Electronic Supplementary Material (ESI) for Catalysis Science & Technology.

Supporting information

The identification and application of a robust ω-transaminase with high tolerance of substrate and isopropylamine from a directed soil metagenome

Youyu Xie,[a#] Jiguo Wang,[a#] Lin Yang,[a] Wei Wang,[a] Qinghai Liu,[a] Hualei Wang,[a,*] and Dongzhi Wei,[a]

[a]State Key Laboratory of Bioreactor Engineering, New World Institute of Biotechnology, East China University of Science and Technology, Shanghai 200237, PR China

#The authors contributed equally to this work.

*Corresponding author:

Hua-Lei Wang: hlwang@ecust.edu.cn

Table S1 The information of the 21 recombinant ω-transaminases from the mixed soil metagenomic

Entry	enzyme	Primer sequences（5'-3')	Restriction enzyme cutting site	GenBank accession no.	Identity (%)[a]
1	ATA1001	AACGCGGATCCATGAGCGCTGCCAAACTG	*BamH* I	AAP92672.1	100
		AACCCAAGCTTCTAAGCCACTTCCTTGAG	*Hind* III		
2	ATA1002	AACGCGGATCCATGAACGCCCCCGCCGCCTT	*BamH* I	KGD90311.1	100
		AACCCAAGCTTTCATGCTACTTCCTTCAGGA	*Hind* III		
3	ATA1003	AACGCGGATCCATGCCCTTTACCGCCAAC	*BamH* I	WP_025425250.1	100
		AACCCAAGCTTTTACTTGGCGCGCTTCAA	*Hind* III		
4	ATA1004	AACGCGGATCCATGAACCAGCACACCAAAC	*BamH* I	WP_034802996.1	100
		AACCCAAGCTTTTACTTGGCGCGCTTCAG	*Hind* III		
5	ATA1005	AACGCGGATCCATGCCGAACTCCGAGGCTTA	*BamH* I	WP_043543822.1	100
		AACCCAAGCTTTCAGGGCATGGATCGCAA	*Hind* III		
6	ATA1006	AACGCGGATCCATGCCGCACGCCGCCGAGCT	*BamH* I	WP_043208183.1	100
		AACCCAAGCTTTCAGGCCGTTTTCAGCACCT	*Hind* III		
7	ATA1007	AACGCGGATCCATGAACATGCCCGAAACC	*BamH* I	WP_054572853.1	100
		AACCCAAGCTTTCAGTCGATCAGGTTCAG	*Hind* III		
8	ATA1008	AACCGGAATTCATGTCCTCCTCAGCGCCTCA	*EcoR* I	WP_068979643.1	99
		AACCCAAGCTTTTACGCGTCCGCGCGCAATA	*Hind* III		
9	ATA1009	AACGCGGATCCATGAACGCCCACAACAAG	*BamH* I	WP_037390420.1	100
		AACCCAAGCTTTTACTGCACCCGTTTCAG	*Hind* III		
10	ATA1010	AACGCGGATCCATGCCCGATTTCGGCGCCAA	*BamH* I	WP_110133035.1	96
		AACCCAAGCTTCTAATCCACGGCGGCAAGCA	*Hind* III		
11	ATA1011	AACCGGAATTCATGAGCCACGACGATCCCA	*EcoR* I	WP_162377787.1	92
		AACCCAAGCTTTCACAGCCCGTCCAGCACC	*Hind* III		
12	ATA1012	AACCGGAATTCATGACCGCCCCCCTCCGCA	*EcoR* I	WP_110132869.1	98
		AACCCAAGCTTTCAGTCCTCGCCCTCCTTA	*Hind* III		
13	ATA1013	AACGCGGATCCATGGCCACCCCAAGCAAA	*BamH* I	WP_054572603.1	100
		AACCGCTCGAGTCATCGGCCGTGGTACAG	*Xho* I		
14	ATA1014	AACGCGGATCCATGTCGAACAGCAGTGCA	*BamH* I	WP_023511892.1	100
		AACCCAAGCTTTCAGGCCGTCATCACCTT	*Hind* III		
15	ATA1015	GGGAATTCCATATGATGCTATCCAACCTCGCC	*Nde* I	WP_043238127.1	100
		AACGCGGATCCTCAGGCCGCGAGCAAACCTT	*BamH* I		
16	ATA1016	AACGCGGATCCATGGATGCGGTTCAAACT	*BamH* I	EXA90087.1	100
		AACCGCTCGAGTTAATTCACTTGATAAAGC	*Xho* I		
17	ATA1017	AACGCGGATCCATGAACGCTCCGACGCCTC	*BamH* I	WP_023458938.1	100
		AACCGCTCGAGTCATAGGGCGCGCAGCAC	*Xho* I		
18	ATA1018	AACCGGAATTCATGAGCCGCATCATCCAT	*EcoR* I	WP_013396903.1	100
		AACCCAAGCTTTTATGGCGTATCGTCCGC	*Hind* III		
19	ATA1019	AACGCGGATCCATGAAGCGTCCCGGAAGCGA	*BamH* I	WP_134734931.1	99
		AACCCAAGCTTTCAGGCCGTGGCCCGGATCAT	*Hind* III		
20	ATA1020	AACGCGGATCCATGTTTGATACGGATAAATTCAGTGAC	*BamH* I	EXA90087.1	100
		AACCGCTCGAGTTAATTCACTTGATAAAGCGCATCGCCC	*Xho* I		

21	ATA1021	AACGCGGATCCATGGTTATGTTTGATACGG AACCGCTCGAGTTAATTCACTTGATAAAGC	*BamH* I *Xho* I	WP_023187880.1	100

[a] The identity means the amino acid sequence similarity to NCBI

Table S2 Initial screening results of the recombinant ω-transaminases towards the reductive amination of 1-Boc-3-piperidone with various equivalents of IPA as amine donor

IPA equivalents	Conversion (%)[a]				
	ATA1002	ATA1008	ATA1009	ATA1012	ATA1018
2	9.5	6.1	10.8	~100	4.4
3	10.6	6.3	12.2	~100	4.5
4	11.2	6.4	13.1	~100	4.5

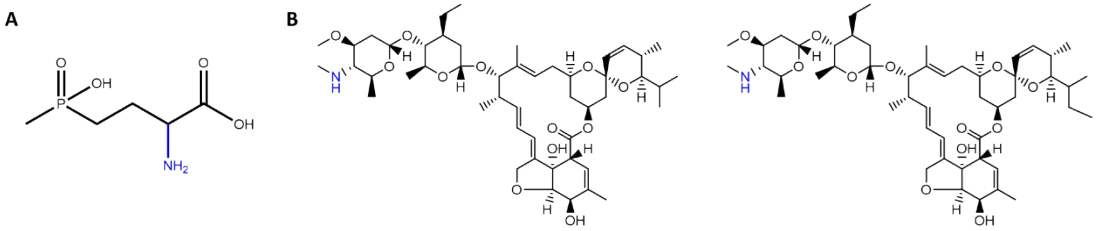
[a] The enzymes with no detectable activities were not shown.

