Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

SUPPORTING INFORMATION

The broad amine scope of pantothenate synthetase enables the synthesis of pharmaceutically relevant amides

Mohammad Z. Abidin,[a,b] Thangavelu Saravanan,*[a,c] Erick Strauss,[d] and Gerrit J. Poelarends*[a]

[a.] *Department of Chemical and Pharmaceutical Biology, Groningen Research Institute of Pharmacy, University of Groningen, Antonius Deusinglaan 1, 9713 AV Groningen, The Netherlands. E-mail: g.j.poelarends@rug.nl*
[b.] *Department of Animal Products Technology, Gadjah Mada University, Bulaksumur, Yogyakarta 55281, Indonesia.*
[c.] *School of Chemistry, University of Hyderabad, P.O. Central University, Hyderabad 500046, India. E-mail: tsaravanan@uohyd.ac.in*
[d.] *Department of Biochemistry, Stellenbosch University, Private Bag X1, Matieland, 7602, South Africa.*

1. Monitoring the enzymatic reaction progress by ^{1}H NMR analysis

^{1}H NMR spectra were recorded in D_2O at 0 h (**A**) and 24 h (**B**) after the start of the reaction. Only the key signals that were used to calculate the conversion are labelled. For better representation of the NMR spectra, the signals for Tris buffer (~3.6 ppm) and ATP/AMP (between 4.2 - 8.6 ppm) were not labelled in each spectrum.

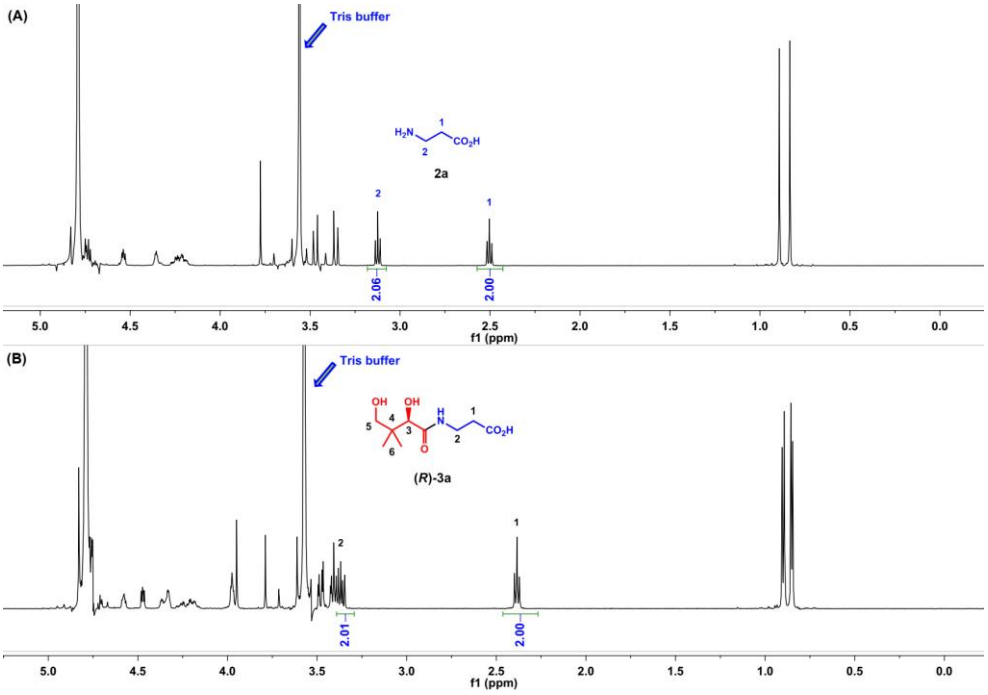


Figure S1. ^{1}H NMR spectra monitoring the $PS_{E.coli}$ catalyzed condensation of (*R*)-**1** and **2a** yielding (*R*)-**3a**

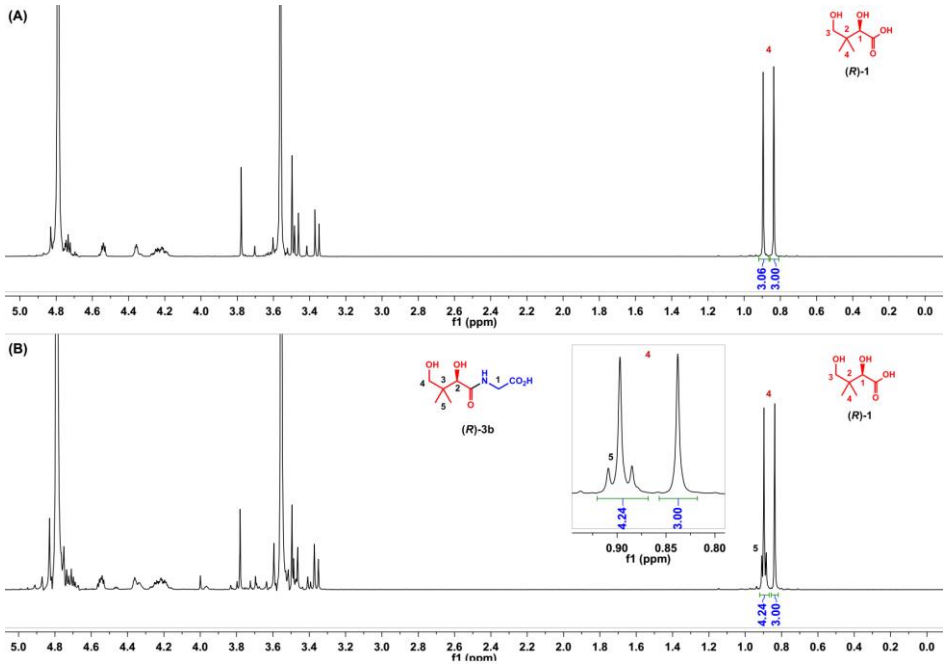


Figure S2. ^{1}H NMR spectra monitoring the $PS_{E.coli}$ catalyzed condensation of (*R*)-**1** and **2b** yielding (*R*)-**3b**

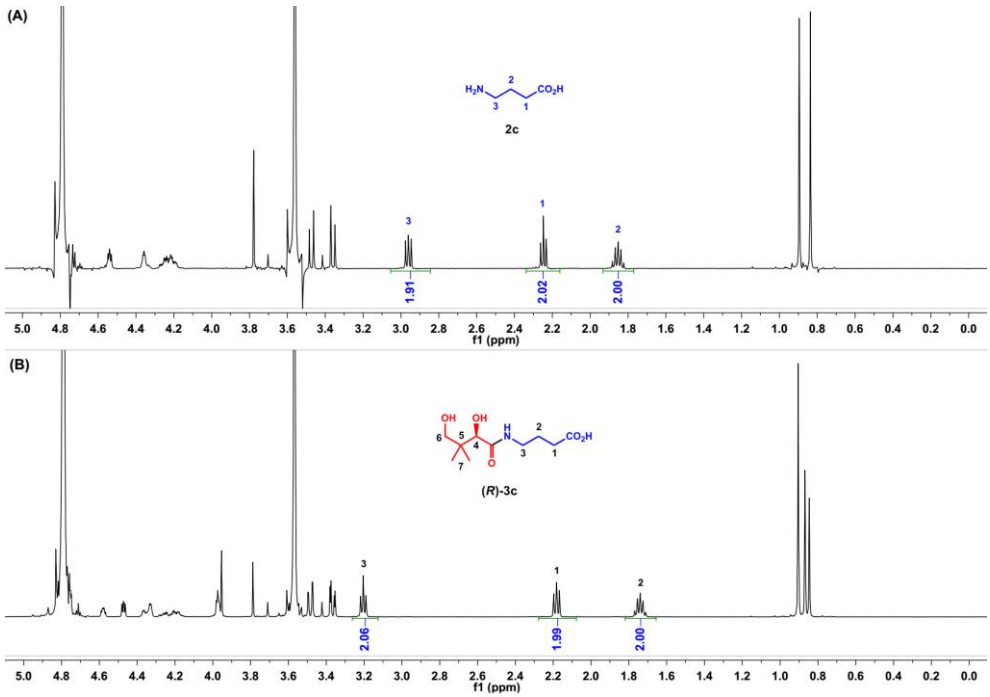


Figure S3. ^{1}H NMR spectra monitoring the $PS_{E.coli}$ catalyzed condensation of (*R*)-**1** and **2c** yielding (*R*)-**3c**

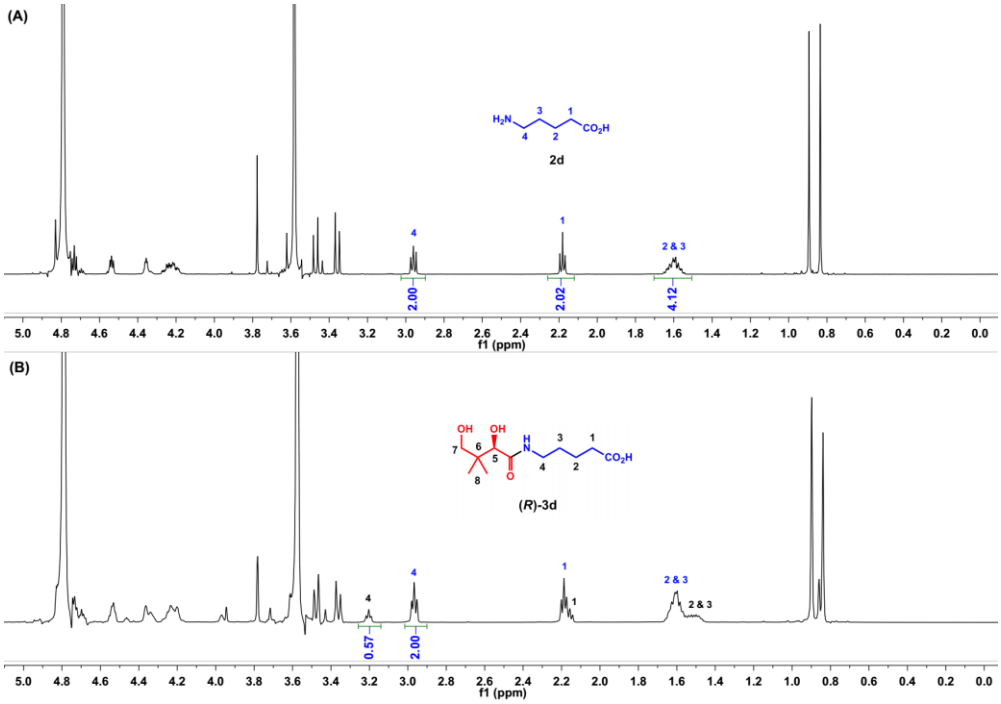


Figure S4. ^{1}H NMR spectra monitoring the $PS_{E.coli}$ catalyzed condensation of (*R*)-**1** and **2d** yielding (*R*)-**3d**

