Supplementary Materials for

Development of a versatile and efficient C-N lyase platform for asymmetric hydroamination via computational enzyme redesign

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1. General information

Reagents

Commercial reagents were used as received: crotonic acid (Acros Organics), (*E*)-2-pentenoic acid (Aldrich), fumaric acid (Sigma), (*E*)-cinnamic acid (Aladdin, Shanghai, China), (*E*)-4-methylcinnamic acid (Macklin, Shanghai, China), (*E*)-3-methylcinnamic acid (Macklin, Shanghai, China), (*E*)-4-fluorocinnamic acid (Macklin, Shanghai, China), (*E*)-3-fluorocinnamic acid (Macklin, Shanghai, China), (*E*)-4-chlorocinnamic acid (Macklin, Shanghai, China), methoxyamine (Leyan, Shanghai, China), methylamine (Sigma), ethylamine (Aladdin, Shanghai, China), ethylenediamine (Alfa), propylamine (Aladdin, Shanghai, China), isopropylamine (ENERGY, Shanghai, China), cyclopropylamine (Aladdin, Shanghai, China), allylamine (Macklin, Shanghai, China), propargylamine (Macklin, Shanghai, China), butylamine (Macklin, Shanghai, China), *sec*-butylamine (ENERGY, Shanghai, China), *tert*-butylamine (Shaoyuan, Shanghai, China), cyclobutylamine (ENERGY, Shanghai, China), pentylamine (Aladdin, Shanghai, China), cyclopentylamine (ENERGY, Shanghai, China), 2,4-dinitrofluorobenzene (TCI Shanghai, China), dansyl chloride (Admas, Shanghai, China), 9-fluorenylmethyl chloroformate (Macklin, Shanghai, China), phosphorus oxychloride (ENERGY, Shanghai, China), trietnylamine (Aladdin, Shanghai, China). The purity of reagents was > 98%.

Authentic standard of products

Authentic standard of products were synthesized by Amatek Scientific (Suzhou, China) and identified by ESI-MS and NMR:

3-(methylamino)butanoic acid (rac-1a): $C_5H_{11}NO_2$: MS (ESI^+): calcd.: [M]=117.1, found: $[M+H]^+$=118.1. ^1H NMR (400 MHz, DMSO-d6) δ 3.13 (m, J = 8.0, 6.5, 4.7 Hz, 1H), 2.47 (s, 3H), 2.22 (dd, J = 16.2, 4.7 Hz, 1H), 2.12 (dd, J = 16.3, 8.1 Hz, 1H), 1.16 (d, J = 6.6 Hz, 3H). ^{13}C NMR (126 MHz, D_2O): δ 177.86, 52.93, 38.95, 29.62, 15.44.

3-(ethylamino)butanoic acid (rac-1b): $C_6H_{13}NO_2$: MS (ESI^+): calcd.: [M]=131.1, found: $[M+H]^+$=132.1. ^1H NMR (400 MHz, DMSO-d6) δ 3.16 (q, J = 6.3 Hz, 1H), 2.92 (dd, J = 12.3, 7.1 Hz, 1H), 2.79 (dd, J = 12.3, 7.1 Hz, 1H), 2.22 (dd, J = 16.2, 4.4 Hz, 1H), 2.04 (dd, J = 16.2, 8.4 Hz, 1H), 1.16 (t, J = 6.8 Hz, 6H). ^{13}C NMR (126 MHz, D_2O): δ 177.86, 51.36, 39.79, 39.30, 15.89, 10.76.

3-(isopropylamino)butanoic acid (rac-1c): $C_7H_{15}NO_2$: MS (ESI^+): calcd.: [M]=145.1, found: $[M+H]^+$=146.1. ^1H NMR (400 MHz, DMSO-d6) δ 2.25 (dd, J = 16.1, 4.7 Hz, 1H), 2.09 (dd, J = 16.1, 7.5 Hz, 1H), 1.23 (d, J = 6.4 Hz, 3H), 1.18 (dd, J = 6.5, 2.4 Hz, 6H). ^{13}C NMR (126 MHz, D_2O): δ 177.89, 48.96, 47.45, 39.33, 18.73, 18.33, 16.09.

3-(propylamino)butanoic acid (rac-1d): $C_7H_{15}NO_2$: MS (ESI^+): calcd.: [M]=145.1, found: $[M+H]^+$=146.1. ^1H NMR (400 MHz, DMSO-d6) δ 3.17 (s, 1H), 2.94 – 2.62 (m,

2H), 2.29 – 2.05 (m, 2H), 1.59 (q, J = 8.3 Hz, 2H), 1.17 (d, J = 6.4 Hz, 3H), 0.93 (t, J = 7.5 Hz, 3H). ^{13}C NMR (126 MHz, D_2O): δ 177.98, 51.77, 46.06, 39.04, 19.35, 15.84, 10.14.

3-(sec-butylamino)butanoic acid (rac-1e): $C_8H_{17}NO_2$: MS (ESI^+): calcd.: [M]=159.1, found: $[M+H]^+$=160.1. ^{1}H NMR (400 MHz, DMSO-d6) δ 3.57 (qd, J = 6.4, 2.3 Hz, 1H), 3.31 – 3.22 (m, 1H), 2.50 – 2.36 (m, 2H), 1.68 (dtd, J = 15.0, 7.5, 5.0 Hz, 1H), 1.52 (dt, J = 13.9, 7.6 Hz, 1H), 1.28 – 1.19 (m, 6H), 0.89 (dd, J = 7.6, 1.0 Hz, 3H). ^{13}C NMR (126 MHz, D_2O): δ 178.00, 52.66, 48.93, 38.59, 25.76, 16.43, 15.58 (d, J = 18.2 Hz), 8.55.

3-(butylamino)butanoic acid (rac-1f): $C_8H_{17}NO_2$: MS (ESI^+): calcd.: [M]=159.1, found: $[M+H]^+$=160.1. ^{1}H NMR (400 MHz, DMSO-d6) δ 3.18 (td, J = 7.4, 4.9 Hz, 1H), 2.89 (dt, J = 11.9, 7.1 Hz, 1H), 2.74 (dt, J = 12.0, 7.5 Hz, 1H), 2.29 – 2.06 (m, 2H), 1.56 (p, J = 7.4 Hz, 2H), 1.36 (h, J = 7.2 Hz, 2H), 1.18 (d, J = 6.5 Hz, 3H), 0.90 (t, J = 7.3 Hz, 3H). ^{13}C NMR (126 MHz, D_2O): δ 177.95, 51.78, 44.21, 39.04, 27.73, 19.06, 15.83, 12.66.

3-(pentylamino)butanoic acid (rac-1g): $C_9H_{19}NO_2$: MS (ESI^+): calcd.: [M]=173.1, found: $[M+H]^+$=174.2. ^{1}H NMR (400 MHz, DMSO-d6) δ 3.07 (ddd, J = 8.7, 6.5, 4.3 Hz, 1H), 2.81 (dt, J = 11.8, 7.1 Hz, 1H), 2.65 (dt, J = 11.9, 7.4 Hz, 1H), 2.16 (dd, J = 16.0, 4.4 Hz, 1H), 2.02 (dd, J = 16.1, 8.7 Hz, 1H), 1.52 (p, J = 7.4 Hz, 2H), 1.30 (dp, J = 7.8, 4.4, 3.9 Hz, 4H), 1.11 (d, J = 6.5 Hz, 3H), 0.93 – 0.82 (m, 3H). ^{13}C NMR (126 MHz, D_2O): δ 178.01, 51.75, 44.45, 39.10, 27.77, 25.37, 21.38, 15.85, 12.99.

3-(allylamino)butanoic acid (rac-1h): $C_7H_{13}NO_2$: MS (ESI^+): calcd.: [M]=143.1, found: $[M+H]^+$=144.1. ^{1}H NMR (400 MHz, DMSO-d6) δ 5.89 (ddt, J = 16.7, 10.4, 6.1 Hz, 1H), 5.41 – 5.22 (m, 2H), 3.53 – 3.30 (m, 2H), 3.17 – 3.07 (m, 1H), 2.31 – 2.08 (m, 2H), 1.14 (d, J = 6.5 Hz, 3H). ^{13}C NMR (126 MHz, D_2O): δ 177.69, 127.67, 123.17, 51.18, 46.54, 39.10, 15.81.

3-(propargylamino)butanoic acid (rac-1i): $C_7H_{11}NO_2$: MS (ESI^+): calcd.: [M]=141.1, found: $[M+H]^+$=142.1. ^{1}H NMR (400 MHz, DMSO-d6) δ 3.53 – 3.34 (m, 2H), 3.16 (q, J = 2.6 Hz, 1H), 3.15 – 3.08 (m, 1H), 2.33 – 2.11 (m, 2H), 1.03 (d, J = 6.3 Hz, 3H). ^{13}C NMR (126 MHz, D_2O): δ 177.62, 77.75, 73.23, 51.21, 38.97, 33.76, 15.64.

3-(cyclopropylamino)butanoic acid (rac-1j): $C_7H_{13}NO_2$: MS (ESI^+): calcd.: [M]=143.1, found: $[M+H]^+$=144.1. ^{1}H NMR (400 MHz, DMSO-d6) δ 3.14 (q, J = 6.5 Hz, 1H), 2.31 (td, J = 7.0, 3.9 Hz, 1H), 2.21 (td, J = 15.8, 6.6 Hz, 2H), 1.14 (d, J = 6.4 Hz, 3H), 0.60 – 0.41 (m, 4H). ^{13}C NMR (126 MHz, D_2O): δ 178.91, 52.81, 40.70, 27.49, 17.03, 3.57 (d, J = 25.2 Hz).

3-(cyclobutylamino)butanoic acid (rac-1k): $C_8H_{15}NO_2$: MS (ESI^+): calcd.: [M]=157.1, found: $[M+H]^+$=158.1. ^{1}H NMR (400 MHz, DMSO-d6) δ 3.56 (p, J = 8.0 Hz, 1H), 3.05 (p, J = 6.3 Hz, 1H), 2.18 (ddt, J = 14.9, 10.1, 4.5 Hz, 3H), 2.08 (dd, J = 16.0, 8.2 Hz, 1H), 1.98 (p, J = 9.6 Hz, 2H), 1.80 – 1.63 (m, 2H), 1.10 (d, J = 6.5 Hz, 3H). ^{13}C NMR (126 MHz, D_2O): δ 177.85, 50.24, 49.27, 39.54, 27.28 – 26.68 (m),

26.52, 16.14, 14.63.

3-((2-aminoethyl)amino)butanoic acid (rac-1m): $C_6H_{14}N_2O_2$: MS (ESI^+): calcd.: [M]=146.1, found: $[M+H]^+$=147.1. ^1H NMR (400 MHz, DMSO-d6) δ 3.12 (dt, J = 13.7, 6.9 Hz, 1H), 2.95 (d, J = 4.4 Hz, 4H), 2.41 – 2.20 (m, 2H), 1.13 (d, J = 6.4 Hz, 3H). ^{13}C NMR (126 MHz, D_2O): δ 179.75, 50.95, 44.92, 42.06, 38.28, 17.64.

3-(methoxyamino)butanoic acid (rac-1n): $C_5H_{11}NO_3$: MS (ESI^+): calcd.: [M]=133.1, found: $[M+H]^+$=134.1. ^1H NMR (400 MHz, DMSO-d6) δ 3.39 (s, 3H), 3.25 (dt, J = 7.2, 6.1 Hz, 1H), 2.43 (dd, J = 15.3, 5.9 Hz, 1H), 2.17 – 2.07 (m, 1H), 0.99 (d, J = 6.4 Hz, 3H). ^{13}C NMR (126 MHz, D_2O): δ 180.58, 61.19, 52.94, 41.91, 16.51.

3-(methylamino)pentanoic acid (rac-2a): $C_6H_{13}NO_2$: MS (ESI^+): calcd.: [M]=131.1, found: $[M+H]^+$=132.1. ^1H NMR (500 MHz, D_2O) δ 3.34 – 3.26 (m, 1H), 2.64 (s, 3H), 2.63 – 2.45 (m, 2H), 1.78 – 1.69 (m, 1H), 1.59 (dt, J = 14.3, 7.6 Hz, 1H), 0.90 (t, J = 7.5 Hz, 3H). ^{13}C NMR (126 MHz, D_2O): δ 177.01, 57.83, 34.95, 29.74, 23.07, 8.76.

3-(ethylamino)pentanoic acid (rac-2b): $C_7H_{15}NO_2$: MS (ESI^+): calcd.: [M]=145.1, found: $[M+H]^+$=146.1. ^1H NMR (400 MHz, DMSO-d6) δ 3.08 (dt, J = 8.0, 3.6 Hz, 1H), 2.98 – 2.78 (m, 2H), 2.37 (dd, J = 16.5, 4.9 Hz, 1H), 2.23 (dd, J = 16.5, 7.6 Hz, 1H), 1.72 (ddd, J = 13.2, 7.6, 5.0 Hz, 1H), 1.49 (dt, J = 14.4, 7.6 Hz, 1H), 1.19 (t, J = 7.2 Hz, 3H), 0.90 (t, J = 7.5 Hz, 3H). ^{13}C NMR (126 MHz, D_2O): δ 177.68, 56.45, 39.93, 35.57, 23.53, 10.83, 8.77.

3-(allylamino)pentanoic acid (rac-2h): $C_8H_{15}NO_2$: MS (ESI^+): calcd.: [M]=157.1, found: $[M+H]^+$=158.1. ^1H NMR (400 MHz, DMSO-d6) δ 5.90 (ddt, J = 16.7, 10.0, 6.1 Hz, 1H), 5.44 – 5.20 (m, 2H), 3.55 – 3.28 (m, 2H), 2.32 (dd, J = 16.1, 4.6 Hz, 1H), 2.13 (dd, J = 16.1, 8.0 Hz, 1H), 1.67 (ddd, J = 13.4, 7.6, 5.3 Hz, 1H), 1.44 (dp, J = 15.0, 7.6 Hz, 1H), 0.88 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, D_2O): δ 178.02, 127.74, 123.27, 56.36, 46.56, 35.63, 23.43, 8.75.

3-(propargylamino)pentanoic acid (rac-2i): $C_8H_{13}NO_2$: MS (ESI^+): calcd.: [M]=155.1, found: $[M+H]^+$=156.1. ^1H NMR (500 MHz, D_2O) δ 3.89 (d, J = 2.5 Hz, 2H), 3.49 (ddt, J = 8.3, 6.1, 5.1 Hz, 1H), 2.88 (t, J = 2.6 Hz, 1H), 2.55 (dd, J = 16.8, 5.4 Hz, 1H), 2.43 (dd, J = 16.8, 6.3 Hz, 1H), 1.74 (dtd, J = 15.1, 7.5, 4.9 Hz, 1H), 1.60 (ddd, J = 14.1, 8.2, 7.3 Hz, 1H), 0.90 (t, J = 7.5 Hz, 3H). ^{13}C NMR (126 MHz, D_2O): δ 177.86, 77.79, 73.28, 56.38, 35.43, 33.84, 23.29, 8.73.

3-(cyclopropylamino)pentanoic acid (rac-2j): $C_8H_{15}NO_2$: MS (ESI^+): calcd.: [M]=157.1, found: $[M+H]^+$=158.1. ^1H NMR (400 MHz, DMSO-d6) δ 2.88 (p, J = 6.4, 5.9 Hz, 1H), 2.21 (d, J = 5.5 Hz, 2H), 2.18 – 2.09 (m, 1H), 1.58 (dt, J = 13.9, 6.9 Hz, 1H), 1.41 (dt, J = 14.1, 7.2 Hz, 1H), 0.84 (t, J = 7.5 Hz, 3H), 0.55 – 0.28 (m, 4H). ^{13}C NMR (126 MHz, D_2O): δ 178.45, 58.57, 36.28, 27.53, 23.77, 8.90, 3.32.

3-((2-aminoethyl)amino)pentanoic acid (rac-2m): $C_7H_{16}N_2O_2$: MS (ESI^+): calcd.: [M]=160.1, found: $[M+H]^+$=161.1. ^1H NMR (400 MHz, D_2O) δ 3.37 (dt, J = 10.2, 5.6 Hz, 5H), 2.59 (dd, J = 17.1, 4.9 Hz, 1H), 2.42 (dd, J = 17.0, 6.9 Hz, 1H), 1.85 – 1.70 (m, 1H), 1.60 (dp, J = 15.2, 7.6 Hz, 1H), 0.91 (t, J = 7.5 Hz, 3H). ^{13}C NMR (126 MHz,

D_2O): δ 178.02, 57.90, 41.30, 35.86, 35.07, 23.59, 8.91.

3-(methoxyamino)pentanoic acid (rac-2n): $C_6H_{13}NO_3$: MS (ESI^+): calcd.: [M]=147.1, found: $[M+H]^+$=148.1. ^1H NMR (500 MHz, D_2O) δ 3.46 (s, 3H), 3.12 (p, J = 6.5 Hz, 1H), 2.27 (dd, J = 14.7, 6.6 Hz, 1H), 2.18 (dd, J = 14.8, 6.8 Hz, 1H), 1.47 – 1.30 (m, 2H), 0.82 (t, J = 7.6 Hz, 3H). ^{13}C NMR (126 MHz, D_2O): δ 180.85, 61.10, 58.81, 39.76, 24.01, 9.35.

2-(methylamino)succinic acid (rac-3a): $C_5H_9NO_4$: MS (ESI^+): calcd.: [M]=147.1, found: $[M+H]^+$=148.0. ^1H NMR (500 MHz, D_2O) δ 3.82 (t, J = 5.3 Hz, 1H), 2.95 (dd, J = 5.4, 2.0 Hz, 2H), 2.69 (s, 3H). ^{13}C NMR (126 MHz, D_2O): δ 176.70, 174.64, 61.67, 35.88, 34.56.

2-(ethylamino)succinic acid (rac-3b): $C_6H_{11}NO_4$: MS (ESI^+): calcd.: [M]=161.1, found: $[M+H]^+$=162.1. ^1H NMR (400 MHz, D_2O) δ 3.84 (dd, J = 6.9, 4.6 Hz, 1H), 3.11 (qd, J = 7.3, 1.6 Hz, 2H), 2.93 – 2.77 (m, 2H), 1.27 (t, J = 7.3 Hz, 3H). ^{13}C NMR (126 MHz, D_2O): δ 175.44, 172.84, 58.12, 42.18, 34.75, 10.66.

2-(isopropylamino)succinic acid (rac-3c): $C_7H_{13}NO_4$: MS (ESI^+): calcd.: [M]=175.1, found: $[M+H]^+$=176.1. ^1H NMR (400 MHz, D_2O) δ 3.88 (dd, J = 8.2, 4.1 Hz, 1H), 3.42 (p, J = 6.6 Hz, 1H), 2.82 (dd, J = 17.6, 4.2 Hz, 1H), 2.72 (dd, J = 17.6, 8.1 Hz, 1H), 1.31 (d, J = 6.6 Hz, 6H). ^{13}C NMR (126 MHz, D_2O): δ 176.18, 173.32, 56.87, 50.73, 35.60, 18.79, 18.23.

2-(propylamino)succinic acid (rac-3d): $C_7H_{13}NO_4$: MS (ESI^+): calcd.: [M]=175.1, found: $[M+H]^+$=176.1. ^1H NMR (400 MHz, D_2O) δ 3.79 (d, J = 3.6 Hz, 1H), 3.02 (d, J = 7.7 Hz, 2H), 2.80 (d, J = 4.3 Hz, 1H), 2.73 (d, J = 7.9 Hz, 1H), 1.70 (q, J = 7.5 Hz, 2H), 0.95 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, D_2O): δ 176.68, 173.15, 58.98, 48.30, 35.17, 19.27, 10.16.

2-(butylamino)succinic acid (rac-3f): $C_8H_{15}NO_4$: MS (ESI^+): calcd.: [M]=189.1, found: $[M+H]^+$=190.1. ^1H NMR (400 MHz, D_2O) δ 3.75 (dd, J = 8.1, 4.2 Hz, 1H), 3.03 (q, J = 7.3 Hz, 2H), 2.77 (dd, J = 17.4, 4.2 Hz, 1H), 2.65 (dd, J = 17.4, 8.1 Hz, 1H), 1.73 – 1.61 (m, 2H), 1.39 (dt, J = 14.9, 7.5 Hz, 2H), 0.91 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, D_2O): δ 174.35, 172.29, 57.94, 46.82, 34.03, 27.47, 19.07, 12.66.

2-(allylamino)succinic acid (rac-3h): $C_7H_{11}NO_4$: MS (ESI^+): calcd.: [M]=173.1, found: $[M+H]^+$=174.1. ^1H NMR (400 MHz, D_2O) δ 5.88 (ddt, J = 17.1, 10.2, 6.9 Hz, 1H), 5.53 – 5.40 (m, 2H), 3.80 (dd, J = 7.6, 4.3 Hz, 1H), 3.74 – 3.59 (m, 2H), 2.86 – 2.68 (m, 2H). ^{13}C NMR (126 MHz, D_2O): δ 176.30, 172.98, 127.49, 123.86, 58.02, 48.69, 35.19.

2-(propargylamino)succinic acid (rac-3i): $C_7H_9NO_4$: MS (ESI^+): calcd.: [M]=171.1, found: $[M+H]^+$=172.0. ^1H NMR (500 MHz, D_2O) δ 3.97 (dd, J = 6.9, 4.3 Hz, 1H), 3.94 (dd, J = 4.2, 2.6 Hz, 2H), 2.90 – 2.87 (m, 1H), 2.86 – 2.76 (m, 2H). ^{13}C NMR (126 MHz, D_2O): δ 175.85, 172.56, 78.05, 73.12, 57.87, 35.73, 34.77.

2-(cyclopropylamino)succinic acid (rac-3j): $C_7H_{11}NO_4$: MS (ESI^+): calcd.:

[M]=173.1, found: $[M+H]^+$=174.1. ^{1}H NMR (400 MHz, D_2O) δ 3.97 (dd, J = 7.3, 4.2 Hz, 1H), 3.30 (s, 1H), 2.96 – 2.76 (m, 2H), 2.76 – 2.69 (m, 1H), 0.94 – 0.80 (m, 4H). ^{13}C NMR (126 MHz, D_2O): δ 176.34, 173.12, 60.14, 34.84, 29.19, 3.29, 3.06.

2-(cyclobutylamino)succinic acid (rac-3k): $C_8H_{13}NO_4$: MS (ESI^+): calcd.: [M]=187.1, found: $[M+H]^+$=188.1. ^{1}H NMR (400 MHz, D_2O) δ 3.89 – 3.72 (m, 2H), 2.98 – 2.85 (m, 2H), 2.34 – 2.11 (m, 4H), 1.92 – 1.71 (m, 2H). ^{13}C NMR (126 MHz, D_2O): δ 174.43, 172.51, 56.21, 51.18, 34.24, 26.28, 14.31.

2-((2-aminoethyl)amino)succinic acid (rac-3m): $C_6H_{12}N_2O_4$: MS (ESI^+): calcd.: [M]=176.1, found: $[M+H]^+$=177.1. ^{1}H NMR (500 MHz, D_2O) δ 3.94 (dd, J = 7.0, 4.3 Hz, 1H), 3.46 (dd, J = 7.2, 5.4 Hz, 2H), 3.39 (t, J = 6.3 Hz, 2H), 2.94 (qd, J = 18.1, 5.7 Hz, 2H). ^{13}C NMR (126 MHz, D_2O): δ 174.39, 171.91, 58.85, 44.06, 35.67, 34.31.

3-(ethylamino)-3-phenylpropanoic acid (rac-4b): $C_{11}H_{15}NO_2$: MS (ESI^+): calcd.: [M]=193.1, found: $[M+H]^+$=194.1. ^{1}H NMR (400 MHz, D_2O) δ 7.41 (p, J = 3.5 Hz, 5H), 4.46 (t, J = 7.4 Hz, 1H), 2.97 – 2.87 (m, 2H), 2.87 – 2.72 (m, 2H), 1.16 (t, J = 7.3 Hz, 3H). ^{13}C NMR (126 MHz, D_2O) δ 176.83, 133.92, 129.73, 127.95, 59.42, 40.68, 39.74, 10.57.

3-(allylamino)-3-phenylpropanoic acid (rac-4h): $C_{12}H_{15}NO_2$: MS (ESI^+): calcd.: [M]=205.1, found: $[M+H]^+$=206.1. ^{1}H NMR (400 MHz, D_2O) δ 7.47 (qd, J = 7.7, 6.5, 2.8 Hz, 5H), 5.85 (ddt, J = 17.1, 10.3, 6.7 Hz, 1H), 5.51 – 5.26 (m, 2H), 4.55 (t, J = 7.4 Hz, 1H), 3.50 (d, J = 6.8 Hz, 2H), 2.99 (dd, J = 15.9, 7.3 Hz, 1H), 2.83 (dd, J = 15.9, 7.5 Hz, 1H). ^{13}C NMR (126 MHz, D_2O): δ 176.81, 133.66, 129.81, 129.39, 128.08, 127.38, 123.62, 59.12, 47.34, 39.65.

3-(propargylamino)-3-phenylpropanoic acid (rac-4i): $C_{12}H_{13}NO_2$: MS (ESI^+): calcd.: [M]=203.1, found: $[M+H]^+$=204.1. ^{1}H NMR (400 MHz, D_2O) δ 7.43 (s, 5H), 4.67 (t, J = 7.4 Hz, 1H), 3.74 (dd, J = 16.7, 2.6 Hz, 1H), 3.59 (dd, J = 16.8, 2.7 Hz, 1H), 3.03 – 2.87 (m, 2H), 2.81 (dd, J = 16.0, 7.4 Hz, 1H). ^{13}C NMR (126 MHz, D_2O): δ 176.64 , 133.07 , 129.70 (d, J = 70.9 Hz), 128.16, 78.16, 73.07, 58.85, 39.42, 34.29.

