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Supporting Information

Role of hypoxanthine-guanine phosphoribosyltransferase in metabolism of fairy chemicals in rice†

Hirohide Takemura,‡[ab] Jae-Hoon Choi,‡[acde] Keiji Fushimi,[a] Rei Narikawa,[ac] Jing Wu,[de] Mitsuru Kondo,[c] David C. Nelson,[f] Tomohiro Suzuki,[g] Hitoshi Ouchi,[h] Makoto Inai,[h] Hirofumi Hirai,[acde] and Hirokazu Kawagishi*[de]

[a]*Graduate School of Science and Technology,* [c]*Research Institute of Green Science and Technology,* [d]*Faculty of Agriculture,* [e]*Research Institute for Mushroom Science, Shizuoka University, 836 Ohya, Suruga-ku, Shizuoka 422-8529, Japan*

[b]*Research Fellow of Japan Society for the Promotion of Science, 5-3-1 Kojimachi, Chiyoda-ku, Tokyo 102-0083, Japan*

[f]*Department of Botany and Plant Sciences, University of California, Riverside, California 92521, USA*

[g]*Center for Bioscience Research and Education, Utsunomiya University, 350 Minemachi, Tochigi 321-8505, Japan*

[h]*Department of Pharmaceutical Sciences, University of Shizuoka, 52-1 Yada, Suruga-ku, Shizuoka 422-8526, Japan*

*Corresponding author: kawagishi.hirokazu@shizuoka.ac.jp.

‡ These authors contributed equally to this work.

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Materials and Methods

RNA isolation and cDNA synthesis.

Total RNA was extracted from the shoot and root of rice using the RNeasy plant mini kit (Qiagen). First, Rice seedlings were divided into two parts, shoot and root, these tissues were placed in a mortar. Liquid nitrogen was added, and the tissue was pulverized. The subsequent procedures for the isolation of total RNA were carried out according to the manufacturer's instructions. The RNA was then used for cDNA synthesis using Prime Script RT Reagent Kit (Perfect Real Time) (Takara Bio) following the manufacturer's protocol. The quality and concentration of the RNA samples were determined by gel electrophoresis and NanoDrop One spectrophotometer (Thermo Fisher Scientific).

Cloning of the full-length cDNA of OsHGPRT and construction of expression vector.

The *Oryza sativa* hypoxanthine-guanine phosphoribosyltransferase gene (*Oshgprt*) was searched based on The Rice Annotation Project Database (RAP-DB: https://rapdb.dna.affrc.go.jp/index.html). Primers were designed to replicate *Oshgprt* and to clone *Oshgprt* into the pET-28a(+) vector (Novagen) with a histidine tag at the N-terminal. The DNA fragment corresponding to *Oshgprt* (783 bp) was amplified from cDNA using the primers 5'-CGC GGC AGC CAT ATG CAG AAT TTG GGA ATC CCA-3' (forward primer) and 5'-CTC GAA TTC GGA TCC CTA ATT GCT TGT ATC TTT TTT GTA-3' (reverse primer) and PrimeSTAR Max DNA polymerase (Takara Bio) following cycling conditions: 40 cycles of denaturation at 98 °C for 10 sec, annealing at 45 °C for 15 sec, and elongation at 72 °C for 20 sec in a thermal cycler (Bio-Rad). The amplification of the DNA fragment for constructing a protein expression vector was performed using the primers 5'-GGA TCC GAA TTC GAG CTC-3' (forward primer) and 5'-CAT ATG GCT GCC GCG CGG-3' (reverse primer), and PrimeSTAR Max DNA polymerase. This PCR product was ligated into the pET-28a(+) vector digested with the restriction enzyme *Bam*H I and *Nde* I using the Gibson assembly system (New England BioLabs). The expression vector was then introduced into *E. coli* JM109 competent cells (Takara Bio) by heat shock at 42°C for 45 sec and transformants were screened at 37°C on LB agar medium with 50 μg/mL kanamycin. Constructs plasmids were verified by electrophoresis on an agarose gel and nucleotide sequencing (Macrogen) after the isolation of the plasmids by using HiYield Plasmid Mini Kit (RBC Bioscience) as manufacturer's instruction.

Heterologous Expression and Purification of OsHGPRT.

For heterologous expression of OsHGPRT, the recombinant plasmid was transformed into *E. coli* BL21-CodonPlus (DE3)-RIPL competent cells (Novagen) by heat shock at 42°C for 45 sec, and positive colonies were screened at 37°C on LB agar medium containing 50 μg/mL kanamycin, 20 μg/mL spectinomycin, and 20 μg/mL chloramphenicol (LB-KAN/SPT/CHL). Individual positive

colonies were grown overnight at 37 °C with shaking at 220 rpm in 2 mL of LB-KAN/SPT/CHL medium. The overnight culture was then inoculated into 1 L of fresh LB-KAN/SPT/CHL medium. The cells were grown at 37 °C with shaking at 220 rpm until the optical density at 600 nm (OD_{600}) of the cells reached between 0.4–0.8, and then isopropyl β-D-1-thiogalactopyranoside (IPTG) was added to the medium at a final concentration of 0.1 mM to induce protein expression. Subsequently, the cells were cultured at 18 °C for 20 h, harvested by centrifugation at 8,000 × *g* for 10 min at 4 °C, and stored at −80 °C for further experimental use.

The cells were resuspended in 10 mL (g cells) of buffer A (20 mM HEPES-NaOH buffer, 500 mM NaCl and 10% (v/v) glycerol, pH 7.0) and were disrupted by sonication for 20 min (output 60, on/off = 10 sec/10 sec) by TOMY sonicator UD-100 (Tomy Seiko) on ice. Then, cell debris was pelleted by centrifuging at 10,000 × *g* for 30 min at 4 °C and the supernatant was filtered with 0.45 μm cellulose acetate membrane filter. The filtrate was subjected to Ni affinity column chromatography with an NGC Chromatography System (Bio-Rad) using His-trap HP column (1 mL, GE Healthcare) and eluted with a linear gradient of 30–500 mM imidazole in the buffer A at the flow rate of 1.0 mL/min. The elution was monitored at 280 nm to detect the protein peak. Peak protein fractions, pellets, and supernatant were used to analyze by 12.5% (w/v) polyacrylamide SDS-PAGE. The protein eluted fractions were mixed and dialyzed three times against a 100-fold volume of buffer A at 4 °C for a total of 12 h. Protein concentration was determined using Pierce 660 nm protein assay reagent (Thermo Fisher Scientific) according to the manufacturer's instructions with bovine serum albumin as a standard.

Enzyme activity of recombinant OsHGPRT.

The phosphoribosylation reaction was performed by the following procedure. 200 μM substrates (hypoxanthine, AHX, AOH, ICA, and AICA) were preincubated with 1 mM PRPP, 10 mM $MgCl_2$ in 20 mM HEPES-NaOH buffer (pH 7.0) for 10 min at 30 °C before initiating the enzymatic reaction. 5 μM (final concentration) rOsHGPRT or buffer A (negative control) were added to each solution to start the reaction and incubated at 37 °C for 10 min. The total reaction volume was 100 μL. The reactions were stopped by adding half volume of MeOH, centrifuged at 15,000 × *g* for 15 min, and analyzed by LC-MS. In the dephosphoribosylation reaction, 200 μM substrates (IMP, AHXR, **1**, ICAR, AICAR), 1 mM pyrophosphate, 10 mM $MgCl_2$ were used, and the reaction was performed under the same conditions as described above.

Further assay for substrate specificity, eleven different substrates (hypoxanthine, guanine, adenine, xanthine, uric acid, 8-azahypoxanthine, allopurinol, AHX, AOH, ICA, and AICA) were used. The reactions were proceeded at 10 nM enzyme (final concentration) at a reaction temperature of 30°C

and reaction time of 1 h.

The conversion rates were calculated from the decrease in substrate relative to the negative control using a Waters ACQUITY H-class UPLC system (Waters) connected to a Waters Xevo TQ-S micro mass spectrometer equipped with an electrospray ionization probe (Waters). MS and MS/MS spectra were detected by tandem quadrupole mass spectrometer (UPLC-ESI-qMS/MS). A CAPCELL PAK ADME column (ϕ 2.1 mm × 150 mm; Shiseido) was used in the analysis (injection volume, 2 μL; column oven, 40°C; solvent, 2% MeOH with 10 mM ammonium formate; flow rate, 0.4 mL/min). The MS was set to acquire a full MS scan simultaneously in the positive and negative ion modes with a mass range of *m/z* 50–500. Ion source conditions were as follows: capillary voltage, 3.50 kV (+) / 2.50 kV (−); cone voltage, 30 V (+) /30 V (−); desolvation temperature, 500°C; desolvation gas flow, 1000 L/Hr; cone gas flow, 50 L/Hr. Data acquisition and analysis were performed using MassLinx software (Waters).

Synthesis of AOH-ribonucleotide by recombinant OsHGPRT.

5 μM rOsHGPRT was incubated with 600 μM AOH (4.6 mg), 1 mM PRPP, 10 mM $MgCl_2$ in 20 mM HEPES-NaOH buffer (pH 7.0) for 12 h at 30 °C. The reaction was performed in a total volume of 50 mL. The enzyme was inactivated by the addition of half volume of MeOH and centrifuged at 15,000 × *g* for 15 min. The supernatant was applied to the Oasis HLB column (6 cc/150 mg; Waters) activated with MeOH. After removal the solvent under reduced pressure, the residue was extracted three times with MeOH and loaded to the HPLC column. Preparative HPLC was performed with a Jasco Gulliver system equipped with a PDA detector (Jasco), using a reverse-phase HPLC column (Develosil C30-UG-5; ϕ 20 mm × 250 mm; Nomura Chemical) with 2% MeOH in 0.1% TFA at 40 °C to obtain 5 fractions (fractions 1–5). All the fractions were subsequently subjected to UPLC-ESI-qMS/MS and fraction 2 was further purified as the same conditions to yield **1** (2.1 mg).

Structure determination of compound 1.

