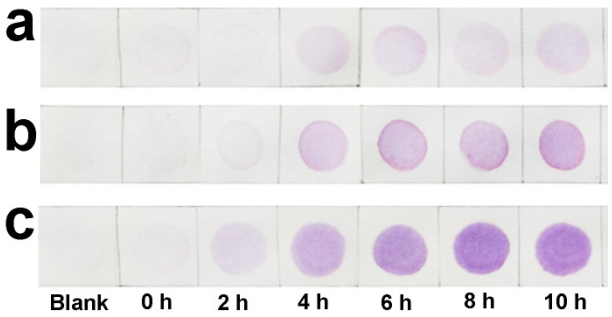

Figure S1. Asymmetric synthesis of (S)-cyclopropylglycine catalyzed by whole-cell *E. coli* (KLT) (a), a combination of the two native enzymes *E. coli* (*Ti*-LDH) and *E. coli* (*Kp*-FDH) (b), and *E. coli* (TLK) (c). The reaction was performed in phosphate-buffered saline (PBS, pH8.0) containing Na_2HPO_4, KH_2PO_4, NaCl and KCl with use of lyophilized cells (75 mg), potassium cyclopropyl-glyoxylate (4 mmol), ammonium formate (12 mmol), 0.6 mM NADH at total volume of 10 mL and 40 °C. pH was kept at 8.0-9.0 with NaOH.

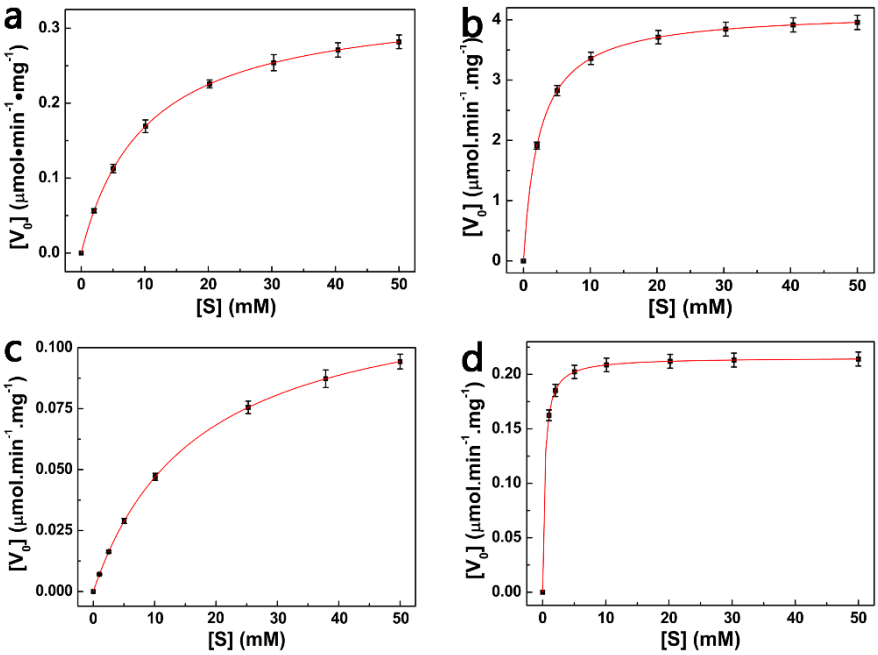

Figure S2. Steady-state kinetic of activities for different recombinant enzymes. a, c), Dependence of coenzyme regeneration activity of *Kp*-FDH and *Kp*-FDH domains in the fusion enzyme TLK on ammonium formate concentration, respectively. b, d), Dependence of reductive amination activity of *Ti*-LDH and *Ti*-LDH domains in the fusion enzyme TLK on potassium cyclopropylglyoxylate concentration, respectively. Analysis was done using Origin 2016.

