α]$_{D}^{24}$ –42.7 (c = 1.07, CHCl₃). IR (nujol): 1612, 1512, 1249, 1087, 1018 cm$^{-1}$. $^1$H NMR (500 MHz, DMSO-d$_6$) δ 3.55 (1H, S11
dd, J = 10.3, 7.8, H-5a), 3.68 (1H, dd, J = 10.3, 7.2, H-5b), 3.70 (1H, dd-like, J = ca. 10.5, 10.5, H-4'a), 3.72 (3H, s, OCH₃), 3.73 (6H, s, OCH₃), 3.81 (1H, dd, J = 13.2, 4.0, H-1'a), 3.94 (1H, dd, J = 13.8, 7.2, H-1'a), 3.99 (1H, dd, J = 13.2, 2.6, H-1'b), 4.10 (1H, dd, J = 13.8, 2.9, H-1'b), 4.16 (1H, ddd, J = 10.5, 10.5, 5.5, H-3'), 4.26/4.29 (each 1H, d, J = 11.5 Hz, OCH₂PMP), 4.28 (1H, ddd-like, J = ca. 10.5, 7.2, 2.9, H-2'), 4.34 (1H, dd, J = 10.5, 5.5, H-4'b), 4.36 (1H, ddd-like, J = ca. 2.3, 2.3, H-3), 4.39/4.45 (each 1H, d, J = 11.2 Hz, OCH₂PMP), 4.43 (1H, br dd, J = 7.8, 7.2, H-4), 4.49/4.51 (each 1H, d, J = 11.5, OCH₂PMP), 4.62 (1H, ddd, J = 4.0, 2.6, 2.3, H-2), 5.60 (1H, s, CHPh), 6.84–7.24 (12H, m, arom.), 7.35–7.54 (5H, m, arom.). ¹³C NMR (125 MHz, DMSO-d₆) δ 46.7 (C-1), 47.0 (C-1'), 55.3 (OCH₃), 63.3 (C-4), 66.1 (C-5), 67.3 (C-3'), 69.0 (C-4'), 70.97/71.10/72.2 (OCH₂PMP), 75.7 (C-2'), 82.4 (C-2), 82.5 (C-3), 100.6 (CHPh), 113.9/114.0(2C)/126.5/128.4/129.39/129.6/129.7/130.0 (d, arom.), 129.1(2C)/129.40/137.2/159.1/159.22/159.24 (s, arom.). HRMS (FAB) m/z: [M+H]⁺ Calcd for C₄₀H₄₇O₁₂S₂ 783.2509; Found 783.2522.

With cyclic sulfate (14). In a similar manner, 1,4-dideoxy-2,3,5-tri-O-(p-methoxybenzyl)-1,4-{[(S)-2,4-O-benzylidene-1-deoxy-3-O-sulfo-D-threitol-1-yl]episulfoniumylidene}-D-arabinitol inner salt (18 438 mg, 76%) was obtained by the condensation between 14 (234 mg, 0.86 mmol) and 17 (376 mg, 0.74 mmol) as colorless amorphous, [α]²₀ D +26.5 [c = 1.00, CHCl₃]. IR (nujol): 1612, 1512, 1246, 1069, 1029 cm⁻¹. ¹H NMR (500 MHz, DMSO-d₆) δ: 5.35 (1H, dd, J = 10.0, 10.0, 5.5a), 3.70/3.74/3.75 (each 3H, s, OCH₃), 3.70–3.77 (2H, m, H-1a and 1'a), 3.79 (1H, dd, J = 10.0, 5.2, H-5b), 3.89 (1H, dd, J = 13.5, 8.6, H-1'b), 3.98 (1H, dd, J = 12.0, 1.4, H-4'a), 4.05 (1H, dd, J = 13.2, 3.0, H-1'b), 4.21 (1H, ddd-like, J = 3.0, 1.4, H-3'), 4.35 (1H, dd-like, J = 3.0, 2.0, H-3), 4.37 (1H, br d-like, J = ca. 12.1 Hz, H-4'b), 4.38/4.46 (each 1H, d, J = 11.7, OCH₂PMP), 4.43/4.48 (each 1H, J = 11.5, OCH₂PMP), 4.51 (1H, ddd-like, J = 10.0, 5.2, 2.0, H-4), 4.52–4.55 (1H, m, H-2'), 4.55/4.58 (2H, dd, J = 11.2, OCH₂PMP), 4.62 (1H, ddd, J = 3.0, 3.0, 3.0, H-2), 5.38 (1H, s, CHPh), 6.81/6.88/6.91/7.15/7.16/7.27 (each 2H, d-like, J = ca. 8.6, arom.), 7.33–7.39 (5H, m, arom.). ¹³C NMR (125 MHz, DMSO-d₆) δ 46.5 (C-1), 47.0 (C-1'), 55.2/55.3(2C) (OCH₃), 63.9 (C-4), 66.5 (C-5), 68.3 (C-3'), 69.5 (C-4'), 70.9/71.3/72.2 (OCH₂PMP), 73.4 (C-2'), 81.8 (C-2), 82.5 (C-3), 100.1 (CHPh), 113.9/114.0(2C)/126.2/128.1/129.0/129.6/130.0 (d, arom.), 129.2/129.3/137.9/159.1/159.21/159.24 (s, arom.). HRMS (FAB) m/z: [M+H]⁺ Calcd for C₄₀H₄₇O₁₂S₂ 783.2509; Found 783.2488.

