Characterization of the *lnmKLM* Genes Unveiling Key Intermediates for β-Alkylation in Leinamycin Biosynthesis

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Supporting Information

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Experiment procedures

General

E. coli DH5α, *E. coli* S17-1, and *Streptomyces atroolivaceus* S-140 were used in this work.[S1] PBS3005 and pSET151 were described previously.[S2-3] Psp72 was from Promega (Madison, WI). Plasmids preparation was carried out using commercial kits (Qiagen, Valencia, CA). *Streptomyces* chromosomal DNA was isolated according to literature protocols.[S4] Restriction enzymes and other molecular biology reagents were from commercial sources, and standard methods for DNA digestion and ligation were followed.[S5] For Southern analysis, digoxigenin labeling of DNA probes, hybridization, and detection were performed according to the protocols provided by the manufacturer (Boehringer Mannheim Biochemicals, Indianapolis, IN). Automated DNA sequencing was provided by the University of Wisconsin-Madison Biotechnology Center (Madison, WI).

^{1}H and ^{13}C NMR spectra were recorded at 25 °C on Varian Unity Inova 400 or 500 instruments operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C nuclei or 500 MHz for ^{1}H and 125 MHz for ^{13}C nuclei respectively. ^{1}H and ^{13}C NMR chemical shifts were referenced to residual solvent signals: δ_H 7.27 and δ_C 77.23 for $CDCl_3$, and δ_H 2.50 and δ_C 39.51 for d_6-DMSO. ^{1}H-^{1}H COSY, ^{1}H-^{1}H TOCSY (mixing time = 80 ms), HMQC ($^{1}J_{CH}$ = 140 Hz), and gHMBC ($^{2\text{-}3}J_{XH}$ = 8.0 Hz) were performed using standard VARIAN pulse sequences. Electrospray ionization-mass spectrometry (ESI-MS) and LC-MS were carried out on an Agilent 1100 HPLC-MSD SL quadrupole mass spectrometer equipped with both orthogonal pneumatically assisted electrospray and atmospheric pressure chemical ionization sources. High-resolution MS analyses were acquired on an IonSpec HiResMALDI FT-Mass spectrometer with a 7 tesla superconducting magnet. A saturated solution of 2,5-dihydroxybenzoic acid in methanol was used for matrix preparation, and the spectra were peak-matched using *m*/*z* 273.03936 ($[2M - 2H_2O + H]^+$) as a reference peak. Optical rotations were measured in MeOH on a Perkin-Elmer 241 instrument at the sodium D line (589 nm).

HPLC was carried out on a Varian system equipped with Prostar 210 pumps and a photodiode array (PDA) detector. The mobile phase used comprised of buffer A (5% CH_3CN in H_2O containing 0.1% CH_3COOH) and buffer B (80% CH_3CN in H_2O containing 0.1% CH_3COOH). Analytical HPLC and LC-MS was conducted using an Alltima C-18 column (5 μm, 250 x 4.6 mm, Alltech, Deerfield, IL), eluted with a linear gradient of 100% buffer A and 0% buffer B to 20% buffer A and 80% buffer B over 45 min, followed by 5 min at 20% buffer A and 80% buffer B at a flow rate of 1.0 mL/min with UV detection at 320 nm. Semi-preparative HPLC was conducted using a C-18 column (5 μm , 250 x 10 mm, Microsob, Varian) eluted with a linear gradient of 40% buffer A and 60% buffer B to 100% buffer B over 20 min at a flow rate of 3.0 mL/min with UV detection at 320 nm.

Silica gel 60A (200-425 mesh, Fisher Chemical) was used for flash column chromatography. Amberlite XAD-16, and Diaion HP-20 resins were purchased from Sigma (St. Louis, MO). Crude fermentation extracts, fractions obtained during isolation, compounds finally purified, and reaction mixtures were all monitored by either analytical HPLC or TLC on Merck silica gel-60F plates using $CHCl_3$/MeOH (9:1) as developing solvents and iodide as visualizing agent.

Gene inactivation of lnmK, lnmL and lnmM

To inactivate *lnmK*, a 0.6 kb SalI-BamHI fragment and a 1.1-kb HindIII-KpnI fragment were

both PCR amplified from pBS3005 using the primers TG68 (5'- TAT AGT CGA CAC GGT CAT TCC GCA G –3') and TG69 (5'- ATG GAT CCT CGT TGC TGC CCG GC –3'), or TG-X (5'- GCT AAG CTT GCC GTC CGG GGA GTC-3' and TG-Y (5'-CTC GGT ACC CGG CCG ACG GGG CC-3'), inserted to the SalI-BamHI or HindIII-KpnI sites of pSP72 to generate pBS3071 or pBS3072, and then confirmed by sequencing respectively. A 0.54 kb PstI-NotI fragment from pBS3071, an 1.5 kb NotI-BamHI fragment containing *aac(3)IV* and a 0.6 kb BglII-HindIII fragment from pBS3072 were ligated to the PstI-HindIII sites of pSET151 to generate pBS3073.

To inactivate *lnmL*, a 1.06 kb SalI-BamHI fragment and a 0.7 kb XbaI-EcoRI fragment were both PCR amplified from pBS3005 using the primers TG68 (5'- TAT AGT CGA CAC GGT CAT TCC GCA G –3') and TG70 (5'- ATG GAT CCA CAC CGG GCA GGA TC –3'), or TG71 (5'- TAT CTA GAC CGG GTC GAG ATC ATC G –3') and TG72 (5'- TAG AAT TCG TAG CTG TAG TTG CCG AAC –3'), inserted to the SalI-BamHI or XbaI-EcoRI sites of pSP72 to generate pBS3074 or pBS3075, and then confirmed by sequencing respectively. The 1.06 kb XhoI-BamHI fragment, a 1.5 kb BglII-XbaI fragment containing *aac(3)IV* and the 0.7 kb XbaI-EcoRI fragment were ligated to the EcoRI-BamHI sites of pSTE151 to generate pBS3076.

To inactivate *lnmM*, a 0.8 kb EcoRI-HindIII fragment and a 1.1 kb BglII-HindIII fragment were both PCR amplified from pBS3005 using the primers TG49 (5- ATG AAT TCT TCT CCA TGG TCG ACA AG –3') and TG50 (5'- ATA AGC TTC ATC ATG AGG TTG TCG AGG –3'), or TG4 (5'- ATA AGC TTT CCA CAA GTA CCT GGG CCT –3') and TG5 (5'- ATA GAT CTT GGA AGA AGG AGC TTC CGC CGC C –3'), inserted to the EcoRI-HindIII or BglII-HindIII sites of pSP72 to generate pBS3077 or pBS3078, and then confirmed by sequencing respectively. The 0.8 kb EcoRI-HindIII fragment from pBS3077, a 1.5 kb HindIII-NcoI fragment containing *aac(3)IV* and a 0.9 kb NcoI-BglII DNA fragment from pBS3078 were ligated to the EcoRI-BamHI sites of pSTE151 to generate pBS3079.

pBS3073, pBS3076 and pBS3079 were introduced into the *S. atroolivaceus* wild-type strain by conjugation, and the double crossover mutants SB3029 (Δ*lnmK*), SB3030 (Δ*lnmL*), SB3031 (Δ*lnmM*) were obtained through an established protocol, and then confirmed by Southern analysis.[S2]

Fermentation, isolation and structure elucidation of ***2-7***

LNM fermentation, production and isolation conditions from the *S. atroolivaceus* wild-type, as well as the initial fermentation of SB3029 (Δ*lnmK*), SB3030 (Δ*lnmL*), SB3031 (Δ*lnmM*) mutants, were taken from the literature with modifications.[S2] The fermentation was carried in two stages: the seed medium (25 mL in a 250-mL baffled flask), consisting of 10 g of glucose, 10 g of soluble starch, 3 g of beef extract, 5 g of yeast extract, 5 g of Bacto-Tryptone, and 2 g of $CaCO_3$ per liter, pH 7.2, was inoculated with 10 μL of spore suspension and incubated at 28°C, 250 rpm, for 48 h. The resultant seed inoculum (5 mL) was then used to inoculate the production medium (50 mL in a 250-mL baffled flask), consisting of 20 g of soluble starch, 10 g of corn steep solid, 0.5 g of KH_2PO4, 0.25 g of $MgSO_4$, 40 mg of $ZnSO_4 \cdot 7H_2O$, 0.1 g of methionine, 0.1 mg of vitamin B_{12}, 5 g of $CaCO_3$ and 20 g Dianion HP-20 resins (Supelco, Bellefonte, Pa.) per liter, pH 7.0, and continue fermentation for 96 h. Then the HP-20 resins were harvested by filtration through two layers of cheesecloth and were then lyophilized to dryness. Crude LNM was eluted off the HP-20 resins with methanol (10 resin volumes), and the methanol elute was concentrated in vacuum.

Since no obvious new peaks with similar UV spectrum with LNM were observed under the

conditions used above for the SB3029 (Δ*lnmK*), SB3030 (Δ*lnmL*), SB3031 (Δ*lnmM*) mutants, the fermentation condition of the three mutants was modified with no HP-20 resin added in the production medium. Instead, the supernatant from the fermentation culture was obtained by centrifugation and extracted by ethyl acetate, the crude extract was dried under vacuum, and a small faction was used for the HPLC analysis. Alternatively the supernatant was applied to a small HP-20 or XAD-16 column, and the column was eluted by ethanol. The two methods yield the similar HPLC profile.

