Supporting Information

Rapid entry to phenanthroindolizidine alkaloids via an acid-catalysed acyliminium ion-electrocyclization cascade

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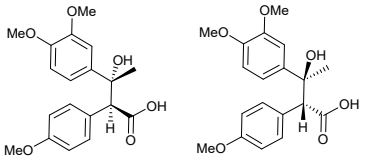
Contents

General Information

All moisture sensitive reactions were conducted in flame dried flasks under nitrogen atmosphere. Solvents were distilled over drying agents under nitrogen atmosphere. Reagents were obtained from commercial sources and used as received without further purification. ^{1}H NMR and ^{13}C NMR were recorded on Bruker 600 MHz nuclear magnetic resonance spectrometer. Data for ^{1}H NMR spectra were reported relative to deuterated chloroform ($CDCl_3$) as an internal standard (7.26 ppm) and were reported as follows: chemical shift (δ ppm), multiplicity, coupling constant (Hz) and integration. Multiplicities are denoted as follows: s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublets and m = multiplet. Data for ^{13}C NMR spectra were reported relative to CDCl3 as an internal standard (77.16 ppm) and were reported in terms of chemical shift (δ ppm). High resolution mass spectra (HRMS) were performed on an Agilent G1969 TOF mass spectrometer (ESI).

3-(3,4-dimethoxyphenyl)-3-hydroxy-2-(4-methoxyphenyl)butanoic acid (9a):

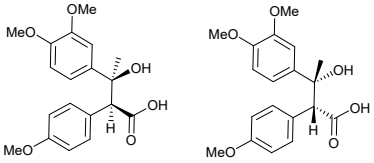
To a flame dried 50 mL two neck round bottom flask connected to a condenser under a nitrogen atmosphere, 4-methoxyphenyl acetic acid (**8a**) (665 mg, 4 mmol, 1.0 eq) was dissolved in 10 mL of anhydrous THF and cooled in an ice bath. To another 10 mL round bottom flask, 3',4'-dimethoxyacetophenone (**7**) (757 mg, 4.2 mmol, 1.05 eq) was dissolved in 2 mL of anhydrous THF under N_2 atmosphere. Under medium stirring, 2.0 M isopropyl magnesium chloride in THF (4.0 mL, 8.0 mmol, 2.0 eq) was added slowly dropwise to the chilled phenyl acetic acid solution. After addition, the ice bath was removed, and the opaque brown solution was heated to 60 °C in an oil bath for 20 minutes. Then, the solution containing the acetophenone was added dropwise to the reaction mixture and continually heated to 60 °C for another 30 minutes. The resulting clear yellow solution was cooled to 0 °C and quenched slowly with 20 mL H_2O followed by 2 M HCl until a pH of 1. The product was extracted 3 x 15 mL EtOAc, the organics were collected, dried over Na_2SO_4, and concentrated by rotary evaporation until approximately 5 mL of solution remained. To the remaining solution, 30 mL of hexanes were added, and the resulting light pink precipitate was collected via vacuum filtration and washed with 3 x 15 mL hexanes to give a white solid (1.321 g, 95%) as a mixture of diastereomers.



(2S/2R,3R/3S)-3-(3,4-dimethoxyphenyl)-3-hydroxy-2-(4-methoxyphenyl)butanoic acid

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.36 – 7.33 (m, 1H), 7.05 (d, *J* = 2.2 Hz, 1H), 6.99 – 6.96 (m, 1H), 6.90 – 6.87 (m, 1H), 6.83 (d, *J* = 8.4 Hz, 1H), 4.12 (s, 1H), 3.89 (s, 2H), 3.87 (s, 3H), 3.82 (s, 3H), 1.27 (s, 3H).

^{13}C NMR (151 MHz, $CDCl_3$) δ 176.39, 159.62, 148.88, 148.12, 139.95, 131.16, 126.14, 116.96, 114.04, 110.96, 108.70, 77.16, 75.61, 58.81, 56.08, 56.01, 55.43, 28.25.



(2S/2R,3S/3R)-3-(3,4-dimethoxyphenyl)-3-hydroxy-2-(4-methoxyphenyl)butanoic acid

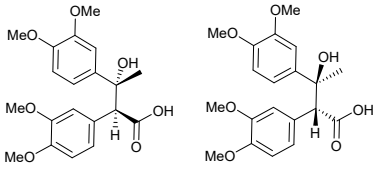
^{1}H NMR (600 MHz, $CDCl_3$) δ 6.97 – 6.94 (m, 1H), 6.76 (dd, *J* = 8.4, 2.1 Hz, 1H), 6.72 (d, *J* = 8.4 Hz, 1H), 6.69 – 6.66 (m, 1H), 6.62 (d, *J* = 2.0 Hz, 1H), 3.82 (s, 1H), 3.81 (s, 1H), 3.72 (s, 1H), 3.65 (s, 1H), 1.78 (s, 1H).

^{13}C NMR (151 MHz, $CDCl_3$) δ 176.94, 159.21, 148.25, 147.82, 136.83, 130.78, 126.07, 117.30, 113.48, 110.40, 109.52, 75.32, 60.58, 55.92, 55.81, 55.31, 29.17.

HRMS (ESI) m/z $[M-H]^-$: Calcd for $C_{19}H_{21}O_6$: 345.1344 Found: 345.1334

2,3-bis(3,4-dimethoxyphenyl)-3-hydroxybutanoic acid (9b):

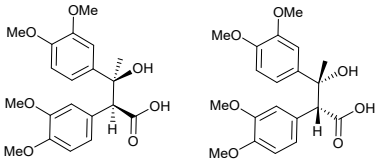
To a 50 mL two neck round bottom flask connected to a condenser under a nitrogen atmosphere, 3,4-dimethoxyphenyl acetic acid (**8b**) (785 mg, 4 mmol, 1.0 eq) was dissolved in 10 mL of anhydrous THF cooled in an ice bath. To another 10 mL round bottom flask, 3',4'-dimethoxyacetophenone (**7**) (757 mg, 4.2 mmol, 1.05 eq) was dissolved in 2 mL of anhydrous THF under N_2 atmosphere. Under medium stirring, 2.0 M isopropyl magnesium chloride in THF (4.0 mL, 8.0 mmol, 2.0 eq) was added dropwise to the chilled phenyl acetic acid solution. After addition, the ice bath was removed, and the opaque brown solution was heated to 60 °C in an oil bath for 30 minutes. Then, the solution containing the acetophenone was added dropwise to the reaction mixture and continually heated to 60 °C for another 30 minutes. The resulting clear yellow solution was cooled to 0 °C in an ice bath and quenched slowly with 20 mL H_2O followed by 2 M HCl until a pH of 2. The product was extracted 3 x 15 mL EtOAc, the organics were collected, dried over Na_2SO_4, and the solvents were removed by rotary evaporation until approximately 5 mL of solution remained. To the remaining solution, 30 mL of hexanes were added, and the resulting light pink precipitate was collected via vacuum filtration and washed with 3 x 15 mL hexanes to give a white solid (1.293g, 86%) as a mixture of diastereomers.



(2S/2R,3R/3S)-2,3-bis(3,4-dimethoxyphenyl)-3-hydroxybutanoic acid

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.07 (d, J = 2.1 Hz, 1H), 6.98 (dd, J = 8.2, 2.0 Hz, 1H), 6.96 – 6.94 (m, 2H), 6.85 (d, J = 8.2 Hz, 1H), 6.83 (d, J = 8.4 Hz, 1H), 4.11 (s, 1H), 3.89 (s, 3H), 3.89 (s, 3H), 3.87 (s, 3H), 3.86 (s, 3H), 1.30 (s, 3H).

^{13}C NMR (151 MHz, $CDCl_3$) δ 176.64, 149.13, 148.85, 148.16, 147.93, 139.91, 126.53, 122.66, 117.00, 113.10, 111.07, 110.95, 108.71, 75.67, 59.15, 56.07, 56.02, 55.98, 55.91, 28.23.



(2S,3S)-2,3-bis(3,4-dimethoxyphenyl)-3-hydroxybutanoic acid

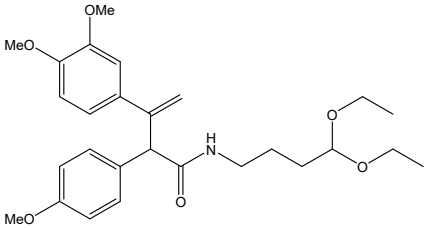
^{1}H NMR (600 MHz, $CDCl_3$) δ 6.76 (dd, J = 8.4, 2.1 Hz, 1H), 6.72 (d, J = 8.4 Hz, 1H), 6.68 (d, J = 2.0 Hz, 1H), 6.66 – 6.61 (m, 2H), 6.56 (d, J = 1.5 Hz, 1H), 3.82 (s, 3H), 3.81 (s, 1H), 3.80 (s, 3H), 3.68 (s, 3H), 3.67 (s, 3H), 1.78 (s, 3H).

^{13}C NMR (151 MHz, $CDCl_3$) δ 177.07, 148.91, 148.69, 148.37, 148.35, 136.87, 126.39, 122.07, 117.48, 113.07, 110.58, 110.47, 109.51, 75.36, 60.85, 56.10, 56.07, 56.02, 55.98, 29.22.

HRMS (ESI) m/z $[M+NH_4]^+$: Calcd for $C_{20}H_{28}NO_7$: 394.1860 Found: 394.1869

N-(4,4-diethoxybutyl)-3-(3,4-dimethoxyphenyl)-2-(4-methoxyphenyl)but-3-enamide (10a):

To a 10 mL two neck round bottom flask, 3-(3,4-dimethoxyphenyl)-3-hydroxy-2-(4-methoxyphenyl)butanoic acid (**9a**) (111 mg, 0.32 mmol, 1.0 eq) was dissolved in 5 mL of anhydrous DCM under a N_2 atmosphere cooled to -78 °C in a dry ice acetone bath. Under strong stirring, triethylamine (187 µL, 1.35 mmol, 4.2 eq) followed by methyl chloroformate (50 µL, 0.656 mmol, 2.05 eq) were both added to the reaction mixture. After 30 minutes, 4-aminobutyraldehyde diethyl acetal (62 mg, 0.384 mmol, 1.2 eq) dissolved in 1 mL of DCM was added dropwise to the cloudy mixture, the flask was removed from the cooling bath, and let warm to room temperature. After 3 hours, the reaction was quenched with 10 mL H_2O, and the product was extracted with 2 x 10 mL DCM. The organics were collected, dried over Na_2SO_4, and the DCM was removed by rotary evaporation. The resulting yellow oil was purified by silica gel column chromatography (0-60% EtOAc/Hexanes) to give a clear oil (67 mg, 44%) as racemic mixture.



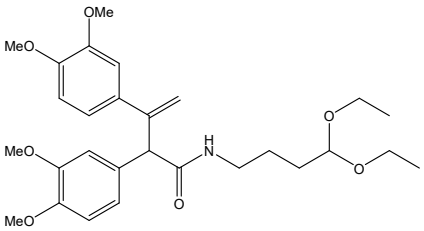
^{1}H NMR (600 MHz, $CDCl_3$) δ 7.25 – 7.20 (m, 2H), 6.96 – 6.93 (m, 2H), 6.88 – 6.84 (m, 2H), 6.78 – 6.74 (m, 1H), 5.83 (t, *J* = 5.7 Hz, 1H), 5.61 (s, 1H), 5.07 (s, 1H), 4.77 (s, 1H), 4.42 (t, *J* = 5.0 Hz, 1H), 3.85 (s, 3H), 3.83 (s, 3H), 3.79 (s, 3H), 3.61 – 3.56 (m, 2H), 3.46 – 3.40 (m, 2H), 3.32 – 3.21 (m, 2H), 1.55 – 1.49 (m, 4H), 1.17 (t, *J* = 7.0 Hz, 6H).

^{13}C NMR (151 MHz, $CDCl_3$) δ 171.72, 159.03, 148.98, 148.79, 146.74, 133.61, 130.21, 130.09, 118.81, 115.28, 114.42, 110.97, 109.71, 102.66, 61.42, 58.34, 55.99, 55.98, 55.39, 39.57, 30.92, 24.72, 15.46.

HRMS (ESI) m/z $[M+Na]^+$ Calcd for $C_{27}H_{37}NO_6Na$: 494.2513 Found: 494.2501

N-(4,4-diethoxybutyl)-2,3-bis(3,4-dimethoxyphenyl)but-3-enamide (10b):

To a 10 mL two neck round bottom flask, 2,3-bis(3,4-dimethoxyphenyl)-3-hydroxybutanoic acid (**9b**) (340 mg, 0.9 mmol, 1.0 eq) was dissolved in 5 mL of anhydrous DCM under a nitrogen atmosphere cooled to -78 °C in a dry ice acetone bath. Under strong stirring, triethylamine (565 µL, 4.05 mmol, 4.5 eq) followed by methyl chloroformate (142 µL, 1.84 mmol, 2.05 eq) were both added to the reaction mixture. After 30 minutes, 4-aminobutyraldehyde diethyl acetal (217 mg, 1.35 mmol, 1.5 eq) was added dropwise to the mixture, the flask was removed from the cooling bath, and let warm to room temperature. After 3 hours, the reaction was quenched with 10 mL H_2O, and the product was extracted with 2 x 10 mL DCM. The organics were collected, dried over Na_2SO_4, and the DCM was removed by rotary evaporation. The resulting clear yellow oil was purified by silica gel column chromatography (0-60% EtOAc/Hexanes) to give a clear oil (158 mg, 35%) as a racemic mixture.



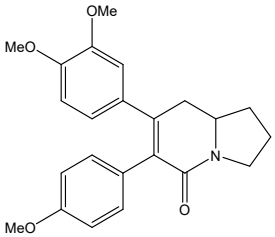
^{1}H NMR (600 MHz, $CDCl_3$) δ 6.96 – 6.93 (m, 2H), 6.86 (dd, J = 8.2, 1.9 Hz, 1H), 6.84 – 6.81 (m, 2H), 6.77 (d, J = 9.0 Hz, 1H), 5.85 (t, J = 5.7 Hz, 1H), 5.62 (s, 1H), 5.10 (s, 1H), 4.76 (s, 1H), 4.42 (t, J = 5.0 Hz, 1H), 3.86 (s, 3H), 3.85 (s, 3H), 3.84 (s, 3H), 3.84 (s, 3H), 3.61 – 3.57 (m, 4H), 3.47 – 3.41 (m, 2H), 3.33 – 3.23 (m, 2H), 1.55 – 1.49 (m, 4H), 1.17 (t, J = 6.9 Hz, 6H).

