Supplementary Information

Genetic incorporation of unnatural amino acids biosynthesized from α-keto acids by an aminotransferase

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Materials and Methods

General

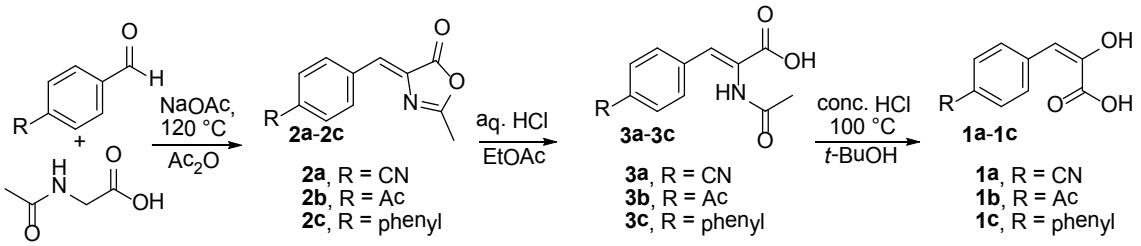
All chemicals and DNA oligomers were obtained from commercial sources and used without further purification. ^{1}H NMR and ^{13}C NMR spectra were obtained using a Varian Gemini-400 (400 MHz for ^{1}H, and 100 MHz for ^{13}C), or a Varian Inova-500 (500 MHz for ^{1}H, and 125 MHz for ^{13}C) spectrometer with chemical shifts recorded relative to tetramethylsilane.

Synthesis of α-keto acids

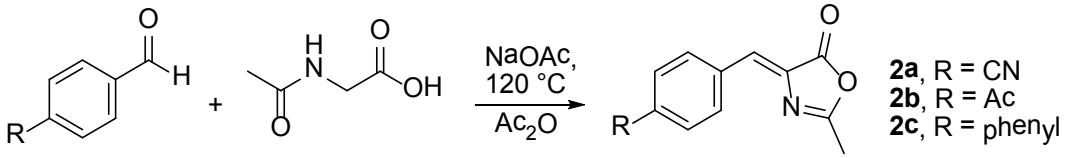
The stereochemistry of double bond at **1a–1d** was assigned by an analogy of ^{1}H NMR chemical shift of their methine proton with that of theoretical value. Also, small amount (~ 5%) of keto isomer, pyruvic acid, was shown in ^{1}H NMR spectrum of all four compounds (aromatic region and at around 4.2 ppm for benzylic α-methylene peak).

Synthetic routes for 2-hydroxyacrylic acids **1a**–**1c** were followed from literature procedure (Scheme S1).[1]

Scheme S1. Synthetic scheme for 2-hydroxyacrylic acids **1a**–**1c**.



General procedure for compounds 2a–2c.



Samples of commercially available corresponding 4-substituted benzaldehyde (1.0 equiv), *N*-acetylglycine (1.2 equiv) and sodium acetate (1.3 equiv) in a flask equipped with refluxing condenser were treated with acetic anhydride (5.0 equiv). The reaction mixture was warmed at 120 °C for 8 h under Ar (gray-white precipitate formed as the reaction progresses) and cooled down to 23 °C. The reaction mixture was diluted with EtOAc and 10% aqueous NaCl. Phases were separated, and the organic phase was washed with water and saturated aqueous NaCl, dried (Na_2SO_4) and concentrated under reduced pressure to provide crude product. Crude product was subject to recrystalization over

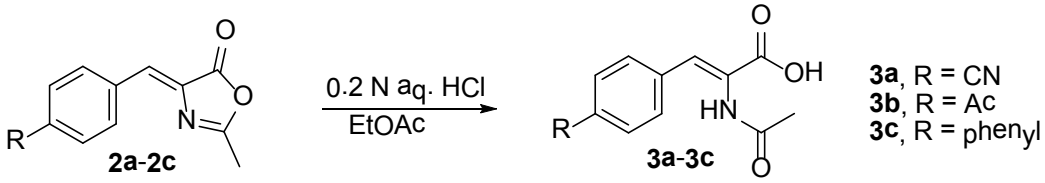
acetone, and the mother liquid was further purified with flash chromatography (SiO_2, 20% EtOAc–hexanes) to afford compounds **2a**–**2c**.

(*Z*)-4-(4-Cyanobenzylidene)-2-methyloxazol-5(*4H*)-one (2a). Compound **2a** was obtained starting from 4-cyanobenzaldehyde: white solid (910 mg, 74%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 (d, J = 8.4 Hz, 2H), 7.71(d, J = 8.4 Hz, 2H), 7.08 (s, 1H), 2.44 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.2, 167.1, 135.3, 132.5 (2C),132.3 (2C), 128.3, 128.2, 118.5, 113.8, 16.0; Anal. Calcd for $C_{12}H_8N_2O_2$: C, 67.92; H, 3.80; N, 13.20. Found: C, 67.97; H, 3.83; N, 13.13.

(*Z*)-4-(4-Acetylbenzylidene)-2-methyloxazol-5(*4H*)-one (2b). Compound **2b** was obtained starting from 4-acetylbenzaldehyde: white solid (2.14 g, 82%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.16 (d, J = 8.0 Hz, 2H), 8.00 (d, J = 8.0 Hz, 2H), 7.14 (s, 1H), 2.63 (s, 3H), 2.44 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 197.5, 167.6, 167.4, 138.3, 137.4, 134.6, 132.3 (2C), 129.5, 128.7 (2C), 26.9, 15.9; Anal. Calcd for $C_{13}H_{11}NO_3$: C, 68.11; H, 4.84; N, 6.11. Found: C, 68.17; H, 5.00; N, 5.95.

