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Biological evaluation of de-O-sulfonated analogs of salacinol, the role of sulfate anion in the side chain on the α-glucosidase inhibitory activity

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Abstract—De-O-sulfonated analogs (**10a**, $Y^- = CH_3OSO_3$ and **10b**, $Y^- = Cl$) of salacinol, a naturally occurring glycosidase inhibitor, and its diastereomer (**12a**, $Y^- = CH_3OSO_3$) with L-thiosugar moiety (1,4-dideoxy-1,4-epithio-L-arabinitol) were prepared. Their inhibitory activities against intestinal maltase and sucrase were examined and compared with those of the parent α-glycosidase inhibitor, salacinol (**1a**). Compounds **10a** and **10b** showed a potent inhibitory activity equal to that of **1a** against both enzymes, although **12a** was a weak inhibitor against sucrase and maltase. These results indicated that the *O*-sulfonate anion moiety of **1a** is not essential for the inhibitory activity.

1. Introduction

Salacinol (**1a**, $X = S^+$) is a potent glycosidase inhibitor isolated from aqueous extracts of roots and stems of *Salacia reticulata* WIGHT (known as 'Kotala himbutu' in Singhalese), which is traditionally used for the treatment of diabetes in Sri Lanka and the south region of India.[1] The structure of **1a** established by the X-ray crystallographic analysis is quite unique, the ring sulfonium ion being stabilized by the sulfate counteranion by forming a spirobicyclic-like configuration comprised of 1-deoxy-4-thio-D-arabinofranosyl cation and 1-deoxy-L-erythrosyl-3-sulfate anion, as shown in Figure 1.[1] The α-glucosidase inhibitory activities of **1a** are potent, and have been revealed to be as strong as those of voglibose and acarbose, which are widely used clinically these days.[1] Thus, glycosidase inhibitors containing sulfonium ions are of interest as mimics of oxacarbenium intermediates in glycosidase hydrolysis reactions,[2] and much attention has been focused on this novel class of inhibitors **1a** and related compounds.[3] Total synthesis of **1a** was accomplished by two groups.[4] Extensive structure–activity relationship studies on **1a** have also been reported, its enantiomer[4c,5f] (**2a**), diastereomers

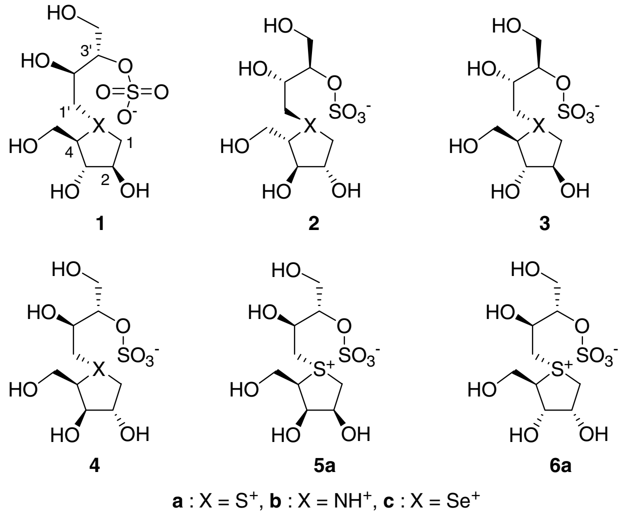

Figure 1.

Keywords: α-Glucosidase inhibitor; Salacinol; De-O-sulfonated salacinol; Thiosugar.

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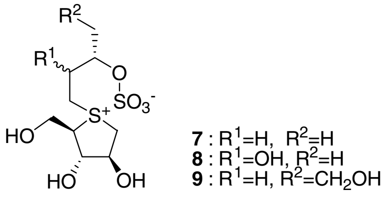


Figure 2.

(**3a**,[4a,c,5b,d,f] **4a**,[5f] **5a**,[5k] and **6a**[5k]) as well as their heterocyclic analogs (**1b**,[5a,c] **1c**,[5e,j] **2b**,[5c,f] **3c**,[5e] and **4b**[5f]) and related compounds[5l–p] having been synthesized and evaluated.

The authors also synthesized analogs of **1a** lacking the hydroxyl and/or hydroxymethyl groups (**7**, **8**, and **9**), and have found that both functionalities (C-2′ hydroxyl and C-3′ hydroxylmethyl) in the side chain are essential to maintain the inhibitory activity. Thus, structural determinant for the glycosidase inhibitory activity has been revealing to a certain extent (Fig. 2). On the other hand, all these structure–activity studies have been focusing on the onium sulfate inner salt structure so far,[5,6] and less consideration has been given to the role of the sulfate anion moiety. This partial structure has been considered to be essential for the inhibitory activities.[5d]

As a part of our continuing study on the structure–activity relationship of **1a**, the role of the sulfonate anion moiety was explored in this study. The analogs (**10a** and **10b**) lacking the *O*-sulfate anion of **1a** were prepared and their inhibitory activity was examined. A diastereomer (**11**) of **1a** and its de-O-sulfonated analogs (**12a**) were also synthesized in order to assess structure–activity relationship (Fig. 3).

Recently, Gallienne and co-workers[5h] have reported the one-pot synthesis of the cyclic sulfate, 2,4-*O*-isopropylidene-L-erythritol-1,3-cyclic sulfate (**13**), the key intermediate for the synthesis of **1a** and related compounds, in which they claimed that the yield at the stage of the cyclic sulfination reaction of the diol, 2,4-*O*-isopropylidene-L-erythritol (**14**) leading to the cyclic sulfite, 2,4-*O*-isopropylidene-L-erythritol-1,3-cyclic sulfite (**15**), was irreproducible when they tried our[5a] protocol published as a communication.[5a] Here, we also present full details on the synthesis of the cyclic sulfate **13** including points to notice (Scheme 2).

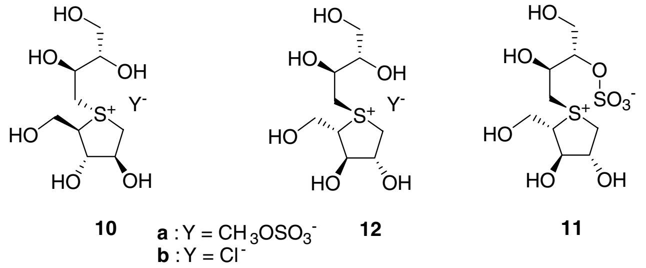


Figure 3.

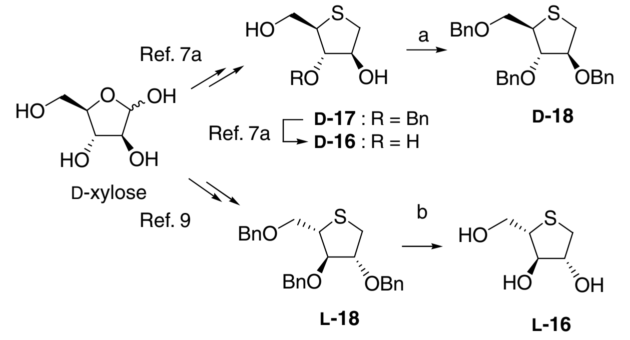
2. Chemistry

2.1. Preparation of thiosugars (16 and 18) and cyclic sulfate (13)

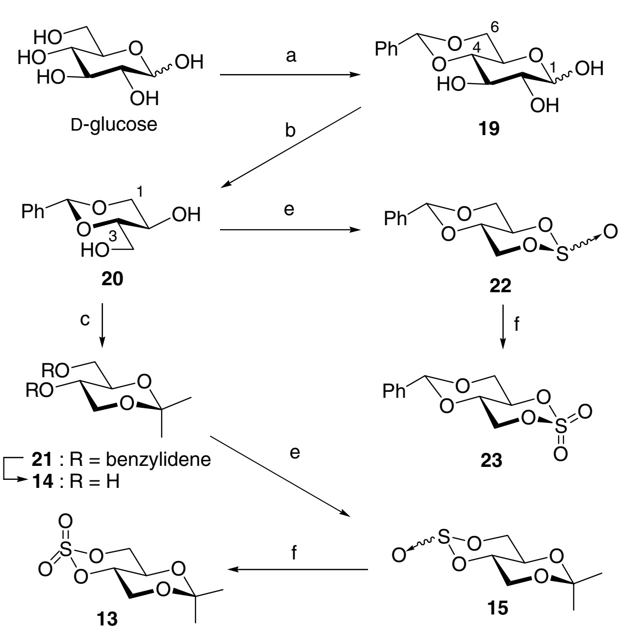
According to the lit.[7a] thio-arabinitol (D-**16**) was prepared starting from D-xylose via 3-*O*-benzyl-1,4-dideoxy-1,4-epithio-D-arabinitol[7a,8] (D-**17**). The perbenzylated compound, 2,3,5-tri-*O*-benzyl-1,4-dideoxy-1,4-epithio-D-arabinitol (D-**18**), was obtained in good yield by treatment of D-**17** with excess benzyl bromide. Its L-isomer, 2,3,5-tri-*O*-benzyl-1,4-dideoxy-1,4-epithio-L-arabinitol (L-**18**), was synthesized starting also from D-xylose according to the lit.[9] The Birch reduction of L-**18** gave L-**16** in 85% yield (Scheme 1).