With cyclic sulfate (15). In a similar manner, 1,4-dideoxy-2,3,5-tri-O-(p-methoxybenzyl)-1,4-{[(S)-2,4-O-benzylidene-1-deoxy-3-O-sulfo-L-threitol-1-yl]episulfoniumylidene}-D-arabinitol inner salt (19 503 mg, 87%) was obtained by the condensation between 15 (234 mg, 0.86 mmol) and 17 (376 mg, 0.74 mmol) as colorless amorphous, [α]²₀ D +48.6 [c = 1.01, CHCl₃]. IR (nujol): 1612, 1512, 1246, 1068, 1026 cm⁻¹. ¹H NMR (500 MHz, DMSO-d₆) δ: 3.60 (1H, dd, J = 10.0, 8.9, H-5a), 3.73/3.75/3.743 (each 3H, s, OCH₃), 3.78 (1H, dd, J = 10.0, 6.6, H-5b), 3.82 (2H, d-like, J = 6.0, H-1'a and H-1'b), 3.95 (1H, dd, J = 13.5, 3.2, H-1'a), 3.96 (1H, dd-like, J = ca. 12.0, 1.4, H-4'a), 3.98 (1H, dd, J = 13.5, 4.0, H-1'b), 4.17 (1H, ddd, J = 1.8, 1.4, 1.4, H-3'), 4.29 (1H, dd, J = 12.0, 1.4, H-4'b), 4.34 (2H, s, OCH₂PMP), 4.39 (1H, dd-like, J = ca. 2.3, 2.3, H-3'), 4.41/4.47 (each 1H, d, J = 11.5, OCH₂PMP), 4.42–4.47 (1H, m H-4), 4.50/4.55 (each 1H, d, J = 11.2, OCH₂PMP), 4.55 (1H, td-like, J = 6.0, 1.8, H-2'), 4.63 (1H, ddd, J = 4.0, 3.2, 2.3, H-2), 5.49 (1H, s, CHPh), 6.84–6.92 (6H, m, arom.), 7.14/7.18/7.26 (each 2H, d-like, J = 8.6 Hz, arom.), 7.35–7.42 (5H, m, arom.). ¹³C
NMR (125 MHz, DMSO-d$_6$) $\delta$ 45.7 (C-1'), 45.9 (C-1), 55.2 (OCH$_3$), 63.6 (C-4), 66.5 (C-5), 68.1 (C-3'), 69.6 (C-4'), 70.9/71.1/72.2 (OCH$_2$PMP), 73.9 (C-2'), 82.5 (C-3), 82.6 (C-2), 100.3 (CHPh), 113.9/113.95/114.01/126.3/128.3/129.2/129.67/129.69/130.0 (d, arom.), 128.97/129.03/129.4/137.9/159.1/159.2/159.3 (s, arom.). HRMS (FAB) m/z: [M+H]$^+$ Calcd for C$_{46}$H$_{45}$O$_{12}$S$_2$ 783.2509; Found 783.2488.

De-protection of PMB and benzylidene moieties of coupling products (8, 10 and 12) by aqueous TFA.

