

# Supporting Information

## Ribosides and Ribotide of a Fairy Chemical, Imidazole-4-carboxamide, as Its Metabolites in Rice

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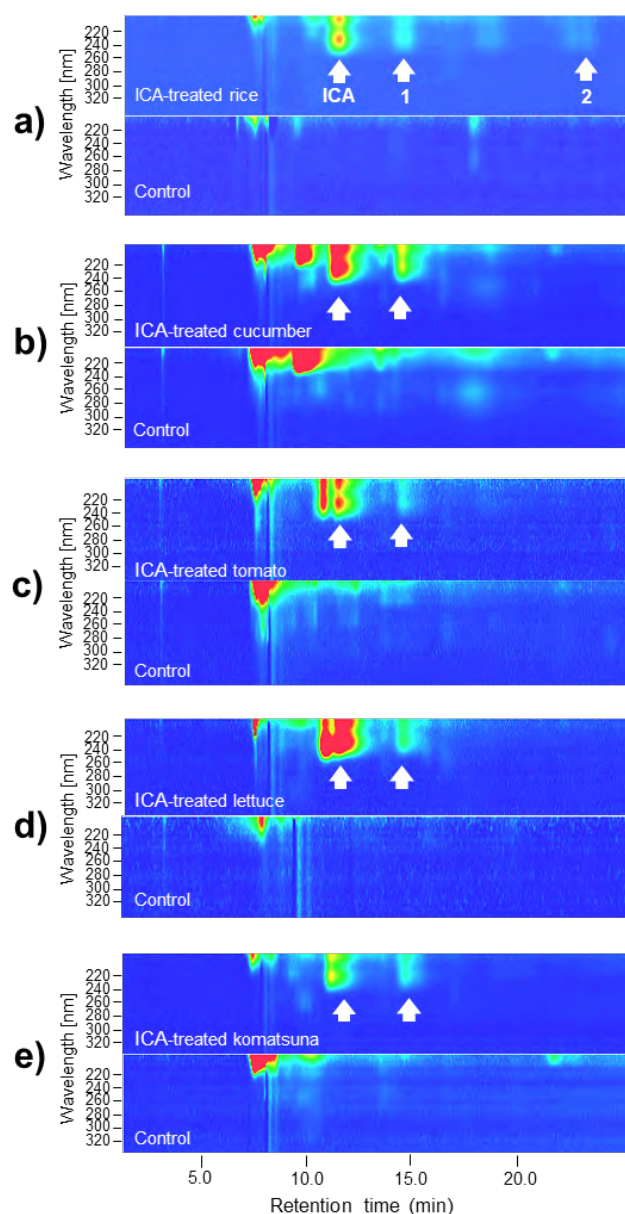
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## EXPERIMENTAL SECTION

**Materials and Chemicals.** Rice cultivar (*Oryza sativa* L. cv. Nipponbare) , cucumber (*Cucumis sativus* L. cv. Ajisango), lettuce (*Lactuca sativa* L. cv. Cisco), komatsuna (*Brassica rapa* L. cv. Rakuten) and tomato (*Solanum lycopersicum* L. cv. House-Momotaro) were used in this study. All solvents used throughout the experiments were obtained from Kanto Chemical Co. (Tokyo, Japan).

**General Experimental Procedures.** <sup>1</sup>H NMR spectra (one- and two-dimensional) were recorded on a Jeol lambda-500 spectrometer at 500 MHz, while <sup>13</sup>C NMR spectra were recorded on the same instrument at 125 MHz (JEOL, Tokyo, Japan). The HRESIMS spectra were measured on a JMS-T100LC mass spectrometer. Infrared (IR) spectra were recorded on a Jasco FT/IR-4100 spectrometer and the specific rotation values were measured by a Jasco DIP-1000 polarimeter. HPLC separations were performed with Jasco Gulliver system using reverse-phase HPLC columns (Develosil C30-UG-5, Nomura chemical, Japan; Cosmosil 5PYE waters and Cosmosil  $\pi$ NAP waters Nacalai tesque, Japan; InertSustain AQ-C18, GL Sciences, Tokyo, Japan). An ODS gel (Cosmosil 140 C18-OPN, Nacalai Tesque, Kyoto, Japan) were used for flash column chromatography.

**Detection of ICA and its metabolites 1 and 2 by HPLC.** Treated seedlings were divided into two parts, all plants were divided into two parts, shoot and root. The shoot and root were extracted with ethanol. After removing the solvent of each extracts under reduced pressure, they were dissolved in 5% MeOH and subjected to HPLC analysis. The extracts were analyzed by reversed-phase HPLC using a Develosil C30-UG-5 column ( $\phi$  4.6  $\times$  250 mm, 5  $\mu$ m) with a gradient manner (5% methanol (B) in (A) for 30 min, 5%-100% B in A for 45 min and 100% B for 30 min) at a flow rate of 0.5 mL/min at a multiple wavelength using a photo-diode array detector.

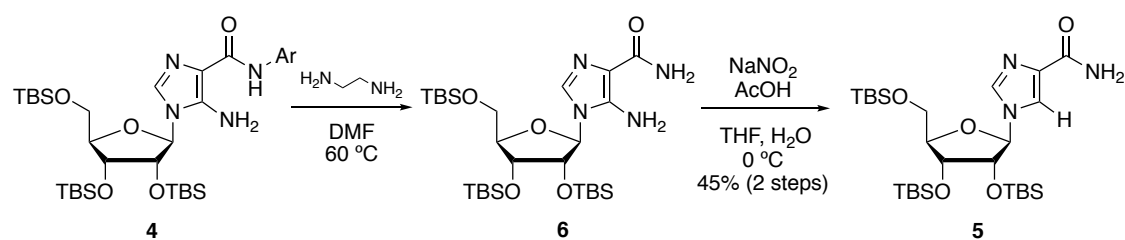


**Figure S1.** HPLC profiles of the extracts of ICA-treated plants, rice (a), cucumber (b), tomato (c), lettuce (d) and komatsuna (e) for one month. The seeds were put on filter paper (Advantec No. 2,  $\phi$  5 mm; Toyo Roshi Kaisha, Ltd., Japan), soaked half-strength of nutrient solution containing ICA (1 mM), in a Petri dish ( $\phi$  60  $\times$  20 mm), and incubated under 18 hours photoperiod at 23°C for one month. The treated plants were extracted by EtOH. After being prepared with 5% MeOH at concentration of 20 mg/mL, each sample was injected into HPLC. The extracts were analyzed by reversed-phase HPLC using a Develosil C30-UG-5 column ( $\phi$  4.6  $\times$  250 mm, 5  $\mu$ m) with a gradient manner (5% methanol (B) in (A) for 30 min, 5%-100% B in A for 45 min and 100% B for 30 min) at a flow rate of 0.5 mL/min at a multiple wavelength.

**Purification of 1 and 2 from ICA-treated rice.** Rice seeds were germinated in nursery boxes, incubated for a week and then treated with ICA (0.5 mM) in a growth chamber under 16 h photoperiod at 28°C for two weeks. The treated seedlings were divided into two parts, shoot and root. The root (1.9 kg) was extracted with EtOH and then acetone. After combining the solution and concentrating under reduced pressure, the concentrate was extracted with CH<sub>2</sub>Cl<sub>2</sub> to remove lipophilic substances and dried under reduced pressure. The residue (14.6 g) was fractionated by ODS gel flash column chromatography (Cosmosil 140 C18-OPN, Nacalai Tesque, Japan; 5%, 10%, 25%, 50% MeOH, MeOH) to obtain 5 fractions. Fraction 2 (263.5 mg) was further separated by HPLC with a Develosil C30-UG-5 column ( $\phi$  20 × 250 mm, 5  $\mu$ m; flow rate 5 mL/min; injection volume 1 mL; 5% MeOH; UV detection 210 nm) to obtain 21 fractions (fraction 2-1 to 2-21). Fraction 2-3 was further fractionated by HPLC with Cosmosil  $\pi$ NAP ( $\phi$  10 × 250 mm, 5  $\mu$ m; flow rate 2 mL/min; injection volume 0.5 mL; 2% MeOH; UV detection 210 nm; retention time 11.0 min) column to afford **1** (2.7 mg). Fractions 2-11 were separated by HPLC with Cosmosil 5PYE ( $\phi$  10 × 250 mm, 5  $\mu$ m; flow rate 2 mL/min; injection volume 0.5 mL; 2% MeOH; UV detection 210 nm; retention time 11.0 min) to afford **2** (3.8 mg). **1**: Pale yellow amorphous;  $[\alpha]_D^{25} - 39$  ( $c$  0.28, MeOH); IR (film, cm<sup>-1</sup>): 3342; <sup>1</sup>H and <sup>13</sup>C NMR data, see Table 1; ESIMS  $m/z$  242 [M – H]<sup>-</sup>; HRESIMS  $m/z$  266.0724 [M + Na]<sup>+</sup> (calcd for C<sub>9</sub>H<sub>13</sub>N<sub>3</sub>NaO<sub>5</sub>, 266.0753). **2**: Pale yellow amorphous;  $[\alpha]_D^{25} - 50$  ( $c$  0.38, MeOH); IR (film, cm<sup>-1</sup>): 3345; <sup>1</sup>H and <sup>13</sup>C NMR data, see Table 1; ESIMS  $m/z$  404 [M – H]<sup>-</sup>; HRESIMS  $m/z$  428.1281 [M + Na]<sup>+</sup> (calcd for C<sub>15</sub>H<sub>23</sub>N<sub>3</sub>NaO<sub>10</sub>, 428.1281).

