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Supplemental Data

Molecular Genetic Mining of the *Aspergillus* Secondary Metabolome: Discovery of the Emericellamide Biosynthetic Pathway

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Supplemental Results

Detailed structural Characterization of Emericellamide C-F

Emericellamide C (**2**) was isolated as a white powder. The molecular formula $C_{30}H_{53}N_5O_7$ was established by its ^{13}C NMR, DEPT and HRFABMS data, representing seven indices of hydrogen deficiency (IHD). The ^1H NMR spectrum in DMSO-d_6 (Table S3) exhibited five typical amide NH signals (δ_H 8.20, 8.11, 7.82, 7.61, and 7.53), one ester carbinol proton (δ_H 4.81), and six α–amino protons [δ_H 4.38 (1H), 4.01-4.09 (3H), 3.91 (1H), and 3.58 (1H)]. ^{13}C NMR and DEPT spectral (Table S4) data exhibited six amide or ester carbonyl groups (δ_C 172.9, 171.6, 171.4, 171.2, 170.9, and 169.4) and one oxygenated carbon [δ_C 75.0 (d)], which correlated with δ_H 4.81 in the gHMQC spectrum. These results, together with the IR absorption band at 3307 cm^{-1} (amide N-H stretching), 1758 cm^{-1} (ester C=O stretching), and 1636 cm^{-1} (amide C=O stretching), suggested that emericellamide C (**2**) is a monocyclic depsipeptide containing five amino acids and one ester functional group. Emericellamide A (**1**), a known compound isolated from marine-derived *Emericella* sp. [1] and also found in this study, exhibited similar ^1H and ^{13}C NMR spectral features with emericellamide C (**2**), as it also contained two alanines (Ala-1 and Ala-2), one leucine (Leu), one valine (Val), and one glycine (Gly). The spectral differences between emericellamide A and C are that emericellamide A (**1**) has an additional methyl group [δ_H 0.82; δ_C 12.9, CH_3-31] and the carbinol proton in emericellamide A (**1**) [δ_H 4.93 (br d, J = 9.6 Hz, H-22)] is shifted upfield to δ_H 4.81 (ddd, J = 9.2, 8.4, 3.6 Hz, H-22) in emericellamide C (**2**). These results suggested that Me-31 in emericellamide A is replaced by H in emericellamide C (**2**).

Emericellamide D (**3**), a constitutional isomer of emericellamide C (**2**), also has similar ^1H and ^{13}C NMR spectral features with emericellamide A (**1**) (Tables S3 and S4). Emericellamide A (**1**) and D (**3**) differ only in that CH_3-30 in emericellamide A (**1**) is replaced by H in emericellamide D (**3**). This generated one methylene [δ_H 2.68 (1H, dd, J = 13.6, 9.6 Hz) and 2.18 (1H, d, J = 13.6 Hz);

δ_C 30.8 (t), CH_2-21] in emericellamide D (**3**) instead of one methine [δ_H 2.87 (1H, dq, *J* = 9.6, 6.8 Hz); δ_C 41.1 (d), CH-21] in emericellamide A (**1**). Emericellamides E (**4**) and F (5), another pair of constitutional isomers, both have the molecular formula $C_{32}H_{57}N_5O_7$. The ^{1}H NMR spectra of emericellamides E (**4**) and F (**5**) are superimposable with emericellamides C (**2**) and D (**3**), respectively. This result, together with the ^{13}C and DEPT spectra exhibiting two more CH_2 carbons, suggested that the structures of emericellamide E (**4**) and F (**5**) extended two more carbons on the aliphatic side chains of emericellamides C (**2**) and D (**3**), respectively. 2D NMR (gHMQC, gHMBC, gCOSY, and ROESY) data led to full assignments of emericellamides C – F (**2** – **5**) (Tables S3 and S4) and also confirmed the assigned structures. The absolute configurations of the amino acids in emericellamides C (**2**) and D (**3**) were determined as all L-isomers by Marfey's reagent after hydrolysis as described by Oh et al [1]. The configurations of C-21 and C-22 in emericellamides C (**2**) and E (**4**) were same as emericellamide A (**1**) because they all have large coupling constants (> 9.0 Hz) between H-21 and H-22. The configurations of C-22 and C-23 in emericellamides D (**3**) and F (**5**) were also the same as emericellamide A (**1**) because all have small coupling constants (< 4.0 Hz) between H-22 and H-23 [1]. The similar optical rotation values of these compounds also support these configurations.

Supplemental Methods

Optical rotations were measured on a JASCO P-1010 digital polarimeter, whereas IR spectra were recorded on a Perkin–Elmer Spectrum BX. ^{1}H and ^{13}C NMR spectra were run on a Varian Unity Plus 400 spectrometer, whereas HRFABMS spectra were obtained on a JEOL MSRoute mass spectrometer. LC/MS was carried out using a ThermoFinnigan LCQ Advantage ion trap mass spectrometer with a RP C_{18} column (Alltech Prevail C18 3 μm 2.1 X 100mm) at a flow rate of 125 μL/min. The solvent gradient for HPLC was 95 % MeCN/H_2O (solvent B) in 5 % MeCN/H_2O (solvent A) both containing 0.05 % formic acid: 0 % B from 0 to 5 min, 0 to 100 % B from 5 to 35 min, 100 to 0 % B from 35 to 40 min, and re-equilibration with 100 % B from 40 to 45 min. Positive ion electrospray ionization (ESI) was used for the detection of the analytes. Conditions for MS included a capillary voltage 5.0 kV, a sheath gas flow rate at 60 arbitrary units, an auxiliary gas flow rate at 10 arbitrary units, and the ion transfer capillary temperature at 350 °C.

Spectral Data of Emericellamides

Emericellamide A (**1**) characterization: white powder; $[\alpha]_D^{24}$ -50.2 (MeOH, c 0.1); IR ν_{max}^{ZnSe} 3306, 2930, 1743, 1652, 1549 cm^{-1}; For ^{1}H and ^{13}C NMR data, see Table S3 and S4. HRFABMS, $[M+H]^+$ *m*/*z* found 610.4154; calc. for $C_{31}H_{56}N_5O_7$: 610.4180.

Emericellamide C (**2**) characterization: white powder; $[\alpha]_D^{24}$ -37.4 (MeOH, c 0.1); IR ν_{max}^{ZnSe} 3307, 2962, 1758, 1636, 1551 cm^{-1}; For ^{1}H and ^{13}C NMR data, see

Table S3 and S4. HRFABMS, $[M+H]^+$ *m*/*z* found 596.4026; calc. for $C_{30}H_{54}N_5O_7$: 596.4023.

Emericellamide D (**3**) characterization: white powder; $[\alpha]_D^{25}$ -65.3 (MeOH, c 0.1); IR ν_{max}^{ZnSe} 3306, 2930, 1745, 1656, 1529 cm^{-1}; For ^1H and ^{13}C NMR data, see Table S3 and S4. HRFABMS, $[M+H]^+$ *m*/*z* found 596.4016; calc. for $C_{30}H_{54}N_5O_7$: 596.4023.

Emericellamide E (**4**) characterization: white powder; $[\alpha]_D^{25}$ -35.2 (MeOH, c 0.1); IR ν_{max}^{ZnSe} 3307, 2962, 1758, 1636, 1551 cm^{-1}; For ^1H and ^{13}C NMR data, see Table S3 and S4. HRFABMS, $[M+H]^+$ *m*/*z* found 624.4324; calc. for $C_{32}H_{58}N_5O_7$: 624.4336.

Emericellamide F (**5**) characterization: white powder; $[\alpha]_D^{25}$ -46.4 ($MeOH/CHCl_3$ 1:1, c 0.05); IR ν_{max}^{ZnSe} 3306, 2930, 1758, 1637, 1549 cm^{-1}; For ^1H and ^{13}C NMR data, see Table S3 and S4. HRFABMS, $[M+H]^+$ *m*/*z* found 624.4312; calc. for $C_{32}H_{58}N_5O_7$: 624.4336.

