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Synthetic inhibitor of leaf-closure that reveals the biological importance of leaf-movement for the survival of leguminous plants

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Abstract—Nyctinasty has been known since pre-Christian era, whereas the question 'Why do leguminous plants sleep?' has always puzzled scientists. This paper gives a clue to the historical mystery: by using synthetic inhibitors for nyctinasty, we found that legumes cannot survive without nyctinasty.

1. Introduction

Plants are unable to move from one place to another. However, the folding and opening movements of the leaves according to circadian rhythm have been widely observed in leguminous plants (Fig. 1). This periodic leaf-movement is called nyctinasty and has been known since the age of Alexander the Great.[1]

On the other hand, the question 'Why do leguminous plants sleep?' has always puzzled many scientists studying nyctinasty. Darwin concluded that nyctinasty might protect plants from chilling or from frost.[2] However, later studies revealed that nyctinasty failed to provide soybeans with significant protection from freezing. This result suggested that Darwin's results were probably artifactual.[3,4] Bünning proposed that nyctinasty protected the photoperiodic time-keeping system from moonlight, because moonlight falling on leaves during the night might prevent accurate measurement of night length.[5] Leaves that were folded together intercepted much less light. However, no experimental evidence to date has been reported that explains the biological significance of nyctinasty. Research has been hindered because the leaf movement could not be inhibited. Also, no mutant without nyctinasty has been reported so far. This result strongly suggests that the mutant that lacks leaf-movement is lethal and cannot survive. Thus, a genetic approach to this issue will be difficult. Now we have succeeded in inhibiting leaf movement using a synthetic inhibitor of leaf closure based on the substance that naturally induces leaf-opening.[6] Our result provides the first experimental evidence to answer the question 'Why do leguminous plants sleep?'

Figure 1. Nyctinastic leaf-movement of *Cassia mimosoides* L. (left: at 10:00 a.m., right: at 9:00 p.m.).

Schildknecht's turgorin had been widely believed to be an endogeneous factor controlling leaf movement,[1] but we revealed that it did not show any bioactivity under physiological conditions.[6,7] Nyctinasty is controlled by two endogenous factors of contrasting bioactivities: leaf-closing substance which makes the leaf close and leaf-opening substance which makes the leaf open.[6] The bioactivity of these factors is extremely specific to the original plant from which they were isolated. In the plant body, the rhythm of nyctinasty is generated by change in balance of concentrations between two leaf-movement factors according to circadian rhythm.[6] This change in balance can be attributed to hydrolysis of the glucoside-type leaf-movement factor into the corresponding aglycon by β-glucosidase, whose activity is controlled by a biological clock (Fig. 2).[6]

According to the mechanism shown in Figure 2, it was

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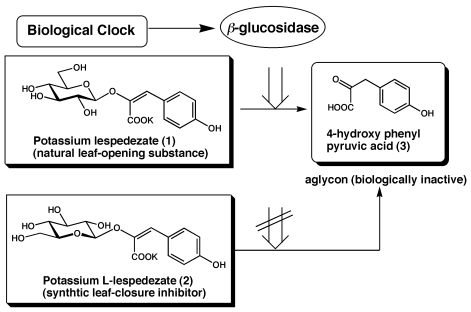


Figure 2. The control of internal concentration of **1** by a biological clock that causes the nyctinastic leaf-movement.

expected that a structurally modified leaf-opening substance that cannot be hydrolyzed by β-glucosidase would keep the leaf open constantly, and, thus, inhibit the leaf-closure (causing 'insomnia').

2. Results and discussion

Potassium lespedezate (**1**) was isolated as a glucoside-type leaf-opening substance that is effective for the leaf of a leguminous plant, *Cassia mimosoides* L.[6] As shown in Figure 2, the molecular design of a leaf-closure inhibitor requires both retention of leaf-opening activity and resistance to hydrolysis by β-glucosidase. Structure–activity relationship studies were carried out using some analogs of **1**, such as those with various glycons (**2**, **4**, and **5**),[8,9] one with a reduced double bond (**6a** and **6b**),[10] one with a phenolic methyl ether (**7**),[10] and one with a methyl ester (**8**).[8,9] Bioassays using them showed that structural modification on the sugar moiety of **1** (**2**, **4**, and **5**) caused no decrease in bioactivity (Table 1).[8–10] In contrast, bioactivity was greatly diminished by modification of the aglycon moiety in **6a**,**6b**, **7** and **8** (Table 1). These results strongly suggest that the aglycon part of **1** would be recognized by the receptor of **1**, and the recognition of the glycon part in **1** would be very weak.

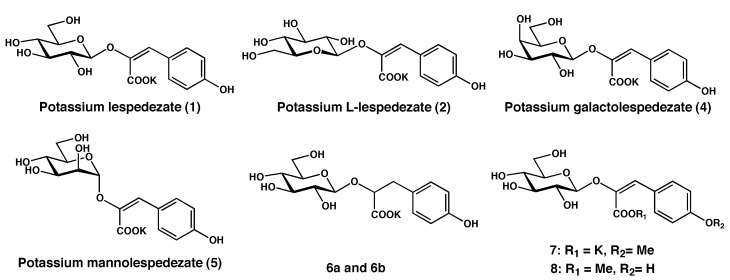
Also, we examined the thioglucoside-type analogs of **1** (**9a** and **9b**) that are expected to be best as an inhibitor because those glucoside linkages containing heteroatom could not be cleaved by β-glucosidase. We synthesized thioglucoside (**9a** and **9b**) by using S_N2 reaction with 1-bromo-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside and thioenol (**14**) which was prepared according to Cline's method (Scheme 1).[11] The separation of the resulting two geometrical isomers was achieved by HPLC equipped with a recycle unit using a high-resolution column packed with 3 μm particles (Cadenza CD-C18). The assignment of geometry on the olefin was determined by NOE. However, NOE between 2′-H in the glycon and 2-H in the aromatic ring which was used for the assignment of geometry in **1** and its analogs,[7–9] was not observed because of the larger atomic radius of the sulfur atom compared to that of oxygen. Thus, we prepared methyl ester of **9a** and **9b** (**16a** and **16b**) by TMS-diazomethane, and the geometry was determined from NOE (0.3%) between the methyl proton in the methoxy group (3.75 ppm) and aromatic H-2 proton (7.14 ppm) for **16a** and NOE (0.7%) between methyl proton in the methoxy group (3.83 ppm) and the olefinic proton (7.864 ppm) for **16b** (Scheme 2).

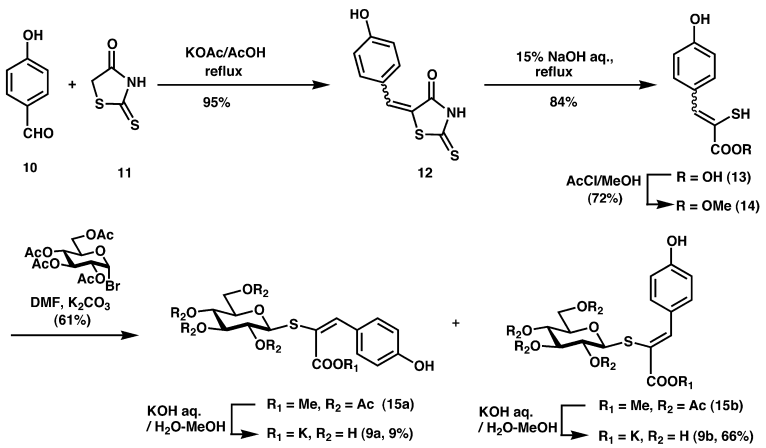
Interestingly, the thioglucoside-type analogs of **1** (**9a** and **9b**) did not show leaf-opening activity at all. There would be two possibilities to explain this phenomenon. The first hypothesis is that the oxygen atom in the glycosidic linkage is important for the leaf-opening activity, and the second one is that thioglucoside-type analogs (**9a** and **9b**) would be susceptible to the hydrolysis by β-glucosidase compared to the glucoside-type ones. However, the latter was ruled out by the hydrolysis of **9a** and **9b** by commercially available β-glucosidase which is described later. These results showed the importance of the oxygen atom in the glucoside linkage of **1** for the development of leaf-opening activity. We showed that some receptor of **1** would be concerned with the recognition of **1** by plant motor cell.[11] Our result suggests that this receptor would recognize the oxygen atom in the glycosidic linkage of **1**. The results of structure-activity relationship study are summarized in Table 1. From these results, the potentially most effective leaf-movement inhibitor that has both leaf-opening activity and resistance against hydrolysis by β-glucosidase would be potassium L-lespedezate (**2a**) and potassium L-isolespedezate (**2b**) (Fig. 2).[6,9,10] Thus, we synthesized **2a** and **2b** (Scheme 3).