### Synthesis of ICA-riboside (**1**)

1-((2*R*,3*R*,4*R*,5*R*)-3,4-bis((tert-butyldimethylsilyl)oxy)-5-(((tert-butyldimethylsilyl)oxy)methyl)tetrahydrofuran-2-yl)-1*H*-imidazole-4-carboxamide (**5**)



To a stirred solution of **4** (3.00 g, 3.91 mmol) in DMF (40 mL) was added ethylenediamine (10 mL) at room temperature. After being stirred at 60 °C for 2.5 hours the resulting mixture was poured into saturated aqueous NH<sub>4</sub>Cl at 0 °C, and extracted with EtOAc. The organic layer was washed with 1 M HCl, brine, dried over anhydrous MgSO<sub>4</sub>, filtered. The filtrate was concentrated under reduced pressure to afford a crude material including **6**. To a stirred solution of the crude material including **6** in a 4 : 1 : 1

mixture of AcOH, THF and H<sub>2</sub>O (total 90 mL) was added NaNO<sub>2</sub> (1.89 g, 27.4 mmol) at 0 °C under an argon atmosphere. After being stirred at the same temperature for 4 hours, the resulting mixture was poured into 25% ammonia water, and extracted with EtOAc. The organic layer was washed with saturated aqueous NaHCO<sub>3</sub>, brine, dried over anhydrous MgSO<sub>4</sub>, filtered. The filtrate was concentrated under reduced pressure. The residue was passed through a short silica gel column chromatography (*n*-hexane/EtOAc = 3/1 to 1/2) to afford **5** (1.03 g, 1.76 mmol, 45%, 2 steps) as an orange solid.

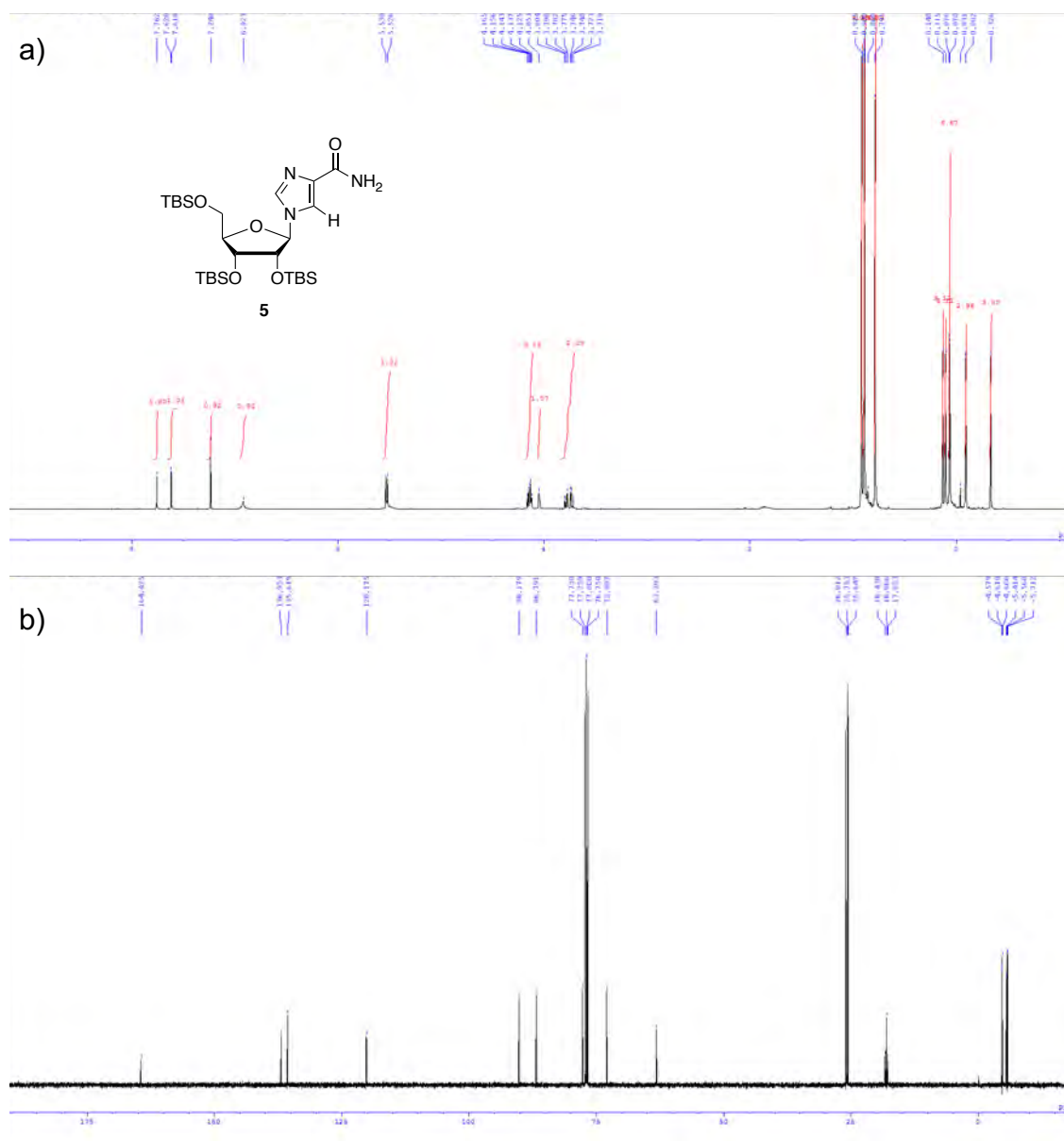
Analytical data for compound **5**

$[\alpha]_D^{23} -72$  (c 0.34, CHCl<sub>3</sub>).

IR (film, cm<sup>-1</sup>): 3470, 3183, 2930, 2859, 1682, 1597, 1472, 1344, 1258, 1161, 1111.

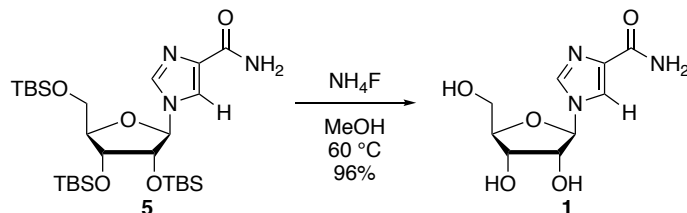
<sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>): δ 7.79 (s, 1H), 7.65 (d, *J*=1.2 Hz, 1H), 6.96 (br s, 1H), 5.56 (d, *J*= 6.3 Hz, 1H), 5.54 (br s, 1H), 4.15-4.20 (m, 2H), 4.08-4.10 (m, 1H), 3.82 (dd, *J*= 11, 3.4 Hz, 1H), 3.76 (dd, *J*= 11, 2.3 Hz, 1H), 0.96 (s, 9H), 0.93 (s, 9H), 0.83 (s, 9H), 0.17 (s, 3H), 0.15 (s, 3H), 0.11 (s, 3H), 0.10 (s, 3H), -0.05 (s, 3H), -0.30 (s, 3H).

<sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>): δ 164.4, 137.0, 135.6, 120.2, 90.2, 86.8, 77.7, 73.0, 63.3, 26.0, 25.8, 25.6, 18.4, 18.0, 17.9, -4.58, -4.62, -4.7, -5.4, -5.6, -5.7. HRMS (ESI): Calculated for C<sub>27</sub>H<sub>55</sub>N<sub>3</sub>O<sub>5</sub>Si<sub>3</sub>Na 608.3342 [M + Na]<sup>+</sup>, found 608.3343.



**Figure S2.**  $^1\text{H}$  NMR (a) and  $^{13}\text{C}$  NMR (b) spectra for **5**.

1-((2*R*,3*R*,4*S*,5*R*)-3,4-dihydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-1*H*-imidazole-4-carboxamide  
(ICA-riboside; **1**)



To a stirred solution of **5** (100 mg, 171  $\mu$ mol) in MeOH (2 mL) was added  $\text{NH}_4\text{F}$  (63 mg, 1.71 mmol) at room temperature under an argon atmosphere. After being stirred at 60  $^\circ\text{C}$  for 28 hours, the resulting mixture was directly charged on the top of silica gel column and eluted with  $\text{CHCl}_3/\text{MeOH} = 4/1$  to 1/1 to afford ICA-riboside (**1**) (40 mg, 164  $\mu$ mol, 96%) as a yellow amorphous.

