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Supporting Information Appendix

Biocatalytic Dynamic Reductive Kinetic Resolution of Aryl α-Chloro β-Keto Esters: Divergent, Stereocontrolled Synthesis of Diltiazem, Clentiazem, and Siratiazem

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General information

Unless otherwise specified, all reagents and solvents were purchased from commercial sources and used as received. Codon-optimized synthetic genes (pET22b-LaADH and pET28a-LfSDR1) were purchased from Genewiz (China) (Table S1). The rest of the enzymes listed in Table S1 were prepared as we previously described.[1,2] Chemically competent cells of *E. coli* BL21 (DE3) were purchased from Weidi Biotech (Shanghai, China). LB medium contained yeast extract (5 g/L), tryptone (10 g/L), NaCl (10 g/L). Melting points were measured using an MP450 fully automatic melting-point apparatus. NMR spectra were recorded on a Bruker Avance 400 spectrometer in $CDCl_3$ or DMSO-d_6 using tetramethylsilane (TMS) as the internal standard. Coupling constant (*J*) values are given in Hz. Optical rotations were measured by a Rudolph AUTOPOL I Automatic Polarimeter. HRMS were recorded on a Bruker micrOTOF spectrometer. Chiral-HPLC analysis was performed with Daicel Chiralpak IA column (25 cm × 4.6 mm × 5 μm), Chiralpak IF column (25 cm × 4.6 mm × 5 μm), and Chiracel OJ-H column (25 cm × 4.6 mm × 5 μm).

Protein expression and preparation of cell-free extract (CFE) of enzymes

An approximately 12 h culture of *E. coli* BL21 (DE3) cells freshly transformed with the appropriate plasmid and grown in LB medium supplemented with kanamycin (50 μg/mL) or ampicillin (100 μg/mL) was diluted 1:100 into 0.5 L of the same medium in a 2 L flask. The culture was shaken at 37 ℃ until the optical density at 600 nm reached 0.6-0.8, and then the flask was placed in an ice/water bath for 30 minutes before the addition of isopropylthio-β-D-galactoside (IPTG) to a final concentration of 0.1 mM. The culture was shaken for an additional 16-18 h at 18 ℃. The cells were collected by centrifugation and then resuspended in an appropriate amount of NaP_i buffer to make a 15% w/v suspension. The cells were lysed by sonication on ice, and debris was removed by centrifugation at 15 000 rpm for 30 minutes at 4 ℃. This collected supernatant (cell-free extract (CFE)) was used as the biocatalyst. To quantify the cell-free extract, a portion of the cell-free extract similarly prepared in water was lyophilized and weighed, and thus obtained information was used as the reference for the calculation of the enzyme loading (g/L).

Table S1. The details of genes used in this study.

Name	Accession No.	Source	aa
RasADH	EU485985	*Ralstonia sp.* DSMZ 6428	250
KmCR2	XP_022675166.1	*Kluyveromyces marxianus CBS4857*	341
YCR107w	NP_010032.1	*Saccharomyces cerevisiae*	363
KRED-F42	WP_023468191.1	*Exiguobacterium sp.* MH3	249
KdoADH	CDO95209.1	*Kluyveromyces dobzhanskii*	342
LaADH	WP_021765610.1	*Leifsonia aquatica*	283
SyADH	EU427523.1	*Sphingobium yanoikuyae*	263
YNL331c	NP_014068.1	*Saccharomyces cerevisiae*	376
YBR149w	NP_009707.3	*Saccharomyces cerevisiae*	344
YJR096w	NP_012630.1	*Saccharomyces cerevisiae*	282
YNL274c	NP_014125.1	*Saccharomyces cerevisiae*	350
KRED-Pglu	AKP95857.1	*Pichia glucozyma*	252
KRED-Bt	WP_103592444.1	*Bacillus thuringiensis*	253
BYueD	WP_134982026.1	*Bacillus subtilis*	243
LkADH	WP_054768785.1	*Lactobacillus kefiri*	252
*Kp*ADH	XP_001644505.1	*Kluyveromyces polyspora*	342
LtCR	XP_002554048.1	*Lachancea thermotolerans*	281
CgCR	XP_447302.1	*Candida glabrata*	310
LfSDR1	WP_015638890.1	*Lactobacillus fermentum*	247
*Ch*KRED20	AHC30841.1	*Chryseobacterium sp.* CA49	244

Nucleotide sequence of LfSDR1 (codon optimized for expression in *E. coli*)

ATGGGTCAGTTCGATAACAAAGTGGCTTTAGTGACTGGTGGTACCAAAGGTATTGG
TTTAGCAATTGCCGAACTGTTTTTAAAGGAAGGTGCCAAAGGCGTTGCCTTTACCG
GTCGTCACGAAGATGAAGGCAAAGCCGTGCAAGAACGTTTAGGTGAACGCTCTTT
ATTTATTACCCAAGATGTGAGCAAAGAAGAAGACTGGCAGAACGCAACCAAAGCA
GTGGTGGACAAGTTCGGTCAGCTGGATGCCATCGTGAATAACGCCGGCATTGGCA
CCCCGCTGGGCATTGAAGAGATGACTTTAGATCACTGGAACCGTGAAATCGCCATC
GATCTGACCGGTACCATGCTGGGTTGCAAATATGGCGTGAAGGCCATGAAGGAGC
ATGGTGGTGCCATTGTGAACATCAGCAGCATCGAAGGTATGATCGGCGATCCGACC
GTGCCGGCATACAATGCCGCAAAAGGTGGCGTGCGTTTACTGACCAAGAGCGTTG
CTTTAGAATGCGCCGAAAAGGGCTACGCCATCCGCGTTAATAGCATCTATCCGGGC
GTGATCGCCACCCCGCTGATCGACCATCTGGACGACGCAACCAAACAGTTTTATAT
TGATAAACATCCGATGGGTCGTTTAGGTAAACCGGAAGAGGTTGCCAAAATGGCC
GTGTTCGTTGCCAGTGATGGTGCCAGCTTTAGCACCGGCAGCGAATTTGTGGTGGA
CGGTGGTTATACCGCCCAGTAA

Amino acid sequence of LfSDR1

MGQFDNKVALVTGGTKGIGLAIAELFLKEGAKGVAFTGRHEDEGKAVQERLGERSLFI
TQDVSKEEDWQNATKAVVDKFGQLDAIVNNAGIGTPLGIEEMTLDHWNREIAIDLTG
TMLGCKYGVKAMKEHGGAIVNISSIEGMIGDPTVPAYNAAKGGVRLLTKSVALECAE
KGYAIRVNSIYPGVIATPLIDHLDDATKQFYIDKHPMGRLGKPEEVAKMAVFVASDGA
SFSTGSEFVVDGGYTAQ

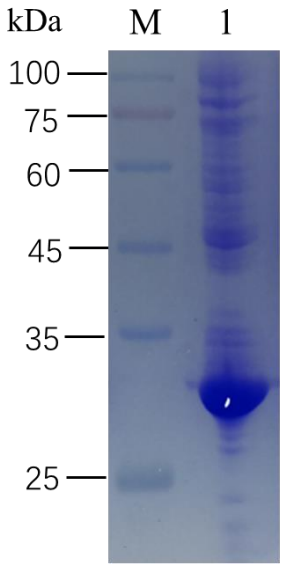
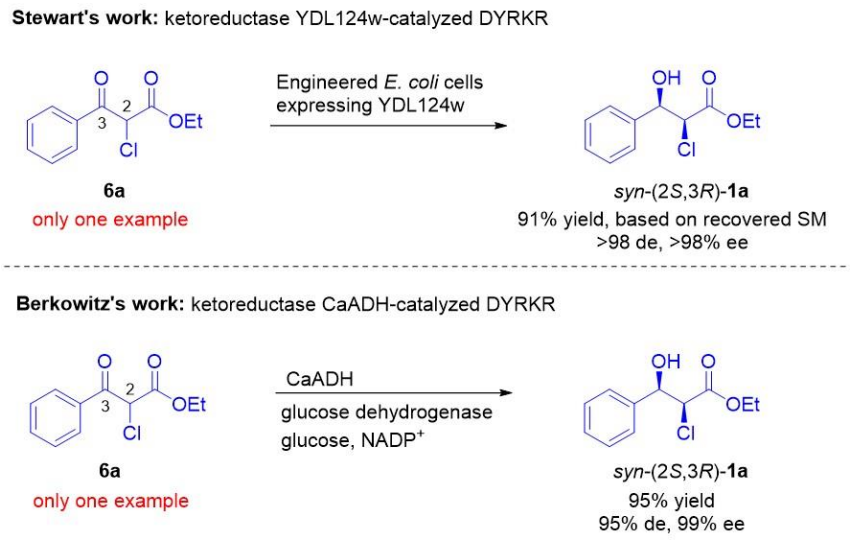
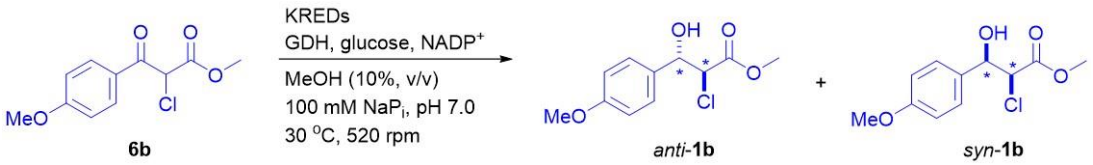


Figure S1. SDS-PAGE analysis of LfSDR1. Coomassie staining. M: RealBand 3-color Regular Range Protein Marker **(**Sangon Biotech, China). Lane 1: soluble cell fraction of LfSDR1.



Scheme S1. Ketoreductases YDL124w- and CaADH-catalyzed synthesis of *syn*-(2*S*,3*R*)-1a via DYRKR.

Table S2. Screening KREDs for the stereoselective reduction of α-chloro β-keto ester 6b.[a]



Entry	Enzyme	Conv. (%)[b]	Isomer I (%)[c]	Isomer II (%)[c]	Isomer III (%)[c]	Isomer IV (%)[c]	dr (*anti*:*syn*)[c]	ee *anti* (%)[c]	ee *syn* (%)[c]
1	RasADH	88.9	88.48	0.83	1.62	9.07	8.4:1	98.1	69.7
2	KmCR2	98.4	47.70	0.37	21.40	30.53	1:1.1	98.5	17.6
3	YCR107w	7.0	0	100	0	0	>99:1	>99	/
4	KRED-F42	92.0	9.29	4.65	0.26	85.80	1:6.2	33.3	>99
5	KdoADH	93.0	11.33	39.98	5.92	42.77	1:1.1	55.8	75.7
6	LaADH	<5.0				n.d.[d]			
7	SyADH	<5.0				n.d.			
8	YNL331c	10.0	15.33	22.61	47.15	14.91	1:1.6	19.2	46.8
9	YBR149w	21.2	1.41	14.50	84.09	0	1:5.3	82.3	>99
10	YJR096w	<5.0				n.d.			
11	YNL274c	<5.0				n.d.			
12	KRED-Pglu	<5.0				n.d.			
13	KRED-Bt	18.3	27.75	0.67	0.14	71.44	1:2.5	95.3	>99
14	BYueD	11.0	15.68	52.86	0	31.46	2.2:1	54.2	>99
15	LkADH	75.0	3.04	74.99	2.57	19.40	3.6:1	92.2	76.6
16	*Kp*ADH	13.5	2.79	92.23	2.26	2.72	19.1:1	94.1	n.d.
17	LtCR	7.0	33.49	12.24	51.21	3.06	1:1.2	46.5	88.7
18	CgCR	6.0	16.00	61.00	7.50	15.50	3.3:1	58.4	34.8
19	LfSDR1	98.0	0.73	97.24	0.92	1.11	48.2:1	98.5	n.d.
20	*Ch*KRED20	65.9	70.39	14.49	0.96	14.16	5.6:1	65.9	87.3

[a] A reaction mixture (60 mL) composed of **6b** (10 mM), glucose (20 mM), $NADP^+$ (0.2 mM), 10 mL 15% (w/v) cell-free extract (CFE) of KREDs in NaP_i buffer (100 mM, pH 7.0), 1 mL 15% (w/v) CFE of GDH in NaP_i buffer (100 mM, pH 7.0), 6 mL MeOH, and 43 mL NaP_i buffer (100 mM, pH 7.0) was stirred at 30 °C with 520 rpm for 12 h. [b] The conversions were determined based on uncorrected integrals of UV/Vis responses of the products and substrate in HPLC analysis using a chiral stationary phase. [c] The percentages of different product isomers, dr, and ee values were determined and calculated based on uncorrected integrals of UV/Vis responses in HPLC analysis using a chiral stationary phase. [d] n.d.: not determined.

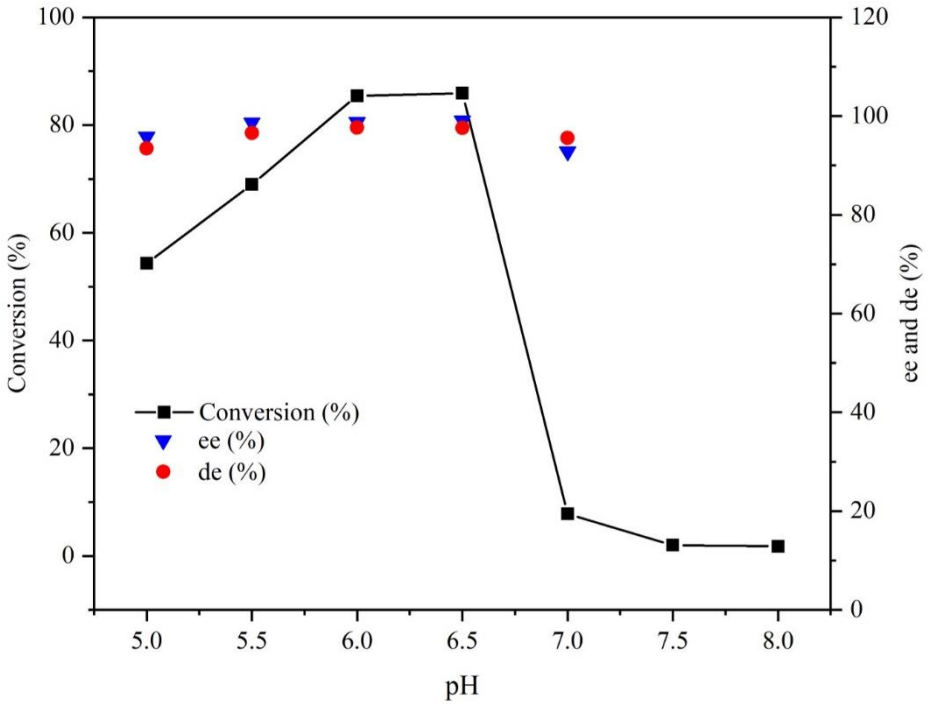
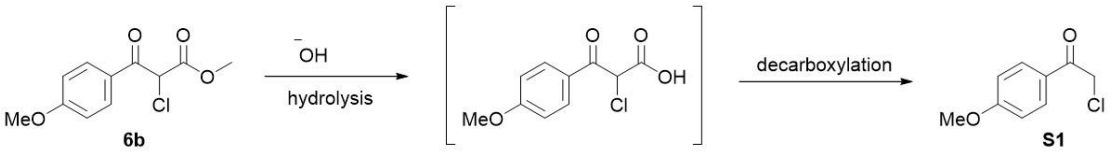
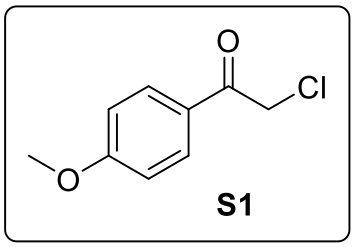


Figure S2. Effect of pH on the reaction conversion. Reaction conditions (60 mL): **6b** (10 mM), glucose (20 mM), $NADP^+$ (0.2 mM), 10 mL of 15% (w/v) cell-free extract (CFE) of LfSDR1 in NaP_i buffer (100 mM, pH 7.0), 0.2 mL of 15% (w/v) CFE of GDH in NaP_i buffer (100 mM, pH 7.0), and 6 mL of MeOH, in 43 mL of different buffers, including 100 mM sodium citrate buffer (pH 5.0, 5.5, and 6.0), 100 mM NaP_i buffer (pH 6.5, 7.0, and 7.5), and 100 mM Tris buffer (pH 8.0). Reaction mixtures were incubated at 30 ℃ with 520 rpm stirring for 1.5 h. Each data point represents the mean result of duplicate assays.



Scheme S2. Possible mechanism for the formation of side product S1.

2-chloro-1-(4-methoxyphenyl)ethan-1-one (S1)



Pale yellow oil. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.99-7.91 (m, 2H), 7.00-6.93 (m, 2H), 4.66 (s, 2H), 3.89 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 189.7, 164.2, 131.0, 127.2, 114.1, 55.6, 45.7.

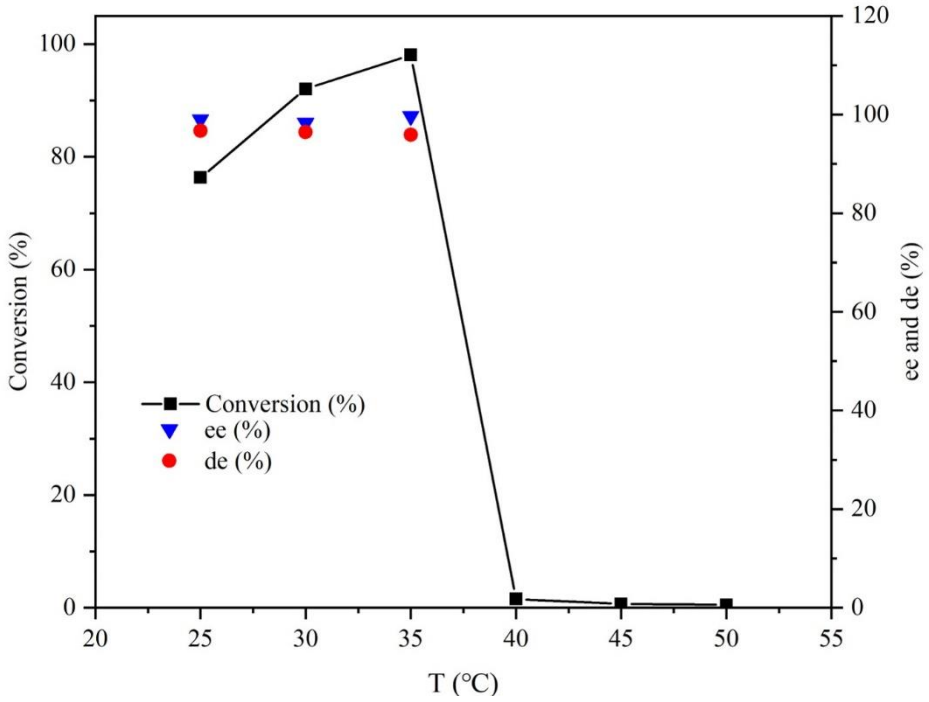


Figure S3. Effect of temperature on the reaction conversion. Reaction conditions (60 mL): **6b** (10 mM), glucose (20 mM), $NADP^+$ (0.2 mM), 10 mL of 15% (w/v) CFE of LfSDR1 in NaP_i buffer (100 mM, pH 6.5), 0.2 mL of 15% (w/v) CFE of GDH in NaP_i buffer (100 mM, pH 6.5), and 6 mL of MeOH, in 43 mL of NaP_i buffer (100 mM, pH 6.5). Reaction mixtures were incubated at different temperatures with 520 rpm stirring for 1.5 h. Each data point represents the mean result of duplicate assays.

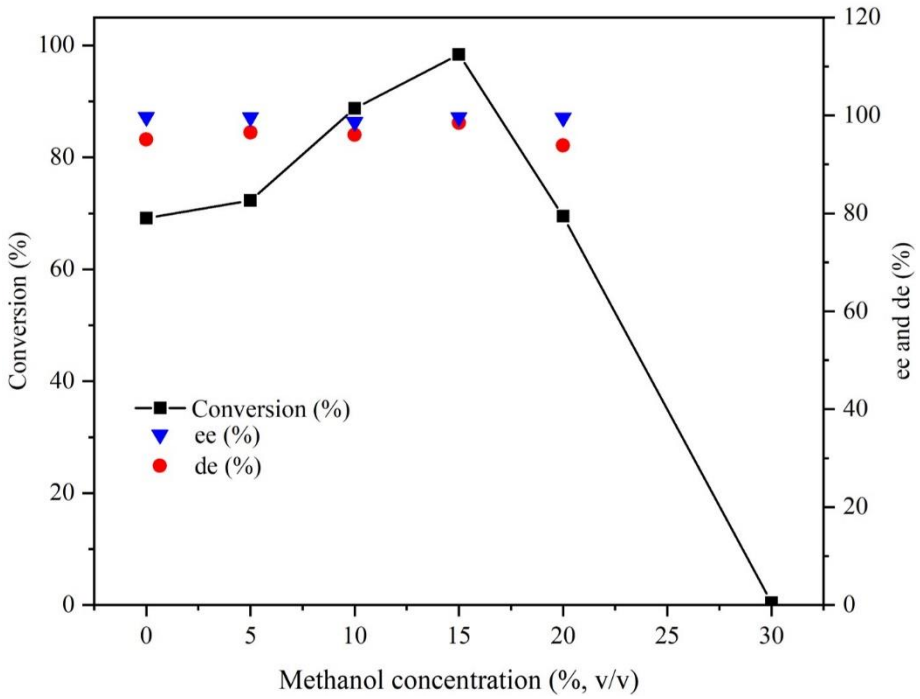


Figure S4. Effect of the amount of methanol on the reaction conversion. Reaction conditions (60 mL): **6b** (10 mM), glucose (20 mM), $NADP^+$ (0.2 mM), 10 mL of 15% (w/v) CFE of LfSDR1 in NaP_i buffer (100 mM, pH 6.5), 0.2 mL of 15% (w/v) CFE of GDH in NaP_i buffer (100 mM, pH 6.5), and 0 to 18 mL of MeOH, in 32 to 50 mL of NaP_i buffer (100 mM, pH 6.5). Reaction mixtures were incubated at 35 ℃ with 520 rpm stirring for 1.5 h. Each data point represents the mean result of duplicate assays.

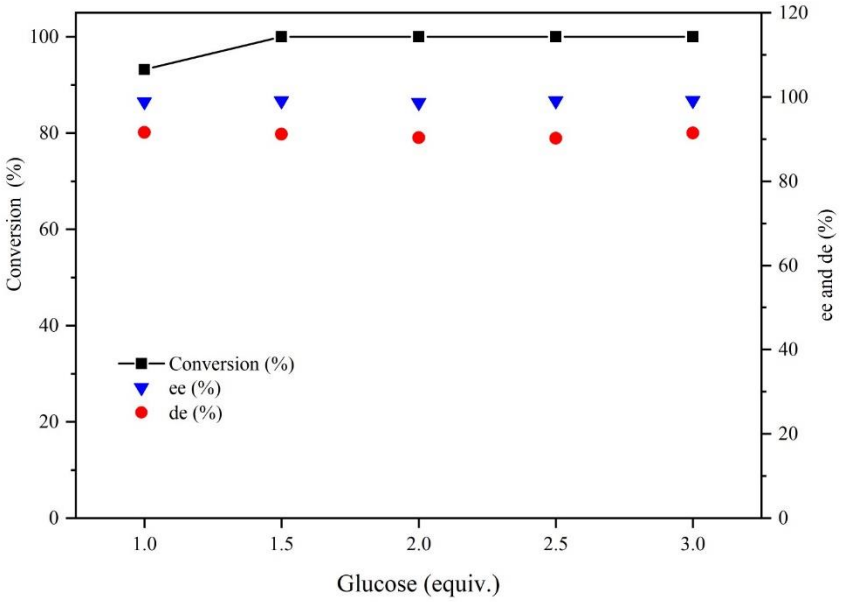


Figure S5. Effect of the loading of glucose on the reaction conversion. Reaction conditions (60 mL): **6b** (10 mM), glucose (10 to 30 mM), $NADP^+$ (0.2 mM), 10 mL of 15% (w/v) CFE of LfSDR1 in NaP_i buffer (100 mM, pH 6.5), 0.2 mL of 15% (w/v) CFE of GDH in NaP_i buffer (100 mM, pH 6.5), and 9 mL of MeOH, in 41 mL of NaP_i buffer (100 mM, pH 6.5). Reaction mixtures were incubated at 35 ℃ with 520 rpm stirring for 1.5 h. Each data point represents the mean result of duplicate assays.

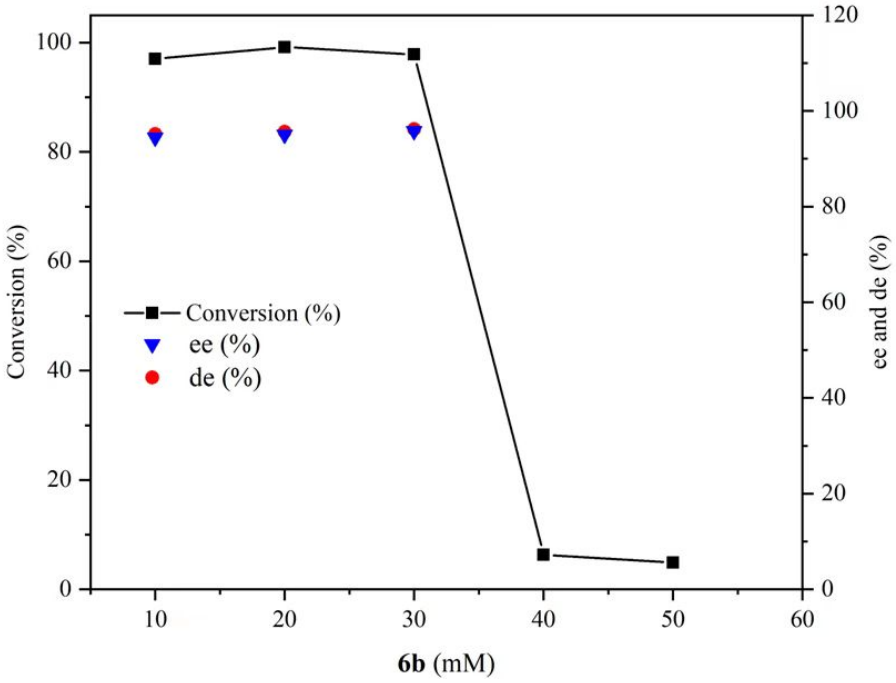
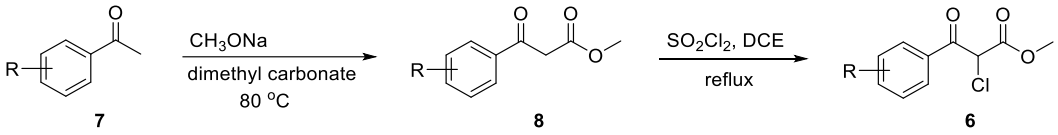


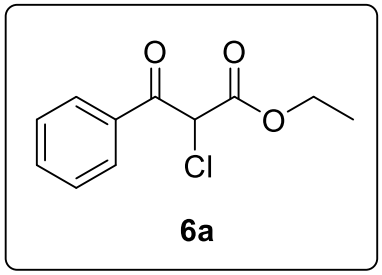
Figure S6. Effect of substrate concentration on the reaction conversion. Reaction conditions (60 mL): **6b** (10 to 50 mM), glucose (1.5 equiv. relative to **6b**), $NADP^+$ (0.2 mM), 10 mL of 15% (w/v) CFE of LfSDR1 in NaP_i buffer (100 mM, pH 6.5), 0.2 mL of 15% (w/v) CFE of GDH in NaP_i buffer (100 mM, pH 6.5), and 9 mL of MeOH, in 41 mL of NaP_i buffer (100 mM, pH 6.5). Reaction mixtures were incubated at 35 °C with 520 rpm stirring for 1.5 h. Each data point represents the mean result of duplicate assays.



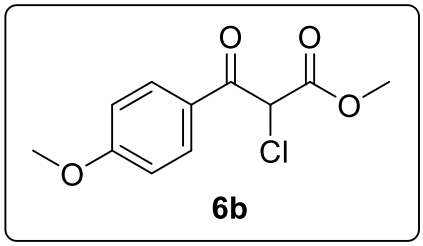
Scheme S3. General procedure for the synthesis of α-chloro β-keto esters 6.[3]

This is a modified literature procedure.[3] A mixture of CH_3ONa (0.81 g, 15 mmol, 1.5 equiv.) and dimethyl carbonate (12 mL) was stirred for 10 minutes at room temperature and then heated at reflux, and a solution of **7** (10 mmol) in dimethyl carbonate (3 mL) was added dropwise over 4 h. After refluxing for 2 h at 80 ℃, the reaction was cooled down and neutralized by HCl (4 N). The organic layer was separated and the aqueous layer was extracted with ethyl acetate. The combined organic layers were dried with anhydrous Na_2SO_4, concentrated *in vacuo* to give keto ester **8**, which was used directly in the next step without further purification.

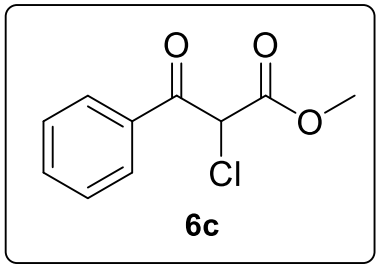
A mixture of **8** and 1,2-dichloroethane (15 mL) was stirred for 15 minutes at room temperature and then heated at reflux, and sulfuryl chloride (10 mmol, 1.0 equiv.) was added dropwise over 2 h. The mixture was then cooled down, and quenched in ice water and the solvent was evaporated. The residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate = 5:1) to give α-chloro β-keto ester **6**.



Ethyl 2-chloro-3-oxo-3-phenylpropanoate (6a) was prepared in 88% yield (1.98 g) as pale yellow oil starting directly from 10 mmol of **8a**. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.03-7.98 (m, 2H), 7.68-7.60 (m, 1H), 7.56-7.47 (m, 2H), 5.61 (s, 1H), 4.29 (q, *J* = 7.1 Hz, 2H), 1.24 (t, *J* = 7.1 Hz, 3H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 188.4, 165.4, 134.5, 133.5, 129.4, 129.1, 63.4, 58.1, 14.0. **HRMS** (ESI, m/z) calcd for $C_{11}H_{12}ClO_3$ $[M + H]^+$ 227.0469, found 227.0470.

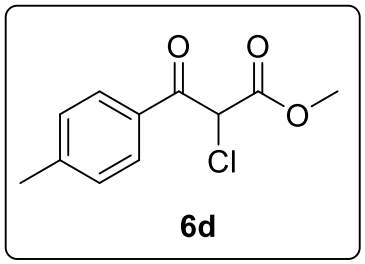


Methyl 2-chloro-3-(4-methoxyphenyl)-3-oxopropanoate (6b) was prepared in 98% yield (66.9 g) as white solid starting from 280 mmol of **7b**. **m.p.** = 44-45 ℃. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.00 (d, *J* = 9.0 Hz, 2H), 6.98 (d, *J* = 9.0 Hz, 2H), 5.63 (s, 1H), 3.90 (s, 3H), 3.84 (s, 3H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 186.7, 166.0, 164.6, 131.8, 126.1, 114.2, 57.6, 55.7, 53.8. **HRMS** (ESI, m/z) calcd for $C_{11}H_{12}ClO_4$ $[M + H]^+$ 243.0419, found 243.0420.



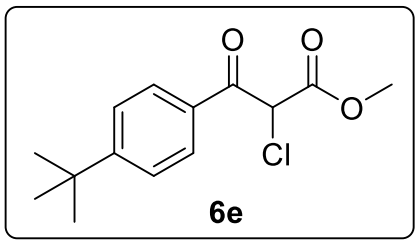
6c

Methyl 2-chloro-3-oxo-3-phenylpropanoate (6c) was prepared in 93% yield (1.98 g) as pale yellow oil starting from 10 mmol of **7c**. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.06-7.95 (m, 2H), 7.68-7.61 (m, 1H), 7.56-7.44 (m, 2H), 5.64 (s, 1H), 3.83 (s, 3H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 188.3, 165.9, 134.6, 133.3, 129.4, 129.1, 57.8, 54.0. **HRMS** (ESI, m/z) calcd for $C_{10}H_{10}ClO_3$ $[M + H]^+$ 213.0313, found 213.0317.



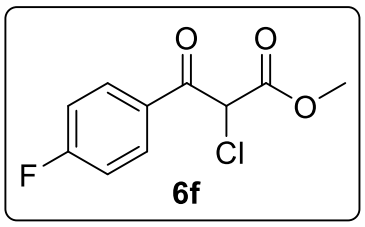
6d

Methyl 2-chloro-3-oxo-3-(p-tolyl)propanoate (6d) was prepared in 93% yield (47.0 g) as white solid starting from 223 mmol of **7d**. **m.p** = 46.8-48.3 ℃. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.86 (d, *J* = 8.0 Hz, 2H), 7.25 (d, *J* = 8.0 Hz, 2H), 5.67 (s, 1H), 3.76 (s, 3H), 2.37 (s, 3H).**^{13}C NMR** (100 MHz, $CDCl_3$) δ 187.8, 165.8, 145.6, 130.6, 129.6, 129.3, 57.5, 53.6, 21.6. **HRMS** (ESI, m/z) calcd for $C_{11}H_{12}ClO_3$ $[M + H]^+$ 227.0469, found 227.0471.



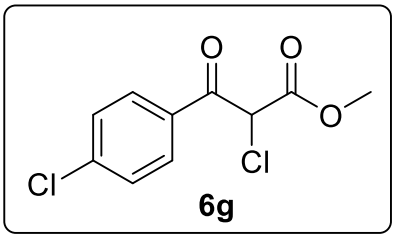
6e

Methyl 3-(4-(*tert*-butyl)phenyl)-2-chloro-3-oxopropanoate (6e) was prepared in 75% yield (2.01 g) as pale yellow oil starting from 10 mmol of **7e**. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.97-7.92 (m, 2H), 7.55-7.49 (m, 2H), 5.63 (s, 1H), 3.83 (s, 3H), 1.35 (s, 9H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 187.7, 165.9, 158.6, 130.6, 129.3, 126.0, 57.7, 53.8, 35.4, 31.0. **HRMS** (ESI, m/z) calcd for $C_{14}H_{18}ClO_3$ $[M + H]^+$ 269.0939, found 269.0942.



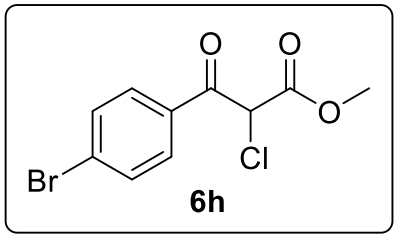
6f

Methyl 2-chloro-3-(4-fluorophenyl)-3-oxopropanoate (6f) was prepared in 86% yield (1.97 g) as pale yellow oil starting from 10 mmol of **7f**. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.08-8.01 (m, 2H), 7.22-7.15 (m, 2H), 5.58 (s, 1H), 3.84 (s, 3H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 186.8, 166.5 (d, *J* = 256.3 Hz), 165.7, 132.3 (d, *J* = 9.5 Hz), 129.7 (d, *J* = 3.0 Hz), 116.3 (d, *J* = 22.2 Hz), 57.8, 54.0. **HRMS** (ESI, m/z) calcd for $C_{10}H_8ClFO_3Na$ $[M + Na]^+$ 253.0038, found 253.0038.



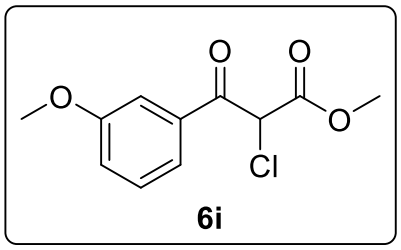
6g

Methyl 2-chloro-3-(4-chlorophenyl)-3-oxopropanoate (6g) was prepared in 87% yield (2.12 g) as pale yellow oil starting from 10 mmol of **7g**. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.08-8.00 (m, 2H), 7.23-7.14 (m, 2H), 5.58 (s, 1H), 3.84 (s, 3H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 187.2, 165.7, 141.3, 131.6, 130.8, 129.5, 57.8, 54.1. **HRMS** (ESI, m/z) calcd for $C_{10}H_8Cl_2O_3Na$ $[M + Na]^+$ 268.9743, found 268.9741.



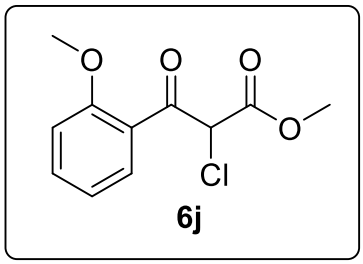
6h

Methyl 3-(4-bromophenyl)-2-chloro-3-oxopropanoate (6h) was prepared in 90% yield (2.61 g) as pale yellow oil starting from 10 mmol of **7h**. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.88-7.84 (m, 2H), 7.67-7.63 (m, 2H), 5.57 (s, 1H), 3.83 (s, 3H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 187.4, 165.7, 132.5, 132.0, 130.9, 130.1, 57.8, 54.1. **HRMS** (ESI, m/z) calcd for $C_{10}H_9BrClO_3$ $[M + H]^+$ 290.9418, found 290.9414.



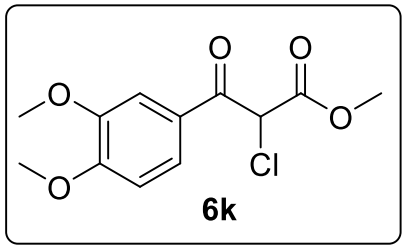
6i

Methyl 2-chloro-3-(3-methoxyphenyl)-3-oxopropanoate (6i) was prepared in 83% yield (2.01 g) as pale yellow oil starting from 10 mmol of **7i**. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.56 (ddd, *J* = 7.7, 1.7, 0.9 Hz, 1H), 7.52 (dd, *J* = 2.7, 1.6 Hz, 1H), 7.41 (t, *J* = 8.0 Hz, 1H), 7.18 (ddd, *J* = 8.3, 2.7, 0.9 Hz, 1H), 5.63 (s, 1H), 3.86 (s, 3H), 3.83 (s, 3H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 188.1, 165.9, 160.1, 134.6, 130.0, 121.8, 121.2, 113.5, 57.8, 55.6, 53.9. **HRMS** (ESI, m/z) calcd for $C_{11}H_{12}ClO_4$ $[M + H]^+$ 243.0419, found 243.0416.



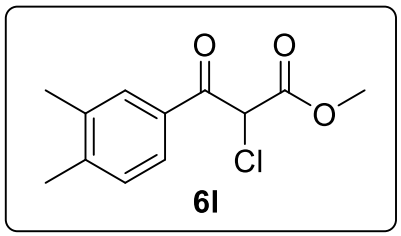
6j

Methyl 2-chloro-3-(2-methoxyphenyl)-3-oxopropanoate (6j) was prepared in 86% yield (2.08 g) as pale yellow oil starting from 10 mmol of **7j**. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.87 (dd, *J* = 7.8, 1.8 Hz, 1H), 7.53 (ddd, *J* = 8.4, 7.3, 1.8 Hz, 1H), 7.03 (ddd, *J* = 8.0, 7.3, 1.0 Hz, 1H), 6.97 (dd, *J* = 8.5, 1.0 Hz, 1H), 5.71 (s, 1H), 3.88 (s, 3H), 3.76 (s, 3H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 188.2, 166.3, 158.7, 135.6, 131.9, 123.9, 121.3, 111.8, 62.5, 55.5, 53.3. **HRMS** (ESI, m/z) calcd for $C_{11}H_{12}ClO_4$ $[M + H]^+$ 243.0419, found 243.0414.



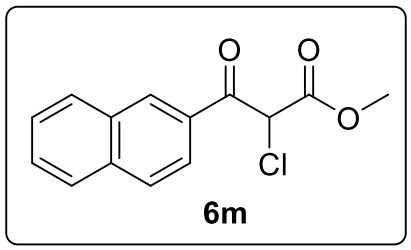
6k

Methyl 2-chloro-3-(3,4-dimethoxyphenyl)-3-oxopropanoate (6k) was prepared in 29% yield (0.78 g) as pale yellow oil starting from 10 mmol of **7k**. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.64 (dd, *J* = 8.4, 2.1 Hz, 1H), 7.55 (d, *J* = 2.1 Hz, 1H), 6.92 (d, *J* = 8.5 Hz, 1H), 5.62 (s, 1H), 3.97 (s, 3H), 3.94 (s, 3H), 3.83 (s, 3H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 186.8, 166.2, 154.6, 149.5, 126.4, 124.5, 111.3, 110.3, 57.8, 56.4, 56.2, 54.0. **HRMS** (ESI, m/z) calcd for $C_{12}H_{13}ClNaO_5$ $[M + Na]^+$ 295.0344, found 295.0349.



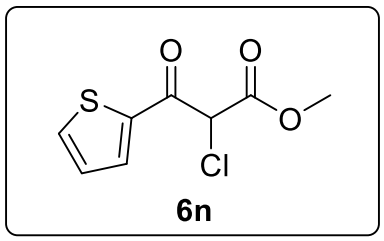
6l

Methyl 2-chloro-3-(3,4-dimethylphenyl)-3-oxopropanoate (6l) was prepared in 36% yield (0.86 g) as pale yellow oil starting from 10 mmol of **7l**. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.75 (d, *J* = 2.0 Hz, 1H), 7.70 (dd, *J* = 7.9, 2.0 Hz, 1H), 7.23 (d, *J* = 7.9 Hz, 1H), 5.64 (s, 1H), 3.80 (s, 3H), 2.31 (s, 3H), 2.30 (s, 3H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 188.0, 166.0, 144.6, 137.6, 131.1, 130.3, 130.2, 127.1, 57.6, 53.8, 20.2, 19.8. **HRMS** (ESI, m/z) calcd for $C_{12}H_{13}ClNaO_3$ $[M + Na]^+$ 263.0445, found 263.0439.



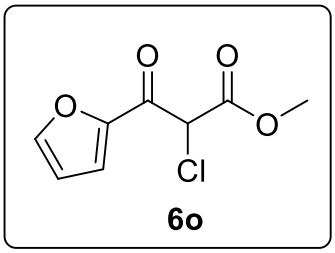
6m

Methyl 2-chloro-3-(naphthalen-2-yl)-3-oxopropanoate (6m) was prepared in 77% yield (2.01 g) as off white solid starting from 10 mmol of **7m**. **m.p** = 79.2-80.1 ℃. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.54 (d, *J* = 1.9 Hz, 1H), 8.08-7.98 (m, 2H), 7.92 (dd, *J* = 14.8, 8.4 Hz, 2H), 7.68-7.57 (m, 2H), 5.80 (s, 1H), 3.85 (s, 3H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 188.3, 166.0, 136.2, 132.5, 131.7, 130.7, 130.0, 129.6, 129.1, 128.0, 127.4, 124.4, 57.9, 54.0. **HRMS** (ESI, m/z) calcd for $C_{14}H_{12}ClO_3$ $[M + H]^+$ 263.0469, found 263.0468.



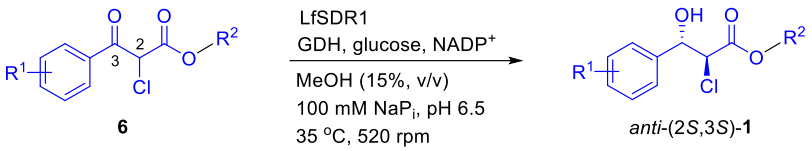
6n

Methyl 2-chloro-3-oxo-3-(thiophen-2-yl)propanoate (6n) was prepared in 79% yield (1.72 g) as pale yellow oil starting from 10 mmol of **7n**. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.87 (dd, *J* = 3.9, 1.1 Hz, 1H), 7.77 (dd, *J* = 4.9, 1.1 Hz, 1H), 7.17 (dd, *J* = 5.0, 3.9 Hz, 1H), 5.49 (s, 1H), 3.82 (s, 3H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 181.1, 165.5, 140.0, 136.4, 134.6, 128.7, 58.6, 54.0. **HRMS** (ESI, m/z) calcd for $C_8H_8ClO_3S$ $[M + H]^+$ 218.9877, found 218.9880.



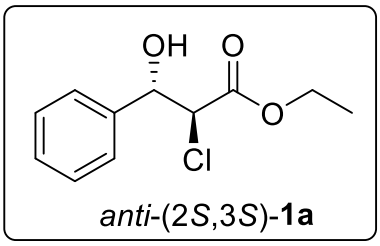
6o

Methyl 2-chloro-3-(furan-2-yl)-3-oxopropanoate (6o) was prepared in 93% yield (1.87 g) as pale yellow oil starting from 10 mmol of **7o**. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.67 (dd, *J* = 1.7, 0.7 Hz, 1H), 7.42 (dd, *J* = 3.7, 0.8 Hz, 1H), 6.62 (dd, *J* = 3.7, 1.7 Hz, 1H), 5.50 (s, 1H), 3.83 (s, 3H).**^{13}C NMR** (100 MHz, $CDCl_3$) δ 176.8, 165.4, 149.7, 148.1, 120.6, 113.3, 57.6, 54.0. **HRMS** (ESI, m/z) calcd for $C_8H_8ClO_4$ $[M + H]^+$ 203.0106, found 203.0103.

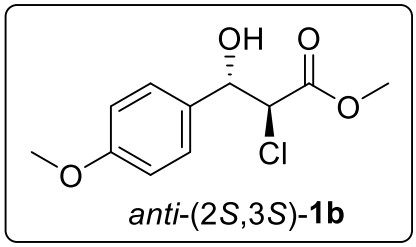


Scheme S4. Preparative-scale (0.6 mmol) synthesis of *anti*-(2*S*,3*S*)-1 catalyzed by LfSDR1.

To a solution of α-chloro β-keto ester **6** (0.6 mmol) in MeOH (9 mL) were added glucose (0.9 mmol, 1.5 equiv.), $NADP^+$ (0.2 mM), 200 μL 15% (w/v) CFE of GDH in NaP_i buffer (100 mM, pH 6.5), 10 mL 15% (w/v) CFE of LfSDR1 in NaP_i buffer (100 mM, pH 6.5), and 41 mL of NaP_i buffer (100 mM, pH 6.5). After stirring at 35 ℃ (referred to the setting temperature of the heating apparatus, applied to all the biocatalytic reactions) with 520 rpm for 6 h, the reaction mixture was extracted with ethyl acetate and subjected to centrifugation for three times. The organic layers were combined, dried with Na_2SO_4, and filtered. An aliquot of the filtrate was taken for ^{1}H NMR analysis to determine the diastereoselectivity. The filtrate was concentrated *in vacuo* and the residue was purified by preparative thin layer chromatography on silica gel (petroleum ether/ethyl acetate) to afford *anti*-(2*S*,3*S*)-**1**.

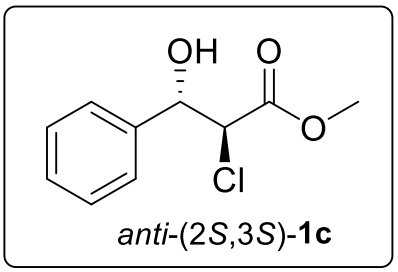


Ethyl (2*S*,3*S*)-2-chloro-3-hydroxy-3-phenylpropanoate (*anti*-(2*S*,3*S*)-1a) was prepared in 95% yield (130.3 mg) as pale yellow oil. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.45-7.32 (m, 5H), 5.02 (dd, *J* = 7.9, 5.1 Hz, 1H), 4.37 (d, *J* = 7.9 Hz, 1H), 4.23 (dq, *J* = 7.1, 1.0 Hz, 2H), 3.38 (d, *J* = 5.2 Hz, 1H), 1.26 (t, *J* = 7.2 Hz, 3H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 169.0, 138.9, 128.8, 128.5, 127.0, 75.3, 62.4, 59.3, 13.9. **HRMS** (ESI, m/z) calcd for $C_{11}H_{13}ClO_3Na$ $[M + Na]^+$ 251.0445, found 251.0447. $[\alpha]_D^{20}$ = +28.40 (c = 1.0, MeOH). **HPLC** Chiracel® OJ-H, 250 × 4.6 mm column, hexane/2-propanol 90:10, 1.0 mL/min flow rate, 220 nm UV lamp, 25 ℃, t_1 = 15.106 min, t_2 = 16.956 min (major), t_3 = 24.619 min, t_4 = 27.492 min. ee = >99%, dr = >99:1 (dr = >99:1, after purification).

