Electronic Supplementary Material (ESI) for Catalysis Science & Technology.

Supporting Information

Reshaping the Active Pocket of Esterase Est816 for Resolution of Economically Important Racemates

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1. Supplementary Figures and Tables

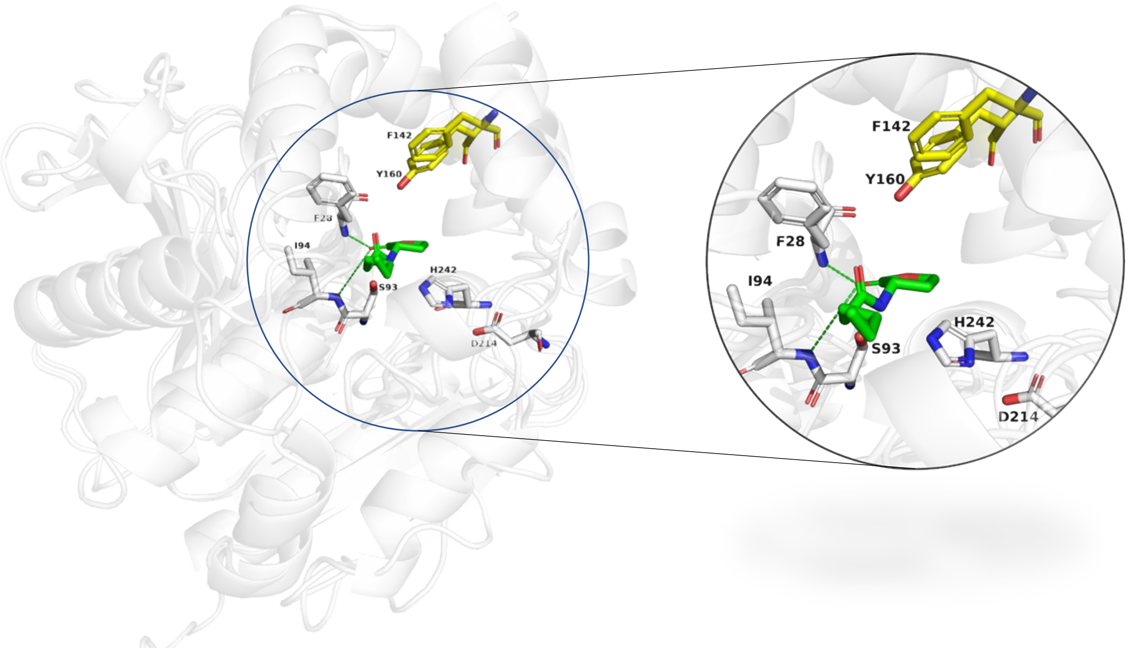


Fig. S1. Structural analyses of Est816 (5EGN) and AidH (4G8B). The catalytic residues (S93, D214 and H242) and the oxyanion hole residues (F28 and I94) of Est816 were shown as white sticks. The residues F142 of Est816 and Y160 of AidH were shown as yellow sticks. The substrate of AidH (N-hexanoyl-L-homoserine lactone) was shown as green sticks.

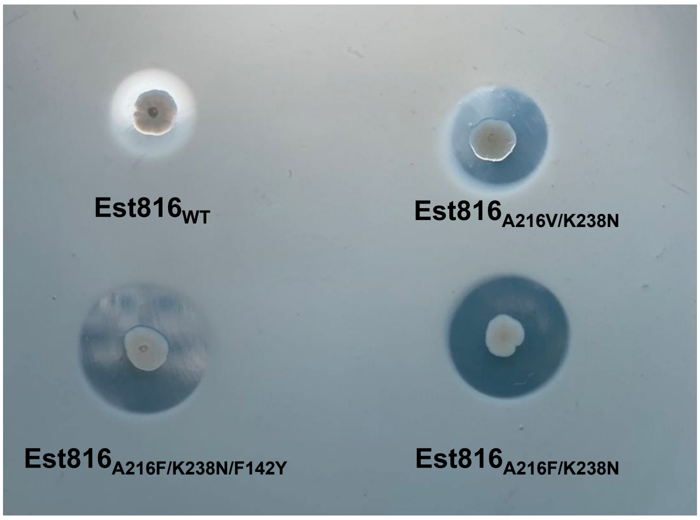


Fig. S2. The hydrolytic activity of Est816$_{WT}$ and variants. Recombinant cells with wide type and different mutant genes were inoculated on LB plate containing 10 mM naproxen ethyl ester and cultured at 37 °C for 24 hours.

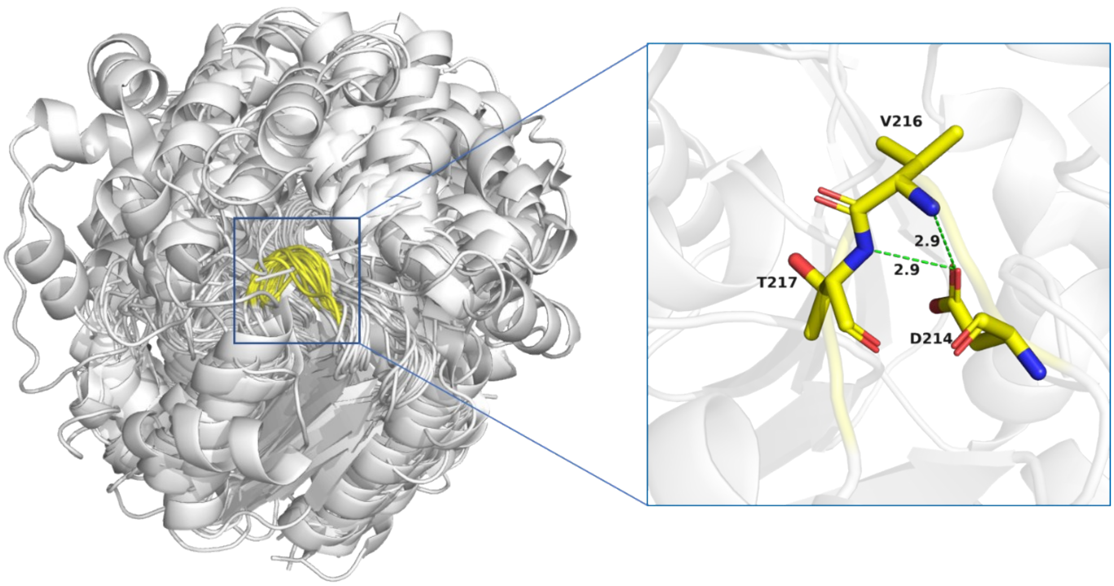


Fig. S3. Superimposed of the crystal structures of Est816 and 6_AlphaBeta_hydrolase family esterases. The β7/α10 loops (Est816 numbering) were shown as yellow, and a hydrogen bond between the backbone N-H of A216 and the O_δ of catalytic D214 was 2.9 Å.

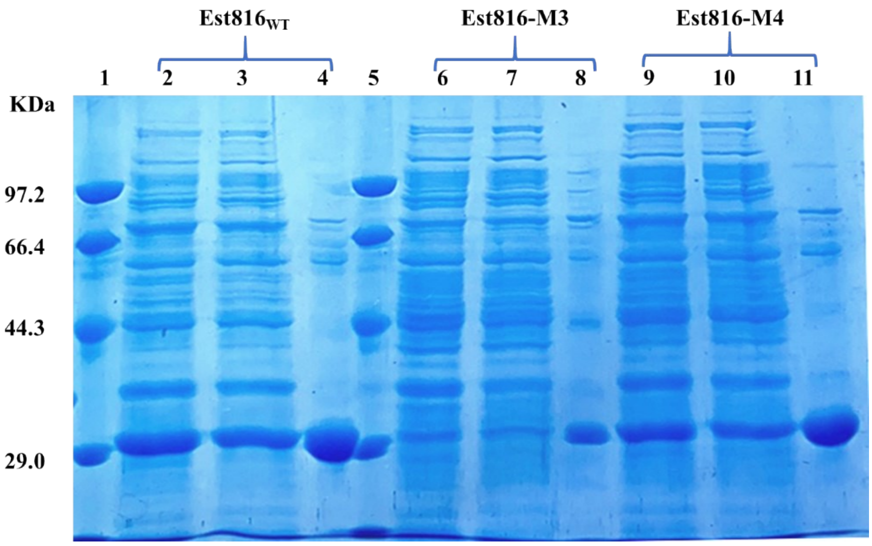


Fig. S4. SDS-PAGE analysis of the purified recombinant $Est816_{WT}$, Est816-M3, and Est816-M4. Protein markers (lane 1 and 5) stained with Coomassie blue, cell-free lysates (lane 2, 6, and 9), the supernatant of *E. coli* BL21 (DE3) cell lysates (lane 3, 7 and 10), purified target protein (lane 4, 8 and 11). The molecular mass of the enzyme subunit was estimated using the following protein markers as standards: rabbit muscle phosphorylase B (97,200 Da); bovine serum albumin (66,409 Da); ovalbumin (44,287 Da); carbonic anhydrase (29,000 Da).

Table S1. Enantiomeric excess, conversion and the corresponding *E* values of ethyl 2-arylpropionates using Est816$_{WT}$.

List	Substrate	Conversion / %	Enantiomeric excess / %	*E* value
1	**1a**	73%	13%ee_S	1.7
2	**2a**	82%	14%ee_S	2.2
3	**3a**	75%	16%ee_S	2
4	**4a**	87%	12%ee_S	2.5
5	**5a**	71%	39%ee_S	7.6
6	**6a**	65%	45.5%ee_R	6.7
7	**7a**	91%	3%ee_R	1.3
8	**8a**	51%	73.5%ee_R	15
9	**9a**	97%	0	1

[a]Conditions: Sodium phosphate buffer (0.2 M, pH 8.0) with substrate (10-50 mM), 5% (v/v) DMSO, and 150 μg of enzyme. The reactions were conducted at 40 °C for 15 min to 12 h. The conversions and enantiomeric excess values were determined by HPLC and chiral HPLC, respectively.

Table S2. Sequences of mutagenesis primers used for the construction of Est816 variants. Red letters indicate nucleotides that were exchanged.