The cyclic sulfate **13** was synthesized starting from D-glucose according to the method which the authors previously reported as a communication.[5a] D-Glucose was first led to 4,6-*O*-benzylidene-D-glucose (**19**) by the tosic acid catalyzed transacetalation.[11] Compound **19** was then oxidized with sodium metaperiodate[12] in the presence of excess sodium bicarbonate, and subsequent sodium borohydride reduction without isolation of the resulting D-erythrose derivative gave 1,3-*O*-benzylidene-L-erythritol (**20**) in 94% yield. Protection of the dihydroxy group of **20** with 2-methoxypropene followed by hydrogenolysis of the benzylidene moiety of the resulting 1,3-*O*-benzylidene-2,4-*O*-isopropylidene-L-erythritol (**21**) provided the diol **14** in good yield. Finally, **14** was converted to the desired cyclic sulfate **13** via the catalytic ruthenium tetroxide oxidation of the cyclic sulfite **15** (Scheme 2).

It is important to note that the condensation of the diol **14** with thionyl chloride to give the cyclic sulfite **15** must be carried out under completely anhydrous conditions. Thus, the employment of the freshly distilled diol **14** was preferred. The reaction proceeded cleanly at 0 °C in the presence of triethylamine to give the corresponding cyclic sulfite **15** in almost quantitative yield. ^{13}C NMR spectrum of the crude product showed no significant peaks other than those corresponding to the desired cyclic sulfite (see Fig. 4). On the other hand, when the diol **14** purified by the column chromatography was subjected to the condensation, highly polar side products often formed to a considerable extent, and



Scheme 1. Reagents and conditions: (a) BnBr, NaH, DMF, 0 °C; (b) Na, liq. NH$_3$, THF, −60 °C.



Scheme 2. Reagents and conditions: (a) $PhCH(OCH_3)_2$, *p*-TsOH, DMF, 50–60 °C; (b) $NaIO_4$, $NaHCO_3$, H_2O, THF, rt, then $NaBH_4$, rt; (c) $CH_3(CH_3O)C{=}CH_2$, *p*-TsOH, DMF, 0–5 °C; (d) H_2, Pd–C, EtOH, rt; (e) $SOCl_2$, Et_3N, CH_2Cl_2, 0 °C; (f) $NaIO_4$, $RuCl_3$, $NaHCO_3$, CCl_4, CH_3CN, H_2O, 0 °C to rt.

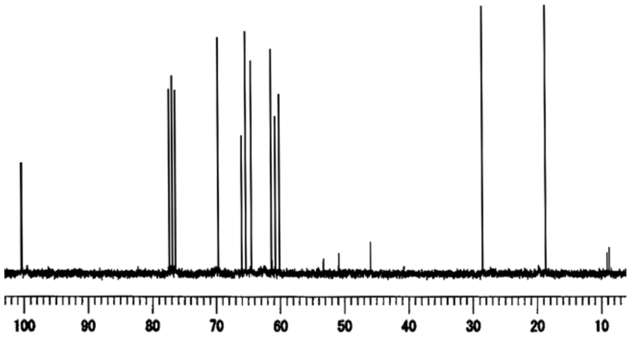


Figure 4. ^{13}C NMR spectrum (67.5 MHz, $CDCl_3$) of the crude sulfite **15**.

resulted in the reduction of the yield of the cyclic sulfite **15**. The formation of these undesired products would be attributable to the facile hydrolysis of the isopropylidene moiety of **15**, although triethylamine was added to trap the hydrogen chloride formed during the reaction. Moreover, quenching the reaction with water caused formation of considerable amount of highly polar and water-soluble materials, the yield of the cyclic sulfite **15** being reduced to ca. <60%. Rapid quenching of the reaction with excess ice-cooled aqueous sodium bicarbonate under vigorous stirring was needed to prevent the hydrolysis of the product **15**. When the quenching with aqueous sodium bicarbonate failed, the yields of **15** varied irregularly (ca. 75–90%).

The crude cyclic sulfite **15** was immediately oxidized to the cyclic sulfate **13** because of its instability even at room temperature. This oxidation stage was also problematic due probably to the acidity of sodium periodate in aqueous solution. When the oxidation of **15** was tried under the conventional conditions, the yield of the cyclic sulfate **13** ended up to be low or moderate (ca. < 65%). Addition of sodium bicarbonate to the reaction mixture was found to be effective, and substantial improvement of the yield of the cyclic sulfate **13** up to around 90–95% from the diol **14** was achieved. No noticeable signals due to side products other than to the desired oxidated product **13** were observed in its ^{13}C NMR spectrum, supporting that **13** was pure enough for the next reaction (see Fig. 5). The cyclic sulfate **13** thus obtained was labile on storing even in a freezer, and purification by column chromatography was needed to keep this compound for a few months. The yield after column chromatography was around 78–83%.

Gallienne and co-workers[5h] also described that the yield of the cyclic sulfination of another diol **20** was moderate, and the reported yield (82%)[4c] of 2,4-*O*-benzylidene-D-erythritol-1,3-cyclic sulfite (**22**) was irreproducible after their numerous attempts. The condensation of **20** with thionyl chloride proceeded without the formation of side products and gave reproducibly and almost quantitatively the cyclic sulfite **22** as reported by Ghavami et al.[4c] Its ^{13}C NMR spectrum suggested that the crude product of this reaction was also pure enough for the next reaction (see Fig. 6). The crude product was then subjected to the modified oxidation conditions used for the preparation of the sulfate **13** to give the practically pure cyclic sulfate, 2,4-*O*-benzylidene-D-erythritol-1,3-cyclic sulfate (**23**), in around 92% yield from the diol **20** (see Fig. 7). The yield of **23** after column

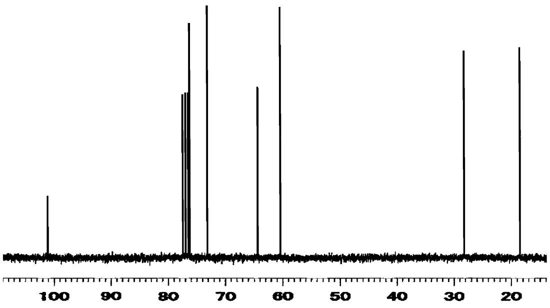


Figure 5. ^{13}C NMR spectrum (67.5 MHz, $CDCl_3$) of **13**.

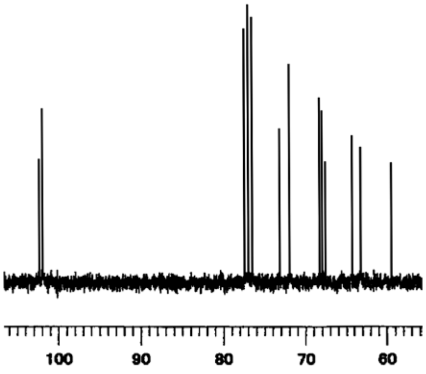


Figure 6. ^{13}C NMR spectrum (67.5 MHz, $CDCl_3$) of the crude **22**.

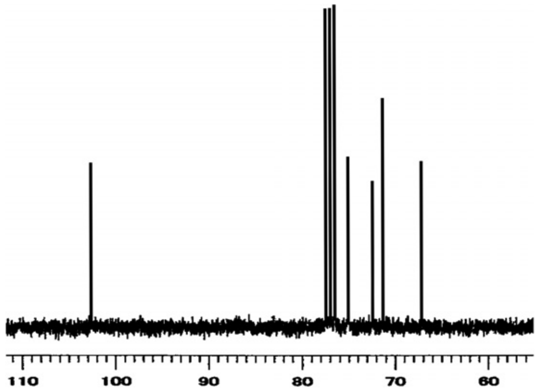


Figure 7. ^{13}C NMR spectrum (67.5 MHz, $CDCl_3$) of the crude **23**.

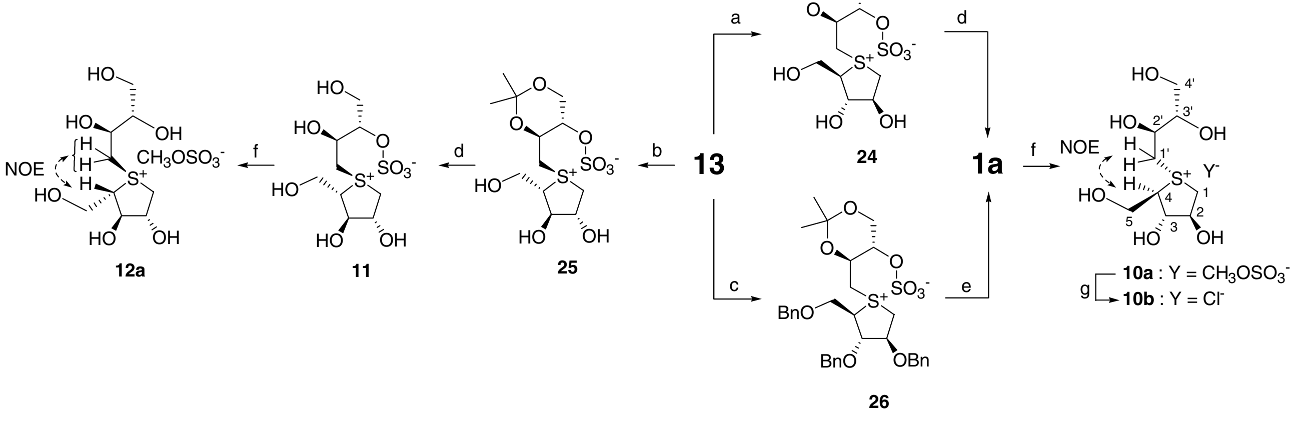
chromatography was slightly superior to that of **13** and was around 88% from the diol **20**.