2. Computational protein redesign

An ensemble of different conformations of the substrate was generated by enumerating these under Yasara. Substrate rotamers were sampled around the canonical minimum dihedral angles (60°, -60° and 180°) with 5° intervals over 35° around the minima (for example, 42.5° to 77.5°). The Rosetta Enzyme Design[1] application positions substrates optimally for catalysis by applying forces between the bound substrate and catalytically important groups in the enzyme. The substrate geometry corresponded to near attack conformation (NAC) criteria and was based on published QM/MM calculations[2] and the crystal structure with bound substrate (PDB 3R6V[3]) (**Table S1**). Rosetta enzyme design oriented the substrate optimally for deamination by applying these forces *in silico*.

To generate an ensemble of different starting points for Rosetta Enzyme Design, 40 molecular dynamics (MD) simulations of each complex structure were performed. The complex structures were positioned in a rectangular simulation cell with at least 7.5 Å between protein and the periodic boundary of the simulation cell. The salt ions were positioned at electrostatically favorable positions by an algorithm implemented under Yasara. An energy minimization was carried out before the MD simulation. MD simulation was carried out under Yasara with a leapfrog algorithm, with a time-step of 1.33 fs and a Berendsen thermostat to preserve constant pressure and temperature. The LINCS and SETTLE algorithms were used to constrain hydrogen atoms. The simulations were carried out using the Yamber3 force field. The temperature was increased from 5 to 298 K over 3 ps, followed by equilibration (2 ps) and production (5 ps). For each complex structure, we ran Rosetta Enzyme Design 10 times for the 40 starting points generated by MD simulations and 100 times for the initial complex structure, resulting in a total of 500 designs. Rosetta Enzyme Design uses a Monte Carlo algorithm in which it selects mutations and structural changes that decrease overall energy to generate 3D structures of designs. The following command line options[4] were used for Rosetta Enzyme Design: -enzdes:: -cst_predock -cst_design -detect_design_interface -cut1 0.0 -cut2 0.0 -cut3 8.0 -cut4 10.0 -cst_min -chi_min -bb_min -cst_opt -include_catres_in_interface_detection -packing:: -use_input_sc -soft_rep_design -extrachi_cutoff 1 -design_min_cycles 3 -ex1:level 4 -ex2:level 4 -ex1aro:level 4 -ex2aro:level 4.

The *in silico* predicted designs were subsequently screened with 5 ps MD simulations. The NAC frequencies corresponding to the reactivity of the bound substrate were calculated. Finally, the obtained 500 designs were selected for experimental characterization on the basis of the following guidelines: (1) the overall energy should be negative, (2) the sum of the penalty energies for the above constrains should not exceed 20 REU, and (3) The NAC frequencies should be larger than 50%. The active site must be organized, and maintain the original β-carboxylate hydrogen-bonding network.

Table S1. Design specifications for maintaining the catalytic machinery of AspB[a]

distances			**angles**		
descriptor	center (Å)	tolerance (Å)	descriptor	center (°)	tolerance (°)
d_1	2.0	0.6	θ_1	120	20
d_2	2.1	0.2	θ_2	150	30
d_3	2.0	0.4	θ_3	150	30
d_4	1.6	0.15	θ_4	150	30
d_5	2.0	0.4	θ_5	150	30
d_6	1.6	0.15	θ_6	150	30
d_7	2.0	0.4	θ_7	150	30
d_8	2.0	0.4	θ_8	150	30
d_9	1.6	0.15	θ_9	150	30

[a] The constraining force (applied outside the tolerances) was always 10 REU $Å^{-2}$ for distances and 10 REU $degree^{-2}$.

3. Detailed experimental procedures

Construction of AspB variants

For BA1-BA22 and BC1-BC16, plasmid pET-21a(+), containing the AspB sequence, was used as a template for QuikChange mutagenesis with Q5 PCR MasterMix (NEB). PCR was performed in 0.5 mL microcentrifuge tubes and the DpnI-treated PCR products were transformed into *E. coli* TOP10. Incorporation of the mutations was confirmed by DNA sequencing.

Table S2. The sequences of the computationally predicted variants for **1i** and **1j**.

1i					**1j**				
Enzyme	Sequence				Enzyme	Sequence			
	99	142	358	362		99	142	358	362
BA1	G	A	A	A	BC1	G	A	C	A
BA2	G	A	A	S	BC2	G	A	C	M
BA3	G	A	I	A	BC3	G	A	C	S
BA4	G	A	M	L	BC4	G	A	H	A
BA5	G	A	M	M	BC5	G	A	T	A
BA6	G	A	S	A	BC6	G	A	V	L
BA7	G	A	S	L	BC7	G	S	A	A
BA8	G	A	V	A	BC8	G	S	A	M
BA9	G	A	V	M	BC9	G	S	A	S
BA10	G	A	V	S	BC10	G	S	C	S
BA11	G	C	C	M	BC11	G	S	H	A
BA12	G	C	C	N	BC12	G	S	H	M
BA13	G	S	C	N	BC13	G	S	H	N
BA14	G	S	I	A	BC14	G	S	S	A
BA15	G	S	V	M	BC15	G	S	S	M
BA16	G	S	M	A	BC16	G	S	V	M
BA17	G	S	M	L					
BA18	G	S	M	M					
BA19	G	S	S	L					
BA20	G	S	S	N					
BA21	G	S	V	L					
BA22	G	S	V	S					

Expression and purification of AspB variants

The enzymes were expressed by *E. coli* BL21(DE3) in auto-induction medium[5] (comprising 1% tryptone, 0.5% yeast extract, 0.33% $(NH_4)_2SO_4$, 0.68% KH_2PO_4, 0.71% Na_2HPO_4, 0.024% $MgSO_4$, 0.2% glycerol (v/v), 0.05% glucose, 0.2% lactose and 50 μg/mL ampicillin) at 30 °C for 24 h. The final OD_{600} typically reached ~4. After

centrifugation (8,000 g, 10 min), the cells were lysed by sonication in a buffer containing 50 mM Tris·HCl (pH 7.5). Enzymes were purified by heat treatment at 60 °C for 30 min and centrifuged (12,000 g, 30 min) to remove the precipitates. The purity of the protein obtained by this method was generally >90% (**Figure S1**). The enzyme was stored in aliquots at -20 °C until use. Protein concentrations were determined with the BCA assay kit (Takara, Beijing, China) and bovine serum albumin as the standard. The purified enzymes were used to measure hydroamination activities.

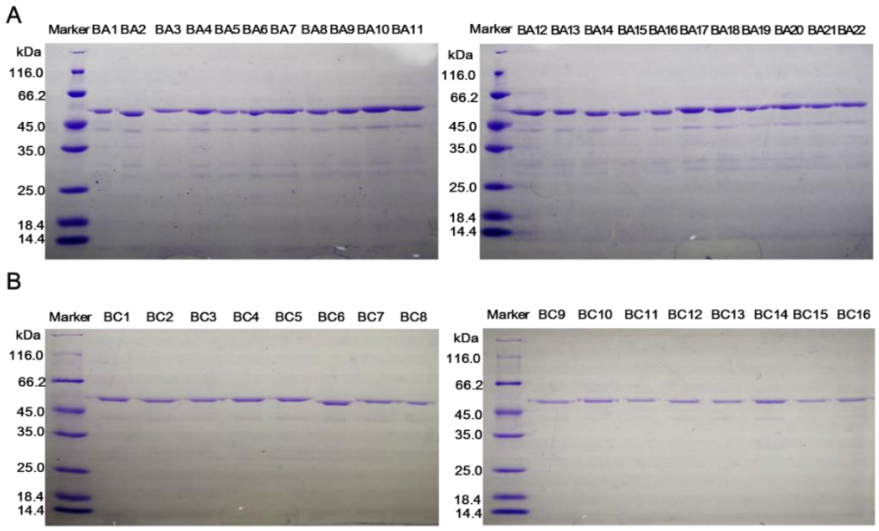


Figure S1. Expression and purification of B19 variants from cells grown in auto-induction medium. The panel shows Coomassie stained SDS-PAGE of (A) heat treated bacterial extracts of BA1-22, and (B) heat treated bacterial extracts of BC1-16.

Method of sample derivatization

DNFB derivatization[5]**:** A mixture of 25 μL amino acid standard solutions or reaction solutions were mixed with 10 μL 1 M $NaHCO_3$, 40 μL DNFB solution (36.7 mM 2,4-dinitrofluorobenzene in acetone) and incubated at 60 °C for 30 min. The reaction was stopped by adding 20 μL of 1 M HCl and the precipitates were subsequently removed by centrifugation.

DNS-Cl derivatization[6]**:** A mixture of 25 μL amino acid standard solutions or reaction solutions were mixed with 15 μL 50 mM $NaHCO_3$ (pH 8.5), 50 μL DNS-Cl solution (10 mM dansyl chloride in acetonitrile) and incubated at 37 °C for 30 min. The precipitates were subsequently removed by centrifugation.

FMOC derivatization[7]**:** A mixture of 25 μL amino acid standard solutions or reaction solutions were mixed with 25 μL borate buffer (0.4 M boric acid dissolved in 0.4 M potassium chloride solution and adjusted with 6 M NaOH to pH 9.0), 50 μL FMOC solution (20 mM 9-fluorenylmethyl chloroformate in acetonitrile) and incubated at room temperature for 20 min. The reaction was stopped by adding 10 μL of 0.1 M HCl (acetonitrile : 0.2 M HCl = 1:1, v/v) and the precipitates were subsequently removed by centrifugation.

Hydroamination activities of computational designs for 1i and 1j

A solution of 110 g/L crotonic acid and 2.2 M allylamine or cyclopropylamine was prepared and the pH was adjusted to 8.5 by adding 6 M HCl. To this solution (450 μL), purified B19 or mutant BA1-BA22 and BC1-BC16 (50 μL, concentration of the purified enzyme was shown in **Table S3**) was added and the reactions were performed at 50 °C for 30 min. The specific activity was determined according to the reduction of crotonic acid monitored by HPLC (254 nm). One unit (U) of enzyme activity is defined as the amount that converts 1 μmol of substrate per minute.

Table S3. The hydroamination activities of the designed variants for **1i** and **1j**[a].

1i				**1j**			
Enzyme	Purified enzyme conc. (mg/mL)	Specific activity (U/mg)	Relative activity[b]	Enzyme	Purified enzyme conc. (mg/mL)	Specific activity (U/mg)	Relative activity[c]
BA1	15.2	1.9	10%	BC1	11.2	< 0.1	< 1%
BA2	14.4	1.4	8%	BC2	11.2	0.2	1%
BA3	10.9	1.6	9%	BC3	11.6	0.3	2%
BA4	12.1	2.2	12%	BC4	11.9	0.2	1%
BA5	12.5	0.7	4%	BC5	12.6	< 0.1	< 1%
BA6	14.6	1.0	5%	BC6	14.6	0.9	7%
BA7	13.9	3.0	16%	BC7	10.0	3.1	23%
BA8	12.9	2.2	12%	BC8	8.8	11.4	85%
BA9	9.4	6.5	36%	BC9	9.3	3.8	29%
BA10	11.7	2.2	12%	BC10	10.7	0.6	4%
BA11	9.5	1.2	7%	BC11	12.6	0.7	5%
BA12	13.2	1.6	9%	BC12	11.3	< 0.1	< 1%
BA13	14.5	1.6	9%	BC13	9.6	0.3	2%
BA14	10.9	1.8	10%	BC14	12.4	0.6	5%
BA15	11.4	18.3	100%	BC15	12.8	2.6	19%
BA16	13.1	0.6	3%	BC16	11.4	13.4	100%
BA17	10.3	0.8	5%				
BA18	7.1	2.4	13%				
BA19	9.3	1.5	8%				
BA20	10.9	0.9	5%				
BA21	11.4	8.6	47%				
BA22	8.8	2.8	15%				

[a] Data are from one experiment.

[b] The specific activity of BA15 was as the standard.

[c] The specific activity of BC16 was as the standard.

Determination of biocatalytic conversion and enantiomeric excess for 1a-1n

Solutions of 22-166 g/L crotonic acid and 0.44-3.33 M amines were prepared and the pH were adjusted to 9.0 by adding 6 M HCl. Purified BA15 (0.5 mL) were added to the solution (4.5 mL), and the reactions were performed at 50 °C. The conversion was determined by measuring the concentration of the crotonic acid through HPLC analysis (254 nm). The products were identified by using ESI-MS. The enantiomeric excess of the products were determined by HPLC analysis. Due to lack of racemic standard compounds, **1l** was identified by NMR, and e.e. was not determined. The final concentrations of substrates, reaction conditions and results are shown in **Table S4**.

Table S4. Biocatalytic conversion and enantiomeric excess for **1a-1n**.

Product	Unsaturated acid Conc. (g/L)	Amine Conc. (M)	BA15 Conc. (mM)	Time （h）	Conversion	*e.e.*
1a	20	0.40	0.19	120	99%	99%
1b	150	3.00	0.19	48	99%	99%
1c	20	0.40	0.19	120	46%	99%
1d	100	2.00	0.19	48	98%	99%
1e	20	0.40	0.19	127	51%	99%
1f	50	1.00	0.21	24	99%	99%
1g	20	0.40	0.19	72	98%	99%
1h	100	2.00	0.19	2	99%	99%
1i	86	1.50	0.19	1	99%	99%
1j	86	1.20	0.27	6	99%	99%
1k	80	1.60	0.19	48	99%	99%
1l	20	0.40	0.19	96	96%	n.d.
1m	100	2.00	0.21	19	97%	90%
1n	80	1.50	0.19	48	99%	99%

(*R*)-3-(methylamino)butanoic acid (1a): $C_5H_{11}NO_2$, MS (ESI^+) calcd.: [M]=117.1, found: $[M+H]^+$=118.1.

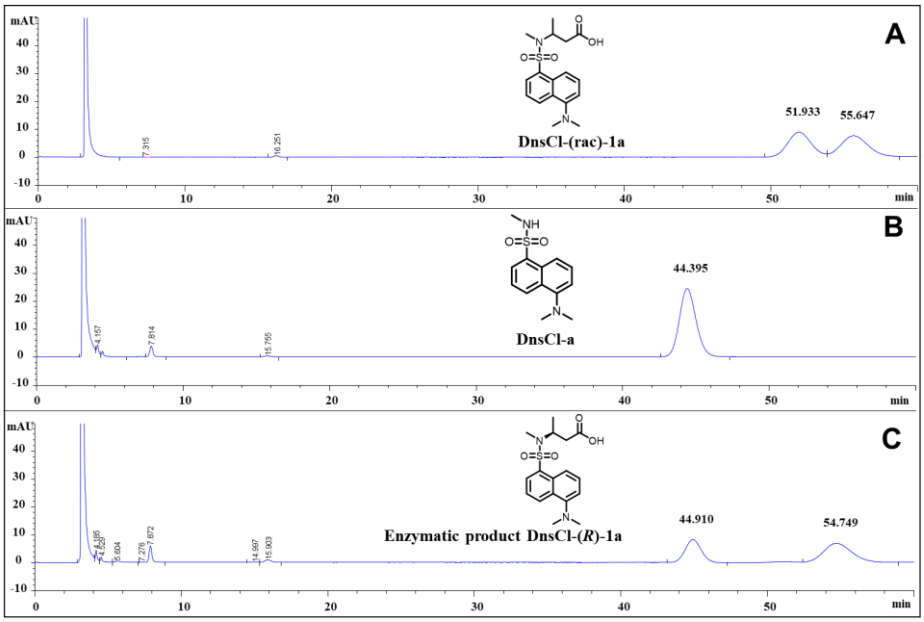


Figure S2. Chiral HPLC analysis of (A) racemic standard compound, (B) amine and (C) enzymatic product **1a**. Chiral HPLC conditions: Chiralpak IG column (5 µm, 250×4.6 mm, Daicel). Phase A: 0.1% formic acid in water, phase B: acetonitrile, A:B = 80:20 (v/v). Flow rate 1.0 mL/min, 25 °C, UV detection at 254 nm.

(*R*)-3-(ethylamino)butanoic acid (1b): $C_6H_{13}NO_2$: MS (ESI^+): calcd.: [M]=131.1, found: $[M+H]^+$=132.1.

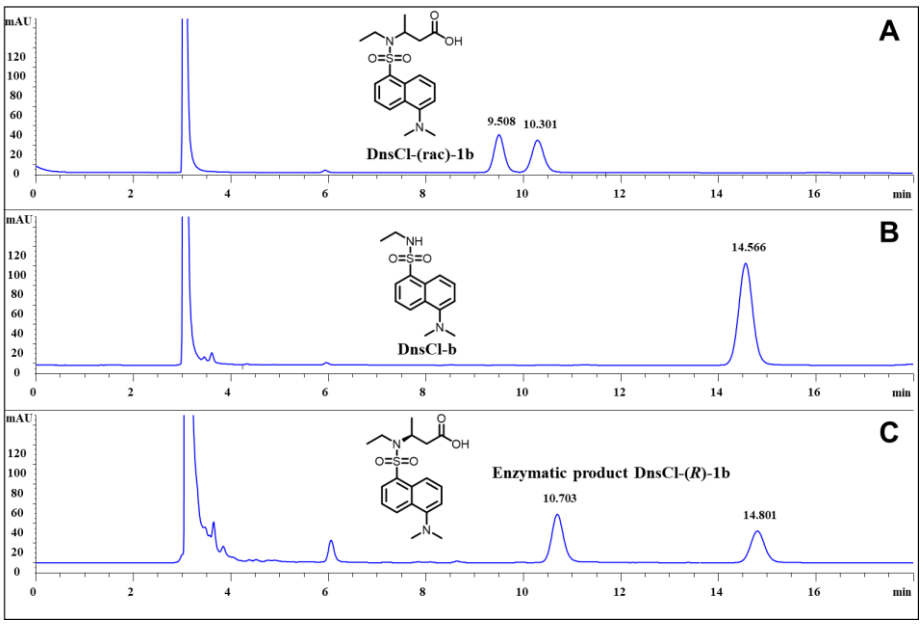


Figure S3. Chiral HPLC analysis of (A) racemic standard compound, (B) amine and (C) enzymatic product **1b**. Chiral HPLC conditions: Chiralpak IG column (5 µm, 250×4.6 mm, Daicel). Phase A: 0.1% formic acid in water, phase B: acetonitrile, A:B = 55:45 (v/v). Flow rate 1.0 mL/min, 25 °C, UV detection at 254 nm.

(*R*)-3-(isopropylamino)butanoic acid (1c): $C_7H_{15}NO_2$: MS (ESI^+): calcd.: [M]=145.1, found: $[M+H]^+$=146.1.

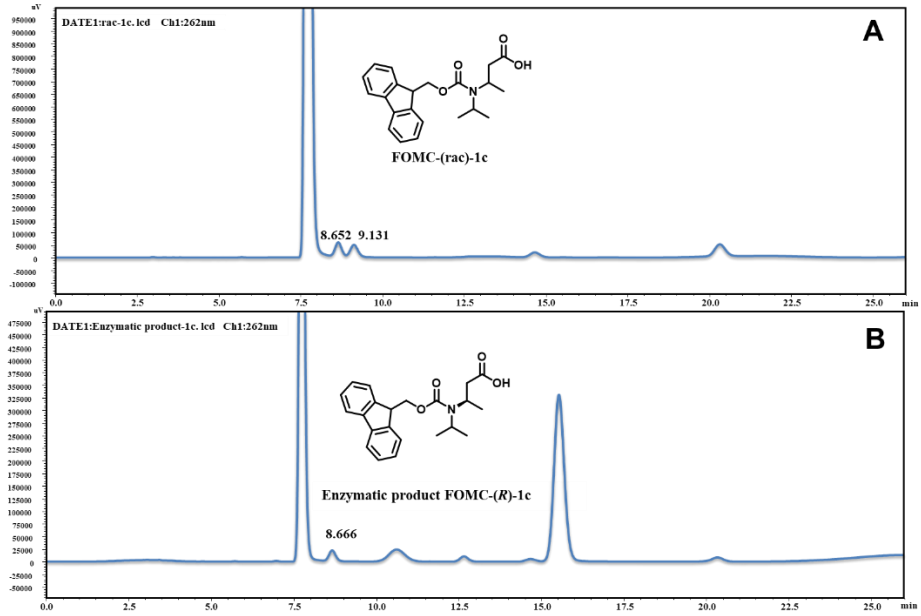


Figure S4. Chiral HPLC analysis of (A) racemic standard compound and (B) enzymatic product **1c**. Chiral HPLC conditions: Chiralpak IG column (5 μm, 250×4.6 mm, Daicel). Phase A: 0.1% formic acid in water, phase B: acetonitrile, A:B = 45:55 (v/v). Flow rate 1.0 mL/min, 25 °C, UV detection at 262 nm.

(*R*)-3-(propylamino)butanoic acid (1d): $C_7H_{15}NO_2$: MS (ESI^+): calcd.: [M]=145.1, found: $[M+H]^+$=146.1.

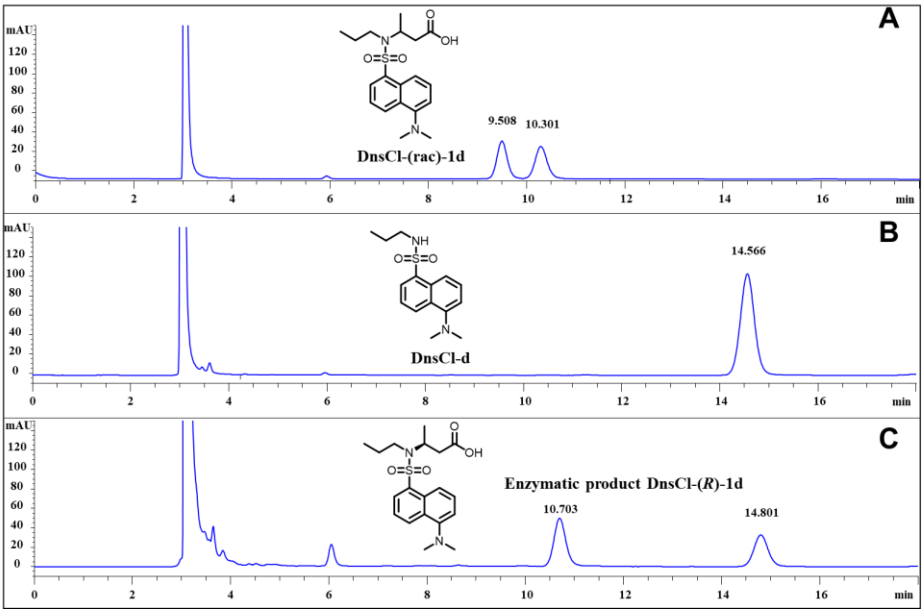


Figure S5. Chiral HPLC analysis of (A) racemic standard compound, (B) amine and (C) enzymatic product **1d**. Chiral HPLC conditions: Chiralpak IG column (5 μm, 250×4.6 mm, Daicel). Phase A: 0.1% formic acid in water, phase B: acetonitrile, A:B = 55:45 (v/v). Flow rate 1.0 mL/min, 25 °C, UV detection at 254 nm.

(*R*)-3-(*sec*-butylamino)butanoic acid (1e): $C_8H_{17}NO_2$: MS (ESI^+): calcd.: [M]=159.1, found: $[M+H]^+$=160.1.

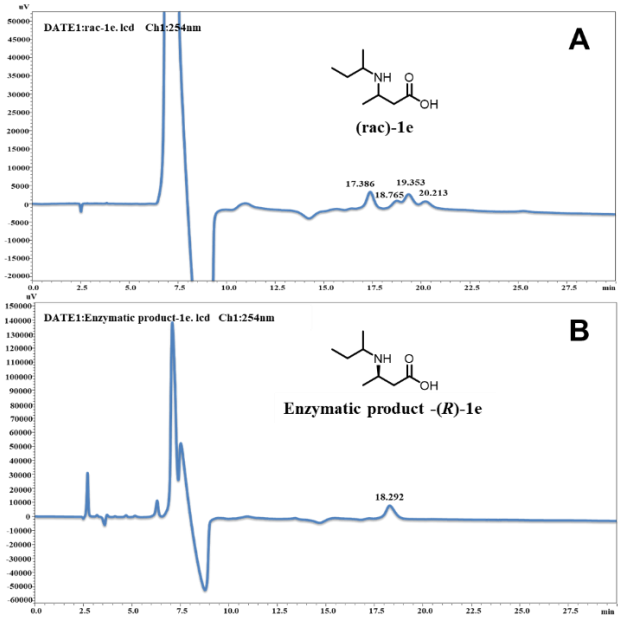


Figure S6. Chiral HPLC analysis of (A) racemic standard compound and (B) enzymatic product **1e**. Chiral HPLC conditions: Chirex 3126-Dpenicillamine column (250 mm x 4.6 mm). Phase: 2.0 mM $CuSO_4$ aqueous solution. Flow rate 1.0 mL/min, 25 °C, UV detection at 254 nm.

(*R*)-3-(butylamino)butanoic acid (1f): $C_8H_{17}NO_2$: MS (ESI^+): calcd.: [M]=159.1, found: $[M+H]^+$=160.1.