For large scale production of compounds **2-7**, the seed medium (50 mL in 12 x 250-mL baffled flasks) was inoculated with 20 uL SB3029 (Δ*lnmK*) spore suspension and grew for 48 hr, then all the 12 x 50 mL seed culture were used to inoculate 12 x 500 mL production medium in 2-L flasks. After 96 hours fermentation, the supernatant was obtained by centrifugation and applied to a XAD-16 column, and the column was eluted by ethanol. The eluates were dried and underwent silica gel flash chromatography (3.5 x 40 cm), eluted with a stepped gradient from $CHCl_3$ to $CHCl_3$-MeOH (9:1), yielding 10 fractions and analyzed by HPLC. Fractions containing **2** and **5**, **3** and **6, 4** and **7** were combined, respectively, and further purified by repeated silica gel flash chromatography. The fractions were further resolved by semi-preparative HPLC to afford **3** (8.3 mg), **4** (1.5 mg), and mixtures of **2** and **5** (1.3 mg), **3** and **6** (2.5 mg), **4** and **7** (1.1 mg).

LNM K-1 **(2**): ESI-MS (positive) for an $[M + H]^+$ ion at *m/z* 265, an $[M + Na]^+$ ion at *m/z* 287, and ESI-MS (negative) for an $[M - H]^-$ ion at *m/z* 263.

LNM K-1/LNM K-1′ (**2/5**): 1.3 mg, yellow oil; $[\alpha]\ ^{25}_D$ +3.0 (*c* 0.118, MeOH); HR MALDI MS for an $[M + H]^+$ ion at *m/z* 265.1001 (calculated $[M + H]^+$ ion for $C_{13}H_{16}N_2O_2S$ at *m/z* 265.1005); ^1H NMR and ^{13}C NMR, see Table S1.

LNM K-2 (**3**): 8.3 mg, yellow oil; $[\alpha]^{25}_D$ +2.0 (*c* 0.12, MeOH); ESI-MS (positive) for an $[M + H]^+$ ion at *m/z* 391, an $[M + Na]^+$ ion at *m/z* 413, and ESI-MS (negative) for an $[M - H]^-$ ion at *m/z* 389; HR MALDI MS for an $[M + Na]^+$ ion at *m/z* 413.1480 (calculated $[M + Na]^+$ ion for $C_{20}H_{26}N_2O_4S$ at *m/z* 413.1505; and ^1H NMR and ^{13}C NMR, see Table S1.

LNM K-3 (**4**): 1.5 mg, yellow oil; $[\alpha]^{25}_D$ +1.0 (*c* 0.17, MeOH); ESI-MS (positive) for an $[M + H]^+$ ion at *m/z* 393, an $[M + Na]^+$ ion at *m/z* 415; ESI-MS (negative) for an $[M + Cl]^-$ ion at *m/z* 427; HR MALDI MS for $[M + Na]^+$ ion at *m/z* 415.1653 (calculated $[M + Na]^+$ ion for $C_{20}H_{28}N_2O_4S$ at *m/z* 415.1667); and ^1H NMR and ^{13}C NMR, see Table S1.

LNM K-1′ (**5**): ESI-MS (positive) for an $[M + H]^+$ ion at *m/z* 265, an $[M + Na]^+$ ion at *m/z* 287, and ESI-MS (negative) for an $[M - H]^-$ ion at *m/z* 263.

LNM K-2′ (**6**): ESI MS (positive) for an $[M + H]^+$ ion at *m/z* 391, an $[M + Na]^+$ ion at *m/z* 413, and ESI-MS (negative) for an $[M - H]^-$ ion at *m/z* 389.

LNM K-3′ (**7**): ESI MS (positive) for an $[M + H]^+$ ion at *m/z* 393, an $[M + Na]^+$ ion at *m/z* 415, and ESI MS (negative) for an $[M + Cl]^-$ ion at *m/z* 427.

*Feeding experiment of [1-^{13}C]sodium acetate to the SB3029 (ΔlnmK) mutant and isolation of ^{13}C labeled compounds **2-4***

The culture of the SB3029 (*ΔlnmK*) mutant and isolation of the ^{13}C-enriched metabolites were carried as described above with the following modifications: (1) 10 x 50 mL seed medium was inoculated with 20 μL SB3029 spore suspension, and the seed culture were used to inoculate 10

x 500 mL production medium; (ii) 250 mg [1-^{13}C]sodium acetate was added to the SB3029 culture after 24 hrs fermentation in the production medium. The isolation of compounds **2-4** was similarly carried out as above by column chromatography on silica gel and semi-preparative HPLC.

References:

S1. Hara, M.; Asano, K.; Kawamoto, I.; Takiguchi, T.; Katsumata, S.; Takahashi, K.; Nakano, H. *J. Antibiot.* **1989**, *42*, 1768-1774.

S2. Cheng, Y.-Q., Tang, G.-L., and Shen, B. *J. Bacteriol*. **2002**, *184*, 7013-7024.

S3. Bierman, M.; Logan, R.; O'Brien, K.; Seno, E.T.; Rao, R.N.; Schoner, B.E. *Gene* **1992**, *116*, 43-49.

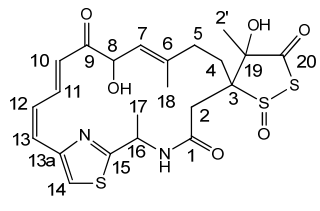
S4. Kieser, T.; Bibb, M.J.; Buttner, M.J.; Chater, K.F.; Hopwood, D.A. Practical *Streptomyces* genetics. **2000**, The John Innes Foundation, Norwich, England.

S5. Sambrook, J.; Russell, D.W. Molecular Cloning: A Laboratory Manual, pp. 1–170, **2001**, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Table S1 Bacterial strains and plasmids used in the study

	Description	Reference
S. atroolivaceus		
S-140	LNM producing wild-type	S2
SB3029	*ΔlnmK* mutant resulted from double crossover homologous recombination of pBS3073 into the S-140 strain	This work
SB3030	*ΔlnmL* mutant resulted from double crossover homologous recombination of pBS3076 into the S-140 strain	This work
SB3031	*ΔlnmM* mutant resulted from double crossover homologous recombination of pBS3079 into the S-140 strain	This work
Plasmids		
pBS3005	A cosmid clone harboring a part of the *lnm* gene cluster	S2
pBS3071	A 0.6-kb *Sal*I-*BamH*I PCR fragment from pBS3005 into the same sites of pSP72	This work
pBS3072	A 1.1-kb *Hind*III-*BamH*I PCR fragment from pBS3005 into the same sites of pSP72	This work
pBS3073	A 0.54-kb *Pst*I-*Not*I fragment from pBS3071, a 1.5-kb *Not*I-*BamH*I fragment containing *aac(3)IV* and a 0.6-kb *Bgl*II-*Hind*III fragment from pBS3072 into the *Pst*I-*Hind*III sites of pSET151	This work
pBS3074	A 1.06-kb *Sal*I-*BamH*I PCR fragment from pBS3005 into the same sites of pSP72	This work
pBS3075	A 0.7-kb *Xba*I-*EcoR*I PCR fragment from pBS3005 into the same sites of pSP72	This work
pBS3076	A 1.06-kb *Xho*I-*BamH*I fragment from pBS3075, a 1.5-kb *Bgl*II-*Xba*I fragment containing *aac(3)IV* and the 0.7-kb *Xba*I-*EcoR*I fragment from pBS3076 into the *EcoR*I-*BamH*I sites of pSTE151	This work
pBS3077	A 0.8-kb *EcoR*I-*Hind*III PCR fragment from pBS3005 into the same sites of pSP72	This work
pBS3078	A 0.7-kb *Xba*I-*EcoR*I PCR fragment from pBS3005 into the same sites of pSP72	This work
pBS3079	A 0.8-kb *EcoR*I-*Hind*III fragment from pBS3077, a 1.5-kb *Hind*III-*Nco*I fragment containing *aac(3) IV* and a 0.9-kb *Nco*I-*Bgl*II DNA fragment from pTG3078 into the *EcoR*I-*BamH*I sites of pSTE151	This work

Table S2 ^{1}H and ^{13}C NMR data of **1-5**



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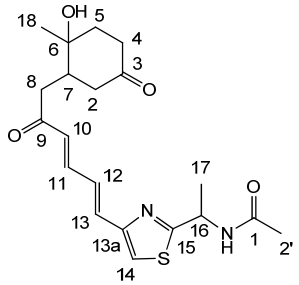

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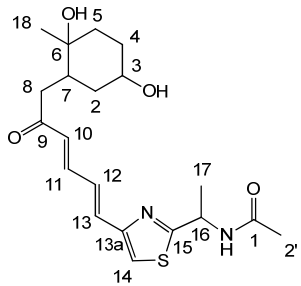

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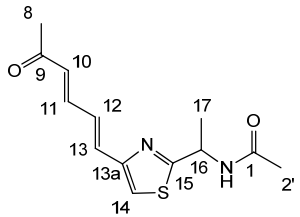

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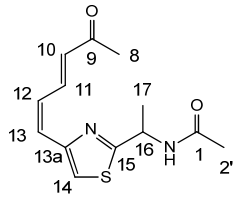
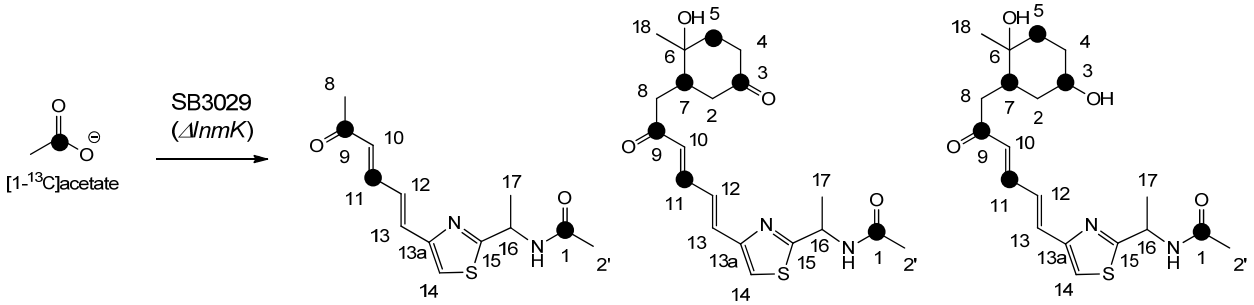


