

Identification of PKS-NRPS Hybrid Metabolites in Marine-Derived *Penicillium oxalicum*

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Table S1. Phylogenetic affiliations of the representative isolates of 32 species

Isolate ID	Order	Genus	Accession number	Closest identified relative	Identity (%)	Overlap (bp)
MEFC001	Eurotiales	<i>Aspergillus</i>	MK732089	<i>Aspergillus</i> sp. (MK775950.1)	99	1112
MEFC009			MK732091	<i>Aspergillus fumigatus</i> (GU566242.1)	99	1153
MEFC090			MK732125	<i>Aspergillus versicolor</i> (MH509421.1)	100	1014
MEFC124			MK732140	<i>Aspergillus sydowii</i> (MH813340.1)	100	1026
MEFC058		<i>Penicillium</i>	MK732107	<i>Penicillium</i> sp. (HM535390.1)	100	1048
MEFC104			MK732132	<i>Penicillium oxalicum</i> (MH705349.1)	100	1044
MEFC106			MK732134	<i>Penicillium polonicum</i> (KU847876.1)	100	1051
MEFC031			MK732096	<i>Acremonium sclerotigenum</i> (MF716849.1)	100	1037
MEFC050	Hypocreales	<i>Acremonium</i>	MK732103	<i>Engyodontium</i> sp. (MH102090.1)	100	1123
MEFC137			MK732148	<i>Uncultured Engyodontium</i> (KF768338.1)	100	1127
MEFC073		<i>Trichoderma</i>	MK732113	<i>Trichoderma harzianum</i> (KY209922.1)	100	1131
MEFC149			MK732155	<i>Hypocreales</i> sp. (GU017486.1)	99	1020
MEFC044	Pleosporales	<i>Alternaria</i>	MK732097	<i>Alternaria tenuissima</i> (KY315929.1)	100	1050
MEFC045			MK732098	<i>Alternaria alternate</i> (MK979371.1)	99	1020
MEFC139		<i>Cochliobolus</i>	MK732149	<i>Pleosporales</i> sp. (KR002678.1)	100	968
MEFC148			MK732154	<i>Bipolaris sorokiniana</i> (MH209068.1)	100	1059
MEFC077	Capnodiales	<i>Cladosporium</i>	MK732115	<i>Cladosporium xanthochromaticum</i> (MF473323.1)	100	1061
MEFC134			MK732146	<i>Cladosporium</i> sp. (LT603047.1)	100	996
MEFC132		<i>Xenosonderhenia</i>	MK732144	<i>Xenosonderhenia syzygii</i> (NR_111763.1)	94	785
MEFC133			MK732145	<i>Xenosonderhenia eucalypti</i> (NR_137937.1)	94	782
MEFC052	Tritirachiales	<i>Tritirachium</i>	MK732104	<i>Tritirachium</i> sp. (AB109761.1)	100	1123
MEFC054			MK732105	<i>Parengyodontium album</i> (MK834516.1)	99	1077
MEFC101	Saccharomycetales	<i>Geotrichum</i>	MK732130	<i>Galactomyces candidum</i> (KP132254.1)	97	623
MEFC063	Polyporales	<i>Irpex</i>	MK732109	<i>Irpex lacteus</i> (KX588111.1)	100	1208
MEFC085		<i>Trametes</i>	MK732121	<i>Trametes hirsute</i> (MH142007.1)	100	1118
MEFC112		<i>Emmia</i>	MK732136	<i>Emmia lacerate</i> (MH793591.1)	100	1158
MEFC081	Agaricales	<i>Schizophyllum</i>	MK732118	<i>Schizophyllum commune</i> (MH863418.1)	100	1147
MEFC116			MK732139	<i>Coprinellus radians</i> (MK087751.1)	100	1225
MEFC082	Auriculariales	<i>Auricularia</i>	MK732119	<i>Auricularia polytricha</i> (MG732993.1)	100	1133
MEFC147	Corticiales	<i>Limonomyces</i>	MK732153	<i>Limonomyces</i> sp. (MH347299.1)	100	1162
MEFC150	Cantharellales	<i>Sistotrema</i>	MK732156	<i>Sistotrema brinkmannii</i> (DQ899095.1)	99	1273
MEFC146	Trichosporonales	<i>Apiotrichum</i>	MK732152	<i>Apiotrichum domesticum</i> (MK267418.1)	100	965

Table S2. Antimicrobial activities of the crude extracts from the marine-derived fungi

Number	Species	<i>S. aureus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>B. subtilis</i>	<i>M. luteus</i>	<i>A. niger</i>	<i>S. cerevisiae</i>	<i>C. albicans</i>
MEFC008	<i>Aspergillus terreus</i>	++	-	-	+	-	-	+	-
MEFC011	<i>Aspergillus fumigatus</i>	++	-	-	-	-	-	-	++
MEFC012	<i>Aspergillus fumigatus</i>	++	-	-	-	-	-	-	++
MEFC013	<i>Aspergillus fumigatus</i>	++	-	-	-	-	-	-	++
MEFC030	<i>Aspergillus fumigatus</i>	-	-	-	-	-	-	-	++
MEFC044	<i>Alternaria alternata</i>	+	-	-	-	-	-	-	-
MEFC056	<i>Tritirachium</i>	-	-	-	+	-	-	-	++
MEFC059	<i>Penicillium chrysogenum</i>	-	-	+	-	+	-	-	-
MEFC063	<i>Irpex lacteus</i>	-	-	+++	-	++	-	-	-
MEFC064	<i>Irpex lacteus</i>	-	-	-	-	-	-	++	++
MEFC091	<i>Aspergillus versicolor</i>	-	-	+++	-	++	-	-	-
MEFC094	<i>Aspergillus versicolor</i>	+	-	++	-	-	-	-	++
MEFC097	<i>Trametes hirsuta</i>	+	-	-	-	-	-	-	++
MEFC104	<i>Penicillium oxalicum</i>	-	-	+++	-	+	-	-	-
MEFC105	<i>Penicillium oxalicum</i>	-	-	+	++	+	-	-	-
MEFC116	<i>Coprinellus radians</i>	-	-	-	+++	++	-	-	-
MEFC129	<i>Aspergillus sydowii</i>	-	-	-	+	-	-	-	-

Positive control: Ampicillin (bacterial, 4.0 mg/mL); Amphotericin B (fungi, 4.0 mg/mL); negative control: dimethyl sulfoxide (DMSO); – no antimicrobial activity; + weak inhibitory activity (inhibition zone between 5 and 10 mm); ++ moderate inhibitory activity (inhibition zone between 10 and 15 mm); +++ strong inhibitory activity (inhibition zone > 15 mm).

Table S3. BGCs in the genome of *P. oxalicum* MEFC104

No.	Locus tag	Type	Products	No.	Locus tag	Type	Products
1	PDE_00149	Terpene		21	PDE_04074	NRPS	
2	PDE_00404	Betalactone		22	PDE_04252	PKS-NRPS	
	PDE_00405	Betalactone		23	PDE_04436	NRPS-like	
3	PDE_00789	NRPS			PDE_04438	NRPS-like	
	PDE_00793	NRPS			PDE_04441	T1 PKS	
	PDE_00797	Indole		24	PDE_04543	NRPS	
	PDE_00807	Indole		25	PDE_05564	NRPS-like	
	PDE_00810	NRPS		26	PDE_05889	Indole	
4	PDE_01066	Indole	Roquefortine C	27	PDE_06013	Terpene	
	PDE_01071	NRPS		28	PDE_06094	NRPS-like	
	PDE_01075	Terpene		29	PDE_06154	Terpene	
	PDE_01077	NRPS		30	PDE_06206	NRPS	
5	PDE_01185	NRPS-like		31	PDE_06630	NRPS-like	
6	PDE_01212	NRPS		32	PDE_07005	NRPS	
7	PDE_01235	PKS-NRPS		33	PDE_07163	NRPS	
8	PDE_01430	Terpene		34	PDE_07205	Terpene	
	PDE_01432	NRPS		35	PDE_07373	NRPS-like	
9	PDE_01655	Terpene		36	PDE_08027	Terpene	
10	PDE_01717	Terpene		37	PDE_08071	Terpene	
11	PDE_02026	Siderophore		38	PDE_08155	NRPS-like	
12	PDE_02110	T3 PKS		39	PDE_08329	NRPS-like	
13	PDE_02131	NRPS		40	PDE_08437	NRPS-like	
	PDE_02141	T1 PKS		41	PDE_09198	PKS-NRPS	
14	PDE_02203	NRPS-like		42	PDE_09237	T1 PKS	
15	PDE_02610	NRPS		43	PDE_09491	T1 PKS	Naphthopyrone
16	PDE_03304	NRPS-like		44	PDE_09561	Terpene	Clavaric acid
17	PDE_03416	NRPS		45	PDE_09740	Indole	Emericellin
18	PDE_03455	T1 PKS			PDE_09743	NRPS-like	
19	PDE_03926	T1 PKS		46	PDE_10001	Terpene	
20	PDE_04017	PKS-NRPS	Oxaleimide C		PDE_10006	T1 PKS	

Table S4. The strains used in gene deletion experiments

Strain	Genotype	Reference
MEFC104-WT	<i>P. oxalicum</i> MEFC104	This study
MEFC104- Δ apnA	<i>P. oxalicum</i> MEFC104, Δ apnA::hph	This study
MEFC104- Δ opdA	<i>P. oxalicum</i> MEFC104, Δ opdA::hph	This study
MEFC104- Δ opdB	<i>P. oxalicum</i> MEFC104, Δ opdB::hph	This study
MEFC104- Δ opdC	<i>P. oxalicum</i> MEFC104, Δ opdC::hph	This study
MEFC104- Δ opdD	<i>P. oxalicum</i> MEFC104, Δ opdD::hph	This study
MEFC104- Δ opdE	<i>P. oxalicum</i> MEFC104, Δ opdE::hph	This study
MEFC104- Δ opdF	<i>P. oxalicum</i> MEFC104, Δ opdF::hph	This study
MEFC104- Δ opdG	<i>P. oxalicum</i> MEFC104, Δ opdG::hph	This study
MEFC104- Δ opdH	<i>P. oxalicum</i> MEFC104, Δ opdH::hph	This study
MEFC104- Δ opdI	<i>P. oxalicum</i> MEFC104, Δ opdI::hph	This study
MEFC104- Δ opdJ	<i>P. oxalicum</i> MEFC104, Δ opdJ::hph	This study
MEFC104- Δ opdK	<i>P. oxalicum</i> MEFC104, Δ opdK::hph	This study
MEFC104- Δ opdL	<i>P. oxalicum</i> MEFC104, Δ opdL::hph	This study
MEFC104- Δ opdM	<i>P. oxalicum</i> MEFC104, Δ opdM::hph	This study
MEFC104- Δ opdN	<i>P. oxalicum</i> MEFC104, Δ opdN::hph	This study
MEFC104- Δ opdO	<i>P. oxalicum</i> MEFC104, Δ opdO::hph	This study
MEFC104- Δ opdR	<i>P. oxalicum</i> MEFC104, Δ opdR::hph	This study

Table S5. The oligonucleotides used in this study

Primers	Sequence (5'-3')
hph-F	TTCGGGATCGCAAGCGTAAAG
hph-R	CAATTATCTTTGCGAACCCAGG
U-01235-F1	AGGAGGCCACCTTTACCAAT
U-01235-R1	CTTTACGCTTGCGATCCCGAATCTCTGTGACTGATTCCCAC
D-01235-F1	CCTGGGTTTCGCAAAGATAATTGGCTCTACAGGCCATCATTCT
D-01235-R1	TTGAACAGTGGGAGGTCACC
U-01235-F	ATCCAATCAAGGATCGCTGC
D-01235-R	GGGGCTGAACAACAAGAGTC
U-09198-F1	CTCGCCATCAAGAATCGTGT
U-09198-R1	CTTTACGCTTGCGATCCCGAATAAGCAGTATCGGTCCTCAGG
D-09198-F1	CCTGGGTTTCGCAAAGATAATTGCGGGCTGTCGATTCTTATTG
D-09198-R1	GCAACAAACGGTTCCAAGCC
U-09198-F	GTACGTCGGATCCGTGAAGAC
D-09198-R	CAGTTGTTTCACATCTGTCGC
U-01228-F1	ATGCTGAGCGCAATGATCTC
U-01228-R1	CTTTACGCTTGCGATCCCGAATTTAGACTTGGTTGGTTTCT
D-01228-F1	CCTGGGTTTCGCAAAGATAATTGGGATAAATTCGTTCTC
D-01228-R1	GAGAATCGCGAAGTCTCTGG
U-01228-F	AAGCGGCATGTAGCCCTGAT
D-01228-R	TATGCGACGGAAATCACCGG
U-01229-F1	TCCATTATGCCGGGAGTTCA
U-01229-R1	CTTTACGCTTGCGATCCCGAAATTGAGAGAGTTGAACGTGA
D-01229-F1	CCTGGGTTTCGCAAAGATAATTGGCTTCTATGAGCGTCAAGCC
D-01229-R1	CAAAGGTGCGATGAGCAAGC
U-01229-F	TTGAAAGGCTCATATGGCTC
D-01229-R	AACTTCATCACCTTGCAGAC
U-01230-F1	ATAGCTGGAATGGCCCACC
U-01230-R1	CTTTACGCTTGCGATCCCGAACCCTTCAACTGGTTTCGTTT
D-01230-F1	CCTGGGTTTCGCAAAGATAATTGATCGGCCTTGGGCCCCGTCC
D-01230-R1	CCTTGTTGGTCTCATATCCA
U-01230-F	CAGCGGGTCCGATTCATACCT
D-01230-R	CTATATGTCTTCACCTGTCG
U-01231-F1	CCTCGTTGCCTTTCTCTAAA
U-01231-R1	CTTTACGCTTGCGATCCCGAATGAAACTGGAATTTGACGT
D-01231-F1	CCTGGGTTTCGCAAAGATAATTGACCCTCGATTCTACCTGT
D-01231-R1	GTTTACATTGCTGCCTGCAA

U-01231-F	CAATTGACCCAGCCAAATGAG
D-01231-R	TTGTTGGACAGGAGTTGTGC
U-01232-F1	ACGCAGCTTGAAGAAGAGGCTT
U-01232-R1	CTTTACGCTTGCGATCCCCGAATGTATCGGAGCCCATATTAT
D-01232-F1	CCTGGGTTTCGCAAAGATAATTGAGCTTCTCAGTCAGGAGA
D-01232-R1	CGATCACCATCGATGAGATC
U-01232-F	TCAGACAACTTGGATGACCTC
D-01232-R	ATACGTGGACAGTGCATGAC
U-01233-F1	TGACAGCTGATTGCATCACG
U-01233-R1	CTTTACGCTTGCGATCCCCGAACCCTGTGTGGGGATTTCGG
D-01233-F1	CCTGGGTTTCGCAAAGATAATTGGACCGTCATCTGGTGGGTC
D-01233-R1	GCAGGCAGCAATGTAAACTG
U-01233-F	CGGTTTCGACTGTGTCAAAG
D-01233-R	AAACGTGACAGGAGAGATGA
U-01234-F1	TGCAACGGTTCGAACAGACCA
U-01234-R1	CTTTACGCTTGCGATCCCCGAATTCGGGTGCTAGAGCTGCC
D-01234-F1	CCTGGGTTTCGCAAAGATAATTGGGCATTAAATAGCGTCCTG
D-01234-R1	GTGGTGATTGGTGACCAAGCA
U-01234-F	ATGGGCGGAGCGTCAAAGA
D-01234-R	GTGCGGAAGGTTATACCGCA
U-01236-F1	TTTGACATCGGTCGAGCCAT
U-01236-R1	CTTTACGCTTGCGATCCCCGAAAATCACACGTGCTCCCTGG
D-01236-F1	CCTGGGTTTCGCAAAGATAATTGGATATAGTCCAGGTTACC
D-01236-R1	AAGCTTCCGAGCCAGAGTCT
U-01236-F	CCAGAAGGATACGTTGTTGG
D-01236-R	AATGCACCGCGGATGCGAA
U-01237-F1	ACCATTATCGCGCCGGTCT
U-01237-R1	CTTTACGCTTGCGATCCCCGAAGAGTCACCAGGAATCTCTG
D-01237-F1	CCTGGGTTTCGCAAAGATAATTGAACCTAGCAAGCAATCCC
D-01237-R1	TGGGTGTGGAGAGAGACCAT
U-01237-F	TGGCCAGACCACAAGGTAGT
D-01237-R	CCGCGATGAGAATCCGTTCA
U-01238-F1	AAATCATGGACCTGGTCAGG
U-01238-R1	CTTTACGCTTGCGATCCCCGAAAACCTCGGGTAACGGGTGCCCT
D-01238-F1	CCTGGGTTTCGCAAAGATAATTGTGGTGGGTTGTCCAGTGT
D-01238-R1	TGTTACGCGGAGTAGCGTAT
U-01238-F	ATCGTTCGCCAATGGTCACC
D-01238-R	ACGAGGTCGGATATCGGATG