Table S1. *A. nidulans* Strains Used in This Study

strain	genotype	reference
WT (TN02A3)	*pyrG*89; *pyroA*4, *nkuA::argB*	[2]
ΔAN0607	*pyrG*89; *pyroA*4, *nkuA::argB*; AN0607::*pyrG A. fumigatus*	this study
ΔAN1242	*pyrG*89; *pyroA*4, *nkuA::argB*; AN1242::*pyrG A. fumigatus*	this study
ΔAN2545	*pyrG*89; *pyroA*4, *nkuA::argB*; AN2545::*pyrG A. fumigatus*	this study
ΔAN2621	*pyrG*89; *pyroA*4, *nkuA::argB*; AN2621::*pyrG A. fumigatus*	this study
ΔAN8412	*pyrG*89; *pyroA*4, *nkuA::argB*; AN8412::*pyrG A. fumigatus*	this study
ΔAN9244	*pyrG*89; *pyroA*4, *nkuA::argB*; AN9244::*pyrG A. fumigatus*	this study
ΔAN2542	*pyrG*89; *pyroA*4, *nkuA::argB*; AN2542::*pyrG A. fumigatus*	this study
ΔAN2543	*pyrG*89; *pyroA*4, *nkuA::argB*; AN2543::*pyrG A. fumigatus*	this study
ΔAN2544	*pyrG*89; *pyroA*4, *nkuA::argB*; AN2544::*pyrG A. fumigatus*	this study
ΔAN2546	*pyrG*89; *pyroA*4, *nkuA::argB*; AN2546::*pyrG A. fumigatus*	this study
ΔAN2547	*pyrG*89; *pyroA*4, *nkuA::argB*; AN2547::*pyrG A. fumigatus*	this study
ΔAN2548	*pyrG*89; *pyroA*4, *nkuA::argB*; AN2548::*pyrG A. fumigatus*	this study
ΔAN2549	*pyrG*89; *pyroA*4, *nkuA::argB*; AN2549::*pyrG A. fumigatus*	this study
ΔAN2550	*pyrG*89; *pyroA*4, *nkuA::argB*; AN2550::*pyrG A. fumigatus*	this study
ΔAN10325	*pyrG*89; *pyroA*4, *nkuA::argB*; AN10325::*pyrG A. fumigatus*	this study

Table S2. Primers Used in This Study

primer	Sequence (5′→3′)
AN0607.3P1	GGT GTC GTA TAG GGA TCG G
AN0607.3P2	CAG TGA AGT GGA ACG AGT CG
AN0607.3P3	CGA AGA GGG TGA AGA GCA TTG CGA TGC CAA ACA CAA GGC C
AN0607.3P4	GCA TCA GTG CCT CCT CTC AGA CAG TGC ATA CAT CGC ATT GAA TGG
AN0607.3P5	GCA GTC ACC CAG CAT GGG
AN0607.3P6	CGC ATG CGA GAG ATA AGA CC
AN1242.3P1	CCT TGC GTT GGC GAC TGG
AN1242.3P2	GCG ACT GGC CTA TCC TGG
AN1242.3P3	CGA AGA GGG TGA AGA GCA TTG CGT CAA TTA GGT ATT AGT AAC C
AN1242.3P4	GCA TCA GTG CCT CCT CTC AGA CAG TCG TTG TTC CAT TTG TTT GGC
AN1242.3P5	CCT AGT CTA TAA AGC TCG CC
AN1242.3P6	CGA CGA GTC TTA TCC AAA CAC
AN2545.3P1	GTC ATT ATT GCA TCA GGG G
AN2545.3P2	GAT CGG TTT CCA TCA GTC C
AN2545.3P3	CGA AGA GGG TGA AGA GCA TTG GAG GCC GCT ATC AAA GGC G
AN2545.3P4	GCA TCA GTG CCT CCT CTC AGA CAG CAA ACC CCT GTA GAA GCA GG
AN2545.3P5	GCT GTT GGC AAA AGG TAA AGG
AN2545.3P6	CCG TTT ATT CCA GAG TCA CC
AN2621.3P1	GCG GCG TCG ATT TGC TGG
AN2621.3P2	GCT ACT CTC ATT TTG GCT GC
AN2621.3P3	CGA AGA GGG TGA AGA GCA TTG GGA GGG CAA AAG CCT GAG G
AN2621.3P4	GCA TCA GTG CCT CCT CTC AGA CAG AAC GTA TGC ATA ATG AGG
AN2621.3P5	CAG CTT GAC ACC ACT ATA TGC
AN2621.3P6	GTG ACC AAA ACG GTT ATT TGC
AN8412.3P1	GAC ACG GTC AAC ATG CAG G
AN8412.3P2	GGT ATC TGT GAA TGT CTC CC
AN8412.3P3	CGA AGA GGG TGA AGA GCA TTG GCA AGT ATC AAG TAA AAC TGG
AN8412.3P4	GCA TCA GTG CCT CCT CTC AGA CAG GTA TGT CTA ACT CAG CAT CCC
AN8412.3P5	GGA TAT ATA CGA CGT TCC ATC
AN8412.3P6	GGG AGT AGC TTT GTT TGA GC
AN9244.3P1	GTT GCG GAT GAA GGA AAC C
AN9244.3P2	GCT CCA CCA GCT TTA GCC G
AN9244.3P3	CGA AGA GGG TGA AGA GCA TTG CGA TGC TAA CGT ACG GAC C
AN9244.3P4	GCA TCA GTG CCT CCT CTC AGA CAG GCT TGA TAG AGA TCA TTG
AN9244.3P5	CCT GCA TTC CCC AGA ATG C
AN9244.3P6	CCT GGT AGT TTG GCA CGC
AN2542.3P1	TGG ACC GGA TCT GTA CTA TGG
AN2542.3P2	CGG AGT TTT ACA GAG GAC AAG C
AN2542.3P3	CGA AGA GGG TGA AGA GCA TTG AAG AGT GTG TGT GCG AAA GG
AN2542.3P4	GCA TCA GTG CCT CCT CTC AGA CAG ACA GCT GCA GCC AAT AAA CC
AN2542.3P5	CTT TCT CCC GAT TCA GTT GG
AN2542.3P6	GAA GCC GTG GAA CTT CTA ACC
AN2543.3P1	GCG CAA TGA GAC AAC AAA GG
AN2543.3P2	GTA AAC CCG CTG GTA CAT GG
AN2543.3P3	CGA AGA GGG TGA AGA GCA TTG TGA AGA CGG GAT GTT CTT GG
AN2543.3P4	GCA TCA GTG CCT CCT CTC AGA CAG AAC ATG CTA GCC CTG ACA CC
AN2543.3P5	GGC ATC AGA CGA TCA GAA GG
AN2543.3P6	GGA GGA GTA ATG GGA TGA AGG
AN2544.3P1	CGC ACC GTG TTT ACT TGT CC
AN2544.3P2	TCT GTG GCT GAA TTG ACA GG
AN2544.3P3	CGA AGA GGG TGA AGA GCA TTG TGA GGG CTC TGA AGA CAT GG
AN2544.3P4	GCA TCA GTG CCT CCT CTC AGA CAG CTG CTT CTA CAG GGG TTT GG

AN2544.3P5	GGC TTC CCC CAG TAT ATT TCC
AN2544.3P6	AGA TCT ACC AGC GCC AGT CC
AN2546.3P1	ACG GCA GTA AGC AGA AGA GC
AN2546.3P2	ACA ACT CGA CGA AGC TGT CC
AN2546.3P3	CGA AGA GGG TGA AGA GCA TTG GGA TAG CCG ATG CTA GTT CC
AN2546.3P4	GCA TCA GTG CCT CCT CTC AGA CAG CGT CAT TCT GGG GTA AAA CG
AN2546.3P5	CGA CGA GTC CAA TAT TGT CG
AN2546.3P6	GGG ACG CCT AGA TCA TTT GG
AN2547.3P1	ATC ACG TCC TCG CTT AGA GG
AN2547.3P2	GAG GTA CGC TGA GCT TGA CG
AN2547.3P3	CGA AGA GGG TGA AGA GCA TTG TGA CCG TCT GAA TAG CAT GG
AN2547.3P4	GCA TCA GTG CCT CCT CTC AGA CAG GTG CTA CCT CTA ATG TGT CTA CGC
AN2547.3P5	CAC CTA CGC GAG AAA AGT GG
AN2547.3P6	TGG AAA CTA TGA GCG ACA CC
AN2548.3P1	GTT GCG CTT CCT GAT AGT CG
AN2548.3P2	AGC CTC TCA CTG TCT GAC TCG
AN2548.3P3	CGA AGA GGG TGA AGA GCA TTG CCT GAG ATT TGG TCA CAG TCC
AN2548.3P4	GCA TCA GTG CCT CCT CTC AGA CAG GGA AAT GGA CCT GTA TGT GC
AN2548.3P5	CCT TCC AAG TTT CGA GAT CC
AN2548.3P6	AAC TCT CAC AAG CCC ACA CG
AN2549.3P1	CTC AGC AGT GTG GTC TCA GC
AN2549.3P2	AGC AGT CGG TCT CCA CGT CG
AN2549.3P3	CGA AGA GGG TGA AGA GCA TTG GCA AGG CGA GTT GTA GAA GG
AN2549.3P4	GCA TCA GTG CCT CCT CTC AGA CAG TTT TAT GGA GCC AGC GTA GC
AN2549.3P5	CCA ACT GGA CAG ATC CAA CC
AN2549.3P6	CCA CCC AGT TCA CTA AAG TCG
AN2550.3P1	CAG AAT GAC TGA GGG TCA TGG
AN2550.3P2	TTC TGG TAG AGG TCT GCT TCG
AN2550.3P3	CGA AGA GGG TGA AGA GCA TTG CCT AAG CTG CAC AGT GAG AGG
AN2550.3P4	GCA TCA GTG CCT CCT CTC AGA CAG TCG TGG TCT GCT AGT TCT TGG
AN2550.3P5	TTC ACA GCA CAG CGT AAA GC
AN2550.3P6	CTA GGC CGC TAT TTC TCA GG
AN10325.3P1	CGC ATG TCT TTA TGC AGA GC
AN10325.3P2	GGT GGA AGA CGG TTT GAA GA
AN10325.3P3	CGA AGA GGG TGA AGA GCA TTG GGG TTG TGA AGA AGG TTT GC
AN10325.3P4	GCA TCA GTG CCT CCT CTC AGA CAG TCA TGA CGT GAC CAC TCA CC
AN10325.3P5	TCG CCA ATC CTC TCT TTA CC
AN10325.3P6	ATG ATC TTC ACC CCC AGT GC

