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This article is dedicated to Professor Satoshi Ōmura in celebration of his 2015 Nobel Prize.

Note

Simple Synthesis of Sakuranetin and Selinone *via* a Common Intermediate, Utilizing Complementary Regioselectivity in the Deacetylation of Naringenin Triacetate

Yasunobu Yamashita, Kengo Hanaya, Mitsuru Shoji, and Takeshi Sugai*

Department of Pharmaceutical Sciences, Keio University; 1–5–30 Shibakoen, Minato-ku, Tokyo 105–8512, Japan.

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Sakuranetin and selinone were successfully synthesized utilizing the regioselective deacetylation of naringenin triacetate. Deacetylation of the latter at C-7 with imidazole in 1,4-dioxane at 40°C furnished the corresponding diacetate in 80% yield. Methylation of the obtained free hydroxy group and subsequent removal of the remaining two acetyl groups gave sakuranetin, which was previously isolated as a phytoalexin against rice blast disease fungus, *Pyricularia oryzae*, in 71% overall yield. The same intermediate, naringenin triacetate, was subjected to transesterification with 2-propanol in tetrahydrofuran, catalyzed by *Candida antarctica* lipase B. A contrasting regioselective preference for C-4′ deacetylation was observed, giving an isomeric diacetate in 82% yield. Prenylation of the free hydroxy group under Mitsunobu conditions and subsequent deprotection furnished selinone, which was previously isolated from *Monotes engleri* and exhibits antifungal activity against *Candida albicans*, in 55% overall yield.

Key words flavanone; regioselective deprotection; lipase-catalyzed transesterification

Partially alkylated forms of flavanones are widespread in plants. Among them, sakuranetin (**1b**) (naringenin (**1a**) methylated at C-7) was first isolated from cherry tree bark as a sakuranin aglycone by Asahina in 1908,[1] and its antifungal activity was reported later.[2] Recently, its additional antimutagenic[3] and antiinflammatory activities[4] were also demonstrated. Agricultural biology and chemistry constitute other fields for the application of **1b**. Kodama *et al.* isolated **1b** from UV-irradiated rice leaves as a phytoalexin against the pathogenic fungus *Pyricularia oryzae*, which causes rice blast disease, and also detected **1b** in rice leaves infected with *P. oryzae*.[5]

Selinone (**1c**) (naringenin prenylated at C-4′) was first isolated by Seshadri and Sood from *Selinum vaginatum* Clarke, an endemic high-altitude Indian medicinal plant of the Umbelliferae family, locally known as "Bhootkeshi."[6] Later, it was also isolated from *Monotes engleri*,[7] a plant of the Dipterocarpaceae family in Zimbabwe, and showed antifungal activity against *Candida albicans* (Chart 1).

Sakuranetin (**1b**) has previously been synthesized by methylation of naringenin (**1a**), as shown in Chart 2. The formation of **1b** in 87% yield by methylation of **1a** with dimethyl sulfate in acetone was reported.[8] The formation of the regioisomeric monoether **1d** under the above condition was also reported.[9] These results suggest that sakuranetin (**1b**) was isolated from a mixture of mono- and dimethylated products by silica gel column chromatography. Furthermore, it was shown that methylation of **1a** by treatment with 1.5 eq of methyl iodide in *N*,*N*-dimethylformamide (DMF) led to the formation of not only **1b** (76%) but also dimethyl ether **1e** (3%).[10] To selectively methylate the C-7 hydroxy group, which is the most acidic of the three, **1a** was treated with diazomethane solution in diethyl ether under almost neutral conditions, to give **1b** in 75% yield after silica gel column chromatography.[11] This reaction, however, must be carried out at very low concentrations in diethyl ether (1.0 g/400 mL), due to the low solubility of **1a**.

In order to address the solubility issue and reduce the amount of regioisomeric by-products, we altered the substrate of methylation from **1a** to **g**, where the C-5 and C-4′ hydroxy groups were protected by acetyl groups. Acetylation of nar-

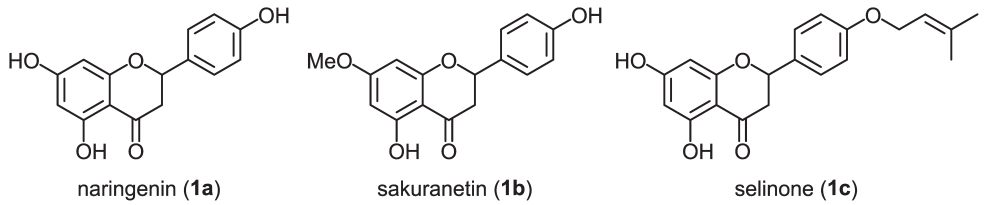

Chart 1. Naringenin (**1a**) and Its Partially Alkylated Derivatives **1b** and **c**

