Chemically guided single-cell transcriptomics reveals sulfotransferase-mediated scaffold remodeling in securinine biosynthesis

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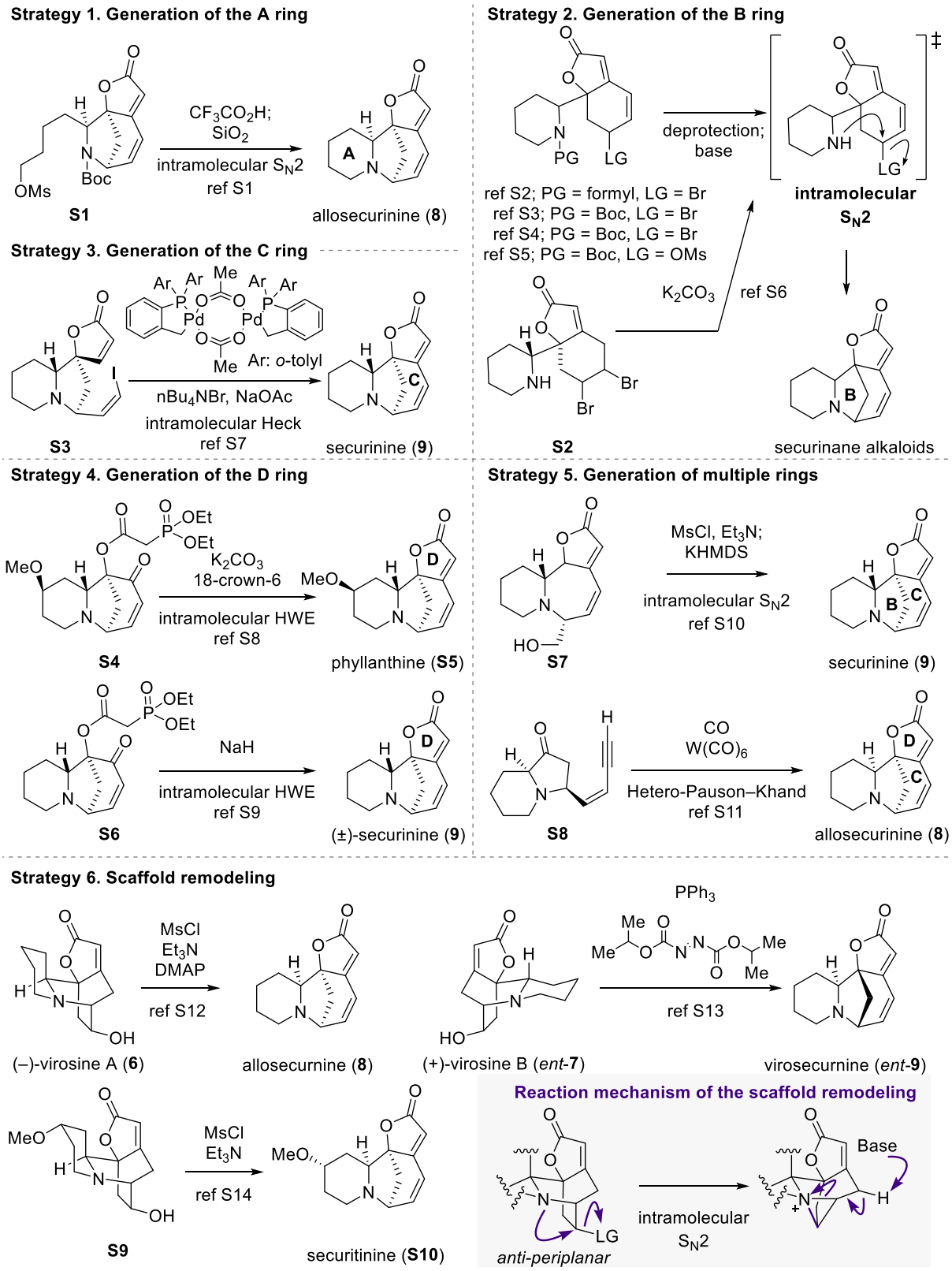
Supplementary Information

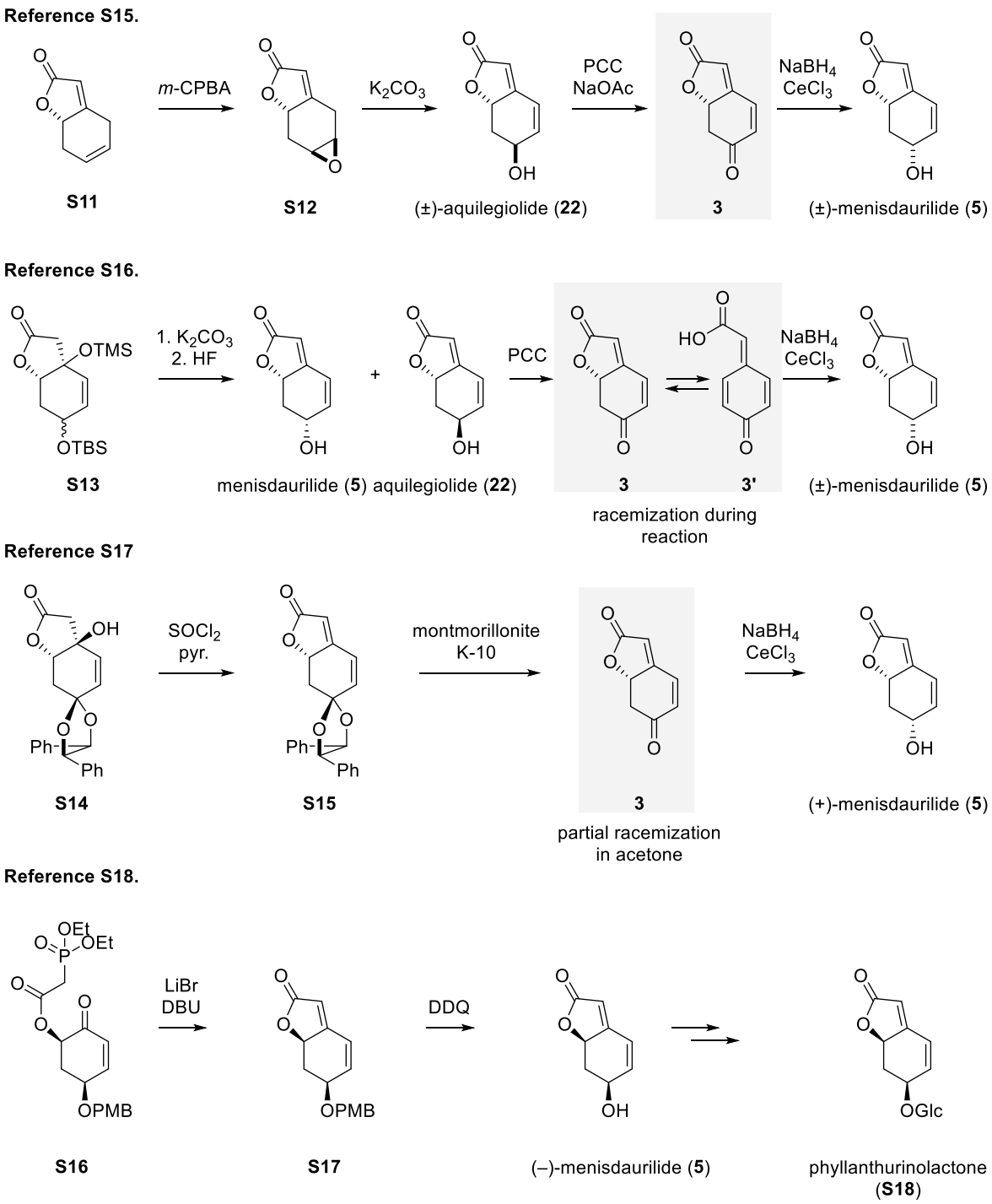
1. Supplementary Figures

1.1. Summary of the final step in the total synthesis of securinane alkaloids



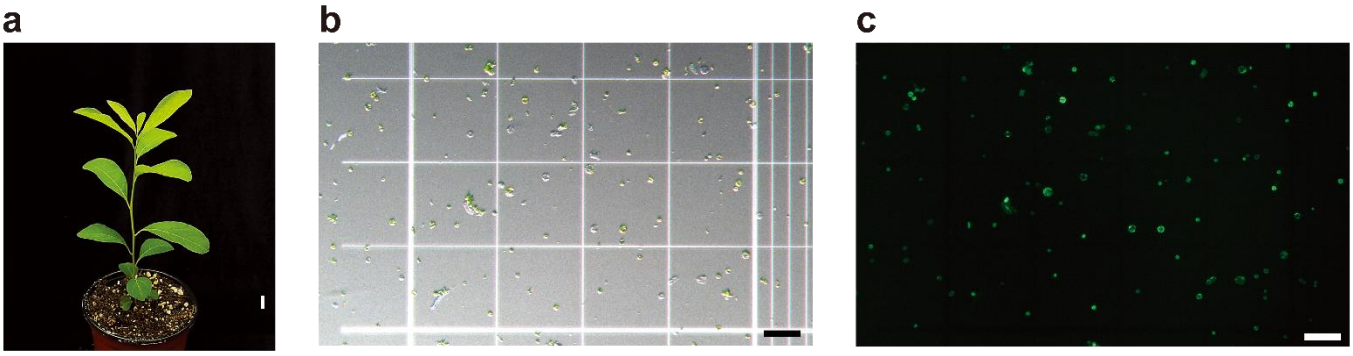
Supplementary Fig. 1. Summary of the final step in the total synthesis of securinane alkaloids. Various strategies have been developed to synthesize the securinane scaffold. Strategy 1 to 5 generates securinane scaffold via the formation of one or more rings, while strategy 6 affords securinane alkaloids through scaffold remodeling of neosecurinane precursors[S1–14].

1.2. Summary of the Synthesis of Menisdaurilide



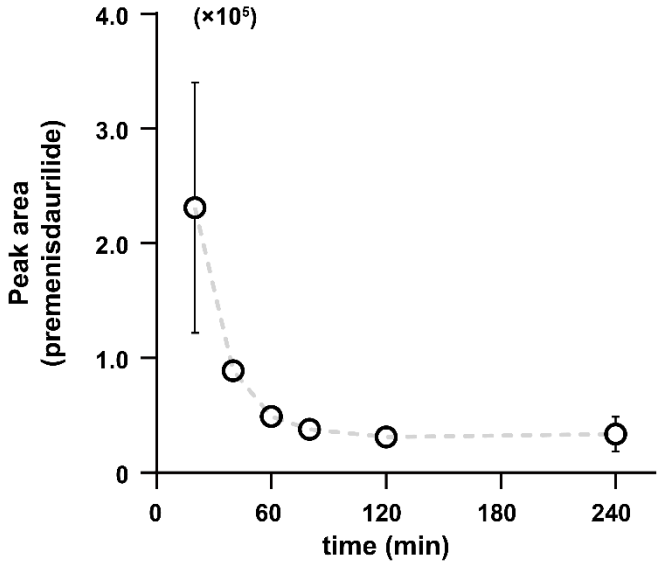
Supplementary Fig. 2. Summary of the synthesis of menisdaurilide (5). Except for one synthesis[S18], premenisdaurilide (**3**) was employed as a key synthetic intermediate[S15–S18].

1.3. Isolated Intact *F. suffruticosa* Leaf Protoplasts



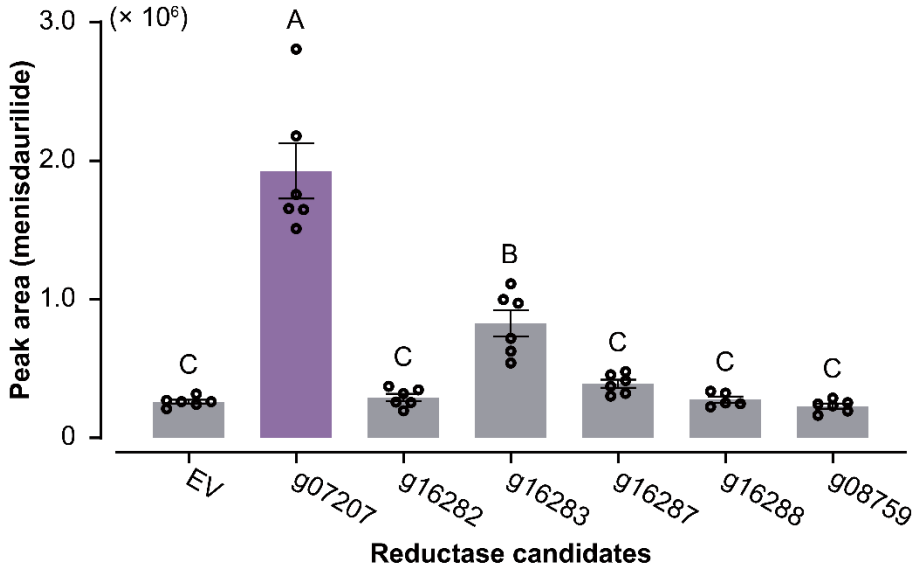
Supplementary Fig. 3. Isolated intact *F. suffruticosa* protoplasts were observed through FDA staining. a representative image of the five-week-old *F. suffruticosa*. Young leaves (the 10^{th} to 12^{th} from the base of the main stem) were used for each replicate in single-cell RNA sequencing. Scale bar, 1 cm. **b**, **c** Protoplast viability and integrity evaluation. Enzymatically isolated protoplasts were observed through light microscopy (**b**) and fluorescent microscopy after the fluorescein diacetate staining (**c**). Scale bar, 100 μm.

1.4. Chemical Stability of Premenisdaurilide



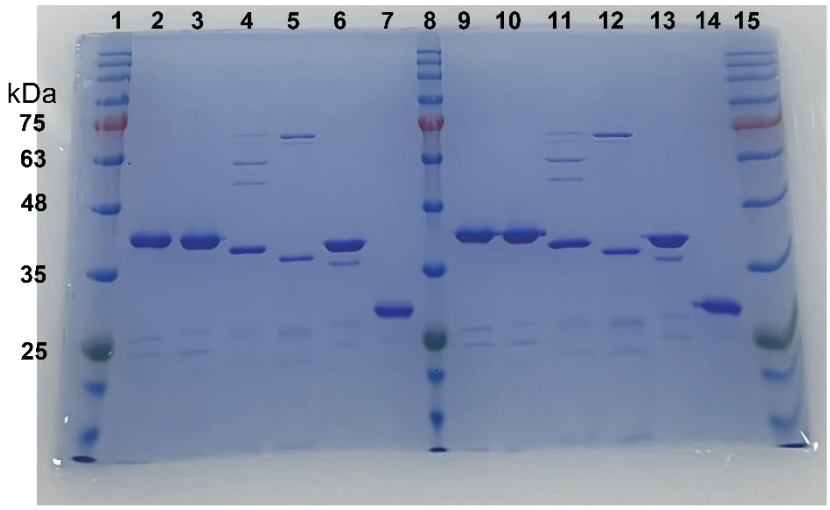
Supplementary Fig. 4. Chemical stability of premenisdaurilide in aqueous solution. Premenisdaurilide was dissolved in water and incubated for 20, 40, 60, 80, 120, and 240 minutes. After incubation, samples were diluted and immediately analyzed by HPLC-MS/MS to quantify the amount of premenisdaurilide (N = 3, Mean ± SEM).

1.5 Functional Characterization of Menisdaurilide Synthases *in planta*



Supplementary Figure 5. Functional characterization of menisdaurilide synthase (*FsMS*, g07207) with additional ketoreductase candidates in *N. benthamiana*, related to Fig. 4. Candidate genes were transiently expressed in *N. benthamiana*, followed by infiltration of premenisdaurilide. The production of menisdaurilide was subsequently measured to assess enzyme activity (N = 6, Mean ± SEM, One-way ANOVA, *post-hoc* Tukey's HSD).

1.5 Functional Characterization of Menisdaurilide Synthases *in planta*



Supplementary Fig. 6. Raw gel image of SDS-PAGE showing purified His-tagged candidate sulfotransferases, related to Fig. 5. A total of 1 μg of each purified protein was loaded onto a 10% polyacrylamide gel. Lanes: 1, 8, 15, ladder; 2, 9, NSST1-8×His; 3, 10, NSST2-8×His; 4, 11, g21405-8×His; 5, 12, g00640-8×His; 6, 13, g01123-8×His; 7, 14, eGFP-8×His. Expected sizes based on the amino acid sequences are as follows: NSST1-8×His, 40.031 kDa; NSST2-8×His, 39.111 kDa; g21405-8×His, 40.478 kDa; g00640-8×His, 37.373 kDa; g01123-8×His, 40.184 kDa; eGFP-8×His, 28.039 kDa.

2. Supplementary Tables

2.1. Supplementary Table 1. Multiple reaction monitoring transitions and MS parameters

Compound	Molecular weight	Precursor ion (m/z)	Mass transitions (m/z)	Q1 pre bias (V)	Collision cell energy (V)	Q3 pre bias (V)
menisdaurilide	152.05	152.90	152.90→ 135.20	-11.0	-15.0	-14.0
			152.90→ 107.00	-20.0	-15.0	-20.0
			152.90→ 121.00	-11.0	-13.0	-24.0
1-piperideine (dimer form)	166.15	167.15	167.15→ 150.15	-12.0	-17.0	-16.0
			167.15→ 138.10	-12.0	-19.0	-14.0
			167.15→ 122.25	-12.0	-22.0	-13.0
premenisdaurilide	150.13	149.00	149.00→ 121.05	30.0	21.0	29.0
			149.00→ 104.90	11.0	13.0	17.0
			149.00→ 77.10	11.0	18.0	12.0
			149.00→ 92.90	10.0	24.0	13.0
allosecurinine	217.11	218.10	218.10→ 129.10	-15.0	-23.0	-13.0
			218.10→ 162.20	-24.0	-28.0	-29.0
			218.10→ 200.25	-23.0	-21.0	-22.0
			218.10→ 172.00	-16.0	-22.0	-29.0
securinine	217.11	218.10	218.10→ 190.20	-11.0	-20.0	-21.0
			218.10→ 162.20	-24.0	-25.0	-30.0
			218.10→ 172.20	-23.0	-22.0	-29.0
			218.10→ 115.05	-20.0	-47.0	-25.0
(–)-virosine A	235.12	236.10	236.10→ 84.00	-12.0	-30.0	-15.0
			236.10→ 107.00	-17.0	-25.0	-21.0
			236.10→ 190.05	-10.0	-25.0	-21.0
(–)-virosine B	235.12	236.00	236.00→ 84.00	-12.0	-32.0	-15.0
			236.00→ 106.95	-17.0	-28.0	-20.0
			236.00→ 136.15	-16.0	-33.0	-30.0
(+)-securinol A	235.12	236.00	236.00→ 84.10	-12.0	-30.0	-16.0
			236.00→ 190.10	-16.0	-28.0	-21.0
			236.00→ 107.10	-12.0	-33.0	-11.0
(+)-episecurinol A	235.12	236.00	236.00→ 84.00	-12.0	-32.0	-16.0
			236.00→190.15	-16.0	-27.0	-21.0
			236.00→135.95	-11.0	-32.0	-14.0
[$^{13}C_2$]-menisdaurilide	154.05	155.20	155.20→ 108.00	-10.0	-15.0	-11.0
			155.20→ 137.00	-14.0	-14.0	-28.0
			155.20→ 122.95	-13.0	-10.0	-26.0
[$^{13}C_2$]-allosecurinine	219.11	220.10	220.10→ 177.10	-20.0	-20.0	-20.0
			220.10→ 130.10	-24.0	-24.0	-25.0
			220.10→ 202.20	-23.0	-21.0	-20.0
			220.10→ 136.15	-16.0	-29.0	-27.0
[$^{13}C_2$]-securinine	219.11	220.10	220.10→ 163.15	-17.0	-28.0	-17.0
			220.10→ 173.20	-21.0	-21.0	-25.0
			220.10→ 136.10	-23.0	-28.0	-15.0
			220.10→ 116.20	-11.0	-47.0	26.0
[$^{13}C_2$]-(–)-virosine A	237.12	238.10	238.10→ 137.10	-11.0	-21.0	-14.0
			238.10→ 108.10	-12.0	-26.0	-20.0
			238.10→ 112.25	-19.0	-19.0	-23.0
			238.10→ 109.25	-18.0	-27.0	-20.0
			238.10→ 155.20	-16.0	-18.0	-16.0
[$^{13}C_2$]-(–)-virosine B	237.12	238.30	238.30→ 137.05	-11.0	-21.0	-14.0
			238.30→ 108.15	-12.0	-26.0	-20.0
			238.30→ 112.25	-15.0	-18.0	-24.0
			238.30→ 155.10	-12.0	-17.0	-30.0
			238.30→ 109.20	-12.0	-28.0	-24.0

2.2. Supplementary Table 2. Primers used in this study

No.	Primer name	Primer sequence
1	FsActin_qPCR_F	AGGCTGTTCTCTCGTTGTATGC
2	FsActin_qPCR_R	GACGGAGGATAGCATGTGGA
3	FsBBE2_qPCR_F	CCAAAAATCCAAGAGAAGCACA
4	FsBBE2_qPCR_R	TAGTCTTCACCCGAACCAAC
5	FsPS_qPCR_F	AGTATCAGAGAAAGCCTGGGTA
6	FsPS_qPCR_R	TCCGCTCACAACAATTCCTTT
7	FsMS_qPCR_F	AGGAAATGAACTGGTAGAAGGG
8	FsMS_qPCR_R	GTAAATCCACCATCAACGCAAA
9	FsNSST1_qPCR_F	ACATGTTCTCGGGTATTGGAAA
10	FsNSST1_qPCR_R	TCCTTTTCTTCCTCTAGCGAAA
11	FsNSST2_qPCR_F	TGTTCGTTTCTTTCTGGCATTT
12	FsNSST2_qPCR_R	AGCTTGCTTTCCAGTATCCTAA
13	pHCF_FsMS_F	GCCACCTCCGTCGACCACATCACATATGACTATAG
14	pHCF_FsMS_R	ACGCTGCCACCTCCGCTAGTAATAGTTTGGTGGGAAT
15	pHCF_g23559_F	AGAACACGGGGGACTATGACTATTTCTCCTTCAATTCCC
16	pHCF_g23559_R	ACGCTGCCACCTCCGCTAGCCGCAGGTTGCTGTTAT
17	pHCF_g21280_F	AGAACACGGGGGACTATGGATGGTCAATCTCCAGTT
18	pHCF_g21280_R	ACGCTGCCACCTCCGTCAATGAAGTGAGAATGAGAGA
19	pHCF_g14770_F	AGAACACGGGGGACTATGGCAAAAGCGATTCGAT
20	pHCF_g14770_R	ACGCTGCCACCTCCGTCAGATTTCACCATCCCAGA
21	pHCF_g11417_F	AGAACACGGGGGACTATGGCAGCACGAATTGATCA
22	pHCF_g11417_R	ACGCTGCCACCTCCGTTAATTCTTGATGAAGGAAACGAAC
23	pHCF_g16282_F	AGAACACGGGGGACTATGAGTGGAGAAAGAAGAGTA
24	pHCF_g16282_R	ACGCTGCCACCTCCGTCAGATTTTCAGAAATCCCTTCT
25	pHCF_g16283_F	AGAACACGGGGGACTATGAGTGGAGAAGGAAGACT
26	pHCF_g16283_R	ACGCTGCCACCTCCGTCACACTTGAAGGAATCCT
27	pHCF_g16287_F	AGAACACGGGGGACTATGGGTGGAGAAGGAAG
28	pHCF_g16287_R	ACGCTGCCACCTCCGTCATATATTAAGAAATCCCTTC
29	pHCF_g16288_F	AGAACACGGGGGACTATGAGTGGAGAAGGAAGAG
30	pHCF_g16288_R	ACGCTGCCACCTCCGTCATACTTTAAGAAACCCCTT
31	pHCF_g08759_F	AGAACACGGGGGACTATGGCAAAGAGCAAGGTTC
32	pHCF_g08759_R	ACGCTGCCACCTCCGTTATGCATAAGCTTTCAGATAT
33	pHCF_NSST1_F	AGAACACGGGGGACTATGGAATCAGTACTACCATC
34	pHCF_NSST1_R	ACGCTGCCACCTCCGTTAATCAAAACTCAAACCATG
35	pHCF_NSST2_F	AGAACACGGGGGACTATGGAAAAAGTTTTAGCACCAC
36	pHCF_NSST2_R	ACGCTGCCACCTCCGTCAACAATCCGAGTCGAAGC
37	pHCF_g00640_F	AGAACACGGGGGACTATGCAGAACTATAGTGTTGTAG
38	pHCF_g00640_R	ACGCTGCCACCTCCGCTAAATTCCATACGTGAAACC
39	pHCF_g21405_F	AGAACACGGGGGACTATGGAATCAGTCTCTCCATCA
40	pHCF_g21405_R	ACGCTGCCACCTCCGTTAAGAACAAGAATTAAAAGTCAAGC
41	pHCF_g01123_F	AGAACACGGGGGACTATGGAATCATCTCCAACCGT
42	pHCF_g01123_R	ACGCTGCCACCTCCGTTAATTAAAACTCAAGCCAGATG
43	pET50_NSST1_F	AGAAGGAGATATACAATGGAATCAGTACTACCATCTG
44	pET50_NSST1_R	TGGTGGTGGTGGTGGTGCCCATCAAAACTCAAACCATGATTACC
45	pET50_NSST2_F	AGAAGGAGATATACAATGGAAAAAGTTTTAGCACCAC
46	pET50_NSST2_R	GTGGTGGTGGTGGTGGTGCCCACAATCCGAGTCGAAGCTC
47	pET50_g00640_F	AGAAGGAGATATACAATGCAGAACTATAGTGTTGTAG
48	pET50_g00640_R	TGGTGGTGGTGGTGCCCAATTCCATACGTGAAACCTGAA
49	pET50_g21405_F	AGAAGGAGATATACAATGGAATCAGTCTCTCCATCA
50	pET50_g21405_R	TGGTGGTGGTGGTGCCCAGAACAAGAATTAAAAGTCAAGCC
51	pET50_g01123_F	AGAAGGAGATATACAATGGAATCATCTCCAACCGT
52	pET50_g01123_R	TGGTGGTGGTGGTGCCCATTAAAACTCAAGCCAGATGAAC
53	pET50_eGFP_F	TTTAAGAAGGAGATATACAATGGTGAGCAAGGGCG
54	pET50_eGFP_R	CGGAGCTCGCCTGCACCTTGTACAGCTCGTCCAT

3. Preparation of Compounds

3.1. General Information

All reactions were performed in oven-dried or flame-dried round-bottomed flasks and vials. Unless otherwise noted, the flasks were fitted with rubber septa and reactions were conducted under a positive pressure of argon, and vials were tightly sealed with plastic septa, Teflon tape, and parafilm. Stainless steel syringes were used to transfer air- and moisture sensitive liquids. Flash column chromatography was performed as described by Still et al. using silica gel (60-Å pore size, 40–63 μm, 4-6% H_2O content, Merck)[S19]. Analytical thin–layer chromatography (TLC) was performed using glass plates pre-coated with 0.25 mm silica gel impregnated with a fluorescent indicator (254 nm). Thin layer chromatography plates were visualized by exposure to ultraviolet light, an aqueous ammonium cerium nitrate/ammonium molybdate (Seebach's staining)[S20], and/or a basic aqueous potassium permanganate ($KMnO_4$).