Blue and red sequences are tails that anneal to the *A. fumigatus pyrG* fragment (AfpyrG) during fusion PCR

Table S3. ^{1}H-NMR Data for Emericellamides A and C – F (400 MHz in DMSO-d_6)[a]

	position	A	C	D	E	F
NRP (L-Ala-1)	2	4.00–4.14[b]	4.03–4.09[b]	4.04–4.11[b]	4.01–4.09[b]	4.04–4.11[b]
	3	1.24 (d, 6.8)	1.22 (d, 6.8)	1.24 (d, 6.8)	1.22 (d, 6.8)	1.24 (d, 6.8)
	2-NH	8.04 (br s)	7.82 (d, 4.4)	8.15 (d, 4.4)	7.85 (br s)	8.16 (d, 4.0)
NRP (L-Ala-2)	5	4.00–4.14[b]	4.03–4.09[b]	4.09–4.17[b]	4.01–4.09[b]	4.09–4.17[b]
	6	1.21 (d, 6.8)	1.23 (d, 6.8)	1.22 (d, 6.8)	1.23 (d, 6.8)	1.22 (d, 6.8)
	5-NH	7.44 (d, 7.2)	7.53 (d, 7.2)	7.42 (d, 7.6)	7.55 (d, 7.2)	7.45 (d, 7.2)
NRP (L-Leu)	8	4.00–4.14[b]	4.01–4.04[b]	4.04–4.11[b]	4.01–4.09[b]	4.04–4.11[b]
	9	1.54–1.62[b]	1.50–1.62[b]	1.51–1.61[b]	1.50–1.62[b]	1.51–1.61[b]
	10	1.54–1.62[b]	1.50–1.62[b]	1.51–1.61[b]	1.50–1.62[b]	1.51–1.61[b]
	11	0.80 (d, 6.8)	0.82 (d, 6.8)	0.82 (d, 6.8)	0.82 (d, 6.8)	0.82 (d, 6.8)
	12	0.89 (d, 6.8)	0.89 (d, 6.8)	0.90 (d, 6.8)	0.89 (d, 6.8)	0.90 (d, 6.8)
	8-NH	8.29 (d, 8.4)	8.20 (d, 7.2)	8.19 (d, 8.4)	8.32 (d, 7.2)	8.31 (d, 7.6)
NRP (L-Val)	14	3.98 (t, 7.6)	3.91 (t, 7.2)	3.93 (t, 7.6)	3.91 (t, 7.2)	3.93 (t, 7.6)
	15	1.90 (m)	1.91 (m)	1.93 (m)	1.93 (m)	1.94 (m)
	16	0.87 (d, 6.8)	0.89 (d, 6.8)	0.90 (d, 6.8)	0.89 (d, 6.8)	0.90 (d, 6.8)
	17	0.88 (d, 6.8)	0.91 (d, 6.8)	0.88 (d, 6.8)	0.90 (d, 6.8)	0.88 (d, 6.8)
	14-NH	8.22 (d, 8.4)	8.11 (d, 7.2)	8.10 (d, 7.6)	8.26 (d, 7.6)	8.25 (d, 7.6)
NRP (Gly)	19	3.62 (dd, 17.6, 2.4); 4.31 (dd, 17.6, 5.2)	3.58 (dd, 17.6, 2.0); 4.38 (dd, 17.6, 6.0)	3.75 (dd, 17.2, 4.0); 4.09–4.17[b]	3.59 (dd, 17.6, 2.4); 4.35 (dd, 17.6, 6.0)	3.76 (dd, 17.2, 4.0); 4.09–4.17[b]
	19-NH	7.45 (br s)	7.61 (dd, 6.0, 2.0)	7.54 (t, 4.0)	7.56 (br, s)	7.51 (br s)
PK	21	2.87 (dq, 9.6, 6.8)	2.73 (dq, 9.2, 6.8)	2.18 (d, 13.6); 2.68 (dd, 13.6, 9.6)	2.72 (dq, 9.2, 6.8)	2.18 (d, 13.6); 2.68 (dd, 13.6, 9.6)
	22	4.93 (br d, 9.6)	4.81 (ddd, 9.2, 8.4, 3.6)	5.04 (dd, 9.6, 3.6)	4.82 (ddd, 9.2, 8.4, 3.6)	5.05 (dd, 9.6, 2.4)
	23	1.67 (m)	1.42 (m); 1.50–1.62[b]	1.54–1.68[b]	1.42 (m); 1.50–1.62[b]	1.54–1.68[b]
	24	1.05–1.16[b]	1.14–1.30[b]	1.02 (m); 1.18–1.34[b]	1.14–1.30[b]	1.02 (m); 1.18–1.34[b]
	25	0.98–1.06[b]	1.14–1.30[b]	1.18–1.34[b]	1.14–1.30[b]	1.18–1.34[b]
	26~28	1.18–1.34[b]	1.14–1.30[b]	1.18–1.34[b]	1.14–1.30[b]	1.18–1.34[b]
	29	0.84 (t, 6.8)	0.85 (t, 6.8)	0.86 (t, 7.6)	1.14–1.30[b]	1.18–1.34[b]
	30	0.90 (d, 6.8)	0.93 (d, 6.8)	0.83 (d, 7.6)	1.14–1.30[b]	1.18–1.34[b]
	31	0.82 (d, 6.8)			0.85 (t, 6.8)	0.86 (t, 7.6)
	32				0.93 (d, 6.8)	0.83 (d, 7.6)

[a] Figures in parentheses are multiplicities and coupling constants (*J*) in Hz.

[b] Data obtained from gHMQC spectrum and/or compared with the spectral data of emericellamide A (**1**) from literature [1].

Table S4. ^{13}C-NMR Data for Emericellamides A and C – F (100 MHz in DMSO-d_6)[a]

	position	A	C	D	E	F
NRP (L-Ala-1)	1	171.4 (s)	171.6 (s)	171.8 (s)	171.4 (s)	171.9 (s)
	2	48.2 (d)	48.2 (d)	48.4 (d)	48.3 (d)	48.4 (d)
	3	16.3 (q)	16.2 (q)	16.1 (q)	16.2 (q)	16.1 (q)
NRP (L-Ala-2)	4	171.5 (s)	171.4 (s)	171.4 (s)	171.6 (s)	171.4 (s)
	5	47.2 (d)	47.8 (d)	47.4 (d)	47.7 (d)	47.3 (d)
	6	18.3 (q)	17.4 (q)	18.1 (q)	17.5 (q)	18.2 (q)
NRP (L-Leu)	7	170.9 (s)	170.9 (s)	170.8 (s)	171.0 (s)	170.9 (s)
	8	51.7 (d)	51.8 (d)	51.6 (d)	51.8 (d)	51.6 (d)
	9	39.4 (t)	38.9 (t)	39.4 (t)	39.0 (t)	39.5 (t)
	10	24.5 (d)	24.5 (d)	24.4 (d)	24.5 (d)	24.4 (d)
	11	20.7 (q)	20.7 (q)	20.7 (q)	20.7 (q)	20.7 (q)
	12	23.2 (q)	23.3 (q)	23.2 (q)	23.2 (q)	23.2 (q)
NRP (L-Val)	13	171.2 (s)	171.2 (s)	171.2 (s)	171.2 (s)	171.3 (s)
	14	60.2 (d)	60.2 (d)	60.2 (d)	60.3 (d)	60.3 (d)
	15	30.1 (d)	29.8 (d)	29.8 (d)	29.8 (d)	29.8 (d)
	16	18.8 (q)	18.7 (q)	18.6 (q)	18.7 (q)	18.7 (q)
	17	19.1 (q)	19.0 (q)	19.1 (q)	19.0 (q)	19.1 (q)
NRP (Gly)	18	168.7 (s)	169.4 (s)	169.0 (s)	169.4 (s)	169.0 (s)
	19	42.5 (t)	42.2 (t)	42.4 (t)	42.2 (t)	42.3 (t)
PK	20	172.9 (s)	172.9 (s)	169.3 (s)	172.8 (s)	169.3 (s)
	21	41.1 (d)	42.7 (d)	38.0 (t)	42.8 (d)	38.1 (t)
	22	76.6 (d)	75.0 (d)	74.4 (d)	75.0 (d)	74.4 (d)
	23	33.2 (d)	28.8 (t)	36.4 (d)	28.8 (t)	36.5 (d)
	24	33.5 (t)	31.1 (t)	32.0 (t)	31.3 (t)	32.0 (t)
	25	26.6 (t)	23.5 (t)	26.3 (t)	23.5 (t)	26.4 (t)
	26	28.9 (t)	28.6 (t)	28.9 (t)	28.9[b] (t)	29.2[b] (t)
	27	31.2 (t)	30.9 (t)	31.2 (t)	28.8[b] (t)	28.7[b] (t)
	28	22.1 (t)	22.1 (t)	22.1 (t)	28.7[b] (t)	28.9[b] (t)
	29	14.0 (q)	14.0 (q)	14.0 (q)	30.9 (t)	31.3 (t)
	30	14.3 (q)	14.4 (q)	14.7 (q)	22.1 (t)	22.1 (t)
	31	12.9 (q)			14.0 (q)	14.0 (q)
	32				14.4 (q)	14.7 (q)

[a] Figures in parentheses are multiplicities assigned by DEPT NMR methods.
[b] Values in the same column may be interchanged.

