Journal of Molecular Catalysis B: Enzymatic 128 (2016) 19–26

Contents lists available at ScienceDirect

Journal of Molecular Catalysis B: Enzymatic

journal homepage: www.elsevier.com/locate/molcatb

Synthesis of acacetin and resveratrol 3,5-di-*O*-β-glucopyranoside using lipase-catalyzed regioselective deacetylation of polyphenol glycoside peracetates as the key step

Shun Hanamura, Kengo Hanaya, Mitsuru Shoji, Takeshi Sugai *

Department of Pharmaceutical Science, Keio University, 1-5-30, Shibakoen, Minato-ku, Tokyo 105-8512, Japan

ARTICLE INFO

Article history:
Received 6 December 2015
Received in revised form 12 February 2016
Accepted 7 March 2016
Available online 19 March 2016

Keywords:
Lipase
Transesterification
Regioselective reaction
Polyphenol
Glycosylated form

ABSTRACT

Acacetin and resveratrol 3,5-di-*O*-β-glucopyranoside were synthesized from naturally abundant naringin and piceid in 65% and 62% overall yield, respectively. The key steps were the regioselective deacetylation of the peracetates of the glycosylated forms with *Candida antarctica* lipase B (Novozym 435) and *Burkholderia cepacia* lipase (Amano PS-IM). Deacetylation occurred exclusively at the least hindered position of the aromatic moieties and all acetyl groups in the sugar side chain remained intact. This excellent selectivity enabled regiospecific transformation of the liberated phenolic hydroxy groups, resulting in efficient synthesis of the target molecules.

1. Introduction

Acacetin (5,7-dihydroxy-4′-methoxyflavone, **1a**) has been isolated from a variety of plants [1–3] and is attracting interest due to its ability to stimulate melanogenesis [4] and thus potentially help prevent gray hair. Resveratrol 3,5-di-*O*-β-glucopyranoside (**2a**) was first isolated from cultured cells of *Vitis vinifera* [5]. The aglycone of **2a**, resveratrol (**2b**), is a well-known natural antioxidant, but the diglucoside **2a** is more water-soluble [6] than **2b**. The current supply of **1a** and **2a** is insufficient for industrial applications such as in cosmetics or food supplements, making the development of a synthetic route towards large quantities of both **1a** and **2a** from readily available natural resources are very important. Herein we report the chemo-enzymatic syntheses of **1a** and **2a** from the glycosylated naturally abundant materials, naringin (**3a**) and piceid (**2c**), respectively (Fig. 1). Naringin is produced as a by-product in the citrus beverage industry and piceid is isolated from *Fallopia japonica* [7], a weed that grows vigorously in Japan.

2. Experimental

Melting points were measured by Yanaco MP-J3 and uncorrected. IR spectra were measured on a Jeol FT-IR/ATR SPX60 spectrometer. ^{1}H NMR spectra were measured in $CDCl_3$ at 500 MHz on a VARIAN 500-MR spectrometer, unless otherwise stated. ^{13}C NMR spectra were measured in $CDCl_3$ at 125 MHz on a VARIAN 500-MR spectrometer, unless otherwise stated. Optical rotation values were recorded on a Jasco P-1010 polarimeter. High resolution mass spectra (HRMS) were recorded on JEOL JMS-T100LP AccuTOF. Silica gel 60 N (spherical, neutral, 63–210 μm, 37565-84) from Kanto Chemical Co. was used for column chromatography.

2.1. 4′,5-Diacetoxy-7-[hexa-O-acetyl-(2-O-α-L-rhamnopyranosyl-β-D-glucopyranosyl)oxy]flavone (*1c*)

Rhoifolin (**1b**) was prepared from naringin (**3a**, hydrate form, 91% purity) by a slight modification of the reported procedure [8]. To a mixture of **3a** (6.38 g, 10.0 mmol) in pyridine (30 mL) was added iodine (2.78 g, 11.0 mmol), and the mixture was stirred and heated for 6 h at 95 °C. Crude **1b** involved in the reaction mixture was directly acetylated. After cooling, acetic anhydride (20 mL) was added and the mixture was further stirred and heated for 6 h at 95 °C. After cooling, phosphate buffer solution (pH 7.2, 1.0 M, 20 mL) was added. The mixture was concentrated in vacuo. The

* Corresponding author.
E-mail address: sugai-tk@pha.keio.ac.jp (T. Sugai).

http://dx.doi.org/10.1016/j.molcatb.2016.03.001
1381-1177/

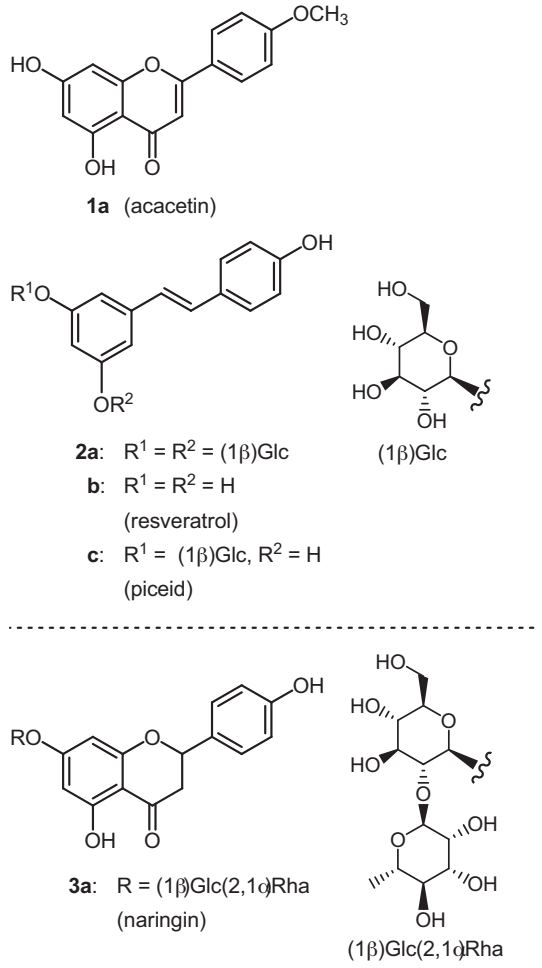


Fig. 1. Acacetin (**1a**), resveratrol 3,5-di-*O*-β-glucopyranoside (**2a**), their starting materials **3a** and **2c**, and related compound in the present study.

residue was diluted with water and concentrated again. The residue was diluted with water containing sodium thiosulfate (8.0 g), and the resulted mixture was sonicated. The precipitates were collected by filtration, and washed with water and dried. The residue (9.55 g) was purified by silica gel column chromatography (150 g, $CHCl_3$/ethyl acetate = 1:1) to afford the desired fraction, which was solidified upon standing at room temperature. Soluble materials were removed from this residue, by washing with diethyl ether to give **1c** (7.25 g) as slightly yellow amorphous solid. These washings were concentrated in vacuo to give brown oily residue. This was further chromatographed to give **1c** (836 mg). The combined yield was 88%. IR: 1749, 1641, 1617, 1433, 1367, 1225, 1043, 912, 842 cm^{-1}; ^1H NMR: δ 1.21 (d, *J* = 5.7 Hz, 3H, rhamnose-H-6), 1.96, 1.98, 2.04, 2.05, 2.12, 2.15 (each s, total 18H, sugar-OAc), 2.34, 2.43 (each s, total 6H, 4′-OAc, 5-OAc), 3.96–4.04 (total 3H, glucose-H-2, glucose-H-5, rhamnose-H-5), 4.17 (dd, *J* = 2.2, 12.5 Hz, 1H, glucose-H-6a), 4.26 (dd, *J* = 5.9, 12.5 Hz, 1H, glucose-H-6b), 5.02–5.08 (total 4H, glucose-H-4, rhamnose-H-1, rhamnose-H-2, rhamnose-H-4), 5.17 (dd, *J* = 2.7, 10.0 Hz, 1H, rhamnose-H-3), 5.23 (d, *J* = 7.6 Hz, 1H, glucose-H-1), 5.36 (dd, *J* = 9.4, 9.4 Hz, seemingly t, 1H, glucose-H-3), 6.61 (s, 1H, H-3), 6.70 (d, *J* = 2.5 Hz, 1H, H-8), 7.02 (d, *J* = 2.5 Hz, 1H, H-6), 7.25 (d, *J* = 8.8 Hz, 2H, H-3′, H-5′), 7.87 (d, *J* = 8.8 Hz, 2H, H-2′, H-6′); ^{13}C NMR: δ 17.67, 20.74, 20.79, 20.84, 21.02, 21.23, 21.28, 62.08, 67.18, 68.41, 68.48, 70.14, 70.88, 72.47, 74.03, 77.04, 98.42, 98.64, 102.58, 108.62, 109.67, 112.93, 122.54, 127.68, 128.76, 150.90, 153.44, 158.56, 160.04, 161.83, 169.05, 169.53, 169.76, 169.90, 170.12, 170.21, 170.29, 170.57, 176.42; the signals 20.74, 20.79, 122.54 and 127.68 included two carbons. HRMS (ESI+): calculated for [M ($C_{43}H_{46}O_{22}$) + Na]$^+$, 937.2378; found, 937.2403.

