Supporting Information

Elucidation of the Concise Biosynthetic Pathway of the Communesin Indole Alkaloids

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Supplementary Experimental Procedures

Strains and Culture Conditions. *Penicillium expansum* NRRL 976 was a kind gift obtained from Professor Jens C. Frisvad from Technical University of Denmark. The strain was maintained on YG-agar (4 g/L yeast extract, 4 g/L dextrose, 16 g/L agar) or glucose minimal media (GMM)[1] media and stored as 30% glycerol stocks at -70°C. For production of **1**, **2** and other intermediates, *P. expansum* and mutant strains were cultured in YG-agar. Total RNA for RT-PCR is extracted from *P. expansum* grown on Czapek-Dox liquid medium with 5 g/L yeast extract (CYB) after 4 days of cultivation. For chemical complementation of Δ*cnsA*, Δ*cnsB* and Δ*cnsF* mutants, tryptamine or purified **10** was supplemented to the YG-agar at 100 or 40 μg/nL, respectively. *Escherichia coli* BL21 (DE3) (Novagen) was used as the *E. col*i expression strain.

Chemicals and Chemical Analysis. All solvents and other chemicals used were of analytical grade. All LC-MS analyses were performed on a Shimadzu 2010 EV LC-MS (Phenomenex® Luna, 5μ, 2.0 × 100 mm, C18 column) using positive and negative mode electrospray ionization with a linear gradient of 5–95% MeCN-H_2O in 30 minutes followed by 95% MeCN for 15 minutes with a flow rate of 0.1 mL/min. ^{1}H, ^{13}C and 2D NMR spectra were obtained on Bruker AV500 spectrometer with a 5 mm dual cryoprobe or a Bruker DRX500 spectrometer with a 5 mm broadband probe at the UCLA Molecular Instrumentation Center.

General Molecular Biology Experiments. General molecular cloning techniques were as described elsewhere.[2] PCR was performed using Phusion® DNA Polymerase (New England Biolabs) or Takara Primer star HS polymerase (Clonetech, USA). DNA restriction enzymes were used as recommended by the manufacturer (New England Biolabs). PCR products were confirmed by DNA sequencing. *E. coli* TOP10 (Invitrogen) and XL1-Blue were used for cloning, following standard recombinant DNA techniques. RNA extraction was performed using a RiboPure Yeast Kit (Ambion) and ImProm-II™ Reverse Transcription System for RT-PCR (Invitrogen) was used to synthesize complementary DNA (cDNA) from total RNA.

Whole Genome Sequence and Analysis. Genomic sequencing of *P. expansum* NRRL 976 was performed by Ambry Genetics Corp. (USA) using the next-generation sequencing technology Illumina HiSeq2000 system. The genome sequence was assembled by the software SOAPdenovo[3] using the UCLA Hoffman2 computer cluster to yield 181 contigs covering approximately 32.9 Mb. Gene structure predictions were performed using the FGENESH program (Softberry) and manually checked by comparing with homologous gene/proteins in the GenBank database using BLASTX analysis. Functional domains in the translated protein sequences were predicted using Conserved Domain Search (NCBI) or InterproScan (EBI). CLUSTALW or BIOEDIT software package is used for multiple sequence alignment.

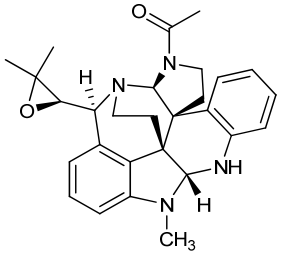
Fungal Transformation and Gene Deletion in *P. expansum*. Deletion cassettes were generated using a fusion PCR technique[4] and followed the hygromycin-resistance split-marker approach for gene deletion previously described using *hph* cassette encodes hygromycin B phosphotransferase as the selectable marker.[5] Briefly, approximately 3.0 kb of 5'flanking region and 3'flanking region were PCR amplified from each targeted loci in the *P. ezpansum* genomic DNA using the corresponding primer pairs p1 & p3 and p4 & p6. *hph* cassette was PCR amplified from pAN7.1 using the corresponding primer pairs p3R and p4F. The two initial fragments containing 5'flanking region and 3'flanking region were fused to the *hph* fragment using the corresponding primer pairs p2 & HY-R, and YG-F & p5, respectively. All primers used in this study are listed in Table S1. Conidia from *P. expansum* was inoculated to 250 mL liquid GMM for 16 hours at 25°C, 250 rpm for germination. The harvested germlings were then digested with 3 mg/mL lysing enzymes (Sigma-Aldrich) and 2 mg/mL Yatalase (Takara Bio) to obtain the protoplasts, which were then transformed with the two split marker-knockout cassettes. After polyethylene glycol (PEG)-mediated transformation, the protoplasts were inoculated into GMM media supplemented with 1.2 M sorbitol, agar and 250 μg/mL of hygromycin B (A.C. Scientific Inc.) as a selective agent. Genomic DNA from the transformants was isolated using Carlson lysis buffer and chloroform extraction followed by precipitation of DNA in the aqueous phase by addition of an equal volume of isopropanol. Gene replacements were confirmed by the PCR screening method (Figure S1–S8), using the primers listed in Table S1.

Overexpression and Purification of His6-tagged CnsF in *E. coli*. The cDNA of *cnsF* was obtained by RT-PCR. The intron-less DNA fragment was inserted into the pET30 Xa/LIC vector (EMD Millipore). Primers used for the amplification and cloning are listed in Table S1. Expression plasmid pET30-*cns*F was transformed into electrocompetent *E. coli* BL21 (DE3). The cells were cultured at 37°C, 250 rpm in 500 mL of LB medium with 35 μg/mL kanamycin. 0.1 mM isopropylthio-β-D-galactoside (IPTG) to induce protein expression was added at OD_{600} between 0.4 to 0.6 and the cells were further culture for 12−16 h at 16°C. The cells were then harvested by centrifugation (3500 rpm, 15 min, 4°C), resuspended in ~25 mL lysis buffer (100 mM Tris-HCL, pH 7.4, 0.1 M NaCl, 20 mM imidazole), and lysed by sonication on ice. Cell debris was removed by centrifugation (15000 rpm, 30 min, 4°C). The His6-tagged proteins were purified by using Ni-NTA agarose (Qiagen) according to manufacturer's instructions. Purified enzyme was checked by SDS-PAGE (Figure S9), concentrated and exchanged into buffer A (50 mM Tris-HCl, pH 7.9, 2 mM EDTA, 2 mM DTT) + 10% glycerol with the centriprep filters (Amicon) and stored at −8°C for enzyme assays.

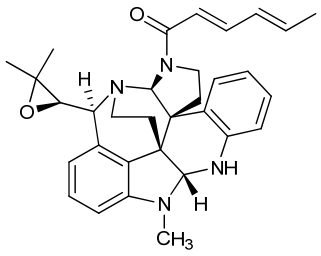
In vitro assay of CnsF. For the in vitro synthesis of 4-L-DMAT (**11**), 20 μM CnsF was incubated with 50 μM L-tryptophan, 50 μM dimethylallyl pyrophosphate (DMAPP) and 10 mM $MgCl_2$ in 100 mM Tri-HCl (pH 7.5). The same condition was performed as described above when taking 20 μM tryptamine as substrate. The reaction was incubated at 14 h and extracted

twice with ethyl acetate. The organic phases were dried and dissolved in 20 µL MeOH and subjected for analysis by LC-MS as described in Chemical Analysis (Figure S10).

Isolation and Purification of communesin A (1) and communesin B (2). *P. expansum* NRRL 976 was cultivated on YG agar (4L) at 25 °C for 4 d, then extracted with acetone (4 L). The acetone extract was concentrated *in vacuo* and the resultant aqueous mixture (800 mL) was extracted with EtOAc (4 × 500 mL). The combined organics were dried over $MgSO_4$ and concentrated *in vacuo* The crude products were purified by flash chromatography (3:1 → 3:2 hexanes : acetone) to provide communesin A (**1**) (8.2 mg), which was further purified by preperative thin layer chromatography (1 : 1 hexanes : acetone), and communesin B (**2**) (8.4 mg), which was further purified by preperative thin layer chromatography (2 : 1 hexanes : acetone). All spectral data for **1** and **2** were consistent with that in the literature.[6, 7]

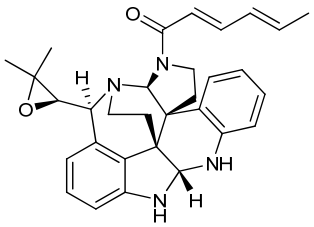


communesin A (**1**)



communesin B (**2**)

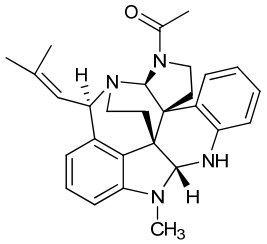
Isolation and Purification of communesin C (3). The Δ*cnsE* mutant strain was cultivated on YG agar (4L) at 25°C for 4d and extracted with acetone (4 L). The acetone extract was concentrated *in vacuo* and the resultant aqueous mixture (900 mL) was extracted with EtOAc (2 × 700 mL) and concentrated *in vacuo*. The crude products were purified by RP-18 column (Redi*Sep* Rf Gold C18 Column, 20–40µ, 15.5g) with CombiFlash Rf200 system and further purified by a Sephadex LH-20 column (35 × 1.2 cm, MeOH) to yield communesin C (**3**) (1.2 mg).[7]



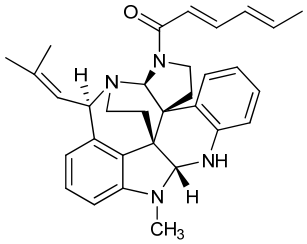
communesin C (**3**)

Isolation and Purification of communesin F (6), communesin J (13) and communesin K (14). The Δ*cnsJ* mutant strain was cultivated on YG agar (6L) at 25 °C for 4 d, and extracted with acetone (6 L). The acetone extract was concentrated *in vacuo* and the resultant aqueous mixture (1000 mL) was extracted with EtOAc (3 × 1000 mL). The combined organics were washed with brine (1500 mL), dried over $MgSO_4$, and concentrated *in vacuo*. The crude products were

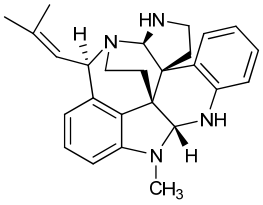
separated by a Sephadex LH-20 column (35 × 1.2 cm, MeOH) followed by purification on a Silica gel column (RediSep®, 40 g Flash Column, 9 : 1 → 11 : 9 hexane : acetone gradient) to yield three fractions (Fr.1−3). Fr2 was purified by flash chromatography (5 : 1 → 4 : 1 hexane : acetone) to provide communesin F (**6**) (8.3 mg) and communesin J (**13**) (7.4 mg). All spectral data for **6** was consistent with that in the literature.[8] Fr.3 was further purified a semi-preparative RP-18 HPLC column (Luna®, ODS-3, 5 μm, 250 × 10 mm) eluted by 27% $MeCN_{aq}$ with flow rate of 2.7 mL/min to give communesin K (**14**) (0.7 mg, t_R: 41.0 min).



communesin F (**6**)

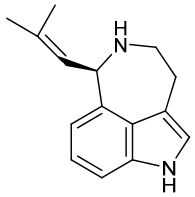


communesin J (**13**)



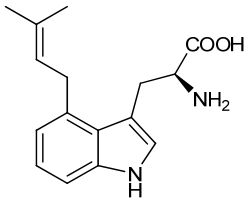
communesin K (**14**)

Isolation and Purification of aurantioclavine (10). The Δ*cnsC* mutant strain was cultivated on YG agar (14L) at 25°C for 4 d and extracted with acetone (14 L). The acetone extract was concentrated *in vacuo* and the resultant aqueous mixture (6000 mL) was split into three 2000 mL batches and extracted with EtOAc (4 × 600 mL). The combined organics were washed with brine (1000 mL), dried over $MgSO_4$, and concentrated *in vacuo*. The crude product was purified by flash chromatography (33 : 17 : 1 hexanes : EtOAc : Et_3N) followed by preperative thin layer chromatography (25 : 25 : 1 hexanes : EtOAc : Et_3N) to provide aurantioclavine (**10**) (69.2 mg). All spectral data for **10** was consistent with that reported in the literature.[9]



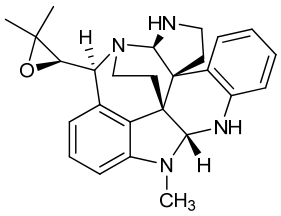
(-)-aurantioclavine (**10**)

Purification of 4-L-DMAT (11). A solution with a total volume of 10 mL comprised of 20 μM CnsF, 50 μM L-tryptophan, 50 μM dimethylallyl pyrophosphate (DMAPP) (Echelon Biosciences, or synthesized according to McIntosh et. al.[10]) and 10 mM $MgCl_2$ in 100 mM Tri-HCl (pH 7.5) was incubated at room temperature. After a 14h-incubation, the reaction was extracted with ethyl acetate (2 × 10 mL). The combined organic phases were dried *in vacuo* and subjected to a semi-preparative RP-18 HPLC column (Luna®, ODS-3, 5 μm, 250 × 10 mm) elutaed by 30% $MeCN_{aq}$ with flow rate of 2.7 mL/min to give **11** (0.2 mg, t_R: 21.6 min). All spectral data for **11** was consistent with that reported in the literature.[11]



4-L-DMAT (**11**)

Isolation and Purification of communesin I (12). The Δ*cnsK* mutant strain was cultivated on YG agar (4L) at 25 °C for 4 d and extracted with acetone (4 L). The acetone extract was concentrated *in vacuo* and the resultant aqueous mixture (1000 mL) was extracted with EtOAc (3 × 200 mL). The combined organics were washed with brine (300 mL), dried over $MgSO_4$, and concentrated *in vacuo*. The crude product was purified by a Sephadex LH-20 column (35 × 1.2 cm, MeOH) followed by flash chromatography (97 : 2 : 1 → 94 : 5 : 1 CH_2Cl_2 : MeOH : Et_3N) followed by a second Sephadex LH-20 column (35 × 1.2 cm, MeOH : CH_2Cl_2 = 7 : 3) to afford communesin I (**12**).



communesin I (**12**)

Supplementary Tables

Table S1. Primers used in this study

Primer name	Sequence (5'→ 3')
cnsA-KO-p1	ggagacggtatggttggtacgac
cnsA-KO-p2	gaactcgatgcgctggaccag
cnsA-KO-p3	ccgtccgtctctccgcatggatgctgaggtggacggcac
cnsA-KO-p4	ccactccacatctccactcgagagcgattgctggctctcaagc
cnsA-KO-p5	gtccgagggatgggagcag
cnsA-KO-p6	ccatgtgctatgtgtgccggatc
cnsA-KO-p3F	gtgccgtccacctcagcatccatgcggagagacggacgg
cnsA-KO-p4R	gcttgagagccagcaatcgctctcgagtggagatgtggagtgg
cnsB-KO-p1	cgacccggctcaatgtaccggag
cnsB-KO-p2	ctcaccgctatgctctctctaagg
cnsB-KO-p3	gtccgtccgtctctccgcatgccaacacaatgcccacttgcg
cnsB-KO-p4	cccactccacatctccactcgaggcatccagtgttcggacaatagg
cnsB-KO-p5	ccaatacacgttgcggaagtcgg
cnsB-KO-p6	caaacctctcaggaccacagtgcg
cnsB-KO-p3F	cgcaagtgggcattgtgttggcatgcggagagacggacggac
cnsB-KO-p4R	cctattgtccgaacactggatgcctcgagtggagatgtggagtggg
cnsC-KO-p1	ggaaagtctccacgctcgatagc
cnsC-KO-p2	gcatggcccaggtacgcac
cnsC-KO-p3	ccgtccgtctctccgcatggaagagcaactttggagtgggcc
cnsC-KO-p4	cccactccacatctccactcgagcgcacgtcggccaaagtc
cnsC-KO-p5	gatgctgaggtggacggcac
cnsC-KO-p6	ggccggaccgaaatcaagtttcc
cnsC-KO-p3F	ggcccactccaaagttgctcttccatgcggagagacggacgg
cnsC-KO-p4R	gactttggccgacgtgcgctcgagtggagatgtggagtggg
cnsE-KO-p1	ggacatgctaggcacaagccttg
cnsE-KO-p2	ccgtctccaacgtctgaagatac
cnsE-KO-p3	ccgtccgtctctccgcatgggtcaagggcaatagctctcag
cnsE-KO-p4	cccactccacatctccactcgacttatcactgcagagcgcctctag
cnsE-KO-p5	gcatttgtgccgaaggttggcag
cnsE-KO-p6	gtaggtgcgtgtgcattatcgg
cnsE-KO-p3F	ctgagagctattgcccttgacccatgcggagagacggacgg
cnsE-KO-p4R	ctagaggcgctctgcagtgataagtcgagtggagatgtggagtggg
cnsF-KO-p1	gatatgggtgacttgaggggcagg
cnsF-KO-p2	gcagcaggcagtacagccaag
cnsF-KO-p3	gtccgtccgtctctccgcatgcctactcccccaagcggcaatacc
cnsF-KO-p4	cccactccacatctccactcgaggatgcattaggggacatgtcatg
cnsF-KO-p5	cttggtgcgccccaagacag
cnsF-KO-p6	gctactggccagcggtcaacg
cnsF-KO-p3F	ggtattgccgcttgggggagtaggcatgcggagagacggacggac
cnsF-KO-p4R	catgacatgtcccctaatgcatcctcgagtggagatgtggagtggg
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cnsI-KO-p2	cggaccgctgtgacagtgc
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cnsI-KO-p4	ccactccacatctccactcgacttgctggaggtcccaggtag
cnsI-KO-p5	gcgtaccccaggaatcactatgc
cnsI-KO-p6	gagcccagatgggataagtcactc
cnsI-KO-p3F	cgtgcagccttgagaaacgaccatgcggagagacggacg
cnsI-KO-p4R	ctacctgggacctccagcaagtcgagtggagatgtggagtgg
cnsJ-KO-p1	gctcctcggaaaccatgtgc
cnsJ-KO-p2	ggcttggaatgtatgccactgatgc
cnsJ-KO-p3	ccgtccgtctctccgcatggacatcgtcggtggcggatg
cnsJ-KO-p4	cccactccacatctccactcgacgaggacgatggtggtatgaagc
cnsJ-KO-p5	gcatcaccagatccagcctactag
cnsJ-KO-p6	ctcaaggctgcacgttcacctc

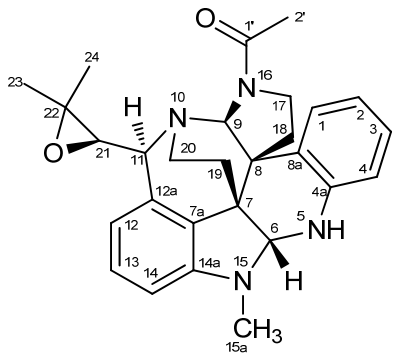
cnsJ-KO-p3F	catccgccaccgacgatgtccatgcggagagacggacgg
cnsJ-KO-p4R	gcttcataccaccatcgtcctcgtcgagtggagatgtggagtggg
cnsK-KO-p1	ggtgttacagccgatgcagctgtg
cnsK-KO-p2	ggccttgataaaacggccatctcg
cnsK-KO-p3	gtccgtccgtctctccgcatgggtggtgccgcagatatagggac
cnsK-KO-p4	cccactccacatctccactcgaggcagggacagggtagaggac
cnsK-KO-p5	catcggcatggcactgtacgg
cnsK-KO-p6	gtgcatcagcatcgccgatcgtc
cnsK-KO-p3F	gtccctatatctgcggcaccacccatgcggagagacggacggac
cnsK-KO-p4R	gtcctctaccctgtccctgcctcgagtggagatgtggagtggg
CnsF-XALIC-F	ggtattgagggtcgcatgggcacacatgacatgtccc
CnsF-XALIC-R	agaggagagttagagccctaggcacaagccttggcg
anchored oligo-dT reverse primer	TTTTTTTTTTTTTTTTTTTTTVN

Table S2. Deduced functions of genes within the *cns* cluster.