U-01239-F1	ACTCCTAAGCCATTTCCCGT
U-01239-R1	CTTTACGCTTGCGATCCCCGAAACAGAGCACGGAGGATATC
D-01239-F1	CCTGGGTTTCGCAAAGATAATTGTTACCCCTCCTGTTTGGC
D-01239-R1	CCGCATTCGACGATGCCTAT
U-01239-F	AGGTCAGCAGCGACATCAAG
D-01239-R	ATTCCTTGATCCTCAACGG
U-01240-F1	TCAAGGCTGGACTCGCTATG
U-01240-R1	CTTTACGCTTGCGATCCCCGAAAAAGCGCGCAGATATTCTTC
D-01240-F1	CCTGGGTTTCGCAAAGATAATTGCTCCCAGCGGCTTGATCAT
D-01240-R1	TGGGATCGGATTCCAGAAGA
U-01240-F	TGGCACCTTGCTTCTTAGGG
D-01240-R	CCAACAGCATCAAAAACACG
U-01241-F1	AATGGGTGATCTGGATTGC
U-01241-R1	CTTTACGCTTGCGATCCCCGAAGAAGAAAGAGAGGAAGGTC
D-01241-F1	CCTGGGTTTCGCAAAGATAATTGGCTACGAATCTTTGGTAGG
D-01241-R1	CGCGATAGAGGGATTCTCAA
U-01241-F	ATGGGGAATGATGATGCAGAAGC
D-01241-R	GGAGTTGAGATACAGGAAGTACC
U-01242-F1	GGTAAGTGGAATTGTGGG
U-01242-R1	CTTTACGCTTGCGATCCCCGAAGGCGTAATGAAATTTGGCG
D-01242-F1	CCTGGGTTTCGCAAAGATAATTGAGGGCGATATTCTCCCGT
D-01242-R1	AGTACGACCGTAAGAAGGTG
U-01242-F	TCCATATCTGTCTCAACGCC
D-01242-R	TGTGGTACTTCCTGTATCTC
U-01243-F1	ACTCCCGCAGAAGTGCTGTC
U-01243-R1	CTTTACGCTTGCGATCCCCGAATGGAATGGATGAGCTTTGC
D-01243-F1	CCTGGGTTTCGCAAAGATAATTGGCTCAAGAAGCTGAGGTG
D-01243-R1	GGTAGGAGCAGCCTCCTATATA
U-01243-F	GTAGTTTCTGCCGTATGCTG
D-01243-R	TCCTCTTAGTATCGATCGGC

Table S6. Functional annotation of genes in *opd* gene cluster

Gene tag	Locus tag	Putative function	Characterized homolog	Protein identity
<i>opdG</i>	PDE_01228	Hypothetical protein		
<i>opdH</i>	PDE_01229	Antifungal protein		
<i>opdF</i>	PDE_01230	MFS transporter	<i>apdF</i> (ANIA_08413)	32.86%
<i>opdR</i>	PDE_01231	Transcription factor	<i>apdR</i> (ANIA_08414)	30.79%
<i>opdI</i>	PDE_01232	-	-	
<i>opdD</i>	PDE_01233	Hypothetical protein	<i>apdD</i> (ANIA_08410)	51.08
<i>opdJ</i>	PDE_01234	Transcription factor	-	
<i>opdA</i>	PDE_01235	PKS-NRPS	<i>apdA</i> (ANIA_08412)	45.60%
<i>opdE</i>	PDE_01236	Cytochrome P450	<i>apdE</i> (ANIA_08411)	54.98%
<i>opdB</i>	PDE_01237	Cytochrome P450	<i>apdB</i> (ANIA_08408)	37.36%
<i>opdC</i>	PDE_01238	Enoyl reductase	<i>apdC</i> (ANIA_08409)	49.27%
<i>opdK</i>	PDE_01239	MFS transporter	<i>apdF</i> (ANIA_08413)	36.19%
<i>opdL</i>	PDE_01240	Transcription factor	-	
<i>opdM</i>	PDE_01241	MFS transporter	-	
<i>opdN</i>	PDE_01242	Short-chain dehydrogenase	-	
<i>opdO</i>	PDE_01243	Ligand-binding SRPBCC	-	

Figure S1. (A) The HPLC profile of crude extracts in *P. oxalicum* MEFC104. (B) The UV absorbs of compounds at 15-20 min.

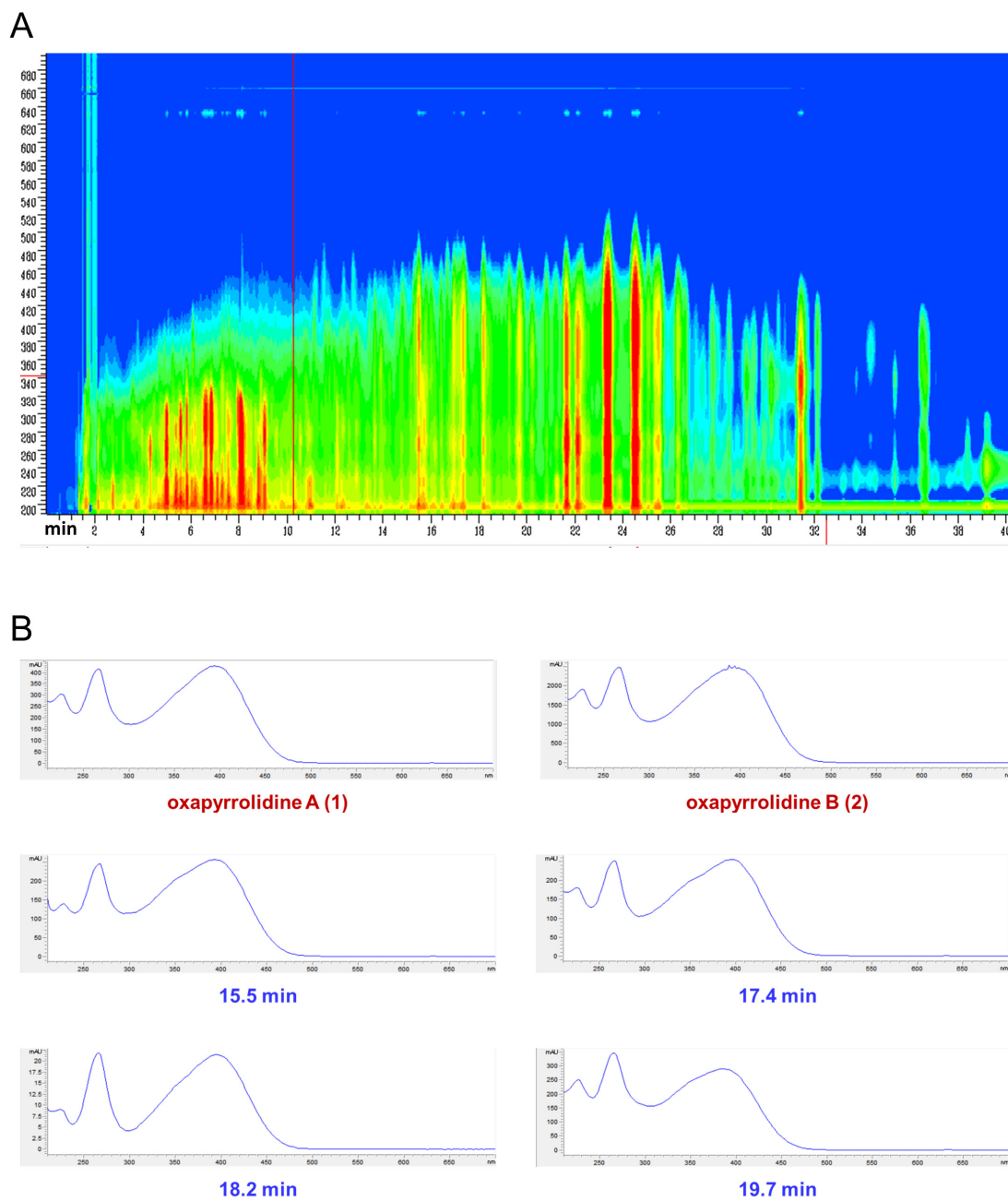


Figure S2. HRESIMS spectrum of oxapyrrolidine A (**1**)