Compound **1** was dissolved in D_2O for nuclear magnetic resonance (NMR) spectroscopic analysis. ^{1}H-NMR (500 MHz), ^{13}C-NMR (125 MHz), distortionless enhancement by polarization transfer (DEPT), correlation spectroscopy (COSY), heteronuclear multiple bond correlation (HMBC), and heteronuclear multiple quantum correlation (HMQC) spectra were recorded on a JEOL lambda-500 spectrometer (JEOL). Coupling constants (J values) were measured in Hertz (Hz) and chemical shifts (δ values) are given in parts per million (ppm).

Compound **1**: colorless liquid; HRESIMS *m/z* 366.0480 $[M+H]^+$; ^{1}H-NMR (in D_2O) δ_H: 3.81 (1H, m), 3.91 (1H, m), 4.12 (1H, m), 4.43 (1H, br dd), 4.65 (1H, dd), 6.35 (1H, d); ^{13}C-NMR (in D_2O) δ_C: 65.6, 70.9, 74.4, 84.0, 91.5, 114.0, 142.5, 150.3, 154.9.

ALP enzyme reaction.

Alkaline phosphatase (ALP) enzyme reaction was performed under the following procedures. ALP with activities of 0.5 U was added to the 10 mL of 100 mM Tris-HCl buffer (pH 9.0) containing 0.55 mM AOHR (2.0 mg) and 2 M NaCl. The mixture was incubated for 6 h at 37 °C and then half volume of MeOH was added to stop the reaction. The solution was passed through an Oasis HLB column (6 cc/150 mg; Waters) activated with MeOH and **2** (1.2 mg) was purified by reverse-phase HPLC as described above.

X-ray crystallographic analysis of 2.

Compound **2** was crystallized in H_2O. Single crystal was mounted on MiTeGen loop with Paraton-N (Hampton Research), and was flash frozen to 173 K in a liquid nitrogen cooled stream of nitrogen. Data collection was carried out on a Rigaku XtaLAB Synergy-S diffractometer using multi-layer mirror monochromated Cu microfocus sealed X-ray source (50 W) and a HyPix-6000 Hybrid Photon Counting (HPC) detector. Data collection and reduction were made using CrysAlis PRO softwater package. The structure was solved by direct method, ShelXT, and refined using the SHELX97 tool. All of the non-hydrogen atoms were refined anisotropically. The hydrogen atoms were placed in calculated positions and allowed to ride on the carrier atoms. The absolute structure was estimated based on the Flack parameter. Crystallographic data have been deposited at The Cambridge Crystallographic Data Centre and allocated the deposition number CCDC2091606. The data can be obtained free of charge via www. ccdc.cam.ac.uk/produts/csd/request. The size of the crystal used for measurements was 0.32×0.28×0.08 mm. Crystal data and the detailed structure determination of **2** is summarized in Table S3.

Cell culture and plant growth conditions.

The rice cell line *Oryza sativa* L. (C5928) was obtained from the RIKEN Bioresource Center (Tsukuba, Japan). Cells were cultivated in 50 mL of liquid medium (Murashige and Skoog (MS) salts, 30 g/L sucrose, 0.5 mg/L nicotinic acid, 0.5 mg/L pyridoxine·HCl, 0.1 mg/L thiamine·HCl, 2.0 mg/L glycine, 0.1 g/L *myo*-inositol, 1.0 mg/L 2,4-dichlorophenoxyacetic acid, pH 5.7) at 25 °C in the dark on an orbital shaker at 120 rpm. The cells were subcultured once a week. The harvested cells were

weighed and stored at −80 °C.

Threshed seeds of rice (*Oryza sativa* L. cv. Nipponbare) were sterilized with 70% (v/v) ethanol for 2 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. Germination was performed by incubating at 28 °C for 3 days in the dark. The germinated seeds were sown in a culture pot (φ 87 × 176.5 mm) with 5 g of perlite and 100 mL of half-strength of nutrient solution (1 mM NH_4NO_3, 0.6 mM Na_2HPO_4, 0.3 mM K_2SO_4, 0.4 mM $MgCl_2$, 0.2 mM $CaC1_2$, 45 μM Fe-ethylenediaminetetraacetic acid, 50 μM H_3BO_3, 9 μM $MnSO_4$, 0.3 μM $CuSO_4$, 0.7 μM $ZnSO_4$ and 0.1 μM Na_2MoO_4) contained with or without 100 μM AHXR. The culture pots were 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.

Extraction, fractionation, and LC-MS/MS analysis for AHXR, AHXr, AOH *N*1-R, and AOH *N*1-r from rice tissue.

The extraction and fractionation were performed according to the method as reported previously.[20] The Frozen tissues (about 100 mg FW) were added to a 2 mL centrifuge tube filled 1 mL of extraction solvent (MeOH: formic acid: water = 15: 1: 4) and crushed tissues using a Micro Smash (4,500 rpm, 3 min) with 3 stainless beads (diameter, 3.2 mm). After centrifugation at 10,000 × g for 10 min, the supernatant was transferred to a new 2 mL microtube. The pellet was re-extracted with 1 mL of extraction solvent and combined with the first supernatant (approximately 2.0 mL). The medium after incubation was concentrated under reduced pressure and extracted with extraction solvent. To remove interfering compounds, the extract was first passed through an Oasis HLB 1 cc/30 mg (Waters) equilibrated with an extraction solvent. After removing the solvent of the extracts under reduced pressure, the residue was reconstituted with 0.5 mL of 2% formic acid. The solution was loaded an Oasis MCX 1 cc/30 mg (Waters) conditioned with MeOH and equilibrated with 0.1 N hydrochloric acid, and sequentially eluted with 1 mL of 0.1 N hydrochloric acid (fraction 1), 1 mL of MeOH (fraction 2), and then 1 mL of 5% ammonia (fraction 3), and each fraction was evaporated to dryness. All the fractions were dissolved in 120 μL of 0.05% formic acid in 80% acetonitrile and subjected to LC-MS/MS analysis.

A Shimadzu UPLC system (Shimadzu) coupled to an LTQ Orbitrap mass spectrometry (Thermo Fisher Scientific) equipped with an electrospray ionization probe was used. MS spectra were detected by Orbitrap Fourier transform mass spectrometer and MS/MS spectra were detected by linear ion trap mass spectrometer (UPLC-ESI-Orbitrap MS/MS). Compounds were identified by the exact mass and characteristic transitions (precursor ion to daughter ion). A CAPCELL PAK ADME column (ϕ 2.1

mm × 150 mm; Shiseido) was used in the analysis (injection volume, 5 μL; column oven, 40°C; solvent, 2% MeOH with 10 mM ammonium formate; flow rate, 0.4 mL/min). The source parameters of MS and MS/MS analysis were performed as follows. For AHXR; analysis in the positive ion 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, 70 V; capillary voltage, 24 V; ion spray voltage, 3 kV. The characteristic transition was monitored at *m/z* 350 > 138. For AHXr; analysis in the negative ion 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, −67 V; capillary voltage, −17 V; ion spray voltage, 3 kV. The characteristic transition was monitored at *m/z* 268 > 240. Data acquisition and analysis were performed using Xcalibur software (Thermo Fisher Scientific).

Enzyme preparation, assay, and LC-MS/MS analysis.

Ten grams of frozen rice cells was lysed with 10 mL of lysis 20 mM Tris-HCl buffer (pH 7.4) and sonicated for 20 min (output 60, on/off = 10 sec/10 sec) by TOMY sonicator UD-100 on ice on ice. Then, cell debris was pelleted by centrifuging at 10,000 × *g* for 30 min at 4 °C and the supernatant was filtered with 0.45 μm cellulose acetate membrane filter. The filtrate was subjected to anion exchange chromatography with an NGC Chromatography System using Macro-Prep DEAE Cartridge (Bio-Rad) and eluted from the column using increasing ionic strengths by a step gradient of NaCl (0.2, 0.5, and 1.0 M) in the Tris-HCl buffer at the flow rate of 5.0 mL/min. The elution was monitored at 280 nm to detect the protein peak, protein eluted fractions were dialyzed three times against a 100-fold volume of Tris-HCl buffer (pH 7.4) at 4 °C for a total of 12 h. Protein concentration was determined using Pierce 660 nm protein assay reagent according to the manufacturer's instructions with bovine serum albumin as a standard.

Assays were performed to confirm the enzymatic activity production of AHXR and AHXr. 0.2 mM AHX or AHXR were preincubated with 1 mM PRPP, 10 mM $MgCl_2$ in 20 mM Tris-HCl buffer (pH 7.4) for 10 min at 30 °C before initiating the enzymatic reaction. 10 mg of enzyme solution or Tris-HCl buffer (negative control) was added to each solution to start the reaction and incubated for 30 min or 12 h at 30 °C. The total reaction volume was 100 μL. The reactions were stopped by adding an equal volume of MeOH, centrifuged at 15,000 × *g* for 15 min, and analyzed by UPLC-ESI-qMS/MS and UPLC-ESI-Orbitrap MS/MS.

A CAPCELL PAK ADME column (ϕ 2.1 mm × 150 mm; Shiseido) was used in the UPLC-ESI-qMS/MS analysis (injection volume, 2 μL; column oven, 40°C; solvent, 2% MeOH with 10 mM ammonium formate; flow rate, 0.2 mL/min). The source parameters of MS/MS analysis were performed as follows; capillary voltage, 3.50 kV (ESI+) and 2.50 kV (ESI−); desolvation temperature,

500℃; desolvation gas flow, 1000 L/Hr; cone gas flow, 50 L/Hr. The characteristic transition was monitored for AHX (ESI+, *m/z* 138 > 67; cone voltage, 26 V; collision energy, 16 eV), for AHXr (ESI−, *m/z* 268 > 240; cone voltage, 2 V; collision energy, 6 eV), for AHXR (ESI+, *m/z* 350 > 138; cone voltage, 16 V; collision energy, 16 eV). Data acquisition and analysis were performed using MassLinx software (Waters).

Non-enzymatic assembly of the triazine ring.