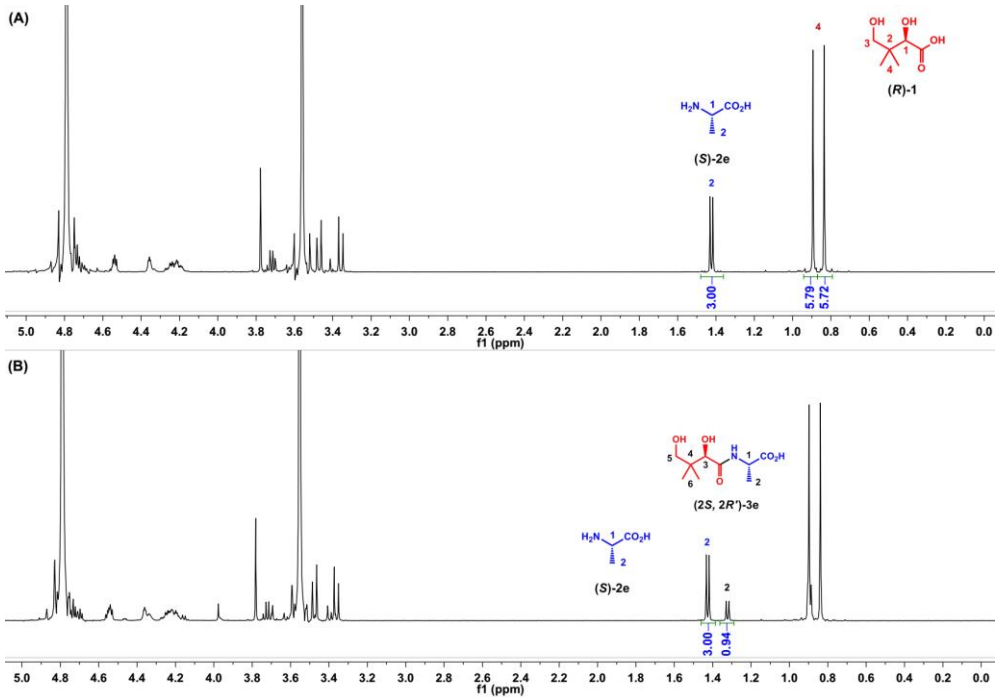


Figure S5. ^{1}H NMR spectra monitoring the $PS_{E.coli}$ catalyzed condensation of (*R*)-**1** and (*S*)-**2e** yielding (2*S*, 2*R'*)-**3e**

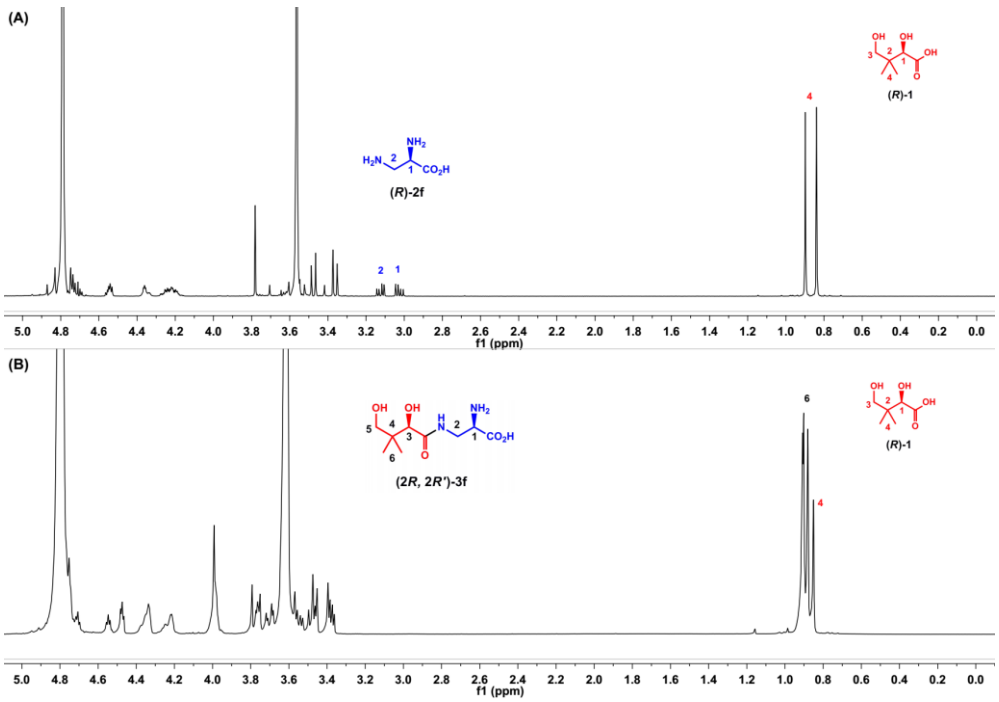


Figure S6. ^{1}H NMR spectra monitoring the $PS_{E.coli}$ catalyzed condensation of (*R*)-**1** and (*R*)-**2f** yielding (2*R*, 2*R'*)-**3f**

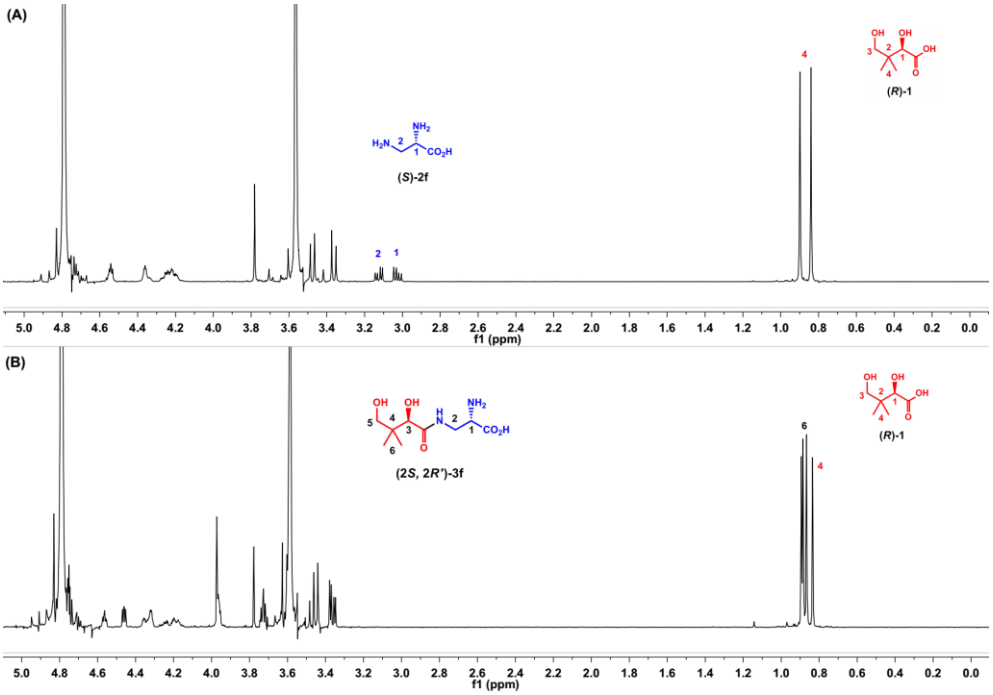


Figure S7. ^{1}H NMR spectra monitoring the $PS_{E.coli}$ catalyzed condensation of (*R*)-**1** and (*S*)-**2f** yielding (2*S*, 2*R*')-3f

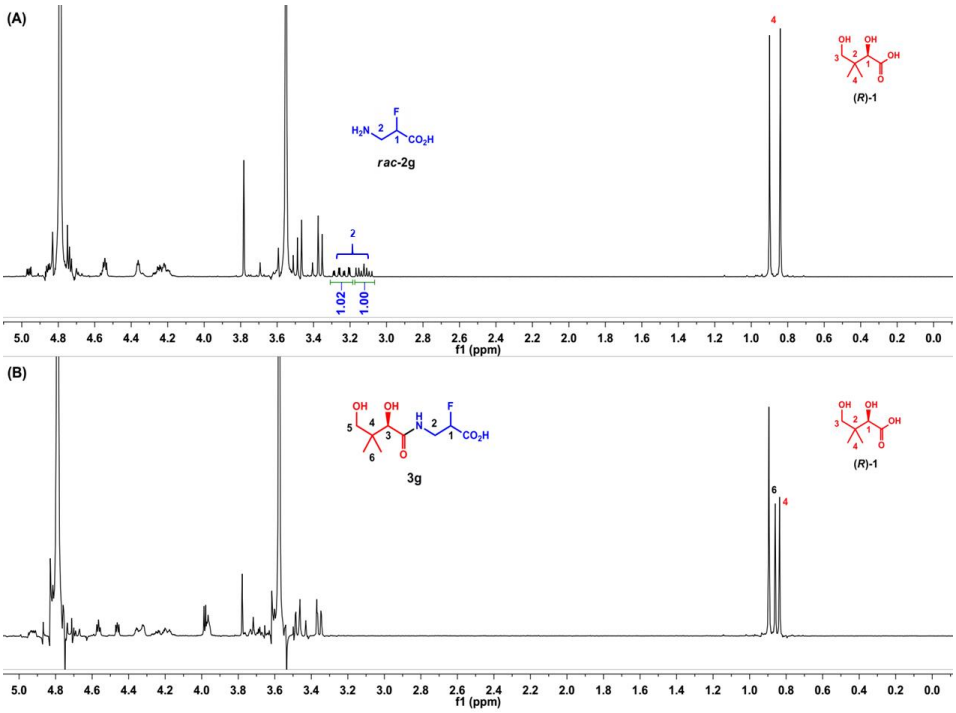


Figure S8. ^{1}H NMR spectra monitoring the $PS_{E.coli}$ catalyzed condensation of (*R*)-**1** and *rac*-**2g** yielding **3g**