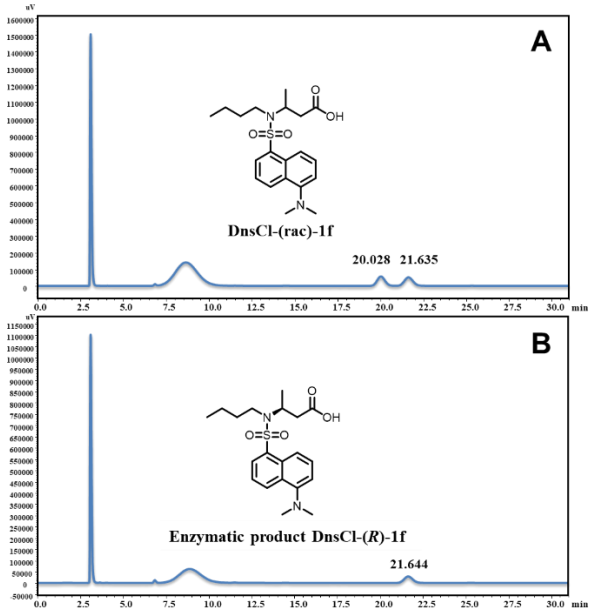


Figure S7. Chiral HPLC analysis of (A) racemic standard compound and (B) enzymatic product **1f**. Chiral HPLC conditions: Chiralpak IG column (5 μm, 250×4.6 mm, Daicel). Phase A: 0.1% formic acid in water, phase B: acetonitrile, A:B = 60:40 (v/v). Flow rate 1.0 mL/min, 25 °C, UV detection at 254 nm.

(*R*)-3-(pentylamino)butanoic acid (1g): $C_9H_{19}NO_2$: MS (ESI^+): calcd.: [M]=173.1, found: $[M+H]^+$=174.2.

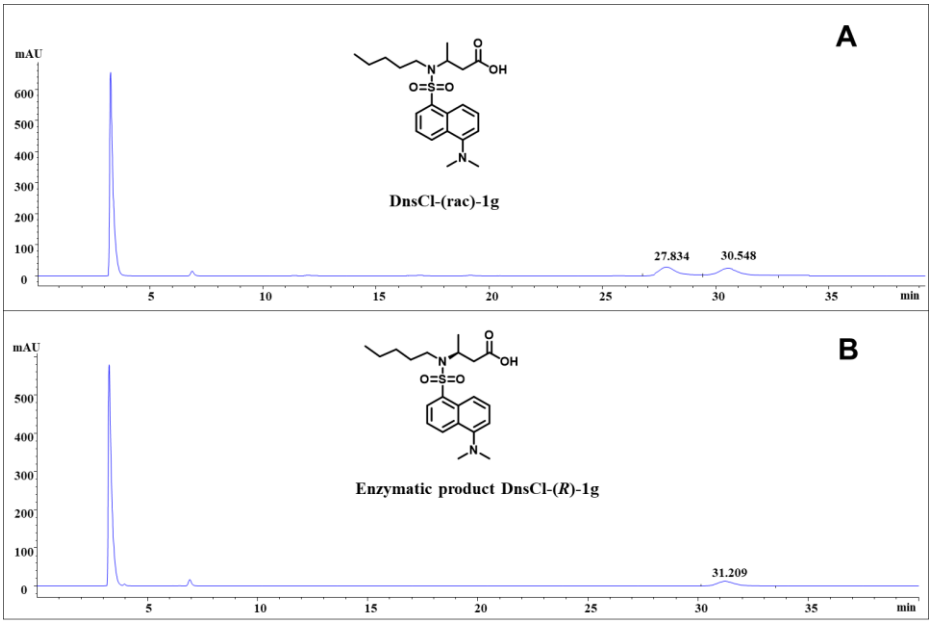


Figure S8. Chiral HPLC analysis of (A) racemic standard compound and (B) enzymatic product **1g**. Chiral HPLC conditions: Chiralpak IG column (5 µm, 250×4.6 mm, Daicel). Phase A: 0.1% formic acid in water, phase B: acetonitrile, A:B = 55:45 (v/v). Flow rate 1.0 mL/min, 25 °C, UV detection at 254 nm.

(*R*)-3-(allylamino)butanoic acid (1h): $C_7H_{13}NO_2$: MS (ESI^+): calcd.: [M]=143.1, found: $[M+H]^+$=144.1.

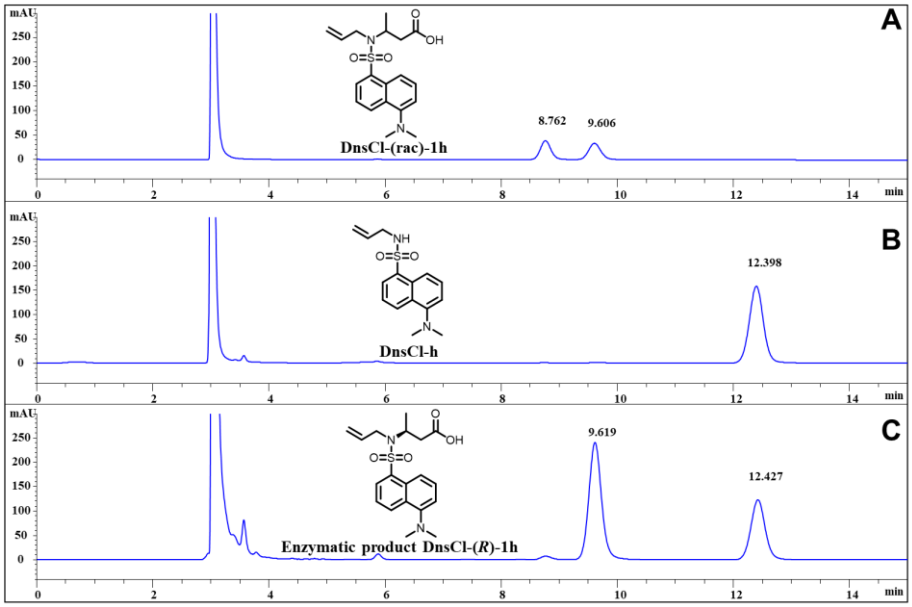


Figure S9. Chiral HPLC analysis of (A) racemic standard compound, (B) amine and (C) enzymatic product **1h**. Chiral HPLC conditions: Chiralpak IG column (5 µm, 250×4.6 mm, Daicel). Phase A: 0.1% formic acid in water, phase B: acetonitrile, A:B = 55:45 (v/v). Flow rate 1.0 mL/min, 25 °C, UV detection at 254 nm.

(*R*)-3-(propargylamino)butanoic acid (1i): $C_7H_{11}NO_2$: MS (ESI^+): calcd.: [M]=141.1, found: $[M+H]^+$=142.1.

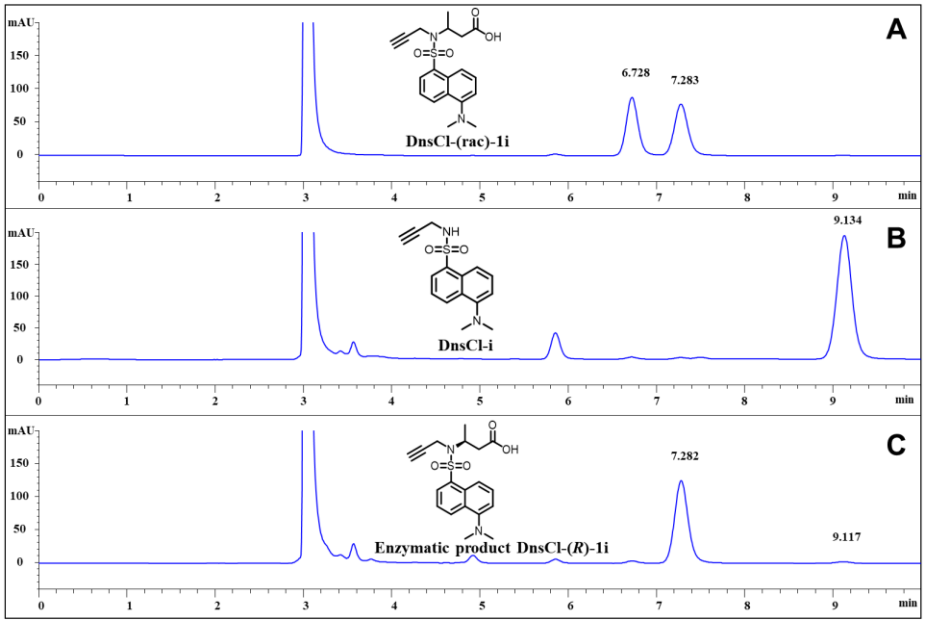


Figure S10. Chiral HPLC analysis of (A) racemic standard compound, (B) amine and (C) enzymatic product **1i**. Chiral HPLC conditions: Chiralpak IG column (5 µm, 250×4.6 mm, Daicel). Phase A: 0.1% formic acid in water, phase B: acetonitrile, A:B = 55:45 (v/v). Flow rate 1.0 mL/min, 25 °C, UV detection at 254 nm.

(*R*)-3-(cyclopropylamino)butanoic acid (1j): $C_7H_{13}NO_2$: MS (ESI^+): calcd.: [M]=143.1, found: $[M+H]^+$=144.1.

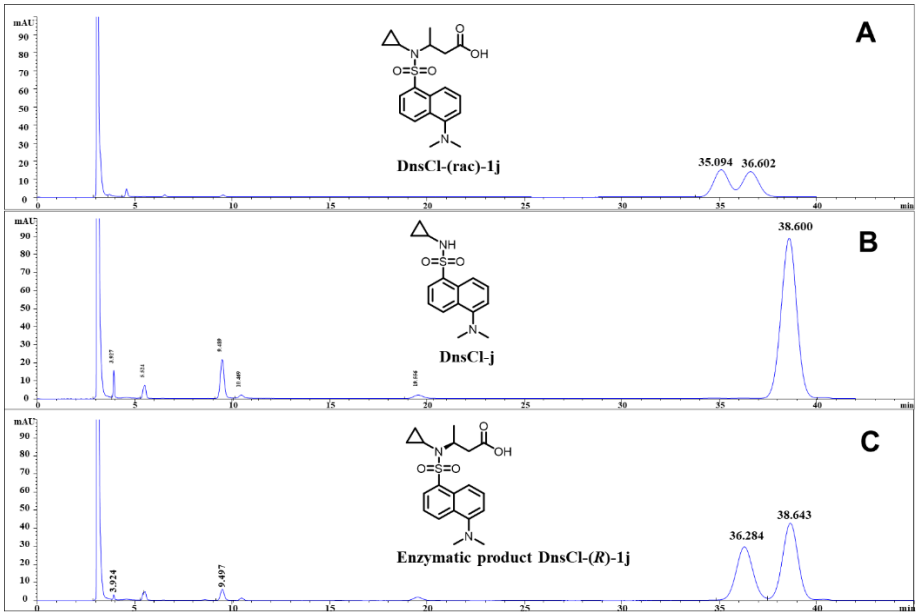


Figure S11. Chiral HPLC analysis of (A) racemic standard compound, (B) amine and (C) enzymatic product **1j**. Chiral HPLC conditions: Chiralpak IG column (5 µm, 250×4.6 mm, Daicel). Phase A: 0.1% formic acid in water, phase B: acetonitrile, A:B = 70:30 (v/v). Flow rate 1.0 mL/min, 25 °C, UV detection at 254 nm.

(*R*)-3-(cyclobutylamino)butanoic acid (1k): $C_8H_{15}NO_2$: MS (ESI^+): calcd.: [M]=157.1, found: $[M+H]^+$=158.1.

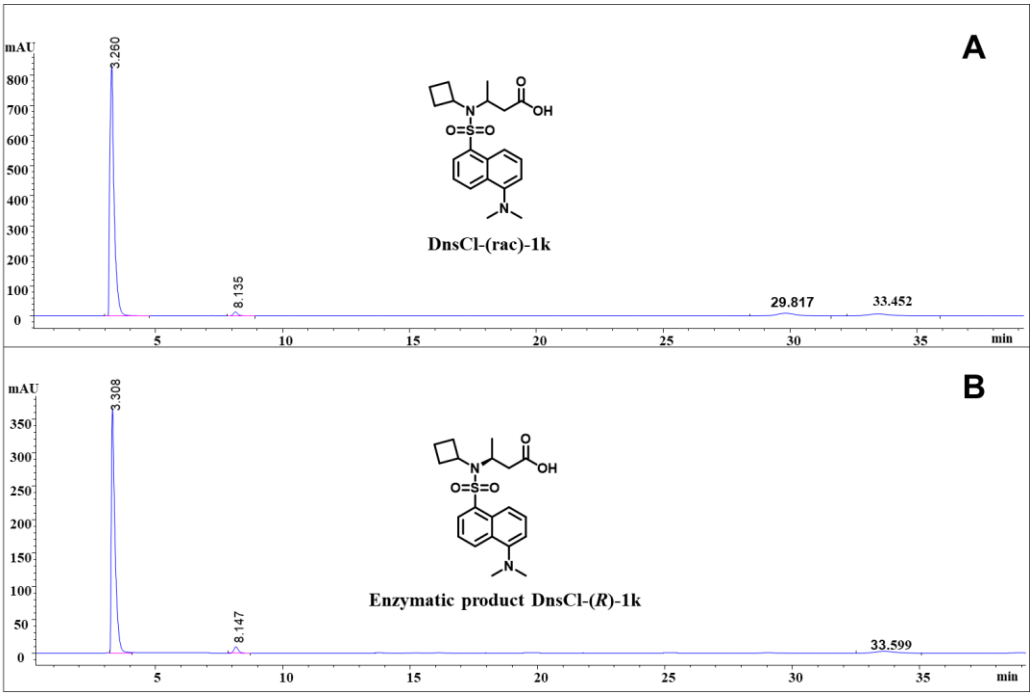


Figure S12. Chiral HPLC analysis of (A) racemic standard compound and (B) enzymatic product **1k**. Chiral HPLC conditions: Chiralpak IG column (5 μm, 250×4.6 mm, Daicel). Phase A: 0.1% formic acid in water, phase B: acetonitrile, A:B = 55:45 (v/v). Flow rate 1.0 mL/min, 25 °C, UV detection at 254 nm.

3-(cyclopentylamino)butanoic acid (1l): $C_9H_{17}NO_2$: MS (ESI^+): calcd.: [M]=171.1, found: $[M+H]^+$=172.1. ^1H NMR (500 MHz, D_2O) δ 3.64 (p, J = 7.1 Hz, 1H), 3.49 (p, J = 6.6 Hz, 1H), 2.53 – 2.38 (m, 2H), 2.06 – 1.94 (m, 2H), 1.72 – 1.62 (m, 2H), 1.62 – 1.51 (m, 4H), 1.25 (d, J = 6.6 Hz, 3H). ^{13}C NMR (126 MHz, D_2O): δ 177.40, 56.23, 50.49, 39.14, 29.72, 29.08, 23.36.

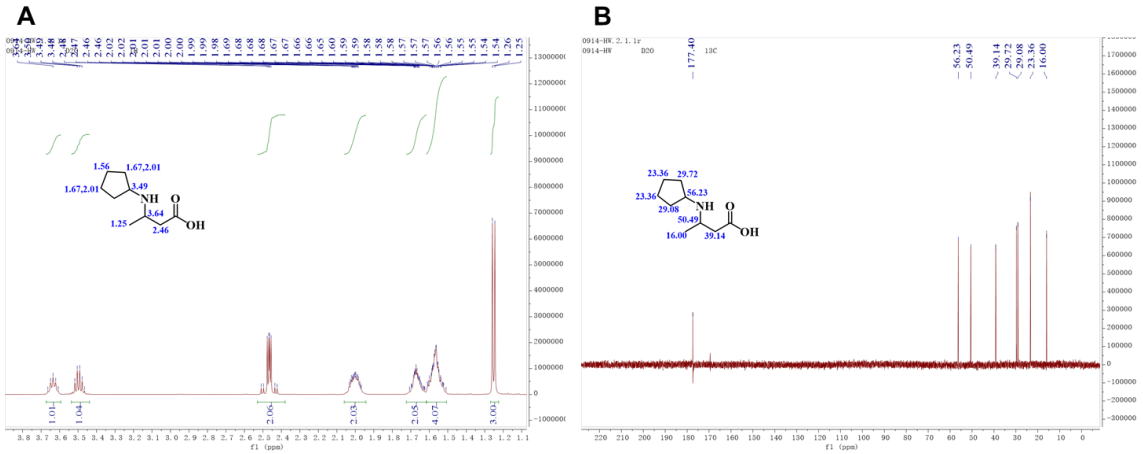


Figure S13. (A) ^1H NMR spectrum and (B) ^{13}C NMR spectrum of the product **1l**.

(*R*)-3-((2-aminoethyl)amino)butanoic acid (1m): $C_6H_{14}N_2O_2$: MS (ESI^+): calcd.: [M]=146.1, found: $[M+H]^+$=147.1.

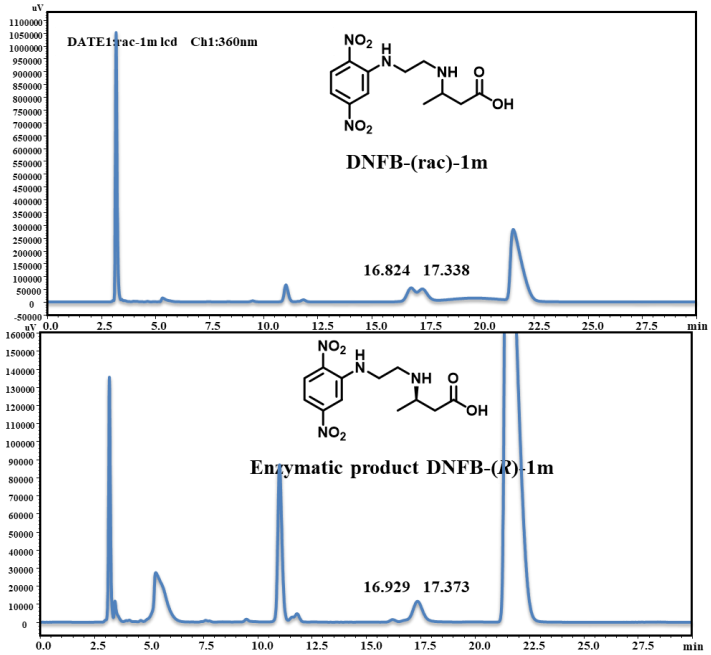


Figure S14. Chiral HPLC analysis of (A) racemic standard compound and (B) enzymatic product **1m**. Chiral HPLC conditions: Chiralpak IG column (5 μm, 250×4.6 mm, Daicel). Phase A: 0.1% formic acid in water, phase B: acetonitrile, A:B = 55:45 (v/v). Flow rate 1.0 mL/min, 25 °C, UV detection at 360 nm.

(*R*)-3-(methoxyamino)butanoic acid (1n): $C_5H_{11}NO_3$: MS (ESI^+): calcd.: [M]=133.1, found: $[M+H]^+$=134.1.

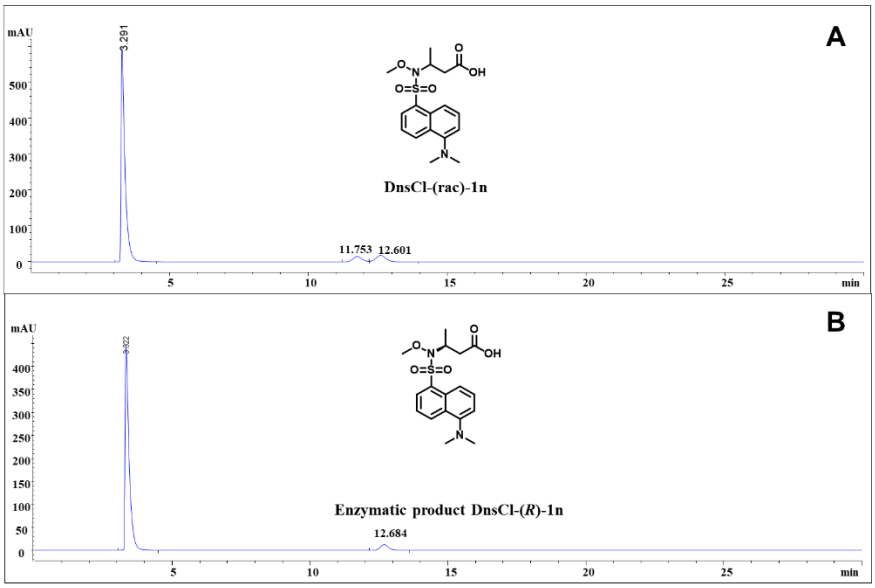


Figure S15. Chiral HPLC analysis of (A) racemic standard compound and (B) enzymatic product **1n**. Chiral HPLC conditions: Chiralpak IG column (5 μm, 250×4.6 mm, Daicel). Phase A: 0.1% formic acid in water, phase B: acetonitrile, A:B = 75:25 (v/v). Flow rate 1.0 mL/min, 25 °C, UV detection at 254 nm.

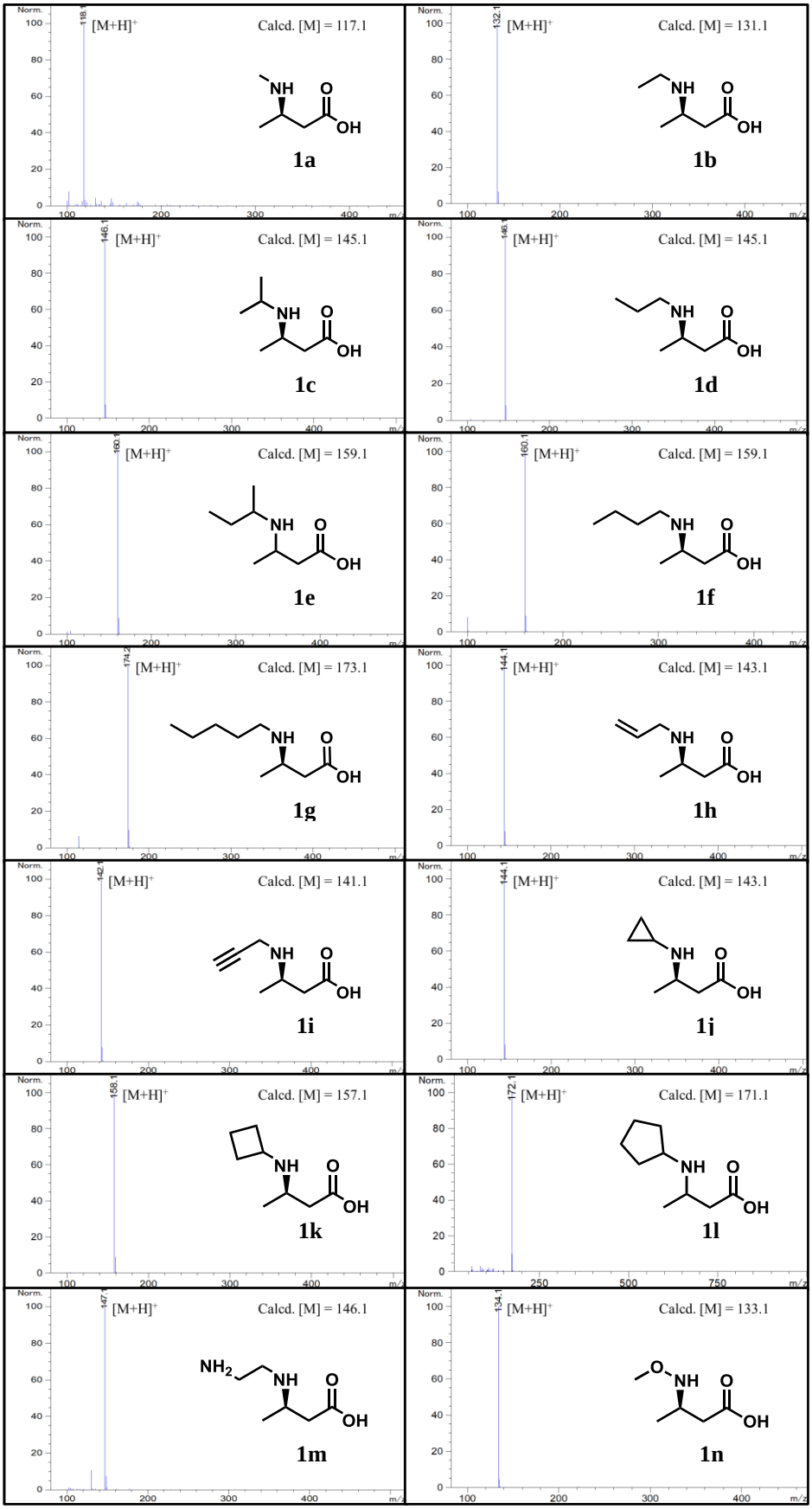


Figure S16. Mass spectrum of **1a-1n**.

Determination of biocatalytic conversion and enantiomeric excess for 2a-2n

Solutions of 22-110 g/L (*E*)-2-pentenoic acid and 0.44-2.22 M amines were prepared and the pH were adjusted to 9.0 by adding 6 M HCl. Purified PA15 (0.5 mL) were added to the solution (4.5 mL), and the reactions were performed at 50 °C. The conversions were determined by measuring the concentrations of the (*E*)-2-pentenoic acid through HPLC analysis (254 nm). The products were identified by using ESI-MS. The enantiomeric excess of the products were determined by HPLC analysis. Due to lack of racemic standard compounds, **2d**, **2f** and **2k** were identified by NMR, and e.e. was not determined. The final concentrations of substrates, reaction conditions and results are shown in **Table S5**.

Table S5. Biocatalytic conversion and enantiomeric excess for **2a-2n**.

Product	Unsaturated acid Conc. (g/L)	Amine Conc. (M)	PA15 Conc. (mM)	Time （h）	Conversion	*e.e.*
2a	20	0.40	0.24	72	94%	99%
2b	50	1.00	0.24	72	98%	99%
2d	20	0.40	0.18	24	96%	n.d.
2f	20	0.40	0.18	74	94%	n.d.
2h	50	1.00	0.24	4	98%	99%
2i	100	1.50	0.27	26	96%	99%
2j	50	1.00	0.27	3	99%	99%
2k	20	0.40	0.18	24	95%	n.d.
2m	100	2.00	0.19	24	99%	97%
2n	50	1.00	0.19	48	98%	99%

(*R*)-3-(methylamino)pentanoic acid (2a): $C_6H_{13}NO_2$: MS (ESI^+): calcd.: [M]=131.1, found: $[M+H]^+$=132.1.

