Supporting Information

Engineered Artificial Biosynthesis Pathway Enables Simultaneous production and In-Situ Bio-dyeing of Indigoids for Textiles

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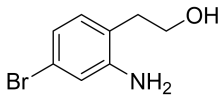
Table of contents

Characterization of substrates and products

2-(2-amino-4-bromophenyl)ethan-1-ol (1a)

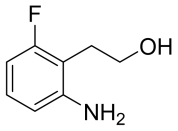


$C_8H_{10}BrNO$: 216.08 g/mol

^{1}H NMR (400 MHz, DMSO-d_6) δ 6.00 (d, *J* = 8.0 Hz, 1H), 5.94 (d, *J* = 2.1 Hz, 1H), 5.75 (dd, *J* = 7.9, 2.1 Hz, 1H), 4.31 (s, 2H), 3.78 (t, *J* = 5.1 Hz, 1H), 2.76 – 2.65 (m, 2H), 1.70 (t, *J* = 6.8 Hz, 2H).

^{13}C NMR (101 MHz, DMSO-d6) δ 132.14, 122.85, 119.82, 118.68, 116.85, 60.76.

2-(2-amino-6-fluorophenyl)ethan-1-ol (2a)

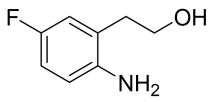


$C_8H_{10}FNO$: 155.17 g/mol

^{1}H NMR (400 MHz, DMSO-d_6) δ 6.06 – 5.96 (m, 1H), 5.58 (d, *J* = 8.0 Hz, 1H), 5.42 (t, *J* = 9.0 Hz, 1H), 4.30 (s, 2H), 3.86 (t, *J* = 5.2 Hz, 1H), 2.69 – 2.59 (m, 2H), 1.79 (td, *J* = 7.0, 1.9 Hz, 2H).

^{13}C NMR (101 MHz, DMSO-d6) δ 161.06, 149.46, 127.66, 111.01, 110.23, 102.64, 60.50, 27.32.

2-(2-amino-5-fluorophenyl)ethan-1-ol (3a)

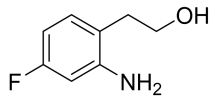


$C_8H_{10}FNO$: 155.17 g/mol

^{1}H NMR (400 MHz, DMSO-d_6) δ 5.91 (dd, *J* = 9.9, 3.1 Hz, 1H), 5.85 (td, *J* = 8.6, 3.1 Hz, 1H), 5.73 (dd, *J* = 8.7, 5.3 Hz, 1H), 3.85 (s, 2H), 3.80 (t, *J* = 5.1 Hz, 1H), 2.80 – 2.66 (m, 2H), 1.72 (t, *J* = 6.7 Hz, 2H).

^{13}C NMR (101 MHz, DMSO-d6) δ 153.76, 143.46, 116.44, 60.80, 34.72.

2-(2-amino-4-fluorophenyl)ethan-1-ol (4a)

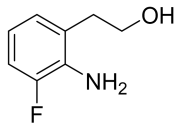


$C_8H_{10}FNO$: 155.17 g/mol

^{1}H NMR (400 MHz, DMSO-d_6) δ 6.10 – 5.98 (m, 1H), 5.52 (dd, *J* = 11.7, 2.7 Hz, 1H), 5.38 (td, *J* = 8.5, 2.7 Hz, 1H), 4.30 (s, 2H), 3.76 (t, *J* = 5.1 Hz, 1H), 2.74 – 2.63 (m, 2H), 1.70 (t, *J* = 6.8 Hz, 2H).

^{13}C NMR (101 MHz, DMSO-d6) δ 160.96, 148.96, 131.49, 119.61, 102.24, 101.05, 61.09, 34.26.

2-(2-amino-3-fluorophenyl)ethan-1-ol (5a)

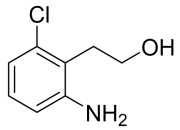


$C_8H_{10}FNO$: 155.17 g/mol

^{1}H NMR (400 MHz, DMSO-d_6) δ 6.00 (dd, *J* = 11.4, 8.1 Hz, 1H), 5.94 (d, *J* = 7.5 Hz, 1H), 5.63 (td, *J* = 7.8, 5.4 Hz, 1H), 3.97 (s, 2H), 3.84 (t, *J* = 5.1 Hz, 1H), 2.80 – 2.68 (m, 2H), 1.82 (t, *J* = 6.7 Hz, 2H).

^{13}C NMR (101 MHz, DMSO-d6) δ 150.29, 134.89, 126.87, 125.98, 116.03, 112.85, 61.15, 34.73.

2-(2-amino-6-chlorophenyl)ethan-1-ol (6a)

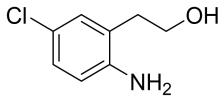


$C_8H_{10}ClNO$: 171.62 g/mol

^{1}H NMR (400 MHz, DMSO-d_6) δ 6.04 (t, *J* = 8.0 Hz, 1H), 5.74 (d, *J* = 8.0 Hz, 2H), 4.33 (s, 2H), 3.90 (t, *J* = 5.2 Hz, 1H), 2.74 – 2.62 (m, 2H), 1.96 (t, *J* = 7.2 Hz, 2H).

^{13}C NMR (101 MHz, DMSO-d6) δ 149.36, 134.55, 128.05, 120.82, 117.18, 114.03, 59.91, 31.93.

2-(2-amino-5-chlorophenyl)ethan-1-ol (7a)

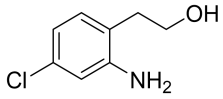


$C_8H_{10}ClNO$: 171.62 g/mol

^{1}H NMR (400 MHz, DMSO-d_6) δ 6.18 – 5.98 (m, 2H), 5.76 (d, *J* = 8.4 Hz, 1H), 4.17 (s, 2H), 3.81 (t, *J* = 5.1 Hz, 1H), 2.82 – 2.67 (m, 2H), 1.73 (t, *J* = 6.6 Hz, 2H).

^{13}C NMR (101 MHz, DMSO-d6) δ 146.13, 129.66, 126.63, 125.69, 119.59, 116.27, 60.64, 34.55.

2-(2-amino-4-chlorophenyl)ethan-1-ol (8a)

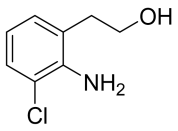


$C_8H_{10}ClNO$: 171.62 g/mol

^{1}H NMR (400 MHz, DMSO-d_6) δ 6.05 (d, *J* = 8.0 Hz, 1H), 5.78 (d, *J* = 2.2 Hz, 1H), 5.62 (dd, *J* = 8.0, 2.2 Hz, 1H), 4.31 (s, 2H), 3.78 (t, *J* = 5.1 Hz, 1H), 2.78 – 2.63 (m, 2H), 1.71 (t, *J* = 6.8 Hz, 2H).

^{13}C NMR (101 MHz, DMSO-d6) δ 148.73, 131.76, 131.31, 122.45, 115.80, 113.99, 60.85, 34.33.

2-(2-amino-3-chlorophenyl)ethan-1-ol (9a)

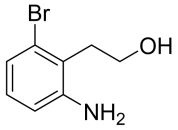


$C_8H_{10}ClNO$: 171.62 g/mol

^{1}H NMR (400 MHz, DMSO-d_6) δ 6.23 (d, *J* = 6.4 Hz, 1H), 6.09 (d, *J* = 7.4 Hz, 1H), 5.67 (t, *J* = 7.7 Hz, 1H), 4.19 (s, 2H), 3.86 (t, *J* = 5.1 Hz, 1H), 2.83 – 2.67 (m, 2H), 1.83 (t, *J* = 6.6 Hz, 2H).

^{13}C NMR (101 MHz, DMSO-d6) δ 142.97, 129.32, 127.32, 126.00, 118.30, 117.22, 60.99, 35.47.

2-(2-amino-6-bromophenyl)ethan-1-ol (10a)

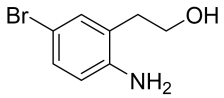


$C_8H_{10}BrNO$: 216.08 g/mol

^{1}H NMR (400 MHz, DMSO-d_6) δ 5.95 (t, *J* = 7.8 Hz, 1H), 5.89 (d, *J* = 6.5 Hz, 1H), 5.76 (d, *J* = 6.3 Hz, 1H), 4.33 (s, 2H), 3.91 (t, *J* = 5.2 Hz, 1H), 2.74 – 2.60 (m, 2H), 1.96 (t, *J* = 7.2 Hz, 2H).

^{13}C NMR (101 MHz, DMSO-d6) δ 149.33, 128.60, 125.84, 122.35, 120.51, 114.65, 59.89, 34.69.

2-(2-amino-5-bromophenyl)ethan-1-ol (11a)

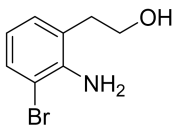


$C_8H_{10}BrNO$: 216.08 g/mol

^{1}H NMR (400 MHz, DMSO-d_6) δ 6.22 (d, *J* = 2.4 Hz, 1H), 6.18 (d, *J* = 8.4 Hz, 1H), 5.72 (d, *J* = 8.4 Hz, 1H), 4.20 (s, 2H), 3.80 (t, *J* = 5.1 Hz, 1H), 2.78 – 2.69 (m, 2H), 1.73 (t, *J* = 6.6 Hz, 2H).

^{13}C NMR (101 MHz, DMSO-d6) δ 146.54, 132.43, 129.48, 126.25, 116.81, 107.09, 60.65, 34.53.

2-(2-amino-3-bromophenyl)ethan-1-ol (12a)

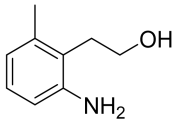


$C_8H_{10}BrNO$: 216.08 g/mol

^{1}H NMR (400 MHz, DMSO-d_6) δ 6.38 (d, *J* = 6.5 Hz, 1H), 6.12 (d, *J* = 7.5 Hz, 1H), 5.62 (t, *J* = 7.7 Hz, 1H), 4.16 (s, 2H), 3.88 (t, *J* = 5.1 Hz, 1H), 2.84 – 2.67 (m, 2H), 1.84 (t, *J* = 6.6 Hz, 2H).

^{13}C NMR (101 MHz, DMSO-d6) δ 143.95, 130.56, 130.00, 126.08, 117.97, 109.11, 60.98, 35.75.

2-(2-amino-6-methylphenyl)ethan-1-ol (14a)

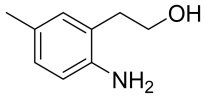


$C_9H_{13}NO$: 151.21 g/mol

^{1}H NMR (400 MHz, DMSO-d_6) δ 5.90 (t, *J* = 7.7 Hz, 1H), 5.61 (d, *J* = 8.0 Hz, 1H), 5.52 (d, *J* = 7.4 Hz, 1H), 3.87 (s, 2H), 3.80 (t, *J* = 5.2 Hz, 1H), 2.74 – 2.56 (m, 2H), 1.79 (t, *J* = 7.2 Hz, 2H), 1.32 (s, 3H).

^{13}C NMR (101 MHz, DMSO-d6) δ 147.27, 136.92, 126.55, 122.05, 119.09, 113.51, 60.28, 31.25, 20.23.

2-(2-amino-5-methylphenyl)ethan-1-ol (15a)

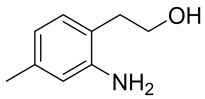


$C_9H_{13}NO$: 151.21 g/mol

^{1}H NMR (400 MHz, DMSO-d_6) δ 5.87 (s, 1H), 5.84 (d, *J* = 7.9 Hz, 1H), 5.65 (d, *J* = 7.9 Hz, 1H), 3.74 (s, 3H), 2.79 – 2.62 (m, 2H), 1.71 (t, *J* = 6.9 Hz, 2H), 1.26 (s, 3H).

^{13}C NMR (101 MHz, DMSO-d6) δ 144.47, 130.94, 127.53, 124.99, 123.79, 115.37, 61.38, 35.11, 20.63.

2-(2-amino-4-methylphenyl)ethan-1-ol (16a)

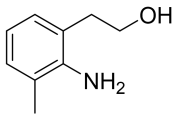


$C_9H_{13}NO$: 151.21 g/mol

^{1}H NMR (400 MHz, DMSO-d_6) δ 5.92 (d, *J* = 7.5 Hz, 1H), 5.56 (s, 1H), 5.44 (d, *J* = 5.9 Hz, 1H), 3.86 (s, 2H), 3.73 (t, *J* = 5.1 Hz, 1H), 2.76 – 2.59 (m, 2H), 1.69 (t, *J* = 7.0 Hz, 2H), 1.26 (s, 3H).

^{13}C NMR (101 MHz, DMSO-d6) δ 146.81, 135.85, 130.28, 120.78, 117.61, 115.81, 61.45, 34.77, 21.31.

2-(2-amino-3-methylphenyl)ethan-1-ol (17a)

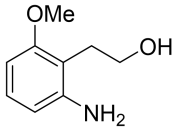


$C_9H_{13}NO$: 151.21 g/mol

^{1}H NMR (400 MHz, DMSO-d_6) δ 6.80 (d, *J* = 7.5 Hz, 2H), 6.43 (t, *J* = 7.4 Hz, 1H), 4.63 (t, *J* = 5.1 Hz, 1H), 4.55 (s, 2H), 3.64 – 3.51 (m, 2H), 2.64 (t, *J* = 6.8 Hz, 2H), 2.07 (s, 3H).

^{13}C NMR (101 MHz, DMSO-d6) δ 144.70, 128.46, 128.28, 123.23, 121.61, 116.48, 61.38, 35.46, 18.42.

2-(2-amino-6-methoxyphenyl)ethan-1-ol (18a)

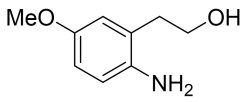


$C_9H_{13}NO_2$: 167.21 g/mol

^{1}H NMR (400 MHz, DMSO-d_6) δ 5.99 (t, *J* = 8.0 Hz, 1H), 5.43 (d, *J* = 7.0 Hz, 1H), 5.35 (d, *J* = 8.2 Hz, 1H), 3.96 (s, 2H), 3.74 (t, *J* = 5.2 Hz, 1H), 2.84 (s, 3H), 2.60 (td, *J* = 7.2, 5.1 Hz, 2H), 1.80 (t, *J* = 7.2 Hz, 2H).

^{13}C NMR (101 MHz, DMSO-d6) δ 158.49, 148.33, 127.18, 111.24, 108.94, 99.82, 60.69, 55.69, 27.96.

2-(2-amino-5-methoxyphenyl)ethan-1-ol (19a)

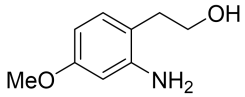


$C_9H_{13}NO_2$: 167.21 g/mol

^{1}H NMR (400 MHz, DMSO-d_6) δ 5.72 (dd, *J* = 5.6, 3.1 Hz, 2H), 5.68 (dd, *J* = 8.7, 2.5 Hz, 1H), 3.78 (s, 1H), 3.57 (s, 2H), 2.77 (s, 3H), 2.74 (t, *J* = 6.9 Hz, 2H), 1.74 (t, *J* = 6.9 Hz, 2H).

^{13}C NMR (101 MHz, DMSO-d6) δ 151.46, 140.68, 125.37, 116.27, 116.14, 112.47, 61.30, 55.66, 35.23.

2-(2-amino-4-methoxyphenyl)ethan-1-ol (20a)

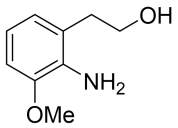


$C_9H_{13}NO_2$: 167.21 g/mol

^{1}H NMR (400 MHz, DMSO-d_6) δ 5.94 (d, *J* = 8.2 Hz, 1H), 5.35 (d, *J* = 2.6 Hz, 1H), 5.22 (dd, *J* = 8.2, 2.6 Hz, 1H), 4.00 (s, 2H), 3.72 (t, *J* = 5.2 Hz, 1H), 2.78 (s, 3H), 2.72 – 2.62 (m, 2H), 1.68 (t, *J* = 7.0 Hz, 2H).

^{13}C NMR (101 MHz, DMSO-d6) δ 159.00, 148.05, 130.99, 116.29, 102.25, 100.65, 61.57, 55.04, 34.41.

2-(2-amino-3-methoxyphenyl)ethan-1-ol (21a)

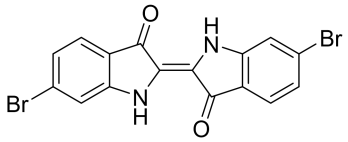


$C_9H_{13}NO_2$: 167.21 g/mol

^{1}H NMR (400 MHz, DMSO-d_6) δ 5.85 (d, *J* = 6.5 Hz, 1H), 5.76 (d, *J* = 7.6 Hz, 1H), 5.66 (t, *J* = 7.8 Hz, 1H), 3.81 (t, *J* = 5.1 Hz, 1H), 3.61 (s, 2H), 2.91 (s, 3H), 2.80 – 2.68 (m, 2H), 1.79 (t, *J* = 6.9 Hz, 2H).

^{13}C NMR (101 MHz, DMSO-d6) δ 147.00, 135.85, 124.19, 122.81, 116.49, 108.96, 61.39, 55.88, 35.05.

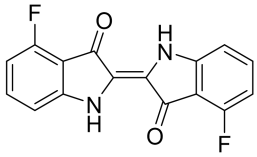
6,6'-dibromo-[2,2'-biindolinylidene]-3,3'-dione (1f)



$C_{16}H_8Br_2N_2O_2$: 420.06 g/mol

^{1}H NMR (400 MHz, D_2O) δ 7.42 (d, *J* = 11.3 Hz, 4H), 7.02 (d, *J* = 7.8 Hz, 2H).

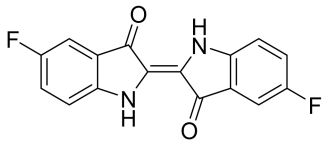
4,4'-difluoro-[2,2'-biindolinylidene]-3,3'-dione (2f)



$C_{16}H_8F_2N_2O_2$: 298.25 g/mol

^{1}H NMR (400 MHz, D_2O) δ 7.05 (d, *J* = 8.1 Hz, 2H), 6.88 (td, *J* = 7.9, 5.2 Hz, 2H), 6.54 (dd, *J* = 12.1, 7.9 Hz, 2H).

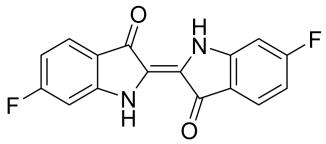
5,5'-difluoro-[2,2'-biindolinylidene]-3,3'-dione (3f)



$C_{16}H_8F_2N_2O_2$: 298.25 g/mol

^{1}H NMR (400 MHz, D_2O) δ 7.40 – 7.02 (m, 4H), 6.85 – 6.69 (m, 2H).

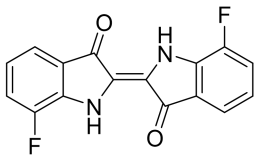
6,6'-difluoro-[2,2'-biindolinylidene]-3,3'-dione (4f)



$C_{16}H_8F_2N_2O_2$: 298.25 g/mol

^{1}H NMR (400 MHz, D_2O) δ 7.43 (dd, *J* = 8.7, 5.7 Hz, 2H), 6.99 (dd, *J* = 10.6, 2.3 Hz, 2H), 6.79 – 6.64 (m, 2H).

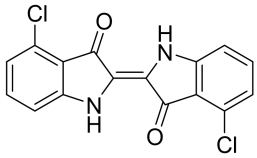
7,7'-difluoro-[2,2'-biindolinylidene]-3,3'-dione (5f)



$C_{16}H_8F_2N_2O_2$: 298.25 g/mol

^{1}H NMR (400 MHz, D_2O) δ 7.34 (d, *J* = 7.8 Hz, 1H), 6.91 – 6.82 (m, 1H), 6.82 – 6.72 (m, 1H).

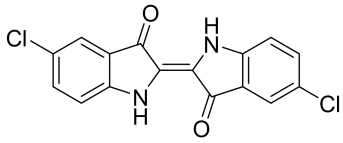
4,4'-dichloro-[2,2'-biindolinylidene]-3,3'-dione (6f)



$C_{16}H_8Cl_2N_2O_2$: 331.15 g/mol

^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ 9.05 (s, 2H), 7.39 (t, *J* = 7.9 Hz, 2H), 7.05 – 6.84 (m, 4H).

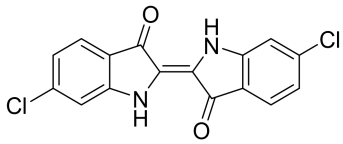
5,5'-dichloro-[2,2'-biindolinylidene]-3,3'-dione (7f)



$C_{16}H_8Cl_2N_2O_2$: 331.15 g/mol

^{1}H NMR (400 MHz, D_2O) δ 7.52 (d, *J* = 22.9 Hz, 2H), 7.25 (dd, *J* = 26.0, 8.1 Hz, 2H), 6.98 (dd, *J* = 33.1, 8.2 Hz, 2H).

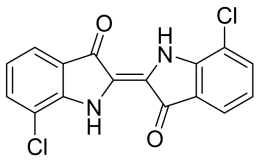
6,6'-dichloro-[2,2'-biindolinylidene]-3,3'-dione (8f)



$C_{16}H_8Cl_2N_2O_2$: 331.15 g/mol

^{1}H NMR (400 MHz, D_2O) δ 7.45 (d, *J* = 8.4 Hz, 2H), 7.27 (s, 2H), 6.89 (d, *J* = 8.5 Hz, 2H).

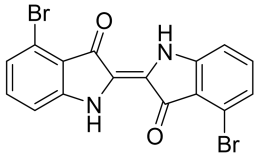
7,7'-dichloro-[2,2'-biindolinylidene]-3,3'-dione (9f)



$C_{16}H_8Cl_2N_2O_2$: 331.15 g/mol

^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ 7.68 (dd, *J* = 7.7, 1.1 Hz, 2H), 7.52 (dd, *J* = 7.7, 1.1 Hz, 2H), 6.98 (t, *J* = 7.7 Hz, 2H).

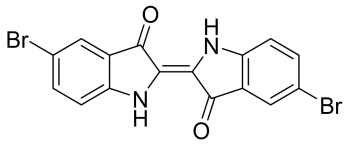
4,4'-dibromo-[2,2'-biindolinylidene]-3,3'-dione (10f)



$C_{16}H_8Br_2N_2O_2$: 420.06 g/mol

^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ 7.31 (t, *J* = 7.9 Hz, 2H), 7.14 (d, *J* = 7.7 Hz, 2H), 6.98 (d, *J* = 8.2 Hz, 2H).

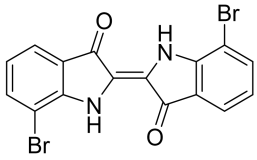
5,5'-dibromo-[2,2'-biindolinylidene]-3,3'-dione (11f)



$C_{16}H_8Br_2N_2O_2$: 420.06 g/mol

^{1}H NMR (400 MHz, D_2O) δ 7.69 – 7.59 (m, 2H), 7.18 (dd, *J* = 8.5, 3.5 Hz, 2H), 7.10 – 7.04 (m, 2H).

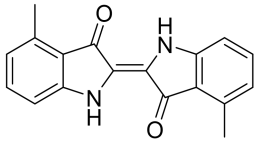
7,7'-dibromo-[2,2'-biindolinylidene]-3,3'-dione (12f)



$C_{16}H_8Br_2N_2O_2$: 420.06 g/mol

^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ 7.71 (dd, *J* = 7.6, 1.1 Hz, 2H), 7.67 (dd, *J* = 7.7, 1.1 Hz, 2H), 6.93 (t, *J* = 7.7 Hz, 2H).

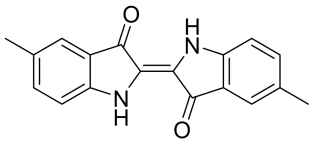
4,4'-dimethyl-[2,2'-biindolinylidene]-3,3'-dione (14f)



$C_{16}H_8Br_2N_2O_2$: 290.32 g/mol

^{1}H NMR (400 MHz, D_2O) δ 7.10 (d, *J* = 8.1 Hz, 2H), 6.90 (dd, *J* = 8.1, 7.0 Hz, 2H), 6.67 (d, *J* = 7.1 Hz, 2H), 2.62 (s, 6H).

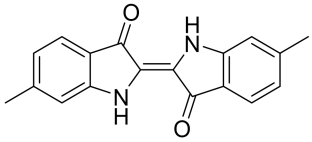
5,5'-dimethyl-[2,2'-biindolinylidene]-3,3'-dione (15f)



$C_{16}H_8Br_2N_2O_2$: 290.32 g/mol

^{1}H NMR (400 MHz, D_2O) δ 7.32 (s, 2H), 7.17 (d, *J* = 8.1 Hz, 2H), 6.84 (dd, *J* = 8.2, 1.7 Hz, 2H), 2.33 (s, 6H).

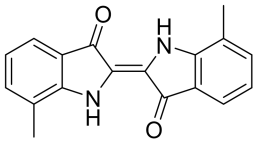
6,6'-dimethyl-[2,2'-biindolinylidene]-3,3'-dione (16f)



$C_{16}H_8Br_2N_2O_2$: 290.32 g/mol

^{1}H NMR (400 MHz, D_2O) δ 7.42 (t, *J* = 6.4 Hz, 2H), 7.08 (s, 2H), 6.79 (d, *J* = 7.8 Hz, 2H), 2.34 (s, 6H).

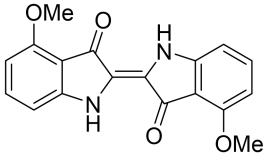
7,7'-dimethyl-[2,2'-biindolinylidene]-3,3'-dione (17f)



$C_{16}H_8Br_2N_2O_2$: 290.32 g/mol

^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ 7.60 (d, *J* = 7.3 Hz, 2H), 7.32 (d, *J* = 7.0 Hz, 2H), 6.93 (t, *J* = 7.3 Hz, 2H), 2.37 (s, 6H).