(*Z*)-4-(4-Phenylbenzylidene)-2-methyloxazol-5(*4H*)-one (2c). Compound **2c** was obtained starting from 4-phenylbenzaldehyde: white solid (1.78 g, 61%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.16 (d, J = 8.4 Hz, 2H), 7.69 (d, J = 8.4 Hz, 2H), 7.65 (m, 2H), 7.47 (m, 2H), 7.40 (m, 1H), 7.19 (s, 1H), 2.43 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.9, 166.1, 143.8, 140.1, 132.8 (2C), 132.6, 132.3, 131.2, 131.1, 129.1 (2C), 128.2, 127.6 (2C), 127.3 (2C), 15.8; Anal. Calcd for $C_{17}H_{13}NO_2$: C, 77.55; H, 4.98; N, 5.32. Found: C, 77.57; H, 5.01; N, 5.15.

General procedure for compounds 3a–3c.



A solution of compounds **2a–2c** in EtOAc (0.5 M) was treated with aqueous 0.2 N HCl (0.1 M). The reaction mixture was stirred at 30 ºC for 16 h under Ar and diluted with EtOAc to 0.02 M. Phases were separated, and organic phase was washed with 1:1 (aqueous 2 N HCl):(aqueous saturated NaCl), dried (Na_2SO_4) and concentrated under reduced pressure to provide crude product. Crude product was subject to recrystalization over EtOH, and the mother liquid was further purified with flash chromatography [SiO_2, gradient 0 to 7% MeOH/(0.1% TFA in CH_2Cl_2) to afford corresponding 2-acetamido acrylic acids **3a**–**3c**.

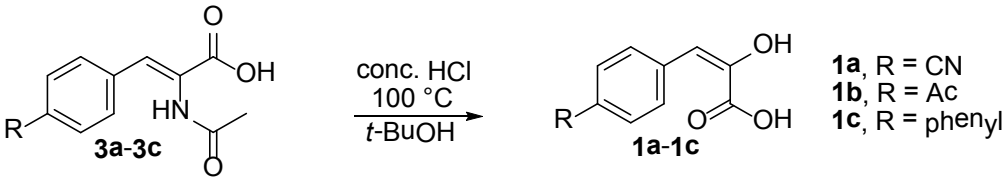
(*Z*)-2-Acetamido-3-(4-cyanophenyl)acrylic acid (3a). Compound **3a** was obtained starting from **2a**: white solid (960 mg, 63%); ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.95 (br s, 1H), 9.66 (s, 1H), 7.85 (d,

J = 8.0 Hz, 2H), 7.75 (d, *J* = 8.0 Hz, 2H), 7.19 (s, 1H), 1.98 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 169.0, 166.0, 138.7, 132.2 (2C), 130.1 (2C), 129.9, 127.9, 118.6, 110.8, 22.5; Anal. Calcd for $C_{12}H_{10}N_2O_3$: C, 62.60; H, 4.38; N, 12.17. Found: C, 62.60; H, 4.48; N, 12.25.

(*Z*)-2-Acetamido-3-(4-acetylphenyl)acrylic acid (3b). Compound **3b** was obtained starting from **2b**: gray solid (630 mg, 62%); ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.80 (br s, 1H), 9.54 (s, 1H), 7.94 (d, *J* = 8.4 Hz, 2H), 7.67 (d, *J* = 8.4 Hz, 2H), 7.20 (s, 1H), 2.58 (s, 3H), 1.99 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 197.3, 169.0, 166.1, 138.4, 136.4, 129.6 (2C), 129.2, 129.0, 128.2 (2C), 26.7, 22.5; Anal. Calcd for $C_{13}H_{13}NO_4$: C, 63.15; H, 5.30; N, 5.67. Found: C, 63.18; H, 5.31; N, 5.54.

(*Z*)-2-Acetamido-3-(biphenyl-4-yl)acrylic acid (3c). Compound **3c** was obtained starting from **2c**: gray solid (610 mg, 66%); ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.70 (br s, 1H), 9.53 (s, 1H), 7.69-7.72 (m, 6H), 7.48 (t, *J* = 7.6 Hz, 2H), 7.39 (t, *J* = 7.6 Hz, 1H), 7.26 (s, 1H), 2.01 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 169.1, 166.3, 140.5, 139.2, 132.9, 130.5, 130.3 (2C), 129.0 (2C), 127.8, 127.3, 126.62 (2C), 126.57 (2C), 22.5; Anal. Calcd for $C_{13}H_{13}NO_4$: C, 72.58; H, 5.37; N, 4.98. Found: C, 72.59; H, 5.37; N, 4.87.

General procedure for compounds 1a–1c.



A mixture of compounds **3a**–**3c** in *t*-BuOH (0.5 M) was treated with concentrated HCl (0.5 M). The reaction mixture was stirred at 100 ºC for 8 h (**2b** and **2c**) or for 2 h (**2a**) in a sealed condition. Reaction mixture was diluted with EtOAc until being homogeneous, washed with 1:1 (aqueous 2 N HCl):(saturated aqueous NaCl), dried (Na_2SO_4) and concentrated under reduced pressure to provide the crude product. Flash chromatography [SiO_2, gradient 0 to 7% MeOH/(0.1% TFA in CH_2Cl_2)] afforded corresponding 2-hydroxy acrylic acids **1a**–**1c**.

(*E*)-3-(4-Cyanophenyl)-2-hydroxyacrylic acid (1a). Compound **1a** was obtained starting from **3a**: white solid (120 mg, 67%); ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.52 (br s, 1H), 9.96 (s, 1H), 7.92 (d, *J* = 6.8 Hz, 2H), 7.78 (d, *J* = 6.8 Hz, 2H), 6.44 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 165.7, 144.7, 139.9, 132.1 (2C), 129.5 (2C), 119.0, 108.7, 107.4; Anal. Calcd for $C_{10}H_7NO_3$: C, 63.49; H, 3.73; N, 7.40. Found: C, 63.41; H, 3.77; N, 7.55.

