Supplementary Information

Engineering industrial fungus *Aspergillus oryzae* for sustainable biosynthesis of ergot alkaloids

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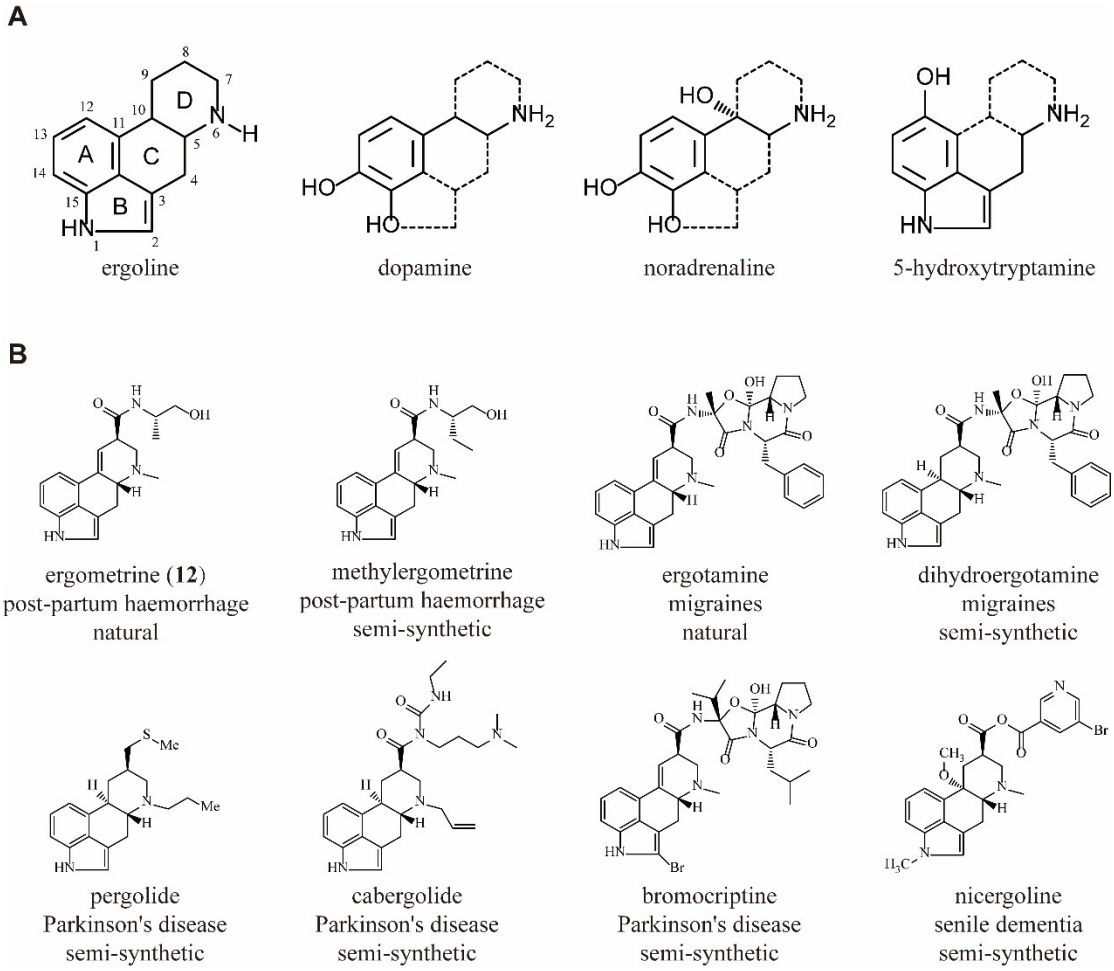

Fig. S1 Structures of ergot alkaloids. (A) Structural analogy between the ergoline ring and different neurotransmitters (dopamine, noradrenaline and 5-hydroxytryptamine). (B) Natural and semi-synthetic drugs of ergot alkaloids with diverse bioactivities.

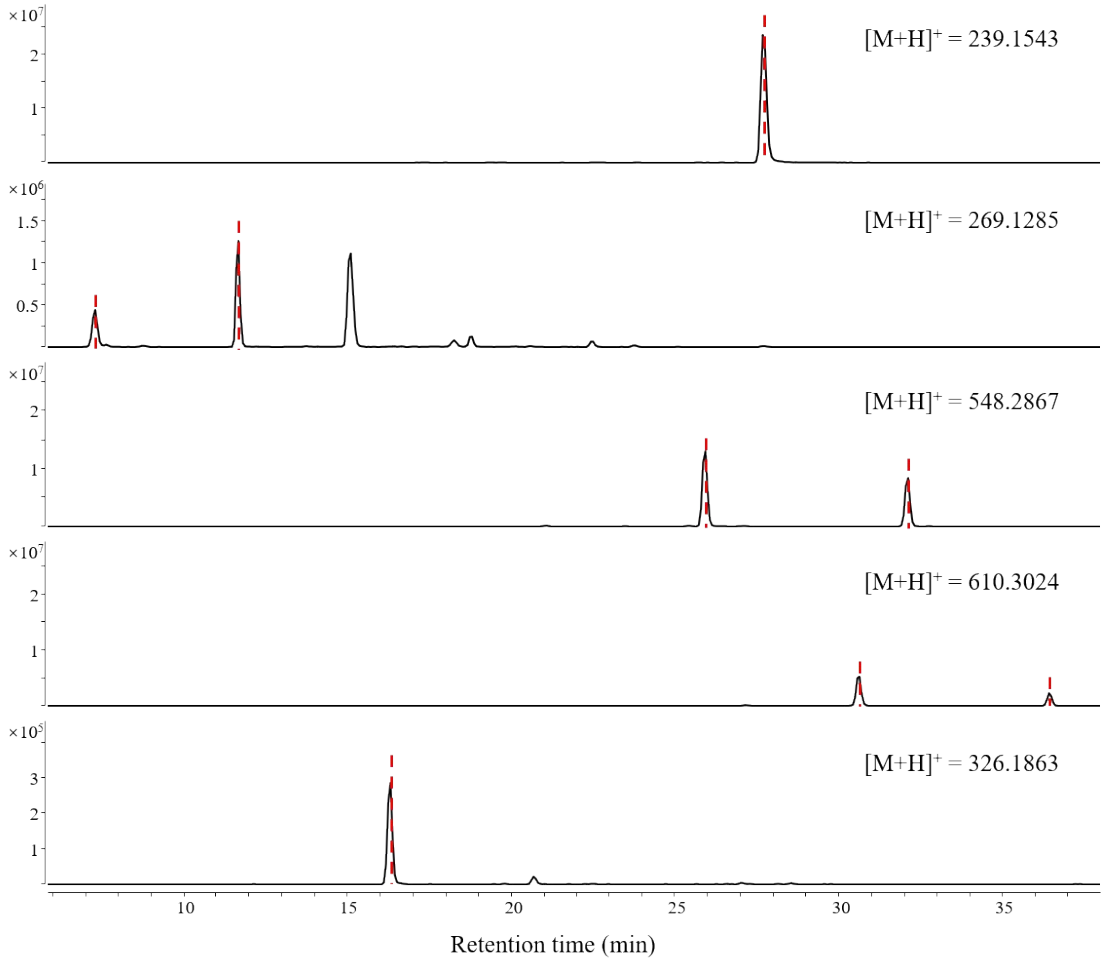

Fig. S2 LC-MS detection of culture extract of a four-weeks culture of *Claviceps purpurea* 22.07. The extracted ion chromatogram of $[M+H]^+$ = 239.1543 (agroclavine), $[M+H]^+$ = 269.1285 (lysergic acid and isolysergic acid), $[M+H]^+$ = 548.2867 (ergosine), $[M+H]^+$ = 610.3024 (ergocristine), and $[M+H]^+$ = 326.1863 (ergometrine), respectively.

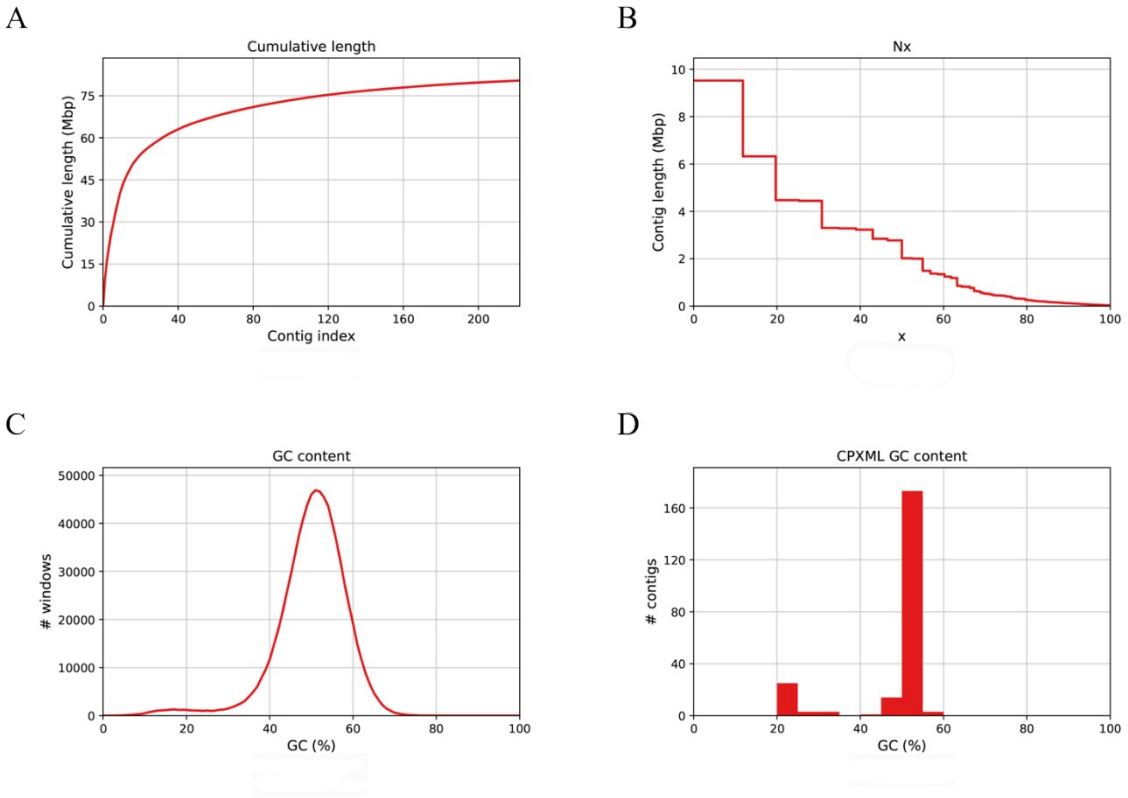

Fig. S3 The distribution figure of assembled *C. purpurea* 22.07 genome. (A) Cumulative length of contigs. Contigs are indexed (x) from longest to shortest. The cumulative length of the first x contigs are plotted as a function of x for the assembly using x-axis. (B) Nx plot displaying the percentage of the genome (x-axis) covered by contigs of a specific length. (C) Contigs are broken into nonoverlapping 100 bp windows. Plot shows number of windows for each GC percentage. (D) Plot shows number of contigs with GC percentage in a certain range.

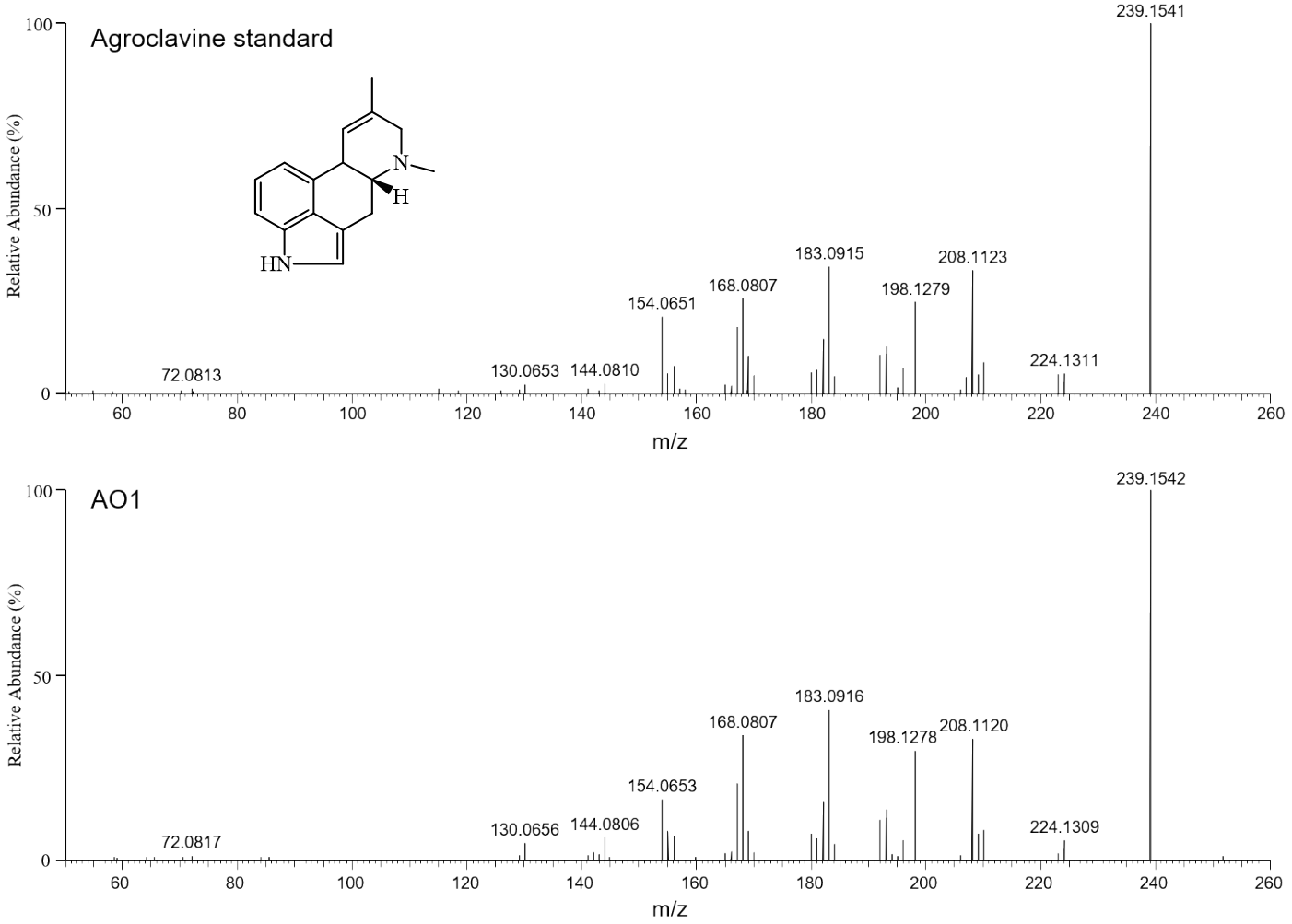

Fig. S4 LC-MS/MS fragmentation spectra of agroclavine (AG, **8**) standard (top) and peak eluted at 43.2 min from AO1 (bottom).

