Strategy for Polymorphic Control by Enzymatic Reaction and Antisolvent Crystallization: Effect of Aminoacylase on Metastable β-Glycine Formation

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Materials

Tetrahydrofuran (THF) (C_4H_8O, 100% purity, Lot: E704198), acetone (CH_3COCH_3, 99.5% purity, Lot: SAC200605), *n*-heptane ($CH_3(CH_2)_5CH_3$, ≥99.0% purity, Lot: EJ2L110), methyl *tert*-butyl ether (MTBE) ($(CH_3)_3COCH_3$, 99.0% purity, Lot: EME181226), 2-propanol (IPA) ($(CH_3)_2CHOH$, 99.9% purity, Lot: EK5Q112), acetonitrile (CH_3CN, 99.9% purity, Lot: E20115099), and dimethyl sulfoxide (DMSO) ($(CH_3)_2SO$, 99.9% purity, Lot: 19100009) were received from Echo Chemical Co. Ltd. (Taiwan). Xylene ($C_6H_4(CH_3)_2$, 98.5% purity, Lot: E03B34) and chloroform ($CHCl_3$, 99.8% purity, Lot: 0000220906) were purchased from J. T. Baker Chemical Co. (USA). *p*-Xylene ($C_6H_4(CH_3)_2$, 99.5% purity, Lot: 802551), ethyl acetate ($CH_3CO_2C_2H_5$, 99.9% purity, Lot: SEA130116), benzene (C_6H_6, 99.0% purity, Lot: 310008), *n*-butyl alcohol ($CH_3(CH_2)_3OH$, 99.4% purity, Lot: 205027) and *N,N*-dimethylformamide (DMF) ($HCON(CH_3)_2$, 99.8% purity, Lot: 19100009) were obtained from TEDIA Company, Inc. (USA). Toluene ($C_6H_5CH_3$, 100% purity, Lot: B46755) was received from Mallinckrodt Baker, Inc. (USA). Methyl ethyl ketone (MEK) ($C_2H_5COCH_3$, 99.0% purity, Lot: SME190429) was obtained from Seedchem Company Pty. Ltd. (Australia). 1,4-dioxane ($C_4H_8O_2$, 98.0% purity, Lot: SP-3432R) was purchased from Showa Chemical Industry Co., Ltd. (Japan). Nitrobenzene ($C_6H_5NO_2$, 99% purity, Lot: A0282673), *N*,*N*-dimethylaniline (DMA) ($C_6H_5N(CH_3)_2$, 99% purity, Lot: A0213203001) and deuterium oxide (D_2O, 99.8 atom % D, Lot: A0348477) were purchased from Acros Organics (USA). Benzyl alcohol ($C_6H_5CH_2OH$, 99.-100.5% purity, Lot: 70950) was received from Riedel-de Haën Ltd. (USA).

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Experiments Procedures for Solvent Initial Solvent Screening Form Space Establishment

23 common solvents: *n*-heptane, xylene, *p*-xylene, ethyl acetate, toluene, MTBE, benzene, MEK, chloroform, THF, DMA, acetone, 1,4-dioxane, nitrobenzene, *n*-butyl alcohol, IPA, benzyl alcohol, acetonitrile, DMF, ethanol, DMSO, methanol, and water, were selected for initial solvent screening. About 5 mg of glycine was weighed in a 7 mL scintillation vial. Each solvent was added dropwise into the vial with intermittent manual shaking until all glycine solids were totally dissolved at 25°C. The solubility was calculated by dividing the weight of glycine with the total volume of solvent added. The solvents giving the solubility values of glycine of ≧5 mg/mL were classified as good solvents, or else they were deemed as bad solvents. The Form space of the purchased α-glycine at 25°C and 1 atm was constructed based on the categories of good and bad solvents.[1]

23 common solvents were listed in the left column and upper row. All single solvents were represented by the diagonal boxes. A good solvent for α-glycine was denoted by a yellow box, and a bad solvent by a red box. Moreover, the solvent pairs of a good co-solvent pair, a bad co-solvent pair and antisolvent could be deduced by combining two good solvents, two bad solvents, and one good solvent with one bad solvent, and represented by blue, white and green boxes, respectively. Good solvents, usually featured higher solvent power, could be served as reaction media, and used for

crystallization, extraction, and equipment cleaning. On the other hand, bad solvents could be used for rinsing the filter cake or as an antisolvent if it is also miscible with the solvent medium to selectively crystallize the desired products and enhance the product yield further. As for the 1:1 (v/v) immiscible solvent pairs at 25°C, they were symbolized by the gray boxes.

Recrystallization of Glycine by Cooling without pH Control.