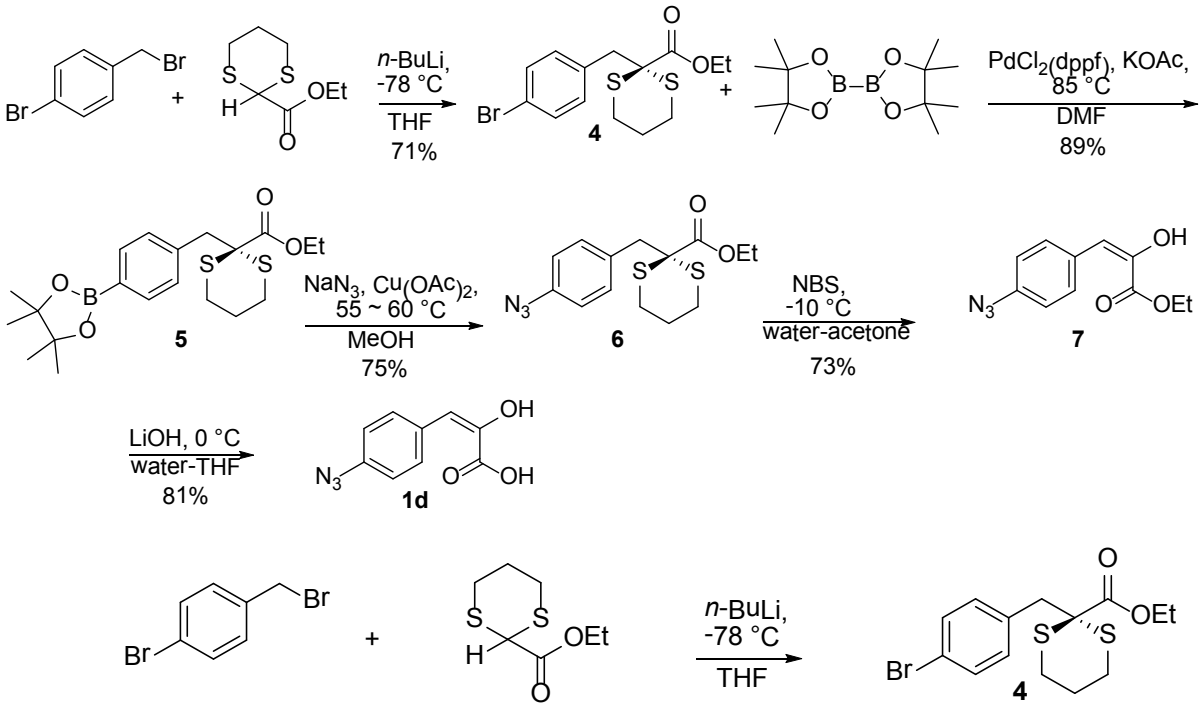
(*E*)-3-(4-Acetylphenyl)-2-hydroxyacrylic acid (1b). gray solid (70 mg, 98%); ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.43 (br s, 1H), 9.74 (s, 1H), 7.92 (d, *J* = 6.8 Hz, 2H), 7.87 (d, *J* = 6.8 Hz, 2H), 6.45 (s,

1H), 2.56 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 197.2, 165.9, 143.9, 139.7, 134.8, 129.1 (2C), 128.2 (2C), 108.1, 26.6; Anal. Calcd for $C_{11}H_{10}O_4$: C, 64.07; H, 4.89. Found: C, 64.08; H, 4.95.

(*E*)-3-(Biphenyl-4-yl)-2-hydroxyacrylic acid (1c). gray solid (60 mg, 71%); ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.25 (br s, 1H), 9.37 (s, 1H), 7.85 (d, J = 8.4 Hz, 2H), 7.69 (m, 4H), 7.46 (t, J = 7.6 Hz, 2H), 7.36 (m, 1H), 6.45 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 166.2, 142.0, 139.6, 138.5, 134.2, 129.8 (2C), 128.9 (2C), 127.4, 126.43 (2C), 126.42 (2C), 109.1; Anal. Calcd for $C_{15}H_{12}O_3$: C, 74.99; H, 5.03. Found: C, 74.93; H, 5.11.

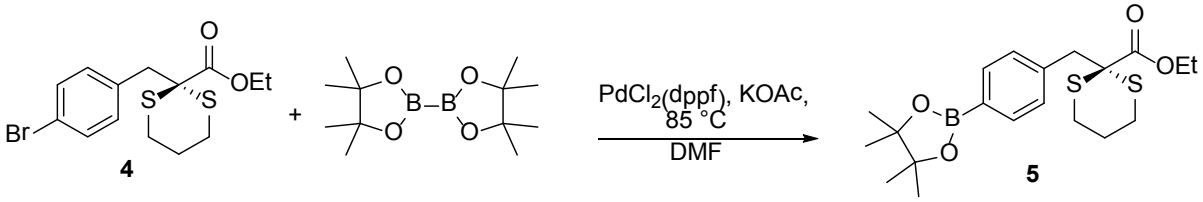
Synthetic route of (*E*)-3-(4-azidophenyl)-2-hydroxyacrylic acid (1d). Compound **1d** was prepared from multistep processes starting with the alkylation between both commercially available ethyl 1,3-dithiane-2-carboxlate and 4-bromobenzyl bromide to afford compound **4**,[2] and its 4-bromo-substituent at phenyl ring was converted to a boronic ester **5**,[3] compound **5** was exposure to sodium azide in presence of Cu(II) afforded the key intermediate 4-azidophenyl compound **6**.[4] Oxidative removal of 1,3-dithiane ring with NBS resulted an acrylic ester **7**,[5] and hydrolysis of the ester afforded desired compound **1d** (Scheme S2).[6]

Scheme S2. Synthetic Scheme for **1d**.