^{13}C NMR (151 MHz, $CDCl_3$) δ 171.63, 149.35, 149.02, 148.81, 148.52, 146.58, 133.62, 130.62, 121.09, 118.82, 115.31, 112.26, 111.49, 110.98, 109.73, 102.66, 61.45, 58.77, 56.05, 56.03, 56.01, 56.00, 39.58, 30.96, 24.75, 15.47.

HRMS (ESI) m/z $[M+Na]^+$: Calcd for $C_{28}H_{39}NO_7Na$: 524.2619 Found: 524.2614

7-(3,4-dimethoxyphenyl)-6-(4-methoxyphenyl)-2,3,8,8a-tetrahydroindolizin-5(1H)-one (11a):

To a 25 mL round bottom flask, N-(4,4-diethoxybutyl)-3-(3,4-dimethoxyphenyl)-2-(4-methoxyphenyl)but-3-enamide (**10a**) (122 mg, 0.258 mmol, 1.0 eq) and para-toluene sulfonic acid monohydrate (49 mg, 0.258 mmol, 1.0 eq) were dissolved in 10 mL toluene. The reaction mixture was refluxed (oil bath temp 115 °C) for 2 hours. The resulting bright yellow solution was cooled to room temperature, quenched with 10 mL of a saturated $NaHCO_3$ solution, and extracted with 2 x 10 mL EtOAc. The organics were collected, dried over Na_2SO_4, and solvents were removed by rotary evaporation. The resulting mixture was purified using silica gel column chromatography (10-80% EtOAc/Hexanes) to give an off-white solid (98 mg, 92%) as a racemic mixture.



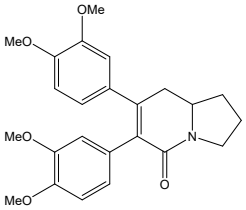
^{1}H NMR (600 MHz, $CDCl_3$) δ 7.08 – 7.00 (m, 2H), 6.76 – 6.70 (m, 4H), 6.44 (d, *J* = 1.9 Hz, 1H), 3.94 (qd, *J* = 9.9, 5.0 Hz, 1H), 3.83 (s, *J* = 4.2 Hz, 3H), 3.74 (s, 3H), 3.70 (ddd, *J* = 11.4, 9.0, 2.1 Hz, 1H), 3.63 – 3.57 (m, 1H), 3.49 (s, 3H), 2.85 (dd, *J* = 16.2, 4.4 Hz, 1H), 2.72 (dd, *J* = 16.2, 13.9 Hz, 1H), 2.30 (ddd, *J* = 12.0, 6.6, 1.3 Hz, 1H), 2.12 – 2.06 (m, 1H), 1.92 – 1.82 (m, 1H), 1.76 – 1.67 (m, 1H).

^{13}C NMR (151 MHz, $CDCl_3$) δ 164.46, 158.56, 148.47, 148.05, 144.20, 132.84, 132.32, 132.05, 129.04, 120.96, 113.46, 112.90, 110.52, 55.89, 55.74, 55.64, 55.34, 44.95, 37.45, 33.99, 23.34.

HRMS (FAB) m/z $[M+H]^+$: Calcd for $C_{23}H_{26}NO_4$: 380.1856 Found 380.1851. [17]

6,7-bis(3,4-dimethoxyphenyl)-2,3,8,8a-tetrahydroindolizin-5(1H)-one (11b):

To a 25 mL round bottom flask, N-(4,4-diethoxybutyl)-2,3-bis(3,4-dimethoxyphenyl)but-3-enamide (**10b**) (92 mg, 0.184 mmol, 1.0 eq) and para-toluene sulfonic acid monohydrate (35 mg, 0.184 mmol, 1.0 eq) were dissolved in 7 mL toluene. The reaction mixture was refluxed (oil bath temp 115 °C) for 2 hours. The resulting bright yellow solution was cooled to room temperature, quenched with 10 mL of a saturated $NaHCO_3$ solution, and extracted with 2 x 10 mL EtOAc. The organics were collected, dried over Na_2SO_4, and solvents were removed by rotary evaporation. The resulting mixture was purified using silica gel column chromatography (10-80% EtOAc/Hexanes) to give an off-white solid (63 mg, 84%) as a racemic mixture.



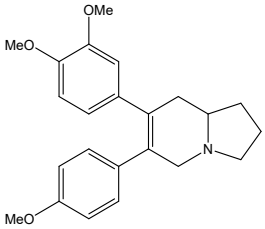
^{1}H NMR (600 MHz, $CDCl_3$) δ 6.75 – 6.68 (m, 4H), 6.66 (dd, *J* = 8.3, 1.7 Hz, 1H), 6.48 (d, *J* = 1.7 Hz, 1H), 3.99 – 3.91 (m, 1H), 3.83 (s, 3H), 3.82 (s, 3H), 3.74 – 3.67 (m, 1H), 3.70 (s, 3H), 3.64 – 3.57 (m, 1H), 3.52 (s, 3H), 2.87 (dd, *J* = 16.3, 4.4 Hz, 1H), 2.71 (dd, *J* = 16.2, 14.0 Hz, 1H), 2.31 (dt, *J* = 11.4, 5.6 Hz, 1H), 2.12 – 2.06 (m, 1H), 1.93 – 1.83 (m, 1H), 1.72 (ddd, *J* = 21.9, 11.8, 7.1 Hz, 1H).

^{13}C NMR (151 MHz, $CDCl_3$) δ 164.35, 148.55, 148.38, 148.18, 148.07, 144.53, 132.85, 132.09, 129.24, 123.83, 121.01, 114.71, 112.64, 110.84, 110.57, 55.96, 55.93, 55.74, 44.97, 37.50, 33.98, 23.37.

HRMS (EI) m/z $[M+H]^+$: Calcd for $C_{24}H_{27}NO_5$: 409.1889 Found 409.1874 [22]

(±)-Seco-Antofine (2):

To a two neck 10 mL round bottom flask attached to a condenser, 7-(3,4-dimethoxyphenyl)-6-(4-methoxyphenyl)-2,3,8,8a-tetrahydroindolizin-5(1H)-one (**11a**) (18.2 mg, 0.0479 mmol, 1.0 eq) was dissolved in 3 mL of anhydrous toluene under a N_2 atmosphere. Under stirring, Red-Al (100 µL, 0.512 mmol, 10 eq) was added dropwise to the solution at room temperature and the reaction was heated to 60 °C for 2 hours. After cooling to room temperature, the mixture was quenched with addition of EtOAc followed by a few drops of water. The mixture was washed with 10 mL H_2O and extracted with 2 x 10 mL EtOAc. The organics were collected, dried over Na_2SO_4, and solvents were removed by rotary evaporation. The resulting mixture was purified using silica gel column chromatography (EtOAc) to give (±)-Seco-Antofine (13 mg, 74%) as a pale-yellow solid.



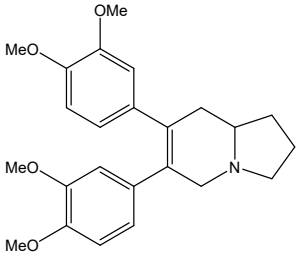
^{1}H NMR (600 MHz, $CDCl_3$) δ 6.98 – 6.94 (m, 2H), 6.70 – 6.64 (m, 4H), 6.47 (d, *J* = 1.6 Hz, 1H), 3.86 (d, *J* = 16.0 Hz, 1H), 3.81 (s, 3H), 3.72 (s, 3H), 3.54 (s, 3H), 3.30 (td, *J* = 8.7, 1.9 Hz, 1H), 3.10 – 3.04 (m, 1H), 2.77 – 2.69 (m, 1H), 2.45 – 2.35 (m, 2H), 2.25 (q, *J* = 9.0 Hz, 1H), 2.15 – 2.06 (m, 1H), 1.99 – 1.89 (m, 1H), 1.88 – 1.79 (m, 1H), 1.61 – 1.51 (m, 1H).

^{13}C NMR (151 MHz, $CDCl_3$) δ 158.14, 148.03, 147.31, 135.17, 132.76, 130.35, 120.81, 113.53, 113.23, 110.59, 60.59, 57.97, 55.83, 55.63, 55.28, 54.38, 38.63, 30.95, 21.66.

HRMS (ESI) m/z $[M+H]^+$: Calcd for $C_{23}H_{28}NO_3$: 366.2069. Found: 366.2082.

(±)-Septicine (3):

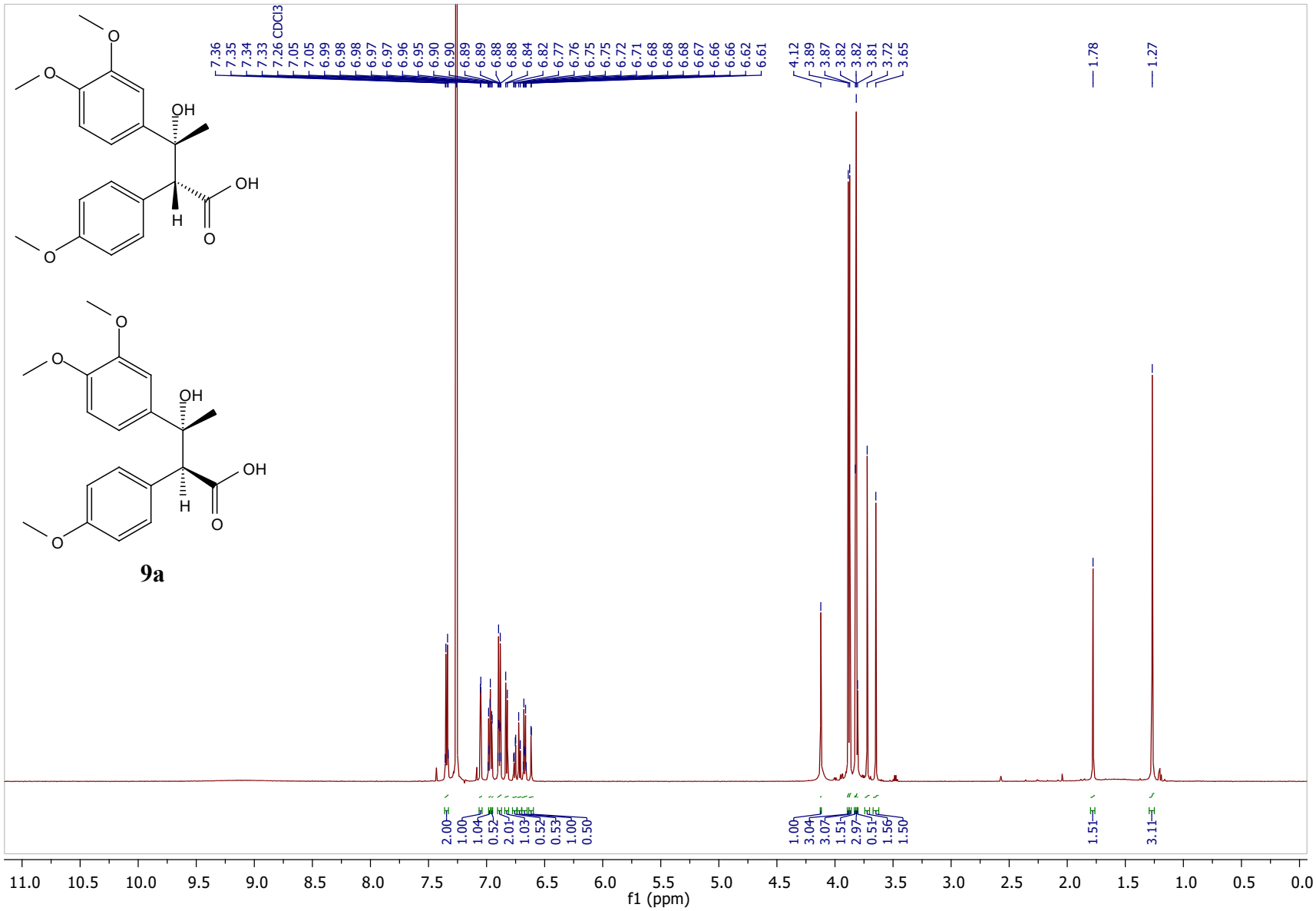
To a two neck 10 mL round bottom flask attached to a condenser, 6,7-bis(3,4-dimethoxyphenyl)-2,3,8,8a-tetrahydroindolizin-5(1H)-one (**11b**) (12.2 mg, 0.0297 mmol, 1.0 eq) was dissolved in 3 mL of anhydrous toluene under a N_2 atmosphere. Under stirring, Red-Al (100 µL, 17 eq) was added dropwise to the solution at room temperature and the reaction was heated to 60 °C for 2 hours. After cooling to room temperature, the mixture was quenched with addition of EtOAc followed by a few drops of water. The mixture was washed with 10 mL H_2O and extracted with 2 x 10 mL EtOAc. The organics were collected, dried over Na_2SO_4, and solvents were removed by rotary evaporation. The resulting mixture was purified using silica gel column chromatography (0-5% MeOH/EtOAc) to give (±)-Septicine (8 mg, 68%) as a pale-yellow solid.



^{1}H NMR (600 MHz, $CDCl_3$) δ 6.67 (dd, *J* = 8.3, 2.3 Hz, 2H), 6.65 (dd, *J* = 8.3, 1.1 Hz, 2H), 6.53 (dd, *J* = 10.4, 1.8 Hz, 2H), 3.89 (d, *J* = 16.4 Hz, 1H), 3.81 (s, 3H), 3.80 (s, 3H), 3.60 (s, 3H), 3.57 (s, 3H), 3.32 (td, *J* = 8.6, 1.3 Hz, 1H), 3.12 – 3.06 (m, 1H), 2.78 – 2.69 (m, 1H), 2.45 – 2.37 (m, 2H), 2.26 (q, *J* = 9.0 Hz, 1H), 2.15 – 2.07 (m, 1H), 2.00 – 1.91 (m, 1H), 1.87 – 1.79 (m, 1H), 1.60 – 1.51 (m, 1H).

^{13}C NMR (151 MHz, $CDCl_3$) δ 148.38, 148.23, 147.59, 147.43, 135.39, 133.92, 133.04, 132.94, 121.16, 120.88, 113.17, 112.99, 110.79, 110.72, 60.57, 57.84, 55.91, 55.78, 55.73, 54.41, 38.80, 30.99, 21.67.

HRMS (ESI) m/z $[M+H]^+$: Calcd for $C_{24}H_{30}NO_4$: 396.2175. Found: 396.2180.


