Supporting Information

Enantioselective Synthesis of Chiral Vicinal Amino Alcohols Using Amine Dehydrogenases

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TABLE OF CONTENTS

Experimental Section

General information

1-Hydroxy-2-butanone was purchased from Fluorochem. L-Leucinol was obtained from Acros Organics. (*S*)-2-Amino-1-butanol and 4-methyl-1-pentene were purchased from Alfa Aesar. 3-Ethylbenzothiazolium bromide was obtained from Fisher Scientific. Sodium hypochlorite solution (active chlorine 16% (w/v)) was purchased from VWR International. Other chemicals were purchased from Sigma-Aldrich used without further purification.

The leucine dehydrogenase *Lf*LeuDH from *Lysinibacillus fusiformis* was previously cloned into plasmid pET-28a (+) between restriction sites of *Eco*R I and *Xho* I and overexpressed with N-terminally positioned His-tag in *E. coli* BL21 (DE3) cells.[1] The formate dehydrogenase gene from *Candida boidinii* (a variant with mutation C23S)[2] was synthesised after codon optimization using OptimumGene™ algorithm, incorporated into pET-28a (+) plasmid between restriction sites of *Nde* I and *Bam*H I in GenScript (Nanjing, China), and subsequently expressed with N-terminally positioned His-tag in *E. coli* BL21 (DE3) cells. ^{1}H NMR and ^{13}C NMR analyses were performed on a Bruker Avance 400 MHz spectrometer. Chemical shifts (δ) and coupling constants (J) are reported in parts per million (ppm) and Hz, respectively. HRMS analyses were performed on an Agilent 6510 Q-TOF mass spectrometer coupled with an Agilent 1200 series LC system. HPLC analyses were conducted using a Phenomenex Luna 5μ C18(2) column (100 Å, 250 × 2.00 mm, 5 μm). GC analyses were conducted using an Agilent HP-1 column (30 m × 0.32 mm × 0.25 μm).

Gene sequence of *Lf*LeuDH

ATGGAAATCTTCAAGTATATGGAAAAGTATGATTACGAACAATTGGTATTTTGCCAAGATGAAGCATCAGGGTTAAAAGCGGTTAT
CGCTATCCATGATACAACACTTGGACCAGCACTAGGTGGAGCACGTATGTGGACGTATGCGTCGGAAGAAAATGCAATTGAAGATG
CATTACGTTTAGCACGAGGAATGACTTACAAAAATGCTGCAGCTGGTTTAAACCTTGGCGGTGGAAAAACGGTCATTATTGGTGAC
CCATTTAAAGATAAAAATGAAGAGATGTTCCGTGCGTTAGGTCGTTTCATCCAAGGATTAAATGGCCGCTATATTACAGCTGAAGAT
GTAGGTACAACAGTATTGGATATGGATTTAATCCATGAAGAAACAAATTATGTAACAGGAATTTCTCCTGCATTCGGTTCTTCAGGT
AACCCATCTCCAGTAACAGCTTATGGTGTTTACCGTGGTATGAAGGCAGCAGCAAAAGAAGCATTCGGTTCAGACTCACTAGAAGG
ATTAAAAGTTTCTGTACAGGGTCTAGGAAATGTAGCCTACAAGCTTTGCGAGTATTTACATAATGAAGGGGCAAAACTTGTTGTAAC
GGATATCAATCAGGCAGCTATTGATCGAGTTGTTAATGACTTCGACGCGATTGCAGTGGCACCAGATGAAATCTATGCGCAAGAAG
TAGATATCTTCTCACCTTGTGCATTAGGGGCAATTTTAAATGATGAAACAATTCCGCAATTAAAAGCGAAAGTGATCGCTGGATCTG
CAAATAACCAATTAAAAGATTCACGTCATGGTGACTACCTACATGAATTAGGTATTGTCTATGCACCAGATTATGTAATCAATGCTG
GTGGCGTTATTAATGTTGCGGATGAATTATATGGCTATAATCGTGAACGTGCAATGAAACGCGTTGATGGCATTTATGATAGTATTG
AAAAAATCTTTGCTATTTCAAAACGAGATGGCATTCCAACATATGTGGCAGCGAACCGCTTAGCGGAGGAACGTATCGCTCGCGTA
GCAAAATCTCGCAGCCAATTTTTAAAAAATGAAAAAAATATTTTACACGGTCGCTAA

Construction and screening of mutagenesis libraries

The two site combinatorial mutagenesis libraries of *Lf*AmDH were constructed via two different techniques: overlap extension PCR method[5] and two-step QuikChange PCR method.[6]

For the overlap extension PCR method (**Figure S1**), gene fragments A, B and C (where the whole *Lf*LeuDH gene is divided at codon sites for K68 and N261) were individually amplified in the first round PCR using respective primer pair *Lf*LeuDH-For & 68NNK-Rev, 68NNK-For & 261NNK-Rev, and 261NNK-For & *Lf*AmDH-Rev (**Table S1**). The PCR products of fragments A, B and C were respectively extracted from agarose gel after electrophoresis. Fragment B was then fused and amplified with fragments A and C respectively through a second round PCR. The fused fragments A+B and B+C were respectively purified after agarose gel electrophoresis and gel extraction. Afterwards, fragments A+B and B+C were fused together via a third round PCR, and the final fused gene was purified again by agarose gel electrophoresis and gel extraction. The final fused gene was inserted into pET-28a (+) between restriction sites of *Eco*R I and *Xho* I, and the recombinant plasmids were transformed into *E. coli* DH5α cells. After that, the resulting *E. coli* DH5α colonies were scraped from agar plates and gathered. Afterwards, plasmids were extracted from the *E. coli* DH5α cells to generate a gene pool of mutagenesis, which was then used to transform *E. coli* BL21 (DE3) cells for protein expression.

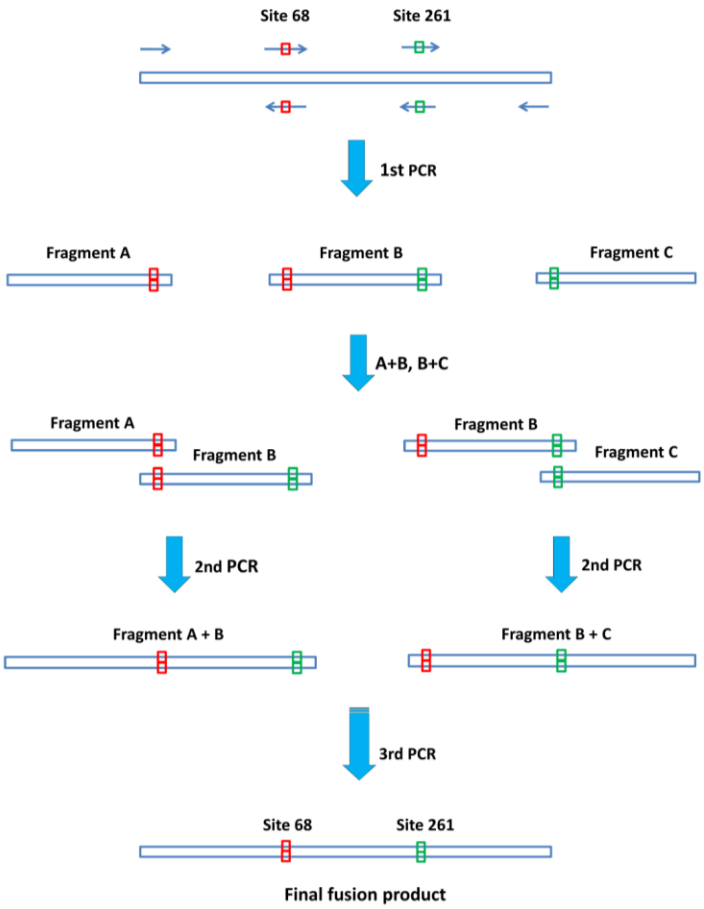


Figure S1. Process of introducing combinatorial mutagenesis at the 68th and 261st amino acid sites of *Lf*AmDH via overlap extension PCR.

Table S1. PCR primers used for construction of the mutagenesis libraries.

Entry	Primer	Sequence (5' to 3')
1	*Lf*LeuDH-For	CCGGAATTCATGGAAATCTTCAAGTATATGG
2	*Lf*LeuDH-Rev	CCGCTCGAGTTAGCGACCGTGTAAAAT
3	68NNK-For	CGAGGAATGACTTACNNKAATGCTGCAGCTGGT
4	68NNK-Rev	ACCAGCTGCAGCATTMNNGTAAGTCATTCCTCG
5	261NNK-For	ATCGCTGGATCTGCANNKAACCAATTAAAAGATTC
6	261NNK-Rev	GAATCTTTTAATTGGTTMNNTGCAGATCCAGCGAT

For the two-step QuikChange PCR method, saturation mutagenesis at K68 was first introduced via a whole-plasmid PCR amplification using primer pair 68NNK-For & 68NNK-Rev (**Table S1**). The PCR product was used to transform *E. coli* DH5α cells after digestion with *Dpn* I (37 ºC, 2 h), and the plasmids from the resulting *E. coli* DH5α colonies were extracted as mentioned above. Afterwards, the prepared plasmid pool was used as the templates of a second round whole-plasmid PCR amplification using primer pair 261NNK-For & 261NNK-Rev (**Table S1**). Then, the PCR product was used to transform *E. coli* DH5α cells after digestion with *Dpn* I (37 ºC, 2 h), and the plasmids of the resulting *E. coli* DH5α colonies were extracted and used to transform *E. coli* BL21 (DE3) cells for protein expression as mentioned before.