5

	1		**3**		**4**		**2**		**5**
No.	δ_H mult. (*J*, Hz)	δ_C	δ_H mult. (*J*, Hz)	δ_C	δ_H mult. (*J*, Hz)	δ_C	δ_H mult. (*J*, Hz)	δ_C	δ_H mult. (*J*, Hz)
1		169.7		169.1		169.6		169.7	
2	3.37, 1H, d (16.0)	43.8	2.10, 1H, dd (14.0, 5.5)	32.9	1.64, 1H, m				
	2.73, 1H, d (16.0)				0.80, 1H, m				
3		210.3		68.6	3.40, 1H, overlap				
4	1.86, 1H, dt (12.7, 4.7)	38.4	2.58, 1H, (overlap)	38.6	1.66, 1H, m				
	1.50, 1H, (overlap)		2.30, 1H, (overlap)		1.18, 1H, m				
5	2.36, 1H, dt (12.7, 4.7)	38.8	1.96, 2H, m	40.1	1.56, 1H, m				
	1.96, 1H, dt (12.7, 3.7)				1.40, 1H, m				
6		71.2		70.1					
7	5.47, 1H, d (9.3)	43.0	2.55, 1H, (overlap)	41.8	1.90, 1H, (overlap)				
8	5.03, 1H, dd (9.3, 3.7)	42.4	2.88, 1H, dd (16.5, 4.5)	40.7	2.88, 1H, dd (16.5, 4.5)	27.8	2.33, 3H, s	27.5	2.36, 3H, s
			2.44, 1H, dd (16.5, 8.0)		2.28, 1H, dd (16.5, 8.0)				
9		199.1		200.3		198.6		199.2	
10	6.19, 1H, d (16.4)	130.2	6.89, 1H, d (15.5)	130.8	6.37, 1H, d (15.5)	132.9	6.33, 1H, d (15.0)	132.4	6.59, 1H, d (14.0)
11	8.03, 1H, dd (16.4, 11.2)	143.0	7.32, 1H, dd (15.5, 11.5)	141.9	7.36, 1H, dd (15.5, 9.5)	142.9	7.27, 1H, overlap	141.4	8.83, 1H, dd (14.0, 11.0)
12	6.40, 1H, t (11.2)	129.3	7.16, 1H, dd (15.0, 11.5)	128.7	7.12, 1H, overlap	129.6	7.18, 1H, dd (15.0, 11.0)	128.7	6.24, 1H, dd (14.0, 11.0)
13	6.71, 1H, d (11.2)	133.7	6.33, 1H, d (15.0)	133.4	7.11, 1H, overlap	131.5	6.88, 1H, d (15.0)	128.0	7.19, 1H, d (11.0)
13a		152.9		152.4		153.0		152.7	
14	7.21, 1H, s	119.1	7.21, 1H, s	119.9	7.69, 1H, s	118.7	7.21, 1H, s	121.2	7.25, 1H, s
15		173.0		175.4		172.9		172.8	
16	5.48, 1H, m	47.6	5.39, 1H, m	47.0	5.14, 1H, m	47.6	5.41, 1H, m	47.5	5.47, 1H, m
17	1.74, 3H, d (6.8)	22.0	1.62, 3H, d (6.5)	20.5	1.48, 3H, d (7.0)	22.2	1.64, 3H, d (7.0)	21.8	1.69, 3H, d (7.0)
18	1.64, 3H, br s	22.9	1.35, 3H, s	20.5	1.01, 3H, s				
2′	1.58, 3H, s	23.5	2.07, 3H, s	22.5	1.89, 3H, s	23.6	2.07, 3H, s	23.5	2.11, 3H, s

Chemical shits are reported in ppm. All signals were determined by ^{1}H-^{1}H COSY, HMBC, HMQC correlations. ^{1}H NMR, 500 MHz; ^{13}C NMR, 125 MHz.

Table S3 ^{13}C Chemical shifts and ^{13}C-incorporation of [1-^{13}C]acetate into **2-4**



2 **3** **4**

	3		**4**		**2**	
No	δ_c	Enrichment*	δ_c	Enrichment*	δ_c	Enrichment*
1	169.7	**1.9**	169.1	**2.2**	169.6	**3.3**
2	43.8	1.1	32.9	0.8		
3	210.3	**2.8**	68.6	**2.7**		
4	38.4	1.3	38.6	1		
5	38.8	**2.9**	40.1	**3.0**		
6	71.2	0.9	70.1	0.5		
7	43.0	**2.9**	41.8	**3.3**		
8	42.4	1.2	40.7	1.1	27.8	1.1
9	199.1	**1.5**	200.3	**4.3**	198.6	**4.1**
10	130.2	1.3	130.8	1.1	132.9	1
11	143.0	**3.4**	141.9	**4.3**	142.9	**2.5**
12	129.3	1.2	128.7	1.1	129.6	0.9
13	133.7	1.3	133.4	1	131.5	0.6
13a	152.9	0.8	152.4	1	153.0	1.2
14	119.1	0.8	119.9	1.1	118.7	1.1
15	173.0	1.2	175.4	1.4	172.9	1.5
16	47.6	1.2	47.0	1	47.6	0.9
17	22.0	1.1	20.5	1	22.2	0.9
18	22.9	1.2	20.5	1.1		
2’	23.5	1.1	22.5	0.9	23.6	1.5

*Relative enrichments were normalized to peak intensities of the C-13a, -14, -16, -17 signals, and these four carbons were selected to represent four types of carbonds from the two amino acids, L-Cys and D-Ala, derived moiety that were not enriched by [1-^{13}C]acetate.

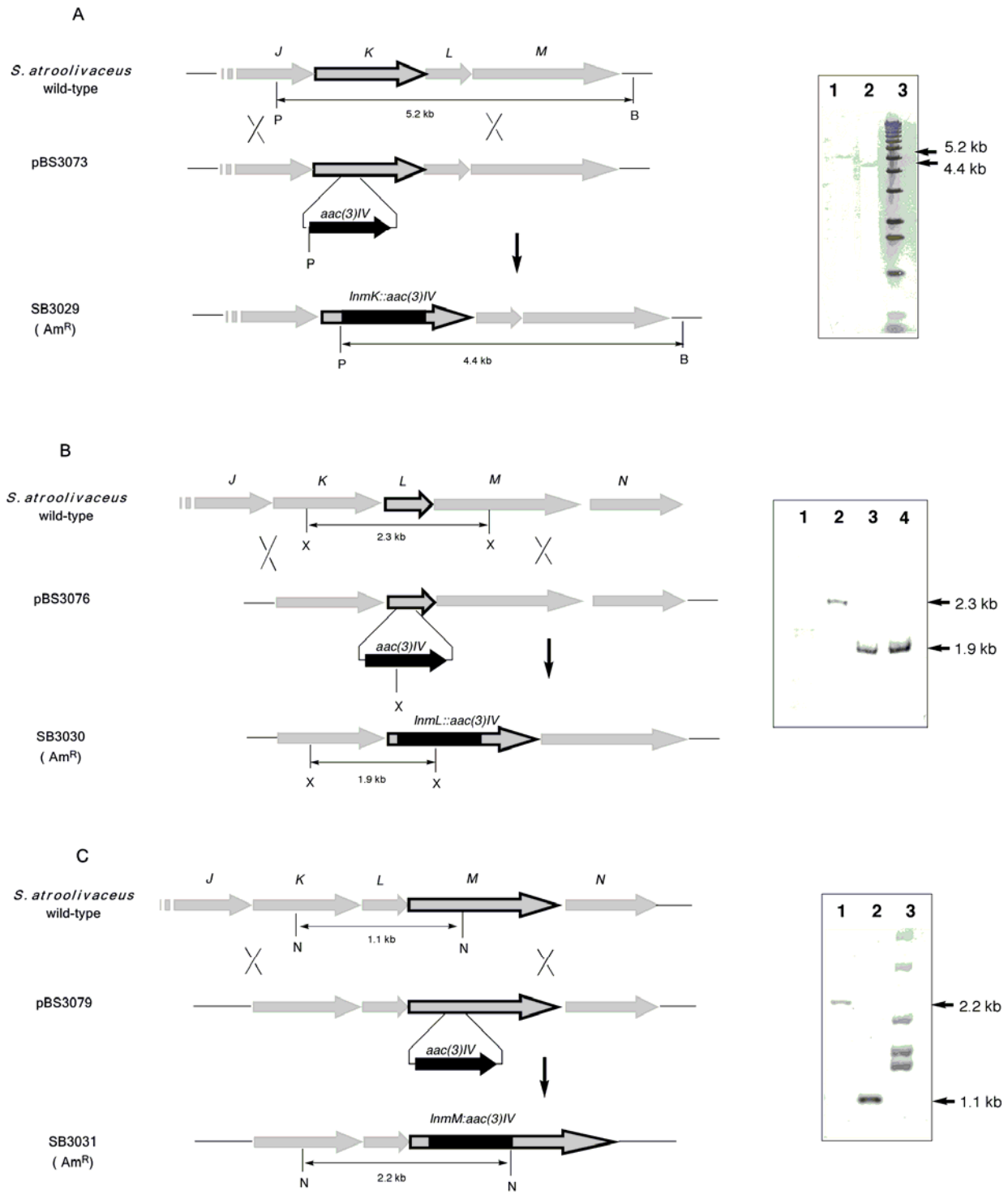


Figure S1 Gene inactivation of *lnmK*, *lnmL* and *lnmM* and Southern analysis. (A) The *lnmK* gene replacement construct pBS3073 and restriction maps of the *S. atroolivaceus* wild-type and the *ΔlnmK*

mutant strain SB3029 showing predicted fragment sizes upon PstI and BamHI digestion. Wild-type (*lane* 1) and Δ*lnmK* (*lane* 2) genomic DNAs digested with PstI and BamHI with the 1.0-kb XhoI-AatII fragment of the *lnmK* right flanking gene as a probe. *Lane* 3, molecular weight marker. A, AatII; B, BamHI; P, PstI. X, XhoI. (B) The *lnmL* gene replacement construct pBS3076 and restriction maps of the *S. atroolivaceus* wild-type and the *ΔlnmL* mutant strain SB3030 showing predicted fragment sizes upon XhoI digestion. Wild-type (*lane* 2) and Δ*lnmL* (*lanes* 3 and 4) genomic DNA digested with XhoI with the 1.2-kb HindIII-BamHI fragment containing the *lnmL* right flanking gene as a probe; *lane* 1, molecular weight marker. X, XhoI. (C) The *lnmM* gene replacement construct pBS3079 and restriction maps of the *S. atroolivaceus* wild-type and the *ΔlnmM* mutant strain SB3031 showing predicted fragment sizes upon NcoI digestion. Δ*LnmM* (*lane* 1) and wild-type (*lane* 2) genomic DNA digested with NcoI with the 0.8-kb EcoRI-HindIII fragment containing the partial *lnmM* gene as a probe. *Lane* 3, molecular weight. N, NcoI.