7.36
7.35
7.34
7.33
CDCl3
7.26
7.05
7.05
6.99
6.98
6.98
6.97
6.97
6.96
6.95
6.90
6.90
6.89
6.89
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3.81
3.72
3.65
1.78
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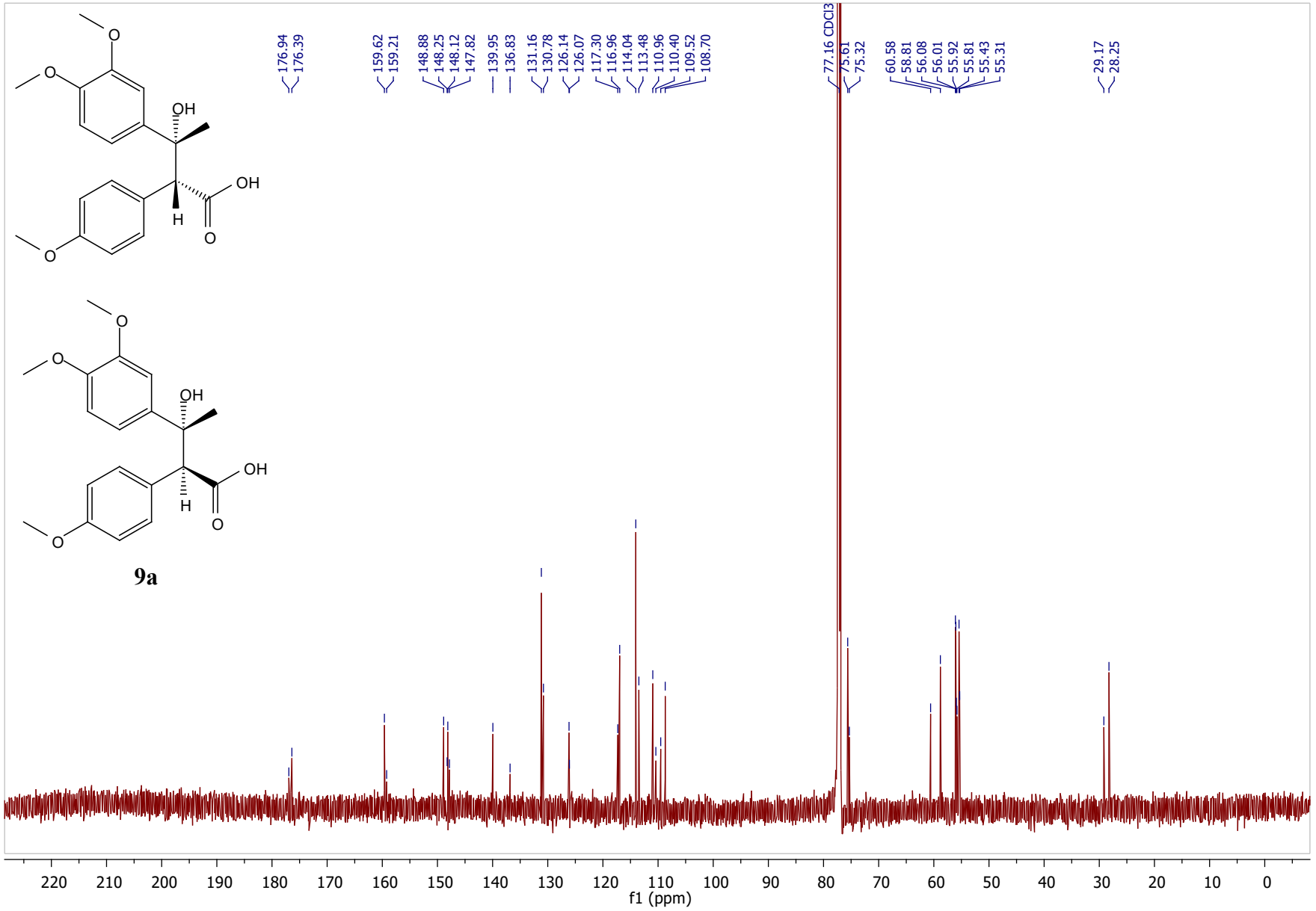
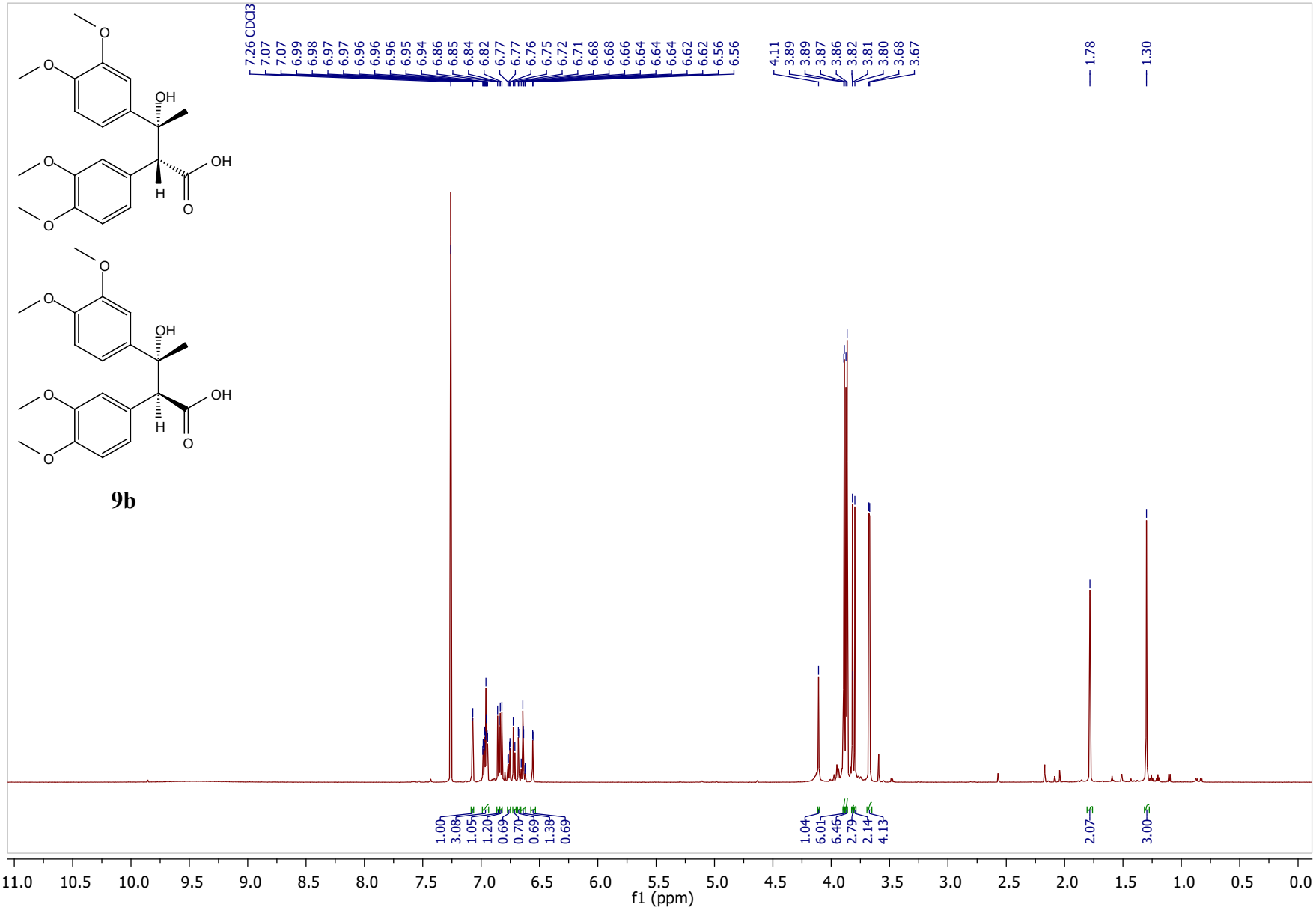
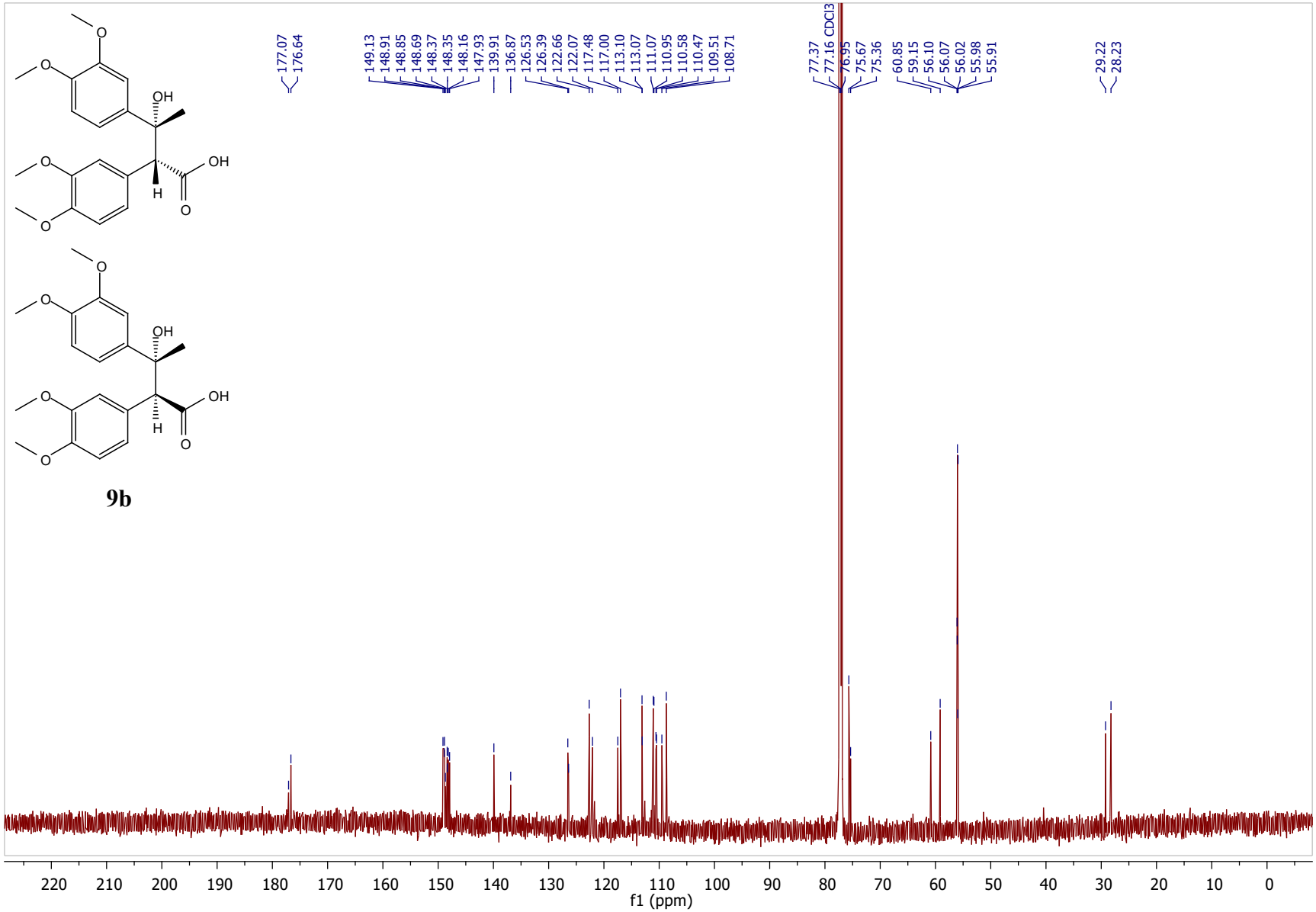
O
O
9a
2.00
1.00
1.04
0.52
2.01
1.03
0.52
0.53
1.00
0.50
1.00
3.04
3.07
1.51
2.97
0.51
1.56
1.50
1.51
3.11
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

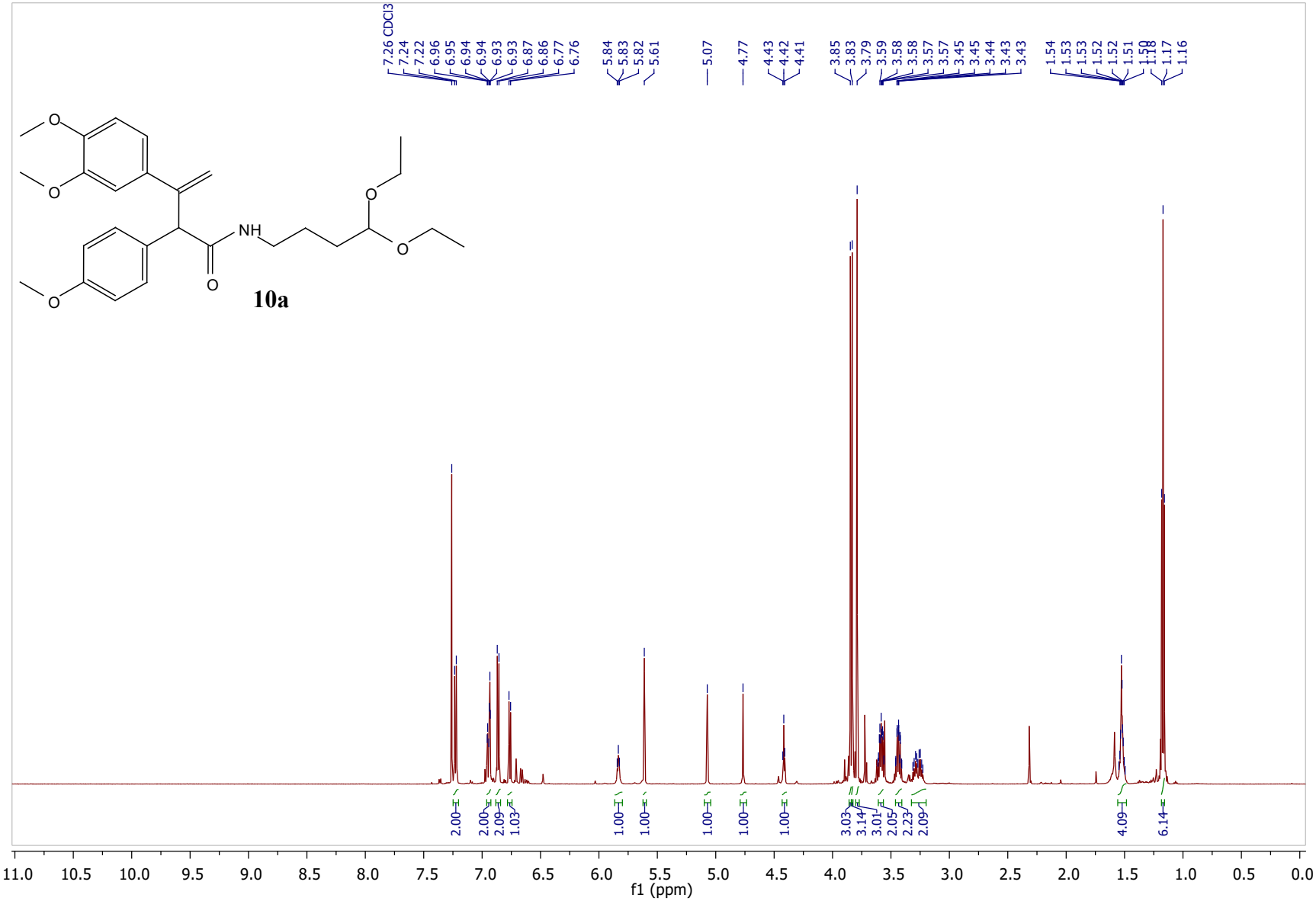

176.94
176.39
159.62
159.21
148.88
148.25
148.12
147.82
139.95
136.83
131.16
130.78
126.14
126.07
117.30
116.96
114.04
113.48
110.96
110.40
109.52
108.70
77.16 CDCl3
75.61
75.32
60.58
58.81
56.08
56.01
55.92
55.81
55.43
55.31
29.17
28.25
OH
OH
H
O
O
O
O
OH
OH
H
O
O
O
9a
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