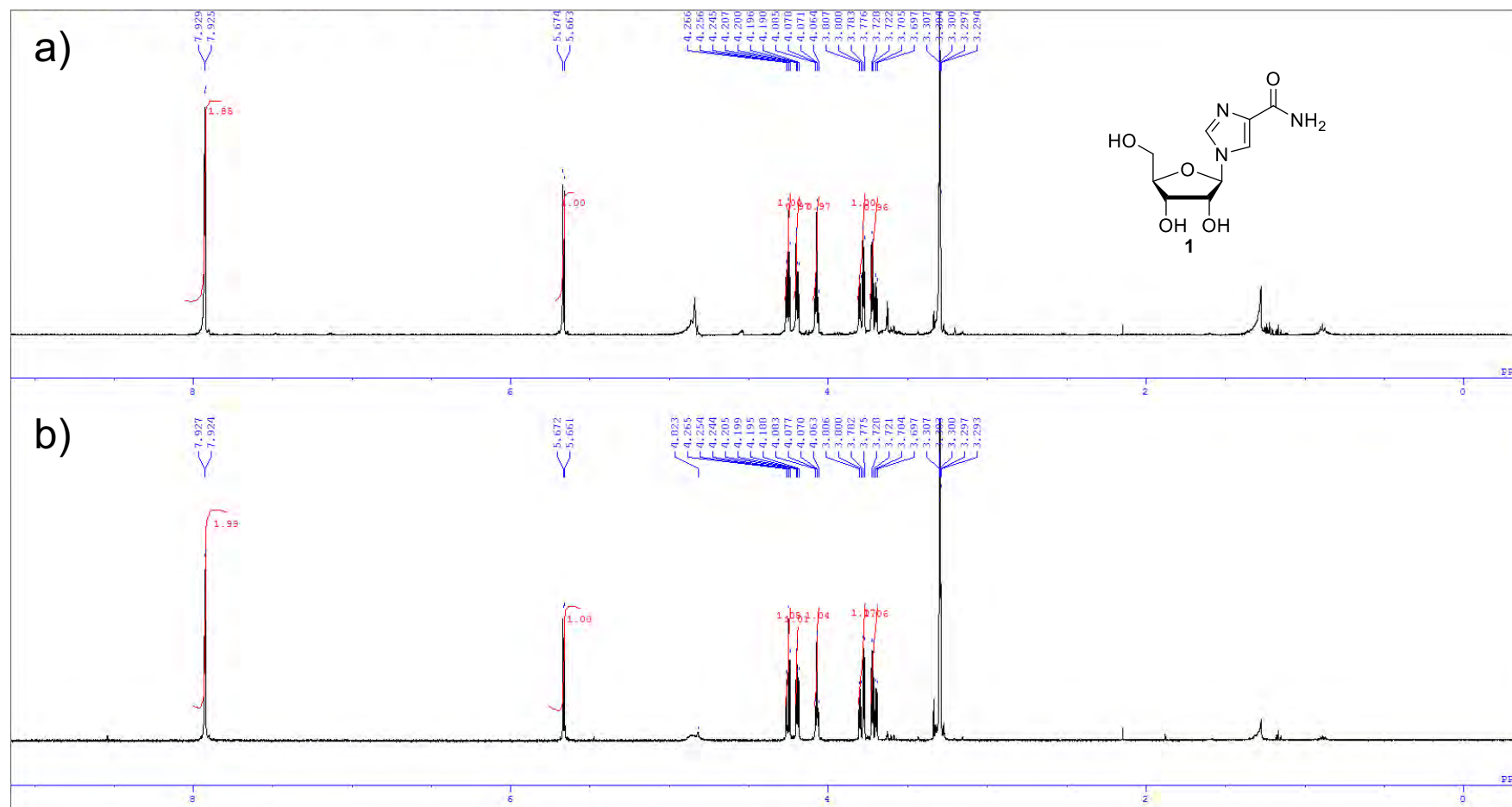
*Analytical data for ICA-riboside (1)*

$$[\alpha]_D^{26} -41 \text{ (} c \text{ 0.28, MeOH).}$$

IR (ATR,  $\text{cm}^{-1}$ ): 3214, 2930, 1649, 1592, 1422, 1345, 1264, 1200.

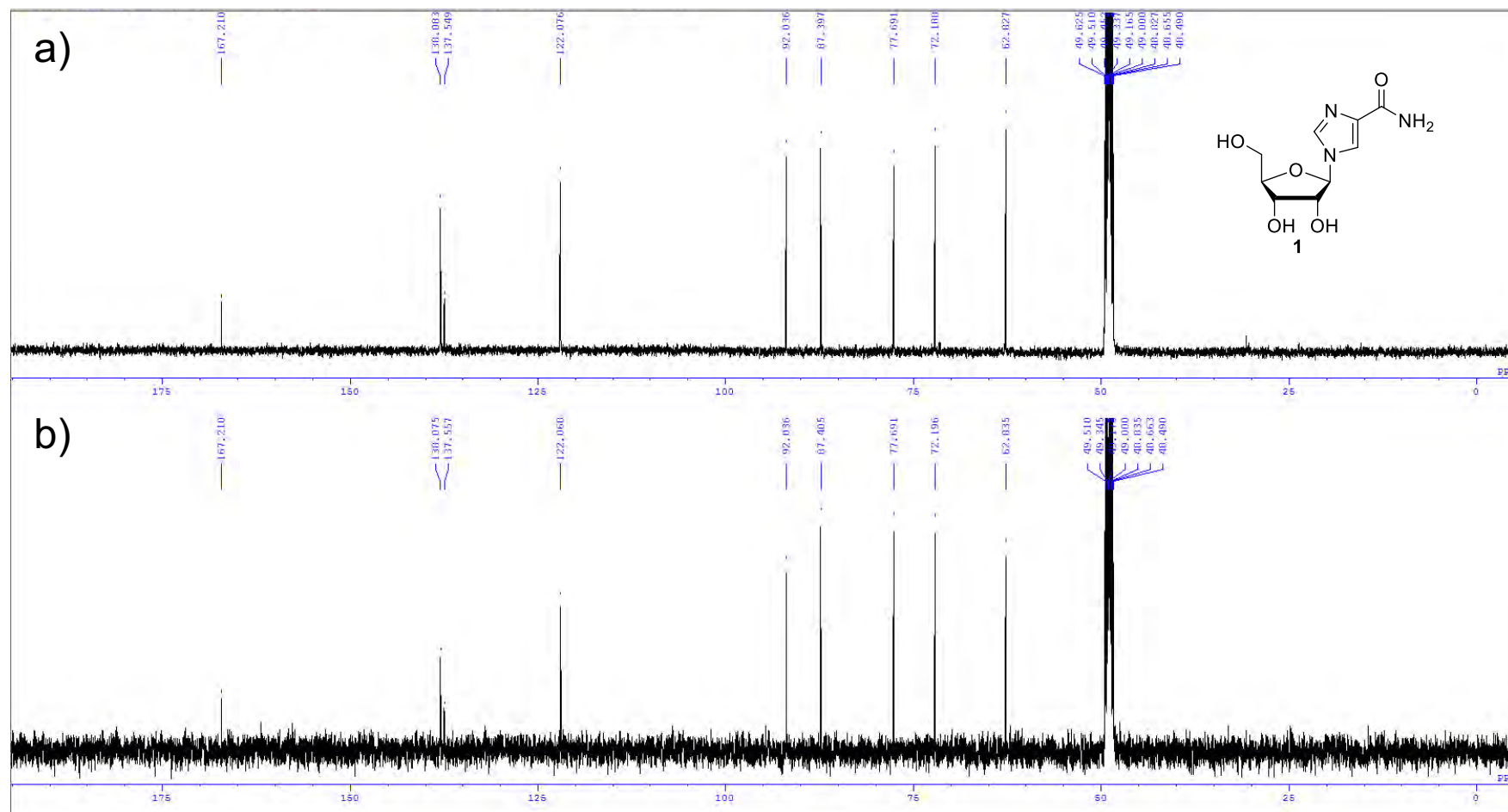
<sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>OD): δ 7.929 (s, 1H), 7.925 (s, 1H), 5.67 (d, *J* = 5.5 Hz, 1H), 4.26 (t, *J* = 5.5 Hz, 1H), 4.20 (dd, *J* = 5.1, 3.4 Hz, 1H), 4.07 (dd, *J* = 6.8, 3.4 Hz, 1H), 3.79 (dd, *J* = 12.2, 3.4 Hz, 1H), 3.71 (dd, *J* = 12.2, 3.4 Hz, 1H). <sup>13</sup>C NMR (125 MHz, CD<sub>3</sub>OD): δ 167.2, 138.1, 137.6, 122.1, 92.0, 87.4, 77.7, 72.2, 62.8.

HRMS (ESI): Calculated for  $C_9H_{13}N_3O_5Na$  266.0747  $[M + Na]^+$ , found 266.0747.