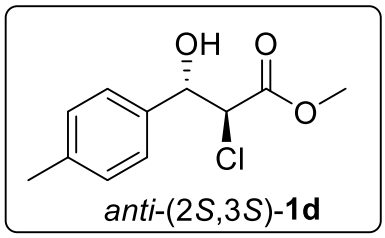


Methyl (2*S*,3*S*)-2-chloro-3-hydroxy-3-(4-methoxyphenyl)pr opanoate (*anti*-(2*S*,3*S*)-1b) was prepared in 96% yield (140.2 mg) as white solid. **m.p.** = 82-83 ℃. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.34-7.30 (m, 2H), 6.93-6.89 (m, 2H), 4.99 (dd, *J* = 8.1, 3.9 Hz, 1H), 4.36 (d, *J* = 8.2 Hz, 1H), 3.811 (s, 3H), 3.808 (s, 3H), 2.97 (d, *J* = 4.8 Hz, 1H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 169.5, 159.9, 130.8, 128.2, 114.0, 75.0, 59.1, 55.3, 53.2. **HRMS** (ESI, m/z) calcd for $C_{11}H_{13}ClO_4Na$ $[M + Na]^+$ 267.0395, found 267.0392. $[\alpha]_D^{20}$ = +32 (c = 0.94, $CHCl_3$). (lit.[3]: $[\alpha]_D^{20}$ = +36 (c = 0.94, $CHCl_3$)). **HPLC** Chiracel® OJ-H, 250 × 4.6 mm column, hexane/2-propanol 70:30, 1.0 mL/min flow rate, 220 nm UV

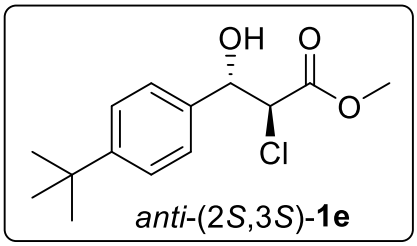
lamp, 25 ℃, t_1 = 11.528 min, t_2 = 12.825 min (major), t_3 = 16.463 min, t_4 = 19.876 min. ee = 99%, dr = 43:1, (dr = 99:1, after purification).



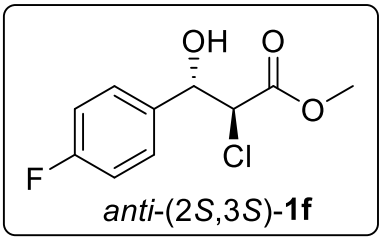
Methyl (2*S*,3*S*)-2-chloro-3-hydroxy-3-phenylpropanoate (*anti*-(2*S*,3*S*)-1c) was prepared in 86% yield (110.0 mg) as pale yellow oil. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.42-7.32 (m, 5H), 5.02 (dd, *J* = 8.0 Hz, 4.7 Hz, 1H), 4.38 (d, *J* = 8.0 Hz, 1H), 3.78 (s, 3H), 3.32 (d, *J* = 4.9 Hz, 1H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 169.5, 138.8, 128.9, 128.6, 127.0, 75.4, 59.1, 53.2. **HRMS** (ESI, m/z) calcd for $C_{10}H_{11}ClO_3Na$ $[M + Na]^+$ 237.0289, found 237.0286. $[\alpha]_D^{20}$ = +8.67 (c = 0.7, MeOH). **HPLC** Chiracel® OJ-H, 250 × 4.6 mm column, hexane/2-propanol 70:30, 0.6 mL/min flow rate, 220 nm UV lamp, 25 ℃, t_1 = 14.275 min, t_2 = 18.251 min (major), t_3 = 21.604 min, t_4 = 24.258 min. ee = >99%, dr = 43:1 (dr = 42:1, after purification).



Methyl (2*S*,3*S*)-2-chloro-3-hydroxy-3-(p-tolyl)propanoate (*anti*-(2*S*,3*S*)-1d) was prepared in 96% yield (131.1 mg) as pale yellow oil. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.30-7.25 (m, 2H), 7.19 (d, *J* = 7.9 Hz, 2H), 5.00 (dd, *J* = 8.0, 4.9 Hz, 1H), 4.38 (d, *J* = 8.0 Hz, 1H), 3.80 (s, 3H), 2.99 (d, *J* = 5.0 Hz, 1H), 2.36 (s, 3H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 169.5, 138.8, 135.9, 129.4, 126.9, 75.3, 59.1, 53.2, 21.3. **HRMS** (ESI, m/z) calcd for $C_{11}H_{13}ClO_3Na$ $[M + Na]^+$ 251.0445, found 251.0442. $[\alpha]_D^{20}$ = +20.16 (c = 1.0, MeOH). **HPLC** Chiracel® OJ-H, 250 × 4.6 mm column, hexane/2-propanol 90:10, 1.0 mL/min flow rate, 220 nm UV lamp, 25 ℃, t_1 = 15.777 min, t_2 = 19.254 min (major), t_3 = 24.427 min, t_4 = 32.646 min. ee = 92%, dr = 17:1 (dr = 16:1, after purification).

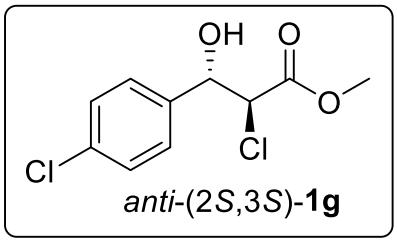


Methyl (2*S*,3*S*)-3-(4-(*tert*-butyl)phenyl)-2-chloro-3-hydrox ypropanoate (*anti*-(2*S*,3*S*)-1e) was prepared in 93% yield (150.1 mg) as pale yellow oil. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.43-7.38 (m, 2H), 7.35-7.30 (m, 2H), 5.02 (dd, *J* = 8.1, 4.5 Hz, 1H), 4.40 (d, *J* = 8.1 Hz, 1H), 3.82 (s, 3H), 2.88 (d, *J* = 4.9 Hz, 1H), 1.32 (s, 9H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 169.6, 152.0, 135.8, 126.8, 125.7, 75.3, 59.0, 53.3, 34.8, 31.4. **HRMS** (ESI, m/z) calcd for $C_{14}H_{19}ClO_3Na$ $[M + Na]^+$ 293.0915, found 293.0918. $[\alpha]_D^{20}$ = +13.40 (c = 0.2, MeOH). **HPLC** Chiracel® IF, 250 × 4.6 mm column, hexane/2-propanol 90:10, 0.8 mL/min flow rate, 220 nm UV lamp, 25 ℃, t_1 = 8.921 min, t_2 = 10.760 min, t_3 = 12.024 min (major), t_4 = 13.162 min. ee = >99%, dr = 37:1 (dr = 35:1, after purification).



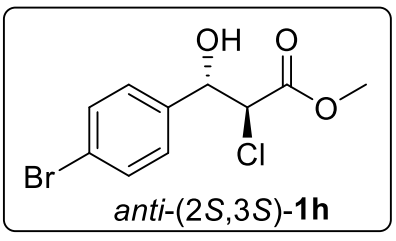
anti-(2*S*,3*S*)-**1f**

Methyl (2*S*,3*S*)-2-chloro-3-(4-fluorophenyl)-3-hydroxyprop anoate (*anti*-(2*S*,3*S*)-1f) was prepared in 79% yield (110.1 mg) as pale yellow oil. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.37-7.30 (m, 2H), 7.08-7.00 (m, 2H), 4.99 (dd, *J* = 8.2, 4.9 Hz, 1H), 4.33 (d, *J* = 8.2 Hz, 1H), 3.83 (dd, *J* = 5.1, 2.4 Hz, 1H), 3.75 (s, 3H). **^{13}C NMR** (100 MHz, CDCl3) δ 169.3, 162.7 (d, *J* = 245.6 Hz), 134.6 (d, *J* = 3.0 Hz), 128.7 (d, *J* = 8.3 Hz), 115.3 (d, *J* = 21.4 Hz), 74.4, 59.1, 53.1. **HRMS** (ESI, m/z) calcd for $C_{10}H_{10}ClFO_3Na$ $[M + Na]^+$ 255.0195, found 255.0195. $[\alpha]_D^{20}$ = +23.54 (c = 1.0, MeOH). **HPLC** Chiracel® IF, 250 ×4.6 mm column, hexane/2-propanol 85:15, 0.8 mL/min flow rate, 220 nm UV lamp, 35 ℃, t_1 = 13.765 min, t_2 = 14.514 min (major), t_3 = 17.810 min, t_4 = 22.580 min. ee = 96%, dr = 9.2:1 (dr = 9:1, after purification).



anti-(2*S*,3*S*)-**1g**

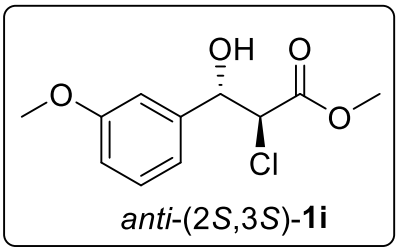
Methyl (2*S*,3*S*)-2-chloro-3-(4-chlorophenyl)-3-hydroxypropanoate (*anti*-(2*S*,3*S*)-1g) was prepared in 87% yield (130.0 mg) as pale yellow oil. *Anti/syn* = 5:1; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.38-7.28 (m, 4H), 5.13 (d, *J* = 6.0 Hz, 1H, *syn*), 5.01 (d, *J* = 7.9 Hz, 1H, *anti*), 4.41 (d, *J* = 6.0 Hz, 1H, *syn*), 4.32 (d, *J* = 7.9 Hz, 1H, *anti*), 3.79 (s, 3H, *anti*), 3.69 (s, 3H, *syn*), 3.29 (s, 1H, *anti*), 3.20 (s, 1H, *syn*). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 169.3 (*anti*), 168.4 (*syn*), 137.2 (*anti*), 136.7 (*syn*), 134.6 (*anti*), 134.6 (*syn*), 128.8 (*syn*), 128.7 (*anti*), 128.4 (*anti*), 128.0 (*syn*), 74.6 (*anti*), 73.7 (*syn*), 62.4 (*syn*), 58.9 (*anti*), 53.3 (*anti*), 53.2 (*syn*). **HRMS** (ESI, m/z) calcd for $C_{10}H_{10}Cl_2O_3Na$ $[M + Na]^+$ 270.9899, found 270.9892; $[\alpha]_D^{20}$ = +17.17 (c = 0.6, MeOH). **HPLC** Chiracel® OJ-H, 250 × 4.6 mm column, hexane/2-propanol 90:10, 1.0 mL/min flow rate, 220 nm UV lamp, 25 ℃, t_1 = 15.358 min, t_2 = 17.291 min (major), t_3 = 24.481 min, t_4 = 26.659 min. ee = 77%, dr = 7.2:1 (dr = 6.7:1, after purification).



anti-(2*S*,3*S*)-**1h**

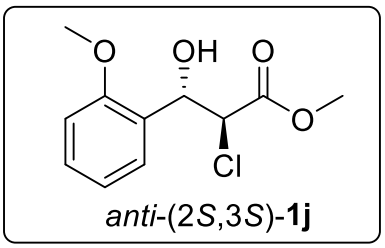
Methyl (2*S*,3*S*)-3-(4-bromophenyl)-2-chloro-3-hydroxypr opanoate (*anti*-(2*S*,3*S*)-1h) was prepared in 95% yield (167.1 mg) as pale yellow oil. *Anti/syn* = 5:1; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.55-7.46 (m, 2H), 7.31-7.20 (m, 2H), 5.13 (dd, *J* = 5.9, 3.9 Hz, 1H, *syn*), 5.01 (dd, *J* = 7.8, 4.7 Hz, 1H, *anti*), 4.41 (dd, *J* = 5.9, 1.4 Hz, 1H, *syn*), 4.33 (dd, *J* = 7.9, 1.3 Hz, 1H, *anti*), 3.80 (s, 3H, *anti*), 3.71 (s, 3H, *syn*), 3.10 (d, *J* = 4.8 Hz, 1H, *anti*), 3.06 (d, *J* = 4.0 Hz, 1H, *syn*). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 169.3 (*anti*), 168.5 (*syn*), 137.8 (*anti*), 137.3 (*syn*), 131.9 (*syn*), 131.8 (*anti*), 128.8 (*anti*), 128.4 (*syn*), 123.0, 74.8 (*anti*), 73.9 (*syn*), 62.4 (*syn*), 58.9 (*anti*), 53.4. **HRMS** (ESI, m/z) calcd for $C_{10}H_{10}BrClO_3Na$ $[M + Na]^+$ 314.9394, found 314.9391; $[\alpha]_D^{20}$ = +19.90 (c = 0.4, MeOH). **HPLC** Chiracel® OJ-H, 250 × 4.6 mm column, hexane/2-propanol 90:10, 1.0 mL/min flow rate, 220 nm UV lamp, 25 ℃, t_1 = 15.989 min, t_2 =

18.266 min (major), t_3 = 24.612 min, t_4 = 26.567 min. ee = 75%, dr = 5.3:1 (dr = 6.1:1, after purification).



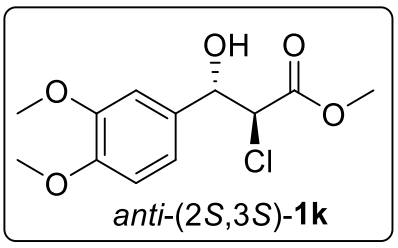
anti-(2*S*,3*S*)-**1i**

Methyl (2*S*,3*S*)-2-chloro-3-hydroxy-3-(3-methoxypheny- l)propanoate (*anti*-(2*S*,3*S*)-1i) was prepared in 95% yield (138.7 mg) as pale yellow oil. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.29 (t, *J* = 7.9 Hz, 1H), 7.00-6.93 (m, 2H), 6.88 (dd, *J* = 8.2, 2.7 Hz, 1H), 5.00 (dd, *J* = 7.9, 4.9 Hz, 1H), 4.38 (d, *J* = 7.9 Hz, 1H), 3.81 (s, 3H), 3.80 (s, 3H), 3.12 (d, *J* = 5.0 Hz, 1H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 169.5, 159.8, 140.4, 129.7, 119.3, 114.4, 112.6, 75.4, 59.0, 55.4, 53.3. **HRMS** (ESI, m/z) calcd for $C_{11}H_{13}ClO_4Na$ $[M + Na]^+$ 267.0395, found 267.0395. $[\alpha]_D^{20}$ = +16.94 (c = 1.0, MeOH). **HPLC** Chiracel® OJ-H, 250 × 4.6 mm column, hexane/2-propanol 70:30, 1.0 mL/min flow rate, 220 nm UV lamp, 25 ℃, t_1 = 9.608 min, t_2 = 11.958 min (major), t_3 = 13.447 min, t_4 = 15.076 min. ee = >99%, dr = >99:1 (dr = >99:1, after purification).



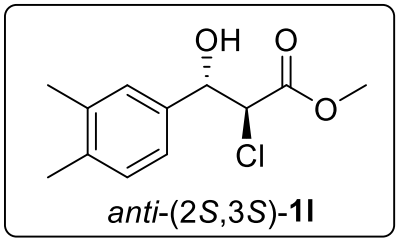
anti-(2*S*,3*S*)-**1j**

Methyl (2*S*,3*S*)-2-chloro-3-hydroxy-3-(2-methoxyphenyl) pr opanoate (*anti*-(2*S*,3*S*)-1j) was prepared in 93% yield (136.0 mg) as pale yellow oil. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.32 (t, *J* = 7.5 Hz, 2H), 6.98 (dt, *J* = 7.5, 1.1 Hz, 1H), 6.95-6.90 (m, 1H), 5.10 (t, *J* = 7.9 Hz, 1H), 4.67 (d, *J* = 7.8 Hz, 1H), 3.89 (s, 3H), 3.77 (s, 3H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 169.4, 156.8, 129.9, 129.5, 126.1, 121.0, 110.8, 74.2, 57.5, 55.5, 53.0. **HRMS** (ESI, m/z) calcd for $C_{11}H_{13}ClO_4Na$ $[M + Na]^+$ 267.0395, found 267.0396. $[\alpha]_D^{20}$ = +19.23 (c = 1.2, MeOH). **HPLC** Chiracel® OJ-H, 250 × 4.6 mm column, hexane/2-propanol 90:10, 0.6 mL/min flow rate, 220 nm UV lamp, 25 ℃, t_1 = 34.598 min (major), t_2 = 37.889 min, t_3 = 39.877 min, t_4 = 55.606 min. ee = >99%, dr = >99:1 (dr = >99:1, after purification).

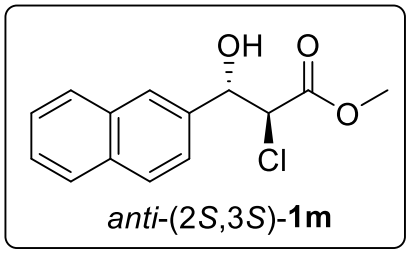


anti-(2*S*,3*S*)-**1k**

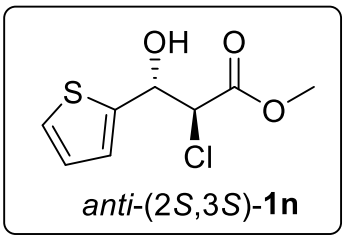
Methyl (2*S*,3*S*)-2-chloro-3-(3,4-dimethoxyphenyl)-3-hydro xypropanoate (*anti*-(2*S*,3*S*)-1k) was prepared in 89% yield (147.3 mg) as pale yellow oil. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 6.94-6.84 (m, 3H), 4.98 (d, *J* = 8.0 Hz, 1H), 4.36 (d, *J* = 8.0 Hz, 1H), 3.88 (s, 3H), 3.87 (s, 3H), 3.81 (s, 3H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 169.6, 149.5, 149.2, 131.3, 119.7, 110.9, 109.7, 75.3, 59.2, 56.03, 56.01, 53.3. **HRMS** (ESI, m/z) calcd for $C_{12}H_{15}ClNaO_5$ $[M + Na]^+$ 297.0500, found 297.0491. $[\alpha]_D^{20}$ = +19.85 (c = 0.4, MeOH). **HPLC** Chiracel® OJ-H, 250 × 4.6 mm column, hexane/2-propanol 85:15, 1.0 mL/min flow rate, 220 nm UV lamp, 25 ℃, t_1 = 27.216 min (major), t_2 = 32.609 min, t_3 = 50.917 min, t_4 = 55.043 min. ee = >99%, dr = 24:1 (dr = >99:1, after purification).



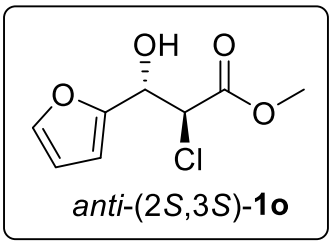
Methyl (2*S*,3*S*)-2-chloro-3-(3,4-dimethylphenyl)-3-hydroxy propanoate (*anti*-(2*S*,3*S*)-1l) was prepared in 74% yield (107.6 mg) as pale yellow oil. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.19-7.08 (m, 3H), 4.97 (dd, *J* = 8.1, 4.5 Hz, 1H), 4.39 (d, *J* = 8.1 Hz, 1H), 3.82 (s, 1H), 2.91 (d, *J* = 4.9 Hz, 1H), 2.28 (s, 3H), 2.27 (s, 3H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 169.5, 137.4, 137.0, 136.1, 129.8, 128.0, 124.4, 75.3, 58.9, 53.1, 19.9, 19.6. **HRMS** (ESI, m/z) calcd for $C_{12}H_{15}ClNaO_3$ $[M + Na]^+$ 265.0602, found 265.0594. $[\alpha]_D^{20}$ = +38.8 (c = 0.15, MeOH). **HPLC** Chiracel® IF, 250 ×4.6 mm column, hexane/2-propanol 90:10, 1.0 mL/min flow rate, 220 nm UV lamp, 25 ℃, t_1 = 8.155 min, t_2 = 8.620 min (major), t_3 = 9.213 min, t_4 = 10.091 min. ee = 98%, dr = 17:1 (dr = 15:1, after purification).



Methyl (2*S*,3*S*)-2-chloro-3-hydroxy-3-(naphthalen-2-yl) pro panoate (*anti*-(2*S*,3*S*)-1m) was prepared in 95% yield (150.2 mg) as pale yellow solid. **m.p.** = 82-83 ℃. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.88-7.82 (m, 4H), 7.58-7.42 (m, 3H), 5.20 (dd, *J* = 7.9, 4.8 Hz, 1H), 4.51 (d, *J* = 7.9 Hz, 1H), 3.79 (s, 3H), 3.33 (d, *J* = 4.9 Hz, 1H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 169.5, 136.1, 133.5, 133.1, 128.6, 128.3, 127.8, 126.8, 126.6, 126.5, 124.1, 75.6, 59.0, 53.3. **HRMS** (ESI, m/z) calcd for $C_{14}H_{13}ClO_3Na$ $[M + Na]^+$ 287.0445, found 287.0444. $[\alpha]_D^{20}$ = +30.46 (c = 1.0, MeOH). **HPLC** Chiracel® OJ-H, 250 × 4.6 mm column, hexane/2-propanol 70:30, 1.0 mL/min flow rate, 220 nm UV lamp, 25 ℃, t_1 = 15.039 min (major), t_2 = 18.509 min, t_3 = 22.030 min, t_4 = 27.397 min. ee = >99%, dr = 85:1 (dr = >99:1, after purification).

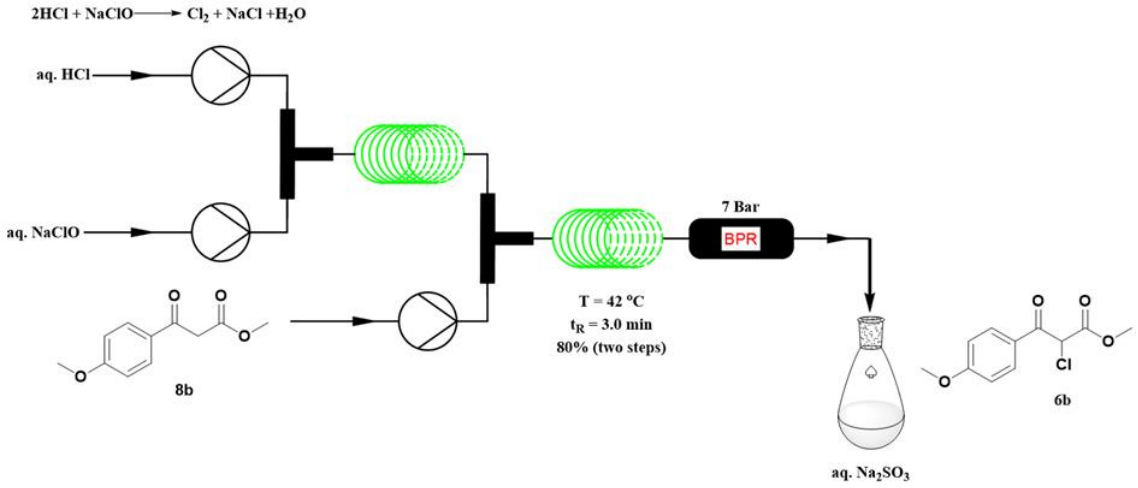


Methyl (2*S*,3*S*)-2-chloro-3-hydroxy-3-(thiophen-2-yl) propa noate (*anti*-(2*S*,3*S*)-1n) was prepared in 97% yield (128.1 mg) as pale yellow oil. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.33 (dd, *J* = 5.0, 1.2 Hz, 1H), 7.11 (dt, *J* = 3.6, 0.9 Hz, 1H), 7.01 (dd, *J* = 5.1, 3.5 Hz, 1H), 5.33 (dd, *J* = 7.6, 5.6 Hz, 1H), 4.45 (d, *J* = 7.6 Hz, 1H), 3.82 (s, 3H), 3.22 (d, *J* = 5.7 Hz, 1H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 169.1, 142.1, 127.0, 126.4, 126.1, 71.7, 59.4, 53.4. **HRMS** (ESI, m/z) calcd for $C_8H_9ClO_3SNa$ $[M + Na]^+$ 242.9853, found 242.9853. $[\alpha]_D^{20}$ = +8.98 (c = 1.0, MeOH). **HPLC** Chiracel® OJ-H, 250 × 4.6 mm column, hexane/2-propanol 90:10, 1.0 mL/min flow rate, 220 nm UV lamp, 25 ℃, t_1 = 19.735 min, t_2 = 24.631 min (major), t_3 = 34.485 min, t_4 = 45.821 min. ee = >99%, dr = >99:1 (dr = >99:1, after purification).



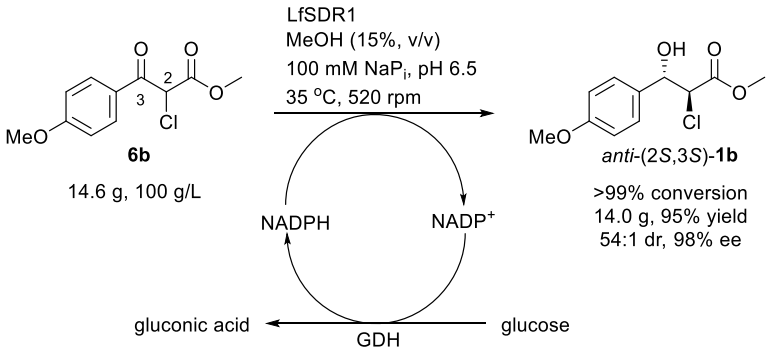
anti-(2*S*,3*S*)-**1o**

Methyl (2*S*,3*S*)-2-chloro-3-(furan-2-yl)-3-hydroxypropanoate (*anti*-(2*S*,3*S*)-1o) was prepared in 98% yield (120.1 mg) as pale yellow oil. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.40 (dt, *J* = 1.9, 1.0 Hz, 1H), 6.45-6.32 (m, 2H), 5.06 (d, *J* = 8.0 Hz, 1H), 4.59 (d, *J* = 8.0, 1H), 3.80 (s, 3H), 3.44 (s, 1H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 169.0, 151.2, 142.9, 110.5, 109.2, 69.1, 56.7, 53.3. **HRMS** (ESI, m/z) calcd for $C_8H_9ClO_4Na$ $[M + Na]^+$ 227.0082, found 227.0080; $[\alpha]_D^{20}$ = +22.80 (c = 0.6, MeOH). **HPLC** Chiracel® IF, 250 ×4.6 mm column, hexane/2-propanol 90:10, 1.0 mL/min flow rate, 220 nm UV lamp, 25 ℃, t_1 = 10.034 min, t_2 = 11.553 min (major), t_3 = 15.735 min, t_4 = 24.604 min. ee = >99%, dr = >99:1 (dr = >99:1, after purification).



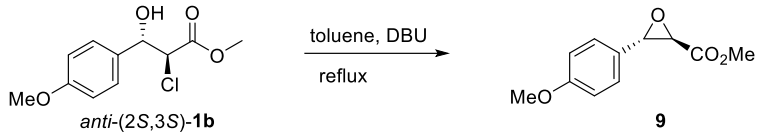
Scheme S5. Synthesis of α-chloro β-keto ester 6b by chlorination of keto ester 8b in flow using chlorine gas generated *in situ*.[4]

This is a modified literature procedure.[4] HCl (6 M, 50 mL, 300 mmol) and NaClO (1.5 M, 50 mL, 75 mmol) were placed in 50 mL gas tight syringes and introduced by the syringe pumps to the first micromixer with 24 mL/h and 30 mL/h flow rates, respectively. The mixture was then passed through the 5 mL PTFE reactor coil ($^{1}/_{16}$, i.d. = 0.8 mm), which was connected to the second micromixer. A solution of the crude product of β-keto ester **8b** (6.0 g, 28.8 mmol), synthesized in the previous step and used without further purification, in dichloroethane (30 mL) was placed in a 50 mL gas-tight syringe and introduced to the second micromixer with 45 mL/h flow rate. The resulting mixture was then passed through the second 5 mL PTFE reactor coil ($^{1}/_{16}$, i.d. = 0.8 mm) at 42 ℃ (water bath) and 7 bar back-pressure with 3-minute residence time. After pumping the above **8b** solution for 6.7 minute (corresponding to 1.0 g of **8b**), the mixture eluted from the outlet of the reaction device was collected in a flask containing cooled 30% Na_2SO_3. The organic layer was easily separated from the aqueous layer, and the aqueous layer was extracted with ethyl acetate for two times. The combined organic layer was concentrated *in vacuo* to give crude product, and the residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate = 5:1) to afford α-chloro β-keto ester **6b** (0.93 g, 80% yield over two steps).



Scheme S6. LfSDR1-catalyzed ten-gram scale synthesis of *anti*-(2*S*,3*S*)-1b at a 100 g/L of substrate concentration.

To a stirred mixture of glucose (16.3 g, 1.5 equiv.), $NADP^+$ (21.7 mg, 0.2 mM), cell-free extracts of LfSDR1 (12 g/L), and cell-free extracts of GDH (0.6 g/L) in NaP_i buffer (124.7 mL, 100 mM, pH 6.5) at 35 ℃ was continuously fed of a solution of **6b** (14.6 g, 100 g/L) in methanol (21.9 mL) at a rate of 0.15 mL/min through a syringe pump. This feeding process lasted for 4 h, followed by an additional 20 h of reaction time which was applied to guarantee a complete reduction of all the substrate added. The pH of the biotransformation during the entire process was maintained between 6.0 and 6.5 by titrating a NaOH solution. Complete conversion (>99%) was achieved as judged by the chiral HPLC analysis. Celite was then added and the resulting mixture was filtered. Ethyl acetate was employed to wash the filter cake for three times, and the two layers of filtrate were separated. The aqueous layer was extracted further with ethyl acetate for two times, and the combined organic layer was then washed with brine, dried, and concentrated *in vacuo* to furnish *anti*-(2*S*,3*S*)-**1b** (14.0 g, 95% yield) with a chemical purity of 94%, as well as an optical purity of 54:1 dr and 98% ee.

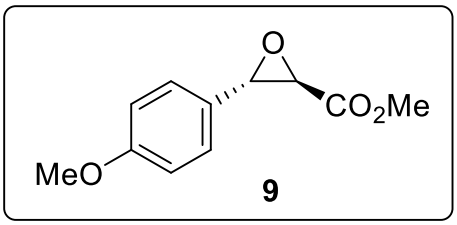


anti-(2*S*,3*S*)-**1b** **9**

Scheme S7. Synthesis of *trans* methyl (2*R*,3*S*)-3-(4-methoxyphenyl) glycidate 9.[3]

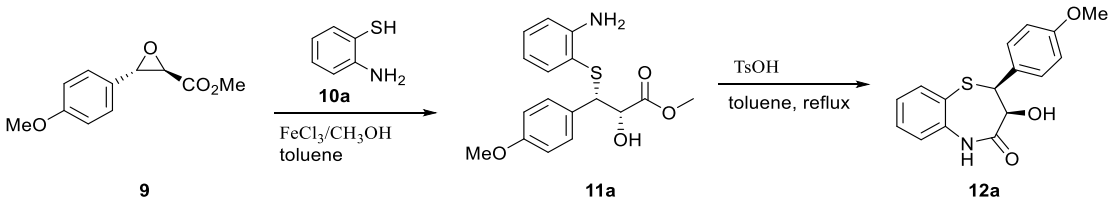
This is a modified literature procedure.[3] To a solution of chlorohydrin *anti*-(2*S*,3*S*)-**1b** (50 g, 205 mmol) in toluene (500 mL) was added DBU (46.8 g, 307.5 mmol, 1.5 equiv.). After stirring under reflux for 1 h, the mixture was diluted with water (1.5 L) and extracted with ethyl acetate for three times. The combined organic layers were washed with water, dried with anhydrous Na_2SO_4. and concentrated *in vacuo* to give compound **9** as pale yellow oil (40.31 g, 95% yield).

Methyl (2R,3S)-3-(4-methoxyphenyl)oxirane-2-carboxylate



9

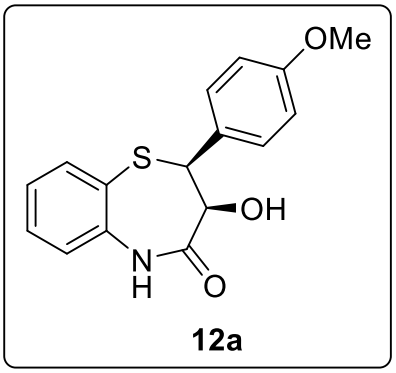
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.22 (d, *J* = 8.7 Hz, 2H), 6.91 (d, *J* = 8.7 Hz, 2H), 4.07 (d, *J* = 1.6 Hz, 1H), 3.84 (s, 3H), 3.82 (s, 3H), 3.53 (d, *J* = 1.8 Hz, 1H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 168.9, 160.3, 127.2, 126.7, 114.1, 58.0, 56.6, 55.4, 52.6. **HRMS** (ESI, m/z) calcd for $C_{11}H_{13}O_4$ [M + H]$^+$ 209.0808, found 209.0810. $[\alpha]_D^{20}$ = -187 (c = 1.0, MeOH). (lit.[3]: $[\alpha]_D^{20}$ = -196 (c = 1.0, MeOH)).



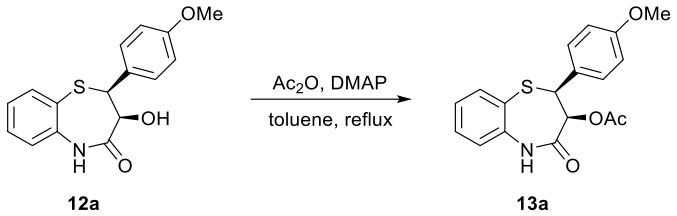
Scheme S8. Synthesis of benzothiazepinones 12a.[3,5,6]

This is a modified literature procedure.[3,5,6] A solution of *trans*-methyl glycidate **9** (10 g, 48 mmol) in toluene (200 mL) was heated at reflux. 2-Aminothiophenol **10a** (6.61 g, 52.8 mmol, 1.1 equiv.) and a solution of $FeCl_3$ $6H_2O$ (1.28 mg, 4.8×10^{-3} mmol, 10^{-4} equiv.) in MeOH (480 μL) were added, and the resulting mixture was stirred at reflux for 4 h. Then, *p*-toluenesulfonic acid (165 mg, 0.96 mmol, 0.02 equiv.) was added and the reflux continued for an additional 7 h. After cooling down, the solvent was concentrated *in vacuo*, and the residue was purified by recrystallization (petroleum ether/ethyl acetate = 2:1) to give compound **12a** as off white solid (10.5 g, 73% yield).

(2*S*,3*S*)-3-hydroxy-2-(4-methoxyphenyl)-2,3-dihydrobenzo[b][1,4]thiazepin-4(5 H)-one



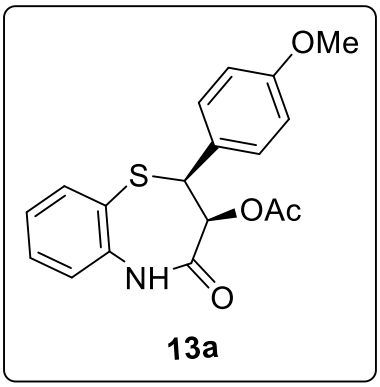
m.p. = 210-212 ℃. **^{1}H NMR** (400 MHz, DMSO-d_6) δ 10.32 (s, 1H), 7.60 (d, *J* = 7.6 Hz, 1H), 7.47-7.33 (m, 3H), 7.21-7.07 (m, 2H), 6.88 (d, *J* = 8.8 Hz, 2H), 5.05 (d, *J* = 6.7 Hz, 1H), 4.75 (d, *J* = 6.2 Hz, 1H), 4.30 (t, *J* = 6.4 Hz, 1H), 3.75 (s, 3H). **^{13}C NMR** (100 MHz, DMSO-d_6) δ 172.5, 159.0, 142.0, 133.9, 130.9, 130.0, 128.5, 126.2, 125.6, 122.6, 113.3, 69.6, 57.1, 55.1. **HRMS** (ESI, m/z) calcd for $C_{16}H_{16}NO_3S$ $[M + H]^+$ 302.0845, found 302.0845. $[\alpha]_D^{20}$ = +120 (c = 1.0, DMF). (lit.[3]: $[\alpha]_D^{20}$ = +100 (c = 0.53, DMF)).



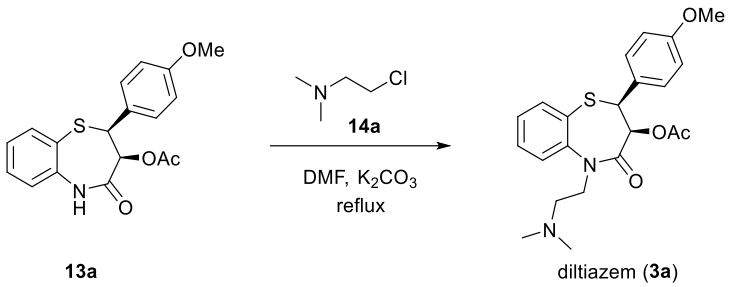
Scheme S9. Synthesis of compound 13a.

A mixture of benzothiazepinone **12a** (140 mg, 0.46 mmol), DMAP (5.7 mg, 0.046 mmol, 0.1 equiv.), and Ac_2O (53 μL, 0.56 mmol, 1.2 equiv.) in toluene (14 mL) was heated at reflux for 2 h. After cooling down, the toluene layer was separated and the aqueous layer was extracted with ethyl acetate. The combined organic layers were dried with anhydrous Na_2SO_4 and concentrated *in vacuo*, and the residue was purified by recrystallization (petroleum ether/ethyl acetate = 2:1) to give compound **13a** as off white solid (150.6 mg, 94% yield).

(2*S*,3*S*)-2-(4-methoxyphenyl)-4-oxo-2,3,4,5-tetrahydrobenzo[b][1,4]thiazepin-3-yl acetate



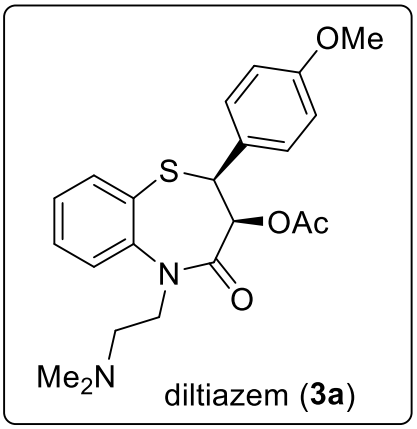
m.p. = 90.1-91.2 ℃. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.32 (s, 1H), 7.69 (d, *J* = 7.6 Hz, 1H), 7.49-7.38 (m, 3H), 7.28-7.17 (m, 2H), 6.85 (d, *J* = 8.7 Hz, 2H), 5.33 (d, *J* = 7.0 Hz, 1H), 5.16 (d, *J* = 7.0 Hz, 1H), 3.80 (s, 3H), 1.92 (s, 3H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 169.8, 168.8, 159.8, 140.7, 135.0, 130.60, 130.56, 127.1, 127.0, 126.9, 123.2, 113.8, 71.2, 55.3, 55.0, 20.4. **HRMS** (ESI, m/z) calcd for $C_{18}H_{18}NO_4S$ $[M + H]^+$ 344.0951, found 344.0956. $[\alpha]_D^{20}$ = +92.8 (c = 1.0, MeOH).



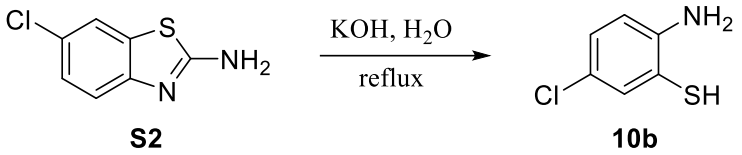
Scheme S10. Synthesis of diltiazem (3a).

To a solution of benzothiazepinone **13a** (100 mg, 0.29 mmol) in DMF (10 mL) was added 2-(dimethylamino) ethyl chloride (**14a**) (37.4 mg, 0.35 mmol, 1.2 equiv.). Under vigorous stirring was then added potassium carbonate (161.2 mg, 1.2 mmol, 4 equiv.). The resulting mixture was heated at reflux for 2 h. After cooling down, the reaction mixture was poured into iced water, and brine was added. This aqueous solution was extracted with ethyl acetate for three times. The combined organic layers were dried with anhydrous Na_2SO_4 and concentrated *in vacuo*, and the residue was purified by recrystallization (petroleum ether/ethyl acetate = 2:1) to give diltiazem (**3a**) as off white solid (108.1 mg, 90% yield).

(2*S*,3*S*)-5-(2-(dimethylamino)ethyl)-2-(4-methoxyphenyl)-4-oxo-2,3,4,5-tetrahydr -obenzo[b][1,4]thiazepin-3-yl acetate



m.p. = 100-102 ℃. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.69 (d, *J* = 7.4 Hz, 1H), 7.51-7.39 (m, 4H), 7.29-7.21 (m, 1H), 6.88 (d, *J* = 8.7 Hz, 2H), 5.14 (d, *J* = 7.5 Hz, 1H), 5.00 (d, *J* = 7.5 Hz, 1H), 4.47-4.39 (m, 1H), 3.81 (s, 3H), 3.74-3.68 (m, 1H), 2.74-2.67 (m, 1H), 2.49-2.42 (m, 1H), 2.27 (s, 6H), 1.89 (s, 3H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 170.0, 166.9, 159.8, 145.5, 135.4, 131.1, 130.9, 128.7, 127.5, 126.8, 124.8, 113.8, 71.2, 56.6, 55.3, 54.4, 48.1, 45.7, 20.6. **HRMS** (ESI, m/z) calcd for $C_{22}H_{27}N_2O_4S$ $[M + H]^+$ 415.1686, found 415.1694. $[\alpha]_D^{20}$ = +118 (c = 1.0, $CHCl_3$). (lit.[3]: $[\alpha]_D^{20}$ = +112 (c = 0.56, $CHCl_3$)).

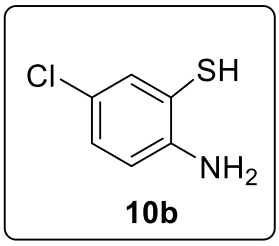


S2 **10b**

Scheme S11. Synthesis of thiol 10b.[7]

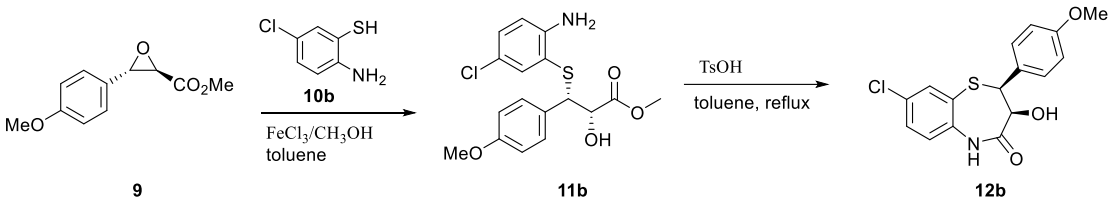
This is a modified literature procedure.[7] To a solution of 2-amino-6-chlorobenzothiazole (**S2**) (5 g, 27.2 mmol) in water (50 mL) was added KOH (25 g). The resulting mixture was heated under reflux until evolution of ammonia ceased. Filtered, and the filtrate was diluted with ice-cold water. Addition of acetic acid with vigorous stirring to neutralize the filtrate. The temperature of the solution was maintained at room temperature or below by adding ice to avoid the formation of decomposed, greenish mass. The precipitate thus formed was extracted three times with petroleum ether. The combined organic layers were dried with anhydrous Na_2SO_4, concentrated *in vacuo*, and the residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate = 20:1) to afford thiol **10b** as pale yellow solid (1.39 g, 32% yield).

2-amino-5-chlorobenzenethiol



10b

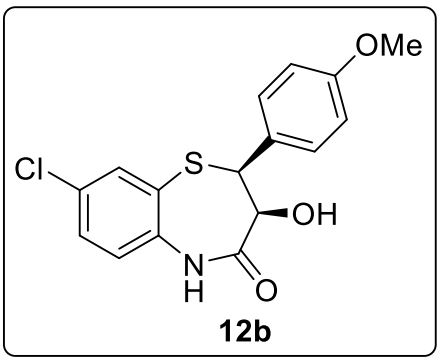
m.p. = 109-111 ℃. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.16-7.09 (m, 2H), 6.65 (dt, *J* = 9.0, 1.3 Hz, 1H), 4.32 (s, 2H), 1.57 (s, 1H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 147.3, 135.8, 131.8, 122.4, 119.5, 116.4.



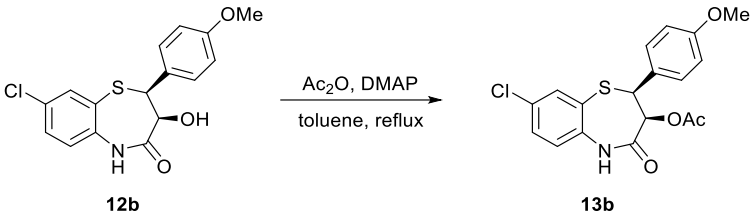
Scheme S12. Synthesis of benzothiazepinones 12b.[3,5,6]

This is a modified literature procedure.[3,5,6] A solution of *trans*-methyl glycidate **9** (100 mg, 0.48 mmol) in toluene (10 mL) was heated at reflux. Aminothiophenol **10b** (84.2 mg, 0.53 mmol, 1.1 equiv.) and a solution of $FeCl_3$ $6H_2O$ (0.013 mg, 4.8×10^{-5} mmol,10^{-4} equiv.) in MeOH (4.8 μL) were added, and the resulting mixture was stirred at reflux for 4 h. Then, *p*-toluenesulfonic acid (1.72 mg, 0.01 mmol, 0.02 equiv.) was added and the reflux continued for an additional 7 h. After cooling down, the solvent was concentrated *in vacuo*, and the residue was purified by recrystallization (petroleum ether/ethyl acetate = 2:1) to give compound **12b** as pale yellow solid (109.8 mg, 68% yield).

(2*S*,3*S*)-8-chloro-3-hydroxy-2-(4-methoxyphenyl)-2,3-dihydrobenzo[b][1,4] thiaz- -epine-4(5H)-one



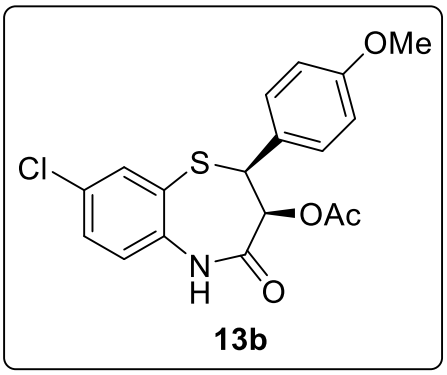
m.p. = 238.7-239.4 ℃. **^{1}H NMR** (400 MHz, DMSO-d_6) δ 10.34 (s, 1H), 7.64 (d, *J* = 2.4 Hz, 1H), 7.48 (dd, *J* = 8.5, 2.5 Hz, 1H), 7.41-7.29 (m, 2H), 7.15 (d, *J* = 8.6 Hz, 1H), 6.95-6.78 (m, 2H), 5.08 (d, *J* = 6.5 Hz, 1H), 4.91 (s, 1H), 4.32 (d, *J* = 6.5 Hz, 1H), 3.75 (s, 3H). **^{13}C NMR** (100 MHz, DMSO-d_6) δ 173.0, 159.5, 141.5, 133.2, 131.3, 130.1, 129.1, 128.9, 128.8, 124.4, 113.8, 70.6, 57.6, 55.6. **HRMS** (ESI, m/z) calcd for $C_{16}H_{14}ClNNaO_3S$ $[M + Na]^+$ 358.0275, found 358.0281. $[\alpha]_D^{20}$ = +51.9 (c = 0.2, DMSO).



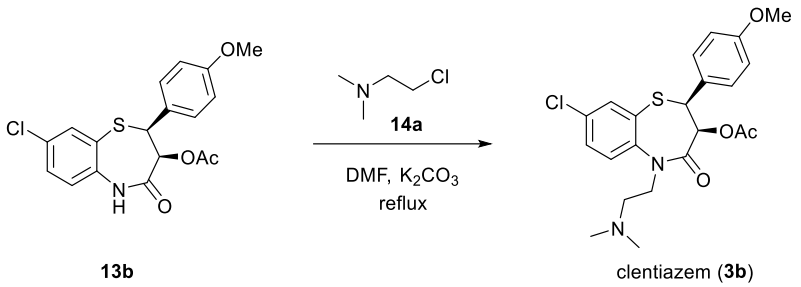
Scheme S13. Synthesis of compound 13b.

A mixture of benzothiazepinone **12b** (234.5 mg, 0.7 mmol), DMAP (8.56 mg, 0.07 mmol, 0.1 equiv.), and Ac_2O (78.9 μL, 0.84 mmol, 1.2 equiv.) in toluene (25 mL) was heated at reflux for 2 h. After cooling down, the toluene layer was separated and the aqueous layer was extracted with ethyl acetate. The combined organic layers were dried with anhydrous Na_2SO_4 and concentrated *in vacuo*, and the residue was purified by recrystallization (petroleum ether/ethyl acetate = 2:1) to give compound **13b** as pale yellow solid (202.9 mg, 77% yield).

(2*S*,3*S*)-8-chloro-2-(4-methoxyphenyl)-4-oxo-2,3,4,5-tetrahydrobenzo[b][1,4] thia -zepin-3-yl acetate



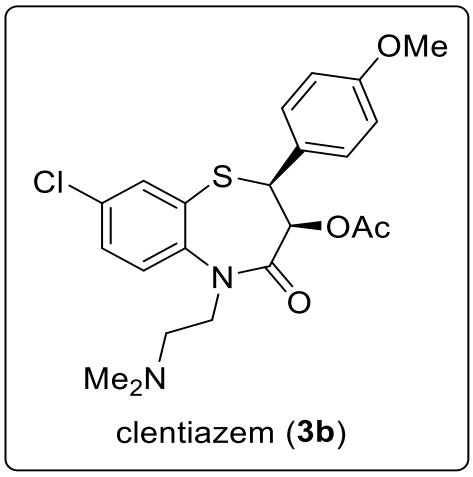
m.p. = 111.3-114.0 ℃. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.52 (s, 1H), 7.69 (d, *J* = 2.4 Hz, 1H), 7.47-7.38 (m, 2H), 7.37 (dd, *J* = 8.5, 2.4 Hz, 1H), 7.13 (d, *J* = 8.5 Hz, 1H), 6.88-6.77 (m, 2H), 5.30 (d, *J* = 6.9 Hz, 1H), 5.16 (d, *J* = 7.0 Hz, 1H), 3.79 (s, 3H), 1.93 (s, 3H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 169.9, 168.9, 160.1, 139.4, 134.5, 132.0, 130.7, 130.7, 128.9, 126.5, 124.4, 114.0, 71.2, 55.4, 55.1, 20.6. **HRMS** (ESI, m/z) calcd for $C_{18}H_{16}ClNNaO_4S$ $[M + Na]^+$ 400.0381, found 400.0374. $[\alpha]_D^{20}$ = +28.23 (c = 0.4, MeOH).