Penicillium expansum NRRL 976, Scaffold 19 (27,965 – 74,677), 46.7 kbp							
Gene name	Size (gene/protein)	BLASTP homologs[a]	Identity/ similarity (%)	Conserved domain[b]	Function[c]	Gene locus in NCBI database (*P. expansum* strain d1, contig 72)	Identity/ similarity (%)
cnsP	1113/370	PROQFM164_S01g000406	95/98	Taurine catabolism dioxygenase TauD, TfdA family, pfam02668	alpha-ketoglutarate-dependent dioxygenase	PEXP_030610	100/100
cnsO	1491/496	CGGC5_280	64/76	The Major Facilitator Superfamily (MFS), cd06174	MFS transporter	PEXP_030600	96/95
cnsN	2580/859	PROQFM164_S01g000403	80/87	GAL4-like Zn2Cys6 binuclear cluster DNA-binding domain, cd00067	Zn2Cys6 fungal-type transcriptional factor	PEXP_030590	86/85
cnsM	1080/359	UCRPA7_4326	85/92	Taurine catabolism dioxygenase TauD, TfdA family, pfam02668	alpha-ketoglutarate-dependent dioxygenase	PEXP_030580	100/100
cnsL	1530/509	UCRPA7_4325	78/88	The Major Facilitator Superfamily (MFS), cd06174	MFS transporter	PEXP_030570	94/94
cnsK	1401/466	AFUA_2G18020	34/51	Transferase family, pfam02458	*N*-acyltransferase[†]	PEXP_030560	100/100
cnsJ	1002/333	NFIA_093740, FtmF	26/40	Phytanoyl-CoA dioxygenase (PhyH), pfam05721	phytanoyl-CoA dioxygenase[†]	PEXP_030550	100/100
cnsI	7071/2356	ANI_1_1948094	54/69	KS-AT-DH-ER-KR-ACP	polyketide synthase[†]	PEXP_030540	99/99
cnsH	837/278	MPH_06429	30/45	Serine hydrolase (FSH1), pfam03959	Serine hydrolase	PEXP_030530	100/100
cnsG	1092/363	HMPREF1120_08694	51/67	Uncharacterized subfamily of fatty acid CoA ligase (FACL), cd05917	long-chain fatty-acid-CoA ligase	PEXP_030520	98/98
cnsF	1362/453	MCYG_06055	61/77	cd13929, aromatic prenyltransferases (PTases) of the DMATS/CymD familiy	4-dimethylallyl tryptophan synthase[†]	PEXP_030510	100/100
cnsE	831/276	*Streptomyces clavuligerus* StaMa	44/57	*S*-adenosylmethionine-dependent methyltransferases (SAM or AdoMet-MTase), class I, cd02440	methyltransferase[†]	PEXP_030500	100/100
cnsD	1431/476	*Epichloe canadensis*, EasC	59/72	cd08157, Fungal catalases similar to yeast catalases A and T	aurantioclavine catalase protein	PEXP_030490	100/100
cnsC	1272/423	MCYG_04917	36/55	Cytochrome P450, pfam00067	cytochrome P450[†]	PEXP_030480	96/96
cnsB	1365/454	*Catharanthus roseus*, TDC	27/46	DOPA decarboxylase family, cd06450	tryptophan decarboxylase[†]	PEXP_030470	100/100
cnsA	1767/588	*Epichloe canadensis*, EasE	50/67	FAD binding domain, pfam01565	aurantioclavine synthase/oxidoreductase [†]	PEXP_030460	100/100

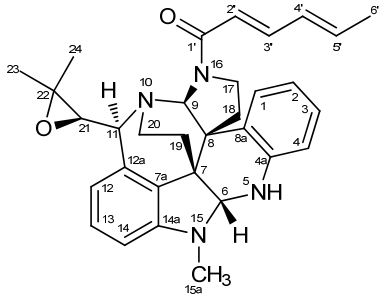
[a] If available, the closest characterized homologs is shown or the locus tag of the closest BLASTP homologs if there is no characterized ones.
[b] Conserved domain is based on analysis by NCBI Conserved Domain Search. For CnsI, the abbreviations for the conserved domains are KS, beta-ketoacyl synthase; AT, malonyl-CoA:transacylase; DH, dehydratase; ER, enoyl reductase; KR; ketoreductase; ACP, acyl carrier protein.
[c] Dagger (†) indicates the function of the encoded protein has been verified by gene deletion and/or in vitro experiments.

Table S3. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectroscopic data of communesin A (**1**) in $CDCl_3$.



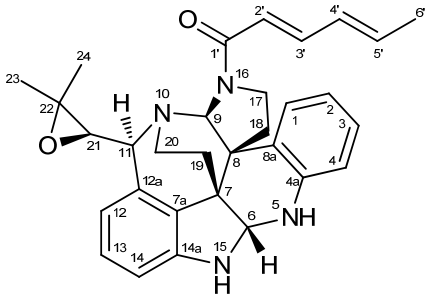
no.	δ_H (mult, *J* (Hz))	δ_C (mult)
1	6.61–6.74 (m)	123.2 d
2	6.61–6.74 (m)	120.6 d
3	7.01 (dt, 7.6, 1.8)	127.4 d
4	6.71 (br.d, 7.6)	116.9 d
4a		142.6 s
6	4.70 (s)	82.4 d
7		51.4 s
7a		132.4 s
8		51.9 s
8a		132.2 s
9	5.02 (s)	79.6 d
11	4.08 (d, 9.1)	65.4 d
12	6.06 (d, 7.6)	113.2 d
12a		136.8 s
13	6.88 (t, 7.6)	128.9 d
14	5.95 (d, 7.6)	101.8 d
14a		150.5 s
15a	2.84 (s)	29.6 d
17A	3.89 (dd, 11.6, 8.7)	44.1 t
17B	3.01 (dt, 11.6, 7.5)	
18A	2.74 (ddd, 13.2, 11.7, 9.0)	30.8 t
18B	1.97 (dd, 13.2, 7.1)	
19A	2.36 (m)	38.0 t
19B	2.27 (m)	
20A	3.47 (br.dd, 15.5, 10.0)	36.3 t
20B	3.36 (dt, 15.5, 8.5)	
21	2.87 (d, 8.8)	64.0 d
22		59.8 s
23	1.38 (s)	24.8 q
24	1.53 (s)	20.5 q
1'		172.0 s
2'	2.33 (s)	22.6 q

Table S4. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectroscopic data of communesin B (**2**) in $CDCl_3$.



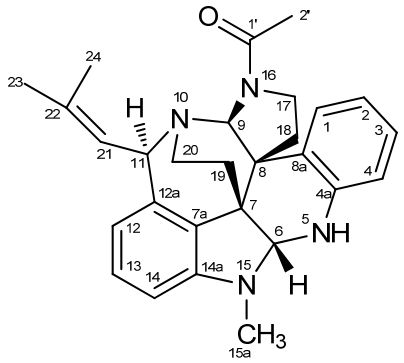
no.	δ_H (mult, *J* (Hz))	δ_C (mult)
1	6.62–6.71 (m)	123.4 d
2	6.62–6.71 (m)	120.5 d
3	6.98 (m)	127.5 d
4	6.62–6.71 (m)	116.8 d
4a		142.6 s
6	4.70 (s)	82.3 d
7		51.3 s
7a		132.3 s
8		52.1 s
8a		132.2 s
9	5.11 (s)	78.9 d
11	4.18 (d, 9.0)	65.5 d
12	6.08 (br.d, 7.7)	113.2 d
12a		136.5 s
13	6.88 (t, 7.7)	128.8 d
14	5.95 (br.d, 7.7)	101.8 d
14a		150.5 s
15a	2.85 (s)	29.6 q
17A	3.87 (dd, 12.1, 8.4)	44.2 t
17B	3.07 (m)	
18A	2.72 (m)	30.4 t
18B	2.00 (dd, 13.1, 6.9)	
19A	2.37 (m)	37.8 t
19B	2.27 (dt, 12.8, 9.1)	
20A	3.47 (m)	36.0 t
20B	3.41 (dd, 15.5, 8.2)	
21	2.90 (d, 9.0)	63.9 d
22		59.7 s
23	1.65 (s)	24.9 q
24	1.42 (s)	20.5 q
1'		168.4 s
2'	6.55 (d, 15.0)	121.2 d
3'	7.31 (dd, 15.0, 10.6)	141.8 d
4'	6.19 (m)	130.7 d
5'	6.11 (m)	137.1 d
6'	1.85 (d, 6.5)	18.7 q

Table S5. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectroscopic data of communesin C (**3**) in CD_3OD.



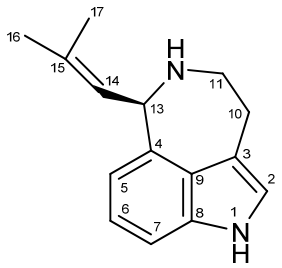
no.	δ_H (mult, *J* (Hz))	δ_C (mult)
1	6.59–6.66 (m)	123.8 d
2	6.59–6.66 (m)	120.4 d
3	6.95 (m)	128.3 d
4	6.71 (br.d, 7.7)	117.9 d
4a		145.1 s
6	4.92 (s)	80.8 d
7		53.0 s
7a		134.0 s
8		53.2 s
8a		132.6 s
9	5.23 (s)	78.2 d
11	4.26 (d, 9.0)	66.8 d
12	6.13–6.21 (m)	115.5 d
12a		137.9 s
13	6.79 (t, 7.7)	129.7 d
14	6.13–6.21 (m)	106.8 d
14a		151.8 s
17A	3.85 (dd, 11.9, 8.1)	45.5 t
17B	2.99 (m)	
18A	2.83 (m)	31.3 t
18B	1.95 (dd, 13.1, 7.1)	
19A	2.41 (m)	38.8 t
19B	2.26 (m)	
20A	3.45 (m)	37.0 t
20B	3.35 (m)	
21	2.95 (d, 9.0)	65.6 d
22		61.6 s
23	1.66 (s)	25.0 q
24	1.40 (s)	20.7 q
1'		170.6 s
2'	6.59–6.66 (m)	122.4 d
3'	7.21 (dd, 15.0, 10.4)	143.1 d
4'	6.28 (m)	131.9 d
5'	6.13–6.21 (m)	138.8 d
6'	1.86 (d, 6.8)	18.8 q

Table S6. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectroscopic data of communesin F (**6**) in $CDCl_3$.



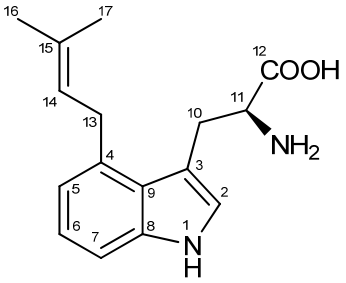
no.	δ_H (mult, *J* (Hz))	δ_C (mult)
1	6.65–6.75 (m)	123.2 d
2	6.65–6.75 (m)	120.6 d
3	7.00 (dt, 7.5, 1.0)	127.3 d
4	6.65–6.75 (m)	117.0 d
4a		142.7 s
6	4.66 (s)	82.6 d
7		51.2 s
7a		132.7 s
8		51.8 s
8a		131.3 s
9	5.30 (s)	79.6 d
11	5.05 (d, 8.7)	64.4 d
12	6.08 (d, 7.6)	114.7 d
12a		140.6 s
13	6.82 (t, 7.7)	128.4 d
14	5.86 (d, 7.6)	100.8 d
14a		150.1 s
15a	2.82 (s)	29.7 q
17A	3.85 (dd, 11.6, 8.9)	44.2 t
17B	3.03 (m)	
18A	2.74 (m)	30.9 t
18B	1.97 (m)	
19A	2.29 (m)	37.8 t
19B	2.21 (m)	
20A	3.34 (br.dd, 14.2, 10.0)	36.3 t
20B	3.14 (m)	
21	5.23 (br.d, 8.7)	124.6 d
22		136.1 s
23	1.79 (s)	26.0 q
24	1.85 (s)	18.5 q
1'		171.6 s
2'	2.41 (s)	22.7 q

Table S7. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectroscopic data of (−)-aurantioclavine (**10**) in $CDCl_3$.



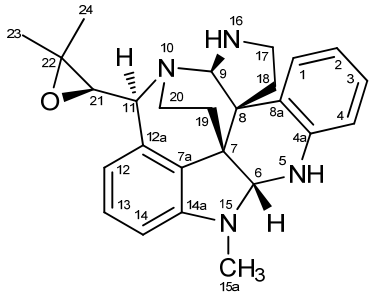
no.	δ_H (mult, *J* (Hz))	δ_C (mult)
2	7.02 (br.d, 1.4)	127.8 d
3		115.8 s
4		137.1 s
5	7.23 (d, 8.1)	120.9 d
6	7.10 (t, 7.7)	121.5 d
7	6.84 (dt, 7.3, 0.9)	109.1 d
8		138.6 s
9		133.2 s
10	2.96–3.16 (m)	31.0 t
11a	3.56 (m)	48.9 t
11b	2.96–3.16 (m)	
13	4.89 (d, 9.0)	62.6 d
14	5.47 (br.d, 9.0)	117.8 d
15		125.4 s
16	1.84 (d, 1.2)	25.8 q
17	1.85 (d, 1.2)	18.3 q

Table S8. ^{1}H NMR (500 MHz) spectroscopic data of 4-L-DMAT (**11**) in $CDCl_3$.



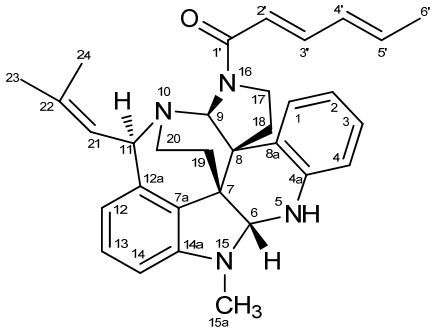
no.	δ_H (mult, *J* (Hz))
1	–
2	7.16 (s)
3	–
4	–
5	7.19 (d, 8.0)
6	7.00 (t, 7.9)
7	6.78 (d, 7.2)
8	–
9	–
10	3.11–3.86 (m)
11	3.11–3.86 (m)
12	–
13	3.11–3.86 (m)
14	5.33 (m)
15	–
16	1.77 (br.s)
17	1.75 (br.s)

Table S9. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectroscopic data of communesin I (**12**) in $CDCl_3$.



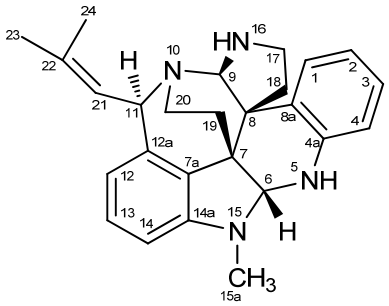
no.	δ_H (mult, *J* (Hz))	δ_C (mult)
1	6.68 (dt, 8.8, 7.5, 1.2)	123.9 d
2	6.60 (dd, 7.5, 1.2)	119.8 d
3	6.96–6.92 (m)	126.8 d
4	6.96–6.92 (m)	116.3 d
4a		143.5 s
6	4.65 (s)	82.2 d
7		51.3 s
7a		134.2 s
8		51.5 s
8a		132.9 s
9	4.88 (s)	80.1 d
11	4.03 (d, 8.8)	65.2 d
12	6.02 (d, 7.8)	113.7 d
12a		136.7 s
13	6.84 (t, 7.8)	128.6 d
14	5.96 (d, 7.8)	102.2 d
25	2.86 (s)	29.8 d
14a		150.4 s
17a	3.62 (ddd, 15.0, 9.2, 6.5)	44 t
17b	2.72–2.62 (m)	
18a	2.72–2.62 (m)	34.6 t
18b	1.83–1.78 (m)	
19a	2.41–2.36 (ddd, 12.7, 9.2, 3.3)	35.5 t
19b	2.18–2.12 (m)	
20a	3.32–3.26 (m)	35.8 t
20b	3.19–3.14 (m)	
21	2.90 (d, 8.8)	64.8 d
22		60.2 s
23	1.34 (s)	25.1 q
24	1.51 (s)	20.2 q

Table S10. ^{1}H NMR (500 MHz), ^{13}C NMR (125 MHz), and 2D-NMR spectroscopic data of communesin J (**13**) in $CDCl_3$.



no.	δ_H (mult, *J* (Hz))	δ_C (mult)	COSY	HMBC
1	6.68–6.65 (m)	123.5		127.4, 52.1
2	6.68–6.65 (m)	120.8	6.99	131.5, 117
3	6.99 (ddd, 7.6, 5.3, 2.3)	127.4	6.68–6.65	142.8, 123.5
4	6.68–6.65 (m)	117	6.99	120.8
4a		142.8		
6	4.67 (s)	82.8		150.2, 142.8, 131.5, 52.1, 37.9, 36.3
7		51.3		
7a		132.8		
8		52.1		
8a		131.5		
9	5.21 (m)	78.6		132.8, 64.5, 51.3, 44.6, 36.3
11	5.15 (m)	64.5	5.22	140.8, 136.5, 132.8, 124.9, 114.8, 26.1
12	6.11 (d, 7.5)	114.8	6.83	132.8, 100.9, 64.5
12a		140.8		
13	6.83 (t, 7.5)	128.5	6.11, 5.87	150.2, 140.8, 100.9
14	5.87 (d, 7.5)	100.9	6.83	132.8, 114.8
14a		150.2		
15a	2.82 (s)	30.8		150.2, 82.8
17a	3.90 (dd, 12.1, 8.2)	44.6	2.75, 1.99	51.3
17b	3.09 (dd, 12.1, 8.2)			
18a	2.75 (td, 8.8, 11.6, 13.1)	29.9	3.90, 3.09	132.8, 30.5
18b	1.99 (dd, 8.0, 13.1)			
19a	2.31–2.25 (m)	37.9	3.35, 3.22–3.14	132.8, 82.8, 51.3
19b	2.24–2.21 (m)			
20a	3.35 (dd, 15.8, 9.3), 3.22-3.14 (m)	36.3	2.31–2.25, 2.24–2.21	78.6, 64.5
20b				
21	5.22 (m)	124.9	5.15	
22		136.5		
23	1.79 (d, 1.0)	26.1		136.5, 124.9
24	1.94 (d, 1.0)	18.8		136.5, 124.9, 18.8
1'		167.6		
2'	6.50 (d, 15.0)	120.5	7.39	167.6, 130.4
3'	7.39 (dd, 15.0, 11.0)	142.3	6.5, 6.26	167.6, 137.9
4'	6.26 (dd, 13.0, 10.8)	130.4	7.39, 6.15	137.9, 18.8
5'	6.15 (dq, 6.5, 13.0)	137.9	6.26, 1.86	142.3, 130.4, 18.8
6'	1.86 (d, 6.5)	18.8	6.15	137.9, 130.4

Table S11. ^{1}H NMR (500 MHz), ^{13}C NMR (125 MHz), and 2D-NMR spectroscopic data of communesin K (**14**) in CD_3OD.



no.	δ_H (mult, *J* (Hz))	δ_C (mult)	HMBC
1	6.87 (m)	124.0 d	145.5, 129.1, 52.2
2	6.72 (m)	120.9 d	132.4, 118.9
3	7.04 (m)	129.1 d	124.0, 145.5
4	6.84 (br.d, 7.8)	118.8 d	132.4, 121.0
4a		145.5 s	
6	4.68 (s)	83.2 d	152.0, 145.5, 131.7, 52.2, 36.7
7		52.2 s	
7a		131.7 s	
8		52.2 s	
8a		132.4 s	
9	5.46 (s)	83.2 d	132.4, 36.6, 34.0, 66.8, 52.2, 44.3
11	5.23 (m)	66.8 d	124.8, 36.5, 18.7
12	6.02 (d, 7.8)	115.3 d	131.7, 102.3, 66.8
12a		139.4 s	
13	6.78 (t, 7.8)	130.0 d	152.0, 139.4
14	5.87 (d, 7.8)	102.3 d	131.7, 115.3
14a		152.0 s	
15a	2.80 (s)	29.9 q	152.0, 83.2
17	3.36 (m)	44.2 t	83.2, 52.2
17b	2.74 (m)		
18a	2.81 (m)	34.0 t	132.4, 83.2
18b	2.17 (m)		
19a	2.39 (m)	36.5 t	131.7, 83.2, 52.2
19b	2.22 (m)		52.2
20a	3.49 (m)	36.5 t	83.2
20b	3.26 (m)		145.5, 66.8
21	5.23 (m)	124.8 d	
22			
23	1.81 (br.s)	25.9 q	140.6, 124.7, 18.7
24	1.88 (br.s)	18.7 q	140.6, 124.7, 26.0

