Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.
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Supporting information for:

Deracemization of Racemic Alcohols Combining Photooxidation and Biocatalytic Reduction

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1. General information

Unless otherwise noted, all reagents were obtained commercially and used without further purification. The ^{1}H and ^{13}C NMR spectra were recorded with a Bruker AMX400 MHz spectrometer using TMS as an internal standard in $CDCl_3$. All known products were characterized by comparison of ^{1}H and ^{13}C NMR data with those reported in the literature. Yields and stereoselectivity were determined by chiral GC with Agilent CP-chirasil-Dex CB column or chiral HPLC with a Chiralpak OJ-H column (250 mm×4.6 mm, n-hexane/2-propanol as the mobile phase) and a UV detector (220 nm). Absolute configuration was confirmed by comparison with literature values. The power of the *LEDs* is 8W and the source spectrum is 450-455 nm.

Preparation of reductases（KtCR and Ras-ADH）

100 μL stored bacteria was first incubated in 5 mL LB media with Kanamycin (50 μg/mL) and then shaking at 37 °C for overnight. 5 mL preculture was added to 500 mL fresh LB medium with 50 μg/mL Kanamycin. The cultures were shaken at 37 °C until OD_{600} at 0.6 and cooled at 4 °C for 30 min, then isopropyl β-thiogalactopyranoside (IPTG) was added to a final concentration of 0.5 mM to induce reductases expression at 25 °C. Cells were harvested by centrifugation, and resuspended in buffer (50 mM sodium phosphate buffer, pH 6.5 for KtCR and Ras-ADH) for whole-cell reaction. In order to obtain the crude enzyme solution and determine the protein concentration, the cell suspension was then repeated freezing and thawing for 3 times, and released the target proteins by sonication. The cell lysate was removed by centrifugation. The supernatant was used for SDS-PAGE analysis of KtCR and Ras-ADH, and the result was shown in Figure S1.

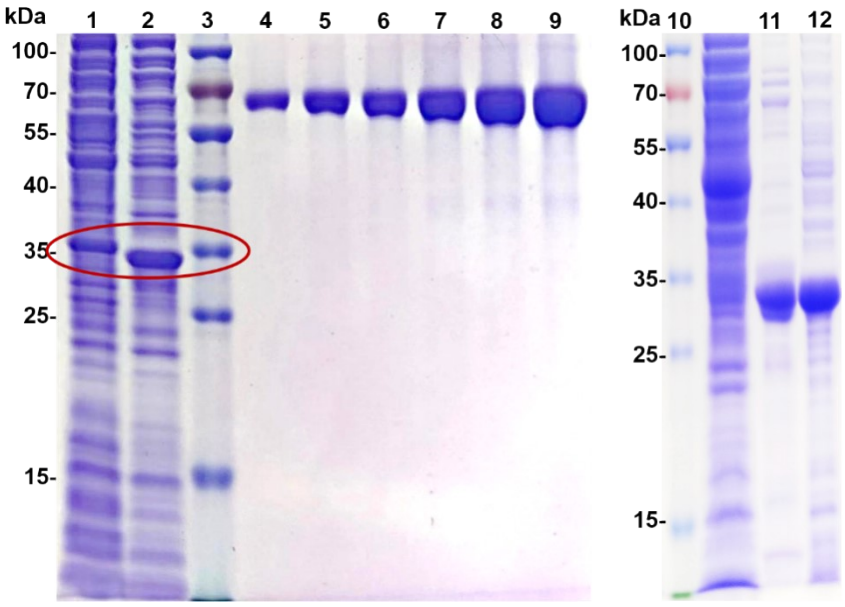


Figure S1 SDS-PAGE analysis of KtCR and Ras-ADH crude extract and purified protein. Lane 1: cell free extract of KtCR; Lane 2: cell free extract of Ras-ADH; Lane 3 and 10: protein markers; Lane 4-9: BAS 0.25, 0.50, 0.75, 1.00, 1.50, 2.00 mg/ml; Lane 11: purified protein of KtCR; Lane 12: purified protein of Ras-ADH.

Preparation of racemic alcohols

The given ketone (2 mmol) was added into a stirred solution of $NaBH_4$ (5 mmol) in methanol (10 mL) at room temperature (20 °C). The solution was stirred until the complete disappearance of ketone substrate indicated by TLC. The crude product was evaporated in vacuo and diluted in dichloromethane (20 mL), and then washed with water (10 mL). The organic phase was separated and dried over anhydrous sodium sulfate, and then evaporated in vacuum.

General procedure for one-pot reaction

SAS (5.0 mM, 20% mol) was dissolved in 2 ml mixture solution of acetonitrile and water (30% v/v acetonitrile as cosolvent) containing substrate (25mM) in a 25 ml conical flask. After irradiation with blue *LED*s (5 cm away from the conical flask) for 12 hours (the reaction time could be extended to ensure no residual alcohols), 10 mL whole cell culture of ketoreductase (resuspended in 50 mM sodium phosphate buffer, pH 6.5) with glucose (250 mM) was added into the photocatalytic mixture and shaken at 30 °C for overnight. The bio-reduction was performed until the complete consumption of phenethyl alcohols determined by GC which ensure the incomplete optical yields of products are due to enzyme specificity. The solution was extracted with equal volume of ethyl acetate for three times, and the stereoselectivity was then determined by chiral GC. The turnover numbers of the two reductase (KtCR and Ras-ADH) used for the model substrate was 2000 and 1250, respectively.

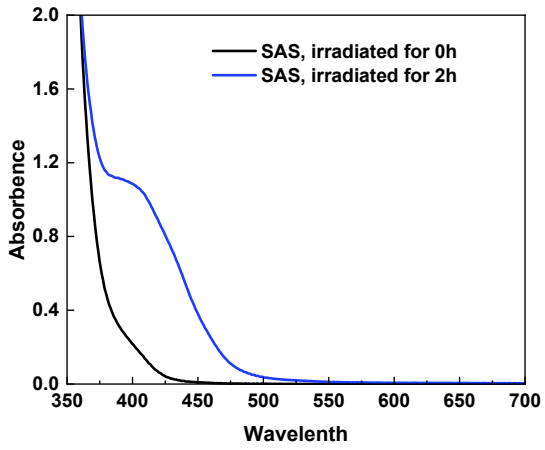


Figure S2 UV-Vis spectra of non-irradiated SAS and irradiated SAS in water for 2 h.

Scaling-up one-pot reaction catalyzed by photocatalyst and ketoreductase

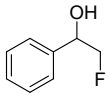
The scale-up reaction was performed as follows: SAS (0.2 mmol, 20 mol%) and 1 mmol **1a** was

dissolved in 40 mL mixture solution of acetonitrile and water (30% v/v acetonitrile as cosolvent) in a 1000 ml conical flask. The reaction mixture was irradiated with blue *LED*s (8W×4, 450-455 nm, 5 cm away from the conical flask) for 20 hours (the reaction time could be extended to ensure no residual alcohols). 400 ml whole-cell culture of ketoreductase (resuspended in 50 mM sodium phosphate buffer, pH 6.5) with glucose (10 mmol) was added into the photocatalytic reaction mixture and shaken at 30 °C for overnight. The reaction solution was extracted with ethyl acetate for three times, then the organic phase was dried over anhydrous sodium sulfate and concentrated in vacuum. The obtained crude product was further separated and purified by flash column chromatography.

2. Characterization data

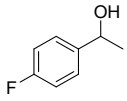
1-phenylethanol (1a)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44-7.31 (m, 4H), 7.31-7.22 (m, 1H), 4.89 (q, *J* = 6.5 Hz, 1H), 1.49 (d, *J* = 6.5 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 145.8, 128.5, 127.5, 125.4, 70.4, 25.2. HRMS (EI-TOF): *m/z* $[M]^+$ calc. for $C_8H_{10}O^+[M]^+$: 122.0732, found:122.0733.



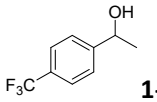
2-fluoro-1-phenylethanol (1b)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39-7.29 (m, 5H), 5.10-4.90 (m, 1H), 4.61-4.26 (m, 2H), 2.64 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.1 (d, *J* = 8.5 Hz), 128.8-128.35 (m), 126.4 (s), 88.1 (s), 86.4 (s), 73.0 (d, *J* = 19.4 Hz). HRMS (EI-TOF): *m/z* $[M]^+$ calc. for C_8H_9FO: 140.0637, found: 140.0636.



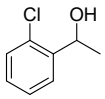
1-(4-fluorophenyl)ethanol[1] (1c)

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.37-7.30 (m, 2H), 7.06-6.99 (m, 2H), 4.88 (q, J = 6.3 Hz, 1H), 1.47 (d, J = 6.4 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 162.2(d, $J_{C\text{-}F}$ = 243.0 Hz), 141.5(d, $J_{C\text{-}F}$ = 3.0 Hz), 127.0(d, $J_{C\text{-}F}$ = 8.0 Hz), 115.3(d, $J_{C\text{-}F}$ = 21.0 Hz), 69.8, 25.3. HRMS (EI-TOF): *m/z* $[M]^+$ calc. for C_8H_9FO: 140.0637, found: 140.0636.



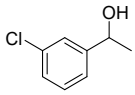
1-(4-trifluoromethyl)ethanol (1d)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.61 (d, *J* = 8.2 Hz, 2H), 7.49 (d, *J* = 8.2 Hz, 2H), 4.96 (q, *J* = 6.5 Hz, 1H), 1.50 (d, *J* = 6.5 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 149.7, 129.6(q, $J_{C\text{-}F}$ = 64 Hz), 128.2 125.7, 125.5(d, $J_{C\text{-}F}$ = 3.7 Hz), 122.8, 120.1, 69.8, 25.4. HRMS (EI-TOF): *m/z* $[M]^+$ calc. for $C_9H_9F_3O$: 190.0605, found: 190.0606.



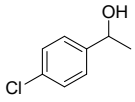
1-(2-chlorophenyl)ethanol (1e)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.59 (dd, *J* = 7.7, 1.6 Hz, 1H), 7.35 – 7.24 (m, 2H), 7.20 (td, *J* = 7.6, 1.7 Hz, 1H), 5.29 (q, *J* = 6.4 Hz, 1H), 1.49 (d, *J* = 6.4 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 143.0, 131.6, 129.4, 128.4, 127.2, 126.4, 67.0, 23.5. HRMS (EI-TOF): *m/z* $[M]^+$ calc. for C_8H_9ClO: 156.0342, found: 156.0342.



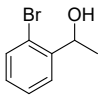
1-(3-chlorophenyl)ethanol (1f)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30 – 7.22 (m, 3H), 4.87 (q, *J* = 6.5 Hz, 1H), 1.48 (d, *J* = 6.5 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 147.9, 134.4, 129.8, 127.6, 125.6, 123.6, 69.8, 25.3. HRMS (EI-TOF): *m/z* $[M]^+$ calc. for C_8H_9ClO: 156.0342, found: 156.0342.



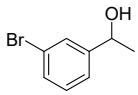
1-(4-chlorophenyl)ethanol (1g)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 (s, 3H), 4.88 (q, *J* = 6.5 Hz, 1H), 1.47 (d, *J* = 6.5 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 144.3, 133.1, 128.6, 126.8, 69.8, 25.3. HRMS (EI-TOF): *m/z* $[M]^+$ calc. for C_8H_9ClO: 156.0342, found: 156.0343.



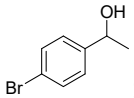
1-(2-bromophenyl)ethanol[2](1h)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58 (dd, J = 7.8, 1.6 Hz, 1H), 7.50 (dd, J = 8.0, 1.1 Hz, 1H), 7.37 – 7.29 (m, 1H), 7.11 (td, J = 7.7, 1.7 Hz, 1H), 5.22 (qd, J = 6.3, 3.2 Hz, 1H), 2.23 (d, J = 3.1 Hz, 1H), 1.47 (d, J = 6.4 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 144.6, 132.7, 128.8, 127.9, 126.7, 121.7, 69.2, 23.6.^{13}C NMR (100 MHz, $CDCl_3$) δ 144.6, 132.7, 128.8, 127.9, 126.7, 121.7, 69.2, 23.6. HRMS (EI-TOF): *m/z* $[M]^+$ calc. for C_8H_9OBr: 199.9837, found: 199.9835.



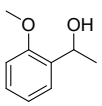
1-(3-bromophenyl)ethanol (1i)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53 (d, *J* = 1.7 Hz, 1H), 7.45 – 7.34 (m, 1H), 7.29 (d, *J* = 7.7 Hz, 1H), 7.21 (t, *J* = 7.8 Hz, 1H), 4.86 (q, *J* = 6.4 Hz, 1H), 1.48 (d, *J* = 6.5 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 148.1, 130.5, 130.1, 128.6, 124.0, 122.6, 69.8, 25.3. ^{13}C NMR (100 MHz, $CDCl_3$) δ 148.1, 130.5, 130.1, 128.6, 124.0, 122.6, 69.8, 25.3. HRMS (EI-TOF): *m/z* $[M]^+$ calc. for C8H9BrO: 199.9837, found: 199.9837.



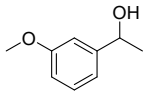
1-(4-bromophenyl)ethanol[3] (1j)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49 – 7.42 (m, 2H), 7.24 (t, J = 7.4 Hz, 2H), 4.85 (q, J = 6.4 Hz, 1H), 2.05 (s, 1H), 1.46 (d, J = 6.5 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 144.8, 131.6, 127.2, 121.2, 69.8, 25.3. HRMS (EI-TOF): *m/z* $[M]^+$ calc. for C_8H_9OBr: 199.9837, found: 199.9839.