Table S3 Analytic methods of the reductive amination activities toward various substrate ketones

Analyte	elution conditions	retention time (min)	
		amine	ketone
2g	40% A/ 60% B, 1mL/min, 37 °C, 210nm	4.0	7.5
2p	40% A/ 60% B, 1mL/min, 37 °C, 210nm	5.6	12
2q	60% A/ 40% B, 1mL/min, 37 °C, 210nm	4.9	19.4
2r	60% A/ 40% B, 1mL/min, 37 °C, 210nm	5.2	17.1
2m	60% A/ 40% B, 1mL/min, 37 °C, 210nm	4.5	8.5
2n	60% A/ 40% B, 1mL/min, 37 °C, 210nm	5.4	14
2o	60% A/ 40% B, 1mL/min, 37 °C, 210nm	7.2	16.4
2k	48% A/ 52% B, 1mL/min, 37 °C, 210nm	5.7	12.8
2i	48% A/ 52% B, 1mL/min, 37 °C, 210nm	4.3	10.4
2h	48% A/ 52% B, 1mL/min, 37 °C, 210nm	5	14
2j	48% A/ 52% B, 1mL/min, 37 °C, 210nm	4.5	11.8
2l	48% A/ 52% B, 1mL/min, 37 °C, 210nm	4.5	7.6
2s	28% C/ 72% B, 0.8mL/min, 37 °C, 210nm	4.2	14
2t	28% C/ 72% B, 0.8mL/min, 37 °C, 210nm	4.7	12.5
2a	GC Method A	3.2	3.7
2c	GC Method A	4.1	4.7
2e	GC Method A	7.2	8.9
2b	GC Method A	5.7	3.8
2d	GC Method A	3.8	4.4

2f	GC Method B	4.5	5.4

A: MeOH (0.1 % TFA), B: water (0.1 % TFA), C: Acetonitrile (0.1 % TFA)
GC Method A: GC program parameters; injector 250 °C; flow rate 1mL/min; temperature program 60°C/hold 2 min.; 90 °C/rate 10 °C per min./hold 2 min; 160 °C/rate 10 °C per min./hold 2 min; 200 °C/rate 10 °C per min./hold 2 min.
GC Method B: GC program parameters; injector 250 °C; flow rate 1.2mL/min; temperature program 100°C/hold 0.5 min.; 250 °C/rate 10 °C per min./hold 2 min.



Scheme S1 Overview of the structures of the amine group contained pesticides of (A) glufosinate and (B) methylamino abamectin, respectively.