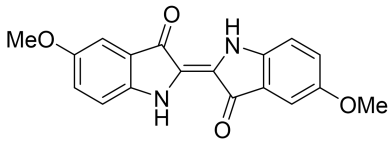
4,4'-dimethoxy-[2,2'-biindolinylidene]-3,3'-dione (18f)



$C_{16}H_8Br_2N_2O_2$: 322.32 g/mol

^{1}H NMR (400 MHz, D_2O) δ 6.97 – 6.88 (m, 4H), 6.43 (dd, *J* = 5.9, 2.6 Hz, 2H), 3.84 (s, 6H).

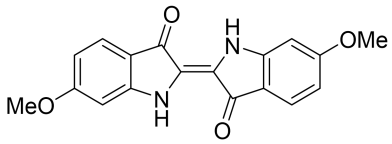
5,5'-dimethoxy-[2,2'-biindolinylidene]-3,3'-dione (18f)



$C_{16}H_8Br_2N_2O_2$: 322.32 g/mol

^{1}H NMR (400 MHz, D_2O) δ 7.20 (dd, *J* = 8.7, 1.9 Hz, 2H), 7.10 (t, *J* = 2.3 Hz, 2H), 6.68 (dt, *J* = 8.6, 2.4 Hz, 2H), 3.93 – 3.76 (m, 6H).

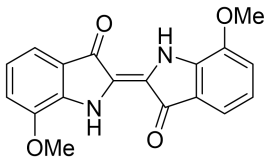
6,6'-dimethoxy-[2,2'-biindolinylidene]-3,3'-dione (20f)



$C_{16}H_8Br_2N_2O_2$: 322.32 g/mol

^{1}H NMR (400 MHz, D_2O) δ 7.40 (d, *J* = 8.6 Hz, 2H), 6.88 (d, *J* = 2.3 Hz, 2H), 6.62 (dd, *J* = 8.6, 2.3 Hz, 2H), 3.79 (s, 6H).

7,7'-dimethoxy-[2,2'-biindolinylidene]-3,3'-dione (21f)



$C_{16}H_8Br_2N_2O_2$: 322.32 g/mol

^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ 7.34 (d, *J* = 7.6 Hz, 2H), 7.05 – 6.86 (m, 4H), 3.95 (s, 6H).

Supplementary Tables

Supplementary Table 1. List of primers used in this study.

Name	Sequence (5'-3')
HLADH-F (MCS1)	ctgcaggtcgacaagcttATGACTGGTGGACAGCAAAT
HLADH-R (MCS1)	ttaagcattatgcggccgcTCAAAAGGTCAGAAT
HLADH-F (MCS2)	gtcggtaccctcgagATGACTGGTGGACAGCAAAT
HLADH-R (MCS2)	gcagcggtttctttaccagaTCAAAAGGTCAGAAT
mFMO-F (MCS1)	gcctgcaggtcgacaagcttATGGCAACCCGCATT
mFMO-R (MCS1)	taagcattatgcggccgcTTAGGCTTCTTTGGC
mFMO-F (MCS2)	cgtcggtaccctcgagATGGCAACCCGCATTG
mFMO-R (MCS2)	gcggtttctttaccagaTTAGGCTTCTTTGGCAA
Duet-1-F (MCS1)	gcggccgcataatgcttaagtcgaacagaa
Duet-1-R (MCS1)	aagcttgtcgacctgcaggcgcgccgagct
Duet-1-F (MCS2)	tctggtaaagaaaccgctgctgcgaaattt
Duet-1-R (MCS2)	ctcgagggtaccgacgtcagcgatcgcgtg
tnaA-KO-F	ATCACCAGTAACTCTGCAGGgttttagagctagaaatagcaagttaaaataaggctag
tnaA-KO-R	CCTGCAGAGTTACTGGTGATactagtattatacctaggactgagctagctgtcaa
tnaA-UP-F	tgactgtcagatgcggcttcgcttcattgttagcactcctgttat
tnaA-UP-R	gcgccgccttccaggccgccatatgttattttaattacagtgatccctgt
tnaA-DOWN-F	acagggatcactgtaattaaaataacatatggcggcctggaaggcggcgc
tnaA-DOWN-R	ttaaacttctttcagttttgcggtgaagtgacgcaatactttgg
KO-sequencing-F	ccagcttctgtattggtaagtaaccgcgcttacgaagccgtattc
KO-sequencing-R	ggtaagagagtggctaacatccttatagccactctgtagtattaa

Supplementary Table 2. List of plasmids used in this study.

Plasmid	Description	Source
pET-28a(+)	T7 promoter, pBR332 ori, Kan^R	Novagen
pRSFDuet-1	double T7 promoters, RSF ori, Kan^R , >100 copy number	Novagen
pETDuet-1	double T7 promoters, pBR322 ori, Amp^R , ~40 copy number	Novagen
pACYCDuet-1	double T7 promoters, ACYC ori, Cm^R , <20 copy number	Novagen
pEcCas	Native cas9 promoter, rhaB promoter, araBAD promoter, pSC101 ori, Kan^R	Addgene
pTargetF	pij23119 promoter, pBR332 ori, $Spec^R$	Addgene
pTargetF-*tnaA*	pTargetF carring sgRNA of gene *tnaA*	This study
pET-28a(+)-APDH	pET-28a(+) carring APDH (6His in N-terminum)	This study
pET-28a(+)-AaADH	pET-28a(+) carring AaADH (6His in N-terminum)	This study
pET-28a(+)-PpADH	pET-28a(+) carring PpADH (6His in N-terminum)	This study
pET-28a(+)-SyADH	pET-28a(+) carring SyADH (6His in N-terminum)	This study
pET-28a(+)-YsADH	pET-28a(+) carring YsADH (6His in N-terminum)	This study
pET-28a(+)-ScADH	pET-28a(+) carring ScADH (6His in N-terminum)	This study
pET-28a(+)-CoADH	pET-28a(+) carring CoADH (6His in N-terminum)	This study
pET-28a(+)-TeSADH	pET-28a(+) carring TeSADH (6His in N-terminum)	This study
pET-28a(+)-HLADH	pET-28a(+) carring HLADH (6His in N-terminum)	This study
pET-28a(+)-ADH-A	pET-28a(+) carring ADH-A (6His in N-terminum)	This study
pET-28a(+)-TeADH	pET-28a(+) carring TeADH (6His in N-terminum)	This study
pET-28a(+)-gtADH	pET-28a(+) carring gtADH (6His in N-terminum)	This study
pET-28a(+)-smNOX	pET-28a(+) carring smNOX (6His in N-terminum)	This study
pET-28a(+)-mFMO	pET-28a(+) carring mFMO (6His in N-terminum)	This study
pET-28a(+)-cFMO	pET-28a(+) carring cFMO (6His in N-terminum)	This study

pET-28a(+)-NiFMO	pET-28a(+) carring NiFMO (6His in N-terminum)	This study
pRSFDuet-1-HLADH	pRSFDuet-1 carring HLADH	This study
pRSFDuet-1-mFMO	pRSFDuet-1 carring mFMO	This study
pETDuet-1-HLADH	pETDuet-1 carring HLADH	This study
pETDuet-1-mFMO	pETDuet-1 carring mFMO	This study
pACYCDuet-1-HLADH	pACYCDuet-1 carring HLADH	This study
pACYCDuet-1-mFMO	pACYCDuet-1 carring mFMO	This study
pRSFDuet-1-HLADH-mFMO	pRSFDuet-1 carring HLADH and mFMO in turn	This study
pRSFDuet-1-mFMO-HLADH	pRSFDuet-1 carring mFMO and HLADH in turn	This study
pETDuet-1-HLADH-mFMO	pETDuet-1 carring HLADH and mFMO in turn	This study
pETDuet-1-mFMO-HLADH	pETDuet-1 carring mFMO and HLADH in turn	This study
pACYCDuet-1-HLADH-mFMO	pACYCDuet-1 carring HLADH and mFMO in turn	This study
pACYCDuet-1-mFMO-HLADH	pACYCDuet-1 carring mFMO and HLADH in turn	This study

Supplementary Table 3. List of strains used in this study.

Strain	Description	Source
E. coli BL21 (*DE3*)	*E. coli* str. B F^- *ompT gal dcm lon hsdS$_B$ (r_B^- m_B^-) λ*(*DE3* [*lacI lacUV5-T7p07 ind1 sam7 nin5*]) [*malB*$^+$]$_{K\text{-}12}$(λ^S)	Novagen
E. coli BL21 (*DE3*) Δ*tnaA*	*E. coli* BL21 (DE3), Δ*tnaA*	This study
M1	pRSFDuet-1-HLADH-mFMO was expressed in *E. coli* BL21 (*DE3*) Δ*tnaA*	This study
M2	pRSFDuet-1-mFMO-HLADH was expressed in *E. coli* BL21 (*DE3*) Δ*tnaA*	This study
M3	pETDuet-1-HLADH-mFMO was expressed in *E. coli* BL21 (*DE3*) Δ*tnaA*	This study
M4	pETDuet-1-mFMO-HLADH was expressed in *E. coli* BL21 (*DE3*) Δ*tnaA*	This study
M5	pACYCDuet-1-HLADH-mFMO was expressed in *E. coli* BL21 (*DE3*) Δ*tnaA*	This study
M6	pACYCDuet-1-mFMO-HLADH was expressed in *E. coli* BL21 (*DE3*) Δ*tnaA*	This study
M7	pRSFDuet-1-HLADH and pETDuet-1-mFMO were coexpressed in *E. coli* BL21 (*DE3*) Δ*tnaA*	This study
M8	pRSFDuet-1-HLADH and were pACYCDuet-1-mFMO coexpressed in *E. coli* BL21 (*DE3*) Δ*tnaA*	This study
M9	pETDuet-1-HLADH and pRSFDuet-1-mFMO were coexpressed in *E. coli* BL21 (*DE3*) Δ*tnaA*	This study
M10	pETDuet-1-HLADH and pACYCDuet-1-mFMO were coexpressed in *E. coli* BL21 (*DE3*) Δ*tnaA*	This study
M11	pACYCDuet-1-HLADH and pRSFDuet-1-mFMO were coexpressed in *E. coli* BL21 (*DE3*) Δ*tnaA*	This study
M12	pACYCDuet-1-HLADH and pETDuet-1-mFMO were coexpressed in *E. coli* BL21 (*DE3*) Δ*tnaA*	This study

Supplementary Table 4. List of enzymes used in this study.

Enzyme	Source	UniProtKB
HLADH	*Horse liver*	P00327
ADH-A	*Rhodococcus ruber DSM 44541*	Q8KLT9
YsADH	*Yokenella sp. WZY002*	W6CX26
TeSADH	*Thermoanaerobacter ethanolicus*	P77990
CoADH	*Candida orthopsilosis*	H8WVM2
ScADH	*Saccharomyces cerevisiae*	P00331
SyADH	*Sphingobium yanoikuyae*	B9U359
PpADH	*Paracoccus pantotrophus*	B9U358
AaADH	*Aromatoleum aromaticum*	Q5P5I4
APDH	*Mycolicibacterium smegmatis*	A0QP46
TeADH	*Thermoanaerobacter ethanolicus*	Q9F282
gtADH	*Geobacillus thermodenitrificans*	A4ISB9
mFMO	*Methylophaga sp. strain SK1*	Q83XK4
cFMO	*Corynebacterium glutamicum*	A0A1Q6BNV7
NiFMO	*Nitrincola lacisaponensis*	A0A063Y6V3

Supplementary Table 5. The analytical conditions for different compounds.

Compound	Analytic methods	Test conditions
1a-21a, 1d-21d	GC-MS	GC-MS analyses were performed on an Agilent 7890B GC/5977A MS detector and equipped with a HP-5 MS capillary column (30 m*0.25 mm*0.25 μm). The injection volume was 1.0 μL with an autosampler and helium was used as a carrier gas with column flow rate of 1.5 $mL \cdot min^{-1}$. The electron ionization (EI) mass spectra in the range of 35-700 (m/z) were recorded in the full-scan mode.
1a-21a, 1d-21d	HPLC	HPLC analysis was conducted as follows: HPLC-DAD, ZORBAX Eclipse Plus C18, MeCN:H_2O = 1:1 for 30min, flow rate: 0.4 mL/min, injection volume: 10 μL. Analytes were detected by using a diode array detector (DAD) at a wavelength of 254 nm.
1a-21a	NMR	The sample was dissolved by DMSO-d_6 and determined. NMR spectra were recorded on a Bruker Ascend 400 MHz NMR spectrometer at 400 MHz (^{1}H NMR). Chemical shifts are reported in parts per million (ppm). Tetramethylsilane (TMS) was used as the reference substance for ^{1}H and ^{13}C chemical shifts.
1f-21f	NMR	**6f**, **9f**, **10f**, **12f**, **17f** and **21f** was dissolved by $CDCl_3$-*d* and the rest was reduced in D_2O and measured. NMR spectra were recorded on a Bruker Ascend 400 MHz NMR spectrometer at 400 MHz (^{1}H NMR). Chemical shifts are reported in parts per million (ppm). Tetramethylsilane (TMS) was used as the reference substance for ^{1}H chemical shifts.
1f-21f	UV–vis	Ultraviolet–visible light (UV–vis) spectroscopyanalysis was conducted as follows: UV 2700, wavelength: 300-800nm, absorbance: 0-5, volume = 3ml, optical path: 1 cm.

Supplementary Table 6. Comparison of Tyrian purple production by *E. coli* strains using diverse methods.

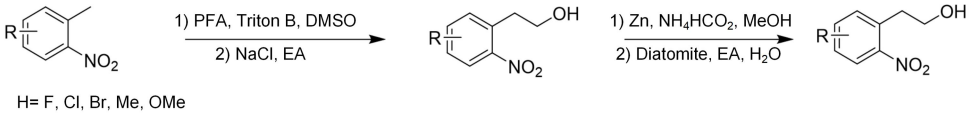
Cell	Step	Enzyme	Reaction	Reaction time (h)	6,6'-DBI production	Yield (%)	References
One	One	HLADH + MaFMO	Whole-cell reaction	24	367.5 mg L^{-1} (15.31 mg L^{-1} h^{-1})	72	This study
One	Two	Fre-L3-SttH + TnaA-L3-*GS*-CD + GroEL/GroES	*In vivo*	27	44.5 mg L^{-1} (1.64 mg L^{-1} h^{-1})	42	Li et al., 2024
Two	Two	Fre-L3-SttH + TnaA+ MaFMO	Whole-cell reaction	36	315.0 mg L^{-1} (8.75 mg L^{-1} h^{-1})	60	Lee et al., 2021
Three	Four	Thal+ TnaA + PTDH-MaFMO + GroEL/GroES	Crosslinked enzyme aggregates (CLEAs)	144	>50.5 mg L^{-1} (0.35 mg L^{-1} h^{-1})	>24	Schnepel et al., 2021

Supplementary Table 7. The color depths of the dyed fabrics dyed by in situ dyeing process were measured in *K/S* values.

Dyeing strategy	Dyeing time (h)	*K/S*					
		Wool	**Acrylic**	**Polyester**	**Nylon**	**Cotton**	**Acetate**
Bio-in-situ	1	0.87	0.12	0.10	1.42	0.23	0.67
	6	2.83	0.31	0.42	3.46	0.84	2.65
	12	3.86	0.73	0.96	5.24	1.31	3.93
	24	4.64	1.87	1.77	6.61	2.21	5.41

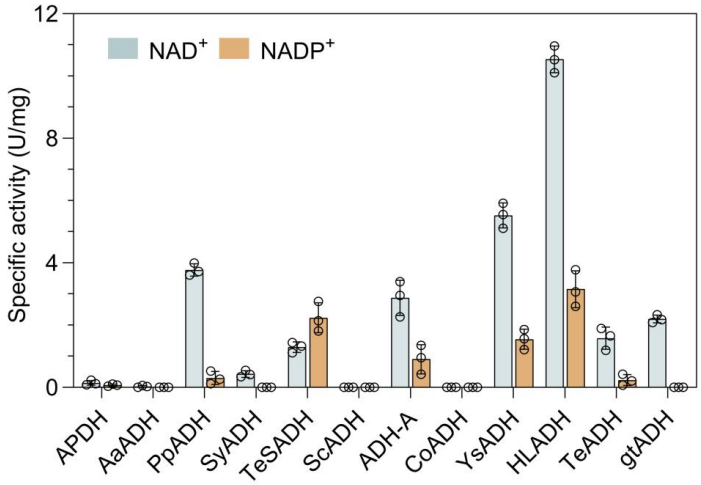
Supplementary Figures

a Chemical synthesis of substituted 2-aminophenethanol (**1a**-**12a, 14a**-**21a**)

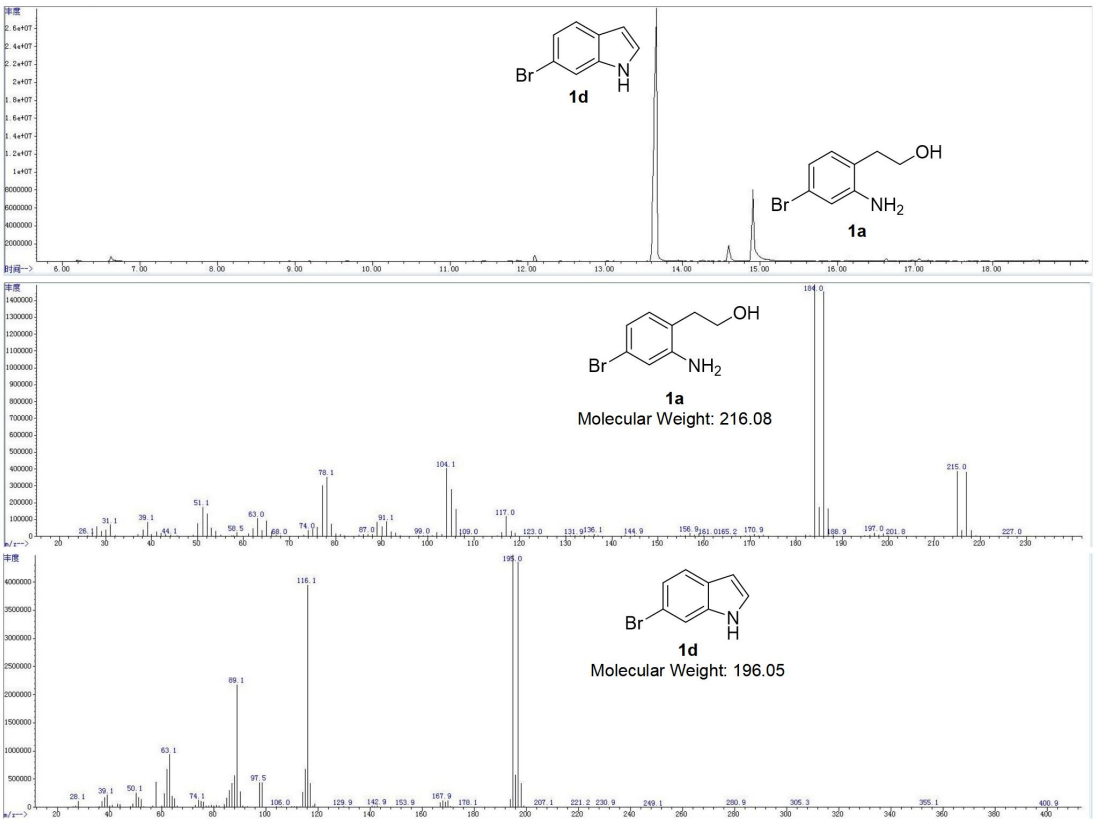


H= F, Cl, Br, Me, OMe

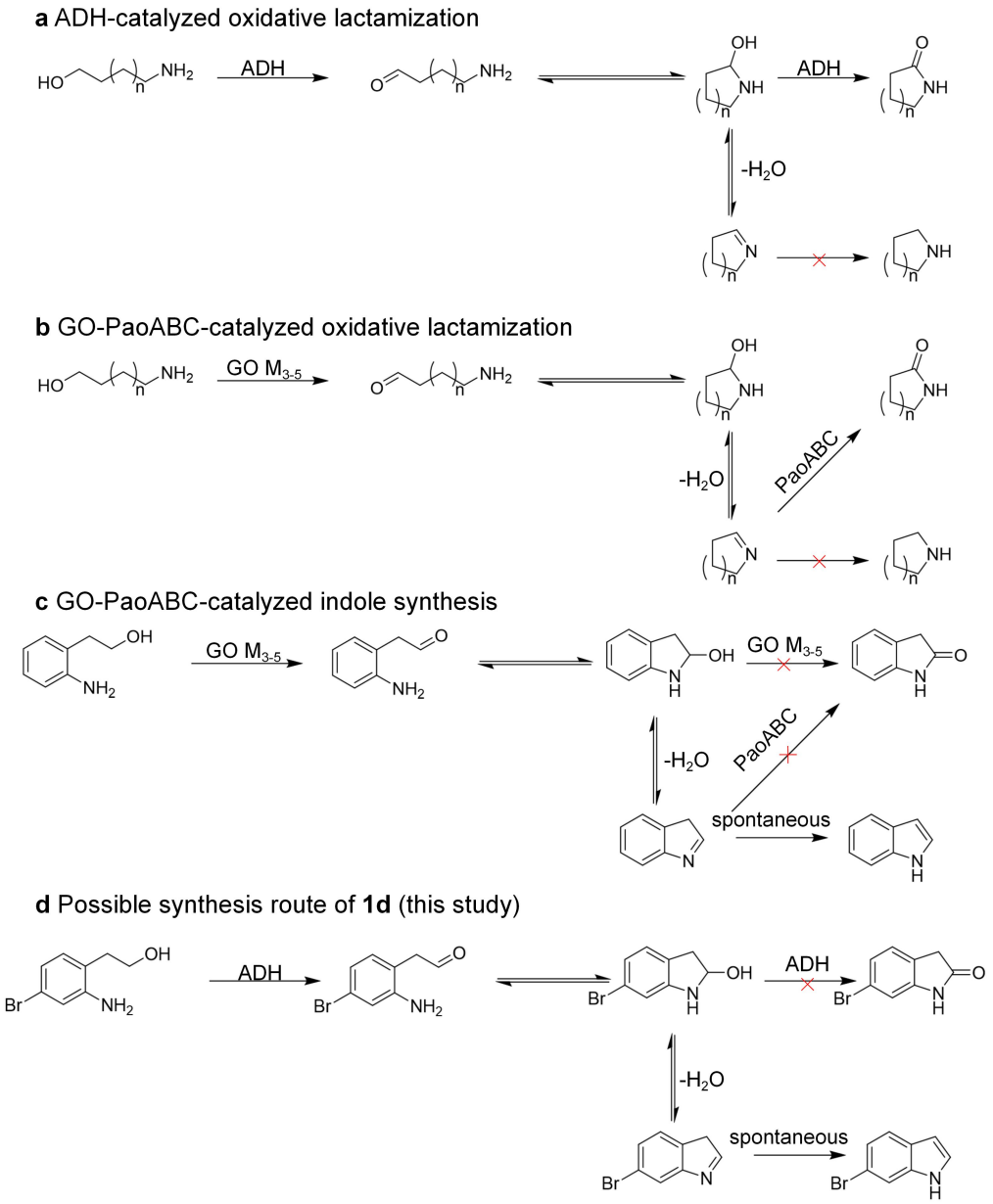
Supplementary Figure 1. General procedures for chemical synthesis of substituted 2-aminophenethanol (**1a**-**12a**, **14a**-**21a**).



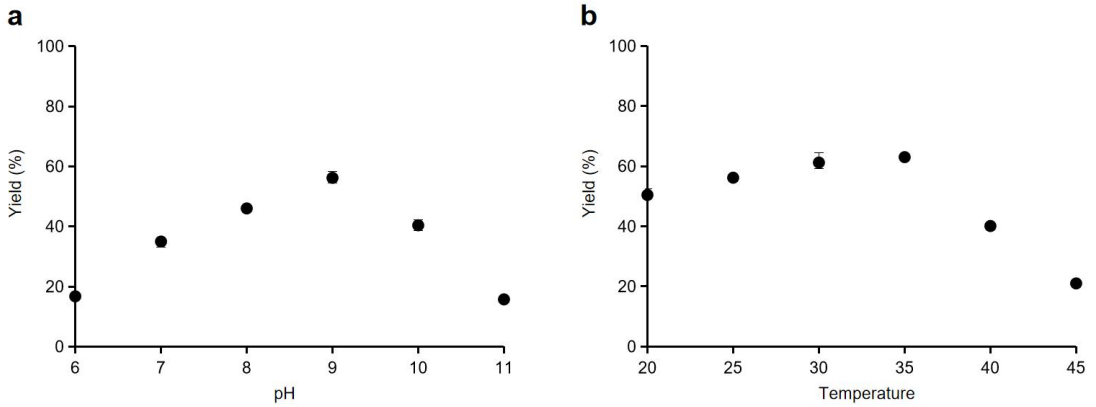
Supplementary Figure 2. Enzymatic activity assay of ADHs with NAD^+ or $NADP^+$ as cofactors.



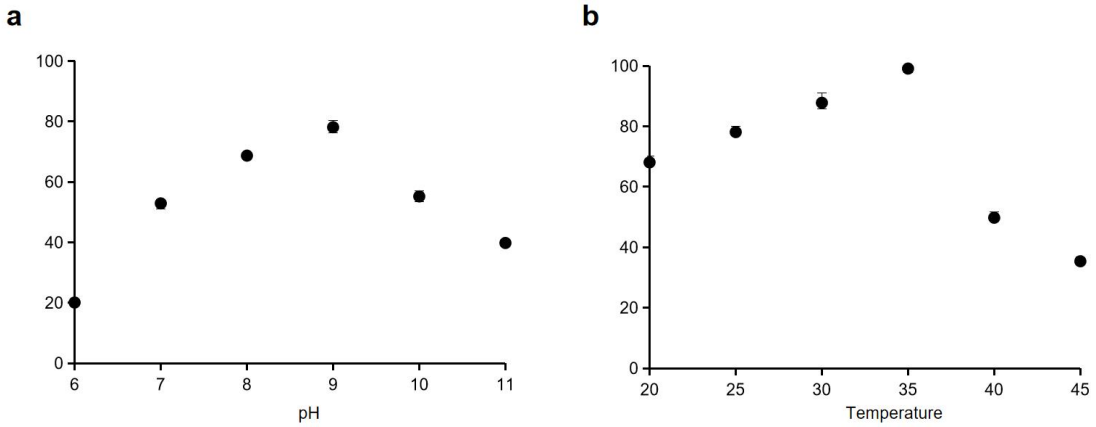
Supplementary Figure 3. GC-MS spectrum of the reaction with the addition of five equivalents of NAD^+ catalyzed by HLADH. Reaction conditions: 1mM **1a**, 5mM NAD^+ and 0.2 mg purified HLADH in 50mM KPi buffer (pH 7.0) at 25°C.