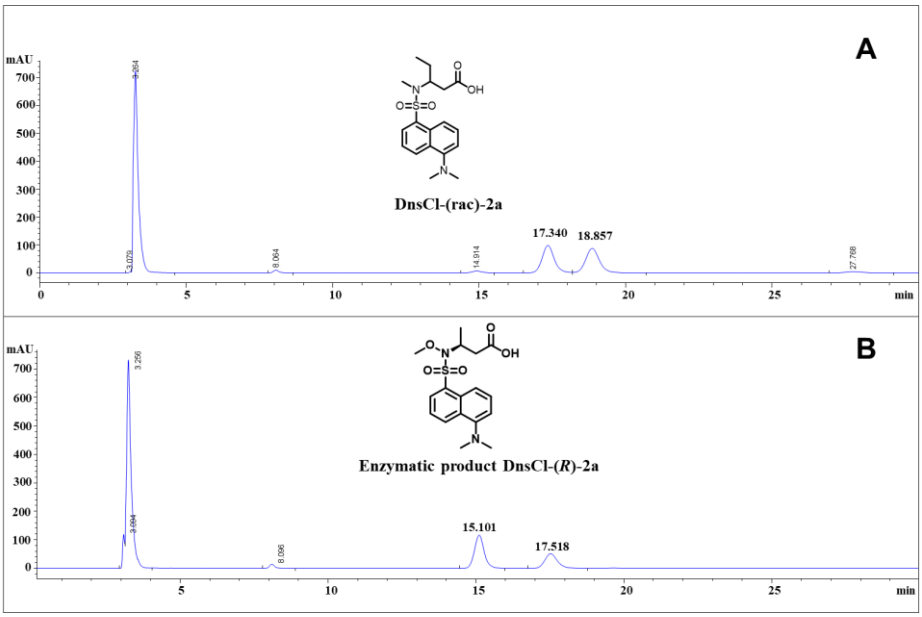


Figure S17. Chiral HPLC analysis of (A) racemic standard compound and (B) enzymatic product **2a**. Chiral HPLC conditions: Chiralpak IG column (5 μm, 250×4.6 mm, Daicel). Phase A: 0.1% formic acid in water, phase B: acetonitrile, A:B = 60:40 (v/v). Flow rate 1.0 mL/min, 25 °C, UV detection at 254 nm.

(*R*)-3-(ethylamino)pentanoic acid (2b): $C_7H_{15}NO_2$: MS (ESI^+): calcd.: [M]=145.1, found: $[M+H]^+$=146.1.

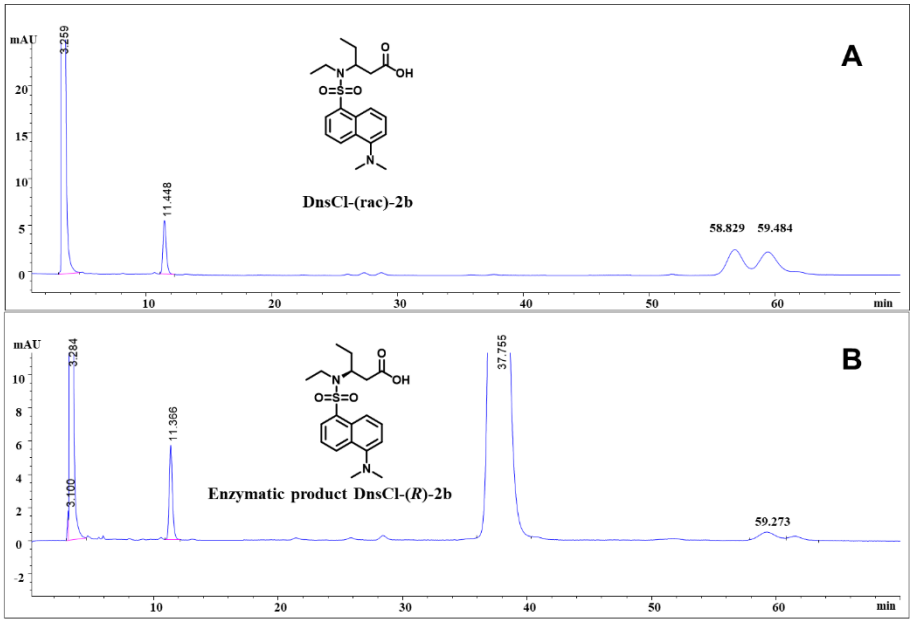


Figure S18. Chiral HPLC analysis of (A) racemic standard compound and (B) enzymatic product **2b**. Chiral HPLC conditions: Chiralpak IG column (5 μm, 250×4.6 mm, Daicel). Phase A: 0.1% formic acid in water, phase B: acetonitrile, A:B = 73:27 (v/v). Flow rate 1.0 mL/min, 25 °C, UV detection at 254 nm.

3-(propylamino)pentanoic acid (2d): $C_8H_{17}NO_2$: MS (ESI^+): calcd.: [M]=159.1, found: $[M+H]^+$=160.1. ^1H NMR (500 MHz, D_2O) δ 3.28 (ddt, J = 8.5, 7.1, 4.9 Hz, 1H), 3.01 – 2.88 (m, 2H), 2.53 (dd, J = 16.8, 5.1 Hz, 1H), 2.39 (dd, J = 16.9, 7.2 Hz, 1H), 1.77 – 1.51 (m, 4H), 0.88 (td, J = 7.5, 6.3 Hz, 6H). ^{13}C NMR (126 MHz, D_2O): δ 178.10, 56.92, 46.00, 35.49, 23.40, 19.37, 10.13, 8.80.

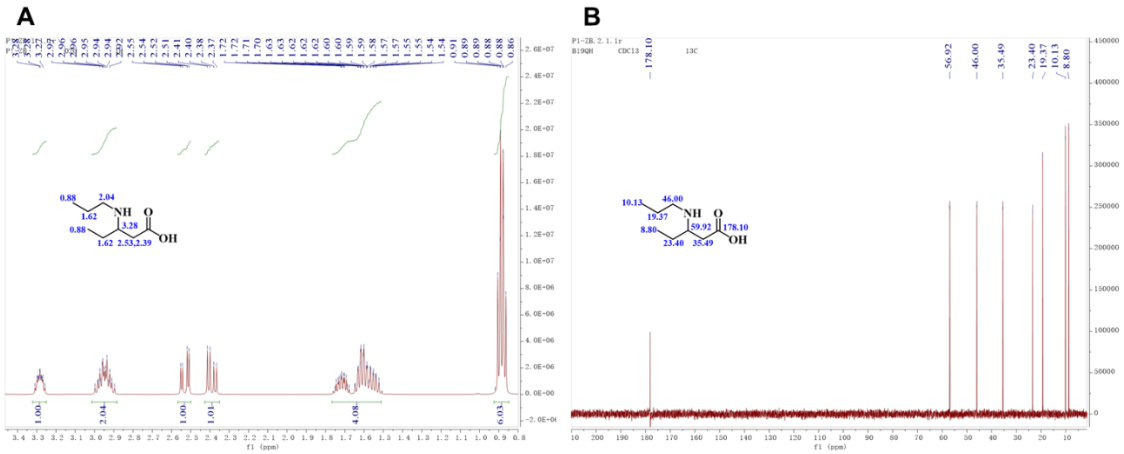


Figure S19. (A) ^1H NMR spectrum and (B) ^{13}C NMR spectrum of the product **2d**.

3-(butylamino)pentanoic acid (2f): $C_9H_{19}NO_2$: MS (ESI^+): calcd.: [M]=173.1, found: $[M+H]^+$=174.1. ^1H NMR (500 MHz, D_2O) δ 3.30 (ddt, J = 9.3, 7.1, 4.9 Hz, 1H), 3.05 – 2.93 (m, 2H), 2.56 (dd, J = 16.9, 5.0 Hz, 1H), 2.42 (dd, J = 16.9, 7.1 Hz, 1H), 1.72 (dtd, J = 15.0, 7.4, 4.7 Hz, 1H), 1.58 (p, J = 7.6 Hz, 3H), 1.31 (h, J = 7.4 Hz, 2H), 0.88 (t, J = 7.5 Hz, 3H), 0.84 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, D_2O): δ 180.33, 59.40, 46.75, 37.89, 30.28, 25.92, 21.61, 15.20, 11.33.

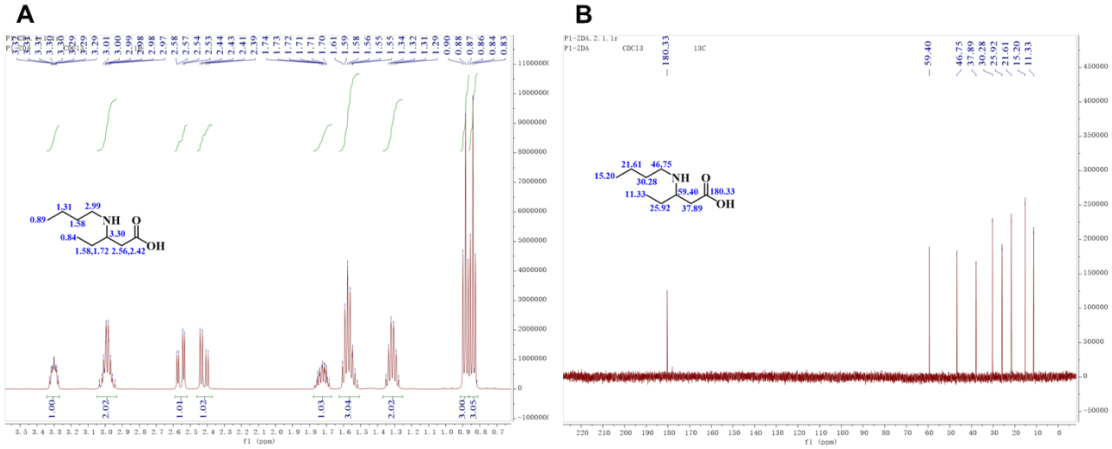


Figure S20. (A) ^1H NMR spectrum and (B) ^{13}C NMR spectrum of the product **2f**.

(*R*)-3-(allylamino)pentanoic acid (2h): $C_8H_{15}NO_2$, MS (ESI^+) calcd.: [M]=157.1, found: $[M+H]^+$=158.1.

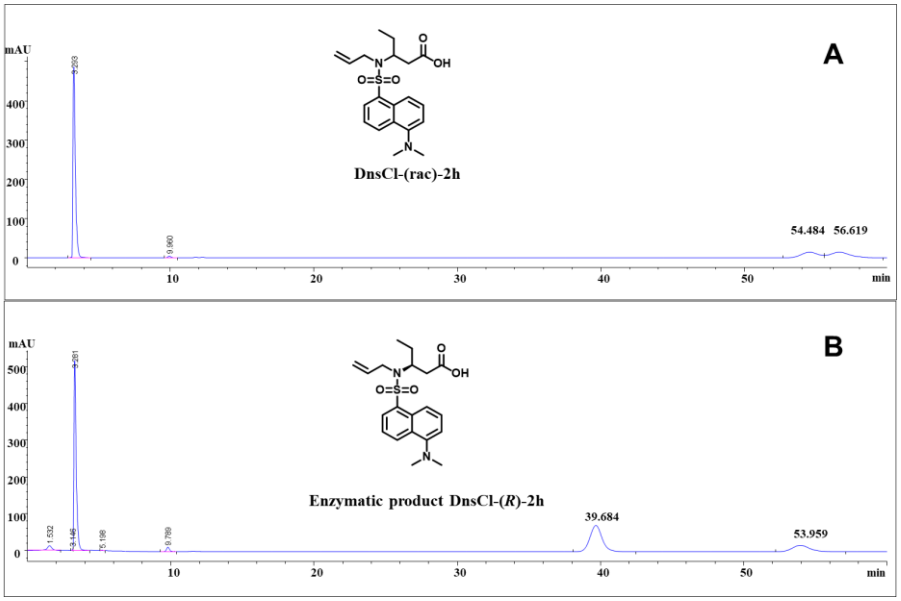


Figure S21. Chiral HPLC analysis of (A) racemic standard compound and (B) enzymatic product **2h**. Chiral HPLC conditions: Chiralpak IG column (5 μm, 250×4.6 mm, Daicel). Phase A: 0.1% formic acid in water, phase B: acetonitrile, A:B = 70:30 (v/v). Flow rate 1.0 mL/min, 25 °C, UV detection at 254 nm.

(*R*)-3-(propargylamino)pentanoic acid (2i): $C_8H_{13}NO_2$: MS (ESI^+): calcd.: [M]=155.1, found: $[M+H]^+$=156.1.

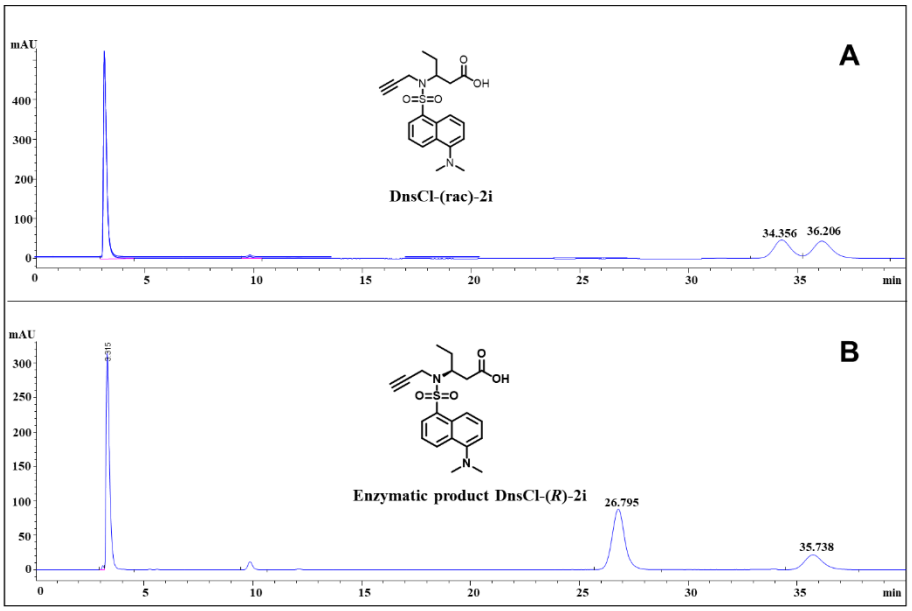


Figure S22. Chiral HPLC analysis of (A) racemic standard compound and (B) enzymatic product **2i**. Chiral HPLC conditions: Chiralpak IG column (5 μm, 250×4.6 mm, Daicel). Phase A: 0.1% formic acid in water, phase B: acetonitrile, A:B = 70:30 (v/v). Flow rate 1.0 mL/min, 25 °C, UV detection at 254 nm.

(*R*)-3-(cyclopropylamino)pentanoic acid (2j): $C_8H_{15}NO_2$: MS (ESI^+): calcd.: [M]=157.1, found: $[M+H]^+$=158.1.

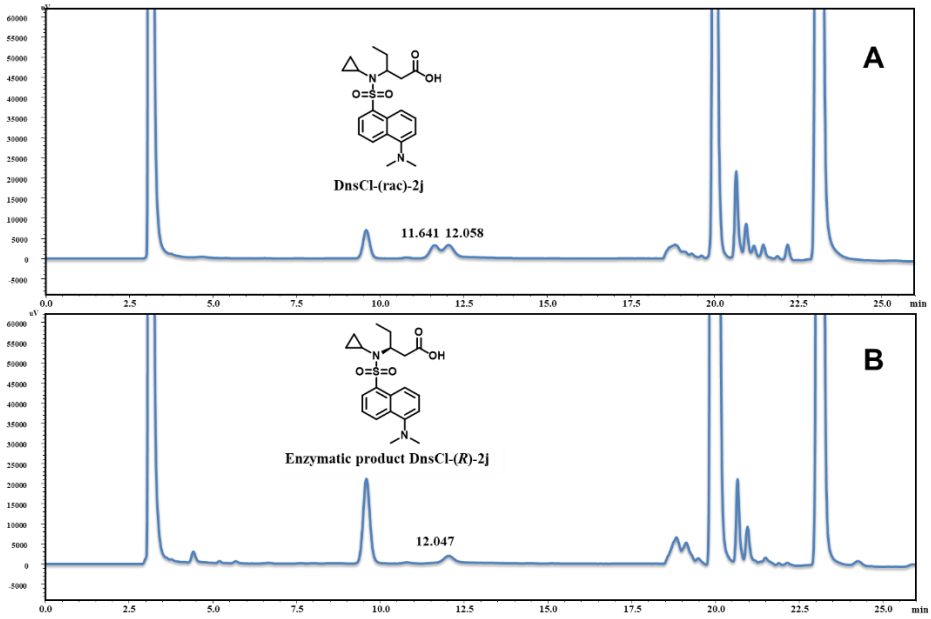


Figure S23. Chiral HPLC analysis of (A) racemic standard compound and (B) enzymatic product **2j**. Chiral HPLC conditions: Chiralpak IG column (5 μm, 250×4.6 mm, Daicel). Phase A: 0.1% formic acid in water, phase B: acetonitrile, A:B = 70:30 (v/v). Flow rate 1.0 mL/min, 25 °C, UV detection at 254 nm.

3-(cyclobutylamino)pentanoic acid (2k): $C_9H_{17}NO_2$: MS (ESI^+): calcd.: [M]=171.1, found: $[M+H]^+$=172.1. ^1H NMR (500 MHz, D_2O) δ 3.81 (p, J = 8.2 Hz, 1H), 3.29 – 3.19 (m, 1H), 2.48 (dd, J = 16.7, 5.4 Hz, 1H), 2.36 (dd, J = 16.7, 6.7 Hz, 1H), 2.24 (dddt, J = 10.9, 7.7, 5.1, 3.1 Hz, 2H), 2.17 – 2.04 (m, 2H), 1.78 (dtd, J = 18.7, 11.1, 10.6, 3.0 Hz, 2H), 1.68 (dtd, J = 15.1, 7.4, 4.6 Hz, 1H), 1.60 – 1.48 (m, 1H), 0.87 (t, J = 7.5 Hz, 3H). ^{13}C NMR (126 MHz, D_2O): δ 178.11 , 55.43 , 49.27 , 36.00 , 26.82 , 26.58 , 23.59 , 8.67 .

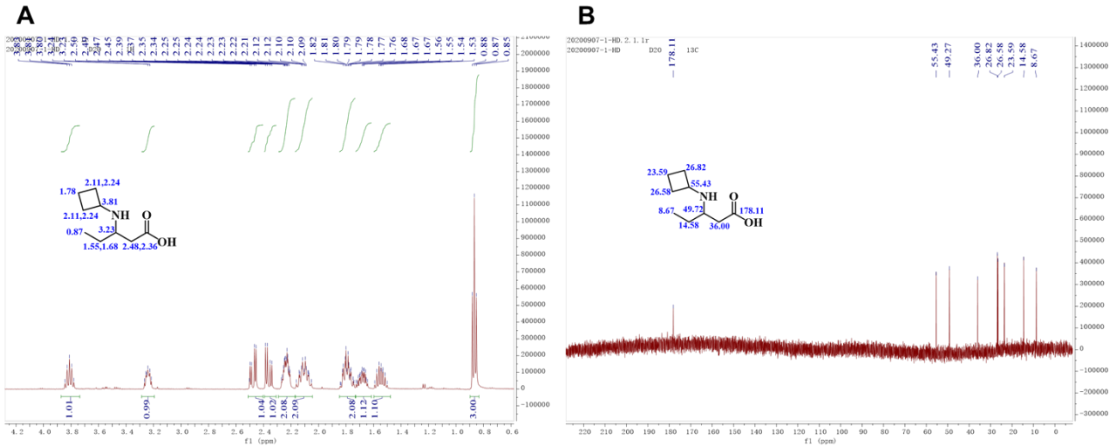


Figure S24. (A) ^1H NMR spectrum and (B) ^{13}C NMR spectrum of the product **2k**.

(*R*)-3-((2-aminoethyl)amino)pentanoic acid (2m): $C_7H_{16}N_2O_2$: MS (ESI^+): calcd.: [M]=160.1, found: $[M+H]^+$=161.1.

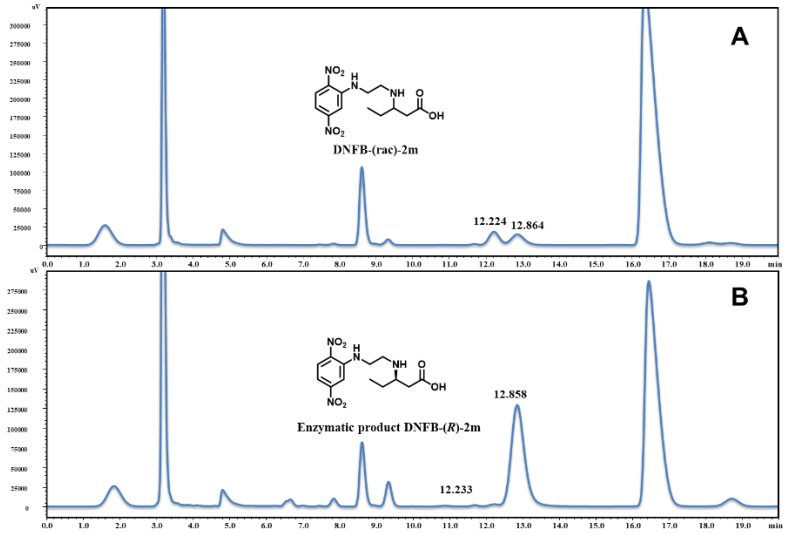


Figure S25. Chiral HPLC analysis of (A) racemic standard compound and (B) enzymatic product **2m**. Chiral HPLC conditions: Chiralpak IG column (5 μm, 250×4.6 mm, Daicel). Phase A: 0.1% formic acid in water, phase B: acetonitrile, A:B = 50:50 (v/v). Flow rate 1.0 mL/min, 25 °C, UV detection at 360 nm.

(*R*)-3-(methoxyamino)pentanoic acid (2n): $C_6H_{13}NO_3$: MS (ESI^+): calcd.: [M]=147.1, found: $[M+H]^+$=148.1.

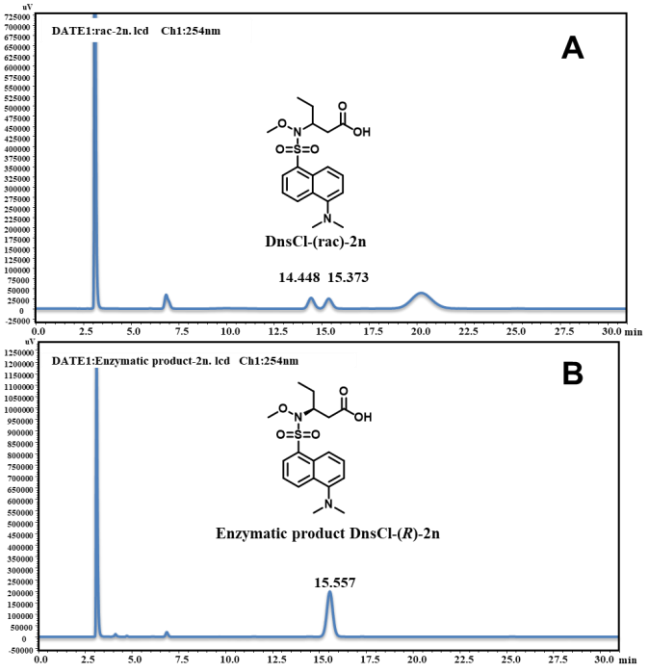


Figure S26. Chiral HPLC analysis of (A) racemic standard compound and (B) enzymatic product **2n**. Chiral HPLC conditions: Chiralpak IG column (5 μm, 250×4.6 mm, Daicel). Phase A: 0.1% formic acid in water, phase B: acetonitrile, A:B = 50:50 (v/v). Flow rate 1.0 mL/min, 25 °C, UV detection at 360 nm.

