Supplementary Materials

Immobilized Enzymatic Alcohol Oxidation as A Versatile Reaction Module for Multienzyme Cascades

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Contents

Characterization

Scanning electron microscopy (SEM) images were recorded on Nova Nano SEM450 field-emission microscope at an accelerating voltage of 200 kV. Transmission electron microscopy (TEM) micrographs of samples were obtained with a JEM-2100 microscope operated at an acceleration voltage of 1.0 kV. Samples powders were dispersed in ethanol for 30 min with sonication. Then the mixture was dropped onto copper grid using a micropipette. Nitrogen adsorption-desorption experiments were carried out on a micromeritics ASAP 2020 gas sorptometer at 77 K. The samples were outgassed at 120 °C for 12 h. The specific surface area was calculated by Brunauer-Emmett-Teller (BET) method. The total pore volume of the samples was estimated from the amount adsorbed at the highest P/P_0 (ca. 0.99) by Barrett–Joyner–Halenda (BJH) model. Fourier transform infrared spectroscopy (FT-IR) spectra were measured with a Bruker VECTOR22 spectrometer. Confocal laser scanning microscopy (CLSM) micrographs were recorded on a Leica TCS SP5 optical microscope with excitation wavelength of 488 nm and emission wavelength of 525 nm. Before tested, *Cps*ADH and laccase were labeled with fluorescein isothiocyanate (FITC) and Rhodamine B, respectively, and then fixed to MSNs.

Experimental section

Determination of enzyme activity

1-Phenylethanol was used as a substrate to determine the enzyme activity of free *Cps*ADH and *Cps*ADH@MSNs. Simply, the reaction mixture consisting of free *Cps*ADH (0.1 mL) and *Cps*ADH@MSNs (10 mg) were evenly dispersed in 0.85 and 0.95 mL PBS buffer (50 mM, pH = 8.5), respectively. Then 1 μL 1-phenylethanol and 50 μL 20 mM NAD^+ (NAD^+ dissolved in 50 mM PBS buffer, pH = 8.5) were added, incubated at 25 °C and shaken violently for 1 min. A change in absorbance at 340 nm was monitored using a UV/vis spectrophotometer to detect the generation of NADH. One unit of *Cps*ADH activity was defined as the amount of enzyme required to produce 1 μmol of NADH per minute.

ABTS was used as a substrate to determine the activity of the free or immobilized laccase. In brief, the reaction mixture consisting of free (0.1 mL) or immobilized laccase (5 mg) and 0.9 mL of 1 mM laccase substrate (ABTS dissolved in 0.1 M acetate buffer, pH = 5) was prepared and incubated at 25 °C and shaken violently for 1 min. A change in absorbance at 420 nm was monitored using a UV/vis spectrophotometer and the laccase activity was calculated using the molar extinction coefficient of ABTS (ε_{420} =36,000 M^{-1} cm^{-1}). One unit of laccase activity was defined as the amount of enzyme required to oxidize 1 μmol of ABTS per minute. The enzyme activity is calculated as follows: $\Delta c/\Delta t=\Delta A/(36\times\Delta t)$, where $\Delta c/\Delta t$ is the absorbance change in unit time. Δt is the reaction time. ΔA is the change value of absorbance. The molar absorptivity of ABTS oxidation at 420 nm is 3.6×10^4 M^{-1} cm^{-1}.

Preparation of MSNs

Mesoporous silica nanoflowers (MSNs) were prepared by the continuous phase microemulsion method. The specific steps for preparing MSNs are as follows: First, the mixture of the CTAB (3 g) and urea solution (0.4 M, 90 g) was ultrasonicated at 25 °C for 30 min. Cyclohexane (36 g) and n-butanol (3 g) were then added and ultrasonicated to form a microemulsion solution. With slow stirring, 6 g TEOS was added to the mixture and stirred at 25 °C for 30 min. Lastly, the mixture was stirred at 70 °C for 20 h. The products were collected and calcined at 550 °C for 5 h to remove the template.

Functionalization of MSNs

The preprocessing steps for MSNs were as follows: First, 0.3 g MSNs were dispersed

in 100 mL n-hexane and ultrasonicated for 30 min. Then, 1 mL of APTES was added and the mixture was refluxed at 80 °C for 12 h. The products were collected by centrifugation and washed with ethanol, dried under vacuum at 60 °C for 12 h, obtaining the amino-functionalized MSNs (MSN-NH_2). Before immobilizing the enzymes, 0.1 g MSN-NH_2 was added to 15 mL of glutaraldehyde aqueous solution (glutaraldehyde/water: 1-8 wt%), and crosslinked at room temperature for different times (1-7 h). The product was then washed three times with phosphate buffer solution (PBS, 50 mM, pH = 8.5) and dried at 60 °C overnight.

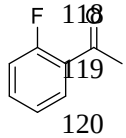
Recycling procedure

After each reaction cycle, the immobilized enzymes were separated by centrifugation at 4 °C. The *Cps*ADH@MSNs and laccase@MSNs were washed 3 times with PBS (50 mM, pH = 8.5) and deionized water, respectively. The separated immobilized enzymes were used in next reaction cycle. The reaction solution was extracted by ethyl acetate, and the organic phase was dried with anhydrous Na_2SO_4. The solvent was concentrated in a vacuum to obtain crude products, which was then purified by column chromatography.

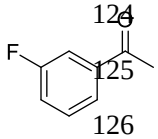
Preparation of the whole-cell catalysts[3]

Recombinant *E. coli* cells were inoculated to 3.0 mL LB (Luria-Bertani) broth (10 g L^{-1} tryptone, 5 g L^{-1} yeat extract, 5 g L^{-1} NaCl) containing 50 μg mL^{-1} kanamycin and grown at 37 °C for 6-8 h. The pre-culture (500 μL) was transferred into 50 mL TB (Terrific Broth) medium (4 mL L^{-1} glycerol, 12 g L^{-1} tryptone, 24 g L^{-1} yeast extract, 17 mM KH_2PO_4, and 72 mM K_2HPO_4) containing kanamycin (50 μg mL^{-1}). Cells were grown at 37 °C and 220 rpm to OD_{600} about of 0.6-0.8, and then induced by addition of isopropyl β-D-thiogalactoside (IPTG) to a concentration of 0.5 mM. The cells continued to grow for another 14-16 h at 25 °C. After cultivation, the cells were harvested by centrifugation (5000 g, 4 °C, 10 min), washed twice with potassium phosphate buffer (KPB, 100 mM, pH = 7.0) and then used as catalysts in the subsequent biotranformations.

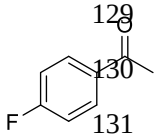
NMR and GC/HPLC data



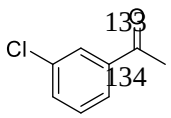
^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.87 (td, *J* = 7.62, 1.90 Hz, 1H), 7.51 (ddd, *J* = 7.31, 3.78, 2.09 Hz, 1H), 7.25 – 7.20 (m, 1H), 7.13 (dd, *J* = 10.09, 8.28 Hz, 1H), 2.64 (d, *J* = 4.90 Hz, 3H). **^{13}C NMR** (126 MHz, Chloroform-*d*) δ 196.1 (d, *J* = 3.5 Hz), 162.4 (d, *J* = 254.5 Hz), 134.8 (d, *J* = 9.1 Hz), 130.7 (d, *J* = 2.6 Hz), 125.9 (d, *J* = 12.7 Hz), 124.5 (d, *J* = 3.4 Hz), 116.8 (d, *J* = 24.0 Hz), 31.6 (d, *J* = 7.3 Hz).



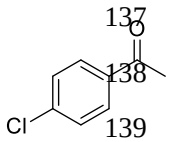
^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.89 (d, *J* = 7.75 Hz, 1H), 7.79 (d, *J* = 9.45 Hz, 1H), 7.60 (q, *J* = 6.37 Hz, 1H), 7.43 (s, 1H), 2.76 (s, 3H). **^{13}C NMR** (126 MHz, Chloroform-*d*) δ 196.9, 163.0 (d, *J* = 248.0 Hz), 139.3 (d, *J* = 6.3 Hz), 130.4 (d, *J* = 7.3 Hz), 124.2 (d, *J* = 3.3 Hz), 120.2 (d, *J* = 21.3 Hz), 115.1 (d, *J* = 22.0 Hz), 26.8.



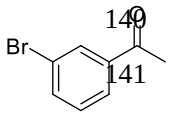
^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.96 (dt, *J* = 8.43, 5.38 Hz, 2H), 7.10 (t, *J* = 7.29 Hz, 2H), 2.57 (d, *J* = 5.65 Hz, 3H). **^{13}C NMR** (126 MHz, Chloroform-*d*) δ 196.6, 165.9 (d, *J* = 254.3 Hz), 133.7 (d, *J* = 3.2 Hz), 131.0 (d, *J* = 9.2 Hz), 115.7 (d, *J* = 21.8 Hz), 26.6.



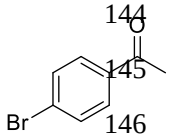
^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.92 (s, 1H), 7.82 (d, *J* = 7.82 Hz, 1H), 7.53 (dd, *J* = 8.13, 1.37 Hz, 1H), 7.40 (t, *J* = 7.86 Hz, 1H), 2.59 (s, 3H). **^{13}C NMR** (126 MHz, Chloroform-*d*) δ 196.9, 138.7, 135.1, 133.2, 130.1, 128.5, 126.5, 26.8.



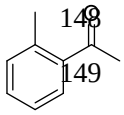
^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.87 (td, *J* = 5.97, 2.14 Hz, 2H), 7.41 (td, *J* = 6.69, 2.07 Hz, 2H), 2.57 (d, *J* = 5.43 Hz, 3H). **^{13}C NMR** (126 MHz, Chloroform-*d*) δ 196.9, 139.6, 135.5, 129.8, 129.0, 26.6.



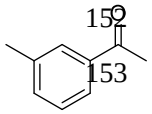
^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.05 (s, 1H), 7.85 (d, *J* = 7.94 Hz, 1H), 7.66 (d, *J* = 8.35 Hz, 1H), 7.32 (t, *J* = 7.83 Hz, 1H), 2.57 (s, 3H). **^{13}C NMR** (126 MHz, Chloroform-*d*) δ 196.7, 138.9, 136.0, 131.4, 130.3, 126.9, 123.0, 26.7.



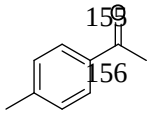
^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.75 (d, *J* = 8.58 Hz, 2H), 7.53 (d, *J* = 8.57 Hz, 2H), 2.52 (s, 3H). **^{13}C NMR** (126 MHz, Chloroform-*d*) δ 196.9, 135.8, 131.8, 129.8, 128.2, 26.5.



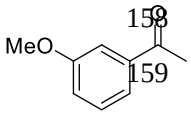
^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.69 (dd, *J* = 7.73, 1.45 Hz, 1H), 7.37 (td, *J* = 7.49, 1.48 Hz, 1H), 7.28 – 7.22 (m, 2H), 2.58 (s, 3H), 2.53 (s, 3H). **^{13}C NMR** (126 MHz, Chloroform-*d*) δ 201.8, 138.5, 137.7, 132.1, 131.6, 129.5, 125.8, 29.6, 21.7.



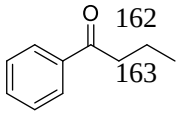
^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.79 – 7.72 (m, 2H), 7.40 – 7.33 (m, 2H), 2.59 (s, 3H), 2.41 (s, 3H). **^{13}C NMR** (126 MHz, Chloroform-*d*) δ 198.6, 138.5, 137.3, 134.0, 128.9, 128.6, 125.7, 26.8, 21.4.



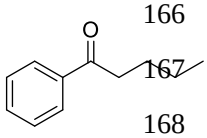
^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.87 (dd, *J* = 8.24, 1.85 Hz, 2H), 7.28 – 7.25 (m, 2H), 2.59 (s, 3H), 2.42 (s, 3H). **^{13}C NMR** (126 MHz, Chloroform-*d*) δ 198.0, 144.0, 134.8, 129.4, 128.6, 26.6, 21.7.



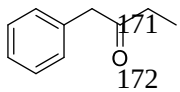
^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.48 – 7.38 (m, 2H), 7.29 (d, *J* = 8.01 Hz, 1H), 7.02 (dd, *J* = 8.33, 2.70 Hz, 1H), 3.76 (s, 3H), 2.51 (s, 3H). **^{13}C NMR** (101 MHz, Chloroform-*d*) δ 197.8, 159.7, 138.4, 129.5, 121.0, 119.4, 112.3, 55.3, 26.5.



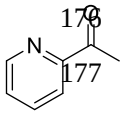
^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.98 – 7.91 (m, 2H), 7.55 – 7.50 (m, 1H), 7.47 – 7.39 (m, 2H), 2.92 (t, *J* = 7.29 Hz, 2H), 1.76 (h, *J* = 7.34 Hz, 2H), 0.99 (t, *J* = 7.46 Hz, 3H). **^{13}C NMR** (101 MHz, Chloroform-*d*) δ 200.4, 137.1, 132.9, 128.6, 128.1, 40.5, 17.8, 13.9.



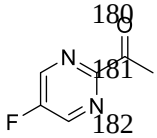
^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.98 – 7.90 (m, 2H), 7.56 – 7.47 (m, 1H), 7.47 – 7.38 (m, 2H), 2.98 – 2.90 (m, 2H), 1.70 (p, *J* = 7.47 Hz, 2H), 1.46 – 1.32 (m, 2H), 0.94 (t, *J* = 7.38 Hz, 3H). **^{13}C NMR** (101 MHz, Chloroform-*d*) δ 200.5, 137.1, 132.9, 128.6, 128.1, 38.3, 26.5, 22.5, 14.0.



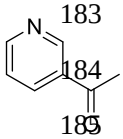
^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.38 – 7.27 (m, 3H), 7.26 – 7.17 (m, 2H), 3.70 (s, 2H), 2.49 (q, *J* = 7.30 Hz, 2H), 1.05 (t, *J* = 7.33 Hz, 3H). **^{13}C NMR** (101 MHz, Chloroform-*d*) δ 208.9, 134.5, 129.4, 128.6, 126.9, 49.7, 35.1, 7.7.



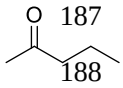
^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.58 – 8.51 (m, 1H), 7.89 (dd, *J* = 7.93, 1.35 Hz, 1H), 7.69 (td, *J* = 7.74, 1.77 Hz, 1H), 7.38 – 7.30 (m, 1H), 2.58 (s, 3H). **^{13}C NMR** (101 MHz, Chloroform-*d*) δ 199.9, 153.5, 148.9, 136.7, 127.0, 121.5, 25.6.



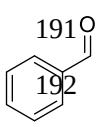
^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.72 (s, 2H), 2.73 (s, 3H). **^{13}C NMR** (101 MHz, Chloroform-*d*) δ 195.9, 158.2 (d, *J* = 272.5 Hz), 156.5 (d, *J* = 5.9 Hz), 145.5 (d, *J* = 20.4 Hz), 27.0.



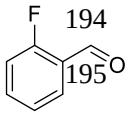
^{1}H NMR (600 MHz, Chloroform-*d*) δ 9.13 (s, 1H), 8.76 – 8.72 (m, 1H), 8.19 (d, *J* = 8.04 Hz, 1H), 7.40 (dd, *J* = 8.51, 4.48 Hz, 1H), 2.61 (s, 3H). **^{13}C NMR** (151 MHz, Chloroform-*d*) δ 196.5, 153.3, 149.7, 135.2, 132.0, 123.4, 26.5**.**



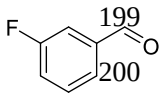
^{1}H NMR (600 MHz, Chloroform-*d*) δ 2.31 (dt, *J* = 9.33, 4.64 Hz, 2H), 2.02 (d, *J* = 2.90 Hz, 3H), 1.50 (dq, *J* = 9.56, 6.97, 5.91 Hz, 2H), 0.81 (dd, *J* = 8.69, 6.07 Hz, 3H). **^{13}C NMR** (151 MHz, Chloroform-*d*) δ 209.0, 45.5, 29.7, 17.2, 13.6.



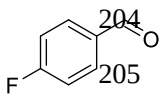
^{1}H NMR (600 MHz, Chloroform-*d*) δ 9.95 (s, 1H), 7.81 (d, *J* = 7.87 Hz, 2H), 7.56 (t, *J* = 7.66 Hz, 1H), 7.46 (t, *J* = 7.75 Hz, 2H). **^{13}C NMR** (151 MHz, Chloroform-*d*) δ 192.3, 136.3, 134.4, 129.6, 128.9.



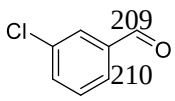
^{1}H NMR (500 MHz, Chloroform-*d*) δ 10.38 (s, 1H), 7.88 (td, *J* = 7.35, 1.92 Hz, 1H), 7.65 – 7.56 (m, 1H), 7.28 (d, *J* = 7.49 Hz, 1H), 7.21 – 7.14 (m, 1H). **^{13}C NMR** (126 MHz, Chloroform-*d*) δ 187.3 (d, *J* = 6.4 Hz), 164.8 (d, *J* = 258.2 Hz), 136.5 (d, *J* = 9.2 Hz), 128.8, 124.8 (d, *J* = 3.6 Hz), 124.3 (d, *J* = 8.1 Hz), 116.6 (d, *J* = 20.2 Hz).



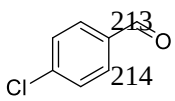
^{1}H NMR (400 MHz, Chloroform-*d*) δ 10.00 (d, *J* = 1.90 Hz, 1H), 7.68 (dt, *J* = 7.58, 1.27 Hz, 1H), 7.59 – 7.50 (m, 2H), 7.33 (tdd, *J* = 8.30, 2.68, 1.09 Hz, 1H). **^{13}C NMR** (101 MHz, Chloroform-*d*) δ 190.7 (d, *J* = 2.5 Hz), 162.9 (d, *J* = 248.8 Hz), 138.3 (d, *J* = 6.5 Hz), 130.7 (d, *J* = 7.4 Hz), 125.9 (d, *J* = 2.9 Hz), 121.3 (d, *J* = 21.9 Hz), 114.9 (d, *J* = 21.9 Hz).



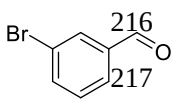
^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.97 (s, 1H), 7.96 – 7.86 (m, 2H), 7.25 – 7.17 (m, 2H). **^{13}C NMR** (101 MHz, Chloroform-*d*) δ 190.4, 166.3 (d, *J* = 256.1 Hz), 132.9 (d, *J* = 3.0 Hz), 132.0 (d, *J* = 10.0 Hz), 116.1 (d, *J* = 22.6 Hz).



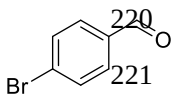
^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.98 (s, 1H), 7.86 (t, *J* = 1.85 Hz, 1H), 7.77 (dt, *J* = 7.58, 1.33 Hz, 1H), 7.60 (ddd, *J* = 7.96, 2.16, 1.13 Hz, 1H), 7.49 (t, *J* = 7.76 Hz, 1H). **^{13}C NMR** (101 MHz, Chloroform-*d*) δ 190.6, 137.6, 135.0, 134.0, 130.2, 128.8, 127.8.



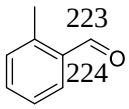
^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.98 (s, 1H), 7.86 – 7.78 (m, 2H), 7.55 – 7.47 (m, 2H). **^{13}C NMR** (101 MHz, Chloroform-*d*) δ 190.7, 140.7, 134.6, 130.8, 129.3.



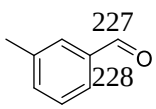
^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.96 (s, 1H), 8.01 (t, *J* = 1.78 Hz, 1H), 7.81 (dt, *J* = 7.63, 1.33 Hz, 1H), 7.75 (ddd, *J* = 7.96, 2.06, 1.09 Hz, 1H), 7.42 (t, *J* = 7.81 Hz, 1H). **^{13}C NMR** (101 MHz, Chloroform-*d*) δ 190.5, 137.7, 137.0, 131.9, 130.4, 128.2, 123.1.



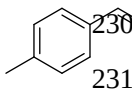
^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.97 (s, 1H), 7.77 – 7.72 (m, 2H), 7.71 – 7.64 (m, 2H). **^{13}C NMR** (101 MHz, Chloroform-*d*) δ 191.1, 135.1, 132.4, 131.0, 129.8.



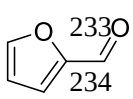
^{1}H NMR (400 MHz, Chloroform-*d*) δ 10.22 (s, 1H), 7.76 (dd, *J* = 7.72, 1.58 Hz, 1H), 7.44 (td, *J* = 7.51, 1.55 Hz, 1H), 7.32 (t, *J* = 7.54 Hz, 1H), 7.22 (d, *J* = 7.67 Hz, 1H), 2.63 (s, 3H). **^{13}C NMR** (101 MHz, Chloroform-*d*) δ 192.8, 140.6, 134.1, 133.6, 132.0, 131.8, 126.3, 19.5.



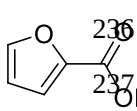
^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.85 (s, 1H), 7.54 (dt, *J* = 3.95, 2.20 Hz, 2H), 7.29 (d, *J* = 7.31 Hz, 2H), 2.29 (s, 3H).**^{13}C NMR** (101 MHz, Chloroform-*d*) δ 192.5, 138.8, 136.4, 135.2, 129.9, 128.8, 127.1, 21.1.



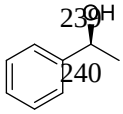
^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.95 (s, 1H), 7.79 – 7.74 (m, 2H), 7.32 (d, *J* = 7.90 Hz, 2H), 2.42 (s, 3H). **^{13}C NMR** (101 MHz, Chloroform-*d*) δ 191.9, 145.5, 134.2, 129.8, 129.7, 21.8.



^{1}H NMR (600 MHz, Chloroform-*d*) δ 9.52 (s, 1H), 7.58 (s, 1H), 7.15 (d, *J* = 4.13 Hz, 1H), 6.49 (s, 1H). **^{13}C NMR** (151 MHz, Chloroform-*d*) δ 177.7, 152.8, 148.0, 121.2, 112.5.

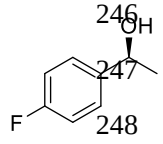


^{1}H NMR (400 MHz, Chloroform-*d*) δ 12.38 (s, 1H), 7.64 (dd, *J* = 1.73, 0.85 Hz, 1H), 7.34 (dd, *J* = 3.56, 0.87 Hz, 1H), 6.56 (dd, *J* = 3.54, 1.71 Hz, 1H). **^{13}C NMR** (101 MHz, Chloroform-*d*) δ 164.0, 147.6, 143.9, 120.4, 112.4.

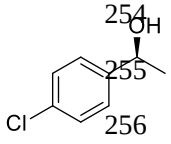


Colorless oil, bp 224 °C, 97% yield, 99% ee. **^{1}H NMR** (600 MHz, Chloroform-*d*) δ 7.34 (t, *J* = 4.06 Hz, 5H), 4.85 (t, *J* = 6.57 Hz, 1H), 2.34 (s, 1H), 1.46 (dd, *J* = 6.63, 2.37 Hz, 3H). **^{13}C NMR** (151 MHz, Chloroform-*d*) δ 145.9, 128.5, 127.5, 125.5, 70.4, 25.2. HPLC conditions: Chiralpak

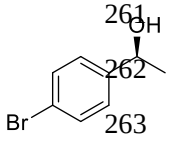
OJ column (4.6 mm I. D. × 250 mmL), UV detection at 210 nm, eluent: *n*-hexane/2-propanol =99:1, flow 1 mL/min, 35 °C using racemic compounds as references. t_R = 27.7 min (major) and 30.8 min (minor).



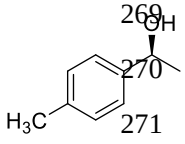
Colorless oil, bp 216.2 °C, 94% yield, 99% ee. **^{1}H NMR** (600 MHz, Chloroform-*d*) δ 7.29 (t, *J* = 6.63 Hz, 2H), 7.00 (t, *J* = 8.51 Hz, 2H), 4.85 – 4.80 (m, 1H), 2.59 (s, 1H), 1.43 (d, *J* = 6.65 Hz, 3H). **^{13}C NMR** (151 MHz, Chloroform-*d*) δ 162.2 (d, *J* = 244.7 Hz), 141.6 (d, *J* = 3.8 Hz), 127.1 (d, *J* = 7.8 Hz), 115.3 (d, *J* = 21.2 Hz), 69.7, 25.3. HPLC conditions: Chiralpak AS column (4.6 mm I. D. × 250 mmL), UV detection at 210 nm, eluent: *n*-hexane/2-propanol =99:1, flow 1 mL/min, 35 °C using racemic compounds as references. t_R = 24.2 min (minor) and 28.8 min (major).



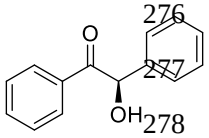
Colorless oil, bp 240.6 °C, 92% yield, 99% ee. **^{1}H NMR** (600 MHz, Chloroform-*d*) δ 7.27 – 7.20 (m, 4H), 4.78 (q, *J* = 6.67 Hz, 1H), 2.44 (s, 1H), 1.39 (d, *J* = 6.67 Hz, 3H). **^{13}C NMR** (151 MHz, Chloroform-*d*) δ 144.3, 133.1, 128.7, 126.9, 69.7, 25.3. HPLC conditions: Chiralpak OD column (4.6 mm I. D. × 250 mmL), UV detection at 210 nm, eluent: *n*-hexane/2-propanol =99:1, flow 1 mL/min, 35 °C using racemic compounds as references. t_R = 25.1 min (major) and 28.1 min (minor).



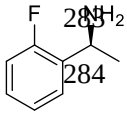
Colorless oil, bp 253.3 °C, 91% yield, 99% ee. **^{1}H NMR** (600 MHz, Chloroform-*d*) δ 7.46 – 7.41 (m, 2H), 7.19 (d, *J* = 8.58 Hz, 2H), 4.79 (q, *J* = 6.58 Hz, 1H), 2.56 (s, 1H), 1.42 (d, *J* = 6.69 Hz, 3H). **^{13}C NMR** (151 MHz, Chloroform-*d*) δ 144.9, 131.6, 127.3, 121.2, 69.7, 25.3. GC conditions: CP-Chirasil Dex CB (df = 0.25 μm, 0.32 mm i.d. × 25 m); carrier gas, N_2 (flow 30 mL/min); injection temp, 250 °C; initial column temperature 80 °C, then progress rate, 1 °C/min to 160 °C, then progress rate, 10 °C/min; final column temperature, 190 °C for 8 min; t_R = 52.9 (minor) and 55.1 min (major).



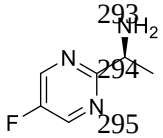
Colorless oil, bp 216 °C, 95% yield, 99% ee. **^{1}H NMR** (600 MHz, Chloroform-*d*) δ 7.25 (s, 2H), 7.16 (d, *J* = 7.77 Hz, 2H), 4.83 (q, *J* = 6.48 Hz, 1H), 2.35 (s, 3H), 2.28 (s, 1H), 1.47 (d, *J* = 6.53 Hz, 3H). **^{13}C NMR** (151 MHz, Chloroform-*d*) δ 143.0, 137.2, 129.2, 125.5, 70.2, 25.1, 21.2. HPLC conditions: Chiralpak AS column (4.6 mm I. D. × 250 mmL), UV detection at 210 nm, eluent: *n*-hexane/2-propanol =99:1, flow 0.5 mL/min, 30 °C using racemic compounds as references. t_R = 36.9 min (minor) and 39.5 min (major).



White solid, bp 343 °C, 91% yield, 99% ee. **^{1}H NMR** (600 MHz, Chloroform-*d*) δ 7.92 – 7.90 (m, 2H), 7.52 – 7.49 (m, 1H), 7.38 (t, *J* = 7.86 Hz, 2H), 7.34 – 7.27 (m, 5H), 5.95 (s, 1H), 4.56 (s, 1H). **^{13}C NMR** (151 MHz, Chloroform-*d*) δ 199.1, 139.1, 134.0, 133.7, 129.3, 129.3, 128.8, 128.7, 127.9, 76.3. HPLC conditions: Chiralpak AD column, UV detection at 254 nm, eluent: *n*-hexane/2-propanol =95:5, flow 1 mL/min, 35 °C using racemic compounds as references. t_R = 29.4 min (minor) and 37.5 min (major).

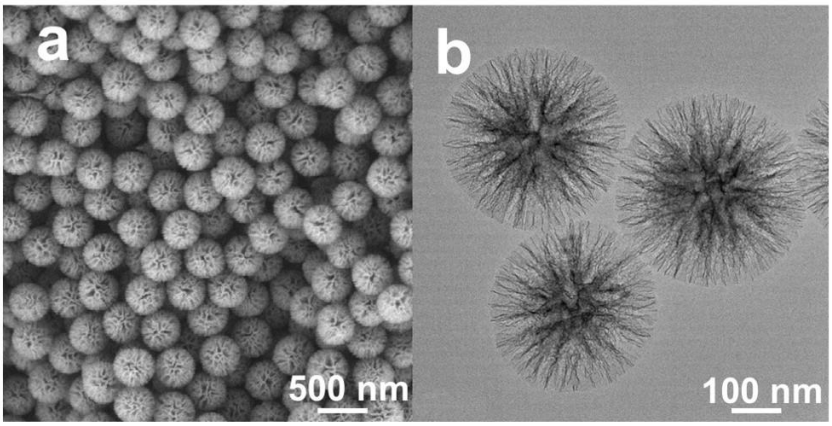


White solid, bp 179.8 °C, 93% yield, 98% ee. **^{1}H NMR** (600 MHz, Chloroform-*d*) δ 7.39 (t, *J* = 7.89 Hz, 1H), 7.18 (t, *J* = 7.01 Hz, 1H), 7.10 (t, *J* = 7.77 Hz, 1H), 6.99 (t, *J* = 9.64 Hz, 1H), 4.36 (q, *J* = 7.04 Hz, 1H), 1.68 (s, 2H), 1.40 (d, *J* = 6.92 Hz, 3H). **^{13}C NMR** (151 MHz, Chloroform-*d*) δ 160.5 (d, *J* = 245.2 Hz), 134.5 (d, *J* = 13.2 Hz), 128.2 (d, *J* = 8.0 Hz), 126.8 (d, *J* = 5.3 Hz), 124.3 (d, *J* = 4.1 Hz), 115.5 (d, *J* = 22.2 Hz), 45.5 (d, *J* = 3.1 Hz), 24.1. GC conditions: CP-Chirasil Dex CB (df = 0.25 μm, 0.32 mm i.d. × 25 m); carrier gas, N_2 (flow 30 mL/min); injection temp, 250 °C; initial column temperature 80 °C, then progress rate, 1 °C/min to 160 °C, then progress rate, 10 °C/min; final column temperature, 190 °C for 8 min; t_R = 36.7 min (minor) and 37.8 min (major).