Coupling of L-acetobromogalactose (**17**) and **18** was carried out with AgOTf-Molecular sieves 4 Å to give mainly β-glycoside (**19**). A racemic mixture of **18** was used in the glycosidation reaction, and the following reactions were carried out using both diastereomers without separation. A trace amount of an α-anomer was also observed in the reaction mixture; however, it was separated from **19** by silica gel column chromatography. In the glycosidation

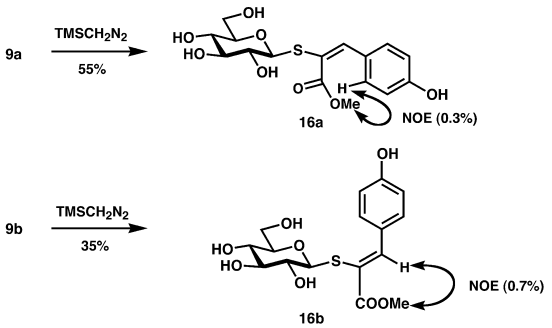
Table 1. Bioactivity of **1** and its analogs

Compound	Bioactivity (mol/l)	Compound	Bioactivity (mol/l)
1a (or **1b**)	1×10^{-6}	**6a** and **6b**	$>1\times10^{-3}$
2a (or **2b**)	1×10^{-6}	**7**	$>1\times10^{-3}$
4	1×10^{-6}	**8**	$>1\times10^{-3}$
5	1×10^{-6}	**9a** and **9b**	$>1\times10^{-3}$





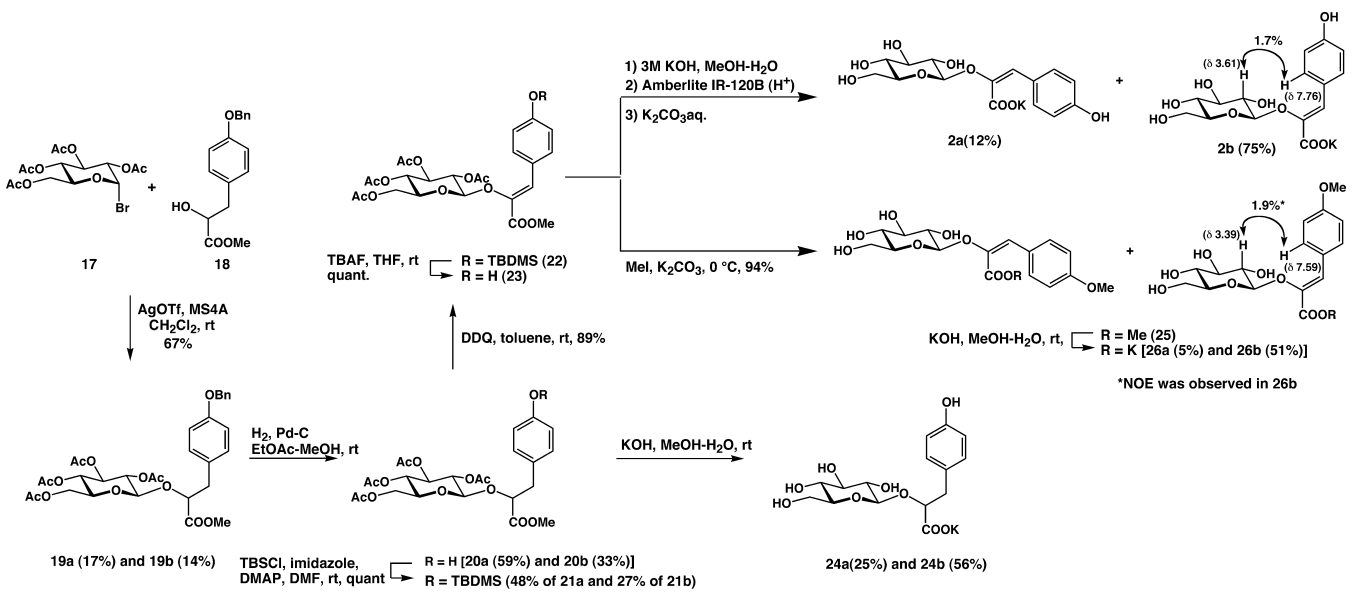
Scheme 1. Synthesis of thioglucoside (**9**).



Scheme 2. NOE experiment using **16a** and **16b**.

reaction, both diastereomers at the α-position of the carbonyl group were equally formed and the following reactions were carried out using a mixture of both diastereomers. After deprotection by catalytic hydrogenation, the phenolic hydroxyl group of **20** was protected with TBDMSCl, and following DDQ oxidation of **21** using 4 equiv. of DDQ for 42 h gave mainly one isomer, **22**, which was deprotected with nBu$_4$NF in THF at 0°C to give the corresponding (*Z*)-**23**. Finally, potassium L-lespedezate (**2a**) and L-isolespedezate (**2b**) were obtained from **23** on treatment with KOH in MeOH at 0°C. The separation of **2a** and **2b** was achieved by HPLC under acidic condition. The reaction mixture was acidified by ion exchange resin and separated with HPLC under acidic condition. The isolated acids were then neutralized with potassium carbonate to give **2a** and **2b**, respectively. The synthetic route is summarized in Scheme 3. The geometry of the double bond of **2a** and **2b** was confirmed by the NOE experiments: NOE (1.7%) was observed between $H_{2'}$ (3.61 ppm) and H_2 (7.76 ppm) in **2b**. L-Glucose type analogs (**2a** and **2b**) were as effective as **1** in the bioassay.

It was already known that **1a** and **1b** afforded a mixture of these two compounds on standing at room temperature as an aqueous solution.[8] And no difference could be observed in the result of bioassay using **1** and related analogs as a single geometrical isomer or a mixture of two isomers.[8,9] Thus, **2a** and **2b** that are optical isomers of **1a** and **1b** should give the same result in the bioassay. Thus, we used a mixture of **2a** and **2b** in the following experiments.



Scheme 3. Syntheses of biologically inactive analogs of **2**.

Figure 3. The effect of a leaf-movement inhibitor (**2a** and **2b**) on the leaf of *C. mimosoides* (From the left, 'insomnia' at second day, fourth day, ninth day, and control on the ninth day).

Table 2. Time course change of the leaf-opening activities in **1**, **2a** and **2b**

	Status of the leaves at 21:00					
	First day	Second day	Third day	Fourth day	Fifth day	Sixth day
1	++	++	−−	−−	−−	−−
2a	++	++	++	++	++	++
2b	++	++	++	++	++	++

Movement of the leaf was represented by the following marks: ++ completely open; + nearly open; − nearly closed; −− completely closed.

Leaf-movement inhibitor (**2a** and **2b**) showed novel bioactivities in bioassay; the leaves detached from the stem of *C. mimosoides* and placed in H_2O continued the circadian rhythmic leaf movement (Fig. 3). Both of leaf-opening substance (**1**) and leaf-movement inhibitor (**2a** and **2b**) can keep the leaves open even at night at 1×10^{-6} mol/l. When the leaves were treated with 3×10^{-6} mol/l of **1**, its leaf-opening activity lasted for only 2 days (Table 2). After that, the leaves closed at night again. This is because **1** is gradually hydrolyzed into **3** within a few days in the plant body (Fig. 2). On the other hand, leaf-opening activity of **2a** and **2b** lasted even after 6 days (Table 2). The leaves treated with 3×10^{-6} mol/l of **2a** and **2b** remained open until it completely withered and died after 9 days (Fig. 3). These results clearly showed that leaf closure is essential for the survival of this plant. This is because no mutant without nyctinasty has been reported so far.

