Supporting Information

Cascade bio-hydroxylation and dehalogenation for one-pot enantioselective synthesis of optically active β-halohydrins from halohydrocarbons

Authors:

Hai-Bo Cui,[a,†] Ling-Zhi Xie,[a,†] Nan-Wei Wan,[a] Qing He,[a] Zhi Li,[b] and Yong-Zheng Chen*[,a]

Affiliations:

[a] Key Laboratory of Biocatalysis & Chiral Drug Synthesis of Guizhou Province, Generic Drug Research Center of Guizhou Province, Green Pharmaceuticals Engineering Research Center of Guizhou Province, School of Pharmacy, Zunyi Medical University, Zunyi, 563000, China.

[b] Department of Chemical and Biomolecular Engineering, National University of Singapore, 4 Engineering Drive 4, Singapore 117585, Singapore

[†] These authors contributed equally to this work.

***Corresponding author:**

Dr. Prof. Yong-Zheng Chen, E-mail: yzchen@zmu.edu.cn

Table of Contents

1. General experimental information

Column chromatography was performed on silica gel (200-400 mesh). ^{1}H NMR (400 MHz) chemical shifts were reported in ppm (δ) relative to tetramethylsilane (TMS) with the solvent resonance employed as the internal standard. Data were reported as follows: chemical shift, multiplicity (s = singlet, br s= broad singlet, d = doublet, t = triplet, dd = doublet of doublets, dt =double of triplet, td = triplet of doublets, m = multiplet), coupling constants (Hz) and integration. ^{13}C NMR (100 MHz) chemical shifts were reported in ppm (δ) from tetramethylsilane (TMS) with the solvent resonance as the internal standard.

Enantiomeric excess was determined by chiral HPLC analysis which was performed on SPD-M20A equipped with Chiralcel OJ-H chiral column (4.6 mmΦ×250 mmL), Chiralcel OD-H chiral column (4.6 mmΦ×250 mmL) or Chiralpak AD-H chiral column (4.6 mmΦ×250 mmL) purchased from Daicel Chemical Industries.

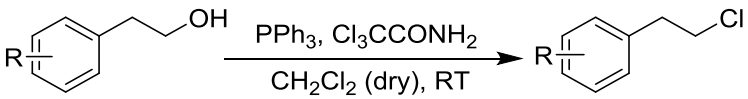
Isopropyl-β-D-thiogalactopyranoside (IPTG, >99%), ampicillin, streptomycin sulfate salt (98%), kanamycin sulfate (>99%) salt were purchased from Solarbio (Beijing, China). Chemicals (2-chloroethyl)benzene (**1a**), (2-bromoethyl)benzene (**1g**), 2-chloro-1-phenylethan-1-ol (**2a**) and (*R*)-**2a** were purchased from J&K Chemical (Shanghai, China). All the other biological and chemical reagents and solvents were obtained from commercial suppliers and used without further purification. Water was distilled before use.

2. Strains culture and enzymes preparation

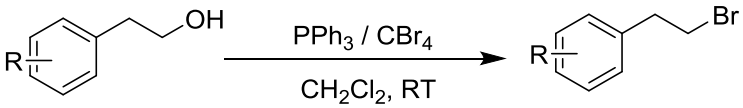
The P450 and HheA10 enzymes were expressed in *E. coli* BL21 (DE3). The corresponding antibiotics were used in whole culture process to the final concentration: ampicillin (100 μg/mL), kanamycin (50 μg/mL) and streptomycin (50 μg/mL). A single colony of each *E. coli* BL21 strain was taken from the agar plate and it was inoculated in Luria-Bertani medium and grown overnight at 37 °C, 200 rpm, for 16 h. Then, a large flask (1000 mL) containing 100 mL LB was inoculated with 5 mL of the overnight culture. The flask culture was grown overnight at 37 °C, 200 rpm, until the OD_{600} up to 0.6-0.8. Then, culture was induced with IPTG (final concentration 0.1 mM) and further shaken at 200 rpm, at 28 °C, for 12-14 h. The cultures were centrifuged and harvested to obtain *E. coli* cells. The cell-free extract of HheA10 was prepared by ultrasonication of the cells suspension (30 g cdw/L) and centrifugal separation.

3. Synthesis of halohydrocarbons 1b-1f, 1h-1m

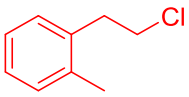
The halohydrocarbon substrates **1b-1f** and **1h-1m** were synthesized from the corresponding phenethyl alcohol derivatives. The procedure described by Pluempanupat *et al.* for chlorohydrocarbon compounds was used.[1] In a typical experiment, a 50 mL Schlenk tube was thoroughly flame-dried and put under an atmosphere of N_2, after which the following compounds were added, respectively: alcohol (1 mmol), PPh_3 (2 mmol), dry CH_2Cl_2 (2 mL), and Cl_3CCONH_2 (2 mmol). The mixture was stirred at room temperature (30 ºC) under an N_2 atmosphere for 1 h. The reaction was quenched with cold water and the product was isolated by purification through silica gel column chromatography.



The procedure described by Han *et al.* for bromohydrocarbon compounds was used.[2] Alcohol (2 mmol) and CBr_4 (2.2 mmol) was dissolved in CH_2Cl_2 (2 mL). After cooled to 0 °C, PPh3 (2.2 mmol) was added in portions to the mixture, then was stirred overnight at room temperature. The solvent was removed under reduced pressure and the residue was added 5 mL of petroleum ether. The precipitated white solid was filtered and washed with 20 mL of petroleum ether. The filtrate was concentrated under reduced pressure, and purified by column chromatography.

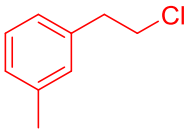


1-(2-chloroethyl)-2-methylbenzene (1b)



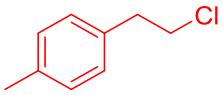
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.20 (s, 4 H), 3.71 (t, *J* = 7.8 Hz, 2 H), 3.12 (t, *J* = 7.8 Hz, 2 H), 2.38 (s, 3 H).

1-(2-chloroethyl)-3-methylbenzene (1c)



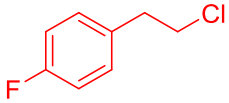
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.23 (d, J = 7.5 Hz, 1 H), 7.14–7.01 (m, 3 H), 3.73 (dd, J_1= 7.8, J_2=7.3 Hz, 2 H), 3.06 (t, J = 7.5 Hz, 2 H), 2.37 (s, 3 H).

1-(2-chloroethyl)-4-methylbenzene (1d)



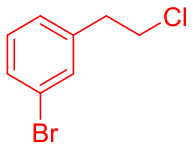
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.23–7.13 (m, 4 H), 3.60 (td, J_1= 7.8, J_2= 2.7 Hz, 2 H), 3.19 (dd, J_1 = 10.5, J_2=4.8 Hz, 2 H), 2.40 (s, 3 H).

1-(2-chloroethyl)-4-fluorobenzene (1e)



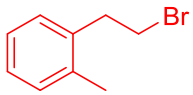
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.24 – 7.14 (m, 2 H), 7.01 (m, 2 H), 3.70 (m, 2 H), 3.04 (t, *J* = 7.1 Hz, 2 H).

1-bromo-3-(2-chloroethyl)benzene (1f)



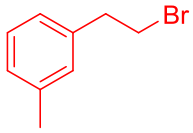
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 (m, 2 H), 7.23–7.13 (m, 2 H), 3.71 (t, *J* = 7.2 Hz, 2 H), 3.04 (t, *J* = 7.2 Hz, 2 H).

1-(2-bromoethyl)-2-methylbenzene (1h)



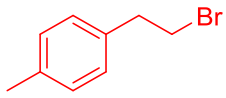
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.19 (s, 4 H), 3.54 (t, *J* = 8.0 Hz, 2 H), 3.20 (t, *J* = 8.0 Hz, 2 H), 2.36 (s, 3 H).

1-(2-bromoethyl)-3-methylbenzene (1i)



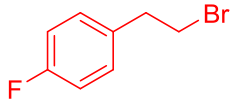
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.23 (t, *J* = 7.5 Hz, 1 H), 7.10 (d, *J* = 7.5 Hz, 1 H), 7.03 (d, *J* = 8.6 Hz, 2 H), 3.58 (t, *J* = 7.7 Hz, 2 H), 3.15 (t, *J* = 7.7 Hz, 2H), 2.37 (s, 3 H).

1-(2-bromoethyl)-4-methylbenzene (1j)



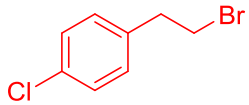
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.23–7.07 (m, 4 H), 3.72 (t, *J* = 7.5 Hz, 2 H), 3.06 (t, *J* = 7.5 Hz, 2 H), 2.36 (s, 3 H).

1-(2-bromoethyl)-4-fluorobenzene (1k)



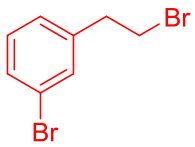
^{1}H NMR (400 MHz, DMSO-d_6) δ 7.32 – 7.26 (m, 2 H), 7.10 (t, *J* = 8.9 Hz, 2 H), 3.67 (t, *J* = 7.2 Hz, 2 H), 3.09 (t, *J* = 7.2 Hz, 2 H).

1-(2-bromoethyl)-4-chlorobenzene (1l)



^{1}H NMR (400 MHz, DMSO-d_6) δ 7.33 (m, 4 H), 3.71 (td, J_1 = 7.0, J_2 =2.3 Hz, 2 H), 3.11 (t, *J* = 7.0 Hz, 2 H).

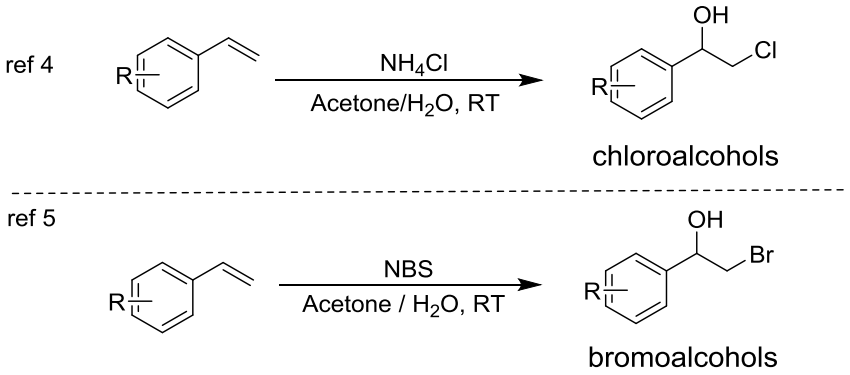
1-bromo-3-(2-bromoethyl)benzene (1m)



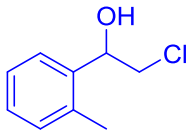
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 (m, 2 H), 7.23–7.13 (m, 2 H), 3.55 (t, J = 7.4 Hz, 2 H), 3.13 (t, J = 7.4 Hz, 2 H).

4. Synthesis of β-haloalcohols 2b-2m

The β-haloalcohols were prepared from the corresponding olefins following the procedure described in our previous paper.[3] Chloroalcohols were synthesized and purified according to the method reported by Swamy,[4] and bromoalcohols were synthesized and purified according to the revised method reported by Hatton.[5]

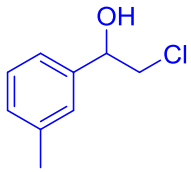


2-chloro-1-(o-tolyl)ethan-1-ol (2b)



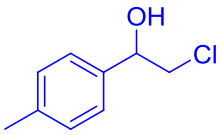
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52–7.48 (m, 1 H), 7.25–7.19 (m, 2 H), 7.16 (d, J = 6.5 Hz, 1 H), 5.09 (dd, J_1= 9.0, J_2 = 3.0 Hz, 1 H), 3.68 (dd, J_1 = 11.3, J_2 = 3.1 Hz, 1 H), 3.58 (dd, J_1= 11.1, J_2= 9.3 Hz, 1 H), 2.63 (s, 1 H), 2.34 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 137.2 (s), 136.2 (s), 130.6 (s), 129.4 (s), 127.2 (s), 126.3 (s), 37.1 (s), 31.8 (s), 19.4 (s).

2-chloro-1-(m-tolyl)ethan-1-ol (2c)



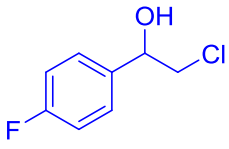
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26 (t, J = 7.5 Hz, 1 H), 7.16 (dd, J_1 = 17.6, J_2 = 8.9 Hz, 3 H), 4.84 (dd, J_1 =8.8, J_2 =3.3 Hz, 1 H), 3.72 (dd, J_1 = 11.2, J_2 = 3.4 Hz, 1 H), 3.63 (dd, J_1 = 11.2, J_2 = 8.8 Hz, 1 H), 2.66 (s, 1H), 2.36 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 134.0 (s), 138.5 (s), 129.3 (s), 128.6 (s), 126.8 (s), 123.2 (s), 74.2 (s), 51.0 (s), 21.5 (s).

2-chloro-1-(p-tolyl)ethan-1-ol (2d)



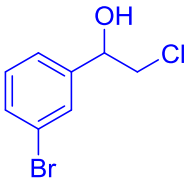
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27 (d, J = 7.9 Hz, 2 H), 7.18 (d, J = 7.7 Hz, 2 H), 4.86 (dd, J_1 = 8.5, J_2 = 3.0 Hz, 1 H), 3.78–3.68 (m, 1 H), 3.68–3.58 (m, 1 H), 2.51 (s, 1H), 2.36 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.4 (s), 137.1 (s), 129.4 (s), 126.1 (s), 74.0 (s), 51.0 (s), 21.3 (s).

2-chloro-1-(4-fluorophenyl)ethan-1-ol (2e)



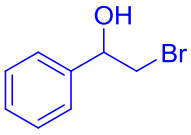
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40–7.33 (m, 2 H), 7.07 (dd, J_1 = 12.0, J_2 = 5.2 Hz, 2 H), 4.89 (dd, J_1 = 8.7, J_2 = 3.4 Hz, 1 H), 3.71 (dd, J_1= 11.2, J_2 = 3.5 Hz, 1 H), 3.65–3.57 (m, 1 H), 2.73 (s, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.4 (d, J = 245.0 Hz), 135.9 (d, J = 3.0 Hz), 127.8 (d, J = 8.0 Hz), 115.3 (d, J = 21.0 Hz), 73.3 (s), 50.2 (s).

1-(3-bromophenyl)-2-chloroethan-1-ol (2f)



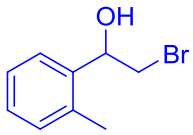
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54 (s, 1 H), 7.44 (dd, J_1 = 7.8, J_2 = 1.1 Hz, 1 H), 7.29 (d, J = 7.7 Hz, 1 H), 7.23 (t, J = 7.6 Hz, 1 H), 4.85 (dd, J_1 = 8.6, J_2 = 2.9 Hz, 1 H), 3.75–3.67 (m, 1 H), 3.64–3.55 (m, 1 H), 2.76 (s, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 142.2 (s), 131.6 (s), 130.3 (s), 129.3 (s), 124.8 (s), 122.9 (s), 73.4 (s), 50.8 (s).

2-bromo-1-phenylethan-1-ol (2g)



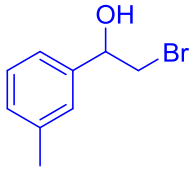
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41–7.30 (m, 5 H), 4.92 (dd, J_1= 8.9, J_2= 3.4 Hz, 1 H), 3.64 (dd, J_1= 10.5, J_2 = 3.4 Hz, 1 H), 3.54 (dd, J_1= 10.4, J_2 = 8.9 Hz, 1 H), 2.59 (s, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 140.4 (s), 128.8 (s), 128.5 (s), 126.1 (s), 73.9 (s), 40.2 (s).

2-bromo-1-(o-tolyl)ethan-1-ol (2h)



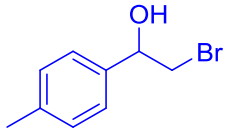
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.50 (d, J = 6.8 Hz, 1 H), 7.29–7.18 (m, 2 H), 7.15 (d, J = 6.8 Hz, 1 H), 5.12 (dd, J_1 = 9.3, J_2 = 2.5 Hz, 1 H), 3.57 (dd, J_1 = 10.6, J_2 =2.8 Hz, 1 H), 3.47 (t, J = 10.0 Hz, 1 H), 2.42 (s, 1 H), 2.34 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.4 (s), 134.8 (s), 130.7 (s), 128.3 (s), 126.6 (s), 125.5 (s), 70.8 (s), 39.2 (s), 19.2(s).

2- bromo -1-(m-tolyl)ethan-1-ol (2i)



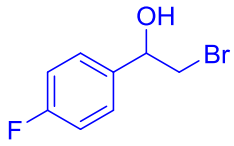
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26 (t, J = 7.4 Hz, 1 H), 7.16 (dd, J_1 = 15.3, J_2 = 8.2 Hz, 3 H), 4.86 (dd, J_1 = 8.9, J_2 = 3.0 Hz, 1 H), 3.61 (dd, J_1 = 10.4, J_2 = 3.2 Hz, 1 H), 3.52 (t, J = 9.7 Hz, 1H), 2.66 (s, 1 H), 2.36 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 140.3 (s), 138.5 (s), 129.3 (s), 128.6 (s), 126.7 (s), 123.1 (s), 73.9 (s), 40.2 (s), 21.5 (s).

2- bromo-1-(p-tolyl)ethan-1-ol (2j)



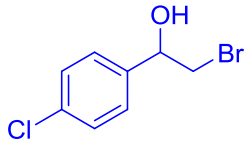
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 (d, J = 8.0 Hz, 2 H), 7.25 (d, J = 8.0 Hz, 2 H), 4.93 (dd, J_1= 8.7, J_2 = 3.5 Hz, 1 H), 3.62 (ddd, J_1 = 19.2, J_2 = 10.4, J_3 = 6.3 Hz, 2 H), 2.82 (s, 1H), 2.42 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.2 (s), 137.5 (s), 129.3 (s), 126.0 (s), 73.7 (s), 40.1 (s), 21.2 (s).

2-bromo-1-(4-fluorophenyl)ethan-1-ol (2k)



^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38–7.30 (m, 2H), 7.0 –7.00 (m, 2H), 4.92–4.84 (m, 1H), 3.58 (m, 1H), 3.54–3.46 (m, 1 H), 2.79 (s, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.3 (d, J = 245.0 Hz), 136.3 (d, J = 3.0 Hz), 127.7 (d, J = 8.0 Hz), 115.3 (d, J = 22.0 Hz), 72.9 (s), 39.2 (s).

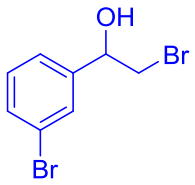
2-bromo-1-(4-chlorophenyl)ethan-1-ol (2l)



^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37–7.27 (m, 4 H), 4.87 (dd, J_1 =

8.6, J_2 = 3.4 Hz, 1H), 3.58 (m, 1H), 3.54 – 3.44 (m, 1 H), 2.84 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.8 (s), 134.2 (s), 128.9 (s), 127.4 (s), 73.2 (s), 39.9 (s).

2-bromo-1-(3-bromophenyl)ethan-1-ol (2m)



^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53 (s, 1H), 7.44–7.39 (m, 1 H), 7.24 (m, 2 H), 4.86 (d, J = 8.8 Hz, 1H), 3.59 (ddd, J_1 = 10.5, J_2 = 3.3, J_3 = 1.1 Hz, 1 H), 3.50–3.43 (m, 1 H), 2.72 (d, J = 2.4 Hz, 1 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 142.5 (s), 131.2 (s), 130.1 (s), 128.9 (s), 124.6 (s), 122.5 (s), 72.8 (s), 39.1 (s).

5. Biotransformation of 1a-1m using *E. coli* (P450$_{PL2}$-4)

To a 25 mL shake flask, 5 mL PBS buffer (50 mM, pH 8.5) containing 30 g cdw/L of *E. coli* (P450$_{PL}$2-4) cells was added. Subsequently, 15-25 uL of substrate **1** stock solution was added to a final concentration of 2 mM. The mixture was stirred at 35 ºC, 200 rpm. After reaction for 12 h, the mixture was centrifuged at 8,000 g for 5 min. 3 mL supernatant was taken and extracted with 3 mL ethyl acetate. The organic phase was separated, dried on anhydrous $NaSO_4$, and analyzed by chiral HPLC.

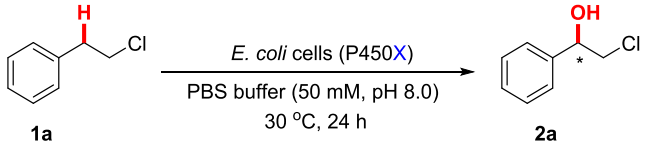
6. Cascade biotransformation of 1a-1m using *E. coli* (P450PL2-4) and cell-free extract (HheA10)

To a 25 mL shake flask, 5 mL PBS buffer (50 mM, pH 8.5) containing 30 g cdw/L of *E. coli* (P450$_{PL}$2-4) cells was added. Subsequently, 15-25 uL ethanol stock solution of substrate **1** was added to a final concentration of 2 mM. The mixture was stirred at 35 ºC, 200 rpm. After reaction for 8 h, 2 mL cell-free extract of HheA10 enzyme was added to the mixture. The mixture continued to react for 4 h (for chlorohydrocarbon substrates) or 1 h (for bromohydrocarbon substrates). Then, the mixture was centrifuged at 8,000 g for 5 min. 3 mL supernatant was taken and extracted with 3 mL ethyl acetate. The organic phase was separated, dried on anhydrous $NaSO_4$, and analyzed by chiral HPLC.

Table S1. Information for the recombinant *E. coli* strains.

Recombinant strain	P450, Fdx-Fdr
E. coli ($P450_{PL2}$-1)	pET28-$P450_{PL2}$; pCDFDuet-**Fdx1**-Fdr
E. coli ($P450_{PL2}$-2)	pET28-$P450_{PL2}$; pCDFDuet-**Fdx2**-Fdr
E. coli ($P450_{PL2}$-3)	pET28-$P450_{PL2}$; pCDFDuet-**Fdx3**-Fdr
E. coli ($P450_{PL2}$-4)	pET28-$P450_{PL2}$; pCDFDuet-**Fdx4**-Fdr
E. coli ($P450_{PL2}$-5)	pET28-$P450_{PL2}$; pCDFDuet-**Fdx5**-Fdr
E. coli ($P450_{PL7}$-1)	pET28-$P450_{PL7}$; pCDFDuet-**Fdx1**-Fdr
E. coli ($P450_{PL7}$-2)	pET28-$P450_{PL7}$; pCDFDuet-**Fdx2**-Fdr
E. coli ($P450_{PL7}$-3)	pET28-$P450_{PL7}$; pCDFDuet-**Fdx3**-Fdr
E. coli ($P450_{PL7}$-4)	pET28-$P450_{PL7}$; pCDFDuet-**Fdx4**-Fdr
E. coli ($P450_{PL7}$-5)	pET28-$P450_{PL7}$; pCDFDuet-**Fdx5**-Fdr
E. coli (P450pyrM1)	pRSFDuet-P450pyr**(N100S)**; pETDuet-Fdx-Fdr
E. coli (P450pyrM2)	pRSFDuet-P450pyr**(N100S/F403I)**; pETDuet-Fdx-Fdr
E. coli (P450pyrM3)	pRSFDuet-P450pyr**(N100S/F403M);** pETDuet-Fdx-Fdr
E. coli (P450pyrM4)	pRSFDuet-P450pyr**(N100S/T186I/M305Q)**; pETDuet-Fdx-Fdr
E. coli (P450pyrM5)	pRSFDuet-P450pyr**(N100S/F403I/L302V)**; pETDuet-Fdx-Fdr
E. coli (P450pyrM6)	pRSFDuet-P450pyr**(N100S/F403I/T186I/D183E)**; pETDuet-Fdx-Fdr
E. coli (P450pyrM7)	pRSFDuet-P450pyr**(N100S/F403I/T186I/L302V/I83F)**; pETDuet-Fdx-Fdr
E. coli (P450pyrM8)	pRSFDuet-P450pyr**(N100S/F403I/T186I/L302V/M305Q)**; pETDuet-Fdx-Fdr
E. coli (P450pyrM9)	pRSFDuet-P450pyr**(N100S/F403I/T186I/L302V/T259A)**; pETDuet-Fdx-Fdr
E. coli (P450pyrM10)	pRSFDuet-P450pyr**(N100S/F403I/T186I/L302V/I83F/I102P)**; pETDuet-Fdx-Fdr
E. coli (P450pyrM11)	pRSFDuet-P450**(pyrT185K)**, pETDuet-Fdx-Fdr
E. coli (P450pyrM12)	pRSFDuet-P450**(pyrT259A)**, pETDuet-Fdx-Fdr
E. coli (P450pyrM13)	pRSFDuet-P450**(pyrT185V)**, pETDuet-Fdx-Fdr
E. coli (pET-28b)	pET-28b(+)
E. coli (pCDFDuet-1)	pCDFDuet-1
E. coli (pRSFDuet-1)	pRSFDuet-1
E. coli (pETDuet-1)	pETDuet-1

Table S2. Screening biocatalysts used for asymmetric hydroxylation of **1a**.



1a **2a**

Entry[a]	Biocatalyst	Yield (%) **2a**[b]	*ee* (%) **2a**[b]	Absolute configuration[c]
1	*E. coli* ($P450_{PL2}$-1)	47.8±2.9	74.9±1.6	*R*
2	*E. coli* ($P450_{PL2}$-2)	25.6±2.1	76.4±0.9	*R*
3	*E. coli* ($P450_{PL2}$-3)	32.6±1.1	76.8±0.2	*R*
4	*E. coli* ($P450_{PL2}$-4)	49.1±2.2	77.0±0.5	*R*
5	*E. coli* ($P450_{PL2}$-5)	19.8±1.3	76.8±0.3	*R*
6	*E. coli* ($P450_{PL7}$-1)	48.3±3.2	76.5±0.1	*R*
7	*E. coli* ($P450_{PL7}$-2)	31.5±2.7	76.4±0.4	*R*
8	*E. coli* ($P450_{PL7}$-3)	32.7±1.8	76.4±0.3	*R*
9	*E. coli* ($P450_{PL7}$-4)	48.9±1.3	75.9±0.2	*R*
10	*E. coli* ($P450_{PL7}$-5)	17.2±0.9	75.9±1.6	*R*
11	*E. coli* (P450pyr-M1)	n.d.	n.d.	n.d.
12	*E. coli* (P450pyr-M2)	n.d.	n.d.	n.d.
13	*E. coli* (P450pyr-M3)	n.d.	n.d.	n.d.
14	*E. coli* (P450pyr-M4)	2.8±0.1	34.5±7.3	*S*
15	*E. coli* (P450pyr-M5)	n.d.	n.d.	n.d.
16	*E. coli* (P450pyr-M6)	17.3±0.8	89.5±1.9	*S*
17	*E. coli* (P450pyr-M7)	n.d.	n.d.	n.d.
18	*E. coli* (P450pyr-M8)	n.d.	n.d.	n.d.
19	*E. coli* (P450pyr-M9)	8.0±1.0	57.1±4.9	*S*
20	*E. coli* (P450pyr-M10)	n.d.	n.d.	n.d.
21	*E. coli* (P450pyr-M11)	n.d.	n.d.	n.d.
22	*E. coli* (P450pyr-M12)	n.d.	n.d.	n.d.
23	*E. coli* (P450pyr-M13)	n.d.	n.d.	n.d.
24	*E. coli* (pET28-b)	n.d.	n.d.	n.d.
25	*E. coli* (pCDFDuet-1)	n.d.	n.d.	n.d.
26	*E. coli* (pRSFDuet-1)	n.d.	n.d.	n.d.
27	*E. coli* (pETDuet-1)	n.d.	n.d.	n.d.