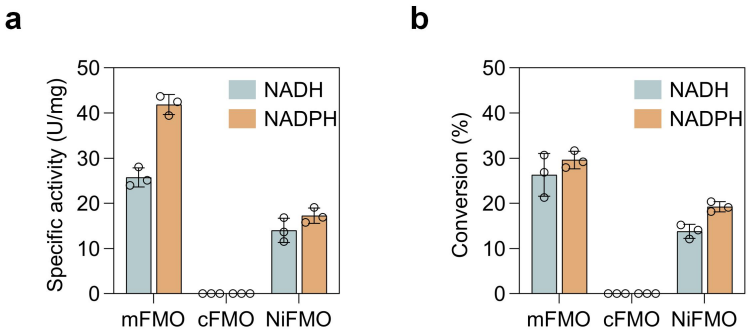
Supplementary Figure 4. Feasibility analysis of **1b** synthesis from **1a**.



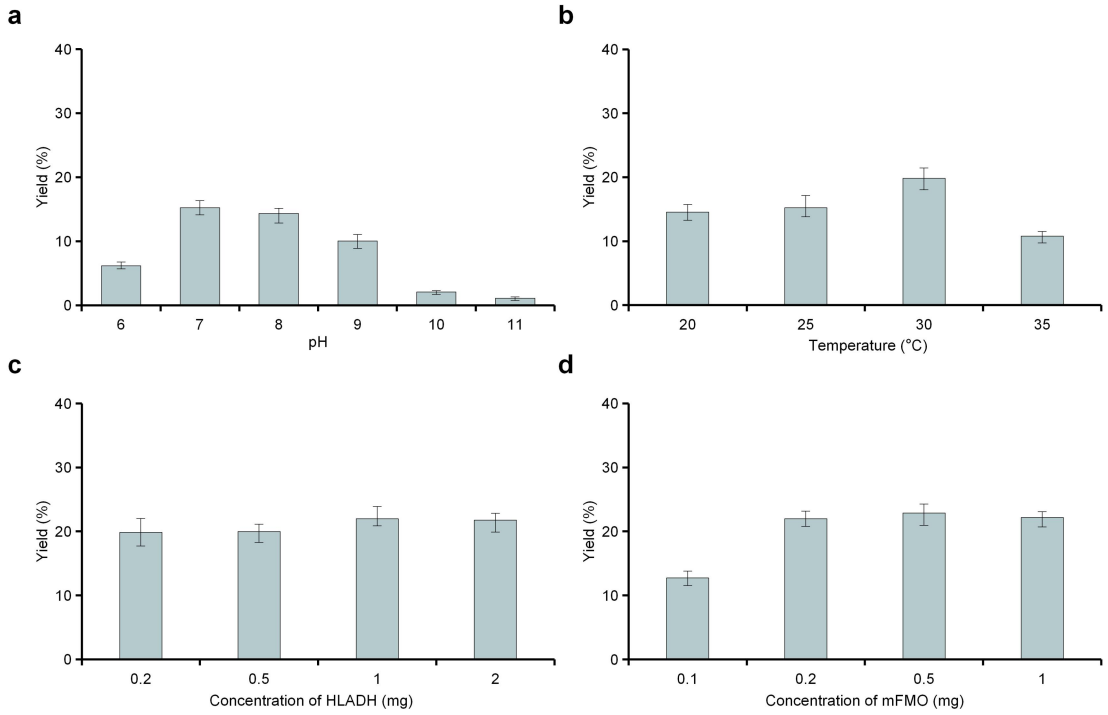
Supplementary Figure 5. Optimization of reaction conditions catalyzed by HLADH with equivalent NAD^+ concentration. **a** Optimization of pH ranging from pH6 to pH11. **b** Optimization of temperature ranging from 20°C to 35°C. Reaction conditions: 2.5 mM **1a**, 0.2 mg purified HLADH and 2.5 mM NAD^+ in buffer solutions under different pH and temperature.



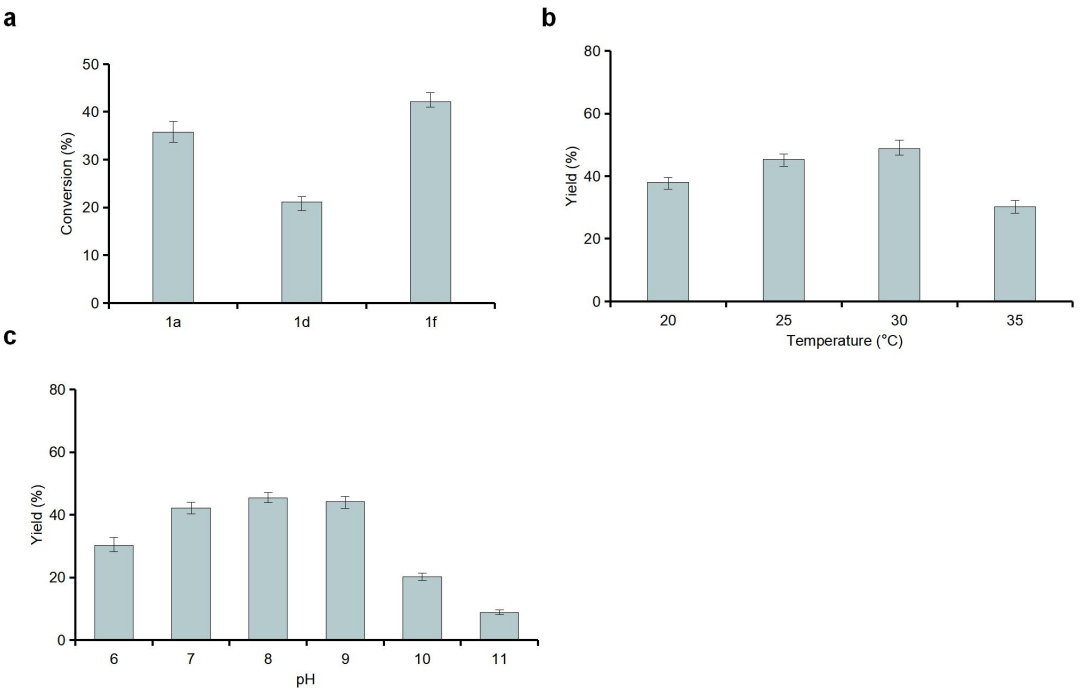
Supplementary Figure 6. Optimization of reaction conditions catalyzed by HLADH with NOX-promoted NAD^+ regeneration system. **a** Optimization of pH ranging from pH 6 to pH 11. **b** Optimization of temperature ranging from 20 °C to 35 °C. Reaction conditions: 2.5 mM **1a**, 0.2 mg purified HLADH and 0.5 mM NAD^+ coupling with 0.2 mg smNOX in buffer solutions under different pH and temperature in 4 hours.



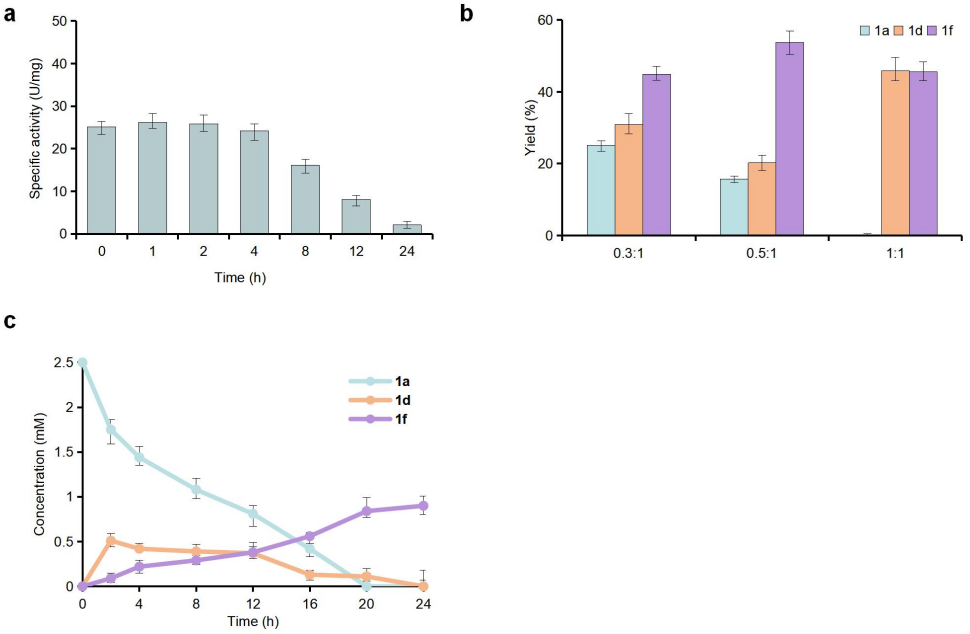
Supplementary Figure 7. a Enzymatic activity assay of mFMO, cFMO and NiFMO with NADH and NADPH as cofactors, respectively. **b** Conversion of 6,6'-DBI catalyzed by FMO with NADH and NADPH as cofactors. Reaction conditions: 2.5 mM **1d**, 2.5 mM NADH/NADPH and 0.2 mg purified FMOs in 50mM KPi buffer (pH 7.0) at 25°C.



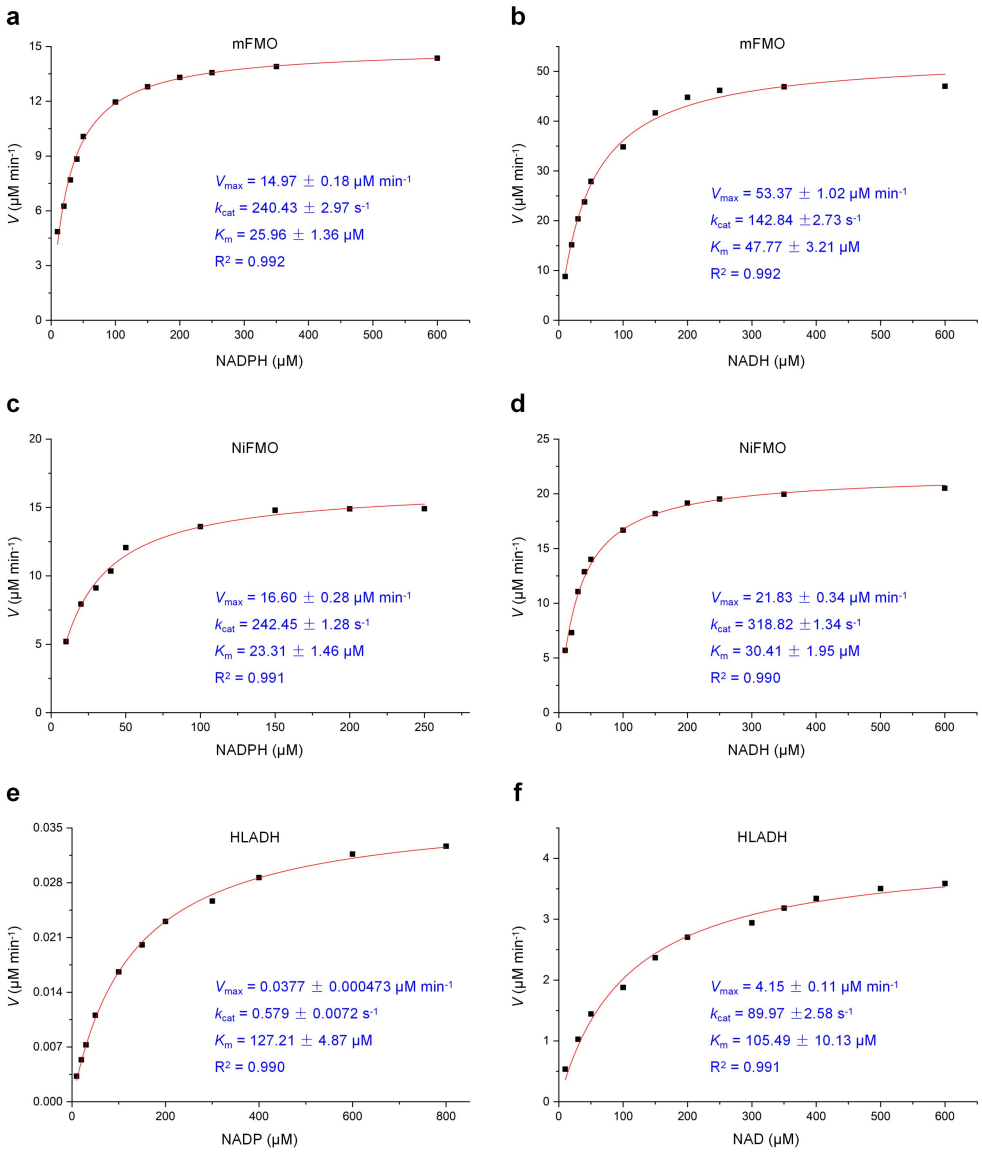
Supplementary Figure 8. Optimization of the effect of **(a)** pH, **(b)** temperature and the amount of **(c)** HALDH and **(d)** mFMO on the yield of tyrian purple in HLADH-mFMO cascade with $NADP^+$ as cofactor. Reaction conditions: 2.5 mM **1a**, 1 mM $NADP^+$, 0.2-2 mg purified HLADH and 0.1-1 mg purified mFMO in 50mM buffer pH 6.0-11.0 at 20-35°C.



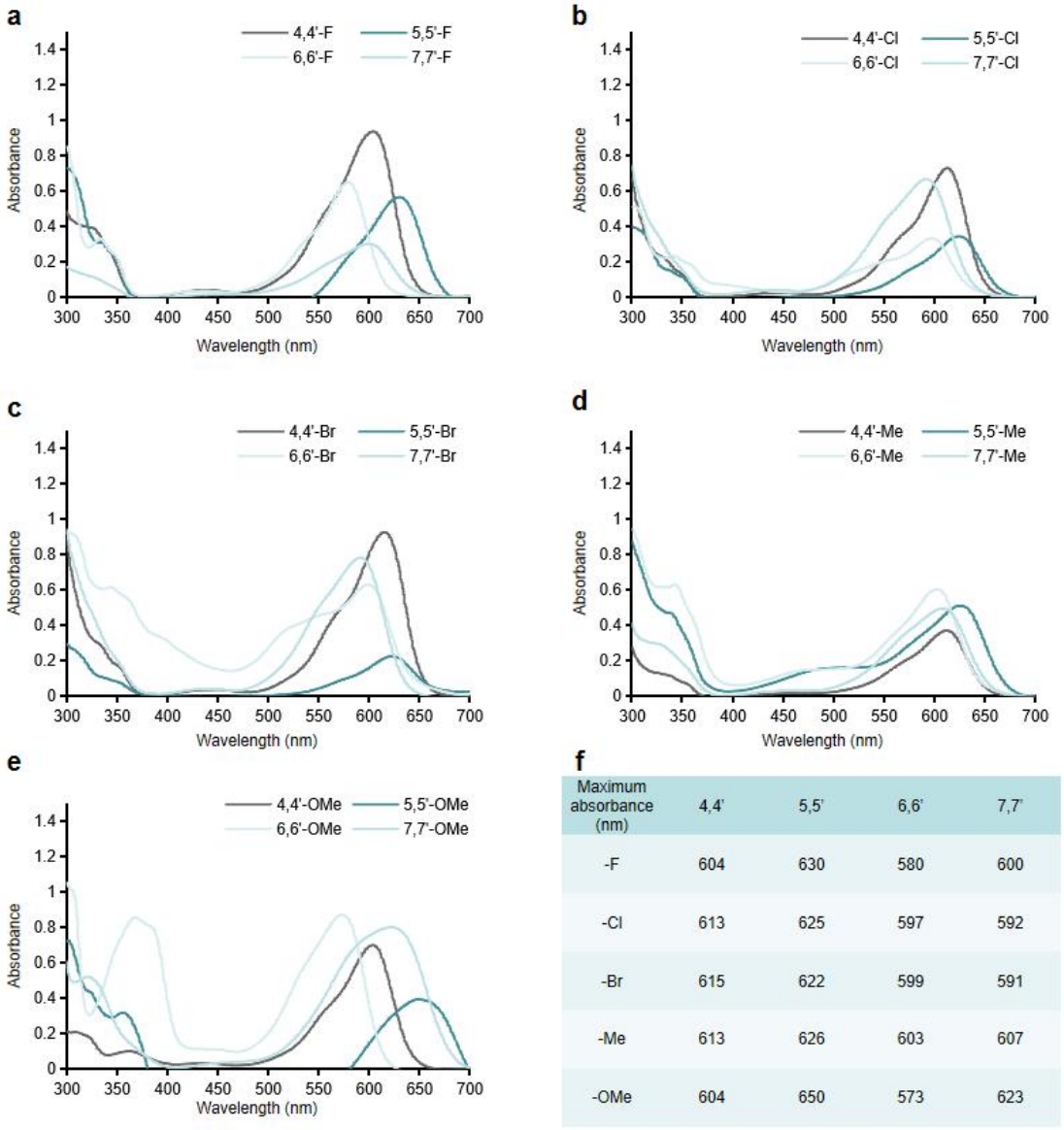
Supplementary Figure 9. a Conversion of **1a**, **1d** and **1f** in the cascade process with NAD+ as cofactor. Optimization of the effect of **(b)** temperature, **(c)** pH on the yield of tyrian purple in HLADH-mFMO cascade with NAD^+ as cofactor. Reaction conditions: 2.5 mM **1a**, 1 mM NAD^+, 0.2 mg purified HLADH and 0.2 mg purified mFMO in 50 mM buffer pH 6.0-11.0 at 20-35°C.



Supplementary Figure 10. a Activity of mFMO over 24h. **b** Analysis of the assay of the components of the reaction process with reaction time that the ratios of HLADH and mFMO were examined as 0.3: 1, 0.5: 1 and 1: 1. **c** Time-course analysis of reaction components.



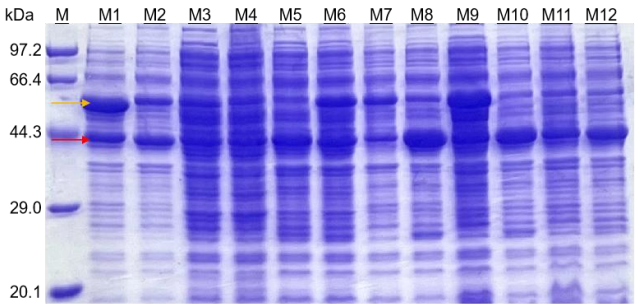
Supplementary Figure 11. The kinetic plots of HLADH towards NAD^+ and $NADP^+$ and mFMO and NiFMO towards NADH and NADPH. **a** The kinetic plots of HLADH towards NAD^+. **b** The kinetic plots of HLADH towards $NADP^+$. **c** The kinetic plots of mFMO towards NADH. **d** The kinetic plots of mFMO towards NADPH. **e** The kinetic plots of mFMO towards NADH. **f** The kinetic plots of mFMO towards NADPH.



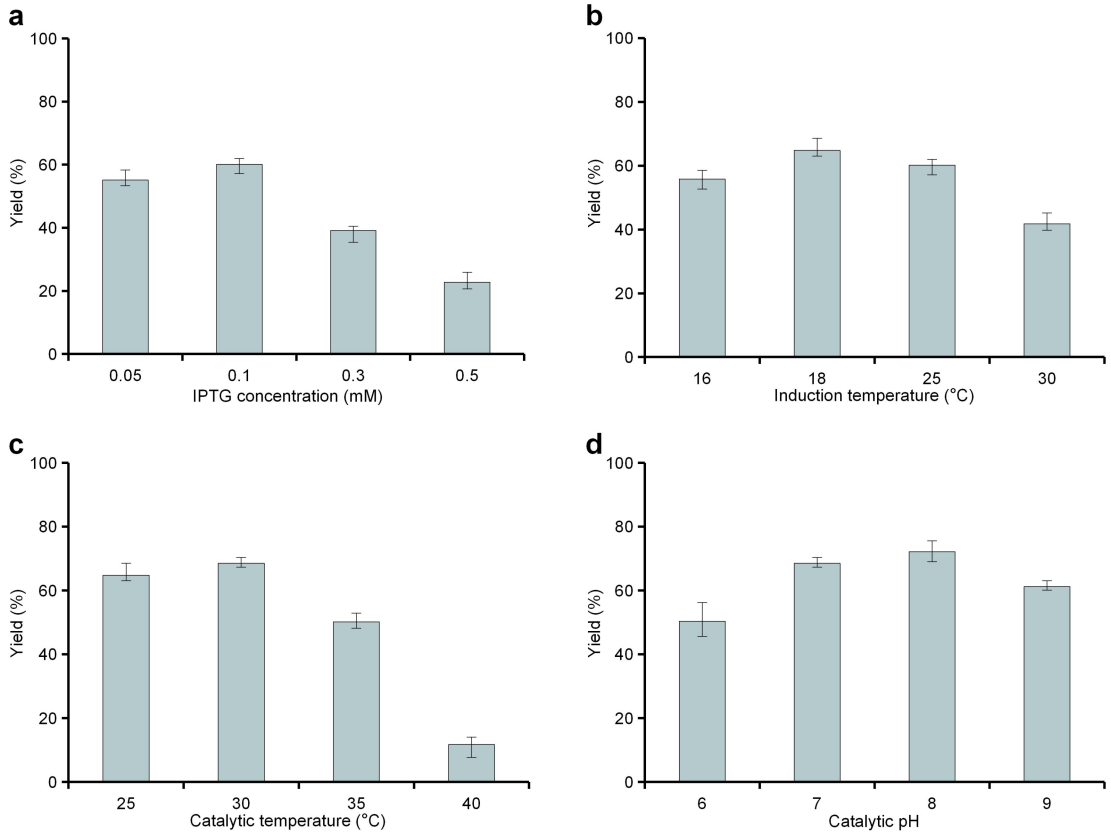
f

Maximum absorbance (nm)	4,4'	5,5'	6,6'	7,7'
-F	604	630	580	600
-Cl	613	625	597	592
-Br	615	622	599	591
-Me	613	626	603	607
-OMe	604	650	573	623

Supplementary Figure 12. The **(a-e)** absorption spectra and **(f)** maximum absorption of the indigoids in DMF solution.

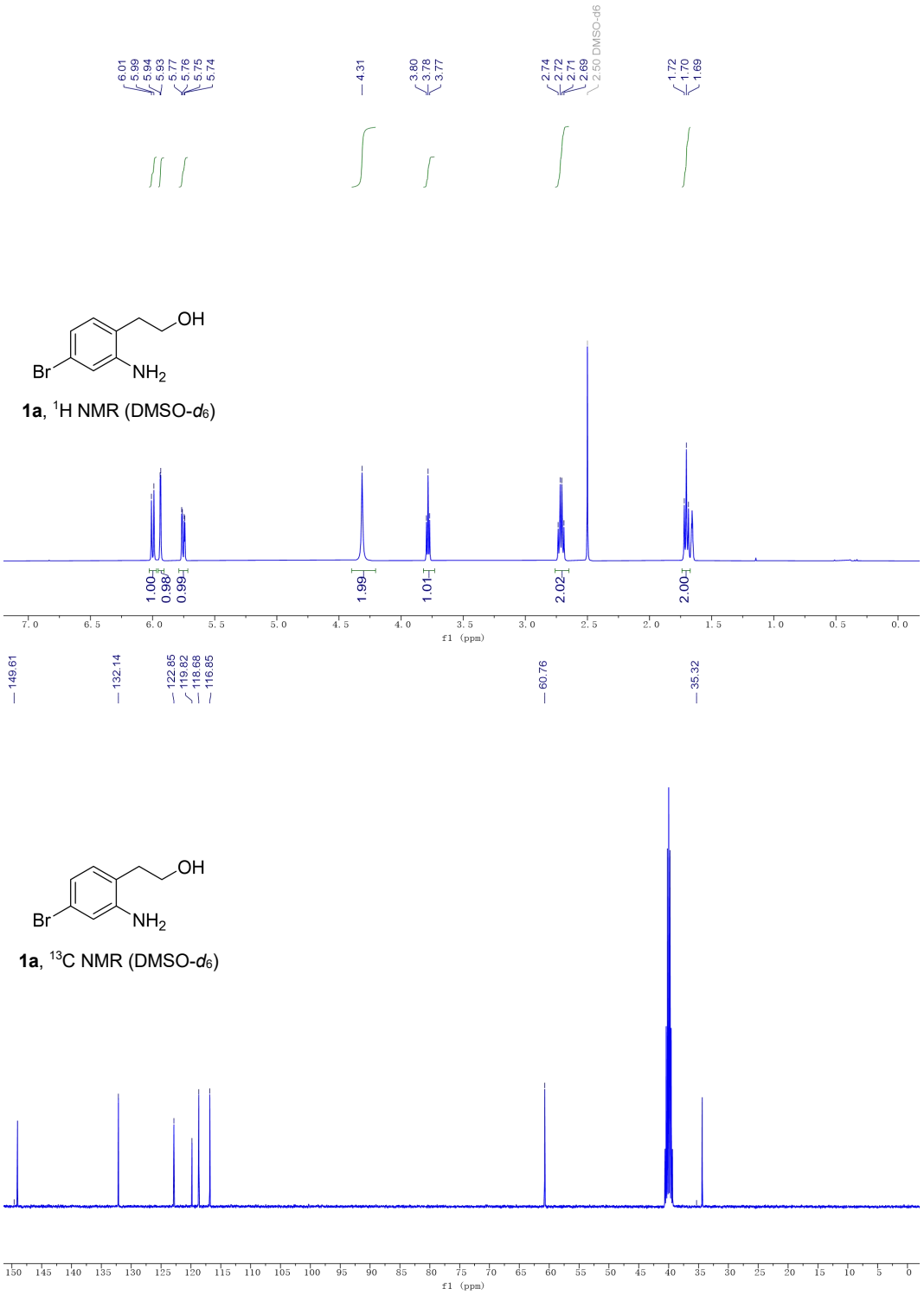


Supplementary Figure 13. SDS-PAGE analysis of M1-M12. Yellow arrow: mFMO; Red arrow: HLADH.