Unless otherwise stated, all commercial reagents and solvents were used without additional purification with the following exceptions as indicated below. Dichloromethane and tetrahydrofuran were purchased from Merck and Daejung Inc., respectively and were purified by the method of Grubbs *et al.* under positive argon pressure[S21]. ^1H and ^{13}C nuclear magnetic resonance spectra were recorded with Bruker Avance NEO (500 MHz) and calibrated by using the residual undeuterated chloroform (δ_H= 7.24 ppm) and $CDCl_3$ (δ_C= 77.23 ppm), undeuterated acetone-d_6 (δ_H= 2.05 ppm) and acetone-d_6 (δ_C= 29.84 ppm), undeuterated CD_3OD (δ_H= 3.31 ppm) and CD_3OD (δ_C= 49.00 ppm) as internal references. Data are reported in the following manners: chemical shift in ppm [multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, p = quintet, m = multiplet), coupling constant(s) in Hertz, integration]. The NMR solvent $CDCl_3$ was taken from a stock containing anhydrous K_2CO_3 to remove residual DCl. High resolution mass spectra were obtained from KAIST Analysis Center for Research (Daejeon) by using ESI method.

3.2. Preparation of Securinega Alkaloids

3.2.1 Isolation of Allosecurinine (8) and Securinine (9) from the Roots of *Flueggea suffruticosa*

1.0 kg of dried roots of *F. suffruticosa* were cut into pieces, placed in 5 L glass bottle, and 1,2-dichloroethane (4 L), 28 % aqueous ammonium hydroxide (400 mL) was added to the bottle. The bottle was sealed and stirred at 23 °C. After 36 h, the resulting mixture was filtered, dried over anhydrous sodium sulfate. The filtrate was concentrated under reduced pressure. The extraction process was repeated three times. The resulting crude residue was purified by iterative flash column chromatography (silica gel: diam: 8 cm, ht = 12 cm; eluent: diethyl ether (1% NH_4OH)) to yield allosecurinine (**8**, overall 4.7 g, calculated 0.47% from the root material) as a yellow powder. Also fraction containing impure securinine collected from the previous purification process was further purified by iterative flash column chromatography (silica gel: diam: 4 cm, ht = 19 cm; eluent: diethyl ether : hexane = 1 : 1) to yield securinine (**9**, overall 1.7 g, calculated 0.17% from the root material) as a yellow powder.

Note: The isolation protocol was originally reported in J. Am. Chem. Soc. ***2022****, 144, 8932–8937*[S22].

Allosecurinine (8):

^{1}H NMR (500 MHz, $CDCl_3$): δ 6.78 (dd, *J* = 9.1, 5.3 Hz, 1H), 6.61 (d, *J* = 9.0 Hz, 1H), 5.68 (s, 1H), 3.86 (t, *J* = 4.8 Hz, 1H), 3.61 (dd, *J* = 13.2, 3.5 Hz, 1H), 2.78 – 2.69 (m, 2H), 2.63 (dd, *J* = 9.8, 4.4 Hz, 1H), 1.88 (d, *J* = 9.8 Hz, 1H), 1.70–1.61 (m, 3H), 1.43–1.38 (m, 1H), 1.34 – 1.28 (m, 1H), 1.11 (qd, *J* = 13.1, 5.7 Hz, 1H).

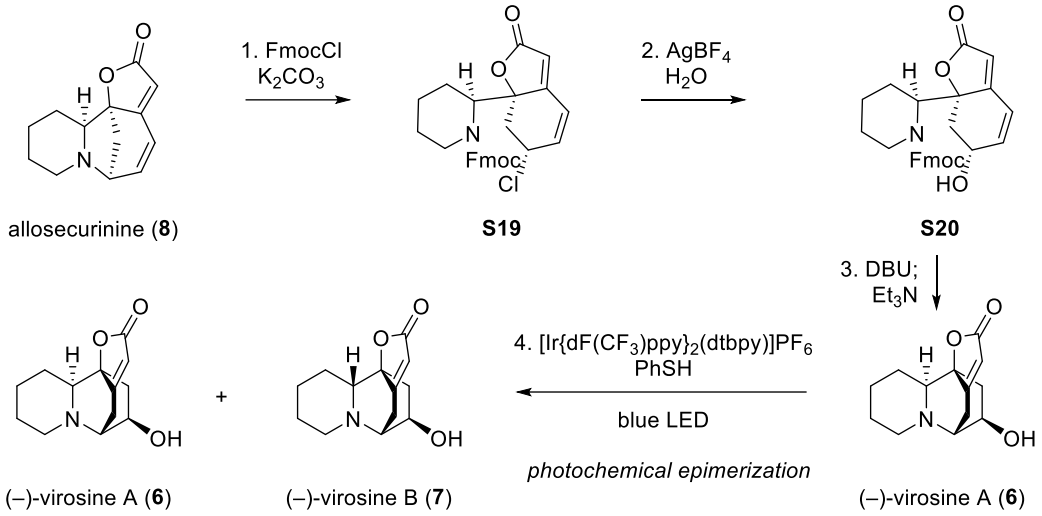
^{13}C NMR (126 MHz, $CDCl_3$): δ 172.8, 167.7, 148.9, 122.8, 109.1, 91.9, 60.9, 59.0, 43.8, 42.8, 22.3, 21.2, 18.6.

Securinine (9):

^{1}H NMR (500 MHz, $CDCl_3$): δ 6.57 (d, *J* = 9.2 Hz, 1H), 6.38 (dd, *J* = 9.3, 5.3 Hz, 1H), 5.51 (s, 1H), 3.78 (t, *J* = 4.7 Hz, 1H), 2.93 (dt, *J* = 10.5, 3.8 Hz, 1H), 2.47 (dd, *J* = 9.3, 4.1 Hz, 1H), 2.39 (dt, *J* = 14.3, 7.6 Hz, 1H), 2.07 (d, *J* = 11.4 Hz, 1H), 1.85 (dt, *J* = 13.7, 3.6 Hz, 1H), 1.75 (d, *J* = 9.3 Hz, 1H), 1.66 – 1.47 (m, 4H), 1.21 (tdd, *J* = 17.9, 10.9, 6.7 Hz, 1H).

^{13}C NMR (126 MHz, $CDCl_3$): δ 173.9, 170.3, 140.4, 121.7, 105.3, 89.7, 63.2, 59.0, 49.0, 42.5, 27.4, 26.1, 24.7.

3.2.2. Synthesis of (–)-Virosine A (6) and (–)-Virosine B (7)



Supplementary Fig. 7. Summary of the synthesis of (–)-virosines A (6) and B (7). (–)-Virosine A(**6**) and (–)-virosine B (**7**) were prepared according to the previously reported method [S14,S23].

(–)-**Virosine A (6):**

^{1}H NMR (500 MHz, $CDCl_3$): δ 5.68 (t, *J* = 1.9 Hz, 1H), 4.37 (dt, *J* = 8.8, 3.9 Hz, 1H), 2.97 (d, *J* = 18.4 Hz, 1H), 2.92–2.85(m, 2H), 2.84 – 2.64 (m, 4H), 1.79 (dt, *J* = 13.6, 3.7 Hz, 1H), 1.58 – 1.52 (m, 2H), 1.51–1.40 (m, 2H), 1.27 (qt, *J* = 12.4, 4.6 Hz, 1H), 0.84 (qd, *J* = 12.1, 4.1 Hz, 1H).

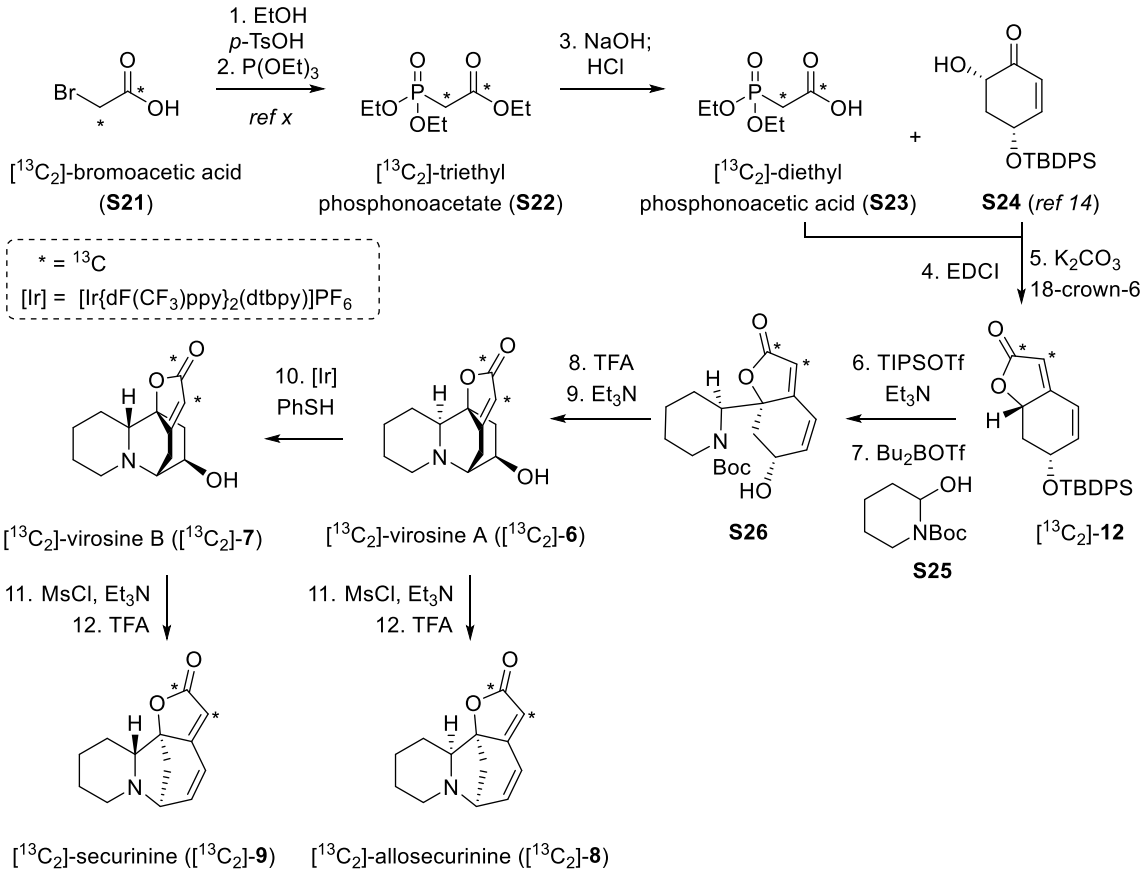
^{13}C NMR (126 MHz, $CDCl_3$): δ 174.2, 174.0, 111.8, 84.5, 65.3, 65.1, 59.1, 52.7, 41.0, 29.4, 26.5, 25.6, 23.9.

(–)-**Virosine B (7):**

^{1}H NMR (500 MHz, $CDCl_3$): δ 5.59 (t, *J* = 1.9 Hz, 1H), 4.19 (dd, *J* = 8.5, 5.1 Hz, 1H), 3.09 (dt, *J* = 19.2, 2.0 Hz, 1H), 2.89 (dt, *J* = 5.1, 2.7 Hz, 1H), 2.80–2.69 (m, 3H), 2.63 (td, *J* = 10.4, 4.4 Hz, 1H), 2.19 (d, *J* = 10.6 Hz, 1H), 1.83 (dt, *J* = 13.3, 3.4 Hz, 1H), 1.61 (d, *J* = 10.6 Hz, 1H), 1.57–1.47 (m, 2H), 1.34 (qd, *J* = 11.6, 3.4 Hz, 1H), 1.30 – 1.20 (m, 1H), 1.17 (d, *J* = 13.2 Hz, 1H).

^{13}C NMR (126 MHz, $CDCl_3$): δ 176.9, 174.7, 108.8, 85.2, 66.8, 63.3, 57.7, 52.6, 36.5, 26.8, 25.9, 24.8, 23.2.

3.2.3. Synthesis of [$^{13}C_2$]-Labeled Securinega Alkaloids



Supplementary Fig. 8. Summary of the synthesis of [$^{13}C_2$]-labeled securinega alkaloids. [$^{13}C_2$]-labeled securinega alkalodis were prepared according to the previously reported method[S5,S14] , using [$^{13}C_2$]-diethylphosphonoacetic acid (**S23** derived from [$^{13}C_2$]-triethylphosphonoacetate (**S22**)[S24].

[$^{13}C_2$]-Allosecurinine ([$^{13}C_2$]-8):

^{1}H NMR (500 MHz, $CDCl_3$): δ 6.79 (dd, *J* = 9.1, 5.3 Hz, 1H), 6.62 (dt, *J* = 9.2, 1.5 Hz, 1H), 5.69 (dd, *J* = 180.8, 8.4 Hz, 1H), 3.87 (t, *J* = 4.9 Hz, 1H), 3.63 (dd, *J* = 13.2, 3.5 Hz, 1H), 2.76–2.67 (m, 2H), 2.65 (dd, *J* = 9.8, 4.4 Hz, 1H), 1.89 (d, *J* = 9.8 Hz, 1H), 1.70–1.61 (m, 3H), 1.43–1.35 (m, 1H), 1.34–1.28 (m, 1H), 1.12 (qd, *J* = 13.0, 5.8 Hz, 1H).

^{13}C NMR (126 MHz, $CDCl_3$): 172.9 (d, *J* = 68.4 Hz), 167.7 (dd, *J* = 69.0, 4.7 Hz), 148.9 (d, *J* = 6.9 Hz), 122.9 (dd, *J* = 7.8, 1.4 Hz), 109.2 (d, *J* = 68.2 Hz), 91.9 (d, *J* = 3.7 Hz), 61.0, 59.0, 43.8, 42.8, 22.4, 21.2, 18.7.

HRMS (ESI): Calculated for $C_{11}{}^{13}C_2H_{15}NO_2$ $[M+H]^+$: 220.1243, found: 220.1255.

[$^{13}C_2$]-Securinine ([$^{13}C_2$]-9):

^{1}H NMR (500 MHz, $CDCl_3$): δ 6.56 (d, *J* = 9.3 Hz, 1H), 6.38 (dd, *J* = 9.2, 5.2 Hz, 1H), 5.50 (dd, *J* = 181.2, 8.8 Hz, 1H), 3.78 (t, *J* = 4.7 Hz, 1H), 2.93 (ddd, *J* = 10.5, 4.1, 3.0 Hz, 1H), 2.47 (dd, *J* = 9.2, 4.1 Hz, 1H), 2.41–2.34 (m, 1H), 2.07 (dd, *J* = 11.4, 2.5 Hz, 1H), 1.88–1.80 (m, 1H), 1.74 (d, *J* = 9.3 Hz, 1H), 1.64 – 1.46 (m, 4H), 1.26–1.14 (m, 1H).

^{13}C NMR (126 MHz, $CDCl_3$): δ 173.9 (d, *J* = 68.5 Hz), 170.3 (dd, *J* = 69.7, 5.3 Hz), 140.4 (d, *J* = 6.4 Hz), 121.6 (dd, *J* = 7.8, 1.7 Hz), 105.3 (d, *J* = 68.3 Hz), 89.7 (d, *J* = 3.5 Hz), 63.2, 59.0, 49.0, 42.5, 27.4, 26.1, 24.7.

HRMS (ESI): Calculated for $C_{11}{}^{13}C_2H_{15}NO_2$ $[M+H]^+$: 220.1243, found: 220.1252.

[$^{13}C_2$]-Virosine A ([$^{13}C_2$]-6):

^{1}H NMR (500 MHz, $CDCl_3$): δ 5.65 (ddt, *J* = 180.4, 9.4, 2.0 Hz, 1H), 4.37 (dddd, *J* = 9.5, 4.7, 3.2, 1.4 Hz, 1H), 2.95 (ddd, *J* = 18.4, 3.5, 1.7 Hz, 1H), 2.89–2.83 (m, 2H), 2.78 – 2.62 (m, 4H), 1.80–1.73 (m, 1H), 1.58 – 1.48 (m, 2H), 1.47–1.37 (m, 2H), 1.25 (qt, *J* = 12.9, 4.6 Hz, 1H), 0.81 (tdd, *J* = 12.3, 11.0, 4.0 Hz, 1H).

^{13}C NMR (126 MHz, $CDCl_3$): δ 174.51 (d, *J* = 67.6 Hz), 174.46 (dd, *J* = 9.8, 4.8 Hz), 111.5 (d, *J* = 67.8 Hz), 84.9 (d, *J* = 4.0 Hz), 65.4, 64.9, 59.1 (d, *J* = 2.3 Hz), 52.8, 40.9, 29.6 (d, *J* = 6.1 Hz), 26.7, 25.8, 24.1.

HRMS (ESI): Calculated for $C_{11}{}^{13}C_2H_{17}NO_3$ $[M+H]^+$: 238.1348, found: 238.1357.

[$^{13}C_2$]-Virosine B ([$^{13}C_2$]-7):

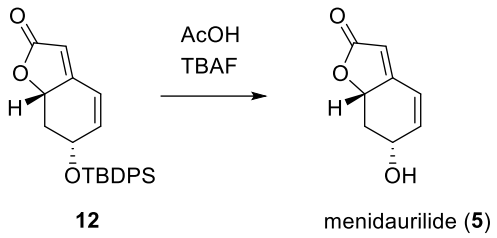
^{1}H NMR (500 MHz, $CDCl_3$): δ 5.60 (ddt, *J* = 180.8, 9.3, 2.0 Hz, 1H), 4.21 (dd, *J* = 8.6, 5.1 Hz, 1H), 3.09 (dt, *J* = 19.2, 2.4 Hz, 1H), 2.96–2.85 (m, 1H), 2.82–2.72 (m, 3H), 2.65 (td, *J* = 10.5, 4.2 Hz, 1H), 2.21 (d, *J* = 10.6 Hz, 1H), 1.84 (dt, *J* = 13.3, 3.5 Hz, 1H), 1.66–1.60 (m, 1H), 1.60–1.48 (m, 2H), 1.41–1.33 (m, 1H), 1.30 – 1.20 (m, 1H), 1.19 (d, *J* = 13.3 Hz, 1H).

^{13}C NMR (126 MHz, $CDCl_3$): δ 174.5 (d, *J* = 67.2 Hz), 108.8, 85.1, 66.8, 63.3, 57.7 (d, *J* = 2.3 Hz), 52.6, 36.5, 26.8, 25.9, 24.8, 23.2 (d, *J* = 6.1 Hz).

Note: Signal at 176.9 ppm could not be detected plausibly due to its multiplicity (doublet of doublets), overall broadness of peaks, and adjacent strong peak at 174.5 ppm.

HRMS (ESI): Calculated for $C_{11}{}^{13}C_2H_{17}NO_3$ $[M+H]^+$: 238.1348, found: 238.1356.

3.3. Synthesis of Menisdaurilide (5) and [$^{13}C_2$]-Menisdaurilide ([$^{13}C_2$]-5)



12 menidaurilide (**5**)

Supplementary Fig. 9. Synthesis of menisdaurilide. Menisdaurilide was prepared through the previously reported method[S23]. [$^{13}C_2$]-menisdaurilide was prepared analogously, using [$^{13}C_2$]-**12** as a starting material.

Menisdaurilide (5):

^{1}H NMR (500 MHz, $CDCl_3$): δ 6.55 (dd, *J* = 9.9, 2.5 Hz, 1H), 6.30 (d, *J* = 10.2 Hz, 1H), 5.80 (s, 1H), 4.86 (ddd, *J* = 13.4, 4.9, 1.8 Hz, 1H), 4.65 – 4.59 (m, 1H), 2.92 (dt, *J* = 10.8, 5.2 Hz, 1H), 2.37 (br s, 1H), 1.65 (dt, *J* = 13.3, 10.7 Hz, 1H).

^{13}C NMR (126 MHz, $CDCl_3$): δ 173.6, 163.2, 143.7, 120.2, 111.7, 78.3, 67.0, 40.2.

[$^{13}C_2$]-Menisdaurilide ([$^{13}C_2$]-5):

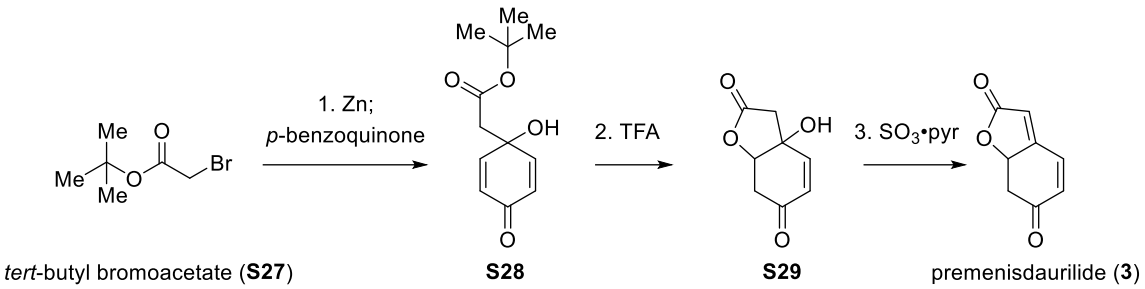
^{1}H NMR (500 MHz, $CDCl_3$): δ 6.55 (dt, *J* = 9.9, 2.3 Hz, 1H), 6.30 (dt, *J* = 10.1, 1.7 Hz, 1H), 5.79 (dd, *J* = 181.9, 8.6 Hz, 1H), 4.85 (ddd, *J* = 13.4, 3.9, 1.7 Hz, 1H), 4.64 – 4.58 (m, 1H), 2.92 (dtt, *J*= 10.3, 5.2, 1.1 Hz, 1H), 1.67 (ddd, *J* = 13.4, 11.1, 10.3 Hz, 1H).

^{13}C NMR (126 MHz, $CDCl_3$): δ 173.6 (d, *J* = 67.8 Hz), 163.1 (dd, *J* = 69.9, 5.9 Hz), 143.8 (d, *J* = 6.3 Hz), 120.2 (dd, *J* = 7.5, 1.6 Hz), 111.8 (d, *J* = 68.1 Hz), 78.1 (d, *J* = 3.7 Hz), 67.0, 40.1.

HRMS (ESI): Calculated for $C_6{}^{13}C_2H_8O_3$ $[M+H]^+$: 155.0613, found: 155.0622.

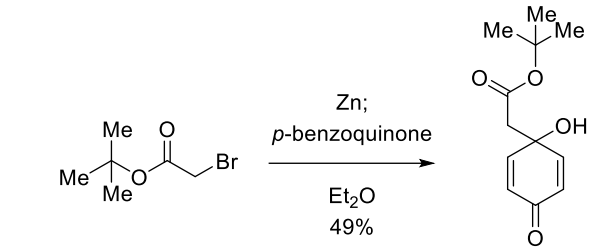
3.4. Synthesis of Plausible Candidates for the Penultimate Precursor of Menisdaurilide

3.4.1. Synthesis of Premensdaurilide and [$^{13}C_2$]-Premenisdaurilide



tert-butyl bromoacetate (**S27**) **S28** **S29** premenisdaurilide (**3**)

Supplementary Fig. 10. Synthesis of premenisdaurilide. Reformatsky reaction between *p*-benzoquinone and organozinc intermediate derived from *tert*-butyl bromoacetate (**S27**) resulted in the formation of quinol **S28**, which was further converted into bicycle **S29** through acid-mediated deprotection of *tert*-butyl ester and sequential *oxa*-Michael cyclization. Finally, premenisdaurilide (**3**) was prepared via dehydration mediated by SO_3•pyr from bicycle **S29**. [$^{13}C_2$]-premenisdaurilide ([$^{13}C_2$]-**3**) was prepared analogously, using [$^{13}C_2$]-*tert*-butyl bromoacetate ([$^{13}C_2$]-**S27**)[S25] as a starting material.



tert-butyl bromoacetate (**S27**) **S28**

Quinol (S28):

Preparation of alkylzinc:

Tert-butyl bromoacetate (**S27**, 2.65 mL, 17.9 mmol, 1.00 equiv) and methylmagnesium iodide (3.0 M in diethyl ether, 20 µL, 0.06 mmol, 0.34 mol%) was sequentially added dropwise to a suspension of zinc (2.30 g, 35.2 mmol, 1.97 equiv; activated by heating with a crystal of I_2) in diethyl ether (100 mL) at 23 °C under argon atmosphere. The resulting mixture was refluxed for 90 min in a silicone oil bath until the bromide was fully consumed based on the TLC. The reaction vessel was removed from the slowly cooled to 23 °C without stirring, and the supernatant was carefully transferred to the new, flame-dried round bottom flask via cannula under an argon atmosphere.