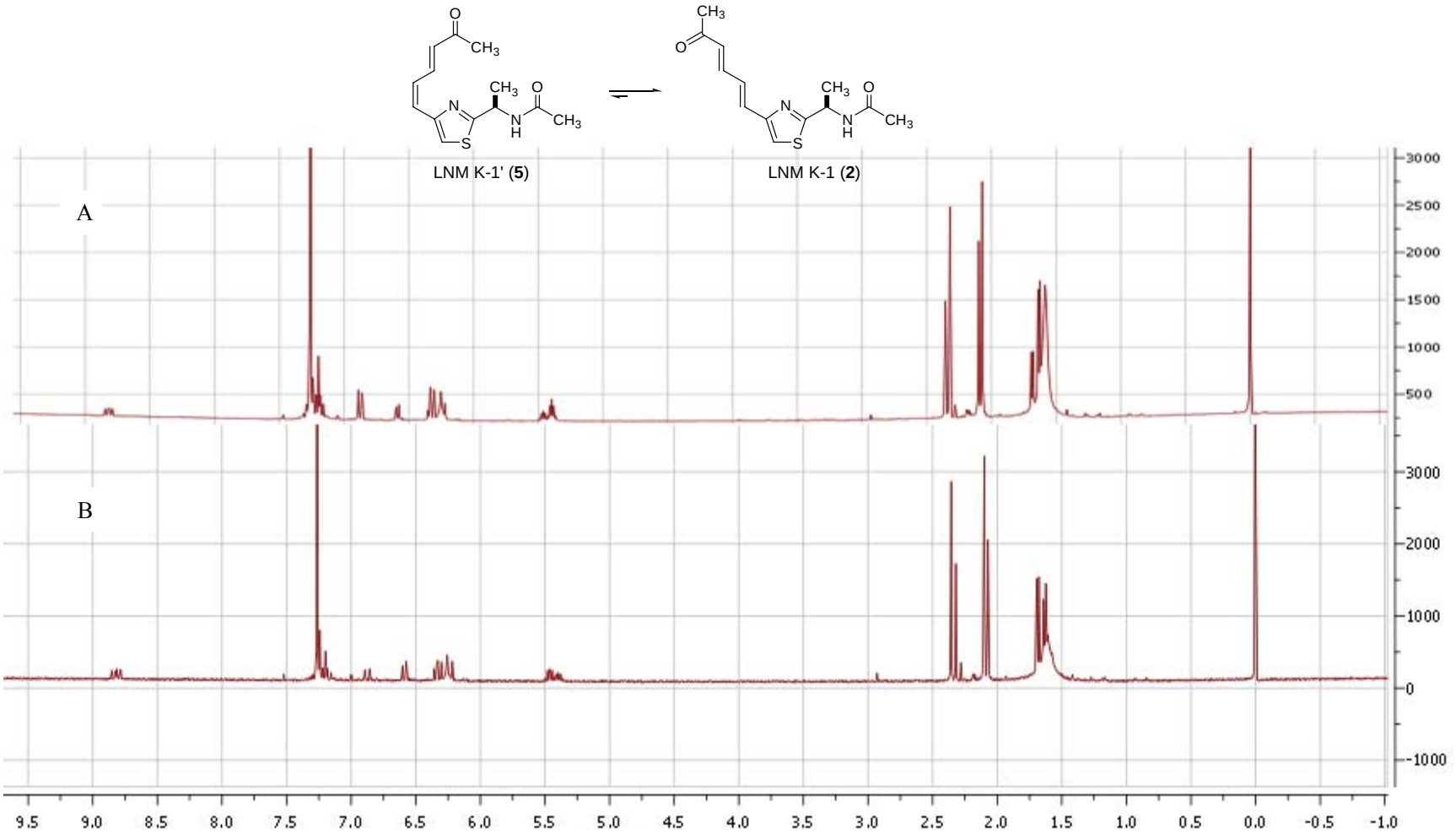


Figure S2 Isomerization of **5** to **2** under UV light. ^{1}H NMR spectra of the mixture of **2** and **5** (A) after exposure under UV (254nm) for 30 min and the mixture of **2** and **5** (B) before exposure.

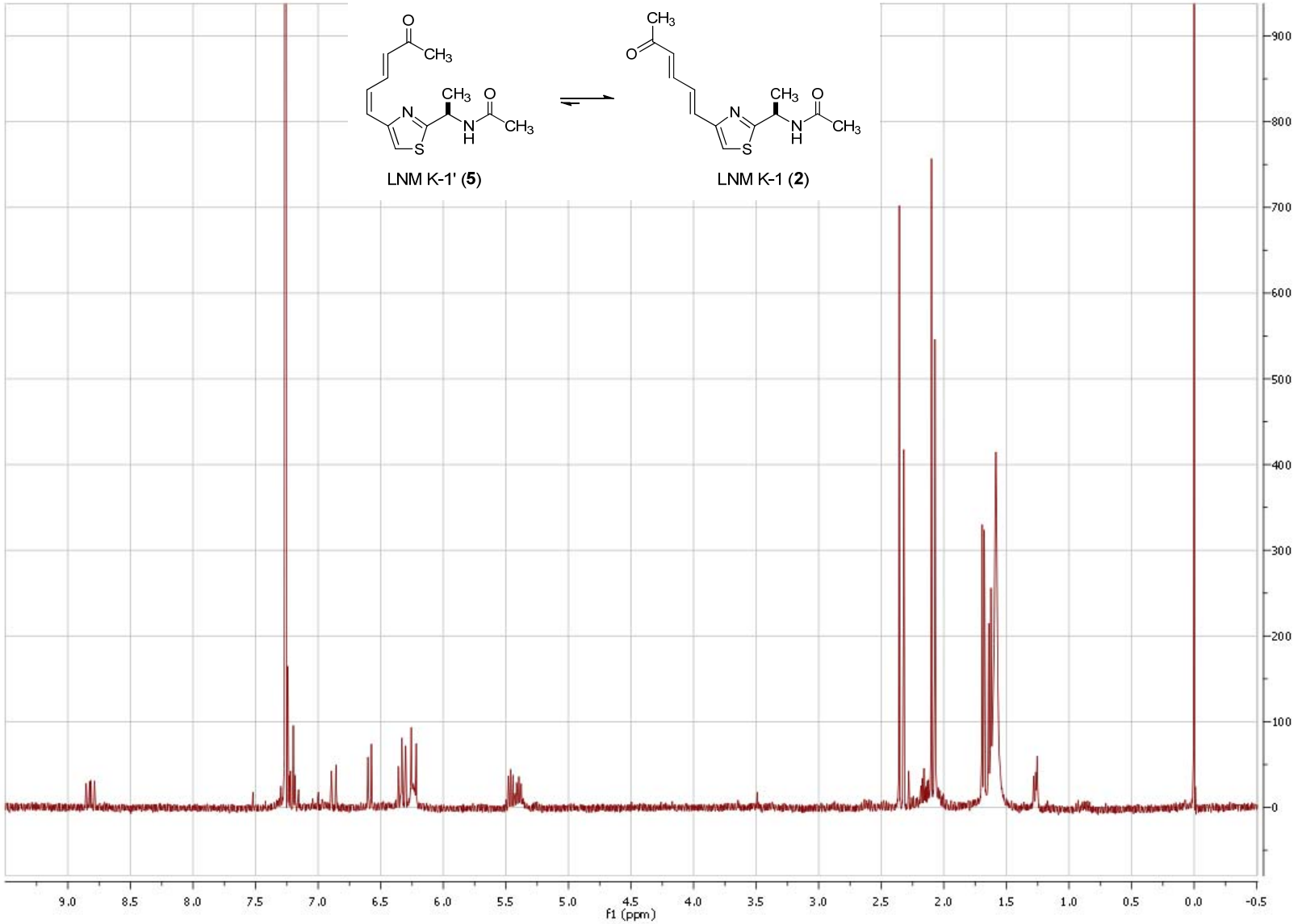


Figure S3 ^{1}H NMR spectrum of a mixture of **2** and **5**.