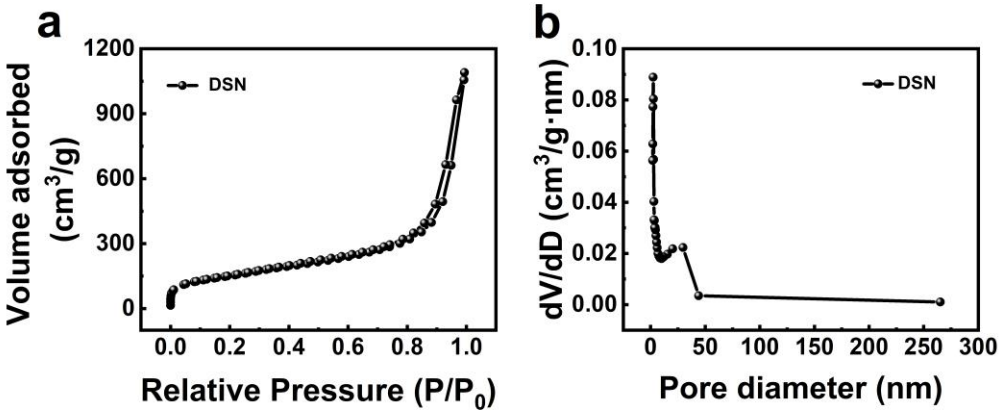


Colorless oil, bp 183.4 °C, 91% yield, 84% ee. **^{1}H NMR** (600 MHz, Deuterium Oxide) δ 8.80 (s, 2H), 4.77 (d, *J* = 7.25 Hz, 1H), 1.70 (d, *J* = 7.04 Hz, 3H). **^{13}C NMR** (151 MHz, Deuterium Oxide) δ 161.2 (d, *J* = 5.4 Hz), 157.3 (d, *J* = 262.8 Hz), 146.1 (d, *J* = 21.9 Hz), 51.2, 18.3. GC conditions: CP-Chirasil Dex CB (df = 0.25 μm, 0.32 mm i.d. × 25 m); carrier gas, N_2 (flow 30 mL/min); injection temp, 250 °C; initial column temperature 100 °C, then progress rate, 5 °C/min to 160 °C, then progress rate, 10 °C/min; final column temperature, 190 °C for 8 min; t_R = 9.1 min (minor) and 9.5 min (major).

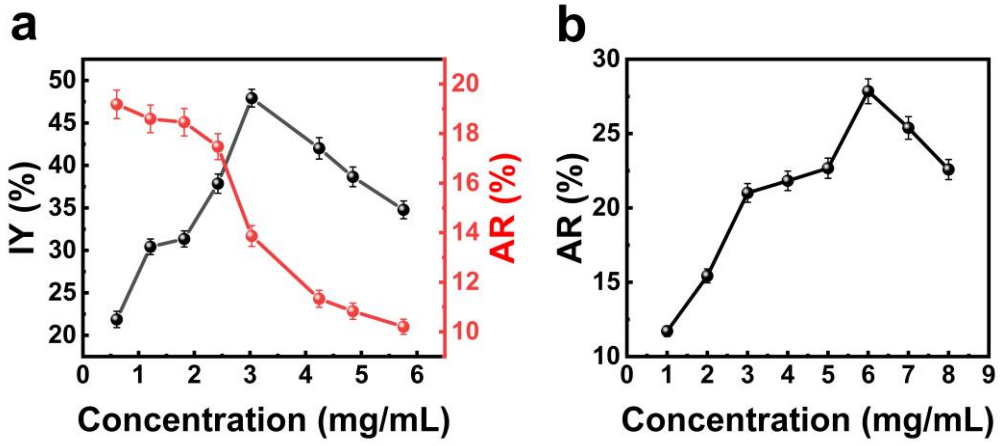
Figures



Supplementarty Figure 1. SEM (a) and TEM (b) images of MSNs

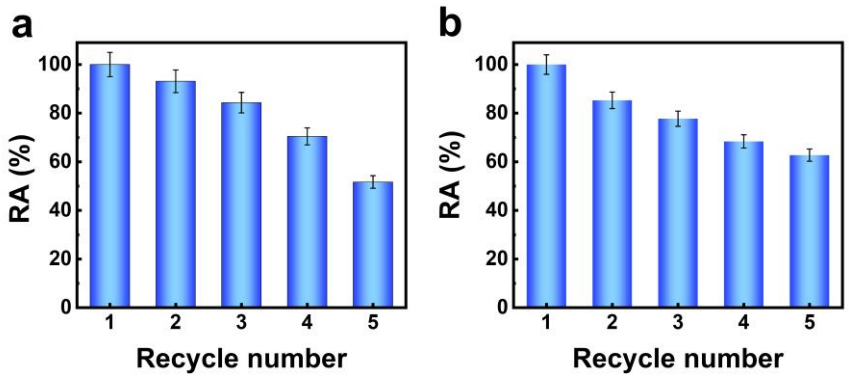


Supplementarty Figure 2. N_2 adsorption-desorption isotherm curves (a) and pore size distribution (b) of MSNs

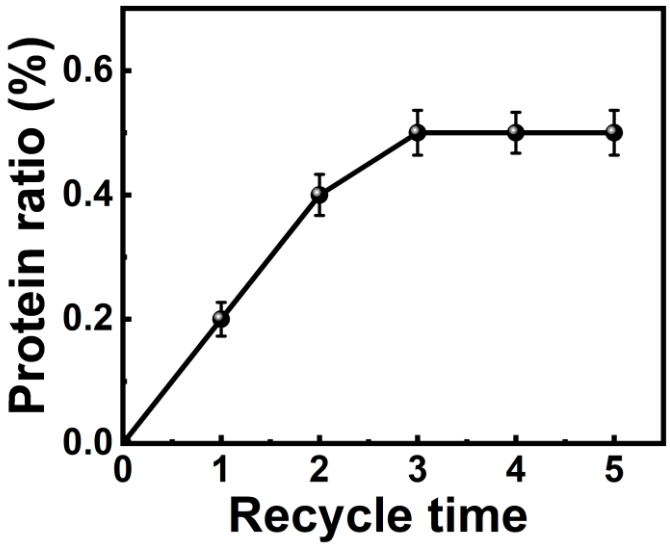


Supplementarty Figure 3. Effect of enzyme concentration on immobilization yield

(IY) and enzyme activity recovery (AR) of *Cps*ADH@MSNs (a) and laccase@MSNs (b).



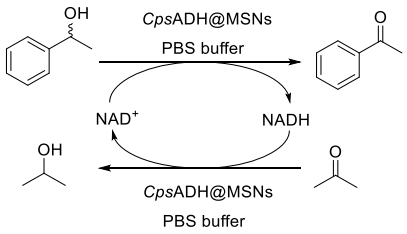
Supplementarty Figure 4. The reusability of *Cps*ADH@MSNs (a) and laccase@MSNs (b)



Supplementarty Figure 5. The leaching of *Cps*ADH during the reaction

Tables

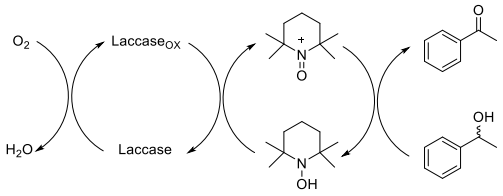
Supplementarty Table 1. Optimizing reaction conditions for enzymatic oxidation of 1-phenylethanol by *Cps*ADH@MSNs.[a]



Entry	pH	Temperature (°C)	Substrate (mM)	Time (h)	Conversion (%)
1	8.5	20	20	6	73
2	8.5	30	20	6	89
3	8.5	40	20	6	99
4	7	40	20	6	79
5	9.5	40	20	6	93
6[b]	8.5	40	20	6	99
7[c]	8.5	40	20	6	99
8[d]	8.5	40	20	6	91
9[d]	8.5	40	20	8	99
10	8.5	40	30	6	74
11	8.5	40	30	12	99
12[e]	8.5	40	50	12	99
13[e]	8.5	40	100	36	99
14[f]	8.5	40	300	36	81
15[f]	8.5	40	300	48	92
16[g]	8.5	40	350	72	91

[a] Reaction conditions: 10 mg *Cps*ADH@MSNs, substrate (20 mM), PBS buffer (1 mL), acetone (200 mM), NAD^+ (1% equiv.). [b] NAD^+ (1 equiv.); [c] NAD^+ (5% equiv.); [d] NAD^+ (1% equiv.). [e] 30 mg *Cps*ADH@MSNs, NAD^+ (1% equiv.). [f] 50 mg *Cps*ADH@MSNs, NAD^+ (1% equiv.). [g] Substrate (1.06 g, 350 mM, 25 mL), 800 mg *Cps*ADH@MSNs, NAD^+ (1% equiv.), acetone (3.5 M), PBS buffer (25 mL).

Supplementarty Table 2. Optimizing reaction conditions for enzymatic oxidation of 1-phenylethanol by laccase@MSNs [a]



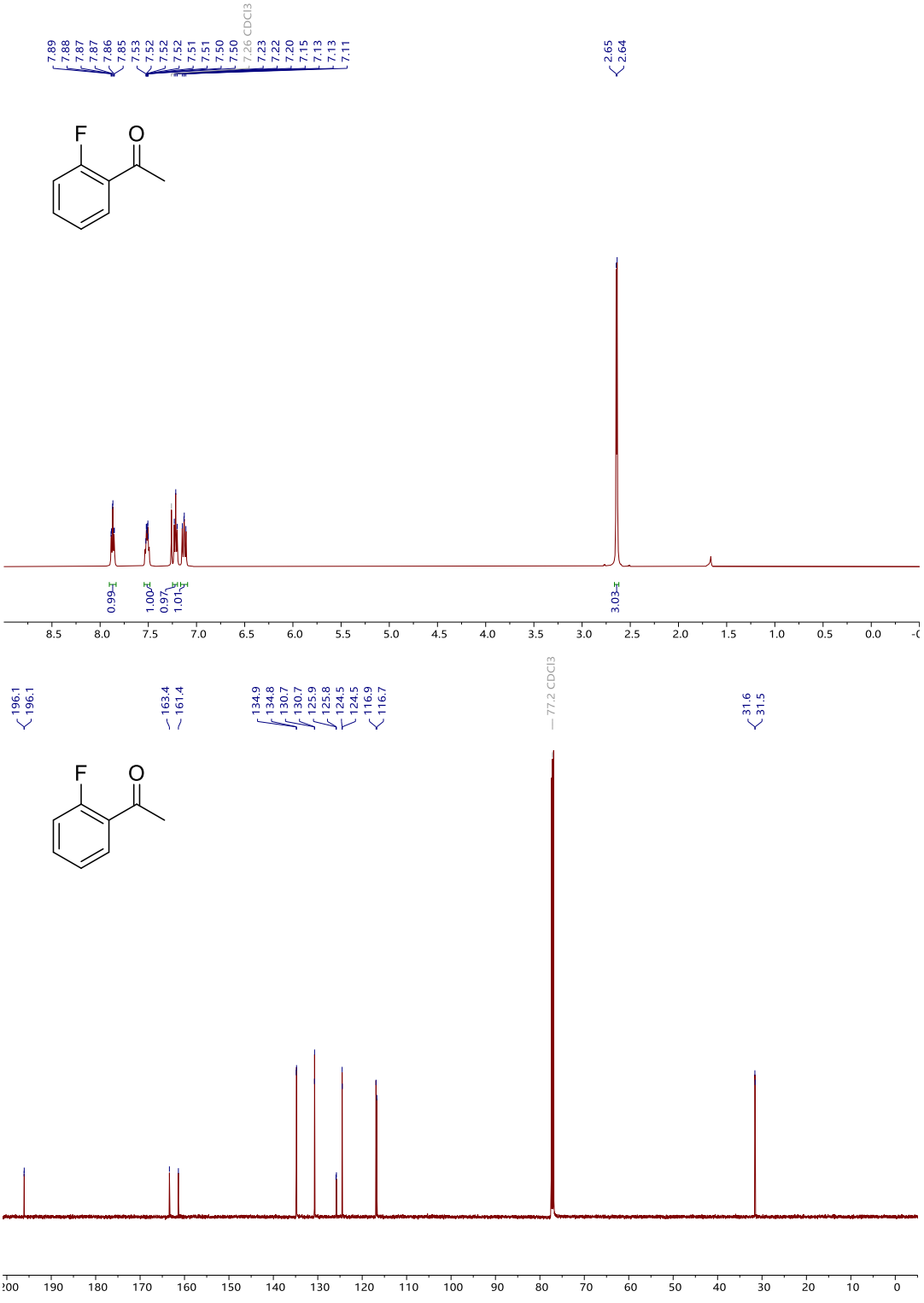
Entry	pH	Temperature (°C)	TEMPO (mol%)	Conversion (%)
1	4.5	40	20	24
2	4.5	40	40	76
3[b]	4.5	40	40	99
4	4.5	40	60	99
5	4.5	30	60	92
6	4.5	50	60	78
7	3.5	40	60	94
8	6	40	60	92
9	7	40	60	74
10[c]	4.5	40	60	99
11[d]	4.5	40	60	99
12[e]	4.5	40	60	99
13[f]	4.5	40	60	91
14[g]	4.5	40	60	89
15	4.5	40	-	-
16[h]	4.5	40	60	-

[a] Typical conditions: 24 h reaction time, 40 °C; substrate (20 mM), TEMPO (0.6 equiv.), 10 mg laccase@MSNs, in 1 mL water solution at pH = 4.5 (0.1 M acetate). [b] 2 days reaction time. [c] Substrate (40 mM), 3 days reaction time. [d] Substrate (60 mM), 5 days reaction time. [e] 30 mg laccase@MSNs, substrate (60 mM), 2 days reaction time. [f] 60 mg laccase@MSNs, substrate (200 mM), 4 days reaction time. [g] Substrate (1.06 g, 350 mM, 25 mL), 500 mg laccase@MSNs, 5 days reaction time. [h] Without laccase@MSNs.

Supplementary Table 3. Alcohol oxidation by different catalytic systems.

Entry	catalysts	Reaction condition	Yield (%)	Ref.
1	$[CuNi(bz)_3(bpy)_2]ClO_4$	H_2O, H_2O_2, 70 °C, 3 h	78-97	4
2	G-750 (N-doped nanosheets)	1,4-dioxane, HNO_3 , 90 °C, 5 h	16-99	5
3	Pt@MSgILg-PMMA	H_2O, K_2CO_3, 80 °C, 2 h	60-99	6
4	$Pd/Cu_2(BDC)_2DABCOF$	Solvent free, Na_2CO_3, 120 °C, 24 h	93-99	7
5	$PdAu@APTES@SiO_2$	DEGDME, TBHP,80 °C, 1 h	94.3	8
6	Au/U_3O_8	TBHP, 94 °C, 0.5 h	85	9
7	Co-BTC	DMF, 95 °C, 10 h	92.9	10
8	Cu-BTC	DMF, 95 °C, 10 h	23.9	10
9	Fe-BTC	DMF, 95 °C, 10 h	11.3	10
10	$ZnO_x(1\%)–MnCO_3$	Toluene, 100 °C, 4 min	99	11
13	*Cps*ADH@MSNs	H_2O, NAD^+, 40 °C, 12 h	91-99	This work
14	laccase@MSNs	H_2O, TEMPO, 40 °C, 24 h	91-99	This work

