Electronic Supplementary Material (ESI) for ChemComm.

Total Biosynthesis of Fungal Tetraketide Pyrones

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Electronic Supplementary Information

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1. Experimental Procedures

1.1 Bioinformatic Analyses

1.1.1 Transcriptome Sequencing and Data Analysis

In this study, the transcriptome data had a dual purpose. Firstly, it helped assess the levels of functional gene expression by comparing conditions of production and non-production. This analysis aided in identifying boundaries of the muitiforisin H biosynthetic gene cluster (*mfn*BGC). Moreover, the transcriptome data facilitated the identification of intron positions. The processed RNASeq data was incorporated into Geneious and aligned with a reference dataset. During alignment, instances where reads spanned intron regions were divided strategically, assisting in accurately pinpointing intron positions. In conjunction with intron predictions from tools like antiSMASH[1] and FGENESH[2], DNA fragments lacking introns were cloned. These fragments had 50-bp overlaps and were later combined through yeast-mediated recombination methods for plasmid construction.

H. monticulosa MUCL 54604 underwent cultivation in both producing conditions (PDB medium) and non-producing conditions (DPY medium). Following cultivation, mycelia were harvested and employed for the extraction of total RNA utilizing the Quick-RNA Fungal/Bacterial Miniprep Kit (Zymo Research). Subsequent to extraction, the RNA samples underwent DNase I treatment (Zymo Research). The generation of cDNA was achieved by employing the High Capacity RNA-to-cDNA™ kit (Thermo Fisher Scientific). To ascertain the absence of genomic DNA contamination in the extracted RNA, a PCR targeting a housekeeping gene was performed.

Three sets of high-quality RNA samples, verified to be free from genomic DNA contamination, were prepared for each experimental condition. These prepared RNA samples were subsequently forwarded to CeBiTec for cDNA sequencing after the reverse transcription procedure was successfully conducted. For library construction, RNA was employed in conjunction with the TruSeq mRNA Sample Preparation Kit (stranded, Illumina). The sequencing of the resulting cDNA libraries was performed on the Illumina HiSeq 1500 platform using the 'Rapid Mode,' with a read configuration of 2 x 75 bp. The subsequent stages of data analysis and base calling were executed using proprietary in-house software.

1.1.2 Identification of *mfn*BGC

The genomic sequences of *H. monticulosa*, *H. submonticulosa*, and *H. spongiphila* were utilized to establish a local database within the Geneious software. This local database was then employed for the manual BLASTp analysis of candidate *mfn*BGC. To serve as a template, the solanapyrone synthase [3] (D7UQ44) associated with solanapyrone biosynthesis was chosen due to its involvement in the α-pyrone backbone. This process resulted in the identification of three clusters exhibiting substantial similarities for each fungus. Utilizing the online BLASTp, genes encompassed within an extended gene cluster were searched for and manually annotated (Table S1.1). This extensive cluster was found to encode a diverse array of enzymes including DNA ligase (*mfnL9*), epimerase (*mfnL8)*, transcriptional regulator (*mfnL7, mfnR1*), SDRs (*mfnL6, mfnR4*), DNA polymerase (*mfnL5*), hydrolase (*mfnL4*), hrPKS (*mfnPKS2, mfnPKS1*), O-acetyltransferase (*mfnL3*), P450s (*mfnL2, mfnR3*), FMO (*mfnR2*), O-methyltransferase (*mfnL1*) and membrane protein (*mfnR6*).

To refine the BGC arrangement, transcriptome data was employed to adjust the borders of the cluster. The analysis was to elucidate the expression patterns of functional genes within the *mfn*BGC while simultaneously delineating its boundaries by the transcriptome data from *H. monticulosa*. The findings

also unveiled a distinct expression pattern within the *mfn*BGC. Specifically, genes from *mfnPKS2* to *mfnR4* (blue area) exhibited significant upregulation under conditions conducive to production (Table 1.1, Figure 1.1). In contrast, genes located beyond this defined region exhibited either downregulation or remained unexpressed altogether.

MfnPKS1 was expressed and produced tetraketide pyrone **5**. Our focus then shifted to *Penicillium islandicum*'s genome, the producer of islandic acid [4], where we used the *mfn* genes as a reference. While *ilaPKS1* and most tailoring genes have similarilies to the *mfn* genes, *ilaPKS2* showed no similarity to *mfnPKS2*. Subsequently, a thorough comparison was performed, encompassing the *sol* BGC, *mfn* BGCs from the three *Hypomontagnella* organisms, *ila*BGC, and *amp*BGC. This analysis was visualized through a cluster map showcasing all six gene clusters [5] (Figure S1.2).

Table S1.1 Proposed functions of genes of muitiforisin H BGC (blue area) from *H. monticulosa* MUCL 54604. Average expression levels from conditions of production (A) and non-production (B) to calculate the log_2-fold change (B/A).

Gene	Locus_tag	AA	Putative Function	Predicted Cofactor	A non-producing	B producing	Log_2-fold change B/A
mfnL9	3645	906	DNA ligase		-	-	-
mfnL8	3646	343	Epimerase		462.24	286.16	-0.69
mfnL7	3647	364	Transcriptional regulator		32.13	26.61	-0.27
mfnL6	3648	186	SDR	NAD(P)	-	-	-
mfnL5	3649	2248	DNA polymerase		-	-	-
mfnL4	3650	163	Hydrolase		1.04	0.40	-1.39
mfnPKS2	3651	2504	hrPKS	NAD(P)	101.57	290.21	1.51
mfnL3	3652	487	O-acetyltransferase		-	-	-
mfnL2	3653	537	P450		100.53	147.30	0.55
mfnL1	3654	427	O-methyltransferase	SAM	169.97	307.35	0.85
mfnPKS1	3655	2591	hrPKS	NAD(P)	60.11	86.35	0.52
mfnR1	3656	654	Transcriptional regulator		-	-	-
mfnR2	3657	526	FMO	FAD	203.14	703.65	1.79
mfnR3	3658	580	P450		23.84	50.29	1.08
mfnR4	3659	274	SDR	NAD(P)	386.59	529.38	0.45
mfnR5	3660	279	Unknown		-	-	-
mfnR6	3661	333	membrane protein		9.33	9.33	-0.72

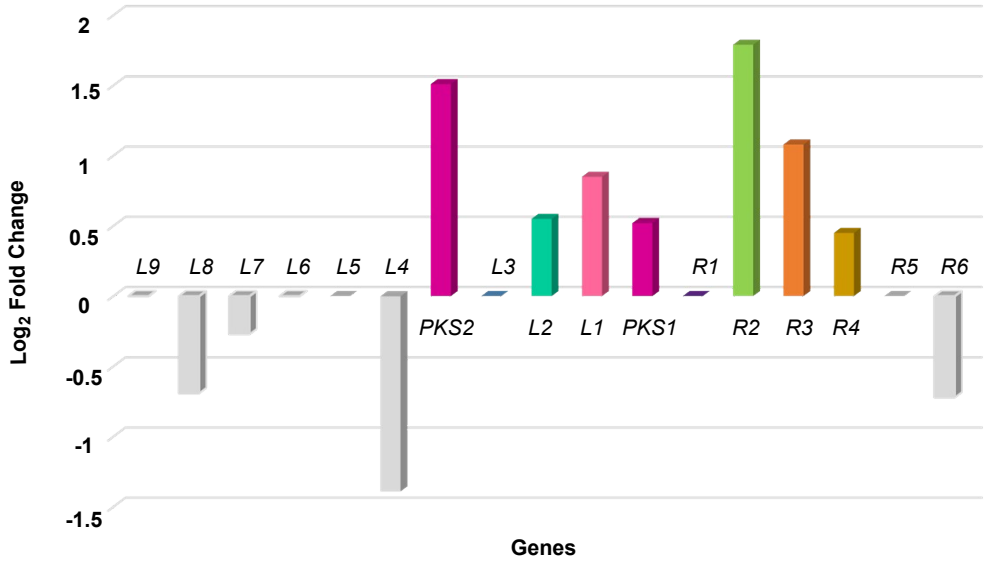


Figure S1.1 Bar chart of Log_2-fold changes represents the expression level of the predicted multiforisin H BGC from *H. monticulosa* MUCL 54604 transcriptome data. The genes from proposed BGC are coloured as shown in table 1.1.

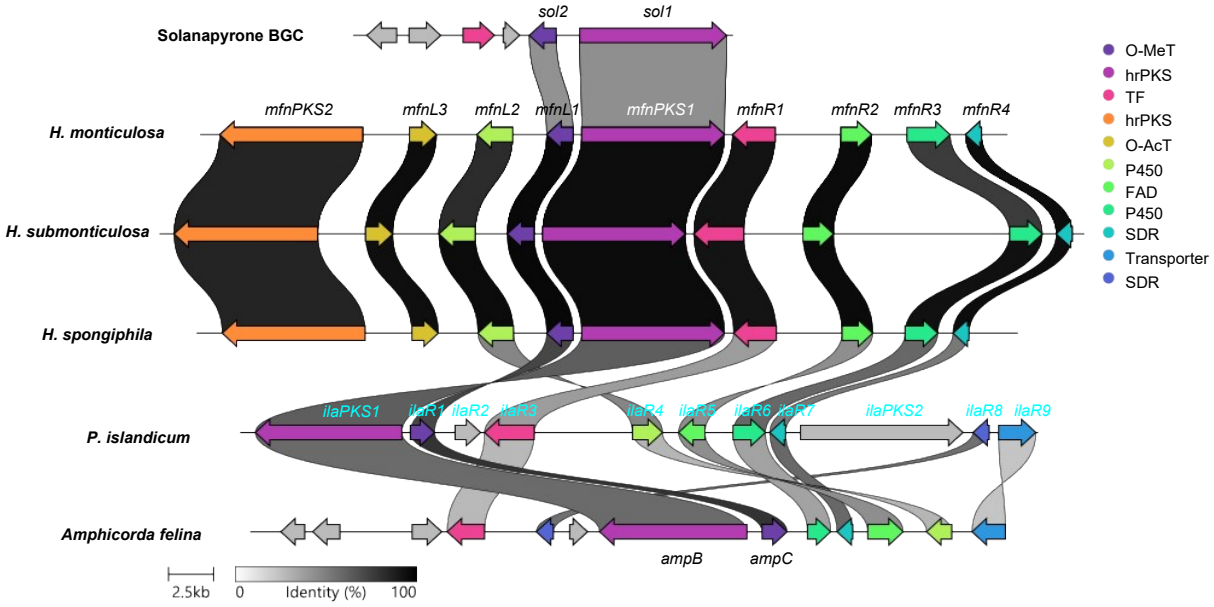


Figure S1.2 BGC alignment of *sol* BGC, *mfn* BGCs from the three *Hypomontagnella* organisms, *ila*BGC, and *amp*BGC by Clinker.[5]

1.1.3 Intron Analysis of *mfn*BGC

The processed RNASeq data was integrated into Geneious and aligned with the *mfn*BGC reference. By examining the RNA reads, we could accurately determine intron positions given the condition of having sufficiently high read quality. For instance, in the case of *mfnL1, mfnL2, mfnR4*, and *mfnR2, mfnL3* (Figure S1.3, S1.4, S1.5, S1.6, S1.7), the high-quality reads allowed us to precisely identify intron positions.

However, for *mfnPKS1, mfnPKS2*, and *mfnR3* (Figure S1.8, S1.9, S1.10), while some reads aided in intron localization, prediction tools (antiSMASH [1]and FGENESH [2]) were necessary for confirming intron positions in certain segments.

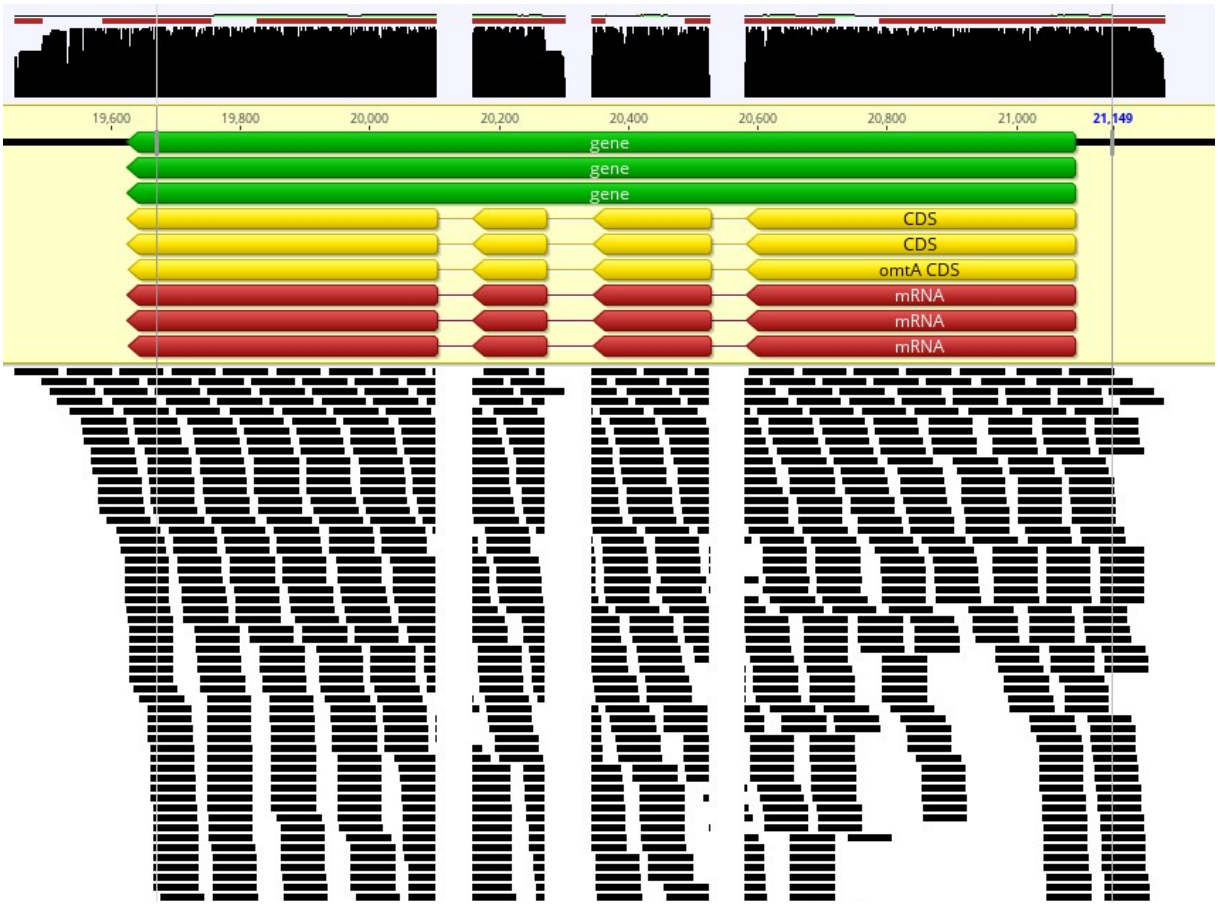


Figure S1.3 The RNASeq mapping of *mfnL1*. The gaps represent the intron positions. Black vertical bars represent the mapped reads

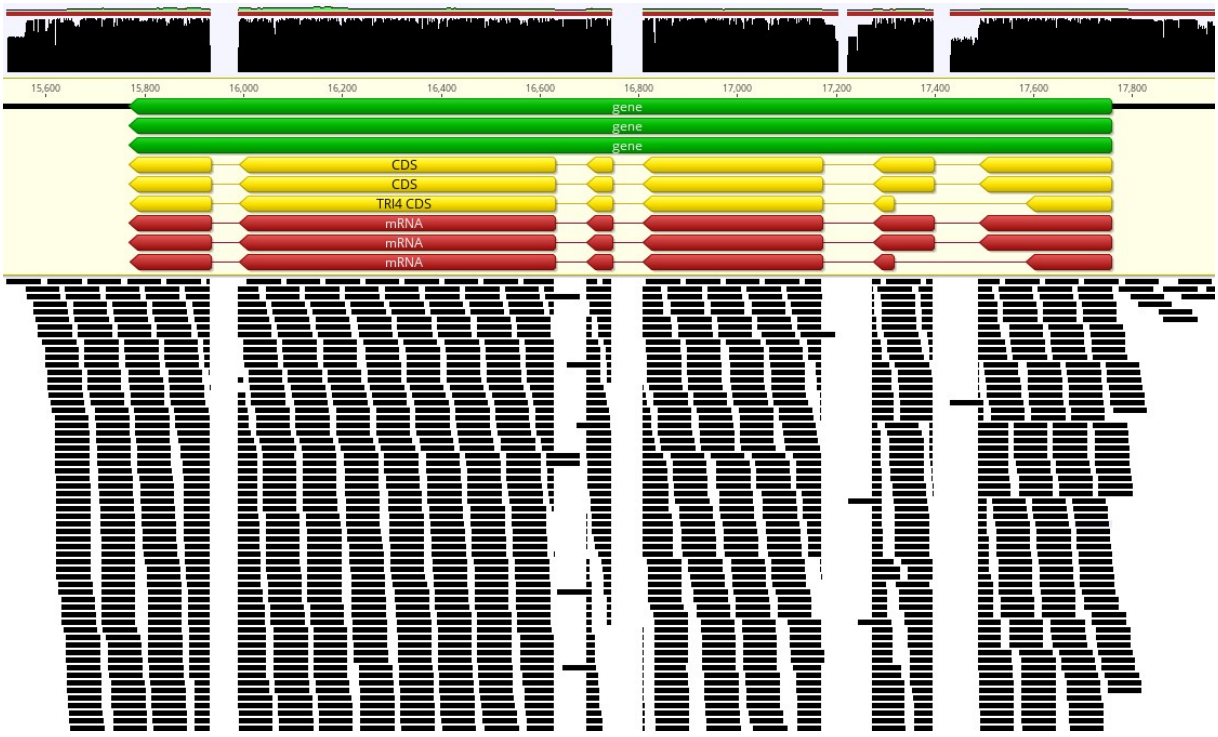


Figure S1.4 The RNASeq mapping of *mfnL2*. The gaps represent the intron positions. Black vertical bars represent the mapped reads

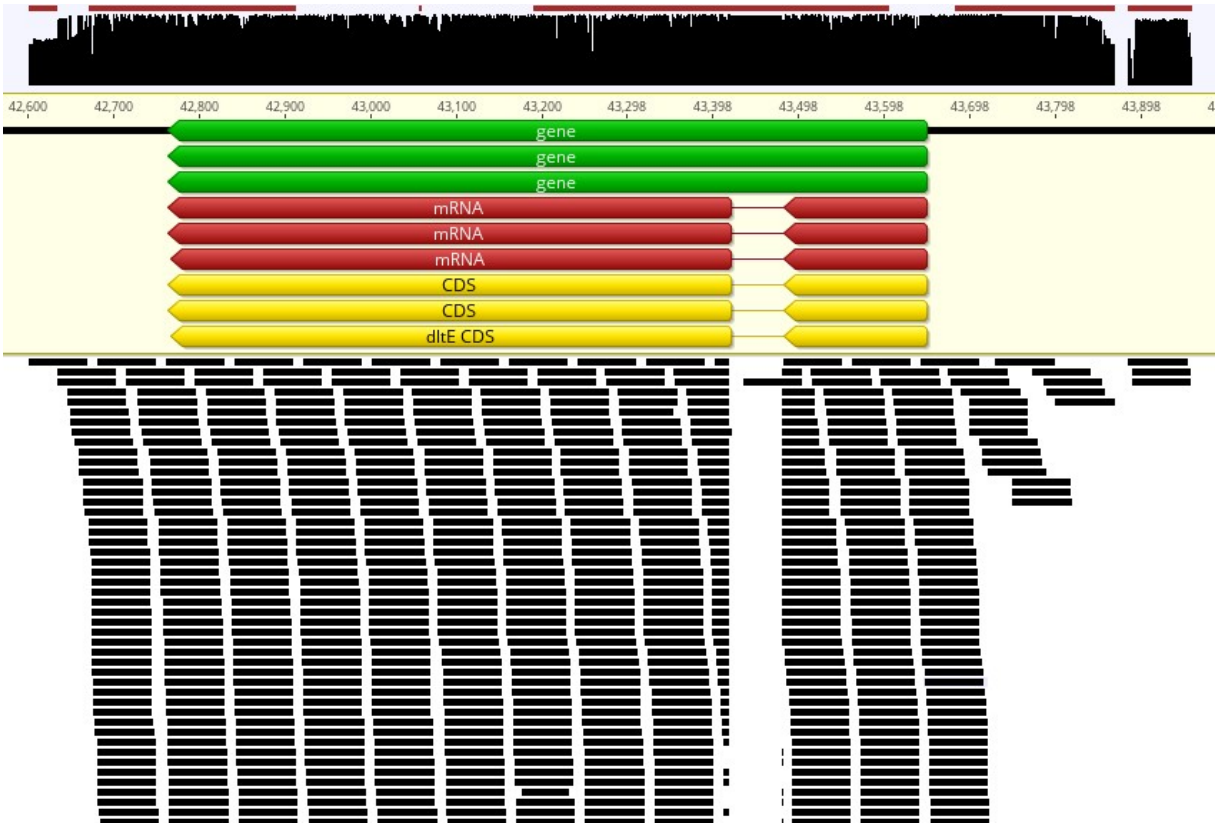


Figure S1.5 The RNASeq mapping of *mfnR4*. The gaps represent the intron positions. Black vertical bars represent the mapped reads

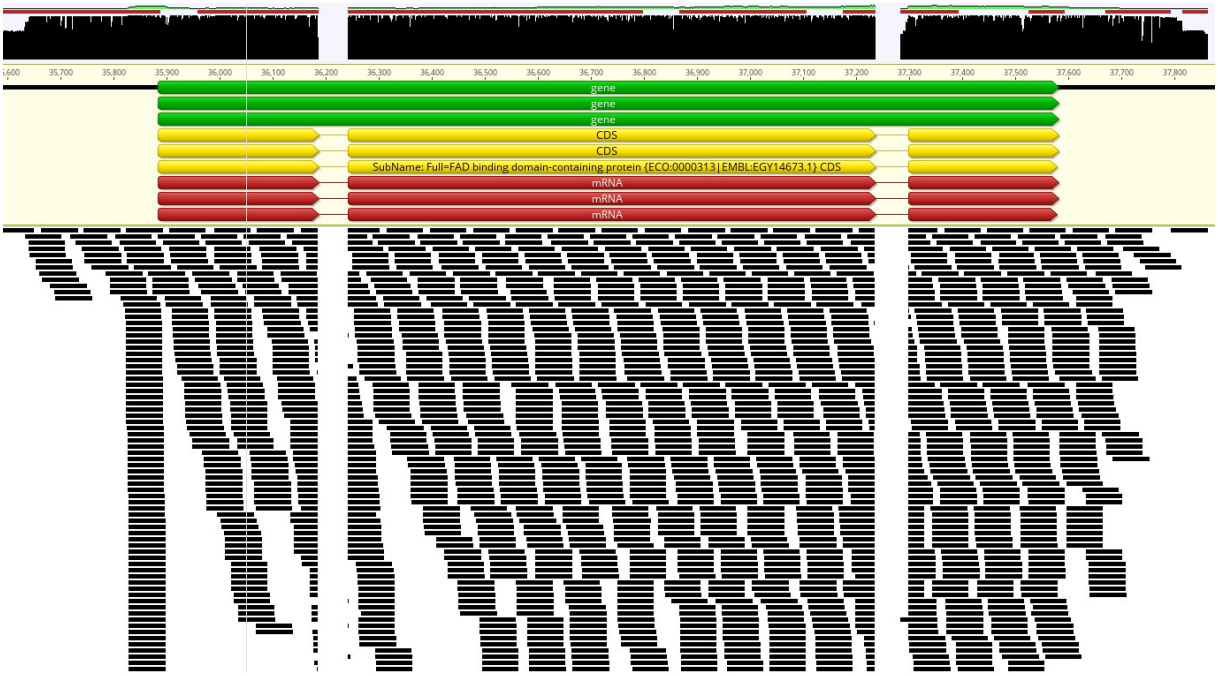


Figure S1.6 The RNASeq mapping of *mfnR2*. The gaps represent the intron positions. Black vertical bars represent the mapped reads

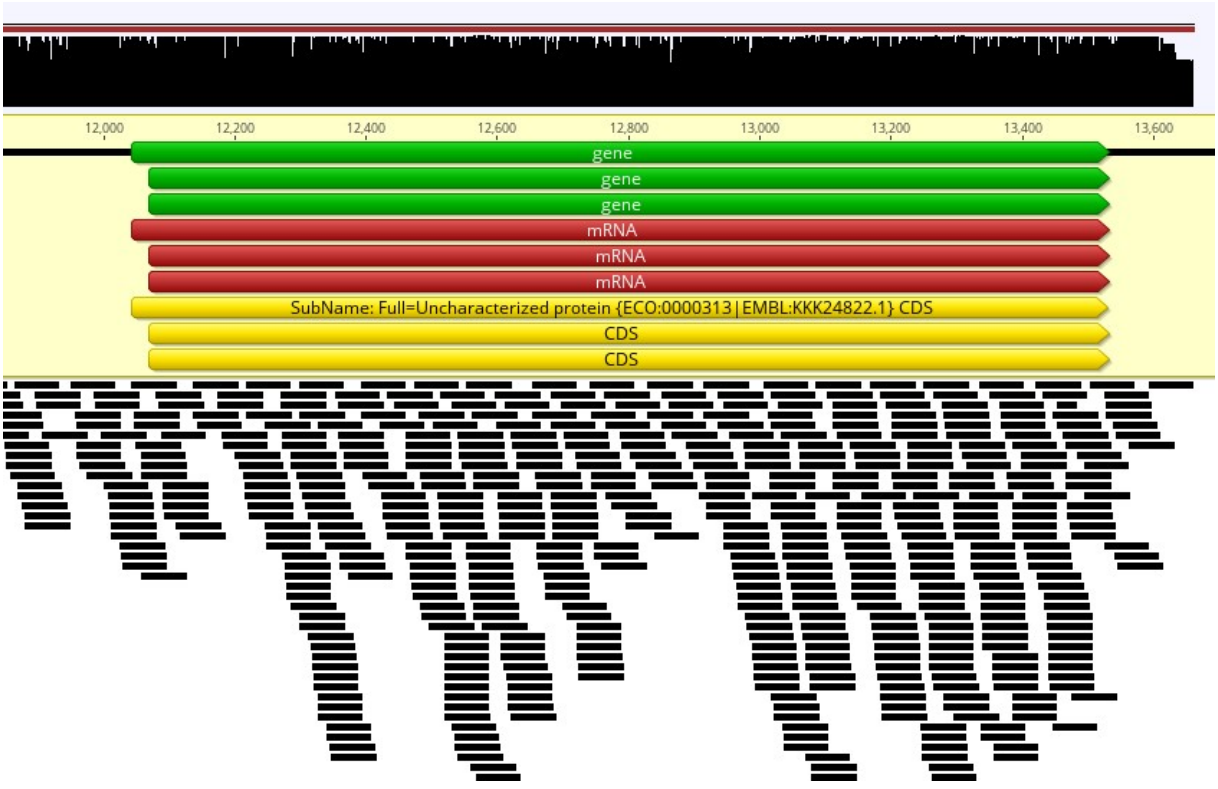


Figure S1.7 The RNASeq mapping of *mfnL3*. Black vertical bars represent the mapped reads.

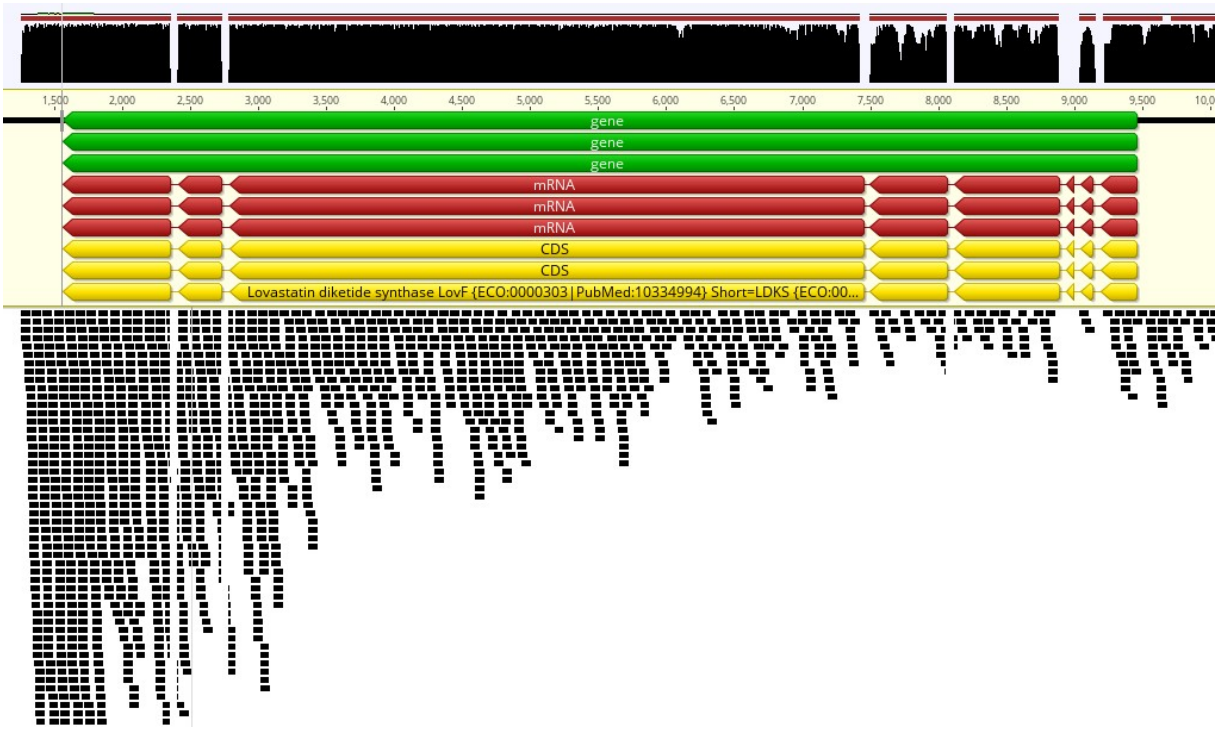


Figure S1.8 The RNASeq mapping of *mfnPKS2*. The gaps represent the intron positions. Black vertical bars represent the mapped reads

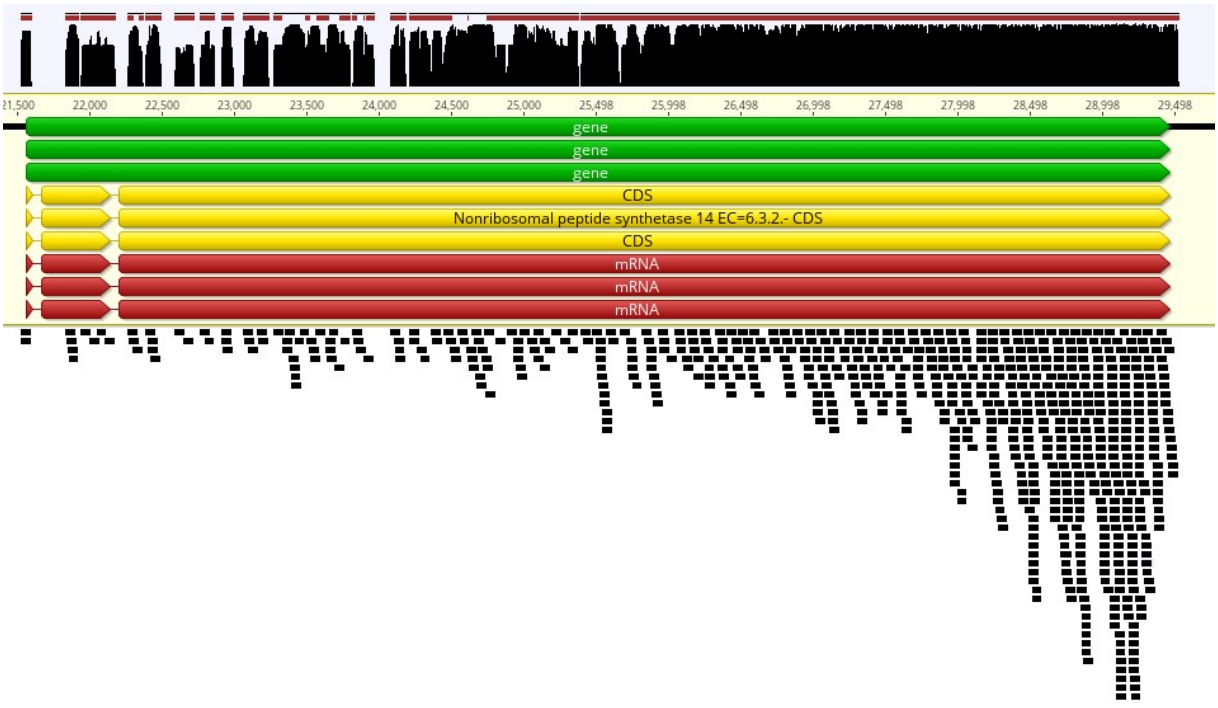


Figure S1.9 The RNASeq mapping of *mfnPKS1*. The gaps represent the intron positions. Black vertical bars represent the mapped reads

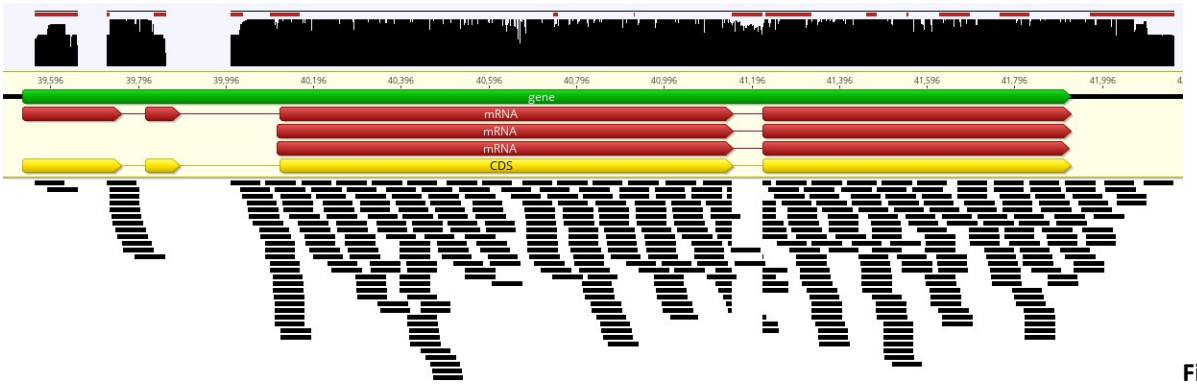


Figure S1.10 The RNASeq mapping of *mfnR3*. The gaps represent the intron positions. Black vertical bars represent the mapped reads

1.1.4 Analysis of *ila*BGC

Before embarking on the experimental procedures, an in-depth analysis of the potential functions inherent in this gene cluster was undertaken (Table S1.2). The scrutiny identified a range of probable functions within the cluster, encompassing two hrPKS (*IlaPKS1, IlaPKS2*), two P450 (*IlaR4* and *IlaR6*) two SDRs (*IlaR7* and *IlaR8*), along with an O-MeT (*IlaR1*), an O-AcT (*IlaR2*), a FMO (*IlaR5*), a transcription factor (*IlaR3*), and a transporter (*IlaR9*). The domain analysis of ilaPKS2 was performed on NCBI, a choline/carnitine o-acyltransferase was found after the ACP [6] (Figure S1.11).

The genes *ilaPKS2, ilaR2,* and *ilaR8* were cloned from the genomic DNA of a *Penicillium islandicum* strain iBT20602, generously provided by Professor Thomas Ostenfeld Larsen from the Danish Technical University. Following the extraction, all DNA inserts were subjected to sequencing, revealing a complete correspondence with the respective sequences found in *Talaromyces islandicus* WF-38-12 [7](ATCC 26535), which is recognized as the source organism for producing islandic acid [4].

Table S1.2 Proposed functions of *ila*BGC

Gene	Locus_tag (PISL3812_)	AA	Protein BLAST	Putative Function	Predicted cofactor	Best hit accession
IlaPKS1	09789	2630	Prosolanapyrone synthase	hrPKS	NAD(P), SAM	D7UQ44
IlaR1	09788	427	O-methyltransferase sol2	O-MeT	SAM	D7UQ43
IlaR2	09787	468	Probable acetyltransferase tazD	O-AcT		Q0CS99
IlaR3	09786	649	Probable transcription factor sol4	TF		D7UQ41
IlaR4	09785	473	Cytochrome P450 monooxygenase tpcC	P450		M2UJ60
IlaR5	09784	466	FAD-linked oxidoreductase anuG	FMO	FAD	W6QEK0
IlaR6	09783	581	Cytochrome P450 monooxygenase TRI13	P450		Q9C1I4
IlaR7	09782	273	Short-chain dehydrogenase/reductase family 32C member 1	SDR	NAD(P)	Q0IH28
IlaPKS2	09781	2972	Highly reducing polyketide synthase SAT13	hrPKS	NAD(P), SAM	A0A084API3
IlaR8	09780	249	Short-chain dehydrogenase/reductase atnB	SDR	NAD(P)	A0A455LLX2
IlaR9	09779	563	MFS-type transporter calB	Transporter		A0A1V6PBC8

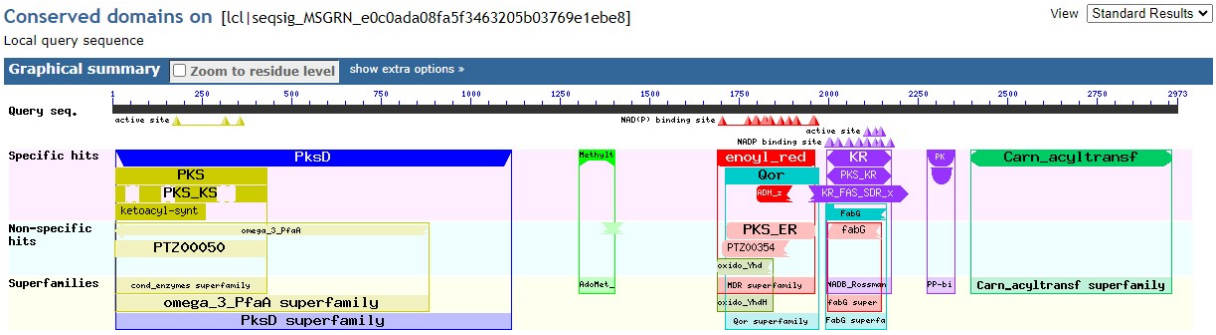


Figure S1.11 Conserved domains analysis of ilaPKS2.

1.1.5 Sequence Analysis of MfnPKS2 KS domain

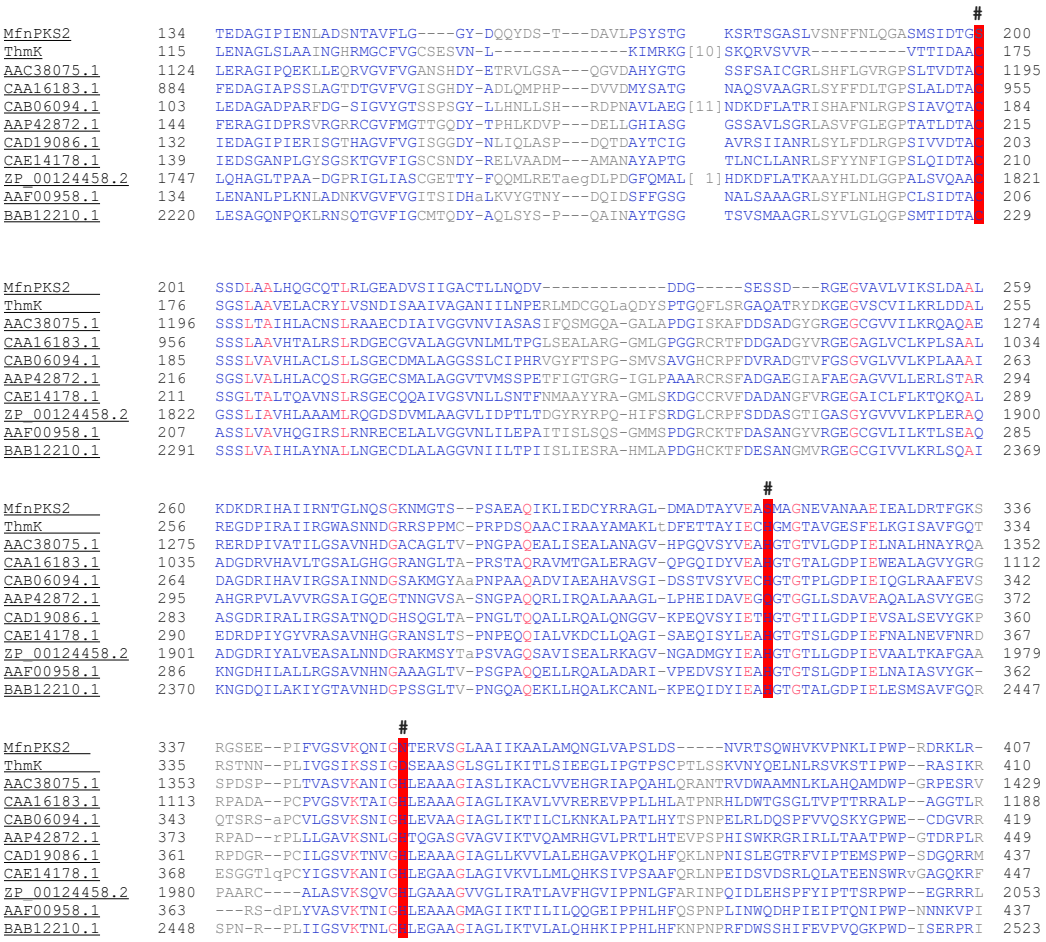


Figure S 1.12. Blastp multiple sequence alignment of the KS domain of mfnPKS2 with that of other PKS enzymes indicated that the amino acid residues in all three active sites were mutated.[8] # Active site in red.

Table S1.3 Descriptions and the accessions of the candidate proteins for Figure S1.12

Accession	Description
KAI0382397.1 (MfnPKS2)	Hypothetical protein F5Y04DRAFT_45677 [*Hypomontagnella monticulosa*]
XP_026611136.1 (ThmK) [8]	Hypothetical protein CDV56_101535 [*Aspergillus thermomutatus*]
AAC38075.1	Polyketide synthase type I [Pseudomonas protegens Pf-5]
CAA16183.1	Polyketide synthase [Streptomyces coelicolor A3(2)]
CAB06094.1	Phenolpthiocerol synthesis type-I polyketide synthase Ppse [Mycobacterium tuberculosis H37Rv]
AAP42872.1	NanA9 [Streptomyces nanchangensis]
CAD19086.1	StiB protein [Stigmatella aurantiaca Sg a15]
CAE14178.1	Unnamed protein product [Photorhabdus laumondii subsp. laumondii TTO1]
ZP_00124458.2	Non-ribosomal peptide synthetase modules and related proteins [Pseudomonas syringae pv. syringae B728a]
AAF00958.1	McyE [Microcystis aeruginosa PCC 7806]
BAB12210.1	polyketide synthase [Microcystis aeruginosa]

1.2 DNA Cloning

Oligonucleotides employed for PCR were designed using the Geneious software platform and subsequently synthesized by Sigma Genosys and Eurofins. The PCR investigations were orchestrated using the high-fidelity DNA polymerase, Q5® (New England Biolabs), for amplifying the DNA fragment destined for heterologous expression. In the context of colony PCR, the OneTaq® DNA polymerase was the enzyme of choice. The genomic DNA (gDNA) was procured from the pool of potential fungal candidates via the utilization of the GeneElute™ Plant Genomic DNA Miniprep Kit (Sigma Life Science). The exonic DNA fragments constituting the multiforisin H biosynthetic gene cluster (BGC) were derived from the gDNA of *Hypomontagnella monticulosa* and subsequently joined to a coding sequence by yeast recombination. Likewise, the exonic DNA fragments characterizing the islandic acid BGC were cloned directly from the genomic DNA of *Penicillium islandicum* (also known as *Talaromyces islandicus*). Subsequently, these fragments were linked to a coding sequence using yeast recombination techniques.