The *E. coli* BL21 (DE3) colonies obtained after transformation were picked using toothpicks and transferred into 96-deep well plates (containing 300 μL LB with 50 μg/mL kanamycin in each well), and incubated overnight at 37 ºC and 250 rpm. Then, 50 μL culture from each well was transferred into a corresponding well in a second 96-deep well plate (containing 400 μL LB with 50 μg/mL kanamycin in each well), and the second 96-deep well plates were incubated at 37 ºC and 250 rpm for 3−4 h. Afterwards, 50 μL LB containing 2 mM β-D-1-thiogalactopyranoside (IPTG) was added into each well to induce protein expression and the plates were then incubated at 20 ºC and 250 rpm for another 16−18 h. The cells were harvested in the 96-deep well plates via centrifugation and stored at −80 °C overnight. After melting at 37 ºC, 200 μL of Tris-HCl buffer (5 mM, pH 8.0), containing 0.75 mg/mL lysozyme and 2 U/mL DNase I, was added into each well of the 96-deep well plates, and the plates were incubated at 37ºC for 1 h. Subsequently, each well was supplemented with 200 μL of Tris-HCl buffer (5 mM, pH 8.0) and the supernatant in each well was used for high throughput activity screening after centrifugation.

The reductive amination activity of each hit was assayed at 25 ºC, by measuring the initial rate of change in absorbance at 340 nm (corresponding to the amount of NADH (ε = 6220 $M^{-1}cm^{-1}$) using a microplate reader. An assay reaction mixture (200 μL) contained 16 μL lysate, 5 mM 1-hydroxy-2-butanone, 0.2 mM NADH, and 2 M NH_4Cl/NH_4OH buffer (pH 9.5). In total, 2444 hits were screened (1222 hits were screened from each mutagenesis library constructed via either of the aforementioned two different techniques), which corresponds to a >90% library coverage according to the equation for fractional library completeness developed by Patrick *et al*.[7] The hits with activity more than 20 mU/mL cell-free extract are listed in **Table S2**.

Table S2. The variants with detectable activity towards 1-hydroxyl-2-butanone.

Entry	Mutation	Activity (mU/mL CFE)[a]	Entry	Mutation	Activity (mU/mL CFE)[a]
*Lf*LeuDH	Wild-type	0	18C8	K68A/N261L	20
1F1	K68S/N261L	55	18D4	K68S/N261L	29
3G4	K68T/N261L	67	18D7	K68A/N261L	21
5E6	K68S/N261C	20	19A6	K68A/N261L	29
5F6	K68S/N261L	64	19A10	K68C/N261L	55
5G9	K68A/N261L	35	20G9	K68T/N261L	85
6B2	K68S/N261L	48	21C12	K68A/N261L	42
7D8	K68A/N261L	31	22D12	K68C/N261L	51
9D12	K68T/N261L	93	22G3	K68C/N261L	41
9F11	K68T/N261L	90	23H7	K68C/N261L	46
12D9	K68T/N261L	76	23H8	K68T/N261L	83
15G1	K68S/N261L	44	25D6	K68C/N261L	39
17F9	K68A/N261L	24	25G7	K68S/N261L	45
18B11	K68T/N261L	62	25H10	K68T/N261L	81

[a] CFE = cell-free extract.

Site-directed mutagenesis

Site-directed mutageneses were performed using QuikChange PCR method.[6] Mutation at a certain site was introduced via whole-plasmid PCR amplification using mutagenesis primers.

Enzyme preparation and purification

*Lf*LeuDH, FDH and all the developed amine dehydrogenases were overexpressed in 500-mL flasks using the method described previously.[8] To induce expression, IPTG at a final concentration of 0.3 mM was added to each culture when the optical density (OD_{600}) reached 0.6–0.8 and then each culture was incubated at 25 ºC and 180 rpm for 12 h. After that, the cells were harvested by centrifugation, suspended in potassium phosphate buffer (10 mM, pH 7.0) and disrupted via ultrasonication in an ice bath. After centrifugation (4 ºC, 12,000 rpm, 20 min), the supernatant was used for protein purification or the preparation of lyophilised cell-free extract. For the protein purification, the supernatant was loaded onto a Ni-NTA agarose column and subsequently eluted with a gradient of 20–500 mM imidazole in 50 mM Tris-HCl buffer (pH 8.0) containing 500 mM NaCl. The eluted fractions containing the target protein were collected, concentrated and buffer-exchanged to remove imidazole via ultrafiltration with a Vivaspin® 20 (MWCO 30 000 Da) centrifugal filter. For the preparation of lyophilised cell-free extract, the supernatant of lysate was freeze-dried for 3 d.

Activity assay and kinetic analysis

The activity of amine dehydrogenase (AmDH) was assayed by measuring the initial rate of change in absorbance at 340 nm (corresponding to the amount of NADH (ε = 6220 $M^{-1}cm^{-1}$) using a microplate reader as mentioned above. One unit (1 U) of activity is defined as the amount of the enzyme catalysing the conversion of 1 μmol substrate within one minute. All the reactions for activity assay were performed at 25 ºC in triplicate. For a standard activity assay, an assay reaction mixture (200 μL) contained a certain amount of purified enzyme, 20 mM substrate, 0.2 mM NADH, and 2 M NH_4Cl/NH_4OH buffer (pH 9.5). Kinetic parameters (k_{cat} and K_m) and respective standard errors were determined by measuring the activities at different substrate concentrations and fitting the activity versus substrate concentration data to the Michaelis–Menten equation using Origin 8.6.

Gene sequencing revealed that the AmDH hits possessed five different mutations: K68S/N261C, K68A/N261L, K68C/N261L, K68S/N261L, and K68T/N261L. Thus, the five variants were purified and their activity towards **1a** was determined (**Table S3**). Among them, K68C/N261L, K68S/N261L, and K68T/N261L displayed remarkable reductive amination activity (171 mU/mg, 180 mU/mg, and 233 mU/mg, respectively) towards **1a**, whereas K68S/N261C and K68A/N261L displayed lower activity (27 mU/mg and 97 mU/mg, respectively).

Table S3. Activity assay of the five screened variants towards 1-hydroxyl-2-butanone (**1a**).

Entry	Mutation	Activity (mU/mg protein)
1	K68S/N261C	27 ± 6
2	K68A/N261L	97 ± 2
3	K68C/N261L	171 ± 5
4	K68S/N261L	180 ± 2
5	K68T/N261L	233 ±16

The activity of the purified AmDH variants (K68S/N261C, K68A/N261L, K68C/N261L, K68S/N261L, and K68T/N261L) towards different methyl ketones was measured using the same method described above. The results were shown below (**Figure S2**).

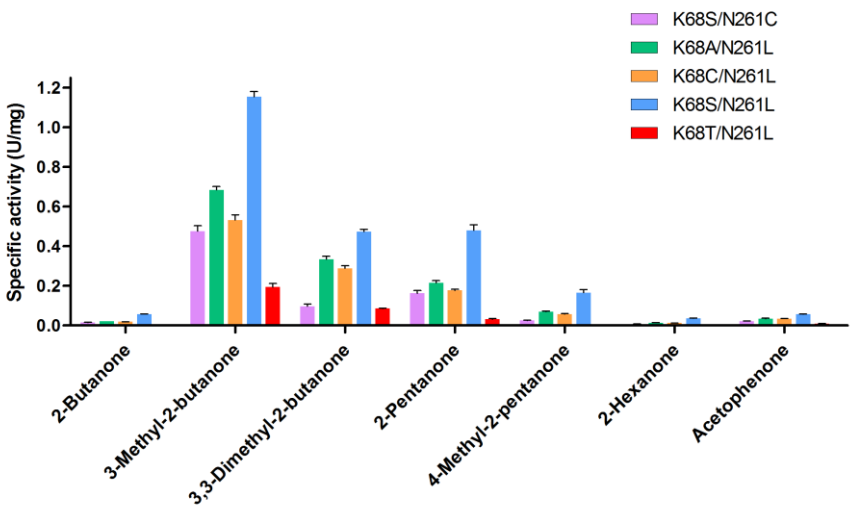


Figure S2. Specific activity comparison of purified AmDH variants over various methyl ketones.

Homology modelling and molecular docking

The 3D homology modelling structures of AmDH-M_0 and AmDH-M_3 were built using the SWISS-MODEL server (http://swissmodel.expasy.org/) with the crystal structure of the subunit A of a LeuDH from *Bacillus sphaericus* (PDB ID: 1LEH) as the template.[3] Molecular dockings were performed with AutoDock Vina. Both the input and the output of molecular structures were performed in PDBQT format. Appropriate substrate-binding modes were explored in a grid box with centre coordinate (-22.049, -17.072, -20.609) and spacing of 1.0 Å (20 × 20 × 20 and 22 × 22 × 22 for AmDH-M_0 and AmDH-M_3, respectively) embracing the substrate-binding site of the enzyme.[4] The docking results are shown below (**Figure S3**).