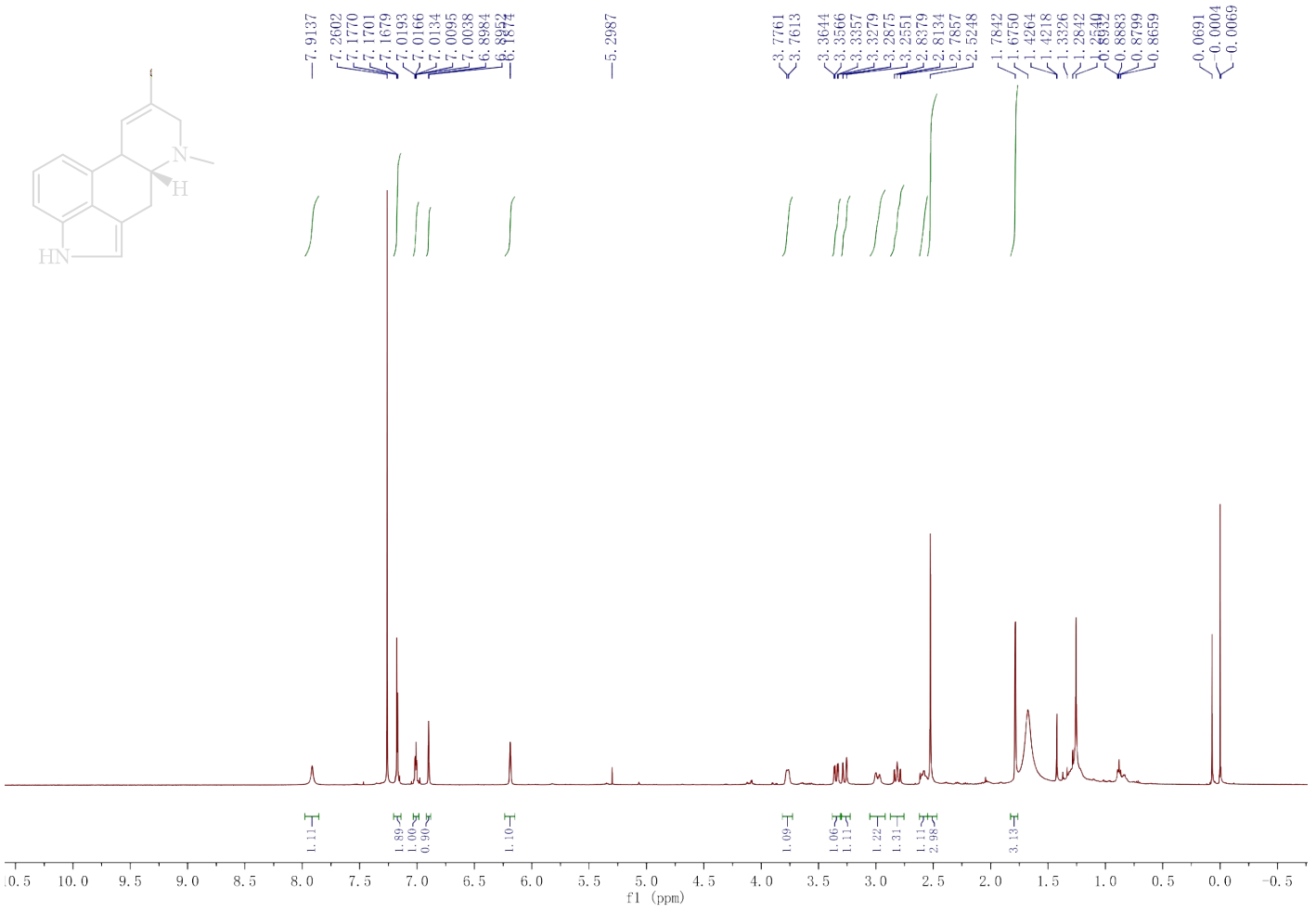

Fig. S5 ^{1}H NMR spectrum of agroclavine (**8**) in $CDCl_3$ at 500 MHz.

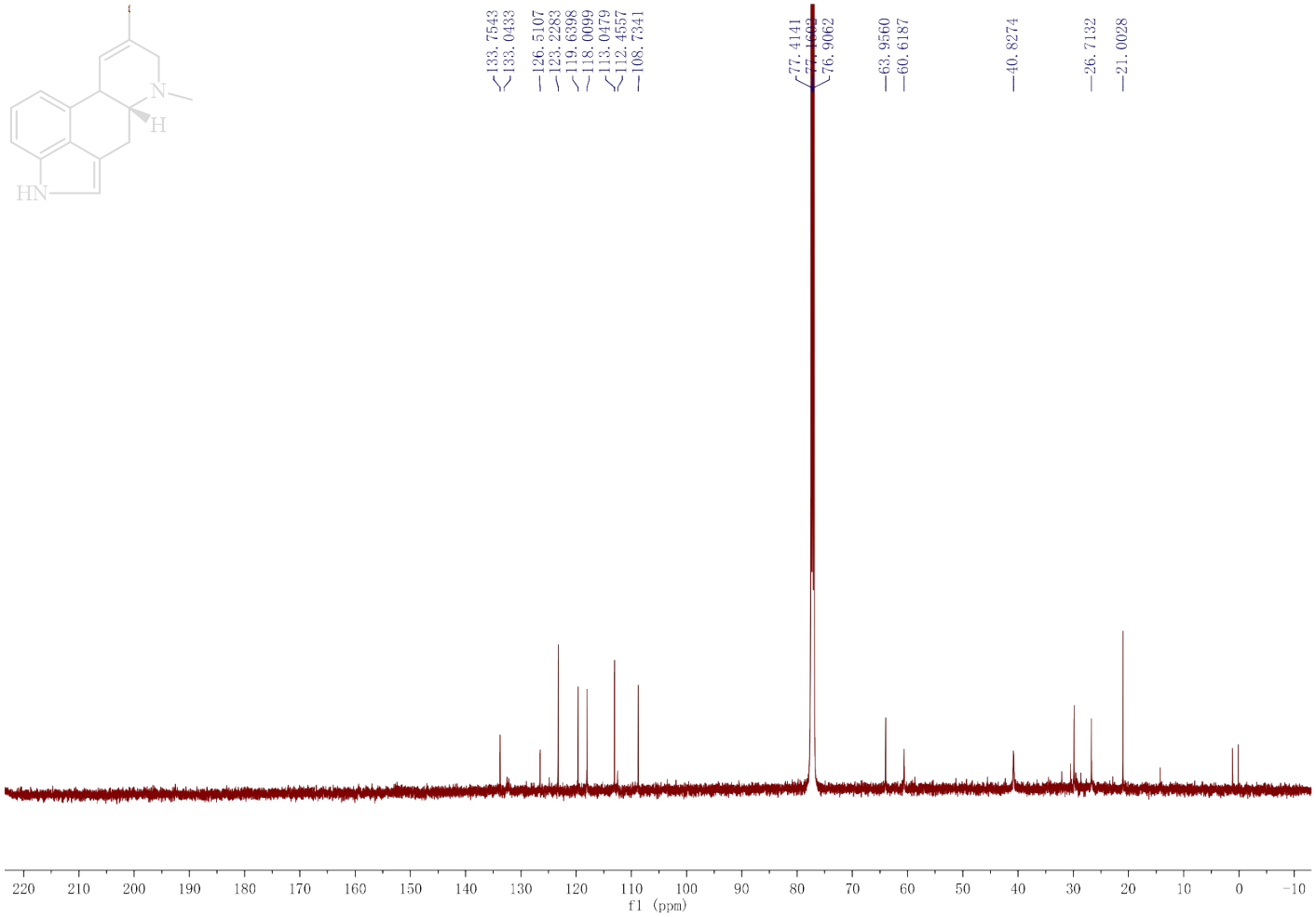

Fig. S6 ^{13}C NMR spectrum of agroclavine (**8**) in $CDCl_3$ at 500 MHz.

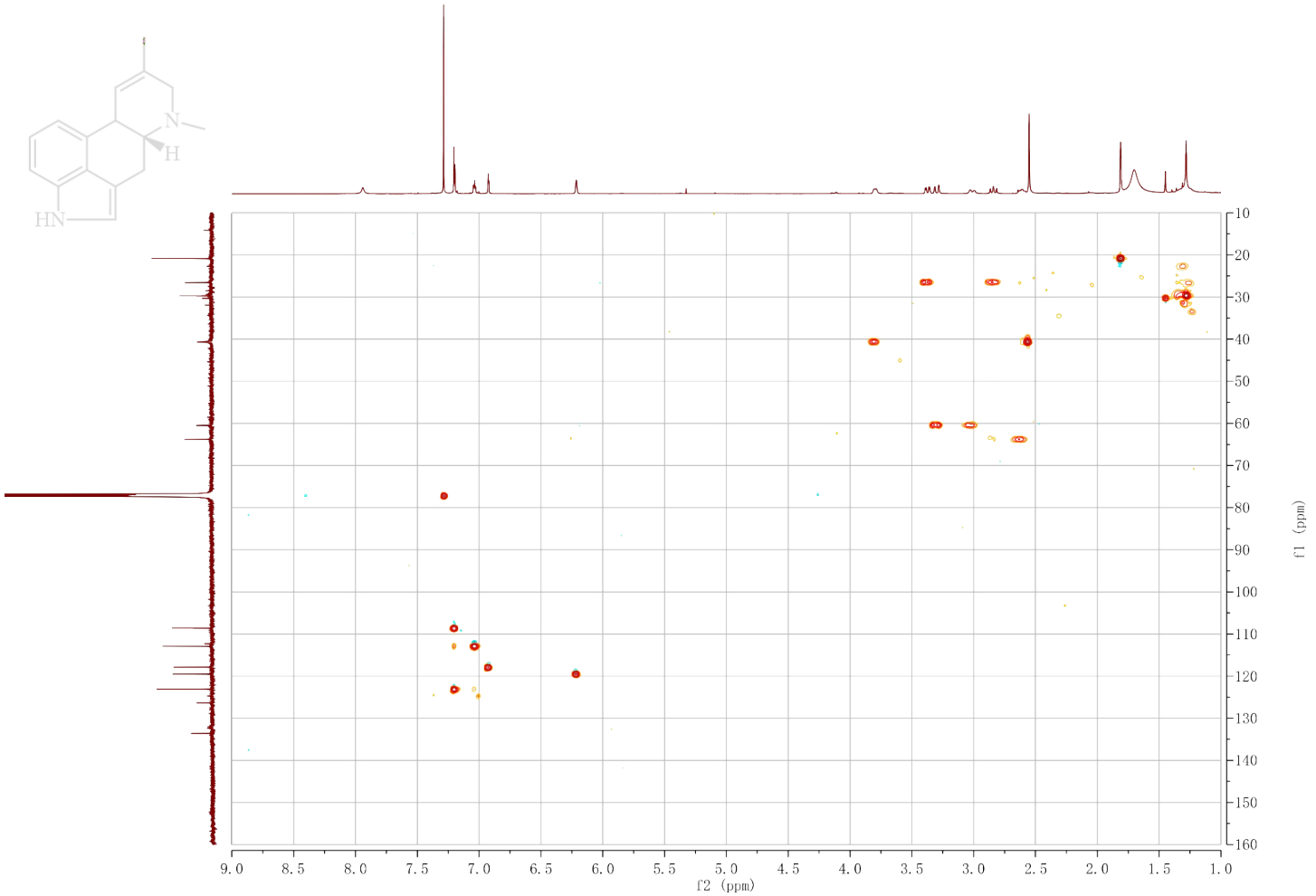

Fig. S7 HSQC spectrum of agroclavine (**8**) in $CDCl_3$.