A nearly saturated glycine aqueous solution of 350 mg/mL was prepared in a 7 mL scintillation vial at 60°C controlled by a water bath. The glycine aqueous solution was further heated to 70°C to avoid the presence of any invisible particles before nucleation. Glycine crystals were formed by rapidly moving the vial from the water bath at 70°C to the other one at 25°C through swift cooling. Once the crystals appeared, the slurry was quickly filtered by vacuum. All of the filtered solids generated by rapidly cooling recrystallization were oven dried at 40°C for 8 h. The pH value of glycine solution at 25°C was 6 to 6.5 indicated by pH indicator strips before crystallization happening, and pH was still 6 to 6.5 after crystallization.

Recrystallization of Glycine by Antisolvent Addition without pH Control.

In the case of antisolvent recrystallization, antisolvents must possess the solubility lower than 5 mg/mL at 25°C with respect to glycine, and be miscible in water. THF,

acetone, 1,4-dioxane, IPA, benzyl alcohol, DMF, ethanol, DMSO and methanol were used as antisolvents. About 225 mg of the purchased α-glycine solids was dissolved in 1 mL of water in a 7 mL scintillation, and then 1 mL of each kind of antisolvent was added at 25°C, to produce glycine crystals. The resulted solids were immediately separated by filtration. All of the filtered solids generated by antisolvent recrystallization were oven dried at 40°C for 8 h.

The pH value was not controlled in this experiment. However, the pH value of glycine aqueous solution should be ranging from 6 to 6.5 before antisolvent addition based on the previous cooling crystallization experiment. Since the concentration of glycine aqueous solution equal to its solubility at 25°C in water (i.e. 225 ± 5 mg/mL), the glycine aqueous solution was saturated before antisolvent addition.

The presence of glycine itself had made the two originally miscible THF/water and acetonitrile/water pairs become immiscible, indicating that these two solvent systems were inappropriate for glycine production. In general, only the diagonal boxes of the form space were based on experiments containing glycine. The remaining solvent combinations in the form space were concocted in equal volumes without any glycine, so the miscibility/immiscibility results might be altered when glycine was actually present in those systems at 25°C.

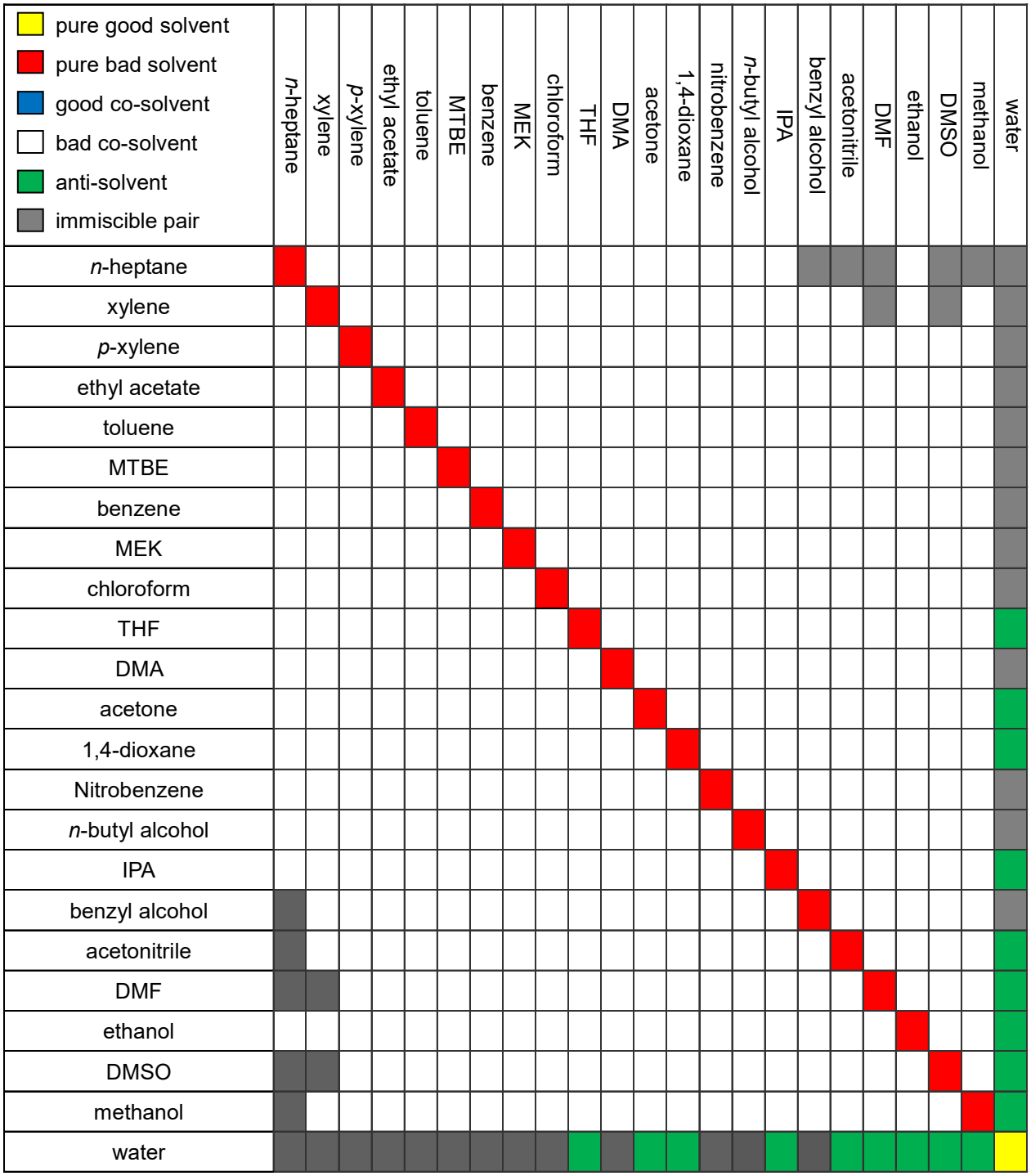
Nuclear Magnetic Resonance (NMR) Spectrometer