2.2. Sulfonium salts (10, 11, and 12)

The coupling reactions were carried out by Sharpless's ring-opening method,[13] which was applied for the synthesis of salacinol (**1a**) by Yuasa[4a] and Ghavami[4b,c] (Scheme 3). The reaction of the thiosugars D- and L-**16b** with the cyclic sulfate **13** in DMF proceeded at 45 °C to give the coupled products **24** or **25** in 65% and 58% yield, respectively. The coupled products **24** and **25** were then subjected to acid-catalyzed deprotection to give **1a** and the diastereomer **11** in 74% and 89% yield, respectively. The specific rotation of **11** ($[\alpha]_D$ +35.5) implied that **11** was the antipode of the levorotatory isomer **3a** ($[\alpha]_D$ −35.6) reported.[4c] The coupling reaction of the perbenzylated thiosugar D-**18** with **13** was performed in gently refluxed 1,1,1,3,3,3-hexafluoroisopropanol (HFIP) according to the lit.[4d] to give the desired product **26** quantitatively. Hydrogenolysis of **26** with palladium on carbon in 90% acetic acid proceeded smoothly to give **1a** in 82% yield. De-O-sulfonation of salacinol (**1a**) and the diastereomer **11** with methanolic hydrogen chloride gave the desired sulfonium salts **10a** and **12a** in 86% and 79% yield, respectively. Finally, the monomethyl sulfate anion of **10a** was exchanged to the chloride anion by the treatment with ion exchange resin IRA-400J (Cl^- form) to give **10b** in 96% yield. The FAB mass spectrum of **10a** run in the positive mode showed a peak at *m/z* 255 due to the sulfonium cation structure, and one in the negative mode showed a peak at *m/z* 111 due to methyl sulfate anion moiety, supporting the desired structure. Observed downfield shift signals [δ_H 3.86 (H-1), 3.73 (H-1′a), 3.85 (H-1′b) and 4.02 (H-4)] corresponding to α-protons of the sulfur in its ^{1}H NMR spectrum supported the sulfonium ion structure of **10a**. The ^{13}C NMR spectroscopic properties of **10a** were similar to those of **1a** except for the signal due to C-3′ methine carbon (δ_C 75.3), which is significantly shifted upfield in comparison with that of **1a** (δ_C 80.6[1b]), supporting the depicted de-O-sulfonated structure. The relative stereochemistry of the side chain on the sulfur atom was detected on the basis of nuclear Overhauser effect spectroscopy (NOESY) experiments, as shown in Scheme 3. The observed NOE correlations between the ring proton (H-4) α to the sulfur atom and methylene protons on C-1′ suggested the depicted configuration of the side chain. The corresponding chloride **10b** showed very similar ^{1}H and ^{13}C NMR spectral properties to those of **10a**. Disappearance of the peak at *m/z* 111 in the FAB mass spectrum of **10a** run in the negative mode also supported the exchange of the methyl sulfate anion into the chloride. Compound **12a** also displayed similar ^{1}H spectroscopic properties to those of **10a**, with deshielded signals of five protons α to the sulfonium ion. The relative stereochemistry of the side chain on the sulfur atom of **12a** was also confirmed by means of NOESY experiments.

3. α-Glucosidase inhibitory activity

The glycosidase inhibitory activities of synthesized compounds **10a**, **10b**, **11**, and **12a** were tested for the intestinal α-glucosidase in vitro[14] and compared with that of salacinol (**1a**), as shown in Table 1. It is noteworthy that desulfonated compounds (**10a** and **10b**) maintained



Scheme 3. Reagents and conditions: (a) D-**16**, DMF, Na_2CO_3, 45 °C; (b) L-**16**, DMF, Na_2CO_3, 45 °C; (c) D-**18**, HFIP, K_2CO_3, reflux; (d) 0.2% aq HCl, 40 °C; (e) H_2, Pd–C, 90% aq AcOH, 50 °C; (f) 5% methanolic HCl, 45 °C; (g) IRA-400J (Cl^- form), MeOH–H_2O, rt.

Table 1. IC_{50} values (μM) of compounds **1a**, **10a**, **10b**, **11**, and **12a** against disaccharidase

Entry	Compound	Maltase	Sucrase
1	**1a**	9.6	2.5
2	**10a**	15.6	3.7
3	**10b**	14.0	3.5
4	**11**	>1197 (18)[a]	>1197 (21)[a]
5	**12a**	>1092[b]	>1092 (48)[a]

[a] Values in parentheses indicate inhibition (%) at 400 μg/ml (**11**, 1197 μM; **12a**, 1092 μM).

[b] Compound **12a** showed no inhibition at 1092 μM.

almost equal activities to salacinol (**1a**), irrespective of species of the counteranion. Thus, characteristic spirobicyclic structure of **1a** is found no longer to be essential for the potent α-glucosidase activity.

Upon substitution of the thiosugar moiety (D-form) of **1a** to its enantiomer (L-form, **11**), the inhibitory activities against both enzymes were reduced to a large extent. Same tendency had been observed with respect to aza-analogs of **1a**, where aza-isomer composed of L-azasugar showed less activity than the one composed of D-azasugar (**1b**, X = NH^+).[7b]

The de-O-sulfonated version (**12a**) of **11** showed no inhibitory activity against maltase, though **12a** sustained modest activity against sucrase (48% inhibition at ca. 1.1 mM, entry 5). Further structure–activity relationship studies on the strong α-glucosidase inhibitory activity of **1a** and selectivity to glycosidases are in progress.

4. Experimental

Mps were determined on a Yanagimoto MP-3S micromelting point apparatus, and mps and bps are uncorrected. IR spectra were measured on either a Shimadzu IR-435 grating spectrophotometer or a Shimadzu FTIR-8600PC spectrophotometer. NMR spectra were recorded on a JEOL JNM-GSX 270 (270 MHz ^{1}H, 67.5 MHz ^{13}C), a JEOL JNM- ECA 500 (500 MHz ^{1}H, 125 MHz ^{13}C) or a JEOL JNM-ECA 600 (600 MHz ^{1}H, 150 MHz ^{13}C) spectrometer. Chemical shifts (δ) and coupling constants (J) are given in parts per million and Hertz, respectively. Low-resolution and high-resolution mass spectra (electron impact) were recorded on either a Shimadzu QP 1000EX spectrometer or a JEOL JMS-HX 100 spectrometer. Optical rotations were determined with a JASCODIP-370 digital polarimeter. Column chromatography was effected over Merck Kieselgel 60 (70–230 mesh). All the organic extracts were dried over either anhydrous magnesium sulfate or anhydrous sodium sulfate prior to evaporation.

4.1. 2,3,5-Tri-*O*-benzyl-1,4-dideoxy-1,4-epithio-D-arabinitol (D-18)

A solution of 3-*O*-benzyl-1,4-dideoxy-1,4-epithio-D-arabinitol[7a,8] (D-**17**, 8.27 g, 34.5 mmol) in DMF (100 ml) was added dropwise to a mixture of sodium hydride (3.2 g, 80 mmol, 60% in liquid paraffin, washed with benzene), benzyl bromide (8.4 ml, 70.7 mmol), and DMF (100 ml) at 0 °C. After being stirred at 0 °C for 30 min, the mixture was poured into ice-water (1 L) and extracted with diethyl ether. The extract was washed with brine and evaporated to give a colorless oil (15.5 g), which on column chromatography (*n*-hexane/ethyl acetate, 10:1) gave the title compound D-**18** (13.1 g, 90%) as a colorless oil, bp 221–224 °C/1 mmHg; $[\alpha]_D^{20}$ +6.03 (c = 1.44, $CHCl_3$) lit.[4c] +5 (c = 1.6, $CHCl_3$). The spectral properties of D-**18** were in accord with those reported.[4c]

4.2. 1,4-Dideoxy–1,4-epithio-L-arabinitol (D-16)

