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

Supporting information

Preparation of domoic acid analogues using bioconversion system, and their toxicity in mice

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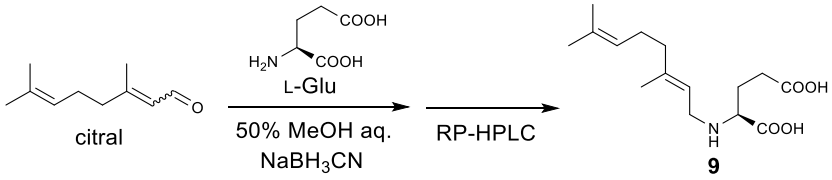
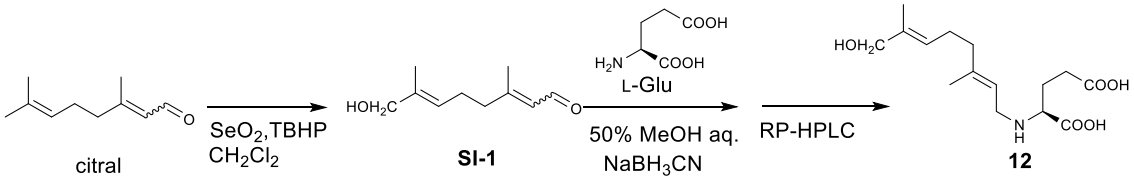
Synthetic procedures of substrates 9 and 12

***N*-geranyl-L-glutamic acid (NGG, 9)**



This molecule was synthesized following a literature procedure.[1] A solution of citral (89 mg, 0.59 mmol, mixture of geranial and neral, approx. 1:1 mol/mol) in MeOH (3 mL) was added to an aqueous solution (3 mL) of L-glutamic acid (193 mg, 1.31 mmol) adjusted to neutral pH using NaOH and the reaction mixture was stirred for 3 h at room temperature. $NaBH_3CN$ (32 mg, 0.51 mmol) was added at once and stirred for 1 h. 1 M HCl was added until pH 3, and the reaction mixture was concentrated in vacuo. The dried reaction mixture was loaded onto a Cosmosil 140C_{18}-OPN column (1.5 mL) pre-equilibrated with H_2O-HCOOH (100:0.1, v/v). The column was washed with H_2O-HCOOH (100:0.1, v/v, 10 mL). Crude **9** was eluted with H_2O-MeOH-HCOOH (20:80:0.1, v/v/v, 15 mL) and purified using an InertSustain C18 column (10 × 250 mm, 5 μm) with H_2O-MeOH-HCOOH (50:50:0.1, v/v/v). Compound **9** (14 mg, 0.049 mmol, yield 17%) was obtained as a white powder. HRESIMS $[M+H]^+$ *m/z* 284.1848 (calcd for $C_{15}H_{26}NO_4^+$ 284.1856). ^{1}H NMR (CD_3OD, 600 MHz) δ5.29 (1H, t, J = 7.3 Hz), 5.10 (1H, t, J = 6.5 Hz), 3.67 (1H, dd, J = 13.4, 7.3 Hz), 3.62 (1H, dd, J = 13.9, 7.9 Hz), 3.53 (1H, t, J = 5.9 Hz), 2.53 (2H, m), 2.14 (2H, dt, J = 13.2, 7.1 Hz), 2.11 (2H, t, J = 7.0), 2.05 (2H, m), 1.75 (3H, s), 1.67 (3H, s), 1.61 (3H, s,); ^{13}C NMR (CD_3OD, 151 MHz) δ177.7, 173.6, 148.3, 133.8, 125.5, 115.9, 62.7, 46.1, 41.5, 32.2, 28.0, 27.4, 26.7, 18.6, 17.5.

7′-hydroxy-*N*-geranyl-L-glutamic acid (12)



This molecule was synthesized following a literature procedure.[2] To a suspension of selenium dioxide (0.089 g, 0.80 mmol) in CH_2Cl_2 (5 mL), a solution of citral (0.36 mL, 95%, 2.00 mmol) in CH_2Cl_2 (5 mL), and aqueous *t*-butyl hydrogen peroxide (0.51 mL, 70%, 4.00 mmol) were sequentially added and stirred for 18 h at room temperature. The reaction mixture was diluted with CH_2Cl_2 (20 mL), filtered to remove particulates, and washed with saturated aqueous $NaHCO_3$ (25 mL) and brine (25 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated in vacuo. The crude reaction mixture

was purified by silica gel column chromatography, yielding **SI-1** as a light-yellow oil (115 mg, 0.68 mmol, 68%). A solution of **SI-1** (115 mg, 0.68 mmol) in MeOH (2.5 mL) was added to an aqueous solution (2.5 mL) of L-glutamic acid (175 mg, 1.19 mmol) adjusted neutral pH using NaOH and the reaction mixture was stirred for 3 h at room temperature. $NaBH_3CN$ (32 mg, 0.51 mmol) was added at once and stirred for 1 h. 1 M HCl was added until pH 3, and the reaction mixture was concentrated in vacuo. Allylic alcohols were removed by extraction with EtOAc, and the aqueous layer containing **12** was purified using an InertSustain C18 column (10 × 250 mm, 5 μm) with H_2O-MeOH-HCOOH (70:30:0.1, v/v/v). Compound **12** (12 mg, 0.040 mmol, yield 6%) was obtained as a white powder. HRESIMS $[M+H]^+$ *m/z* 298.1682 (calcd for $C_{15}H_{26}NO_4^+$ 298.1649). ^1H NMR (CD_3OD, 600 MHz) δ5.38 (1H, t, J = 6.8 Hz), 5.30 (1H, t, J = 7.3 Hz), 3.91 (2H, s), 3.68 (1H, dd, J = 13.5, 7.3 Hz), 3.63 (1H, dd, J = 13.2, 7.9 Hz), 3.53 (1H, t, J = 5.9 Hz), 2.53 (2H, m), 2.14 (2H, q, J = 7.1 Hz), 2.16 (2H, t, J = 7.0), 2.12 (1H, m), 2.05 (1H, m), 1.76 (3H, s), 1.65 (3H, s); ^{13}C NMR (CD_3OD, 151 MHz) δ177.0, 173.0, 147.3, 136.8, 125.6, 115.5, 68.8, 61.9, 45.4, 40.5, 31.5, 26.8, 26.7, 16.8, 13.9.

Supplementary Figures and Tables

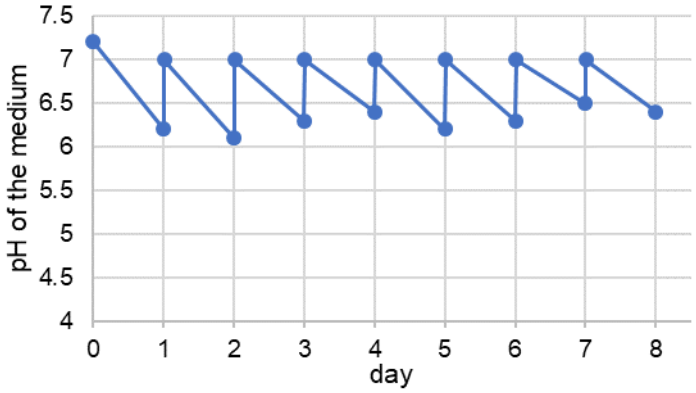


Fig. S1 Time course of pH of the medium, which it was adjusted to 7.0 by the addition of NaOH aq. every day.

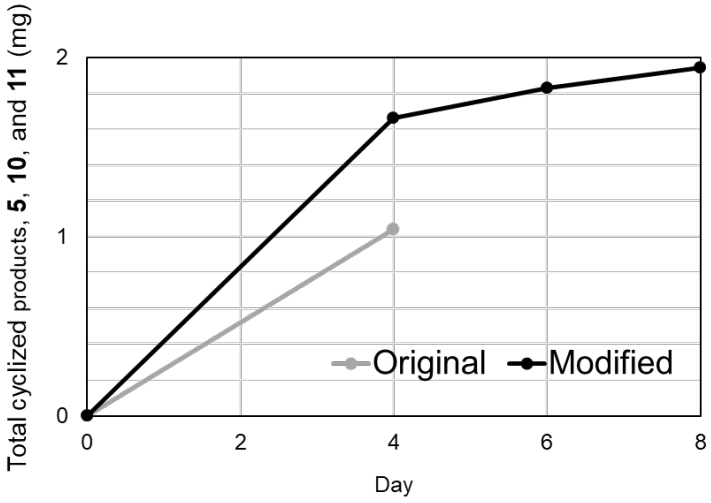


Fig. S2 Time course of bioconversion of **9** (4 mg) to **5**, **10**, and **11** under original and modified conditions.

Table S1. The summary of DabC bioconversion reactions

substrate	**9**	**12**	**13**	**14**
substrate amount (mg)*	4.0	3.0	1.6	1.0
cyclized product	**5**	**6**	**7**	**23**
cyclized products amount (mg)**	1.9	0.97	0.050	N.D.
yield (%)	49	32	3.6	0

*Added to 100 mL medium.

**The cyclized products include all geometric isomers.

N.D. = Not detected

Table S2. ^{1}H (600 MHz) NMR Data of natural **5**, **6**[1] and synthetic **5**, **6** in CD_3OD.

	natural **5**[1]	synthetic **5**	natural **6**[1]	synthetic **6**
position	δ_H (*J* in Hz)	δ_H (*J* in Hz)	δ_H (*J* in Hz)	δ_H (*J* in Hz)
2	3.85, d (7.6)	3.86, d (7.4)	3.87, d (6.8)	3.86, d (6.7)
3	2.98, m	2.98, m	2.99, m	2.99, m
4	3.61, dd (15.4, 7.3)	3.61, dd (15.3, 7.1)	3.62, m	3.62, m
5α	3.33, m	3.33, m	3.34, m	3.33, m
5β	3.56, dd (11.7, 8.2)	3.56, dd (11.8, 8.2)	3.56, m	3.56, m
6a	2.68, dd (16.7, 5.9)	2.67, dd (16.4, 6.0)	2.66, m	2.68, m
6b	2.42, dd (16.7, 8.8)	2.43, dd (16.5, 8.7)	2.41, dd (16.6, 8.4)	2.42, dd (16.6, 8.3)
2'	5.38, t (7.3)	5.38, t (7.1)	5.42, t (7.3)	5.41, t (7.1)
3'a	2.73, m	2.73, m	2.80, m	2.80, m
3'b	2.61, m	2.61, m	2.69, m	2.70, m
4'	5.04, t (7.3)	5.04, t (7.1)	5.32, t (6.8)	5.32, t (6.8)
6'	1.61, s	1.61, s	1.65, s	1.65, s
7'	1.67, s	1.67, s	3.90, s	3.90, s
8'	1.71, s	1.71, s	1.72, s	1.72, s

The signal of CHD_2OD was adjusted at 3.30 ppm for ^{1}H NMR

Table S3. ^{1}H (600 MHz) and ^{13}C (151 MHz) NMR Data of IA[4] and **7** in D_2O

	IA (**2**)[4]	7′-amide-IA (**7**)		
position	δ_H (J in Hz)	δ_H (J in Hz)	δ_C	HMBC
2	3.96, d (7.8)	3.89, d (7.0)	66.1	5β
3	2.99, dddd	2.93, m	44.4	2, 5β, 6b
4	3.63, ddd (7.2, 7.8, 7.2)	3.53, q (7.6)	41.3	5α, 2′, 8′
5α	3.47, dd (11.4, 7.8)	3.39, dd (11.9, 7.6)	47.9	4
5β	3.69, dd (12.0, 7.8)	3.62, dd (12.2, 8.1)		
6a	2.36, dd (15.6, 9.6)	2.31, dd (16.0, 8.8)	37.3	2
6b	2.52, dd (15.6, 6.0)	2.46, dd (15.7, 6.2)		
7			N.D.	
8			175.2	2
1′			131.6	4, 8′
2′	5.51, dd (7.2, 6.0)	5.43, t (7.3)	128.8	3′a, 3′b, 8′
3′a	2.76, m	2.79, m	27.5	
3′b	2.99, m	2.91, m		
4′	6.31, dd (7.8, 6.6)	6.28, t (7.0)	137.5	3′a, 3′b, 6′
5′			130.4	3′a, 3′b, 6′
6′	1.81, s	1.79, s	13.2	4′
7′			176.8	4′, 6′
8′	1.76, s	1.69, s	22.7	4, 2′

N.D. = Not detected because of the limited amounts

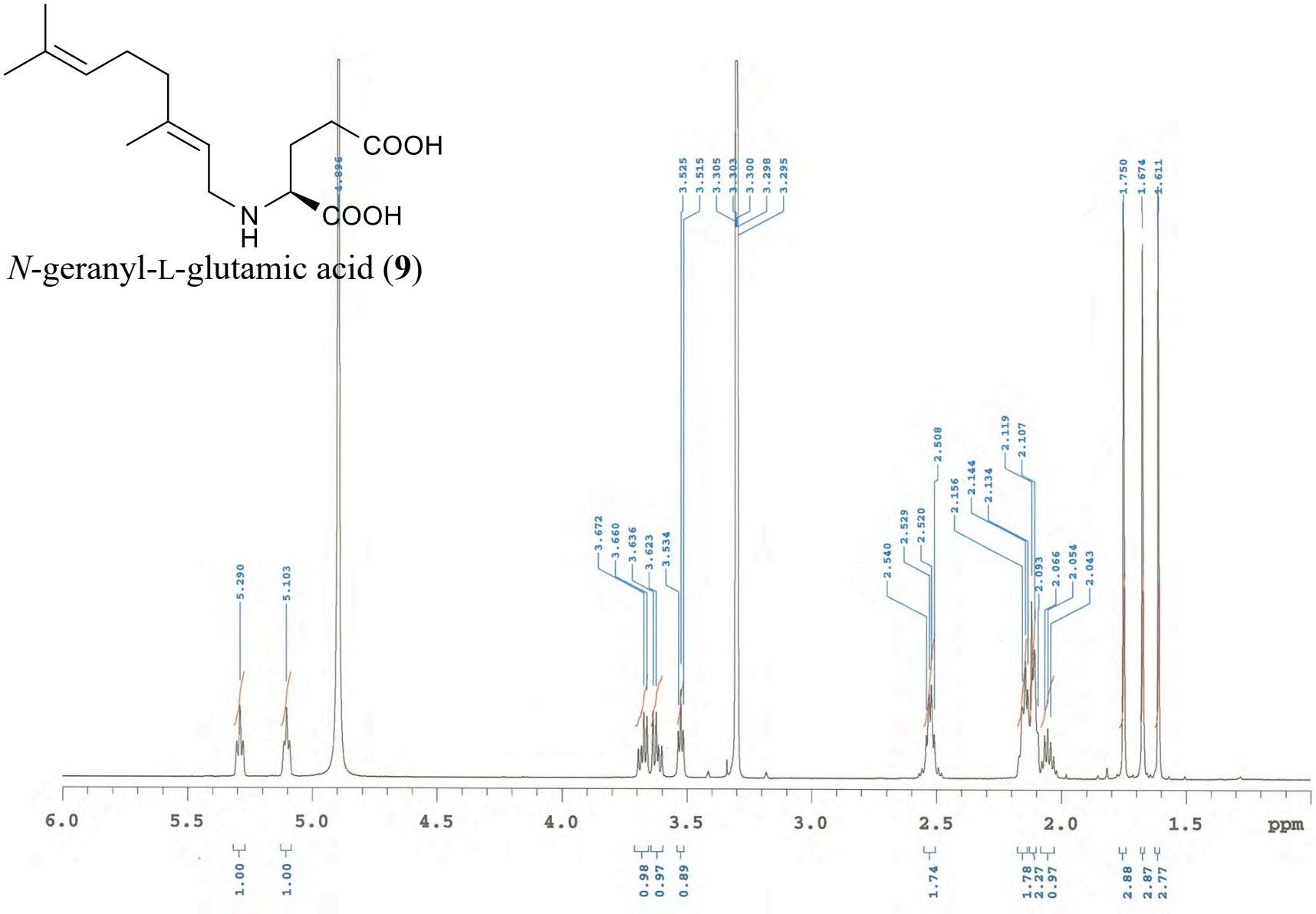
The NMR data of IA[4] are listed for comparison. The signal of residual CH_3OD was adjusted at 3.30 ppm for ^{1}H NMR, and that of $^{13}CH_3OD$ was adjusted at 49.0 ppm for ^{13}C NMR as the internal references.

Table S4. Primers used in this study.

DabC For	ATGACTGTGGCAATAAATAACGA
DabC Rev	CTAATCAGCGTAGTATCCGG
pET-DabC For	CCGCGCGGCAGCCATATGACTGTGGCAATAAATAACGA
pET-DabC Rev	GTGGTGGTGCTCGAGCTAATCAGCGTAGTATCCGC
pET28 up	CATATGGCTGCCGCGCGGCACC
pET28 down	CTCGAGCACCACCACCACCACCACTGAG

References

1. Y. Maeno, Y. Kotaki, R. Terada, Y. Cho, K. Konoki and M. Yotsu-Yamashita, *Sci. Rep*. **2018**, 8, 356.
2. J. K. Brunson, S. M. K. McKinnie, J. R. Chekan, J. P. McCrow, Z. D. Miles, E. M. Bertrand, V. A. Bielinski, H. Luhavaya, M. Oborník, G. J. Smith, D. A. Hutchins, A. E. Allen and B. S. Moore, *Science* **2018**, 361, 1356−1358.
3. M. H. Nanao, T. Green, Y. Stern-Bach, S. F. Heinemann and S. Choe, *Proc. Natl. Acad. Sci. U.S.A.* **2005**, 102, 1708−1713.
4. Y. Kotaki, E. F. Furio, M. Satake, N. Lundholmd, T. Katayama, K. Koike, V. P. Fulgueras, F. A. Bajarias, Y. Takata, K. Kobayashi, S. Sato, Y. Fukuyo and M. Kodama, *Toxicon* **2005**, 46, 946−953.



N-geranyl-L-glutamic acid (**9**)

Fig. S3 ^{1}H NMR spectrum of **9** (CD_3OD, 600 MHz).

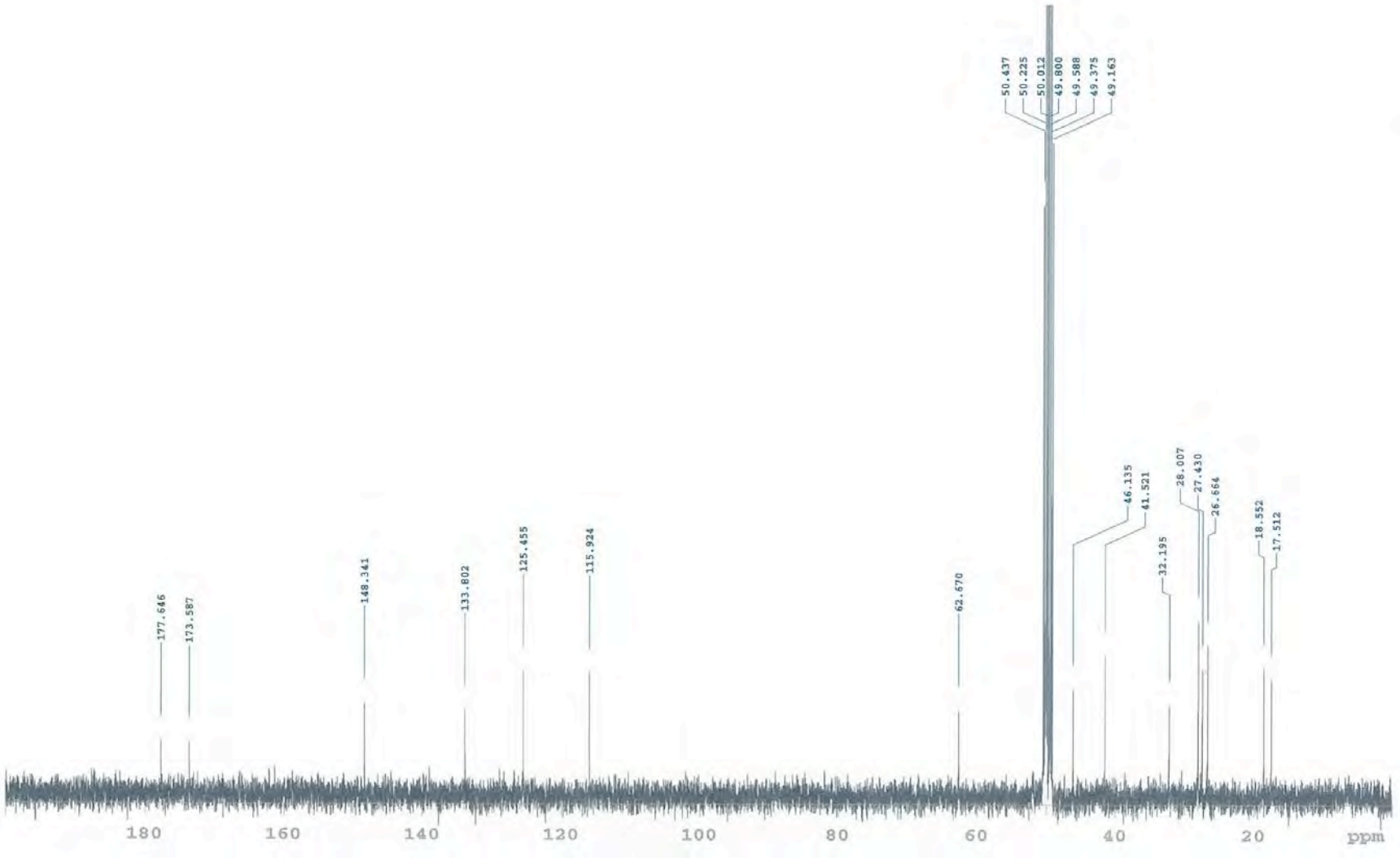
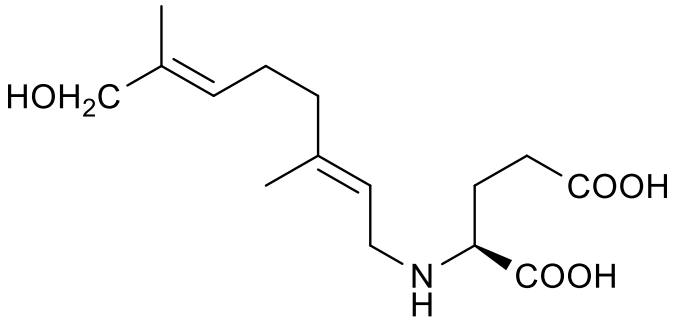


Fig. S4 ^{13}C NMR spectrum of **9** (CD_3OD, 151 MHz).