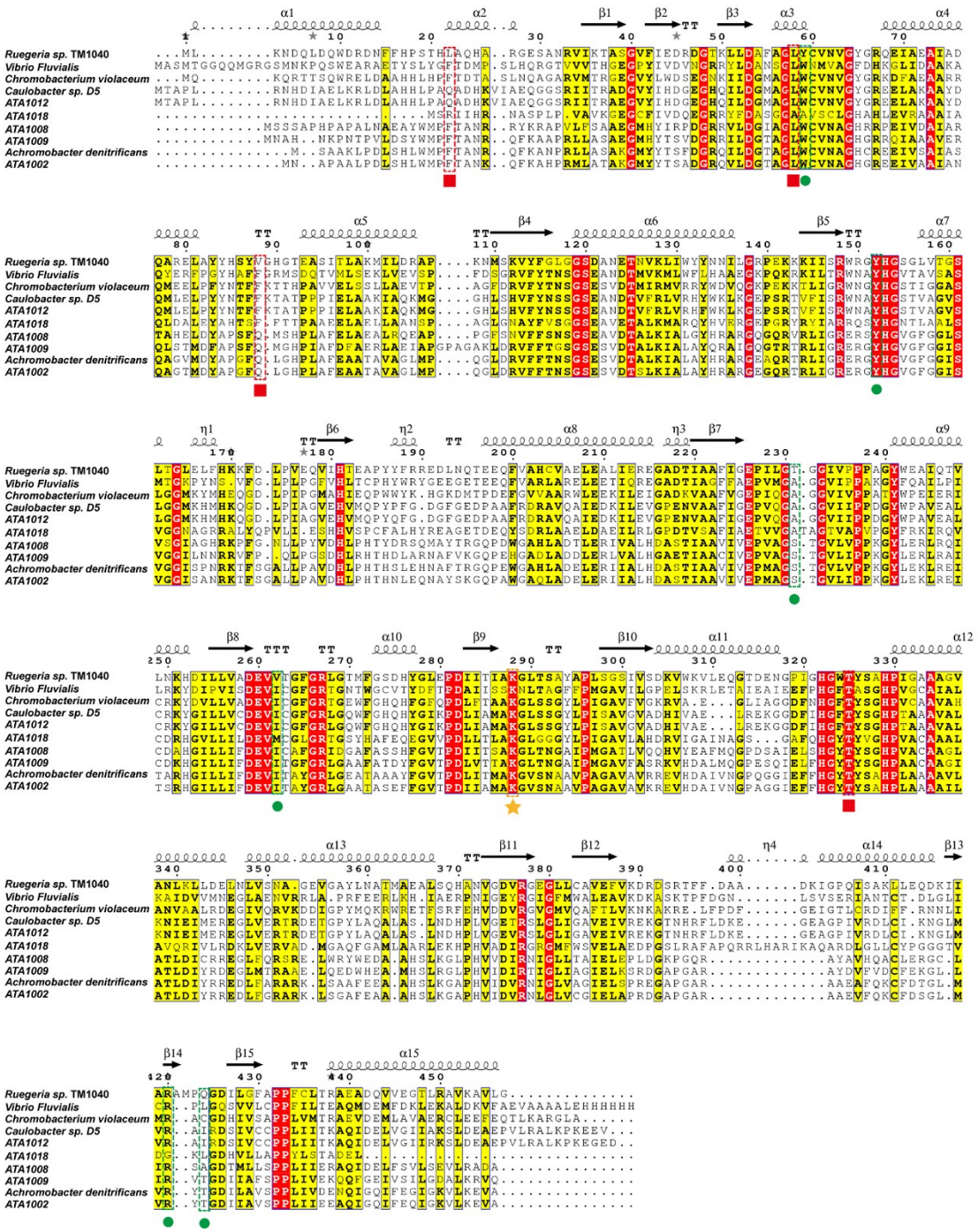


Fig. S1 Multiple sequence alignment analysis. Alignment of multiple amino acid sequences of the ω-transaminases from *Vibro fluvialis* (GenBank accession no. 3NUI_A), *Chromobacterim violaceum* (GenBank accession no. WP_011135573.1), *Ruegeria sp* TM1040 (GenBank accession no. WP_011540005.1), *Achromobacter denitrificans* (GenBank accession no. AAP92672.1), ATA1002, ATA1008, ATA1009, ATA1012 and ATA1018. Red rectangles with dash line and labeled with the red rectangles represent residues in small binding pocket of the enzyme, and green cycle with dash line and marked with a round represent the residues in large binding pocket of the enzyme; The orange rectangles with dash line and labeled with the orange star represent catalytic residue in the active of the enzyme.

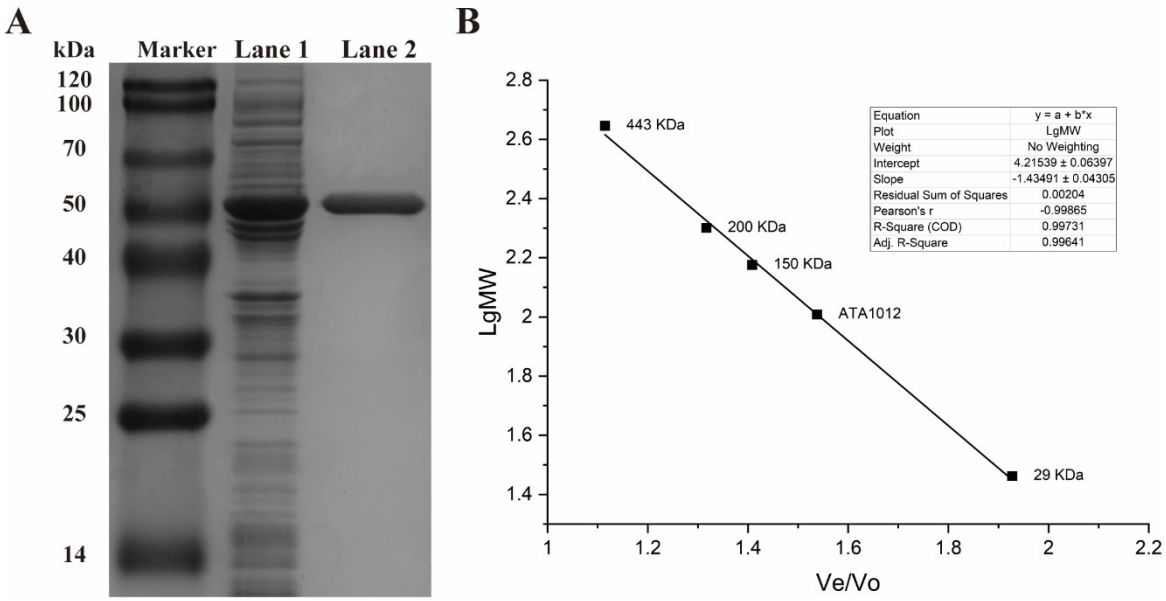


Fig. S2 Molecular mass of ATA1012. (A) SDS-PAGE analysis of cell free extracts and the purified enzyme of ATA1012. The lane 1 was the cell free extract of the ATA1012, lane 2 was the purified enzyme of ATA1012. (B) Calibration curve obtained with standard proteins from Apoferritin from horse spleen (443 kDa), β-Amylase from sweet potato (200 kDa), Alcohol Dehydrogenase from yeast (150 kDa), Carbonic Anhydrase from bovine erythrocytes (29 kDa). The molecular mass determination of purified enzyme of ATA1012 was calculated as referred in the reference [1].