200 μM AICA analogues (AICA, AICAr, AICAR) were preincubated in ultrapure water (pH not adjusted) for 10 min at 30 °C before initiating the chemical reaction. 1 mM NOC7 (solved in 0.1 M NaOH) or 0.1 M NaOH (negative control), 20 mM carboxy-PTIO (cPTIO) or ultrapure water (negative control) were added to each solution. The reaction was started by adding 0.1 M HCl and rotated for 1 h at 30 °C. The total reaction volume was 100 μl. The reaction solutions were directly analyzed by HPLC. Analytical HPLC was performed with a Jasco Gulliver system equipped with a PDA detector (Jasco), using a reverse-phase HPLC column (Develosil C30-UG-5; ϕ 4.6 mm × 250 mm; Nomura Chemical) with 2% MeOH in 0.1% TFA at 40 °C.

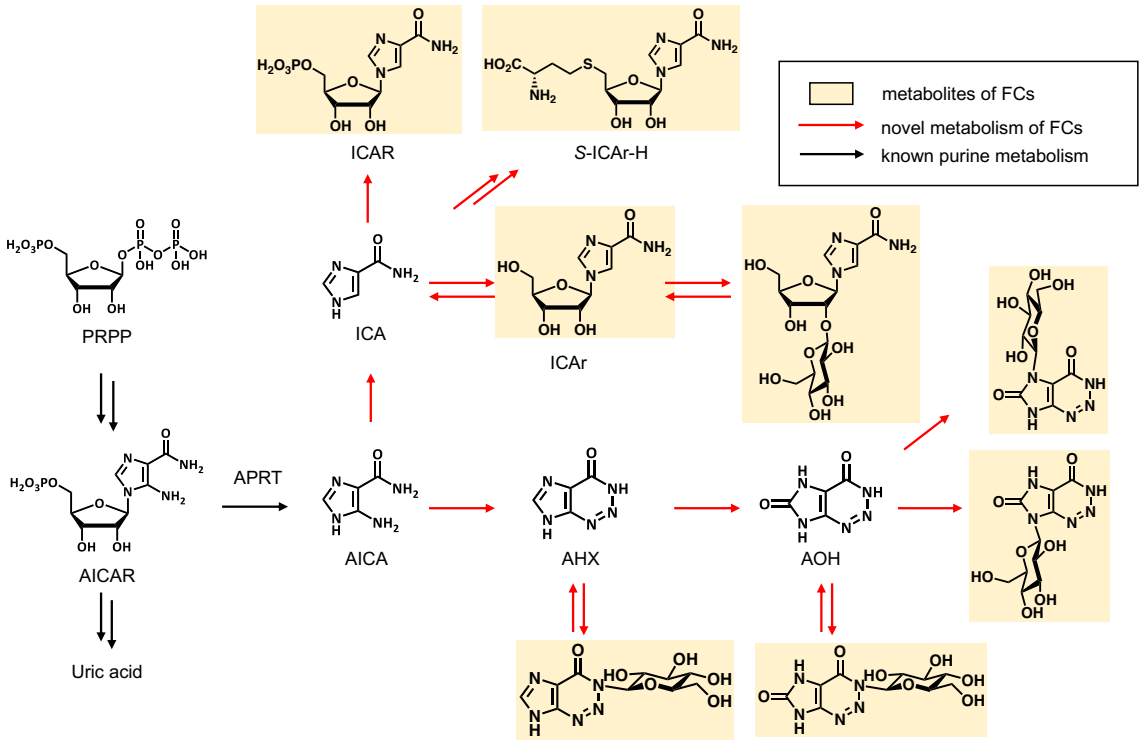


Fig. S1. Biosynthetic and metabolic pathways of fairy chemicals in rice.
Fairy chemicals indicate 2-azahypoxanthine (AHX), imidazole-4-carboxamide (ICA) and 2-aza-8-oxohypoxanthine (AOH). PRPP, 5-phosphoribosyl-1-pyrophosphate; AICARFT, AICAR formyltransferase; FAICAR, *N*-formyl-AICAR; IMP, inosine monophosphate; XMP, xanthosine monophosphate; XOD, xanthine oxidase; XDH, xanthine dehydrogenase.

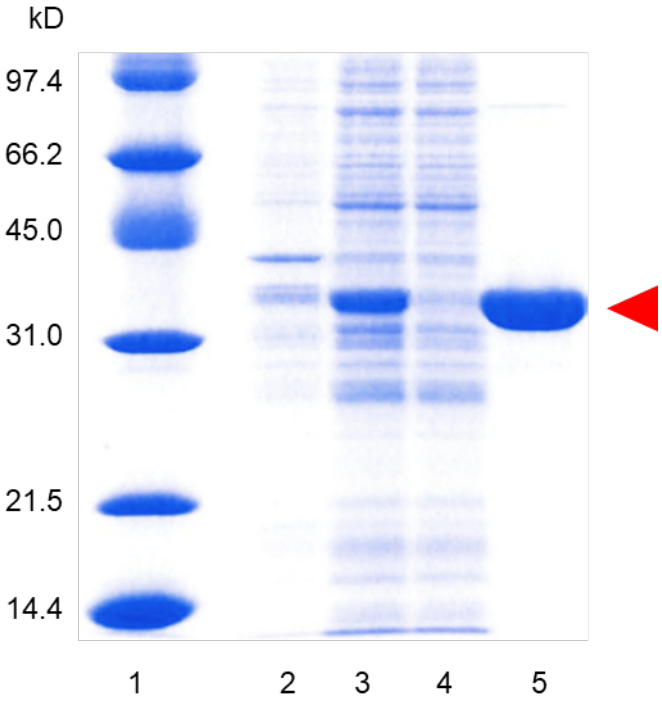
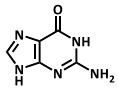
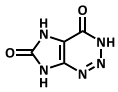
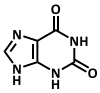
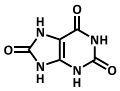


Fig. S2. Expression and purification of recombinant OsHGPRT.
rOsHGPRT was expressed as a protein with His-tag added to the N-terminal and was purified by Ni^{2+} affinity column chromatography. The total cell lysate and samples at each purification step were electrophoresed on SDS-PAGE using 10% polyacrylamide gel and stained by CBB. The enzyme corresponded to theoretical prediction based on cDNA sequences. Lane 1, protein marker; lane 2, insoluble fraction of cells expressing *OsHGPRT*; lane 3, soluble fraction of cells expressing *OsHGPRT* ; lane 4, unbound fraction of the Ni^{2+} affinity column; lane 5 bound fraction of the Ni^{2+} affinity column. The red triangle shows the protein of recombinant OsHGPRT.

Table S1. Conversion rates of phosphoribosylation for the substrates by rOsHGPRT.
Data are presented as means ± S.D. (n = 3).

substrates	conversion rate	structure
allopurinol	100.0 ± 0.0	
8-azahypoxanthine	94.7 ± 0.7	
hypoxanthine	93.3 ± 0.5	
guanine	92.8 ± 1.0	
AHX	75.7 ± 3.4	
AOH	38.0 ± 3.5	
xanthine	19.9 ± 3.0	
ICA	6.0 ± 2.0	
AICA	5.8 ± 1.7	
uric acid	2.9 ± 1.3	
adenine	1.4 ± 0.9	

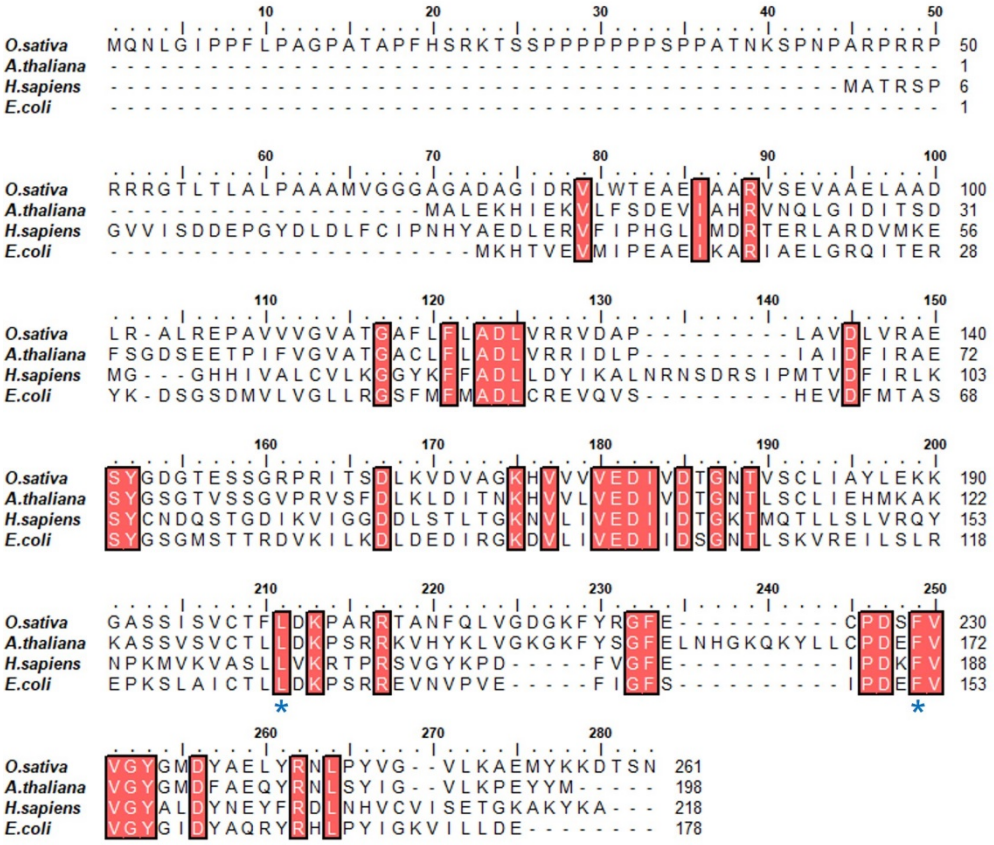


Fig. S3. Multiple alignment of amino acid sequences of HGPRT.
The alignment was carried out with ClustalW. Conserved identical amino acid residues are indicated by red boxes and amino acid residues that specifically affect substrate recognition are marked by blue asterisks.