A solution of 2,3,5-tri-*O*-benzyl-1,4-dideoxy-1,4-epithio-L-arabinitol[9] (L-**18**, 7.05 g, 16.8 mmol) in THF (50 ml) was treated with a solution of sodium (1.54 g, 67 mg-atom) in liquid ammonia (ca. 140 ml) at −60 °C for 1 h. After addition of a mixture of methanol (5 ml) and THF (15 ml) to the mixture, ammonia was gradually removed by increasing the temperature of the mixture and neutralized with concd hydrochloric acid. The resulting precipitates were filtered off and washed with methanol. The combined filtrate and washings were evaporated to give a pale yellow oil (3.5 g), which on column chromatography ($CHCl_3$/EtOH, 5:1) gave the title compound L-**16** (2.14 g, 85%) as a pale yellow oil, $[\alpha]_D^{22}$ −41.8 (c = 1.2, MeOH). The spectral properties of L-**16** were in accord with those of the corresponding D-isomer D-**16** reported.[10]

4.3. 4,6-*O*-Benzylidene-D-glucose (19)

According to the method reported by Barili et al.[11] tosic acid catalyzed transacetalation of D-glucose (15 g, 83.3 mmol) with benzaldehyde dimethyl acetal (13.8 ml, 91.7 mmol) in DMF (70 ml) was carried out. Work-up in a manner similar to that described in the lit.[11] gave a viscous syrup, which was triturated with a mixture of diethyl ether (40 ml) and water (40 ml). The deposited solid was collected by filtration, and then washed with diethyl ether and water to give the title compound **19** (13 g, 58%, α/β = ca. 2/1) as colorless needles. The combined filtrate and washings were separated, and organic layer was extracted with water. Deposited solid during evaporation of the water layer was collected by filtration to give **19** (1.9 g, 9%) as colorless needles. The filtrate was evaporated again, and the residue was extracted with hot ethyl acetate to give **19** (3.4 g, 15%) as a colorless solid. Mp 181–183 °C, lit.[11] 178–179 °C. The spectral properties of **19** were in accord with those reported.[11] Compound **19** was used in the next step without further purification.

4.4. 1,3-*O*-Benzylidene-L-erythritol (20)

A mixture of **19** (6.0 g, 22.4 mmol), sodium metaperiodate (9.8 g, 45.9 mmol), sodium hydrogen carbonate (5.0 g, 60 mmol), water (160 ml), and THF (70 ml) was stirred at room temperature for 1 h. The mixture was cooled to 0 °C, and sodium borohydride (1.74 g, 45.9 mmol) was added, and the resulting mixture was stirred for another 1 h. The precipitates were filtered

Table 2. ^{1}H NMR data of **20** in CD_3OD

	δ observed (500 MHz)	δ lit.[4c] (400 MHz)
H-1ax	3.58 (dd, J = 10.0, 10.0)	3.55-3.67 (m)
H-1eq	4.20 (dd, J = 10.0, 4.0)	4.20 (m)
H-2	3.60–3.66 (m)	3.55–3.67 (m)
H-3	3.60–3.66 (m)	3.55–3.67 (m)
H-4a	3.74 (dd, J = 12.0, 5.5)	3.74 (dd, J = 12.1, 5.7)
H-4b	3.91 (dd, J = 12.0, 2.0)	3.92 (dd, J = 12.1, 1.7)
Others	5.52 (1H, s, PhC*H*), 7.27–7.35 (3H, m, arom.), 7.45–7.51 (2H, m, arom.)	5.53 (1H, s, PhC*H*), 7.28–7.53 (5H, m, arom.)

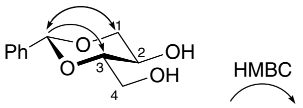
Compound **20** was named as 2,4-*O*-benzylidene-D-erythritol in the literature.[4c]

off, and the filtrate was extracted with ethyl acetate. The extract was washed with aqueous sodium thiosulfate–sodium hydrogen carbonate, and evaporated to give the title compound **20** (4.41 g, 94%) as a colorless solid, which was pure enough for further reaction. For analytical purpose a small portion was recrystallized from a mixture of dichloromethane and methanol.

Compound **20**. Colorless needles; Mp 133–134 °C, lit.[4c] mp 138–139 °C. $[\alpha]_D^{19}$ −46.8 (c = 1.0, CH_3OH), lit.[4c] $[\alpha]_D$ −44.0 (c = 1.0, CH_3OH).

Although ^{1}H and ^{13}C NMR spectroscopic properties of **20** were in good accordance with those reported,[4c] in which assigned signals due to C-2 and C-3 carbons[4c] were found to be interchanged with each by two-dimensional spectroscopic studies. All the signals were unambiguously assigned as shown in Tables 2 and 3.

Long-range correlation was detected between the signals due to the benzylidene methine ptoton (δ_H 5.52) and C-3 methine carbon (δ_C 84.2), and no correlation between the signal due to C-2 methine carbon (δ_C 62.6) and that due to the benzylidene methine proton (δ_H 5.52) as shown below.



20

4.5. 1,3-*O*-Benzylidene-2,4-*O*-isopropylidene-L-erythritol (21)

A mixture of **20** (3.0 g, 14.3 mmol), 2-methoxypropene (2.07 g, 28.8 mmol), tosic acid (30 mg, 0.17 mmol), and DMF (60 ml) was stirred at 0–5 °C for 12 h. The reaction mixture was neutralized by the addition of sodium carbonate (700 mg), and the resulting mixture was filtered. The filtrate was poured into water (300 ml) and extracted with *n*-hexane. The extract was washed with brine, and evaporated to give quantitatively **21** (3.6 g) as a colorless solid, which was pure enough for further reaction. For analytical purpose a small portion was recrystallized from ethanol to give colorless needles.

Compound **21**. Mp 76.5–78 °C. $[\alpha]_D^{22}$ +3.1 (c = 1.0, $CHCl_3$), lit.[5h] $[\alpha]_D$ +2 (c = 1.2, $CHCl_3$).

Table 3. ^{13}C NMR data of **20** in CD_3OD

	δ observed (125 MHz)	δ lit.[4c] (100 MHz)
C-1	72.2	72.21
C-2	62.6	84.22
C-3	84.2	62.59
C-4	62.8	62.76
Others	102.4 [PhCH], 127.5/129.0/ 129.8 (d, arom.), 139.5 (s, arom.)	102.36 [PhCH], 127.49/128.99/129.77 (d, arom.), 139.52 (s, arom.)

Compound **20** was named as 2,4-*O*-benzylidene-D-erythritol in the literature.[4c]

Although ^{1}H and ^{13}C NMR spectroscopic properties of **21** were in good accordance with those reported,[5h] there appeared some incorrect assignments in the lit.[5h] On the basis of two-dimensional spectroscopic studies, all the signals were unambiguously assigned as shown in Tables 4 and 5.

Long-range correlations were detected with the benzylidene methine proton (δ_H 5.61) between both C-1 (δ_C 69.6) and C-3 (δ_C 75.0) carbons in HMBC experiments. Between the signals due to the equatorial protons (δ_H 4.23) on C-1 and the tertiary carbon (δ_C 102.0) of the

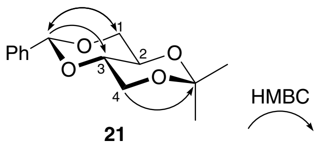
Table 4. ^{1}H NMR data of**21** in $CDCl_3$

	δ observed (600 MHz)	δ lit.[5h] (400 MHz)
H-1ax	3.77 (dd, J = 10.1, 9.8)	3.95–4.01 (m)
H-1eq	4.23 (dd, J = 10.1, 4.6)	3.95–4.01 (m)
H-2	3.98 (ddd, J = 9.8, 9.2, 4.6)	3.73–3.80 (m)
H-3	3.76 (ddd, J = 10.1, 9.2, 5.2)	3.88–3.93 (m)
H-4ax	3.90 (dd, J = 10.6, 10.1)	3.73–3.80 (m) as H-4b
H-4eq	3.96 (dd, J = 10.6, 5.2)	4.23 (dd J = 10.3, 4.0) as H-4a
Others	1.43/1.56 [s, $(CH_3)_2C$], 5.61 (s, PhC*H*), 7.33–7.39 (3H, m, arom.), 7.46–7.49 (2H, m, arom.)	1.57/1.43 [s, $(CH_3)_2C$], 5.62 (s, PhC*H*), 7.35–7.38 (3H, m, arom.), 7.47–7.80 (2H, m, arom.)

Table 5. ^{13}C NMR data of **21** in $CDCl_3$

	δ observed (150 MHz)	δ lit.[5h] (100 MHz)
C-1	69.6	62.3
C-2	66.6	75.0
C-3	75.0	66.6
C-4	62.3	69.6
Others	19.3/29.1 [$(CH_3)_2C$], 100.0 [$(CH_3)_2C$], 102.0 [PhCH], 126.1/128.3/129.1 (d, arom.), 137.2 (s, arom.)	19.3/29.1 [$(CH_3)_2C$], 100.0 [$(CH_3)_2C$], 102.0 [PhCH], 126.1/128.3/129.2 (d, arom.), 137.2 (s, arom.)

benzylidene moiety were also observed. Furthermore, the equatorial proton (δ_H 3.96) on C-4 also correlated with the quaternary carbon (δ_C 100.0) of the isopropylidene moiety.