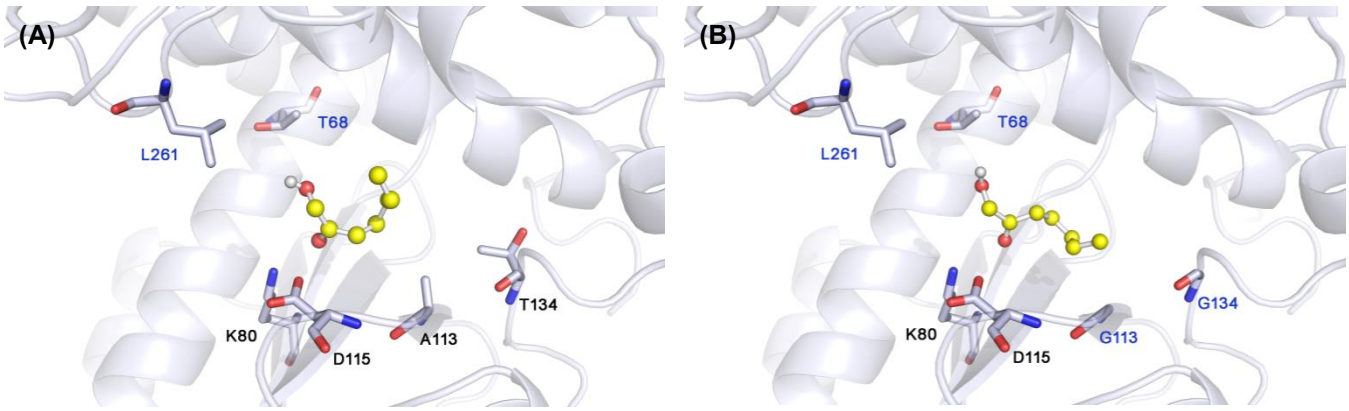


Figure S3. Structural analysis of the active sites of AmDH-M_0 (K68T/N261L) (A) and the pocket-expanded mutant AmDH-M_3 (K68T/N261L/A113G/T134G) (B) with the docked substrate 1-hydroxy-2-heptanone. 1-Hydroxy-2-heptanone was shown as ball-and-stick model. The mutated residues (the 68th, 261st, 113th, and 134th residues) and the two catalytic residues (K80 and D115) are shown as sticks.

Synthesis of α-hydroxy ketone substrates

α-Hydroxy ketone **1b** was synthesised via cross-acyloin condensation (**Figure S4** (i)).[9] Isobutyraldehyde (3.6 g, 50 mmol), paraformaldehyde (1.5 g, 50 mmol), 3-ethylbenzothiazolium bromide (1.2 g, 5 mmol), and triethylamine (0.5 g, 5 mmol) were dissolved in ethanol (50 mL). The mixture was stirred at 60 ºC for 48 h. The resulting reaction mixture was concentrated via evaporation under reduced pressure and then purified by column chromatography (silica gel, petroleum: ethyl acetate = 15:1 or 10:1) to afford 0.56 g (11%) of α-hydroxy ketone **1b**.

α-Hydroxy ketones **1c**, **1d** and **1f** were synthesised via selective oxidation of the corresponding diols with NaClO (**Figure S4** (ii)).[10] The corresponding diol (48 mmol, 5.7 g, 5.0 g and 5.7 g for **1c**, **1d** and **1f** respectively) was dissolved in acetic acid (39 mL), to which an aqueous 2.3 M NaClO solution was added dropwise at room temperature (RT). The mixture was stirred continuously until TLC showed complete conversion of the diol substrate. After that, water was added into the mixture, which was then extracted with dichloromethane (3 x 100 mL). The organic phase was neutralised by washing with aqueous $NaHCO_3$ solution (1 M, 3 x 100 mL), dried over anhydrous $MgSO_4$ and evaporated under reduced pressure to give the α-hydroxy ketone products (4.0 g (71%) of **1c**, 2.4 g (49%) of **1d**, and 3.5 g (63%) of **1f**).

α-Hydroxy ketones **1e** and **1g** were synthesised via direct ketohydroxylation of the corresponding olefins (**Figure S4** (iii)).[10] The corresponding olefin (25.5 mmol, 2.1 g and 2.5 g for **1e** and **1g** respectively) was dissolved in acetone (206 mL), water (46 mL) and acetic acid (9.7 mL), to which a $KMnO_4$ solution (43 mmol, 6.8 g in 81 mL acetone and 27 mL water) was added dropwise at RT. The mixture was stirred continuously until TLC showed complete conversion of the starting olefin substrate. After that, the reaction mixture was filtered through a celite pad, and then the pad was washed with a small amount of acetone. The combined filtrate was evaporated under reduced pressure to remove the acetone and then extracted with dichloromethane (3 x 100 mL). The combined organic layers were neutralised by repeated washings with aqueous $NaHCO_3$ solution (1 M, 3 x 100 mL), dried over anhydrous $MgSO_4$, and evaporated under reduced pressure to give the α-hydroxy ketone product (0.56 g (19%) of **1e** and 1.1 g (32%) of **1g**).

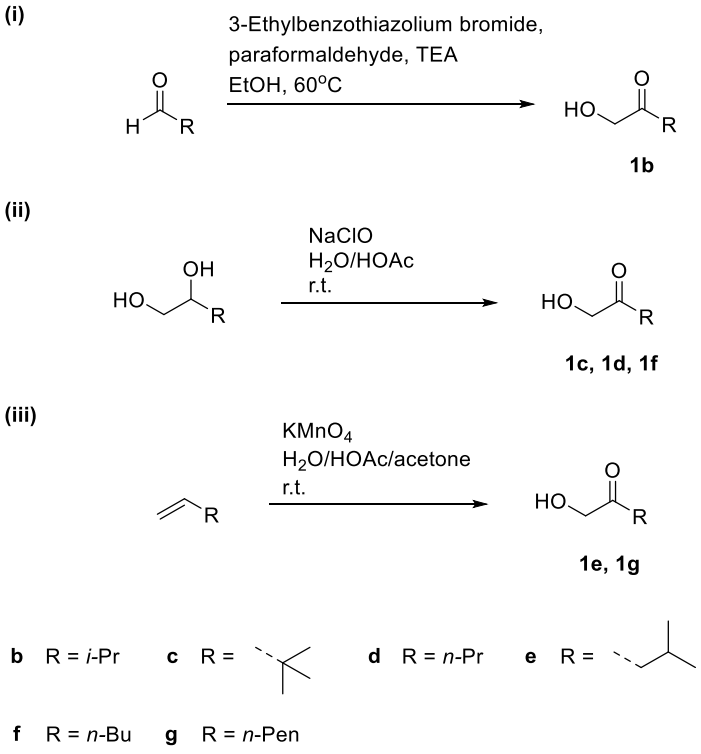


Figure S4. Synthetic routes of α-hydroxy ketone substrates used in this study.

1-Hydroxy-3-methyl-2-butanone (1b)

Yield 11%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 4.33 (s, 2H), 3.16 (br s, 1H), 2.66 (hept, 1H, *J* = 7.0 Hz), 1.18 (d, 6H, *J* = 7.0 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 213.5, 66.3, 37.3, 18.1.

1-Hydroxy-3,3-dimethyl-2-butanone (1c)

Yield 71%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 4.32 (s, 2H), 3.12 (br s, 1H), 1.11 (s, 9H). ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 215.2, 63.9, 42.1, 26.2.

1-Hydroxy-2-pentanone (1d)

Yield 49%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 4.25 (s, 2H), 3.00 (br s, 1H), 2.40 (t, 2H, *J* = 7.4 Hz), 1.68 (m, 2H), 0.96 (t, 3H, *J* = 7.4 Hz). ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 209.8, 68.1, 40.3, 17.2, 13.7.

1-Hydroxy-4-methyl-2-pentanone (1e)

Yield 19%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 4.14 (s, 2H), 3.06 (br s, 1H), 2.22 (d, 2H, *J* = 7.1 Hz), 2.12 (m, 1H), 0.88 (d, 6H, *J* = 6.6 Hz). ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ. 209.5, 68.7, 47.3, 24.9, 22.5.

1-hydroxy-2-hexanone (1f)

Yield 63%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 4.25 (s, 2H), 3.14 (br s, 1H), 2.42 (t, 2H, *J* = 7.5 Hz), 1.63 (m, 2H), 1.35 (m, 2H), 0.93 (t, 3H, *J* = 7.3 Hz). ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 209.9, 68.1, 38.1, 25.8, 22.3, 13.7.

1-Hydroxy-2-heptanone (1g)

Yield 32%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 4.26 (s, 2H), 3.14 (br s, 1H), 2.42 (t, 2H, *J* = 7.5 Hz), 1.66 (m, 2H), 1.32 (m, 4H), 0.91 (t, 3H, *J* = 6.8 Hz). ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 209.9, 68.1, 38.4, 31.3, 23.4, 22.3, 13.9.