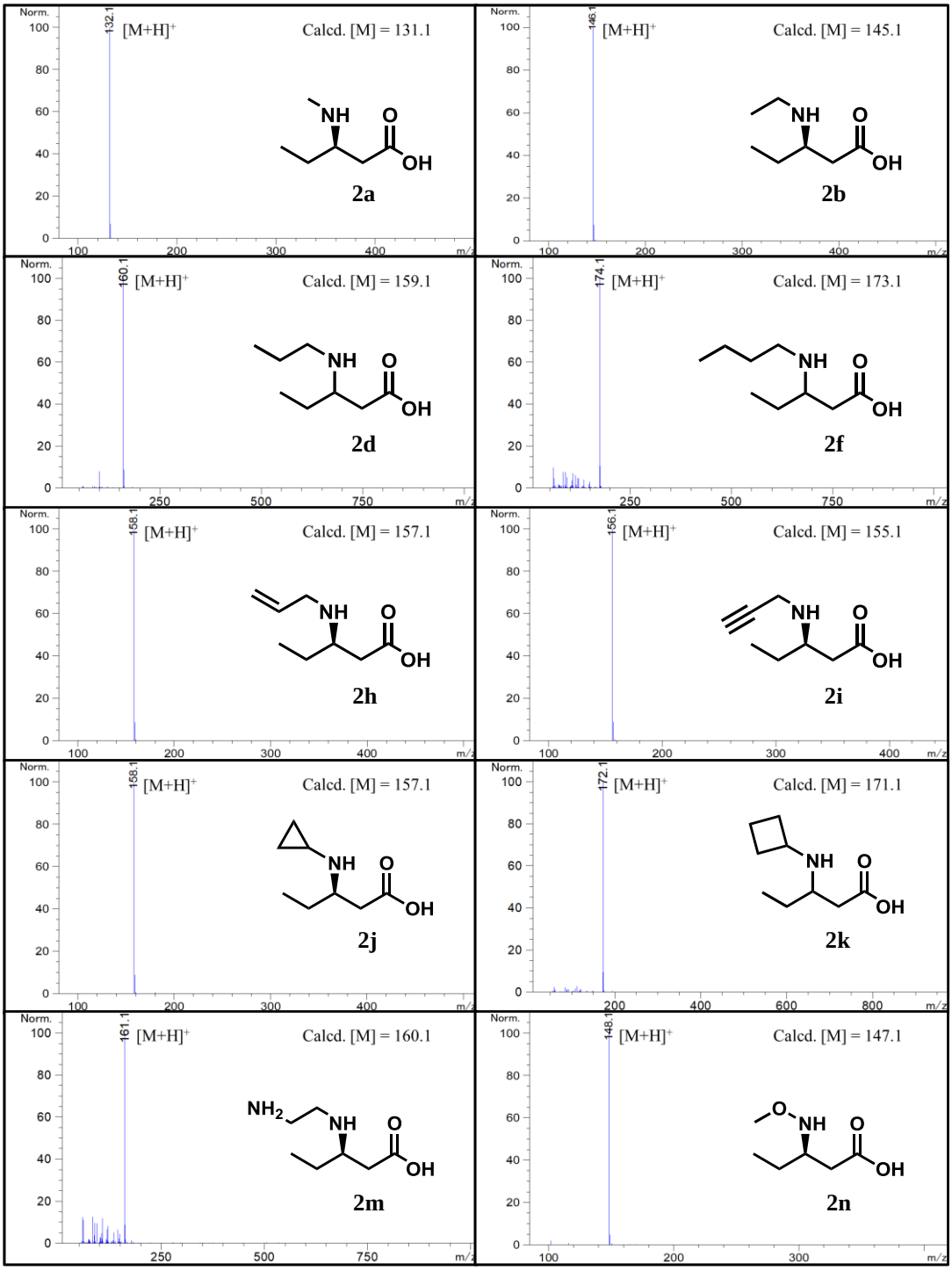


Figure S27. Mass spectrum of **2a-2n**.

Determination of biocatalytic conversion and enantiomeric excess for 3a-3n

Solutions of 88-144 g/L fumaric acid and 1.1-2.0 M amines were prepared and the pH were adjusted to 9.0 by adding 6 M HCl. Purified AA15 (0.5 mL) were added to the solution (4.5 mL), and the reactions were performed at 37 °C. The conversions were determined by measuring the concentrations of the fumaric acid through HPLC analysis (254 nm). The products were identified by using ESI-MS. The enantiomeric excess of the products were determined by HPLC analysis. Due to lack of racemic standard compounds, **3n** was identified by NMR, and e.e. was not determined. The final concentrations of substrates, reaction conditions and results are shown in **Table S6**.

Table S6. Biocatalytic conversion and enantiomeric excess for **3a-3n**.

Product	Unsaturated acid Conc. (g/L)	Amine Conc. (M)	AA15 Conc. (mM)	Time （h）	Conversion	*e.e.*
3a	130	1.8	0.12	9	99%	99%
3b	130	1.8	0.12	2	96%	99%
3c	130	1.8	0.12	120	88%	99%
3d	130	1.8	0.12	2	96%	99%
3f	116	1.5	0.12	6	97%	99%
3h	80	1.0	0.12	9	94%	99%
3i	130	1.8	0.12	9	90%	99%
3j	130	1.8	0.12	4	97%	99%
3k	100	1.4	0.17	2	99%	99%
3m	100	1.4	0.17	2	99%	94%
3n	80	1.0	0.12	2	99%	n.d.

(*S*)-2-(methylamino)succinic acid (3a): $C_5H_9NO_4$: MS (ESI^+): calcd.: [M]=147.1, found: $[M+H]^+$=148.0.

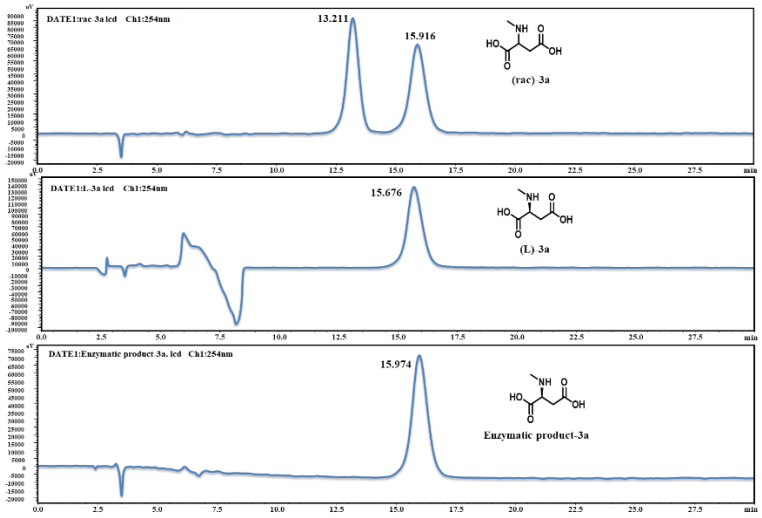


Figure S28. Chiral HPLC analysis of (A) racemic standard compound, (B) standard compound and (C) enzymatic product **3a**. Chiral HPLC conditions: Chirex 3126-Dpenicillamine column (250 mm x 4.6 mm). Phase A: 2.0 mM $CuSO_4$ aqueous solution, phase B: isopropanol, A:B = 90:10 (v/v). Flow rate 1.0 mL/min, 25 °C, UV detection at 254 nm.

(*S*)-2-(ethylamino)succinic acid (3b): $C_6H_{11}NO_4$: MS (ESI^+): calcd.: [M]=161.1, found: $[M+H]^+$=162.1.

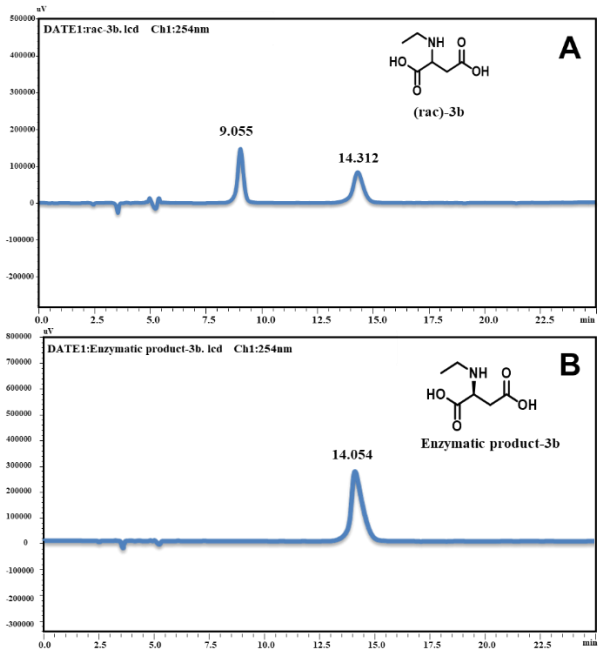


Figure S29. Chiral HPLC analysis of (A) racemic standard compound and (B) enzymatic product **3b**. Chiral HPLC conditions: Chirex 3126-Dpenicillamine column (250 mm x 4.6 mm). Phase A: 2.0 mM $CuSO_4$ aqueous solution, phase B: isopropanol, A:B = 90:10 (v/v). Flow rate 1.0 mL/min, 25 °C, UV detection at 254 nm.

(*S*)-2-(isopropylamino)succinic acid (3c): $C_7H_{13}NO_4$: MS (ESI^+): calcd.: [M]=175.1, found: $[M+H]^+$=176.1.

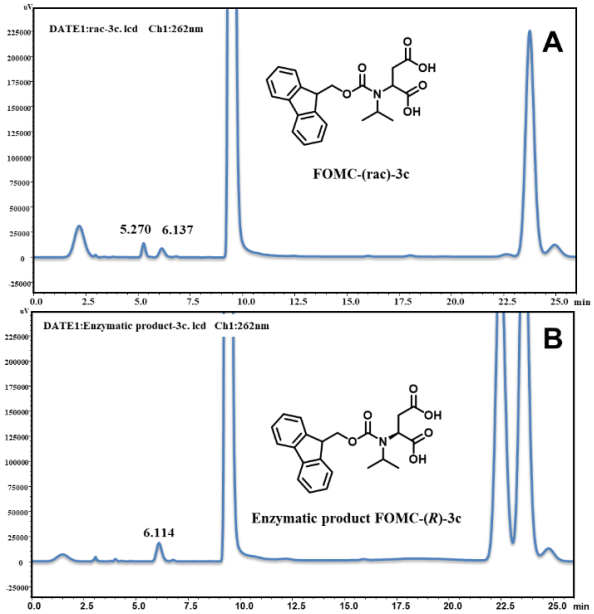


Figure S30. Chiral HPLC analysis of (A) racemic standard compound and (B) enzymatic product **3c**. Chiral HPLC conditions: Chiralpak IG column (5 μm, 250×4.6 mm, Daicel). Phase A: 0.1% formic acid in water, phase B: acetonitrile, A:B = 45:55 (v/v). Flow rate 1.0 mL/min, 25 °C, UV detection at 262 nm.

(*S*)-2-(propylamino)succinic acid (3d): $C_7H_{13}NO_4$: MS (ESI^+): calcd.: [M]=175.1, found: $[M+H]^+$=176.1.

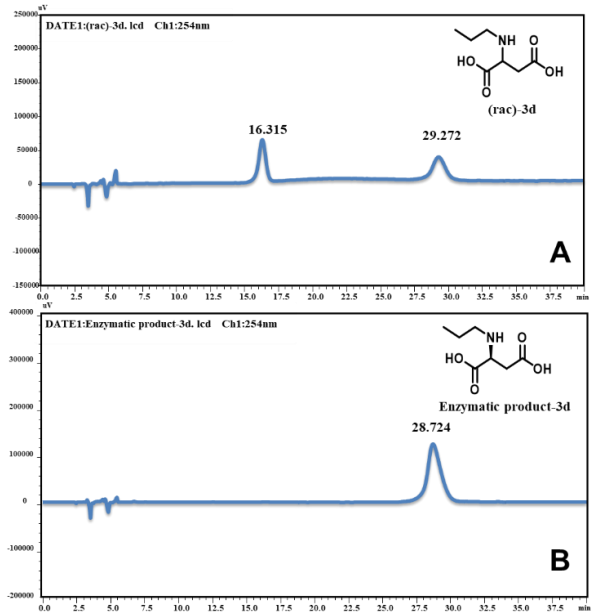


Figure S31. Chiral HPLC analysis of (A) racemic standard compound and (B) enzymatic product **3d**. Chiral HPLC conditions: Chirex 3126-Dpenicillamine column (250 mm x 4.6 mm). Phase A: 2.0 mM $CuSO_4$ aqueous solution, phase B: isopropanol, A:B = 90:10 (v/v). Flow rate 1.0 mL/min, 25 °C, UV detection at 254 nm.

(*S*)-2-(butylamino)succinic acid (3f): $C_8H_{15}NO_4$: MS (ESI^+): calcd.: [M]=189.1, found: $[M+H]^+$=190.1.

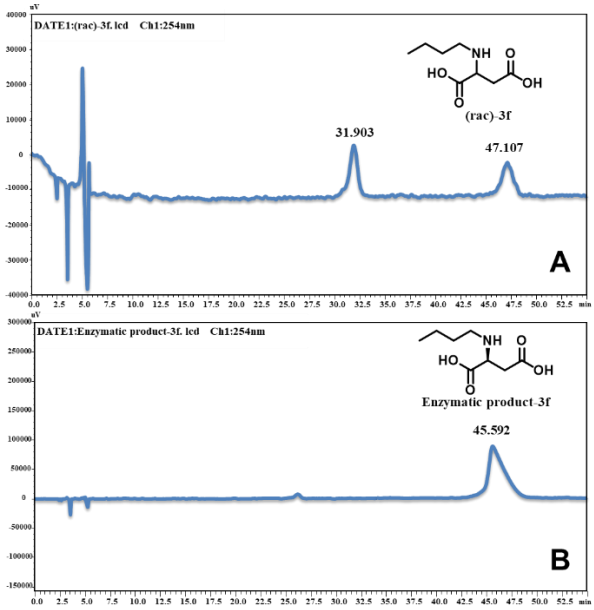


Figure S32. Chiral HPLC analysis of (A) racemic standard compound and (B) enzymatic product **3f**. Chiral HPLC conditions: Chirex 3126-Dpenicillamine column (250 mm x 4.6 mm). Phase A: 2.0 mM $CuSO_4$ aqueous solution, phase B: isopropanol, A:B = 90:10 (v/v). Flow rate 1.0 mL/min, 25 °C, UV detection at 254 nm.

(S)-2-(allylamino)succinic acid (3h): $C_7H_{11}NO_4$: MS (ESI^+): calcd.: [M]=173.1, found: $[M+H]^+$=174.1.

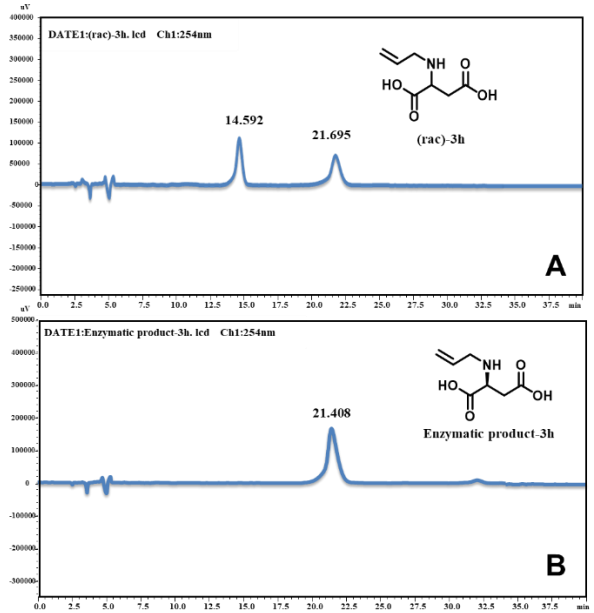


Figure S33. Chiral HPLC analysis of (A) racemic standard compound and (B) enzymatic product **3h**. Chiral HPLC conditions: Chirex 3126-Dpenicillamine column (250 mm x 4.6 mm). Phase A: 2.0 mM $CuSO_4$ aqueous solution, phase B: isopropanol, A:B = 90:10 (v/v). Flow rate 1.0 mL/min, 25 °C, UV detection at 254 nm.

(S)-2-(propargylamino)succinic acid (3i): $C_7H_9NO_4$: MS (ESI^+): calcd.: [M]=171.1, found: $[M+H]^+$=172.0.

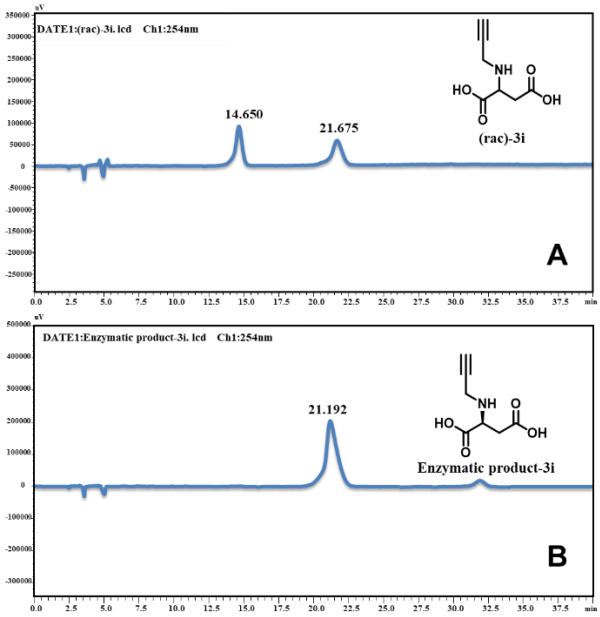


Figure S34. Chiral HPLC analysis of (A) racemic standard compound and (B) enzymatic product **3i**. Chiral HPLC conditions: Chirex 3126-Dpenicillamine column (250 mm x 4.6 mm). Phase A: 2.0 mM $CuSO_4$ aqueous solution, phase B: isopropanol, A:B = 90:10 (v/v). Flow rate 1.0 mL/min, 25 °C, UV detection at 254 nm.

(*S*)-2-(cyclopropylamino)succinic acid (3j): $C_7H_{11}NO_4$: MS (ESI^+): calcd.: [M]=173.1, found: $[M+H]^+$=174.1.

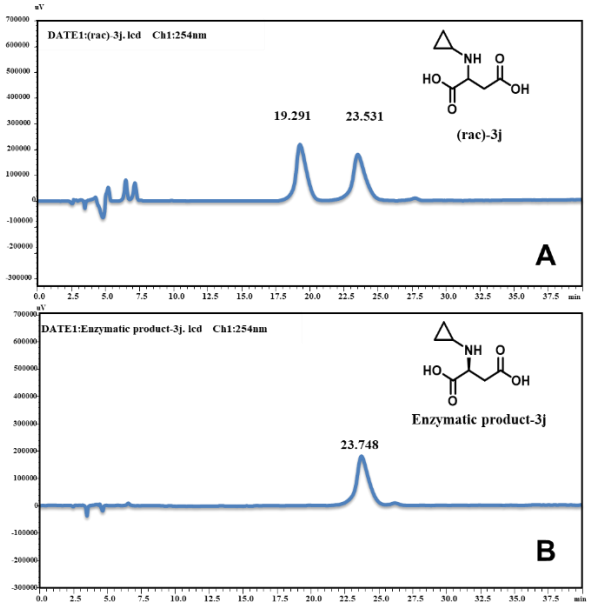


Figure S35. Chiral HPLC analysis of (A) racemic standard compound and (B) enzymatic product **3j**. Chiral HPLC conditions: Chirex 3126-Dpenicillamine column (250 mm x 4.6 mm). Phase A: 2.0 mM $CuSO_4$ aqueous solution, phase B: isopropanol, A:B = 90:10 (v/v). Flow rate 1.0 mL/min, 25 °C, UV detection at 254 nm.

(*S*)-2-(cyclobutylamino)succinic acid (3k): $C_8H_{13}NO_4$: MS (ESI^+): calcd.: [M]=187.1, found: $[M+H]^+$=188.1.

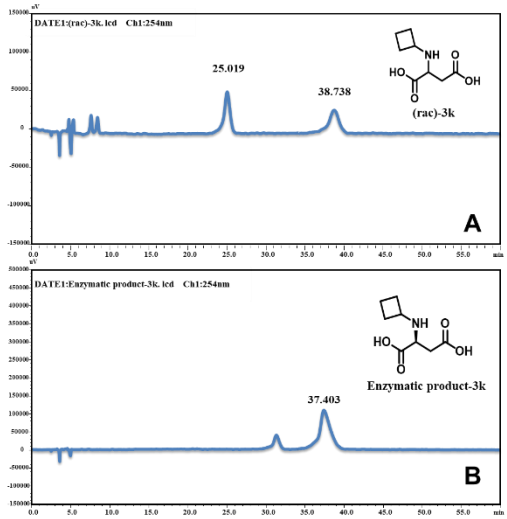


Figure S36. Chiral HPLC analysis of (A) racemic standard compound and (B) enzymatic product **3k**. Chiral HPLC conditions: Chirex 3126-Dpenicillamine column (250 mm x 4.6 mm). Phase A: 2.0 mM $CuSO_4$ aqueous solution, phase B: isopropanol, A:B = 90:10 (v/v). Flow rate 1.0 mL/min, 25 °C, UV detection at 254 nm.

(S)-2-((2-aminoethyl)amino)succinic acid (3m): $C_6H_{12}N_2O_4$: MS (ESI^+): calcd.: [M]=176.1, found: $[M+H]^+$=177.1.

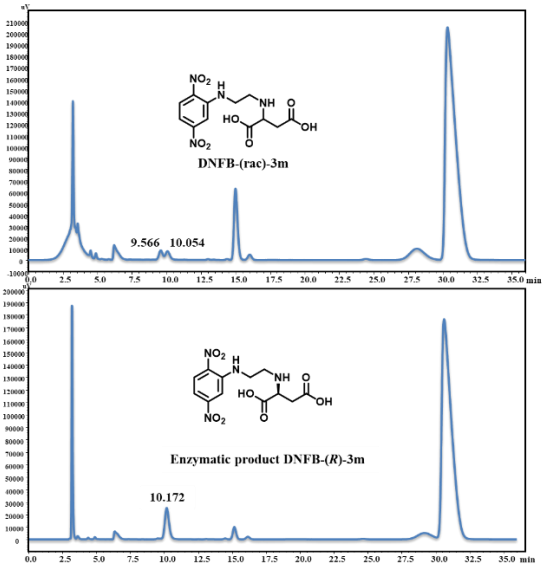


Figure S37. Chiral HPLC analysis of (A) racemic standard compound and (B) enzymatic product **3m**. Chiral HPLC conditions: Chiralpak IG column (5 μm, 250×4.6 mm, Daicel). Phase A: 0.1% formic acid in water, phase B: acetonitrile, A:B = 55:45 (v/v). Flow rate 1.0 mL/min, 25 °C, UV detection at 360 nm.

2-(methoxyamino)succinic acid (3n): $C_5H_9NO_5$: MS (ESI^+): calcd.: [M]=163.0, found: $[M+H]^+$=164.0. Results of NMR were shown in **Figure S49**.

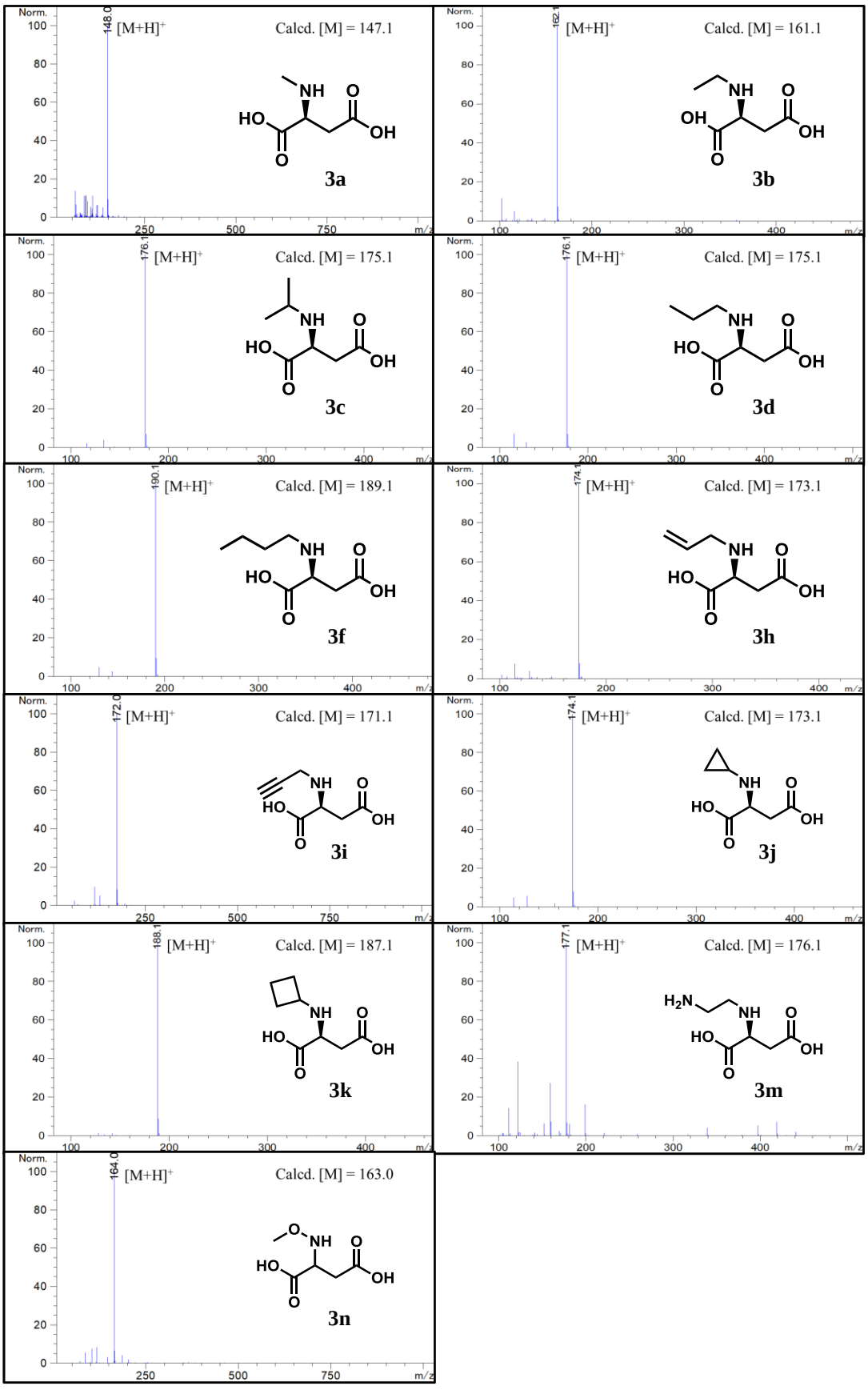


Figure S38. Mass spectrum of **3a-3n**.