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4.6. 2,4-*O*-Isopropylidene-L-erythritol (14)

A suspension of 10% palladium-on-carbon (1.0 g) in ethanol (25 ml) was pre-equilibrated with hydrogen. To the suspension was added a solution of **21** (2.08 g, 8.3 mmol) in ethanol (50 ml), and hydrogenation was continued at room temperature under atmospheric pressure for 6 days. The catalyst was filtered off, and the filtrate was evaporated to give an oil (1.41 g), which was triturated with *n*-hexane to give the title compound **14** (1.33 g, 99%) as a colorless oil. A mixture of the oil and a small amount of dichloromethane solidified on keeping in a refrigerator to give colorless needles.

The hydrogenation of **21** was very slow, and 4 days was also taken up to complete the reduction when the hydrogenolysis was preformed at 50 °C. When reaction progress is hardly recognized by TLC analysis, replacement of the used catalyst to new one was effective to complete the hydrogenation. Significant improvement of reaction efficiency was also not observed under 4.5 atm pressure.

Compound **14**. Mp 41–42 °C. Bp 138–140 °C/2 mmHg. $[\alpha]_D^{22}$ +46.6 ($c = 1.0$, CH_3OH), lit.[5h] $[\alpha]_D$ +45 ($c = 1.1$, CH_3OH).

Although ^1H and ^{13}C NMR spectroscopic properties of **14** were in good accordance with those reported, there appeared some incorrect assignments in the lit.[5h] On the basis of two-dimensional spectroscopic studies, all the signals were unambiguously assigned as shown in Tables 6 and 7.

Long-range correlations, between the signals due to both methylene protons (δ_H 3.60 and 3.79) on C-4 and the quaternary carbon (δ_C 99.8) of the isopropylidene moiety were detected in HMBC experiments.

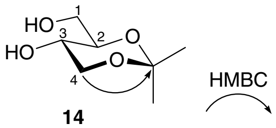

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Table 6. ^1H NMR data of **14** in $CDCl_3$

	δ observed (500 MHz)	δ lit.[5h] (400 MHz)
H-1a	3.59 (dd, $J = 11.5, 6.0$)	3.76–3.82 (m)
H-1b	3.77 (dd, $J = 11.5, 2.3$)	3.57–3.69 (m)
H-2	3.66 (ddd, $J = 9.5, 6.0, 2.3$)	3.48 (td, $J = 9.2, 9.2, 5.6$)
H-3	3.47 (ddd, $J = 9.5, 9.5, 5.5$)	3.57–3.69 (m)
H-4ax	3.60 (dd, $J = 11.5, 9.5$)	3.57–3.69 (m) as H-4b
H-4eq	3.79 (1dd, $J = 11.5, 5.5$)	3.76–3.82 (m) as H-4a
Others	1.34/1.46 [s, $(CH_3)_2C$]	1.35/1.48 [s, $(CH_3)_2C$]

Table 7. ^{13}C NMR data of **14** in $CDCl_3$

	δ observed (125 MHz)	δ lit.[5h] (100 MHz)
C-1	63.2	65.5
C-2	76.5	64.0
C-3	64.0	76.5
C-4	65.5	63.3
Others	19.6/28.9 [$(CH_3)_2C$], 99.8 [$(CH_3)_2C$]	19.7/28.9 [$(CH_3)_2C$], 99.9 [$(CH_3)_2C$]

4.7. 2,4-*O*-Isopropylidene-L-erythritol 1,3-cyclic sulfite (15)

A solution of freshly distilled thionyl chloride (476 μl, 6.55 mmol) in dichloromethane (20 ml) was added dropwise to a stirred mixture of **14** (810 mg, 5.0 mmol), triethylamine (1.74 ml, 12.6 mmol), and dichloromethane (25 ml) at 0 °C. After being stirred at 0 °C for 10 min, the mixture was poured into ice-cooled and vigorously stirred aqueous sodium hydrogen carbonate (50 ml), and extracted with dichloromethane. The extract was washed with brine, and evaporated to give **15** (1.05 g) as a pale yellow oil, whose ^{13}C NMR spectrum suggested that the crude product was pure enough for the next reaction (Fig. 4). The crude mixture was immediately used in the next step without purification.

A mixture of the sulfite **15**: FABMS *m/z*: 209 $[M+H]^+$ (pos.), FABHRMS *m/z*: 209.0491 ($C_7H_{13}O_5S$ requires 209.0483), ^1H NMR (600 MHz, $CDCl_3$) δ: 1.40 [3H, s, $(CH_3)_2C$], 1.54 [1.8H, s, $(CH_3)_2C$], 1.55 [1.2H, s, $(CH_3)_2C$], 3.83 (0.4H, dd, $J = 10.8$, 5.8 Hz, H-β4eq), 3.86 (0.4H, dd, $J = 10.8$, 10.0 Hz, H-β4ax), 3.890 (0.6H, dd, $J = 11.0$, 10.5 Hz, H-α4ax), 3.892 (0.4H, dd, $J = 10.0$, 4.6 Hz, H-β1eq), 3.97 (0.6H, dd, $J = 11.0$, 5.3 Hz, H-α4eq), 4.13 (0.6H, dd, $J = 10.5$, 8.6 Hz, H-α1ax), 4.16 (0.6H, ddd, $J = 10.5$, 9.6, 5.3 Hz, H-α3), 4.18 (0.4H, ddd, $J = 10.3$, 10.0, 4.6 Hz, H-β2), 4.44 (0.6H, ddd, $J = 9.6$, 8.6, 5.8, H-α2), 4.47 (0.6H, dd, $J = 10.5$, 5.8 Hz, H-α1eq), 4.67 (0.4H, dd, $J = 10.3$, 10.0 Hz, H-β1ax), 4.89 (0.4H, ddd, $J = 10.0$, 10.0, 5.8 Hz, H-β3), ^{13}C NMR (150 MHz, $CDCl_3$, α/β) δ: 65.6/60.4 (C-1), 61.6/61.1 (C-4), 69.8/64.7 (C-3), 64.9/66.2 (C-2), 100.4/100.5 [$(CH_3)_2C$].

4.8. 2,4-*O*-Isopropylidene-L-erythritol 1,3-cyclic sulfate (13)

To a well-stirred mixture of the crude **15** (1.05 g), sodium hydrogen carbonate (1.39 g mg, 16.5 mmol), carbon tetrachloride (20 ml), acetonitrile (20 ml), and water (10 ml) was added dropwise a brown mixture of sodium metaperiodate (3.21 g, 15 mmol), ruthenium chloride *n*-hydrate (70 mg), and water (20 ml) at 0 °C. After being stirred at room temperature for 30 min, the reaction was quenched by the addition of aqueous sodium thiosulfate–sodium hydrogen carbonate (20 ml). The resulting purple mixture was filtered off, and the filtrate was extracted with diethyl ether. The extract was washed with brine, and evaporated to give a colorless solid (1.1 g), to which was added successively diethyl ether and *n*-hexane, and the deposited solid was filtered off. The filtrate was evaporated to give the title compound **13** (1.06 g, 95%) as a colorless solid, which was pure enough for further reaction. This was supported by the ^{13}C NMR spectrum of the solid (Fig. 5). Compound **13** decomposed gradually on keeping even in a freezer. Column chromatography (*n*-hexane/acetone, 5:1, *n*-hexane/ethyl acetate, 7:1 or *n*-hexane/dichloromethane, 1:2) gave **13** in around 78–83%, and pure **13** could be kept in a freezer for few months.

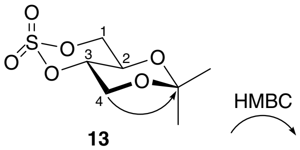
Compound **13**. Colorless prisms. Mp 91–92.5 °C (dec). The value of the specific rotation for **13** was $[\alpha]_{\mathrm{D}}^{24}$ +2.2 (c = 1.15, $CHCl_3$), being inconsistent with that reported [lit.[5h] $[\alpha]_{\mathrm{D}}$ = −4 (c = 1.1, $CHCl_3$)]. In order to reconfirm the $[\alpha]_{\mathrm{D}}$ value of **13**, the enantiomer of **13**, 2,4-*O*-isopropylidene-D-erythritol-1,3-cyclic sulfate (**28**) was synthesized as follows (Scheme 4). According to the modified method used for the preparation of the cyclic sulfate **13**, 2,4-*O*-isopropylidene-D-erythritol[15] (**27**, 1.0 g, 6.17 mmol) was converted to **28**[4a,b] (1.1 g, 80%), the specific rotation of which is lacking in the lit.[4a,b] The specific rotation of enantiomer **28** was $[\alpha]_{\mathrm{D}}^{27}$ −2.43 (c = 1.25, $CHCl_3$), indicating that the value of the specific rotation reported by Gallienne et al.[5h] was incorrect.

Although ^{1}H and ^{13}C NMR spectroscopic properties of **13** were in good accordance with those reported, there appeared some incorrect assignments in the lit.[5h] On the basis of two-dimensional spectroscopic studies, all the signals were unambiguously assigned as shown in Tables 8 and 9.