NMR spectra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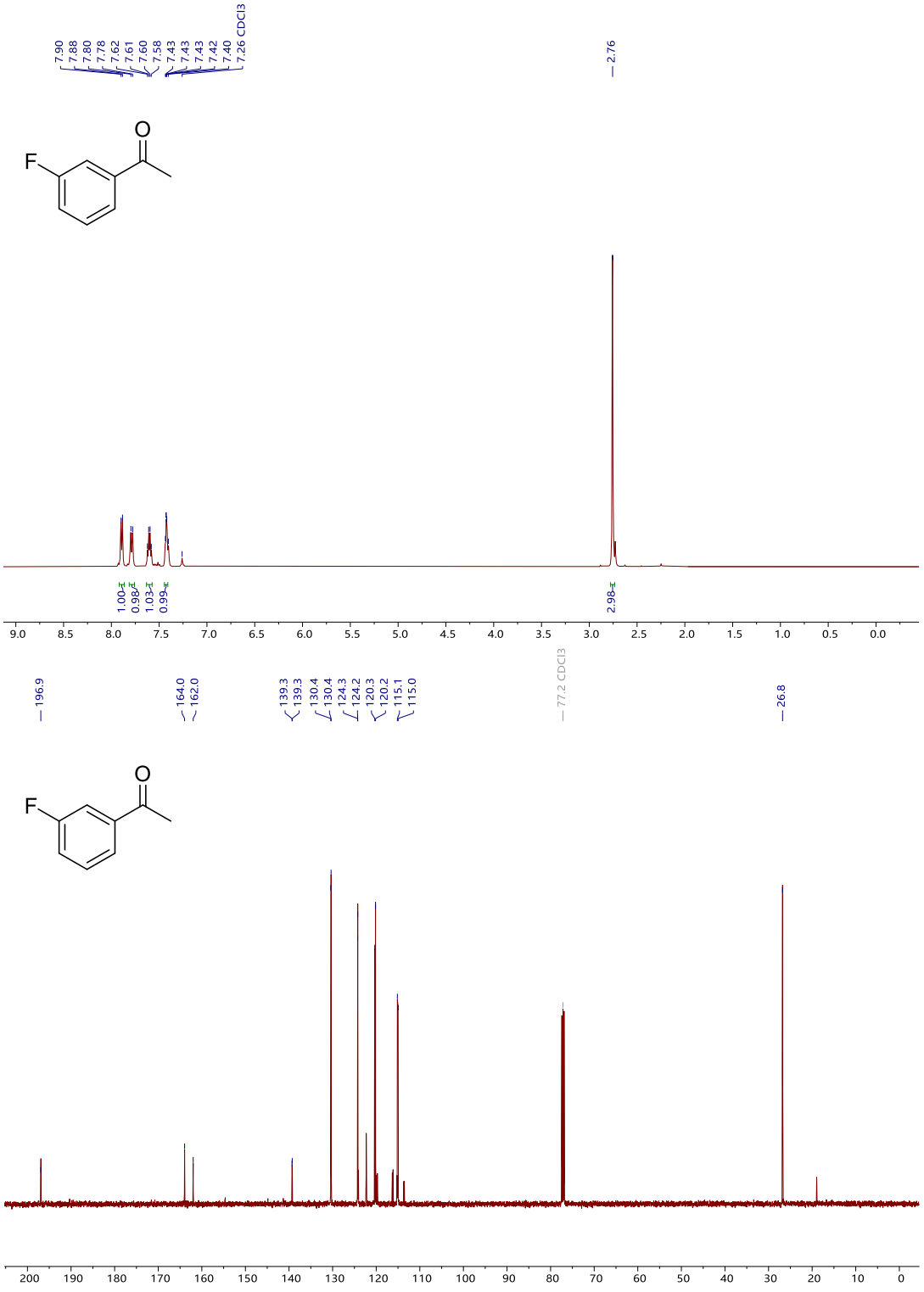
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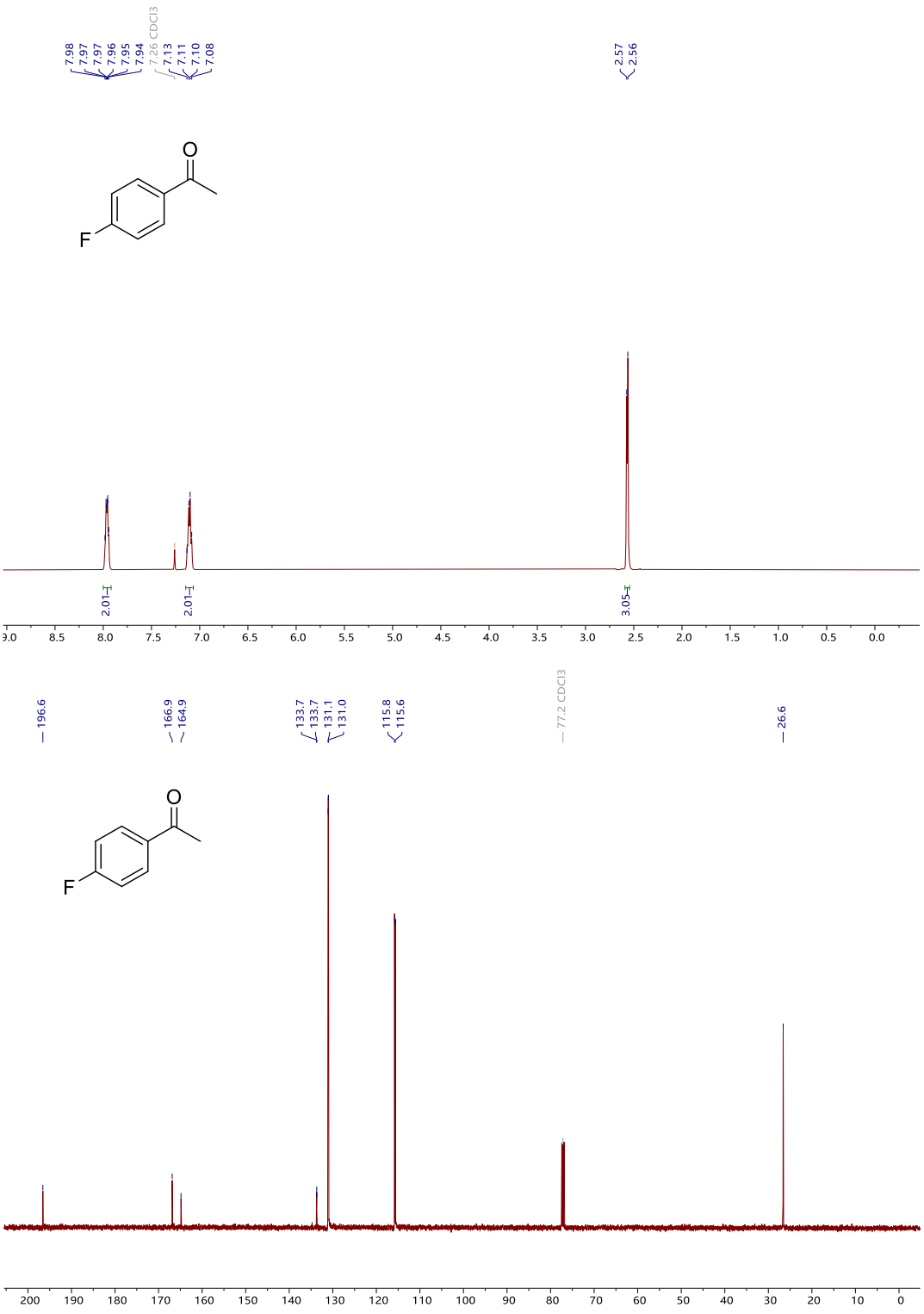
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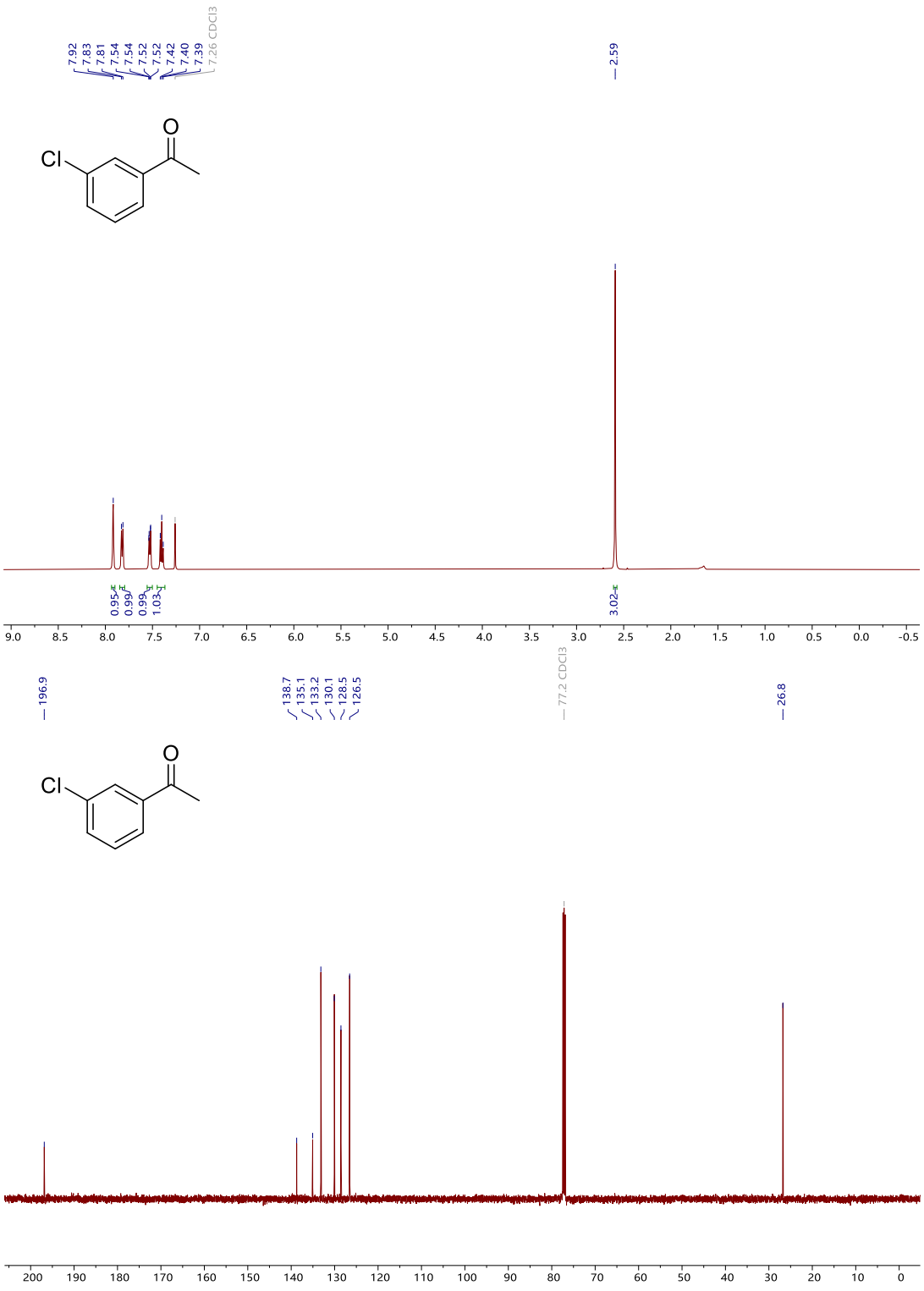
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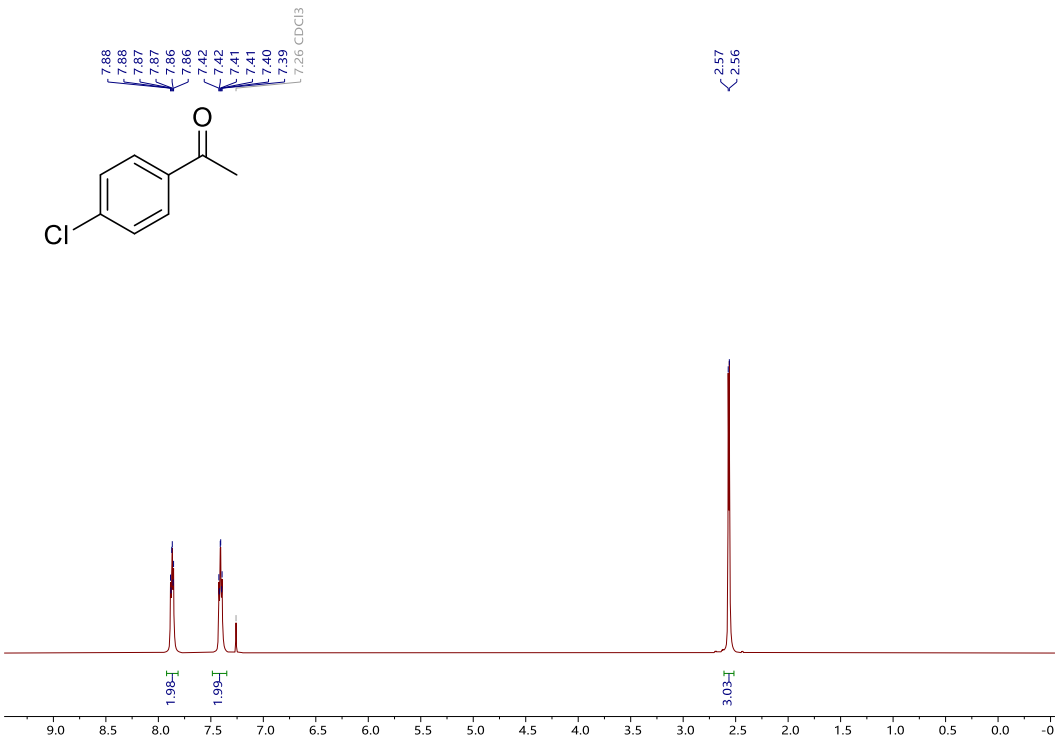
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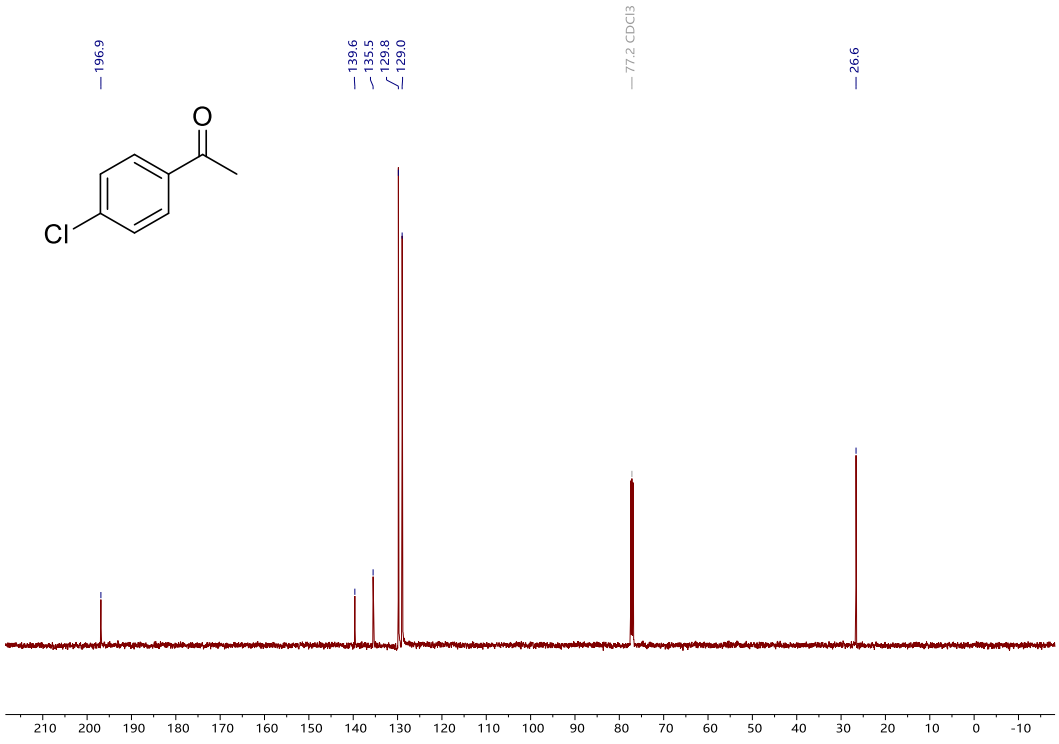
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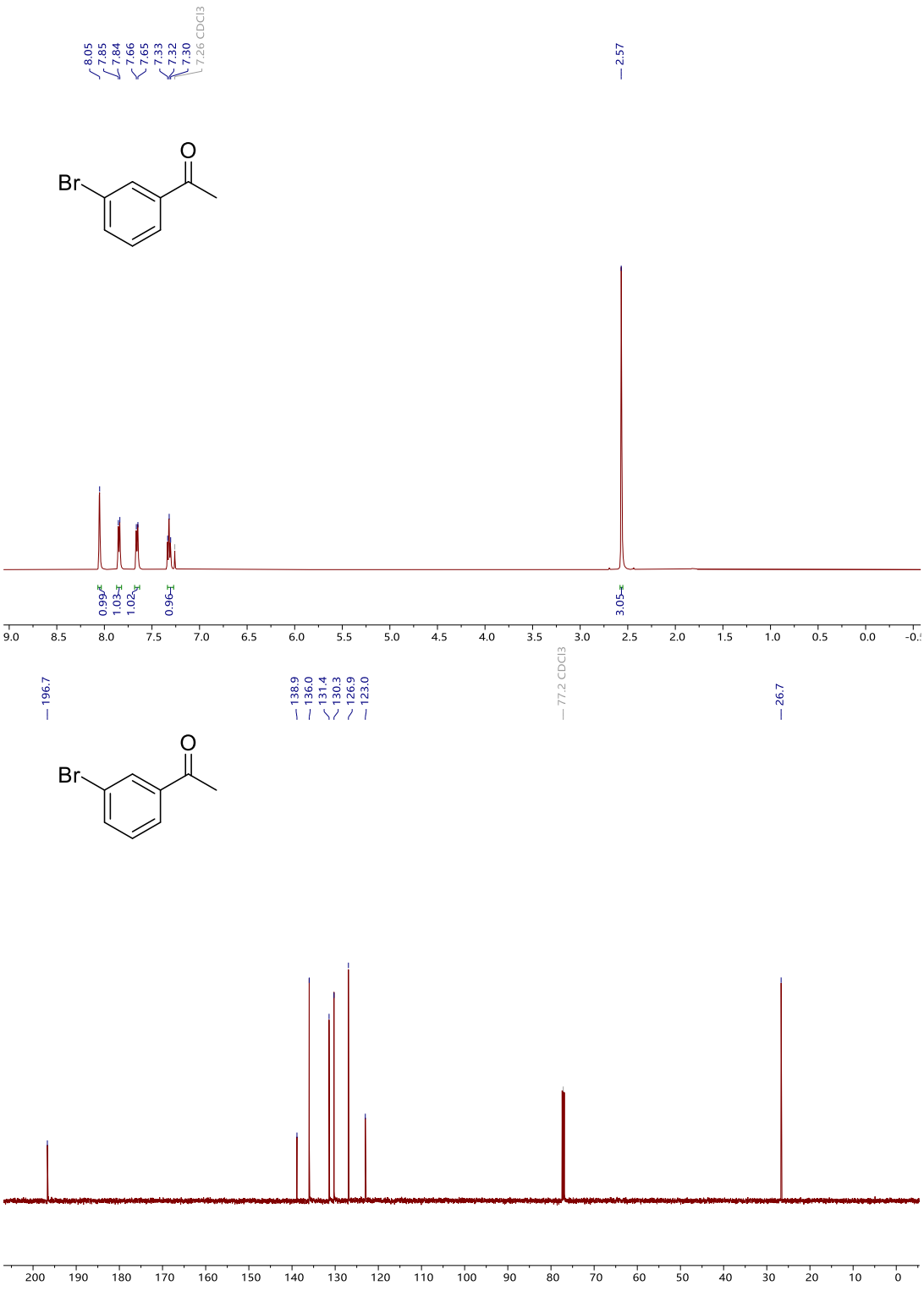
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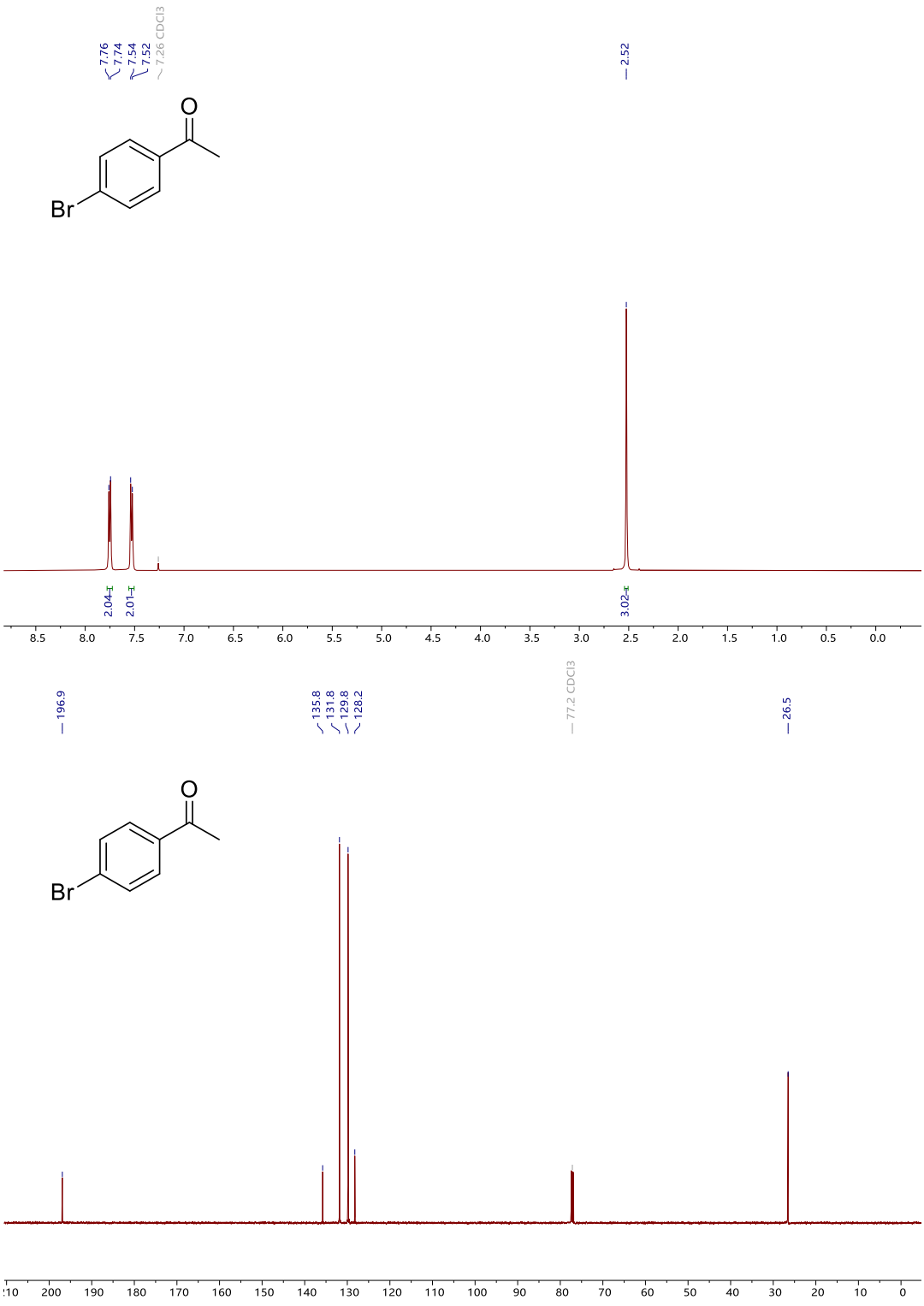
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135.8
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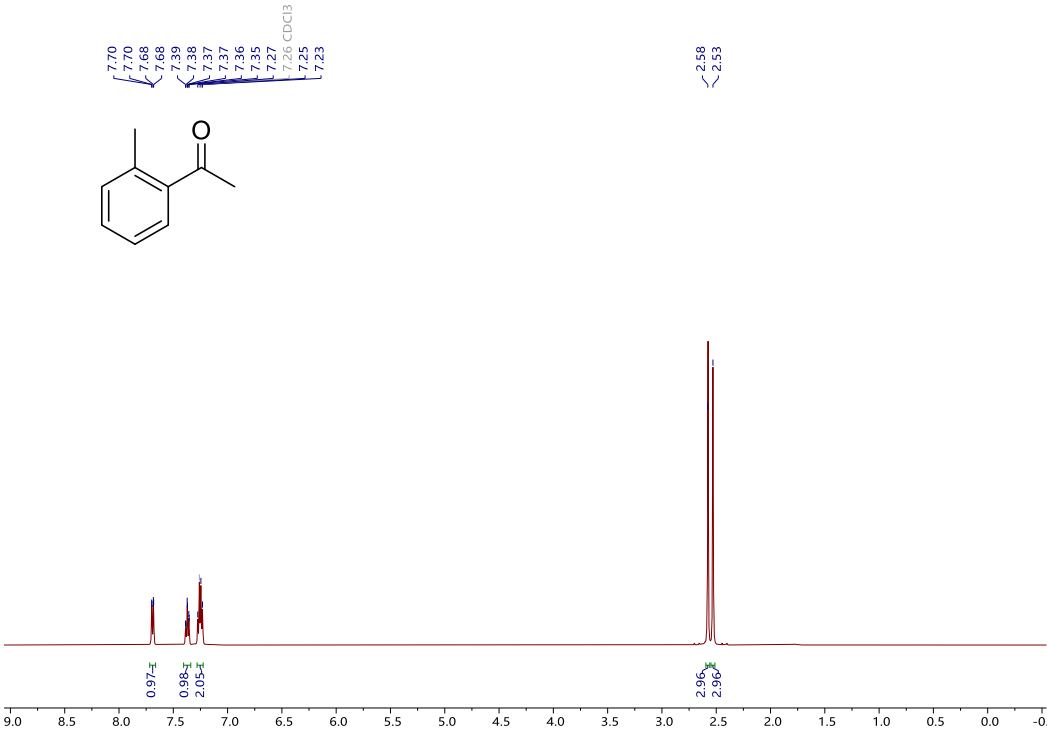
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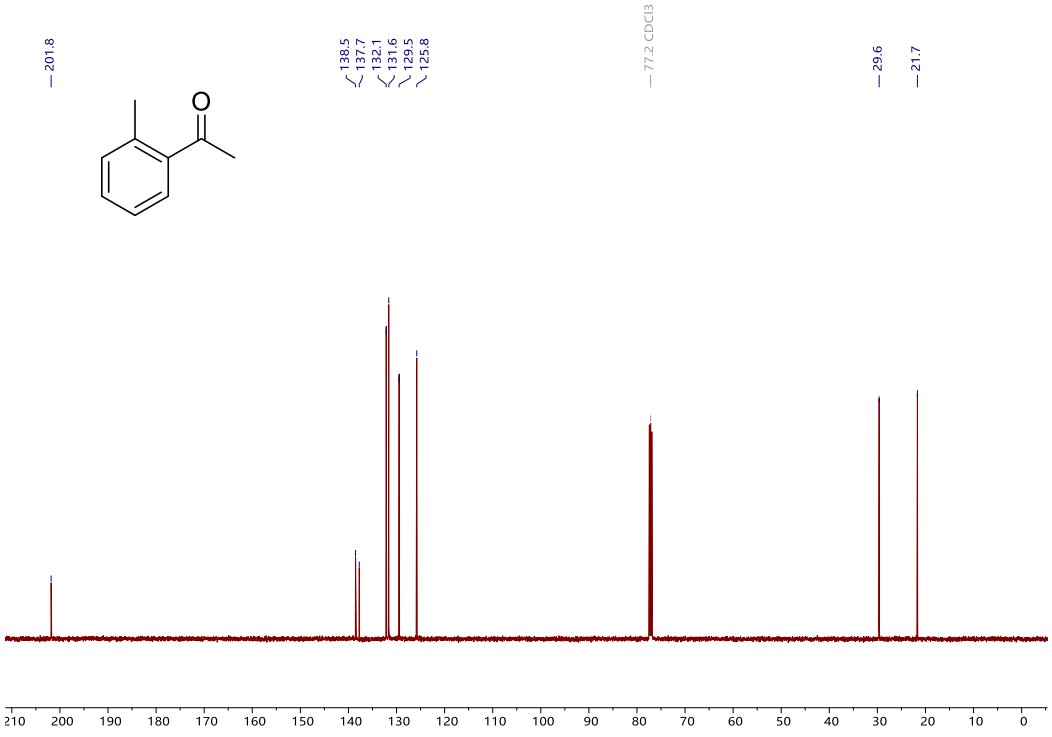
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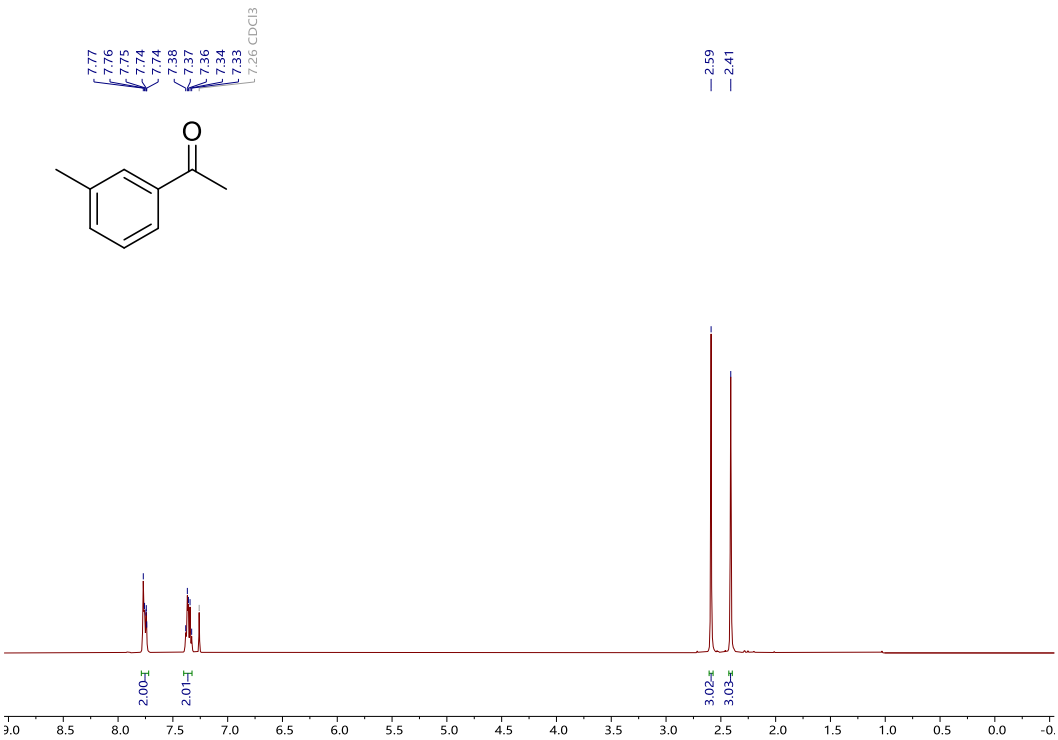
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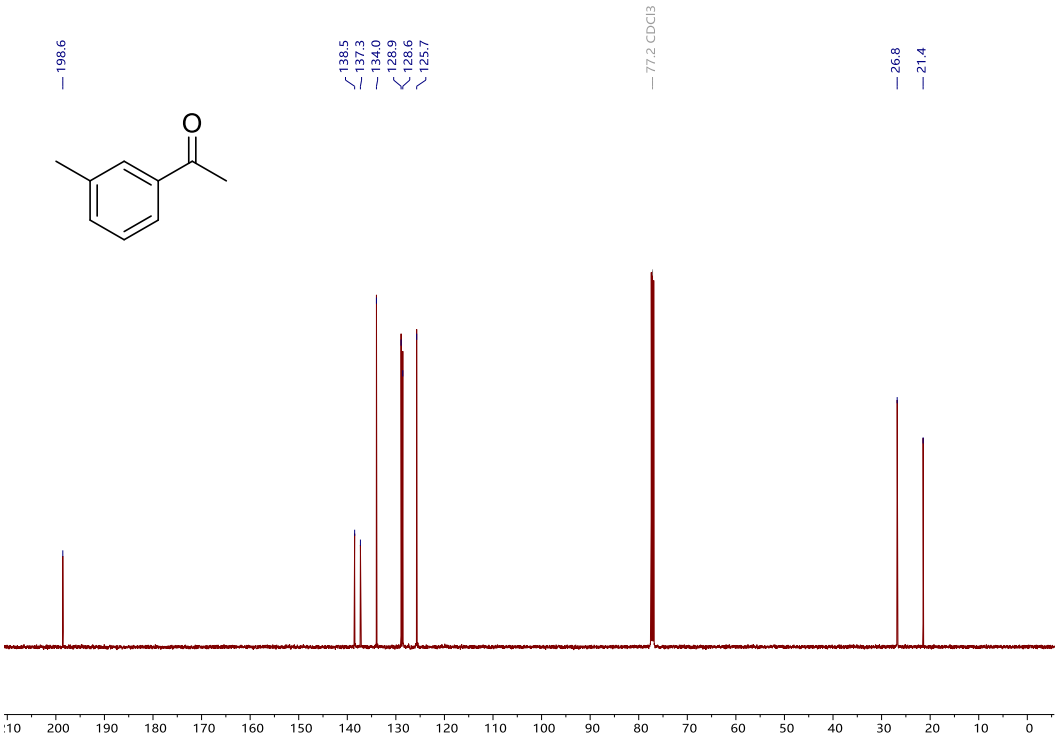
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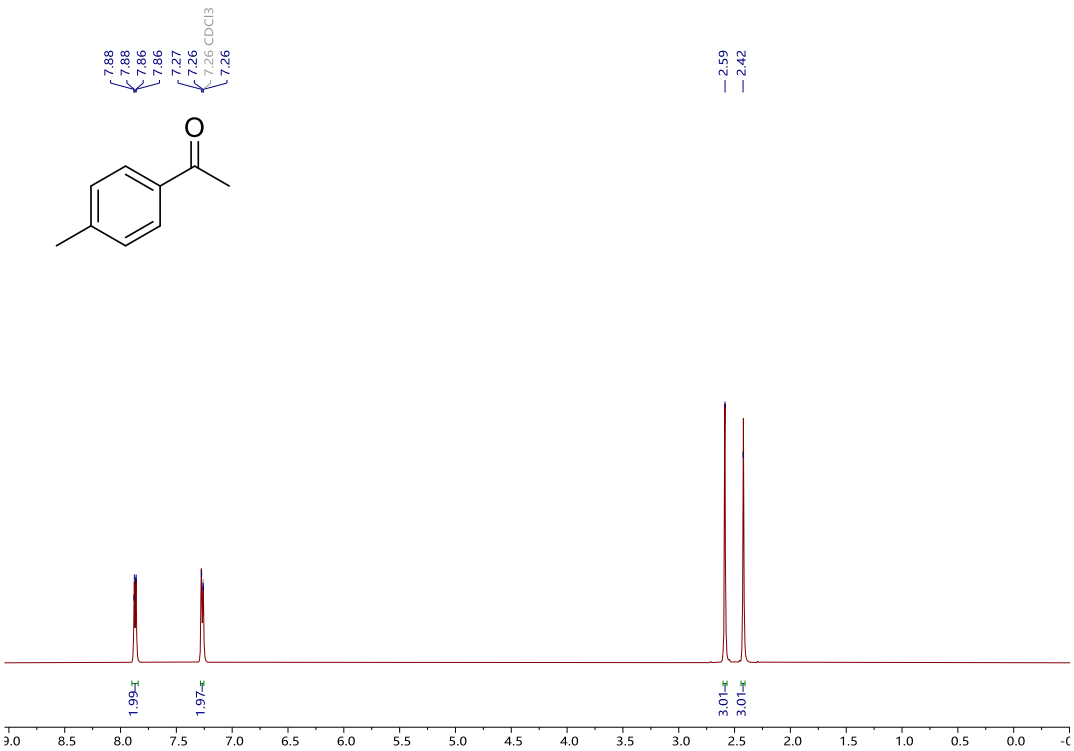
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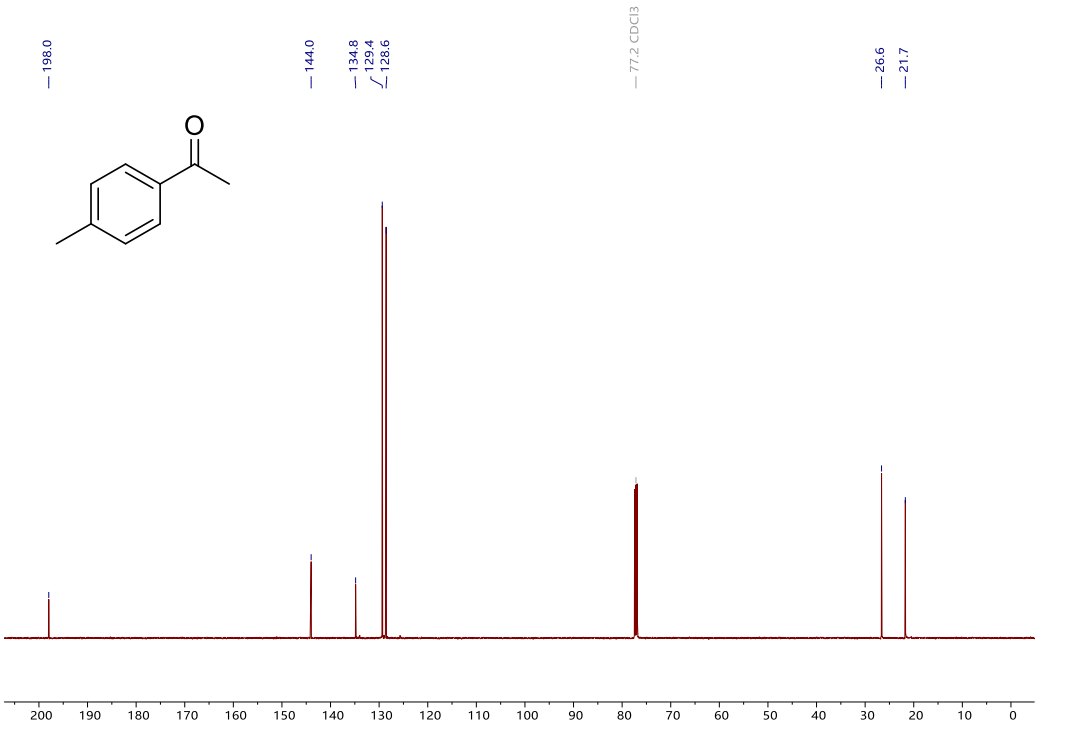
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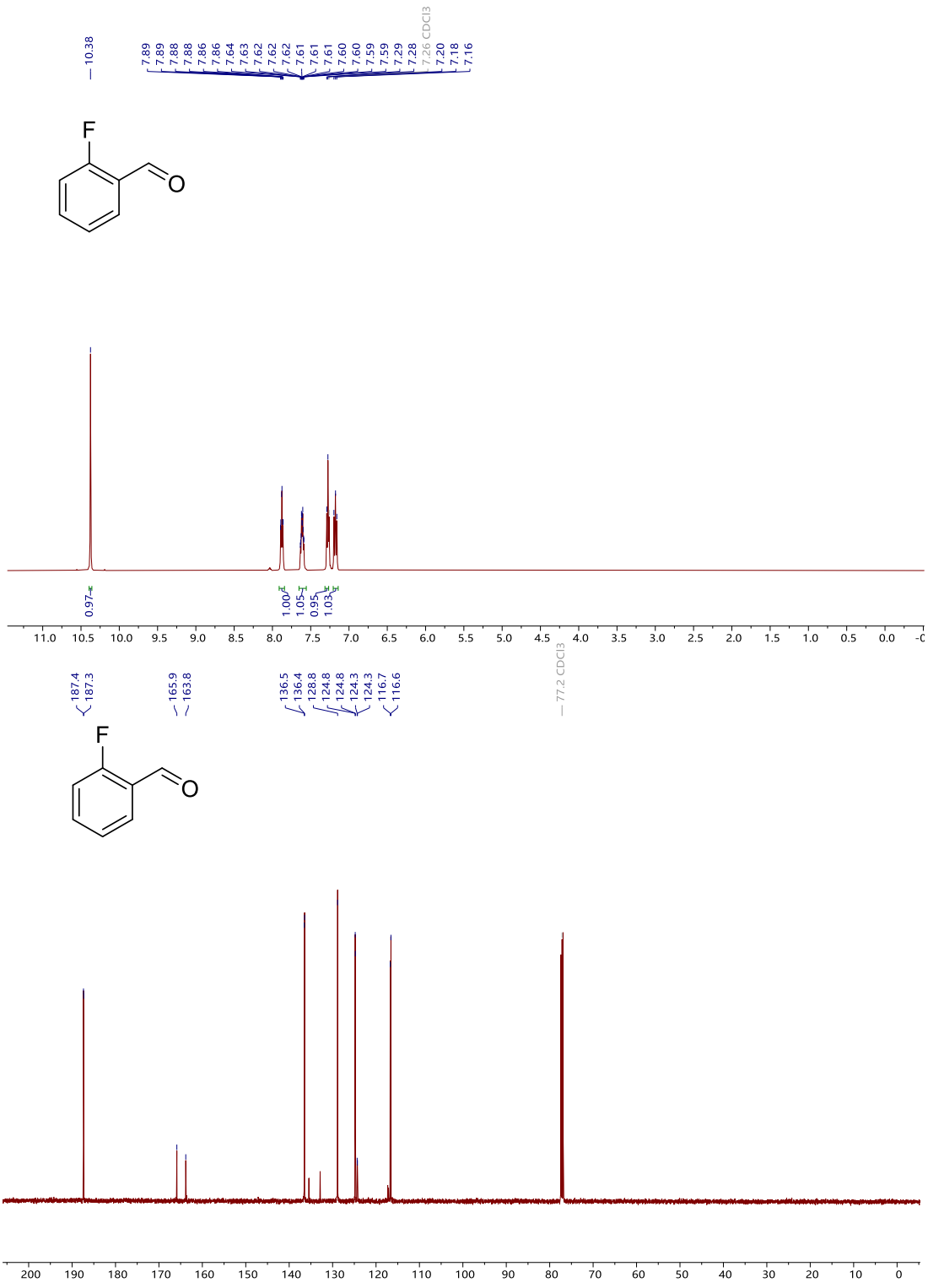
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351