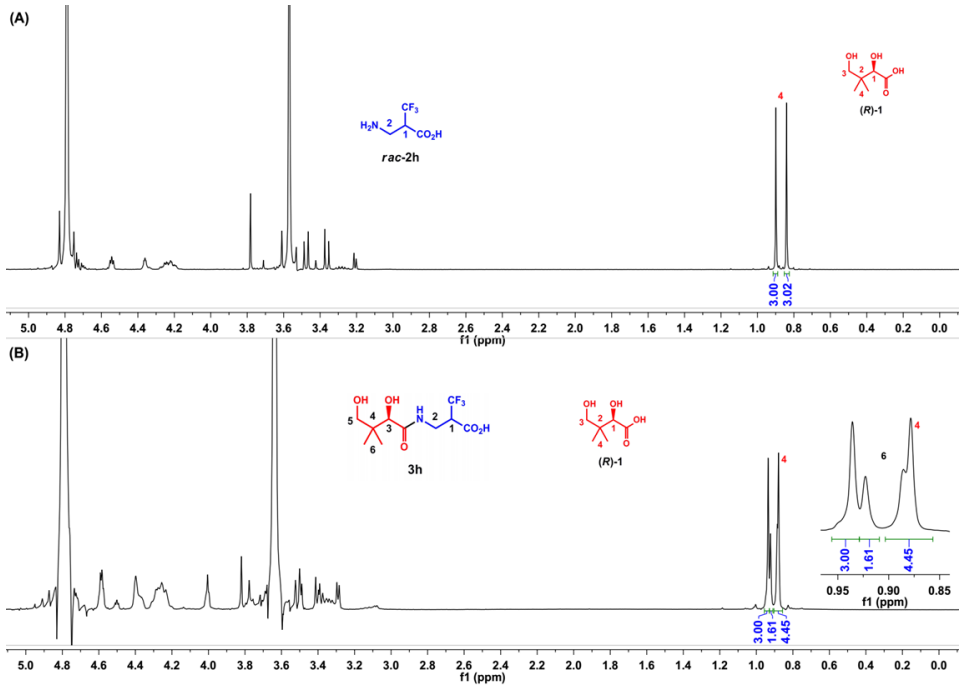


Figure S9. ^{1}H NMR spectra monitoring the $PS_{E.coli}$ catalyzed condensation of (*R*)-**1** and *rac*-**2h** yielding **3h**

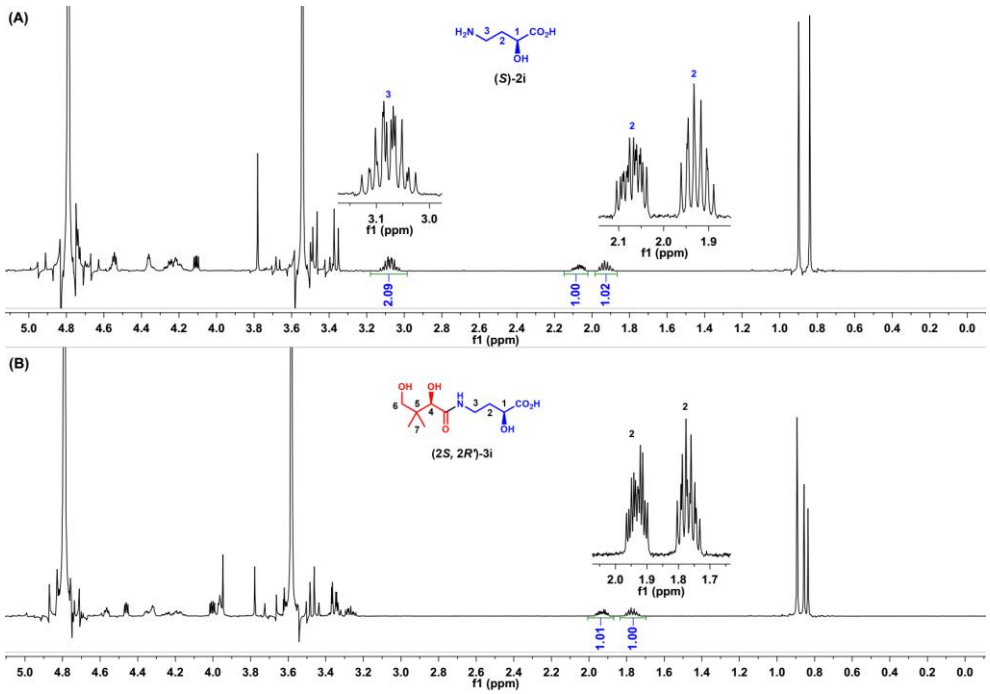


Figure S10. ^{1}H NMR spectra monitoring the $PS_{E.coli}$ catalyzed condensation of (*R*)-**1** and (*S*)-**2i** yielding (2*S*, 2*R'*)-**3i**

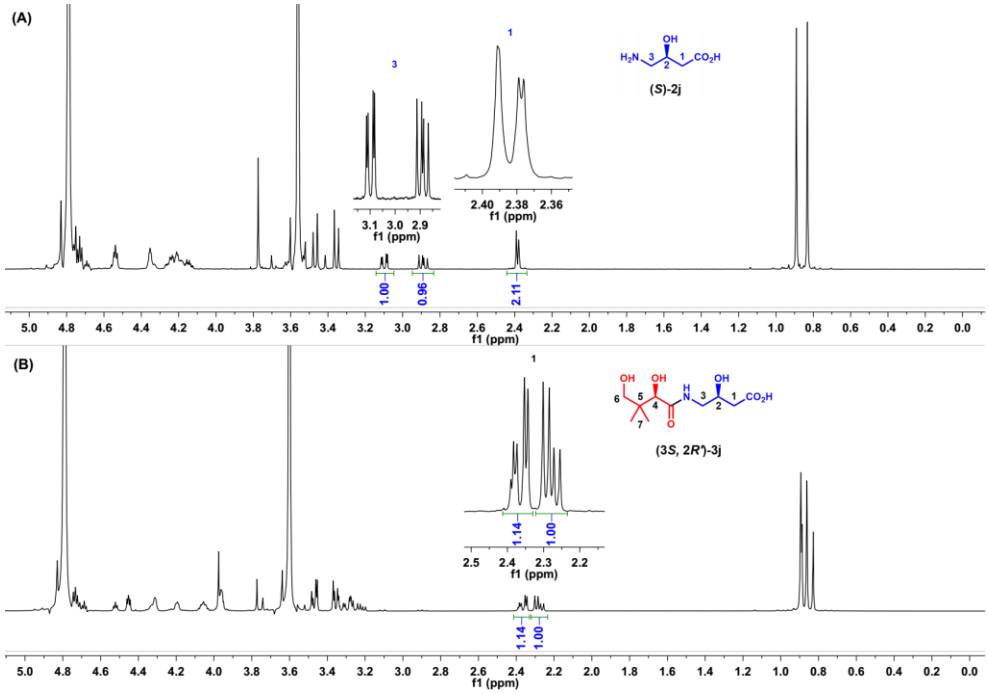


Figure S11. ^{1}H NMR spectra monitoring the $PS_{E.coli}$ catalyzed condensation of (*R*)-**1** and (*S*)-**2j** yielding (2*S*, 2*R'*)-**3j**

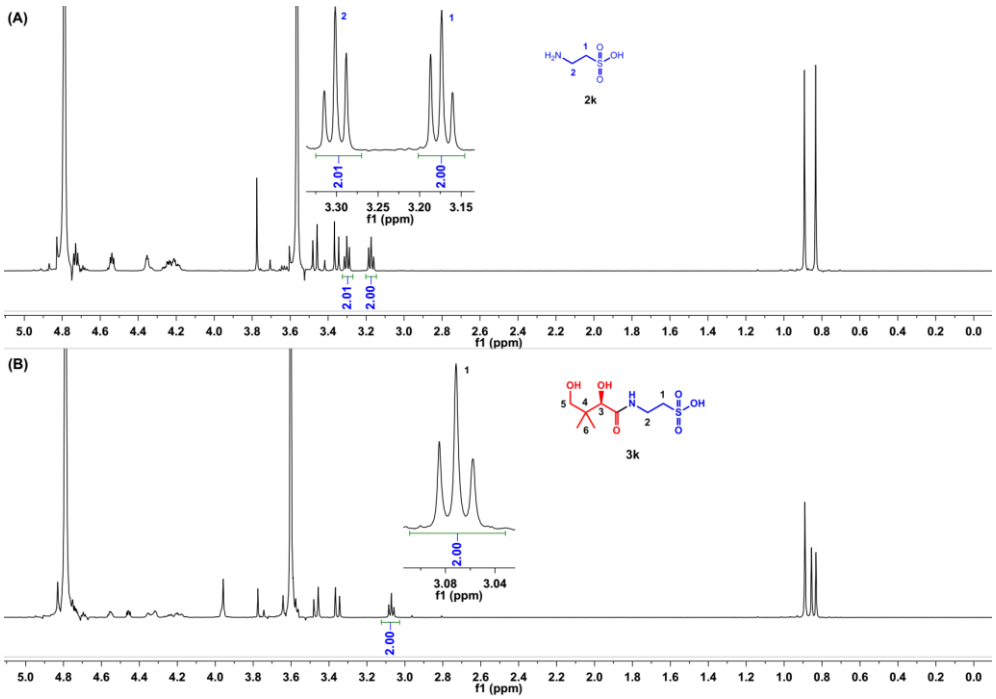


Figure S12. ^{1}H NMR spectra monitoring the $PS_{E.coli}$ catalyzed condensation of (*R*)-**1** and **2k** yielding **3k**