NMR was used to identify the molecular identity of produced glycine crystals for product verification. NMR was conducted on Bruker Ascend^{TM} 600 MHz NMR. The glycine products obtained by enzymatic reaction using pristine and recycled aminoacylase were weighed to about 20 mg, then dissolved in 1 mL of deuterium oxide. 0.8 to 1 mL of the glycine solution was added into an NMR glass tube for ^{1}H NMR analysis. The spectra were treated by Top Spin 3.5 software from Bruker.

Table S1. Experimental conditions for recrystallized glycine by antisolvent addition with and without aminoacylase.

Exp	**Aminoacylase (mg)**	**pH**	**Antisolvent**	**Water-to- Antisolvent ratio** (v/v)
1	-	4	EtOH	3 : 5
2	-	7	EtOH	3 : 5
3	-	10	EtOH	3 : 7
4	-	4	MeOH	3 : 7
5	-	7	MeOH	3 : 7
6	-	10	MeOH	3 : 9
7	13.3	4	EtOH	3 : 5
8	13.3	7	EtOH	3 : 5
9	13.3	10	EtOH	3 : 7
10	13.3	4	MeOH	3 : 7
11	13.3	7	MeOH	3 : 7
12	13.3	10	MeOH	3 : 9

Table S2. Form space of purchased α-glycine at 25°C and 1 atm.



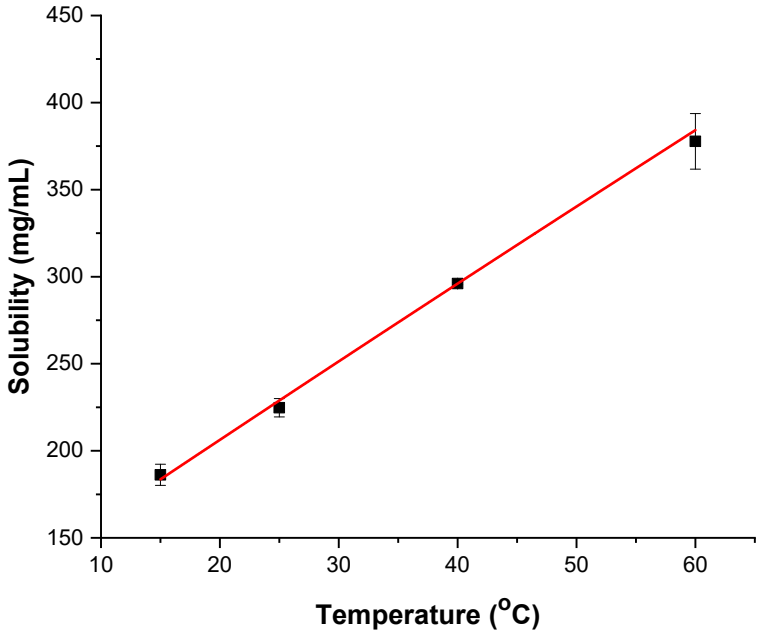


Fig S1. Solubility curve of purchased α-glycine in water.

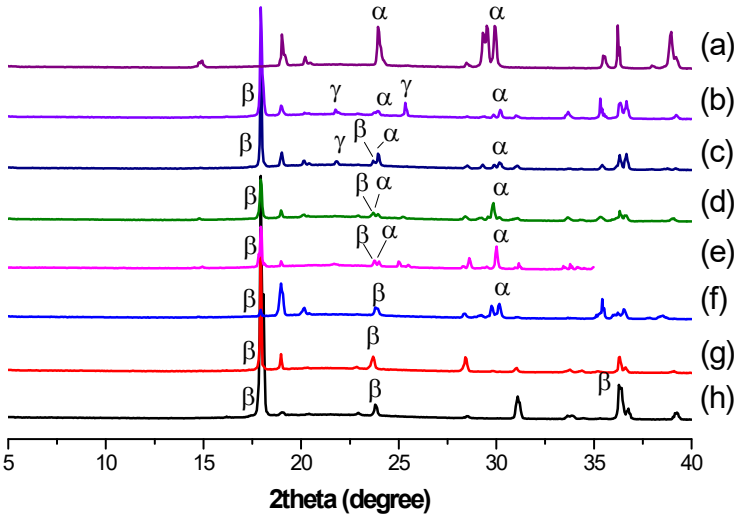


Figure S2. PXRD patterns of glycine crystals harvested from (a) water by cooling, water with antisolvent addition giving a binary mixture of 1:1 (v/v): (b) water/acetone, (c) water/1,4-dioxane, (d) water/IPA, (e) water/DMF, (f) water/ethanol, (g) water/ DMSO, and (h) water/methanol.