The ^{1}H NMR and ^{13}C NMR spectra of the synthesised products are in agreement with the reference.[10]

Synthesis of racemic 2-amino-1-heptanol 2g

Racemic 2-amino-1-heptanol **2g** was synthesised via reductive amination of α-hydroxy ketone **1g** using $NaBH_3CN$.[11] **1g** (2 mmol), NH_4NO_3 (10 mmol), $NaBH_3CN$ (4 mmol), and acetic acid (400 μL) were dissolved in methanol (40 mL), and the mixture was stirred at RT for 48 h. After that, aqueous HCl (1M, 8 mL) was added. After stirring for another 0.5 h, the mixture was diluted with water (92 mL), evaporated under reduced pressure to remove the methanol, and then washed with ethyl acetate (4 x 100 mL). The aqueous layer was basified with aqueous NaOH solution (10 M, 20 mL) and extracted with ether (3 x 120 mL). The organic layers were combined, dried over anhydrous $MgSO_4$, and evaporated under reduced pressure to afford 97 mg (37%) of racemic **2g** as a yellow solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.63 (dd, 1H, J_1 = 3.7 Hz, J_2 = 10.6 Hz), 3.31 (dd, 1H, J_1 = 8.0 Hz, J_2 = 10.6 Hz), 2.87 (m, 1H), 1.47-1.21 (m, 8H), 0.91 (t, 3H, *J* = 6.7 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 66.4, 52.9, 34.3, 31.9, 25.7, 22.6, 14.0. HRMS (ESI): calcd. for $C_7H_{18}NO^+$ 132.1383 $[M+H]^+$, found 132.1384.

Analytical scale synthesis of chiral 1,2-amino alcohols using AmDHs

An analytical scale biocatalytic reaction was conducted in a 2-mL Eppendorf tube containing 0.5 mL NH_4COOH/NH_4OH buffer (1 M, pH 8.9) with substrate (50 mM), DMSO (2% v/v), NAD^+ (1 mM), lyophilised cell-free extract of FDH (3 mg) and purified AmDH (0.75 mg). The reaction mixture was shaken at 30 ºC and 250 rpm for 24 h. After that, the reaction mixture was analysed by HPLC or GC. Work-up for HPLC analysis: 100 μL of the reaction mixture was taken, quenched by mixing with 500 μL acetonitrile and filtered. Then, 20 μL of the filtrate was mixed with 20 μL of 1-fluoro-2,4-dinitrophenyl-5-L-alanine amide (Marfey's reagent) solution (14 mM in acetonitrile), 36 μL of aqueous $NaHCO_3$ (1 M) and 100 μL of DMSO for derivatization. The derivatization mixture was incubated at 40 ºC for 2 h, after which 40 μL of aqueous HCl (1 M) was added to quench the reaction. The resulting mixture was analysed by HPLC. Work-up for GC analysis: 100 μL of aqueous NaOH solution (10 M) was added to the 0.5 mL reaction mixture, which was then extracted with dichloromethane (600 μL). The organic phase was dried with anhydrous $MgSO_4$ and then analysed by GC.

Preparative scale synthesis of (*S*)-2-amino-1-hexanol (*S*)-2f using AmDH-M$_3$

A 100-mL scale biocatalytic reaction was conducted in NH_4COOH/NH_4OH buffer (1 M, pH 8.9) containing substrate **1f** (100 mM), DMSO (3.7% v/v), NAD^+ (1 mM), lyophilised cell-free extract of FDH (0.4 g) and lyophilised cell-free extract of AmDH (1.0 g). The reaction mixture was incubated at 30 ºC and 200 rpm for 24 h. During the reaction, 50 μL portions of the reaction mixture were taken at time points to determine reaction progress (**Table S4**). Each portion was mixed with 50 μL NH_4COOH/NH_4OH buffer (1 M, pH 8.9) and 500 μL acetonitrile, and then filtered. Afterwards, 20 μL of the filtrate was used for derivatization with Marfey's reagent using the method mentioned above, and then analysed by HPLC. The 100-mL scale biocatalytic reaction was then quenched by addition of aqueous NaOH (20 mL, 10 M) and extracted with dichloromethane (2 x 120 mL). The organic layers were combined and washed with aqueous HCl solution (100 mL, 1 M). After that, the aqueous layer was isolated, washed with dichloromethane (3 x 100 mL). The aqueous phase was basified by addition of aqueous NaOH (30 mL, 10 M) and extracted with dichloromethane (3 x 100 mL). Afterwards, the combined organic phases were washed with brine, dried over anhydrous $MgSO_4$ and then evaporated to afford the final product (*S*)-**2f** (662 mg, 5.6 mmol, 56%, >99% *ee*). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.60 (dd, 1H, J_1 = 3.8 Hz, J_2 =

10.5 Hz), 3.28 (dd, 1H, J_1 = 8.0 Hz, J_2 = 10.5 Hz), 2.83 (m, 1H), 1.35 (m, 6H), 0.93 (t, 3H, J = 6.9 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 66.8, 52.8, 34.3, 28.3, 22.8, 14.0. HRMS (ESI): calcd. for $C_6H_{16}NO^+$ 118.1226 $[M+H]^+$, found 118.1230.

Table S4. Reaction time-course of the preparative scale synthesis of (*S*)-2-amino-1-hexanol (*S*)-**2f**.

Time (h)	2	4	6	10
Conversion (%)	74	85	88	88

Preparative scale synthesis of (*S*)-2-amino-1-butanol (*S*)-2a using AmDH-M$_0$

A 100-mL scale biocatalytic reaction was conducted in NH_4COOH/NH_4OH buffer (1 M, pH 8.9) containing substrate **1a** (100 mM), DMSO (3.7% v/v), NAD^+ (1 mM), lyophilised cell-free extract of FDH (0.4 g) and lyophilised cell-free extract of AmDH (1.0 g). The reaction mixture was incubated at 30 ºC and 200 rpm for 24 h. During the reaction, 50 µL portions of the reaction mixture were taken at time points to determine reaction progress (**Table S5**). Each portion was mixed with 50 µL NH_4COOH/NH_4OH buffer (1 M, pH 8.9) and 500 µL acetonitrile, and then filtered. Afterwards, 20 µL of the filtrate was used for derivatization with Marfey's reagent using the method mentioned above, and then analysed by HPLC. The 100-mL scale biocatalytic reaction was then quenched by acidification to pH <2 by addition of 50% H_2SO_4, centrifuged to pellet insoluble material and purified via ion exchange column.[12] DOWEX® 50WX8 hydrogen form (25 g) was prepared by washing with 70 mL deionised water followed by 50 mL 5% w/v H_2SO_4. The acidified reaction supernatant was applied to the resin at 1 mL/min, and then washed with deionised water until the eluent was pH ~7. The column was eluted with 60 mL 9% w/v NH_4OH and dried via rotary evaporation to afford the final product (*S*)-**2a** (748 mg, 8.4 mmol, 84%, >99% *ee*).[13] ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.58 (dd, 1H, J_1 = 10.7 Hz, J_2 = 3.8 Hz), 3.27 (dd, 1H, J_1 = 10.7 Hz, J_2 = 7.9 Hz), 2.73 (tdd, 1H, J_1 = 7.9 Hz, J_2 = 5.6 Hz, J_3 = 3.8 Hz,), 2.07 (br. s, 3H), 1.53-1.37 (m, 1H), 1.35-1.19 (m, 1H), 0.93 (t, 3H, J_1 = 7.5 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 66.5, 54.5, 27.3, 10.6. HRMS (ESI): calcd. for $C_4H_{12}NO^+$ 90.0913 $[M+H]^+$, found 90.0914.

Table S5. Reaction time-course of the preparative scale synthesis of (*S*)-2-amino-1-butanol (*S*)-**2a**.