*2.2. 5-Acetoxy-7-[hexa-*O*-acetyl-(2-*O*-α-*L*-rhamnopyranosyl-β-*D*-glucopyranosyl)oxy]-4′-hydroxyflavone (**1d**)*

To a solution of **1c** (1.83 g, 2.0 mmol) in a mixture of 2-propanol (10 mL) and tetrahydrofuran (THF, 20 mL), which was pre-dried over anhydrous Na_2SO_4 at room temperature overnight, was added an immobilized form of *Candida antarctica* lipase B (Novozymes, Novozym 435, 91 mg). The mixture was stirred for 3 days at 22 °C. The mixture was filtered to remove insoluble materials with a pad of Celite. The precipitates were washed with ethyl acetate. The combined filtrate and washings were concentrated in vacuo. The residue was purified by silica gel column chromatography (40 g, hexane/ethyl acetate = 1:1) to afford **1d** (1.71 g, 98%) as white amorphous solid. IR: 1747, 1633, 1606, 1437, 1369, 1222, 1041, 839 cm^{-1}; ^1H NMR: δ 1.23 (d, *J* = 6.3 Hz, 3H, rhamnose-H-6), 1.97, 1.98, 2.04, 2.05, 2.12, 2.15 (each s, total 18H, sugar-OAc), 2.43 (s, 3H, 5-OAc), 3.97–4.05 (total 3H, glucose-H-2, glucose-H-5, rhamnose-H-5), 4.18 (dd, *J* = 1.9, 12.2 Hz, 1H, glucose-H-6a), 4.27 (dd, *J* = 5.7, 12.2 Hz, 1H, glucose-H-6b), 5.04–5.09 (total 4H, glucose-H-4, rhamnose-H-1, rhamnose-H-2, rhamnose-H-4), 5.19 (dd, *J* = 2.3, 10.8 Hz, 1H, rhamnose-H-3), 5.24 (d, *J* = 7.3 Hz, 1H, glucose-H-1), 5.38 (dd, *J* = 9.5, 9.5 Hz, seemingly t, 1H, glucose-H-3), 6.57 (s, 1H, H-3), 6.70 (d, *J* = 2.4 Hz, 1H, H-8), 6.87 (d, *J* = 8.8 Hz, 2H, H-3′, H-5′), 6.99 (d, *J* = 2.4 Hz, 1H, H-6), 7.67 (d, *J* = 8.8 Hz, 2H, H-2′, H-6′); ^{13}C NMR: δ 17.68, 20.71, 20.75, 20.80, 20.83, 21.01, 21.31, 62.07, 67.20, 68.36, 68.53, 70.14, 70.91, 72.45, 74.07, 76.96, 98.37, 98.63, 102.46, 106.12, 109.86, 112.43, 116.35, 122.11, 128.34, 150.59, 158.51, 160.13, 160.65, 163.53, 169.81, 170.07, 170.20, 170.23, 170.27, 170.39, 170.71, 176.84; the signals 20.75, 116.35 and 128.34 included two carbons. HRMS (ESI+): calculated for [M ($C_{41}H_{44}O_{21}$) + Na]$^+$, 895.2273; found, 895.2290.

*2.3. 5-Acetoxy-7-[hexa-*O*-acetyl-(2-*O*-α-*L*-rhamnopyranosyl-β-*D*-glucopyranosyl)oxy]-4′-methoxyflavone (**1e**)*

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), 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 **1d** (872 mg, 1.00 mmol) in CH_2Cl_2 (9 mL) placed in the receiver flask at 0 °C. The mixture was left to stand overnight at room temperature, and then concentrated in vacuo. The residue was purified by silica gel column chromatography (10 g, hexane/ethyl acetate = 1:1) to afford **1e** (869 mg, 98%) as white amorphous solid. IR: 1747, 1635, 1610, 1369, 1223, 1035, 836 cm^{-1}; ^1H NMR: 1.21 (d, *J* = 6.1 Hz, 3H, rhamnose-H-6), 1.96, 1.98, 2.04, 2.12, 2.15 (each s, total 18H, sugar-OAc), 2.43 (s, 3H, 5-OAc), 3.88 (s, 3H, −OMe), 3.96 (ddd, *J* = 2.2, 6.1, 9.8 Hz, 1H, glucose-H-5), 4.00–4.04 (total 2H, glucose-H-2, rhamnose-H-5), 4.17 (dd, *J* = 2.2, 12.2 Hz, 1H, glucose-H-6a), 4.26 (dd, *J* = 6.0, 12.2 Hz, 1H, glucose-H-6b), 5.03–5.08 (total 4H, glucose-H-4, rhamnose-H-1, rhamnose-H-3, rhamnose-H-4), 5.17 (dd, *J* = 2.9, 10.0 Hz, 1H, rhamnose-H-2), 5.23 (d, *J* = 7.6 Hz, 1H, glucose-H-1), 5.36 (dd, *J* = 9.4, 9.4 Hz, seemingly t, 1H, glucose-H-3), 6.61 (s, 1H, H-3), 6.69 (d, *J* = 2.4 Hz, 1H, H-8), 7.01 (d, *J* = 9.0 Hz, 2H, H-3′, H-5′), 7.02 (d, *J* = 2.4 Hz, 1H, H-6), 7.81 (d, *J* = 9.0 Hz, 2H, H-2′, H-6′); ^{13}C NMR: δ 17.65, 20.69, 20.71, 20.76, 20.82, 20.99, 21.14, 20.22, 55.63, 62.09, 67.14, 68.42, 68.46, 70.11, 70.86, 72.43, 74.01, 77.02, 98.40, 98.65, 102.53, 107.04, 109.49, 112.80, 114.61, 123.37, 128.05, 150.83, 158.50, 159.88, 162.67, 169.46, 169.72, 169.83, 170.10, 170.16, 170.23, 170.54, 176.41; the signals 114.61, 128.05

and 150.83 included two carbons. HRMS (ESI+): calculated for [M ($C_{42}H_{46}O_{21}$) + Na]$^+$, 909.2429; found, 909.2432.