**Figure S3.**  $^1\text{H}$  NMR spectrum for natural **1** (a) and synthetic **1** (b).

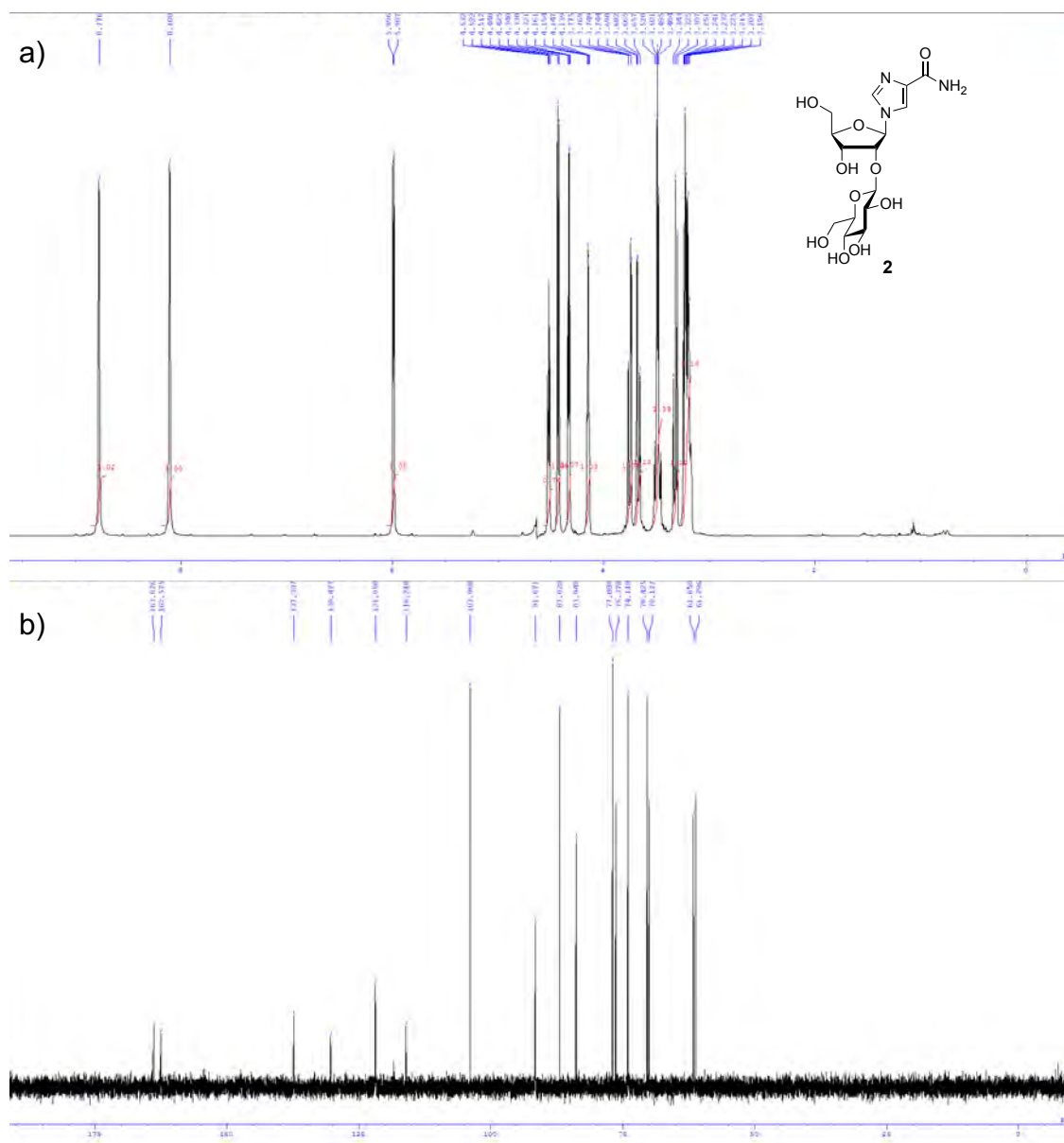




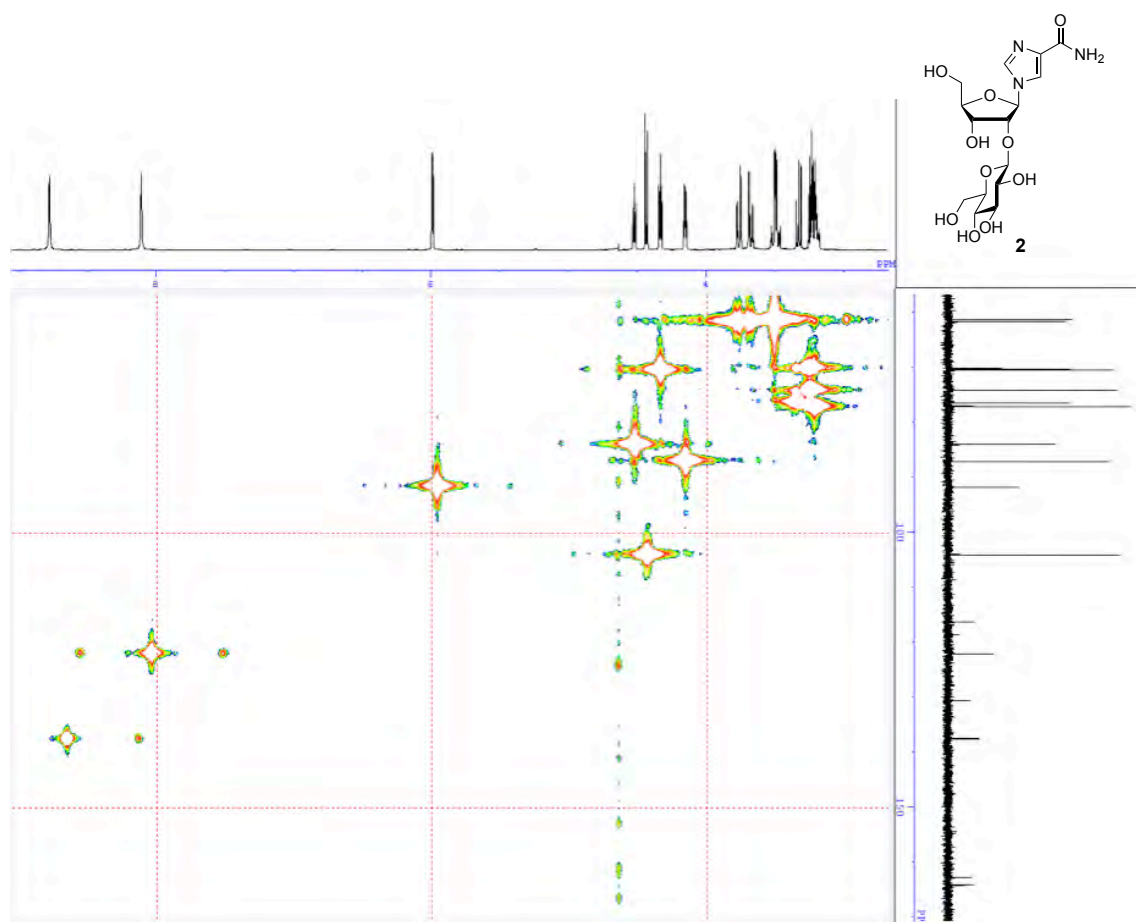
**Figure S4.**  $^{13}\text{C}$  NMR spectrum for natural **1** (a) and synthetic **1** (b).

**X-ray Crystallography Analysis.** Single crystal of the compound **2** was mounted on MicroMount loop (MiTeGen) with Paraton-N (Hampton Research), and was flash frozen to 173 K in a liquid nitrogen cooled stream of nitrogen. Data collections were carried out on a Rigaku VariMax Saturn (Mo K $\alpha$  radiation, 1.2-kW rotating anode). Eighteen preliminary data frames were measured at 0.5° increments of  $\omega$ , to assess the crystal quality and preliminary unit cell parameters. The intensity images were also measured at 0.5° intervals of  $\omega$ . The intensity images were integrated using the CrystalClear program package (Rigaku), and the empirical absorption correction was applied for the data. The structure was solved by direct method, SHELXL-97 (1), and refined using the SHELXL-97 tool. Crystallographic data have been deposited at The Cambridge Crystallographic Data Centre and allocated the deposition number CCDC 1531773. The data can be obtained free of charge via [www.ccdc.cam.ac.uk/products/csd/request](http://www.ccdc.cam.ac.uk/products/csd/request).