7′-hydroxy-*N*-geranyl-L-glutamic acid (**12**)

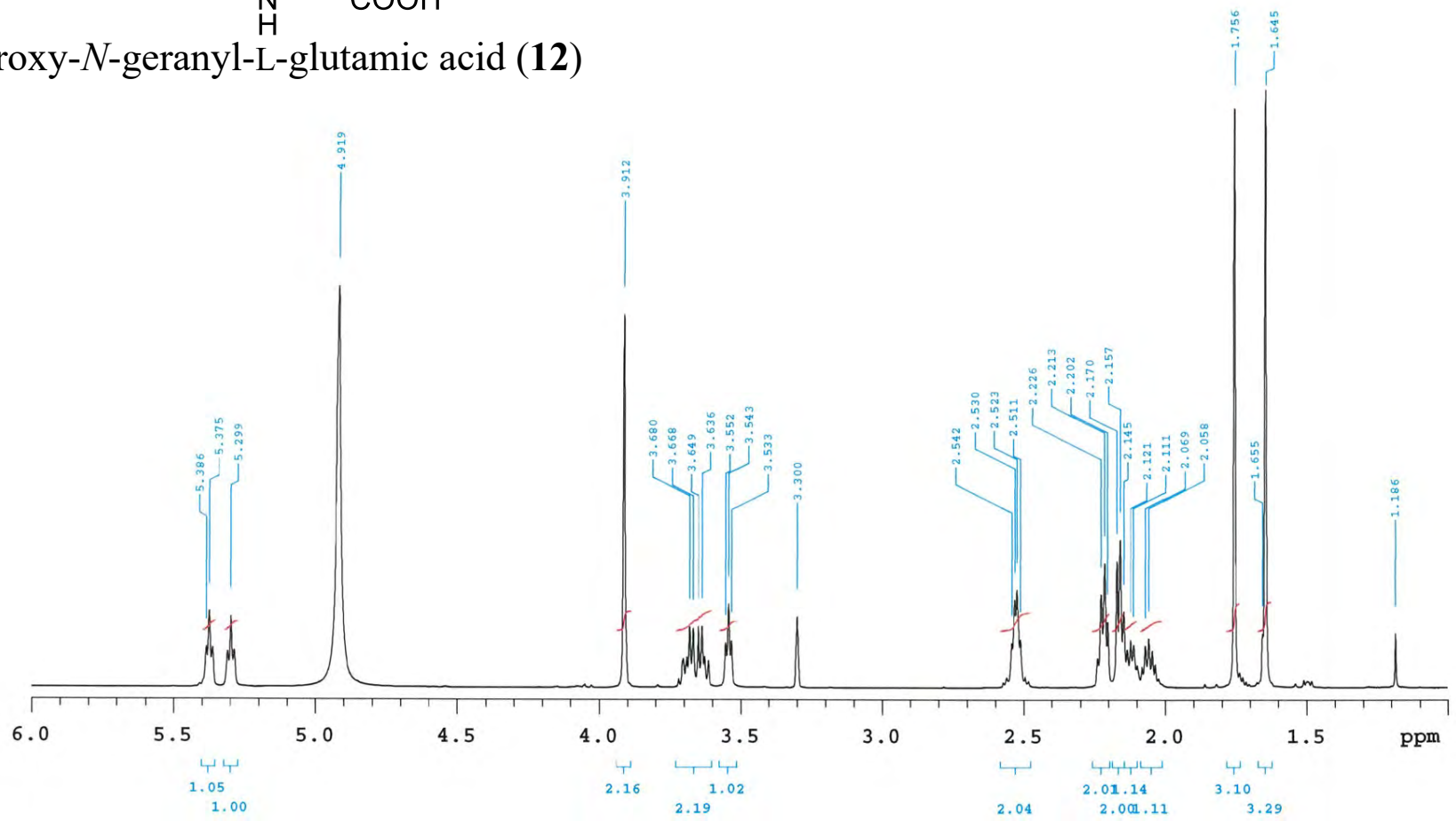


Fig. S5 ^{1}H NMR spectrum of **12** (CD_3OD, 600 MHz).

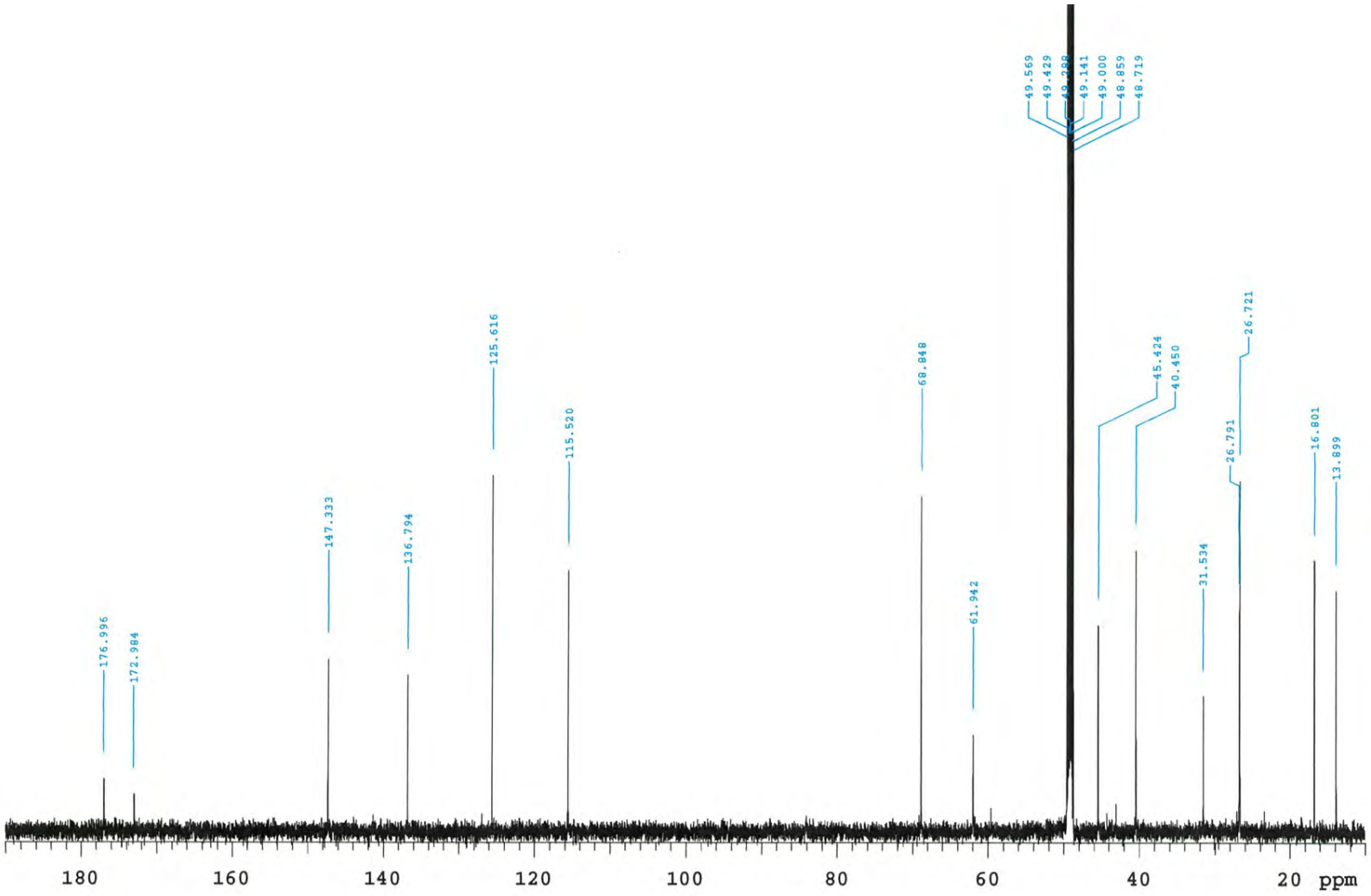


Fig. S6 ^{13}C NMR spectrum of **12** (CD_3OD, 151 MHz).

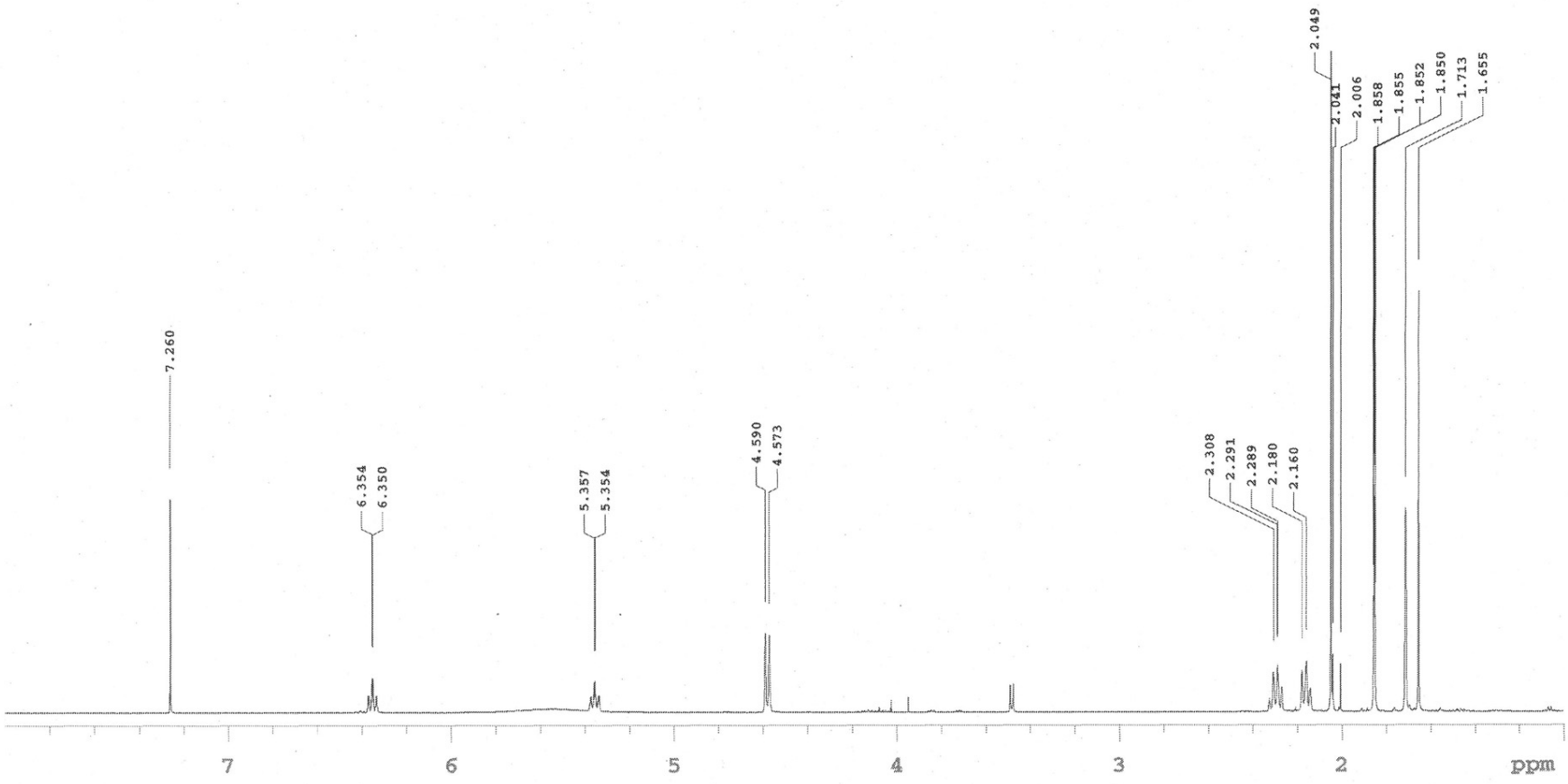


Fig. S7 ^{1}H NMR spectrum of **17** ($CDCl_3$, 400 MHz).

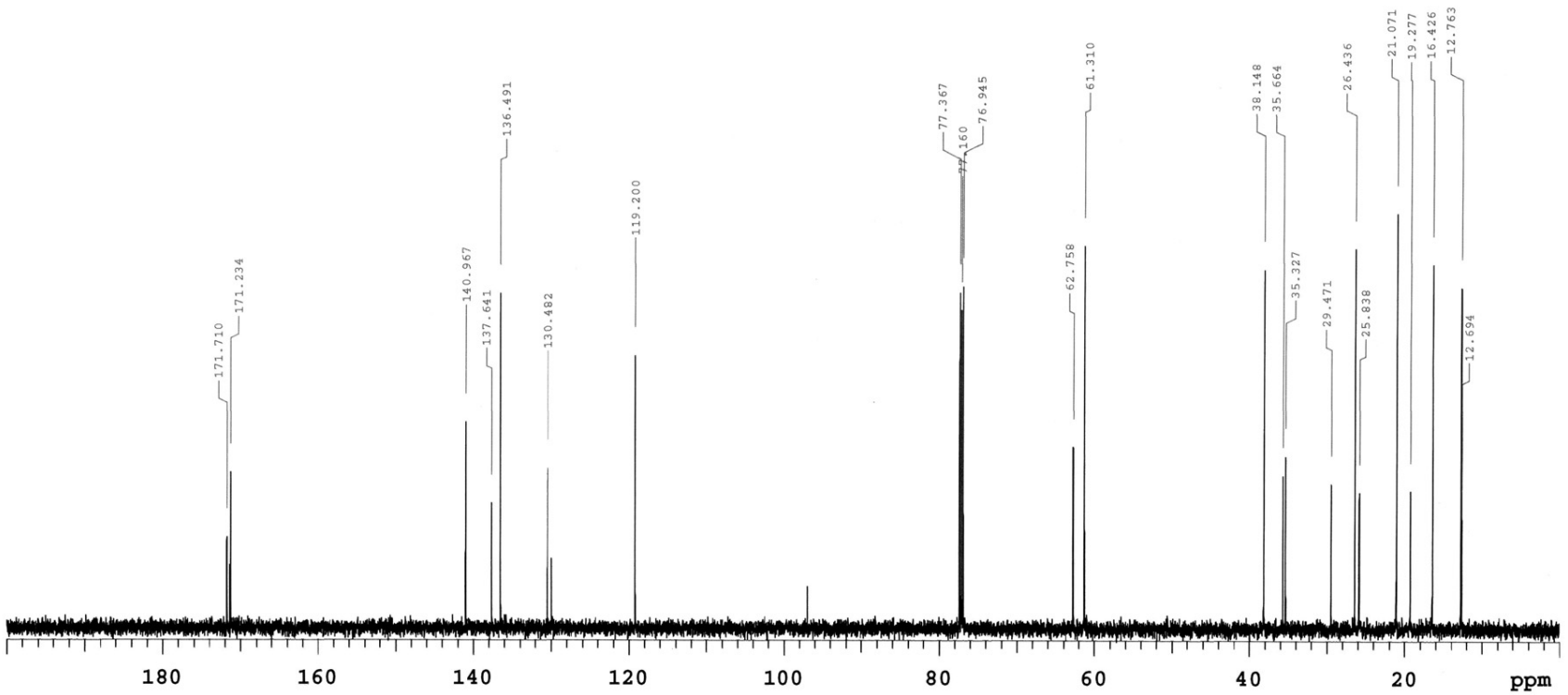


Fig. S8 ^{13}C NMR spectrum of **17** ($CDCl_3$, 151 MHz).
The signals of impurity from the starting material (geranyl acetate, purity 70%) were also shown in the NMR spectra of **17**. The signals of **17** were assigned based on HSQC and HMBC spectra.

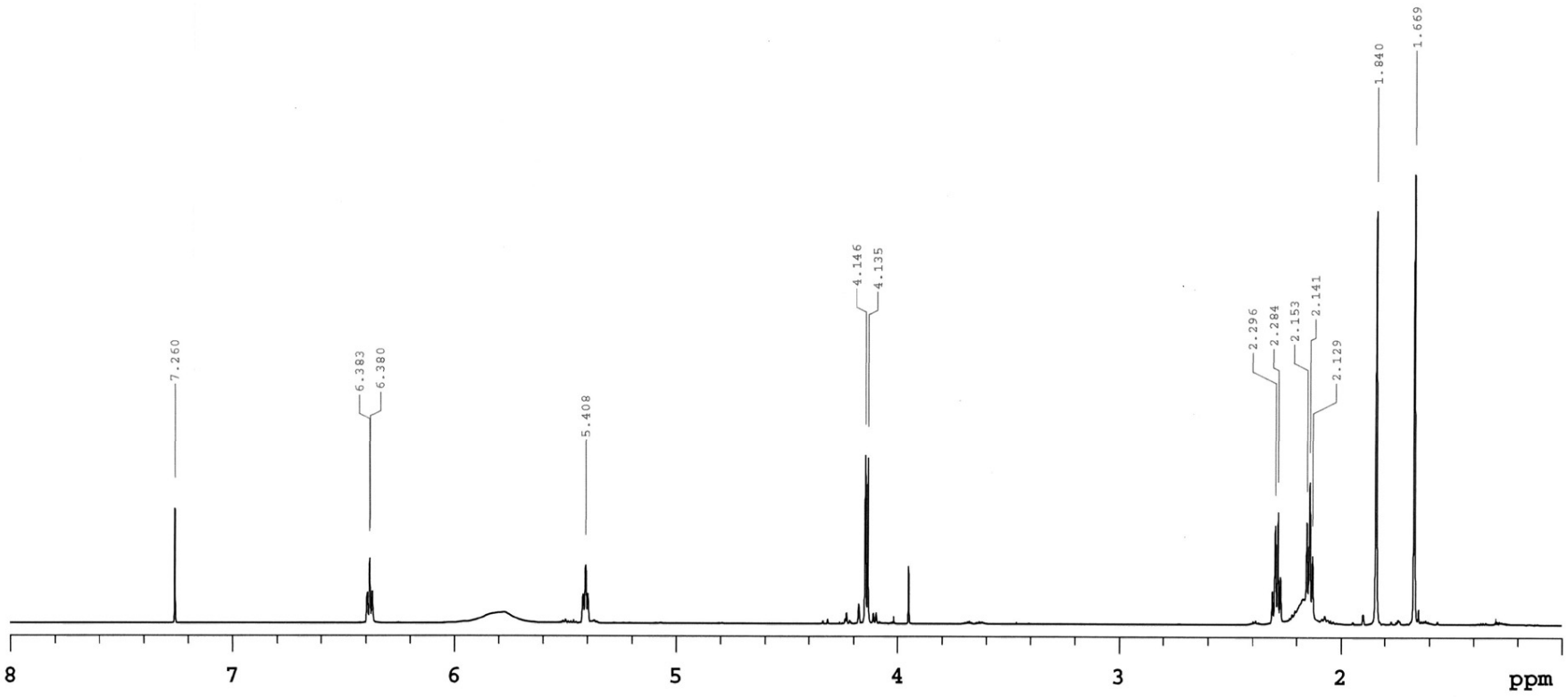


Fig. S9 ^{1}H NMR spectrum of **19** ($CDCl_3$, 400 MHz).

