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Supporting Information

Data mining of amine dehydrogenases for the synthesis of enantiopure amino alcohols

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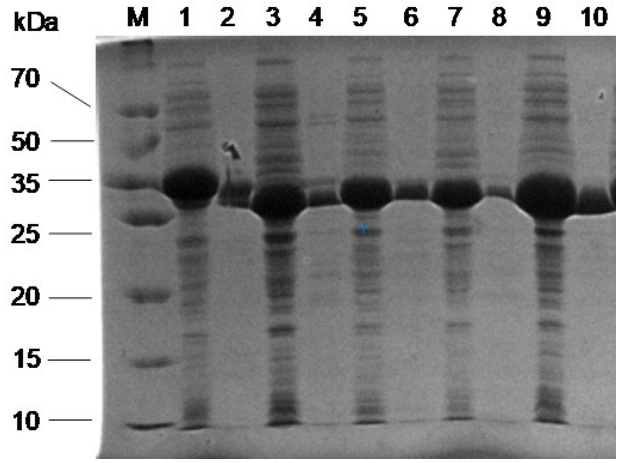


Figure S1. SDS-PAGE analysis of the protein expression and purification of AmDHs. M: protein marker. Lane 1-2: the crude extract and purified enzyme of *Gs*AmDH; Lane 3-4: the crude extract and purified enzyme of *Ti*AmDH; Lane 5-6: the crude extract and purified enzyme of *Bs*AmDH; Lane 7-8: the crude extract and purified enzyme of *Sp*AmDH; Lane 9-10: the crude extract and purified enzyme of *Ls*AmDH.

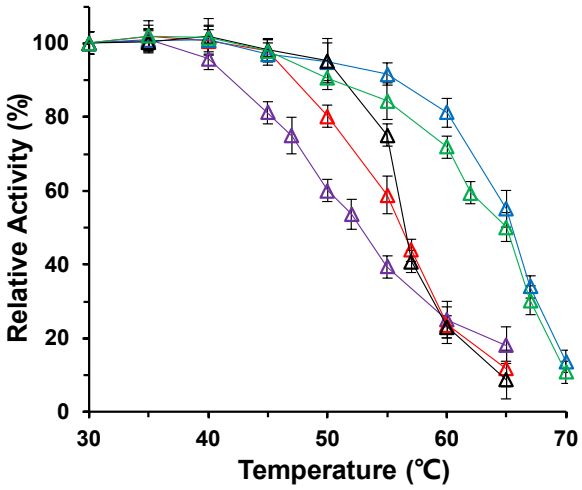


Figure S2. The thermostability analysis of AmDHs (▲: *Gs*AmDH, △ : *Bs*AmDH, △ : *Ls*AmDH, △ : *Sp*AmDH, △: *Ti*AmDH), purified AmDHs (1 mg/mL) were incubated at different temperatures (30~70 °C) for 15 min, followed by measuring the residual activity towards substrate **1a** (10 mM). The initial activities of *Gs*AmDH, *Bs*AmDH, *Ls*AmDH, *Sp*AmDH and *Ti*AmDH were determined to be 0.38 U/mg, 0.30 U/mg, 0.35 U/mg, 0.28 U/mg, 0.14 U/mg, respectively, towards substrate **1a**, after incubating at 30 °C for 15 min.

