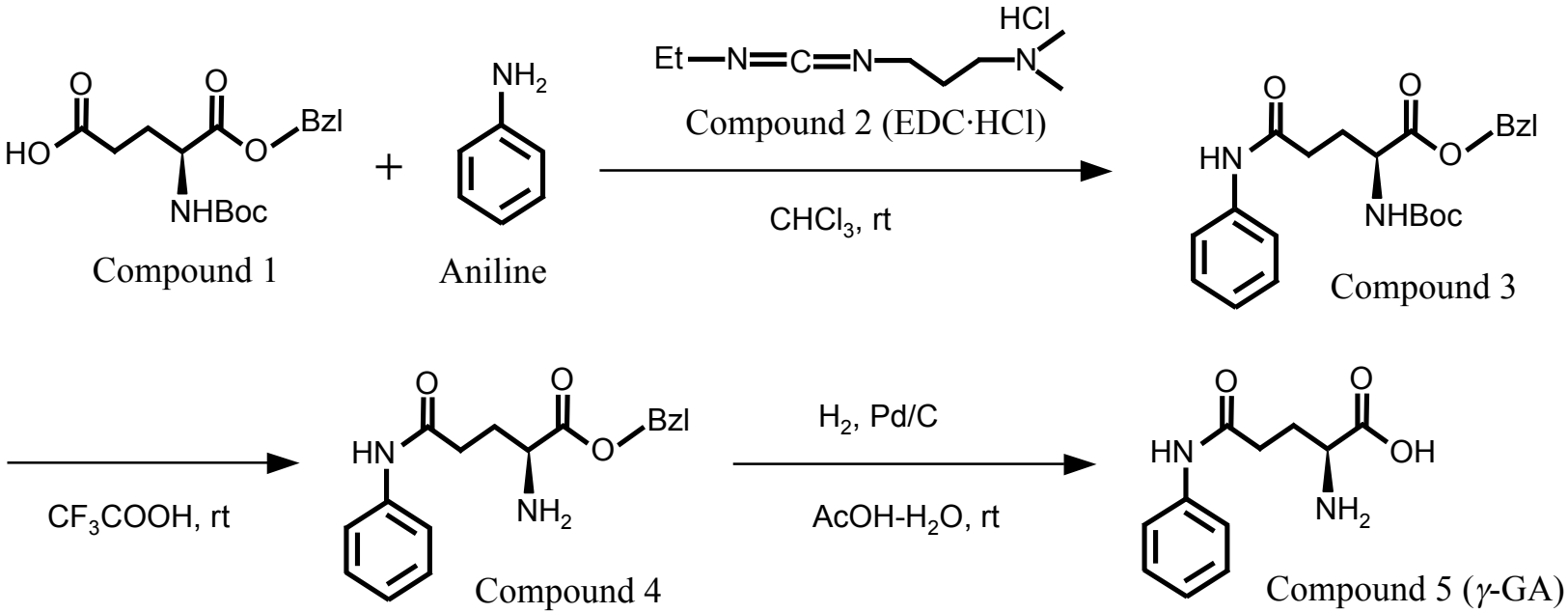


Fig. S1 Scheme for chemical synthesis of γ-glutamylanilide (γ-GA).

Fig. S1 continued

(i) Amidation of aniline with Boc-Glu-OBzl.

To an ice-cooled solution of *N*-(*tert*-butoxycarbonyl)-*L*-glutamic acid 1-benzyl ester (Boc-Glu-OBzl, Compound **1**, 1.602 g, 4.75 mmol) (Tokyo Chemical Industry, Tokyo, Japan) and aniline (0.5 ml, 5.49 mmol) in 20 ml of chloroform, a solution of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC·HCl, Compound **2**, 1.055 g, 5.50 mmol) in 10 ml of chloroform was added dropwise under an N_2 gas atmosphere. After removal of the ice bath, the reaction mixture was agitated at room temperature for 12 h. The resulting mixture was transferred into a separatory funnel and washed with 1M HCl, 5% (w/v) $NaHCO_3$ aqueous solution, and saturated NaCl aqueous solution. The organic layer was dried over Na_2SO_4. After removal of Na_2SO_4 by filtration, the chloroform was removed under reduced pressure using a rotary evaporator. After fractionation by silica gel column chromatography using $CHCl_3$-MeOH (10:1) as the eluent, the solvents were removed under reduced pressure using a rotary evaporator. The residue was dried using a vacuum pump to obtain the product (Compound **3**) as a light yellow solid.