We also examined the hydrolysis of **2a** and **2b** by commercially available β-glucosidase. We treated **2a** and **2b** with β-glucosidase to examine the reactivity against this enzyme. The potential product of this reaction, **3**, existed mainly as an enol form in aqueous solutions: about 95% of **3** isomerized to an enol form within an hour under the reaction conditions used for the enzymatic reaction. Therefore, we observed the content of an enol form of **3** by the HPLC analyses of the reaction mixture. After 30 min of incubation in 0.1 M citrate buffer (pH 5.0) at 37°C with commercially available β-glucosidase, 0% of **2a** and **2b** was hydrolyzed into its aglycon; on the other hand, 78% of **1** was easily hydrolyzed into its aglycon under the same conditions. Therefore, remarkable difference was observed in the rate of enzymatic hydrolysis of **1** and **2a** (or **2b**) by using β-glucosidase. Also, no hydrolysis was observed in the experiment using thioglucoside-type analogs (**9a** and **9b**). These results strongly suggested that **2a** and **2b** were not hydrolyzed by β-glucosidase in the plant body during bioassay and the leaves were kept open.

However, the death of leaf could be also attributable to the potential toxic feature of **2**. However, we have two experimental proofs that **2** operates as a leaf-movement inhibitor, and not as toxins in the plant body: Strong correlation was observed between the leaf-opening activity and the death of plant leaf.

We synthesized two biologically inactive analogs of **2** from **21** and **23** (Scheme 3) such as one with a reduced double bond (**24a** and **24b**) and one with phenolic methyl ether (**26a** and **26b**). The geometry of the double bond was confirmed by the NOE experiments: NOE (1.9%) was observed between $H_{2'}$ (3.39 ppm) and H_2 (7.59 ppm) in **26b**. Compounds **24**, **26a** and **26b** did not show leaf-opening activity even at 1×10^{-3} mol/l. When the leaves were treated with (a) a diastereomeric mixture of **24**, (b) **26a**, and (c) **26b**, the leaves suffered no damage and did not wither and die even after 2 weeks in all cases.

Moreover, **2** showed extremely specific bioactivity to the leaf of *C. mimosoides*, that is a special feature also observed in **1**.[6] And **2** showed no leaf-opening activity with leaves of other plants, such as *Mimosa pudica* L., *Albizzia julibrissin* Durazz, *Aeschynomene indica* L., and *Phyllanthus urinaria* L., even at 3×10^{-5} mol/l. Also, after 2 weeks, no damage to the leaf was observed in these plants. This specific bioactivity cannot be interpreted if **2** operated as some toxin in the plant body of *C. mimosoides*. These results strongly suggested that **2** operated as a leaf-movement inhibitor in the plant body and caused withering and death of *C. mimosoides* by inhibiting leaf closure.

Our result gives an important clue for to the historic mystery, 'Why do leguminous plants sleep?' We showed that nyctinastic leaf-movement is essential for the survival of leguminous plants by using a synthetic inhibitor of leaf-closure that was designed on the chemical mechanism of nyctinasty. This result clearly showed the effectiveness of a chemical approach using a synthetic inhibitor of some biological phenomenon that would be appropriate for solving biological problems to which a genetic knockout approach cannot be applied.

3. Experimental

3.1. General

NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a Jeol GX400 spectrometer or a Jeol ALPHA 400 spectrometer equipped with microprobe NTH3-FG (NAROLAC Co., Ltd) using TMS in $CDCl_3$ or *t*-BuOH (^1H; 1.23 ppm, ^{13}C; 32.1 ppm) in D_2O as internal standards at various temperatures. The FAB-MS and HR FAB-MS spectra were recorded on a Jeol JMS-700 spectrometer, using glycerol or *m*-nitrobenzylalcohol as a matrix. The IR spectra were measured by JASCO FT/IR-410. The specific rotations were measured by JASCO DIP-360 polarimeter.

The HPLC purification was carried out with a JASCO LC 2000 system equipped with RV-2080-02 recycle unit using COSMOCIL $5C_{18}$-AR column (ϕ 20×250 mm) (Nakalai Tesque Co. Ltd) or Cadenza CD-C18 column (ϕ 10×250 mm) (Intact Co. Ltd). The solvents used for HPLC were available from Kanto Chemical Co. and were filtered through a Toyo Roshi membrane filter (cellulose acetate of 0.45 μm pore size, 47 mm dia.) before use. Silica gel column chromatography was performed on Silica Gel 60N (Kanto Chemical Co. Ltd). Reversed-phase open-column chromatography was performed on Cosmosil 75C18-OPN (Nakalai Tesque Co. Ltd). TLC was performed on Silica gel F_{254} (0.25 mm or 0.5 mm, MERCK) or RP-$18F_{254S}$ (0.25 mm, MERCK).

3.1.1. 5-(4-Hydroxybenzylidene)-rhodanine (12). 4-Hydroxybenzaldehyde (**10**, 506.6 mg, 4.15 mmol), rhodanine (**11**, 555.9 mg, 4.18 mmol) were dissolved in acetic acid (20 ml). To this solution, potassium acetate (2.44 g, 24.9 mmol) was added and refluxed for 18 h. After adding 80 ml of water, the solution was cooled over ice bath and resulting orange crystal was collected and dried to give 5-(4-hydroxybenzylidene)-rhodanine (**12**) (938.1 mg, 95%).

^{1}H NMR (400 MHz, acetone-d_6, rt): 7.56 (1H, s), 7.50 (2H, d, J=8.4 Hz), 7.02 (2H, d, J=8.4 Hz) ppm; ^{13}C NMR (100 MHz, acetone-d_6, rt): 195.7, 169.5, 160.9, 133.7 (2C), 133.0, 125.6, 122.4, 117.2 (2C) ppm; HR FAB MS (positive): $[M+H]^+$ found m/z 238.0012, $C_{10}H_8O_2NS_2$ requires m/z 237.9996; IR (film) ν: 3427, 1695, 1564, 1516 cm^{-1}.

3.1.2. 3-*p*-Hydroxyphenyl-2-mercaptoacrylic acid (13). Compound **12** (430.1 mg, 1.81 mmol) was dissolved in 18 ml of 15% NaOH aq. After refluxed for 10 min, the solution was cooled to room temperature and neutralized with 6N HCl aq. The resulting precipitate was filtered and dried to give **13** (299.1 mg, 84%).

^{1}H NMR (400 MHz, CD_3OD, rt): 7.73 (1H, s), 7.54 (2H, d, J=8.6 Hz), 6.84 (2H, d, J=8.6 Hz) ppm; ^{13}C NMR (100 MHz, CD_3OD, rt): 168.5, 159.4, 135.9, 132.9 (2C), 127.9, 120.7, 116.3 (2C) ppm; HR FAB MS (negative): $[M-H]^-$ found m/z 195.0090, $C_9H_7O_3S$ requires m/z 195.0116; IR (film) ν: 3313, 1682, 1604, 1570, 1508 cm^{-1}.

3.1.3. Methyl 3-*p*-hydroxyphenyl-2-mercaptoacrylate (14). Compound **13** (322.5 mg, 1.65 mmol) was dissolved in methanol (5 ml). To this solution, acetyl chloride (1 ml) in methanol (10 ml) was added, and then the solution was stirred for 34 h. After evaporation, the residue was purified with silica gel column chromatography with $CHCl_3$/MeOH=10/1 to give **14** (249.7 mg, 72%).

^{1}H NMR (400 MHz, CD_3OD, rt): 7.69 (1H, s), 7.54 (2H, d, J=8.8 Hz), 6.84 (2H, d, J=8.8 Hz), 3.84 (3H, s) ppm; ^{13}C NMR (100 MHz, CD_3OD, rt): 167.5, 159.6, 136.4, 133.1 (2C), 127.7, 119.8, 116.4 (2C), 53.8 ppm; HR FAB MS (positive): $[M+H]^+$ found m/z 211.0408, $C_{10}H_{11}O_3S$ requires m/z 211.0429; IR (film) ν: 3392, 1672, 1603, 1577, 1508 cm^{-1}.