1-(2-methoxyphenyl)ethanol (1k)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34 (dd, *J* = 7.5, 1.5 Hz, 1H), 7.25 (ddd, *J* = 9.4, 7.0, 1.7 Hz, 1H), 6.96 (td, *J* = 7.5, 0.9 Hz, 1H), 6.88 (d, *J* = 8.2 Hz, 1H), 3.86 (s, 3H), 1.50 (d, *J* = 6.5 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.7, 133.4, 128.3, 126.1, 120.8, 110.4, 66.6, 55.3, 22.8. HRMS (EI-TOF): *m/z* $[M]^+$ calc. for $C_9H_{12}O_2$: 152.0837, found: 152.0837.

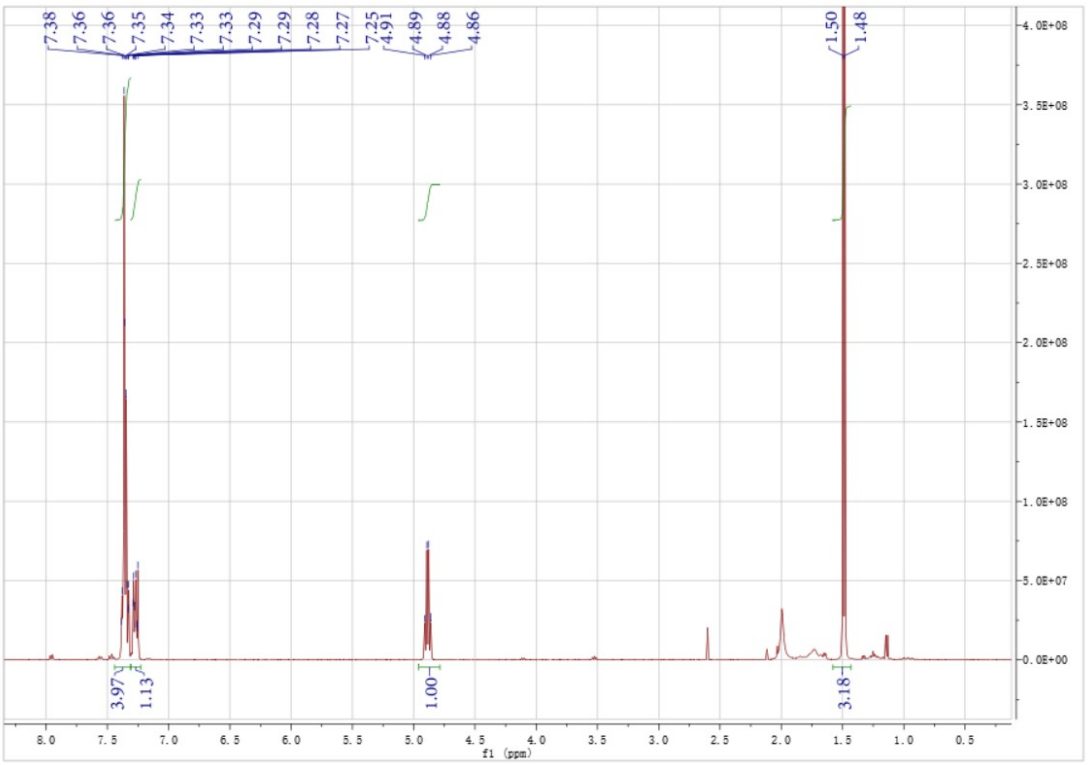


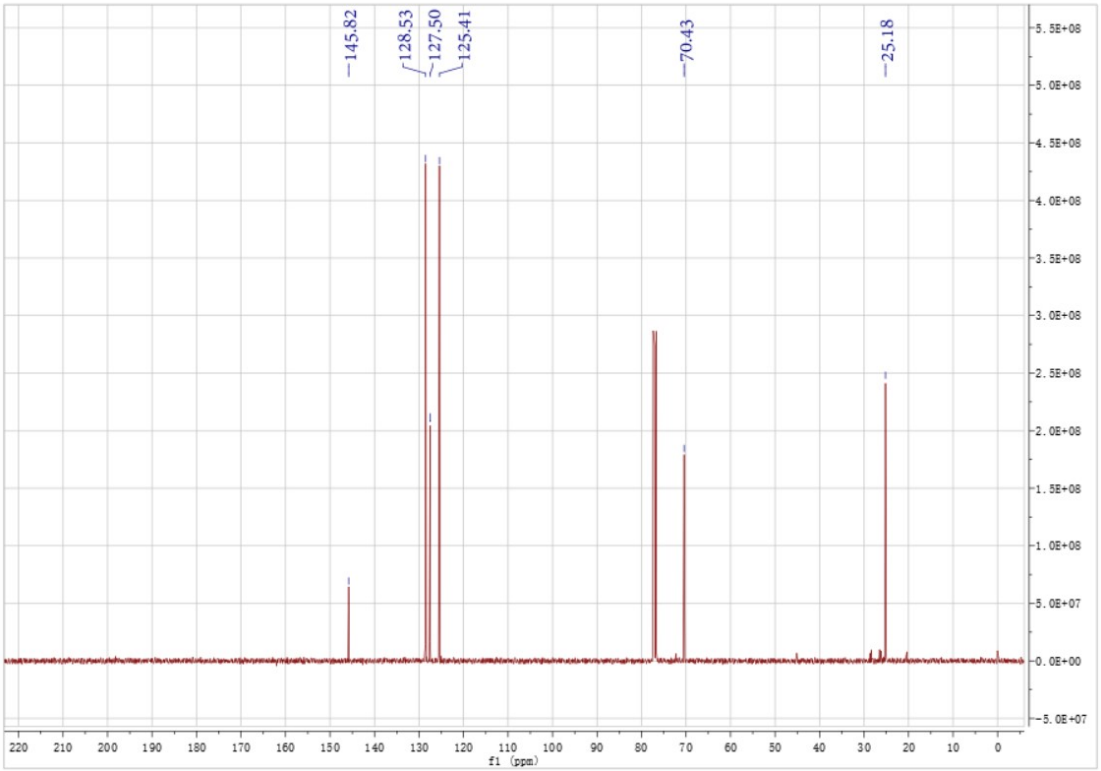
1-(3-methoxyphenyl)ethanol (1l)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28 – 7.23 (m, 1H), 7.02 – 6.88 (m, 2H), 6.86 – 6.76 (m, 1H), 3.81 (s, 3H), 1.48 (d, *J* = 6.5 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.8, 147.6, 129.6, 117.7, 112.9, 110.9, 70.4, 55.2, 25.2. HRMS (EI-TOF): *m/z* $[M]^+$ calc. for $C_9H_{12}O_2$: 152.0837, found: 152.0838.

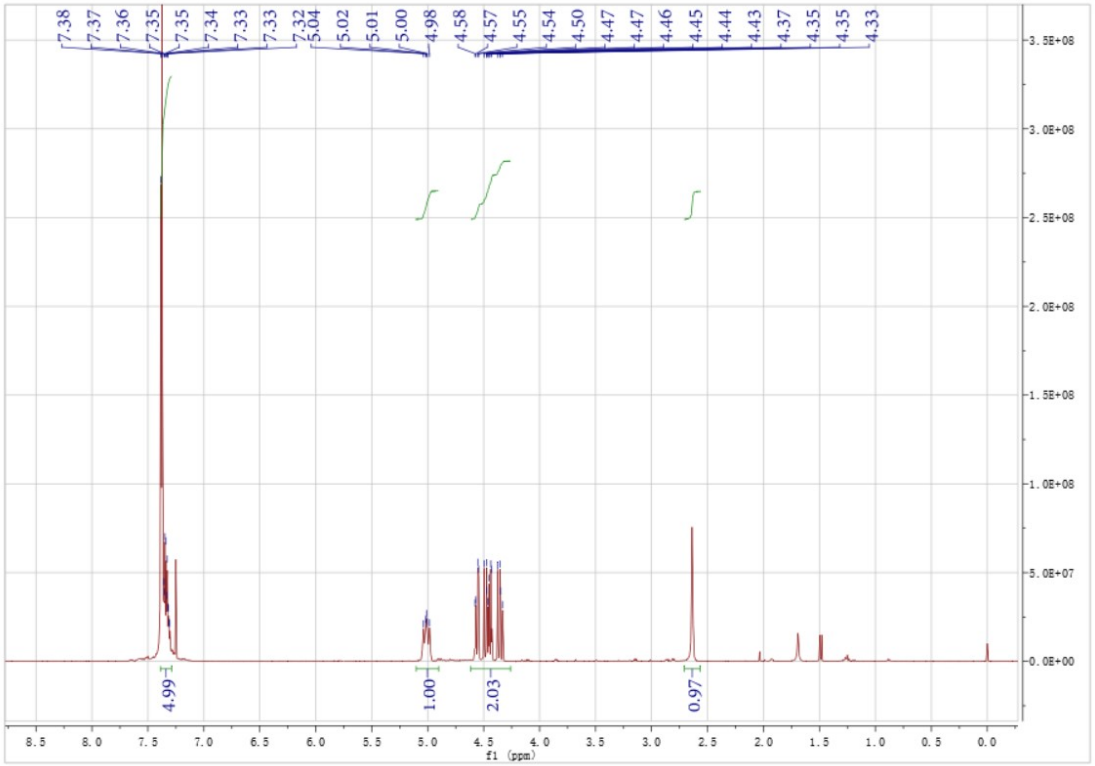
3. NMR spectra

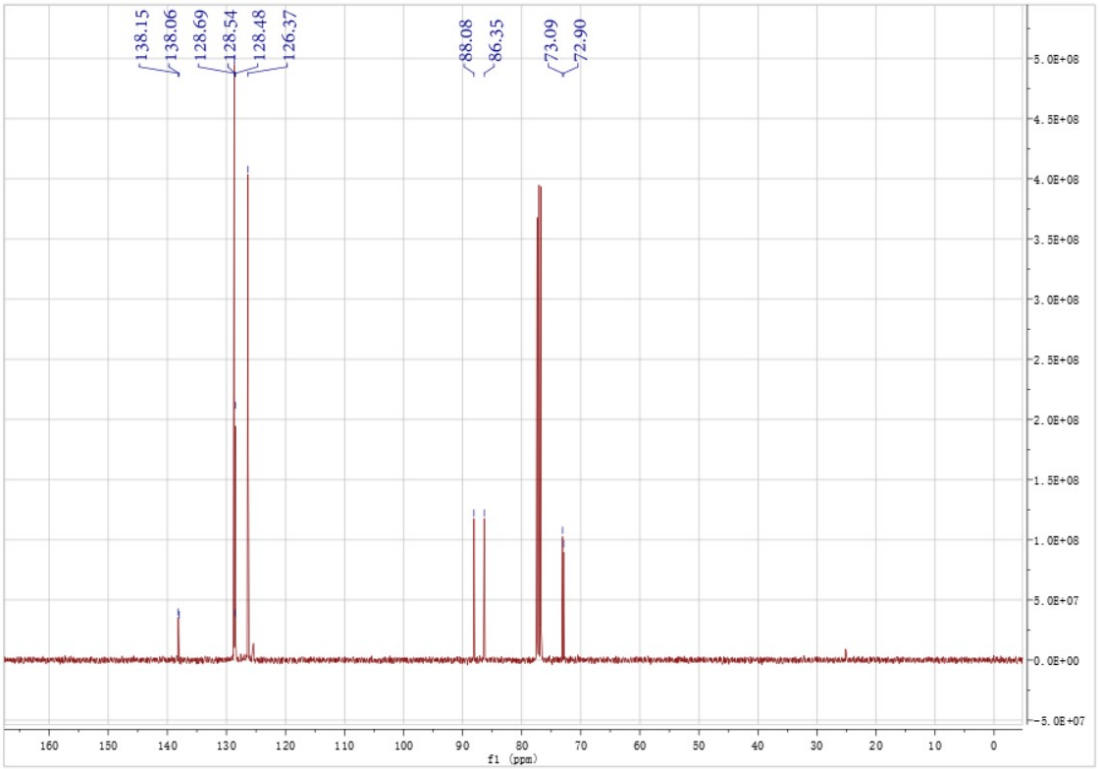
1-phenylethanol (1a)