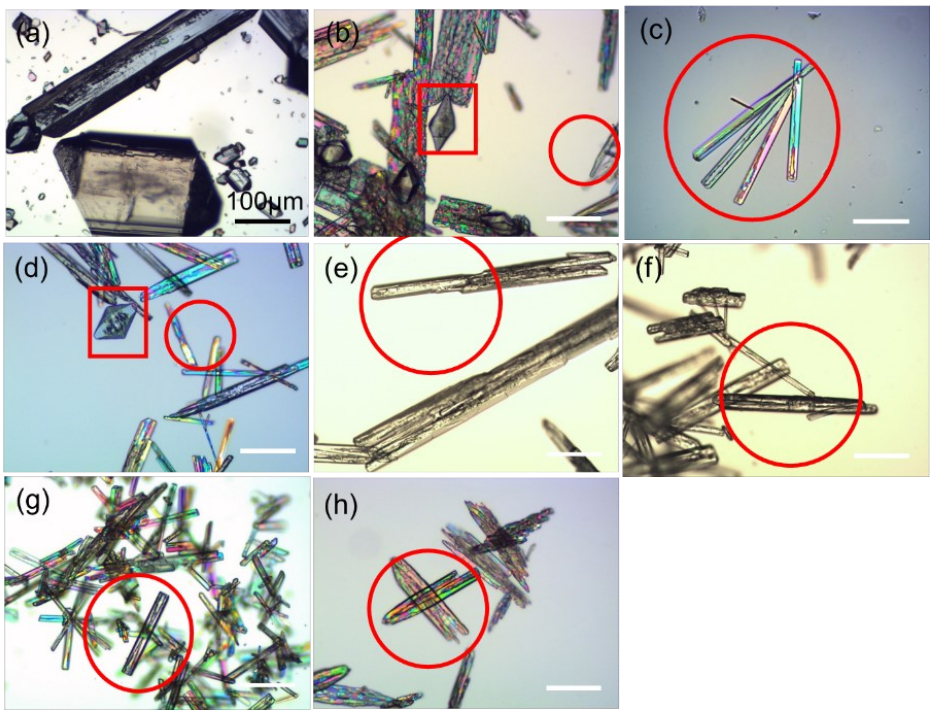


Figure S3. Optical micrographs of glycine crystals harvested from (a) water by cooling, water with antisolvent addition giving a binary mixture of 1:1 (v/v): (b) water/acetone, (c) water/1,4-dioxane, (d) water/IPA, (e) water/DMF, (f) water/ethanol, (g) water/DMSO, and (h) water/methanol. Rod-like crystals and bipyramid crystals were marked with circles and squares. (Scale bar = 100 μm)