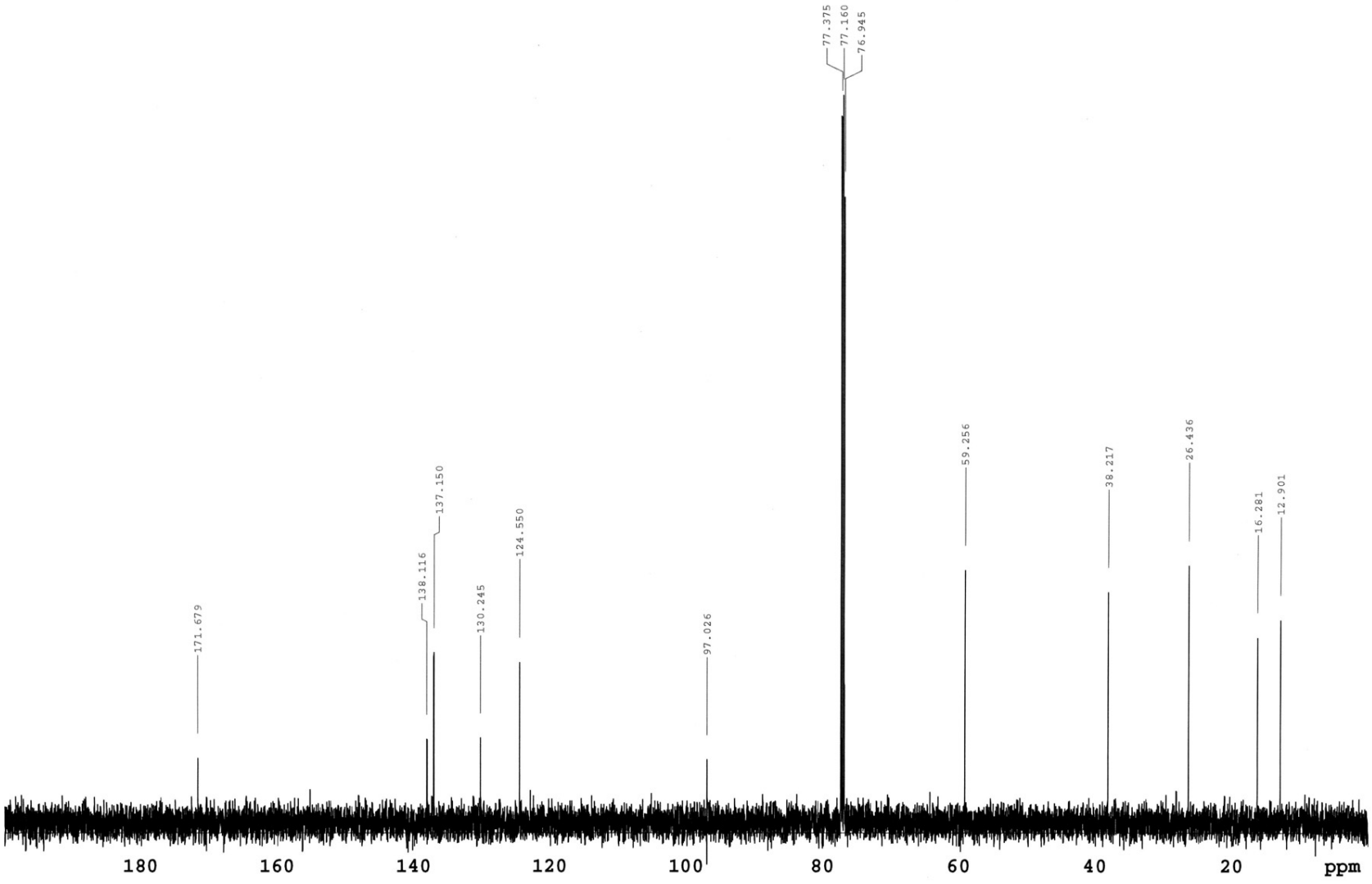


Fig. S10 ^{13}C NMR spectrum of **19** ($CDCl_3$, 151 MHz).

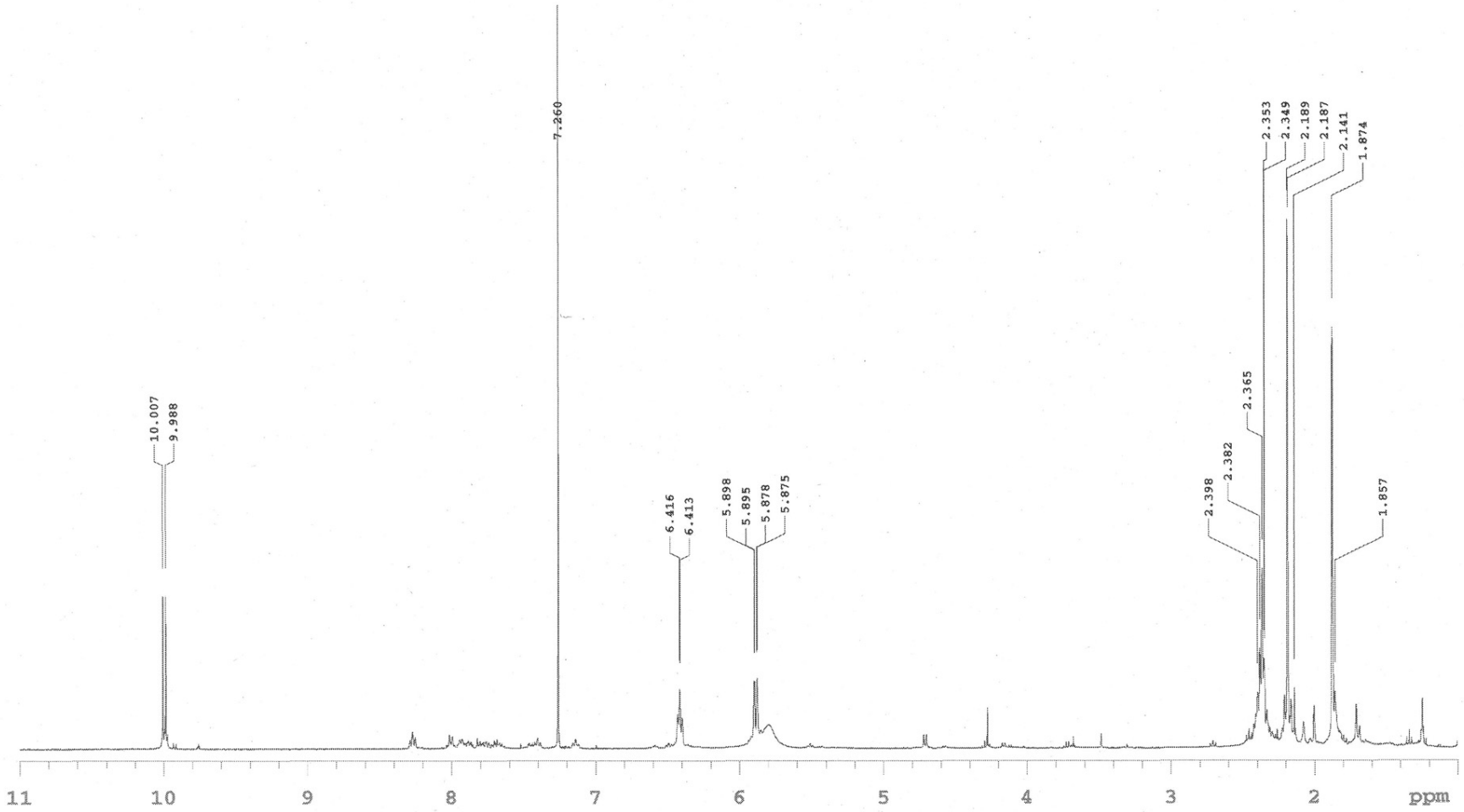


Fig. S11 ^{1}H NMR spectrum of **21** ($CDCl_3$, 400 MHz).

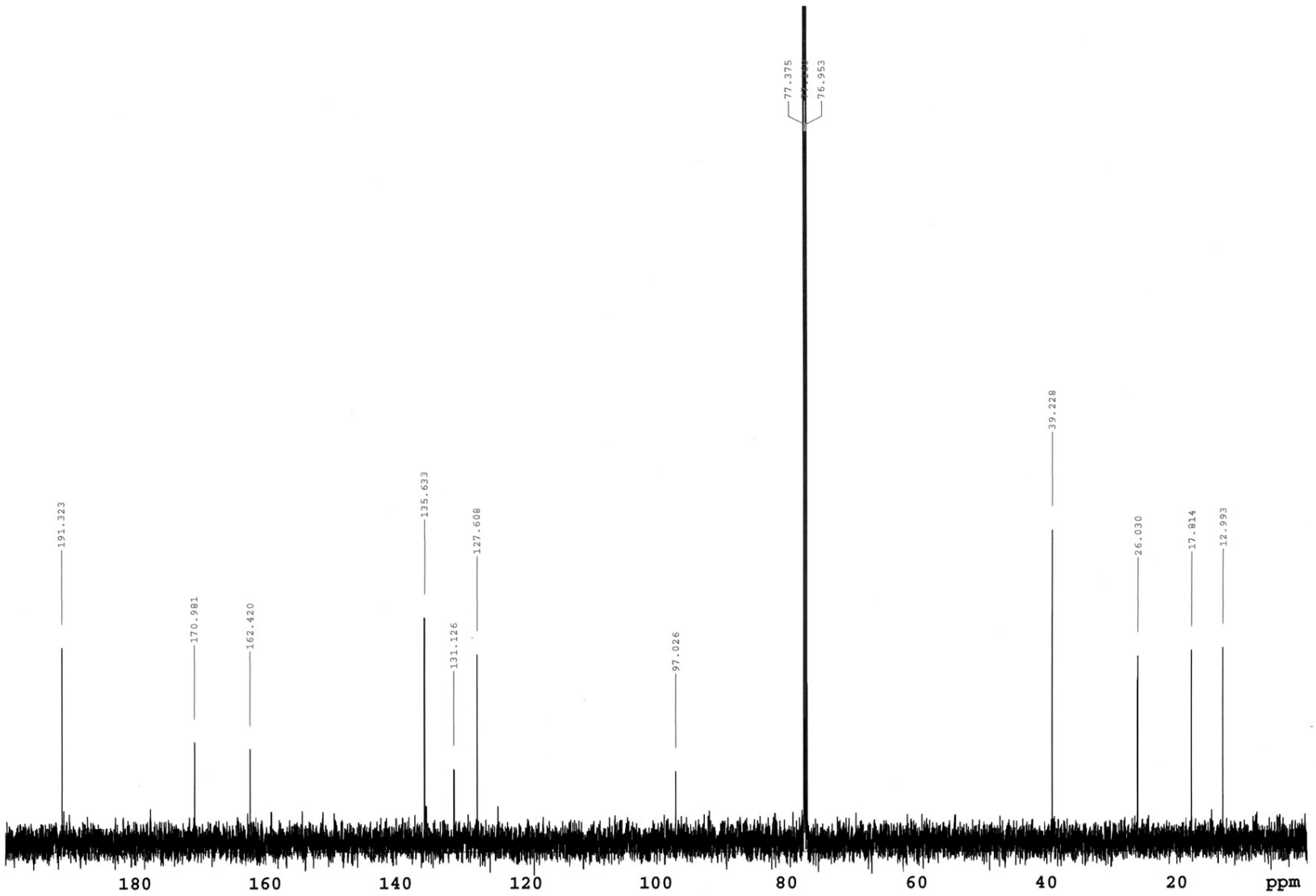
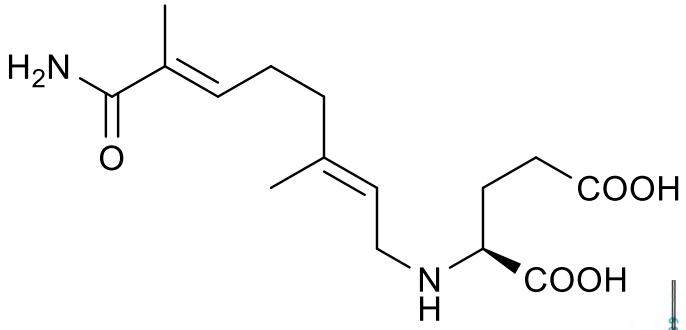


Fig. S12 ^{13}C NMR spectrum of **21** ($CDCl_3$, 151 MHz).



7′-amide-*N*-geranyl-L-glutamic acid (**13**)

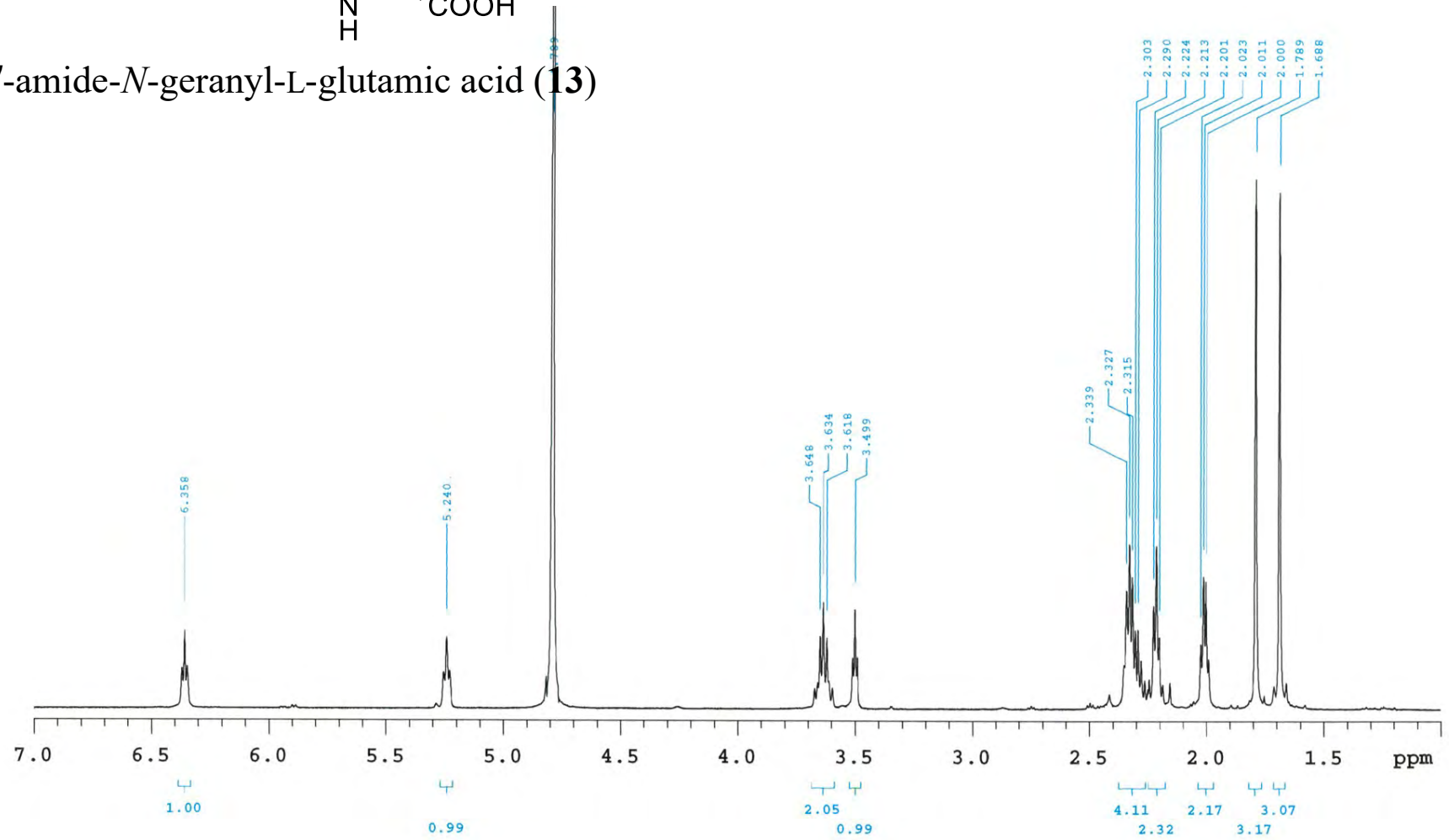


Fig. S13 ^{1}H NMR spectrum of **13** (D_2O, 600 MHz).

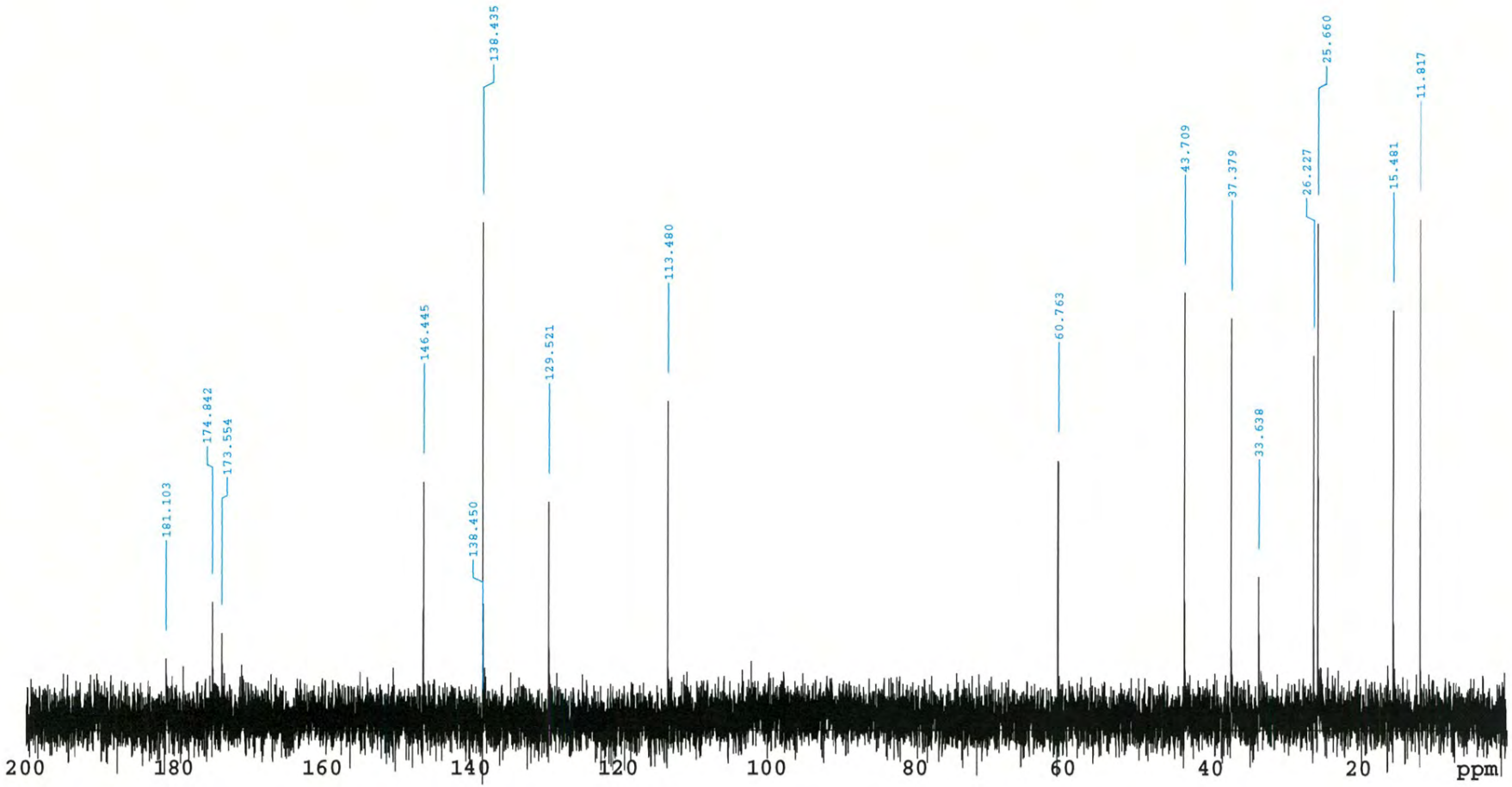


Fig. S14 ^{13}C NMR spectrum of **13** (D_2O, 151 MHz).