Supplementary Figures

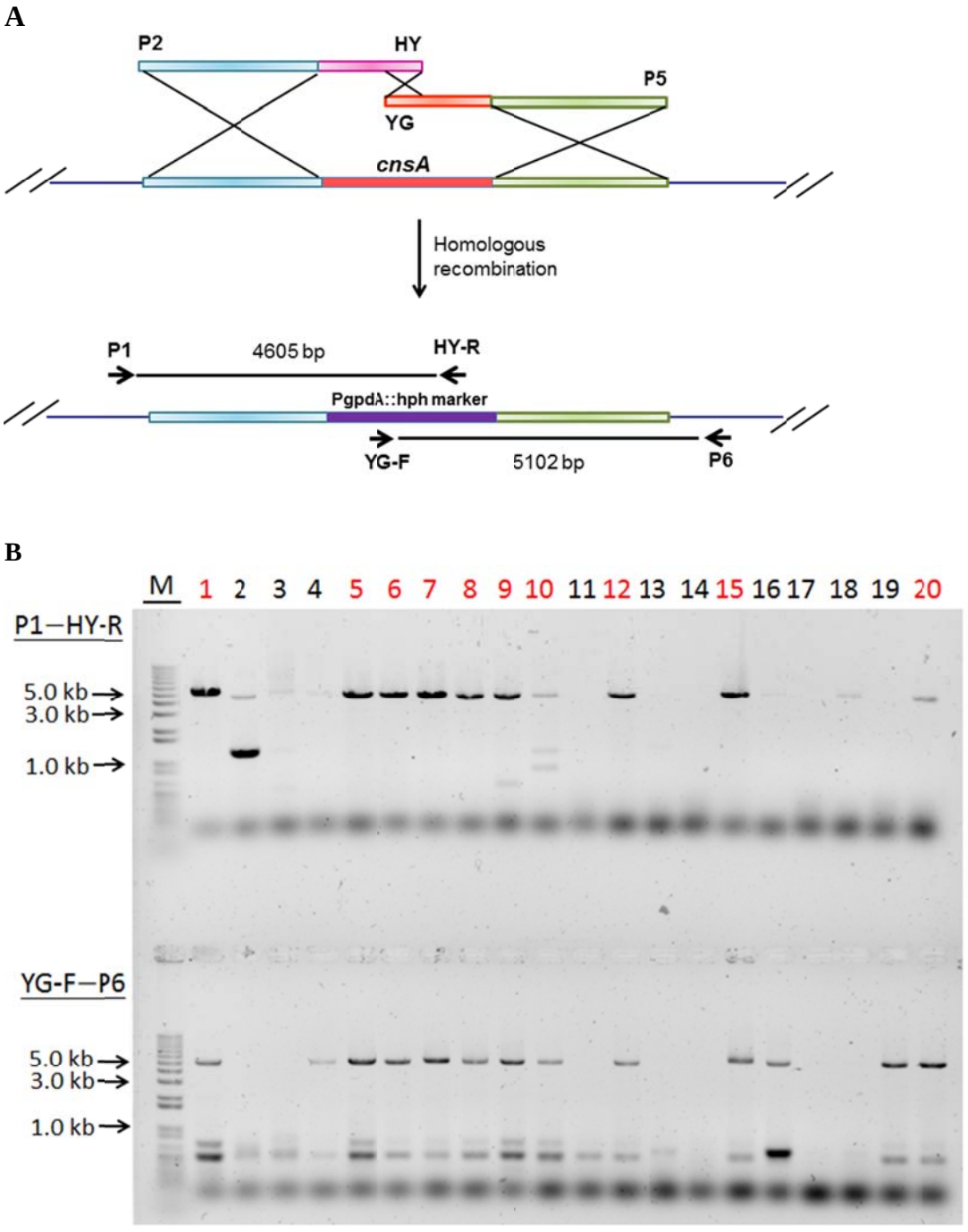


Figure S1. (A) Targeted deletion of *cnsA* with knockout cassette with split marker (B) PCR screening using primer pairs of P1 and HY-R, YG-F amd P6. Colony number shown in red indicates that it passed the screening by using both primer pairs

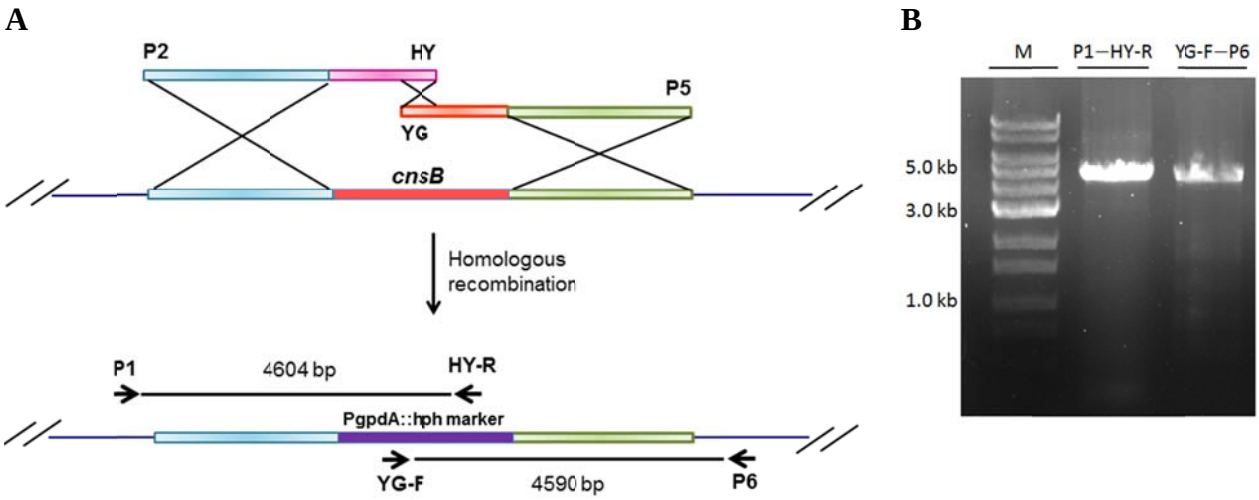
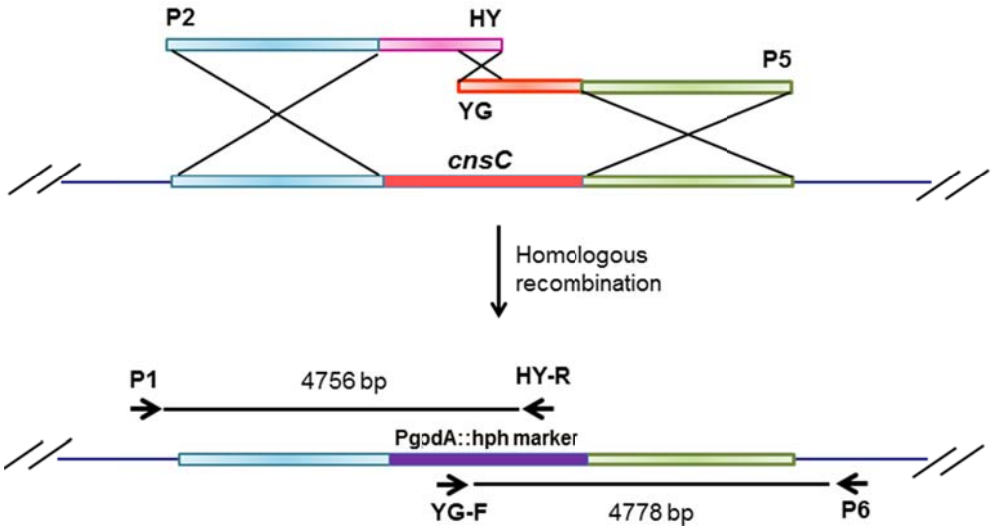


Figure S2. (A) Targeted deletion of *cnsB* with knockout cassette with split marker (B) PCR screening using primer pairs of P1 and HY-R, YG-F amd P6.

A



B

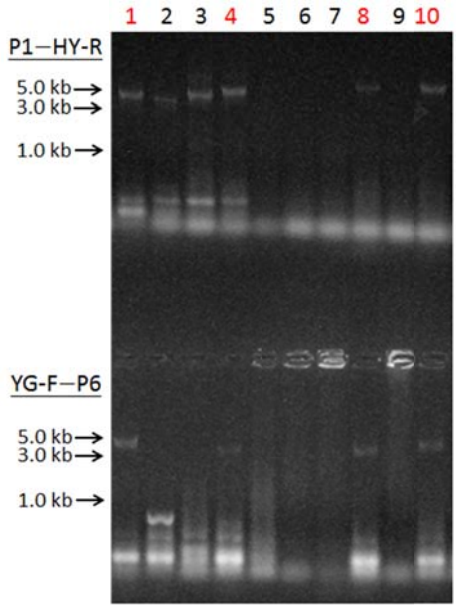


Figure S3. (A) Targeted deletion of *cnsC* with knockout cassette with split marker (B) PCR screening using primer pairs of P1 and HY-R, YG-F amd P6. Colony number shown in red indicates that it passed the screening by using both primer pairs

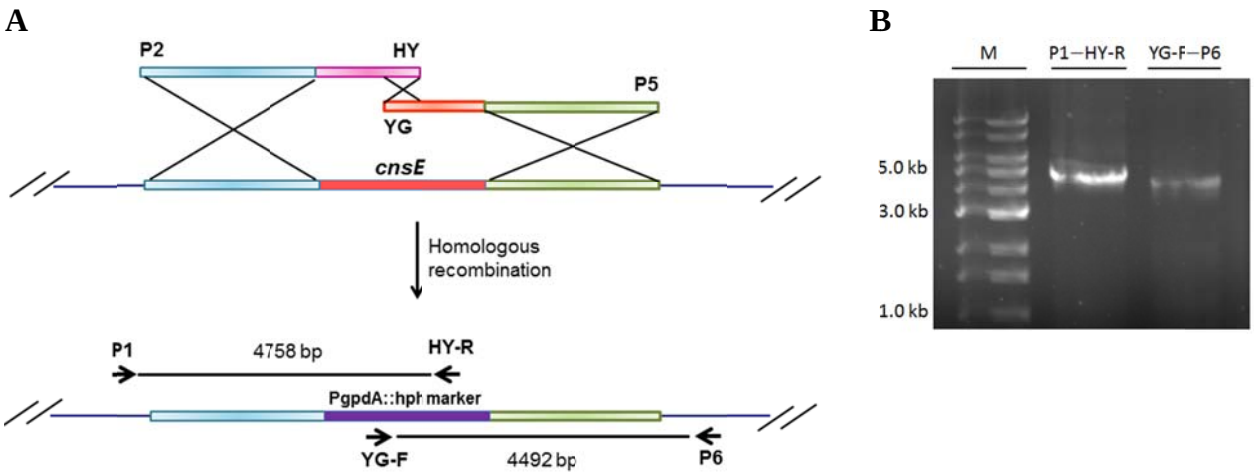


Figure S4. (A) Targeted deletion of *cnsE* with knockout cassette with split marker (B) PCR screening using primer pairs of P1 and HY-R, YG-F amd P6.

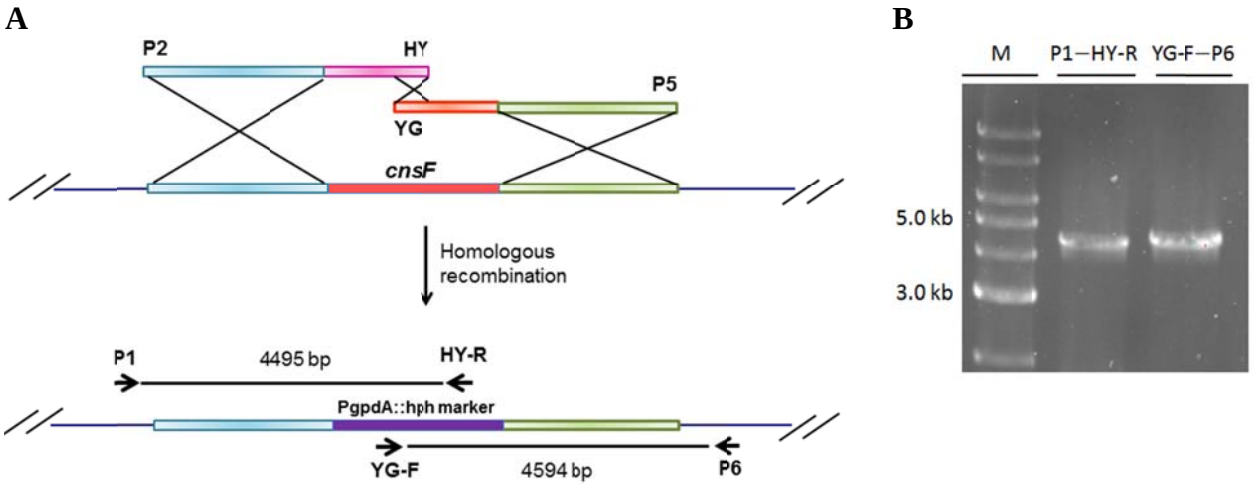


Figure S5. (A) Targeted deletion of *cnsF* with knockout cassette with split marker (B) PCR screening using primer pairs of P1 and HY-R, YG-F amd P6.

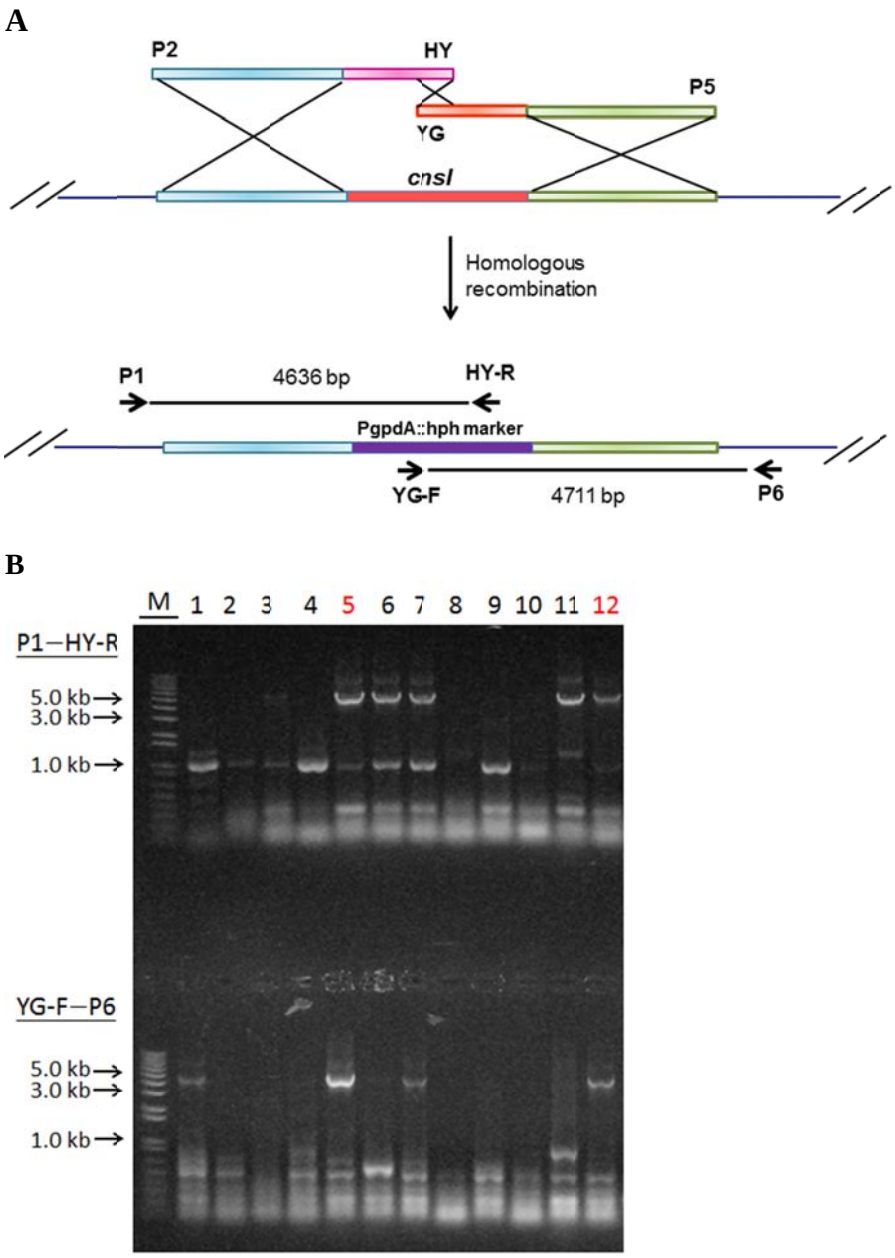


Figure S6. (A) Targeted deletion of *cnsI* with knockout cassette with split marker (B) PCR screening using primer pairs of P1 and HY-R, YG-F amd P6. Colony number shown in red indicates that it passed the screening by using both primer pairs.

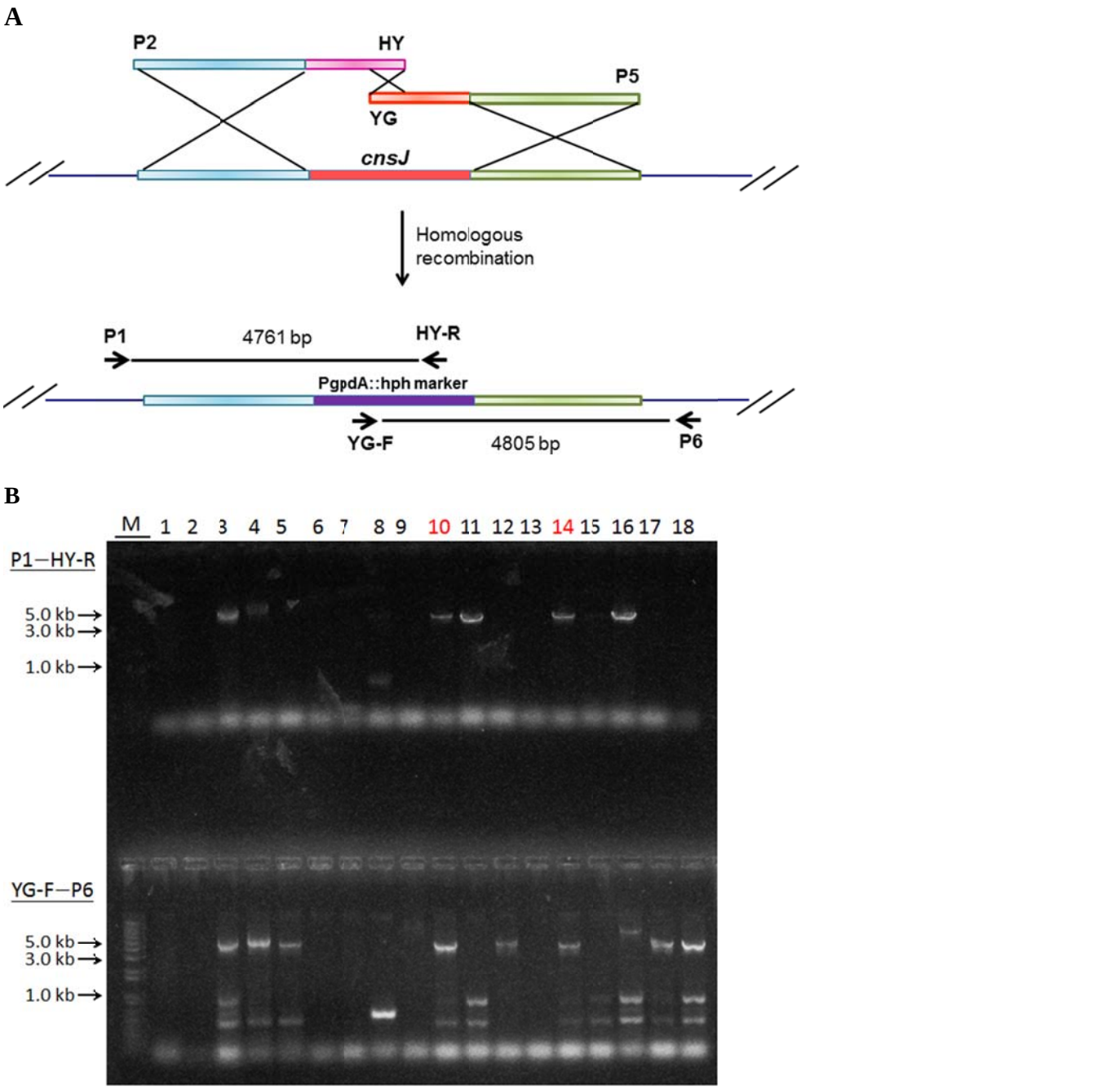


Figure S7. (A) Targeted deletion of *cnsJ* with knockout cassette with split marker (B) PCR screening using primer pairs of P1 and HY-R, YG-F amd P6. Colony number shown in red indicates that it passed the screening by using both primer pairs.