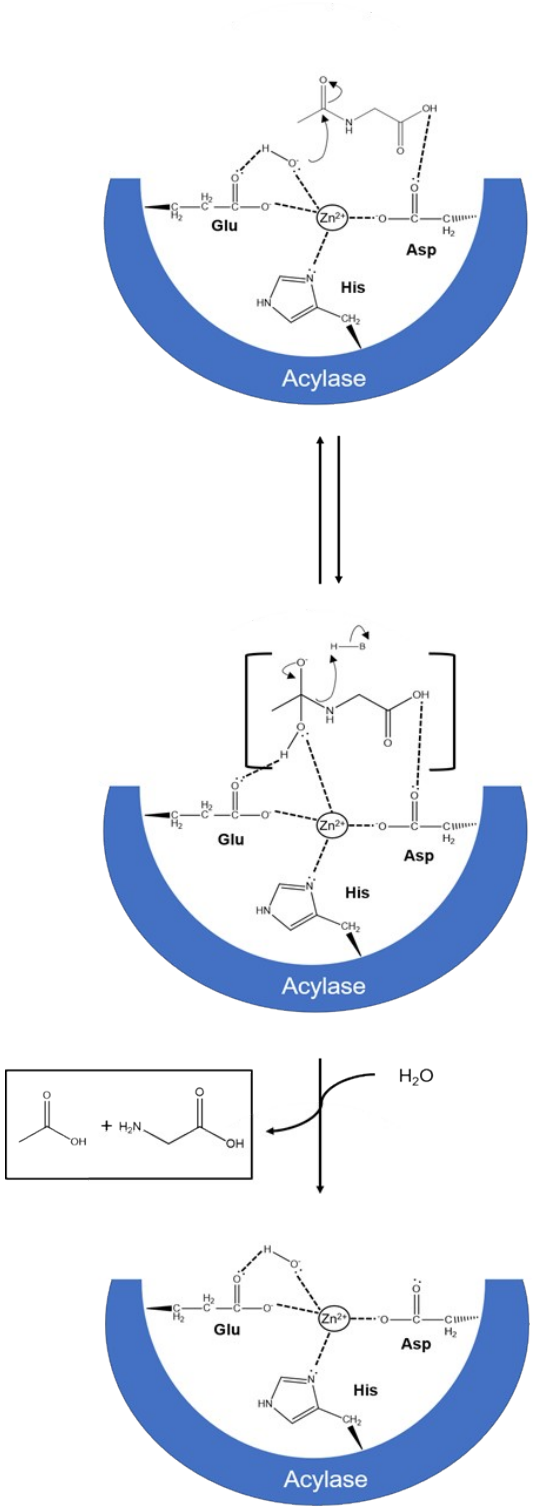
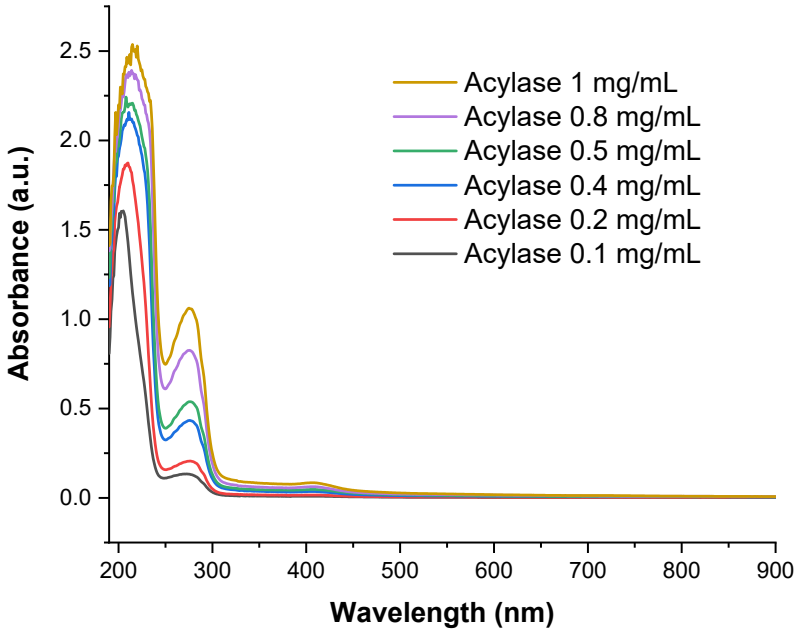
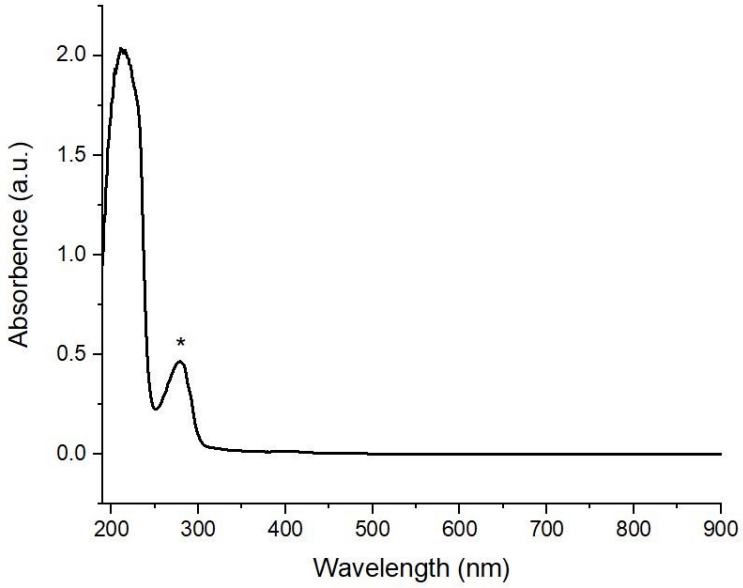


Fig. S4 Schematic diagram for the hydrolysis of N-acetylglycine to glycine by aminoacylase. H-B is a basic residue.



(a)



(b)

Fig. S5 UV-Vis spectra of aminoacylase dispersed in water with different concentrations of 0.1, 0.2, 0.4, 0.5, 0.8 and 1 mg/mL, and (b) centrifuged supernatant obtained at 9000 rpm for 40 min upon 10x dilution.