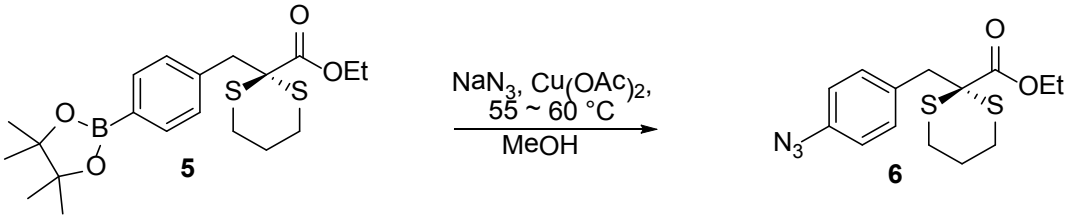


Ethyl 2-(4-bromobenzyl)-1,3-dithiane-2-carboxylate (4). A stirred solution of commercially available ethyl 1,3-dithiane-2-carboxylate (962 mg, 5.00 mmol) in anhydrous THF (13 mL) at -78 ºC under Ar was treated dropwise with a solution of *n*-BuLi in hexanes (3.3 mL, 1.6 M). The reaction mixture was stirred for 30 min and treated with a solution of commercially available 4-bromobenzyl

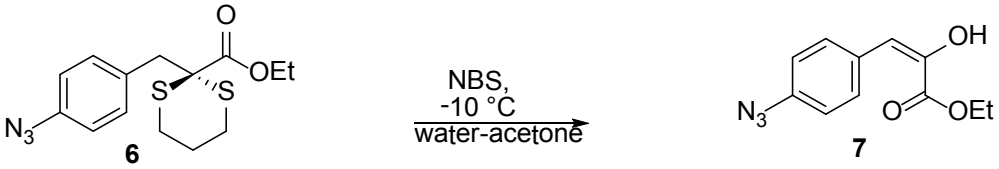
bromide (1.50 g, 5.25 mmol) in THF (10 mL) over 10 min. The reaction mixture was stirred at -78 ºC for 4 h and quenched with saturated aqueous NH_4Cl (10 mL). Resulting mixture was allowed to warm to 23 ˚C, diluted with EtOAc (100 mL), washed with water (3 x 20 mL) and saturated aqueous NaCl, dried (Na_2SO_4) and concentrated under reduced pressure to provide the crude product. Flash chromatography (SiO_2, 5% EtOAc–hexanes) afforded the alkylation compound **4**: white solid (1.29 g, 71%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 (d, J = 8.4 Hz, 2H), 7.19 (d, J = 8.4 Hz, 2H), 4.25 (q, J = 7.2 Hz, 2H), 3.30 (s, 2H), 3.20 (m, 2H), 2.67 (dt, J = 14.4, 3.8 Hz, 2H), 2.15-2.07 (m, 1H), 1.87-1.74 (br m, 1H), 1.32 (t, J = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.5, 133.6, 132.5 (2C), 131.1 (2C), 121.6, 62.2, 53.1, 43.8, 28.1 (2C), 24.4, 14.3; Anal. Calcd for $C_{14}H_{17}BrO_2S_2$: C, 46.54; H, 4.74; S, 17.75. Found: C, 46.65; H, 4.64; S, 17.76.



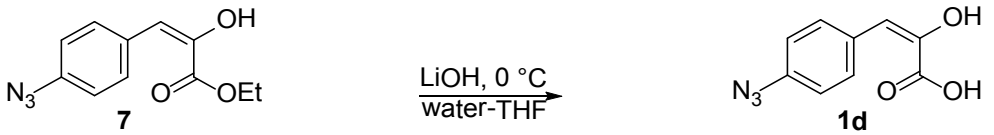
Ethyl 2-(4-(4,4,5,5-tetramethyl-1,3-dioxa-2-borolan-2-yl)benzyl)-1,3-dithiane-2-carboxylate (5). A solution of compound **4** (1.80 g, 4.97 mmol) in DMF (33 mL) was treated with bis(pinacolato)diboron (1.39 g, 5.47 mmol), $PdCl_2$(dppf) (406 mg, 0.50 mmol), KOAc (1.46 g, 14.91 mmol). Reaction mixture was degassed for 10 min and then warmed at 85 ºC for 3 h under Ar. The reaction was quenched with water (10 mL), and the resulting mixture was diluted with EtOAc (300 mL), washed with aqueous 1 N HCl (30 mL), water (5 x 30 mL) and saturated aqueous NaCl (30 mL), dried (Na_2SO_4) and concentrated under reduced pressure to provide the crude product. Flash chromatography (SiO_2, 10% EtOAc–hexanes) afforded the alkylation compound **5**: colorless oil (1.81 g, 89%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71 (d, J = 8.0 Hz, 2H), 7.31 (d, J = 8.0 Hz, 2H), 4.24 (q, J = 7.2 Hz, 2H), 3.38 (s, 2H), 3.23 (m, 2H), 2.66 (dt, J = 14.0, 3.6 Hz, 2H), 2.14-2.06 (m, 1H), 1.89-1.77 (br m, 1H), 1.34-1.30 (m 15H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.6, 137.8, 134.4 (2C), 130.1 (2C), 83.7, 62.1 (2C), 53.6, 44.6, 28.1 (2C), 24.9 (4C), 24.4, 14.2 (one phenylic carbon was not identified); Anal. Calcd for $C_{20}H_{29}BO_4S_2$: C, 58.82; H, 7.16; S, 15.70. Found: C, 58.88; H, 7.16; S, 15.72.