(ii) Deprotection of the butoxycarbonyl group (BOC).

Compound **3** (1.746 g, 4.23 mmol) was completely dissolved in 9 ml of CF_3COOH. Confirming the generation of fine bubbles (CO_2 gas), the solution was agitated at 30 ºC for 30 min and the reaction mixture was concentrated under reduced pressure using a rotary evaporator. Chloroform (30 ml) was added to the residue, and the resulting solution was transferred into a separatory funnel, washed with 5% (w/v) $NaHCO_3$ aqueous solution and saturated NaCl aqueous solution, and dried over Na_2SO_4. After removal of the solvent under reduced pressure using a rotary evaporator and being dried with a vacuum pump, the product (Compound **4**) was obtained as a light yellow oil, which was used in the next reaction without further purification.

(iii) Deprotection of the benzyl group (Bzl).

A solution of Compound **4** (1.261 g, 4.04 mmol, 95%) in 30 ml of 50% (v/v) acetic acid was hydrogenated over 100 mg of 10% Pd/C by bubbling hydrogen gas through the solution at room temperature for 6 h. The catalyst was removed by filtration and washed with warm water. The combined filtrate was neutralized with 28% aqueous ammonia solution. At approximately pH 6, a white solid precipitated. After filtration of the precipitate, washing with water, and drying using a vacuum pump, the product (Compound **5**, *γ*-GA**)** was obtained as a white solid. The above sequence was repeated to recover the product from the filtrate.