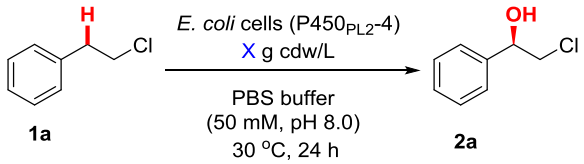
[a] Reactions were carried out in 5 mL PBS buffer (50 mM, pH 8.0) containing 2 mM of substrate **1a** and 10 g cdw/L of recombinant *E. coli* cells.

[b] Yield and ee values were measured with three parallel samples by chiral HPLC analysis after incubation at 30 °C for 24 h.

[c] Absolute configuration was confirmed using commercial (*R*)-**2a** and (*R*,*S*)-**2a**.

n.d. = not determined.

Table S3. Optimization of the cell density of the *E. coli* ($P450_{PL2}$-4) catalyzed asymmetric hydroxylation of **1a**.

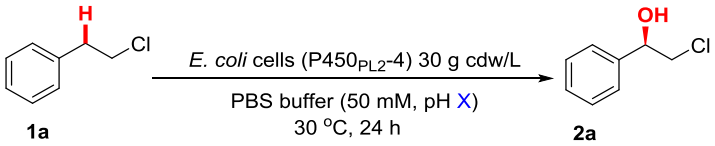


Entry[a]	biocatalyst loading (g cdw/L)	Yield (%) **2a**[b]	ee (%) (*R*)-**2a**[b]
1	5	33.6±0.4	76.5±0.3
2	10	40.5±1.2	77.1±0.1
3	20	49.1±0.7	77.9±0.3
4	30	61.5±2.7	84.8±0.1
5	50	54.9±5.8	84.7±0.4

[a] Reactions were carried out in 5 mL PBS buffer (50 mM, pH 8.0) containing 2 mM of substrate **1a** and X g cdw/L recombinant *E. coli* ($P450_{PL2}$-4) cells;

[b] Yield and ee values were measured with three parallel samples by chiral HPLC analysis after incubation at 30 °C for 12 h.

Table S4. Optimization of the reaction pH of the *E. coli* ($P450_{PL2}$-4) catalyzed asymmetric hydroxylation of **1a**.

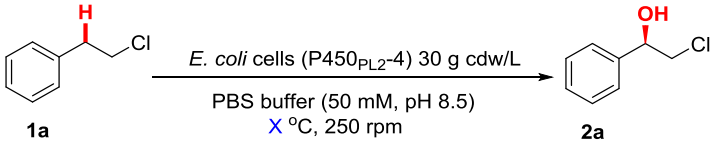


Entry[a]	pH value	Yield (%) **2a**[b]	*ee* (%) (*R*)-**2a**[b]
1	7.0	39.3±2.1	93.1±1.0
2	7.5	37.0±2.2	94.9±0.3
3	8.0	48.9±3.5	93.4±0.2
4	8.5	63.1±1.0	89.1±0.2
5	9.0	55.5±1.9	91.4±0.4

[a] Reactions were carried out in 5 mL PBS buffer (50 mM, pH X) containing 2 mM of substrate **1a** and 30 g cdw/L of recombinant *E. coli* ($P450_{PL2}$-4) cells;

[b] Yield and ee values were measured with three parallel samples by chiral HPLC analysis after incubation at 30 °C for 12 h.

Table S5. Optimization of the reaction temperature of the *E. coli* ($P450_{PL2}$-4) catalyzed asymmetric hydroxylation of **1a**.



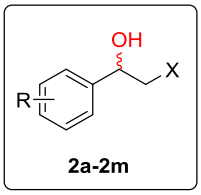
Entry [a]	Reaction temperature (°C)	Yield (%) **2a**[b]	ee (%) (*R*)-**2a**[b]
1	20	33.5±0.3	95.5±1.5
2	30	60.2±0.6	88.8±0.9
3	35	80.3±0.09	81.6±0.6
4	40	72.5±0.06	76.4±0.04
5	45	46.4±0.5	74.7±0.04
6	50	15.8±0.3	73.4±1.1

[a] Reactions were carried out in 5 mL PBS buffer (50 mM, pH 8.5) containing 2 mM of substrate **1a** and 30 g cdw/L of recombinant *E. coli* ($P450_{PL2}$-4) cells;

[b] Yield and ee values were measured with three parallel samples by chiral HPLC analysis after incubation at X °C for 12 h.

Table S6. Chiral HPLC methods for analysis of halohydrins **2a-2m**.

2a: X=Cl, R=H,
2b: X=Cl, R=2-CH_3
2c: X=Cl, R=3-CH_3
2d: X=Cl, R=4-CH_3
2e: X=Cl, R=4-F
2f: X=Cl, R=3-Br,



2a-2m

2g: X=Br, R=H
2h: X=Br, R=2-CH_3
2i: X=Br, R=3-CH_3
2j: X=Br, R=4-CH_3
2k: X=Br, R=4-F
2l: X=Br, R=4-Cl
2m: X=Br, R=3-Br

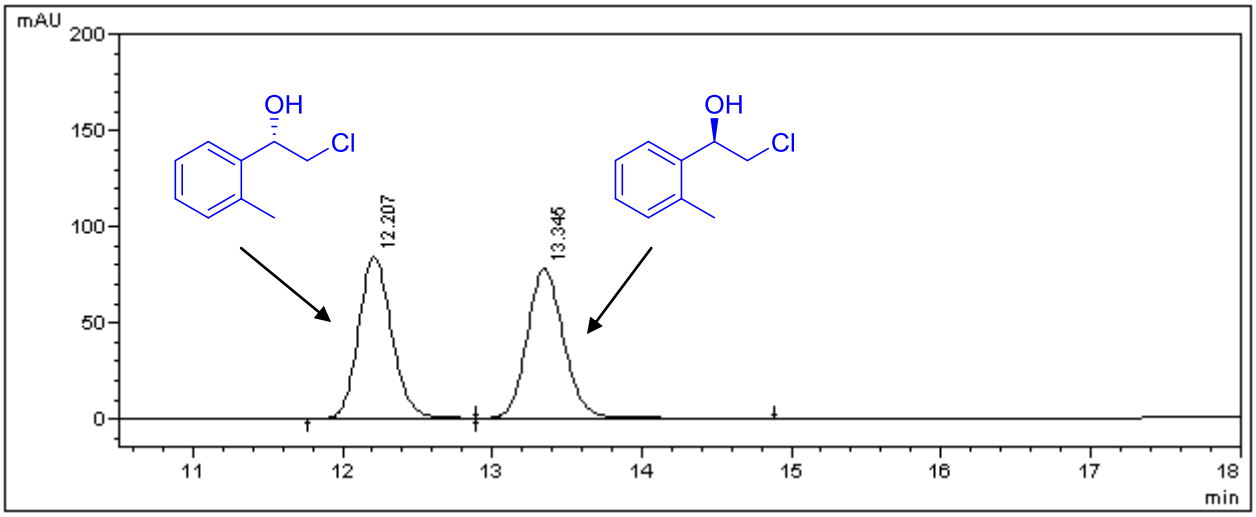
Entry[a]	Halohydrin	Analysis method[a]	Standard curve[b]	Retention time (min)
1	**2a**	A	y=1.2924x-0.0295, R^2=0.9997	t_S=12.0, t_R=13.0
2	**2b**	A	y=1.4855x-0.0005, R^2=0.9999	t_S=11.0, t_R=12.2
3	**2c**	A	y=33619x-460.94, R^2=0.9996	t_S=10.2, t_R=11.9
4	**2d**	A	y=2.4973x+0.0086, R^2=0.9999	t_S=10.2, t_R=10.9
5	**2e**	B	y=0.4896x+0.0063, R^2=0.9999	t_R=14.7, t_S=15.4
6	**2f**	A	y=2.2953x+0.0116, R^2=0.9999	t_R=12.6, t_S=15.1
7	**2g**	A	y=1.8283x+0.0083, R^2=0.9999	t_S=12.4, t_R=13.4
8	**2h**	A	y=1.9742x+0.0029, R^2=0.9997	t_R=11.4, t_S=12.9
9	**2i**	A	y=2.3756x+0.0026, R^2=0.9999	t_S=10.7, t_R=12.6
10	**2j**	A	y=2.8416x+0.0573, R^2=0.9989	t_S=10.5, t_R=11.5
11	**2k**	B	y=76482x-15985, R^2=0.9998	t_R=14.0, t_S=14.7
12	**2l**	C	y=1.0345x+0.0088, R^2=0.9999	t_R=13.2, t_S=14.5
13	**2m**	A	y=3.3972x-0.1156, R^2=0.9994	t_R=13.0, t_S=15.4

[a] Analysis method: (A) OD-H column, 2-propanol:hexane = 5:95, 1 mL/min, 220 nm; (B) OJ-H column, 2-propanol:hexane = 10:90, 0.8 mL/min, 220 nm; (C) OJ-H column, 2-propanol:hexane = 10:90, 0.8 mL/min, 215 nm; (D) AD-H column, 2-propanol:hexane = 5:95, 1 mL/min, 220 nm.

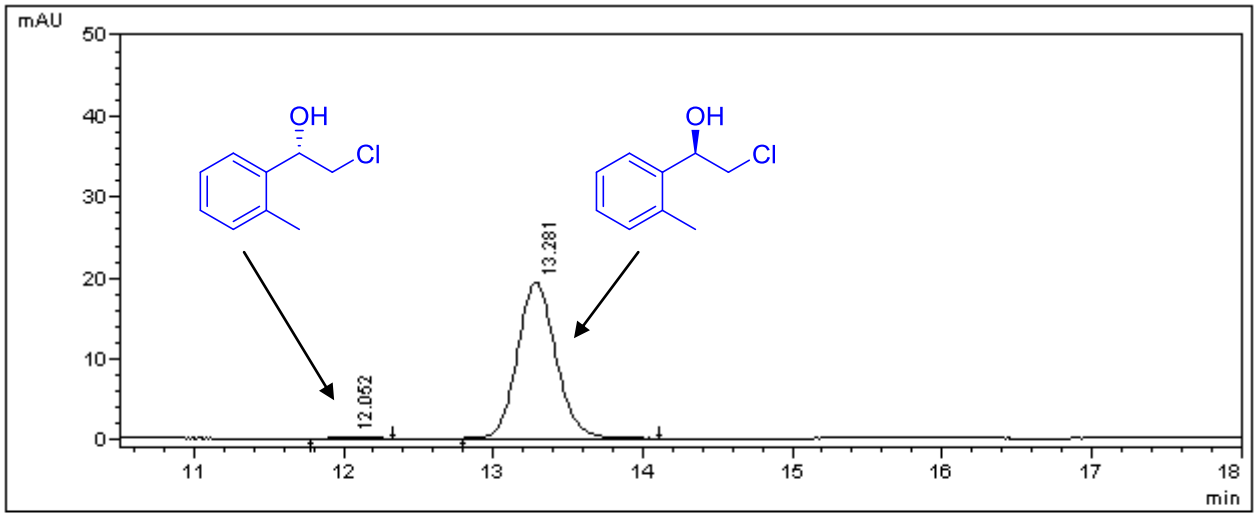
[b] The standard curves of **2c** and **2k** were drew using external standard method, x = the concentration of halohydrin, y = the peak area of halohydrin; The other standard curves were drew using internal standard method, x = the concentration of halohydrin, y = the ration of the peak area of halohydrin to the peak area of internal standard (2 mM phenethylol).

References

1. Pluempanupat W, Chavasiri W. An efficient method for chlorination of alcohols using PPh_3/Cl_3CCONH_2. Tetrahedron letters, 2006, 47(38): 6821-6823.
2. Han Y, Zheng B, Peng Y. Construction of Chiral 2-Substituted Octahydroindoles from Cyclic Ketones and Nitroolefins Bearing only One α-Substituent. Advanced Synthesis & Catalysis, 2015, 357(6): 1136-1142.
3. Wan N, Tian J, Wang H, et al. Identification and characterization of a highly S-enantioselective halohydrin dehalogenase from Tsukamurella sp. 1534 for kinetic resolution of halohydrins. Bioorganic chemistry, 2018, 81: 529-535.
4. Swamy P, Kumar M A, Reddy M M, et al. The vicinal functionalization of olefins: a facile route to the direct synthesis of β-chlorohydrins and β-chloroethers. RSC Advances, 2014, 4(50): 26288-26294.
5. Zhang X, Li J, Tian H, et al. Catalytic Asymmetric Bromination of Unfunctionalized Olefins with H_2O as a Nucleophile. Chemistry-A European Journal, 2015, 21(33): 11658-11663.



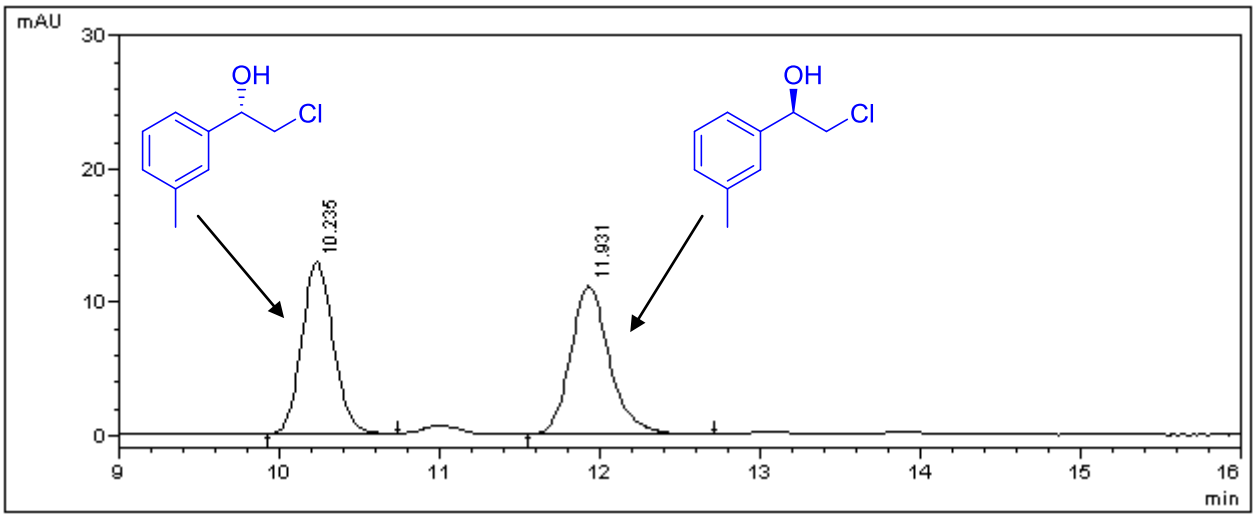
Synthesized by chemical method



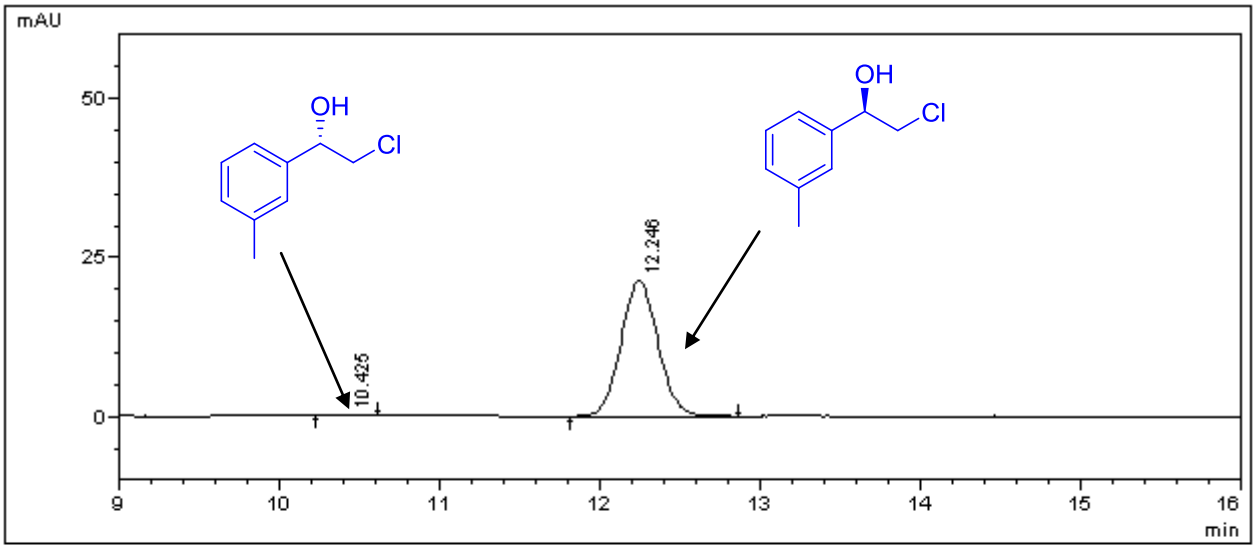
PDA

ID#	Rt. Time	Area	Height	Area %
1	12.052	4000	271	1.145
2	13.281	345291	19249	98.855

Synthesized by cascade bio-hydroxylation and dehalogenation



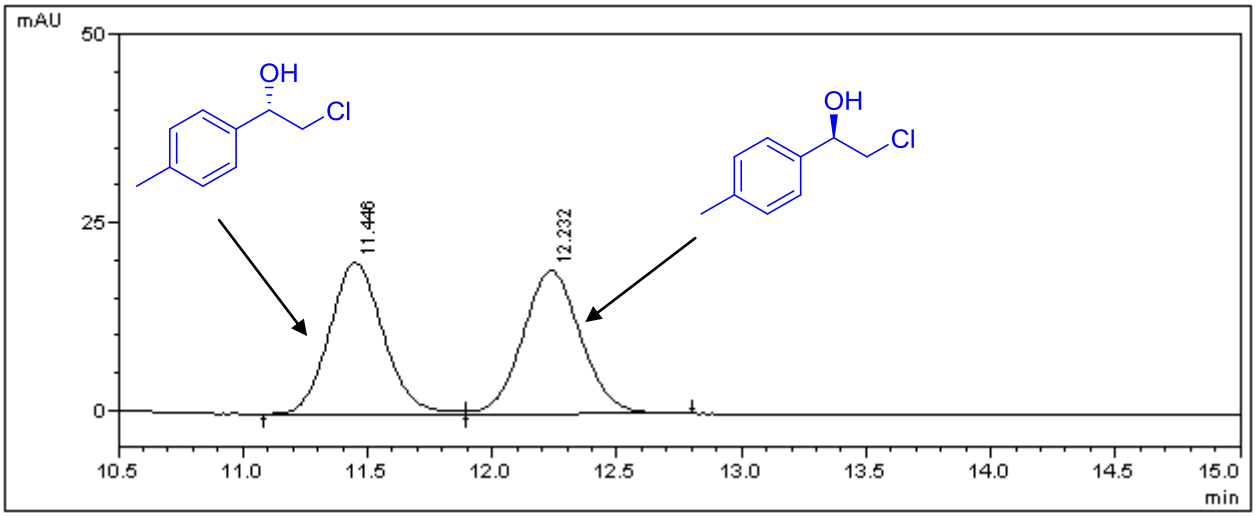
Synthesized by chemical method



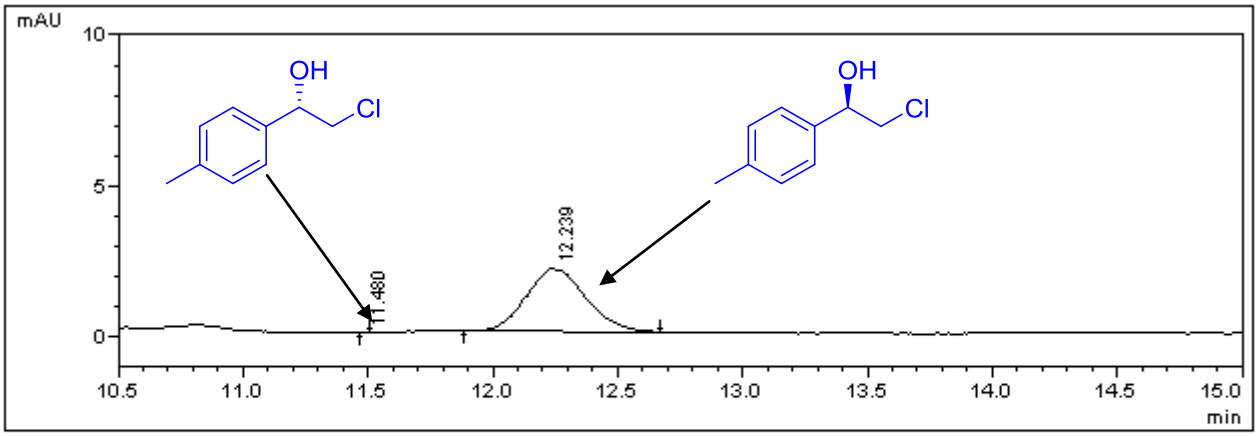
PDA

ID#	Rt. Time	Area	Height	Area %
1	10.425	2667	231	0.775
2	12.246	341474	21306	99.225

Synthesized by cascade bio-hydroxylation and dehalogenation



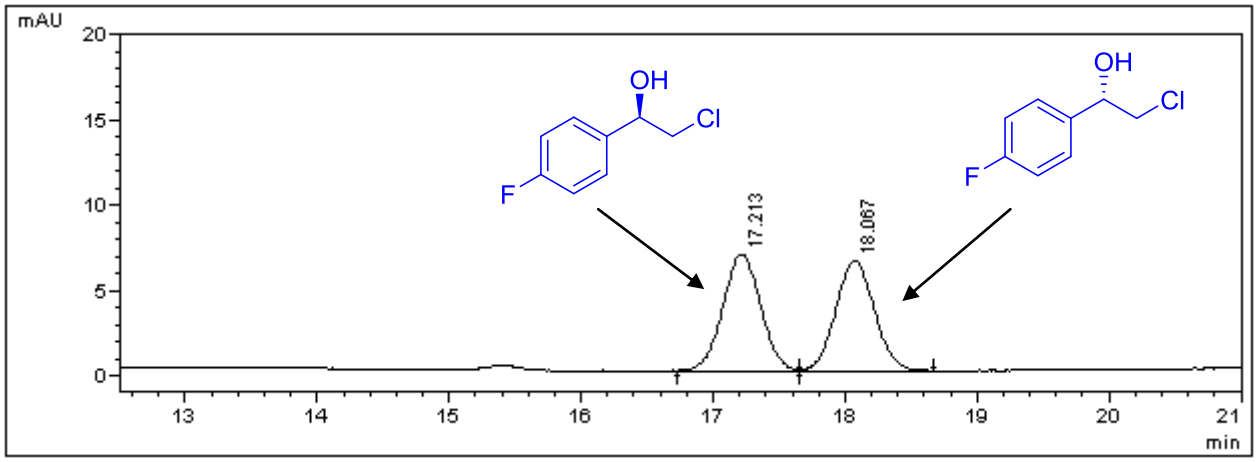
Synthesized by chemical method



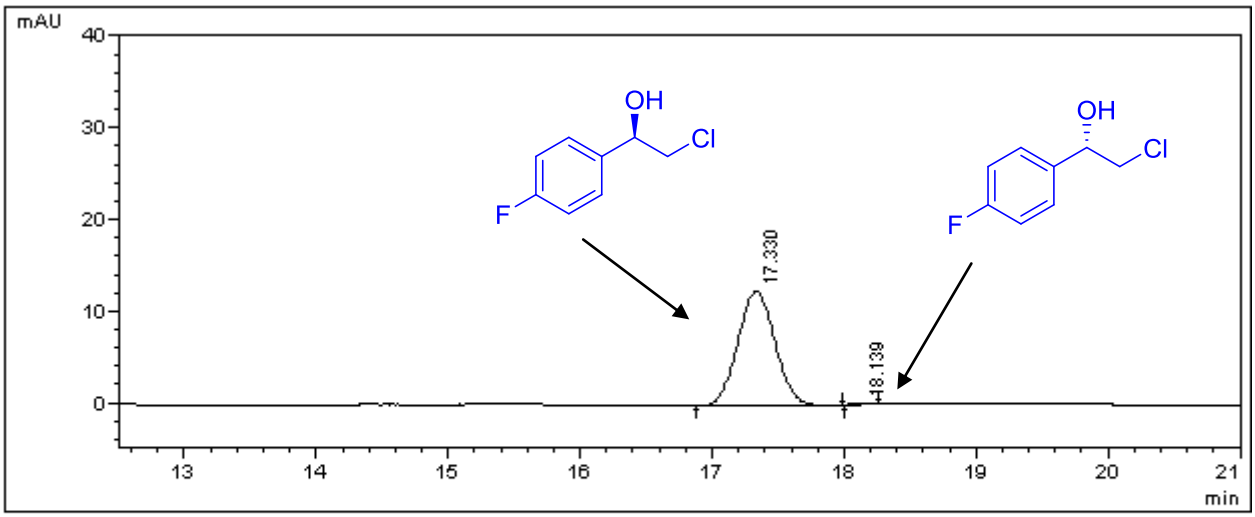
PDA

ID#	Rt. Time	Area	Height	Area %
1	11.480	-5	3	-0.016
2	12.239	34440	2063	100.016

Synthesized by cascade bio-hydroxylation and dehalogenation



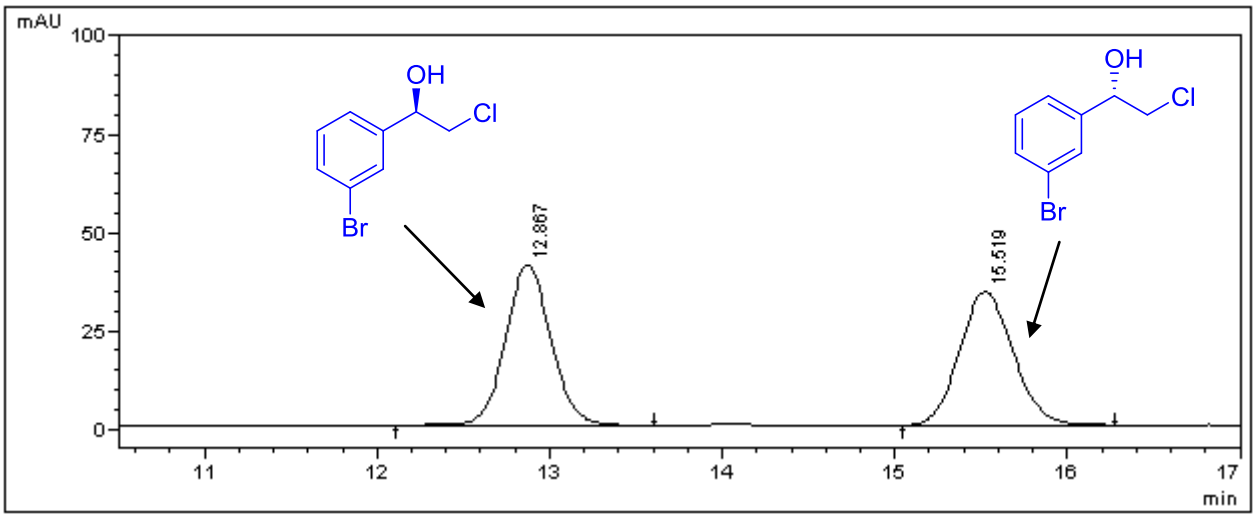
Synthesized by chemical method



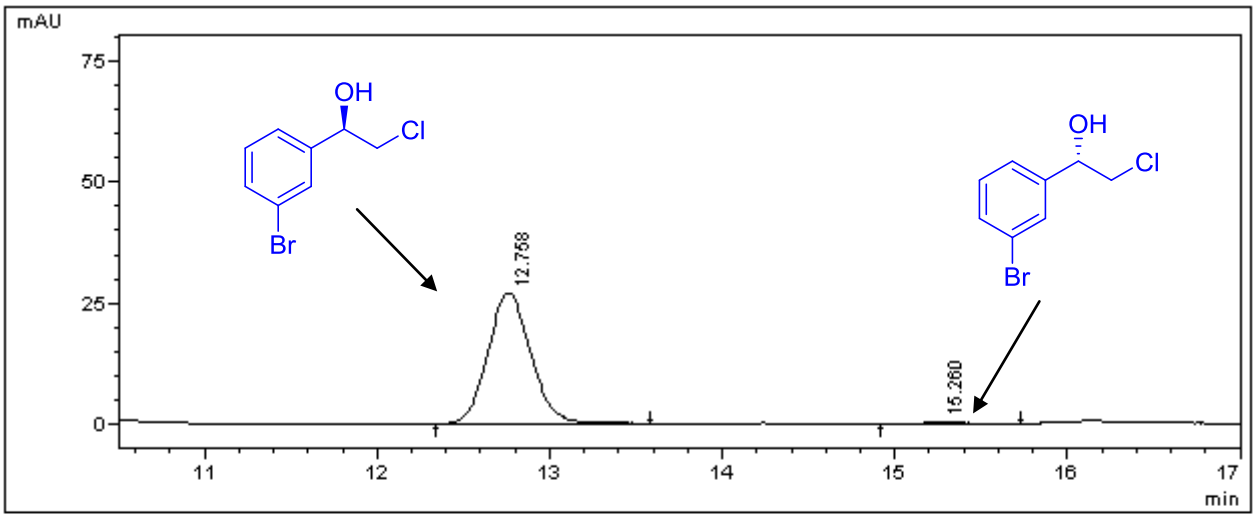
PDA

ID#	Rt. Time	Area	Height	Area %
1	17.330	244362	12516	99.823
2	18.139	432	45	0.177