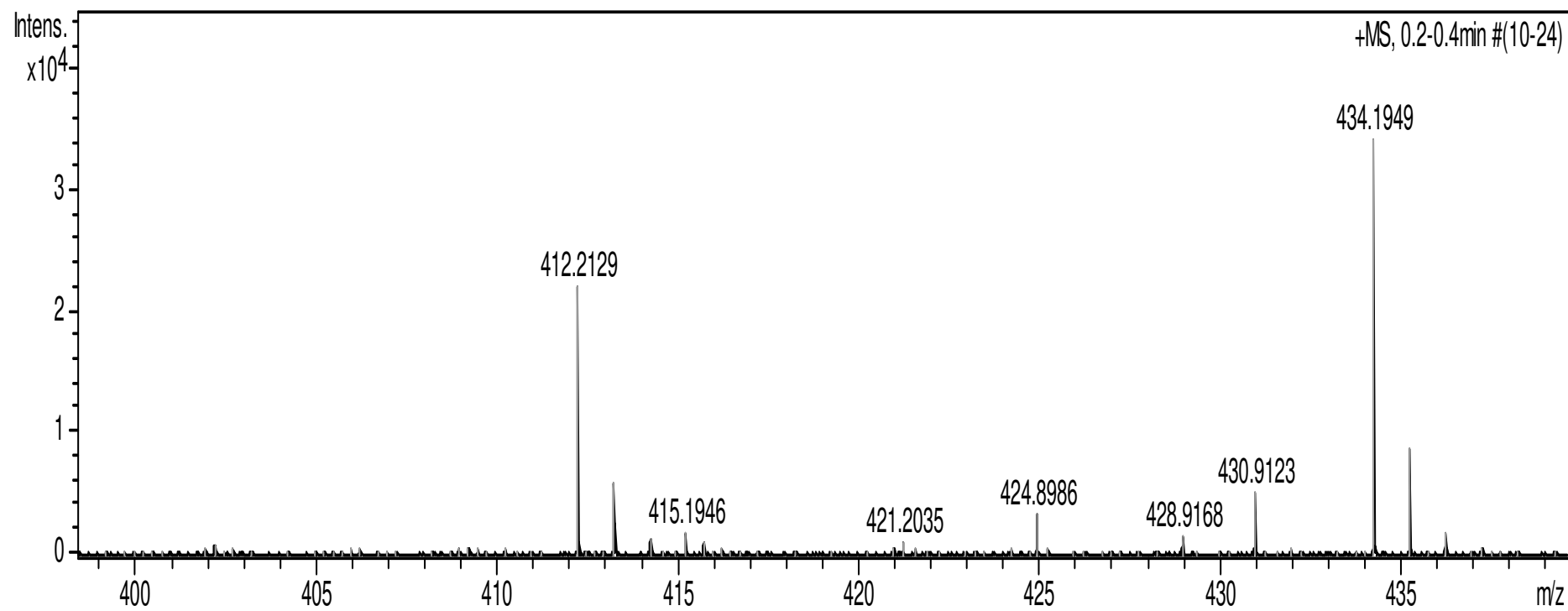


Figure S3. ^1H NMR spectrum of oxapyrrolidine A (**1**) in $\text{DMSO}-d_6$

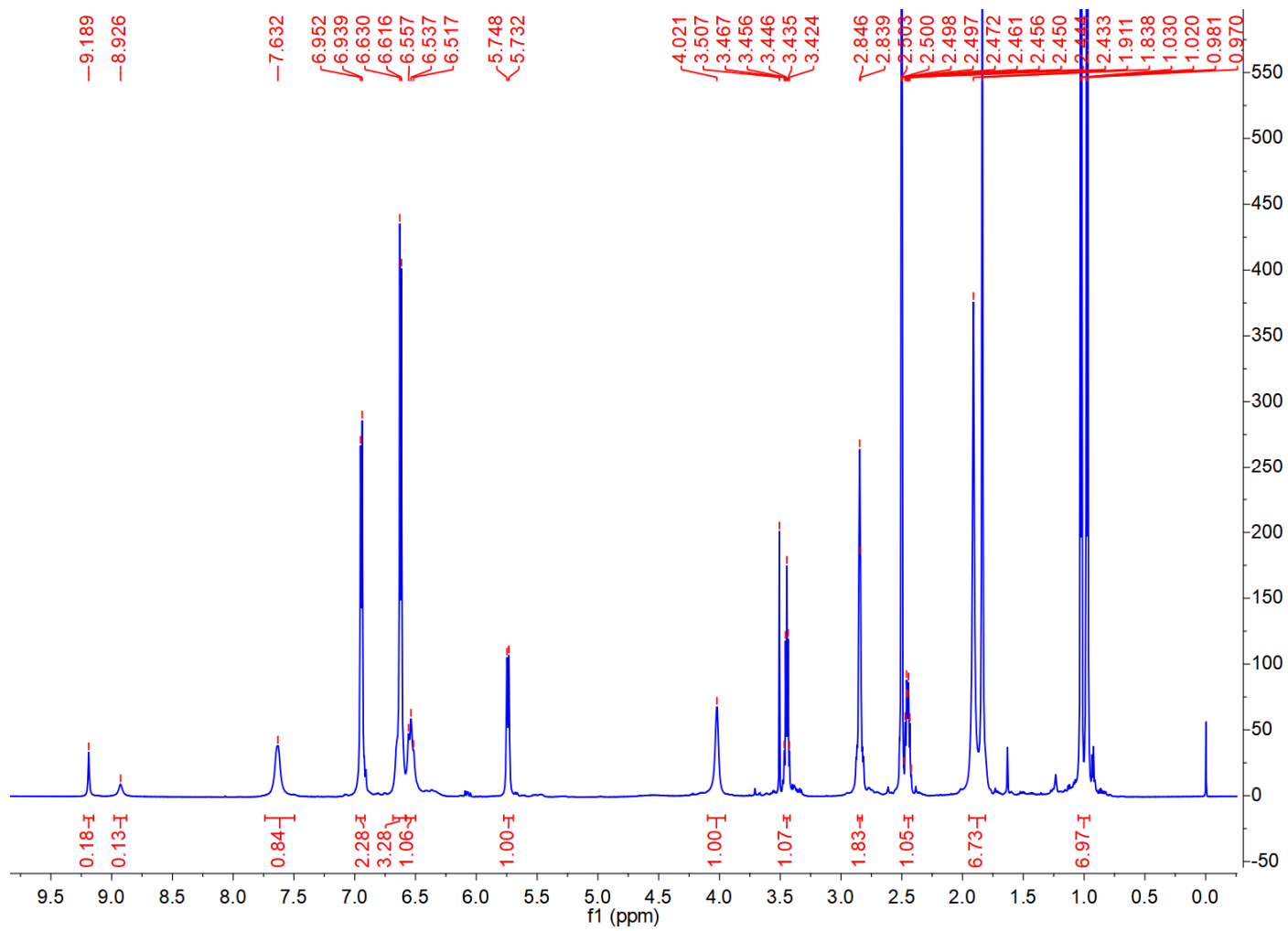


Figure S4. ^{13}C NMR spectrum of oxapyrrolidine A (**1**) in $\text{DMSO-}d_6$

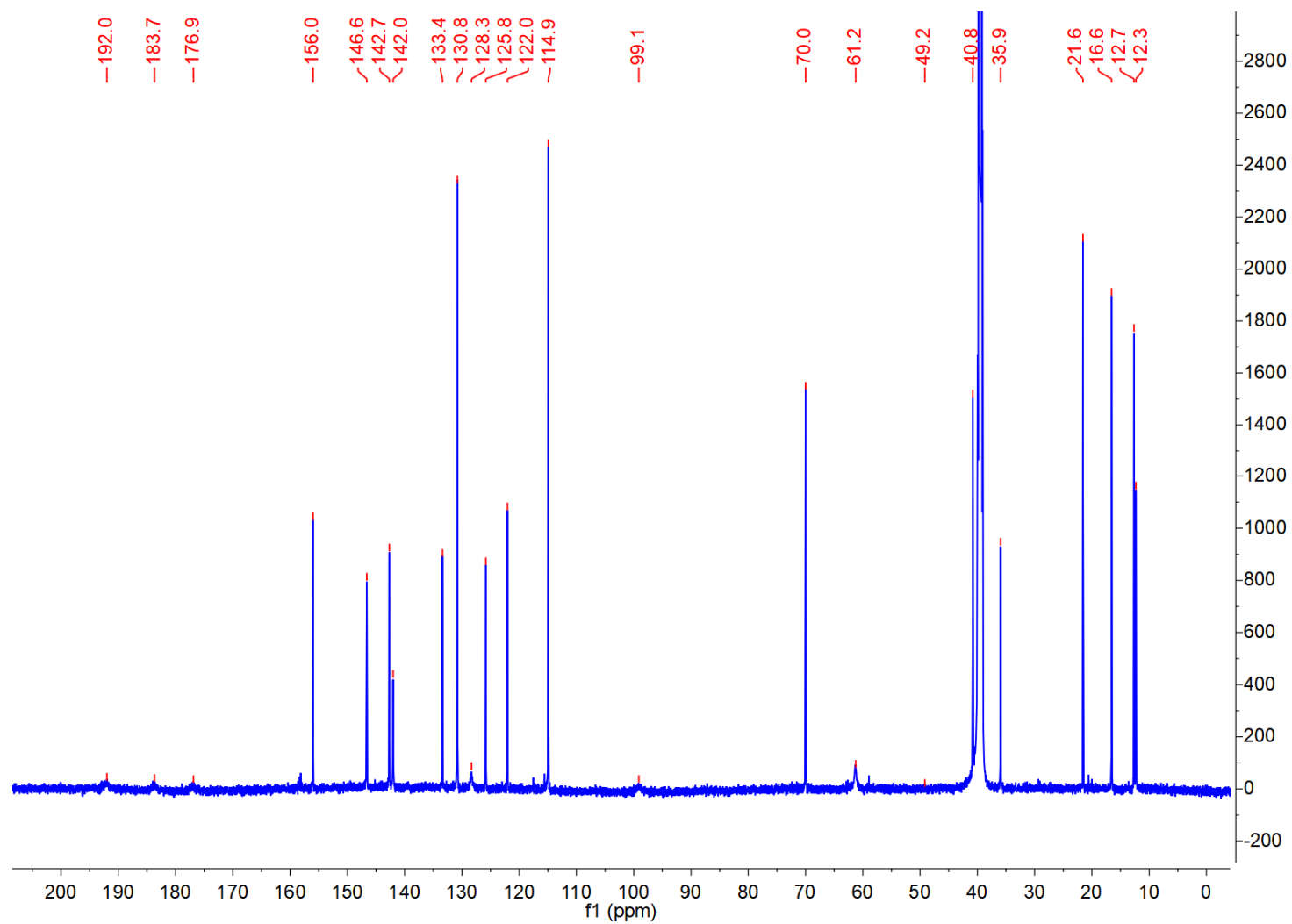


Figure S5. DEPT 135 spectrum of oxapyrrolidine A (**1**) in DMSO-*d*₆

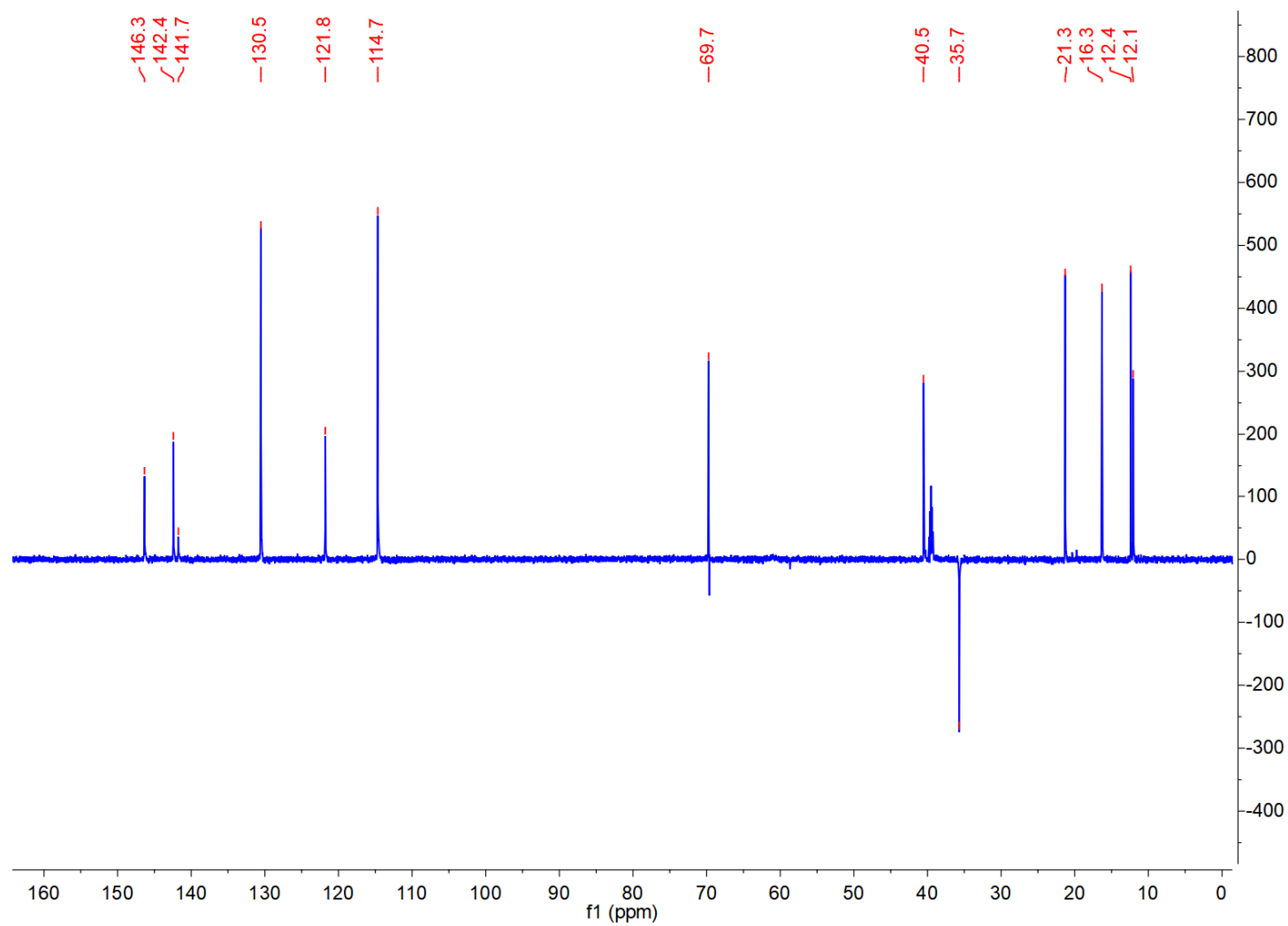


Figure S6. HSQC spectrum of oxapyrrolidine A (**1**) in DMSO- d_6

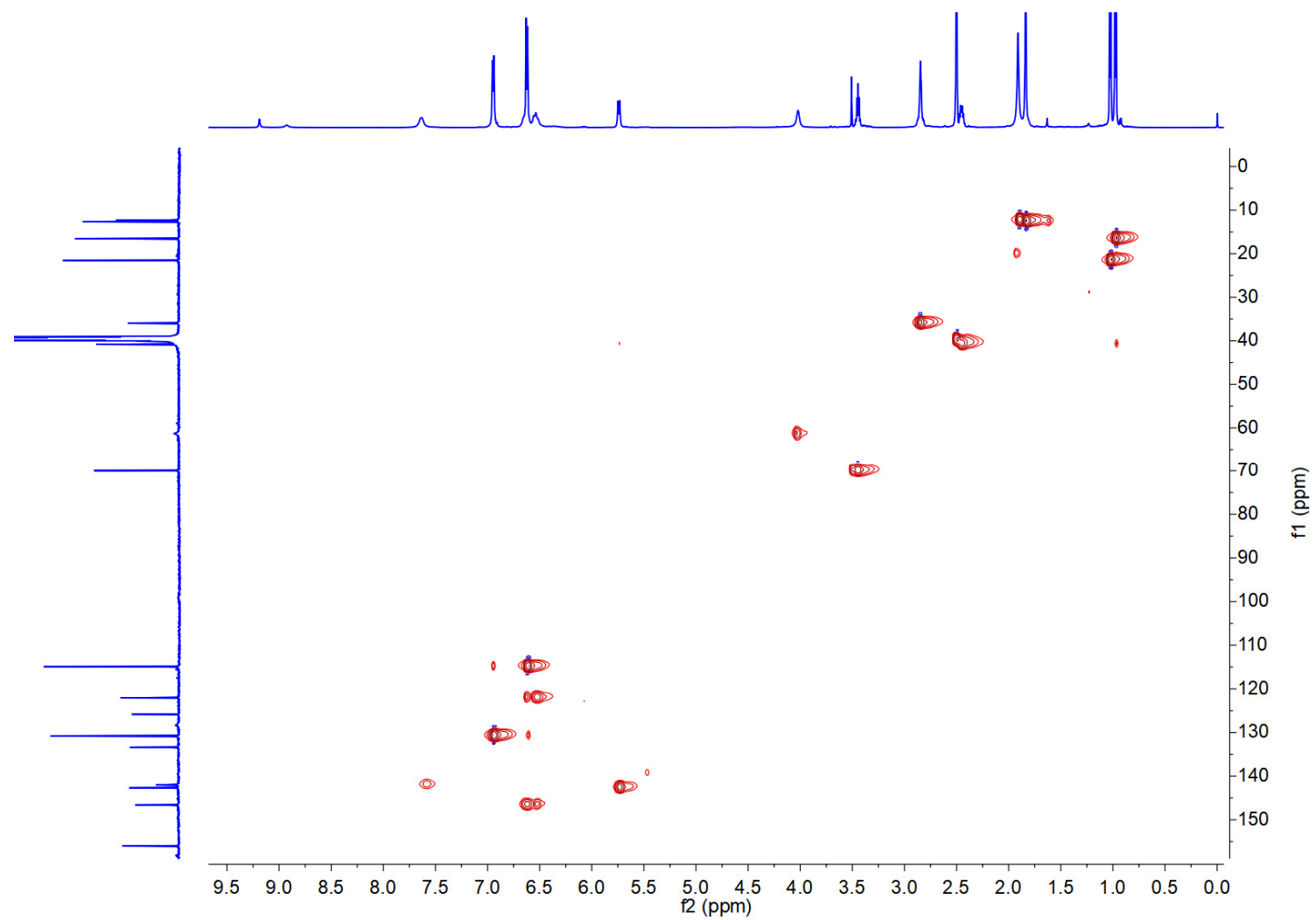


Figure S7. ^1H - ^1H COSY spectrum of oxapyrrolidine A (**1**) in $\text{DMSO-}d_6$