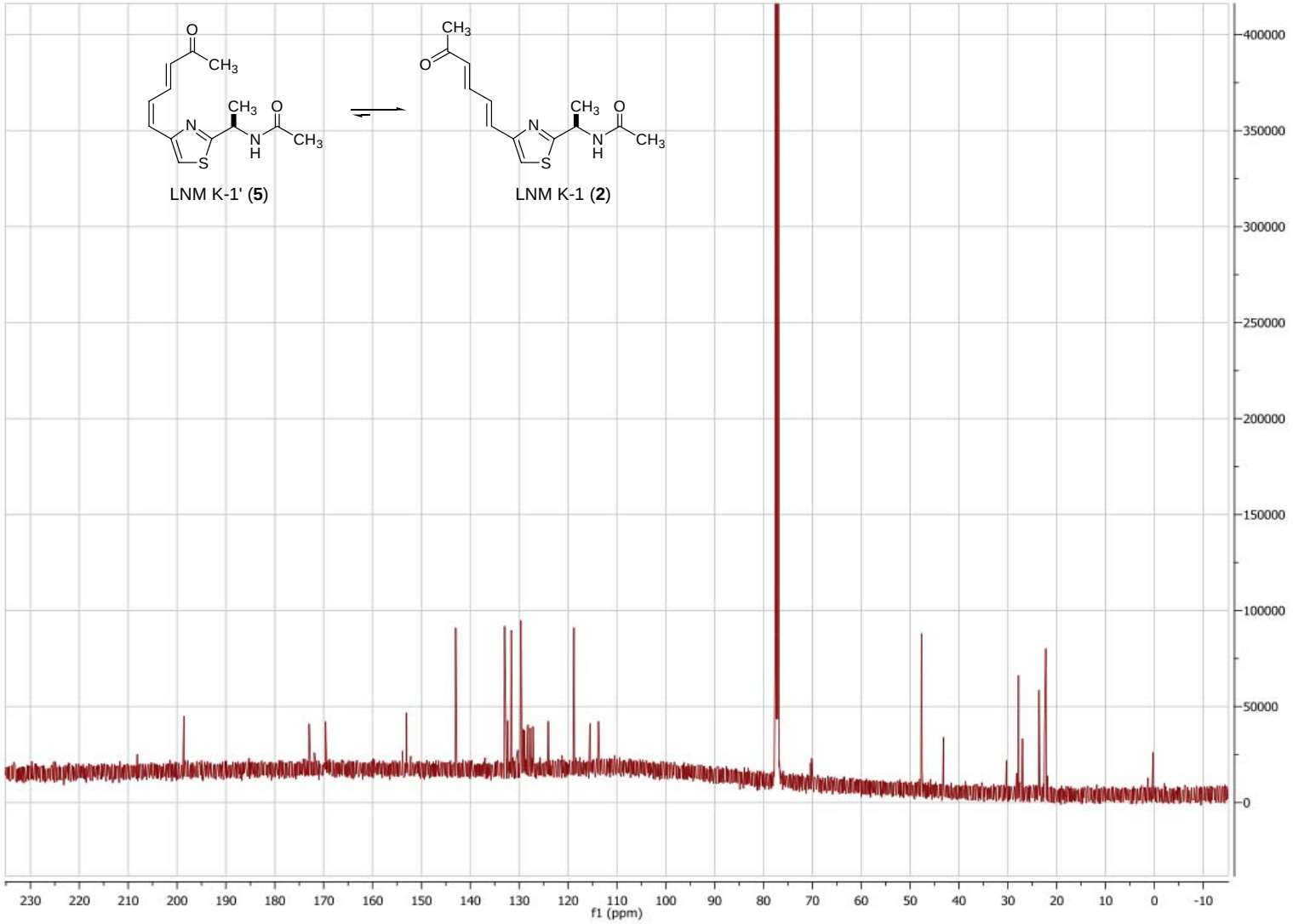


Figure S4 ^{13}C NMR spectrum of a mixture of **2** and **5**.

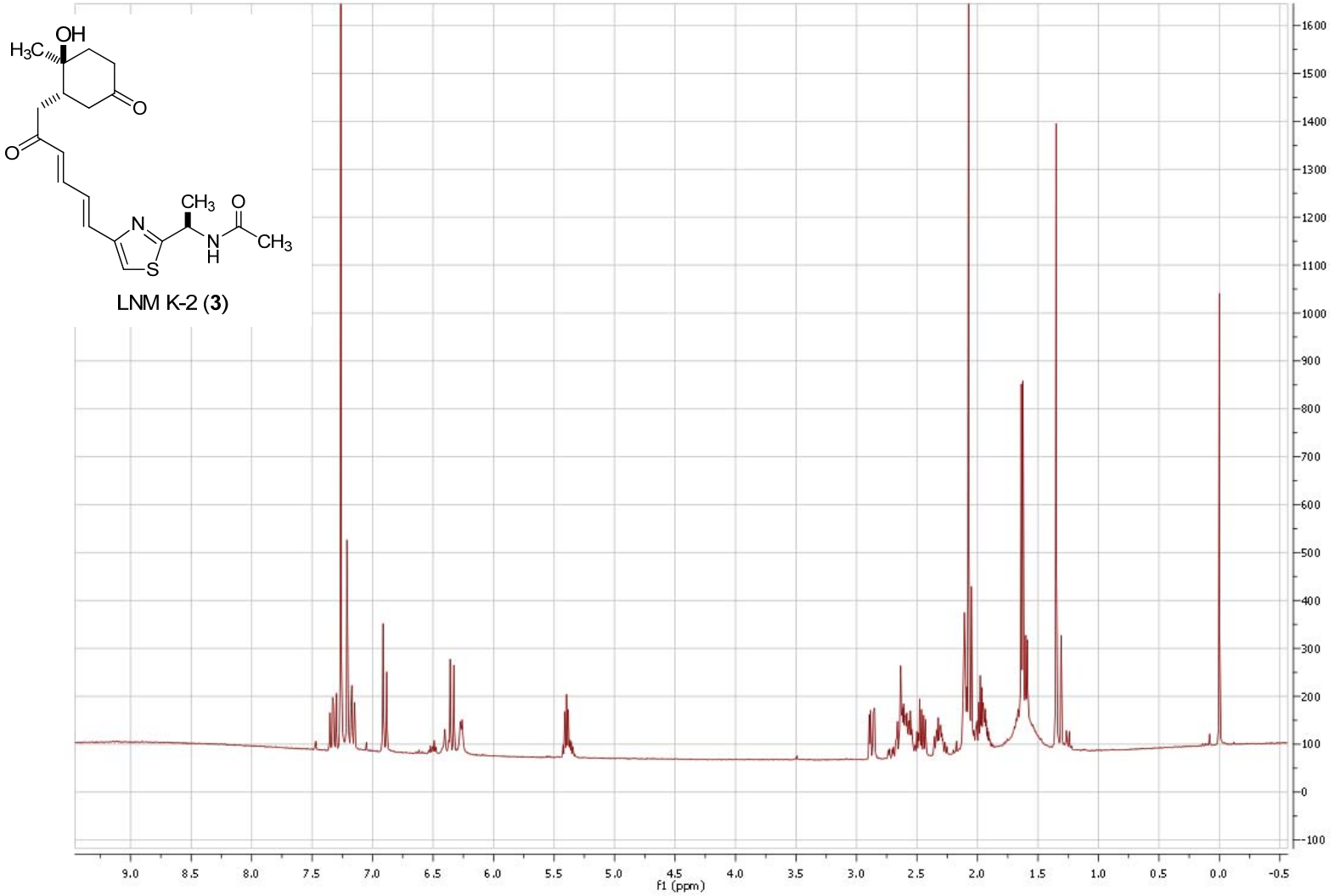


Figure S5 ^{1}H NMR spectrum of **3**.

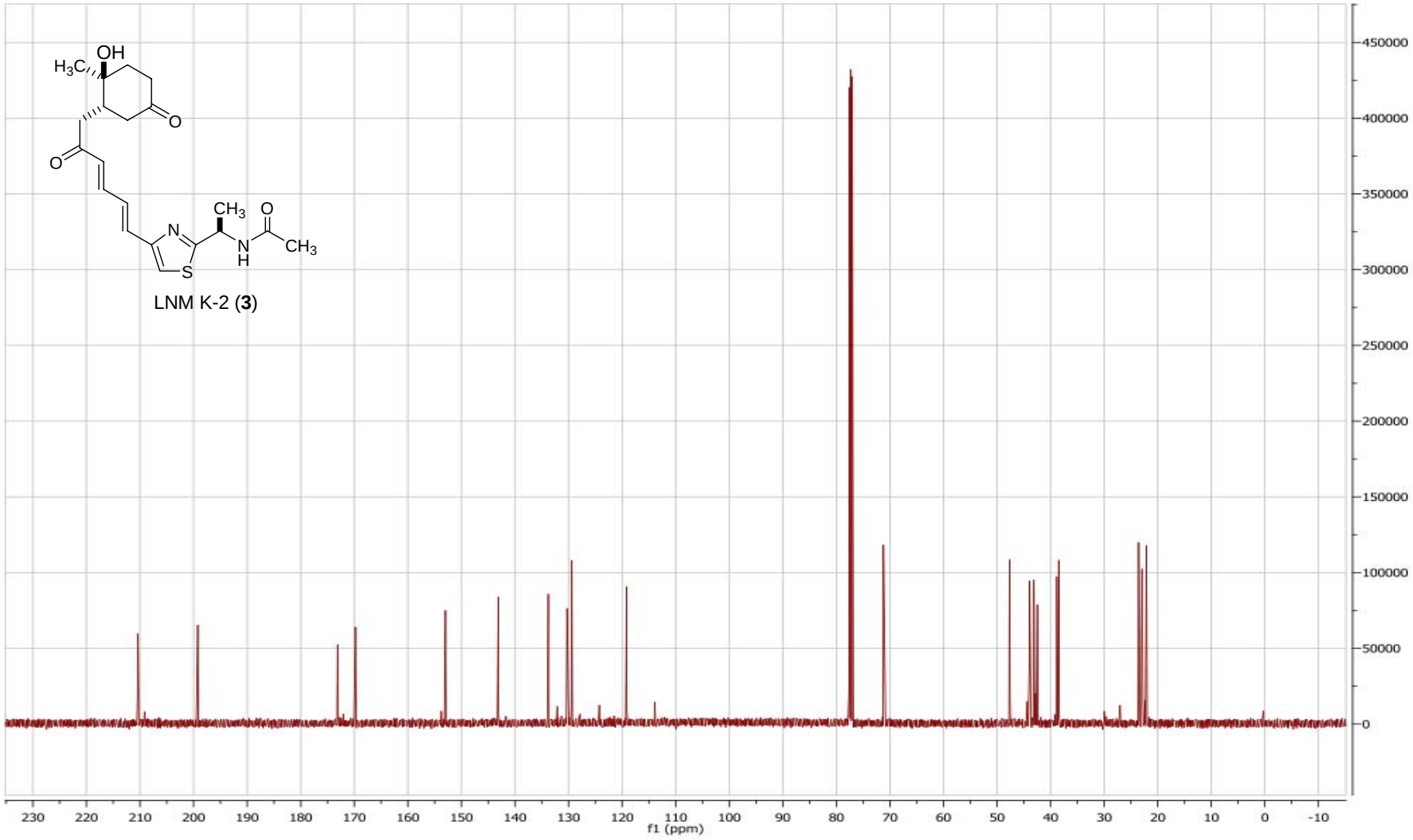


Figure S6 ^{13}C NMR spectrum of **3**.