Reaction between alkylzinc and p-benzoquinone:

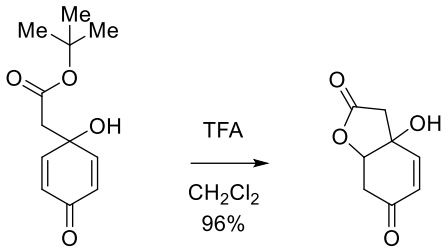
Solution of *p*-Benzoquinone (1.55 g, 14.3 mmol, 0.80 equiv) in diethyl ether (50 mL) was added dropwise to an alkylzinc solution at 23 °C under argon atmosphere. The resulting mixture was stirred at at 23 °C for 40 min, quenched carefully with aqueous hydrogen chloride (1.0 M), then extracted with ethyl acetate (3 × 100 mL). The combined organic layer was washed with saturated aqueous sodium bicarbonate (100 mL) and brine (100 mL), dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The resulting crude residue was purified by flash column chromatography (silica gel: diam. 4 cm, ht. 10 cm; eluent: ethyl acetate : hexanes = 1 : 2 → 2:3) to afford quinol with impurities. Recrystallization with chloroform/hexane of impure quinol afforded analytically pure quinol **S28** (1.57 g, 49%) as a colorless needle.

^{1}H NMR (500 MHz, $CDCl_3$): δ 6.90 (d, *J* = 10.2 Hz, 2H), 6.16 (d, *J* = 10.2 Hz, 2H), 4.28 (br s, 1H), 2.57 (s, 2H), 1.45 (s, 9H).

^{13}C NMR (126 MHz, $CDCl_3$): δ 185.3, 170.7, 149.3, 128.4, 83.2, 67.8, 44.4, 28.2.

HRMS (ESI): Calculated for $C_{12}H_{16}O_4$ $[M+Na]^+$: 247.0941, found: 247.0942

TLC (ethyl acetate : hexanes = 1 : 3) R*f*: 0.12 (UV, $KMnO_4$, CAM).



S28 **S29**

Bicycle (S29):

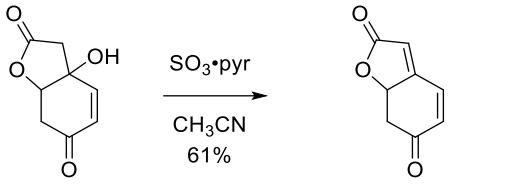
Trifluoroacetic acid (2 mL, 0.4 M) was added to a solution of quinol **S28** (175.4 mg, 0.7821 mmol, 1.0 equiv.) in dichloromethane (2 mL, 0.4 M) at 23 °C under argon atmosphere. After stirring at 23 °C for 1 h, the reaction mixture was concentrated over reduced pressure, and the resulting crude residue was purified by flash column chromatography (silica gel: diam. 4 cm, ht. 14 cm; acetone : hexanes = 3 : 7) to afford bicycle **S29** (125.7 mg, 96%) as a white solid.

^{1}H NMR (500 MHz, acetone-d_6): δ 6.88 (dd, J = 10.3, 1.3 Hz, 1H), 6.03 (d, J = 10.2 Hz, 1H), 5.43 (s, 1H), 4.89 (ddd, J = 6.4, 4.9, 1.3 Hz, 1H), 2.99 – 2.95 (m, 2H), 2.74 (dd, J = 16.8, 6.5 Hz, 1H).

^{13}C NMR (126 MHz, acetone-d_6): δ 194.7, 173.3, 147.7, 129.3, 83.1, 72.7, 42.7, 39.9.

HRMS (ESI): Calculated for $C_8H_8O_4$ $[M+Na]^+$: 191.0315, found: 191.0327.

TLC (acetone : hexanes = 1 : 1) R*f*: 0.28 (UV, $KMnO_4$).



S29 premenisdaurilide (**3**)

Premenisdaurilide (3):

Sulfur trioxide pyridine complex (238 mg, 1.4951 mmol, 2 equiv.) was added to a solution of bicycle **S29** (125.7 mg, 0.7476 mmol, 1.0 equiv.) in acetonitrile (12 mL, 0.06 M) at 23 °C under argon atmosphere. After stirring at 23 °C for overnight, the reaction mixture was poured to a saturated aqueous sodium bicarbonate (15 mL) and extracted with dichloromethane (5 × 15 mL). The combined organic layers were dried over anhydrous sodium sulfate. The filtrate was concentrated over reduced pressure, and the resulting crude residue was purified by flash column chromatography (silica gel: diam. 3 cm, ht. 15 cm; acetone : hexanes = 1 : 4) to afford premenisdaurilide (**3**) (97.2 mg, 61%) as a white solid.

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.51 (d, *J* = 9.9 Hz, 1H), 6.32 (d, *J* = 9.9 Hz, 1H), 6.19 (s, 1H), 5.28 (ddd, *J* = 12.3, 6.5, 1.9 Hz, 1H), 3.35 (dd, *J* = 15.5, 6.4 Hz, 1H), 2.58 (dd, *J* = 15.4, 12.3 Hz, 1H).

^{13}C NMR (126 MHz, $CDCl_3$): δ 194.1, 171.7, 159.2, 135.4, 134.9, 117.7, 78.1, 45.4.

HRMS (ESI): Calculated for $C_8H_6O_3$ $[M–H]^–$: 149.0244, found**:** 149.0239.

TLC (acetone : hexanes = 1 : 4) R*f*: 0.23 (UV, $KMnO_4$).

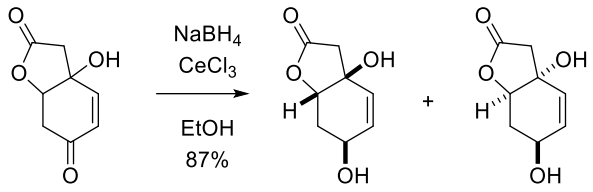
[$^{13}C_2$]-Premenisdaurilide ([$^{13}C_2$]-3):

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.51 (dd, *J* = 9.9, 2.2 Hz, 1H), 6.34 (d, *J* = 9.9 Hz, 1H), 6.20 (ddd, *J* = 183.4, 8.4, 2.0 Hz, 1H), 5.28 (dt, *J* = 12.1, 4.1 Hz, 1H), 3.37 (dd, *J* = 15.5, 6.4 Hz, 1H), 2.59 (dd, *J* = 15.5, 12.3 Hz, 1H).

^{13}C NMR (126 MHz, $CDCl_3$): δ 194.0, 171.7 (d, *J* = 67.4 Hz), 159.1 (dd, *J* = 70.8, 5.4 Hz), 135.4 (d, *J* = 5.6 Hz), 134.9 (dd, *J* = 7.5, 1.7 Hz), 117.7 (d, *J* = 67.4 Hz), 78.1 (d, *J* = 3.6 Hz), 45.5.

HRMS (ESI): Calculated for $C_6{}^{13}C_2H_6O_3$ $[M+Na]^+$: 175.0276, found: 175.1480.

3.4.2. Synthesis of diol



syn-**4** : *anti*-**4** = 1 : 4

Diol (4):

Cerium(III) chloride heptahydrate (59.0 mg, 0.1582 mmol, 1 equiv.) was added to a solution of bicycle **S29** (26.6 mg, 0.1582 mmol, 1.0 equiv.) in ethanol (1.6 mL, 0.1 M) at 23 °C under argon atmosphere. After stirring at 23 °C for 30 min, Sodium borohydride (6.3 mg, 0.1661 mmol, 1 equiv.) was added to a solution. After stirring at 23 °C for 30 min, the reaction mixture was quenched with acetone (5 mL) and concentrated over reduced pressure. The resulting crude residue was purified by flash column chromatography (silica gel: diam. 2 cm, ht. 16 cm; acetone : hexanes = 1 : 1) to afford diol **4** (97.2 mg, 87%, dr = 1 : 4) as a colorless sticky oil.

(Major diastereomer)

^{1}H NMR (500 MHz, acetone-d_6): δ 5.83 (dt, *J* = 10.2, 1.6 Hz, 1H), 5.73 (dd, *J* = 10.1, 2.1 Hz, 1H), 4.65 (s, 1H), 4.51 (dd, *J* = 12.2, 4.9 Hz, 1H), 4.40 – 4.34 (m, 1H), 4.19–4.13 (m, 1H), 2.77 (d, *J* = 17.5 Hz, 1H), 2.53 (d, *J* = 17.5 Hz, 1H), 2.42 (dt, J = 12.1, 4.7 Hz, 1H), 1.61 (td, *J* = 12.1, 9.8 Hz, 1H).

^{13}C NMR (126 MHz, acetone-d_6): δ 175.1, 134.8, 129.1, 84.6, 74.4, 65.1, 42.7, 38.8.

HRMS (ESI): Calculated for $C_8H_{10}O_4$ [M+Na]$^+$: 193.0472, found: 193.0484.

TLC (acetone : hexanes = 1: 1) R*f*: 0.33 ($KMnO_4$).

*Note: Diol **4** was obtained as an inseparable mixture composed of syn-**4** : anti-**4** = 1: 4, based on the NMR, and spectra of the major diastereomer was reported.*

[$^{13}C_2$]-Diol ([$^{13}C_2$]-4):

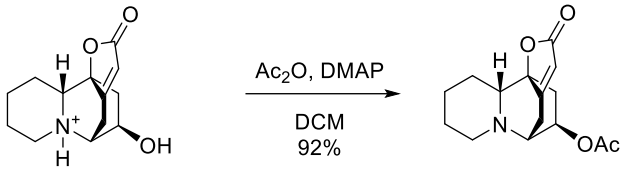
^{1}H NMR (500 MHz, Acetone): δ 5.83 (dt, *J* = 10.2, 1.7 Hz, 1H), 5.73 (dd, *J* = 10.6, 2.1 Hz, 1H), 4.65 (d, *J* = 4.6 Hz, 1H), 4.51 (dt, *J* = 11.7, 5.3 Hz, 1H), 4.40 – 4.31 (m, 1H), 4.19–4.16 (m, 1H), 2.79 (ddd, *J* = 113.8, 17.5, 6.5 Hz, 1H), 2.63–2.40 (overlapped, 1H), 2.45 – 2.38 (m, 1H), 1.61 (td, *J* = 12.2, 10.0 Hz, 1H).

^{13}C NMR (126 MHz, Acetone): δ 175.1 (d, *J* = 50.2 Hz), 134.8 (d, *J* = 2.4 Hz), 129.1 (d, *J* = 4.8 Hz), 84.6 (d, *J* = 2.45 Hz), 74.4 (d, *J* = 38.4 Hz), 65.1, 42.7 (d, *J* = 50.3 Hz), 38.8.

HRMS (ESI): Calculated for $C_6{}^{13}C_2H_{10}O_4$ [M+H]$^+$: 173.0719, found: 173.0739.

3.5. Chemical Transformations of (–)-virosine B (7)

3.5.1. Acetylation of (–)-virosine B (7)



(–)-virosine B•HCl
(**7**•HCl)

28

***O*-Acetylvirosine B (28):**

Acetic anhydride (0.02 mL, 0.2208 mmol, 3 equiv.) and 4-Dimethylaminopyridine (18.0 mg, 0.1472 mmol, 2 equiv.) was added to a solution of (–)-virosine B hydrochloride (**7**•HCl) (20 mg, 0.0736 mmol, 1.0 equiv.) in dichloromethane (3.7 mL, 0.02 M) at 23 °C under argon atmosphere. After stirring at 23 °C for 20 min, the reaction mixture was poured to a saturated aqueous sodium bicarbonate (5 mL) and extracted with dichloromethane (3 × 10 mL). The combined organic layers were dried over anhydrous sodium sulfate. The filtrate was concentrated over reduced pressure, and the resulting crude residue was purified by flash column chromatography (silica gel: diam. 2 cm, ht. 13 cm; acetone : hexanes = 1 : 9) to afford *O*-acetylvirosine B (**28**) (18.7 mg, 92%) as a white solid.

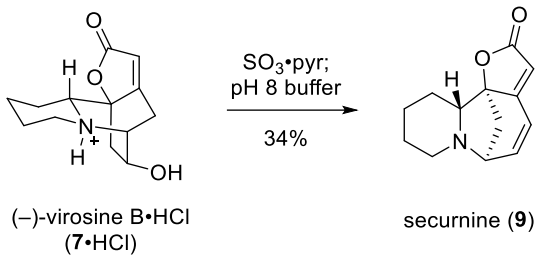
^{1}H NMR (500 MHz, $CDCl_3$): δ 5.63 (t, *J* = 2.0 Hz, 1H), 4.99 (dd, *J* = 8.8, 5.1 Hz, 1H), 3.11 (dt, *J* = 5.1, 2.6 Hz, 1H), 2.92 – 2.77 (m, 4H), 2.67 – 2.60 (m, 1H), 2.47 (dt, *J* = 10.8, 1.4 Hz, 1H), 1.99 (s, 3H), 1.85 (dt, *J* = 13.1, 3.4 Hz, 1H), 1.67 – 1.60 (m, 1H), 1.58 – 1.50 (m, 2H), 1.38 (tdd, *J* = 11.9, 10.8, 3.7 Hz, 1H), 1.28 (d, *J* = 13.9 Hz, 1H), 1.28 – 1.19 (m, 1H).

^{13}C NMR (126 MHz, $CDCl_3$): δ 175.3, 174.0, 170.4, 109.5, 84.4, 68.7, 63.4, 54.9, 52.4, 33.3, 26.8, 25.8, 24.7, 23.8, 21.3.

HRMS (ESI): Calculated for $C_{15}H_{19}NO_4$ $[M+H]^+$: 278.1387, found: 278.1401.

TLC (acetone : hexanes = 6 : 4) R*f*: 0.60 (UV, $KMnO_4$).

3.5.2. Sulfation-mediated conversion of (–)-virosine B into securinine



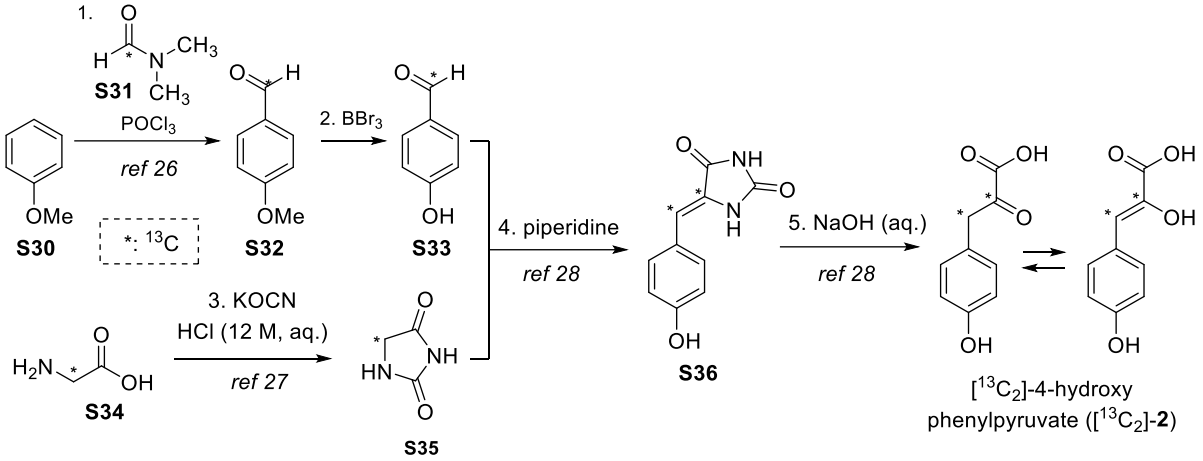
(–)-virosine B•HCl
(**7**•HCl)

securnine (**9**)

Securinine (9):

In a glovebox, sulfur trioxide–pyridine complex (5.1 mg, 3.22×10^{-2} mmol, 1.25 equiv) was added to a solution of (–)-virosine B hydrochloride (**7**•HCl) (7.0 mg, 2.58×10^{-2} mmol, 1.0 equiv) in anhydrous dimethylforamide (0.52 mL, 0.05 M) at 23 °C. The reaction mixture was stirred in the glovebox for 36 h, and 5 ml of pH = 8 phosphate buffer (0.2 M; prepared from Na_2HPO_4 and NaH_2PO_4) was added after taken out from the glovebox. The resulting mixture was stirred vigorously (1500 rpm) for 10 min, then extracted with diethyl ether (3 × 10 mL). Combined organic layer was dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The resulting crude residue was purified by flash column chromatography (silica gel: diam. 1 cm, ht. 6 cm; eluent: acetone : hexanes = 1 : 2) to afford securinine (**9**) (1.9 mg, 34%) as a yellow solid.

3.6. Synthesis of [$^{13}C_2$]-4-Hydroxyphenylpyruvic acid ([$^{13}C_2$]-2)



Supplementary Fig. 11. Synthesis of [$^{13}C_2$]-4-Hydroxyphenylpyruvic acid ([$^{13}C_2$]-2). [$^{13}C_2$]-4-hydroxyphenylpyruvic acid was prepared according to previous literature[S26–S28], using *N*,*N*-dimethylformamide (carbonyl-^{13}C) (**S31**) and glycine-2-^{13}C (**S34**) as a source of isotope.

[$^{13}C_2$]-4-hydroxyphenylpyruvic acid ([$^{13}C_2$]-2):

^{1}H NMR (500 MHz, CD_3OD): δ 7.63 (dd, *J* = 8.7, 4.6 Hz, 2H), 6.75 (d, *J* = 8.7 Hz, 2H), 6.44 (dd, *J* = 157.0, 2.7 Hz, 1H)

^{13}C NMR (126 MHz, CD_3OD): δ 169.1*, 158.2, 140.0 (d, *J* = 84.4 Hz), 132.4 (dd, *J* = 4.1, 2.7 Hz), 128.0 (dd, *J* = 58.7, 1.2 Hz), 116.1 (d, *J* = 4.8 Hz), 112.2 (d, *J* = 84.3 Hz),

** The title compound exists predominantly as the enol tautomer under the measurement conditions. The multiplicity of the peak could not be assigned due to the presence of multiple signals in the 169–170 ppm range.*

HRMS (ESI): Calculated for $C_7{}^{13}C_2H_8O_4$ $[M-H]^-$: 181.0416, found: 181.0420.

4. Supplemental References

S1. Leduc, A. B. & Kerr, M. A. Total Synthesis of (−)-Allosecurinine. *Angew. Chem. Int. Ed.* **47**, 7945–7948 (2008).

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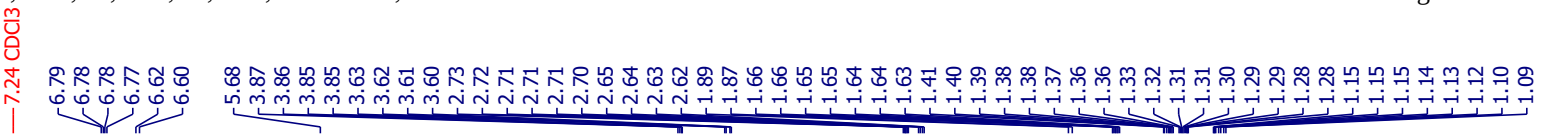
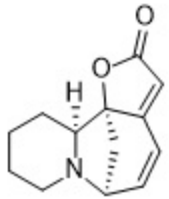
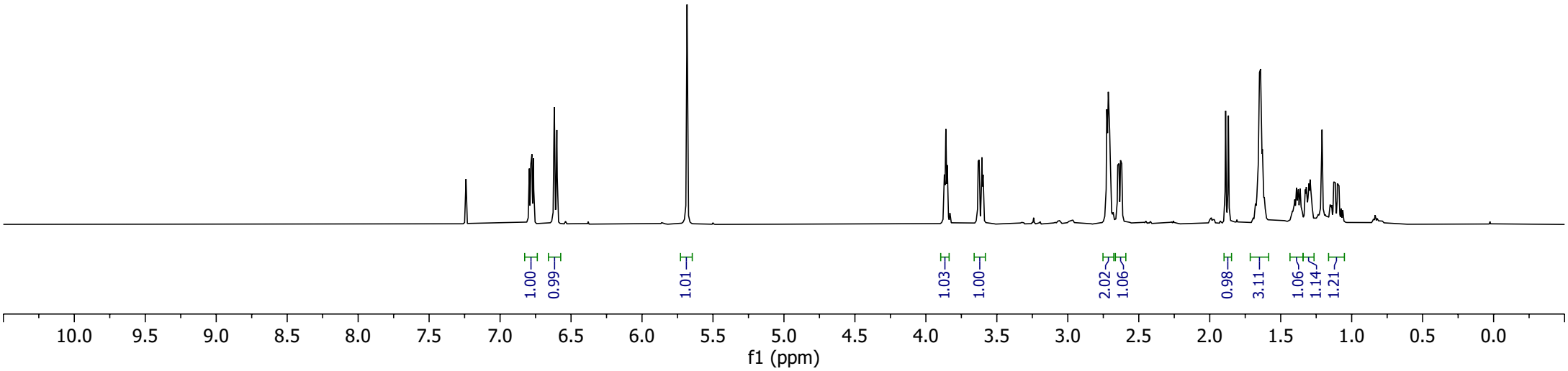
5. Copies of NMR Spectra of Compounds Used in this Study

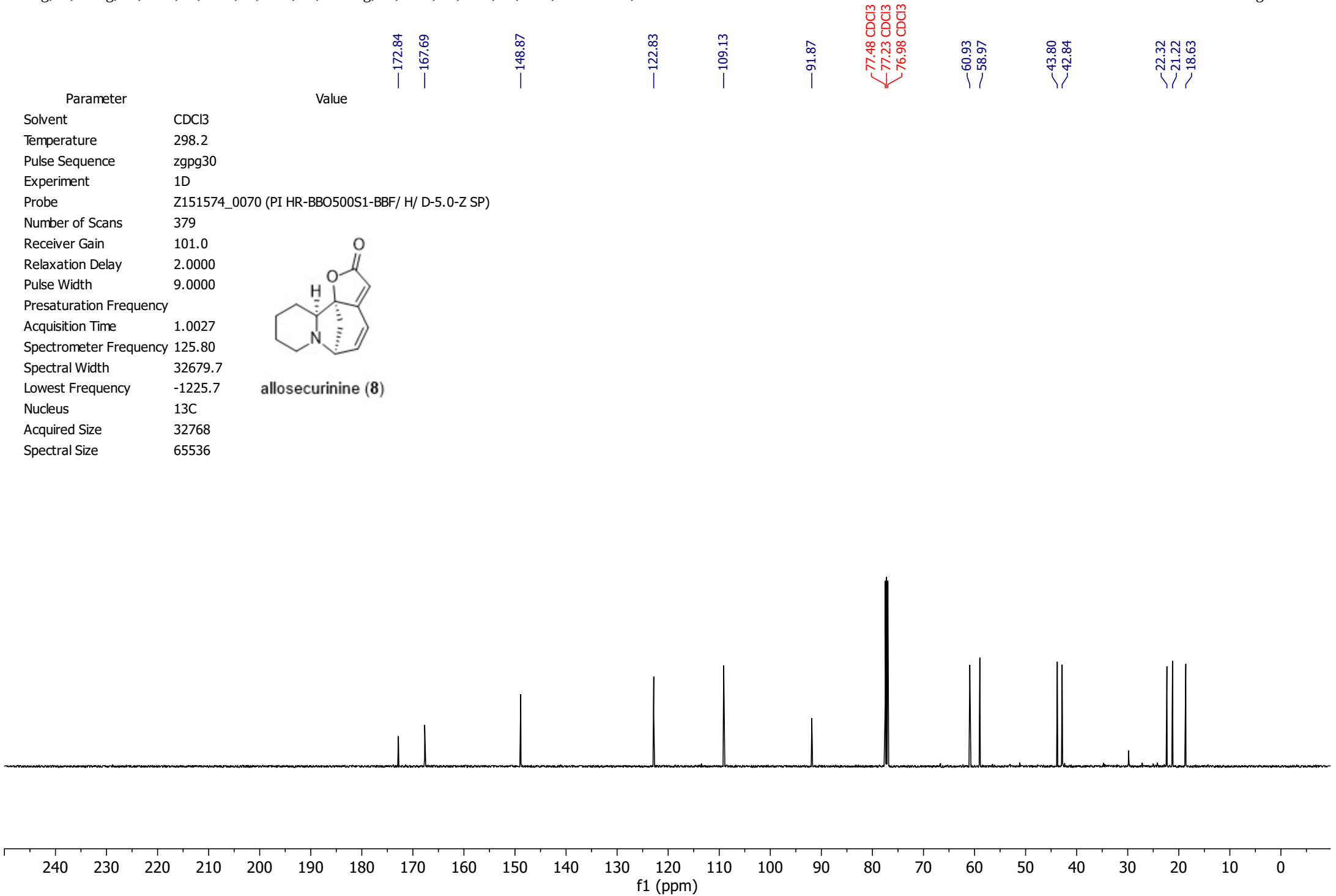


Parameter	Value
Solvent	CDCl3
Temperature	298.0
Pulse Sequence	zg30
Experiment	1D
Probe	Z151574_0070 (PI HR-BBO500S1-BBF/ H/ D-5.0-Z SP)
Number of Scans	16
Receiver Gain	57.0
Relaxation Delay	1.0000
Pulse Width	8.0000
Presaturation Frequency	
Acquisition Time	5.5706
Spectrometer Frequency	500.23
Spectral Width	5882.4
Lowest Frequency	-462.3
Nucleus	1H
Acquired Size	32768
Spectral Size	65536