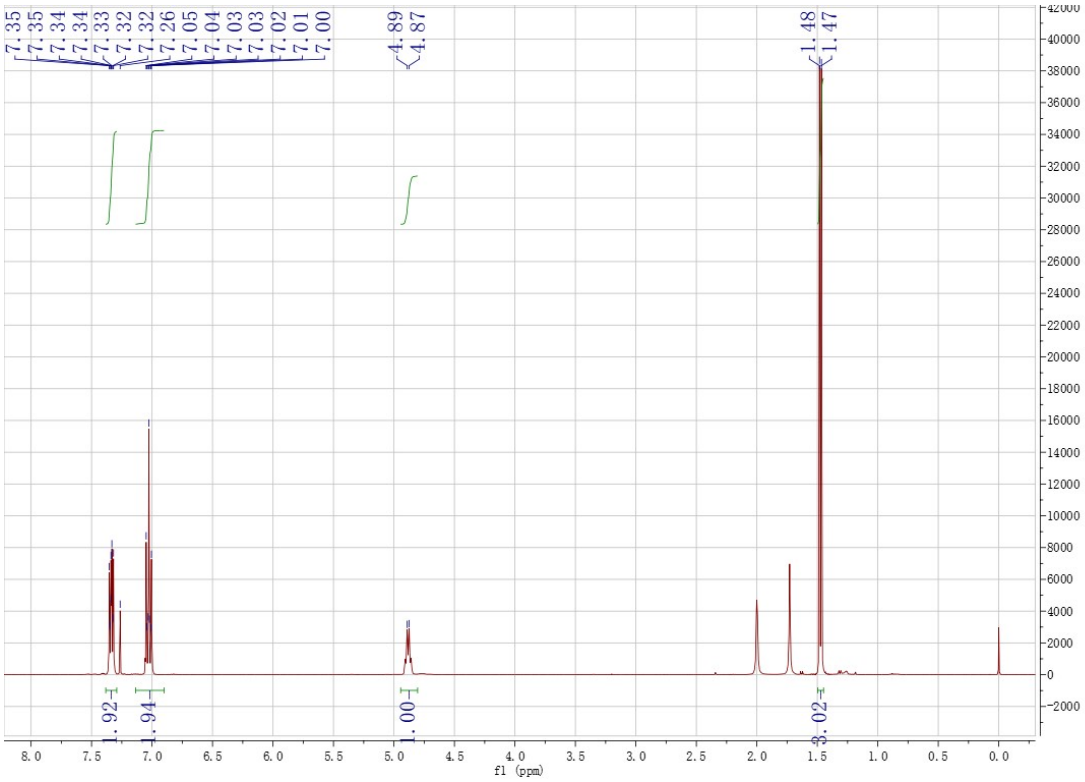


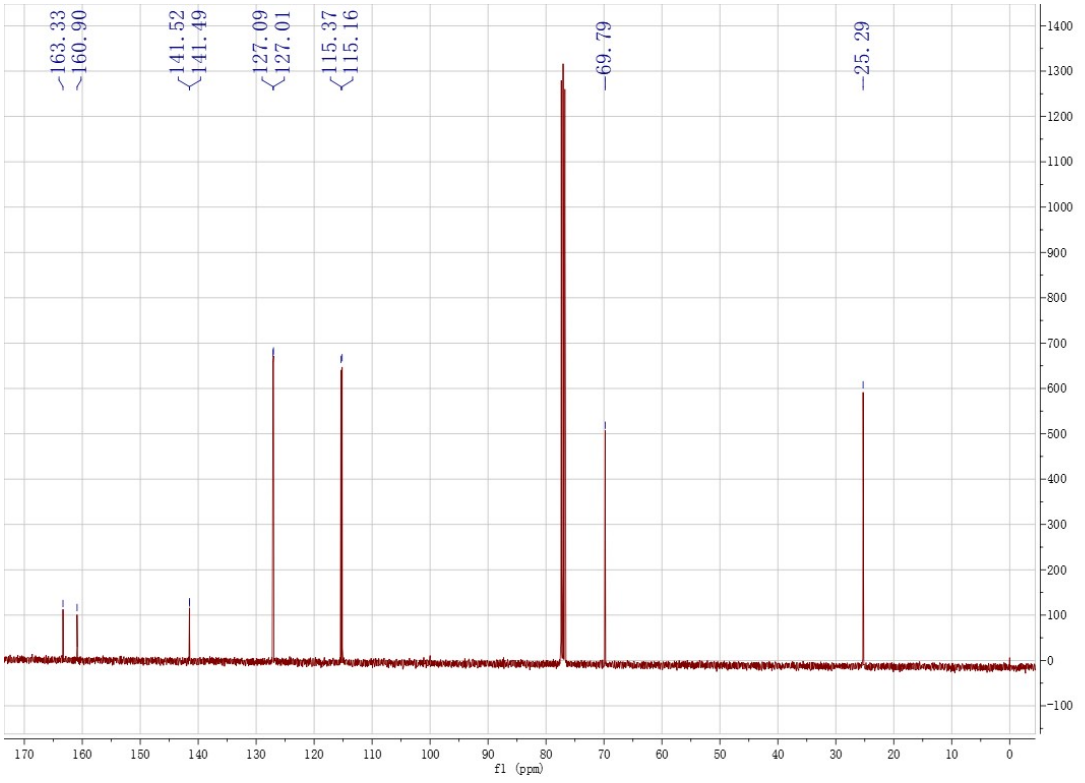
2-fluoro-1-phenylethanol (1b)



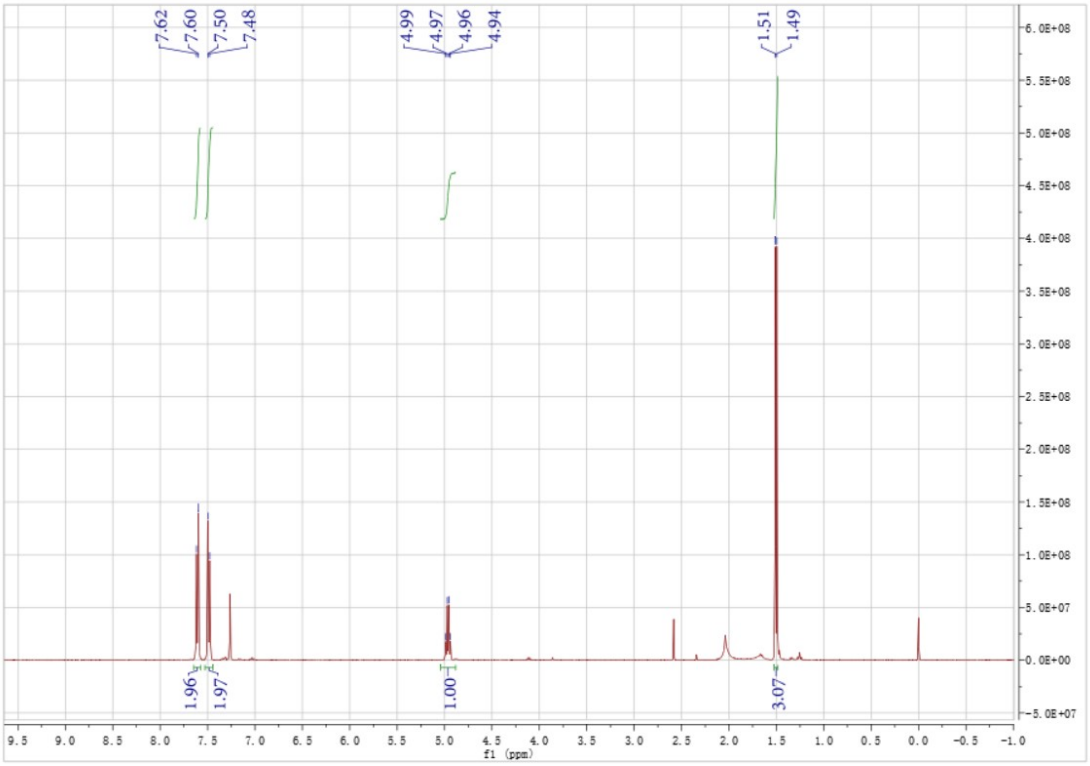


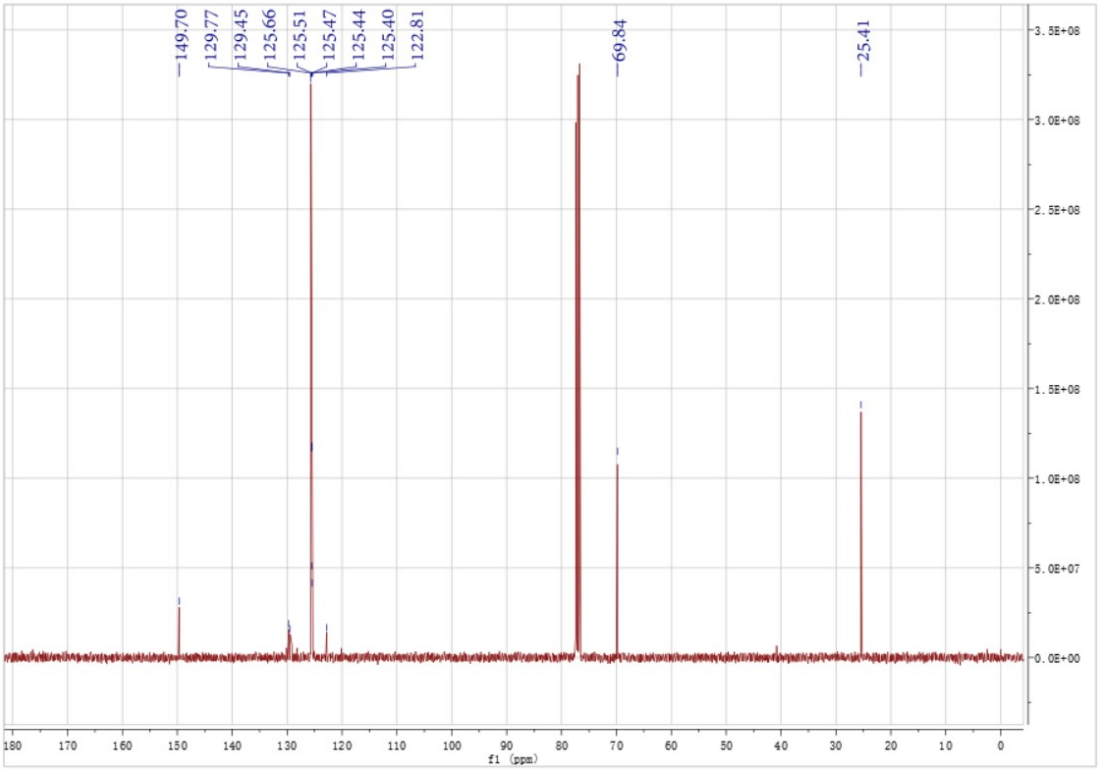
1-(4-fluorophenyl)ethanol (1c)



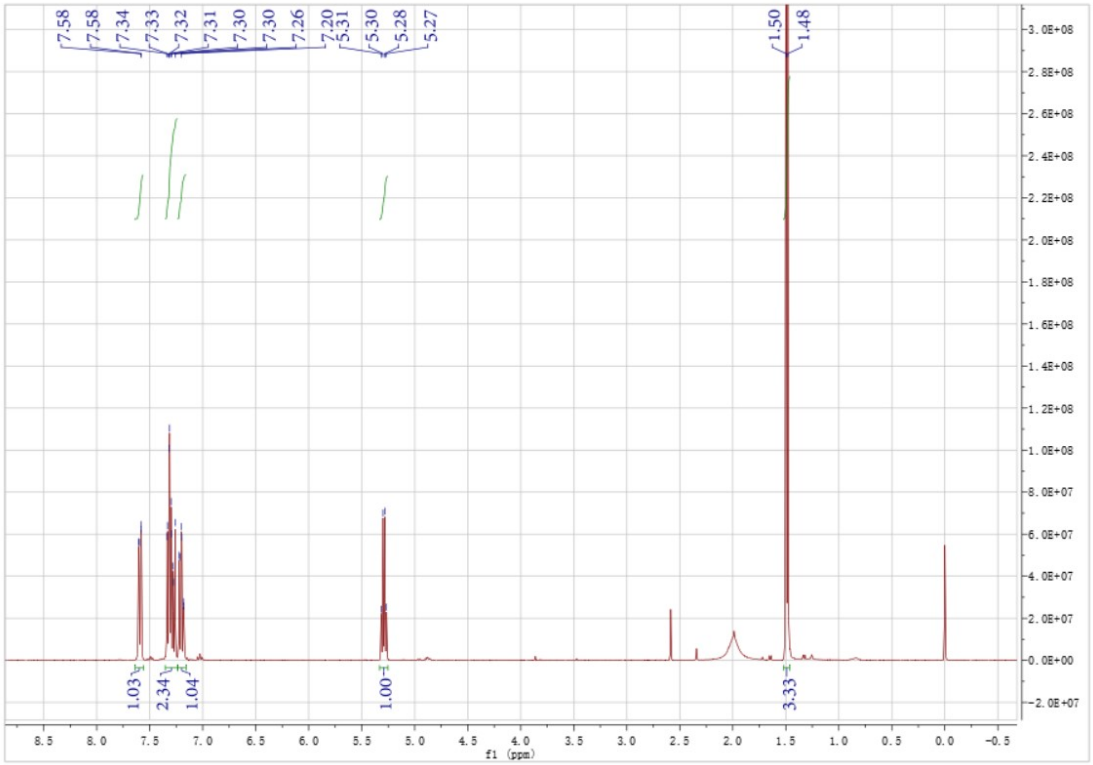


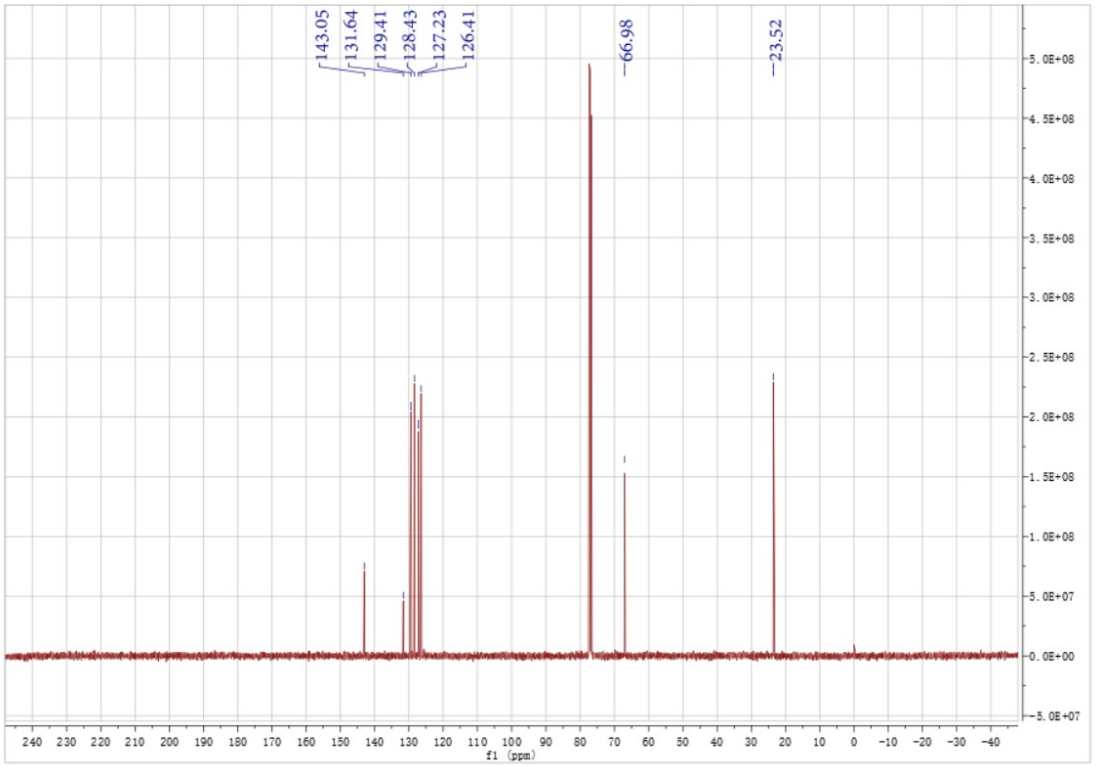
1-(4-trifluoromethyl)ethanol (1d)