1,4-Dideoxy-1,4-{{(S)}-[1-deoxy-3-O-sulfo-D-erythritol-1-yl]episulphoniumyldiene}-D-arabinitol inner salt (12). A mixture of coupling product (20, 460 mg, 0.59 mmol), trifluoroacetic acid (5 mL), and water (0.5 mL) was stirred at room temperature for 1.5 h. After removal of the solvent in vacuo, the residue was washed with dichloromethane to give a colorless solid, which was triturated with methanol to give the title compound (12, 157 mg, 80%) as a white powder, mp 149–151 °C. [α]$^2$$_D$ –27.3 (c = 0.60, H$_2$O), lit.$^a$ [α]$^2$$_D$ –35.6 (c = 0.87, MeOH). IR (KBr): 3645, 1261, 1215, 1049, 1010 cm$^{-1}$. $^1$H NMR (800 MHz, D$_2$O) $\delta$ 3.89 (1H, dd, J = 12.8, 3.2, H-4'a), 3.90 (1H, dd, J = 13.6, 4.0, H-1'a), 3.918 (1H, dd, J = 12.8, 8.8, H-1'a), 3.922 (1H, dd, J = 13.6, 4.8, H-1b), 3.99 (1H, dd, J = 12.8, 3.2, H-1'b), 4.00 (1H, dd, J = 12.8, 3.2, H-4'b), 4.02 (1H, dd, J = 12.8, 8.0, H-5'a), 4.03 (1H, dd, J = 12.8, 5.6, H-5'b), 4.19 (1H, ddd, J = 8.0, 5.6, 3.2, H-4), 4.37 (1H, ddd, J = 7.2, 3.2, 3.2, H-3'), 4.44 (1H, ddd, J = 8.8, 7.2, 3.2, H-2'), 4.51 (1H, ddd-like, J = ca. 3.2, 3.2, 3.2, H-3), 4.78 (1H, ddd, J = 4.8, 4.0, 3.2, H-2). $^{13}$C NMR (200 MHz, D$_2$O) $\delta$ 49.8 (C-1), 52.1 (C-1'), 61.7 (C-5), 62.2 (C-4'), 68.6 (C-2'), 72.3 (C-4), 79.5 (C-2), 80.5 (C-3), 82.8 (C-3'). HRMS (FAB) m/z: [M+H]$^+$ Calcd for C$_{46}$H$_{45}$O$_{12}$S$_2$ 335.0471; Found 335.0472.

1,4-Dideoxy-1,4-{{(S)}-[1-deoxy-3-O-sulfo-D-threitol-1-yl]episulphoniumyldiene}-D-arabinitol inner salt (8). In a similar manner, the title compound (8, 151 mg, 86%) was obtained from coupling product (18, 410 mg, 0.52 mmol) as a white powder, mp. 161–162 °C. [α]$^2$$_D$ +17.5 (c = 0.40, H$_2$O). IR (nujol): 3418, 1249, 1219, 1056, 1007 cm$^{-1}$. $^1$H NMR (500 MHz, D$_2$O) $\delta$ 3.82 (1H, dd, J = 12.0, 6.3, H-4'a), 3.81–3.85 (1H, m, H-1'a), 3.85 (1H, dd, J = 12.0, 6.0, H-4'b), 3.87 (1H, dd, J = 13.5, 4.0, H-1'a), 3.85–3.89 (1H, m, H-1'b), 3.90 (1H, dd, J = 13.5, 3.8, H-1b), 3.92 (1H, dd, J = 11.8, 8.9, H-5'a), 4.07 (1H, ddd, J = 8.9, 4.9, 3.2, H-4), 4.11 (1H, dd, J = 11.8, 4.9, H-5b), 4.38 (1H, ddd-like, J = ca. 6.3, 6.0, 3.0, H-3'), 4.42 (1H, ddd-like, J = ca. 3.5, 3.2, H-3), 4.46 (1H, ddd, J = 9.2, 4.1, 3.0, H-2'), 4.73 (1H, ddd, J = 4.0, 3.8, 3.5, H-2). $^{13}$C NMR (125 MHz, D$_2$O) $\delta$ 50.4 (C-1), 52.0 (C-1'), 61.7 (C-5), 62.2 (C-4'), 68.4 (C-2'), 72.3 (C-4), 79.5 (C-2), 80.1 (C-3), 82.1 (C-3'). HRMS (FAB) m/z: [M+H]$^+$ Calcd for C$_{46}$H$_{45}$O$_{12}$S$_2$ 335.0471; Found 335.0473.