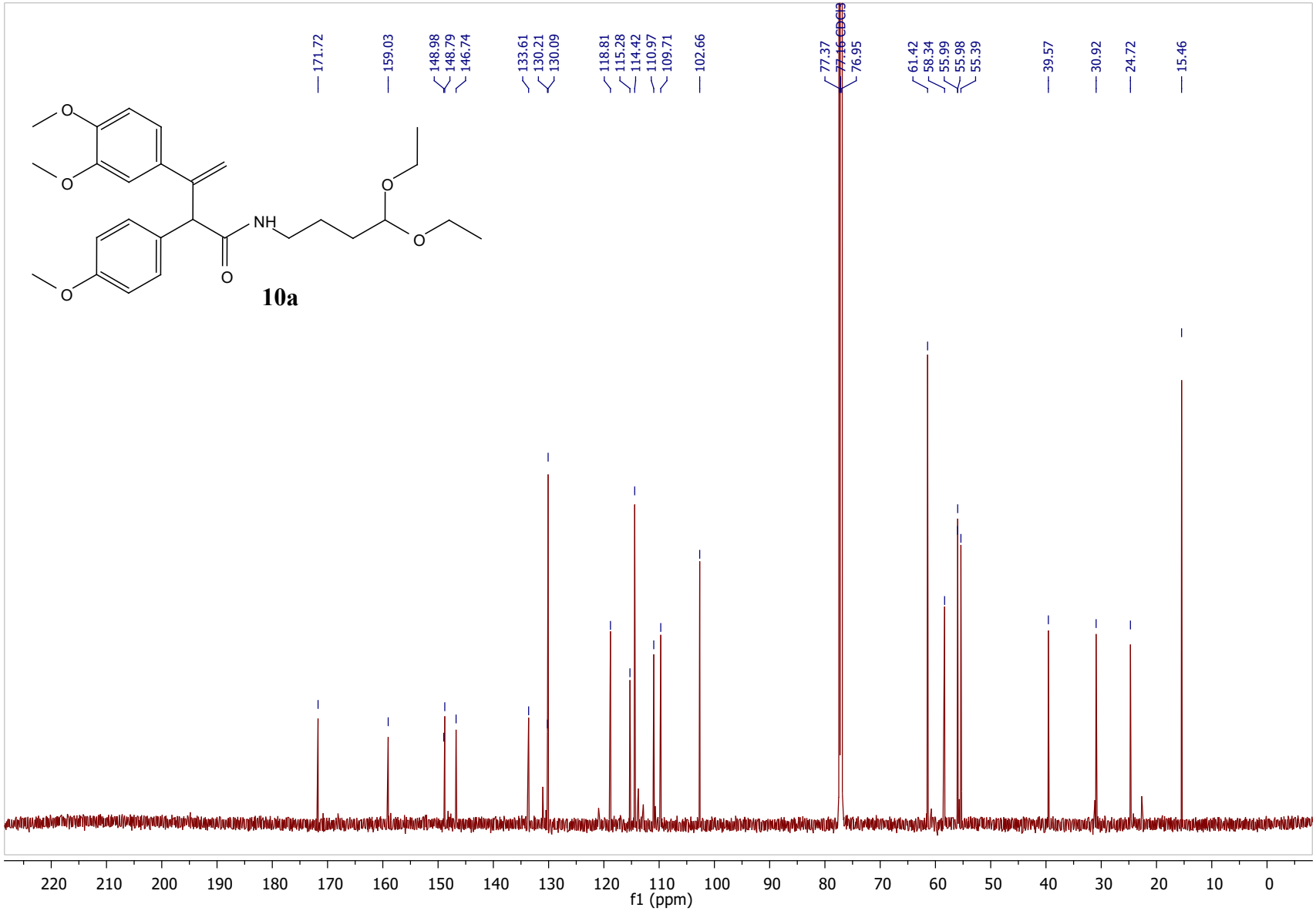

7.26 CDCl3
7.07
7.07
6.99
6.98
6.97
6.97
6.96
6.96
6.96
6.95
6.94
6.86
6.85
6.84
6.82
6.77
6.77
6.76
6.75
6.72
6.71
6.68
6.68
6.66
6.64
6.64
6.64
6.62
6.62
6.56
6.56
4.11
3.89
3.89
3.87
3.86
3.82
3.81
3.80
3.68
3.67
1.78
1.30
OH
OH
H
O
O
O
O
O
O
1.00
3.08
1.05
1.20
0.69
0.70
0.69
1.38
0.69
1.04
6.01
6.46
2.79
2.14
4.13
2.07
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
f1 (ppm)

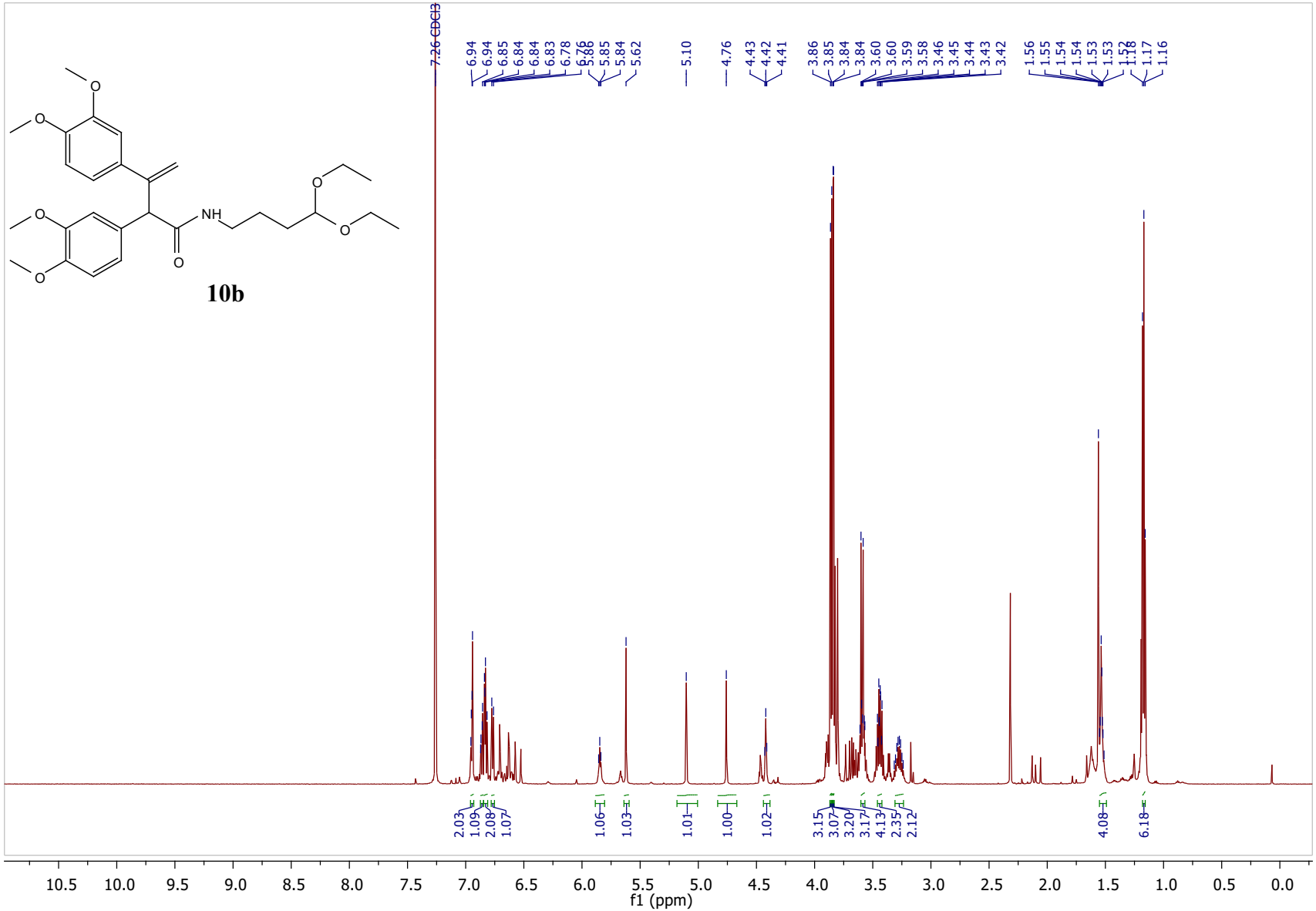
9b


177.07
176.64
149.13
148.91
148.85
148.69
148.37
148.35
148.16
147.93
139.91
136.87
126.53
126.39
122.66
122.07
117.48
117.00
113.10
113.07
111.07
110.95
110.58
110.47
109.51
108.71
77.37
77.16 CDCl3
76.95
75.67
75.36
60.85
59.15
56.10
56.07
56.02
55.98
55.91
29.22
28.23
OH
OH
O
H
O
O
O
O
O
f1 (ppm)