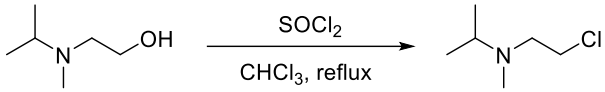
Scheme S14. Synthesis of clentiazem (3b).

To a solution of benzothiazepinone **13b** (109.3 mg, 0.29 mmol) in DMF (10 mL) was added 2-(dimethylamino)ethyl chloride (**14a**) (37.4 mg, 0.35 mmol, 1.2 equiv.). Under vigorous stirring was then added potassium carbonate (161.2 mg, 1.2 mmol, 4 equiv.). The resulting mixture was heated at reflux for 2 h. After cooling down, the reaction mixture was poured into iced water, and brine was added. The combined organic layers were dried with anhydrous Na_2SO_4 and concentrated *in vacuo*, and the residue was purified by recrystallization (petroleum ether/ethyl acetate = 2:1) to give clentiazem (**3b**) as off white solid (111.3 mg, 85% yield).

(2*S*,3*S*)-8-chloro-5-(2-(dimethylamino)ethyl)-2-(4-methoxyphenyl)-4-oxo-2,3,4,5-tetrahydrobenzo[b][1,4]hiazepine-3-yl acetate



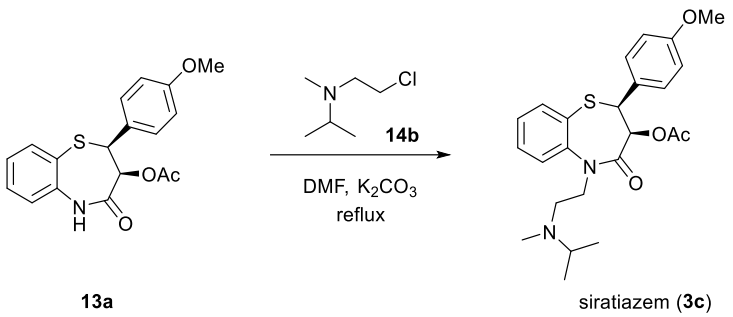
m.p. = 127.4-129.1 ℃. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.67 (s, 1H), 7.50-7.36 (m, 4H), 6.87 (d, *J* = 8.3 Hz, 2H), 5.11 (d, *J* = 7.5 Hz, 1H), 4.99 (d, *J* = 7.5 Hz, 1H), 4.42-4.27 (m, 1H), 3.78 (s, 3H), 3.75-3.65 (m, 1H), 2.75-2.64 (m, 1H), 2.50-2.39 (m, 1H), 2.26 (s, 6H), 1.88 (s, 3H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 169.8, 166.7, 159.9, 144.1, 134.8, 132.4, 131.1, 130.8, 130.2, 126.2, 125.9, 113.8, 70.8, 56.5, 55.2, 54.4, 47.9, 45.4, 20.5. **HRMS** (ESI, m/z) calcd for $C_{22}H_{26}ClN_2O_4S$ $[M + H]^+$ 449.1296, found 449.1281. $[\alpha]_D^{20}$ = +49.25 (c = 0.4, MeOH).



S3 **14b**

Scheme S15. Synthesis of amino chloride 14b.[8]

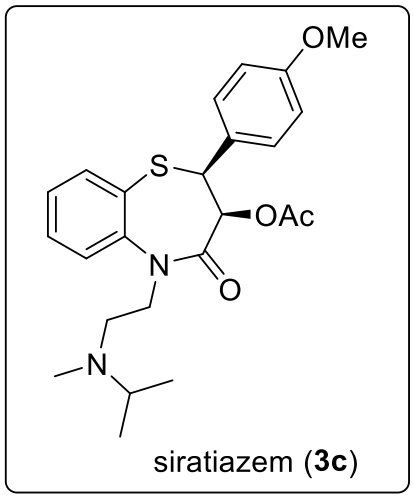
This is a modified literature procedure.[8] 2-(Isopropyl(methyl)amino)ethan-1-ol (**S3**) (936 mg, 8 mmol) was first dissolved in chloroform (12 mL) and stirred in an ice-cold bath, to which 0.84 mL of thionyl chloride (12 mmol, 1.5 equiv.) in chloroform (2.4 mL) was added dropwise over 30 min. The resulting mixture was heated to reflux for 4 h. The solvent was then evaporated *in vacuo* and the solid remained was re-dissolved in ethanol and precipitated into diethyl ether. The resultant light yellow solid was filtrated, washed with diethyl ether, and used for the next reaction without further purification.



Scheme S16. Synthesis of siratiazem (3c).

To a solution of benzothiazepinone **13a** (100 mg, 0.29 mmol) in DMF (10 mL) was added amino chloride (**14b**) (156.6 mg, 1.16 mmol, 4.0 equiv.). Under vigorous stirring was then added potassium carbonate (162 mg, 1.2 mmol, 4 equiv.). The resulting mixture was heated at reflux for 2 h. After cooling down, the reaction mixture was poured into iced water, and brine was added. The combined organic layers were dried with anhydrous Na_2SO_4 and concentrated *in vacuo*, and the residue was purified by recrystallization (petroleum ether/ethyl acetate = 2:1) to give siratiazem (**3c**) as off white solid (93.2 mg, 73% yield).

(2*S*,3*S*)-5-(2-(isopropyl(methyl)amino)ethyl)-2-(4-methoxyphenyl)-4-oxo-2,3,4,5-tetrahydrobenzo[b][1,4]hiazepine-3-yl acetate



^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58 (d, *J* = 7.7 Hz, 1H), 7.43 (d, *J* = 8.1 Hz, 2H), 7.34 (t, *J* = 7.7 Hz, 1H), 7.11 (d, *J* = 7.9 Hz, 1H), 7.03 (t, *J* = 7.6 Hz, 1H), 6.87 (d, *J* = 8.3 Hz, 2H), 5.46-5.23 (m, 2H), 4.55-4.43 (m, 2H), 3.82 (s, 3H), 3.01-2.91 (m, 1H), 2.90-2.80 (m, 2H), 2.33 (s, 3H), 1.77 (s, 3H), 1.05 (d, *J* = 6.6 Hz, 6H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 169.6, 161.8, 159.7, 149.5, 134.1, 130.5, 130.1, 128.6, 124.8, 124.5, 124.4, 113.6, 71.7, 65.0, 60.4, 55.4, 54.1, 51.1, 37.6, 20.4, 18.0. **HRMS** (ESI, m/z) calcd for $C_{24}H_{30}N_2O_4SNa$ $[M + Na]^+$ 465.1818, found 465.1820. $[\alpha]_D^{20}$ = +244.8 (c = 0.2, MeOH).

References

[1] Z. Wang, Y. Zeng, X. Wu, Z. Li, Y. Tao, X. Yu, Z. Huang and F. Chen, *Org. Biomol. Chem.*, 2020, **18**, 2672-2677.

[2] Z. Wang, X. Wu, Z. Li, Z. Huang and F. Chen, *Org. Biomol. Chem.*, 2019, **17**, 3575-3580.

[3] C. Mordant, C. C. de Andrade, R. Touati, V. Ratovelomanana-Vidal, B. B. Hassine and J.-P. Genêt, *Synthesis*, 2003, 2405-2409.

[4] T. Fukuyama, M. Tokizane, A. Matsui and I. Ryu, *React. Chem. Eng.*, 2016, **1**, 613-615.

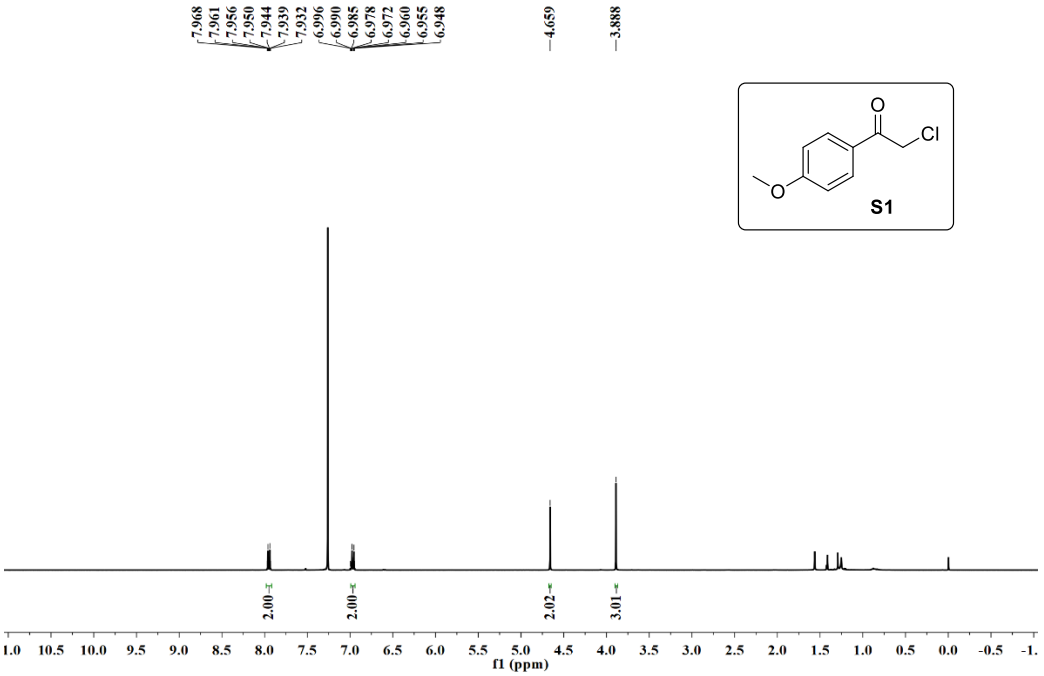
[5] A. Schwartz, P. B. Madan, E. Mohacsi, J. P. O'Brien, L. J. Todaro and D. L. Coffen, *J. Org. Chem.*, 1992, **57**, 851-856.

[6] B. M. Adger, J. V. Barkley, S. Bergeron, M. W. Cappi, B. E. Flowerdew, M. P. Jackson, R. McCague, T. C. Nugent and S. M. Roberts, *J. Chem. Soc., Perkin Trans.*, 1997, **1**, 3501-3507.

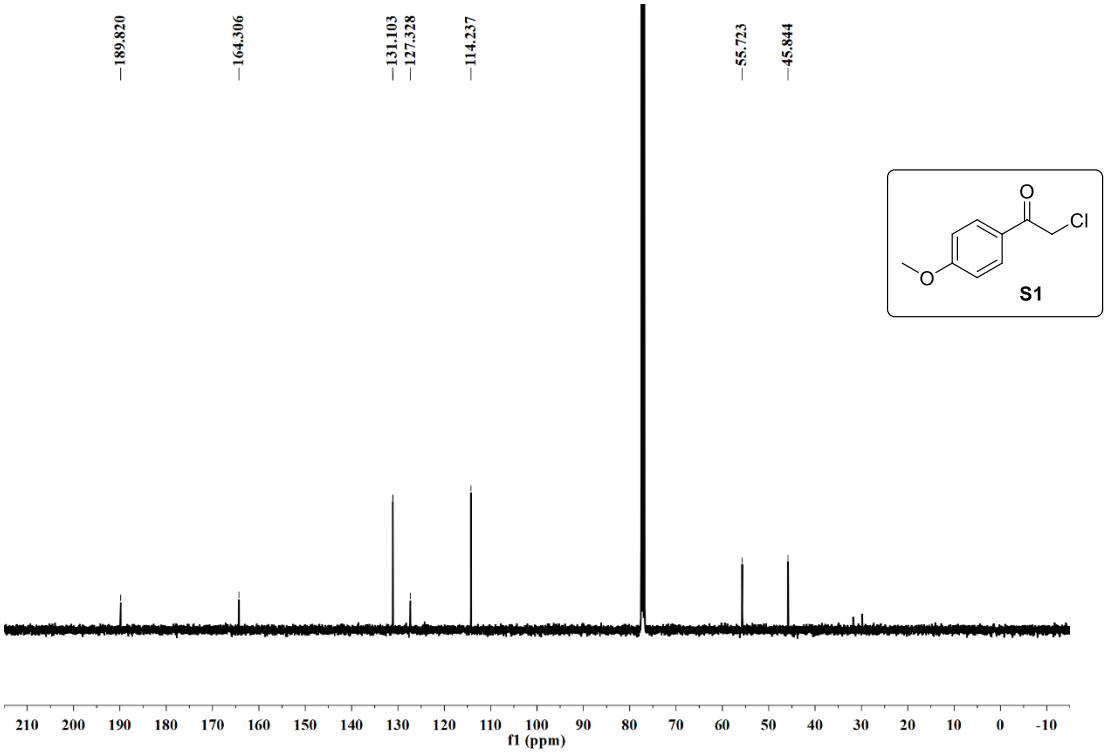
[7] S. Mor and S. Sindhu, *RSC Adv.*, 2019, **9**, 12784-12792.

[8] C. Xiao, Y. Cheng, Y. Zhang, J. Ding, C. He, X. Zhuang and X. Chen, *J. Polym. Sci. Pol. Chem.*, 2014, **52**, 671-679.

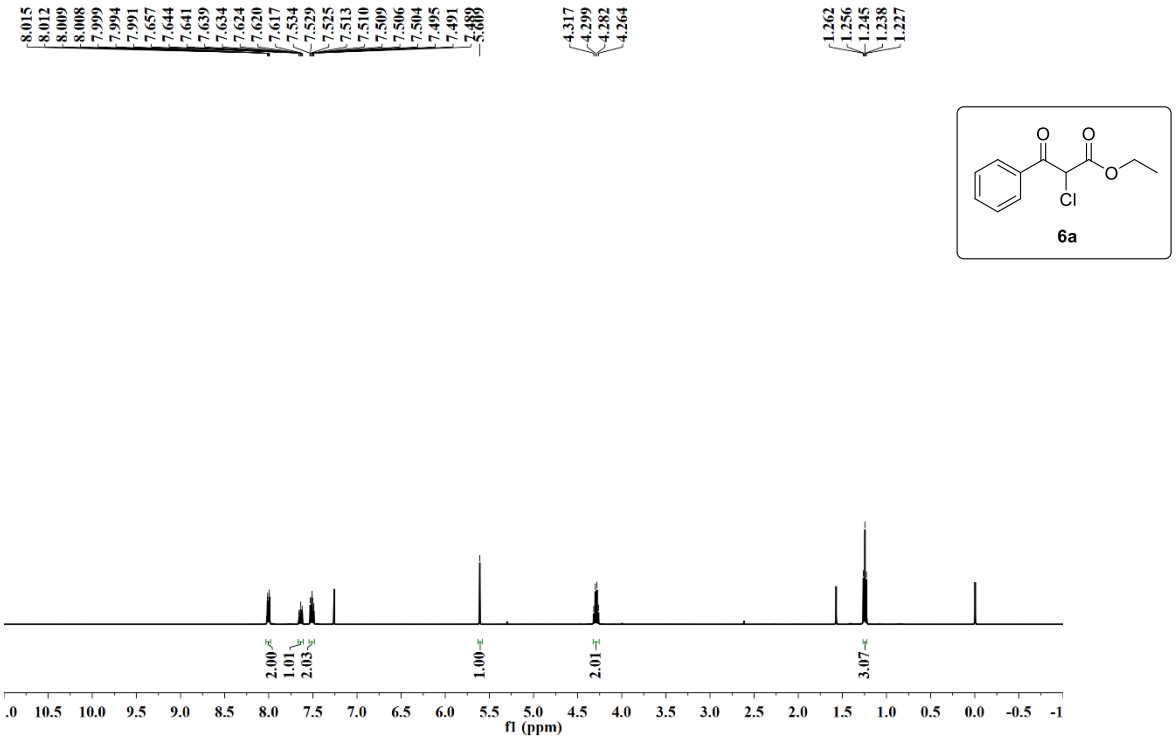
The ^{1}H NMR (400 MHz, $CDCl_3$) Spectrum of **S1**



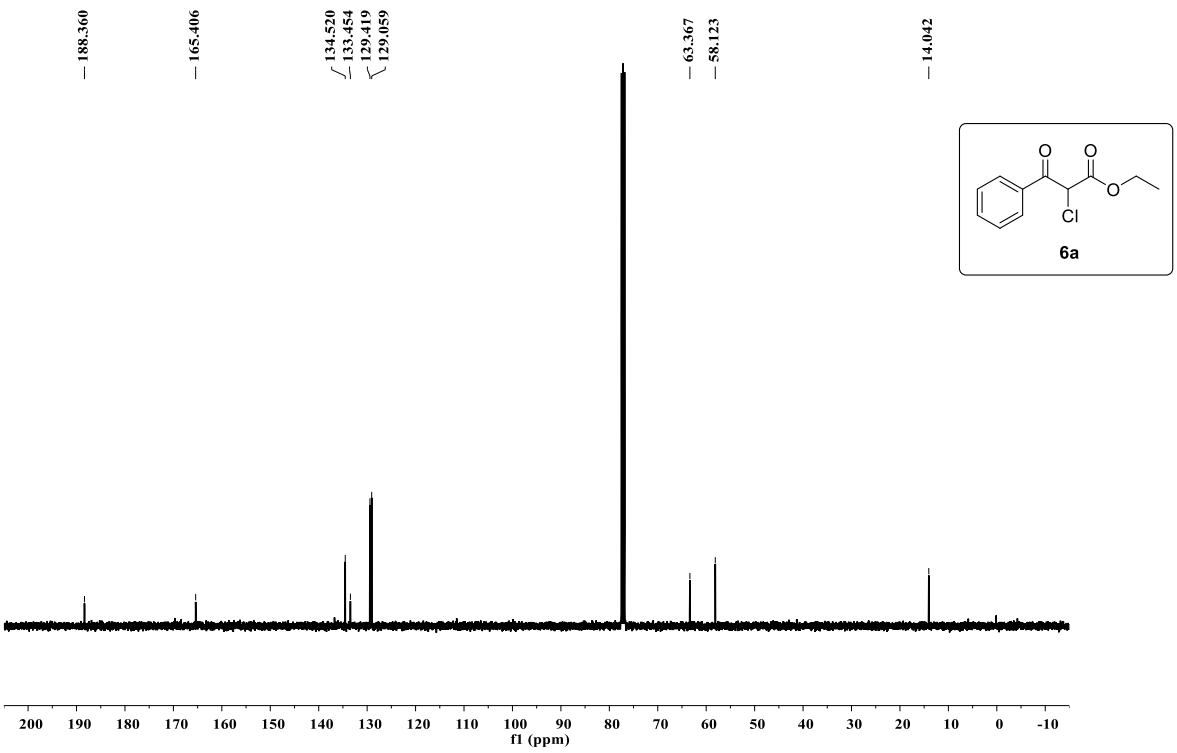
The ^{13}C NMR (100 MHz, $CDCl_3$) Spectrum of **S1**



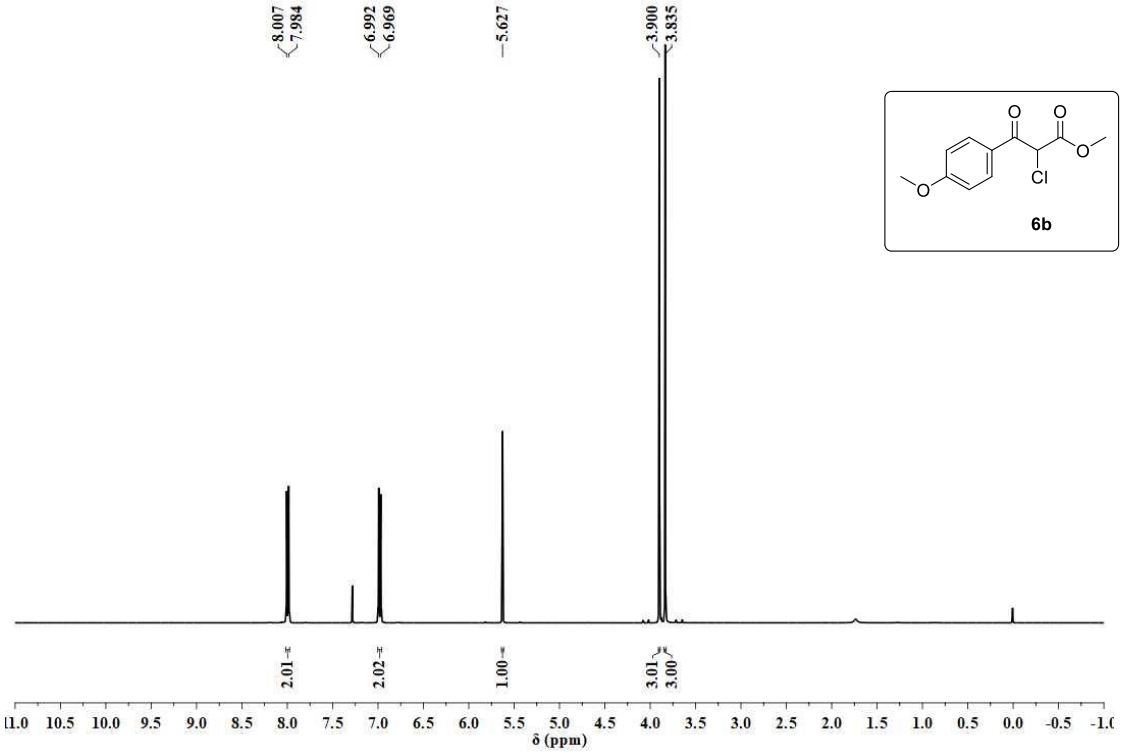
The ^{1}H NMR (400 MHz, $CDCl_3$) Spectrum of **6a**



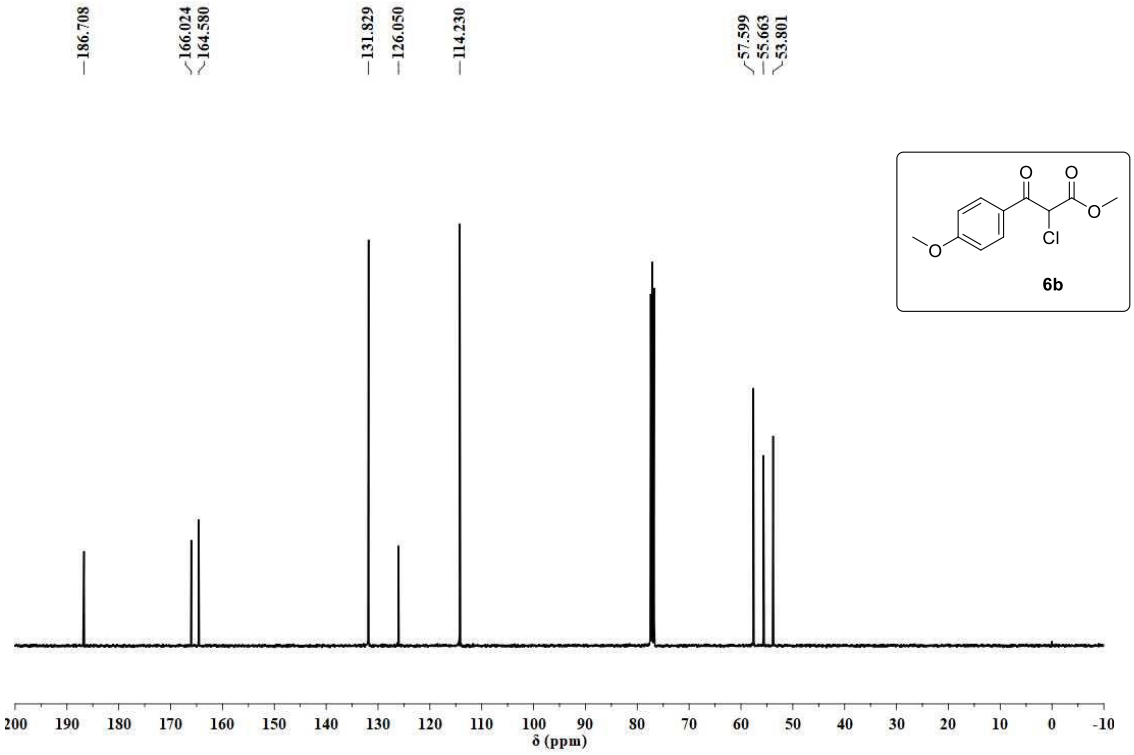
The ^{13}C NMR (100 MHz, $CDCl_3$) Spectrum of **6a**



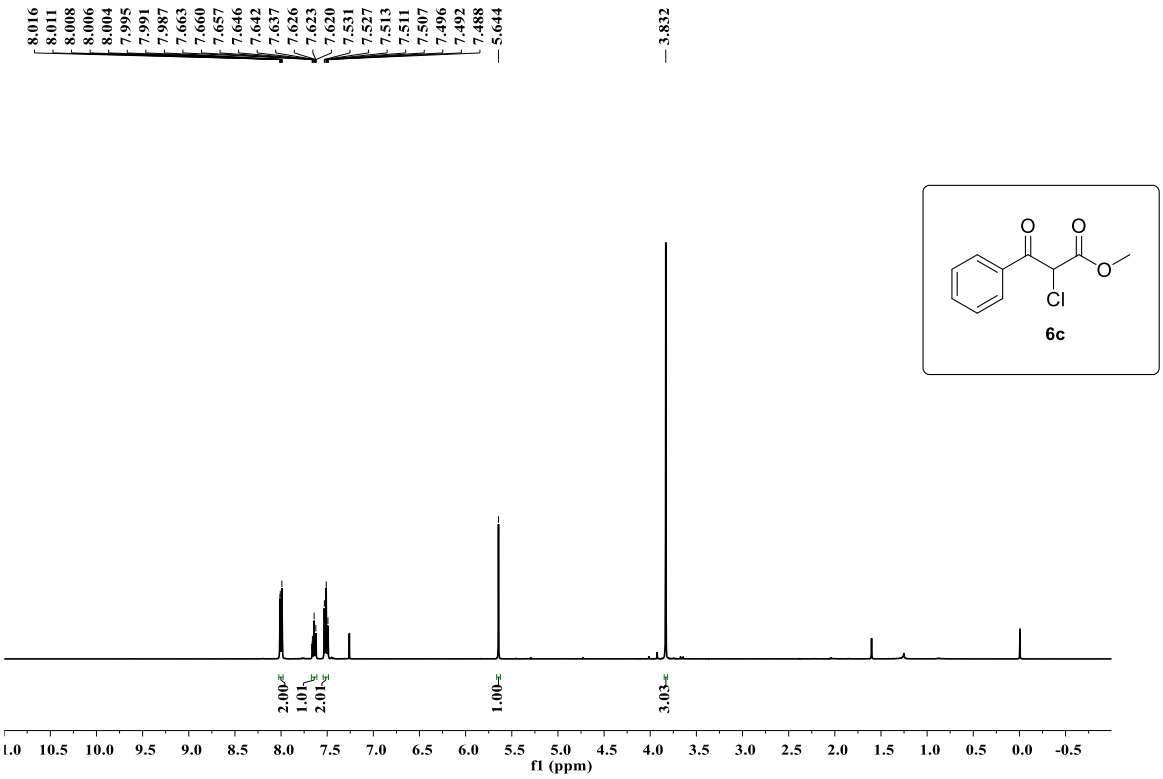
The ^{1}H NMR (400 MHz, $CDCl_3$) Spectrum of **6b**



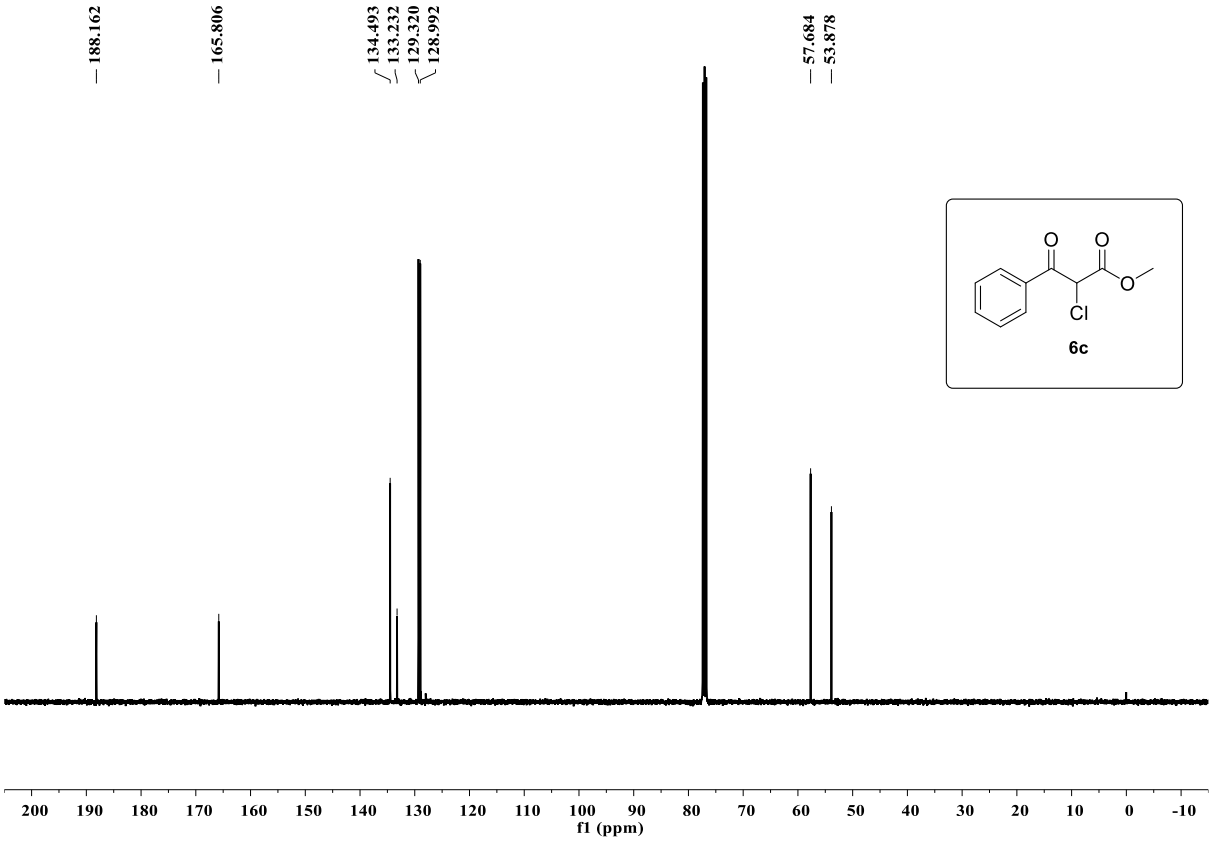
The ^{13}C NMR (100 MHz, $CDCl_3$) Spectrum of **6b**



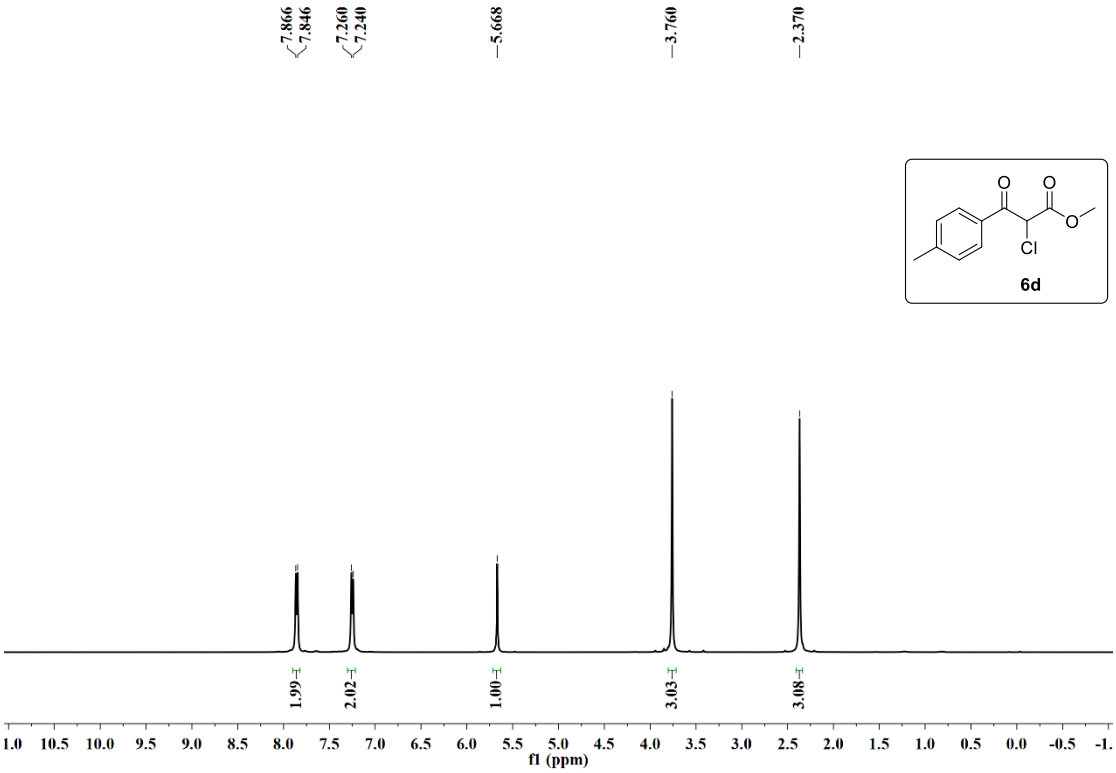
The ^{1}H NMR (400 MHz, $CDCl_3$) Spectrum of **6c**



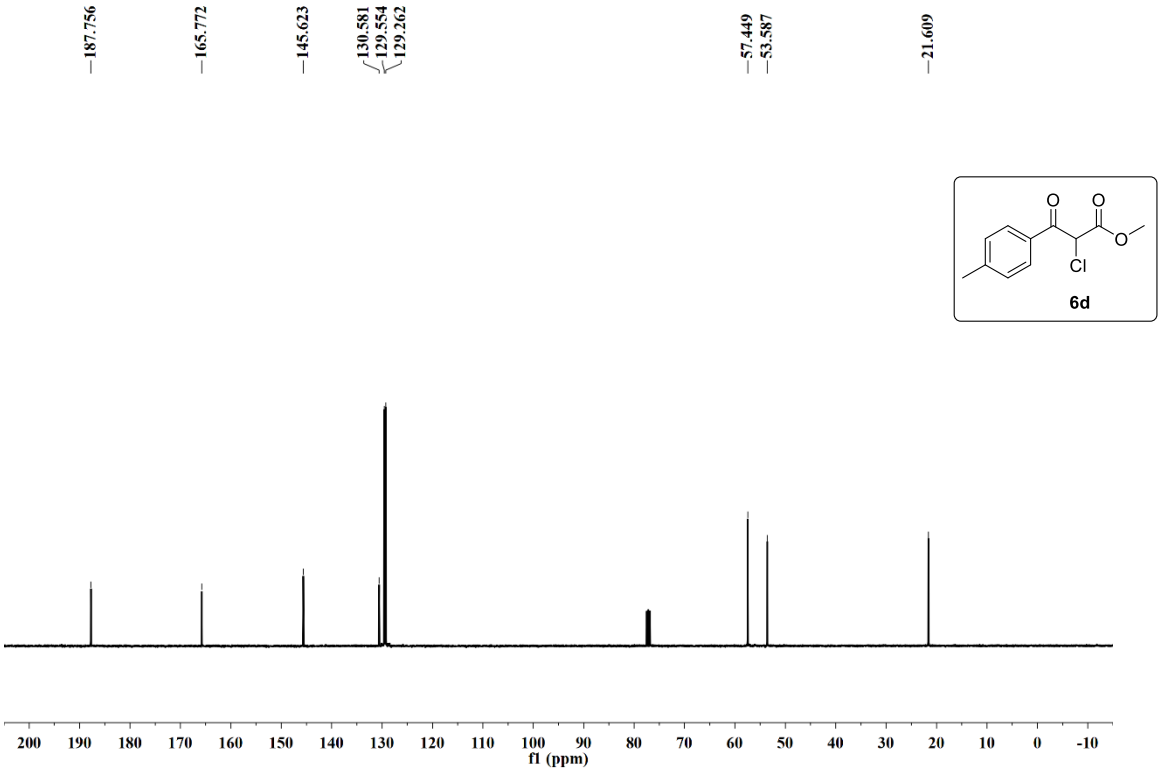
The ^{13}C NMR (100 MHz, $CDCl_3$) Spectrum of **6c**



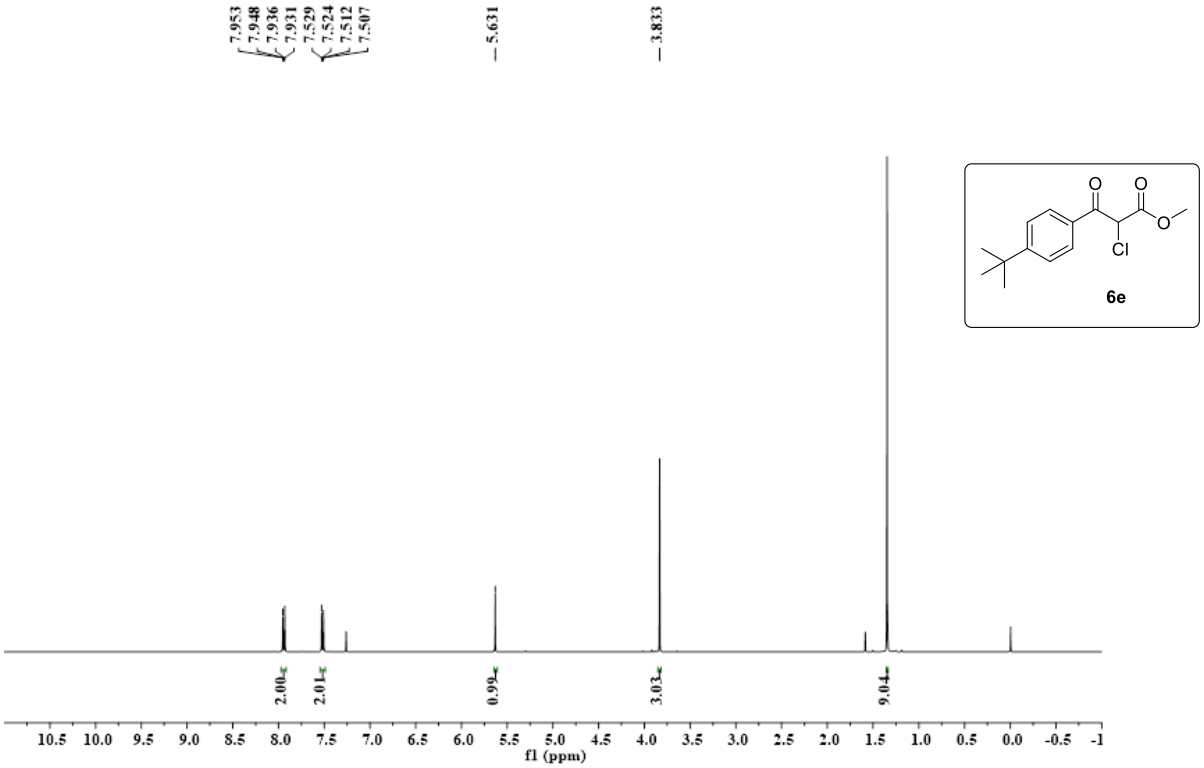
The ^{1}H NMR (400 MHz, $CDCl_3$) Spectrum of **6d**



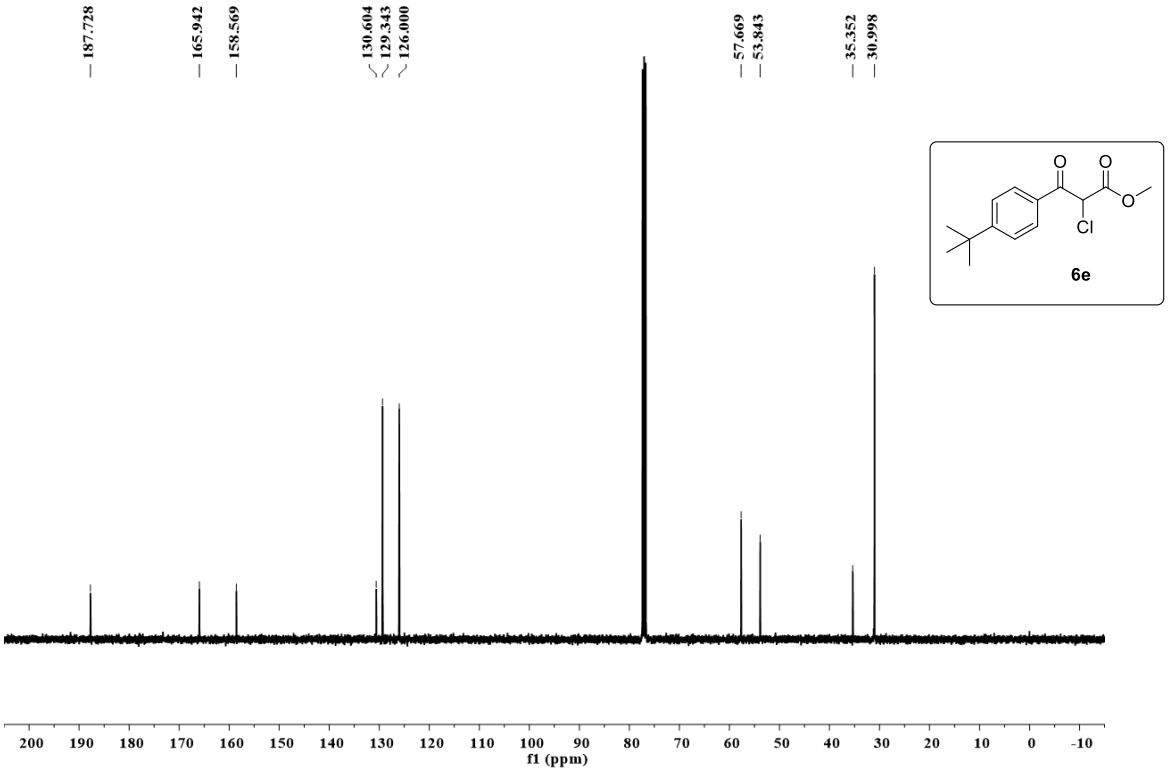
The ^{13}C NMR (100 MHz, $CDCl_3$) Spectrum of **6d**



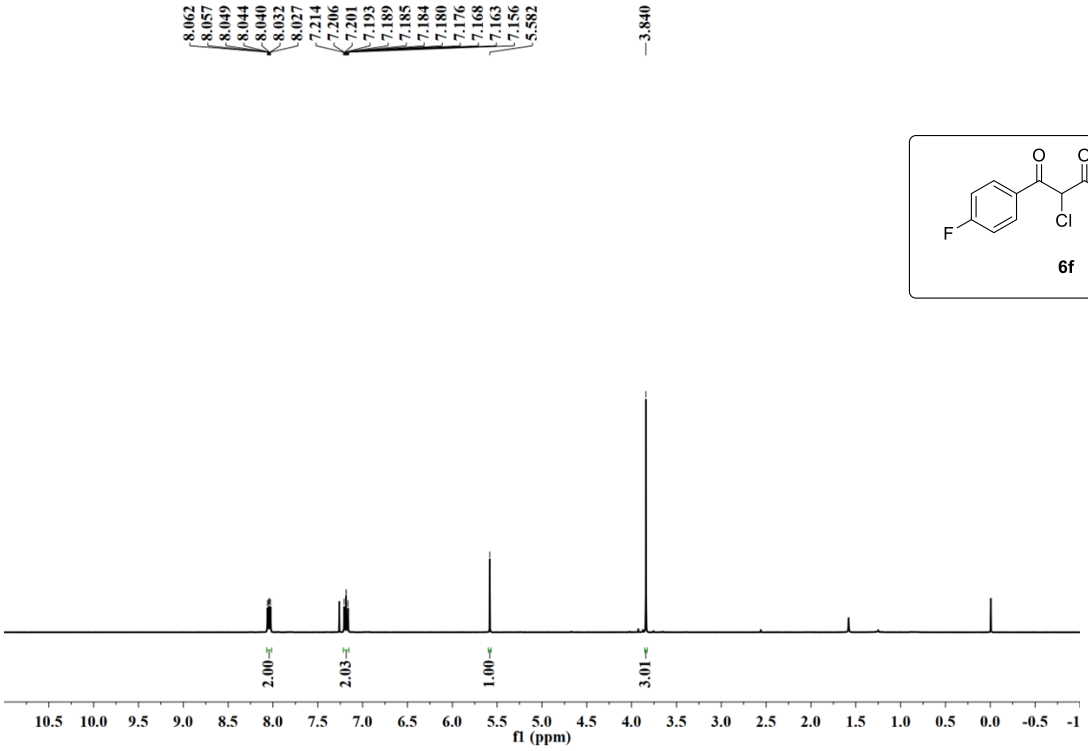
The ^{1}H NMR (400 MHz, $CDCl_3$) Spectrum of **6e**



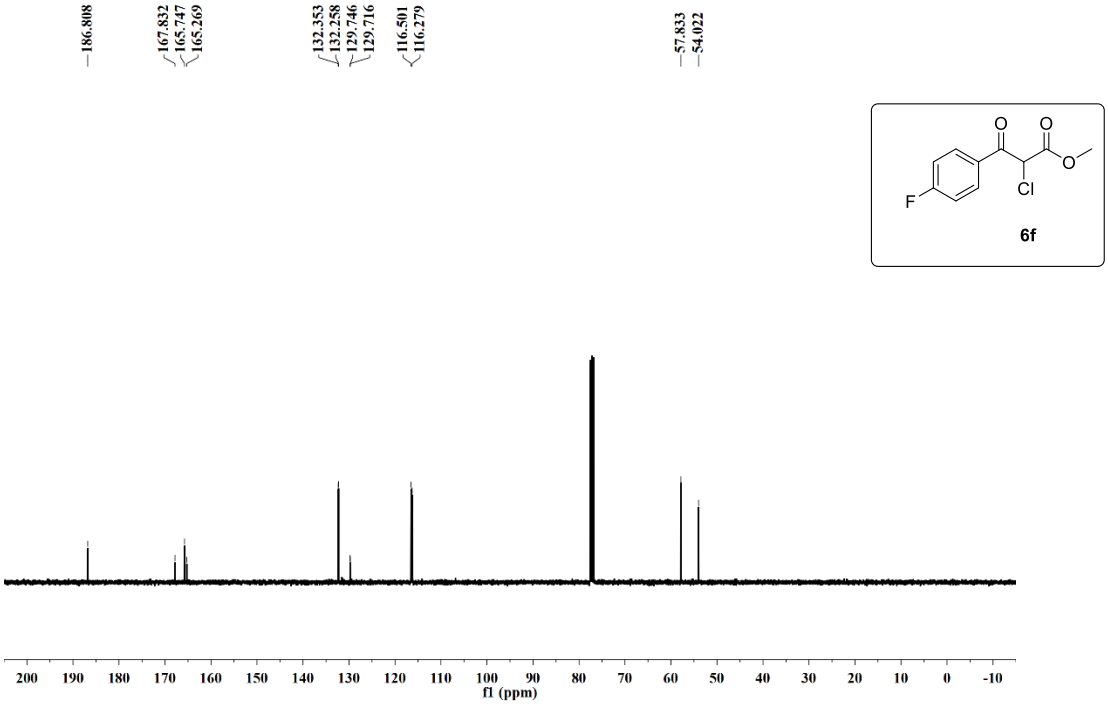
The ^{13}C NMR (100 MHz, $CDCl_3$) Spectrum of **6e**