352


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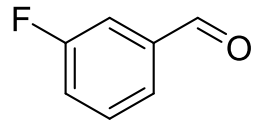
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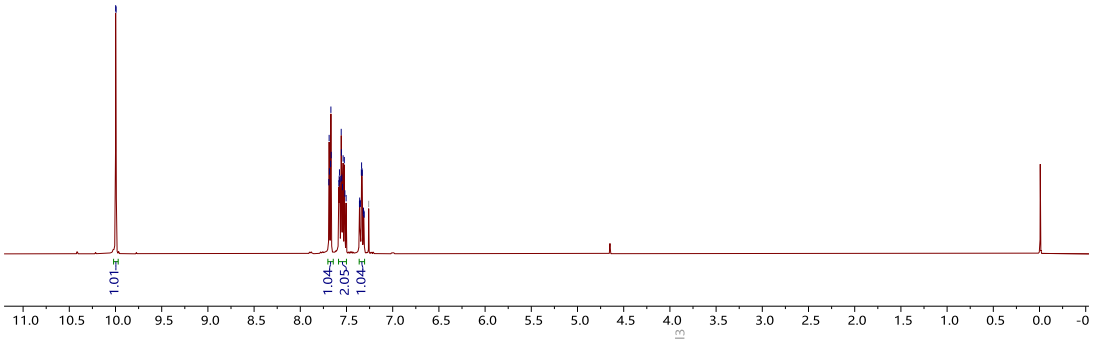
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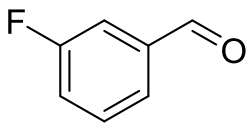
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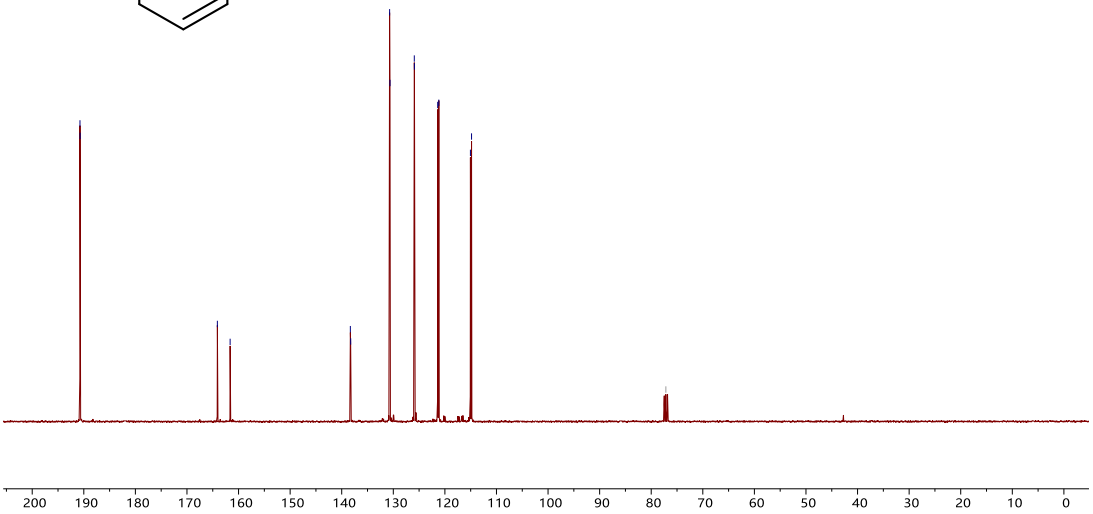
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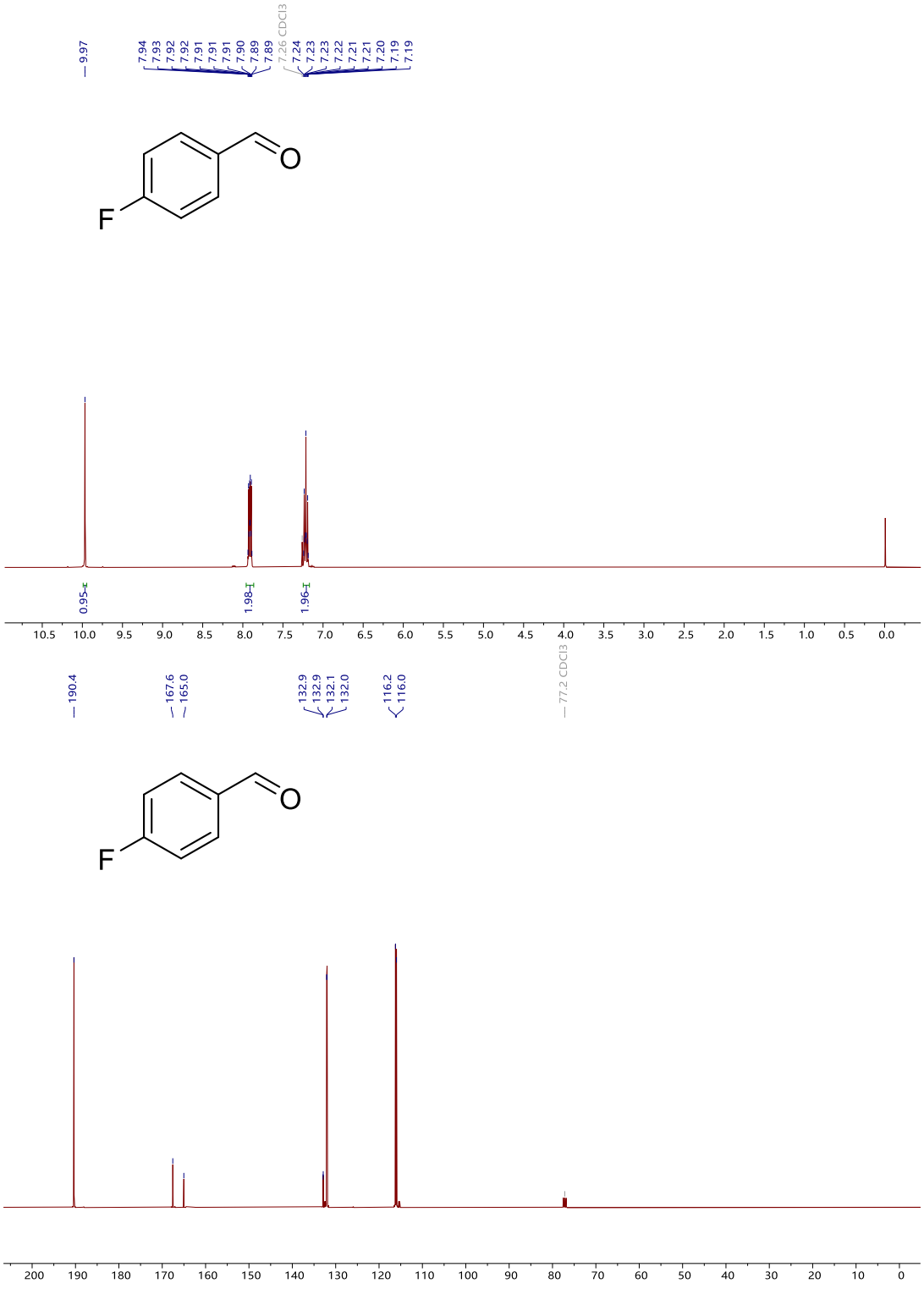
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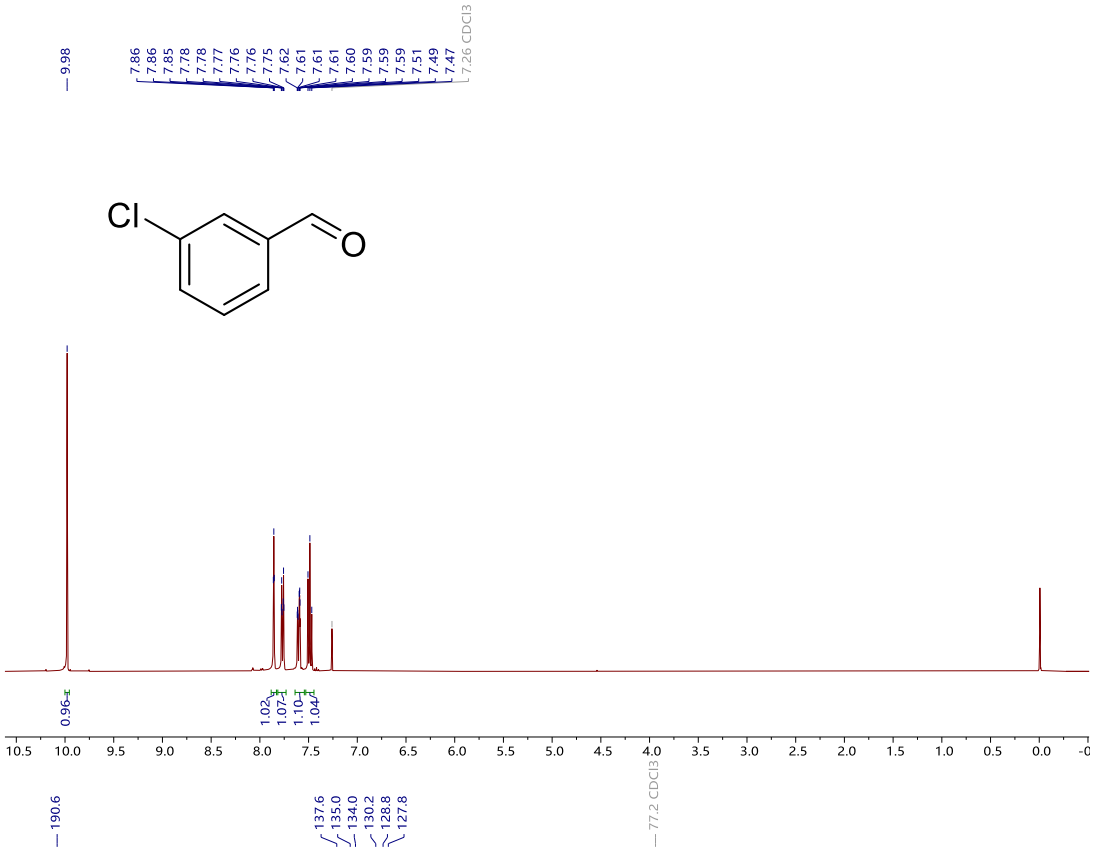
355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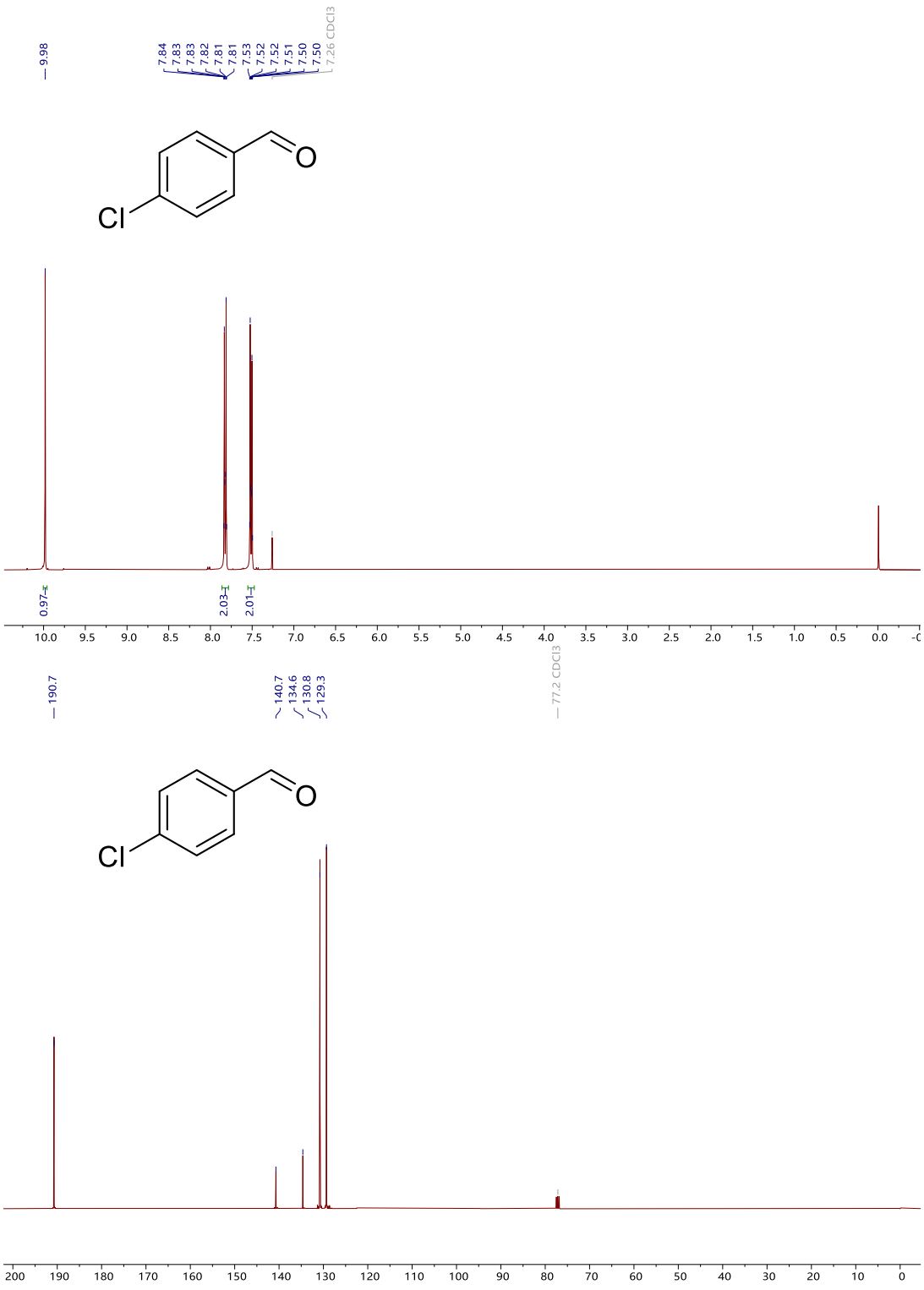
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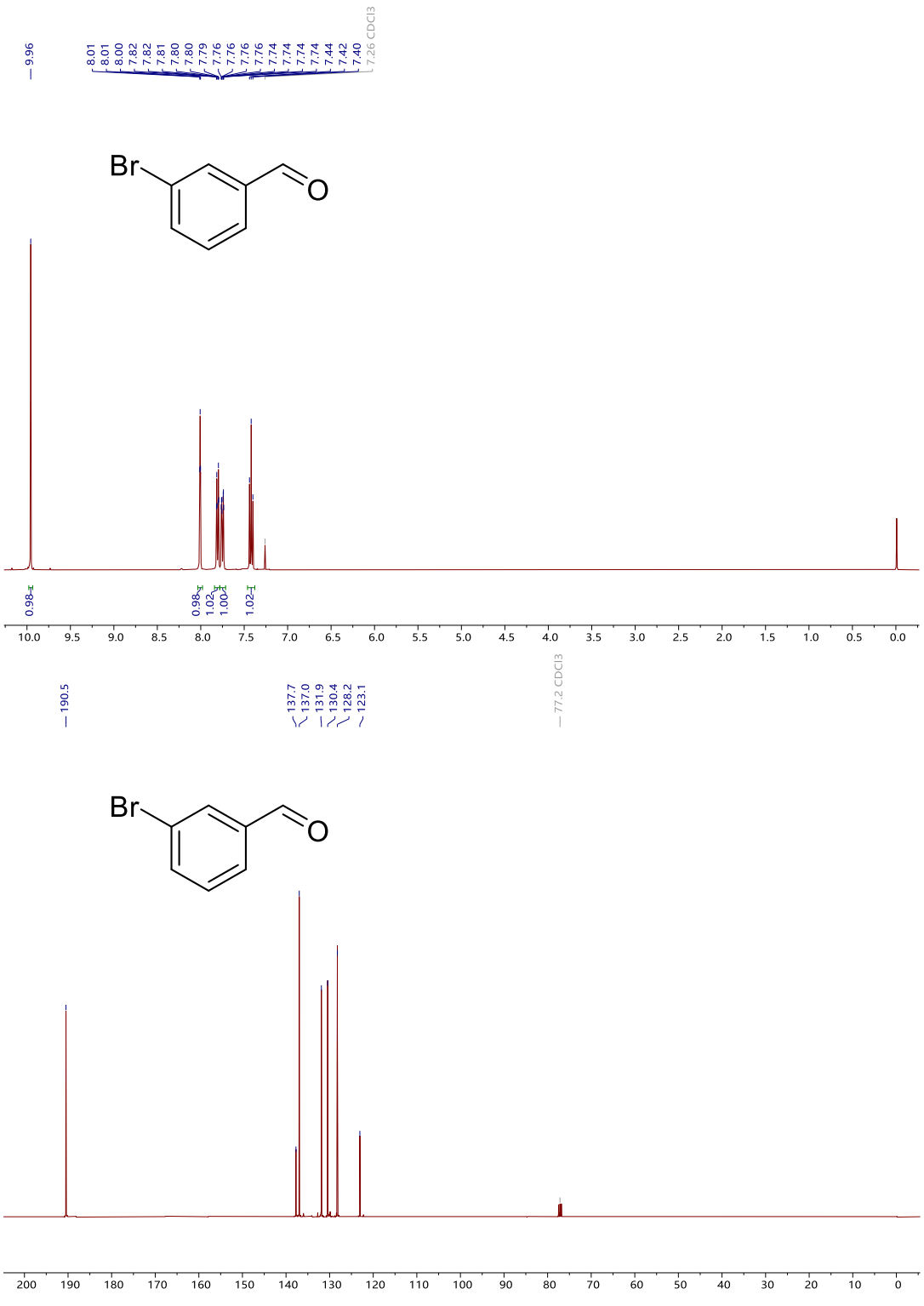
357

358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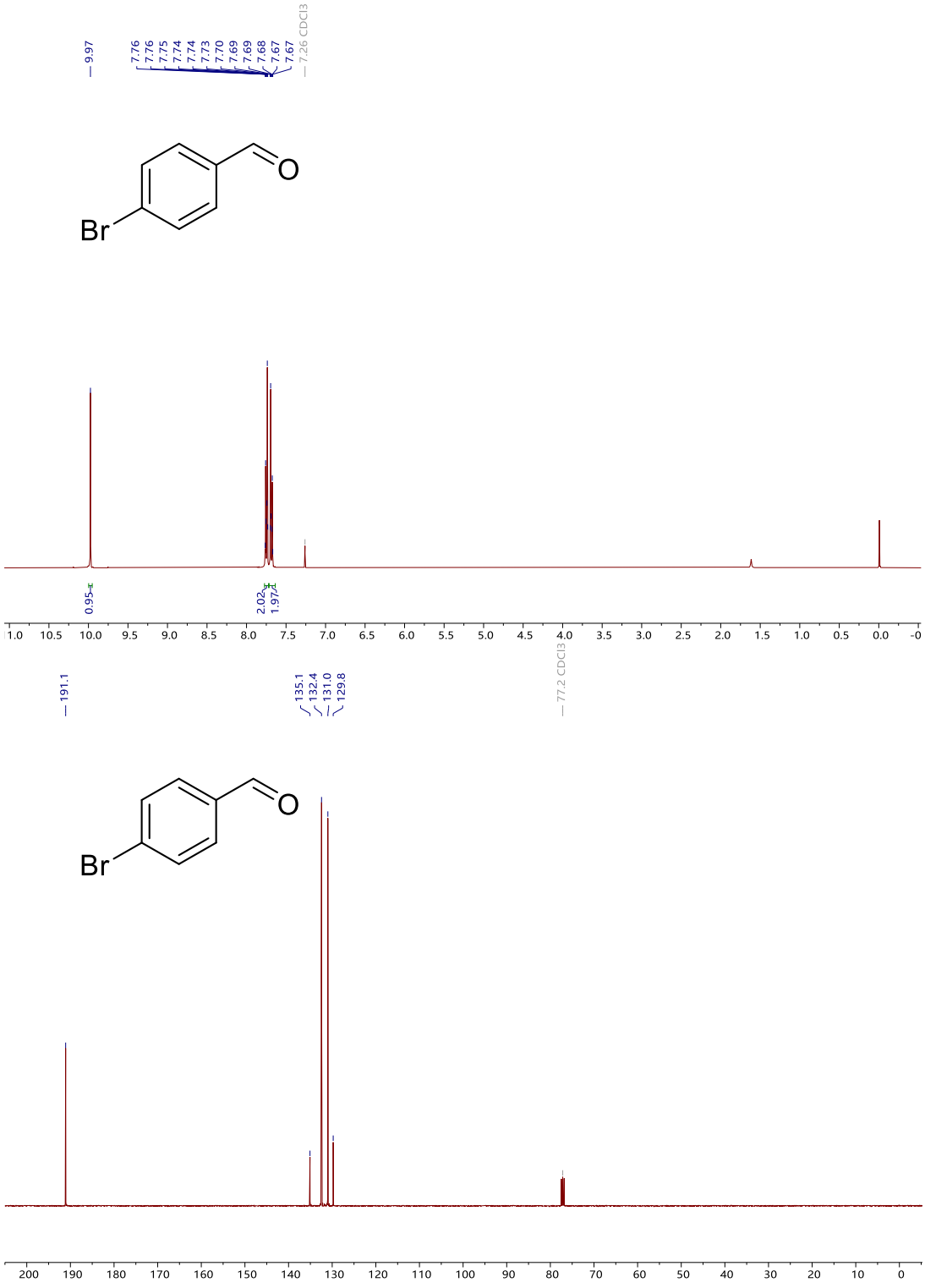
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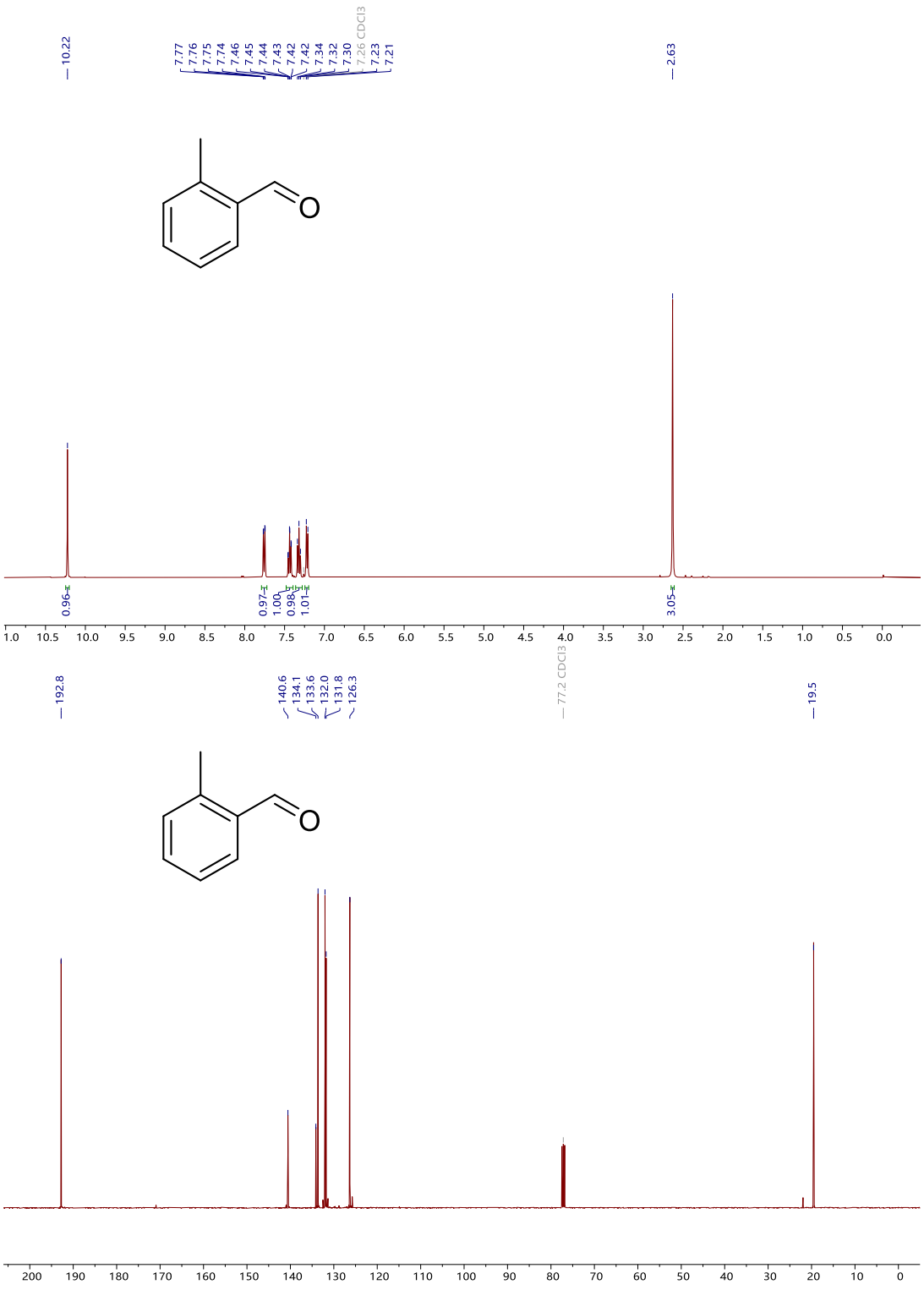
7.26 CDCl3
O
Br
0.95
2.02
1.97
1.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0
191.1
135.1
132.4
131.0
129.8
77.2 CDCl3
O
Br
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

360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7.42
7.42
7.34
7.32
7.30
7.26 CDCl3
7.23
7.21
2.63
O
0.96
0.97
1.00
0.98
1.01
3.05
1.0 10.5 10.0 9.5 9.0 8.5 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 0.5 0.0
192.8
140.6
134.1
133.6
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126.3
77.2 CDCl3
19.5
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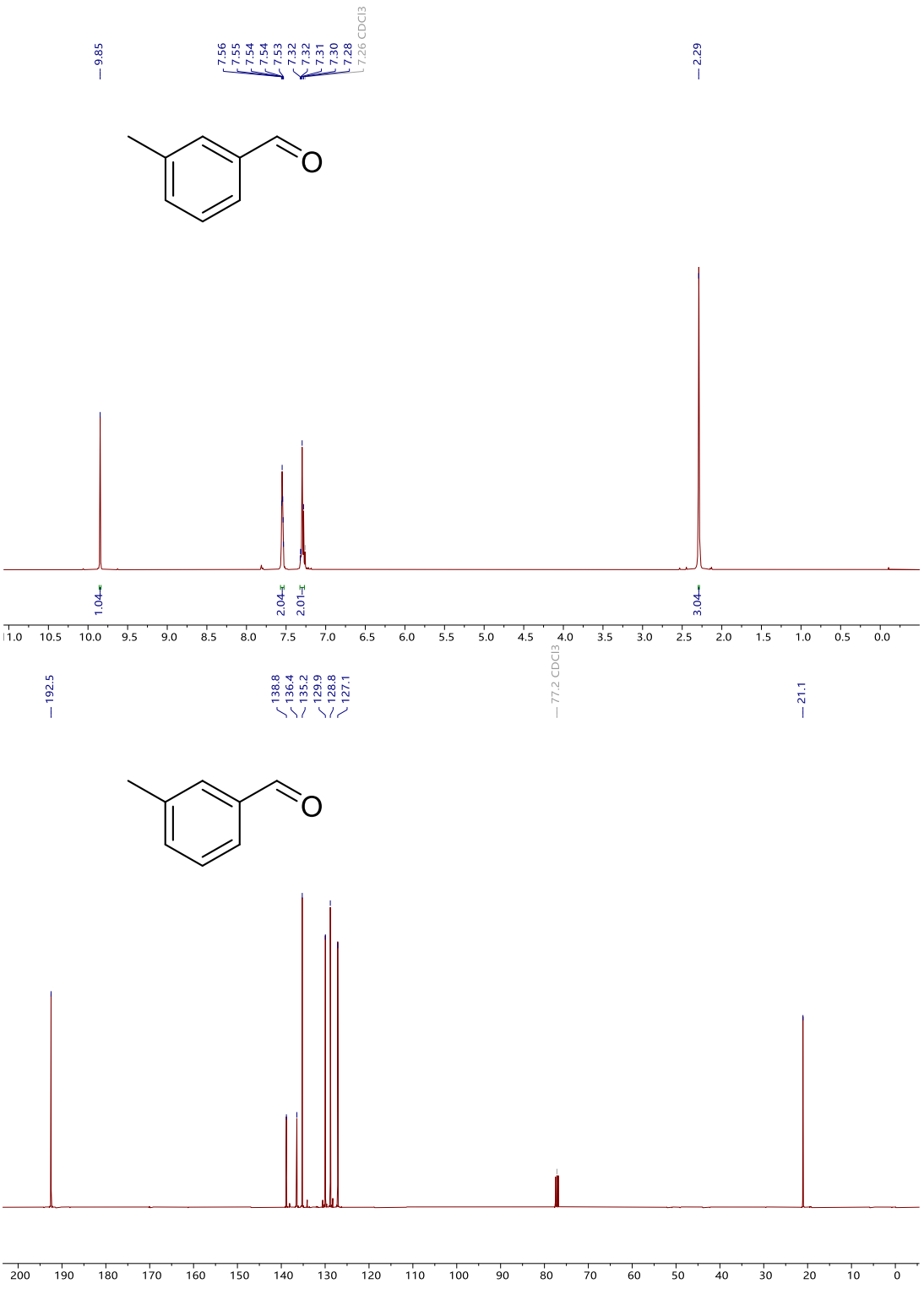
361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7.32
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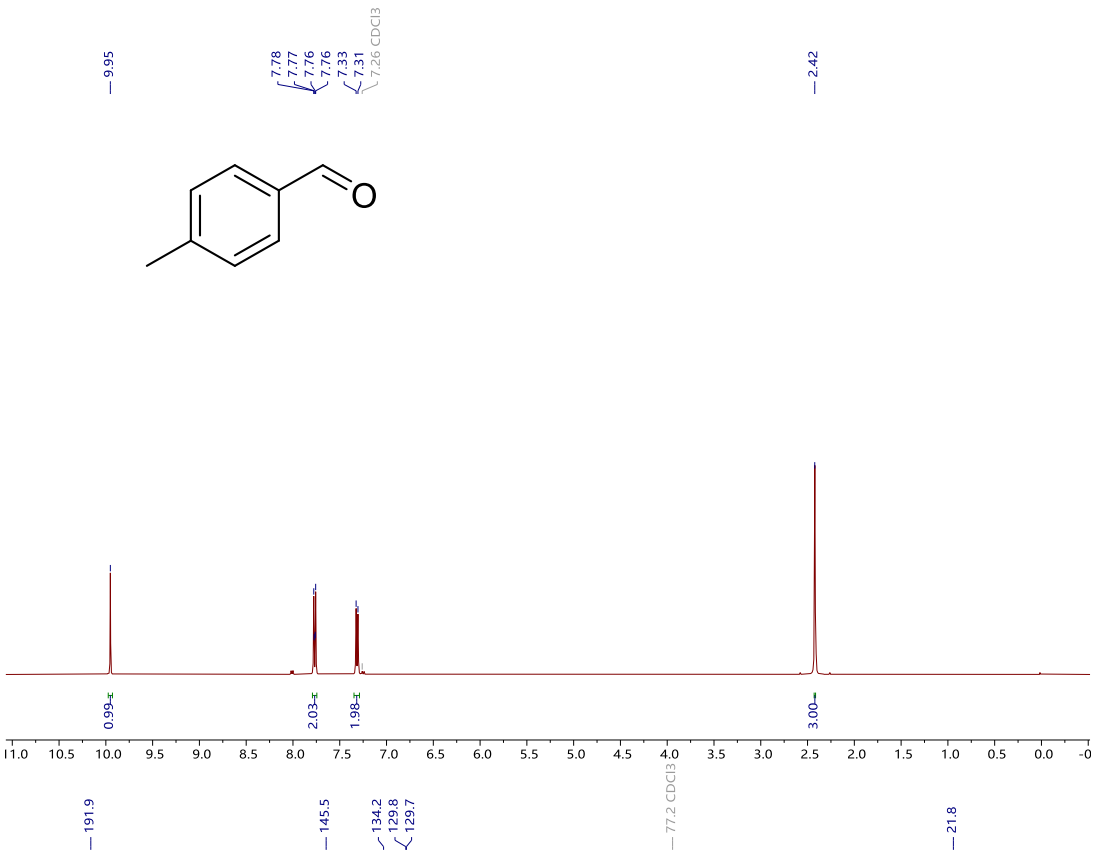
7.30
7.28
7.26 CDCl3
2.29
O
1.04
2.04
2.01
3.04
192.5
138.8
136.4
135.2
129.9
128.8
127.1
77.2 CDCl3
21.1
O