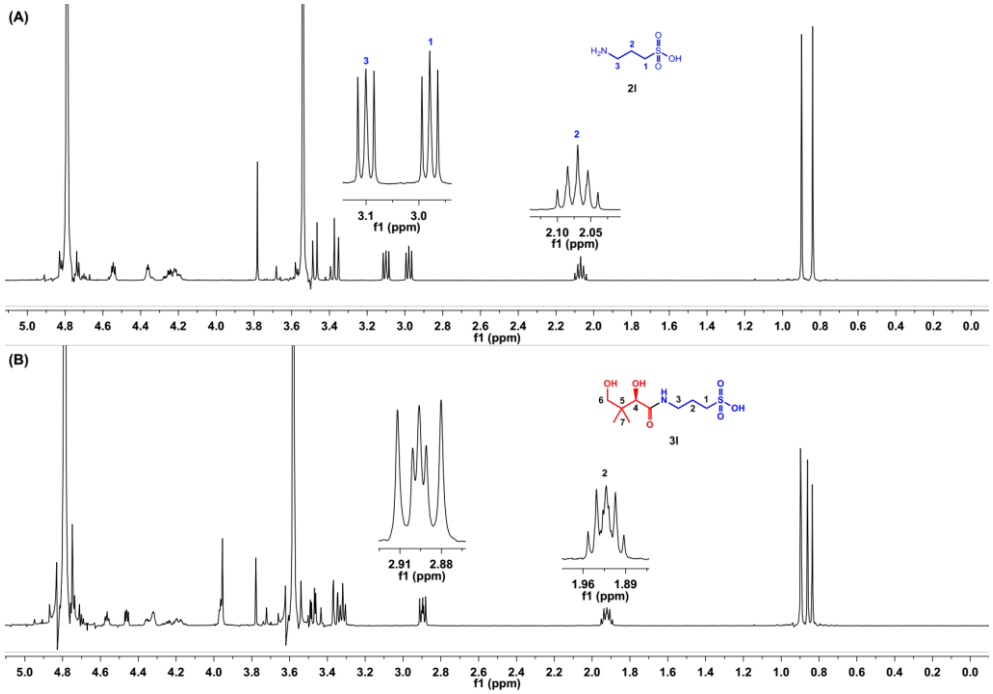


Figure S13. ^{1}H NMR spectra monitoring the $PS_{E.coli}$ catalyzed condensation of (*R*)-**1** and **2l** yielding **3l**

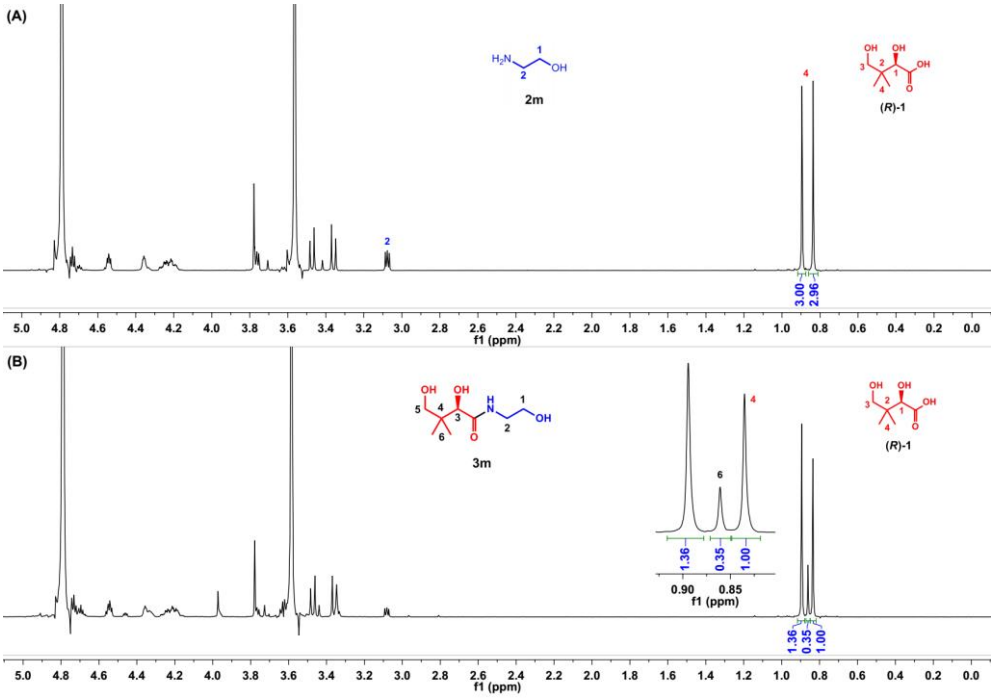


Figure S14. ^{1}H NMR spectra monitoring the $PS_{E.coli}$ catalyzed condensation of (*R*)-**1** and **2m** yielding **3m**

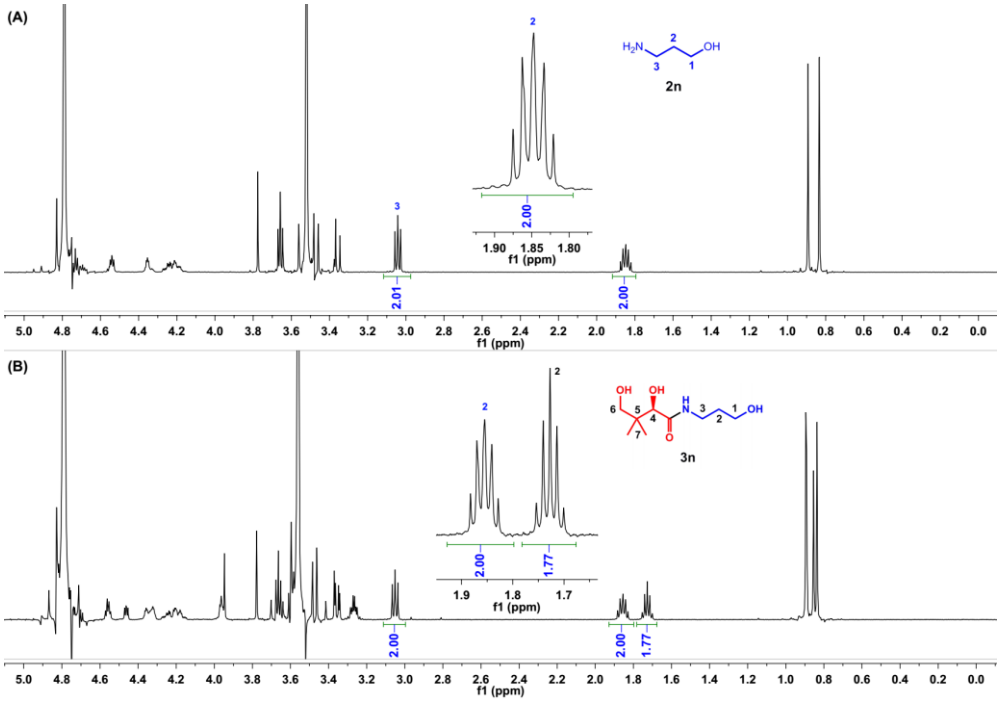


Figure S15. ^{1}H NMR spectra monitoring the $PS_{E.coli}$ catalyzed condensation of (*R*)-**1** and **2n** yielding **3n**

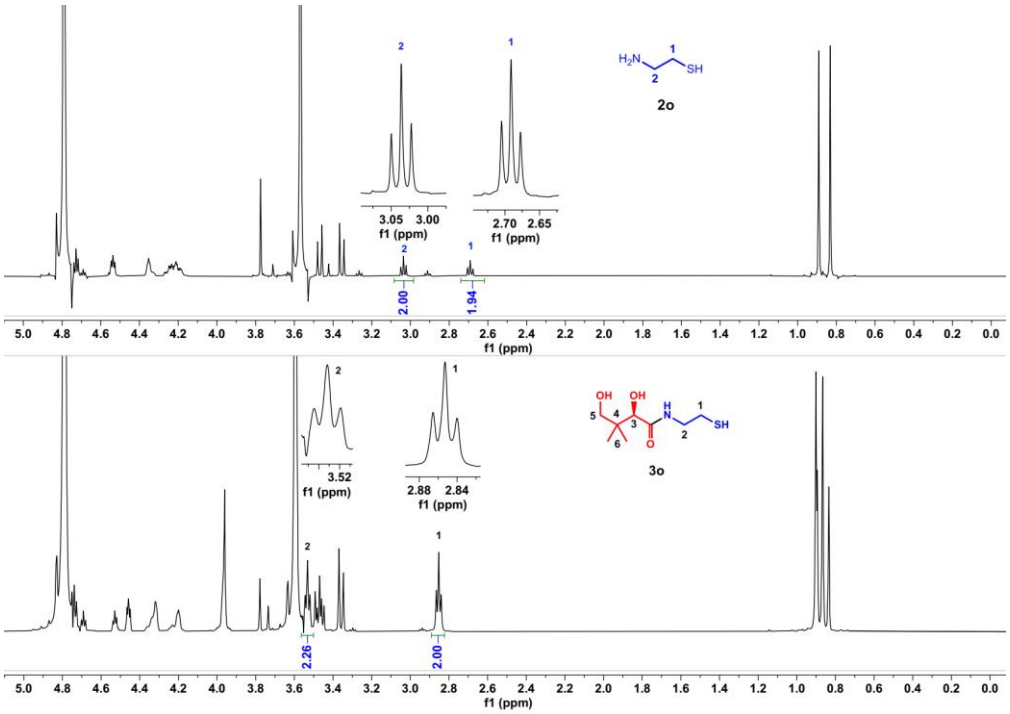


Figure S16. ^{1}H NMR spectra monitoring the $PS_{E.coli}$ catalyzed condensation of (*R*)-**1** and **2o** yielding **3o**

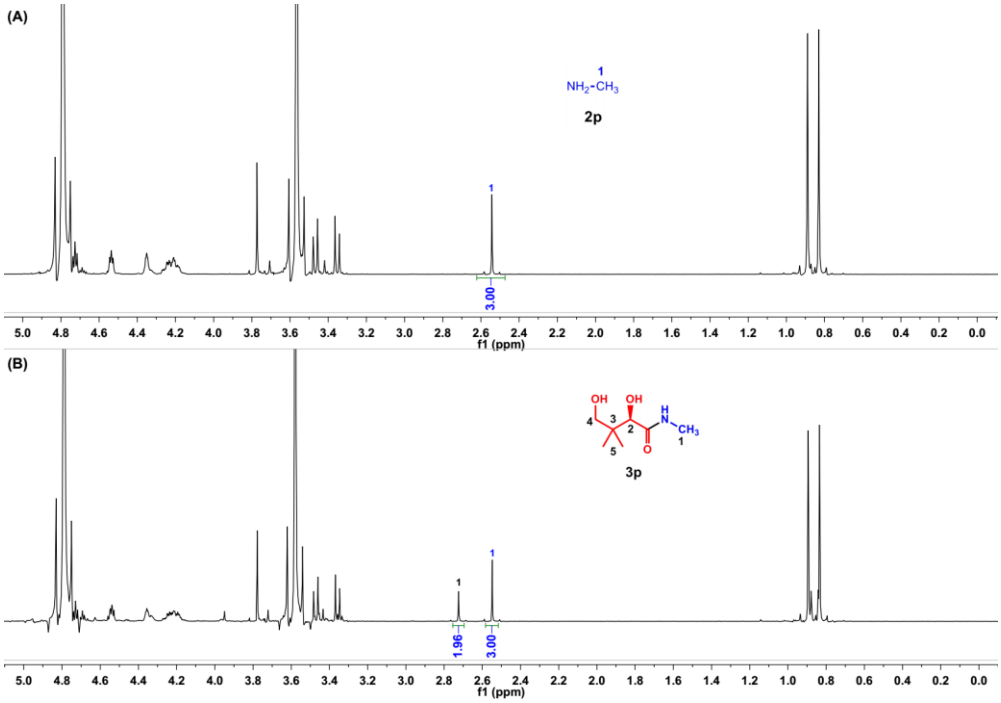


Figure S17. ^{1}H NMR spectra monitoring the $PS_{E.coli}$ catalyzed condensation of (*R*)-**1** and **2p** yielding **3p**