* To whom correspondence should be addressed. e-mail: sugai-tk@pha.keio.ac.jp

ingenin (**1a**) with acetic anhydride and pyridine furnished naringenin triacetate (**1f**) in 97% yield. Then, the obtained **1f** was treated with imidazole[12] in 1,4-dioxane at 40°C. The most electrophilic acetate at C-7[13] was predominantly removed to furnish **1g** in 80% yield. Treatment of **1g** with a diazomethane solution in a mixture of diethyl ether and dichloromethane, followed by subsequent short column chromatography, gave **1h** in 95% yield. As expected from the greater lipophilicity of **1g** compared with **1a**, the reaction could be performed at a higher substrate concentration (400 mg/9 mL). Moreover, the methylation under conventional basic conditions by applying methyl iodide in the presence of potassium carbonate in DMF also furnished **1h** in 95% yield. Removal of two acetyl groups in **1h** by sodium methoxide in methanol gave **1b** in 96% yield (Chart 3).

It should be noted that our present scheme established a more scalable synthesis of **1h**. This methyl ether is a reported precursor for 7-methoxyapigeninidin (**2**), which has been synthesized as an antifungal agent against sorghum fungi, *Gloeocercospora sorghi*.[11]

Selinone (**1c**) was prepared by a prenyl transferase (NovQ)-catalyzed prenylation of **1a**.[14] The prenylation, however, occurred mainly in the aromatic ring, at the carbon atom adjacent to C-3′ (87%), and the *O*-prenylated **1c** was obtained in as low as 11% yield. An efficient multistep synthesis of **1c** was established by Antus and colleagues[15] (Chart 4). In their first attempt, **3** and **4a** were chosen as starting materials for the construction of the carbon skeleton of **1c**. It turned out that the pre-installed prenyl group was unstable under the acidic conditions required for the methoxymethyl (MOM) group removal, such as the transformation of **5a** to **b**. To address this issue, the prenyl group in **4a** was substituted for a benzyl group in **4b**. Furthermore, the MOM group in **1i** was replaced with acetyl in **1j**. Removal of the benzyl group by hydrogenolysis, prenylation under Mitsunobu conditions, and the final removal of all acetyl groups furnished **1c** in 5% total yield over seven steps, starting from **3**.

We next focused our interest on intermediate **1k**, which has a free hydroxy group at C-4′. In order to prepare **1k** effectively, regioselective deacetylation of **1f** at C-4′ is required, unlike in the case of **1g**, where deacetylation occurs at C-7. We have already attempted the regioselective deacetylation of **1f**, catalyzed by *Candida antarctica* lipase B (Novozym 435) in the presence of cyclopentanol as nucleophile and cyclopentyl methyl ether (CPME) as solvent. The first step led to the desired deprotection at C-4′, however, this reaction was immediately followed by further deprotection at C-7 to give a mixture of **1k** and **m** in 33 and 56% yield, respectively[16] (Chart 5). The desired **1k** was obtained as a minor product.

Our task was the improvement of the yield of **1k**. For that purpose, 2-propanol and tetrahydrofuran (THF) were chosen as nucleophile and solvent, respectively,[17] the nucleophilicity of 2-propanol being less than that of cyclopentanol. In addition, compared with the previous attempt, the catalyst loading was lowered by a factor of 20 [10 wt% *vs.* 200 wt%]. After incubation for 28 h at 22°C, the desired **1k** was isolated in 82% yield. Interestingly, the three factor changes (cyclopentanol to 2-propanol, CPME to THF as solvent, and the lowering of the catalyst loading) cumulatively contributed to regioselectivity enhancement. Based on the present observation, the over-deacetylation at C-7 in our previous case would be rationalized by the swelling of the resin after immersion in CPME, which increased the effective concentration of the enzyme. Although **1f** has one chiral center at C-2, kinetic resolution did not occur in the above-mentioned lipase-catalyzed transesterification, and the product **1k** did not show any optical rotation.