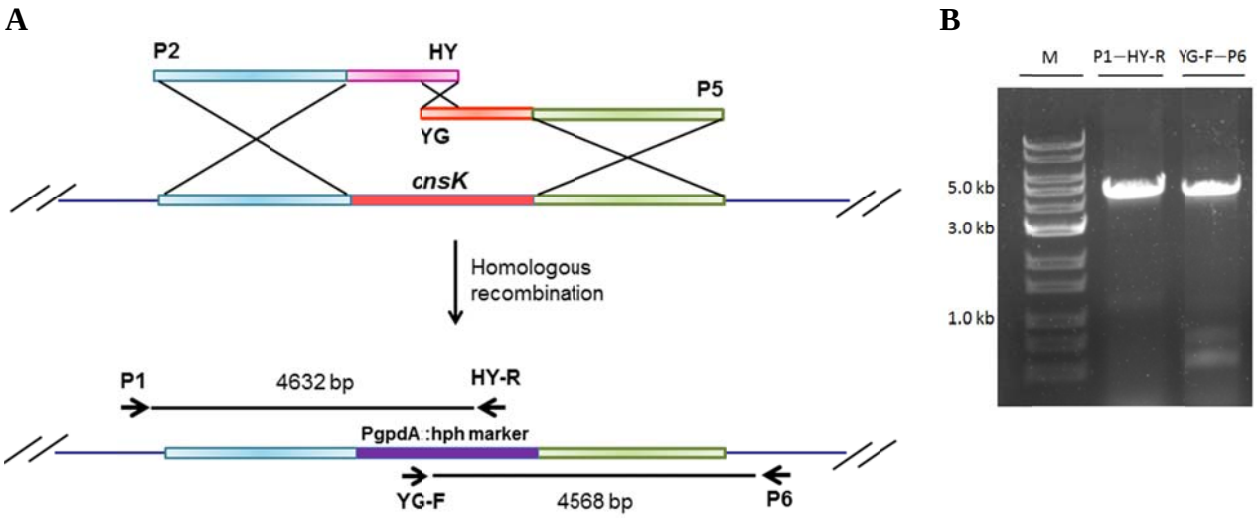


Figure S8. (A) Targeted deletion of *cnsK* with knockout cassette with split marker (B) PCR screening using primer pairs of P1 and HY-R, YG-F amd P6.

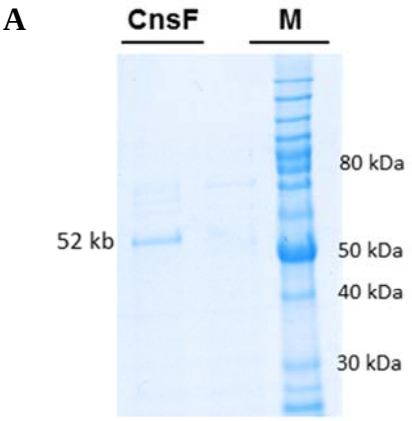


Figure S9. Recombinant CnsF purified from *E.coli* BL21 (DE3). SDS-PAGE analysis of purified CnsF (52 kDa).

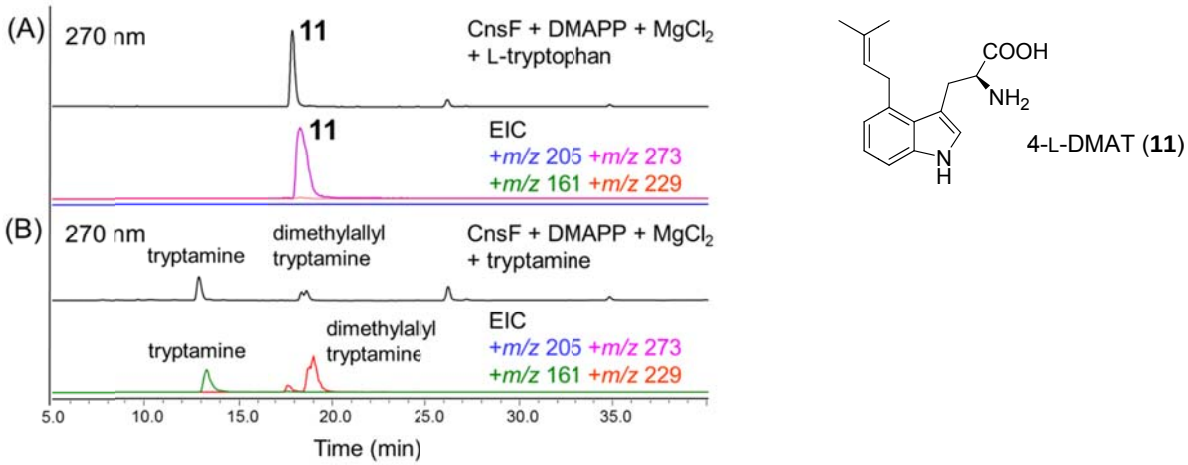


Figure S10. LC-MS analysis of in vitro assays of CnsF with (A) L-tryptophan and (B) tryptamine.

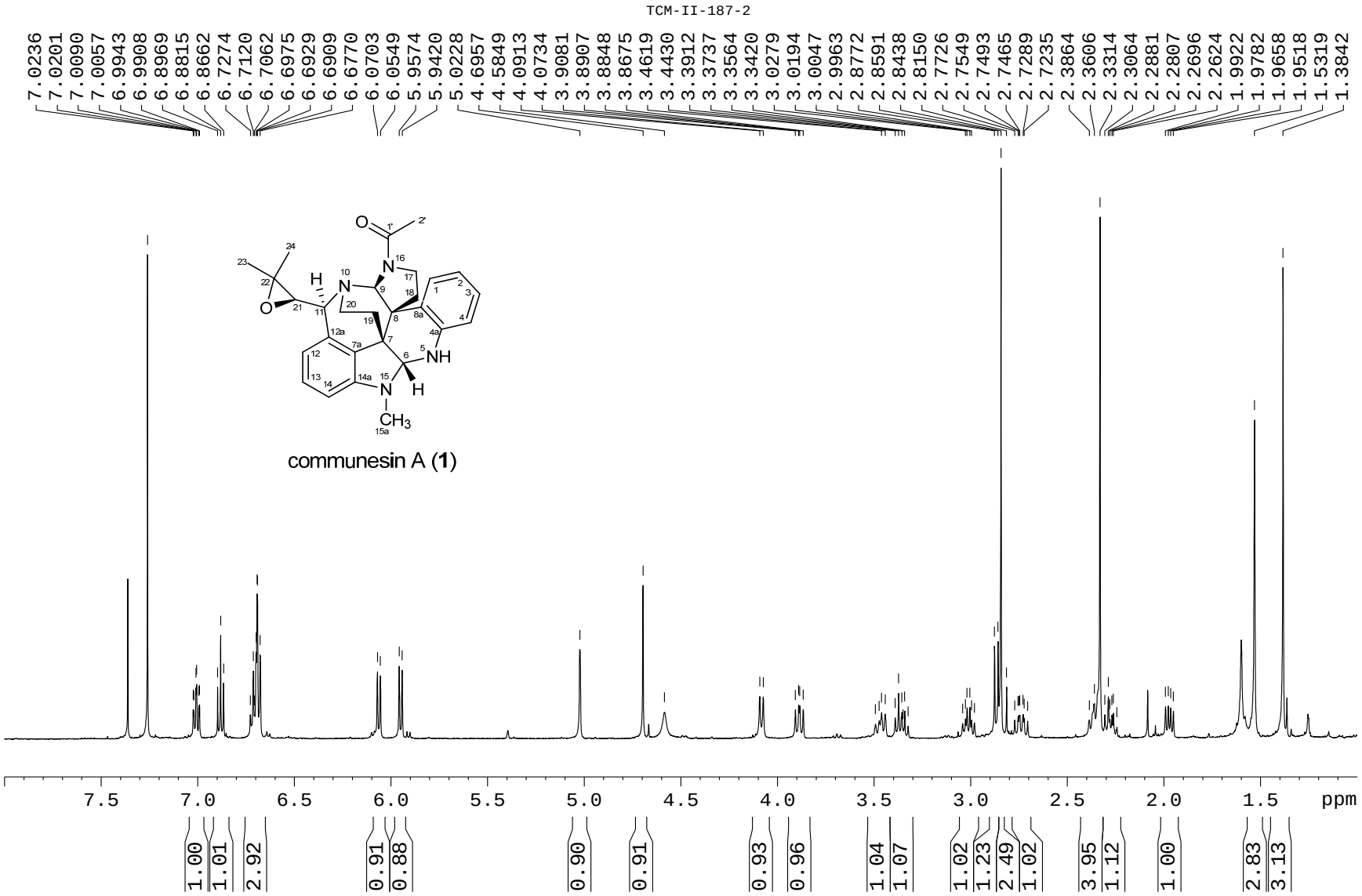


Figure S11. ^{1}H NMR spectrum of **1** ($CDCl_3$, 500 MHz).

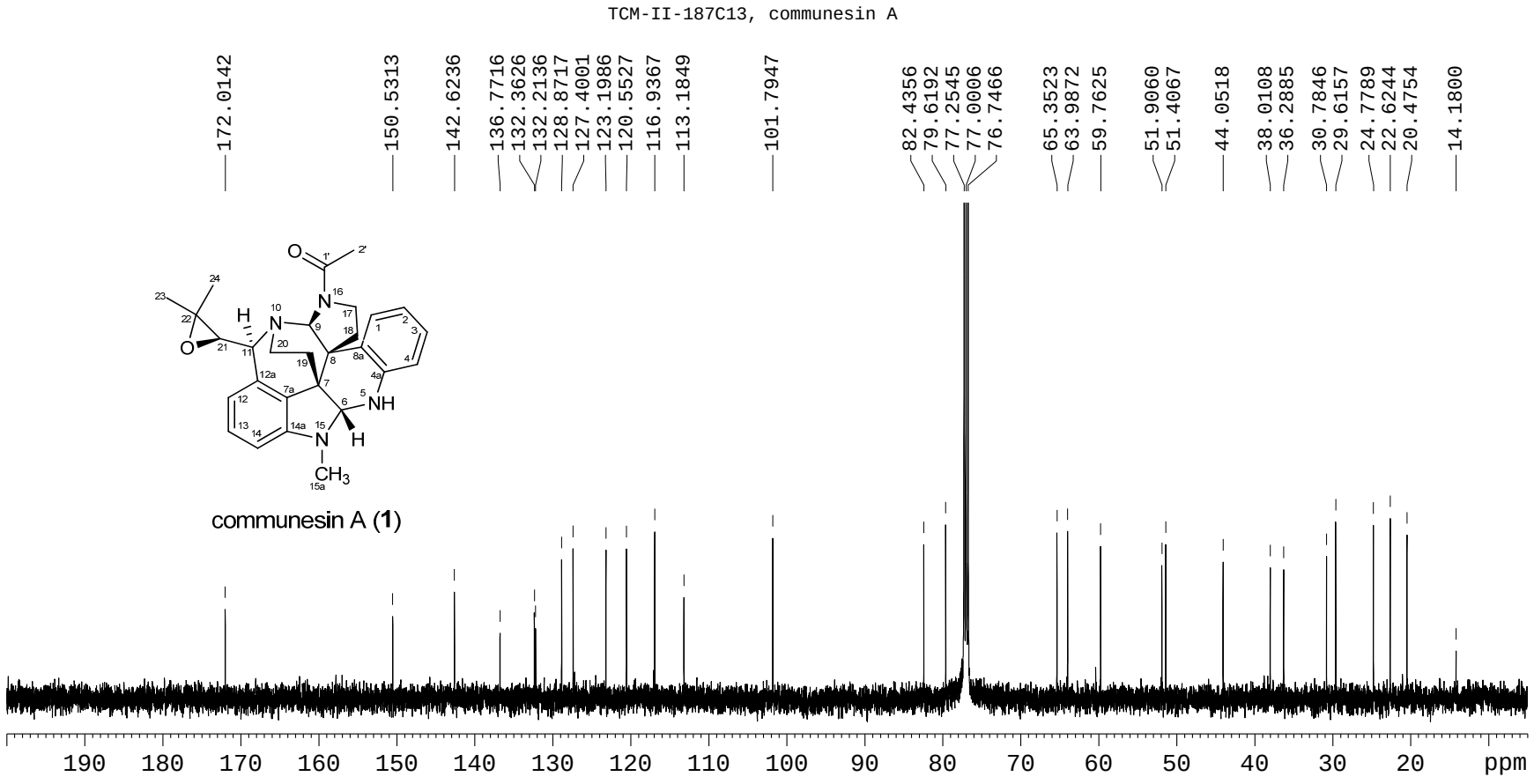


Figure S12. ^{13}C NMR spectrum of **1** ($CDCl_3$, 125 MHz).

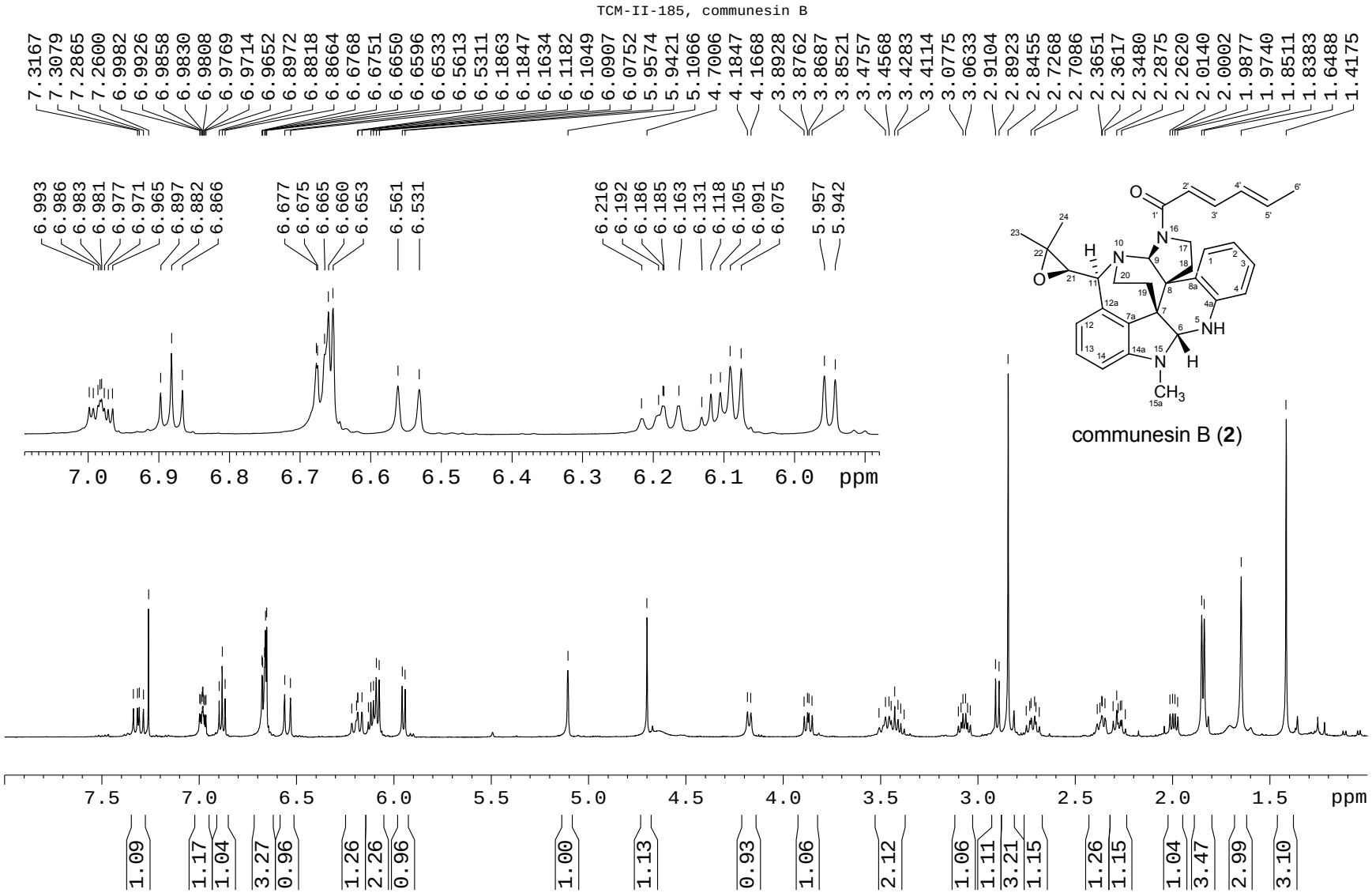


Figure S13. ^{1}H NMR spectrum of **2** ($CDCl_3$, 500 MHz).

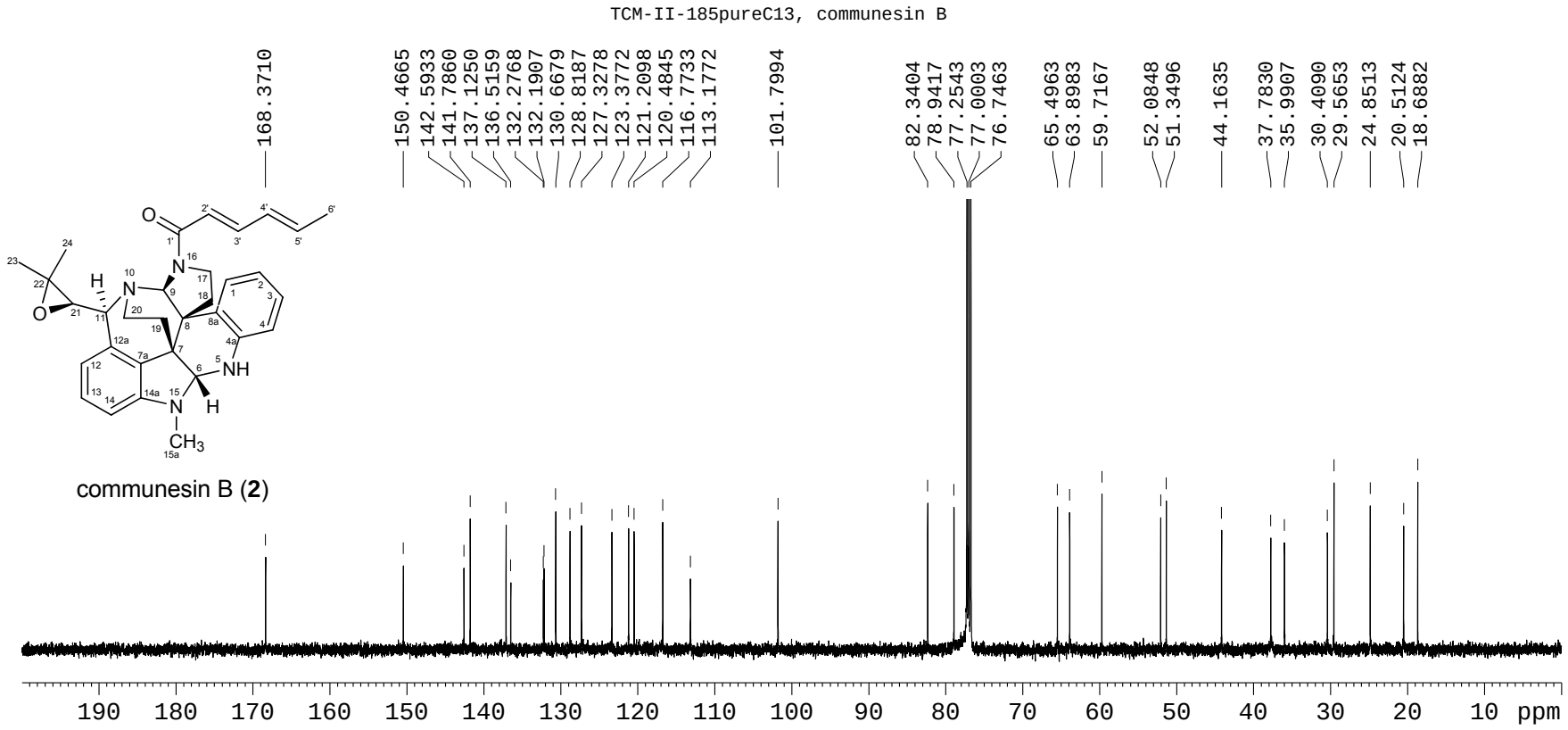


Figure S14. ^{13}C NMR spectrum of **2** ($CDCl_3$, 125 MHz).