Subsequent experiments employed four modified vectors (designated as pTYGs), each tailored with distinct selection markers (△*argB*, *sC*$^{-}$, *ade*A^{-}, *niaD*$^{-}$) to facilitate targeted selection in *A. oryzae* NSAR1 [9,10]. These pTYGs vectors are equipped with the *2μ* origin and the *colE1* gene, optimizing their replication within *Saccharomyces cerevisiae* and *E. coli*, respectively. Selection mechanisms were integrated, employing the auxotrophy *URA3* gene for selection in *Saccharomyces cerevisiae*, while the *carB* resistance gene conferred selection advantage in *E. coli*. Notably, the *ccdB* suicide gene was employed as an additional selection marker in *E. coli*. Each pTYGs vector boasts four distinct promoter and terminator combinations (P/TamyB, P/Tadh, P/TgpdA, and P/Teno). Moreover, all four of these plasmids can be specifically cleaved using *Asc*I between P/Tadh, P/TgpdA, and P/Teno. Furthermore, the P/TamyB region can be precisely cleaved using *Not*I, facilitating yeast recombination processes.

1.3 Yeast Recombination

Yeast cultivation was carried out on YPAD agar at 30 °C for three days. A singular colony was selected and incubated overnight within 10 mL of YPAD media, maintained at 30 °C with shaking at 200 rpm. Following this, the 10 mL YPAD culture was transferred to a 250 mL Erlenmeyer flask preloaded with 40 mL of fresh YPAD medium. This composite culture was then incubated at 30 °C while being continuously shaken at 200 rpm for an additional 4 hours.

Subsequent to this incubation, cell collection was executed by subjecting the culture medium to centrifugation at 3,000 g for five minutes. The resulting pellet was subjected to two cycles of rinsing with 25 mL of double-distilled H_2O, followed by centrifugation after each rinse. This rinsed pellet was then suspended in a Falcon tube, with a total volume of 5 mL of Lithium acetate (LiOAc, 0.1 M). In a further step, aliquots of 50 µL from this suspension were individually transferred into distinct 1.5 mL Eppendorf reaction tubes. For immediate utilization, each aliquot underwent rapid pelleting at 21,000 g for 15 seconds, with the resulting pellet being immediately employed for yeast transformation.

On the other hand, for long-term cell stocking, the initial LiOAc step was substituted with an FCC solution. The harvested pellet was suspended in 5 mL of the FCC solution, prior to being apportioned into 50 µL aliquots, each of which was placed into separate 1.5 mL Eppendorf reaction tubes. These aliquots were then stored at -80 °C. For the purpose of thawing, samples were initially subjected to incubation on ice, followed by centrifugation for 15 seconds at

21,000 g. After the removal of the FCC solution, the cells were deemed ready for employment in the yeast transformation process.

The subsequent steps involved adding the following components to the pellet in a specific order: first, 50 µL of ssDNA, followed by 36 µL of 1 M LiOAc, and then 34 µL of a DNA mixture containing the linearized plasmid and corresponding inserts. This was followed by the addition of 240 µL of the PEG solution, ensuring thorough mixing to achieve a homogenous blend. In this process, the empty plasmid was utilized as a positive control, while the linearized plasmid served as the negative control. The resulting particulate was incorporated into the transformation mixture, and the mixture was incubated at 30 °C for 30 minutes at 300 rpm. Subsequently, the mixture was subjected to further incubation at 42 °C for 40 minutes. Following these incubation steps, the cells underwent centrifugation at 13,000 g for 60 seconds to obtain a pellet, from which the supernatant was removed. The ensuing pellet was then suspended in 200 µL of double-distilled H_2O before being dispensed onto selective SM-Ura plates. These plates were then subjected to an incubation for three days at 30 °C. To perform the extraction of the yeast plasmid, the Zymoprep™ Yeast Plasmid Miniprep II kit (Zymo Research, USA) was employed.

1.4 Construction of Plasmids

After completing the yeast plasmid extraction, the entirety of the plasmids was subsequently introduced into *E. coli* competent cells. A total of 50 µL of *E. coli* competent cells (Top10 or *ccd*B Survival TM 2 T1R, sourced from Thermo Fisher Scientific, USA) were combined with the yeast plasmids, followed by incubation on ice for 25 minutes. Following this, a heat shock was administered at 42 °C for 90 seconds, followed by immediate transfer to an ice bath for 3 minutes. The cell mixture was then introduced to 500 µL of SOC medium. The ensuing cell mixture underwent incubation at 37 °C with gentle shaking at 200 rpm for 1 hour. Subsequently, the cells were spread onto LB agar plates supplemented with appropriate antibiotics. These plates were then allowed to incubate overnight at 37 °C.

Colonies from each plasmid were selected and individually suspended in separate PCR tubes containing 10 µL of double-distilled H_2O, serving as the template for colony PCR. A distinct set of primers was employed to identify all genes present within each plasmid. From each plasmid, three positive colonies were chosen, and they were cultured overnight within a 50 mL LB medium containing the necessary antibiotics. The *E. coli* cells were harvested via centrifugation. For the purification of pure plasmids, a NucleoSpin Plasmid Kit from MACHEREY-NAGEL was utilized. Additionally, the sequences of all plasmids were confirmed using a DNA sequencing kit sourced from Eurofins Genomics.

Table S1.4 Oligonucleotide sequences

Primer	Sequence (5'- 3')	Purpose
HSHE15-P1	GCCAACTTTGTACAAAAAAGCAGGCTCCGCATGGCGCCTCGAGACGAACA	Cloning for *mfnPKS1* into PEYA
HSHE15-P2	CTCGCATCTCCATCTGCGAGAAATGCGCCGCGCATCGCCATGGTTCCCGG	
HSHE15-P3	CTCTAACCGCCCGGGAACCATGGCGATGCGCGGCGCATTTCTCGCAGATG	
HSHE15-P4	GATGACGAGCAAGCTGTGTCGACCGTGAGACTCGGCCCTTGAAGGTTGAA	
HSHE15-P5	AAGCTACATATTCAACCTTCAAGGGCCGAGTCTCACGGTCGACACAGCTT	
HSHE15-P6	TGCCAACTTTGTACAAGAAAGCTGGGTCGGTCACGAACTCTCAGCTGGAG	
PKS3655seq-F1	TCTTCAGACCACGCTTTCAG	
PKS3655seq-R1	ATTCGAAAAAGTGACCCACG	
3653-1F	TTCTTTCAACACAAGATCCCAAAGTCAAAGATGACAGTTCAGGACCCCAT	Cloning for *mfnL2* into pTYGs-*arg* under Padh
3653-2F	GACGGATTCAAGAGTTACATGAAATTTACGGACCAATTGTCCGTATTAGC	
3653-1R	CTTCATTCGGGCTAATACGGACAATTGGTCCGTAAATTTCATGTAACTCT	
3653-3F	GCCTGCCCACCAGGTTAGAGGCAGCGGCTCGGTAGCCCACGCGATCTTCT	
3653-2R	TCGGTTGTTGAGAAGATCGCGTGGGCTACCGAGCCGCTGCCTCTAACCTG	
3653-4F	TTTCACCAAGCACATGTCGGATGATATGGCGCTGCTGATCAAAACTCTCACC GTTGATATGCCGAATCAAAT	
3653-3R	GGTGAGAGTTTTGATCAGCAGCGCCATATCATCCGACATGTGCTTGGTGAAA ACACTAGCCGCAAGACCGAC	
3653-5F	CAAGGGAAGCCGATCTTGCCTTGGCATGAACTTGGCATTCTGTGAGCTGT	
3653-4R	AGTGAGAGATACAGCTCACAGAATGCCAAGTTCATGCCAAGGCAAGATCG	
3653-5R	CAGGTTGGCTGGTAGACGTCATATAATCATTTATACGATAATCGCCCGCA	
3653Tadh-R	TCGTAGCTGTTTTCATTCTATGCGTTATGAACATGTTCCCTTATACGATAAT CGCCCGCA	
3654-1F	TTCTTTCAACACAAGATCCCAAAGTCAAAGATGTCCAGCCAAAACACCAC	Cloning for *mfnL1* into pTYGs-*arg* under Padh and PgpdA
3654-2F	TGAGAACCTGCGATCCGTTGTACACTACTCACTGGATGAGATGTTGAAGG	
3654-1R	TCTGCGGCCGCCTTCAACATCTCATCCAGTGAGTAGTGTACAACGGATCG	
3654-3F	TTGCGAAGGCAATGGCTGGCTTACGGCAAATGGACTACCACTTGGACTAC	
3654-2R	CTTTGAGCAGGTAGTCCAAGTGGTAGTCCATTTGCCGTAAGCCAGCCATT	
3654-4F	AACGGCCACATCTCCAGGTCTTTGGCCAAGCAATTCCCCGACCTGAACTT	
3654-3R	TTGAACAACAAAGTTCAGGTCGGGGAATTGCTTGGCCAAAGACCTGGAGA	
3654-4R	CAGGTTGGCTGGTAGACGTCATATAATCATTTAGGCTCGCTTCAGGTAAA	
Pgpd3654-F	AGCTTGACTAACAGCTACCCCGCTTGAGCAGACATCACCGATGTCCAGCCAA AACACCAC	
3654Tgpd-R	TCTTGCGAACTACGACAATGTCCATATCATCAATCATGACTTAGGCTCGCTT CAGGTAAA	
3658-1F	TTCTTTCAACACAAGATCCCAAAGTCAAAGATGTCAGTAAACGTACACGAAG TTCCC	Cloning for *mfnR3* into pTYGs-*arg* under Padh and Peno
3658-F2	GGTAAAAGCCTCTAAACTCTTCGACCGGATCCGTTTTCTGTCTACCAACG	
3658-R1	TTATCGTCGACGTTGGTAGACAGAAAACGGATCCGGTCGAAGAGTTTAGA	
3658-F3	ACACTAGGGACAAGGCTAATGTCTCACCAATAAACGTACACGAAGTTCCC	
3658-R2	CCTGTGGTAAGGGAACTTCGTGTACGTTTATTGGTGAGACATTAGCCTTG	
3658-F4	AATTTCCAGAGTAACGTTCTAGTCGATGAGATCTTCGGCGATATAATTGG	
3658-R3	ATGGTGACCGCCAATTATATCGCCGAAGATCTCATCGACTAGAACGTTAC	
3658-R4	CAGGTTGGCTGGTAGACGTCATATAATCATTTATAGTTTCTTCGGTCTTA	
007corect2-F	CGACTGACCAATTCCGCAGCTCGTCAAAGGATGTCAGTAAACGTACACGAAG TTCCC	
padh3652-F	TTCTTTCAACACAAGATCCCAAAGTCAAAGATGACGGTGAAAACACCAAA	Cloning for *mfnL3 into* pTYGs-*ade*
padh3652-R	TTTCATTCTATGCGTTATGAACATGTTCCCTTAGGAAGATACATAGGAGG	
pgpda3659-F1	ACAGCTACCCCGCTTGAGCAGACATCACCGATGGCTTCGAGAATCAACAC	Cloning for *mfnR4* into pTYGs-*ade* under PgpdA
pgpda3659-F2	GCTGAACTGAAGGGTGTTGAAACCCGGCAGTTCGACATCGGCGACATTGC	
pgpda3659-R1	CGGAAGAGCGGCAATGTCGCCGATGTCGAACTGCCGGGTTTCAACACCCT	
pgpda3659-R2	TACGACAATGTCCATATCATCAATCATGACTTAAATGGTCATGCCGATAG	
3657-Peno-P1	CGACTGACCAATTCCGCAGCTCGTCAAAGGATGAAGTTCTCTGCTCAGCA	Cloning for *mfnR2* into pTYGs-*ade* under Peno
3657-Peno-P3	CCCGCCACCGAGAAGGATGTTTCAACTATTGTCAAATACTGCAATGACAA	
3657-Peno-P2	CTCAATGCTGTTGTCATTGCAGTATTTGACAATAGTTGAAACATCCTTCT	
3657-Peno-P5	CTTCCCCTTCCGTGCGGACCGTCATCTCATGCTCTTCGATGTCCAGATCC	
3657-Peno-P4	TTCTCAGTGGGGATCTGGACATCGAAGAGCATGAGATGACGGTCCGCACG	
3657-Peno-P6	CAGGTTGGCTGGTAGACGTCATATAATCATTTACGCGGTGGTAGCTTCCG	

Table S1.4 Oligonucleotide sequences (continue)

HSHE13-P1	GCCAACTTTGTACAAAAAAGCAGGCTCCGCATGGCGCGCATCTCAAGGCC	Cloning and sequencing for *mfnPKS2* in PEYA
HSHE13-P2	CTTTAGGAAATGGCCGCTATGTGTGTGATTTCTCTCTGTGCTCTCCTCTT	
HSHE13-P3	GTAGATGGGCAAGAGGAGAGCACAGAGAGAAATCACACACATAGCGGCCA	
HSHE13-P4	GCATCGGTGCTATTGTATTGCTGGTCATAGCCTCCCAGGAAAACAGCGGT	
HSHE13-P5	GGATTCGAATACCGCTGTTTTCCTGGGAGGCTATGACCAGCAATACAATA	
HSHE13-P6	TCGTGGCCAAGGAATTAACTTGTTCGGCACCTTAACGTGCCATTGGCTTG	
HSHE13-P7	AATATCCCCACAAGCCAATGGCACGTTAAGGTGCCGAACAAGTTAATTCC	
HSHE13-P8	GGCTTGAGGACTCATCCCTTAGTAGTTCGTCATATAGGGACCATGTTGCA	
HSHE13-P9	ATGGGTACGGTGCAACATGGTCCCTATATGACGAACTACTAAGGGATGAG	
HSHE13-P10	CAAAGAAAGTAGAGATGATCGGGGGATGACATTGACTTGATCATCCTCAG	
HSHE13-P11	ATTGTTCCAACTGAGGATGATCAAGTCAATGTCATCCCCCGATCATCTCT	
HSHE13-P12	TGCGGAGGATACGTGTTCGGATGCGGTGGCATTTAGAACCAGATCCGTGT	
HSHE13-P13	GGGGTTATCAACACGGATCTGGTTCTAAATGCCACCGCATCCGAACACGT	
HSHE13-P14	TGCCAACTTTGTACAAGAAAGCTGGGTCGGCTATGCAGCAGCCTCTTTGC	
3651Seq-F3A	TCGTAACACTGGTCTCAACC	
3651Seq-F1	ATGGGCCGAGAGCTCATCG	
3651Seq-F4A	ACGCGATCTTGGAAGGATCA	
3651Seq-R1	GGAGGAACCCTTCGTTTGCG	
3651Seq-F5A	TGGCGCTTTATTCCACTACT	
3651Seq-F6A	CTACGCGGACTTGGAGACAA	
3651Seq-F2	AACAACTCGAAGCGCCTGGA	
PiPKS2-F1	CTGAACAATAAACCCCACAGCAAGCTCCGAATGAGCGGAAGAAATCCTAT	Cloning for *ilaPKS2* into pTYGs-*met* under PamyB
PiPKS2-R1	CGTCTCCAGCAAGAGCCGTTGTTGAGGGTCCATTGCTGCAGCCTCCTGCG	
PiPKS2-F2	AATGTTCTGCCGCAGGAGGCTGCAGCAATGGACCCTCAACAACGGCTCTT	
PiPKS2-R2	TAGTGGAAAGTGTATCAAAC	
PiPKS2-F3	CATAGTTTCTCAGTGACTGC	
PiPKS2-R3	CGAACTCTTGAAGCTCTTTC	
PiPKS2-F4	AGTGGCGTCACATATTTAGT	
PiPKS2-R4	ACTCTCCACCCTTCACGAGCTACTACAGATTCAAGCCTTAGATAGACCAC	
PiPKS2-seq-1	CAACCAGGACGGCCACACGG	
PiPKS2-seq-2	TACGTGGAACCTCGTTGAAG	
PiPKS2-seq-3	ATCGGATCAGGGCTTCGAGC	
PiPKS2-seq-4	CCTATTCAATCTTCCGAGGT	
Pgpd-PiAcT-F	ACAGCTACCCCGCTTGAGCAGACATCACCGATGTCTTTCGCCAAAGCTCA	Cloning of *ilaR2*
PiAcT-Teno-R	CAGGTTGGCTGGTAGACGTCATATAATCATCTATAACACACTAGACGGCC	
Padh-SDR2-F	TTCTTTCAACACAAGATCCCAAAGTCAAAGATGGCTTCATATCTCATCAC	Cloning of *ilaR8*
Padh-SDR2-R	TTTCATTCTATGCGTTATGAACATGTTCCCTTACCAGGGAGCATTGGAGC	
SeqPEYA-F	ACGGCCAGTCTTAAGCTCGGG	Public primers located in all plasmids, for sequencing or cloning.
SeqPEYA-R	CTATAGGGGATATCAGCTGGA	
PamyB_S-F1	CATGCTTGGAGGATAGCAACCG	
PamyB_S-R1	ACTCCAACTGTACATCAAACTCA	
Padh plugF	ATTCACCACTATTATTCCCACCCTATAATA	
Padh plugR	GAGACGAAACAGACTTTTTCATCGCTAAAA	
PgdpA plugF	CTTTTCTTTTCTCTTTCTTTTCCCATCTTC	
PgpdA plugR	TACGACAATGTCCATATCATCAATCATGAC	
Peno plugF	CTTCTTAAATATCGTTGTAACTGTTCCTGA	
Peno plugR	CGAAGTATATTGGGAGACTATAGCTACTAG	

Table S1.5 Protein sequences used in this study

MfnPKS1
MAPRDEHEPVAIVGMGCRWPGGVRNAPELWEFLRDRTDGWREFDDPRFSAKGFHHPNSNRPGTMAMRGAFLADGDARLFDHAFFGMTGLEVE TLDPSQRKLLEVTYEALENAGETWDSVSGSRTGVFVGNFCLDHWMIQSRDWDNPRPYAFTGAGTSILANRISYIFNLQGPSLTVDTACSSSM YALHLAMNSIRAGDCDSAIVASSNWIADPGVTIALDKLGALSASARCHTFDARAEGYARGEGFAAIYLKRPSLAIATGSPIRAMIRGTAINA NGRTGGITRPSAAGQEAVIREAYRNAGNLPFSDTNYFECHGTGTYVGDPIEVAAVGRVFAPERSSEDPMLVGSVKSNVGHSEGASALASIMK VVLSLENGAIPPLFNLQTLNPNIDFDGAKVKPVTELTPWPKGRLWRASINSFGYGGANGHCILDHVNNVLPDYIKPGIYRSLTNGSTNGTNG TNGDSKTHRVIVESPKLKSIENATIRKLVLLPLSAHNEPSLKLNVEALSQAINKFPLADVAYTLSARRSRLPQRHFCIVDKDNVVEGLTIEK KPVRAPLNSSNLGFVFTGQGAQWHAMGAELFEYRVFQTAIDHLDHVLSSLPTPPSWTLRDILSGNCDADLIQTAEVSQTACTAVQVGLVDLL ASWSVRPSGVAGHSSGEMAAAYASGRITAAEAIVAAYFRGQAVSKNEQTGAMLAVGLGPEQVSKYLEGLEDQVKLAAINSQGSVTLSGEVPA IDKLSEAMNADSVFNRKLKTGGNAYHSHHMLPLGRNYIEILTNGLEHIQKQGLLDKSQRYALVPWASSVTPDKTIGELENHAAYWRSNLESP VRFSEAITNLVNLEETTINAIVEIGPHPALKSPIDQIVKAAGKTVAYASTLKRQEDARVSLLTLAGTLFGVNSEIDLVAVNAVDGAHGSLEH GCTSIDLPPYQYTYGALNYHESRASKEYRYRKIPRHDLLGSKVVGNAKLRPQWRNILRMKDVPWLGDHRLIPDAVLPAAGYLAMAVEAAGRI YNEFPEPAKITGFSLRDVSIKTSLPIPEDDYGVEVLTSMELVDTATAKSPAWATFSISSVDRESNEWSEHCTGLVKVEISESEDSEKIAFAE DSSRATDSKAWYKKFAAIGLGYGATFQPLSEIRADADKNLATAKVALNTTADLIKGGESPYPLHPASLDGAIQLGLIASHGGRIEEAHTAFV PVQVGQLYLKNGIEGDSCTAVVRGERRGIRAAWLDLQMLGPNGEVLLNVDNLRCISYSSESKSSDHAFSSPFTRLVWKPDIRSLSNRQCRAM FPPPKENVEKSPLWGVMNKLAFMVVYAVYDKFGRTDDGPKPTADVGHFFEWTKRRSQLDMSPEMEEARSLTTAEREAKINELVSQAPDVMEV KIAKLLHDNMADILYQRRTGVDVIIAEGLLTPLYQTGLLMTGVYPQLYNVLDSLSHANPNLRILEVGGGTGGATRIAMKAFRGPNGIKRYRE YTFTDISAGFLGGARESLAEFKDMNFSVFDAEVDPIEQGYEPVYDLVIACQVLHATSSMKNTLTNVRKLLKPGGQLLLVETNKNFMVPGVVV GTFTGYWAGIPDGRVDAPFQSLEAWDKALQNVGFSGLDIVLDDFPEPHNTTSVILSTYKGEPTTKKTASVNLLYSAETAPALLDQLAKELEG RGVSTKVGPLNEAPTSVSQSSRVVVFLDDKHLLQDATEQDITTFQHLARSTSSLVVITSCGTAKGRNPDGALIPGLLRVLSTENPAGQYVSI DIDADNFAVSADDAEDLVRSIVDKEFELHQPPSTDDEEGNPKDREFVWQDGSLCVSRLVPDGGFHSQHGIDSQSVKTEQLPLDSQGAVRAAF ETPGVLSSLYFKAYQELWQPLPADYIDVKVAAVGLNSKDIEHWSGRSDANNLSSEYTGTITAVGSAVTDLKVGDRVYGLGKGQFGNFTRVPA SLAQKLQPDDDLVQMATLPLVYVTAIYAFEHAAHLRKGQNVLVQAATSDLGLAALRFAQAKGANVFALVDTPEKARFLSDELSVPATHVILS SDPSNLRRAAGKTRKGGFDVIINTAQSENLHASLQALAPLGHFIDVGQVDAQSAKAIGSELFQKNANFSSIDPFVLLDSDPELGEELIQAVD KYYRQGLIQPIRPVTATDVSQLSQVLMDFSKGNLIGKLVATFSPESVVRMLPPSPTARFDSEAAYVVTGGLGGLGLSLVRWMADHGARHLAV LSRRSVDAVPEAKKLVETLASRGVQVEPVVCDASSKDQVTSVIQKIASARPIKGVVHAAVSYLDLSFDKLEVDRWRQSLSAKVQGTKNLHEA TLNMPLDFFVMTTSLLSVYALATQGAYTAGNNFQDLFARYRRNLGLPASTASFSLISDAGSPYMDPITVDTFERNKTLTLSEHQFLTLLEPA FLNNTTSVETKPYQWFGQQDDPLSVANLLTCLDPAGMLAKKRDEIEAGVTSTAALPRWYTDGRVSLIMRAFSDAQRHAFDGSQDAAEGSKST VARLRREFDAAIQAGAGERANTITFVQNAITNTVAEMLFVDAEAVDPAKSVADHGVDSLIAAELRNWFHQALGTNISMLDLLDPSMKISALS EKITDDSLNPPAESS
MfnL2
MTVQDPILVVPIIPSNLSSFQPVWYLVVAGSVFLAYQLILALWNISPFHPLSHIPGPKLAAATYIPEFYYDAVLFGRYTRRIQELHEIYGPI VRISPNEVHCNDTRFIDEIYAFGNRKRDKPAHQVRGSGSVAHAIFSTTDHDIHRMRRGALAKFFARSQVSKLEPKIQTLVHRLCDKILRAGE KAPFDITSAYSCFSTDVITDYCFGDSFGFLTQESWEPNFRGPLYALLKPIFLFRFFPFLRYVGLAASVFTKHMSDDMALLIKTLTVDMPNQI VKTKNDLDAGITGKQQTVFGSLLESDLPIEEKSVERLTDEATALLSAATETISWTMTVISYHLLTKPELLKKLTDEVNQAVDSSGQLPQWST LEKLPYMGAVIFEGLRLAYGVGSRTARIAPEEDLVYHGEWTPKGSKKPVTVDYVIPRGFPIGMSSYVTHHDERIYPDSHSFIPERWLDEKMQ RRKDLERSMISFSKGSRSCLGMNLAFCELYLSLAAMTVRVYPRMKLYETTEEDVAYDHDMFNPIPKASSKGVRAIIV
MfnR3
MSVNVHEVPLPQAIGLVSPRVFGIFSIVAVCLYGLYRWLLPKPIPGIPYNQKATTMLFGDAPDMVREVSVTGELRVWCAKQVKKLNSPICQV FIVPFSKPWILIADFREARDILTRRKEFDKSSFLINGMAPMGDFHGIYKTGEAFKANRQLIQDLMTSTFLNNLVGPAAHAKGLELIKLFETK MKLAKGRPFSVKSDFEYASLDVMLSFAFSNNWVKTAIGPQLELLSQMNPSEIPDASPDEPLTLPKAPVDDFLMAIYEAPEVVEKLINAPAPK VTLWWWKKQAWYKKIFDVKDRVLREQVAIAIENYRGGRVESGIEHMLMREEARAEKQGRLPNFQSNVLVDEIFGDIIGGHHTTSGAMMWLVK YLTDHPAVQTKLRAKLHEALPTALEENRLPTFEELRWAKIPYMEAIIEEMLRLNAVTVTREALCDTQILGHHIPKGTQVFLVSNGPGFLSPS MPIDDSLRSETSRAAKIRATWDETQDLTVFDPERWLVYKTDENGVETVEFDGAAGPQLVFGLGPRACWGRRLAHMEMRTIISMLVWHFELLP TPQALSSYSGLEGIARVPQMCYIRPKKL
MfnL1
MSSQNTTTVQGPSILALAQNILELTQDMTKYLQVNGIAAPTFALDAGDPPNTPEYRKIHASLKTNLEDLSRLIDGPRKWLREFCCSGYDLGA LQIALDFEFFTLIPADGGLTLKELAEKAGLDLDRTSRVVRQLMTYKFFHEHTPGFITHSSTSLVMREDENLRSVVHYSLDEMLKAAADSNIS LKANPFEADQNHNPFVTRHGVGIFEFYAKDPAKARRFAKAMAGLRQMDYHLDYLLKDGFDWAGLKGTVVDCGGGNGHISRSLAKQFPDLNFV VQDSNADMLAEGKEQLTDDIRDRVSYLQHSFFDPQPCKDVSAFLIRQCTHNWADKDVVRIFKGFVPGLEGSSPETPLLINDIIIPEPGVWPA HQERVVRQVDMVMLVNCGAKQRTKAEFDALLKEADPRYEIRKVHDNGPLGLLEVYLKRA
MfnR2
MKFSAQQAVLGFSFLQTLVAGSAIPRAPGTPQYFRPHGFTRRDLSVTQVQQELGPQLSNGSLLFGPSDPRWYAAIERYSTHAIPDVEIVVQP ATEKDVSTIVKYCNDNSIEFLAVNRGHSRSYSVAAFKGMQIDMAGLLDITIQPDGKSAWFQGGTYDGQVMEYLWERGYVATTGSCSCVGMMG PGLGGGHGRQEGFYGMISDNLRNLNVVLADGTAVRVNSTSHADLLWGMKGAGHNFGIVTSFELNIYPREVDSWHYHTYTWKGDKLDTVINAL NKLHGNGTTPVNMAVNFGSFLLNTSVSTTEASLWWTFGYKGTAEEANKVLKPFNDIGAEYEEFGDVPYPQISDMQGTGIGGPLCAKNASHTT STVNLLTYNLTAERQIYNRFSDWIKEYPELGPTAQIVHEGYSTEAVDKFPADDSAFPFRADRHLMLFDVQIPTENPRGINFTTVAREWAQEV QTMWNEGQPTRIPGAYVNYANGLEGPKMWYGHEQWRQDRLLALKKKYDPQNRFRFYNPIVSEATTA
MfnR4
MASRINTILIIGATTGIGEGLARRFHALGKKVIITGRRQDRLDALAAELKGVETRQFDIGDIAALPGHVSAILKDYPKLDTVYVNAGIQQCY NIFDNSSITNEKVASEVAINLTAPNLLANLFAPHLLNLAKSGTKTTIFITTSSLAYIPFSFYPTYCATKAGLQAFCKIFRQQLAFAGEGAQN MNVVEIVPPYVDTGLDAAHRDYTIAAQGGKDKAFPPTPLKEFLDAVFAGIEDVGPDGSIKKEIAVGFGELGVGTWRGAFEKVYESIGMTI
MfnL3
MTVKTPNSKMGSMTETTCIPLTPLDHYPPGHYAFFGFFLPLNDGVTFQDAYKVLQKGLLLAFSQLPWLGGKVFYQSPDTPGWRPGQLEMRYE PVDLTVPGPYQLKYRELETDVGYEGLKERGFPLDTWADSSVMWSSGVTDDAKGAEVFVAQANFIPGGCFLTAGLHHCVGDGTSTFDVLKIWA DNCHAVQSESWEPQPIPPESSDRNIMERIWEKENTGHSFSEMAPDAFRLLNLQPPGEESKVEMKSGKINVQDEAMQAGIFYISAANFNKLRQ DCTRDAGDSISISGVDALCALVWRTLIKARRAAAVQRGQETDNFTSTMFLTSDGRPNFSNSMPSPYFGNVVLMQHNQLPLPKLTGSEASVGS VSRTIRTVANRVTAETVLDAYAIARSMDDYSKLTLRLSTLHAFDMLMSVMVMVQEDLVCFRGGIFANGGMPDTIRPLMDDLNRFSRICYLMP RKKSGGVELVVNLFADEMEFLFKDPEFGGYASYVSS

Table S1.5 Protein sequences used in this study (continue)

MfnPKS2

MARISRPTPRPKNGVPFPSKDYTHTNNNGVSYSRSRPVAIVGMACRFAGDATSPSNLWDLCANGQVGRSPIPEAVDSQVDGQEESTERNHTH
SGHFLKDNTSSFDVAFSNLPVDKTGVTDPQARLLLESVYQATEDAGIPIENLADSNTAVFLGGYDQQYNSTDAVLPSYPTGKSRTSGASLVS
NFFNLQGASMSIDTGSSSDLAALHQGCQTLRLGEADVSIVGACTLLNQEFDDSSEGSDRGEGVAVLVIKSLDAALKDKDRIHAIIRNTGLNQ
SGKNTGTSPSAEAQIKLIEDCYRRAGLDMADTAYVEASMAGSEVANAAEIEALDRTFGKSRGSEEPIFVGSVKQNIGNTERVSGLAAIIKAA
LAMQNGLVAPSLNPNIPTSQWHVKVPNKLIPWPRDRKLRASINKFGRDGSNAHVIIDGAPNAVARRLSGNSLREKAAQSPDKSRVFVLSARD
STTAEVMAKNLSAHLRRLLESGQAPGSSSLAYTLATRRSRFPWTVTMRASNVPELATGLGEPVKAVHSTKEPRIGFVFNGQGAQWYAMGREL
IAEYPVFRRAIEDADKVLNGYGATWSLYDELLRDESSSRVSQVILAQSVTVALQICLVRLLESWGIVPHAVSSHSSGEVAAAYAAGLLSFKE
ALGVVYFRDGLLAKLESQSASRPGGMLAAGLGPDQVEPYLANTEGGRAVIACVNSPESVTLAGDLAAINEVLARLEKDGIFARKLKVPLAYH
SHHMLDMAQEYAGALTAILPRRPSWPAKALYASPVTGDIIESPDILTPEYWVQNLTDPVLFSQALEAMCFDTEVSAAQASNVDMLVEIGPHS
TLAGPIRQILKTRMMPYTSCLKRSENAVHTMQALAGELLNRGYPVSLKEVNFPLGDNDGPQTFVPNLPTYPWSHASTTESKATKTIRQRRFA
RHELLGTHLASSSGLVHEWRNSLRLSDIAWLSDHKVDSNVVLPGAGYVAMAVEAVRLLADPAEKSTRGYQLRDVEILNALVIPDSPSSVETH
LRLTPCSEKELDYEGWYDFNISSMNADGDWVSNCKGMVSAAVSEAAAIAAPKAADFNEEAFFPRGTKARRISVSSLQSDLRKMGIEYGPAFQ
NLIGSQAAANKSASSMFIRNPMPKIKNQLKYVLHPTTLDSIIQATYSGLPDDAKRDTTLSLKSFRNLYISRDLGRISGAKLKAFANRTKTEK
KGLTSSVTVLNNDANEGFLQIDGLFCQSIPHIPEEISEESEQTLCYKTHWEPDVRYRVPASVKESMRVILGRDDAEFEKKMVRASYYLIHDA
VAELEGQNPESFASYQKELHKWMKTVVAQAKRGTLAPLSSTWENATSGIKQLVYDQLNTSGVAGRTVVRVGSQLAGIVRGEVSPQELLKNGN
LLSQYFAELPRLRDRTYKQLSKVAEFYAVTSPGANVLEIGAGTGGVVSQVILQAFGARGNGSGTLLGSYTYTDLQDDALHGAAQRLAPWGDM
VQFQKLDIGQDLAKQSFKGGEYDLIVVPLALYSTTSVKNALHTIRSLLKLDGKLILIEPTSNKLDMQLLFGTSPEWWVNDEPDKLSPILSLQ
GWDDTLRETGFTGVDFDIGDCEQPEFQGTSIILTGLQLQSLYLEPVSIVHTAVPDKQWLKHLSSAIRGQTGFAPVVESIENAQPEDRICIFT
AEMLGPYLDSMGEKGFETLRRLFRSSRGVLWLSSGGVIDAAAPSFSKIQGLLRTLRVENPNKRYAHLDFEYGKNPWSNDNIIHIIHVLKHVF
DFGANPAGIDWEYSVKGSVLYVPRIYADLETSAVVSSDRVDPPPEPQPFFQLERSLVWKPLATNDPHNFCFVDNEELTSDIPAGMVEIEPKA
FGLNTRDIPVDGIEETASAHDLSGIVVRLGPDTKQSGLKVGDRVYGLAKGRLANVSRAPWTSIAKVPAEMSFETAAALPTAHITAYYSLLHV
ARLQAGESILIHNAASDVGQAATTLAQYIGAKIFVTCGTEAQKGLLVEKYGIDPTRVLSSKSANFARDIMAQTGGVGVDVALNSLSGSLLKA
TWECIASFGRLVDIGRTNSNNSKRLDMTPFGRSATYTSVDILQLCEFRRSLVQEALQETLRICFTANSGRTIHPIRSYPISELEAAIGHVKE
ETHFGKSIIVPTEDDQVNVIPRSSLLSLNSQYETFMVAGSSGEVNHAITSWLIEKKARNIVVVSHDAESNLSAAYLQQEAAGSGCNIHIRNC
DIADEKSLVKLLKELAGSLPPIRGVINTDLVLNATASEHVSSAGTWNLHKHLPDLSFFIMLSSIAGVTGHPSQATYAADQAFRDALARHRIA
RGLPAVSLDLPAITSAIETAQANEMTTSLDMDKVLRLVEAAVTHSLKHGPDDAQVIVGLQPWDQLSDATIARADPRFGTLQLAVPRATSSST
ATTPEGSVMGVTPTDLLQQALKLSSEDSIKLATEAVAARLAELLNVDAEGIHRDASIMSHGVDSLSAVEIRNWLGTVAKAKVSIAEILRDTP
LPEFSALVLSRSAEGKEAAA

IlaPKS2

MSGRNPIPLAIVGIGCRLPGDATSPEKLWDMLANGKSAWSKVPADRWNEEAFLHPDPDDTNGTHNHAGGHFLKQDIGAFDASFFNVLPQEAA
AMDPQQRLLLETTYEALESAGIRQEDIQKSNTAVYMAMFTRDYDRNVYKDMMSIPKYHVTGTGDAILANRISHLFDLNGPSATMDTGCSGGM
TAISHACQALRSGQSDMALAGAANLILSPDHMISMSNLHMLNADGRSYSFDSRGAGYGRGEGIATLVIKRLDDAIRDNDPIRAVLLDAAVNQ
DGHTAGITLPSGAAQKSLERRVWENLNIHTQDVGYVEAHGTGTLAGDSAELEGISQVFCQNRDPSSPLLVGSIKSNIGHLESVSGLAAMIKS
ILILEHGAIPPNVNFEYPRASLDLEKKKIKVPQALEPWSQPGVARISINSFGYGGANAHAVLERPSRLTATEQEEAVPDEIPRLFILTAASQ
SSLLSMLGTTEEWVSKNFNQKSLRDLSYTLSQRRSIMPWRFSCAASNQAELLEALNKGAKKTDSITRISPDVRISFVFTGQGAQWAGMGCEL
LSDPTYRDSIHQSTKILHGLGATWNLVEELLRGKEQSRLKEAELAQPATTAIQIALVDLVRRWGIIPDAVVGHSSGEIAAAYAAGYLSQHEA
LKISYFRGFSSAISKNKGLGKGGMLAVGVGEHDVAQYIGILKQGVAVVACQNSPSSTTISGDDAAISELSEILTQKAIFNRKLNVDTAYHSH
HMQAAADEYKAGLGYIVQDALPKTTIKMFSSVTGSLKSSGFDGDYWTSNLTNKVRFCDALQSLCREEQTTSRSVQPHRIFIEIGPHSALAGP
ARQCIADLEEPMPYSYTSALVRGTGALQSALTMVGSVFNHGYQVNLAEISASDPTSVNASVLYKLPSYPWDHSKRHWHESRLSRDYRLRKHP
YHDLLGLRMTDNTPLRPAWRHMIGVEGLPWLRDHVVDGLMIFPGAGYLCMAMEAAVQLAGDRHQAKKIRQIQLEDVSFLKGLVIPEGRTRVE
VQLSFYPVEAIDNGKTMQHSFSVTAYTGDEHWNEHCRGLVSVEFASENDQNSFETPITYGEISDQFDTLSTKSIQPDDLYQELERVGNAYGP
TFTGIEEFTLESDRAISLVMIPDVVSVMPARHIRPHIIHPSTLDIILHGSLPLVNQKLGAGSVMPVRIGDLTISSEVENAPGKMLSAVTTLT
STHFRAAEADLVVFPGKAVSTSTPVISVSGMELRSLASNDIEDAGIRGGREICYEMKWGPDERFLSEKQLEPLQAVVSDDPLAHCYALMSQY
LEHKAFKQSKISVIEIGGVSGGATLSFLQALQSYGARPSVYDFGFKDTLGDVESELQDWSDVVTFKPLDIETDTSDQGFEQNSYDVVLVCNT
LPVTKINVALSSMRNLLKPDGVLLLIETTTATQNLLSRSEWSNTLSEASLKLQLAAQVDDSMPKSTFIVARAVDNANIDPLPEIEFIIEPTL
SHTMKNFVTEISMSNALHSKEVQITTTSWESKQTHKDVIHVVIDDGSNPILAGVGPEKFQSVVDLLQRPSKVIWISAQDEEKDMFSPRKHLI
TGLARTAHAENEDLAMVTIDVQQTLDQKTKPAILNFLMEVLQSFNKANIQRREREYVYNGTDVLIPRVIPSGKLNRQVSGNDETITETKMFTA
SRVPLKLDNQKKDSFTHPVFVEDEIHRQDLGKDCVEIEGKAFGIPSQSPQHSNIINEYSGVVTATGSDVSSFKVGDSVVAFSSVPYAQRLRV
PATQVQLIPRGISFIMAAALPISFMNACHALIDIANIQPGETVLIDGAATDIGQAAISVAKHLGAEVIAAVSRVDEVNFLKESFKIPSSHII
PRESYLGRHRIQKLVGPGGLSVVLGCAKSSVSNEIIEFLQPFGTLVQIGGSGKPVKLTKAVSNVTVSTFDLEFLVRAKPQKASQLLQKVMEM
ASQGLTLPSQNITALPLDNIDEALKQARHEDVNKYVLEVQEHSTVRAARPSYTLPKLDSGVTYLVAGGMGDLGRRLLRLMAKAGARYLVTLS
RRGATPAEREKVEKELQEFGPGCSLYCIKCDVSKETSTTAALSEITAKGFPQVKGVIQATVALRDSTLDTMTAEDFNSVLQAKAQGTLNLKK
TFASEDLEFFISFSSAVNIIGTAGQANYNAGNSLQDALAQFDRSPNCFYMSLNIGTIEDATVNNEAIIQSLRRQGLTPVLHNELLALFEYAL
SAEARETGCHQAVIGFTPETIAGTTAINGSAHTPMFTHVRQADEGGVENDATNKAKTFKDIIGETSSKDEISAFVAQVIGKKLAELIAIDPV
DVNLGSSITDFGLDSLIAIELRNWMMREFDAPIQSSEVLDNQNIWTLAQKVTMRSRLANGDGTDSSSSSEGNVASTLPTSRSPSQERQKRAF
EQPPLPIPDLGETLRFFADSRKGICSPEELAETERVIEEFQSSGLELQDALRVNPSGPDSRLEFYENNIHIERREPLQDHALFYIGHLTDGA
PTHSQAERAAIVTVATLDFKKRYESGKLEQNSLNDIPLCMETMKWMFNSVQEPRKELDKAQKYASNNNVIVLRRGHIFEIAVREEDNYTSLT
ALFTDIIASSEHIIPPVSVLTSKRRDHWAELRSKLRAVKANAVLLEAIESAAFVISLDDSSPVTSSERCTSILLNDLHLTNRWLDKILQLTV
AANGVSSILAENSKLDGLSTRQLNEYITDEIFHNPCVTPLNEKPIPASTVRELQFEIPSTISKAIAEQTKNNLSHYNTIGASRHYYSELSRS
FLGSKGLRSKGTVLISILLANRLFYGYFEPIWETVTVSKYAKGRIDWLQNLTPDIVHWIEKALEFMEDGKGDVAELASKLKDAAIGHAQTLR
RVADGRGYVEPLYSLMGTSLAEGKPLPPLFSSSAWRHSDRNLTPKRAKTDCLGSGGYMRMQEGGFLMPNPNSVFVHYEVHHPDPLILVQGRE
DDVARFEDCLNESIRTVRAIIERGLSKA

IlaR8

MASYLITGASRGLGLELTRQLSTRSDVGKIFATARGDAPKLQQLASTSPDKIVVVKLDVTDEASIKQAAAEVESKLA
GKGLDVLINNAGVLQYAPKGVSSMENLQESFNINVLGVHWTTRVFIPLLQKGTQKKIVNISTTIGSLALSRFVHLLPAPAYKITKAALNS
LTVQYALDYEKEGFTIFALSPGWLRTDLGGEQADLPVEQGAEAALVRILGSTPEQNGQFLKIEIKGWDKDKRNQYDGSNAPW

IlaR2

MSFAKAHNYPWRQVTPGTYIQDYDSWQSVQAIWNNVDREGRRLHMLASCIEIQSNITDLESRLRSAWLAARYFHPGL
AIELGEYYKAYRVPTAEELEAWVNDTFIMPACANAEEFQKQHLSTSPDSHLHWFPKTKQLLFTAHHTLFDATALWLFWGAYLDLVISPKI
VTFGDEWKNLPLARDDLLGLPKYPSLAGNVKGLSMITNALKPDAIELPTLNTTTNSDGQVVPNGRSRNEFLRLALSAEQSTAIIQACKRR
GTSITAAFFTAISLTCQKIQREYGSAGRYAIGFHNFDSRPWFPRELASTVNAGNDPHAMIPFTVDLDGKSFEDIAKITDENFKSIRADFG
NDPAGLDAVSHMLKGLLNLDGPIATFPGFTSFGVADRVIKTAYHDEVGGWIKIEDSYHWIQNMVKGMNAVCVYNWKGRMYLGGCFNEAYH
TKEMFHRLFRDSFDLILHTFNLGPMGPSSVL