Table 9. ^{13}C NMR data of **13** in $CDCl_3$

	δ observed (125 MHz)	δ lit.[5h] (100 MHz)
C-1	73.3	60.8
C-2	64.6	76.6
C-3	76.6	64.7
C-4	60.8	73.3
Others	18.8/28.5 [$(CH_3)_2C$], 101.1 [$(CH_3)_2C$]	18.9/28.5 [$(CH_3)_2C$], 101.1 [$(CH_3)_2C$]

Following long-range correlation between signals due to both methylene protons (δ_H 3.92 and 4.03) on C-4 and that of the quaternary carbon (δ_C 101.1) of the isopropylidene moiety was also observed in the HMBC experimental.



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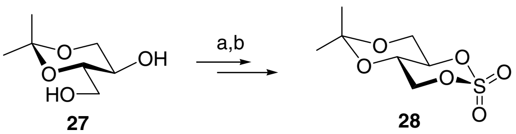

27 **28**

Scheme 4. Reagents and conditions: (a) $SOCl_2$, Et_3N, CH_2Cl_2, 0 °C; (b) $NaIO_4$, $RuCl_3$, $NaHCO_3$, CCl_4, CH_3CN, H_2O, 0 °C to rt.

4.9. 2,4-*O*-Benzylidene-D-erythritol 1,3-cyclic sulfite (22)

Following the method used for the preparation of **15**, the diol **20** (1.6 g, 7.6 mmol) was treated with thionyl chloride (0.7 ml, 9.6 mmol) in dichloromethane (54 ml). Work-up gave a practical pure crude mixture of cyclic sulfite **22** (1.96 g, α/β = ca. 1/1.6) quantitatively. The crude **22** was immediately used in the next step without further purification. The ^{1}H and ^{13}C NMR spectra of the minor isomer **22**α were in good accordance with those of less polar isomer reported.[4c] The downfield shift of signals due to H-1ax and H-3 of **22**α arising from anisotropy effect by the S → O bond suggested the α orientation of the oxygen in S → O group.[16]

A mixture of the sulfite **22**: ^{1}H NMR ($CDCl_3$) δ: 3.88 (0.38H, dd, J = 10.6, 10.3 Hz, H-4αax), 3.91 (0.62H, dd, J = 10.6, 10.3 Hz, H-4βax), 4.05 (0.38H, dd, J = 10.3, 4.9 Hz, H-1αeq), 4.16 (0.38H, ddd, J = 10.9, 9.6, 4.9 Hz, H-α2), 4.26 (0.38H, dd, J = 10.6, 4.9 Hz, H-4αeq), 4.27 (0.62H, dd, J = 11.0, 9.0 Hz, H-β1ax), 4.32 (0.62H, ddd, J = 10.3, 9.8, 4.9 Hz, H-β3), 4.39 (0.62H, dd, J = 10.6, 4.9 Hz, H-β4eq), 4.47 (0.62H, ddd, J = 9.8, 9.0, 6.0, H-β2), 4.63 (0.62H, dd, J = 11.0, 6.0 Hz, H-β1eq), 4.83 (0.38H, dd, J = 10.9, 10.3 Hz, H-α1ax), 5.07 (0.38H, ddd, J = 10.3, 9.6, 4.9 Hz, H-α3), 5.62, (0.62H, s, PhC*H*β), 5.67 (0.38H, s, PhC*H*-α), 7.36–7.48 (5H, m, arom.). ^{13}C NMR ($CDCl_3$, α/β) δ: 59.7/64.4 (C-1), 63.4/68.5 (C-3), 67.8/68.3 (C-4),

Table 8. ^{1}H NMR data of **13** in $CDCl_3$

	δ observed (500 MHz)	δ lit.[5h] (400 MHz)
H-1ax	4.61 (dd, J = 10.6, 10.3),	3.92 (dd, J = 10.3, 10.3) as H-1b
H-1eq	4.47 (dd, J = 10.3, 4.9)	4.03 (dd, J = 10.3, 5.4) as H-1a
H-2	4.22 (ddd, J = 10.6, 10.1, 4.9),	4.66 (td, J = 10.3, 5.4)
H-3	4.66 (ddd, J = 10.6, 10.1, 5.2)	4.22 (td, J = 10.3, 4.8)
H-4ax	3.92 (dd, J = 10.9, 10.6)	4.61 (dd, J = 10.3, 10.3) as H-4a
H-4eq	4.03 (dd, J = 10.9, 5.2)	4.46 (dd, J = 10.3, 4.8) as H-4b
Others	1.43/1.55 [$(CH_3)_2C$]	1.43/1.55 [$(CH_3)_2C$]

72.1/ 73.3 (C-2), 102.0/102.5 (Ph*C*H_2), 126.0/126.1/ 128.4/129.5 (d, arom.), 136.2/136.3 (s, arom.).

4.10. 2,4-*O*-Benzylidene-D-erythritol 1,3-cyclic sulfate (23)

Following the method used for the preparation of **13**, the crude **22** (1.96 g) was oxidized with ruthenium tetroxide generated in situ from sodium metaperiodate (4.8 g, 22.4 mmol) and ruthenium chloride *n*-hydrate (80 mg) in the presence of sodium bicarbonate (1.9 g, 22.6 mmol). Work-up in a manner similar to that used for the preparation of **13** gave the title compound **23** as a colorless solid (1.9 g, 92%), which was pure enough for further reaction. This was supported by the ^{13}C NMR spectrum of the crude **23** as shown in Figure 7. Purification of the crude **23** on column chromatography (*n*-hexane/CH_2Cl_2, 1:2) gave **23** (1.82 g, 88%) as a colorless solid. The ^{1}H and ^{13}C NMR spectroscopic properties of **23** were in good accordance with those reported.[4c]

Compound **23**. Colorless prisms. Mp 129–130 °C (dec). Lit.[4c] 115–125 °C (dec). $[\alpha]_D^{22}$ +6.4 (*c* = 1.14, $CHCl_3$), lit.[4c] $[\alpha]_D$ +4 (*c* = 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ: 3.96 (1H, dd, *J* = 10.5, 10.0 Hz, H-4ax), 4.22 (1H, ddd, *J* = 10.5, 9.5, 5.0 Hz, H-2), 4.45 (1H, dd, *J* = 10.5, 5.0 Hz, H-4eq), 4.62 (1H, dd, *J* = 10.5, 5.0 Hz, H-1eq), 4.76 (1H, dd, *J* = 10.5, 10.5 Hz, H-1ax), 4.85 (1H, ddd, *J* = 10.0, 9.5, 5.0 Hz, H-3), 5.63 (1H, s, PhC*H*$_2$), 7.36–7.47 (5H, m, arom.). ^{13}C NMR ($CDCl_3$) δ: 67.3 (C-4), 71.5 (C-2), 72.5 (C-1), 75.1 (C-3), 102.7 (Ph*C*H_2), 126.2/128.5/ 129.8 (d, arom.), 135.6 (s, arom.).

4.11. Coupling reaction between cyclic sulfate and thiosugars

According to the literature, the cyclic sulfate **13** was treated with thiosugars D-**16**, L-**16**, and L-**18** in either DMF[4a] or 1,1,1,3,3,3-hexafluoroisopropanol[4b,c] (HFIP).

4.11.1. Method A. A mixture of D-**16** (100 mg, 0.67 mmol), **13** (224 mg, 1.0 mmol), sodium carbonate (212 mg, 2.0 mmol), and DMF (300 μl) was stirred at 45 °C for 63 h. The reaction mixture was diluted with methanol (10 ml), and solid material was filtered off. The filtrate was concentrated in vacuo, and the residue was triturated with diethyl ether to give a pale brown oil (310 mg), which on column chromatography (AcOEt/MeOH/H_2O, 20:4:1) gave 1,4-dideoxy-1,4-{(*S*)-[(2*S*,3*S*)-2,4-*O*-isopropylidene-3-(sulfooxy)butyl]episulfoniumylidene}-D-arabinitol inner salt (**24**, 163 mg, 65%).

Compound **24**. Pale yellow oil. $[\alpha]_D^{20}$ +28.1 (*c* = 3.37, CH_3OH). IR (neat): 3499, 3225, 1381, 1281, 1200, 1161, 1126, 1087, 1007 cm^{-1}. ^{1}H NMR (CD_3OD) δ: 1.41/1.52 [each 3H, s, (C*H*$_3$)$_2$C], 3.76–3.89 (4H, m, H-1a, H-1b, H-1′a, H-4′a), 3.93 (1H, dd, *J* = 9.5, 7.7 Hz, H-5a), 3.96–4.06 (3H, m, H-4, H-5b, H-1′b), 4.08 (1H, dd, *J* = 11.5, 5.5 Hz, H-4′b), 4.28 (1H, ddd, *J* = 8.9, 8.9, 5.5 Hz, H-3′), 4.33 (1H, ddd, *J* = 8.9, 6.3, 3.2 Hz, H-2′), 4.41 (1H, br s-like, H-3), 4.63 (1H, br s-like, H-2). ^{13}C NMR (CD_3OD) δ: 19.7/28.4 [(*C*H$_3$)$_2$C], 49.5 (C-1′), 51.4 (C-1), 60.9 (C-5), 63.3 (C-4′), 70.4 (C-2′), 71.1 (C-3′), 74.0 (C-4), 79.3 (C-2), 79.9 (C-3), 101.4 [(CH$_3$)$_2$*C*]. FABMS *m*/*z*: 375 [M+H]$^+$ (pos.), FABHRMS *m*/*z*: 375.0767 ($C_{12}H_{23}O_9S_2$ requires 375.0783).