Mutation	Forward primer	Reverse primer
A216F	GAAGAAGATCTGTTTACGACCGTG GCCAACAATCAACTGC	GCAGTTGATTGTTGGCCACGGTCGTAAACAGATCTTCTTC
A216W	GAAGAAGATCTGTGGACGACCGTG GCCAACAATCAACTGC	GCAGTTGATTGTTGGCCACGGTCGTCCACAGATCTTCTTC
A216R	GAAGAAGATCTGCGTACGACCGTGGCCAACAATCAACTGC	GCAGTTGATTGTTGGCCACGGTCGTACGCAGATCTTCTTC
F142Y	CATCGGCGCATATGGCGGCCTGCTCGAGGGCGCCGTCTC	GAGACGGCGCCCTCGAGCAGGCCGCCATATGCGCCGATG
F28W	CTTCATCCCTGGAGCACGAATGGGGGGATCTGGTAC	GTACCAGATCCCCCCATTCGTGCTCCAGGGATGAAG
F28G	CTTCATCCCGGTAGCACGAATGGGGGGATCTGGTAC	GTACCAGATCCCCCCATTCGTGCTACCGGGATGAAG
S29L	CTTCATCCCTGGCTTACGAATGGGGGGATC	GATCCCCCCATTCGTAAGCCAGGGATGAAG
S118PG119A	CATCCTCAGCCCTGCTACCGGTCTTG	CAAGACCGGTAGCAGGGCTGAGGATG
S118PG119V	CATCCTCAGCCCTGTTACCGGTCTTG	CAAGACCGGTAACAGGGCTGAGGATG
S118PG119S	CATCCTCAGCCCTTCTACCGGTCTTG	CAAGACCGGTAGAAGGGCTGAGGATG
V190L	GATCCCAACGGGTTGTTTGCCTGGAAC	GTTCCAGGCAAACAACCCGTTGGGATC
V190F	GATCCCAACGGGTTCTTTGCCTGGAAC	GTTCCAGGCAAAGAACCCGTTGGGATC
L145G	CATTCGGCGGCGGTCTCGAGGGCGCCGTCTCCGCGCGTTCAAAACG	CGTTTTGAACGCGCGGAGACGGCGCCCTCGAGACCGCCGCCGAATG
L145F	CATTCGGCGGCTTTCTCGAGGGCGCCGTCTCCGCGCGTTCAAAACG	CGTTTTGAACGCGCGGAGACGGCGCCCTCGAGAAAGCCGCCGAATG
A149L	CTGCTCGAGGGCTTAGTCTCCGCGCGTTCAAAACGTGAGC	GCTCACGTTTTGAACGCGCGGAGACTAAGCCCTCGAGCAG
A149F	CTGCTCGAGGGCTTTGTCTCCGCGCGTTCAAAACGTGAGC	GCTCACGTTTTGAACGCGCGGAGACAAAGCCCTCGAGCAG
L122I	CTGGCACCGGTATTGGCGAGGGCATGCCGCCCGAAGCTG	CAGCTTCGGGCGGCATGCCCTCGCCAATACCGGTGCCAG

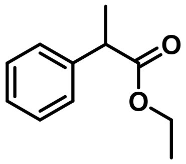
L122F	CTGGCACCGGTTTTGGCGAGGGC ATGCCGCCCGAAGCTG	CAGCTTCGGGCGGCATGCCCTCG CCAAAACCGGTGCCAG
L122Y	CTGGCACCGGTTATGGCGAGGGC ATGCCGCCCGAAGCTG	CAGCTTCGGGCGGCATGCCCTCG CCATAACCGGTGCCAG
L122A	CTGGCACCGGTGCTGGCGAGGGC ATGCCGCCCGAAGCTG	CAGCTTCGGGCGGCATGCCCTCG CCAGCACCGGTGCCAG
M97F	GATCGGCGGATTCATCGCCATGC AAC	GTTGCATGGCGATGAATCCGCCG ATC
Est816-EcoRI-F'	CGGAATTCATGCCGCATGTAGAG AACGACGG	
Est816-SacI-R'	CGAGCTCTCAGGACACCAATGAA GCTTCTCGAGC	

Table S3. Chiral HPLC methods and retention times for ethyl 2-arylpropionates.

List	Substrate	Method	$Rt_{(S)}$ min	$Rt_{(R)}$ min
1	**1a**	Chiralcel OJ-H, hexane/2-propanol (90 : 10) flowing at 0.7 mL min^{-1}, UV 214 nm	9.26	9.92
2	**2a**	Chiralcel OD-H, hexane/2-propanol (98 : 2) flowing at 0.7 mL min^{-1}, UV 214 nm	10.08	9.06
3	**3a**	Chiralcel OJ-H, hexane/2-propanol (99 : 1) flowing at 0.7 mL min^{-1}, UV 214 nm	8.29	10.30
4	**4a**	Chiralcel OJ-H, hexane/2-propanol (99 : 1) flowing at 0.7 mL min^{-1}, UV 214 nm	27.29	28.79
5	**5a**	Chiralcel OD-H, hexane/2-propanol (99 : 1) flowing at 0.3 mL min^{-1}, UV 214 nm	28.70	29.85
6	**6a**	Chiralcel OJ-H, hexane/2-propanol (90 : 10) flowing at 0.7 mL min^{-1}, UV 214 nm	12.85	12.02
7	**7a**	Chiralcel OJ-H, hexane/2-propanol (99 : 1) flowing at 0.7 mL min^{-1}, UV 214 nm	9.28	10.75
8	**8a**	Chiralcel IG, hexane/2-propanol (90 : 10) flowing at 0.7 mL min^{-1}, UV 214 nm	12.17	13.30

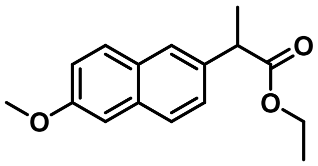
4. spectral data

1a



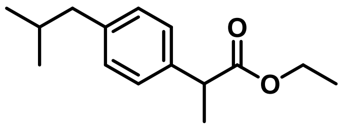
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 – 7.17 (m, 5H), 4.12 (qq, *J* = 10.8, 7.1 Hz, 2H), 3.70 (q, *J* = 7.2 Hz, 1H), 1.49 (d, *J* = 7.2 Hz, 3H), 1.20 (t, *J* = 7.1 Hz, 3H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 174.55, 140.72, 128.57, 127.46, 127.04, 60.70, 45.57, 18.59, 14.11.

2a



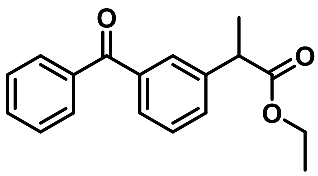
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.73 – 7.62 (m, 3H), 7.41 (dd, *J* = 8.5, 1.7 Hz, 1H), 7.17 – 7.06 (m, 2H), 4.12 (qq, *J* = 10.8, 7.1 Hz, 2H), 3.92 – 3.74 (m, 4H), 1.57 (d, *J* = 7.2 Hz, 3H), 1.19 (t, *J* = 7.1 Hz, 3H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 174.70, 157.65, 135.88, 133.69, 129.29, 128.97, 127.11, 126.26, 125.92, 118.94, 105.64, 60.75, 55.30, 45.51, 18.62, 14.15.

3a



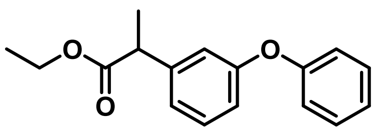
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.20 (d, *J* = 8.1 Hz, 2H), 7.09 (d, *J* = 8.1 Hz, 2H), 4.12 (qq, *J* = 10.8, 7.1 Hz, 2H), 3.67 (q, *J* = 7.2 Hz, 1H), 2.44 (d, *J* = 7.2 Hz, 2H), 1.84 (dp, *J* = 13.7, 6.7 Hz, 1H), 1.48 (d, *J* = 7.2 Hz, 3H), 1.21 (t, *J* = 7.1 Hz, 3H), 0.89 (d, *J* = 6.6 Hz, 6H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 174.77, 140.43, 137.91, 129.28, 127.12, 60.62, 45.18, 45.05, 30.16, 22.38, 18.59, 14.12.

4a



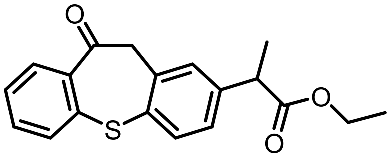
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84 – 7.65 (m, 4H), 7.64 – 7.52 (m, 2H), 7.52 – 7.40 (m, 3H), 4.21 – 4.06 (m, 2H), 3.79 (q, *J* = 7.2 Hz, 1H), 1.53 (d, *J* = 7.2 Hz, 3H), 1.22 (t, *J* = 7.1 Hz, 3H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 196.51, 174.06, 140.98, 137.90, 137.56, 132.47, 131.49, 130.06, 129.22, 128.93, 128.52, 128.29, 60.93, 45.44, 18.51, 14.12.

5a



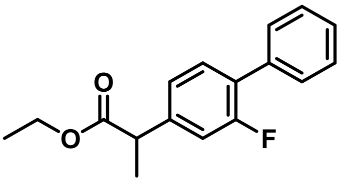
^{1}H NMR (600 MHz, $CDCl_3$) δ 7.36 – 7.29 (m, 2H), 7.29 – 7.23 (m, 1H), 7.09 (tt, *J* = 7.5, 1.1 Hz, 1H), 7.06 – 6.96 (m, 4H), 6.87 (ddd, *J* = 8.1, 2.4, 0.9 Hz, 1H), 4.17 – 4.05 (m, 2H), 3.67 (q, *J* = 7.2 Hz, 1H), 1.47 (d, *J* = 7.2 Hz, 3H), 1.19 (t, *J* = 7.1 Hz, 3H).
^{13}C NMR (151 MHz, $CDCl_3$) δ 174.24, 157.45, 157.20, 142.74, 129.85, 129.80, 123.34, 122.39, 118.96, 118.24, 117.42, 60.86, 45.51, 18.53, 14.19.