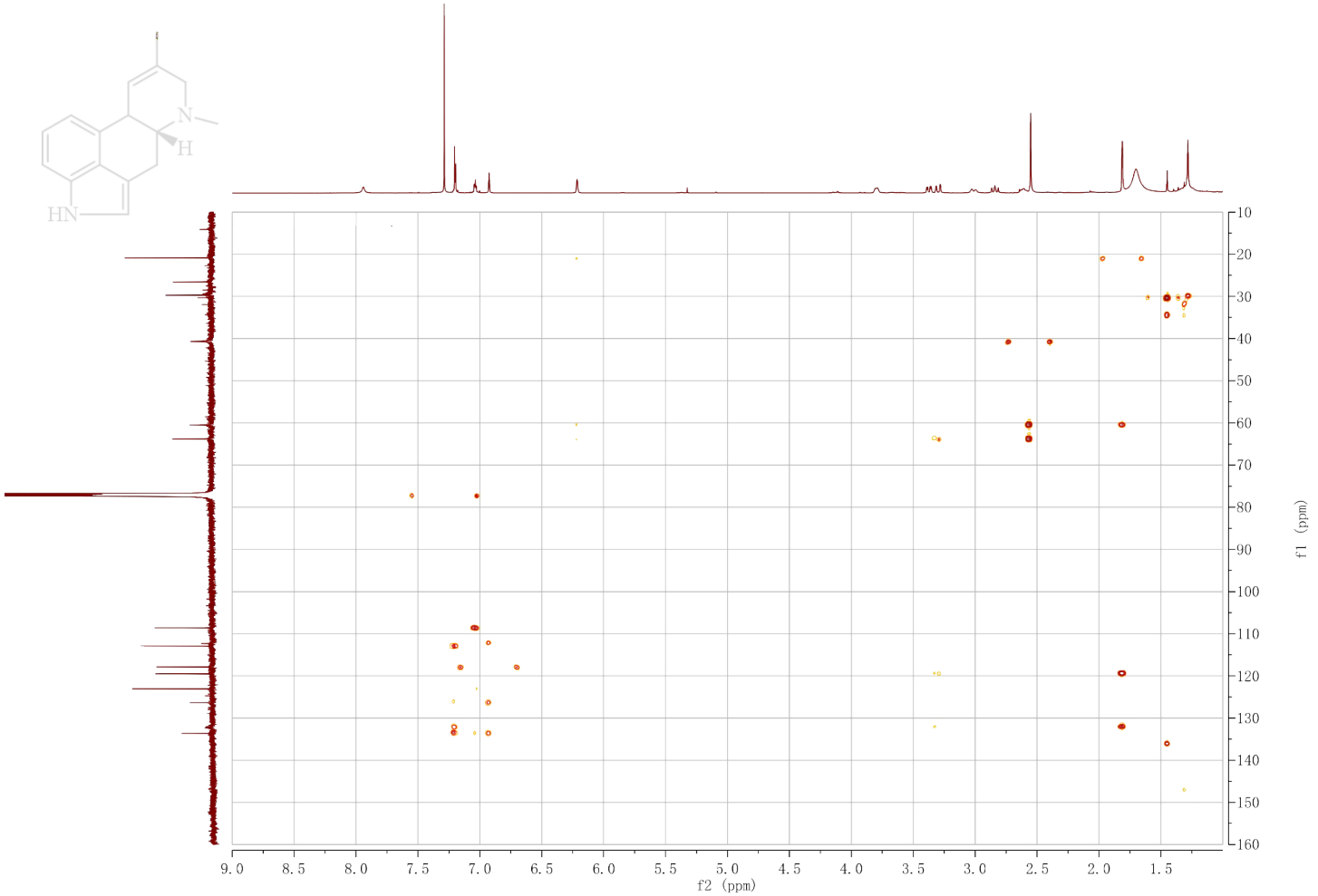

Fig. S8 HMBC spectrum of agroclavine (**8**) in $CDCl_3$.

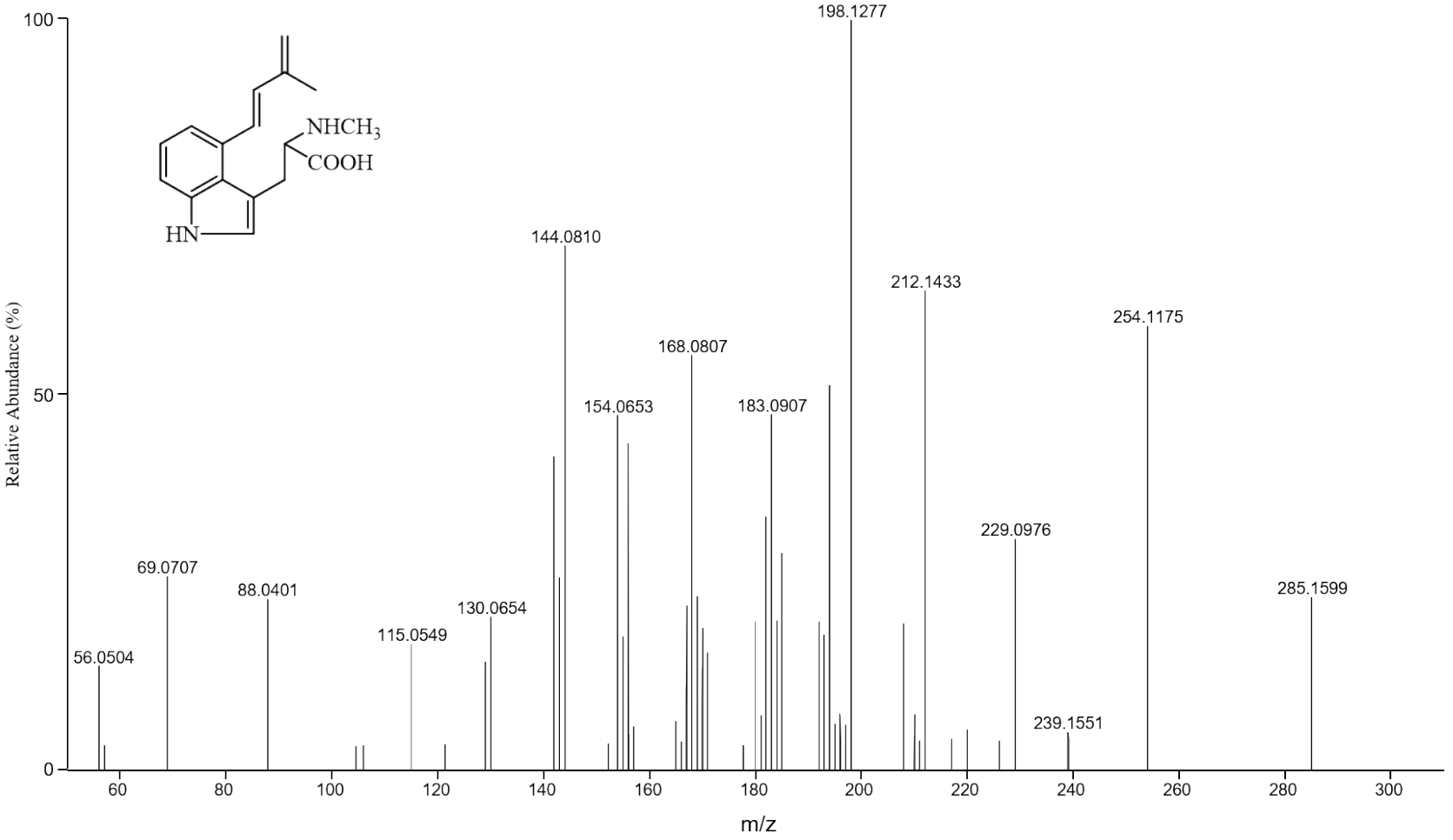

Fig. S9 LC-MS/MS fragmentation spectra of peak eluted at 40.6 min from AO1 (prechanoclavine, PCC, **4**).

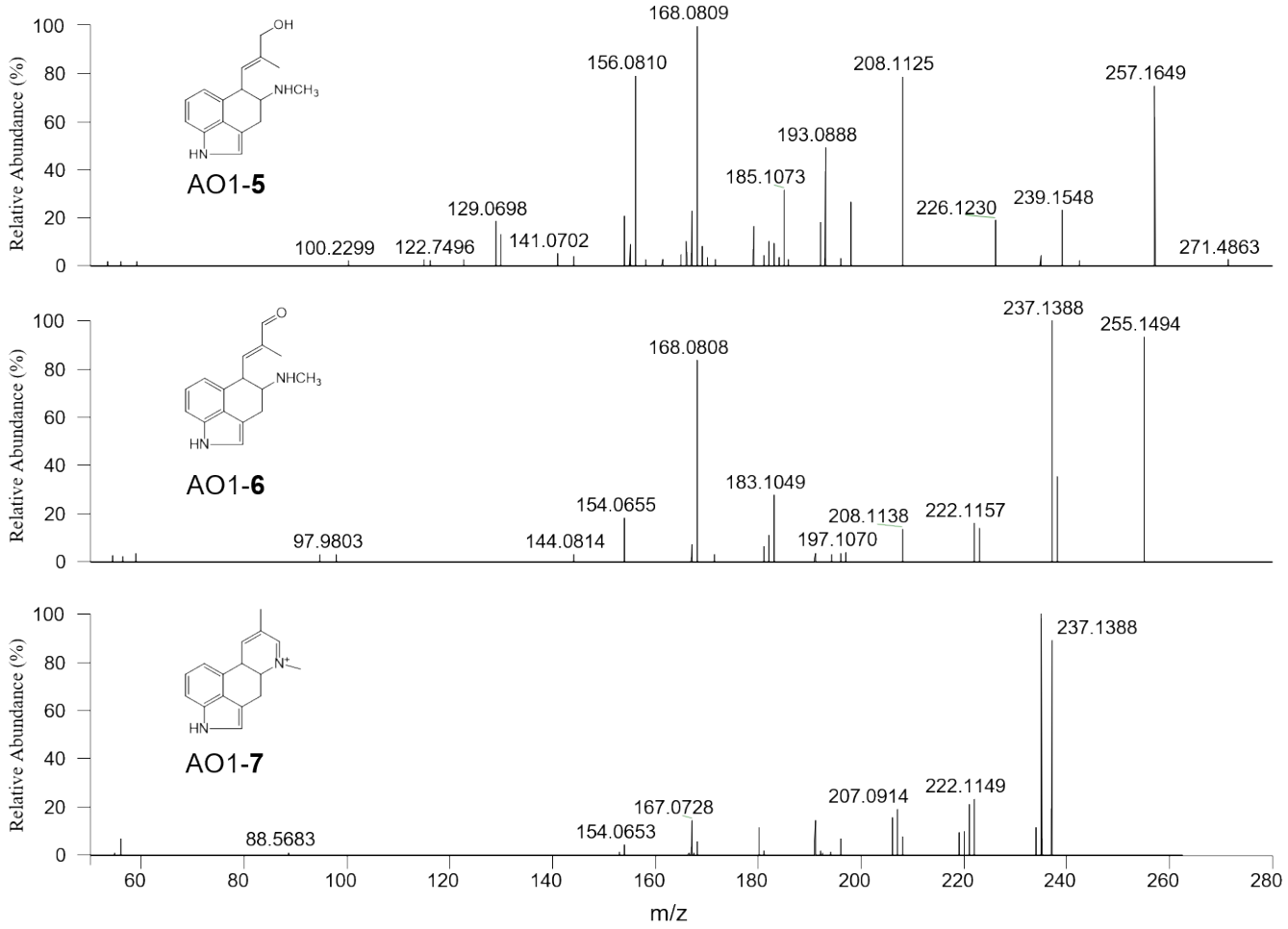

Fig. S10 LC-MS/MS fragmentation spectra of compound **5** (top), **6** (mid) and **7** (bottom)from AO1 (chanoclavine-I, CC, **5**; chanoclavine-I aldehyde, **6**).

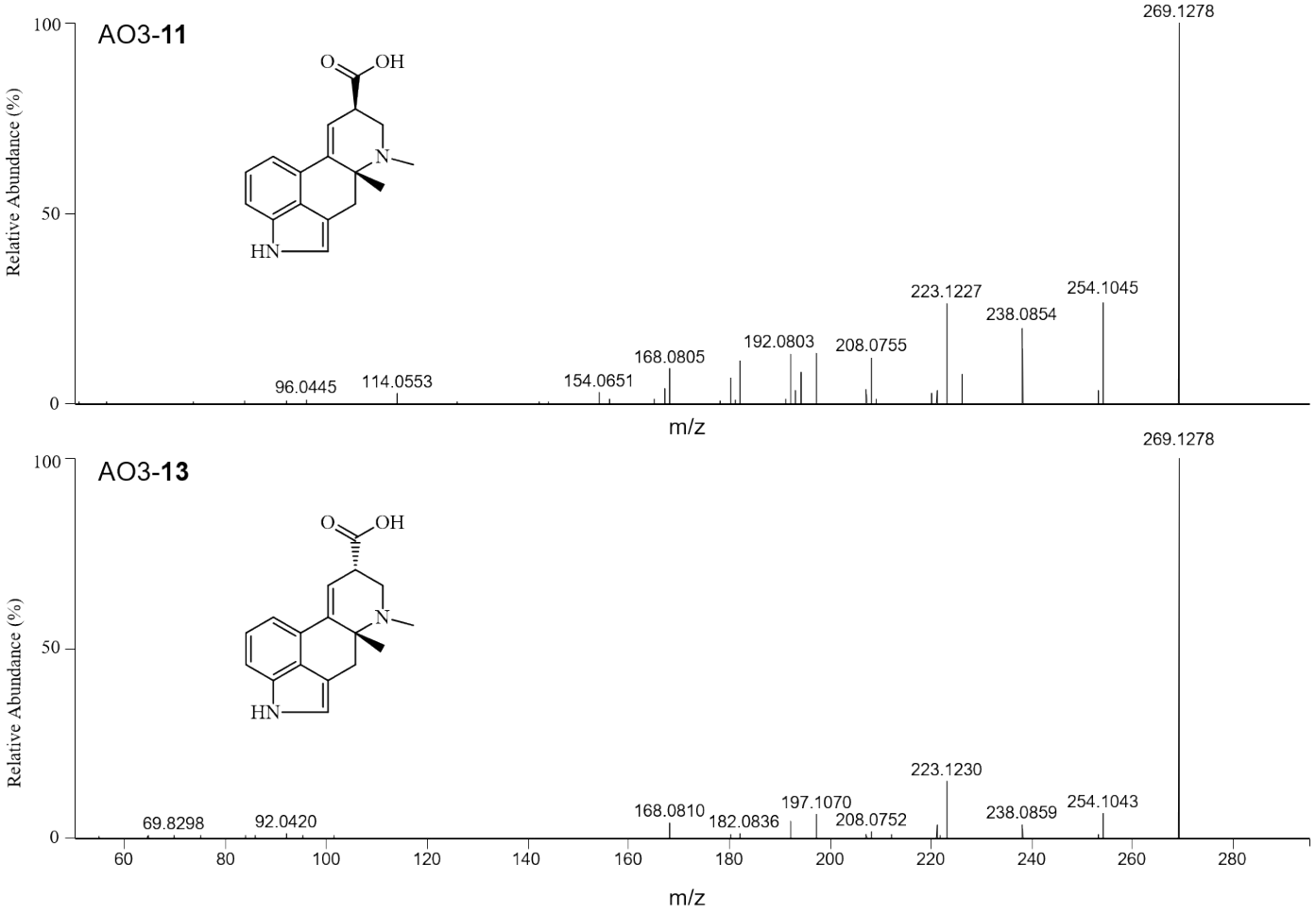

Fig. S11 LC-MS/MS fragmentation spectra of compound **11** (top) and **13** (bottom) from AO1 (lysergic acid, LA, **11**; isolysergic acid, ILA, **13**).