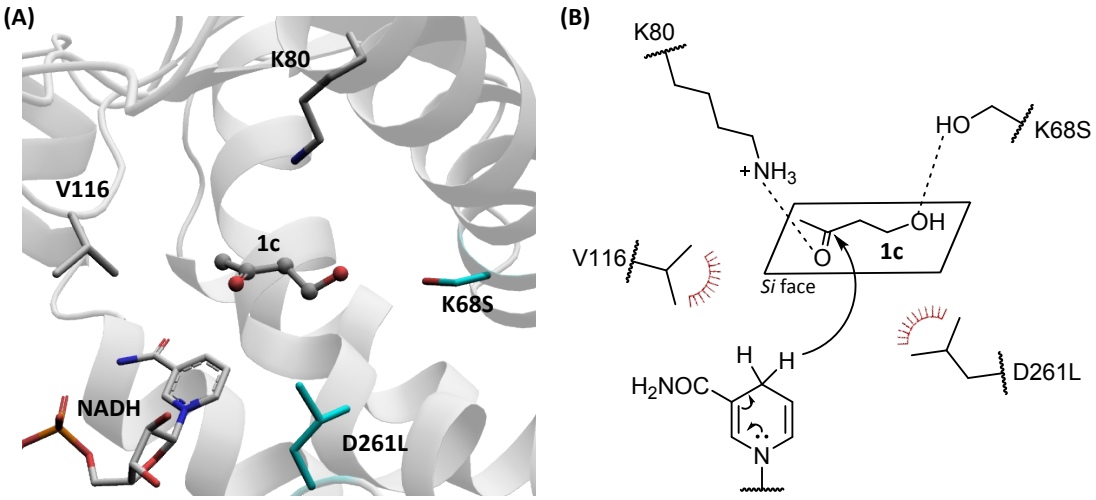


Figure S3. Docking analysis of substrate **1c** in GsAmDH. **(A)** Highest ranked docking pose for substrate **1c** in the catalytic pocket of *Gs*AmDH; **(B)** Schematic representation of the interactions between substrate and surrounding residues. Nucleophilic attack (black arrow) at the *Si* face of the carbonyl group of **1c**, resulting in formation of (*S*)-**2c**. Hydrogen bonds are indicated by black lines, while penitential hydrophobic interactions are shown in red spikes.

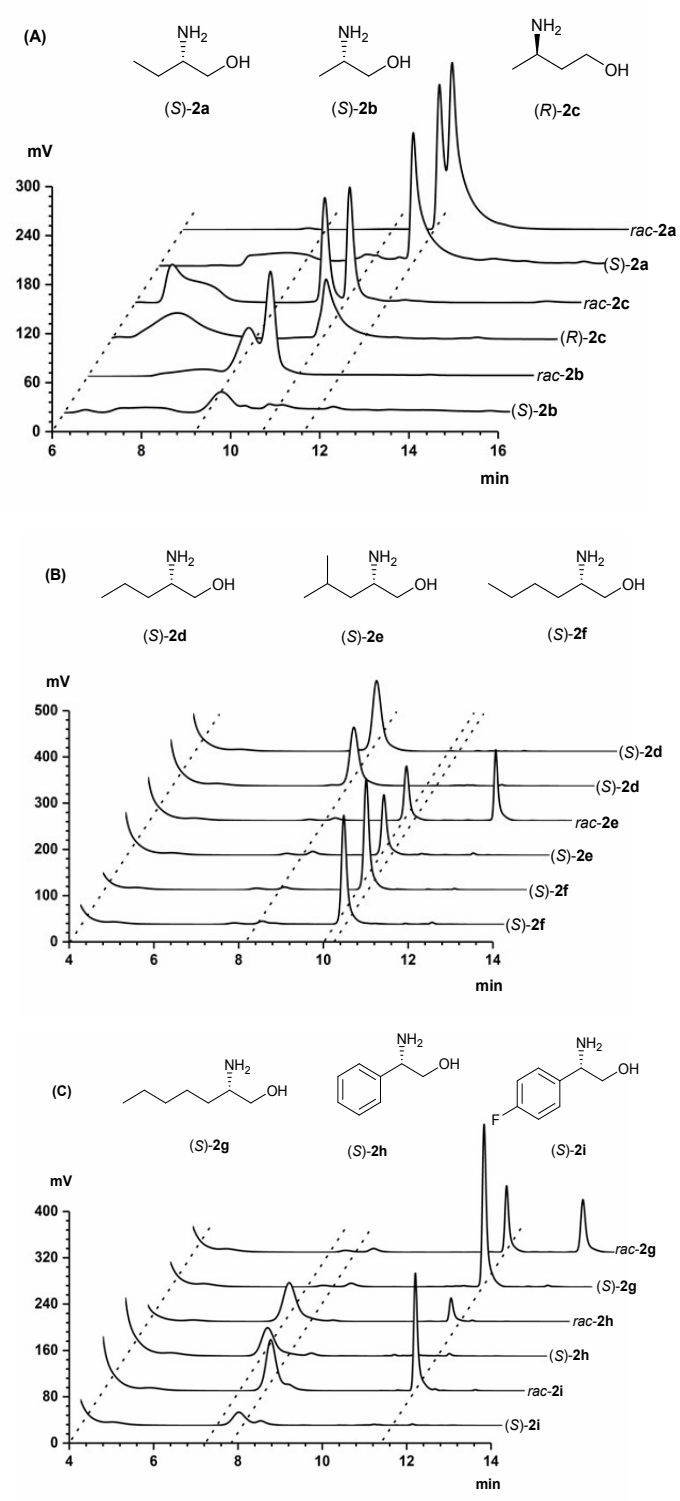
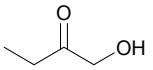
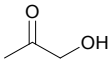
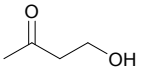
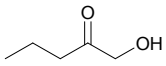
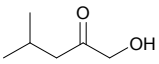
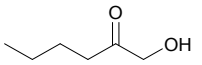
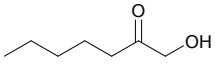
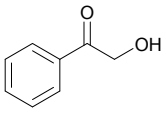
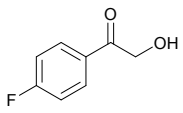