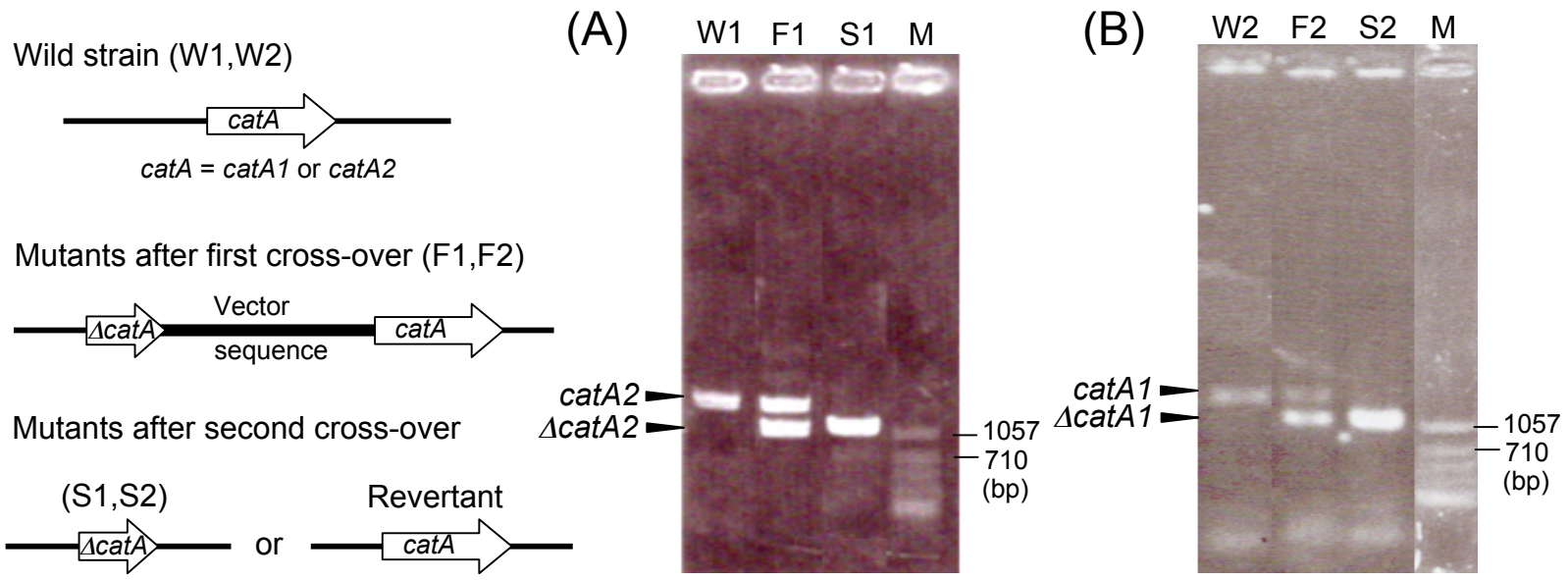


Fig. S2 Analysis of DNA fragments amplified by PCR from the total DNA of *P. putida* KT2440 and its gene disrupted mutants. (A) For the analysis of *catA2*-disrupted mutants. DNA fragments from *P. putida* KT2440 (W1), one mutant after first cross-over (F1), one mutant after second cross-over (S1), and size marker (φX *Hinc*II digest, Takara) (M). (B) For the analysis of *catA1*-disrupted mutants. DNA fragments from *P. putida* KT2440 (*ΔcatA2*) (W2), one mutant after first cross-over (F2), one mutant after second cross-over (S2), and the same size marker (M). For this analysis, PCR was carried out using the total DNA of each strain as the DNA template and the primers cA1F-F and cA1R-R for *catA1* amplification and cA2F-F and cA2R-R for *catA2* amplification.

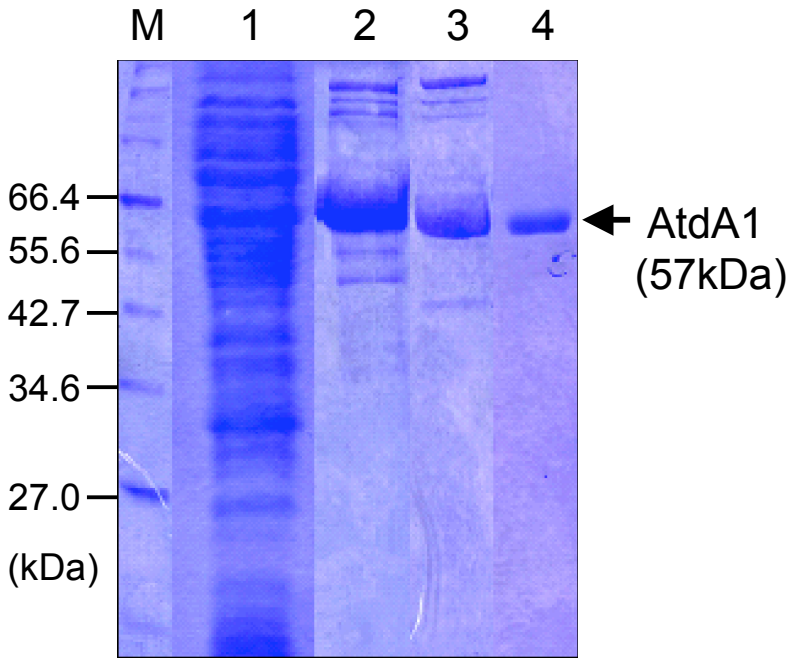


Fig. S3 SDS-PAGE analysis of proteins in purification steps for AtdA1. Lane M: Protein marker (20-212kDa, BioLabs), Lane 1: cell extract of *E. coli* SO58 containing pGS18, Lane 2: after the first anion-exchange chromatography, Lane 3: after the second anion-exchange chromatography, Lane 4: after the hydrophobic chromatography.