Ethyl 2-(4-azidobenzyl)-1,3-dithiane-2-carboxylate (6). A stirred solution of compound **5** (1.47 g, 3.60 mmol) in MeOH (18 mL) was treated with NaN_3 (351 mg, 5.40 mmol) and $Cu(OAc)_2$ (122 mg, 1.08 mmol). Reaction mixture was stirred at 55 ºC for 4 h and cooled down to 23 ºC. Volatiles were removed under reduced pressured to provide crude product. Flash chromatography (SiO_2, 7% EtOAc–hexanes) afforded compound **6**: greenish yellow semi-solid (878 mg, 75%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 (d, J = 8.0 Hz, 2H), 6.94 (d, J = 8.0 Hz, 2H), 4.26 (q, J = 7.2 Hz, 2H), 3.34 (s, 2H), 3.24 (m, 2H), 2.68 (dt, J = 14.1, 3.8 Hz, 2H), 2.17-2.08 (m, 1H), 1.89-1.77 (br m, 1H), 1.34 (t, J = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.7, 139.3, 132.3 (2C), 131.5, 118.7 (2C), 62.3, 53.6, 43.9, 28.2 (2C), 24.5, 14.4; Anal. Calcd for $C_{14}H_{17}N_3O_2S_2$: C, 51.99; H, 5.30; S, 19.83. Found: C, 51.90; H, 5.22; S, 19.93.



(*Z*)-Ethyl 3-(4-azidophenyl)-2-hydroxyacrylate (7). A stirred solution of compound **6** (107 mg, 0.33 mmol) in 95% aqueous acetone (3 mL) at -10 ºC (ice/MeOH) was treated with a cold solution (-10 ºC) of NBS (589 mg, 3.30 mmol) in 95% aqueous acetone (5 mL). The reaction mixture was stirred at -10 ºC for 30 min (product is unstable under prolong reaction period or at temperature above 0 ºC), and the reaction was quenched with aqueous 3 M Na_2SO_3 (3 mL). Organic volatiles were removed with a stream of N_2, and the residue was dissolved in EtOAc (200 mL), washed with water (5 x 40 mL) and saturated aqueous NaCl, dried (Na_2SO_4) and concentrated under reduced pressure to provide the crude product. Flash chromatography (SiO_2, 7% EtOAc–hexanes) afforded compound **7**: yellow solid (56 mg, 73%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (d, J = 8.2 Hz, 2H), 7.03 (d, J = 8.2 Hz, 2H), 6.47 (d, J = 12.42 Hz, 2H), 4.37 (q, J = 7.2 Hz, 2H), 1.40 (t, J = 7.2 Hz, 3H), (one hydroxyl peak was not shown); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.2, 139.4, 139.2, 131.4 (2C), 131.2, 119.2 (2C), 109.6, 62.8, 14.4; HRMS EI-DFA *m/z* Calcd for $C_{11}H_{11}N_3O_3^+$ [M^+] 233.0800. Found 233.0803.



(*E*)-3-(4-Azidophenyl)-2-hydroxyacrylic acid (1d). A solution of compound **7** (62 mg, 0.27 mmol) in 1:1 mixture of THF:water (8 mL) at 0 ºC was treated with $LiOH{\cdot}H_2O$ (113 mg, 2.70 mmol). Reaction mixture was stirred at 0 ºC for 6 h and quenched with aqueous 2 N HCl (2 mL). Resulting

mixture was diluted with EtOAc (50 mL) and water (20 mL). Phases were separated, and the organic phase was washed with saturated aqueous NaCl, dried (Na_2SO_4) and concentrated under reduced pressure to provide the crude product. Flash chromatography [SiO_2, gradient 0 to 7% MeOH/(0.1% TFA in CH_2Cl_2)] afforded **1d**: yellow solid (49 mg, 81%); ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.2 (br s, 1H), 9.33 (s, 1H), 7.80 (d, J = 8.6 Hz, 2H), 7.10 (d, J = 8.6 Hz, 2H), 6.40 (s, 1H), (one hydroxyl peak was not shown); ^{13}C NMR (125 MHz, DMSO-d_6) δ 166.2, 141.7, 137.8, 132.1, 130.8 (2C), 119.1 (2C), 119.0, 108.8; Anal. Calcd for $C_9H_7N_3O_3$: C, 52.69; H, 3.44; N, 20.48. Found: C, 52.64; H, 3.45; N, 20.32.

Expression and purification of ttGlnAT. A C-terminal hexahistidine-tagged ttGlnAT gene was amplified from *Thermus thermophilus* HB8 genomic DNA with primers having NdeI and BglII restriction sites. The sequences of the primers were 5′-ATATCATATGCGTCTCCA CCCCCGCACCGACGAGGCGGCCA-3′ (forward primer) with NdeI site (underlined) and 5′-ATATAGATCTTTATTATCCAGATACCGCACCACCTTCGGCT-3′ (reverse primer) with BglII site. The gene was inserted between the NdeI and BamHI sites of pET20b (Invitrogen) to generate pET20b-ttGlnAT which was then transformed into *E. coli* BL21 (DE3). Starter culture (5 mL) was used to inoculate 200 mL of LB medium. The culture was induced at OD = 0.8 (600 nm) by adding IPTG (1 mM, final concentration). Cells were grown at 37 ºC for 14-16 h and harvested by centrifugation. The protein expressed was purified by Ni-NTA affinity chromatography under native conditions according to the manufacturer's protocol (Qiagen, Hilden, Germany).