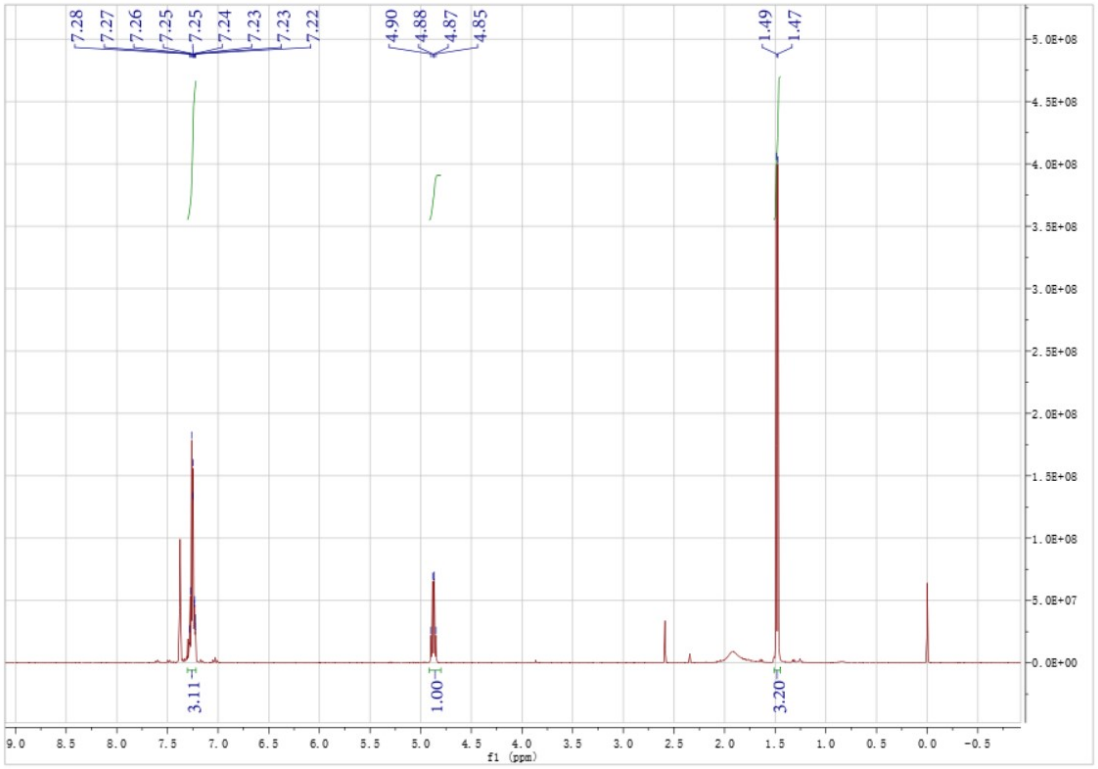


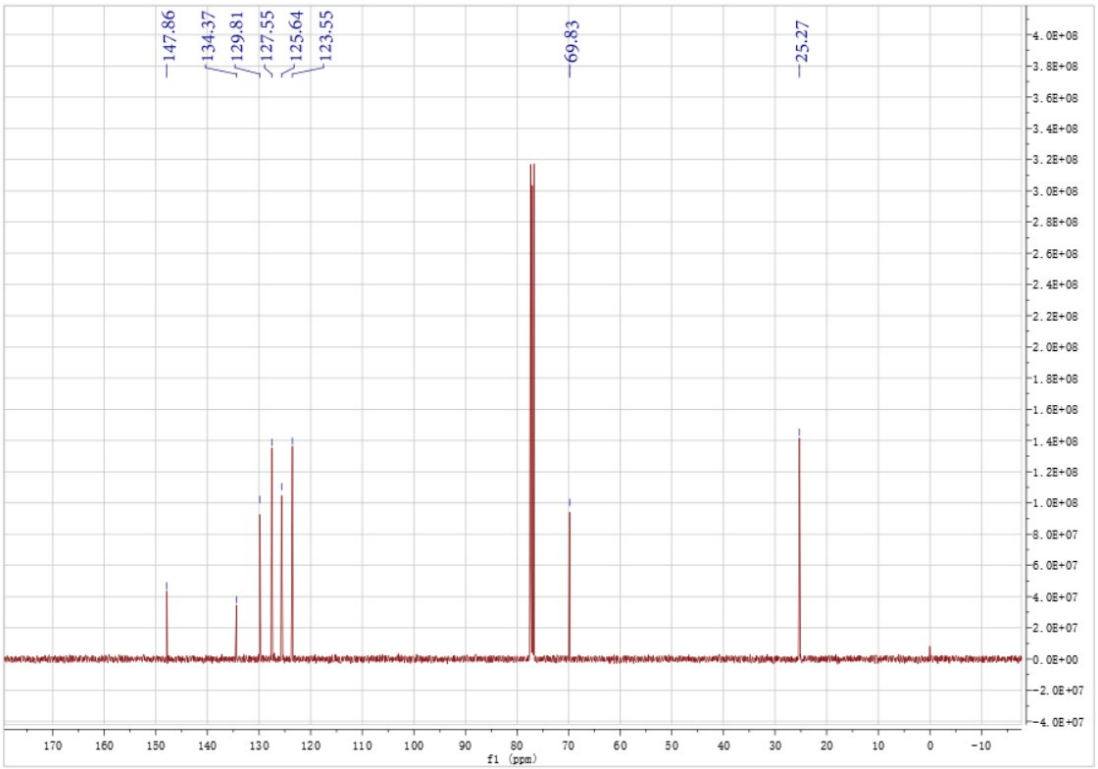
1-(2-chlorophenyl)ethanol (1e)



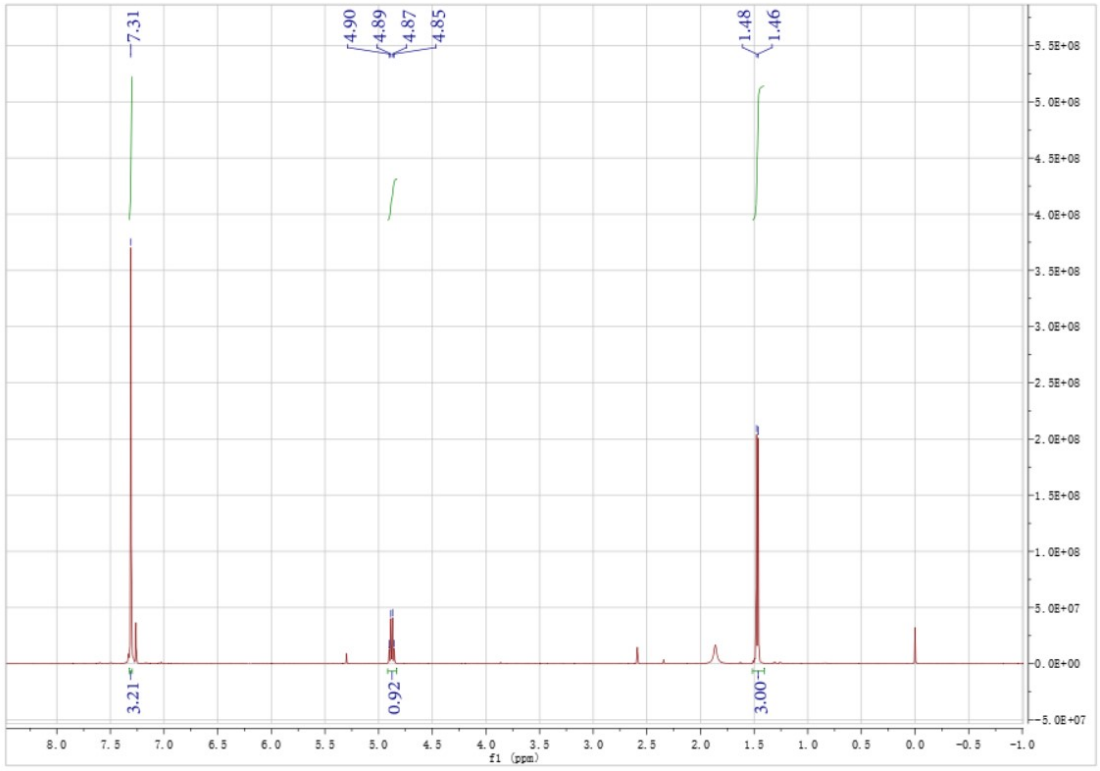


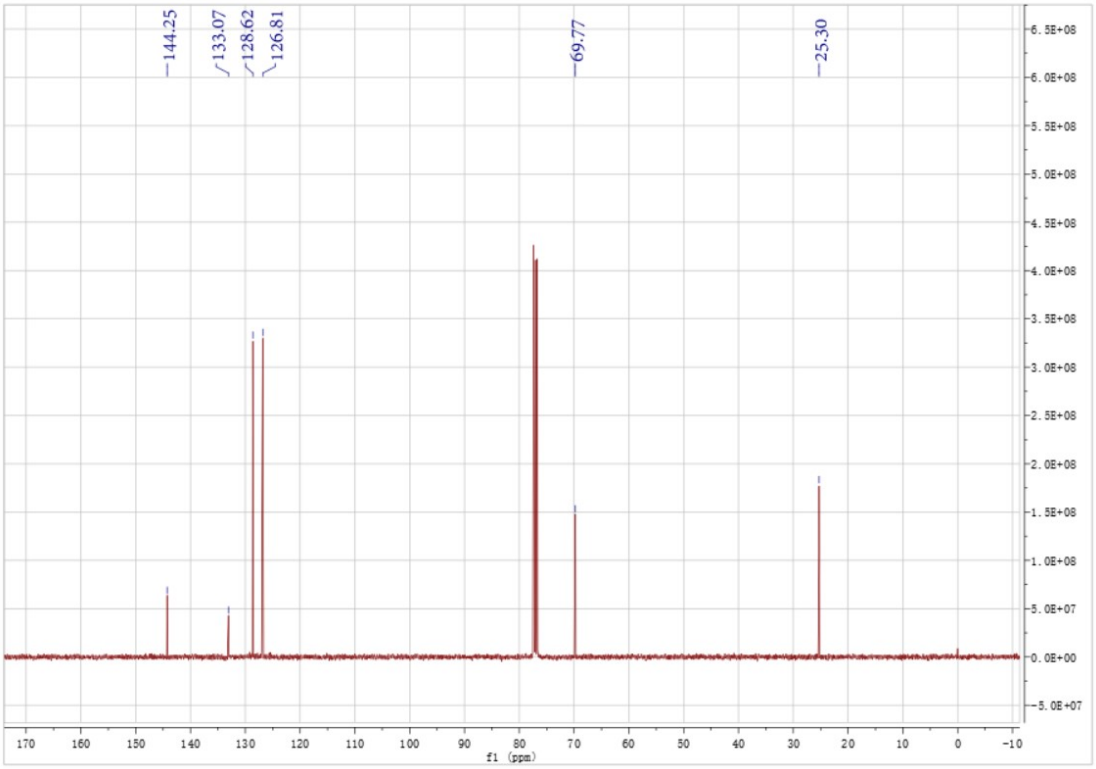
1-(3-chlorophenyl)ethanol (1f)