3.1.4. Methyl (*Z*)-2-(2′,3′,4′,6′-tetra-*O*-acetyl-β-D-glucopyranosyl-thio)-3-*p*-benzyloxyphenyl acrylate (15). Compound **14** (238.8 mg, 1.14 mmol) was dissolved in DMF (11 ml) under argon atmosphere, and then, potassium carbonate (314.6 mg, 2.28 mmol) was added to this solution. After stirring for 10 min, 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (514.0 mg, 1.25 mmol) was added to this solution. Then the solution was stirred for 4 h. Then, 80 ml of water was added to this solution and this mixture was extracted three times with 40 ml each of ethyl acetate. The resulting organic layer was washed by brine and dried over absolute sodium sulfate. After evaporation, the residue was purified by silica gel column chromatography with $CHCl_3$/MeOH=20/1 to give **15** (374.7 mg, 61%).

^{1}H NMR (400 MHz, $CDCl_3$, rt): 8.05 (1H, s), 7.85 (2H, d, J=8.8 Hz), 6.85 (2H, d, J=8.8 Hz), 6.56 (1H, s, -OH), 5.20 (1H, dd, J=9.6, 9.6 Hz), 5.09 (1H, dd, J=9.6, 10.2 Hz), 5.08 (1H, dd, J=9.6, 9.9 Hz), 4.84 (1H, d, J=10.2 Hz), 4.18 (1H, dd, J=5.2, 12.4 Hz), 4.02 (1H, dd, J=2.0, 12.4 Hz), 3.87 (3H, s), 3.63 (1H, ddd, J=2.0, 5.2, 9.9 Hz), 2.02 (3H, s), 2.01 (3H, s), 2.00 (3H, s), 1.96 (3H, s) ppm; ^{13}C NMR (100 MHz, $CDCl_3$, rt): 170.8, 170.2, 169.6, 169.4, 166.9, 158.0, 148.2, 133.7 (2C), 126.3, 118.2, 115.2 (2C), 84.8, 75.7, 73.9, 70.9, 68.2, 62.1, 52.9, 20.68, 20.66, 20.63 (2C) ppm; HR FAB MS (positive): $[M+Na]^+$ found m/z 563.1199, $C_{24}H_{28}O_{12}SNa$ requires m/z 563.1199; IR (film) ν: 3396, 1753, 1716, 1606, 1589, 1512 cm^{-1}; $[\alpha]_D^{26}$=+106.0° (c 1.00, $CHCl_3$).

3.1.5. Potassium (*Z*)-2-β-D-glucopyranosythio-3-*p*-benzyloxy-phenyl-acrylate (9a and 9b). A mixture of **15a** and **15b** (98.3 mg, 0.128 mmol) was dissolved in 2 ml of methanol, and then 1 ml of 4 M NaOH aq. was added to this solution. After stirring for 12 h at rt, the reaction mixture was acidified by Amberlite-IR120B (H^+). After filtration, the filtrate was dried up to give a mixture of isomers of **9a** and **9b** (66.0 mg). This mixture (51.9 mg) was purified by HPLC [COSMOCIL $5C_{18}$-AR, 1% AcOH/40% MeOHaq.], and each fraction was neutralized by potassium carbonate. Another purification by recycle-HPLC [Cadenza CD-C18, 5% CH_3CNaq.] gave **9a** (3.7 mg, 9%) and **9b** (25.4 mg, 66%), respectively.

Compound **9a**. ^{1}H NMR (400 MHz, D_2O, rt): 7.21 (2H, d, J=8.8 Hz), 6.72 (2H, d, J=8.8 Hz), 6.61 (1H, s), 4.55 (1H, d, J=10.0 Hz), 3.75 (1H, dd, J=1.6, 12.8 Hz), 3.56 (1H, dd, J=5.2, 12.8 Hz), 3.39 (1H, t, J=8.6, 8.6 Hz), 3.35–3.21 (3H, m) ppm; ^{13}C NMR (100 MHz, D_2O, rt): 176.8, 157.1, 133.2, 131.1 (2C), 129.4, 128.9, 116.9 (2C), 87.4, 81.6, 78.7, 73.4, 70.9, 62.5 ppm; HR FAB MS (negative): $[M-K]^-$ found m/z 357.0648, $C_{15}H_{17}O_8S$ requires m/z 357.0644; IR (film) ν: 3298, 1568, 1510 cm^{-1}; $[\alpha]_D^{26}$=+13.1° (c 0.62, H_2O).

Compound **9b**. ^{1}H NMR (400 MHz, D_2O, rt): 7.52 (2H, d, J=8.2 Hz), 7.36 (1H, s), 6.79 (2H, d, J=8.2 Hz), 4.61 (1H, d, J=9.6 Hz), 3.66 (1H, broad d, J=12.4 Hz), 3.49 (1H, dd, J=5.0, 12.4 Hz), 3.35 (1H, t, J=8.8, 8.8 Hz), 3.26–3.17 (3H, m) ppm; ^{13}C NMR (100 MHz, D_2O, rt): 175.1, 157.6, 139.2, 133.1 (2C), 128.9, 128.3, 116.2 (2C), 86.3, 81.1, 78.2, 74.0, 70.3, 61.8 ppm; HR FAB MS (negative): $[M-K]^-$ found m/z 357.0648, $C_{15}H_{17}O_8S$ requires m/z

357.0644; IR (film) ν: 3294, 1599, 1568, 1510 cm^{-1}; $[\alpha]_D^{26}$=+20.3° (*c* 1.00, H_2O).

3.1.6. Methyl (*E*)-2-β-D-glucopyranosythio-3-*p*-benzyloxy-phenylacrylate (16a). (*E*)-Thioglucoside (**9a**) (12.0 mg, 0.034 mmol) was dissolved in MeOH and to trimethylsilyldiazomethane (2 M solution in hexane, 50 μl) was added to this solution under argon atmosphere at 0°C. After stirring for 1 h, additional trimethylsilyldiazomethane (2 M solution in hexane, 40 μl) was added. After 1 h stirring, reaction mixture was evaporated in vacuo and purified by prep.TLC with 50% MeOHaq. to give methyl ester (**16a**) (7.0 mg, 55%).

Compound **16a**. ^1H NMR (400 MHz, CD_3OD, rt): 7.17 (1H, s), 7.14 (2H, d, *J*=8.8 Hz), 6.73 (2H, d, *J*=8.8 Hz), 4.46 (1H, d, *J*=9.2 Hz), 3.81 (1H, dd, *J*=2.2, 12.0 Hz), 3.75 (3H, s), 3.61 (1H, dd, *J*=5.2, 12.0 Hz), 3.36–3.25 (4H, m); ^{13}C NMR (100 MHz, CD_3OD, rt): 170.9, 159.7, 142.6, 131.1 (2C), 127.6, 121.3, 116.3 (2C), 88.1, 82.2, 79.4, 73.6, 71.2, 62.9, 53.0 ppm; HR FAB MS (negative): $[M-H]^-$ found *m/z* 371.0796, $C_{16}H_{19}O_8S$ requires *m/z* 371.0801; IR (film) ν: 3379, 1703, 1608, 1512 cm^{-1}; $[\alpha]_D^{21}$=+42.5° (*c* 0.70, MeOH).

3.1.7. Methyl (*Z*)-2-β-D-glucopyranosythio-3-*p*-benzyloxy-phenylacrylate (16b). (*Z*)-Thioglucoside (**9b**) (6.6 mg, 0.018 mmol) was dissolved in MeOH and to trimethylsilyldiazomethane (2 M solution in hexane, 23 μl) was added to this solution under argon atmosphere at 0°C. After stirring for 12 h, reaction mixture was evaporated in vacuo and purified by prep.TLC with 50% MeOHaq. to give methyl ester (**16b**) (2.4 mg, 35%) with recovered **9b** (3.2 mg, 48%).

Compound **16b**. ^1H NMR (400 MHz, CD_3OD, rt): 7.864 (1H, s), 7.862 (2H, d, *J*=8.6 Hz), 6.80 (2H, d, *J*=8.6 Hz), 4.72 (1H, d, *J*=9.2 Hz), 3.83 (3H, s), 3.72 (1H, dd, *J*=2.4, 12.4 Hz), 3.58 (1H, dd, *J*=5.4, 12.4 Hz), 3.34–3.24 (3H, m), 3.15 (1H, ddd, *J*=2.4, 5.4, 9.2 Hz); ^{13}C NMR (100 MHz, CD_3OD, rt): 169.2, 160.6, 146.4, 134.6 (2C), 127.1, 120.5, 116.1 (2C), 87.2, 82.1, 79.4, 75.3, 71.2, 62.7, 53.3 ppm; HR FAB MS (positive): $[M+H]^+$ found *m/z* 373.0928, $C_{16}H_{21}O_8S$ requires *m/z* 373.0957; IR (film) ν: 3335, 1668, 1606, 1568, 1508 cm^{-1}; $[\alpha]_D^{20}$=+59.7° (*c* 1.00, MeOH).