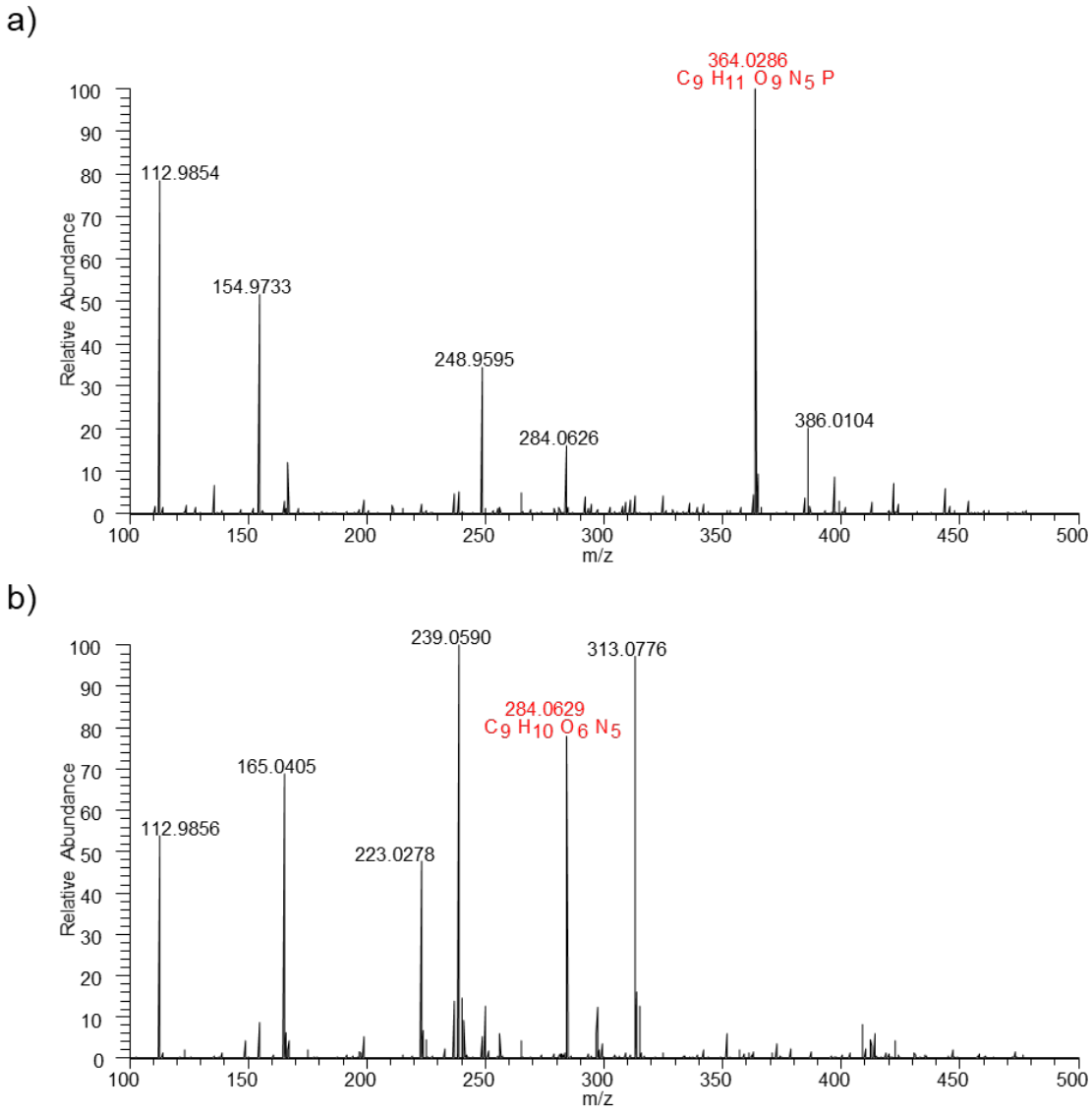


Fig. S4. MS spectrum for compound 1 (a) and compound 2 (b).
MS spectrum was obtained using MS scan (m/z 50–600) in the negative ion mode. The molecular weight in red indicates the molecular weight consistent with AOHR (*m/z* 364.0286 $[M-H]^-$; calcd for $C_9H_{11}N_5O_9P$, 364.0294) and AOHr (*m/z* 284.0629 $[M-H]^-$; calcd for $C_9H_{10}N_5O_6$, 284.0631).

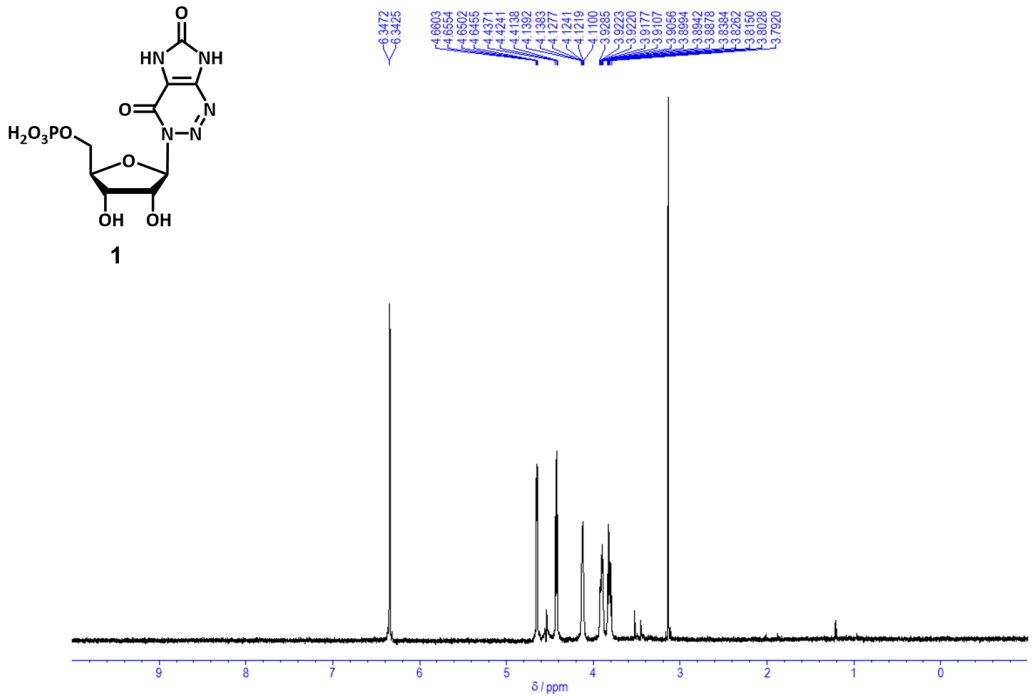


Fig. S5. ^{1}H NMR spectrum for compound 1 (D_2O).

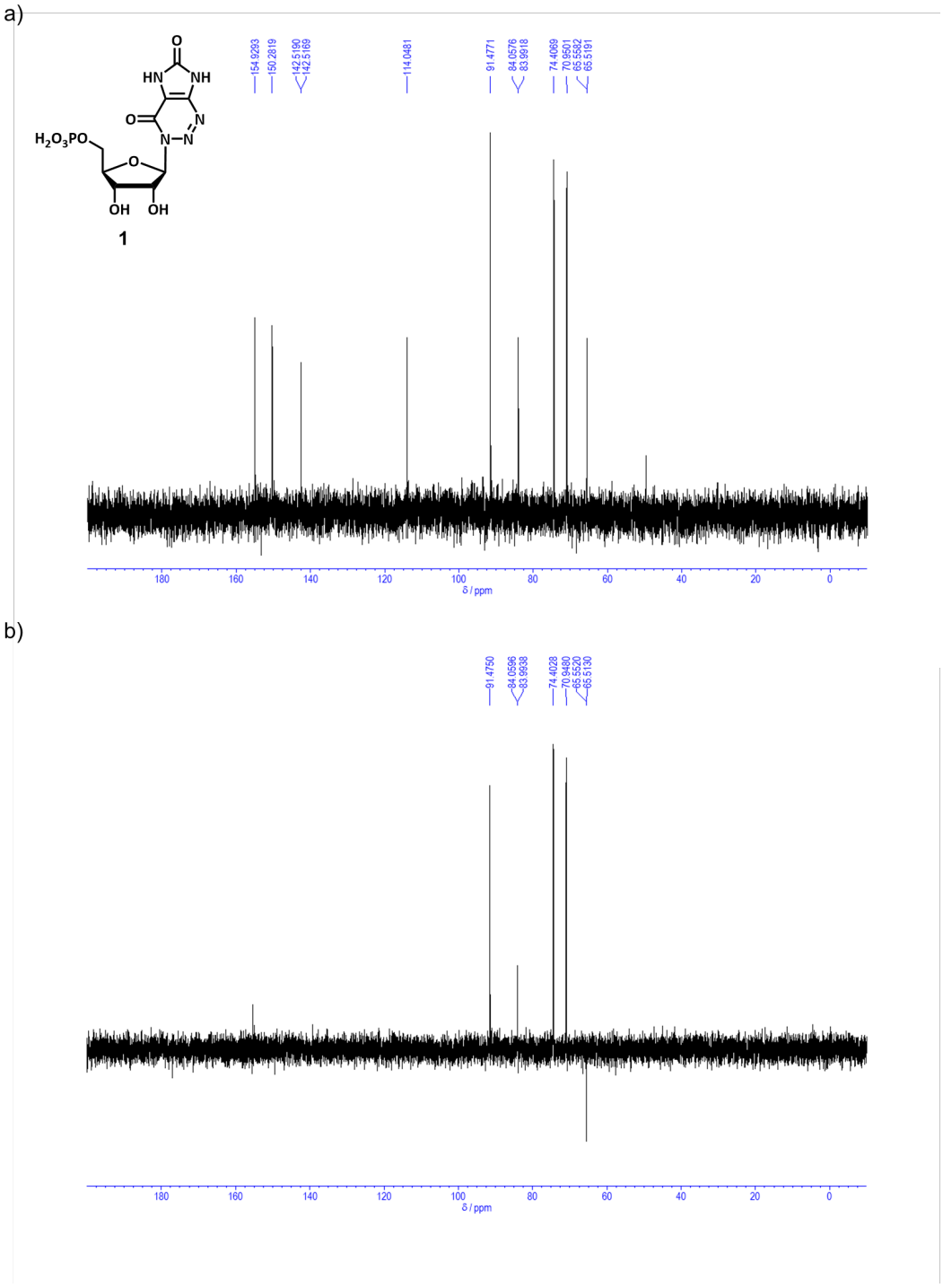


Fig. S6. ^{13}C NMR (a) and DEPT (b) spectra for compound 1 (D_2O).