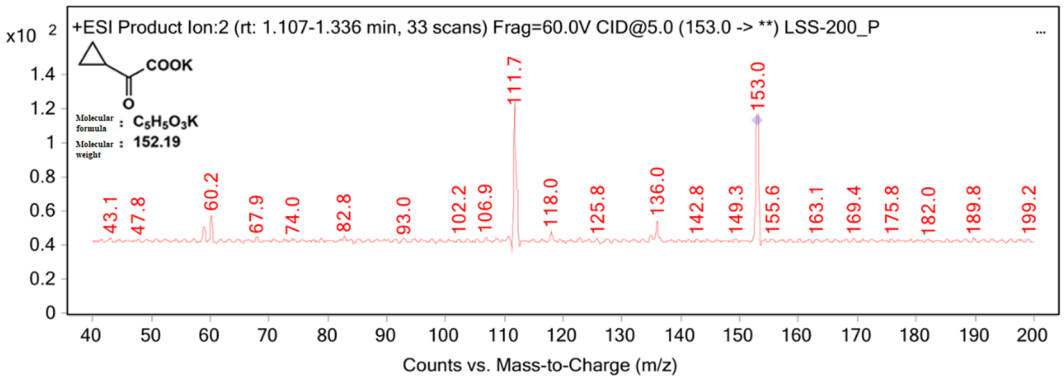

Figure S3. The LC-MS of potassium cyclopropylglyoxylate

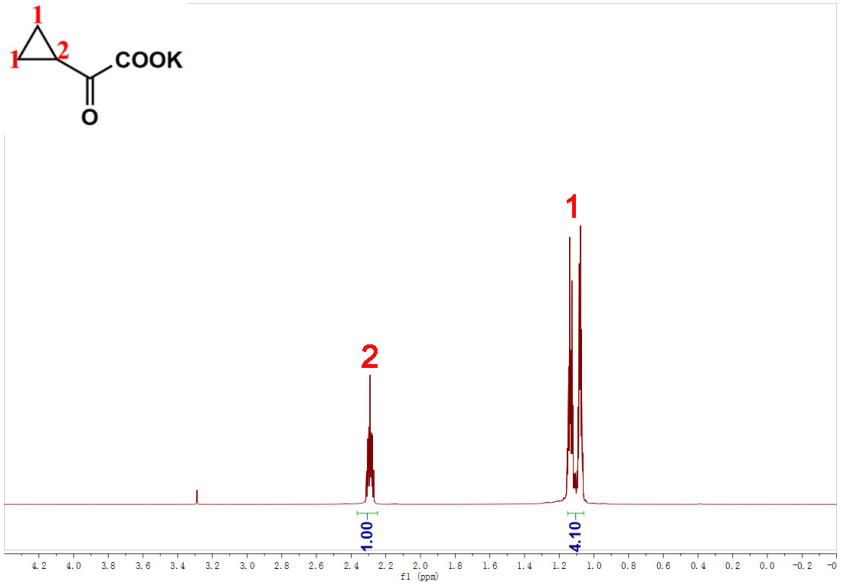

Figure S4. ^{1}H NMR of potassium cyclopropylglyoxylate (600 MHz, D_2O). δ = 1.0-1.2 (4H, m), 2.2-2.4 (1H, m).