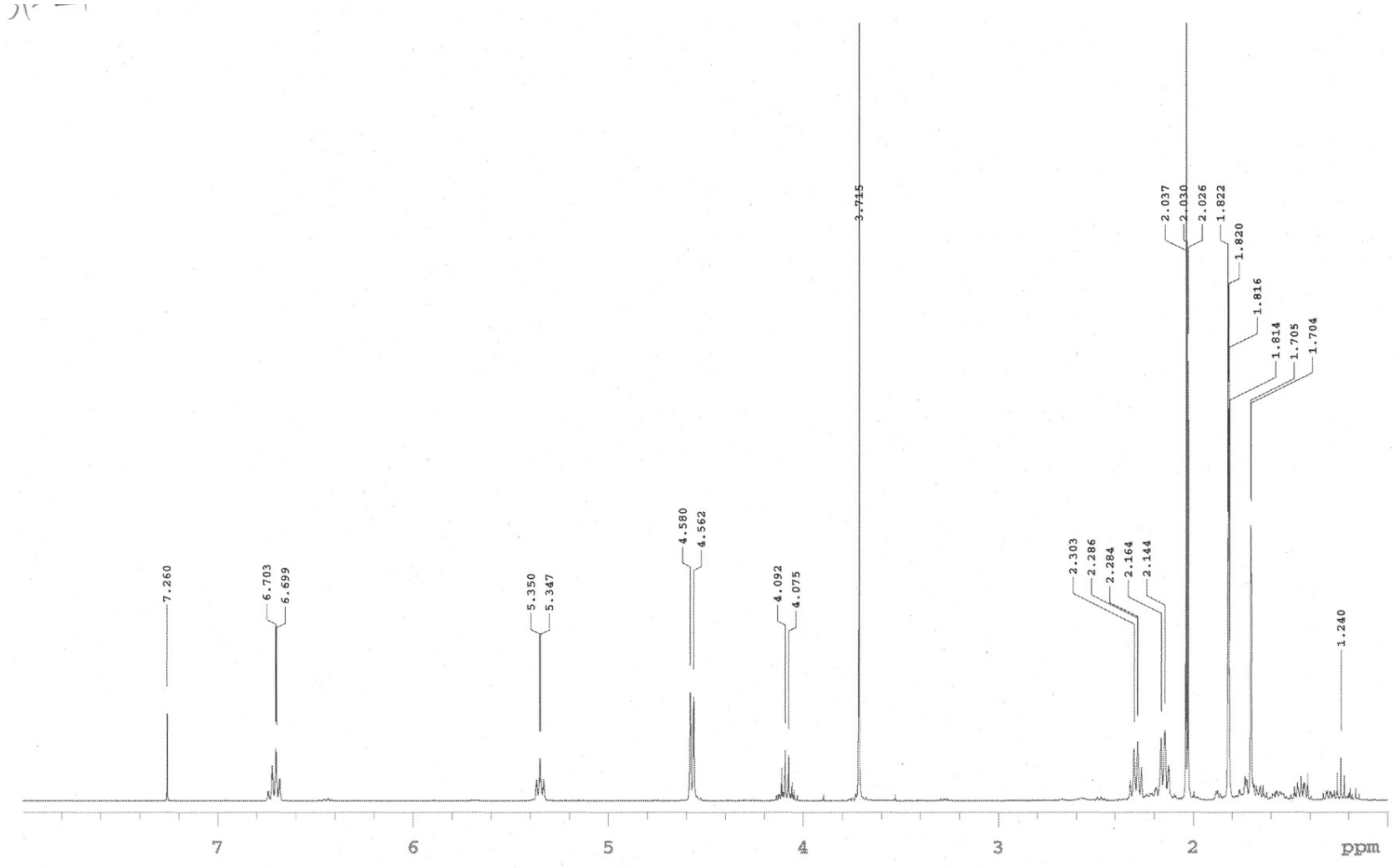


Fig. S15 ^{1}H NMR spectrum of **18** ($CDCl_3$, 400 MHz).

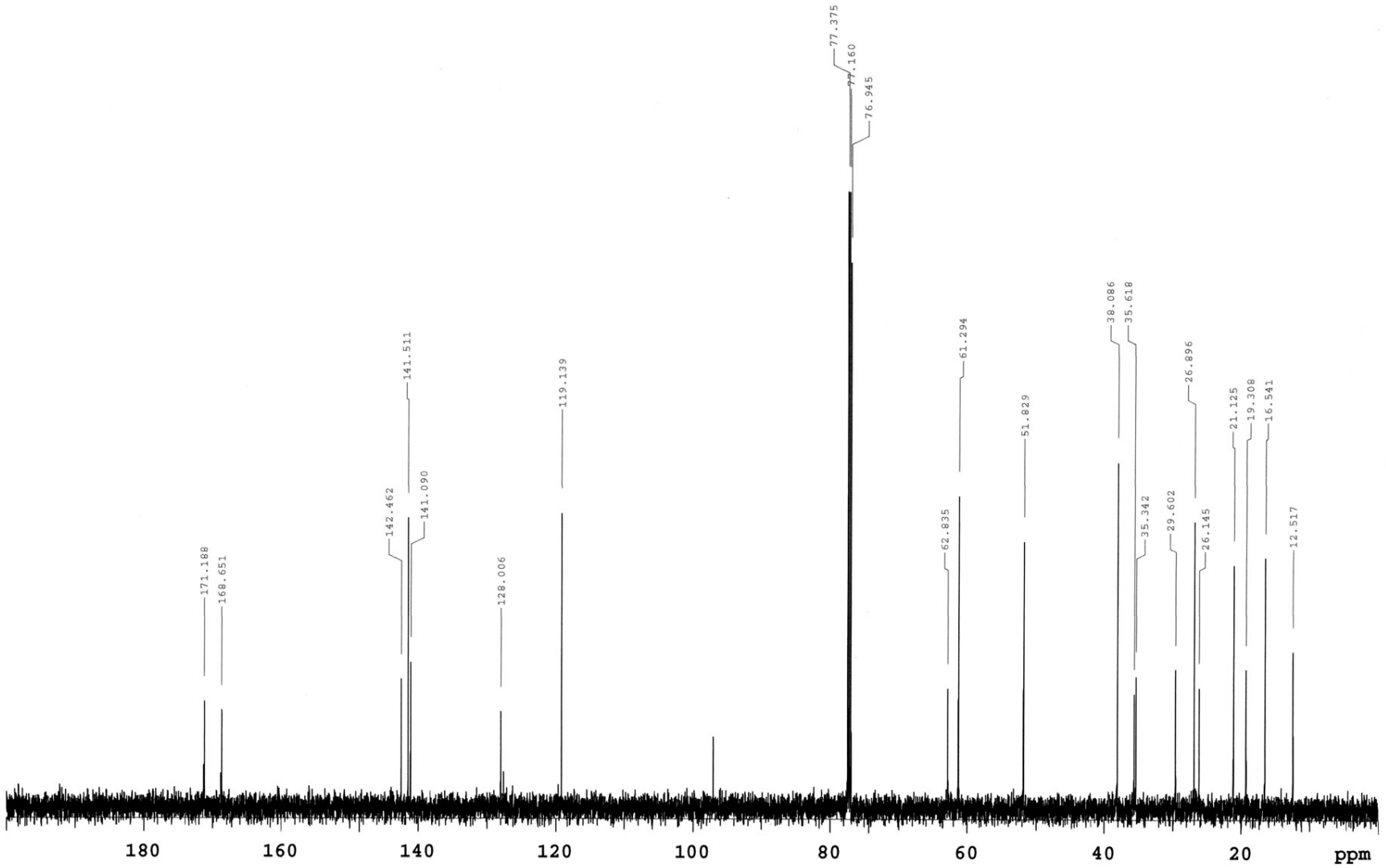


Fig. S16 ^{13}C NMR spectrum of **18** ($CDCl_3$, 151 MHz).
The signals of impurity from the starting material (geranyl acetate, purity 70%) were also shown in the NMR spectra of **18**. The signals of **18** were assigned based on HSQC and HMBC spectra.

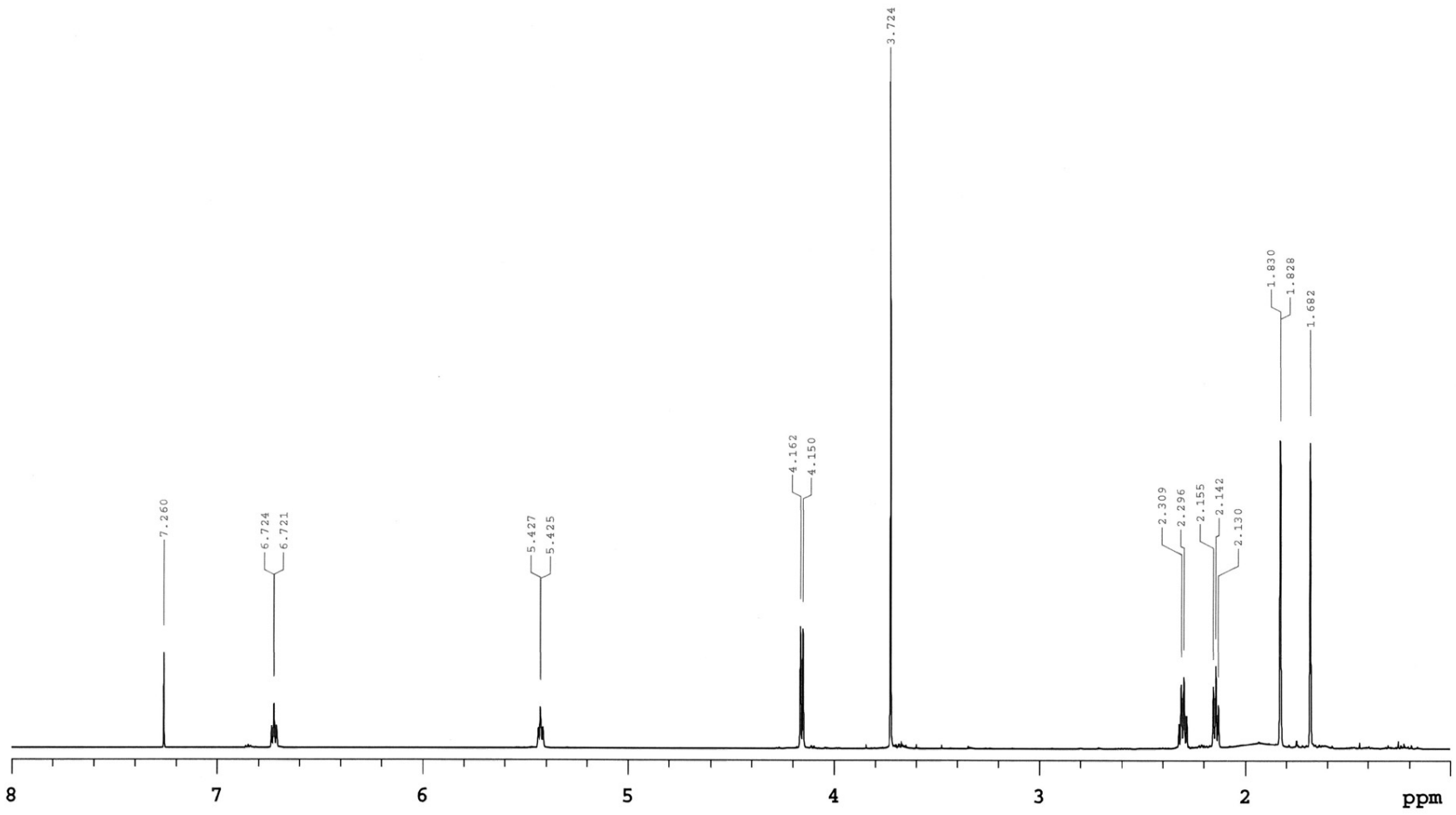


Fig. S17 ^{1}H NMR spectrum of **20** ($CDCl_3$, 400 MHz).

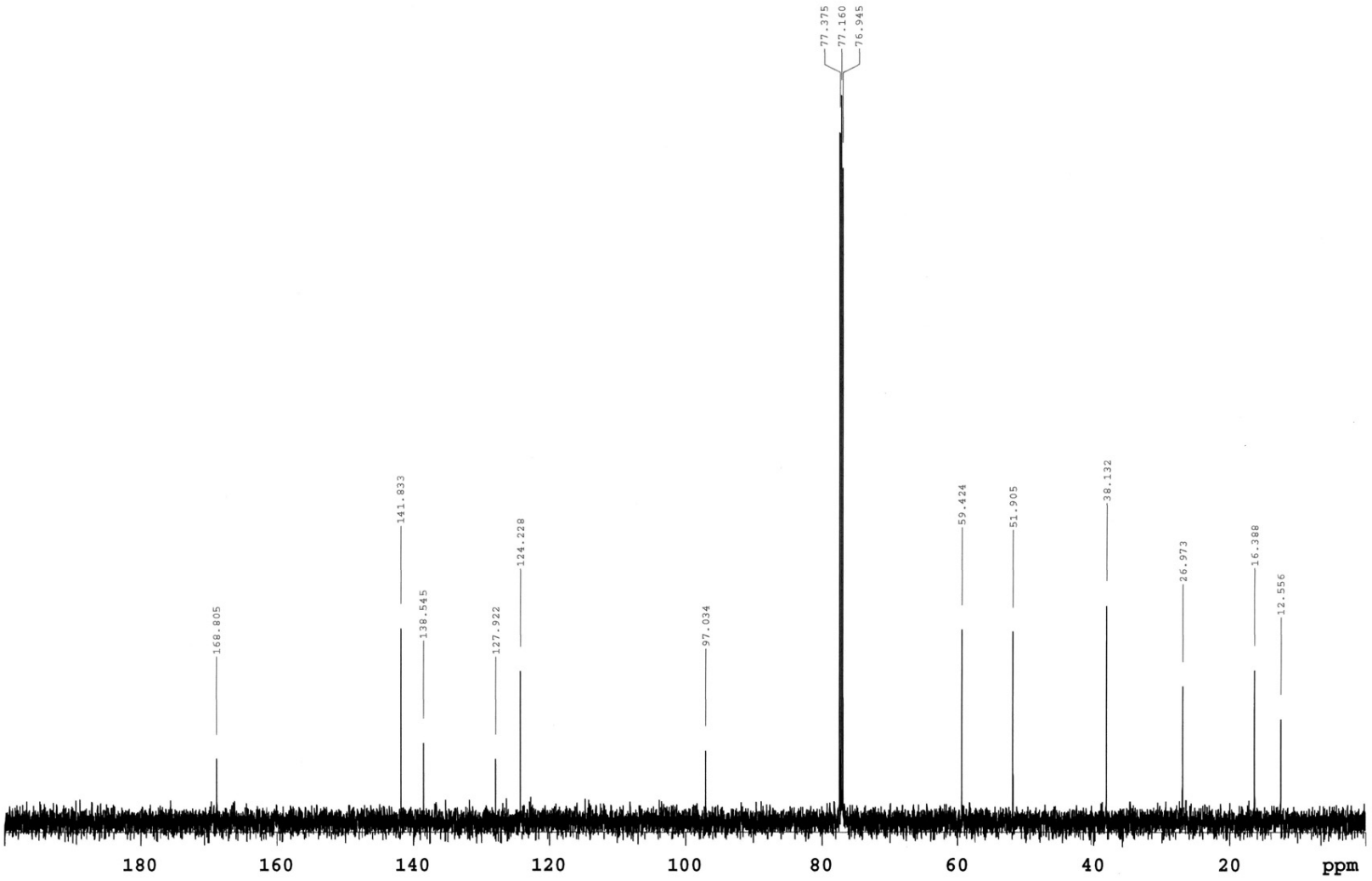


Fig. S18 ^{13}C NMR spectrum of **20** ($CDCl_3$, 151 MHz).

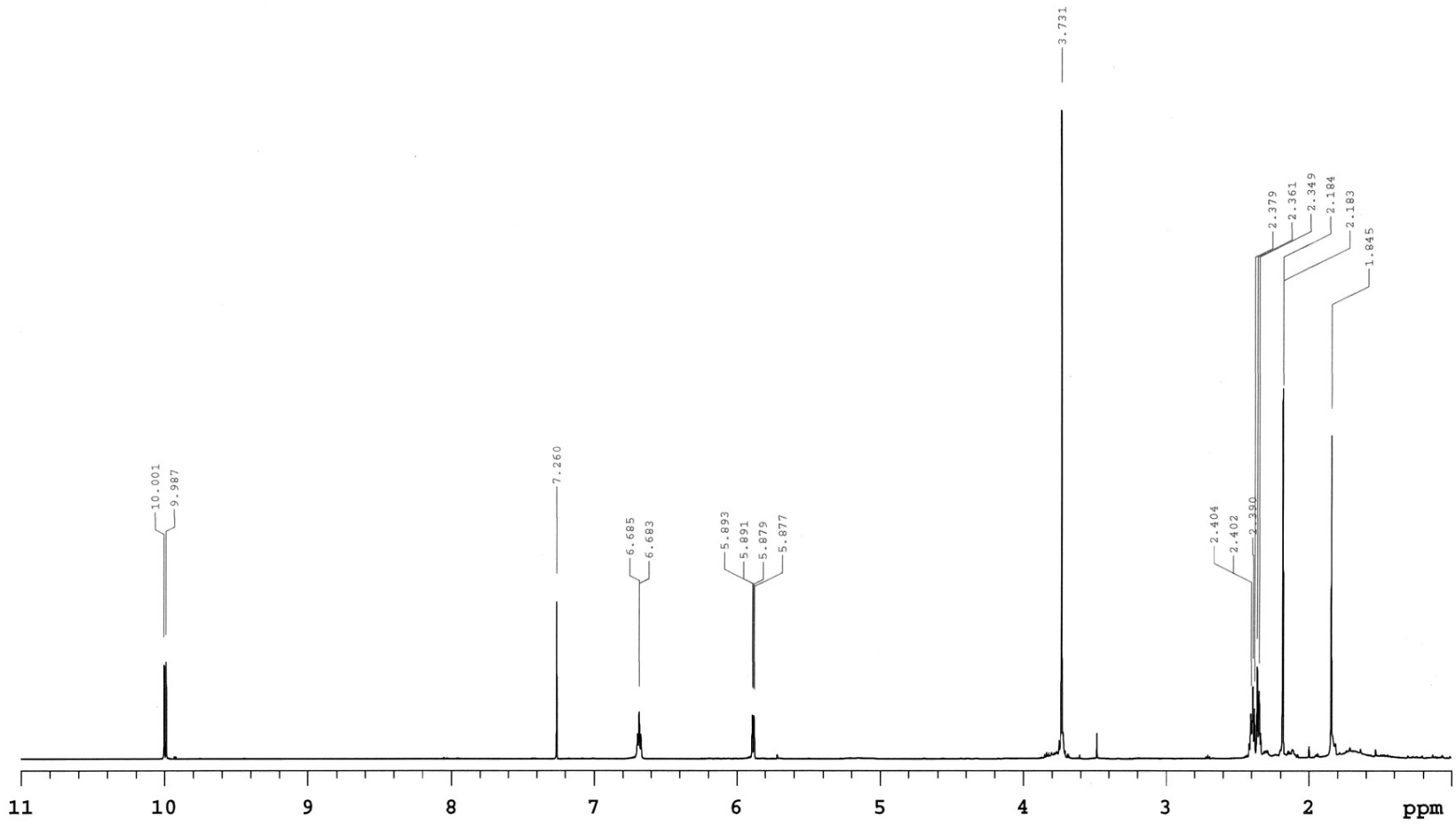


Fig. S19 ^{1}H NMR spectrum of **22** ($CDCl_3$, 400 MHz).