Synthesized by cascade bio-hydroxylation and dehalogenation



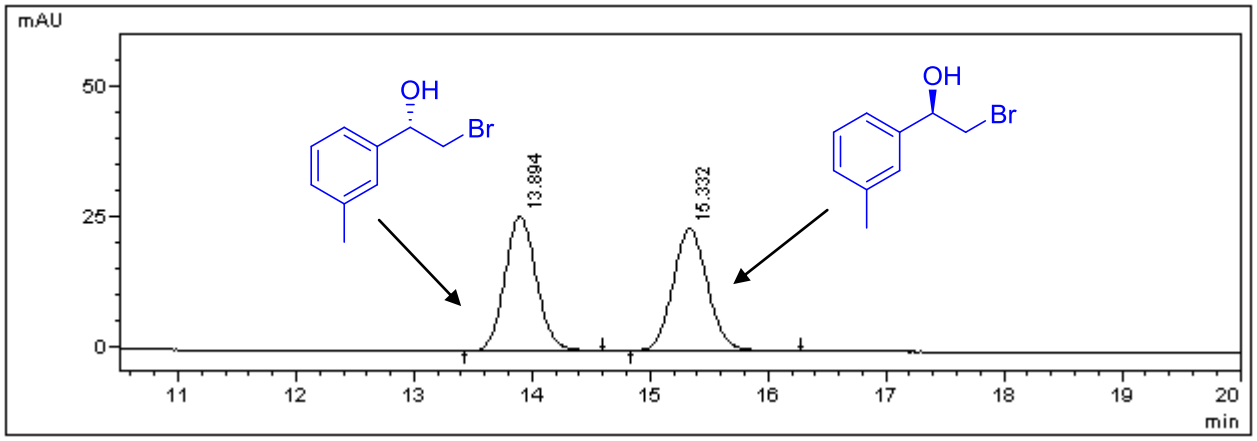
Synthesized by chemical method



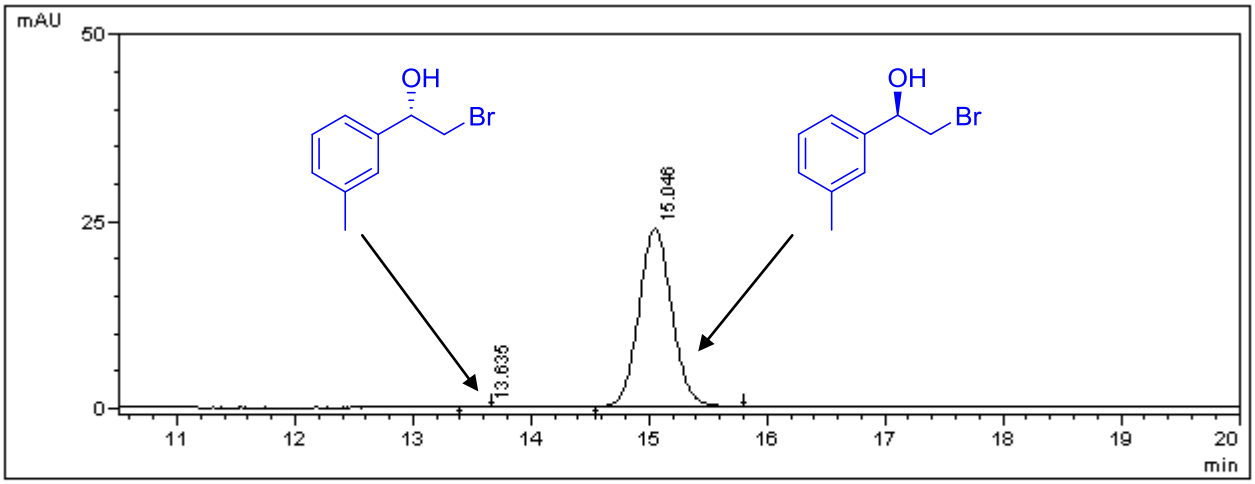
PDA

ID#	Rt. Time	Area	Height	Area %
1	12.758	464824	26869	99.593
2	15.260	1902	119	0.407

Synthesized by cascade bio-hydroxylation and dehalogenation



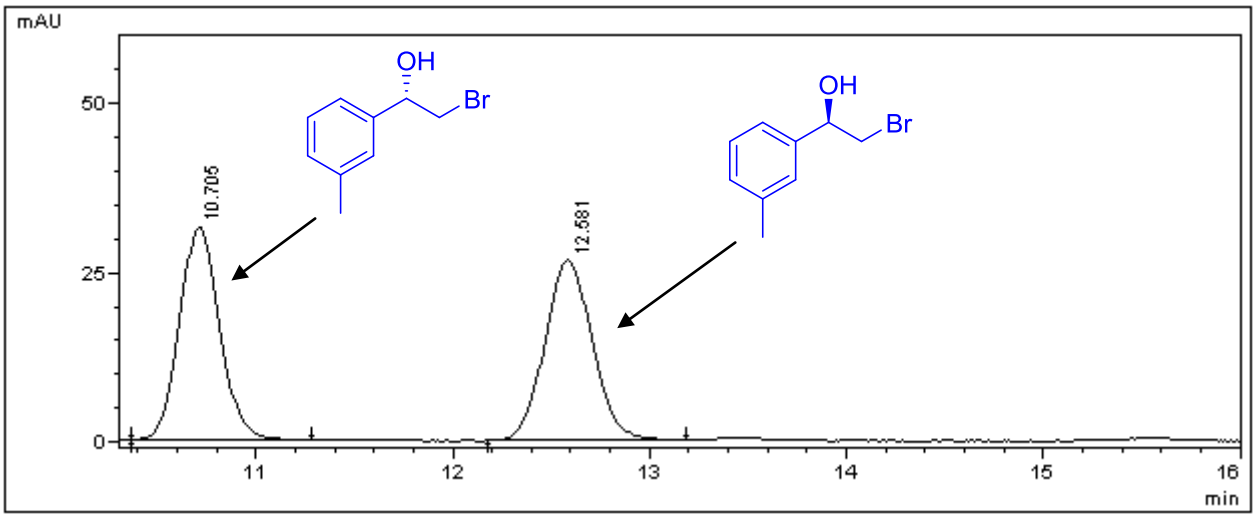
Synthesized by chemical method



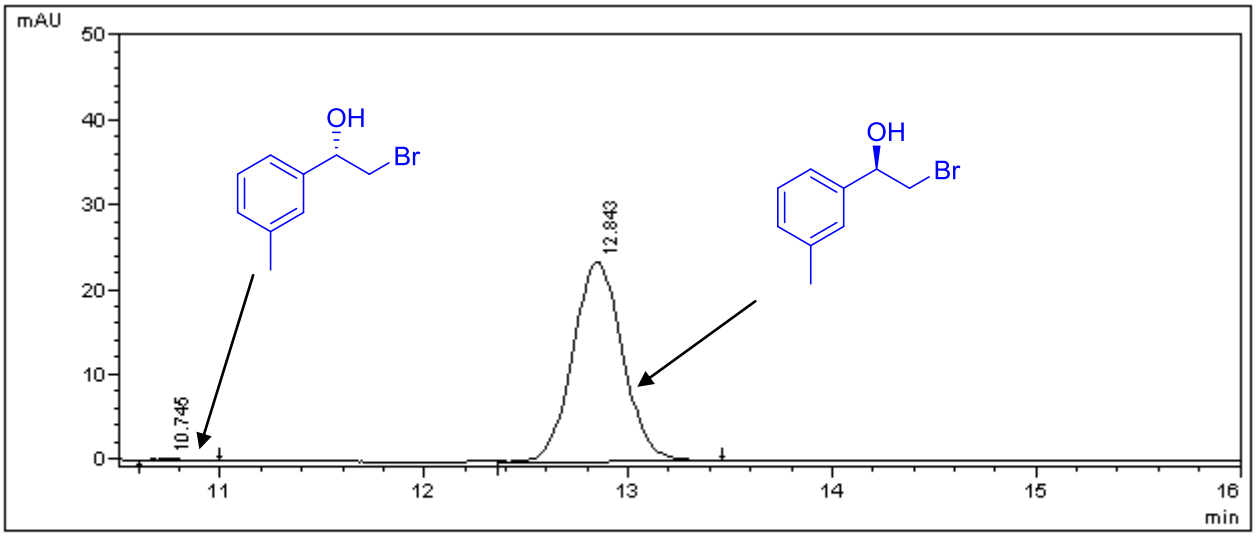
PDA

ID#	Rt. Time	Area	Height	Area %
1	13.635	83	23	0.018
2	15.046	460612	23805	99.982

Synthesized by cascade bio-hydroxylation and dehalogenation



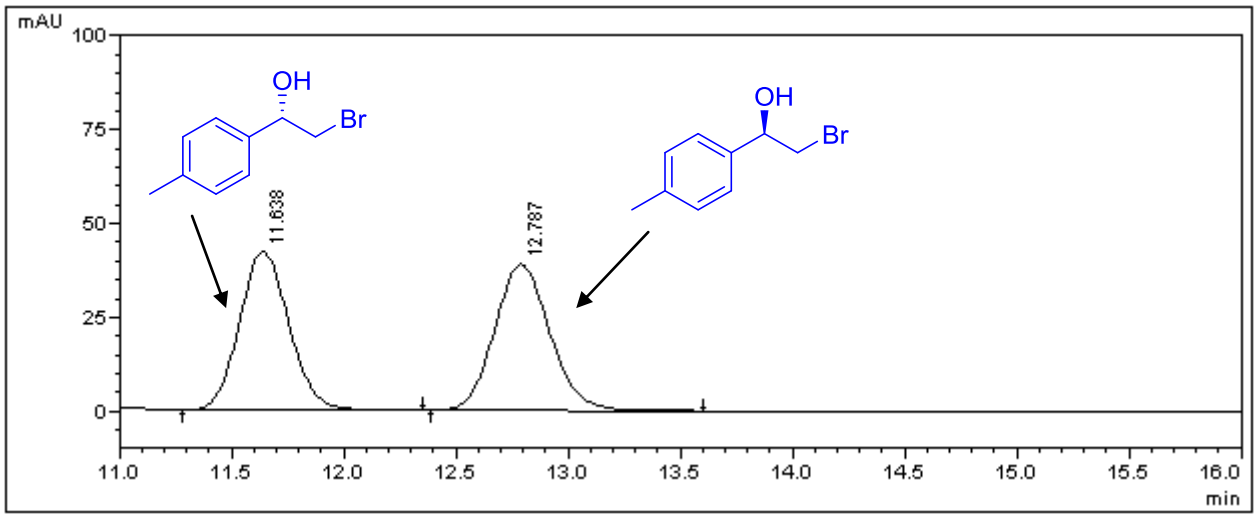
Synthesized by chemical method



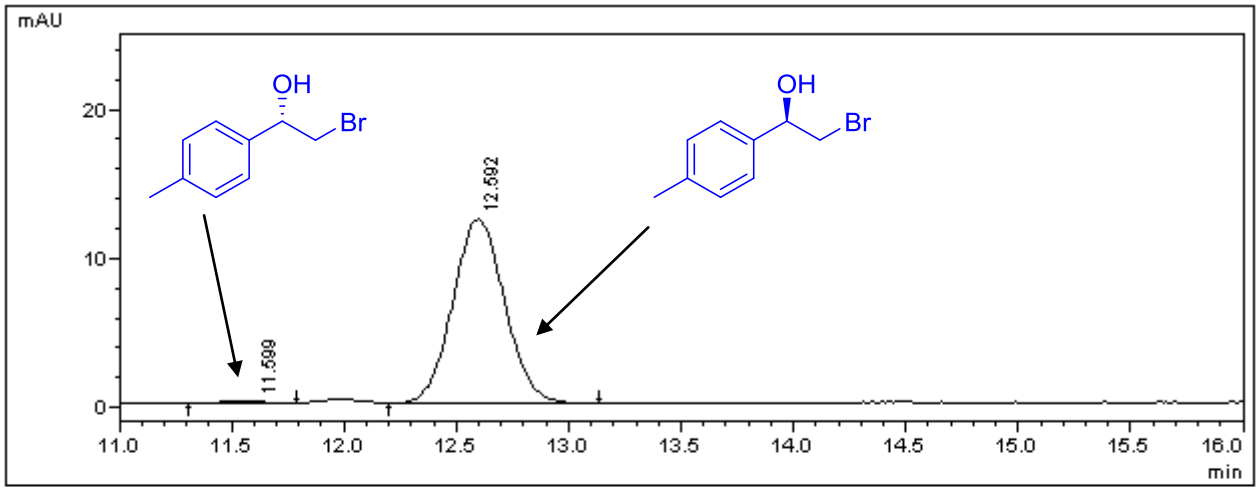
PDA

ID#	Rt. Time	Area	Height	Area %
1	10.745	1026	92	0.257
2	12.843	397711	23542	99.743

Synthesized by cascade bio-hydroxylation and dehalogenation



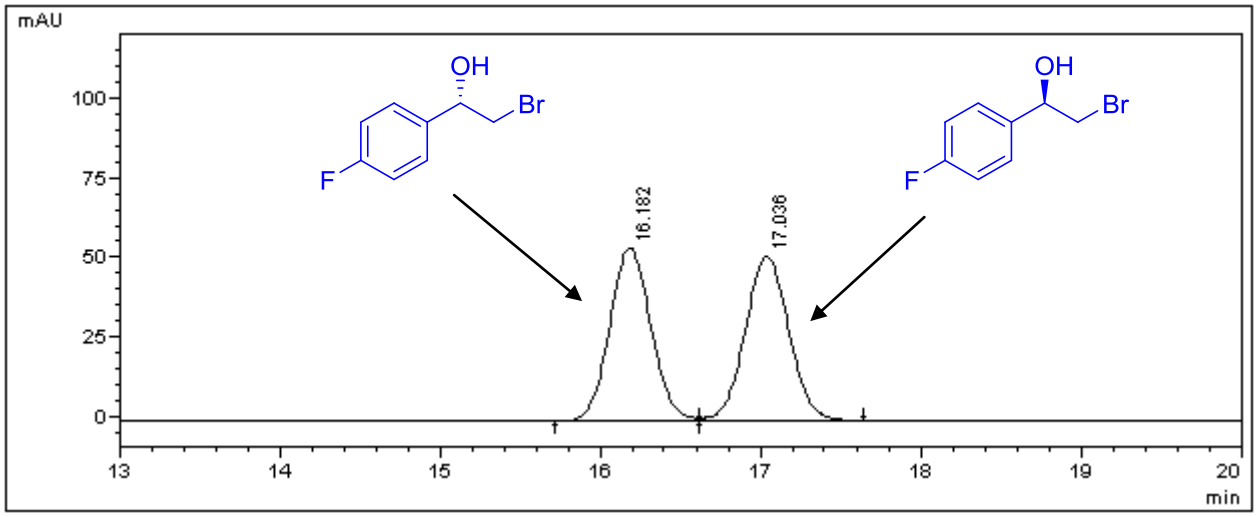
Synthesized by chemical method



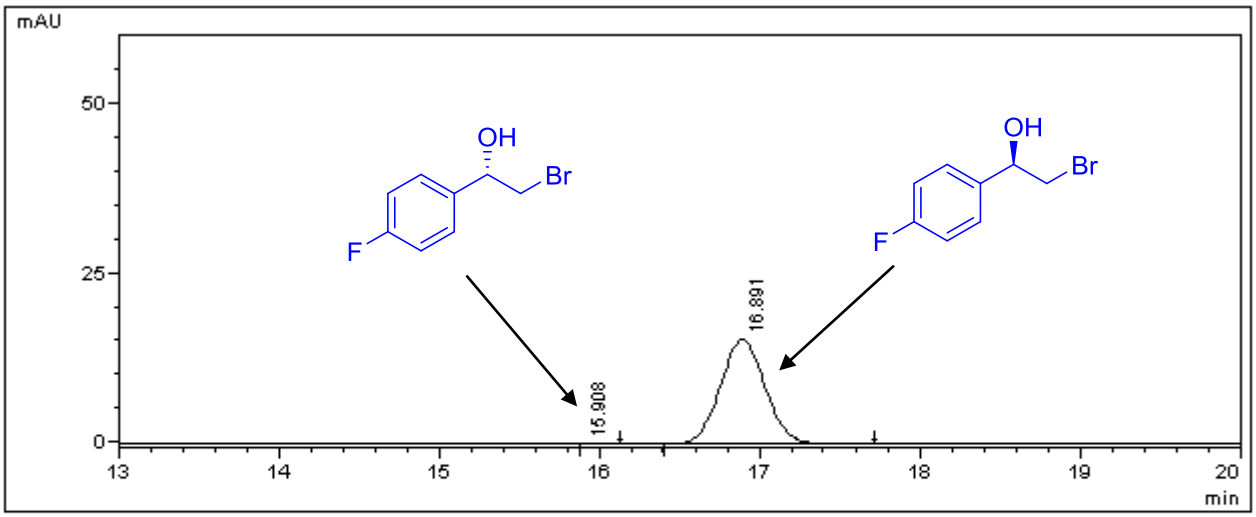
PDA

ID#	Rt. Time	Area	Height	Area %
1	11.599	1215	95	0.606
2	12.592	199242	12379	99.394

Synthesized by cascade bio-hydroxylation and dehalogenation



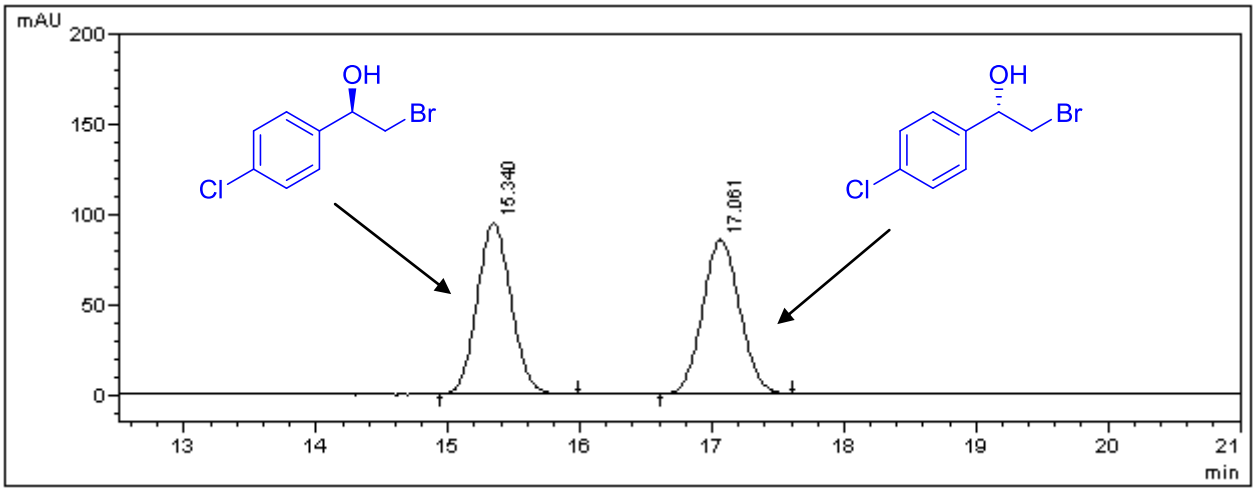
Synthesized by chemical method



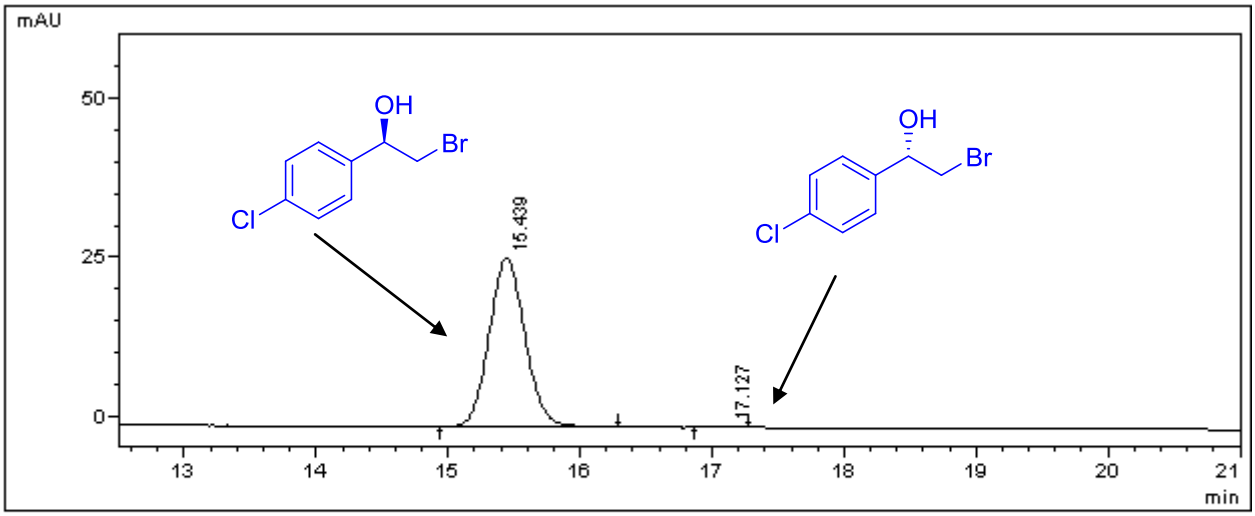
PDA

ID#	Rt. Time	Area	Height	Area %
1	15.908	84	22	0.028
2	16.891	303747	15450	99.972

Synthesized by cascade bio-hydroxylation and dehalogenation



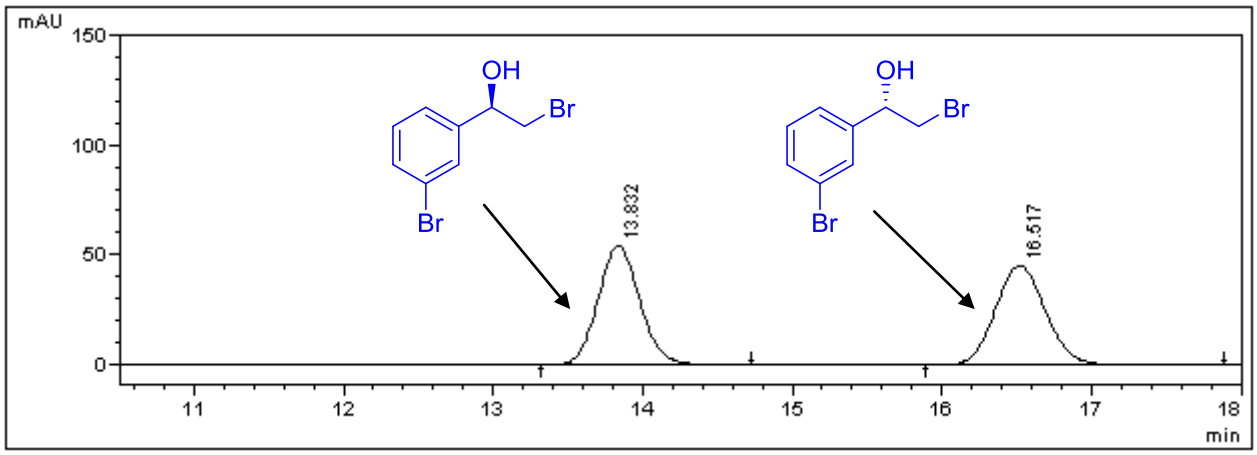
Synthesized by chemical method



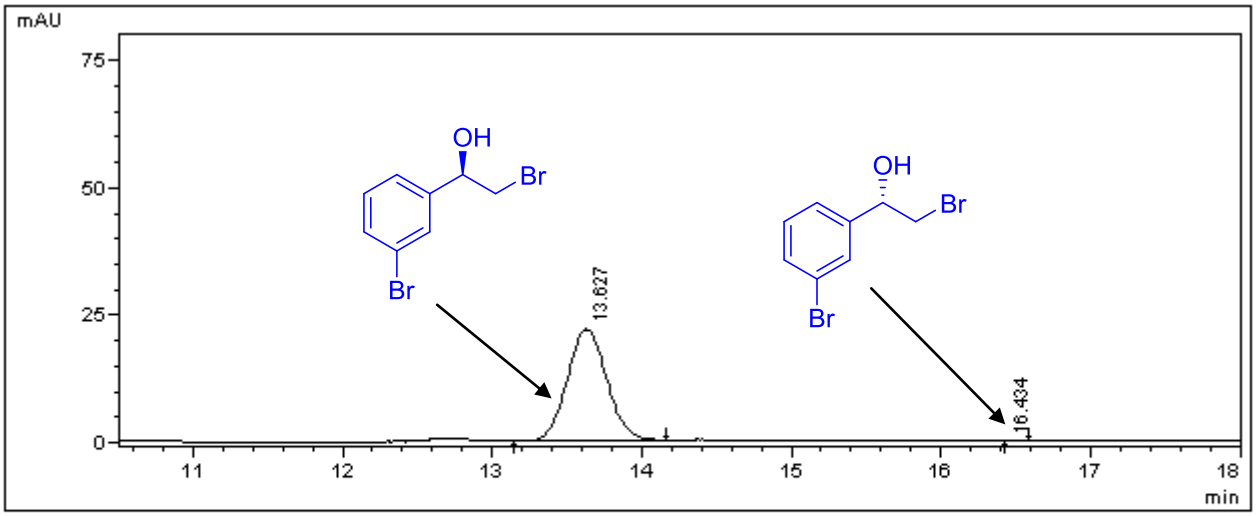
PDA

ID#	Rt. Time	Area	Height	Area %
1	15.439	501505	26505	99.819
2	17.127	909	87	0.181