2.4. 5-Hydroxy-4′-methoxy-7-[(2-O-α-L-rhamnopyranosyl-β-D-glucopyranosyl)oxy]flavone (**1f**)

To a solution of **1e** (301 mg, 0.339 mmol) in methanol (3.39 mL) was added a solution of sodium methoxide in methanol (5.0 M, 13 μL, 0.2 equiv.). The mixture was stirred for 1 h at room temperature, then the reaction was quenched by adding acetic acid. The resulting precipitates were collected by filtration, and washed with water. After drying, **1f** (177 mg, 88%) was obtained as yellow solid. ^{1}H NMR (CD_3OD): δ 1.33 (d, J = 6.1 Hz, 3H, rhamnose-H-6), 3.41 (dd, J = 9.4, 9.4 Hz, seemingly t, 2H, rhamnose-H-4, glucose-H-4), 3.53–3.56 (m, 1H, glucose-H-5), 3.59–3.73 (total 4H, glucose-H-2, glucose-H-3, glucose-H-6a, rhamnose-H-3), 3.89–3.94 (total 6H, glucose-H-6b, rhamnose-H-2, rhamnose-H-5, −OMe), 5.20 (d, J = 7.6 Hz, 1H, glucose-H-1), 5.28 (brs, 1H, rhamnose-H-1), 6.46 (d, J = 2.2 Hz, 1H, H-8), 6.70 (s, 1H, H-3), 6.79 (d, J = 2.2 Hz, 1H, H-6), 7.08 (d, J = 8.8 Hz, 2H, H-3′, H-5′), 7.97 (d, J = 8.8 Hz, 2H, H-2′, H-6′). This was employed for the next step without further purification.

2.5. 5,7-Dihydroxy-4′-methoxyflavone (acacetin, **1a**)

To a solution of **1f** (101 mg, 0.170 mmol) in ethanol (1.70 mL) was added conc. sulfuric acid (170 μL). The mixture was heated under reflux for 2 h, then the reaction was cooled at 0 °C. The resulting precipitates were recovered by filtration and washed with water to afford **1a** (42 mg, 87%) as yellow solid. This was recrystallized from methanol to give an analytical sample of **1a** as yellow needles, mp 256.0–257.8 °C [lit. [3] mp 254.6–257.5 °C]; ^{1}H NMR (DMSO-d_6): δ 3.84 (s, 3H, −OMe), 6.18 (d, J = 2.2 Hz, 1H, H-6), 6.49 (d, J = 2.0 Hz, 1H, H-8), 6.86 (s, 1H, H-3), 7.10 (d, J = 9.0 Hz, 2H, H-3′, H-5′), 8.02 (d, J = 9.0 Hz, 2H, H-2′, H-6′), 10.86 (s, 1H, OH-7), 12.91 (s, 1H, OH-5); ^{13}C NMR (DMSO-d_6): δ 55.58, 94.06, 98.91, 103.56, 103.78, 114.61, 122.84, 128.36, 157.36, 161.46, 162.33, 163.33, 164.24, 181.81; the signals 114.61 and 128.36 included two carbons. The identity of **1a** was further confirmed after derivation to the corresponding diacetate (**1g**) by an acetylation in a conventional manner. This was recrystallized from methanol to give an analytical sample of **1g** as colorless needles, mp 200.1–201.0 °C [lit. [2] mp 199 °C]; ^{1}H NMR: δ 2.35, 2.44 (each s, 6H, 5-OAc, 7-OAc), 3.89 (s, 3H, −OMe), 6.61 (s, 1H, H-3), 6.83 (d, J = 2.2 Hz, 1H, H-6), 7.01 (d, J = 8.8 Hz, 2H, H-3′, H-5′), 7.34 (d, J = 2.2 Hz, 1H, H-8), 7.82 (d, J = 8.8 Hz, 2H, H-2′, H-6′). Its ^{1}H NMR spectrum was in good accordance to that reported previously [3].

2.6. 4′,5-Diacetoxy-3-(tetra-O-acetyl-β-D-glucopyranosyl)oxystilbene (**2d**)

To a solution of piceid (**2c**, 2.00 g, 5.13 mmol) in pyridine (10 mL) were added Ac_2O (10 mL) and 4-*N*,*N*-dimethylaminopyridine (62.5 mg, 0.51 mmol, 0.1 equiv.) under argon atmosphere. The mixture was stirred for 2 h at room temperature, then the reaction was quenched by adding ice. Firstly separated gummy residue gradually solidified by grinding well with water in a mortar. The resulting precipitates were filtered and washed with water and then was dried in vacuo to afford **2d** (3.29 g, quant.) as white solid. ^{1}H NMR: δ 2.04, 2.06, 2.08 (each s, total 12H, sugar-OAc), 2.31 (s, 6H, 5-OAc, 4′-OAc), 3.91 (ddd, J = 2.0, 5.4, 8.8 Hz, 1H, glucose-H-5), 4.18 (dd, J = 2.0, 12.2 Hz, 1H, glucose-H-6a), 4.28 (dd, J = 5.4, 12.2 Hz, 1H, glucose-H-6b), 5.12 (d, J = 7.2 Hz, 1H, glucose-H-1), 5.16 (dd, J = 9.3, 9.3 Hz, seemingly t, 1H, glucose-H-4), 5.26–5.33 (total 2H, glucose-H-2, glucose-H-3), 6.65 (s, 1H, H-4), 6.95 (d, J = 16.4 Hz, 1H, CH=CH), 6.97 (s, 2H, H-2, H-6), 7.01 (d, J = 16.4 Hz, 1H, CH=CH), 7.09 (d, J = 8.5 Hz, 2H, H-3′, H-5′), 7.49 (d, J = 8.5 Hz, 2H, H-2′, H-6′); ^{13}C NMR: δ 20.76, 20.82, 21.29, 62.18, 68.44, 71.21, 72.22, 72.79, 98.94, 109.69, 112.80, 114.63, 121.89, 127.54, 127.76, 129.65, 134.55, 139.84, 150.56, 151.78, 157.67, 169.33, 169.48, 169.55, 170.35, 170.72; the signals 20.76, 20.82, 21.29, 121.89, 127.76 and 169.55 included two carbons. HRMS (ESI+): calculated for [M ($C_{32}H_{34}O_{14}$) + Na]$^+$, 655.1846; found, 665.1855. This was employed for the next step without further purification.

2.7. 5-Acetoxy-3-(tetra-O-acetyl-β-D-glucopyranosyl)oxy-4′-hydroxystilbene (**2e**)

To a solution of **2d** (3.29 g, 5.13 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 *Burkholderia cepacia* lipase (Amano PS-IM, 250 mg). The mixture was stirred for 12 h at 22 °C. The mixture was filtered to remove insoluble materials with a pad of Celite. The precipitates were washed with ethyl acetate. The combined filtrate and washings were concentrated in vacuo to afford **2e** (3.07 g, quant.) as white amorphous solid. ^{1}H NMR: δ 2.04, 2.05, 2.08 (each s, total 12H, sugar-OAc), 2.31 (s, 3H, 5-OAc), 3.90 (ddd, J = 2.0, 5.4, 8.8 Hz, 1H, glucose-H-5), 4.18 (dd, J = 2.0, 12.2 Hz, 1H, glucose-H-6a), 4.28 (dd, J = 5.4, 12.2 Hz, 1H, glucose-H-6b), 5.10 (d, J = 7.1 Hz, 1H, glucose-H-1), 5.16 (dd, J = 9.3, 9.3 Hz, seemingly t, 1H, glucose-H-4), 5.26–5.33 (total 2H, glucose-H-2, glucose-H-3), 6.61 (s, 1H, H-4), 6.81 (d, J = 16.4 Hz, 1H, CH=CH), 6.82 (d, J = 8.6 Hz, 2H, H-3′, H-5′), 6.93 (s, 2H, H-2, H-6), 6.98 (d, J = 16.4 Hz, 1H, CH=CH), 7.34 (d, J = 8.6 Hz, 2H, H-2′, H-6′); ^{13}C NMR: δ 20.69, 20.73, 20.78, 21.26, 62.18, 68.41, 71.23, 72.11, 72.78, 98.88, 109.04, 112.52, 114.31, 115.84, 124.82, 128.24, 129.20, 130.28, 140.42, 151.64, 156.38, 157.58, 169.69, 169.72, 170.48, 170.94; the signals 20.73,115.84, 128.24 and 169.69 included two carbons. HRMS (ESI+): calculated for [M ($C_{30}H_{32}O_{13}$) + Na]$^+$, 623.1741; found, 623.1748. This was employed for the next step without further purification.