Table S1.6 Plasmids constructed in this study

Construct ID	Plasmids	Features
PL01	pTYGS_*arg-mfnPKS1*	P*amyB* promotes *mfnPKS1*
PL02	pTYGS_*arg-mfnPKS1-mfnL2*	P*amyB* promotes *mfnPKS1*, P*adh* promotes *mfnL2*
PL03	pTYGS_*arg-mfnPKS1-mfnR3*	P*amyB* promotes *mfnPKS1*, P*adh* promotes *mfnR3*
PL04	pTYGS_*arg-mfnPKS1-mfnL1*	P*amyB* promotes *mfnPKS1*, P*adh* promotes *mfnL1*
PL05	pTYGS_*arg-mfnPKS1-mfnL2-mfnL1*	P*amyB* promotes *mfnPKS1*, P*adh* promotes *mfnL2,* P*gpdA* promotes *mfnL1*
PL06	pTYGS_*arg-mfnPKS1-mfnL1-mfnR3*	P*amyB* promotes *mfnPKS1*, P*gpdA* promotes *mfnL1,* P*eno* promotes *mfnR3*
PL07	pTYGS_*arg-mfnPKS1-mfnL2-mfnL1-mfnR3*	P*amyB* promotes *mfnPKS1*, P*adh* promotes *mfnL2,* P*gpdA* promotes *mfnL1,* P*eno* promotes *mfnR3*
PL08	pTYGS_*ade-mfnR2*	P*eno* promotes *mfnR2*
PL09	pTYGS_*ade-mfnR4*	P*gpdA* promotes *mfnR4*
PL10	pTYGS_*ade-mfnL3*	P*adh* promotes *mfnL3*
PL11	pTYGS_*ade-mfnPKS2-mfnL3*	P*amyB* promotes *mfnPKS2,* P*adh* promotes *mfnL3*
PL12	pTYGS_*met-ilaPKS2*	P*amyB* promotes *ilaPKS2*
PL13	pTYGS_*met-ilaPKS2-ilaR2*	P*amyB* promotes *ilaPKS2,* P*gpdA* promotes *ilaR2*
PL14	pTYGS_*met-ilaPKS2-ilaR8-ilaR2*	P*amyB* promotes *ilaPKS2,* P*adh* promotes *ilaR8,* P*gpdA* promotes *ilaR2*

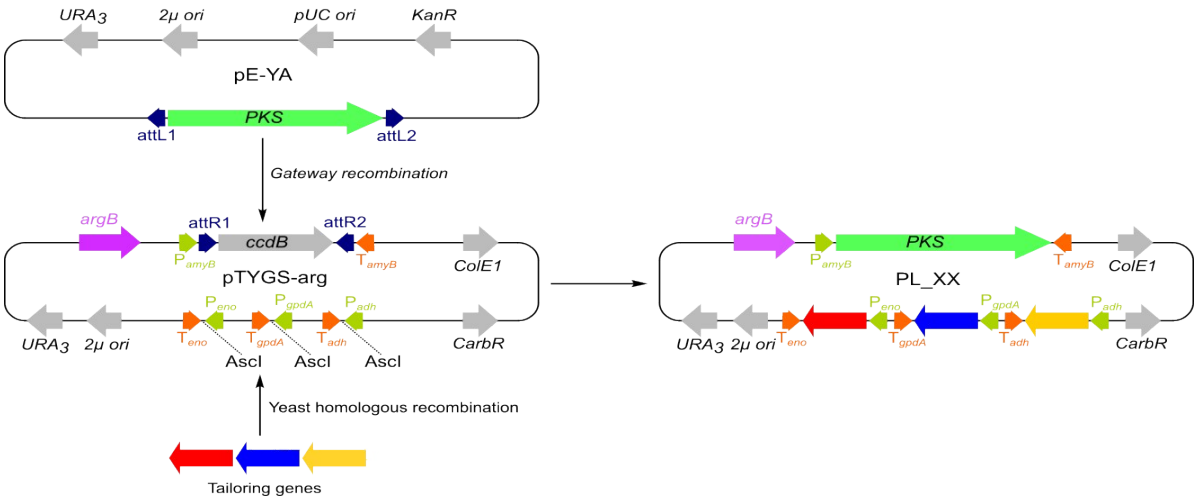


Figure S1.13 The workflow of construction of plasmids

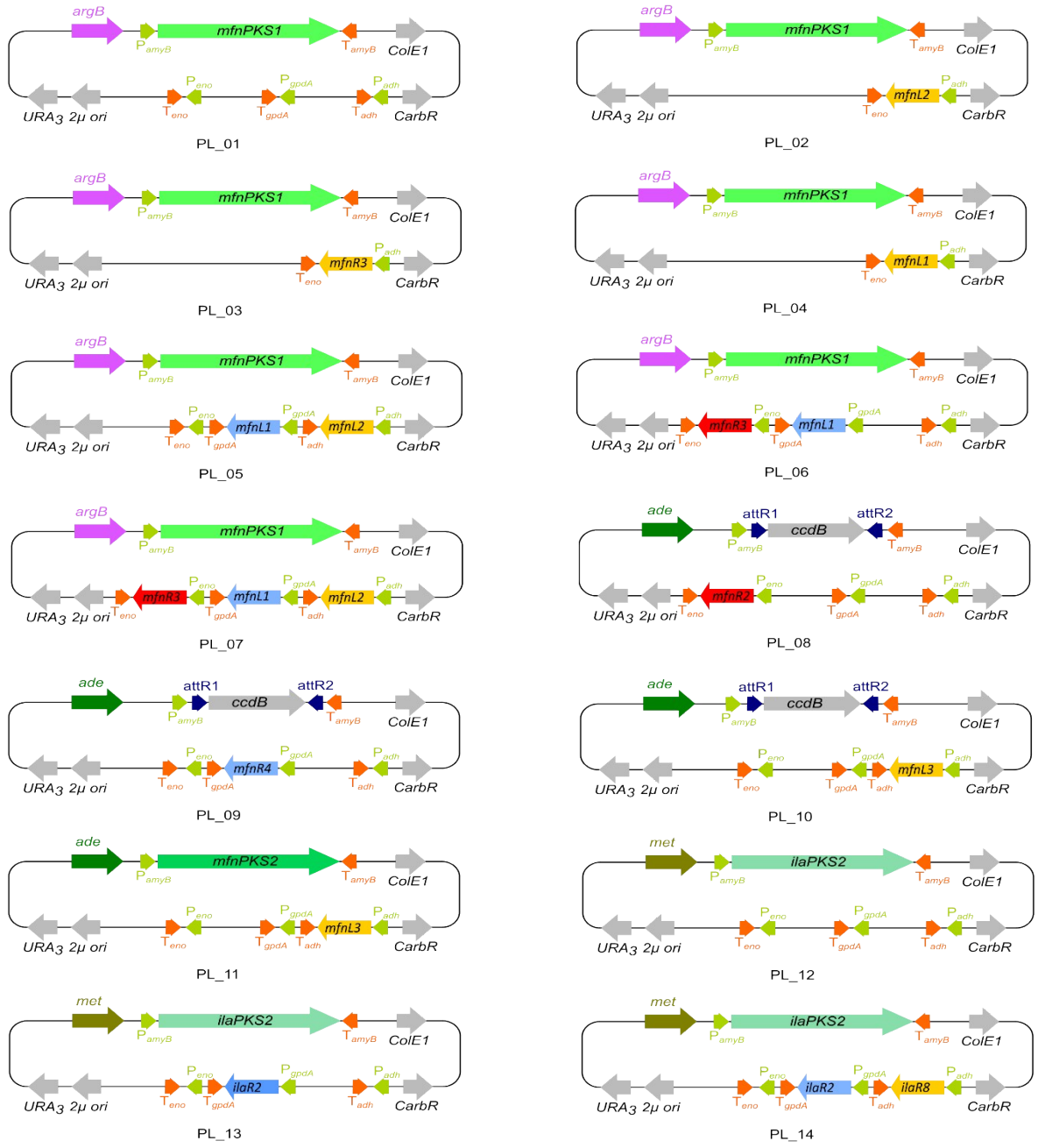


Figure S1.14 The built plasmids for heterologous expression experiments in *A. oryzae*

Table S1.7 Media and buffer

Media / buffer	Ingredient
YPAD Agar or medium	1.00 % (w/v) Yeast extract; 2.00 % (w/v) Tryptone; 2.00 % (w/v) D (+)-Glucose Monohydrate; 0.03 % (w/v) Adenine; 1.50 % (w/v) Agar;
SM-URA Agar	0.17 % (w/v) Yeast nitrogen base; 0.50 % (w/v) Ammonium sulfate; 2.00 % (w/v) D(+)-Glucose Monohydrate; 0.077 % (w/v) Complete supplement mixture minus Uracil; 1.50 % (w/v) Agar
LB Agar or medium	0.50 % (w/v) Yeast extract; 1.00 % (w/v) Tryptone; 0.50 % (w/v) Sodium chloride; 1.50 % (w/v) Agar
SOC medium	0.50 % (w/v) Yeast extract; 2.00 % (w/v) Tryptone; 0.06 % (w/v) Sodium chloride; 0.02 % (w/v) Potassium chloride; 25 mM final concentration Magnesium chloride hexahydrate 2M; 1.0 % final concentration D(+)-Glucose Monohydrate 20 %
DPY agar or medium	2.00 % (w/v) Dextrin from potato starch; 1.00 % (w/v) Polypeptone; 0.50 % (w/v) Yeast extract; 0.50 % (w/v) Monopotassium phosphate; 0.05 % (w/v) Magnesium sulfate hexahydrate
PDB	2.40 % (w/v) Potato dextrose broth
GN medium	2.00 % (w/v) D (+)-Glucose Monohydrate; 1.00 % (w/v) Nutrient broth;
CZD/S Agar	3.50 % (w/v) Czapek Dox broth; 18.22 % (w/v) D-Sorbitol; 0.10 % (w/v) Ammonium sulfate; 0.05 % (w/v) Adenine; 0.15 % (w/v) L-Methionine; 1.50 % (w/v) Agar; or 0.80 % (w/v) Agar for soft agar
CZD/S1 Agar	CZD/S Agar without Adenine
CZD/S1 Agar/ w/o Methionine	CZD/S Agar without Adenine and Methionine
FCC solution	5% (v/v) glycerol; 10% (v/v) DMSO; ddH_2O
PEG solution	50% (w/v) polyethylene glycol 3350; ddH_2O
ssDNA	2 mg/mL salmon sperm DNA; TE buffer
Solution 1	0.8 M Sodium chloride; 10mM Calcium chloride; 50 mM Tris-HCl; pH 7.5.
Solution 2	60% (w/v) PEG3350; 0.8 M Sodium chloride; 10 mM Calcium chloride; 50 mM Tris-HCl; pH 7.5

1.5 Transformation and Selection of *A. oryzae*

A. oryzae NSAR1 was cultivated on a DPY plate for 5-7 days. Conidia were then introduced into 50 mL of GN medium within a 250 mL flask. This flask was subjected to overnight incubation at 28 °C with shaking at 110 rpm. Following this, the grown mycelia were gathered through a sterile Mira-cloth filter. Subsequently, these mycelia were placed within a 25 mL solution of 0.8 M NaCl containing 15 mg/mL of lysing enzyme. This combination was housed within a 50 mL Falcon tube, which in turn was positioned on a Stuart SB3 rotator. The entire assembly was maintained at room temperature and incubated for 4 hours. To release the protoplasts from the hyphal strands, gentle pipetting was employed using a wide-bore pipette. Subsequently, the resulting supernatant was passed through another sterile Mira-cloth filter and collected within a new 50 mL Falcon tube. This collected solution was then subjected to centrifugation at 3000 x g for 5 minutes to gather the protoplasts.

The resulting supernatant was discarded, and the pellet comprising the protoplasts was suspended in 1 mL of solution 1. This resuspension was subsequently partitioned into 10 separate tubes, each housed within a 15 mL Falcon tube. Plasmids were then introduced into the protoplast solution following a protocol: for a single plasmid, 1 µg of the plasmid was utilized per tube; in the case of two plasmids, 3 µg of each plasmid was introduced into a single tube; for three plasmids, 6 µg of each plasmid was added to a single tube. To serve as a negative control, 10 µL of water was introduced into one of the Falcon tubes. Correspondingly, empty plasmids were utilized as positive controls, adapted to the selection media requirements.

The mixture of the protoplast solution and the plasmids underwent incubation on ice for 2 minutes. Following this, 1 mL of solution 2 was added to each tube. Subsequently, the tubes were gently inverted several times to ensure thorough mixing of the protoplasts, solutions, and plasmids. The tubes were then subjected to incubation at 28 °C for 30 minutes. Next, pre-warmed 12 mL of CZD/S soft agar was introduced to each tube and mixed meticulously. The resulting mixture was then overlaid onto

two prepared CZD/S agar plates. These plates were subsequently incubated at 28 °C for 4-5 days, allowing time for colonies to develop. Upon emergence of colonies, these were transferred to another CZD/S selection plate for a day. This process was repeated by streaking single colonies onto new CZD/S plates. Subsequent to this, the colonies were cultivated for 5-7 days on DPY agar. The spores and mycelia were harvested and introduced into DPY medium for fermentation. Meanwhile, the spores were also transferred to create glycerol stocks for future use.

Table S1.8 Combinations of plasmids for each experimental group

Gene	*mfnPKS2*	*mfnL3*	*mfnL2*	*mfnL1*	*mfnPKS1*	*mfnR2*	*mfnR3*	*mfnR4*	*ilaPKS2*	*ilaR2*	*ilaR8*	Plasmids
Exp	hrPKS	O-AcT	P450	O-MeT	hrPKS	FMO	P450	SDR	hrPKS	O-AcT	SDR	
1					✓							PL01
2			✓		✓							PL02
3					✓		✓					PL03
4				✓	✓							PL04
5			✓	✓	✓							PL05
6				✓	✓		✓					PL06
7			✓	✓	✓		✓					PL07
8			✓	✓	✓	✓	✓					Exp 7+ PL08
9			✓	✓	✓		✓	✓				Exp 7+ PL09
10		✓	✓	✓	✓		✓					Exp 7+PL10
11	✓	✓	✓	✓	✓		✓					Exp 7+ PL11
12			✓	✓	✓		✓		✓			Exp 7+ pTYGS-ade + PL12
13	✓	✓	✓	✓	✓		✓		✓	✓		Exp 11+ PL13
14			✓	✓	✓		✓		✓	✓	✓	Exp 11+ pTYGS-ade + PL14

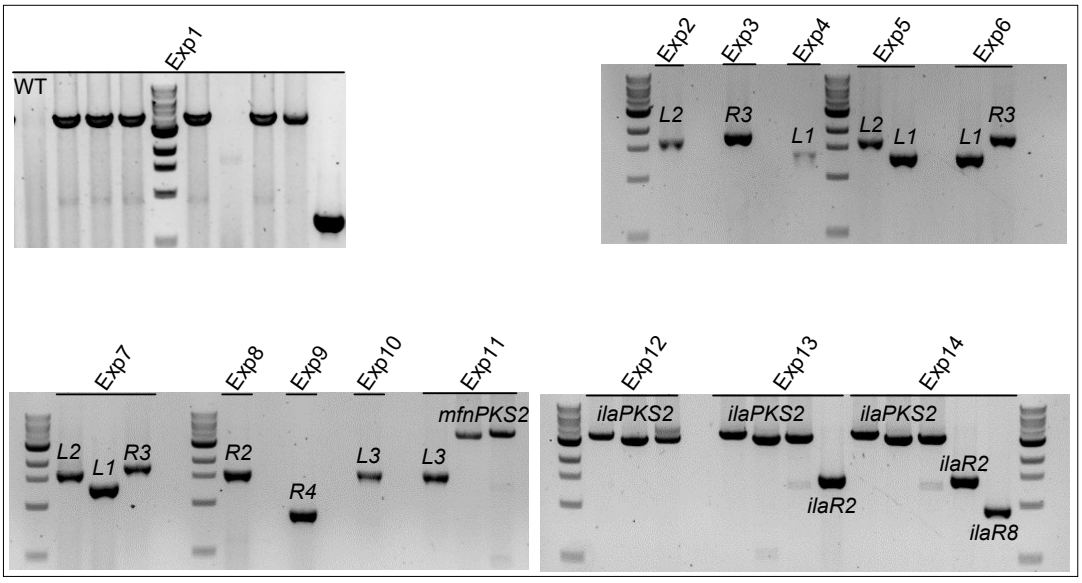


Figure S1.15 PCR amplification using the gDNA as templtes for each experiment. In exp 1, WT represents untransformed strain. The *mfnPKS1* was amplified using primer pair PamyB_S-F1/ PKS3655seq-R1 for 8 transformants. The *mfnPKS2* was amplified using primer pairs PamyB_S-F1/ 3651Seq-R1, 3651Seq-F4A/ PamyB_S-R1. The *ilaPKS2* was amplified using primer pairs PamyB_S-F1/PiPKS2-R2, PiPKS2-F3/ PiPKS2-R3, PiPKS2-F4/ PamyB_S-R1.

1.6 Fermentation and Analysis of Compounds

Transformants were obtained from DPY agar plates through scraping. Subsequently, a 1 mL spore suspension was introduced into a 500 mL baffled flask containing 100 mL of DPY-medium. This mixture was then incubated at 28 °C with shaking at 110 rpm for 5-7 days. The entire culture was blended using a hand blender. After homogenization, a separation process was employed using filtration. Following this, a dual extraction was carried out using ethyl acetate. Once the organic layers were successfully partitioned, they underwent a drying process utilizing $MgSO_4$. Subsequently, the solvent was removed under reduced pressure. The crude extract, upon dissolution in methanol at 10 mg/mL, underwent filtration through glass wool before undergoing testing via LCMS. The purification procedure necessitated a concentration of 50 mg/mL for the crude extract, achieved after cultivating transformants on a larger scale (1 liter) in preparation for the subsequent LCMS analysis.

Analytical LCMS data was generated using a Waters LCMS system comprising a Waters 2767 autosampler, a Waters 2545 pump, and a Phenomenex Kinetex column (2.6 um, C18, 100 Å, 4.6 x 100 mm) equipped with a Phenomenex Security Guard precolumn (Luna, C5, 300 Å). The solvent flow rate was maintained at 1.0 $mL \cdot min^{-1}$. For detection, two instruments were employed: a Waters ZQ mass detector, capable of functioning in both ES^+ and ES^- modes, encompassing a mass range of 100 to 1000 m/z, and a 996 Diode Array detector offering a wavelength range spanning 210 to 600 nm. In this study, the HPLC system employed two solvents: Acetonitrile (B) containing 0.045 % formic acid, and water (A) supplemented with an additional 0.05 % formic acid, effectively ensuring optimal separation and detection conditions.

The purification of all compounds was executed employing a Waters mass-directed autopurification system, comprising a Waters 2767 autosampler, Binary Gradient Module 2545 with 515 HPLC pumps, and System Fluid Organiser. For this process, a Phenomenex Kinetex Axia column (5 µm, C18, 100 Å, 21.2 × 250 mm), coupled with a Security Guard pre-column (Luna C5 300 Å), was utilized. The elution of compounds transpired at a flow rate of 20 $mL \cdot min^{-1}$, maintaining ambient temperature conditions. Fraction collection was facilitated by the Waters Sample Manager 2767 instrument, which triggered fractions either through mass-directed or time-dependent triggers. The fractions derived from the mixture were initially subjected to vacuum evaporation to eliminate organic solvents. Following this, the resultant aqueous phases underwent drying, employing Freeze Dryers and/or a rotary evaporator. The desiccated samples were weighed, dissolved, and subsequently subjected to HPLC analysis, prior to their submission for nuclear magnetic resonance (NMR) analysis.

1.6.1 Presence of 6 in experiment 1

LCMS data from experiment 1 was examined for the presence of **6** *via* the extracted ion chromatogram. Compound **6** was clearly observed in both ES^+ and ES^- data.

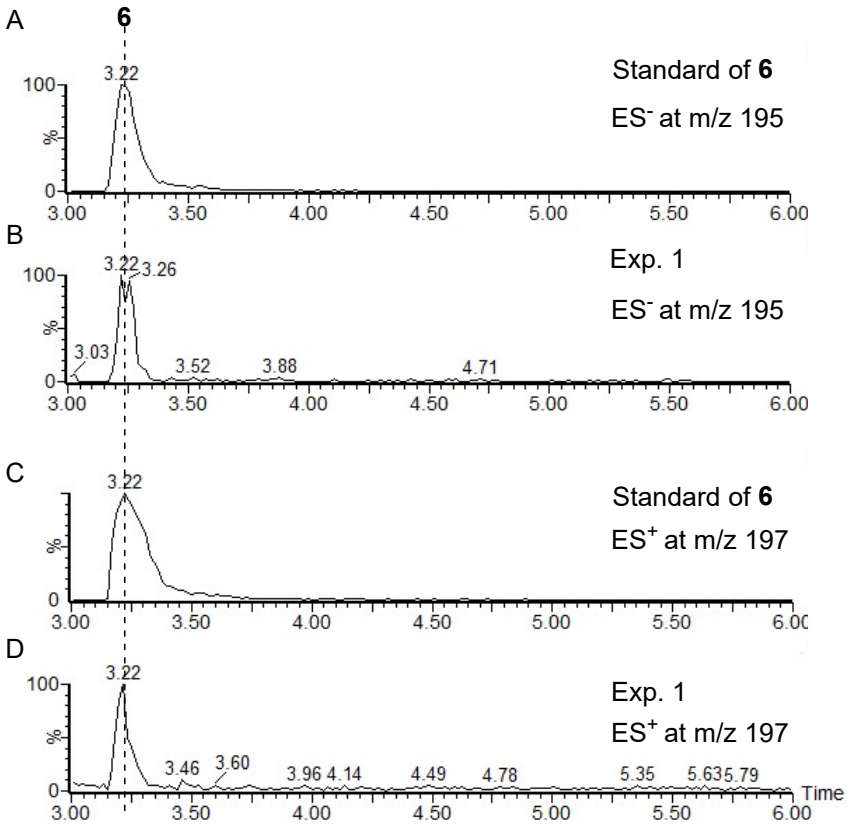
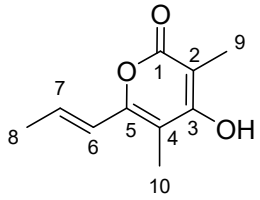


Figure S1.16. **A**, the mass of standard **6** scanned by ES^- at *m/z* 195; **B**, the mass of **6** from exp.1 scanned by ES^- at *m/z* 195; **C**, the mass of standard **6** scanned by ES^+ at *m/z* 197; **D**, the mass of **6** from exp.1 scanned by ES^+ at *m/z* 197

2. Compound Characterization

Compound 5



5

Chemical Formula: $C_{10}H_{12}O_3$
Exact Mass: 180.0786

Compound 5						
Pos.	**δ_C / ppm**	**δ_H / ppm (J/Hz)**	**^{1}H-^{1}H COSY**	**HMBC (H-C)**	**δ_C / ppm literature [11]**	**δ_H / ppm (J/Hz) literature [11]**
1	167.6				163.6	
2	99.9				99.0	
3	167.8				164.3	
4	108.6				106.3	
5	153.5				151.6	
6	121.4	6.41, 1H, dddd (15.3, 1.7, 1.7, 1.7)	7, 8	5, 8	120.6	6.42, dq (15.4, 1.3)
7	134.2	6.6, 1H, dddd (15.4, 6.9, 6.9, 6.9)	6, 8	5, 8	132.0	6.50, dq (15.4, 6.0)
8	18.6	1.92, 3H, m	6, 7	6, 7	17.6	1.90, d (6.0)
9	9.0	1.92, 3H, m		1, 2, 3	8.6	1.94, s
10	9.4	2.0, 3H, s		3, 4, 5	8.4	2.01, s

Table S2.1 Summarized NMR signals for ^{13}C, ^{1}H, ^{1}H-^{1}H COSY, HMBC for **5** recorded in CD_3OD. Literature [11] data was measured in acetone-d_6

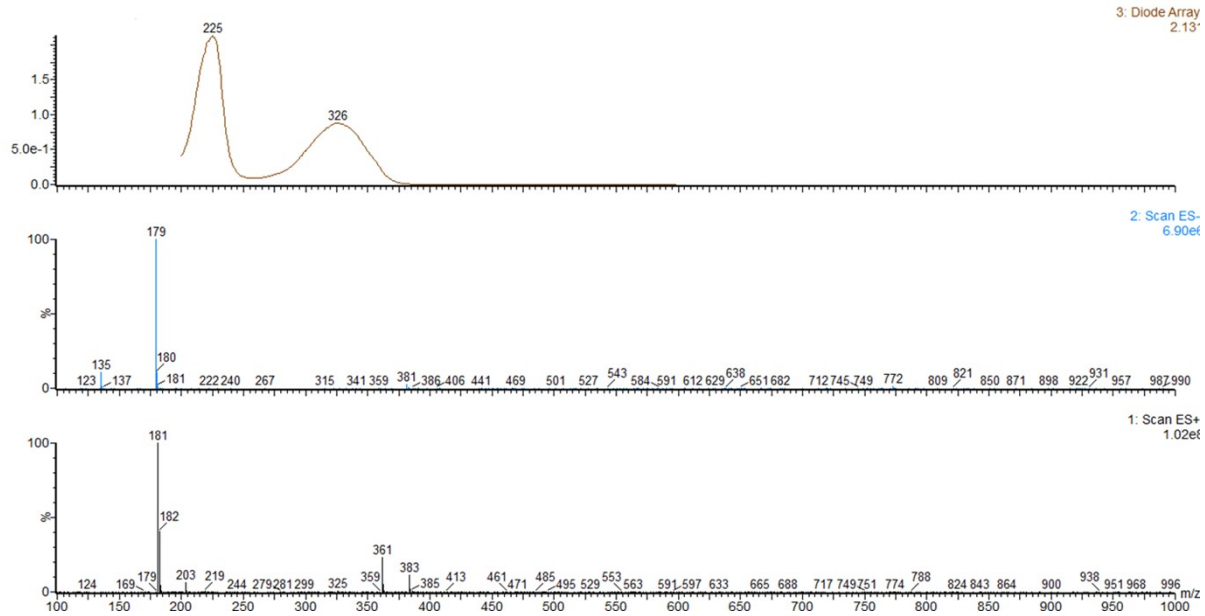


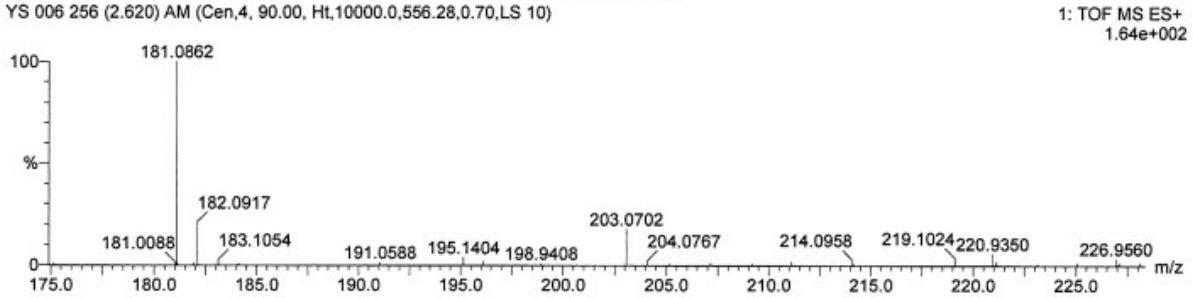
Figure S2.1 UV-absorption (top) and fragmentation pattern of **5** in ES^- (middle) and ES^+ TIC (bottom) by LR-LCMS

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 20.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
38 formula(e) evaluated with 2 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-30 H: 0-50 O: 0-8 Na: 0-1

Sun QTof Premier HAB321
YS 006 256 (2.620) AM (Cen,4, 90.00, Ht,10000.0,556.28,0.70,LS 10)



Minimum:				-1.5			
Maximum:		5.0	20.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
181.0862	181.0865	-0.3	-1.7	4.5	12.0	0.2	C10 H13 O3
	181.0841	2.1	11.6	1.5	13.6	1.8	C8 H14 O3 Na

Figure 2.2 HRMS data for **5**; *m/z* $(M+H)^+$ calc. mass is 181.0865, 181.0862 was found.

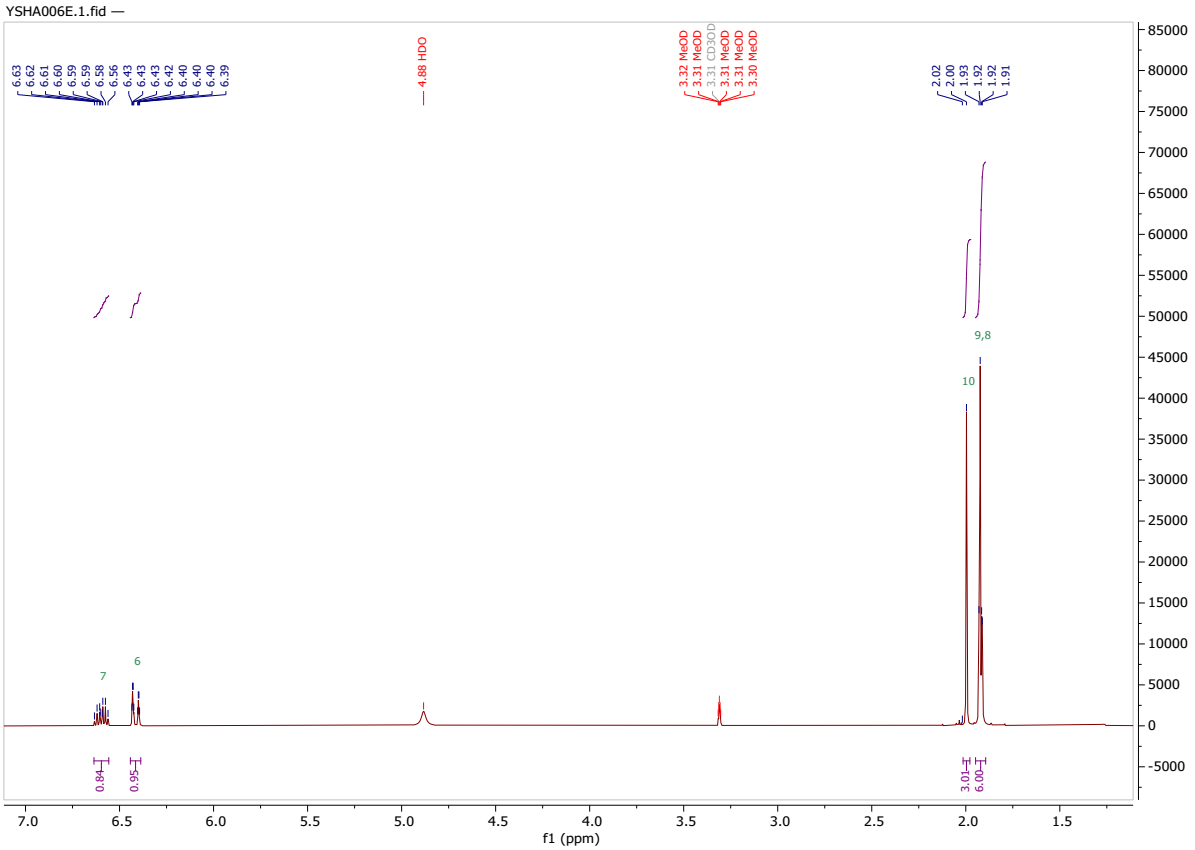


Figure S2.3 ^{1}H-NMR of **5** recorded at 500 MHz in CD_3OD

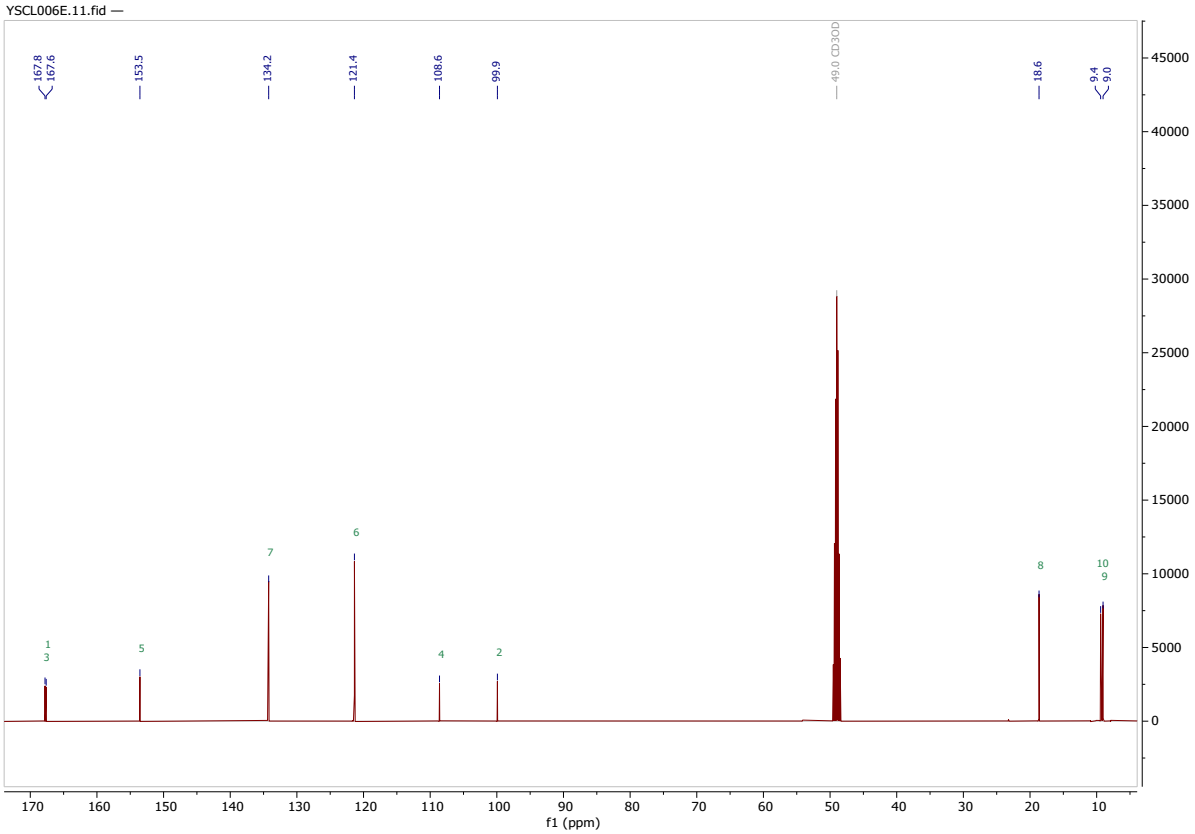


Figure S2.4 ^{13}C-NMR of **5** recorded at 125 MHz in CD_3OD

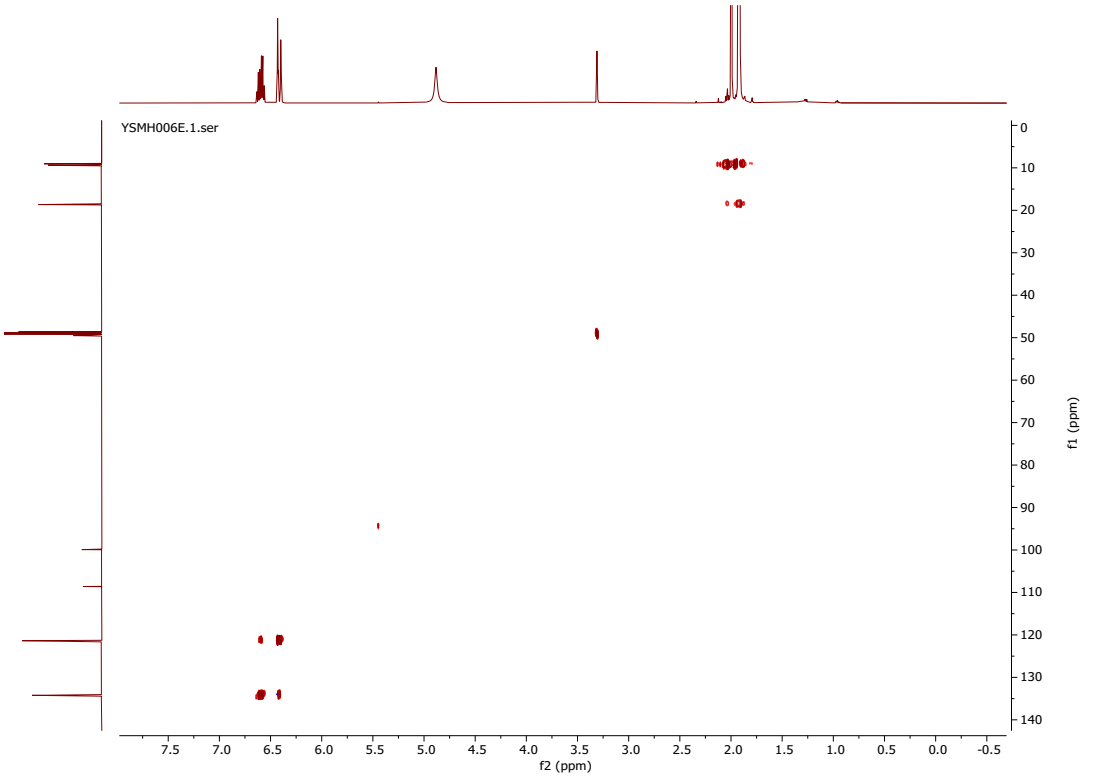


Figure S2.5 HSQC-spectrum of **5** recorded at 500, 125 MHz in CD_3OD

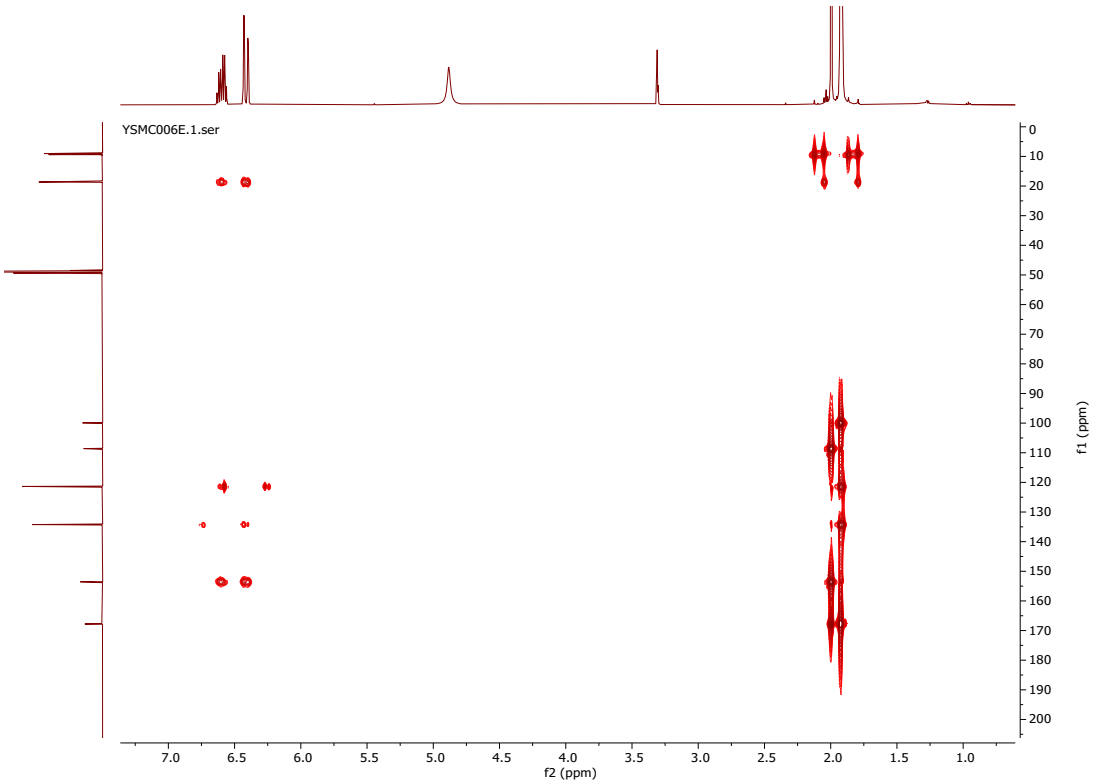


Figure S2.6 HMBC-spectrum of **5** recorded at 500, 125 MHz in CD_3OD

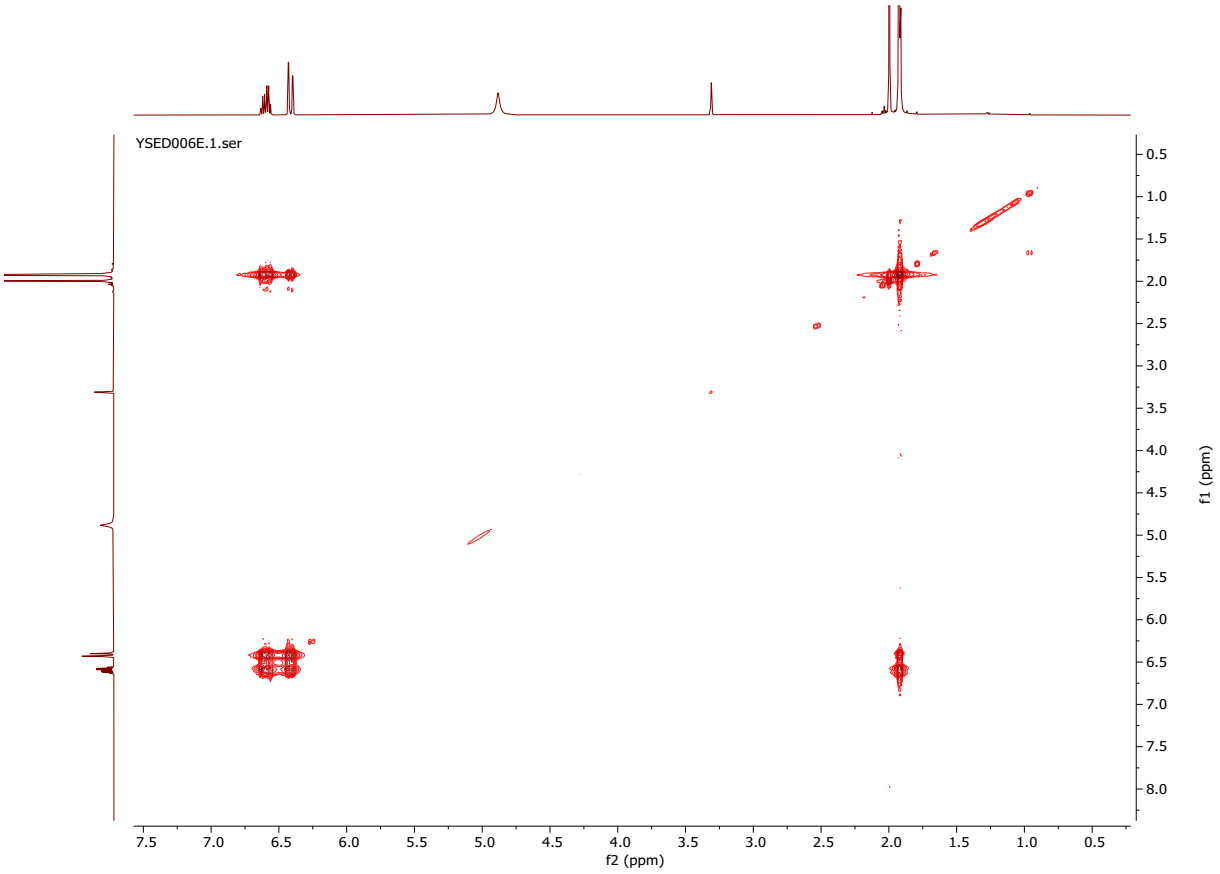
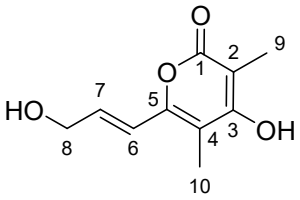


Figure S2.7 ^{1}H, ^{1}H-COSY-spectrum of **5** recorded at 500 MHz in CD_3OD

Compound 6



6

Chemical Formula: $C_{10}H_{12}O_4$
Exact Mass: 196.0736

Compound 6				
Pos.	**δ_C / ppm**	**δ_H / ppm (*J*/Hz)**	**^{1}H-^{1}H COSY**	**HMBC (H-C)**
1	168.1			
2	100.3			
3	167.5			
4	110.1			
5	153.1			
6	118.8	6.65, 1H, m	7, 8	5, 7, 8
7	137.4	6.65, 1H, m	6, 8	5, 6, 8
8	62.7	4.29, 2H, d (2.9)	6, 7	5, 6, 7
9	9.1	1.94, 3H, s		1, 2, 3
10	9.5	2.03, 3H, s		3, 4, 5

Table S2.2 Summarized NMR signals for ^{13}C, ^{1}H, ^{1}H-^{1}H COSY, HMBC for **6** recorded in CD_3OD.