362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7.76
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7.31
7.26 CDCl3
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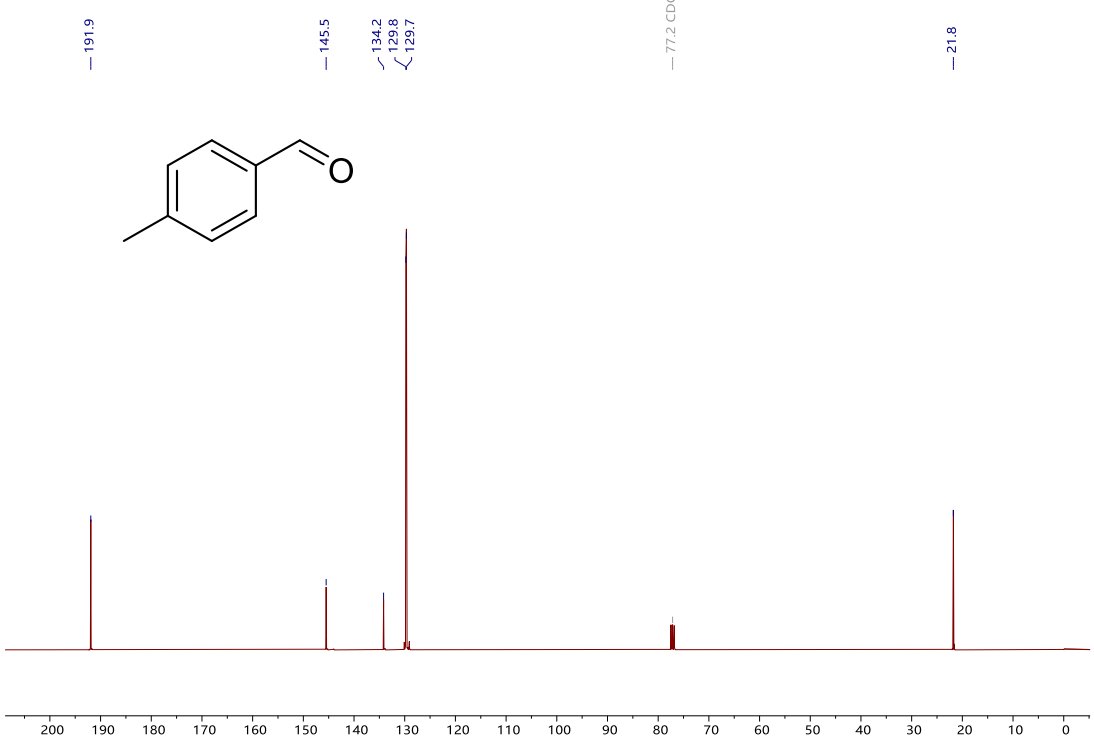
0.99
2.03
1.98
3.00
11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0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145.5
134.2
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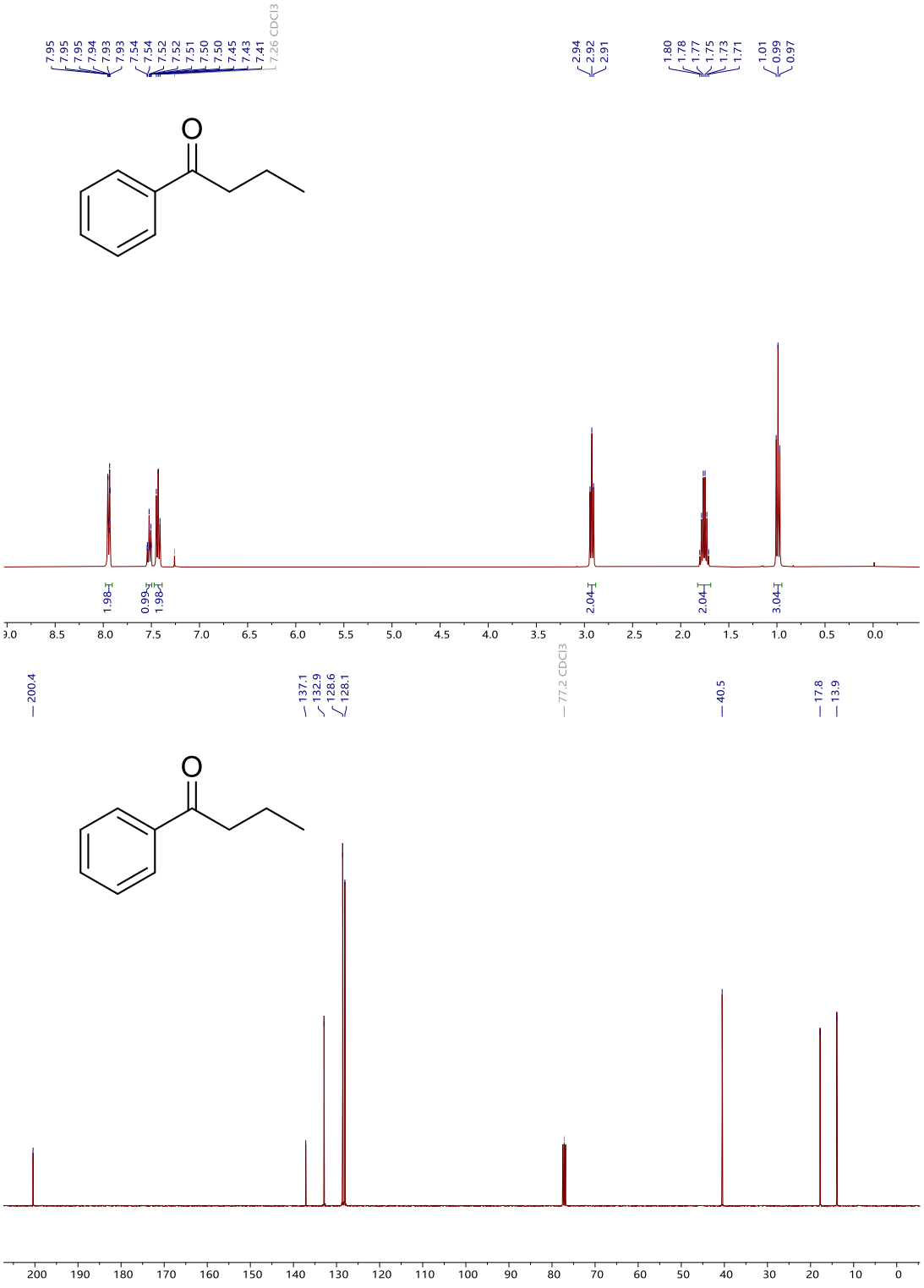
129.7
77.2 CDCl3
21.8
O
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

363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7.93
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7.54
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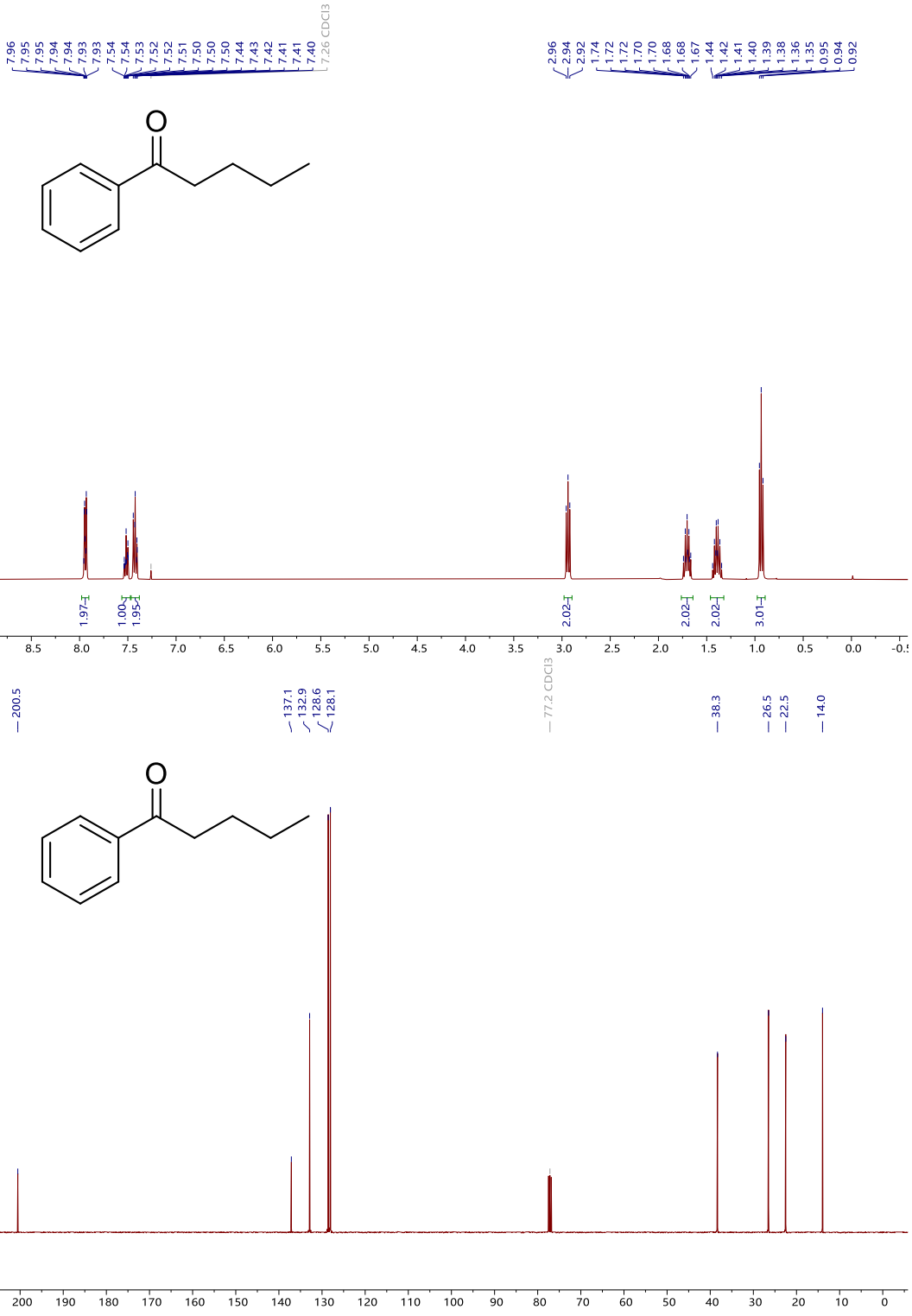
7.52
7.51
7.50
7.50
7.45
7.43
7.41
7.26 CDCl3
2.94
2.92
2.91
1.80
1.78
1.77
1.75
1.73
1.71
1.01
0.99
0.97
O
1.98
0.99
1.98
2.04
2.04
3.04
200.4
137.1
132.9
128.6
128.1
77.2 CDCl3
40.5
17.8
13.9
O

364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7.50
7.50
7.50
7.44
7.43
7.42
7.41
7.41
7.40
7.26 CDCl3
2.96
2.94
2.92
1.74
1.72
1.72
1.70
1.70
1.68
1.68
1.67
1.44
1.42
1.41
1.40
1.39
1.38
1.36
1.35
0.95
0.94
0.92
O
1.97
1.00
1.95
2.02
2.02
2.02
3.01
9.0 8.5 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 0.5 0.0
200.5
137.1
132.9
128.6
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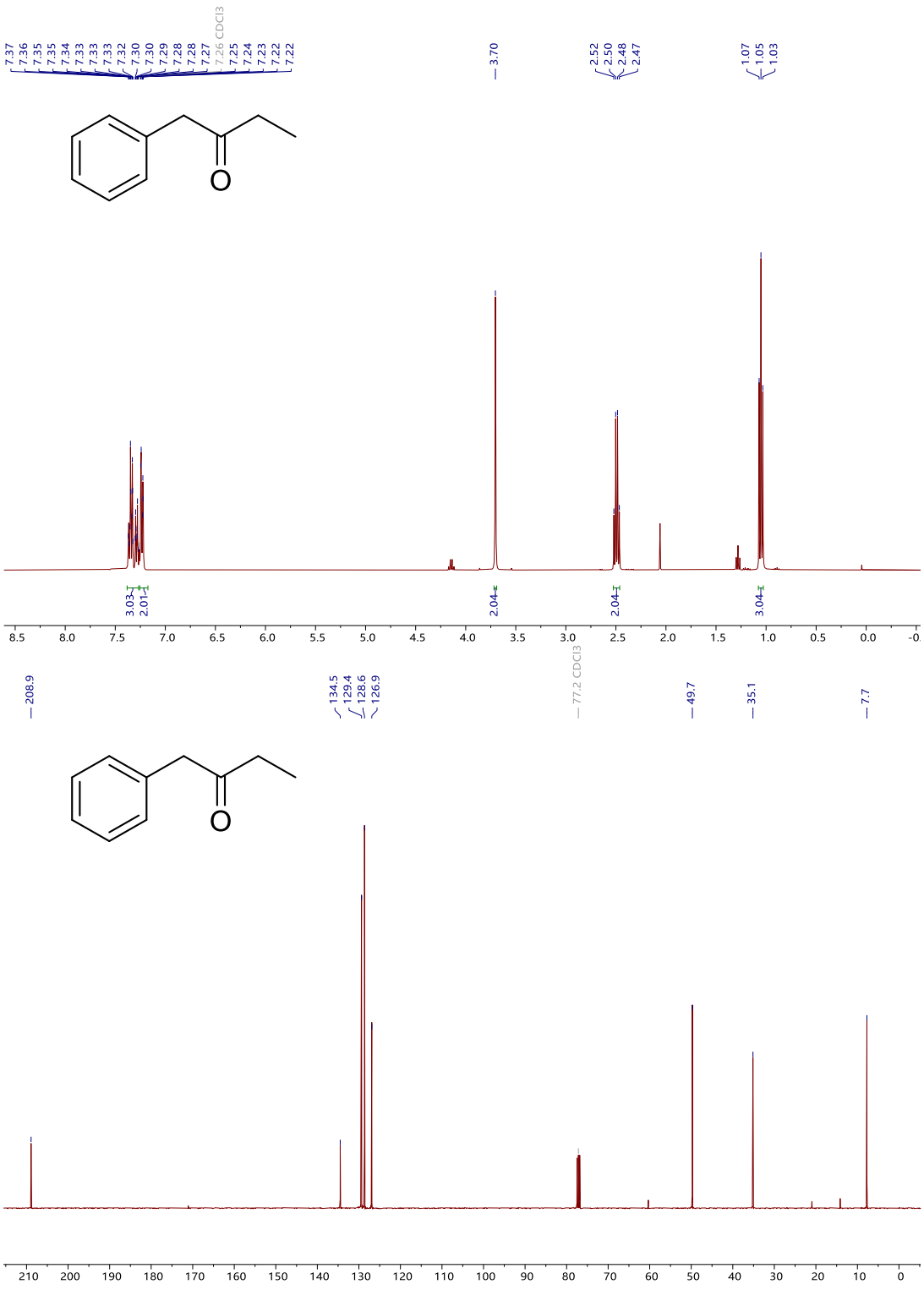
77.2 CDCl3
38.3
26.5
22.5
14.0
O
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

365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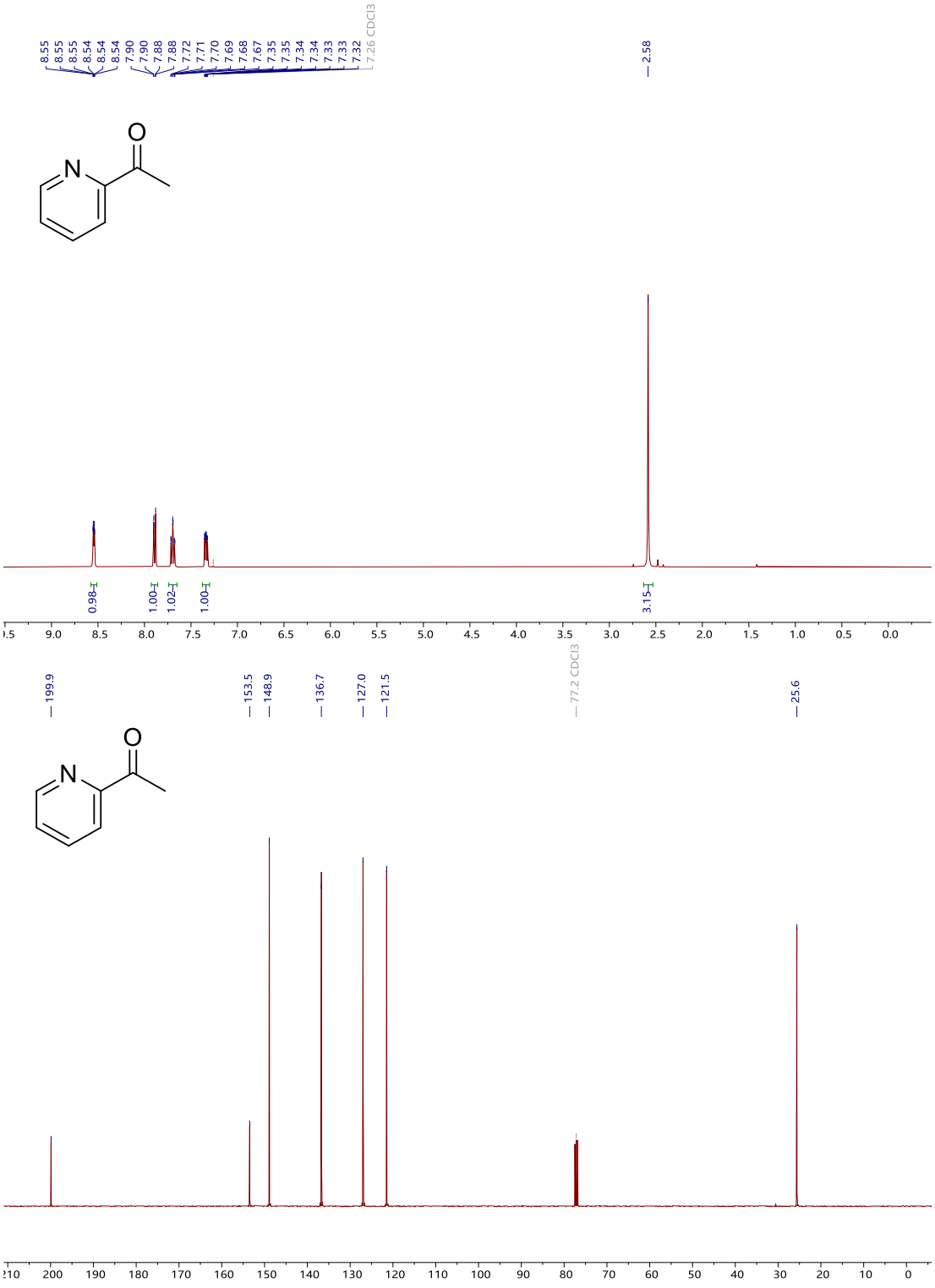
7.33
7.32
7.30
7.30
7.29
7.28
7.28
7.27
7.26 CDCl3
7.25
7.24
7.23
7.22
7.22
3.70
2.52
2.50
2.48
2.47
1.07
1.05
1.03
O
3.03
2.01
2.04
2.04
3.04
208.9
134.5
129.4
128.6
126.9
77.2 CDCl3
49.7
35.1
7.7
O

366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8.54
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8.54
7.90
7.90
7.88
7.88
7.72
7.71
7.70
7.69
7.68
7.67
7.35
7.35
7.34
7.34
7.33
7.33
7.32
7.26 CDCl3
2.58
O
N
0.98
1.00
1.02
1.00
3.15
9.0
8.5
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
0.5
0.0
199.9
153.5
148.9
136.7
127.0
121.5
77.2 CDCl3
25.6
O
N
210
200
190
180
170
160
150
140
130
120
110
100
90
80
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60
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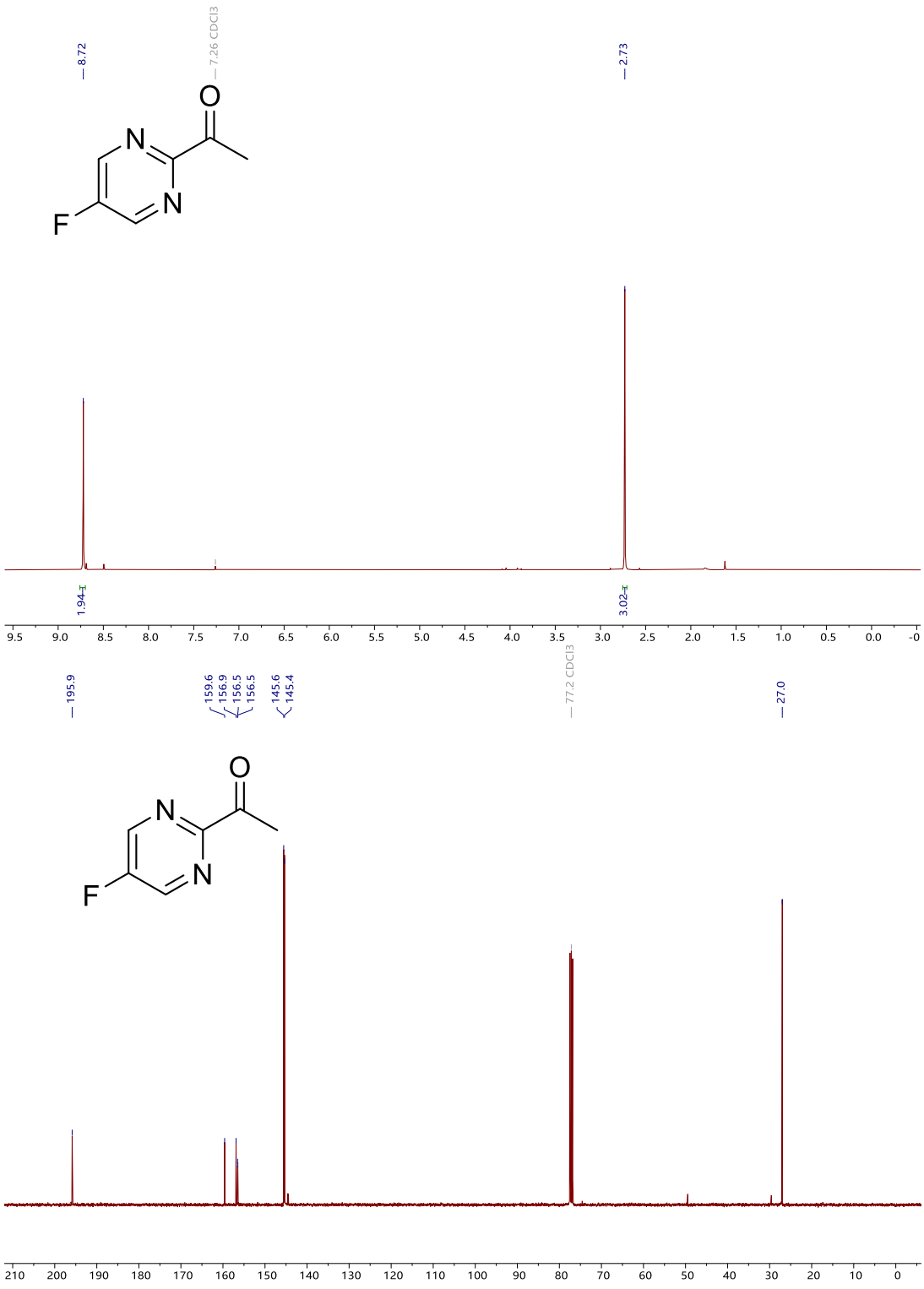
40
30
20
10
0

367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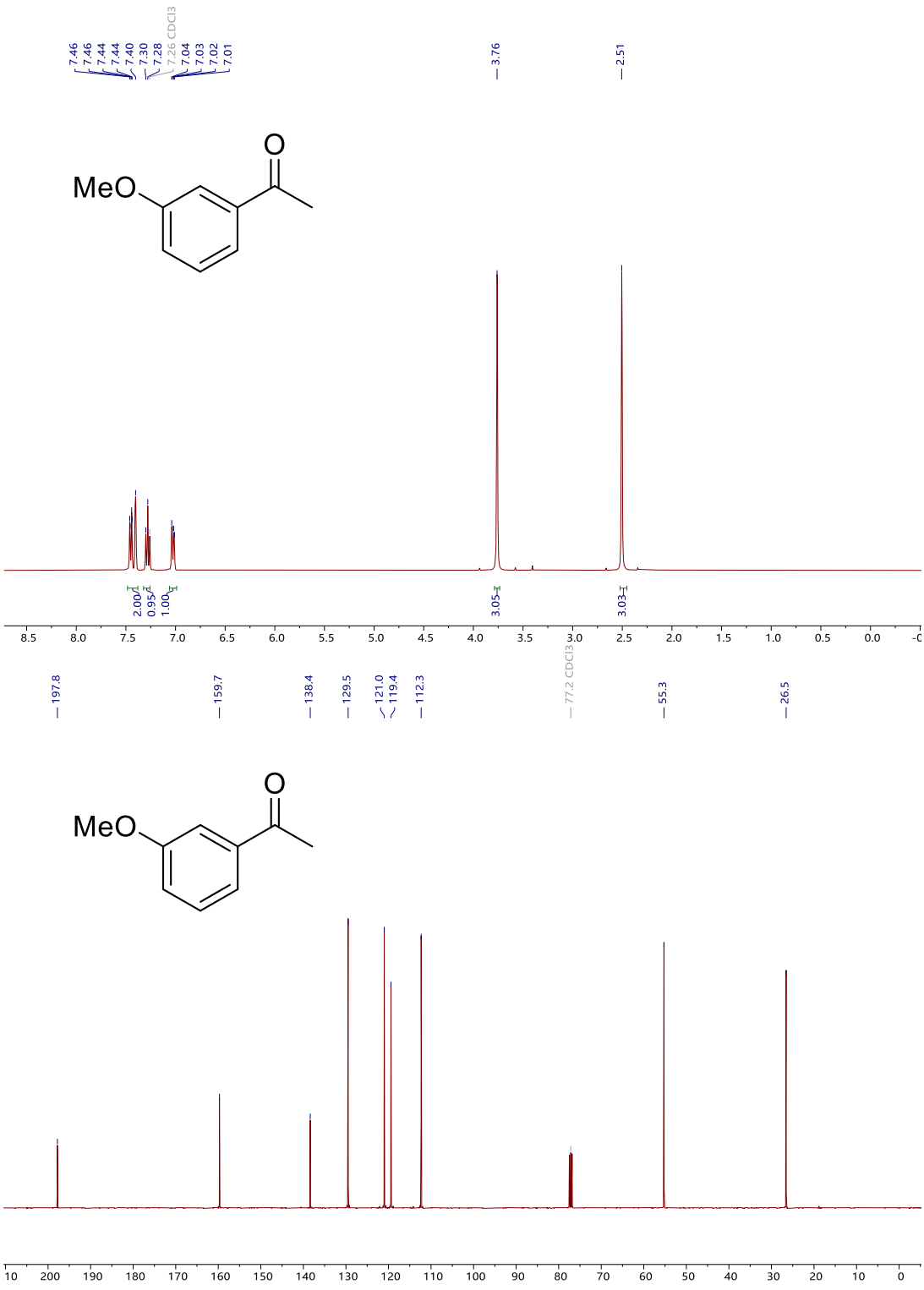
2.73
1.94
3.02
195.9
159.6
156.9
156.5
156.5
145.6
145.4
77.2 CDCl3
27.0
O
N
N
F