2.8. 5-Acetoxy-3-(tetra-O-acetyl-β-D-glucopyranosyl)oxy-4′-[tert-butyl(dimethyl)silyl]oxystilbene (**2f**)

To a solution of **2e** (3.00 g, 5.00 mmol) and imidazole (680 mg, 10.0 mmol, 2.0 equiv.) in anhydrous *N*,*N*-dimethylformamide (25 mL) *tert*-butyldimethylsilyl (TBS) chloride (1.50 g, 10.0 mmol, 2.0 equiv.) was added at 0 °C, then the mixture was warmed to room temperature. After stirring for 4 h, the reaction was quenched by adding cold water. The organic materials were extracted with ethyl acetate twice. The combined extracts were washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (30 g, hexane/ethyl acetate = 2:1) to afford **2f** (3.54 g, 99%) as slightly yellow amorphous solid. ^{1}H NMR: δ 0.21 (s, 6H, Me), 0.99 (s, 9H, *tert*-Bu), 2.04, 2.05, 2.06, 2.08 (each s, total 12H, sugar-OAc), 2.31 (s, 3H, 5-OAc), 3.90 (ddd, J = 2.2, 5.6, 9.1 Hz, 1H, glucose-H-5), 4.18 (dd, J = 2.2, 12.2 Hz, 1H, glucose-H-6a), 4.28 (dd, J = 5.6, 12.2 Hz, 1H, glucose-H-6b), 5.12 (d, J = 7.4 Hz, 1H, glucose-H-1), 5.16 (dd, J = 9.6, 9.6 Hz, seemingly t, 1H, glucose-H-4), 5.26–5.32 (total 2H, glucose-H-2, glucose-H-3), 6.62 (s, 1H, H-4), 6.83 (d, J = 8.5 Hz, 2H, H-3′, H-5′), 6.86 (d, J = 16.4 Hz, 1H, CH=CH), 6.94, 6.96 (each s, total 2H, H-2, H-6), 7.01 (d, J = 16.4 Hz, 1H, CH=CH), 7.36 (d, J = 8.5 Hz, 2H, H-2′, H-6′); ^{13}C NMR: δ −4.29, 18.34, 20.70, 20.72, 20.78, 21.15, 21.24, 25.77, 62.17, 68.42, 71.18, 72.17, 72.78, 98.93, 109.17, 112.44, 114.35, 120.52, 125.30, 127.99, 130.05, 130.30, 140.35, 151.72, 156.02, 157.62, 169.30, 169.44, 169.52, 170.30, 170.69; the signals −4.29, 120.52 and 127.99 included two carbons. the signal 25.77 included

three carbons. HRMS (ESI+): calculated for [M ($C_{36}H_{46}O_{13}Si$) + Na]$^+$, 737.2605; found, 737.2622.

2.9. 3-(Tetra-O-acetyl-β-D-glucopyranosyl)oxy-4′-[tert-butyl(dimethyl)silyl]oxy-5-hydroxystilbene (**2g**)

To a solution of **2f** (3.20 g, 4.48 mmol) in methanol (8.9 mL) sodium perborate (367 mg, 4.48 mmol, 1.0 equiv.) was added at 0 °C, then the mixture was warmed to room temperature. The mixture was further stirred for 3.5 h at room temperature. Insoluble materials were removed by filtration with a pad of Celite. The precipitates were washed with ethyl acetate, and the combined filtrate and washings were concentrated in vacuo. The residue was purified by silica gel column chromatography (90 g, hexane/ethyl acetate = 2:1) to afford **2g** (2.41 g, 80%) as slightly yellow amorphous solid. ^{1}H NMR: δ 0.21 (s, 6H, Me), 0.99 (s, 9H, *tert*-Bu), 2.04, 2.05, 2.06, 2.07 (each s, total 12H, sugar-OAc), 3.89 (ddd, *J* = 2.4, 5.6, 9.0 Hz, 1H, glucose-H-5), 4.19 (dd, *J* = 2.4, 12.2 Hz, 1H, glucose-H-6a), 4.29 (dd, *J* = 5.6, 12.2 Hz, 1H, glucose-H-6b), 5.09 (d, *J* = 7.3 Hz, 1H, glucose-H-1), 5.18 (dd, *J* = 9.1, 9.1 Hz, seemingly t, 1H, glucose-H-4), 5.26–5.32 (total 2H, glucose-H-2, glucose-H-3), 6.41 (s, 1H, H-4), 6.67, 6.68 (each s, total 2H, H-2, H-6), 6.83 (d, *J* = 16.4 Hz, 1H, CH=CH), 6.83 (d, *J* = 8.5 Hz, 2H, H-3′, H-5′), 6.99 (d, *J* = 16.4 Hz, 1H, CH=CH), 7.37 (d, *J* = 8.5 Hz, 2H, H-2′, H-6′); ^{13}C NMR: δ −4.39, 18.24, 20.59, 20.63, 20.67, 25.65, 62.05, 68.38, 71.15, 71.97, 72.73, 98.89, 103.37, 107.34, 108.17, 120.39, 125.80, 127.80, 129.51, 130.14, 140.26, 155.75, 156.97, 158.11, 169.52, 170.38, 170.91; the signals −4.39, 20.67, 120.39, 127.80 and 169.52 included two carbons; the signal 25.65 included three carbons. HRMS (ESI+): calculated for [M ($C_{34}H_{44}O_{12}Si$) + Na]$^+$, 695.2500; found, 695.2509.

2.10. 3,5-Bis-(tetra-O-acetyl-β-D-glucopyranosyl)oxy-4′-[tert-butyl(dimethyl)silyl]oxystilbene (**2h**)