Supplemental References

1. Oh, D.C., Kauffman, C.A., Jensen, P.R., and Fenical, W. (2007). Induced production of emericellamides A and B from the marine-derived fungus Emericella sp. in competing co-culture. J Nat Prod *70*, 515-520.
2. Nayak, T., Szewczyk, E., Oakley, C.E., Osmani, A., Ukil, L., Murray, S.L., Hynes, M.J., Osmani, S.A., and Oakley, B.R. (2006). A versatile and efficient gene-targeting system for Aspergillus nidulans. Genetics *172*, 1557-1566.

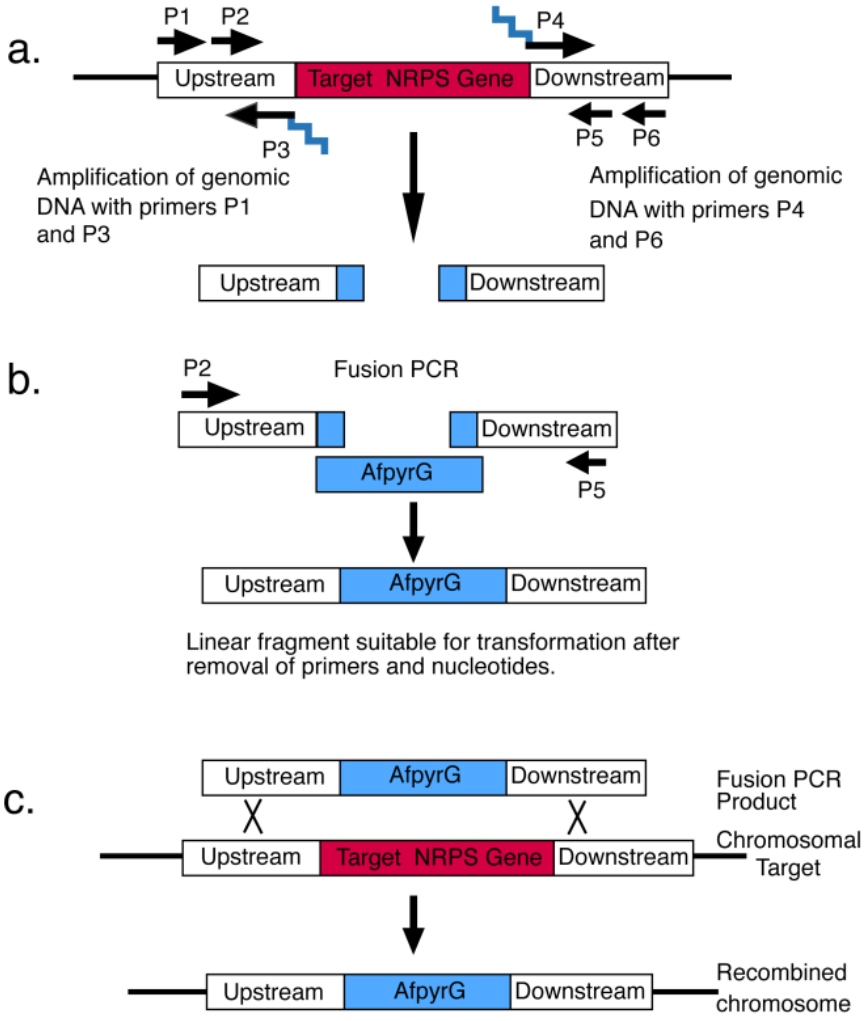


Figure S1. A Schematic Diagram of the Use of Fusion PCR to Target an NRPS Gene in *A. nidulans*

(a) DNA fragments flanking upstream and downstream of the target NRPS genes are amplified with primers P1 and P3 and with P4 and P6. P3 and P4 are designed to have tails that anneal to the *A. fumigatus pyrG* fragment (AfpyrG) during fusion PCR. (b) The two fragments and the AfpyrG cassette are mixed and fused together by PCR using nested primers P2 and P5 creating a linear fragment suitable for transformation. (c) Replacement of the targeted NRPS gene with the AfpyrG gene via homologous recombination.

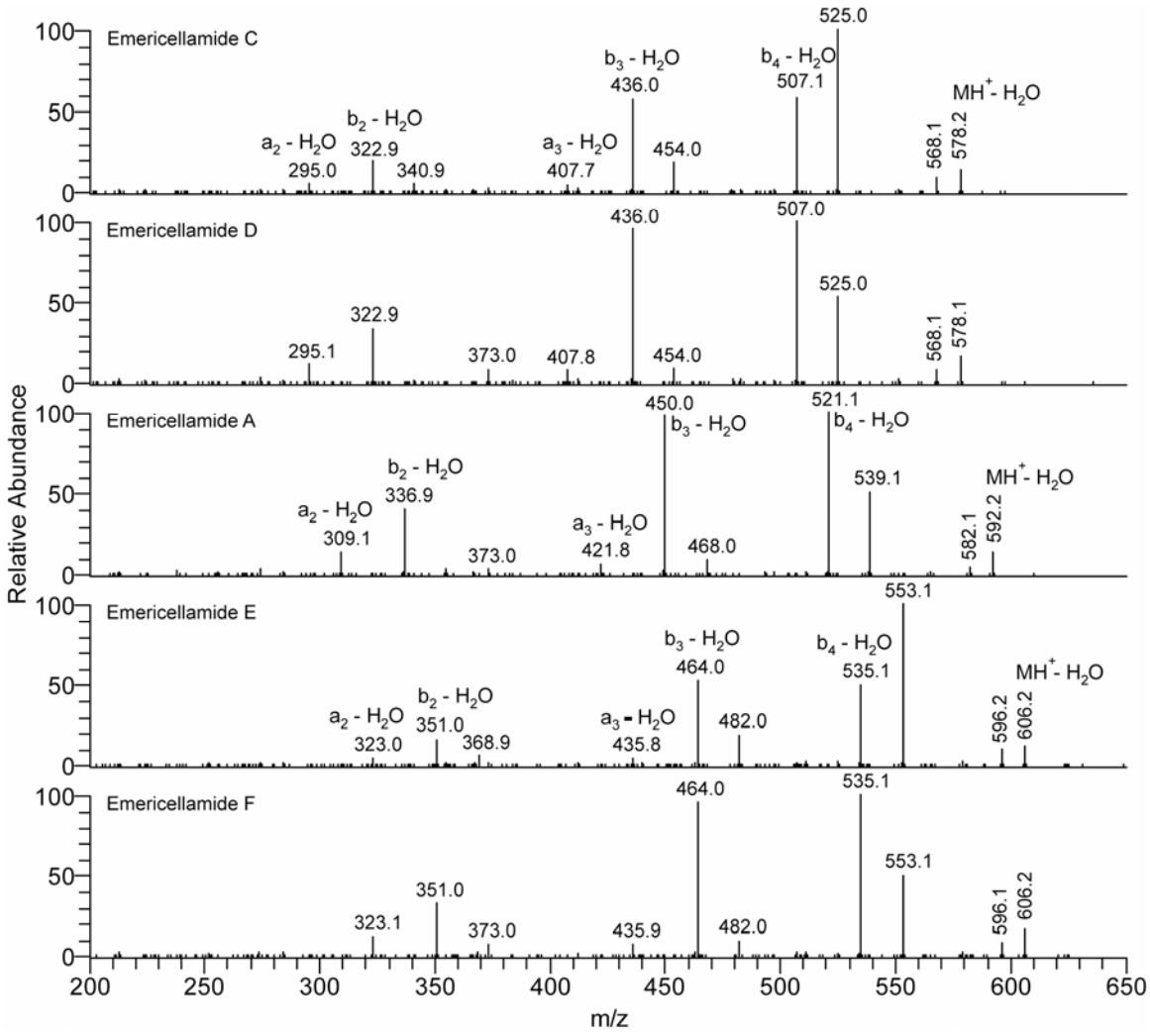
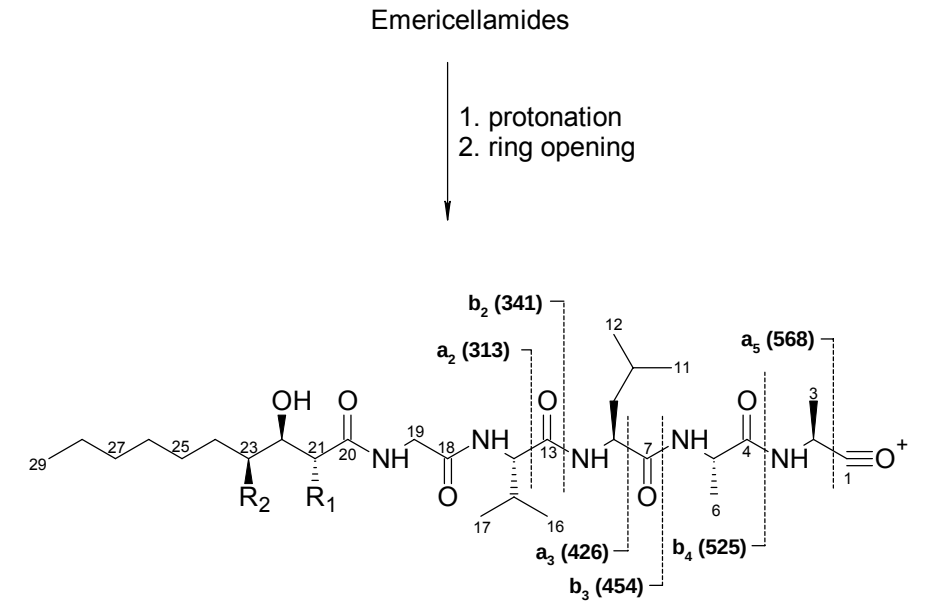
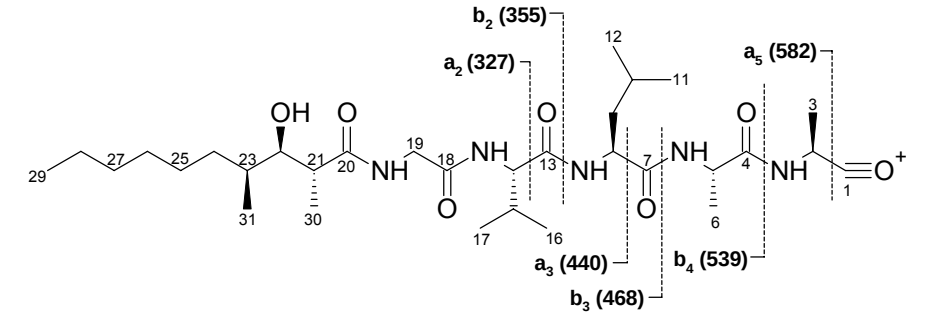