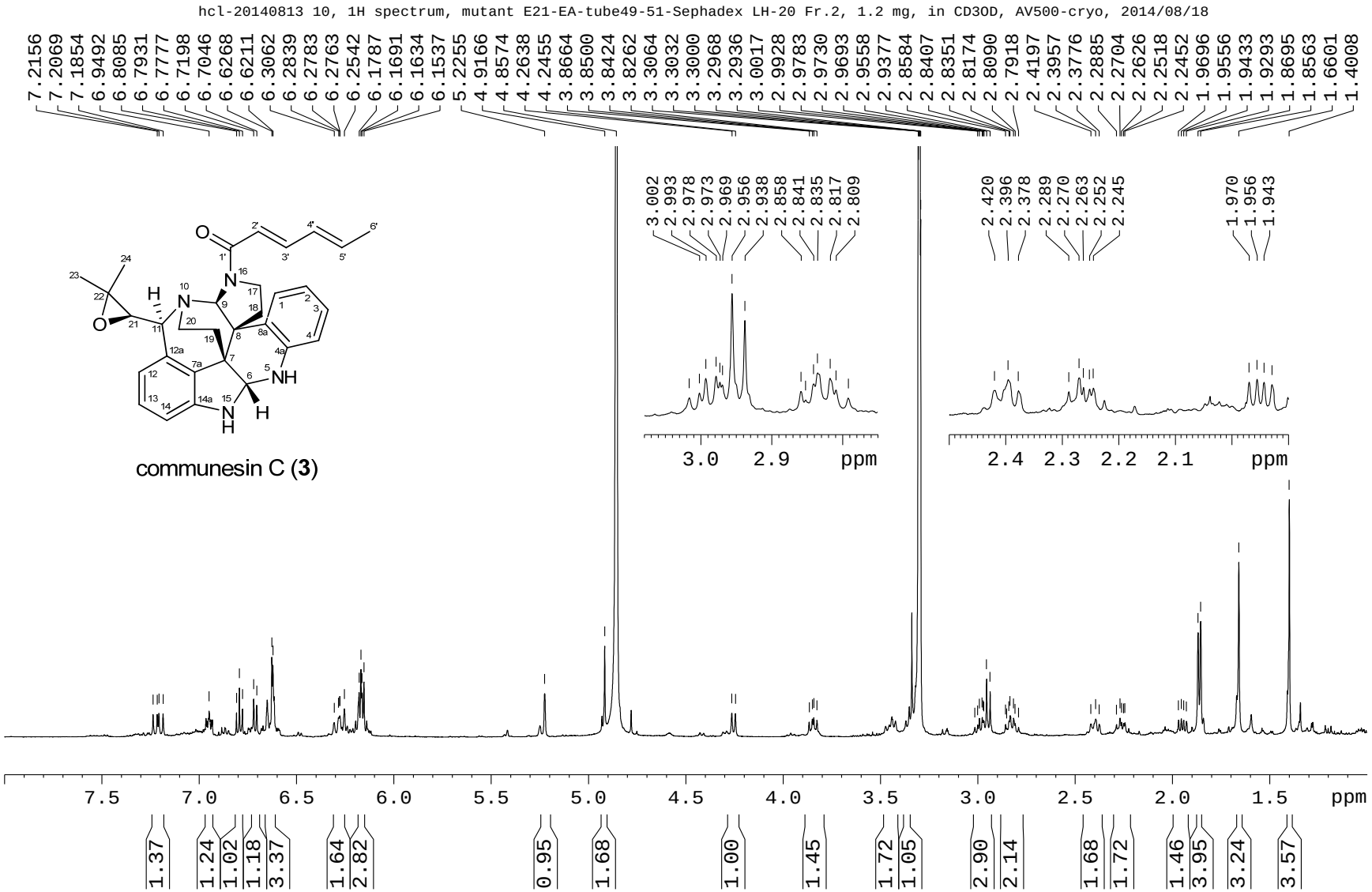
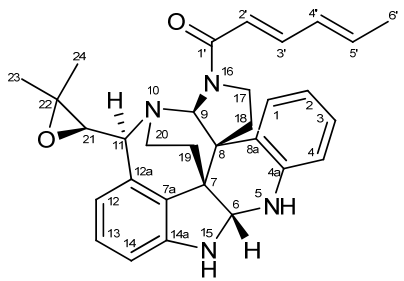


Figure S15. ^{1}H NMR spectrum of **3** ($CDCl_3$, 500 MHz).



communesin C (**3**)

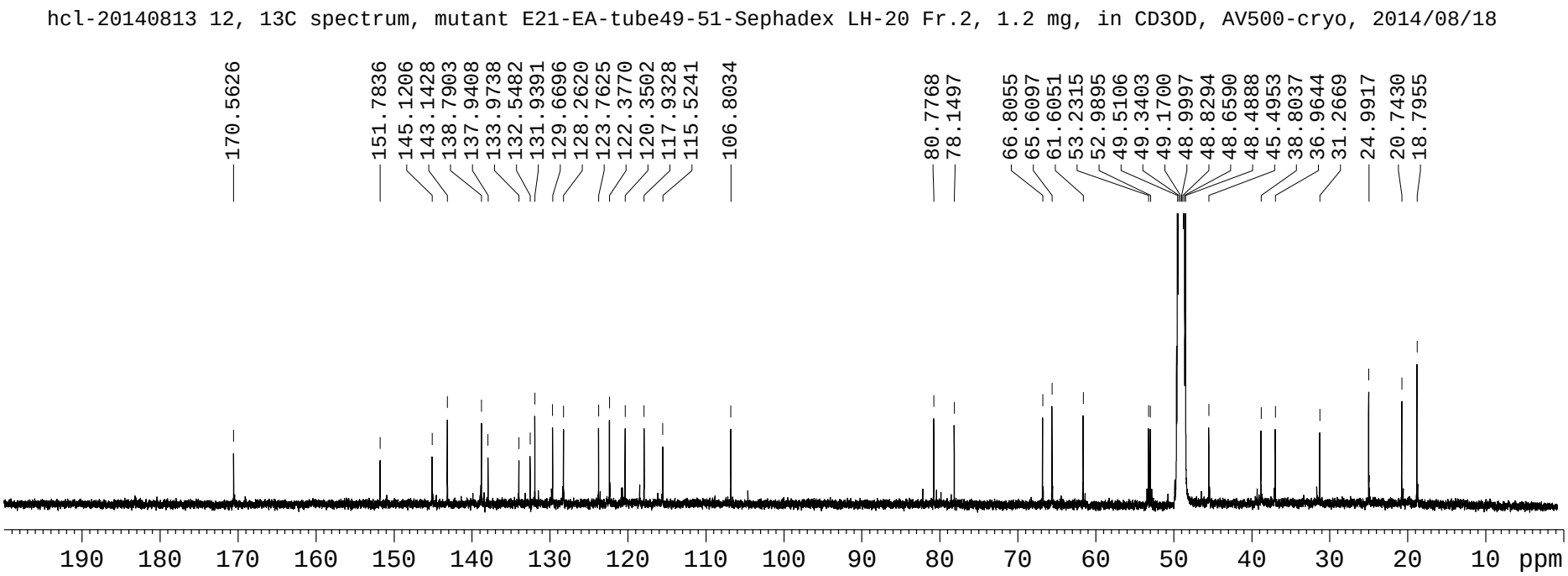


Figure S16. ^{13}C NMR spectrum of **3** ($CDCl_3$, 125 MHz).

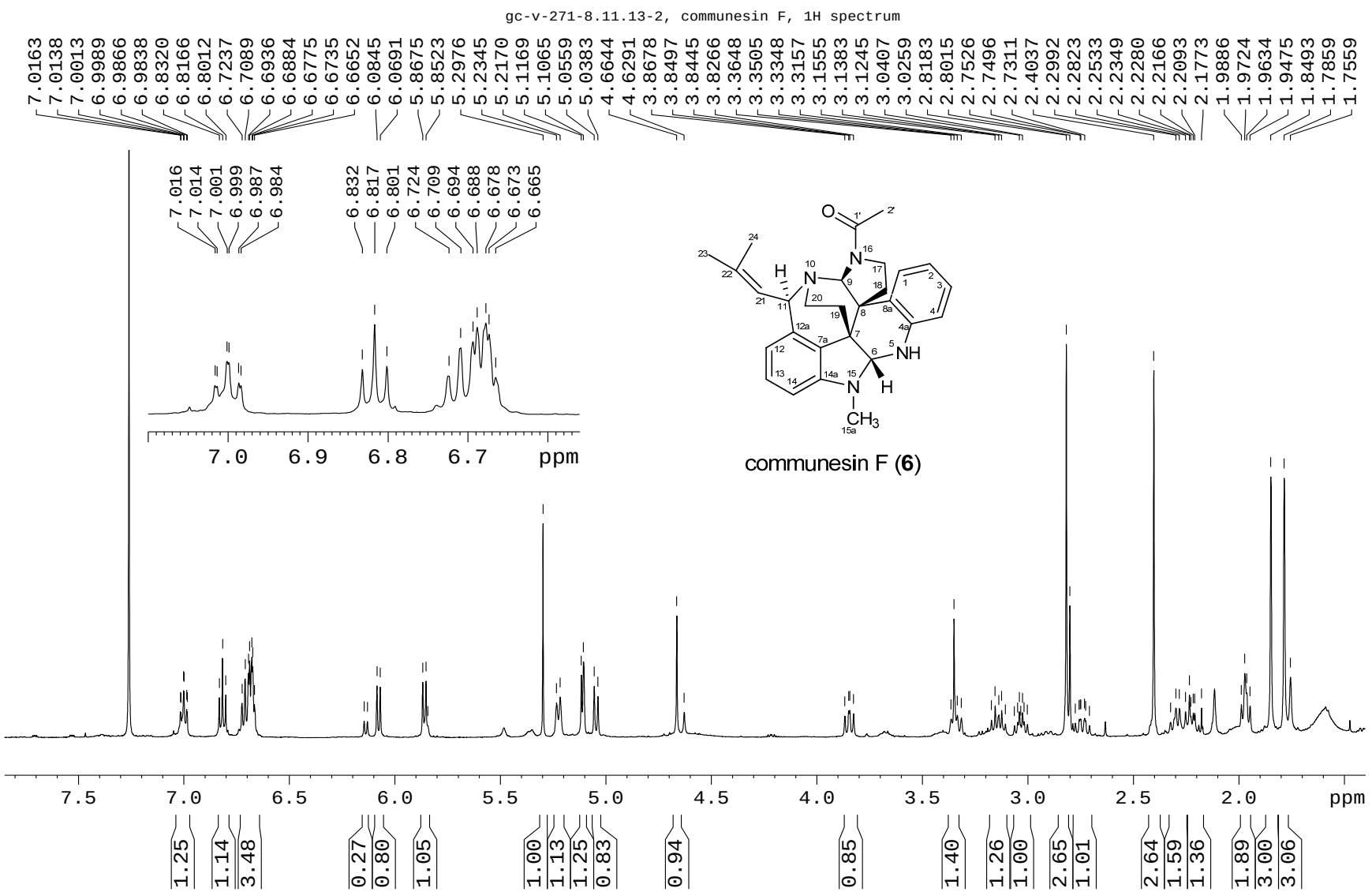


Figure S17. ^{1}H NMR spectrum of **6** ($CDCl_3$, 500 MHz).

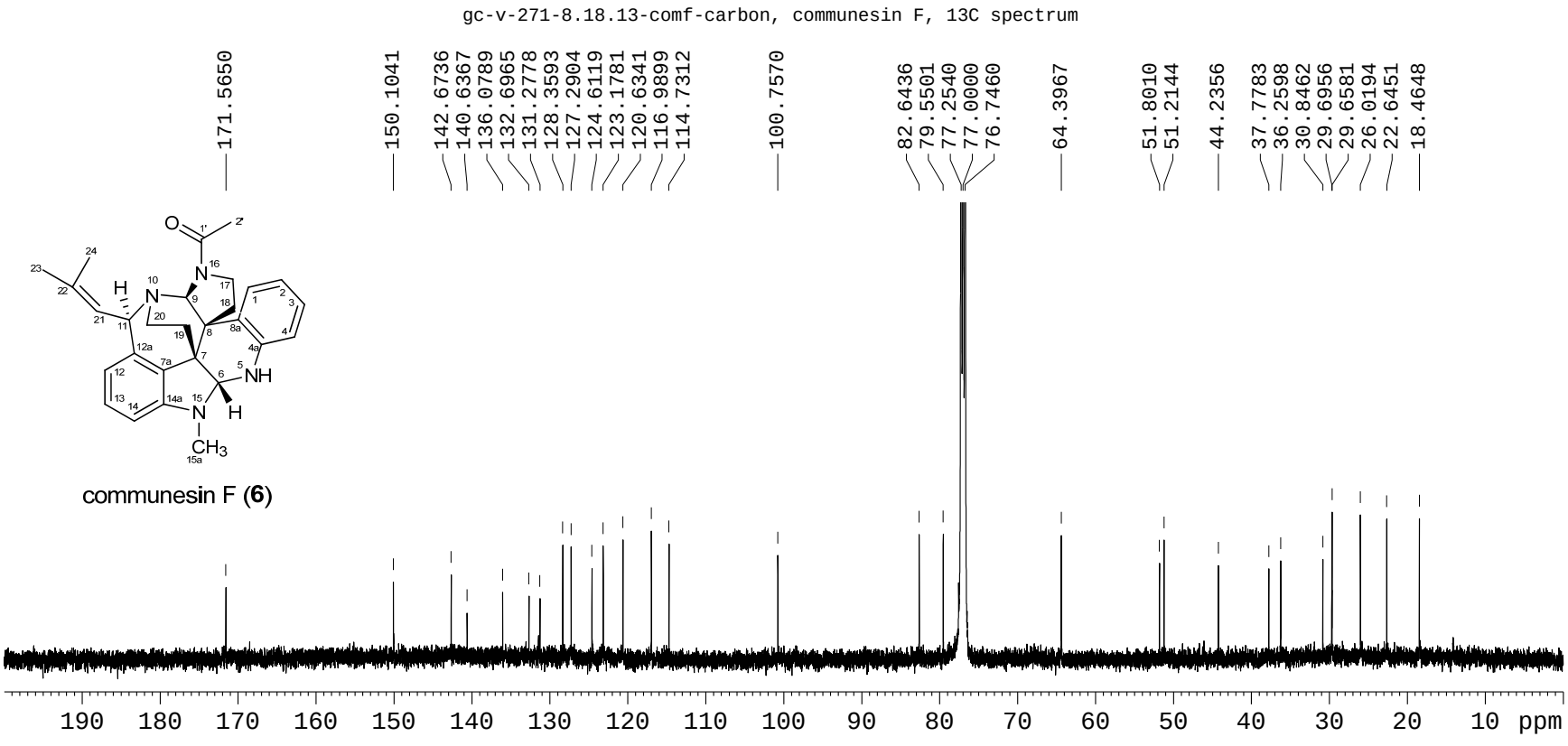


Figure S18. ^{13}C NMR spectrum of **6** ($CDCl_3$, 125 MHz).

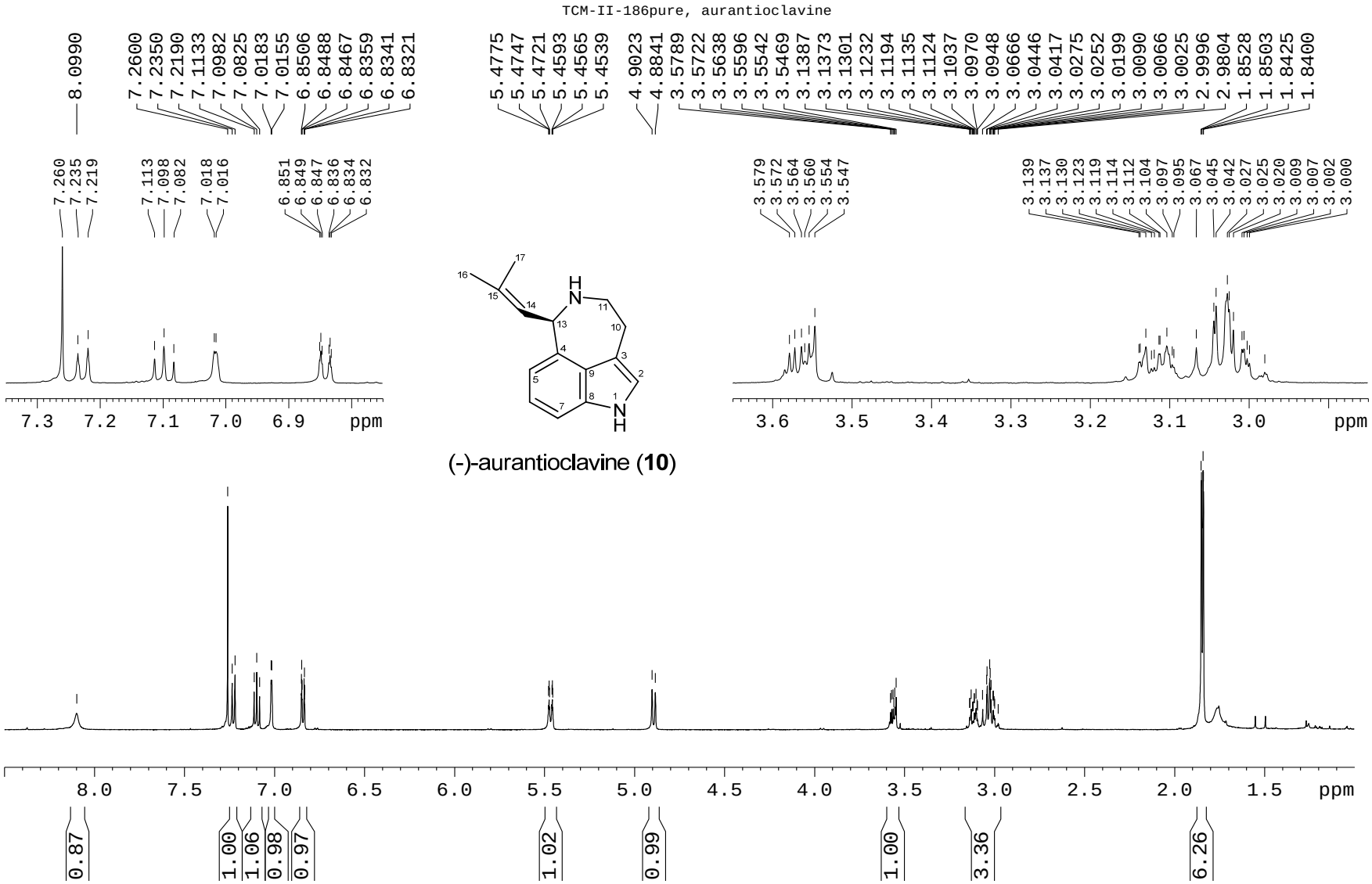


Figure S19. ^{1}H NMR spectrum of **10** ($CDCl_3$, 500 MHz).