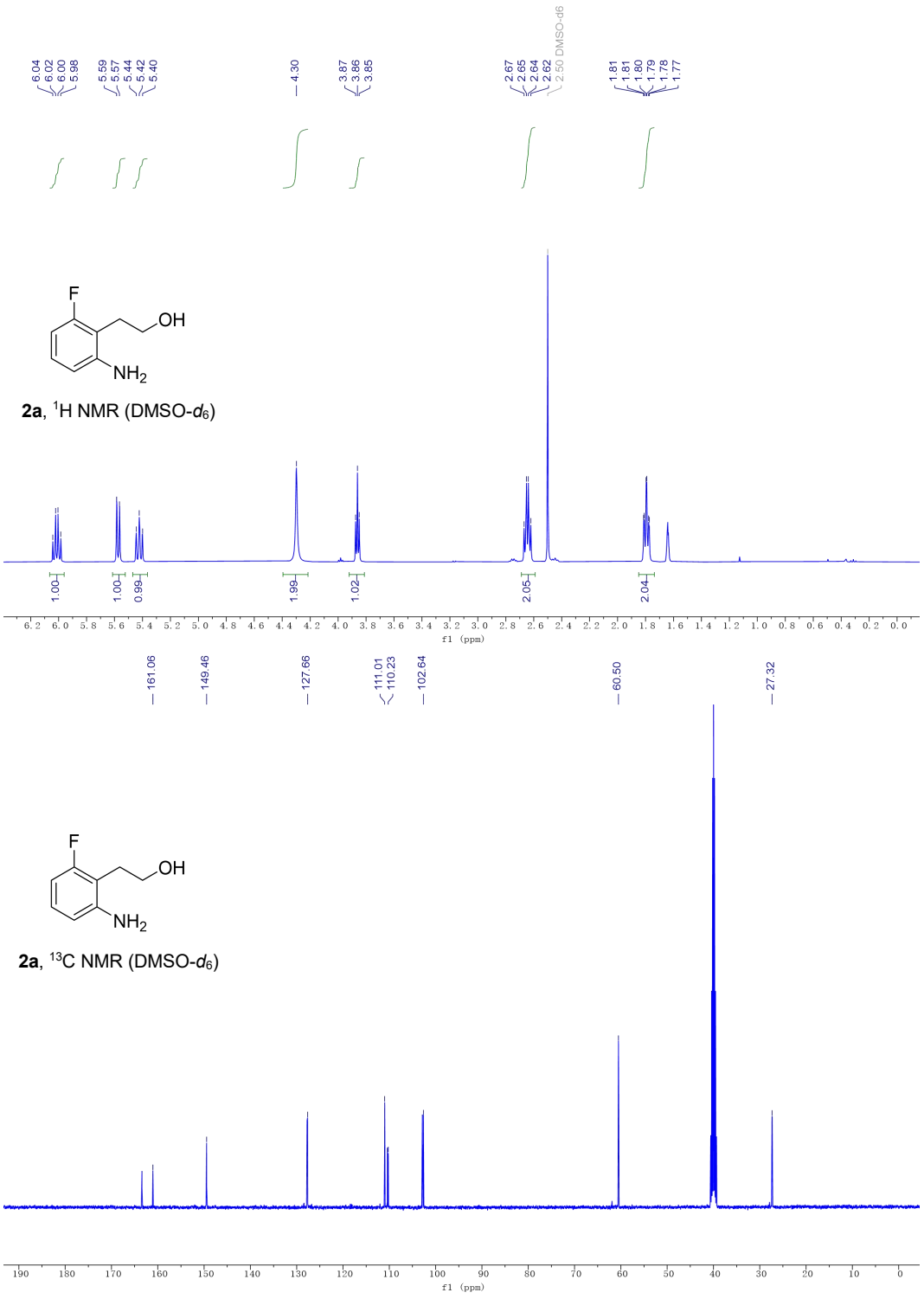


Supplementary Figure 14. Optimization of whole-cell reaction conditions catalyzed by M1. **a** Optimization of IPTG concentration ranging from 0.05 mM to 0.5 mM. **b** Optimization of induction temperature ranging from 16 °C to 30 °C. **c** Optimization of catalytic temperature ranging from 25 °C to 40 °C. **d** Optimization of catalytic pH ranging from pH 6 to pH 9.

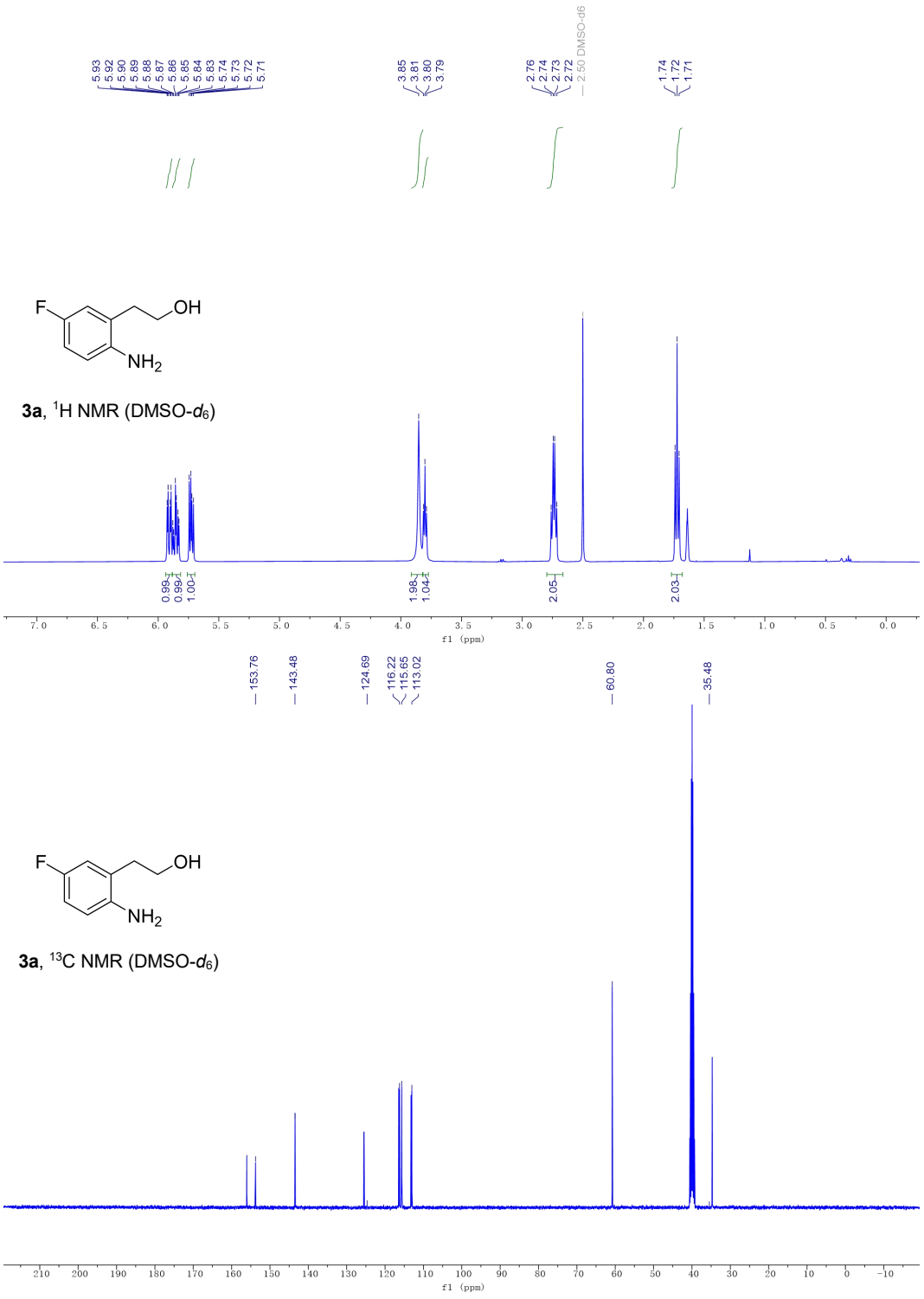
Supplementary Figure 15. Six structurally diverse indigoid dyes were systematically evaluated. The compounds are arranged from left to right as follows: 6,6'-dichloroindigo, 7,7'-dichloroindigo, 7,7'-dibromoindigo, 6,6'-difluoroindigo, 6,6'-dimethylindigo, and 6,6'-dimethoxyindigo.



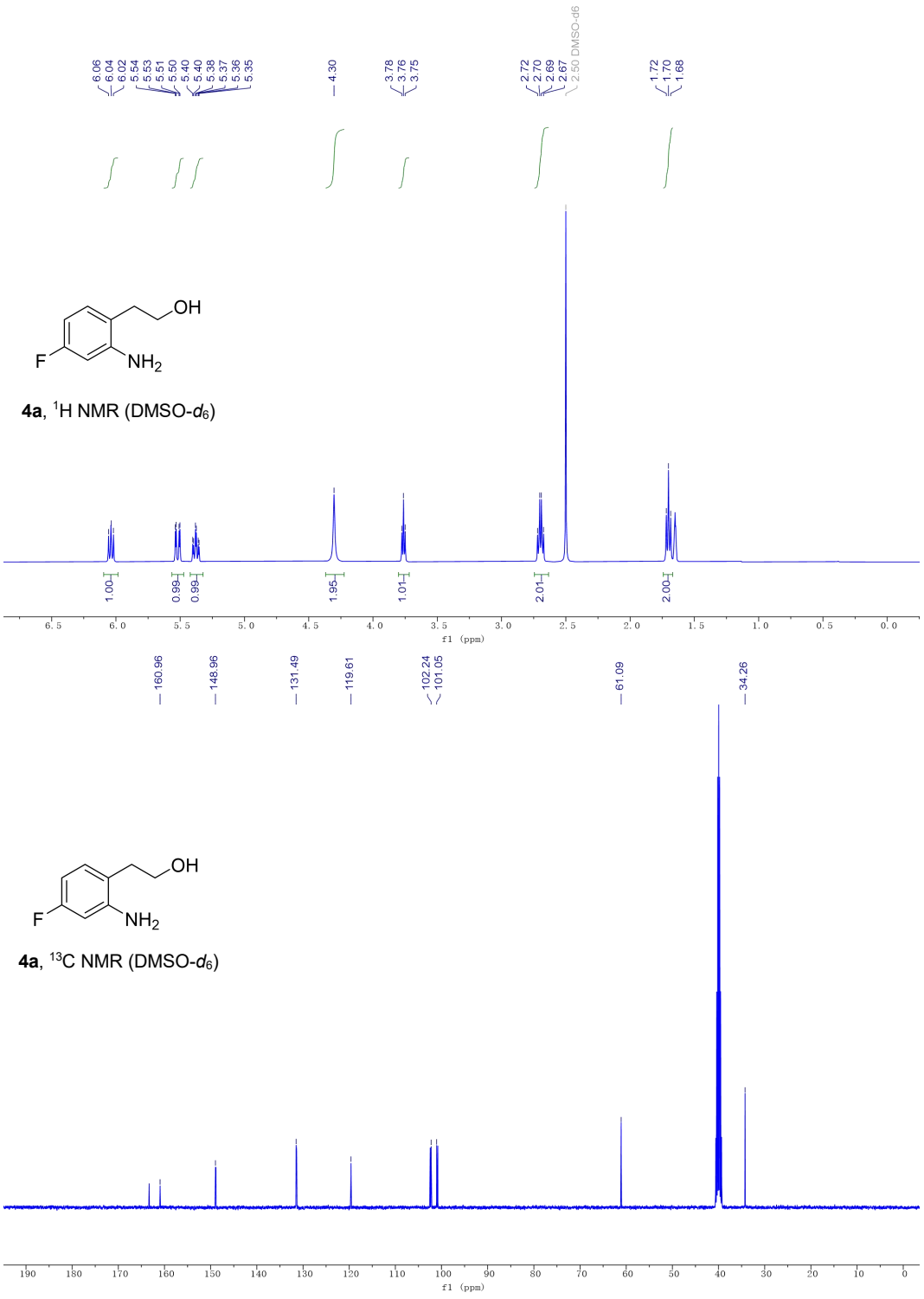
Supplementary Figure 16. ^{1}H NMR and ^{13}C NMR spectra of **1a**.



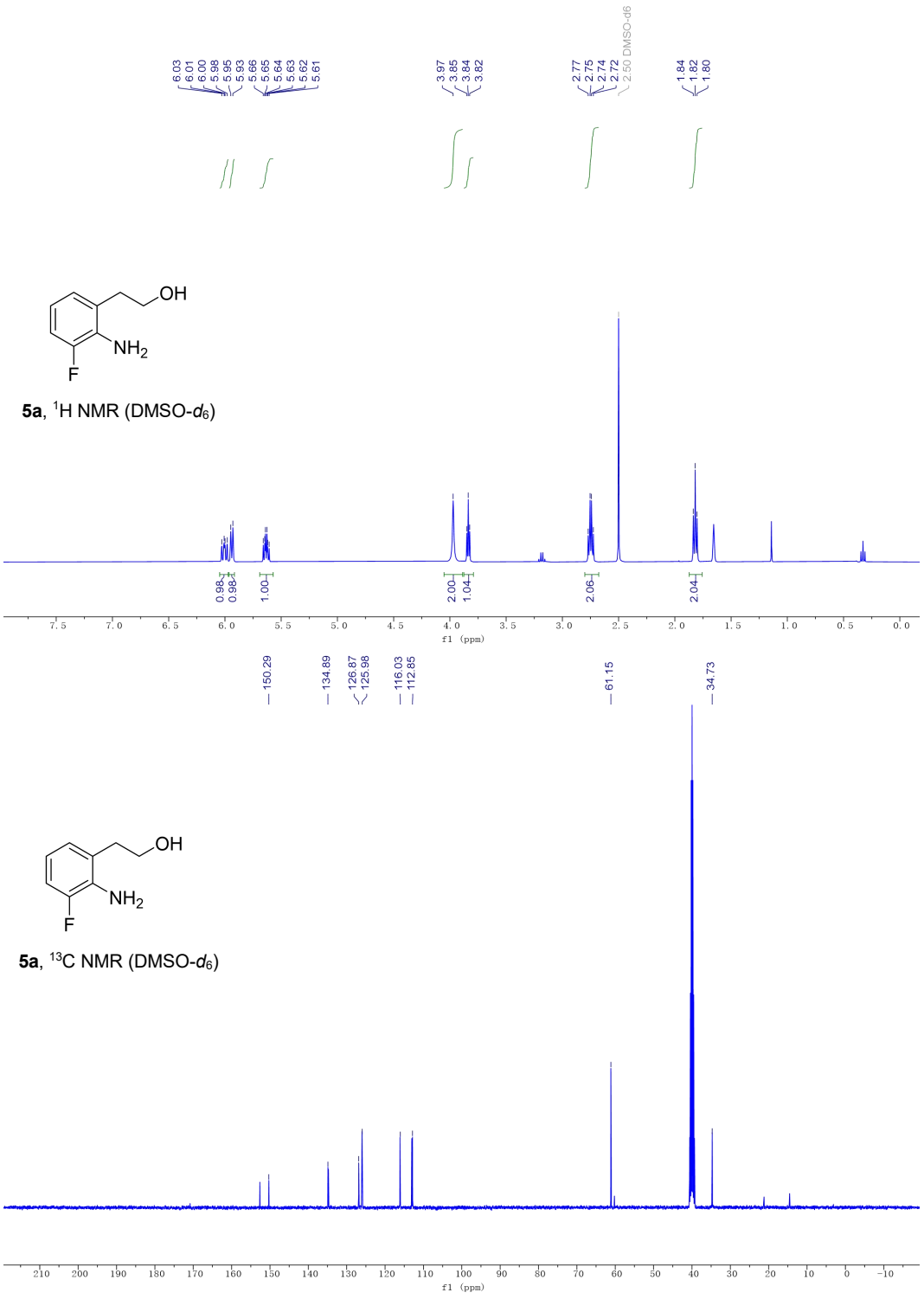
Supplementary Figure 17. ^{1}H NMR and ^{13}C NMR spectra of 2**a**.



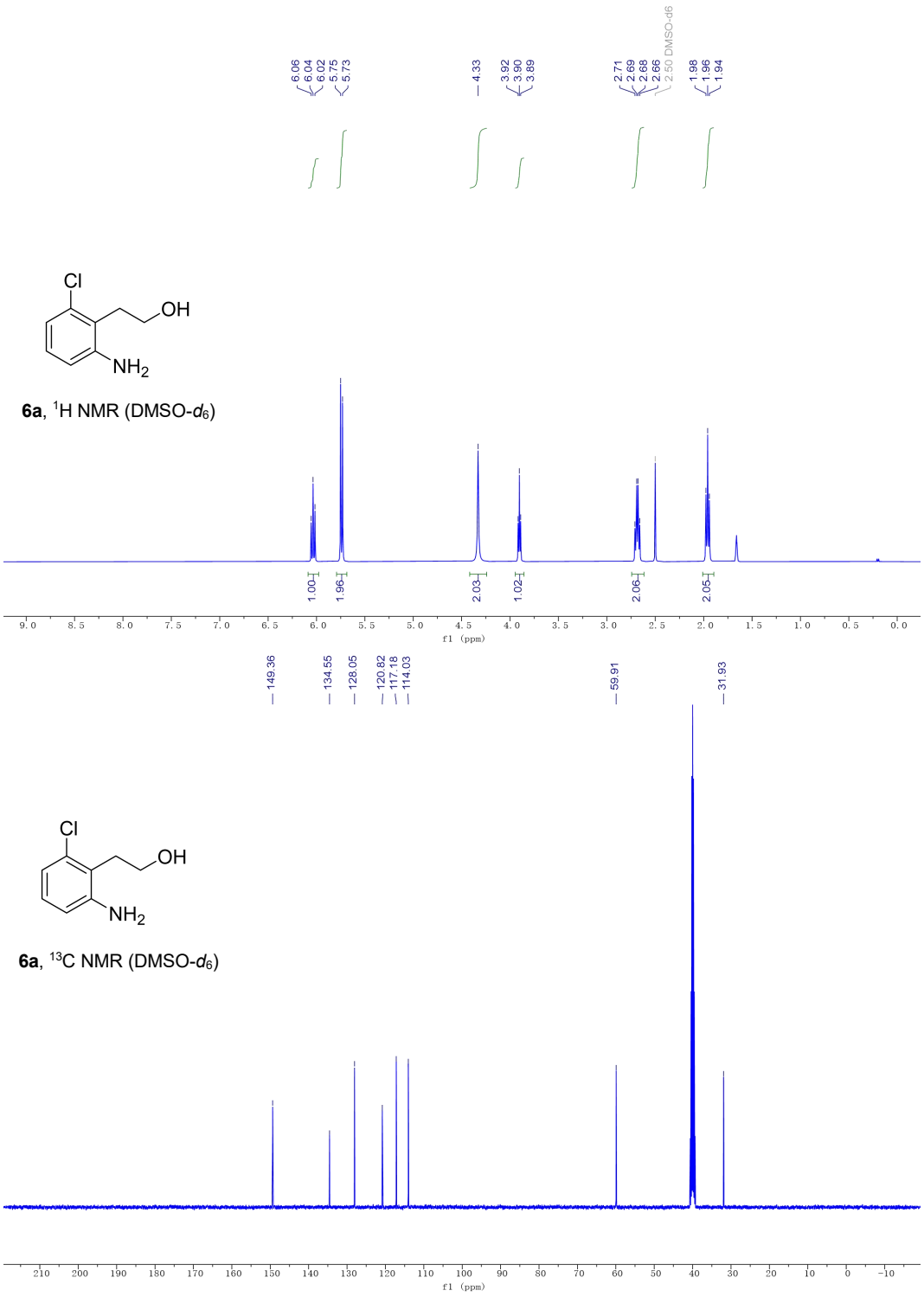
Supplementary Figure 18. ^{1}H NMR and ^{13}C NMR spectra of **3a**.



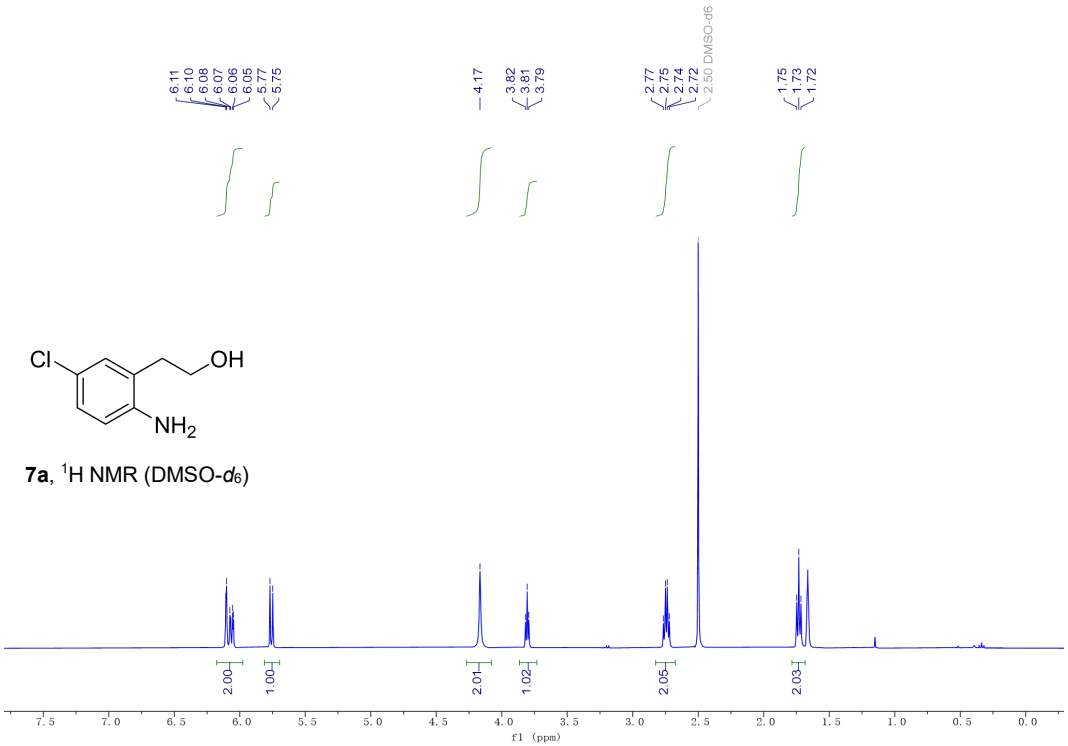
Supplementary Figure 19. ^{1}H NMR and ^{13}C NMR spectra of **4a**.



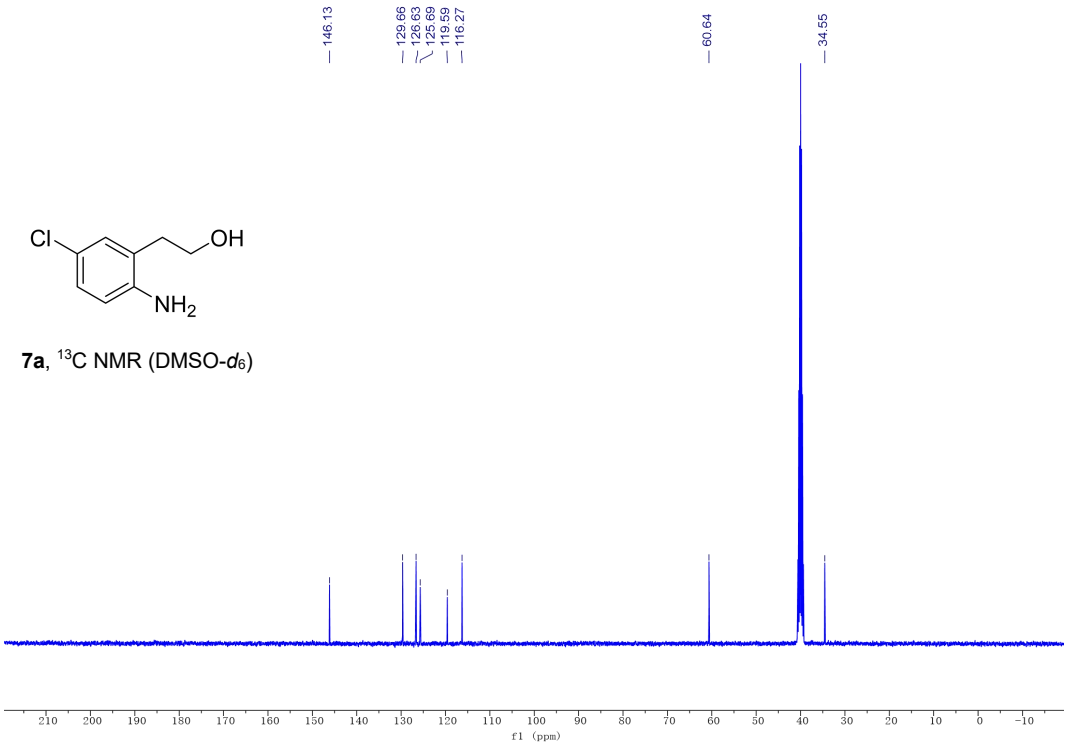
Supplementary Figure 20. ^{1}H NMR and ^{13}C NMR spectra of **5a**.



Supplementary Figure 21. ^{1}H NMR and ^{13}C NMR spectra of **6a**.