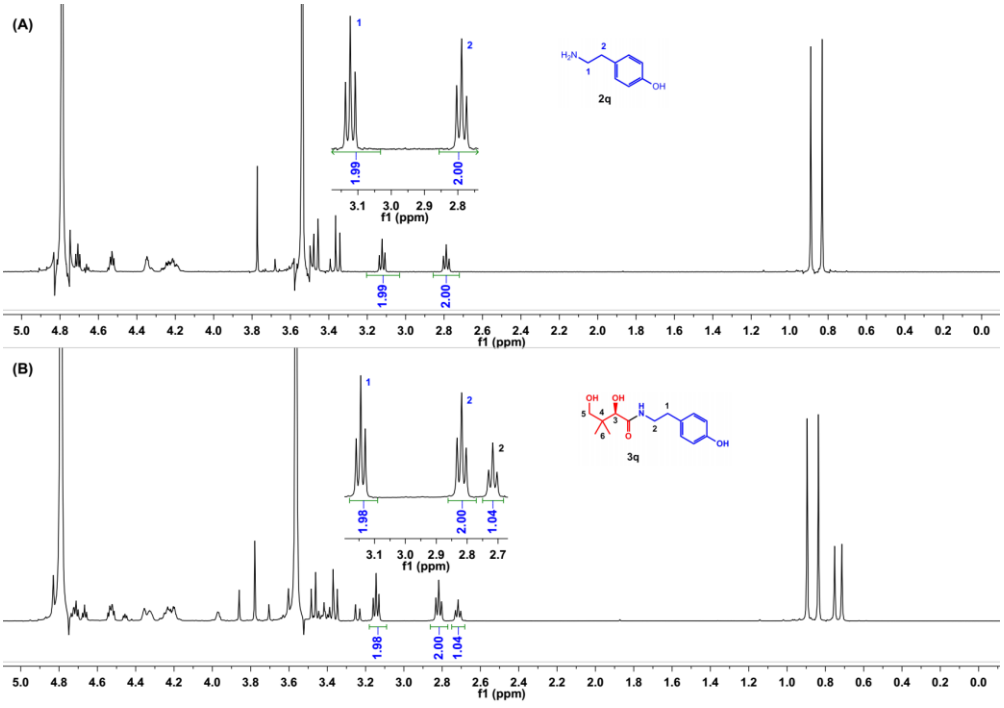


Figure S18. ^{1}H NMR spectra monitoring the $PS_{E.coli}$ catalyzed condensation of (*R*)-**1** and **2q** yielding **3q**

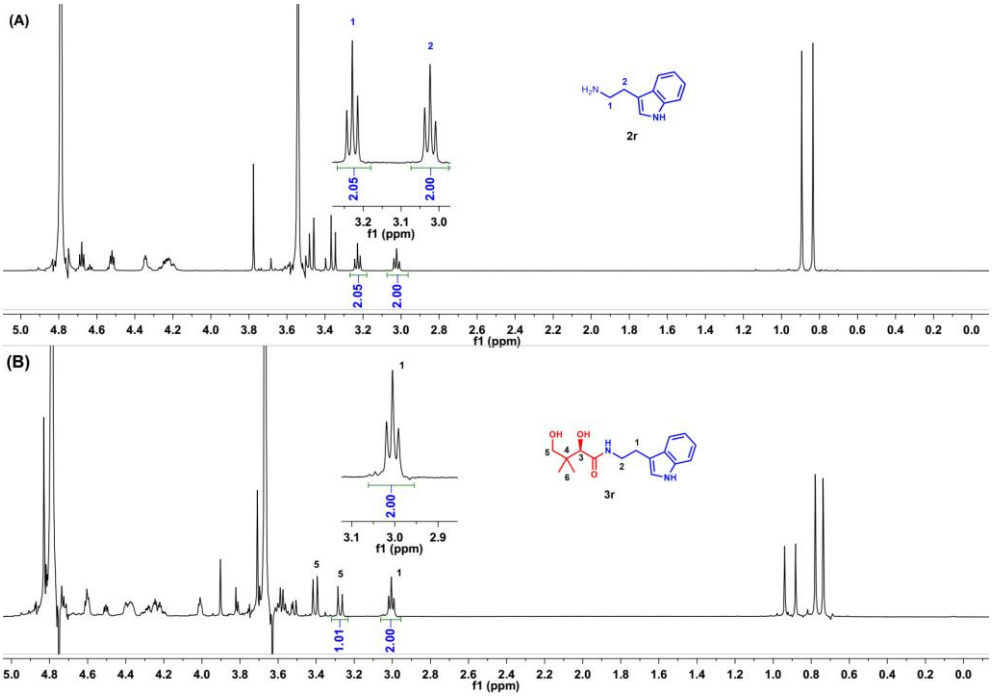
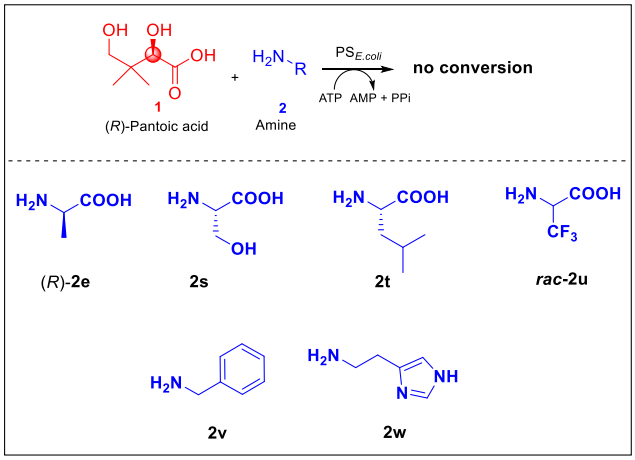


Figure S19. ^{1}H NMR spectra monitoring the PS$_{E.coli}$ catalyzed condensation of (*R*)-**1** and **2r** yielding **3r**

2. Non-substrates

The following amines [(*R*)-**2e**, **2s**–**2w**) were not accepted as substrates by PS$_{E.coli}$ for coupling to **1** (Scheme S1).

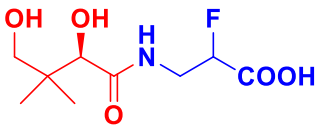


Scheme S1: Amines not accepted as substrates by PS$_{E.coli}$

3. Semi-preparative scale synthesis of selected amides

Semi-preparative scale synthesis of selected amides was performed according to the procedure reported in the Experimental section of the main manuscipt. The purification and chacterization of the amide products are described below.

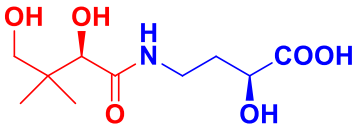
(*R*)-3-(2,4-dihydroxy-3,3-dimethylbutanamido)-2-fluoro-2λ^3-propanoic acid (3g)



The pH of the crude reaction mixture was adjusted to 1.0 by adding 1N HCl. Then, the reaction mixture was concentrated to 2 mL using a rotary evaporator. To this concentrated crude mixture, silica gel was added to make a slurry. This crude material was purified by silica gel column chromatography using ethyl acetate and methanol (9:1) as eluent. The fractions that contained the enzymatic product (analysed by TLC using KMnO4 as staining agent) were combined and concentrated under vacuum to afford the desired product **3g** as a white solid (84 mg; 71 % yield).

^{1}H NMR (500 MHz, D_2O): δ 5.20 (ddd, *J* = 4.7, 3.7, 2.8 Hz, 1H), 5.11 (ddd, *J* = 4.7, 3.7, 2.7 Hz, 1H), 3.99 (dd, *J* = 3.8, 2.0 Hz, 2H), 3.88 (ddd, *J* = 29.3, 14.9, 4.9 Hz, 2H), 3.81 – 3.67 (m, 3H), 3.48 (dt, *J* = 11.2, 1.3 Hz, 2H), 3.35 (dt, *J* = 11.2, 2.3 Hz, 2H), 0.89 (dd, *J* = 4.7, 2.2 Hz, 6H), 0.85 (d, *J* = 1.7 Hz, 6H). **^{13}C NMR** (126 MHz, D_2O): δ 175.6, 175.5, 172.1, 172.0, 88.5, 88.4, 87.1, 87.0, 75.8, 75.8, 75.7, 75.7, 68.3, 68.2, 40.2, 40.0, 38.5, 38.5, 20.4, 20.3, 18.9. **HRMS**: m/z calc/ for. $C_9H_{17}O_5NF$, 238,10125 $[M+H]^+$, found: 238,10847.

(*S*)-4-((*R*)-2,4-dihydroxy-3,3-dimethylbutanamido)-2-hydroxybutanoic acid (3i)



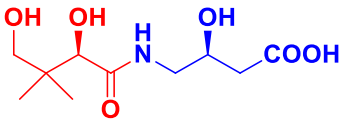
The product **3i** was purified by two steps as follows.

Step 1: The crude reaction mixture was loaded onto a cation-exchange column [5 g of Dowex 50W X8 resin, 100-200 mesh, which was pretreated with 2 M aqueous ammonia (5 column volumes), 1 M HCl (3 column volumes) and water (5 column volumes)] to remove the high concentration of Tris-HCl. The column was washed with water (3 column volumes) until the mixture of **3i** and (*R*)-pantoate was collected. The eluted mixture was concentrated up to 2 mL.