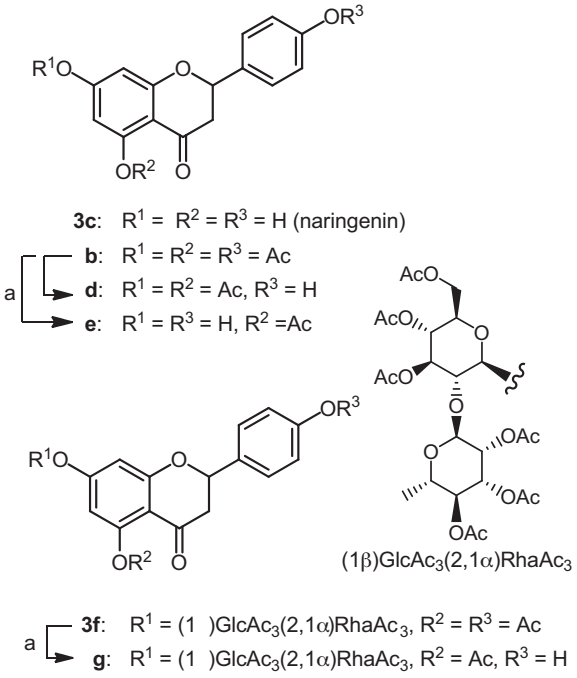
A suspension of the tetra-*O*-acetyl-D-glucopyranosyl trichloroacetimidate (2.00 g, 4.06 mmol, 2.0 equiv.), which was prepared from penta-*O*-acetyl-β-D-glucose by the action of *C. antarctica* lipase B for the revioselective hydrolysis of anomeric acetate [9] and the subsequent trichloroimidation, the acceptor **2g** (1.26 g, 2.03 mmol) and molecular sieves 4A (2.00 g) in anhydrous CH_2Cl_2 (40 mL) was vigorously stirred at room temperature. After 30 min, the suspension was cooled to 0 °C and trimethylsilyl trifluoromethylsulfonate (73 μL, 0.20 mmol, 0.1 equiv.) was slowly added, then the mixture was warmed to room temperature. The mixture was further stirred for 1 h at room temperature. The reaction was quenched by adding trimethylamine. The mixture was filtered with a pad of Celite to remove insoluble materials. The precipitates were washed with ethyl acetate, and the combined filtrate and washings were concentrated. The residue was purified by column chromatography (100 g, hexane/ethyl acetate = 2:1) to afford **2h** (1.52 g, 81%) as slightly yellow amorphous solid. $[\alpha]_D^{21}$ −4.61 (*c* 1.0, $CHCl_3$); ^{1}H NMR: δ 0.21 (s, 6H, Me), 0.99 (s, 9H, *tert*-Bu), 2.04, 2.05, 2.06, 2.07 (each s, total 24H, sugar-OAc), 3.92 (ddd, *J* = 2.4, 5.6, 9.1 Hz, 2H, glucose-H-5), 4.18 (dd, *J* = 2.4, 12.5 Hz, 2H, glucose-H-6a), 4.28 (dd, *J* = 5.6, 12.5 Hz, 2H, glucose-H-6b), 5.15 (d, *J* = 7.8 Hz, 2H, glucose-H-1), 5.15 (dd, *J* = 9.8, 9.3 Hz, seemingly t, 2H, glucose-H-4), 5.25 (dd, *J* = 7.8, 9.7 Hz, 2H, glucose-H-2), 5.32 (dd, *J* = 9.7, 9.8 Hz, 2H, glucose-H-3), 6.50 (s, 1H, H-4), 6.81, 6.82 (each s, total 2H, H-2, H-6), 6.83 (d, *J* = 8.5 Hz, 2H, H-3′, H-5′), 6.84 (d, *J* = 16.4 Hz, 1H, CH=CH), 7.00 (d, *J* = 16.4 Hz, 1H, CH=CH), 7.37 (d, *J* = 8.5 Hz, 2H, H-2′, H-6′); ^{13}C NMR: δ −4.24, 18.39, 20.75, 20.81, 20.84, 25.81, 62.20, 68.52, 71.26, 72.21, 72.84, 98.65, 104.94, 109.27, 120.59, 125.72, 127.99, 130.05, 140.47, 156.09, 157.92, 169.45, 169.58, 170.37, 170.81; the signals −4.24, 20.81, 20.84, 62.20, 68.52, 71.26, 72.21, 72.84, 98.65, 109.27, 120.59, 127.99, 157.92, 169.45, 169.58, 170.37 and 170.81 included two carbons. The signal 25.81 included three carbons. The signal 20.75 included four carbons. HRMS (ESI+): calculated for [M ($C_{48}H_{62}O_{21}Si$) + Na]$^+$, 1025.3451; found, 1025.3428.

2.11. 3,5-Bis-(β-D-glucopyranosyl)oxy-4′-hydroxystilbene (**2a**)

To a solution of **2h** (1.20 g, 1.20 mmol) in a mixture of methanol (8 mL) and THF (4 mL), aq. NaOH (2.0 M, 1.2 mL) was added at 0 °C. The resulting solution was stirred for 5 h at 0 °C. Then it was diluted with water (4 mL), neutralized with Dowex 50W-X4 (H^+) resin and the mixture was filtered with a pad of Celite to remove insoluble materials. The precipitates were washed several times with water. The combined filtrate and washings were concentrated in vacuo. The residue was purified by column chromatography (30 g, $CHCl_3$/methanol = 2:1) to afford **2a** (642 mg, 97%) as slightly yellow amorphous solid. $[\alpha]_D^{22}$ −64.7 (*c* 0.5, methanol); ^{1}H NMR (CD_3OD): δ 3.30–3.60 (m, 8H, glucose-H-2, glucose-H-3, glucose-H-4, glucose-H-5), 3.70 (dd, *J* = 6.3, 12.0 Hz, 2H, glucose-H-6a), 3.95 (dd, *J* = 2.2, 12.0 Hz, 2H, glucose-H-6b), 4.94 (d, *J* = 7.3 Hz, 2H, glucose-H-1), 6.75 (s, 1H, H-4), 6.77 (, *J* = 8.5 Hz, 2H, H-3′, H-5′), 6.89 (d, *J* = 16.4 Hz, 1H, CH=CH), 6.94 (d, *J* = 2.2 Hz, 2H, H-2, H-6), 7.08 (d, *J* = 16.4 Hz, 1H, CH=CH), 7.38 (d, *J* = 8.5 Hz, 2H, H-2′, H-6′); ^{13}C NMR (CD_3OD): δ 62.72, 71.63, 74.91, 78.00, 78.25, 102.23, 104.80, 109.68, 116.48, 126.24, 129.02, 130.19, 130.54, 141.43, 158.59, 160.18; the signals 62.72, 71.63, 74.91, 78.00, 78.25, 102.23, 109.68, 116.48, 129.02 and 160.18 included two carbons. Its ^{1}H and ^{13}C NMR spectra were identical with those reported previously [5]. HRMS (ESI+): calculated for [M ($C_{26}H_{32}O_{13}Si$) + Na]$^+$, 575.1741; found, 575.1750.

2.12. Docking simulations of **1c** and **2d** with C. antarctica lipase B

Relaxation steps of a crystal structure and docking simulations were carried out by using minimization protocol and CDOCKER [10] of the program package Discovery Studio 4.5 (Accerlys, Inc.). All simulations were performed under CHARMm force field [11]. Before applying a crystal structure of *C. antarctica* lipase B (PDB code 1LBS) [12] for a docking simulation, the structure was prepared according to the following methods. The PDB file 1LBS contained the six chains bearing ethyl hexylphosphonate on Ser105, *N*-acetylglucosamine on Asn72 and 92, and adsorbed water molecules. Only one chain and adsorbed water molecules were left intact and others were erased. In the remaining chain, above small molecules except water were removed. Protons were added to the structure and partial charges were assigned under CHARMm force field. The resulting structure was solvated with explicit periodic boundary solvation model. Before applying the structure for docking simulations, the structure was relaxed by the step-wise minimization method under the following conditions [13–16]: (i) In order to eliminate initial strains of the structure, all protein heavy atoms were fixed and all protons and water molecules were allowed to move. (ii) A fixed atom constraint and a harmonic restraint were applied for the main chain and the side chain, respectively and other atoms were allowed to move. (iii) A harmonic restraint was applied for the main chain and other atoms were allowed to move. (iv) The whole system was minimized without any constraint or restraint. Minimizations were carried out with steepest descent algorithm (1000 steps) for a first step and conjugate gradient algorithm for second, third, and forth steps. In second, third, and forth steps, simulations were continued until the root mean square deviation (RMSD) gradient fell down 0.01, 0.001, and 0.0001 kcal mol^{-1} A^{-1}, respectively. The catalytic cavity was defined as a binding site (sphere radius: 12 Å). Possible docking poses of **1c** and **2d** in a binding site were simulated by using CDOCKER. The resulting docking poses were evaluated by using



Scheme 1. Lipase-catalyzed regioselective transesterification on peracetylated naringenin (**3b**) and peracetylated naringin (**3f**). Reagents and conditions: (a) *Candida antarctica* lipase B (Novozymes, Novozym 435), cyclopentanol, cyclopentyl methyl ether.

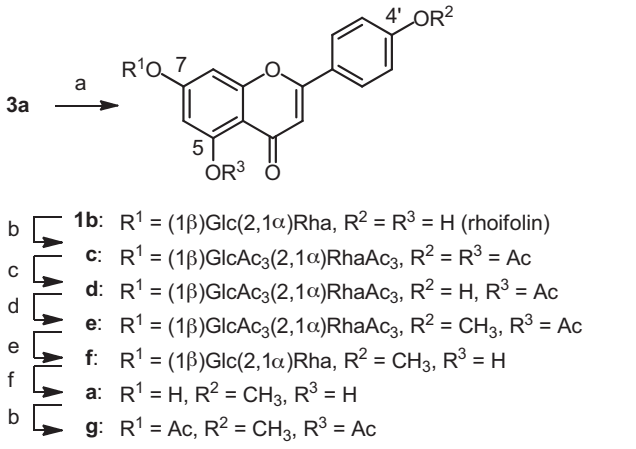
six scoring functions (Ligscore1, Ligscore2, PLP1, PLP2, PMF, and Jain) and ranked based on consensus scoring algorithm [13,14,17]. In the case of **2d**, docking poses containing *cis*-configuration aglycon were excluded by visual judgement. In both cases of **1c** and **2d**, the complexes with the top five consensus score were used for further minimization of resulting complexes. Three step minimizations were carried out under following conditions [13,14]: (i) A fixed atom constraint was applied for the main chains and a harmonic restraint was applied for the side chains and substrates. (ii) A fixed atom constraint was applied for the main chains and a harmonic restraint was applied for the side chains. (iii) A fixed atom constraint was applied for the main chains. Resulting complexes were used for the discussions.