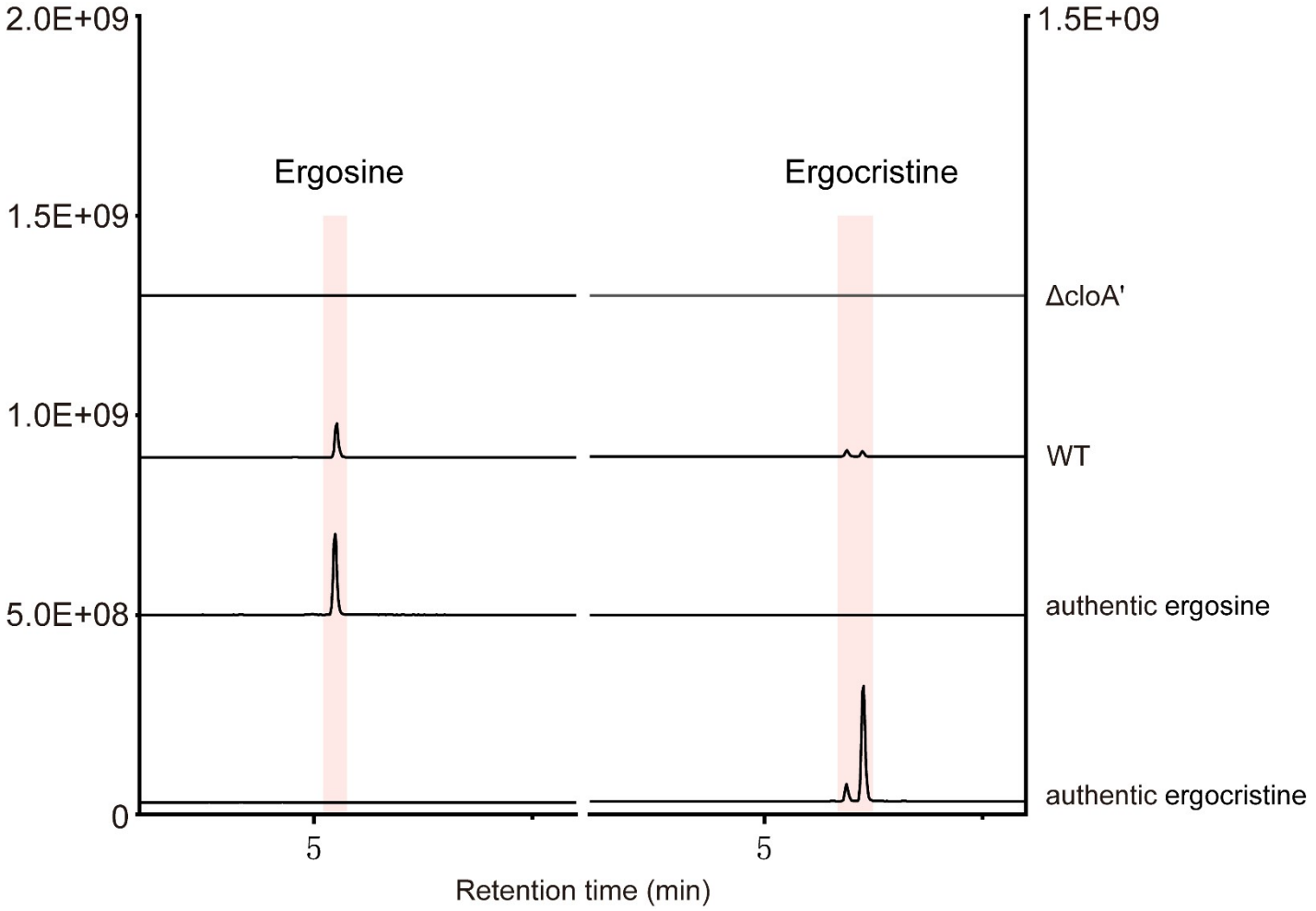

Fig. S12 LC-MS analysis of culture extract from *cloA'* deletion mutant and wild type of *C.purpurea* 22.07.

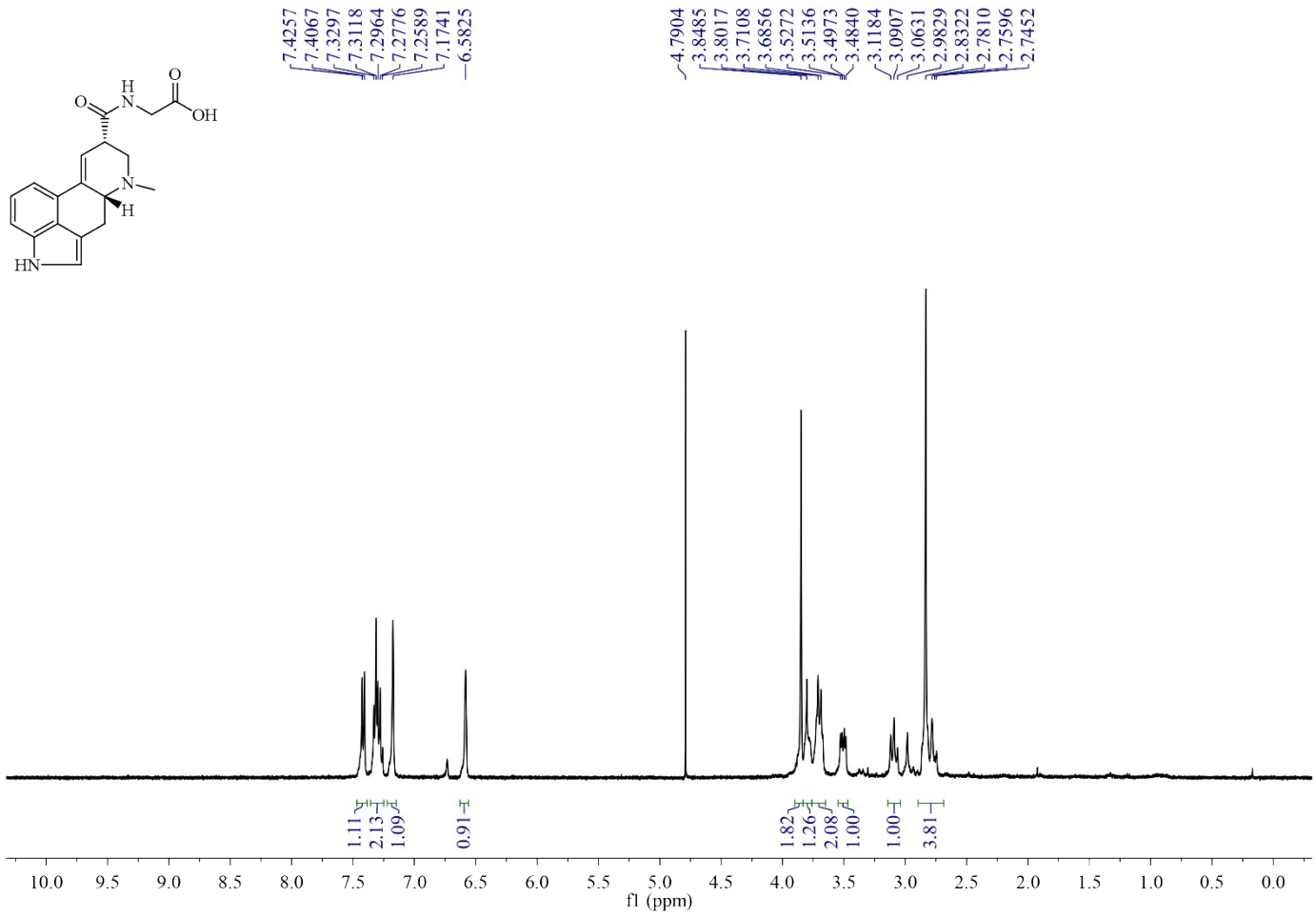

Fig. S13 ^{1}H NMR spectrum of isolysergyl-glycine (**14**) in D_2O at 400 MHz.

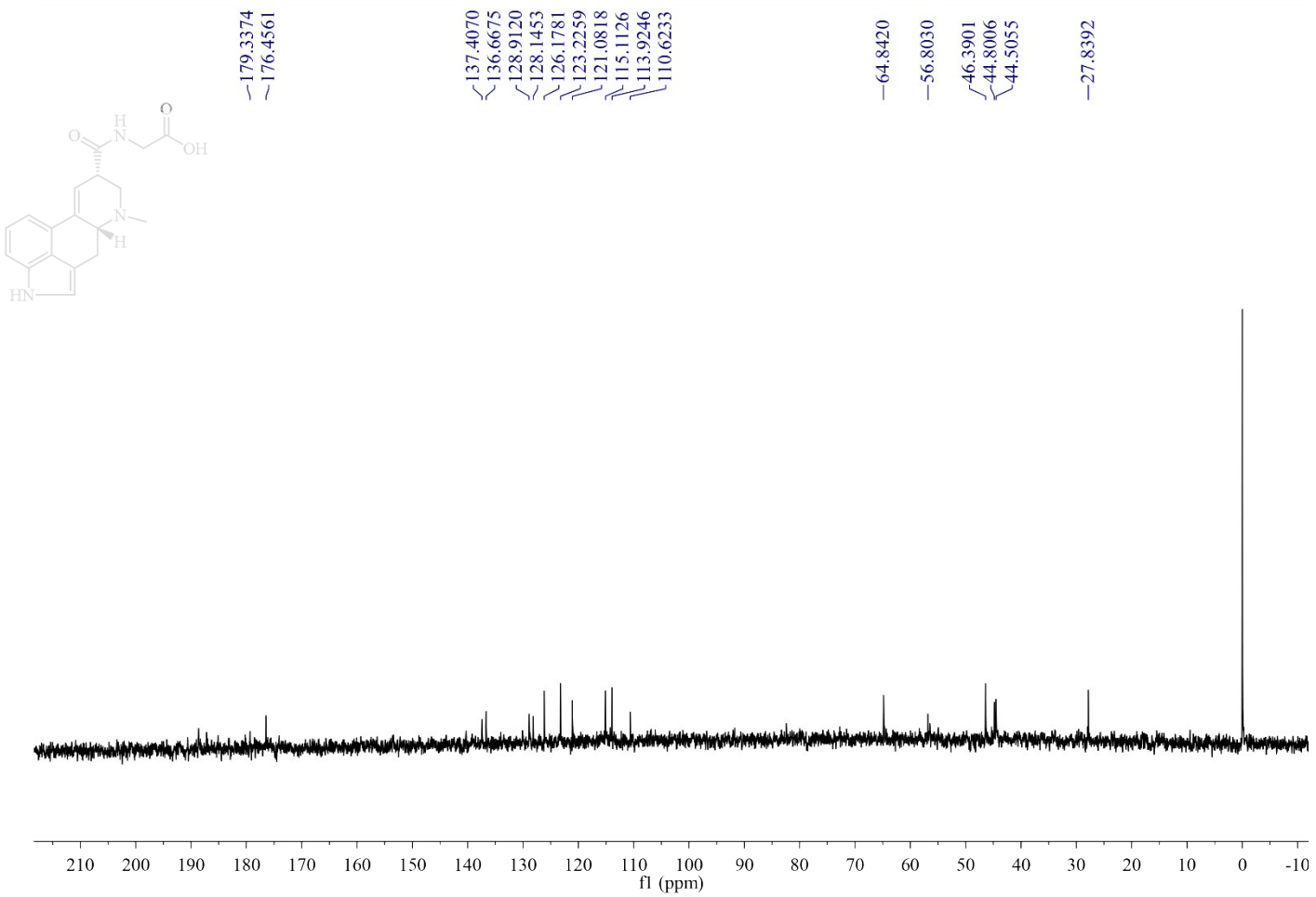

Fig. S14 ^{13}C NMR spectrum of isolysergyl-glycine (**14**) in D_2O at 100 MHz.

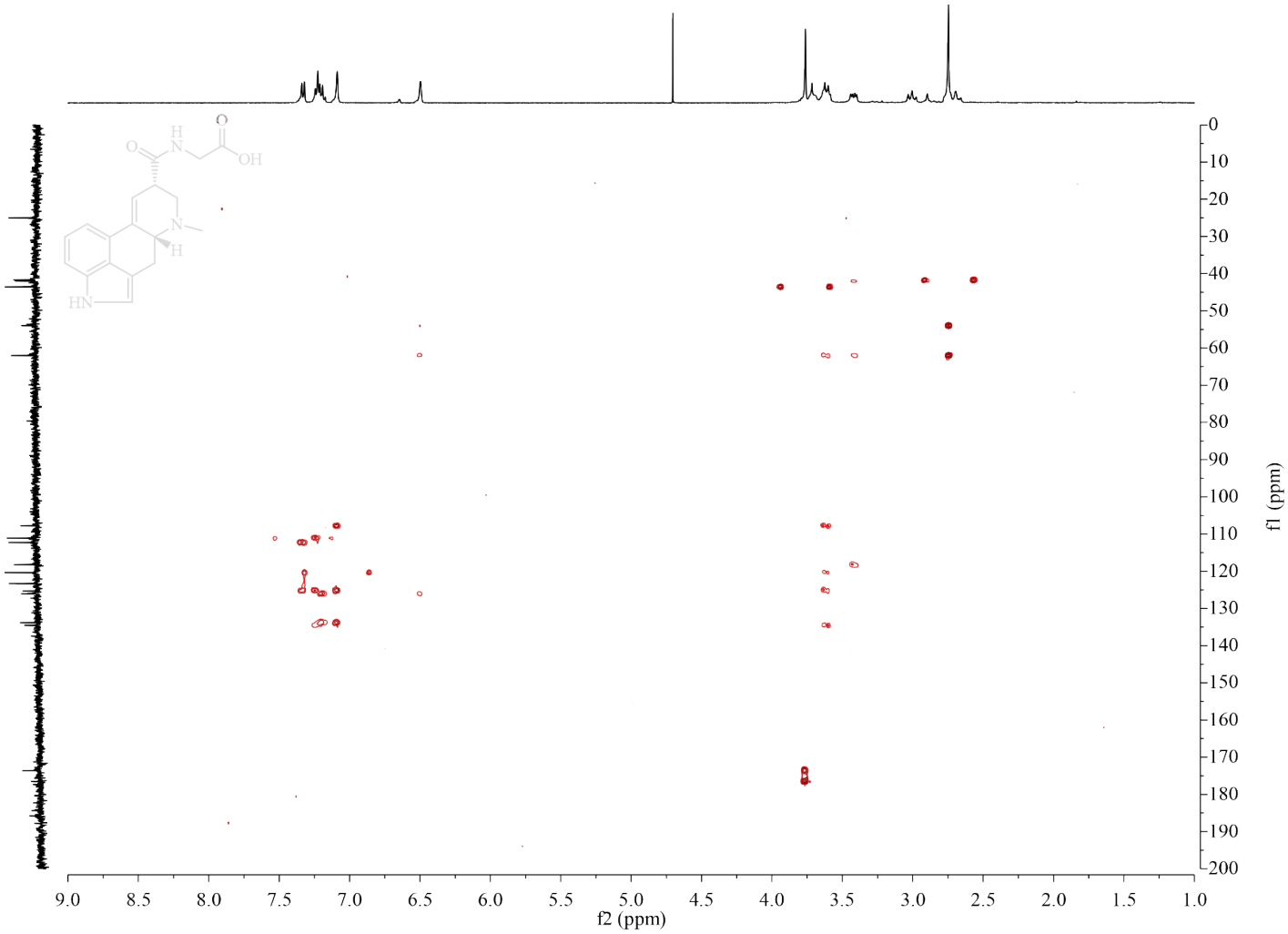

Fig. S15 HMBC spectrum of isolysergyl-glycine (**14**) in D_2O.