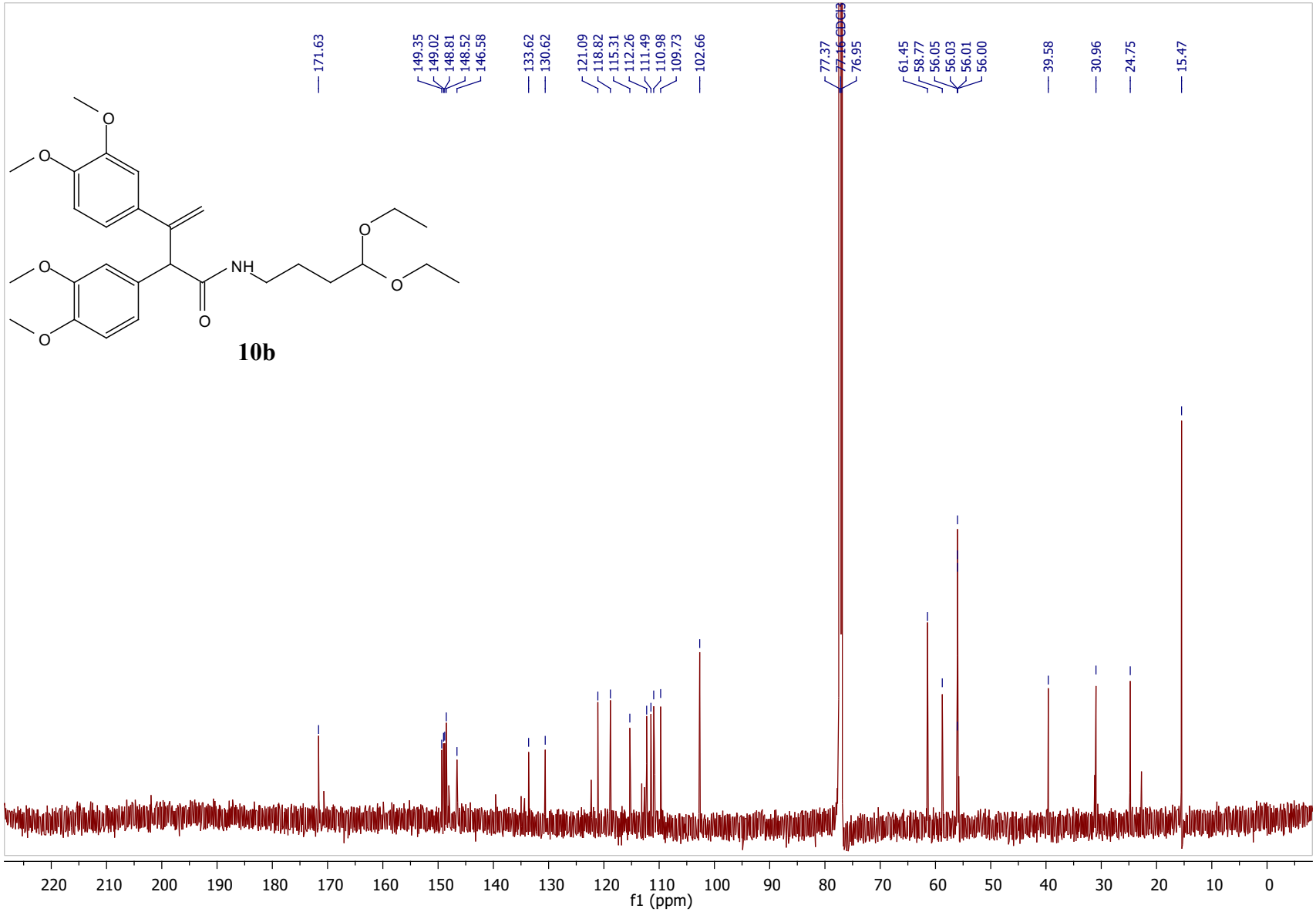
9b

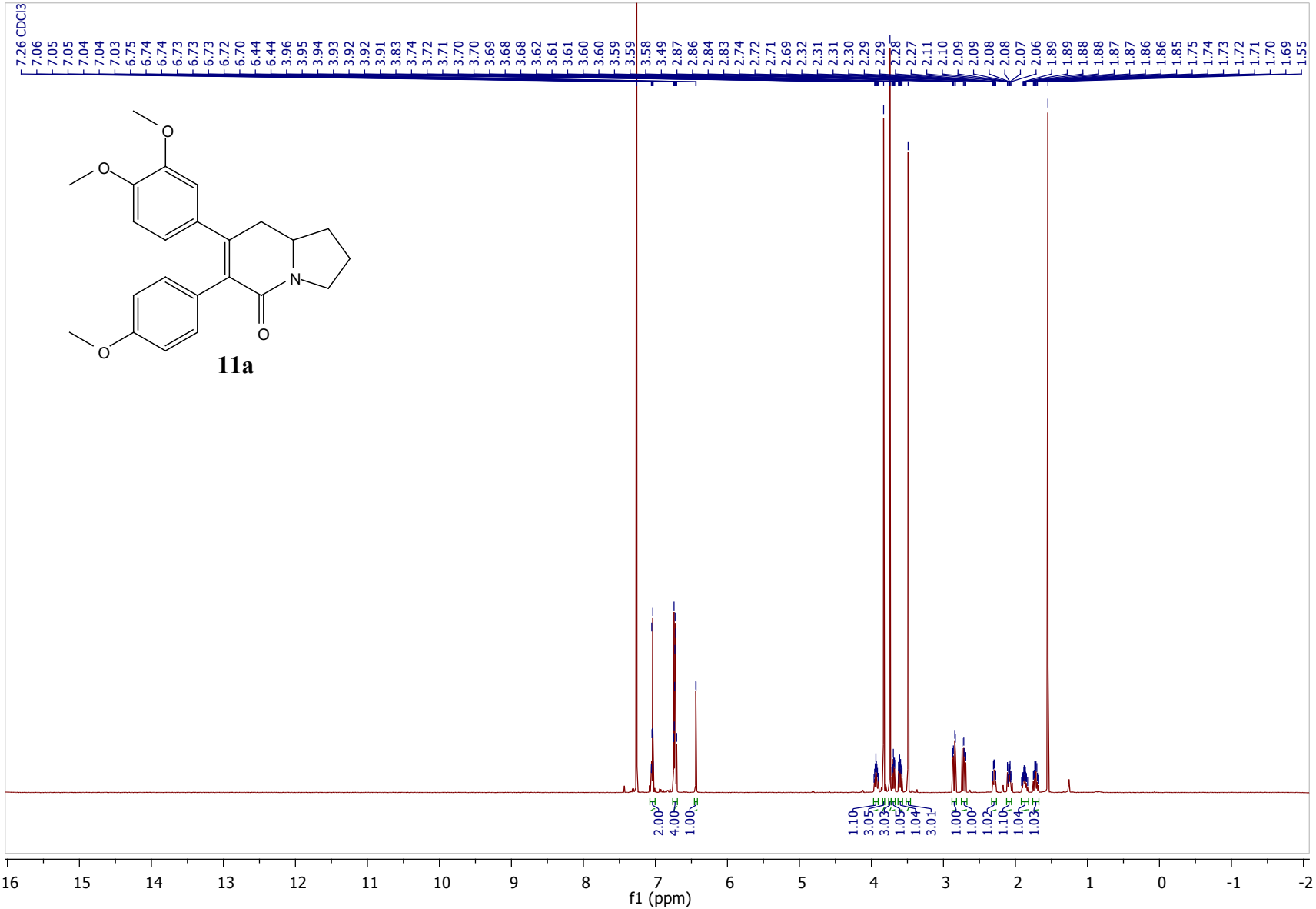

7.26 CDCl3
7.24
7.22
6.96
6.95
6.94
6.94
6.93
6.93
6.87
6.86
6.77
6.76
5.84
5.83
5.82
5.61
5.07
4.77
4.43
4.42
4.41
3.85
3.83
3.79
3.59
3.58
3.58
3.57
3.57
3.45
3.45
3.44
3.43
3.43
1.54
1.53
1.53
1.52
1.52
1.51
1.17
1.16
NH
O
10a
2.00
2.00
2.09
1.03
1.00
1.00
1.00
1.00
1.00
3.03
3.14
3.01
2.05
2.23
2.09
4.09
6.14
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
f1 (ppm)


171.72
159.03
148.98
148.79
146.74
133.61
130.21
130.09
118.81
115.28
114.42
110.97
109.71
102.66
77.37
77.16 CDCl3
76.95
61.42
58.34
55.99
55.98
55.39
39.57
30.92
24.72
15.46
NH
O
10a
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

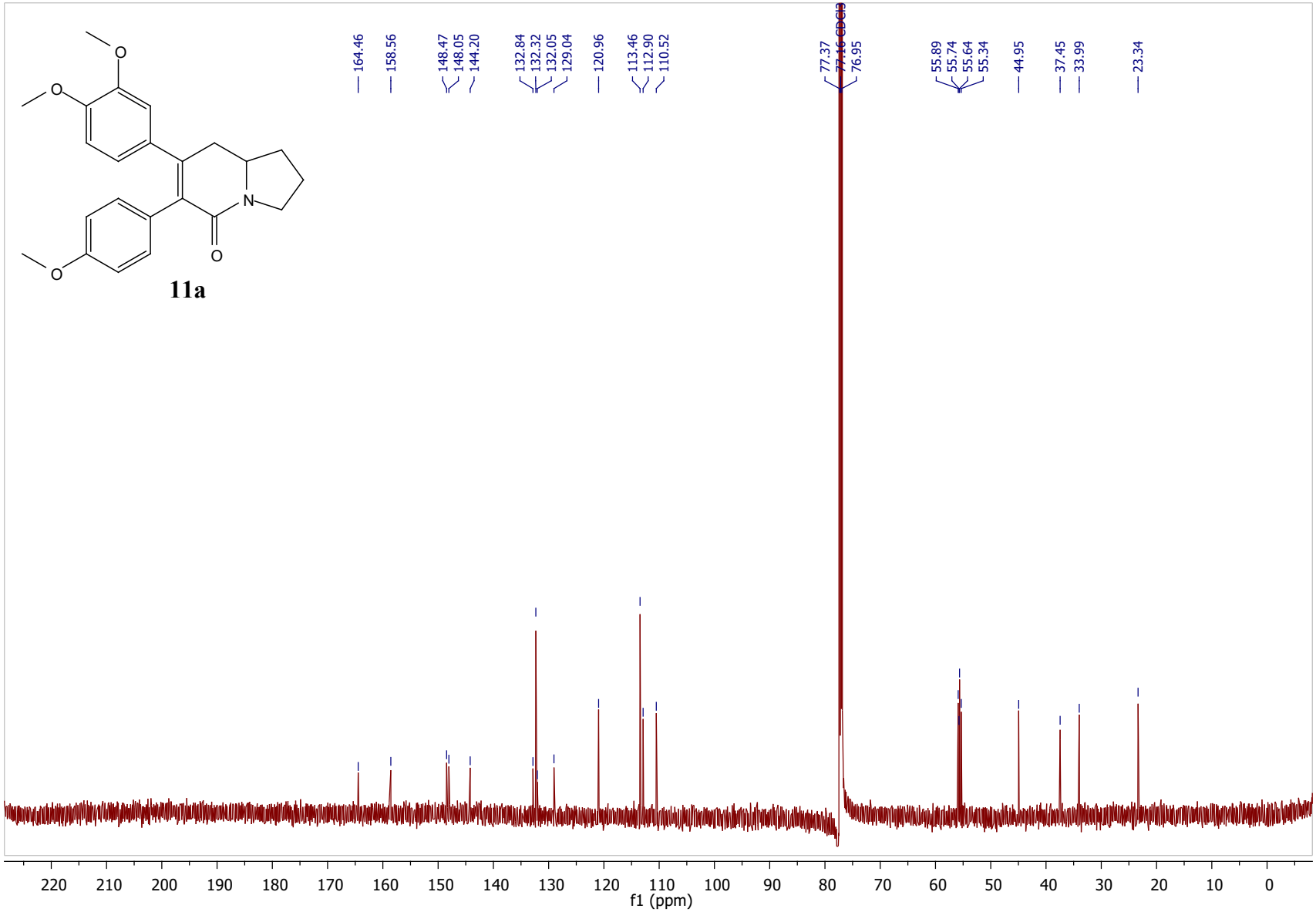

7.26 CDCl3
6.94
6.94
6.85
6.84
6.84
6.83
6.78
5.88
5.85
5.84
5.62
5.10
4.76
4.43
4.42
4.41
3.86
3.85
3.84
3.84
3.60
3.60
3.59
3.58
3.46
3.45
3.44
3.43
3.42
1.56
1.55
1.54
1.54
1.53
1.53
1.18
1.17
1.16
2.03
1.09
2.08
1.07
1.06
1.03
1.01
1.00
1.02
3.15
3.07
3.20
3.17
4.13
2.35
2.12
4.08
6.18
10.5
10.0
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
f1 (ppm)

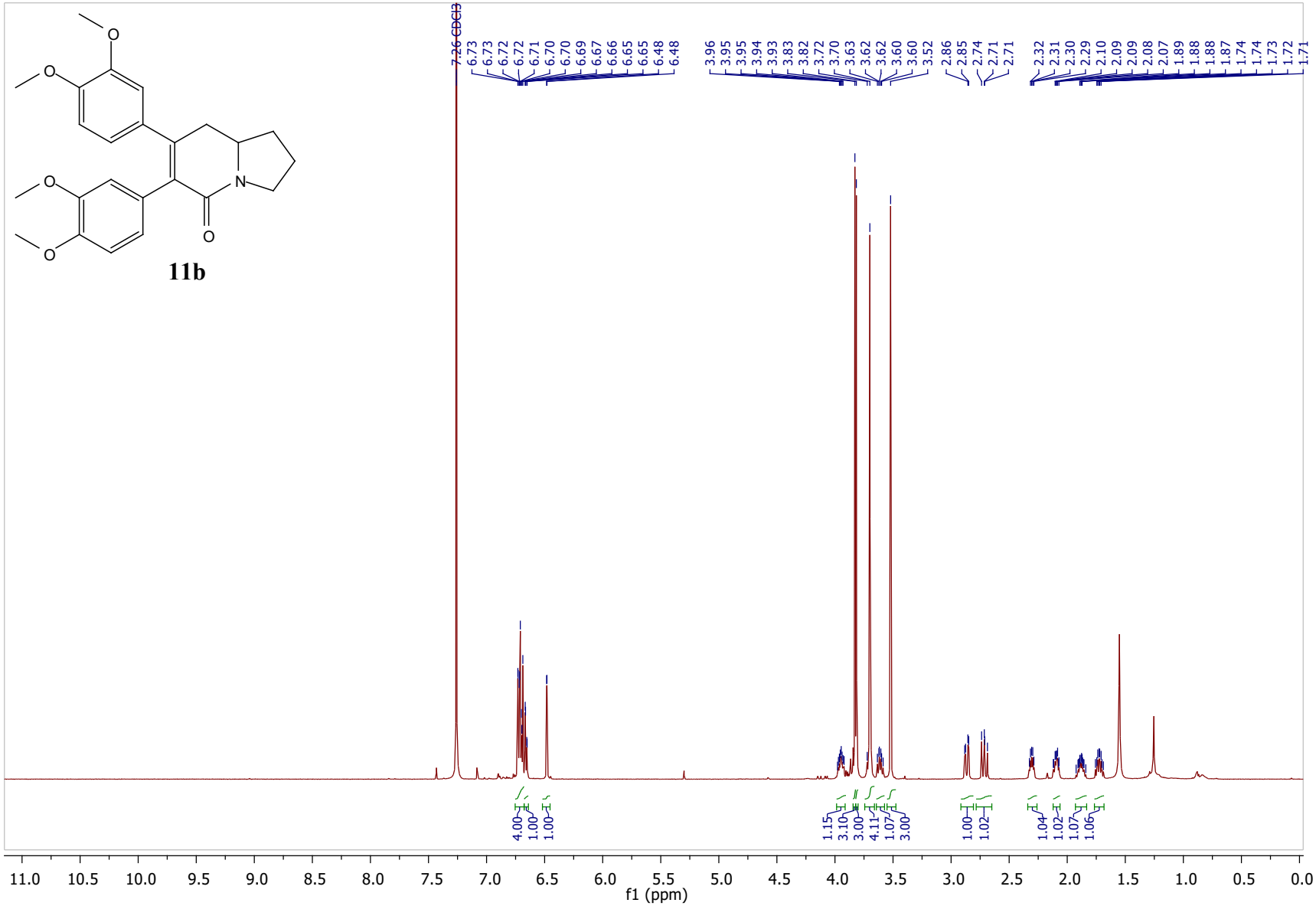
10b

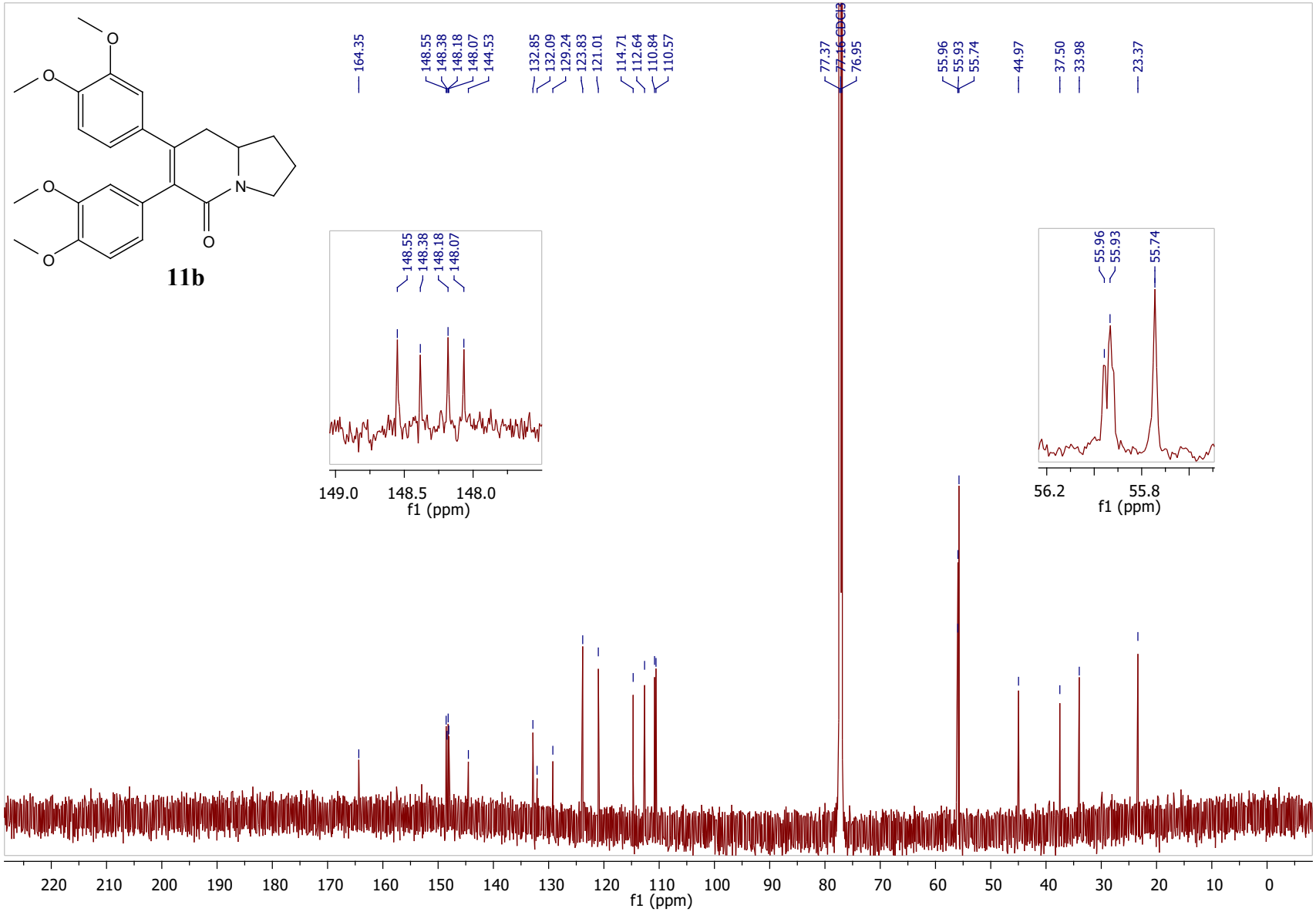

171.63
149.35
149.02
148.81
148.52
146.58
133.62
130.62
121.09
118.82
115.31
112.26
111.49
110.98
109.73
102.66
77.37
77.16 CDCl3
76.95
61.45
58.77
56.05
56.03
56.01
56.00
39.58
30.96
24.75
15.47
O
O
O
NH
O
O
O
O
10b
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