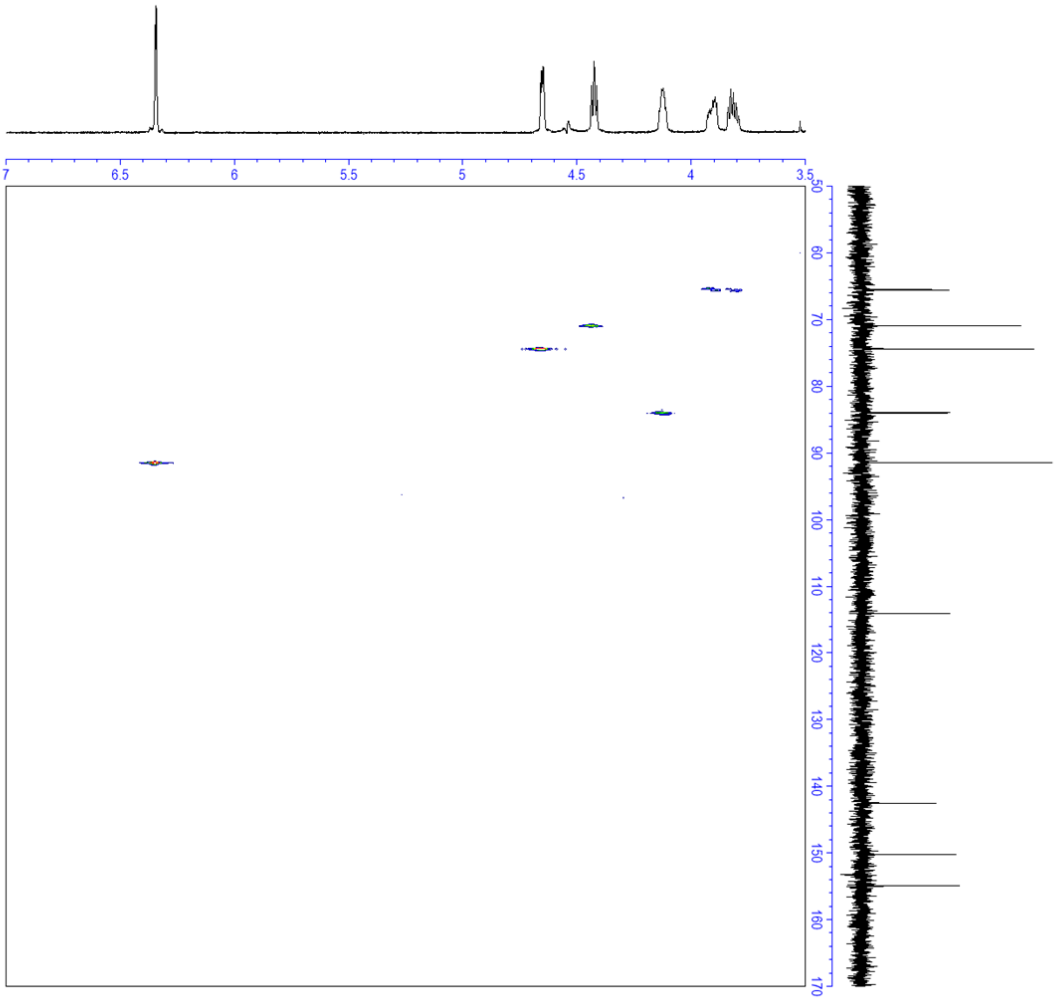


Fig. S7. HMQC spectrum for compound 1 (D_2O).

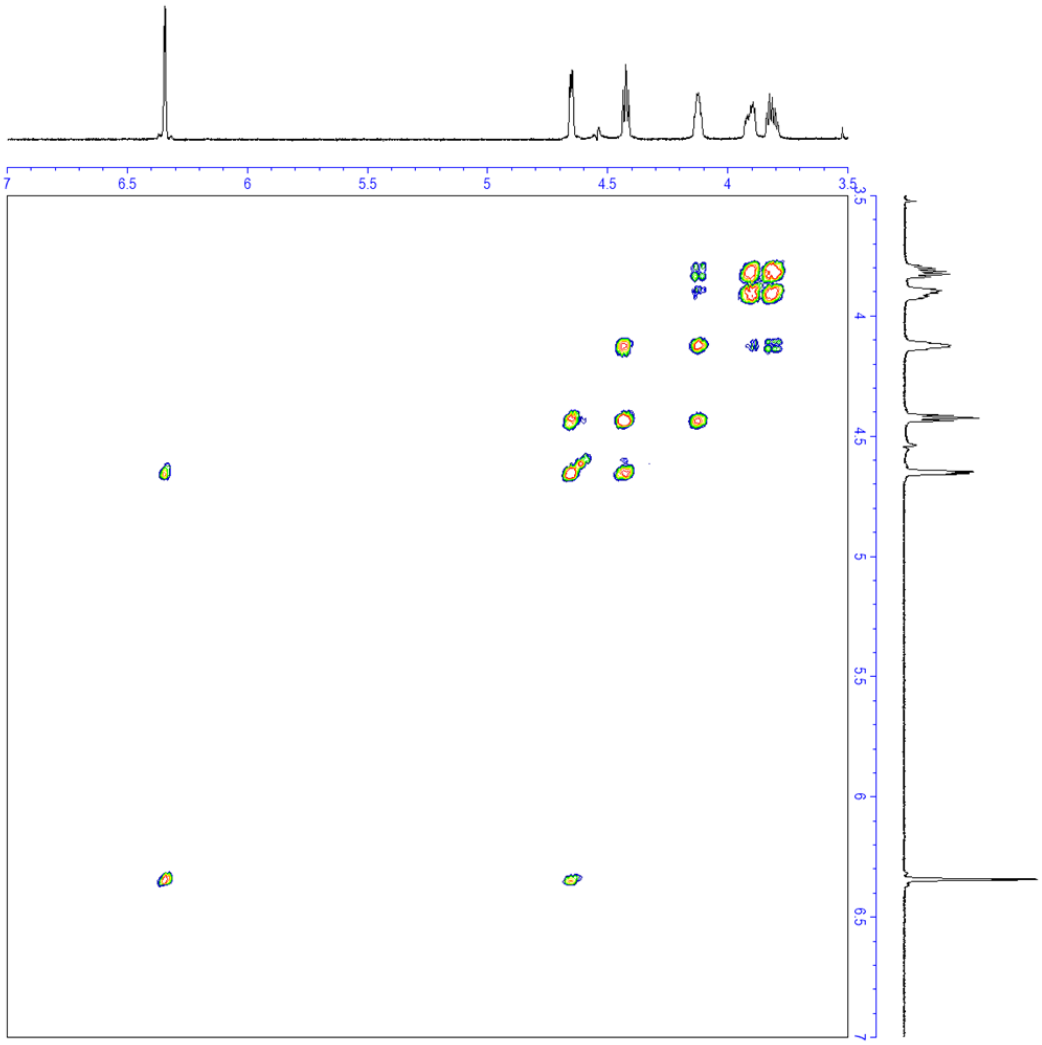


Fig. S8. DQF-COSY spectrum for compound 1 (D_2O).

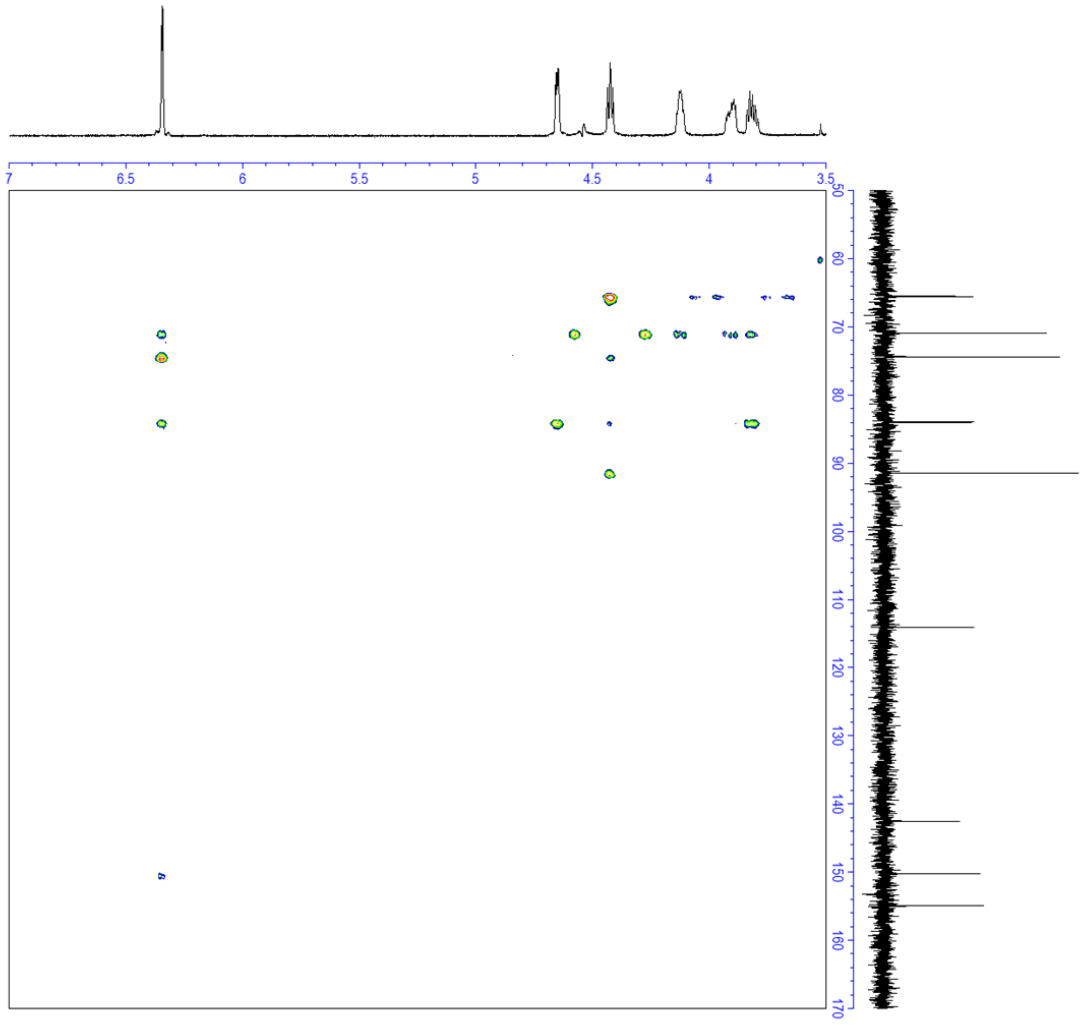


Fig. S9. HMBC spectrum for compound 1 (D_2O).