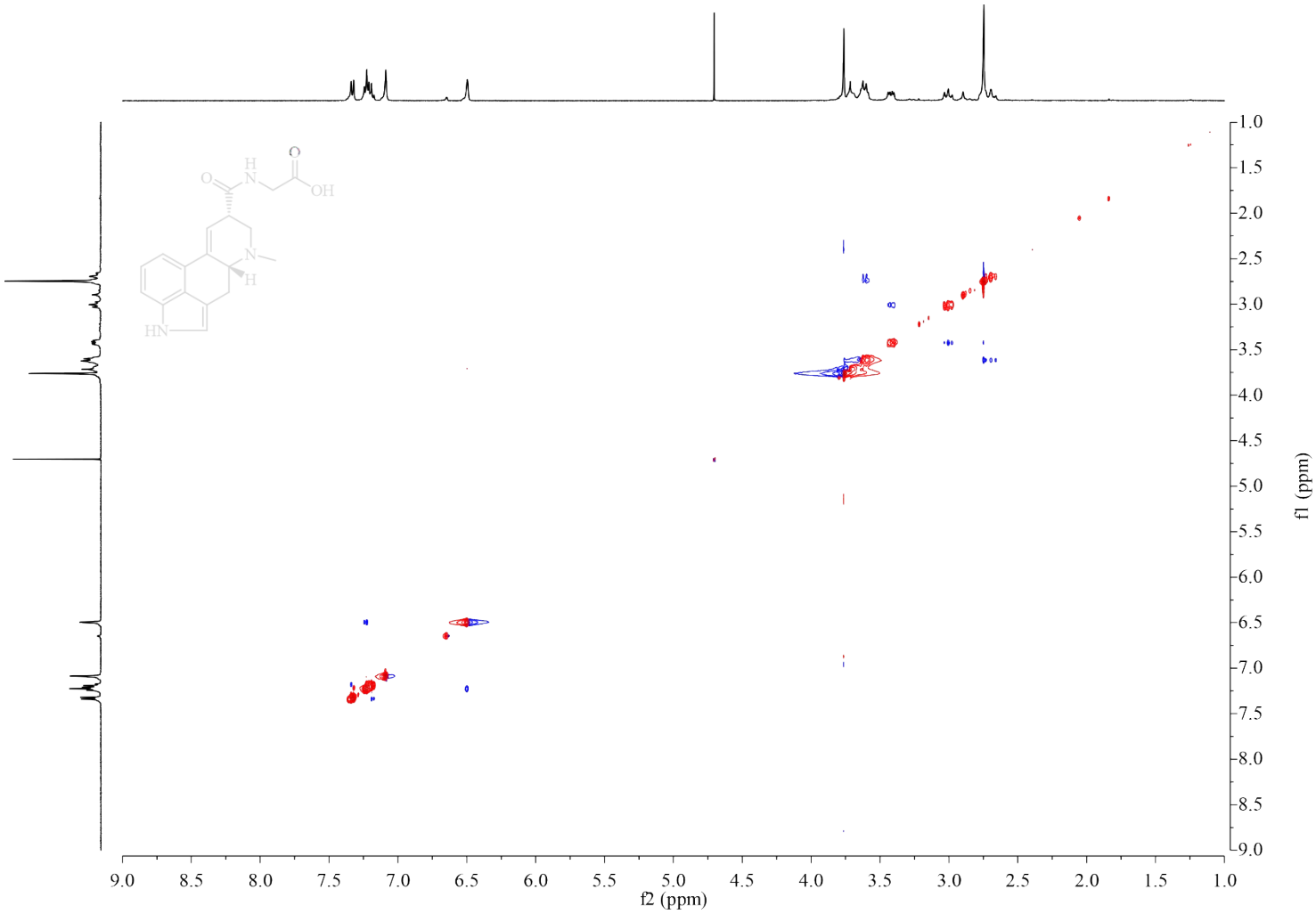

Fig. S16 NOESY spectrum of isolysergyl-glycine (**14**) in D_2O.

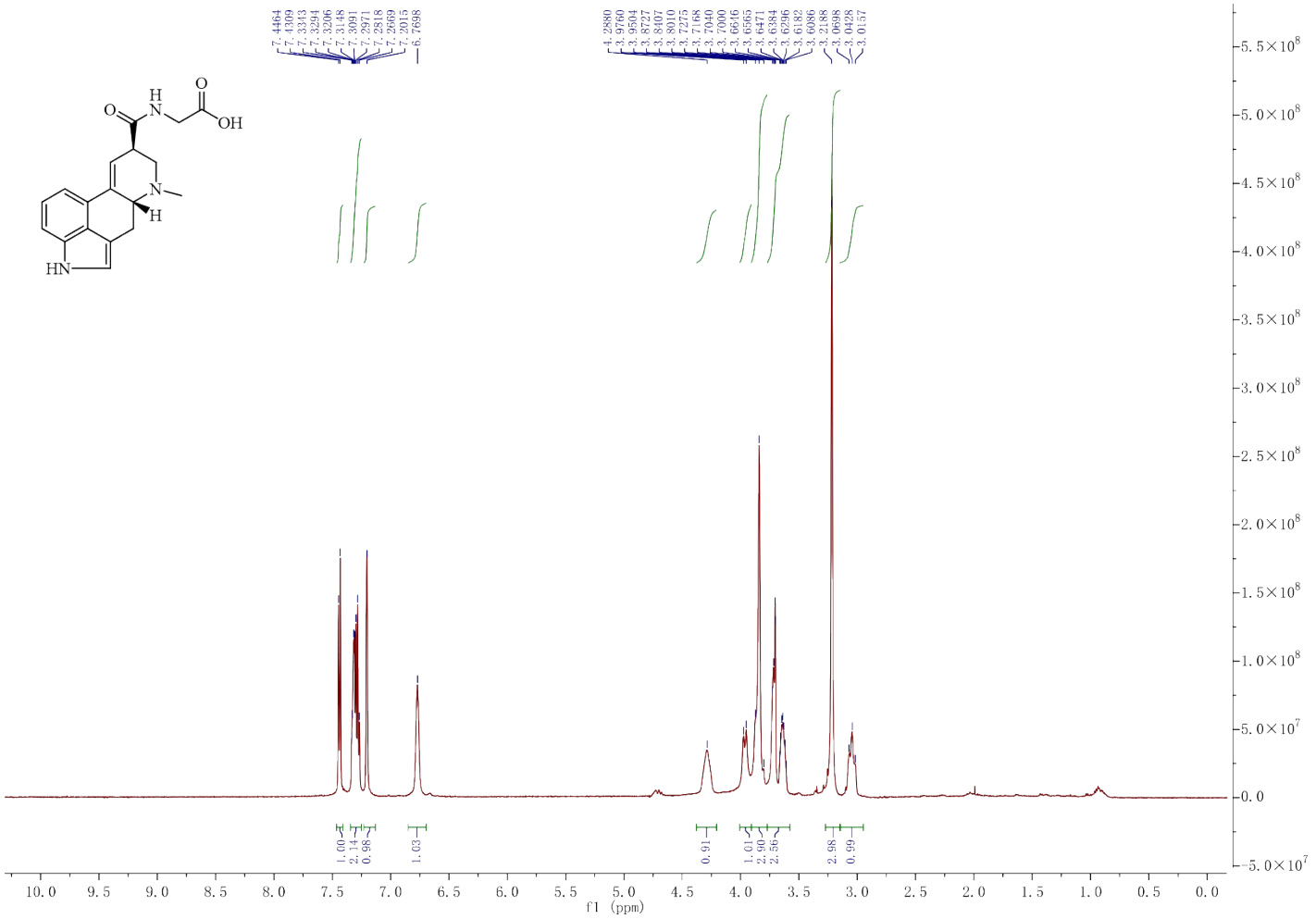

Fig. S17 ^{1}H NMR spectrum of lysergyl-glycine (**15**) in D_2O at 500 MHz.

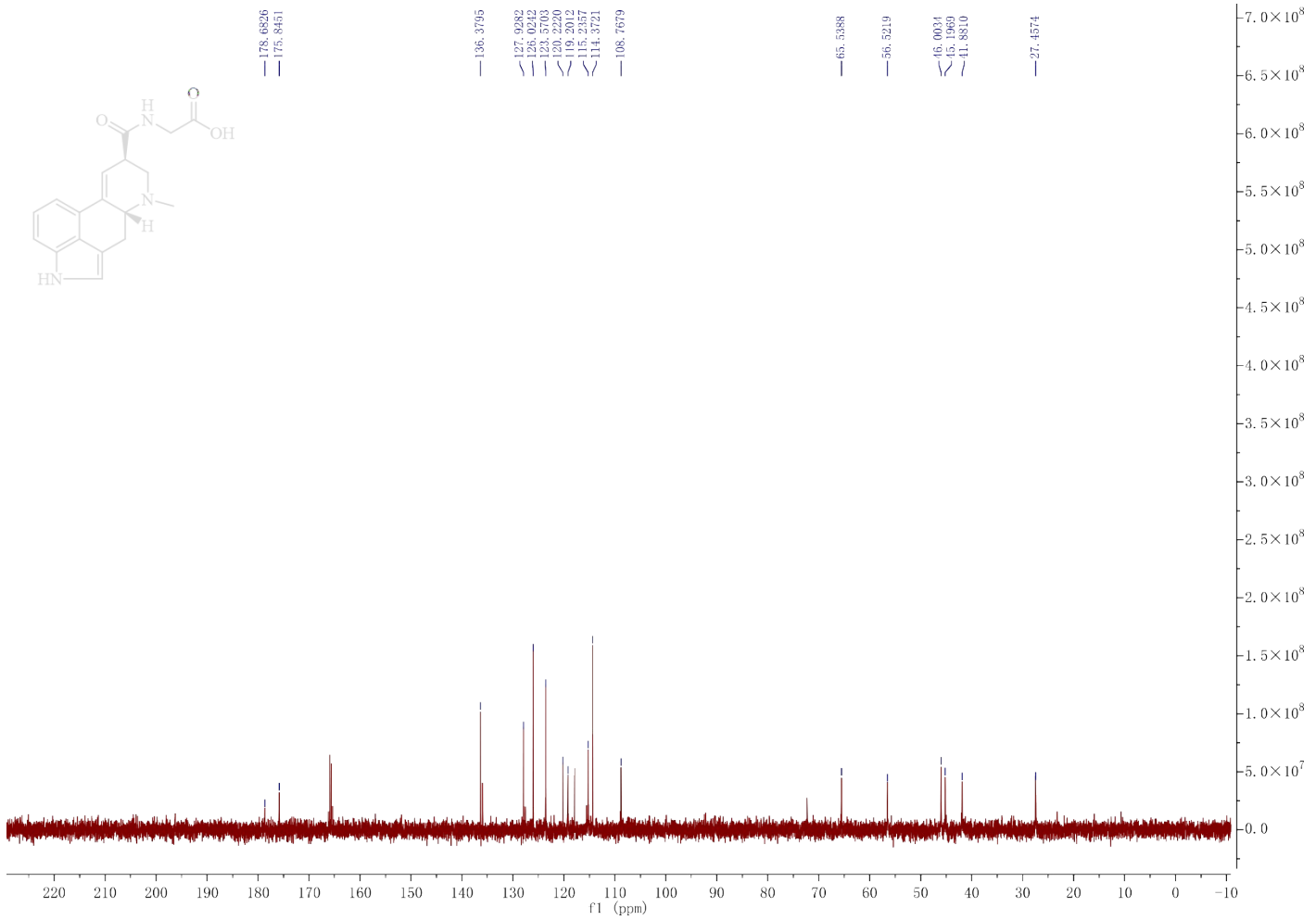

Fig. S18 ^{13}C NMR spectrum of lysergyl-glycine (**15**) in D_2O at 125 MHz.