1,4-Dideoxy-1,4-{{(S)}-[1-deoxy-3-O-sulfo-L-threitol-1-yl]episulphoniumyldiene}-D-arabinitol inner salt (10). In a similar manner, the title compound (10, 166 mg, 78%) was obtained from coupling product (19, 498 mg, 0.64 mmol) as a white powder, mp. 145–147 °C. [α]$^2$$_D$ –39.0 (c = 0.40, H$_2$O). IR (nujol): 3360, 1288, 1200, 1064, 1015 cm$^{-1}$. $^1$H NMR (500 MHz, D$_2$O) $\delta$ 3.82 (1H, dd, J = 11.8, 6.0, H-4'a), 3.83 (1H, dd, J = 13.5 4.3, H-1'a), 3.85 (1H, dd, J = 11.8, 5.2, H-4'b), 3.86 (1H, ddd-like, J = ca. 13.2, 3.7, H-1'a), 3.89 (1H, dd, J = 13.2, 3.7, H-1b), 3.92 (1H, dd, J = 13.2, 9.5, H-1'b), 3.97 (1H, dd, J = 12.3, 8.0, H-5'a), 4.09 (1H, dd, J = 12.3, 5.2, H-5b), 4.15 (1H, ddd, J = 8.0, 5.2, 3.2, H-4), 4.38 (1H, ddd, J = 6.0, 5.2, 2.9, H-3'), 4.460 (1H, ddd-like, J = ca. 9.5, 4.3, 2.9, H-2'), 4.462 (1H, ddd-like, J = ca. 3.2, 3.2, H-3), 4.74 (1H, ddd, J = 3.7, 3.7, 3.2, H-2). $^{13}$C NMR (125 MHz, D$_2$O) $\delta$ 49.4 (C-1), 51.5 (C-1'), 61.7 (C-5), 62.2 (C-4'), 68.7 (C-2'), 72.6 (C-4), 79.4 (C-2), 82.1 (C-3').
80.4 (C-3), 82.0 (C-3'). HRMS (FAB) m/z: [M+H]+ Calcd for C₉H₁₆O₆S₂ 335.0471; Found 335.0476.

Acidic methanolyis of sulfonium inner salts (9, 11, 13).

1,4-Dideoxy-1,4-{[(R)-1-deoxy-D-erythritol-1-yl]episulfoniumylidene}-D-arabinitol chloride (13). A mixture of 12 (75 mg, 0.22 mmol) and 5% methanolic hydrogen chloride (4 ml) was stirred at 50 °C for 3 h. After removal of the solvent in vacuo, the residue (92 mg) was treated with ion exchange resin IRA400J (Cl⁻ form, 2.0 g) in methanol (4 mL) at room temperature for 15 h. The resin was filtered off and washed with methanol. The combined filtrate and washings were condensed to give the title compound 13 (50 mg, 77%) as a colorless oil, [α]²³D −52.9 (c = 0.24, MeOH). IR (neat): 3418, 1643, 1415, 1258, 1222, 1072 cm⁻¹. ¹H NMR (800 MHz, CD₃OD) δ: 3.60–3.63 (1H, m, H-3'), 3.62–3.64 (1H, m, H-4'a), 3.67–3.70 (1H, m, H-4'b), 3.79 (1H, dd, J = 12.8, 4.8, H-1'a), 3.81 (1H, dd, J = 12.8, 8.8, H-5a), 4.03 (1H, dd, J = 12.0, 6.4, H-5b), 4.07 (1H, ddd-like, J = ca. 6.4, 6.4, 4.8, H-2'), 4.09 (1H, br dd, J = 8.8, 6.4, H-4), 4.40 (1H, dd, J = 2.4, 0.8, H-3), 4.62 (1H, ddd-like, J = ca. 3.2, 2.4, 1.6, H-2). ¹³C NMR (200 MHz, CD₃OD) δ: 50.8 (C-1), 51.4 (C-1'), 61.1 (C-5), 64.0 (C-4'), 69.5 (C-2'), 73.5 (C-4), 75.1 (C-3'), 79.4 (C-2), 79.7 (C-3). HRMS (FAB) m/z: [M–Cl]⁺ Calcd for C₉H₁₆O₆S 255.0903; Found 255.0893.