3. Results and discussion

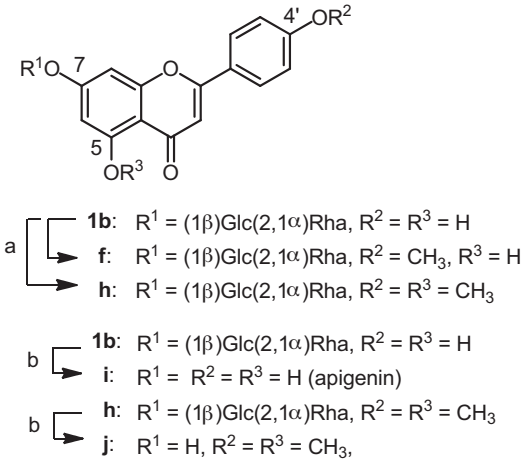
We previously reported treating naringenin triacetate (**3b**) using *C. antarctica* lipase B-catalyzed transesterification conditions [18] and found that cleavage occurred at the C-4′ and C-7 acetates, providing a mixture of **3d** and **3e** (Scheme 1), with **3e** as the major component. In contrast, under the same transesterification conditions, cleavage at C-7 of naringin octaacetate (**3f**), the glycosylated form of **3c**, did not occur: the reaction exclusively occurred on the least hindered phenolic acetate at C-4′ and the sole product was heptaacetate **3g**. Moreover, no transformation was observed of any acetate in the disaccharide side chain [–(1β)GlcAc$_3$(2,1α)RhaAc$_3$], including the primary acetate. This is in contrast to reports of lipase-catalyzed regioselective acylation at the primary position in sugars of glycosylated flavonoids [19,20], rather than on phenolic hydroxy groups.

The introduction of a sugar side chain in **3f** played a crucial role in protecting the C-7 hydroxy group in the aglycone. Methylation should occur only on the free C-4′ hydroxy group, thus facilitating the synthesis of **1a**.

The synthesis of acacetin (**1a**) is summarized in Scheme 2. The first step was the dehydrogenation of **3a** to rhoifolin (**1b**) [8].



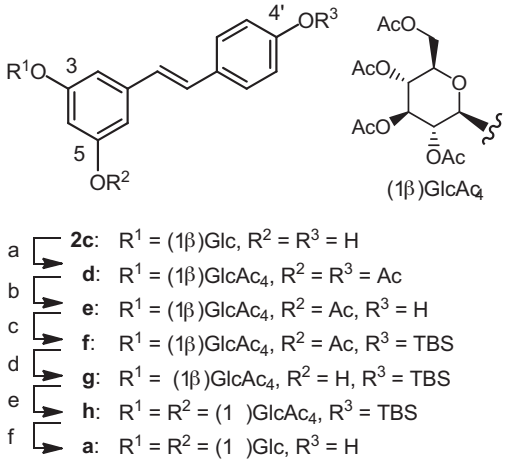
Scheme 2. Chemo-enzymatic synthesis of acacetin (**1a**) from naringin (**3a**). Reagents and conditions: (a) I_2, pyridine, 95 °C; (b) acetic anhydride, pyridine; (c) *C. antarctica* lipase B, 2-propanol, tetrahydrofuran (THF); (d) diazomethane, diethyl ether, CH_2Cl_2; (e) sodium methoxide, methanol, and (f) conc. sulfuric acid, ethanol, reflux.



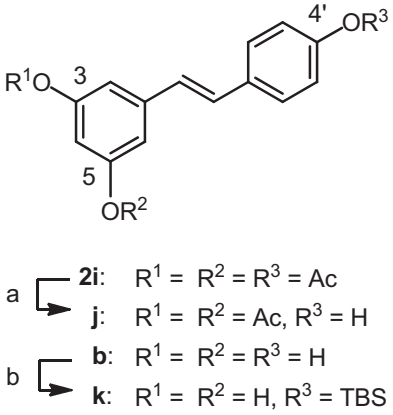
Scheme 3. Key steps in so-far reported chemical synthesis of acacetin (**1a**) by way of rhoifolin (**1b**). Reagents and conditions: (a) aq. KOH, dimethyl sulfate and (b) aq. sulfuric acid, reflux.

The introduction of a conjugated double bond at this stage in the starting material should (1) enhance the electrophilicity of the C-4′ acetyl group for lipase-catalyzed transesterification and (2) increase the acidity of the C-4′ hydroxy group, facilitating subsequent methylation. Acetylation of **1b** resulted in *C. antarctica* lipase-catalyzed transesterification occurring exclusively at the C-4′ acetyl group of the peracetate in **1c** to furnish **1d** in a manner similar to that of **3f** in Scheme 1. In contrast, the use of *B. cepacia* lipase provided lower regioselectivity. Although **1d** was still obtained as the major product and byproducts were also formed. Methylation of the liberated C-4′ hydroxy group in **1d** with diazomethane proceeded smoothly to give **1e**. Finally, deprotection of all acetyl groups and subsequent cleavage of the glycosylic bonds provided **1a** in 65% overall yield from naringin **3a**. The identity of **1a** was confirmed by comparison of melting point [2] and spectral data [3] after conversion to the corresponding diacetate **1g**.

It was previously reported that methylation of **1b** at the C-4′ hydroxy group and subsequent hydrolysis of the disaccharide side chain in **1f** furnished **1a** in 26% yield [21] (Scheme 3). However, the methylation at C-4′ proceeded with poor regioselecivity, and similar reaction conditions provided the dimethyl ether **1h** [22]. Moreover, methylation did not progress sufficiently, and after



Scheme 4. Chemo-enzymatic synthesis of resveratrol 3,5-di-*O*-β-glucopyranoside (**2a**) from piceid (**2c**). Reagents and conditions: (a) acetic anhydride, 4-*N*,*N*-dimethylaminopyridine, pyridine; (b) *Burkholderia cepacia* lipase (Amano, PS-IM), 2-propanol, THF; (c) *tert*-butyldimethylsilyl chloride, imidazole, *N*,*N*-dimethylformamide; (d) sodium perborate, methanol; (e) tetra-*O*-acetyl-D-glucopyranosyl trichloroacetimidate, trimethylsilyl trifluromethylsulfonate, molecular sieves 4A, CH_2Cl_2, and (f) NaOH, methanol, THF.



Scheme 5. Reported transformation of resveratrol. Reagents and conditions: (a) *B. cepacia* lipase, 1-butanol, *tert*-butyl methyl ether and (b) *tert*-butyldimethylsilyl chloride, imidazole, *N*,*N*-dimethylformamide.

cleavage of the sugar side chain, the aglycones were obtained as a mixture of desired **1a**, apigenin (**1i**), and apigenin 4′,7-dimethyl ether (**1j**). In contrast, using our newly developed synthesis, chromatographic separation of **1i** and **1j** from **1a** at the final stage is unnecessary. Although the new synthetic strategy for **1a** involves two more steps compared with the previous route [21], the overall yield is much higher.