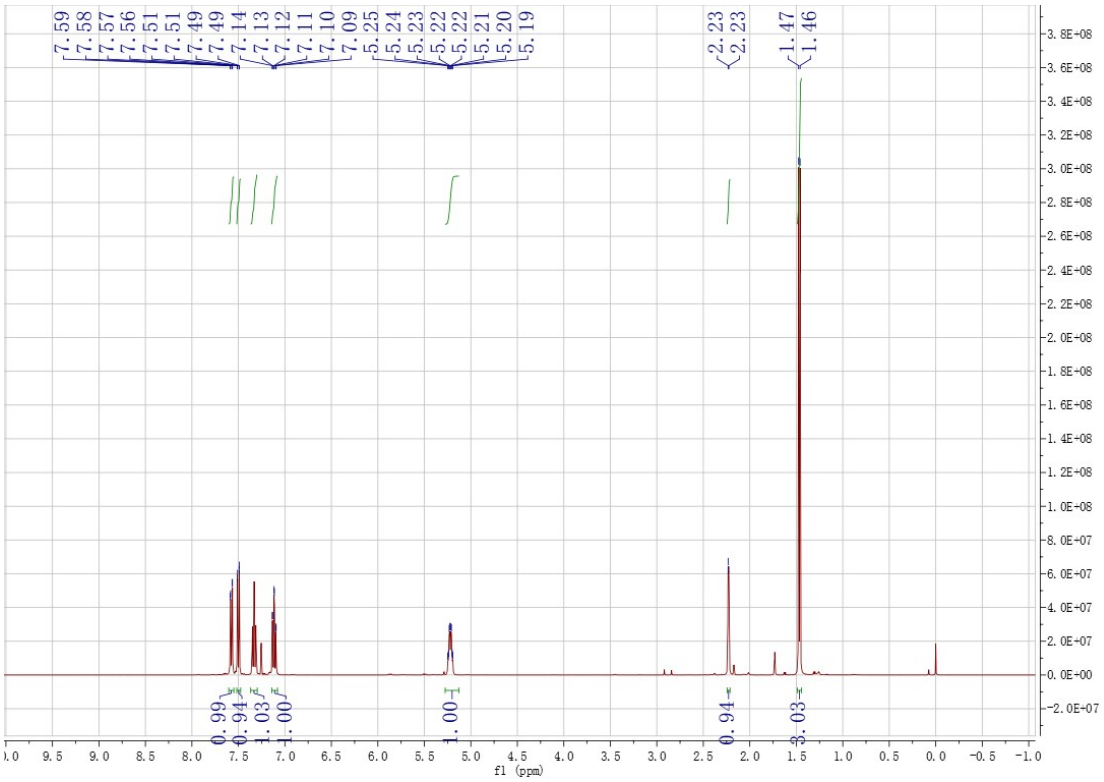


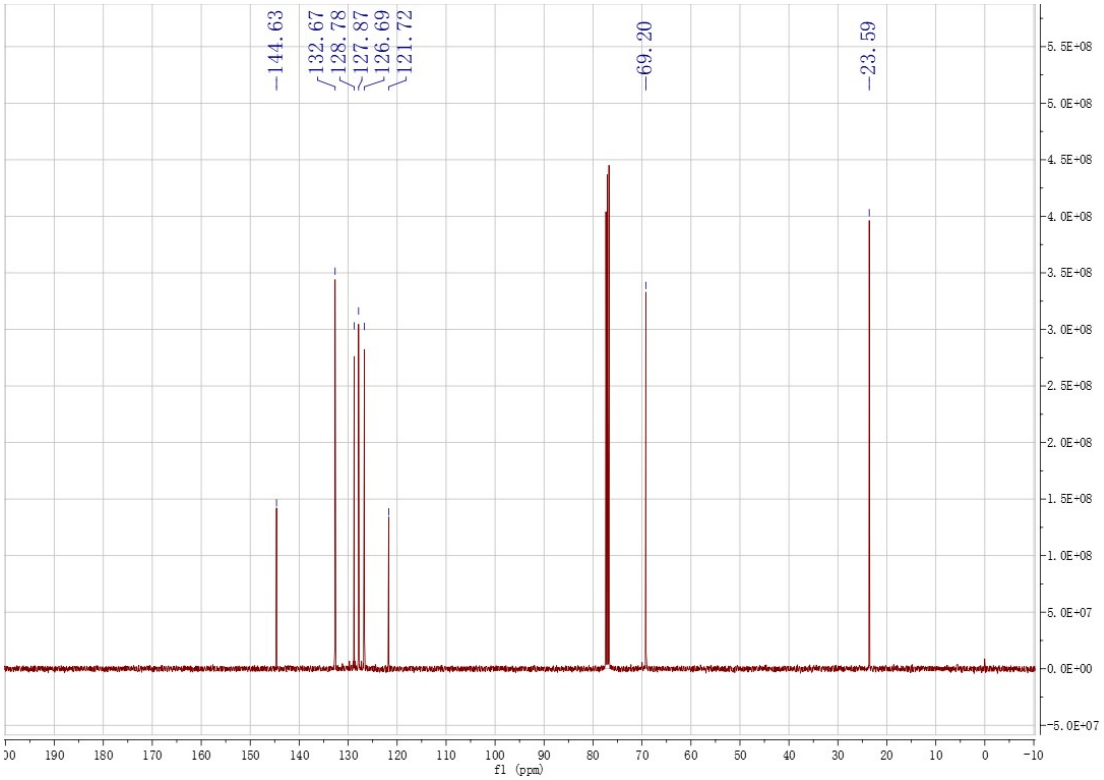
1-(4-chlorophenyl)ethanol (1g)



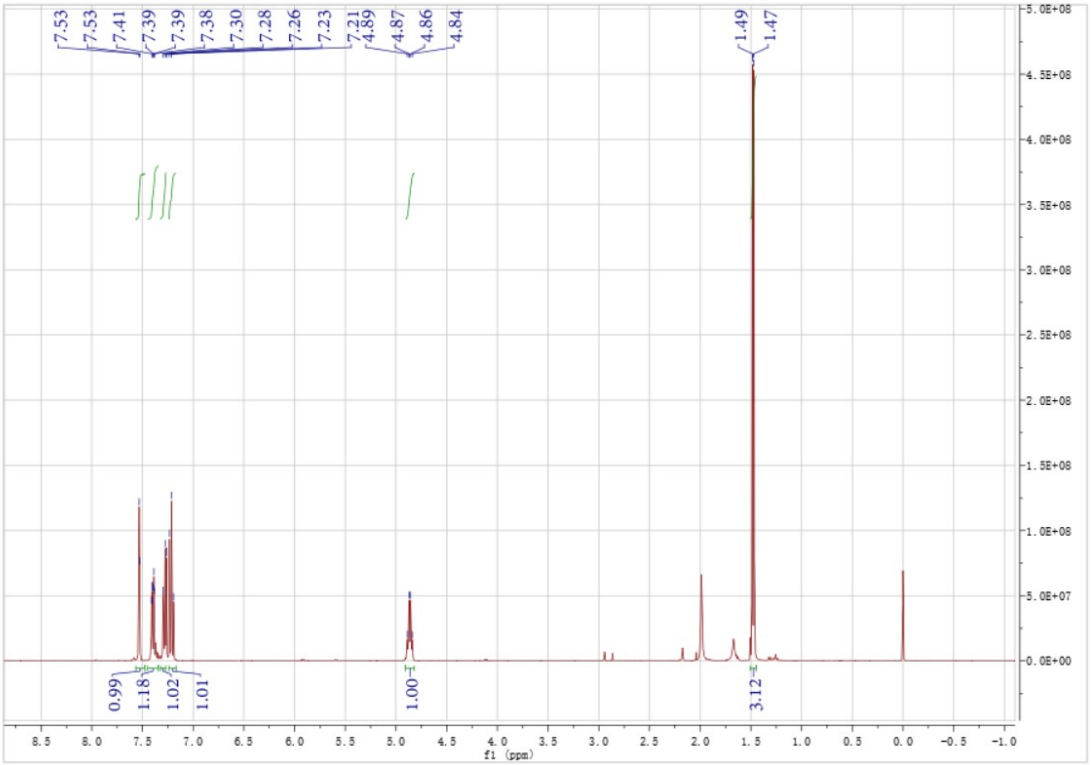


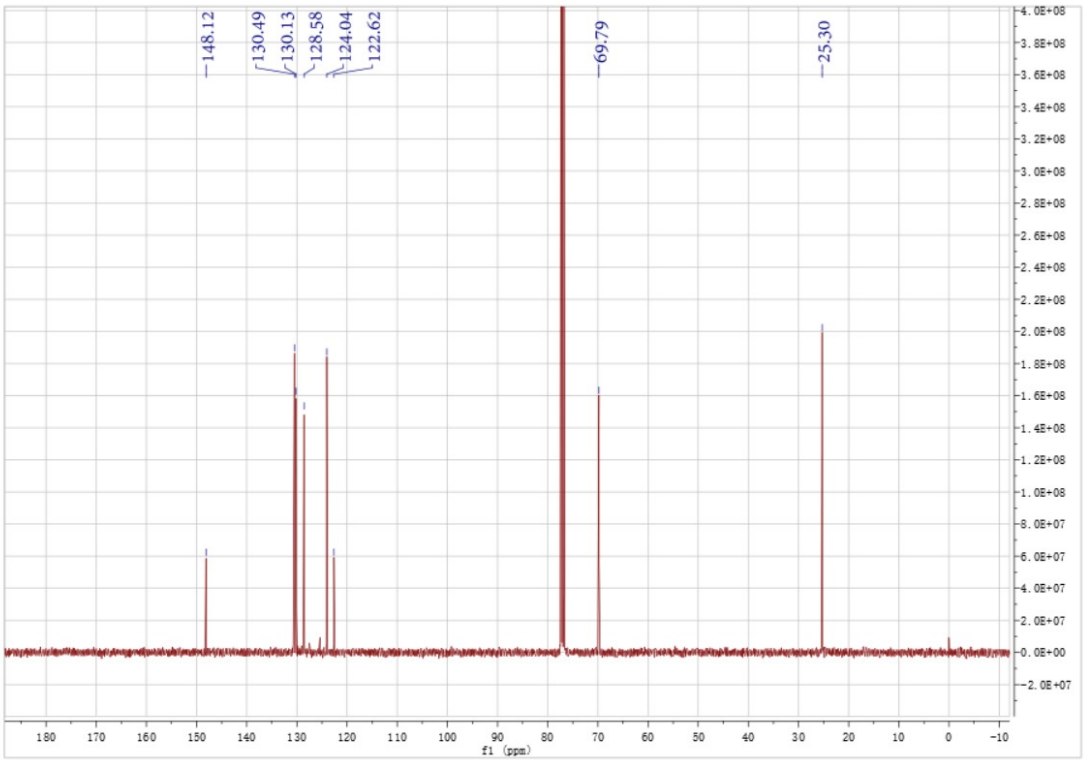
1-(2-bromophenyl)ethanol (1h)



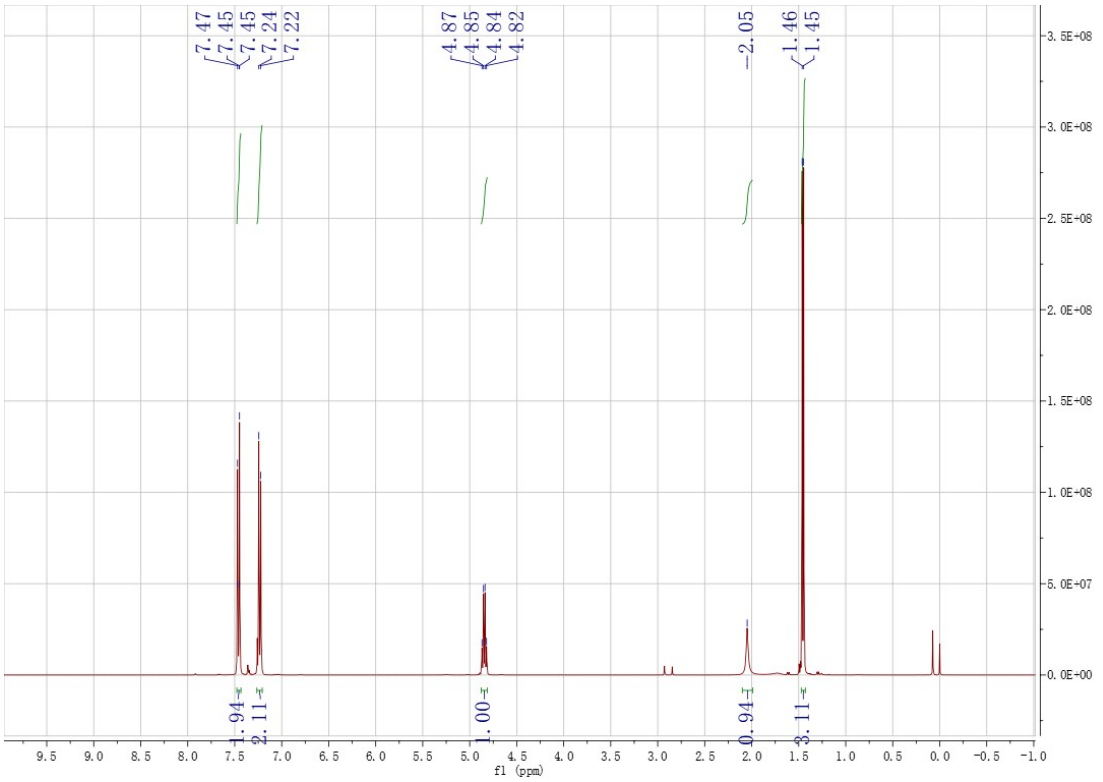


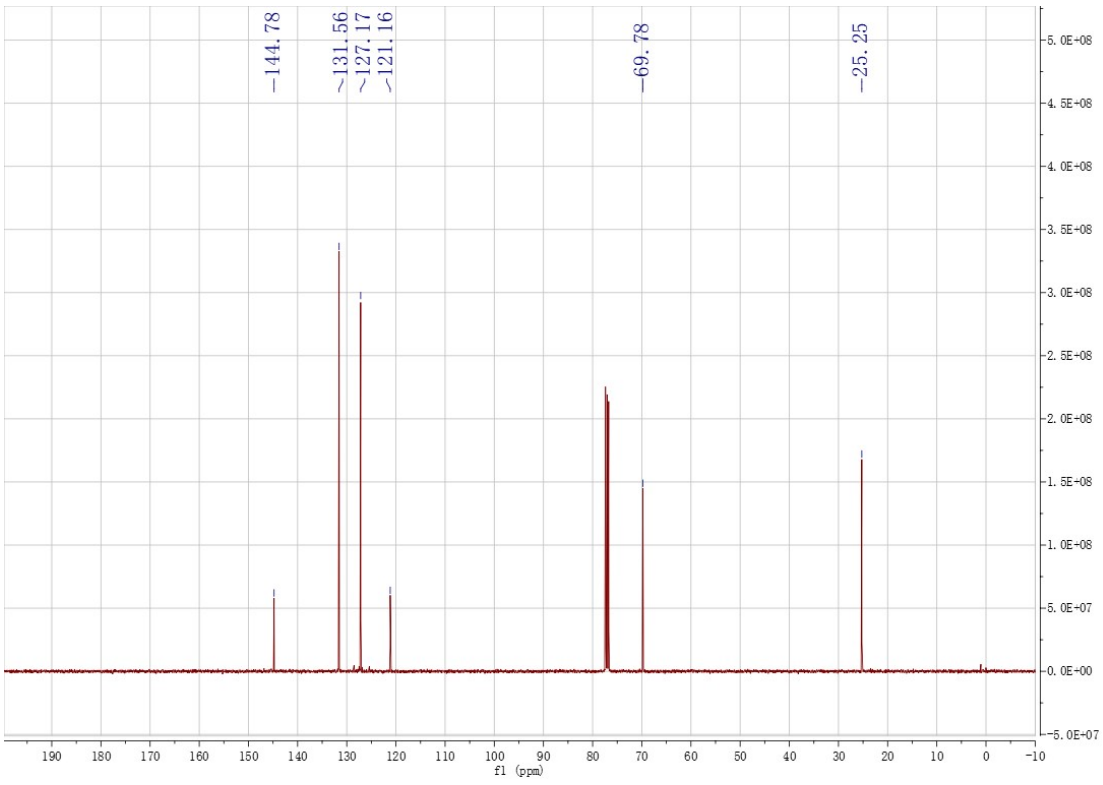
1-(3-bromophenyl)ethanol (1i)