Figure S2. MS/MS Spectra of Emericellamide A (1) , C-F (2-5)

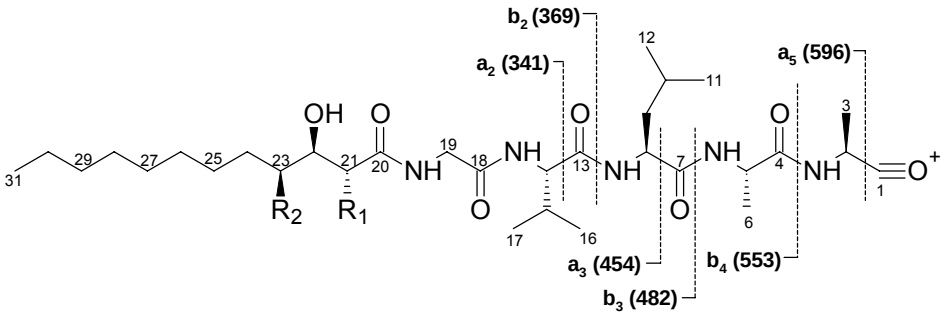


Emericellamide C (**2**): R_1 = Me, R_2 = H

Emericellamide D (**3**): R_1 = H, R_2 = Me



Emericellamide A (**1**)



Emericellamide E (**4**): R_1 = Me, R_2 = H

Emericellamide F (**5**): R_1 = H, R_2 = Me

Figure S3. Assignment of Emercellamide MS/MS Fragmentation

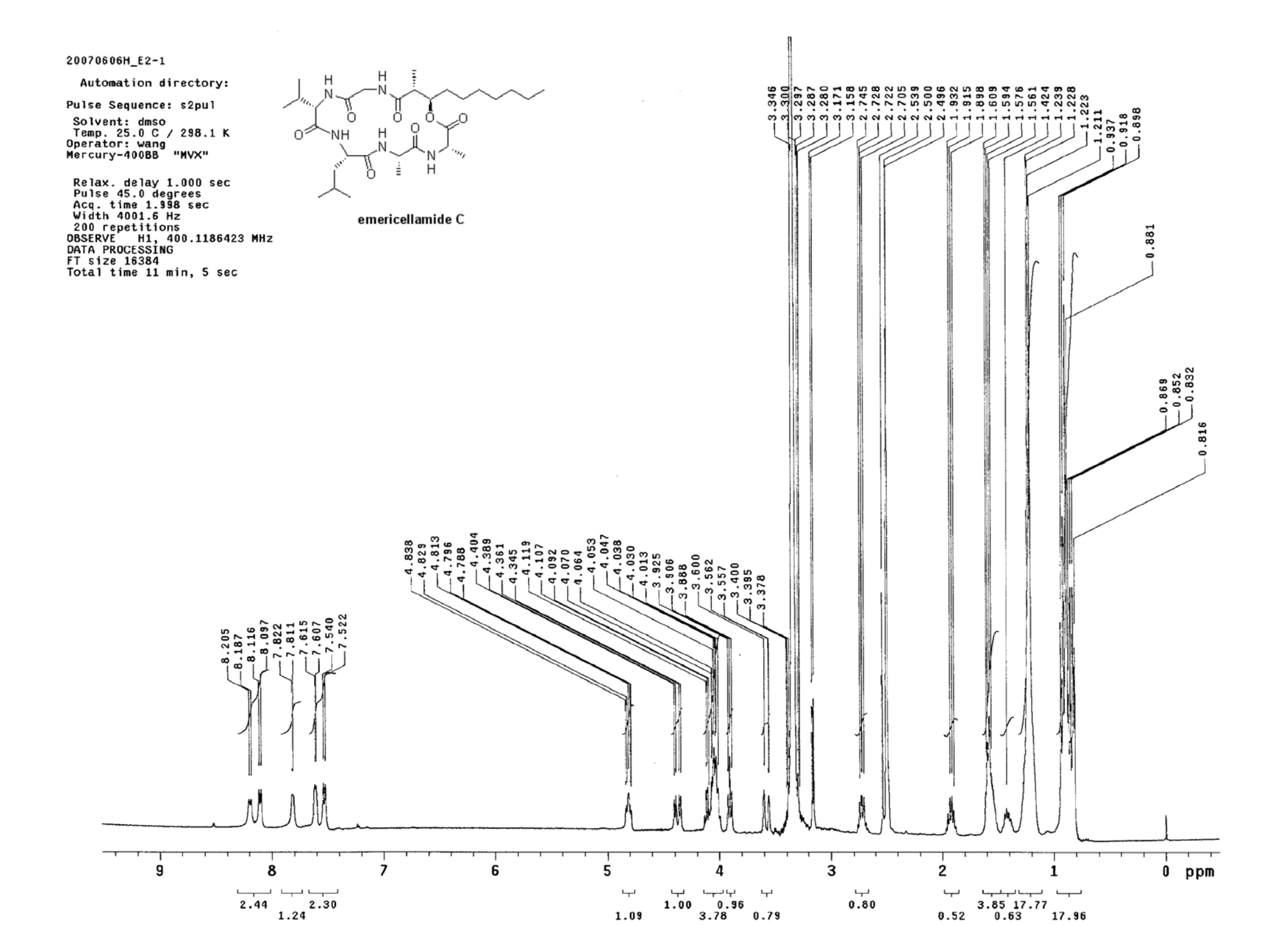


Figure S4. ^{1}H NMR spectrum of emericellamide C (2).