Table S2. ^{1}H and ^{13}C NMR Data for 1.
Data were recorded at 500 MHz (^{1}H) and 125 MHz (^{13}C) in D_2O.

	^{13}C	^{1}H				
Position	δ (ppm)	δ (ppm)	multiplicity	*J* value (Hz)	HMBC correlation	COSY correlation
4	142.5					
5	114.0					
6	150.3					
8	154.9					
1’	91.5	6.35	d	2.4	6, 2’, 3’, 4’	2’
2’	74.4	4.65	dd	2.7 5.2	4’	1’, 3’
3’	70.9	4.43	dd	5.5 6.4	1’, 2’, 4’, 5’	2’, 4’
4’	84.0	4.12	dd	5.5 8.9	3’	3’, 5’
5’	65.5	3.81 3.91	m m		3’, 4’	4’

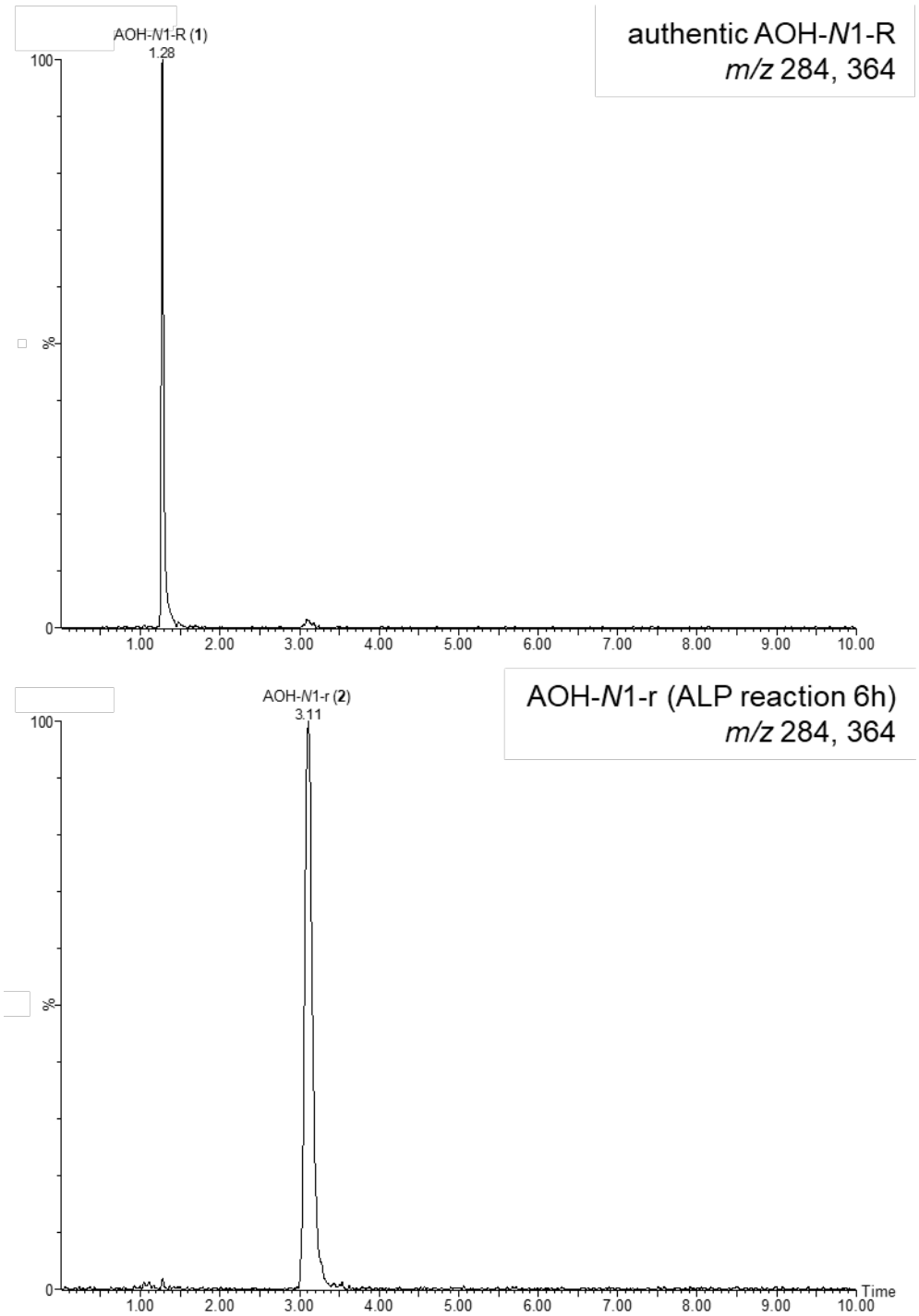


Fig. S10. Extracted ion chromatogram of the reaction solution with alkaline phosphatase (ALP) and AOH-*N*1-R (1).

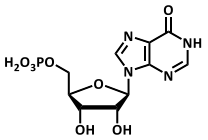
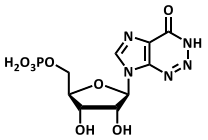
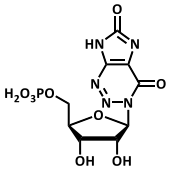
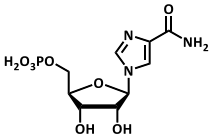
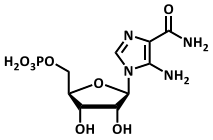
The reaction was carried out with buffer with ALP at 37°C for 6 h. The chromatogram shows AOH-*N*1-R (**1**; *m/z* 364 $[M-H]^-$) and AOH-*N*1-r (**2**; *m/z* 284 $[M-H]^-$).

Table S3. Crystallographic data of 2.

	2
Formula	$C_9H_{11}N_5O_6 \cdot H_2O$
Formula weight	303.23
Crystal system	monoclinic
Space Group	*C*2
a /Å	19.32005(12)
b /Å	5.34244(2)
c /Å	11.81711(4)
α /°	90
β /°	98.3058(4)
γ /°	90
V /Å^3	1206.924(9)
Z	2
D_x, gcm^{-3}	1.669
F(000)	632.0
μ /cm^{-1}	1.263
Temperature/K	173.00(10)
reflections collected	53776
unique reflections	
parameters refined	196
R_1 ($I > 2\sigma(I)$)	0.0288
wR_2 (all data)	0.0784
GOF	1.064

$R_1 = \Sigma||F_o|-|F_c||/\Sigma|F_o|$. $wR_2 = [\Sigma w(F_o^2-F_c^2)^2/\Sigma w(F_o^2)^2]^{1/2}$.

Table S4. Conversion rates of dephosphoribosylation for the substrates by rOsHGPRT.
Data are presented as means ± S.D. (n = 3).

substrates	conversion rate	structure
IMP	1.4 ± 0.3	
AHXR	10.8 ± 2.0	
AOHR (**1**)	8.1 ± 2.2	
ICAR	5.1 ± 1.1	
AICAR	7.2 ± 1.1	