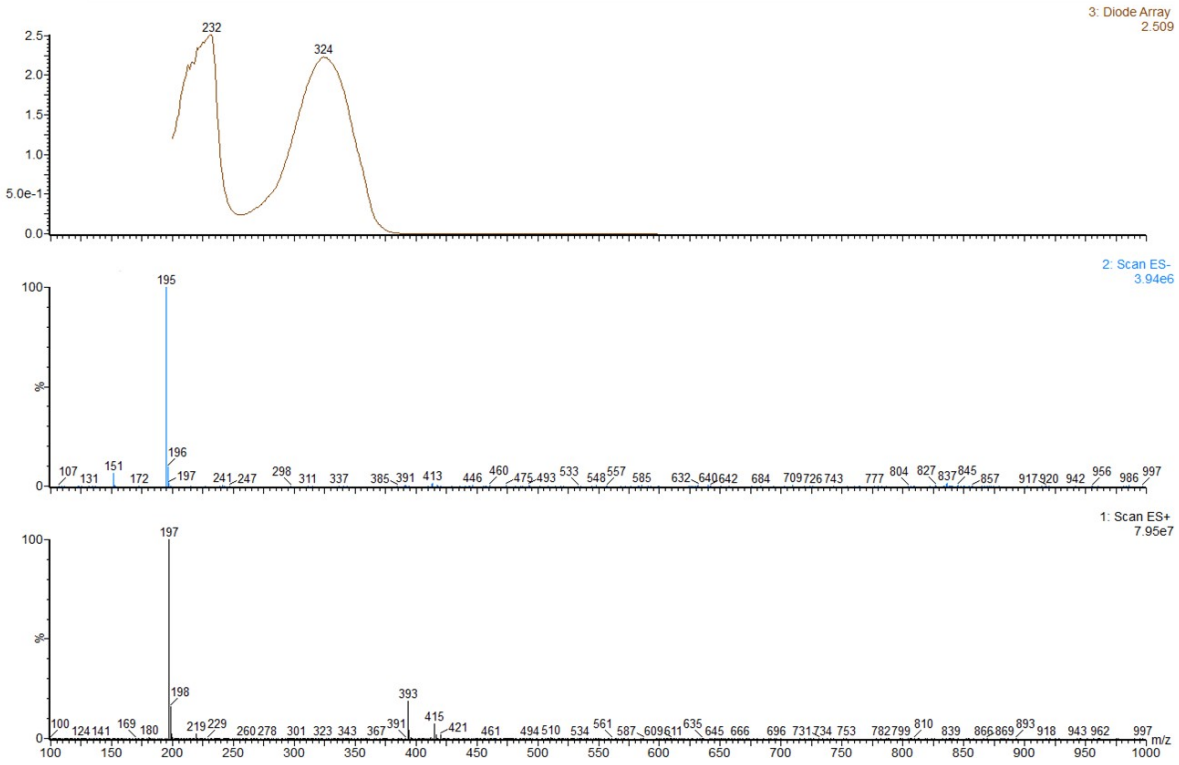


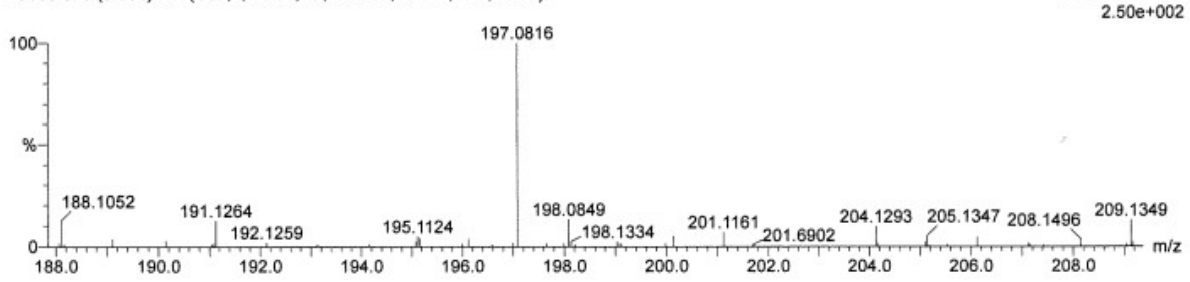
Figure S2.8 UV-absorption (top) and fragmentation pattern of **6** in ES^- (middle) and ES^+ TIC (bottom) by LR-LCMS

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 20.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
199 formula(e) evaluated with 3 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-100 H: 0-160 N: 0-10 O: 0-10

Sun QTof Premier HAB321
YS005 376 (3.836) AM (Cen,4, 70.00, Ht,10000.0,556.28,0.70,LS 10)



Minimum:				-1.5			
Maximum:		5.0	20.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
197.0816	197.0814	0.2	1.0	4.5	24.9	0.8	C10 H13 O4
	197.0827	-1.1	-5.6	9.5	24.9	0.9	C11 H9 N4
	197.0787	2.9	14.7	5.5	26.0	2.0	C6 H9 N6 O2

Figure S2.9 HRMS data for **6**; *m/z* $(M+H)^+$ calc. mass is 197.0814, 197.0816 was found.

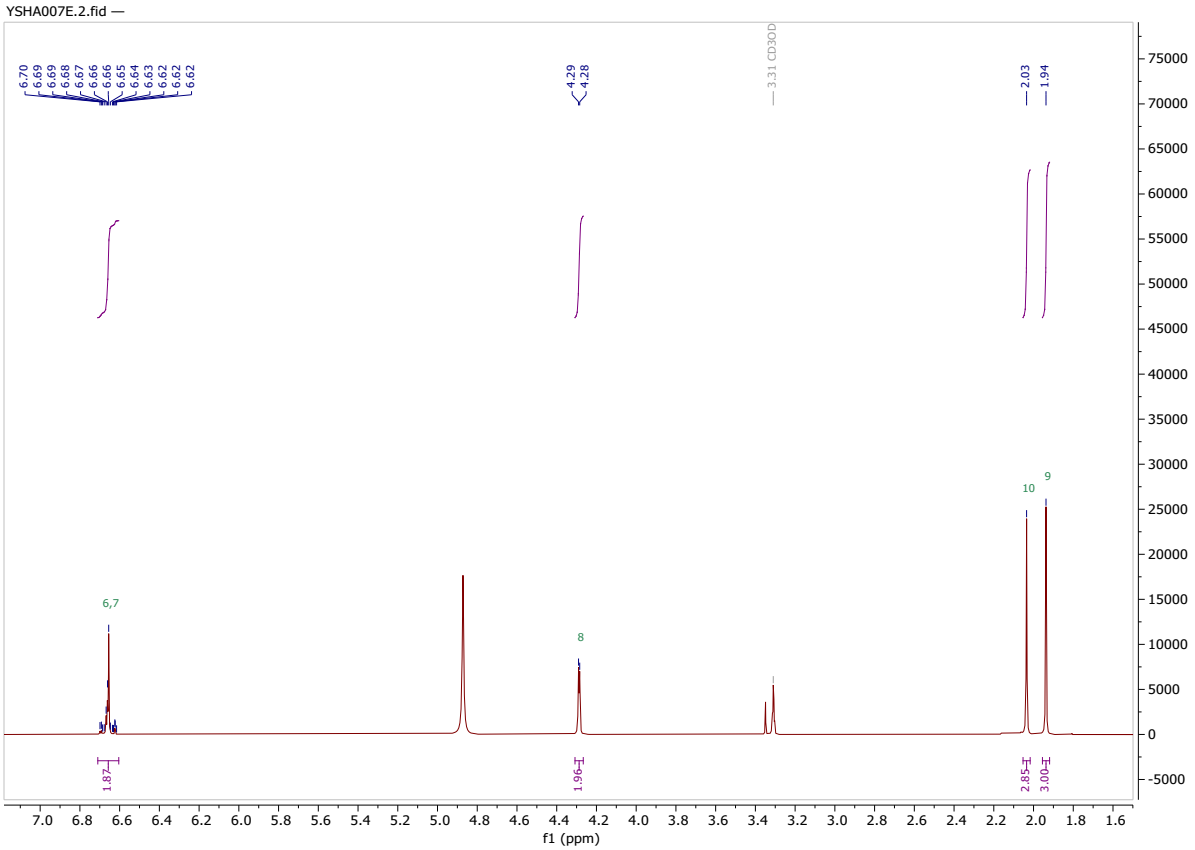


Figure S2.10 ^{1}H-NMR of **6** recorded at 500 MHz in CD_3OD

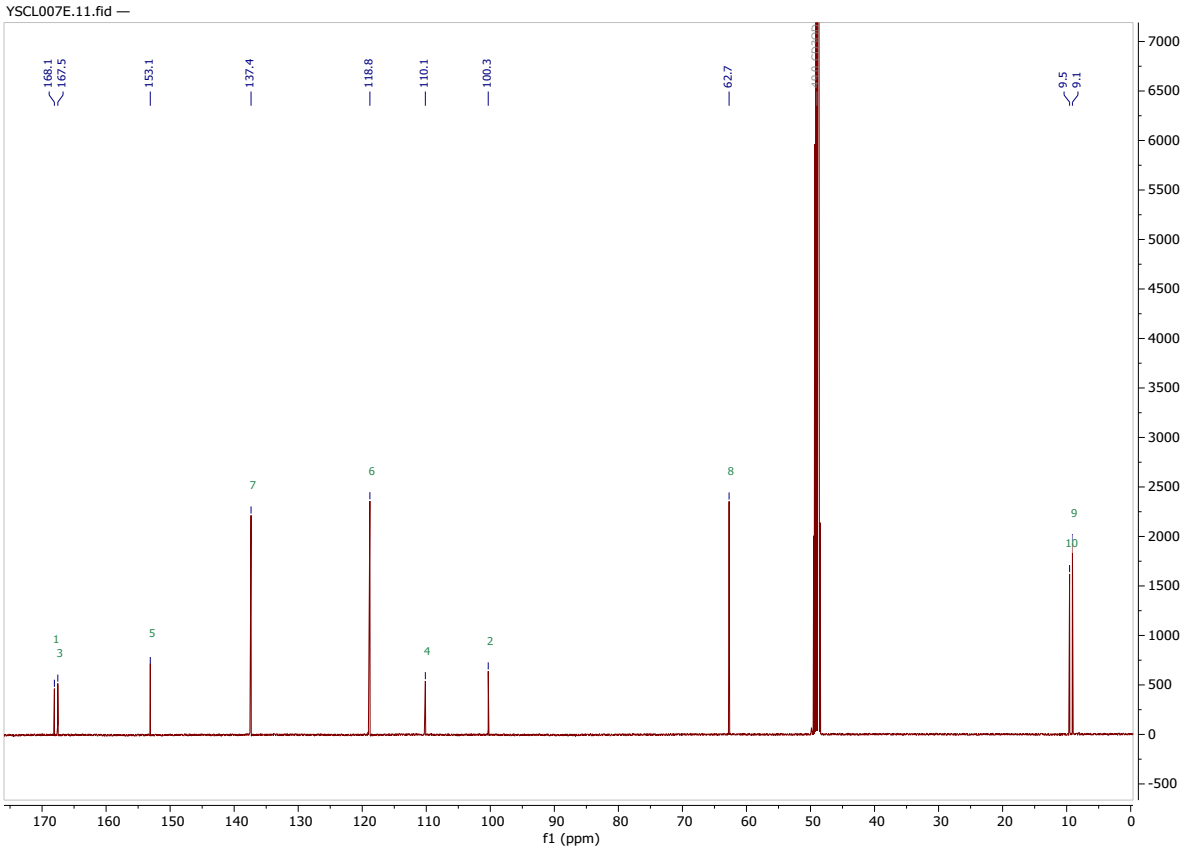


Figure S2.11 ^{13}C-NMR of **6** recorded at 125 MHz in CD_3OD

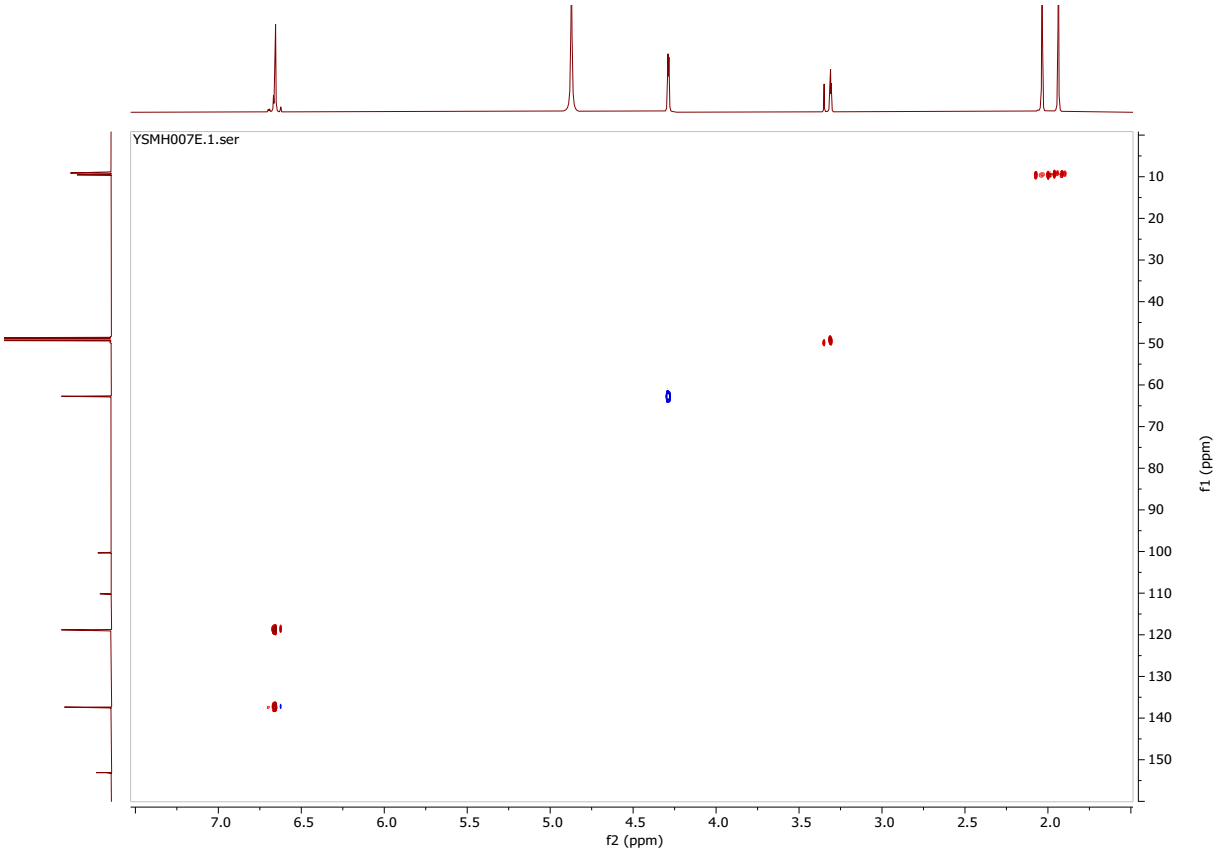


Figure S2.12 HSQC-spectrum of **6** recorded at 500, 125 MHz in CD_3OD

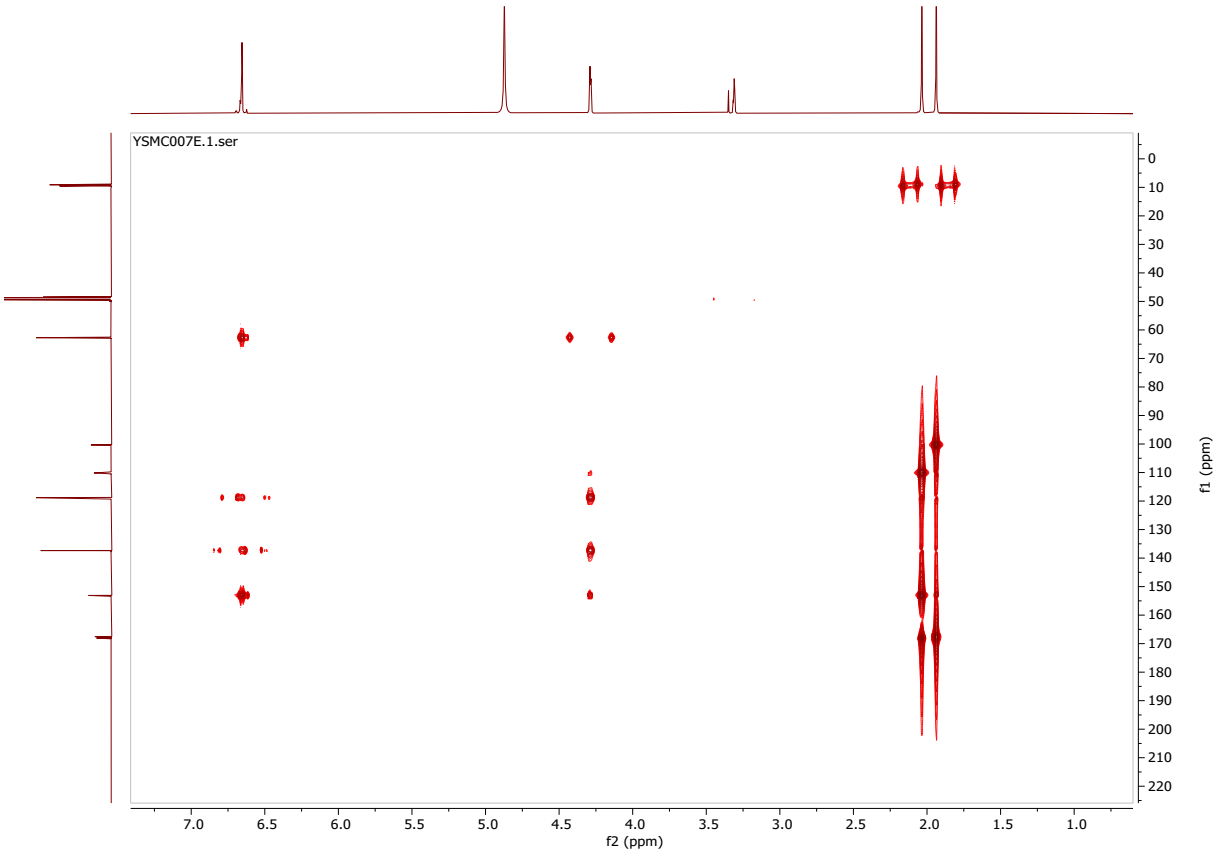


Figure S2.13 HMBC-spectrum of **6** recorded at 500, 125 MHz in CD_3OD

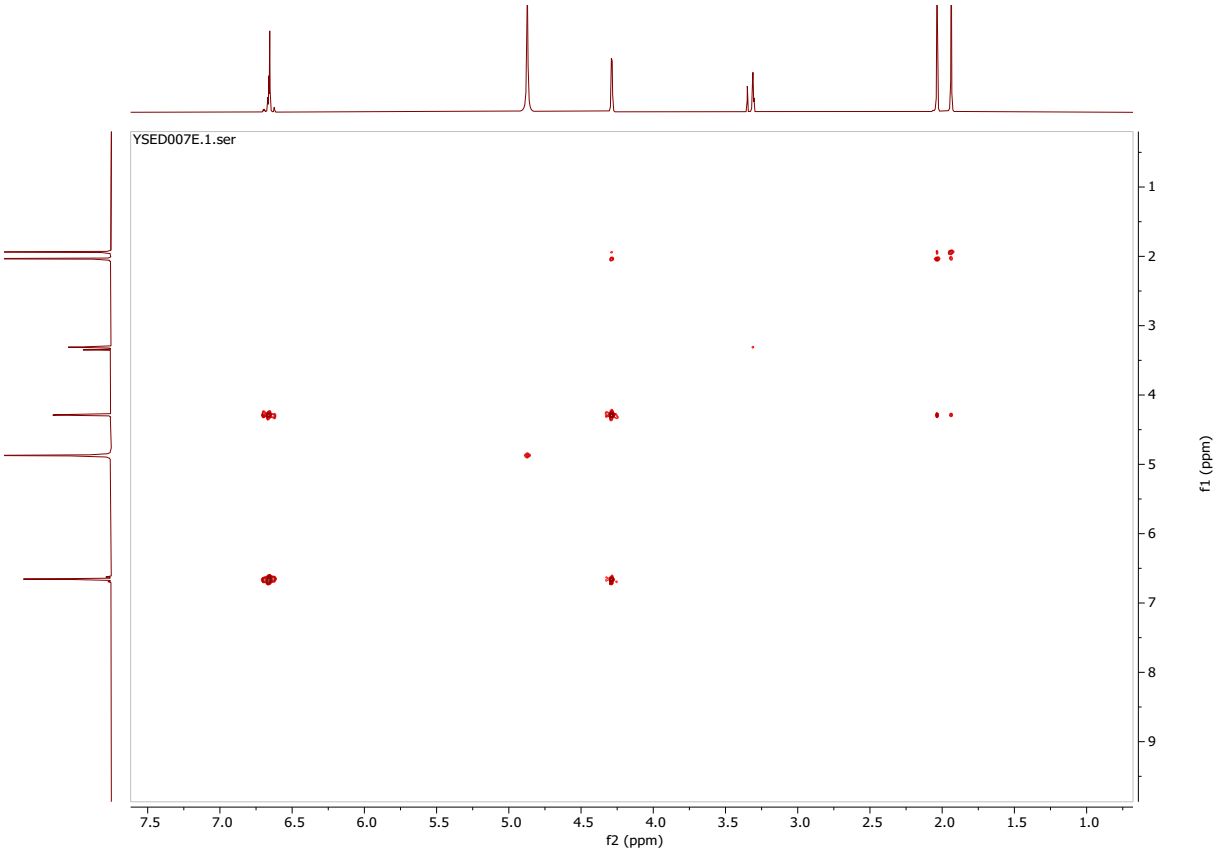
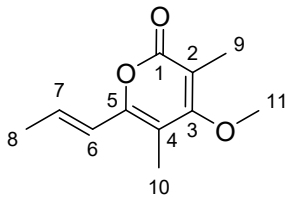


Figure S2.14 ^{1}H, ^{1}H-COSY-spectrum of **6** recorded at 500 MHz in CD_3OD

Compound 7



7

Chemical Formula: $C_{11}H_{14}O_3$
Exact Mass: 194.0943

Compound 7						
Pos.	**δ_C / ppm**	**δ_H / ppm (J/Hz)**	**^{1}H-^{1}H COSY**	**HMBC (H-C)**	**δ_C / ppm literature [11]**	**δ_H / ppm (J/Hz) literature [11]**
1	167.4				164.5	
2	110.7				111.2	
3	170.7				168.7	
4	111.1				109.4	
5	154.0				153.0	
6	121.3	6.41, 1H, dddd (15.30, 1.71, 1.71, 1.69)	7, 8	5, 8	121.4	6.42, dq (15.4, 1.3)
7	134.5	6.61, 1H, dddd (15.41, 6.92, 6.92, 6.90)	6, 8	5, 8	133.1	6.51, dq (15.4, 6.5)
8	18.7	1.93, 3H, dd (7.0, 1.7)	7, 6	6, 7	18.6	1.91, d (6.5)
9	10.3	2.01, 3H, s		1, 2, 3	10.4	1.96, s
10	9.6	2.0, 3H, s		3, 4, 5	9.5	1.98, s
11	61.1	3.85, 3H, s		3	60.7	3.83, s

Table S2.3 Summarized NMR signals for ^{13}C, ^{1}H, ^{1}H-^{1}H COSY, HMBC for **7** recorded in CD_3OD. Literature [11] data was measured in acetone-d_6

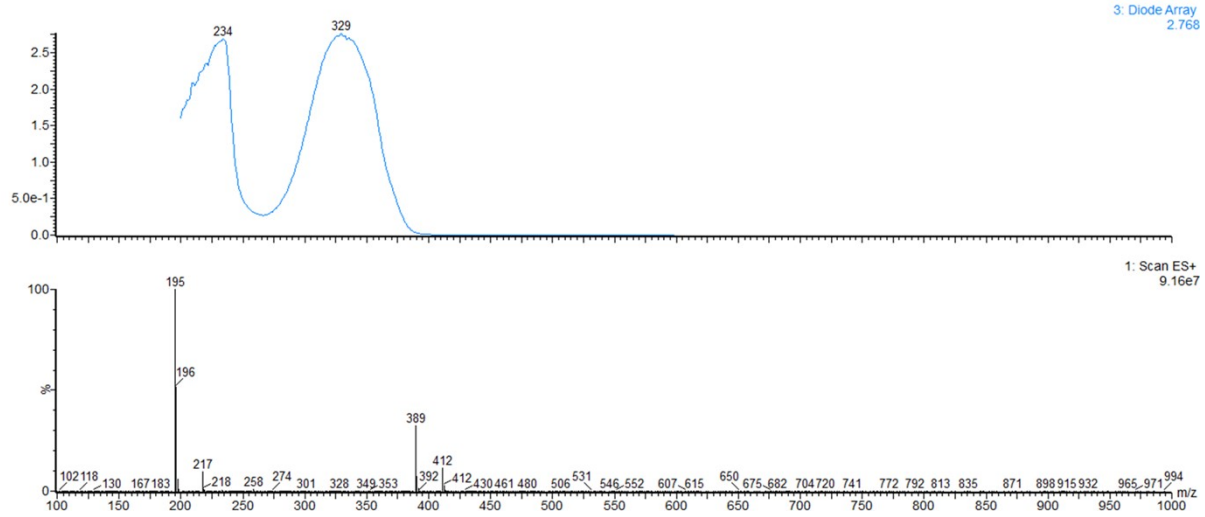


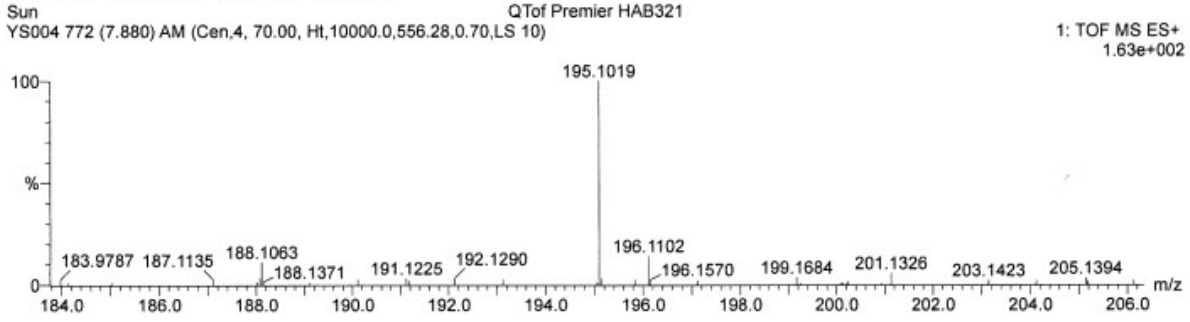
Figure S2.15 UV-absorption (top) and fragmentation pattern of **7** in ES$^+$ TIC (bottom) by LR-LCMS

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 20.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
197 formula(e) evaluated with 3 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-100 H: 0-160 N: 0-10 O: 0-10

Sun QTof Premier HAB321
YS004 772 (7.880) AM (Cen,4, 70.00, Ht,10000.0,556.28,0.70,LS 10)



Minimum:				-1.5			
Maximum:		5.0	20.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
195.1019	195.1021	-0.2	-1.0	4.5	16.6	0.2	C11 H15 O3
	195.0994	2.5	12.8	5.5	18.5	2.2	C7 H11 N6 O
	195.0981	3.8	19.5	0.5	19.3	2.9	C6 H15 N2 O5

Figure S2.16 HRMS data for **7**; *m/z* (M+H) $^+$ calc. mass is 195.1021, 195.1019 was found

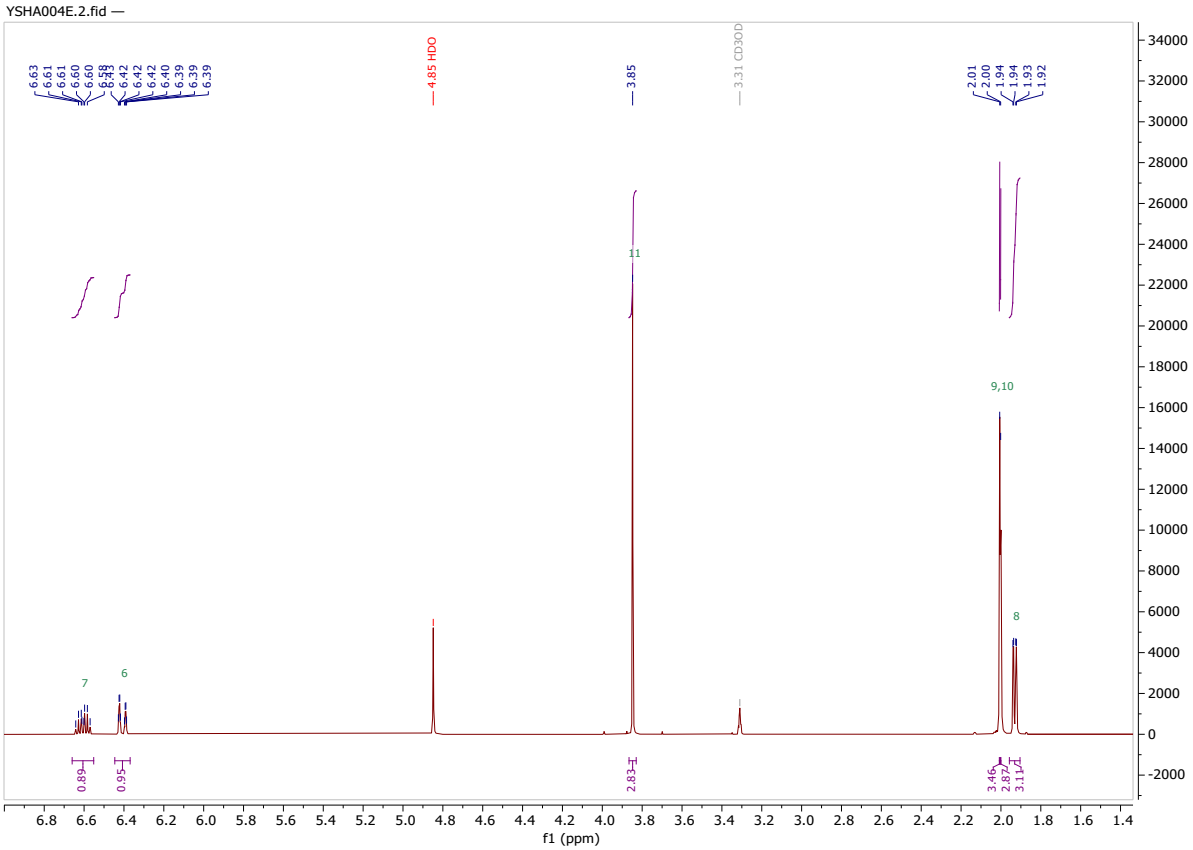


Figure S2.17 ^{1}H-NMR of **7** recorded at 500 MHz in CD_3OD

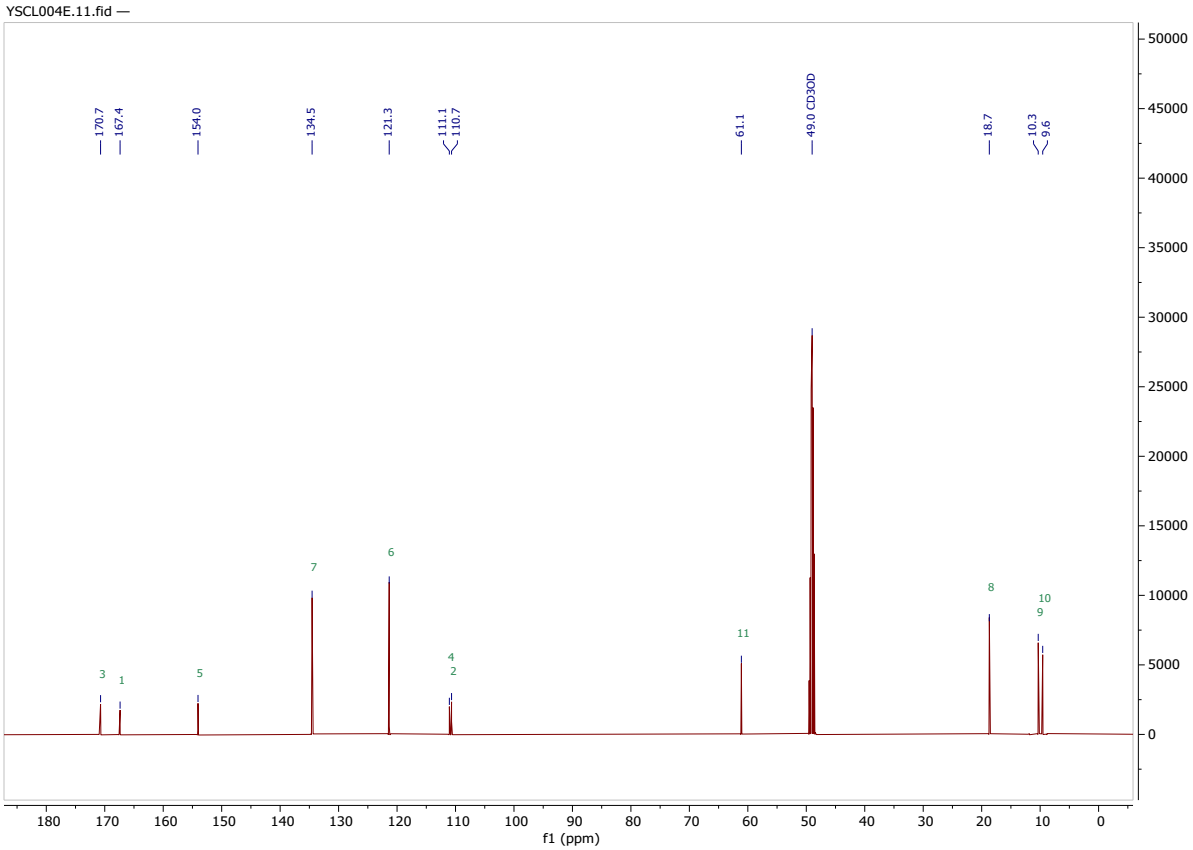


Figure S2.18 ^{13}C-NMR of **7** recorded at 125 MHz in CD_3OD

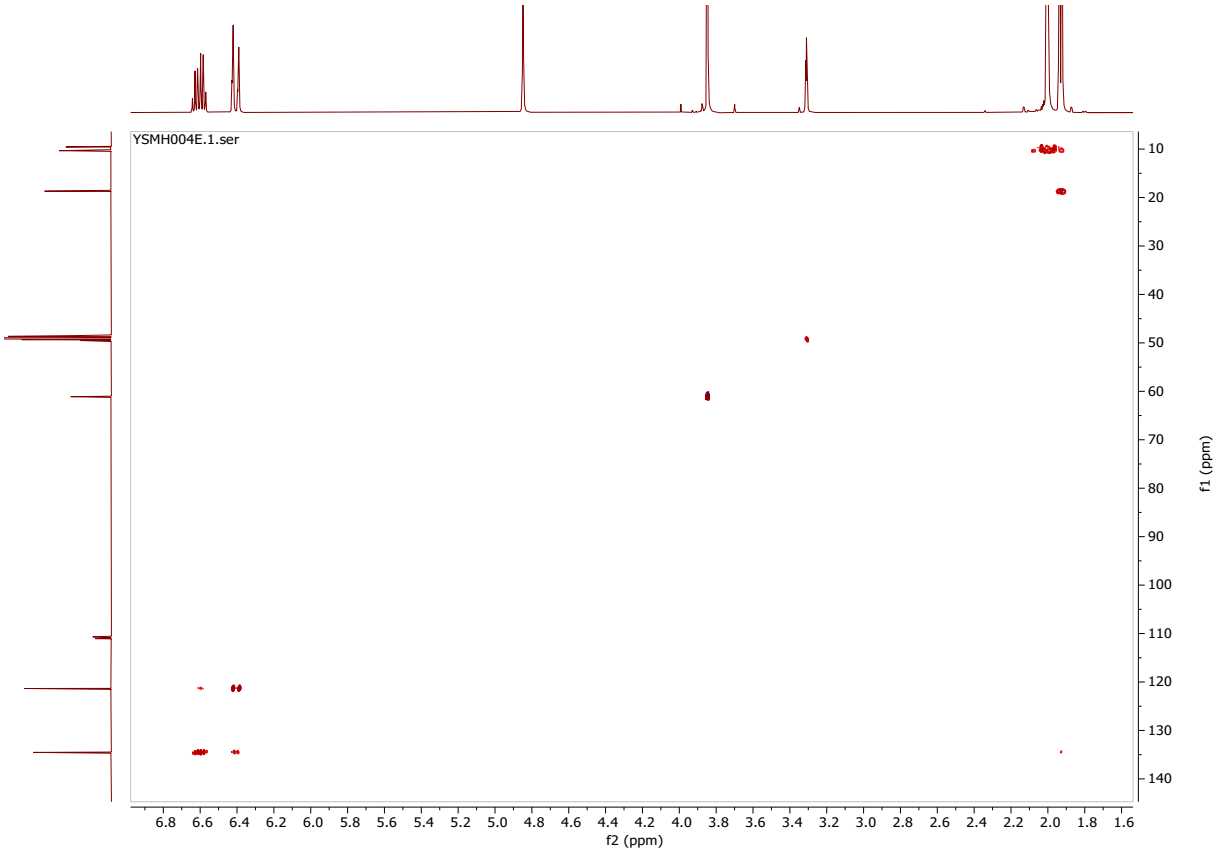


Figure S2.19 HSQC-spectrum of **7** recorded at 500, 125 MHz in CD_3OD

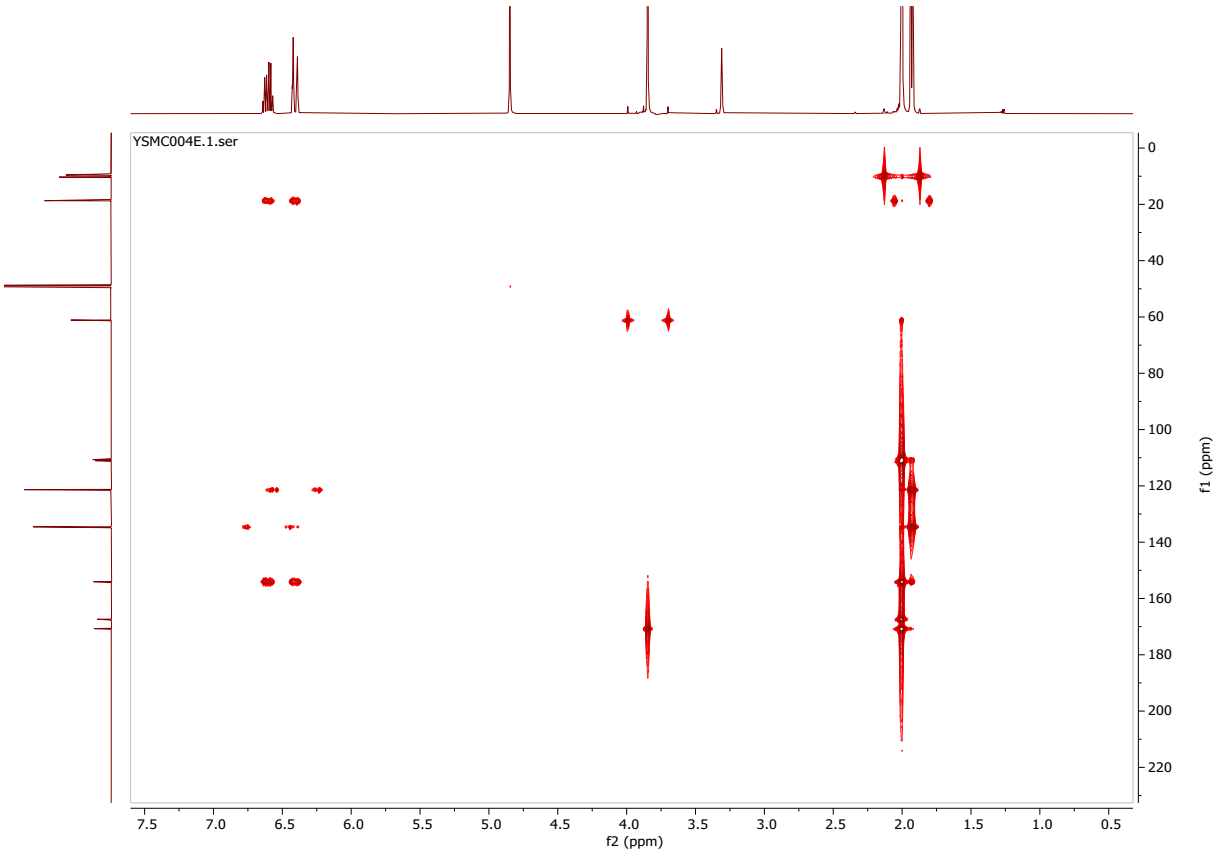


Figure S2.20 HMBC-spectrum of **7** recorded at 500, 125 MHz in CD_3OD

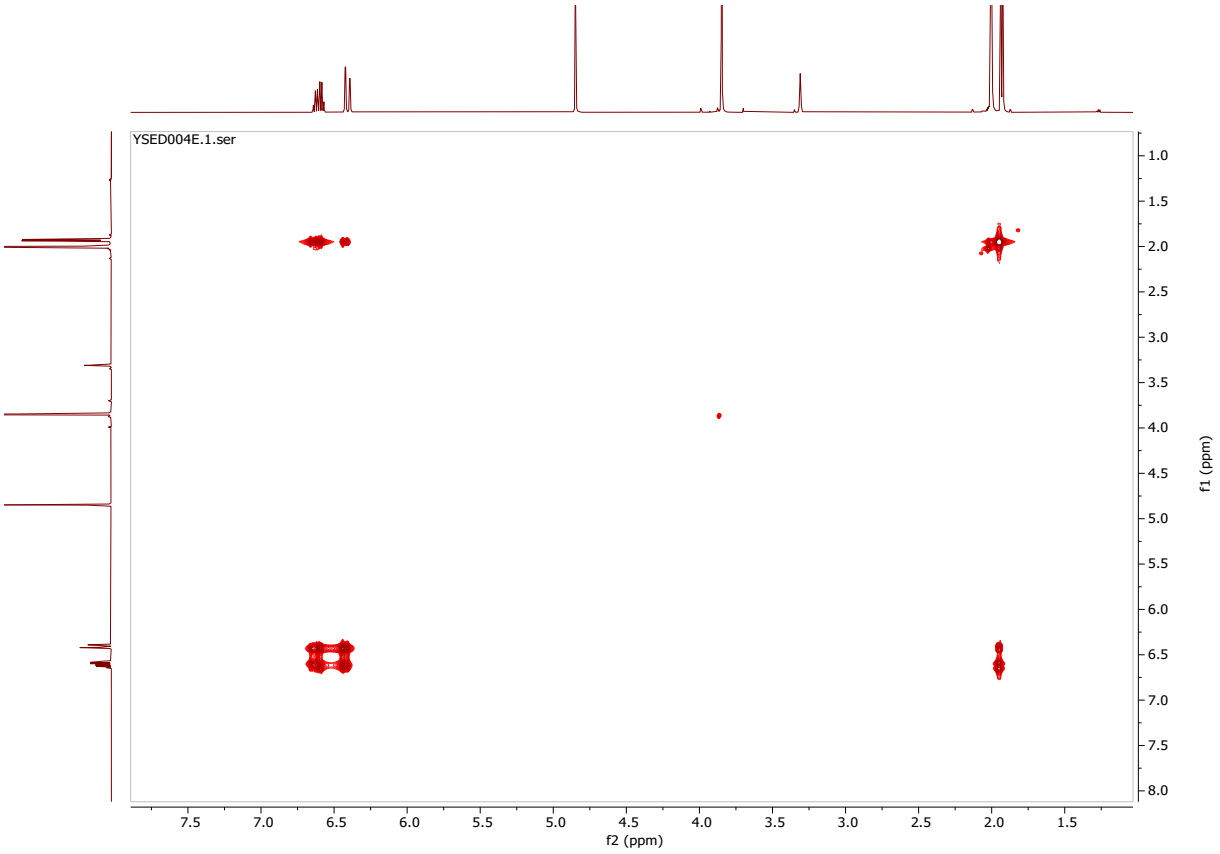
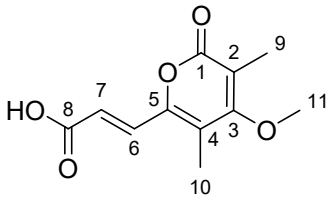