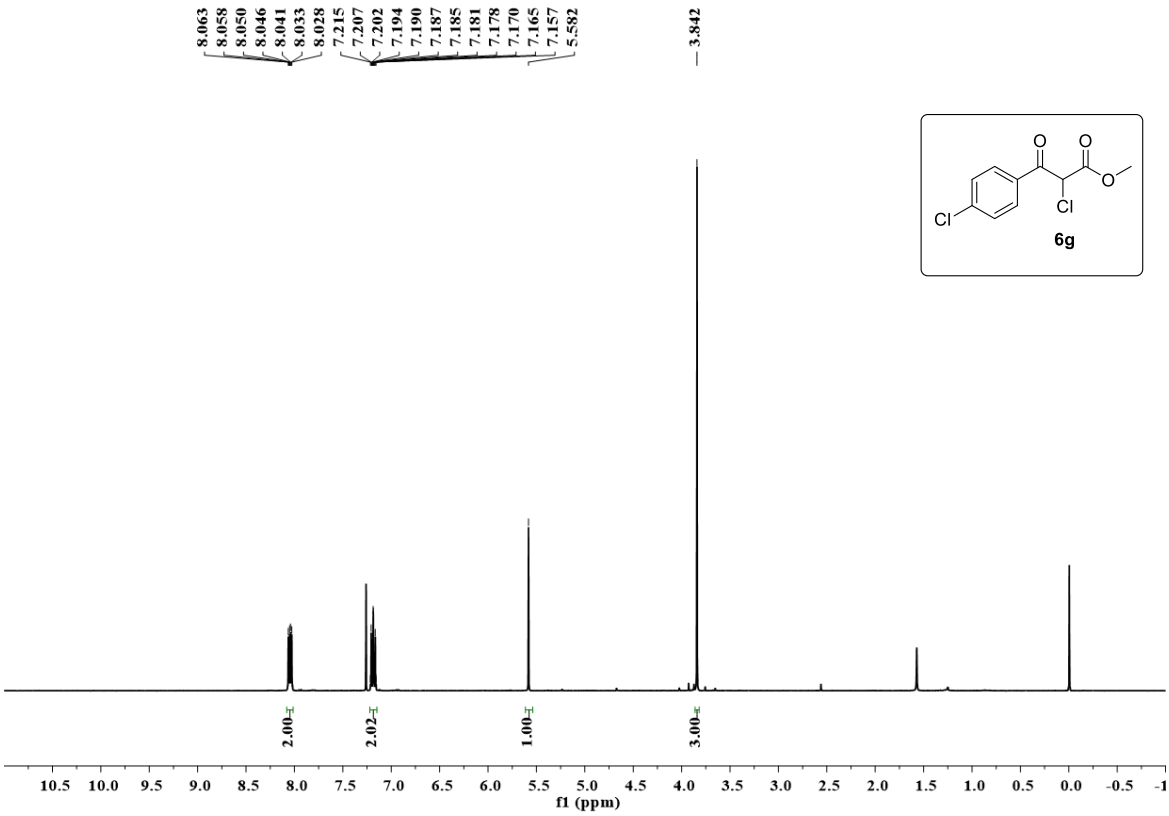
The ^{1}H NMR (400 MHz, $CDCl_3$) Spectrum of **6f**



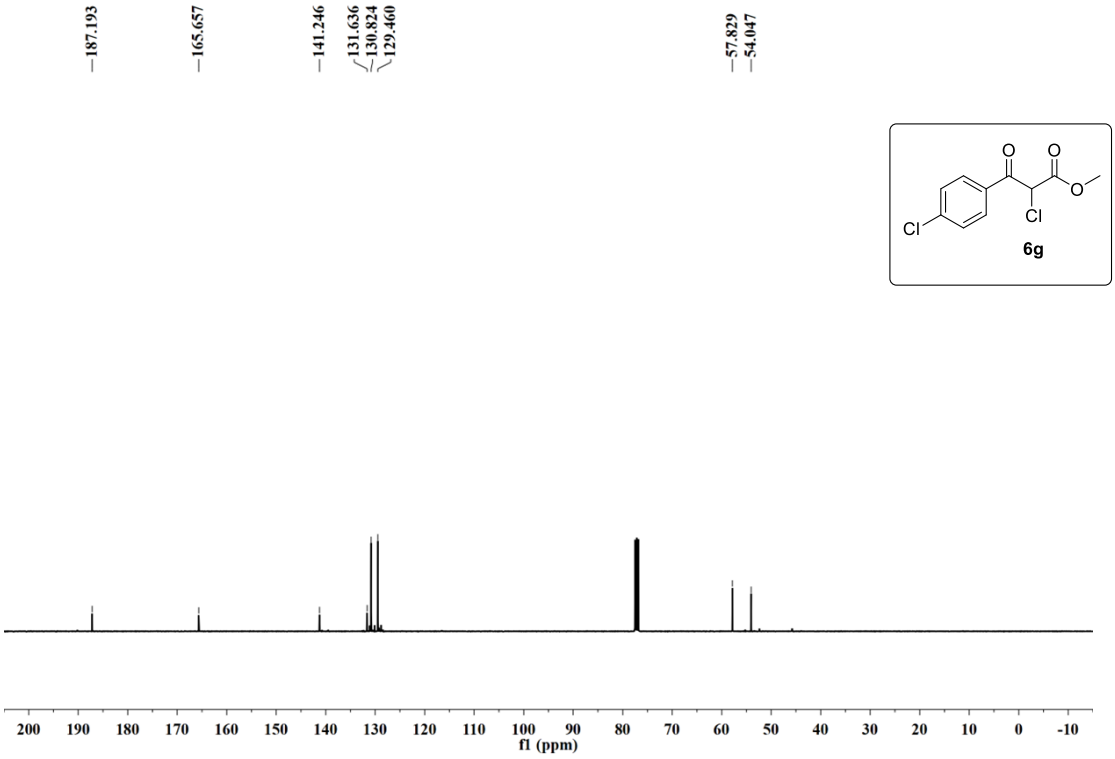
The ^{13}C NMR (100 MHz, $CDCl_3$) Spectrum of **6f**



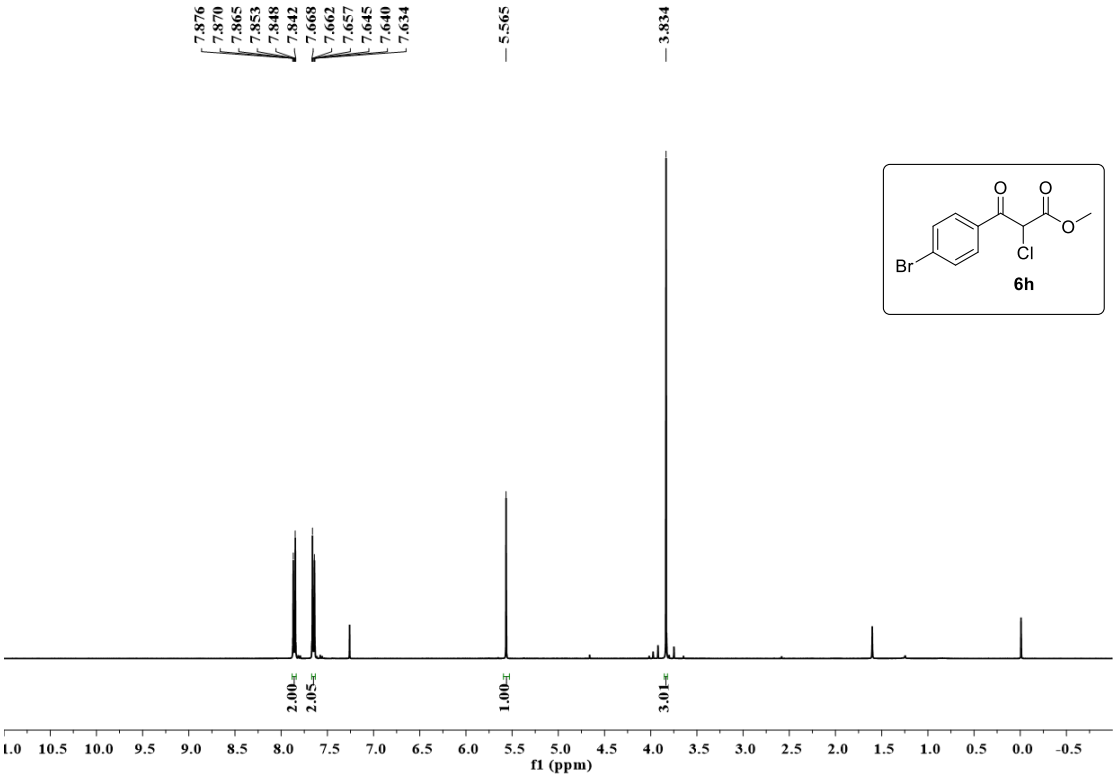
The ^{1}H NMR (400 MHz, $CDCl_3$) Spectrum of **6g**



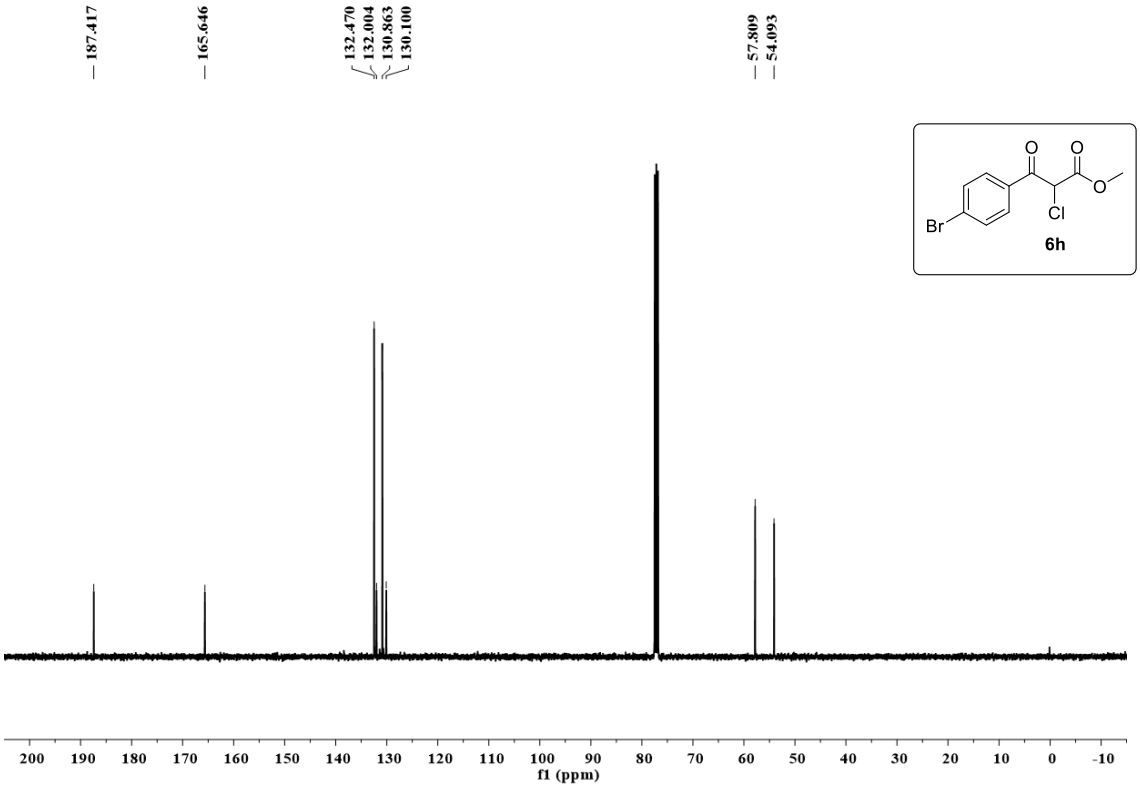
The ^{13}C NMR (100 MHz, $CDCl_3$) Spectrum of **6g**



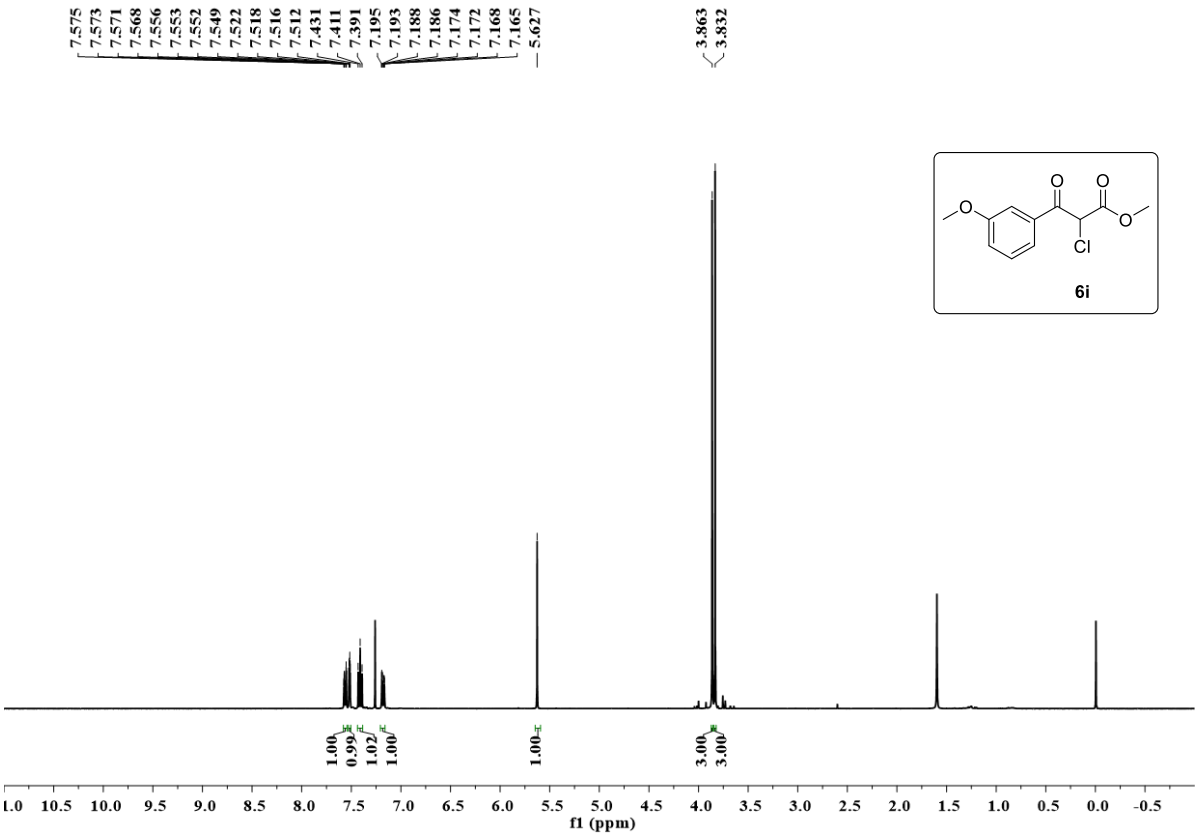
The ^{1}H NMR (400 MHz, $CDCl_3$) Spectrum of **6h**



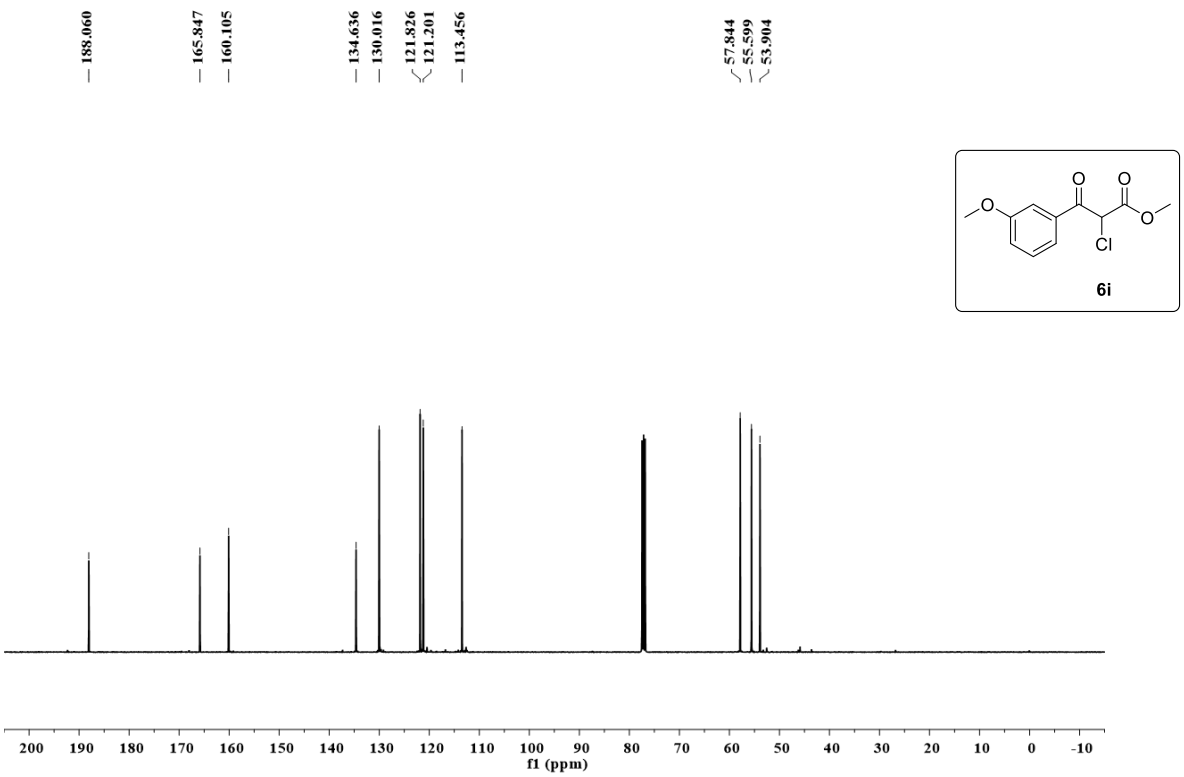
The ^{13}C NMR (100 MHz, $CDCl_3$) Spectrum of **6h**



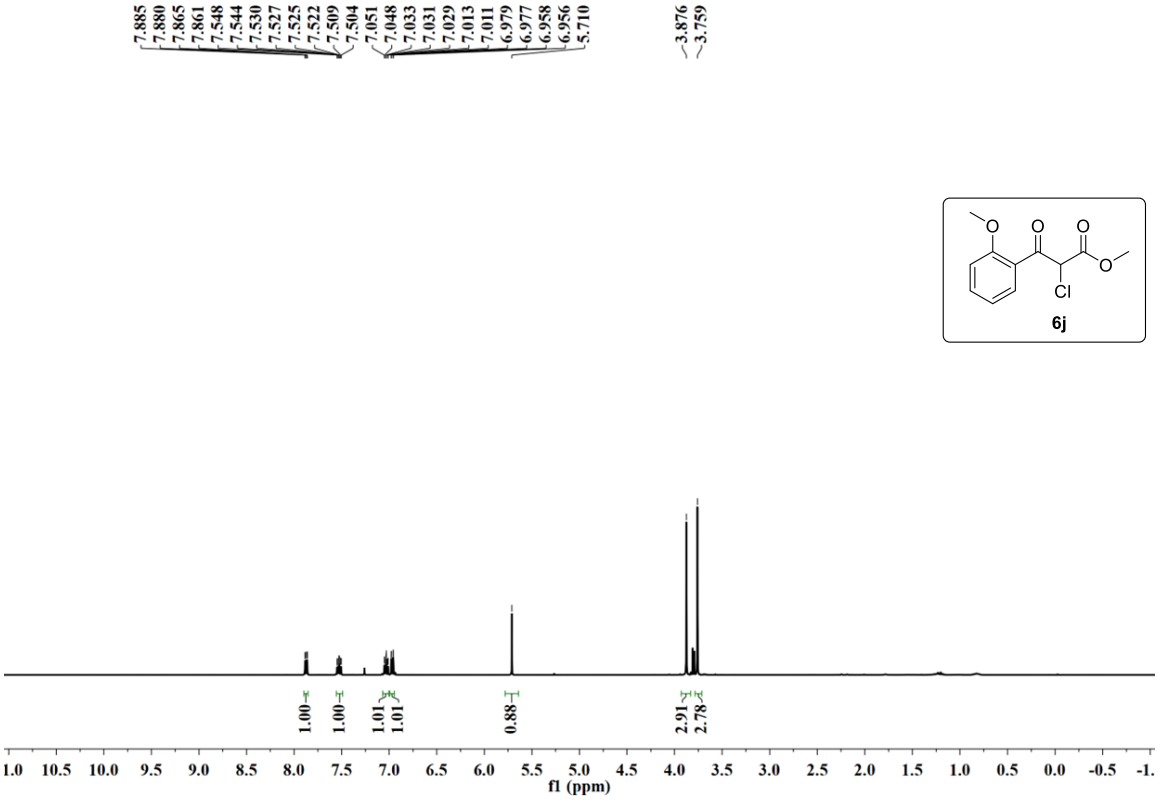
The ^{1}H NMR (400 MHz, $CDCl_3$) Spectrum of **6i**



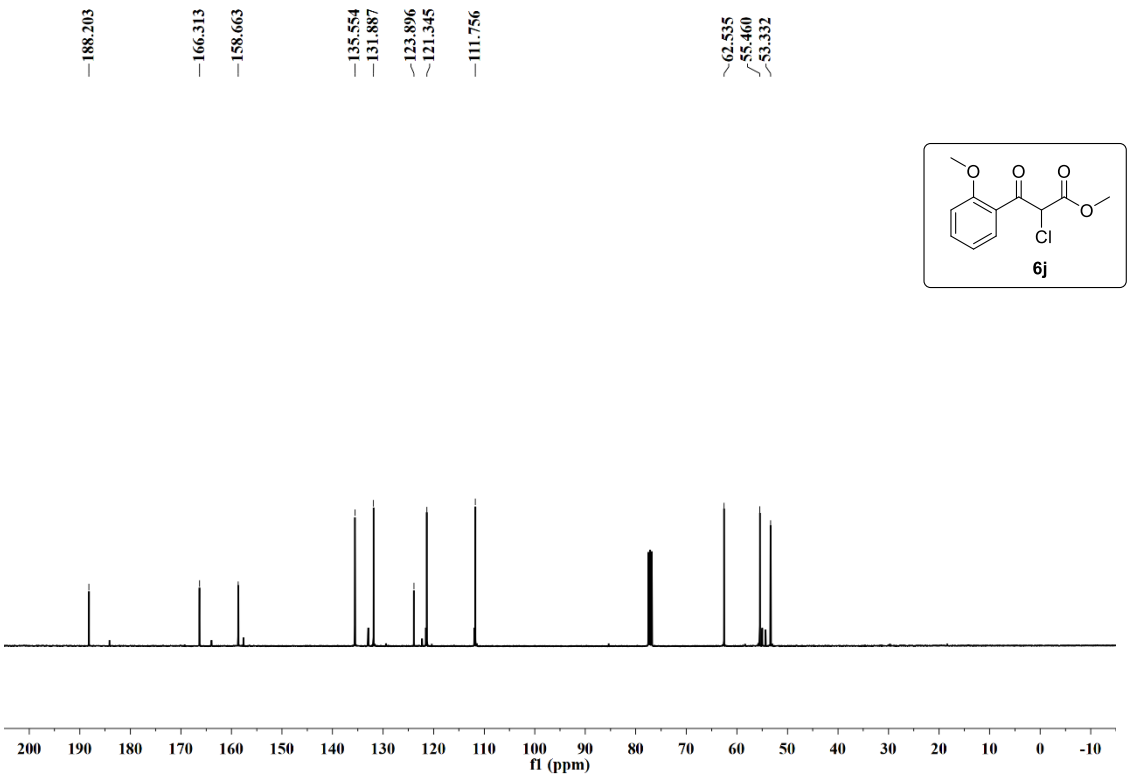
The ^{13}C NMR (100 MHz, $CDCl_3$) Spectrum of **6i**



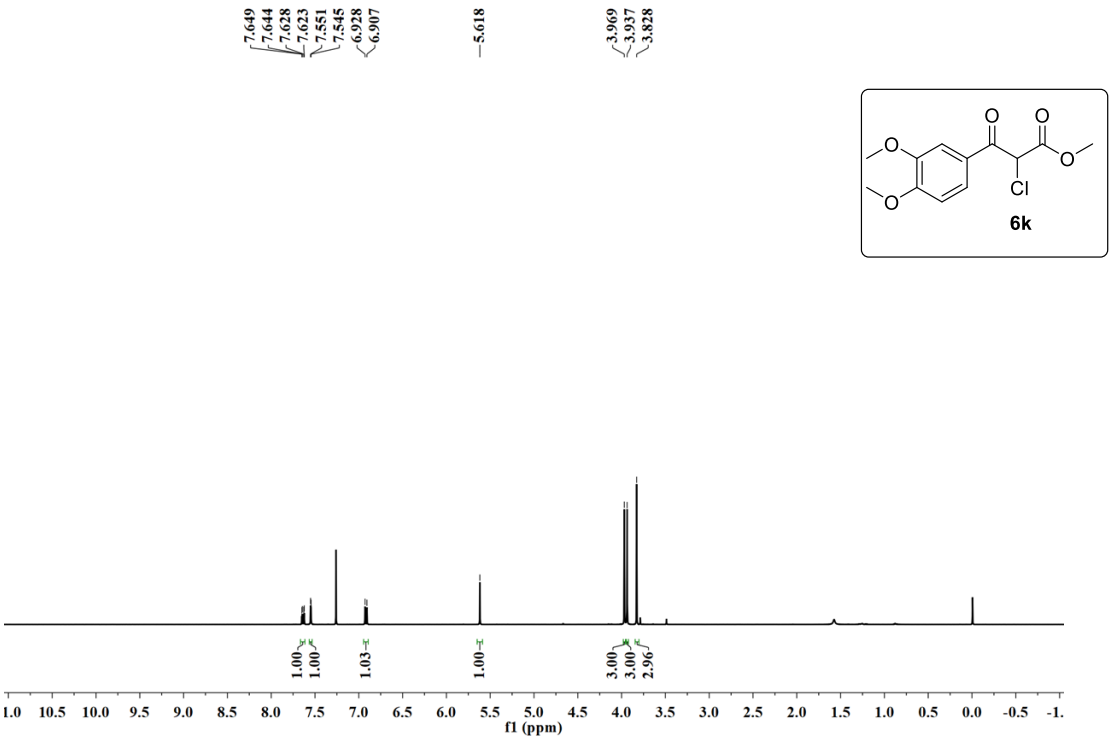
The ^{1}H NMR (400 MHz, $CDCl_3$) Spectrum of **6j**



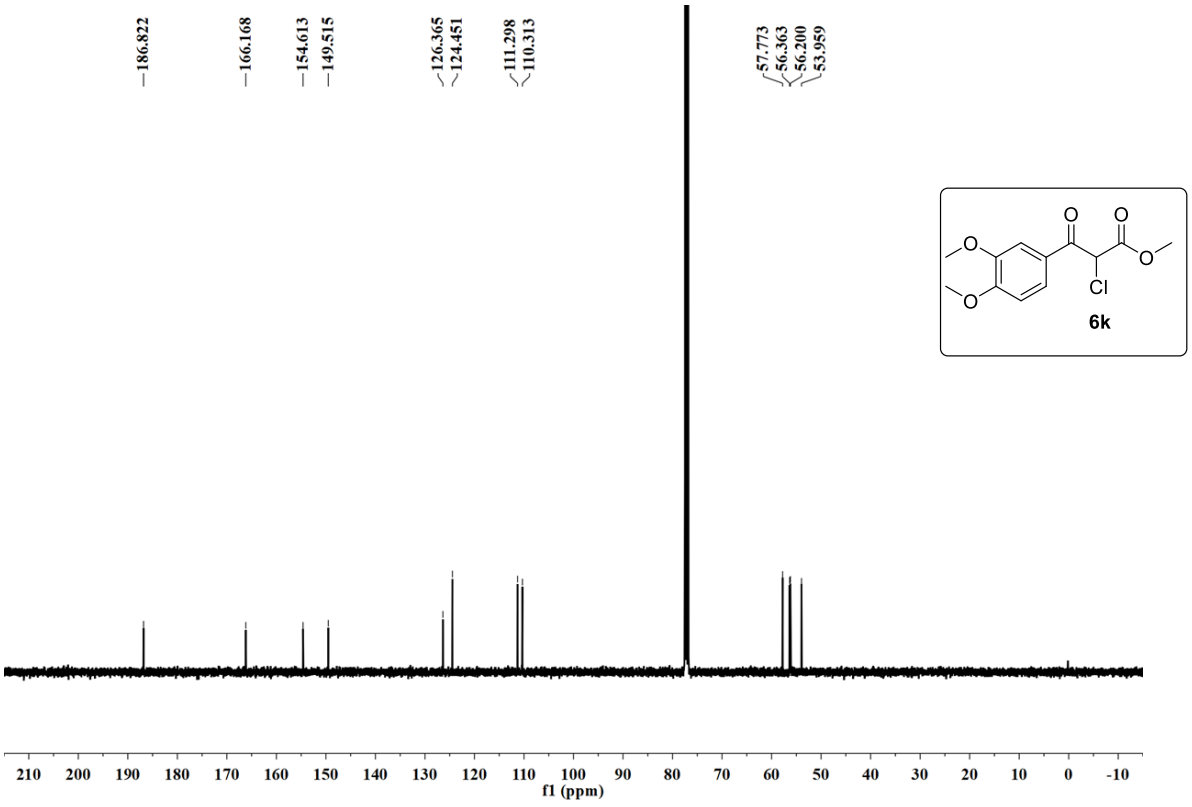
The ^{13}C NMR (100 MHz, $CDCl_3$) Spectrum of **6j**



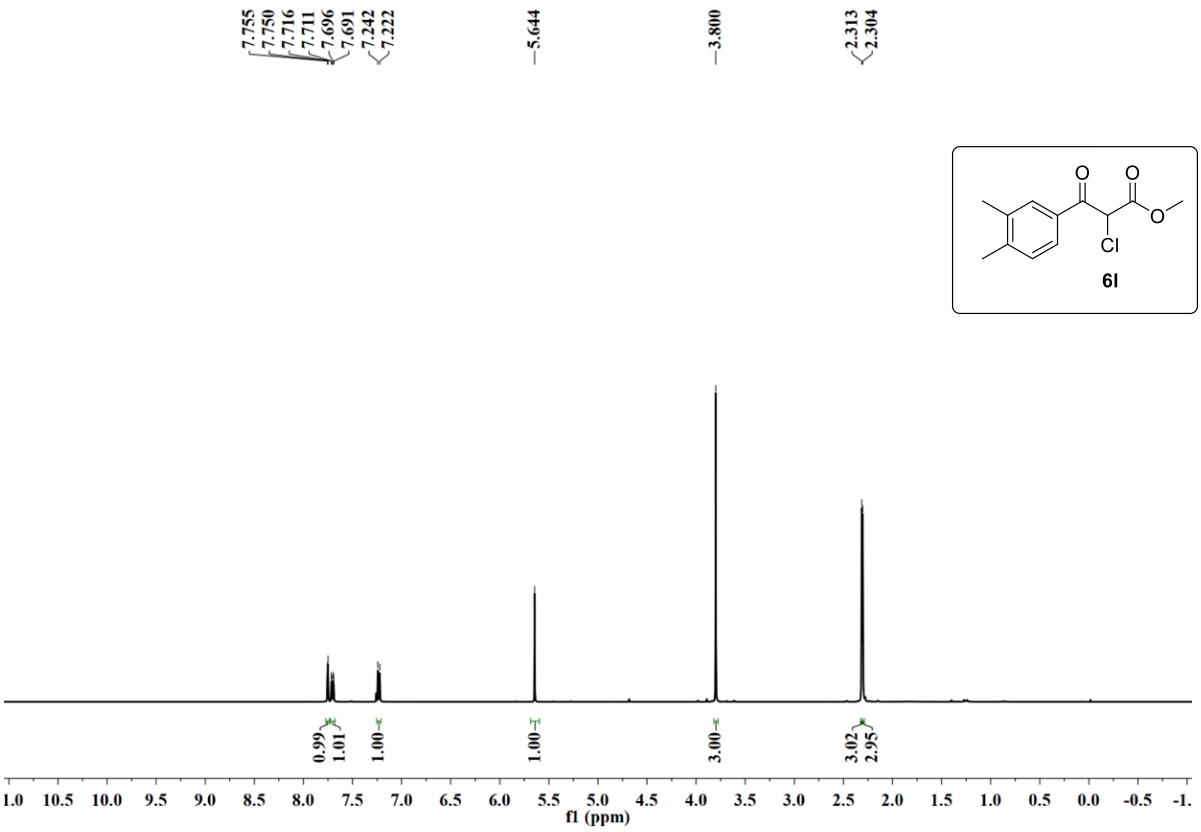
The ^{1}H NMR (400 MHz, $CDCl_3$) Spectrum of **6k**



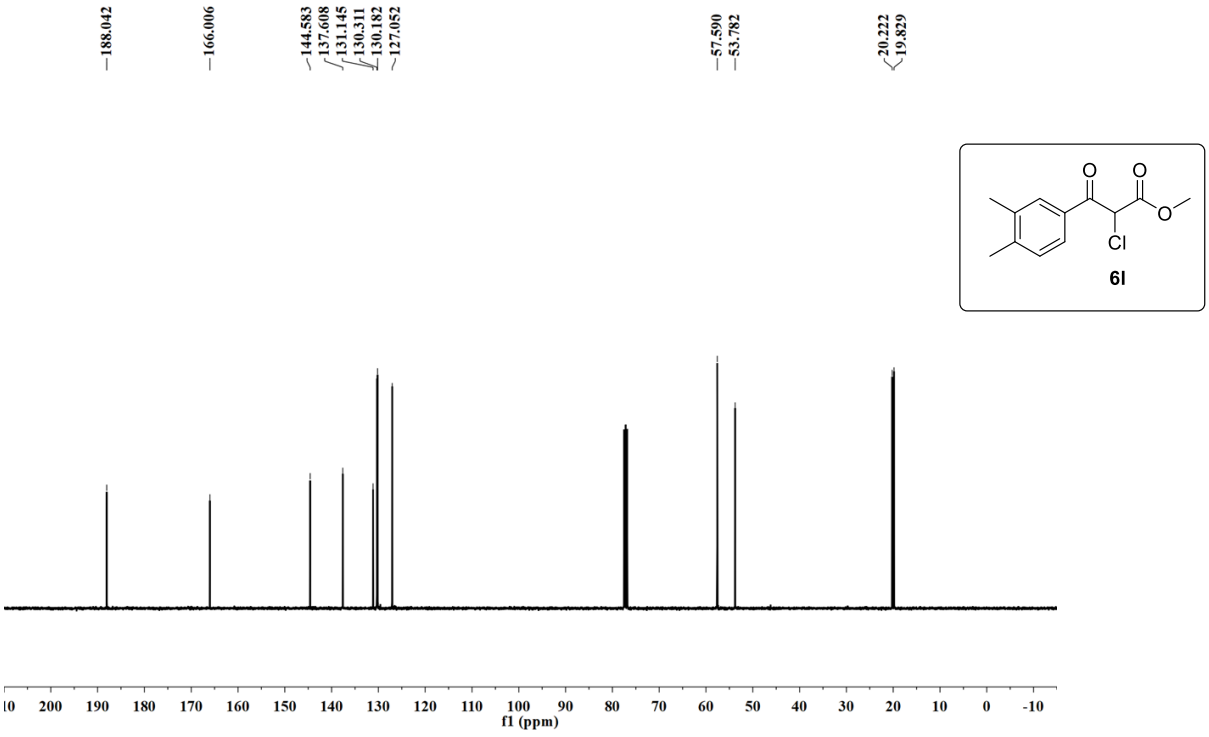
The ^{13}C NMR (100 MHz, $CDCl_3$) Spectrum of **6k**



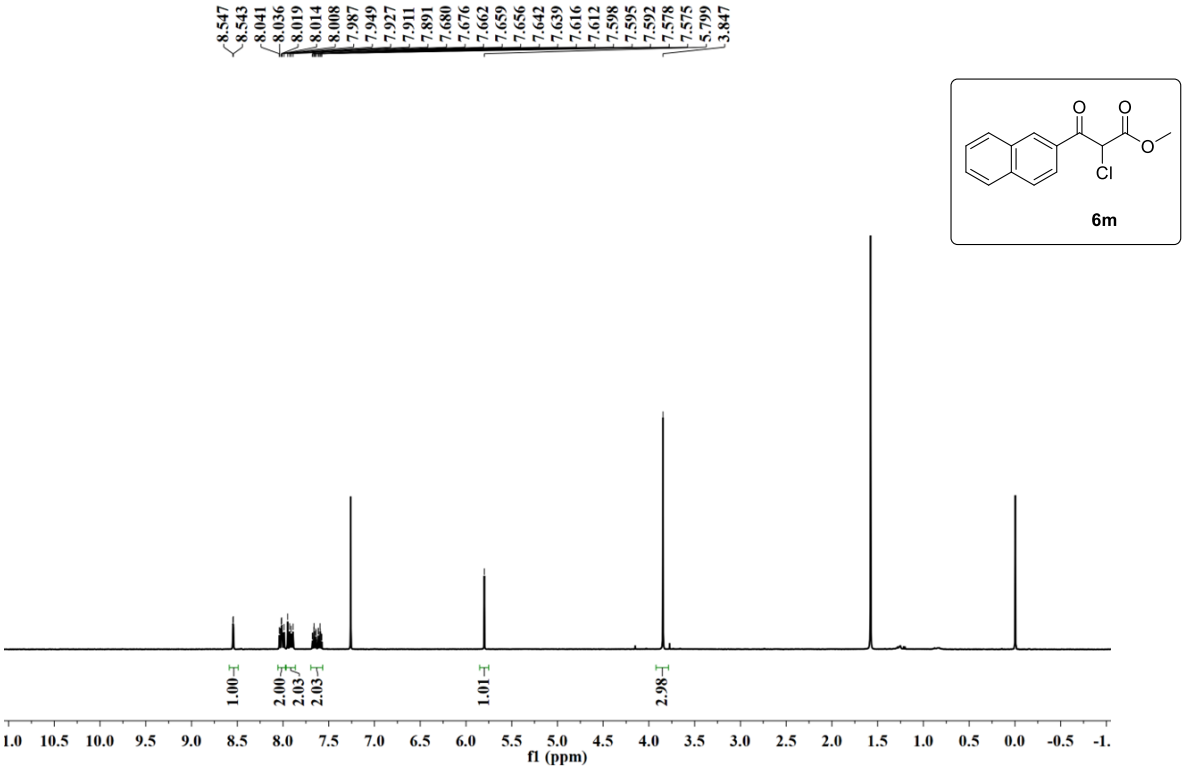
The ^{1}H NMR (400 MHz, $CDCl_3$) Spectrum of **6l**



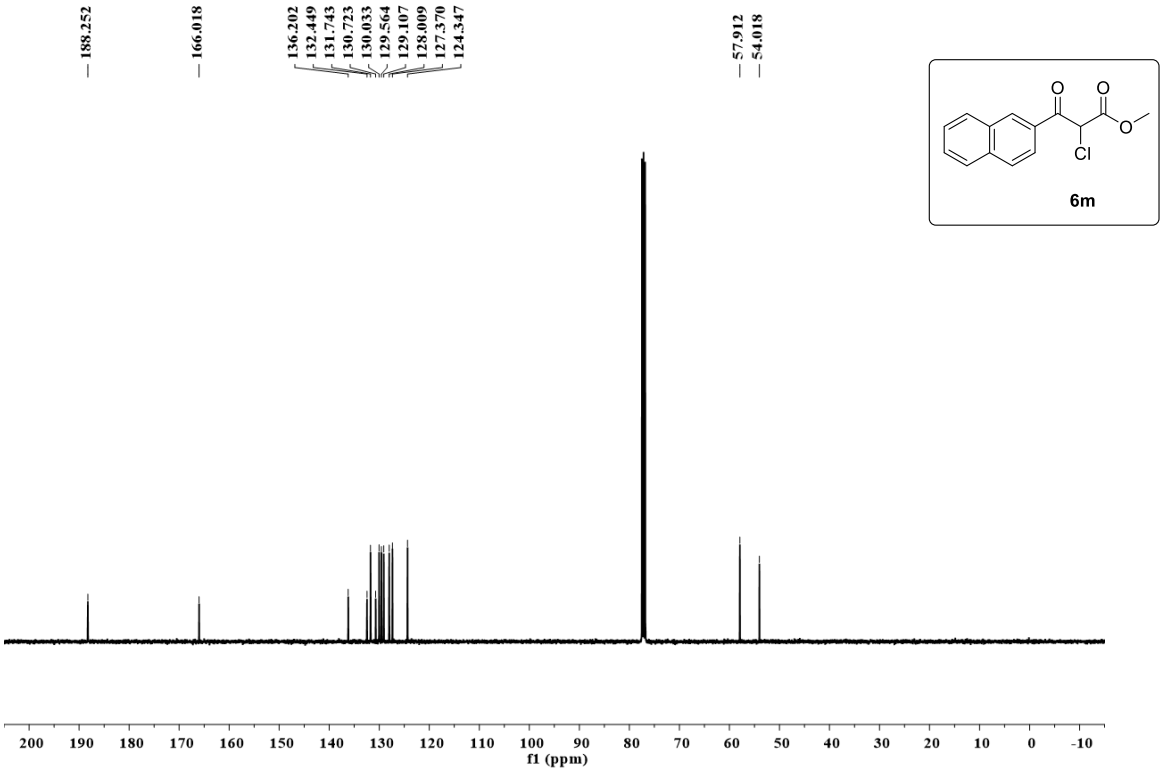
The ^{13}C NMR (100 MHz, $CDCl_3$) Spectrum of **6l**



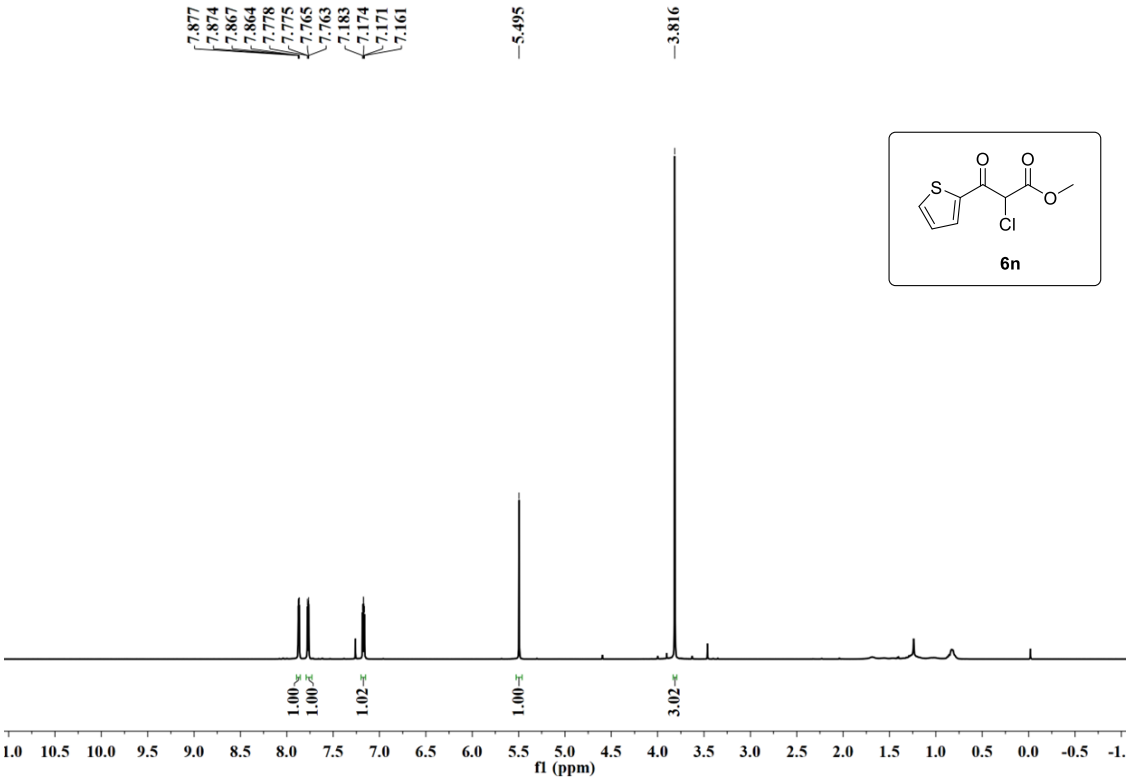
The ^{1}H NMR (400 MHz, $CDCl_3$) Spectrum of **6m**



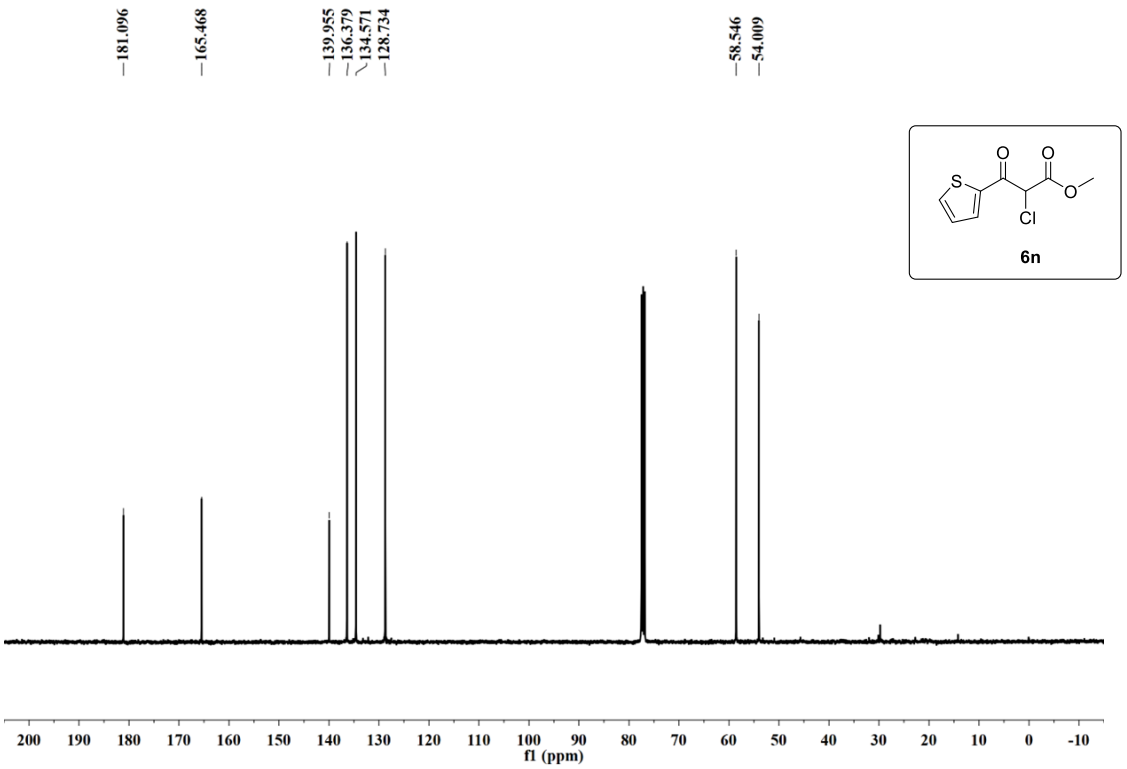
The ^{13}C NMR (100 MHz, $CDCl_3$) Spectrum of **6m**



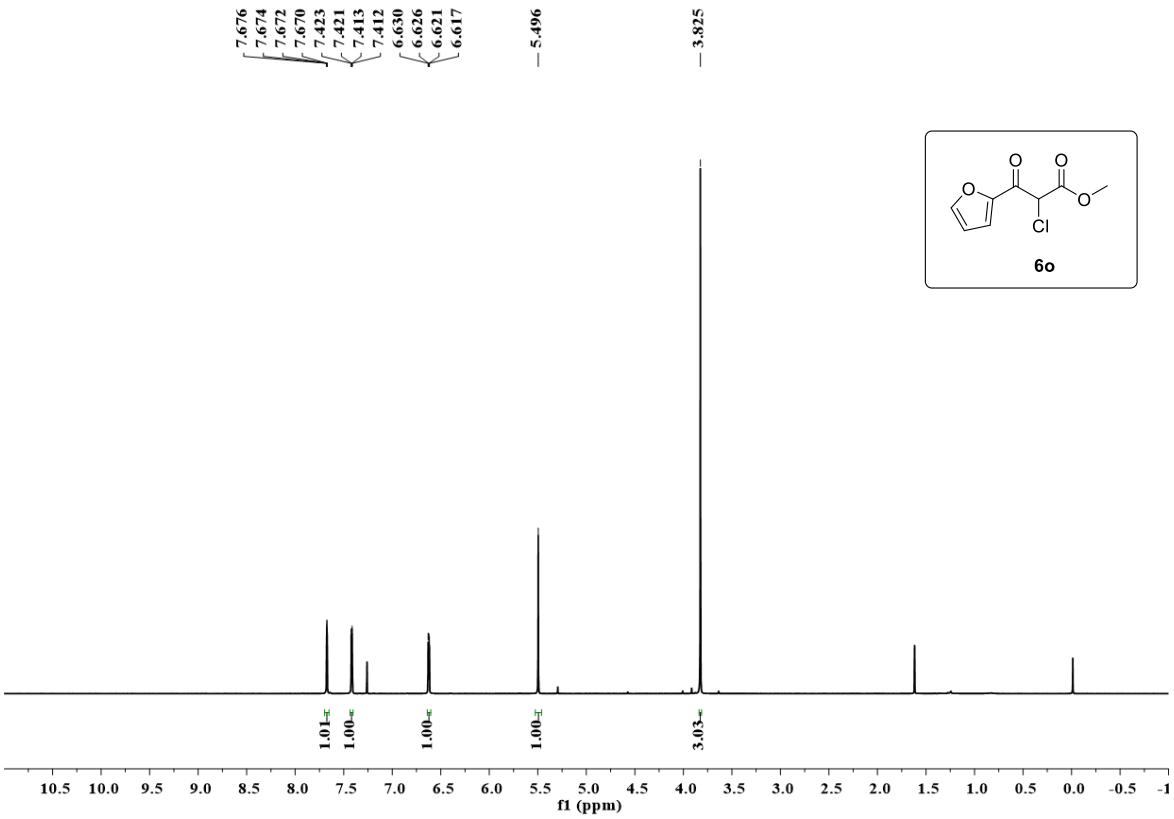
The ^{1}H NMR (400 MHz, $CDCl_3$) Spectrum of **6n**