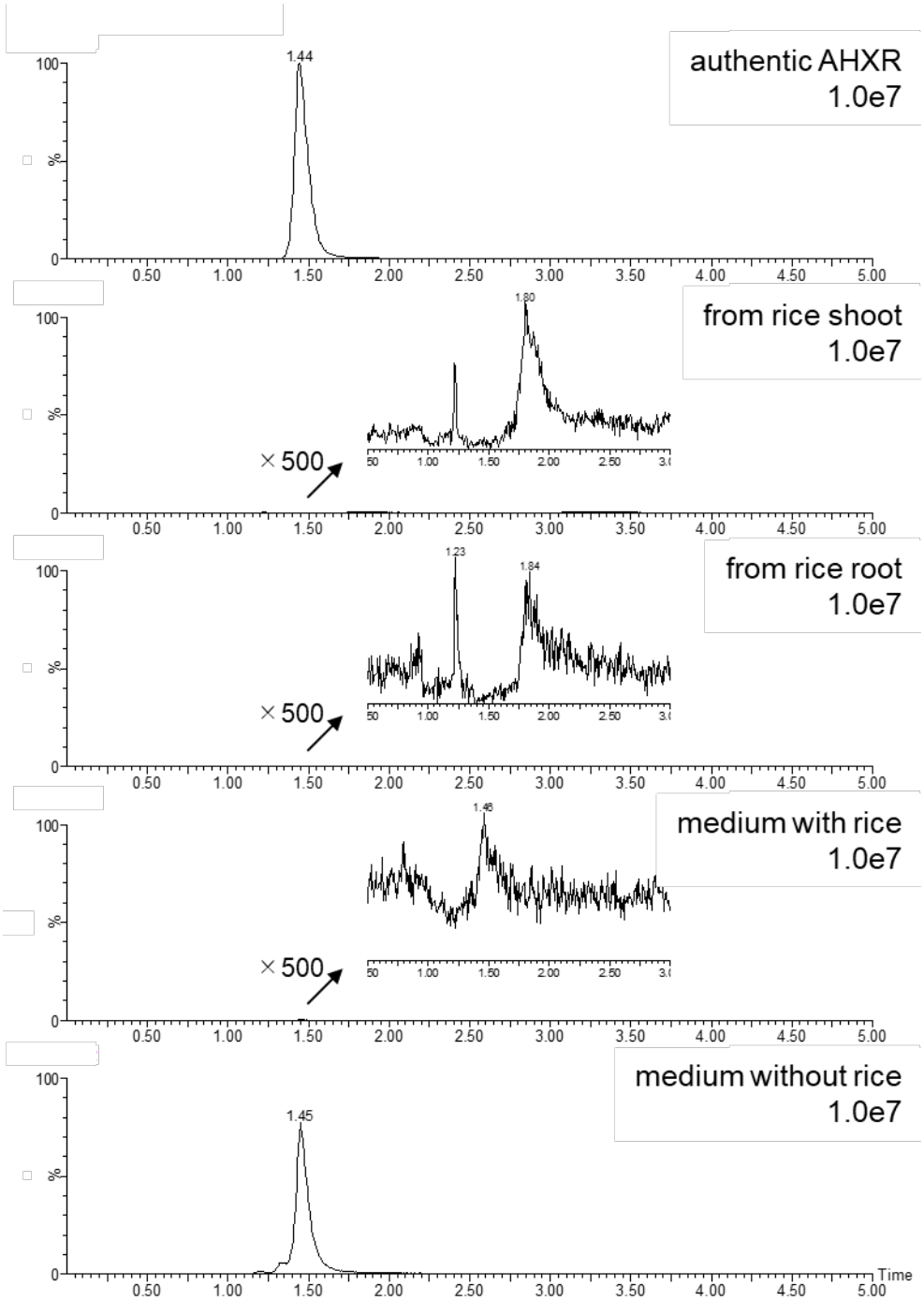


Fig. S11. LC-MS/MS chromatogram of AHXR in rice treated with AHXR.
Rice treated with 100 µM AHXR for a week and the medium after incubation were used. Medium containing 100 µM AHXR (without rice) was incubated for a week and used as control. MS/MS chromatograms are shown for AHXR identified by the characteristic transition at *m/z* 350 > 138.

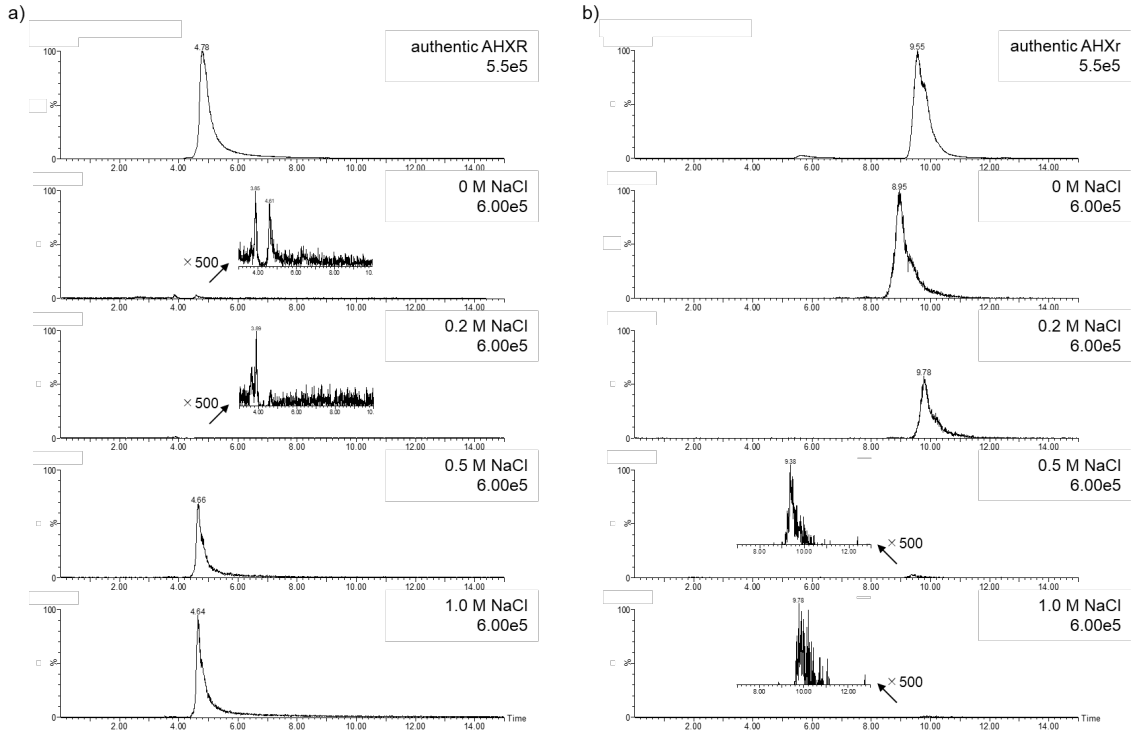
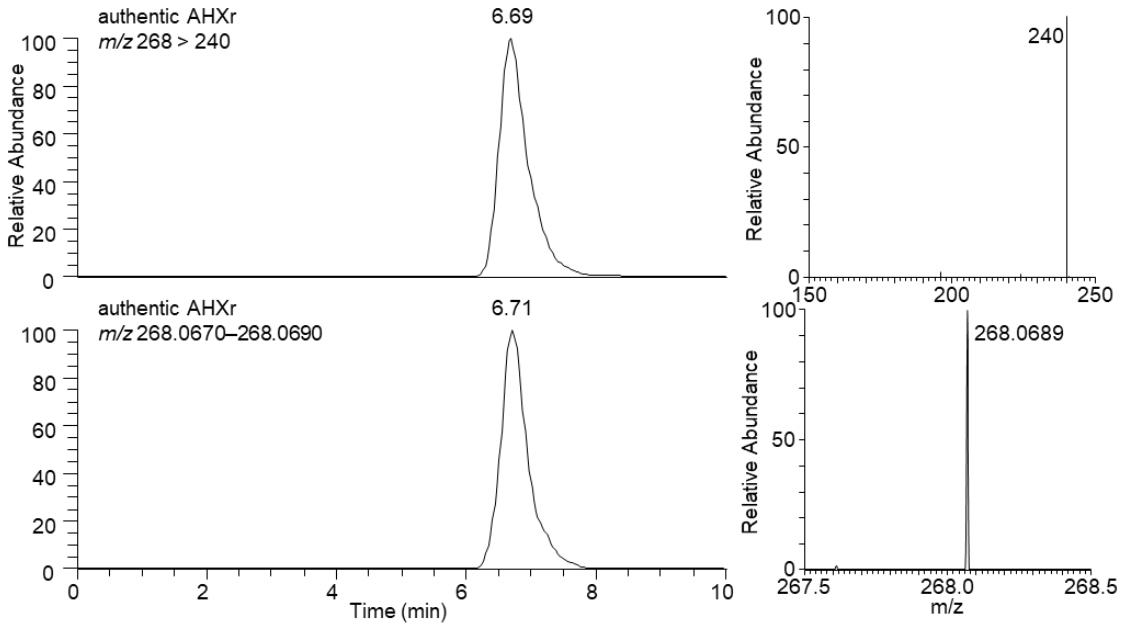
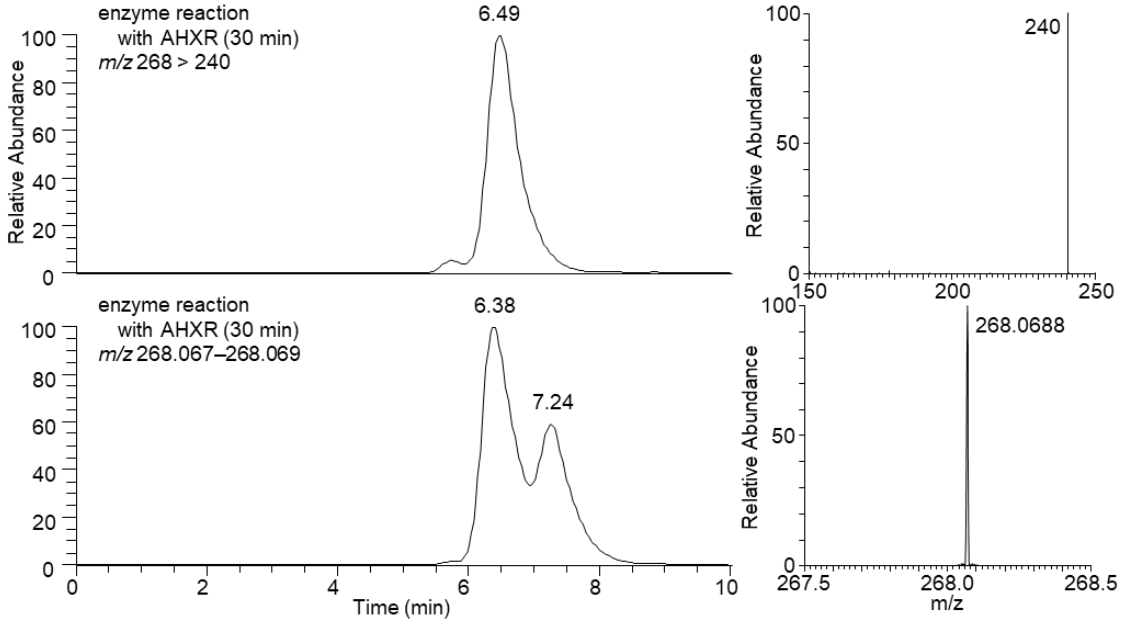


Fig. S12. LC-MS/MS chromatogram of AHXR (a) and AHXr (b) in the reaction solution with DEAE fractions.