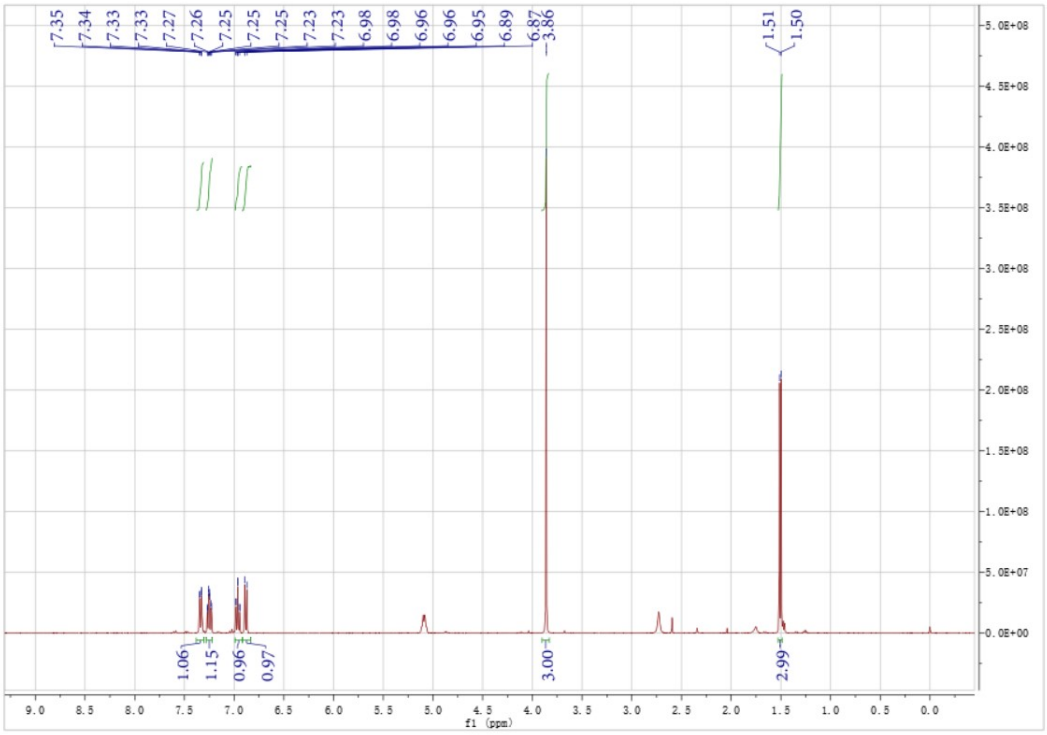


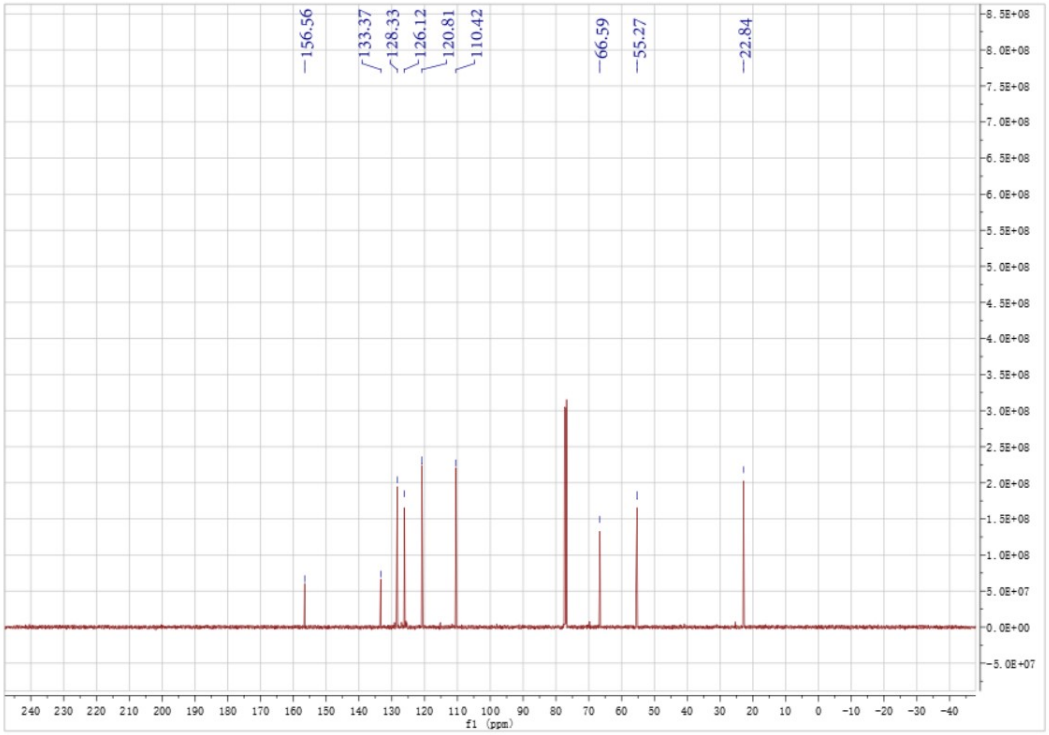
1-(4-bromophenyl)ethanol (1j）



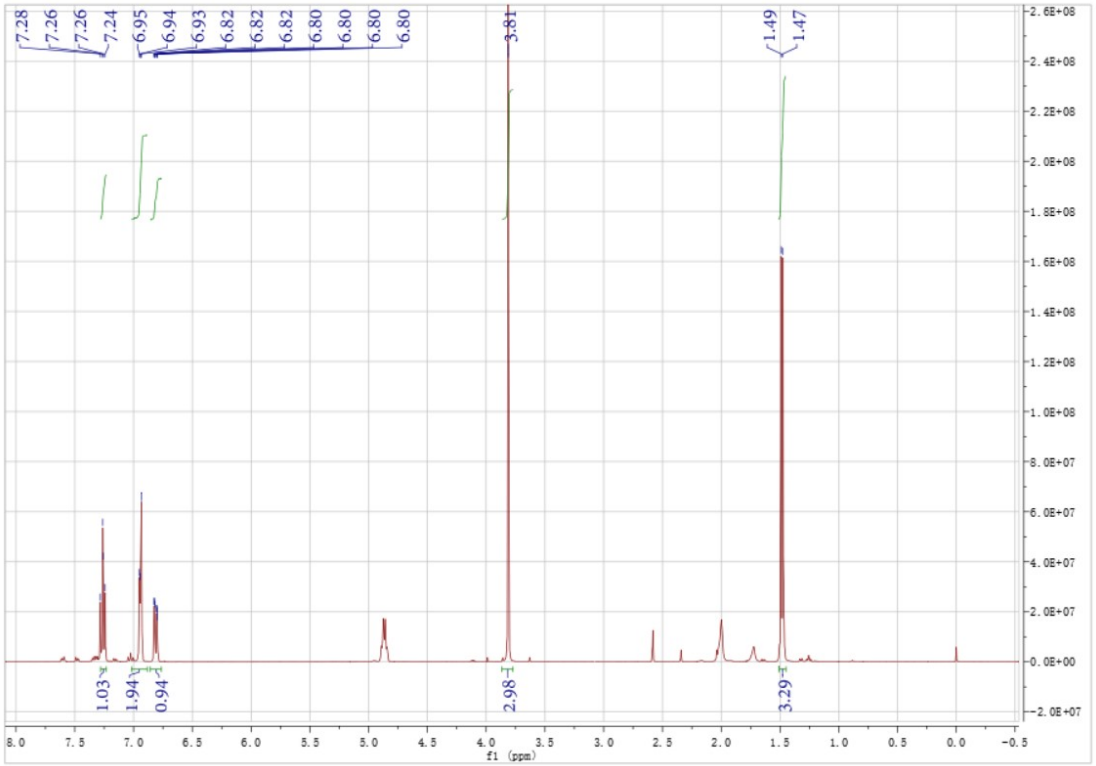


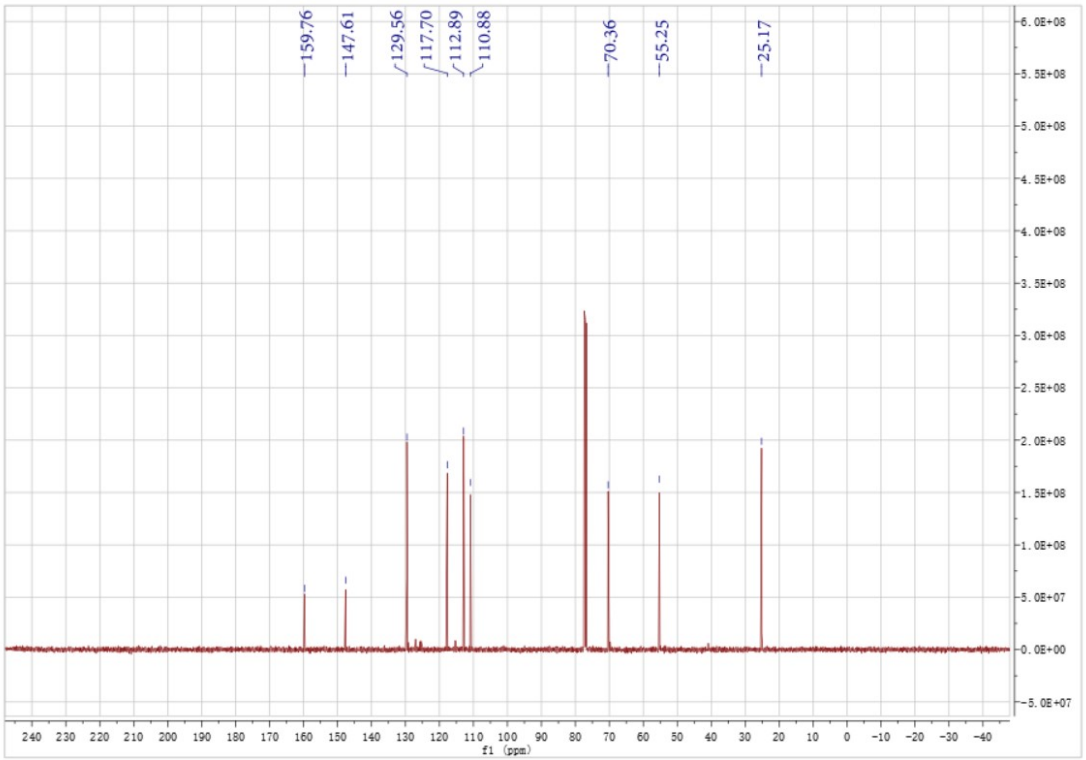
1-(2-methoxyphenyl)ethanol (1k)





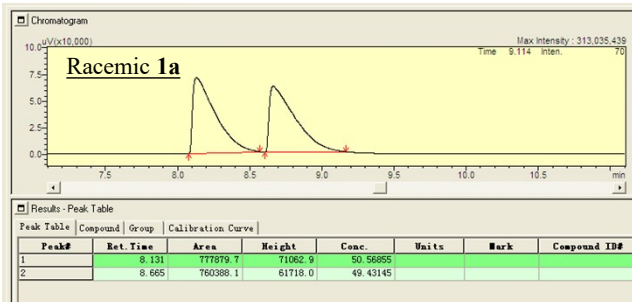
1-(3-methoxyphenyl)ethanol (1l)

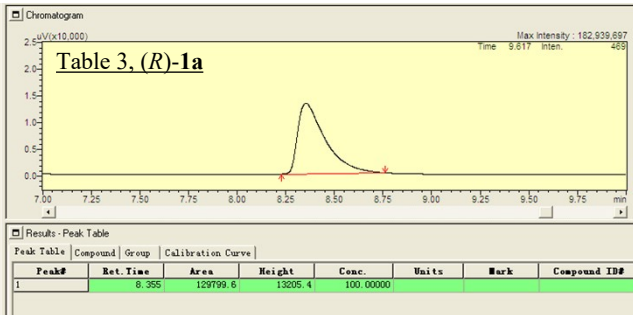


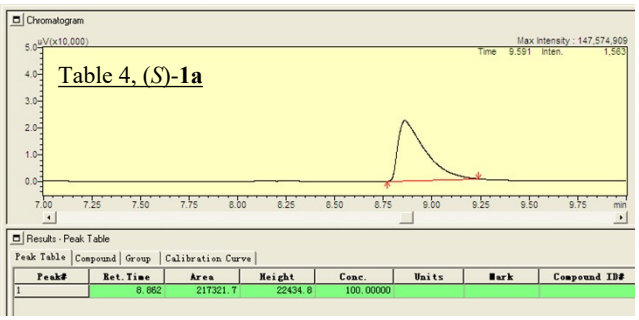


4. GC data

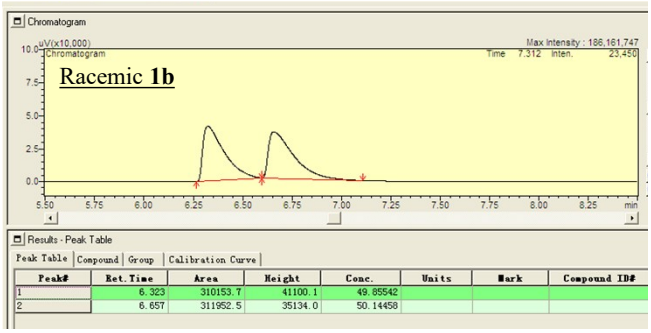
1a, chiral GC (Agilent CP-chirasil-Dex CB, T_R= 8.1 min, T_S= 8.7 min,Temperature conditions: initial temperature 100 °C, 2 °C/min to 140 °C, then 40°C/min to 200 °C, holding 1 min).