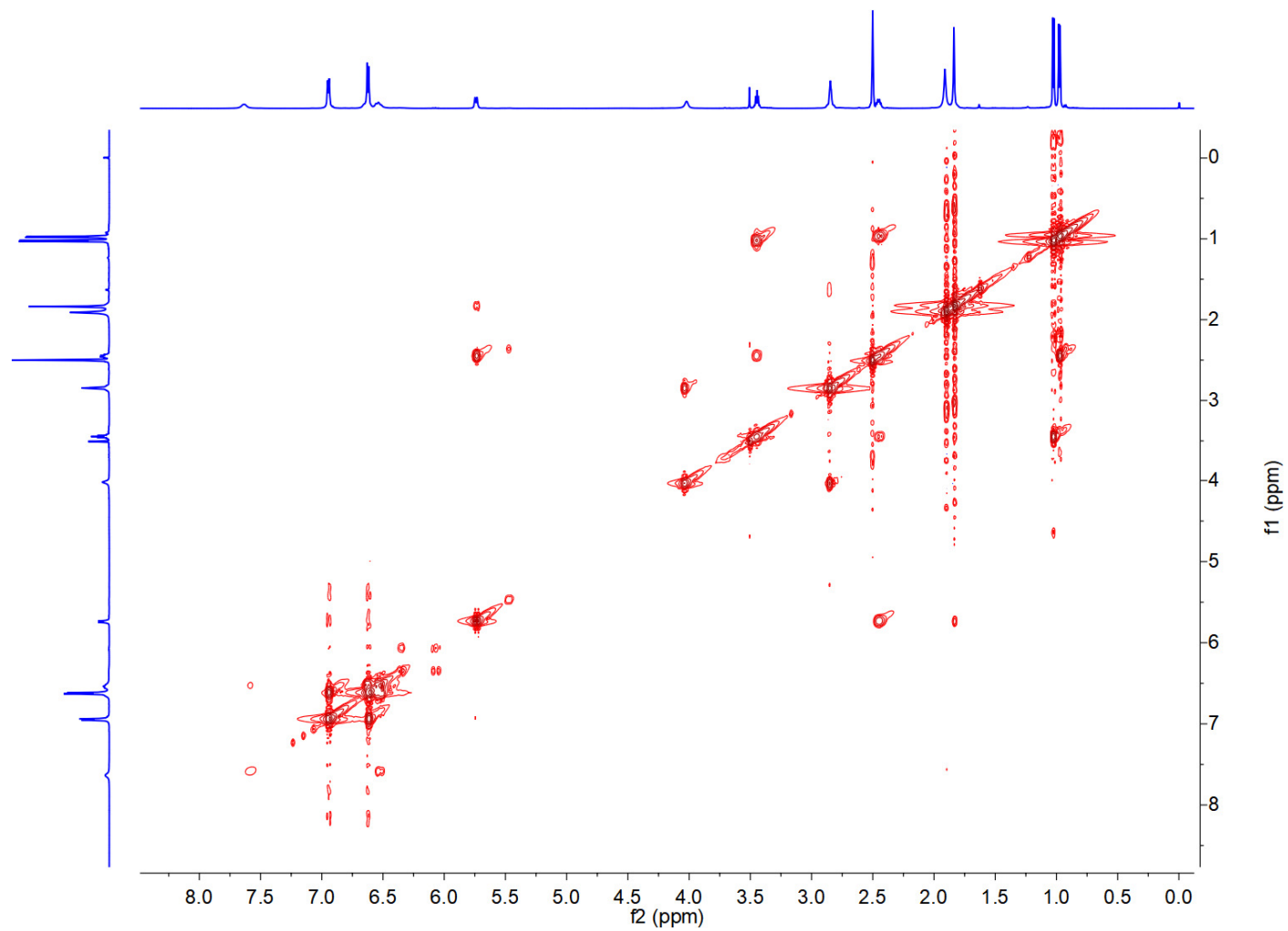


Figure S8. HMBC spectrum of oxapyrrolidine A (**1**) in DMSO- d_6

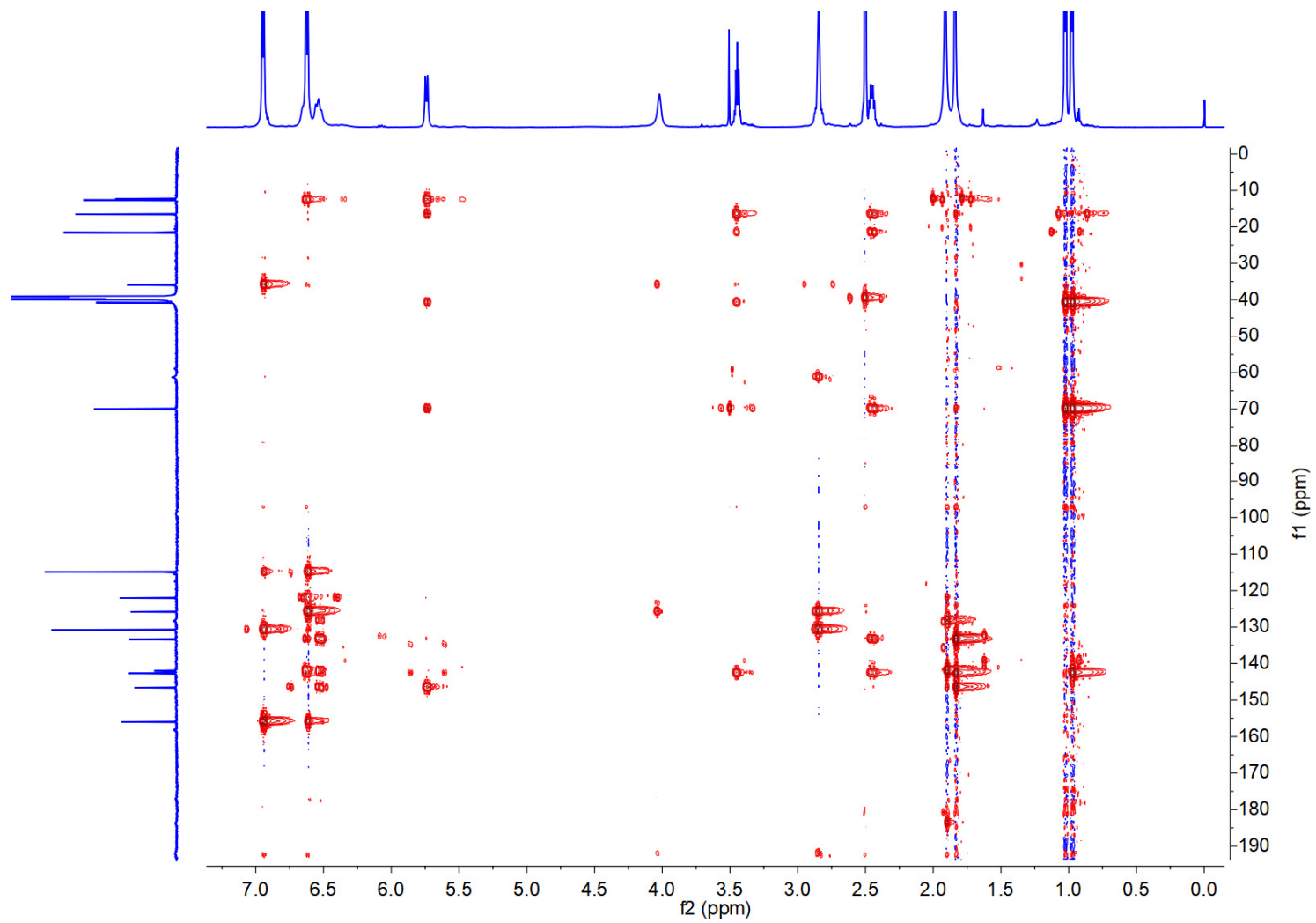


Figure S9. NOESY spectrum of oxapyrrolidine A (**1**) in DMSO- d_6

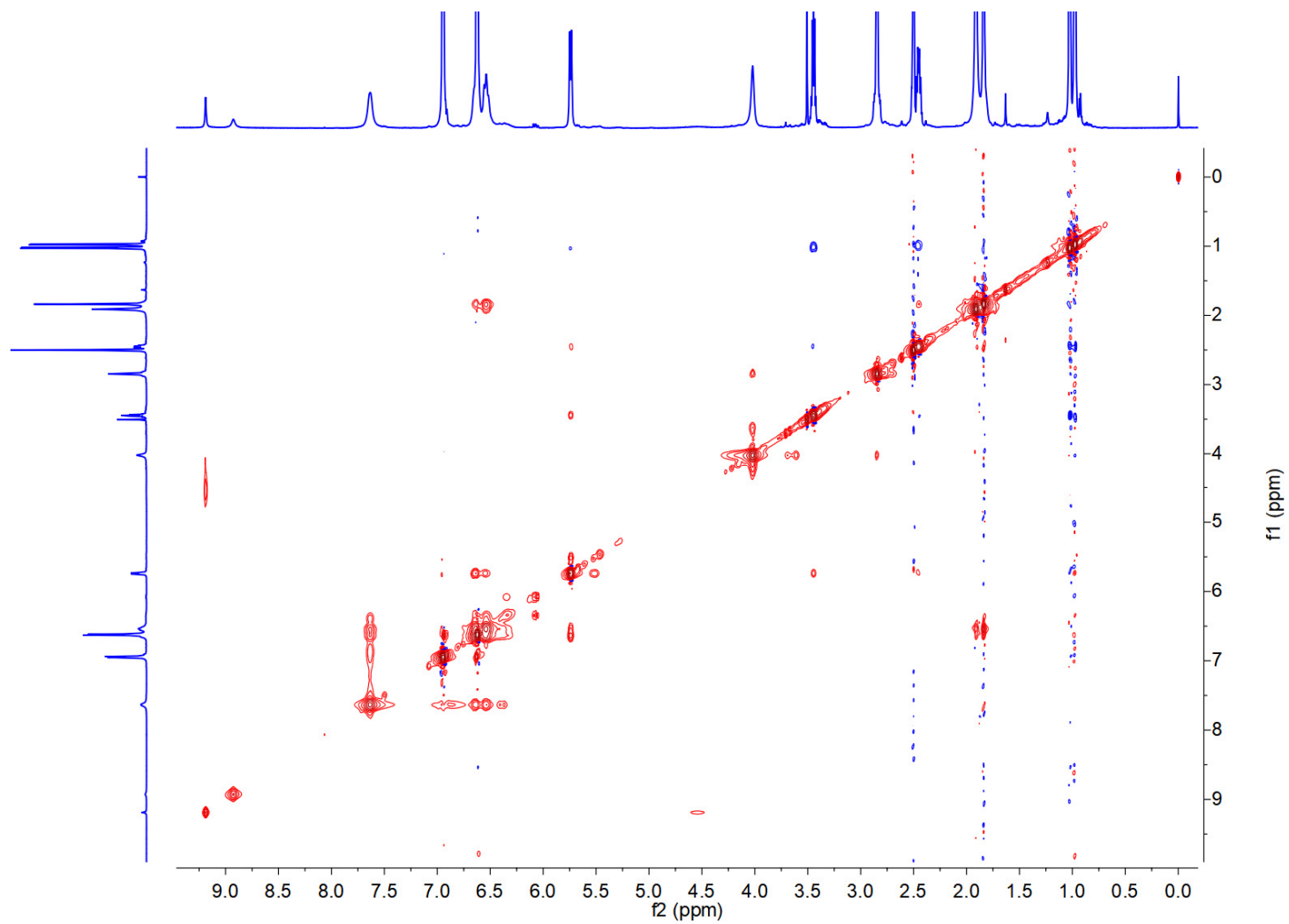


Figure S10. Key regions of ^1H spectrum of Mosher's ester of oxapyrrolidine A (**1**) in $\text{DMSO-}d_6$

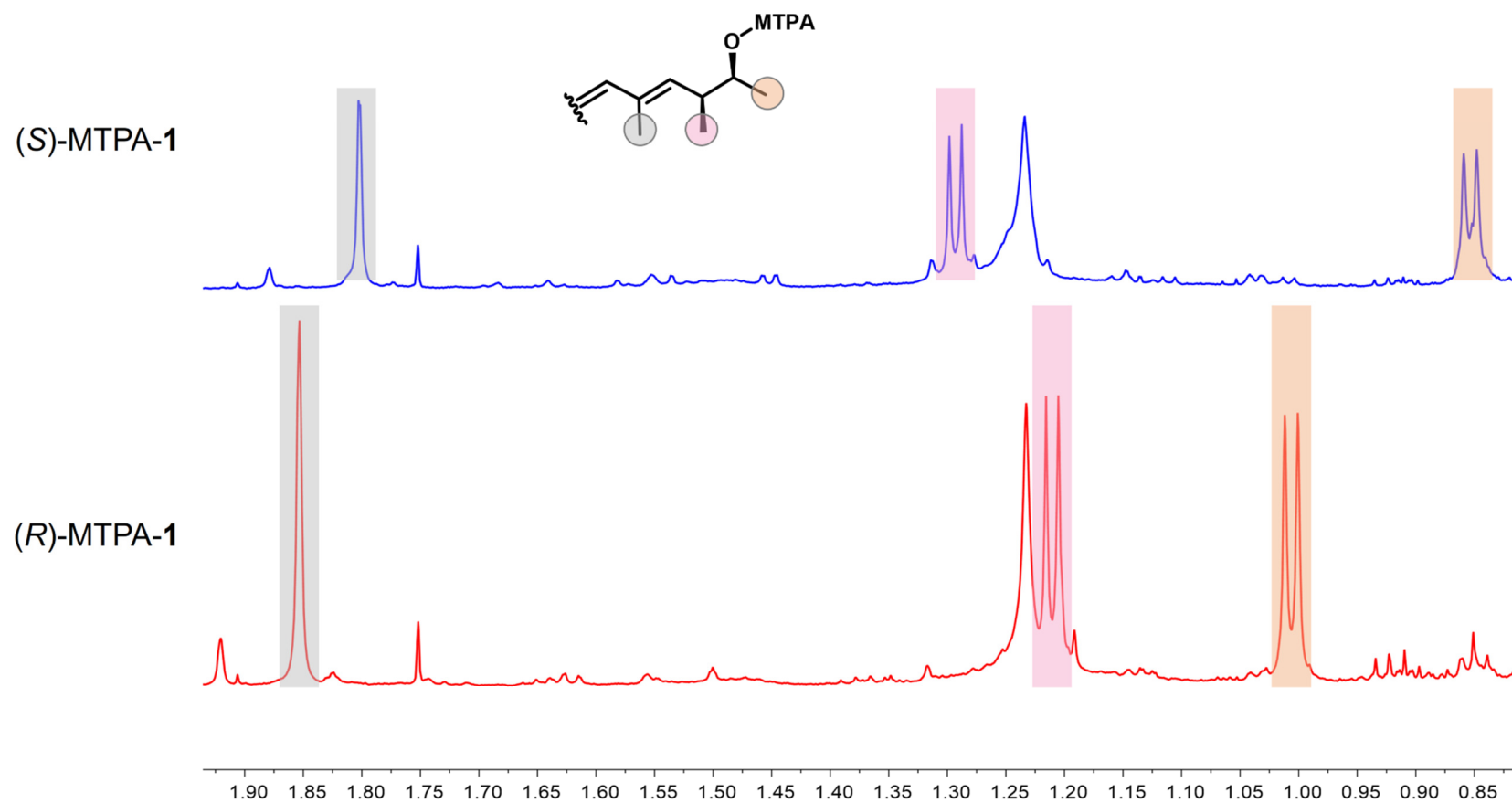


Figure S11. HECADE spectrum of oxapyrrolidine A (**1**) in DMSO- d_6

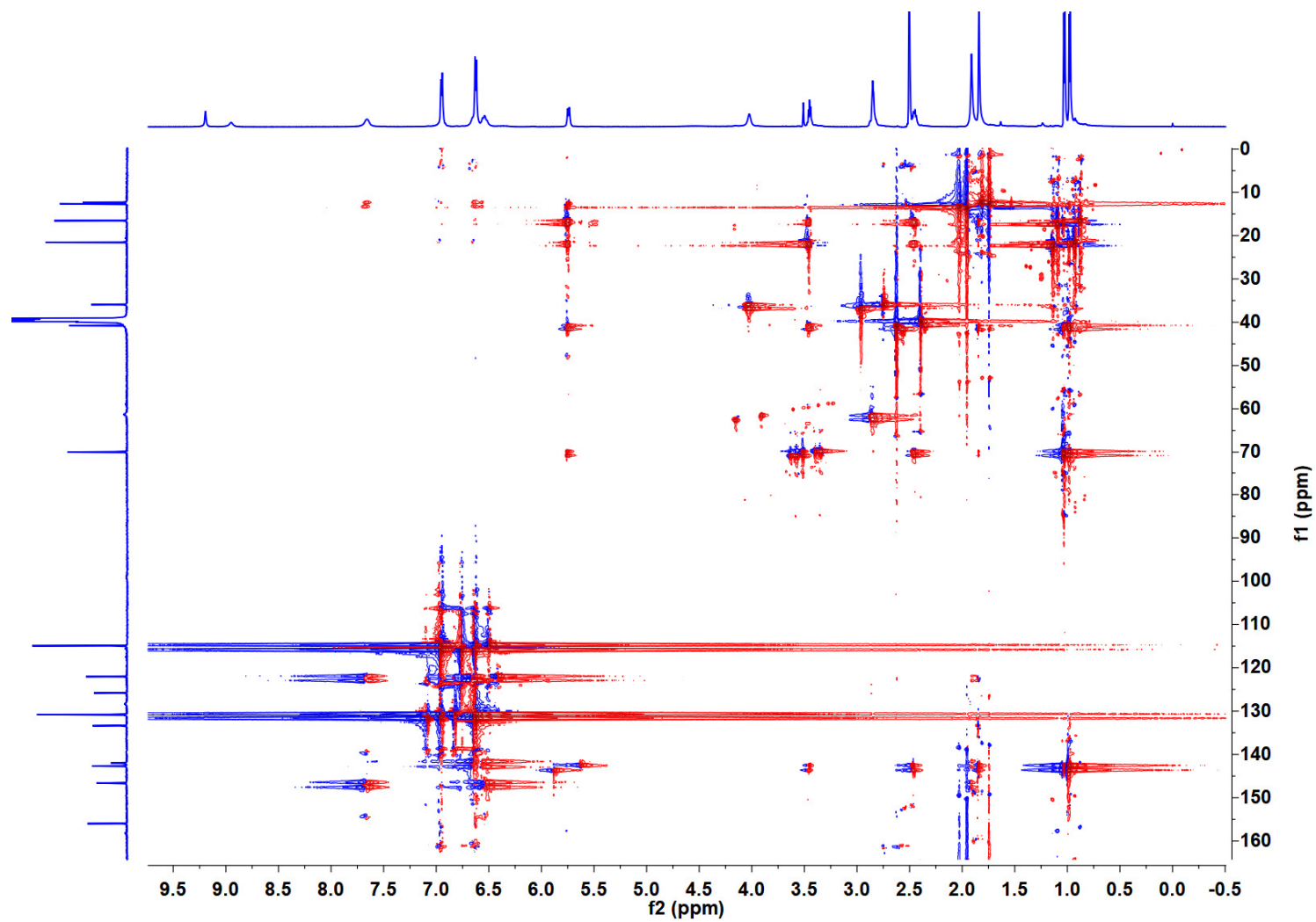


Figure S12. ECD spectrum of oxapyrrolidine A (**1**)

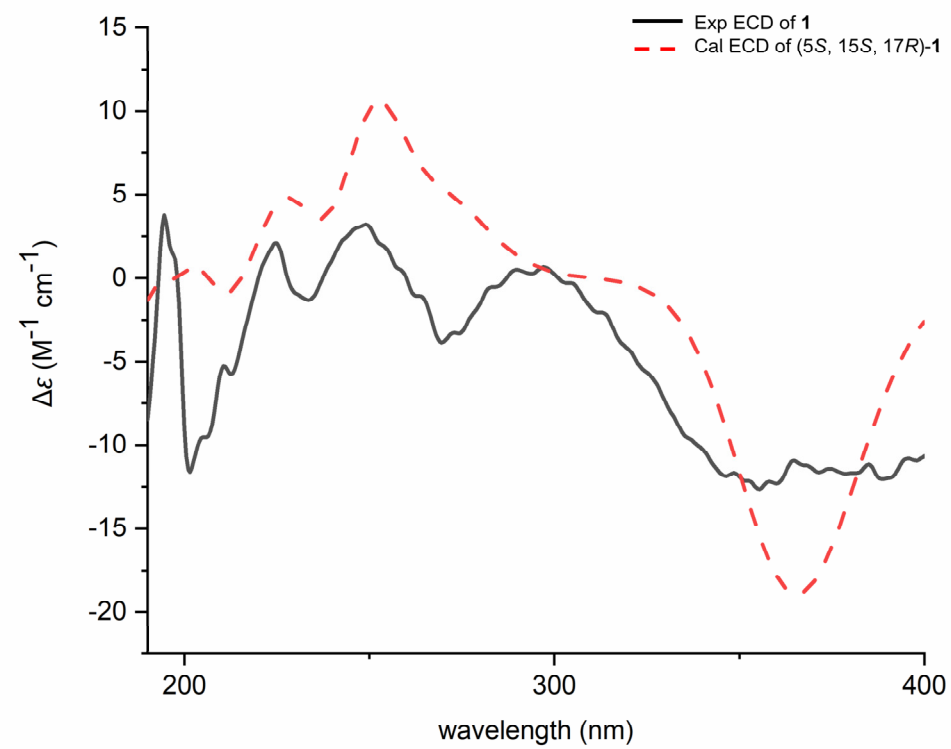


Figure S13. UV spectrum of oxapyrrolidine A (**1**)

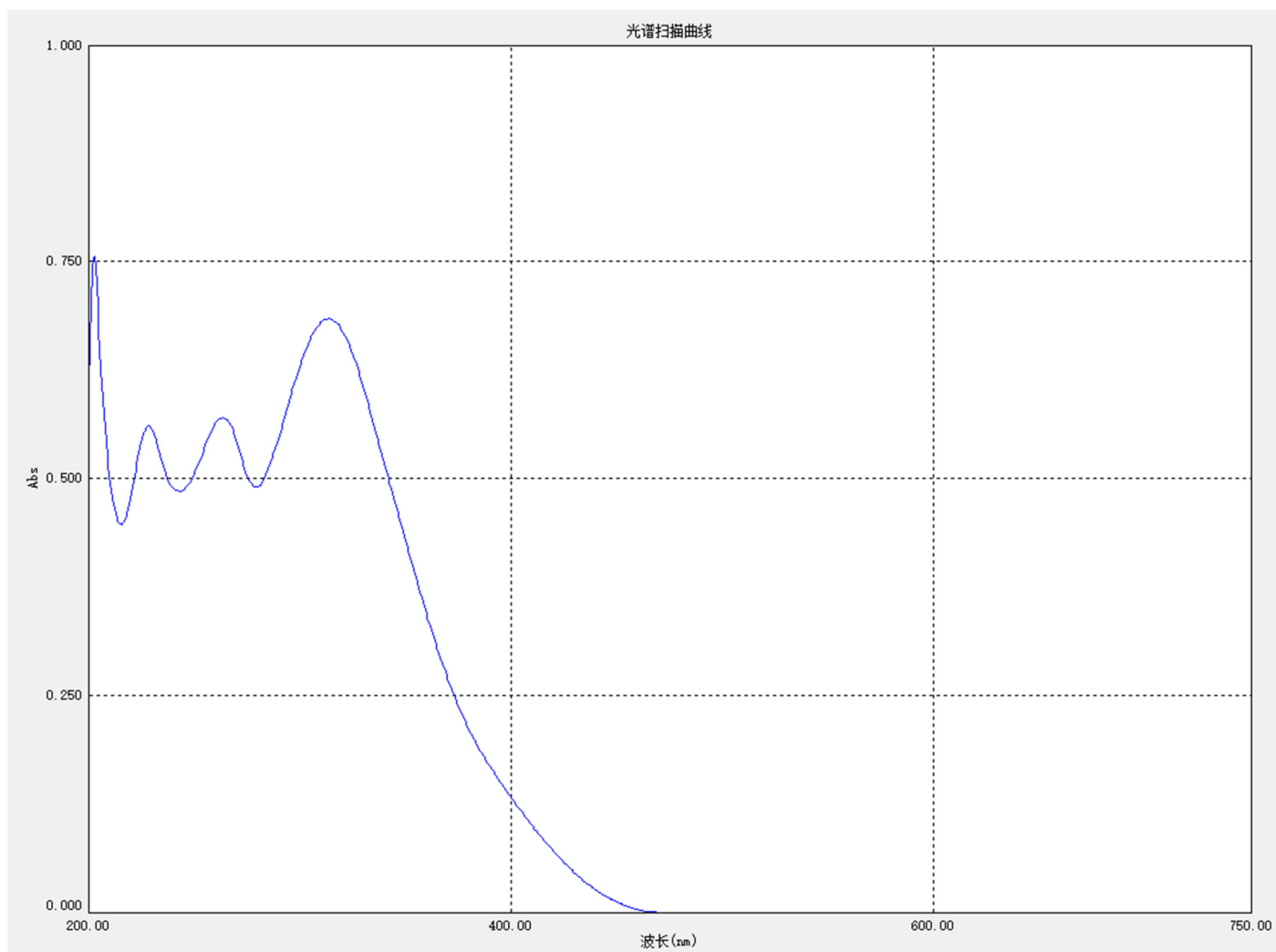


Figure S14. IR spectrum of oxapyrrolidine A (**1**)