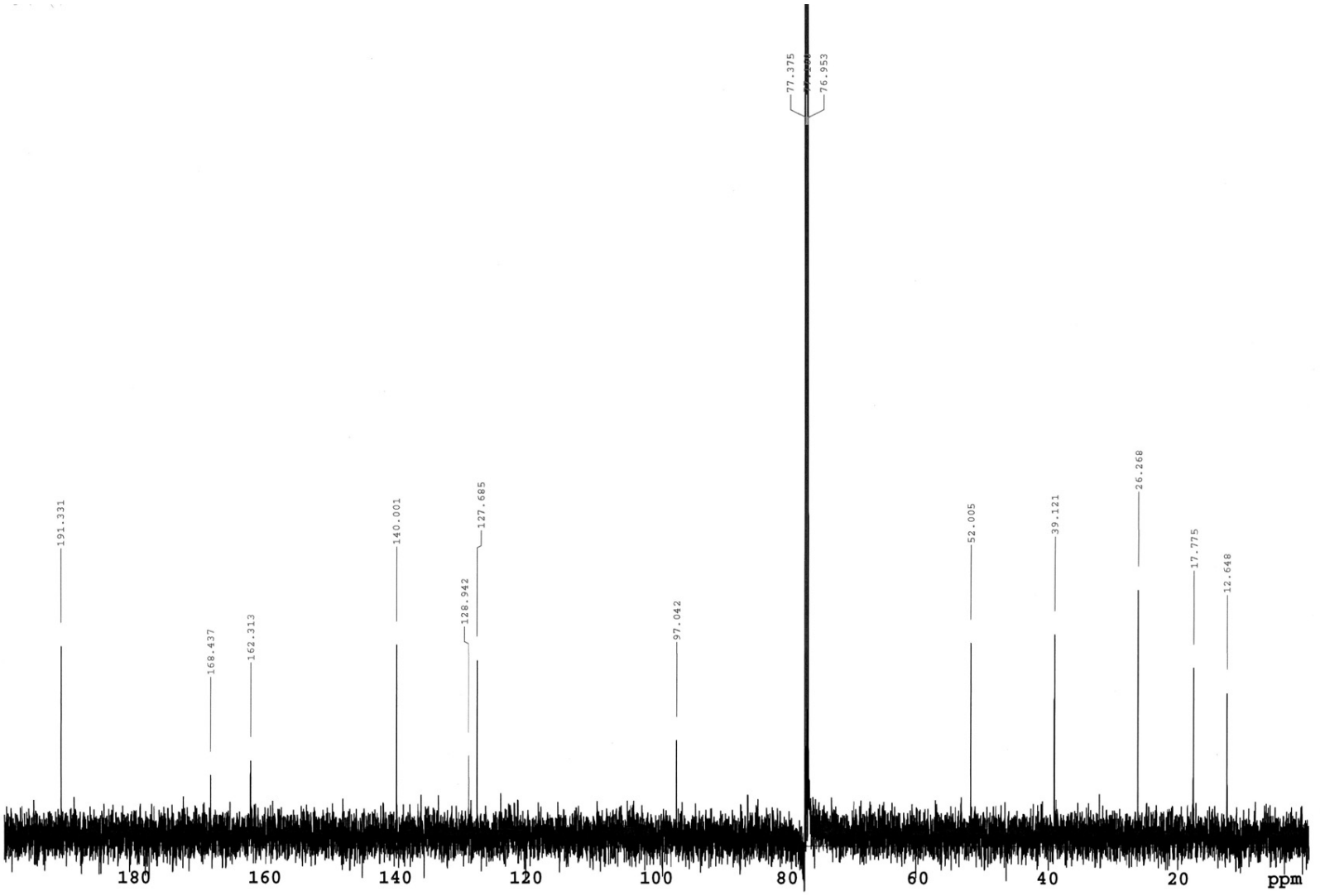
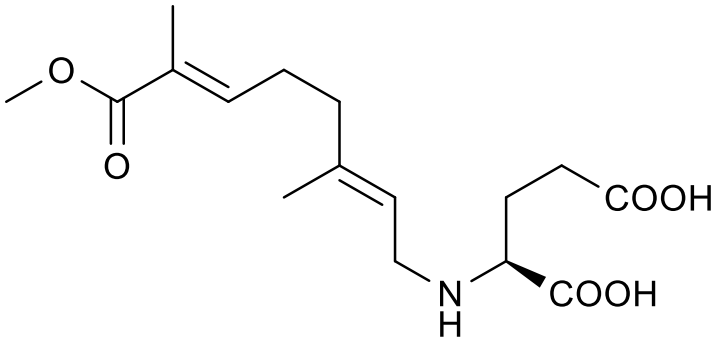


Fig. S20 ^{13}C NMR spectrum of **22** ($CDCl_3$, 151 MHz).



7′-methylester-*N*-geranyl-L-glutamic acid (**14**)

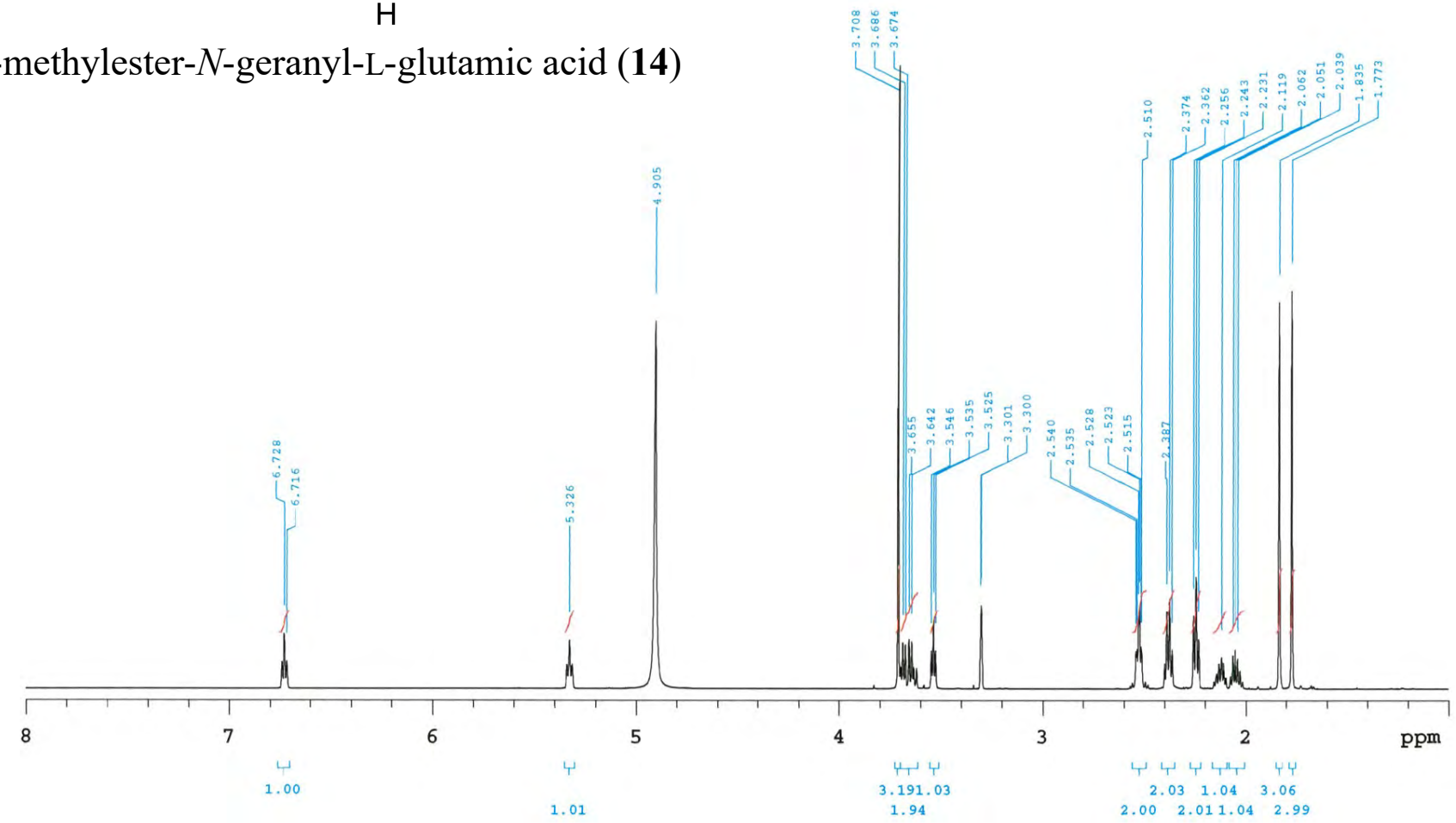


Fig. S21 ^{1}H NMR spectrum of **14** (CD_3OD, 600 MHz).

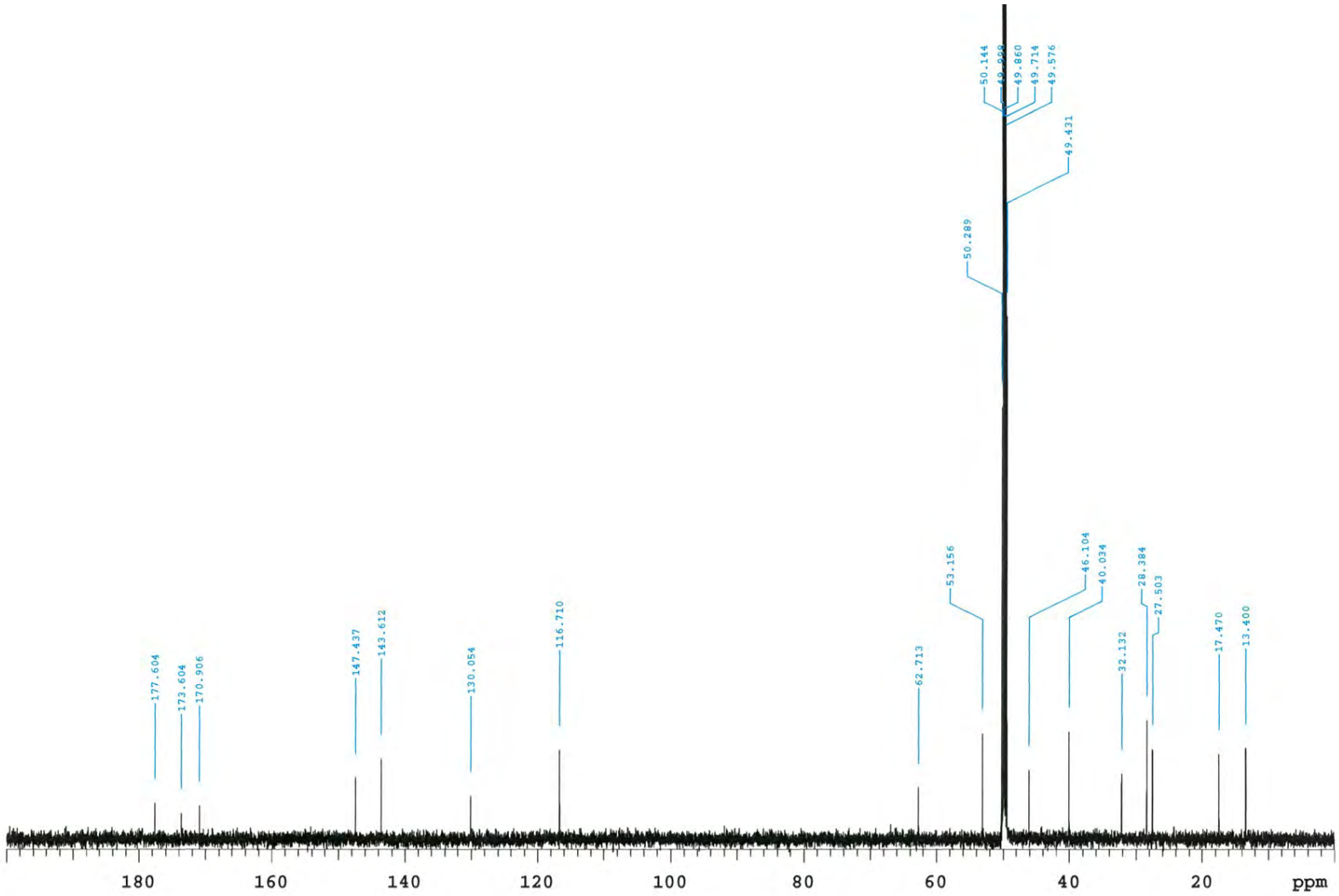


Fig. S22 ^{13}C NMR spectrum of **14** (CD_3OD, 151 MHz).

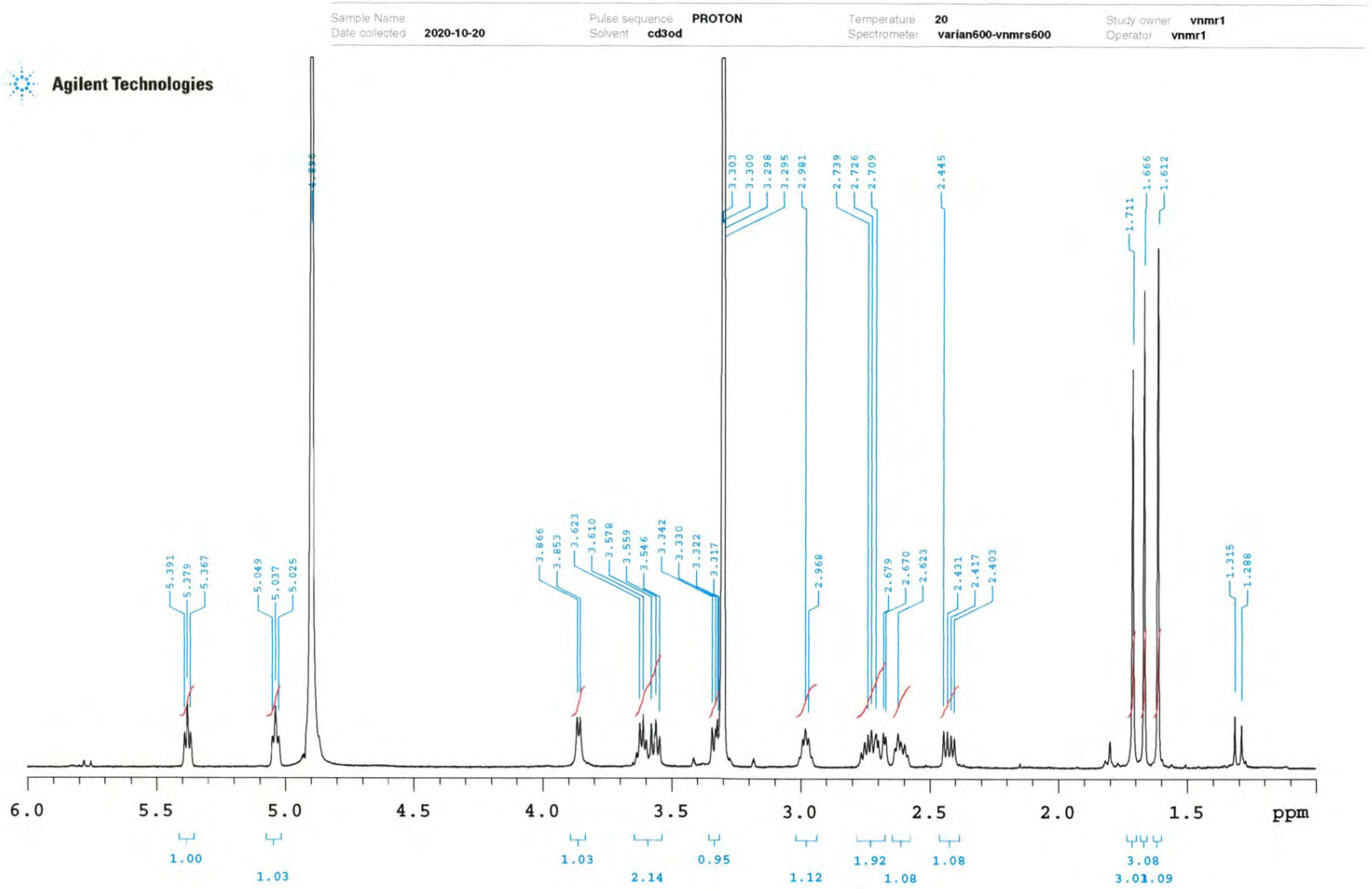


Fig. S23 ^{1}H NMR spectrum of synthetic **5** (CD_3OD, 600 MHz).

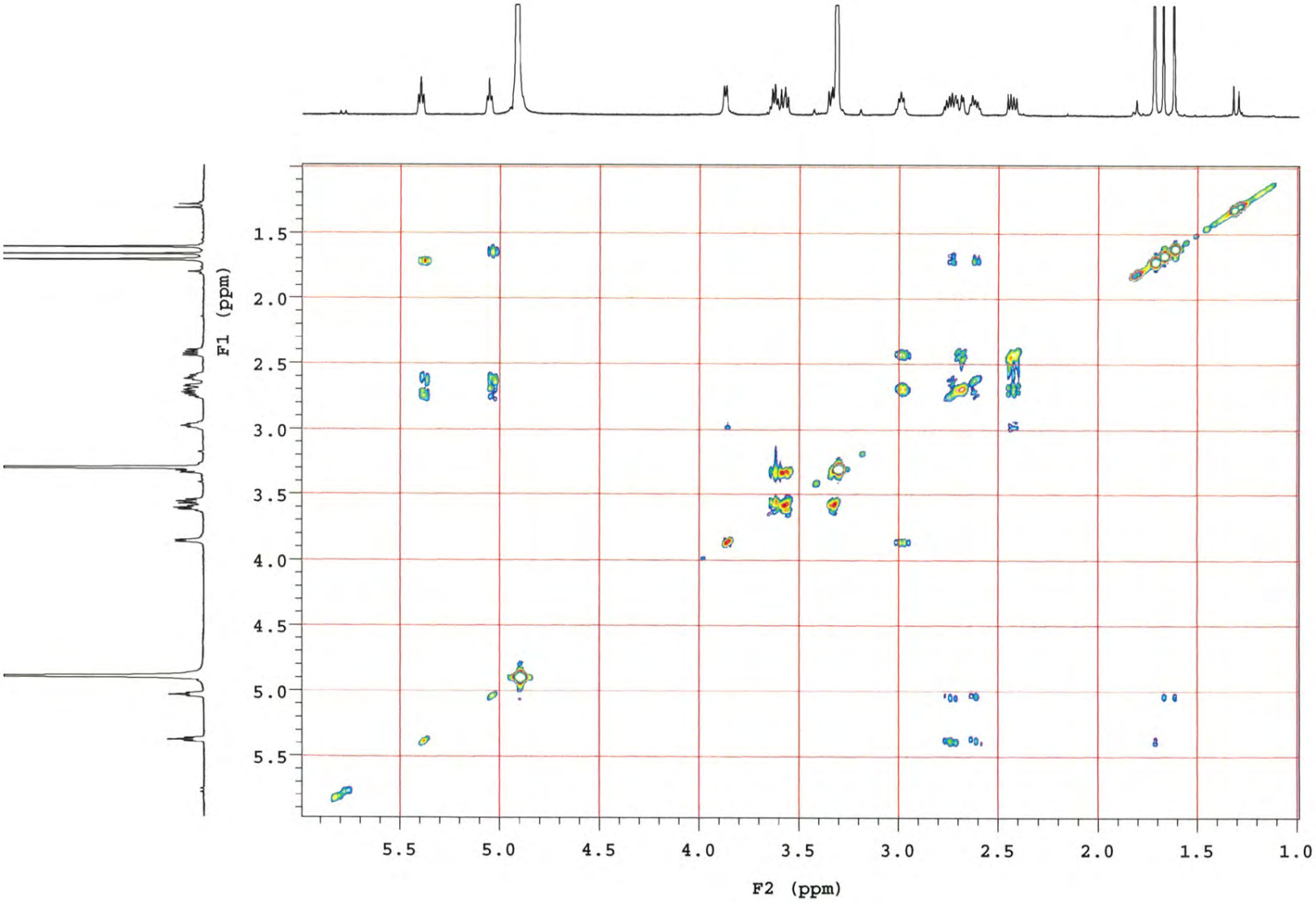


Fig. S24 Gradient COSY spectrum of synthetic **5** (CD_3OD, 600 MHz).