368


7.46
7.46
7.44
7.44
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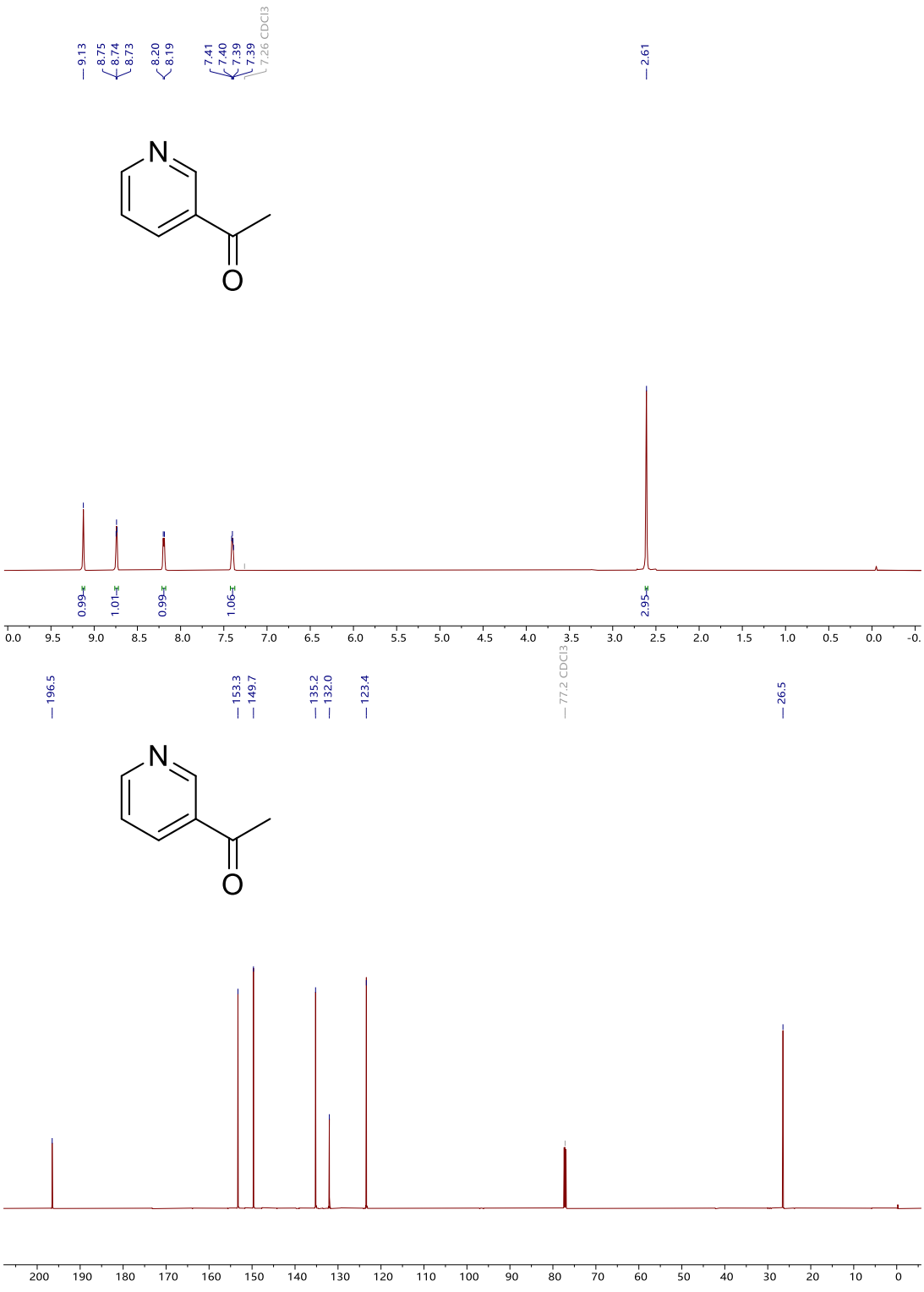
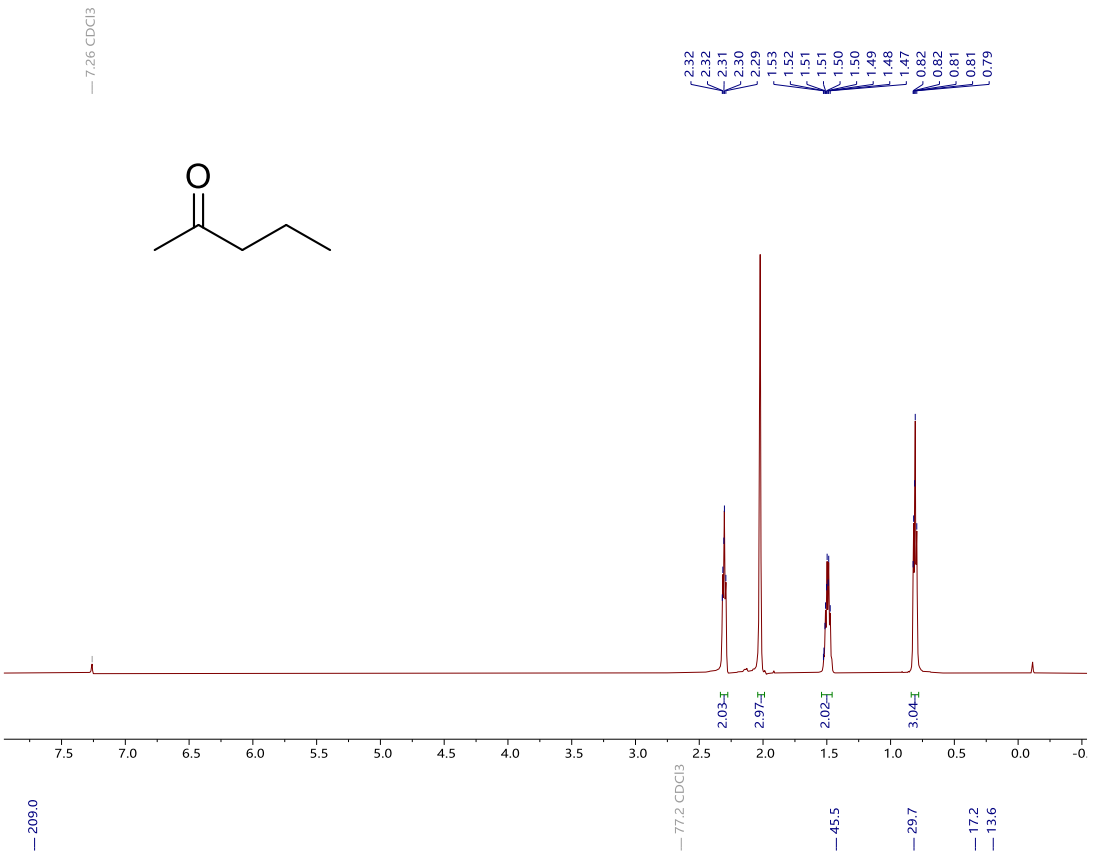
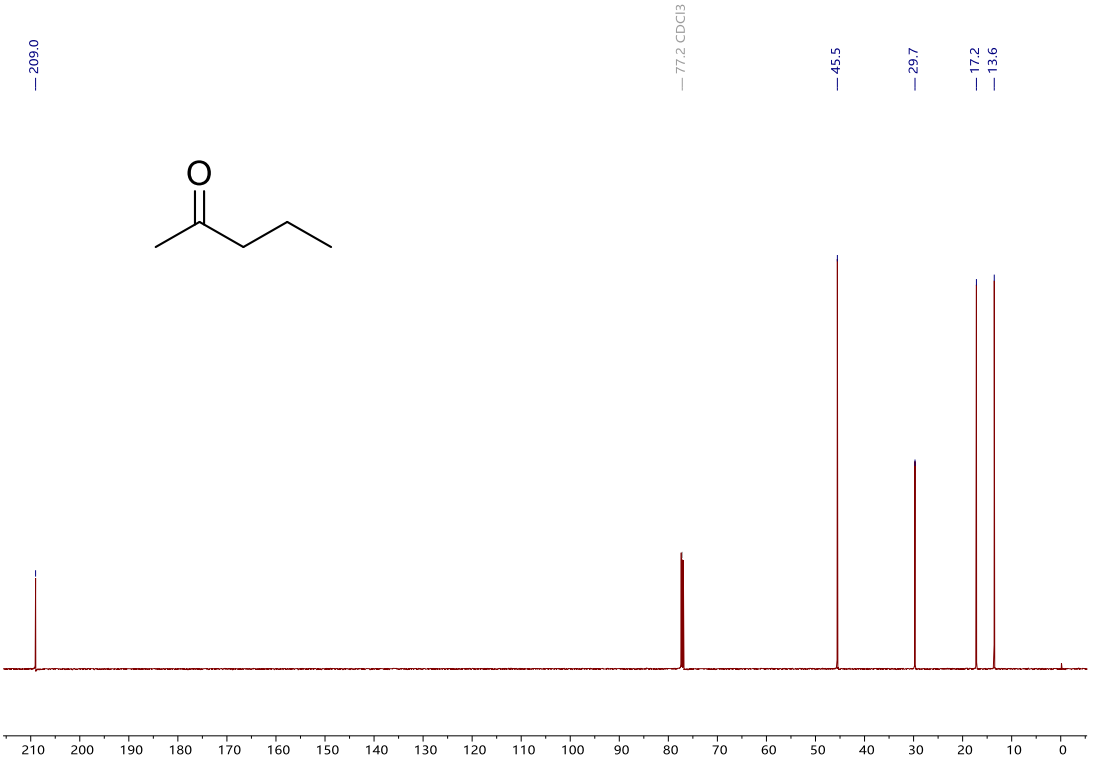
7.30
7.28
7.26 CDCl3
7.04
7.03
7.02
7.01
3.76
2.51
O
MeO
2.00
0.95
1.00
3.05
3.03
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
197.8
159.7
138.4
129.5
121.0
119.4
112.3
77.2 CDCl3
55.3
26.5
O
MeO
10
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0

369

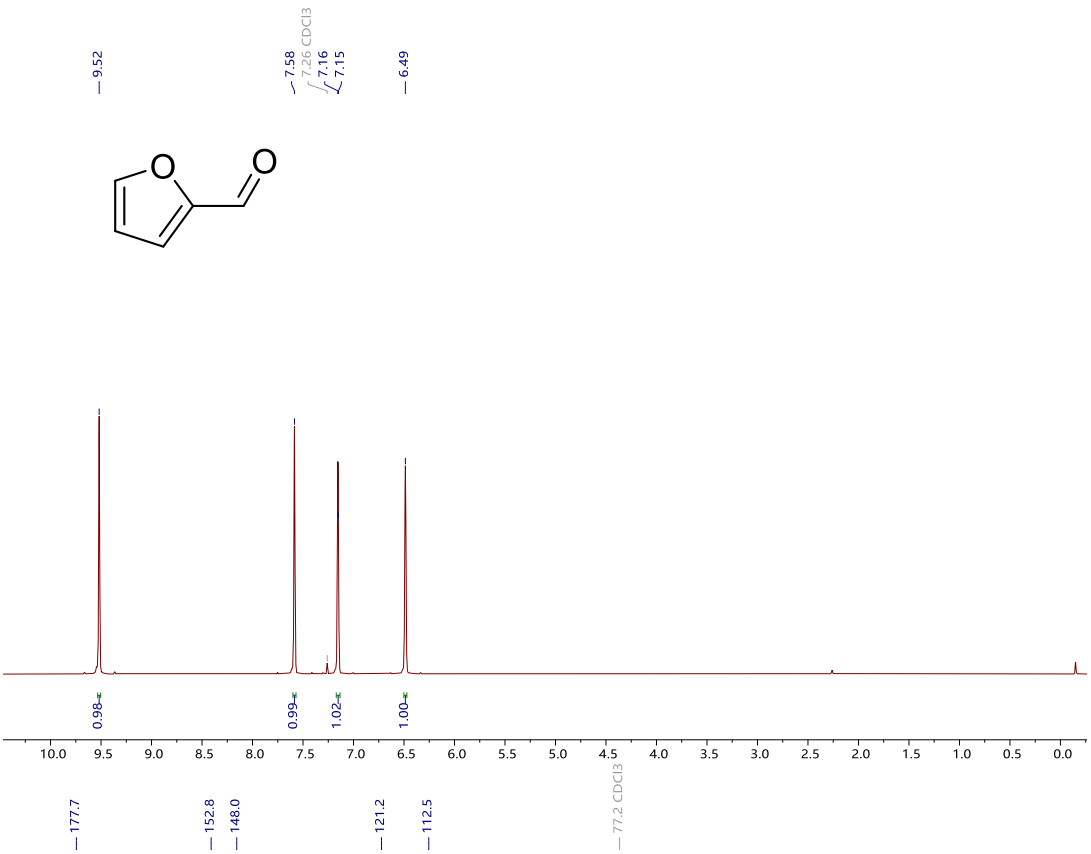

9.13
8.75
8.74
8.73
8.20
8.19
7.41
7.40
7.39
7.39
7.26 CDCl3
2.61
N
O
0.99
1.01
0.99
1.06
2.95
0.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.
196.5
153.3
149.7
135.2
132.0
123.4
77.2 CDCl3
26.5
N
O
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

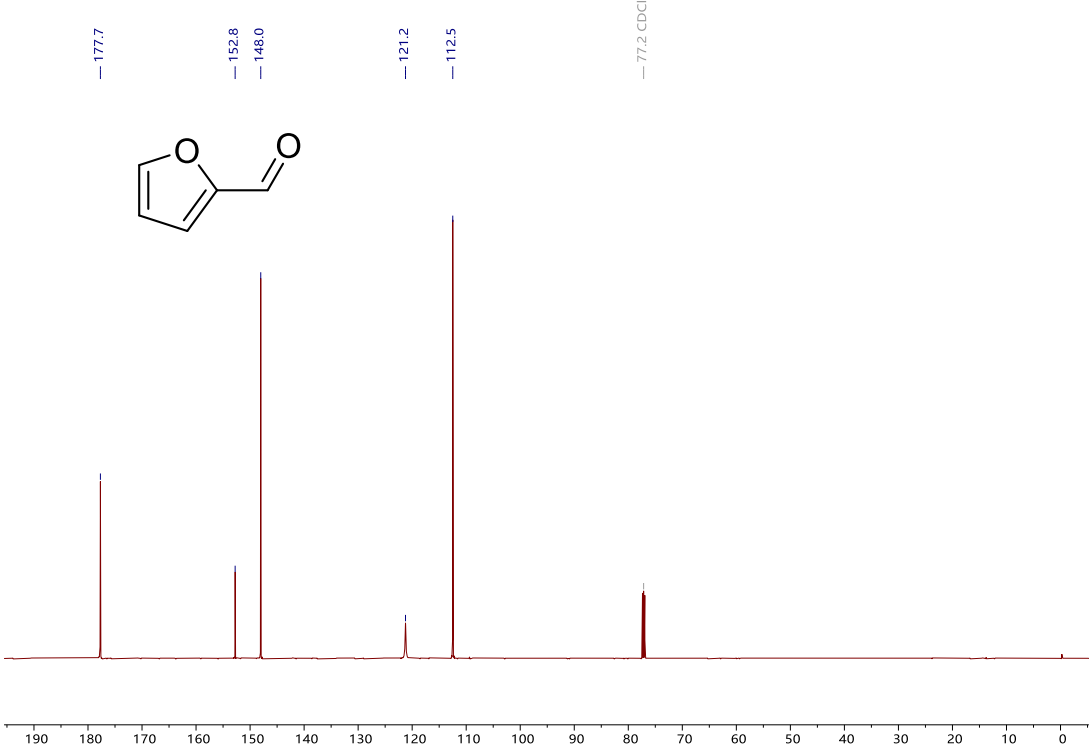
370


7.26 CDCl3
2.32
2.32
2.31
2.30
2.29
1.53
1.52
1.51
1.51
1.50
1.50
1.49
1.48
1.47
0.82
0.82
0.81
0.81
0.79
2.03
2.97
2.02
3.04
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

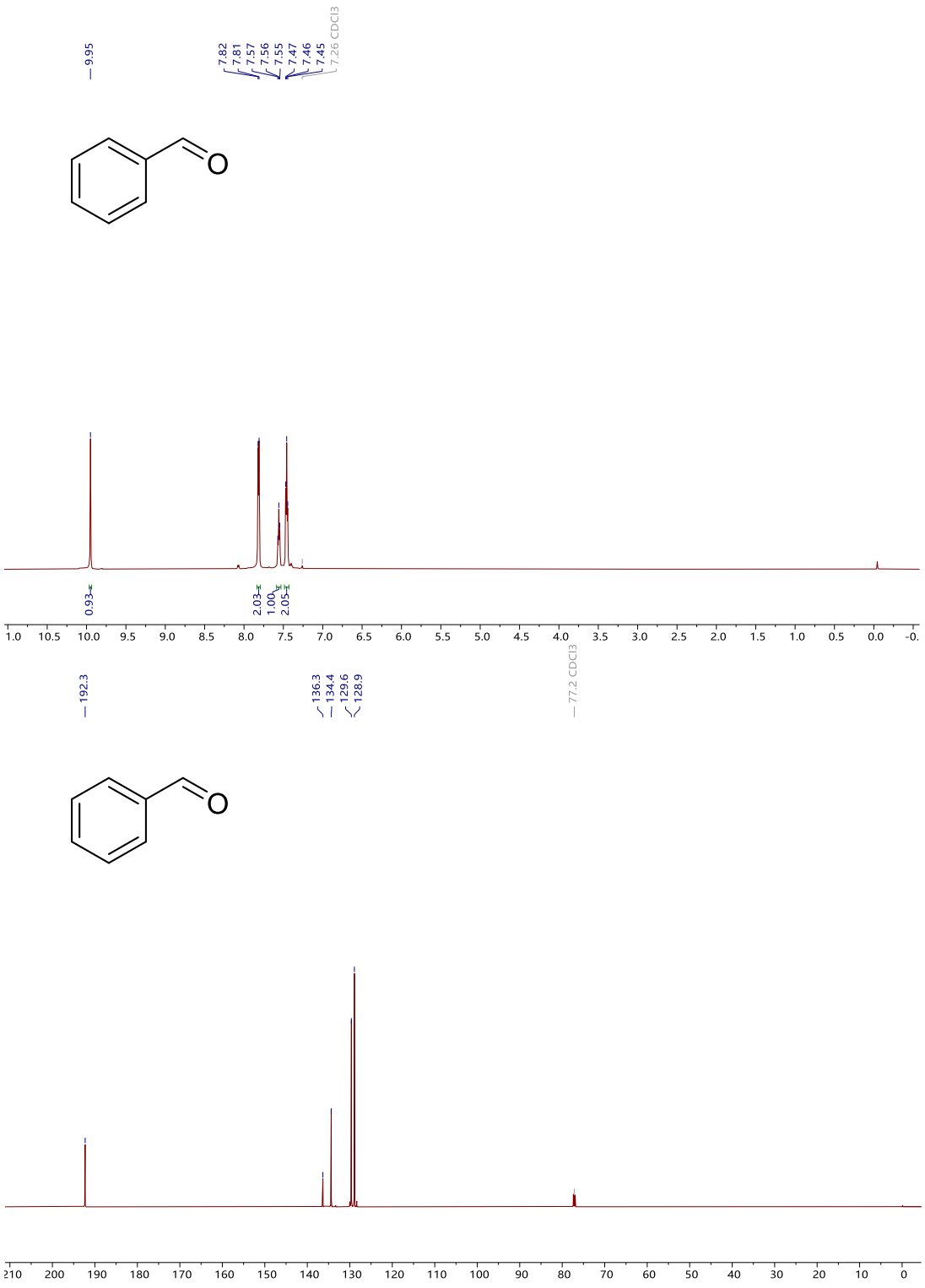

209.0
77.2 CDCl3
45.5
29.7
17.2
13.6
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0

371

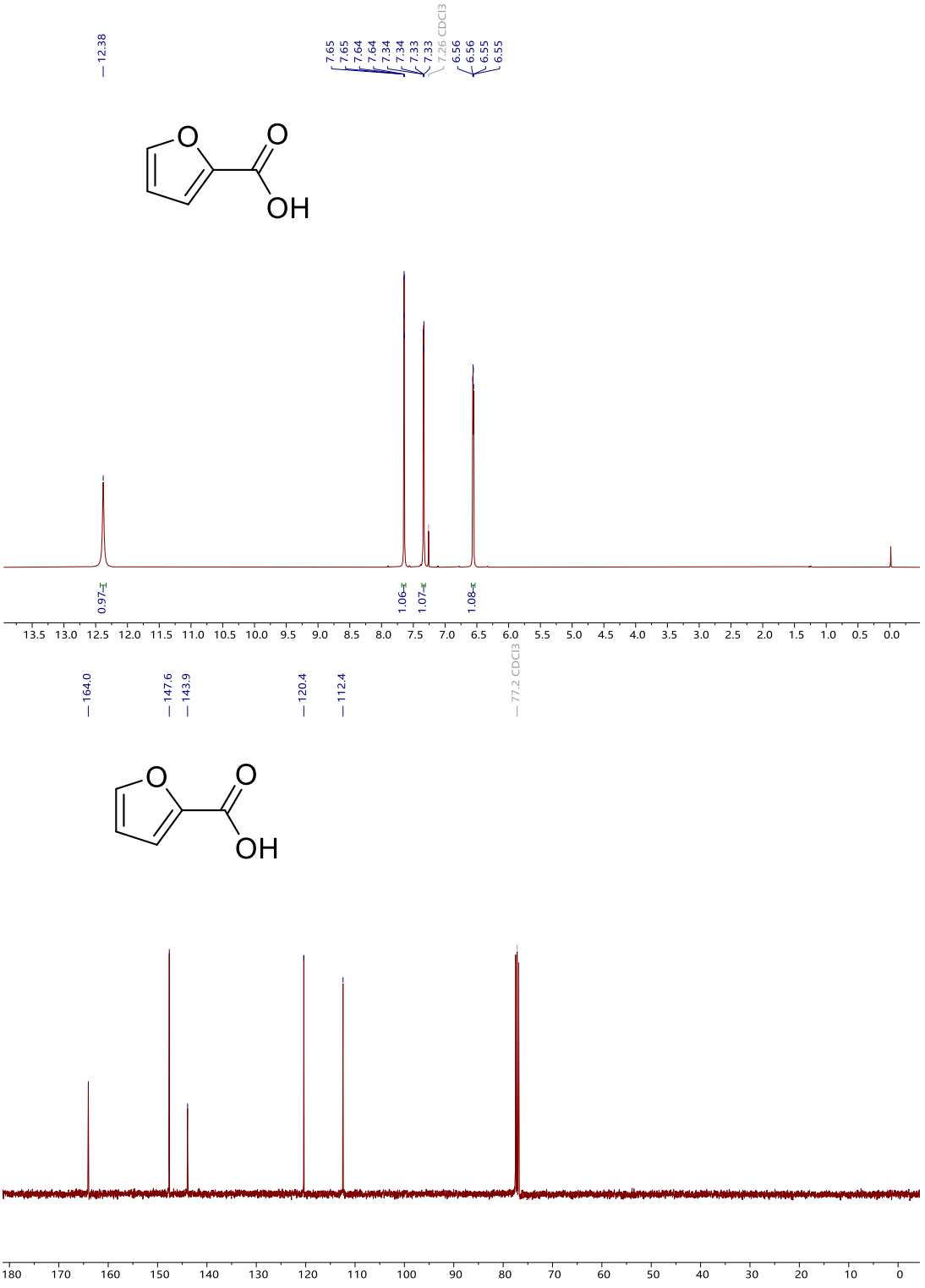

9.52
7.58
7.26 CDCl3
7.16
7.15
6.49
0.98
0.99
1.02
1.00
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0


177.7
152.8
148.0
121.2
112.5
77.2 CDCl3
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

372


9.95
7.82
7.81
7.57
7.56
7.55
7.47
7.46
7.45
7.26 CDCl3
O
0.93
2.03
1.00
2.05
192.3
136.3
134.4
129.6
128.9
77.2 CDCl3
O

373


12.38
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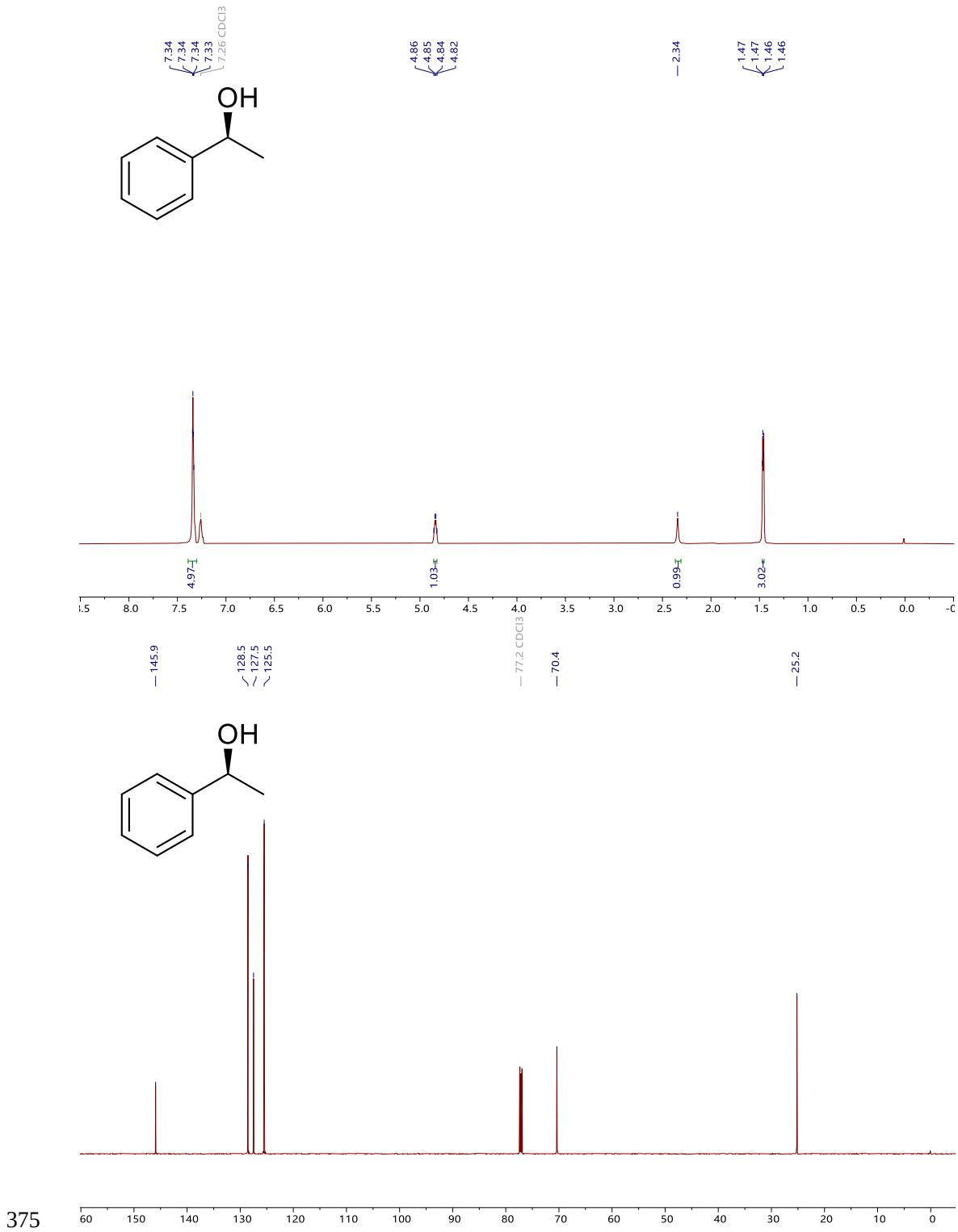
7.65
7.64
7.64
7.34
7.34
7.33
7.33
7.26 CDCl3
6.56
6.56
6.55
6.55
O
O
OH
0.97
1.06
1.07
1.08
13.5 13.0 12.5 12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
164.0
147.6
143.9
120.4
112.4
77.2 CDCl3
O
O
OH
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