3.1.8. Methyl 2-(2′,3′,4′,6′-tetra-*O*-acetyl-β-L-glucopyranosyl-oxy)-3-*p*-benzyloxyphenylpropionate (19). To a suspension of 2,3,4,6-tetra-*O*-acetyl-β-L-glucopyranosyl-bromide (**17**) (789.3 mg, 1.92 mmol), methyl 3-*p*-benzyloxyphenyl-2-hydroxypropionate (**18**) (377.2 mg, 1.32 mmol), dry powdered molecular sieves 4 Å (2.0 g) in CH_2Cl_2 (19 ml), and silver triflate (0.61 g×2.37 mmol) were added at 0°C under argon atmosphere. The reaction mixture was stirred for 18 h at room temperature, and then, filtered on celite and the filtrate was concentrated in vacuo. The residue was separated with silica gel column chromatography (*n*-hexane/EtOAc=11/9) to give a mixture of diastereomers of **19** (306.8 mg×38%). From 36.9 mg of **19**, each diastereomer was separated with preparative TLC (benzene/acetone=10/1) to give **19a** (16.1 mg, 17%), **19b** (14.0 mg, 14%), respectively.

Compound **19a**. ^1H NMR (400 MHz, $CDCl_3$, rt): 7.43–7.30 (5H, m), 7.09 (2H, d, *J*=8.8 Hz), 6.86 (2H, d, *J*=8.8 Hz), 5.20 (1H, dd, *J*=9.5, 9.6 Hz), 5.07 (1H, dd, *J*=9.6, 9.8 Hz), 5.06 (1H, dd, *J*=8.0, 9.5 Hz), 5.03 (2H, s), 4.57 (1H, d, *J*=8.0 Hz), 4.55 (1H, t, *J*=6.0, 6.0 Hz), 4.19 (1H, dd, *J*=4.8, 12.2 Hz), 4.09 (1H, dd, *J*=2.4, 12.2 Hz), 3.65–3.61 (1H, m), 3.64 (3H, s), 3.06 (1H, dd, *J*=6.0, 13.4 Hz), 3.00 (1H, dd, *J*=6.0, 13.4 Hz), 2.08 (3H, s), 2.02 (3H, s), 2.01 (3H, s), 1.98 (3H, s) ppm; ^{13}C NMR (100 MHz, $CDCl_3$, rt): 171.0, 170.6, 170.2, 169.6, 169.4, 157.6, 137.0, 130.6 (2C), 128.5 (2C), 128.0, 127.9, 127.4 (2C), 114.4 (2C), 99.0, 76.5, 72.4, 71.8, 70.8, 69.9, 68.4, 61.8, 51.8, 37.9, 20.7, 20.63, 20.62, 20.59 ppm; HR FAB MS (positive): $[M+Na]^+$ found *m/z* 639.2084, $C_{31}H_{36}O_{13}Na$ requires *m/z* 639.2054; IR (film) ν: 1753, 1512 cm^{-1}; $[\alpha]_D^{26}$=+30.8° (*c* 1.00, $CHCl_3$).

Compound **19b**. ^1H NMR (400 MHz, $CDCl_3$, rt): 7.43–7.30 (5H, m), 7.09 (2H, d, *J*=8.8 Hz), 6.88 (2H, d, *J*=8.8 Hz), 5.10 (1H, dd, *J*=9.4, 9.4 Hz), 5.06–5.00 (2H, m), 5.04 (2H, s), 4.44 (1H, d, *J*=7.6 Hz), 4.16 (1H, dd, *J*=5.3, 12.2 Hz), 4.11 (1H, dd, *J*=4.4, 9.1 Hz), 4.07 (1H, dd, *J*=2.5, 12.2 Hz), 3.72 (3H, s), 3.62 (1H, ddd, *J*=2.4, 5.3, 9.6 Hz), 2.97 (1H, dd, *J*=9.1, 14.5 Hz), 2.90 (1H, dd, *J*=4.4, 14.5 Hz), 2.10 (3H, s), 2.01 (3H, s), 1.98 (3H, s), 1.78 (3H, s) ppm; ^{13}C NMR (100 MHz, $CDCl_3$, rt): 171.8, 170.6, 170.2, 169.3, 169.0, 157.6, 136.9, 130.3 (2C), 128.6 (3C), 128.0, 127.4 (2C), 114.7 (2C), 101.3, 81.5, 72.7, 71.9, 70.9, 69.9, 68.2, 61.8, 52.1, 37.8, 20.7, 20.6 (2C), 20.5 ppm; HR FAB MS (positive): $[M+Na]^+$ found *m/z* 639.2078, $C_{31}H_{36}O_{13}Na$ requires *m/z* 639.2054; IR (film) ν: 1755, 1512 cm^{-1}; $[\alpha]_D^{26}$=−0.7° (*c* 1.00, $CHCl_3$).

3.1.9. Methyl 2-(2′,3′,4′,6′-tetra-*O*-acetyl-β-L-glucopyranosy-loxy)-3-*p*-hydroxyphenylpropionate (20a and 20b). To a solution of **19** (353.5 mg, 0.574 mmol) in EtOAc/MeOH=1/1 (6 ml), 10% Pd–C was added and stirred at rt under hydrogen atomosphere for 9 h. The reaction mixture was filtered on celite and the filtrate was concentrated in vacuo. The residue was purified with silica gel column chromatography (*n*-hexane/EtOAc=2/3) to give a mixture of **20a** and **20b** (293.4 mg×97%). From 25.9 mg of this mixture, each diastereomer was separated with preparative TLC (benzene/acetone=5/1) to give **20a** (15.7 mg, 59%) and **20b** (8.8 mg, 33%), respectively.

Compound **20a**. ^1H NMR (400 MHz, $CDCl_3$, rt): 7.03 (2H, d, *J*=8.6 Hz), 6.72 (2H, d, *J*=8.6 Hz), 5.41 (1H, s, –OH), 5.20 (1H, dd, *J*=9.3, 9.5 Hz), 5.07 (1H, dd, *J*=9.5, 10.0 Hz), 5.05 (1H, dd, *J*=8.1, 9.3 Hz), 4.56 (1H, d, *J*=8.1 Hz), 4.52 (1H, dd, *J*=5.4, 6.1 Hz), 4.17 (1H, dd, *J*=4.5, 12.5 Hz), 4.08 (1H, dd, *J*=2.3, 12.5 Hz), 3.66 (3H, s), 3.63 (1H, ddd, *J*=2.3, 4.5, 10.0 Hz), 3.04 (1H, dd, *J*=5.4, 14.3 Hz), 2.98 (1H, dd, *J*=6.1, 14.3 Hz), 2.09 (3H, s), 2.02 (3H, s), 2.013 (3H, s), 2.005 (3H, s) ppm; ^{13}C NMR (100 MHz, $CDCl_3$, rt): 171.0, 170.7, 170.1, 169.6, 169.4, 154.5, 130.7 (2C), 127.7, 114.9 (2C), 99.1, 76.7, 72.4, 71.7, 70.9, 68.4, 61.9, 51.9, 38.0, 20.8, 20.72, 20.69, 20.66 ppm; HR FAB MS (positive): $[M+Na]^+$ found *m/z* 549.1591, $C_{24}H_{30}O_{13}Na$ requires *m/z* 549.1584; IR (film) ν: 3438, 1753, 1518 cm^{-1}; $[\alpha]_D^{25}$=+23.4° (*c* 1.00, $CHCl_3$).