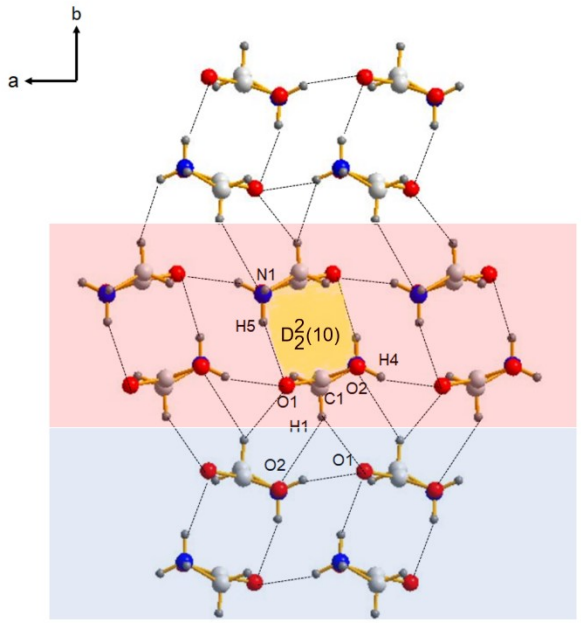


Fig. S6 Hydrogen-bonded layers stacking along the *b*-direction of an *α*-glycine crystal as depicted by a computer software (Diamond 3.1). The $D_2^2(10)$ Synthon was highlighted in yellow.

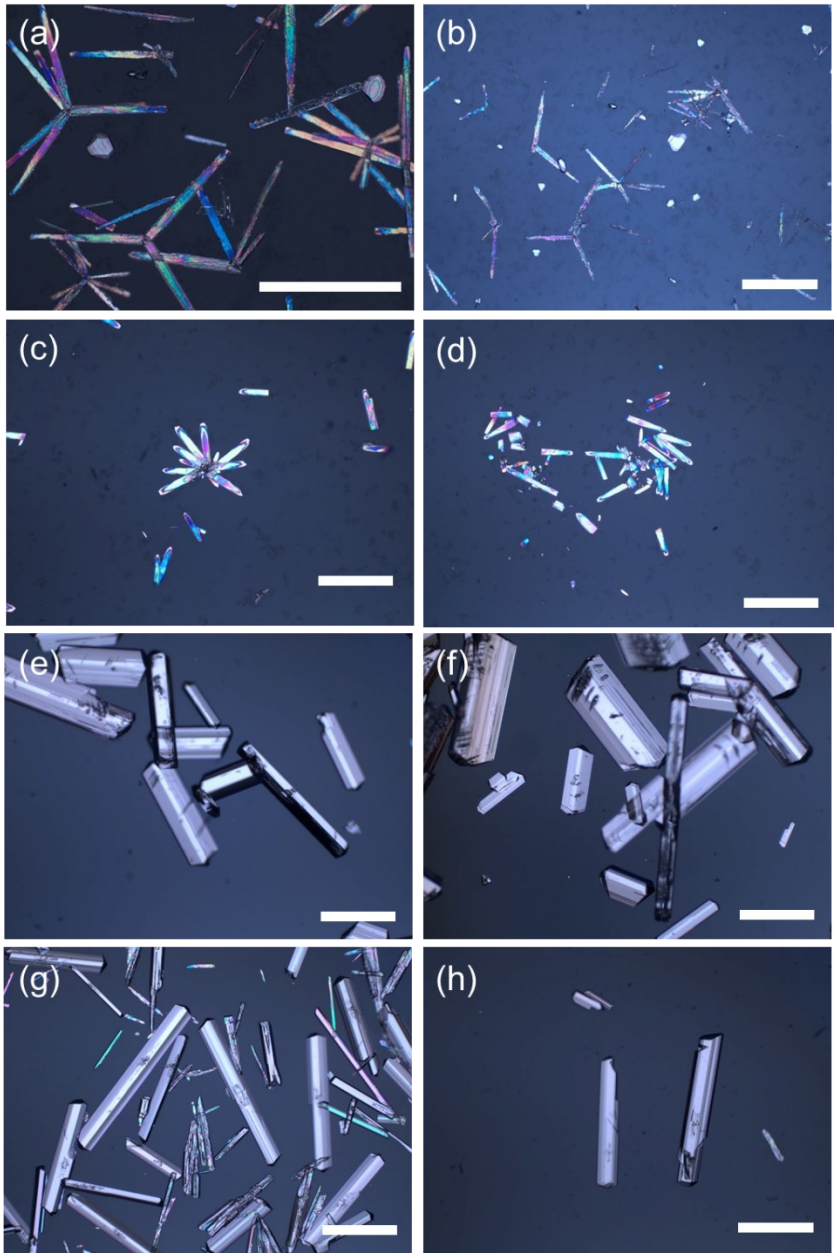


Fig.S7 OM images of glycine produced by enzymatic reaction and ethanol addition aged for (a) 12 h, (b) 24 h, and methanol addition aged for (c) 12h, (d) 24 h, produced by recrystallization without aminoacylase and ethanol addition aged for (e) 12 h, (f) 24 h, and methanol addition aged for (g) 12 h, and (h) 24 h (Scale bar = 200 μm).