6a



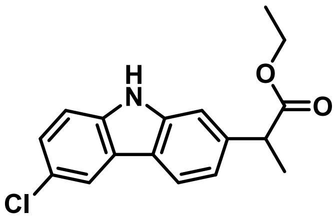
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.22 – 8.14 (m, 1H), 7.62 – 7.53 (m, 2H), 7.45 – 7.33 (m, 2H), 7.33 – 7.21 (m, 1H), 7.15 (dd, J = 8.0, 1.9 Hz, 1H), 4.35 (s, 2H), 4.22 – 3.96 (m, 2H), 3.70 (q, J = 7.2 Hz, 1H), 1.47 (d, J = 7.2 Hz, 3H), 1.20 (t, J = 7.1 Hz, 3H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 191.26, 173.88, 142.86, 140.20, 137.88, 136.15, 133.15, 132.48, 131.50, 131.44, 130.83, 128.64, 126.81, 126.33, 60.95, 51.05, 45.20, 18.54, 14.11.

7a



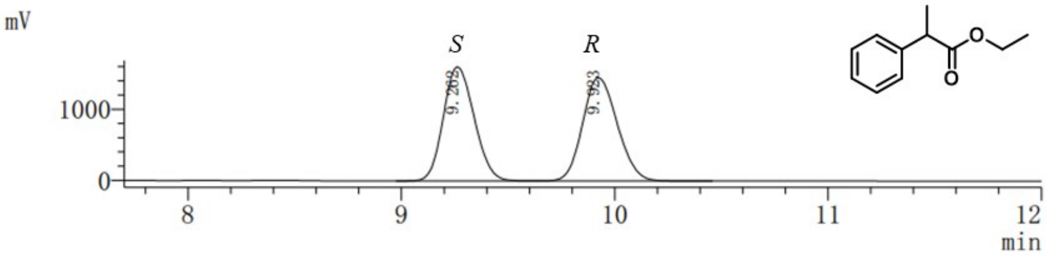
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.57 – 7.50 (m, 2H), 7.48 – 7.14 (m, 5H), 7.14 – 7.08 (m, 1H), 4.25 – 4.03 (m, 2H), 3.74 (q, J = 7.2 Hz, 1H), 1.53 (d, J = 7.2 Hz, 3H), 1.24 (t, J = 7.1 Hz, 3H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 173.99 (s), 160.78 (t, J = 225.2 Hz), 142.00 (d, J = 7.7 Hz), 135.56 (s), 130.76 (d, J = 4.0 Hz), 128.95 (d, J = 3.0 Hz), 128.43 (s), 127.76 (d, J = 13.6 Hz), 127.64 (s), 123.52 (d, J = 3.3 Hz), 115.23 (d, J = 23.7 Hz), 60.99 (s), 45.08 (s), 18.44 (s), 14.13 (s).
^{19}F NMR (377 MHz, $CDCl_3$) δ -117.70.

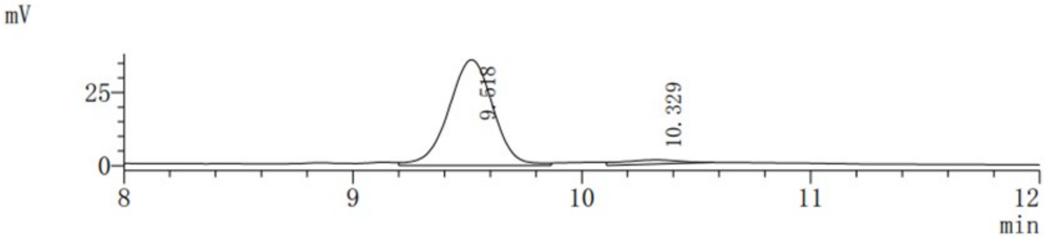
8a



^{1}H NMR (400 MHz, $CDCl_3$) δ 8.23 (s, 1H), 7.94 (d, J = 1.4 Hz, 1H), 7.90 (d, J = 8.1 Hz, 1H), 7.36 – 7.13 (m, 4H), 4.26 – 3.97 (m, 2H), 3.86 (q, J = 7.1 Hz, 1H), 1.57 (d, J = 7.2 Hz, 3H), 1.26 – 1.17 (m, 3H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 175.15, 140.43, 139.28, 138.12, 125.73, 124.83, 124.25, 121.57, 120.55, 119.89, 119.59, 111.62, 109.57, 61.01, 46.01, 18.93, 14.16.

1a





检测器A Ch1 214nm

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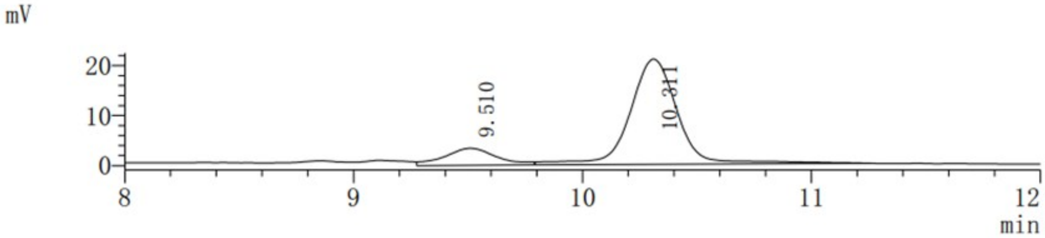

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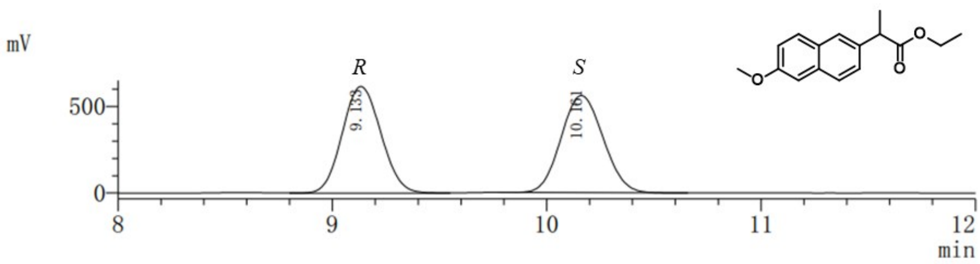
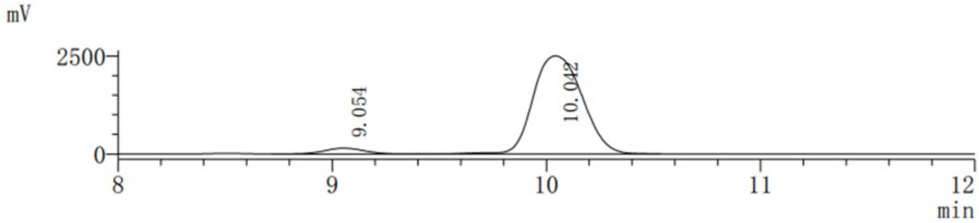
2a





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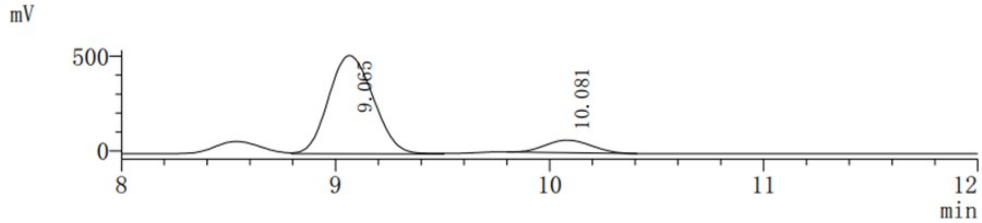
2	10.042	39935729	95.315



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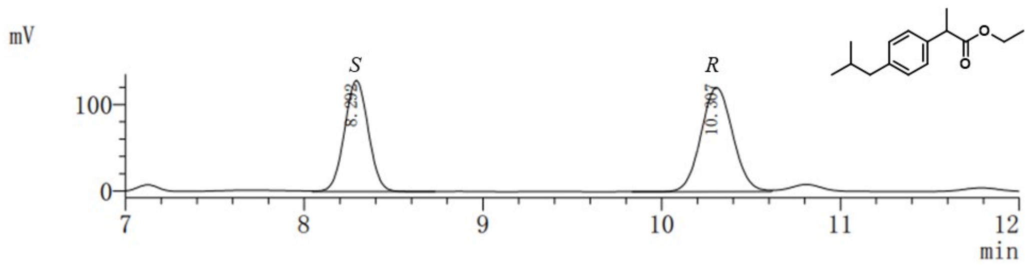
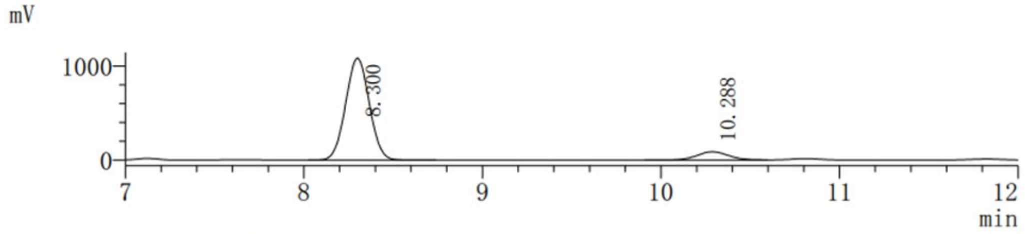
3a





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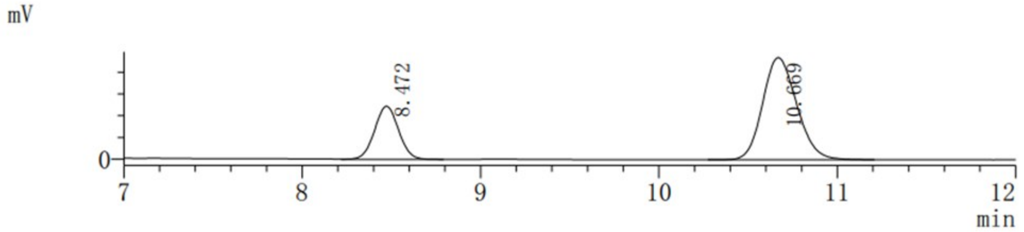