Compound **20b**. ^1H NMR (400 MHz, $CDCl_3$, rt): 7.04 (2H, d, *J*=8.6 Hz), 6.74 (2H, d, *J*=8.6 Hz), 5.25 (1H, s, –OH),

5.11 (1H, dd, J=8.8, 9.1 Hz), 5.04 (1H, dd, J=8.3, 8.8 Hz), 5.03 (1H, dd, J=9.1, 9.3 Hz), 4.46 (1H, d, J=8.3 Hz), 4.15 (1H, dd, J=5.1, 12.3 Hz), 4.11 (1H, dd, J=4.6, 9.3 Hz), 4.08 (1H, dd, J=2.4, 12.3 Hz), 3.72 (3H, s), 3.62 (1H, ddd, J=2.4, 5.1, 9.3 Hz), 2.96 (1H, dd, J=9.3, 14.1 Hz), 2.89 (1H, dd, J=4.6, 14.1 Hz), 2.10 (3H, s), 2.01 (3H, s), 1.98 (3H, s), 1.83 (3H, s) ppm; ^{13}C NMR (100 MHz, $CDCl_3$, rt): 171.8, 170.6, 170.2, 169.3, 169.0, 154.5, 130.4 (2C), 128.2, 115.2 (2C), 101.2, 81.5, 72.7, 71.9, 71.0, 68.2, 61.9, 52.1, 37.9, 20.8, 20.64 (2C), 20.57 ppm; HR FAB MS (positive): $[M+Na]^+$ found m/z 549.1599, $C_{24}H_{30}O_{13}Na$ requires m/z 549.1584; IR (film) ν: 3419, 1753, 1518 cm^{-1}; $[\alpha]_D^{25}$=+2.7° (c 0.81, $CHCl_3$).

3.1.10. Methyl 2-(2′,3′,4′,6′-tetra-*O*-acetyl-β-L-glucopyranosyl-oxy)-3-*p*-*t*-butyldimethylsiloxyphenylpropionate (21a and 21b). To a solution of **20** (293.4 mg×0.558 mmol) in DMF (5 ml), TBDMSCl (179.8 mg, 1.19 mmol), imidazole (153.1 mg×2.25 mmol), and DMAP (7.3 mg, 0.060 mmol) were added at 0°C under argon atmosphere and then the reaction mixture was stirred for 2 h. After the addition of water (35 ml), the reaction mixture was partitioned with EtOAc (20 ml) three times. The organic layer was washed with saturated NaClaq. and dried over absolute Na_2SO_4 and concentrated in vacuo. The residue was purified with silica gel column chromatography (n-hexane/EtOAc=3/2) to give **21** (336.6 mg, 94%). From 29.4 mg of **21**, each diastereomer was separated with preparative TLC (benzene/acetone=10/1) to give **21a** (14.9 mg, 48%) and **21b** (8.5 mg, 27%), respectively.

Compound **21a**. ^{1}H NMR (400 MHz, $CDCl_3$, rt): 7.02 (2H, d, J=8.6 Hz), 6.72 (2H, d, J=8.6 Hz), 5.21 (1H, dd, J=9.5, 9.6 Hz), 5.08 (1H, dd, J=9.6, 10.0 Hz), 5.07 (1H, dd, J=8.0, 9.5 Hz), 4.562 (1H, d, J=8.0 Hz), 4.556 (1H, t, J=5.8 Hz), 4.21 (1H, dd, J=8.8, 12.0 Hz), 4.10 (1H, dd, J=2.4, 12.0 Hz), 3.63 (1H, ddd, J=2.4, 8.8, 10.0 Hz), 3.62 (3H, s), 3.04 (1H, dd, J=5.8, 14.2 Hz), 3.00 (1H, dd, J=5.8, 14.2 Hz), 2.09 (3H, s), 2.019 (3H, s), 2.015 (3H, s), 2.00 (3H, s), 0.97 (9H, s), 0.17 (6H, s) ppm; ^{13}C NMR (100 MHz, $CDCl_3$, rt): 170.9, 170.5, 170.1, 169.4, 169.3, 154.4, 130.5 (2C), 128.3, 119.7 (2C), 99.0, 76.5, 72.4, 71.8, 70.8, 68.4, 61.8, 51.8, 38.1, 25.7 (3C), 20.8, 20.72, 20.70, 20.66, 18.25, −4.4 (2C) ppm; HR FAB MS (positive): $[M+Na]^+$ found m/z 663.2458, $C_{30}H_{44}O_{13}SiNa$ requires m/z 663.2449; IR (film) ν: 1755, 1510 cm^{-1}; $[\alpha]_D^{27}$=+27.0° (c 1.00, $CHCl_3$).

Compound **21b**. ^{1}H NMR (400 MHz, $CDCl_3$, rt): 7.03 (2H, d, J=8.6 Hz), 6.73 (2H, d, J=8.6 Hz), 5.11 (1H, dd, J=9.4, 9.5 Hz), 5.05 (1H, dd, J=7.8, 9.5 Hz), 5.03 (1H, dd, J=9.4, 9.5 Hz), 4.45 (1H, d, J=7.8 Hz), 4.16 (1H, dd, J=5.3, 12.4 Hz), 4.11 (1H, dd, J=4.6, 9.0 Hz), 4.07 (1H, dd, J=2.5, 12.4 Hz), 3.71 (3H, s), 3.62 (1H, ddd, J=2.5, 5.3, 9.5 Hz), 2.95 (1H, dd, J=9.0, 14.6 Hz), 2.89 (1H, dd, J=4.6, 14.6 Hz), 2.10 (3H, s), 2.01 (3H, s), 1.98 (3H, s), 1.85 (3H, s), 0.97 (9H, s), 0.176 (3H, s), 0.173 (3H, s) ppm; ^{13}C NMR (100 MHz, $CDCl_3$, rt): 171.7, 170.5, 170.1, 169.2, 168.9, 154.4, 130.2 (2C), 128.8, 119.9 (2C), 101.2, 81.4, 72.7, 71.9, 71.0, 68.2, 61.8, 52.1, 38.0 25.8 (3C), 20.8, 20.65 (2C), 20.60, 18.2, −4.4 (2C) ppm; HR FAB MS (positive): $[M+Na]^+$ found m/z 663.2458, $C_{30}H_{44}O_{13}SiNa$ requires m/z 663.2449; IR (film) ν: 1757, 1512 cm^{-1}; $[\alpha]_D^{27}$=+2.9° (c 0.85, $CHCl_3$).

3.1.11. Methyl (*Z*)-2-(2′,3′,4′,6′-tetra-*O*-acetyl-β-L-glucopyranosyloxy)-3-*t*-butyldimethylsiloxyphenyl-2-acrylate (22). To a solution of **21** (332.6 mg, 0.519 mmol) in 1,4-dioxane (5 ml) was added 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ, 482.3 mg, 2.12 mmol) and refluxed for 42 h under argon atmosphere. The reaction mixture was mixed with sat. $NaHCO_3$aq. and then extracted with diethyl ether. The organic layer was washed with saturated NaClaq. and dried over anhydrous Na_2SO_4. After evaporation, the residue was purified by silica gel column chromatography to give **22** (295.6 mg, 89%).

^{1}H NMR (400 MHz, $CDCl_3$, rt): 7.67 (2H, d, J=8.8 Hz), 7.05 (1H, s), 6.81 (2H, d, J=8.8 Hz), 5.30–2.25 (3H, m), 5.18–5.14 (1H, m), 4.13 (1H, dd, J=4.4, 12.4 Hz), 4.01 (1H, dd, J=2.6, 12.4 Hz), 3.83 (3H, s), 3.67 (1H, ddd, J=2.6, 4.4, 10.0 Hz), 2.03 (6H, s), 2.02 (3H, s), 1.98 (3H, s), 0.99 (9H, s), 0.22 (6H, s) ppm; ^{13}C NMR (100 MHz, $CDCl_3$, rt): 170.5, 170.2, 169.6, 169.5, 164.2, 157.0, 138.1 (2C), 132.5, 126.9, 125.8, 119.9 (2C), 99.2, 72.8, 71.8, 71.6, 68.3, 61.5, 52.2, 25.6 (3C), 20.65, 20.62, 20.59, 20.52, 18.2, −4.38, −4.42 ppm; HR FAB MS (positive): $[M+Na]^+$ found m/z 661.2282, $C_{30}H_{42}O_{13}SiNa$ requires m/z 661.2292; IR (film) ν: 1757, 1718, 1601, 1508 cm^{-1}; $[\alpha]_D^{25}$=+1.9° (c 1.00, $CHCl_3$).