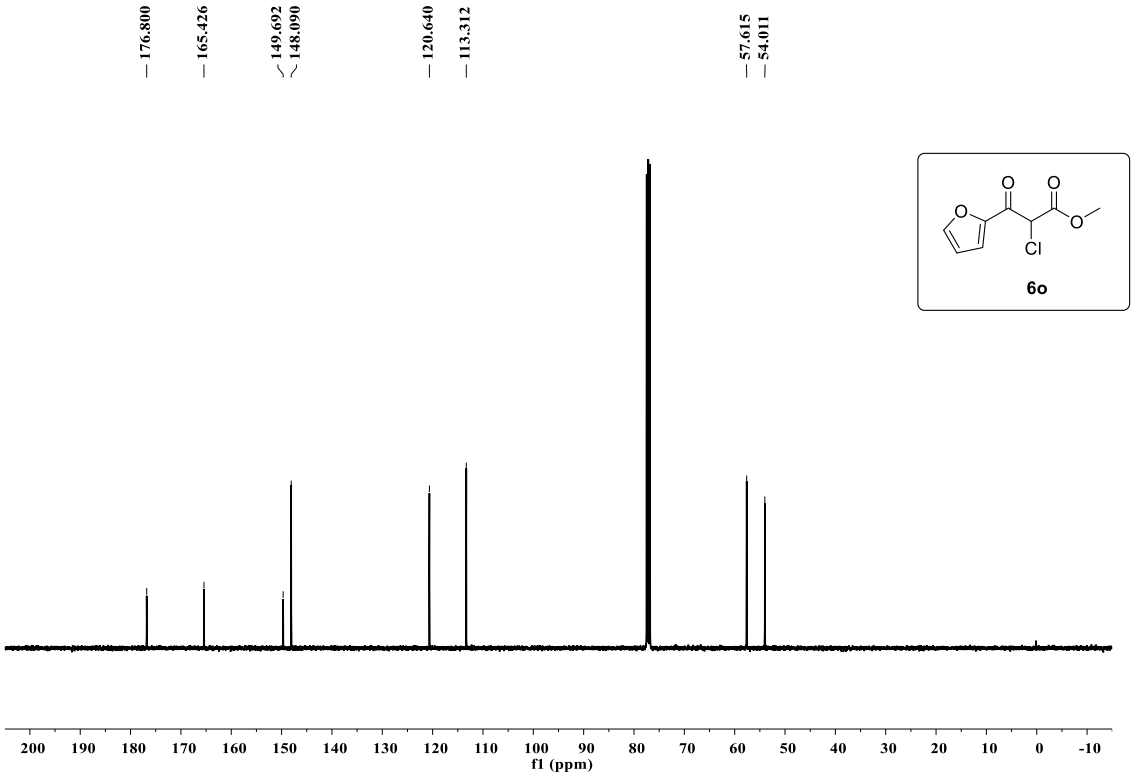
The ^{13}C NMR (100 MHz, $CDCl_3$) Spectrum of **6n**



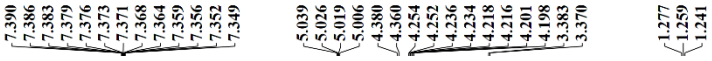
The ^{1}H NMR (400 MHz, $CDCl_3$) Spectrum of **6o**

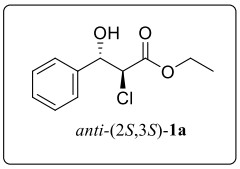


The ^{13}C NMR (100 MHz, $CDCl_3$) Spectrum of **6o**

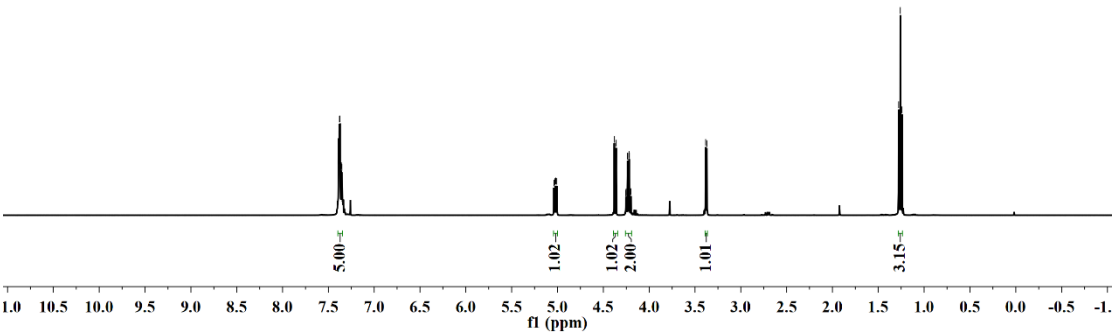


The ^{1}H NMR (400 MHz, $CDCl_3$) Spectrum of **1a**

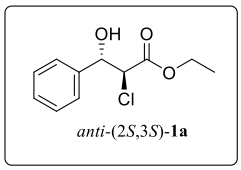




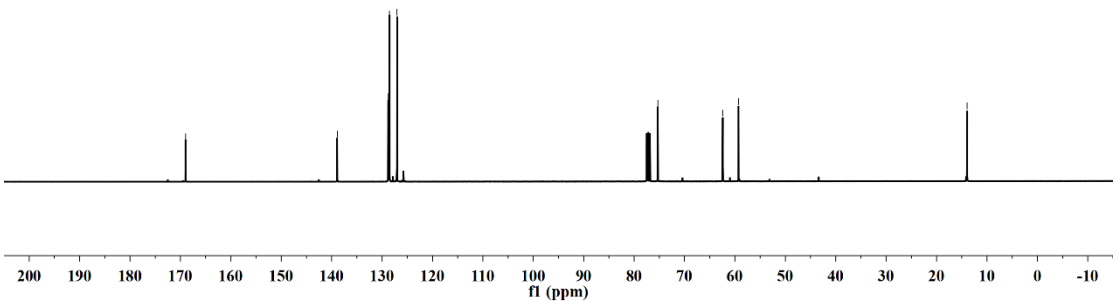
anti-(2*S*,3*S*)-**1a**



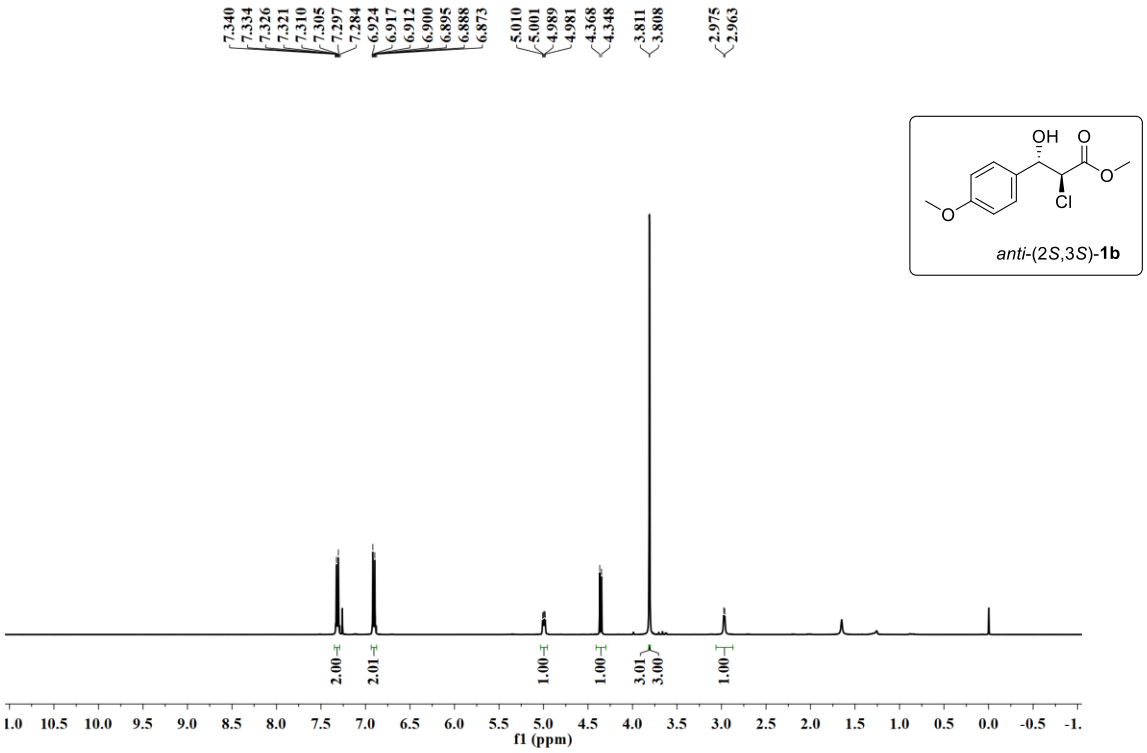
The ^{13}C NMR (100 MHz, $CDCl_3$) Spectrum of **1a**



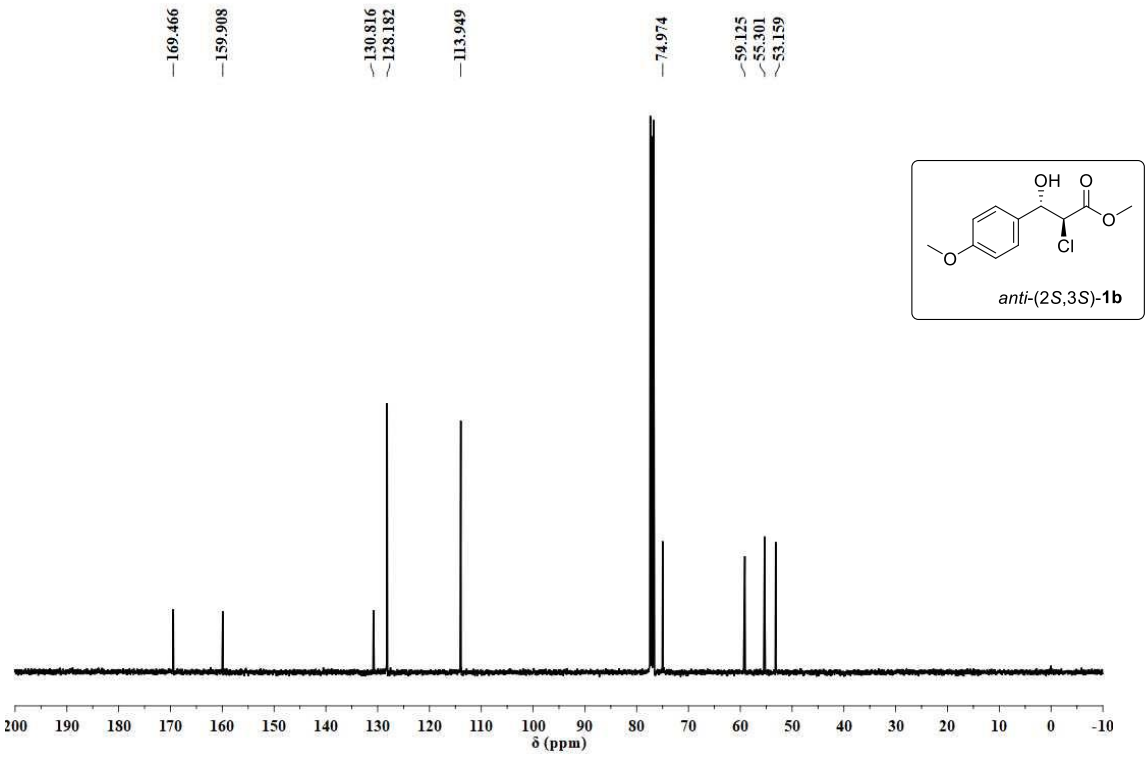
anti-(2*S*,3*S*)-**1a**



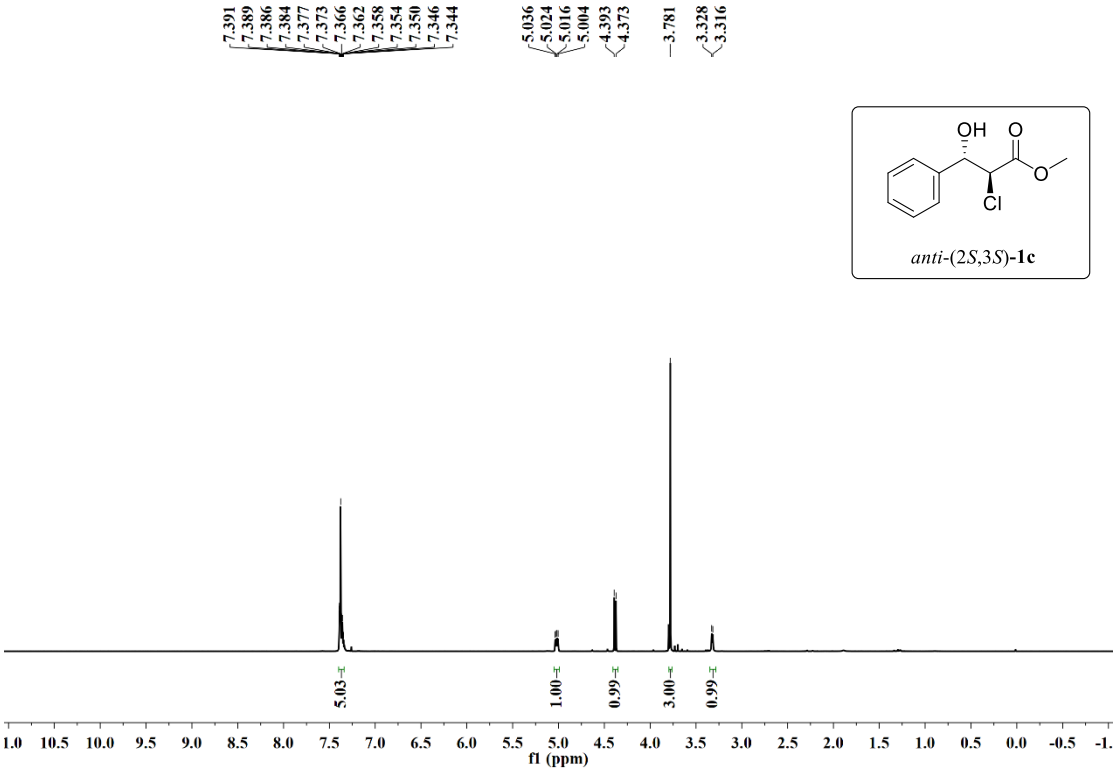
The ^{1}H NMR (400 MHz, $CDCl_3$) Spectrum of **1b**



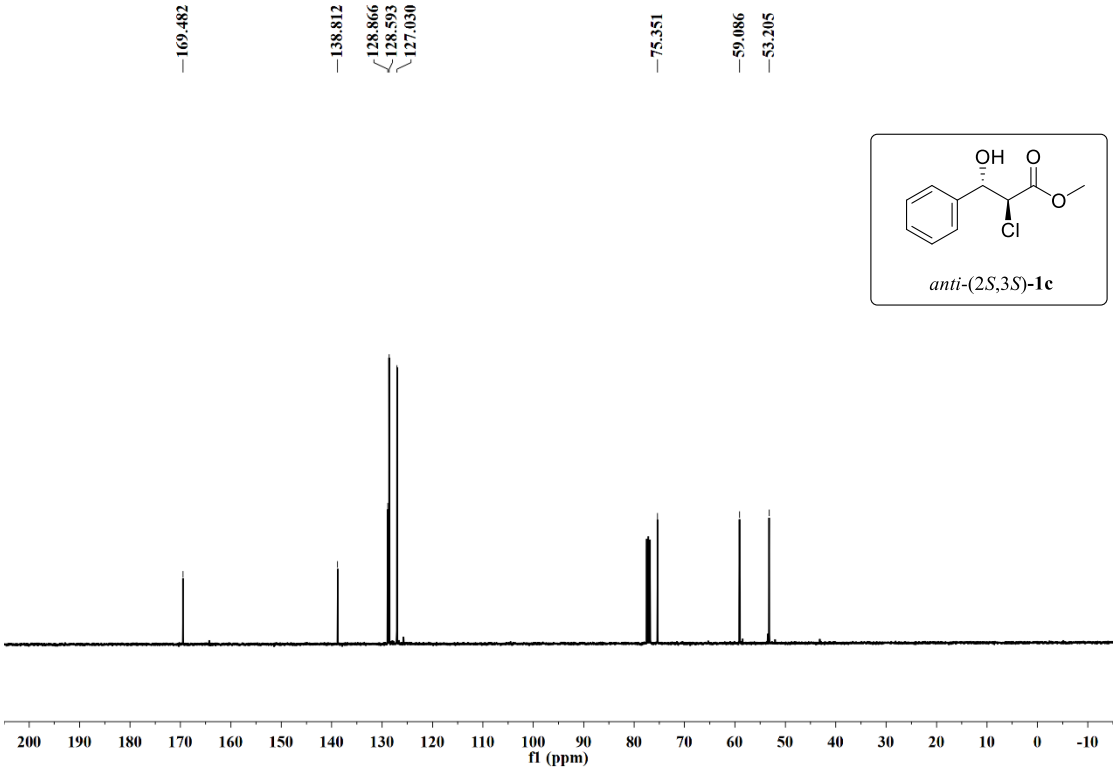
The ^{13}C NMR (100 MHz, $CDCl_3$) Spectrum of **1b**



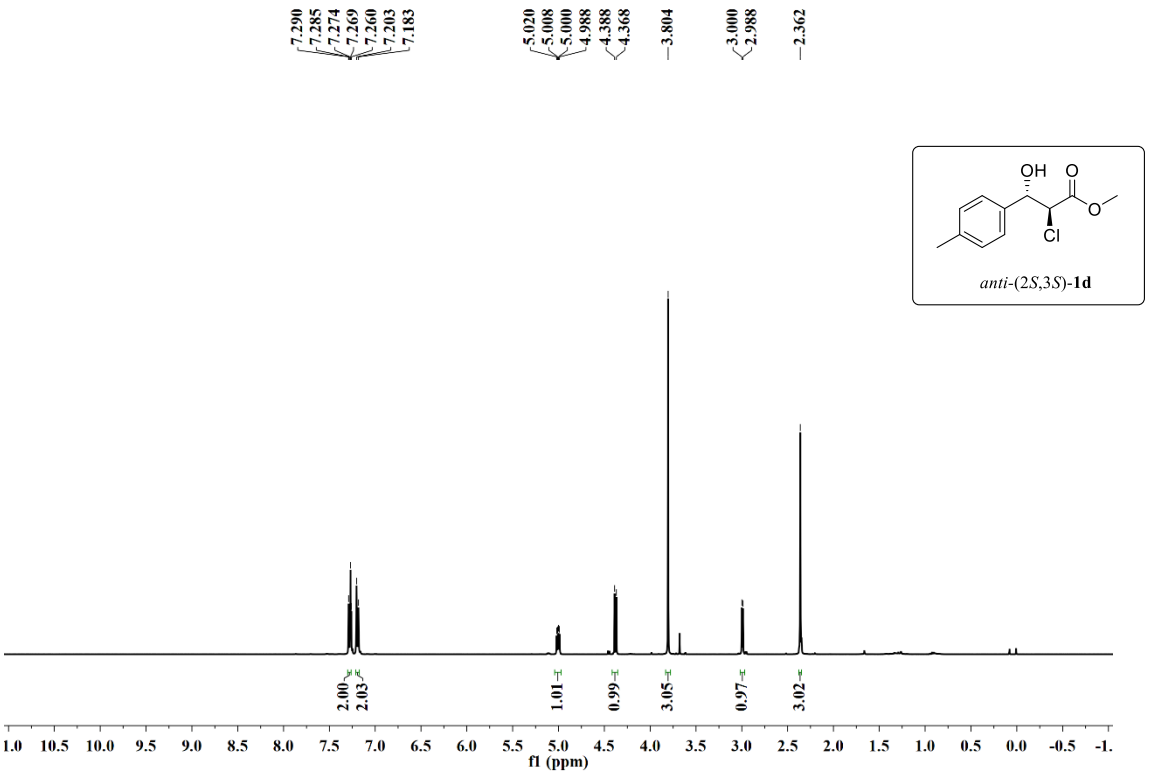
The ^{1}H NMR (400 MHz, $CDCl_3$) Spectrum of **1c**



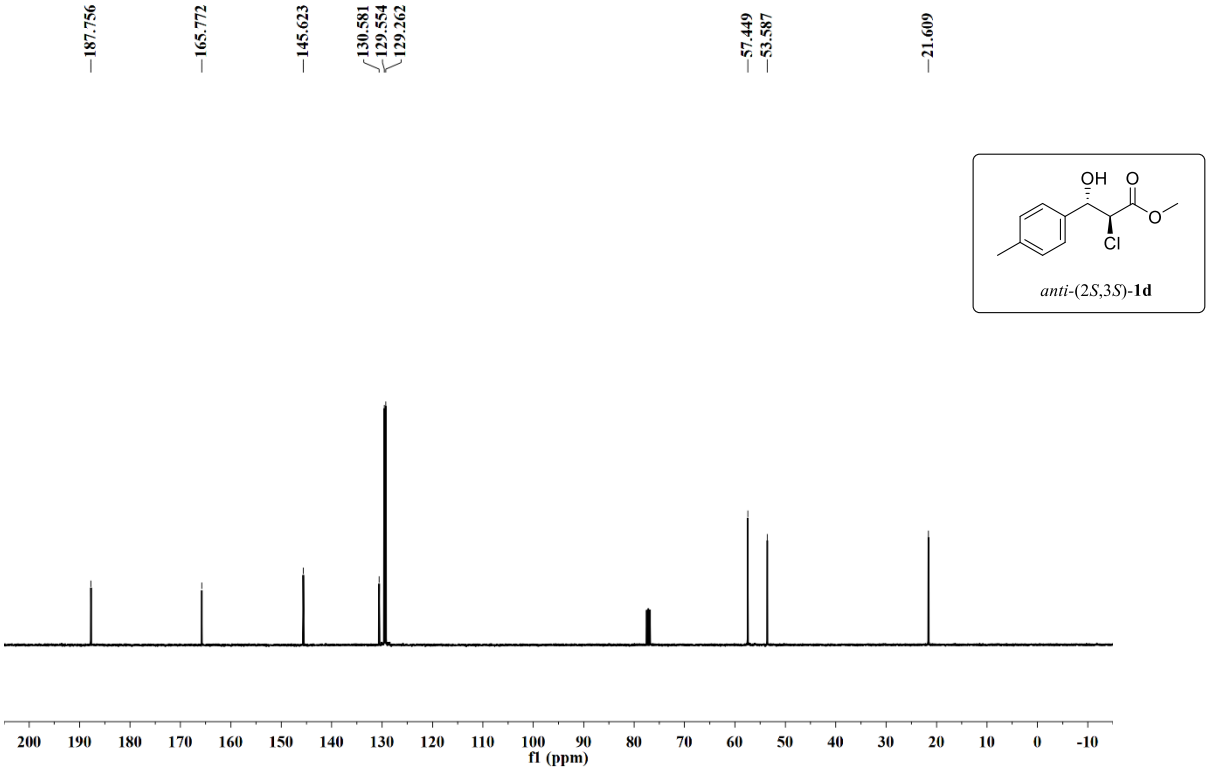
The ^{13}C NMR (100 MHz, $CDCl_3$) Spectrum of **1c**



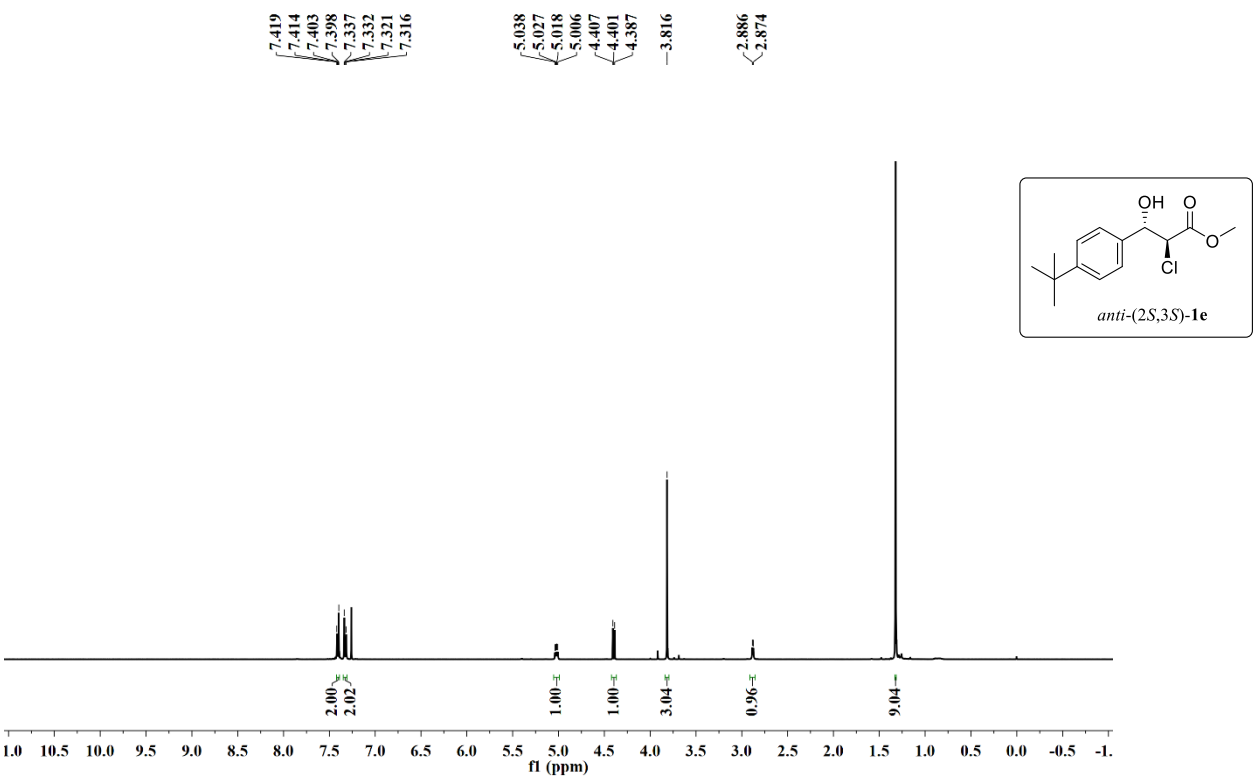
The ^{1}H NMR (400 MHz, $CDCl_3$) Spectrum of **1d**



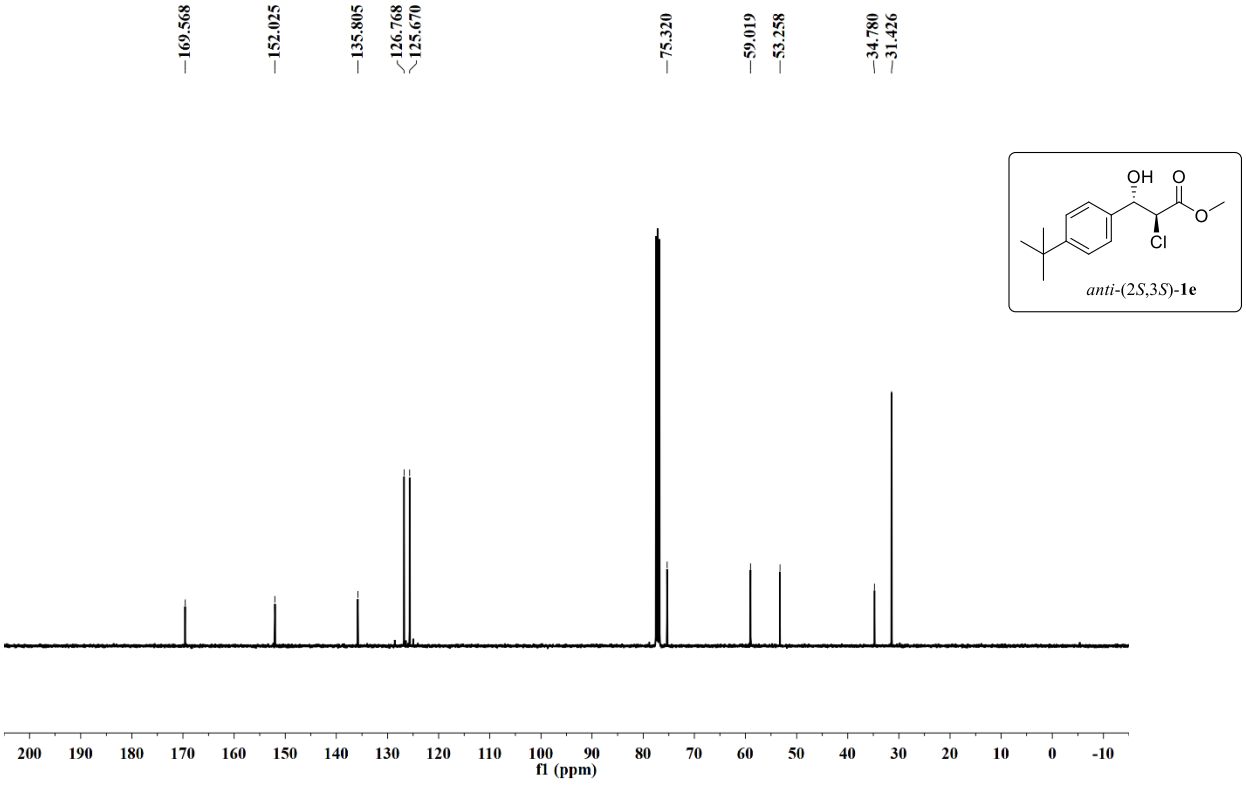
The ^{13}C NMR (100 MHz, $CDCl_3$) Spectrum of **1d**



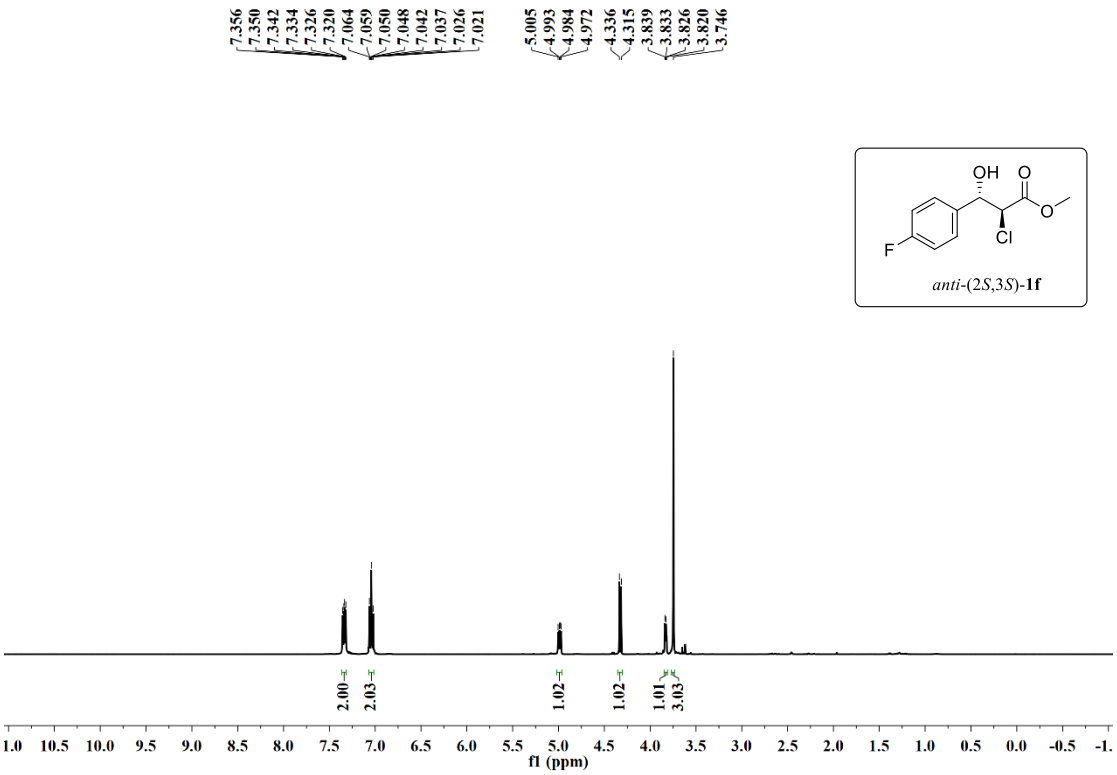
The ^{1}H NMR (400 MHz, $CDCl_3$) Spectrum of **1e**



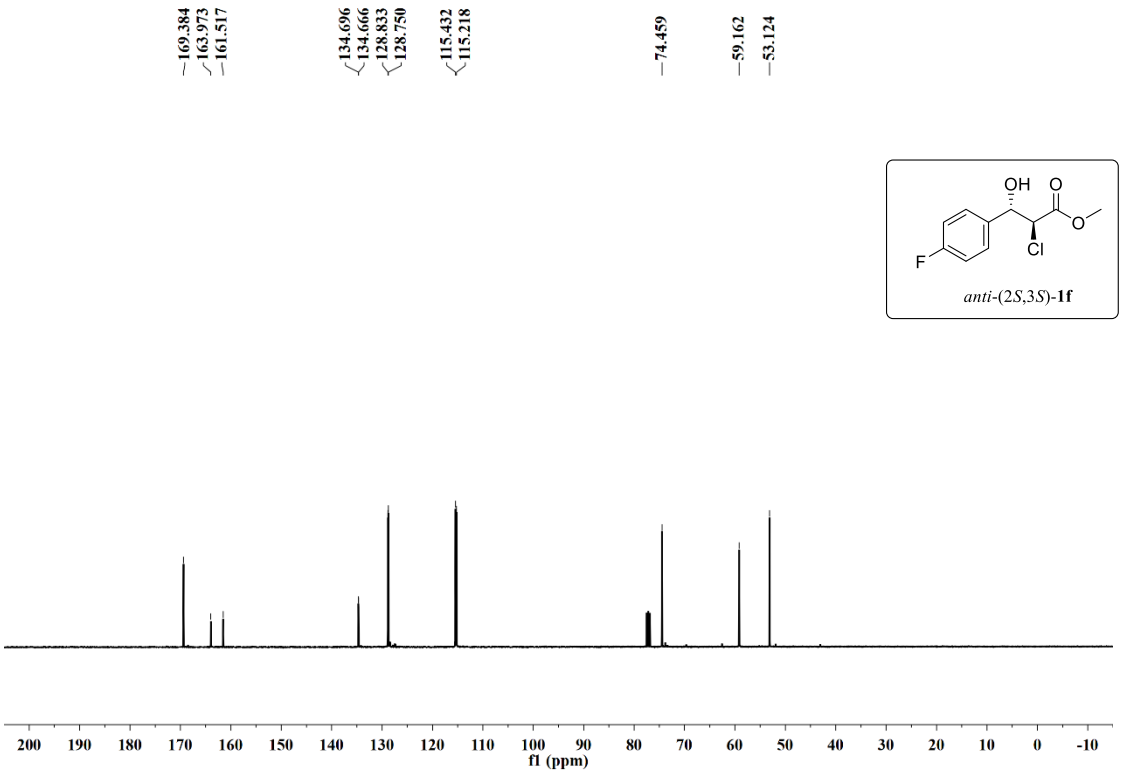
The ^{13}C NMR (100 MHz, $CDCl_3$) Spectrum of **1e**



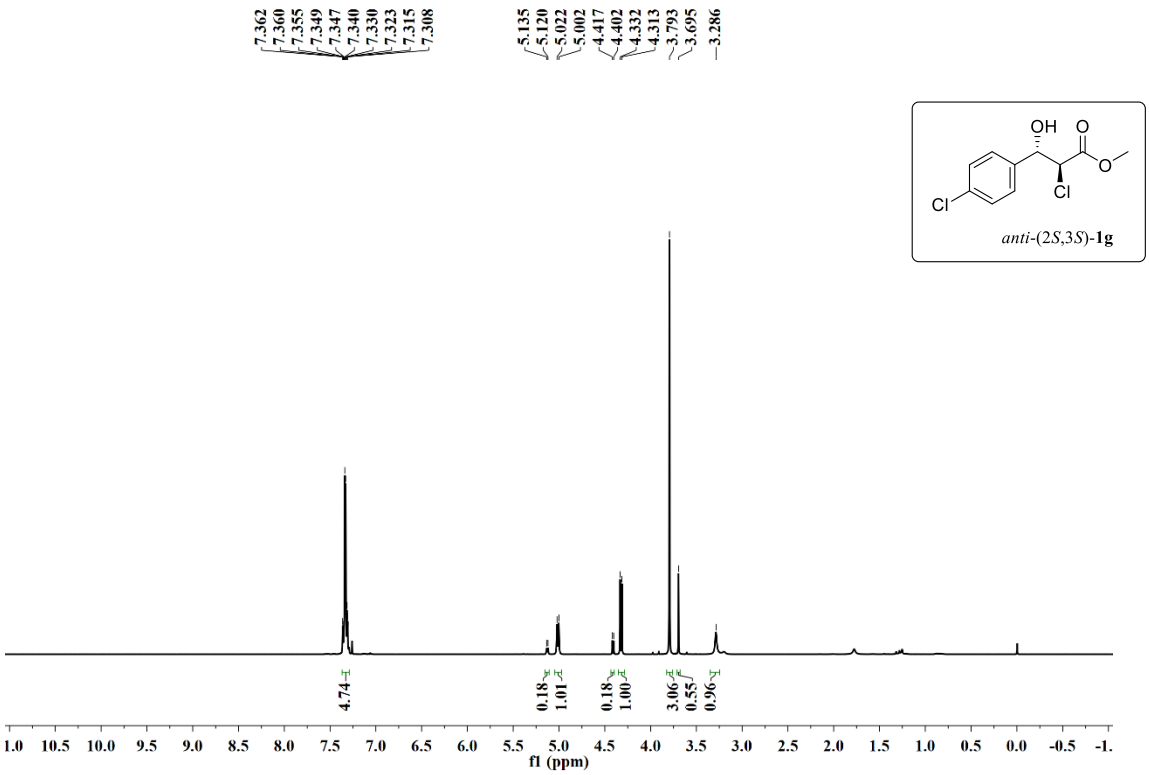
The ^{1}H NMR (400 MHz, $CDCl_3$) Spectrum of **1f**



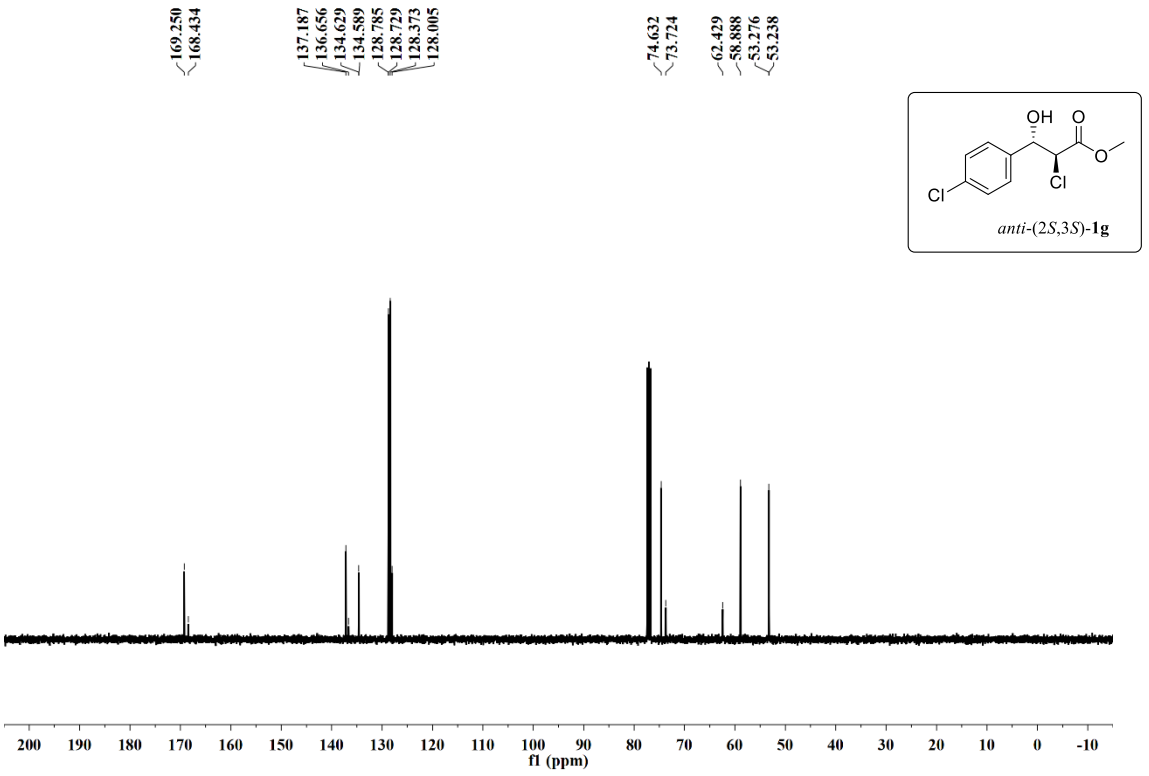
The ^{13}C NMR (100 MHz, $CDCl_3$) Spectrum of **1f**



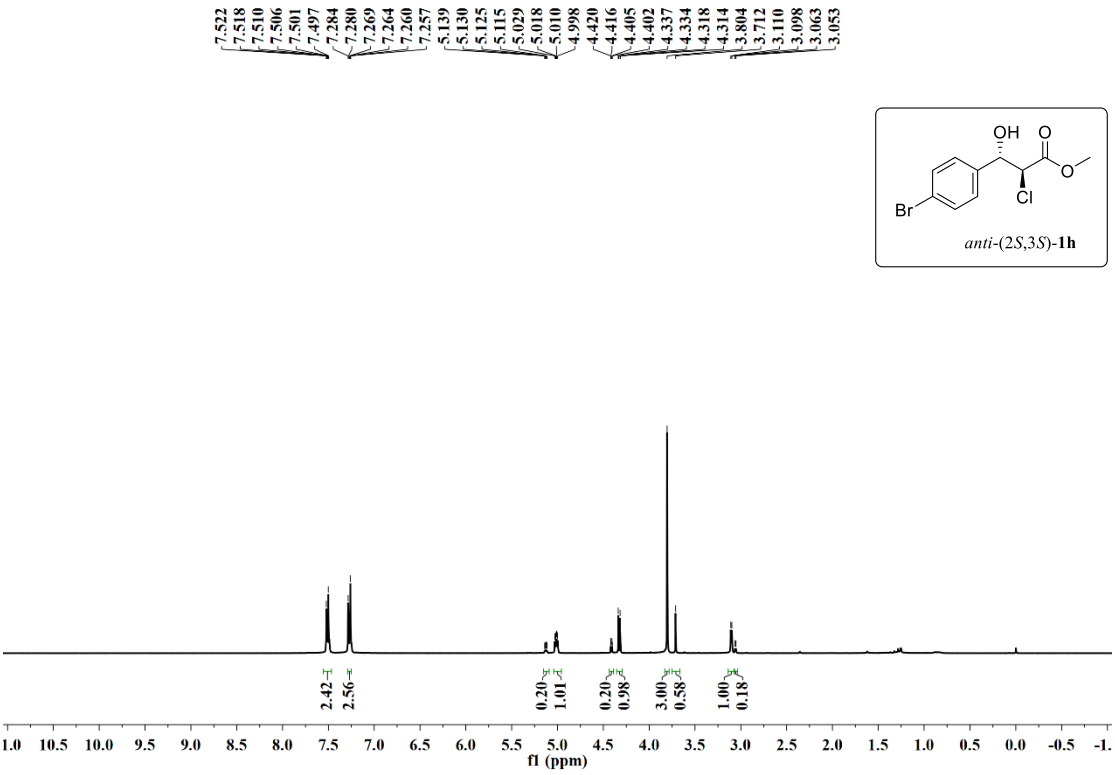
The ^{1}H NMR (400 MHz, $CDCl_3$) Spectrum of **1g**



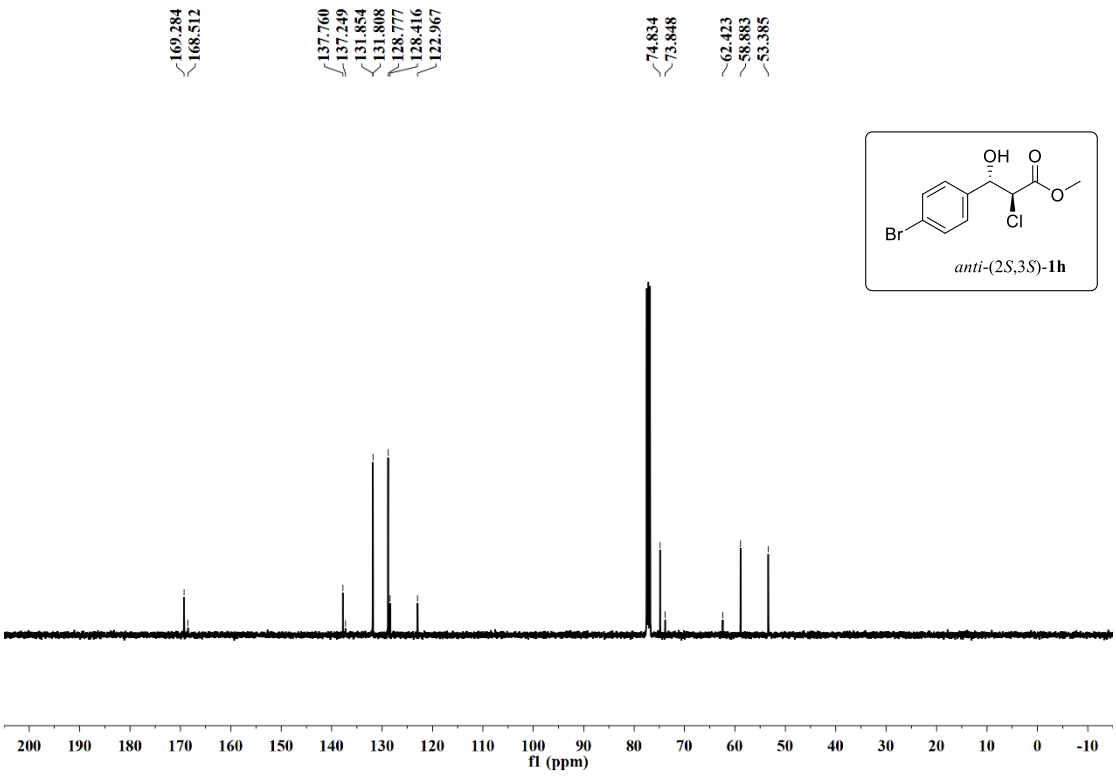
The ^{13}C NMR (100 MHz, $CDCl_3$) Spectrum of **1g**



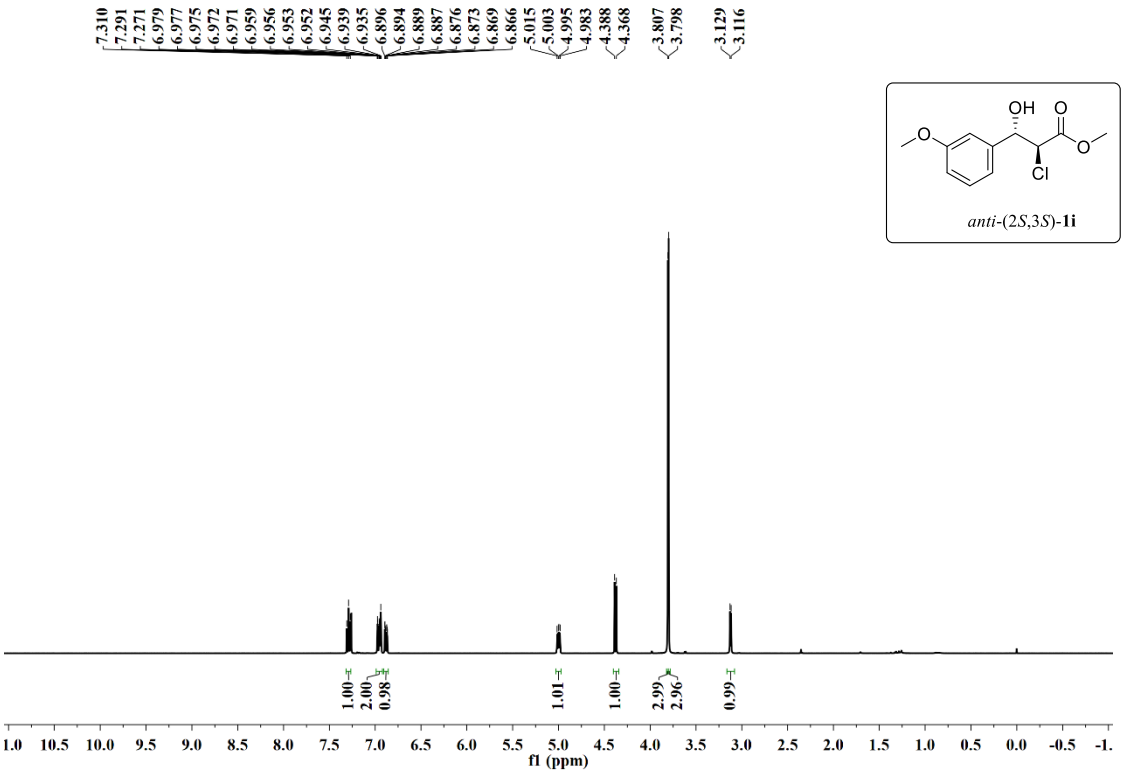
The ^{1}H NMR (400 MHz, $CDCl_3$) Spectrum of **1h**