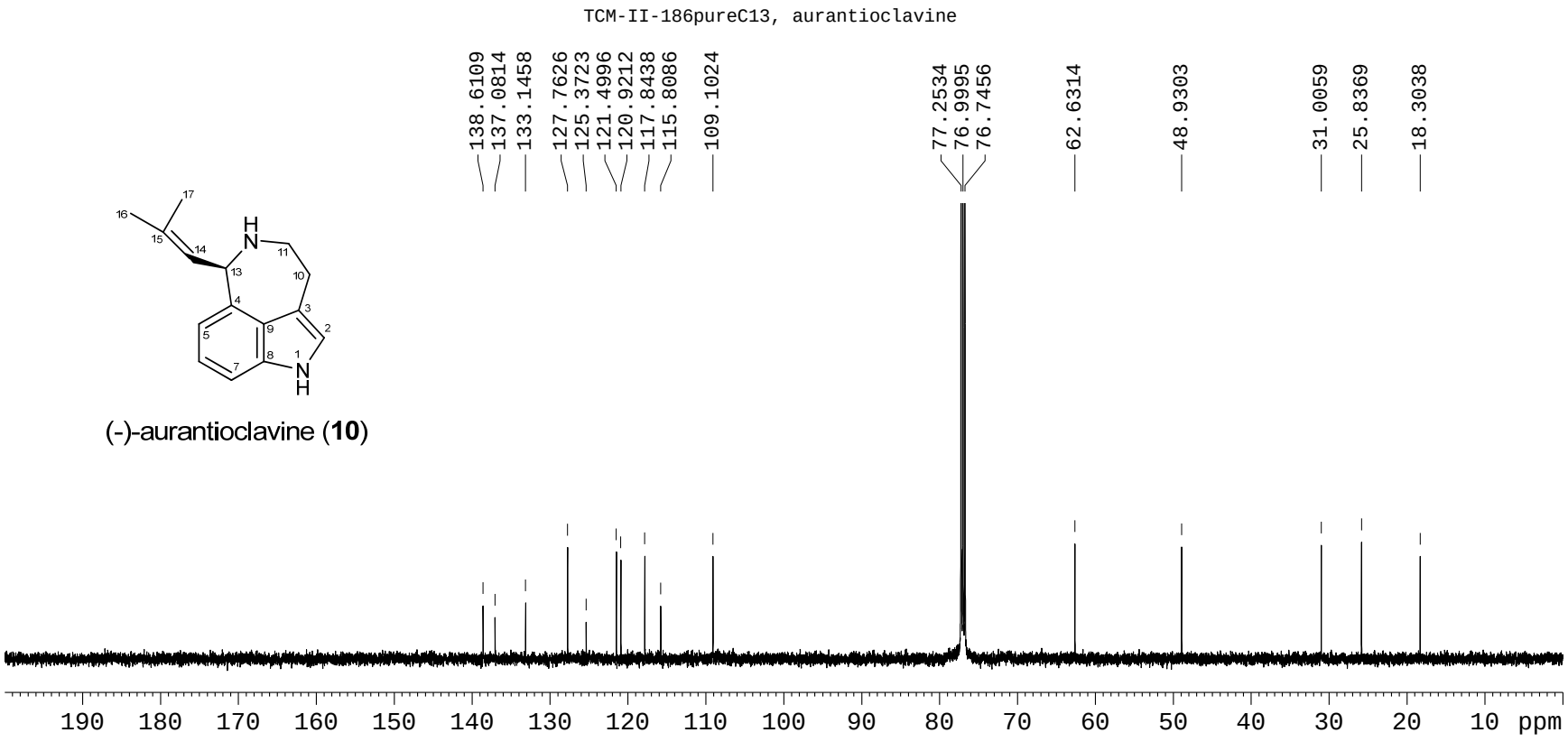


Figure S20. ^{13}C NMR spectrum of **10** ($CDCl_3$, 125 MHz).

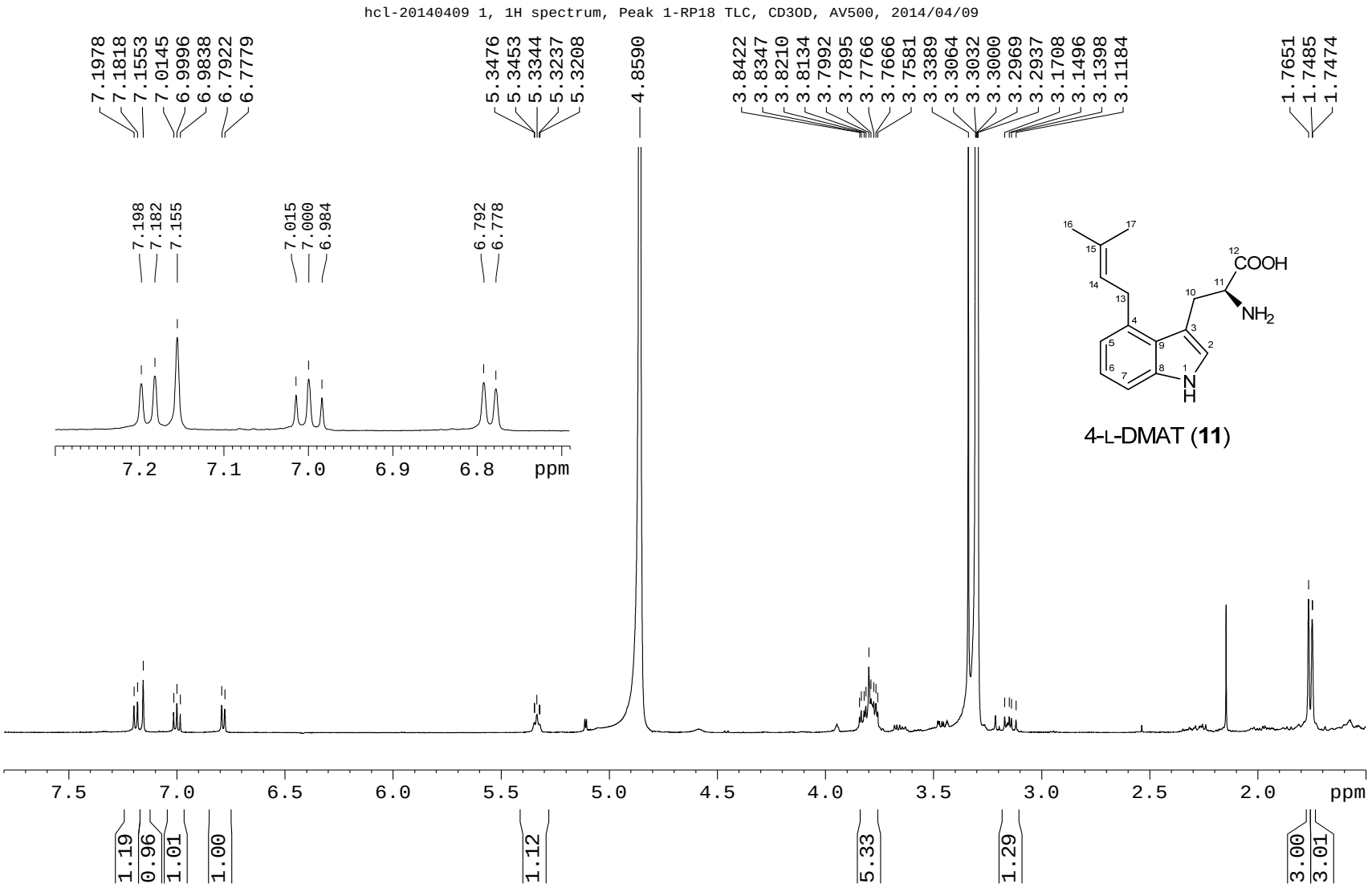


Figure S21. ^{1}H NMR spectrum of **11** (CD_3OD, 500 MHz). 4-L-DMAT.

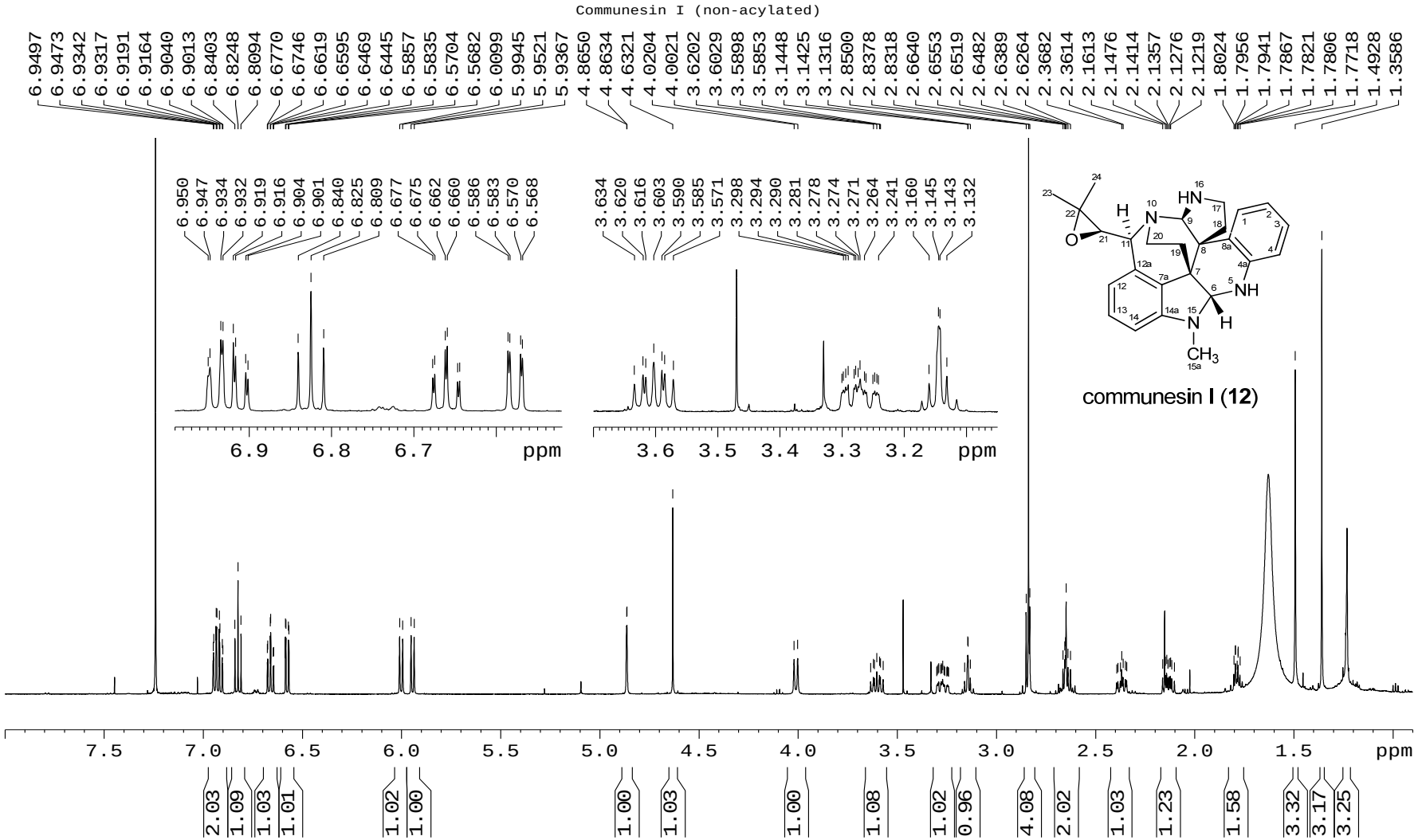


Figure S22. ^{1}H NMR spectrum of **12** ($CDCl_3$, 500 MHz).

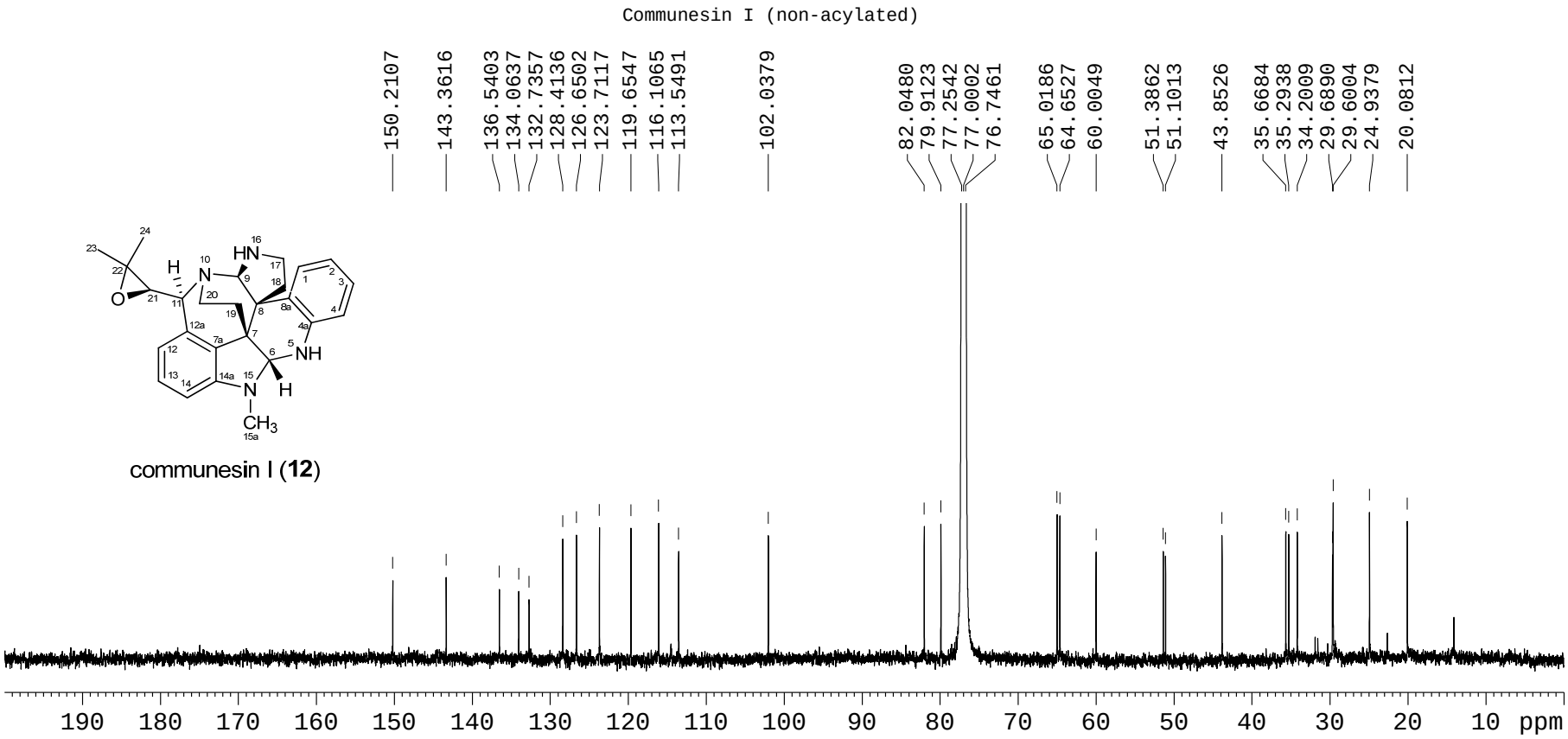


Figure S23. ^{13}C NMR spectrum of **12** ($CDCl_3$, 125 MHz).

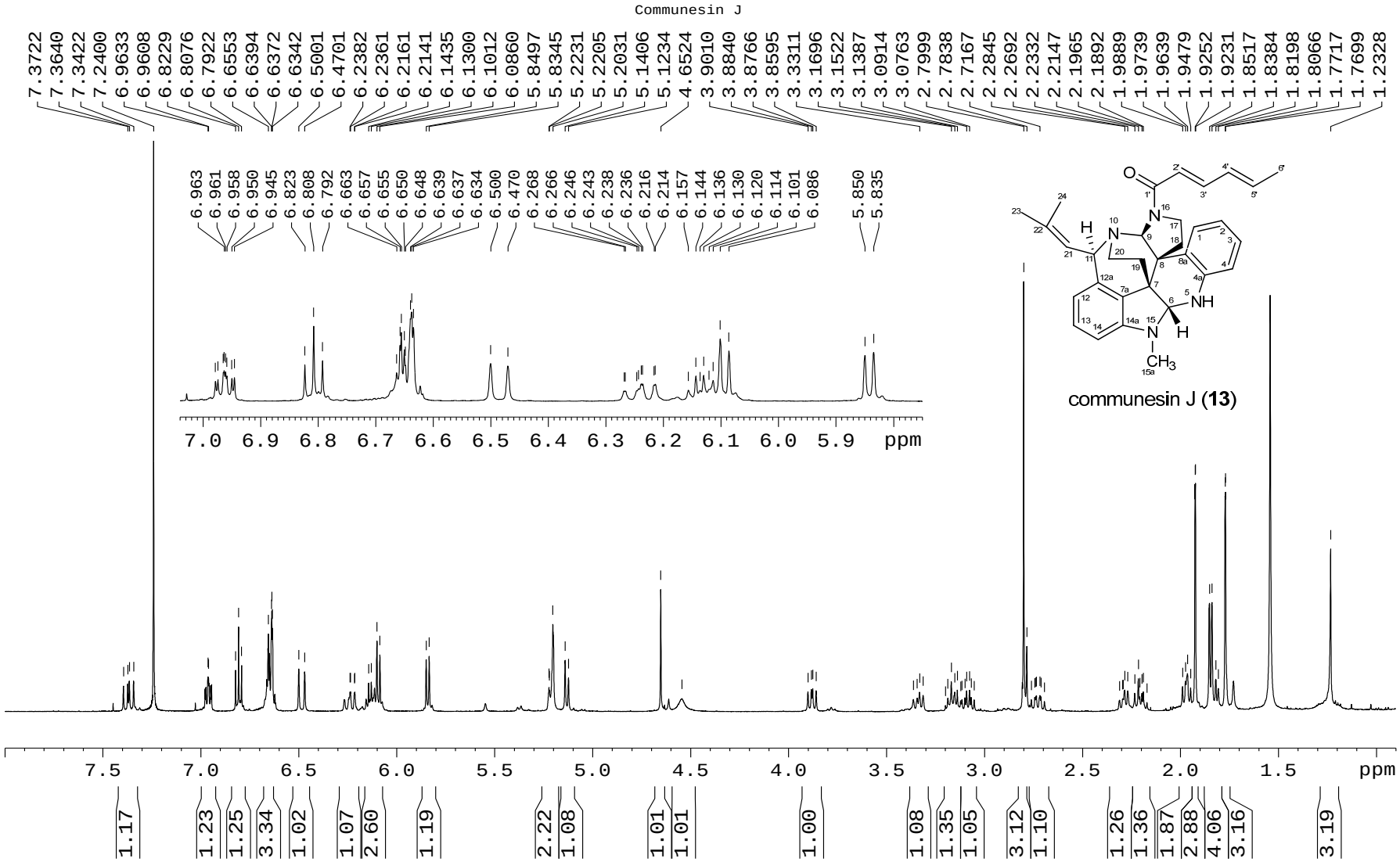


Figure S24. ^{1}H NMR spectrum of **13** ($CDCl_3$, 500 MHz).