3.1.12. Methyl (*Z*)-2-(2′,3′,4′,6′-tetra-*O*-acetyl-β-L-glucopyranosyloxy)-3hydroxyphenyl-2-acrylate (23). To a solution of **22** (290.0 mg, 0.454 mmol) in dry tetrahydrofuran (THF, 4 ml) was added tetra-n-butyl ammonium fluoride (1.0 M in THF, 0.60 ml, 0.60 mmol) at 0°C for 10 min. After adding water, the reaction mixture was partitioned with EtOAc, and washed with $NaHCO_3$aq. The organic layer was evaporated in vacuo and the residue was purified by preparative TLC ($CHCl_3$/acetone=10/1) to afford **23** (243.2 mg, quant.).

^{1}H NMR (400 MHz, $CDCl_3$, rt): 7.67 (2H, d, J=8.8 Hz), 7.06 (1H, s), 6.80 (2H, d, J=8.8 Hz), 5.68 (1H, s, −OH), 5.29–5.26 (3H, m), 5.18–5.12 (1H, m), 4.11 (1H, dd, J=12.2, 4.9 Hz), 4.01 (1H, dd, J=12.2, 2.4 Hz), 3.84 (3H, s), 3.66 (1H, ddd, J=9.8, 4.4, 2.4 Hz), 2.04 (3H, s), 2.03 (6H, s), 2.02 (3H, s) ppm; ^{13}C NMR (100 MHz, $CDCl_3$, rt): 170.5, 170.0, 169.7, 169.4, 164.1, 156.8, 137.9, 132.7, 130.8, 126.9, 125.2, 115.2, 99.3, 72.8, 71.8, 71.6, 68.4, 61.6, 20.8, 20.7, 20.6 ppm; HR FAB MS (positive): $[M+Na]^+$ found m/z 547.1444, $C_{24}H_{28}O_{13}Na$ requires m/z 547.1428; IR (film) ν: 3419, 1756, 1717, 1639, 1606, 1585, 1514 cm^{-1}; $[\alpha]_D^{19}$=+4.3° (c 0.39, $CHCl_3$).

3.1.13. Potassium L-isolespedezate (2a) and potassium L-lespedezate (2b). Compound **23** (72.0 mg, 0.137 mmol) was dissolved in metahol/H_2O=3/1 (6 ml). To this solution was added 3.0 M KOHaq. (0.5 ml, 0.15 mmol) at 0°C, and then the reaction mixture was stirred for 40 min. The reaction mixture was concentrated and then acidified by Amberlite IR-120B(H^+) column. The eluate was concentrated and purified by using HPLC[COSMOSIL 5C_{18}-AR(ϕ 20×250 mm), 1% AcOH/30%MeOHaq.] to afford L-lespedezic acid (5.7 mg) and L-isolespedezic acid (34.9 mg), respectively. Each acid was neutralized by K_2CO_3aq. to afford potassium L-lespedezate (**2a**) (6.3 mg, 12%) and potassium L-isolespedezate (**2b**) (38.8 mg, 75%).

Compound **2a**. ^{1}H NMR (400 MHz, D_2O, 30°C): 7.23 (2H, d, *J*=8.3 Hz), 6.83 (2H, d, *J*=8.3 Hz), 6.19 (1H, s), 4.94 (1H, d, *J*=7.8 Hz), 3.96 (1H, dd, *J*=12.2, 2.0 Hz), 3.77 (1H, dd, *J*=12.2, 5.9 Hz), 3.61 (1H, t, *J*=9.0 Hz), 3.57 (1H, m), 3.53 (1H, dd, *J*=9.3, 7.8 Hz), 3.48 (1H, t, *J*=9.0 Hz) ppm; ^{13}C NMR (100 MHz, D_2O, 30°C): 172.5, 156.6, 149.7, 130.2, 126.7, 116.4, 109.6, 101.0, 77.0, 76.3, 73.7, 70.3, 61.5 ppm; HR FAB MS (negative): $[M-K]^-$ found *m/z* 341.0914, $C_{15}H_{17}O_9$ requires *m/z* 341.0872; IR (film) *ν*: 3247, 1634, 1593, 1513 cm^{-1}; $[\alpha]_D^{21}$=+40.9° (*c* 0.57, H_2O).

Compound **2b**. ^{1}H NMR (400 MHz, D_2O, 30°C): 7.76 (2H, d, *J*=8.3 Hz), 6.93 (2H, d, *J*=8.3 Hz), 6.76 (1H, s), 5.07 (1H, d, *J*=7.4 Hz), 3.82 (1H, dd, *J*=12.7, 2.0 Hz), 3.70 (1H, *J*=12.7, 5.0 Hz), 3.61 (1H, dd, *J*=9.0, 7.4 Hz), 3.56 (1H, t, *J*=9.0 Hz), 3.47 (1H, t, *J*=9.0 Hz), 3.39 (1H, ddd, *J*=9.0, 5.0, 2.0 Hz) ppm; ^{13}C NMR (100 MHz, D_2O, 30°C): 172.1, 156.5, 145.9, 132.4, 126.7, 120.8, 116.1, 101.9, 77.2, 76.7, 74.6, 70.2, 61.3 ppm; HR FAB MS (negative): $[M-K]^-$ found *m/z* 341.0914, $C_{15}H_{17}O_9$ requires *m/z* 341.0872; IR (film) *ν*: 3290, 1642, 1574, 1511 cm^{-1}; $[\alpha]_D^{20}$=−50.7° (*c* 1.0, H_2O).

3.1.14. Potassium 2-β-L-glucopyranosyloxy-3-*p*-hydroxyphenyl-propionate (24a and 24b). A mixture of **21a** and **21b** (101.0 mg, 0.192 mmol) was dissolved in MeOH/H_2O=3/1 (1 ml). To this solution was added 4 M KOHaq. (0.5 ml, 2.0 mmol) at 0°C. After stirring for 19 h, the solution was acidified by Amberlite IR-120, filtered, and then concentrated in vacuo. The resulting residue was neutralized by K_2CO_3, purified by HPLC (Cosmocil 5C18-AR, 1% AcOH–30% MeOH aq.), and neutralized by K_2CO_3 again to give a mixture of **24a** (16.5 mg, 25%) and **24b** (37.1 mg, 56%).

Compound **24a**. ^{1}H NMR (400 MHz, D_2O, rt): 7.19 (2H, d, *J*=8.0 Hz), 6.82 (2H, d, *J*=8.0 Hz), 4.30 (1H, d, *J*=7.8 Hz), 4.17 (1H, dd, *J*=5.2, 7.4 Hz), 3.80 (1H, dd, *J*=2.4, 12.4 Hz), 3.67 (1H, *J*=5.0, 12.4 Hz), 3.39 (1H, dd, *J*=8.0, 8.8 Hz), 3.37 (1H, dd, *J*=8.0, 9.6 Hz), 3.29 (1H, dd, *J*=7.8, 8.8 Hz), 3.27 (1H, ddd, *J*=2.4, 5.0, 9.6 Hz), 2.97 (1H, dd, *J*=5.2, 14.4 Hz), 2.91 (1H, dd, *J*=7.4, 14.4 Hz) ppm; ^{13}C NMR (100 MHz, D_2O, rt): 181.3, 155.4, 132.3 (2C), 130.9, 116.7 (2C), 103.6, 83.4, 77.3, 77.1, 74.6, 70.9, 62.0, 39.1 ppm; HR FAB MS (negative): $[M-K]^-$ found *m/z* 343.1042, $C_{15}H_{19}O_9$ requires *m/z* 343.1029; IR (film) *ν*: 3307, 1597, 1516 cm^{-1}; $[\alpha]_D^{26}$=+22.1° (*c* 1.0, H_2O).