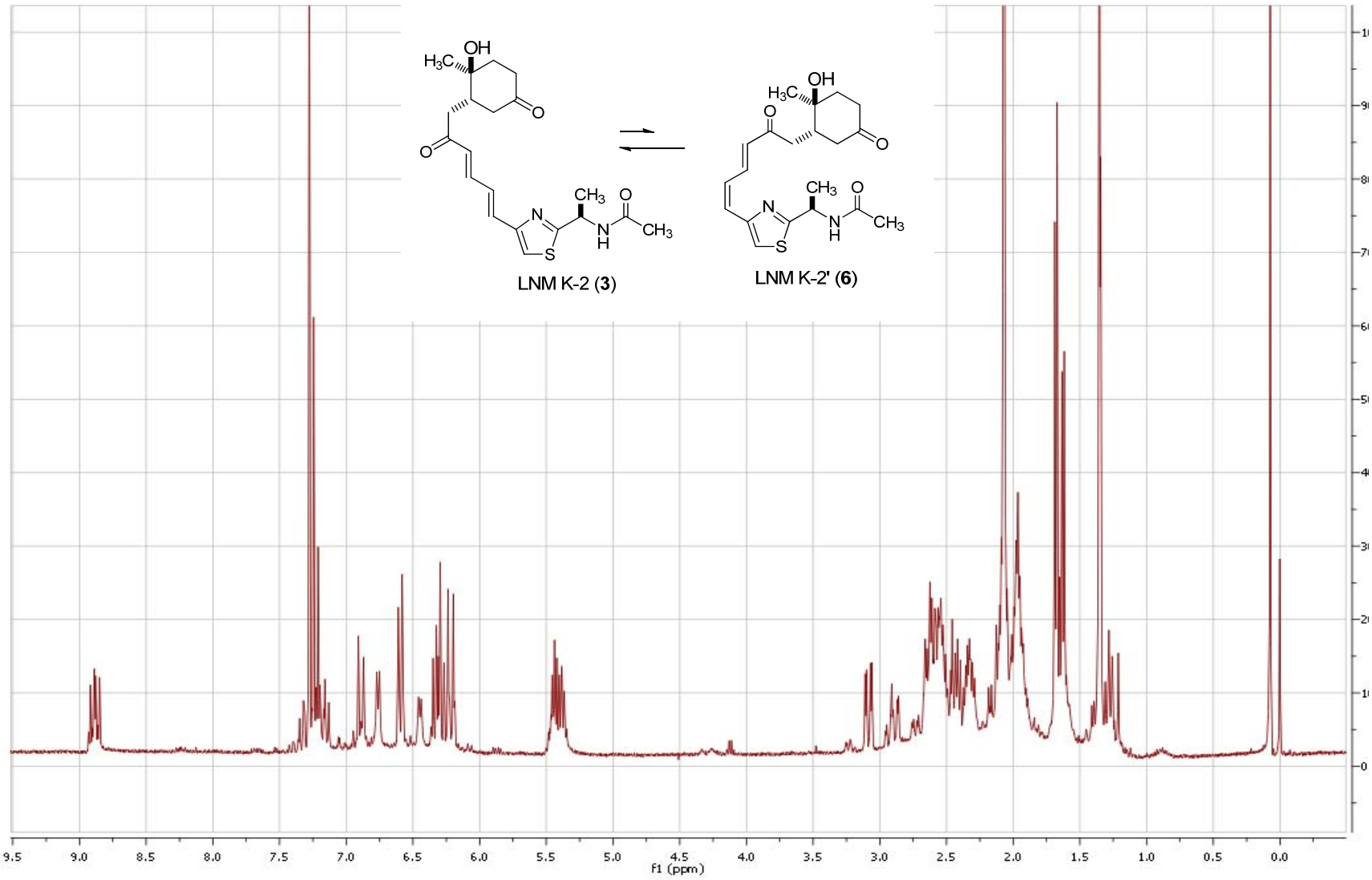


Figure S7 ^{1}H NMR spectrum of a mixture of **3** and **6**.

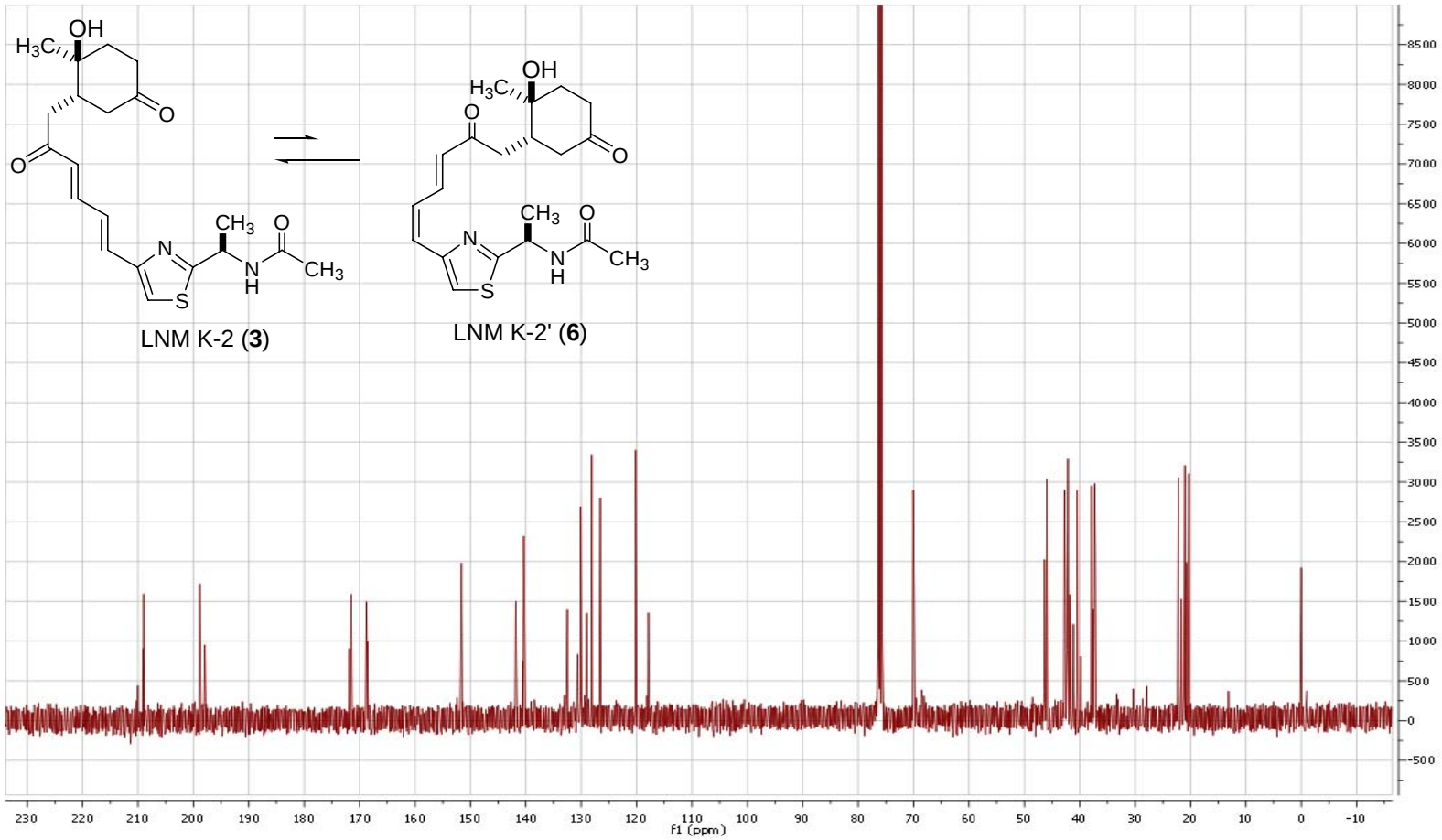


Figure S8. ^{13}C NMR spectrum of a mixture of **3** and **6**.