374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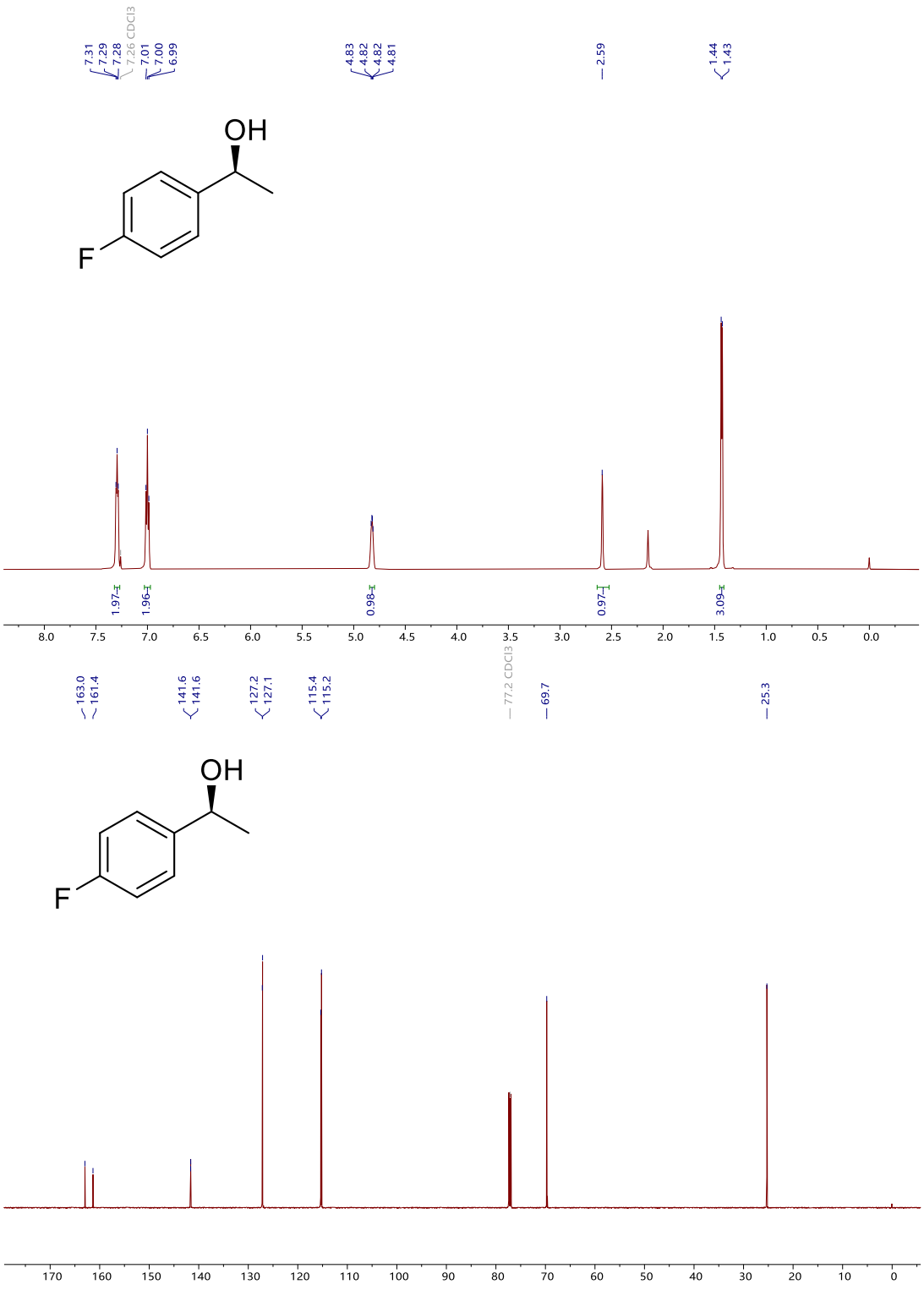
7.34
7.34
7.33
7.26 CDCl3
4.86
4.85
4.84
4.82
2.34
1.47
1.47
1.46
1.46
OH
4.97
1.03
0.99
3.02
145.9
128.5
127.5
125.5
77.2 CDCl3
70.4
25.2
OH

375


7.31
7.29
7.28
7.26 CDCl3
7.01
7.00
6.99
4.83
4.82
4.82
4.81
2.59
1.44
1.43
OH
F
1.97
1.96
0.98
0.97
3.09
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
0.5
0.0
163.0
161.4
141.6
141.6
127.2
127.1
115.4
115.2
77.2 CDCl3
69.7
25.3
OH
F
170
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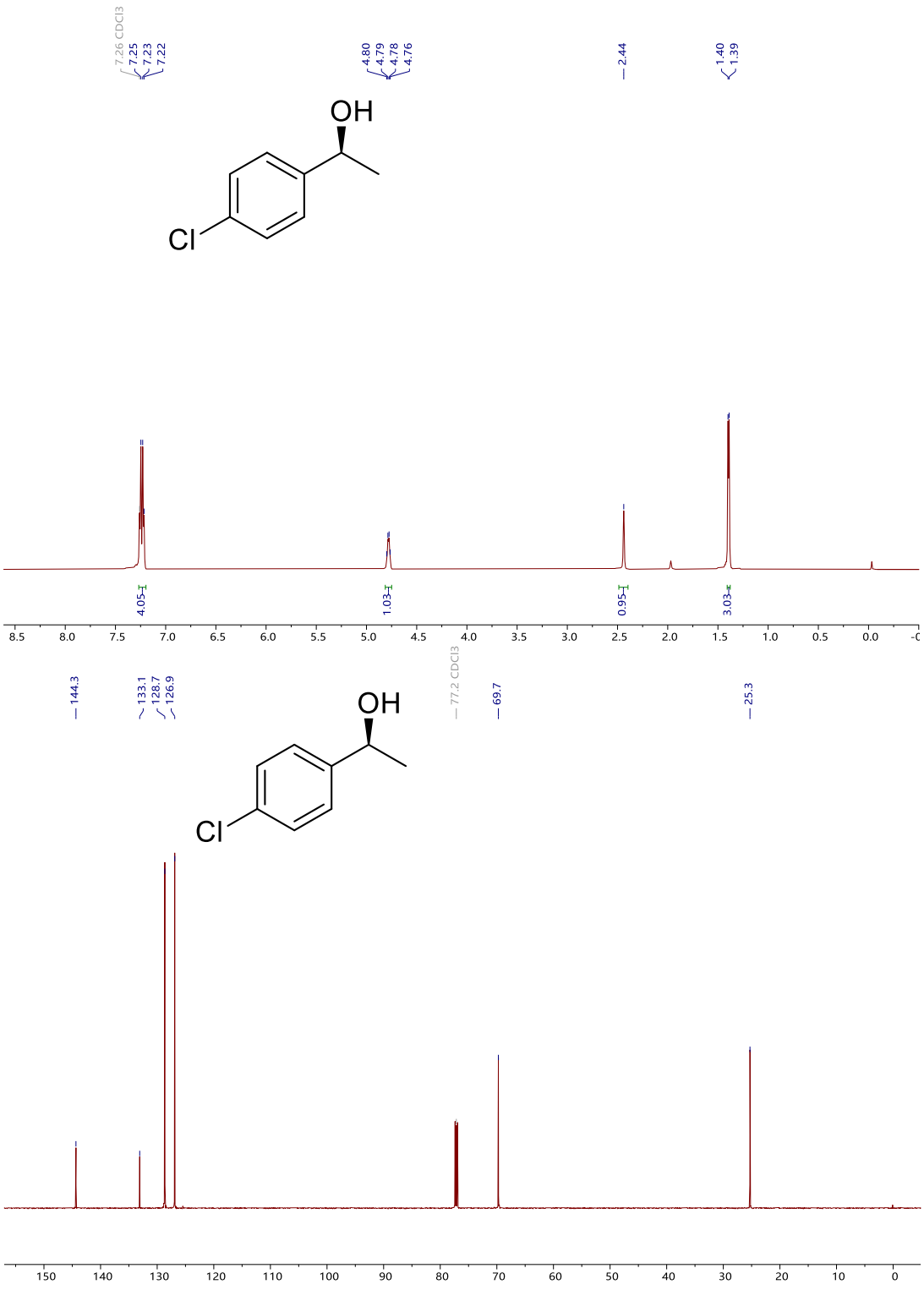
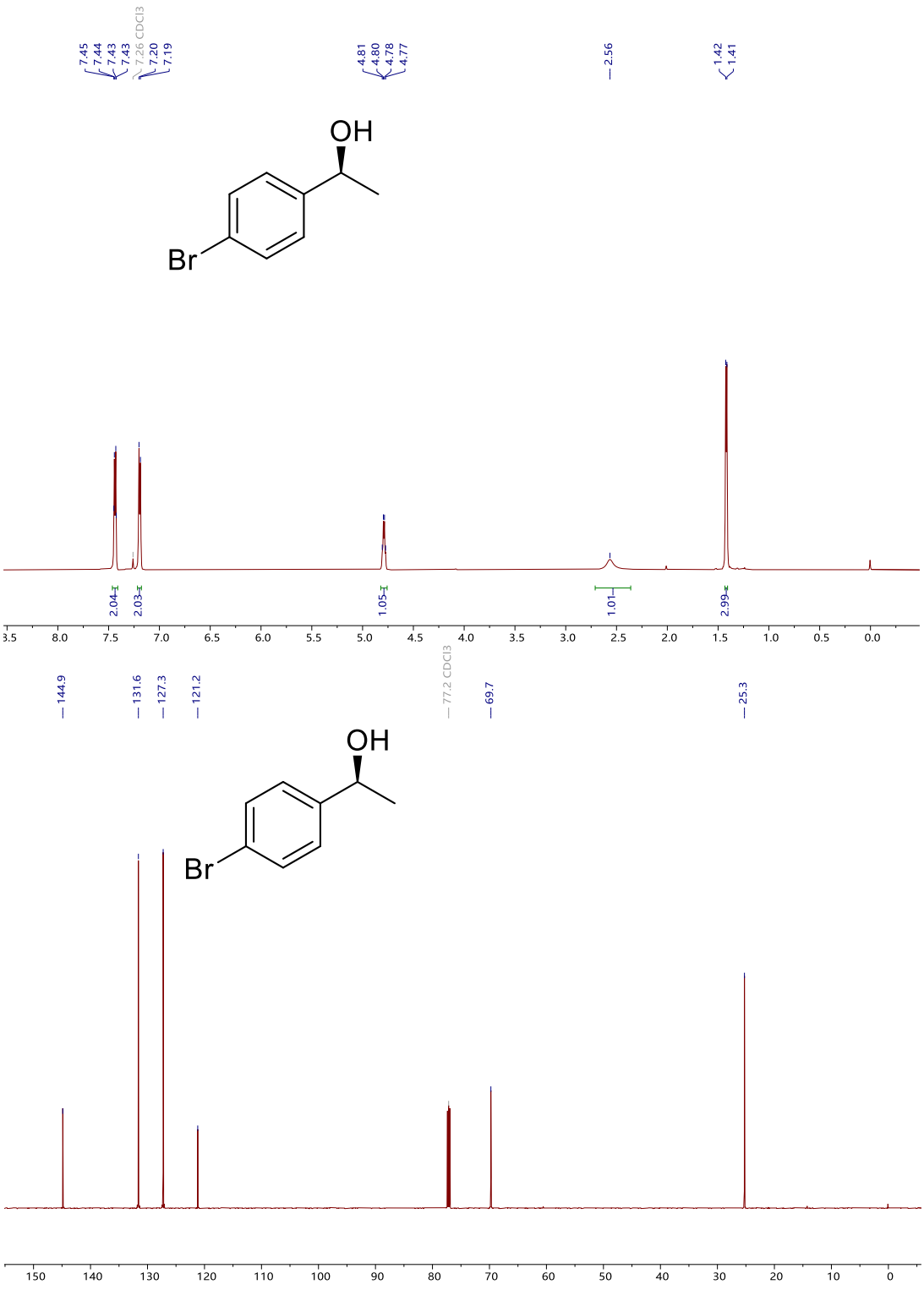
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0

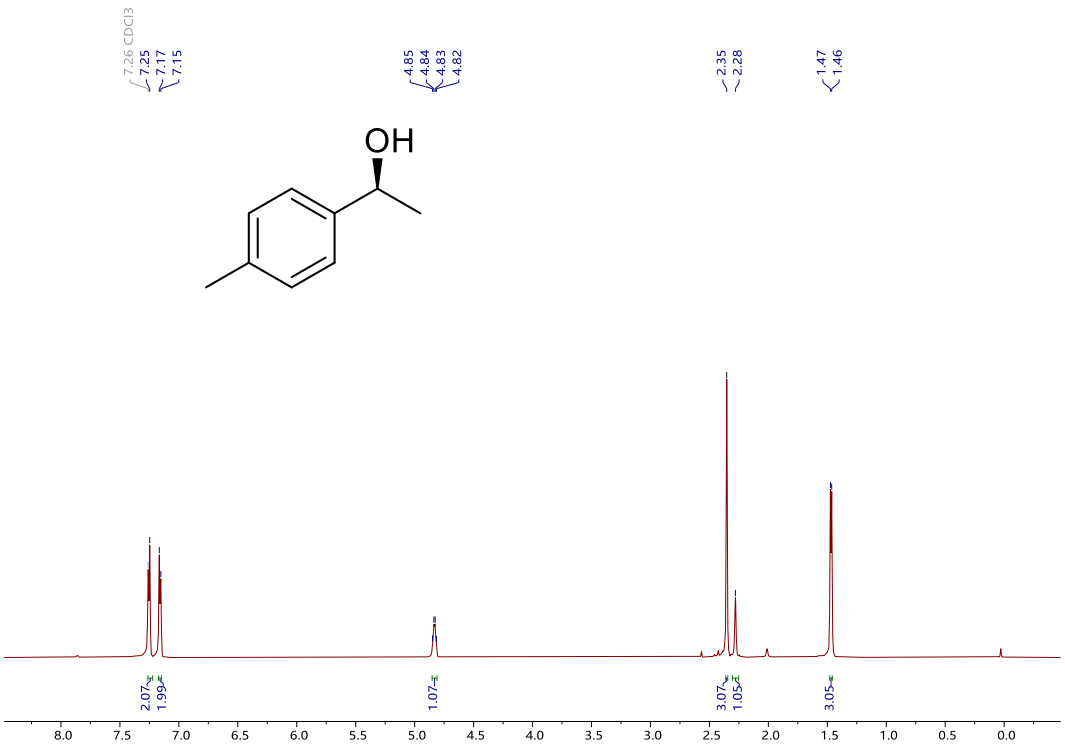
376

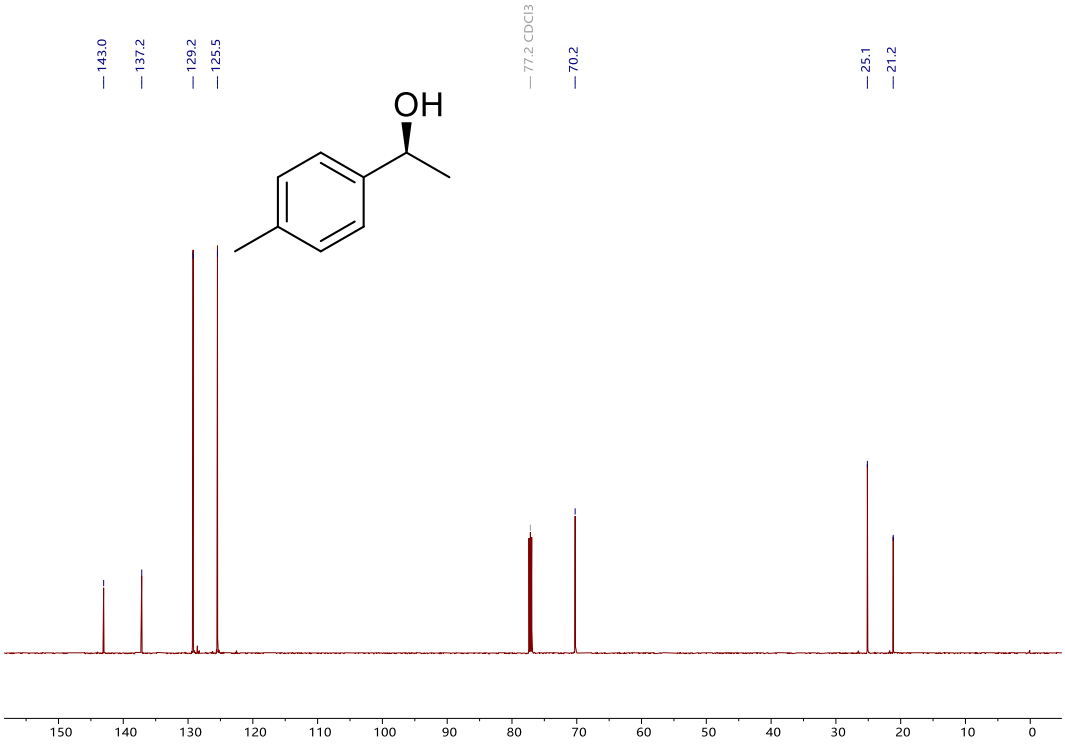

7.26 CDCl3
7.25
7.23
7.22
4.80
4.79
4.78
4.76
2.44
1.40
1.39
OH
Cl
4.05
1.03
0.95
3.03
144.3
133.1
128.7
126.9
77.2 CDCl3
69.7
25.3
OH
Cl

377

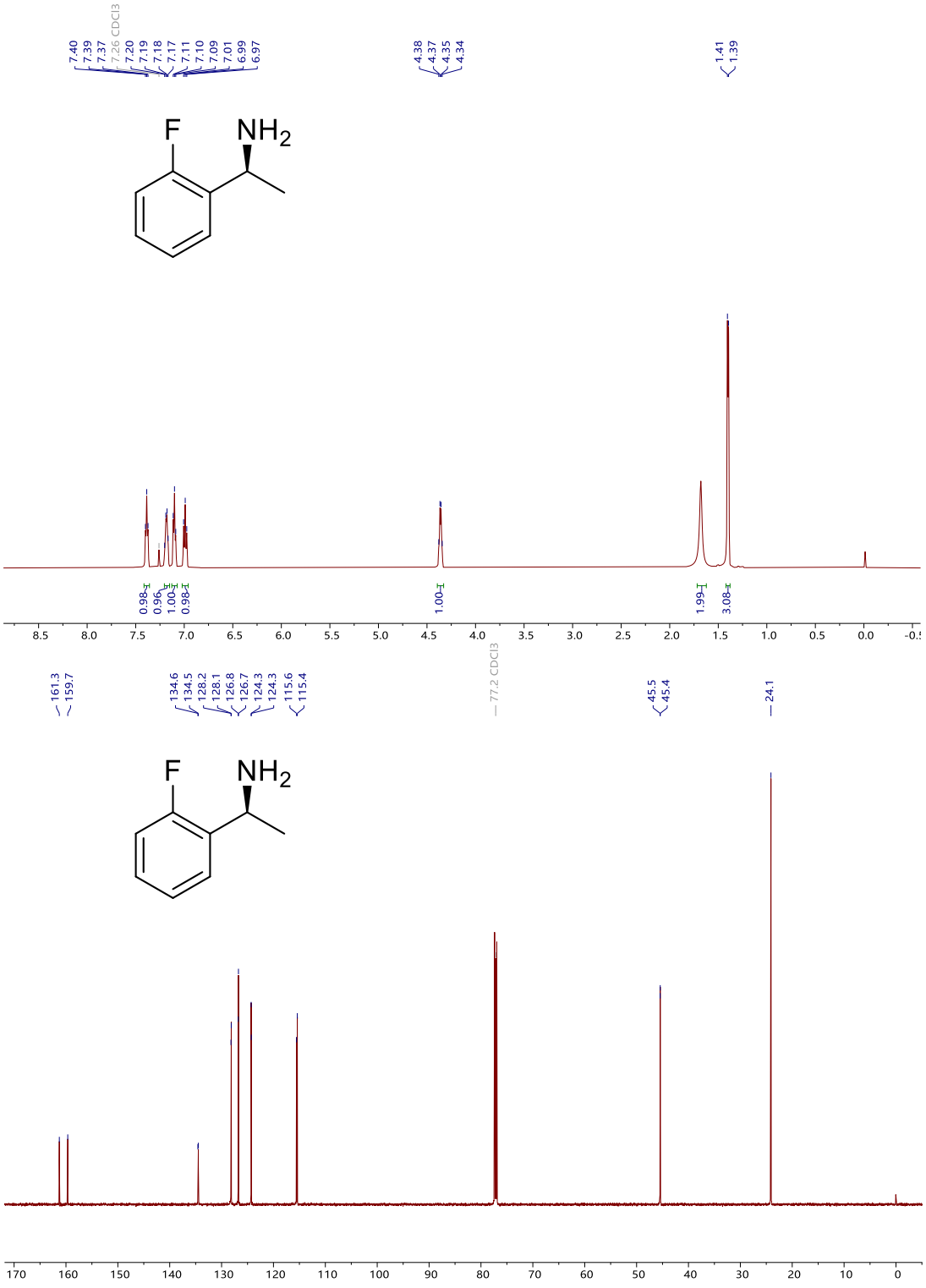

7.45
7.44
7.43
7.43
7.26 CDCl3
7.20
7.19
4.81
4.80
4.78
4.77
2.56
1.42
1.41
OH
Br
2.04
2.03
1.05
1.01
2.99
144.9
131.6
127.3
121.2
77.2 CDCl3
69.7
25.3
OH
Br

378


7.26 CDCl3
7.25
7.17
7.15
4.85
4.84
4.83
4.82
2.35
2.28
1.47
1.46
OH
2.07
1.99
1.07
3.07
1.05
3.05
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
0.5
0.0


143.0
137.2
129.2
125.5
77.2 CDCl3
70.2
25.1
21.2
OH
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0

379


7.40
7.39
7.37
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7.20
7.19
7.18
7.17
7.11
7.10
7.09
7.01
6.99
6.97
4.38
4.37
4.35
4.34
1.41
1.39
F
NH2
0.98
0.96
1.00
0.98
1.00
1.99
3.08
8.5
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
0.5
0.0
161.3
159.7
134.6
134.5
128.2
128.1
126.8
126.7
124.3
124.3
115.6
115.4
77.2 CDCl3
45.5
45.4
24.1
F
NH2
170
160
150
140
130
120
110
100
90
80
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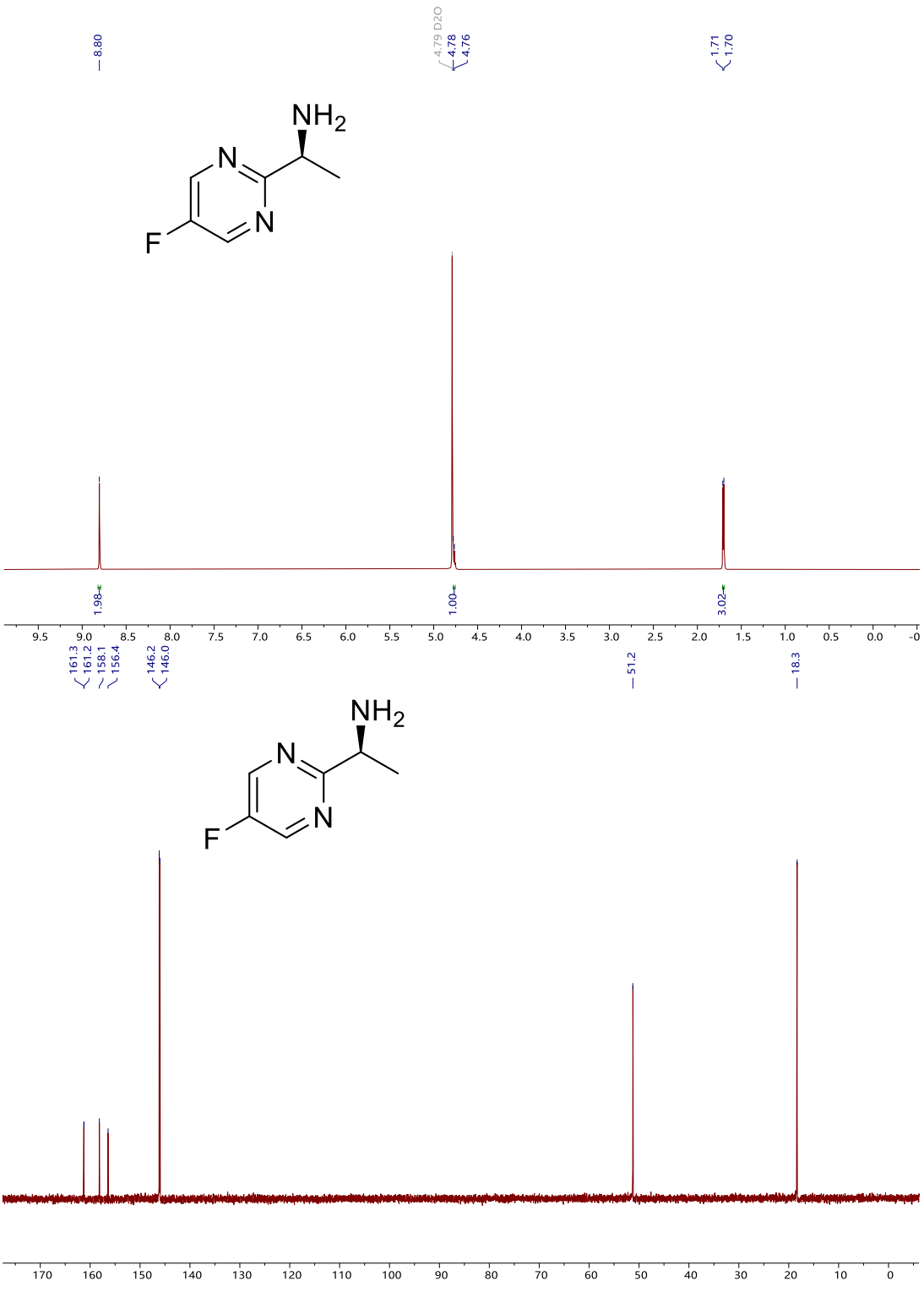
60
50
40
30
20
10
0