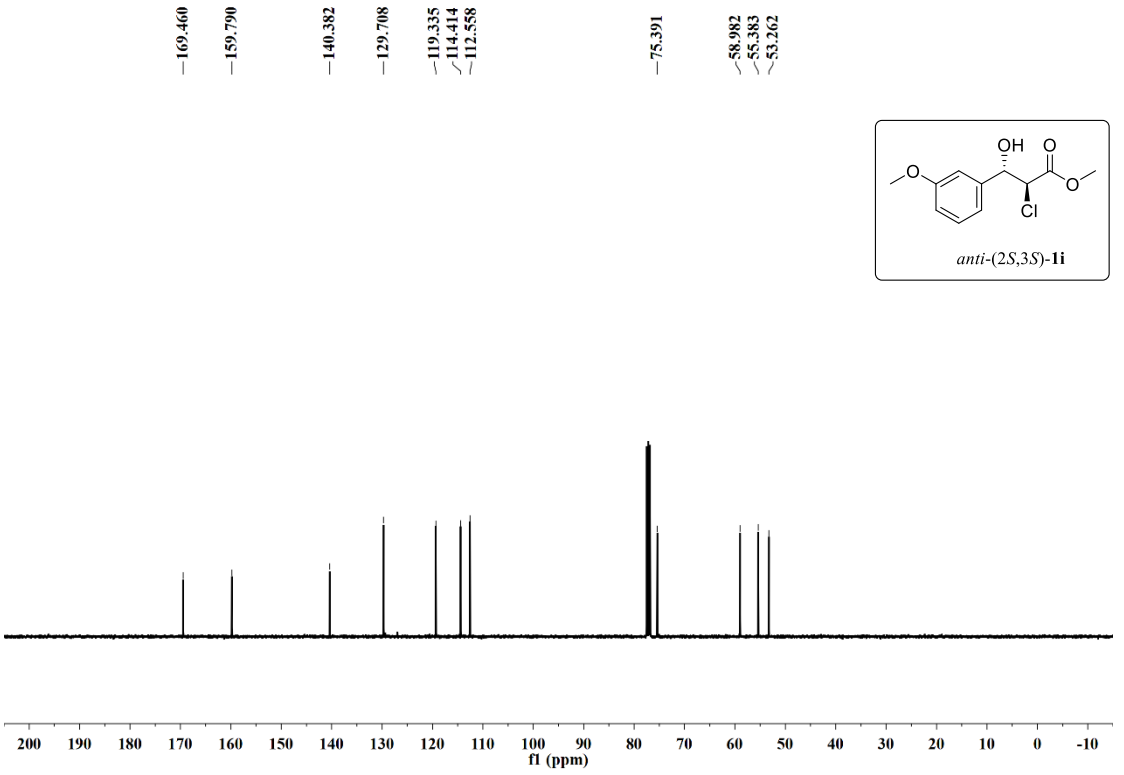
The ^{13}C NMR (100 MHz, $CDCl_3$) Spectrum of **1h**



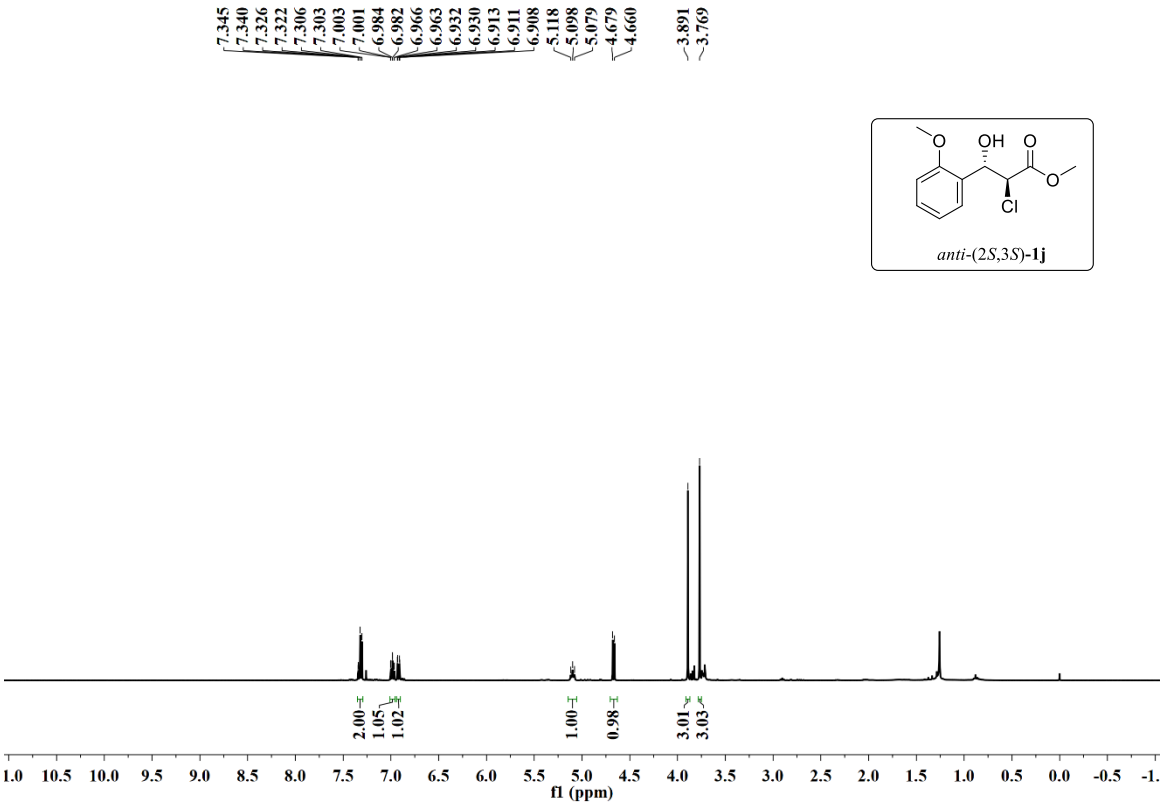
The ^{1}H NMR (400 MHz, $CDCl_3$) Spectrum of **1i**



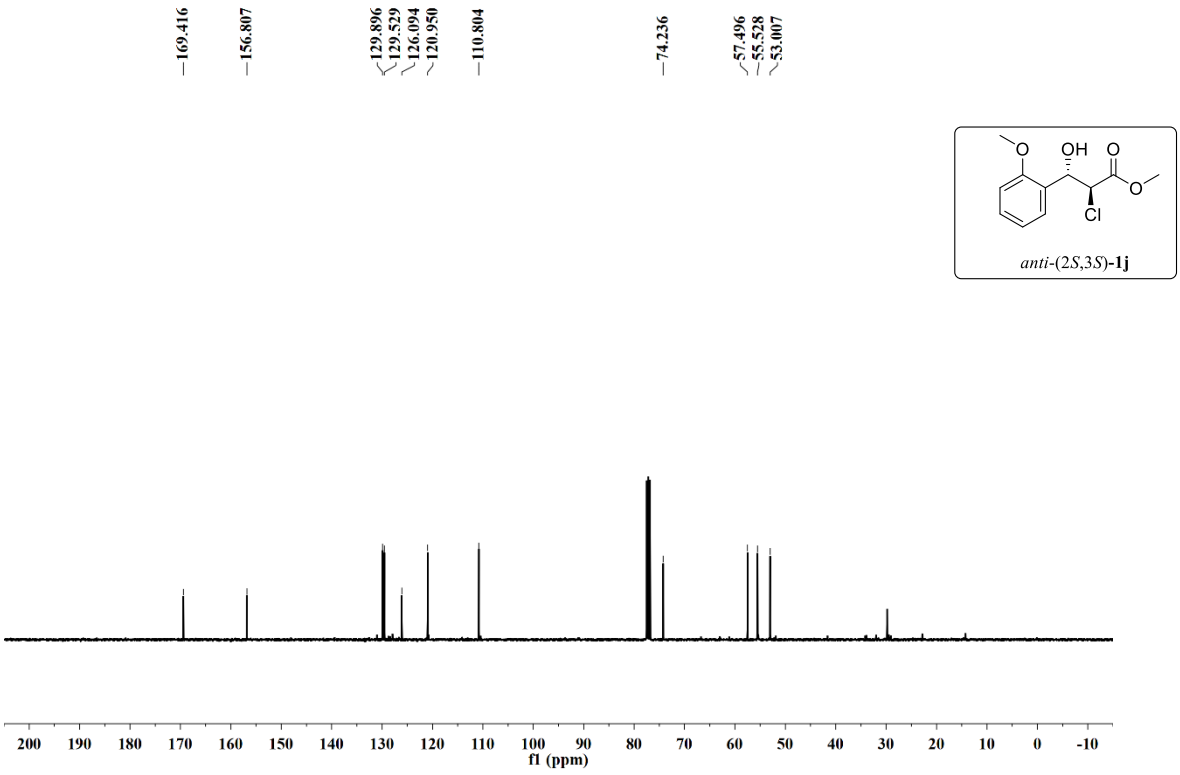
The ^{13}C NMR (100 MHz, $CDCl_3$) Spectrum of **1i**



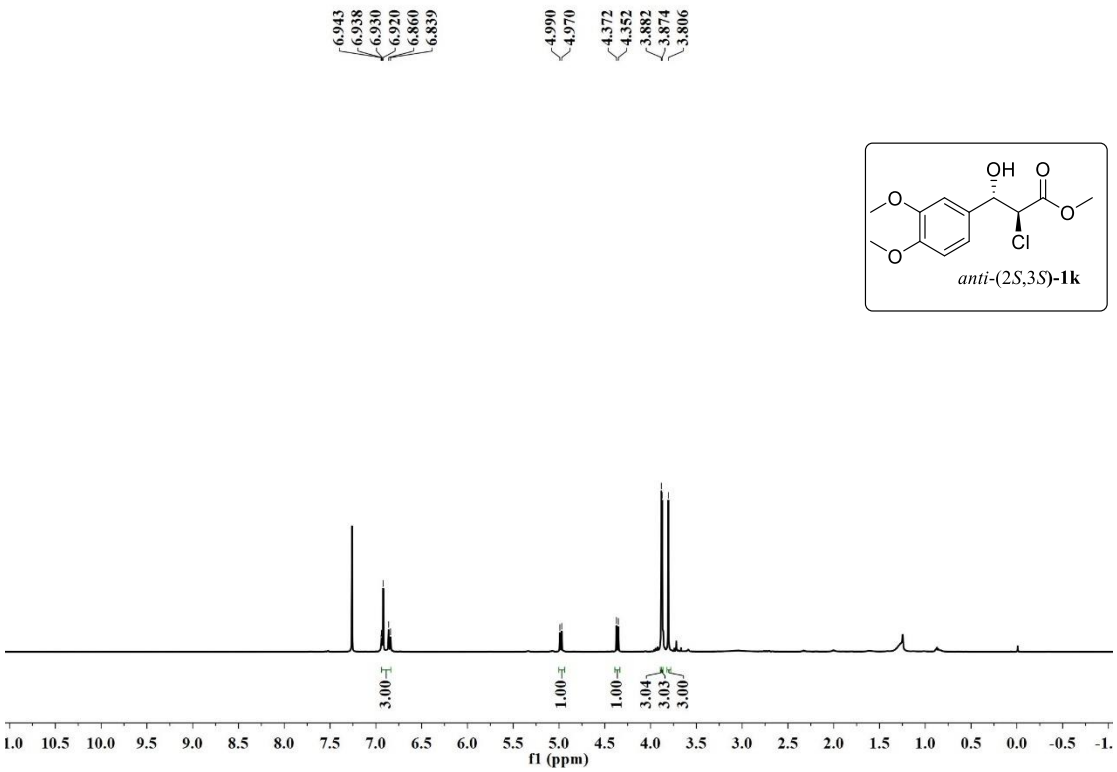
The ^{1}H NMR (400 MHz, $CDCl_3$) Spectrum of **1j**



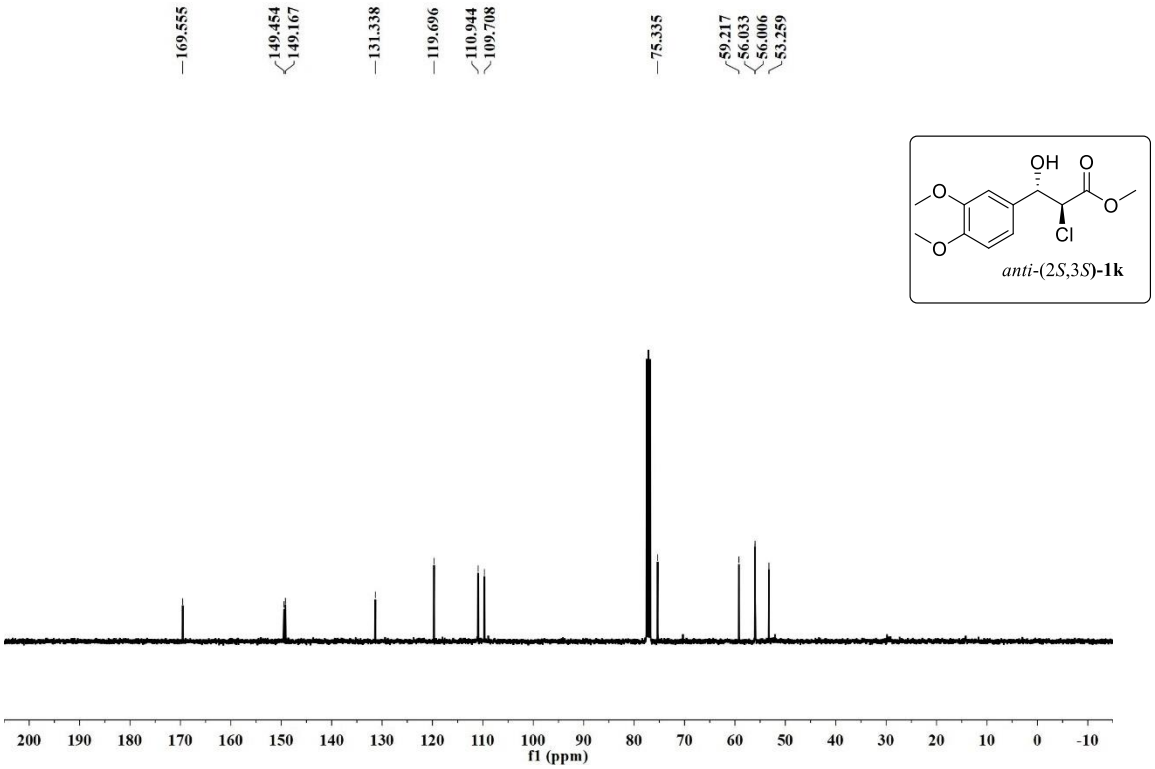
The ^{13}C NMR (100 MHz, $CDCl_3$) Spectrum of **1j**



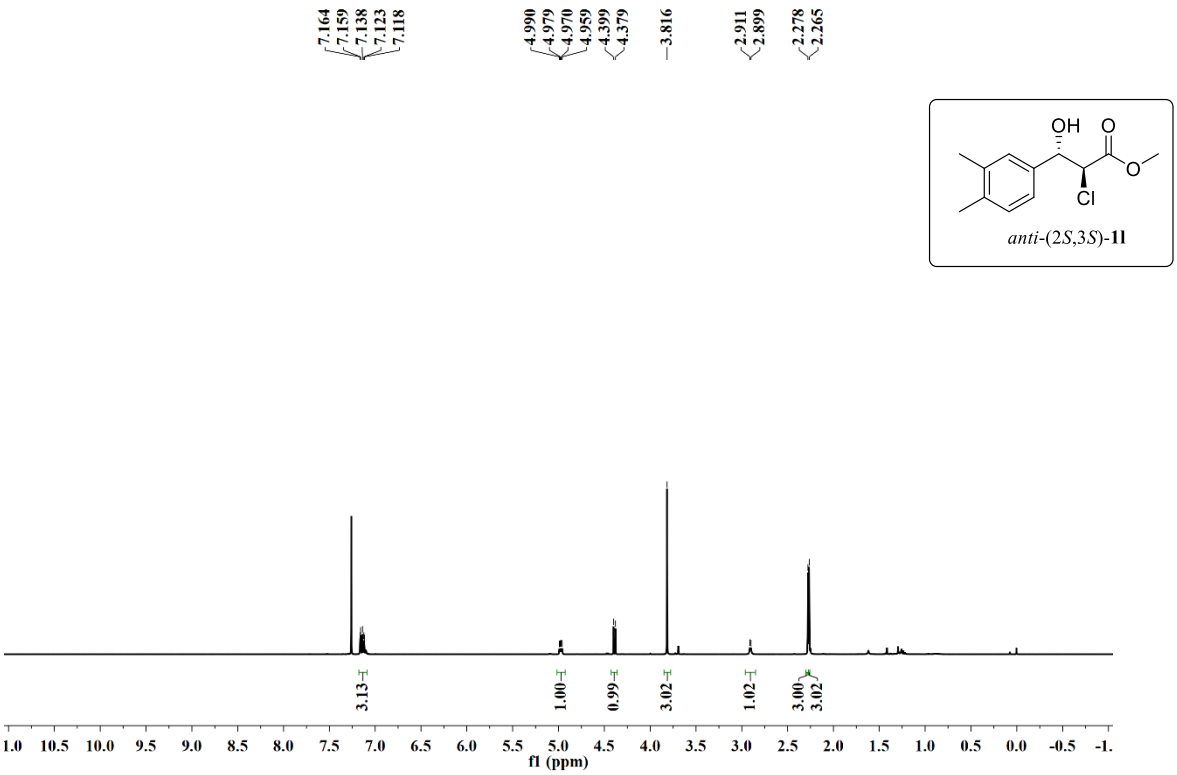
The ^{1}H NMR (400 MHz, $CDCl_3$) Spectrum of **1k**



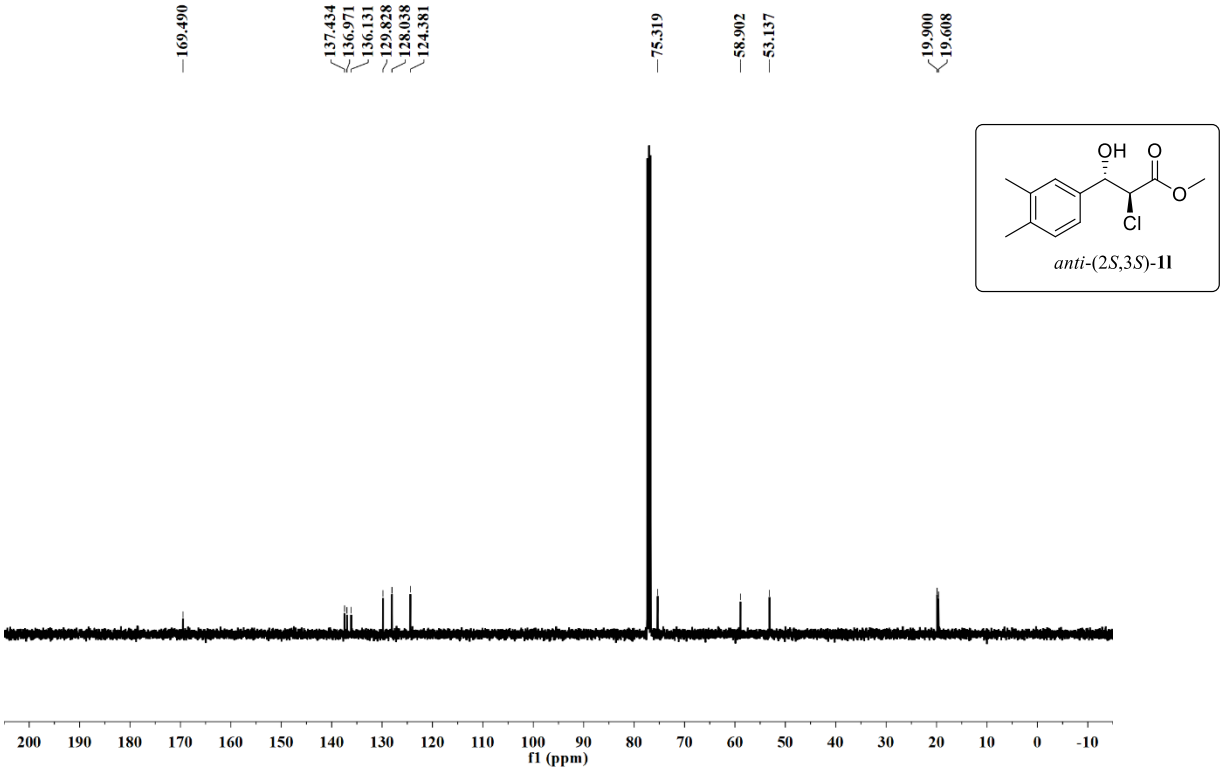
The ^{13}C NMR (100 MHz, $CDCl_3$) Spectrum of **1k**



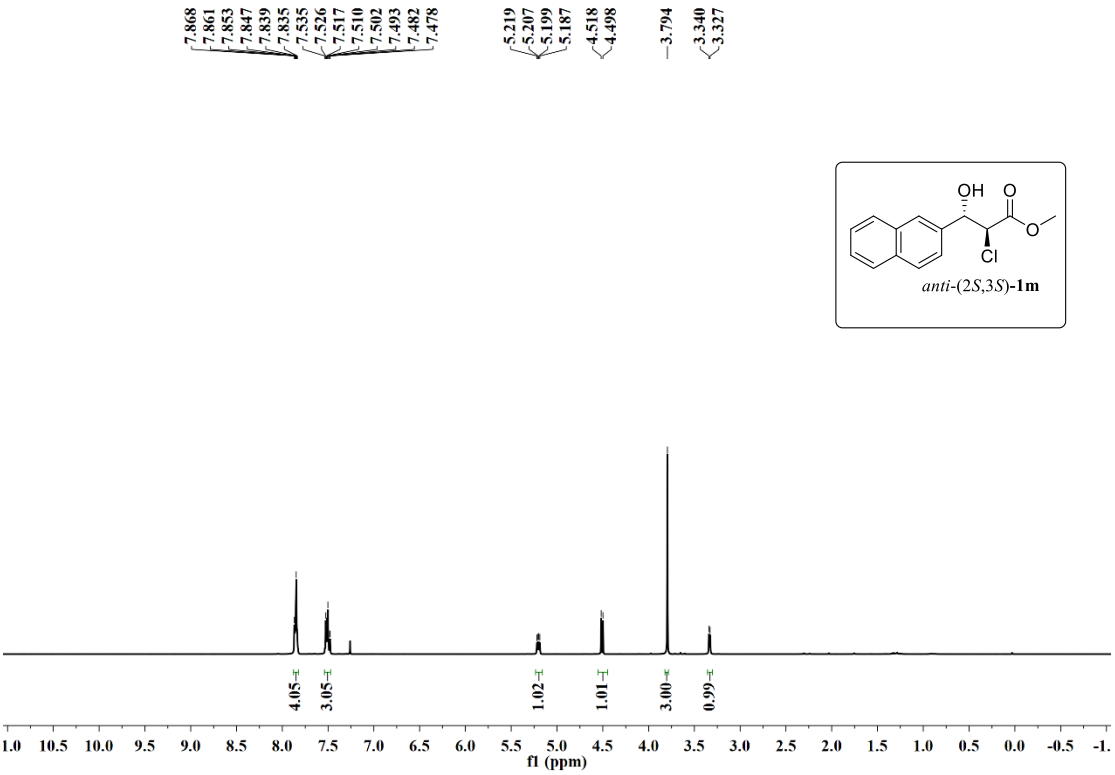
The ^{1}H NMR (400 MHz, $CDCl_3$) Spectrum of **1l**



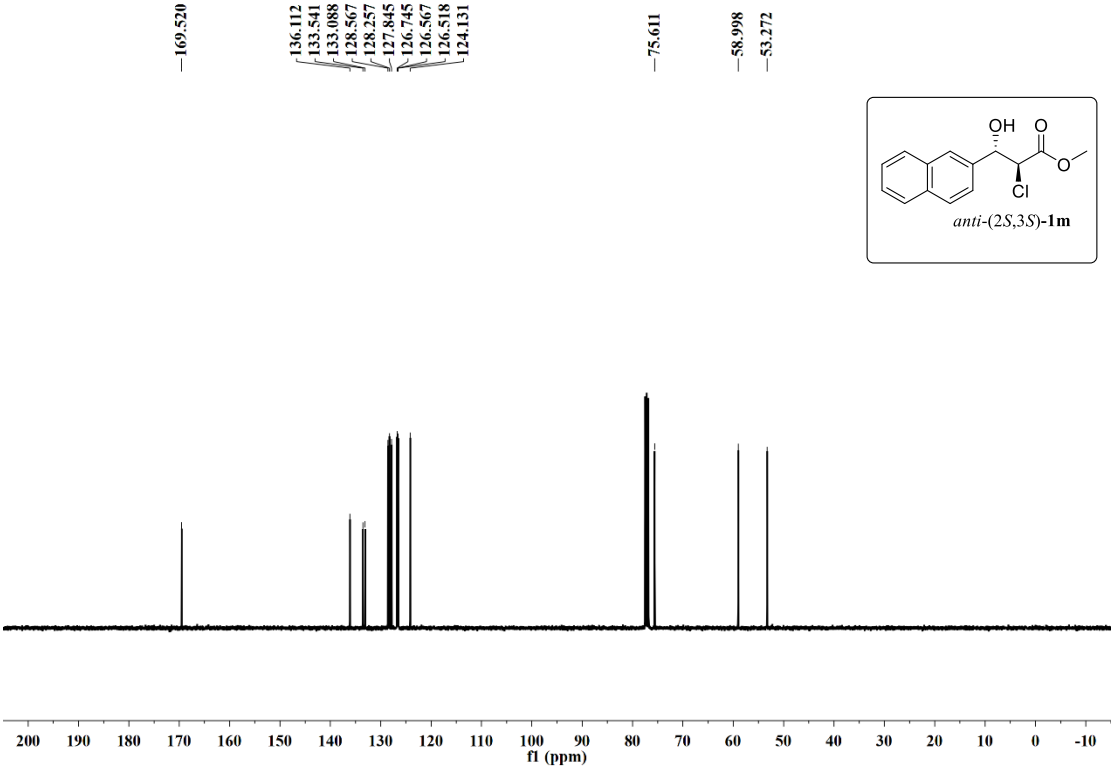
The ^{13}C NMR (100 MHz, $CDCl_3$) Spectrum of **1l**



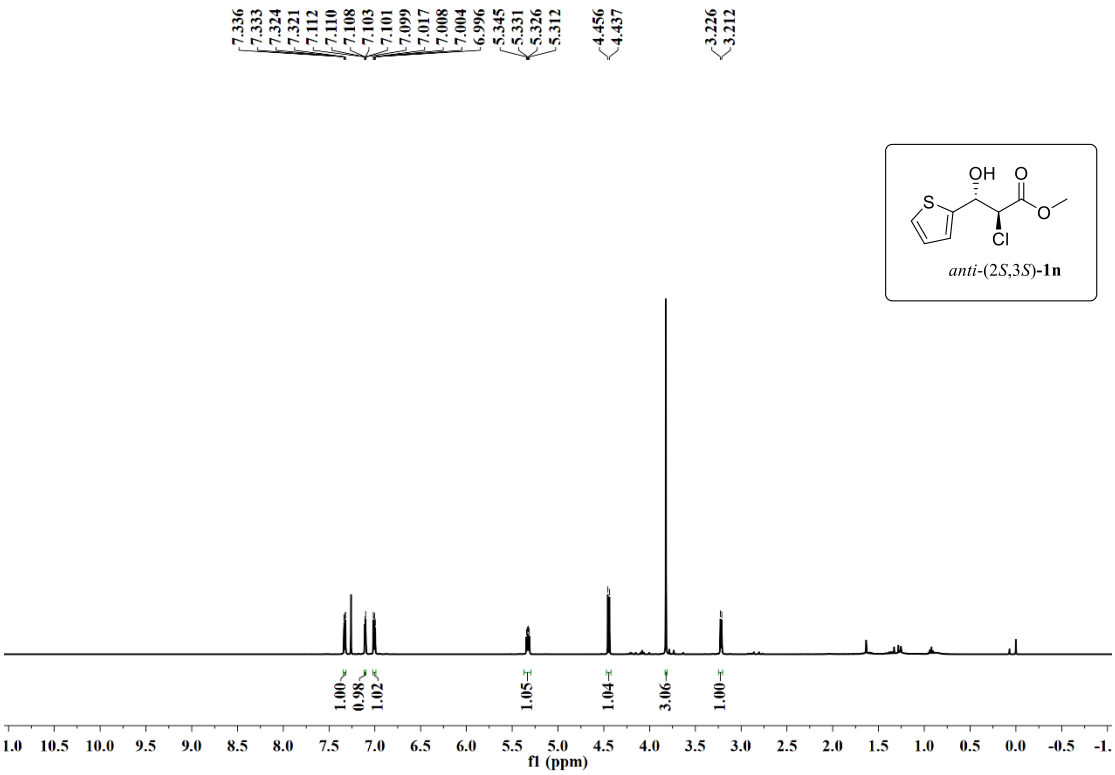
The ^{1}H NMR (400 MHz, $CDCl_3$) Spectrum of **1m**



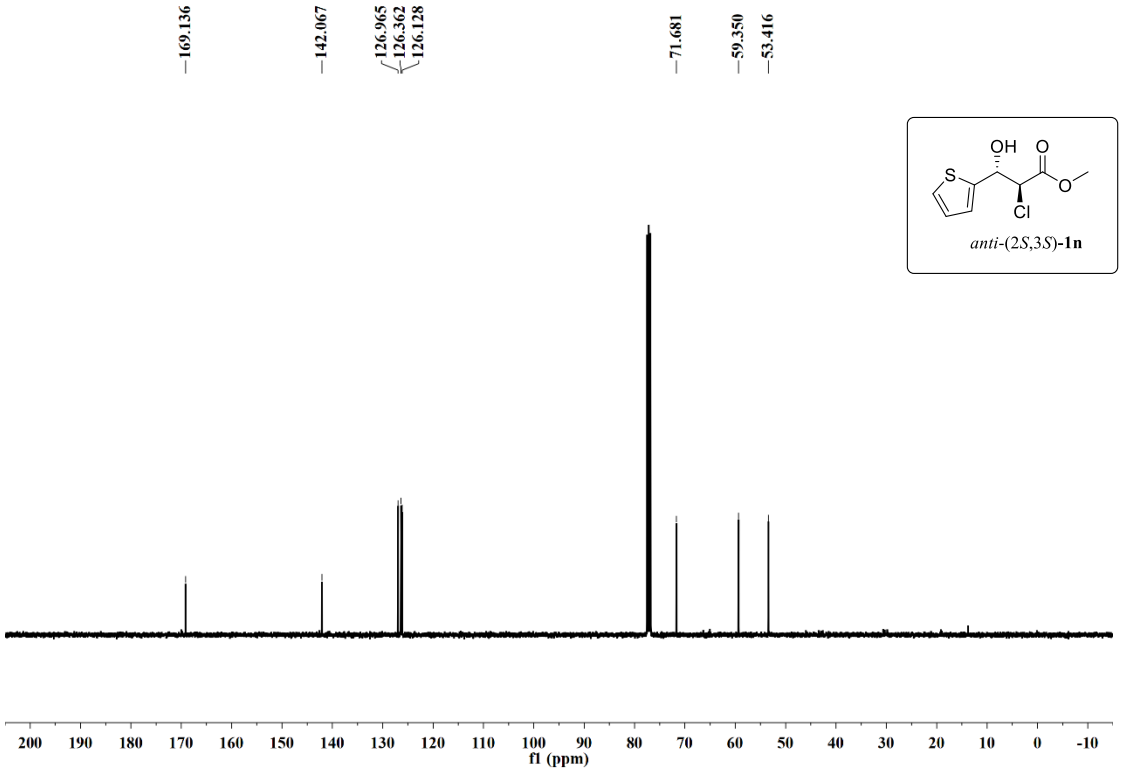
The ^{13}C NMR (100 MHz, $CDCl_3$) Spectrum of **1m**



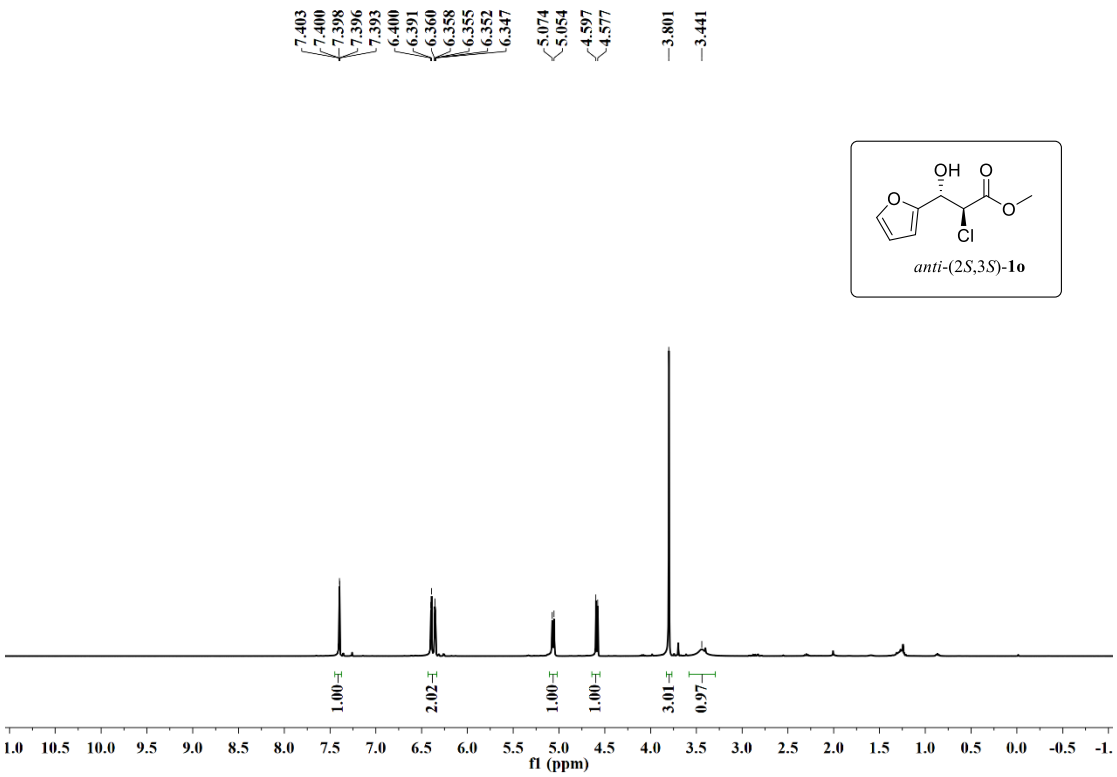
The ^{1}H NMR (400 MHz, $CDCl_3$) Spectrum of **1n**



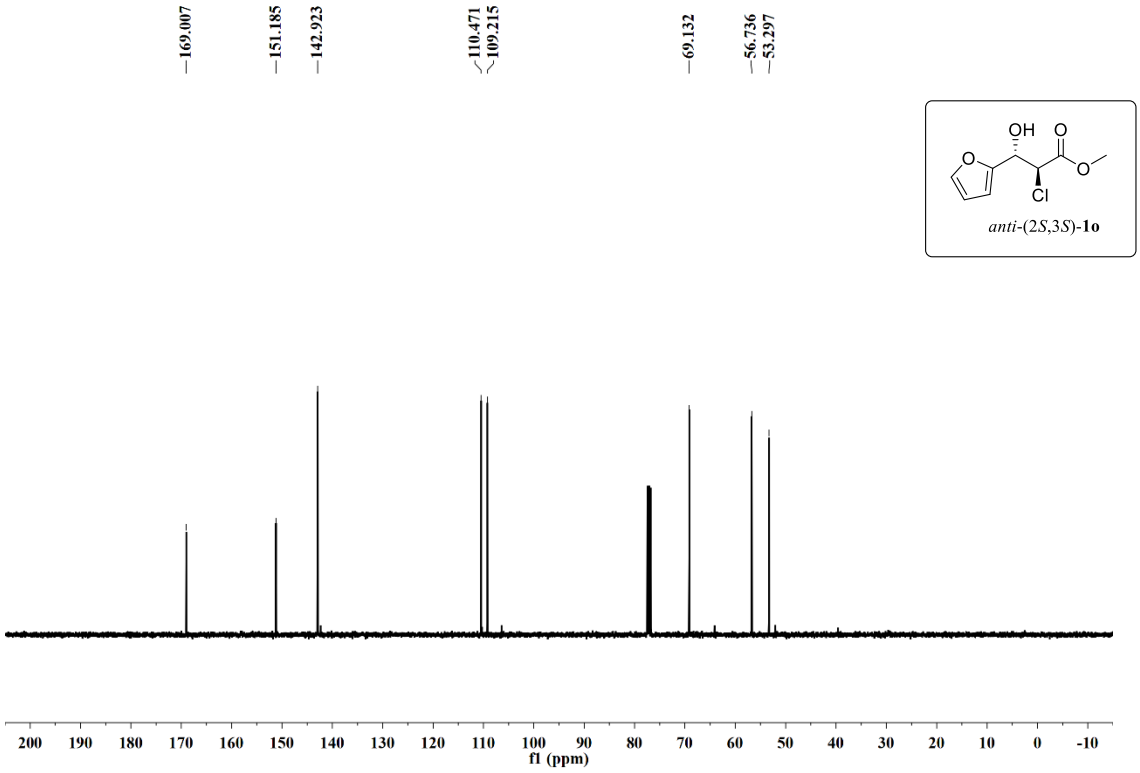
The ^{13}C NMR (100 MHz, $CDCl_3$) Spectrum of **1n**



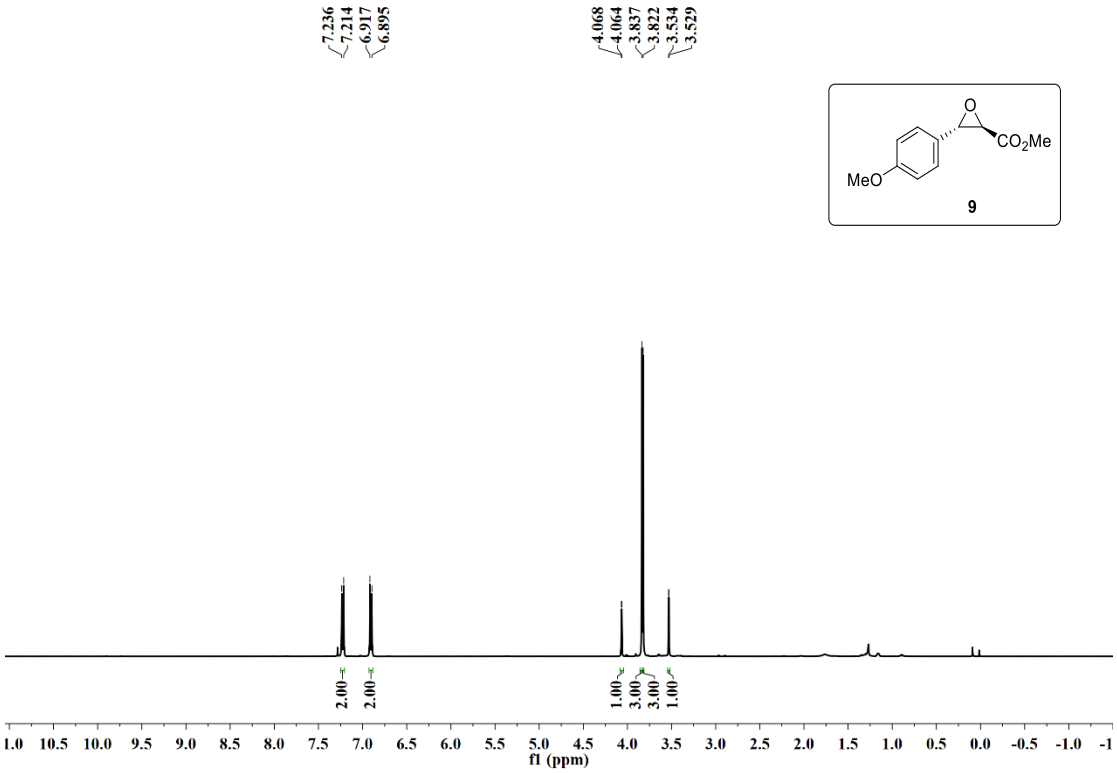
The ^{1}H NMR (400 MHz, $CDCl_3$) Spectrum of **1o**



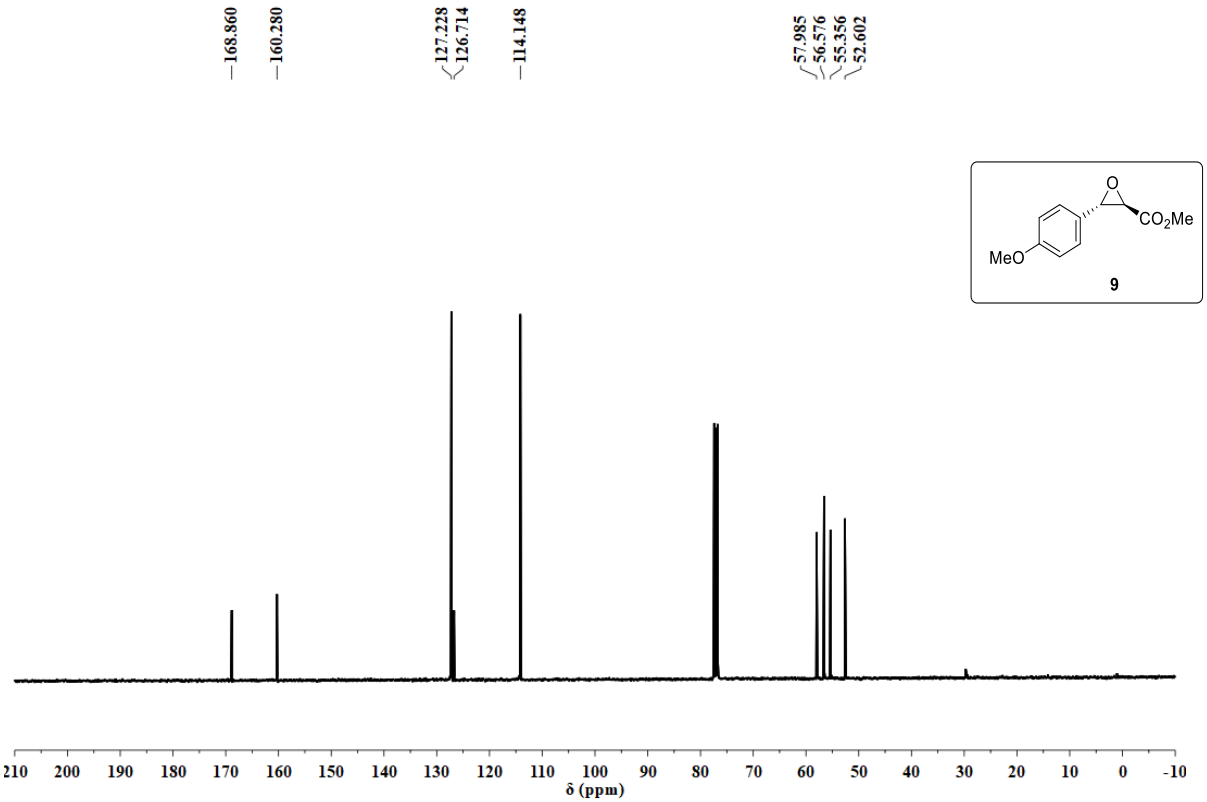
The ^{13}C NMR (100 MHz, $CDCl_3$) Spectrum of **1o**



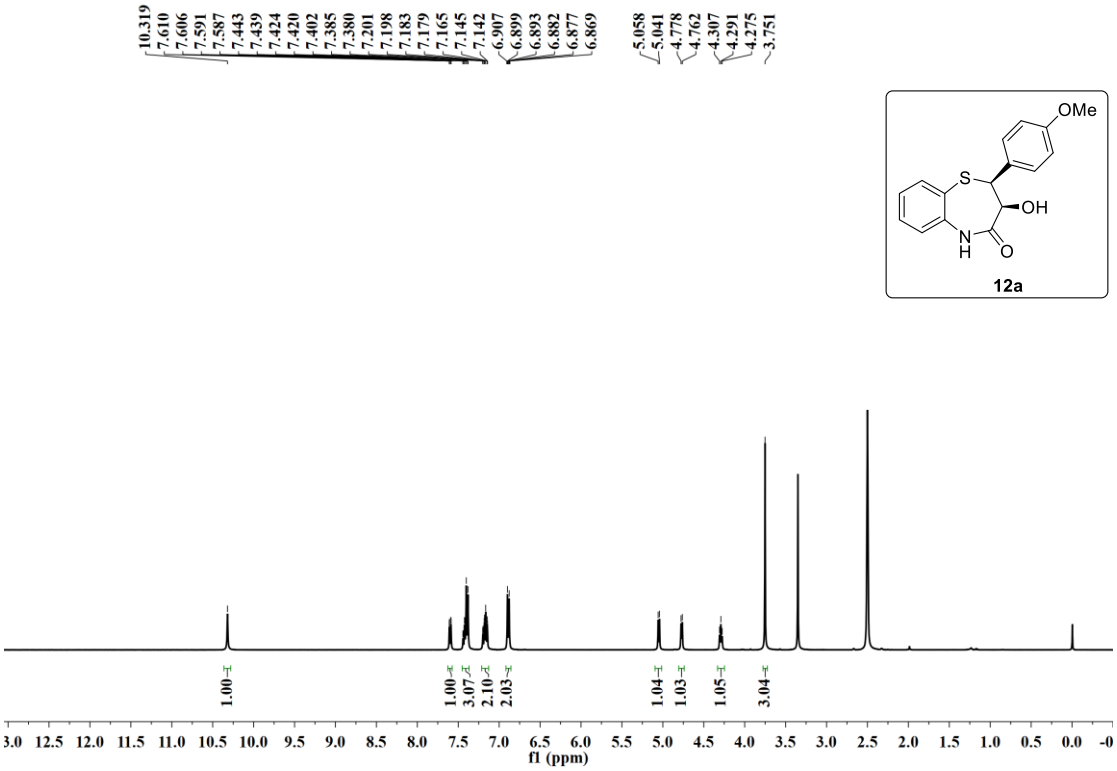
The ^{1}H NMR (400 MHz, $CDCl_3$) Spectrum of **9**



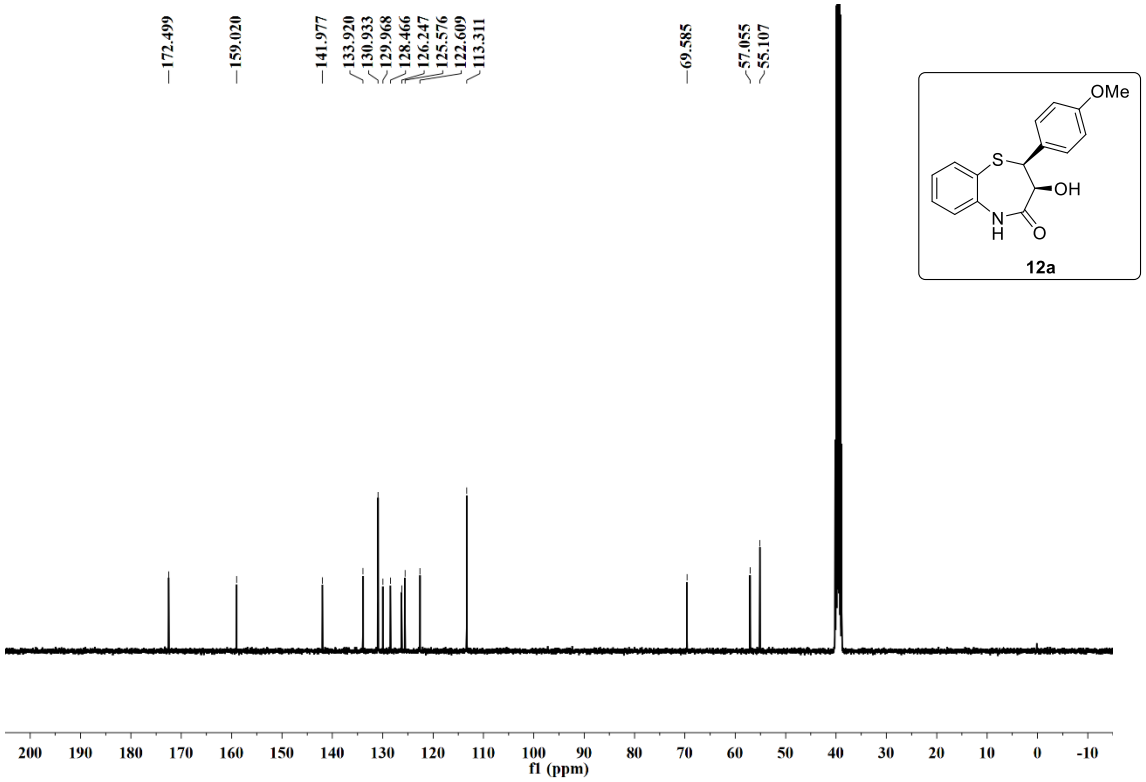
The ^{13}C NMR (100 MHz, $CDCl_3$) Spectrum of **9**