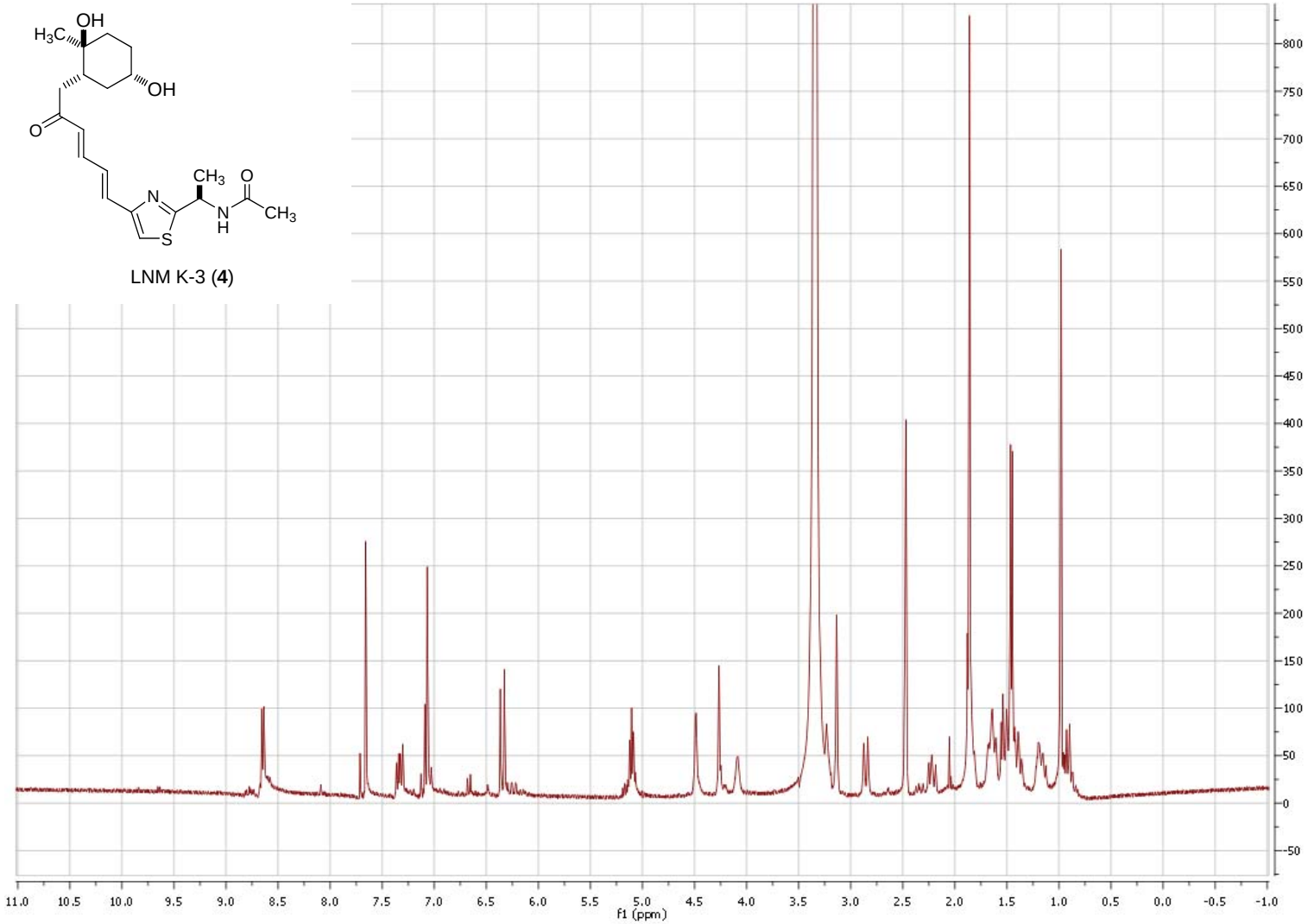
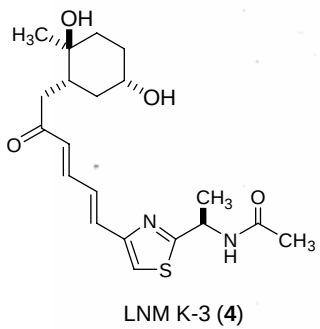


Figure S9 ^{1}H NMR spectrum of **4**.



LNM K-3 (**4**)

Figure S10 ^{13}C NMR spectrum of **4**. Compound **4** was not stable and degraded in the course of acquiring the ^{1}H and ^{13}C NMR spectra. Please refer to Figure S13 for a cleaner ^{13}C spectrum of **4**.

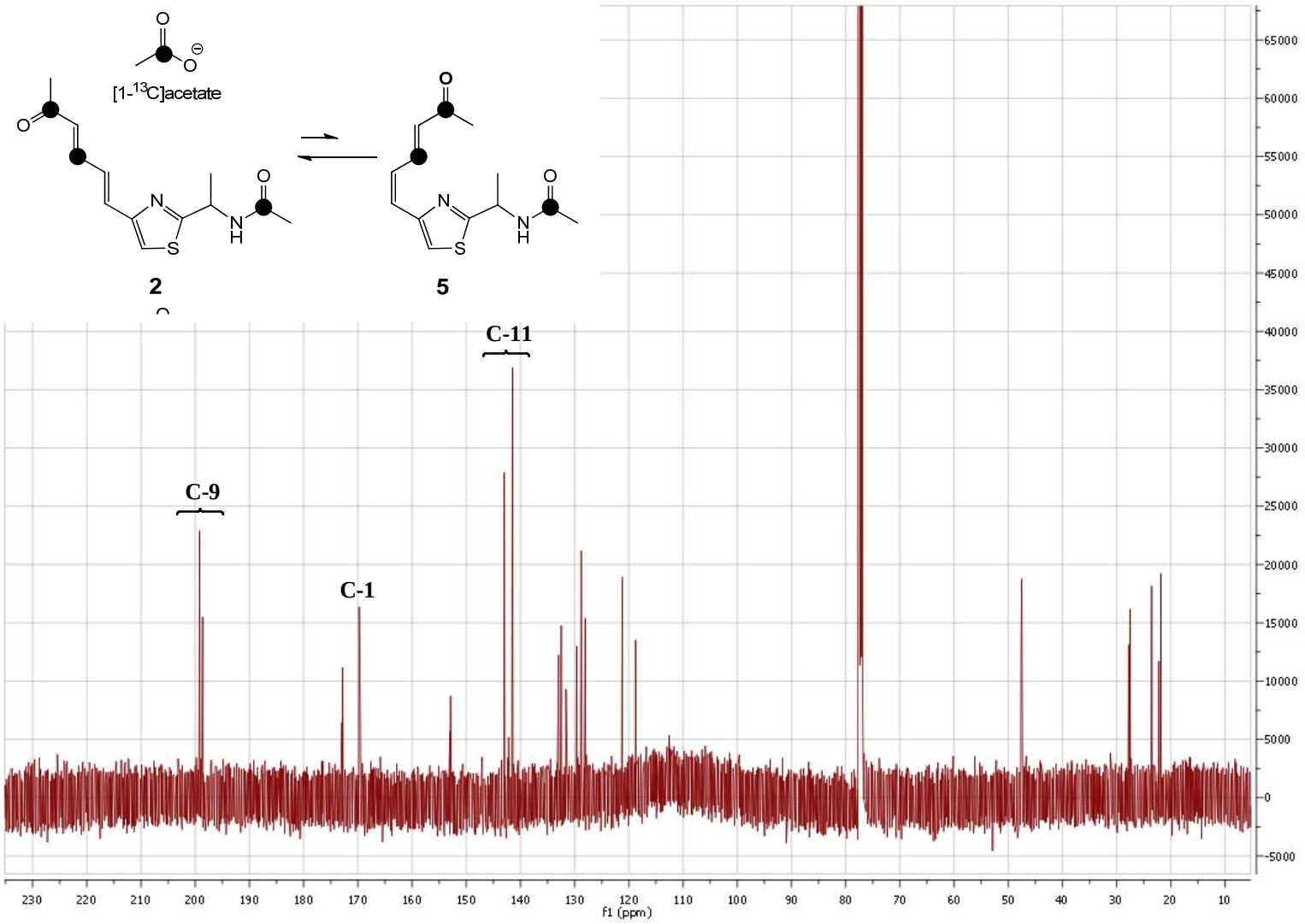


Figure S11 ^{13}C NMR spectrum of **2** and **5** from [1-^{13}C]acetate feeding.