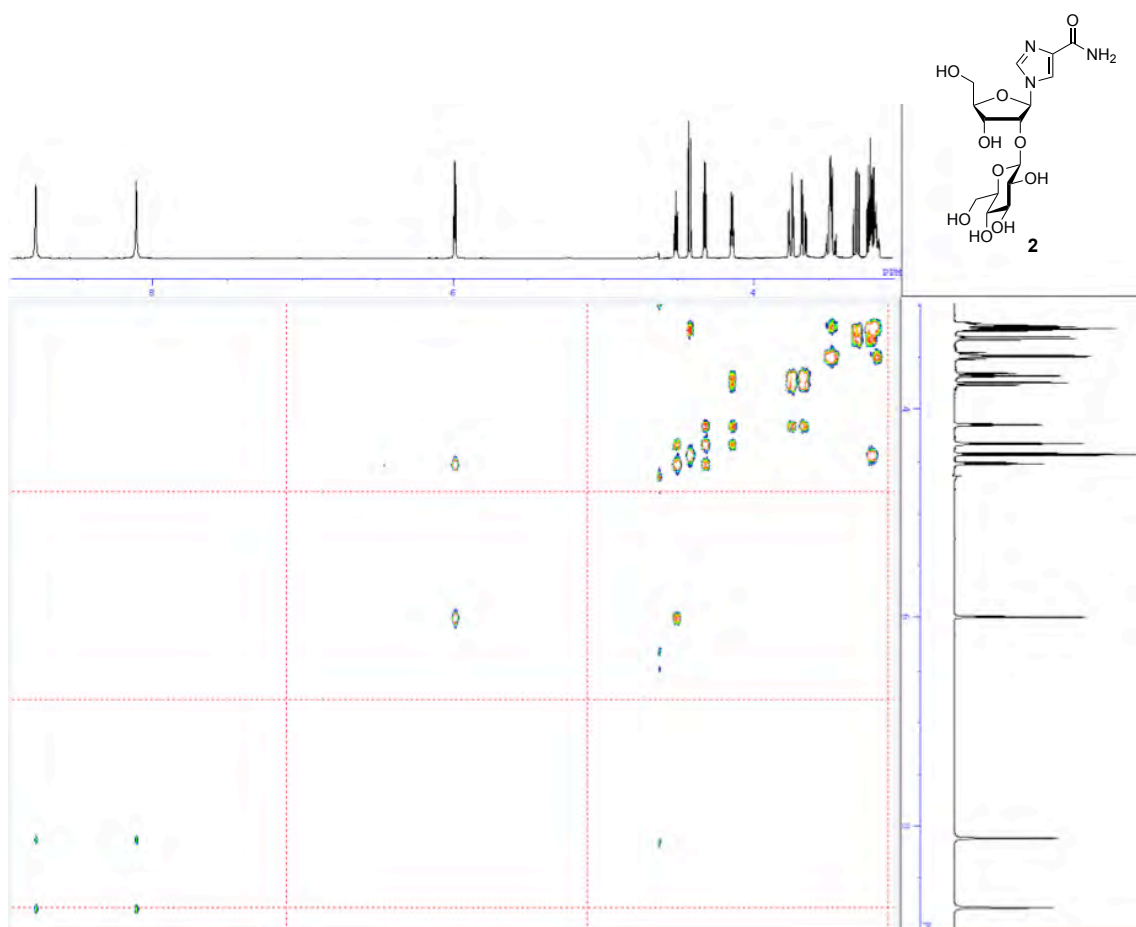
**Hydrolysis of 2 with  $\beta$ -D-glucosidase.** The mixture (100  $\mu$ L) of  $\beta$ -D-glucosidase (22 U, from almond, Wako Pure Chemical Industries, Ltd, Osaka, Japan) and **2** (0.23 mg) in 0.1 M acetic acid-sodium acetate buffer (pH 5.0) was incubated at 37°C for 3 h. The reaction was monitored by HPLC (InertSustain AQ-C18, 5% MeOH in 0.02% TFA) at a flow rate of 0.5 mL/min at a wavelength of 250 nm.



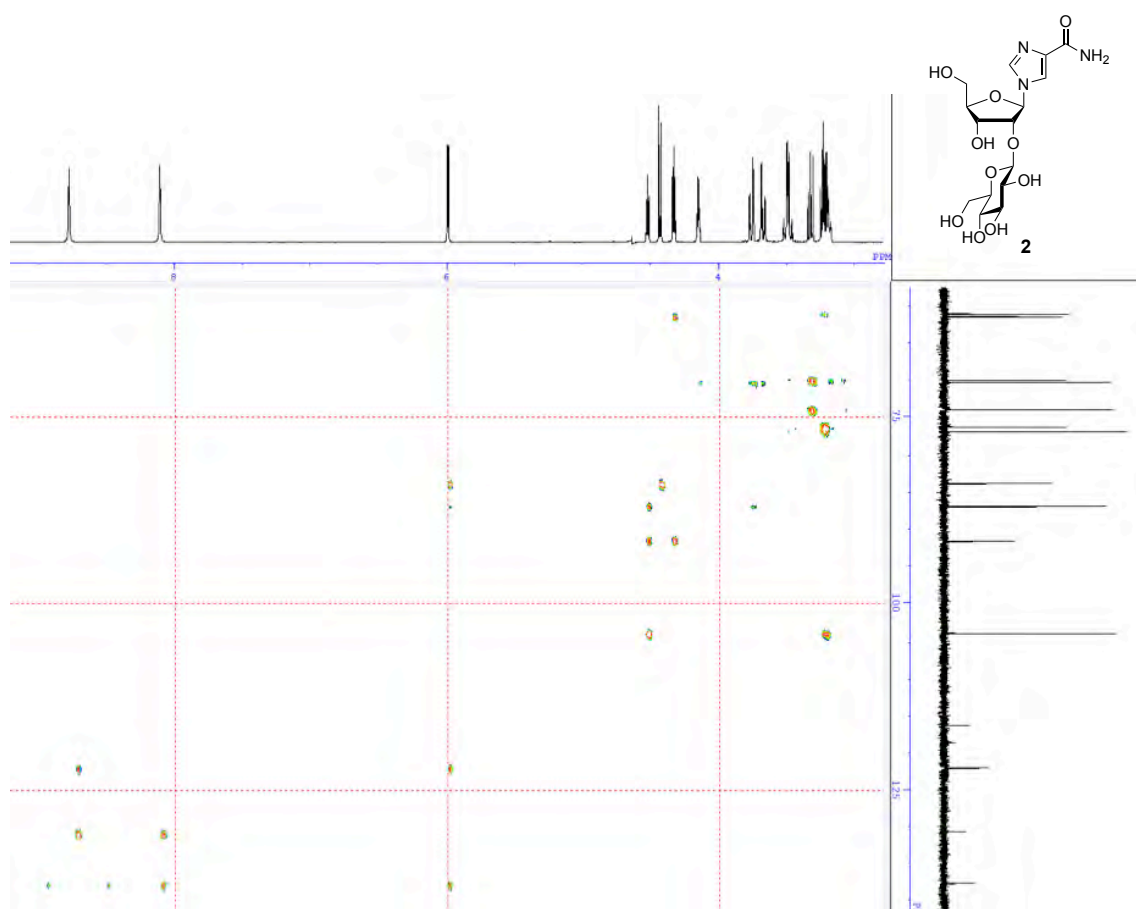
**Figure S5.** <sup>1</sup>H NMR (a) and <sup>13</sup>C NMR (b) spectra for **2**.



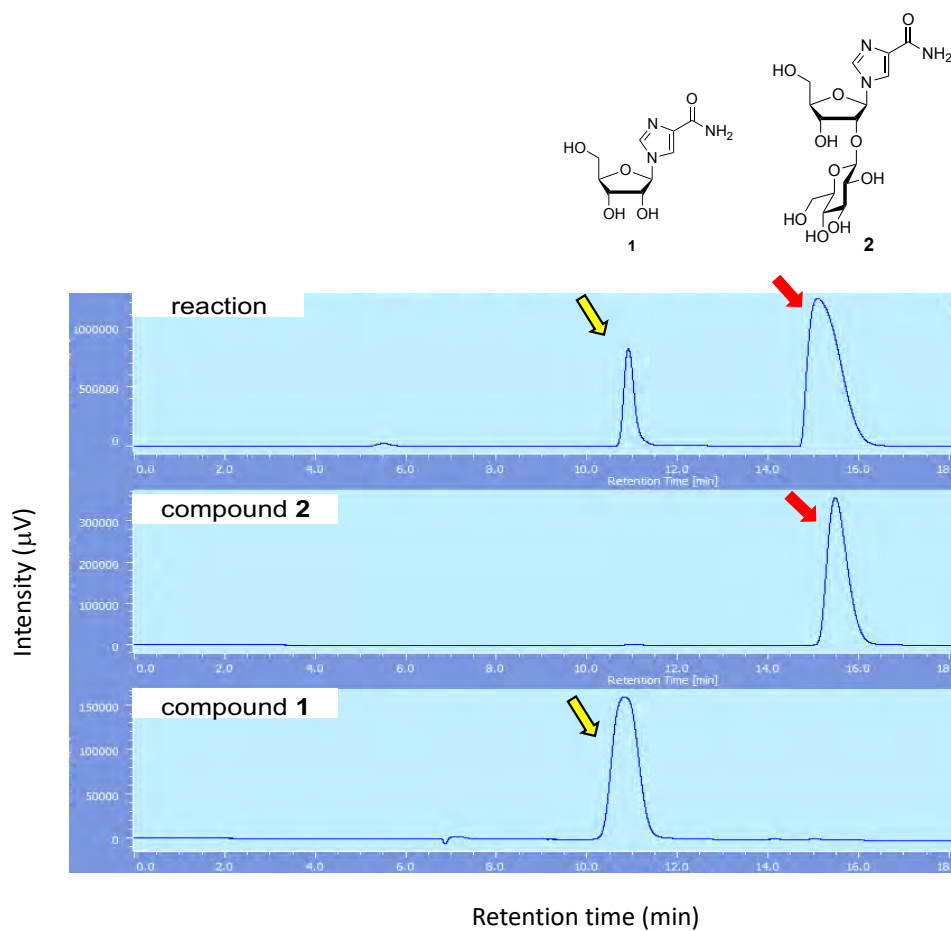
**Figure S6.** HMQC spectrum for **2**.