Time (h)	2	4	6
Conversion (%)	70	89	95

Analytical methods

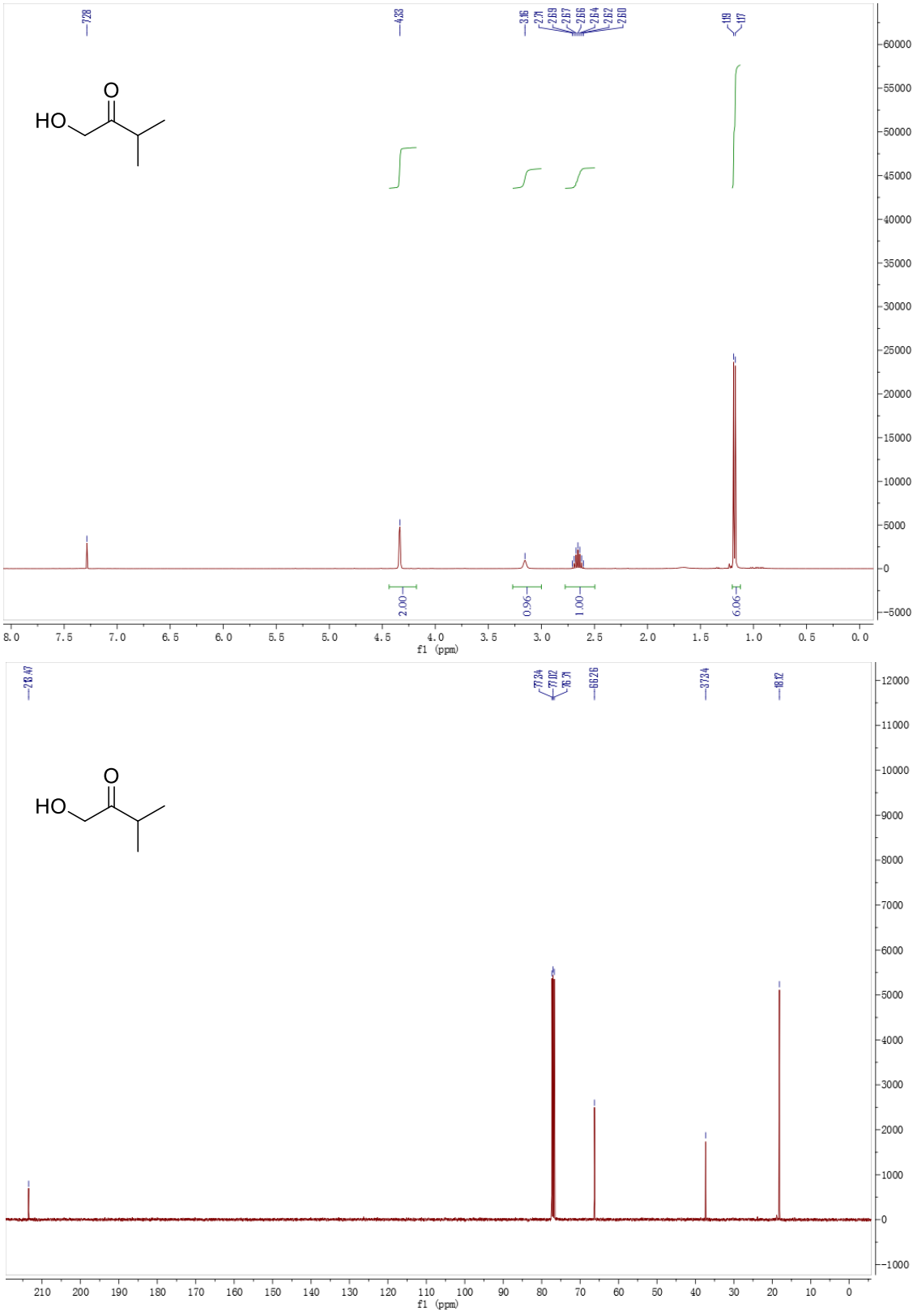
The conversions of α-hydroxy ketones **1a**–**1f** and **1h**, and the absolute configurations and *ee* of all the amino alcohol products were analysed via HPLC with a Phenomenex Luna 5µ C18(2) column (100 Å, 250 × 2.00 mm 5 µm) after derivatisation. For quantitative determination of the conversions of **1a**–**1f** and **1h**, calibration curves of chiral amino alcohols were prepared using external standards. The conversion of **1g** was determined via GC using an Agilent HP-1 column (30 m × 0.32 mm × 0.25 µm). The absolute configurations and *ee* of products **1a**–**1f** and **1h** were determined by co-injection with commercial standard reference materials after derivatisation. The *ee* of product **2g** was measured via HPLC by co-injection with synthesised racemic **2g** after derivatisation. The absolute configuration of product **2g** was assigned by comparison of elution orders, considering that after derivatization with Marfey's reagent the (*R*)-enantiomer formed diastereomer derivative interacts more strongly with the reverse phase column (C18) and thus has a longer retention time than that of the (*S*)-enantiomer.[14] The detailed HPLC/GC analytical conditions are shown in **Table S6**.

Table S6. HPLC/GC conditions used for analysis of the reaction samples.

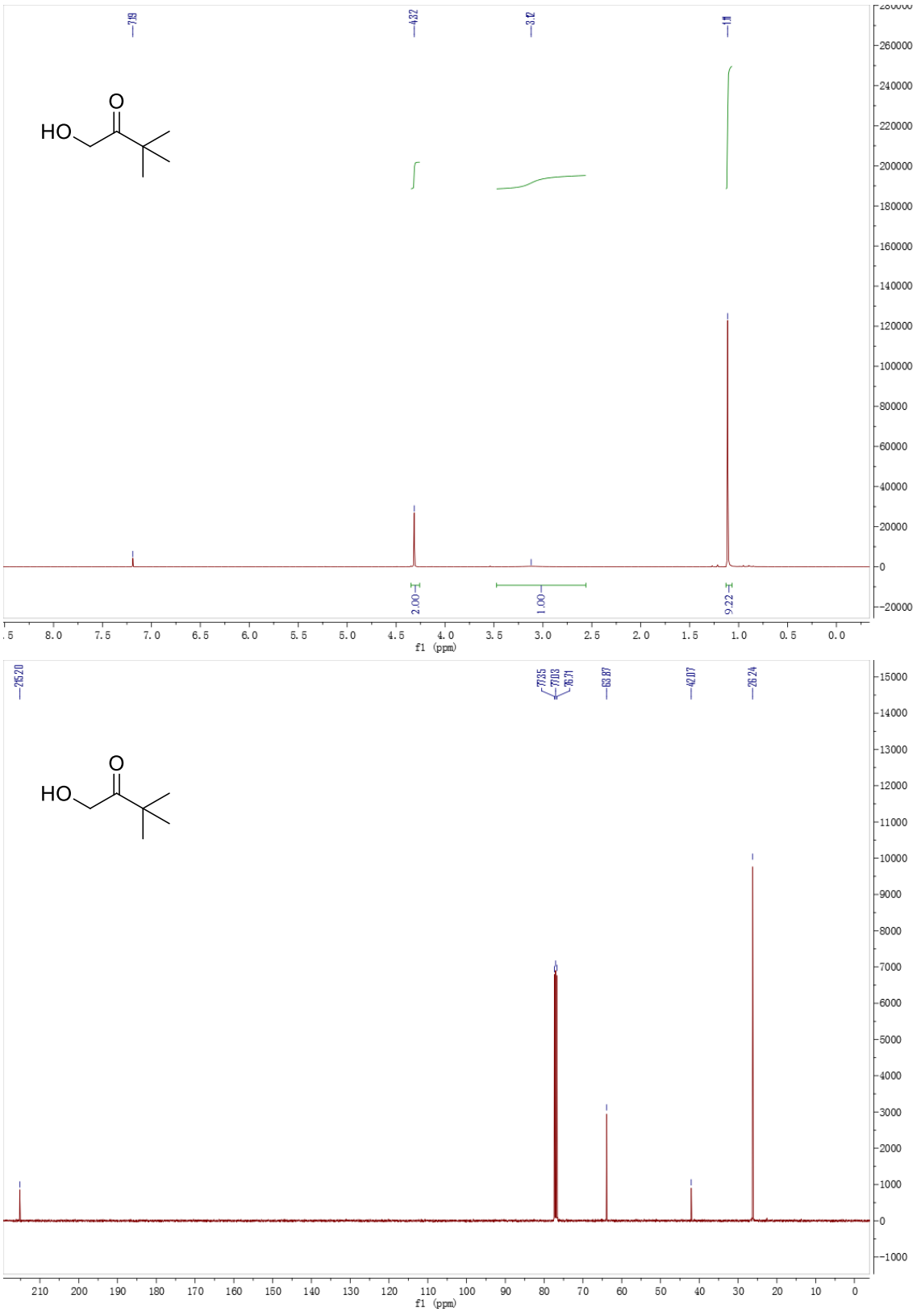
Substrate	Product	Analytical conditions[a]	Retention time (min)		
			Substrate	(*R*)-Product	(*S*)-Product
1a	**2a**	HPLC/C18 column; 75% A/25% B; increase B to 40% in 2 min, hold for 6 min; increase B to 60% in 8 min, hold for 8 min.	n.a.[b]	22.2	16.7
1b	**2b**	HPLC/C18 column; 75% A/25% B; increase B to 40% in 2 min, hold for 2 min; increase B to 60% in 8 min, hold for 17 min.	n.a.	24.1	15.9
1c	**2c**	HPLC/C18 column; 75% A/25% B; increase B to 40% in 2 min, hold for 0 min; increase B to 60% in 2 min, hold for 25 min.	n.a.	27.2	12.7
1d	**2d**	HPLC/C18 column; 40% A/60% B, hold for 12 min; increase B to 80% in 8 min, hold for 10 min.	n.a.	8.5	4.1
1e	**2e**	HPLC/C18 column; 40% A/60% B, hold for 12 min; increase B to 80% in 8 min, hold for 10 min.	n.a.	13.6	5.6
1f	**2f**	HPLC/C18 column; 40% A/60% B, hold for 12 min; increase B to 80% in 8 min, hold for 10 min.	n.a.	14.5	5.9
1g	**2g**	GC/HP-1 column; 50ºC, hold for 2 min; 10ºC/min to 120ºC, hold for 2 min; 20ºC/min to 250ºC, hold for 1 min.	8.2	9.4	9.4
1g	**2g**	HPLC/C18 column; 40% A/60% B, hold for 12 min; increase B to 80% in 8 min, hold for 10 min.	n.a.	20.3	9.3
1h	**2h**	HPLC/C18 column; 75% A/25% B; increase B to 40% in 2 min, hold for 0 min; increase B to 60% in 2 min, hold for 25 min.	n.a.	19.6	10.7

[a]HPLC conditions: injection volume 5 µL, oven temperature 25ºC, flow rate 0.45 mL/min, detection wavelength 340 nm; buffer A: water (0.1% trifluoroacetic acid), buffer B: methanol (0.1% trifluoroacetic acid). GC conditions: injector 250ºC, 6.8 psi, split ratio 30:1, FID detector 250ºC. [b]n.a. = not available.