Determination of biocatalytic conversion and enantiomeric excess for 4b-4n

Solutions of 10.8 g/L (*E*)-cinnamic acid and 1.0-1.4 M amines were prepared and the pH were adjusted to 8.5 by adding 6 M NaOH or HCl. Purified FA15 (0.37 mL) were added to the solution (3.5 mL), and the reactions were performed at 37 °C. After 12 h, 24 h, and 36 h, the same amount of purified mutants were added respectively. The conversions were determined by measuring the concentrations of the (*E*)-cinnamic acid through HPLC analysis (254 nm). The products were identified by using ESI-MS. The enantiomeric excess of the products were determined by HPLC analysis. Due to lack of racemic standard compounds, **4d** and **4n** were identified by NMR, and e.e. was not determined. e.e. of **4b** and **4i** was not determined because the racemic standard β-amino acids could not be distinguished by HPLC analysis after numerous attempts. The final concentrations of substrates, reaction conditions and results are shown in **Table S7**.

Table S7. Biocatalytic conversion and enantiomeric excess for **4b**-**4n**.

Product	Unsaturated acid Conc. (g/L)	Amine Conc. (M)	FA15 Conc. (mM)	Time （h）	Conversion	*e.e.*
4b	7.5	0.70	0.60	52	23%	n.d.
4d	7.5	0.70	0.60	42	19%	n.d.
4h	7.5	0.70	0.60	42	27%	99%
4i	7.5	0.70	0.60	52	19%	n.d.
4n	7.5	1.00	0.60	72	97%	n.d.

3-(ethylamino)-3-phenylpropanoic acid (4b): $C_{11}H_{15}NO_2$: MS (ESI^+): calcd.: [M]=193.1, found: $[M+H]^+$=194.1.

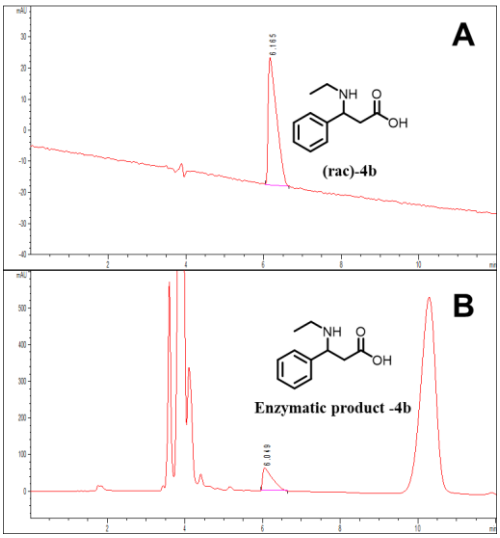


Figure S39. HPLC analysis of (A) racemic standard compound and (B) enzymatic reaction mixture of **4b**. HPLC conditions: C4 reversed-phase column (250×4.6 mm, 5 μm, 300 Å, Nanochrom, Suzhou, China). Phase A: 0.1% formic acid in water, phase B: acetonitrile, gradient from 5% to 30% CH_3CN in 12 min. Flow rate 1.0 mL/min, 25 °C, UV detection at 254 nm.

3-(propylamino)-3-phenylpropanoic acid (4d): $C_{12}H_{17}NO_2$: MS (ESI^+): calcd.: [M]=207.1, found: $[M+H]^+$=208.1. ^1H NMR (500 MHz, D_2O) δ 7.46 – 7.36 (m, 5H), 4.46 (t, J = 7.4 Hz, 1H), 2.94 (dd, J = 16.1, 7.5 Hz, 1H), 2.85 – 2.68 (m, 3H), 1.64 – 1.48 (m, 2H), 0.82 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, D_2O): δ 176.61, 133.74, 129.77, 129.34, 127.99, 59.62, 46.96, 39.24, 19.11, 10.08.

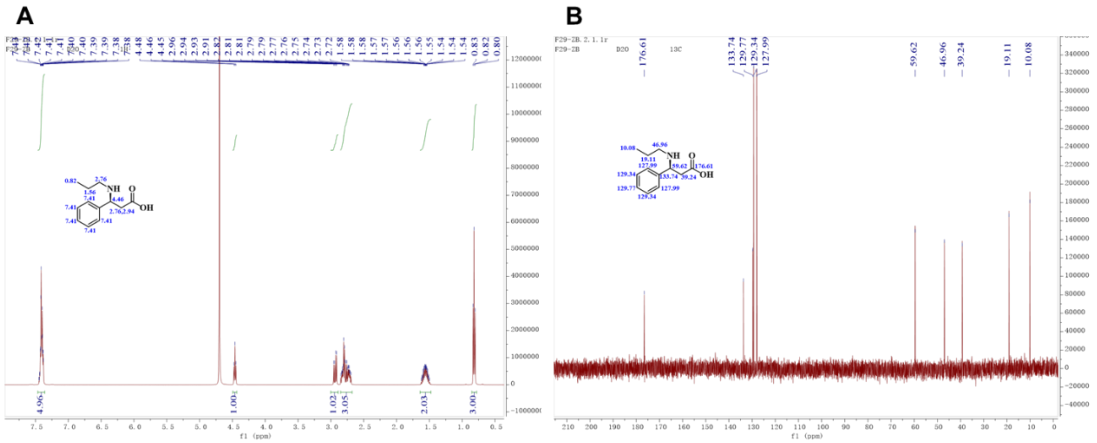


Figure S40. (A) ^1H NMR spectrum and (B) ^{13}C NMR spectrum of the product **4d**.

(*S*)-3-(allylamino)-3-phenylpropanoic acid (4h): $C_{12}H_{15}NO_2$: MS (ESI^+): calcd.: [M]=205.1, found: $[M+H]^+$=206.1.

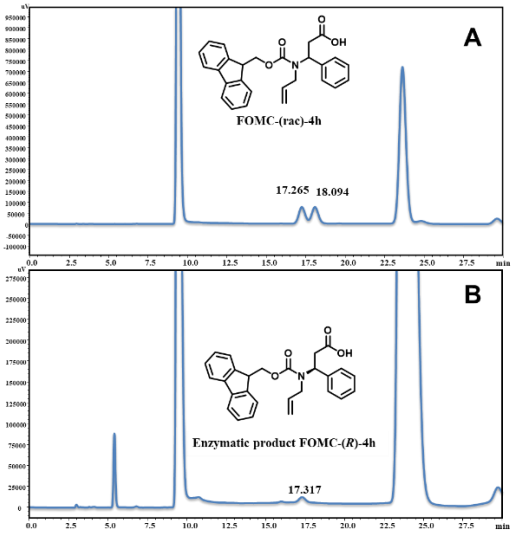


Figure S41. Chiral HPLC analysis of (A) racemic standard compound and (B) enzymatic product **4h**. Chiral HPLC conditions: Chiralpak IG column (5 μm, 250×4.6 mm, Daicel). Phase A: 0.1% formic acid in water, phase B: acetonitrile, A:B = 45:55 (v/v). Flow rate 1.0 mL/min, 25 °C, UV detection at 262 nm.

3-(propargylamino)-3-phenylpropanoic acid (4i): $C_{12}H_{13}NO_2$: MS (ESI^+): calcd.: [M]=203.1, found: $[M+H]^+$=204.1.

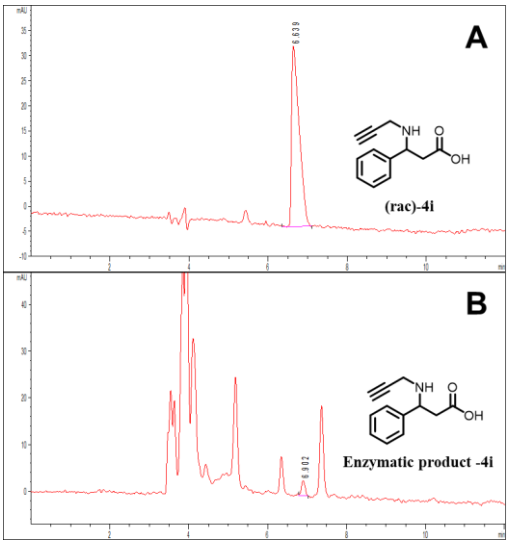


Figure S42. HPLC analysis of (A) racemic standard compound and (B) enzymatic reaction mixture of **4i**. HPLC conditions: C4 reversed-phase column (250×4.6 mm, 5 μm, 300 Å, Nanochrom, Suzhou, China). Phase A: 0.1% formic acid in water, phase B: acetonitrile, gradient from 5% to 30% CH_3CN in 12 min. Flow rate 1.0 mL/min, 25 °C, UV detection at 254 nm.

3-(methoxyamino)-3-phenylpropanoic acid (4n): $C_{10}H_{13}NO_3$: MS (ESI^+): calcd.: [M]=195.1, found: $[M+H]^+$=196.1. Results of NMR were shown in **Figure S50**.

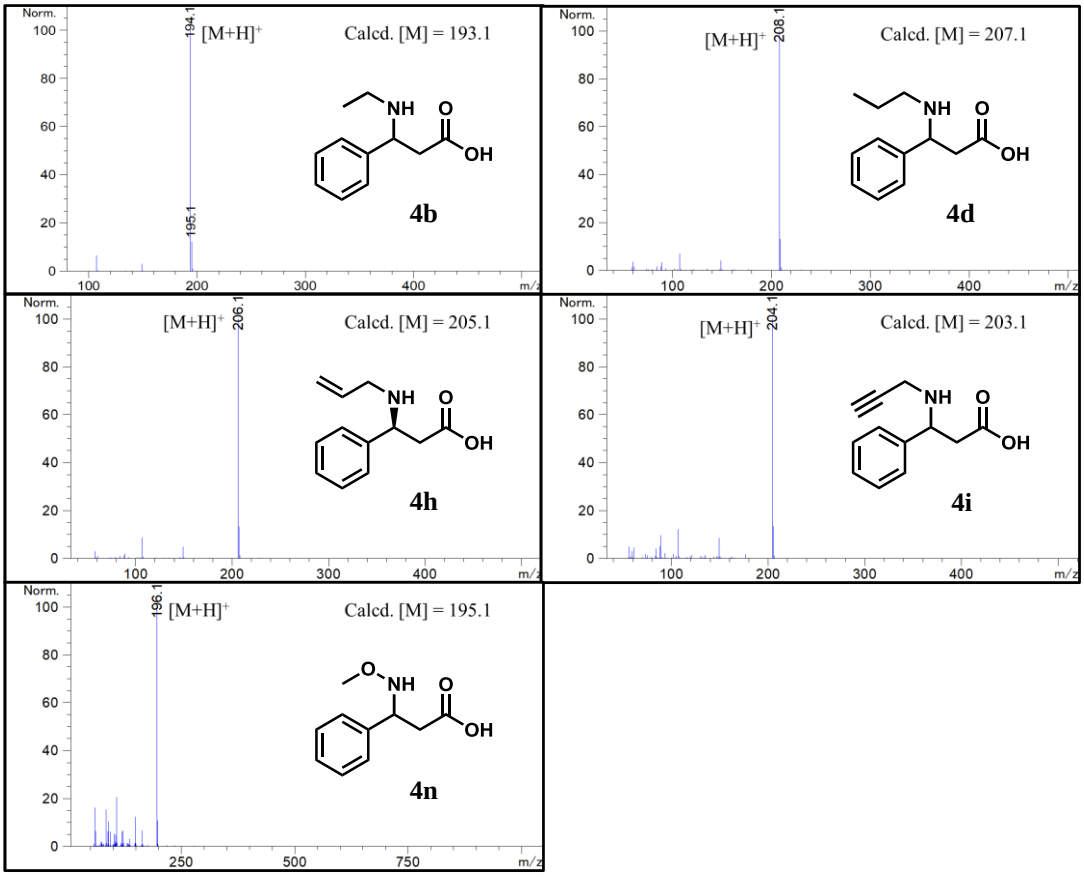


Figure S43. Mass spectrum of **4b-4n**.

Determination of biocatalytic conversion for 5b-9n

Solutions of 6.5-10.8 g/L unsaturated carboxylic acid and 1.0 M amines were prepared and the pH were adjusted to 8.5 by adding 6 M NaOH or HCl. Purified FA15 (0.5 mL) were added to the solution (3.5 mL), and the reactions were performed at 37 °C. After 24 h and 48 h, the same amount of purified mutants were added respectively. The conversions were determined by measuring the concentrations of the unsaturated carboxylic acid through HPLC analysis (254 nm). The products were identified by using ESI-MS. The final concentrations of substrates, reaction conditions and results are shown in **Table S8**.

Table S8. Biocatalytic conversion for **5b-9n**.

Product	Unsaturated acid Conc. (g/L)	Amine Conc. (M)	FA15 Conc. (mM)	Time （h）	Conversion
5b	7.5	0.70	0.54	96	10%
5d	7.5	0.70	0.54	96	7%
5n	4.5	0.70	0.54	96	57%
6n	7.5	0.70	0.54	96	9%
7b	7.5	0.70	0.54	96	8%
7n	4.5	0.70	0.54	96	30%
8n	7.5	0.70	0.54	96	67%
9b	7.5	0.70	0.54	96	10%
9n	4.5	0.70	0.54	96	87%

3-(ethylamino)-3-(*p*-tolyl)propanoic acid (5b): $C_{12}H_{17}NO_2$: MS (ESI^+): calcd.: [M]=207.1, found: $[M+H]^+$=208.1.

3-(propylamino)-3-(*p*-tolyl)propanoic acid (5d): $C_{13}H_{19}NO_2$: MS (ESI^+): calcd.: [M]=221.1, found: $[M+H]^+$=222.1.

3-(methoxyamino)-3-(*p*-tolyl)propanoic acid (5n): $C_{11}H_{15}NO_3$: MS (ESI^+): calcd.: [M]=209.1, found: $[M+H]^+$=210.1.

3-(methoxyamino)-3-(*m*-tolyl)propanoic acid (6n): $C_{11}H_{15}NO_3$: MS (ESI^+): calcd.: [M]=209.1, found: $[M+H]^+$=210.1.

3-(ethylamino)-3-(4-fluorophenyl)propanoic acid (7b): $C_{11}H_{14}FNO_2$: MS (ESI^+): calcd.: [M]=211.1, found: $[M+H]^+$=212.1.

3-(4-fluorophenyl)-3-(methoxyamino)propanoic acid (7n): $C_{10}H_{12}FNO_3$: MS (ESI^+): calcd.: [M]=213.1, found: $[M+H]^+$=214.1.

3-(3-fluorophenyl)-3-(methoxyamino)propanoic acid (8n): $C_{10}H_{12}FNO_3$: MS (ESI^+): calcd.: [M]=213.1, found: $[M+H]^+$=214.1.

3-(4-chlorophenyl)-3-(ethylamino)propanoic acid (9b): $C_{11}H_{14}ClNO_2$: MS (ESI^+): calcd.: [M]=227.1, found: $[M+H]^+$=228.1.

3-(4-chlorophenyl)-3-(methoxyamino)propanoic acid (9n): $C_{10}H_{12}ClNO_3$: MS (ESI^+): calcd.: [M]=229.1, found: $[M+H]^+$=230.1.

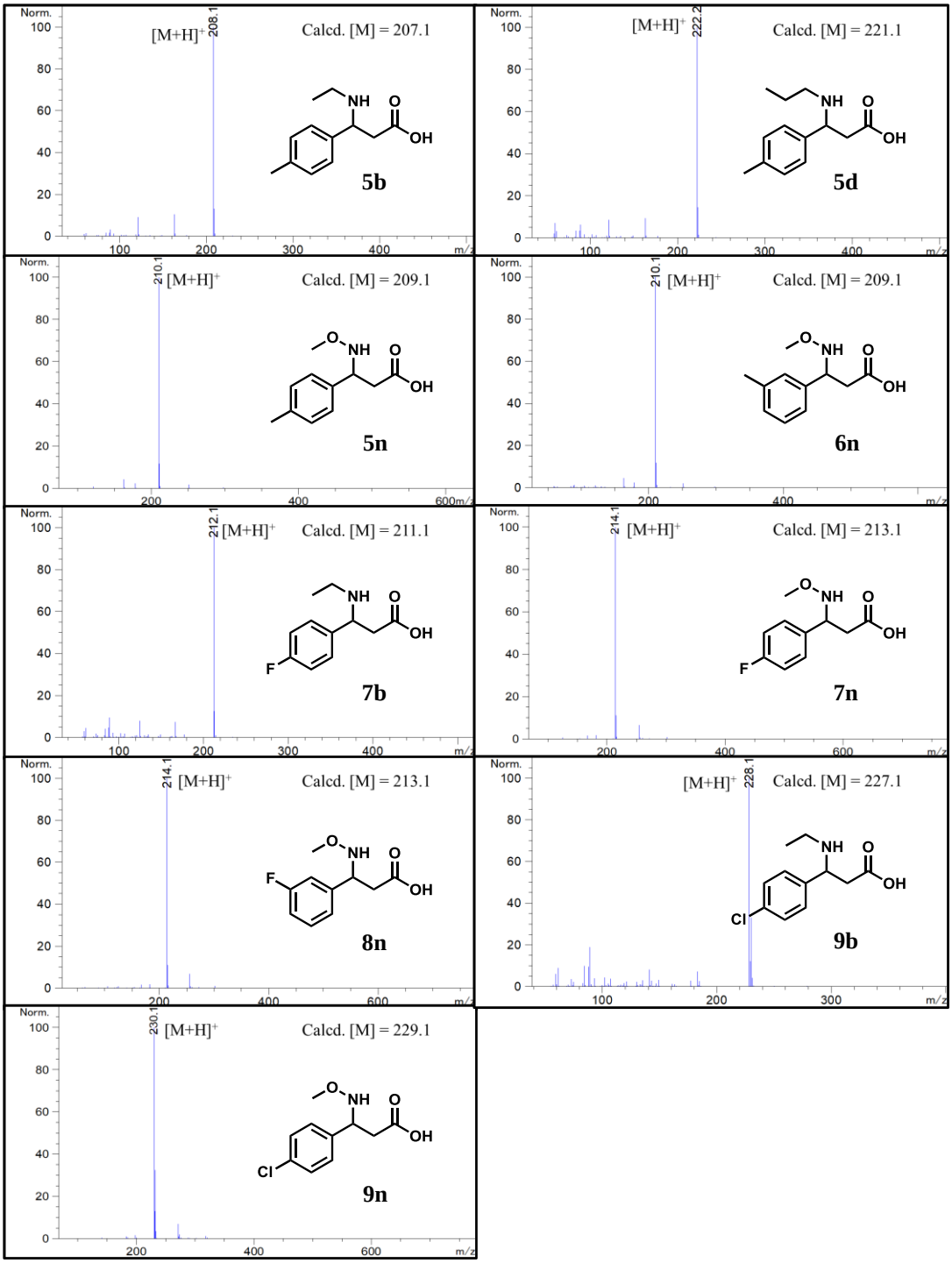
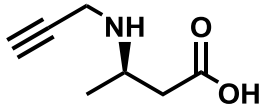


Figure S44. Mass spectrum of **5b**-**9n**.

Ten gram to kilogram scale synthesis of β-amino acids



The reaction consisted of crotonic acid (86 g, 1 mol) and propargylamine (82 g, 1.5 mol) was carried out in 2-L fermenter and the pH of the reaction mixture was adjusted to pH 9.0 with 6 M NaOH. The enzymatic reaction was started by addition of cell (13 g wet weight, approximately 3.2 g dry weight), then the final volume was adjusted to 1 L with the deionized water immediately. The reaction mixture was stirred for 60 minutes at 50 °C. After completion of the reaction, the cells were separated by centrifugation (18,000 g, 30 min). The protein was filtered by ultrafiltration membrane (Dachuan, Tianjin, 10 kD). The filtrate was evaporated to remove the excess propargylamine and desalted with electrodialysis until the conductivity was lower than 1 ms/cm. Then the solution of amino acid were evaporated and crystallized in water. The product was dried and 131 g white crystal was obtained (yield 93%). The structure was confirmed using NMR. ^{1}H NMR (500 MHz, D_2O) δ 4.16 (dd, J = 17.9, 2.6 Hz, 1H), 3.86 – 3.79 (m, 2H), 3.09 (dd, J = 14.5, 5.0 Hz, 1H), 2.54 (ddd, J = 14.5, 2.4, 0.8 Hz, 1H), 2.24 (t, J = 2.6 Hz, 1H), 1.39 (d, J = 6.1 Hz, 3H). ^{13}C NMR (126 MHz, D_2O): δ 177.62, 77.75, 73.23, 51.21, 38.97, 33.76, 15.64.

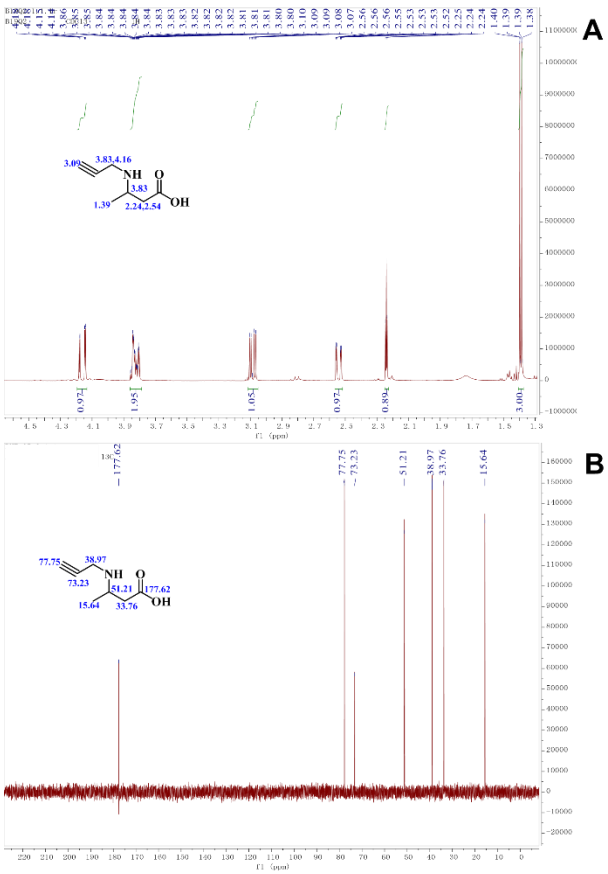
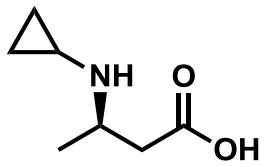


Figure S45. (A) ^{1}H NMR spectrum and (B) ^{13}C NMR spectrum of the product **1i**.



The reaction consisted of crotonic acid (86 g, 1 mol) and cyclopropylamine (69 g, 1.2 mol) was carried out in 2-L fermenter. The enzymatic reaction was started by addition of cell (12.5 g wet weight, approximately 3.2 g dry weight), the final volume was adjusted immediately to 1 L with the deionized water. The reaction mixture was stirred for 6 hours at 50 °C. After completion of the reaction, the cells were separated by centrifugation (18,000 g, 30 min). The protein was filtered by ultrafiltration membrane (Dachuan, Tianjin, 10 kD). Then the solution of amino acid were evaporated and crystallized in water. The product was dried and 130 g white crystal was obtained (yield 91%). The structure was confirmed using NMR. ^{1}H NMR (500 MHz, D_2O) δ 3.49 – 3.39 (m, 1H), 2.55 – 2.45 (m, 2H), 2.33 (dd, J = 15.6, 6.8 Hz, 1H), 1.22 (d, J = 6.6 Hz, 3H), 0.78 – 0.59 (m, 4H). ^{13}C NMR (126 MHz, D_2O): δ 178.91, 52.81, 40.70, 27.49, 17.03, 3.67, 3.47.

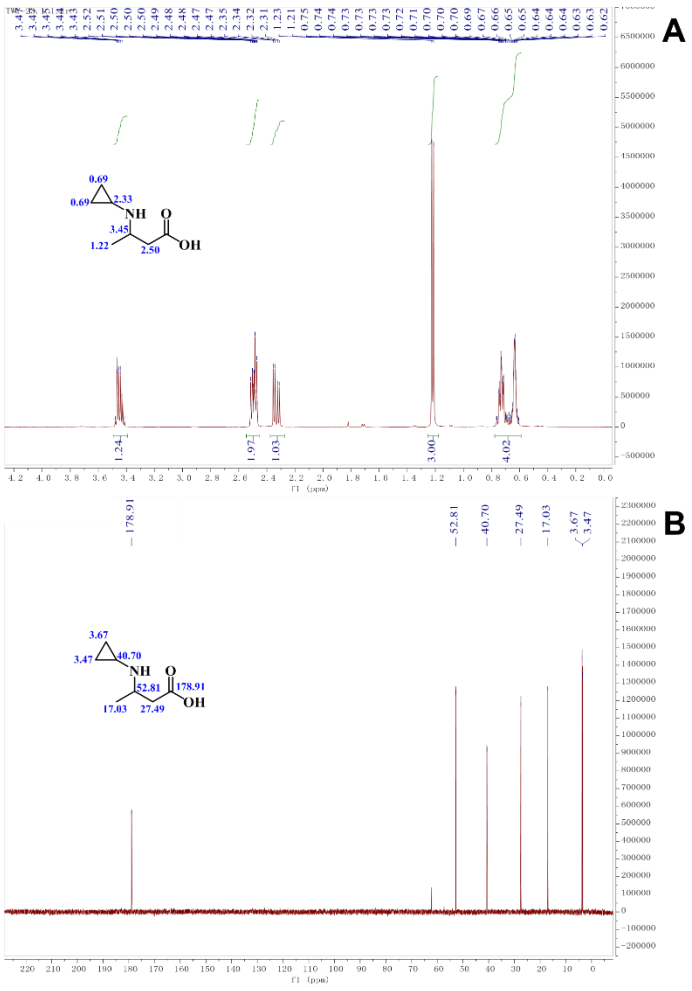
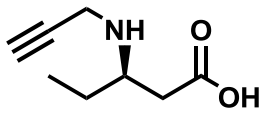


Figure S46. (A) ^{1}H NMR spectrum and (B) ^{13}C NMR spectrum of the product **1j**.