Figure S2.21 ^{1}H, ^{1}H-COSY-spectrum of **7** recorded at 500 MHz in CD_3OD

Compound 8



8

Chemical Formula: $C_{11}H_{12}O_5$
Exact Mass: 224.0685

Compound 8				
Pos.	**δ_C / ppm**	**δ_H / ppm (J/Hz)**	**^{1}H-^{1}H COSY**	**HMBC (H-C)**
1	166.1			
2	115.1			
3	169.2			
4	118.1			
5	151.1			
6	124.6	6.55, 1H, d (15.3)	7	5
7	132.1	7.52, 1H, d (15.4)	6	5, 6, 8
8	169.6			
9	10.8	2.07, 3H, s		1, 2, 3
10	10.1	2.13, 3H, s		3, 4, 5
11	61.4	3.9, 3H, s		3

Table S2.4 Summarized NMR signals for ^{13}C, ^{1}H, ^{1}H-^{1}H COSY, HMBC for **8** recorded in CD_3OD

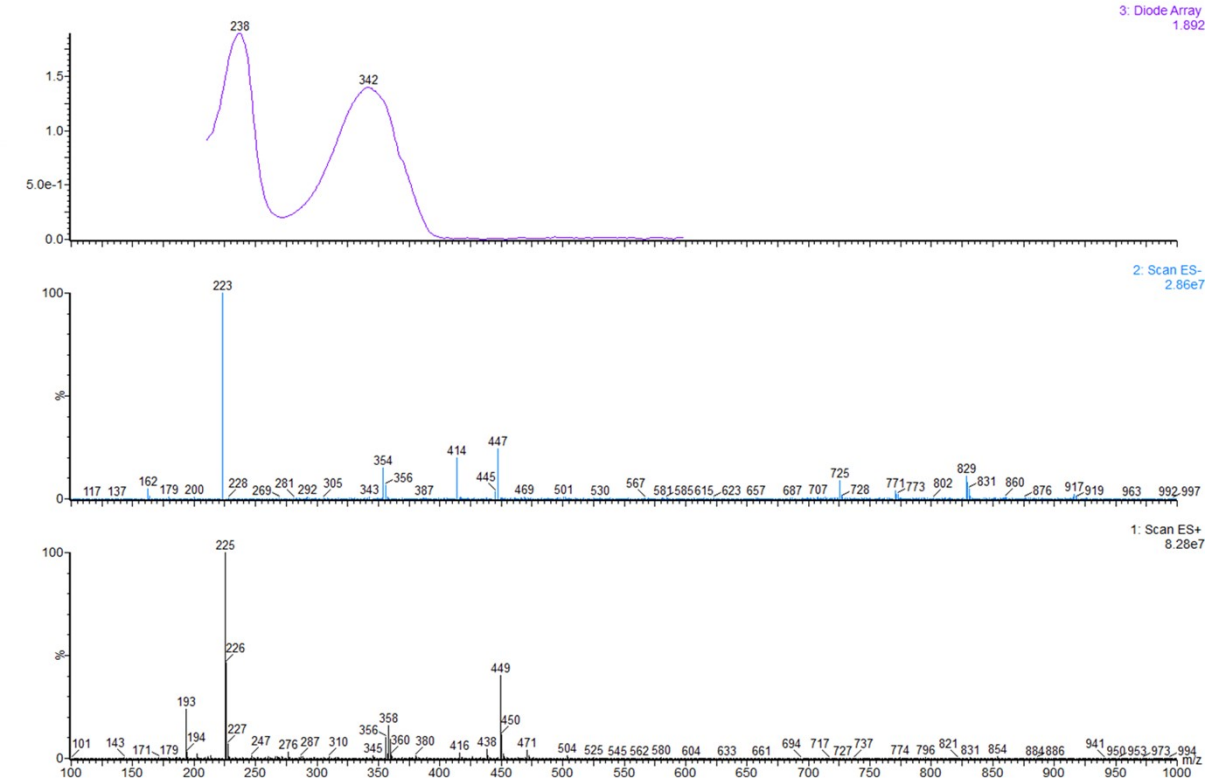


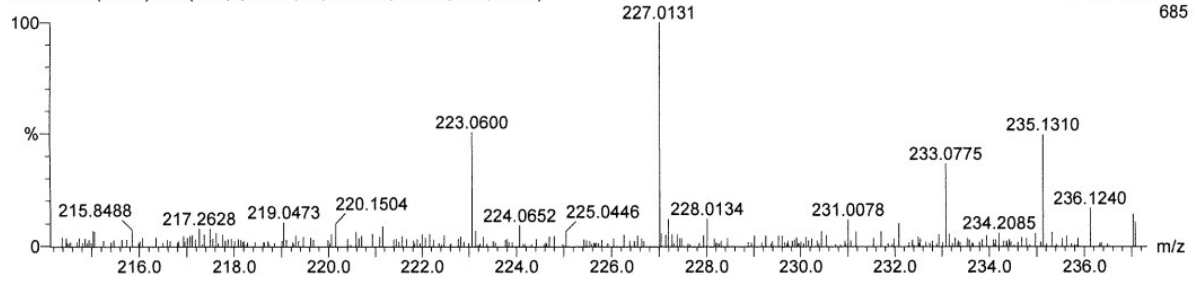
Figure S2.22 UV-absorption (top) and fragmentation pattern of **8** in ES^- (middle) and ES^+ TIC (bottom) by LR-LCMS

Elemental Composition Report **Page 1**

Single Mass Analysis (displaying only valid results)
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Selected filters: None

Monoisotopic Mass, Even Electron Ions
150 formula(e) evaluated with 1 results within limits (up to 40 closest results for each mass)
Elements Used:
C: 0-72 H: 0-50 N: 0-1 O: 0-6 Na: 0-1 S: 0-1

Sun LCT Premier KD070
YS 009 24 (0.531) AM (Cen,4, 90.00, Ar,10000.0,554.26,0.70,LS 10) 1: TOF MS ES-
685



Minimum:				-1.5		
Maximum:		5.0	5.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
223.0600	223.0606	-0.6	-2.7	6.5	19.6	C11 H11 O5

Figure S2.23 HRMS data for **8**; *m/z* $(M-H)^-$ calc. mass is 223.0606, 223.0600 was found.

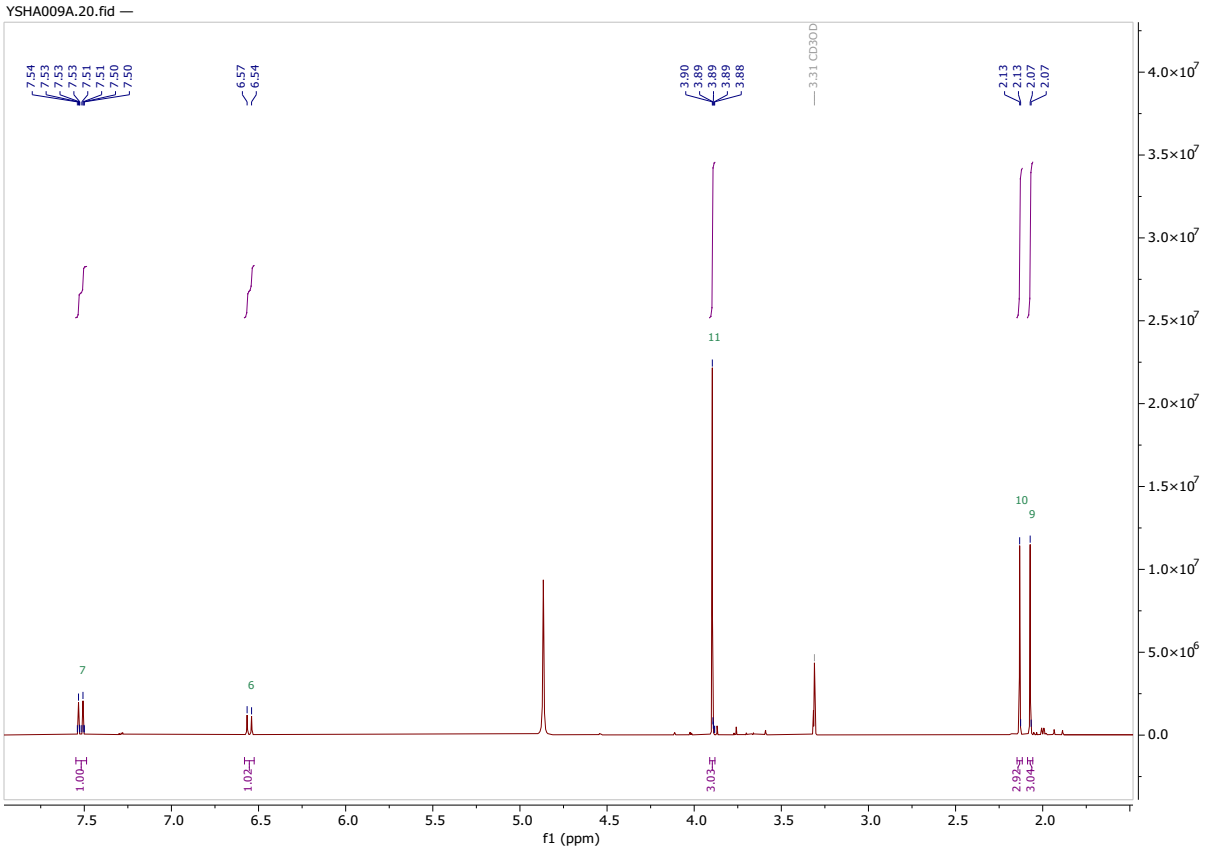


Figure S2.24 ^{1}H-NMR of **8** recorded at 600 MHz in CD_3OD.

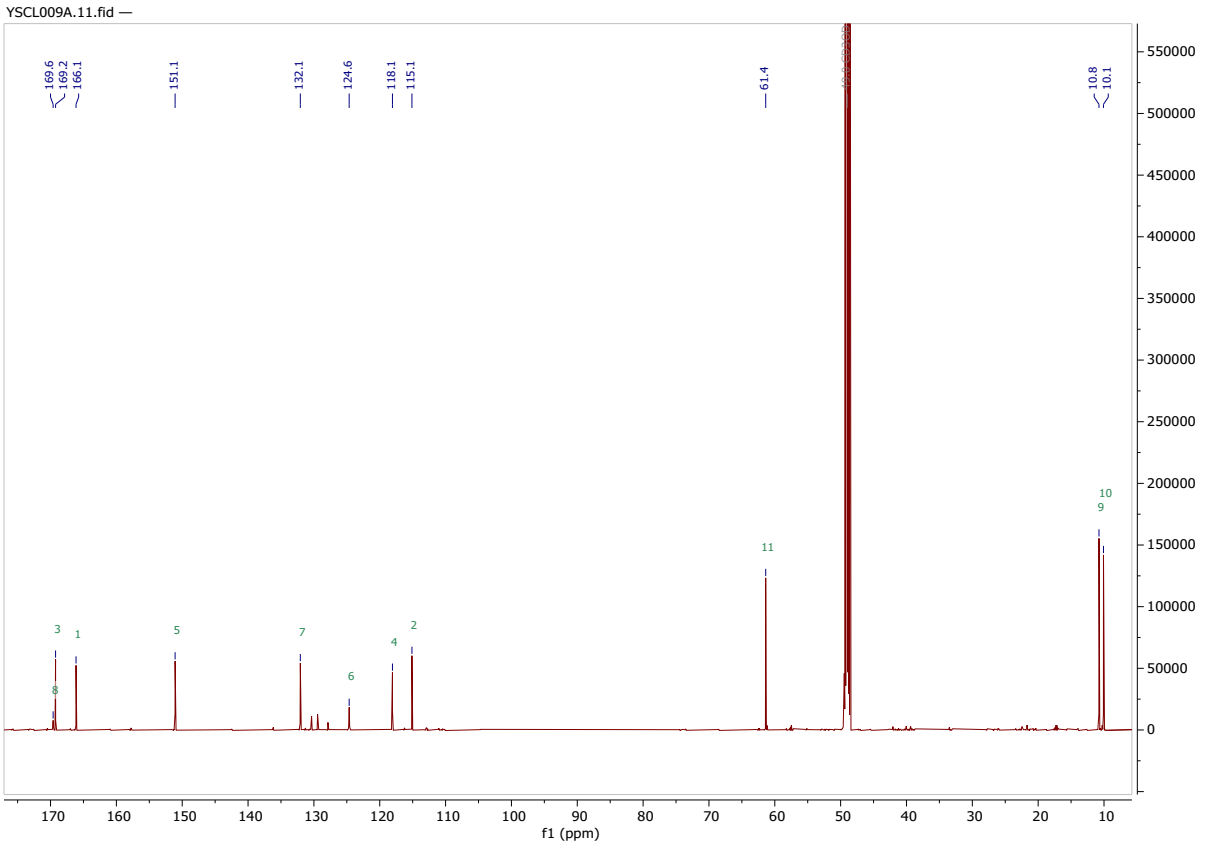


Figure S2.25 ^{13}C-NMR of **8** recorded at 150 MHz in CD_3OD.

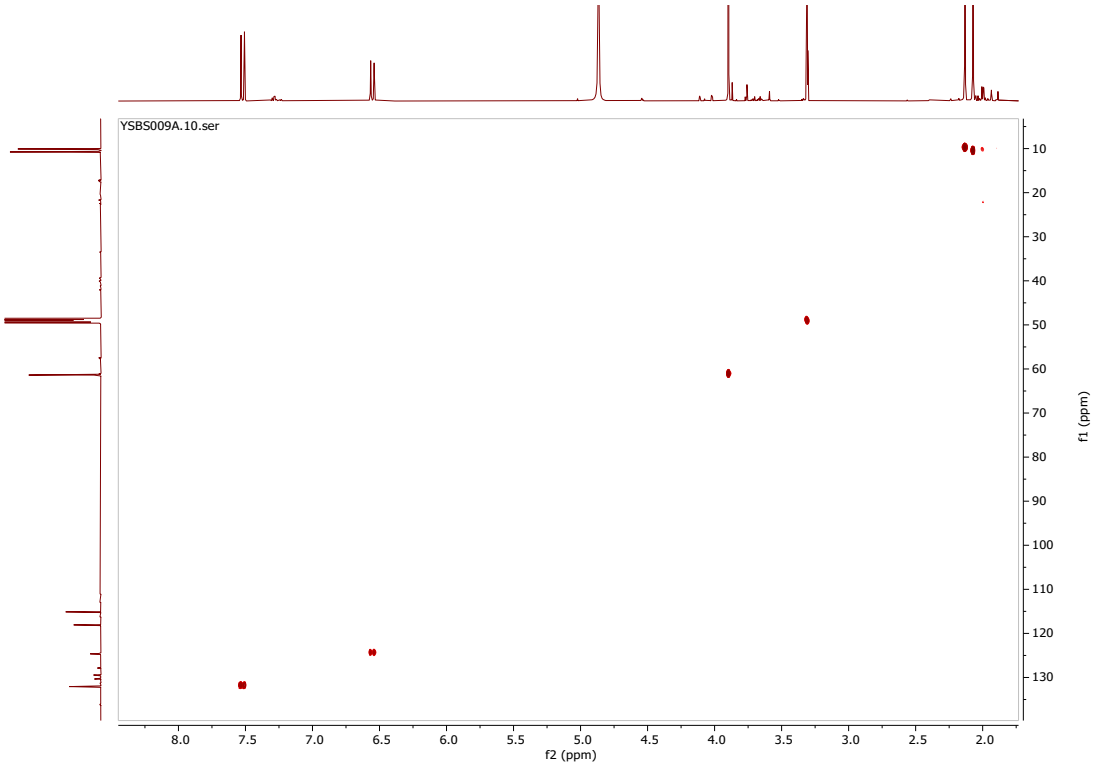


Figure S2.26 HSQC-spectrum of **8** recorded at 600, 150 MHz in CD_3OD.

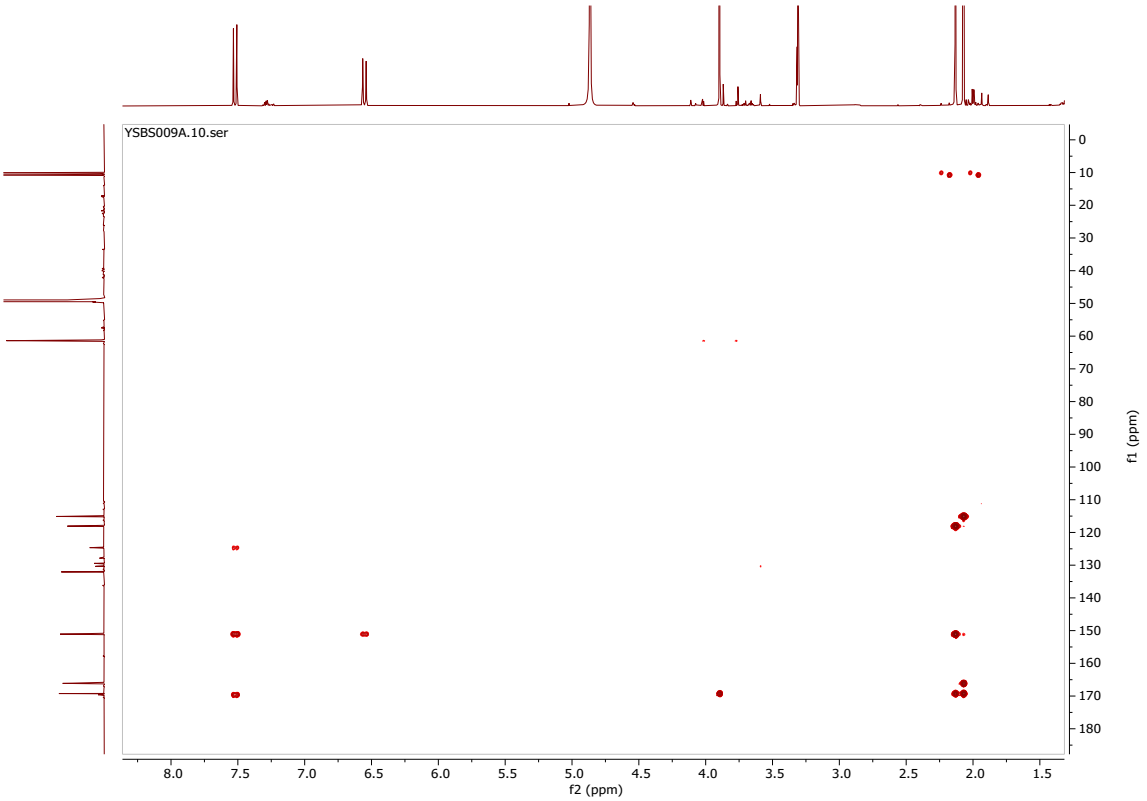


Figure S2.27 HMBC-spectrum of **8** recorded at 600, 150 MHz in CD_3OD.

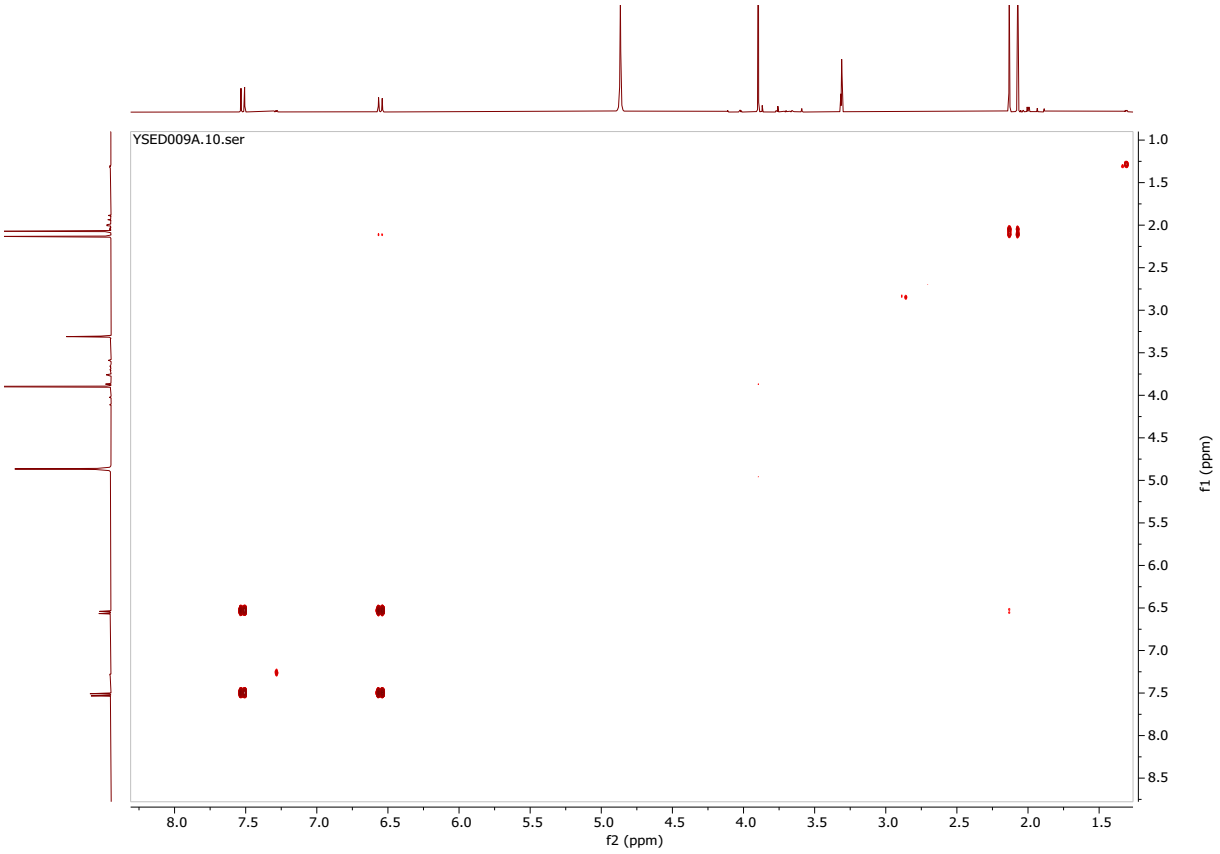
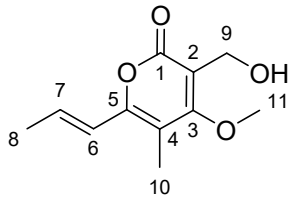


Figure S2.28 ^{1}H, ^{1}H-COSY-spectrum of **8** recorded at 600 MHz in CD_3OD.

Compound 9



9

Chemical Formula: $C_{11}H_{14}O_4$
Exact Mass: 210.0892

Compound 9						
Pos.	**δ_C / ppm**	**δ_H / ppm (J/Hz)**	**^{1}H-^{1}H COSY**	**HMBC (H-C)**	**δ_C / ppm literature** [12]	**δ_H / ppm (J/Hz) literature** [12]
1	166.9				165.1	
2	111.5				111.3	
3	172.2				169.4	
4	110.5				108.6	
5	155.6				154.2	
6	121.4	6.45, 1H, dddd (15.3, 1.7, 1.7, 1.7)	7, 8	5, 8	119.9	6.25, dq (15.3, 1.7)
7	135.9	6.67, 1H, dddd (15.3, 6.9, 6.9, 6.9)	6, 8	5, 8	135.0	6.69, dq (15.4, 7.0)
8	18.7	1.95, 3H, dd (7.48, 1.73)	6, 7	6, 7	9.4	1.91, dd (7.0, 1.7)
9	55.1	4.53, 2H, s		1, 2, 3	61.9	4.56, s
10	9.6	2.0, 3H, s		3, 4, 5	18.7	1.96, s
11	62.4	4.07, 3H,s		3	55.8	3.96, s

Table S2.5 Summarized NMR signals for ^{13}C, ^{1}H, ^{1}H-^{1}H COSY, HMBC for **9** recorded in CD_3OD, Compound from the literature [12] was measured in $CDCl_3$.

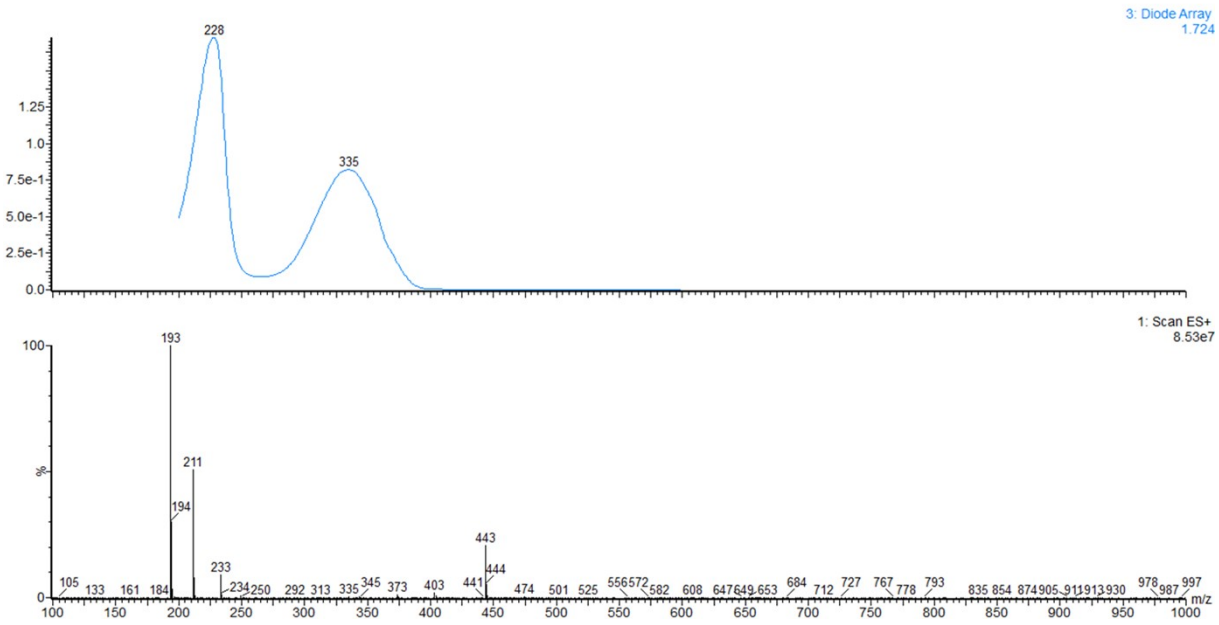


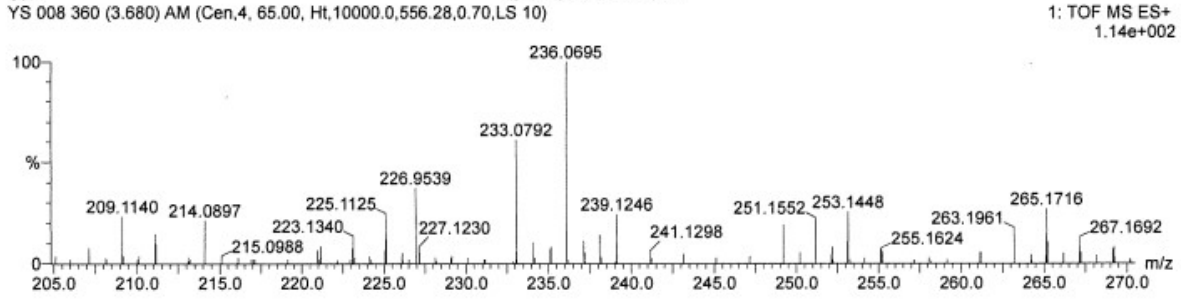
Figure S2.29 UV-absorption (top) and fragmentation pattern of **9** ES$^+$ TIC (bottom) by LR-LCMS.

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 20.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
476 formula(e) evaluated with 10 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-40 H: 0-50 N: 0-10 O: 0-10 Na: 0-1

Sun QTof Premier HAB321
YS 008 360 (3.680) AM (Cen,4, 65.00, Ht,10000.0,556.28,0.70,LS 10)



Minimum:				-1.5			
Maximum:		5.0	20.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
233.0792	233.0790	0.2	0.9	4.5	22.9	1.6	C11 H14 O4 Na
	233.0787	0.5	2.1	8.5	23.1	1.8	C9 H9 N6 O2
	233.0803	-1.1	-4.7	9.5	22.9	1.6	C12 H10 N4 Na
	233.0774	1.8	7.7	3.5	23.8	2.4	C8 H13 N2 O6
	233.0814	-2.2	-9.4	7.5	23.2	1.9	C13 H13 O4
	233.0763	2.9	12.4	5.5	24.4	3.1	C7 H10 N6 O2 Na
	233.0827	-3.5	-15.0	12.5	23.7	2.4	C14 H9 N4
	233.0750	4.2	18.0	0.5	25.2	3.9	C6 H14 N2 O6 Na
	233.0835	-4.3	-18.4	1.5	25.6	4.2	C H10 N10 O3 Na
	233.0747	4.5	19.3	4.5	25.5	4.1	C4 H9 N8 O4

Figure S2.30 HRMS data for **9**; *m/z* (M+Na) calc. mass is 233.0790, 233.0792 was found.

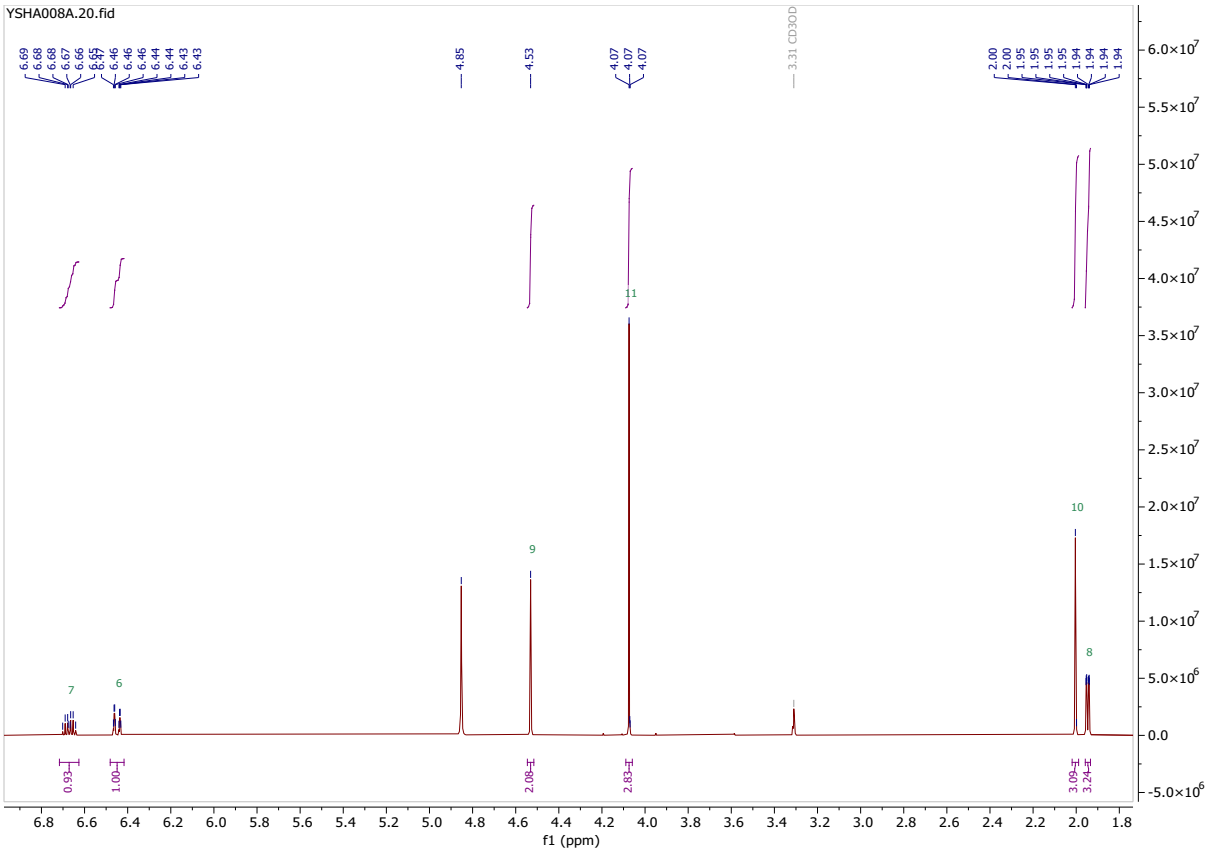


Figure S2.31 ^{1}H-NMR of **9** recorded at 600 MHz in CD_3OD.

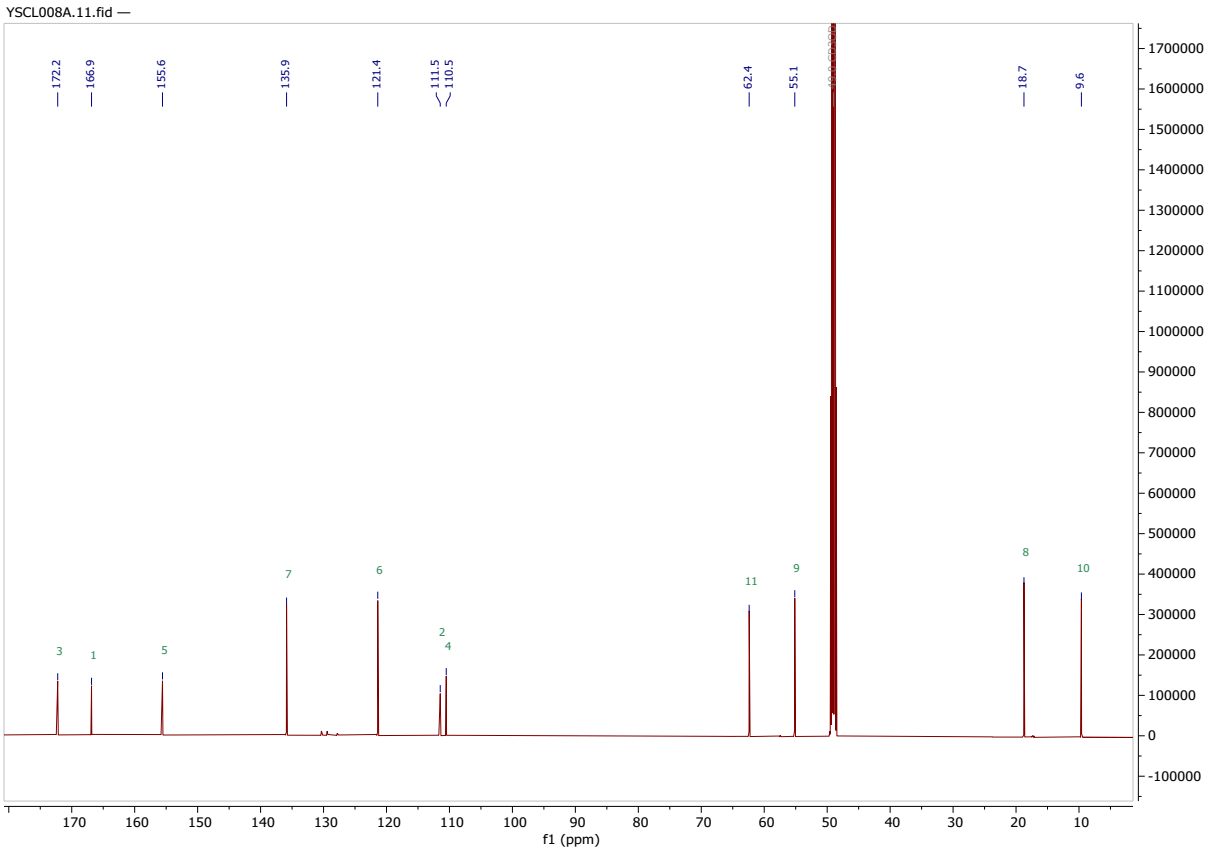


Figure S2.32 ^{13}C-NMR of **9** recorded at 150 MHz in CD_3OD.

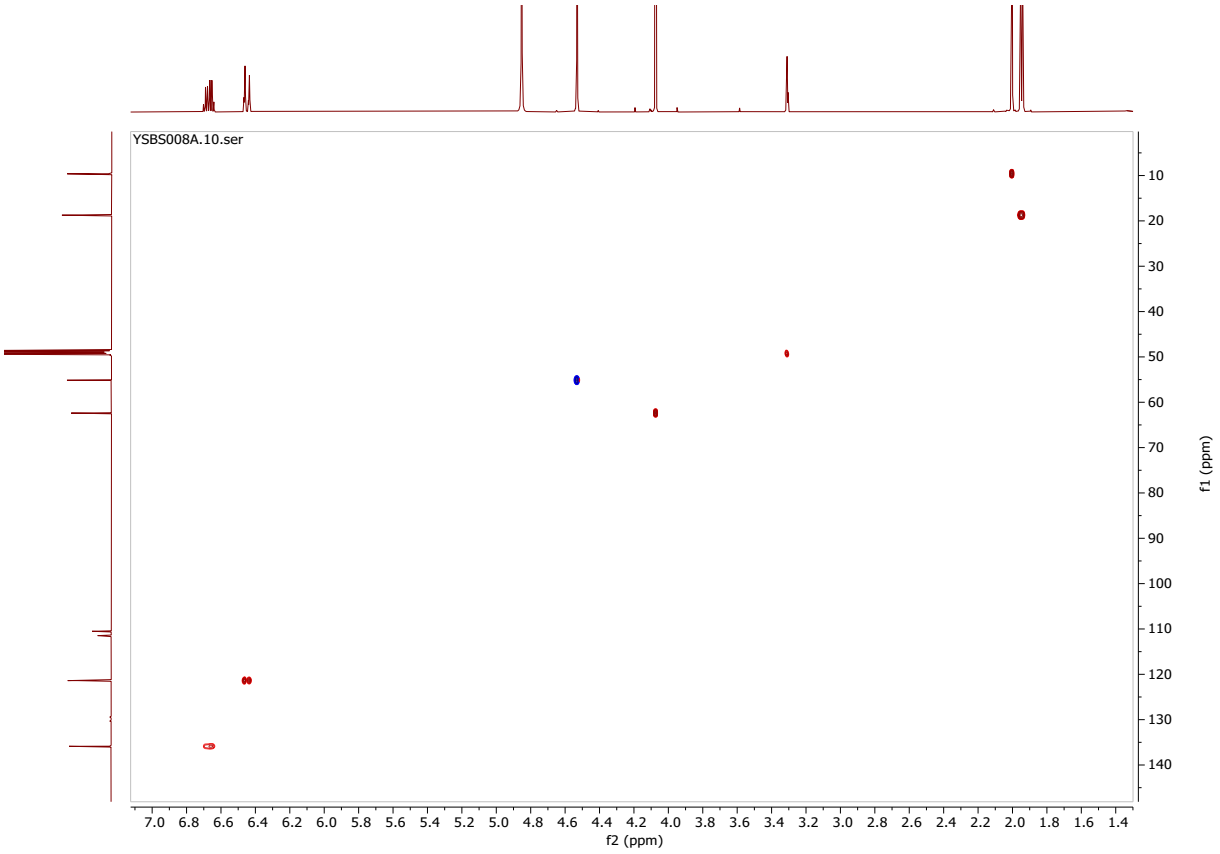


Figure S2.33 HSQC-spectrum of **9** recorded at 600, 150 MHz in CD_3OD.

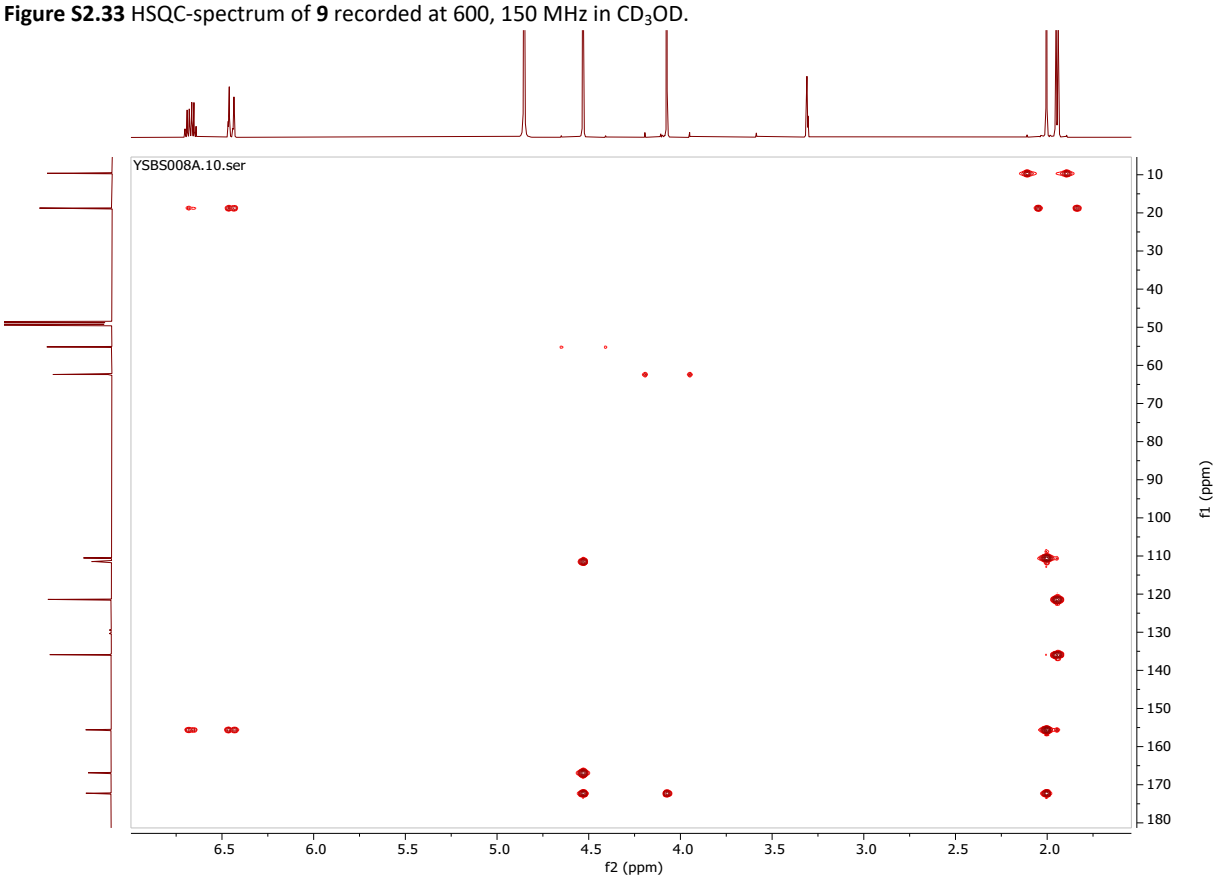


Figure S2.34 HMBC-spectrum of **9** recorded at 600, 150 MHz in CD_3OD.