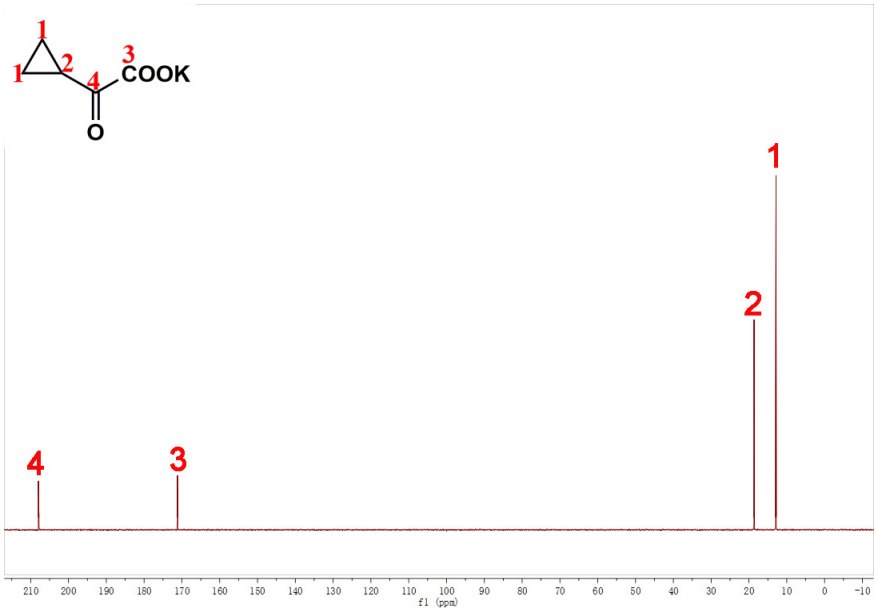

Figure S5. ^{13}C NMR of potassium cyclopropylglyoxylate (600 MHz, D_2O). δ =13.8 ($2CH_2$), 19.6 (CH), 172.3 (C), 209.1 (C).

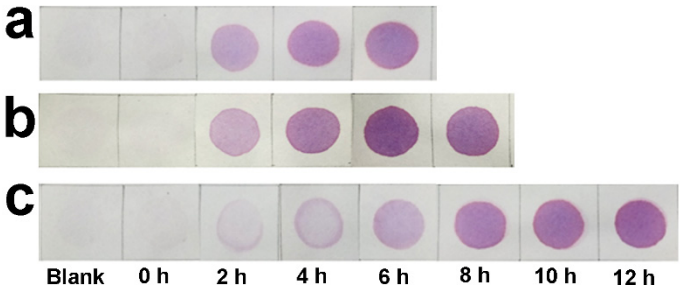

Figure S6. Asymmetric synthesis of (S)-cyclopropylglycine catalyzed by whole-cell E. coli (TLK) in the presence of 7.5 g/L biocatalysts and 0.6 mM cofactor at different concentration of potassium cyclopropylglyoxylate. (a), 100 g/L, (b) 120 g/L, and (c) 140 g/L. Other conditions: reaction medium was PBS (pH8.0), and pH was kept at 8.0-9.0 with NaOH and 40 °C.