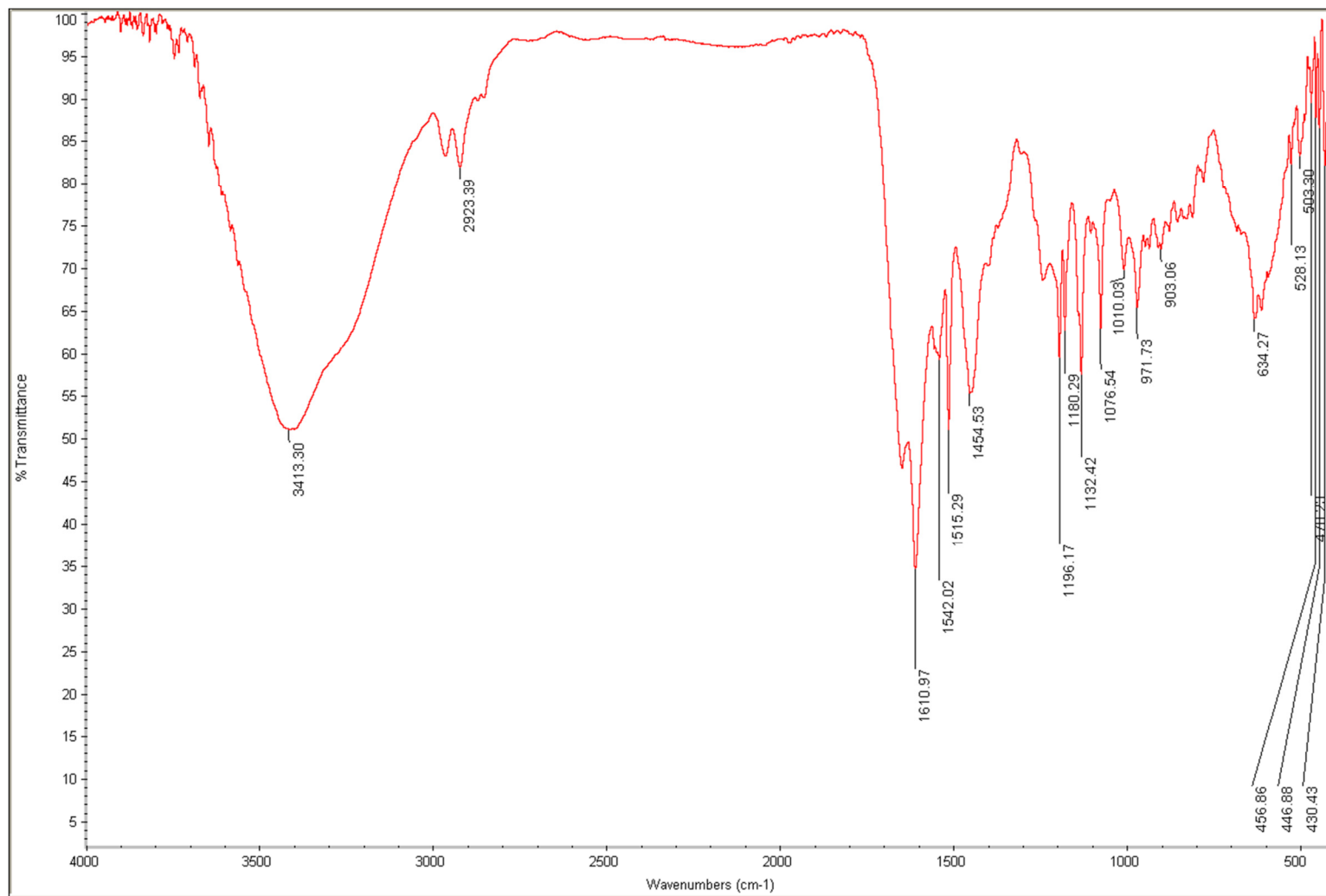


Figure S15. HR-ESI-MS spectrum of oxapyrrolidine B (**2**)