The reaction consisted of (*E*)-2-pentaonic acid (4 g, 40 mmol) and propargylamine (3.3 g, 60 mmol) was carried out in 100 mL shake flask. The enzymatic reaction was started by addition of cell (1.8 g wet weight, approximately 0.4 g dry weight), the final volume was adjusted immediately to 40 mL with the deionized water. The reaction mixture was stirred for 26 hours at 50 °C. After completion of the reaction, the cells were separated by centrifugation (12,000 g, 60 min). The protein was filtered via Amicon filtration (10 kD, Millipore). Then the solution of amino acid were evaporated and crystallized in water. The product was dried and 4.6 g white crystal was obtained (yield 74%). The structure was confirmed using NMR. ^{1}H NMR (500 MHz, D_2O) δ 3.89 (d, J = 2.5 Hz, 2H), 3.49 (ddt, J = 8.3, 6.1, 5.1 Hz, 1H), 2.88 (t, J = 2.6 Hz, 1H), 2.55 (dd, J = 16.8, 5.4 Hz, 1H), 2.43 (dd, J = 16.8, 6.3 Hz, 1H), 1.74 (dtd, J = 15.1, 7.5, 4.9 Hz, 1H), 1.60 (ddd, J = 14.1, 8.2, 7.3 Hz, 1H), 0.90 (t, J = 7.5 Hz, 3H). ^{13}C NMR (126 MHz, D_2O): δ 171.86, 77.79, 73.28, 56.38, 35.43, 33.84, 23.29, 8.73.

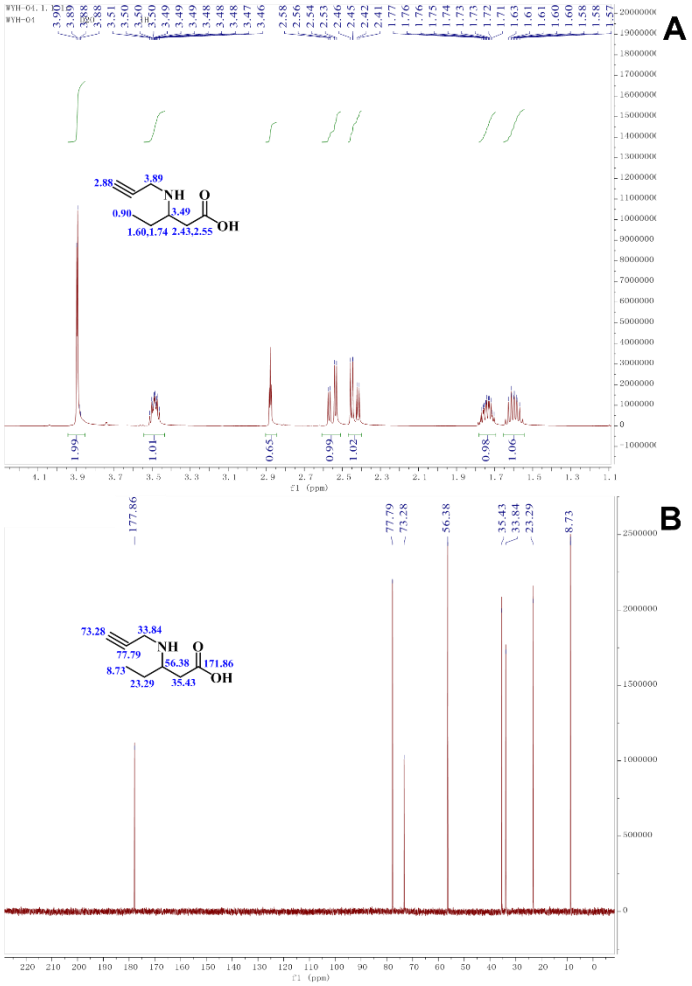
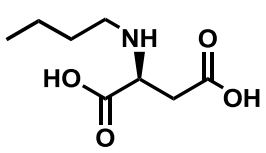


Figure S47. (A) ^{1}H NMR spectrum and (B) ^{13}C NMR spectrum of the product **2i**.



The reaction consisted of fumaric acid (928 g, 8 mol) and *n*-butylamine (872 g, 12 mol) was carried out in 10-L fermenter and the pH of the reaction mixture was adjusted to 9.0 with NaOH powder. The enzymatic reaction was started by addition of cell (120 g wet weight, approximately 26 g dry weight), the final volume was adjusted immediately to 8 L with the deionized water. The reaction mixture was stirred for 6 hours at room temperature. After completion of the reaction, the cells were separated by centrifugation (18,000 g, 30 min). The protein was filtered by ultrafiltration membrane (Dachuan, Tianjin, 10 kD). The filtrate was evaporated to remove the excess *n*-butylamine and desalted with electrodialysis until the conductivity was lower than 1 ms/cm. Then the solution of amino acid were evaporated and crystallized in water. The product was dried and 1402 g white crystal was obtained (yield 92%). The structure was confirmed using NMR. ^{1}H NMR (500 MHz, D_2O) δ 3.85 (dd, J = 6.8, 4.7 Hz, 1H), 3.02 (tt, J = 8.0, 4.1 Hz, 2H), 2.95 – 2.83 (m, 2H), 1.66 – 1.50 (m, 2H), 1.31 (h, J = 7.4 Hz, 2H), 0.84 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, D_2O): δ 174.35, 172.29, 57.94, 46.82, 34.03, 27.47, 19.07, 12.66.

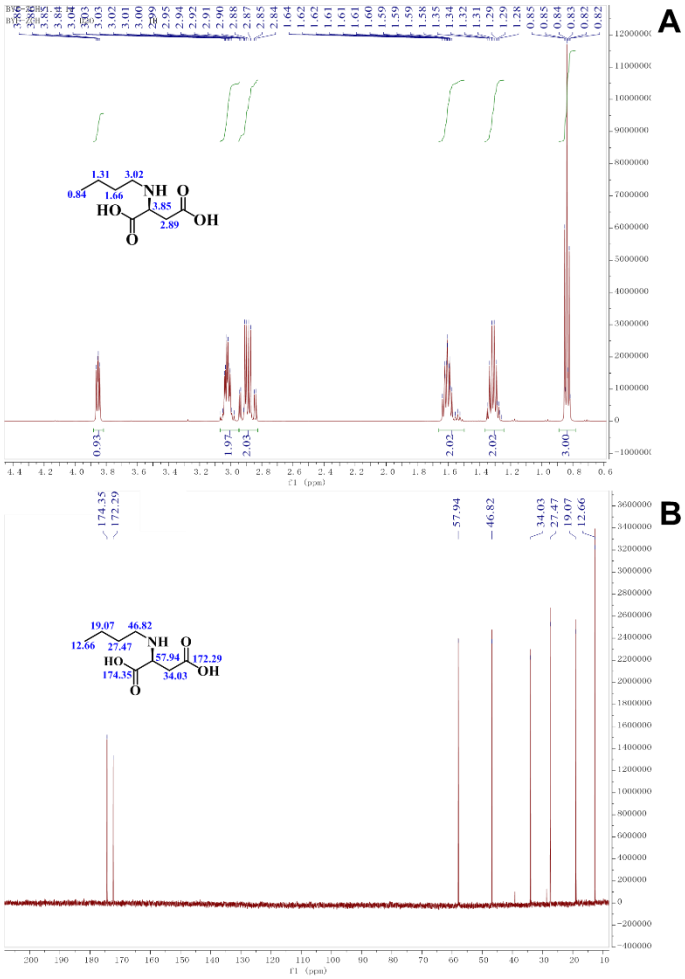
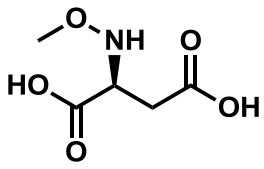


Figure S48. (A) ^{1}H NMR spectrum and (B) ^{13}C NMR spectrum of the product **3f**.



The reaction consisted of fumaric acid (23.2 g, 0.2 mol) and methoxylamine hydrochloride (25 g, 0.3 mol) was carried out in 2-L fermenter and the pH of the reaction mixture was adjusted to 9.0 with NaOH powder. The enzymatic reaction was started by addition of cell (2.4 g wet weight, approximately 0.8 g dry weight), the final volume was adjusted immediately to 0.29 L with the deionized water. The reaction mixture was stirred for 2 hours at 50 °C. After completion of the reaction, the cells were separated by centrifugation (18,000 g, 30 min). The protein was filtered by ultrafiltration membrane (Dachuan, Tianjin, 10 kD). The filtrate was evaporated to remove the excess methoxylamine and desalted with electrodialysis until the conductivity was lower than 1 ms/cm. Then the solution of amino acid were evaporated and lyophilized without further purification. The product of 25 g yellow powder was obtained (yield 78%). The structure was confirmed using NMR. ^{1}H NMR (500 MHz, D_2O): δ 4.03 (dd, J = 6.9, 5.8 Hz, 1H), 3.58 (s, 3H), 2.82 (dd, J = 16.9, 5.8 Hz, 1H), 2.69 (dd, J = 16.8, 6.9 Hz, 1H). ^{13}C NMR (126 MHz, D_2O): δ 175.23, 174.18, 61.51, 60.01, 33.73.

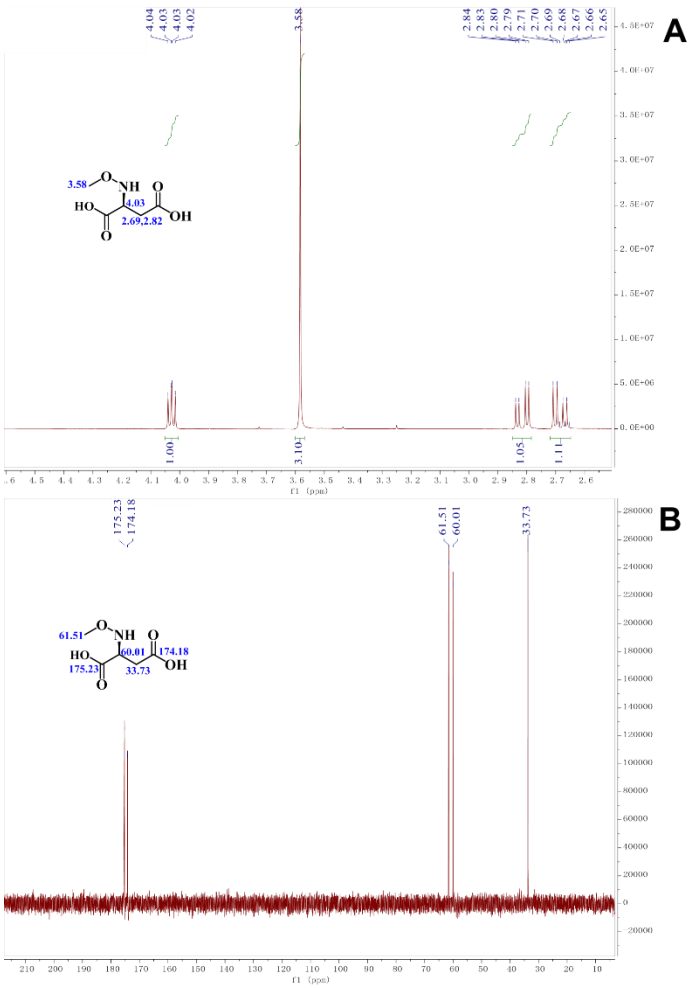
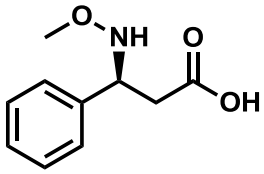


Figure S49. (A) ^{1}H NMR spectrum and (B) ^{13}C NMR spectrum of the product **3n**.



The reaction consisted of cinnamic acid (7.5 g, 50 mmol) and methoxylamine hydrochloride (84 g, 1 mol) was carried out in 2-L fermenter and the pH of the reaction mixture was adjusted to 8.5 with NaOH powder. The enzymatic reaction was started by addition of cell (45 g wet weight, approximately 9.6 g dry weight), the final volume was adjusted immediately to 1 L with the deionized water. The reaction mixture was stirred at 50 °C, the same amount of cell were added for every 12 hours, and the total reaction time was 72 h. After completion of the reaction, the cells were separated by centrifugation (18,000 g, 60 min). The protein was filtered by ultrafiltration membrane (Dachuan, Tianjin, 10 kD). The filtrate was evaporated to remove the excess methoxylamine and desalted with electrodialysis until the conductivity was lower than 1 ms/cm. Then the solution of amino acid were evaporated and lyophilized without further purification. The product of 7.4 g yellow powder was obtained (yield 75%). The structure was confirmed using NMR. ^{1}H NMR (500 MHz, D_2O) δ 7.42 – 7.29 (m, 5H), 4.47 (t, J = 7.3 Hz, 1H), 3.45 (s, 3H), 2.87 (dd, J = 15.5, 6.9 Hz, 1H), 2.69 (dd, J = 15.5, 7.6 Hz, 1H). ^{13}C NMR (126 MHz, D_2O): δ 176.28, 128.88, 128.88, 128.61, 127.73, 127.73, 61.13, 60.92, 38.15.

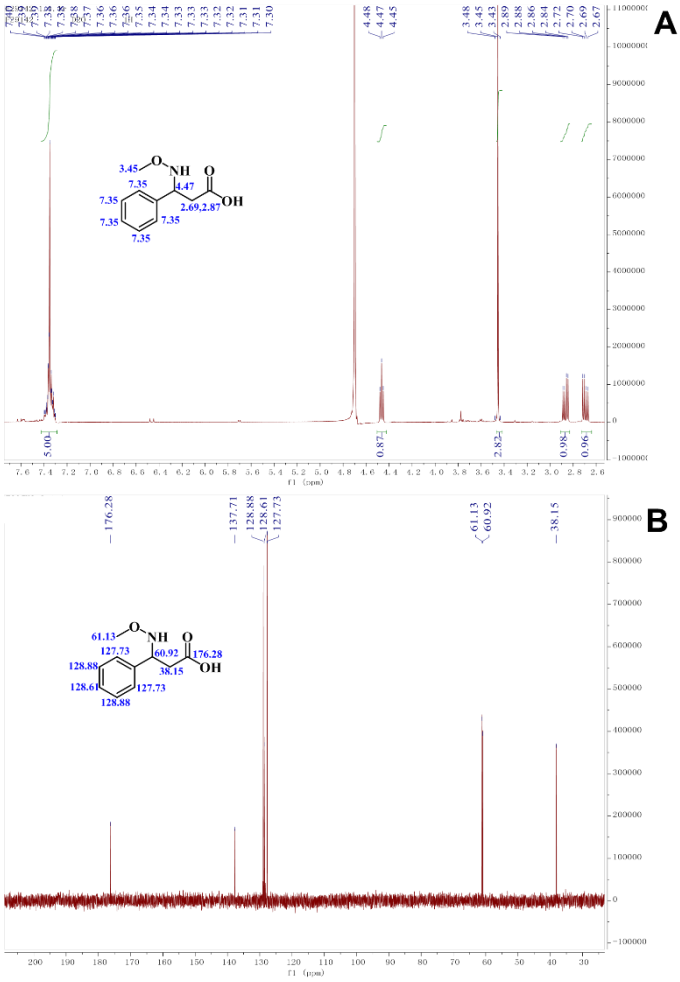


Figure S50. (A) ^{1}H NMR spectrum and (B) ^{13}C NMR spectrum of the product **4n**.

General procedure for one-pot chemoenzymatic synthesis of β-lactam compounds

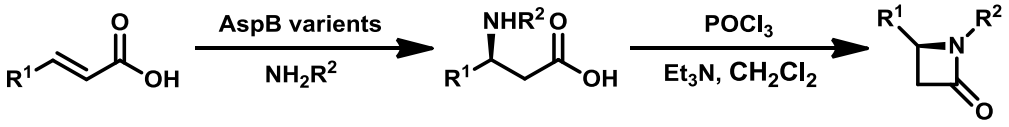
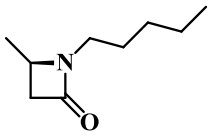


Figure S51. Reaction route of one-pot synthesis of β-lactam compounds.

The reaction conditions of preparation of β-amino acid were as described above. Then the reaction solutions were subsequently vacuum evaporated to remove water. The residues were resolved in 270 mL or 27 mL methylene chloride. Triethylamine (4.5 mL or 0.45 mL) and phosphorus oxychloride (3.05 mL or 0.3 mL) were added to start the reaction[8]. The reaction mixture was stirred for 12 h at room temperature and washed with $NaHCO_3$ (100 g/L, 250 mL or 25 mL) and saturated NaCl solutions (200 mL or 20 mL for twice). β-Lactam compounds were obtained after drying over anhydrous Na_2SO_4 and evaporation of the solvent. The structure was confirmed using MS and NMR. The enantiomeric excess of **1jc** was determined by HPLC analysis.



1-pentyl-4-methyl-2-azacyclobutanone (1gc): 70 mg (0.46 mmol) brown oil liquid was obtained from 86 mg (1 mmol) unsaturated acid (yield 46%). $C_9H_{17}NO$: MS (ESI^+): calcd.: [M]=155.1, found: $[M+H]^+$=156.1. ^1H NMR (500 MHz, $CDCl_3$) δ 3.71 – 3.61 (m, 1H), 3.30 (dt, J = 14.1, 7.6 Hz, 1H), 3.08 – 2.94 (m, 2H), 2.48 (dd, J = 14.5, 2.2 Hz, 1H), 1.58 – 1.48 (m, 2H), 1.31 (dd, J = 6.3, 2.8 Hz, 7H), 0.90 (t, J = 7.1 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$): δ166.89, 47.15, 43.79, 40.24, 29.24, 27.80, 22.27, 18.70, 13.97.

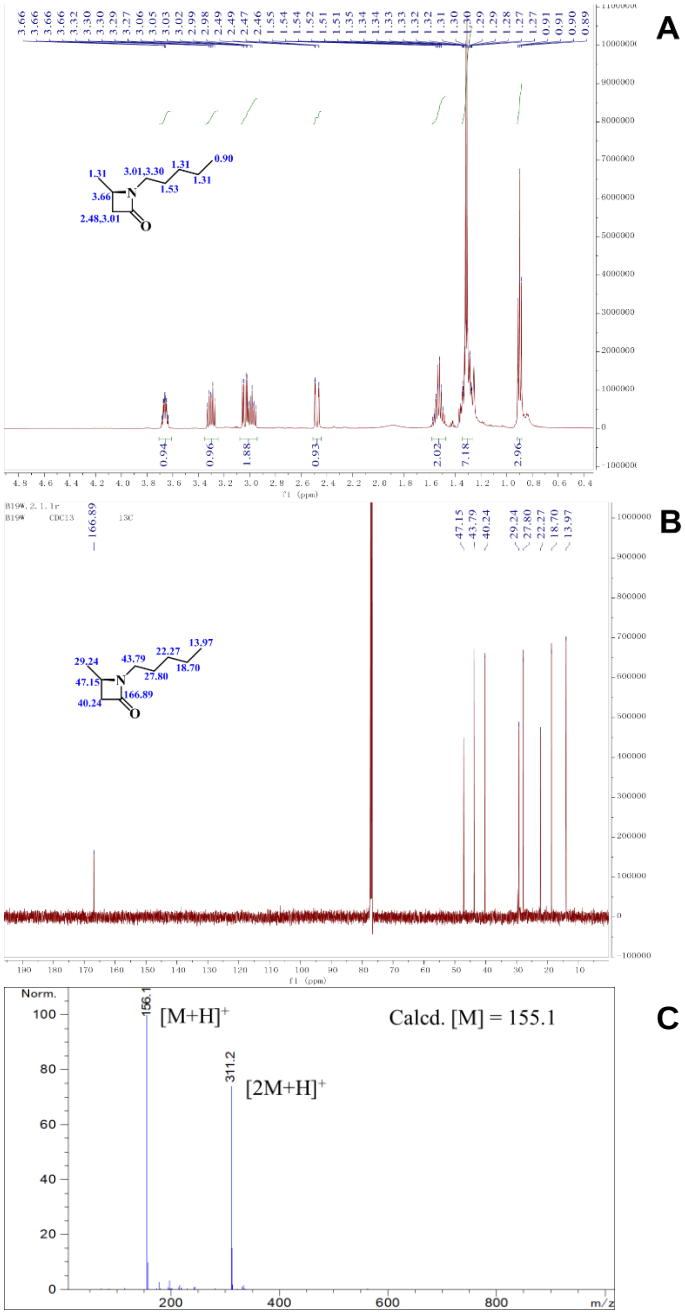
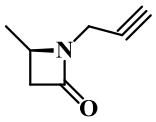


Figure S52. (A) ^1H NMR spectrum, (B) ^{13}C NMR spectrum and (C) MS spectrum of the product **1gc**.



1-propargyl-4-methyl-2-azacyclobutanone (1ic): 0.73 g (5.9 mmol) brown oil liquid was obtained from 0.86 g (10 mmol) unsaturated acid (yield 59%). C_7H_9NO: MS (ESI^+): calcd.: [M]=123.1, found: $[M+H]^+$=124.0. ^1H NMR (500 MHz, $CDCl_3$) δ 4.16 (dd, J = 17.9, 2.5 Hz, 1H), 3.86 – 3.80 (m, 2H), 3.09 (dd, J = 14.5, 5.0 Hz, 1H), 2.56 – 2.51 (m, 1H), 2.24 (t, J = 2.6 Hz, 1H), 1.40 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 166.10, 78.92, 74.77, 47.52, 44.32, 29.52, 18.86.

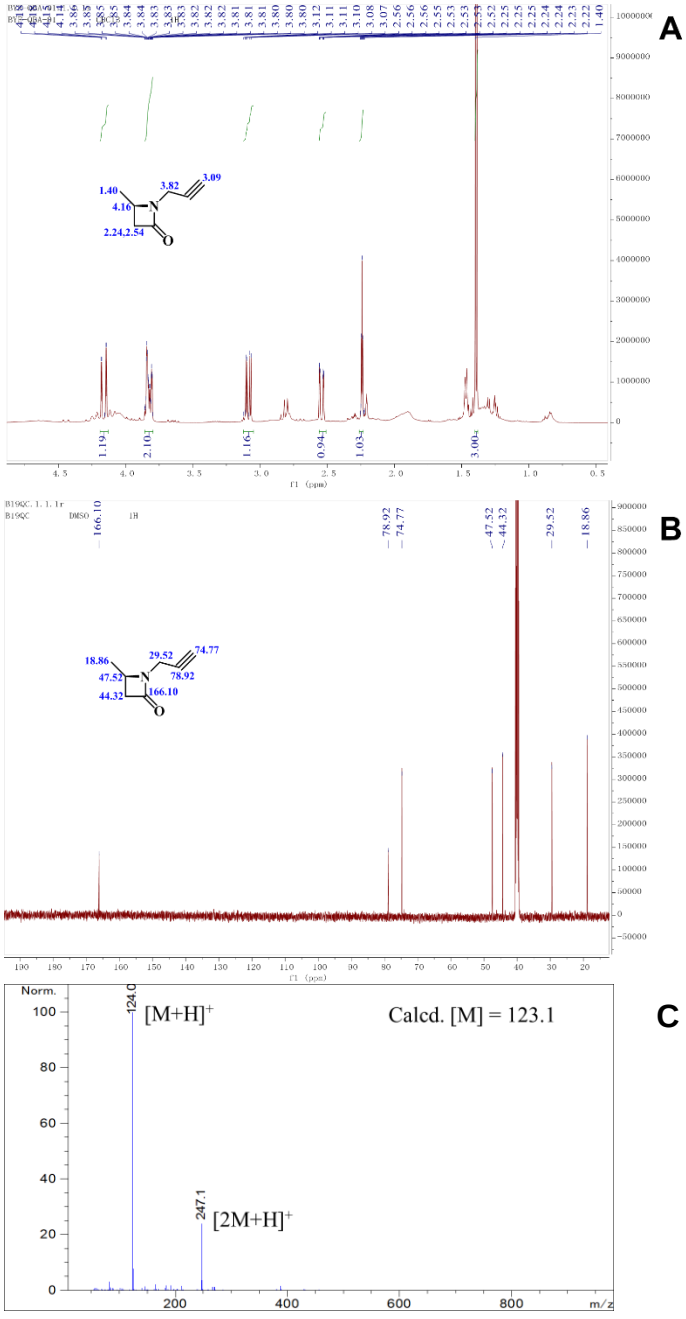
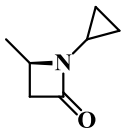


Figure S53. (A) ^1H NMR spectrum, (B) ^{13}C NMR spectrum and (C) MS spectrum of the product **1ic**..