The synthesis of resveratrol 3,5-di-*O*-β-glucopyranoside (**2a**) is shown in Scheme 4. The excellent regioselectivity in deacetylation obtained by introducing a sugar side chain in the synthesis of acacetin (Scheme 2) inspired us to use piceid (**2c**) [7], a glycosylated resveratrol, as the starting material. *C. antarctica* lipase B-catalyzed deacetylation of the corresponding peracetate **2d** proceeded exclusively on the C-4′ acetyl group. However, unfortunately, this reaction was practically slow and unreacted starting material **2d** was difficult to separate from desired product **2c** by column chromatography. To our delight, conducting the reaction using *B. cepacia* lipase resulted in prompt transesterification to furnish **2e**. Silylation at the liberated C-4′ hydroxy group in **2e** proceeded smoothly to give **2f**, and subsequent selective deacetylation on the phenolic acetate [23] at C-5 proceeded without affecting the four acetates in the glucose unit to furnish **2g**. Glycosylation with a trichloroacetimidate donor in which all hydroxy groups were protected as acetates yielded symmetric diglucoside **2h**. Finally, all acetyl groups and the *tert*-butyldimethylsilyl (TBS) group were simultaneously removed under basic conditions to give **2a** in 62% overall yield from **2c**.

For consideration of the high regioselectivity in deacetylation of **1c** and **2d** with *C. antarctica* lipase B, the docking simulation was carried out and docking modes of **1c** and **2d** in the transesterification reactions were estimated. The docking poses of the resulting complexes were ranked based on consensus scoring algorithm using six scoring functions (Ligscore1, Ligscore2, PLP1, PLP2, PMF, and Jain) [13,14,17]. In the both cases of **1c** and **2d**, the complexes with the top five consensus score included two types of docking poses; (1) an acetate of sugar moieties or (2) C-4′ acetate of aglycone moieties directed to a catalytic triad, Ser105, His224, and Asp187, that located in the bottom of catalytic cavity. The results suggested that acetates of not only aglycones but also sugar moieties in both of **1c** and **2d** were reactive under transesterification conditions. However, in our cases, C-4′ acetates of **1c** and **2d** were exclusively reacted due to high leaving group ability of phenol groups. Lipase-catalyzed transesterification is a reversible procedure. Re-acylation of the once liberated primary alcohol in sugar side chain is easy, as suggested by the previous examples [19,20]. On the other hand, any docking poses that C-5 acetate of aglycones directed to a catalytic triad were not obtained, indicating that steric hindrance of peracetylated sugar moieties on C-3 in **1c** and **2d** suppressed deacetylation on C-5.

For the following discussion, the latter cases that aglycones directed to catalytic triad were considered. The catalytic cavity of *C. antarctica* lipase B contains a large hydrophobic pocket on the left side of Ser105 and a medium hydrophobic pocket on the right side of it as shown in Fig. 2(a) [24,25]. In addition to them, a small and shallow pocket constituted by Leu144, Val149, Val154, and Val286 can be found besides a large hydrophobic pocket[Fig. 2(a and b)]. For **1c**, aglycone moiety in **1c** was positioned in a large hydrophobic pocket via hydrophobic interactions with Ala141 and Leu144 in α-helix 5 and Val154 in α-helix 6 [Fig. 2(c and d)]. Acetyl group at 6-position in glucose moiety was placed in a small and shallow pocket. Though rhamnose moiety located outside of catalytic cavity, its methyl group interacted with Ile189 in loop region between β-sheet 6 and 7.

In contrast to **1c**, aglycone moiety of **2d** was positioned in a medium pocket and its acetate at 4′-postion pointed toward catalytic triad [Fig. 2(e and f)]. In a medium pocket, aglycone moiety was held via hydrophobic interactions with hydrophobic residues (Ala281, Ala282, Ile285, and Val286) in α-helix 10 and Ile189. Pyranose ring and acetyl group at 6-position in glucose moiety interacted with Leu144 in α-helix 5 to occupy entrance and upper part of a large hydrophobic pocket, respectively. Furthermore, acetyl group at 3-position in glucose was located in small and shallow pocket next to a large hydrophobic pocket. Surprisingly, these results indicated that docking modes of these two substrates are entirely different, despite of similar regioselectivity in transesterification.

As indicated in Scheme 4 and the results of the docking simulations, the peracetylated glucose unit on C-3 in **2d** worked very well as a bulky substituent on the aromatic ring for preventing overdeacetylation on the C-5 acetate, as the rate of lipase-catalyzed transesterification is affected by the steric hindrance [26–28].

B. cepacia lipase-catalyzed regioselective deprotection of the acetyl group on C-4′ in resveratrol triacetate (**2i**) had previously been reported [29] (Scheme 5). We confirmed that this reaction regioselectively proceeded at the initial stage of the reaction, but we also observed transesterification of the C-3 and C-5 positions and were thus unable to obtain **2j** in reproducible yields. The TBS ether **2k** was the key precursor in the chemical synthesis of **2a** [30].

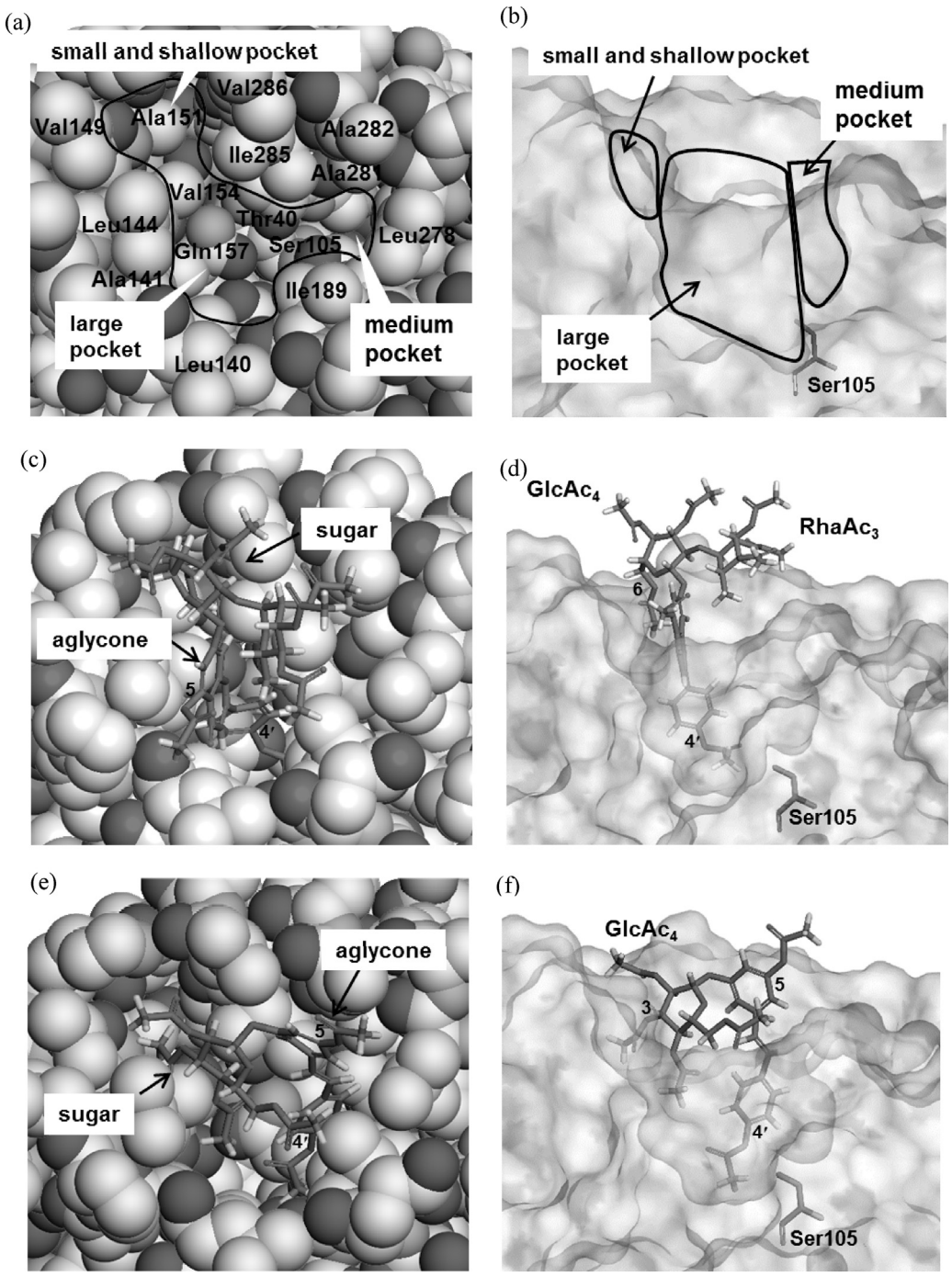


Fig. 2. Catalytic cavity of *C. antarctica* lipase B and plausible docking poses of **1c** and **2d**. Top view of (a) catalytic cavity and its complex with (c) **1c** and (e) **2d**. Side view of (b) catalytic cavity and its complex with (d) **1c** and (f) **2d**.