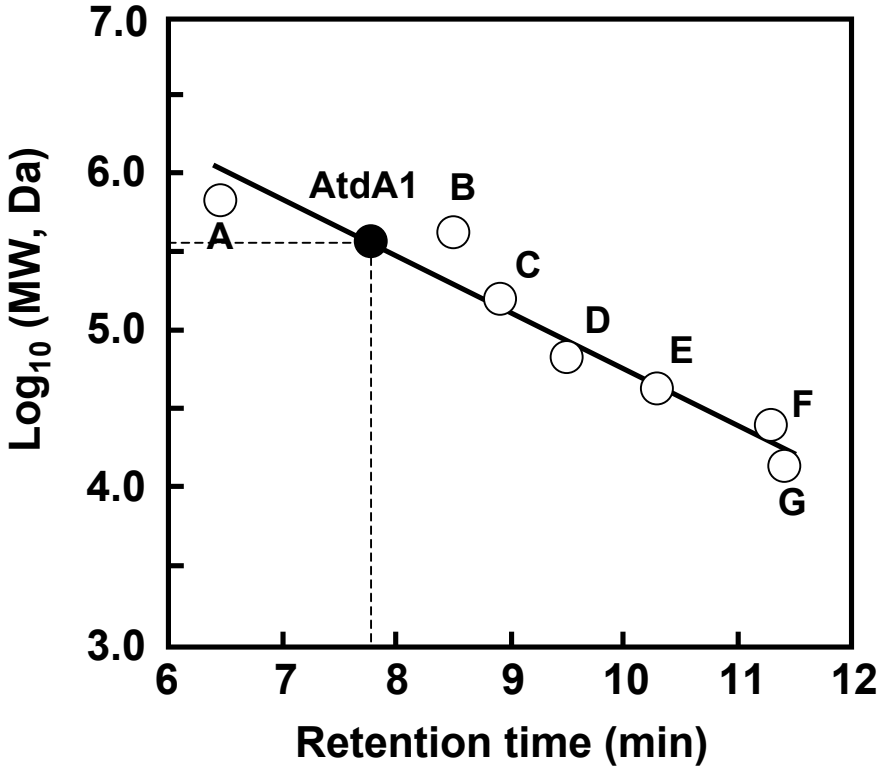


Fig. S4 Determination of native molecular mass of AtdA1 based on a standard curve using Gel Filtration Calibration kit (A, thyroglobulin 669kDa; B, ferritin 440kDa; C, aldolase 158kDa; D, albumin 67kDa; E, ovalbumin 43kDa; F, chymotrypsinogen 25kDa; G, Ribonuclease 13.7kDa).

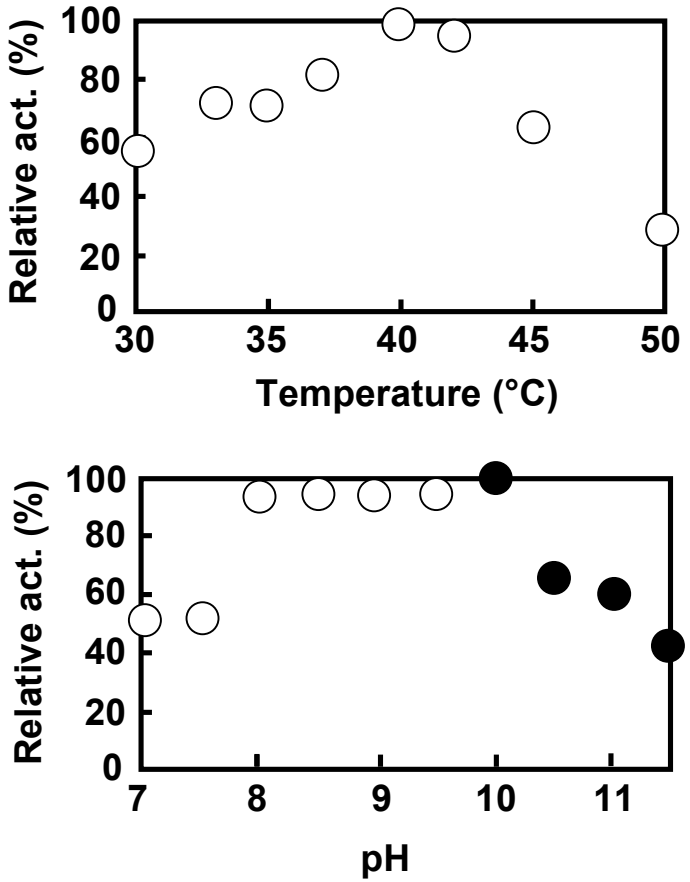


Fig. S5 Effects of temperature and pH on the enzyme activity of AtdA1. The reaction mixture (1 ml) contained 1 mM *L*-glutamate, 0.5 mM ATP, 5 mM $MgCl_2$, and 1μg of AtdA1 in 10 mM phosphate buffer (standard pH = 8.0) (open circles) or 10 mM glycine-NaOH buffer (closed circles). The reactions were performed at various temperatures (standard temperature = 30°C) for 30 min.