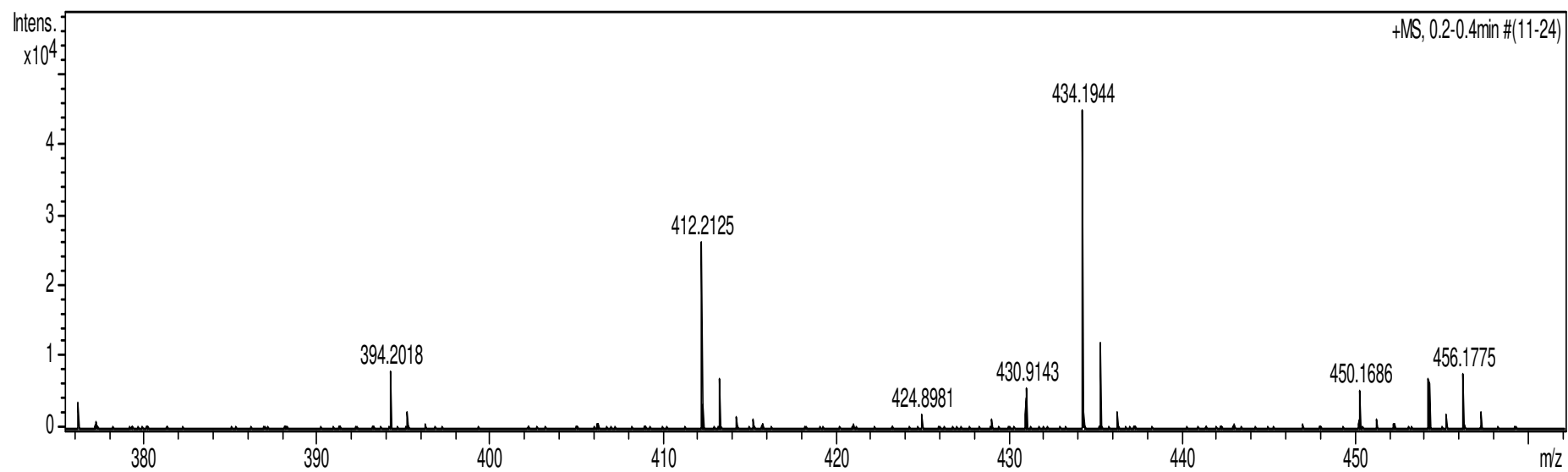


Figure S16. ^1H NMR spectrum of oxapyrrolidine B (**2**) in $\text{DMSO}-d_6$

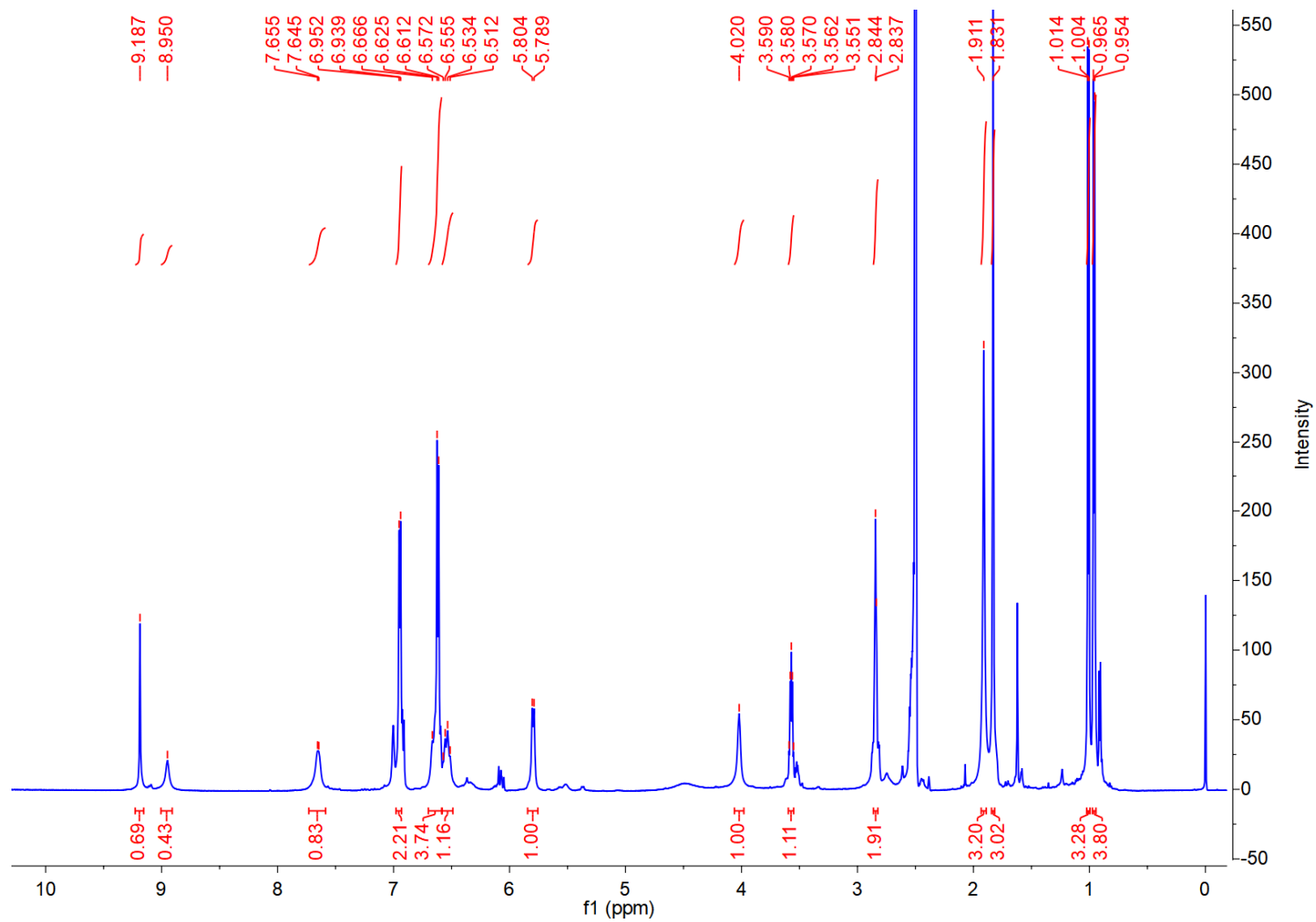


Figure S17. ^{13}C NMR spectrum of oxapyrrolidine B (**2**) in $\text{DMSO-}d_6$

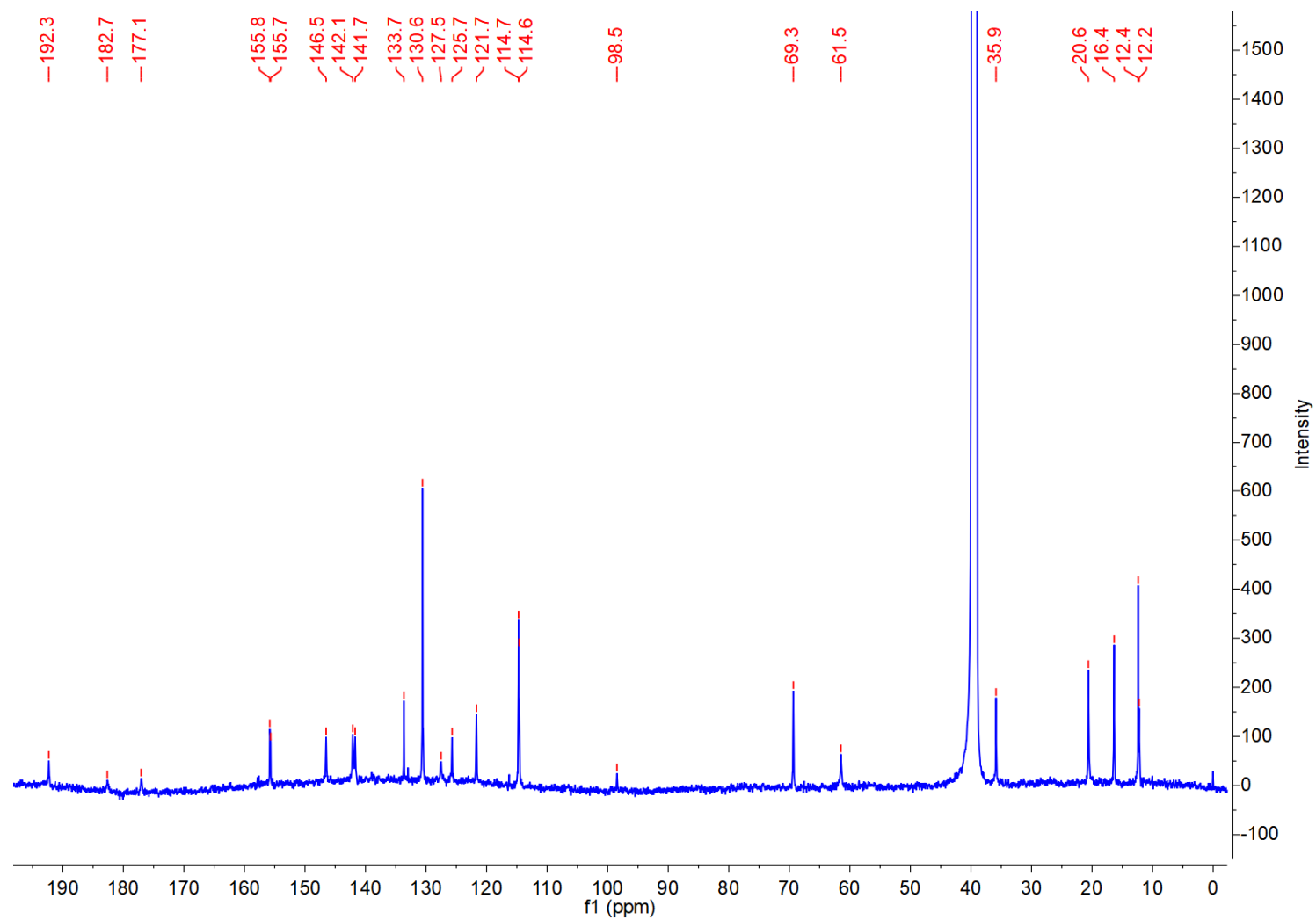


Figure S18. DEPT 135 spectrum of oxapyrrolidine B (**2**) in DMSO-*d*₆

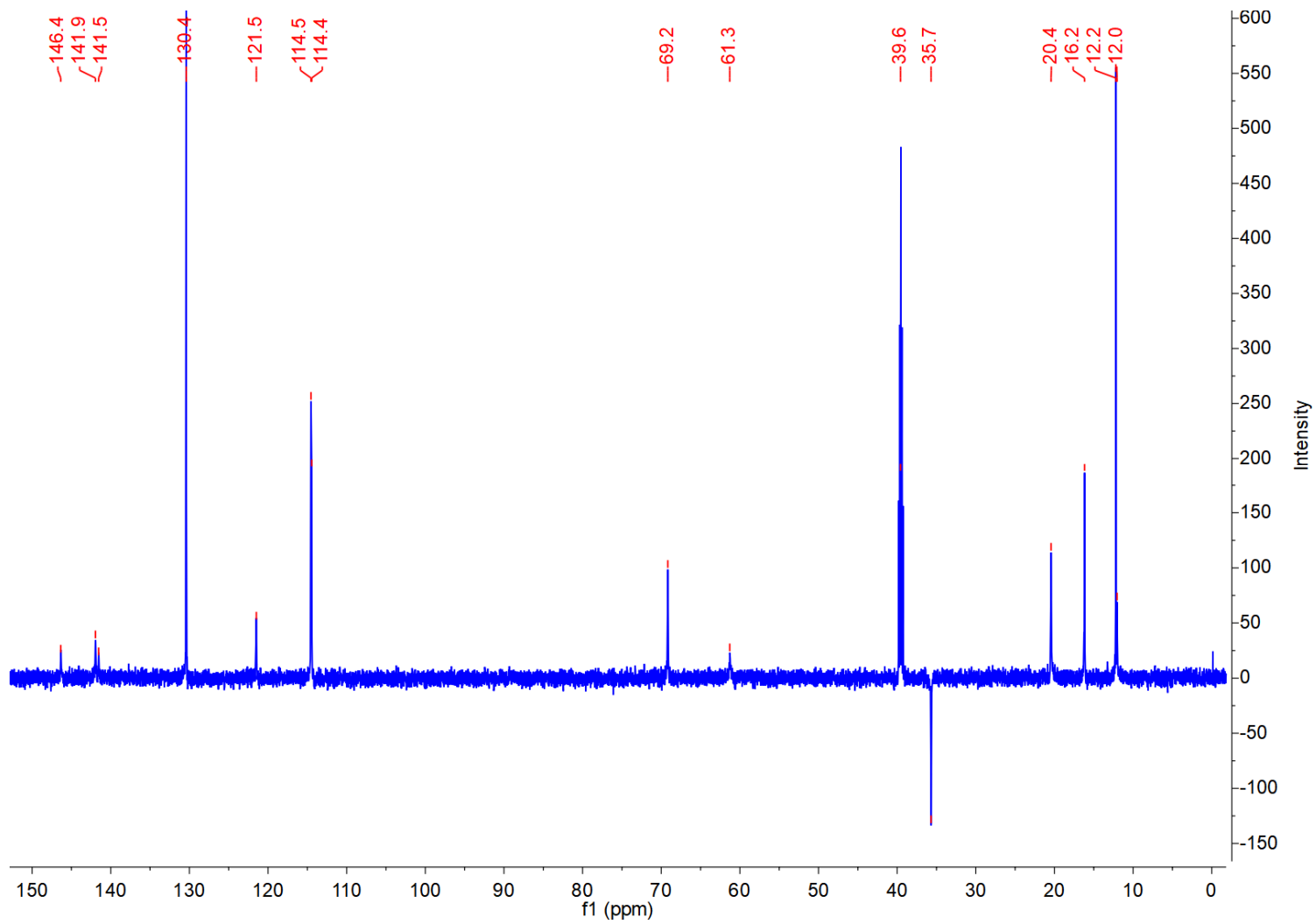


Figure S19. HSQC spectrum of oxapyrrolidine B (**2**) in DMSO- d_6

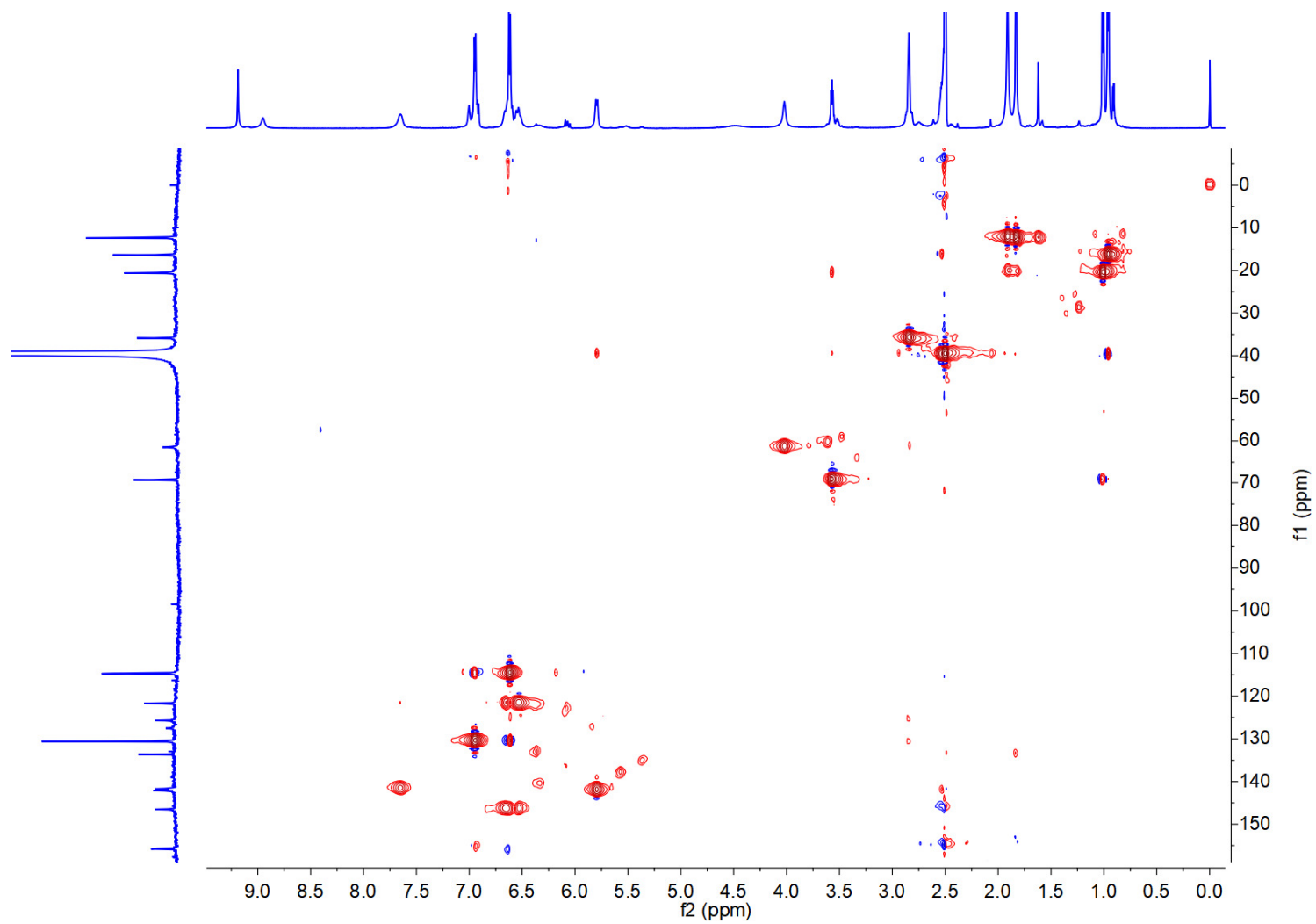


Figure S20. ^1H - ^1H COSY spectrum of oxapyrrolidine B (**2**) in $\text{DMSO-}d_6$

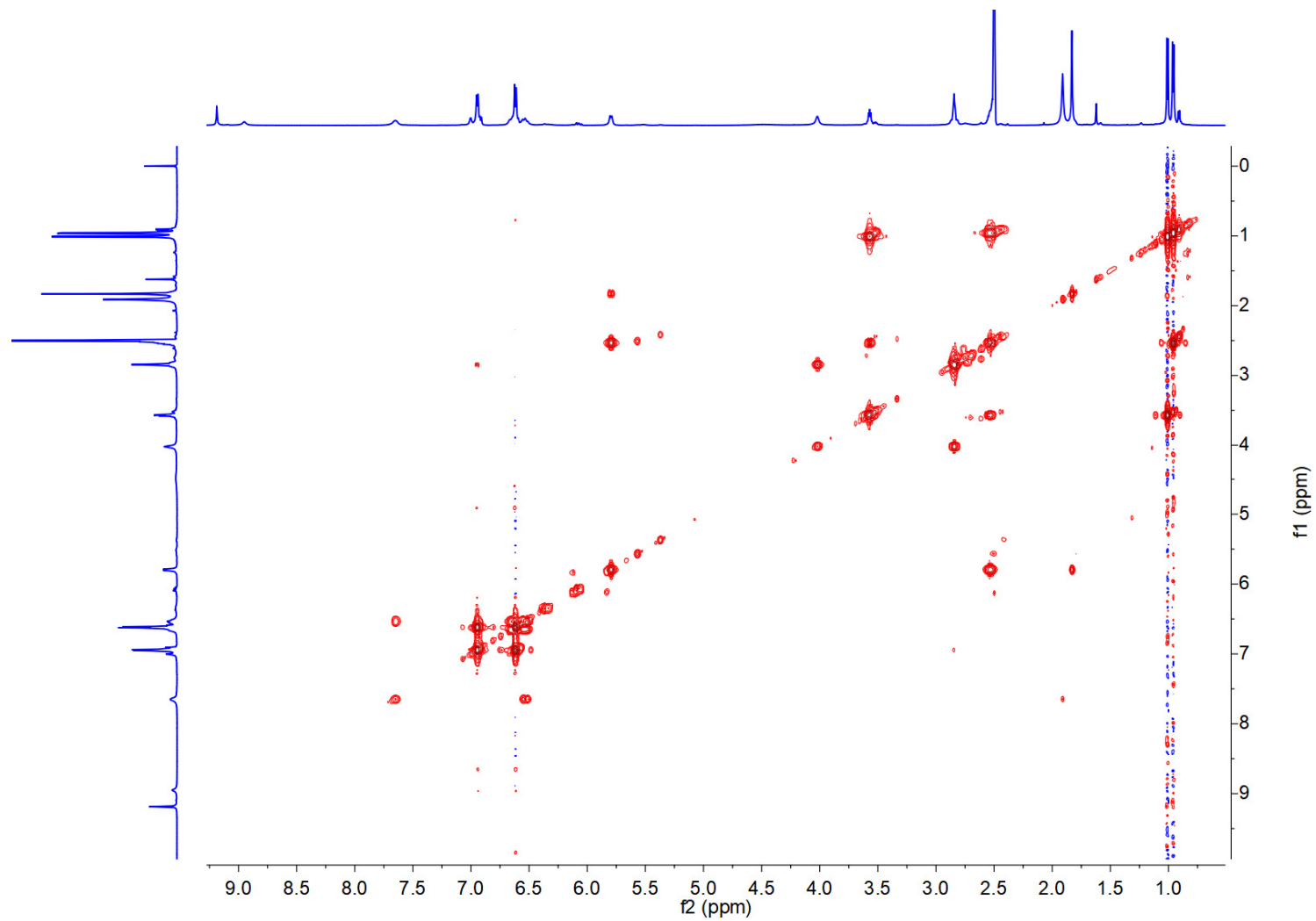


Figure S21. HMBC spectrum of oxapyrrolidine B (**2**) in DMSO-*d*₆

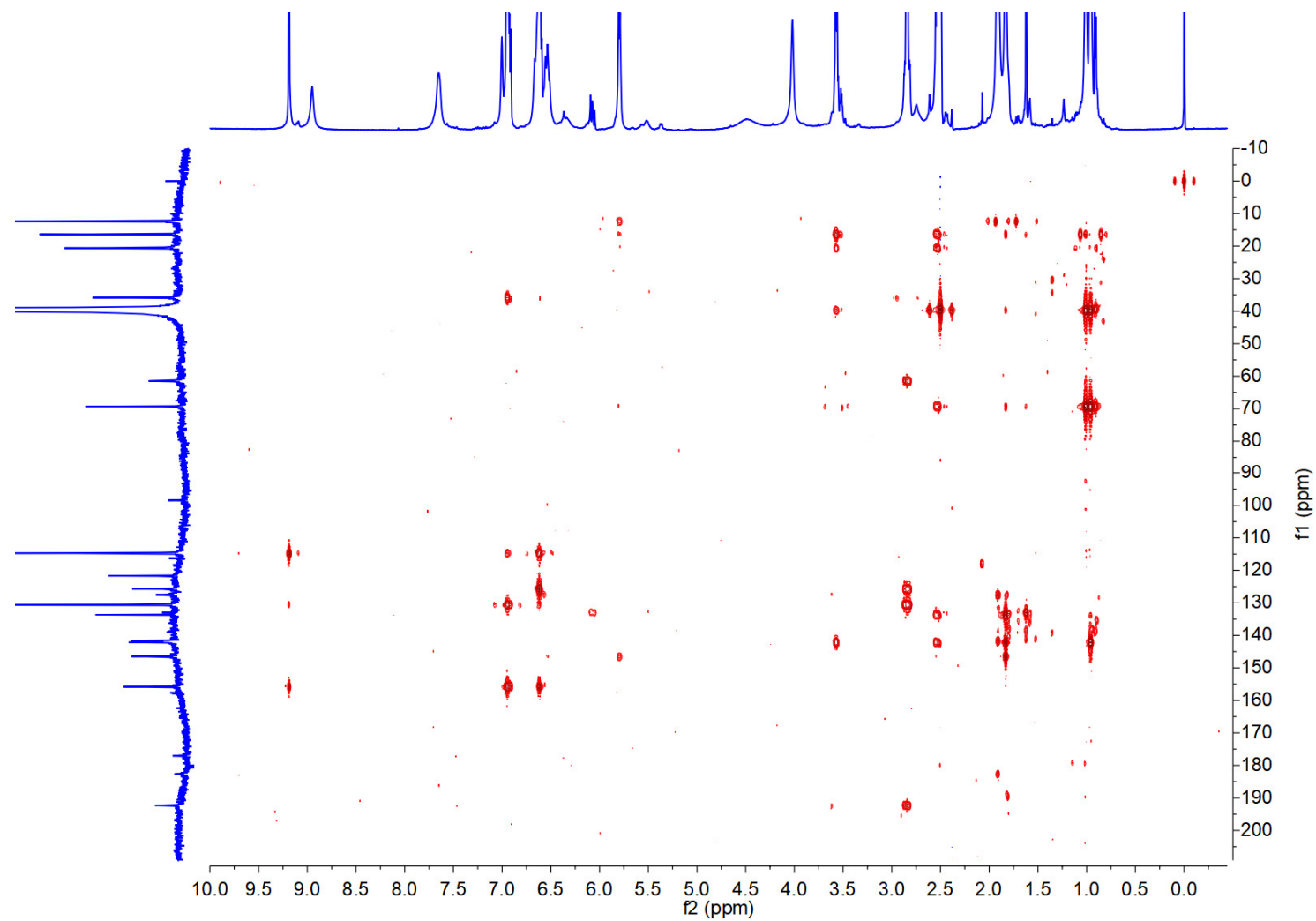


Figure S22. NOESY spectrum of oxapyrrolidine B (**2**) in DMSO-*d*₆

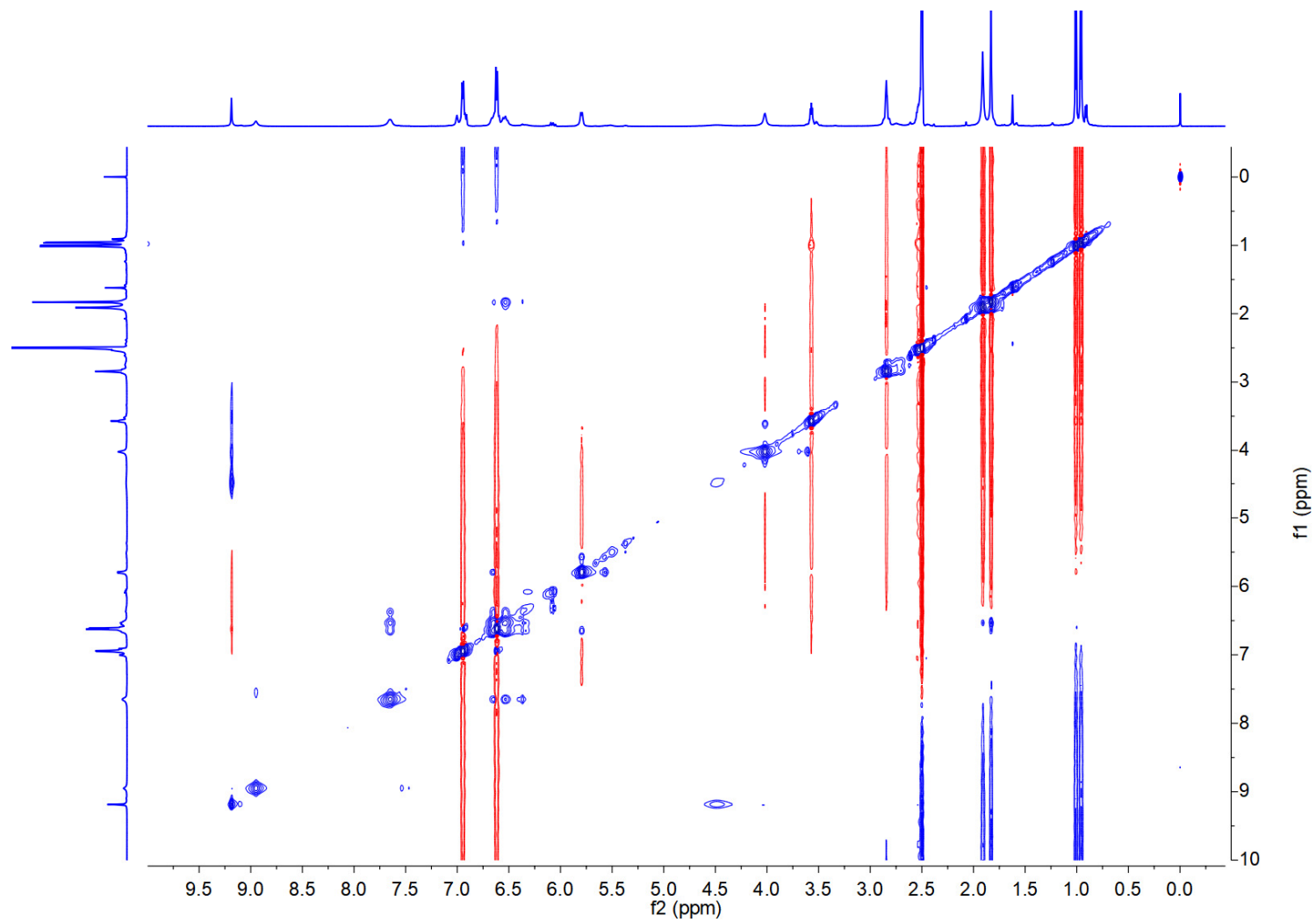


Figure S23. Key regions of ^1H spectrum of Mosher's ester of oxapyrrolidine B (**2**) in $\text{DMSO-}d_6$

