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STEREOSPECIFIC PREPARATION OF MONOGLUCOSIDES OF OPTICALLY ACTIVE *trans*-1,2- CYCLOHEXANEDIOLS BY ENZYMIC TRANS-D-GLUCOSYLATION, AND ^{13}C-N.M.R. SPECTROSCOPY OF THE RESULTING MONO-D-GLUCOPYRANOSIDES

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ABSTRACT

Anomeric mono-D-glucosides of optically active *trans*-1,2-cyclohexanediols [(1*R*, 2*R*) and (1*S*, 2*S*)] were stereoselectively prepared from the racemic mixture by means of enzymic trans-D-glucosylation using crude enzyme preparations that are commercially available or are utilized for food manufacturing. The ^{13}C-n.m.r. spectra of these D-glucosides were characterized, and the stereochemical influence, on the glycosylation shifts of cyclohexanol, of the vicinal hydroxyl substitution was discussed in comparison with that of vicinal alkyl substitution reported previously.

INTRODUCTION

Recent description of enzymic transglycosylation from oligosaccharides, as donor, to substrates other than carbohydrates (aliphatic alcohols[1] or phenolic compounds[2]), as acceptor, suggested the further development of its application to regioselective and stereospecific synthesis of monoglycosides of di-, tri-, and polyhydroxy-aglycons that are extremely difficult and tedious to prepare by chemical procedures. In our serial studies of ^{13}C-n.m.r. spectroscopy for a variety of D-glucosides[3], D-mannosides, L-rhamnosides[4], and L-arabinosides[5], we have attempted to investigate the carbon-resonance displacement of both the glycosyloxy and aglycon moieties by glycosylation (glycosylation shifts) for *trans*-1,2-cyclohexanediol, considered to be a model of the cyclic glycol systems of a monosaccharide unit.

The preparation of α- and β-mono-D-glucosides of optically active, *trans*-1,2-cyclohexanediols from a racemic mixture of the diols by enzymic trans-D-glucosylation is now reported. Stereochemical correlation of the glycosylation shifts for the resulting mono-D-glucosides is also described, in comparison with that of the glycosides of *d*- and *l*-menthol reported previously[3–5].

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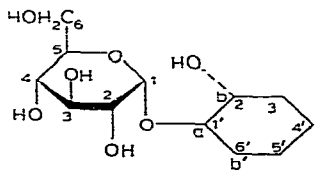
RESULTS AND DISCUSSION

Transglycosylation activities were examined for several crude enzyme preparations that are commercially available, or are used for food manufacturing on an industrial scale. Positive results were obtained for the following enzyme preparations: Takadiastase, a crude enzyme-mixture produced by *Aspergillus oryzae*, and used as a medicine for promotion of carbohydrate digestion; crude hesperidinase, a crude enzyme-mixture produced by *A. niger*, and used for hydrolysis of hesperidin (5,3'-dihydroxyflavanon-7-yl 6-*O*-α-L-rhamnopyranosyl-D-glucoside) in the process of production of canned unshu-mikan (*Citrus unshu* Marc.); crude naringinase[6], a crude enzyme-mixture produced by *A. niger*, and used for hydrolysis of naringin (a flavanone L-rhamnosyl-D-glucoside) in the process of production of canned Natsumikan (*Citrus natsudaidai* Hayata); and β-D-glucosidase (almond emulsin) which is, commercially available. Of these crude enzymes, it was already known that crude hesperidinase and crude naringinase contain unidentified enzymes having high activities for the hydrolysis of such isoprenoid, plant glycosides as saponins and diterpene glycosides[7].

Optimal pH and temperature for the transglycosylation with these enzymes were respectively at pH 4.0–5.0 (McIlvain buffer) and 25–37°. The activity decreased remarkably at pH 6.0. The reaction process depends significantly upon the ratio of the concentration of the enzyme–donor–acceptor. The most appropriate concentrations of an enzyme, a donor (oligosaccharide), and an acceptor were respectively 0.5–1.0, 20–40, and 20 mg/mL.