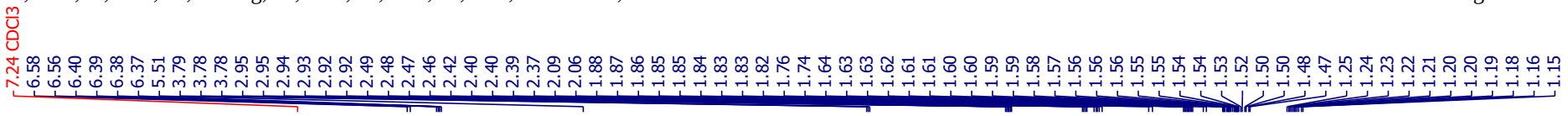
allosecurinine (**8**)



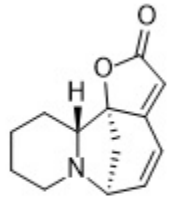


Parameter	Value
Solvent	CDCl3
Temperature	298.2
Pulse Sequence	zgpg30
Experiment	1D
Probe	Z151574_0070 (PI HR-BBO500S1-BBF/ H/ D-5.0-Z SP)
Number of Scans	379
Receiver Gain	101.0
Relaxation Delay	2.0000
Pulse Width	9.0000
Presaturation Frequency	
Acquisition Time	1.0027
Spectrometer Frequency	125.80
Spectral Width	32679.7
Lowest Frequency	-1225.7
Nucleus	13C
Acquired Size	32768
Spectral Size	65536

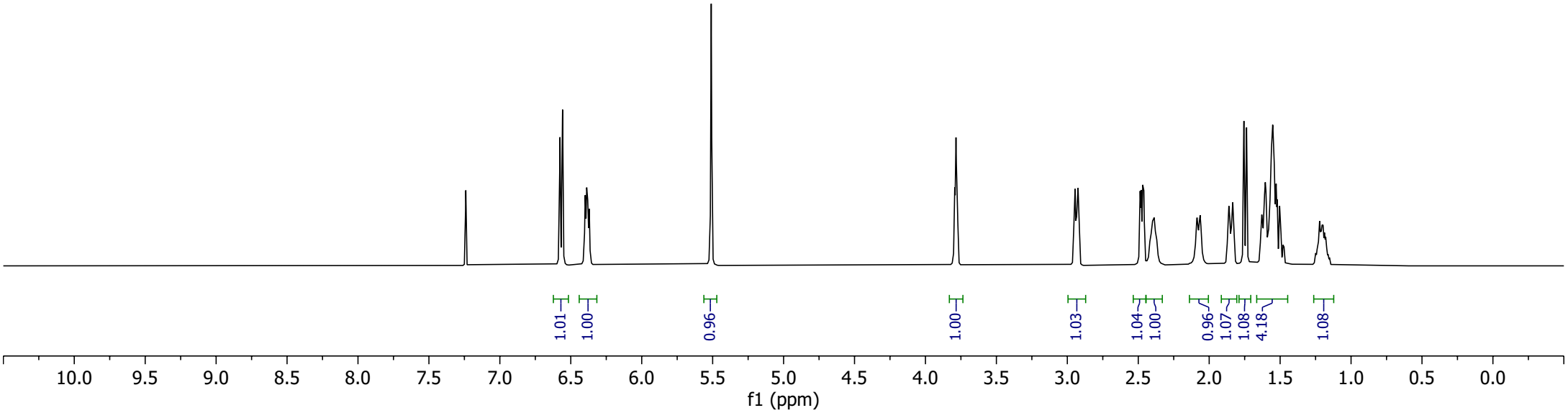
allosecurinine (**8**)

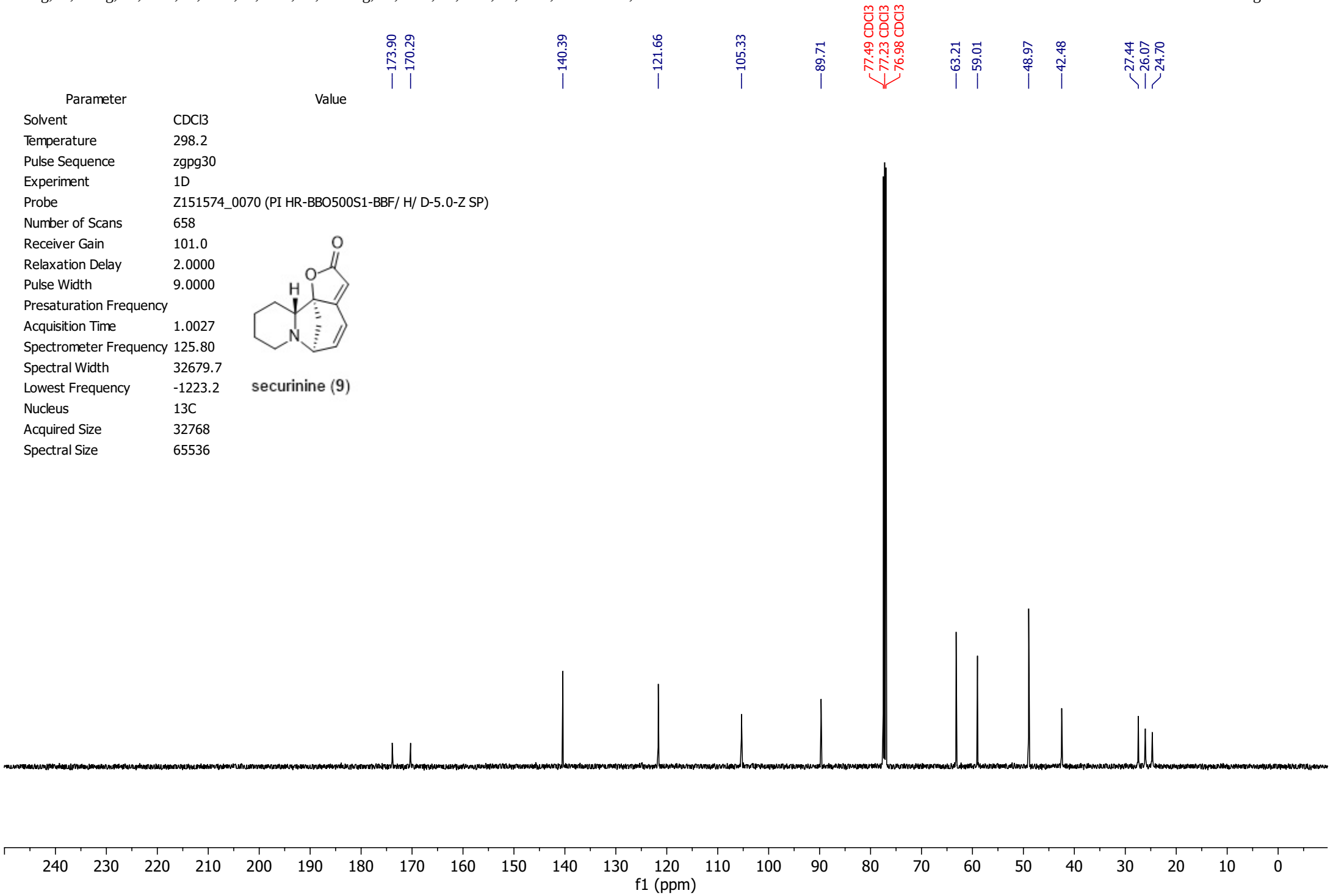


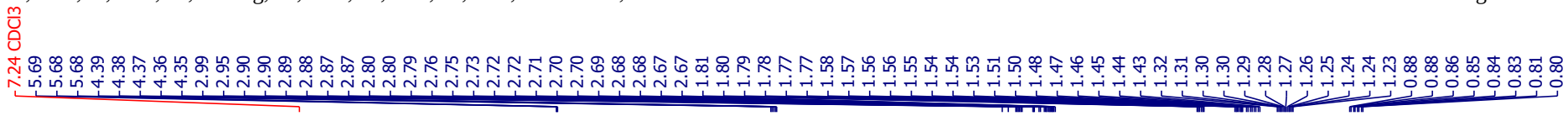
Parameter	Value
Solvent	CDCl3
Temperature	298.0
Pulse Sequence	zg30
Experiment	1D
Probe	Z151574_0070 (PI HR-BBO500S1-BBF/ H/ D-5.0-Z SP)
Number of Scans	16
Receiver Gain	101.0
Relaxation Delay	1.0000
Pulse Width	8.0000
Presaturation Frequency	
Acquisition Time	5.5706
Spectrometer Frequency	500.23
Spectral Width	5882.4
Lowest Frequency	-462.2
Nucleus	1H
Acquired Size	32768
Spectral Size	65536



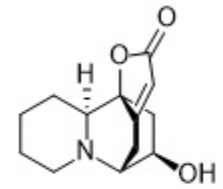
securinine (9)



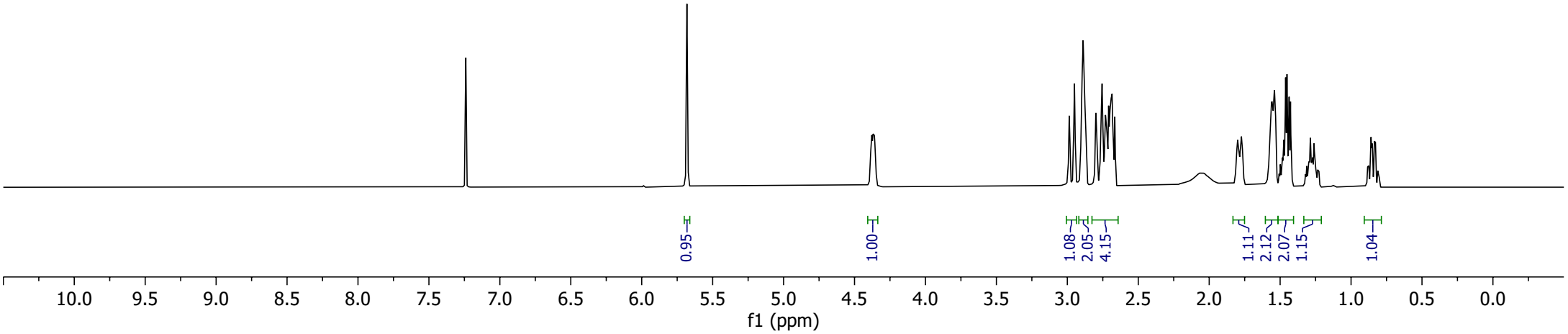

173.90
170.29
140.39
121.66
105.33
89.71
77.49 CDCl3
77.23 CDCl3
76.98 CDCl3
63.21
59.01
48.97
42.48
27.44
26.07
24.70
Parameter
Value
Solvent CDCl3
Temperature 298.2
Pulse Sequence zgpg30
Experiment 1D
Probe Z151574_0070 (PI HR-BBO500S1-BBF/ H/ D-5.0-Z SP)
Number of Scans 658
Receiver Gain 101.0
Relaxation Delay 2.0000
Pulse Width 9.0000
Presaturation Frequency
Acquisition Time 1.0027
Spectrometer Frequency 125.80
Spectral Width 32679.7
Lowest Frequency -1223.2
Nucleus 13C
Acquired Size 32768
Spectral Size 65536
O
O
H
N
securinine (9)
240 230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

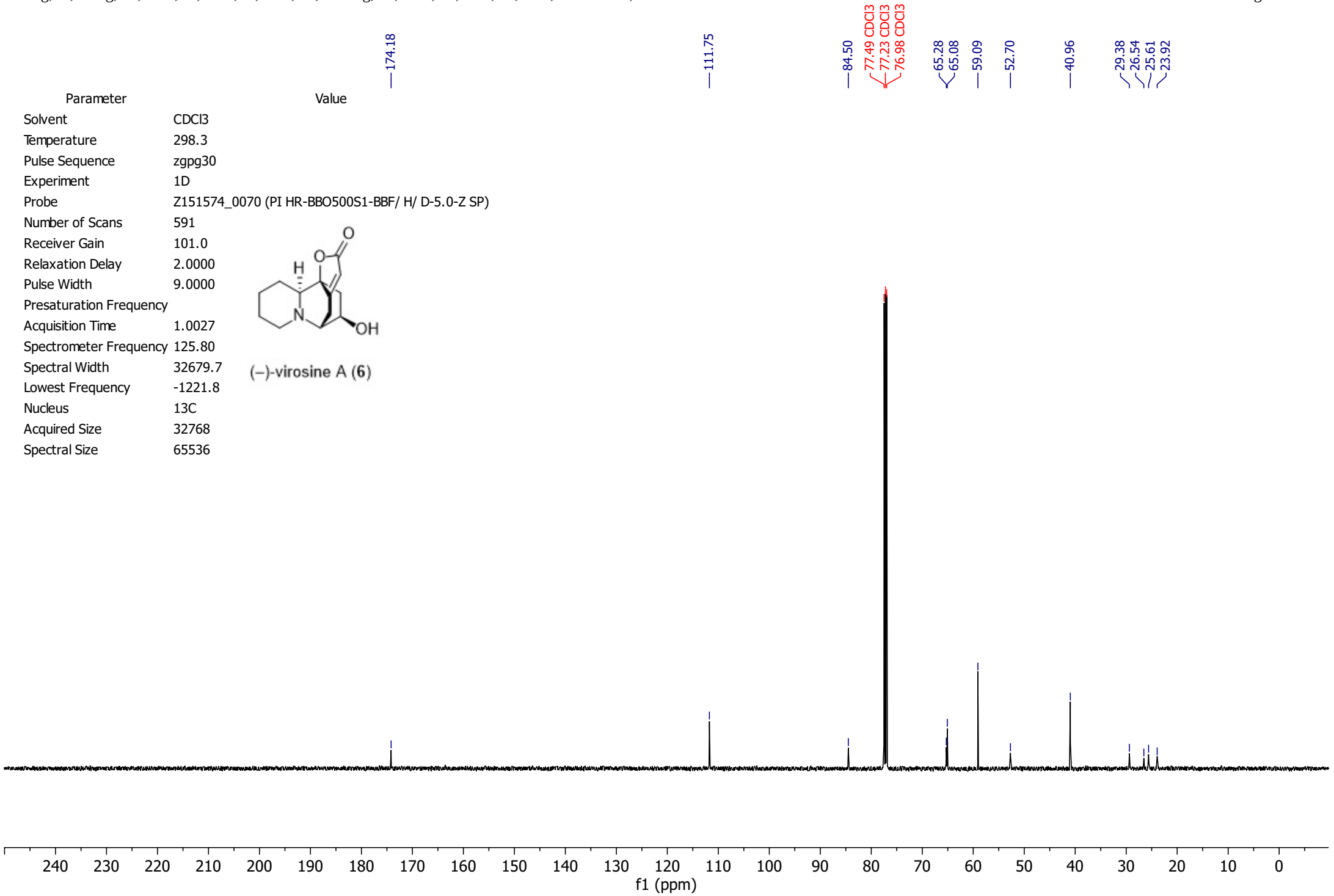


Parameter	Value
Solvent	CDCl3
Temperature	298.0
Pulse Sequence	zg30
Experiment	1D
Probe	Z151574_0070 (PI HR-BBO500S1-BBF/ H/ D-5.0-Z SP)
Number of Scans	16
Receiver Gain	101.0
Relaxation Delay	1.0000
Pulse Width	8.0000
Presaturation Frequency	
Acquisition Time	5.5706
Spectrometer Frequency	500.23
Spectral Width	5882.4
Lowest Frequency	-462.3
Nucleus	1H
Acquired Size	32768
Spectral Size	65536



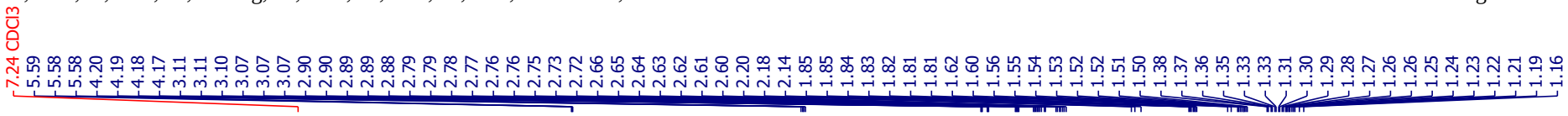
(–)-virosine A (**6**)



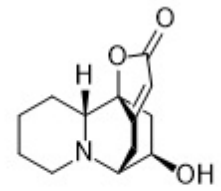


Parameter	Value
Solvent	CDCl3
Temperature	298.3
Pulse Sequence	zgpg30
Experiment	1D
Probe	Z151574_0070 (PI HR-BBO500S1-BBF/ H/ D-5.0-Z SP)
Number of Scans	591
Receiver Gain	101.0
Relaxation Delay	2.0000
Pulse Width	9.0000
Presaturation Frequency	
Acquisition Time	1.0027
Spectrometer Frequency	125.80
Spectral Width	32679.7
Lowest Frequency	-1221.8
Nucleus	13C
Acquired Size	32768
Spectral Size	65536

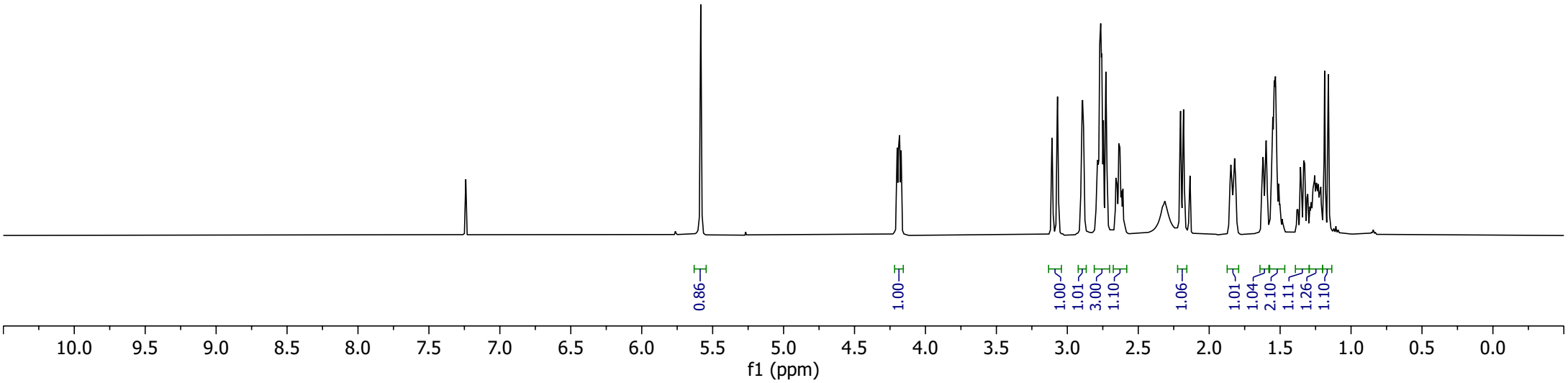
(–)-virosine A (**6**)

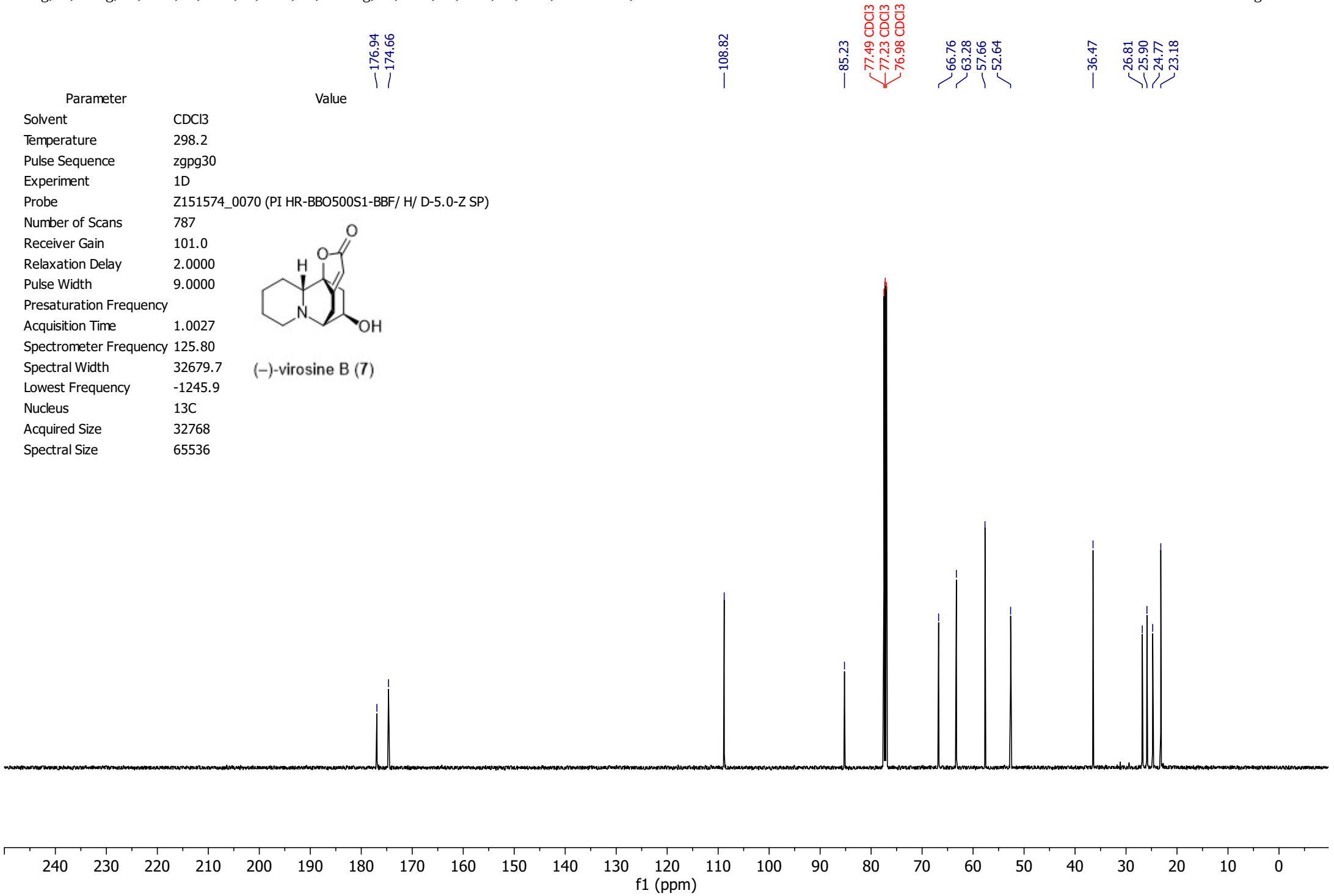


Parameter	Value
Solvent	CDCl3
Temperature	298.0
Pulse Sequence	zg30
Experiment	1D
Probe	Z151574_0070 (PI HR-BBO500S1-BBF/ H/ D-5.0-Z SP)
Number of Scans	16
Receiver Gain	71.8
Relaxation Delay	1.0000
Pulse Width	8.0000
Presaturation Frequency	
Acquisition Time	5.5706
Spectrometer Frequency	500.23
Spectral Width	5882.4
Lowest Frequency	-462.4
Nucleus	1H
Acquired Size	32768
Spectral Size	65536



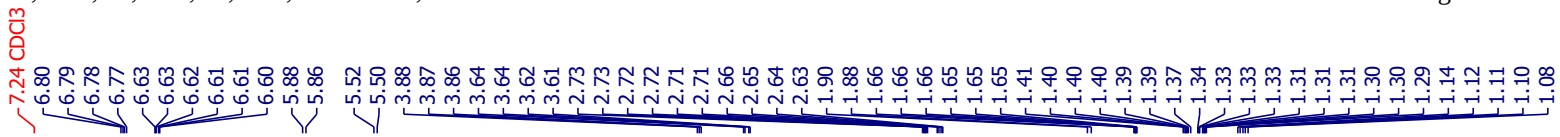
(–)-virosine B (7)



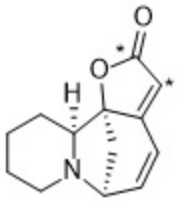


Parameter	Value
Solvent	CDCl3
Temperature	298.2
Pulse Sequence	zgpg30
Experiment	1D
Probe	Z151574_0070 (PI HR-BBO500S1-BBF/ H/ D-5.0-Z SP)
Number of Scans	787
Receiver Gain	101.0
Relaxation Delay	2.0000
Pulse Width	9.0000
Presaturation Frequency	
Acquisition Time	1.0027
Spectrometer Frequency	125.80
Spectral Width	32679.7
Lowest Frequency	-1245.9
Nucleus	13C
Acquired Size	32768
Spectral Size	65536