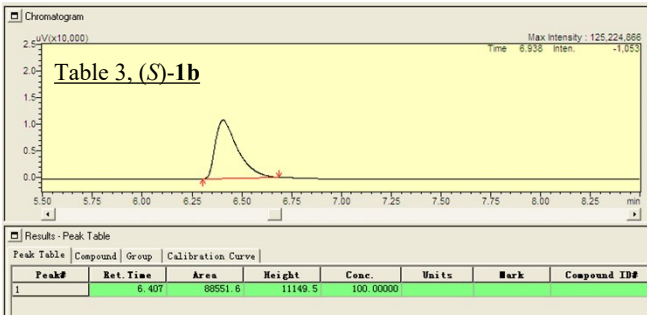


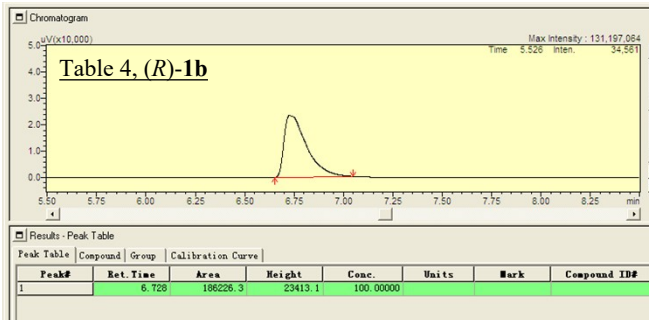




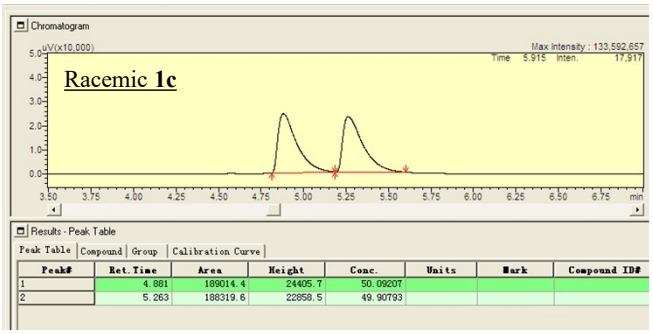
1b, chiral GC (Agilent CP-chirasil-Dex CB, T_S= 6.3 min, T_R= 6.7 min,Temperature conditions: initial temperature 120 °C, 2 °C/min to 160 °C, then 40 °C/min to 200 °C, holding 1 min).

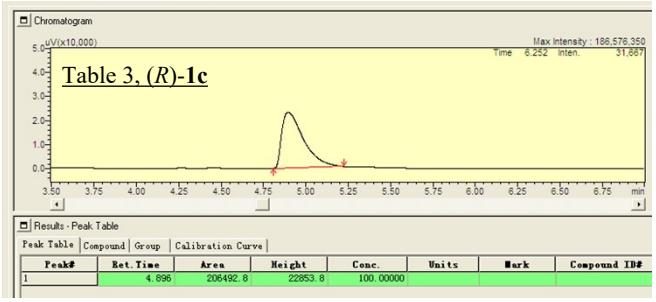


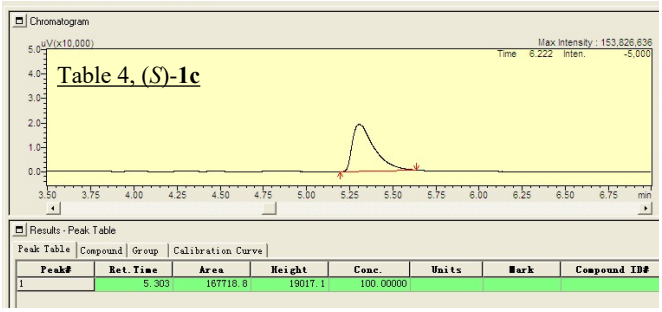




1c, chiral GC (Agilent CP-chirasil-Dex CB, T_R= 4.9 min, T_S= 5.3 min,Temperature conditions: initial temperature 120 °C, 2 °C/min to 160 °C, then 40 °C/min to 200 °C, holding 1 min).

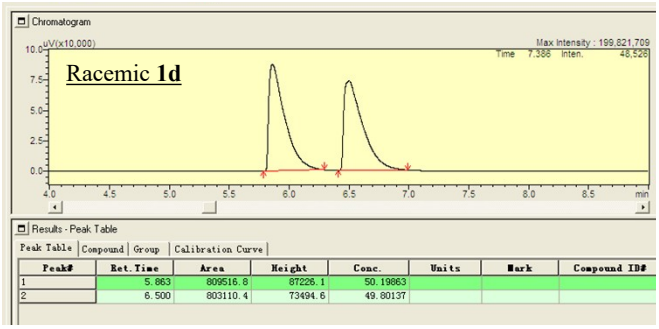


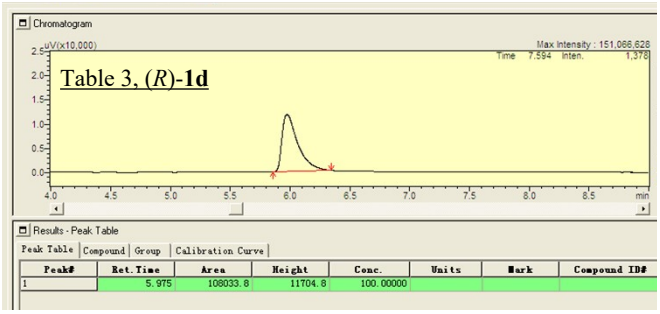


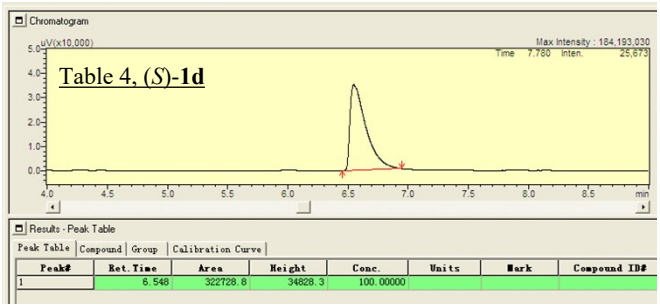


1d, chiral GC (Agilent CP-chirasil-Dex CB, T_R= 5.9 min, T_S= 6.5 min,Temperature conditions: initial

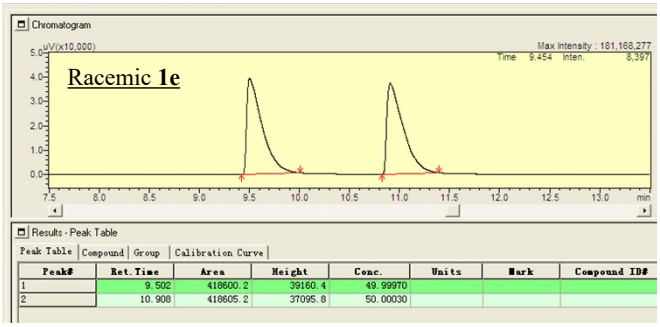
temperature 120 °C, 2 °C/min to 160 °C, then 40 °C/min to 200 °C, holding 1 min).

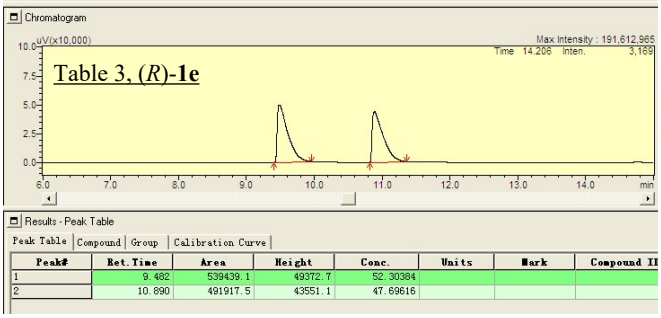


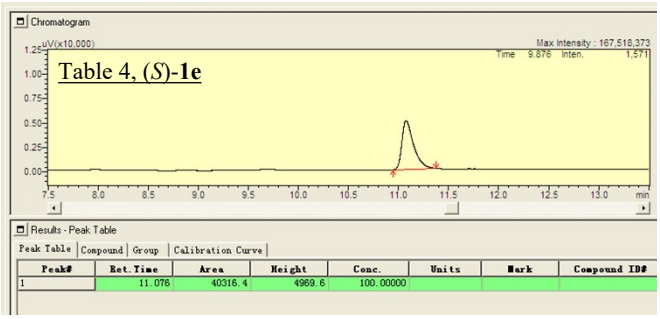




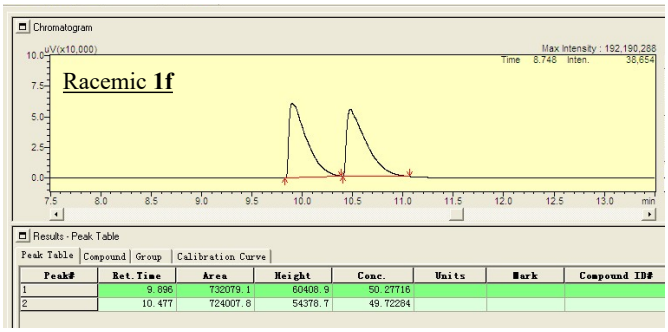
1e, chiral GC (Agilent CP-chirasil-Dex CB, T_R= 9.5 min, T_S= 10.9 min,Temperature conditions: initial temperature 120 °C, 2 °C/min to 160 °C, then 40 °C/min to 200 °C, holding 1 min).

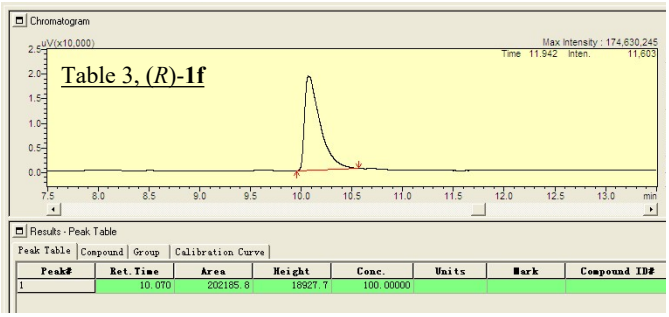


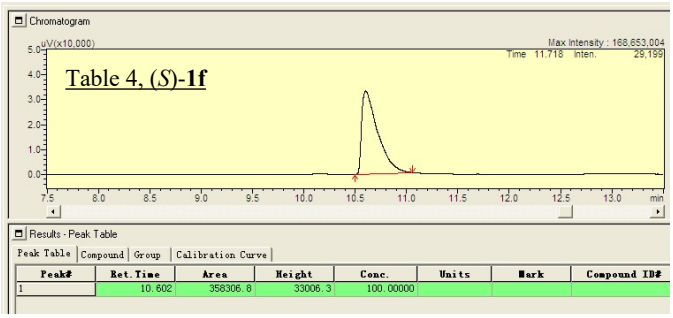




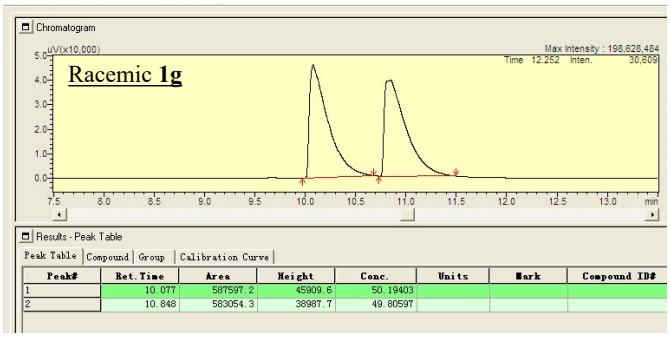
1f, chiral GC (Agilent CP-chirasil-Dex CB, T_R= 9.9 min, T_S= 10.5 min,Temperature conditions: initial temperature 120 °C, 2 °C/min to 160 °C, then 40 °C/min to 200 °C, holding 1 min).

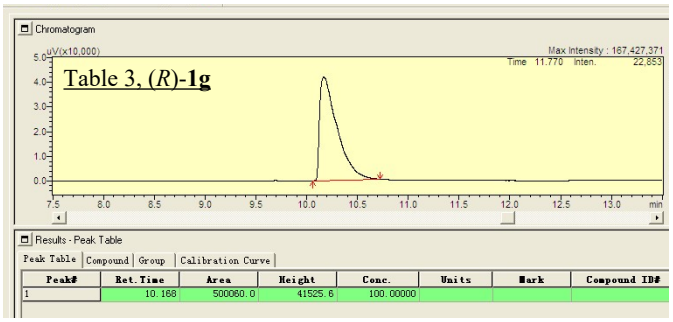


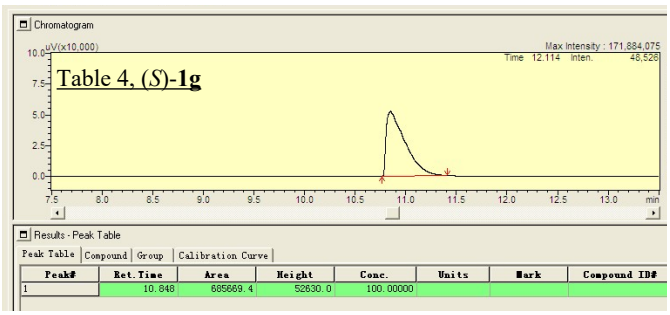




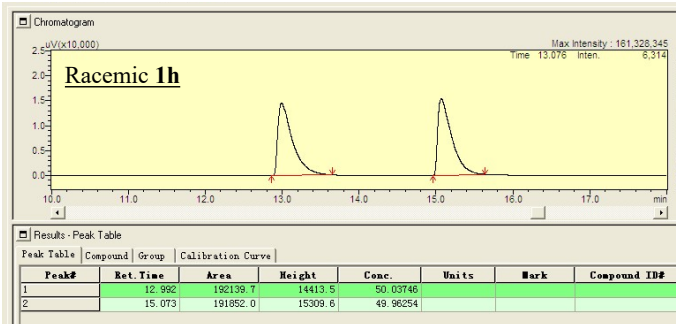
1g, chiral GC (Agilent CP-chirasil-Dex CB, T_R= 10.1 min, T_S= 10.8 min,Temperature conditions: initial temperature 120 °C, 2 °C/min to 160 °C, then 40 °C/min to 200 °C, holding 1 min).