检测器A Ch1 214nm

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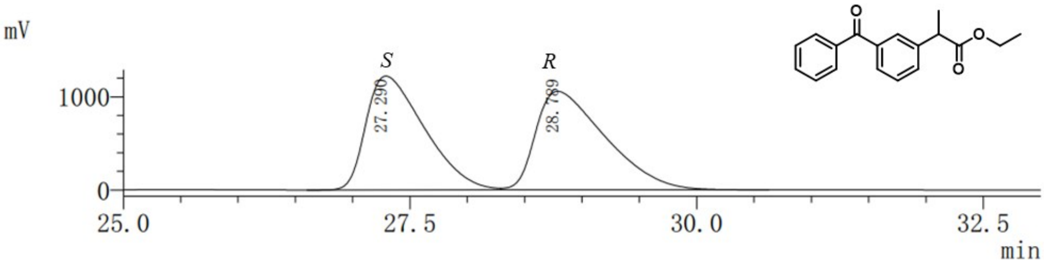
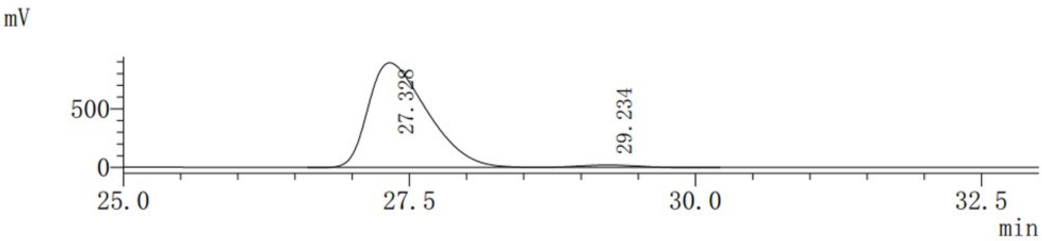
2	10.669	6225088	73.085

4a





检测器A Ch1 214nm

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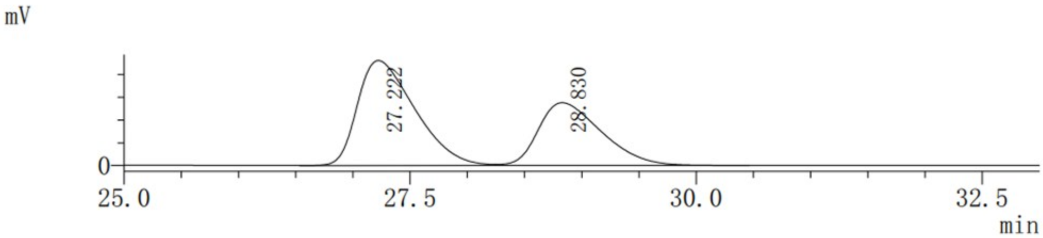

检测器A Ch1 214nm

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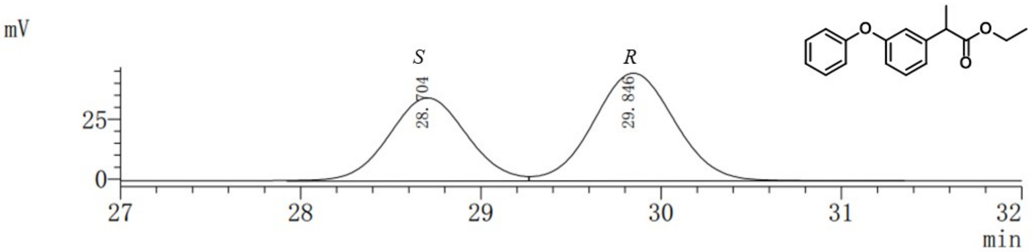
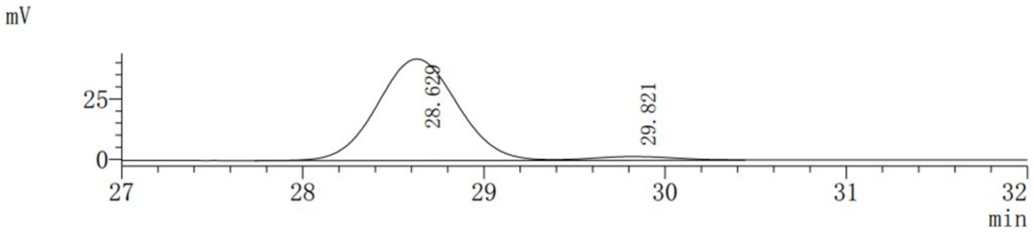
2	28.830	21158831	39.682
总计			100.000

5a





检测器A Ch1 214nm

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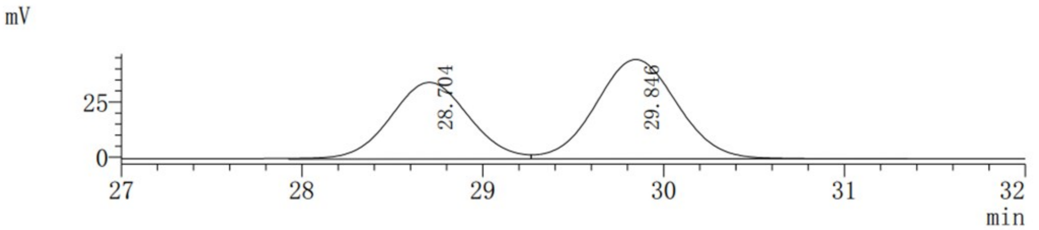

检测器A Ch1 214nm

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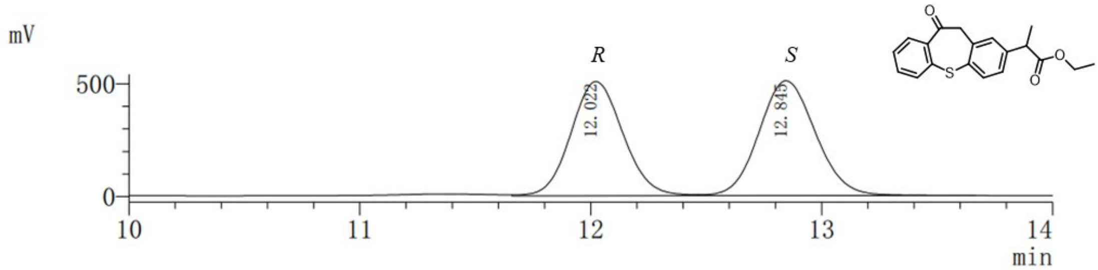
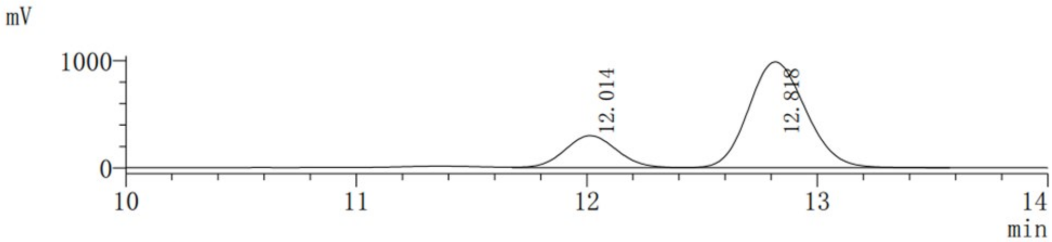
6a





检测器A Ch1 214nm

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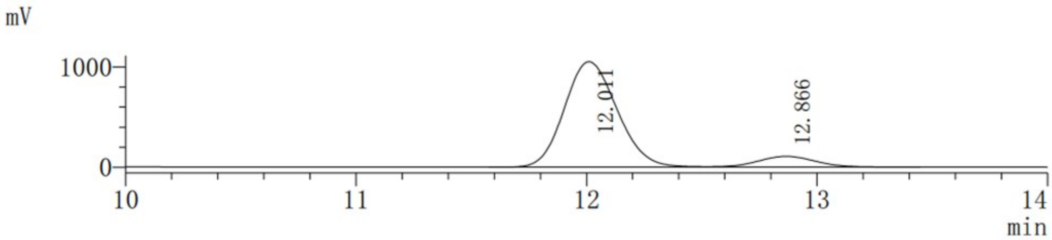
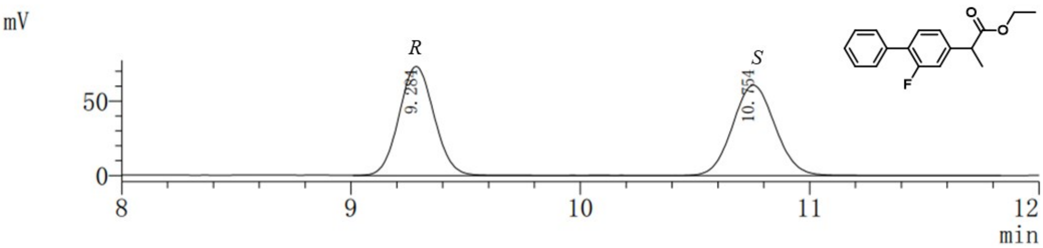
1	12.014	4745575	21.774
2	12.818	17049193	78.226

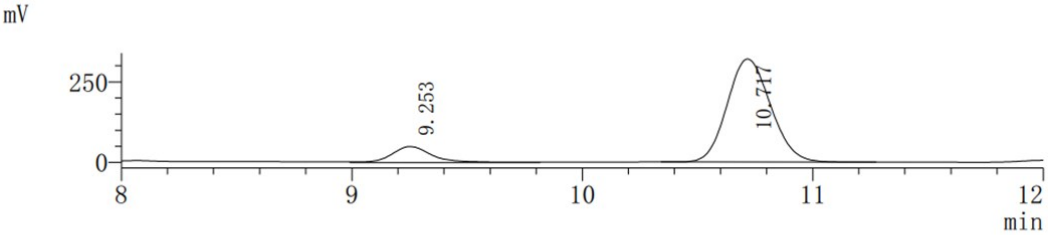


检测器A Ch1 214nm

Peak[#]	RetTime[min]	Area[uV*s]	Area[%]
1	12.011	16740178	89.929
2	12.866	1874603	10.071