A racemic mixture of *trans*-1,2-cyclohexanediol [1(*R*),2(*R*) (**1R**) and 1(*S*),2(*S*) (**1S**)] was incubated with Takadiastase and maltose (donor) for 24 h, to give exclusively one D-glucoside, namely, **2**, $C_{12}H_{22}O_7$, colorless prisms, m.p. 137°. The ^{1}H-n.m.r spectrum of **2** in C_5D_5N exhibited an anomeric-proton signal at δ 5.52 (d, 1 H, *J* 4.0 Hz: α configuration), and acid hydrolysis of **2** yielded levorotatory *trans*-1,2-cyclohexanediol, m.p. 113–114°. $[\alpha]_D^{20}$ −40° (*c* 0.32, $CHCl_3$), whose absolute configuration had already been established[8] to be 1(*R*),2(*R*) (**1R**). This evidence led to formulation of **2** as the mono-α-D-glucoside of **1R**. A similar result was obtained in trans-D-glucosylation with the crude naringinase–maltose system.



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In contrast to the results with Takadiastase, trans-D-glucosylation with crude hesperidinase proceeded less stereospecifically. Incubation of a racemic mixture of **1R** and **1S** with crude hesperidinase and maltose for 5 days afforded a mixture of

two D-glucosides which was separated by chromatography, giving **2** and a new D-glucoside (**3**), $C_{12}H_{22}O_7$, as a hygroscopic, white powder. The D-glucoside **3** in C_5D_5N showed an anomeric-proton signal at δ 5.42 (d, 1 H, J 4.0 Hz; α configuration) in its ^{1}H-n.m.r. spectrum, and afforded dextrorotatory (1*S*)-*trans*-1,2-cyclohexanediol on acid hydrolysis; it was therefore formulated as the mono-α-D-glucoside of **1S**. On monitoring the reaction process by t.l.c., it was found that the formation of **2** was faster than that of **3**.

Stereospecific preparation of the mono-β-D-glucoside (**4**) of **1R** was achieved by use of Takadiastase and cellobiose (donor). Incubation of the racemic mixture with this system for 7 h yielded D-glucoside **4**, $C_{12}H_{22}O_7$, as a hygroscopic, white powder. The ^{1}H-n.m.r. spectrum of **4** exhibited an anomeric-proton signal at δ 5.50 (d, 1 H, J 7.5 Hz; β configuration), and hydrolysis of **4** with almond emulsin yielded **1R**, leading to the structure of **4** as the mono-β-D-glucoside of **1R**. A similar result was obtained in trans-D-glucosylation with a crude hesperidinase–cellobiose system.

Less-stereospecific D-glucosylation was observed in the case of almond emulsin and salicin (donor). Incubation of the racemic mixture with this system for 4 h at 25° afforded a mixture of two D-glucosides; this was separated by column chromatography, to give the D-glucoside **4** and a new D-glucoside (**5**), $C_{12}H_{22}O_7$, colorless prisms, m p. 142–145° The structure of **5** was elucidated as the mono-β-D-glucoside of **1S** on the basis of its anomeric-proton signal at δ 5.18 (d, 1 H, J 7.5 Hz; β configuration) in C_5D_5N, and the formation of **1S** from **5** on hydrolysis with almond emulsin. A similar result was obtained in the trans-D-glucosylation with the same enzyme and amygdalin (donor), and with the crude naringinase–cellobiose system

With regard to the stereospecificity of these trans-D-glucosylations, it is notable that the configuration of the glycol system of **1R** corresponds to that of the 3,4-glycol grouping of the (reducing) D-glucose residue of the donor (maltose, or cellobiose). It was also noted that, in every instance, the resulting mono-D-glucosides retained the same anomeric configuration as that of the D-glucosyl linkage of the donor, and that only a trace of di-D-glucoside was formed.

Preliminary experiments on the specificity of the substrate (acceptor) in some of the foregoing reactions were conducted. By use of the system of Takadiastase–maltose [which afforded **2** from the racemic mixture (**1R** + **1S**) with high stereospecificity], cyclohexanol was not D-glucosylated. Trans-D-glucosylation of *cis*-1,2-cyclohexanediol by use of the same system was found to be slower than that of its trans isomer (**1R**), yielding, after incubation for 50 h, a mono-α-D-glucoside whose stereo-structure has not as yet been established, because of the presence of a conformational equilibrium. In contrast to the case of Takadiastase, which mainly consists of enzymes having activities for hydrolysis of carbohydrates, the lower substrate-specificity was demonstrated for the D-glucosidase–D-glucoside (donor) system. With almond emulsin and salicin (donor), *cis*-1,2-cyclohexanediol was D-glucosylated at a rate similar to that for its trans isomer. Cyclohexanol, 2-butanol, and *tert*-butyl alcohol were also D-glucosylated, although the reaction proceeded relatively slowly compared to that of the 1,2-cyclohexanediols.