1,4-Dideoxy-1,4-{[(R)-1-deoxy-D-threitol-1-yl]episulfoniumylidene}-D-arabinitol chloride (9). In a similar manner, the title compound (9, 58 mg, 78%) was obtained from coupling product (8, 86 mg, 0.26 mmol) as a colorless oil, [α]²³D −52.9 (c = 0.24, MeOH). IR (neat): 3418, 1643, 1415, 1258, 1222, 1072 cm⁻¹. ¹H NMR (800 MHz, CD₃OD) δ: 3.60–3.63 (1H, m, H-3'), 3.62–3.64 (1H, m, H-4'a), 3.67–3.70 (1H, m, H-4'b), 3.79 (1H, dd, J = 12.8, 4.8, H-1'a), 3.81 (1H, dd, J = 12.8, 8.8, H-5a), 4.03 (1H, dd, J = 12.0, 6.4, H-5b), 4.07 (1H, ddd-like, J = ca. 6.4, 6.4, 4.8, H-2'), 4.09 (1H, br dd, J = 8.8, 6.4, H-4), 4.40 (1H, dd, J = 2.4, 0.8, H-3), 4.62 (1H, ddd-like, J = ca. 3.2, 2.4, 2.0, H-2). ¹³C NMR (200 MHz, CD₃OD) δ: 50.8 (C-1'), 52.3 (C-1), 61.1 (C-5), 64.0 (C-4'), 69.5 (C-2'), 73.5 (C-4), 75.1 (C-3'), 79.4 (C-2), 79.7 (C-3). HRMS (FAB) m/z: [M–Cl]⁺ Calcd for C₉H₁₆O₆S 255.0903; Found 255.0893.

1,4-Dideoxy-1,4-{[(R)-1-deoxy-L-threitol-1-yl]episulfoniumylidene}-D-arabinitol Chloride (11). In a similar manner, the title compound (11, 56 mg, 84%) was obtained from coupling product (10, 77 mg, 0.23 mmol) as a colorless oil, [α]²³D −17.0 (c = 0.17, MeOH). IR (neat): 3360, 1643, 1416, 1254, 1072, 1042 cm⁻¹. ¹H NMR (800 MHz, CD₃OD) δ: 3.60 (1H, ddd-like, J = ca. 6.5, 4.8, 3.2, H-3'), 3.62 (1H, dd, J = 12.8, 4.8, H-4'a), 3.64 (1H, dd, J = 11.2, 6.4, H-4'b), 3.74 (1H, dd, J = 12.8, 4.0, H-1'a), 3.79 (1H, dd, J = 12.8, 9., H-1'b), 3.84 (1H, dd, J = 12.8, 3.2, H-1a), 3.86 (1H, dd, J = 12.8, 1.6, H-1b), 3.93 (1H, dd, J = 12.0, 9.6, H-5a), 3.98 (1H, br dd, J = 9.6, 4.8, H-4), 4.05 (1H, dd, J = 12.0, 4.8, H-5b), 4.18 (1H, dd, J = 9.6, 4.0, 3.2, H-2'), 4.37 (1H, dd-like, J = ca. 2.4, 0.8, H-3), 4.61 (1H, dd-like, J = ca. 3.2, 2.4, 1.6, H-2). ¹³C NMR (200 MHz, CD₃OD) δ: 52.2 (C-1'), 52.3 (C-1), 63.6 (C-4'), 69.2 (C-2'), 73.5 (C-4), 74.2 (C-3'), 79.4 (C-2), 79.6 (C-3). HRMS (FAB) m/z: [M–Cl]⁺ Calcd for C₉H₁₆O₆S 255.0903; Found 255.0921.

Biochemistry Procedures

*In vitro* enzymatic assay toward recombinant human GAA (rGAA, Myozyme).
Standard assay conditions: Reactions contained 0.5 µM rGAA (Myozyme, SANOFI GENZYME), 150 mM McIlvaine bufer (pH 5.2), 3 mM 4-nitrophenyl-α-D-glucopyranoside (α-p-NPG), and varying amounts of inhibitors (compounds 1–13, voglibose, and acarbose). The reactions (80 µL) were run in 96-well plates (FALCON, 353072). Absorbance at 405 nm (A₄₀₅) was measured on a Multiskan FC (Thermo Fisher Scientific).