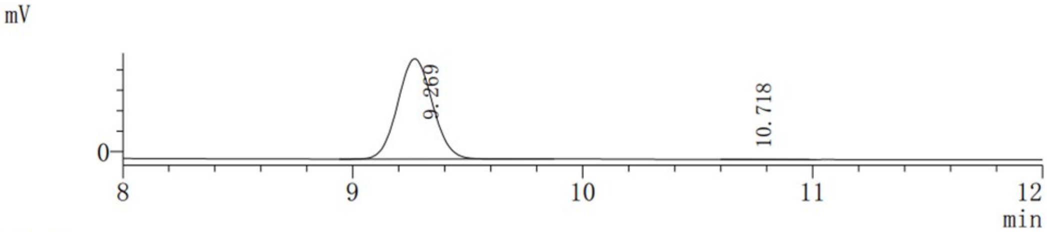
7a





检测器A Ch1 214nm

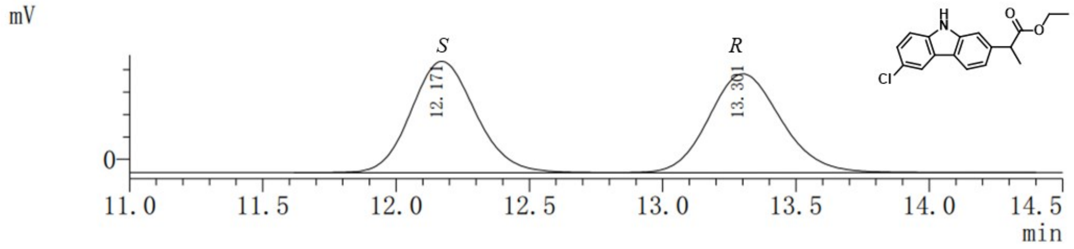
Peak[#]	RetTime[min]	Height[uV]	Width[min]	Area[uV*s]	Area[%]
1	9.253	49704	0.172	607774	12.669
2	10.717	319819	0.204	4189503	87.331

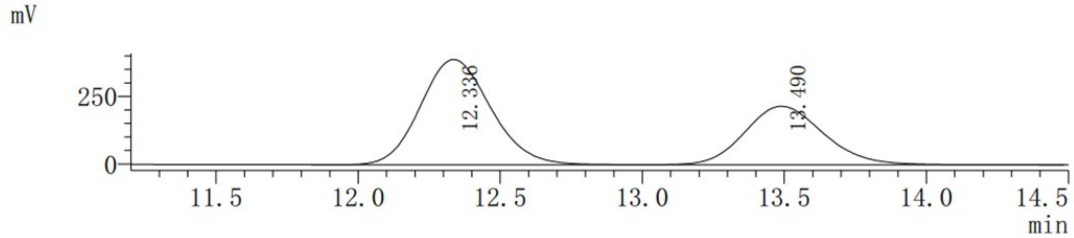


检测器A Ch1 214nm

Peak[#]	RetTime[min]	Height[uV]	Width[min]	Area[uV*s]	Area[%]
1	9.269	98038	0.164	1035567	99.887
2	10.718	126	0.170	1167	0.113

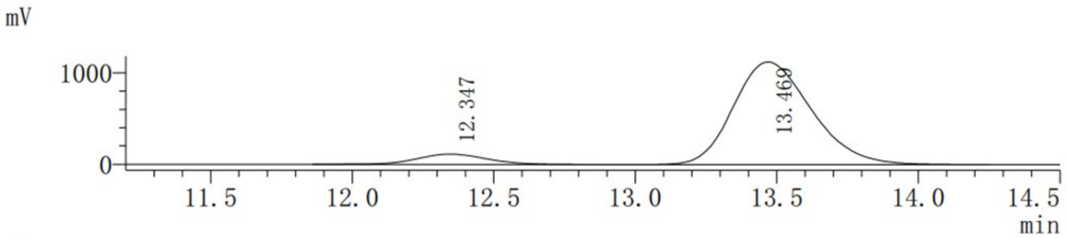
8a





检测器A Ch1 214nm

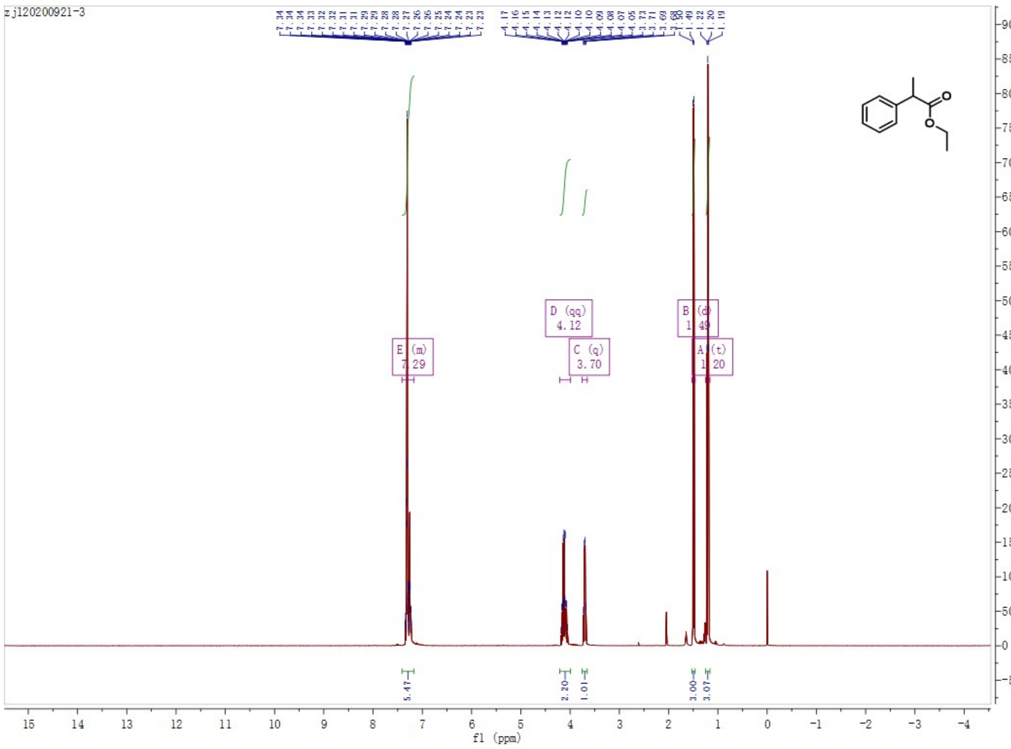
Peak[#]	RetTime[min]	Height[uV]	Width[min]	Area[uV*s]	Area[%]
1	12.336	389234	0.267	6730814	61.587
2	13.490	216585	0.297	4198119	38.413



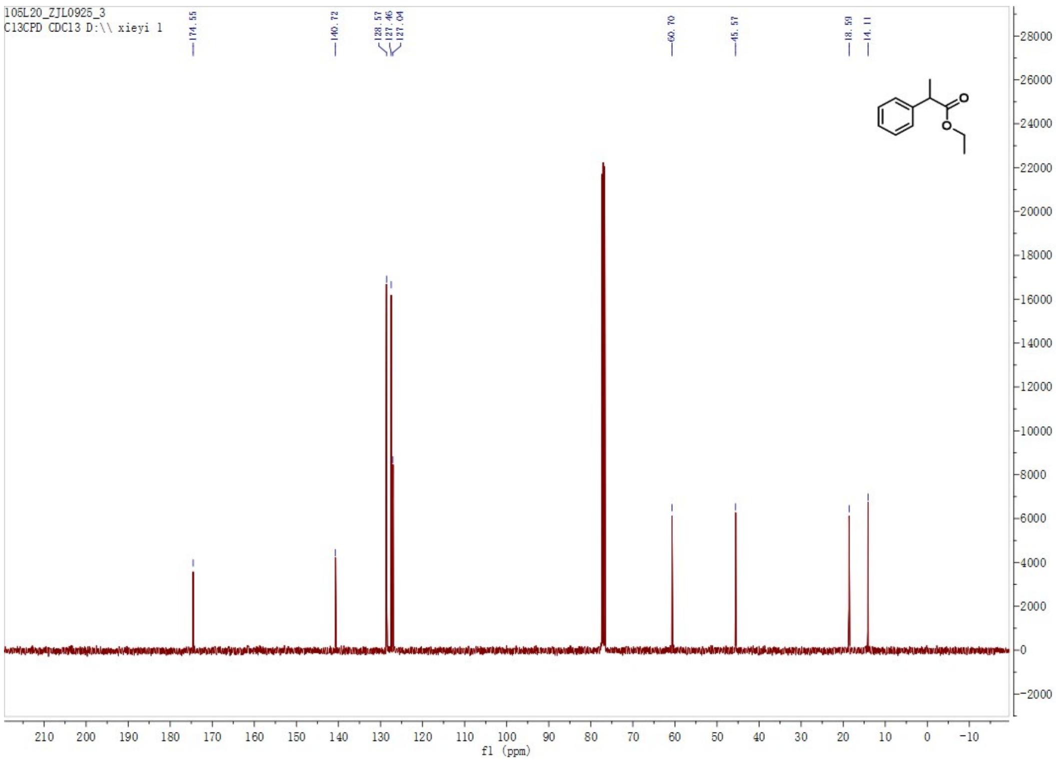
检测器A Ch1 214nm

Peak[#]	RetTime[min]	Height[uV]	Width[min]	Area[uV*s]	Area[%]
1	12.347	113287	0.264	1946680	8.305
2	13.469	1121333	0.297	21494578	91.695