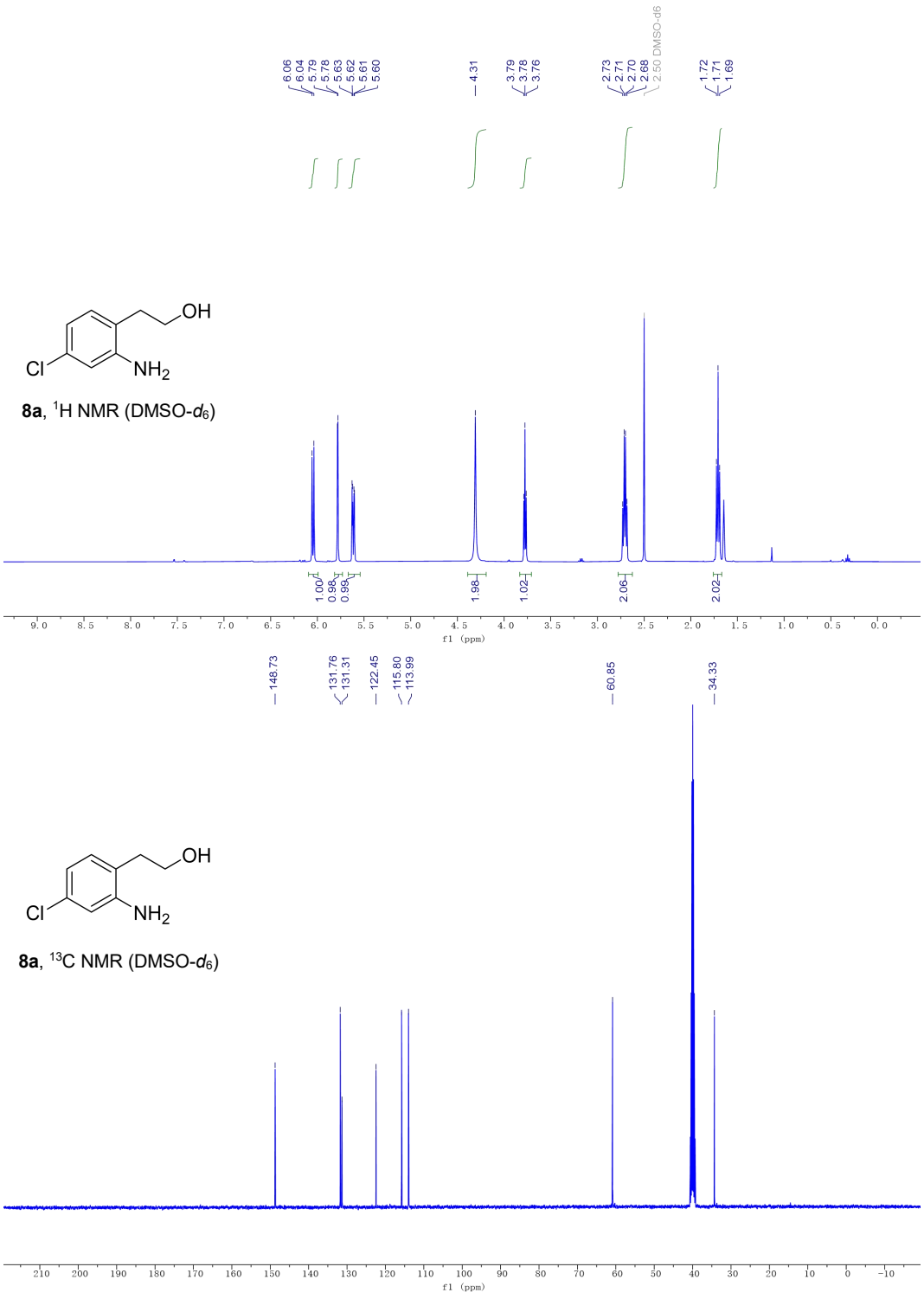


7a, ^{1}H NMR (DMSO-d_6)

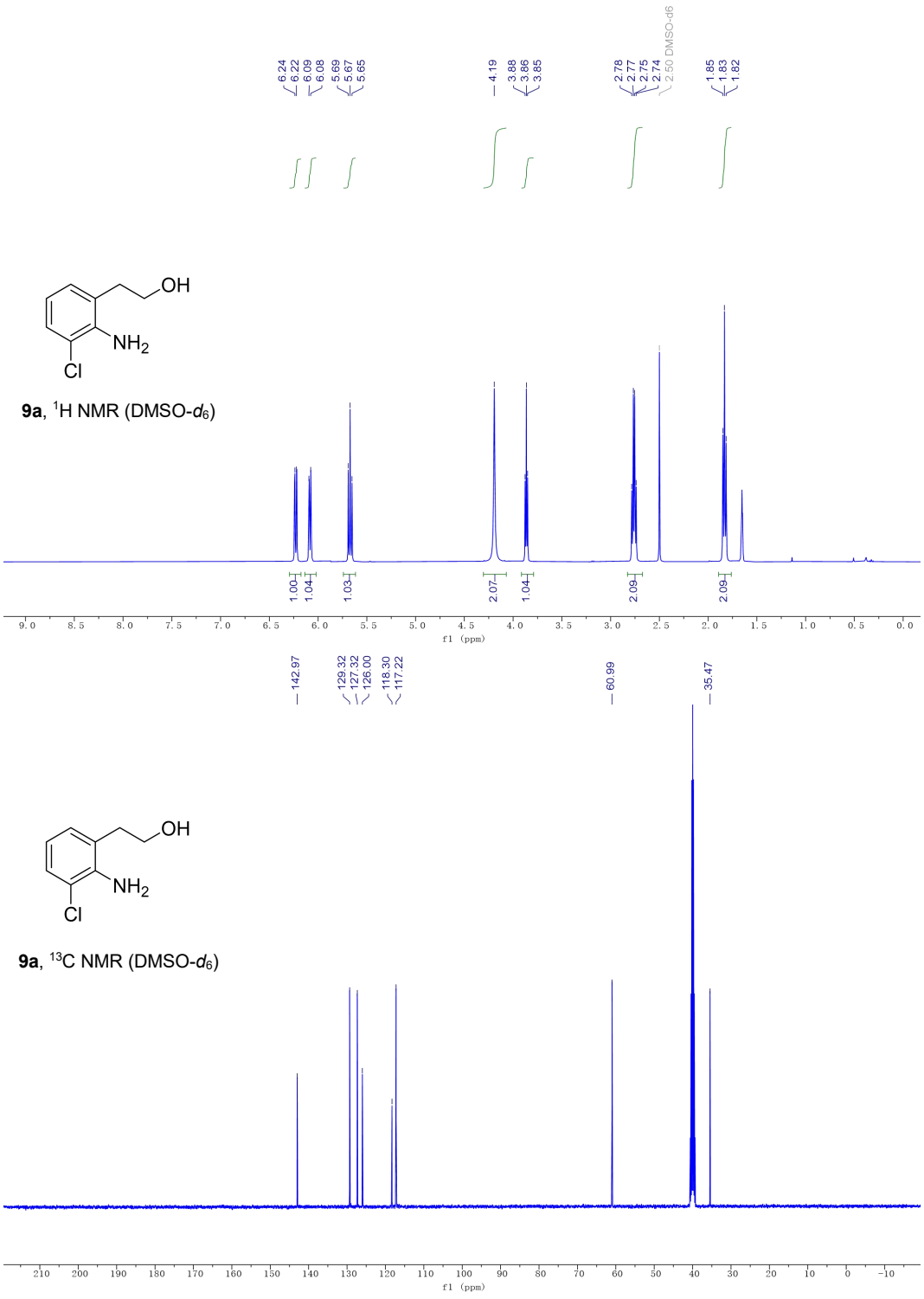


7a, ^{13}C NMR (DMSO-d_6)

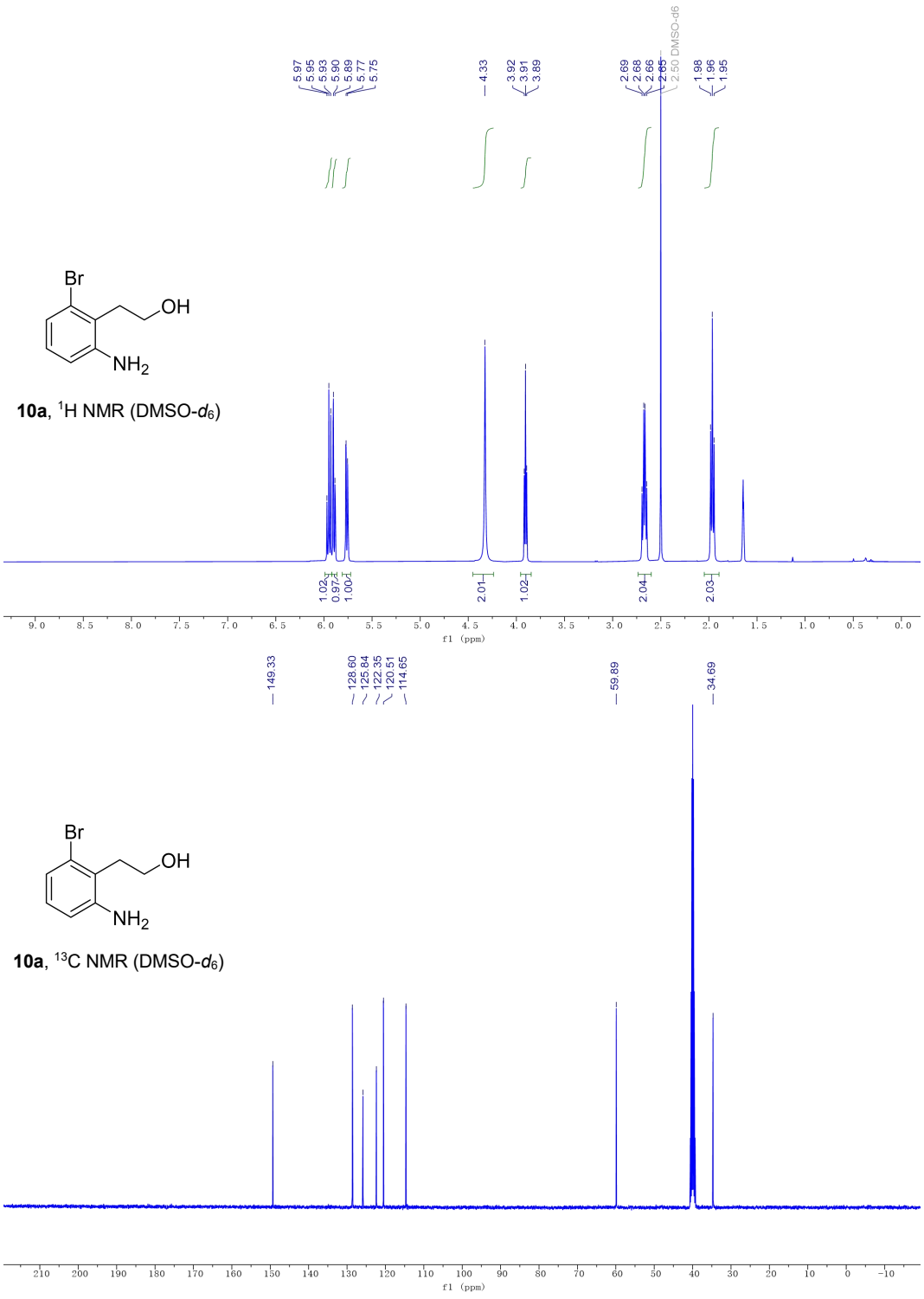
Supplementary Figure 22. ^{1}H NMR and ^{13}C NMR spectra of **7a**.



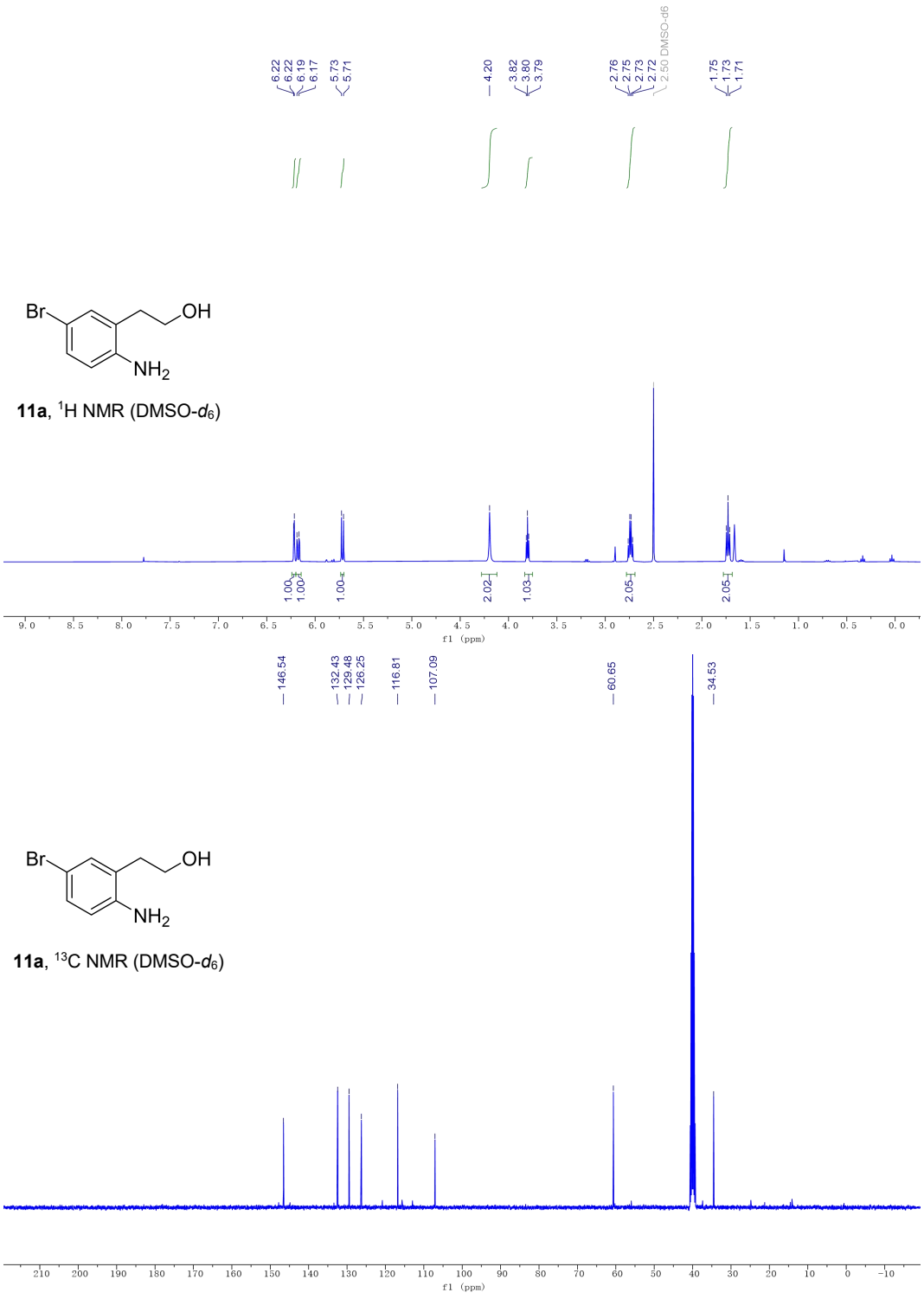
Supplementary Figure 23. ^{1}H NMR and ^{13}C NMR spectra of **8a**.



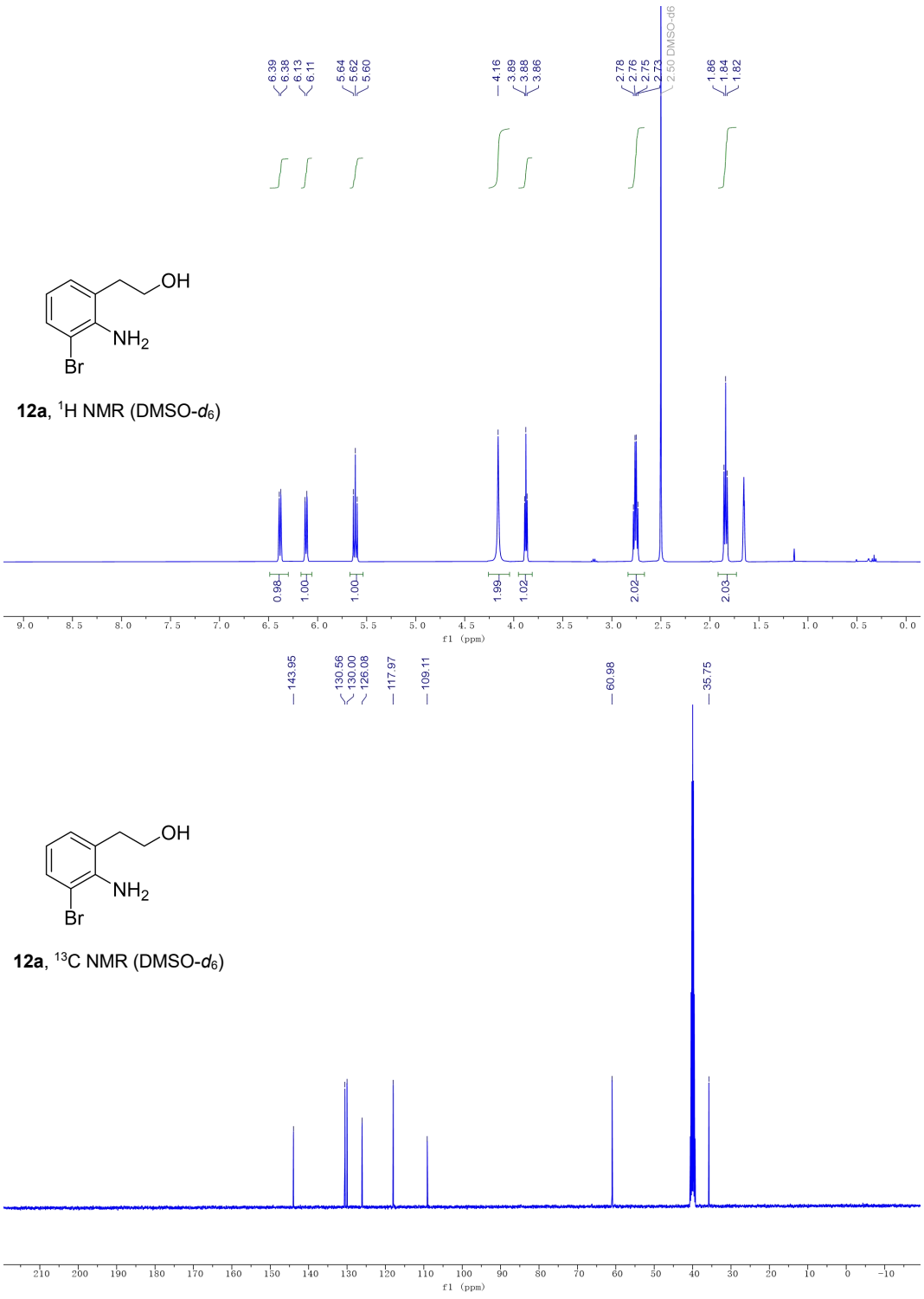
Supplementary Figure 24. ^{1}H NMR and ^{13}C NMR spectra of **9a**.



Supplementary Figure 25. ^{1}H NMR and ^{13}C NMR spectra of **10a**.



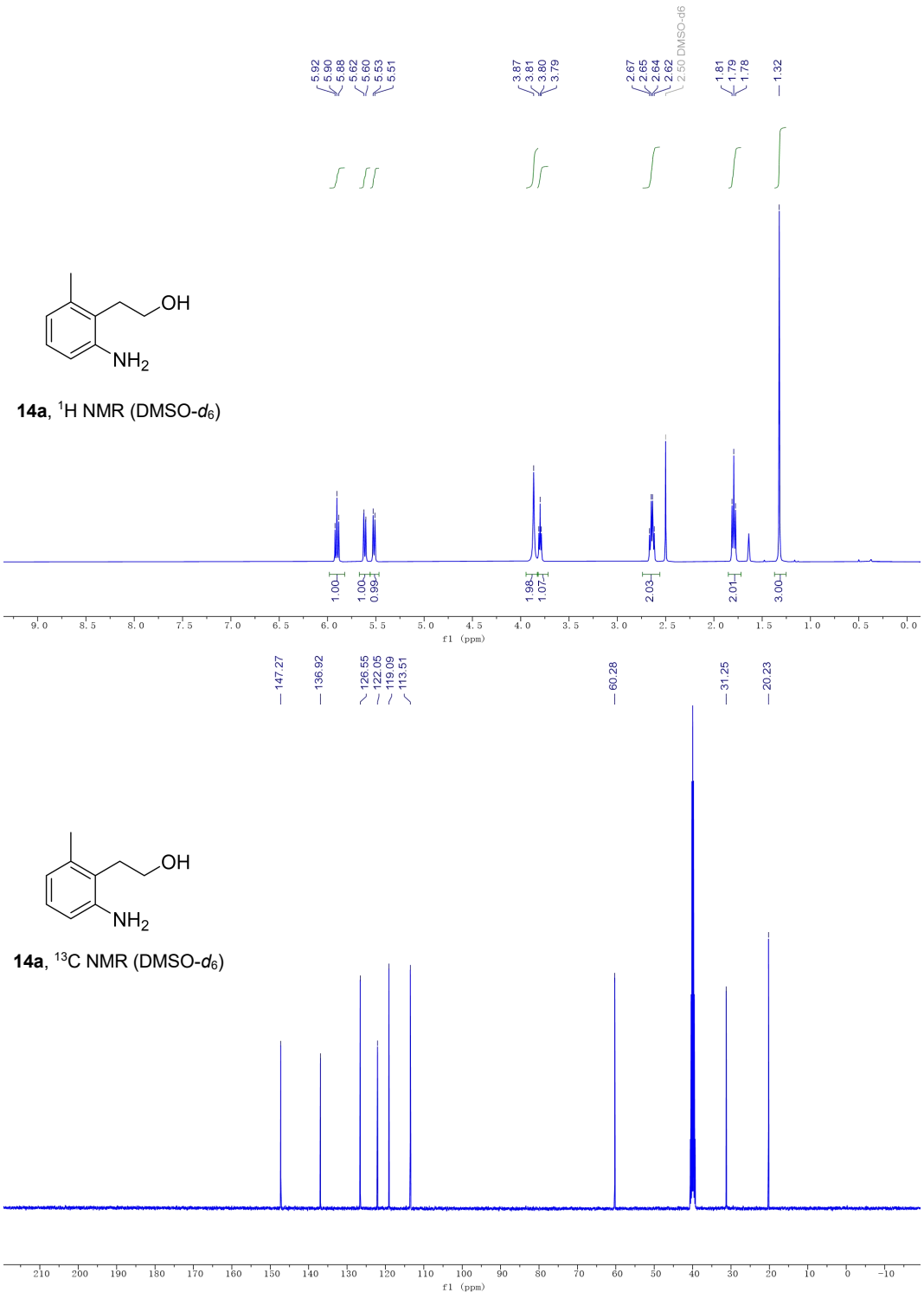
Supplementary Figure 26. ^{1}H NMR and ^{13}C NMR spectra of **11a**.