Determination of apparent Kᵢ (Kᵢₚᵖᵖ) values of compounds 1–13, voglibose, and acarbose: Kᵢₚᵖᵖ determination was performed under standard assay conditions. The enzyme, substrate, and inhibitor concentrations are listed here: rGAA was used at 0.5 µM with 3 mM of α-p-NPG in either the absence or presence of compounds 1–13, voglibose, and acarbose (salacinol (1): 0.031–1000 µM; neosalacinol (2): 0.031–1000 µM; 3 (H): 0.024–100 µM; 4 (o-CH₃): 0.024–100 µM; 5 (o-Cl): 0.024–100 µM; 6 (o-CF₃): 0.024–100 µM; 7 (o-NO₂): 0.024–100 µM; 3’-epi-salacinol (8): 0.031–1000 µM; 3’-epi-neosalacinol (9): 0.031–1000 µM; 2’-epi-salacinol (10): 0.122–4000 µM; 3’-epi-neosalacinol (11): 0.122–4000 µM; 2’,3’-epi-salacinol (12): 0.122–4000 µM; 2’,3’-epi-neosalacinol (13): 0.061–2000 µM; voglibose: 0.031–1000 µM; acarbose: 0.122–4000 µM). Inhibition studies were performed by pre-incubation of rGAA (0.5 µM) with varying amounts of inhibitors (compounds 1–13, voglibose, and acarbose) for 10 min at room temperature. After 10 min at room temperature, the reaction was initiated by adding α-p-NPG (3 mM). After 30 min incubation at 37 °C, the reaction was quenched by adding 120 µL of 80 mM glycine-NaOH buffer (pH 10). Control reactions were treated except no inhibitors (compounds 1–13, voglibose, and acarbose) were added to the reaction mixture. In all experiments, the total DMSO concentration was kept at or below 2.0%. All Kᵢₚᵖᵖ values were determined by replicating each assay twice. Data was fit to the Morrison equation using Prism 7 (GraphPad Software).

In vitro enzymatic assay toward β-glucosidase from Aspergillus niger.

Standard assay conditions: Reactions contained 0.5 U/mL β-glucosidase from Aspergillus niger (Sigma-Aldrich, 49291), 150 mM McIlvaine bufer (pH 4.6), 10 mM 4-nitrophenyl-β-D-glucopyranoside (β-p-NPG), and 1 mM inhibitors (compounds 1–13, voglibose, and acarbose). The reactions (80 µL) were run in 96-well plates (FALCON, 353072). Absorbance at 405 nm (A₄₀₅) was measured on a Multiskan FC (Thermo Fisher Scientific).

Inhibition profile of β-glucosidase from A. niger toward compounds 1–13, voglibose, and acarbose: Inhibition studies were performed by pre-incubation of β-glucosidase from A. niger (0.5 U/mL) with 1 mM of inhibitors (compounds 1–13, voglibose, and acarbose) for 10 min at room temperature. After 10 min at room temperature, the reaction was initiated by adding β-p-NPG (10 mM). After 30 min incubation at 37 °C, the reaction was quenched by adding 120 µL of 80 mM glycine-NaOH buffer (pH 10). Control reactions were treated except no inhibitors (compounds 1–13, voglibose, and acarbose) were added to the reaction mixture. In all experiments, the total DMSO concentration was kept at or below 1.0%.

Inhibitory effects on rat intestinal α-glucosidases.

The experiments were performed according to the method reported. Thus, rat small intestinal brush border membrane vesicles were prepared and their suspensions in 0.1 M maleate buffer (pH 6.0) were used as small intestinal α-glucosidases of maltase, sucrose, and isomaltase. A test sample was dissolved in dimethyl sulfoxide (DMSO), and the resulting solution was diluted with 0.1 M maleate buffer to prepare the test sample solution (concentration of DMSO 10 %). A substrate solution in the maleate buffer (maltose 74 mM, sucrose
74 mM, isomaltase 7.4 mM, 50 µL), the test sample solution (25 µL), and the enzyme solution (25 µL) were mixed at 37 °C for 30 min, and then immediately heated by boiling water for 2 min to stop the reaction. The glucose concentrations were determined by a glucose-oxidase method. The final concentration of DMSO in the test solution was 2.5 % and no influence of DMSO on the inhibitory activity was detected.

**Modeling studies.**

Docking study for salacinol and its derivatives (1–7) to GAA has been performed to derive their structure-activity relationship. The apo structure of GAA (PDB-ID: 5NN3) was retrieved from database, then all amino acid protonation states and the positions of the hydrogen atoms for docking protein were assigned by the Protonate-3D method\(^ {11}\) implemented in MOE\(^ {12}\). The docking site to investigate was defined considering from GAA-acarbose complex (PDB-ID: 5NN8), since salacinol interact with same binding site of acarbose in alpha-glucosidase \( N \)-terminal catalytic subunit (PDB-ID: 3L4Z and 2QMJ, respectively). The compounds were flexibly docked into prepared protein structure. For one compound molecule, top 100 candidate poses ranked by Affinity\(_dG\) docking score\(^ {12}\) were optimized with flexible protein side chain atoms in the truncated pocket structure, then optimized poses were ranked again with same score. Each docked compound of binding pose with best Affinity\(_dG\) score was further optimized geometrically in full protein structure. The interaction energy \( E_{\text{int}} \) between each optimized compound and GAA was calculated by MM-GB/VI method.\(^ {13}\)