Synthesized by cascade bio-hydroxylation and dehalogenation



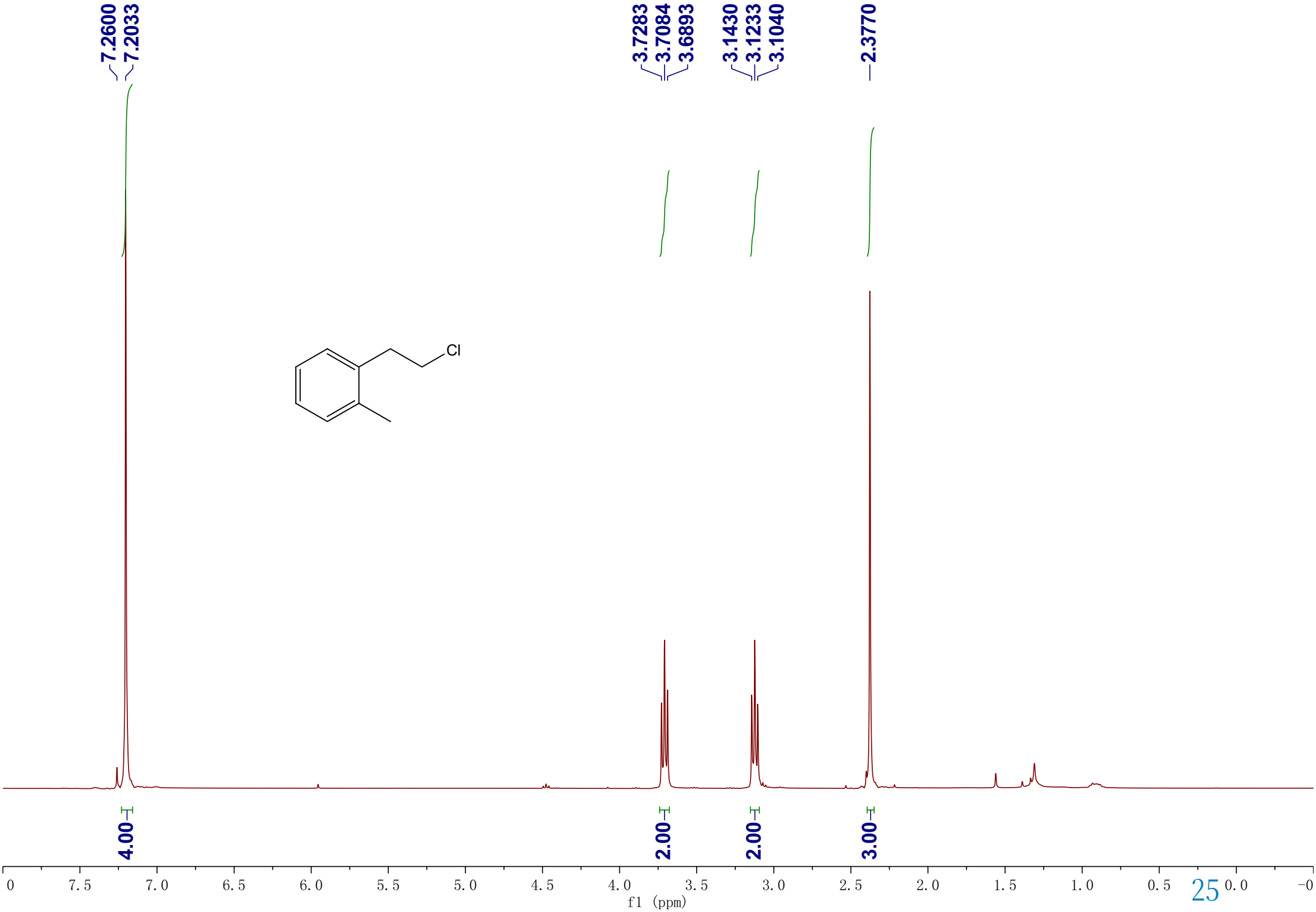
Synthesized by chemical method

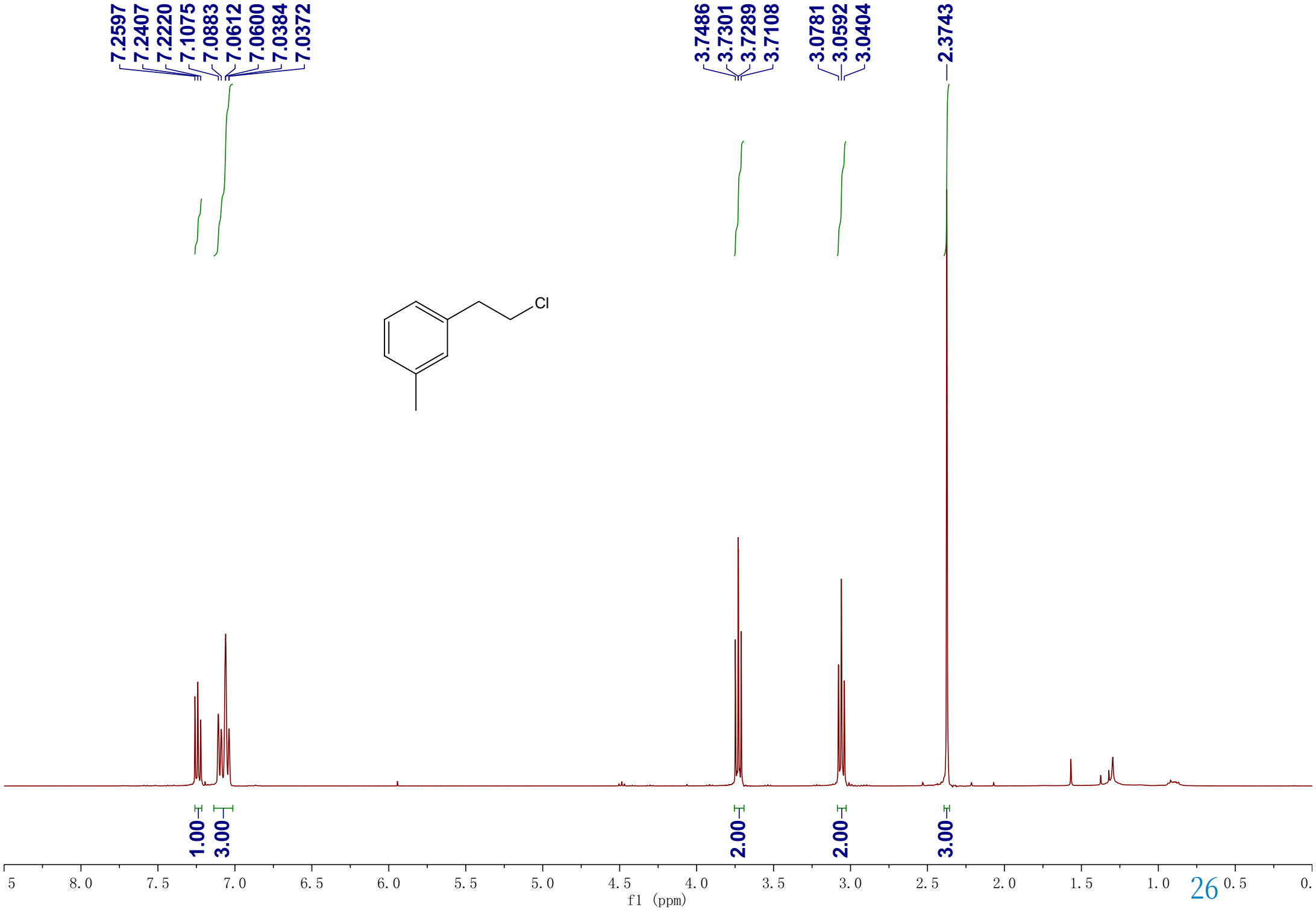


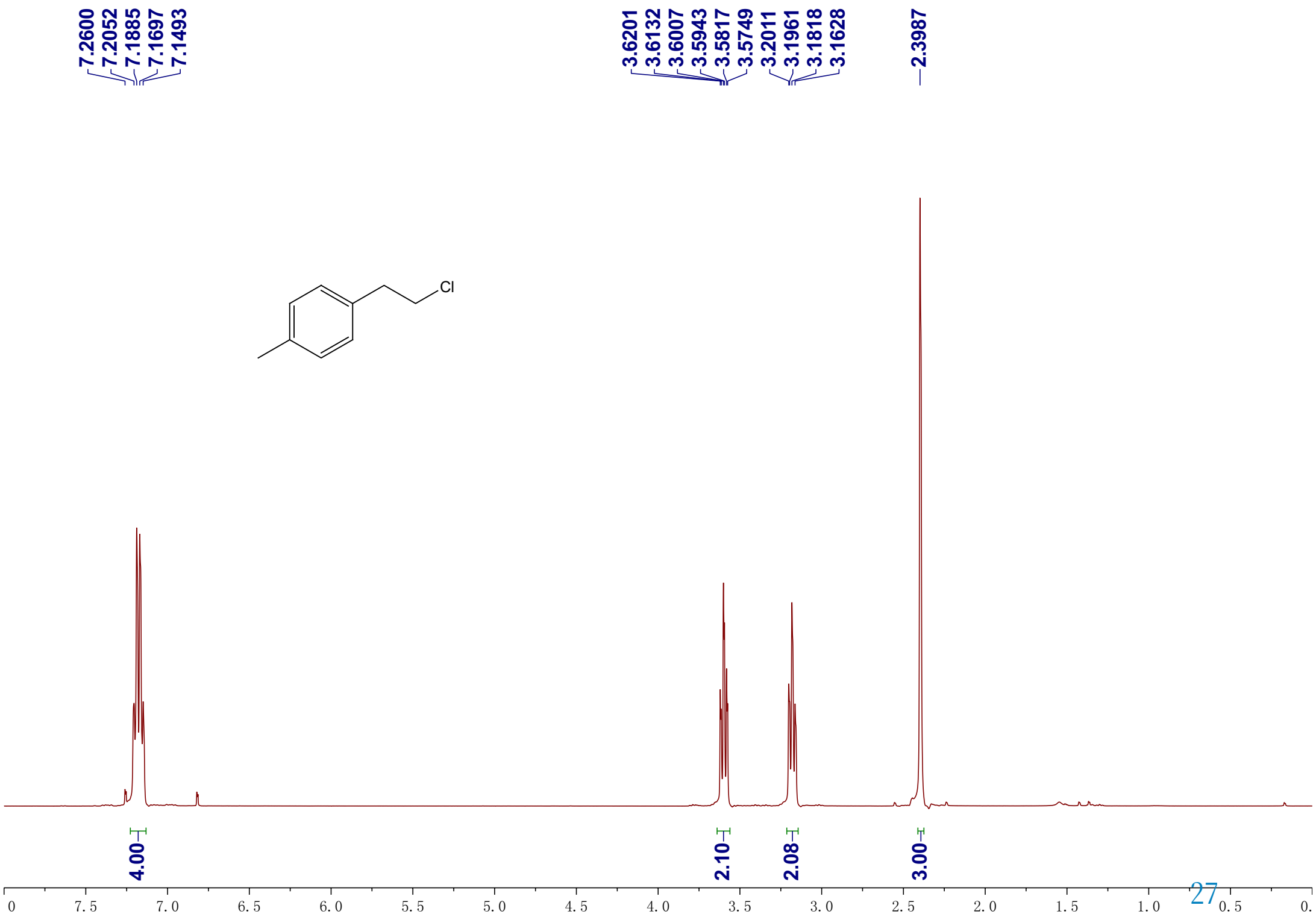
PDA

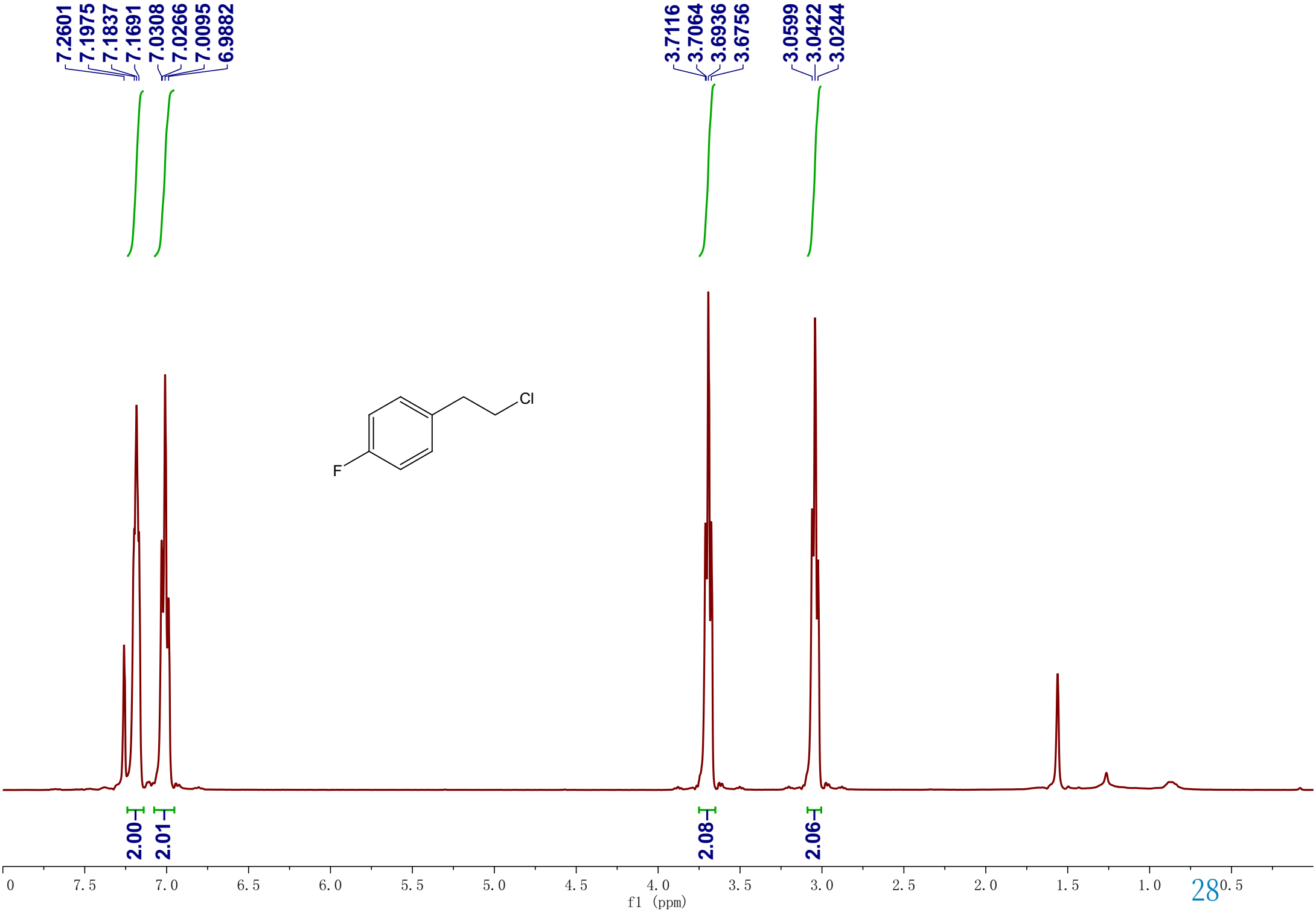
ID#	Rt. Time	Area	Height	Area %
1	13.627	402306	21928	99.991
2	16.434	36	10	0.009

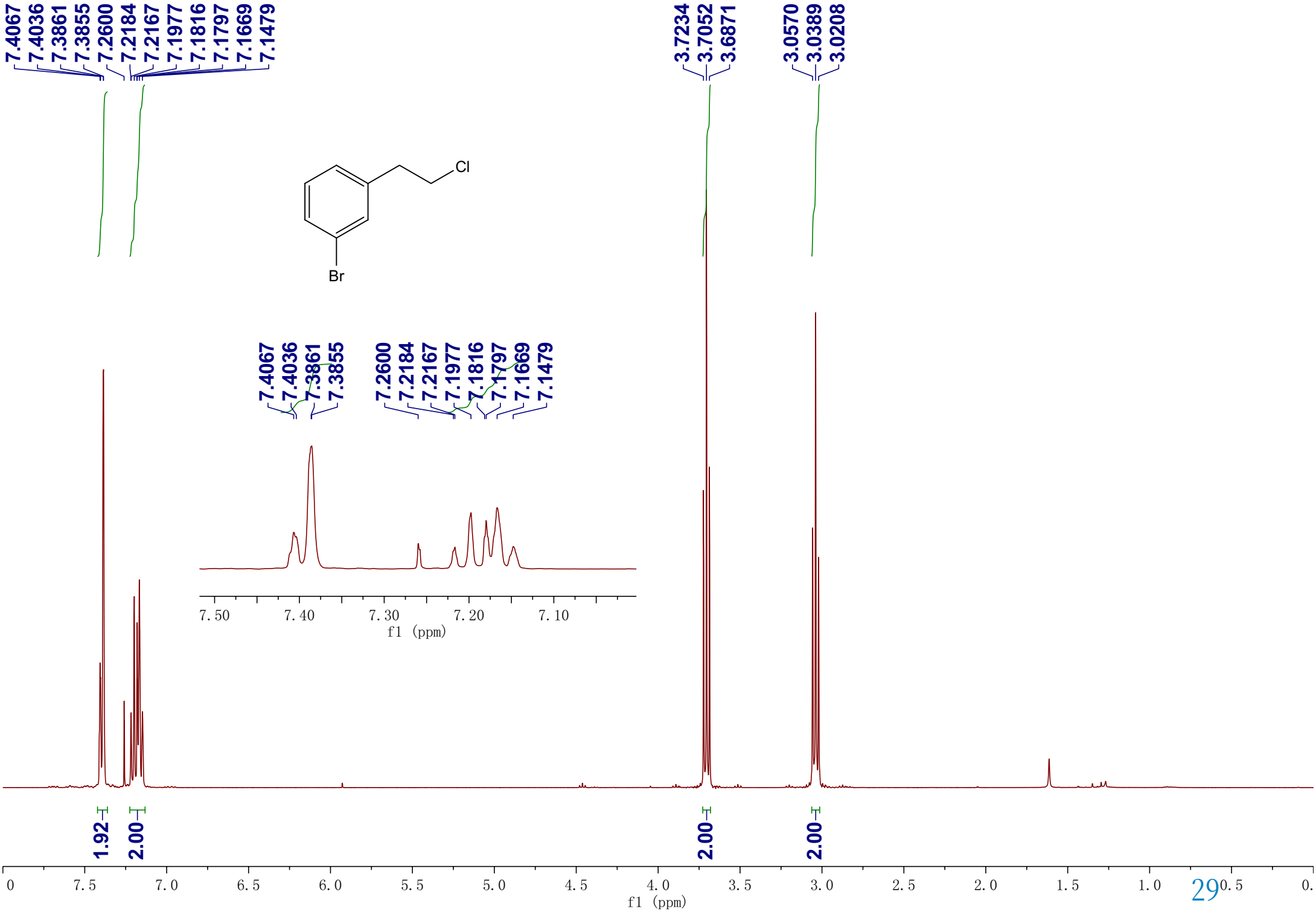
Synthesized by cascade bio-hydroxylation and dehalogenation

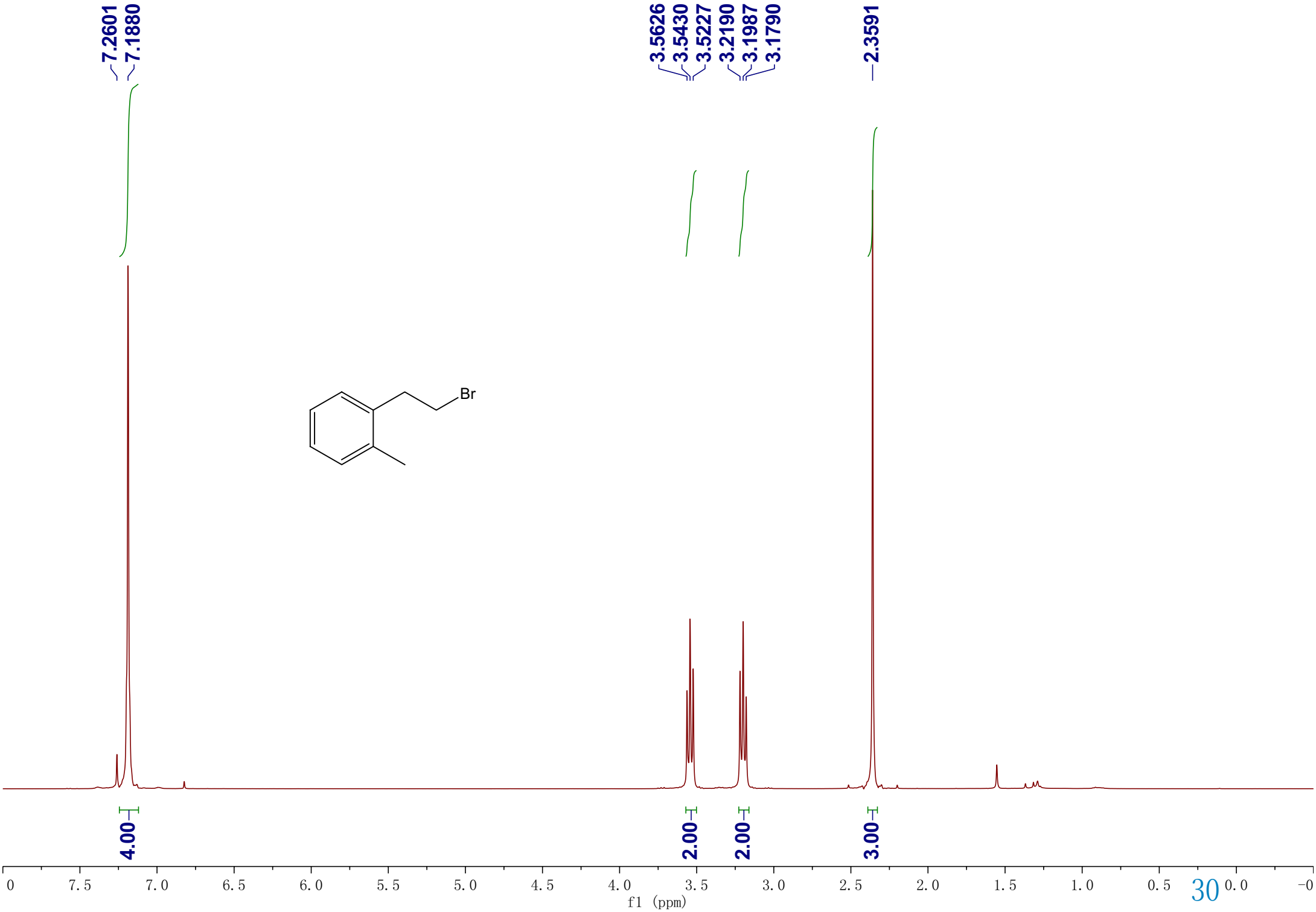

7.2600
7.2033
3.7283
3.7084
3.6893
3.1430
3.1233
3.1040
2.3770
Cl
4.00
2.00
2.00
3.00
0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0
f1 (ppm)

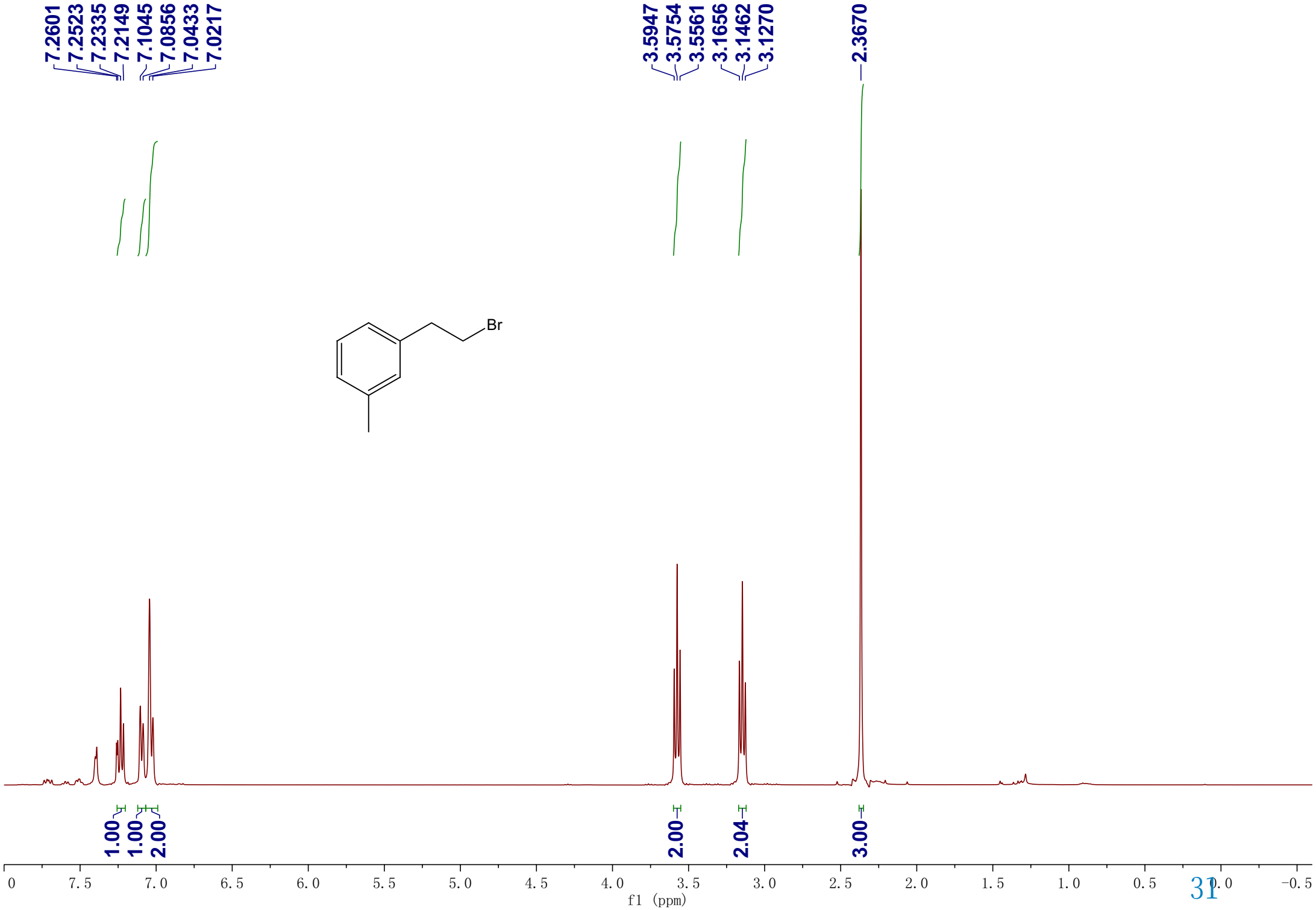

7.2597
7.2407
7.2220
7.1075
7.0883
7.0612
7.0600
7.0384
7.0372
3.7486
3.7301
3.7289
3.7108
3.0781
3.0592
3.0404
2.3743
Cl
1.00
3.00
2.00
2.00
3.00
f1 (ppm)