1-cyclopropyl-4-methyl-2-azacyclobutanone (1jc): 0.78 g (6.3 mmol) brown oil liquid was obtained from 0.86 g (10 mmol) unsaturated acid (yield 63%). $C_7H_{11}NO$: MS (ESI^+): calcd.: [M]=125.1, found: $[M+H]^+$=126.1. ^1H NMR (500 MHz, $CDCl_3$) δ 3.63 – 3.54 (m, 1H), 2.95 (ddd, J = 14.7, 5.0, 1.1 Hz, 1H), 2.45 – 2.37 (m, 2H), 1.35 (d, J = 6.1 Hz, 3H), 0.89 – 0.65 (m, 4H). ^{13}C NMR (126 MHz, $CDCl_3$): δ167.63, 48.16, 43.40, 22.28, 18.95, 5.38, 4.14. e.e.: 99%

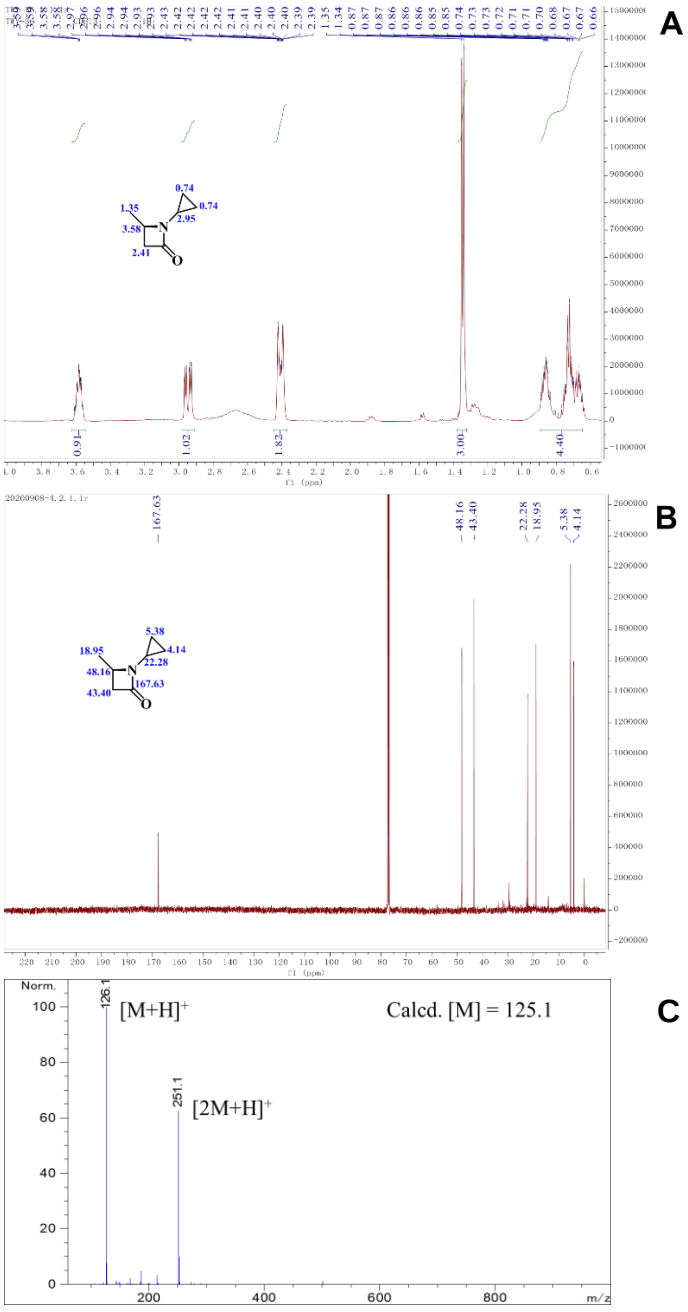


Figure S54. (A) ^1H NMR spectrum, (B) ^{13}C NMR spectrum and (C) MS spectrum of the product **1jc**.

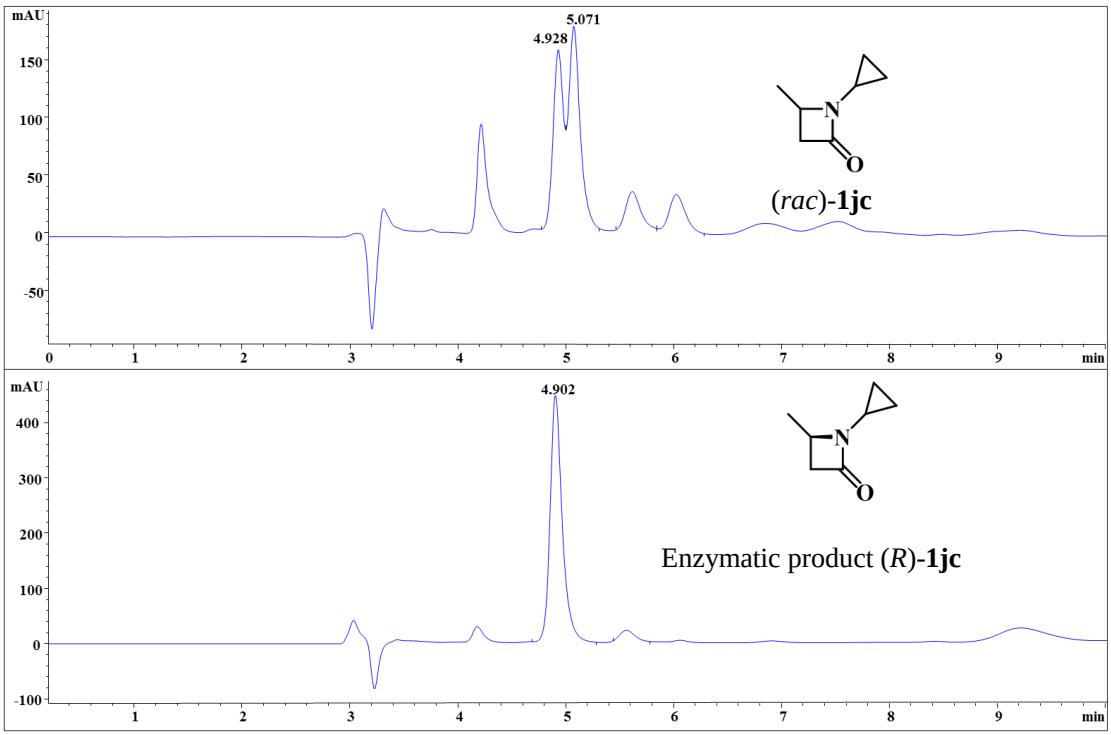
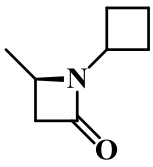


Figure S55. Chiral HPLC analysis of **1jc**. Chiral HPLC conditions: Chiralpak IG column (5 µm, 250×4.6 mm, Daicel). Phase A: 0.1% formic acid in water, phase B: acetonitrile, A:B = 60:40 (v/v). Flow rate 1.0 mL/min, 25 °C, UV detection at 220 nm.



1-cyclobutyl-4-methyl-2-azacyclobutanone (1kc): 99 mg (0.71 mmol) brown oil liquid was obtained from 86 mg (1.0 mmol) unsaturated acid (yield 71%). $C_8H_{13}NO$: MS (ESI^+): calcd.: [M]=139.1, found: $[M+H]^+$=140.1. ^1H NMR (500 MHz, $CDCl_3$) δ 4.05 (ttd, J = 9.0, 7.7, 1.0 Hz, 1H), 3.77 – 3.70 (m, 1H), 2.98 (dd, J = 14.5, 5.0 Hz, 1H), 2.42 (dd, J = 14.4, 2.3 Hz, 1H), 2.37 – 2.22 (m, 2H), 2.23 – 2.12 (m, 2H), 1.78 – 1.63 (m, 2H), 1.38 (d, J = 6.1 Hz, 3H).^{13}C NMR (126 MHz, $CDCl_3$): δ166.45, 46.77, 46.67, 43.33, 29.03, 28.30, 29.03, 28.30, 20.37, 15.49.

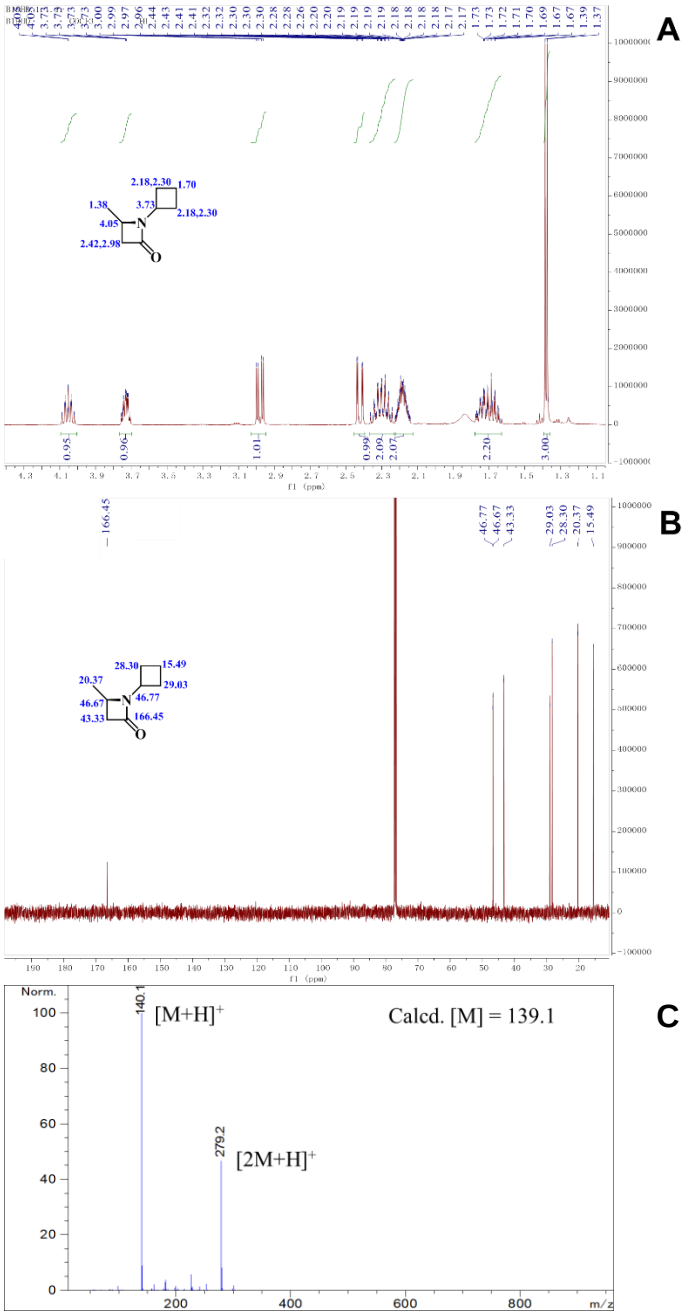
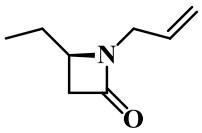


Figure S56. (A) ^1H NMR spectrum, (B) ^{13}C NMR spectrum and (C) MS spectrum of the product **1kc**.



1-allyl-4-ethyl-2-azacyclobutanone (2hc): 82 mg (0.58 mmol) brown oil liquid was obtained from 100 mg (1.0 mmol) unsaturated acid (yield 58%). $C_8H_{13}NO$: MS (ESI^+): calcd.: [M]=139.1, found: $[M+H]^+$=140.1. ^1H NMR (500 MHz, $CDCl_3$): δ 5.77 (m, J = 17.0, 10.2, 6.8, 5.5 Hz, 1H), 5.25 – 5.14 (m, 2H), 3.99 (ddt, J = 15.7, 5.5, 1.6 Hz, 1H), 3.67 – 3.59 (m, 1H), 3.53 (dtd, J = 8.8, 4.6, 2.3 Hz, 1H), 2.99 (dd, J = 14.5, 4.9 Hz, 1H), 2.55 (dd, J = 14.6, 2.3 Hz, 1H), 1.83 (dqd, J = 13.7, 7.5, 4.2 Hz, 1H), 1.50 – 1.41 (m, 1H), 0.93 (t, J = 7.5 Hz, 3H).^{13}C NMR (126 MHz, $CDCl_3$): δ 167.11, 132.38, 118.12, 52.79, 43.35, 41.69, 25.81, 9.39.

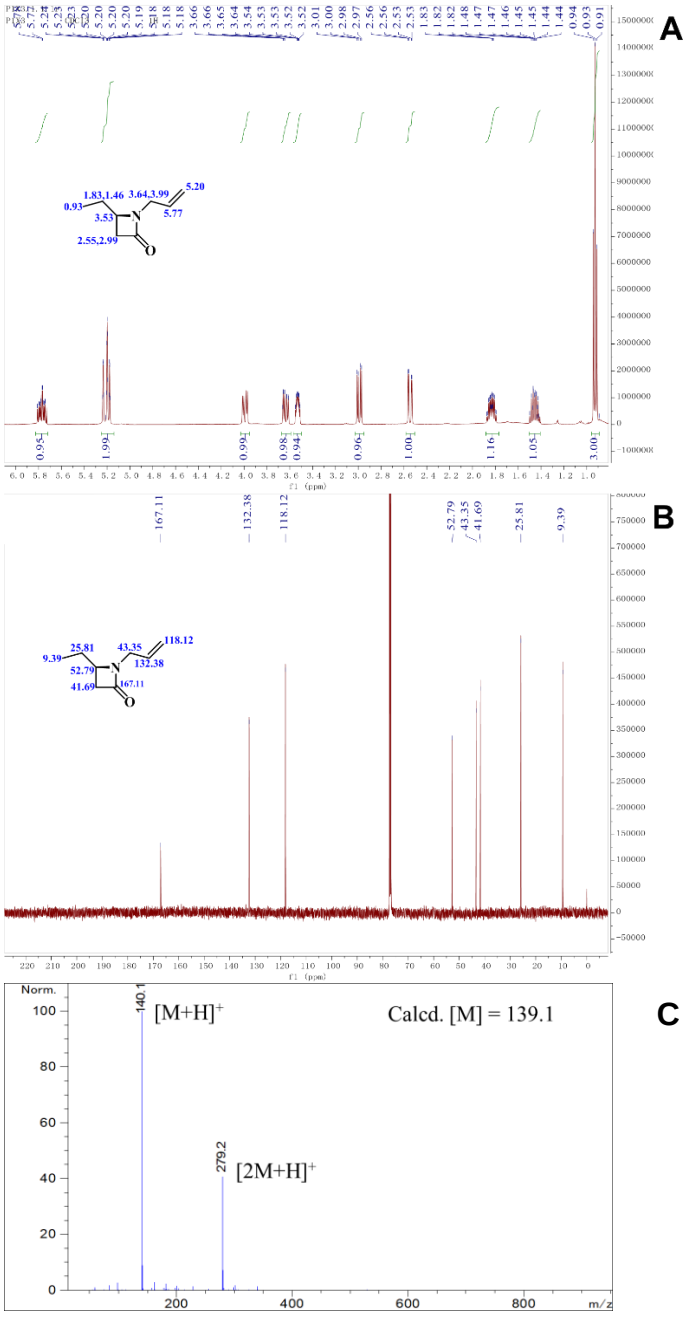
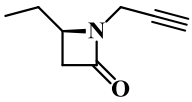


Figure S57. (A) ^1H NMR spectrum, (B) ^{13}C NMR spectrum and (C) MS spectrum of the product **2hc**.



1-propargyl-4-ethyl-2-azacyclobutanone (2ic): 0.89 g (6.4 mmol) brown oil liquid was obtained from 1.00 g (10 mmol) unsaturated acid (yield 64%). $C_8H_{11}NO$: MS (ESI^+): calcd.: [M]=137.1, found: $[M+H]^+$=138.1. ^1H NMR (500 MHz, $CDCl_3$) δ 4.19 (dd, J = 17.9, 2.6 Hz, 1H), 3.83 (ddd, J = 17.9, 2.6, 0.9 Hz, 1H), 3.71 – 3.65 (m, 1H), 3.00 (dd, J = 14.5, 5.1 Hz, 1H), 2.57 (ddd, J = 14.5, 2.5, 0.9 Hz, 1H), 2.25 (t, J = 2.6 Hz, 1H), 1.92 (dqd, J = 13.8, 7.5, 4.5 Hz, 1H), 1.60 – 1.50 (m, 1H), 0.97 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, D_2O): δ 166.40, 78.92, 74.81, 52.87, 41.82, 29.94, 25.67, 9.68.

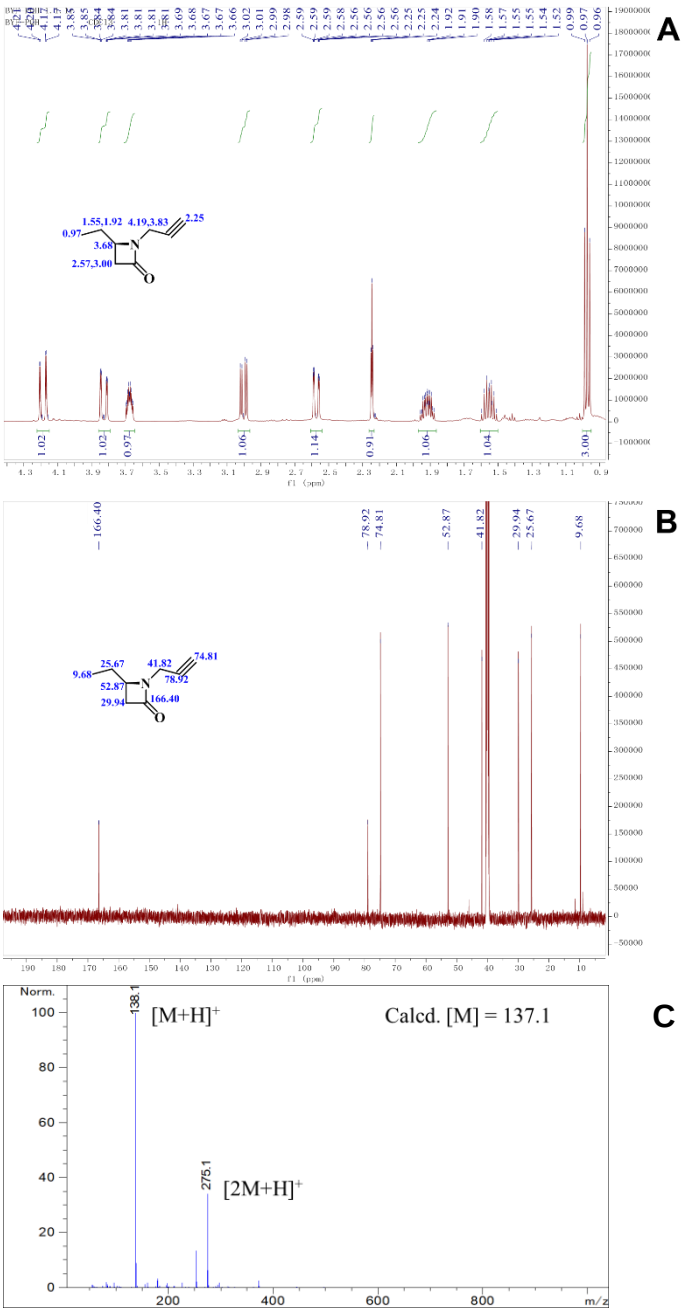
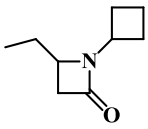


Figure S58. (A) ^1H NMR spectrum, (B) ^{13}C NMR spectrum and (C) MS spectrum of the product **2ic**.



1-cyclobutyl-4-ethyl-2-azacyclobutanone (2kc): 87 mg (0.57 mmol) brown oil liquid was obtained from 100 mg (1.0 mmol) unsaturated acid (yield 57%). $C_9H_{15}NO$: MS (ESI^+): calcd.: [M]=153.1, found: $[M+H]^+$=154.1. ^1H NMR (500 MHz, $CDCl_3$) δ 4.03 (ttd, J = 9.1, 7.8, 1.0 Hz, 1H), 3.57 (m, J = 8.8, 5.0, 3.8, 2.3 Hz, 1H), 2.89 (dd, J = 14.5, 5.0 Hz, 1H), 2.46 (dd, J = 14.5, 2.4 Hz, 1H), 2.39 – 2.23 (m, 2H), 2.22 – 2.14 (m, 2H), 1.94 – 1.89 (m, 1H), 1.72 – 1.63 (m, 2H), 1.55 – 1.48 (m, 1H), 0.93 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 166.70, 52.28, 46.93, 40.82, 29.13, 28.40, 26.88, 15.46, 9.17.

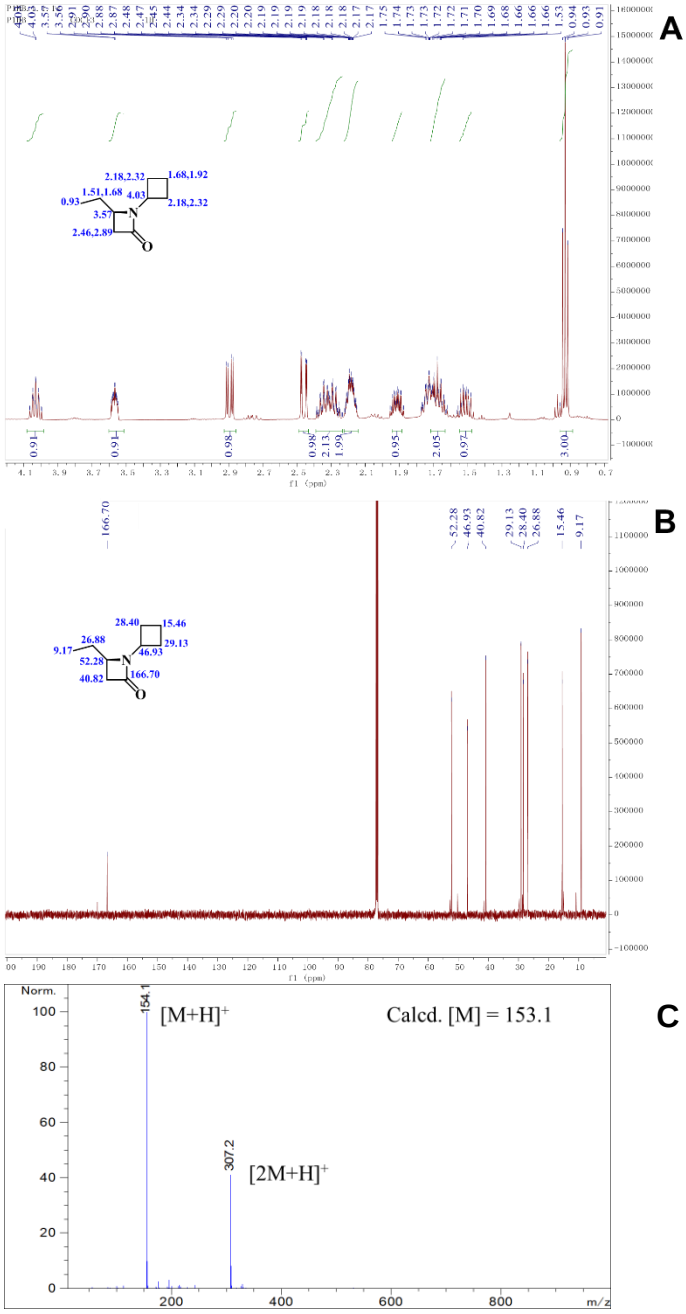


Figure S59. (A) ^1H NMR spectrum, (B) ^{13}C NMR spectrum and (C) MS spectrum of the product **2kc**.

Click reaction for β-lactam compound

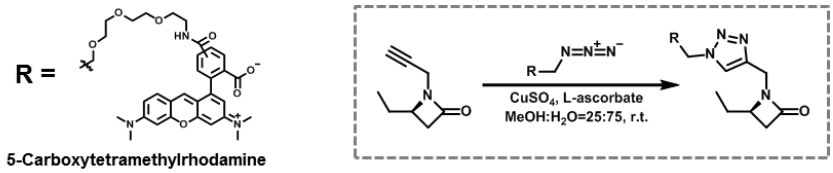


Figure S60. Reaction route of click reaction.

5 μL of 2 mM $CuSO_4$ solution and 5 μL of 300 mM sodium ascorbate solution were pipetted into a 1.5-mL Eppendorf tube and mixed thoroughly. Then 5 μL of 1 mg/mL 1-propargyl-4-ethyl-2-azacyclobutanone (**2ic**) solution (dissolved in methanol) and 5 μL of 10 mg/mL Azide-fluor 545® solution (2.2 equiv., Sigma-Aldrich, two isomers) were added into the mixture. The reaction was carried out at room temperature for 3 h.

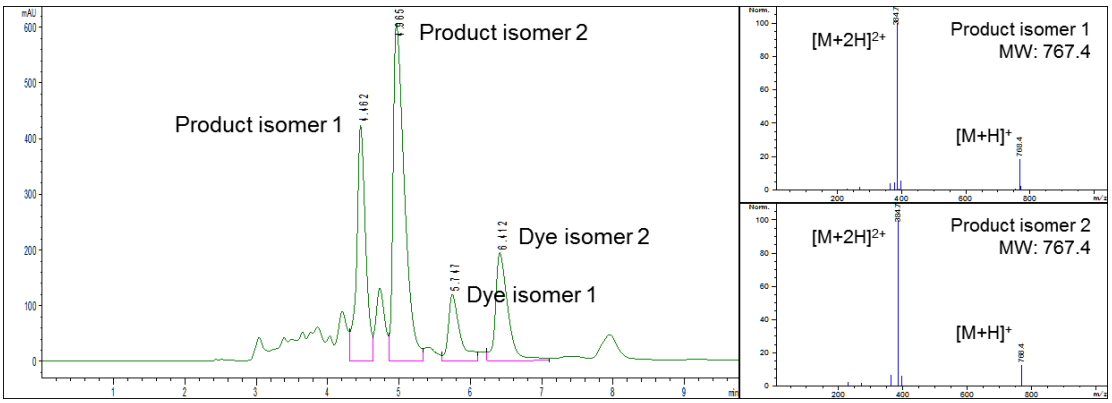


Figure S61. The compounds were identified by LC-MS. Detection method: column: C18 reversed-phase column (4.6×250mm, pore size: 120 Å, particle size 5 μm, Nanochrom, Suzhou, China); temperature: 25°C; eluent A: 0.1% formic acid in water, eluent B: acetonitrile, A:B=65:35; flow rate: 1 mL·min^{-1}; detection: visible light at 545 nm; ESI-MS: positive scan mode (m/z 50~1000).

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