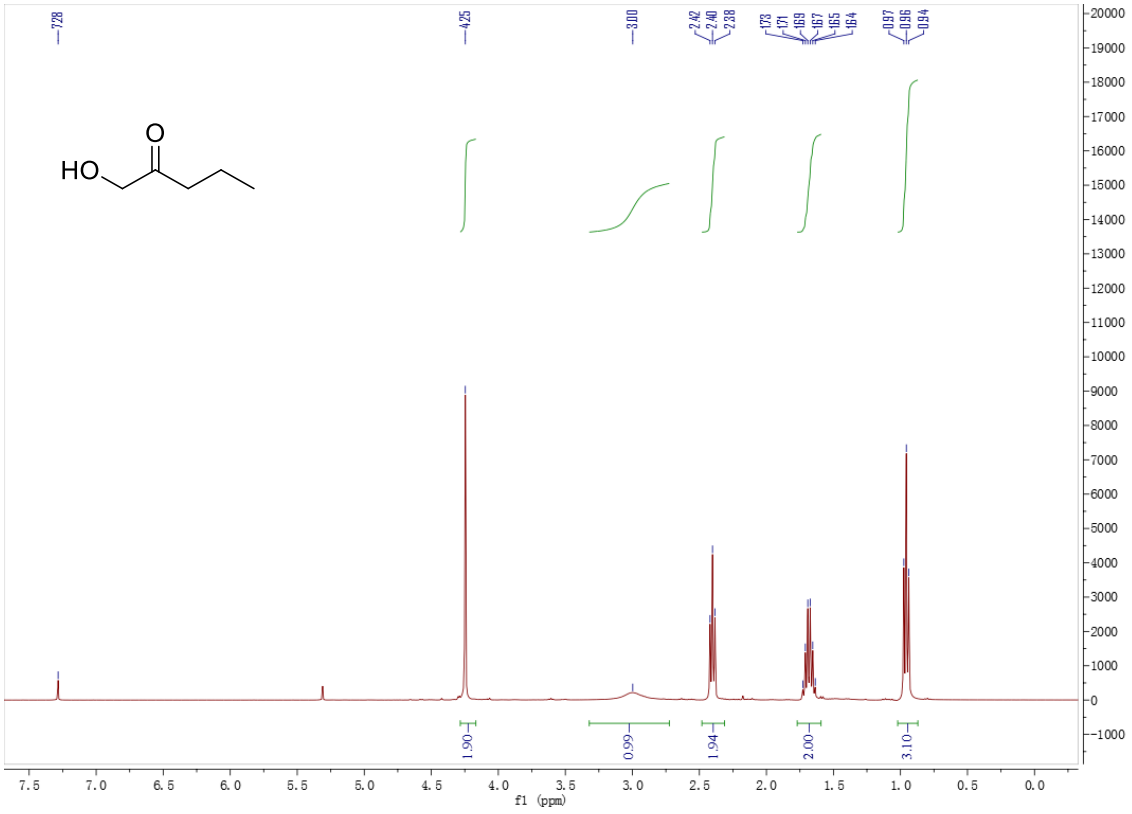
NMR spectra

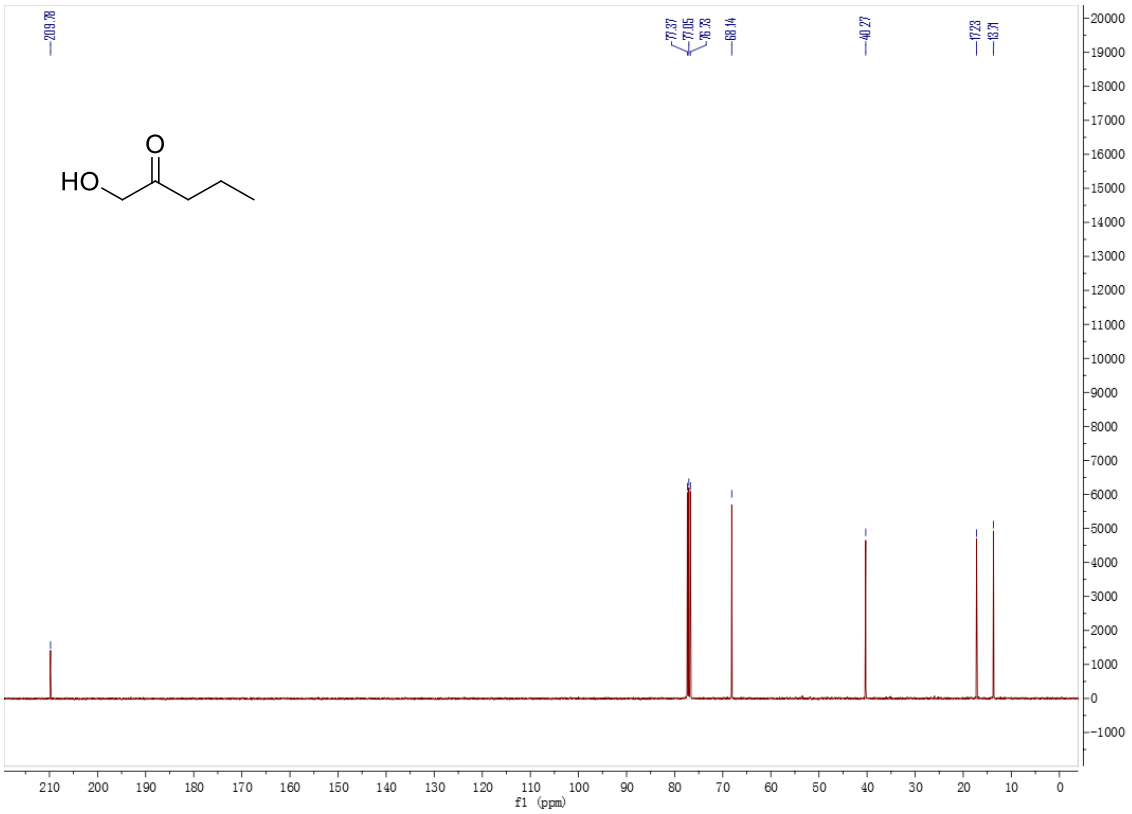


^{1}H NMR and ^{13}C NMR spectra of synthesised 1-hydroxy-3-methyl-2-butanone **1b**.

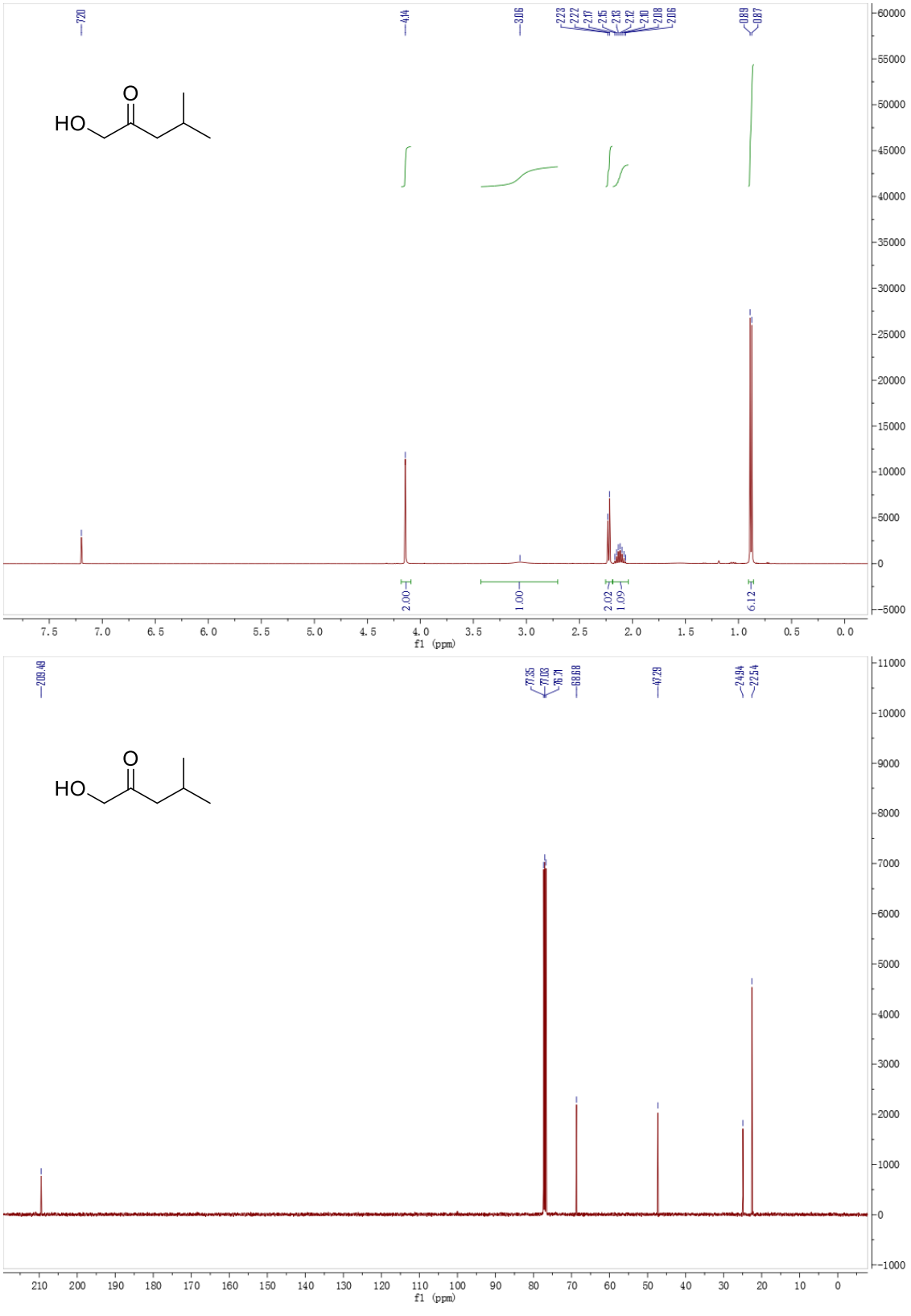


^{1}H NMR and ^{13}C NMR spectra of synthesised 1-hydroxy-3,3-dimethyl-2-butanone **1c**.