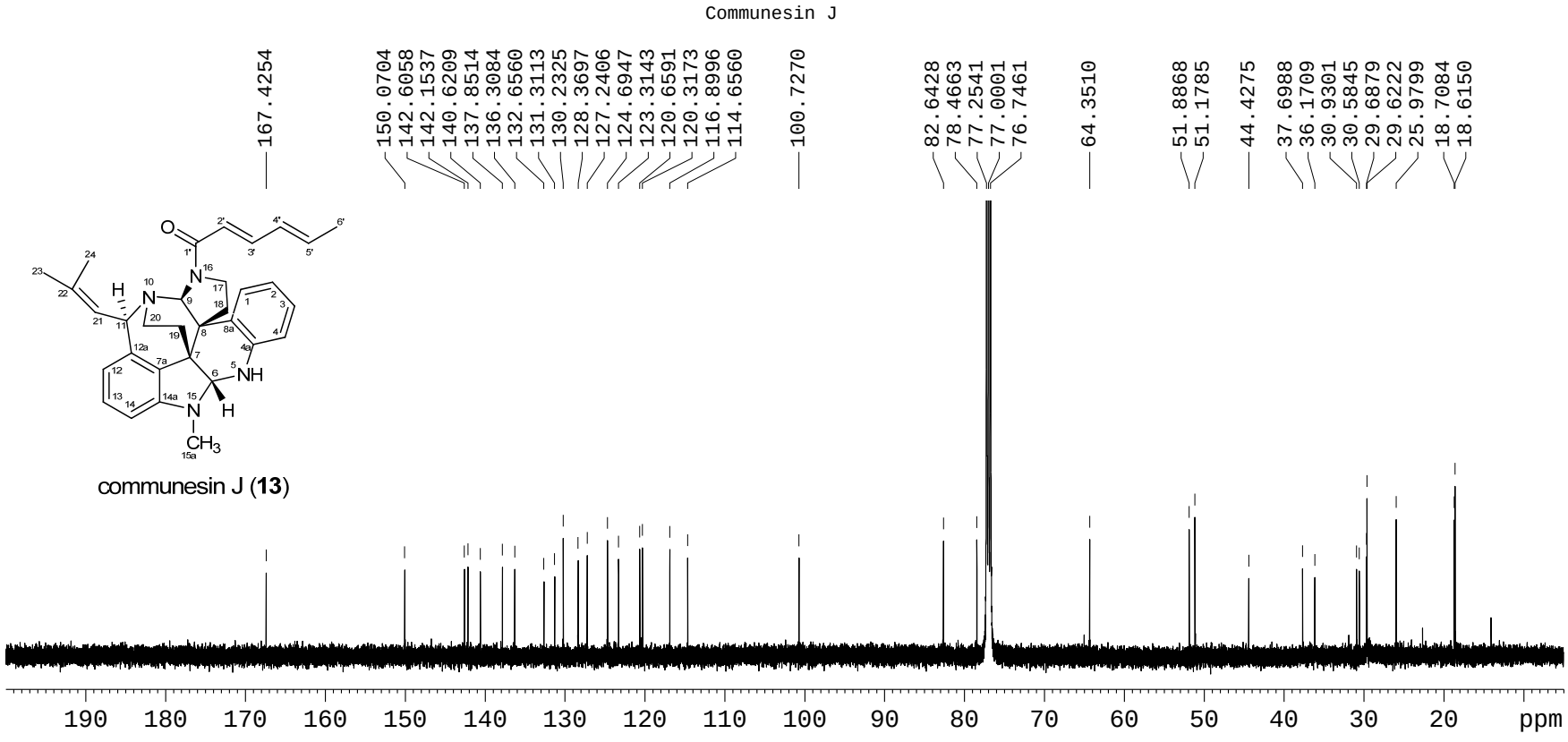


Figure S25. ^{13}C NMR spectrum of **13** ($CDCl_3$, 125 MHz).

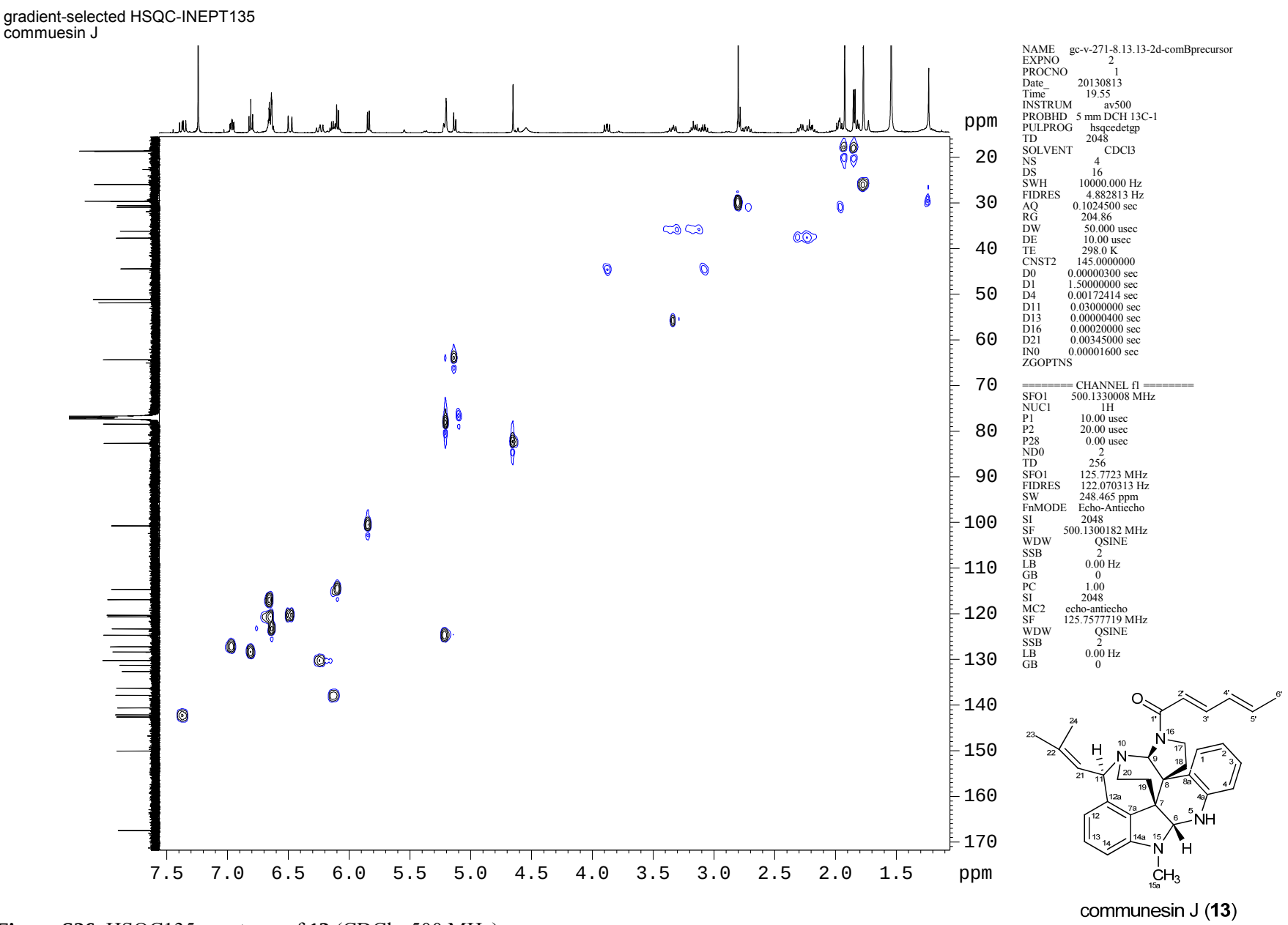


Figure S26. HSQC135 spectrum of **13** ($CDCl_3$, 500 MHz).

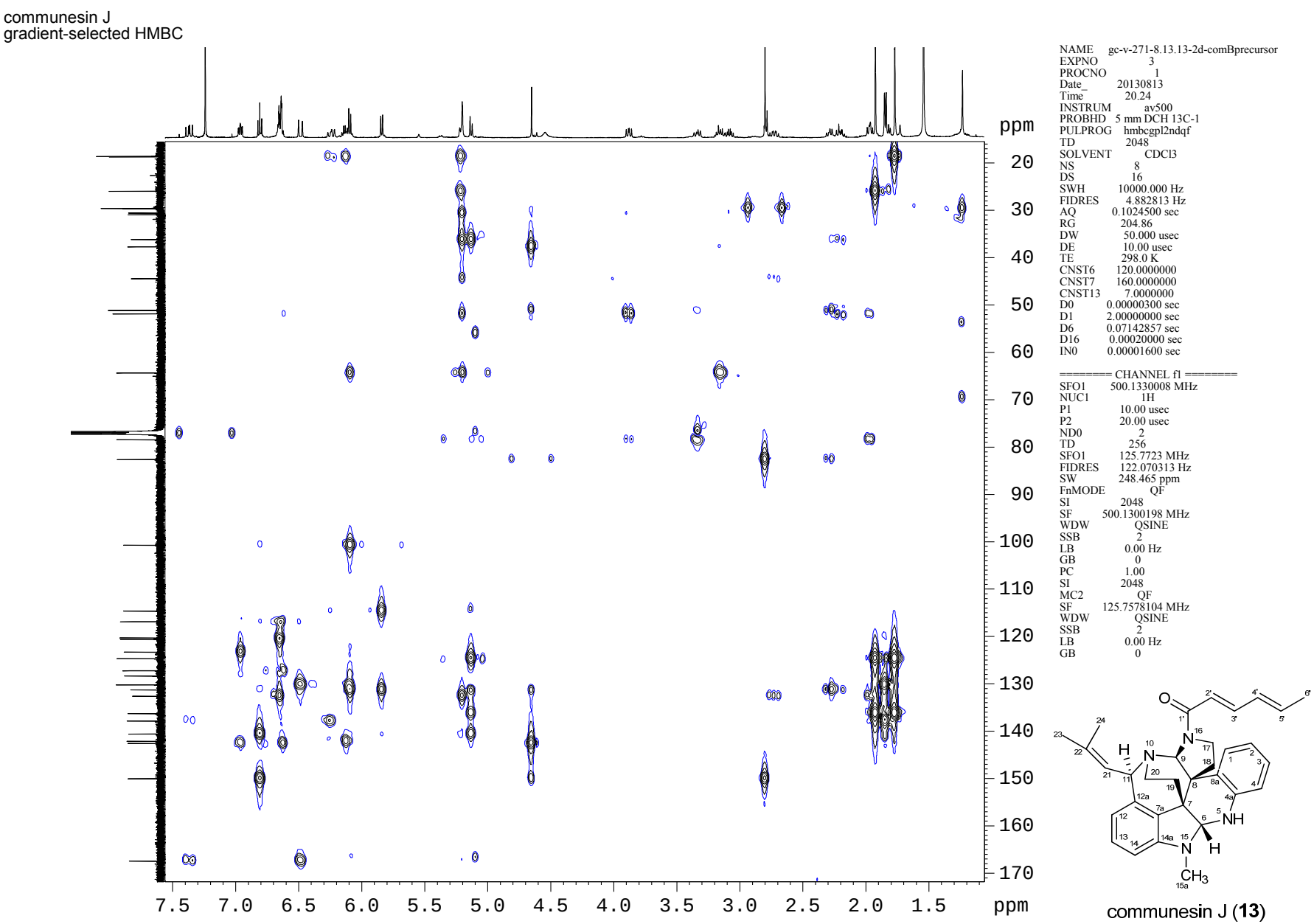


Figure S27. HMBC spectrum of **13** ($CDCl_3$, 500 MHz).

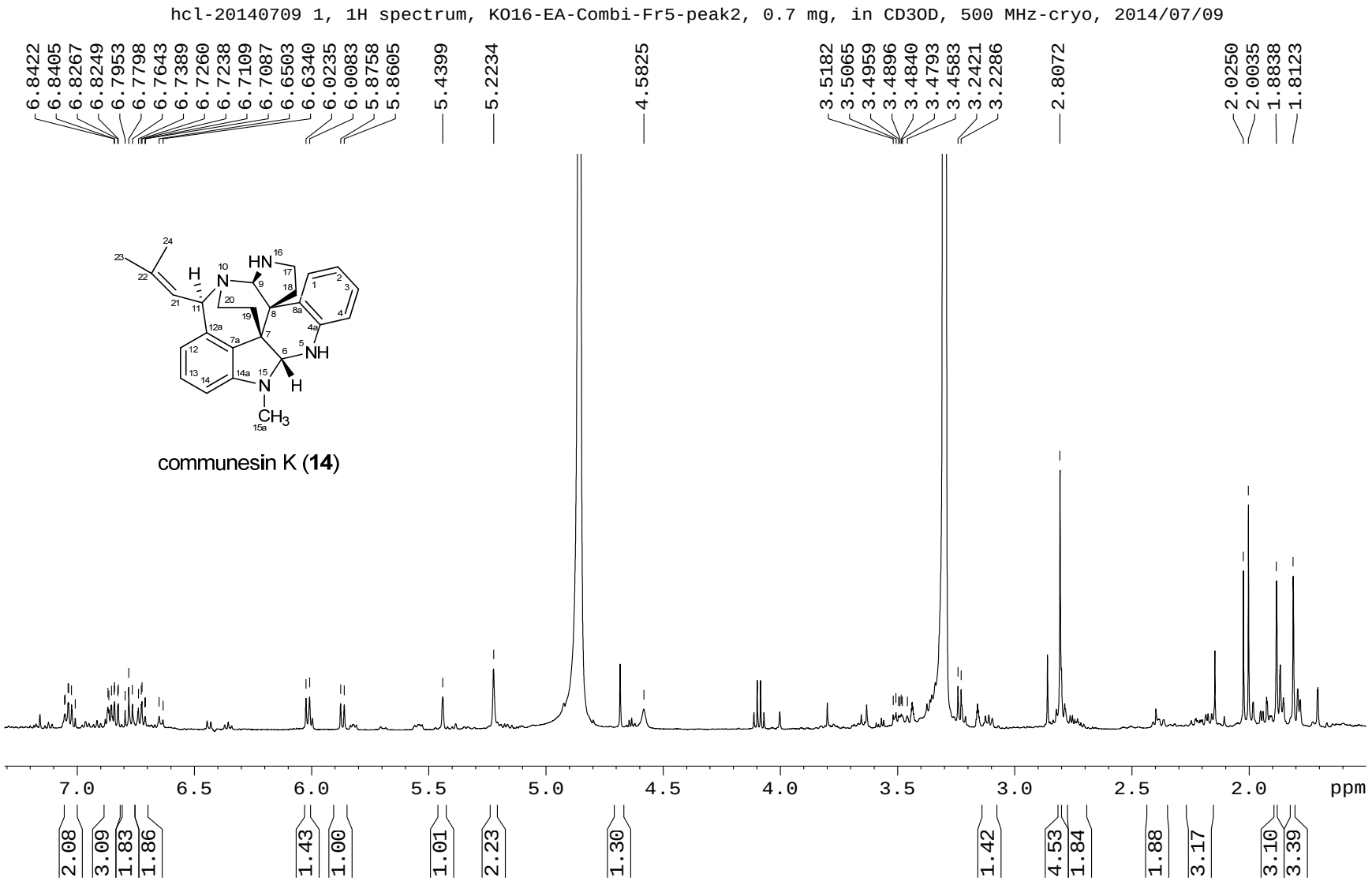


Figure S28. ^{1}H NMR spectrum of **14** ($CDCl_3$, 500 MHz).

communesin K (**14**)

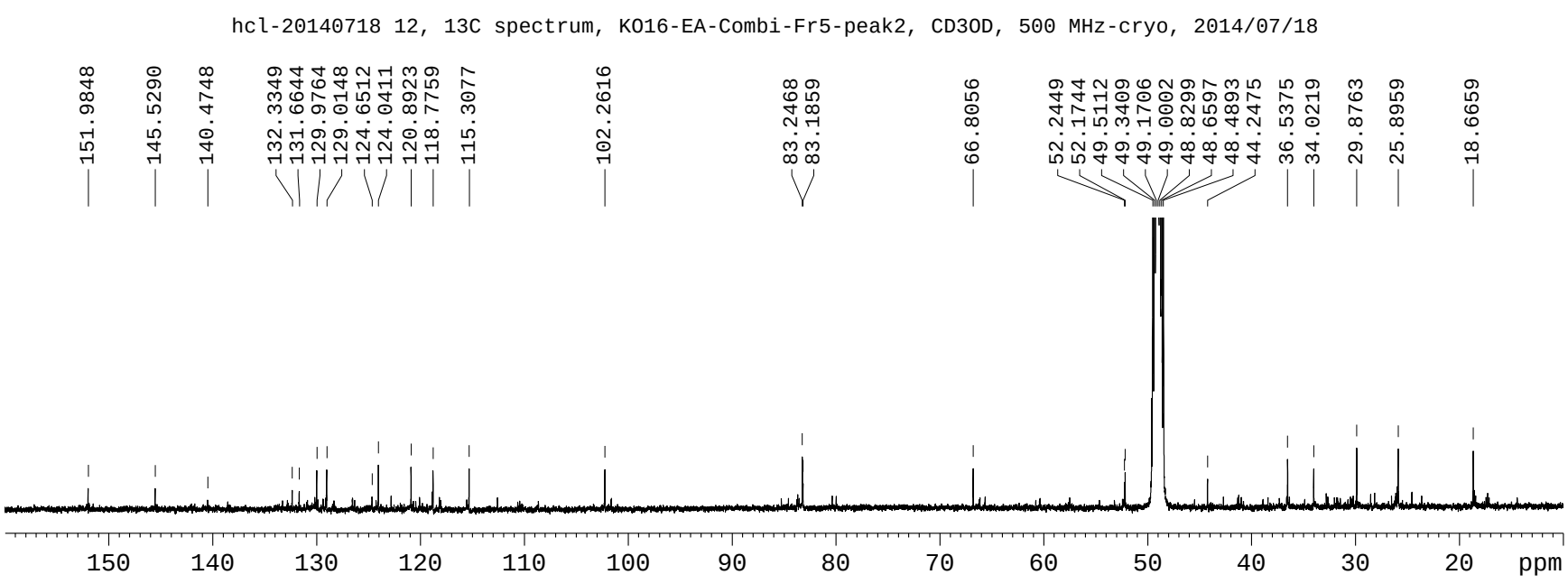


Figure S29. ^{13}C NMR spectrum of **14** ($CDCl_3$, 125 MHz).