Figure S4. HPLC profiles of amino alcohols standards and products catalyzed by *Gs*AmDH. **(A)** HPLC profiles of standards *rac*-**2a**, *rac*-**2c**, *rac*-**2b** and products (*S*)-**2a**, (*R*)-**2c**, (*S*)-**2b**; **(B)** HPLC profiles of standards (*S*)-**2d**, *rac*-**2e**, (*S*)-**2f** and products (*S*)-**2d**, (*S*)-**2e**, (*S*)-**2f**; **(C)** HPLC profiles of standards *rac*-**2g**, *rac*-**2h**, *rac*-**2i** and products (*S*)-**2g**, (*S*)-**2h**, (*S*)-**2i**.

Table S1. Analytical conditions of HPLC.

Substrates	Structures	Products	Retention time (min)		
			Substrate	(*S*)-Product	(*R*)-Product
1a	 1-hydroxybutan-2-one	**2a** [a]	n.a. [c]	11.7	12.0
1b	 1-hydroxypropan-2-one	**2b** [a]	n.a. [c]	9.6	10.1
1c	 4-hydroxybutan-2-one	**2c** [a]	n.a. [c]	10.3	10.8
1d	 1-hydroxypentan-2-one	**2d** [b]	n.a. [c]	8.3	n.a. [c]
1e	 1-hydroxy-4-methylpentan-2-one	**2e** [b]	n.a. [c]	10.1	12.2
1f	 1-hydroxyhexan-2-one	**2f** [b]	n.a. [c]	10.2	n.a. [c]
1g	 1-hydroxyheptan-2-one	**2g** [b]	n.a. [c]	11.4	13.2
1h	 2-hydroxy-1-phenylethan-1-one	**2h** [b]	n.a. [c]	7.4	11.2
1i	 1-(4-fluorophenyl)-2-hydroxy ethan-1-one	**2i** [b]	n.a. [c]	8.0	11.4

[a] HPLC conditions: Agilent SB-Aq C18 column (4.6*250 mm, 5 μm), injection volume 10 μL, column temperature 35 ºC, flow rate 1 mL/min, detection wavelength 334 nm; buffer A: 0.05M sodium acetate, buffer B: methanol, gradient program: 70% A/30% B; 30% B, hold for 6 min, increase B to 45% in 1 min, hold for 8 min; decrease B to 30% in 0.5 min, hold for 4.5 min.

[b] HPLC conditions: Zorbax SB-C18 column (4.6*150 mm, 5 μm), injection volume 10 μL, column temperature 25 ºC, flow rate 1 mL/min, detection wavelength 340 nm; buffer A: ddH_2O (0.1% trifluoroacetic acid), buffer B: methanol (0.1% trifluoroacetic acid), gradient program: 40% A/60% B; decrease B to 40% in 3 min, hold for 0 min; increase B to 60% in 4 min, hold for 0 min; increase B to 80% in 3 min, hold for 3 min, decrease B to 60% in 2 min, hold for 0 min.

[c] n.a. = not available.

Synthesis of α-hydroxy ketone substrates

General information:

^{1}H, ^{13}C spectra were recorded on Bruker AV 400 MHz instrument at 400 MHz (^{1}H NMR), 100 MHz (^{13}C NMR). or Bruker AV 600 MHz instrument at 600 MHz (^{1}H NMR), 150 MHz (^{13}C NMR). Chemical shifts were reported in ppm down field from internal Me_4Si and external CCl_3F, respectively. Multiplicity was indicated as follows: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), dd (doublet of doublet), br (broad). Coupling constants are reported in Hertz (Hz).

General procedure A: synthesis of **1d** and **1f** [1,2]

To a solution of the corresponding diol (37 mmol) in acetic acid (30 mL) was slowly added aqueous 2.8 M NaClO solution (13 mL, 1.1 equiv) at room temperature. After completion of the reaction (monitored by TLC), the mixture was then extracted with dichloromethane (3 x 100 mL). The combined organic phases were washed with aqueous $NaHCO_3$ solution and brine, dried over anhydrous $MgSO_4$ and concentrated in vacuo. Flash chromatography on silica gel with petroleum ether/ethyl acetate (6:1-2:1) afforded the α-hydroxy ketone product.