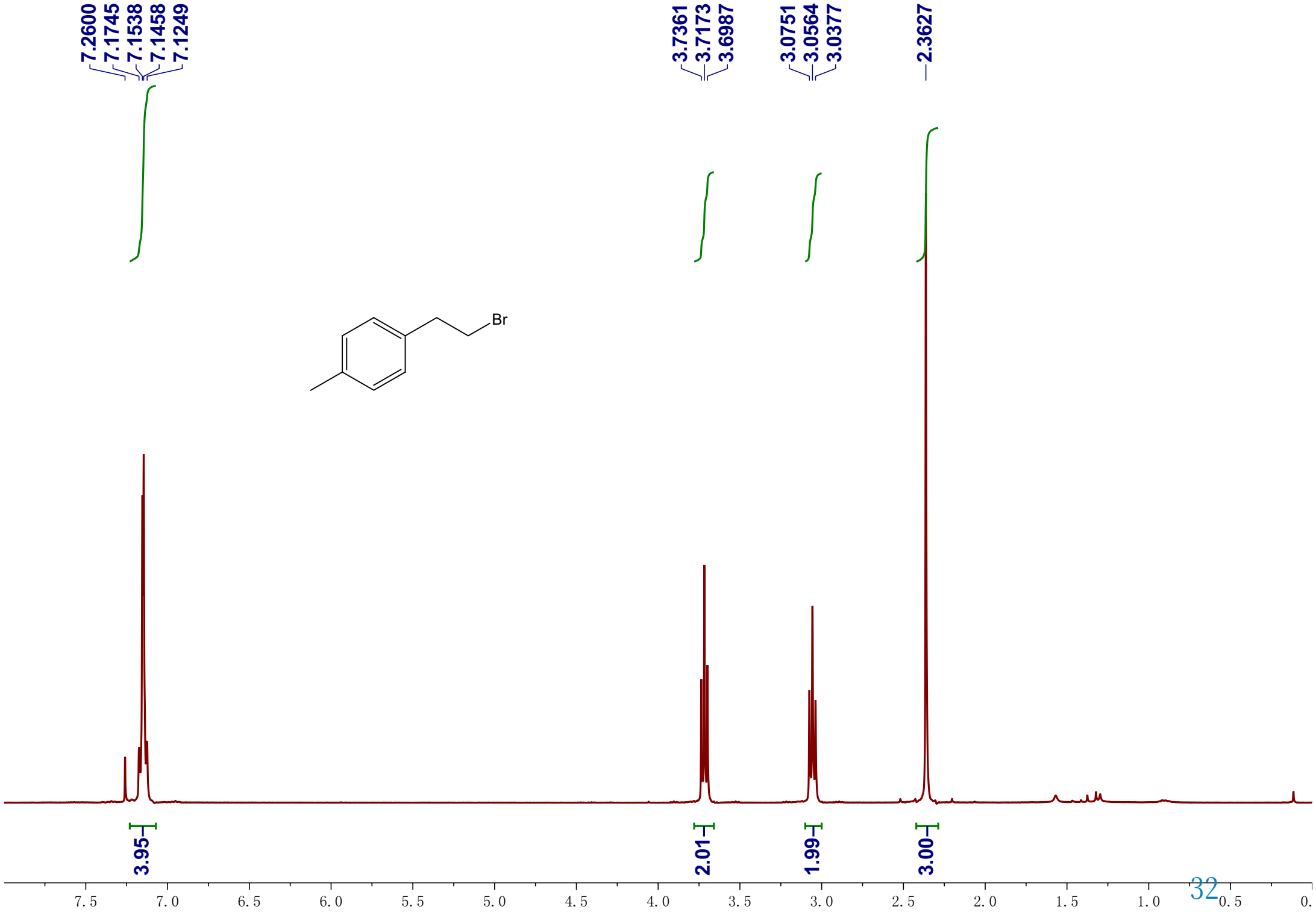

7.2600
7.2052
7.1885
7.1697
7.1493
3.6201
3.6132
3.6007
3.5943
3.5817
3.5749
3.2011
3.1961
3.1818
3.1628
2.3987
Cl
4.00
2.10
2.08
3.00
0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.

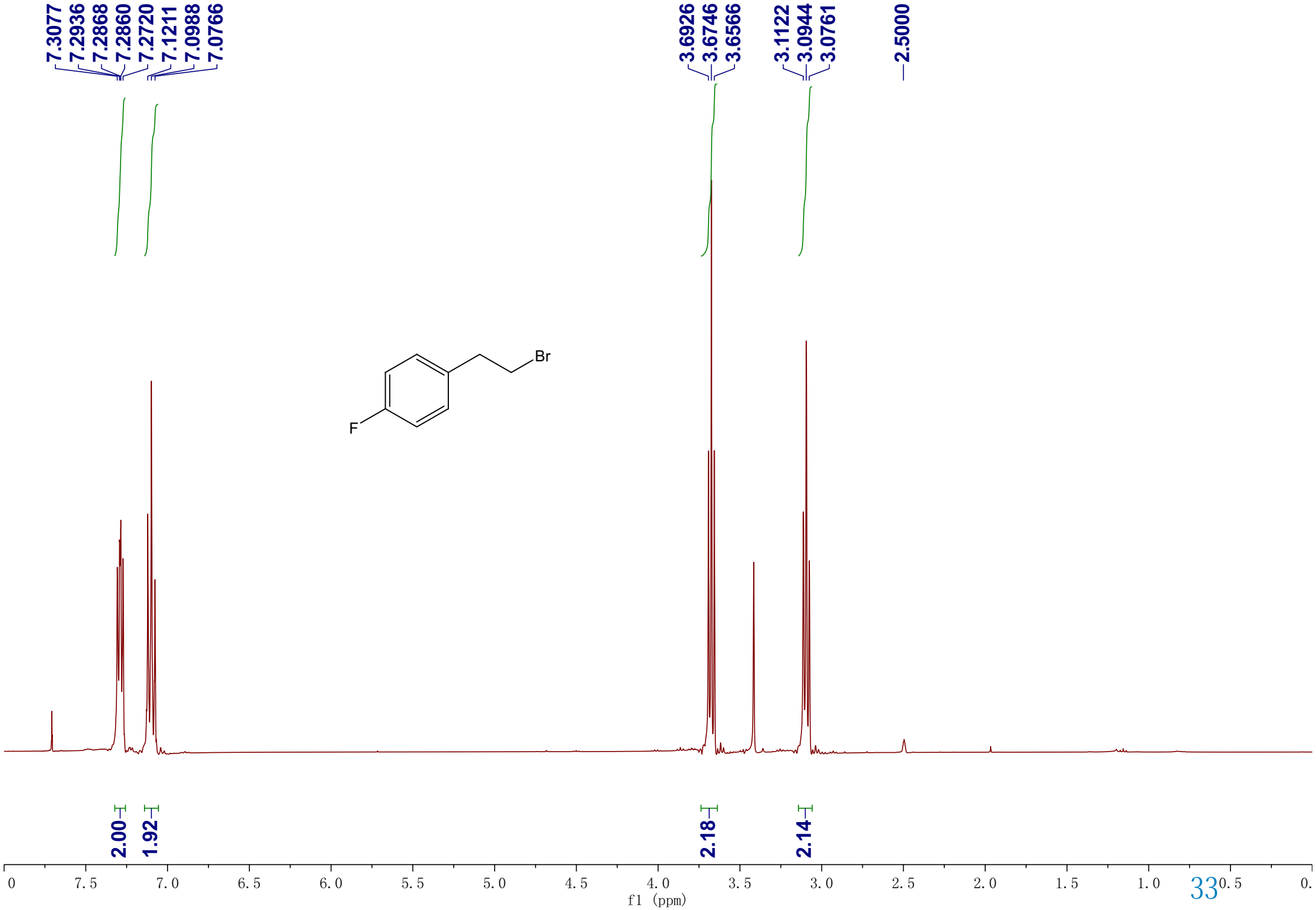

7.2601
7.1975
7.1837
7.1691
7.0308
7.0266
7.0095
6.9882
3.7116
3.7064
3.6936
3.6756
3.0599
3.0422
3.0244
Cl
F
2.00
2.01
2.08
2.06
0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)

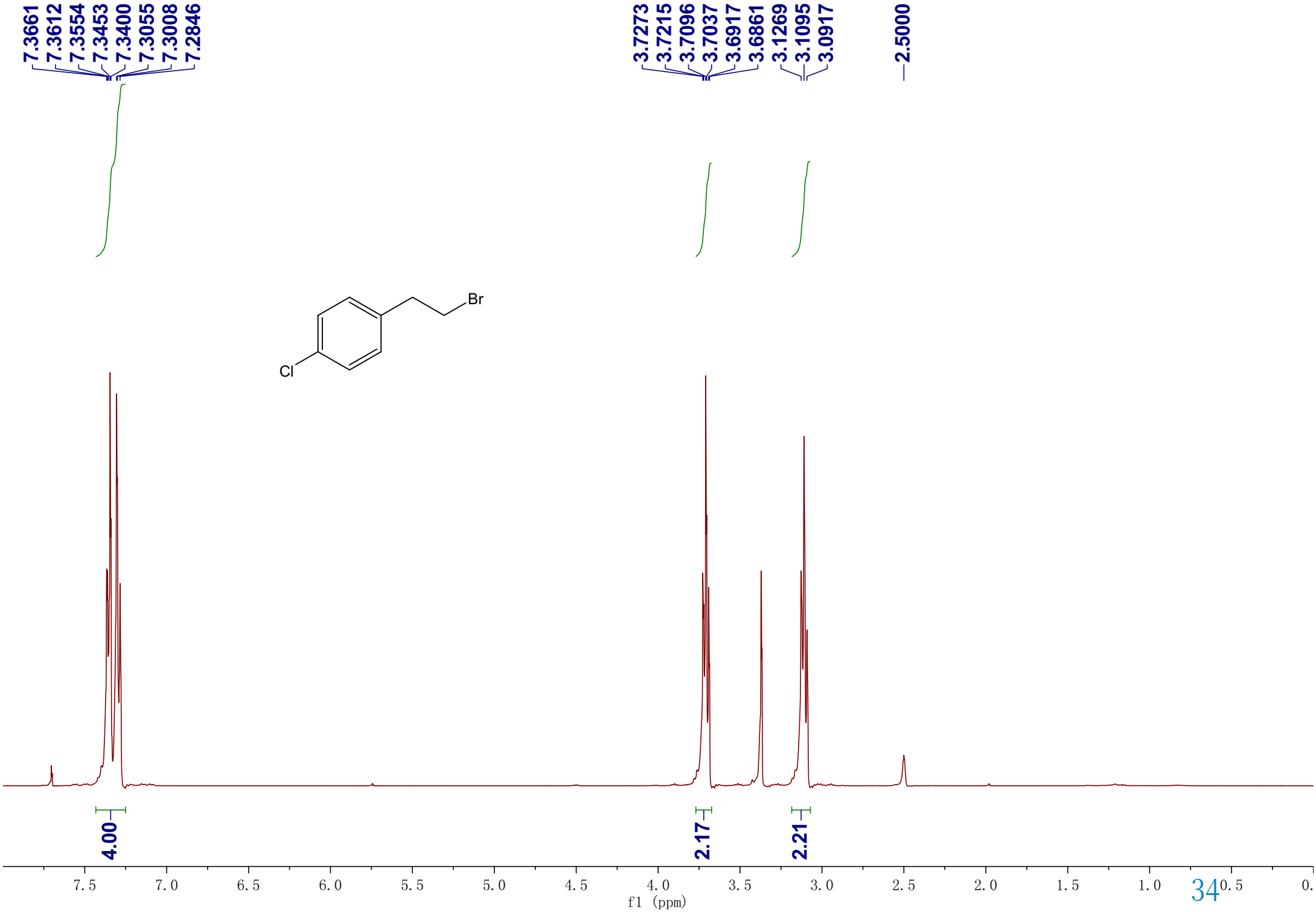

7.4067
7.4036
7.3861
7.3855
7.2600
7.2184
7.2167
7.1977
7.1816
7.1797
7.1669
7.1479
3.7234
3.7052
3.6871
3.0570
3.0389
3.0208
Cl
Br
7.50
7.40
7.30
7.20
7.10
f1 (ppm)
1.92
2.00
2.00
2.00
0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.
f1 (ppm)

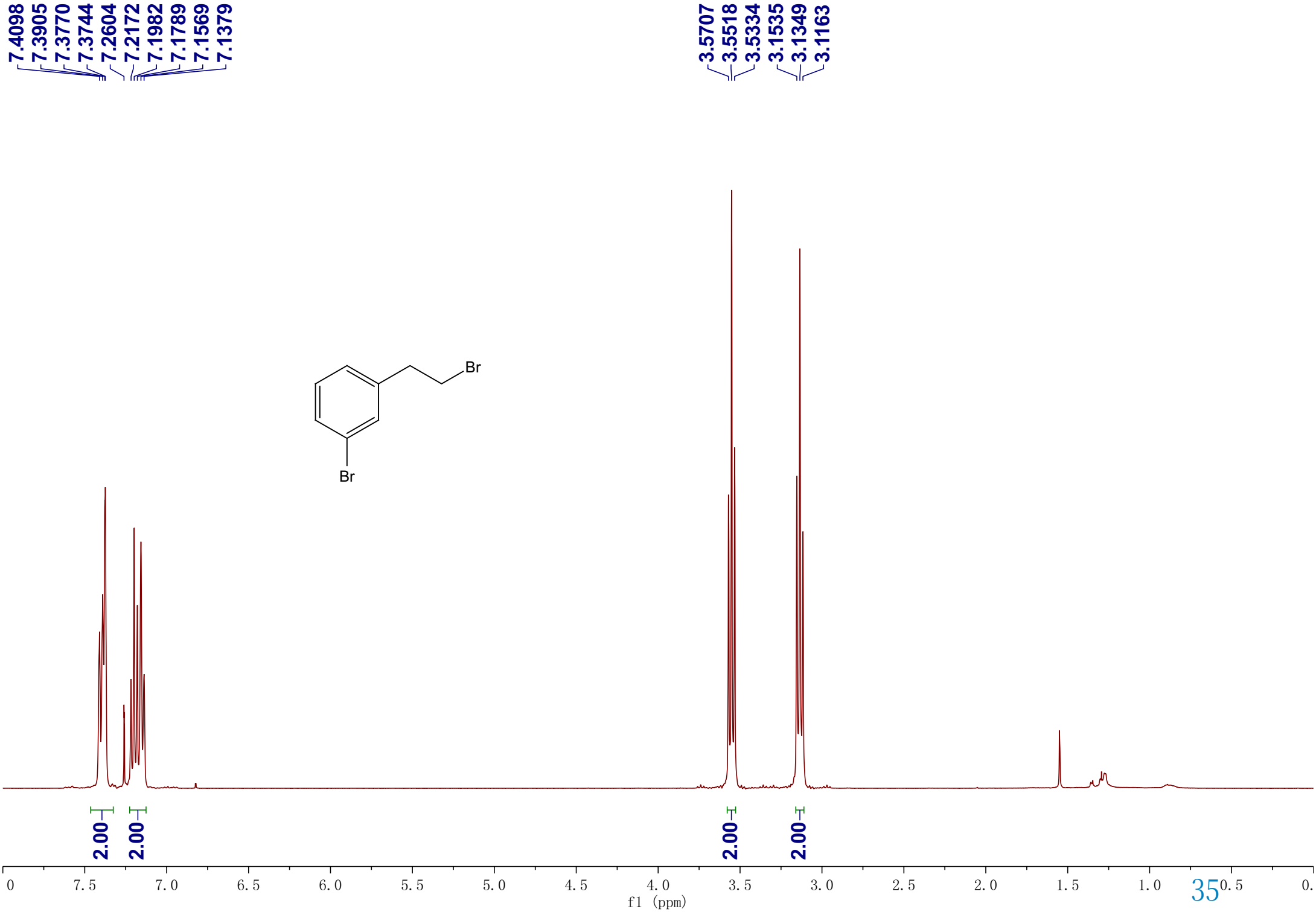

7.2601
7.1880
3.5626
3.5430
3.5227
3.2190
3.1987
3.1790
2.3591
Br
4.00
2.00
2.00
3.00
0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0
f1 (ppm)

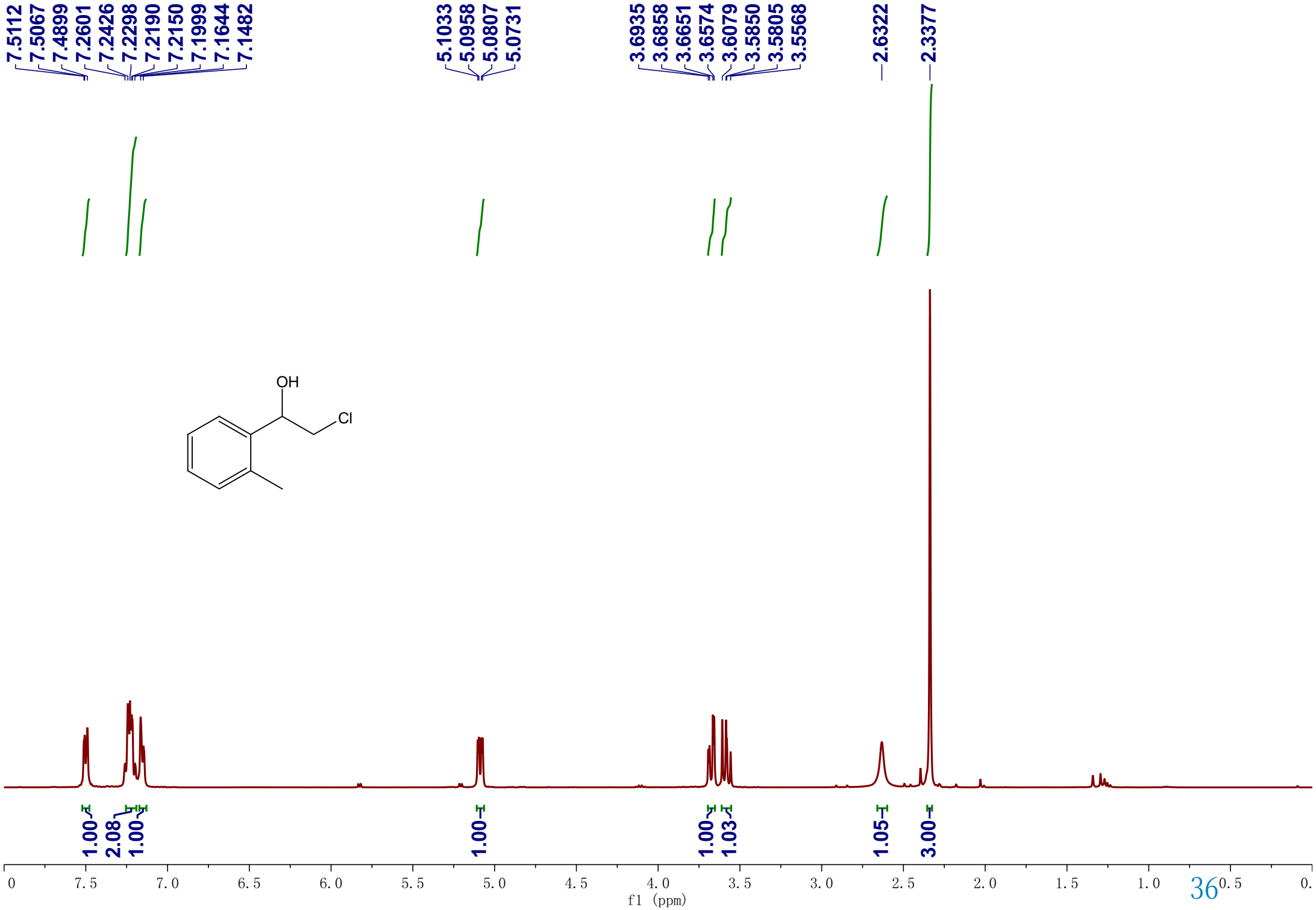

7.2601
7.2523
7.2335
7.2149
7.1045
7.0856
7.0433
7.0217
3.5947
3.5754
3.5561
3.1656
3.1462
3.1270
2.3670
Br
1.00
1.00
2.00
2.00
2.04
3.00
0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

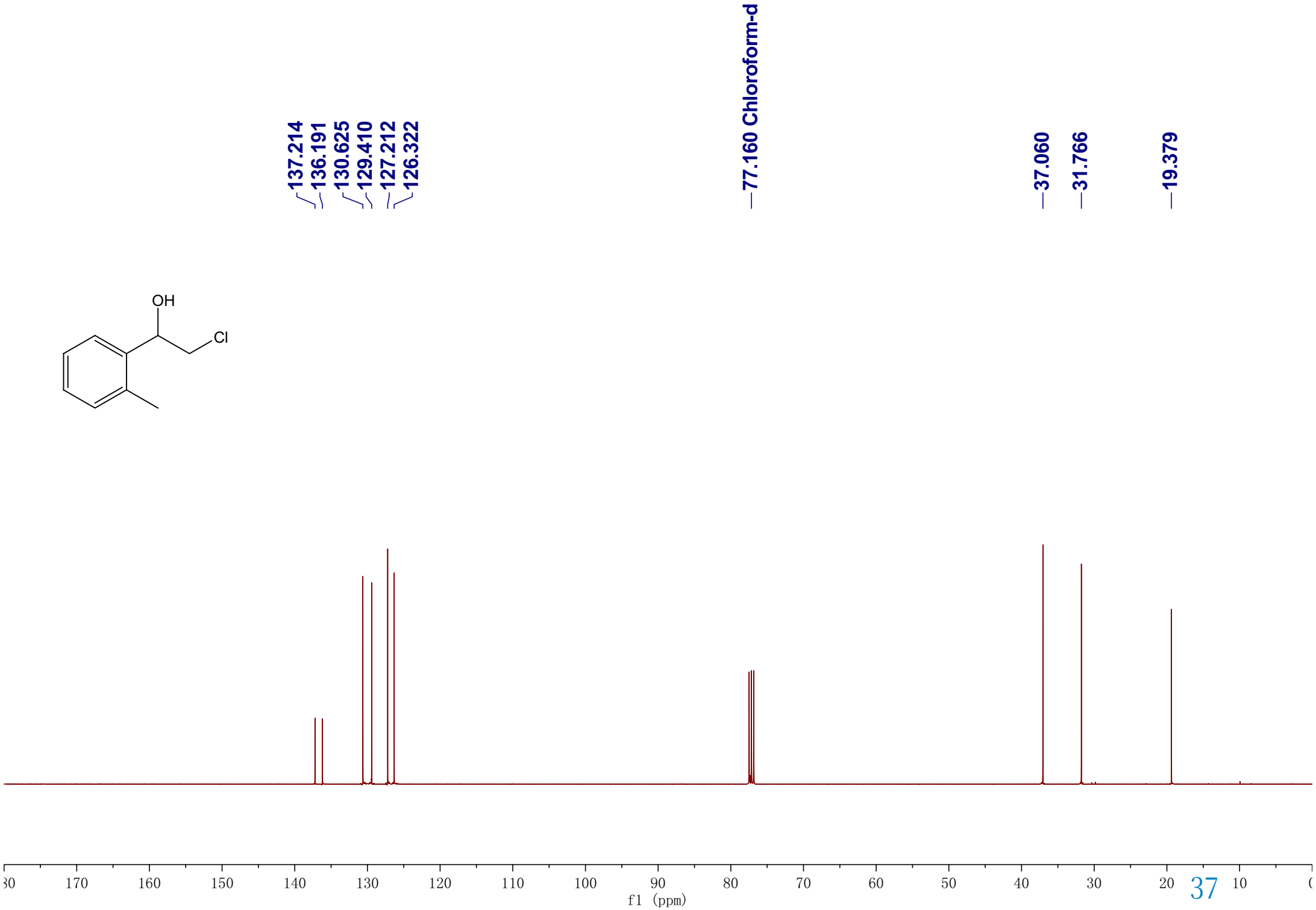

7.2600
7.1745
7.1538
7.1458
7.1249
3.7361
3.7173
3.6987
3.0751
3.0564
3.0377
2.3627
Br
3.95
2.01
1.99
3.00
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.