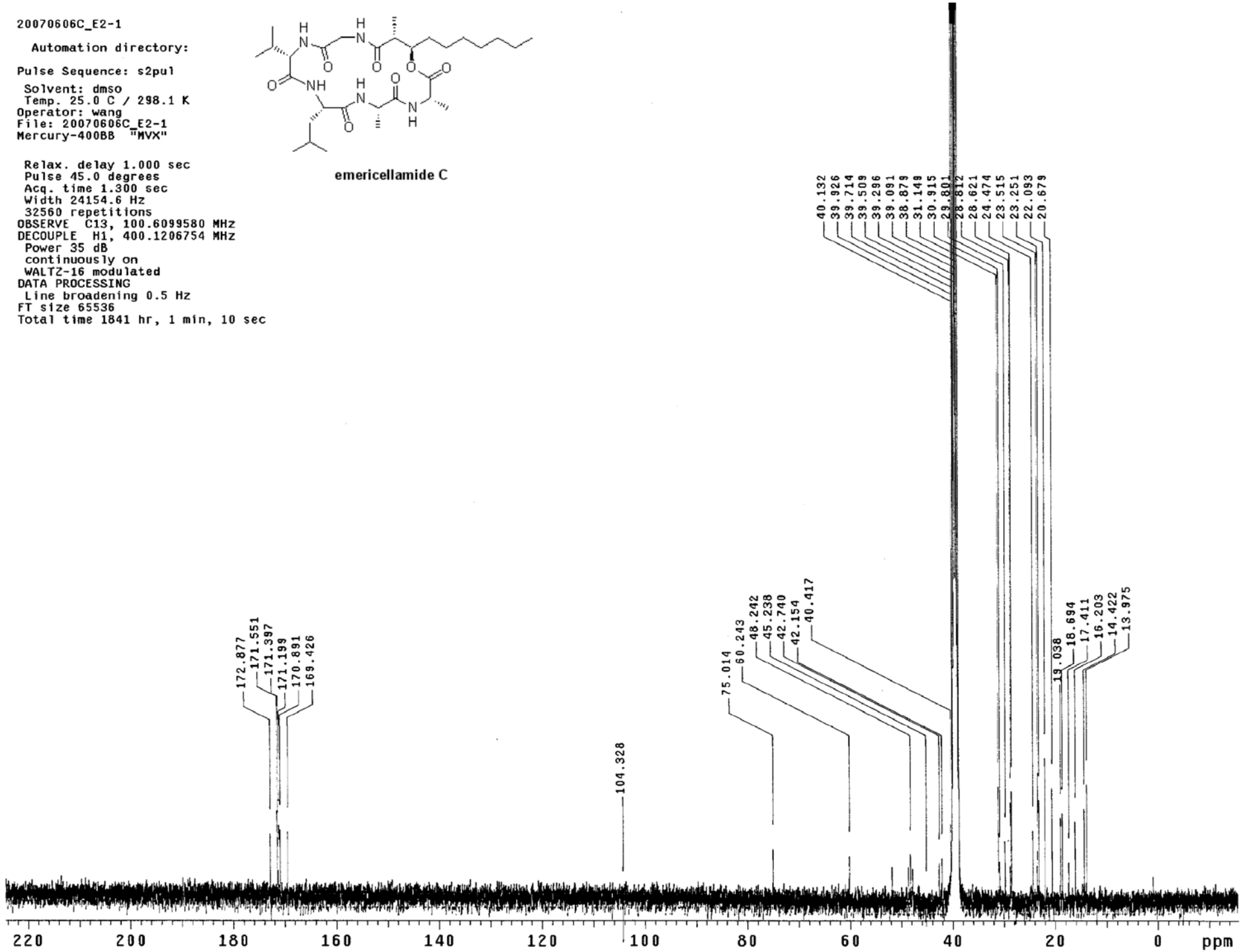


Figure S5. ^{13}C NMR spectrum of emericellamide C (2).

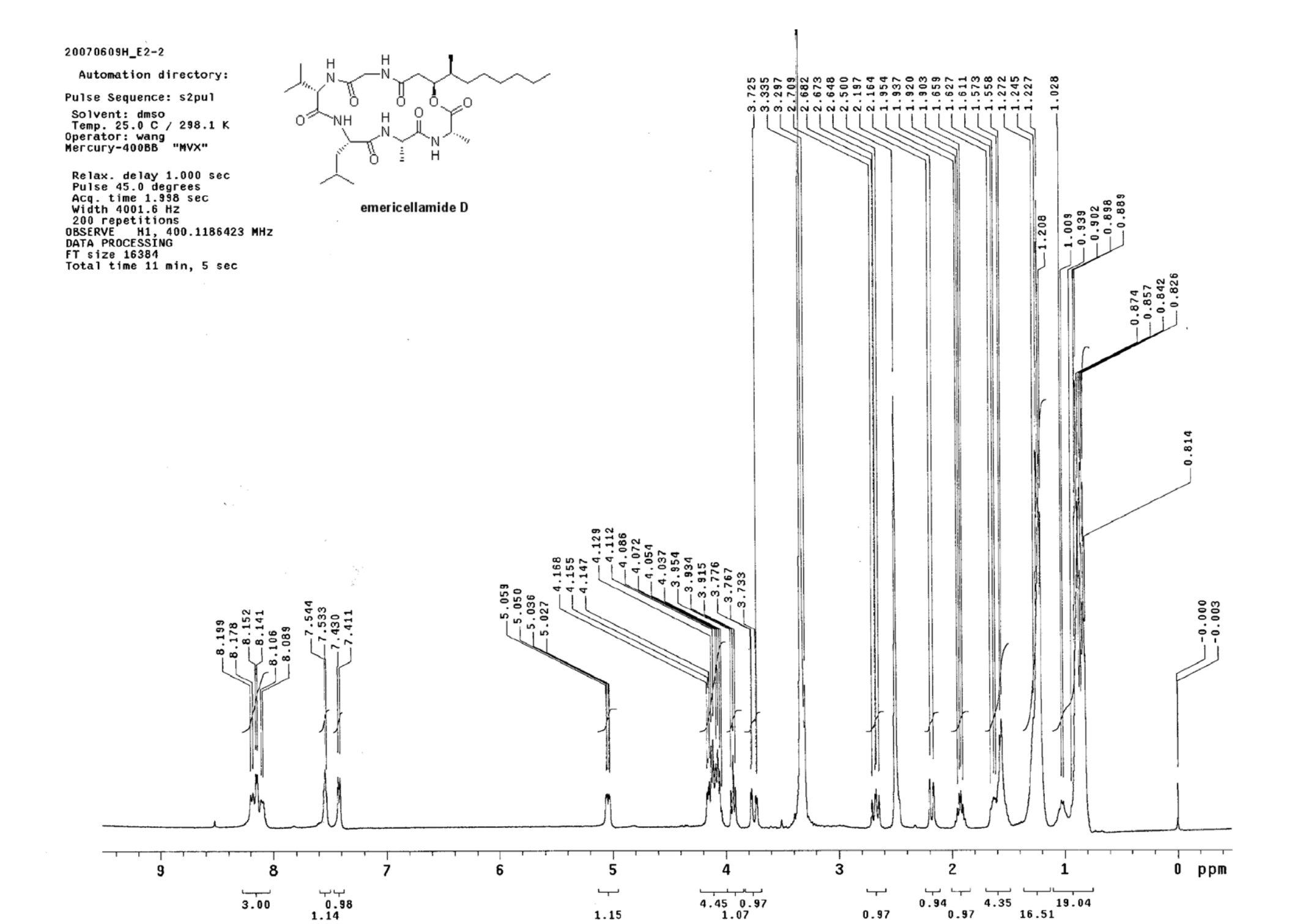


Figure S6. 1H NMR spectrum of emericellamide D (3).