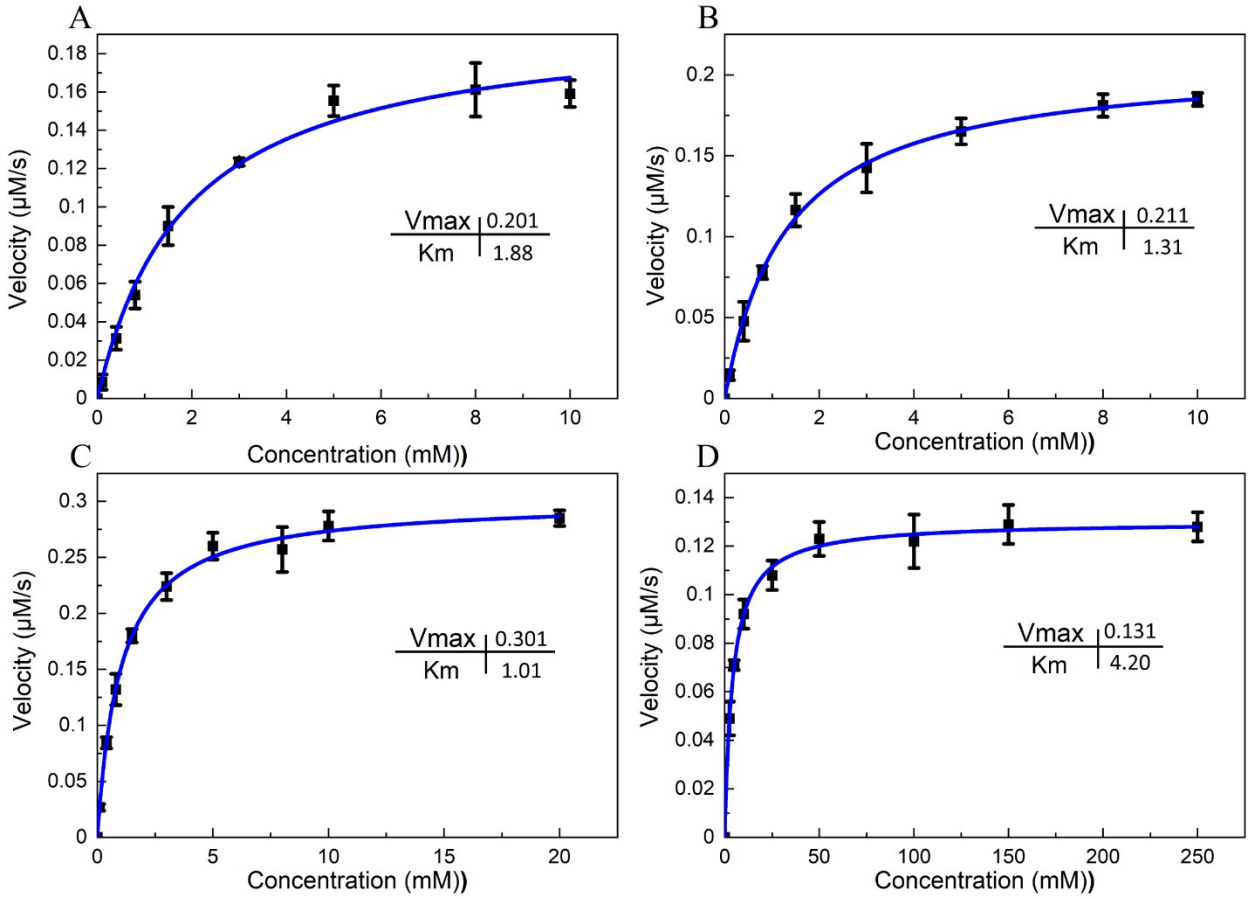


Fig. S3 Measurements of the apparent kinetic parameters by a Michaelis–Menten nonlinear regression of initial velocity vs. substrate concentration using Origin 8.0. (A) Michaelis–Menten plot of pyruvate concentration, K_m = 1.88 mM. (B) Michaelis-Menten plot of (*S*)-MBA concentration, K_m = 1.31 mM. (C) Michaelis-Menten plot of 1-Boc piperidone concentration, K_m = 1.01 mM. (D) Michaelis-Menten plot of IPA concentration, K_m = 4.20 mM.

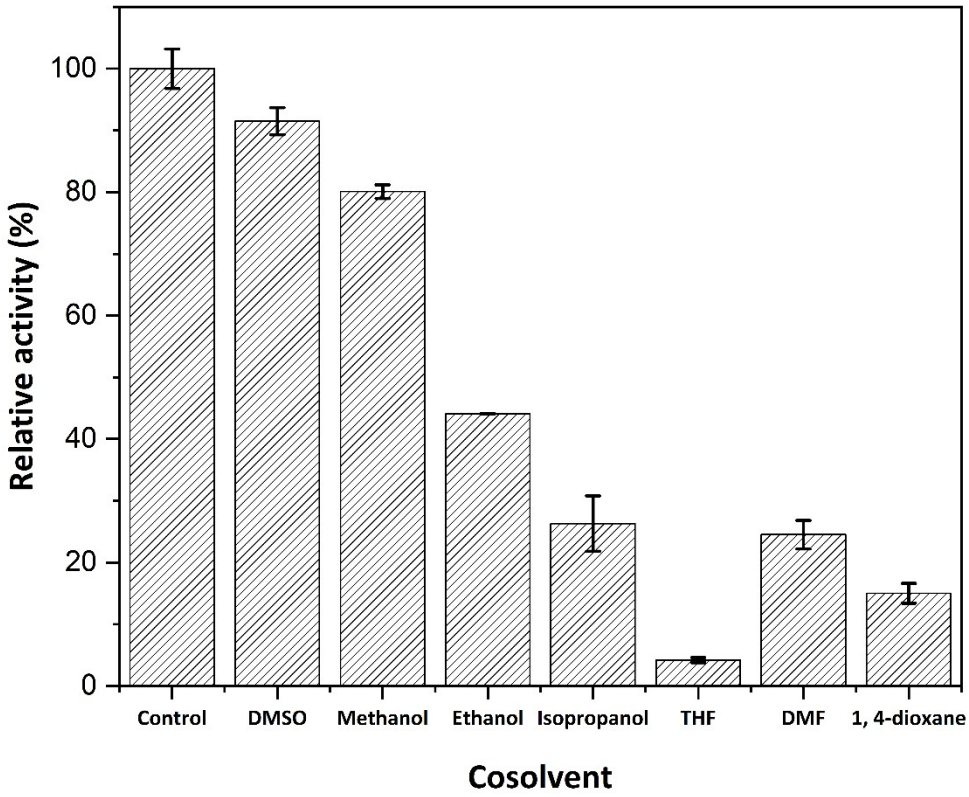
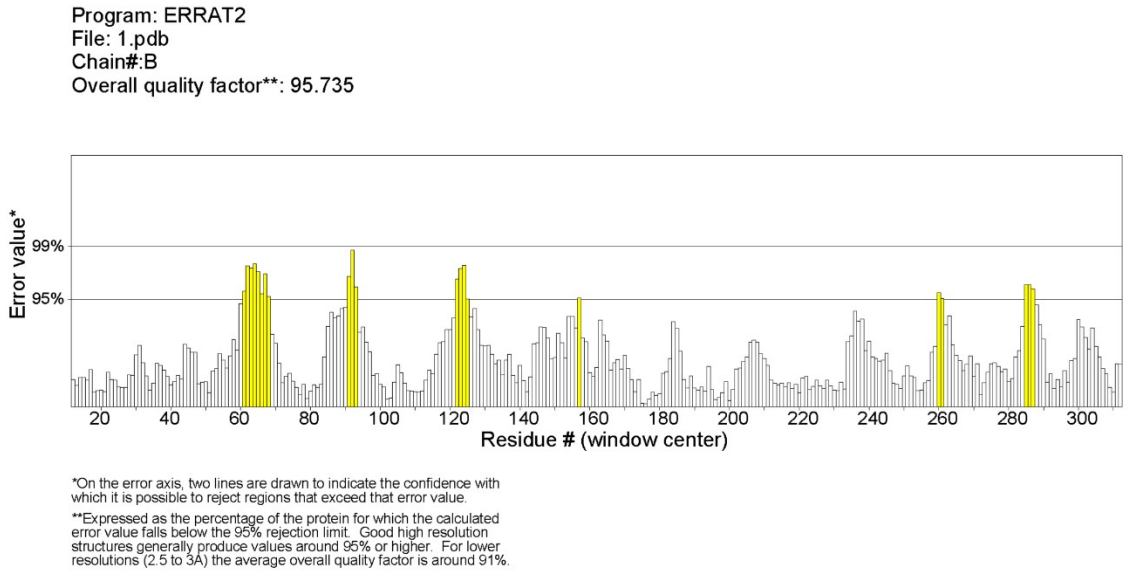


Fig. S4 Effect of various co-solvents on catalytic activity of ATA1012, 10% of different co-solvents were incubated with ATA1012 for 1 h at 37°C.