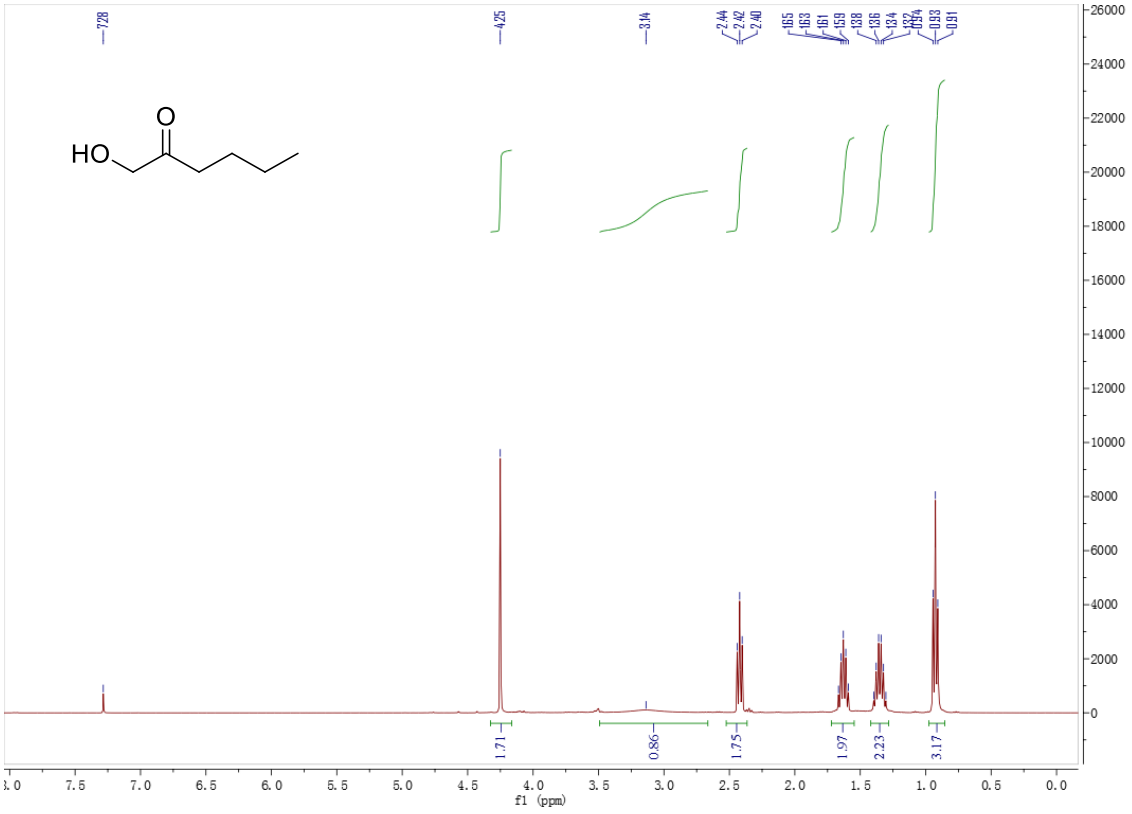


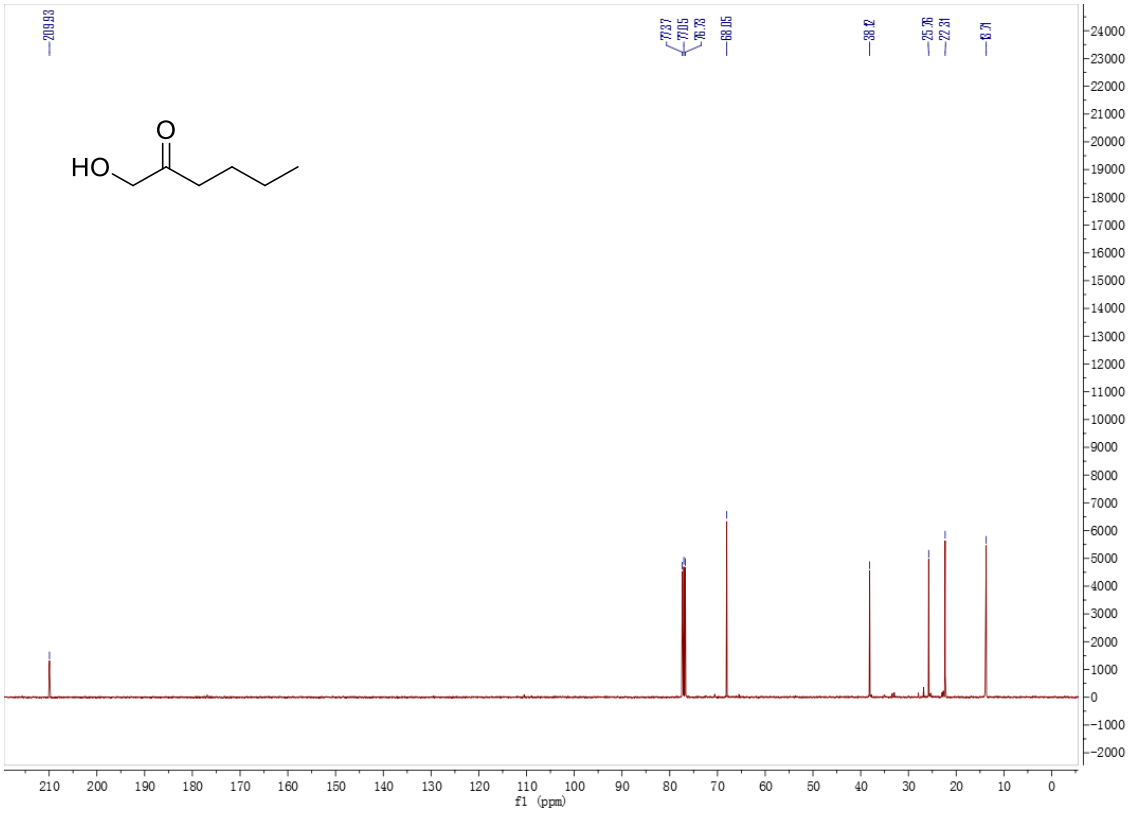


^{1}H NMR and ^{13}C NMR spectra of synthesised 1-hydroxy-2-pentanone **1d**.

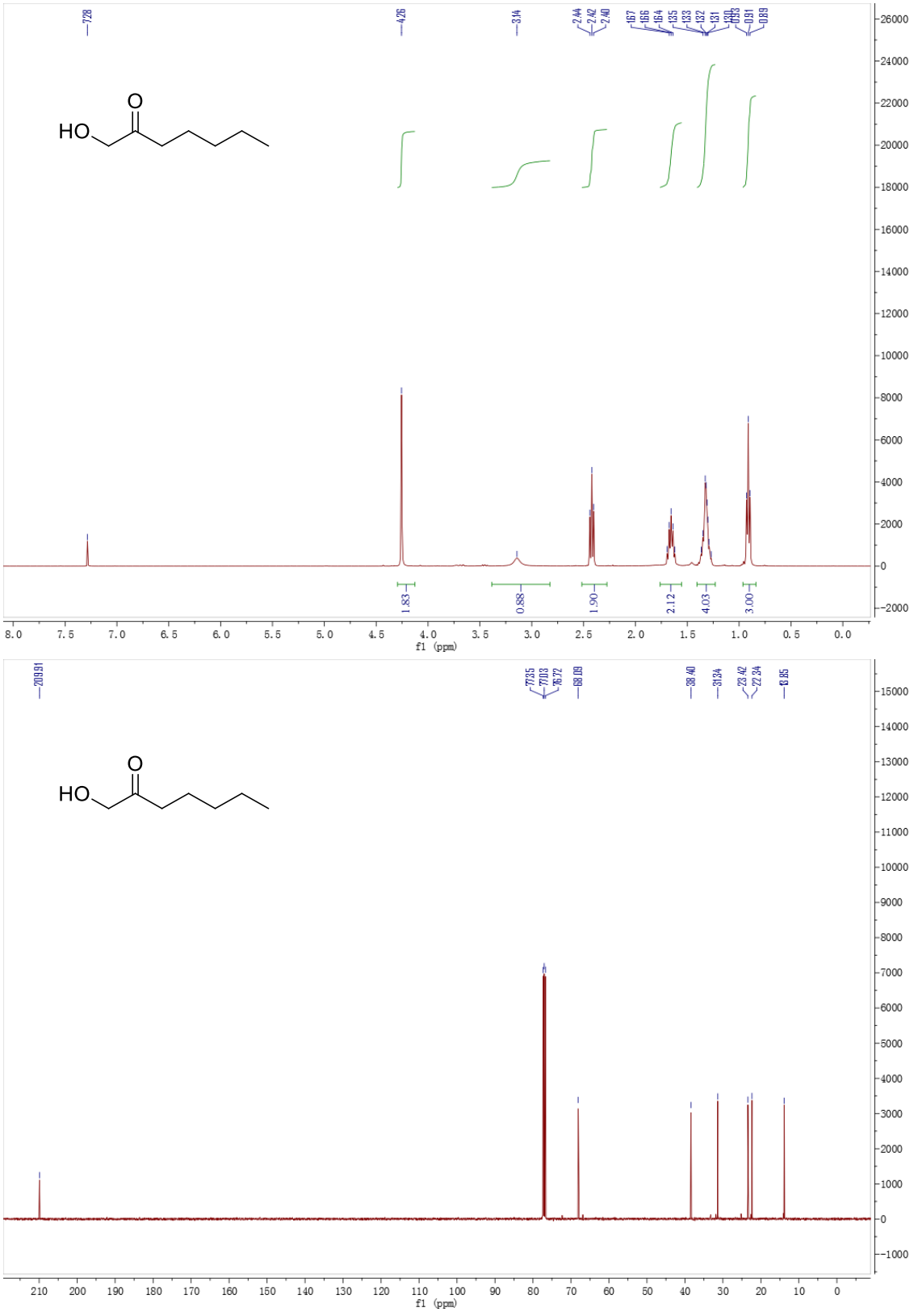


^{1}H NMR and ^{13}C NMR spectra of synthesised 1-hydroxy-4-methyl-2-pentanone **1e**.