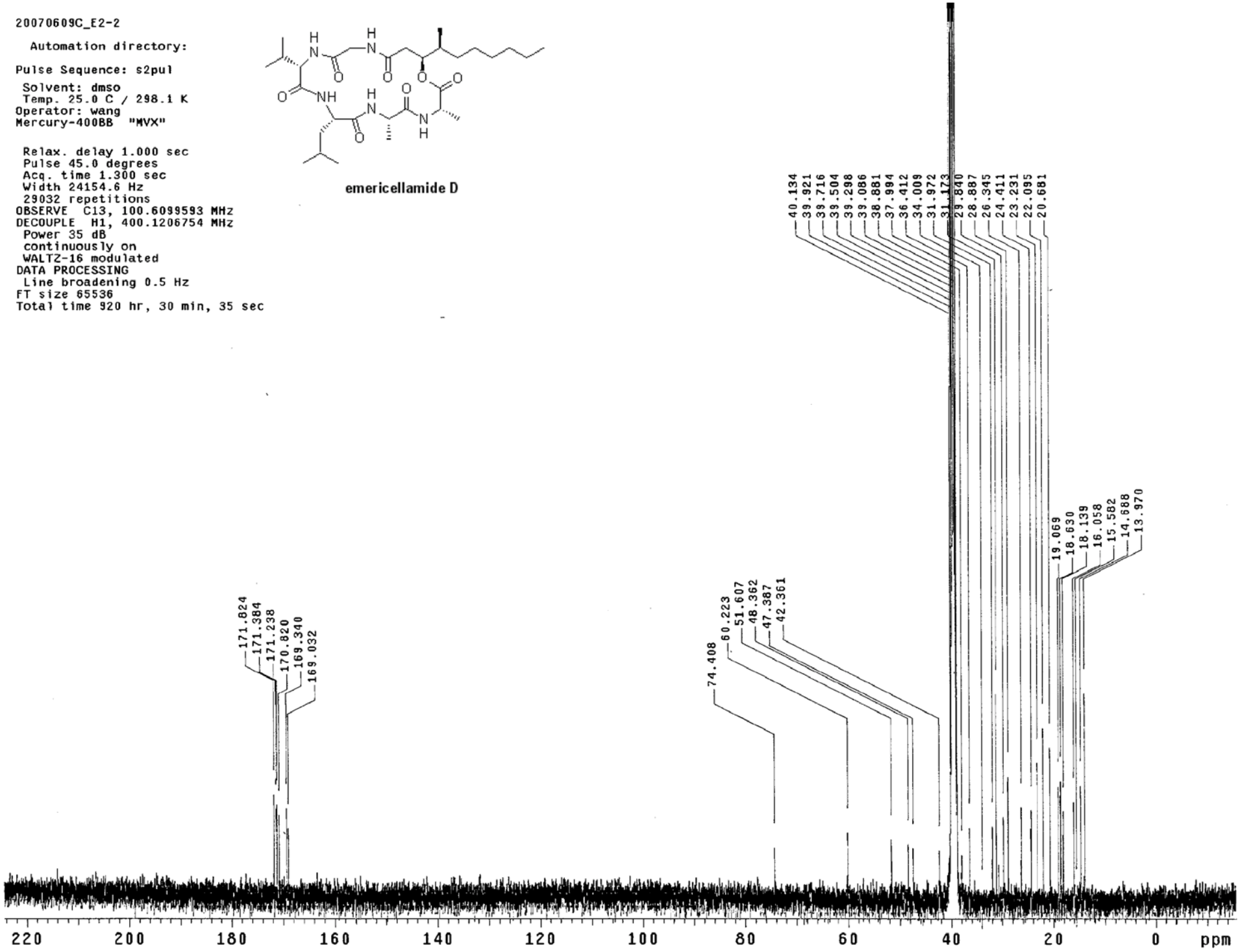


Figure S7. ^{13}C NMR spectrum of emericellamide D (3).

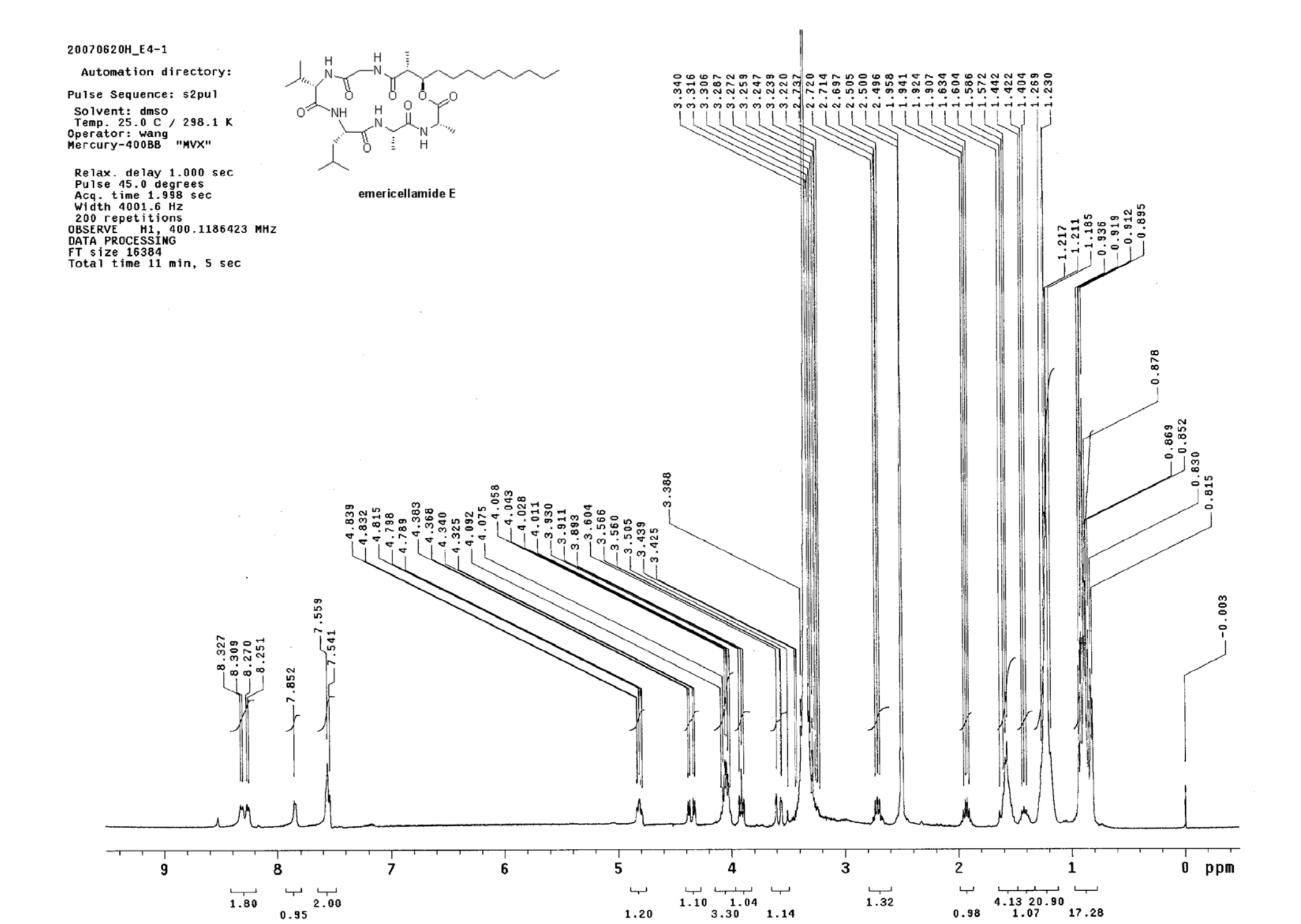


Figure S8. ^{1}H NMR spectrum of emericellamide E (4).

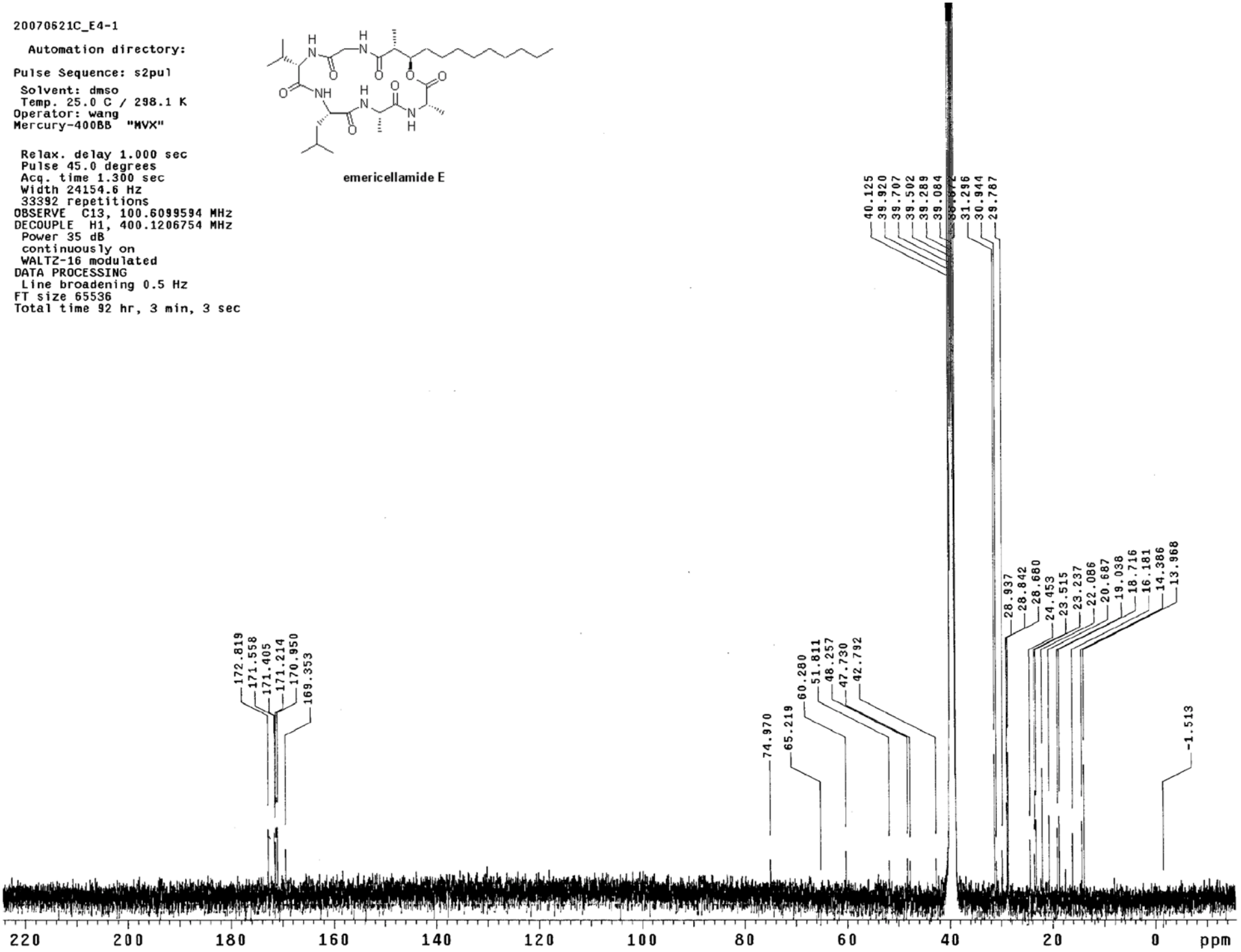


Figure S9. ^{13}C NMR spectrum of emericellamide E (4).