Step 2: To this concentrated mixture, silica gel was added directly to give a slurry. This crude mixture was loaded on a silica gel column and eluted using ethyl acetate and methanol (9:1) as eluent. The fractions that contained the product (analyzed by TLC using $KMnO_4$ as staining agent) were combined and concentrated under vacuum to afford the desired product **3i** as white crystalline solid (22.1 mg; 88 % yield).

^{1}H NMR (500 MHz, D_2O): δ 4.19 (dd, *J* = 8.4, 3.7 Hz, 1H), 3.87 (s, 1H), 3.39 (d, *J* = 11.2 Hz, 1H), 3.27 (h, *J* = 8.0, 7.5 Hz, 3H), 2.00 – 1.90 (m, 1H), 1.78 (dq, *J* = 14.3, 6.7 Hz, 1H), 0.79 (d, *J* = 17.4 Hz, 6H). **^{13}C NMR** (126 MHz, D_2O): δ 177.5, 175.2, 75.8, 68.3, 68.0, 38.6, 35.2, 32.7, 20.5, 19.1. **HRMS:** m/z calc/ for. $C_{10}H_{20}NO_6$, 250.1212 $[M+H]^+$, found: 250,1293.

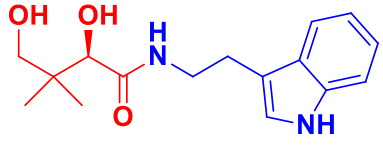
(*S*)-4-((*R*)-2,4-dihydroxy-3,3-dimethylbutanamido)-3-hydroxybutanoic acid (3j)



The product **3j** was purified by following a similar procedure as explained above for **3i**. The product was obtained as white crystalline solid (21.4 mg; 86 % yield).

^{1}H NMR (500 MHz, D_2O): δ 4.15 (ddt, *J* = 8.9, 6.9, 4.5 Hz, 1H), 3.98 (s, 1H), 3.48 (d, *J* = 11.2 Hz, 1H), 3.41 – 3.26 (m, 3H), 2.60 (dd, *J* = 15.8, 4.0 Hz, 1H), 2.45 (dd, *J* = 15.8, 8.9 Hz, 1H), 0.90 (s, 3H), 0.87 (s, 3H). **^{13}C NMR** (126 MHz, D_2O): δ 175.5, 175.4, 75.8, 68.3, 66.6, 43.8, 39.2, 38.6, 20.5, 19.1. **HRMS**: m/z calc/ for. $C_{10}H_{20}NO_6$, 250.1212 $[M+H]^+$, found: 250,1303.

(*R*)-N-(2-(1H-indol-3-yl)ethyl)-2,4-dihydroxy-3,3-dimethylbutanamide (3r)



The crude reaction mixture was concentrated using a rotary evaporator up to 2 mL. To this mixture silica gel was added to make a slurry. This crude material was purified by silica gel column chromatography using ethyl acetate (100%) as eluent. The fractions that contained product (analysed by TLC using $KMnO_4$ as staining agent) were combined and concentrated under vacuum to afford the desired product **3r** as light purple solid. (26 mg; 89 % yield).

^{1}H NMR (500 MHz, D_2O): δ 7.70 (d, *J* = 7.9 Hz, 1H), 7.49 (d, *J* = 8.2 Hz, 1H), 7.27 – 7.21 (m, 2H), 7.16 (t, *J* = 7.5 Hz, 1H), 3.89 (s, 1H), 3.58 (hept, *J* = 6.7 Hz, 2H), 3.39 (d, *J* = 11.2 Hz, 1H), 3.26 (d, *J* = 11.2 Hz, 1H), 3.02 (t, *J* = 6.9 Hz, 2H), 0.77 (s, 3H), 0.73 (s, 3H). **^{13}C NMR** (126 MHz, D_2O): δ 174.8, 136.3, 126.9, 123.6, 121.9, 119.1, 118.6, 111.8, 111.8, 75.9, 68.4, 39.3, 38.5, 24.2, 20.3, 19.0. **HRMS**: m/z calc/ for. $C_{16}H_{23}O_3N_2$, 291.16304 $[M+H]^+$, found: 291,17041.

4. NMR spectra of enzymatically prepared amides

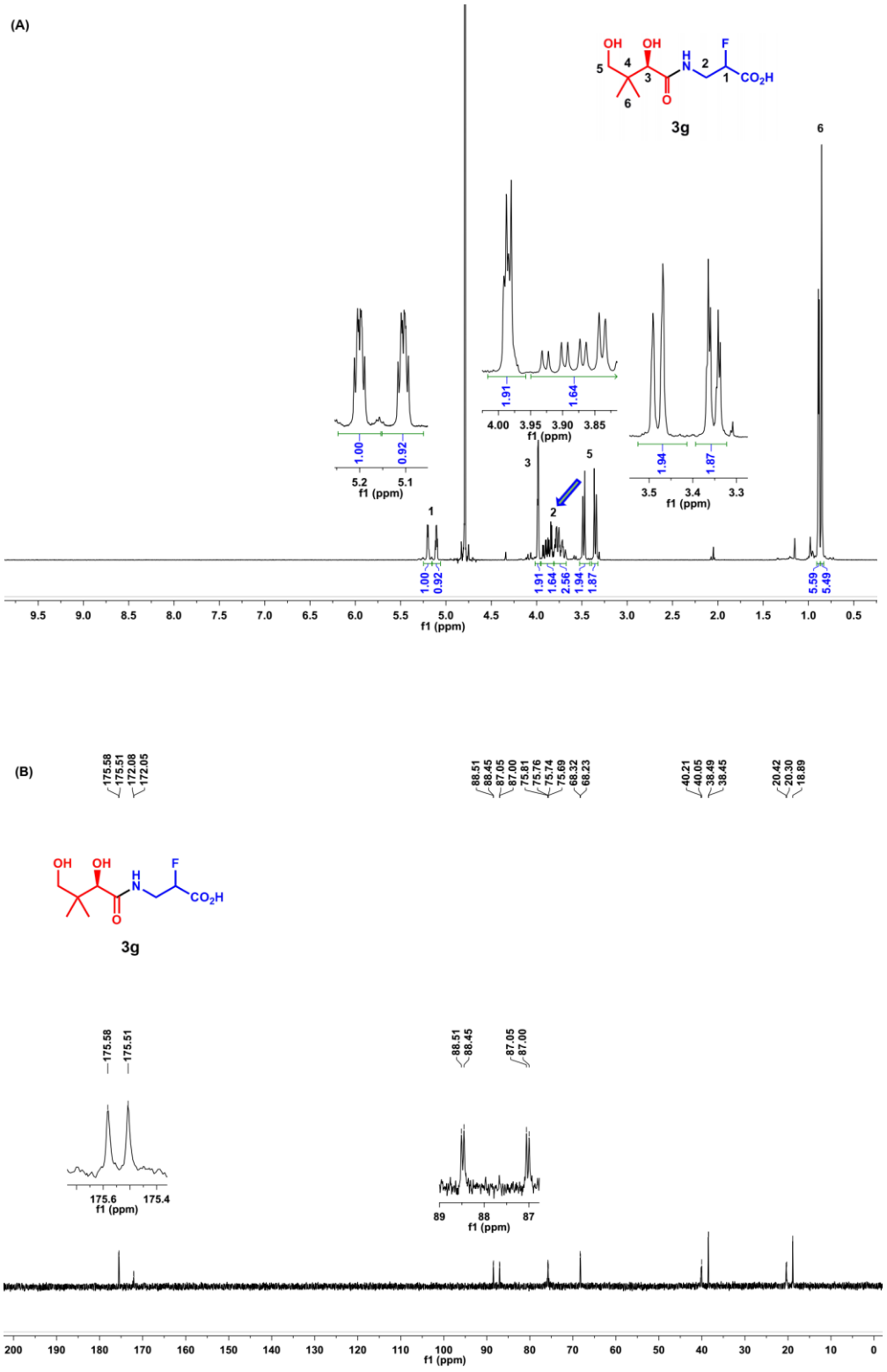


Figure S20. ^{1}H NMR (**A**) and ^{13}C NMR (**B**) of 3-((*R*)-2,4-dihydroxy-3,3-dimethylbutanamido)-2-fluoropropanoic acid [**3g**].