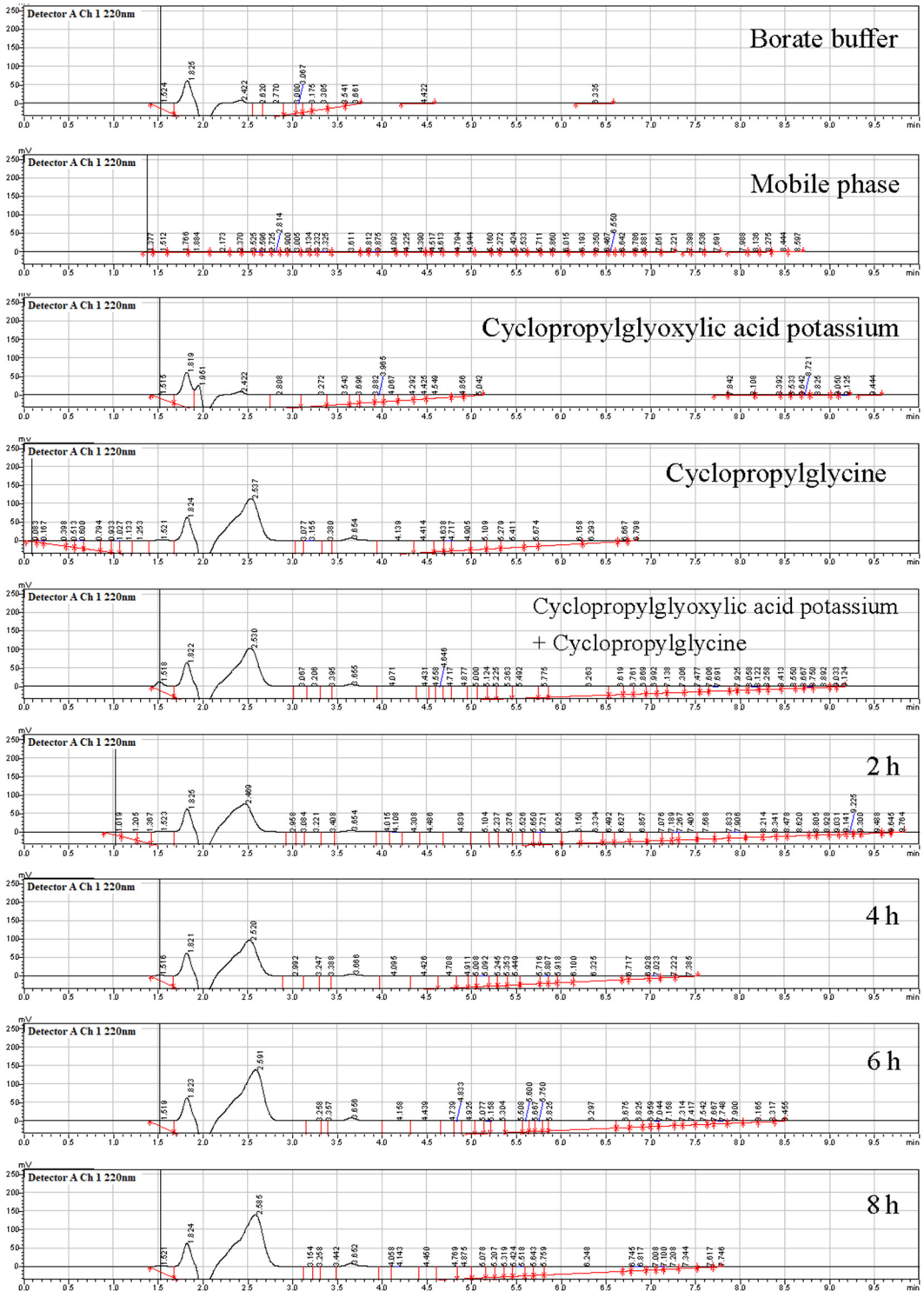

Figure S7. HPLC analysis of the generated (S)-cyclopropylglycine catalyzed by E. coli (KLT) at 120 g/L potassium cyclopropylglyoxylate in the presence of 7.5 g/L biocatalysts and 0.6 mM cofactor. Other conditions: reaction medium was PBS (pH8.0); pH was kept at 8.0-9.0 with NaOH at 40 °C.

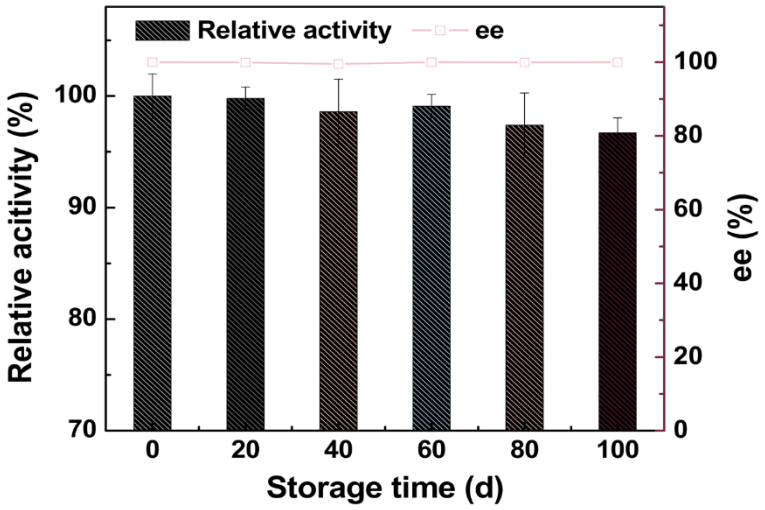

Figure S8. The influence of storage time on the biocatalytic activity of the bifunctional enzyme TLK. The biocatalytic process was conducted under standard conditions.