**Figure S7.** DQF-COSY spectrum for **2**.



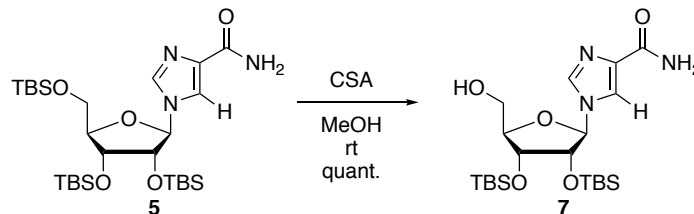
**Figure S8.** HMBC spectrum for **2**.



**Figure S9.** HPLC profile of the reaction mixture of  $\beta$ -D-glucosidase and **2**. The reaction was monitored by HPLC (InertSustain AQ-C18, 5% MeOH in 0.02% TFA) at a flow rate of 0.5 mL/min at a wavelength of 250 nm.

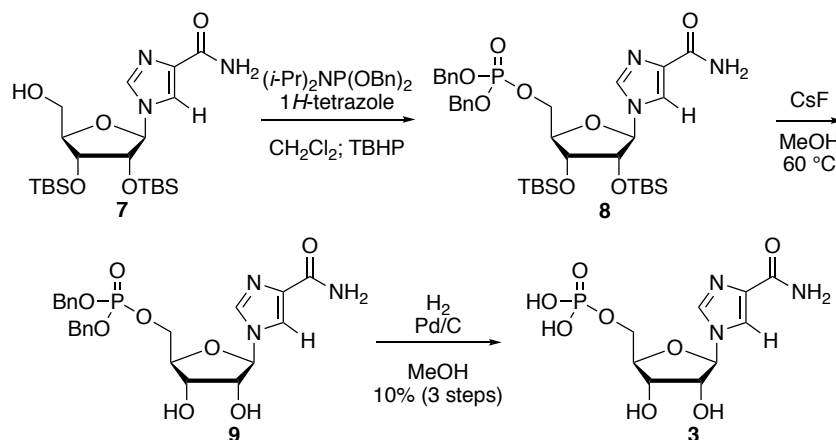
### Synthesis of ICA-ribotide (3)

1-((2*R*,3*R*,4*R*,5*R*)-3,4-bis((tert-butyldimethylsilyl)oxy)-5-(hydroxymethyl)tetrahydrofuran-2-yl)-1*H*-imidazole-4-carboxamide (7)



To a stirred solution of **5** (300 mg, 512  $\mu\text{mol}$ ) in MeOH (1.6 mL) was added CSA (119 mg, 512 mmol) at room temperature under an argon atmosphere. After being stirred for 24 h, the resulting mixture was poured into saturated aqueous  $\text{NaHCO}_3$  at 0  $^\circ\text{C}$ , and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous  $\text{Na}_2\text{SO}_4$ , filtered. The filtrate was concentrated under reduced pressure to afford a crude material including **7** (240 mg, quant) as a white solid. The residue was used in the next step without further purification.

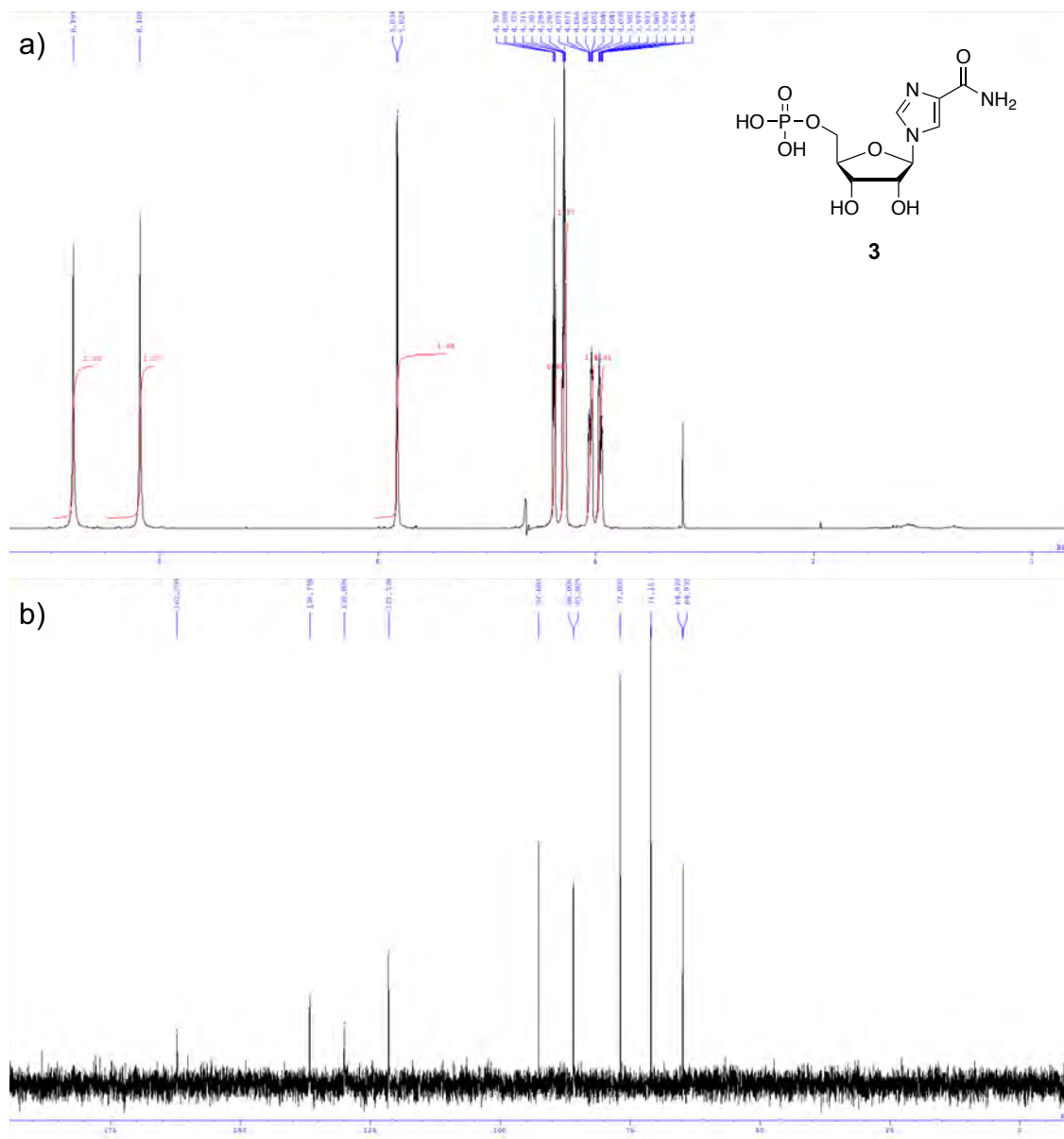
((2*R*,3*S*,4*R*,5*R*)-5-(4-carbamoyl-1*H*-imidazol-1-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl dihydrogen phosphate (ICA-ribotide; **3**)



To a stirred solution of **7** (100 mg, 212  $\mu\text{mol}$ ) in  $\text{CH}_2\text{Cl}_2$  (4 mL) were added 1*H*-tetrazole (90 mg, 1.27 mmol) and dibenzyl *N,N*-diisopropylphosphoramidite (213  $\mu\text{L}$ , 636  $\mu\text{mol}$ ) at 0  $^\circ\text{C}$  under an argon atmosphere. After being stirred at the same temperature for 20 min, TBHP (70% solution of  $\text{H}_2\text{O}$ , 181  $\mu\text{L}$ , 1.27 mmol) was added the reaction mixture at -78  $^\circ\text{C}$ . After being stirred at the same temperature for 2 h, warmed to 0  $^\circ\text{C}$  for 1 h. Then, the resulting mixture was poured into saturated aqueous  $\text{Na}_2\text{S}_2\text{O}_3$  and extracted with EtOAc. The organic layer was dried over anhydrous  $\text{Na}_2\text{SO}_4$ , filtered and concentrated under reduced pressure. The residue was passed through a short silica gel column chromatography (*n*-hexane/EtOAc = 3/1 to 1/2) to afford **8**. To a stirred solution of the crude material including **8** (8 mg, 11  $\mu\text{mol}$ ) in MeOH (500  $\mu\text{L}$ ) was added CsF (60 mg, 110  $\mu\text{mol}$ ) at room temperature under an argon atmosphere. After being stirred at 60  $^\circ\text{C}$  for 24 h, the resulting mixture was concentrated under reduced pressure to afford a crude material including **9**. A mixture of the crude material including **9** and 5% Pd/C (2 mg, 900 nmol) in