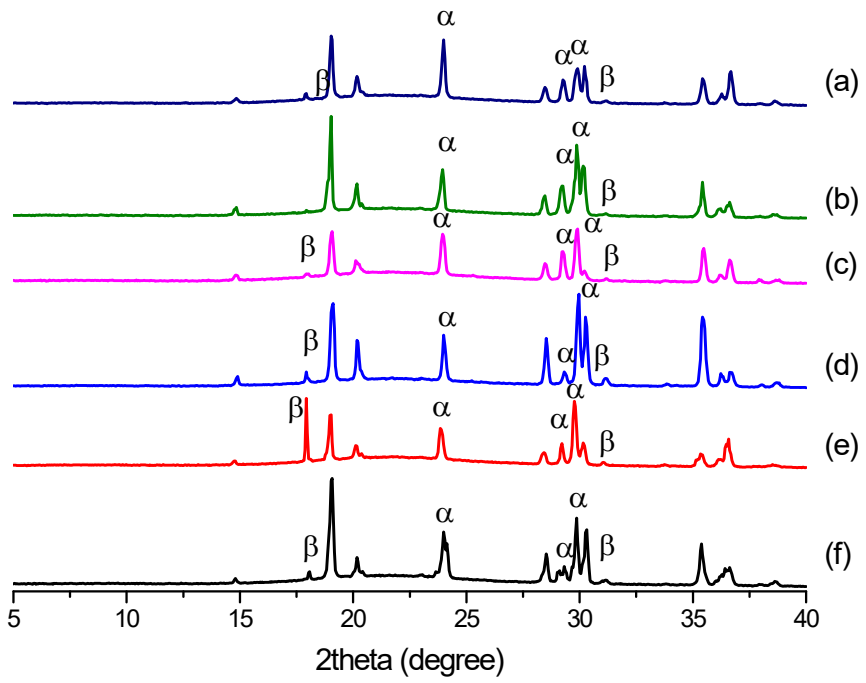
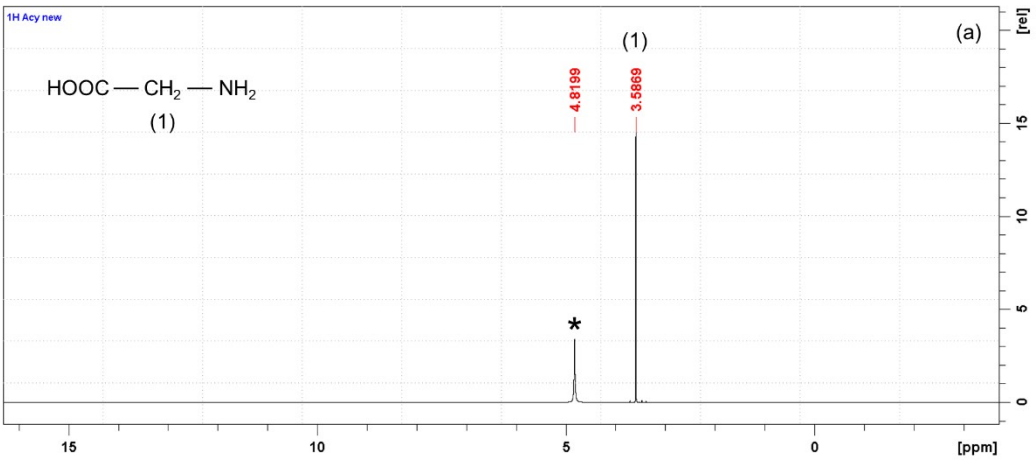
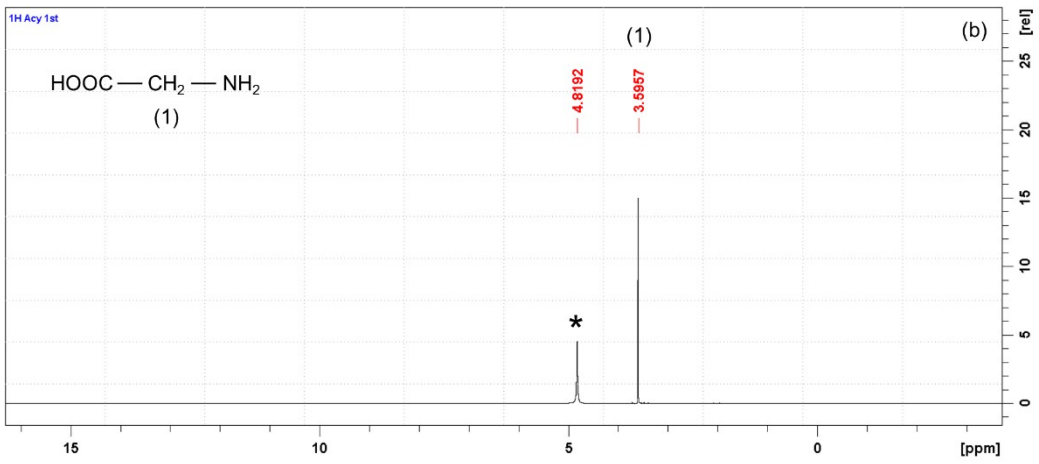