Finally, according to the slight modification of reported procedure,[15] prenylation of the free hydroxy group at C-4′ under Mitsunobu conditions and the subsequent removal of

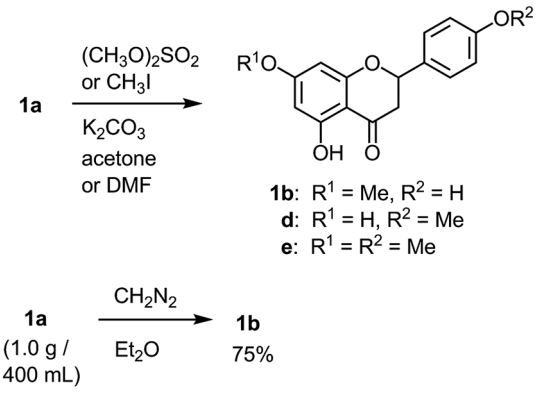

Chart 2. Methylation of Naringenin (**1a**) to Form **1b**

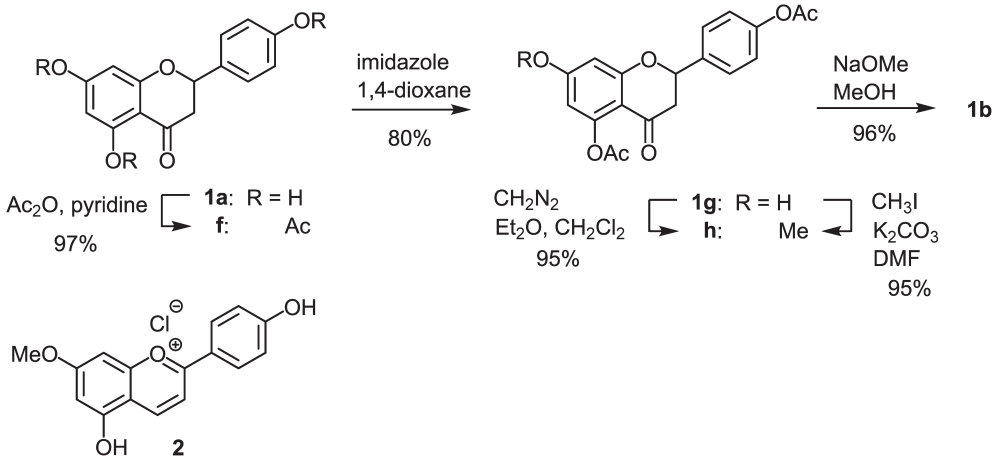

Chart 3. Synthesis of Sakuranetin (**1b**) Based on the Regioselective Deacetylation of **1f** as the Key Step

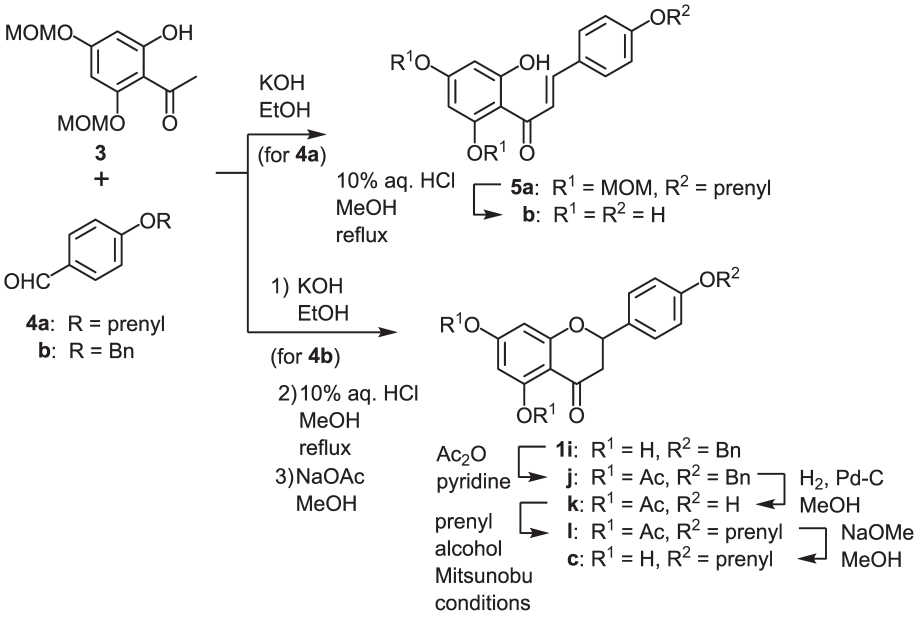

Chart 4. Previous Approaches in the Synthesis of Selinone (**1c**)