General procedure B: synthesis of **1e** and **1g** [1,2]

To a mixture of the corresponding olefin (21 mmol), acetone (170 mL), water (38 mL) and acetic acid (8 mL) at room temperature was added dropwise a $KMnO_4$ solution (24 mmol, 5 g in 64 mL acetone and 21 mL water) and the mixture was stirred at this temperature until the olefin was completely converted (by TLC). Then the reaction mixture was filtered through a celite pad. The combined filtrate was concentrated under reduced pressure to remove the acetone and then extracted with dichloromethane (3 x 100 mL). The combined organic layers were neutralized by repeated washings with aqueous $NaHCO_3$ solution (1 M, 3 x 100 mL), dried over anhydrous $MgSO_4$, and concentrated in vacuo. Purification by flash chromatography on silica get eluting with petroleum ether/ethyl acetate (6:1-2:1) gave the α-hydroxy ketone product.

1-hydroxypentan-2-one (**1d**)

Colorless liquid; 1.80 g; 50% yield; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 4.19 (d, J = 3.6 Hz, 1H), 3.69 – 3.08 (m, 1H), 2.35 (t, J = 7.4 Hz, 1H), 1.76 – 1.52 (m, 1H), 0.90 (t, J = 7.4 Hz, 2H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 209.9, 68.1, 40.3, 17.2, 13.7.

1-hydroxy-4-methylpentan-2-one (**1e**)

Colorless liquid; 0.39 g; 18% yield; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 5.33 (s, 1H), 4.24 (s, 4H), 2.32 (d, *J* = 7.0 Hz, 5H), 2.28 – 2.14 (m, 3H), 0.97 (d, *J* = 6.6 Hz, 14H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 209.5, 68.7, 47.3, 24.9, 22.5.

1-hydroxyhexan-2-one (**1f**)

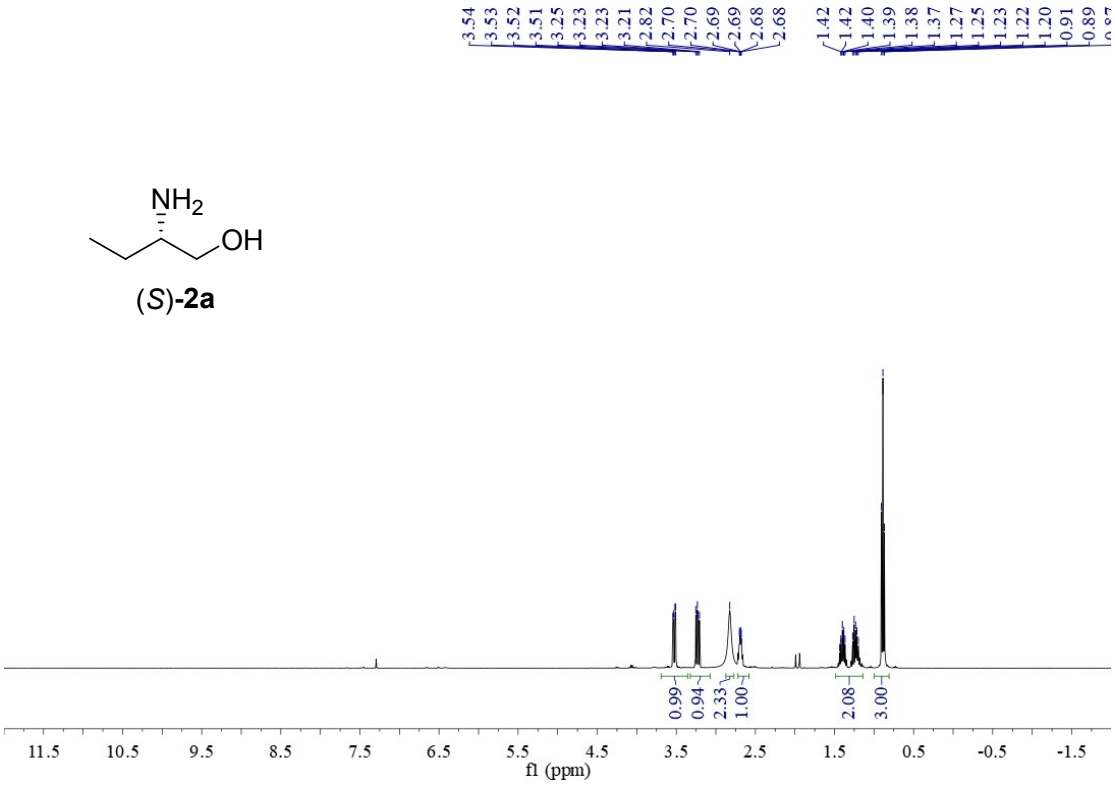
Colorless liquid; 2.56 g; 60% yield; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 4.27 (d, *J* = 3.4 Hz, 1H), 3.19 (s, 1H), 2.44 (t, *J* = 7.5 Hz, 1H), 1.65 (dt, *J* = 15.2, 7.5 Hz, 1H), 1.49 – 1.21 (m, 1H), 0.95 (t, *J* = 7.3 Hz, 2H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 210.0, 68.0, 38.1, 25.7, 22.3, 13.7.