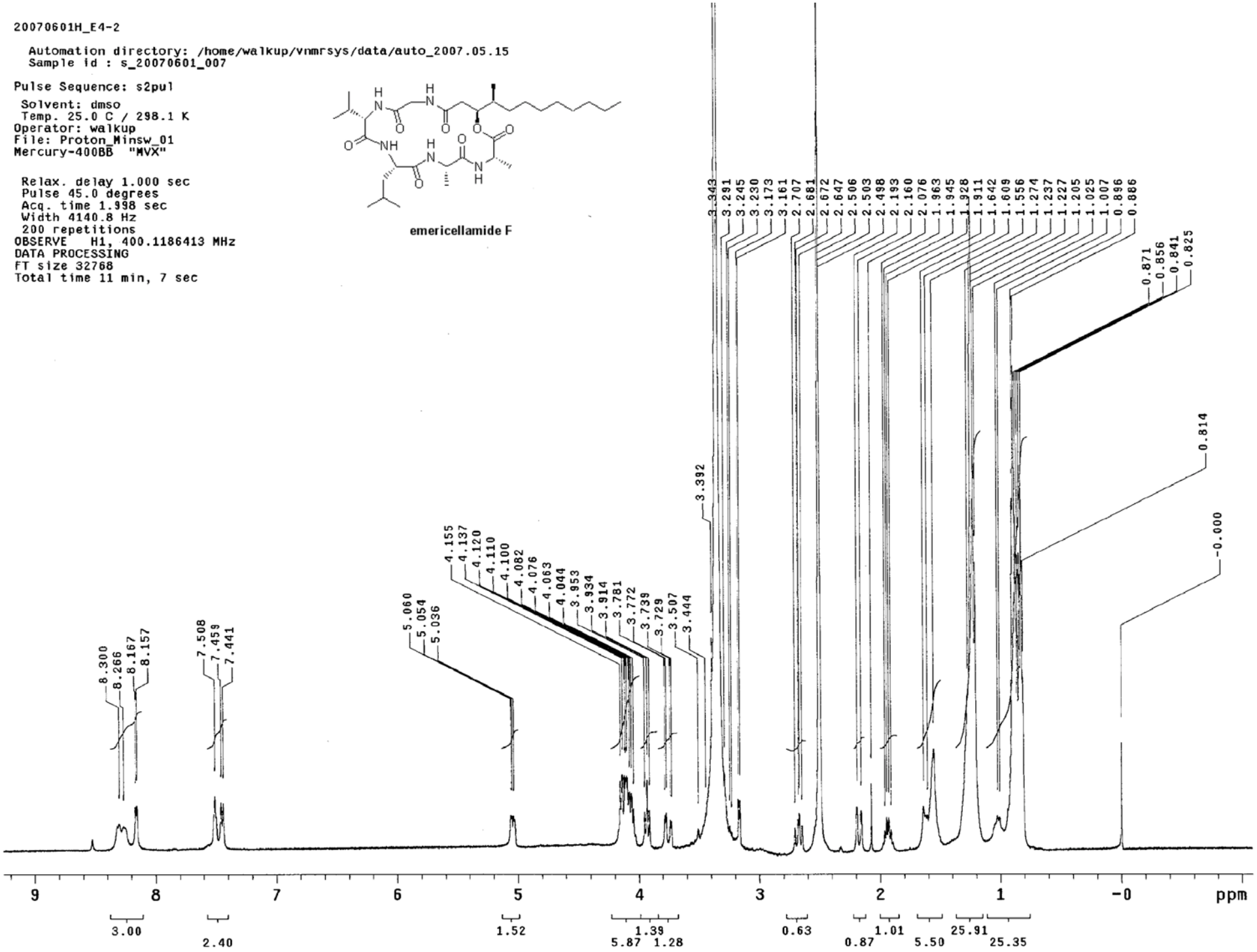


Figure S10. ^{1}H NMR spectrum of emericellamide F (5).

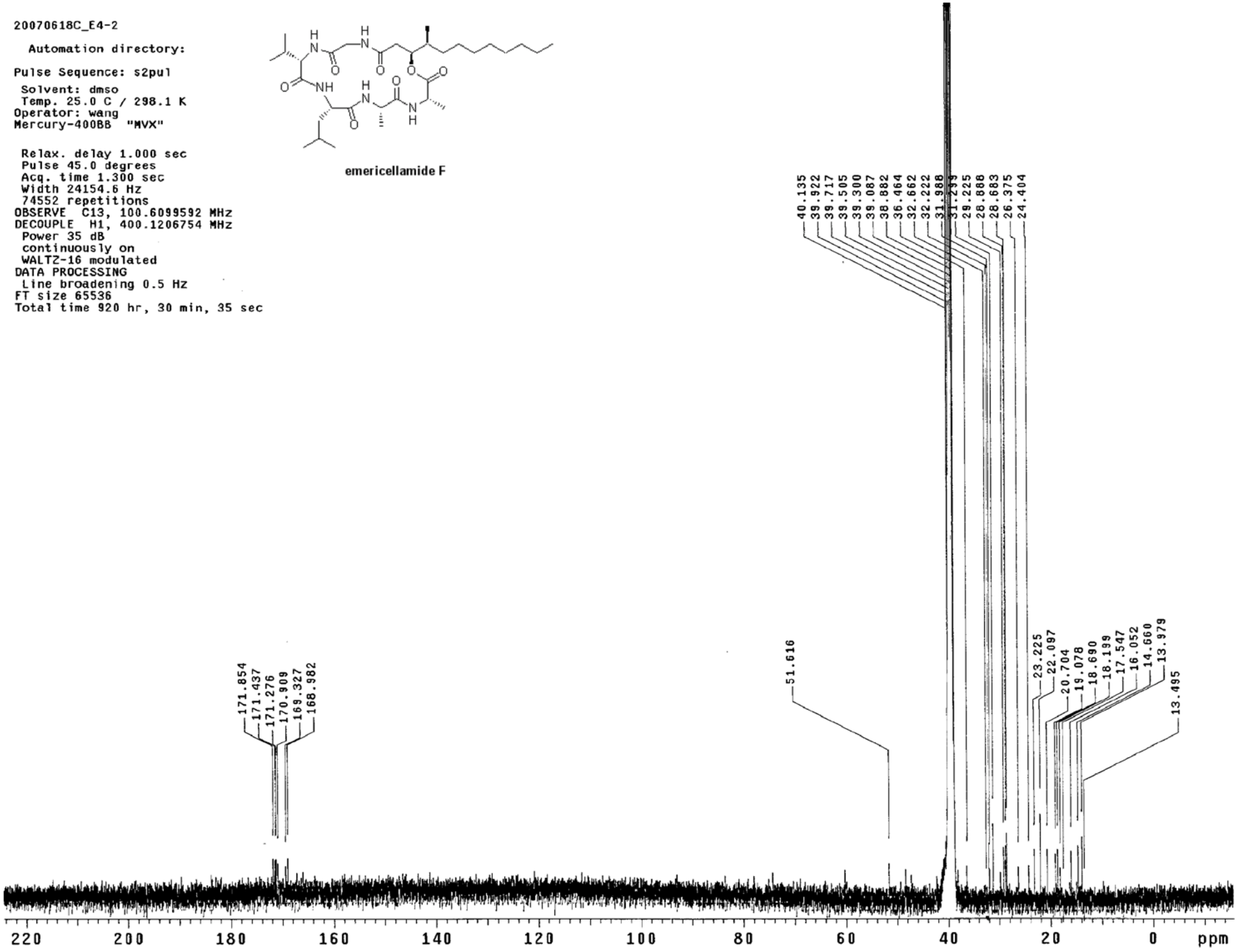


Figure S11. ^{13}C NMR spectrum of emericellamide F (5).