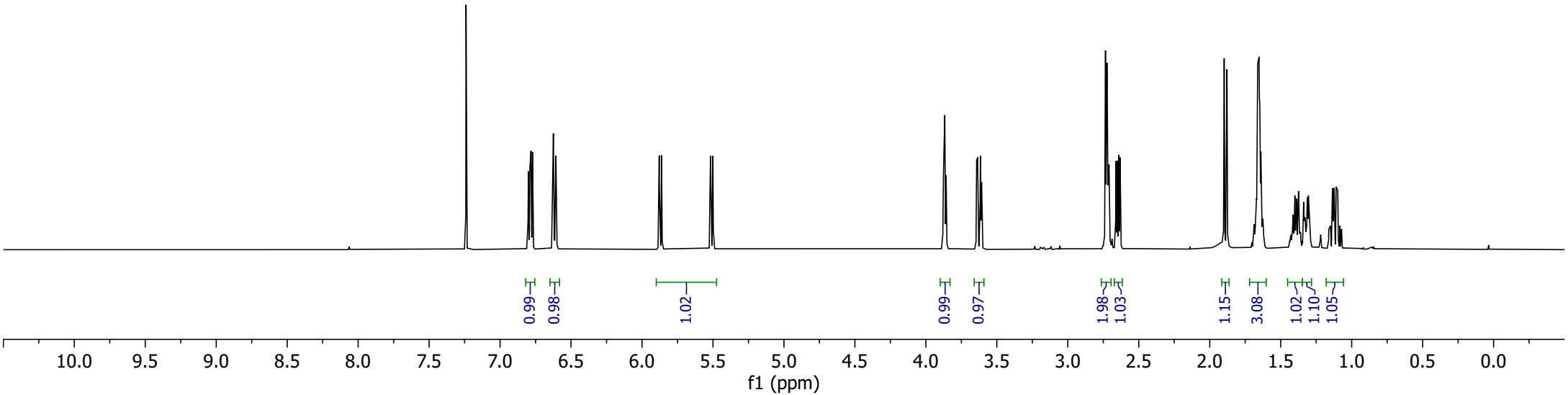
(–)-virosine B (**7**)

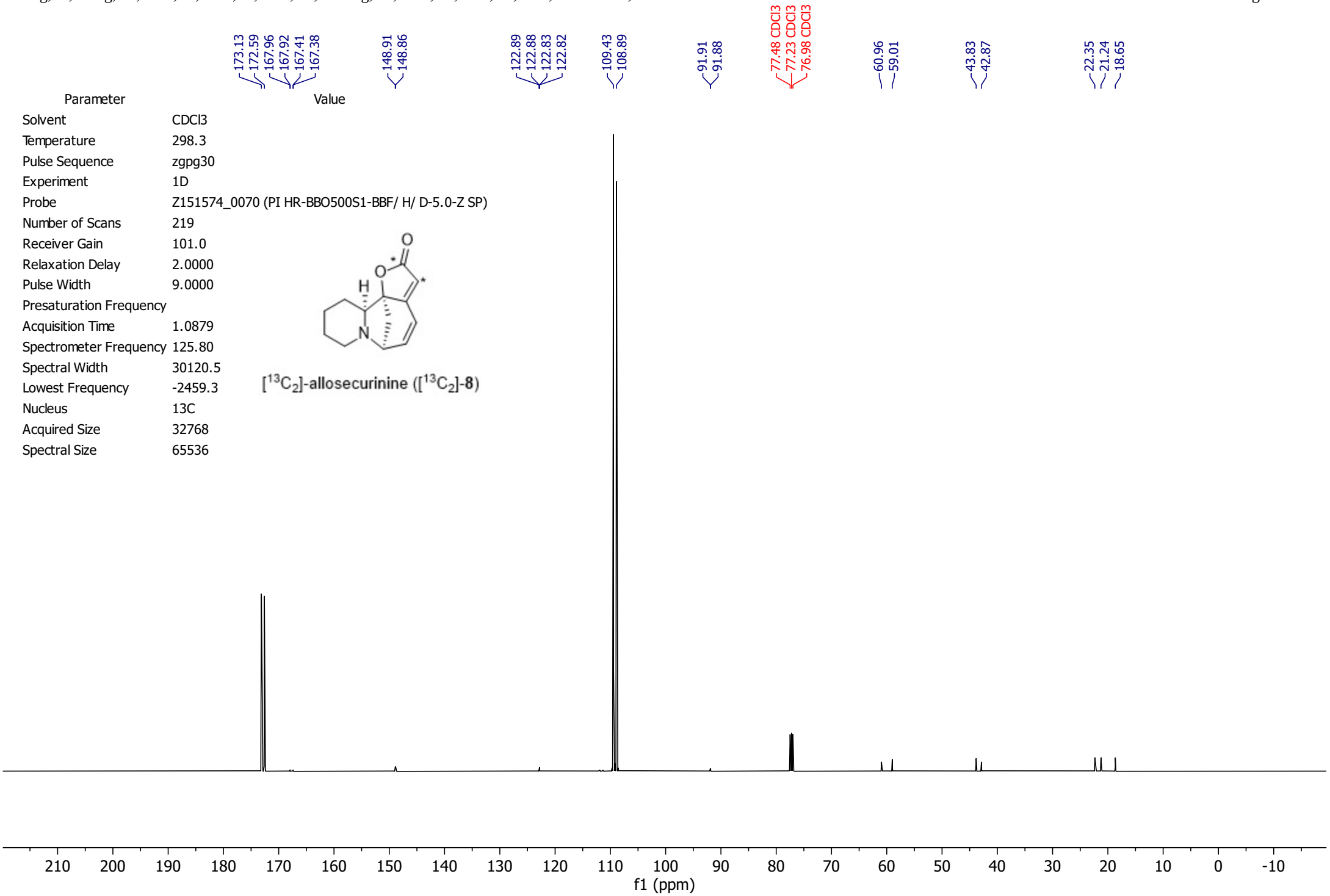


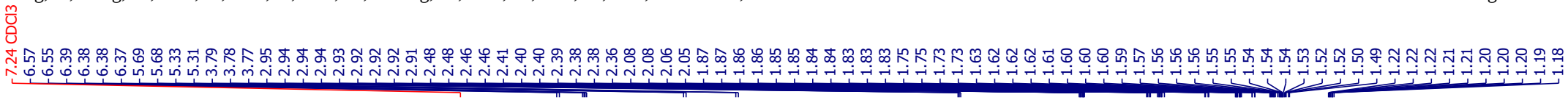
Parameter	Value
Solvent	CDCl3
Temperature	298.0
Pulse Sequence	zg30
Experiment	1D
Probe	Z151574_0070 (PI HR-BBO500S1-BBF/ H/ D-5.0-Z SP)
Number of Scans	32
Receiver Gain	101.0
Relaxation Delay	1.0000
Pulse Width	8.0000
Presaturation Frequency	
Acquisition Time	3.2768
Spectrometer Frequency	500.23
Spectral Width	10000.0
Lowest Frequency	-1933.3
Nucleus	1H
Acquired Size	32768
Spectral Size	65536



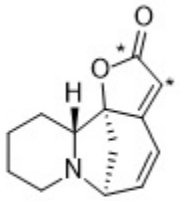
$[^{13}C_2]$-allosecurinine ($[^{13}C_2]$-**8**)



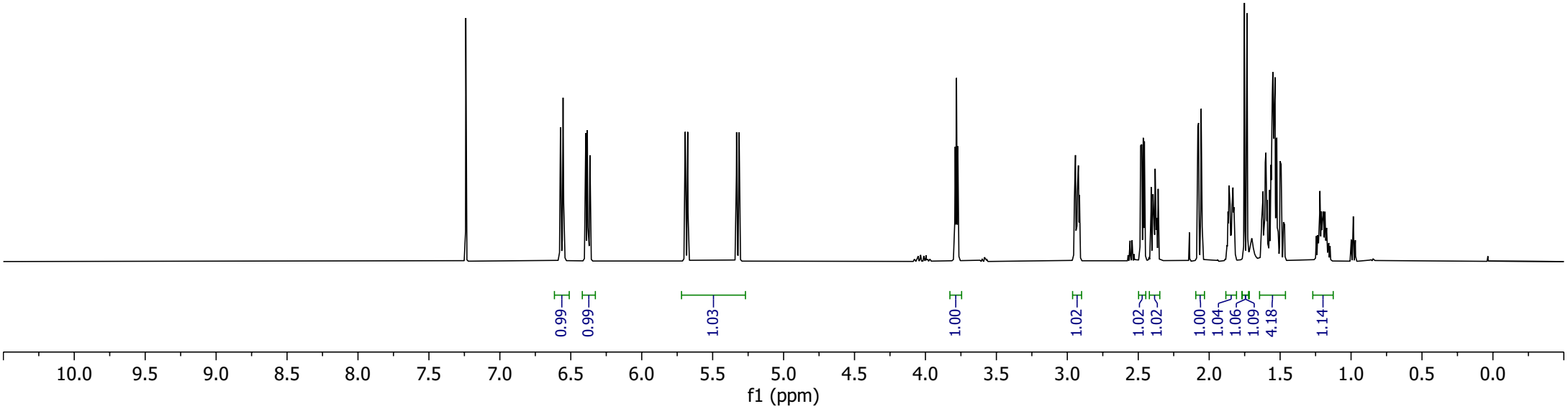

173.13
172.59
167.96
167.92
167.41
167.38
148.91
148.86
122.89
122.88
122.83
122.82
109.43
108.89
91.91
91.88
77.48 CDCl3
77.23 CDCl3
76.98 CDCl3
60.96
59.01
43.83
42.87
22.35
21.24
18.65
Parameter
Value
Solvent CDCl3
Temperature 298.3
Pulse Sequence zgpg30
Experiment 1D
Probe Z151574_0070 (PI HR-BBO500S1-BBF/ H/ D-5.0-Z SP)
Number of Scans 219
Receiver Gain 101.0
Relaxation Delay 2.0000
Pulse Width 9.0000
Presaturation Frequency
Acquisition Time 1.0879
Spectrometer Frequency 125.80
Spectral Width 30120.5
Lowest Frequency -2459.3
Nucleus 13C
Acquired Size 32768
Spectral Size 65536
O
O
H
N
[13C2]-allosecurinine ([13C2]-8)
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

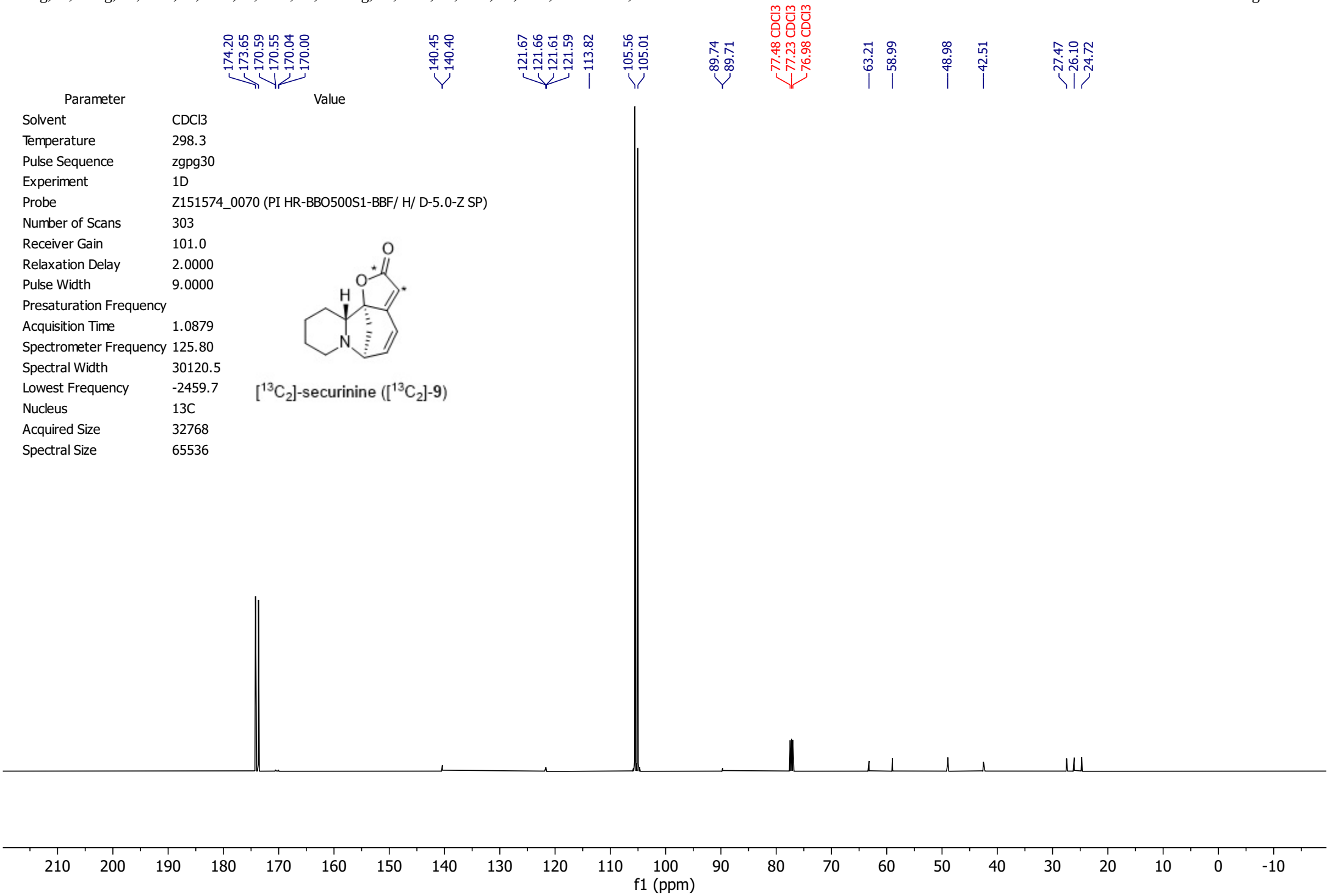


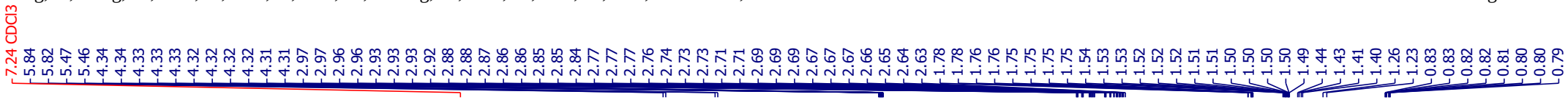
Parameter	Value
Solvent	CDCl3
Temperature	298.0
Pulse Sequence	zg30
Experiment	1D
Probe	Z151574_0070 (PI HR-BBO500S1-BBF/ H/ D-5.0-Z SP)
Number of Scans	17
Receiver Gain	90.5
Relaxation Delay	1.0000
Pulse Width	8.0000
Presaturation Frequency	
Acquisition Time	3.2768
Spectrometer Frequency	500.23
Spectral Width	10000.0
Lowest Frequency	-1933.1
Nucleus	1H
Acquired Size	32768
Spectral Size	65536



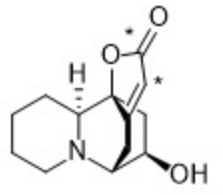
[$^{13}C_2$]-securinine ([$^{13}C_2$]-**9**)



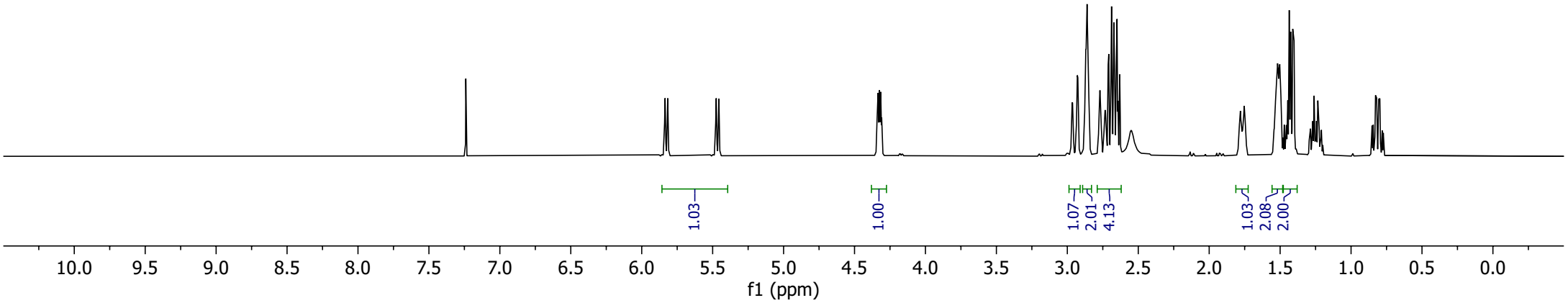

174.20
173.65
170.59
170.55
170.04
170.00
140.45
140.40
121.67
121.66
121.61
121.59
113.82
105.56
105.01
89.74
89.71
77.48 CDCl3
77.23 CDCl3
76.98 CDCl3
63.21
58.99
48.98
42.51
27.47
26.10
24.72
Parameter
Value
Solvent CDCl3
Temperature 298.3
Pulse Sequence zgpg30
Experiment 1D
Probe Z151574_0070 (PI HR-BBO500S1-BBF/ H/ D-5.0-Z SP)
Number of Scans 303
Receiver Gain 101.0
Relaxation Delay 2.0000
Pulse Width 9.0000
Presaturation Frequency
Acquisition Time 1.0879
Spectrometer Frequency 125.80
Spectral Width 30120.5
Lowest Frequency -2459.7
Nucleus 13C
Acquired Size 32768
Spectral Size 65536
O
O
H
N
[13C2]-securinine ([13C2]-9)
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

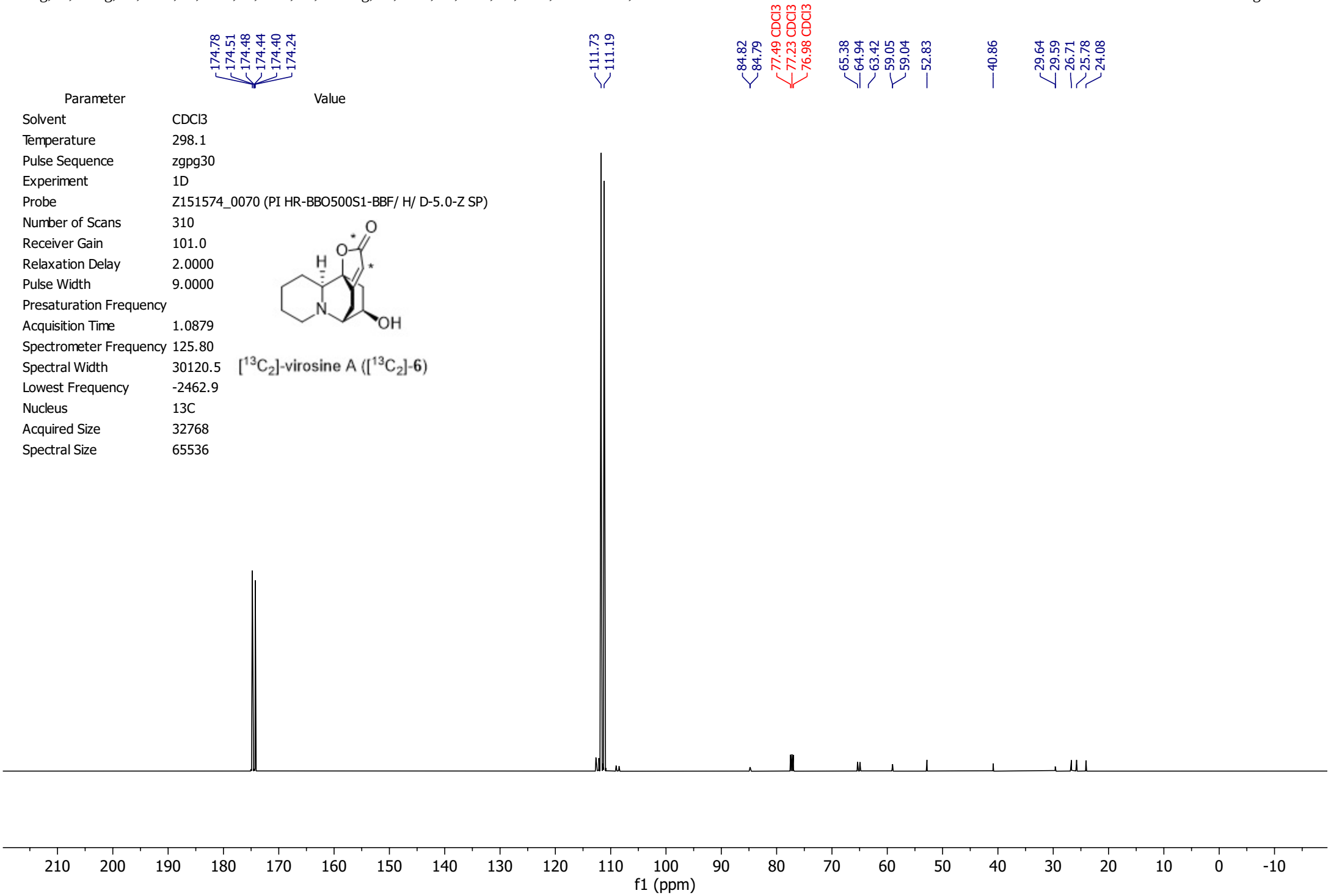


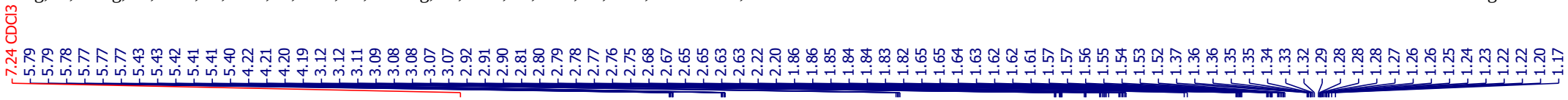
Parameter	Value
Solvent	CDCl3
Temperature	298.0
Pulse Sequence	zg30
Experiment	1D
Probe	Z151574_0070 (PI HR-BBO500S1-BBF/ H/ D-5.0-Z SP)
Number of Scans	12
Receiver Gain	57.0
Relaxation Delay	1.0000
Pulse Width	8.0000
Presaturation Frequency	
Acquisition Time	3.2768
Spectrometer Frequency	500.23
Spectral Width	10000.0
Lowest Frequency	-1933.1
Nucleus	1H
Acquired Size	32768
Spectral Size	65536



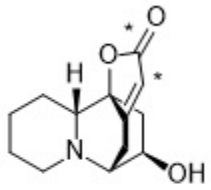
[$^{13}C_2$]-virosine A ([$^{13}C_2$]-**6**)



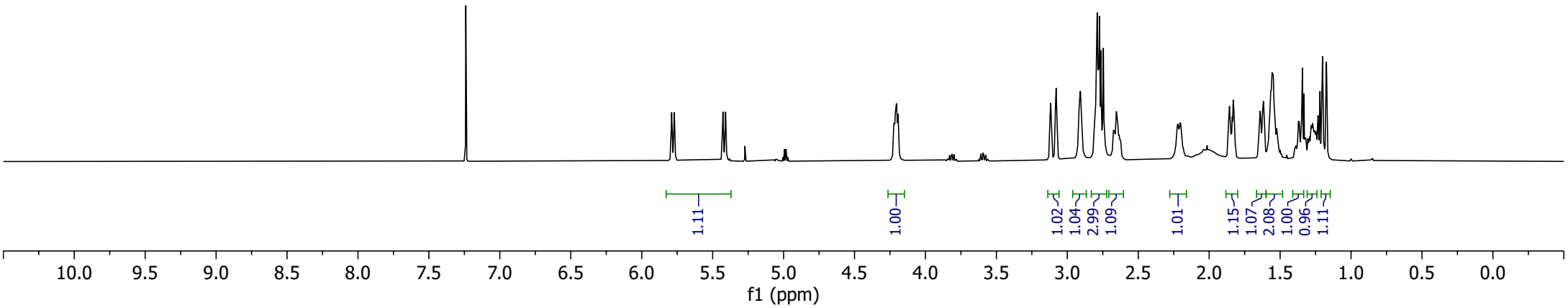

174.78
174.51
174.48
174.44
174.40
174.24
111.73
111.19
84.82
84.79
77.49 CDCl3
77.23 CDCl3
76.98 CDCl3
65.38
64.94
63.42
59.05
59.04
52.83
40.86
29.64
29.59
26.71
25.78
24.08
Parameter
Value
Solvent CDCl3
Temperature 298.1
Pulse Sequence zgpg30
Experiment 1D
Probe Z151574_0070 (PI HR-BBO500S1-BBF/ H/ D-5.0-Z SP)
Number of Scans 310
Receiver Gain 101.0
Relaxation Delay 2.0000
Pulse Width 9.0000
Presaturation Frequency
Acquisition Time 1.0879
Spectrometer Frequency 125.80
Spectral Width 30120.5
Lowest Frequency -2462.9
Nucleus 13C
Acquired Size 32768
Spectral Size 65536
O
O
H
N
OH
[13C2]-virosine A ([13C2]-6)
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