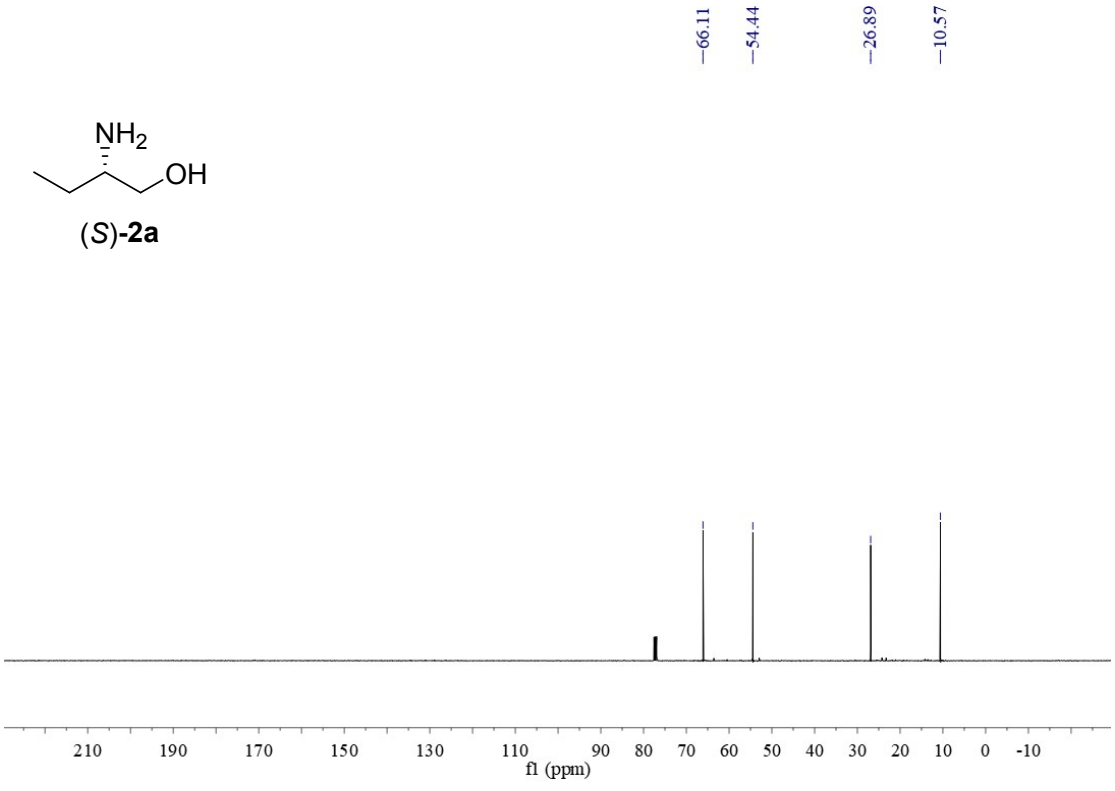
1-hydroxyheptan-2-one (**1g**)

Colorless liquid; 0.93 g; 34% yield; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 4.24 (s, 1H), 3.16 (s, 1H), 2.41 (t, *J* = 7.5 Hz, 1H), 1.78 – 1.54 (m, 1H), 1.31 (dt, *J* = 7.4, 4.8 Hz, 2H), 0.89 (t, *J* = 6.9 Hz, 2H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 210.0, 68.1, 38.4, 31.3, 23.4, 22.3, 13.8.

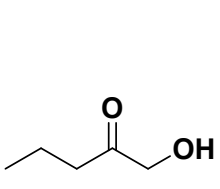
NMR spectra

(*S*)-2-aminobutan-1-ol (2a)





1-hydroxypentan-2-one (1d)



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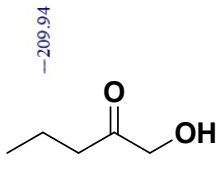

1d

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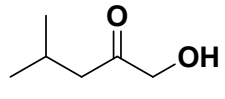