Enzymatic transamination reaction. The reaction initiated by adding the enzyme (10 μM, final concentration) to the reaction mixture containing 5 mM α-keto acid, 10 mM methionine, 50 mM HEPES-NaOH (pH 8.0), 0.1 M KCl, and 0.1 mM EDTA to make the total volume 50 μL, and the reaction mixture was incubated at 25 ºC for 1 h. The enzyme was precipitated by adding methanol (50 μL) and the supernatant was used for HPLC analysis after centrifugation. For a control, the enzyme was replaced with water. For HPLC analysis, C18 column (Zorbax Eclipse Plus C18, Agilent) was used and HPLC traces were taken at 254nm.

Enzyme kinetics. Kinetics was performed with constant concentration of ttGlnAT (0.1 μM) and Met (5 mM) in 50 mM HEPES while varying concentration of α-keto acids (3.5, 7, 15, 30, 60, 120, and 250 μM). Amino acid production was assessed by measuring decrease in absorbance of α-keto acids at absorption maximum (254 nm for 4-acetylphenylpyruvic acid, 310 nm for 4-azidophenylpyruvic

acid, 302 nm for 4-phenylphenylpyruvic acid, and 308 nm for 4-cyanophenylpyruvic acid). Initial reaction velocities, obtained as the slope of best fit to the initial linear portion of the reaction time course, were subsequently fit to the Michaelis–Menten equation.

Transamination reaction in cells. The ttGlnAT-expressing cells containing pET20b-ttGlnAT were grown in LB medium in the presence of 2 mM α-keto acid. The ttGlnAT expression was induced at OD = 0.8 (600 nm) by adding IPTG (1 mM, final concentration). Cells were grown at 37 ºC for 14-16 h, and the medium was analyzed by HPLC after centrifugation.

Genetic incorporation of UAAs synthesized from α-keto acids. ttGlnAT and GFP genes were inserted into pET-duet1 (Invitrogen). The ttGlnAT gene was amplified from pET20b-ttGlnAT with primers having BspHI and EcoRI restriction sites. The sequences of the primers were 5′-ATATTCATGAAACTCCACCCCCGCACC-3′ (forward primer) with BspH1 site (underlined) and 5′-ATATGAATTCTCAATGGTGATGATGGTGATGTCCA GATACC-3′ (reverse primer) with EcoRI site. The gene was inserted between the NcoI and EcoRI sites of the vector. The C-terminal hexahistidine-tagged GFP gene was then inserted between the NdeI and KpnI sites of the same vector to generate pET-duet1-ttGlnAT-GFP. The sequences of primers used for this procedure were 5′- GCACGCATATGAGTAAAGGAGAAGAACTTTTCACTGGAGT-3′ (forward primer) with NdeI site (underlined) and 5′- ATATGGTACCTCAGTGGTGGTGGTGGTGG -3′ (reverse primer) with KpnI site. Site-directed mutagenesis was used to introduce Y151TAG mutation into the GFP gene. (The sequences of the primers used for this mutagenesis: 5′-CTATAACTCACACAATGTATAGATCACGGCAGACAAAC-3′ and 5′-GTCTGCCGTGATCTATACATTGTGTGAGTTATAGTTG-3′). The plasmid, pET-duet1-ttGlnAT-GFP-Y151TAG, was co-transformed with the plasmid containing tRNA and aaRS genes (pEvol-pAcF[7] for 4-acetylphenylpyruvic acid, pEvol-pAzF[7] for 4-azidophenylpyruvic acid, and pUltra-CNF[8] for 4-phenylphenylpyruvic acid and 4-cyanophenylpyruvic acid) into *E. coli* BL21 (DE3). Cells were amplified in LB medium supplemented with ampicillin (100 μg/mL) and chloramphenicol (35 μg/mL). When pUltra-CNF was used, spectinomycin (30 μg/mL) was used instead of chloramphenicol. Starter culture (2.5 mL) was used to inoculate 100 mL of LB medium. The culture was induced at OD = 0.8 (600 nm) by adding IPTG (1 mM, final concentration). Cells were grown at 37 ºC for 14-16 h and harvested by centrifugation. The protein expressed was purified by Ni-NTA affinity chromatography under native conditions according to the manufacturer's protocol (Qiagen, Hilden, Germany).

Conjugation reactions. The conjugation reaction was performed in 10 mM phosphate buffer (pH 7.0) containing 100 mM NaCl by adding GFP WT or GFP Y151AzF (20 μM, final concentration)

and a Cy5.5-linked aza-dibenzocyclooctyne[9] (Cy5.5-ADIBO, 200 μM, final concentration) for 2 h at room temperature. The reaction was quenched by adding excess AzF and the mixture was directly analyzed by SDS-PAGE. Fluorescence images were taken using the Typhoon 9210 variable mode imager (Cy5 mode) and the same gel was then stained with Coomassie Brilliant Blue R-250.

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Figure S1. HPLC traces (UV 254 nm) of transamination reactions by the glutamine aminotransferase from *T. thermophilus* for (A) AcPPA, (B) AzPPA, (C) PhPPA, and (D) CNPPA. From the top, α-keto acids (1 mM), control reactions without the enzyme, authentic amino acid (1 mM), and the reaction mixture. Masses of the reaction products were analyzed by LCMS: for AcPPA, calculated for $C_{11}H_{13}NO_3$ (M-1) 206.2, observed, 206.5; AzPPA, calculated for $C_9H_{10}N_4O_2$ (M-1) 205.2, observed, 205.9; PhPPA, calculated for $C_{15}H_{15}NO_2$ (M-1) 240.3, observed, 240.4; CNPPA, calculated for $C_{10}H_{10}N_2O_2$ (M-1) 189.2, observed, 189.2.