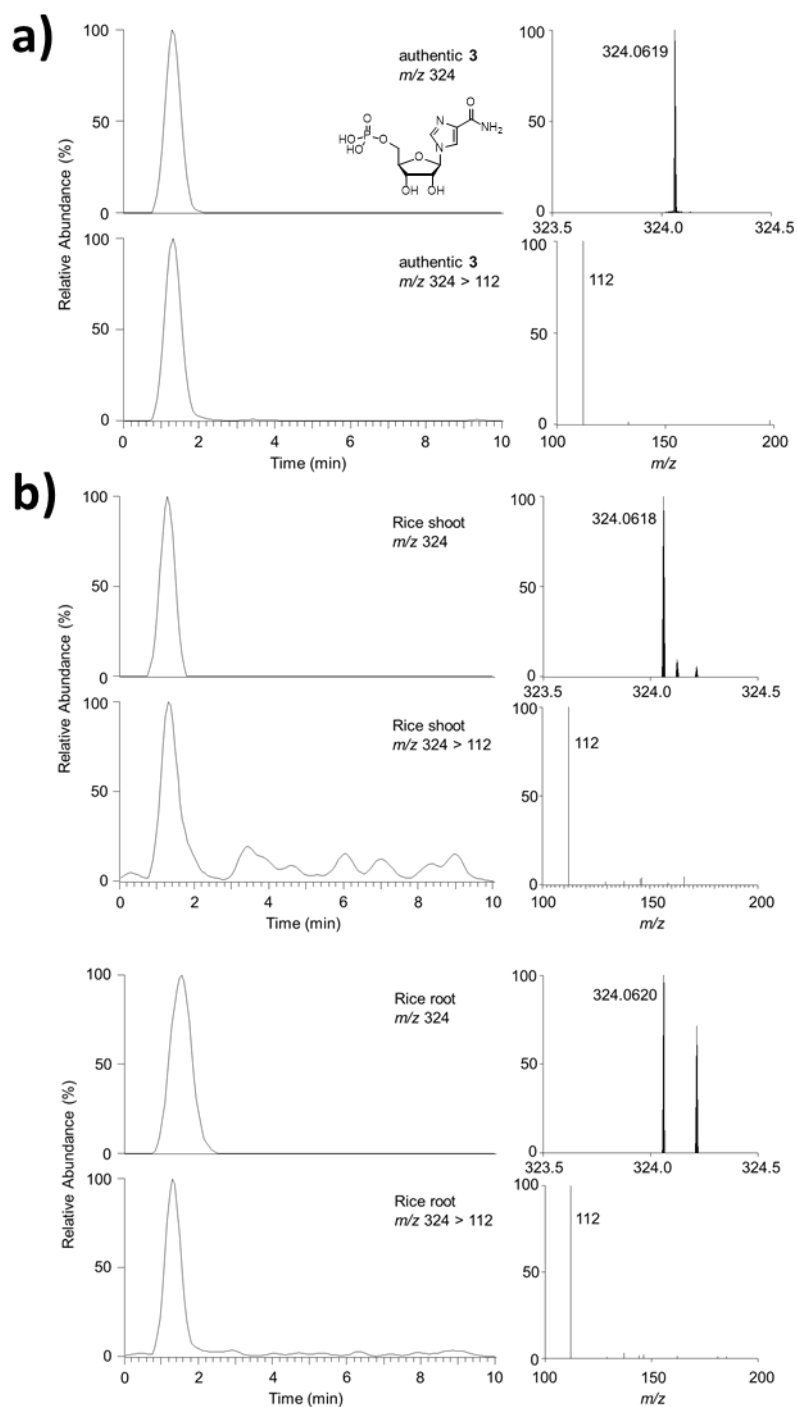
MeOH (1 mL) was stirred under ordinary hydrogen pressure (balloon) at room temperature for 30 min. The resulting mixture was filtered through a pad of Celite and concentrated under reduced pressure to afford ICA-ribotide (**3**) (3 mg, 10.3  $\mu$ mol, 10%, 3 steps) as a colorless solid.



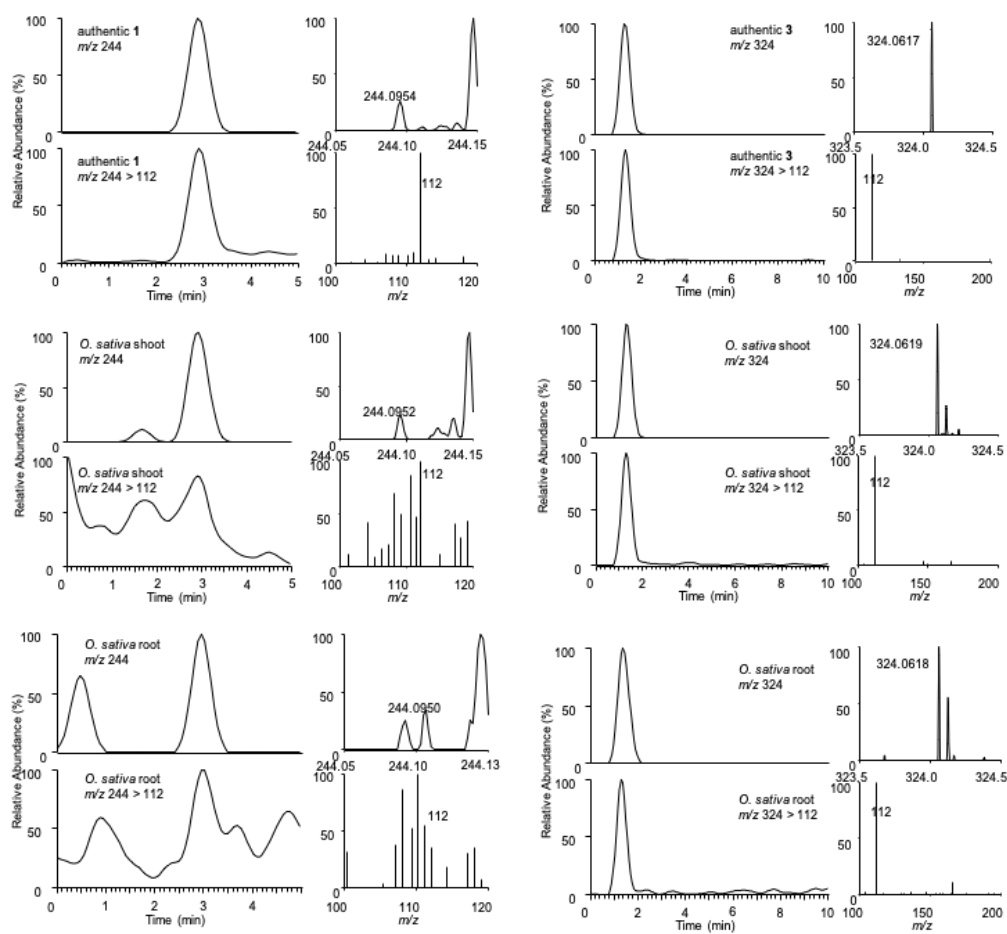
**Figure S10.**  $^1\text{H}$  NMR (a) and  $^{13}\text{C}$  NMR (b) spectra for synthetic **3**.

**Fractionation and UPLC-ESI-Orbitrap MS/MS conditions.** Extraction and fractionation of metabolites **1** and **2** were performed as previously described.<sup>6</sup> The fraction 2 that contained **1** and **3** was dissolved in 120  $\mu$ L of 0.05% formic acid in 80% MeCN and subjected to LC-MS/MS analysis. A Shimadzu UPLC system (Shimadzu, Japan) coupled to an LTQ Orbitrap mass spectrometry (Thermo Fisher Scientific, Waltham, MA, USA) equipped with an electrospray ionization probe was used. MS analysis was performed in the positive FTMS mode at a resolution of 30,000 at  $m/z$  400 with the following source parameters: sheath gas flow, 50; auxiliary gas flow rate, 10; tube lens, 50 V; capillary voltage, 1.0 V; ion spray voltage, 3 kV; capillary temperature, 350 °C. MS spectra were detected by Orbitrap Fourier transform mass spectrometer (Orbitrap FT-MS) and MS/MS spectra were detected by linear ion trap quadrupole mass spectrometer (LTQ-MS). Compounds were identified by exact mass and characteristic transitions (precursor ion to daughter ion). **For 1:** A PC-HILIC column ( $\phi$  2  $\times$  100 mm, 3  $\mu$ m; Shiseido, Japan) was used in the analysis (injection volume, 10  $\mu$ L; solvent, 95% acetonitrile with 0.05% formic acid; flow rate; 0.4 mL/min). **For 3:** A Capcell Pak ADME column ( $\phi$  2.1  $\times$  150 mm, 3  $\mu$ m; Osaka Soda, Japan) was used in the analysis (injection volume, 10  $\mu$ L; solvent, 95% acetonitrile with 0.05% formic acid; flow rate; 0.2 mL/min).