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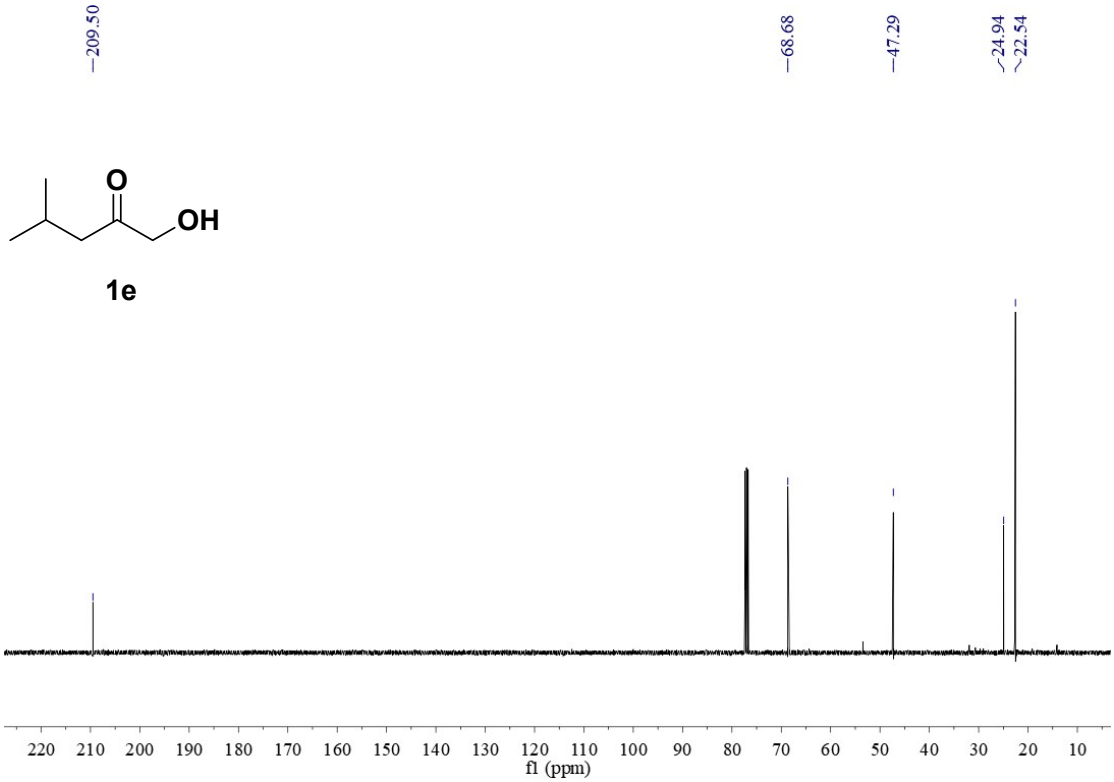

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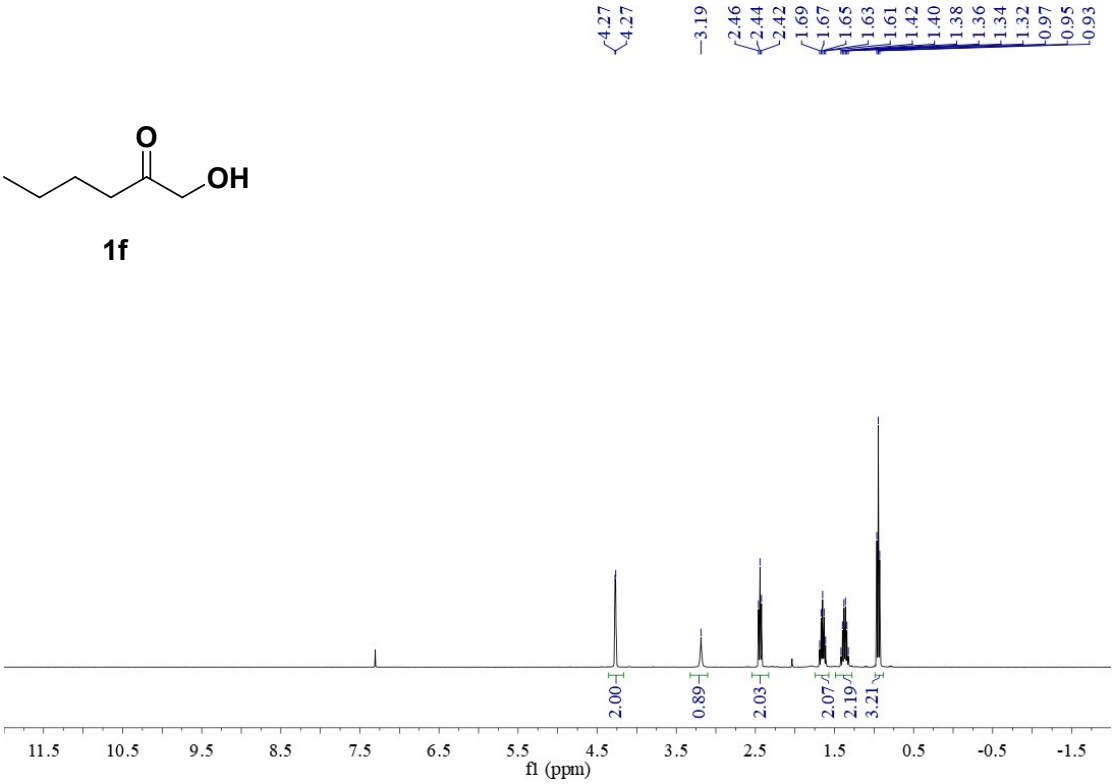