The ^{13}C-n.m.r. spectra of the D-glucosides **2**–**5** in C_5D_5N were recorded, and assigned as shown in Table I. D-Glucosylation shift-values are also listed in Table I, wherein significant shift values (α and β position of the aglycon, and anomeric carbon atom of the sugar moiety) are summarized. and compared with those of corresponding D-glucosides of cyclohexanol derivatives reported previously[3] (see Table II).

It should be emphasized that, in the present study, the use of ^{13}C-n.m.r. spectroscopy was essential for monitoring the reaction and obtaining information on the stereochemical purity of the products, as the signal allocations of the D-glucosides are obviously different from each other, especially as regards the chemical shifts of the anomeric-carbon signals.

In our previous studies on the ^{13}C-n.m.r. spectroscopy of anomeric pairs of D-glucosides[3], D-mannosides, and L-rhamnosides[4], it was discovered that, on glycosylation, an anomeric-carbon signal (C-1) of a sugar moiety and a carbinyl-carbon signal (a-C) of an aglycon alcohol are both displaced downfield, whereas signals due to carbon atoms adjacent to a glycosyl linkage (b-C and b′-C) of an aglycon are shifted upfield. It has further been found that alkyl substitution on b-C or b′-C exerts a characteristic influence upon the magnitude of these glycosylation shifts, and that this influence depends significantly on the combination of the chirality of C-1 and α-C.

For the α-D-glucoside (**7**) of *l*-menthol (C-3′:*R*) (**6R**) and the β-D-glucoside (**10**) of *d*-menthol (C-3′:*S*) (**6S**), C-3′ (a-C) and C-1 are remarkably more shielded on D-glucosylation than those of α- and β-D-glucosides (**11** and **12**) of an achiral,

TABLE I

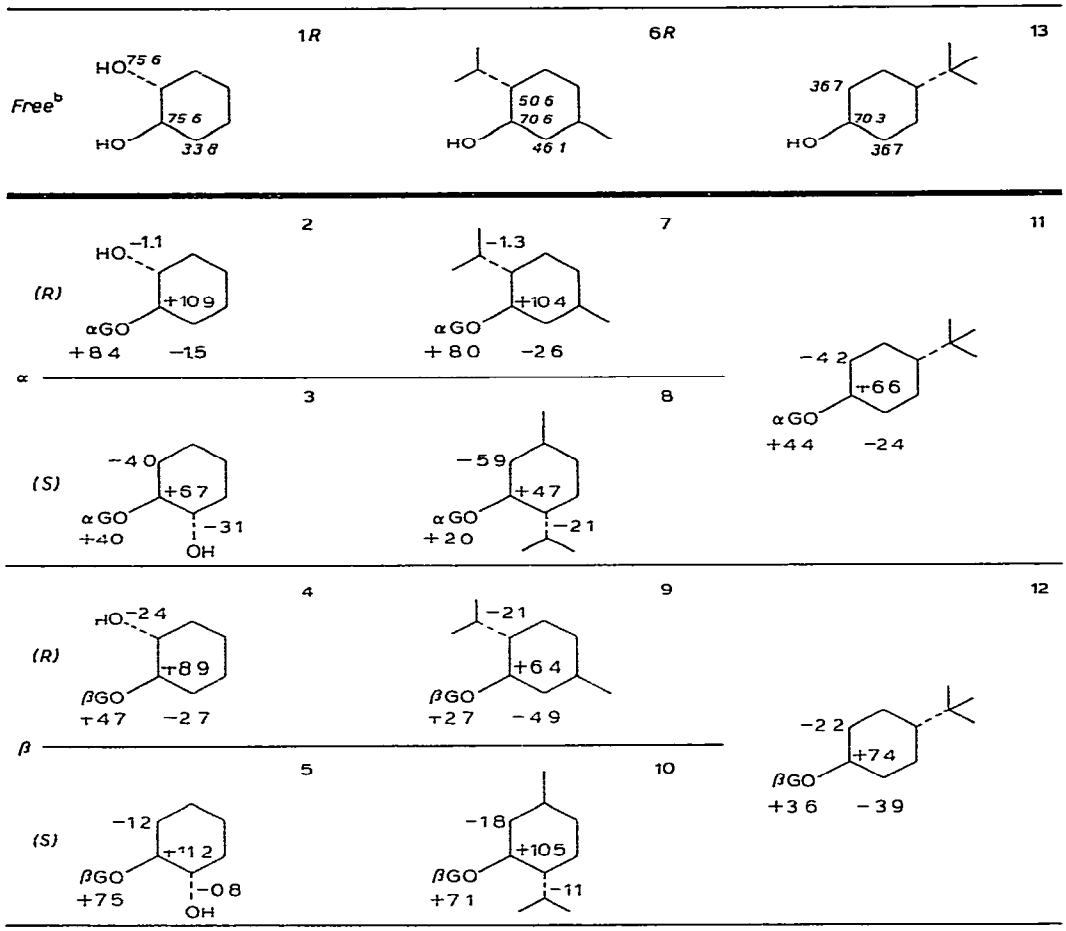
^{13}C CHEMICAL-SHIFT (δ) AND D-GLUCOSYLATION SHIFT ($\Delta\delta$) OF α- AND β-MONOGLUCOSIDES OF *trans*-1,2-CYCLOHEXANEDIOL[a]