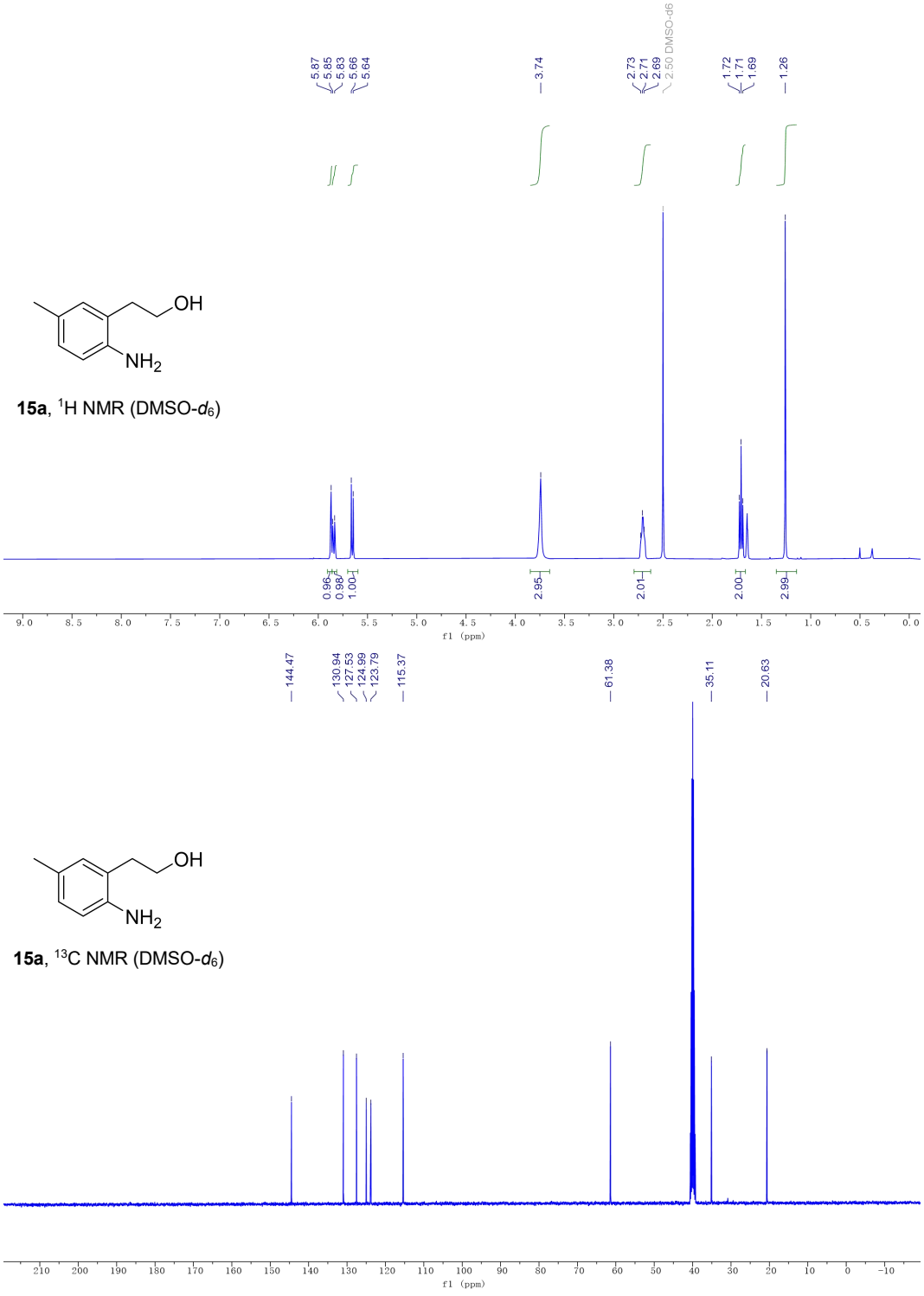
12a, ^{1}H NMR (DMSO-d_6)

12a, ^{13}C NMR (DMSO-d_6)

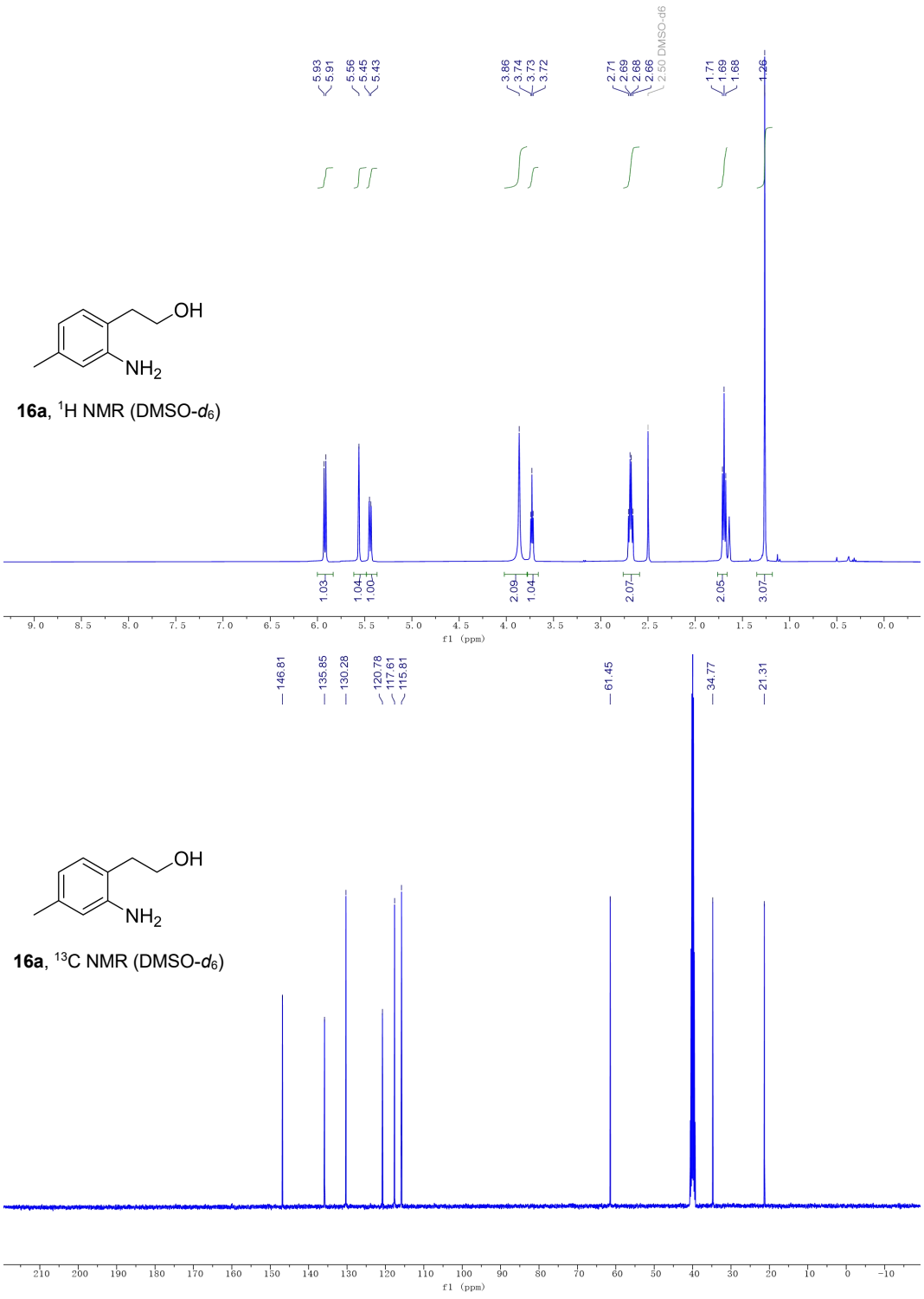
Supplementary Figure 27. ^{1}H NMR and ^{13}C NMR spectra of **12a**.



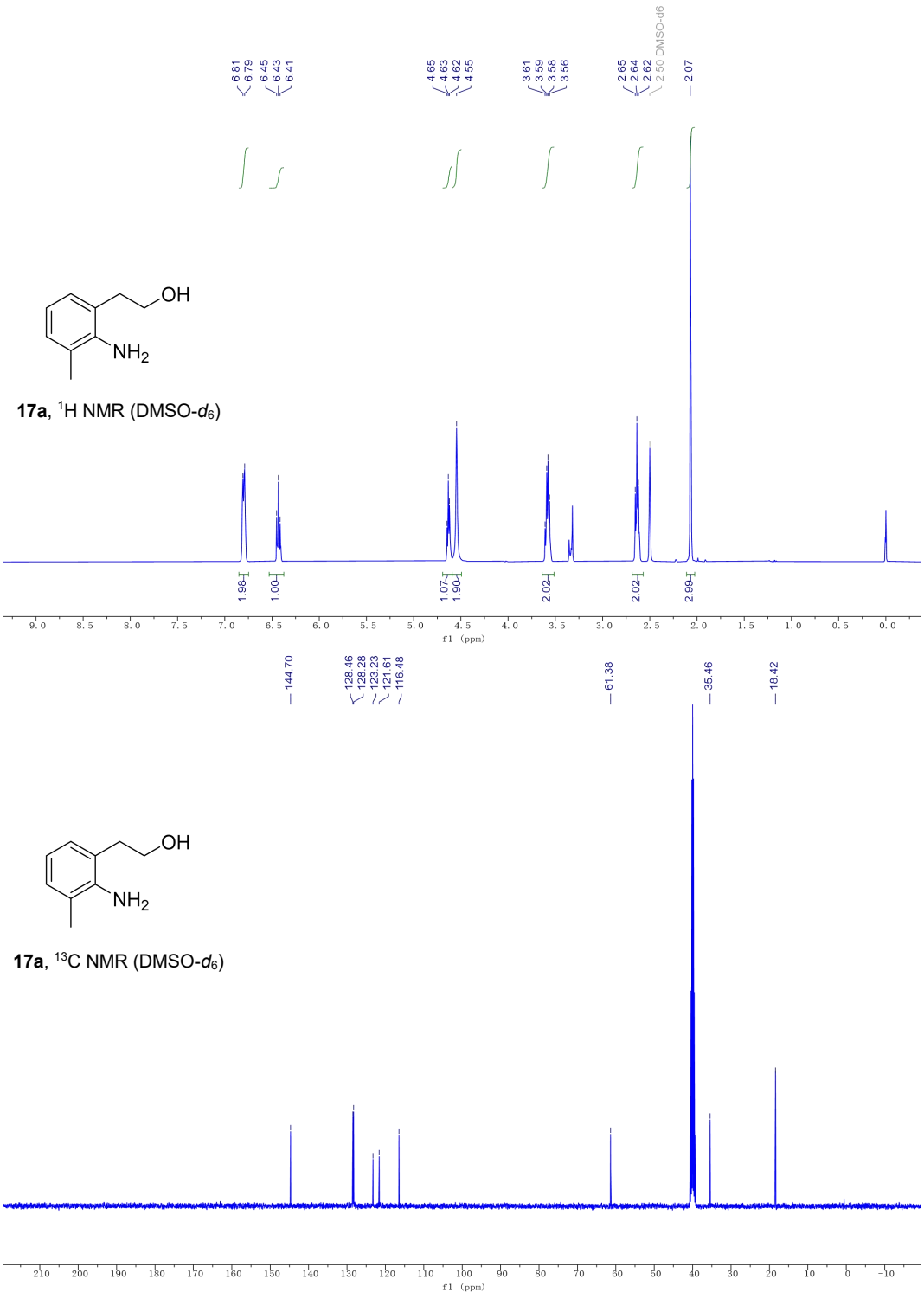
Supplementary Figure 28. ^{1}H NMR and ^{13}C NMR spectra of **14a**.



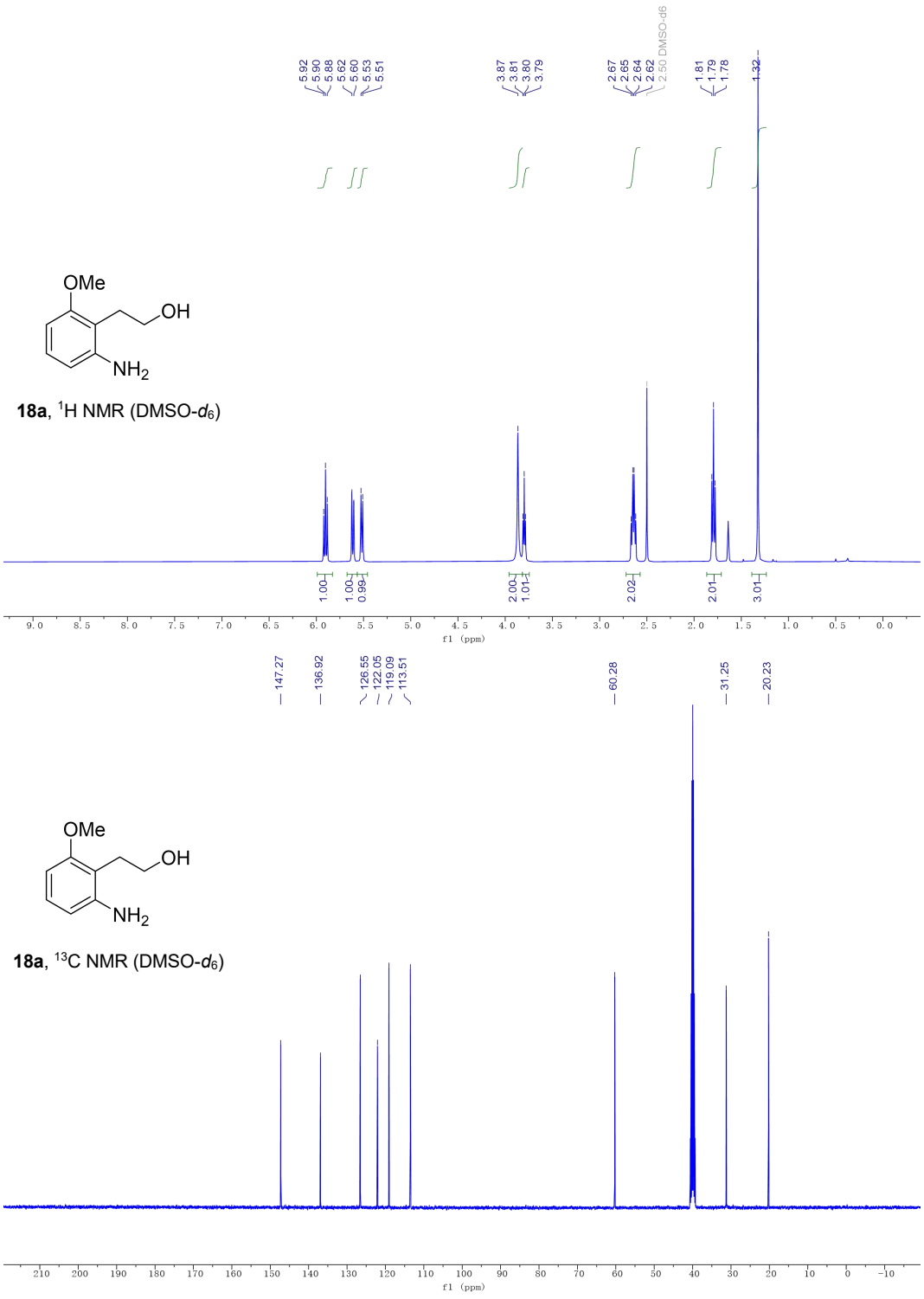
Supplementary Figure 29. ^{1}H NMR and ^{13}C NMR spectra of **15a**.



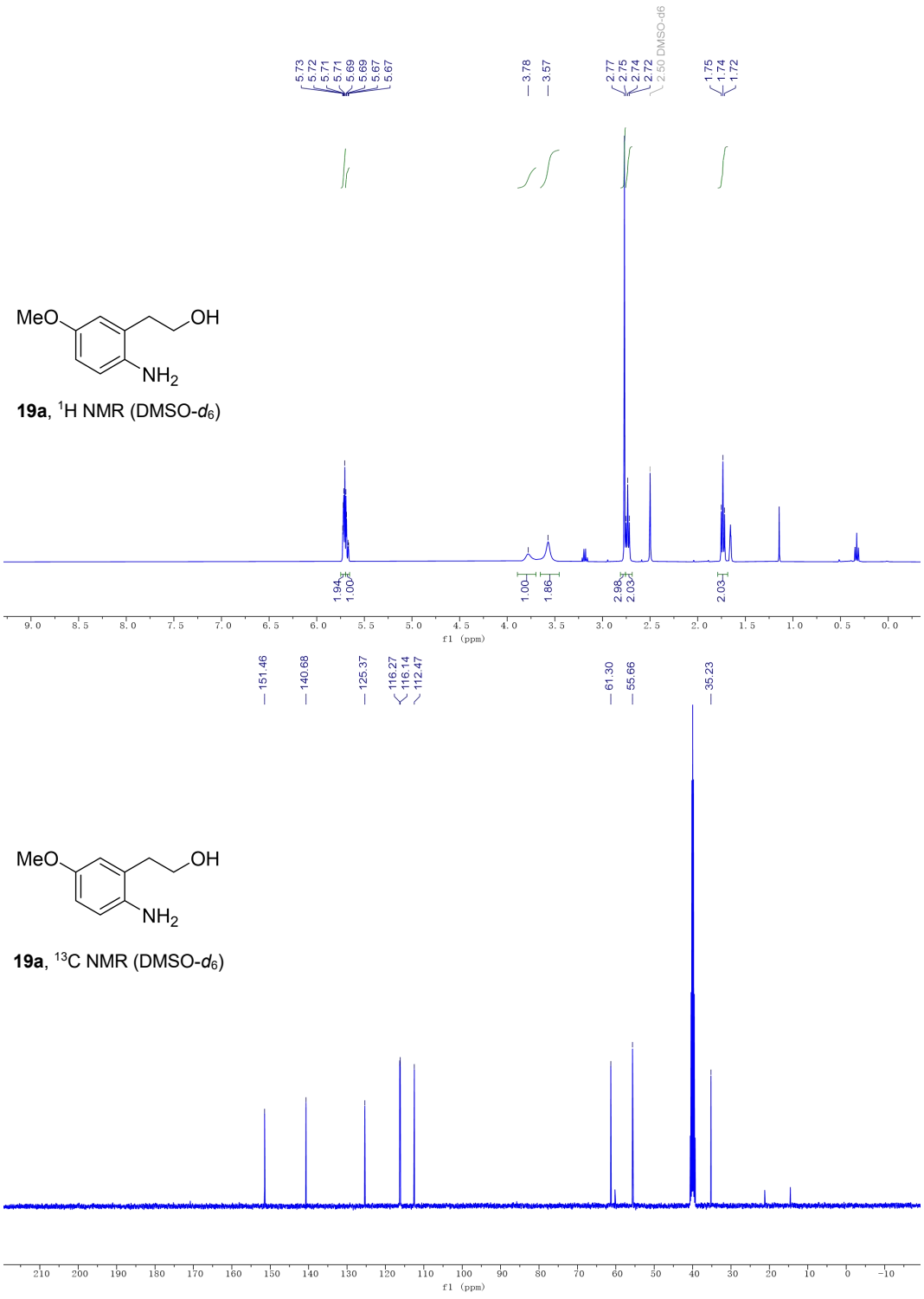
Supplementary Figure 30. ^{1}H NMR and ^{13}C NMR spectra of **16a**.



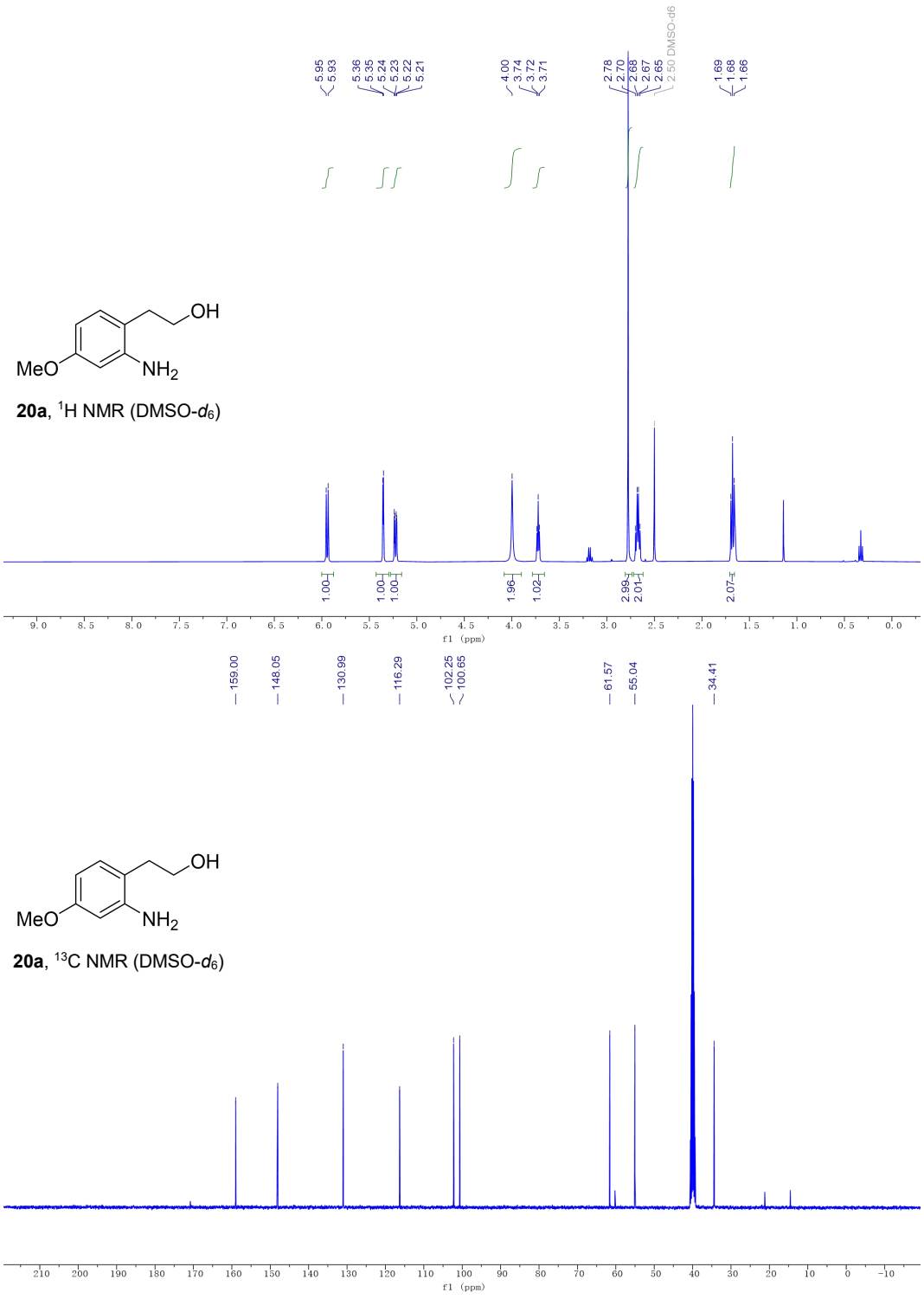
Supplementary Figure 31. ^{1}H NMR and ^{13}C NMR spectra of 1**7a**.



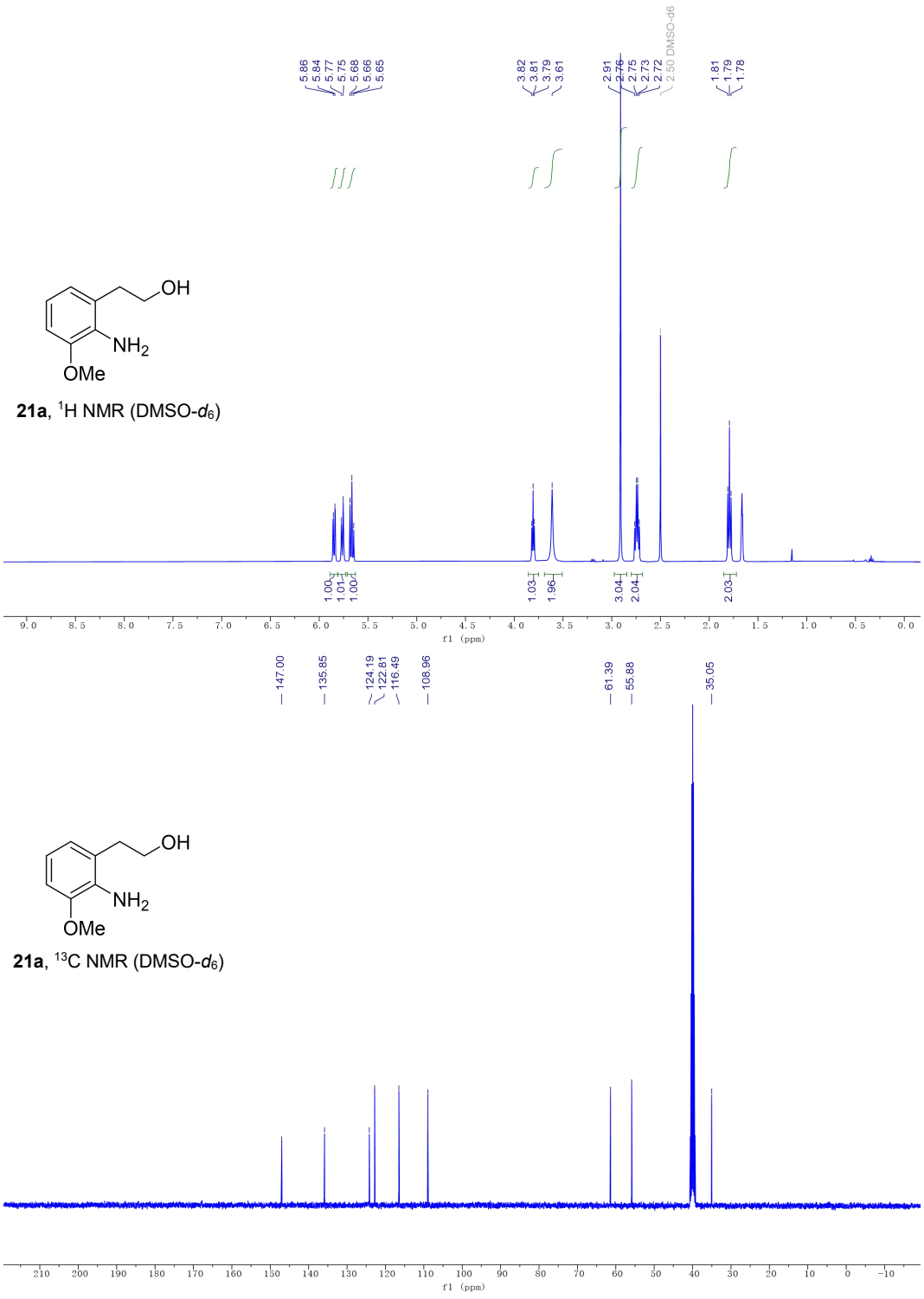
Supplementary Figure 32. ^{1}H NMR and ^{13}C NMR spectra of **18a**.



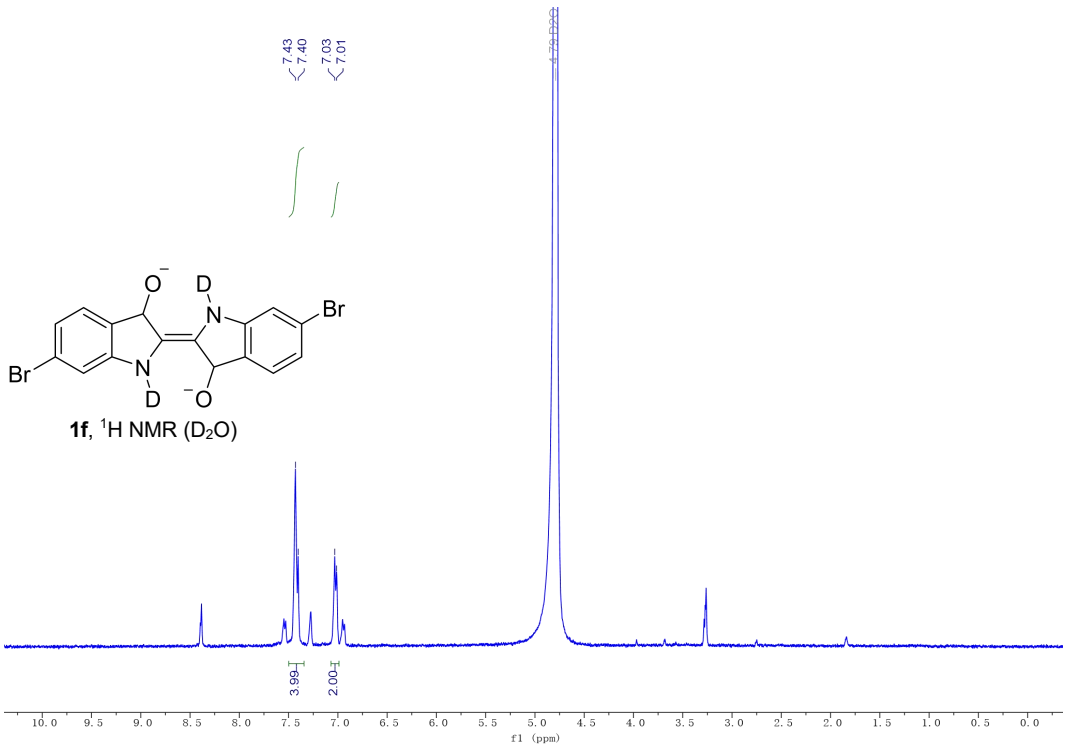
Supplementary Figure 33. ^{1}H NMR and ^{13}C NMR spectra of **19a**.