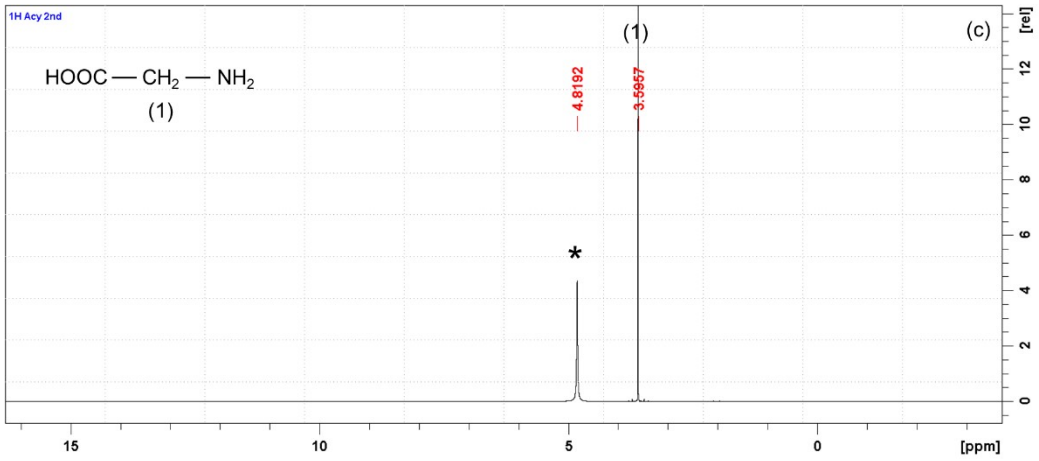
Fig. S8. PXRD patterns of glycine crystals obtained by recrystallization containing recycled aminoacylase by antisolvent ethanol addition at (a) pH 4 , (b) pH 7, and (c) pH 10, and antisolvent methanol addition at (d) pH 4, (e) pH 7, and (f) pH 10.



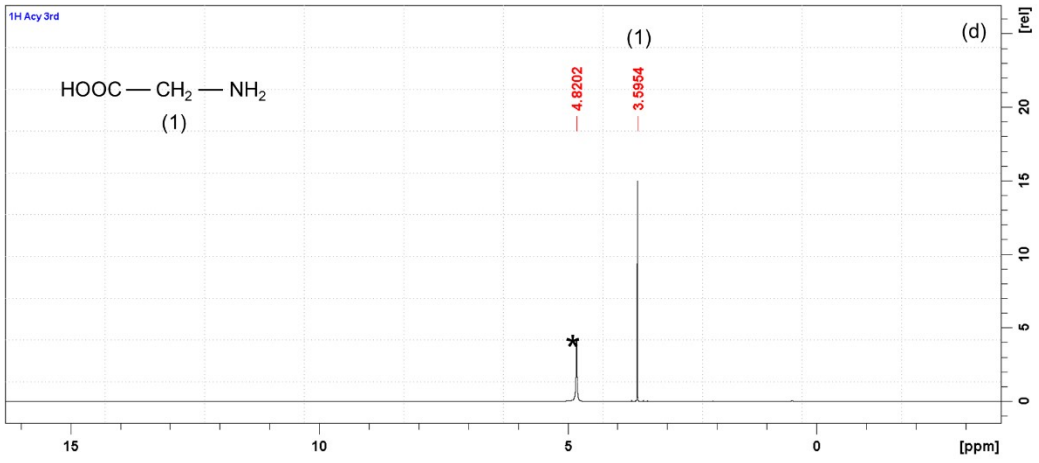
(a)



(b)



(c)



(d)

Fig. S9 ^{1}H NMR spectra of glycine produced by enzymatic reaction using (a) pristine aminoacylase, (b) 1st (c) 2nd and (d) 3rd round of recycled aminoacylase. D_2O was denoted by *.

Table S3. The yields of glycine obtained from enzymatic reactions with different ratios of pristine and recycled aminoacylase.

	Recycle rounds			
	initial	1st	2nd	3rd
Weight of pristine aminoacylase (mg)	30	11.5	5.8	3.0
Weight of recycled aminoacylase (mg)		18.5	24.2	27.0
Yield of glycine (%)	20.9	24.4	24.5	22.5

[1] Lee, T.; Kuo, C. S.; Chen, Y. H. Solubility, Polymorphism, Crystallinity, and Crystal Habit of Acetaminophen and Ibuprofen by Initial Solvent Screening. Pharm. Technol. 2006, 30, 72-93.