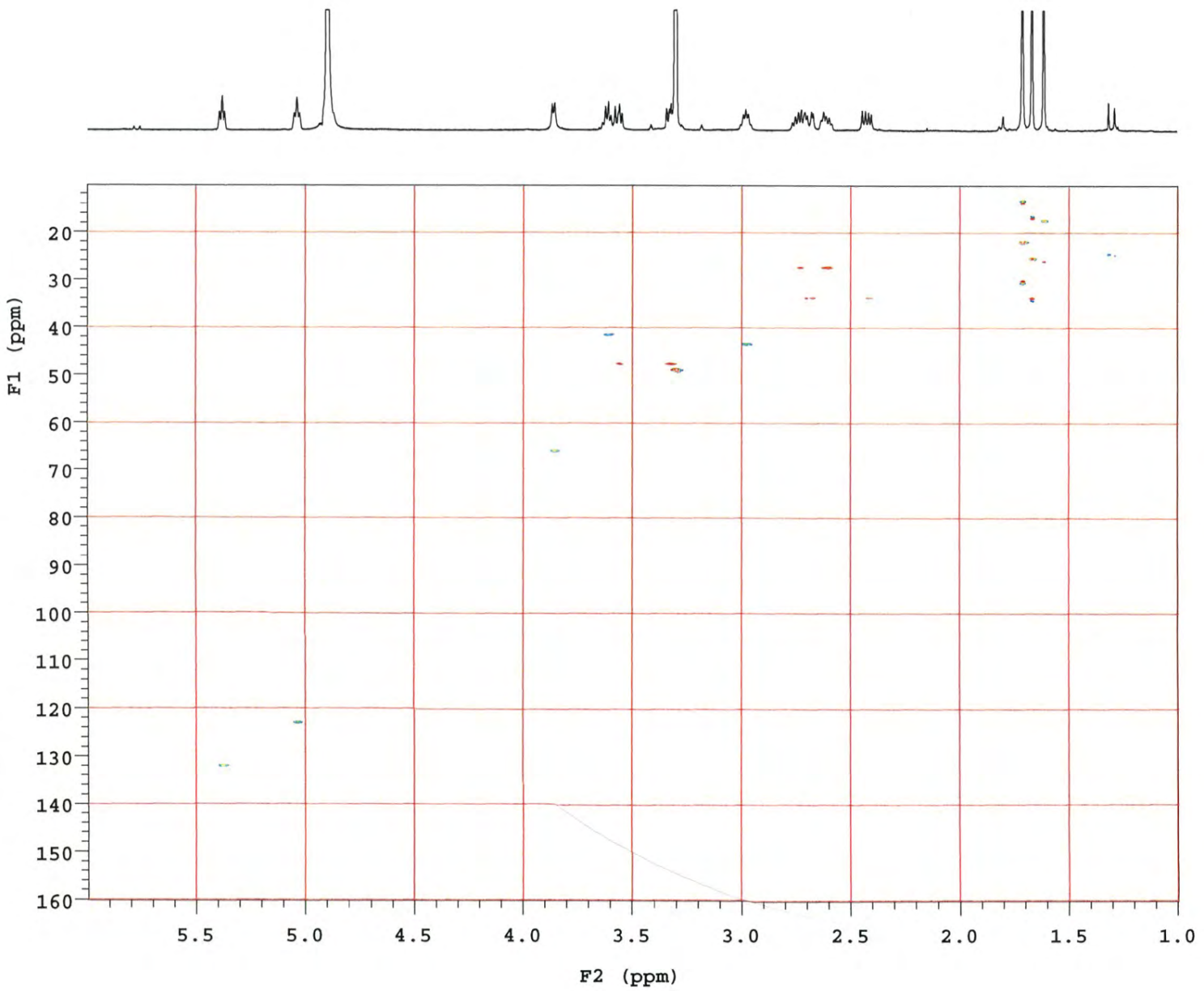


Fig. S25 Gradient HSQC spectrum of synthetic **5** (CD_3OD, 600 MHz).

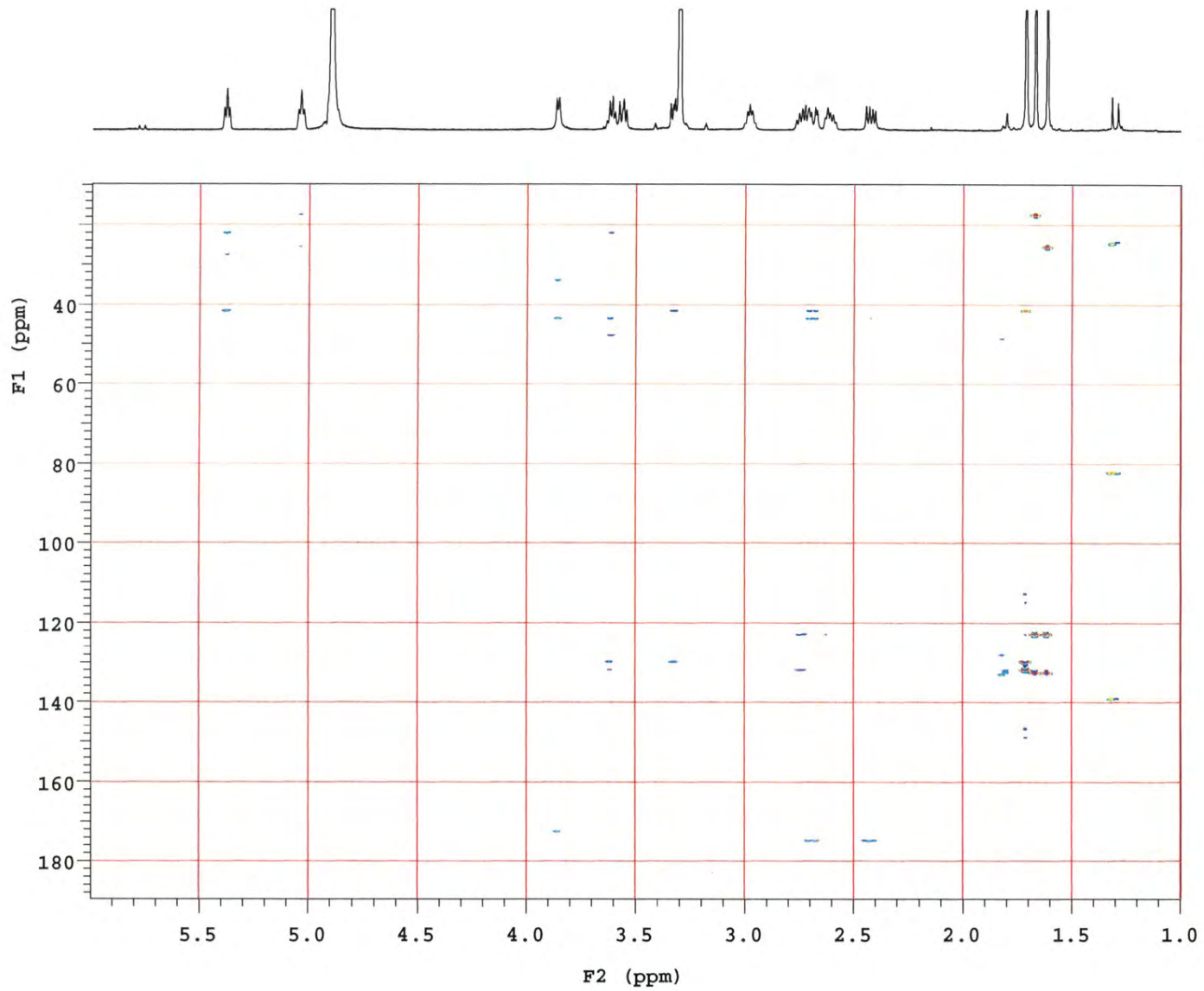


Fig. S26 Gradient HMBC spectrum of synthetic **5** (CD_3OD, 600 MHz).

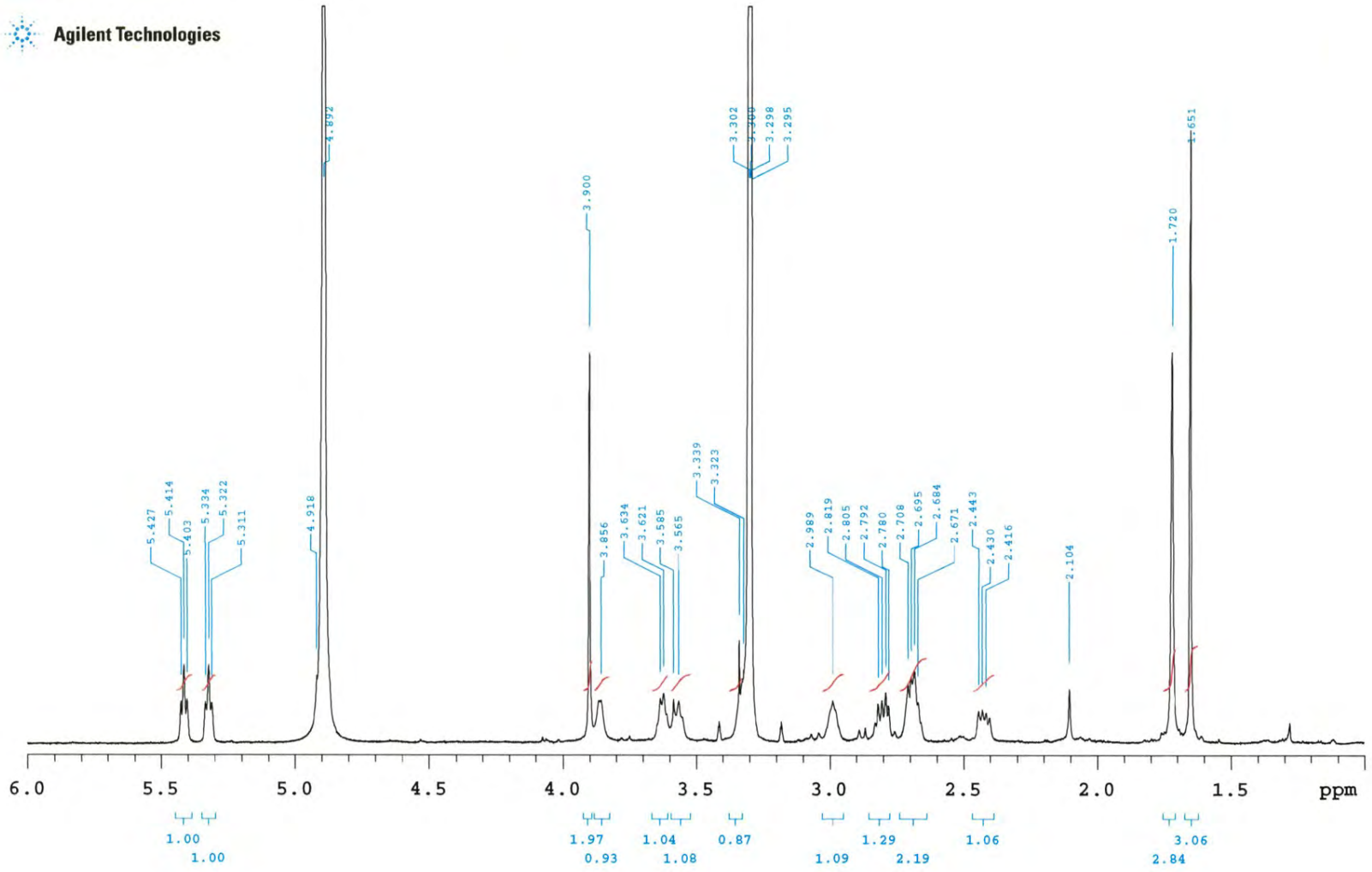


Fig. S27 ^{1}H NMR spectrum of synthetic **6** (CD_3OD, 600 MHz).

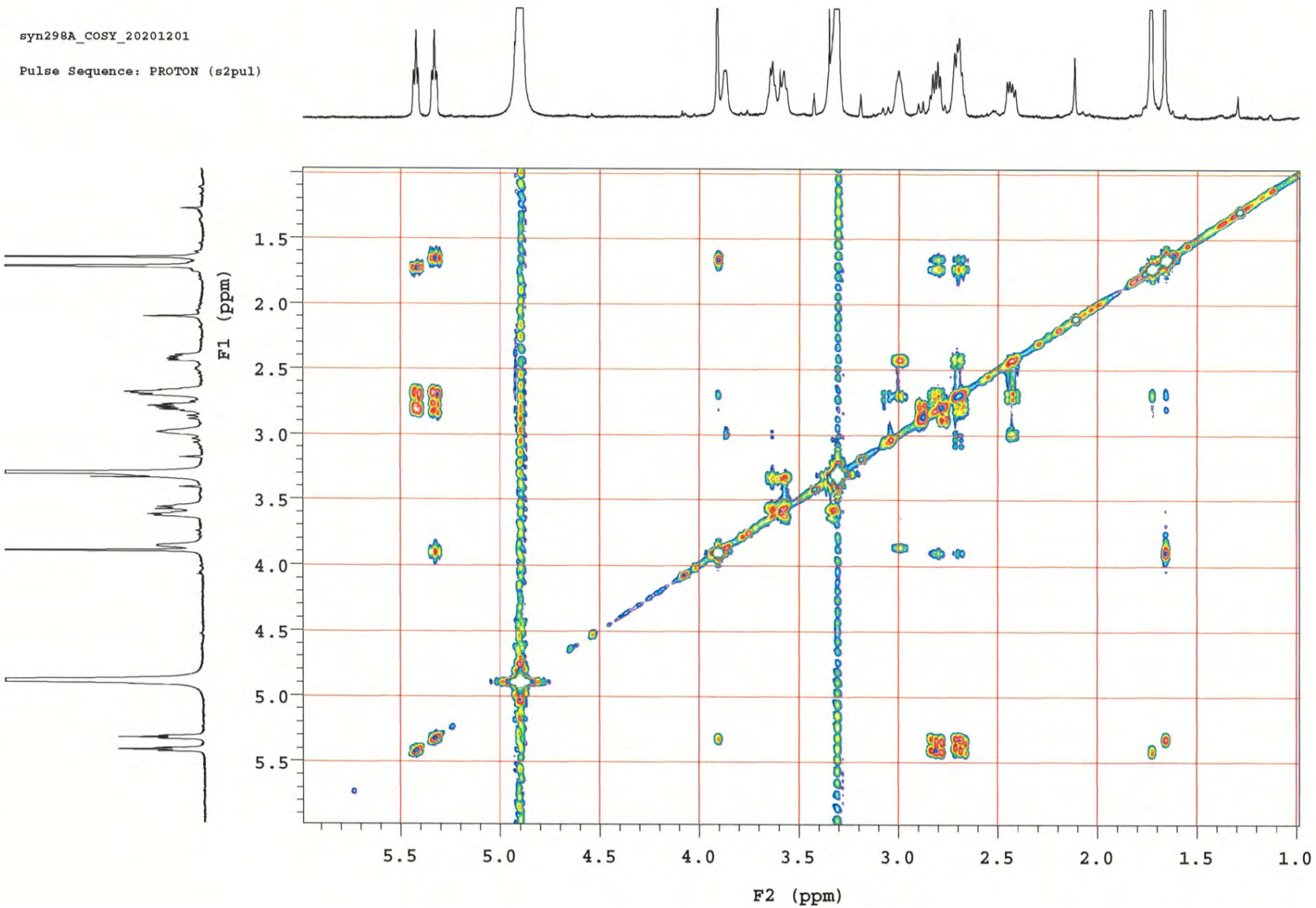


Fig. S28 Gradient COSY spectrum of synthetic **6** (CD_3OD, 600 MHz).

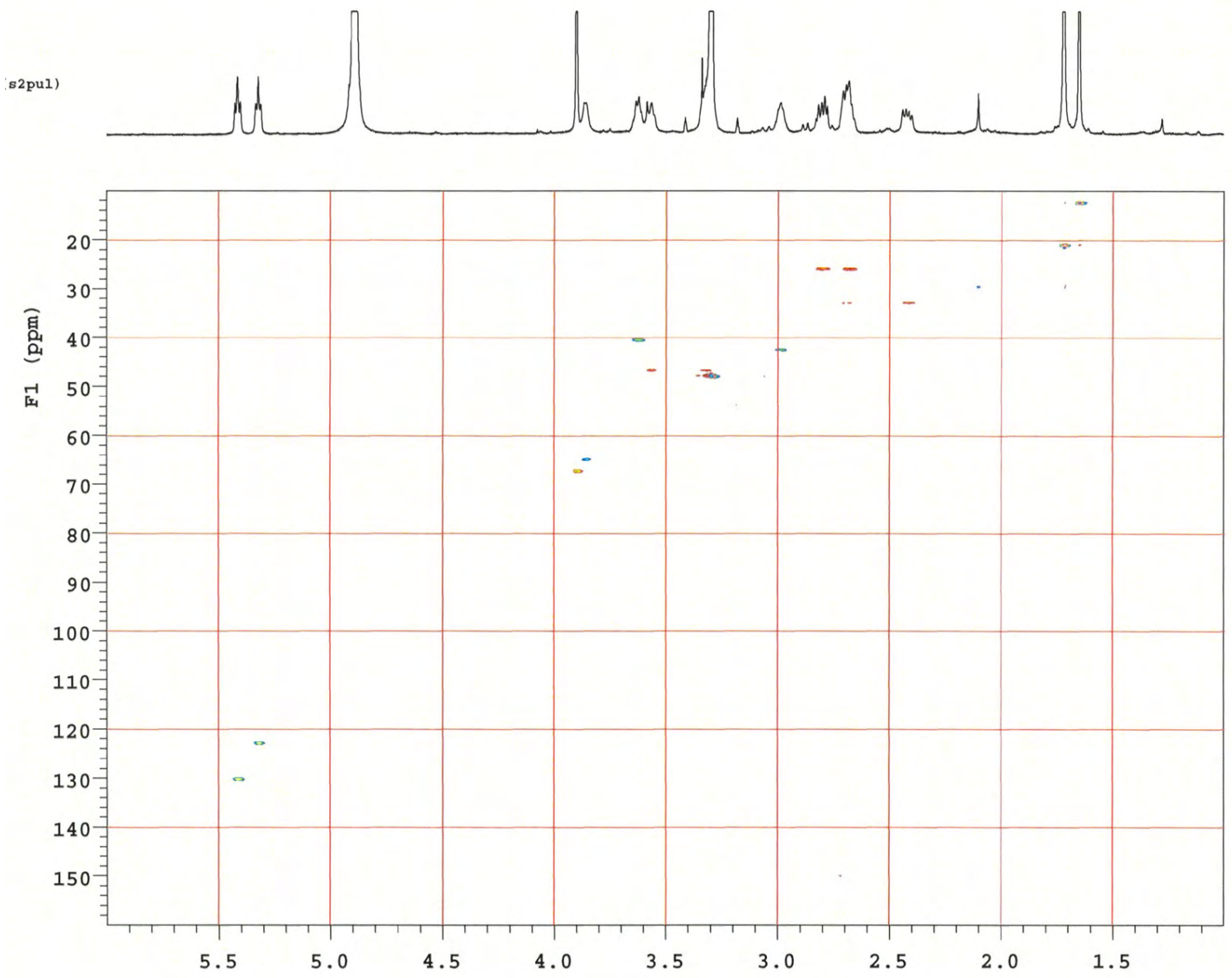


Fig. S29 Gradient HSQC spectrum of synthetic **6** (CD_3OD, 600 MHz).

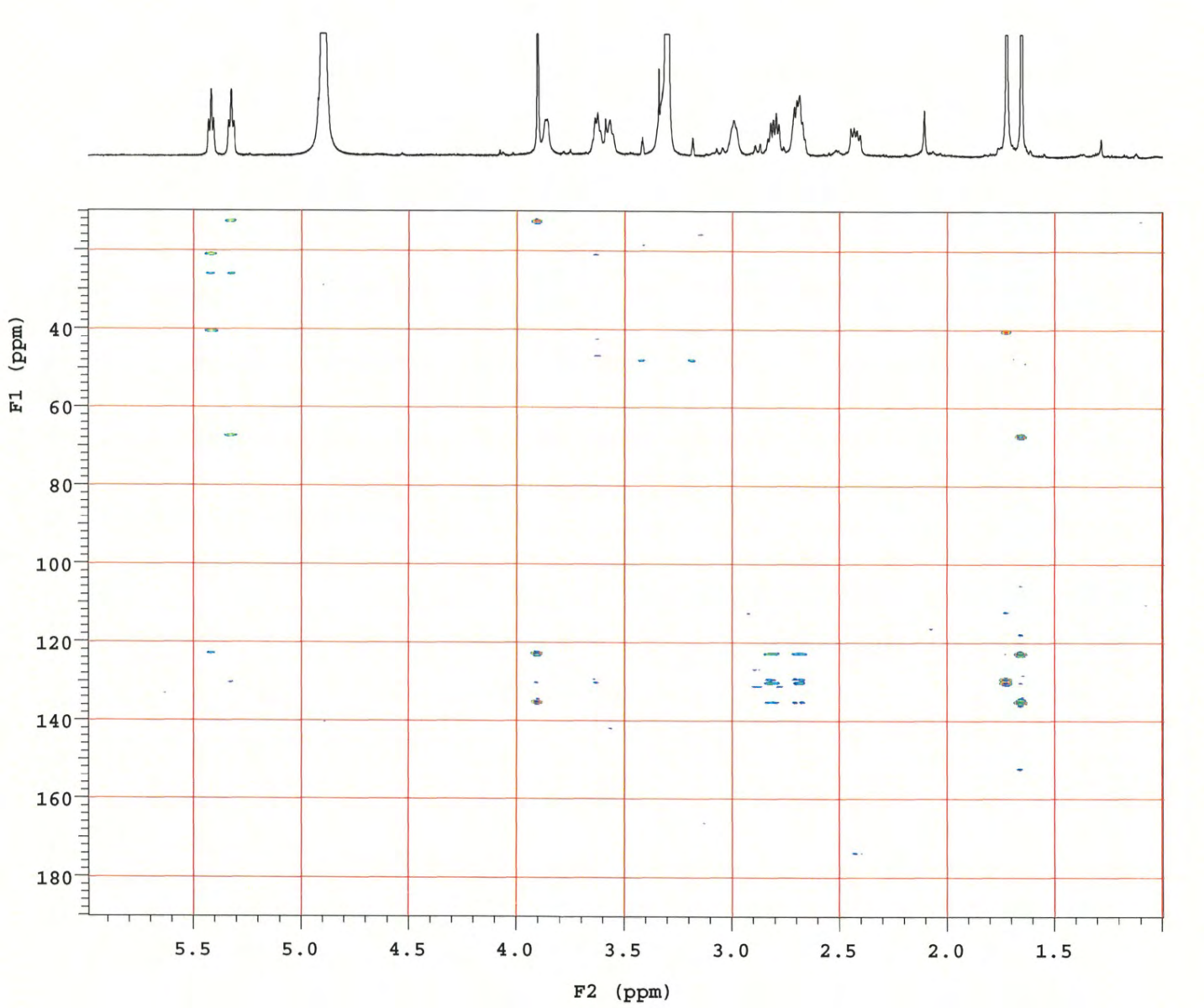


Fig. S30 Gradient HMBC spectrum of synthetic **6** (CD_3OD, 600 MHz).