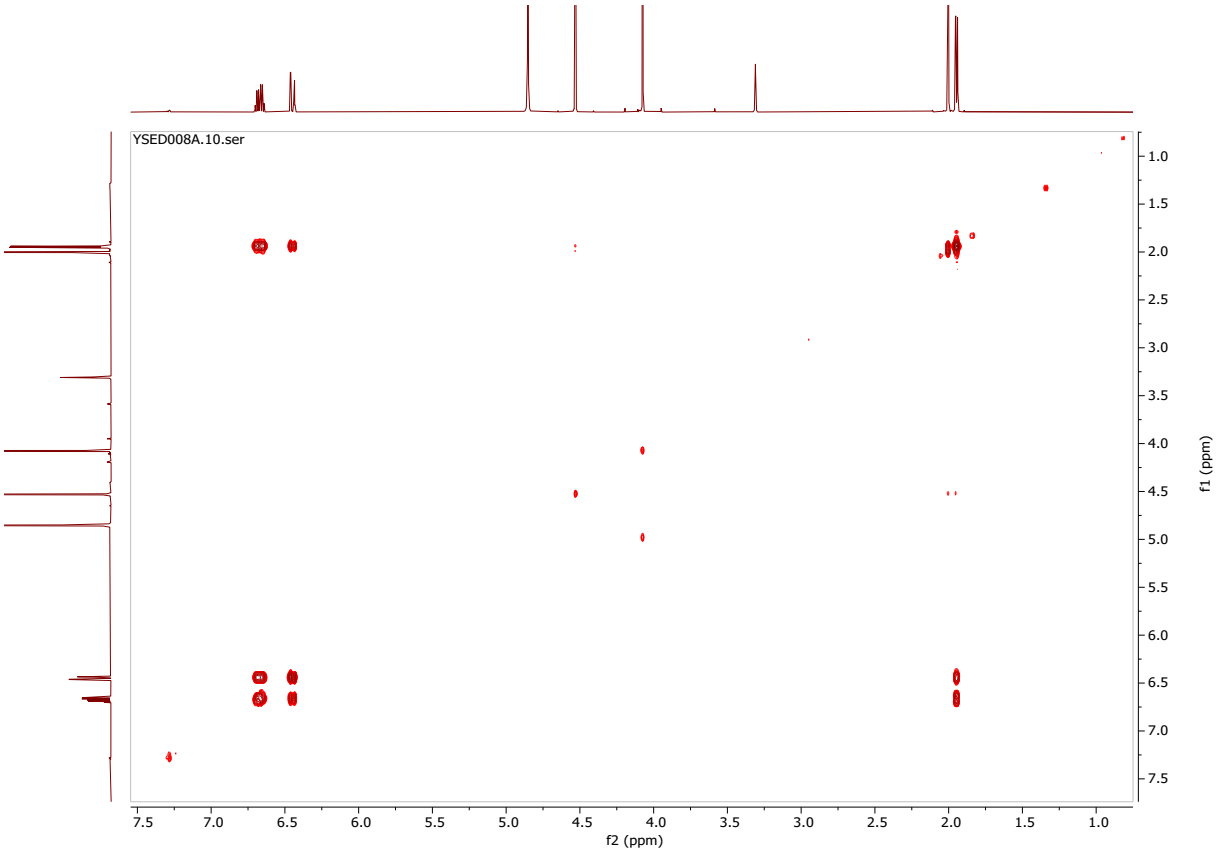
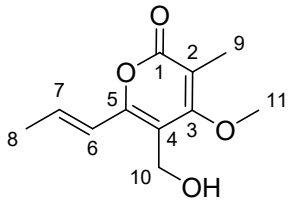


Figure S2.35 ^{1}H, ^{1}H-COSY-spectrum of **9** recorded at 600 MHz in CD_3OD.

Compound 10



10

Chemical Formula: $C_{11}H_{14}O_4$
Exact Mass: 210.0892

Compound 10				
Pos.	**δ_C / ppm**	**δ_H / ppm (J/Hz)**	**^{1}H-^{1}H COSY**	**HMBC (H-C)**
1	167.1			
2	111.0			
3	170.3			
4	114.2			
5	156.9			
6	121.2	6.56, 1H, dddd (15.3, 1.6, 1.6, 1.6)	7, 8	5, 7, 8
7	136.1	6.71, 1H, dddd (15.3, 6.8, 6.8, 6.8)		
8	18.8	1.95, 3H, dd (6.8, 1.6)	6, 7	5, 6, 7
9	10.4	2.03, 3H, s		1, 2, 3
10	54.5	4.46, 2H, s		3, 4, 5
11	62.1	3.94, 3H, s		3

Table S2.6 Summarized NMR signals for ^{13}C, ^{1}H, ^{1}H-^{1}H COSY, HMBC for **10** recorded in CD_3OD.

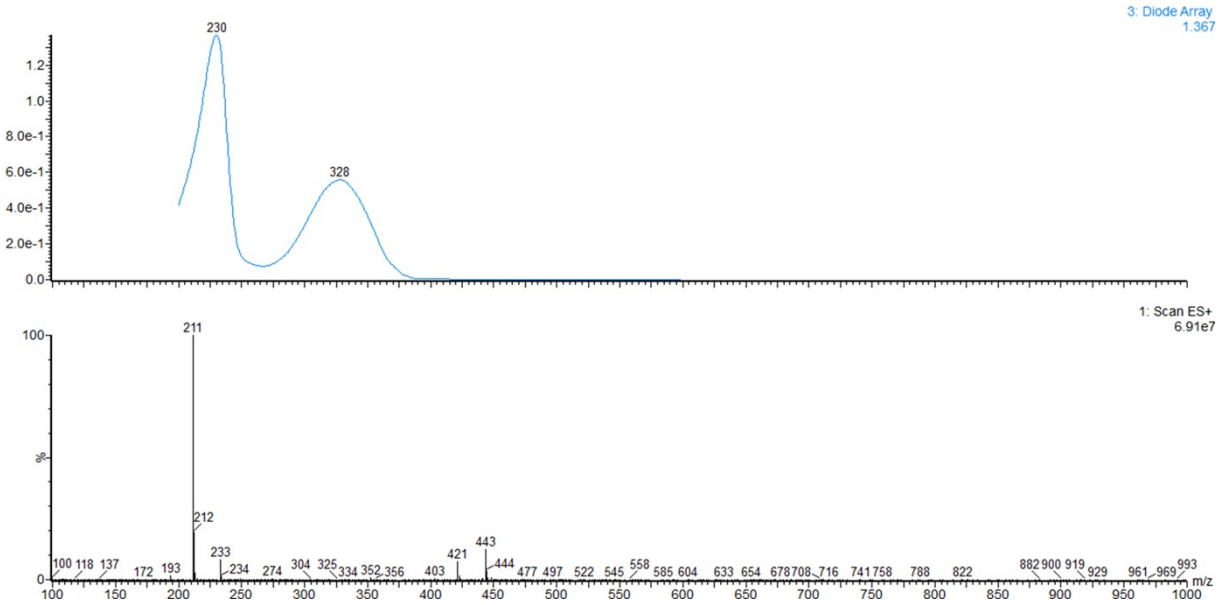


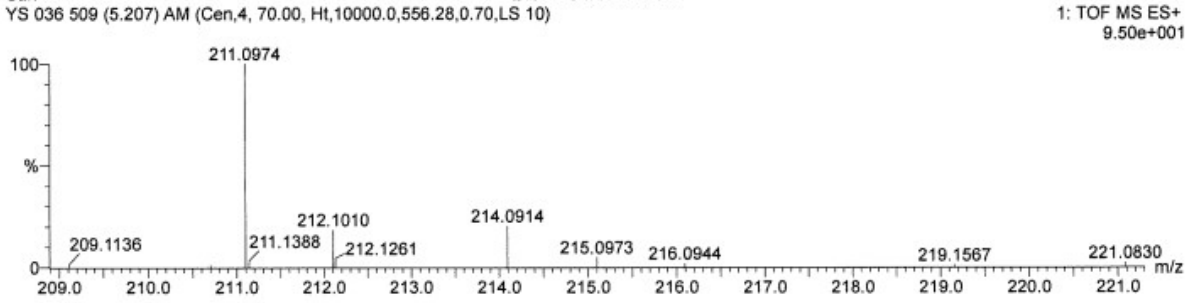
Figure S2.36 UV-absorption (top) and fragmentation pattern of **10** in ES$^+$ TIC (bottom) by LR-LCMS.

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 20.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
57 formula(e) evaluated with 2 results within limits (up to 30 closest results for each mass)
Elements Used:
C: 0-85 H: 0-116 O: 0-12 Na: 0-1

Sun QTof Premier HAB321
YS 036 509 (5.207) AM (Cen,4, 70.00, Ht,10000.0,556.28,0.70,LS 10)



Minimum:				-1.5			
Maximum:		5.0	20.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
211.0974	211.0970	0.4	1.9	4.5	13.3	0.2	C11 H15 O4
	211.0946	2.8	13.3	1.5	14.7	1.7	C9 H16 O4 Na

Figure S2.37 HRMS data for **10**; *m/z* (M+H) $^+$ calc. mass is 211.0970, 211.0974 was found.

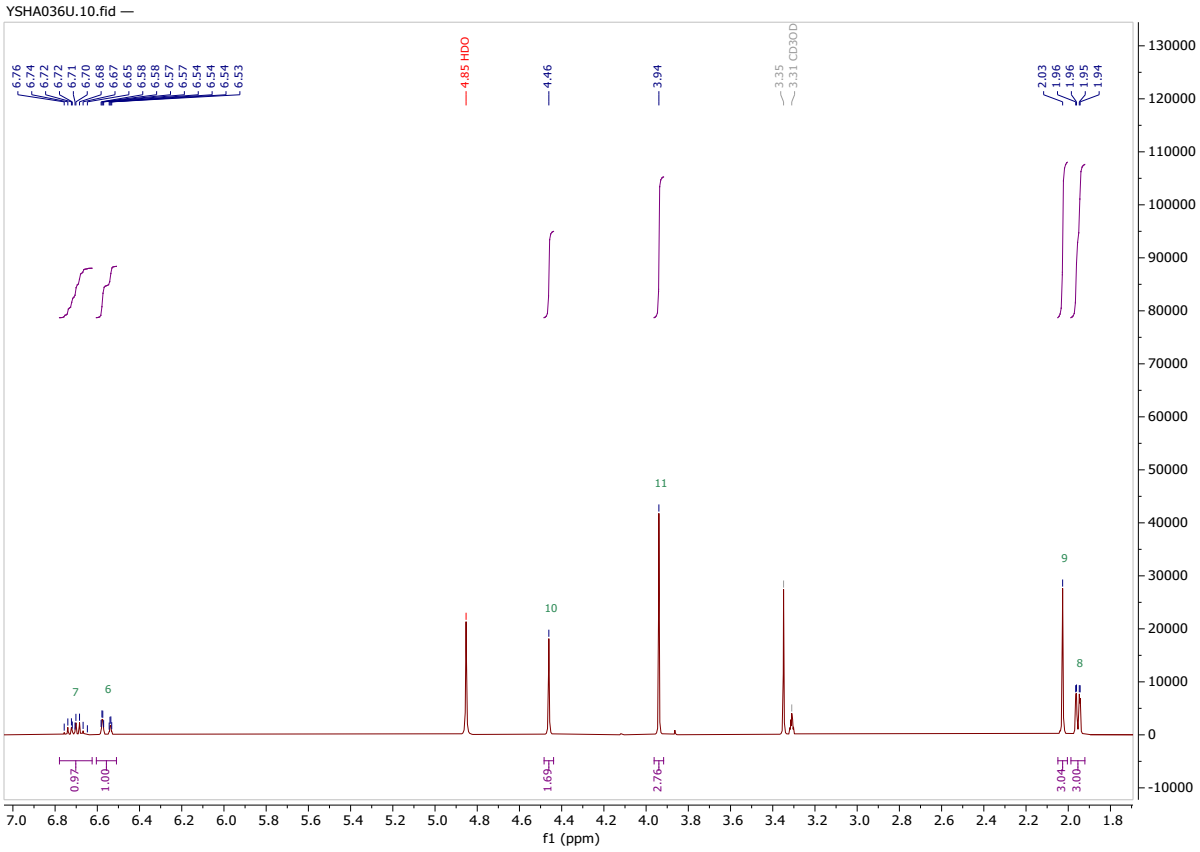


Figure S2.38 ^{1}H-NMR of **10** recorded at 400 MHz in CD_3OD.

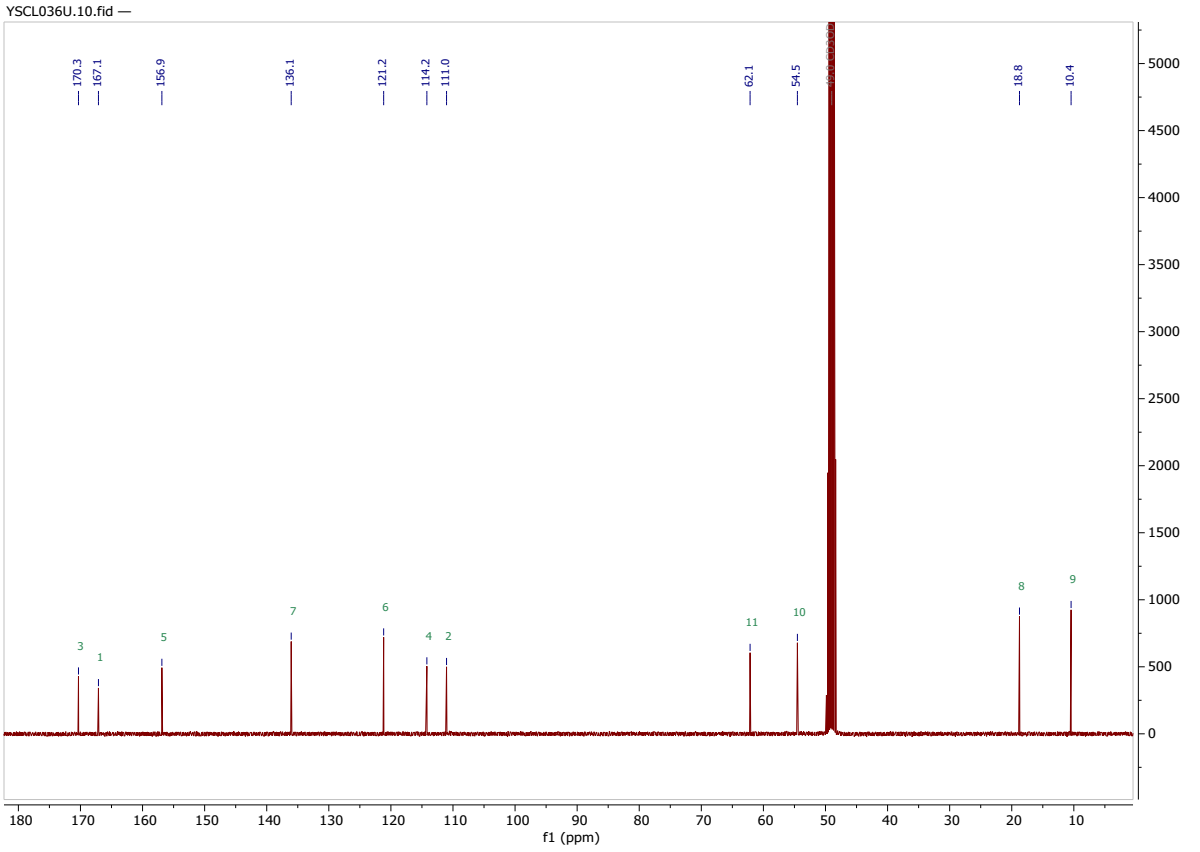


Figure S2.39 ^{13}C-NMR of **10** recorded at 100 MHz in CD_3OD.

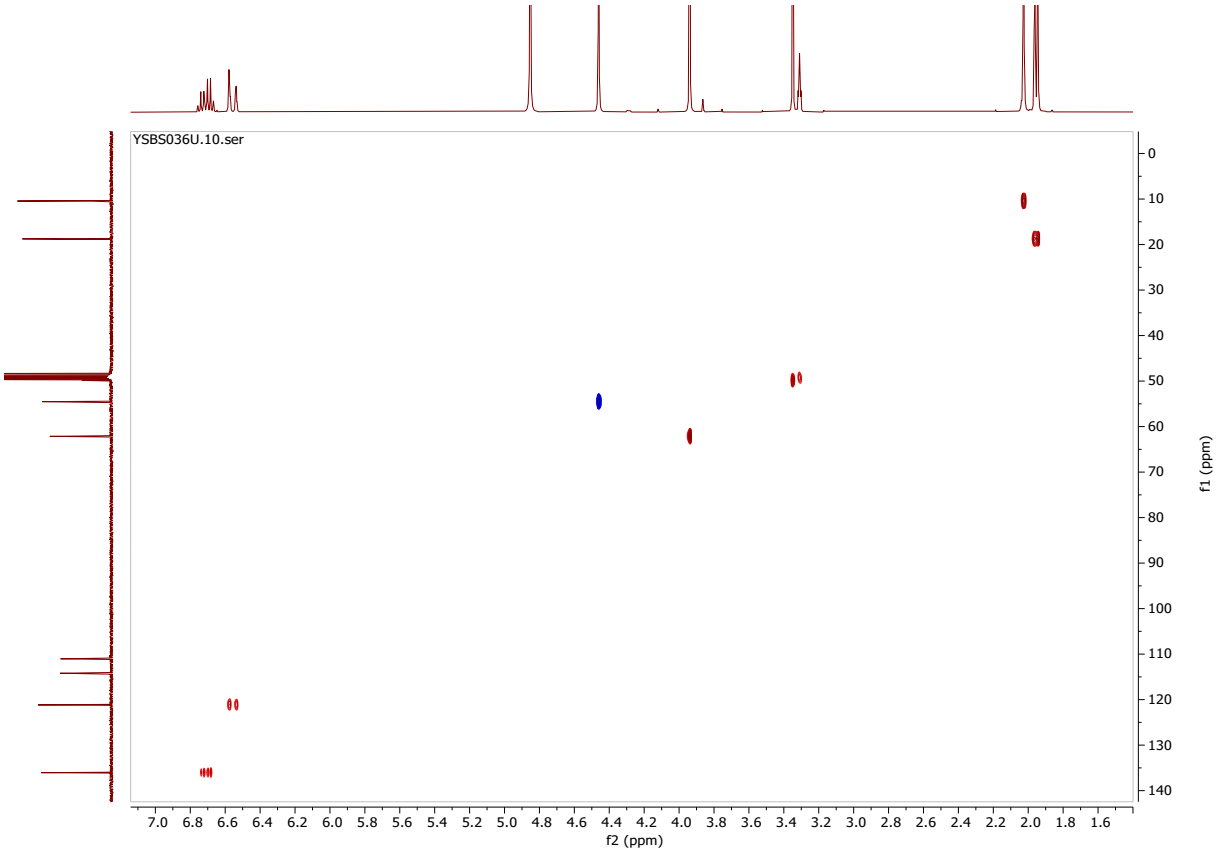


Figure S2.40 HSQC-spectrum of **10** recorded at 400, 100 MHz in CD_3OD.

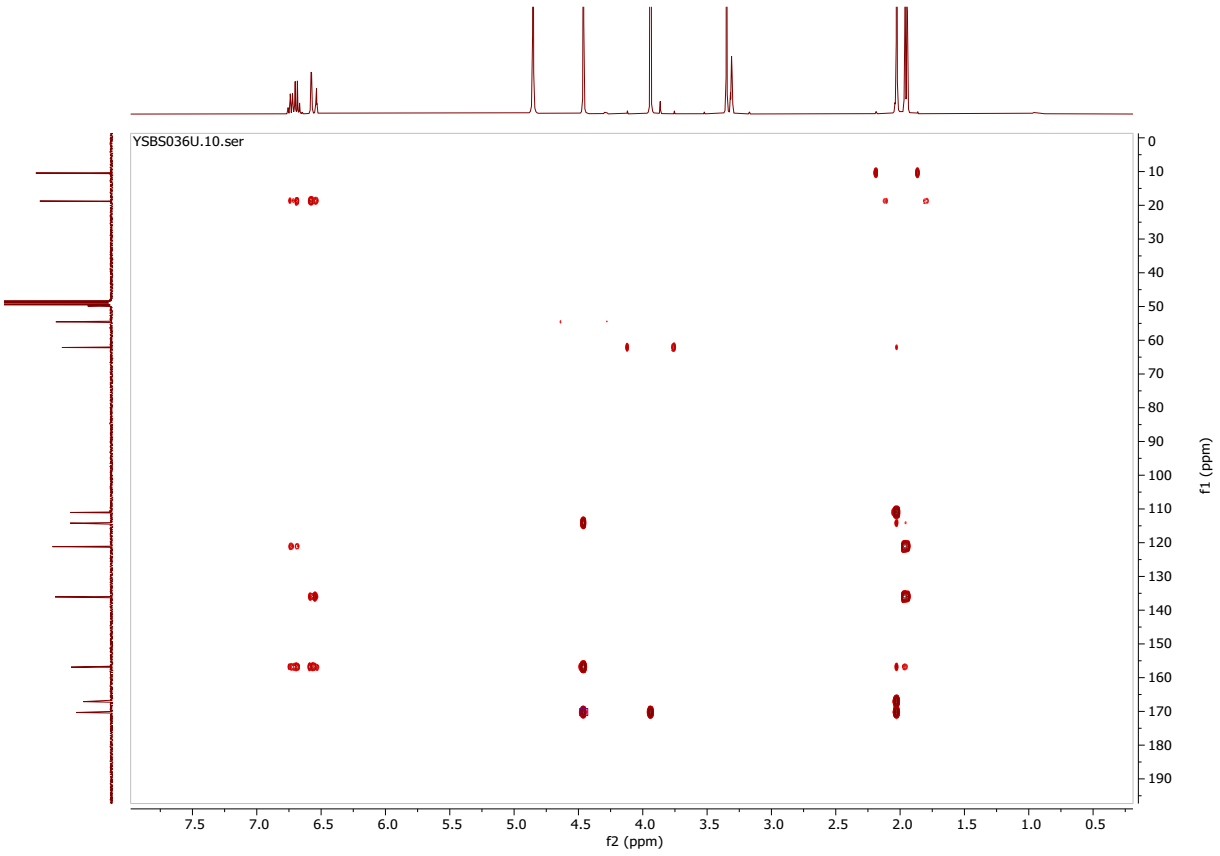


Figure S2.41 HMBC-spectrum of **10** recorded at 400, 100 MHz in CD_3OD.

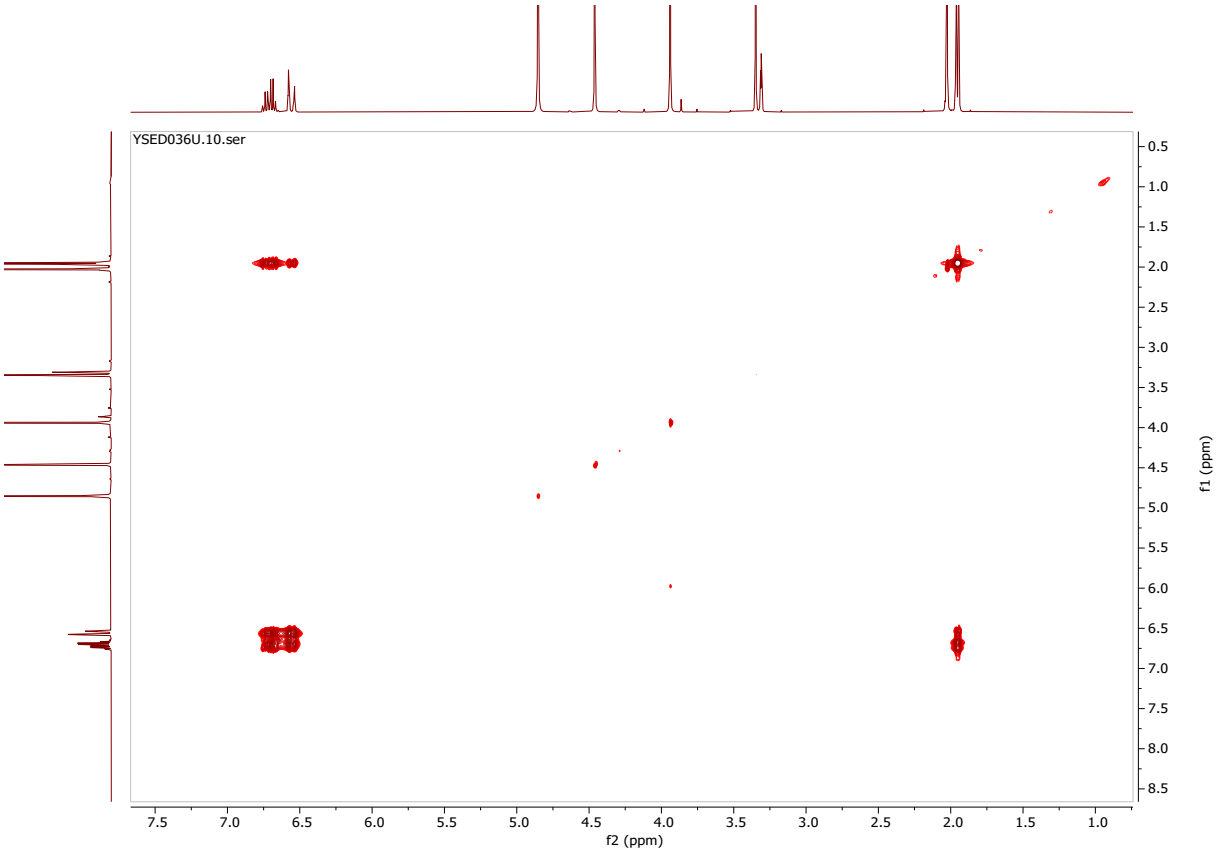
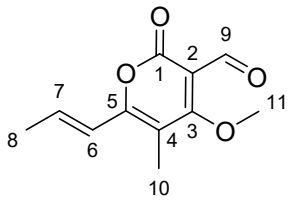


Figure S2.42 ^{1}H, ^{1}H-COSY-spectrum of **10** recorded at 400 MHz in CD_3OD.

Compound 11



11

Chemical Formula: $C_{11}H_{12}O_4$
Exact Mass: 208.0736

Compound 11				
Pos.	**δ_C / ppm**	**δ_H / ppm (*J*/Hz)**	**^{1}H-^{1}H COSY**	**HMBC (H-C)**
1	162.3			
2	104.9			
3	175.1			
4	109.3			
5	157.8			
6	120.6	6.61, 1H, dddd (15.3, 1.5, 1.5, 1.5)	7, 8	5, 7, 8
7	138.7	6.72, 1H, m	6, 8	5, 6, 8
8	18.7	1.95, 3H, m	6, 7	6, 7
9	187.5	9.94, 1H, s		2, 3
10	8.9	1.95, 3H, m		3, 4, 5
11	64.6	4.02, 3H, s		3

Table S2.7 Summarized NMR signals for ^{13}C, ^{1}H, ^{1}H-^{1}H COSY, HMBC for **11** recorded in DMSO-d_6.

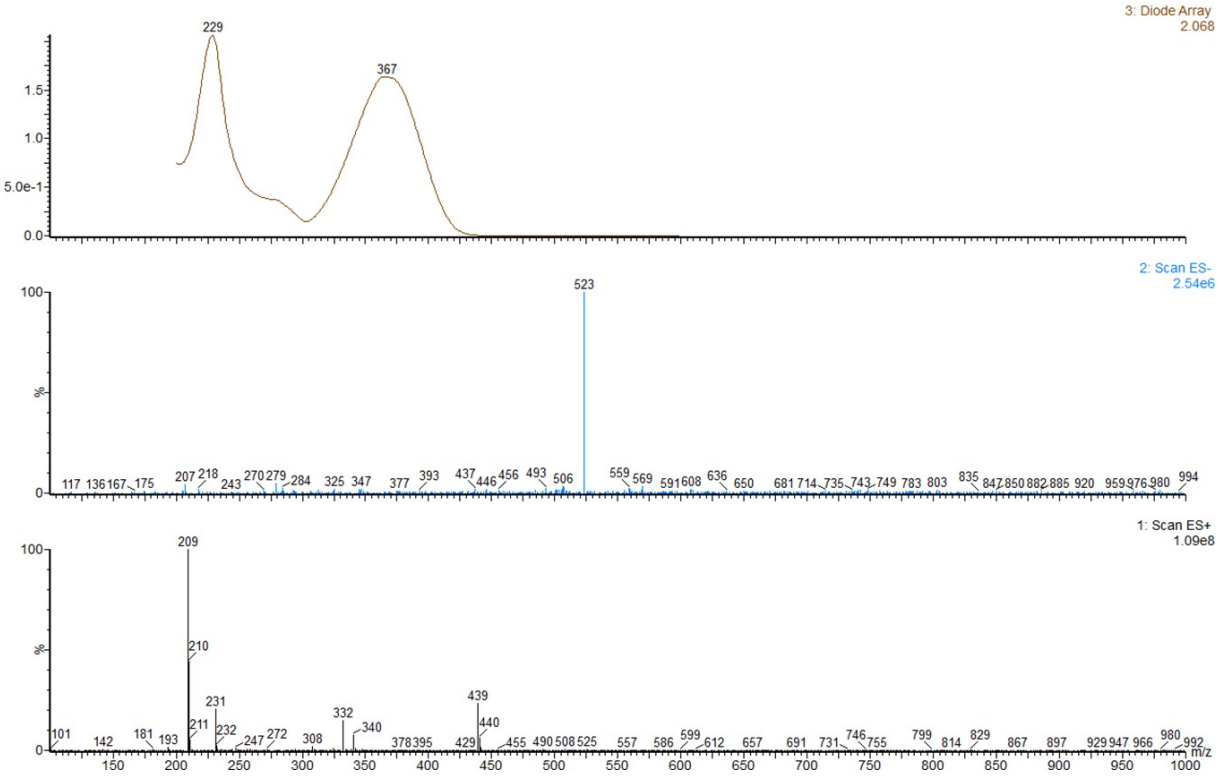


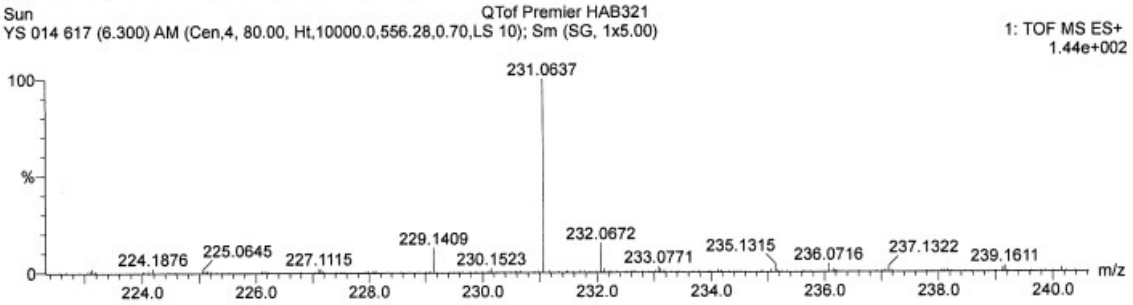
Figure S2.43 UV-absorption (top) and fragmentation pattern of **11** in ES^- (middle) and ES^+ TIC (bottom) by LR-LCMS.

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 20.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Odd and Even Electron Ions
259 formula(e) evaluated with 10 results within limits (up to 30 closest results for each mass)
Elements Used:
C: 0-80 H: 0-100 N: 0-5 O: 0-7 Na: 0-1

Sun QTof Premier HAB321
YS 014 617 (6.300) AM (Cen,4, 80.00, Ht,10000.0,556.28,0.70,LS 10); Sm (SG, 1x5.00)



Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
Minimum:				-1.5			
Maximum:		5.0	20.0	50.0			
231.0637	231.0633	0.4	1.7	5.5	29.3	1.7	C11 H12 O4 Na
	231.0644	-0.7	-3.0	9.0	29.3	1.7	C11 H9 N3 O3
	231.0647	-1.0	-4.3	10.5	29.5	1.8	C12 H8 N4 Na
	231.0620	1.7	7.4	6.0	30.3	2.6	C9 H10 N3 O3 Na
	231.0617	2.0	8.7	4.5	30.6	2.9	C8 H11 N2 O6
	231.0657	-2.0	-8.7	8.5	29.5	1.9	C13 H11 O4
	231.0660	-2.3	-10.0	10.0	29.8	2.1	C14 H10 N O Na
	231.0604	3.3	14.3	5.0	32.1	4.5	C6 H9 N5 O5
	231.0671	-3.4	-14.7	13.5	30.7	3.0	C14 H7 N4
	231.0593	4.4	19.0	1.5	33.0	5.4	C6 H12 N2 O6 Na

Figure S2.44 HRMS data for **1l**; *m/z* (M+Na) calc. mass is 231.0633, 231.0637 was found.

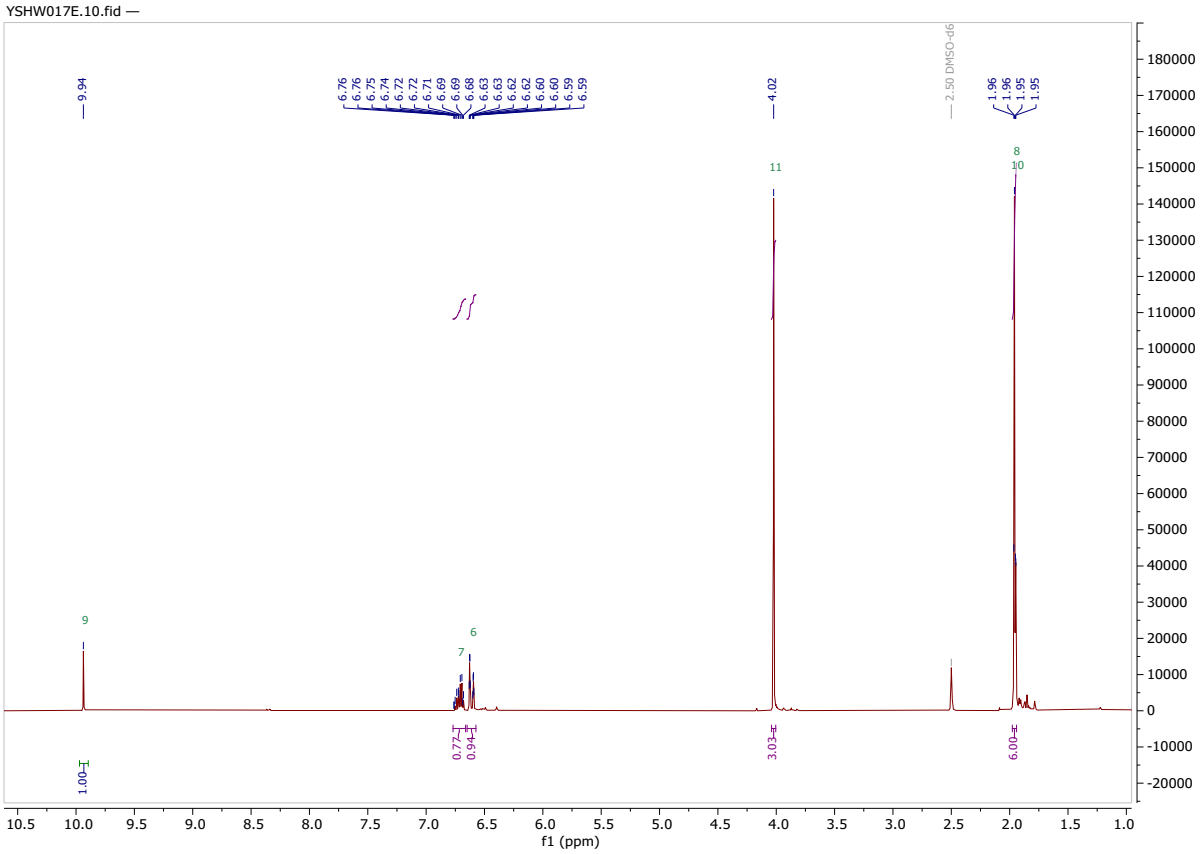


Figure S2.45 ^{1}H-NMR of **11** recorded at 500 MHz in DMSO-d_6.

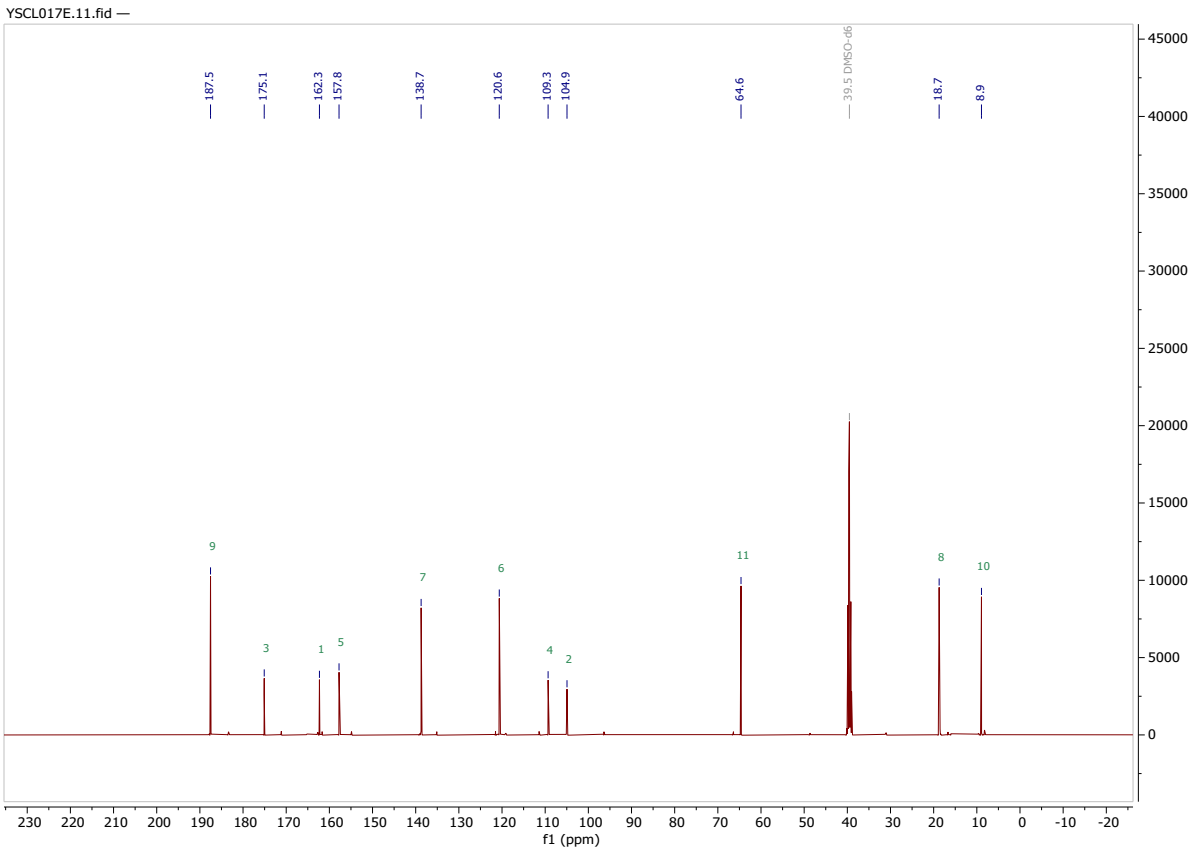


Figure S2.46 ^{13}C-NMR of **11** recorded at 125 MHz in DMSO-d_6.

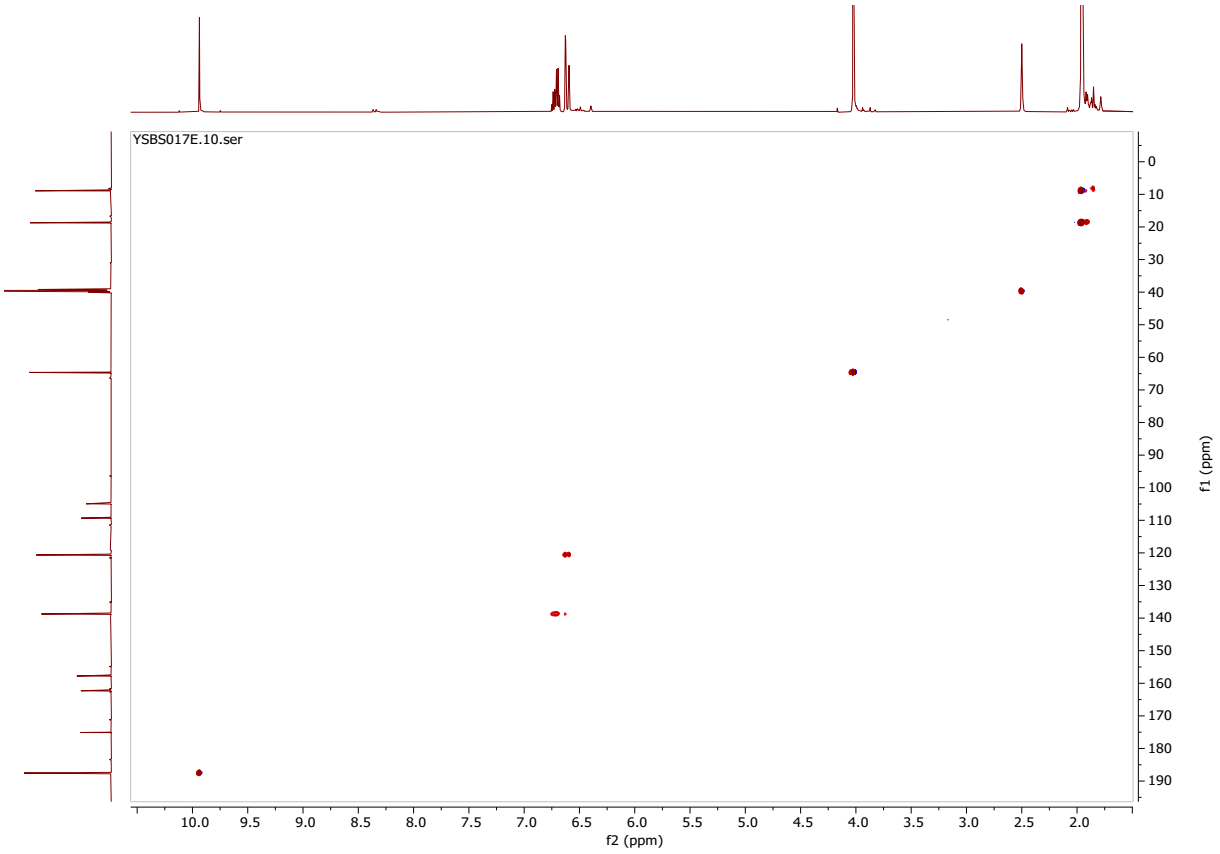


Figure S2.47 HSQC-spectrum of **11** recorded at 500, 125 MHz in DMSO-d_6.

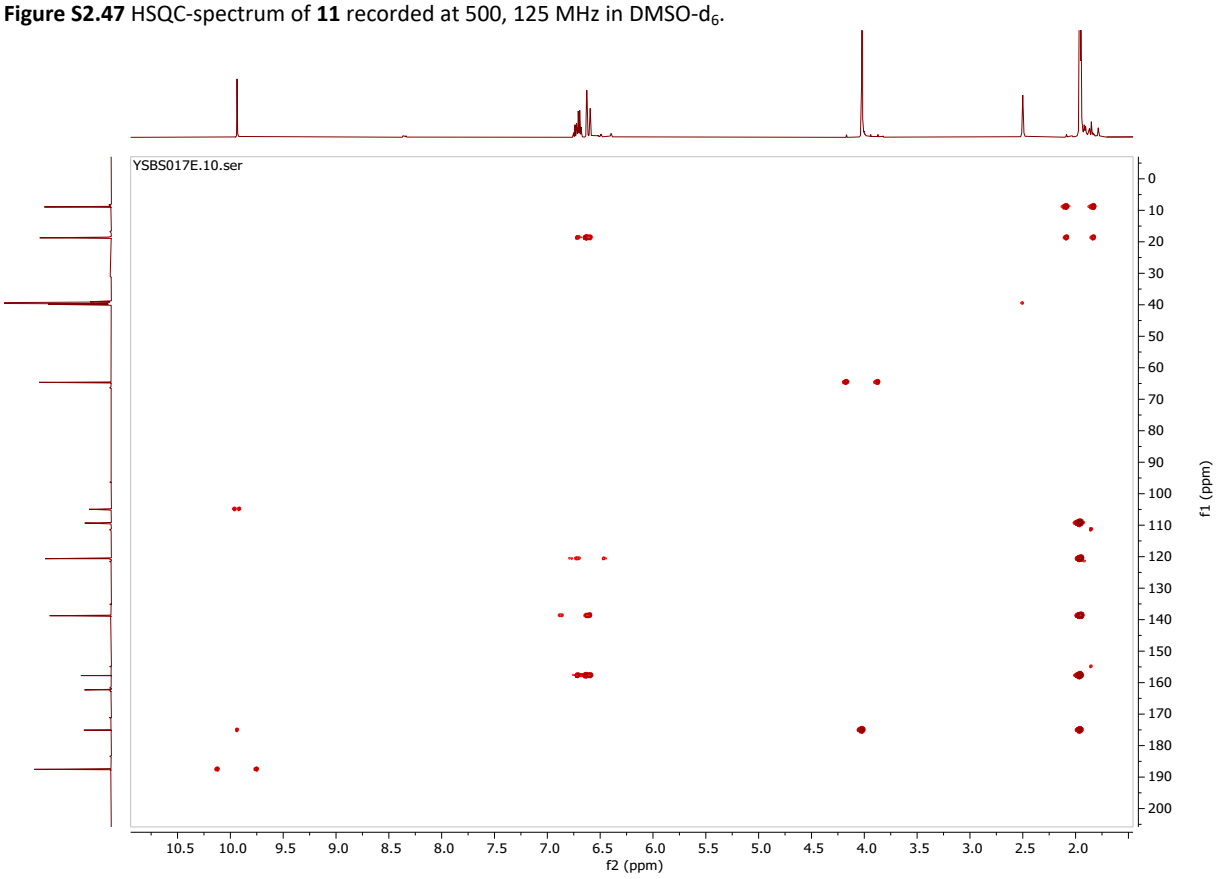


Figure S2.48 HMBC-spectrum of **11** recorded at 500, 125 MHz in DMSO-d_6.