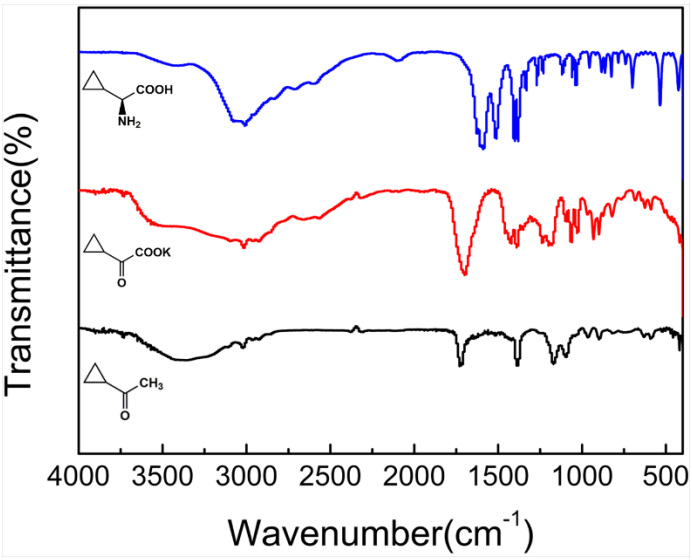

Figure S9. FT-IR spectra of cyclopropyl methyl ketone, potassium cyclopropylglyoxylate and (S)-cyclopropylglycine