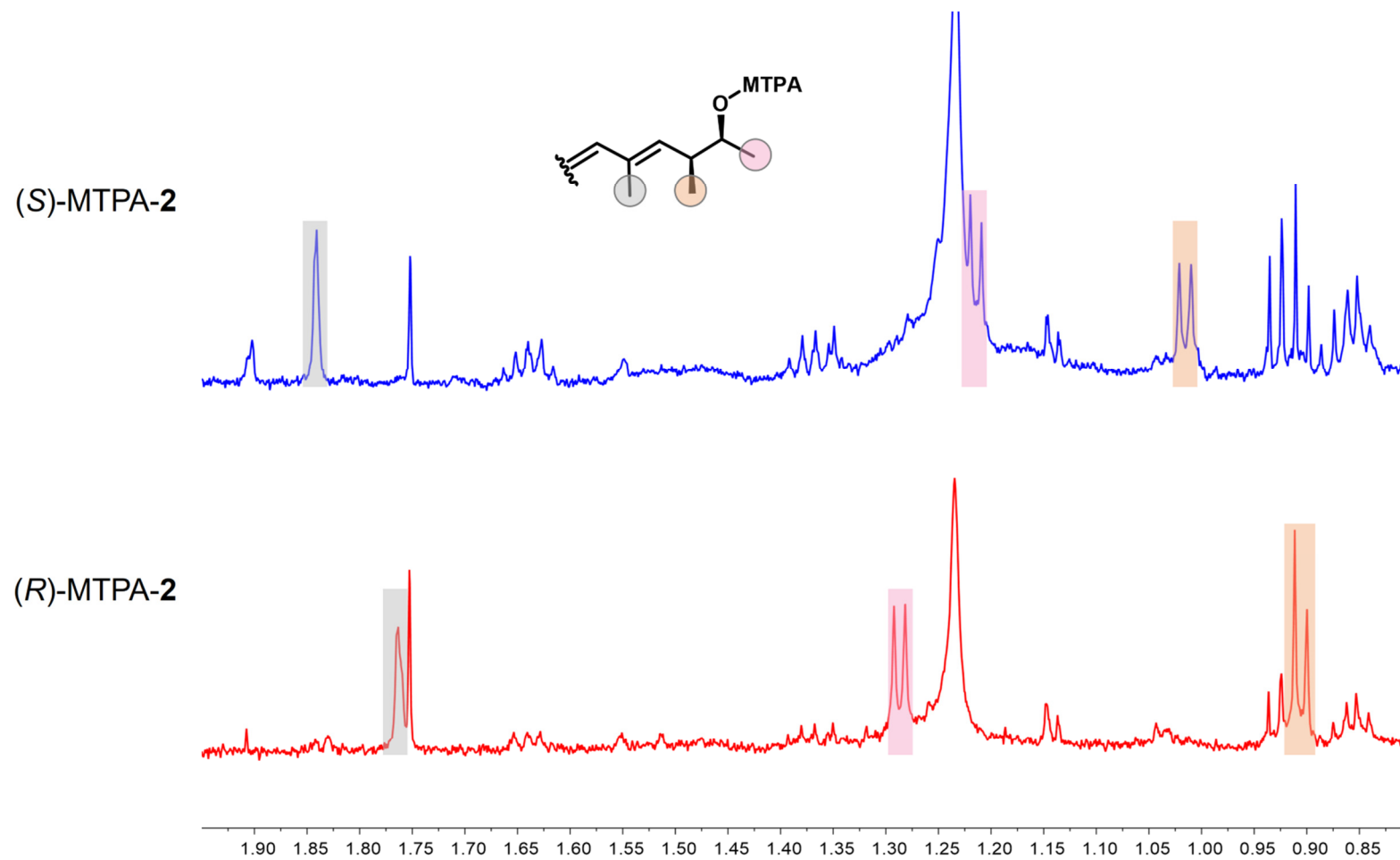


Figure S24. HECADE spectrum of oxapyrrolidine B (**2**) in DMSO- d_6

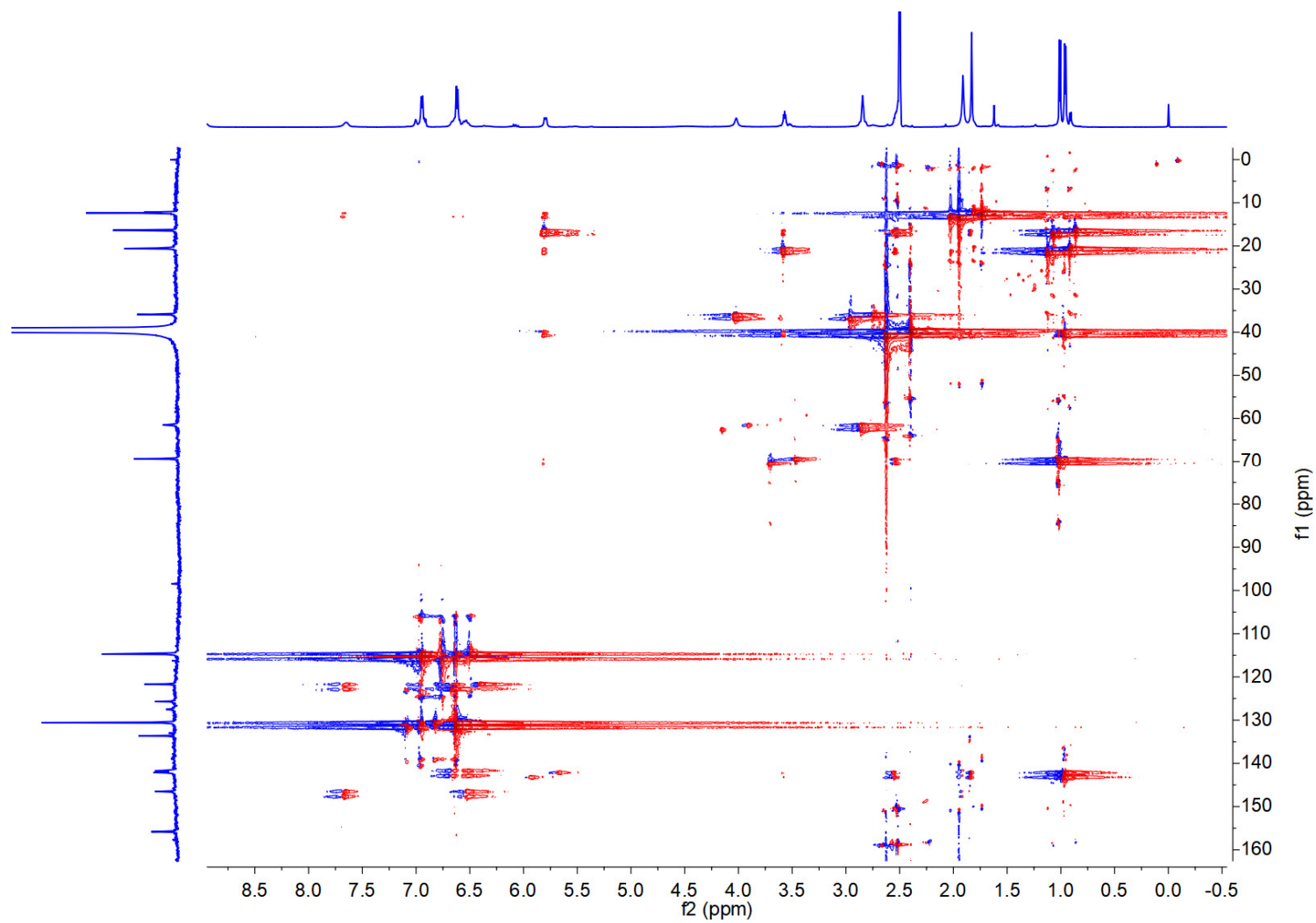


Figure S25. ECD spectrum of oxapyrrolidine B (**2**)

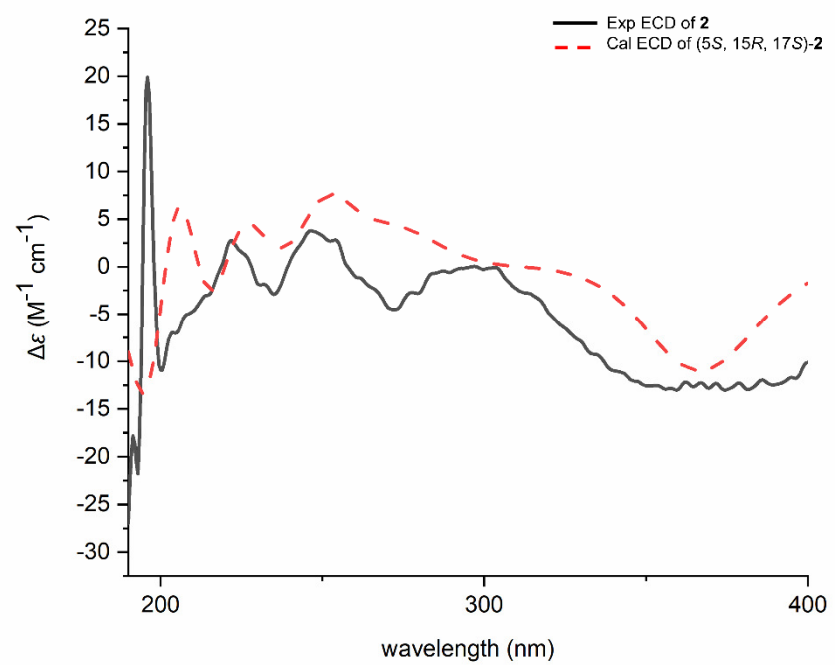


Figure S26. UV spectrum of oxapyrrolidine B (2)

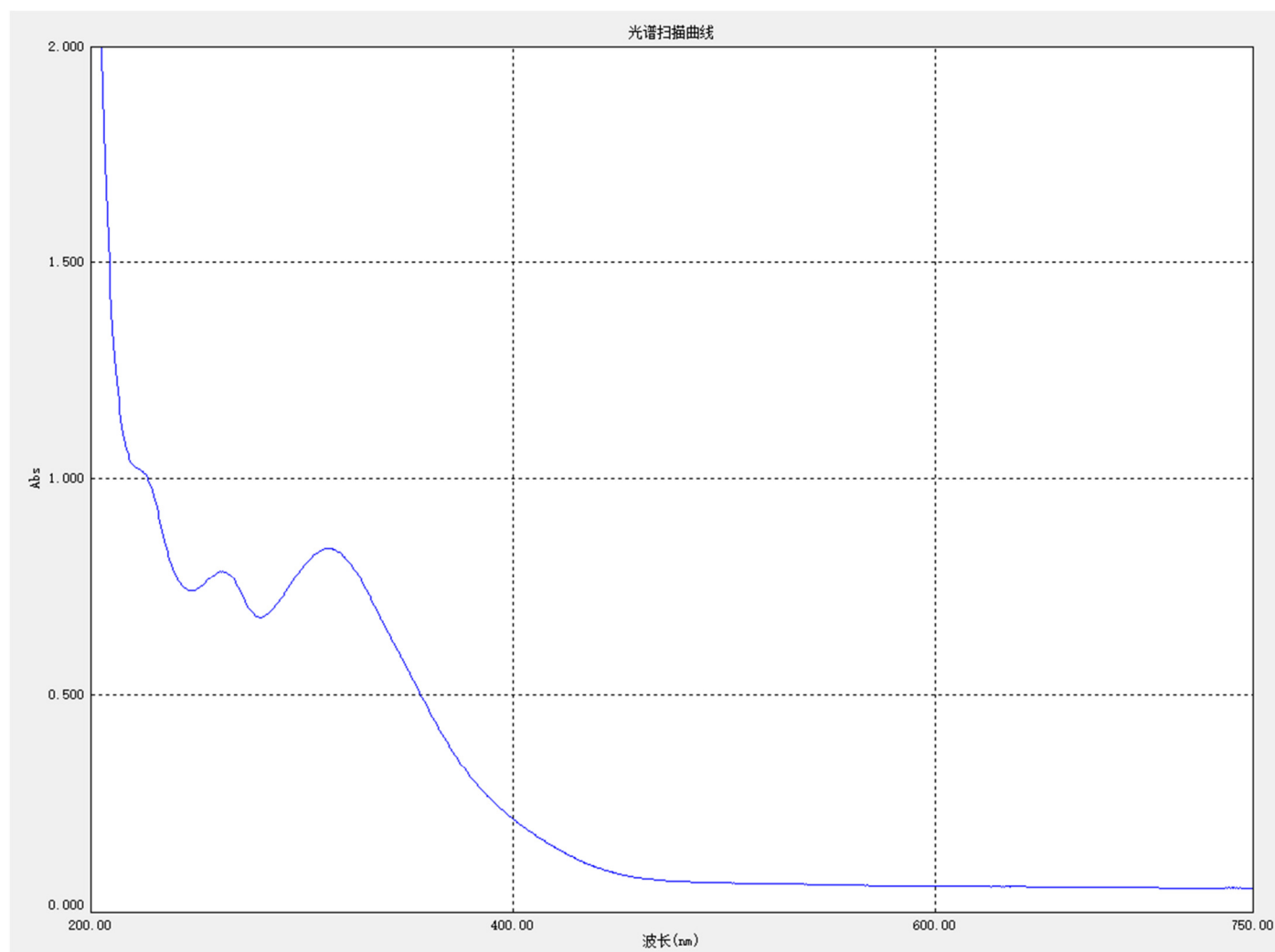


Figure S27. IR spectrum of oxapyrrolidine B (2)

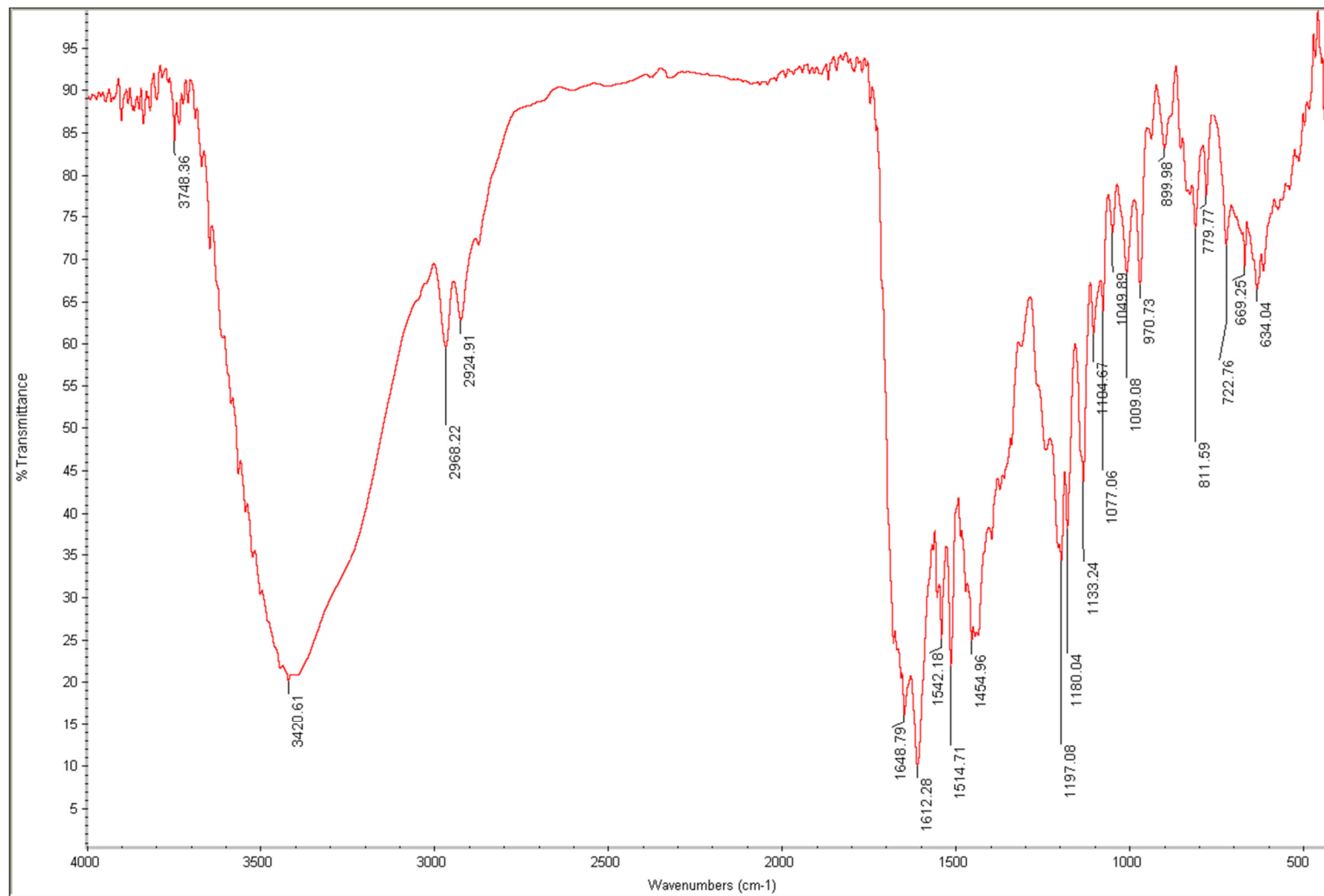
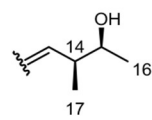
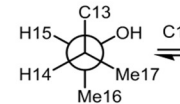
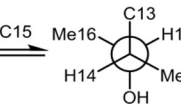
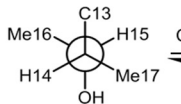
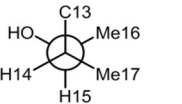
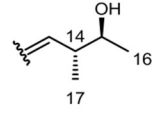
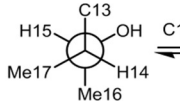
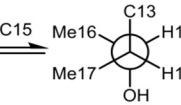
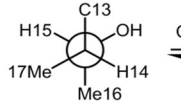
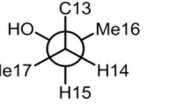
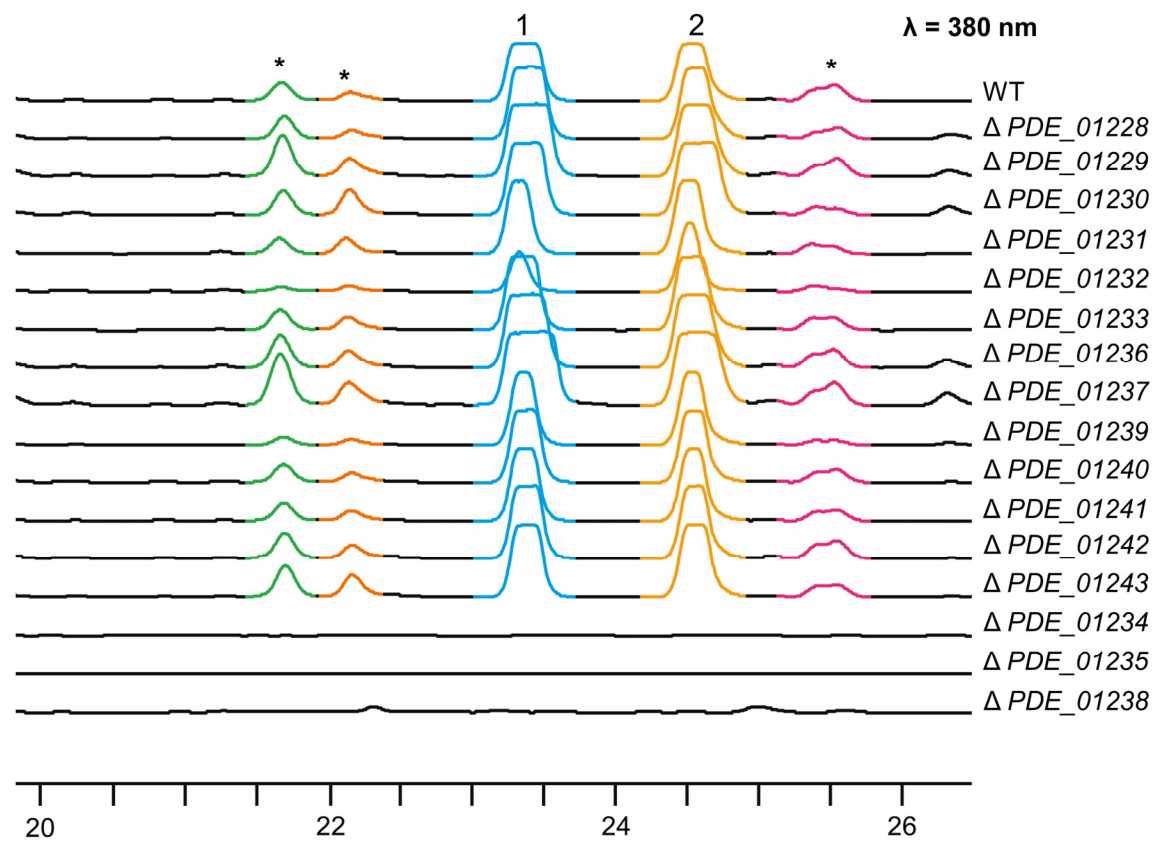