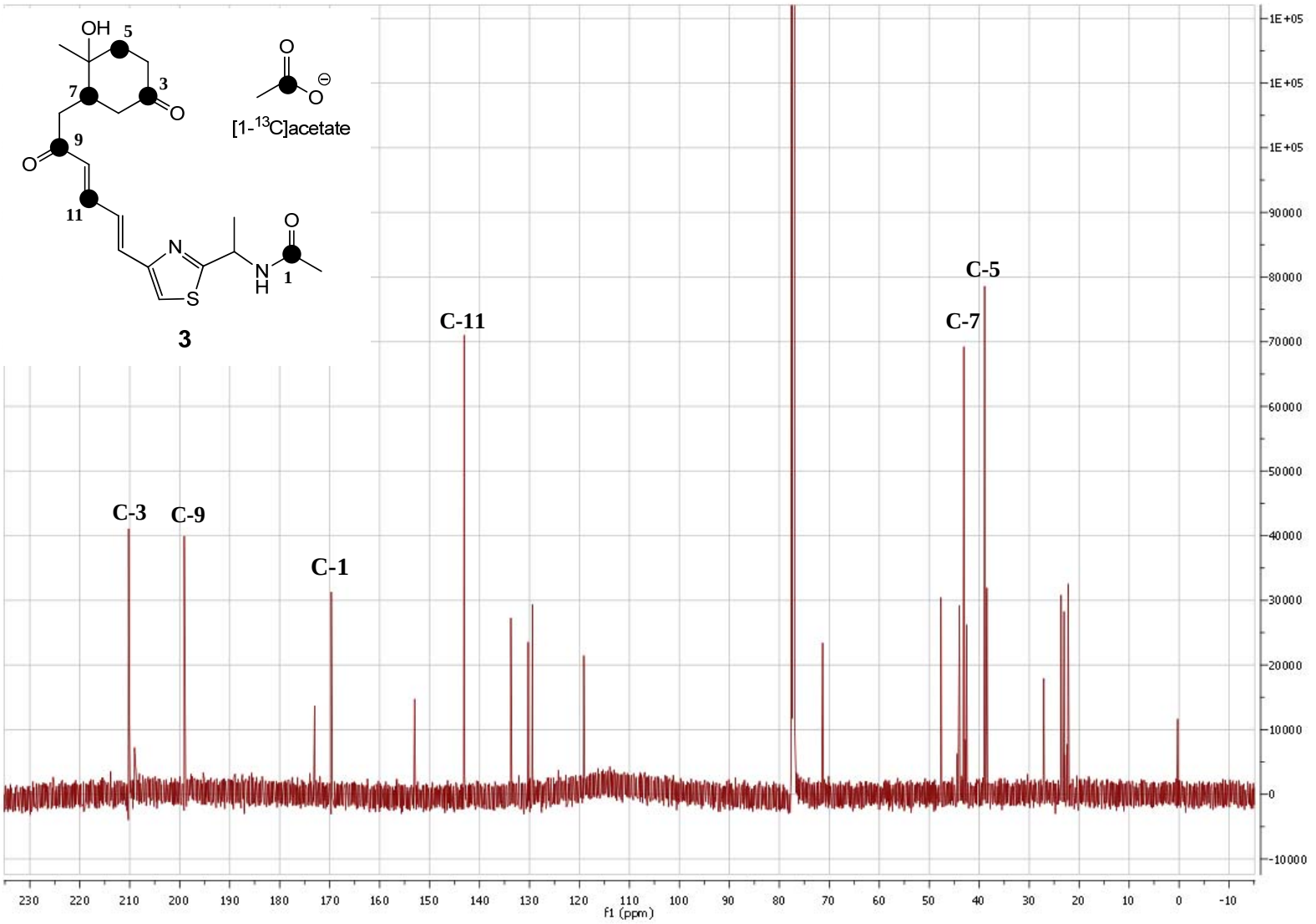


Figure S12 ^{13}C NMR spectrum of **3** from [1-^{13}C]acetate feeding

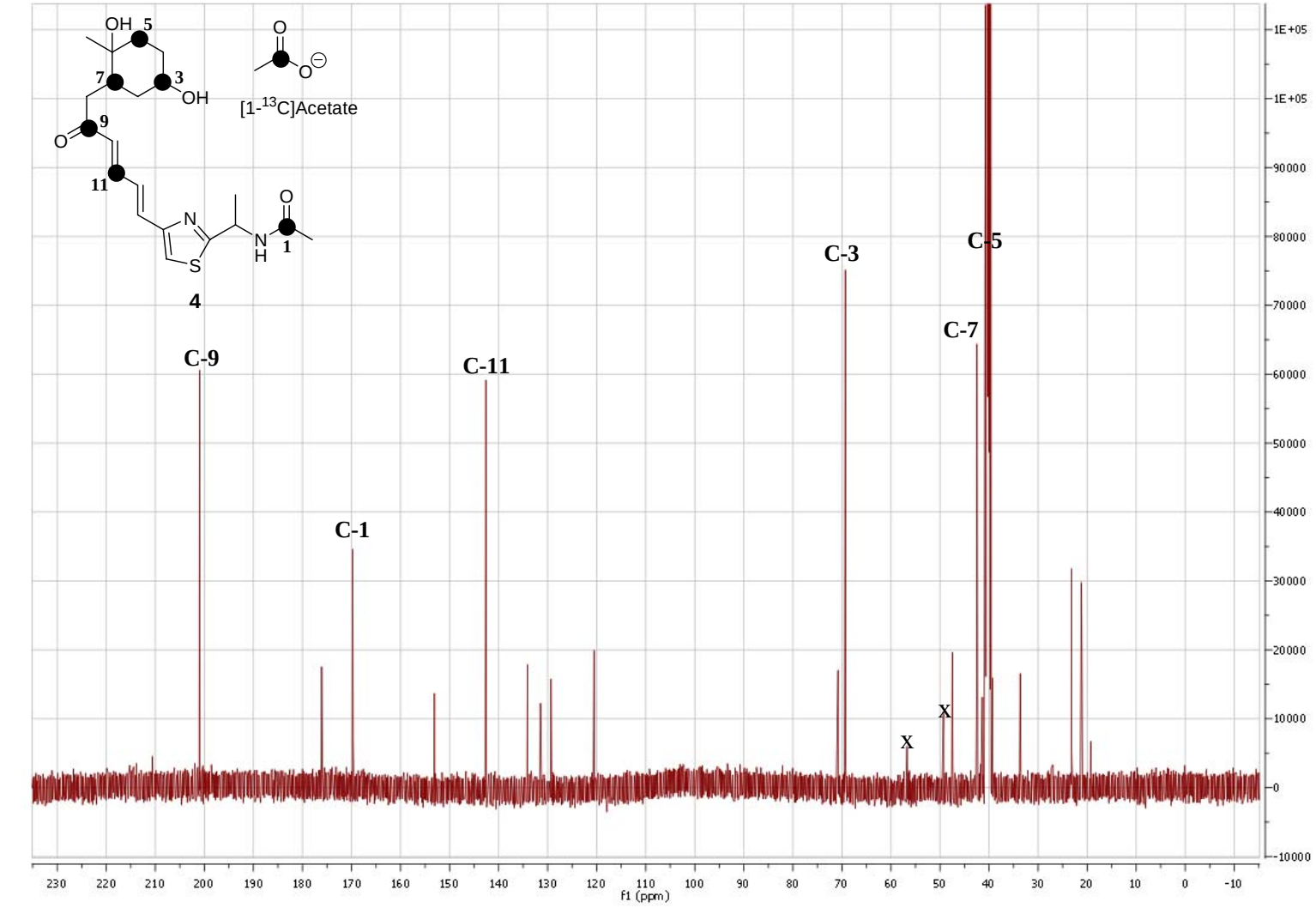


Figure S13 ^{13}C NMR spectrum of **4** from [1-^{13}C]acetate feeding (x, impurities)

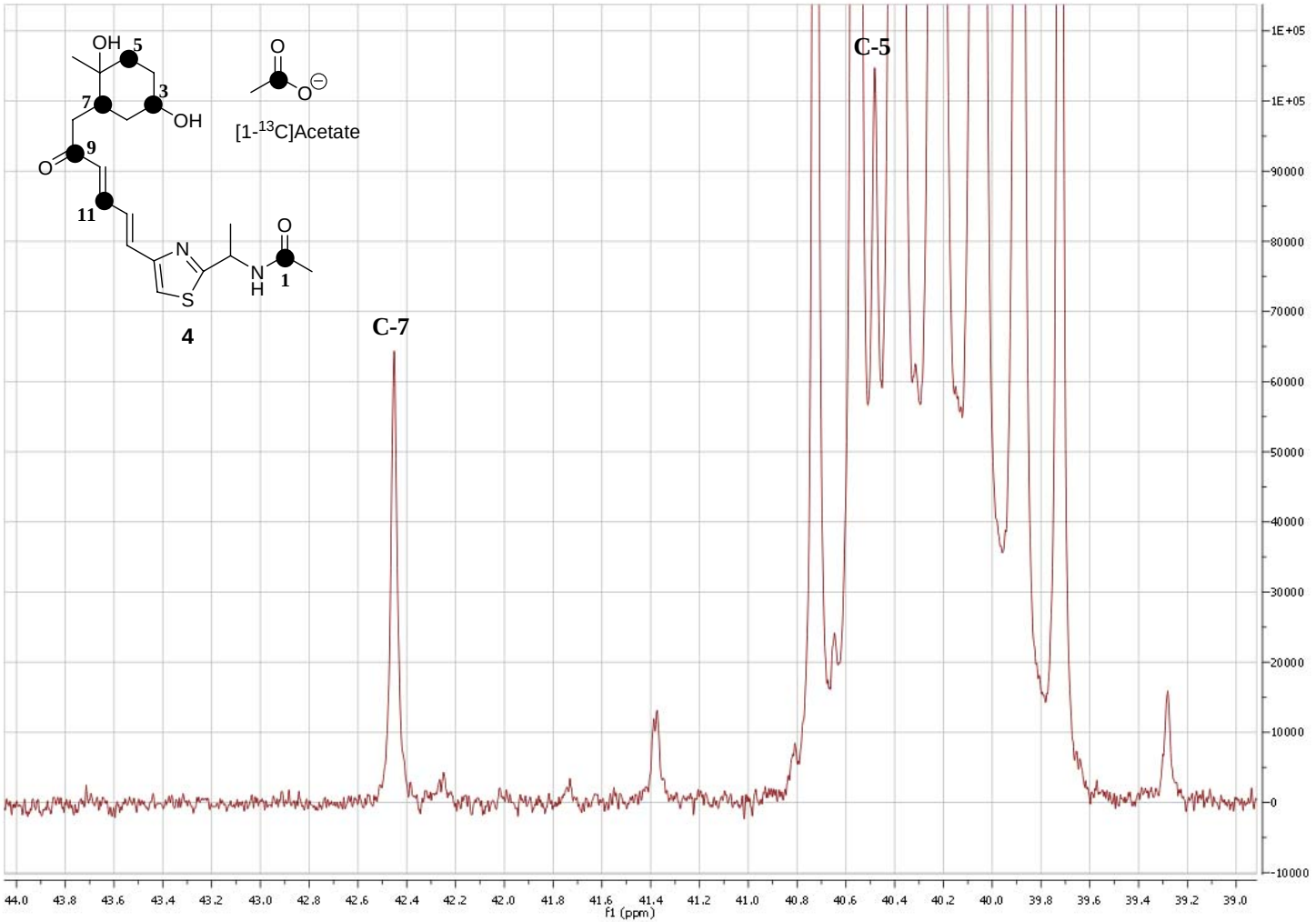


Figure S14 ^{13}C NMR spectrum of **4** from [1-^{13}C]acetate feeding (partial enlarged).