Compound **24b**. ^{1}H NMR (400 MHz, D_2O, rt): 7.17 (2H, d, *J*=9.0 Hz), 6.80 (2H, d, *J*=9.0 Hz), 4.43 (1H, d, *J*=7.8 Hz), 4.41 (1H, dd, *J*=6.1, 7.2 Hz), 3.85 (1H, dd, *J*=2.0, 12.2 Hz), 3.66 (1H, *J*=5.2, 12.2 Hz), 3.46 (1H, dd, *J*=9.0, 9.0 Hz), 3.38 (1H, ddd, *J*=2.0, 5.2, 9.2 Hz), 3.36 (1H, dd, *J*=9.0, 9.2 Hz), 3.30 (1H, dd, *J*=7.8, 9.0 Hz), 3.00 (1H, dd, *J*=6.1, 13.2 Hz), 2.95 (1H, dd, *J*=7.2, 13.2 Hz) ppm; ^{13}C NMR (100 MHz, D_2O, rt): 180.5, 156.2, 132.3 (2C), 130.3, 116.9 (2C), 103.2, 82.7, 77.5, 77.2, 74.8, 71.0, 62.2, 39.6 ppm; HR FAB MS (negative): $[M-K]^-$ found *m/z* 343.1008, $C_{15}H_{19}O_9$ requires *m/z* 343.1029; IR (film) *ν*: 3222, 1597, 1512 cm^{-1}; $[\alpha]_D^{26}$=+14.7° (*c* 1.0, H_2O).

3.1.15. Methyl (*Z*)-2-β-L-glucopyranosyloxy-3methoxy-phenyl-2-acrylate (25). Compound **23** (68.3 mg, 0.130 mmol) was dissolved in DMF (1.3 ml) and K_2CO_3 (34.3 mg, 0.249 mmol) was added to this solution. After adding iodomethane (50 μl, 0.942 mmol) at 0°C under argon atmosphere, the solution was stirred for 2 h. To this solution was added water, and then this mixture was extracted by EtOAc. The organic layer was washed by saturated NaClaq., dried over Na_2SO_4, and evaporated to dryness in vacuo. The residue was separated by preparative TLC (*n*-hexane/EtOAc=1/1) to give methyl ether (**25** 65.8 mg, 94%).

^{1}H NMR (400 MHz, $CDCl_3$, rt): 7.73 (2H, d, *J*=8.8 Hz), 7.07 (1H, s), 6.88 (2H, d, *J*=8.8 Hz), 5.30–5.26 (3H, m), 5.17–5.12 (1H, m), 4.11 (1H, dd, *J*=4.8, 12.4 Hz), 4.01 (1H, dd, *J*=2.4, 12,4 Hz), 3.84 (3H, s), 3.83 (3H, s), 3.67 (1H, ddd, *J*=2.4, 4.8, 9.8 Hz), 2.04 (3H, s), 2.03 (3H, s), 2.02 (3H, s), 1.96 (3H, s) ppm; ^{13}C NMR (100 MHz, $CDCl_3$, rt): 170.3, 170.0, 169.5, 169.3, 164.0, 160.4, 137.9, 132.5 (2C), 126.9, 125.2, 113.6 (2C), 99.2, 72.7, 71.8, 71.6, 68.3, 61.6, 55.3, 52.2, 20.7, 20.64 (2C), 20.57 ppm; HR FAB MS (positive): $[M+Na]^+$ found *m/z*561.1573, $C_{25}H_{30}O_{13}Na$ requires *m/z* 561.1584; IR (film) *ν*: 1755, 1716, 1604, 1512 cm^{-1}; $[\alpha]_D^{26}$=+3.3° (*c* 1.0, $CHCl_3$).

3.1.16. Potassium-2-β-L-glucopyranosyloxy-3methoxy-phenyl-2-acrylate (26a and 26b). To a solution of **25** (54.4 mg, 0.101 mmol) in MeOH/H_2O=3/1 (1 ml) 4 M KOHaq. (0.3 ml, 0.12 mmol) was added at 0°C. The reaction mixture was stirred at rt for 19 h and then acidified with Amberlite IR-120 B (H^+) column chromatography. The eluate was concentrated in vacuo, purified by HPLC using Cosmosil 5C18-AR column (ϕ 20×mm) with 30% MeOHaq. containing 1% AcOH, and neutralized with K_2CO_3, to give **26a** (1.9 mg, 5%) and **26b** (18.4 mg, 51%), respectively.

Compound **26a**. ^{1}H NMR (400 MHz, D_2O, rt): 7.06 (2H, d, *J*=8.6 Hz), 6.73 (2H, d, *J*=8.6 Hz), 5.97 (1H, s), 4.72 (1H, d, *J*=7.8 Hz), 3.72 (1H, dd, *J*=2.0, 12.8 Hz), 3.61 (3H, s), 3.42 (1H, dd, *J*=5.8, 12.8 Hz), 3.37 (1H, dd, *J*=9.4, 9.4 Hz), 3.34 (1H, m), 3.30 (1H, dd, *J*=7.8, 9.4 Hz), 3.24 (1H, dd, *J*=9.4, 9.4 Hz) ppm; ^{13}C NMR (100 MHz, D_2O, rt): 173.2, 159.2, 151.0, 130.8 (2C), 129.0, 115.4 (2C), 109.8, 101.6, 77.7, 77.0, 74.4, 71.0, 62.2, 56.9 ppm; HR FAB MS (negative): $[M-K]^-$ found *m/z*355.1012, $C_{16}H_{19}O_9$ requires *m/z*355.1029; IR (film) *ν*: 3269, 1595, 1512 cm^{-1}; $[\alpha]_D^{26}$=+41.0° (*c* 0.63, H_2O).

Compound **26b**. ^{1}H NMR (400 MHz, D_2O, rt): 7.59 (2H, d, *J*=8.8 Hz), 6.80 (2H, d, *J*=8.8 Hz), 6.55 (1H, s), 4.85 (1H, d, *J*=7.6 Hz), 3.65 (3H, s), 3.59 (1H, dd, *J*=2.2, 12.6 Hz), 3.47 (1H, dd, *J*=5.2, 12.6 Hz), 3.39 (1H, dd, *J*=7.6, 9.2 Hz), 3.34 (1H, dd, *J*=9.0, 9.2 Hz), 3.24 (1H, dd, *J*=9.0, 9.4 Hz), 3.16 (1H, ddd, *J*=2.2, 5.2, 9.4 Hz) ppm; ^{13}C NMR (100 MHz, D_2O, rt): 172.9, 160.5, 147.2, 133.1 (2C), 128.3, 121.3, 115.6 (2C), 102.7, 77.9, 77.5, 75.3, 70.9, 62.1, 57.0 ppm; HR FAB MS (negative): $[M-K]^-$ found *m/z*355.1012, $C_{16}H_{19}O_9$ requires *m/z*355.1029; IR (film) *ν*: 3303, 1604, 1577, 1510 cm^{-1}; $[\alpha]_D^{20}$=−54.9° (*c* 1.0, H_2O).

3.2. Bioassay

C. mimosoides, which was used for the bioassay, was grown

in the greenhouse of Keio University. The young leaves detached from the stem of the plant with a sharp razor blade were used for the bioassay. One leaf was placed in H_2O (ca. 1.0 ml) using a 5-ml glass tube in the greenhouse kept at 25–30°C and allowed to stand overnight. The leaves which opened again the next morning (around 10:00 a.m.) were used for the bioassay. Each test solution was carefully poured into test tubes around 10:00 a.m. The bioactive fraction was judged by the leaf-opening at 21:00 after the leaf-closing of the plant leaf in the blank solution containing no sample. Other nyctinastic plants, *Mimosa pudica* L., *Albizzia julibrissin* Durazz, *Aeschynomene indica* L. and *Phyllanthus urinaria* L.,used in the bioassay were also grown in the greenhouse of Keio University.

3.3. Bioassay using leaf-movement inhibitors

All experiments were carried out in a greenhouse of Keio University maintained within 25–30°C, or in a biotron under the following conditions: 12-hour light/12-hour dark, at 27°C, 50% humidity. Both conditions gave the same results. Each leaf was soaked in a 3×10^{-6} mol/l aqueous solution of **2** or distilled water only for the control sample. These samples were allowed to stand for 10 days, and the status of the leaves was checked every day.

3.4. Enzymatic reaction

A reaction mixture (0.10 ml) containing 1.0 mM of substrate, 0.1 M citrate buffer (pH 5.0), and β-glucosidase (1 unit) was incubated at 37°C for 30 min. After the reaction was stopped by the heating at 100°C for 1 min, the reaction mixture was analyzed by HPLC equipped with a photodiode array detector with 20% MeOH containing 1% AcOH (flow rate: 0.9 ml/min, detection: 220 nm) using Develosil ODS HG-5 column (ϕ 4.6×250 mm).

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