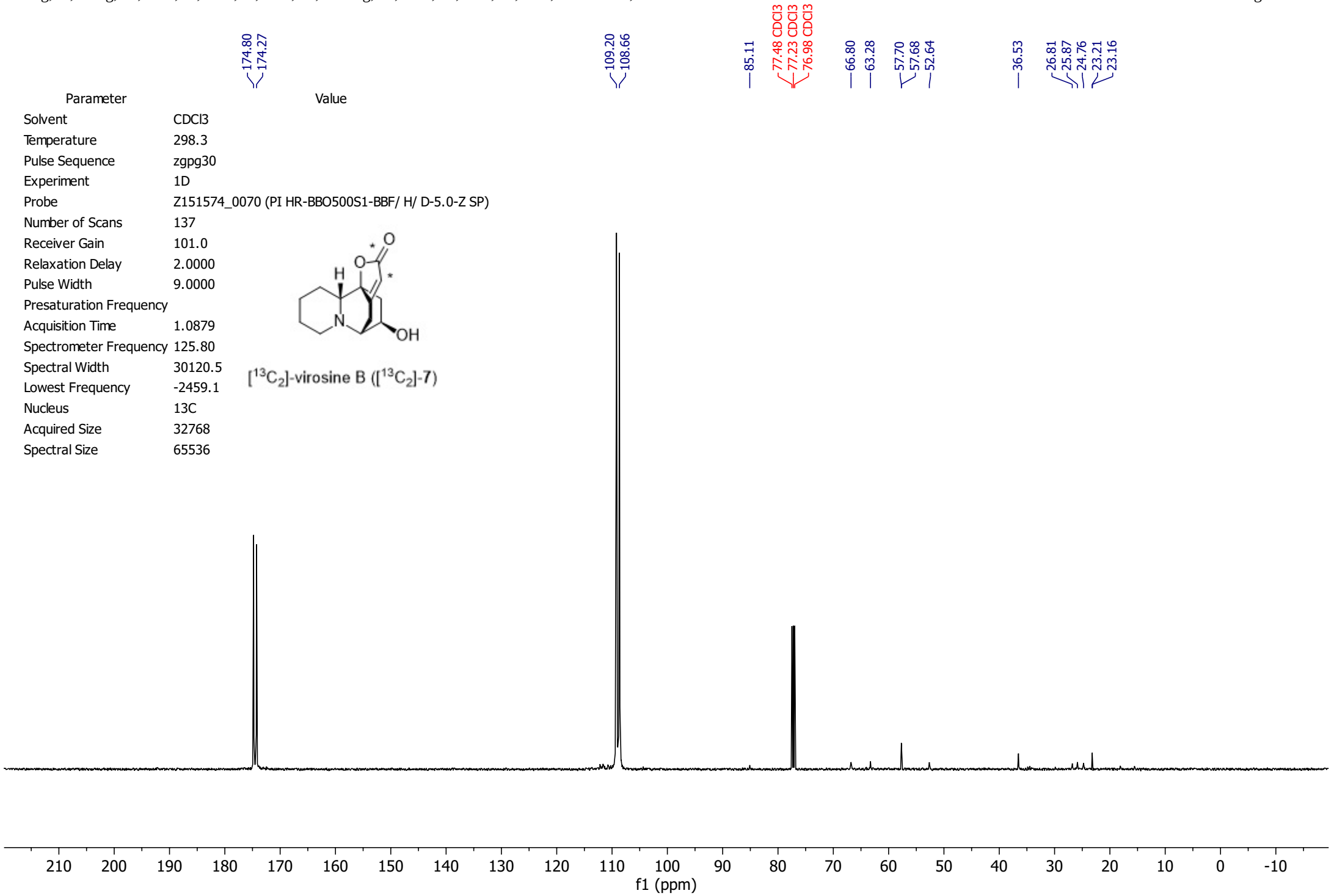


Parameter	Value
Solvent	CDCl3
Temperature	298.0
Pulse Sequence	zg30
Experiment	1D
Probe	Z151574_0070 (PI HR-BBO500S1-BBF/ H/ D-5.0-Z SP)
Number of Scans	26
Receiver Gain	90.5
Relaxation Delay	1.0000
Pulse Width	8.0000
Presaturation Frequency	
Acquisition Time	3.2768
Spectrometer Frequency	500.23
Spectral Width	10000.0
Lowest Frequency	-1932.8
Nucleus	1H
Acquired Size	32768
Spectral Size	65536



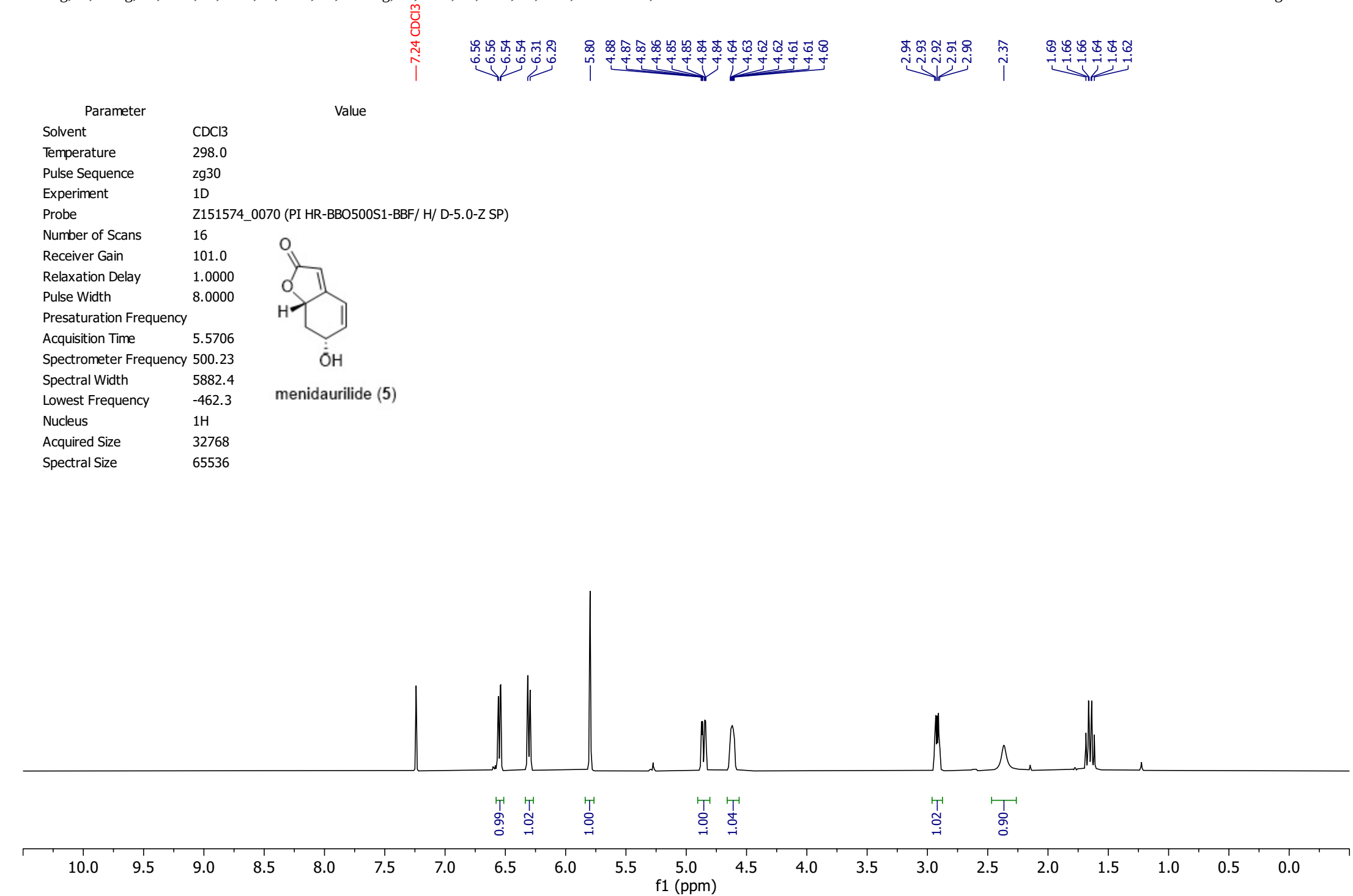
[$^{13}C_2$]-virosine B ([$^{13}C_2$]-7)





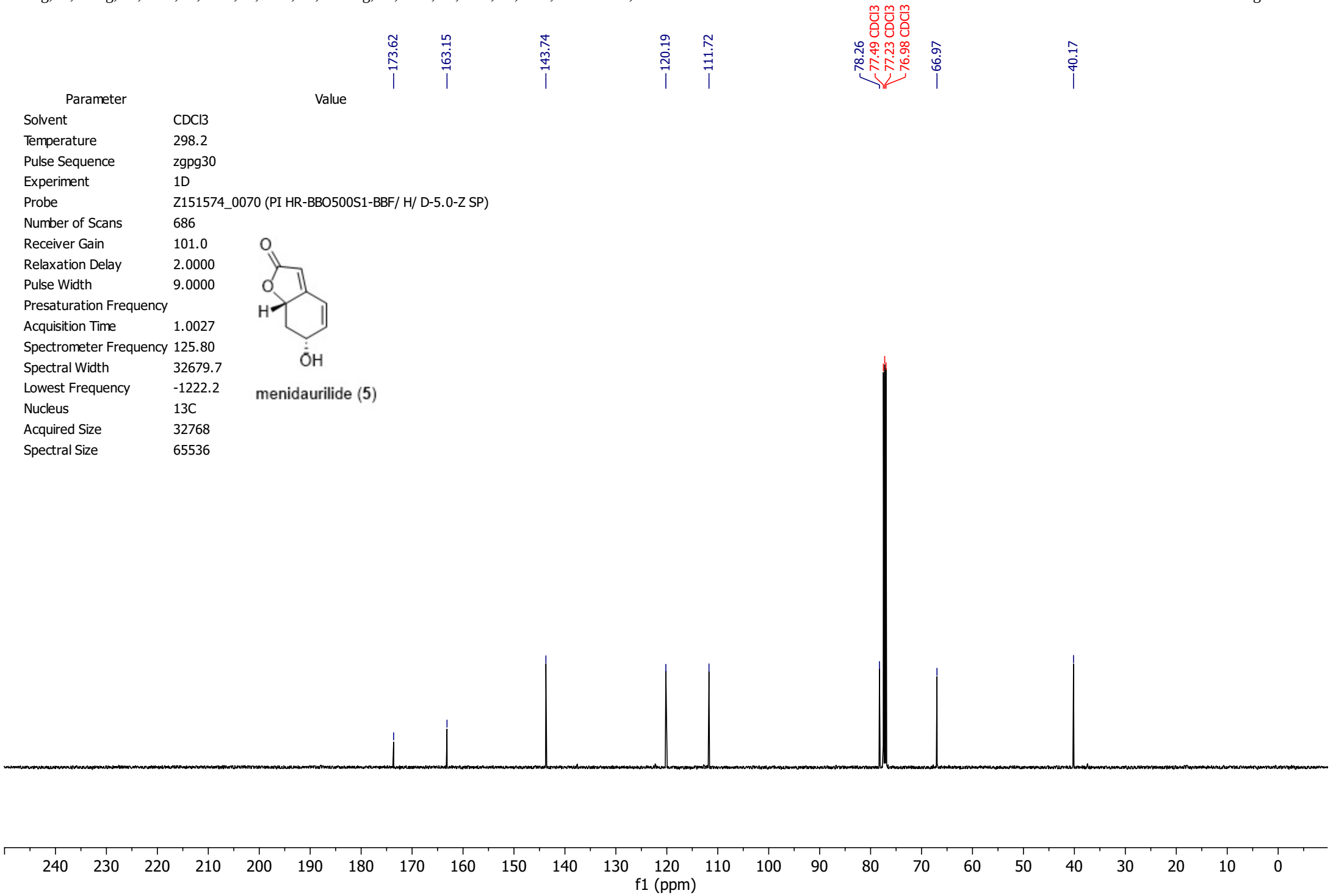
Parameter	Value
Solvent	CDCl3
Temperature	298.3
Pulse Sequence	zgpg30
Experiment	1D
Probe	Z151574_0070 (PI HR-BBO500S1-BBF/ H/ D-5.0-Z SP)
Number of Scans	137
Receiver Gain	101.0
Relaxation Delay	2.0000
Pulse Width	9.0000
Presaturation Frequency	
Acquisition Time	1.0879
Spectrometer Frequency	125.80
Spectral Width	30120.5
Lowest Frequency	-2459.1
Nucleus	13C
Acquired Size	32768
Spectral Size	65536

[$^{13}C_2$]-virosine B ([$^{13}C_2$]-7)



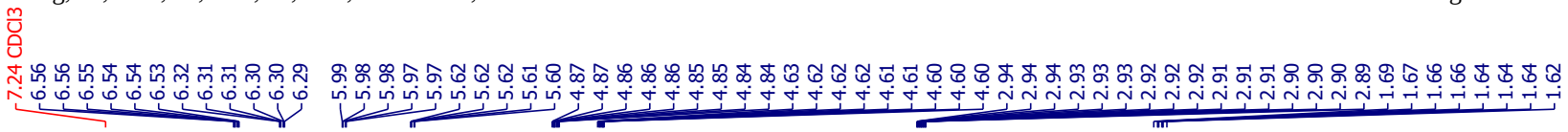
Parameter	Value
Solvent	CDCl3
Temperature	298.0
Pulse Sequence	zg30
Experiment	1D
Probe	Z151574_0070 (PI HR-BBO500S1-BBF/ H/ D-5.0-Z SP)
Number of Scans	16
Receiver Gain	101.0
Relaxation Delay	1.0000
Pulse Width	8.0000
Presaturation Frequency	
Acquisition Time	5.5706
Spectrometer Frequency	500.23
Spectral Width	5882.4
Lowest Frequency	-462.3
Nucleus	1H
Acquired Size	32768
Spectral Size	65536

menidaurilide (5)

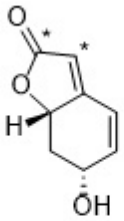


Parameter	Value
Solvent	CDCl3
Temperature	298.2
Pulse Sequence	zgpg30
Experiment	1D
Probe	Z151574_0070 (PI HR-BBO500S1-BBF/ H/ D-5.0-Z SP)
Number of Scans	686
Receiver Gain	101.0
Relaxation Delay	2.0000
Pulse Width	9.0000
Presaturation Frequency	
Acquisition Time	1.0027
Spectrometer Frequency	125.80
Spectral Width	32679.7
Lowest Frequency	-1222.2
Nucleus	13C
Acquired Size	32768
Spectral Size	65536

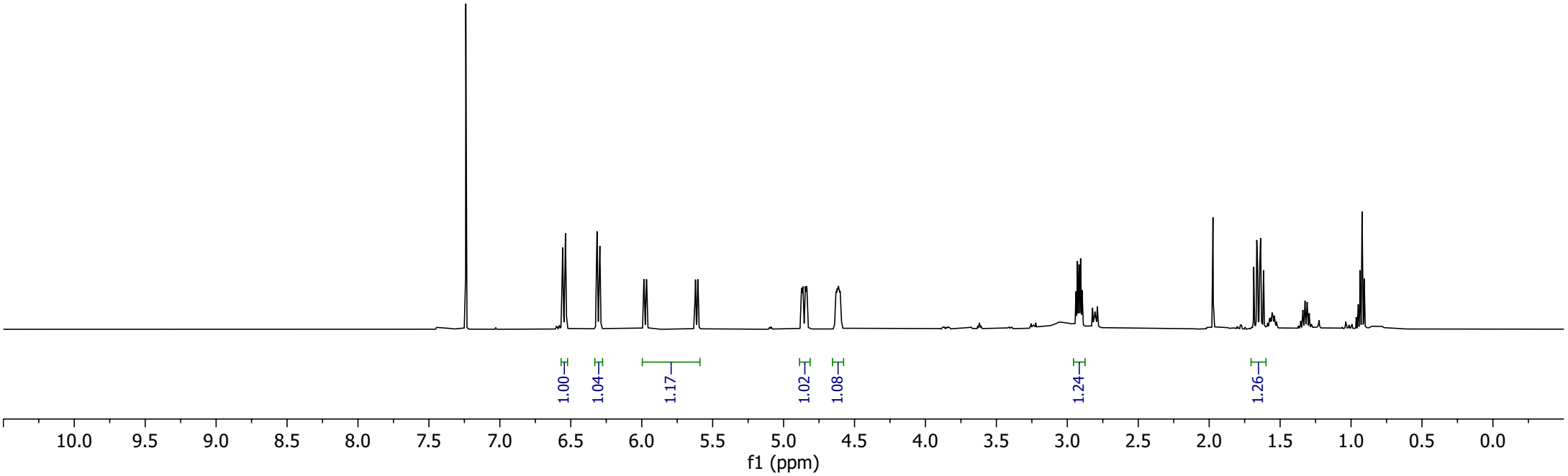
menidaurilide (5)

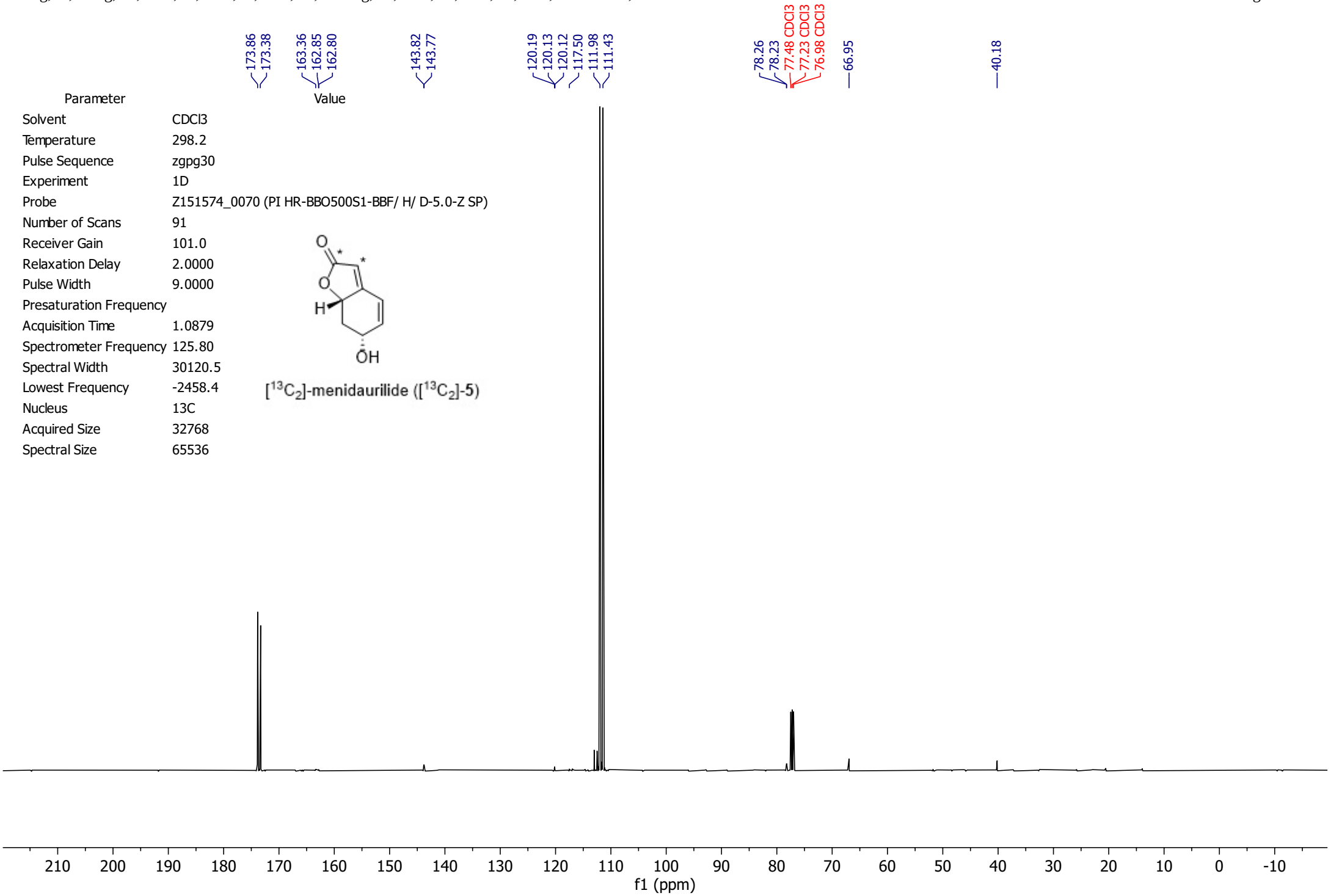


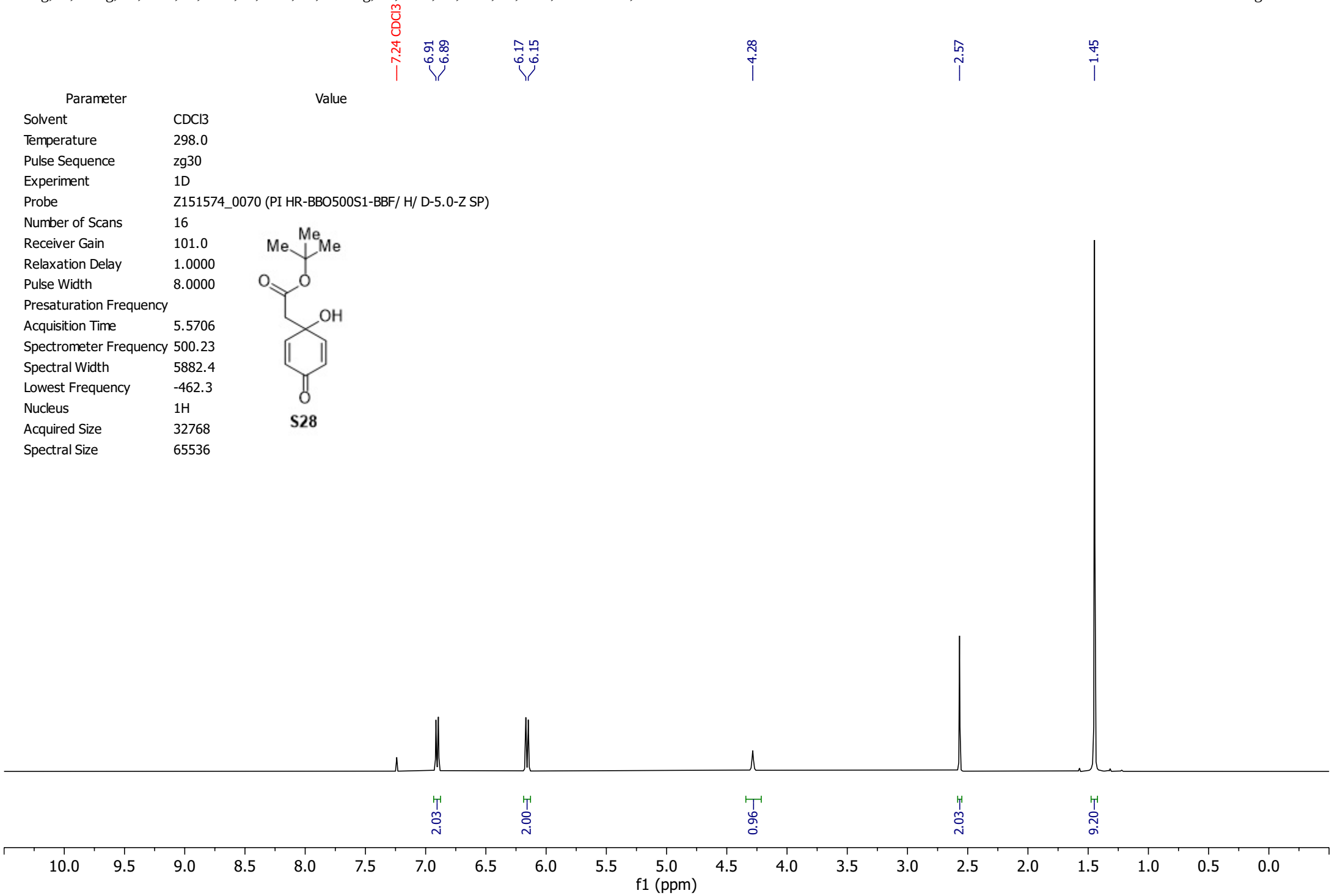
Parameter	Value
Solvent	CDCl3
Temperature	298.0
Pulse Sequence	zg30
Experiment	1D
Probe	Z151574_0070 (PI HR-BBO500S1-BBF/ H/ D-5.0-Z SP)
Number of Scans	29
Receiver Gain	101.0
Relaxation Delay	1.0000
Pulse Width	8.0000
Presaturation Frequency	
Acquisition Time	3.2768
Spectrometer Frequency	500.23
Spectral Width	10000.0
Lowest Frequency	-1933.3
Nucleus	1H
Acquired Size	32768
Spectral Size	65536



[$^{13}C_2$]-menidaurilide ([$^{13}C_2$]-**5**)