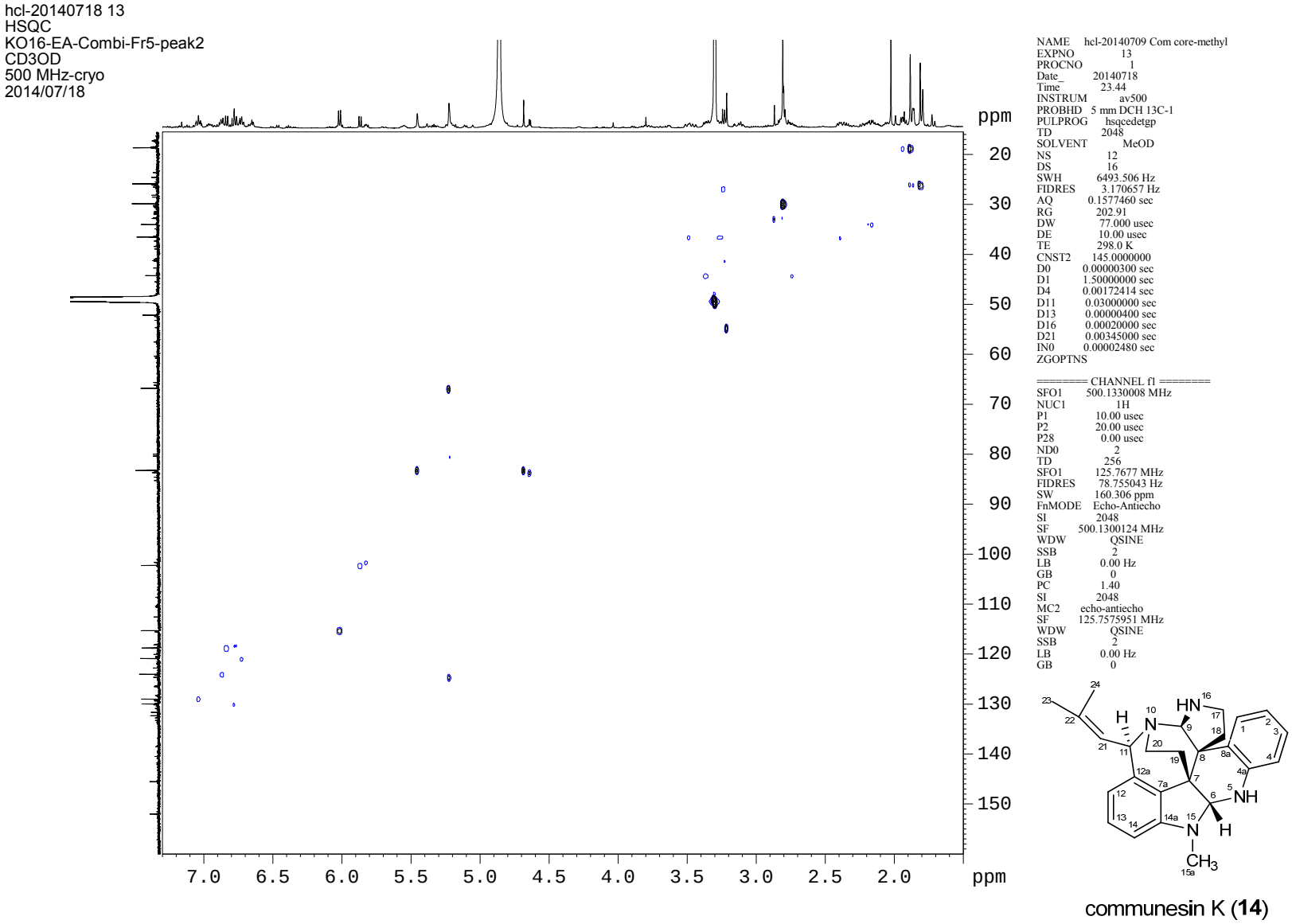


Figure S30. HSQC-135 spectrum of **14** ($CDCl_3$, 500 MHz).

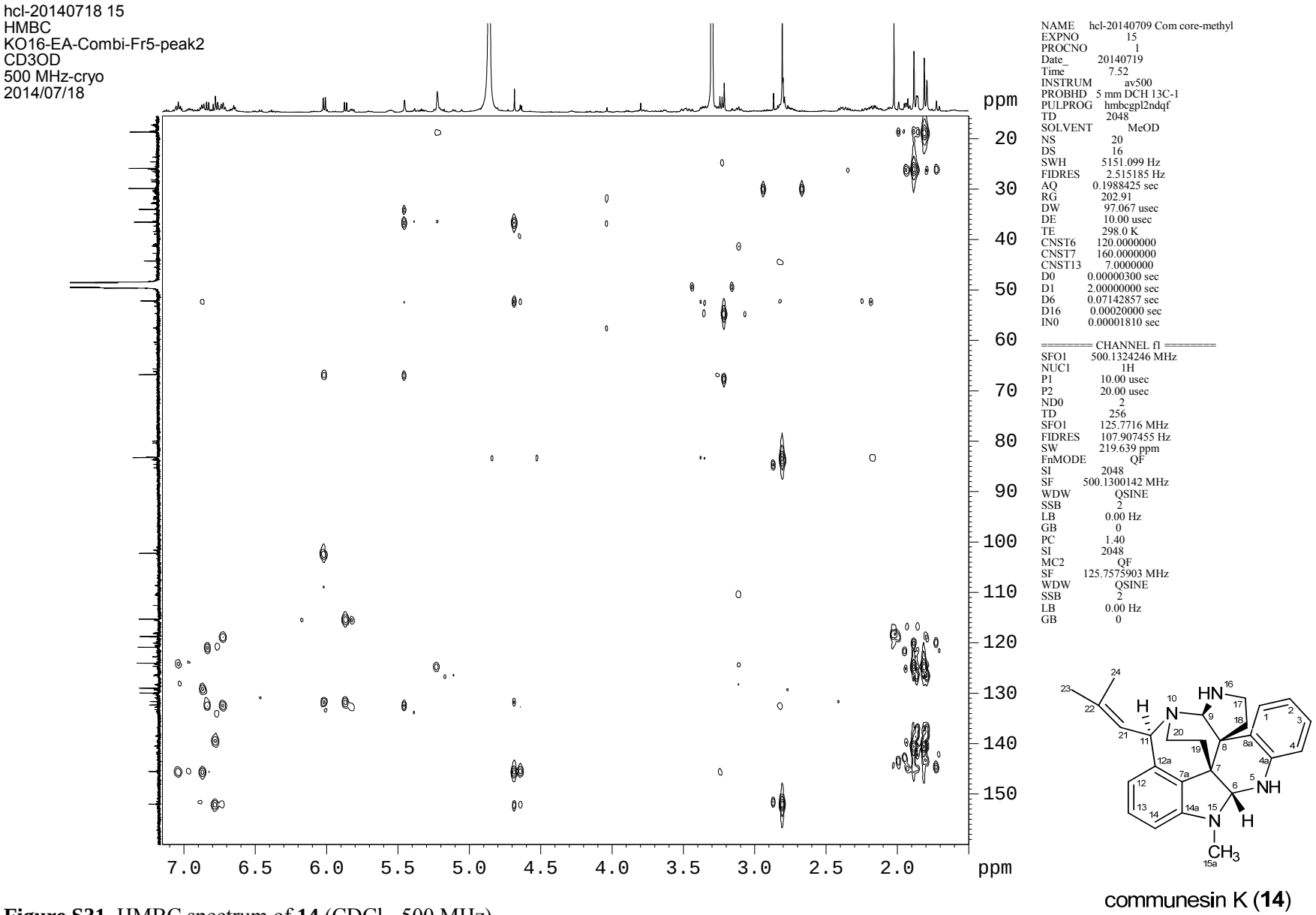


Figure S31. HMBC spectrum of **14** ($CDCl_3$, 500 MHz).

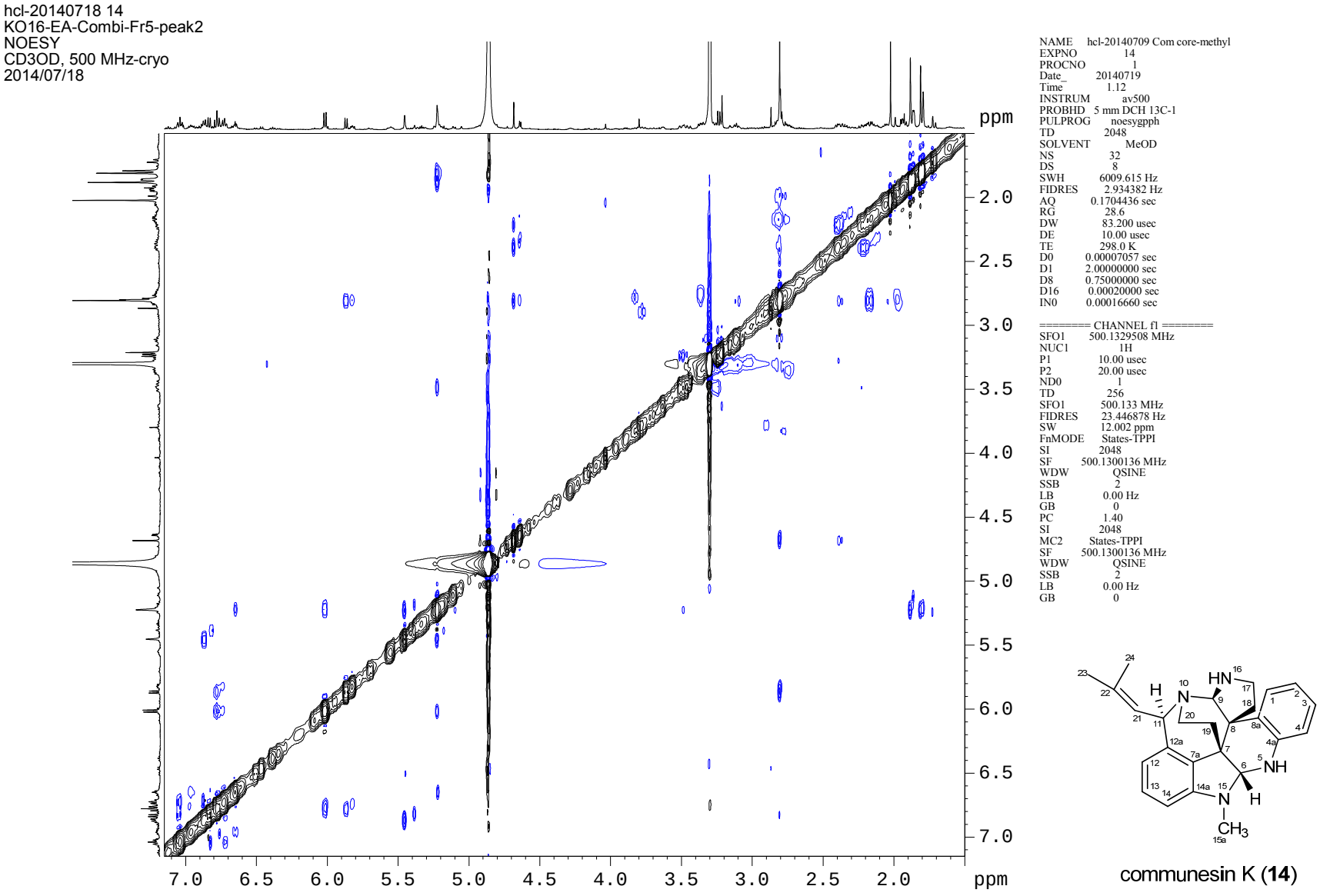


Figure S32. NOESY spectrum of **14** ($CDCl_3$, 500 MHz).

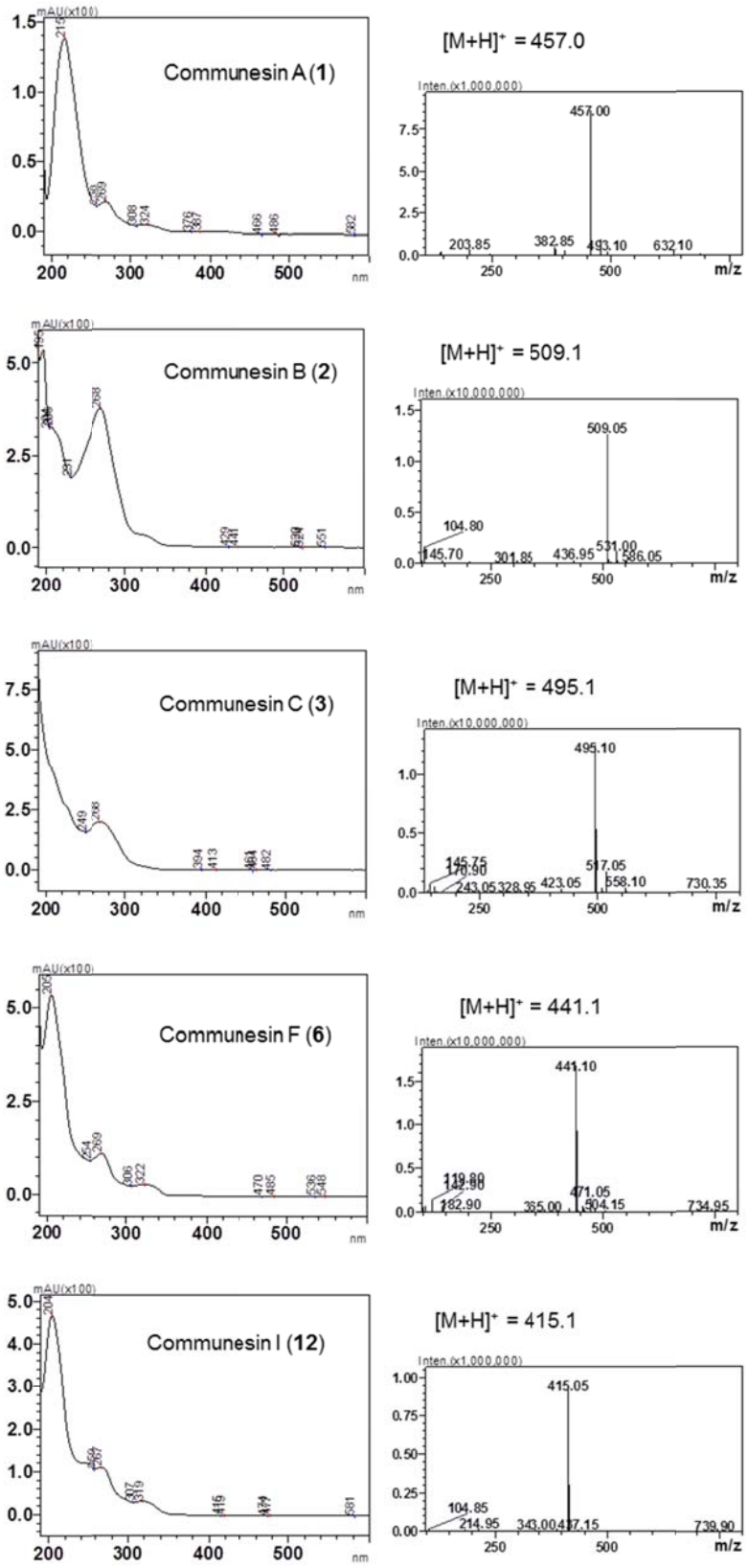


Figure S33. UV and MS spectra of **1**−**3**, **6** and **12**.

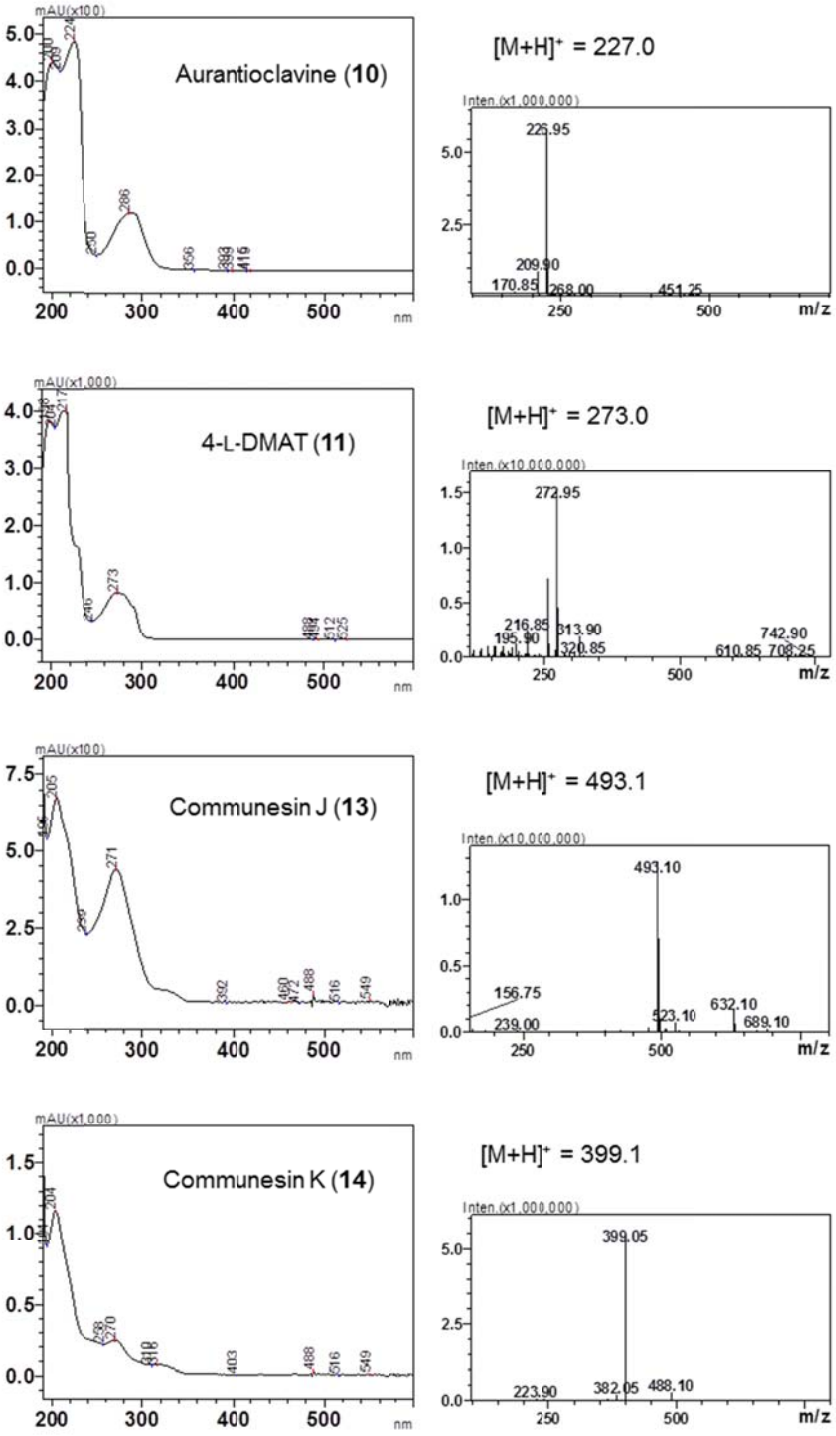


Figure S34. UV and MS spectra of **10**, **11**, **13** and **14**.

Supplementary References

[1] E. Kafer, *Adv. Genet.* **1977**, *19*, 33.

[2] J. Sambrook, D. W. Russell, *Molecular cloning: a laboratory manual, Vol. 1*, CSHL press, **2001**.

[3] R. Q. Li, H. M. Zhu, J. Ruan, W. B. Qian, X. D. Fang, Z. B. Shi, Y. R. Li, S. T. Li, G. Shan, K. Kristiansen, S. G. Li, H. M. Yang, J. Wang, *Genome Res.* **2010**, *20*, 265.

[4] E. Szewczyk, T. Nayak, C. E. Oakley, H. Edgerton, Y. Xiong, N. Taheri-Talesh, S. A. Osmani, B. R. Oakley, *Nat. Protoc.* **2007**, *1*, 3111.

[5] J. B. Kieler, K. L. Duong, W. S. Moye-Rowley, J. S. Klutts, *J. Microbiol. Methods* **2013**, *95*, 373.

[6] A. Numata, C. Takahashi, Y. Ito, T. Takada, K. Kawai, Y. Usami, E. Matsumura, M. Imachi, T. Ito, T. Hasegawa, *Tetrahedron Lett.* **1993**, *34*, 2355; Z. Zuo, D. Ma, *Angewandte Chemie-International Edition* **2011**, *50*, 12008.

[7] H. Hayashi, H. Matsumoto, K. Akiyama, *"Biosci. Biotechnol., Biochem. "* **2004**, *68*, 753; R. Jadulco, R. A. Edrada, R. Ebel, A. Berg, K. Schaumann, V. Wray, K. Steube, P. Proksch, *J. Nat. Prod.* **2004**, *67*, 78.

[8] J. Belmar, R. L. Funk, *J. Am. Chem. Soc.* **2012**, *134*, 16941; Z. W. Zuo, W. Q. Xie, D. W. Ma, *J. Am. Chem. Soc.* **2010**, *132*, 13226.

[9] S. Krishnan, J. T. Bagdanoff, D. C. Ebner, Y. K. Ramtohul, U. K. Tambar, B. M. Stoltz, *J. Am. Chem. Soc.* **2008**, *130*, 13745.

[10] J. A. McIntosh, M. S. Donia, S. K. Nair, E. W. Schmidt, *J. Am. Chem. Soc.* **2011**, *133*, 13698.

[11] H. Hikawa, Y. Yokoyama, Y. Murakami, *Synthesis-Stuttgart* **2000**, 214.