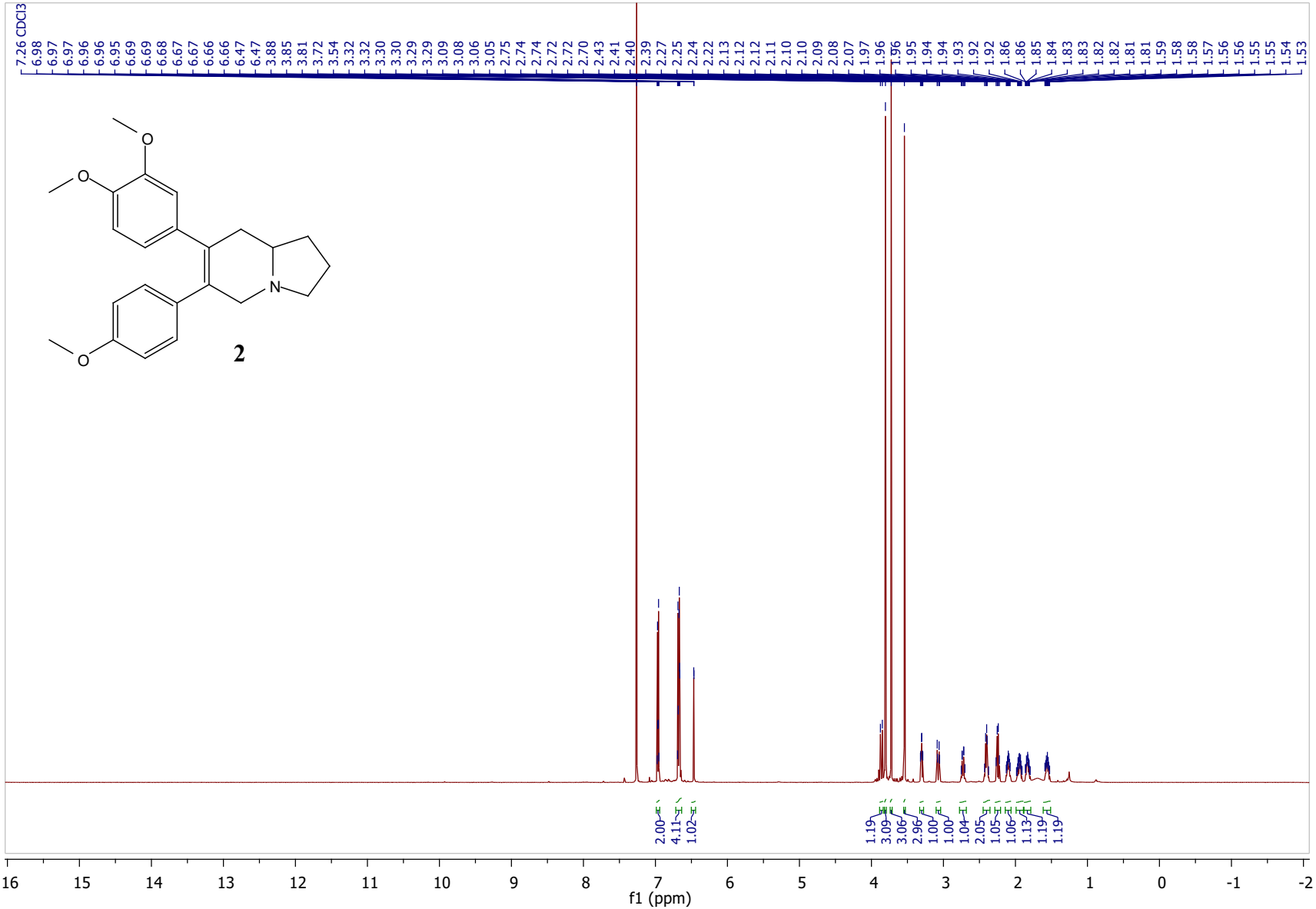

7.26 CDCl3
7.06 7.05 7.05 7.04 7.04 7.03 6.75 6.74 6.74 6.73 6.73 6.73 6.72 6.70 6.44 6.44 3.96 3.95 3.94 3.93 3.92 3.92 3.91 3.83 3.74 3.72 3.71 3.70 3.70 3.69 3.68 3.68 3.62 3.61 3.61 3.60 3.60 3.59 3.59 3.58 3.49 2.87 2.86 2.84 2.83 2.74 2.72 2.71 2.69 2.32 2.31 2.31 2.30 2.29 2.29 2.28 2.27 2.11 2.10 2.09 2.09 2.08 2.08 2.07 2.06 1.89 1.89 1.88 1.88 1.87 1.87 1.86 1.86 1.85 1.75 1.74 1.73 1.72 1.71 1.70 1.69 1.55
O
O
O
N
O
2.00 4.00 1.00 1.10 3.05 3.03 1.05 1.04 3.01 1.00 1.00 1.02 1.10 1.04 1.03
16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2
f1 (ppm)

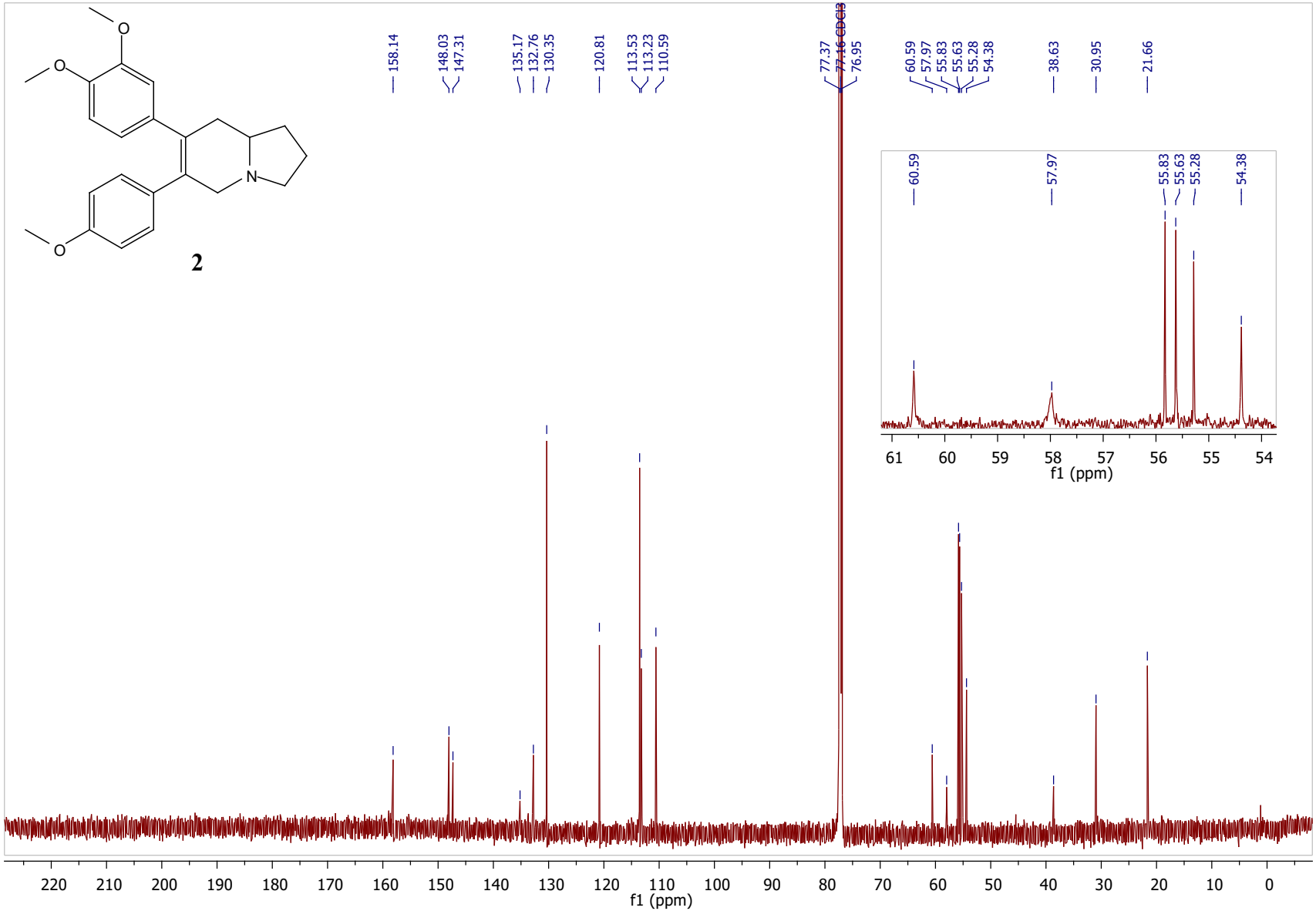
11a

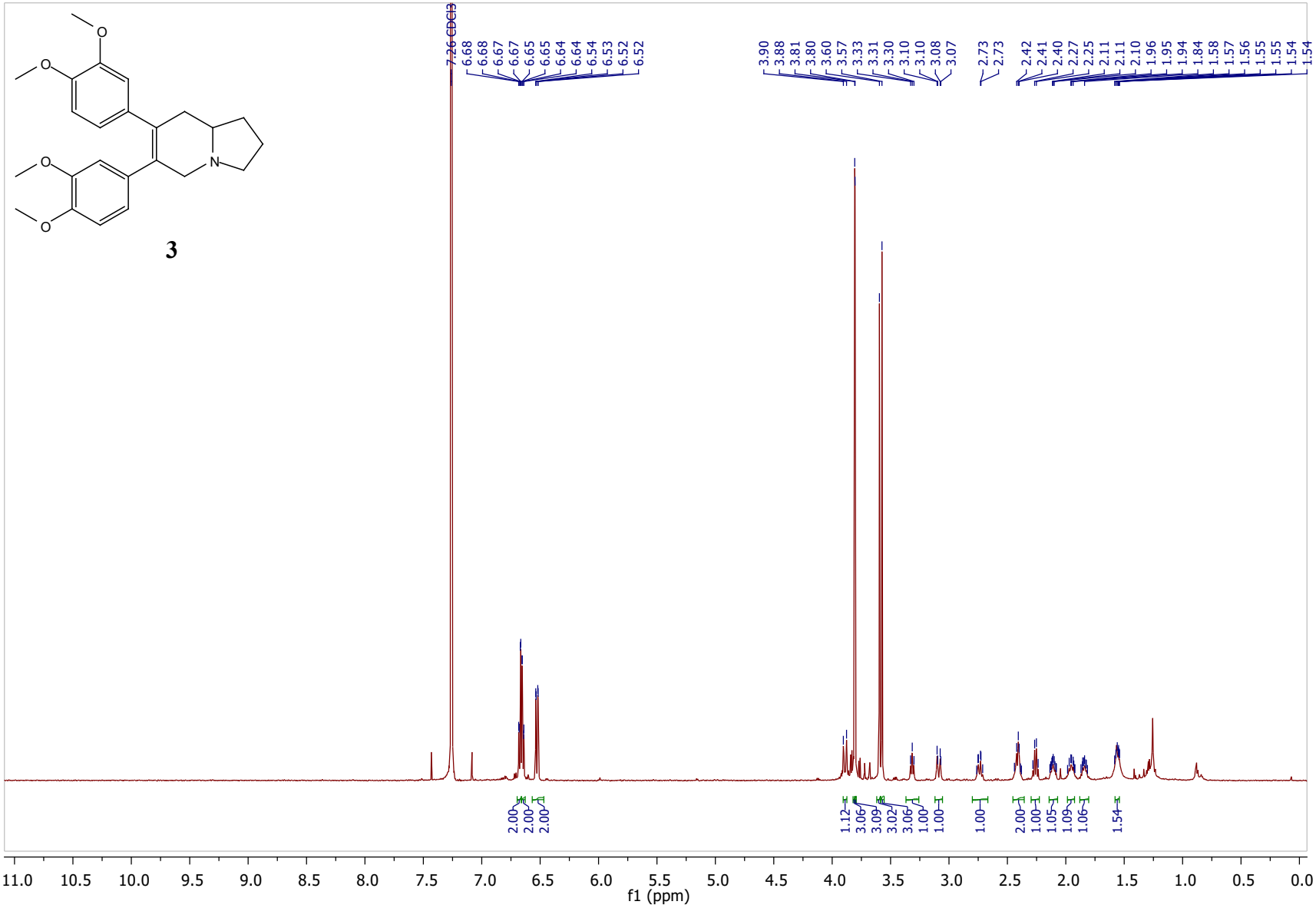

O
O
O
N
O
11a
164.46
158.56
148.47
148.05
144.20
132.84
132.32
132.05
129.04
120.96
113.46
112.90
110.52
77.37
77.16 CDCl3
76.95
55.89
55.74
55.64
55.34
44.95
37.45
33.99
23.34
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