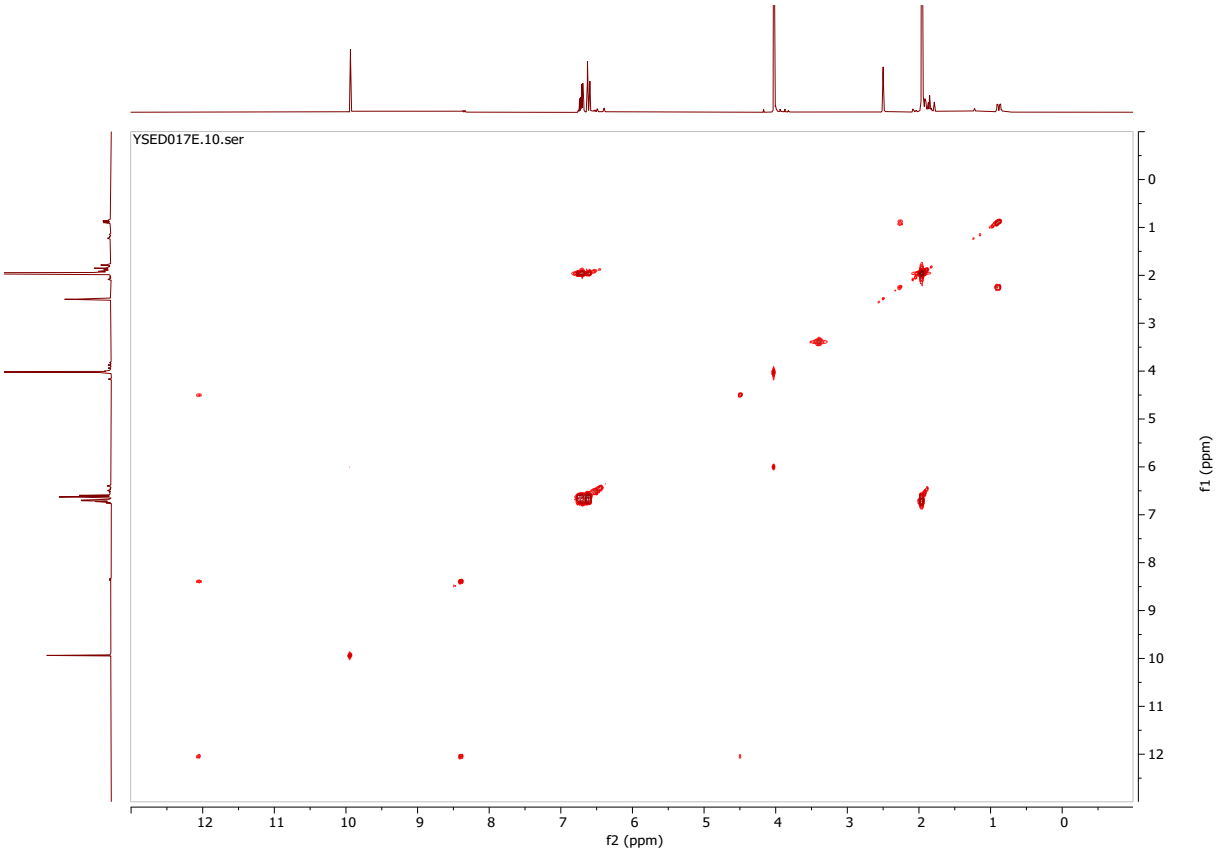
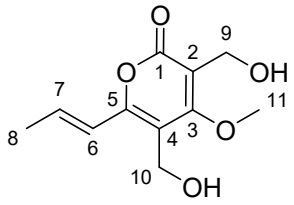


Figure S2.49 ^{1}H, ^{1}H-COSY-spectrum of **11** recorded at 500 MHz in DMSO-d_6.

Compound 1l



1l

Chemical Formula: $C_{11}H_{14}O_5$
Exact Mass: 226.0841

Compound 1l						
Pos.	**δ_C / ppm**	**δ_H / ppm (J/Hz)**	**^{1}H-^{1}H COSY**	**HMBC (H-C)**	**δ_C / ppm literature** [13]	**δ_H / ppm (J/Hz) literature** [13]
1	163.2				164.9	
2	111.0				110.8	
3	169.1				168.8	
4	113.0				111.8	
5	155.7				156.8	
6	120.6	6.57, 1H, m	7, 8	5, 8	119.3	6.42 (dq, 15.3, 1.8)
7	134.6	6.57, 1H, m	6, 8	5, 8	137.5	6.84 (dq, 15.3, 7.0)
8	18.4	1.92, 3H, m	6, 7	5, 6, 7	18.9	1.96(3H, dd, 7.0, 1.8)
9	53.2	4.33, 2H, d (5.1)		1, 2, 3	55.9	4.6 (2H, s)
10	52.6	4.29, 2H, d (4.8)		3, 4, 5	55.1	4.52 (2H, s)
11	62.2	4.05, 3H, s		3	63.1	4.12 (3H, s)

Table S2.8 Summarized NMR signals for ^{13}C, ^{1}H, ^{1}H-^{1}H COSY, HMBC for **1H** recorded in DMSO-d6. Compound from literature [13] was measured in $CDCl_3$.

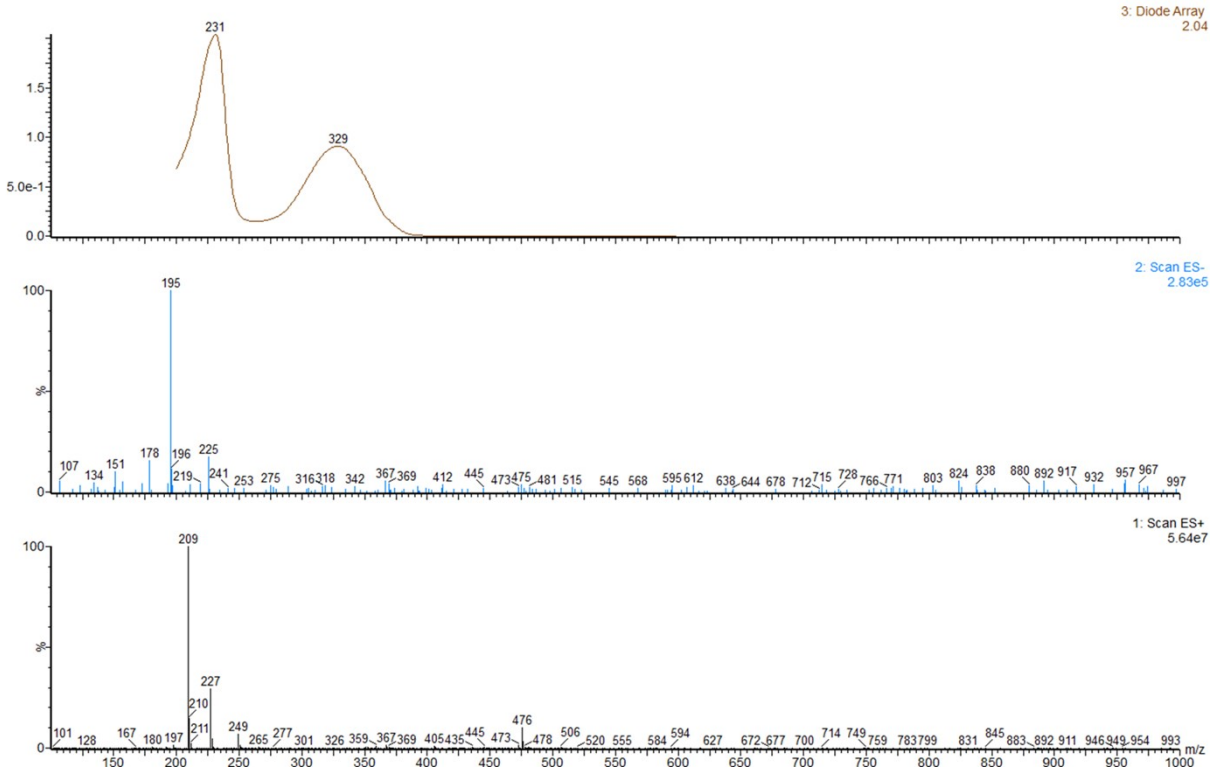


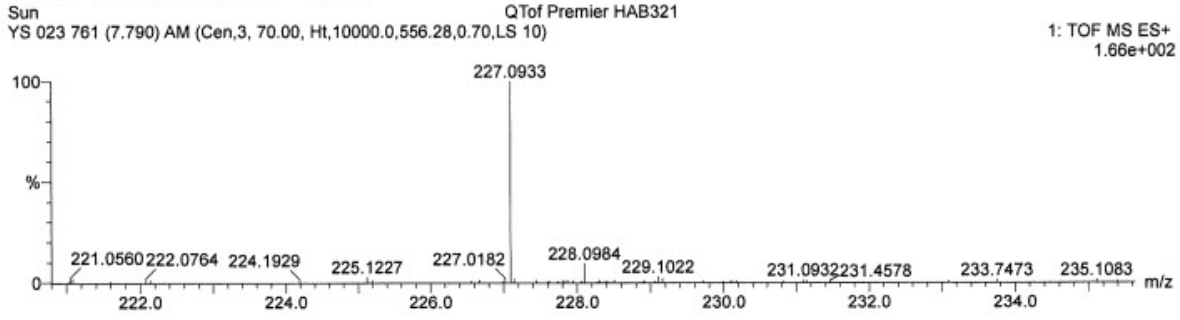
Figure S2.50 UV-absorption (top) and fragmentation pattern of **1H** in ES^- (middle) and ES^+ TIC (bottom) by LR-LCMS.

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 20.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Odd and Even Electron Ions
290 formula(e) evaluated with 9 results within limits (up to 30 closest results for each mass)
Elements Used:
C: 0-80 H: 0-110 N: 0-16 O: 0-10

Sun QTof Premier HAB321
YS 023 761 (7.790) AM (Cen,3, 70.00, Ht,10000.0,556.28,0.70,LS 10)



Minimum:				-1.5			
Maximum:		5.0	20.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
227.0933	227.0933	0.0	0.0	9.5	38.8	1.8	C12 H11 N4 O
	227.0946	-1.3	-5.7	9.0	39.2	2.2	C14 H13 N O2
	227.0919	1.4	6.2	4.5	38.1	1.0	C11 H15 O5

Figure S2.51 HRMS data for **1H**; *m/z* $(M+H)^+$ calc. mass is 227.0919, 227.0933 was found.

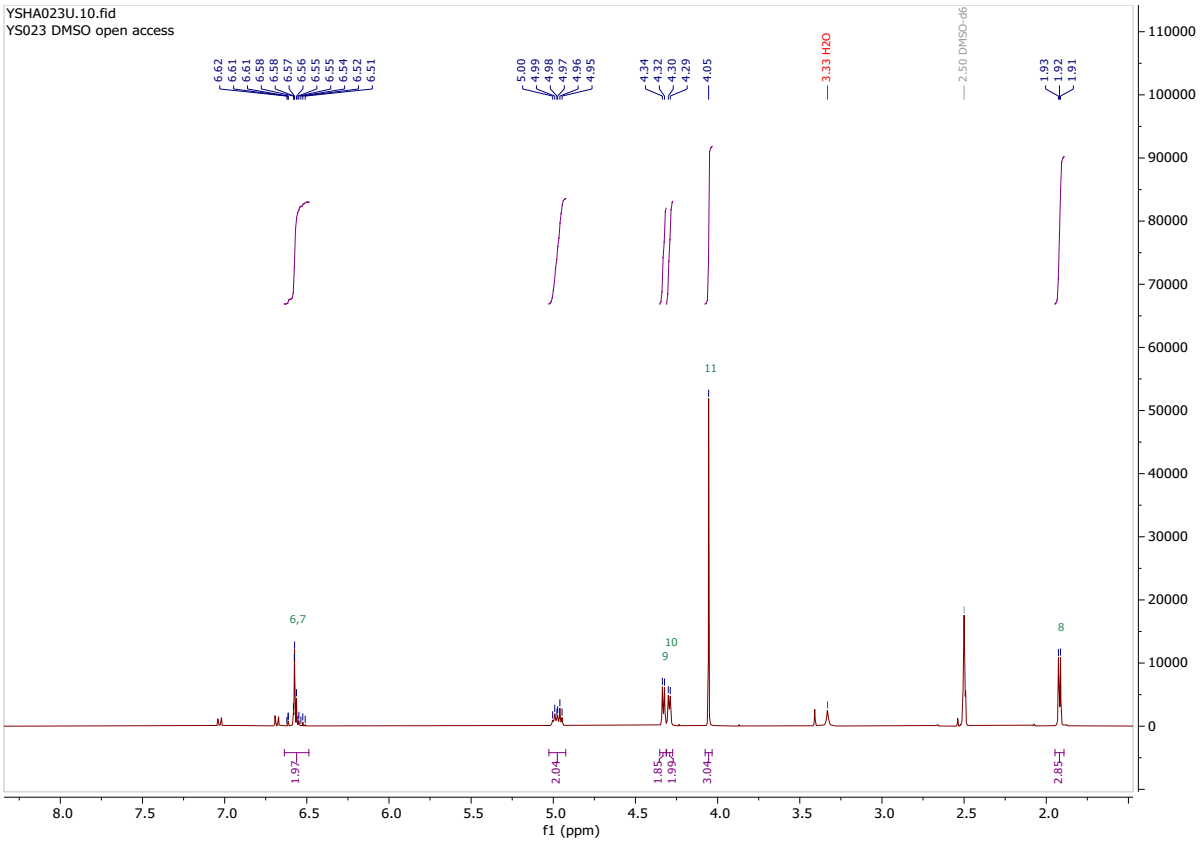


Figure S2.52 ^{1}H-NMR of **1l** recorded at 400 MHz in DMSO-d_6.

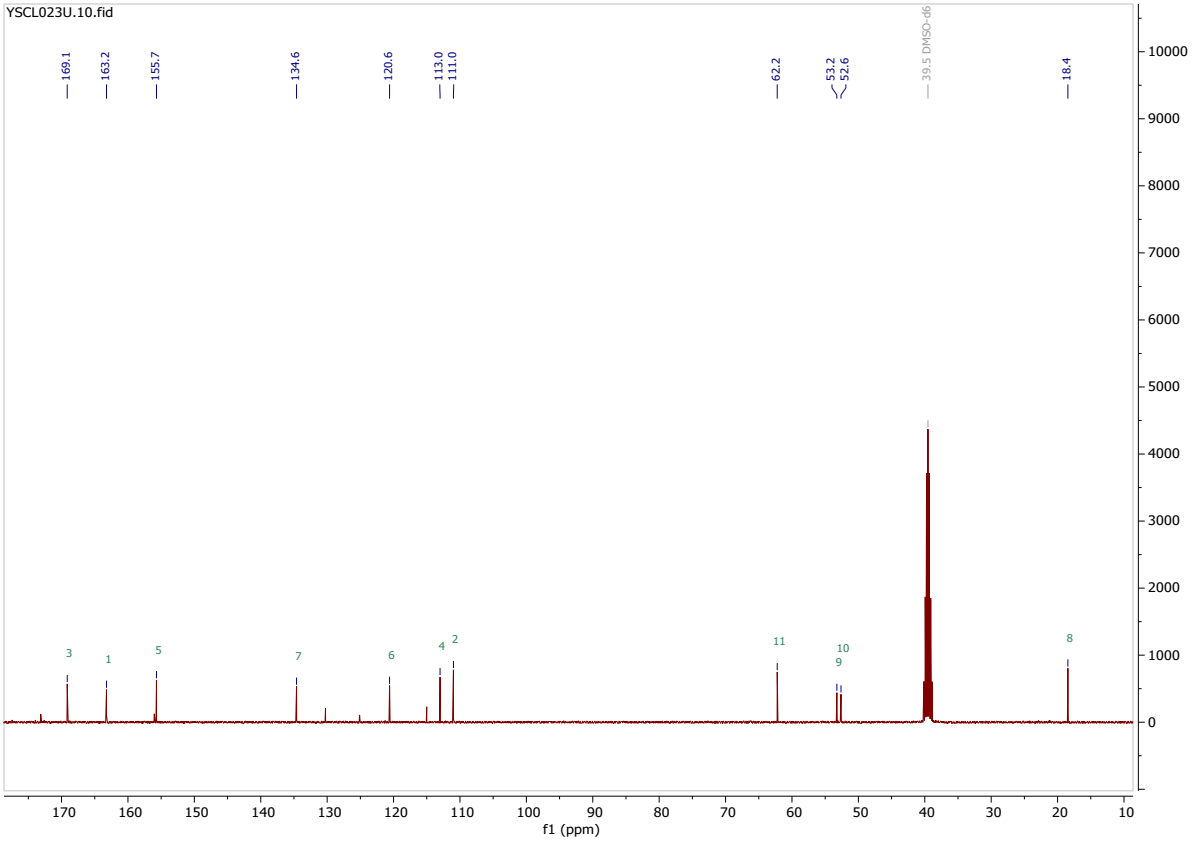


Figure S2.53 ^{13}C-NMR of **1l** recorded at 100 MHz in DMSO-d_6.

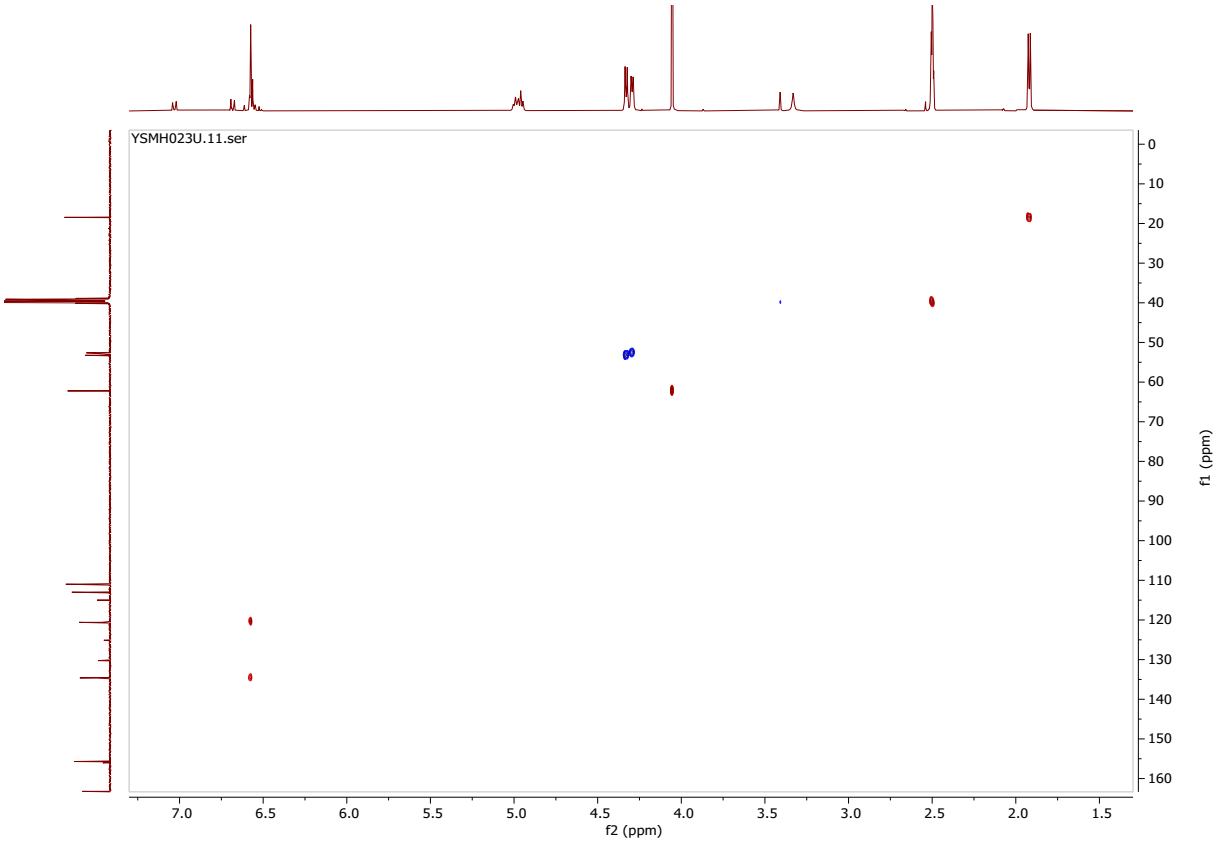


Figure S2.54 HSQC-spectrum of **1l** recorded at 400, 100 MHz in DMSO-d_6.

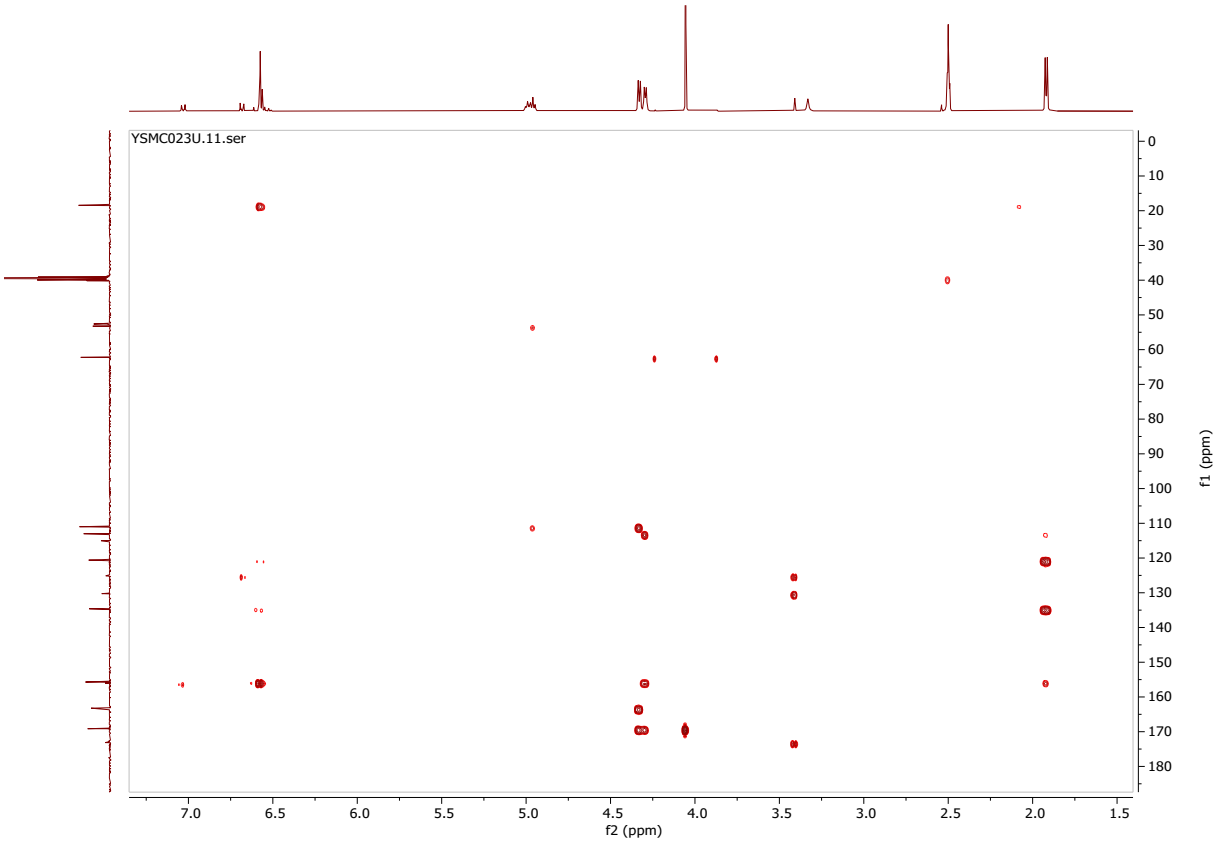


Figure S2.55 HMBC-spectrum of **1l** recorded at 400, 100 MHz in DMSO-d_6.

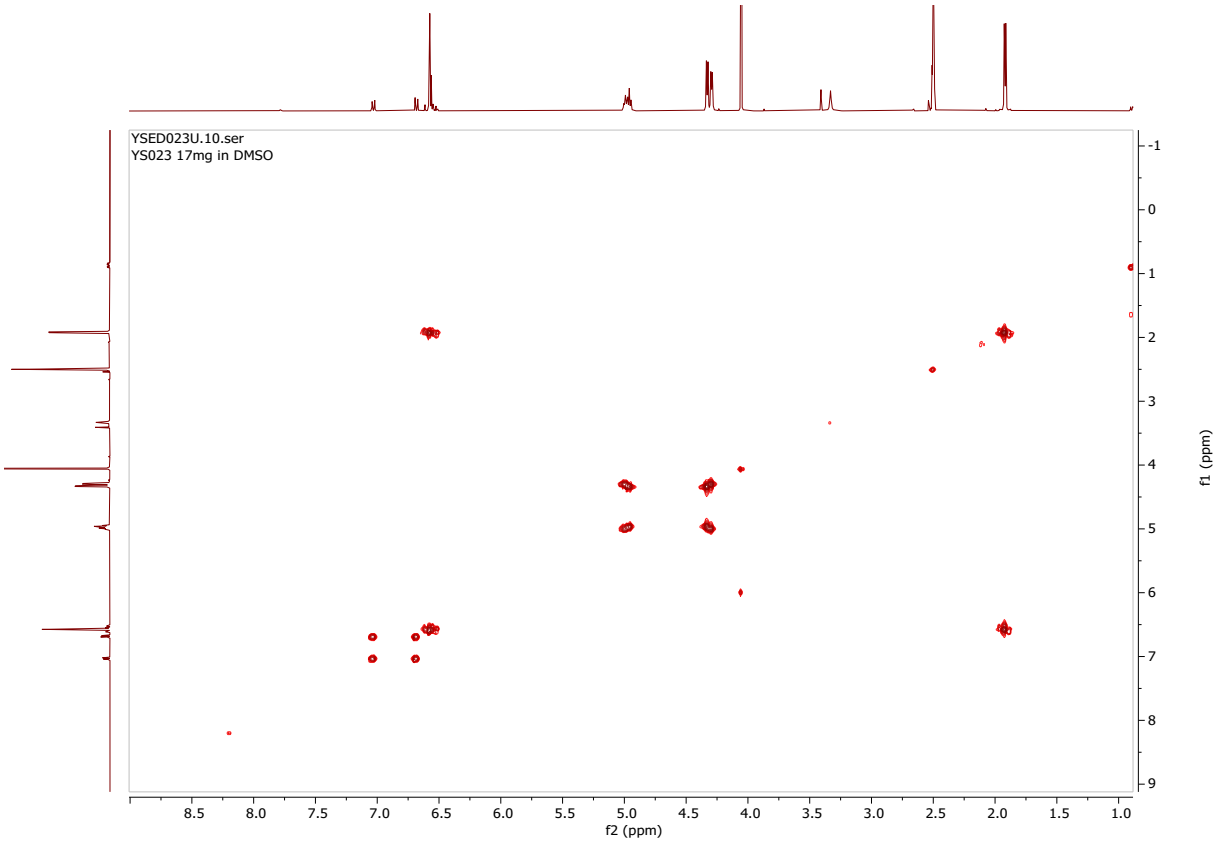
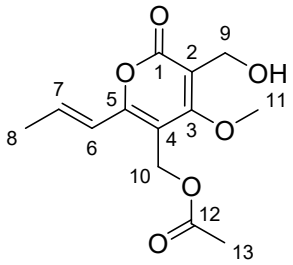


Figure S2.56 ^{1}H, ^{1}H-COSY-spectrum of **1l** recorded at 400 MHz in DMSO-d_6.

Compound 1H



1H

Chemical Formula: $C_{13}H_{16}O_6$
Exact Mass: 268.0947

Compound 1H						
Pos.	**δ_C / ppm**	**δ_H / ppm (J/Hz)**	**^{1}H-^{1}H COSY**	**HMBC (H-C)**	**δ_C / ppm literature** [13]	**δ_H / ppm (J/Hz) literature** [13]
1	164.8				164.7	
2	111.0				110.8	
3	168.9				168.8	
4	108.0				107.9	
5	158.1				158.0	
6	119.5	6.39, 1H, dddd (15.3, 1.7, 1.7, 1.7)	7, 8	5, 7, 8	119.3	6.39 (dq, 15.1, 1.7)
7	138.4	6.86, 1H, dddd (15.3, 7.0, 7.0, 7.0)	6, 8	5, 6, 8	138.2	6.86 (dq, 15.1, 7.0)
8	19.0	1.96, 3H, dd (7.0, 1.7)	6, 7	6, 7	18.9	1.97(3H, dd, 7.0, 1.7)
9	56.1	4.59, 2H, s		1, 2, 3	55.9	4.59, (2H, s)
10	56.3	4.97, 2H, s		3, 4, 5, 12	56.2	4.97, (2H, s)
11	63.3	4.06, 3H, s		3	63.2	4.07 (s)
12	170.9				170.8	
13	21.0	2.08, 3H, s		12	20.9	2.08(3H, s)

Table S2.9 Summarized NMR signals for ^{13}C, ^{1}H, ^{1}H-^{1}H COSY, HMBC for **1H** recorded in $CDCl_3$. Compound from literature [13] was measured in $CDCl_3$.

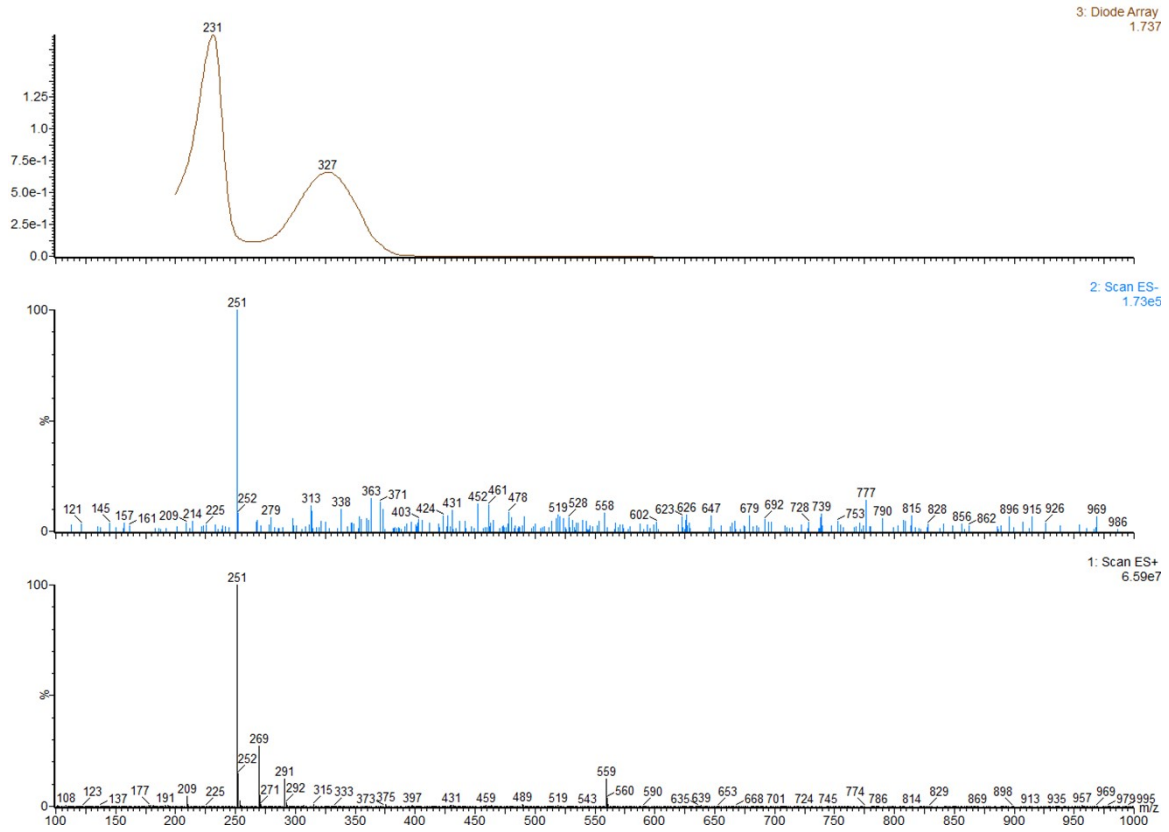


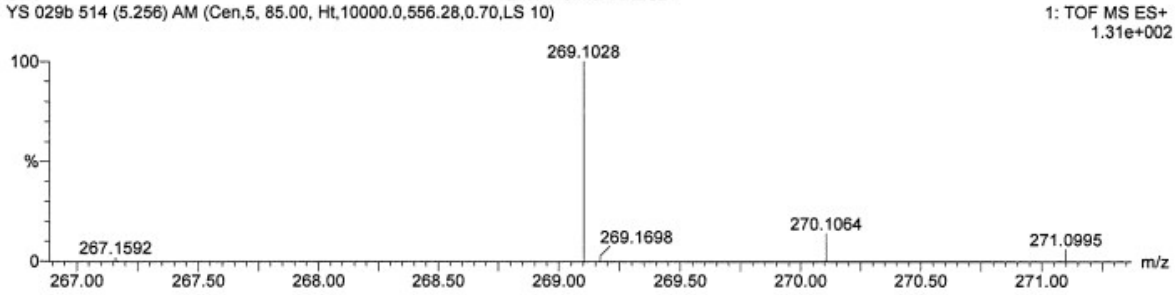
Figure S2.57 UV-absorption (top) and fragmentation pattern of **1H** in ES^- (middle) and ES^+ TIC (bottom) by LR-LCMS.

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 20.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
87 formula(e) evaluated with 4 results within limits (up to 30 closest results for each mass)
Elements Used:
C: 0-85 H: 0-110 O: 0-9 S: 0-2

Sun QTof Premier HAB321
YS 029b 514 (5.256) AM (Cen,5, 85.00, Ht,10000.0,556.28,0.70,LS 10)



Minimum:				-1.5			
Maximum:		5.0	20.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
269.1028	269.1025	0.3	1.1	5.5	8.8	1.9	C13 H17 O6
	269.1034	-0.6	-2.2	4.5	8.1	1.2	C14 H21 O S2
	269.1000	2.8	10.4	9.5	8.5	1.6	C17 H17 O S
	269.1059	-3.1	-11.5	0.5	7.9	1.0	C10 H21 O6 S

Figure S2.58 HRMS data for **1H**; *m/z* $(M+H)^+$ calc. mass is 269.1025, 269.1028 was found.

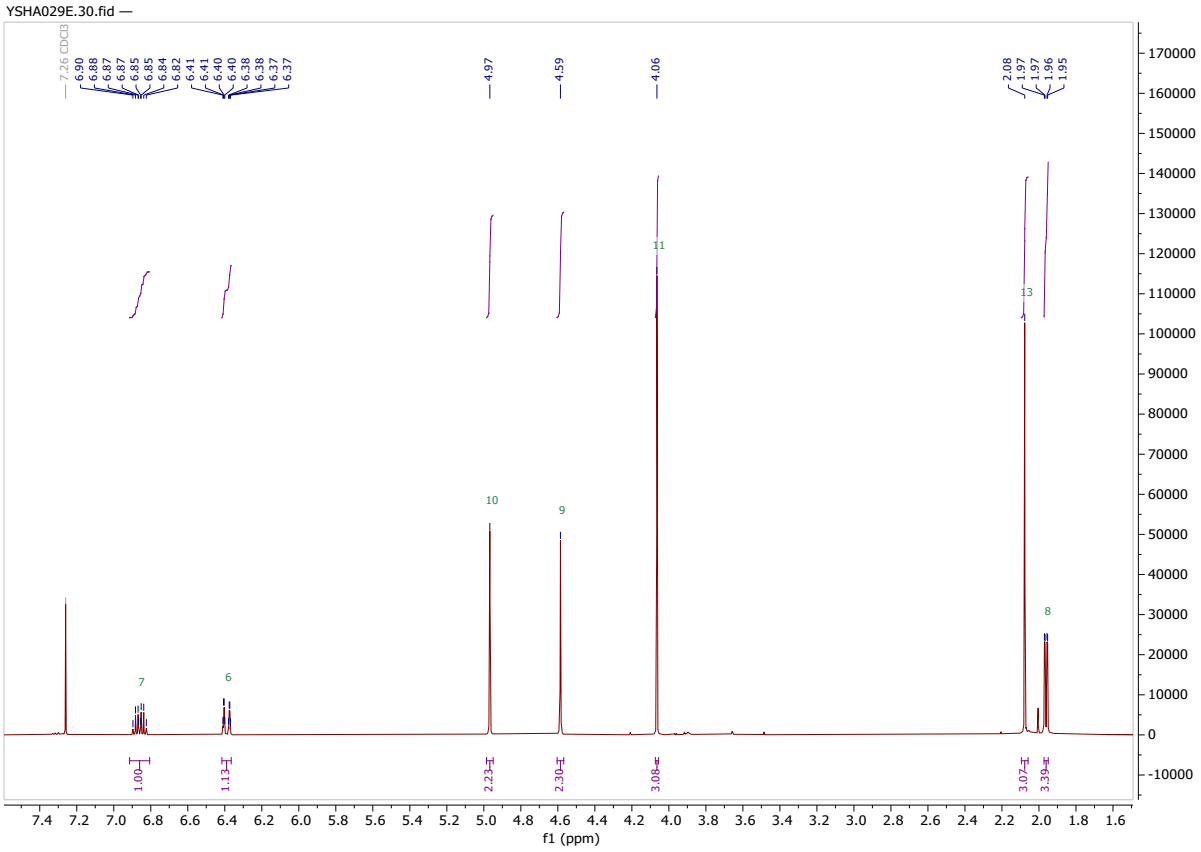


Figure S2.59 ^{1}H-NMR of **1H** recorded at 500 MHz in $CDCl_3$.

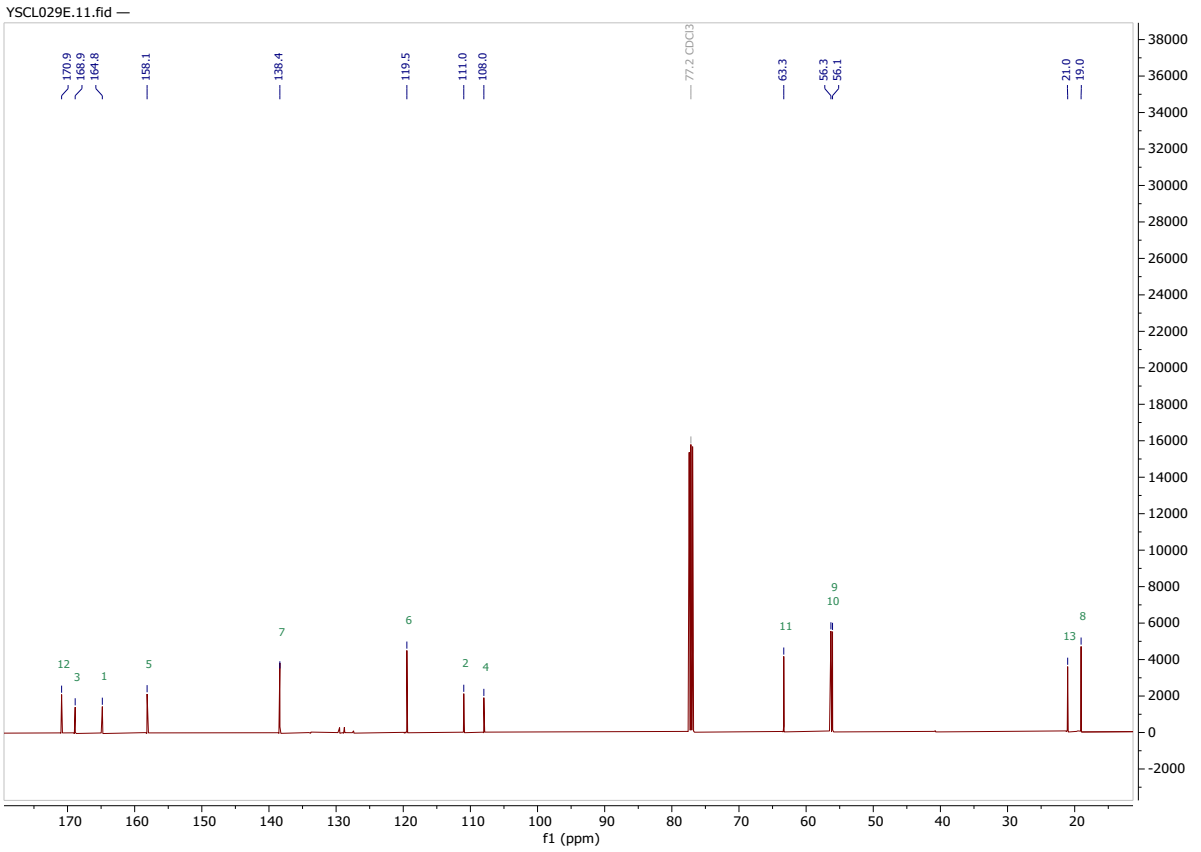


Figure S2.60 ^{13}C-NMR of **1H** recorded at 125 MHz in $CDCl_3$.

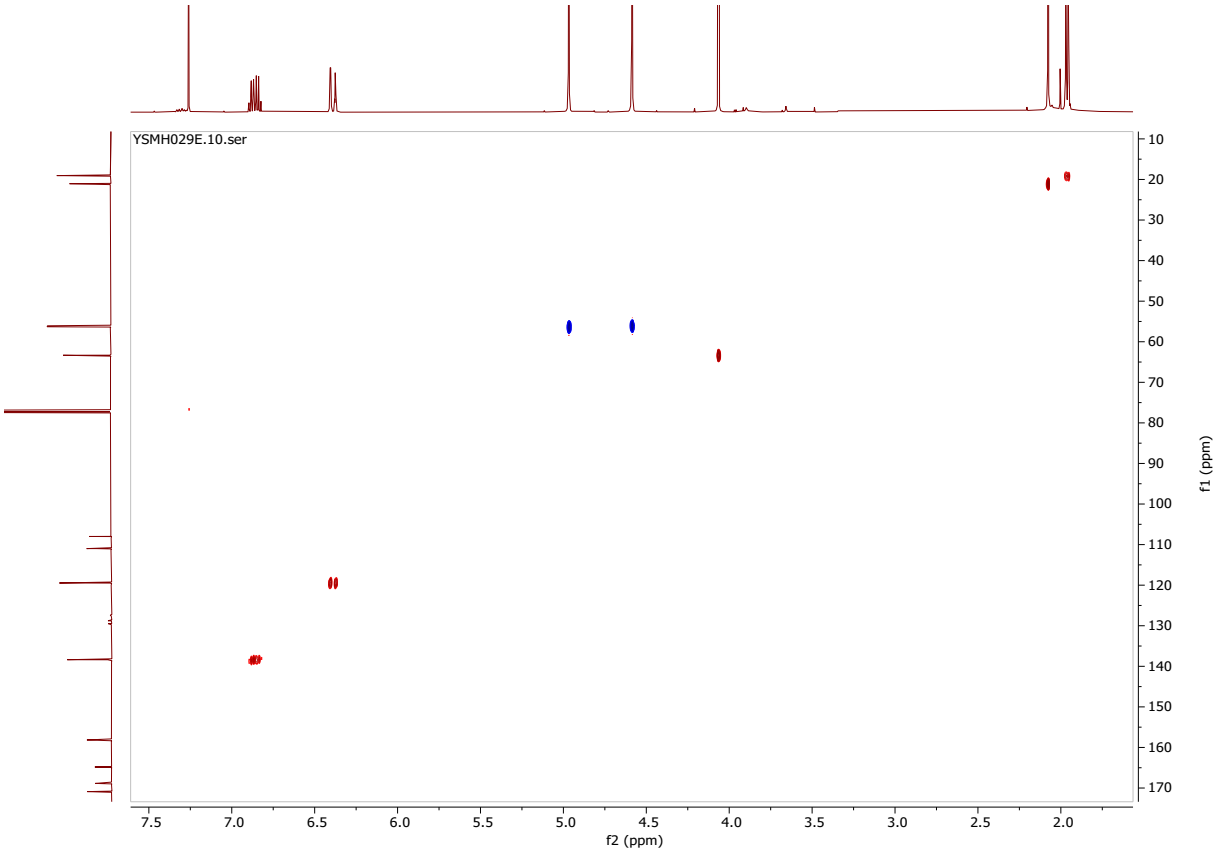


Figure S2.61 HSQC-spectrum of **1H** recorded at 500, 125 MHz in $CDCl_3$.