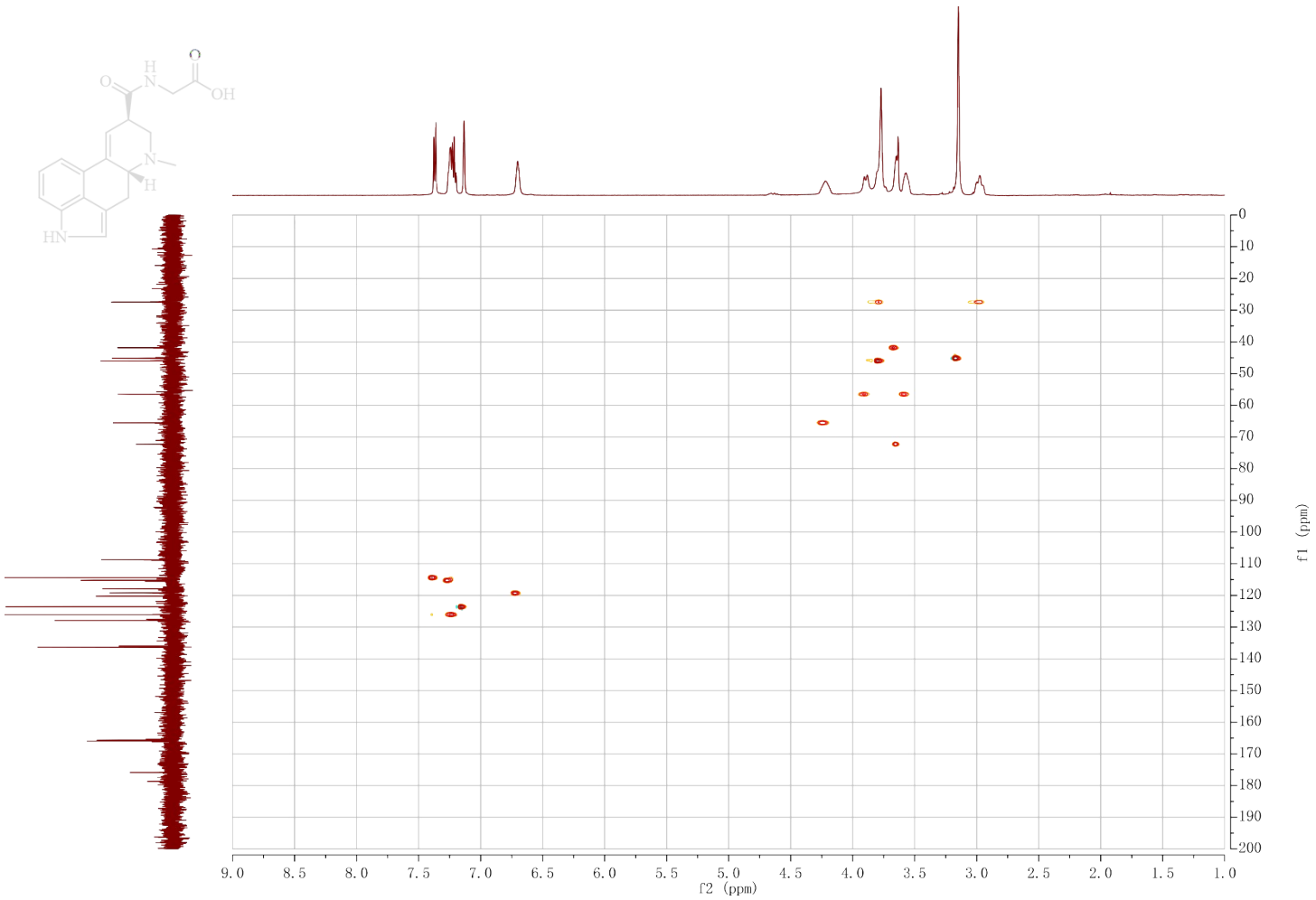

Fig. S19 HSQC spectrum of lysergyl-glycine (**15**) in D_2O.

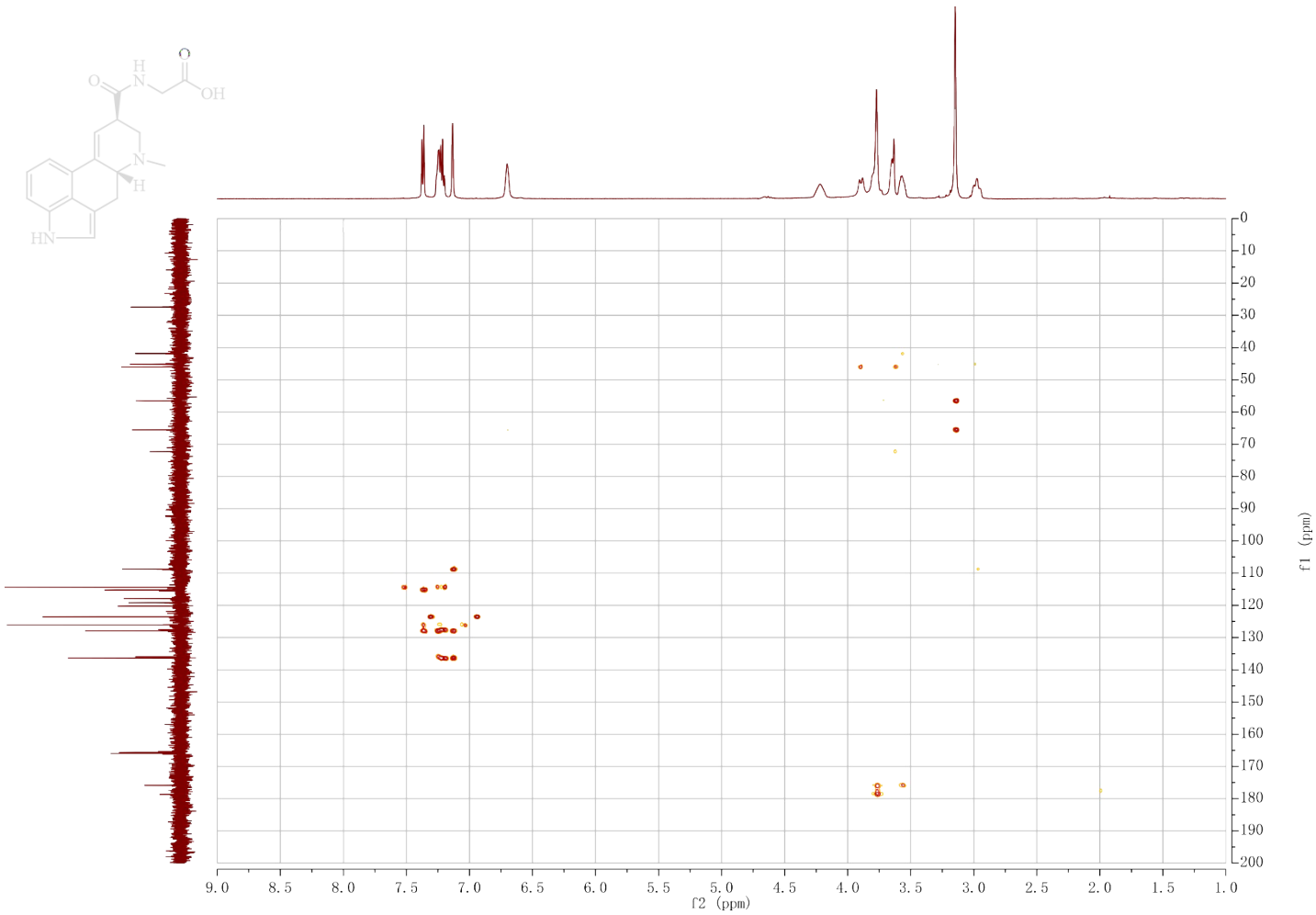

Fig. S20 HMBC spectrum of lysergyl-glycine (**15**) in D_2O.

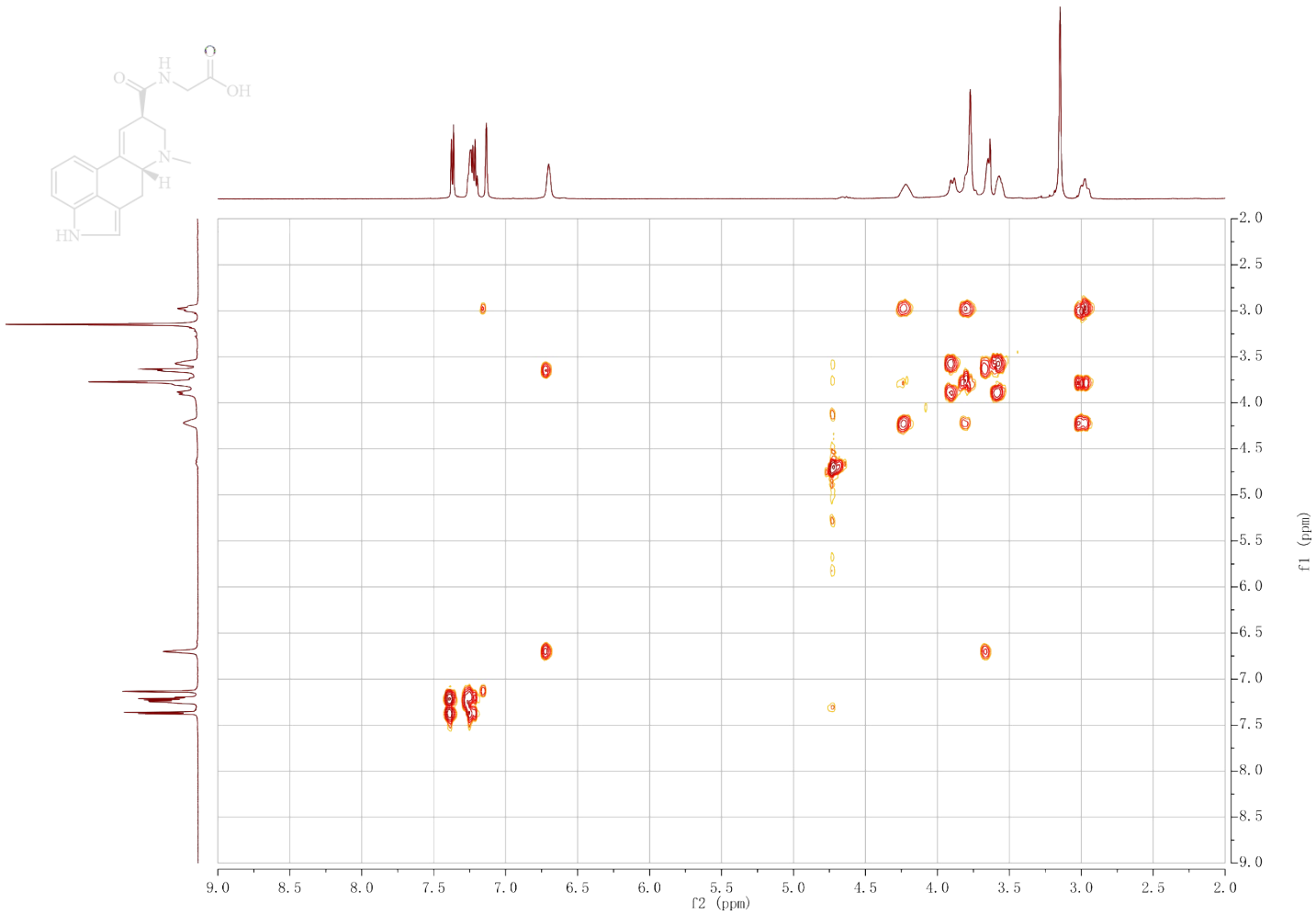

Fig. S21 COSY spectrum of lysergyl-glycine (**15**) in D_2O.

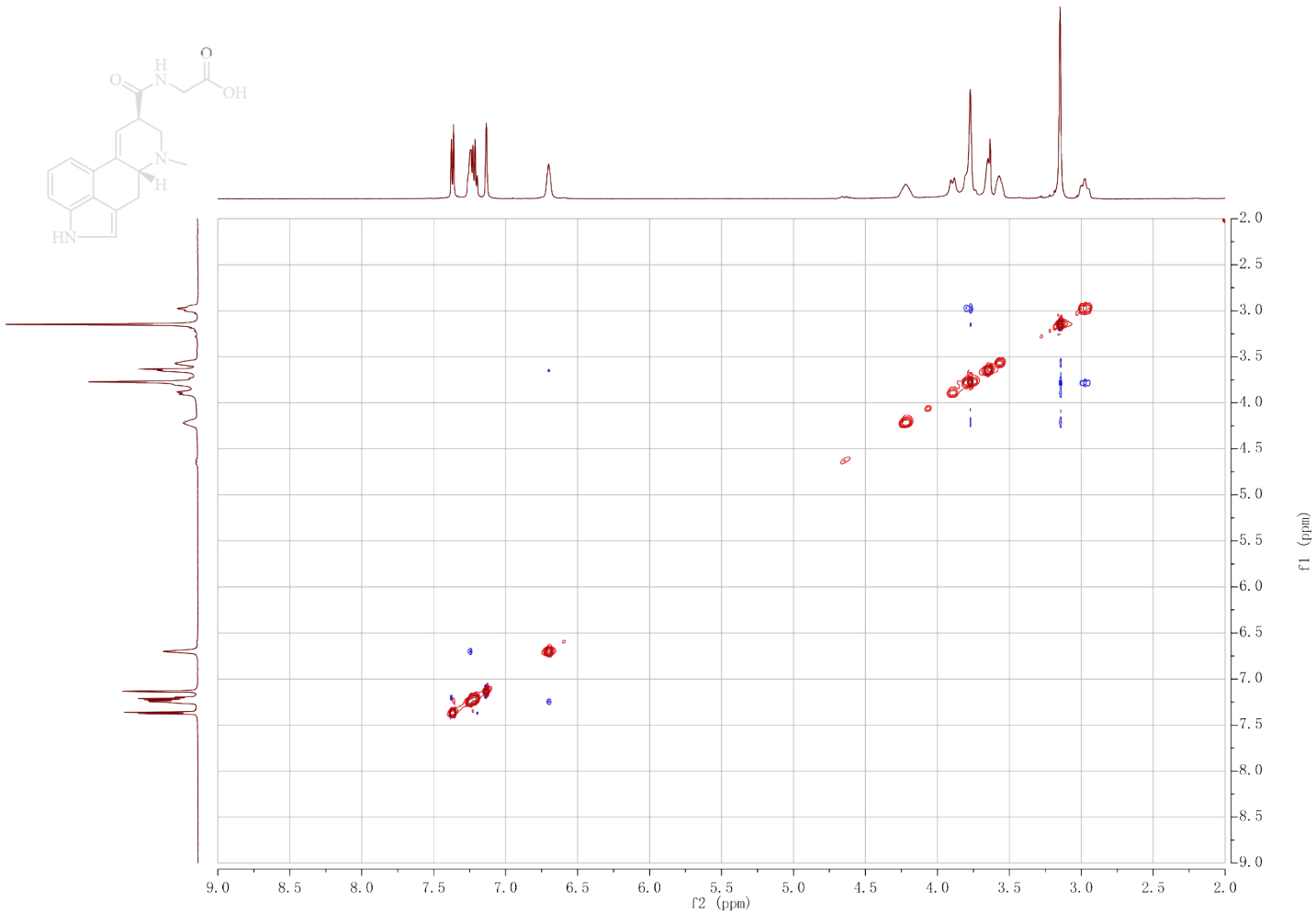

Fig. S22 NOESY spectrum of lysergyl-glycine (**15**) in D_2O.