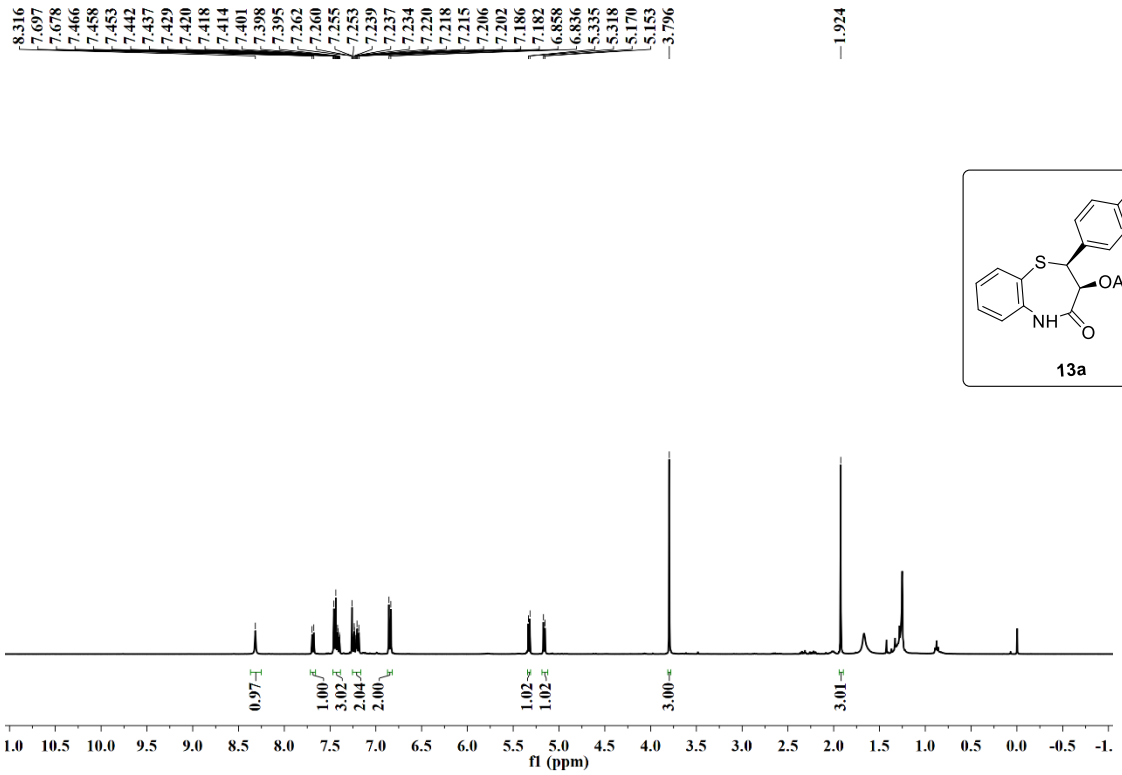
The ^{1}H NMR (400 MHz, DMSO-d_6) Spectrum of **12a**



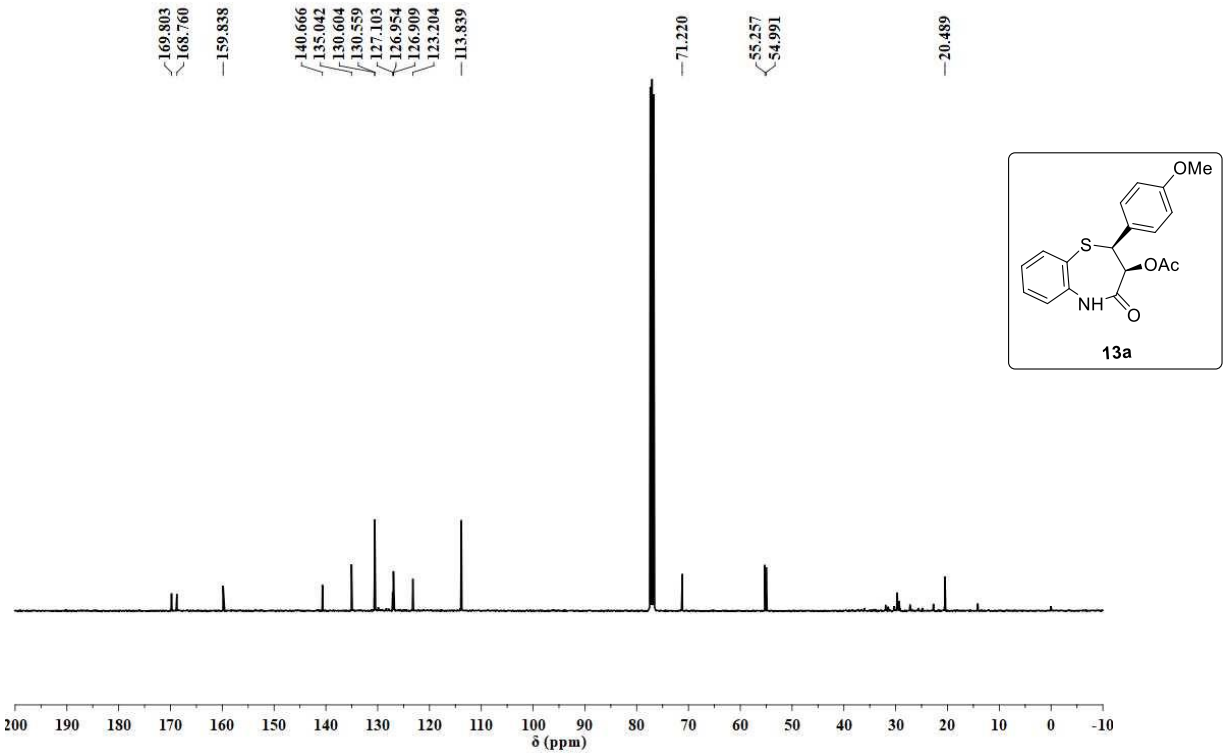
The ^{13}C NMR (100 MHz, DMSO-d_6) Spectrum of **12a**



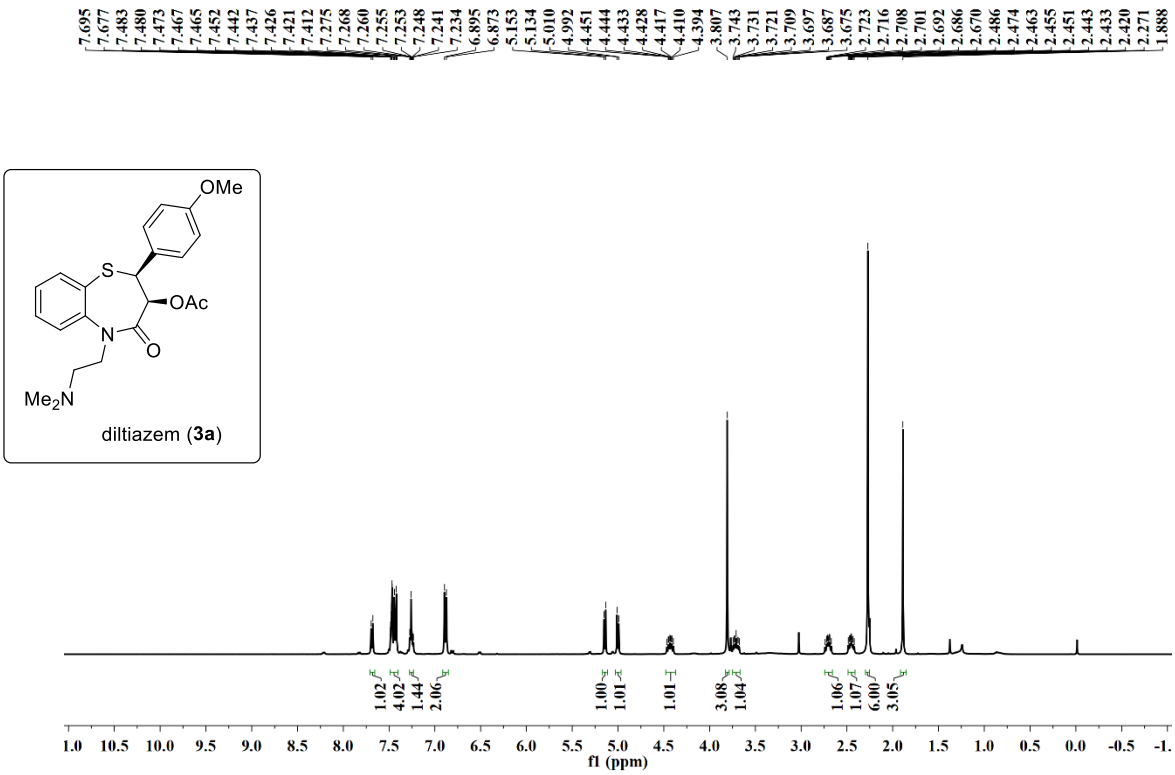
The ^{1}H NMR (400 MHz, $CDCl_3$) Spectrum of **13a**



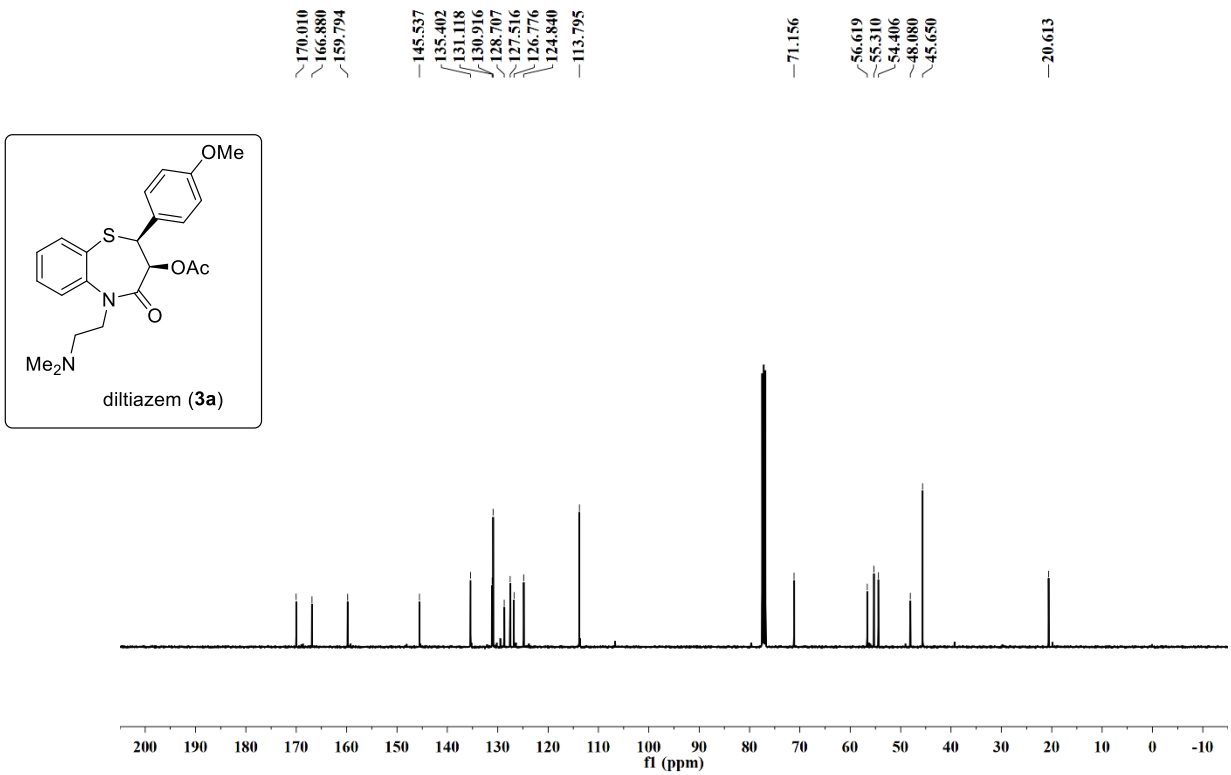
The ^{13}C NMR (100 MHz, $CDCl_3$) Spectrum of **13a**



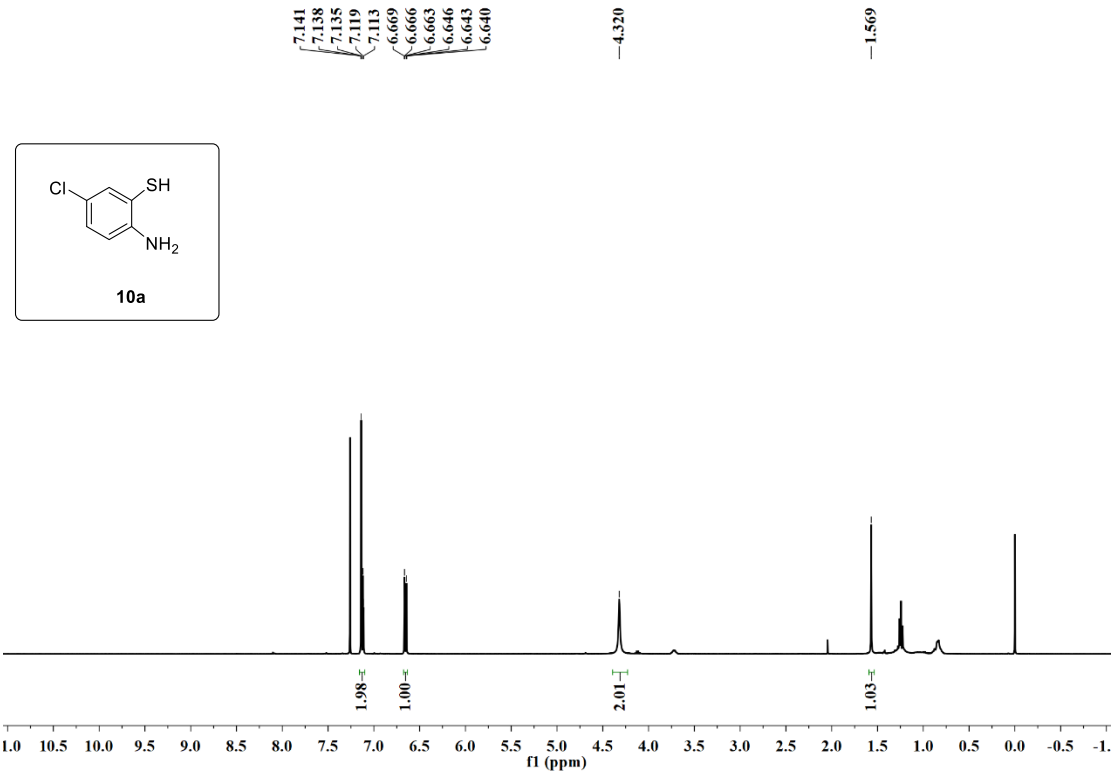
The ^{1}H NMR (400 MHz, $CDCl_3$) Spectrum of **3**



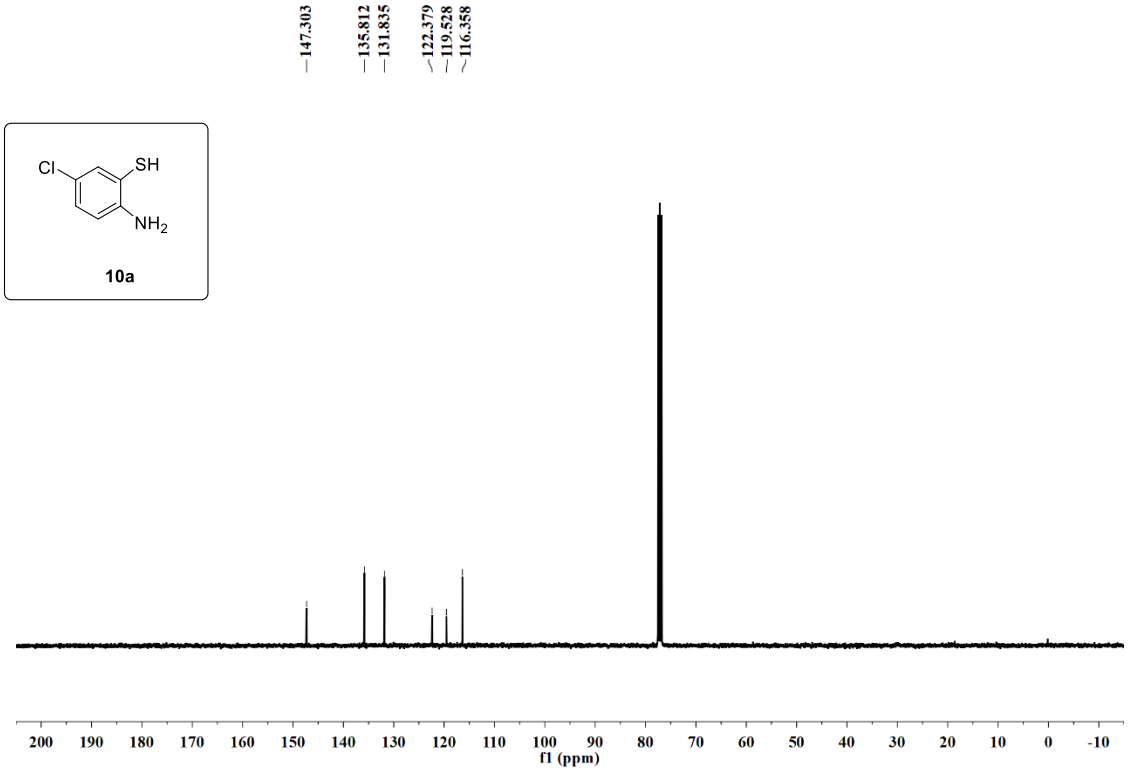
The ^{13}C NMR (100 MHz, $CDCl_3$) Spectrum of **3**



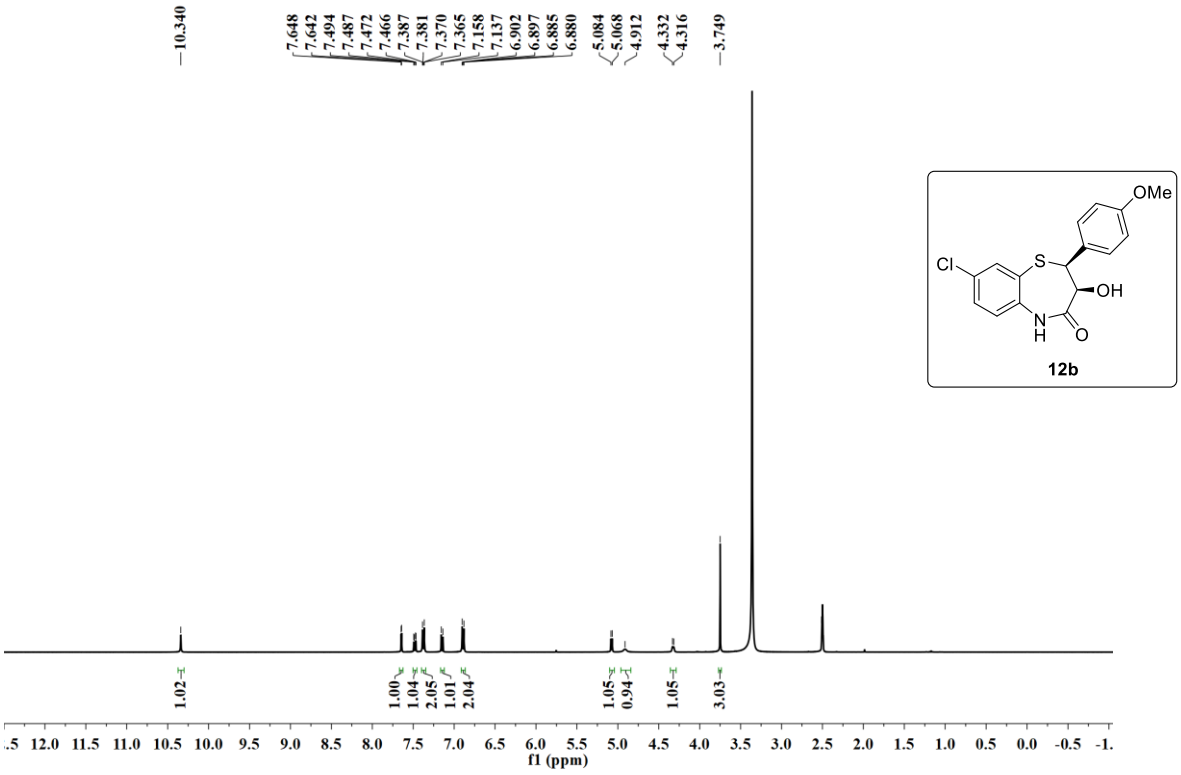
The ^{1}H NMR (400 MHz, $CDCl_3$) Spectrum of **10a**



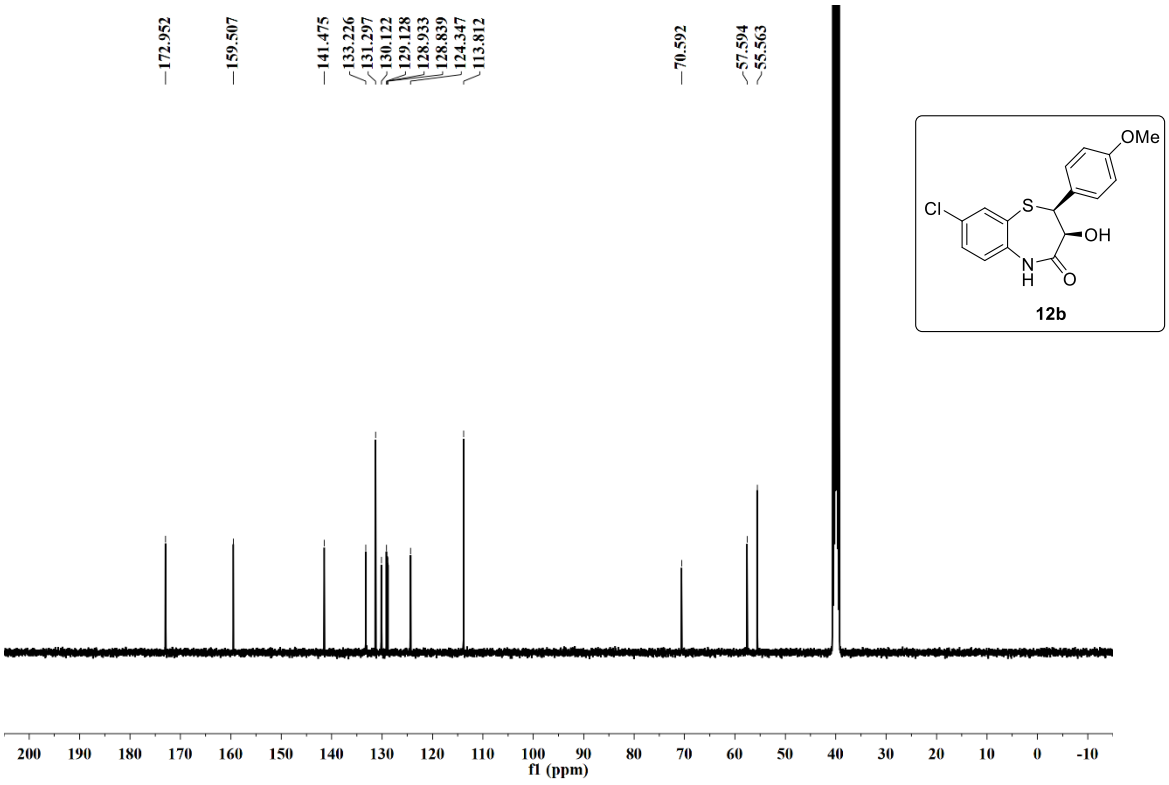
The ^{13}C NMR (100 MHz, $CDCl_3$) Spectrum of **10a**



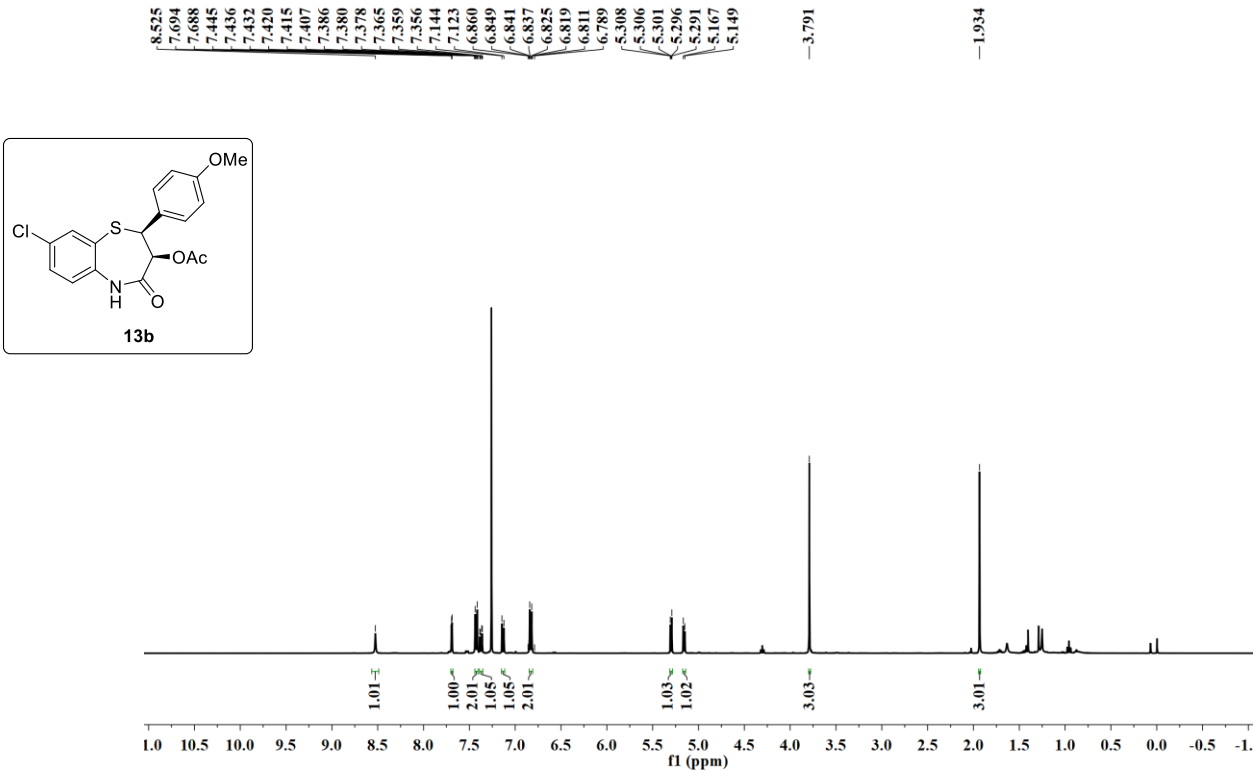
The ^{1}H NMR (400 MHz, DMSO-d_6) Spectrum of **12b**



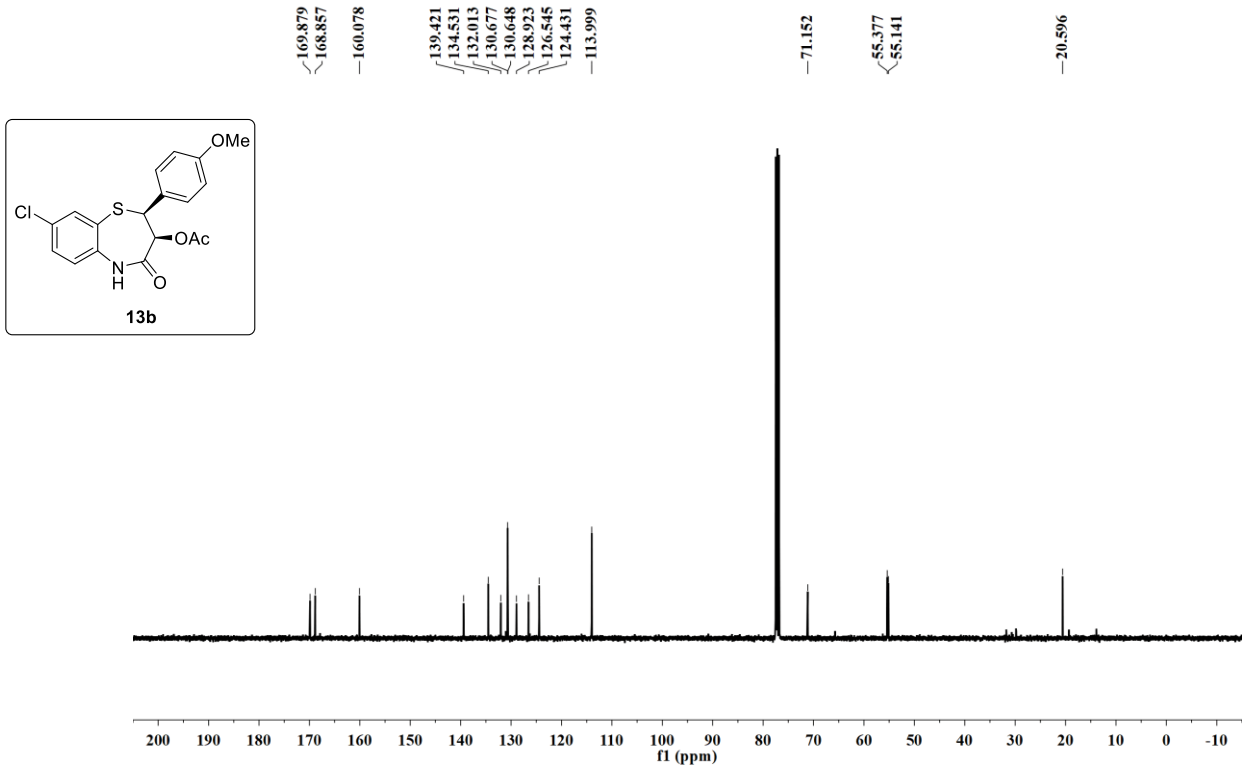
The ^{13}C NMR (100 MHz, DMSO-d_6) Spectrum of **12b**



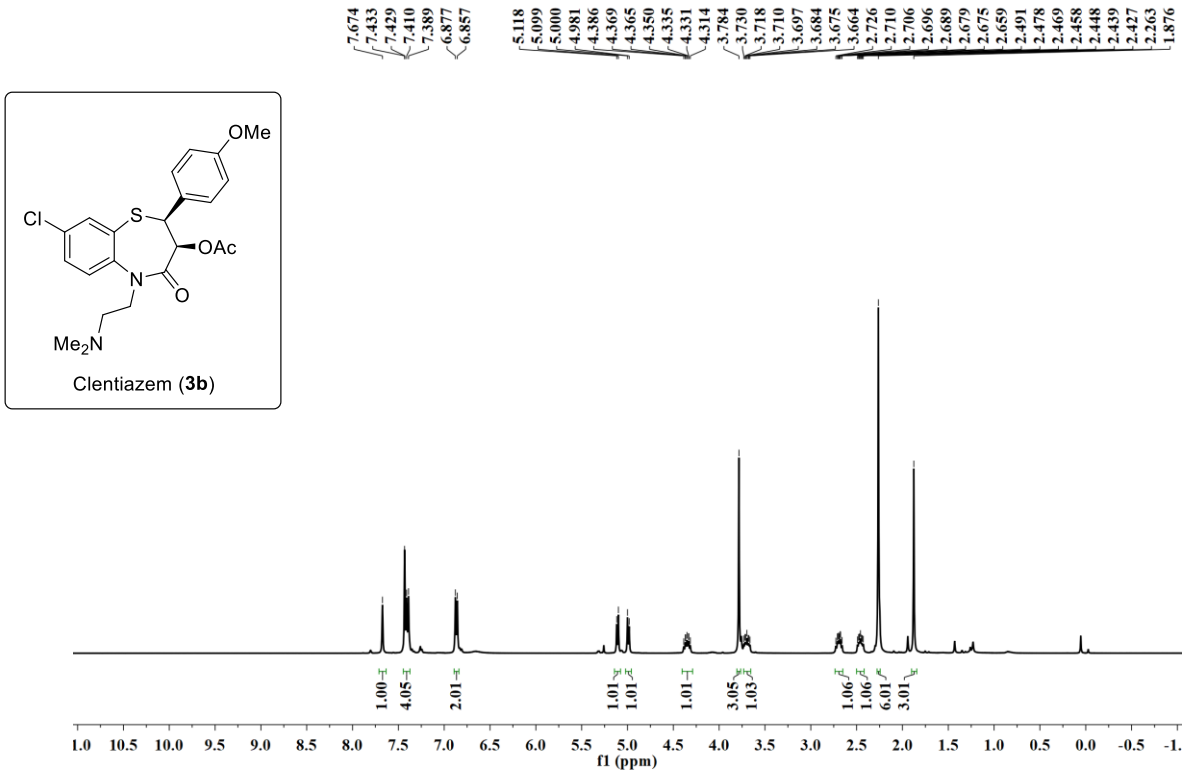
The ^{1}H NMR (400 MHz, $CDCl_3$) Spectrum of **13b**



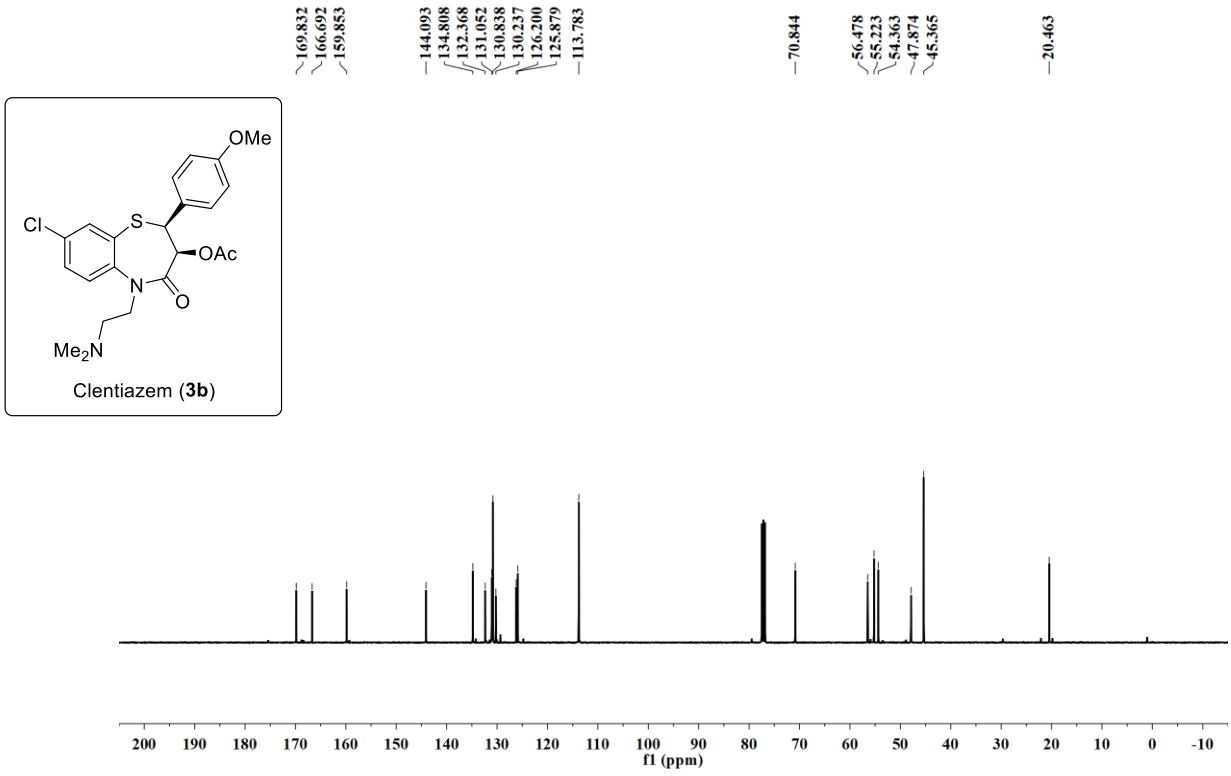
The ^{13}C NMR (100 MHz, $CDCl_3$) Spectrum of **13b**



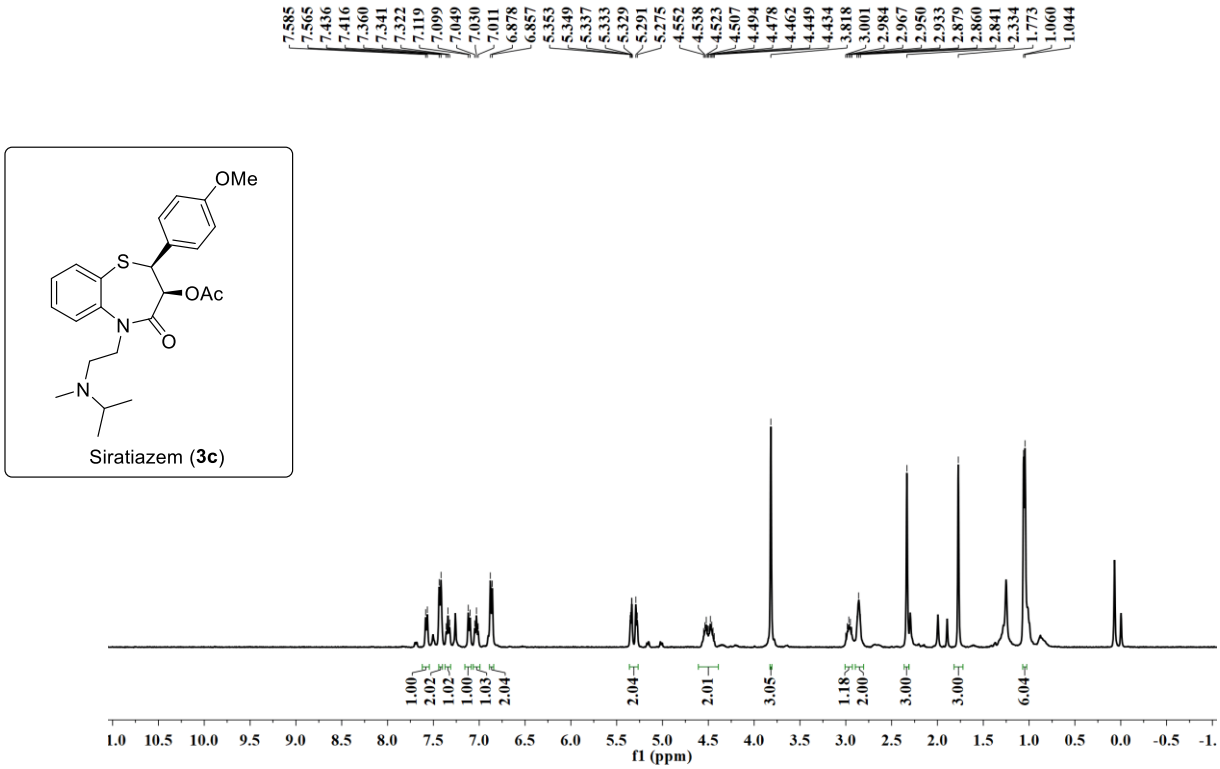
The ^{1}H NMR (400 MHz, $CDCl_3$) Spectrum of **15**



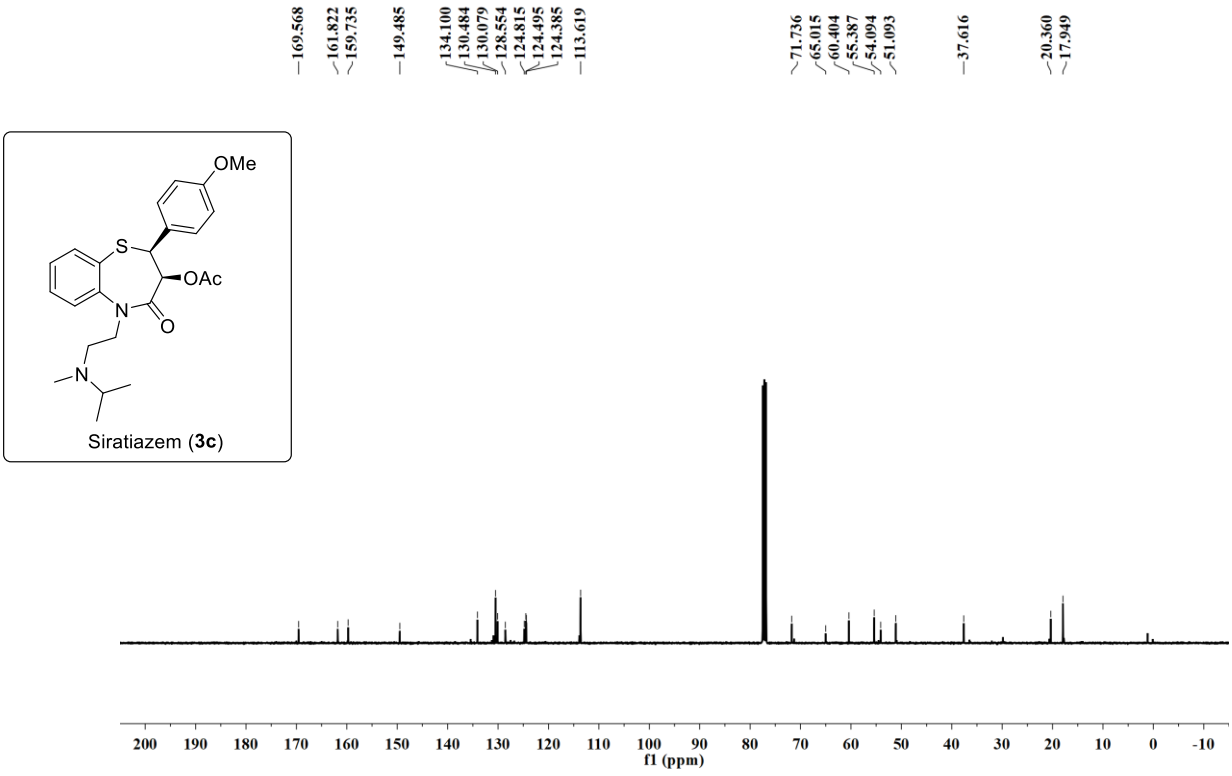
The ^{13}C NMR (100 MHz, $CDCl_3$) Spectrum of **15**



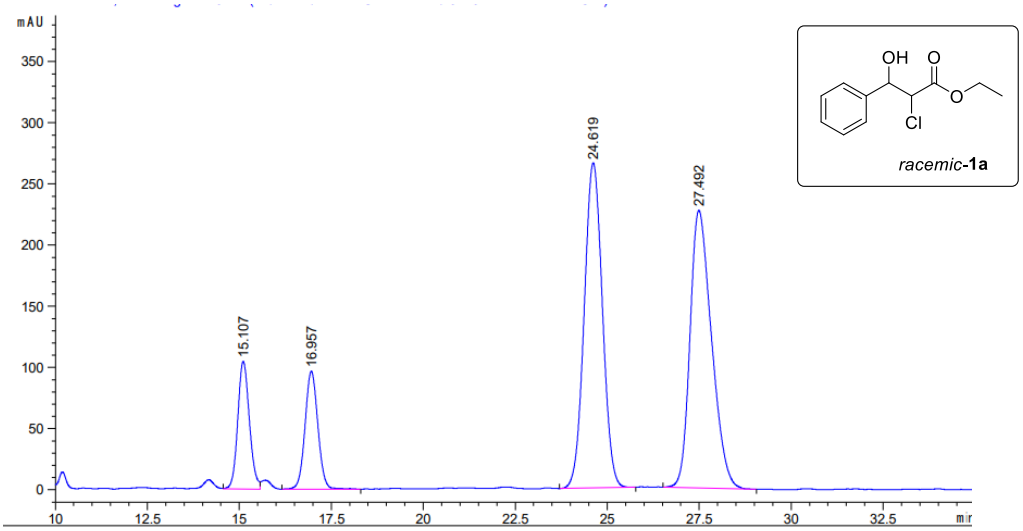
The ^{1}H NMR (400 MHz, $CDCl_3$) Spectrum of **16**



The ^{13}C NMR (100 MHz, $CDCl_3$) Spectrum of **16**

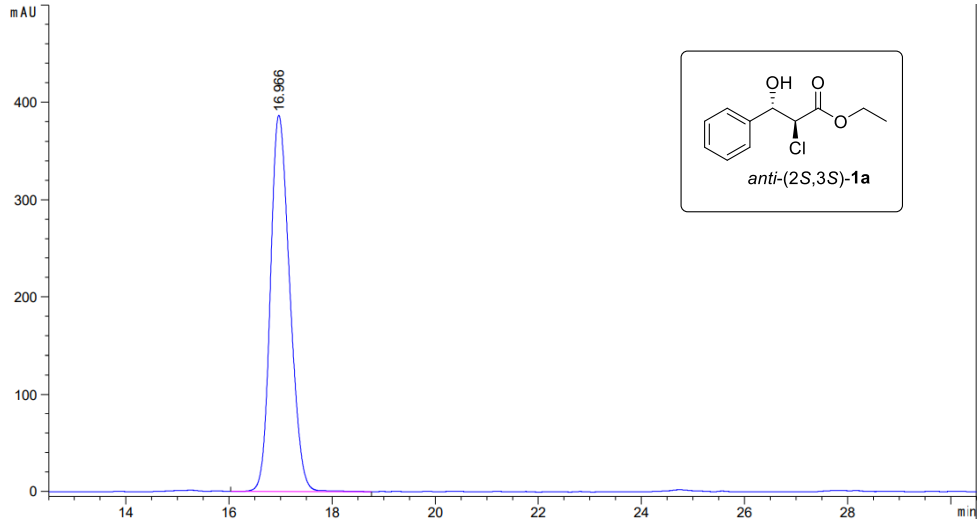


Reduction of **6a** with $NaBH_4$



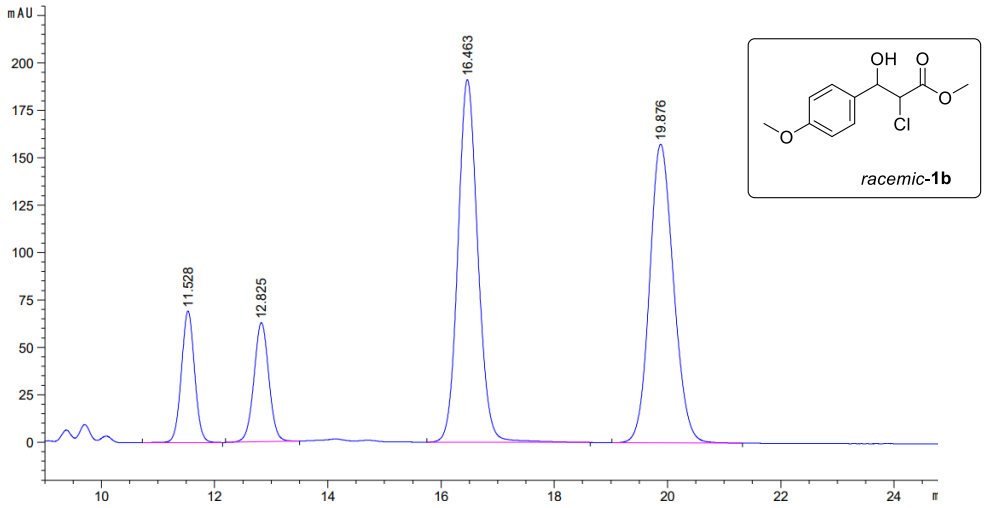
peak #	retention time [min]	type	peak width [min]	peak area [mAU*s]	peak height [mAU]	peak area %
1	15.106	VV	0.3396	2296.13257	104.39244	10.0074
2	16.956	BB	0.3720	2308.53882	96.18604	10.0615
3	24.619	BB	0.5349	9109.49902	265.78278	39.7025
4	27.492	BB	0.6143	9230.21973	227.03760	40.2287