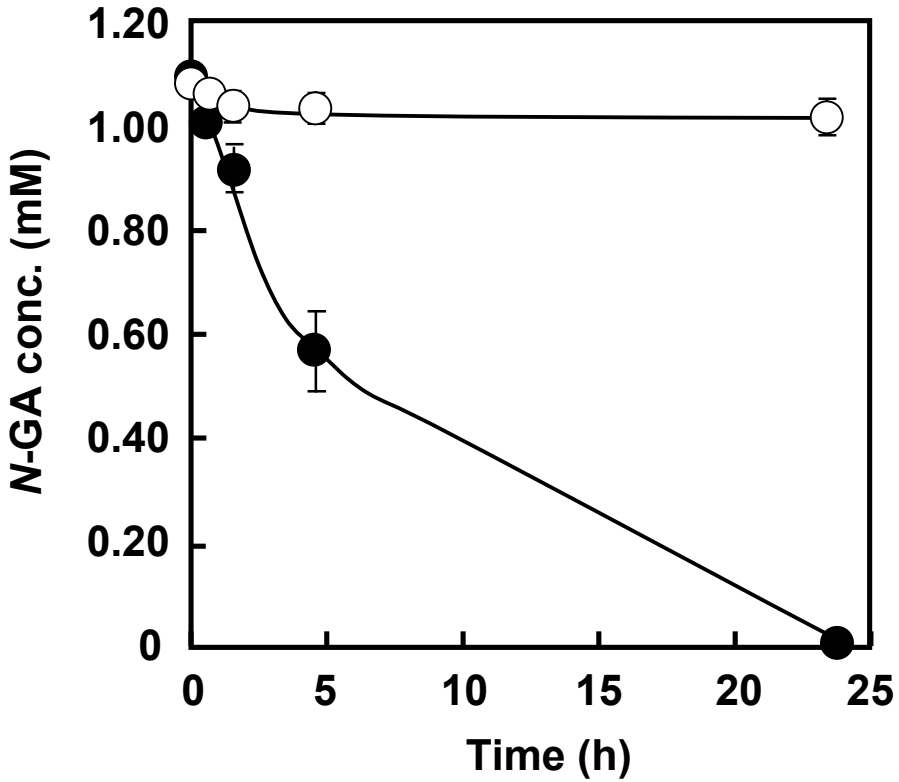
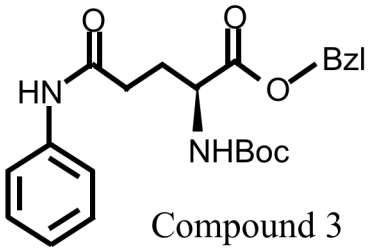
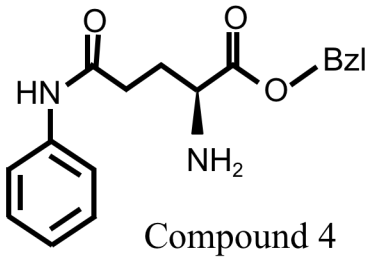
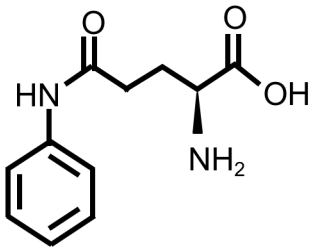


Fig. S6 γ-Glutamylanilide (γ-GA) degradation by the cell suspensions of *P. putida* KT2440-*ΔcatAΔggt* (open circles) and KT2440-*ΔcatA* (closed circles). The experiment was performed in triplicate and the average ± standard deviation is shown.