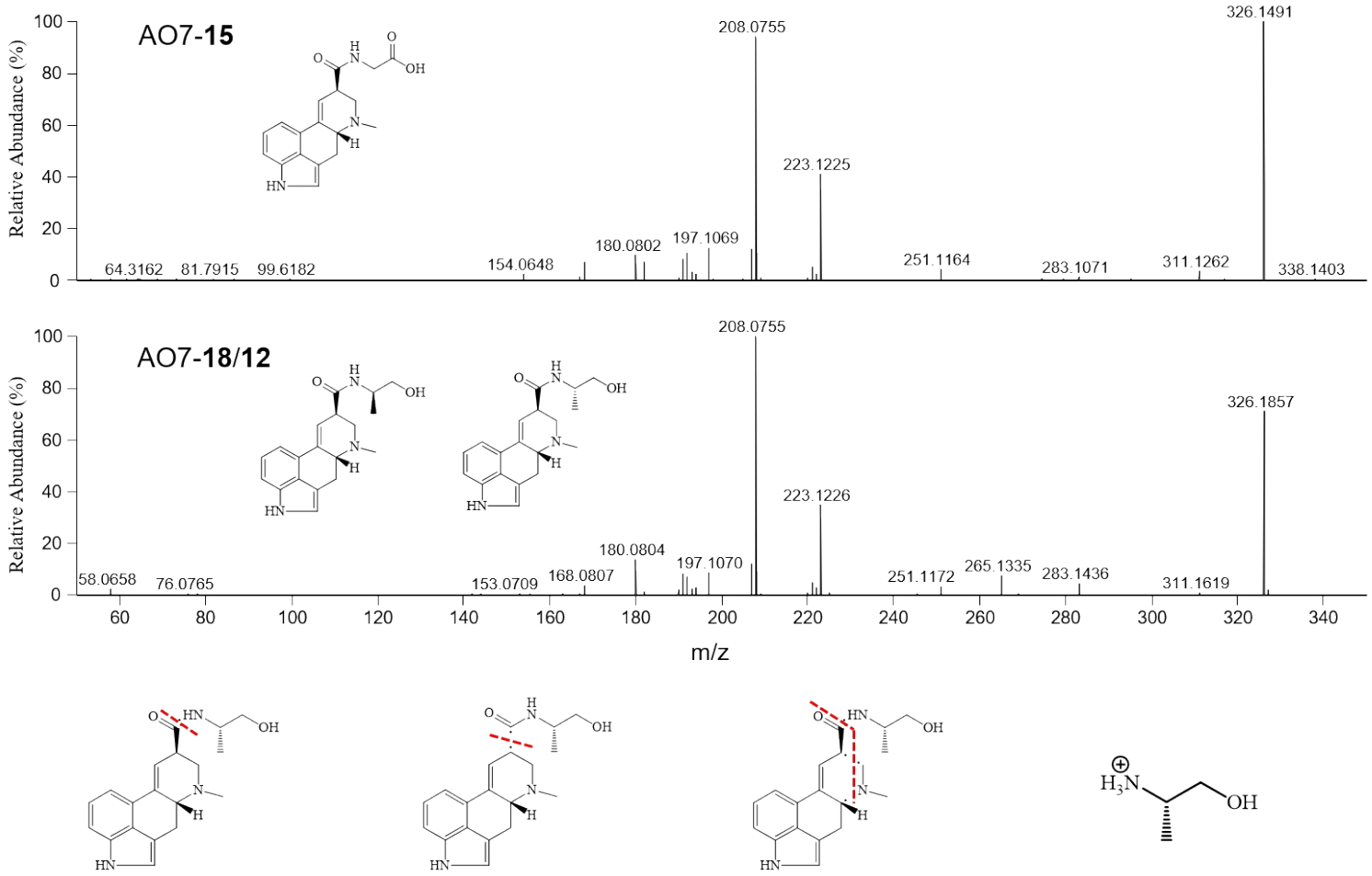

Fig. S23 LC-MS/MS fragmentation spectra of compound **15**, **18**, and **12** from AO7 and proposed structures for the fragment ions, 251, 223, 208 and 76 m/z.

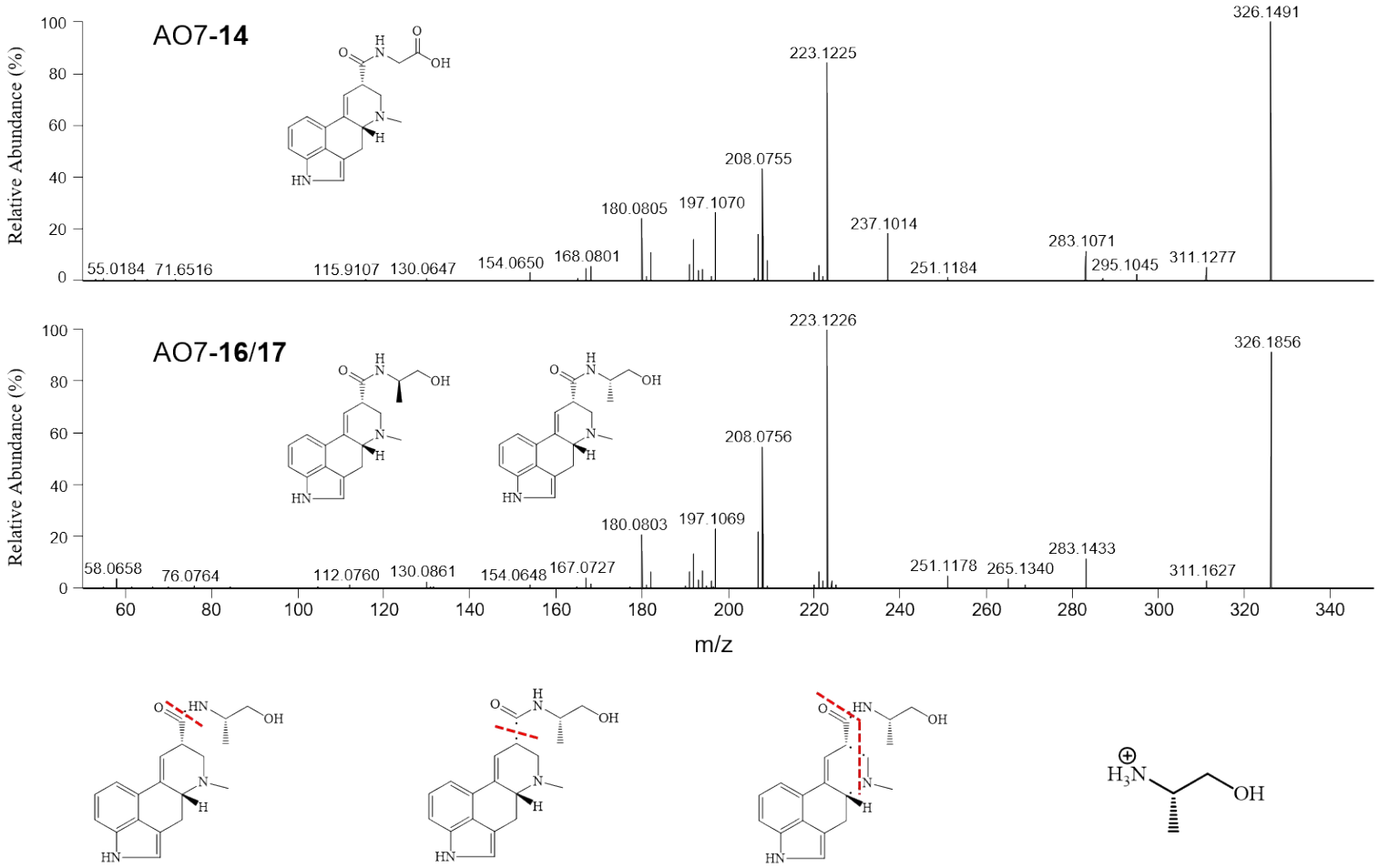

Fig. S24 LC-MS/MS fragmentation spectra of compound **14**, **16** and **17** from AO7 and proposed structures for the fragment ions, 251, 223, 208 and 76 m/z.

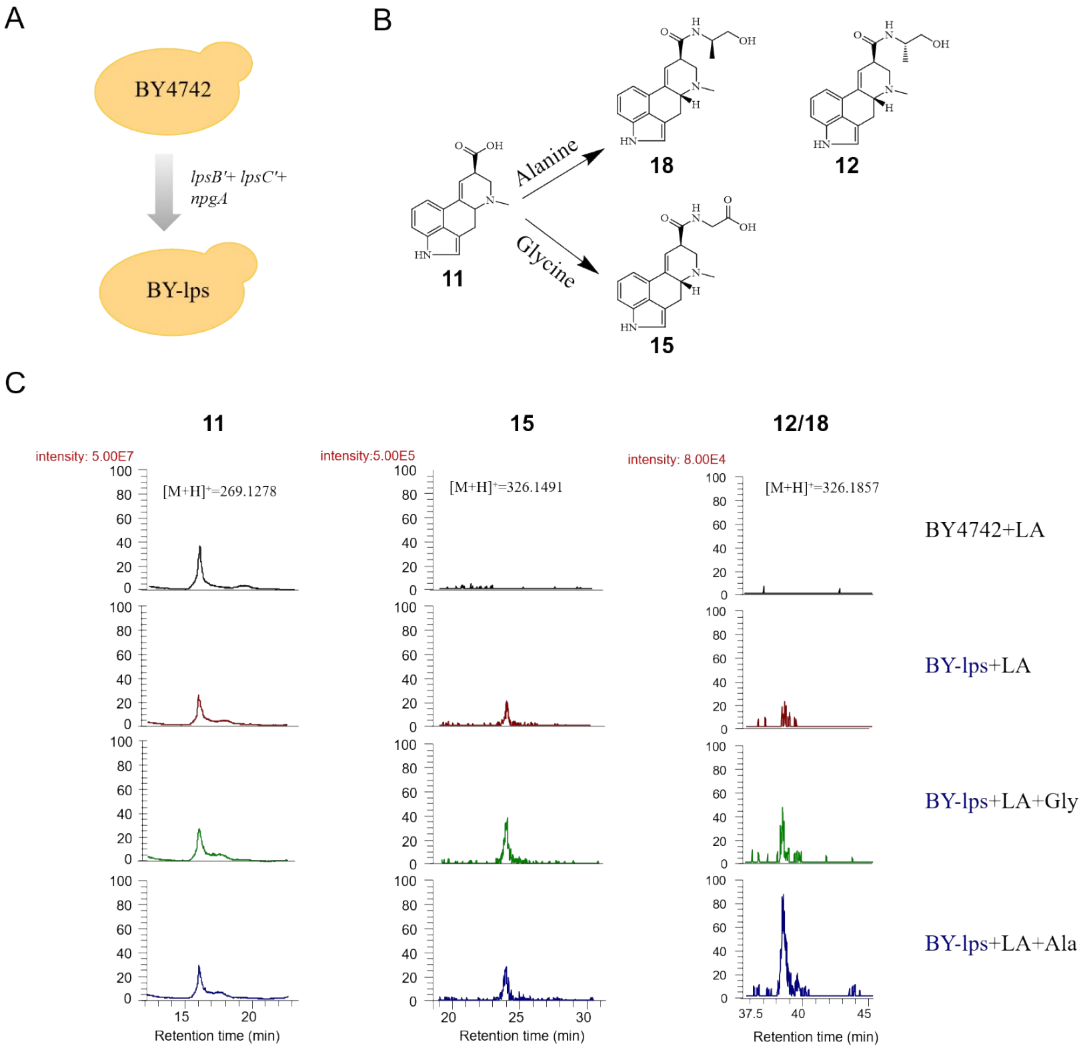

Fig. S25 Functional characterization of LpsC' in yeast. (A) Construction of BY-lps. The BY-lps strain was constructed by introducing *lpsB'*, *lpsC'*, and the *npgA* from *Aspergillus nidulans* into the starting strain BY4742. (B) Biosynthetic reaction producing ergoamide from compound **11**. (C) LC-MS analysis of yeast cell feeding with LA (**11**) and glycine or alanine. **11**, lysergic acid; **15**, lysergyl-glycine; **12**, ergometrine; **18**, diastereoisomer of **12**.