A



B

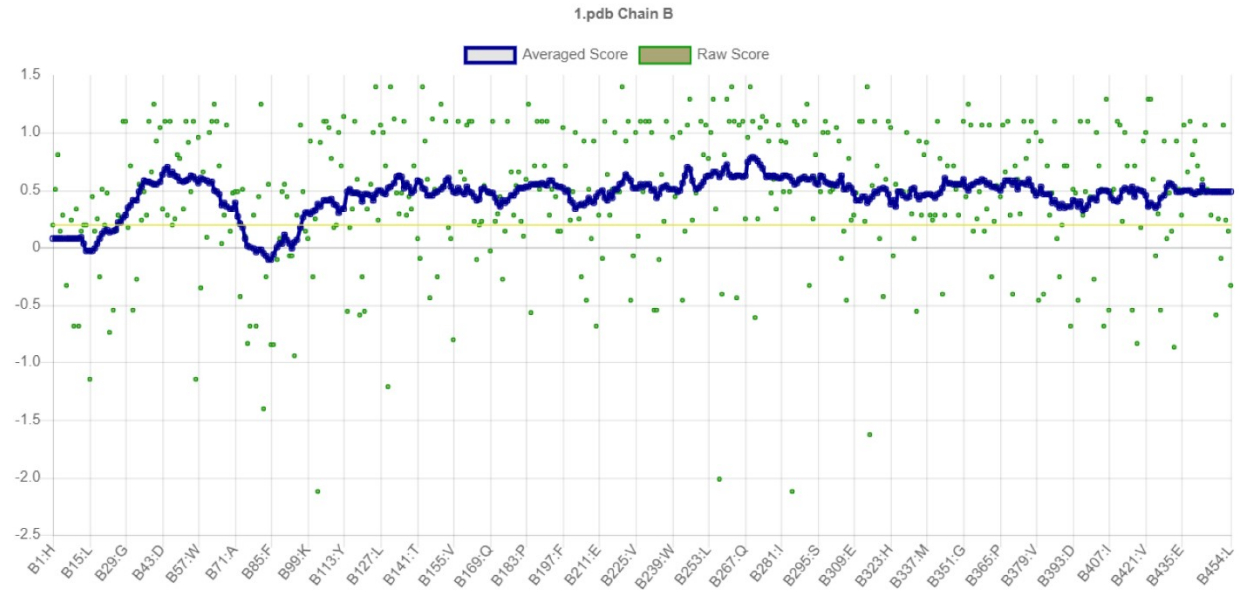


Fig. S5 The result of the model assessment evaluated by the program ERRAT (A) and the program VERIFY3D (B) in the ATA1012.

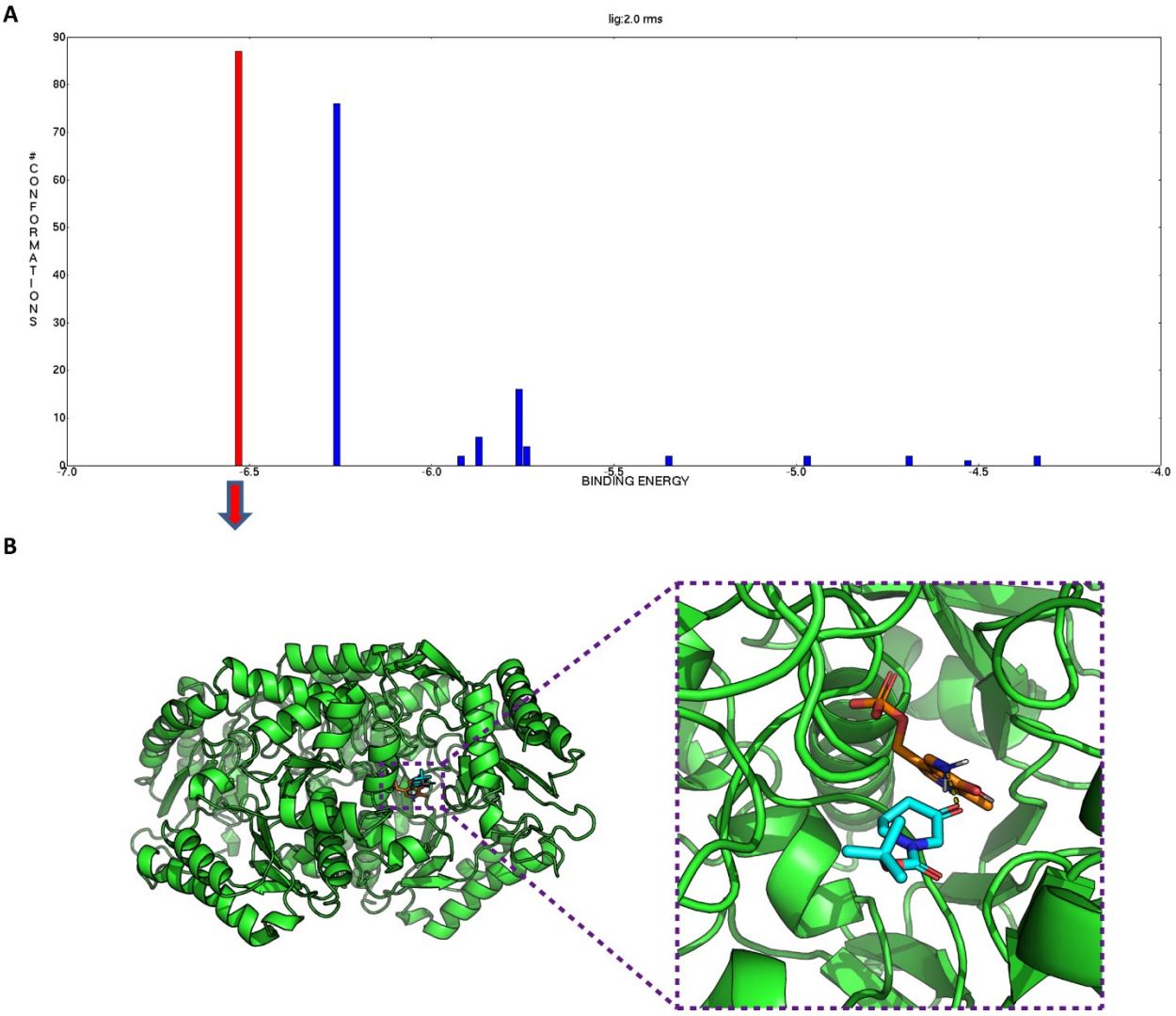


Fig. S6 The molecular docking analysis of the ATA1012. (A) The clustering analysis of docking conformation performed by autodock 4.2.6 suite; (B). Overview of the selected docking conformation with the most populated clustering and the lowest binding energy, which was used for further MD simulation.