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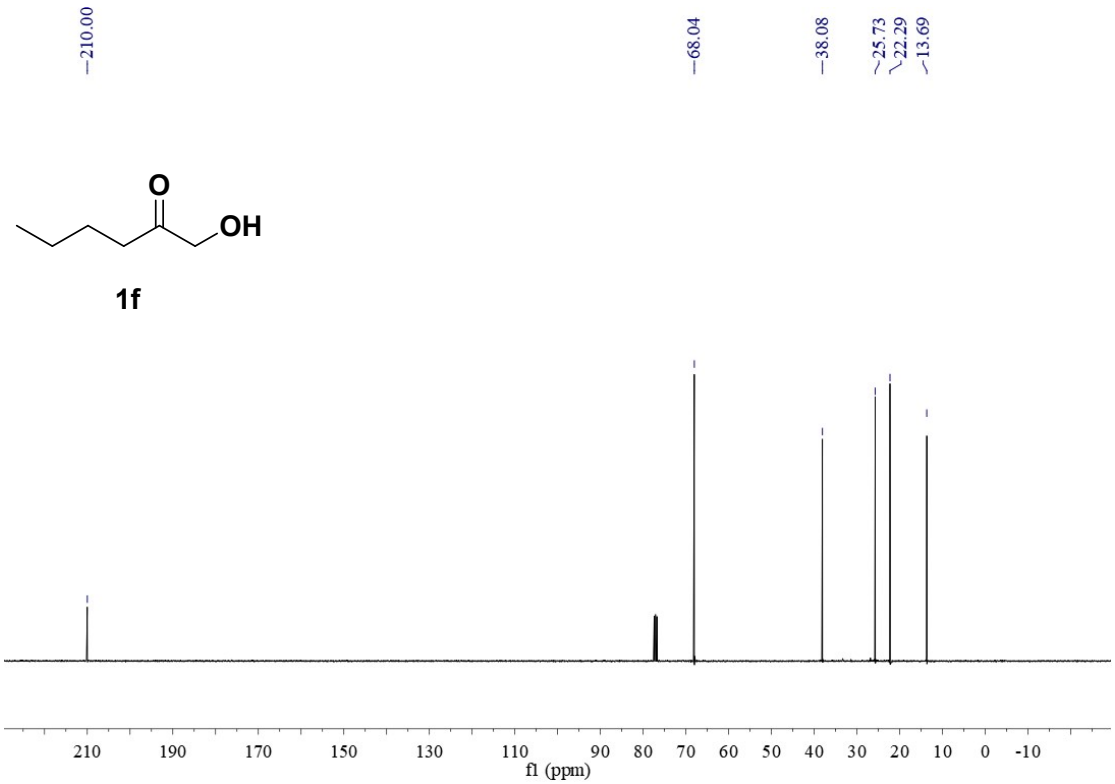
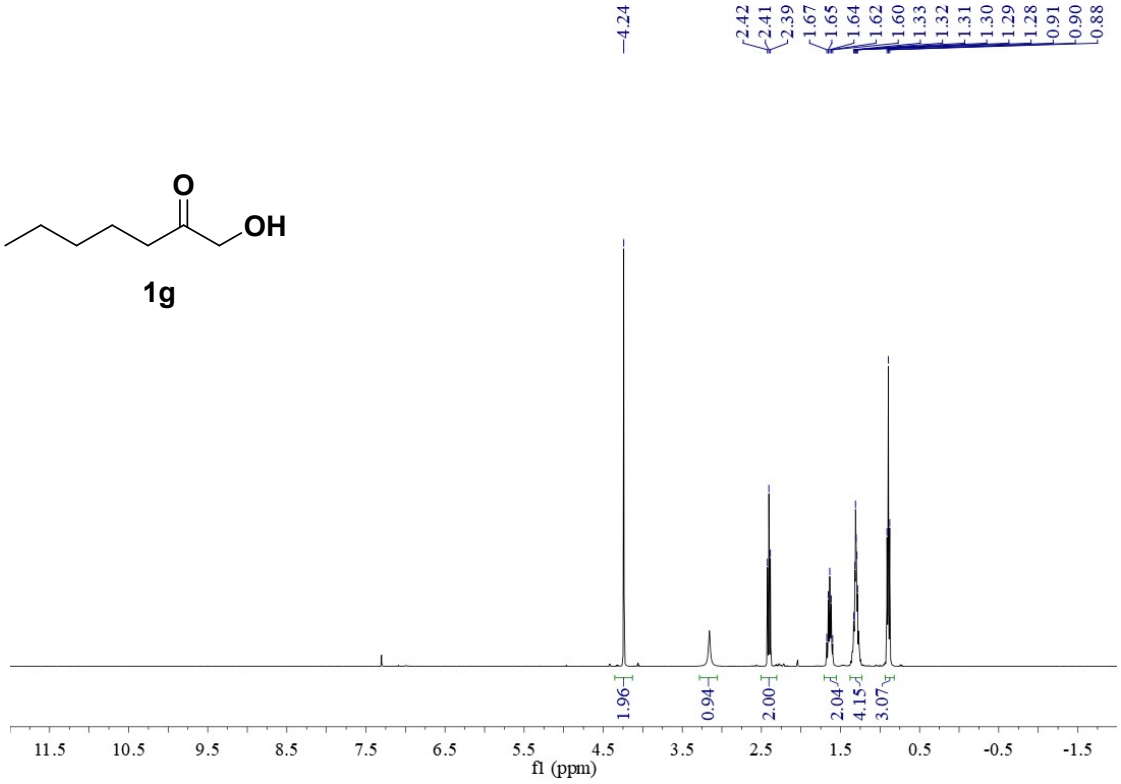