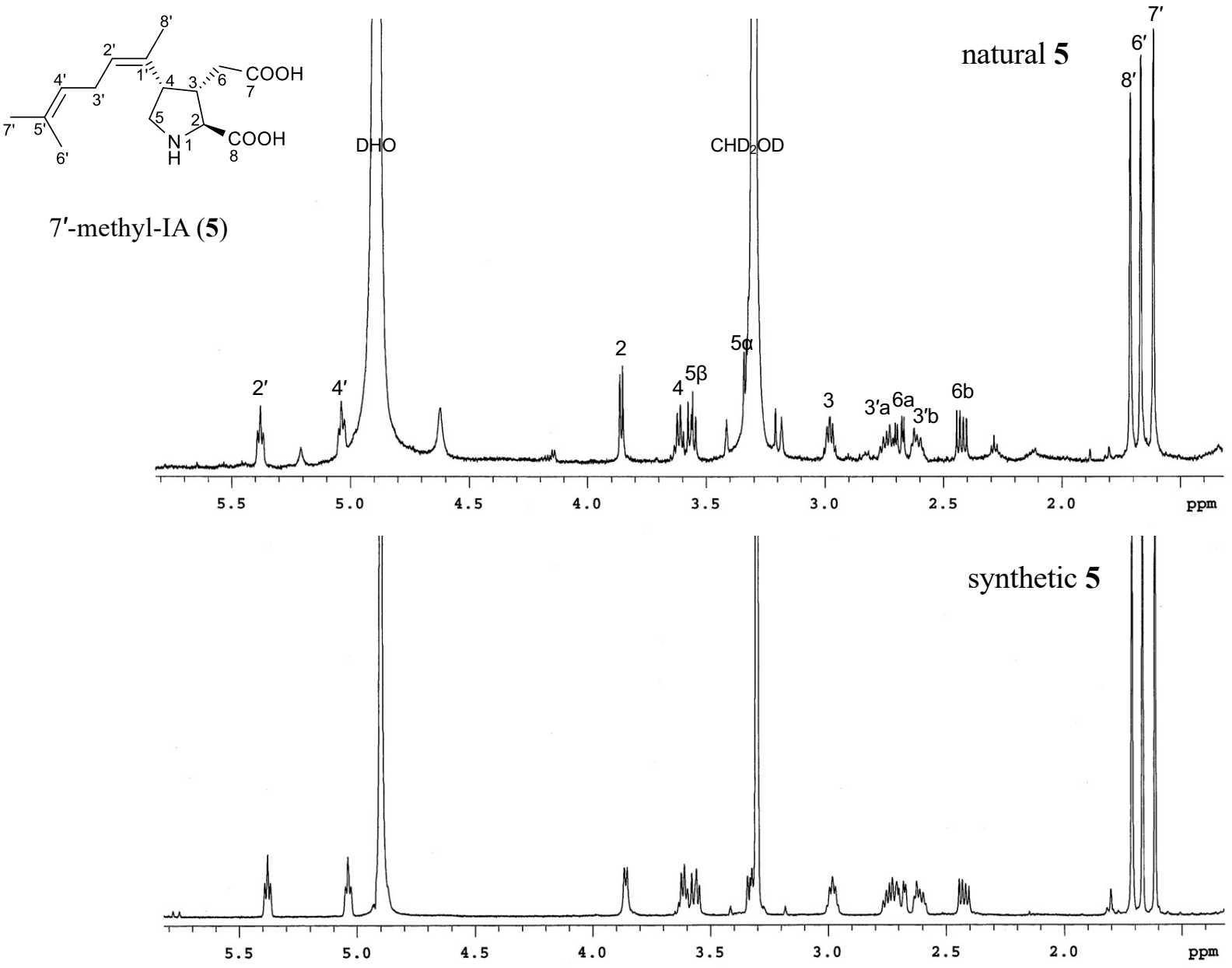
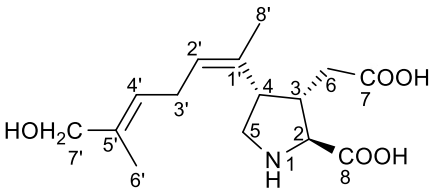


Fig. S31 Comparison of ^{1}H NMR spectra of natural **5**[1)] and synthetic **5** (CD_3OD, 600 MHz).



7′-hydroxy-IA (**6**)

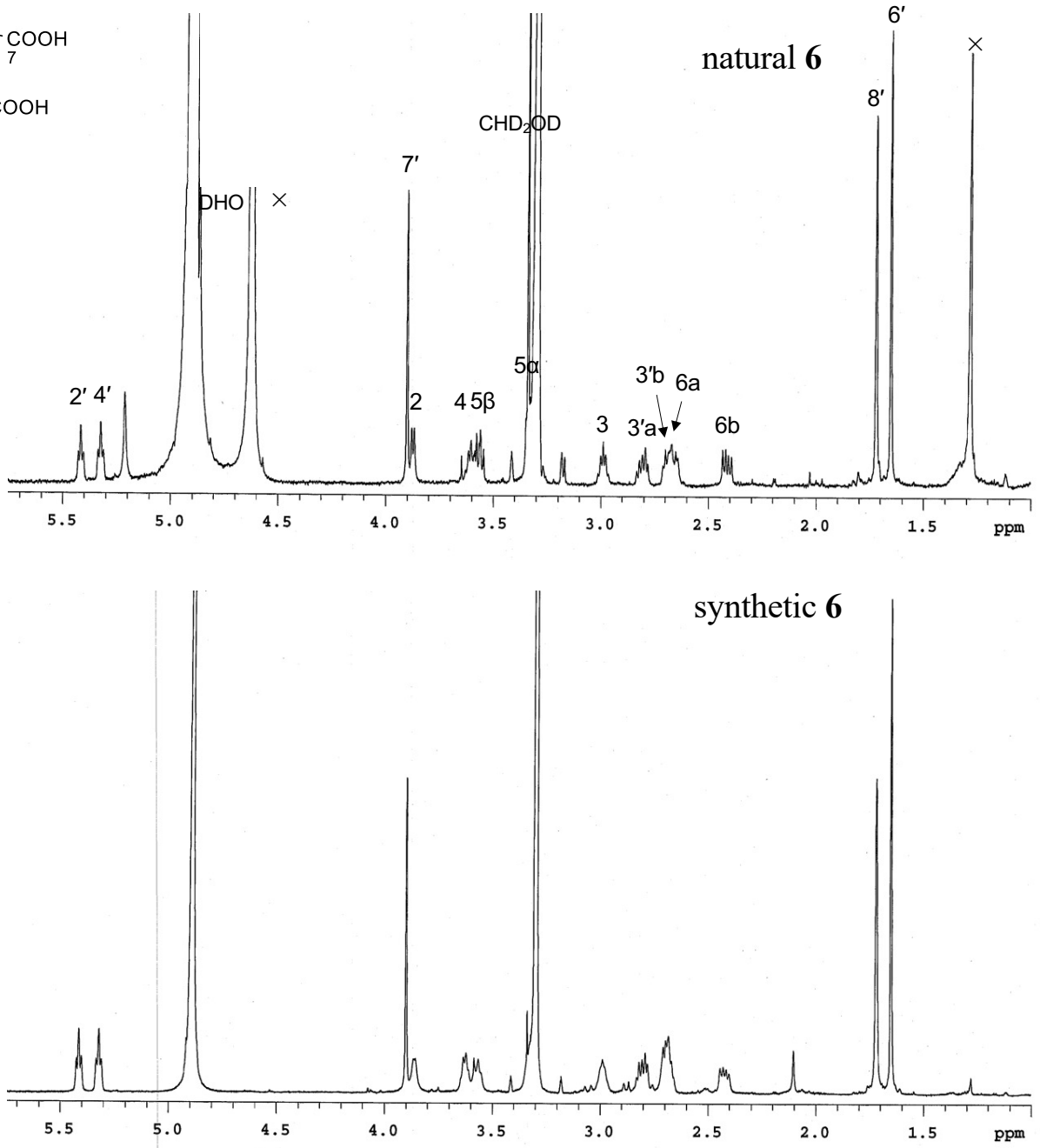


Fig. S32 Comparison of ^{1}H NMR spectra of natural **6**[1)] and synthetic **6** (CD_3OD, 600 MHz).

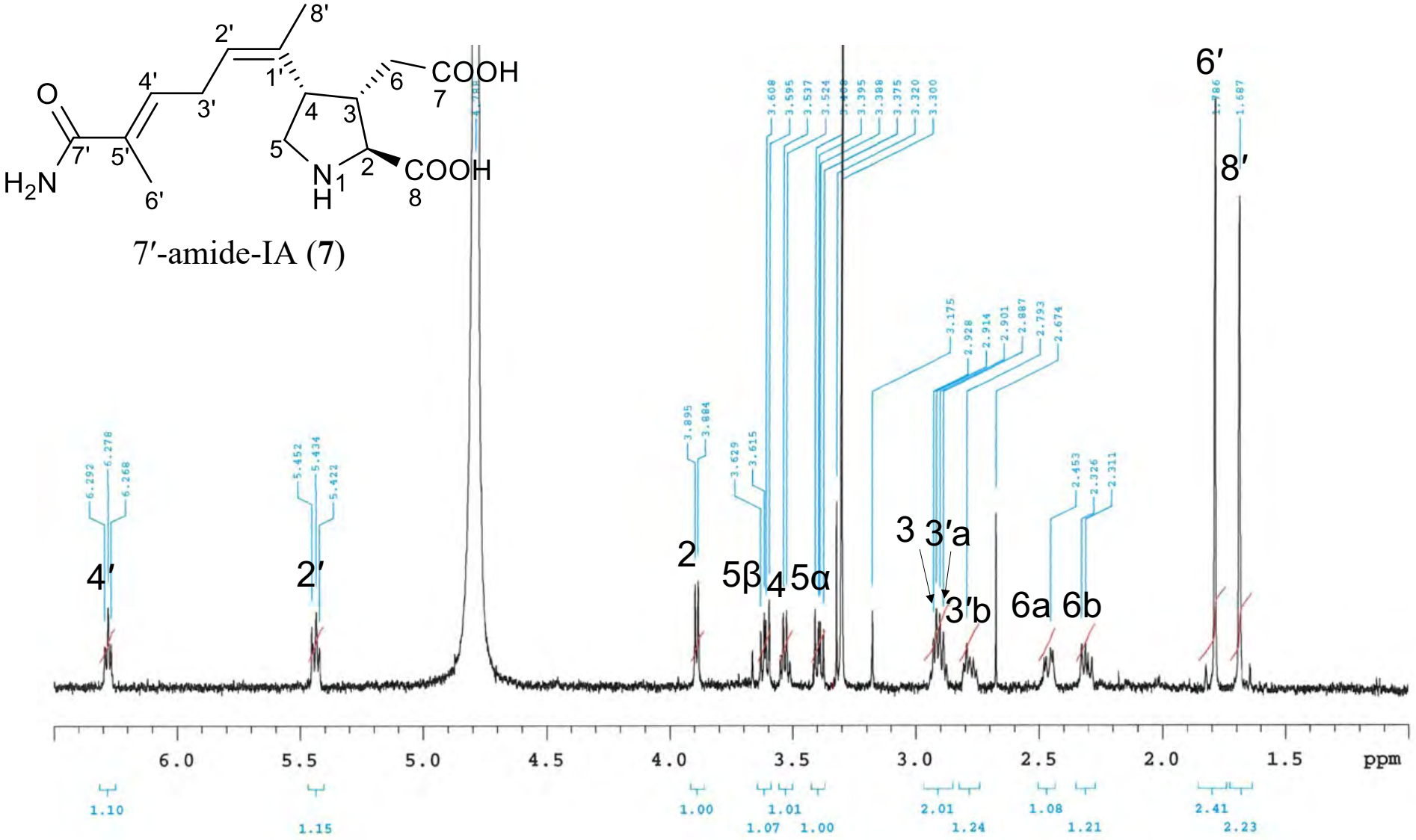


Fig. S33 ^{1}H NMR spectrum of **7** (D_2O, 600 MHz).

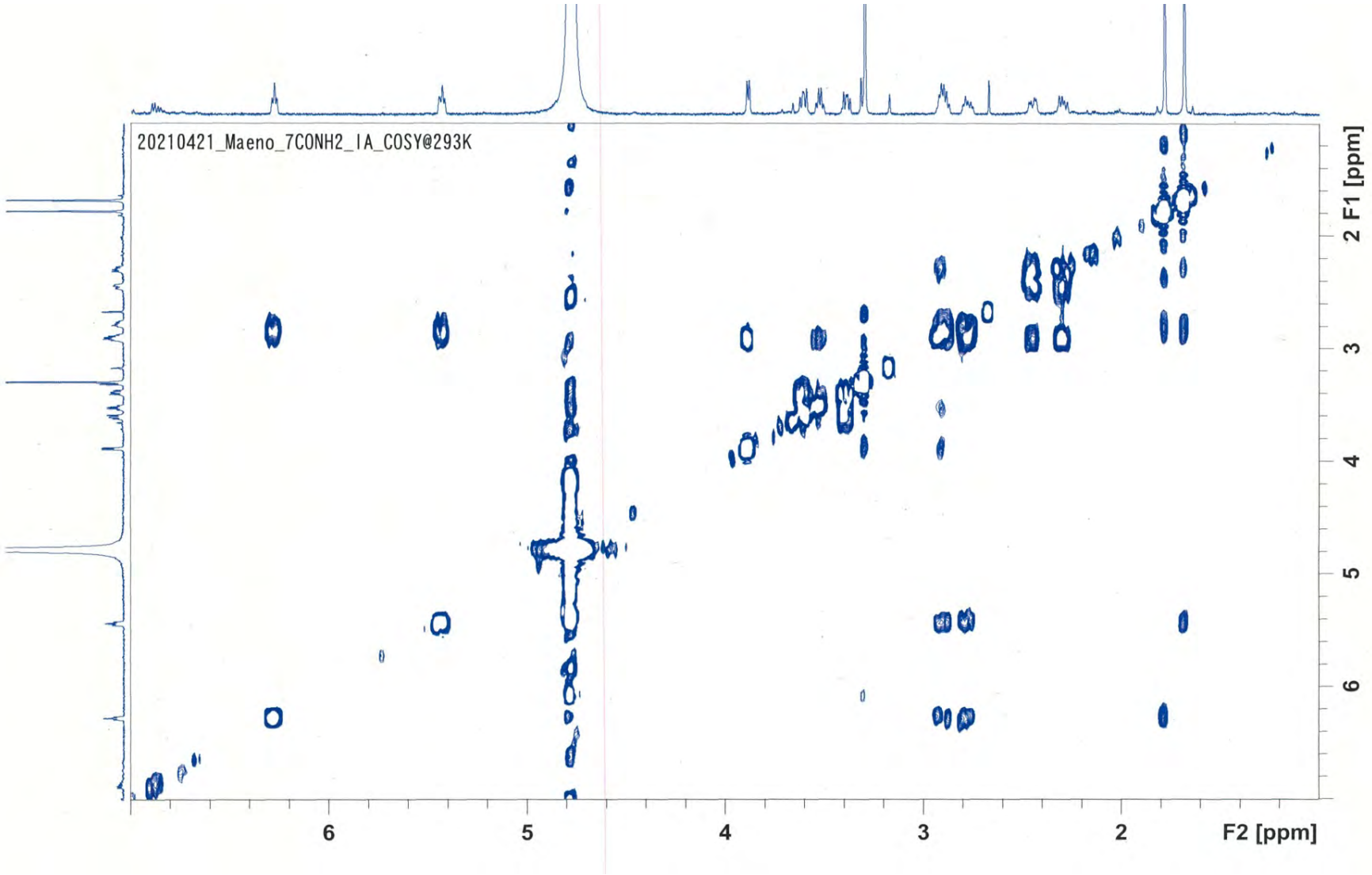


Fig. S34 Gradient COSY spectrum of **7** (D_2O, 600 MHz, CryoProbe).

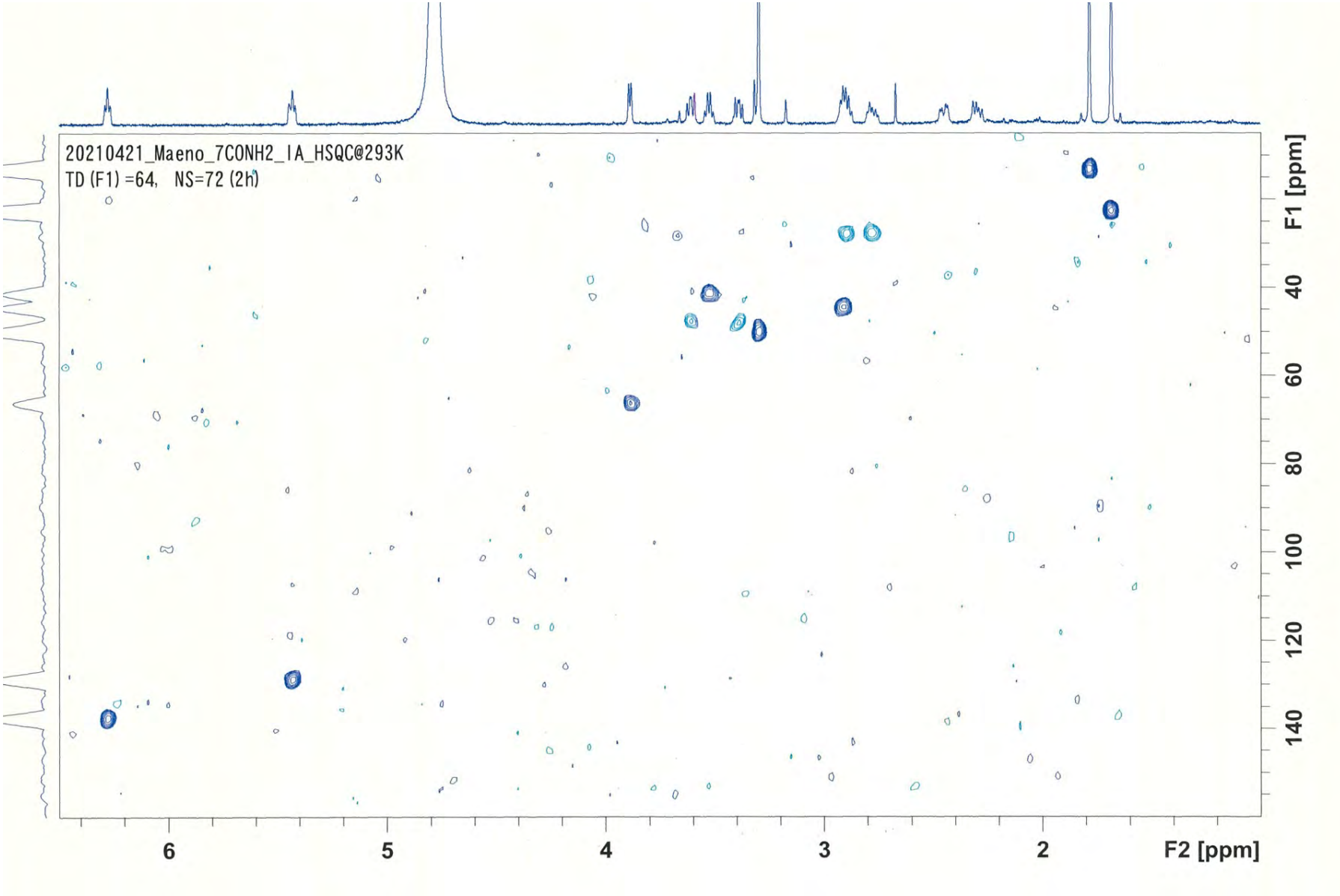


Fig. S35 Gradient HSQC spectrum of **7** (D_2O, 600 MHz, CryoProbe).