Figure S28. *J*-based analysis including all conformer pairs that produce a medium $^3J_{(H14-H15)}$ [1]

	A1		A2		A2		A3			
										
									16 (Hz) 17 (Hz)	
	$^3J_{(H14, H15)}$	Small (0-4 Hz)	Large (6-8 Hz)	Medium (4-6 Hz)	Large (8-12 Hz)	Small (0-4 Hz)	Medium (4-6 Hz)	6.2	6.2	Medium (4-6 Hz)
	$^3J_{(Me16, H14)}$	Small (1-3 Hz)	Small (1-3 Hz)	Small (1-3 Hz)	Small (1-3 Hz)	Large (6-8 Hz)	Medium (3-6 Hz)	2.7	1.6	Small (1-3 Hz)
	$^3J_{(C13, H15)}$	Small (1-3 Hz)	Small (1-3 Hz)	Small (1-3 Hz)	Small (1-3 Hz)	Large (6-8 Hz)	Medium (3-6 Hz)	2.8	2.3	Small (1-3 Hz)
	B1		B2		B1		B3			
										
									16 (Hz) 17 (Hz)	
	$^3J_{(H14, H15)}$	Large (8-12 Hz)	Small (0-4 Hz)	Medium (4-6 Hz)	Large (8-12 Hz)	Small (0-4 Hz)	Medium (4-6 Hz)	6.2	6.2	Medium (4-6 Hz)
	$^3J_{(Me16, H14)}$	Small (1-3 Hz)	Large (6-8 Hz)	Medium (3-6 Hz)	Small (1-3 Hz)	Small (1-3 Hz)	Small (1-3 Hz)	2.7	1.6	Small (1-3 Hz)
	$^3J_{(C13, H15)}$	Small (1-3 Hz)	Small (1-3 Hz)	Small (1-3 Hz)	Small (1-3 Hz)	Large (6-8 Hz)	Medium (3-6 Hz)	2.8	2.3	Small (1-3 Hz)
	$^3J_{(Me17, H15)}$	Small (1-3 Hz)	Large (6-8 Hz)	Medium (3-6 Hz)	Small (1-3 Hz)	Small (1-3 Hz)	Small (1-3 Hz)	3.9	3.4	Medium (3-6 Hz)
	$^2J_{(C15, H14)}$	Large (5-7 Hz)	Large (5-7 Hz)	Large (5-7 Hz)	Large (5-7 Hz)	Small (0-2 Hz)	Medium (2-5 Hz)	4.5	4.2	Medium (2-5 Hz)

1. Neuhaus, G.F.; Adpressa, D.A.; Bruhn, T.; Loesgen, S. Polyketides from marine-derived *Aspergillus porosus*: challenges and opportunities for determining absolute configuration. *J. Nat. Prod.* **2019**, *82*, 2780-2789.

Figure S29. HPLC profiles of extracts from wild-type and mutants.



The asterisk-marked peaks represent the analogs of 1 and 2.

Figure S30. Extracted ion chromatograms of extracts from wild-type and mutants

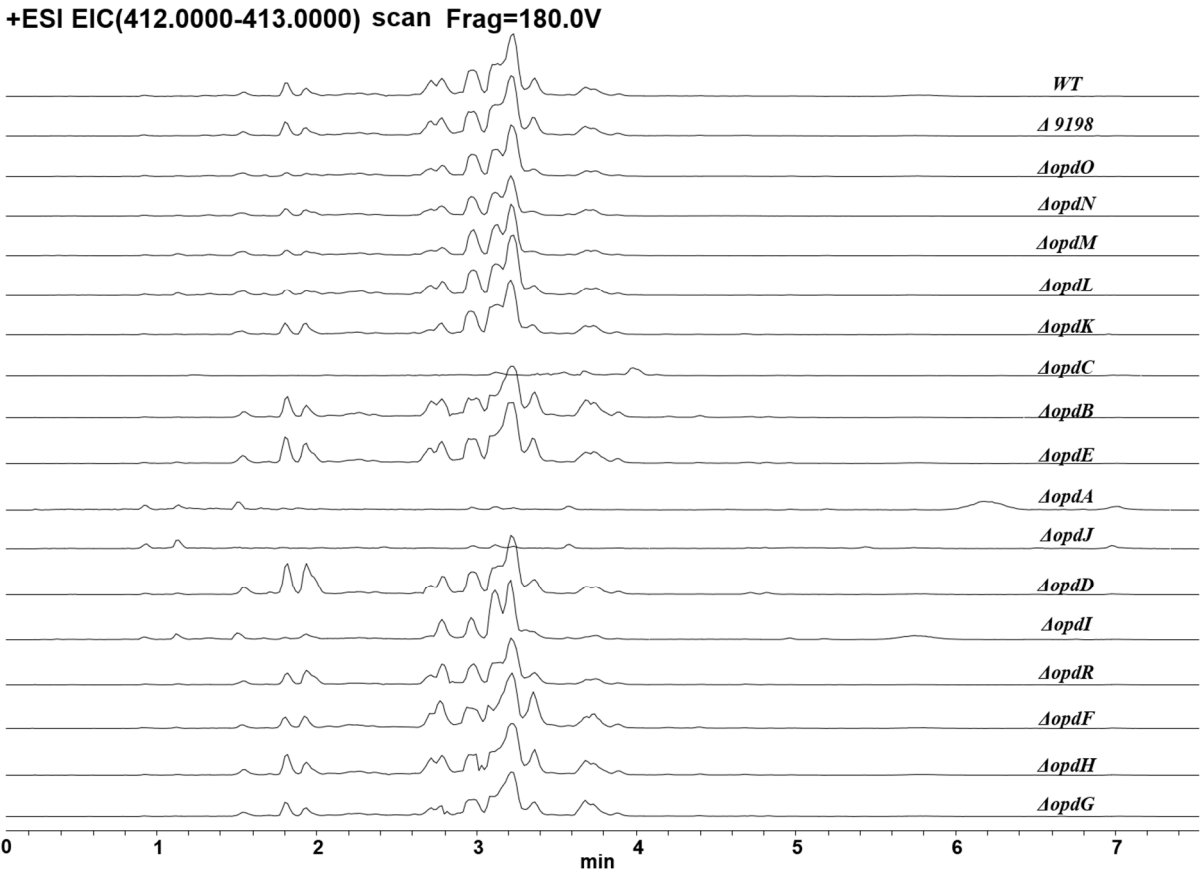
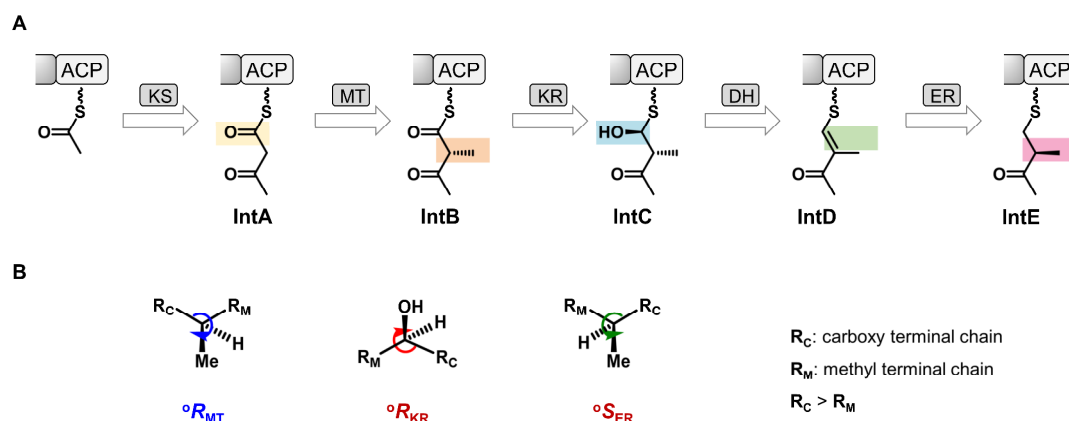


Figure S31. Stereochemical course of fungal reduced polyketides.

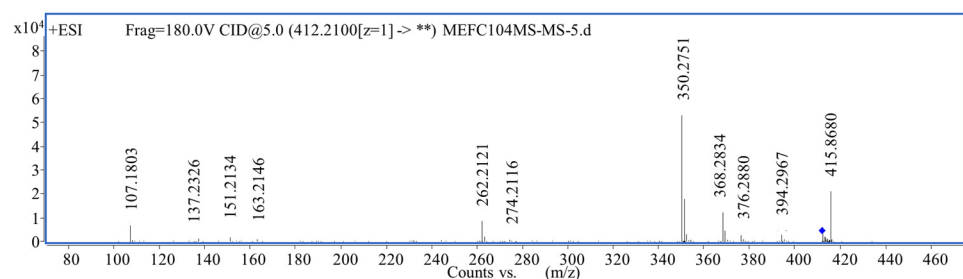


(A) absolute configurations of stereogenic centers in fungal reduced polyketides. (B) Newly defined $^{\circ}R/^{\circ}S$ model. The newly defined $^{\circ}R/^{\circ}S$ model focuses on the direction of the polyketide chain elongation and does not consider the absolute configurations of other nearby substituents. In the stereochemical model, the author defined that a carboxy-terminal chain (R_C) has a higher priority than a methyl terminal chain (R_M).

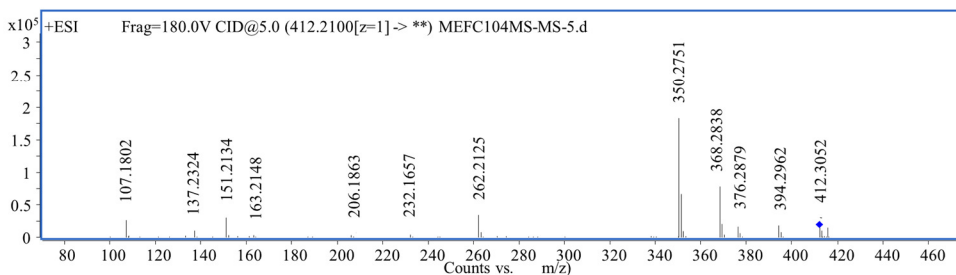
Oikawa and coworkers have proposed a stereochemical rule to explain the absolute configurations of fungal reduced polyketides [1]. During the polyketide elongation, an $^{\circ}R_{MT}$ -methyl group was introduced into IntA to form IntB by the methyltransferase (MT) domain. Then, the ketoreductase (KR) domains reduced IntB to give IntC with an $^{\circ}R_{KR}$ -hydroxy group. Subsequently, dehydratase (DH) domains catalyzed *E*-olefin formation to afford IntD. Finally, enoyl was reduced by enoyl reductases (ER), giving IntE with an $^{\circ}S_{ER}$ -methyl group. This stereochemical rule can explain the absolute configurations of **1**. Meanwhile, Oikawa and coworkers also proposed that a few compounds have mismatched stereochemistry. Therefore, it can explain that compound **2** possesses an $^{\circ}R_{ER}$ -methyl group at C-15 and an $^{\circ}S_{KR}$ -hydroxy group at C-17.

[1] Takino, T.; Kotani, A.; Ozaki, T.; Peng, W.Q.; Yu, J.; Guo, Y.; Mochizuki, S.; Akimitsu, K.; Hashimoto, M.; Ye, T.; Minami, A.; Oikawa, H. Biochemistry-guided prediction of the absolute configuration of fungal reduced polyketides. *Angew. Chem. Int. Ed.* **2021**, 60, 23403–23411.

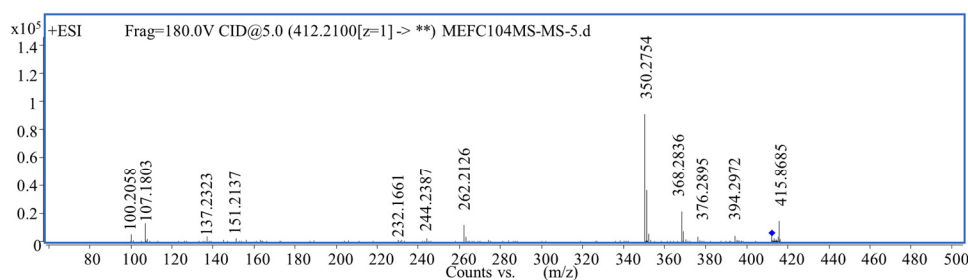
Figure S32. MS/MS data of oxapyrrolidines (**1-2**) and asterisk-marked compounds.



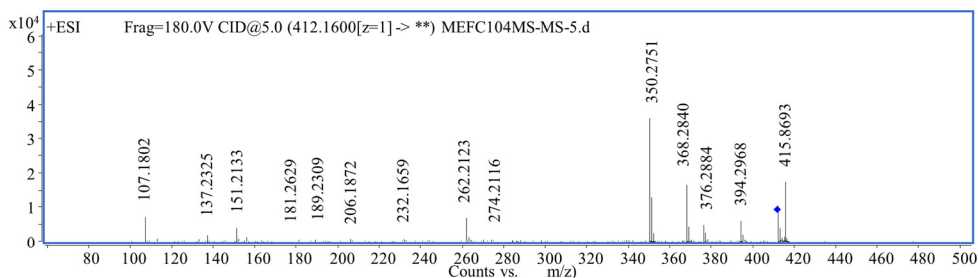
Oxapyrrolidines A (1)



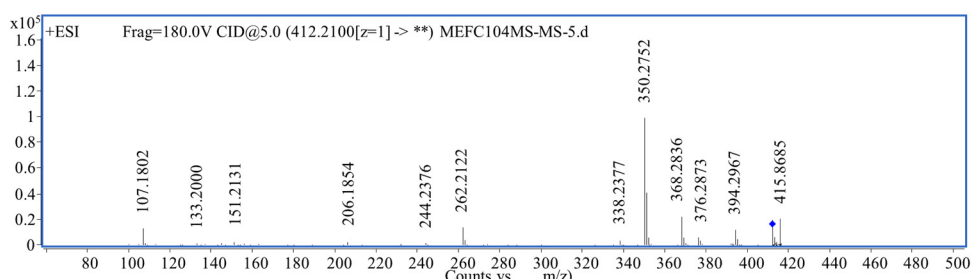
Oxapyrrolidines B (2)



21.6 min (3)

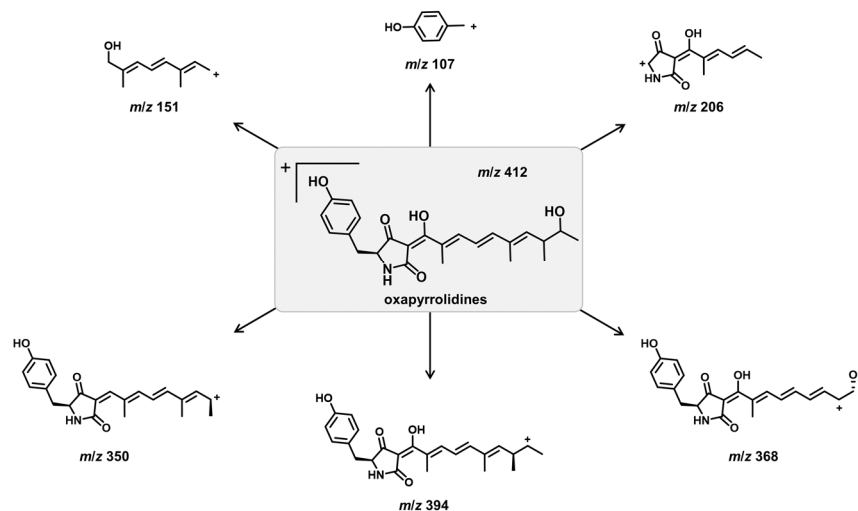


22.3 min (4)



25.6 min (5)

Figure S33. Fragmentation pattern of oxapyrrolidines based on MS/MS data.



Compounds **1-2** and its analogs have the similar MS/MS patterns, such as the ion fragments of m/z = 394, 368, 350, 206, 151, 107 (Figure S32). We have assigned the structural fragments corresponding to daughter ions, including *p*-hydroxy benzyl, α -cleavage of the tetramic acid moiety, and the degradation of the aliphatic side chain. This information suggested that the structures of asterisk-marked in HPLC profiles were similar to that of **1-2**. The difference between **1-2** and its analogs might be the keto-enol tautomerization in tetramic acid moiety and *cis-trans* isomerization in the linkage of the aliphatic side chain and tetramic acid.