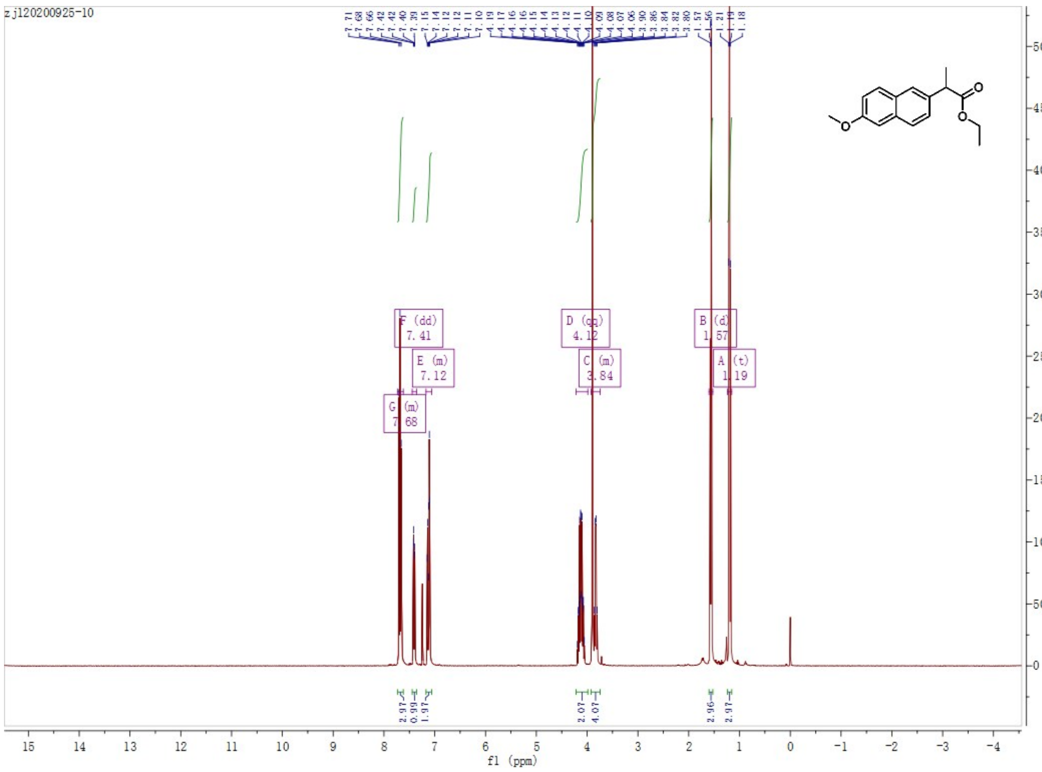
^{1}H NMR of **1a**



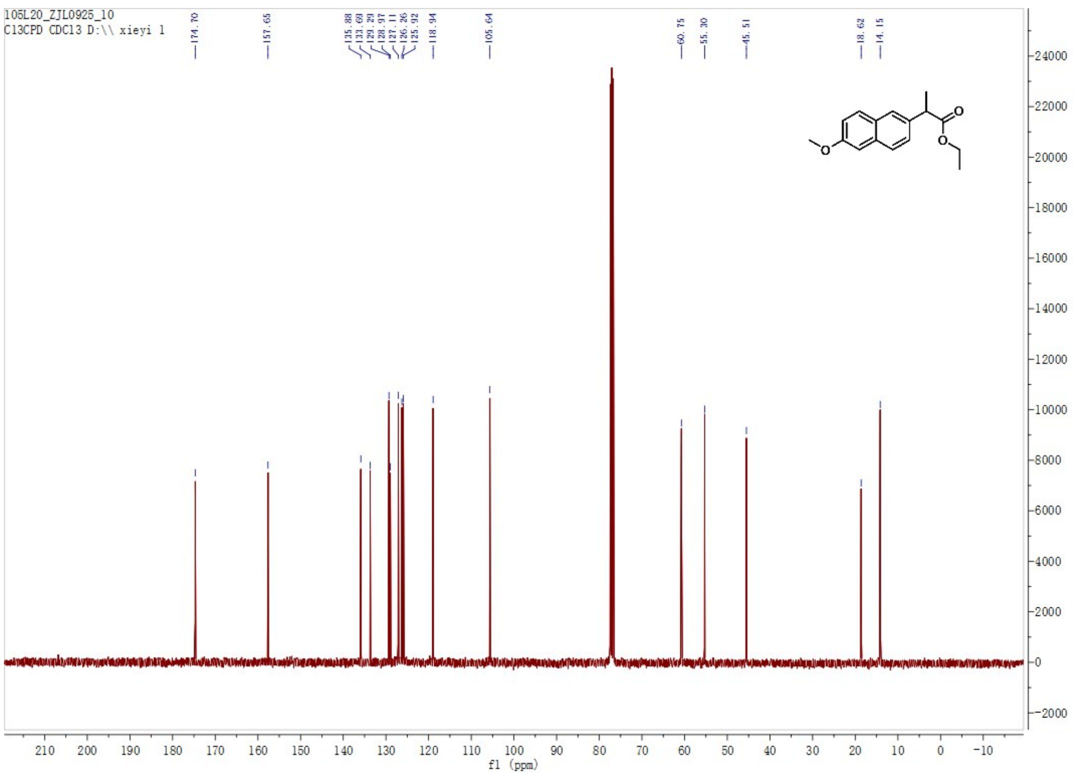
^{13}C NMR of **1a**



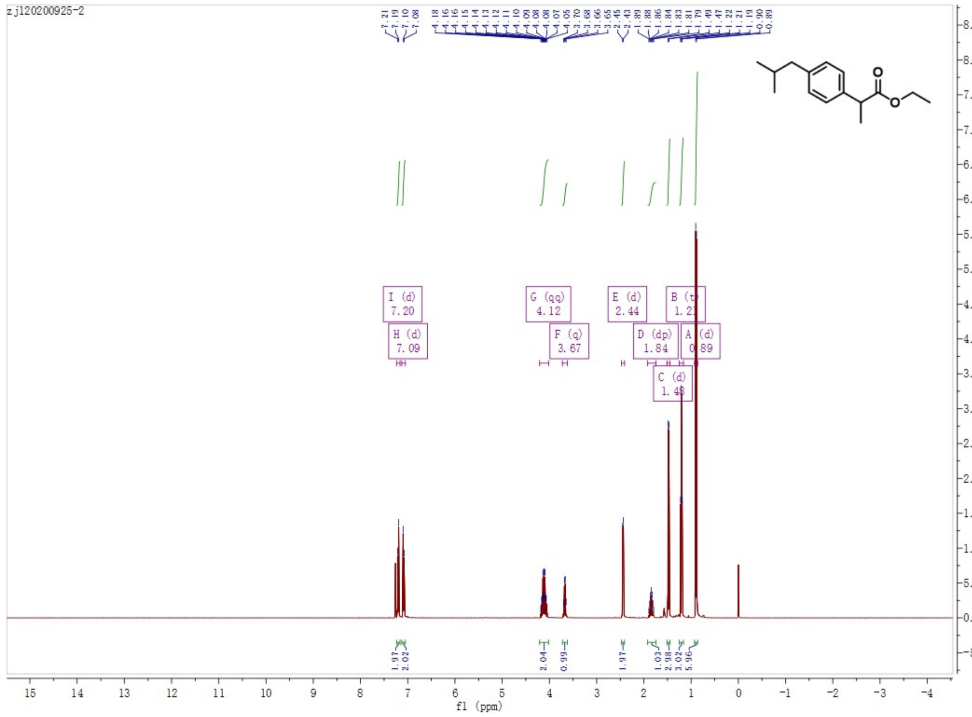
^{1}H NMR of **2a**



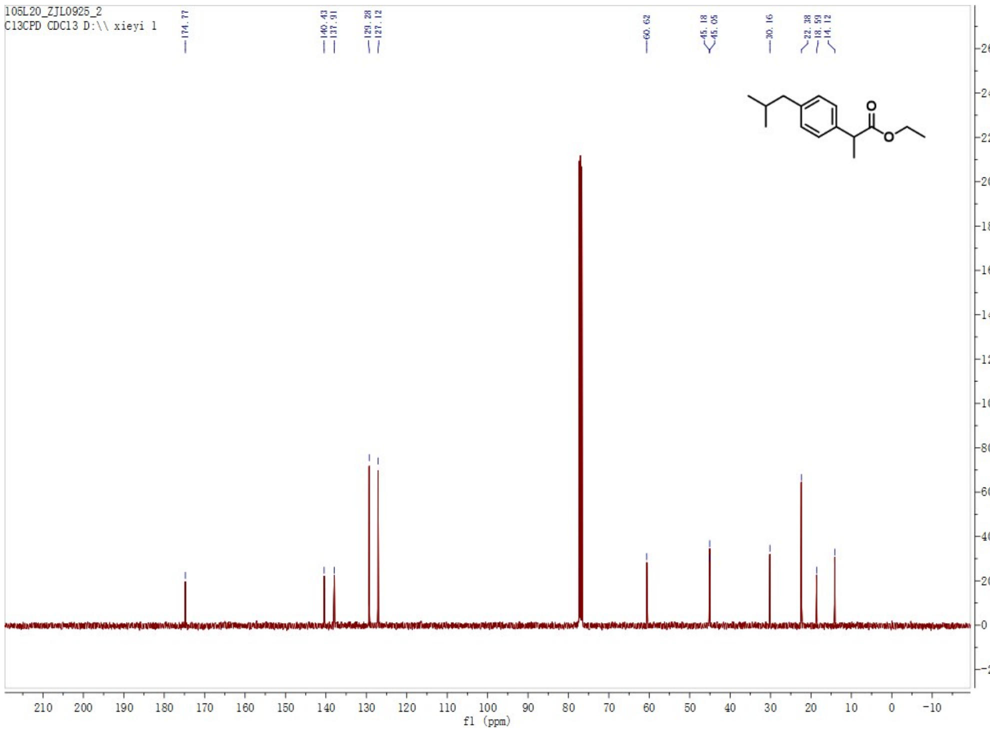
^{13}C NMR of **2a**



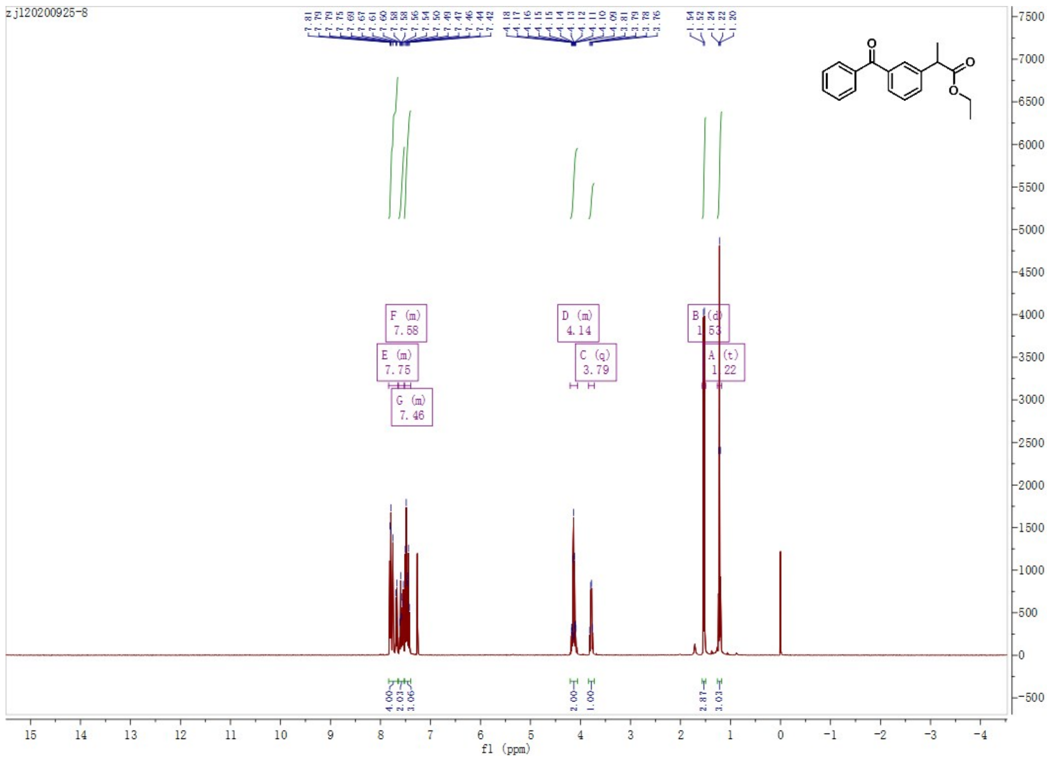