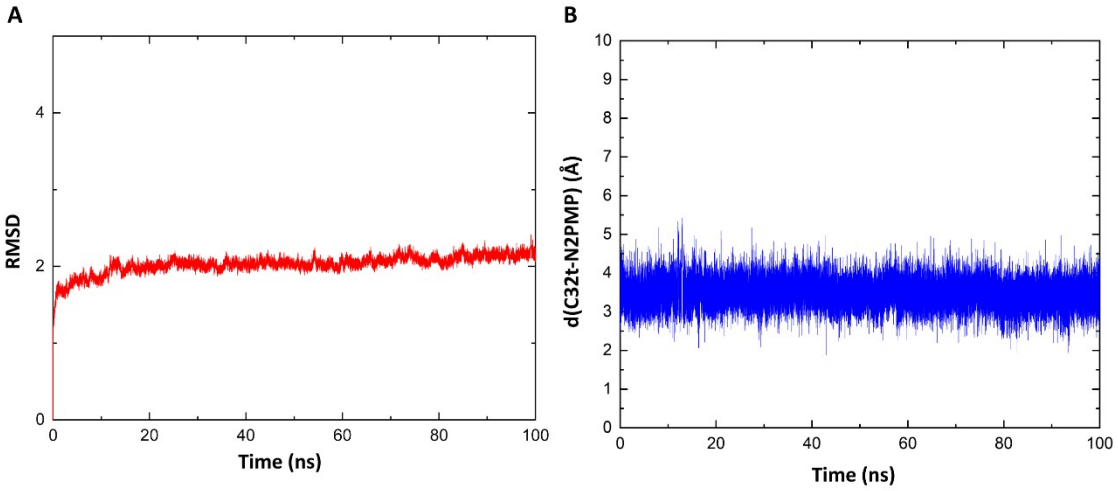


Fig. S7 The molecular dynamic simulation result obtained by using Amber 16 with GAFF and ff99SB force filed. (A) RMSD of alpha-carbon atoms for ATA1012 during 100 ns MD simulation. (B) The distance between carbonyl carbon (C3) of the substrate ketone 2t and the PMP's exocyclic nitrogen atom during the 100 ns of MD simulation.

HPLC spectra of chiral amines

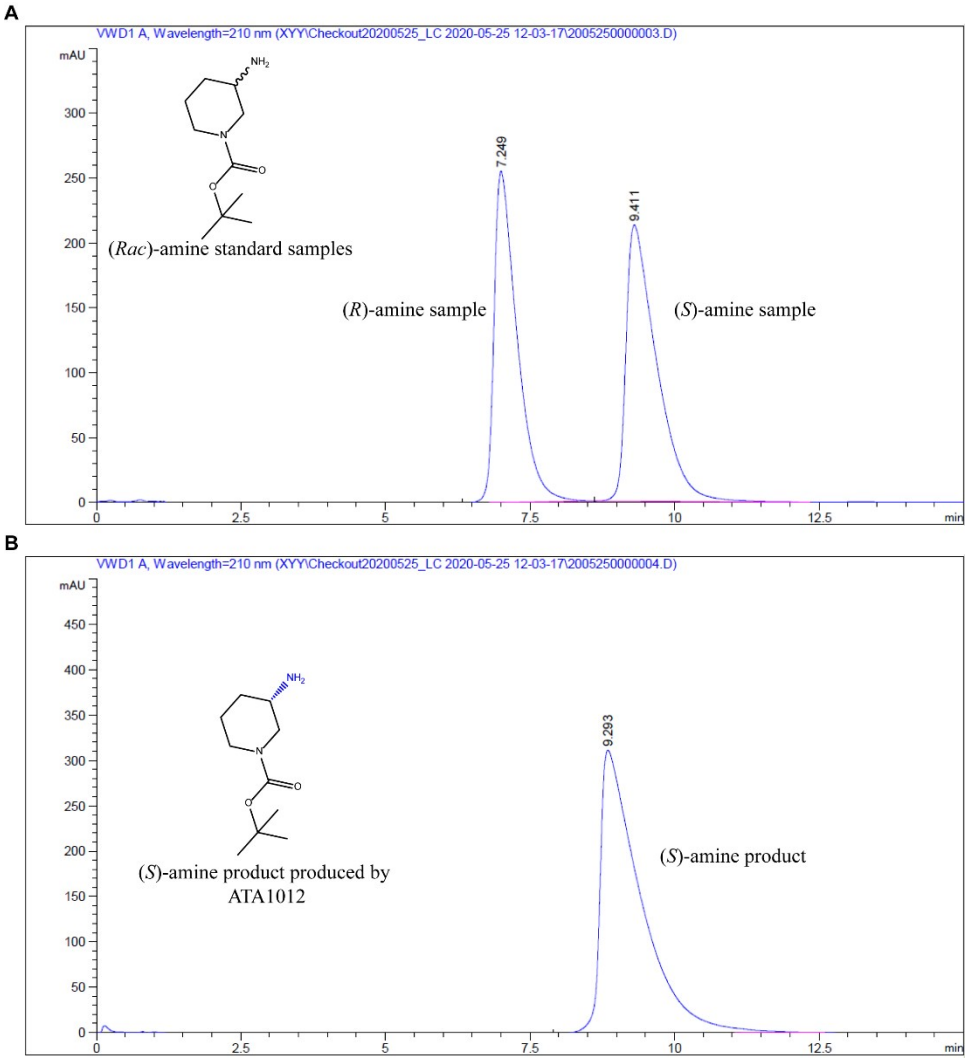


Fig. S8 HPLC analysis of the enantioselectivity of the amine product obtained by asymmetric amination of 2t. (A) The HPLC spectra of the (*Rac*)-amine standard sample of 1t; (B) The HPLC spectra of the amine product produced by ATA1012.