However, attempts to conduct the regioselective silylation of resveratrol (**2b**) resulted in a complex mixture, and the desired product (**2k**) was isolated in as low as 14% yield from **2b** (Scheme 5).

The direct enzyme-catalyzed glycosylation of **2b** [31] and **2c** [6] has also been reported, but the low water-solubility of the starting materials as well as the low availability of overexpressed specific glycosyl transferases hindered scale-up of the reaction. Although the present synthesis requires six steps, it is efficient in that the reactants are readily available and each step utilizes only commercially available reagents.

4. Conclusion

Acacetin (**1a**) and resveratrol 3,5-di-*O*-β-glucopyranoside (**2a**) were synthesized from naringin (**3a**) and piceid (**2c**) in 65% and 62% overall yield, respectively, using only commercially available reagents. In both syntheses, blocking the correct hydroxy group with a sugar side chain enhanced lipase-catalyzed transesterification, providing better regioselectivity than that obtained using the corresponding aglycones.

Acknowledgements

We thank Professor Hiroki Hamada of Okayama Science University for discussion, and Novozymes Japan for generous gift of Novozym 435, and Amano Enzyme Inc. for lipase PS-IM. Mr. Susanta Mandal's contribution in the early phase of this work is appreciated. This work was supported both by a Grant-in-Aid for Scientific Research (No. 26450143) and Platform for Drug Discovery, Informatics, and Structural Life Science from the Ministry of Education, Culture, Sports, Science and Technology, Japan, and is gratefully acknowledged.

References

[1] A.G. Perkin, J. Chem. Soc. 77 (1900) 423.
[2] T. Nakaoki, N. Morita, S. Kishitani, Yakugaku Zasshi 80 (1960) 1743.
[3] S. Sakamura, Y. Terayama, S. Kawakatsu, A. Ichihara, H. Saito, Agric. Biol. Chem. 44 (1980) 2951.
[4] I. Horibe, Y. Satoh, Y. Shiota, A. Kumagai, N. Horike, H. Takemori, S. Uesato, S. Sugie, K. Obata, H. Kawahara, Y. Nagaoka, J. Nat. Med. 67 (2013) 705.
[5] F. Larronde, T. Richard, J.-C. Delaunay, A. Decendit, J.-P. Monti, S. Krista, J.-M. Mérillon, Planta Med. 71 (2005) 888.
[6] A. Lepak, A. Gutmann, S.T. Kulmer, B. Nidetzky, ChemBioChem 16 (2015) 1870.
[7] T. Okada, Y. Konishi, K. Ishihara, Y. Masuoka, Naturalistae 14 (2009) 17.
[8] H.-W. Voigtländer, H. Härtner, Arch. Pharm. 316 (1983) 219.
[9] O. Kirk, M.W. Christensen, F. Beck, T. Damhus, Biocat. Biotrans. 12 (1995) 91.
[10] G. Wu, D.H. Robertson, C.L. Brooks III, M. Vieth, J. Comput. Chem. 24 (2003) 1549.
[11] D. Mackerell Jr., D. Bashford, M. Bellott, R.L. Dunbrack, J.D. Evanseck, M.J. Field, S. Fischer, J. Gao, H. Guo, S. Ha, D. Joseph-McCarthy, L. Kuchnir, K. Kuczera, F.T.K. Lau, C. Mattos, S. Michnick, T. Ngo, D.T. Nguyen, B. Prodhom, W.E. Reiher III, B. Roux, M. Schlenkrich, J.C. Smith, R. Stote, J. Straub, M. Watanabe, J. Wiórkiewicz-Kuczera, D. Yin, M. Karplus, J. Phys. Chem.: B 102 (1998) 3586.
[12] J. Uppenberg, N. Ohrner, M. Norin, K. Hult, G.J. Kleywegt, S. Patkar, V. Waagen, T. Anthonsen, T. Jones, Biochemistry 34 (1995) 16838.
[13] E.B. De Oliveira, C. Humeau, L. Chebil, E.R. Maia, F. Dehez, B. Maigret, M. Ghoul, J.-M. Engasser, J. Mol. Catal. B: Enzym. 59 (2009) 96.
[14] C. Bidouil, D.O.E. Basilio, L. Chebil, E.-R. Maia, B. Maigret, E. Ronat-Heidt, M. Ghoul, J.-M. Engasser, C. Humeau, J. Biotechnol. 156 (2011) 203.
[15] C.K. Andrade, W.A. Silva, E.R. Maia, J. Biomol. Struct. Dyn. 25 (2007) 35.
[16] E.F.F. da Cunha, T.C. Ramalho, R.B. de Alencastro, E.R. Maia, Comput. Math. Methods Med. 8 (2007) 113.
[17] M. Feher, Drug Dis. Today 11 (2006) 421.
[18] R. Kobayashi, T. Itou, K. Hanaya, M. Shoji, N. Hada, T. Sugai, J. Mol. Catal. B: Enzym. 92 (2013) 14.
[19] A. Kontogianni, V. Skouridou, V. Sereti, H. Stamatis, F.N. Kolisis, J. Mol. Catal. B: Enzym. 21 (2003) 59.
[20] M. Ardhaoui, A. Falcimaigne, S. Ognier, J.M. Engasser, P. Moussou, G. Pauly, M. Ghoul, J. Biotechnol. 110 (2004) 265.
[21] J. Liu, L. Chen, S. Cai, Q. Wang, Carbohydr. Res. 357 (2012) 41.
[22] S. Hattori, H. Matsuda, Arch. Biochem. Biophys. 37 (1951) 85.
[23] B.P. Bandgar, L.S. Uppalla, V.S. Sadavarte, S.V. Patil, New. J. Chem. 26 (2002) 1273.
[24] J. Uppenberg, M.T. Hansen, S. Patkar, T. Jones, Structure 2 (1994) 293.
[25] I. Lavandera, S. Fernández, J. Magdalena, M. Ferrero, R.J. Kazlauskas, V. Gotor, ChemBioChem 6 (2005) 1381.
[26] T. Miyazawa, M. Hamada, R. Morimoto, T. Murashima, T. Yamada, Tetrahedron Lett. 48 (2007) 8334.
[27] T. Miyazawa, M. Hamada, R. Morimoto, T. Murashima, T. Yamada, Tetrahedron Lett. 49 (2008) 175.
[28] K. Yashiro, K. Hanaya, M. Shoji, T. Sugai, Biosci. Biotechnol. Biochem. 79 (2015) 1926.
[29] G. Nicolosi, C. Spatafora, C. Tringali, J. Mol. Catal. B: Enzym. 16 (2002) 223.
[30] Z. Zhang, B. Yu, R.R. Schmidt, Synthesis (2006) 1301.
[31] R.P. Pandey, P. Parajuli, J.Y. Shin, J. Lee, S. Lee, Y.-S. Hong, Y. Park II, J.S. Kim, J.K. Sohng, Appl. Environ. Microbiol. 80 (2014) 7235.