**References**

1) Tanabe, G.; Yoshikai, K.; Hatanaka, T.; Yamamoto, M.; Shao, Y.; Minematsu, T.; Muraoka, O.; Wang, T.; Matsuda, H.; Yoshikawa, M. *Bioorg. Med. Chem.* **2007**, *15*, 3926–3937.
2) Haskins, W. T.; Hann, R. M.; Hudson, C. S. *J. Am. Chem. Soc*. **1943**, *65*, 1663–1667.
3) (a) Lehamann, J.; Wagenknecht. H.-A. *Carbohydr. Res.*, **1995**, *276*, 215–218; (b) Urbansky, M.; Davis, C. E.; Surjan, J. D.; Coates, R. M. *Org. Lett*. **2004**, *6*, 135–138.
4) Foster, A. B.; Homer, H. J.; Lehmann, J. *J. Chem. Soc.*, **1961**, *5005–5011*.
5) Ghavami, A.; Sadalapure, K. S.; Johnston, B. D.; Lobera, M.; Snider, B. B.; Pinto, B. M. *Synlett* **2003**, *1259–1262*.
6) Ghavami, A.; Johnston, B. D.; Pinto, B. M. *J. Org. Chem.* **2001**, *66*, 2312–2317.
7) Nakamura, S.; Takahira, K.; Tanabe, G.; Morikawa, T.; Sakano, M.; Ninomiya, K.; Yoshikawa, M.; Muraoka, O.; Nakanishi, I. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 4420–4423.
8) Morikawa, T.; Akaki, J.; Ninomiya, K.; Kinouch, E.; Tanabe, G.; Pongpiriyadacha, Y.; Yoshikawa, M.; Muraoka, O. *Nutrients*, **2015**, *7*, 1480.
9) Morikawa, T.; Ninomiya, K.; Imamura, M.; Akaki, J.; Fujikura, S.; Pan, Y.; Yuan, D.; Yoshikawa, M. Jia, X.; Li, Z.; Muraoka O. *J. Nat. Med.* **2014**, *68*, 561.
10) Kessler, M.; Acuto, O.; Storelli, C.; Murer, H.; Muller, M.; Semenza G. *Biochim. Biophys. Acta* **1978**, *506*, 136.
11) Labute, P. *Proteins* **2009**, *75*, 187.
12) MOE Ver. 2019, *Chemical Computing Group Inc.*, Montreal, Canada.
13) Labute, P. *J. Comput. Chem.* **2008**, *29*, 1693.
$^1$H NMR spectrum (800 MHz, in DMSO-$d_6$) of 1,3-O-benzylidene-D-threitol [2,4-O-benzylidene-D-threitol] (S3)

$^{13}$C NMR spectrum (200 MHz, in DMSO-$d_6$) of 1,3-O-benzylidene-D-threitol [2,4-O-benzylidene-D-threitol] (S3)
$^{1}$H NMR spectrum (500 MHz, in CDCl$_3$) of 2,4-O-benzylidene-D-threitol 1,3-cyclic sulfate (S4)

$^{13}$C NMR spectrum (125 MHz, in CDCl$_3$) of 2,4-O-benzylidene-D-threitol 1,3-cyclic sulfate (S4)
$^1$H NMR spectrum (500 MHz, in DMSO-$d_6$) of 1,4-dideoxy-2,3,5-tri-O-(4-methoxybenzyl)-1,4-\{(S)-[2,4-O-benzylidene-1-deoxy-3-O-sulfo-D-threitol-1-yl]episulfoniumilidene-D-arabinitol inner salt (18)
\[^{13}\text{C}\] NMR spectrum (125 MHz, in DMSO-\text{d}_6) of 1,4-dideoxy-2,3,5-tri-\text{O}(4\text{-methoxybenzyl})-1,4- \{(S)-[2,4-O\text{-benzylidene}-1\text{-deoxy}-3\text{-O-}\text{ sulfo-}\text{D-threitol-1-yl}]\text{episulfoniumilidene-D-arabinitol inner salt (18)}\}

\[^{1}\text{H}\] NMR spectrum (500 MHz, in D\text{2}O) of 1,4-dideoxy-1,4-\{(S)-[1\text{-deoxy}-3\text{-O-}\text{ sulfo-}\text{D-threitol-1-yl}]\text{- episulfoniumilidene-D-arabinitol inner salt (8)}\}
\(^{13}\)C NMR spectrum (125 MHz, in D\(_2\)O) of 1,4-dideoxy-1,4-{(S)-[1-deoxy-3-O-sulfo-d-threitol-1-yl]}episulfoniumidene-D-arabinitol inner salt (8)