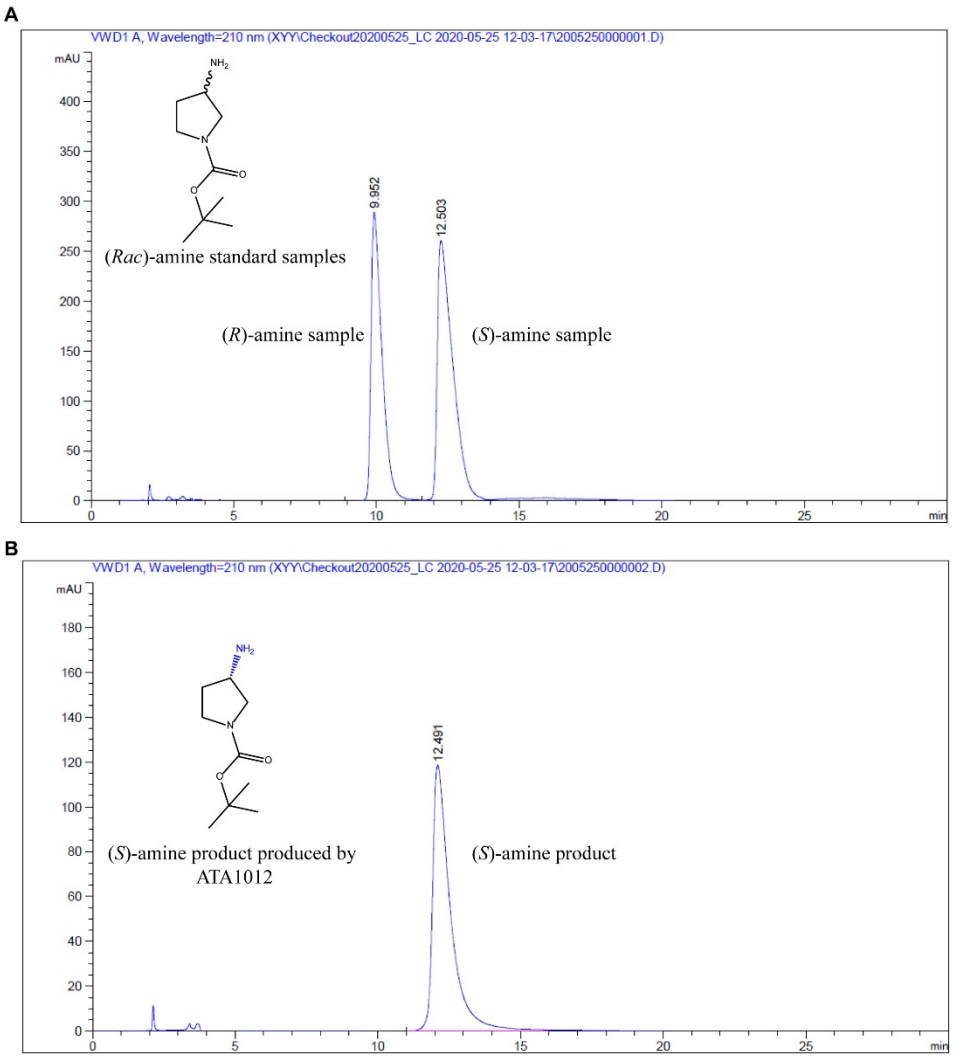


Fig. S9 HPLC analysis of the enantioselectivity of the amine product obtained by asymmetric amination of 2s. (A) The HPLC spectra of the (*Rac*)-amine standard sample of 1s; (B) The HPLC spectra of the amine product produced by ATA1012.

NMR spectra of the chiral amine products

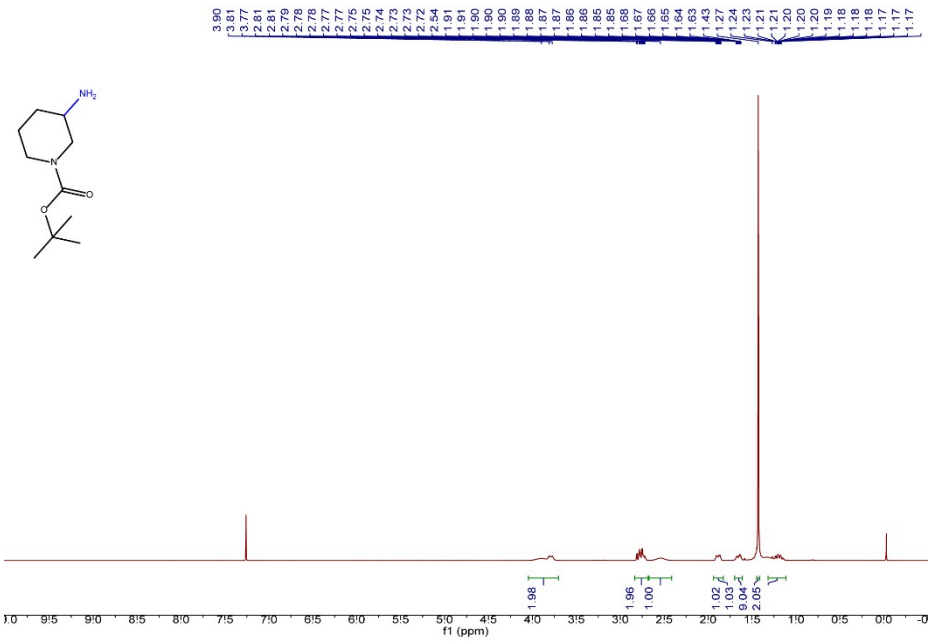


Fig. S10 The ^{1}H NMR spectra of the amine product towards 1t. ^{1}H NMR (400 MHz, $CDCl_3$) δ/ppm: 3.90 (br, 1H), 3.8–3.7 (m, 1H), 2.8–2.7 (m, 2H), 2.5 (br, 1H), 1.9–1.8 (m, 1H), 1.7–1.6 (m, 1H), 1.4 (s, 9H), 1.3–1.1 (m, 2H).