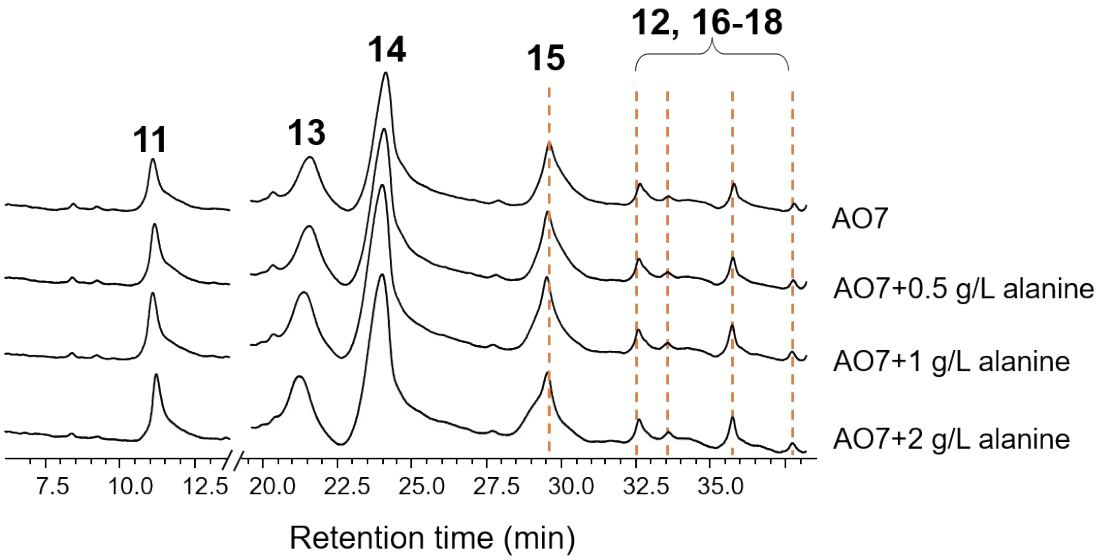

Fig. S26 EAs production of AO7 by feeding with various concentrations of alanine. **11**, D-lysergic acid (LA); **13**, D-isolysergic acid (ILA); **14**, isolysergyl-glycine; **15**, lysergyl-glycine; **12**, ergometrine; **16-18**, analogues of ergometrine.

Table 1 Primers used in the study

Name	Sequences (5'-3')	Usage
PamyB-F	TCATGGTGTTTTGATCATTTT	Amplification of *amyB* promoter from *A. oryzae* genome DNA
Pamy-R	CTGTGGGGTTTATTGTTCAG	
TamyB-F	AGGGTGGAGAGTATATGATG	Amplification of *amyB* terminator from *A. oryzae* genome DNA
TamyB-R	AATTCCGTTCCTTTGCTTTC	
AdargB-F	CCTGCAGGTCGACTCTAGAGTCTGCTCTCCCAGGCTCAG	Amplification of *argB* gene from *A. nidulans* genome DNA
AdargB-R	TGGGGATCCTCTAGACTGATGATGCAGTGGTTC	
AoAdeA-F	GCCTGCAGGTCGACTCTAGACCGTCATGTCCAGGAAGAT	Amplification of *adeA* gene from *A. oryzae* genome DNA
AoAdeA-R	ATGATGGGGATCCTCTAGACTGCGCAACAGCATACGAG	
AdsC-F	GCCTGCAGGTCGACTCTAGAGATCTTGGATATAAAAATC	Amplification of *sC* gene from *A. nidulans* genome DNA
AdsC-R	ATGATGGGGATCCTCTAGAAAGCTTCTCTTGGCAATAG	
AoniaD-F	GCCTGCAGGTCGACTCTAGAAAGCTTAACAGGCCCCAAATTC	Amplification of *niaD* gene from *A. oryzae* genome DNA
AoniaD-R	ATGATGGGGATCCTCTAGAAAGCTTTGGATTTCCTAC	
bar-F	TAGTCATTGCAACTTGAAACATGAGCCCAGAACGACGCC	Amplification of *bar* gene
bar-R	ATTCGGGTAGTGAGTCATTCAGATCTCGGTGACGGGCAG	
itrA-F	GTCATTGCAACTTGAAACATGGCATATCTTGCTGTC	Amplification of *itrA* gene from *A. luchuensis*

itrA-R	ATTCGGGTAGTGAGTCATCTAATTCAAGGACCCTTTG	genome DNA
dmaW'-F	TGAACAATAAACCCCACAGATGTCGACCACGAACGAC	Amplification of *dmaW'* gene from *C. purpurea 22.07* cDNA
dmaW'-R	CATCATATACTCTCCACCCTTTACTTCGTTGAGAGATCACAGCGCCTGTAG	
easF'-F	CTGAACAATAAACCCCACAGATGCCTGCTCTTCCGGTC	Amplification of *easF'* gene from *C. purpurea 22.07* cDNA
easF'-R	CATCATATACTCTCCACCCTCTACGCCAACTTTAATTC	
easC'-F	TGAACAATAAACCCCACAGATGGCTTCTGAGGTCTCTG	Amplification of *easC'* gene from *C. purpurea 22.07* cDNA
easC'-R	CATCATATACTCTCCACCCTTTACTCCACTATCTTTTCG	
easE'-F	GAACAATAAACCCCACAGATGTATCACATTCTCGGCCCA	Amplification of *easE'* gene from *C. purpurea 22.07* cDNA
easE'-R	CATCATATACTCTCCACCCTTCATTTTATCCCGATAAG	
easD'-F	TGAACAATAAACCCCACAGATGCCGTCCATGACGTCC	Amplification of *easD'* gene from *C. purpurea 22.07* cDNA
easD'-R	TCATATACTCTCCACCCTTCAAGGCGGGCAGGCCCCAAC	
easA'-F	GAACAATAAACCCCACAGATGTCCACATCGAACCTTTTC	Amplification of *easA'* gene from *C. purpurea 22.07* cDNA
easA'-R	CATCATATACTCTCCACCCTTCAACCCGCCACTGCTGC	
easG'-F	TGAACAATAAACCCCACAGATGACGGTCTTACTGACAG	Amplification of *easG'* gene from *C. purpurea 22.07* cDNA
easG'-R	CATCATATACTCTCCACCCTTCACTTCCTTGCACGCCAG	
cloA'-F	TGAACAATAAACCCCACAGATGTCGCTACAATGGCTGC	Amplification of *cloA'* gene from *C. purpurea 22.07* cDNA
cloA'-R	CATCATATACTCTCCACCCTTCAGTGGTGATGGTGATG	

EpcloA-F	TGAACAATAAACCCCACAGATGATCCTTCCTTGGCTTTC	Amplification of *EpcloA* gene from synthetic gene
EpcloA-R	TCATATACTCTCCACCCTTTAGTGGTGATGGTGATGATG	
RFP-F	CTGAACAATAAACCCCACAGATGGTGAGCAAGGGCGAG	Amplification of *RFP* gene with protein localized to the cytoplasm
RFP-R	CATCATATACTCTCCACCCTTTACTTGTACAGCTCGTC	
RFP^{SKL}-R	CATCATATACTCTCCACCCTTTACAGCTTCGACTTGTACAGCTCGTCCATG	Amplification of RFP^{SKL} gene with protein localized to the peroxisomes
GFP-F	CTGAACAATAAACCCCACAGGTACCGGTCGCCACCATG	Amplification of G*FP* gene fused to *easC′*
GFP-R	ACAGAGACCTCAGAAGCCATCTTGTACAGCTCGTCCATG	
easC'-F	ATGGCTTCTGAGGTCTCTG	Amplification of *easC′* gene fused to *GFP*
lpsB'-F	TGAACAATAAACCCCACAGATGGCAAGCCTCGACAAG	Amplification of *lpsB′* gene from *C. purpurea 22.07* cDNA
lpsB'-R	TCATATACTCTCCACCCTTCAAGACTCAAGACACTTG	
lpsC'-F	GAACAATAAACCCCACAGATGAATTCAATCAAGCTGAAG	Amplification of *lpsC′* gene from *C. purpurea 22.07* cDNA
lpsC'-R	ATCATATACTCTCCACCCTCTACCACGCCCTCGTAAATG	
up-F	CGGGGATCCTCTAGAGATTCTTGCCTTCTCTCCCTCTC	Amplification of left flanking region for homology-directed repair (HDR) of *C. purpurea cloA′*
up-R	AGTGAGGGTTAATTGCGCCGTGTCCTGACCGGGCGAAAG	
hph-F	GCGCAATTAACCCTCACTAAAG	Amplification of *hph* marker cassette

hph-R	CAGGGCTGGTGACGGAATTTTC	
down-F	AATTCCGTCACCAGCCCTGGGCGTCCAATGCAATACATC	Amplification of right flanking region for HDR of *C. purpurea cloA'*
down-R	ATGCCTGCAGGTCGACGATACTGGCCACGCTGCCTGATG	
YZΔcloA-F	ATCAGGCGAAGCAGATTG	Verification of *cloA'* deletion transformants
YZΔcloA-F	AACCCTATTCTGCGCACG	

Table S2 Plasmids used in the study

Plasmids	Characteristics	Source
pADR	Plasmids containing PamyB promoter and TamyB terminator and *argB* marker gene cassette, (*Amp*^{R})	This work
pAOA	Plasmids containing PamyB promoter and TamyB terminator and *adeA* marker gene cassette, (*Amp*^{R})	This work
pADS	Plasmids containing PamyB promoter and TamyB terminator and *sC* marker gene cassette, (*Amp*^{R})	This work
pAON	Plasmids containing PamyB promoter and TamyB terminator and *niaD* marker gene cassette, (*Amp*^{R})	This work
pPTR	Plasmids containing PamyB promoter and TamyB terminator and *ptr* marker gene cassette, (*Amp*^{R})	This work
pBAR	Plasmids containing PamyB promoter and TamyB terminator and *bar* marker gene cassette, (*Amp*^{R})	This work
pITR	Plasmids containing PamyB promoter and TamyB terminator and *itr* marker gene cassette, (*Amp*^{R})	This work
pADR-*cloA′*	Plasmids containing PmayB-*cloA′*-TmayB cassette and *argB* marker gene cassette served as expression plasmid, (*Amp*^{R})	This work
pADR-*CpcloA*	Plasmids containing PmayB-*CpcloA*-TmayB cassette and *argB* marker gene cassette served as expression plasmid, (*Amp*^{R})	This work
pADR-*EpcloA*	Plasmids containing PmayB-*EpcloA*-TmayB cassette and *argB* marker gene cassette served as expression plasmid, (*Amp*^{R})	This work
pADR-*dmaW′-easF*	Plasmids containing PmayB-*dmaW′*-TmayB-PmayB-*easF′*-TmayB cassette and *argB* marker gene cassette served as expression plasmid, (*Amp*^{R})	This work
pAOA-*easE′-easC*	Plasmids containing PmayB-*easE′*-TmayB-PmayB-*easC′*-TmayB cassette and *adeA* marker gene cassette served as expression plasmid, (*Amp*^{R})	This work
pADS-*easD′-easA′*	Plasmids containing PmayB-*easD′*-TmayB-PmayB-*easA′*-TmayB cassette and *sC* marker gene cassette served as expression	This work

	plasmid, (*Amp*^{R})	
pAON-*easG′*	Plasmids containing PmayB-*easG′*-TmayB cassette and *niaD* marker gene cassette served as expression plasmid, (*Amp*^{R})	This work
pADR-*GFP*-*easC′*	Plasmids containing PmayB-*GFP*-*easG′*-TmayB cassette and *argB* marker gene cassette served as expression plasmid, (*Amp*^{R})	This work
pAOA-*RFP*	Plasmids containing PmayB-*RFP*-TmayB cassette and *adeA* marker gene cassette served as expression plasmid, (*Amp*^{R})	This work
pAOA-*RFP*^{SKL}	Plasmids containing PmayB-*RFP*^{SKL}-TmayB cassette and *adeA* marker gene cassette served as expression plasmid, (*Amp*^{R})	This work
pPTR-*cloA′*	Plasmids containing PmayB-*cloA′*-TmayB cassette and *ptr* marker gene cassette served as expression plasmid, (*Amp*^{R})	This work
pBAR-*lpsB′*-*lpsC′*	Plasmids containing PmayB-*lpsB′*-TmayB-PmayB-*lpsC′*-TmayB cassette and *bar* marker gene cassette served as expression plasmid, (*Amp*^{R})	This work
pPTR-*easC′*	Plasmids containing PmayB-*easC′*-TmayB cassette and *ptr* marker gene cassette served as expression plasmid, (*Amp*^{R})	This work
pITR-*easC′*	Plasmids containing PmayB-*easC′*-TmayB cassette and *itr* marker gene cassette served as expression plasmid, (*Amp*^{R})	This work
pMD18T-hph	Plasmids containing *hph* marker gene cassette served as donor DNA to delete the *cloA′* gene, (*Amp*^{R})	This work

Table S3 Strains used in the study

name	parent strain	genotype	Source
AO1	*A.oryzae* NSAR1	*dmaW′*, *easF′*, *easC′*, *easE′*, *easD′*, *easA′*, *easG′*, *argB*, *adeA*, *sC*, *niaD*	This work
CGC	*A.oryzae* NSAR1	*GFP-easC′*, *RFP*, *argB*, *adeA*	This work
CGP	*A.oryzae* NSAR1	*GFP-easC′*, RFP^{SKL}, *argB*, *adeA*	This work
AO2	*A.oryzae* AO1	*dmaW′*, *easF′*, *easC′*, *easE′*, *easD′*, *easA′*, *easG′*, *easC′*, *argB*, *adeA*, *sC*, *niaD*, *ptr*	This work
AO3	*A.oryzae* AO1	*dmaW′*, *easF′*, *easC′*, *easE′*, *easD′*, *easA′*, *easG′*, *cloA′*, *argB*, *adeA*, *sC*, *niaD*, *ptr*	This work
AO4	*A.oryzae* NSAR1	*cloA′*, *argB*	This work
AO5	*A.oryzae* AO1	*dmaW′*, *easF′*, *easC′*, *easE′*, *easD′*, *easA′*, *easG′*, *CpcloA′*, *argB*, *adeA*, *sC*, *niaD*, *ptr*	This work
AO6	*A.oryzae* AO1	*dmaW′*, *easF′*, *easC′*, *easE′*, *easD′*, *easA′*, *easG′*, *EpcloA′*, *argB*, *adeA*, *sC*, *niaD*, *ptr*	This work
AO7	*A.oryzae* AO3	*dmaW′*, *easF′*, *easC′*, *easE′*, *easD′*, *easA′*, *easG′*, *cloA′*, *lpsB′*, *lpsC′*, *argB*, *adeA*, *sC*, *niaD*, *ptr*, *bar*	This work
AO8	*A.oryzae* AO7	*dmaW′*, *easF′*, *easC′*, *easE′*, *easD′*, *easA′*, *easG′*, *cloA′*, *lpsB′*, *lpsC′*, *easC′*, *argB*, *adeA*, *sC*, *niaD*, *ptr*, *bar*, *itr*	This work
ΔcloA′	*C. purpurea* 22.07	Δ*cloA′* :: *hph*	This work