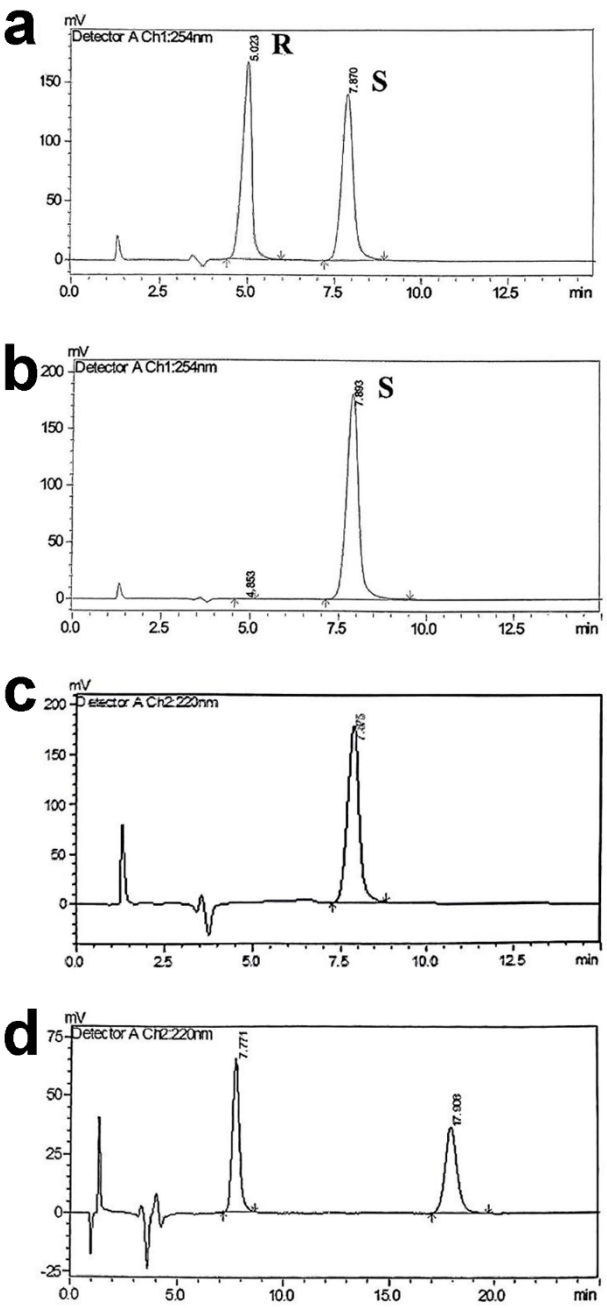

Figure S10. Determination of optical purity of the products by HPLC. a) The retention times for the standard (R/S)-cyclopropylglycine were 5.023 and 7.870 min. b) The retention time for the standard (S)-cyclopropylglycine was 7.893 min. c) The retention time for the synthesized (S)-cyclopropylglycine was 7.905 min. d) The collected reaction mixture supernatant after treated with trifluoroacetic acid and subsequent active carbon. The retention time for (S)-cyclopropylglycine and potassium cyclopropylglyoxylate was 7.771 min and 17.903 min, respectively.

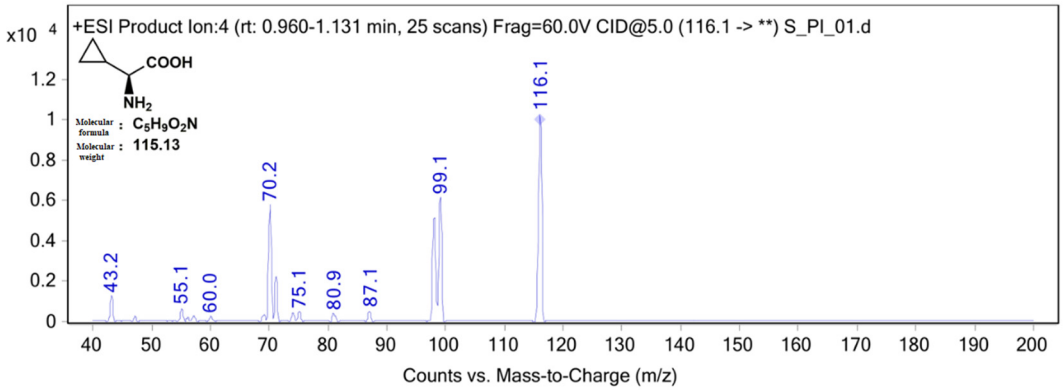

Figure S11. The LC-MS of (S)-Cyclopropylglycine

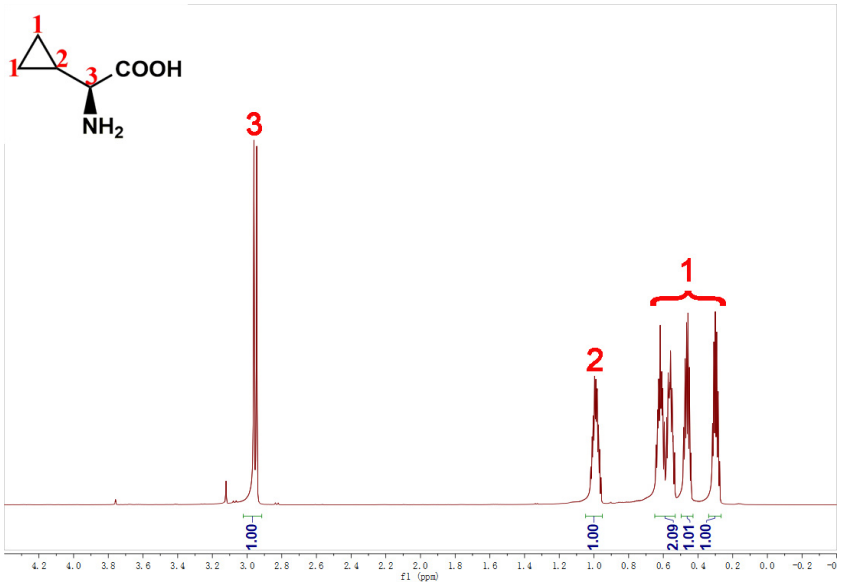

Figure S12. ^{1}HNMR of (S)-Cyclopropylglycine (600 MHz, D_2O). δ = 0.2-0.3 (1H, m), 0.4-0.5 (1H, m), 0.5-0.7 (2H, m), 0.9-1.1 (1H, m), 2.9(1H, s).