Following the method A, a mixture of L-**16** (150 mg, 1.0 mmol), **13** (336 mg, 1.5 mmol), sodium carbonate (318 mg, 2.8 mmol), and DMF (500 μl) was stirred at 45 °C for 60 h. Work-up gave a pale brown oil (501 mg), which on column chromatography (AcOEt/MeOH, 4:1 to 2:1) gave 1,4-dideoxy-1,4-{(*R*)-[(2*S*,3*S*)-2,4-*O*-isopropylidene-3-(sulfooxy)butyl]episulfoniumylidene}-L-arabinitol inner salt (**25**, 218 mg, 58%).

Compound **25**. Pale yellow oil. $[\alpha]_D^{24}$ +64.9 (*c* = 1.0, CH_3OH). IR (neat): 3364, 1651, 1223, 1163, 1081, 1005 cm^{-1}. ^{1}H NMR (CD_3OD) δ: 1.41/1.53 [each 3H, s, (C*H*$_3$)$_2$C], 3.81 (1H, dd, *J* = 12.9, 3.5 Hz, H-1a), 3.84 (1H, dd, *J* = 12.9, 2.3 Hz, H-1b), 3.85 (1H, dd, *J* = 11.8, 9.2 Hz, H-4′a), 3.90 (1H, dd, *J* = 13.5, 6.6 Hz, H-1′a), 3.97 (1H, dd, *J* = 11.8, 8.0 Hz, H-5a), 3.98 (1H, dd, *J* = 13.5, 3.5 Hz, H-1′b), 4.01 (1H, dd, *J* = 11.8, 5.7 Hz, H-5b), 4.07 (1H, br dd, *J* = 8.0, 5.7 Hz, H-4), 4.09 (1H, dd, *J* = 11.8, 5.5 Hz, H-4′b), 4.24 (1H, ddd, *J* = 9.5, 9.2, 5.5 Hz, H-3′), 4.35 (1H, ddd, *J* = 9.5, 6.6, 3.5 Hz, H-2′), 4.44 (1H, dd, *J* = 2.6, 1.7 Hz, H-3), 4.63 (1H, ddd, *J* = 3.5, 2.6, 2.3, H-2). ^{13}C NMR (CD_3OD) δ: 19.7/28.4 [(*C*H$_3$)$_2$C], 49.3 (C-1′), 51.7 (C-1), 60.8 (C-5), 63.3 (C-4′), 70.7 (C-2′), 71.2 (C-3′), 73.0 (C-4), 79.4 (C-2), 79.9 (C-3), 101.4 [(CH$_3$)$_2$*C*]. FABMS *m*/*z*: 375 [M+H]$^+$ (pos.), FABHRMS *m*/*z*: 375.0757 ($C_{12}H_{23}O_9S_2$ requires 375.0783).

4.11.2. Method B. A mixture of D-**18** (840 mg, 2.0 mmol), **13** (672 mg, 3 mmol), potassium carbonate (55 mg, 0.4 mmol), and HFIP (2 ml) was heated under reflux for 6 h. After removal of the solvent in vacuo, the residue (20 mg) was purified on column chromatography ($CHCl_3$/EtOH, 20:1) to give 2,3,5-tri-*O*-benzyl-1,4-dideoxy-1,4-{(*S*)-[(2*S*,3*S*)-2,4-*O*-isopropylidene-3-(sulfooxy)butyl]episulfoniumylidene}-D-arabinitol inner salt (**26**, 1.27 g, 99%).

Compound **26**. Amorphous solid. $[\alpha]_D^{24}$ −16.3 (*c* = 1.56, $CHCl_3$). IR ($CHCl_3$): 1215, 1092, 1018 cm^{-1}. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 1.26/1.45 (each 3H, s, C(C*H*$_3$)$_2$), 3.68 (1H, dd, *J* = 9.8, 8.3 Hz, H-5a), 3.73 (1H, dd, *J* = 9.8, 6.9 Hz, H-5b), 3.78 (1H, dd, *J* = 11.5, 10.0 Hz, H-4′a), 3.90 (2H, br dd-like, *J* = ca. 13.0, 3.4 Hz, H-1a, H-1′a), 3.99 (1H, br dd, *J* = 8.3, 6.9 Hz, H-4), 4.04 (1H, dd, *J* = 13.0, 1.5 Hz, H-1b), 4.14 (1H, dd, *J* = 11.5, 5.8 Hz, H-4′b), 4.28 (ddd, 1H, *J* = 10.0, 3.4, 2.3 Hz, H-2′), 4.33 (1H, dd, *J* = 13.0, 2.3 Hz, H-1′b), 4.34 (1H, br s, H-3), 4.41 (1H, d, *J* = 11.8 Hz, PhC*H*$_2$), 4.44 (1H, d, *J* = 12.0 Hz, PhC*H*$_2$), 4.49 (1H, td, *J* = 10.0, 5.8 Hz, H-3′), 4.50 (1H, d, *J* = 11.8 Hz, PhC*H*$_2$), 4.52 (1H, d, *J* = 12.0 Hz, PhC*H*$_2$), 4.55 (1H, dd, *J* = 12.0 Hz, PhC*H*$_2$), 4.63 (1H br s, H-2), 4.65 (1H, dd, *J* = 12.0 Hz, PhC*H*$_2$), 7.14–7.38 (15H, m, arom.) ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 19.0/28.3 [C(*C*H$_3$)$_2$], 47.4 (C-1), 49.7 (C-1′), 62.3 (C-4′), 65.4 (C-4), 66.6 (C-5), 67.2 (C-3′), 69.7 (C-2′), 72.1/72.3/73.6 (Ph*C*H_2), 82.3 (C-2), 83.1 (C-3), 99.7 [*C*$(CH_3)_2$], 127.9/128.0/128.2/128.3/128.4/128.5/128.58/128.63/128.7 (d, arom.), 136.05/136.14/136.5 (s, arom.). FABMS *m*/*z*: 645 $[M+H]^+$ (pos.). FABHRMS *m*/*z*: 645.2220 ($C_{33}H_{41}O_9S_2$ requires 645.2192).

4.12. 1,4-Dideoxy-1,4-{(*S*)-[(2*S*,3*S*)-2,4-dihydroxy-3-(sulfooxy)butyl]episulfoniumylidene}-D-arabinitol inner salt (salacinol 1a)

4.12.1. Method A. A solution of **24** (77 mg, 0.2 mmol) and 0.2% hydrochloric acid (1 ml) was stirred at 40 °C for 4 h. After neutralization of the reaction mixture with ion exchange resin (IRA-67), the resin was filtered off. The filtrate was concentrated in vacuo. The residue (67 mg) was purified on column chromatography (AcOEt/MeOH/H_2O, 20:4:1) to give salacinol (**1a**, 50 mg, 74%) as a colorless solid.

4.12.2. Method B. A suspension of 10% palladium-on-carbon (100 mg) in 90% aqueous acetic acid (6 ml) was pre-equilibrated with hydrogen. To the suspension was added a solution of **26** (200 mg, 0.31 mmol) in 90% aqueous acetic acid (8 ml), and the mixture was hydrogenated at 50 °C for 5 h. The catalyst was filtered off, and the filtrate was evaporated to give a colorless oil (118 mg), which on column chromatography ($CHCl_3$/MeOH/H_2O, 12:8:1) gave **1a** (84 mg, 82%).

4.13. 1,4-Dideoxy-1,4-{(*R*)-[(2*S*,3*S*)-2,4-dihydroxy-3-(sulfooxy)butyl]episulfoniumylidene}-L-arabinitol inner salt (11)

Compound **25** (120 mg, 0.32 mmol) was treated in a manner similar to that used for hydrolysis of **24**. Work-up gave a colorless oil (117 mg), which on column chromatography (AcOEt/MeOH/H_2O, 20:4:1) gave **11** (95 mg, 89%) as a colorless solid.