\(^1\)H NMR spectrum (800 MHz, in CD\(_3\)OD) of 1,4-dideoxy-1,4-{(R)-[1-deoxy-D-threitol-1-yl]}-episulfoniumidene-D-arabinitol chloride (9)
$^{13}$C NMR spectrum (200 MHz, in CD$_3$OD) of 1,4-dideoxy-1,4-\{(R)-[1-deoxy-D-threitol-1-yl]-episulfoniumidene-D-arabinitol chloride (9)
$^1$H NMR spectrum (500 MHz, in DMSO-$d_6$) of 1,4-dideoxy-2,3,5-tri-O-(4-methoxybenzyl)-1,4- {$(S)$-[2,4-$O$-benzylidene-1-deoxy-3-$O$-sulfo-L-threitol-1-yl]episulfoniumlidene-D-arabinitol inner salt (19)

$^{13}$C NMR spectrum (125 MHz, in DMSO-$d_6$) of 1,4-dideoxy-2,3,5-tri-O-(4-methoxybenzyl)-1,4- {$(S)$-[2,4-$O$-benzylidene-1-deoxy-3-$O$-sulfo-L-threitol-1-yl]episulfoniumlidene-D-arabinitol inner salt (19)
$^1$H NMR spectrum (500 MHz, in D$_2$O) of 1,4-dideoxy-1,4-\{(S)-[1-deoxy-3-O-sulfo-L-threitol-1-yl]-episulfoniumilidene-D-arabinitol inner salt (10)

$^{13}$C NMR spectrum (125 MHz, in D$_2$O) of 1,4-dideoxy-1,4-\{(S)-[1-deoxy-3-O-sulfo-L-threitol-1-yl]-episulfoniumilidene-D-arabinitol inner salt (10)
$\text{yl\episulfoniumilidene-D-arabinitol inner salt (10)}$

$\text{H NMR spectrum (800 MHz, in CD}_3\text{OD) of 1,4-dideoxy-1,4-\{(R)-[1-deoxy-L-threitol-1-yl]-episulfoniumilidene-D-arabinitol chloride (11)}$
$^{13}$C NMR spectrum (200 MHz, in CD$_3$OD) of 1,4-dideoxy-1,4-\{(R)-[1-deoxy-L-threitol-1-yl]-episulfoniumlidene-D-arabinitol chloride (11)

$^1$H NMR spectrum (500 MHz, in DMSO-$d_6$) of 1,4-dideoxy-2,3,5-tri-O-(4-methoxybenzyl)-1,4- \{(S)-[2,4-O-
1H NMR spectrum (500 MHz, in DMSO-\(d_6\)) of 1,4-dideoxy-2,3,5-tri-O-(4-methoxybenzyl)-1,4-\{\(\text{S}\)\}-[2,4-\(\text{O}\)-benzylidene-1-deoxy-3-\(\text{O}\)-sulfo-\(\text{D}\)-erythritol-1-yl\]episulfoniumidene-\(\text{D}\)-arabinitol inner salt (20)
$^1$H NMR spectrum (800 MHz, in D$_2$O) of 1,4-dideoxy-1,4-{$(S)$-[1-deoxy-3-O-sulfo-D-erythritol-1-yl]episulfoniumidene-D-arabinitol inner salt (12)

$^{13}$C NMR spectrum (200 MHz, in D$_2$O) of 1,4-dideoxy-1,4-{$(S)$-[1-deoxy-3-O-sulfo-D-erythritol-1-yl]episulfoniumidene-D-arabinitol inner salt (12)
$^1$H NMR spectrum (800 MHz, in D$_2$O) of 1,4-dideoxy-1,4-{(S)-[1-deoxy-3-O-sulfo-D-erythritol-1-yl]episulfoniumidene-D-arabinitol inner salt (13)
$^{13}$C NMR spectrum (200 MHz, in D$_2$O) of 1,4-dideoxy-1,4-\{(S)-[1-deoxy-3-O-sulfo-D-erythritol-1-yl]episulfoniumidene-D-arabinitol inner salt (13)