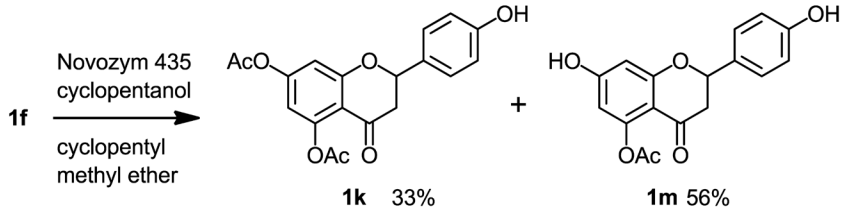

Chart 5. Previously Attempted Deacetylation of **1f** Catalyzed by *C. antarctica* Lipase B

the remaining acetates furnished **1c** in 69% yield over two steps (Chart 6).

In summary, partially alkylated flavanones, namely sakuranetin (**1b**, 71% over four steps), a phytoalexin, and selinone (**1c**, 55% over four steps), an antifungal agent, were conveniently synthesized from a common inexpensive starting material, naringenin (**1a**). Contrasting regioselectivity in the deacetylation of **1f** using imidazole (reaction at C-7 to give **1g**) and *C. antarctica* lipase B (reaction at C-4′ to give **1k**) was a key factor for this success.

Experimental

General Melting points were measured on a Yanaco MP-J3 micromelting apparatus and are uncorrected. ^{1}H-NMR spectra were measured at 400 MHz on a VARIAN 400-MR or at 500 MHz on an Agilent INOVA-500 spectrometers and ^{13}C-NMR spectra were measured at 125 MHz on an Agilent INOVA-500 spectrometers. IR spectra were measured as attenuated total reflectance (ATR) on a Jeol Fourier transform (FT)-IR SPX60 spectrometer. High resolution mass spectra were recorded on Jeol JMS-T100LP AccuTOF. Silica Gel 60 (spherical and neutral; 100–210 μm, 37560–79) from Kanto Chemical Co. (Japan) was used for column chromatography. *C. antarctica* lipase B (Novozym 435) is gift from Novozymes Japan.

(±)-5,4′-Diacetoxy-7-hydroxyflavan-4-one (5,4′-Diacetylnaringenin), 1g To a solution of 5,7,4′-triacetoxyflavan-4-one (**1f**, 210.0 mg, 0.53 mmol) in dioxane (5 mL) was added imidazole (71.7 mg, 1.05 mmol) and the mixture was stirred for 16 h at 40°C. After cooling, the mixture was quenched with saturated aqueous NH_4Cl (15 mL), and the organic materials were extracted with AcOEt. The combined organic layer was washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (1.0 g, hexane–AcOEt=3 : 1) to afford (±)-**1g** as a colorless solid (149.9 mg, 80%). mp 178.1–179.1°C. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 2.33 (3H, s), 2.39 (3H, s), 2.69 (1H, dd, J=2.7, 16.9 Hz), 2.95 (1H, dd, J=13.7, 16.9 Hz), 5.39 (1H, dd, J=2.7, 13.7 Hz), 6.16 (1H, d, J=2.5 Hz), 6.23 (1H, d, J=2.5 Hz), 7.13 (2H, d, J=8.6 Hz), 7.42 (2H, d, J=8.6 Hz). ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 21.1, 21.2, 44.8, 78.7, 102.0, 105.6, 107.4, 122.0, 127.4, 136.0, 150.7, 151.7, 163.2, 164.0, 169.8, 170.6, 189.1. IR cm^{-1}: 3299, 1749, 1616, 1211, 1054. High resolution (HR)-MS electrospray ionization (ESI+) Calcd for $C_{19}H_{16}NaO_7$ $[M+Na]^+$ 379.0794. Found 379.0788. Its NMR spectrum was identical with that reported previously.[13]

(±)-5,4′-Diacetoxy-7-methoxyflavan-4-one (5,4′-Diacetyl-7-methylnaringenin), 1h An *in-situ* formed solution of diazomethane in diethyl ether (6.5 mL), which was prepared from *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide (1.125 g, 5.25 mmol), potassium hydroxide (KOH) (0.25 g), water (0.4 mL) and ethanol (1.25 mL) in a special distilling apparatus, was directly added dropwise to a solution of **1g** (400.2 mg, 1.12 mmol) in CH_2Cl_2 (9 mL) at 0°C. The mixture was stirred for 20 h at room temperature, and then concentrated *in vacuo*. The residue was purified by short column chroma-