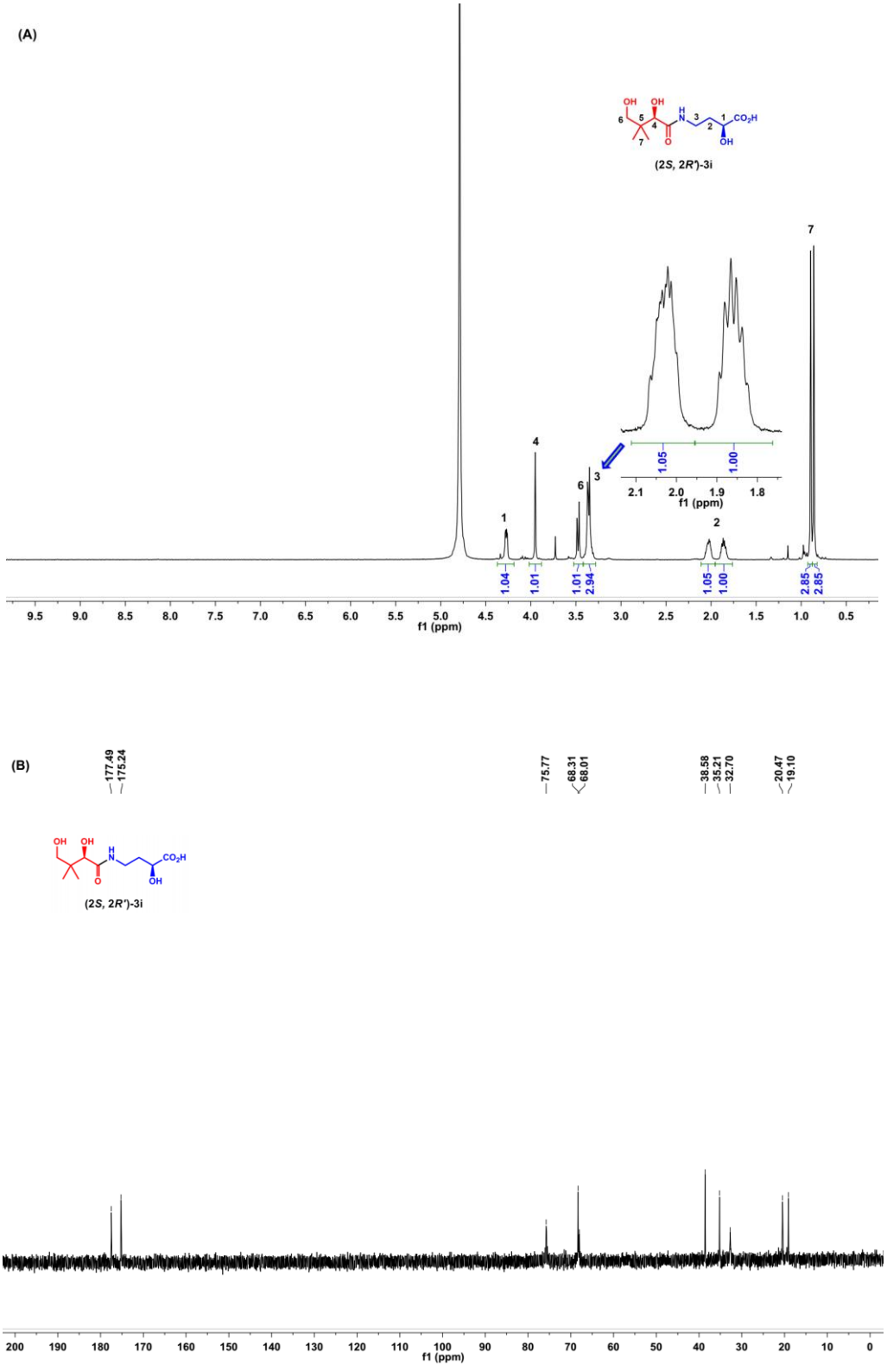


Figure S21. ^{1}H NMR (**A**) and ^{13}C NMR (**B**) of (*S*)-4-((*R*)-2,4-dihydroxy-3,3-dimethylbutanamido)-2-hydroxybutanoic acid [**3i**].

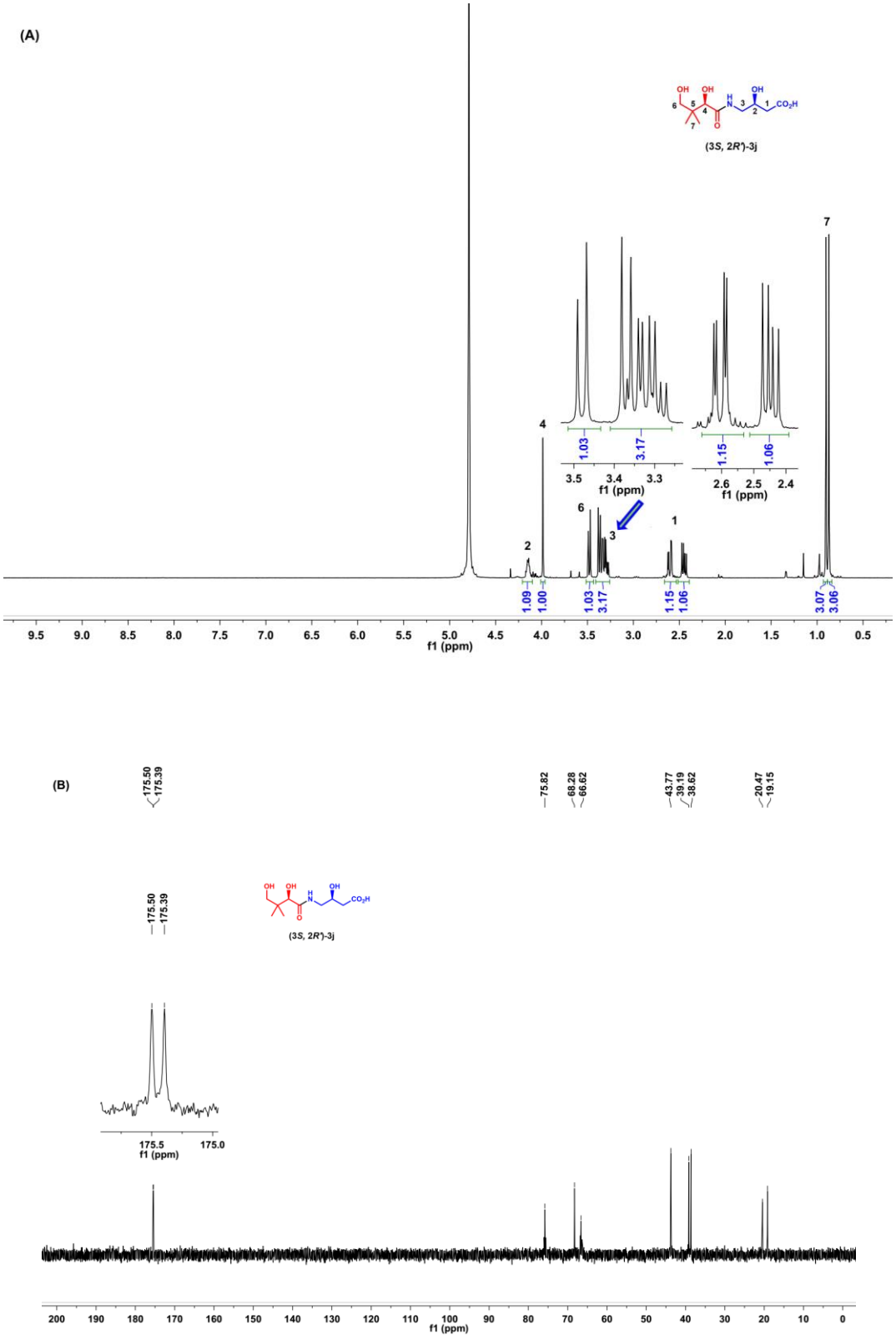


Figure S22. ^{1}H NMR (**A**) and ^{13}C NMR (**B**) of (*S*)-4-((*R*)-2,4-dihydroxy-3,3-dimethylbutanamido)-3-hydroxybutanoic acid [**3j**].

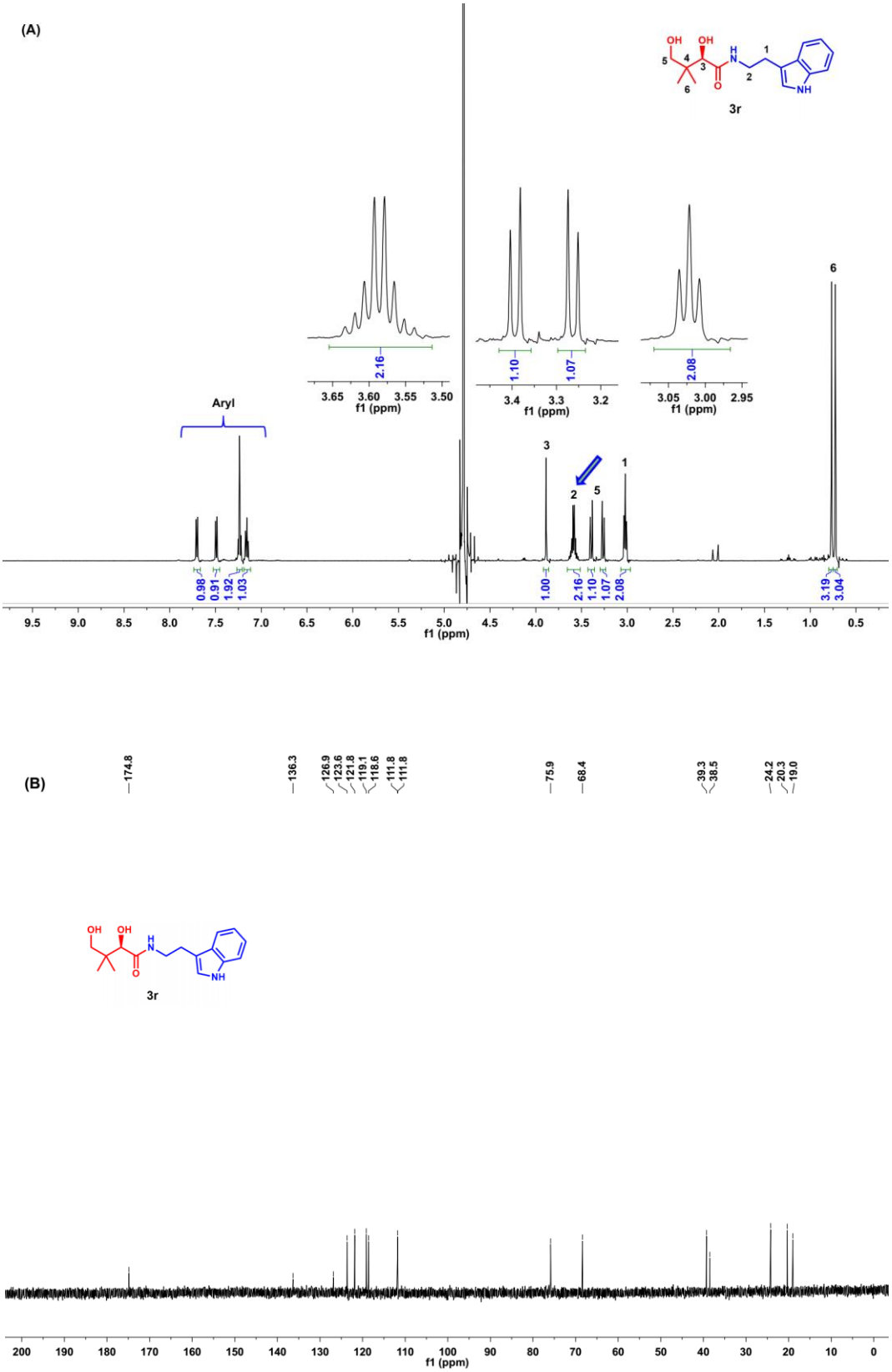


Figure S23. ^{1}H NMR (**A**) and ^{13}C NMR (**B**) of (*R*)-N-(2-(1H-indol-3-yl)ethyl)-2,4-dihydroxy-3,3-dimethylbutanamide [**3r**].