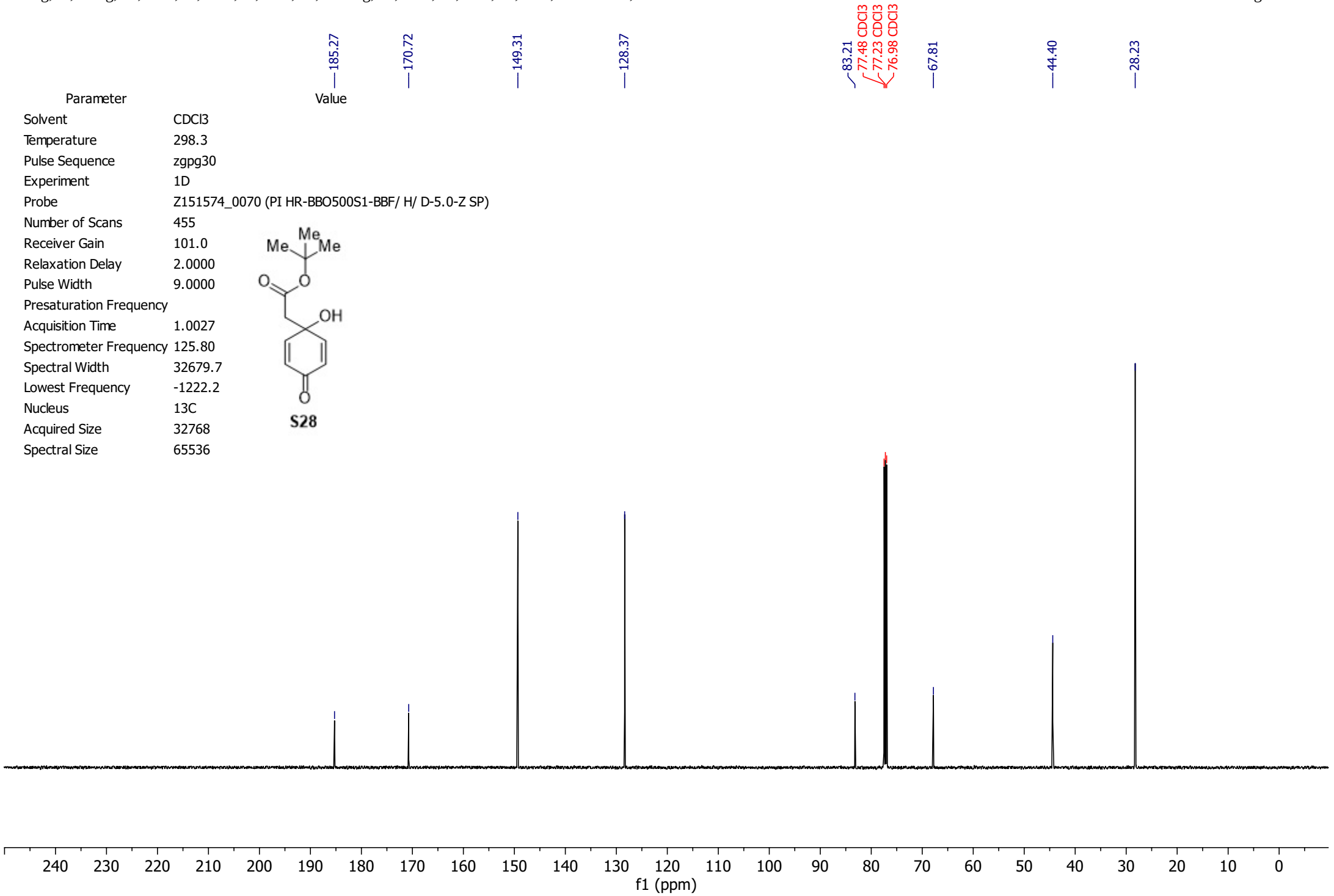



173.86
173.38
163.36
162.85
162.80
143.82
143.77
120.19
120.13
120.12
117.50
111.98
111.43
78.26
78.23
77.48 CDCl3
77.23 CDCl3
76.98 CDCl3
66.95
40.18
Parameter
Value
Solvent CDCl3
Temperature 298.2
Pulse Sequence zgpg30
Experiment 1D
Probe Z151574_0070 (PI HR-BBO500S1-BBF/ H/ D-5.0-Z SP)
Number of Scans 91
Receiver Gain 101.0
Relaxation Delay 2.0000
Pulse Width 9.0000
Presaturation Frequency
Acquisition Time 1.0879
Spectrometer Frequency 125.80
Spectral Width 30120.5
Lowest Frequency -2458.4
Nucleus 13C
Acquired Size 32768
Spectral Size 65536
O
O
H
OH
[13C2]-menidaurilide ([13C2]-5)
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)



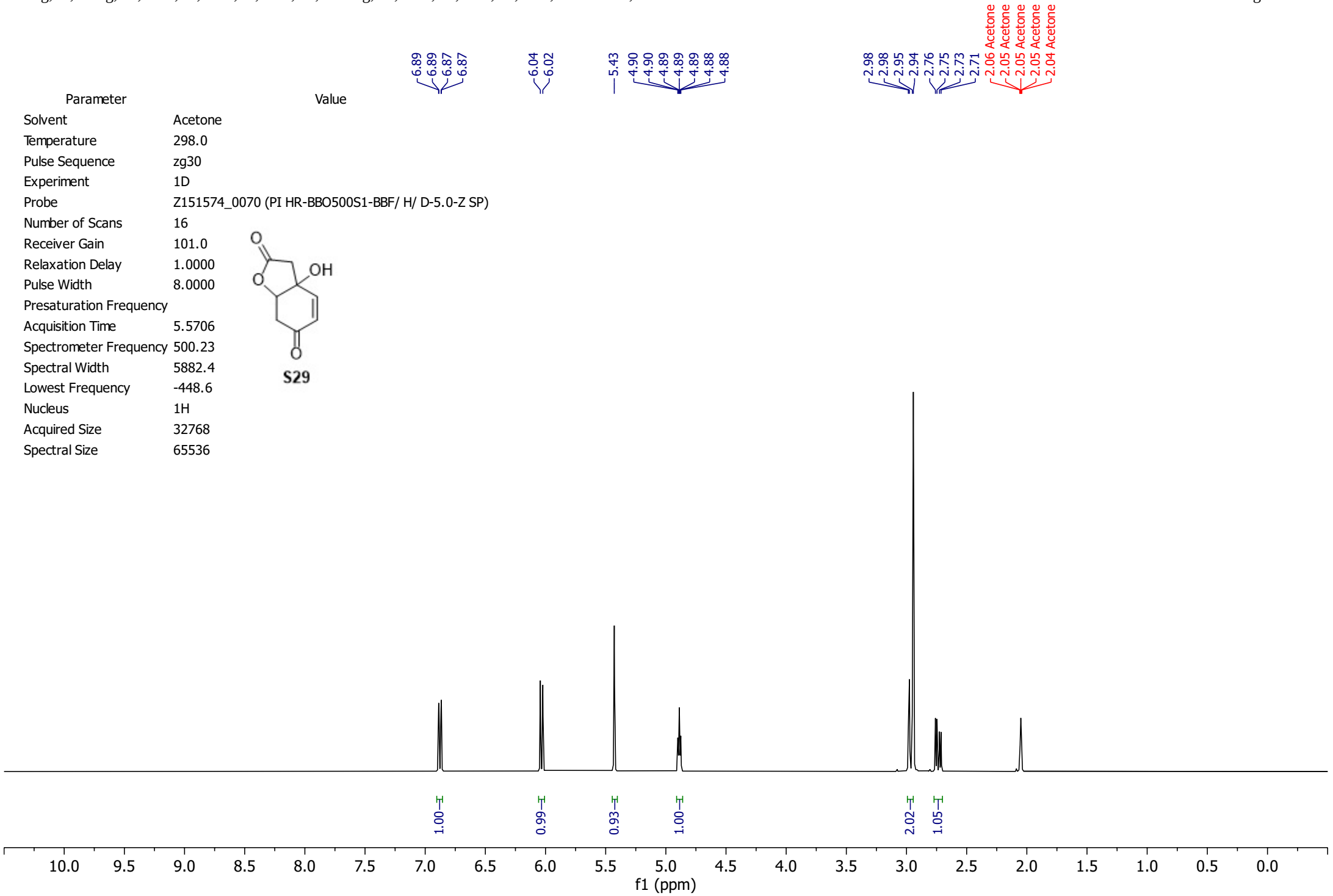
Parameter	Value
Solvent	CDCl3
Temperature	298.0
Pulse Sequence	zg30
Experiment	1D
Probe	Z151574_0070 (PI HR-BBO500S1-BBF/ H/ D-5.0-Z SP)
Number of Scans	16
Receiver Gain	101.0
Relaxation Delay	1.0000
Pulse Width	8.0000
Presaturation Frequency	
Acquisition Time	5.5706
Spectrometer Frequency	500.23
Spectral Width	5882.4
Lowest Frequency	-462.3
Nucleus	1H
Acquired Size	32768
Spectral Size	65536

S28



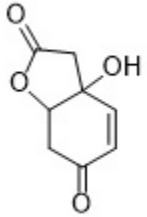
Parameter	Value
Solvent	CDCl3
Temperature	298.3
Pulse Sequence	zgpg30
Experiment	1D
Probe	Z151574_0070 (PI HR-BBO500S1-BBF/ H/ D-5.0-Z SP)
Number of Scans	455
Receiver Gain	101.0
Relaxation Delay	2.0000
Pulse Width	9.0000
Presaturation Frequency	
Acquisition Time	1.0027
Spectrometer Frequency	125.80
Spectral Width	32679.7
Lowest Frequency	-1222.2
Nucleus	13C
Acquired Size	32768
Spectral Size	65536

S28

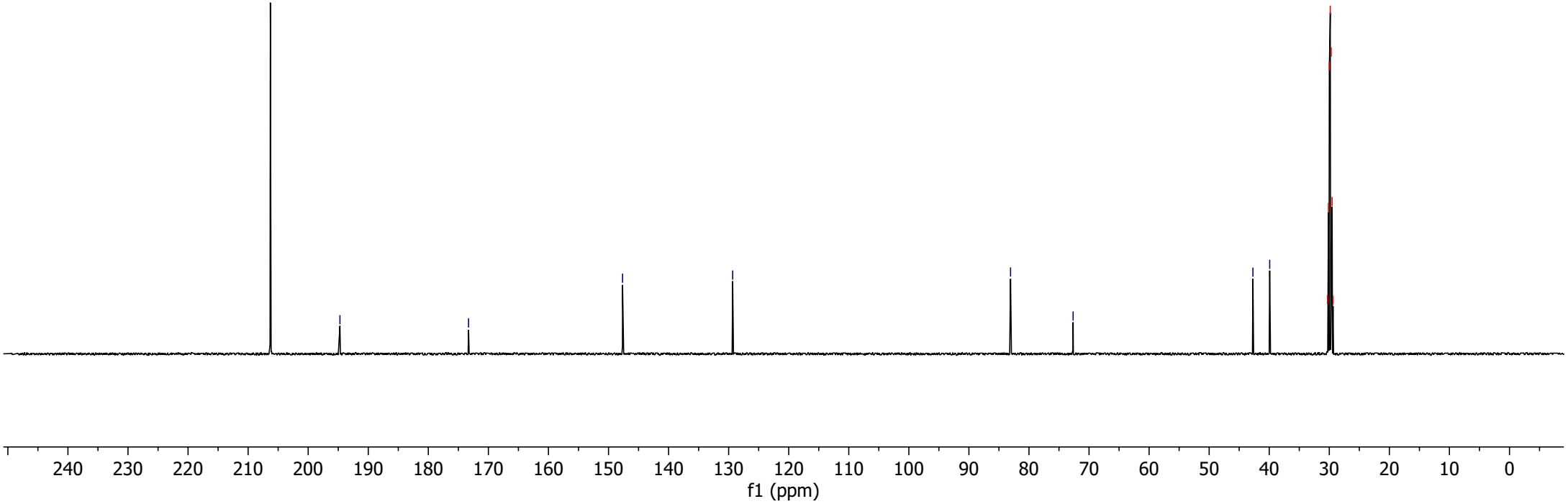

6.89
6.89
6.87
6.87
6.04
6.02
5.43
4.90
4.90
4.89
4.89
4.89
4.88
4.88
2.98
2.98
2.95
2.94
2.76
2.75
2.73
2.71
2.06 Acetone
2.05 Acetone
2.05 Acetone
2.05 Acetone
2.04 Acetone
Parameter
Value
Solvent
Acetone
Temperature
298.0
Pulse Sequence
zg30
Experiment
1D
Probe
Z151574_0070 (PI HR-BBO500S1-BBF/ H/ D-5.0-Z SP)
Number of Scans
16
Receiver Gain
101.0
Relaxation Delay
1.0000
Pulse Width
8.0000
Presaturation Frequency
Acquisition Time
5.5706
Spectrometer Frequency
500.23
Spectral Width
5882.4
Lowest Frequency
-448.6
Nucleus
1H
Acquired Size
32768
Spectral Size
65536
O
O
OH
O
S29
1.00
0.99
0.93
1.00
2.02
1.05
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)

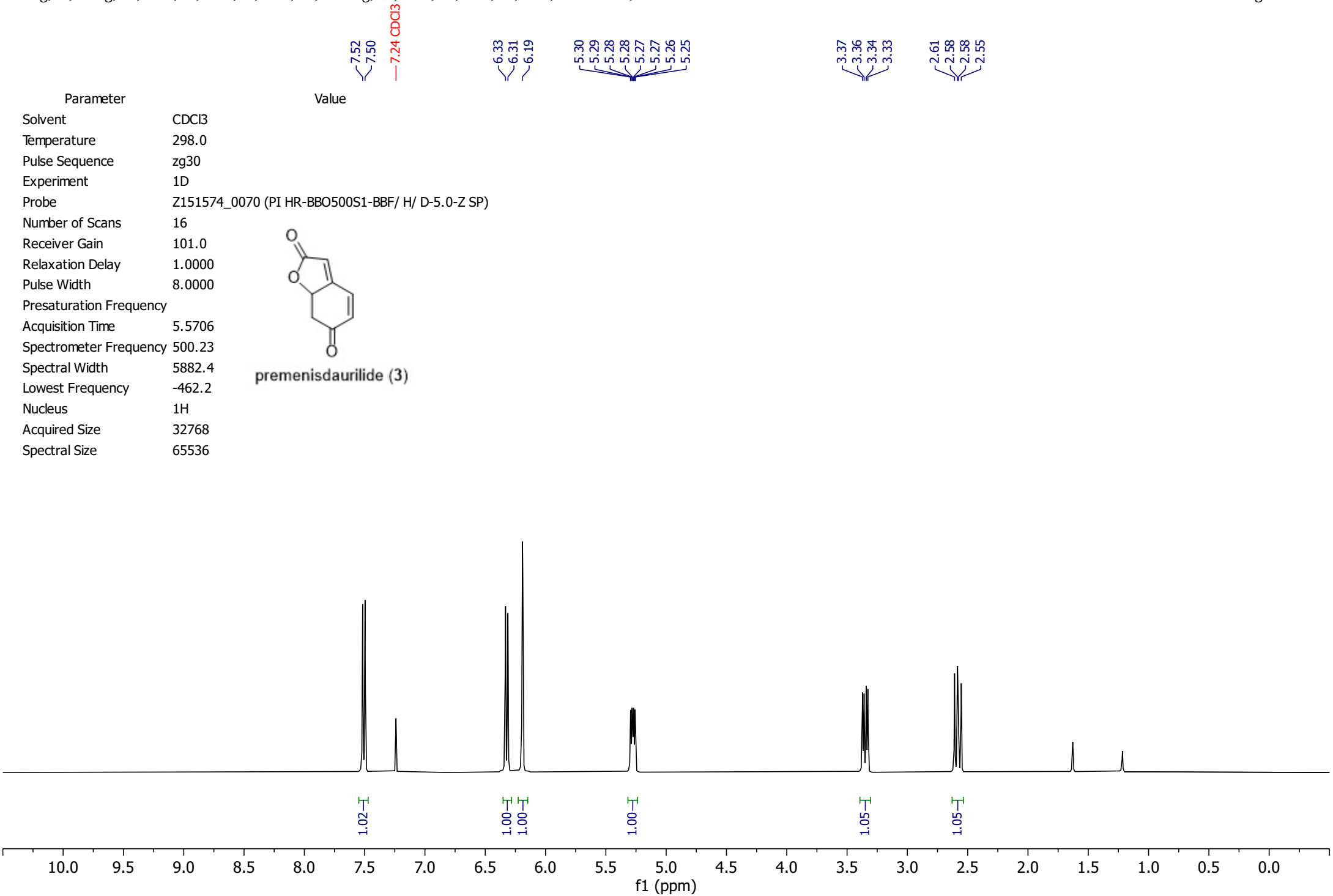
194.72 173.30 147.66 129.33 83.07 72.65 42.72 39.92 30.30 Acetone 30.15 Acetone 29.99 Acetone 29.84 Acetone 29.69 Acetone 29.53 Acetone 29.38 Acetone

Parameter	Value
Solvent	Acetone
Temperature	298.2
Pulse Sequence	zgpg30
Experiment	1D
Probe	Z151574_0070 (PI HR-BBO500S1-BBF/ H/ D-5.0-Z SP)
Number of Scans	82
Receiver Gain	101.0
Relaxation Delay	2.0000
Pulse Width	9.0000
Presaturation Frequency	
Acquisition Time	1.0027
Spectrometer Frequency	125.80
Spectral Width	32679.7
Lowest Frequency	-1138.9
Nucleus	13C
Acquired Size	32768
Spectral Size	65536



S29



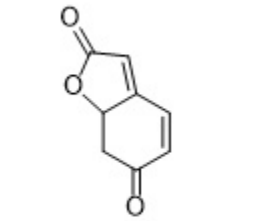


Parameter	Value
Solvent	CDCl3
Temperature	298.0
Pulse Sequence	zg30
Experiment	1D
Probe	Z151574_0070 (PI HR-BBO500S1-BBF/ H/ D-5.0-Z SP)
Number of Scans	16
Receiver Gain	101.0
Relaxation Delay	1.0000
Pulse Width	8.0000
Presaturation Frequency	
Acquisition Time	5.5706
Spectrometer Frequency	500.23
Spectral Width	5882.4
Lowest Frequency	-462.2
Nucleus	1H
Acquired Size	32768
Spectral Size	65536

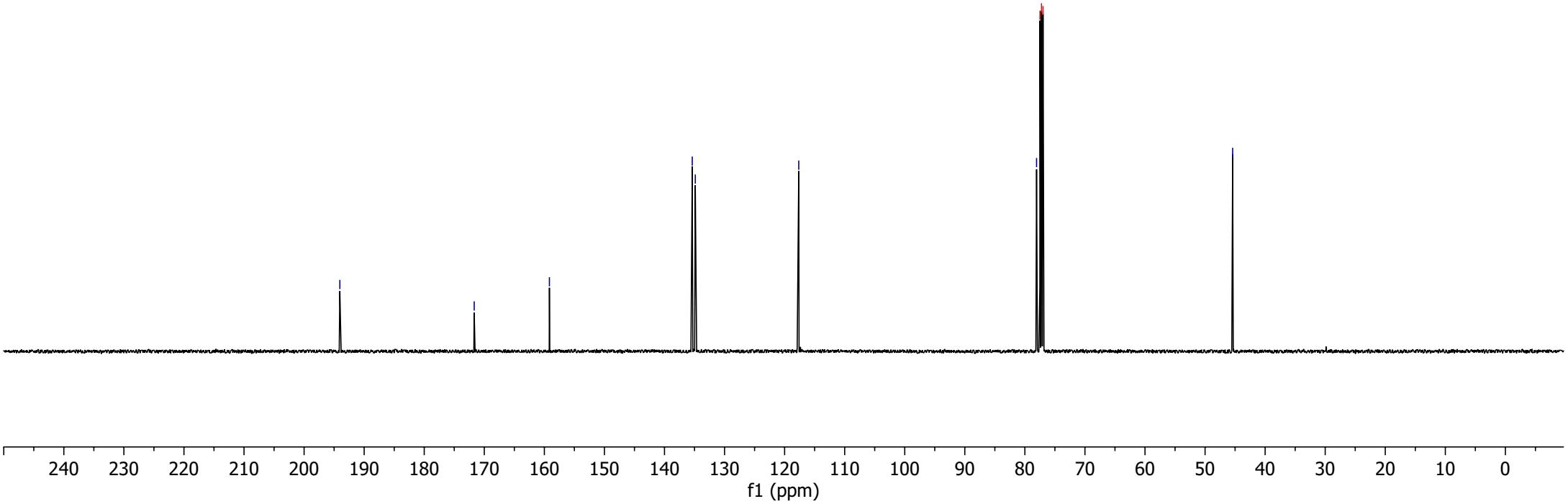
premenisdaurilide (3)

194.05
171.68
159.16
135.38
134.87
117.65
78.05
77.48 CDCl3
77.23 CDCl3
76.98 CDCl3
45.41

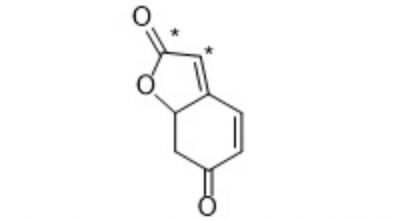
Parameter	Value
Solvent	CDCl3
Temperature	298.2
Pulse Sequence	zgpg30
Experiment	1D
Probe	Z151574_0070 (PI HR-BBO500S1-BBF/ H/ D-5.0-Z SP)
Number of Scans	521
Receiver Gain	101.0
Relaxation Delay	2.0000
Pulse Width	9.0000
Presaturation Frequency	
Acquisition Time	1.0027
Spectrometer Frequency	125.80
Spectral Width	32679.7
Lowest Frequency	-1224.7
Nucleus	13C
Acquired Size	32768
Spectral Size	65536



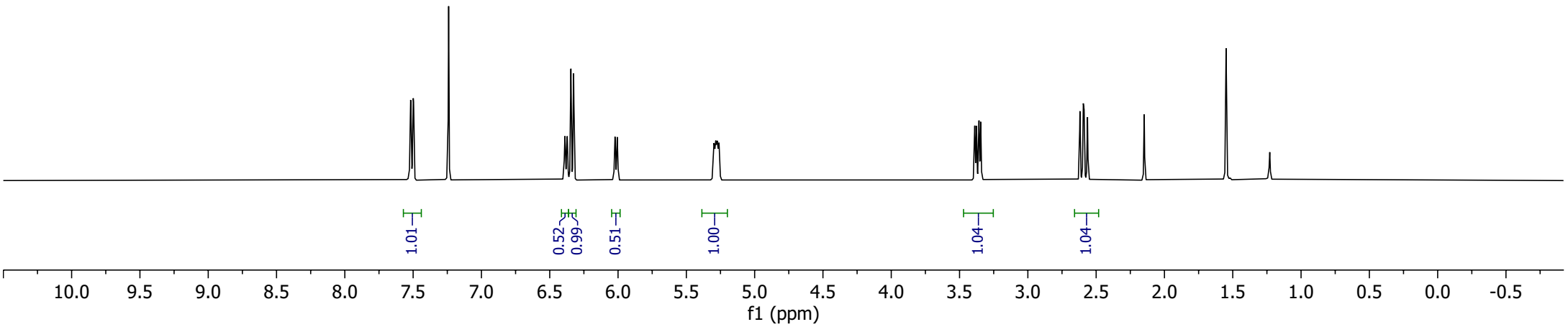
premenisdaurilide (3)



Parameter	Value
Solvent	CDCl3
Temperature	298.0
Pulse Sequence	zg30
Experiment	1D
Probe	Z151574_0070 (PI HR-BBO500S1-BBF/ H/ D-5.0-Z SP)
Number of Scans	16
Receiver Gain	101.0
Relaxation Delay	1.0000
Pulse Width	8.0000
Presaturation Frequency	
Acquisition Time	5.5706
Spectrometer Frequency	500.23
Spectral Width	5882.4
Lowest Frequency	-462.2
Nucleus	1H
Acquired Size	32768
Spectral Size	65536

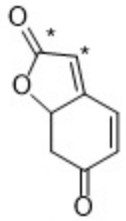


[$^{13}C_2$]-premenisdaurilide ([$^{13}C_2$]-3)

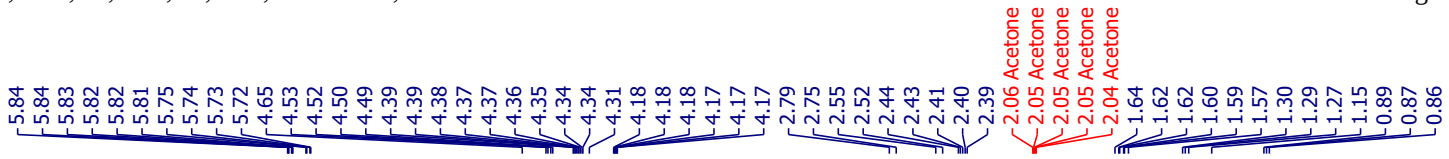


194.04
171.93
171.40
159.42
159.38
158.86
158.81
135.45
135.41
134.89
134.88
134.83
134.82
117.96
117.43
78.09
78.06
77.49 CDCl3
77.23 CDCl3
76.98 CDCl3
45.46

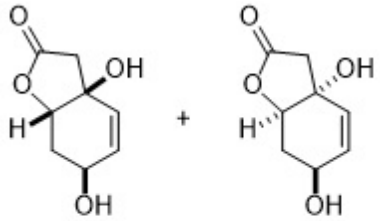
Parameter	Value
Solvent	CDCl3
Temperature	298.2
Pulse Sequence	zgpg30
Experiment	1D
Probe	Z151574_0070 (PI HR-BBO500S1-BBF/ H/ D-5.0-Z SP)
Number of Scans	1718
Receiver Gain	101.0
Relaxation Delay	2.0000
Pulse Width	9.0000
Presaturation Frequency	
Acquisition Time	1.0027
Spectrometer Frequency	125.80
Spectral Width	32679.7
Lowest Frequency	-1221.0
Nucleus	13C
Acquired Size	32768
Spectral Size	65536



[$^{13}C_2$]-premenisdaurilide ([$^{13}C_2$]-**3**)