Compound **11**. Colorless plates. Mp 150–152 °C (from aq MeOH). $[\alpha]_D^{24}$ +35.5 (*c* = 1.0, MeOH), lit.[4a] $[\alpha]_D^{24}$ −25.2 (*c* =1.17, MeOH for the enantiomer), lit.[4c]$[\alpha]_D^{24}$ −35.6 (*c* = 0.86, MeOH for the enantiomer). IR (KBr): 3348, 1265, 1215, 1080, 1049, 1018 cm^{-1}. FABMS *m*/*z*: 335 $[M+H]^+$ (pos.). FABHRMS *m*/*z*: 335.0466 ($C_9H_{19}O_9S_2$ requires 355.0451). ^1H NMR spectroscopic properties of compound **11** were in accord with those of the corresponding emantiomer reported.[4a,c] The two close signals at δ_c 79.2 (C-3′) and 79.3 (C-3) observed in the ^{13}C NMR (150 MHz) spectrum of **13a** were unambiguously assigned on the basis of two-dimensional NMR spectroscopic studies, and the reported assignments[4c] for signals due to two methine carbons [δ_c 79.38 (C-3) and 79.46 (C-3′)] of the corresponding enantiomer were found interchanged with each other. ^{13}C NMR (pyridine-d_5) δ: 51.0 (C-1), 52.7 (C-1′), 60.2 (C-5), 62.0 (C-4′), 67.4 (C-2′), 71.9 (C-4), 79.0 (C-2), 79.2 (C-3′), 79.3 (C-3).

4.14. De-*O*-sulfonation of salacinol (1a) and the Diastereomer (11)

A mixture of salacinol (**1a**, 28 mg, 0.08 mmol) and 5% methanolic hydrogen chloride (0.6 ml) was stirred at 45 °C for 3 h. After removal of the solvent, the residue (29 mg) was purified on column chromatography (AcOEt/MeOH/H_2O, 10:4:1) to give 1,4-dideoxy-1,4-{(*R*)-[(2*S*,3*S*)-2,3,4-trihydroxybutyl]episulfoniumyl-idene}-D-arabinitol methyl sulfate (**10a**, 26.5 mg, 86%) as a colorless oil.

Compound **10a** Colorless oil. $[\alpha]_D^{20}$ +3.6 (*c* = 1.08, CH_3OH). IR (neat): 3360, 1651, 1420, 1207, 1072, 1042, 1007 cm^{-1}. ^1H NMR (CD_3OD) δ: 3.59–3.62 (1H, m, H-3′), 3.62 (1H, dd, *J* = 13.4, 5.2 Hz, H-4′a), 3.68 [3 H, s, ($CH_3OSO_3^-$)], 3.69 (1H, dd, *J* = 13.4, 3.8 Hz, H-4′b), 3.73 (1H, dd, *J* = 13.1, 8.9 Hz, H-1′a), 3.85 (1H, dd, *J* = 13.1, 3.4 Hz, H-1′b), 3.86 (2H, m, H-1a, H-1b), 3.93 (1H, dd, *J* = 11.0, 8.9 Hz, H-5a), 4.02 (1H, br dd, *J* = 8.9, 5.2 Hz, H-4), 4.05 (1H, dd, *J* = 11.0, 5.2 Hz, H-5b), 4.09 (1H, ddd, *J* = 8.9, 6.5, 3.4 Hz, H-2′), 4.38 (1H, dd, *J* = 2.4, 1.4 Hz, H-3), 4.63 (1H, ddd-like, *J* = ca. 2.4, 2.4, 2.4 Hz, H-2). ^{13}C NMR (CD_3OD) δ: 51.8 (C-1′), 52.0 (C-1), 55.2 ($CH_3OSO_3^-$), 61.0 (C-5), 64.0 (C-4′), 69.6 (C-2′), 73.6 (C-4), 75.3 (C-3′), 79.4 (C-2), 79.5 (C-3). FABMS *m*/*z*: 255 $[M-CH_3OSO_3]^+$ (pos.), 111 $[CH_3OSO_3]^-$ (neg.). FABHRMS *m*/*z*: 255.0912 ($C_9H_{19}O_6S$ requires 255.0902).

Following the method used for de-O-sulfonation of salacinol (**1a**), a mixture of **11** (30 mg, 0.09 mmol) and 5% methanolic hydrogen chloride (0.6 ml) was stirred at 40 °C for 4 h. Work-up gave a pale brown oil (34 mg), which on column chromatography (AcOEt/MeOH/H_2O, 10:4:1) gave 1,4-dideoxy-1,4-{(*S*)-[(2*S*,3*S*)-2,3,4-trihydroxybutyl]episulfoniumylidene}-L-arabinitol methyl sulfate (**12a**, 26 mg, 79%) as a colorless oil.

Compound **12a**. Colorless oil. $[\alpha]_D^{23}$ +62.5 (*c* = 1.0, CH_3OH). IR (neat): 3199, 1651, 1421, 1204, 1051, 1003 cm^{-1}. ^1H NMR (CD_3OD) δ: 3.60–3.65 (2H, m, H-3′, H-4′a), 3.68 [3H, s, ($CH_3OSO_3^-$)], 3.69 (1H, dd, *J* = 12.7, 5.5 Hz, H-4′b), 3.77–3.82 (2H, br d-like, *J* = ca. 5.8 Hz, H-1′a, H-1′b), 3.82 (2H, d-like, *J* = 2.8 Hz, H-1a, H-1b), 3.95 (1H, dd, *J* = 11.7, 8.9 Hz, H-5a), 4.03 (1H, dd, *J* = 11.7, 5.8 Hz, H-5b), 4.05–4.13 (2H, m, H-4, H-2′), 4.40 (1H, dd, *J* = 2.4, 1.4 Hz, H-3), 4.62 (1H, ddd-like, *J* = ca. 2.4, 2.4, 2.4 Hz, H-2). ^{13}C NMR (CD_3OD) δ: 50.8 (C-1), 51.4 (C-1′), 55.1 ($CH_3OSO_3^-$), 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). FABMS *m*/*z*: 255 $[M-CH_3OSO_3]^+$ (pos.), 111 $[CH_3OSO_3]^-$ (neg.). FABHRMS *m*/*z*: 255.0891 ($C_9H_{19}O_6S$ requires 255.0902).

4.15. 1,4-Dideoxy-1,4-{(*R*)-[(2*S*,3*S*)-2,3,4-trihydroxy butyl]episulfoniumylidene}-D-arabinitol chloride (10b)

A mixture of **10a** (16 mg, 0.04 mmol), ion exchange resin IRA-400J (Cl^- form, 290 mg), methanol (0.3 ml), and water (0.5 ml) was stirred at room temperature for 12 h. The resin was filtered off, and the filtrate was evaporated to give a pale yellow oil (15 mg), which on column chromatography (AcOEt/MeOH/H_2O, 6:4:1) gave **10b** (12.2 mg, 96%).

Compound **10b**. Colorless oil. $[\alpha]_D^{20}$ +5.9 ($c = 0.8$, CH_3OH). IR (neat): 3325, 1651, 1420, 1261, 1209, 1076 cm^{-1}. ^1H NMR (CD_3OD) δ: 3.58–3.62 (1H, m, H-3′), 3.62 (1H, dd, $J = 12.9$, 5.2 Hz, H-4′a), 3.68 (1H, dd, $J = 12.9$, 5.7 Hz, H-4′b), 3.73 (1H, dd, $J = 13.2$, 8.9 Hz, H-1′a), 3.85 (1H, dd, $J = 13.2$, 3.2 Hz, H-1′b), 3.86 (2H, m, H-1a, H-1b), 3.92 (1H, dd, $J = 10.3$, 8.6 Hz, H-5a), 4.02 (1H, br dd, $J = 8.6$, 5.5 Hz, H-4), 4.05 (1H, dd, $J = 10.3$, 5.5 Hz, H-5b), 4.09 (1H, ddd, $J = 8.9$, 6.3, 3.2 Hz, H-2′), 4.37 (1H, d-like, $J =$ ca. 1.5 Hz, H-3), 4.62 (1H, br d-like, $J =$ ca. 2.3 Hz, H-2). ^{13}C NMR (CD_3OD) δ: 51.8 (C-1′), 52.1 (C-1), 61.0 (C-5), 64.0 (C-4′), 69.6 (C-2′), 73.7 (C-4), 75.3 (C-3′), 79.4 (C-2), 79.5 (C-3). FABMS *m/z*: 255 $[M-Cl]^+$ (pos.). FABHRMS *m/z*: 255.0915 ($C_9H_{19}O_6S$ requires 255.0902).

4.16. Enzyme inhibition assays

Rat small intestinal brush border membrane vesicles were prepared and its suspension in 0.1 M maleate buffer (pH 6.0) was used as small intestinal α-glucosidase of maltase and sucrase. Test compound was dissolved in dimethylsulfoxide DMSO, and the resulting solution was diluted with 0.1 M maleate buffer to prepare the test compound solution (concentration of DMSO: 10%). The substrate solution in the maleate buffer (maltose, 74 mM; sucrose, 74 mM, 100 μl), test compound solution (50 μl), and the enzyme solution (50 μl) were mixed and incubated at 37 °C for 30 min. After incubation, the solution was mixed with water (0.8 ml) and was immediately heated by boiling water for 2 min to stop the reaction. Glucose concentration was then determined by the glucose-oxidase method. Final concentration of DMSO in test solution was 2.5% and no influence of DMSO was detected on the inhibitory activity.

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