5. LC-HRMS of enzymatically prepared amides

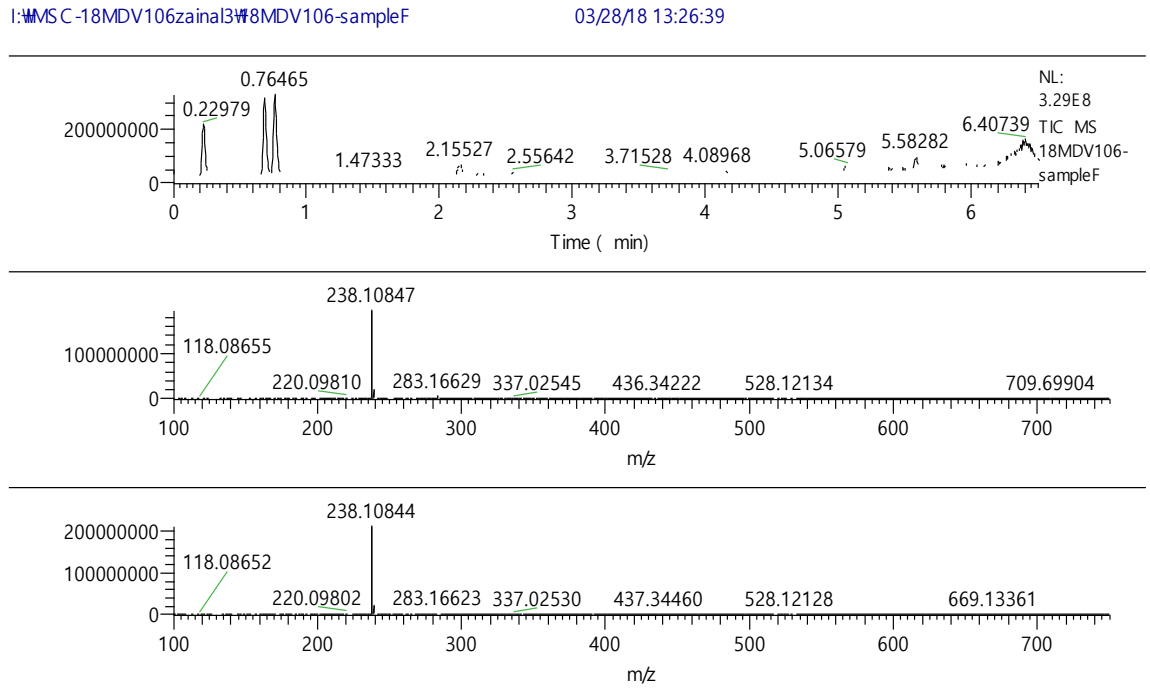


Figure S24. LC-HRMS spectrum of enzymatic product **3g**.

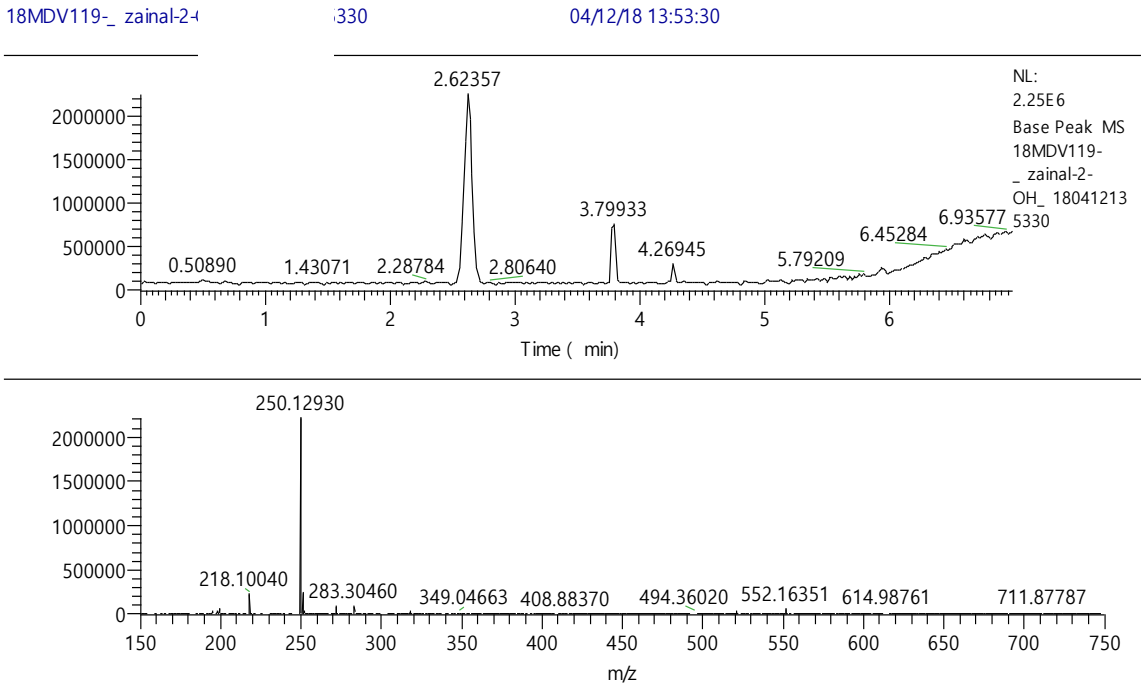


Figure S25. LC-HRMS spectrum of enzymatic product **3i**.

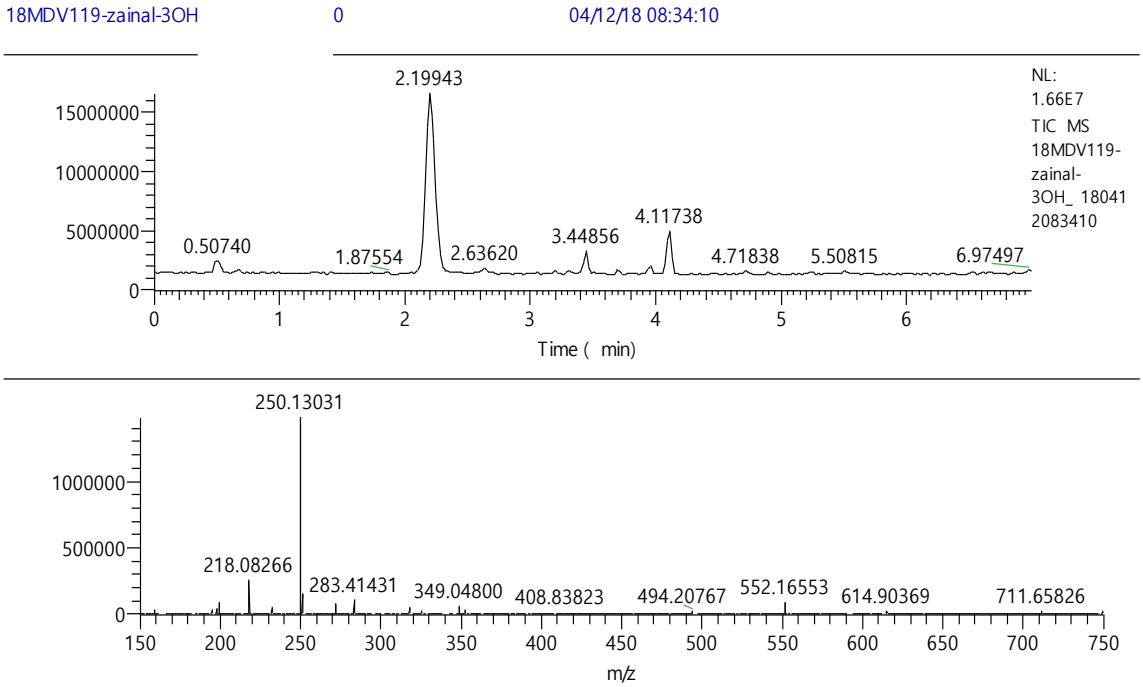


Figure S26. LC-HRMS spectrum of enzymatic product **3j**.

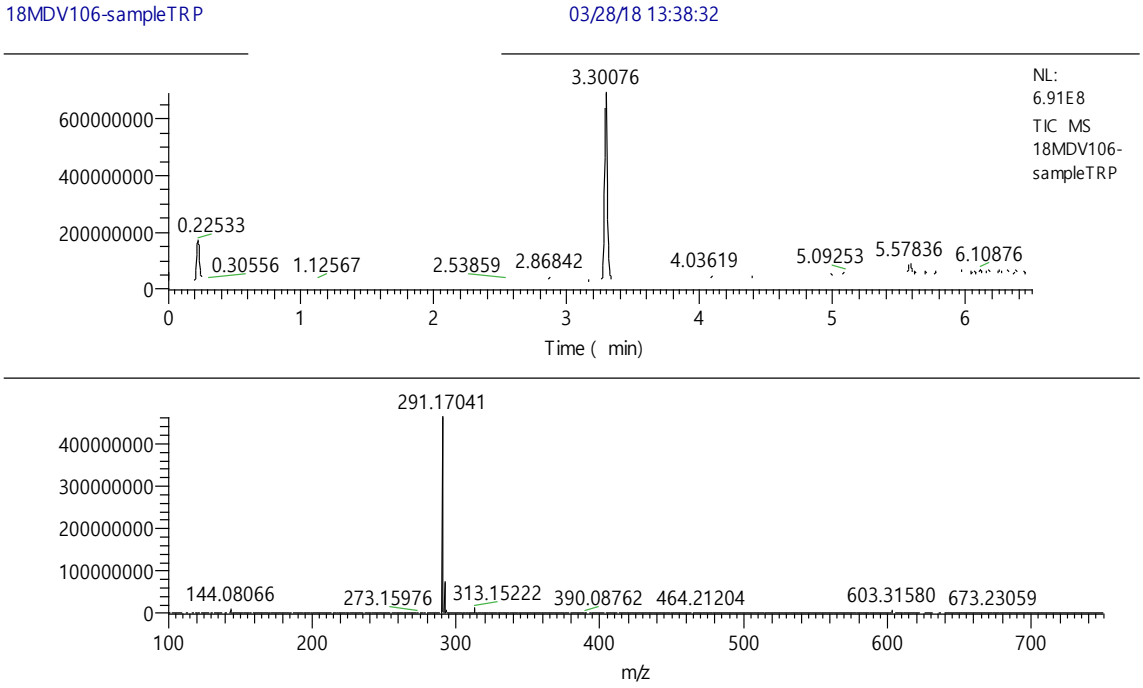


Figure S27. LC-HRMS spectrum of enzymatic product **3r**.