^{1}H NMR of **3a**



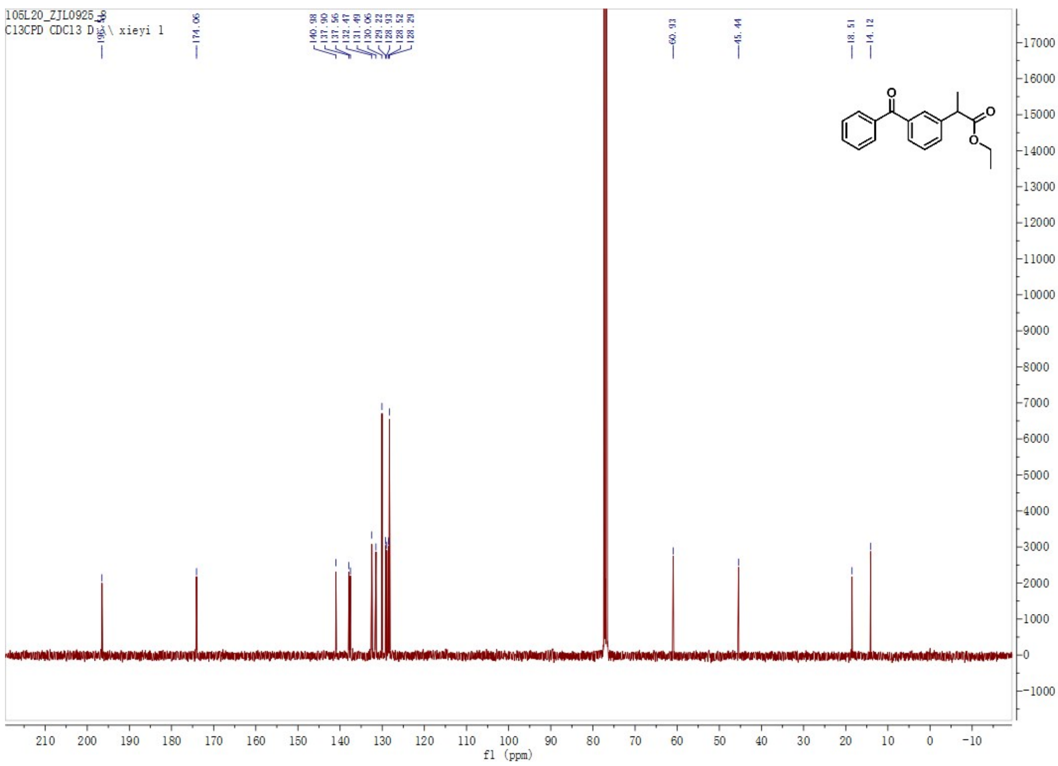
^{13}C NMR of **3a**



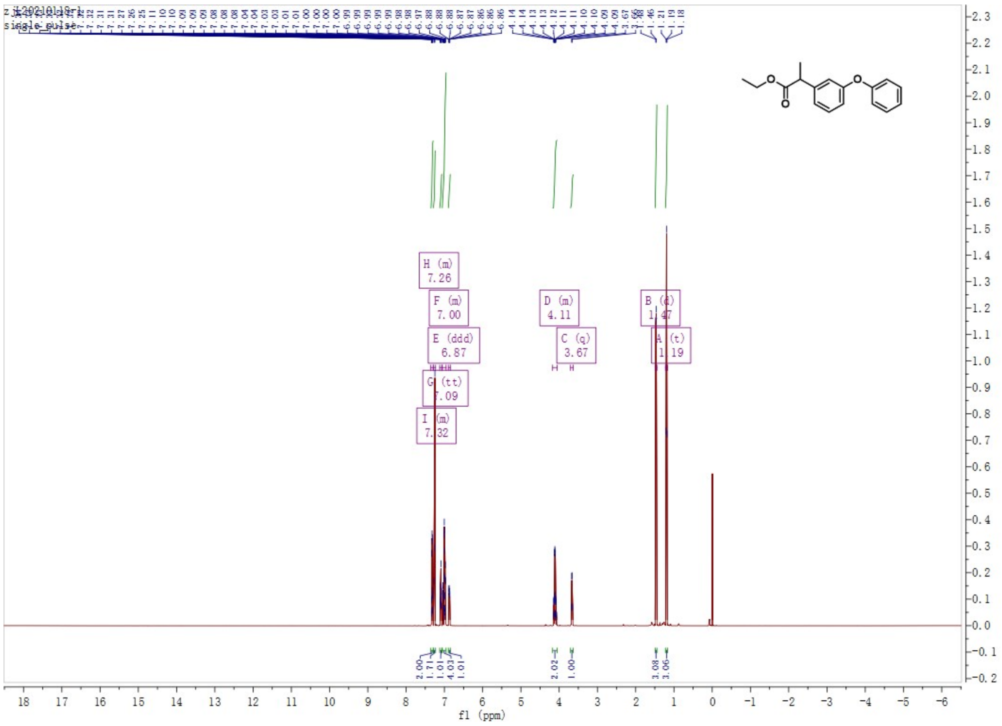
^{1}H NMR of **4a**



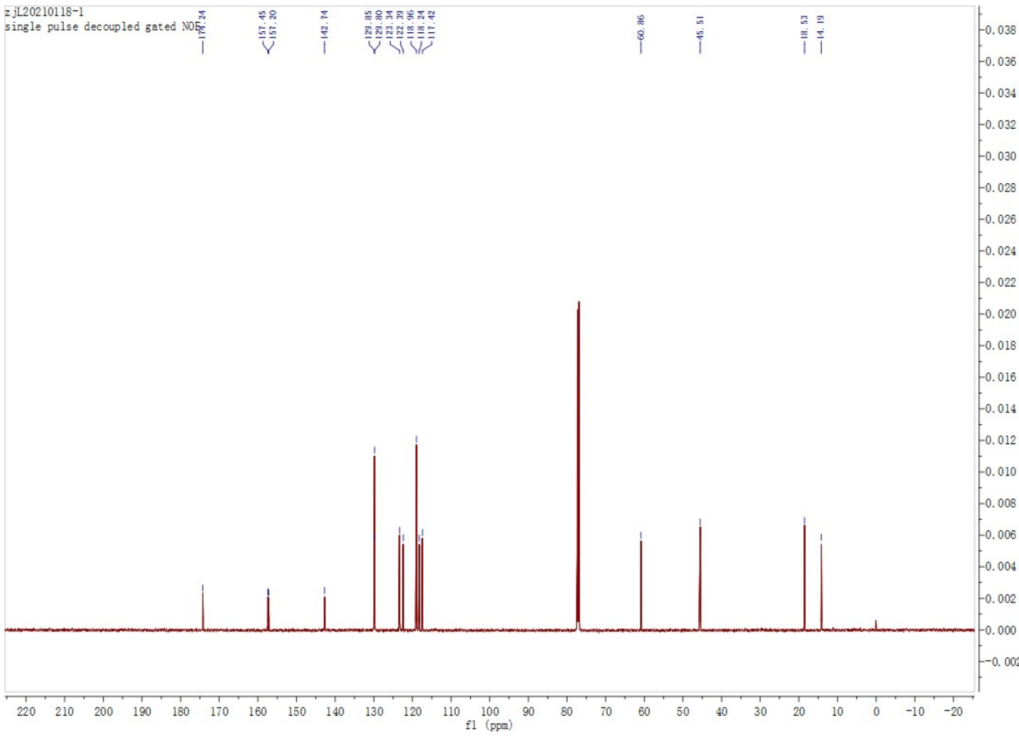
^{13}C NMR of **4a**



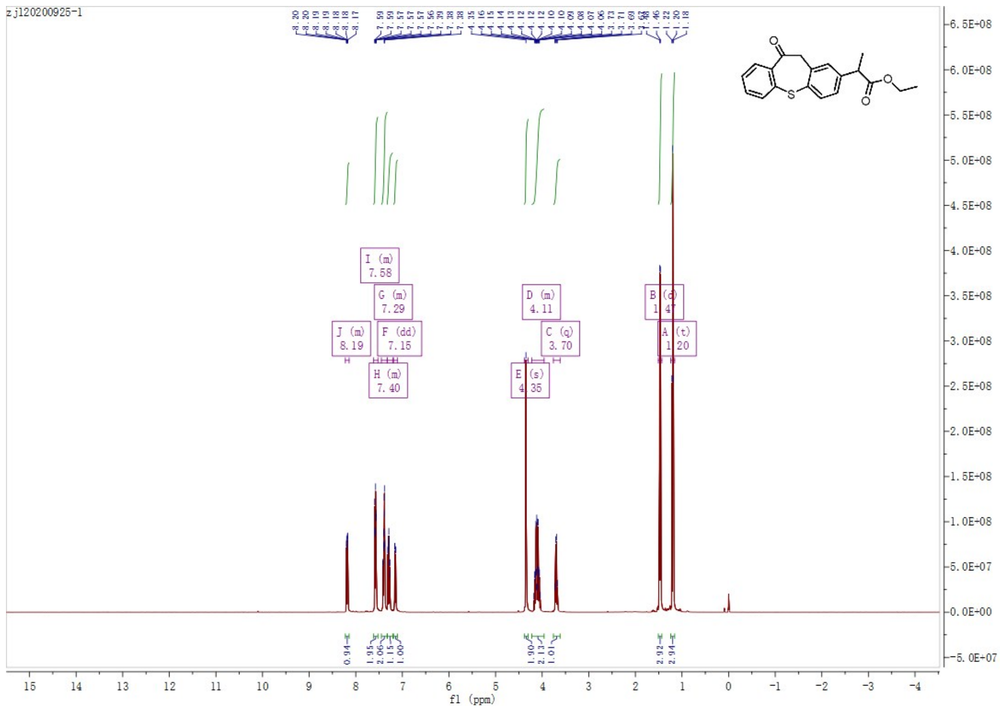
[1]H NMR of **5a**



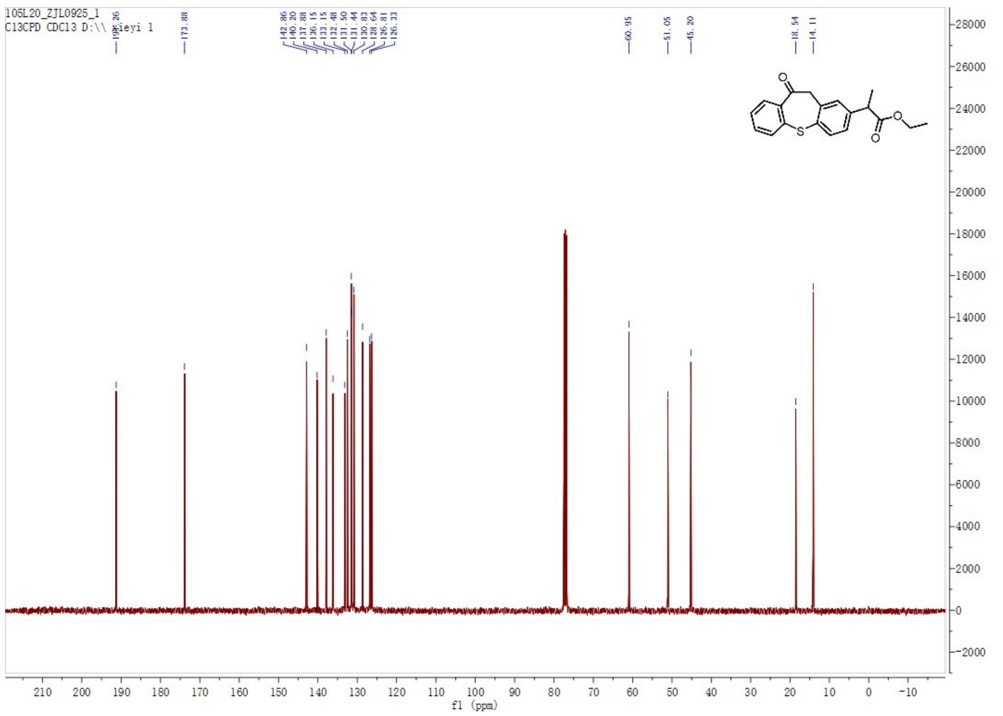