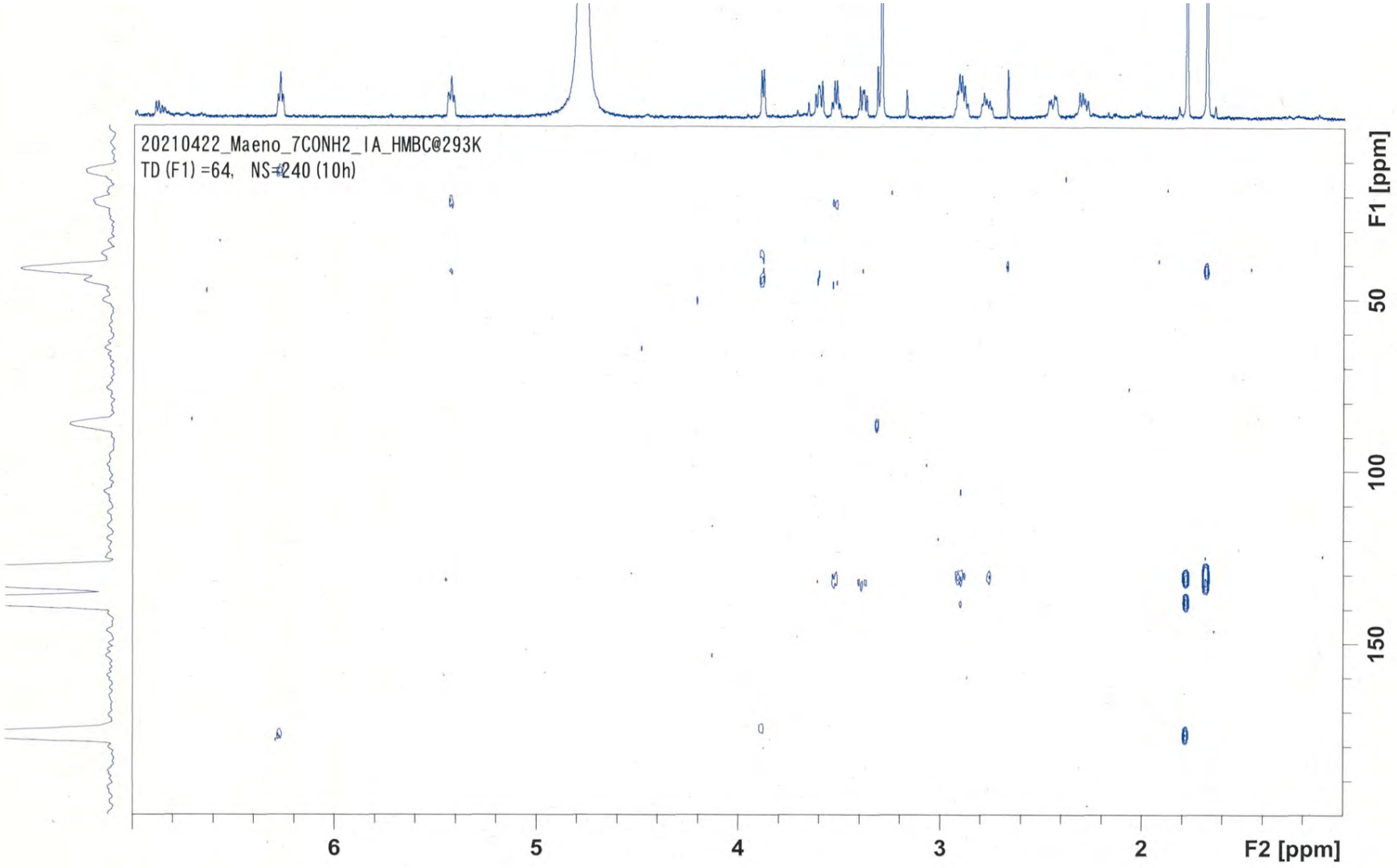


Fig. S36 Gradient HMBC spectrum of **7** (D_2O, 600 MHz, CryoProbe).

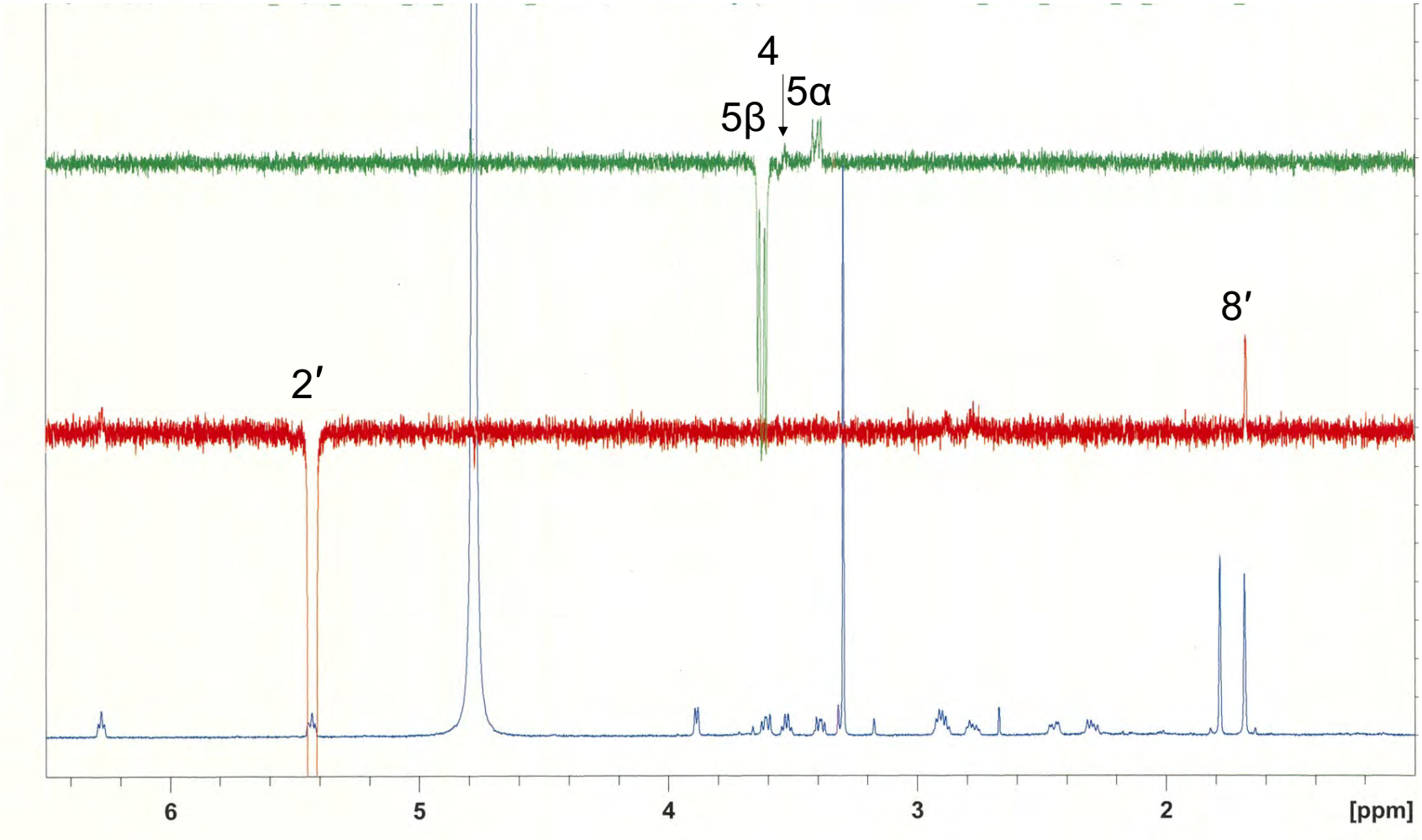
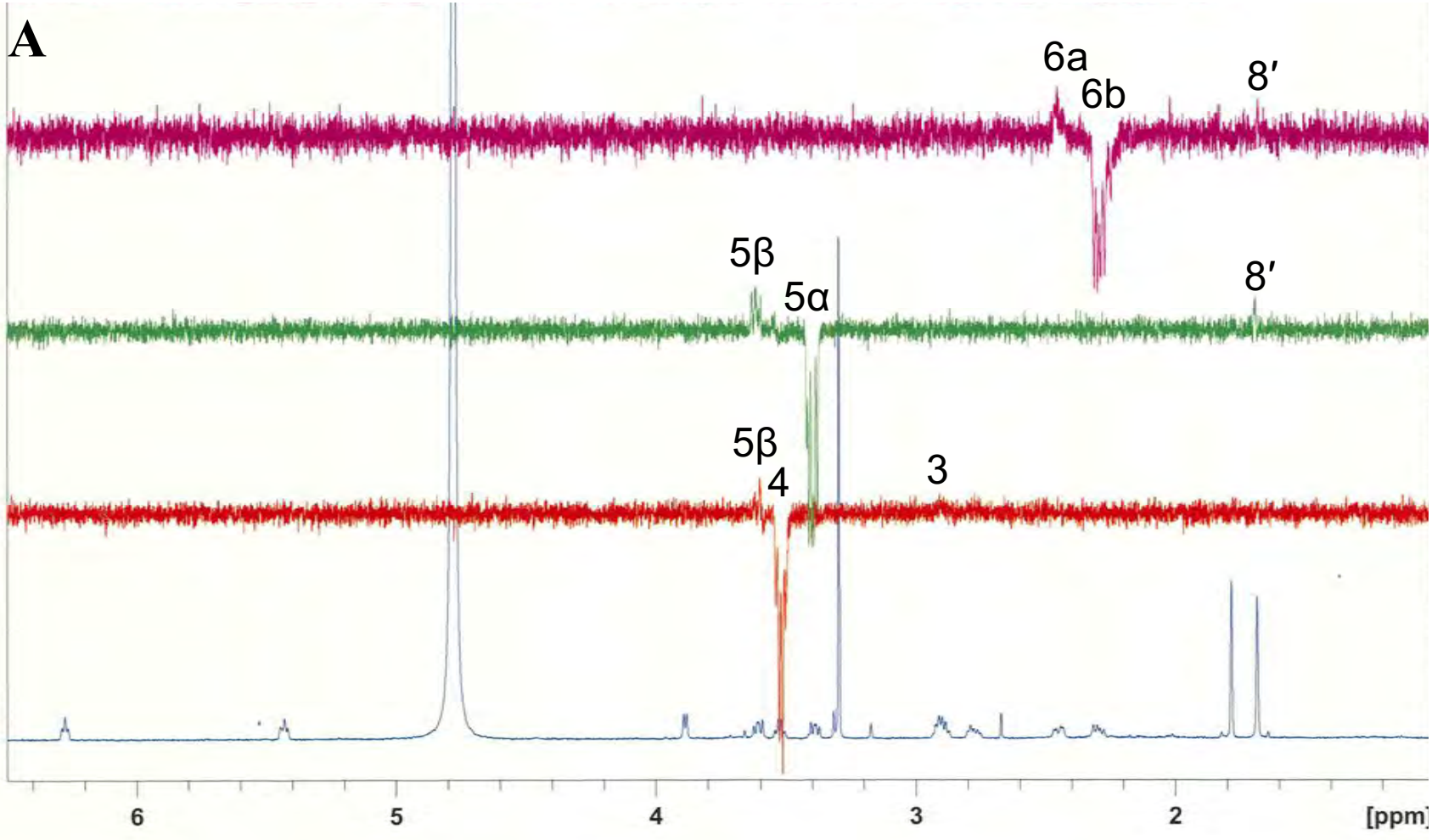


Fig. S37 NOESY1D spectra of **7** (D_2O, 600 MHz, CryoProbe).
Irradiated at δ5.43 ppm (H2′) and δ3.62 ppm (5β).



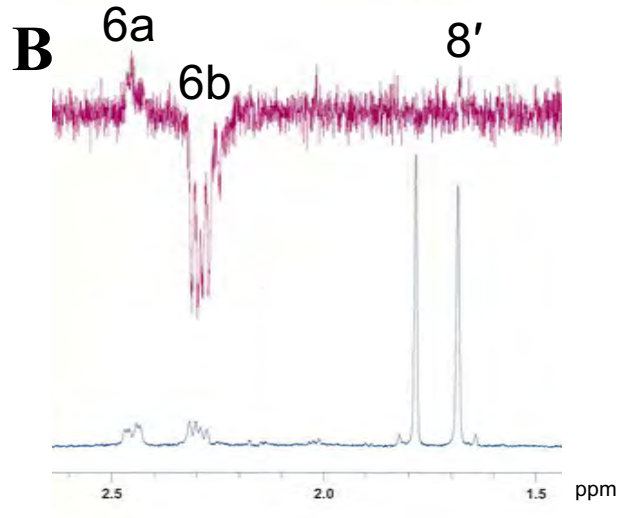
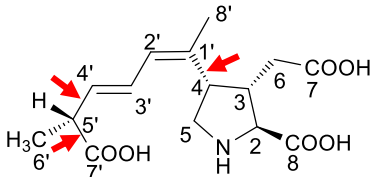


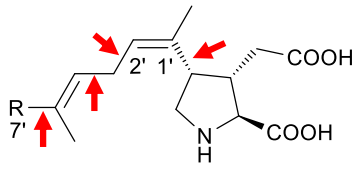
Fig. S38 NOESY1D spectra of **7** (D_2O, 600 MHz, CryoProbe).
A. Irradiated at δ3.53 ppm (H4), δ3.39 ppm (5α), and δ2.31 ppm (6b).
B. Enlarged spectrum irradiated at δ2.31 ppm (6b).

A DA (**1**) Crystal structure

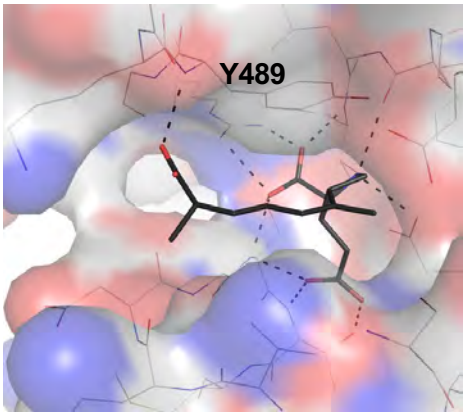
B DA (**1**) Docking

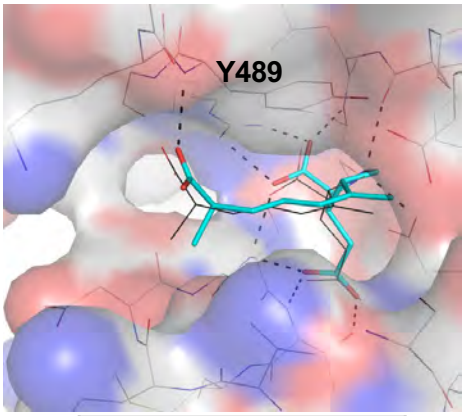


C IA (**2**) Docking

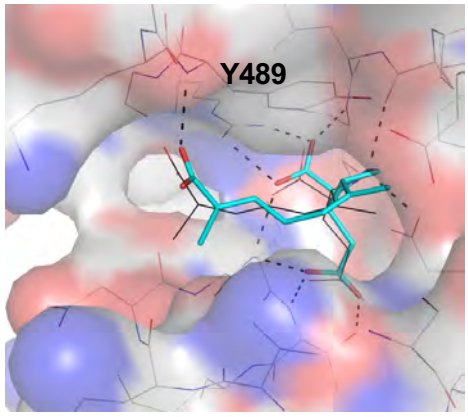


2 : R = COOH (isodomoic acid A,IA)



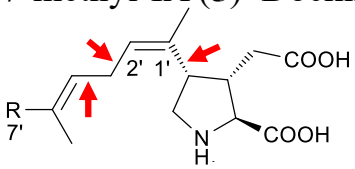


Lowest Binding Energy
–14.0 kcal/mol



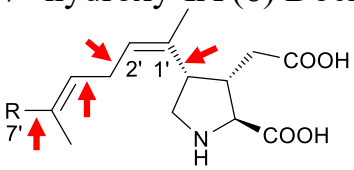
Lowest Binding Energy
–13.6 kcal/mol

D 7'methyl-IA (**5**) Docking



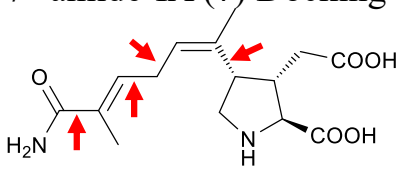
5 : R = CH_3 (7'-methyl-IA)

E 7'-hydroxy-IA (**6**) Docking

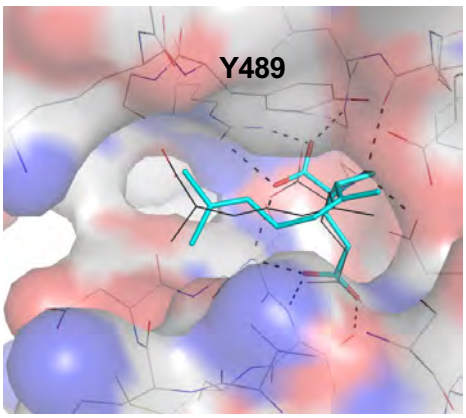


6 : R = CH_2OH (7'-hydroxy-IA)

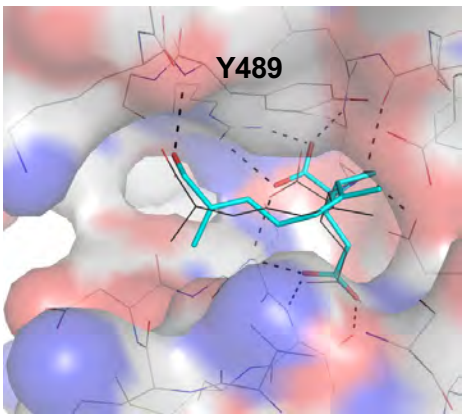
F 7'-amide-IA (**7**) Docking



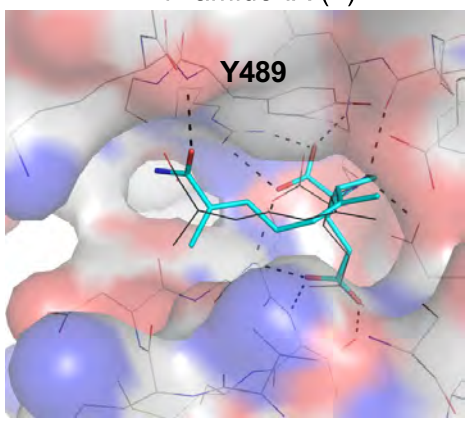
7'-amide-IA (**7**)



Lowest Binding Energy
–12.2 kcal/mol



Lowest Binding Energy
–12.7 kcal/mol



Lowest Binding Energy
–13.4 kcal/mol

Fig. S39 Docking analyses of DA, IA, **5**, **6**, and **7** to GluK1.
Upper column shows the structure of (A and B) DA (**1**), (C) IA (**2**), (D) 7'methyl-IA (**5**), (E) 7'-hydroxy-IA (**6**), and (F) 7'-amide-IA (**7**). Bonds indicated by red arrows are defined as rotatable and were subjected to AutoDock. Lower column shows the results of the docking analysis of AutoDock. The molecular surface of GluK1 (PDB ID; 2pbw) is shown. Structures of (A) crystallographic DA and (B-F) the best docking model of each compound is shown as stick model, and crystallographic DA (black line) is shown as a reference structure. Direct hydrogen bonds between ligands and GluK1 are shown as dashed lines.