Supplementary Figure 34. ^{1}H NMR and ^{13}C NMR spectra of **20a**.

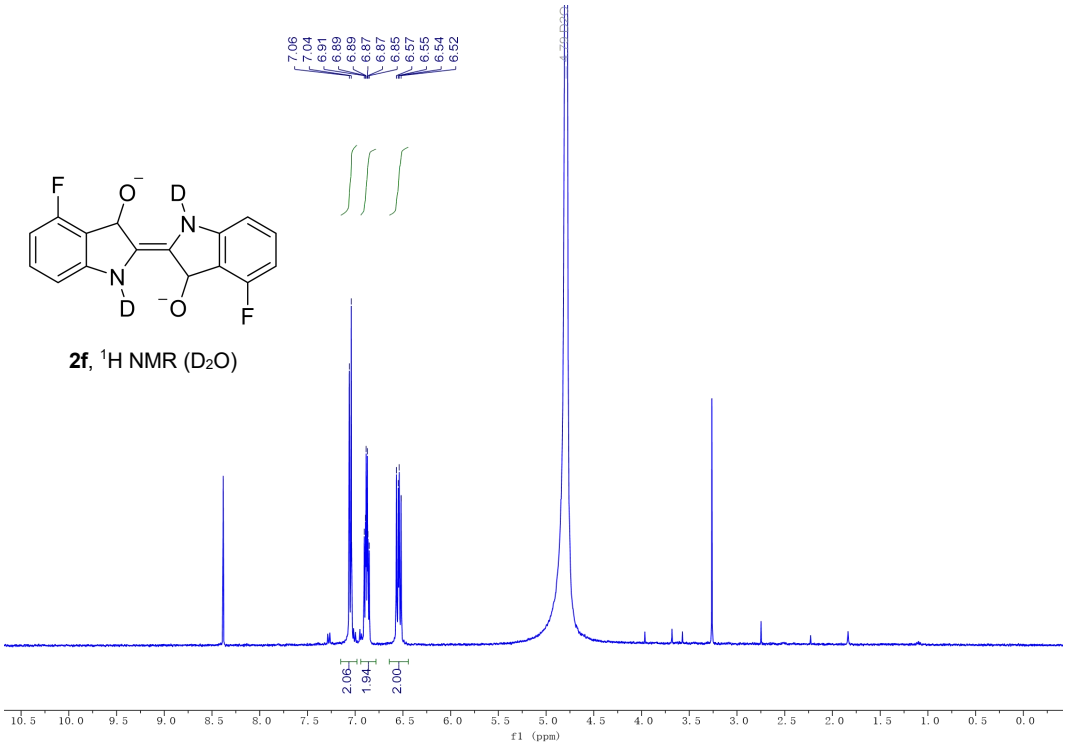


Supplementary Figure 35. ^{1}H NMR and ^{13}C NMR spectra of **21a**.



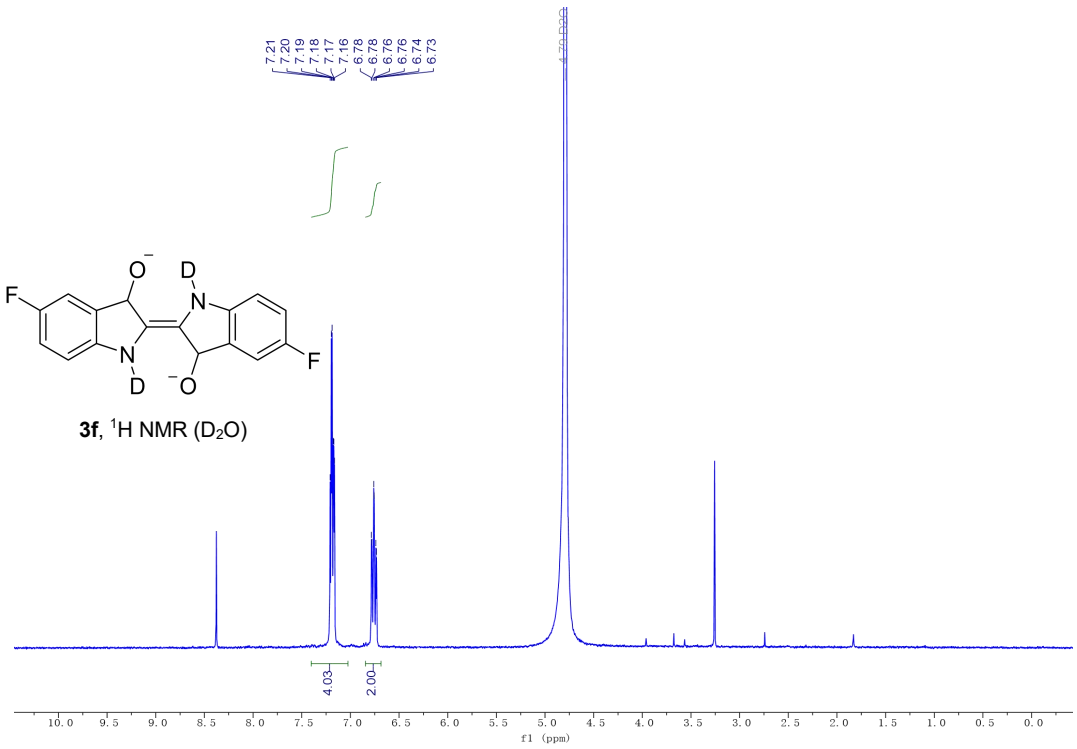
1f, ^{1}H NMR (D_2O)

Supplementary Figure 36. ^{1}H NMR spectra of **1f**.



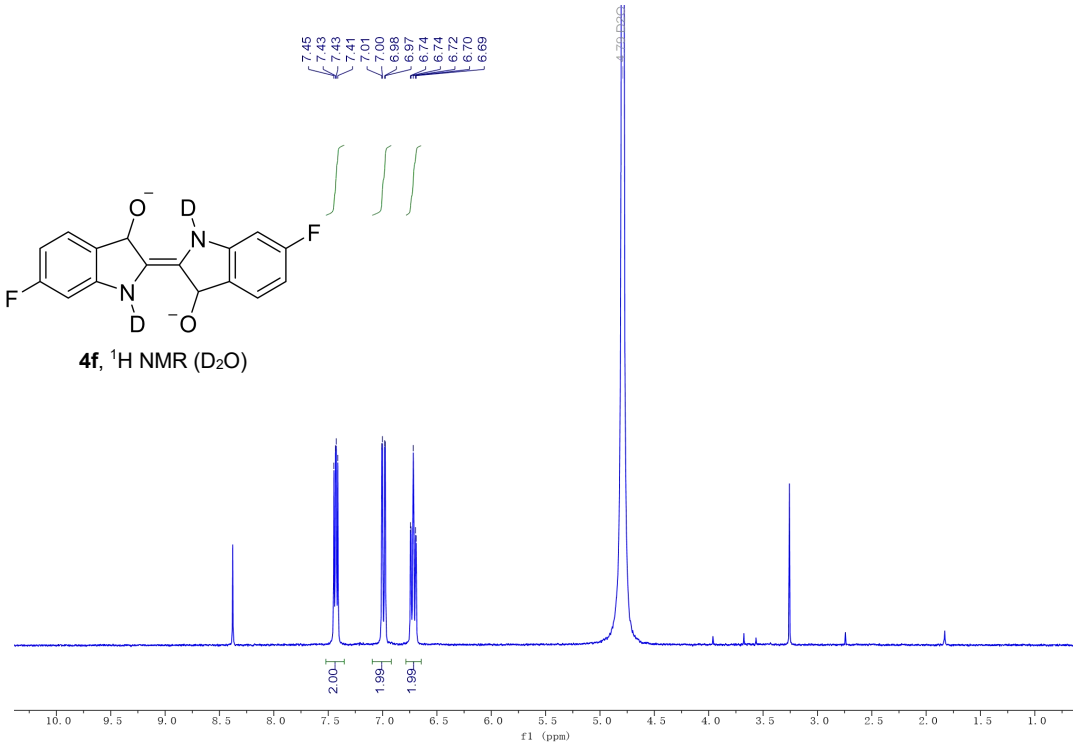
2f, ^{1}H NMR (D_2O)

Supplementary Figure 37. ^{1}H NMR spectra of 2f.



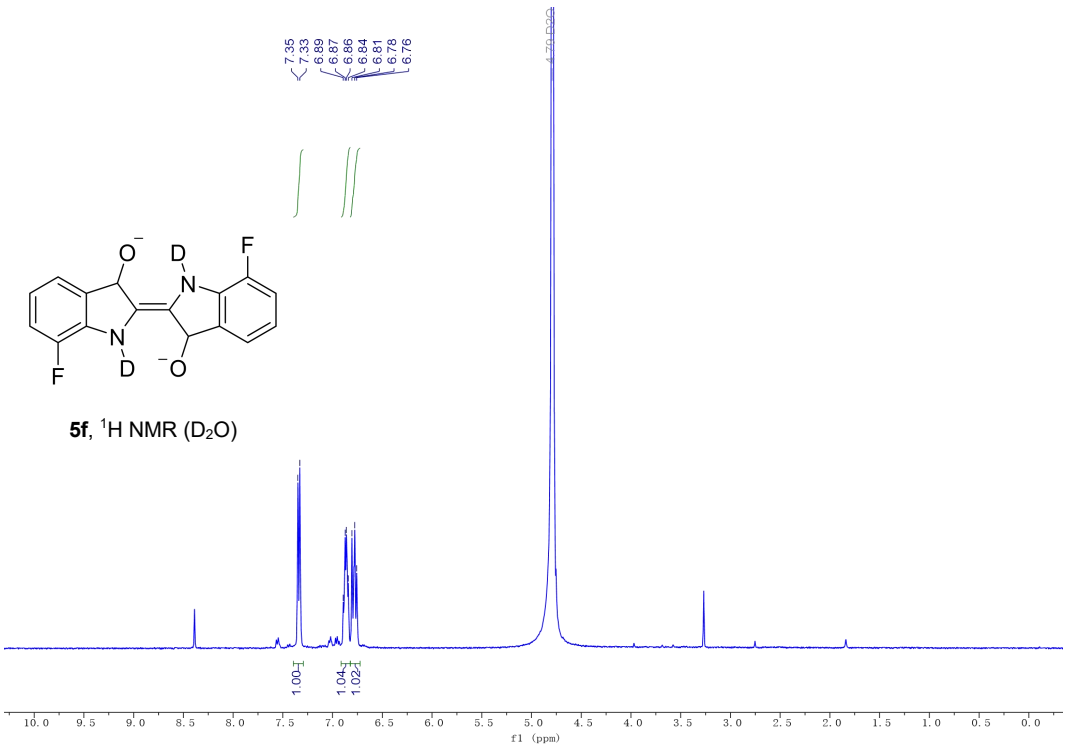
3f, ^{1}H NMR (D_2O)

Supplementary Figure 38. ^{1}H NMR spectra of **3f**.



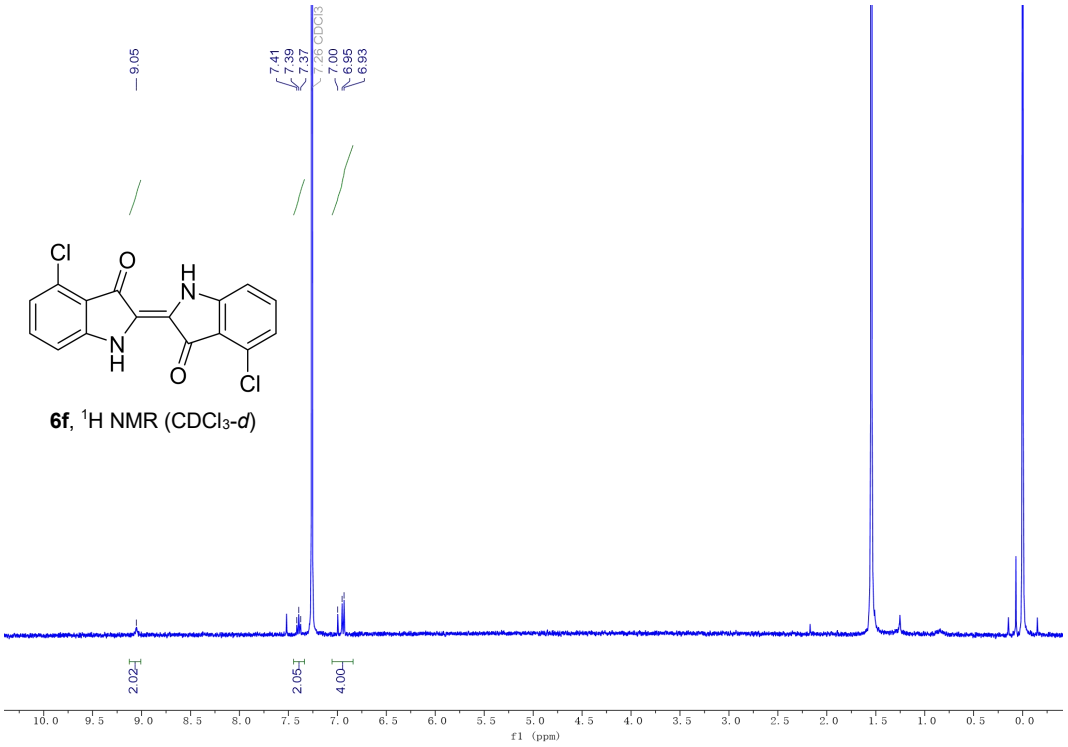
4f, ^{1}H NMR (D_2O)

Supplementary Figure 39. ^{1}H NMR spectra of **4f**.



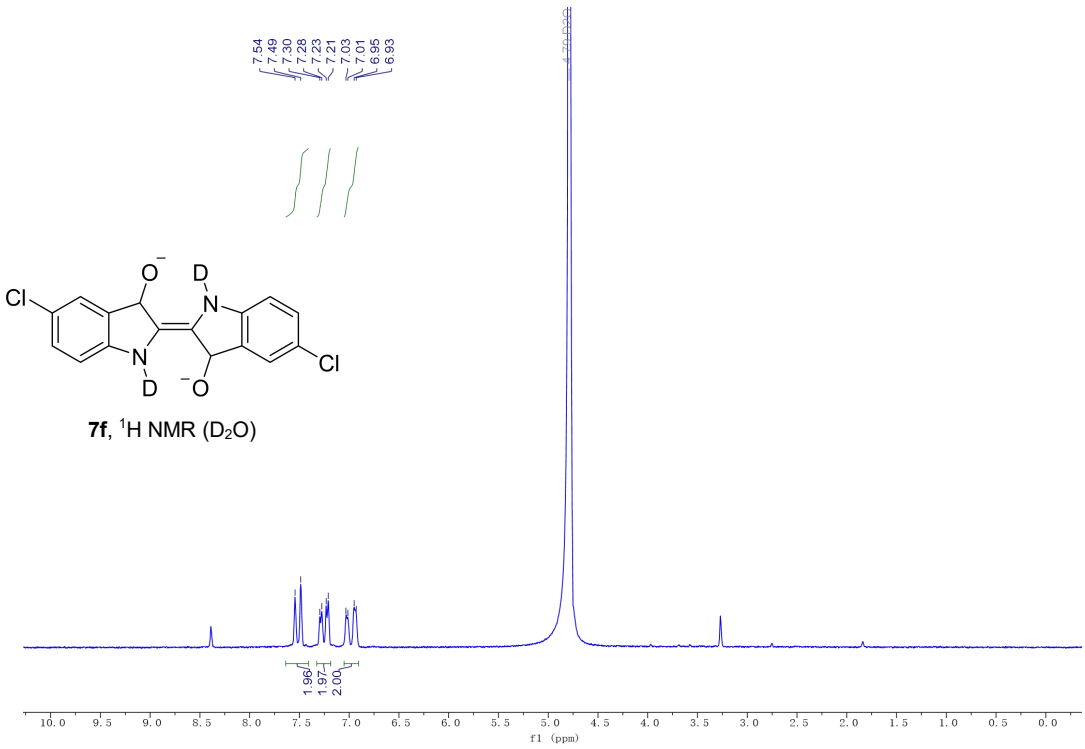
5f, ^{1}H NMR (D_2O)

Supplementary Figure 40. ^{1}H NMR spectra of **5f**.



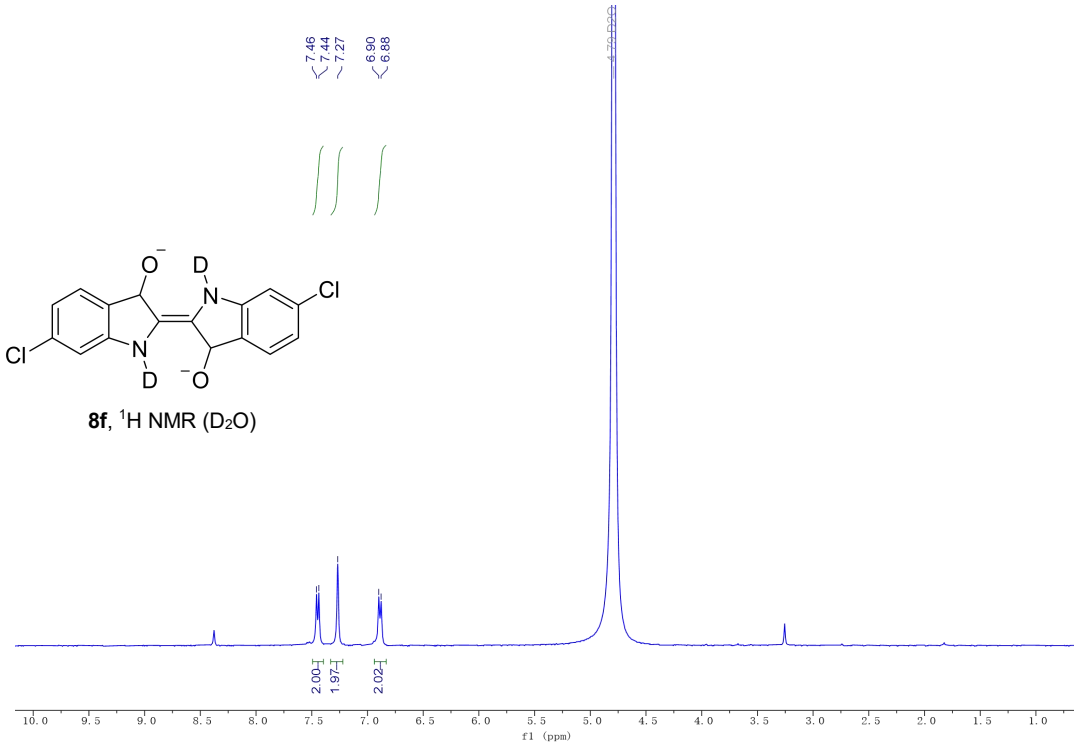
6f, ^{1}H NMR ($CDCl_3$-*d*)

Supplementary Figure 41. ^{1}H NMR spectra of **6f**.



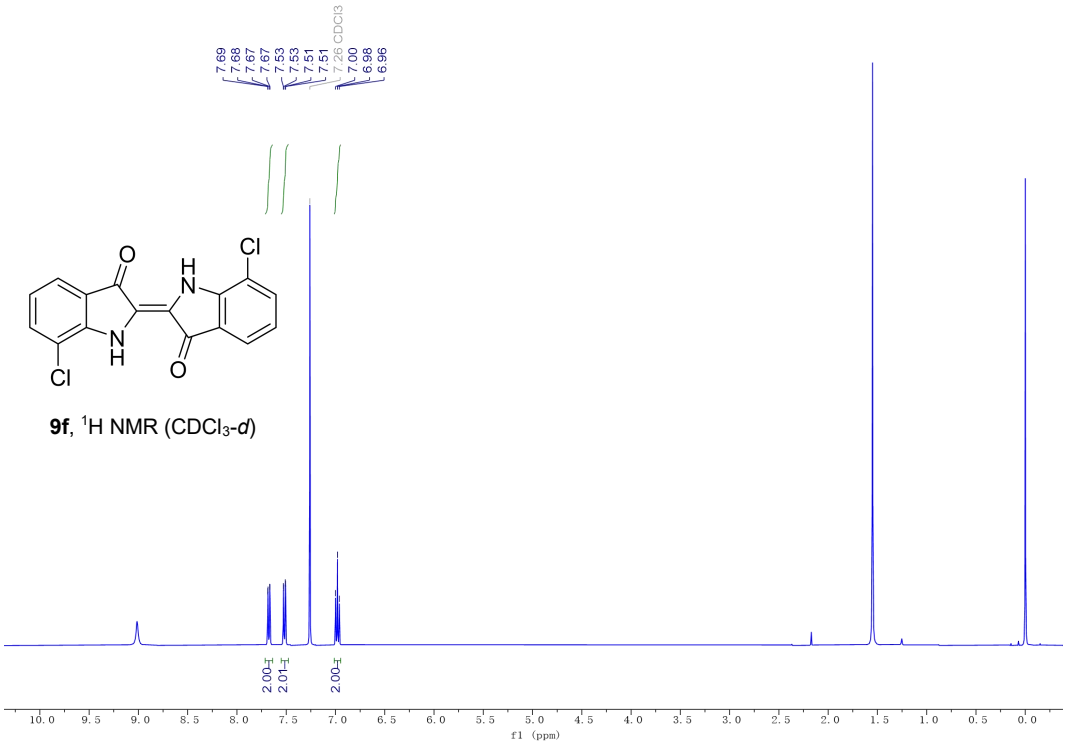
7f, ^{1}H NMR (D_2O)

Supplementary Figure 42. ^{1}H NMR spectra of **7f**.



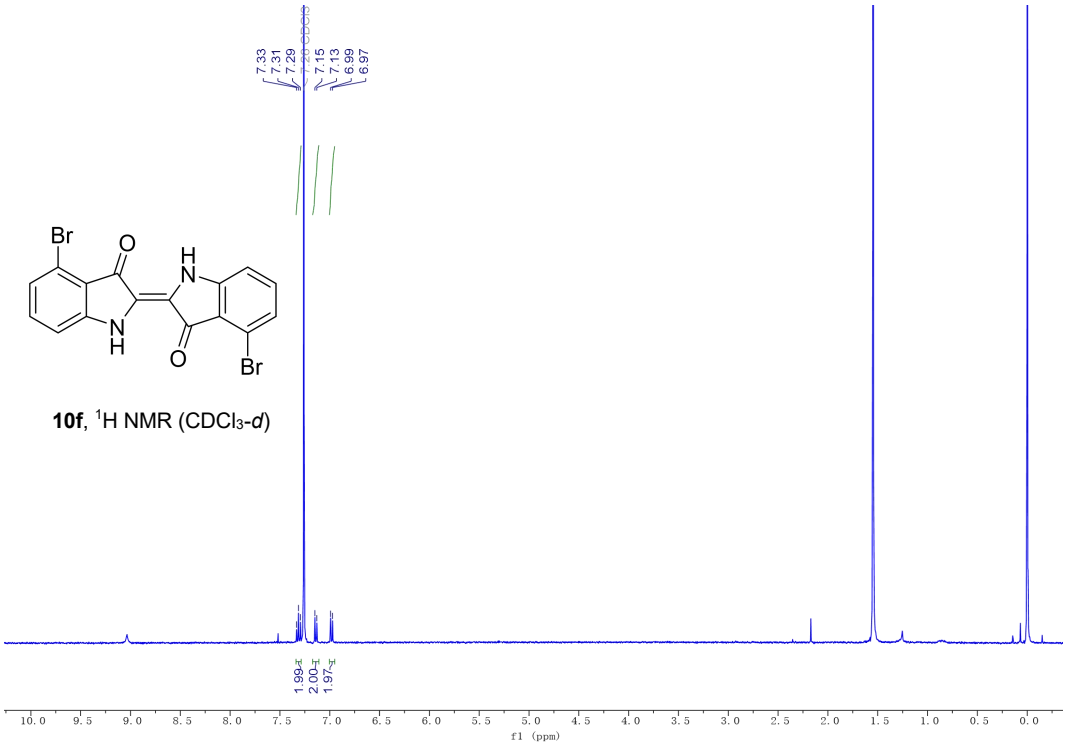
8f, ^{1}H NMR (D_2O)

Supplementary Figure 43. ^{1}H NMR spectra of **8f**.