**Cultivation and Detection of 3 in ICA-treated rice.** Seeds of rice (*Oryza sativa* L. cv. Nipponbare) were sterilized with 70% (v/v) ethanol for 1 min and 2.5% (v/v) sodium hypochlorite for 10 min. After washing with distilled water for three times, the seeds were transferred to a covered petri dish (90 mm) with 20 mL of distilled water in clean bench and covered from light by aluminum foil. These seeds were incubated for germination at 28 °C. After 3 days, germinated seeds were sown on a net about 50 seeds per a culture pot ( $\phi$  87  $\times$  176.5 mm) with 100 mL of 0.2 mM ICA in nutrient solution (0.5 mM  $\text{NH}_4\text{NO}_3$ , 0.3 mM  $\text{Na}_2\text{HPO}_4$ , 0.15 mM  $\text{K}_2\text{SO}_4$ , 0.2 mM  $\text{MgCl}_2$ , 0.1 mM  $\text{CaCl}_2$ , 22.5  $\mu$ M Fe-EDTA, 25  $\mu$ M  $\text{H}_3\text{BO}_3$ , 4.5  $\mu$ M  $\text{MnSO}_4$ , 0.15  $\mu$ M  $\text{CuSO}_4$ , 0.35  $\mu$ M  $\text{ZnSO}_4$  and 0.05  $\mu$ M  $\text{Na}_2\text{MoO}_4$ ). The culture pots were placed in an incubator and maintained in a vertical position under dark/light cycles of 8 h/16 h at 28 °C for a week. Rice seedlings were divided into two parts, shoot and root. All tissues were weighed and stored at  $-80$  °C. Detection of **3** was carried out according to the method described above.

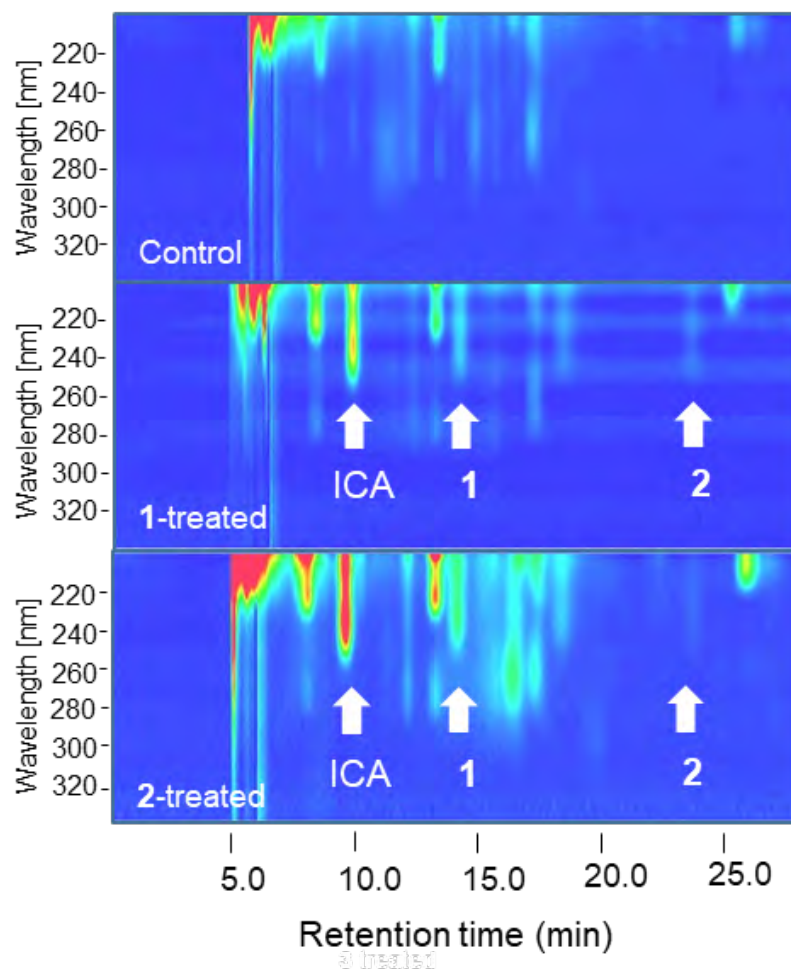


**Figure S11.** Detection of **3** in ICA-treated rice by LC-MS/MS. The charts indicate LC-MS chromatogram for **3** including MS/MS spectra, authentic **3** (a) and shoot and root of ICA-treated rice (b).



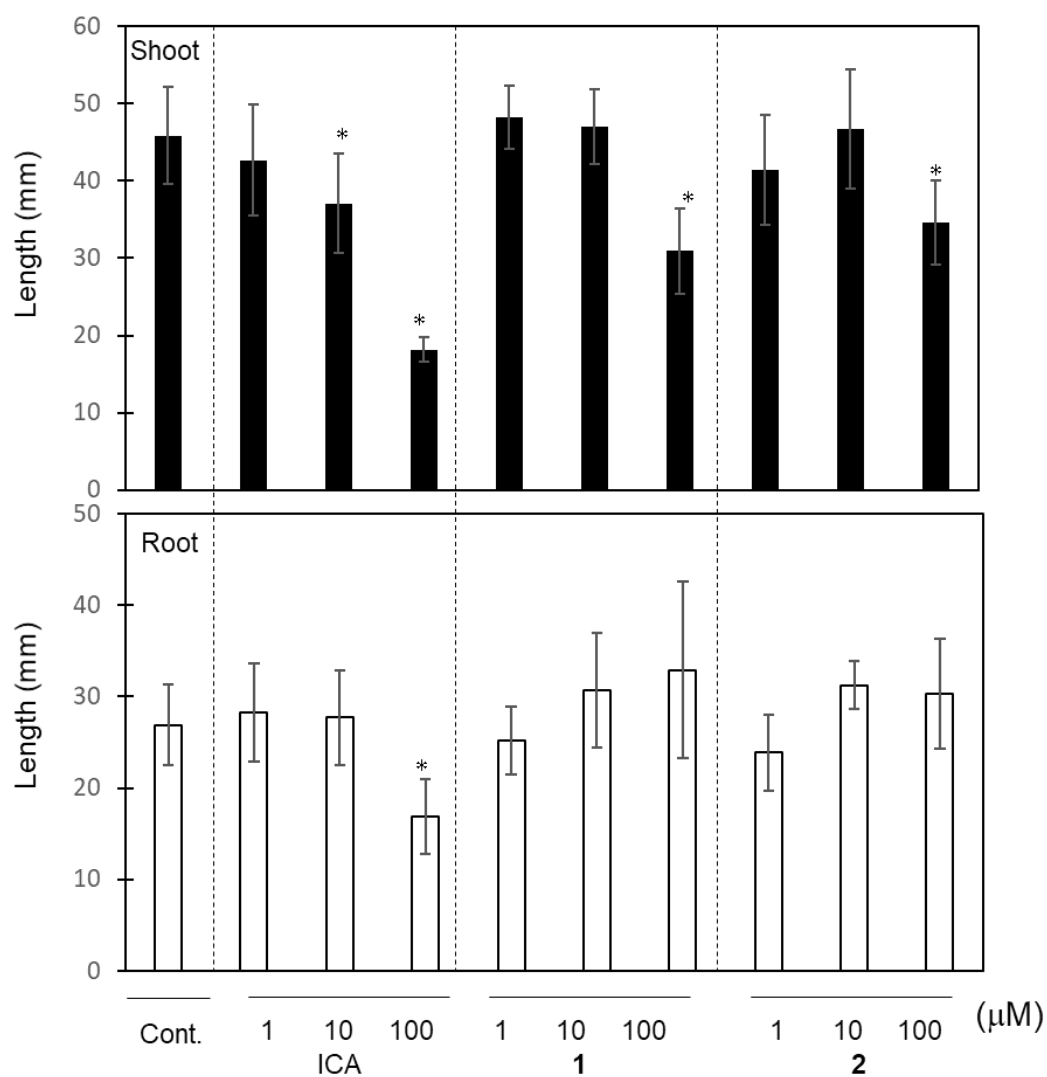
Plant	culture condition	tissue	1	3
rice	aseptic	shoot	d	d
		root	d	d

**Figure S12.** Detection of **1** and **3** in rice by LC-MS/MS analysis.  
d: detected, nd: not detected



**Figure S13.** Metabolism of ICA and its metabolites **1** and **2** in rice. HPLC profiles of extracts of ICA-, **1**- or **2**-treated rice. Treated rice was extracted with EtOH. After being prepared with 5% MeOH at concentration of 20 mg/mL, each sample was injected into HPLC. The extracts were analyzed by reversed-phase HPLC using a Develosil C30-UG-5 column ( $\phi$  4.6  $\times$  250 mm, 5  $\mu$ m) with a gradient manner (5% methanol (B) in (A) for 30 min, 5%-100% B in A for 45 min and 100% B for 30 min) at a flow rate of 0.5 mL/min at a multiple wavelength.

**Bioassay.** Rice seeds (*Oryza sativa* L. cv. Nipponbare) were sterilized in ethanol for 5 min and then 1% sodium hypochlorite for 30 min on a plastic container (18.5 × 14.5 × 4.5 cm). The seeds were washed completely in sterile water and germinated for 2 days at 30 °C with intensive light. The germinated seeds (n = 4) were planted onto test tube (ϕ 5.5 × 10 cm) containing samples (1, 10, 100 μM), and incubated for a week at 30 °C with intensive light. The lengths of the root and shoot were measured to an accuracy of 0.01 mm with an absolute digimatic caliper (Mitutoyo Co., Kawasaki, Kanagawa, Japan).



**Figure S14.** Growth regulatory of ICA, **1** and **2** against rice. Results are the mean ± standard deviation (n = 12). \*p < 0.05