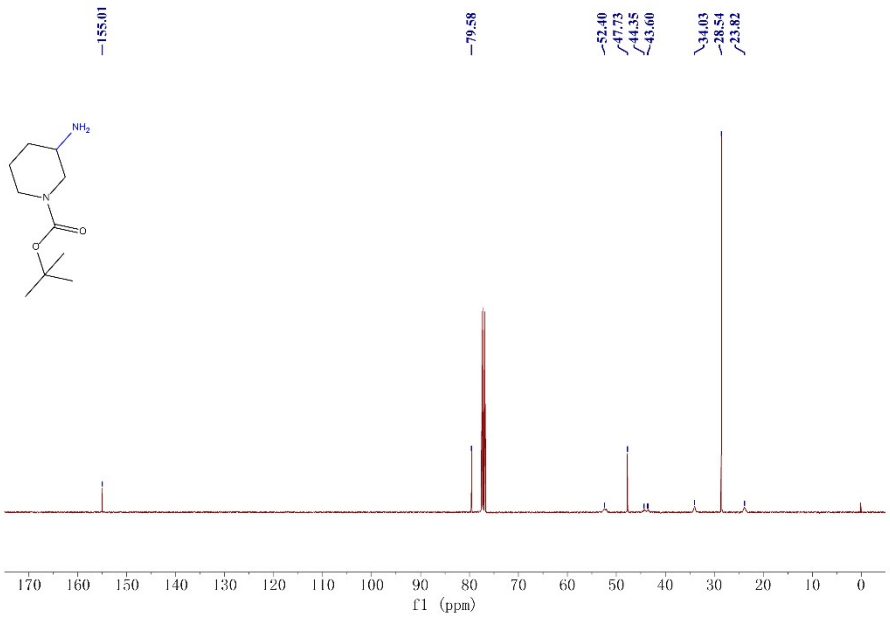


Fig. S11 The ^{13}C NMR spectra of the amine product towards 1t. ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.0, 79.6, 52.4, 47.7, 44.4 & 43.6, 34.0, 28.5, 23.8. (Two peaks in ^{13}C NMR of the amine product seen for each carbon in the piperidine ring due to the restricted rotation around the amide bond).

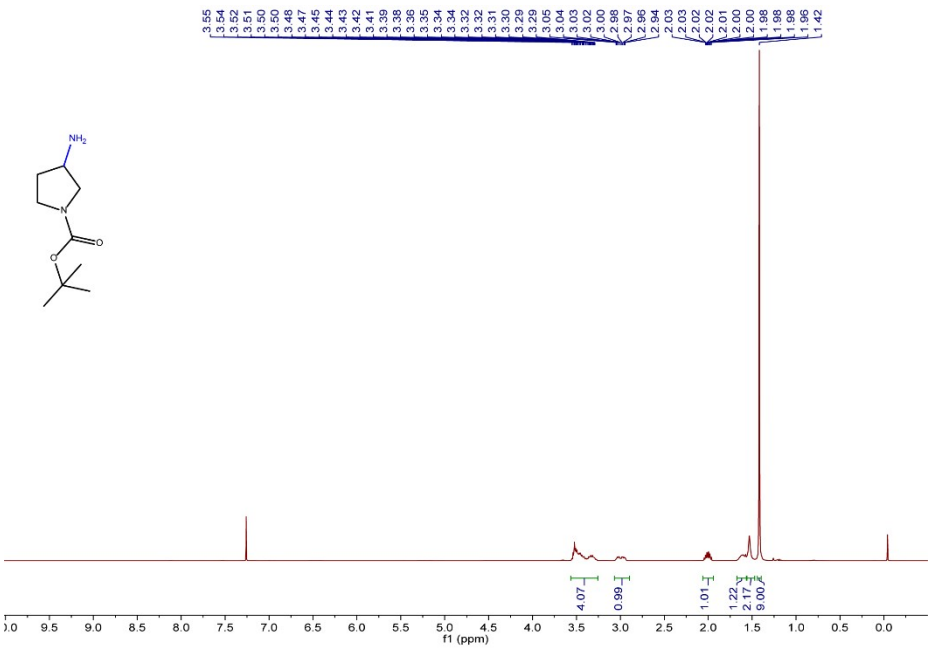


Fig. S12 The ^{1}H NMR spectra of the amine product towards 1s. ^{1}H NMR (400 MHz, $CDCl_3$) δ/ppm: 3.6–3.3 (m, 4H), 3.1–2.9 (m, 1H), 2.1–1.9 (m, 1H), 1.7–1.6 (m, 1H), 1.5 (br, 2H), 1.4 (s, 9H).

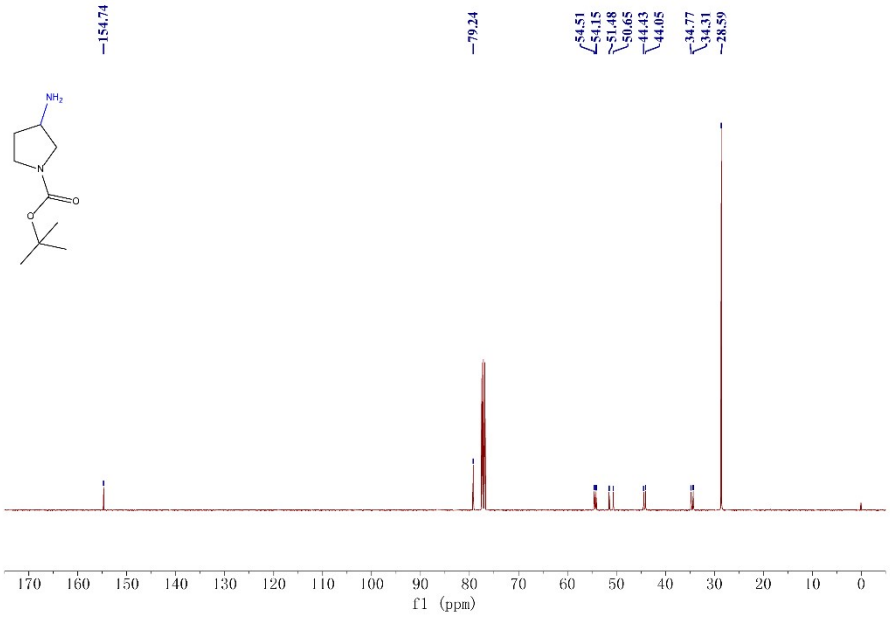


Fig. S13 The ^{13}C NMR spectra of the amine product towards 1s. ^{13}C NMR (100 MHz, $CDCl_3$) δ 154.8, 79.2, 54.5 and 54.2, 51.5 and 50.7, 44.4 and 44.1, 34.8 and 34.3, 28.6. (Two peaks in ^{13}C NMR in pyrrolidine ring due to the restricted rotation around amide bond).

Reference

1. E. Gizis and L. M. Meyer, *Nature*, 1968, **217**, 272-274.