[13]C NMR of **5a**



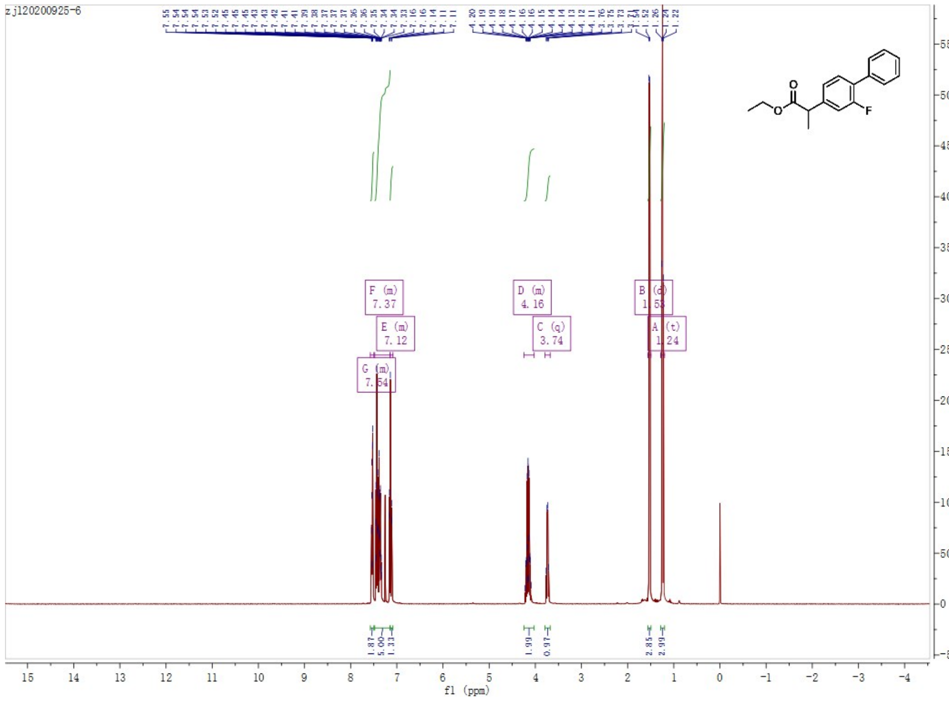
[1]H NMR of **6a**



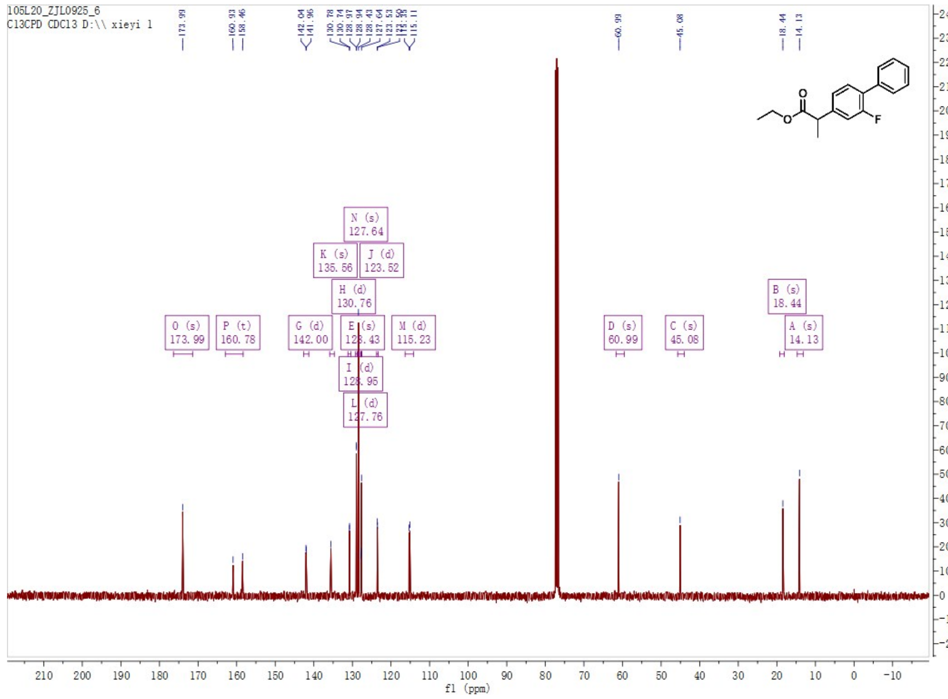
[13]C NMR of **6a**



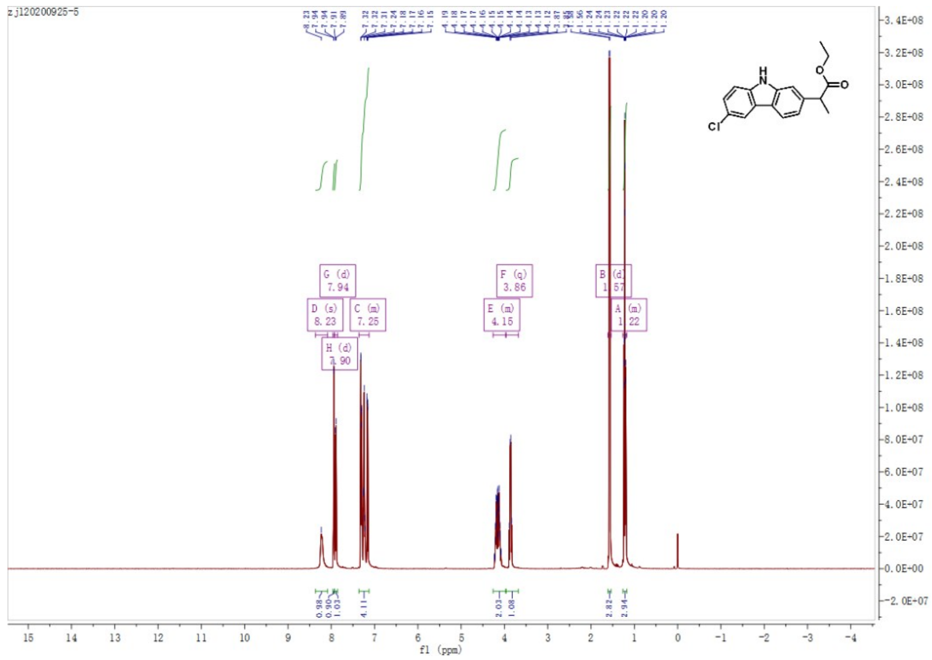
^{1}H NMR of **7a**



^{13}C NMR of **7a**



^{1}H NMR of **8a**



^{13}C NMR of **8a**