The reaction was carried out at 30°C for 12 h. MS/MS chromatograms are shown for AHXR (a) and AHXr (b) identified by the characteristic transition at *m/z* 350 > 138 and *m/z* 268 > 240, respectively.


authentic AHXr
m/z 268 > 240
6.69
Relative Abundance
authentic AHXr
m/z 268.0670–268.0690
6.71
Time (min)
240
268.0689
m/z


enzyme reaction
with AHXR (30 min)
m/z 268 > 240
6.49
Relative Abundance
enzyme reaction
with AHXR (30 min)
m/z 268.067–268.069
6.38
7.24
Time (min)
240
268.0688
m/z

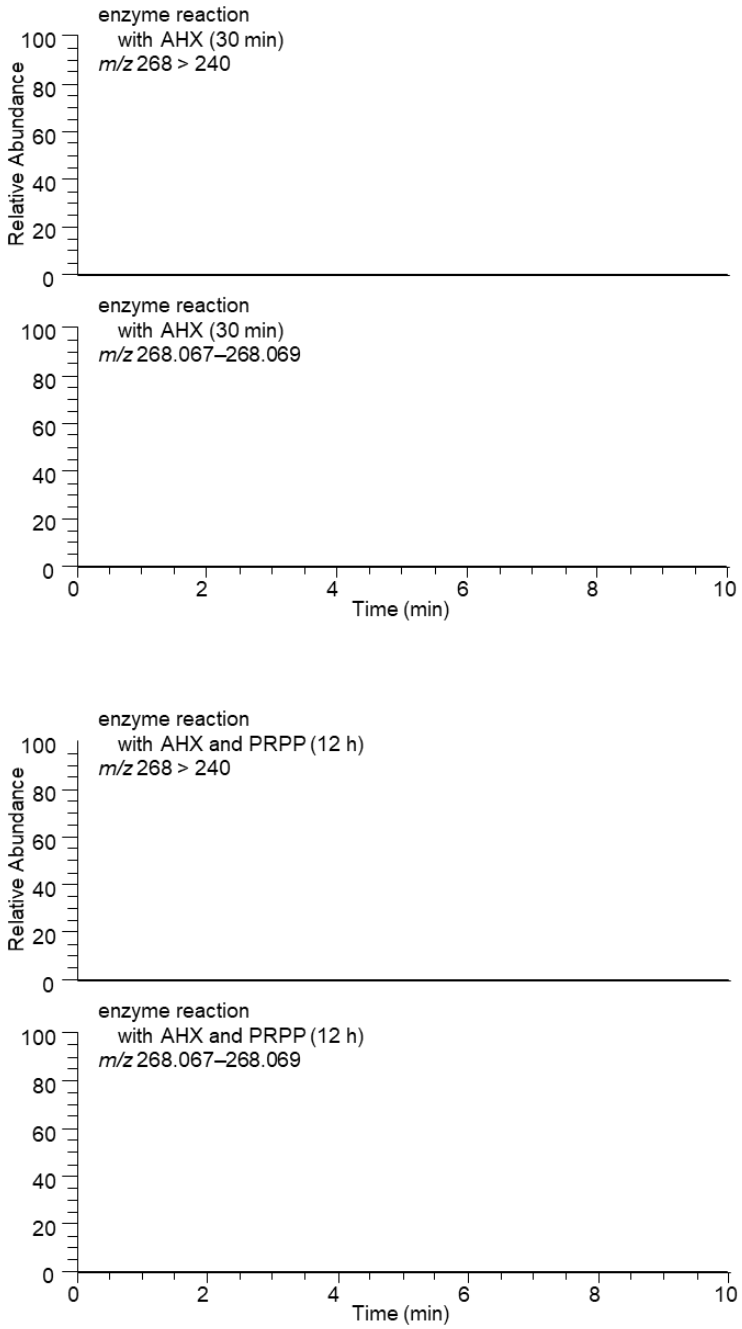


Fig. S13. LC-MS/MS chromatogram of AHXr in the reaction solution with DEAE fraction eluted with 0.2 M NaCl.

The reaction was carried out at 30°C for 30 min or 12 h. AHXr was identified by the characteristic transition at *m/z* 268 > 240 by MS/MS analysis (upper) and the exact mass at *m/z* 268.067–268.090 by MS analysis (lower). Each MS spectrum is shown on the right.

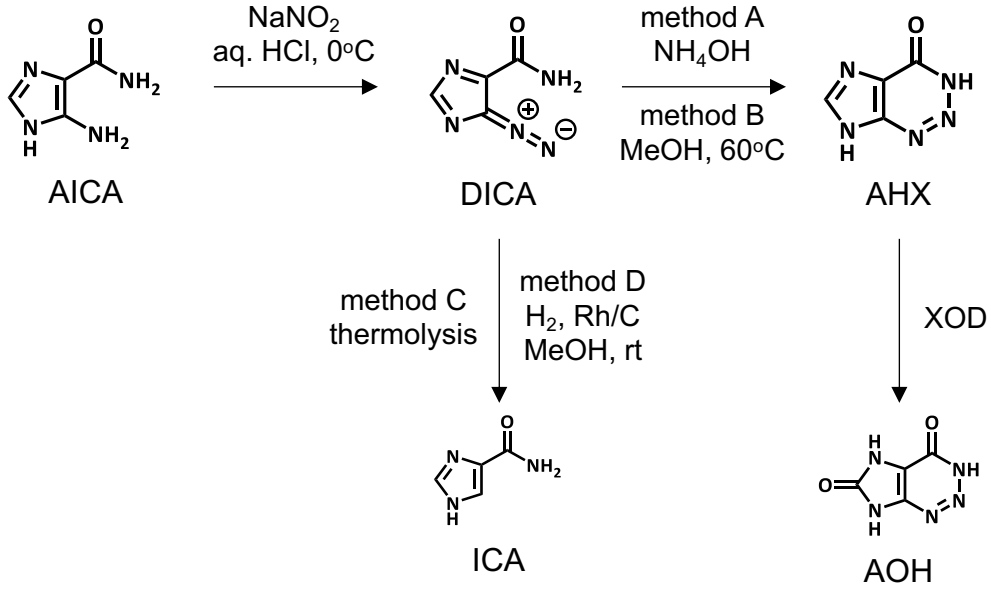


Fig. S14. Chemical synthesis route of FCs.
Conversion of AHX to AOH was carried out with xanthine oxidase (XOD) from buttermilk in 10 mM phosphate-buffered saline, pH 7.4.

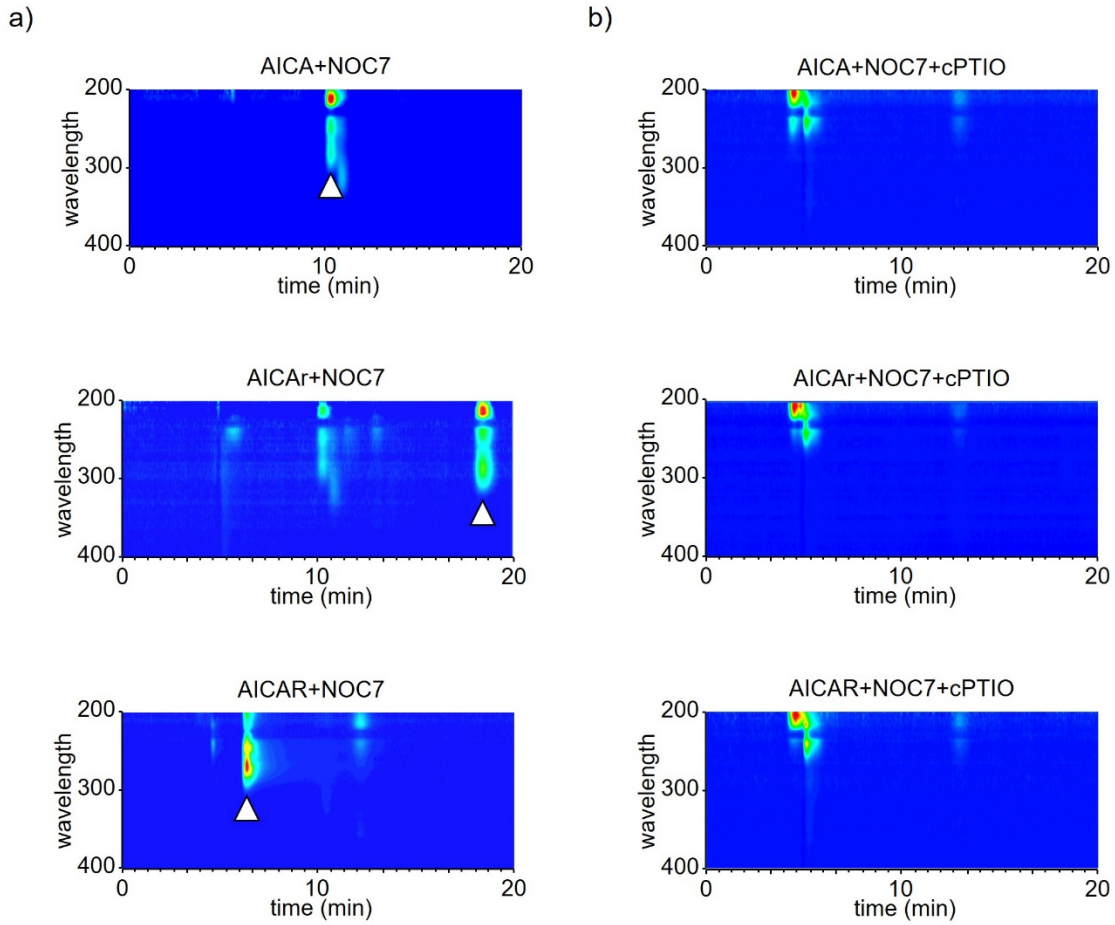


Fig. S15. HPLC profile of the reaction with NO donor (NOC7) and NO scavenger (cPTIO). AICA, AICAr, and AICAR were incubated with NOC7 (a) or NOC7 and cPTIO (b) in the distilled water. Enzyme reaction mixture extracted with methanol was injected into HPLC with a photo-diode array detector.The target products identified by the retention time and absorption spectra of authentic AHX, AHXr, and AHXR are marked by white triangles.