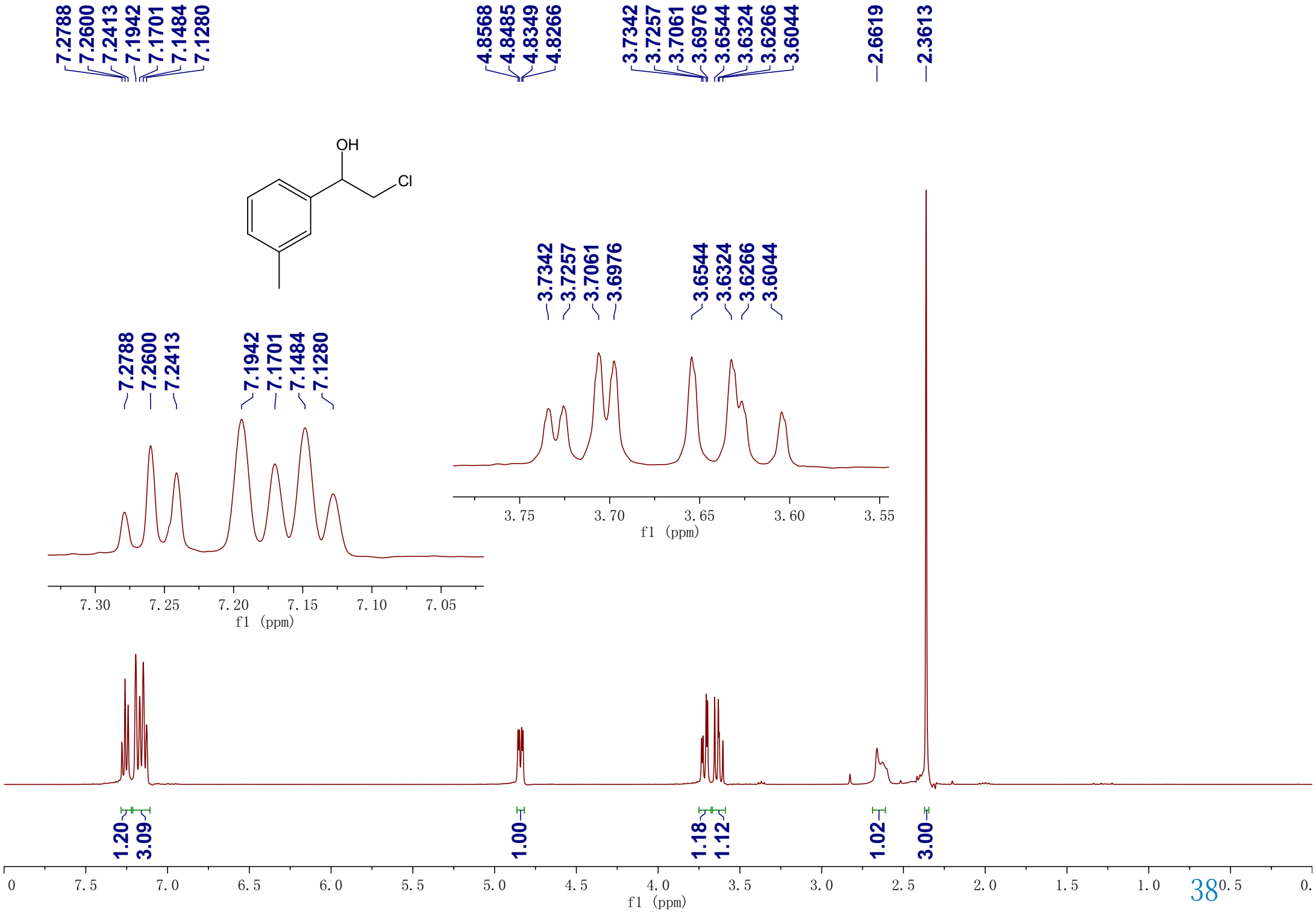

7.3077
7.2936
7.2868
7.2860
7.2720
7.1211
7.0988
7.0766
3.6926
3.6746
3.6566
3.1122
3.0944
3.0761
2.5000
Br
F
2.00
1.92
2.18
2.14
0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.
f1 (ppm)

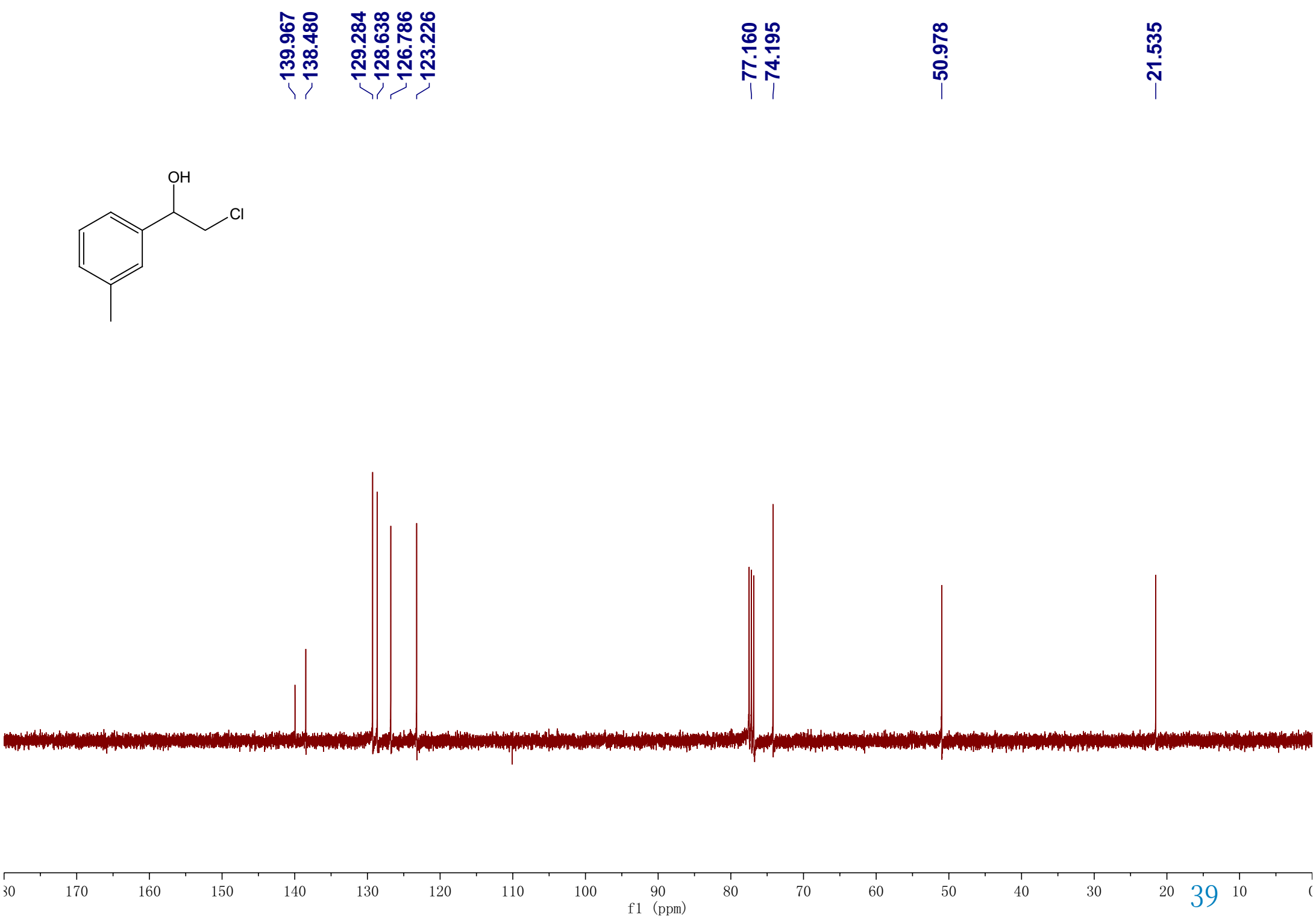

7.3661
7.3612
7.3554
7.3453
7.3400
7.3055
7.3008
7.2846
3.7273
3.7215
3.7096
3.7037
3.6917
3.6861
3.1269
3.1095
3.0917
2.5000
Br
Cl
4.00
2.17
2.21
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.
f1 (ppm)

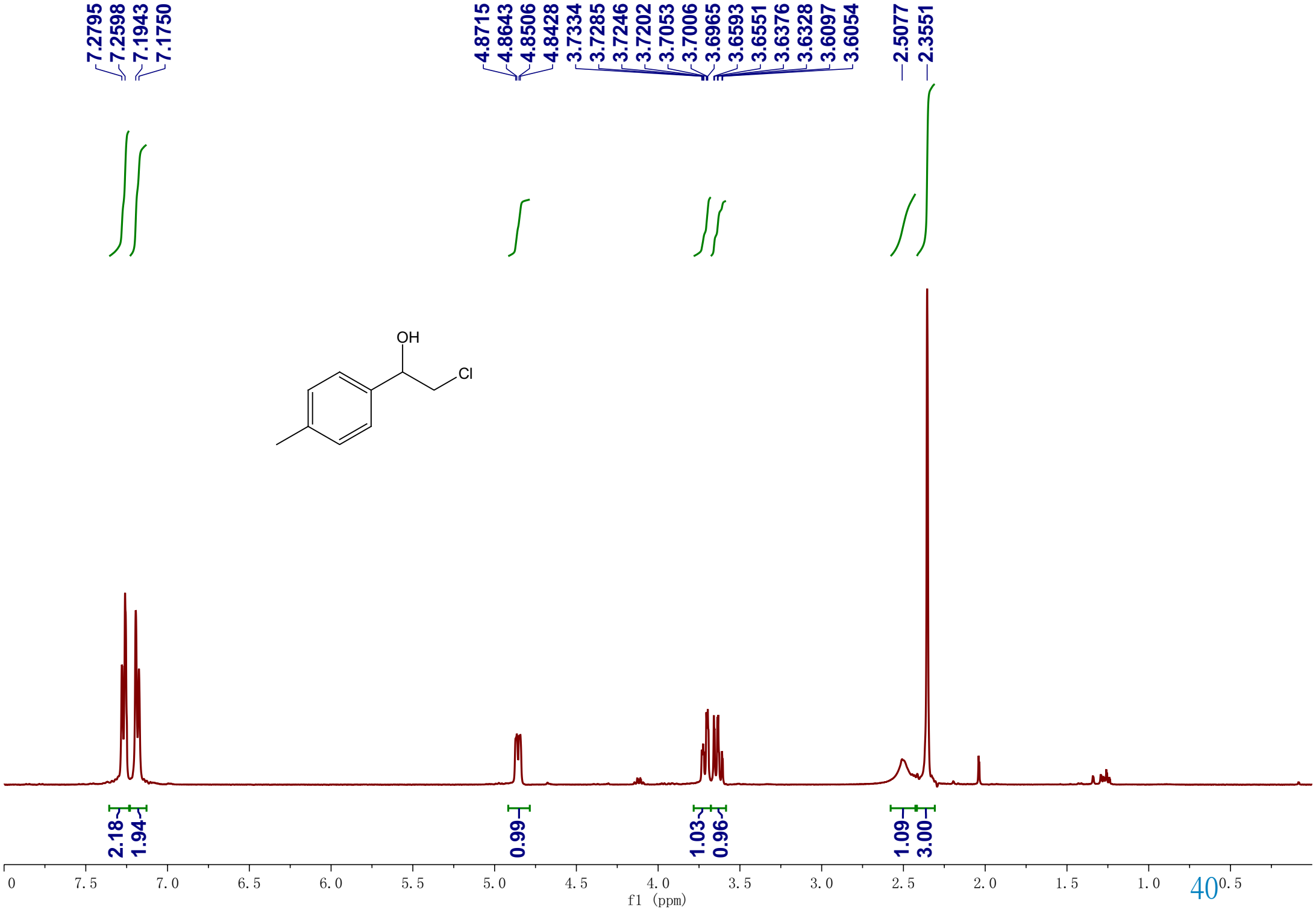

7.4098
7.3905
7.3770
7.3744
7.2604
7.2172
7.1982
7.1789
7.1569
7.1379
3.5707
3.5518
3.5334
3.1535
3.1349
3.1163
Br
Br
2.00
2.00
2.00
2.00
0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.
f1 (ppm)

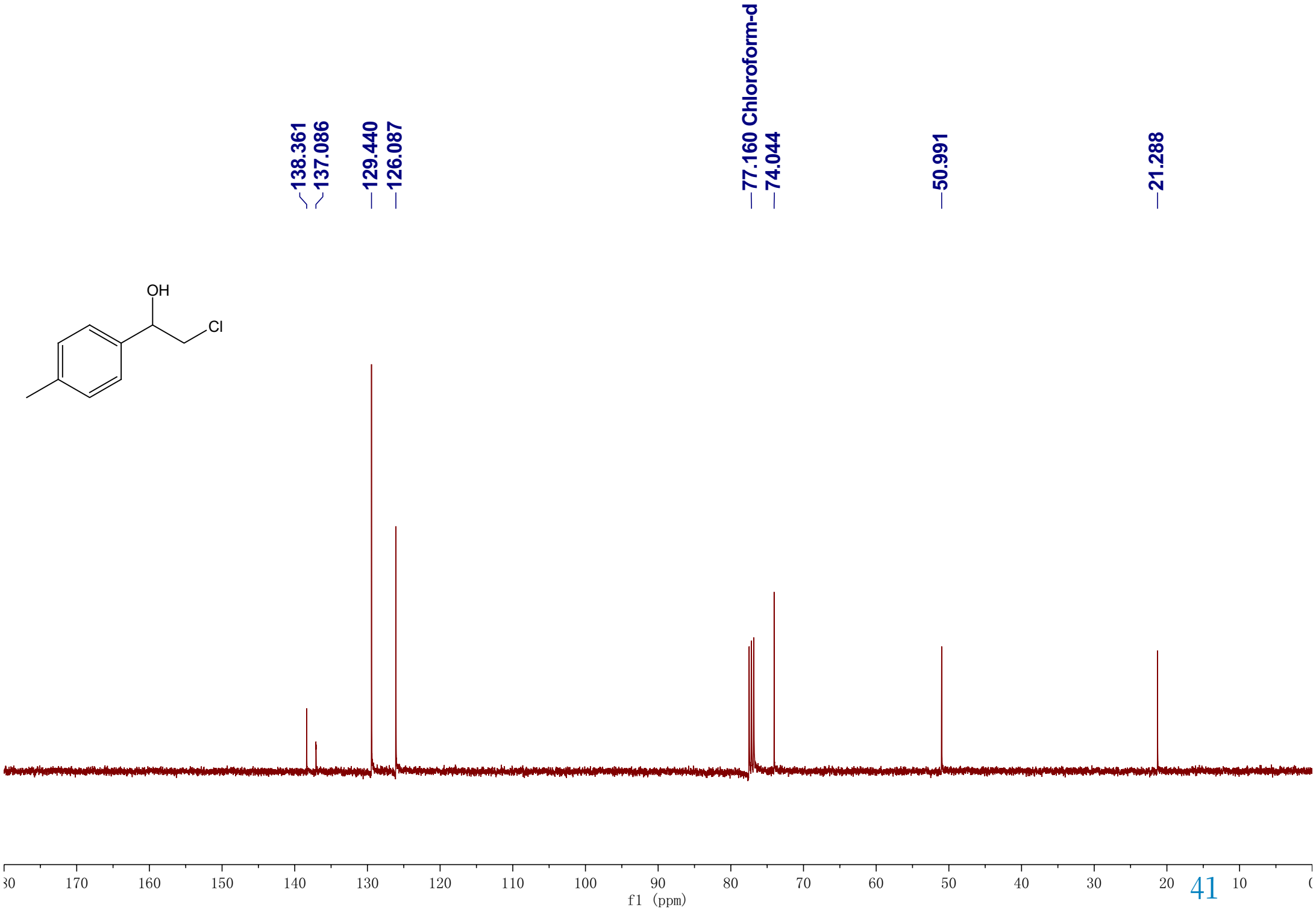

7.5112
7.5067
7.4899
7.2601
7.2426
7.2298
7.2190
7.2150
7.1999
7.1644
7.1482
5.1033
5.0958
5.0807
5.0731
3.6935
3.6858
3.6651
3.6574
3.6079
3.5850
3.5805
3.5568
2.6322
2.3377
OH
Cl
1.00
2.08
1.00
1.00
1.00
1.03
1.05
3.00
0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.
f1 (ppm)

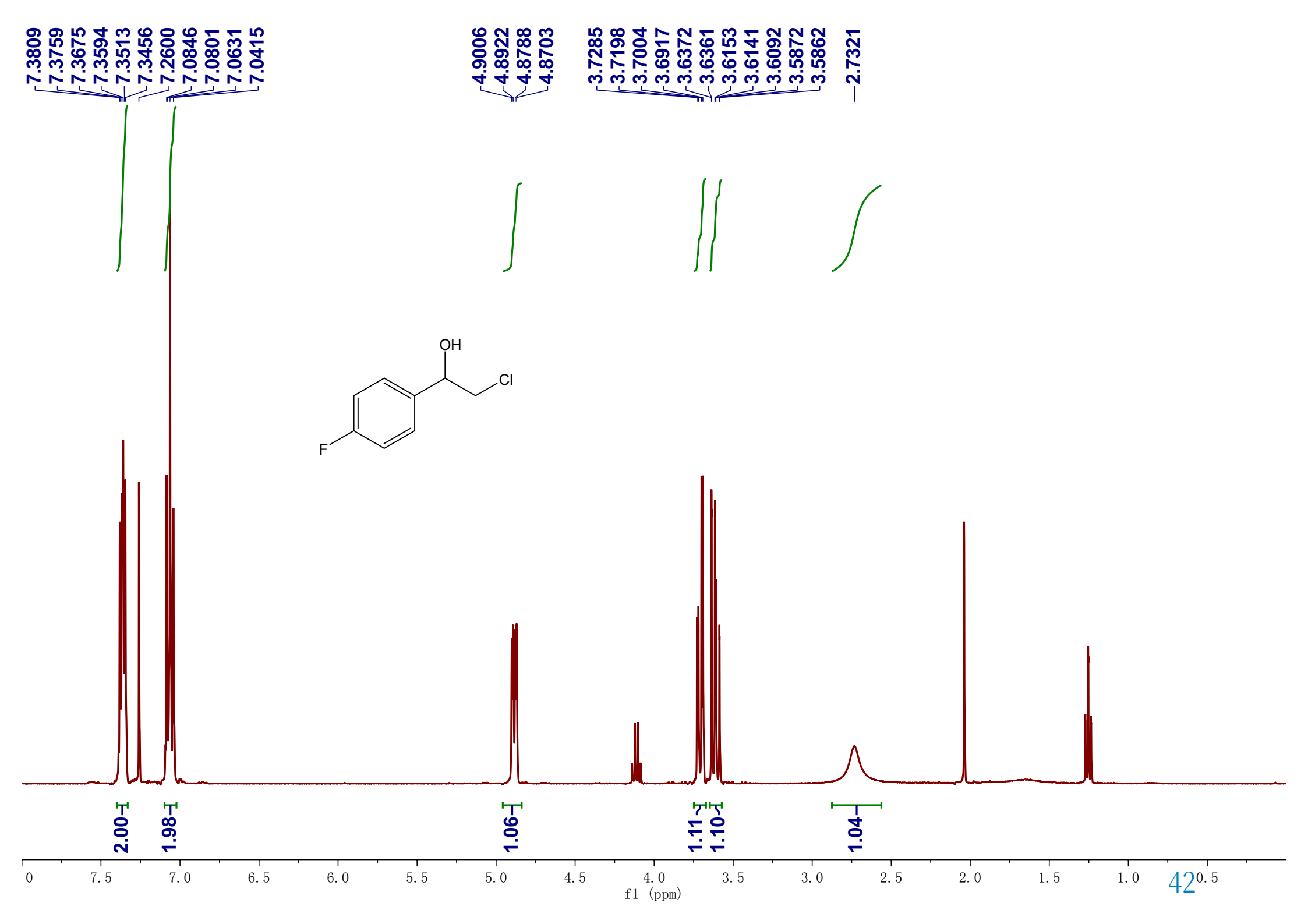

137.214
136.191
130.625
129.410
127.212
126.322
77.160 Chloroform-d
37.060
31.766
19.379
OH
Cl
f1 (ppm)

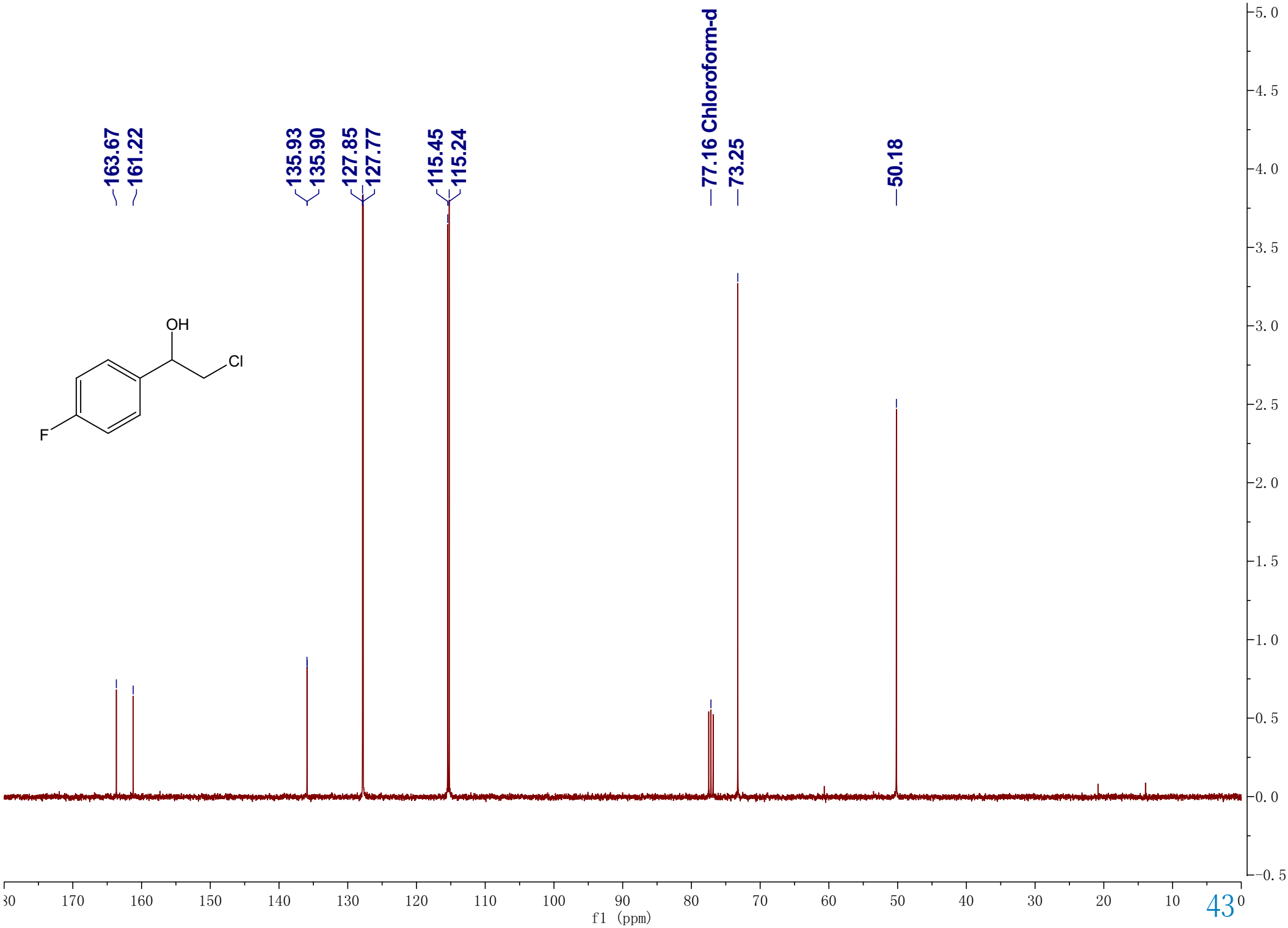

7.2788
7.2600
7.2413
7.1942
7.1701
7.1484
7.1280
4.8568
4.8485
4.8349
4.8266
3.7342
3.7257
3.7061
3.6976
3.6544
3.6324
3.6266
3.6044
2.6619
2.3613
OH
Cl
f1 (ppm)
1.20
3.09
1.00
1.18
1.12
1.02
3.00

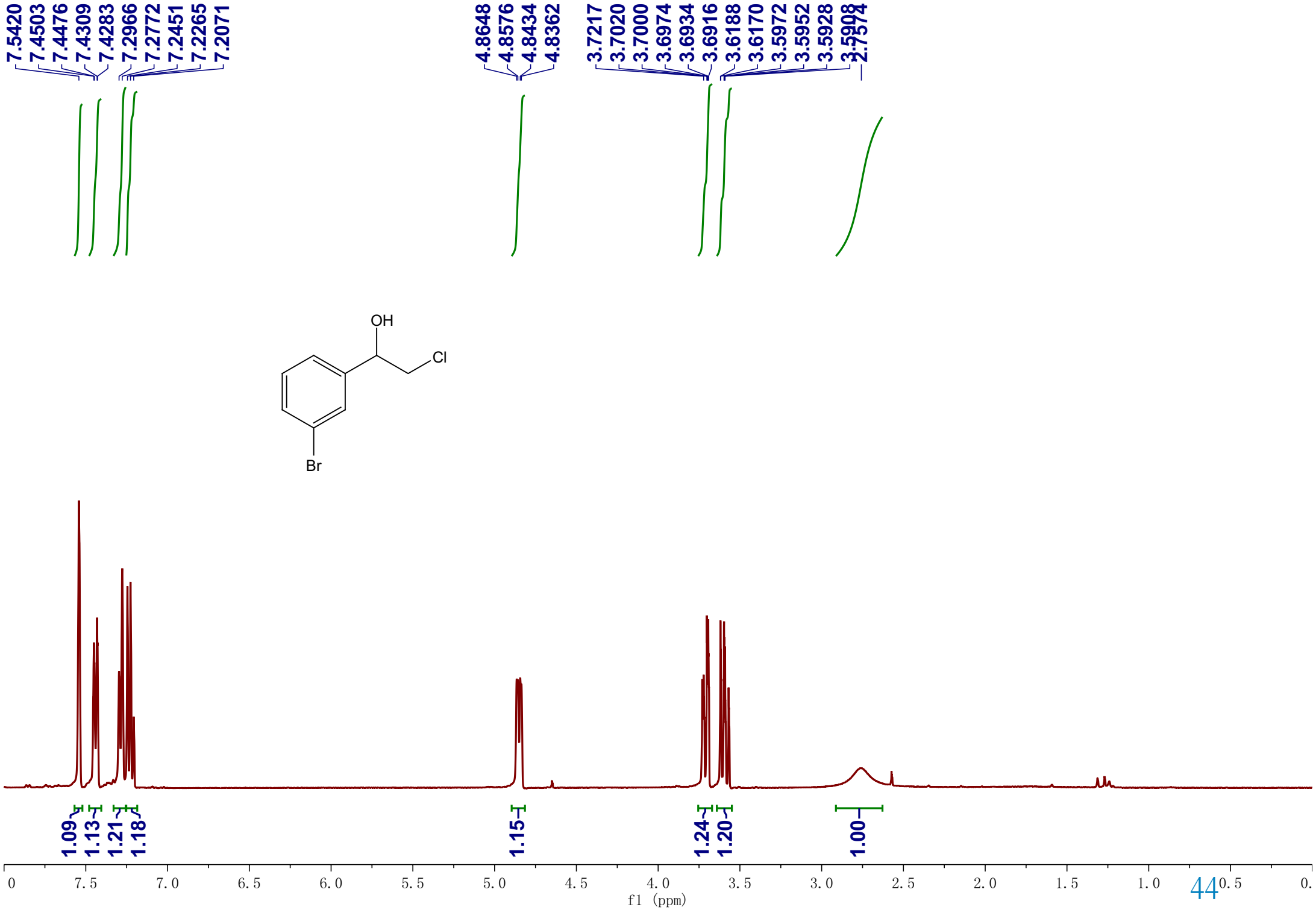

139.967
138.480
129.284
128.638
126.786
123.226
77.160
74.195
50.978
21.535
OH
Cl
f1 (ppm)