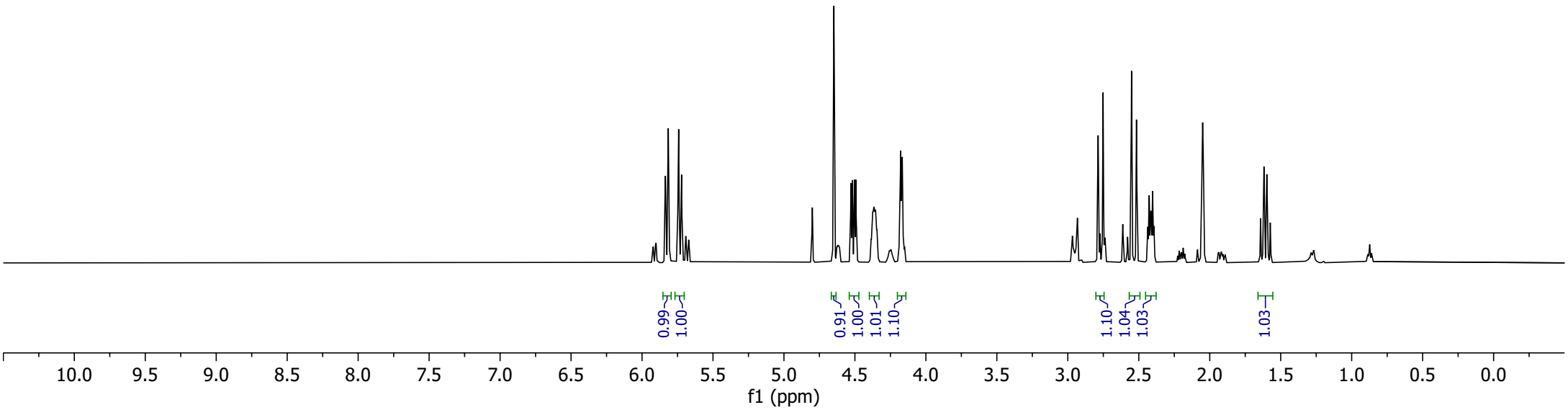


Parameter	Value
Solvent	Acetone
Temperature	298.0
Pulse Sequence	zg30
Experiment	1D
Probe	Z151574_0070 (PI HR-BBO500S1-BBF/ H/ D-5.0-Z SP)
Number of Scans	16
Receiver Gain	90.5
Relaxation Delay	1.0000
Pulse Width	8.0000
Presaturation Frequency	
Acquisition Time	5.5706
Spectrometer Frequency	500.23
Spectral Width	5882.4
Lowest Frequency	-448.7
Nucleus	1H
Acquired Size	32768
Spectral Size	65536

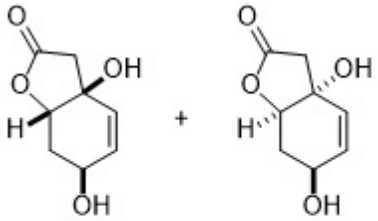


syn-**4** *anti*-**4**

syn-**4** : *anti*-**4** = 1 : 4



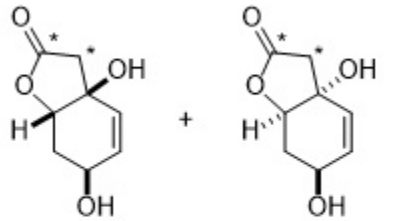
Parameter	Value
Solvent	Acetone
Temperature	298.0
Pulse Sequence	zgpg30
Experiment	1D
Probe	Z151574_0070 (PI HR-BBO500S1-BBF/ H/ D-5.0-Z SP)
Number of Scans	3603
Receiver Gain	101.0
Relaxation Delay	2.0000
Pulse Width	9.0000
Presaturation Frequency	
Acquisition Time	1.0027
Spectrometer Frequency	125.80
Spectral Width	32679.7
Lowest Frequency	-1138.9
Nucleus	13C
Acquired Size	32768
Spectral Size	65536



syn-**4** *anti*-**4**

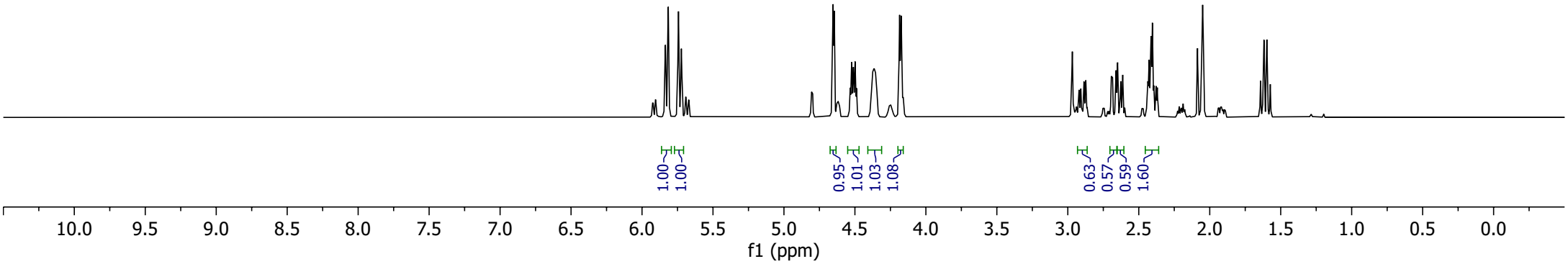
syn-**4** : *anti*-**4** = 1 : 4

Parameter	Value
Solvent	Acetone
Temperature	298.0
Pulse Sequence	zg30
Experiment	1D
Probe	Z151574_0070 (PI HR-BBO500S1-BBF/ H/ D-5.0-Z SP)
Number of Scans	16
Receiver Gain	64.0
Relaxation Delay	1.0000
Pulse Width	8.0000
Presaturation Frequency	
Acquisition Time	5.5706
Spectrometer Frequency	500.23
Spectral Width	5882.4
Lowest Frequency	-448.7
Nucleus	1H
Acquired Size	32768
Spectral Size	65536

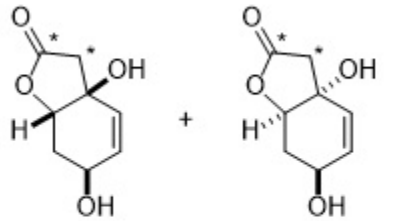


$[^{13}C_2]$-*syn*-**4** + $[^{13}C_2]$-*anti*-**4**

$[^{13}C_2]$-*syn*-**4** : $[^{13}C_2]$-*anti*-**4** = 1 : 4

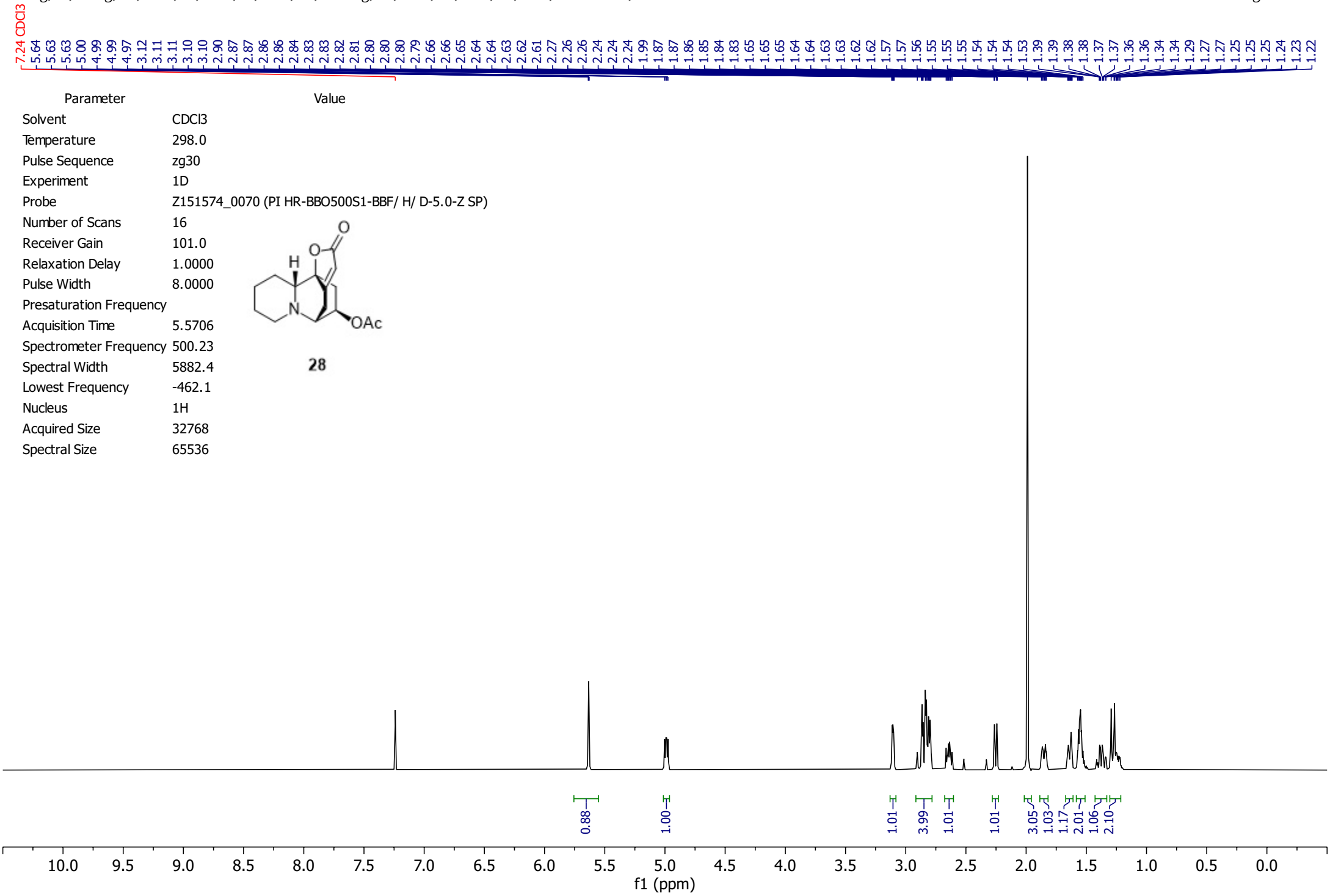


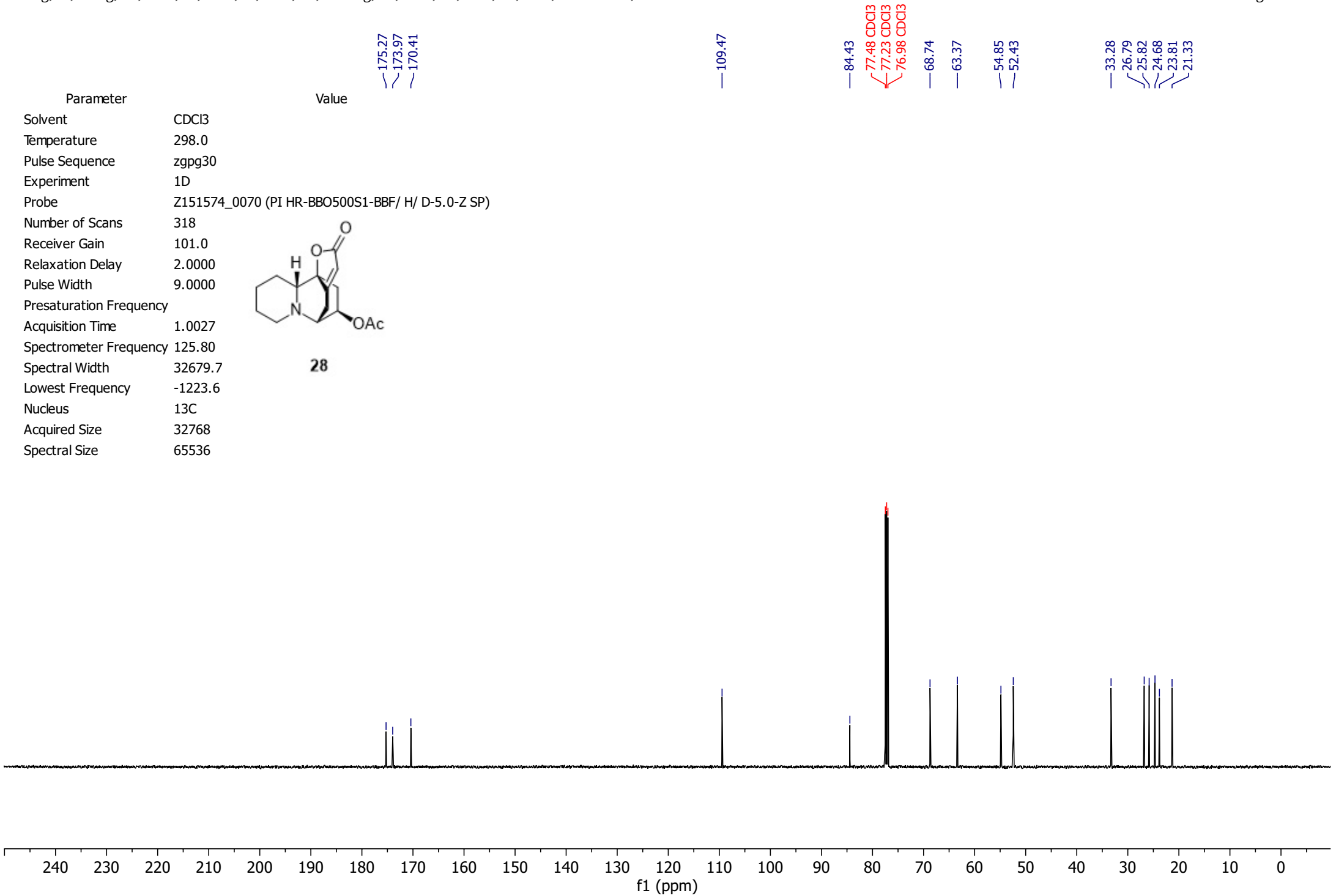
Parameter	Value
Solvent	Acetone
Temperature	298.2
Pulse Sequence	zgpg30
Experiment	1D
Probe	Z151574_0070 (PI HR-BBO500S1-BBF/ H/ D-5.0-Z SP)
Number of Scans	776
Receiver Gain	101.0
Relaxation Delay	2.0000
Pulse Width	9.0000
Presaturation Frequency	
Acquisition Time	1.0027
Spectrometer Frequency	125.80
Spectral Width	32679.7
Lowest Frequency	-1139.4
Nucleus	13C
Acquired Size	32768
Spectral Size	65536



[$^{13}C_2$]-*syn*-**4** + [$^{13}C_2$]-*anti*-**4**

[$^{13}C_2$]-*syn*-**4** : [$^{13}C_2$]-*anti*-**4** = 1 : 4


7.24 CDCl3
5.64 5.63 5.63 5.00 4.99 4.99 4.97 3.12 3.11 3.11 3.10 3.10 2.90 2.87 2.87 2.86 2.86 2.84 2.83 2.83 2.82 2.81 2.80 2.80 2.80 2.79 2.66 2.65 2.64 2.64 2.63 2.62 2.61 2.27 2.26 2.26 2.24 2.24 2.24 1.99 1.87 1.87 1.86 1.85 1.84 1.83 1.65 1.65 1.65 1.64 1.64 1.63 1.63 1.62 1.62 1.57 1.57 1.56 1.55 1.55 1.55 1.54 1.54 1.54 1.53 1.39 1.39 1.38 1.38 1.37 1.37 1.36 1.36 1.34 1.34 1.29 1.27 1.27 1.25 1.25 1.24 1.23 1.22
Parameter Value
Solvent CDCl3
Temperature 298.0
Pulse Sequence zg30
Experiment 1D
Probe Z151574_0070 (PI HR-BBO500S1-BBF/ H/ D-5.0-Z SP)
Number of Scans 16
Receiver Gain 101.0
Relaxation Delay 1.0000
Pulse Width 8.0000
Presaturation Frequency
Acquisition Time 5.5706
Spectrometer Frequency 500.23
Spectral Width 5882.4
Lowest Frequency -462.1
Nucleus 1H
Acquired Size 32768
Spectral Size 65536
O
O
H
N
OAc
28
0.88 1.00 1.01 3.99 1.01 1.01 3.05 1.03 1.17 2.01 1.06 2.10
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)



Parameter	Value
Solvent	CDCl3
Temperature	298.0
Pulse Sequence	zgpg30
Experiment	1D
Probe	Z151574_0070 (PI HR-BBO500S1-BBF/ H/ D-5.0-Z SP)
Number of Scans	318
Receiver Gain	101.0
Relaxation Delay	2.0000
Pulse Width	9.0000
Presaturation Frequency	
Acquisition Time	1.0027
Spectrometer Frequency	125.80
Spectral Width	32679.7
Lowest Frequency	-1223.6
Nucleus	13C
Acquired Size	32768
Spectral Size	65536

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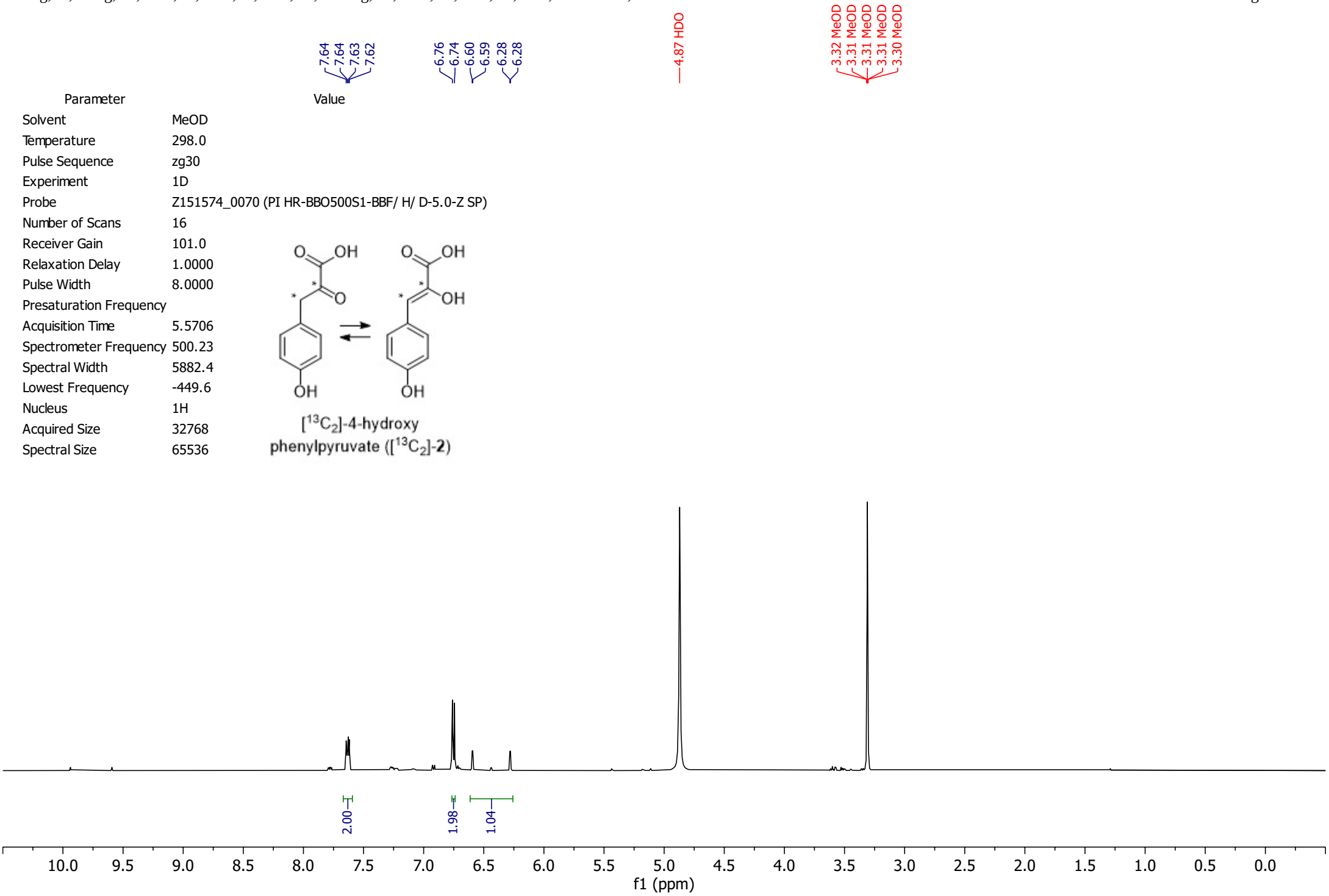
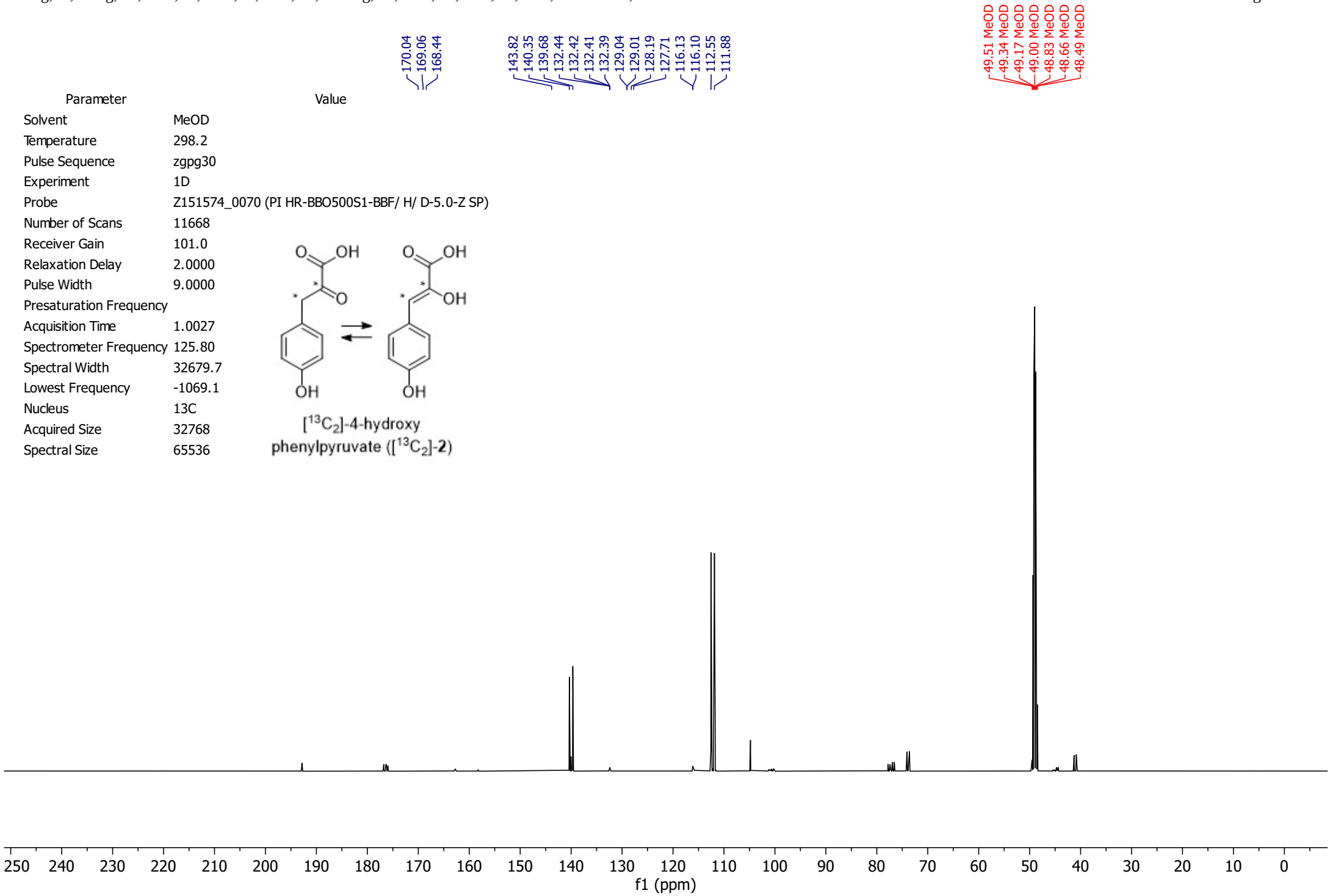


Parameter	Value
Solvent	MeOD
Temperature	298.0
Pulse Sequence	zg30
Experiment	1D
Probe	Z151574_0070 (PI HR-BBO500S1-BBF/ H/ D-5.0-Z SP)
Number of Scans	16
Receiver Gain	101.0
Relaxation Delay	1.0000
Pulse Width	8.0000
Presaturation Frequency	
Acquisition Time	5.5706
Spectrometer Frequency	500.23
Spectral Width	5882.4
Lowest Frequency	-449.6
Nucleus	1H
Acquired Size	32768
Spectral Size	65536

[$^{13}C_2$]-4-hydroxy phenylpyruvate ([$^{13}C_2$]-**2**)



Parameter	Value
Solvent	MeOD
Temperature	298.2
Pulse Sequence	zgpg30
Experiment	1D
Probe	Z151574_0070 (PI HR-BBO500S1-BBF/ H/ D-5.0-Z SP)
Number of Scans	11668
Receiver Gain	101.0
Relaxation Delay	2.0000
Pulse Width	9.0000
Presaturation Frequency	
Acquisition Time	1.0027
Spectrometer Frequency	125.80
Spectral Width	32679.7
Lowest Frequency	-1069.1
Nucleus	13C
Acquired Size	32768
Spectral Size	65536

[$^{13}C_2$]-4-hydroxy phenylpyruvate ([$^{13}C_2$]-**2**)