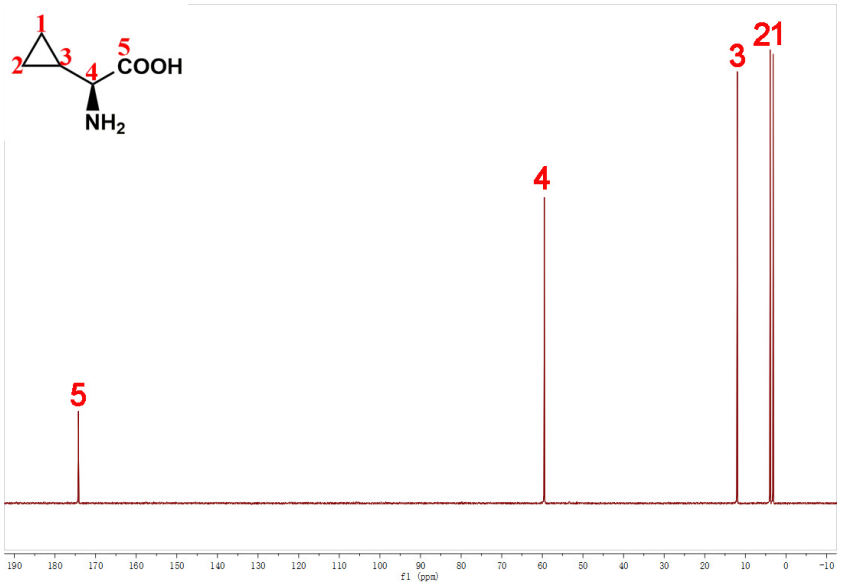

Figure S13. ^{13}C NMR of (S)-Cyclopropylglycine (600 MHz, D_2O). δ = 4.4 (CH_2), 5.8 (CH_2),13.2 (CH), 59.2 (CH), 174.8 (C);

MKIFDYMEKYDYEQLVMCQDKESGLKAIICIHVTTLGPALGGMRMWTYASEEEAIEDALRLGRGMTYKN

AAAGLNLGGGKTVIIGDPRKDKNEAMFRALGRFIQGLNGRYITAEDVGTTVEDMDIIHEETRYVTGVSPAF

GSSGNPSPVTAYGVYRGMKAAAKEAFGDDSLEGKVVAVQGVGHVAYELCKHLHNEGAKLIVTDINKENA

DRAVQEFGAEFVHPDKIYDVECDIFAPCALGAIINDETIERLKCKVVAGSANNQLKEERHGKMLEEKGIVY

APDYVINAGGVINVADELLGYNRERAMKKVEGIYDKILKVFEIAKRDGIPSYLAADRMAEERIEMMRKTR

STFLQDQRNLINFNNKGGGGSMKIVLVLYSAGKHAADEPKLYGCIENELGIRQWLEKGGHELVTTSDKEG

ENSELEKHIPDADVIISTPFHPAYITKERIQKAKKLKLLVVAGVGSDHIDLDYIEQNGLDISVLEVTGSNVVS

VAEHVVMTILNLVRNFVPAHEQIVNHGWDVAAIAKDAYDIEGKTIATIGAGRIGYRVLERLVAFNPKELLY

YDYQGLPKEAEEKVGARRVDTVEELVAQADVVTVNAPLHAGTKGLVNKELLSKFKKGAWLVNTARGAI

CNAQDVADAVASGQLRGYGGDVWFPQPAPKDHPWRDMRNKYGYGNAMTPHYSGTTLDAQVRYAEGTK

NILNSFLTKKFDYRPQDVILLNGKYKTKAYGNDKKVA

Figure 14. The amino acid sequence of TLK.