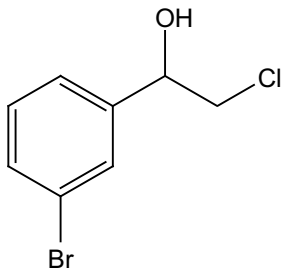

7.2795
7.2598
7.1943
7.1750
4.8715
4.8643
4.8506
4.8428
3.7334
3.7285
3.7246
3.7202
3.7053
3.7006
3.6965
3.6593
3.6551
3.6376
3.6328
3.6097
3.6054
2.5077
2.3551
OH
Cl
2.18
1.94
0.99
1.03
0.96
1.09
3.00
0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)


138.361
137.086
129.440
126.087
77.160 Chloroform-d
74.044
50.991
21.288
OH
Cl
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)


7.3809
7.3759
7.3675
7.3594
7.3513
7.3456
7.2600
7.0846
7.0801
7.0631
7.0415
4.9006
4.8922
4.8788
4.8703
3.7285
3.7198
3.7004
3.6917
3.6372
3.6361
3.6153
3.6141
3.6092
3.5872
3.5862
2.7321
OH
Cl
F
2.00
1.98
1.06
1.11
1.10
1.04
f1 (ppm)

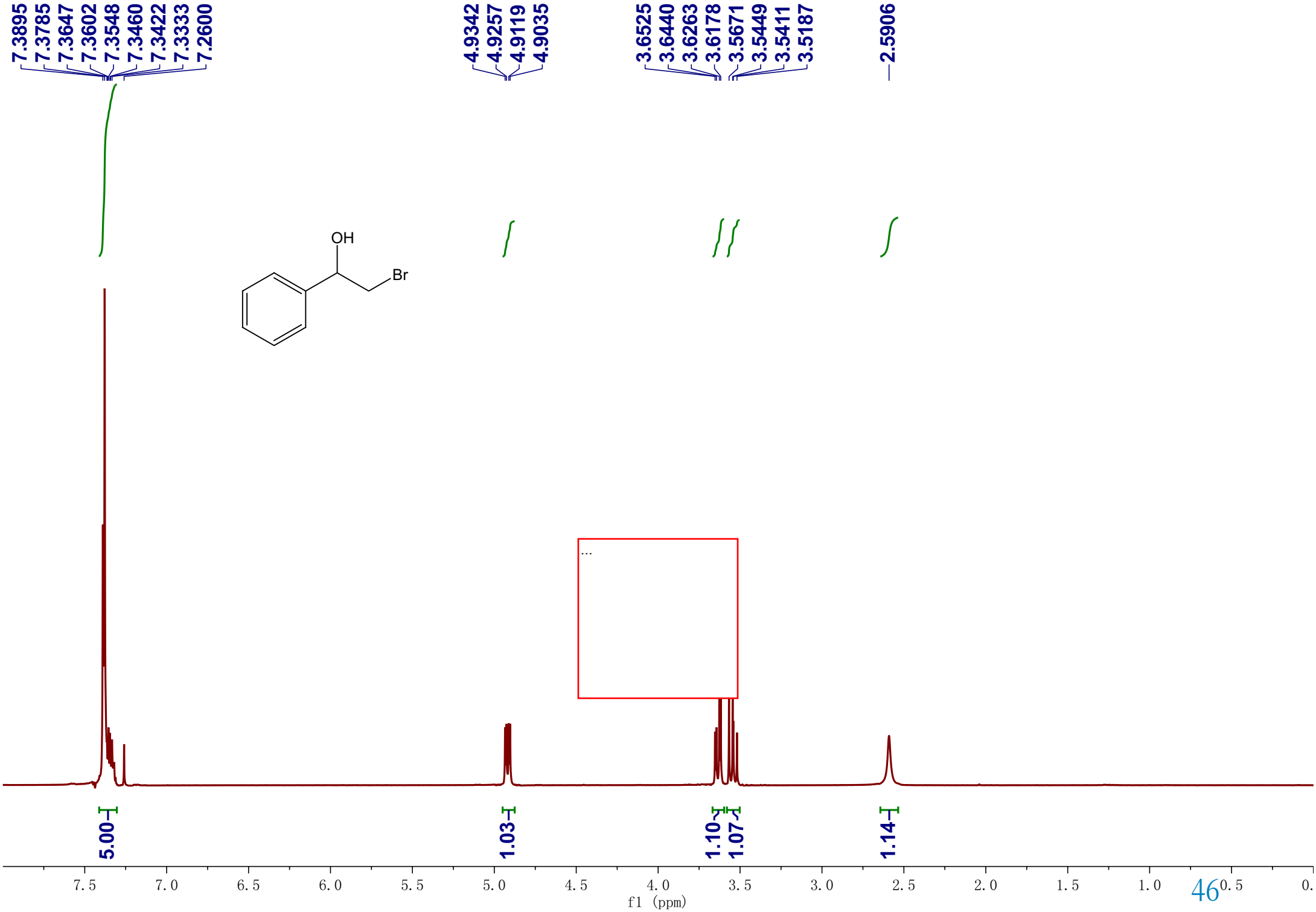

163.67
161.22
135.93
135.90
127.85
127.77
115.45
115.24
77.16 Chloroform-d
73.25
50.18
OH
Cl
F
f1 (ppm)

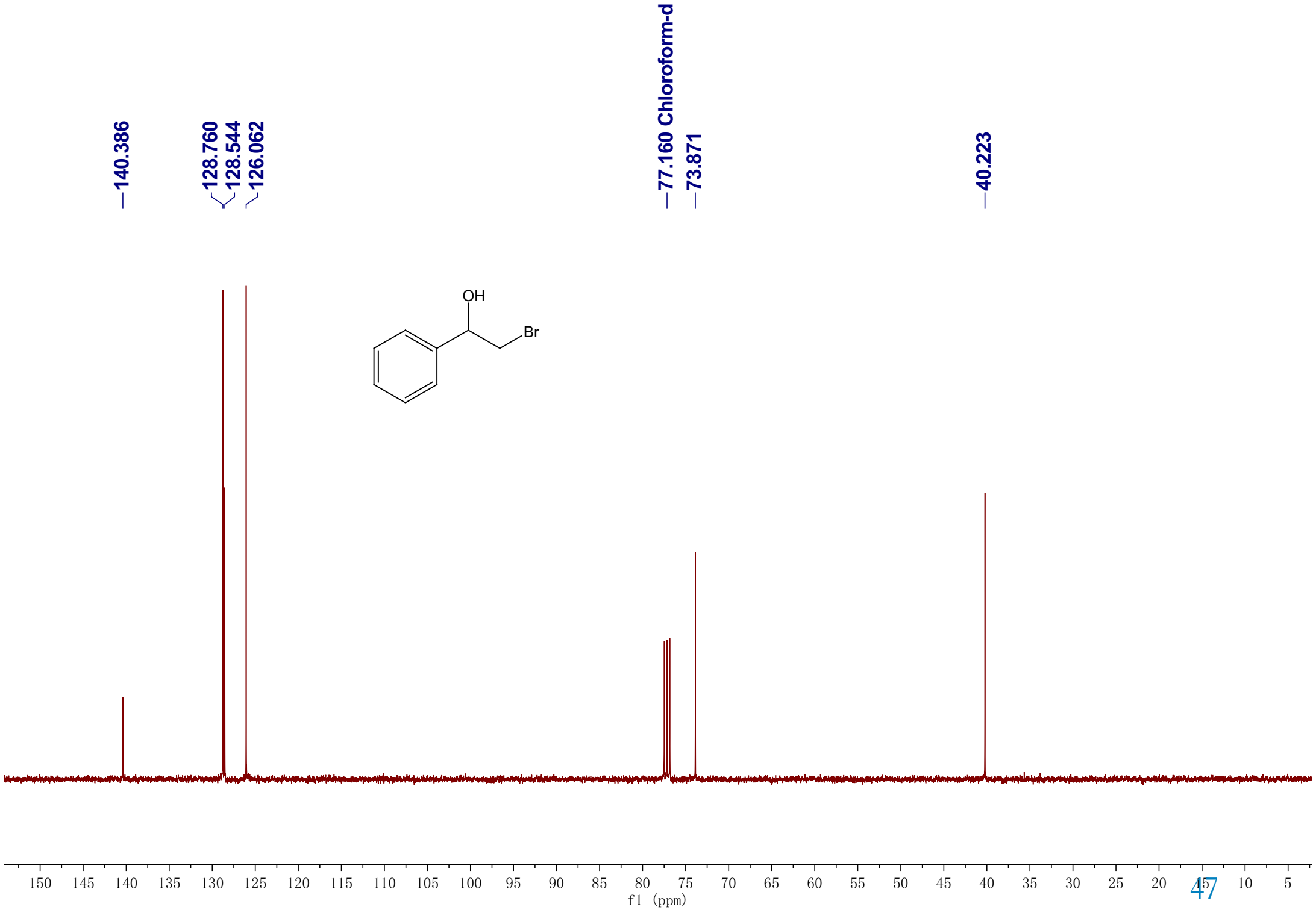

7.5420
7.4503
7.4476
7.4309
7.4283
7.2966
7.2772
7.2451
7.2265
7.2071
4.8648
4.8576
4.8434
4.8362
3.7217
3.7020
3.7000
3.6974
3.6934
3.6916
3.6188
3.6170
3.5972
3.5952
3.5928
3.5908
3.5574
OH
Cl
Br
1.09
1.13
1.21
1.18
1.15
1.24
1.20
1.00
0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.
f1 (ppm)

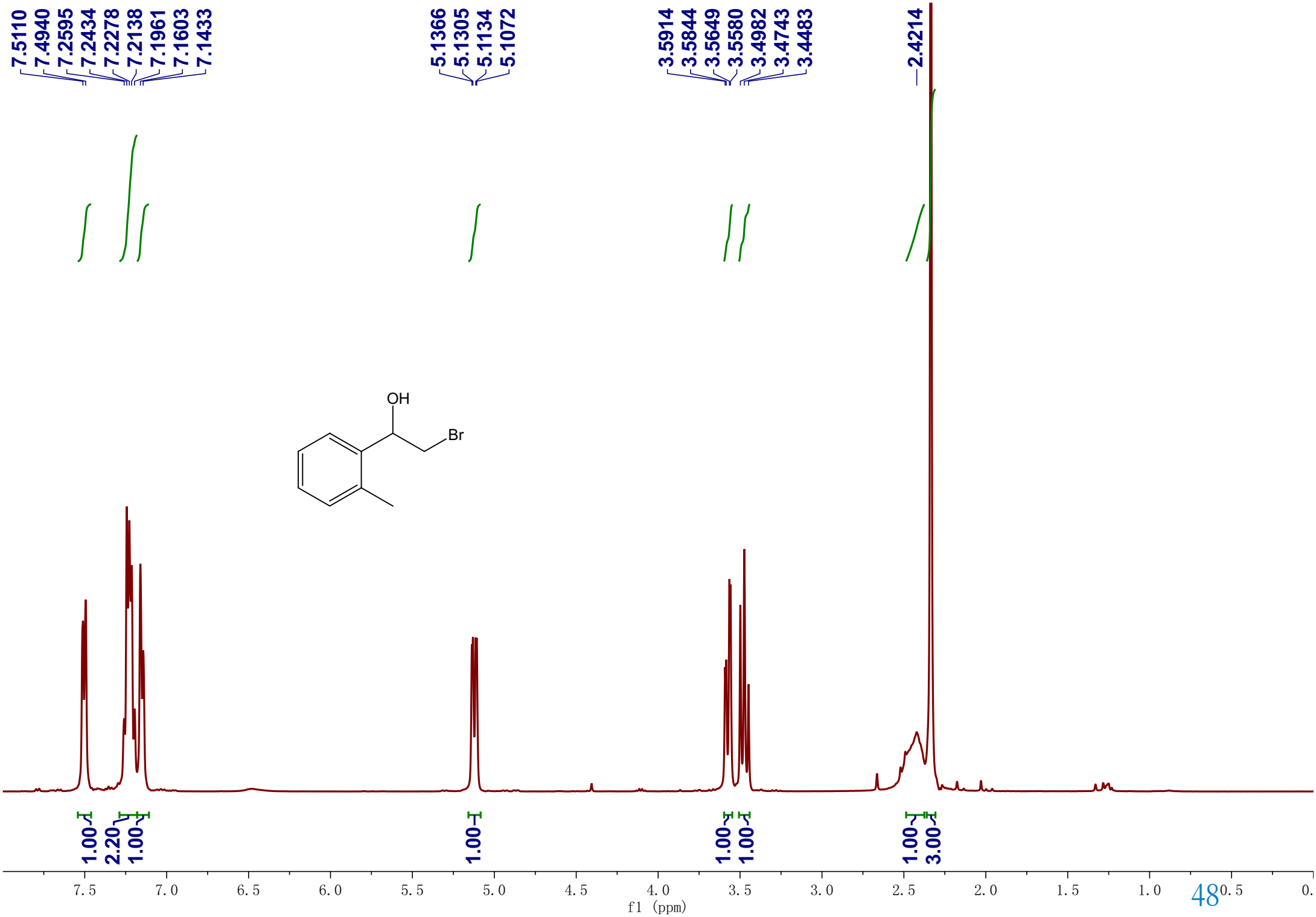


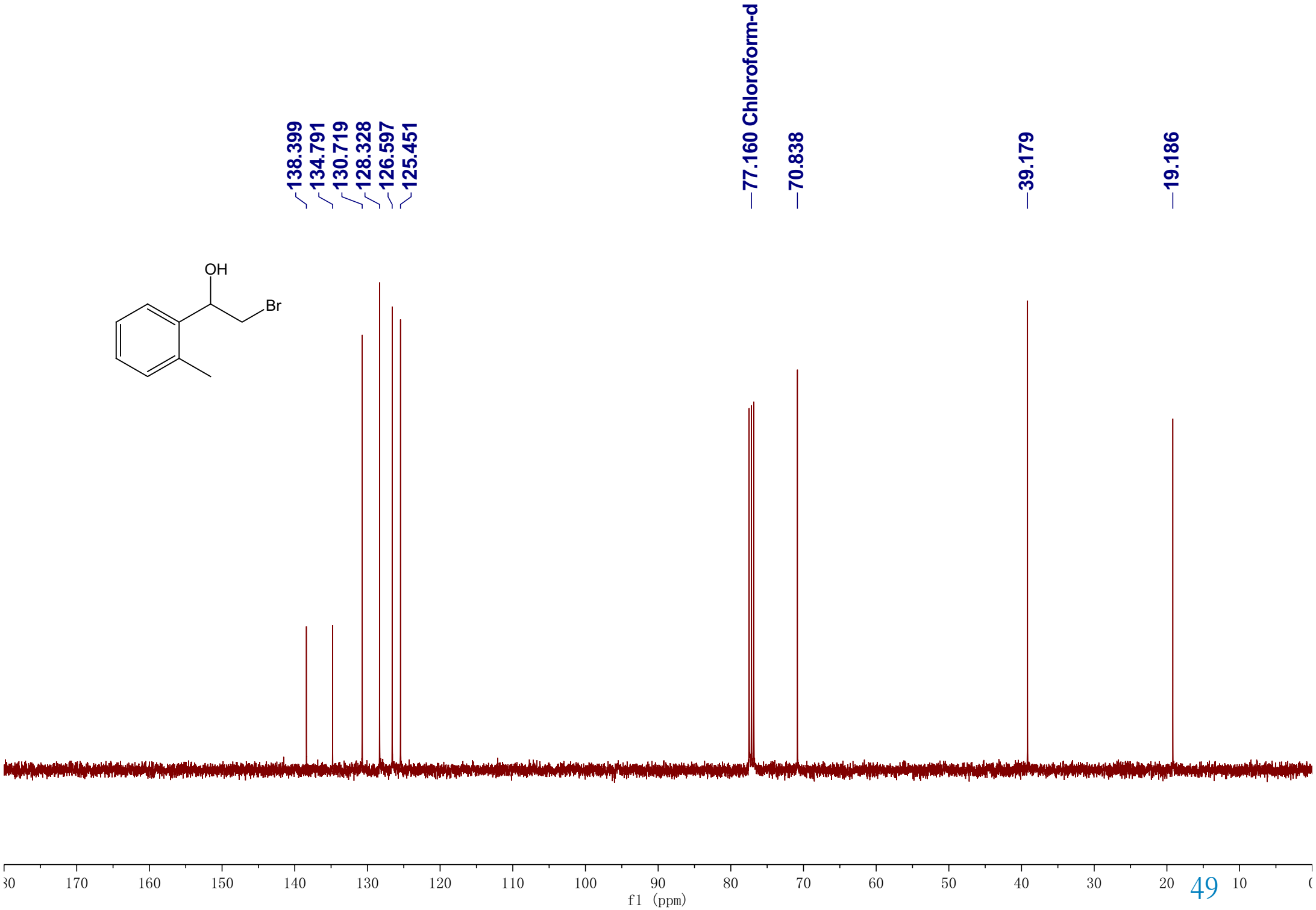
142.215
131.637
130.337
129.313
124.822
122.894
77.160 Chloroform-d
73.420
50.770

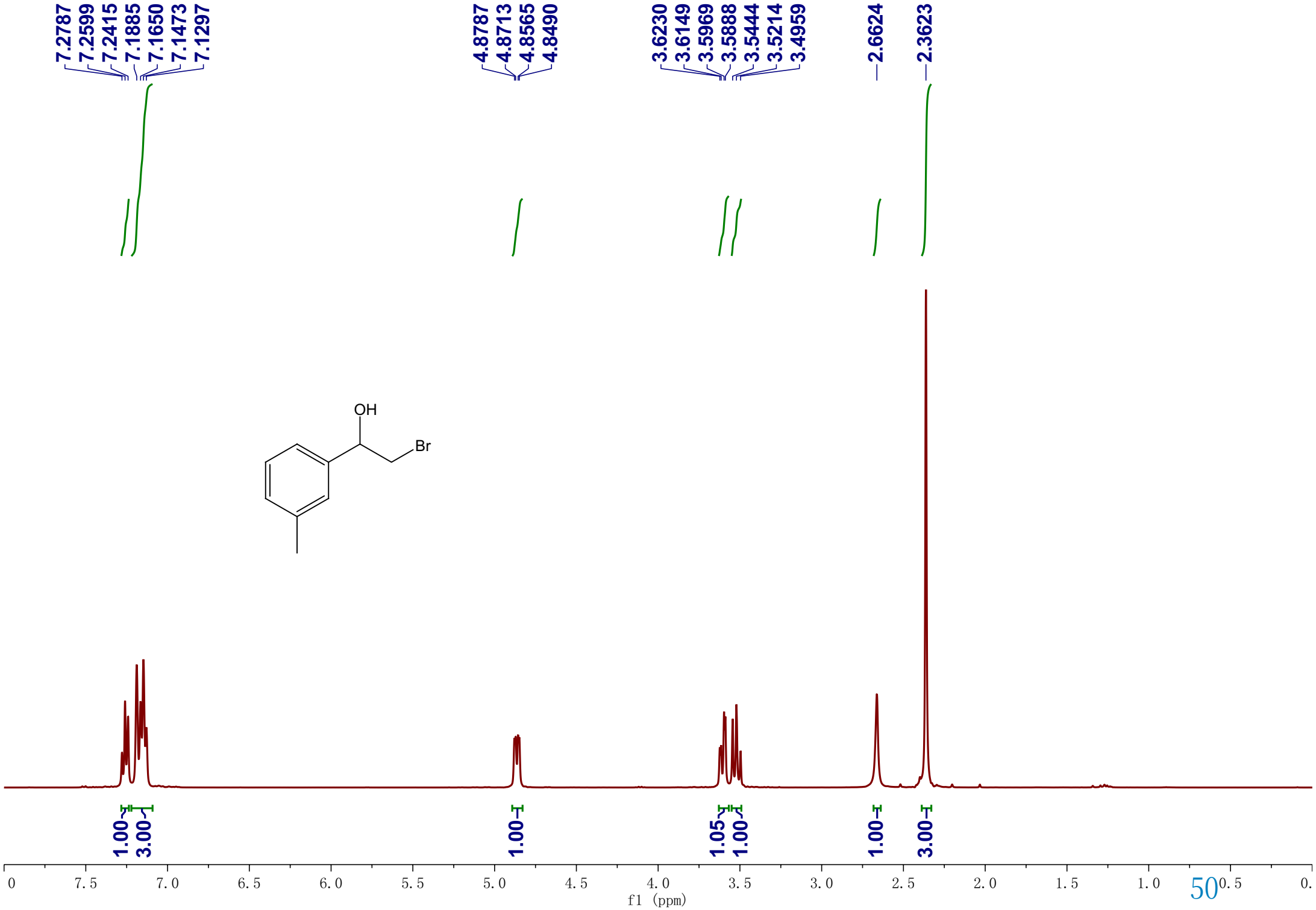
f1 (ppm)

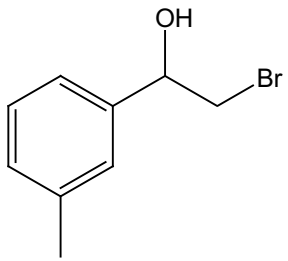

7.3895
7.3785
7.3647
7.3602
7.3548
7.3460
7.3422
7.3333
7.2600
4.9342
4.9257
4.9119
4.9035
3.6525
3.6440
3.6263
3.6178
3.5671
3.5449
3.5411
3.5187
2.5906
OH
Br
5.00
1.03
1.10
1.07
1.14
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.
f1 (ppm)


140.386
128.760
128.544
126.062
77.160 Chloroform-d
73.871
40.223
OH
Br
150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25 20 15 10 5
f1 (ppm)

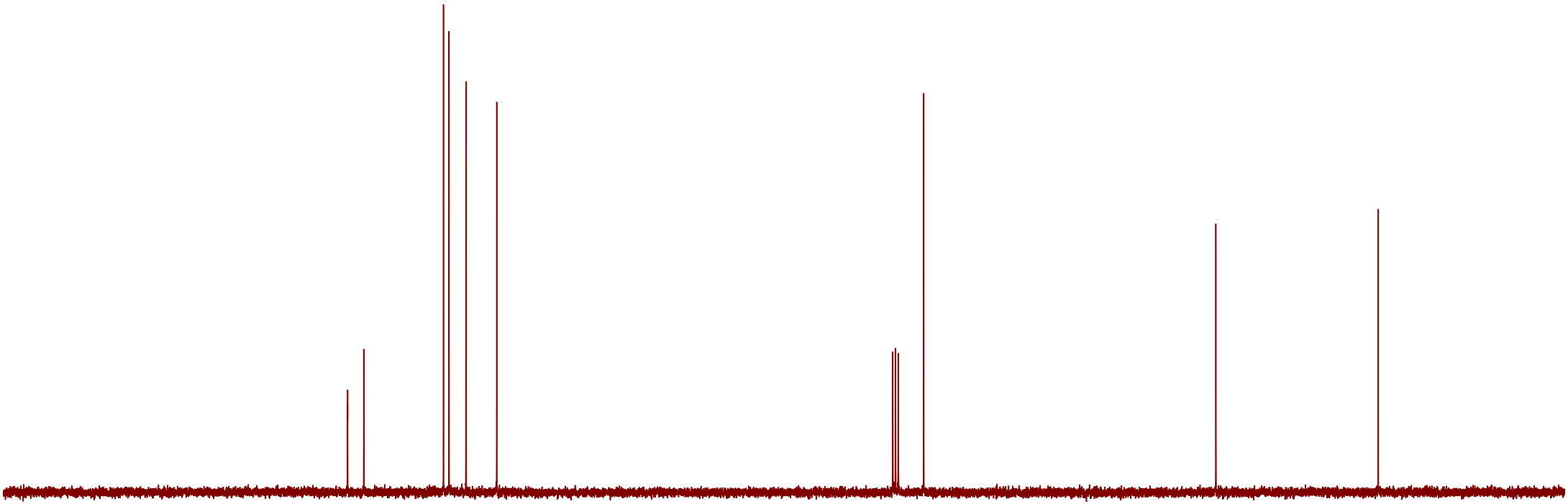

7.5110
7.4940
7.2595
7.2434
7.2278
7.2138
7.1961
7.1603
7.1433
5.1366
5.1305
5.1134
5.1072
3.5914
3.5844
3.5649
3.5580
3.4982
3.4743
3.4483
2.4214
OH
Br
1.00
2.20
1.00
1.00
1.00
1.00
1.00
3.00
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.
f1 (ppm)

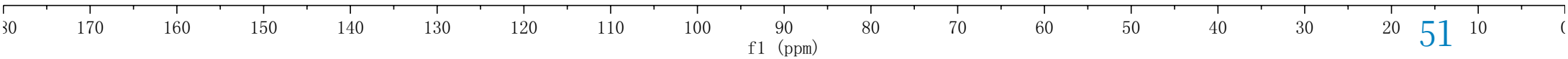

138.399
134.791
130.719
128.328
126.597
125.451
77.160 Chloroform-d
70.838
39.179
19.186
OH
Br
f1 (ppm)

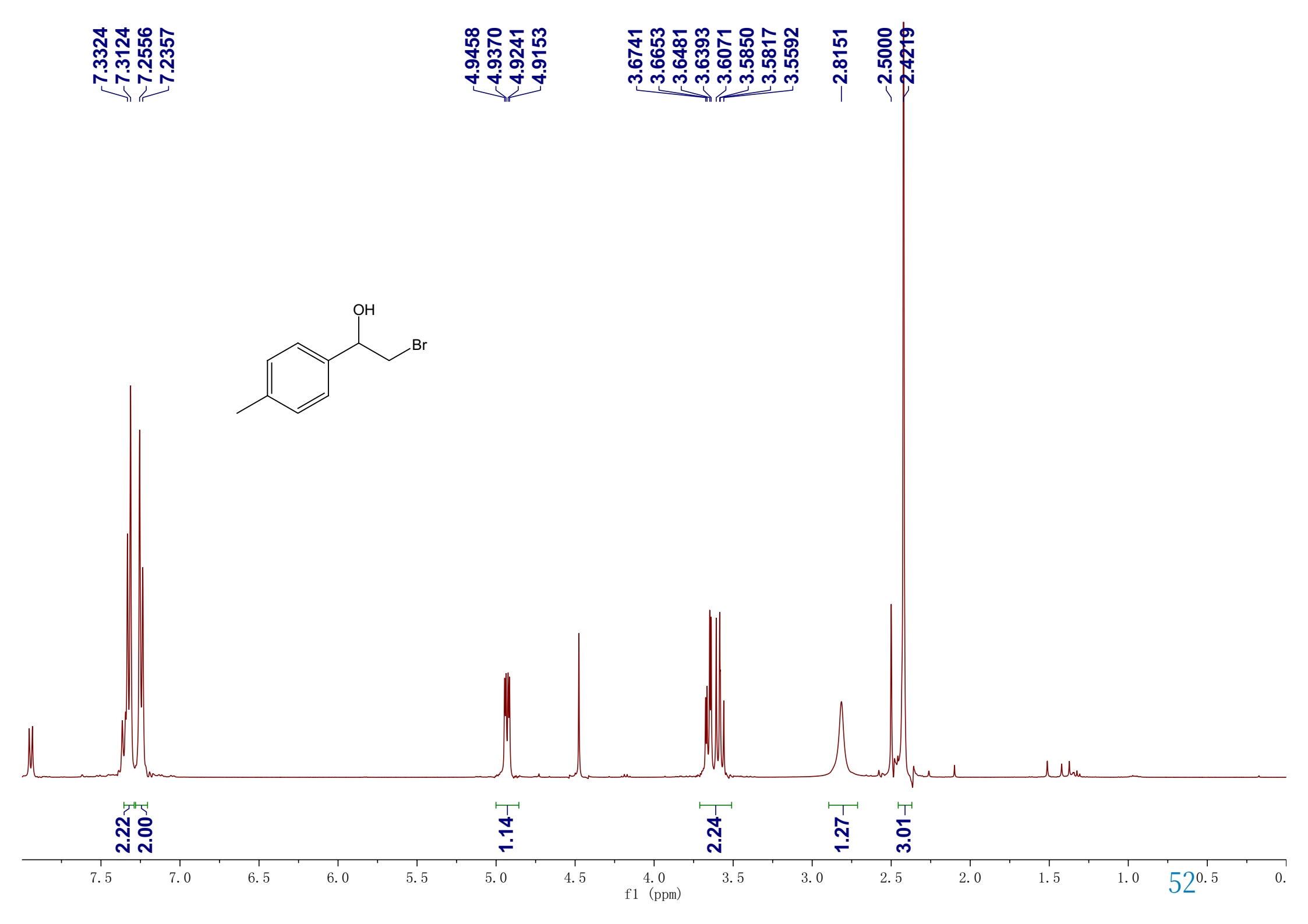

7.2787
7.2599
7.2415
7.1885
7.1650
7.1473
7.1297
4.8787
4.8713
4.8565
4.8490
3.6230
3.6149
3.5969
3.5888
3.5444
3.5214
3.4959
2.6624
2.3623
OH
Br
1.00
3.00
1.00
1.05
1.00
1.00
3.00
0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.
f1 (ppm)