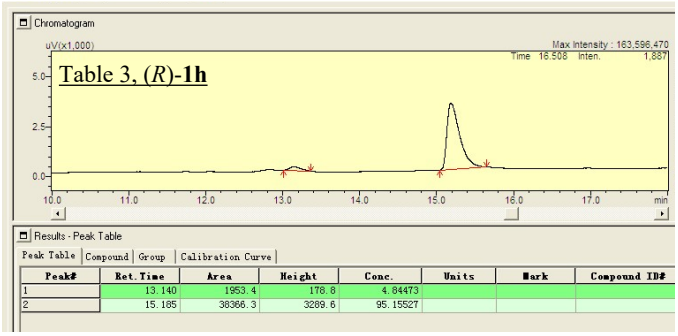


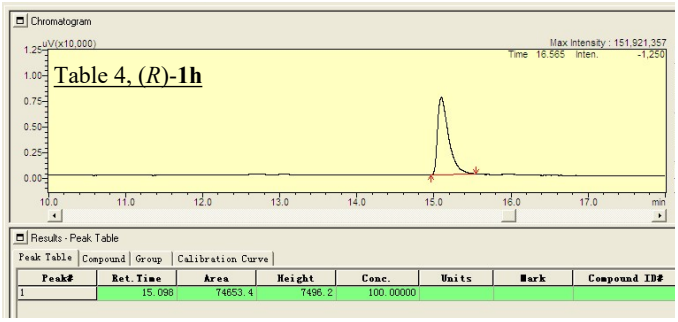




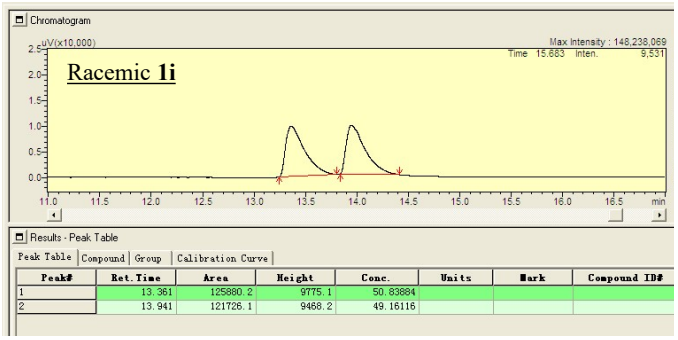
1h, chiral GC (Agilent CP-chirasil-Dex CB, T_R= 13.0 min, T_S= 15.1 min,Temperature conditions: initial temperature 120 °C, 2 °C/min to 160 °C, then 40 °C/min to 200 °C, holding 1 min).

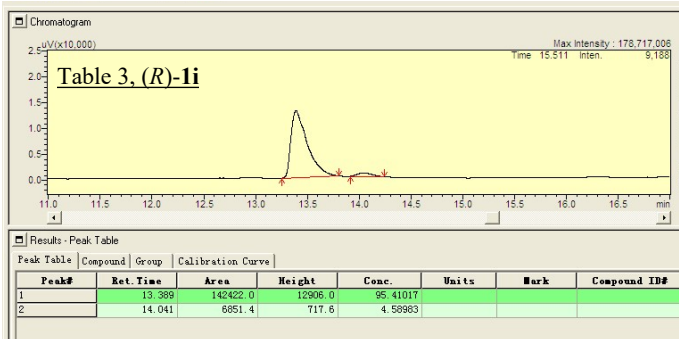


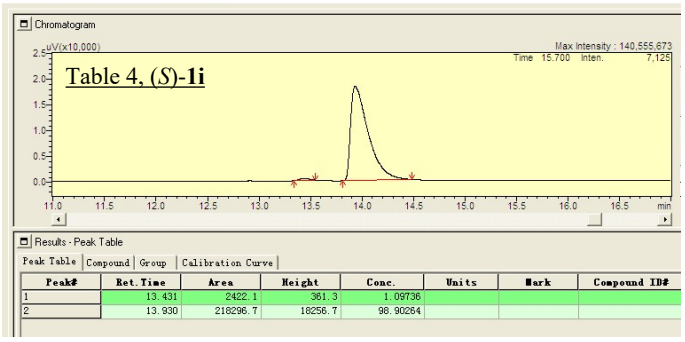




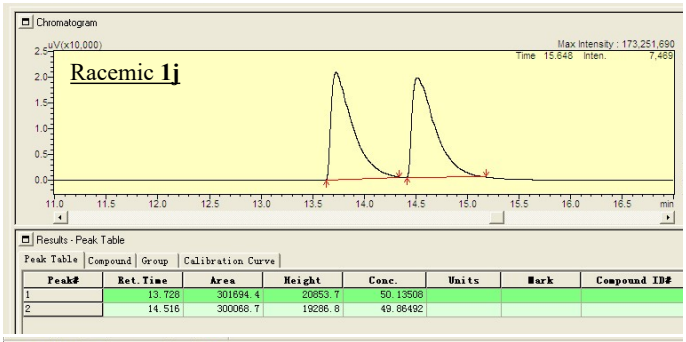
1i, chiral GC (Agilent CP-chirasil-Dex CB, T_R= 13.4 min, T_S= 13.9 min,Temperature conditions: initial temperature 120 °C, 2 °C/min to 160 °C, then 40 °C/min to 200 °C, holding 1 min).

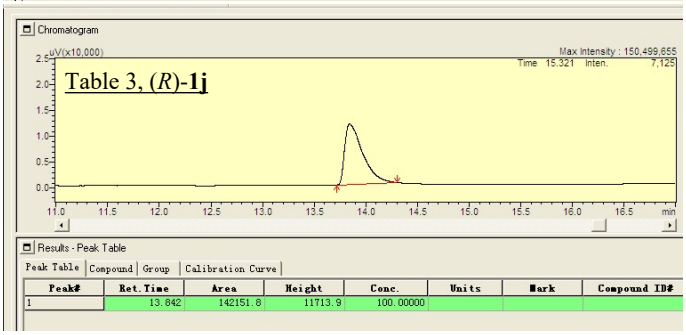


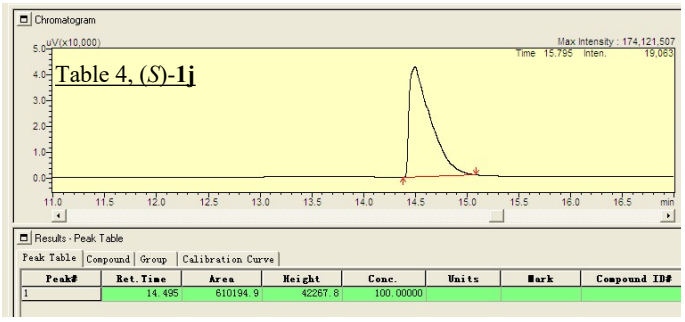




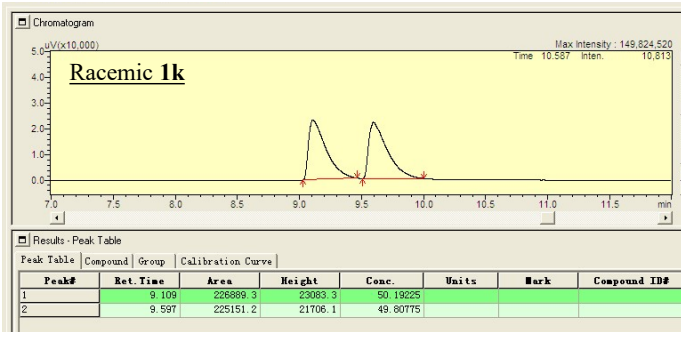
1j, chiral GC (Agilent CP-chirasil-Dex CB, T_R= 13.7 min, T_S= 14.5 min,Temperature conditions: initial temperature 120 °C, 2 °C/min to 160 °C, then 40 °C/min to 200 °C, holding 1 min).

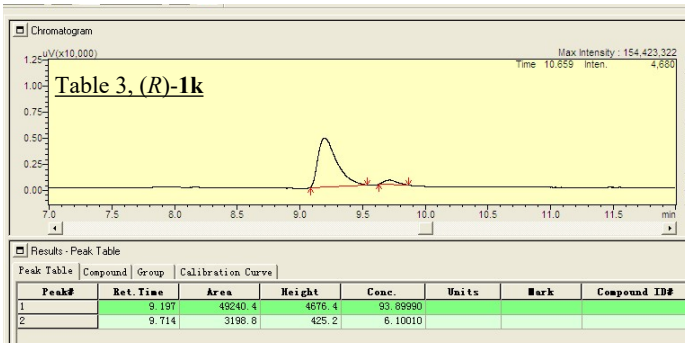


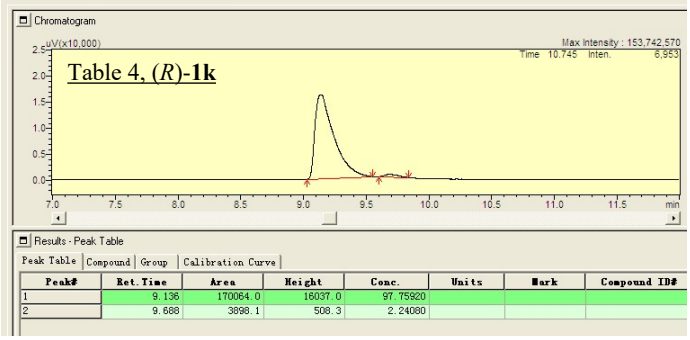




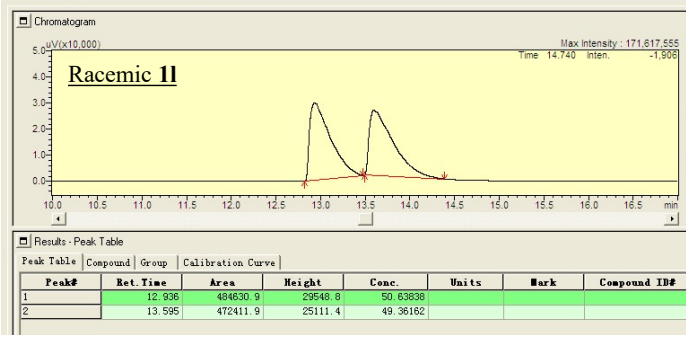
1k, chiral GC (Agilent CP-chirasil-Dex CB, T_R= 9.1 min, T_S= 9.6 min,Temperature conditions: initial temperature 120 °C, 1 °C/min to 160 °C, then 40 °C/min to 200 °C, holding 1 min).

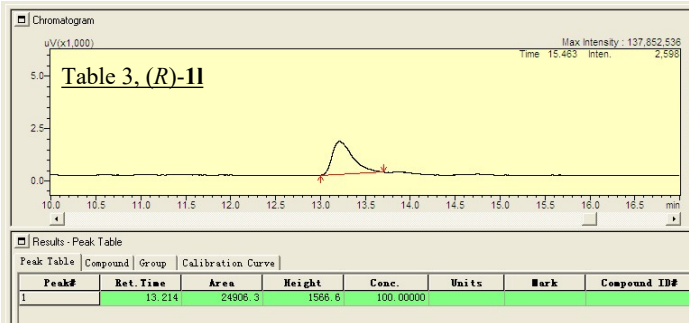


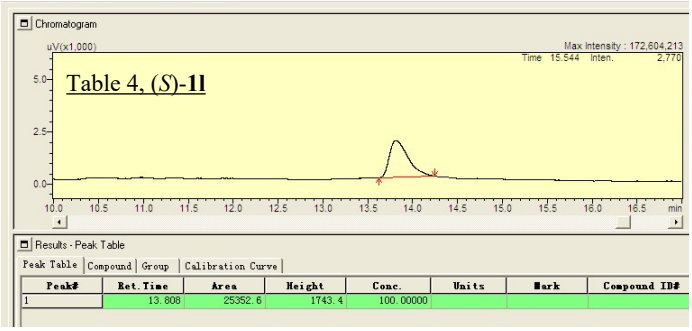




1l, chiral GC (Agilent CP-chirasil-Dex CB, T_R= 12.9 min, T_S= 13.6 min,Temperature conditions: initial temperature 120 °C, 1 °C/min to 160 °C, then 40 °C/min to 200 °C, holding 1 min).







5. References

1 J. Xu, M. Arkin, Y. Z. Peng, et al.W. H. Xu, H. L. Yu, X. F. Lin, Q. Wu, *Green Chem.*,2019, 21: 1907-1911.

2 B. Schulte, R. Fröhlich, A. Studer, *Tetrahedron* 2008, 64(52): 11852-11859.

3 T. Mandal, S. Jana, J. Dash, *Eur. J. Org. Chem.* 2017, 2017(33): 4972-4983.