Bioreduction of **6a** with LfSDR1



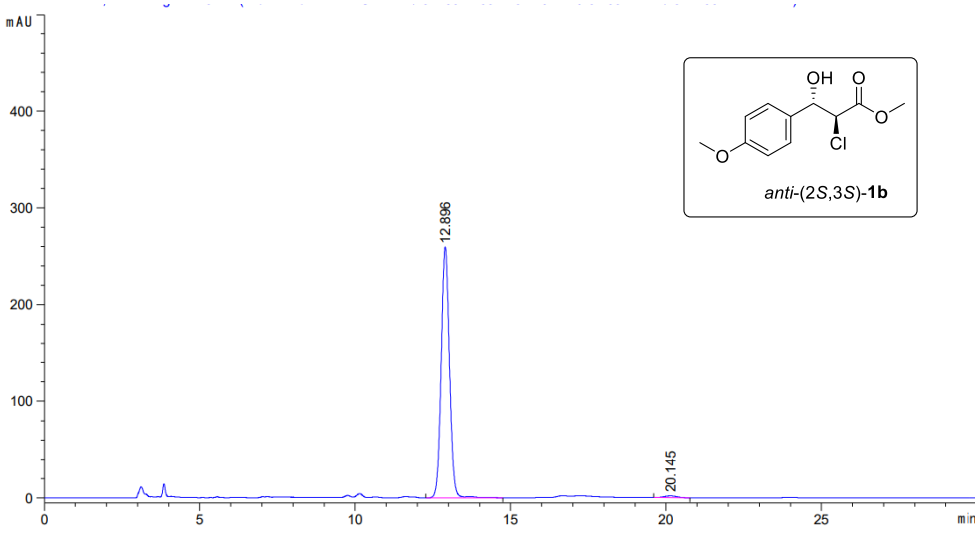
peak #	retention time [min]	type	peak width [min]	peak area [mAU*s]	peak height [mAU]	peak area %
1	16.966	BB	0.4052	1.01099e4	386.51373	100.0000

Reduction of **6b** with $NaBH_4$



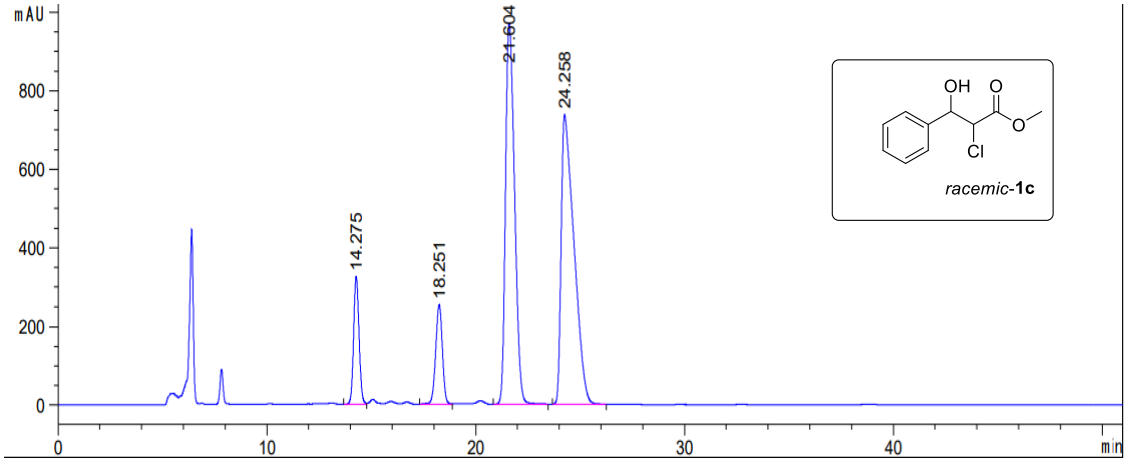
peak #	retention time [min]	type	peak width [min]	peak area [mAU*s]	peak height [mAU]	peak area %
1	11.528	BB	0.2505	1126.33936	69.39761	9.7299
2	12.825	BB	0.2834	1149.53467	62.82388	9.9303
3	16.463	BB	0.3753	4673.19043	191.25017	40.3694
4	19.876	BB	0.4518	4627.00781	157.54982	39.9704

Bioreduction of **6b** with LfSDR1



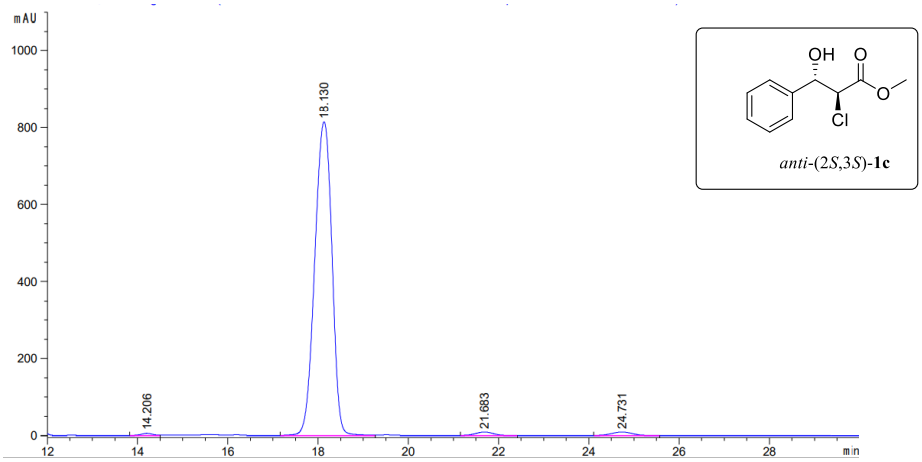
peak #	retention time [min]	type	peak width [min]	peak area [mAU*s]	peak height [mAU]	peak area %
1	12.896	BV R	0.2865	4818.60742	259.58603	98.9218
2	20.145	BB	0.3807	52.52224	1.89147	1.0782

Reduction of **6c** with $NaBH_4$



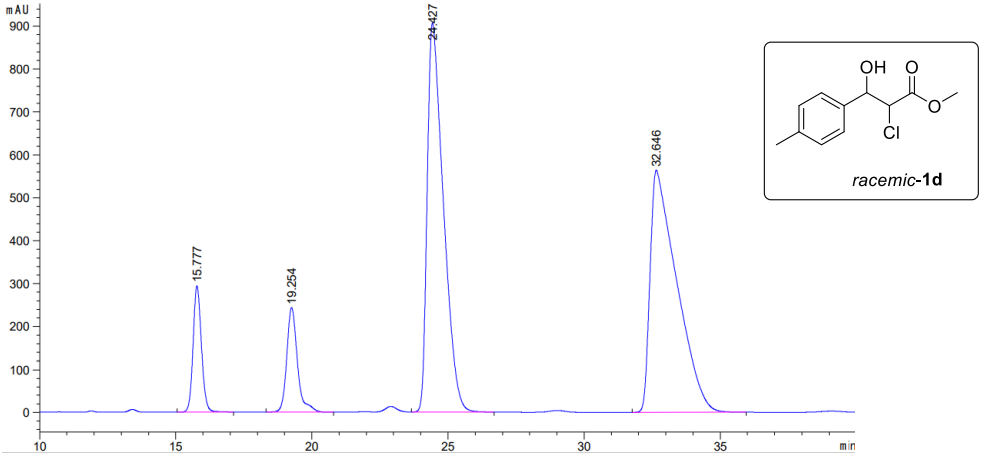
peak retention time 峰 #	type 保留时间 [min]	类型	peak width 峰宽 [min]	peak area 峰面积 [mAU*s]	peak height 峰高 [mAU]	peak area 峰面积 %
1	14.275	BV	0.2834	5929.38086	326.23514	8.1889
2	18.251	BB	0.3545	5748.37305	253.83910	7.9389
3	21.604	VB	0.4801	2.93168e4	971.87659	40.4885
4	24.258	BB	0.6423	3.14131e4	737.81470	43.3837

Bioreduction of **6c** with LfSDR1



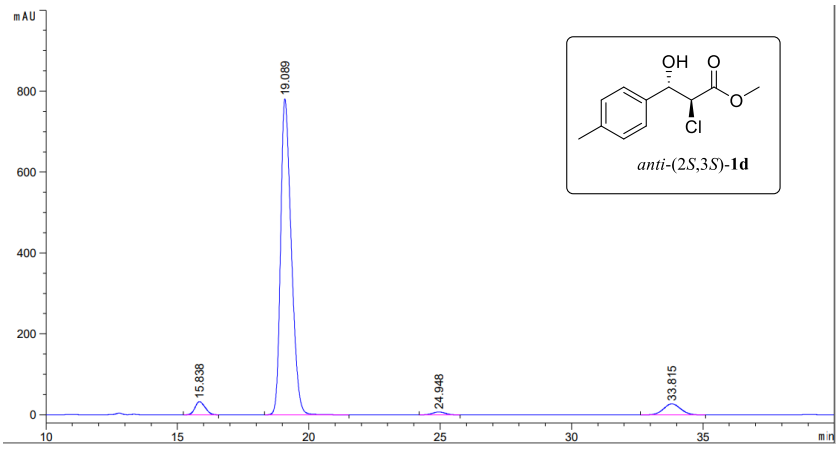
peak retention time 峰 #	type 保留时间 [min]	类型	peak width 峰宽 [min]	peak area 峰面积 [mAU*s]	peak height 峰高 [mAU]	peak area 峰面积 %
1	14.206	BV	0.2794	96.30255	5.36230	0.4355
2	18.130	BB	0.4273	2.15045e4	814.81421	97.2451
3	21.683	BB	0.4066	231.82509	8.87759	1.0483
4	24.731	BB	0.4877	281.08383	8.97549	1.2711

Reduction of **6d** with $NaBH_4$



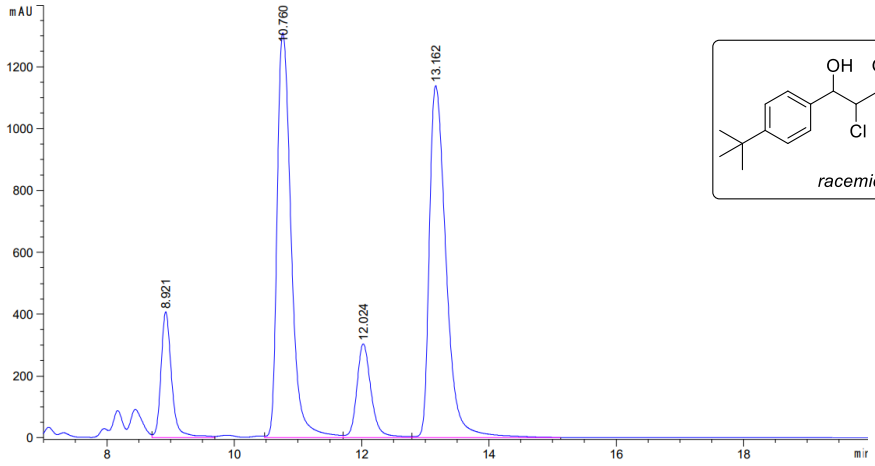
peak retention time	type	peak width	peak area	peak height	peak area
峰 #	保留时间 [min]	类型	峰宽 [min]	峰面积 [mAU*s]	峰高 [mAU]	峰面积 %
1	15.777	BB	0.3279	6287.93311	294.29660	7.0803
2	19.254	BB	0.4154	6667.75391	243.65475	7.5080
3	24.427	BB	0.6243	3.74342e4	906.87238	42.1518
4	32.646	BB	0.9442	3.84183e4	564.30450	43.2599

Bioreduction of **6d** with LfSDR1



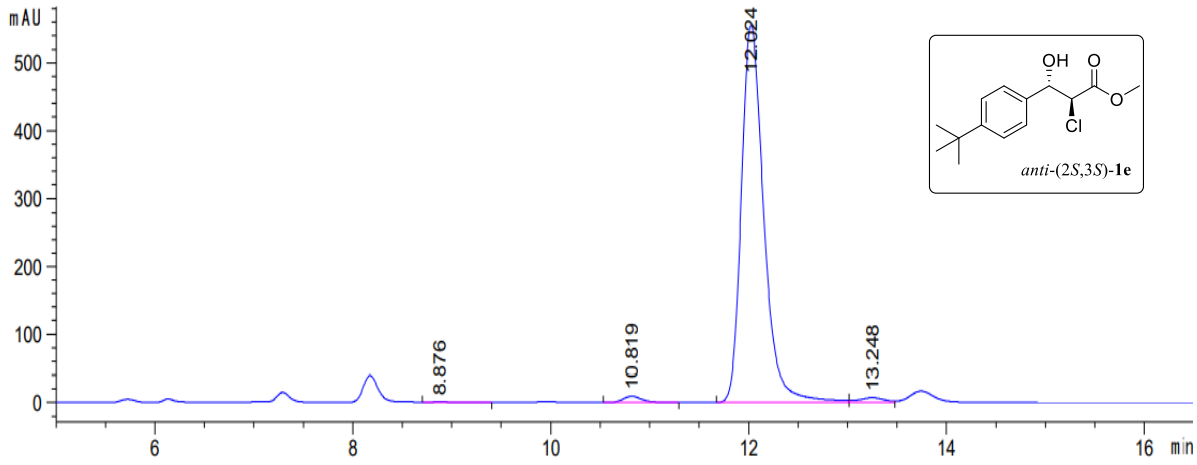
peak retention time	type	peak width	peak area	peak height	peak area
峰 #	保留时间 [min]	类型	峰宽 [min]	峰面积 [mAU*s]	峰高 [mAU]	峰面积 %
1	15.838	BB	0.4274	865.11395	32.46219	3.5586
2	19.089	BB	0.4331	2.19681e4	780.80920	90.3640
3	24.948	BB	0.4818	237.19223	7.17468	0.9757
4	33.815	BB	0.6899	1240.27795	27.31751	5.1018

Reduction of **6e** with $NaBH_4$



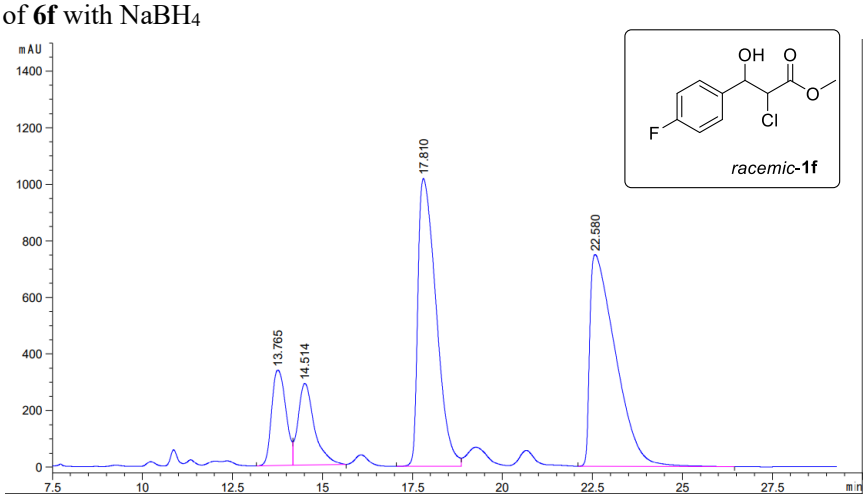
peak 峰 #	retention time 保留时间 [min]	type 类型	peak width 峰宽 [min]	peak area 峰面积 [mAU*s]	peak height 峰高 [mAU]	peak area 峰面积 %
1	8.921	VV	0.1782	4743.37109	406.92874	9.4723
2	10.760	VV	0.2369	1.98831e4	1309.03308	39.7054
3	12.024	VV	0.2367	4714.09766	303.29465	9.4138
4	13.162	VB	0.2824	2.07359e4	1138.20532	41.4085

Bioreduction of **6e** with LfSDR1



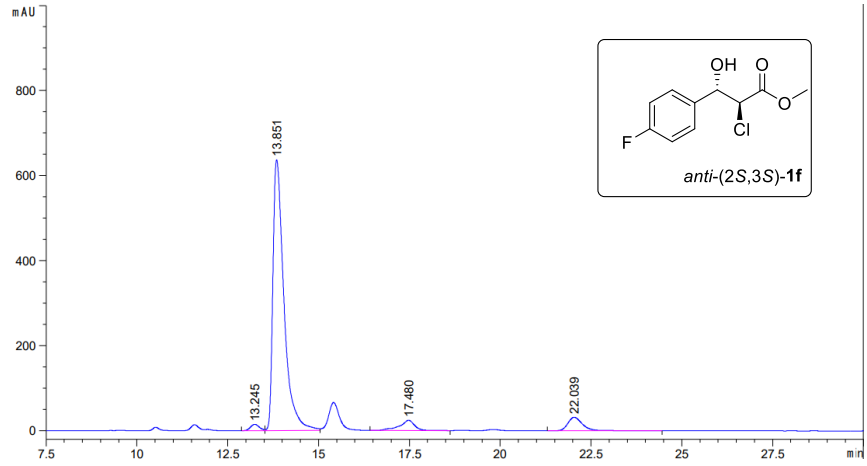
peak 峰 #	retention time 保留时间 [min]	type 类型	peak width 峰宽 [min]	peak area 峰面积 [mAU*s]	peak height 峰高 [mAU]	peak area 峰面积 %
1	8.876	VB	0.2493	17.55627	1.05598	0.1944
2	10.819	VB	0.2083	127.12845	9.33746	1.4079
3	12.024	BV	0.2422	8757.90625	555.36810	96.9872
4	13.248	VV	0.2650	127.37279	7.08786	1.4106

Reduction of **6f** with $NaBH_4$



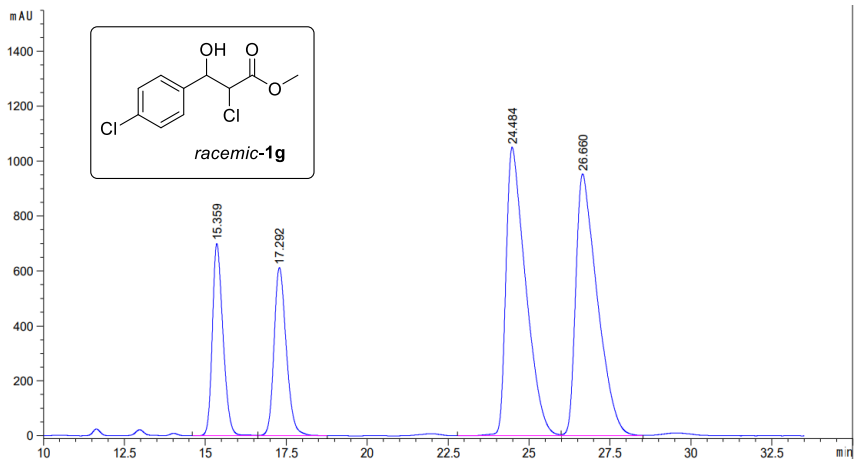
peak retention time 峰 #	保留时间 [min]	type 类型	peak width 峰宽 [min]	peak area 峰面积 [mAU*s]	peak height 峰高 [mAU]	peak area 峰面积 %
1	13.765	BV	0.4370	9295.65723	337.79834	10.1610
2	14.514	VB	0.4742	9155.49609	288.51694	10.0078
3	17.810	BV	0.5667	3.58153e4	1017.40430	39.1493
4	22.580	BB	0.7759	3.72174e4	748.81421	40.6819

Bioreduction of **6f** with LfSDR1



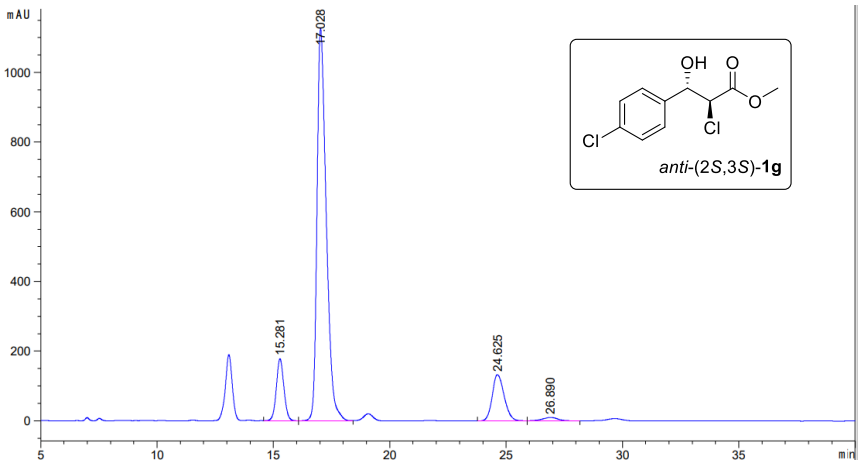
peak retention time 峰 #	保留时间 [min]	type 类型	peak width 峰宽 [min]	peak area 峰面积 [mAU*s]	peak height 峰高 [mAU]	peak area 峰面积 %
1	13.245	BV	0.2606	251.61391	14.93011	1.6402
2	13.851	VV	0.3185	1.34673e4	637.52222	87.7877
3	17.480	BB	0.4179	712.97198	24.04798	4.6476
4	22.039	BB	0.4373	908.86371	31.81002	5.9245

Reduction of **6g** with $NaBH_4$



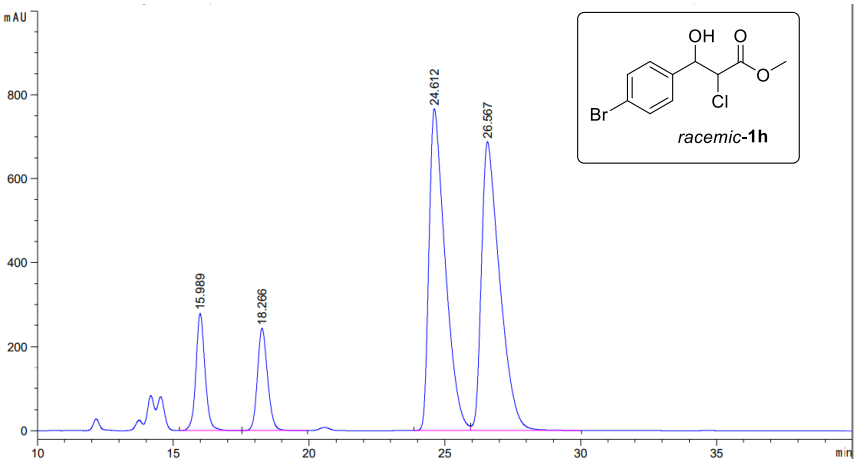
peak #	retention time [min]	type	peak width [min]	peak area [mAU*s]	peak height [mAU]	peak area %
峰 #	保留时间 [min]	类型	峰宽 [min]	峰面积 [mAU*s]	峰高 [mAU]	峰面积 %
1	15.358	BV	0.3473	1.59259e4	700.38245	13.2333
2	17.291	VB	0.4026	1.61540e4	612.73016	13.4229
3	24.481	BV	0.6299	4.43037e4	1050.79602	36.8133
4	26.659	VB	0.6470	4.39634e4	953.41357	36.5306

Bioreduction of **6g** with LfSDR1



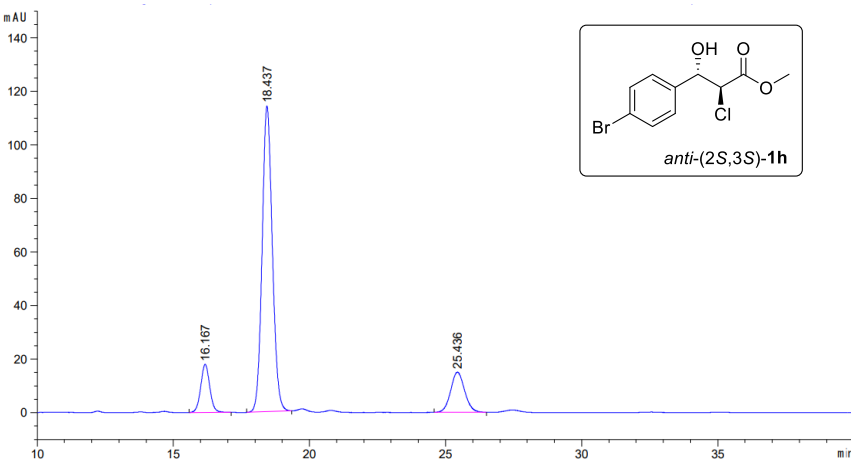
peak #	retention time [min]	type	peak width [min]	peak area [mAU*s]	peak height [mAU]	peak area %
峰 #	保留时间 [min]	类型	峰宽 [min]	峰面积 [mAU*s]	峰高 [mAU]	峰面积 %
1	15.281	VB	0.3464	4014.33447	177.78088	9.9796
2	17.028	BV	0.4173	3.10321e4	1126.63416	77.1455
3	24.624	BB	0.5425	4721.07178	132.89282	11.7365
4	26.890	BB	0.6722	457.92252	9.79846	1.1384

Reduction of **6h** with $NaBH_4$



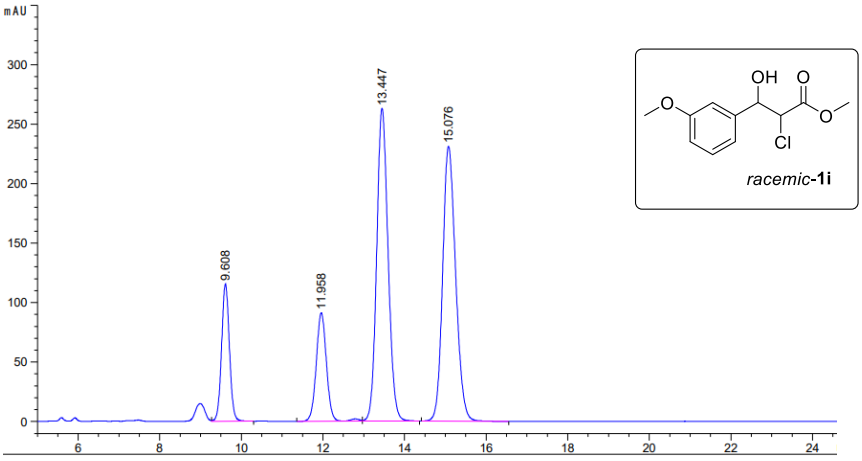
peak retention time 峰 #	type 保留时间 [min]	类型	peak width 峰宽 [min]	peak area 峰面积 [mAU*s]	peak height 峰高 [mAU]	peak area 峰面积 %
1	15.989	BB	0.3533	6470.24512	277.72186	8.4360
2	18.266	BB	0.3961	6270.76123	242.68399	8.1759
3	24.612	BV	0.6175	3.17420e4	765.91992	41.3857
4	26.567	VB	0.6968	3.22148e4	687.32001	42.0023

Bioreduction of **6h** with LfSDR1



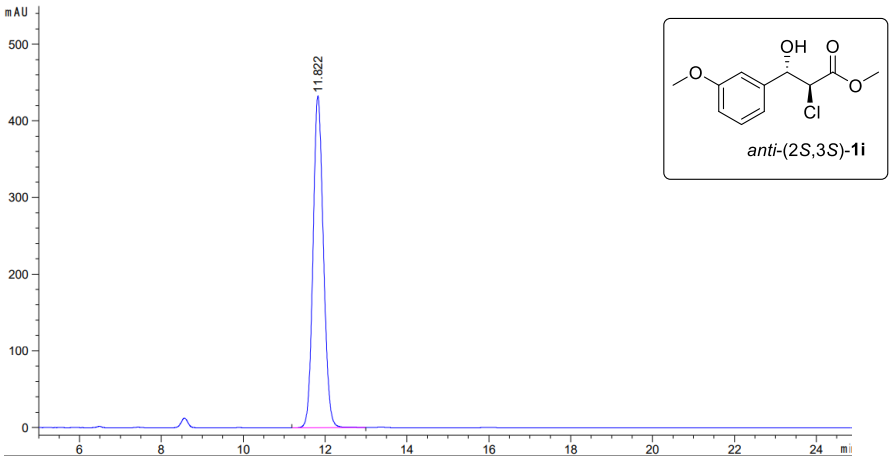
peak retention time 峰 #	type 保留时间 [min]	类型	peak width 峰宽 [min]	peak area 峰面积 [mAU*s]	peak height 峰高 [mAU]	peak area 峰面积 %
1	16.167	BV R	0.3579	415.06476	17.99698	10.5842
2	18.437	BB	0.4013	2972.17798	114.16785	75.7912
3	25.436	BB	0.5363	534.28949	15.00258	13.6245

Reduction of **6i** with $NaBH_4$



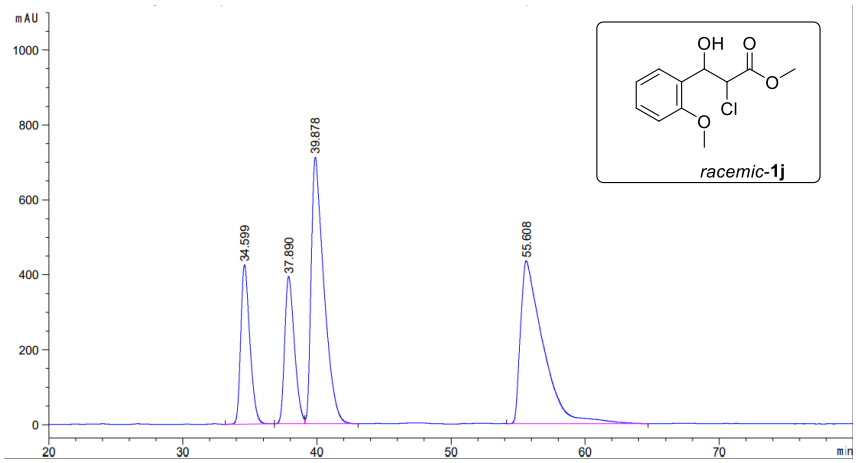
peak retention time	type	peak width	peak area	peak height	peak area
峰 保留时间	类型	峰宽	峰面积	峰高	峰面积
# [min]		[min]	[mAU*s]	[mAU]	%
1 9.608	VB	0.2067	1542.30957	115.53669	11.5715
2 11.958	BV R	0.2666	1602.24683	91.15568	12.0212
3 13.447	VB	0.2983	5082.35938	262.98462	38.1316
4 15.076	BB	0.3391	5101.55859	231.06401	38.2756

Bioreduction of **6i** with LfSDR1



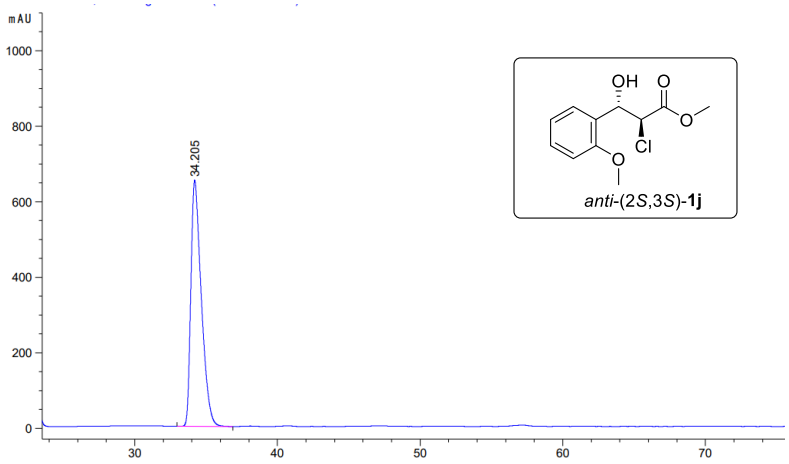
peak retention time	type	peak width	peak area	peak height	peak area
峰 保留时间	类型	峰宽	峰面积	峰高	峰面积
# [min]		[min]	[mAU*s]	[mAU]	%
1 11.822	BV R	0.2727	7630.19531	432.78909	100.0000

Reduction of **6j** with $NaBH_4$



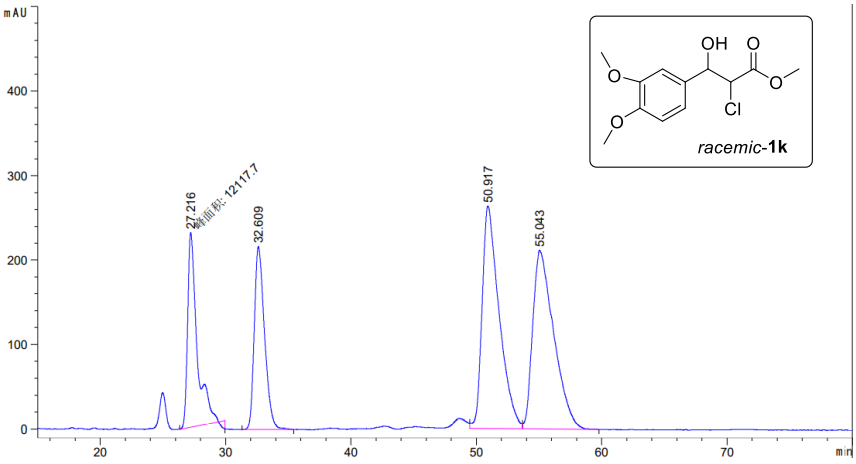
peak retention time #	type	peak width [min]	peak area [mAU*s]	peak height [mAU]	peak area %
峰	保留时间 类型	峰宽	峰面积	峰高	峰面积
1	34.598 BB	0.7194	1.99956e4	425.39532	14.6469
2	37.889 BV	0.7799	1.98363e4	392.94656	14.5302
3	39.877 VB	0.9377	4.70158e4	711.67230	34.4394
4	55.606 BB	1.6332	4.96698e4	433.67389	36.3835

Bioreduction of **6j** with LfSDR1



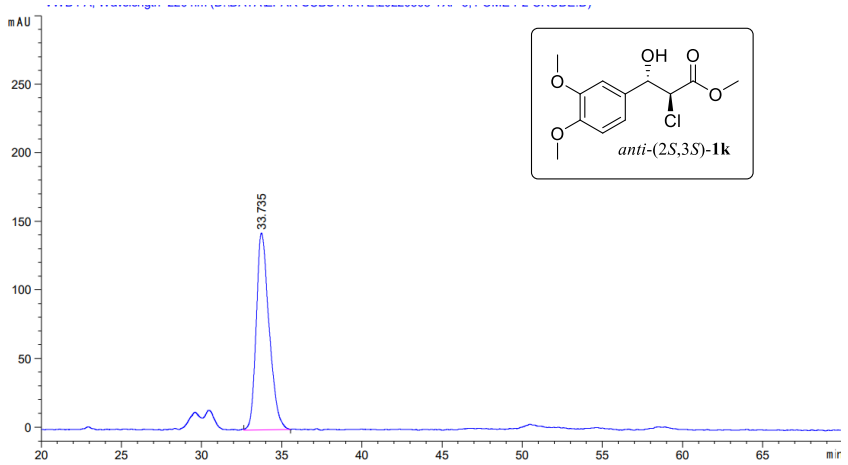
peak retention time #	type	peak width [min]	peak area [mAU*s]	peak height [mAU]	peak area %
峰	保留时间 类型	峰宽	峰面积	峰高	峰面积
1	34.205 BB	0.7651	3.31251e4	651.63519	100.0000

Reduction of **6k** with $NaBH_4$



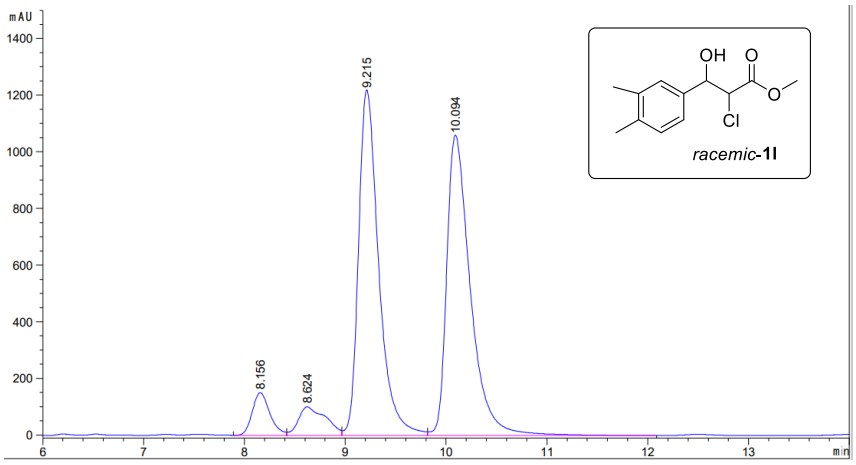
peak retention time 峰 #	type 保留时间 [min]	类型	peak width 峰宽 [min]	peak area 峰面积 [mAU*s]	peak height 峰高 [mAU]	peak area 峰面积 %
1	27.216	MM	0.8754	1.21177e4	230.70847	16.3482
2	32.609	BB	0.8680	1.23436e4	216.87788	16.6529
3	50.917	VV	1.2872	2.49090e4	263.42102	33.6051
4	55.043	VB	1.5876	2.47525e4	211.77254	33.3939

Bioreduction of **6k** with LfSDR1



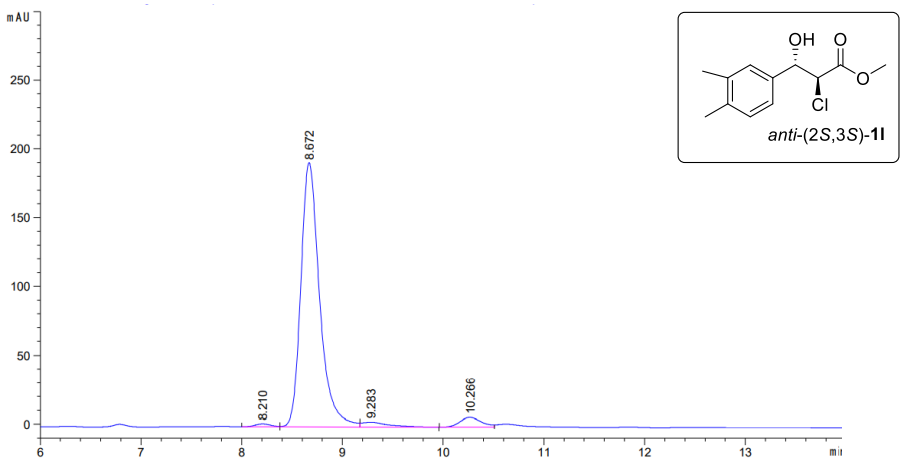
peak retention time 峰 #	type 保留时间 [min]	类型	peak width 峰宽 [min]	peak area 峰面积 [mAU*s]	peak height 峰高 [mAU]	peak area 峰面积 %
1	33.735	VV	0.8027	7987.49902	143.21753	100.0000

Reduction of **6l** with $NaBH_4$



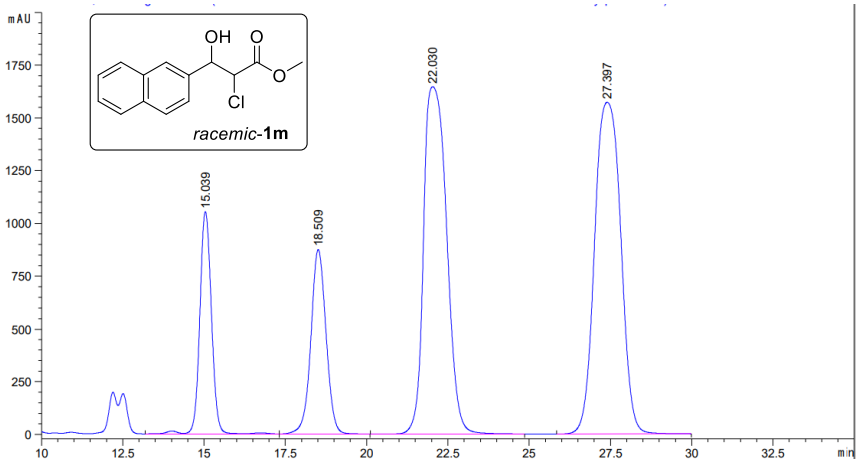
peak retention time	type	peak width	peak area	peak height	peak area
峰 #	保留时间 [min]	类型	峰宽 [min]	峰面积 [mAU*s]	峰高 [mAU]	峰面积 %
1	8.155	BV	0.1854	1825.04822	150.60562	4.8188
2	8.620	VV	0.2565	1881.33569	100.46745	4.9674
3	9.213	VV	0.2101	1.69439e4	1219.62000	44.7377
4	10.091	VB	0.2457	1.72236e4	1059.49792	45.4762

Bioreduction of **6l** with LfSDR1



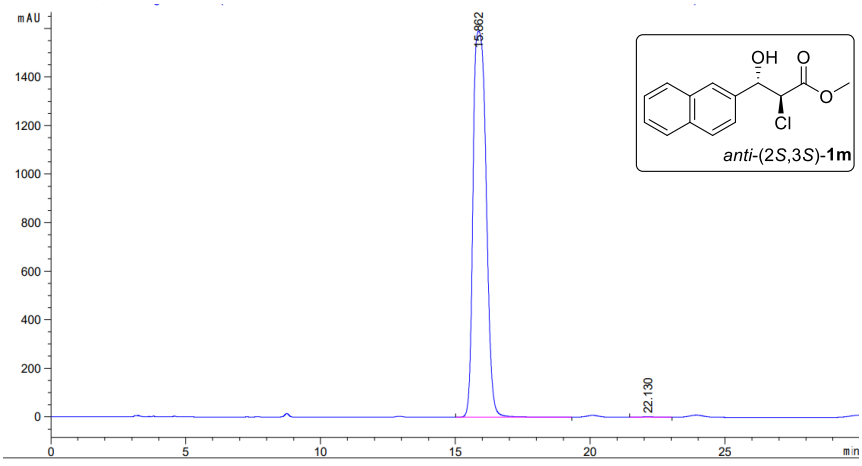
peak retention time	type	peak width	peak area	peak height	peak area
峰 #	保留时间 [min]	类型	峰宽 [min]	峰面积 [mAU*s]	峰高 [mAU]	峰面积 %
1	8.209	BV	0.1666	22.58586	2.11511	0.8456
2	8.671	VV	0.1958	2480.11890	191.93584	92.8523
3	9.285	VB	0.2610	64.44640	3.46542	2.4128
4	10.265	BV	0.2273	103.88685	6.99180	3.8894

Reduction of **6m** with $NaBH_4$



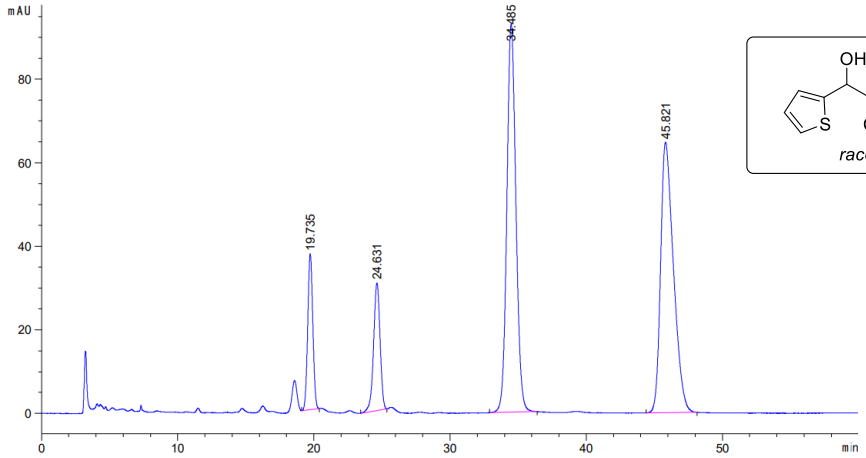
peak retention time	type	peak width	peak area	peak height	peak area
峰 #	保留时间 [min]	类型	峰宽 [min]	峰面积 [mAU*s]	峰高 [mAU]	峰面积 %
1	15.039	VV R	0.3835	2.63330e4	1053.73193	11.7999
2	18.509	BB	0.5008	2.83531e4	873.84961	12.7051
3	22.030	VB R	0.7852	8.07895e4	1646.06580	36.2019
4	27.397	BBA	0.8984	8.76879e4	1571.88257	39.2931

Bioreduction of **6m** with LfSDR1



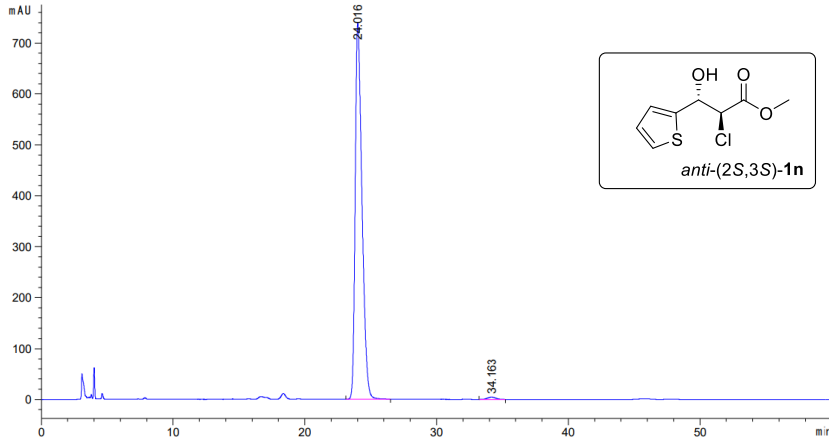
peak retention time	type	peak width	peak area	peak height	peak area
峰 #	保留时间 [min]	类型	峰宽 [min]	峰面积 [mAU*s]	峰高 [mAU]	峰面积 %
1	15.862	BB	0.5414	5.35530e4	1590.35059	99.8874
2	22.130	BB	0.4347	60.36330	2.01071	0.1126

Reduction of **6n** with $NaBH_4$



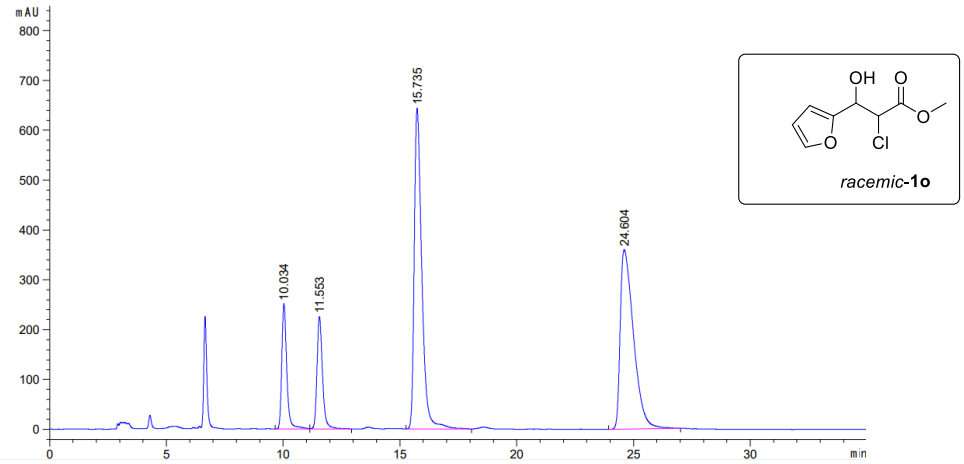
peak retention time 峰 #	type 保留时间 [min]	类型	peak width 峰宽 [min]	peak area 峰面积 [mAU*s]	peak height 峰高 [mAU]	peak area 峰面积 %
1	19.735	BB	0.3996	970.78253	37.32142	9.2264
2	24.631	BB	0.5120	1013.59015	30.57279	9.6333
3	34.485	BB	0.7063	4293.54834	92.96152	40.8064
4	45.821	BB	0.9569	4243.82910	64.74166	40.3339

Bioreduction of **6n** with LfSDR1



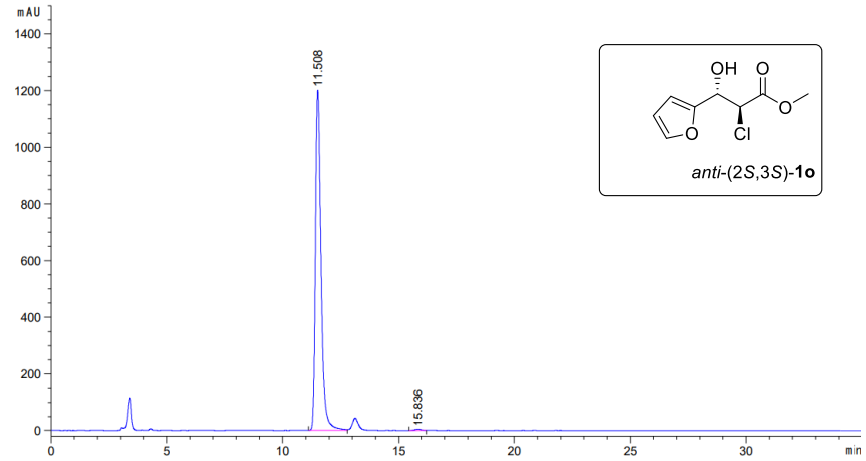
peak retention time 峰 #	type 保留时间 [min]	类型	peak width 峰宽 [min]	peak area 峰面积 [mAU*s]	peak height 峰高 [mAU]	peak area 峰面积 %
1	24.016	BB	0.5444	2.64497e4	738.68207	99.2641
2	34.163	BB	0.5429	196.07498	4.40250	0.7359

Reduction of **6o** with $NaBH_4$



peak retention time	type	peak width	peak area	peak height	peak area
峰 #	保留时间 [min]	类型	峰宽 [min]	峰面积 [mAU*s]	峰高 [mAU]	峰面积 %
1	10.034	BV	0.2266	3764.34277	251.52634	10.3991
2	11.553	VB	0.2499	3693.17749	225.67656	10.2025
3	15.735	BV	0.3398	1.44969e4	643.65771	40.0481
4	24.604	BB	0.6141	1.42443e4	360.44290	39.3503

Bioreduction of **6o** with LfSDR1



peak retention time	type	peak width	peak area	peak height	peak area
峰 #	保留时间 [min]	类型	峰宽 [min]	峰面积 [mAU*s]	峰高 [mAU]	峰面积 %
1	11.508	VV	0.2552	2.02192e4	1202.48682	99.5425
2	15.836	BB	0.2979	92.93533	4.86445	0.4575