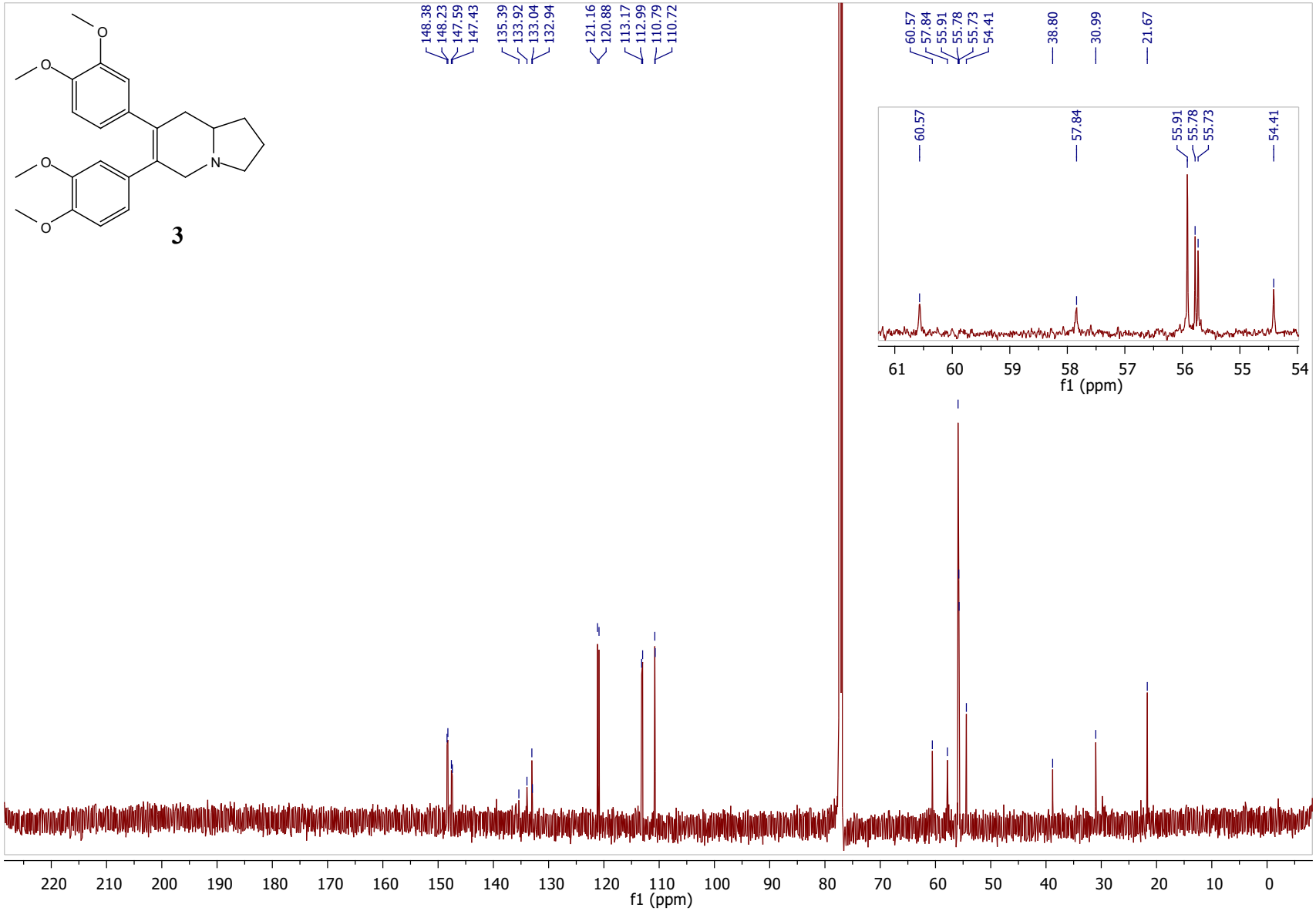

O
O
O
O
N
O
11b
7.26 CDCl3
6.73
6.73
6.72
6.72
6.71
6.70
6.70
6.69
6.67
6.66
6.65
6.65
6.48
6.48
3.96
3.95
3.95
3.94
3.93
3.83
3.82
3.72
3.70
3.63
3.62
3.62
3.60
3.60
3.52
2.86
2.85
2.74
2.71
2.71
2.32
2.31
2.30
2.29
2.10
2.09
2.09
2.08
2.07
1.89
1.88
1.88
1.87
1.74
1.74
1.73
1.72
1.71
4.00
1.00
1.00
1.15
3.10
3.00
4.11
1.07
3.00
1.00
1.02
1.04
1.02
1.07
1.06
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
f1 (ppm)

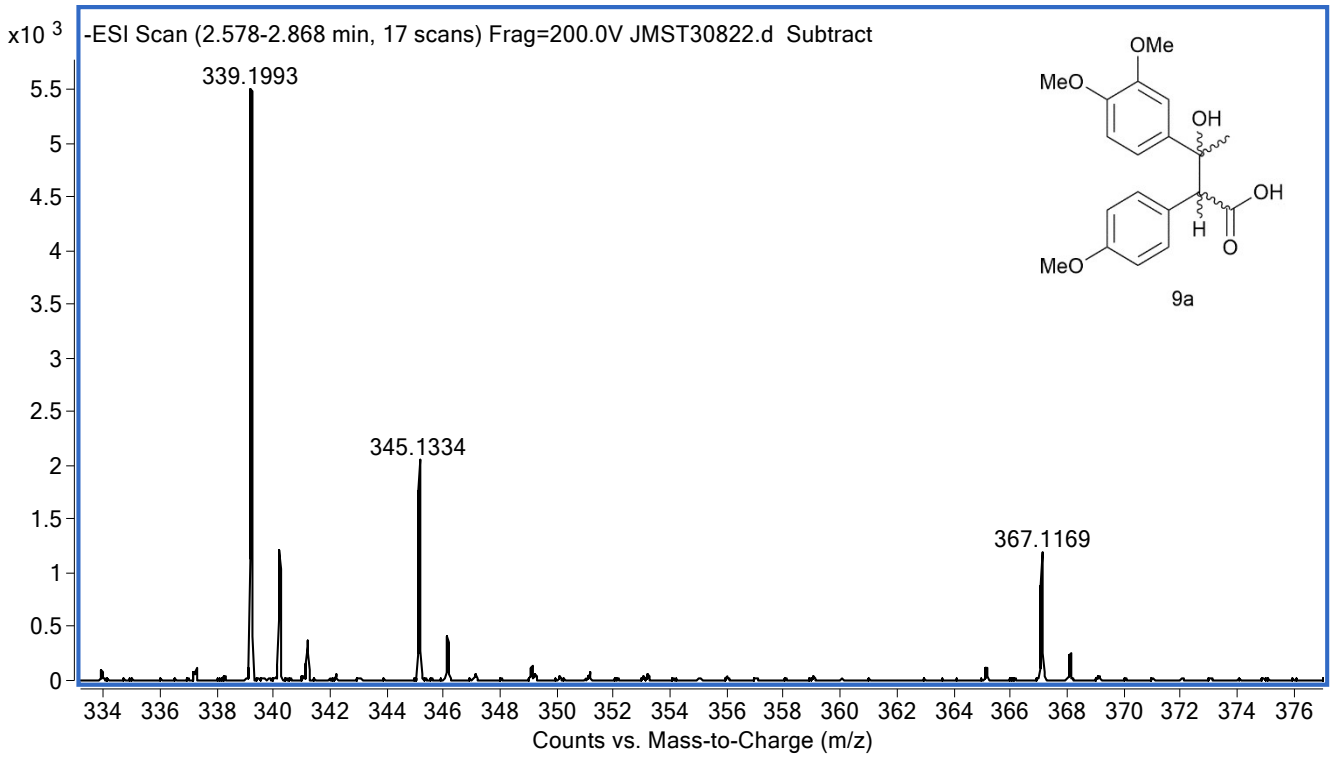

11b
164.35
148.55
148.38
148.18
148.07
144.53
132.85
132.09
129.24
123.83
121.01
114.71
112.64
110.84
110.57
77.37
77.16 CDCl3
76.95
55.96
55.93
55.74
44.97
37.50
33.98
23.37
149.0
148.5
148.0
f1 (ppm)
56.2
55.8
f1 (ppm)
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

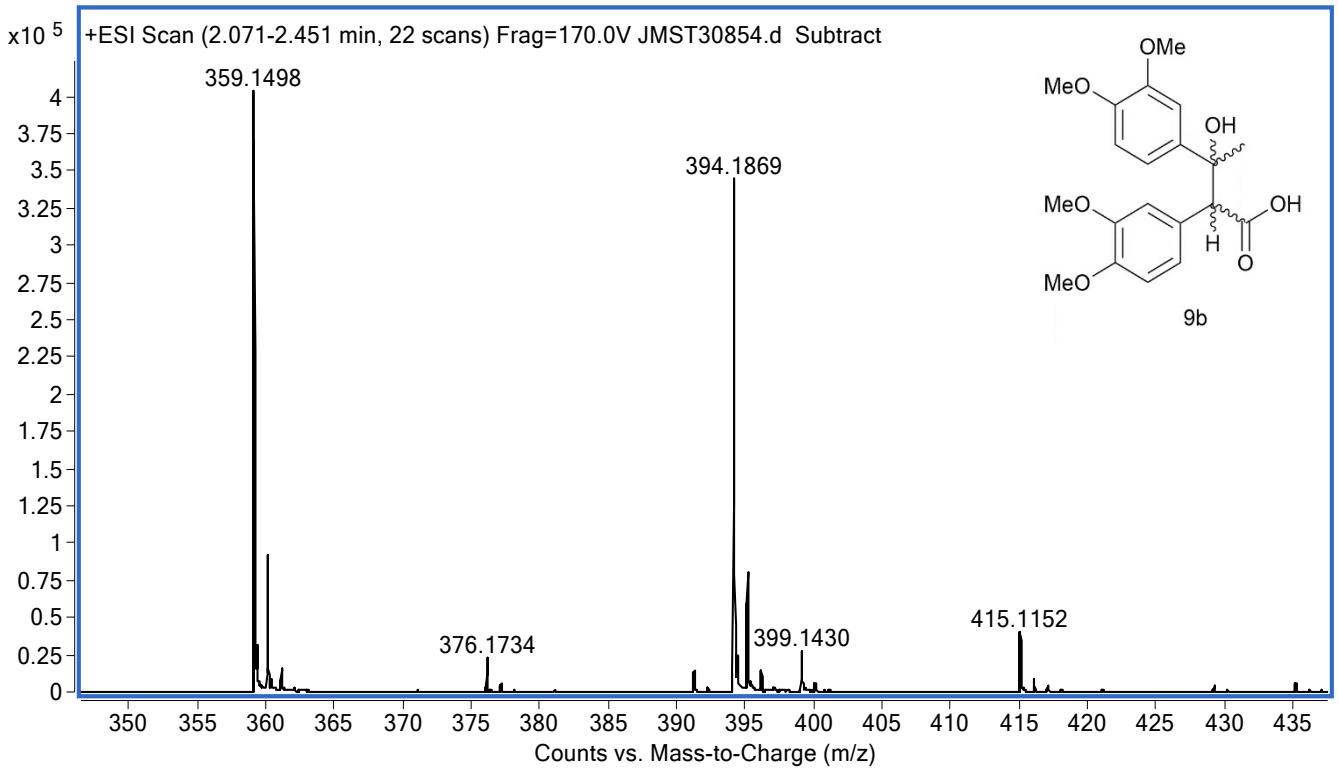

7.26 CDCl3
6.98 6.97 6.97 6.96 6.96 6.95 6.69 6.69 6.68 6.67 6.67 6.66 6.66 6.47 6.47 3.88 3.85 3.81 3.72 3.54 3.32 3.32 3.30 3.30 3.29 3.29 3.09 3.08 3.06 3.05 2.75 2.74 2.74 2.72 2.72 2.70 2.43 2.41 2.40 2.39 2.27 2.25 2.24 2.22 2.13 2.12 2.12 2.11 2.10 2.10 2.09 2.08 2.07 1.97 1.96 1.96 1.95 1.94 1.94 1.93 1.92 1.92 1.86 1.86 1.85 1.84 1.83 1.83 1.82 1.82 1.81 1.81 1.59 1.58 1.58 1.57 1.56 1.56 1.55 1.55 1.54 1.53
O
O
O
N
2
2.00 4.11 1.02
1.19 3.09 3.06 2.96 1.00 1.00 1.04 2.05 1.05 1.06 1.13 1.19 1.19
16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2
f1 (ppm)

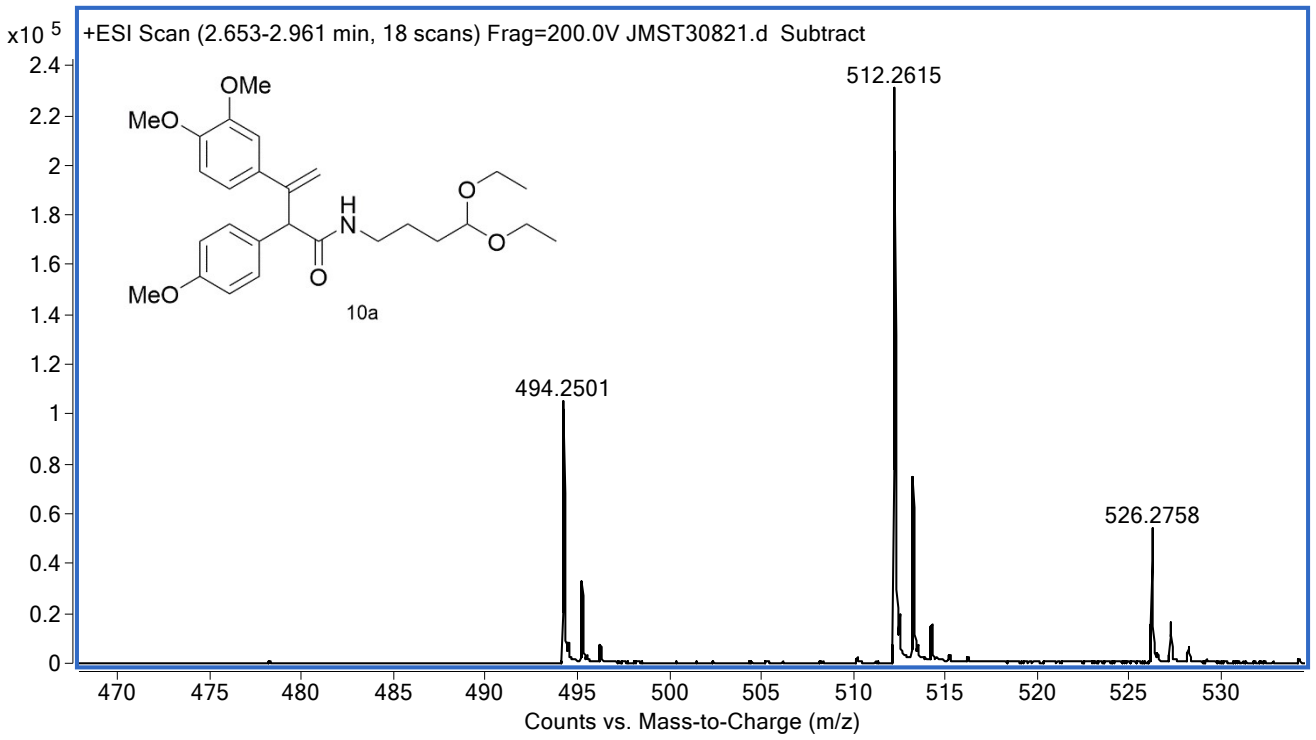

O
O
O
N
2
158.14
148.03
147.31
135.17
132.76
130.35
120.81
113.53
113.23
110.59
77.37
77.16 CDCl3
76.95
60.59
57.97
55.83
55.63
55.28
54.38
38.63
30.95
21.66
f1 (ppm)