Carbon atom	*Aglycon* 1	α-D-*Glucosides* 2	3	β-D-*Glucosides* 4	5	D-*Glucose* α	β
1′	75.6	86.5 (+10.9)	82.3 (+6.7)	84.5 (+8 9)	86 8 (+11.2)		
2′	75 6	74.5 (−1.1)	72 5 (−3.1)	73 2 (−2.4)	74.8 (−0 8)		
3′	33 8	33 1 (−0.7)	33.5 (−0.3)	33 2 (−0 6)	33 2 (−0 6)		
4′	24.8	24.6 (−0 2)	24 2 (−0.6)	24.4 (−0.4)	24 7 (−0.1)		
5′	24.8	24 2 (−0 6)	24.2 (−0 6)	24.1 (−0.7)	24.2 (−0.6)		
6′	33.8	32 3 (−1.5)	29.8 (−4.0)	31.1 (−2.7)	32.6 (−1.2)		
1		102 5 (+8.4)	98.1 (+4.0)	103.5 (+4.7)	106.3 (+7.5)	94.1	98.8
2		74.1 (−0.3)	74.2 (−0.2)	74.7 (−2.1)	75.8 (−1.0)	74.4	76.8
3		75 0 (−0.3)	75.3 (0.0)	78.5 (−0.1)	78.4 (−0.2)	75.3	78.6
4		71 8 (−0.7)	72 5 (0.0)	71.6 (−0.4)	71.4 (−0.6)	72.5	72.0
5		73.7 (+0 2)	73.7 (+0.2)	78.4 (0)	78.4 (0.0)	73 5	78.4
6		62.7 (−0.5)	62.8 (−0.4)	62.6 (−0.4)	62.6 (−0.4)	63.2	63.0

[a] $\Delta\delta$ Values (in parentheses) are relative to the aglycondiol for aglycon carbon atoms, and to corresponding free D-glucose (α or β) for D-glucosyl carbon atoms (1–6) measured in C_5D_5N.

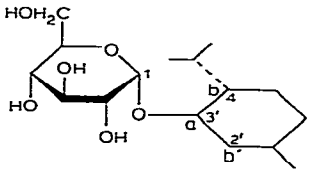
TABLE II

D-GLUCOSYLATION SHIFT-VALUES ($\Delta\delta$)[a] IN C_5D_5N



[a] $\Delta\delta$ = δ (D-glucoside) − δ (aglycon), for a and b carbon atoms; $\Delta\delta$ = δ (D-glucoside) − δ (D-glucose), for anomeric carbon atoms [b] δ Values of aglycon are shown in italics G = D-Glucopyranosyl; **6*R*–13**, data taken from ref. 3

secondary alcohol, *i.e.*, *trans*-4-*tert*-butylcyclohexanol (**13**). In contrast, C-3′ and C-1 of the α-D-glucoside (**8**) of **6S**, as well as of the β-D-glucoside (**9**) of **6R** are rather less shielded than those of the corresponding D-glucosides of **13**. In the spectra of the D-glucosides in the present study, characteristic deshielding of C-1 and a-C, similar to those of **7** and **10**, was observed for **2** and **5**, indicating that, in this case, substitution by a hydroxyl group on b-C or b′-C exhibits an effect on the glycosylation shifts similar to that of alkyl substitution. On the other hand, the spectra of **3** and **4** revealed that, in the case of combination of α-D-glucosyl and the (*S*)-alcohol (to give **1S**), as well as β-D-glucosyl and the (*R*)-alcohol (to give **1R**), the glycosylation shifts of both



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the C-1 and a-C signals were different in magnitude from those of alkyl substitution. On α-D-glycosylation of **1S**, C-1 and a-C were deshielded in a degree similar to that of the α-D-glucoside (**11**) of the achiral alcohol (**13**), rather than that of the α-D-glucoside (**8**) of the corresponding alkyl-substituted, chiral alcohol (**6S**), whereas, on β-D-glucosylation of **1R**, the downfield displacements of C-1 and a-C were more remarkable than that caused on β-D-glucosylation of both the achiral alcohol **13** and the corresponding, alkyl-substituted, chiral alcohol (**6R**).