Table S1 Primers used in this study

Name	Sequence (5' to 3')
A1F	CGGATCCCATGAGTGAGAAATTAG
A1R	CGGATCCATCACAGTAAGTTGAAGTATTC
A2F-salI	GAGGTCGACATGTCTAAACGCTTTGCA
A2R	CGGATCCTTCATACATCACCCACCAAG
cA1F-F	TGCGAATTCGACGGTACTTCTTCAAGATGAAC
cA1F-R	CGAAAGCTTTGTGCAAGGCGAGCGCTTT
cA1R-F	CGAAAGCTTGGCCCTGAAGGAACATCACC
cA1R-R	CATTCTAGAAACTACAGCGTGTTCGATGTGCCC
cA2F-F	CTGGAATTCATCAACCTGCCACTGCAACTC
cA2F-R	CACGGATCCTTCCAGGAACATCACCGTG
cA2R-F	GTGGGATCCGTCGAAGGGCCGGATGGTCG
cA2R-R	CCCTCTAGAGGACCAGGCGATTACTACC
ggt-2FF-H	ACTTCTAGACCCACGAAGATATCCGCACAGG
ggt-2RR	ACGGGATCCACGATCCAGTCGATGAACCA
ggt-2FR	CGCGTCGACCGCGTTGCCGCCCTTTTTCA
ggt-2RF	ATCGTCGACAAGCAGGGCAACGCGGTCA
F-pAS51	GAGGGATCCATGTCTAAACGCTTTGCAT
pTB01-A2-5R	GGAGGATCCTTTGTATGATTTTTCGAGCAC
A1R2	ATTGAATTCTGAAAATCACAGTAAGTTGA
A3F	TTCAGAATTCGGTGGGTGATGAAAAC

Table S2 Analytical data on γ-glutamylanilide (γ-GA) and its synthetic intermediates.

Compound and structure	^{1}H-NMR	IR (cm^{-1}, in KBr)
 Compound 3	(ppm in $CDCl_3$ at 500 MHz) δ 1.45 (s, 9H, tBu), 1.88-1.97 (m, 1H, β-CH), 2.24-2.32 (m, 1H, β-CH), 2.36-2.45 (m, 1H, γ-CH_2), 4.37-4.42 (m, 1H, α-CH), 5.13-5.21 (m, 2H, CH_2 Ph), 5.37 (d, J = 7.8 Hz, 1H, $NHCO_2$), 7.08-7.57 (m, 10H, ArH), 8.43 (brs, 1H, NHPh)	N.A.
 Compound 4	(ppm in $CDCl_3$ at 500 MHz) δ 0.54 (brs, 1H, NH_2), 1.93-1.96 (m, 1H, β-CH), 2.22-2.26 (m, 1H, β-CH), 2.42-2.55 (m, 2H, γ-CH_2), 3.59-3.61 (m, 1H, α-CH), 5.13-5.20 (m, 2H, CH_2 Ph), 7.07-7.49 (m, 10H, ArH), 8.16 (brs, 1H, NHPh).	N.A.
 Compound 5 (γ-GA)	(ppm in D_2O at 500 MHz) δ 2.09-2.13 (m, 2H, γ-CH_2), 2.50-2.54 (m, 2H, β-CH), 3.63-3.65 (m, 1H, α-CH), 7.21 (brs, 1H, NHPh), 7.36-7.38 (m, 5H, ArH).	1407.9, 1444.6, 1533.3, 1583.4, 1662.5, 3317.3

N.A.: Not analyzed

Table S3 Aniline conversion activities in samples after each purification step

Purification process	Vol (ml)	Amt of protein (mg ml^{-1})	Total act (U)	Sp act (U mg^{-1})	Yield (%)
Cell extract	13.5	4.320	4.49	0.1	100
Hi-Trap Q chromatography (1st)	18	0.252	3.08	0.7	68.5
Hi-Trap Q chromatography (2nd)	18	0.058	1.90	1.8	42.3
Hi-Trap Butyl FF chromatography	8	0.036	0.61	2.1	13.6
Final soln.	1	0.208	0.45	2.2	9.9

Abbreviations: Amt : amount, Vol. : volume, act.: activity, Sp : specific. One unit (U) was defined as the activity which converts 1 μmole of aniline per min.