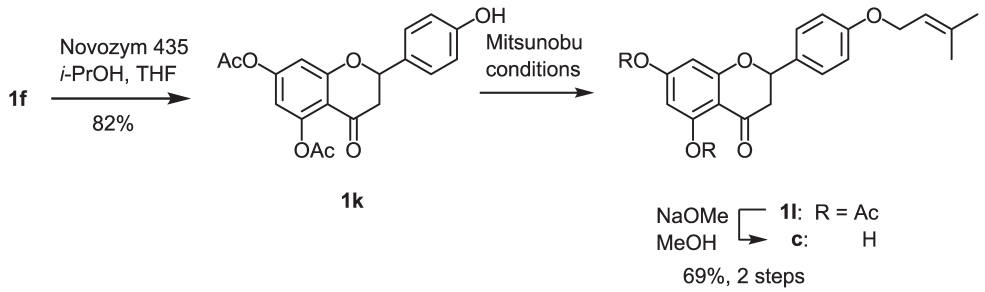

Chart 6. Regioselectivity Improvement of the Deacetylation of **1f** Catalyzed by *C. antarctica* Lipase B, and Its Application to the Synthesis of Selinone (**1c**)

tography to afford (±)-**1h** as a yellow solid (395.0 mg, 95%). mp 98.6–99.5°C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 2.30 (3H, s), 2.36 (3H, s), 2.71 (1H, dd, J=2.9, 16.8 Hz), 2.98 (1H, dd, J=13.3, 16.8 Hz), 3.81 (3H, s), 5.45 (1H, dd, J=2.9, 13.3 Hz), 6.27 (1H, d, J=2.7 Hz), 6.40 (1H, d, J=2.7 Hz), 7.13 (2H, d, J=8.4 Hz), 7.44 (2H, d, J=8.4 Hz). ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 21.1, 21.1, 45.0, 55.8, 79.0, 99.5, 104.8, 107.9, 122.1, 127.4, 136.0, 150.9, 151.9, 164.1, 165.5, 169.3, 169.5, 188.6. IR cm^{-1}: 2977, 1772, 1685, 1616, 1193, 1153. HR-MS (ESI+) Calcd for $C_{20}H_{18}NaO_7$ $[M+Na]^+$ 393.0950. Found 393.0941. Its NMR spectrum was identical with that reported previously.[10] Alternatively, to a solution of **1g** (200.1 mg, 0.56 mmol) in DMF (2 mL) were added methyl iodide (42 μL, 0.67 mmol) and potassium carbonate (77.4 mg, 0.56 mmol) and the mixture was stirred for 17 h at room temperature. The mixture was quenched with saturated aqueous NH_4Cl (2 mL), and the organic materials were extracted with AcOEt. The combined organic layer was washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (5.0 g, hexane–AcOEt=3 : 1) to afford (±)-**1h** as a yellow solid (197.4 mg, 95%).

(±)-5,4′-Dihydroxy-7-methoxyflavan-4-one (Sakuranetin), 1b To a solution of **1h** (50.0 mg, 0.14 mmol) in methanol (2 mL) was added 28% NaOMe in methanol (50 μL, 0.28 mmol) and the mixture was stirred for 2 h at room temperature. The mixture was quenched with saturated aqueous NH_4Cl (5 mL), and the organic materials were extracted with AcOEt. The combined organic layer was washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (1.0 g, hexane–AcOEt=3 : 1) to afford (±)-**1b** as a colorless solid (37.2 mg, 96%). mp 135.2–136.1°C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 2.76 (1H, dd, J=2.9, 17.0 Hz), 3.07 (1H, dd, J=13.1, 17.0 Hz), 3.79 (3H, s), 5.35 (1H, dd, J=2.9, 13.1 Hz), 6.02 (1H, d, J=2.4 Hz), 6.05 (1H, d, J=2.4 Hz), 6.86 (2H, d, J=8.6 Hz), 7.30 (2H, d, J=8.6 Hz). ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 43.1, 55.8, 79.0, 94.2, 95.0, 103.1, 115.6, 127.8, 130.5, 156.1, 162.9, 164.1, 168.0, 196.0. IR cm^{-1}: 3326, 1648, 1515, 1454, 1205, 1149, 1029. HR-MS (ESI+) Calcd for $C_{16}H_{14}NaO_5$ $[M+Na]^+$ 309.0739. Found 309.0754. Its NMR spectrum was identical with that reported previously.[10]