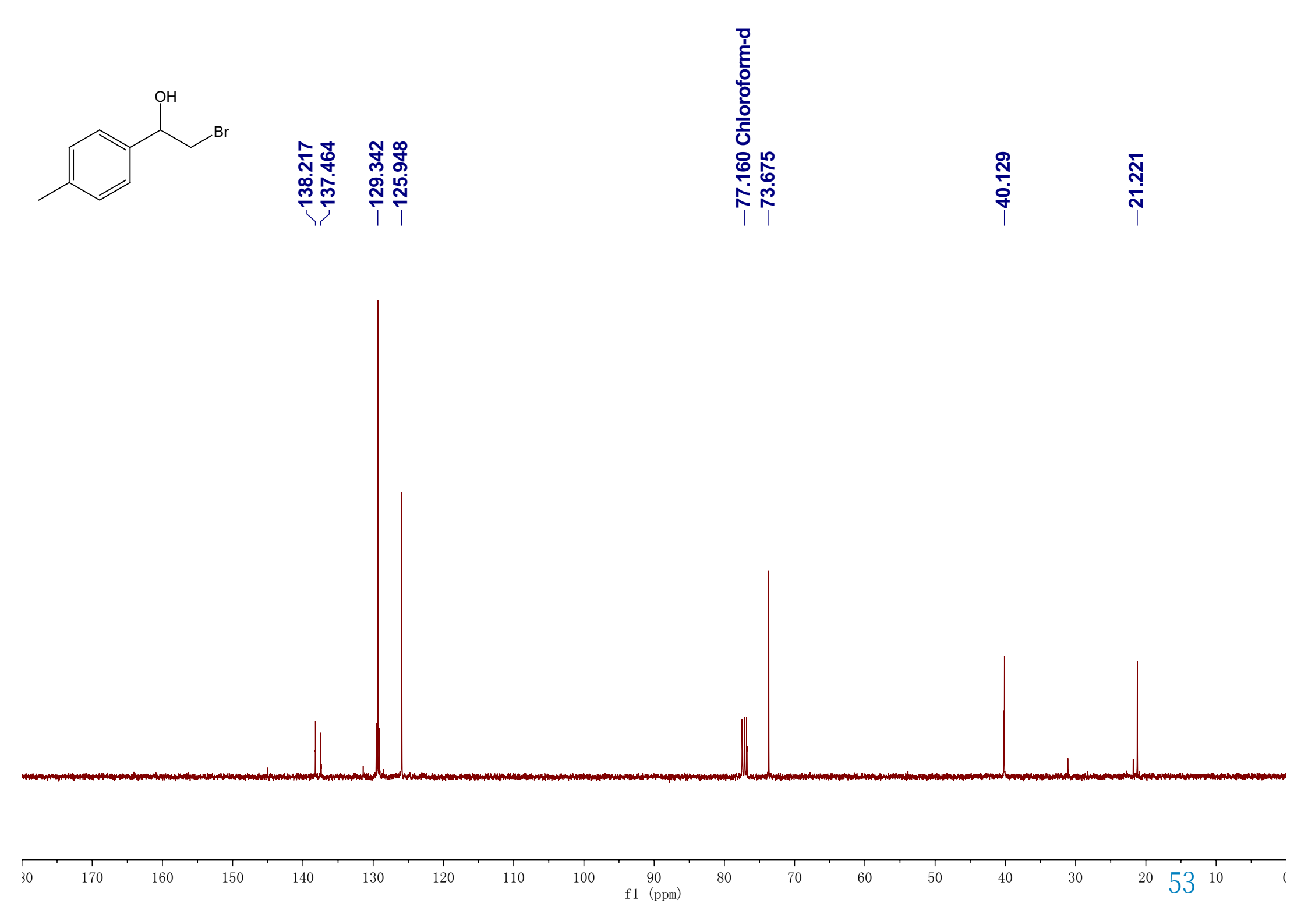


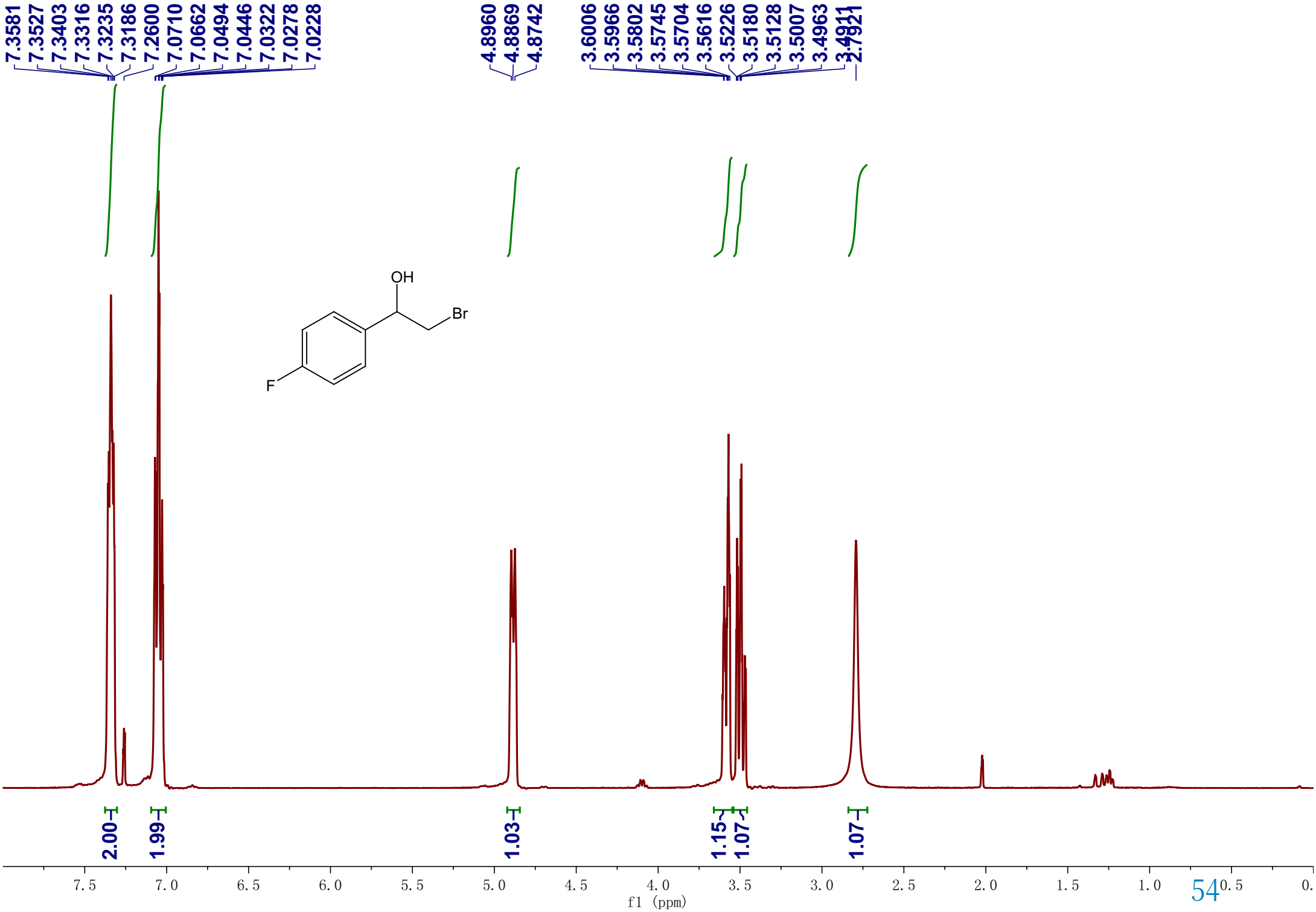
140.313
138.460
129.272
128.629
126.679
123.129
77.160 Chloroform-d
73.914
40.244
21.530

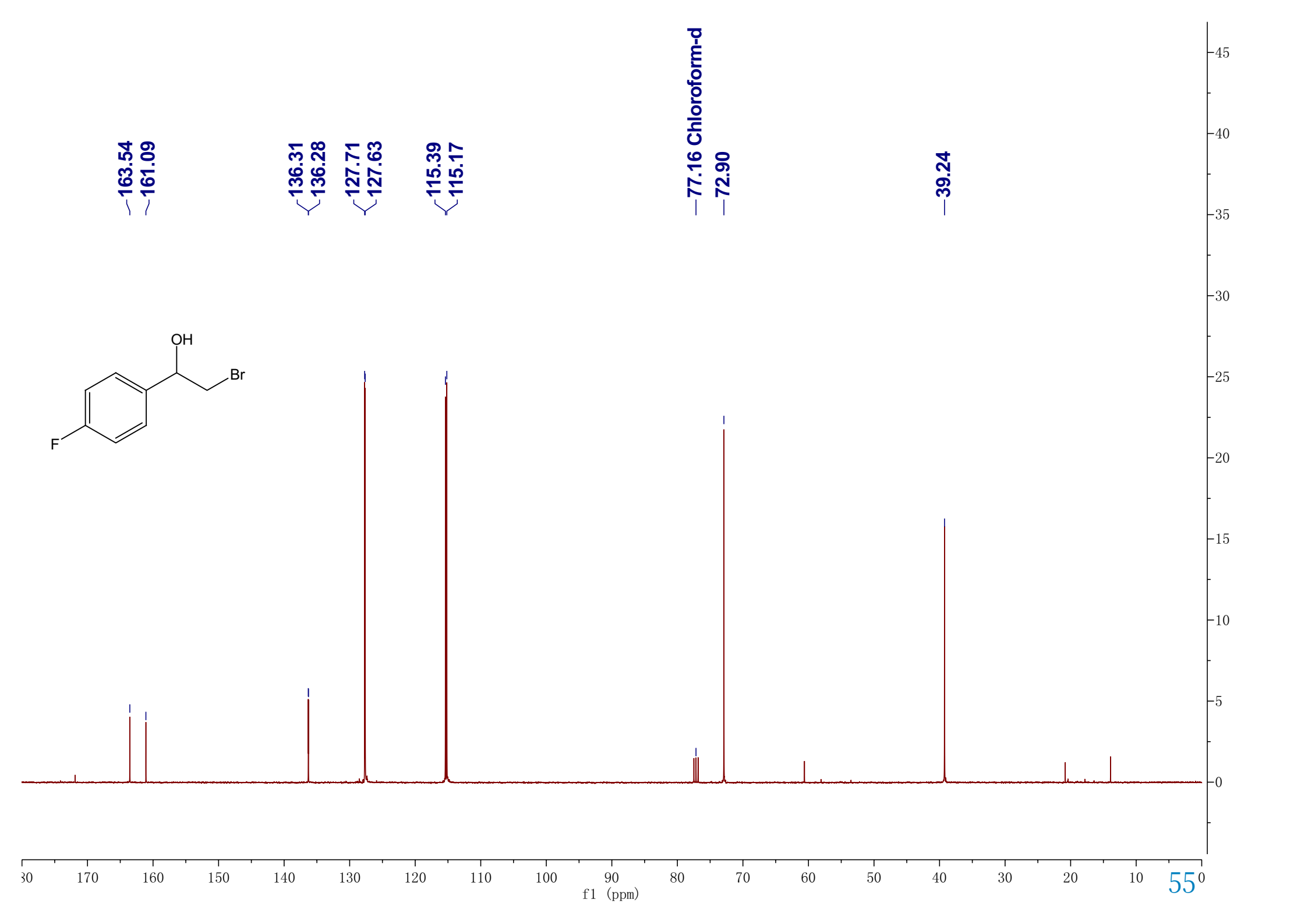


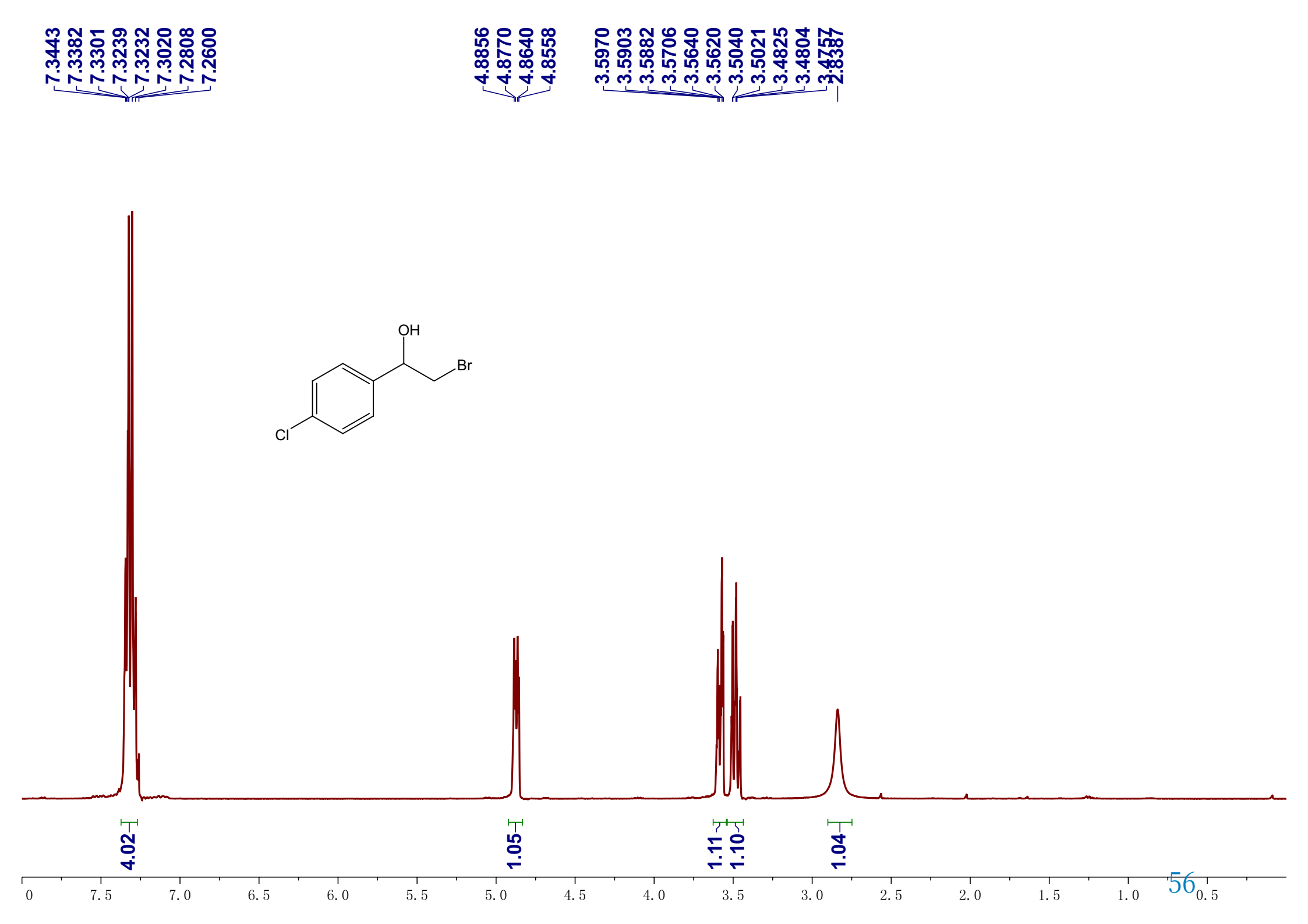


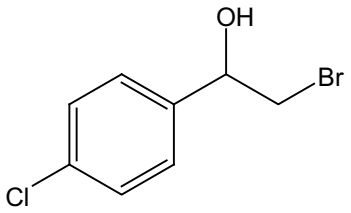

7.3324
7.3124
7.2556
7.2357
4.9458
4.9370
4.9241
4.9153
3.6741
3.6653
3.6481
3.6393
3.6071
3.5850
3.5817
3.5592
2.8151
2.5000
2.4219
OH
Br
2.22
2.00
1.14
2.24
1.27
3.01
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.
f1 (ppm)


OH
Br
138.217
137.464
129.342
125.948
77.160 Chloroform-d
73.675
40.129
21.221
f1 (ppm)


7.3581
7.3527
7.3403
7.3316
7.3235
7.3186
7.2600
7.0710
7.0662
7.0494
7.0446
7.0322
7.0278
7.0228
4.8960
4.8869
4.8742
3.6006
3.5966
3.5802
3.5745
3.5704
3.5616
3.5226
3.5180
3.5128
3.5007
3.4963
3.4911
2.7921
OH
Br
F
2.00
1.99
1.03
1.15
1.07
1.07
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.
f1 (ppm)

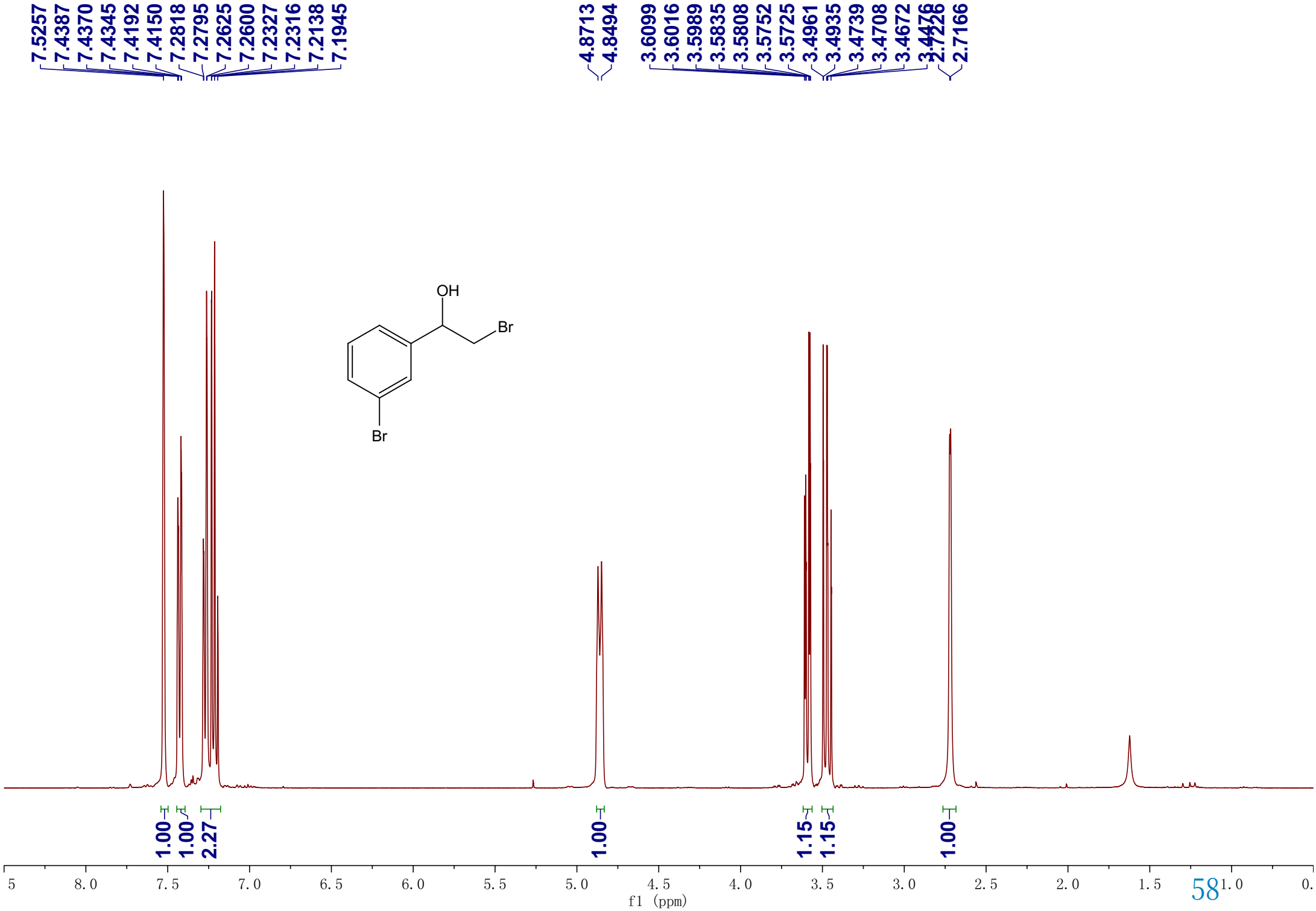

163.54
161.09
136.31
136.28
127.71
127.63
115.39
115.17
77.16 Chloroform-d
72.90
39.24
OH
Br
F
f1 (ppm)

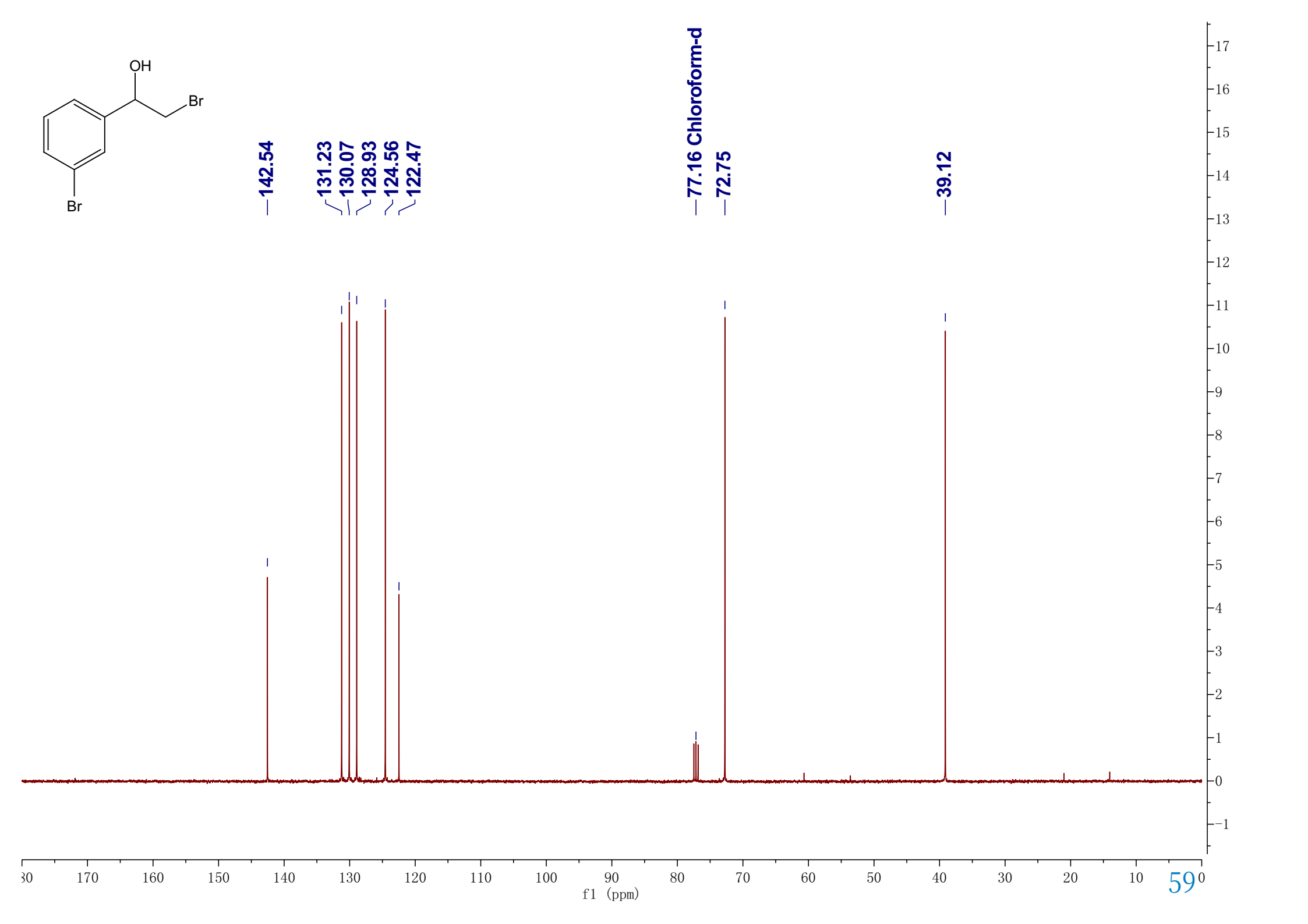

7.3443
7.3382
7.3301
7.3239
7.3232
7.3020
7.2808
7.2600
4.8856
4.8770
4.8640
4.8558
3.5970
3.5903
3.5882
3.5706
3.5640
3.5620
3.5040
3.5021
3.4825
3.4804
2.8387
OH
Br
Cl
4.02
1.05
1.11
1.10
1.04
0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5



138.801
134.196
128.873
127.428
77.160 Chloroform-d
73.170
39.827

f1 (ppm)


7.5257
7.4387
7.4370
7.4345
7.4192
7.4150
7.2818
7.2795
7.2625
7.2600
7.2327
7.2316
7.2138
7.1945
4.8713
4.8494
3.6099
3.6016
3.5989
3.5835
3.5808
3.5752
3.5725
3.4961
3.4935
3.4739
3.4708
3.4672
2.7226
2.7166
OH
Br
Br
1.00
1.00
2.27
1.00
1.15
1.15
1.00
5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.
f1 (ppm)


OH
Br
Br
142.54
131.23
130.07
128.93
124.56
122.47
77.16 Chloroform-d
72.75
39.12
f1 (ppm)