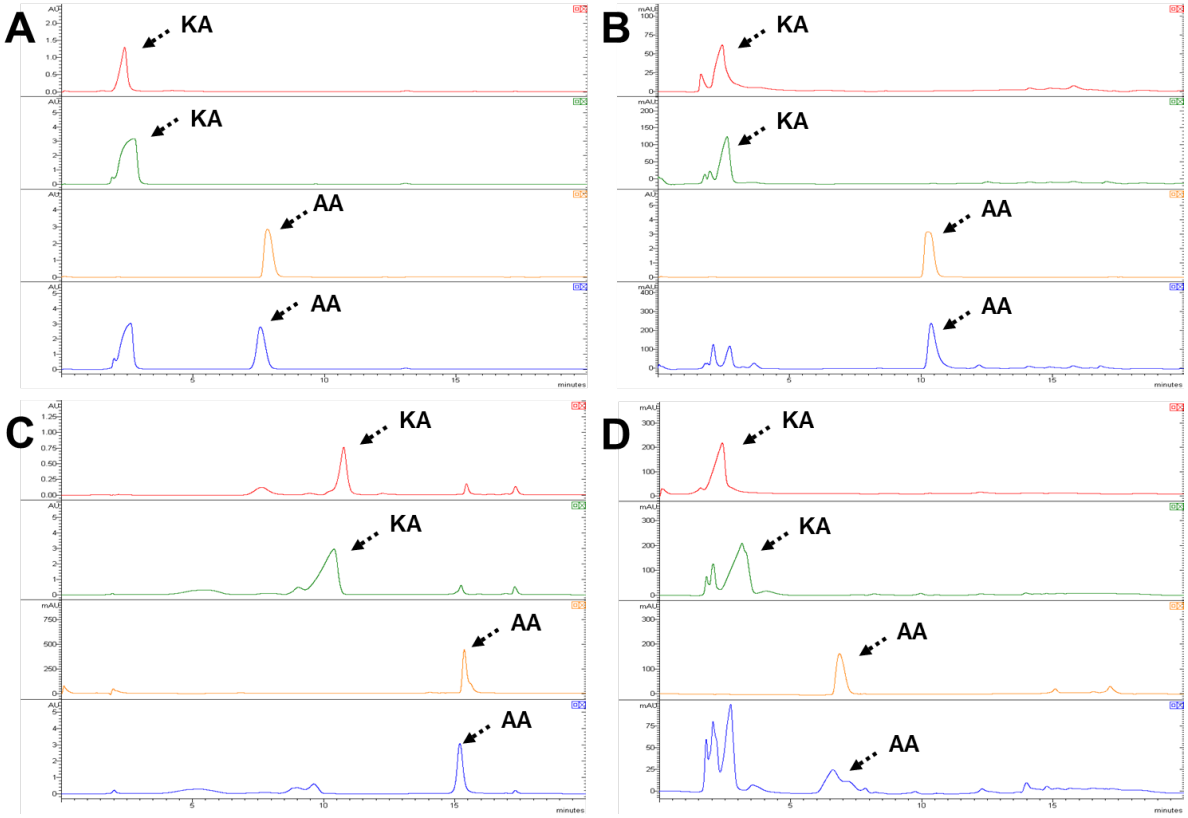

Figure S2. Kinetics results of transamination reactions. Initial reaction rates were plotted against concentrations of α-keto acids. (A) phenylpyruvic acid; (B) 4-acetylphenylpyruvic acid; (C) 4-azidophenylpyruvic acid; (D) 4-phenylphenylpyruvic acid; (E) 4-cyanophenylpyruvic acid.