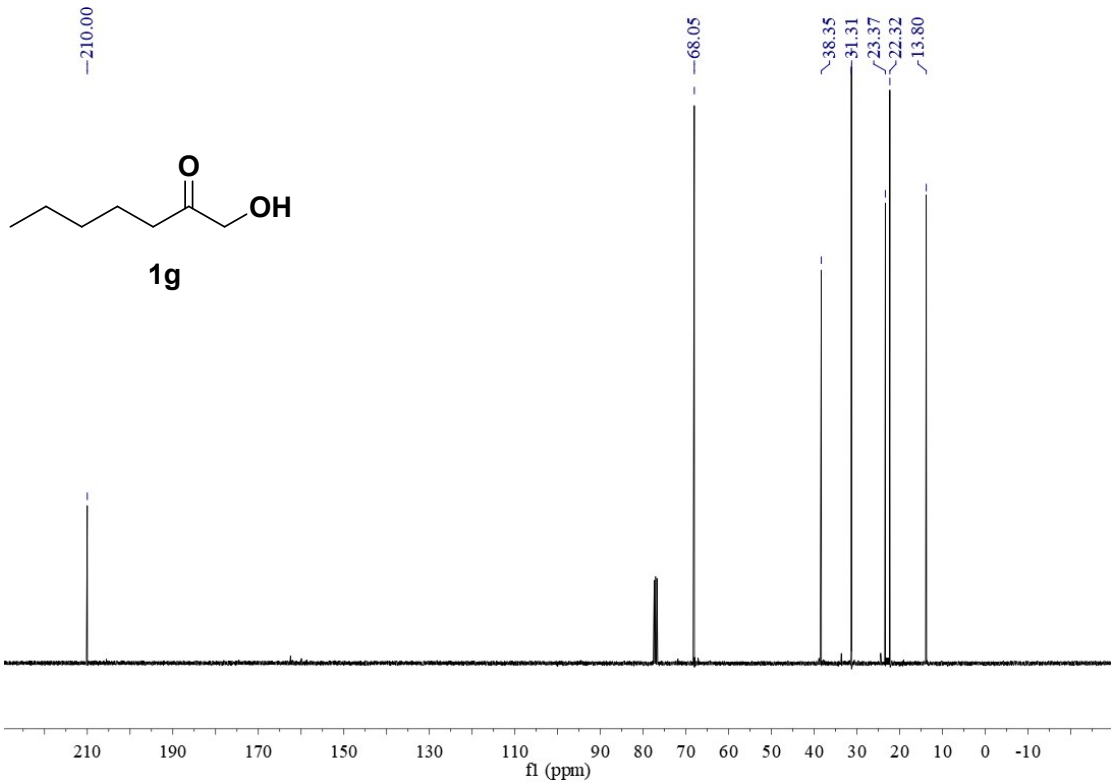


1-hydroxyheptan-2-one (1g)





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