It was also found that the magnitude of the upfield shifts of b-C and b′-C of secondary alcohols on glycosylation differs, depending on the stereochemical combination of C-1 and a-C. These shift values of b-C and b′-C for **1S** and **1R** are also recorded in Table II, in comparison with those of the data reported[3] for **7**–**12**

These findings of the effect of hydroxyl substitution on the ^{13}C-n.m.r. glycosylation shifts will be valuable for chemical study of glycosides of carbohydrates, as well as other polyhydroxy compounds.

EXPERIMENTAL

Melting points are uncorrected. ^{13}C-N.m.r. spectra were recorded with a JEOL PFT-100, Fourier-transform spectrometer operating at 25.15 MHz (spectral width 4 kHz, 4096 data points, 500–10,000 accumulations; pulse length 16–32 μs (45–90°) at a pulse interval of 1 s). The samples (15–70 mg) were dissolved in C_5D_5N (0.5–1 mL). The solvent deuterium provided the lock signal, and Me_4Si was used as the internal standard Chemical shifts were estimated to be accurate to within ±0.1 p.p.m. ^{1}H-N.m.r. spectra were recorded for C_5D_5N solutions with a JEOL PS-100 spectrometer in the CW mode.

Materials. — Takadiastase (Sankyo) was donated by Dr. A Endo of Sankyo Co., Ltd. Crude hesperidinase (Tanabe) was supplied by Dr Y. Egawa of Tanabe Co., Ltd. Crude hesperidinase (Nagase) was supplied by Dr. K. Yoshikawa of Nagase Sangyo Co., Ltd. Crude naringinase (Tanabe) was a gift from Dr. M. Ono of Tanabe Co., Ltd. The following were commercial materials: emulsin (β-D-glucosidase from sweet almond, Tokyo Kasei Co., Ltd.), β-D-glucosidase (from almond, Sigma), cellobiose (Yoneyama Yakuhin Co., Ltd.), maltose (Nakarai Chemicals Co., Ltd.), and salicin (Wako-Junyaku Co., Ltd.).

General procedure for enzymic trans-D-glucosylation. — To a solution of **1(RS)** in McIlvain buffer (pH 4.0–5.0) were added donor and enzyme preparations, and the mixture was divided into 50 parts which were placed in 50 test tubes (1.5 × 13 cm).

The mixtures were shaken in a reciprocal incubator (80 r.p.m.) for an appropriate time at 25–37°. The reaction was monitored by t.l.c. on precoated Silica Gel (5554, Merck) developed with 20:55:4 C_6H_6–Me_2CO–H_2O and made visible by spraying with 10% H_2SO_4 and heating. The mixtures were heated for 10 min at 100°. The mixtures were combined, evaporated *in vacuo*, and chromatographed on silica gel (Silica Gel 7734, Merck; ~70 times the weight of the sample), eluting with solvent *C*, 40:10:1 $CHCl_3$–MeOH–H_2O or solvent *B*, C_6H_6–Me_2CO–H_2O (4:11:3, upper layer), or both, to give a glucoside. When diastereoisomeric glucosides were produced, the chromatography was repeated several times.

(R)-α-D-*Glucoside* (**2**). — Reaction of **1(RS)** (2 g), maltose (2 g), and Takadiastase (50 mg) in the buffer (pH 4.5; 50 mL) for 24 h at 37°, followed by chromatography, gave a solid that crystallized from EtOH-EtOAc, to afford **2** (336 mg), m.p. 137°, $[\alpha]_D^{20}$ +97° (*c* 1.1, MeOH); ^{1}H-n.m.r : δ 5.52 (d, 1 H, *J* 4 Hz); for ^{13}C-n.m.r. data, see Table I; no contamination by any other D-glucoside. The pentaacetate, which was prepared with Ac_2O–C_5H_5N, had m.p. 143–145° (from aq. MeOH); *m/z* 489.196 ($[M + H]^+$, $C_{22}H_{33}O_{12}$ = 489.197). Compound **2** was also prepared by substitution of crude naringinase for Takadiastase.