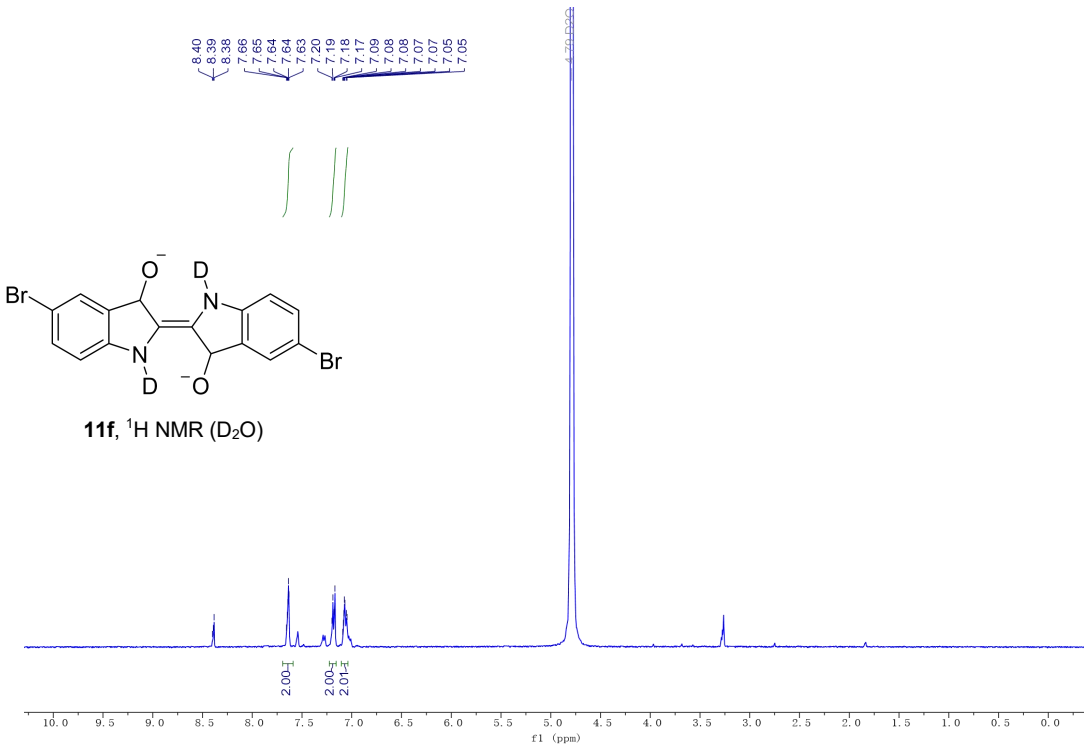
9f, ^{1}H NMR ($CDCl_3$-*d*)

Supplementary Figure 44. ^{1}H NMR spectra of **9f**.



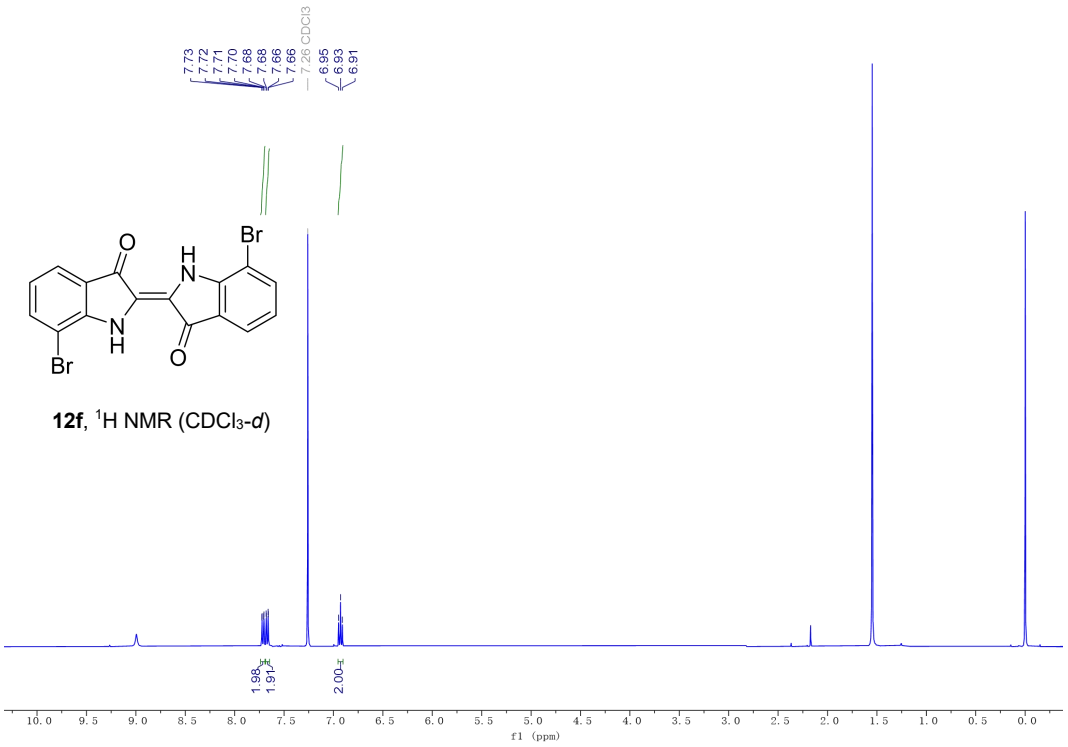
10f, ^{1}H NMR ($CDCl_3$-*d*)

Supplementary Figure 45. ^{1}H NMR spectra of **10f**.



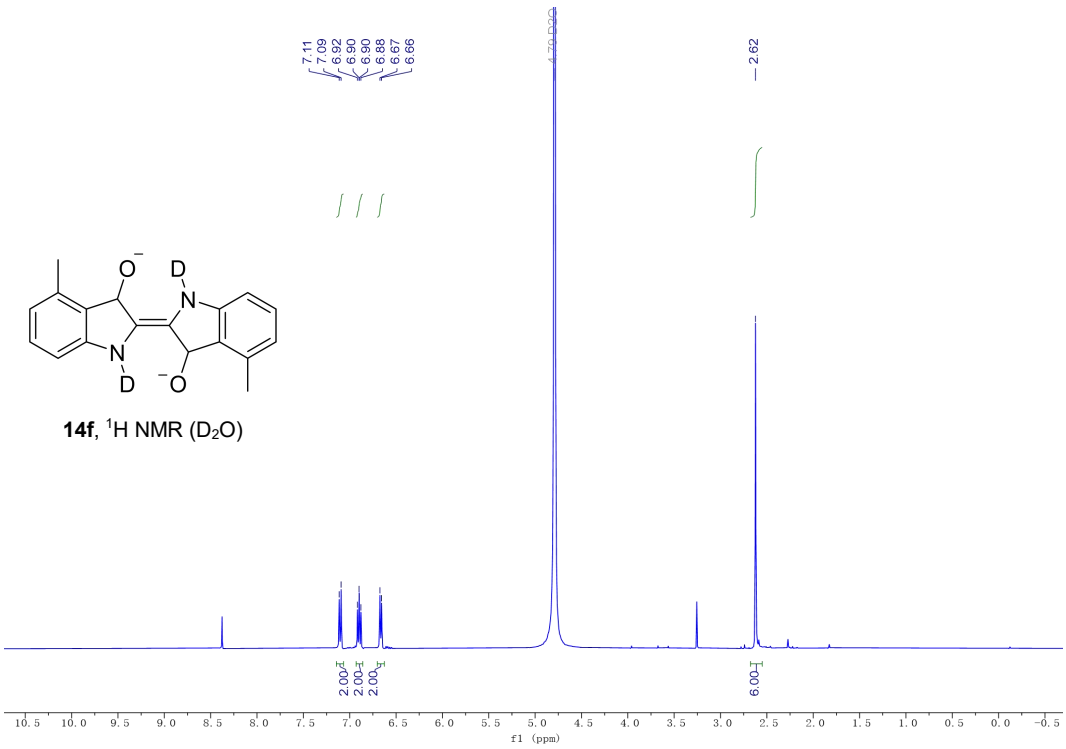
11f, ^{1}H NMR (D_2O)

Supplementary Figure 46. ^{1}H NMR spectra of **11f**.



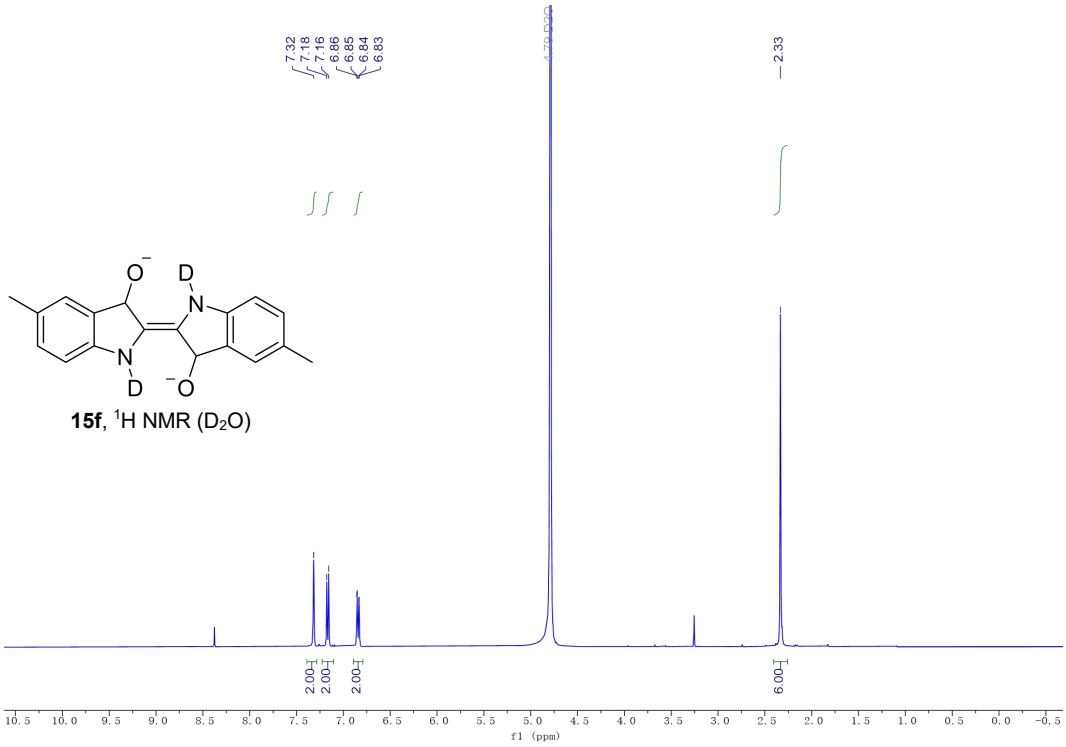
12f, ^{1}H NMR ($CDCl_3$-*d*)

Supplementary Figure 47. ^{1}H NMR spectra of **12f**.



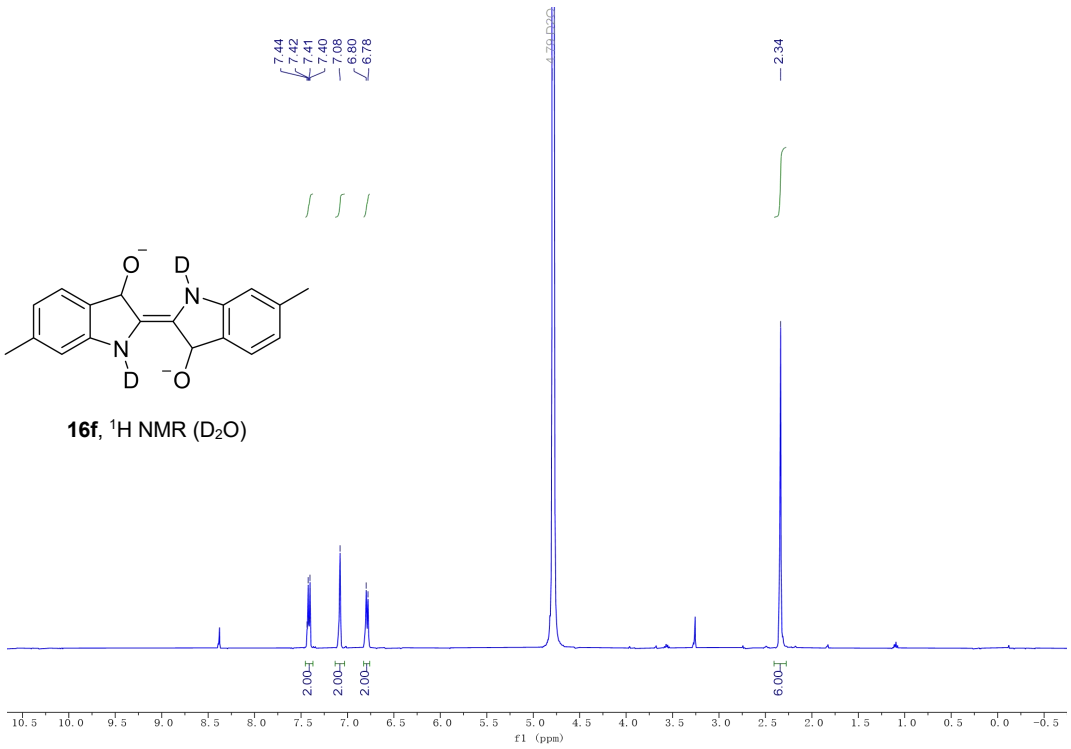
14f, ^{1}H NMR (D_2O)

Supplementary Figure 48. ^{1}H NMR spectra of **14f**.



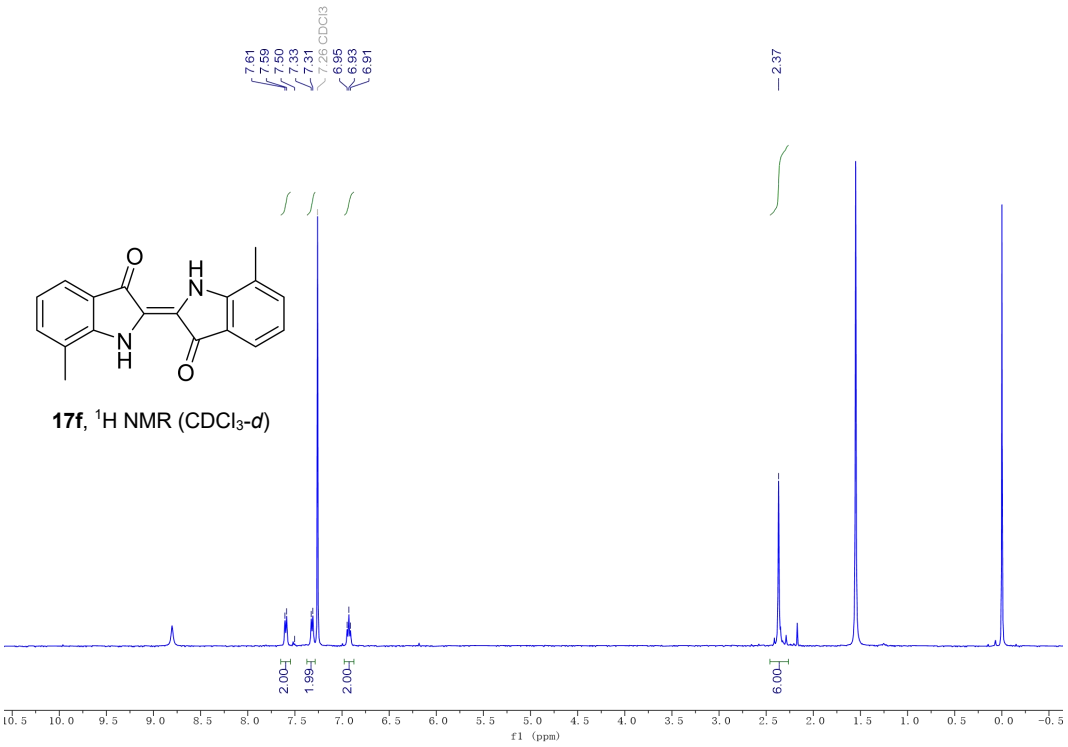
15f, ^{1}H NMR (D_2O)

Supplementary Figure 49. ^{1}H NMR spectra of **15f**.



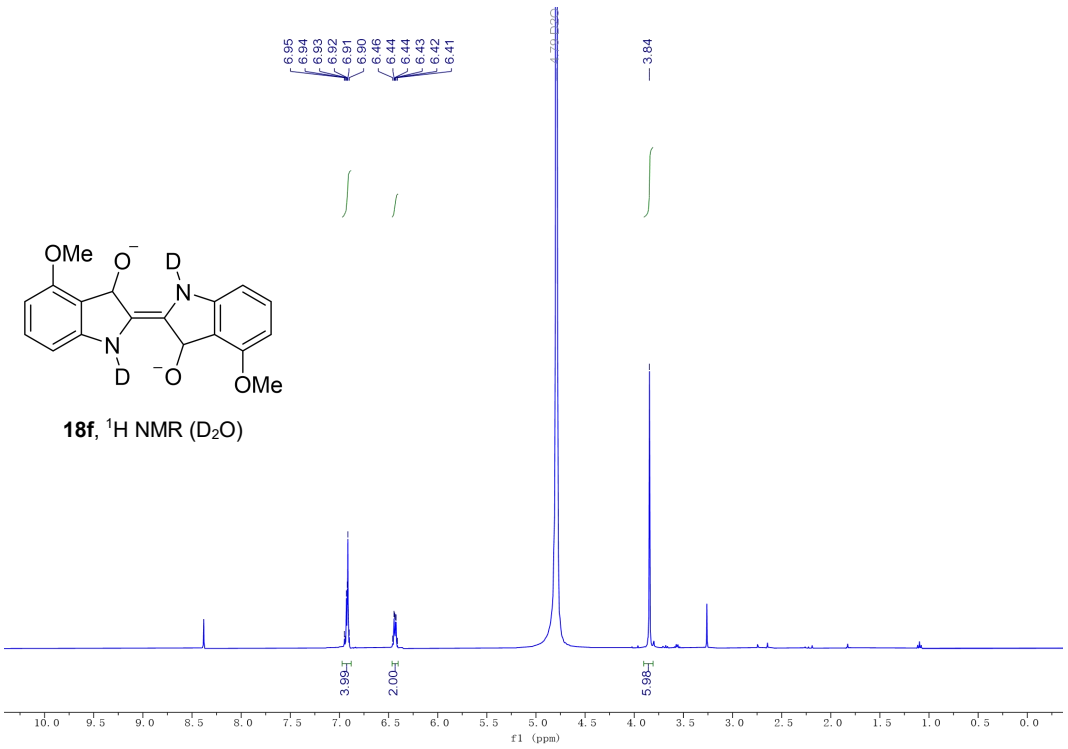
16f, ^{1}H NMR (D_2O)

Supplementary Figure 50. ^{1}H NMR spectra of **16f**.



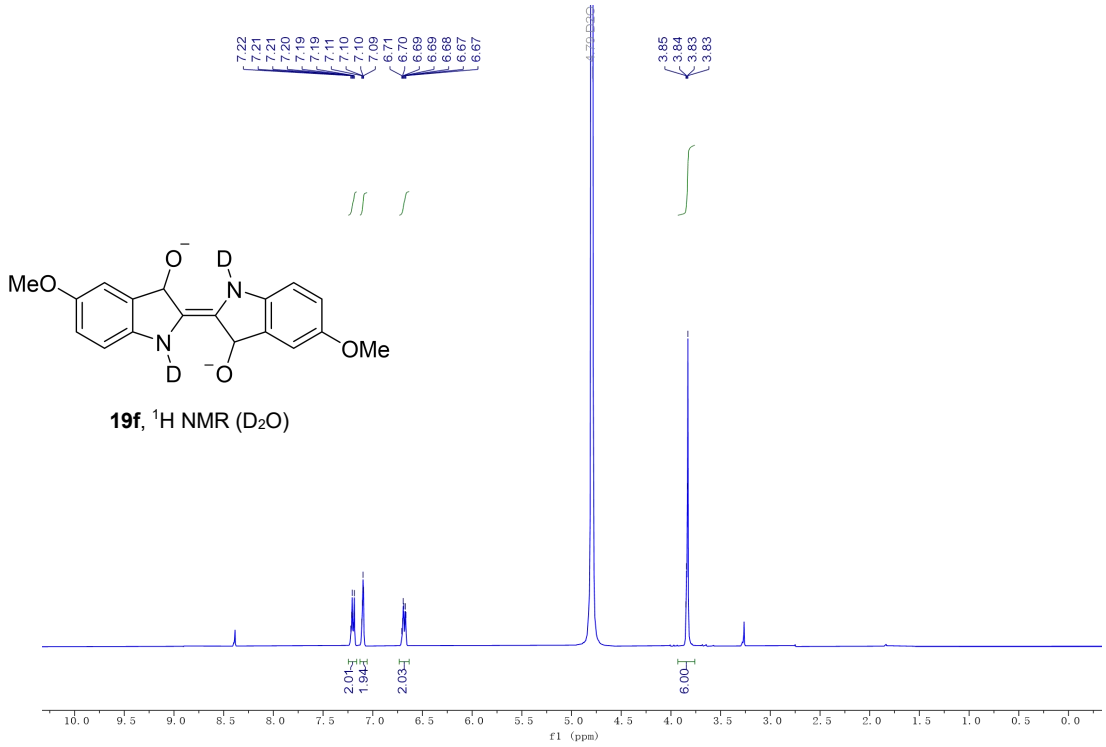
17f, ^{1}H NMR ($CDCl_3$-*d*)

Supplementary Figure 51. ^{1}H NMR spectra of 1**7f**.



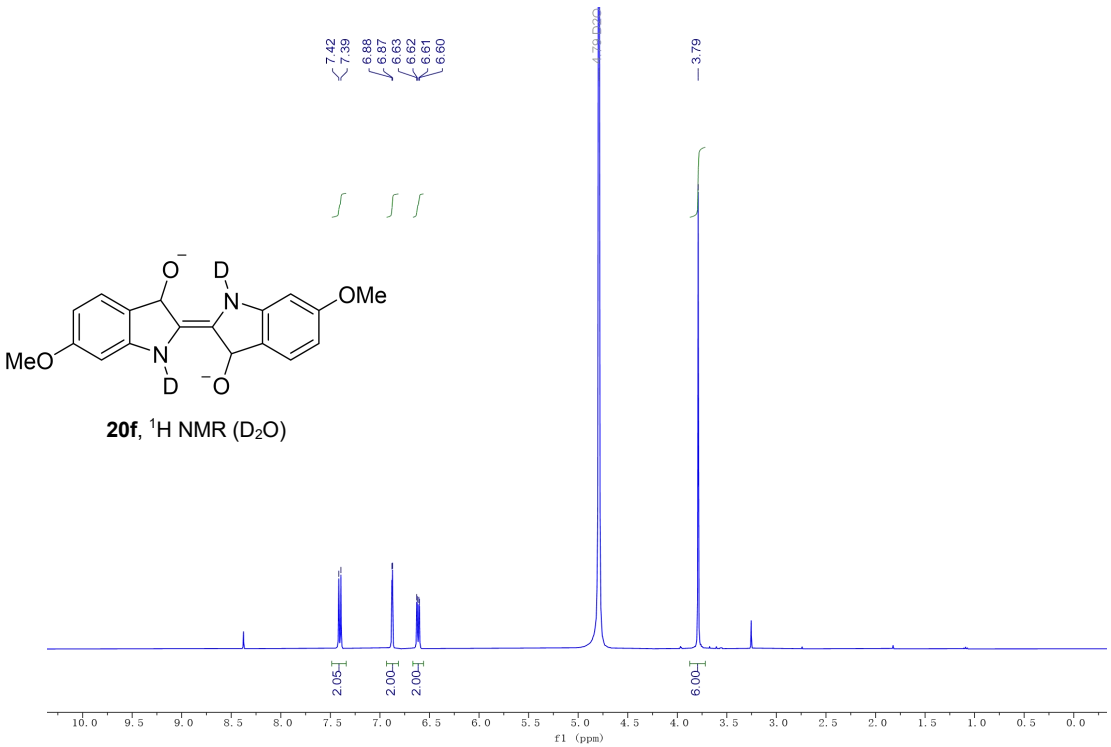
18f, ^{1}H NMR (D_2O)

Supplementary Figure 52. ^{1}H NMR spectra of **18f**.



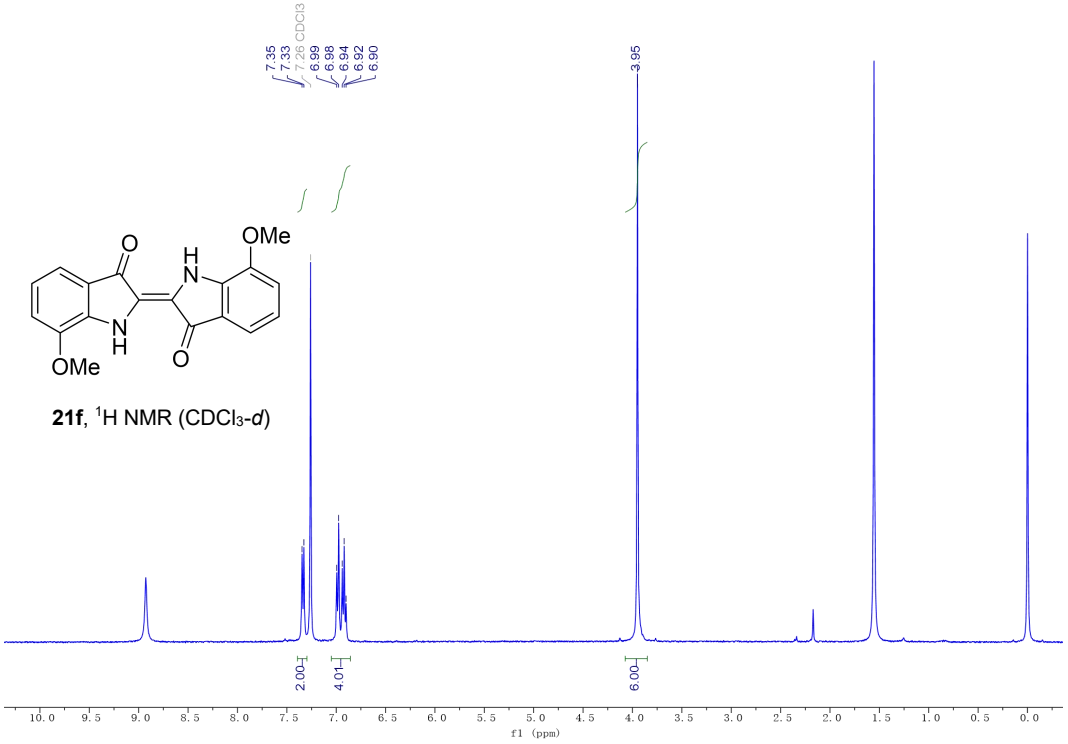
19f, ^{1}H NMR (D_2O)

Supplementary Figure 53. ^{1}H NMR spectra of **19f**.



20f, ^{1}H NMR (D_2O)

Supplementary Figure 54. ^{1}H NMR spectra of **20f**.



21f, ^{1}H NMR ($CDCl_3$-*d*)

Supplementary Figure 55. ^{1}H NMR spectra of **21f**.