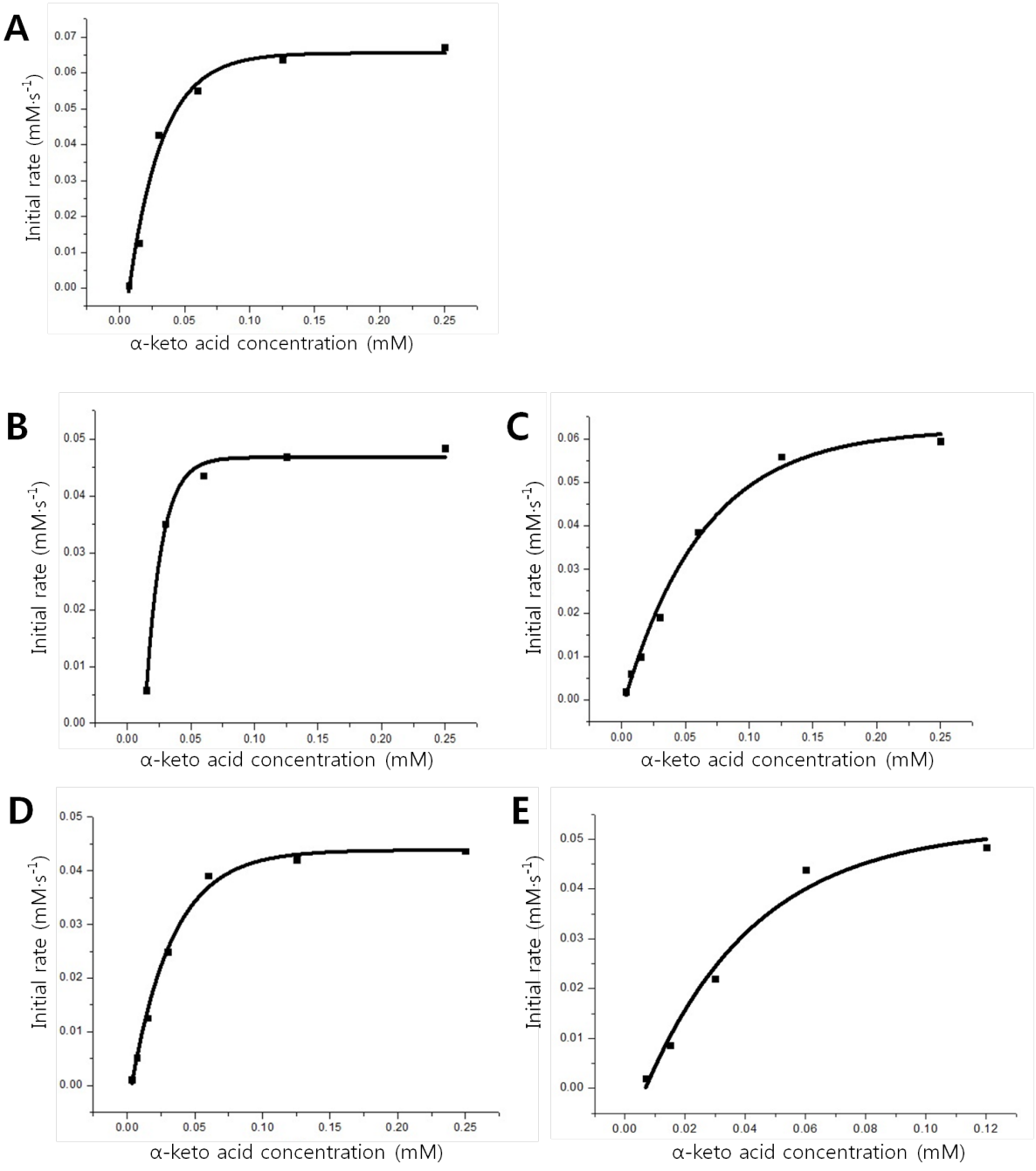

Figure S3. HPLC traces (UV 254nm) of growth medium used to grow ttGlnAT-expressing cells in the presence of α-keto acids. (A) AcPPA, (B) AzPPA, (C) PhPPA, and (D) CNPPA. From the top, initial media containing α-keto acid (2 mM), media after saturation, and authentic amino acid (1 mM).

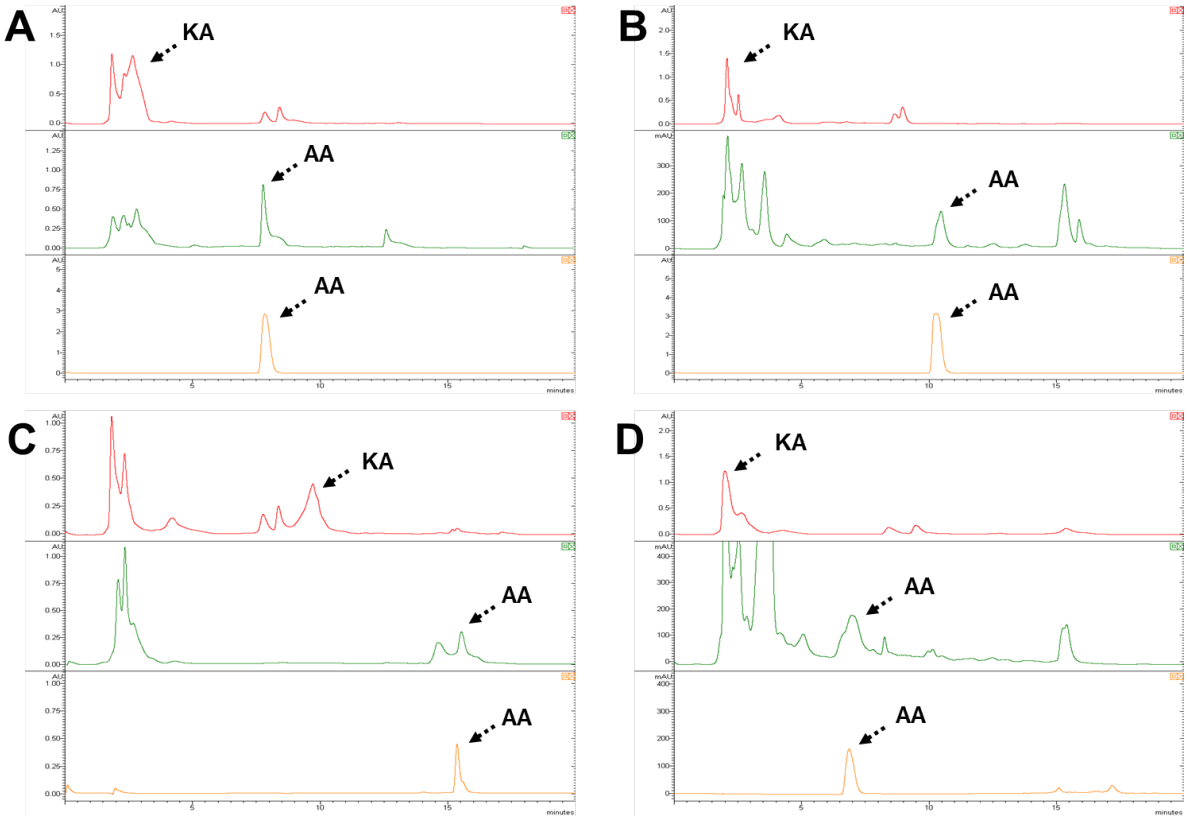

Figure S4. Labeling of a GFP mutant containing an azide group at position 151. The mutant protein was expressed as described above in the presence of AzPPA (2 mM). The reaction mixture containing the mutant protein and Cy5.5-ADIBO was analyzed by SDS-PAGE. The same reaction was performed with GFP WT and analyzed as a control. Reaction condition: 20 μM GFP, 200 μM Cy5.5-ADIBO in 10 mM phosphate buffer (pH 7.0) containing 100 mM NaCl, 10 μL total volume, room temperature, 2 h reaction time. Top, coomassie-stained gel image; bottom, fluorescence gel image.