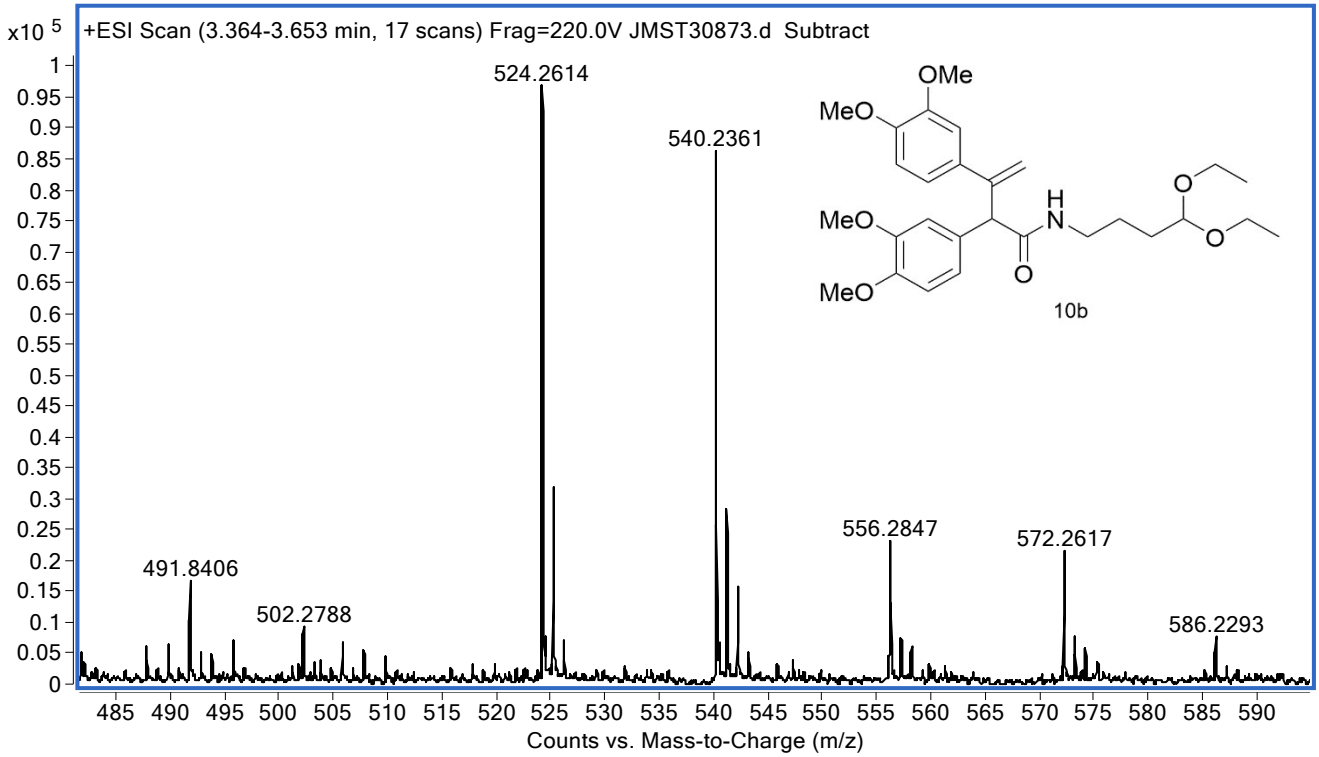

O
O
O
O
N
3
7.26 CDCl3
6.68
6.68
6.67
6.67
6.65
6.65
6.64
6.64
6.54
6.53
6.52
6.52
3.90
3.88
3.81
3.80
3.60
3.57
3.33
3.31
3.30
3.10
3.10
3.08
3.07
2.73
2.73
2.42
2.41
2.40
2.27
2.25
2.11
2.11
2.10
1.96
1.95
1.94
1.84
1.58
1.57
1.56
1.55
1.55
1.54
1.54
2.00
2.00
2.00
1.12
3.06
3.09
3.02
3.06
1.00
1.00
1.00
1.00
2.00
1.00
1.05
1.09
1.06
1.54
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
f1 (ppm)


148.38
148.23
147.59
147.43
135.39
133.92
133.04
132.94
121.16
120.88
113.17
112.99
110.79
110.72
60.57
57.84
55.91
55.78
55.73
54.41
38.80
30.99
21.67
3
f1 (ppm)
f1 (ppm)


x10 3
-ESI Scan (2.578-2.868 min, 17 scans) Frag=200.0V JMST30822.d Subtract
339.1993
345.1334
367.1169
OMe
MeO
OH
OH
H
O
MeO
9a
Counts vs. Mass-to-Charge (m/z)


x10 5
+ESI Scan (2.071-2.451 min, 22 scans) Frag=170.0V JMST30854.d Subtract
359.1498
394.1869
376.1734
399.1430
415.1152
OMe
MeO
OH
MeO
OH
H
O
MeO
9b
Counts vs. Mass-to-Charge (m/z)


x10 5
+ESI Scan (2.653-2.961 min, 18 scans) Frag=200.0V JMST30821.d Subtract
OMe
MeO
MeO
H
N
O
O
O
10a
512.2615
494.2501
526.2758
Counts vs. Mass-to-Charge (m/z)

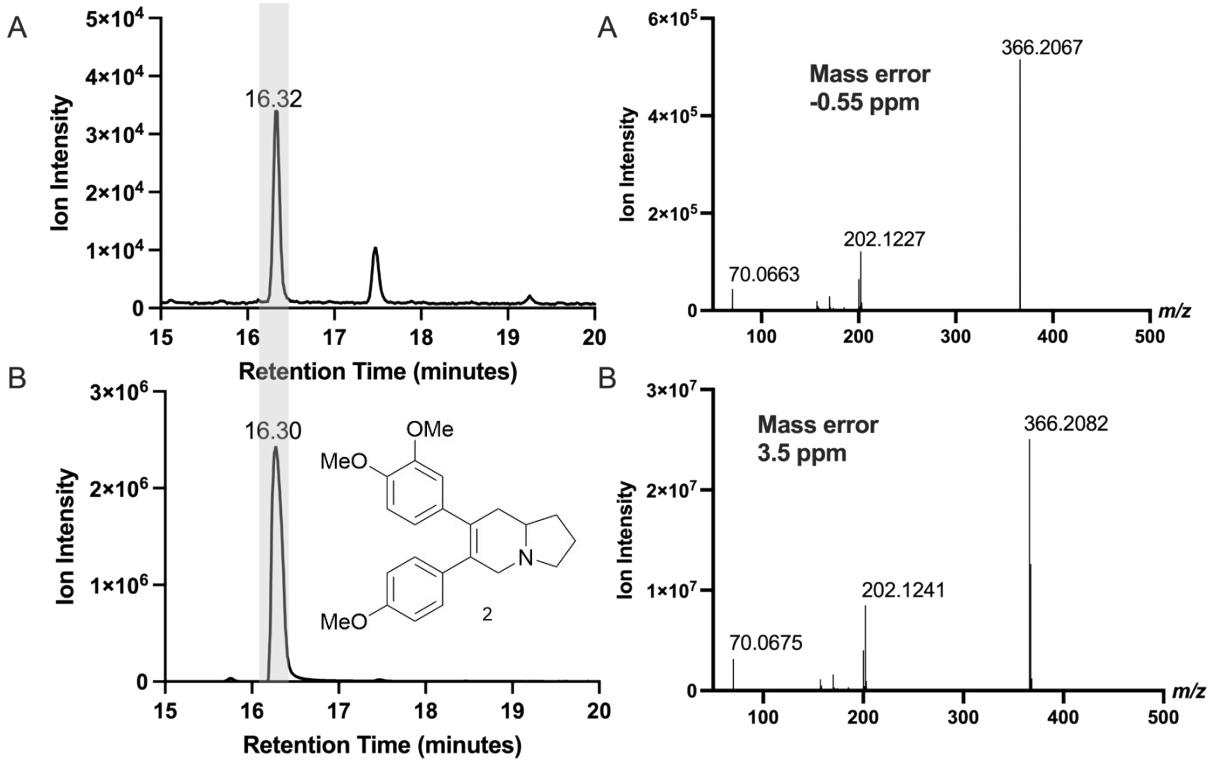

x10 5
+ESI Scan (3.364-3.653 min, 17 scans) Frag=220.0V JMST30873.d Subtract
524.2614
540.2361
OMe
MeO
MeO
MeO
H
N
O
O
O
10b
556.2847
572.2617
491.8406
502.2788
586.2293
Counts vs. Mass-to-Charge (m/z)

UPLC-ESI-QTOF-MS

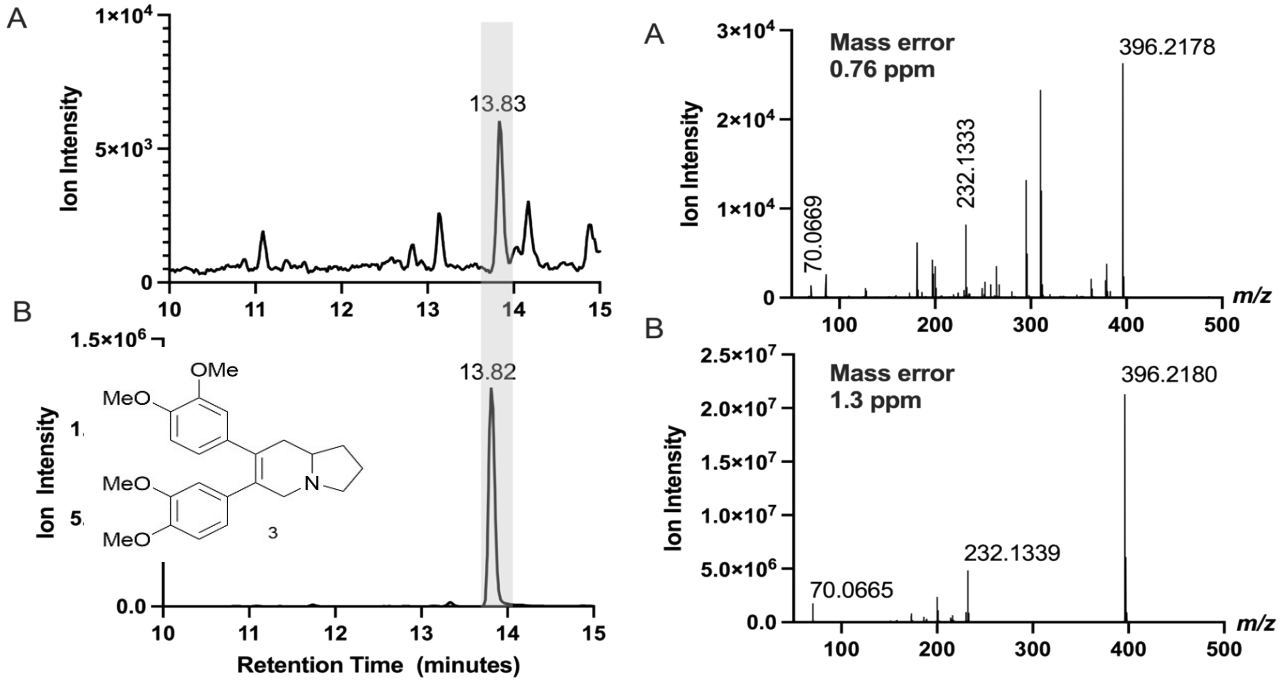
Chromatographic separation was performed on an Acquity I-Class UPLC (Waters, Manchester, UK) equipped with an Acquity UPLC BEH C18 1.7 μm column (Waters) using a binary solvent mixture consisting of solvent A (5% acetonitrile in water with 0.1% formic acid) and solvent B (acetonitrile with 0.1% formic acid), with 1 μL sample injection at an infusion flow rate of 0.3 mL/min. The gradient was set for solvent B at 0-40% for 0.0-22.5 min, 100% over 23.0-24.5 min, decreasing to 0% over 25.0-26.0 min.[1] Analytic data were acquired on a Xevo G2-XS QTOF (Waters) with a capillary voltage of 3 kV (ESI+) and sample cone voltage of 40 eV, with desolvation temperature of 500 °C, cone gas flow of 50 L/hr, and desolvation gas flow of 800 L/hr. MS/MS data was acquired over 5-20 min at a mass range of 50-800 Da using a scan time of 0.25 sec in continuum format at an energy ramp of 10-50 eV collision energy. MS/MS data and theoretical *m/z* were assessed with MassLynx 4.1 (Waters). Leucine enkephelin (200 pg/μL in 50:50 acetonitrile/water with 0.1% formic acid) was used as a reference calibrant with LockSpray ion source (Waters; infusion flow rate 10 μl/min) for exact mass measurement. Seco-antofine and septicine identification was conducted by comparing extracted ion chromatogram (retention time, *m/z*, fragmentation) of natural extracts from freshly prepared *V. rossicum* leaf, stem, and seed extracts with the synthetic standards of seco-antofine and septicine spiked in mobile phase A and tissue extracts. Mass errors were calculated using the formula: (observed *m/z* – theoretical *m/z*)/(theoretical *m/z* * 10^6).

References:

1 I. Rogachev, and A. Aharoni. *Plant Metabolomics,* 2011, 129-144.



Extracted ion chromtaograms (left) and UPLC-ESI-QTOF-MS/MS (right) of seco-antofine (**2**) ([M+H] = *m/z* 366.2069, [$C_{23}H_{27}NO_3$ + H+] in *Vincetoxicum rossicum* extracts (**A**) compared to the authentic standard of synthetic seco-antofine. Samples were run in positive electrospray ionization mode and MS/MS collected with a collision energy profile of 10-50 eV. Synthetic seco-antofine was spiked in the *V. rossicum* extraction matrix (**B**).



Extracted ion chromtaograms (left) and UPLC-ESI-QTOF-MS/MS (right) of septicine (**3**) ([M+H] = *m/z* 396.2175, [$C_{24}H_{29}NO_4$ + H+] in *Vincetoxicum rossicum* extracts (**A**) compared to the authentic standard of synthetic septicine. Samples were run in positive electrospray ionization mode and MS/MS collected with a collision energy profile of 10-50 eV. Synthetic septicine was spiked in the *V. rossicum* extraction matrix (**B**).