380


8.80
4.79 D2O
4.78
4.76
1.71
1.70
NH2
N
N
F
1.98
1.00
3.02
9.5
9.0
8.5
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
0.5
0.0
-0
161.3
161.2
158.1
156.4
146.2
146.0
51.2
18.3
NH2
N
N
F
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80
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50
40
30
20
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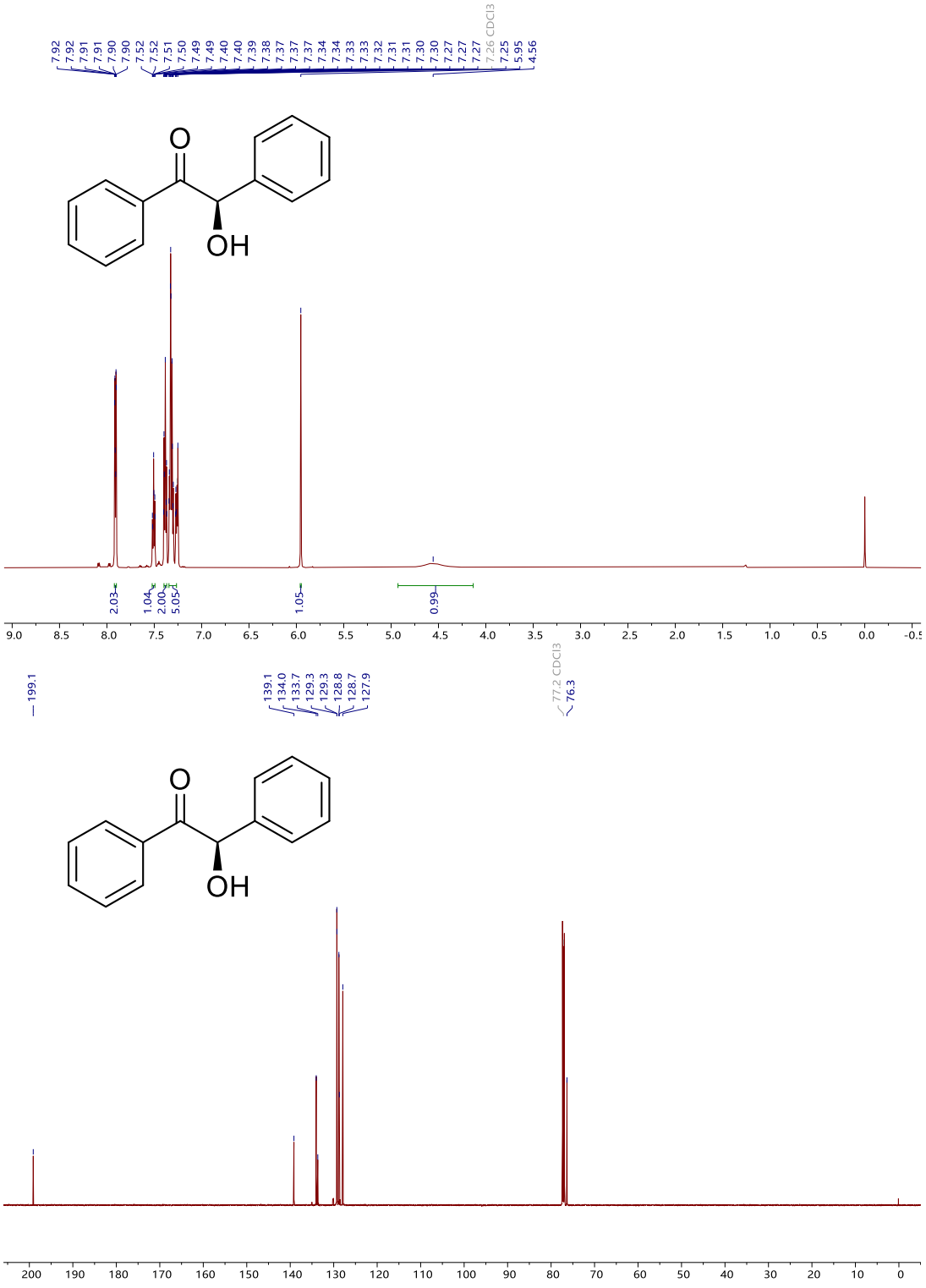
381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7.92
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7.49
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7.33
7.33
7.32
7.31
7.31
7.30
7.30
7.27
7.27
7.27
7.26 CDCl3
7.25
5.95
4.56
O
OH
2.03
1.04
2.00
5.05
1.05
0.99
199.1
139.1
134.0
133.7
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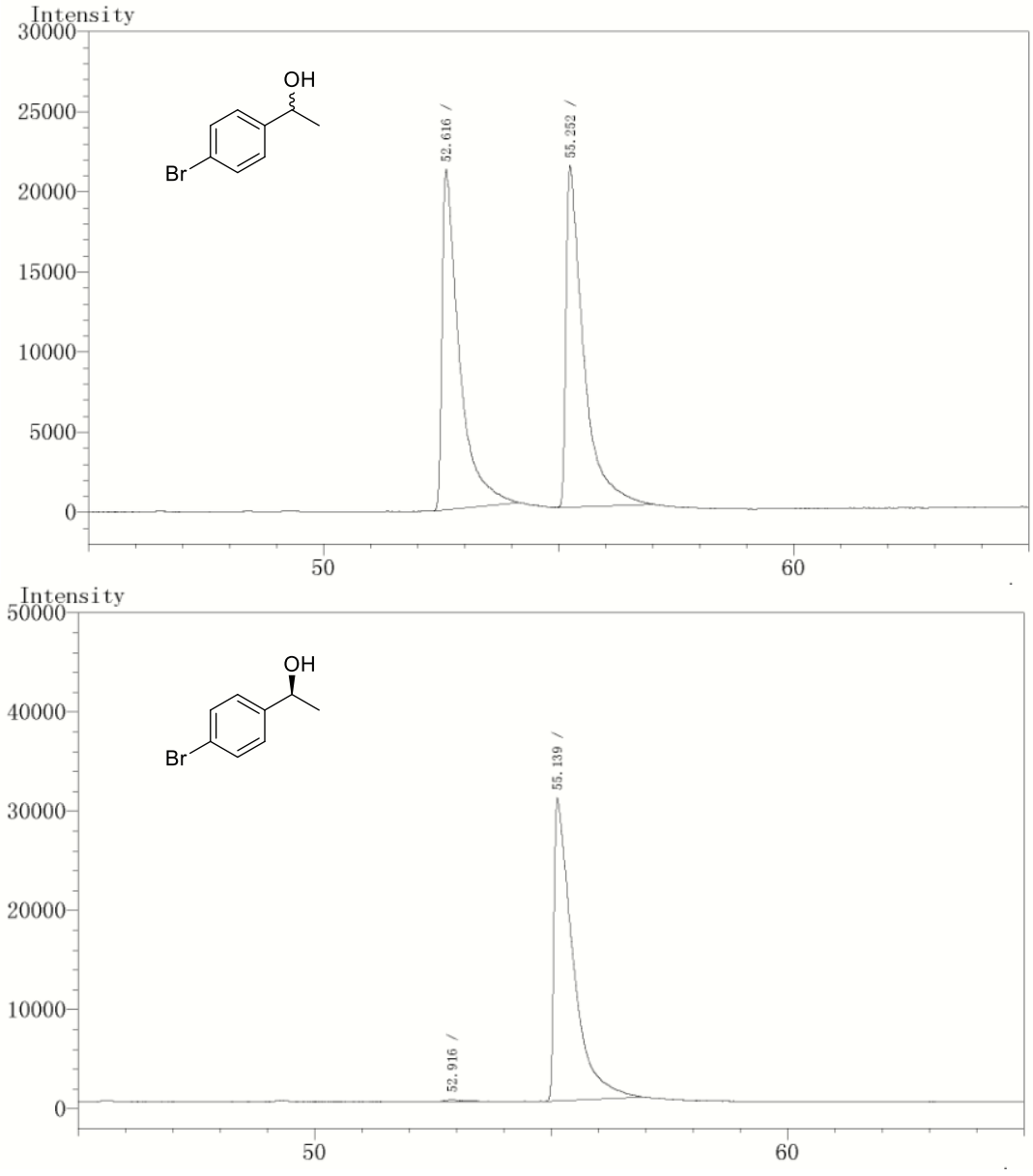
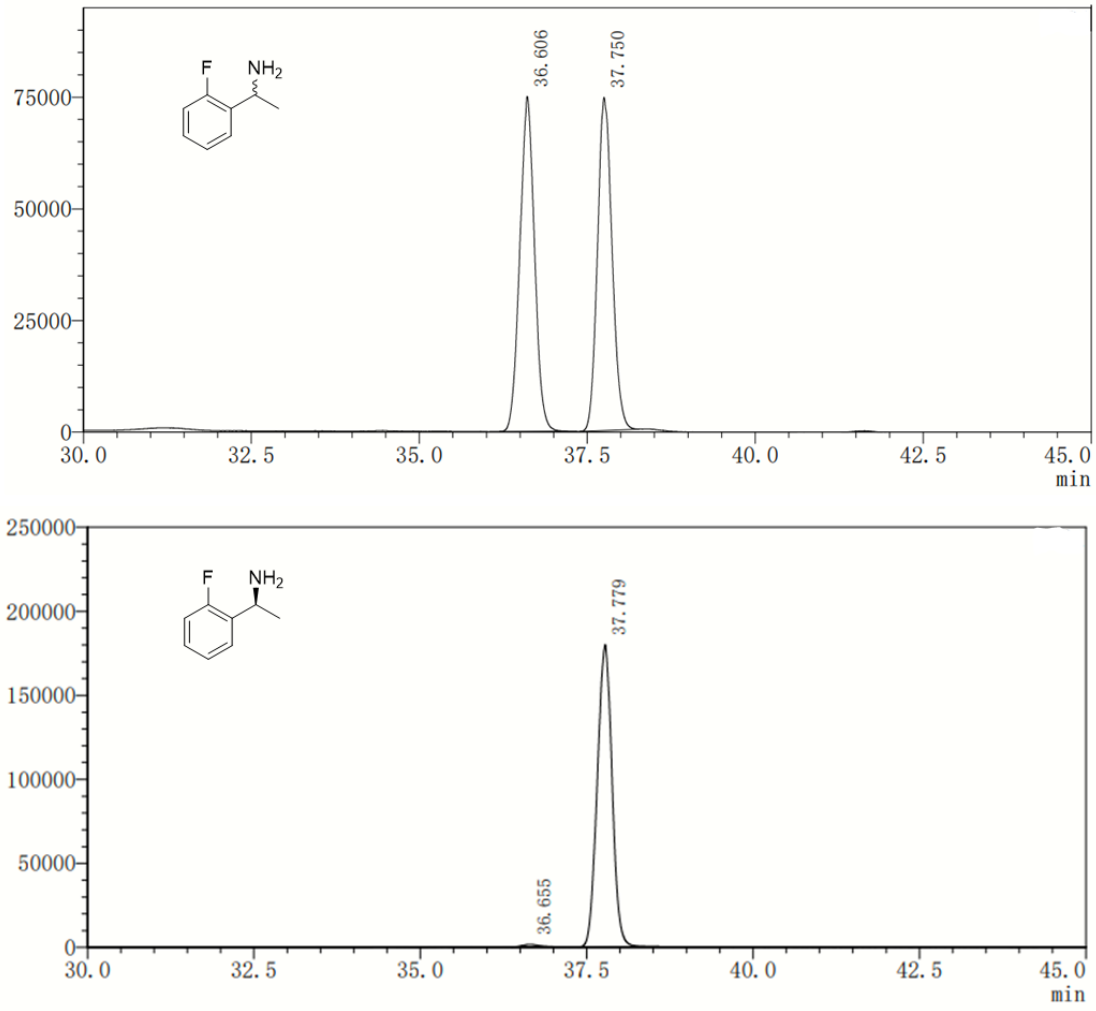
129.3
128.8
128.7
127.9
77.2 CDCl3
76.3
O
OH

382

GC chromatograms



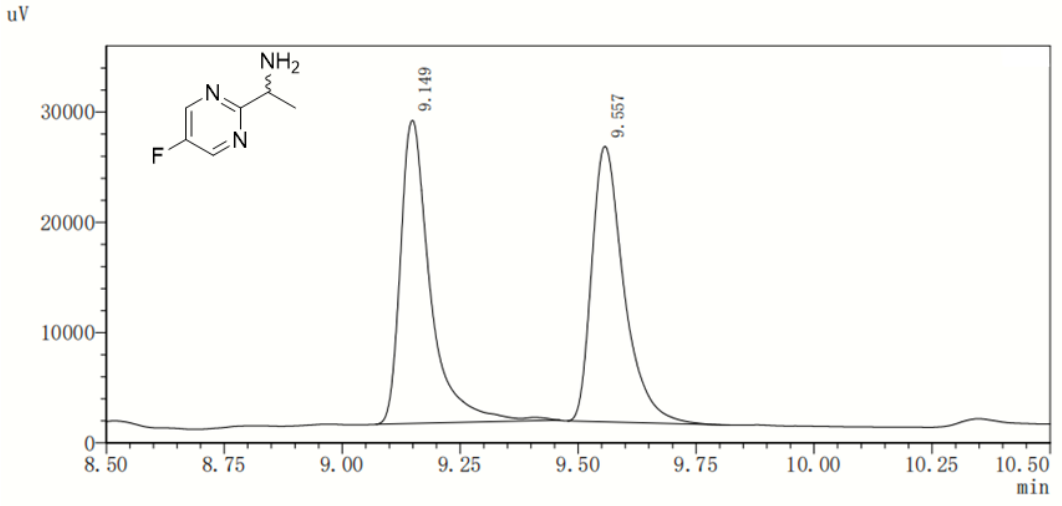
#	Time/min	Area	Height	Area%
1	52.916	4109	681	0.48
2	55.139	850302	181818	99.52

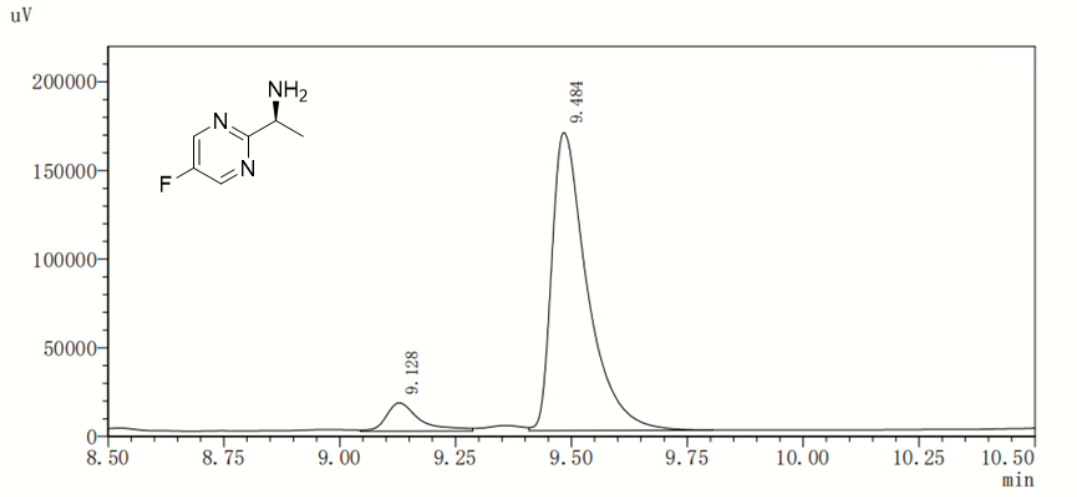


389

#	Time/min	Area	Height	Area%
1	36.655	26625	1480	0.951
2	37.779	2771807	179758	99.049

390



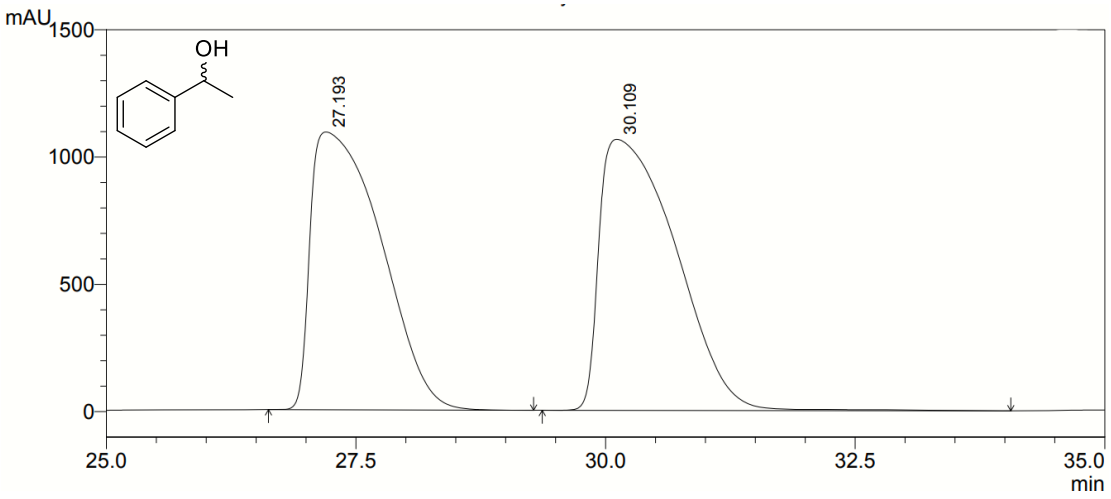


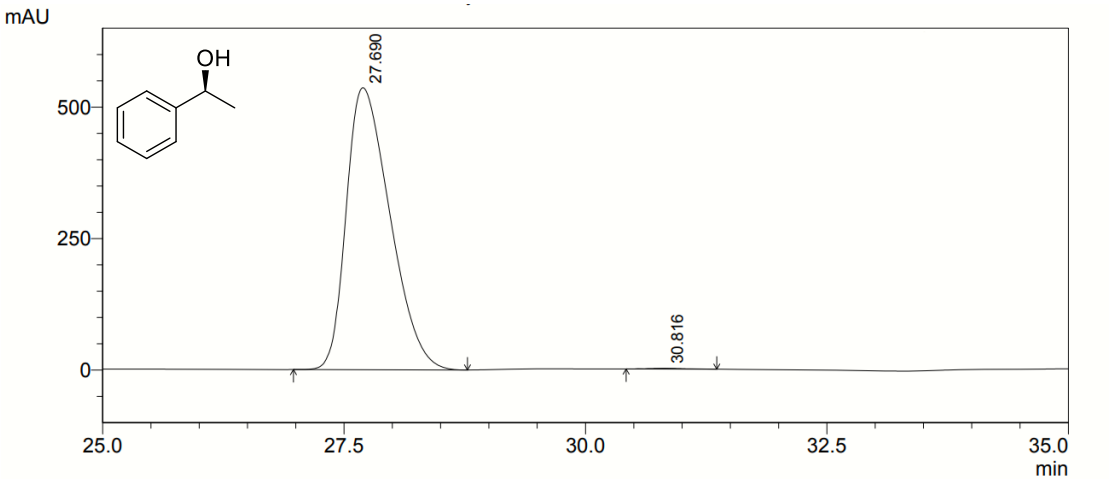
391

#	Time/min	Area	Height	Area%
1	9.128	79333	15863	8.13
2	9.484	896527	167691	91.87

392

HPLC chromatograms





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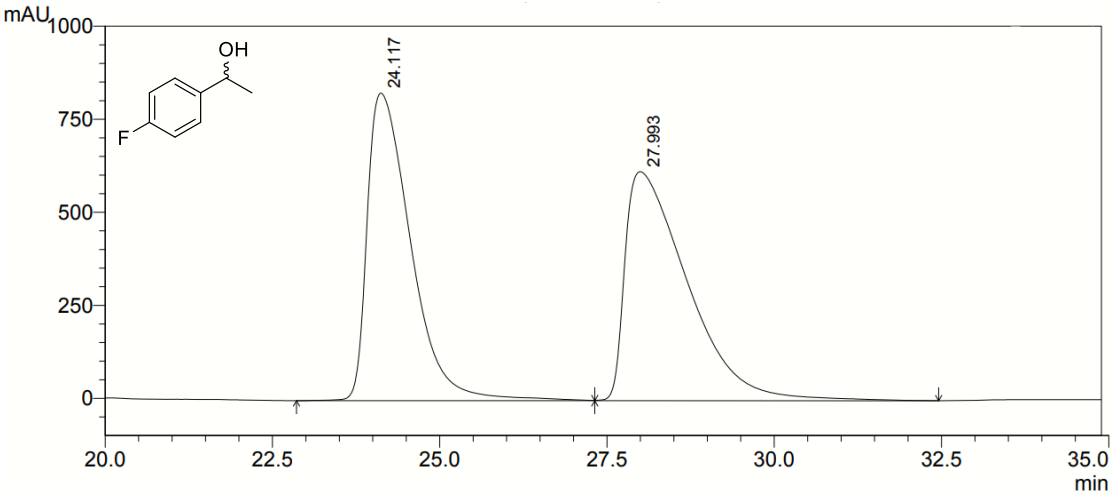
1	27.690	16890070	536137	99.77
2	30.816	39117	1468	0.23



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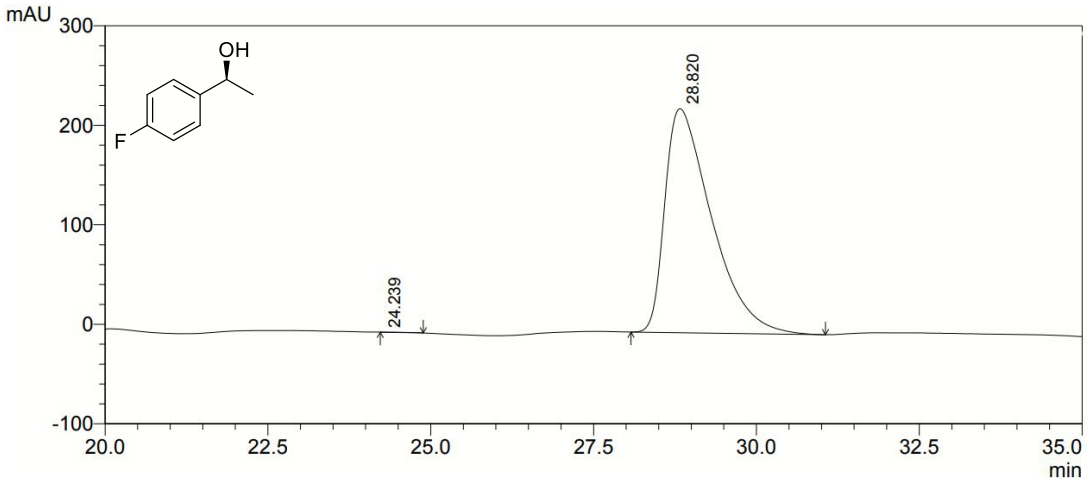
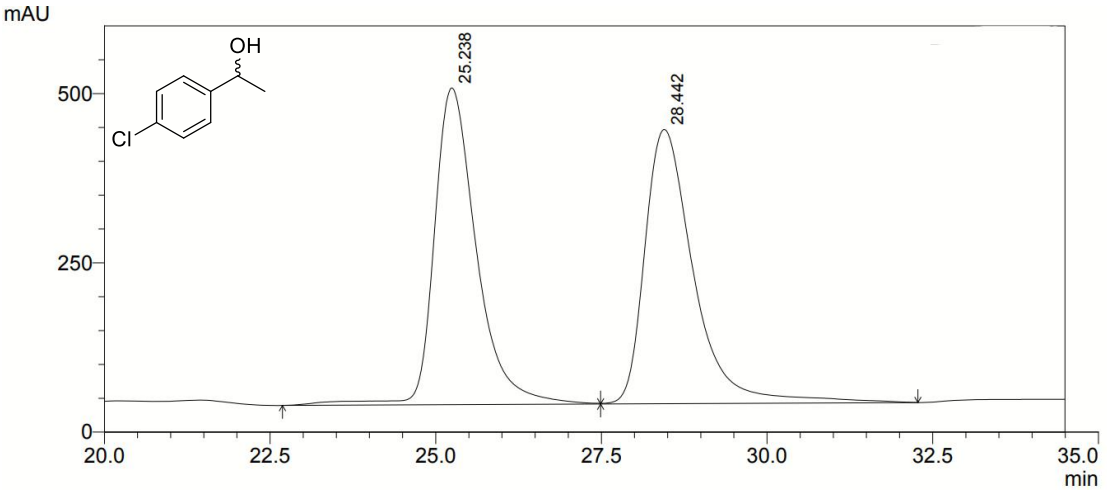


398

#	Time/min	Area	Height	Area%
1	24.239	5891	20	0.01
2	28.820	11248463	225122	99.99

399

400



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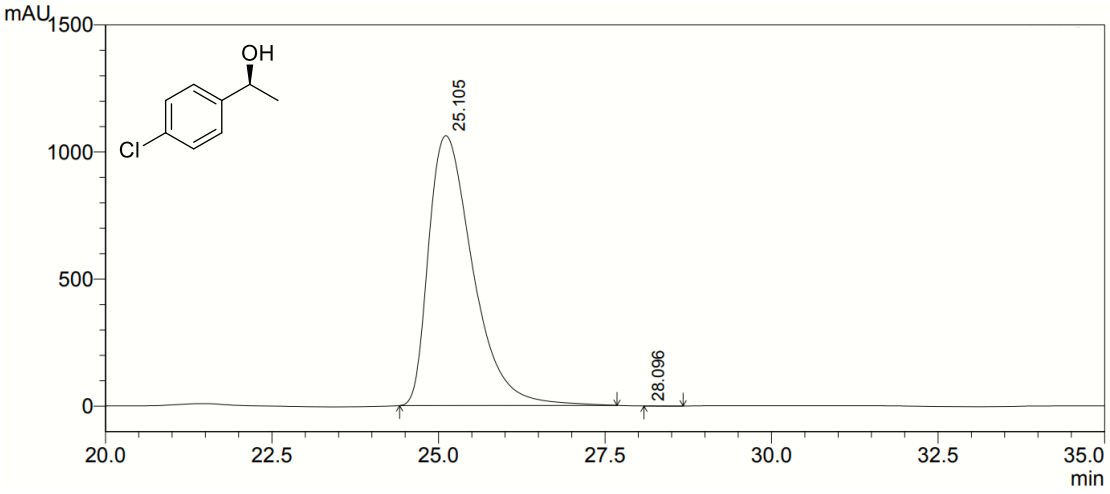
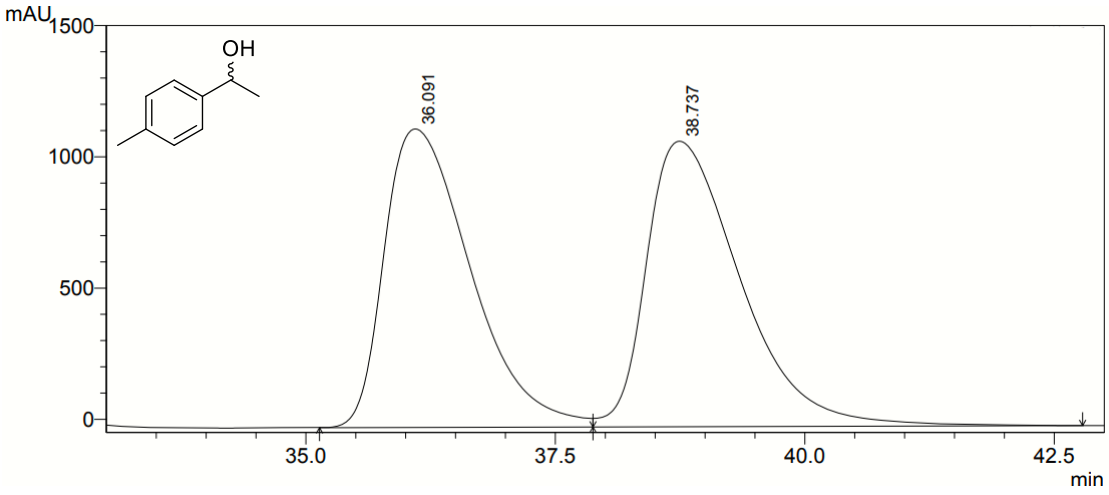


#	Time/min	Area	Height	Area%
1	25.105	49216699	1061775	99.98
2	28.096	11327	43	0.02

402

403



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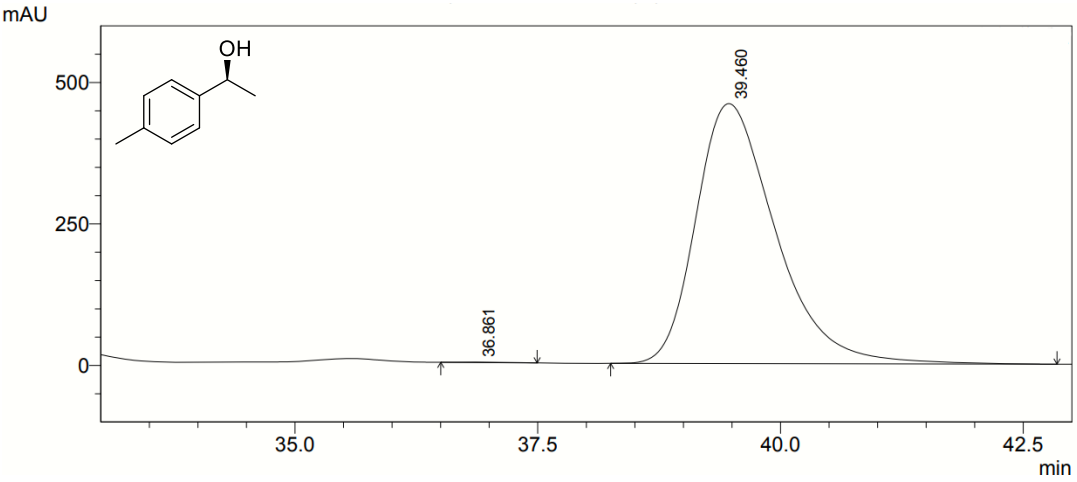

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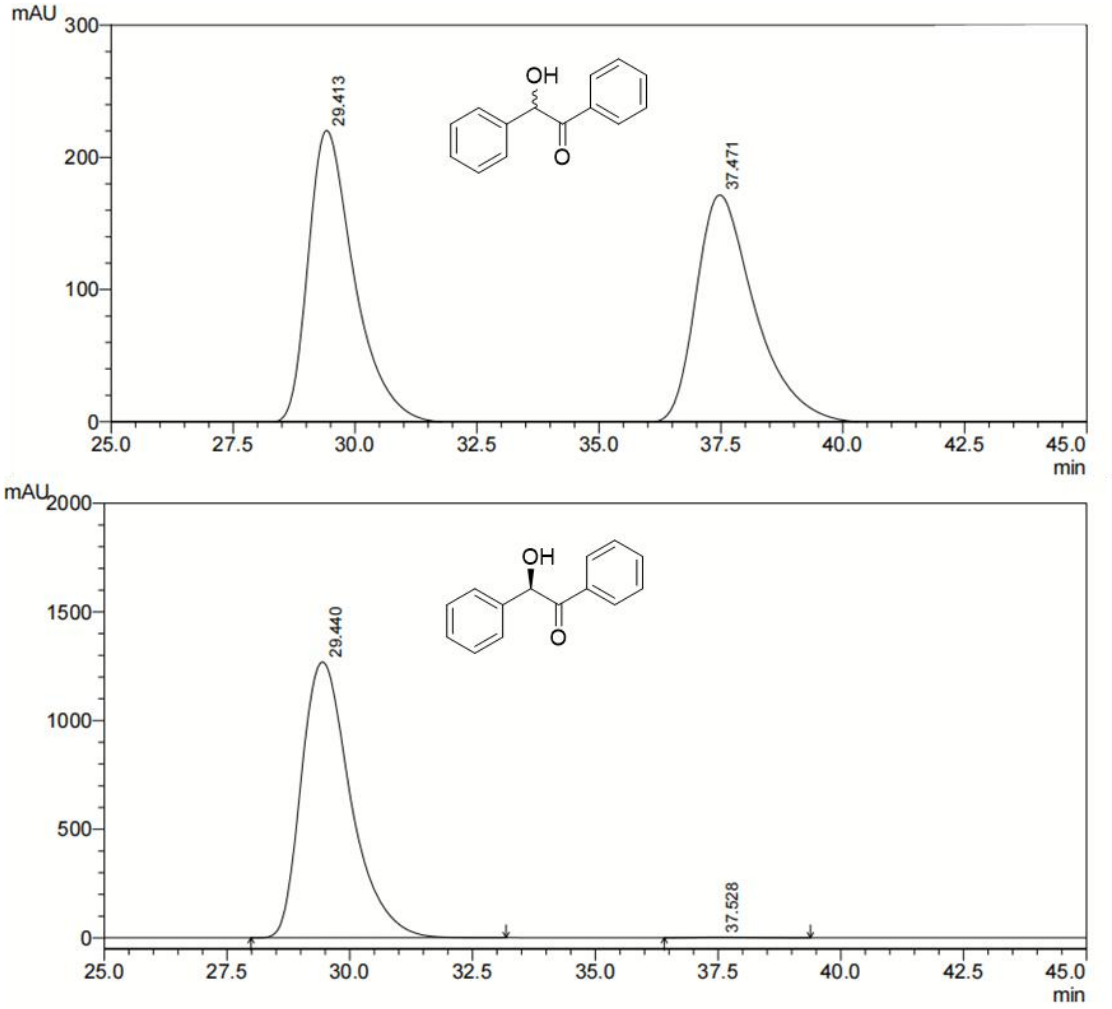
1	36.861	27617	805	0.11
2	39.460	26258631	459634	99.89



406

#	Time/min	Area	Height	Area%
1	29.440	88558398	1268801	99.780
2	37.528	195040	2535	0.22

407

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