Hydrolysis of **2**. — Compound **2** (220 mg) was dissolved in 10% H_2SO_4 in 10% aq. EtOH (9 mL), and the solution was boiled under reflux for 3 h, diluted with water, de-ionized with Amberlite IRA-410 resin, and evaporated *in vacuo*. The residue was chromatographed on silica gel, eluting with 5.1 $CHCl_3$–MeOH, and crystallized from Me_2CO–hexane, to afford **1(R)** (33 mg), m.p. 113–114°, $[\alpha]_D^{20}$ −40° (*c* 0 32, $CHCl_3$); lit.[8] $[\alpha]_D$ −46°.

(S)-α-D-*Glucoside* (**3**) — Reaction of **1(RS)** (2 g), maltose (4 g), and crude hesperidinase (Nagase; 100 mg) in the buffer (pH 4.0; 100 mL) for 5 days at 27°, followed by chromatography with solvents *C* and *B* gave **2** (83 mg), **2** + **3** (34 mg), and **3** (115 mg) as a white powder, $[\alpha]_D^{20}$ +125° (*c* 1.1, MeOH); ^{1}H-n.m.r.: δ 5.42 (d, 1 H, *J* 4 Hz). The peracetate was obtained as colorless prisms, m p. 156–158° (from MeOH–H_2O): *m/z* 489.196 ($[M + H]^+$, $C_{22}H_{33}O_{12}$ = 489.197).

Hydrolysis of **3**. — Compound **3** (90 mg) was hydrolyzed as for **2**, to afford (*S*)-*trans*-1,2-cyclohexanediol (**1S**) (15 mg), m.p. 110°, $[\alpha]_D^{20}$ +34° (*c* 0.12, MeOH).

(R)-β-D-*Glucoside* (**4**). — Reaction of **1(RS)** (1 g), cellobiose (2 g), and Takadiastase (50 mg) in the buffer (50 mL), followed by chromatography with solvent *C*, gave **4** (45 mg) as a hygroscopic, white powder, $[\alpha]_D^{20}$ −17° (*c* 0.33, MeOH); ^{1}H-n.m.r.: δ 5.50 (d, 1 H, *J* 7.5 Hz); for ^{13}C-n.m r., see Table I. Compound **4** was also produced by substitution of crude hesperidinase for Takadiastase. The pentaacetate of **4** had m.p. 140–142° (from aq. MeOH); *m/z* 489.194 ($[M + H]^+$, $C_{22}H_{33}O_{12}$ = 489.197).

Hydrolysis of **4**. — Compound **4** (100 mg) was dissolved in the buffer (pH 5.0) in the presence of β-D-glucosidase (20 mg). The solution was incubated for 2 h at 37°, and extracted with ether. The extract was evaporated, and the residue crystallized from acetone–hexane, to afford (*R*)-*trans*-1,2-cyclohexanediol [**1(R)**], m.p. 108–110°, $[\alpha]_D^{25}$ −36° (*c* 0 97, MeOH)

(S)-*β*-D-*Glucoside* (**5**). — Reaction of **1**(**RS**) (1 g), salicin (2 g), and *β*-D-glucosidase (Sigma, 25 mg) in the buffer (pH 5.0, 50 mL), followed by chromatography with solvent *C*, afforded **5** (35 mg), along with **4** (15 mg) and a mixture of (**4** + **5**) (85 mg). Compound **5** had m.p. 142–145° (from acetone–hexane), $[\alpha]_D^{20}$ −22° (*c* 0.33, MeOH); ^{1}H-n.m.r.: δ 5.18 (d, 1 H, *J* 7.5 Hz). The peracetate of **5** had m.p. 120° (from aq. MeOH): *m/z* 489.195 ($[M + H]^+$, $C_{22}H_{33}O_{12}$ = 489.197). The D-glucoside **5** was also prepared by reaction of **1**(**RS**) (1 g), cellobiose (2 g), and crude naringinase, followed by chromatography (yield, 20 mg).

Hydrolysis of **5**. — The D-glucoside **5** (14 mg) was dissolved in the buffer (pH 5.0), *β*-D-glucosidase (4 mg) was added, and the mixture was incubated for 2 h at 37°, extracted with BuOH, and purified by preparative-layer chromatography, to afford **1**(**S**) (3 mg), $[\alpha]_D^{11}$ +30° (*c* 0.19, MeOH).

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