(±)-5,7-Diacetoxy-4′-hydroxyflavan-4-one (5,7-Diacetylnaringenin), 1k To a solution of **1f** (1.97 g, 4.94 mmol) in a mixture of 2-propanol (10 mL) and THF (20 mL), which was pre-dried over anhydrous Na_2SO_4 at room temperature overnight, was added an immobilized form of *C. antarctica* lipase B (Novozymes, Novozym 435, 192 mg). The mixture was stirred for 28 h at 22°C. The mixture was filtered to remove insoluble materials with a pad of Celite. The precipitates were washed with AcOEt. The combined filtrate and washings were concentrated *in vacuo*. The residue was purified by silica gel column chromatography (40 g, hexane–AcOEt=1 : 1) to afford (±)-**1k** as a colorless solid (1.45 g, 82%). mp 113.9–114.8°C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 2.30 (3H, s), 2.39 (3H, s), 2.70 (1H, dd, J=2.6, 16.7 Hz), 3.00 (1H, dd, J=13.7, 16.7 Hz), 5.32 (1H, dd, J=2.6, 13.7 Hz), 6.52 (1H, d, J=2.4 Hz), 6.75 (1H, d, J=2.4 Hz), 6.82 (2H, d, J=8.7 Hz), 7.25 (2H, d, J=8.7 Hz). ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 21.1, 21.2, 44.8, 79.3, 109.3, 110.4, 115.6, 128.0, 129.7, 151.1, 155.9, 156.4, 156.6, 163.5, 168.3, 169.9, 190.0. IR cm^{-1}: 3382, 1770, 1683, 1508, 1373, 1157, 1016. HR-MS (ESI+) Calcd for $C_{19}H_{16}NaO_7$ $[M+Na]^+$ 379.0794. Found 379.0790. Its NMR spectrum was identical with that reported previously.[15]

(±)-5,7-Dihydroxy-4′-(3-methyl-but-2-enyloxy)flavan-4-one (Selinone), 1c To a solution of **1k** (839.8 mg, 2.38 mmol), triphenylphosphine (749.1 mg, 2.87 mmol), and 3-methyl-2-buten-1-ol (350 μL, 3.50 mmol) in THF (20 mL) was added dropwise a solution of diisopropyl azodicarboxylate in toluene (1.96 mL, 3.85 mmol) at 0°C. The reaction mixture was warm to room temperature and stirred for 24 h. After removal of the solvent *in vacuo*, highly polar substance was removed by short silica gel column chromatography to afford (±)-**1l** as a yellow solid (880.0 mg). This was employed for the next step without further purification.

To a solution of **1l** (880.0 mg, 2.38 mmol) in methanol (30 mL) was added 28% NaOMe in methanol (440 μL, 5.76 mmol) and the mixture was stirred for 2 h at room temperature. The mixture was quenched with saturated aqueous NH_4Cl (20 mL), and the organic materials were extracted with AcOEt. The combined organic layer was washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (20 g, hexane–AcOEt=3 : 1) to afford (±)-**1c** as a yellow solid (600.3 mg, 69% over 2 steps). mp 139.8–140.5°C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.73 (3H, s), 1.78 (3H, s), 2.76 (1H, dd, J=3.1, 17.0 Hz), 3.08 (1H, dd, J=13.1, 17.0 Hz), 4.51 (2H, d, J=6.6 Hz), 5.33 (1H, dd, J=3.1, 13.1 Hz), 5.42 (1H, m), 5.96 (1H, d, J=2.4 Hz), 5.98 (1H, d, J=2.4 Hz), 6.95 (2H, d, J=8.8 Hz), 7.34 (2H, d, J=8.8 Hz). ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 18.2, 25.8, 43.1, 64.9, 79.0, 95.5, 96.7, 103.1, 114.9, 119.3, 127.7, 130.1, 138.6, 159.3, 163.2, 164.3, 164.9, 196.1. IR cm^{-1}: 3282, 1610, 1558, 1540, 1488, 1471, 1361, 1087, 1012. HR-MS (ESI+) Calcd for $C_{20}H_{20}NaO_5$ $[M+Na]^+$ 363.1208. Found 363.1203. Its NMR spectrum was identical with that reported previously.[15]

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Conflict of Interest The authors declare no conflict of interest.

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