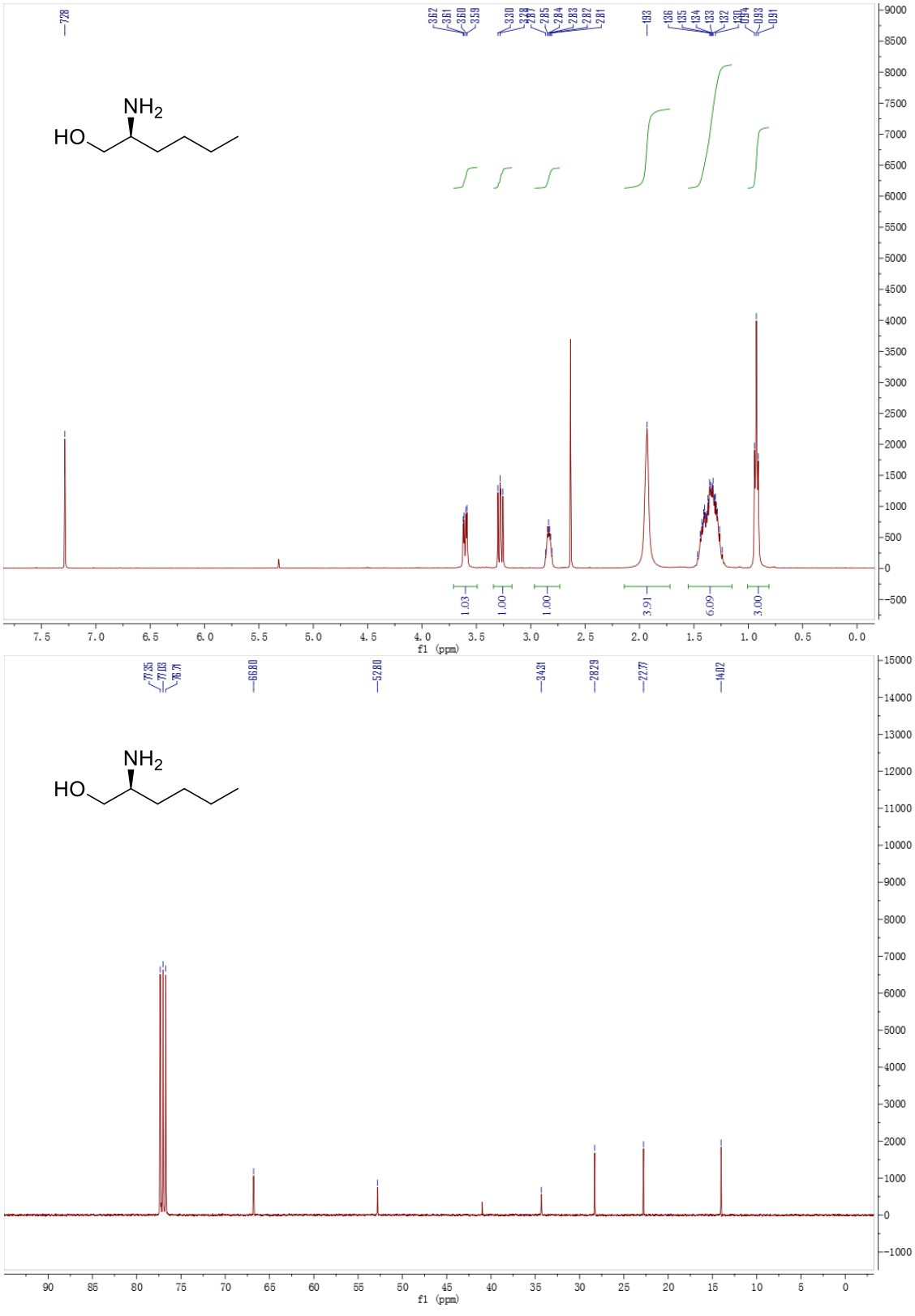




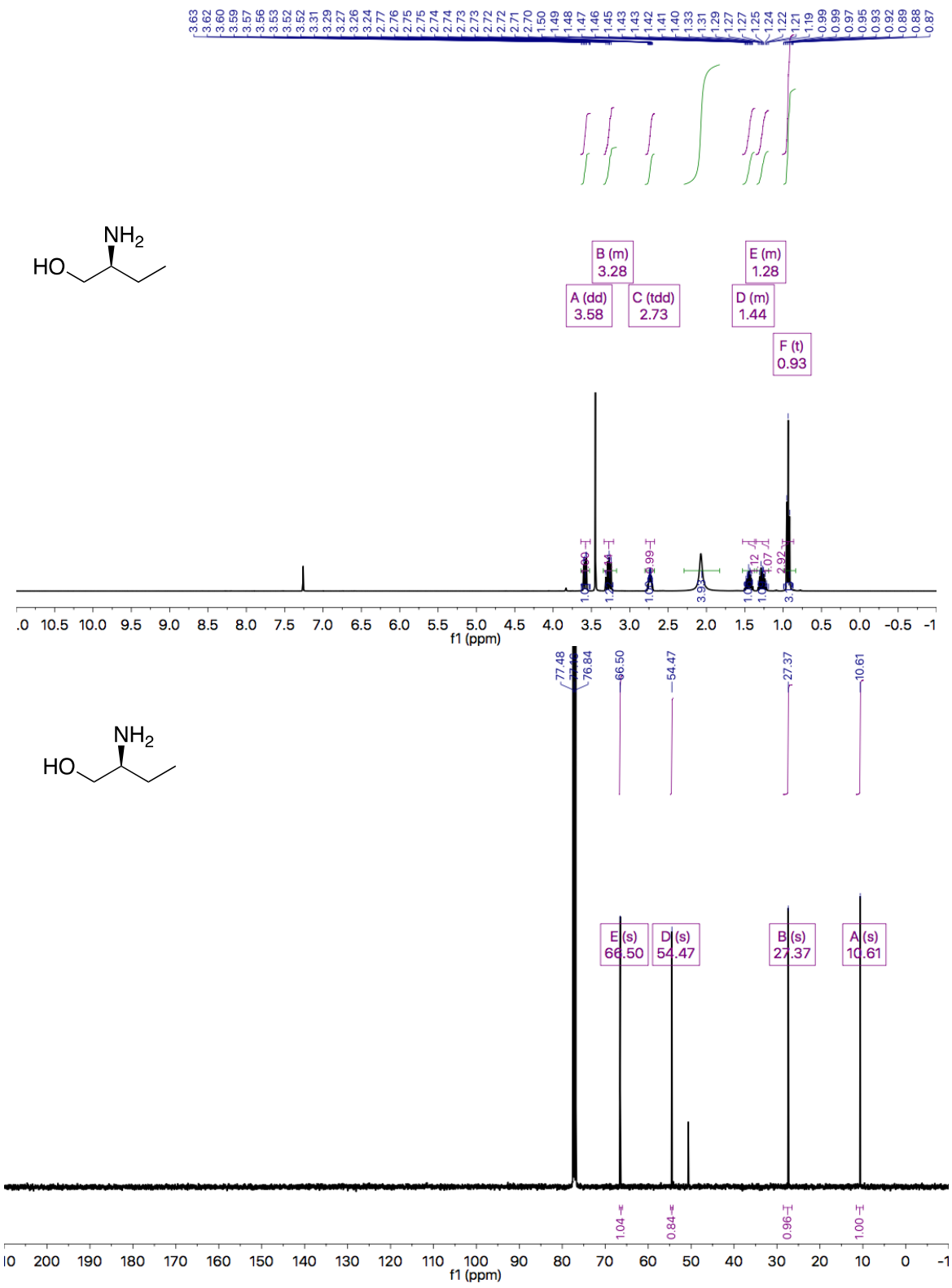
^{1}H NMR and ^{13}C NMR spectra of synthesised 1-hydroxy-2-hexanone **1f**.



^{1}H NMR and ^{13}C NMR spectra of synthesised 1-hydroxy-2-heptanone **1g**.



^{1}H NMR and ^{13}C NMR spectra of product (*S*)-2-amino-1-hexanol (*S*)-**2f** isolated from the preparative scale reaction.



^{1}H NMR and ^{13}C NMR spectra of product (*S*)-2-amino-1-butanol (*S*)-**2a** isolated from the preparative scale reaction.

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