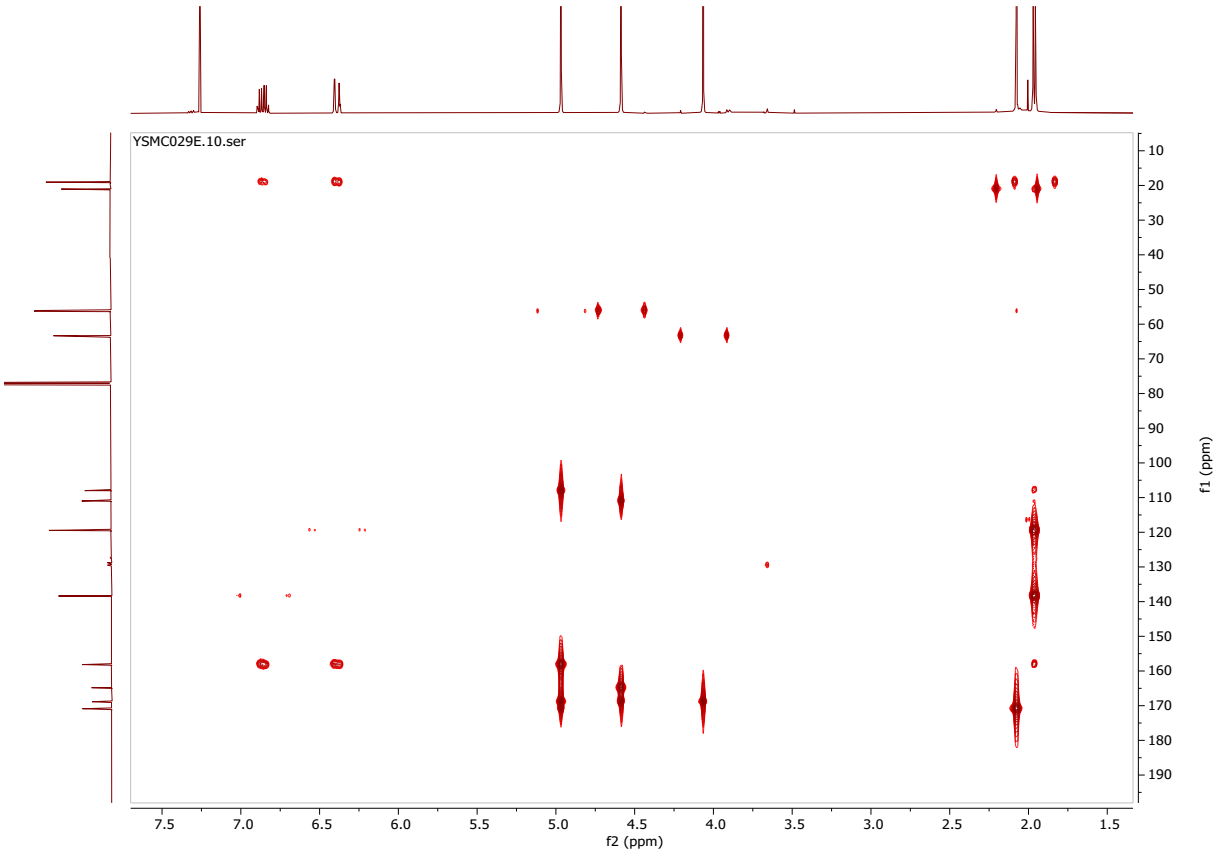


Figure S2.62 HMBC-spectrum of **1H** recorded at 500, 125 MHz in $CDCl_3$.

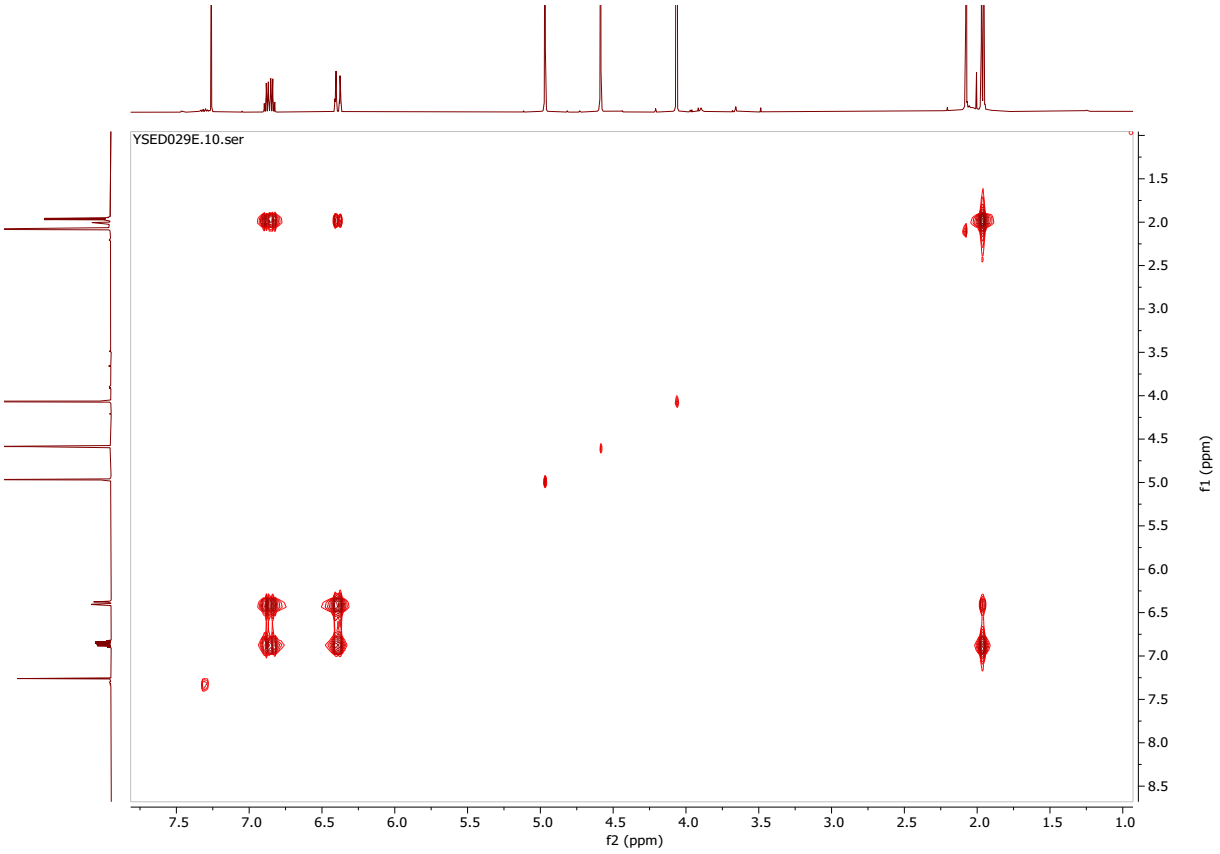
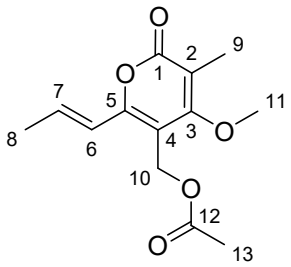


Figure S2.63 ^{1}H, ^{1}H-COSY-spectrum of **1H** recorded at 500 MHz in $CDCl_3$.

Compound 12



12

Chemical Formula: $C_{13}H_{16}O_5$
Exact Mass: 252.0998

Compound 12				
Pos.	**δ_C / ppm**	**δ_H / ppm (J/Hz)**	**^{1}H-^{1}H COSY**	**HMBC (H-C)**
1	166.7			
2	110.9			
3	169.8			
4	110.0			
5	157.7			
6	120.9	6.54, 1H, dddd (15.3, 1.7, 1.7, 1.7)	7, 8	5, 7, 8
7	137.2	6.75, 1H, dddd (15.3, 7.0, 6.9, 6.9)	6, 8	5, 8
8	18.8	1.95, 3H, dd (7.0, 1.7)	6, 7	6, 7
9	10.6	2.04, 3H, s		1, 2, 3
10	57.3	5.01, 2H, s		3, 4, 5, 12
11	62.1	3.92, 3H, s		3
12	172.4			
13	20.7	2.05, 3H, s		12

Table S2.10 Summarized NMR signals for ^{13}C, ^{1}H, ^{1}H-^{1}H COSY, HMBC for **12** recorded in CD_3OD.

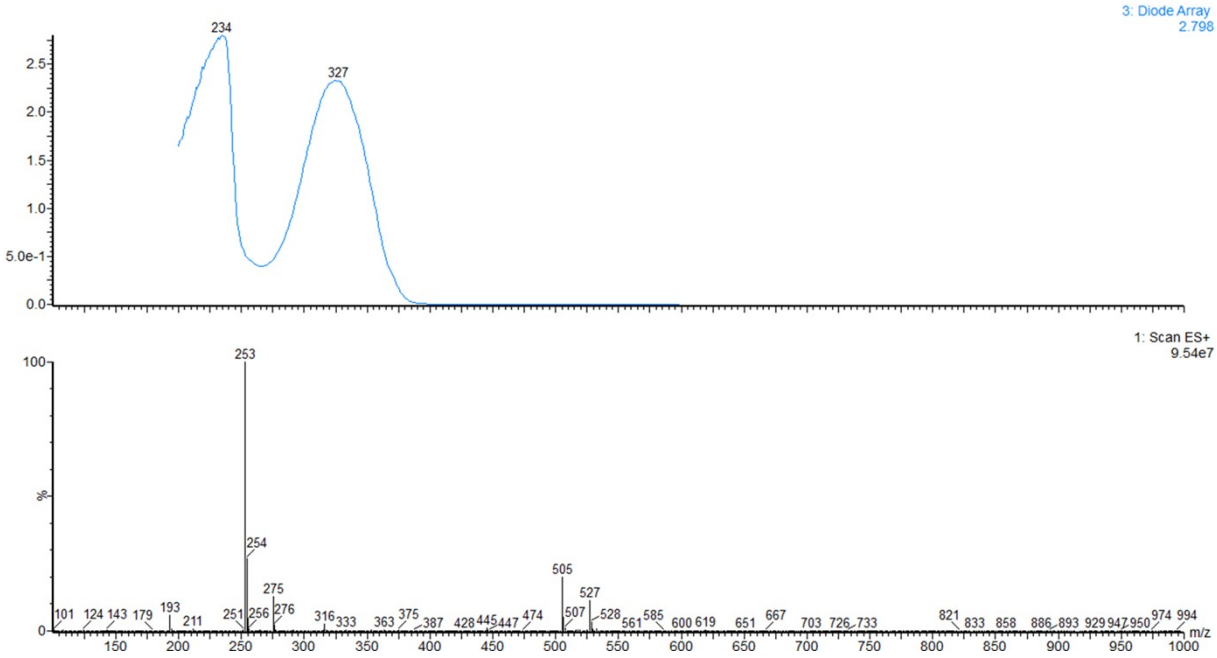


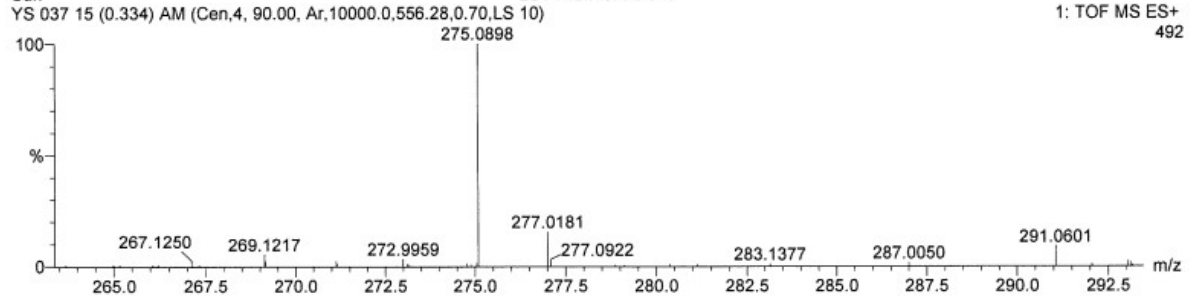
Figure S2.64 UV-absorption (top) and fragmentation pattern of **12** in ES$^+$ TIC (bottom) by LR-LCMS.

Elemental Composition Report **Page 1**

Single Mass Analysis (displaying only valid results)
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Selected filters: None

Monoisotopic Mass, Even Electron Ions
186 formula(e) evaluated with 2 results within limits (up to 40 closest results for each mass)
Elements Used:
C: 0-72 H: 0-50 N: 0-1 O: 0-6 Na: 0-1 S: 0-1

Sun LCT Premier KD070
YS 037 15 (0.334) AM (Cen,4, 90.00, Ar,10000.0,556.28,0.70,LS 10)



Minimum:				-1.5		
Maximum:		5.0	5.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
275.0898	275.0895	0.3	1.1	5.5	2773237.3	C13 H16 O5 Na
	275.0894	0.4	1.5	12.5	2773249.3	C19 H15 S

Figure S2.65 HRMS data for **12**; *m/z* (M+Na) calc. mass is 275.0895, 275.0898 was found.

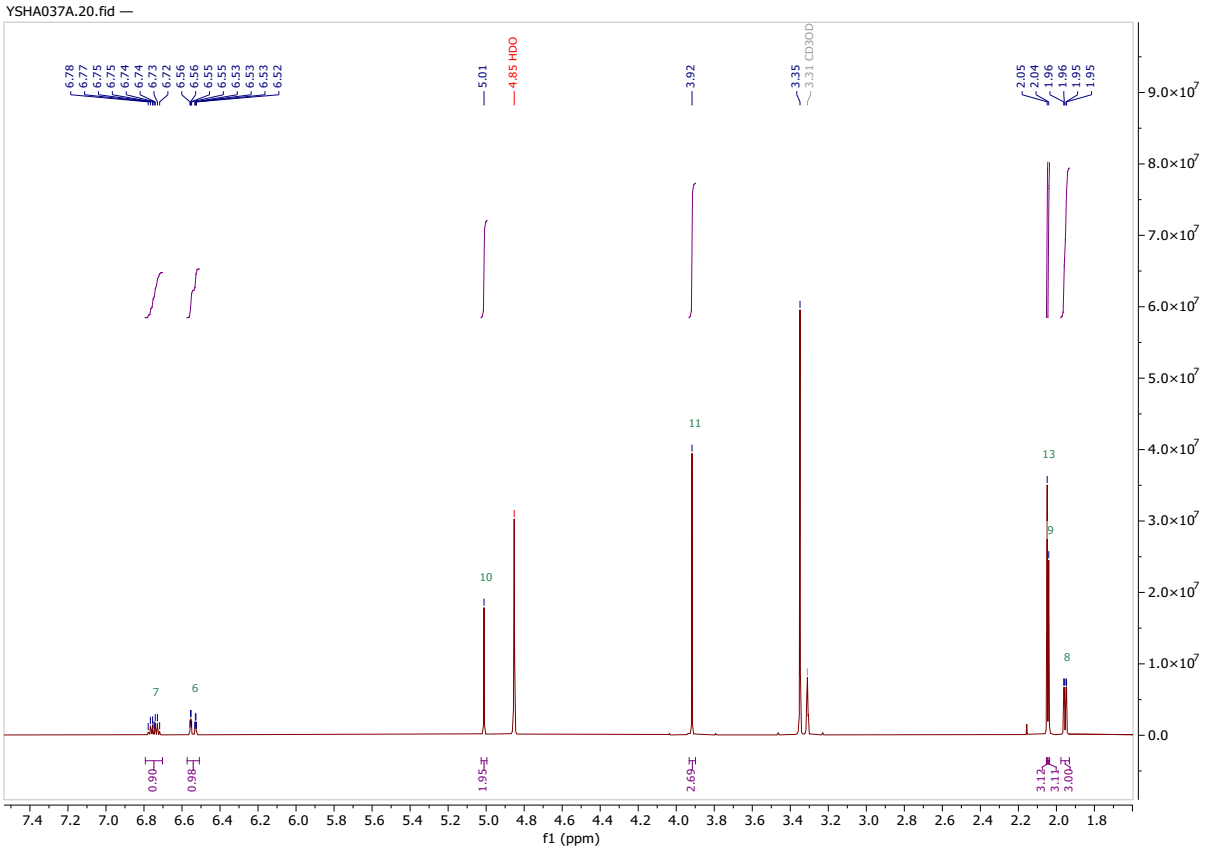


Figure S2.66 ^{1}H-NMR of **12** recorded at 600 MHz in CD_3OD.

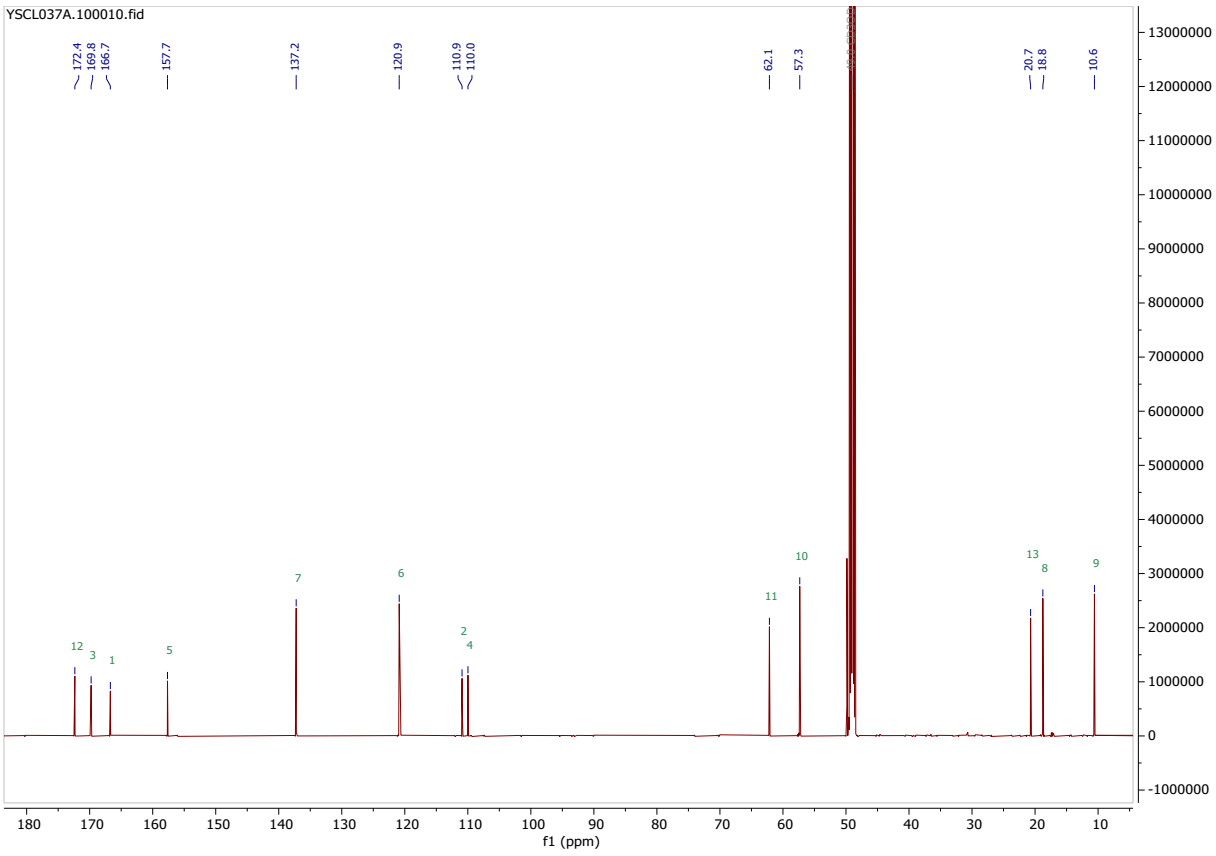


Figure S2.67 ^{13}C-NMR of **12** recorded at 150 MHz in CD_3OD.

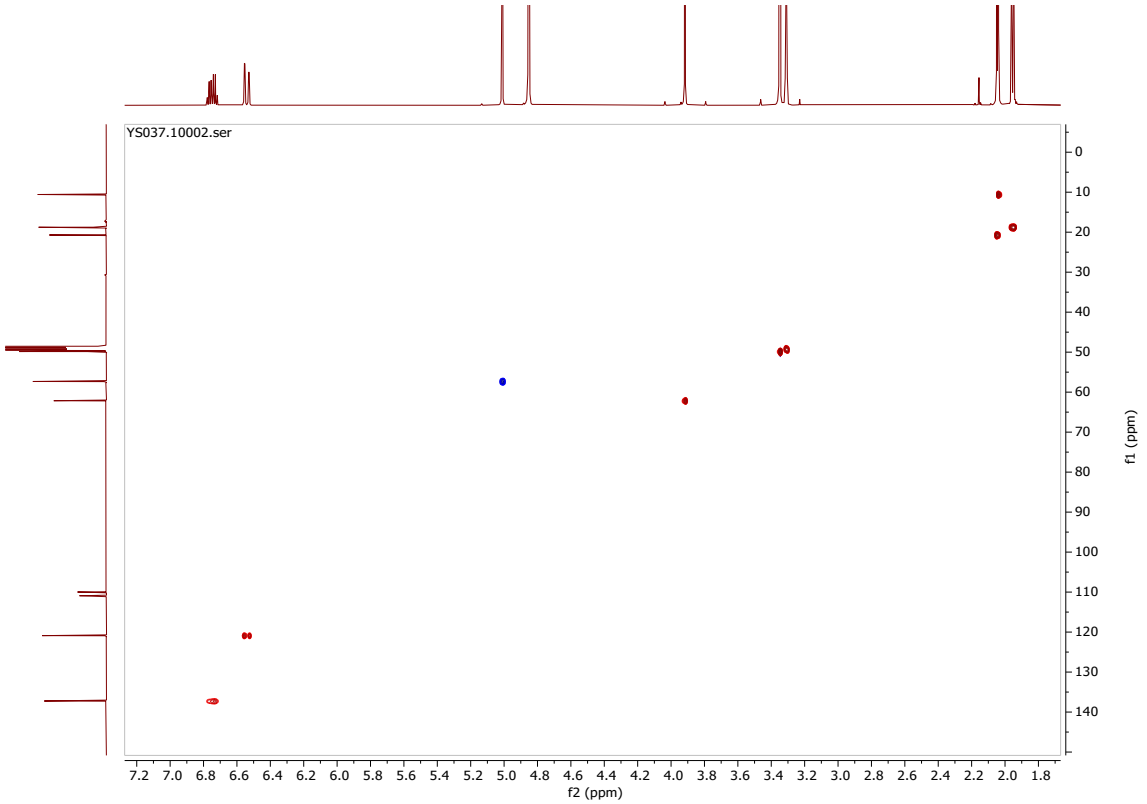


Figure S2.68 HSQC-spectrum of **12** recorded at 600, 150 MHz in CD_3OD.

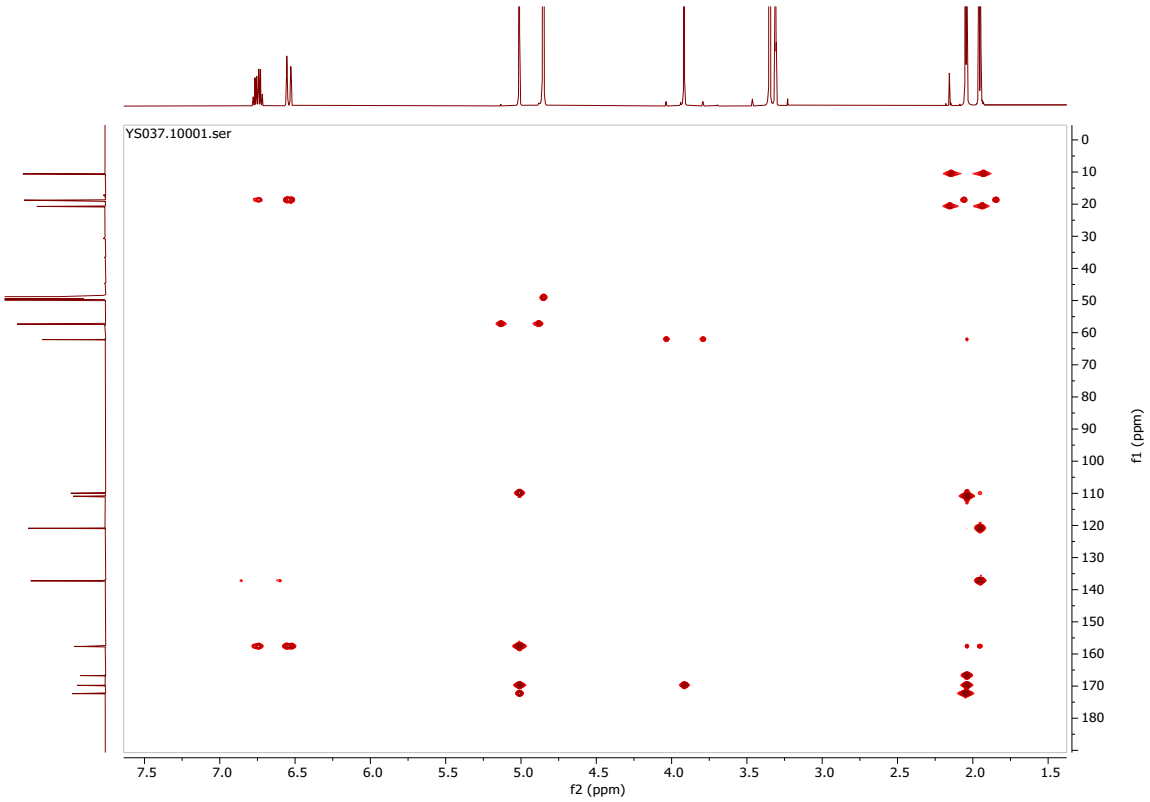


Figure S2.69 HMBC-spectrum of **12** recorded at 600, 150 MHz in CD_3OD.

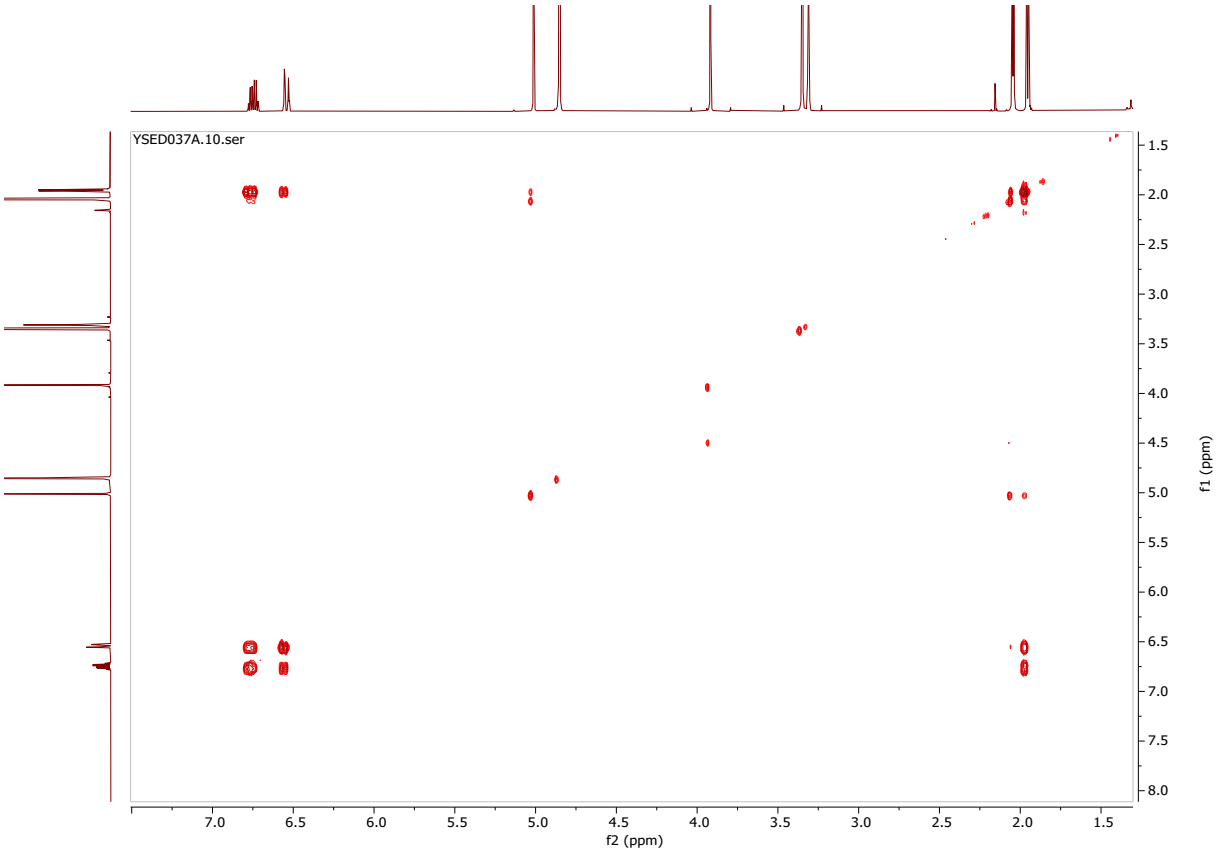
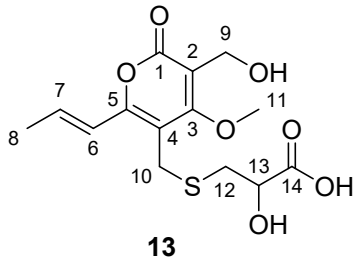


Figure S2.70 ^{1}H, ^{1}H-COSY-spectrum of **12** recorded at 600 MHz in CD_3OD.

Compound 13



13

Chemical Formula: $C_{14}H_{18}O_7S$
Exact Mass: 330.0773

Compound 13				
Pos.	**δ_C / ppm**	**δ_H / ppm (J/Hz)**	**^{1}H-^{1}H COSY**	**HMBC (H-C)**
1	166.5			
2	111.2			
3	171.3			
4	112.2			
5	156.8			
6	121.5	6.62, 1H, dddd (15.2, 1.7, 1.6, 1.6)	7, 8	5, 7, 8
7	137.1	6.73, 1H, dddd (15.3, 6.8, 6.8, 6.8)	6, 8	5, 6, 8
8	18.8	1.97, 3H, dd (6.9, 1.6)	6, 7	5, 6, 7
9	55.3	4.54, 2H, s		1, 2, 3
10	26.8	3.77, 2H, s		3, 4, 5, 12
11	63.7	4.21, 3H,s		3
12	37.3	2.86, 1H, dd (14.1, 6.4) 3.0, 1H, dd (14.2, 4.1)	12, 13 12, 13	10, 13, 14 10, 13, 14
13	72.4	4.38, 1H, dd (6.5, 4.1)	12	12, 14
14	176.2			

Table S2.11 Summarized NMR signals for ^{13}C, ^{1}H, ^{1}H-^{1}H COSY, HMBC for **13** recorded in CD_3OD.

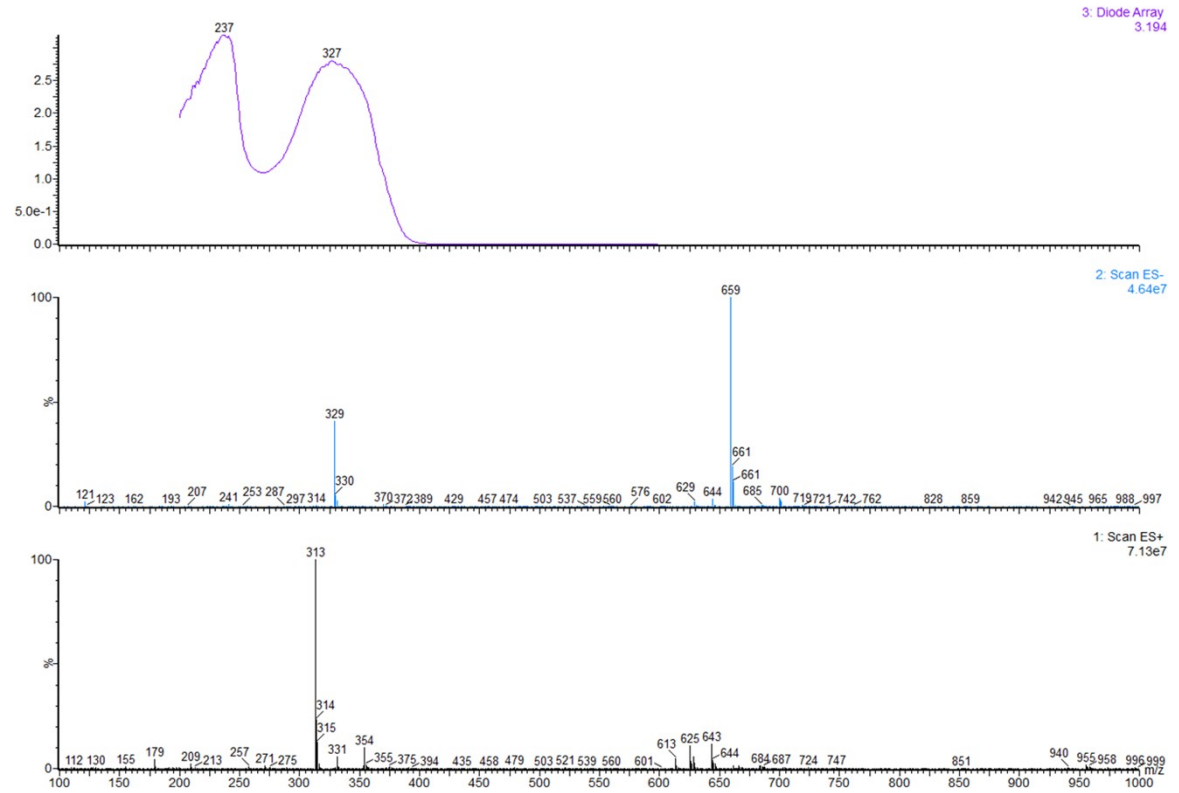


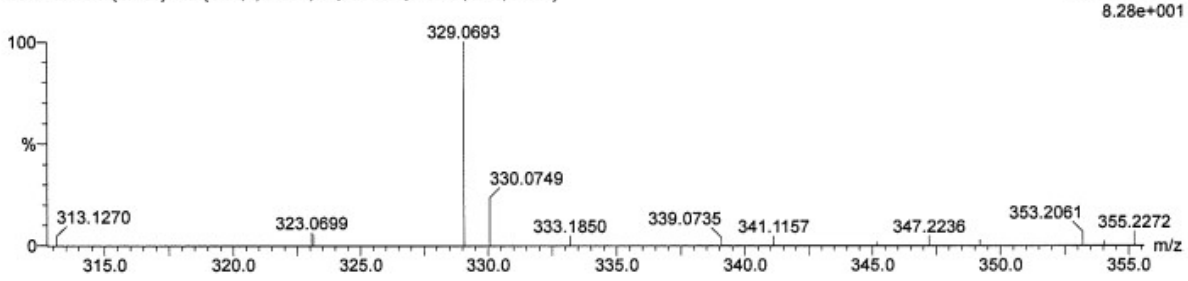
Figure S2.71 UV-absorption (top) and fragmentation pattern of **13** in ES^- (middle) and ES^+ TIC (bottom) by LR-LCMS.

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 20.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
116 formula(e) evaluated with 6 results within limits (up to 30 closest results for each mass)
Elements Used:
C: 0-85 H: 0-110 O: 0-7 S: 0-3

Sun QTof Premier HAB321
YS 019d 433 (4.428) AM (Cen,5, 85.00, Ht,10000.0,554.26,0.70,LS 10)



Minimum:				-1.5			
Maximum:		5.0	20.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
329.0693	329.0695	-0.2	-0.6	6.5	11.7	2.5	C14 H17 O7 S
	329.0704	-1.1	-3.3	5.5	14.0	4.8	C15 H21 O2 S3
	329.0670	2.3	7.0	10.5	13.6	4.4	C18 H17 O2 S2
	329.0661	3.2	9.7	11.5	9.4	0.1	C17 H13 O7
	329.0729	-3.6	-10.9	1.5	13.9	4.6	C11 H21 O7 S2
	329.0636	5.7	17.3	15.5	13.5	4.2	C21 H13 O2 S

Figure S2.72 HRMS data for **13**; *m/z* $(M-H)^-$ calc. mass is 329.0695, 329.0693 was found.

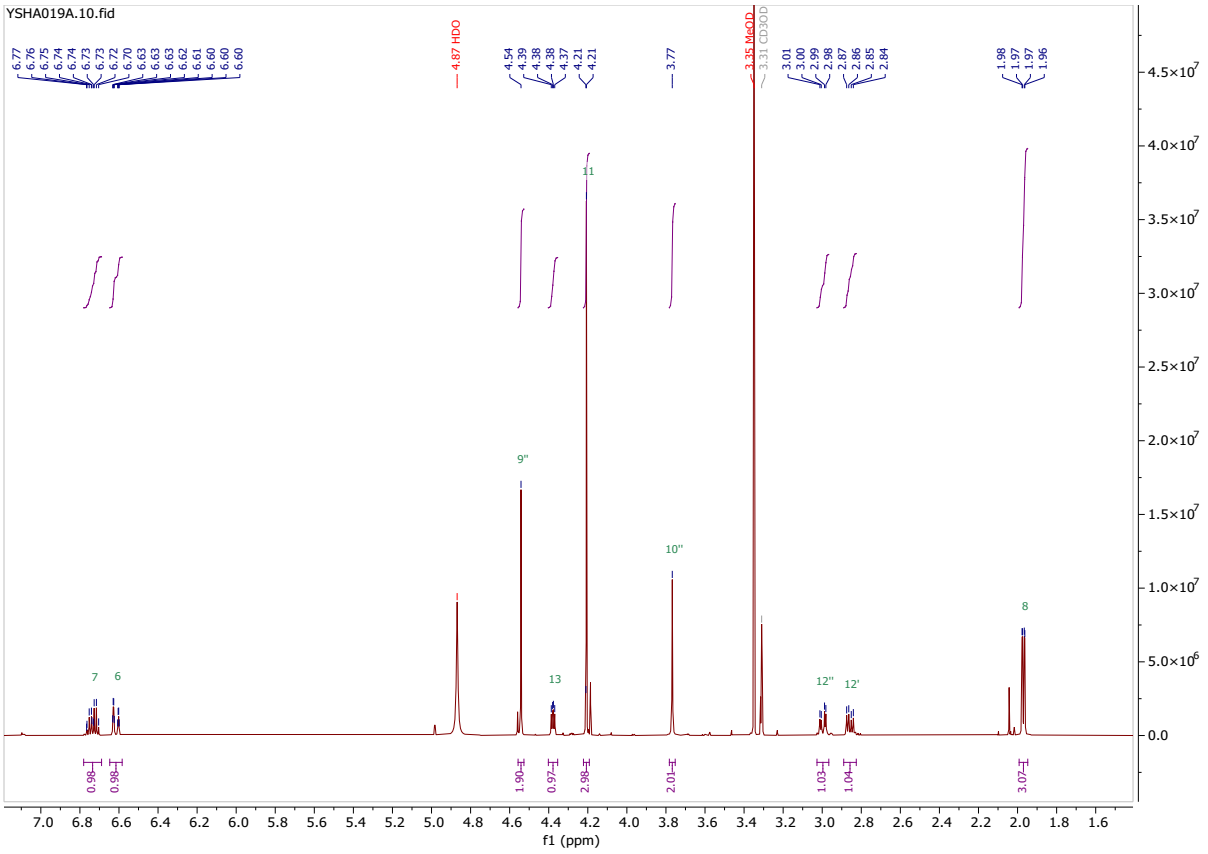


Figure S2.73 ^{1}H-NMR of **13** recorded at 600 MHz in CD_3OD.

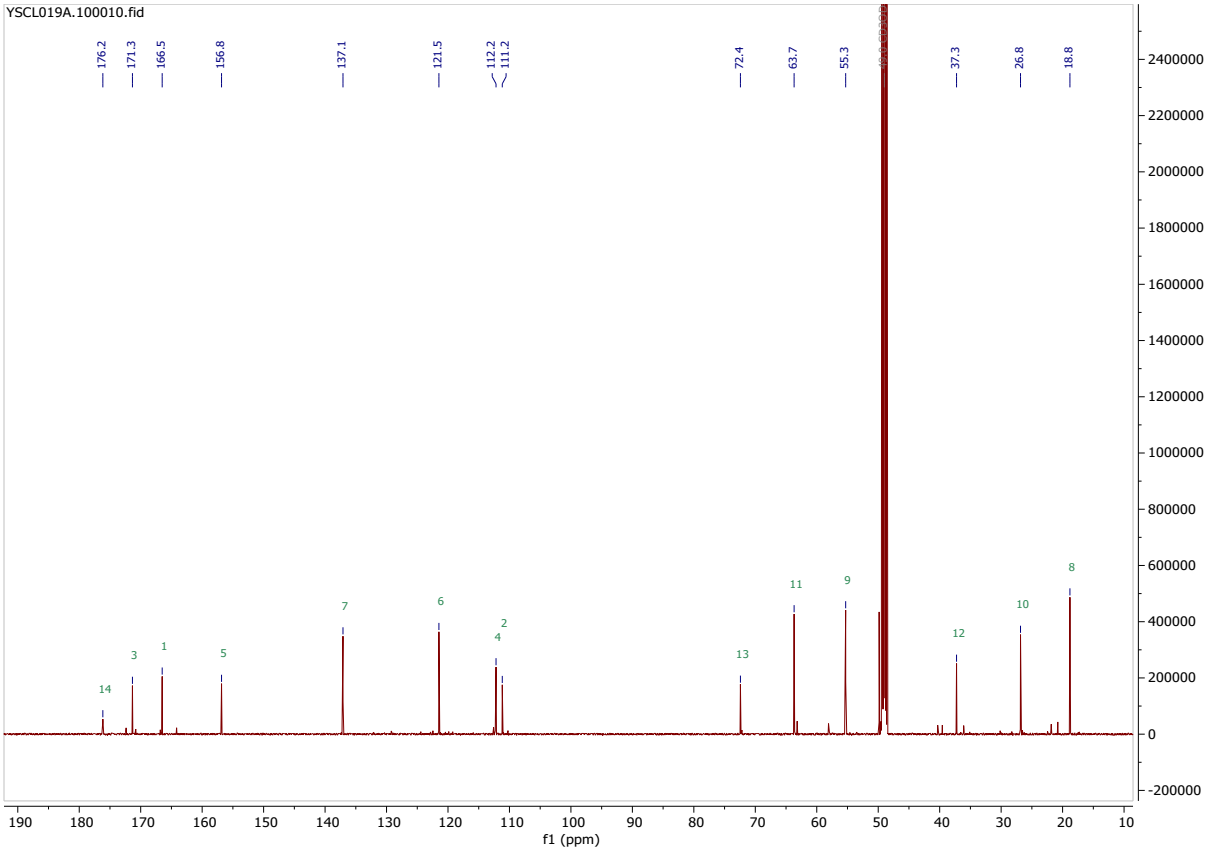


Figure S2.74 ^{13}C-NMR of **13** recorded at 150 MHz in CD_3OD.

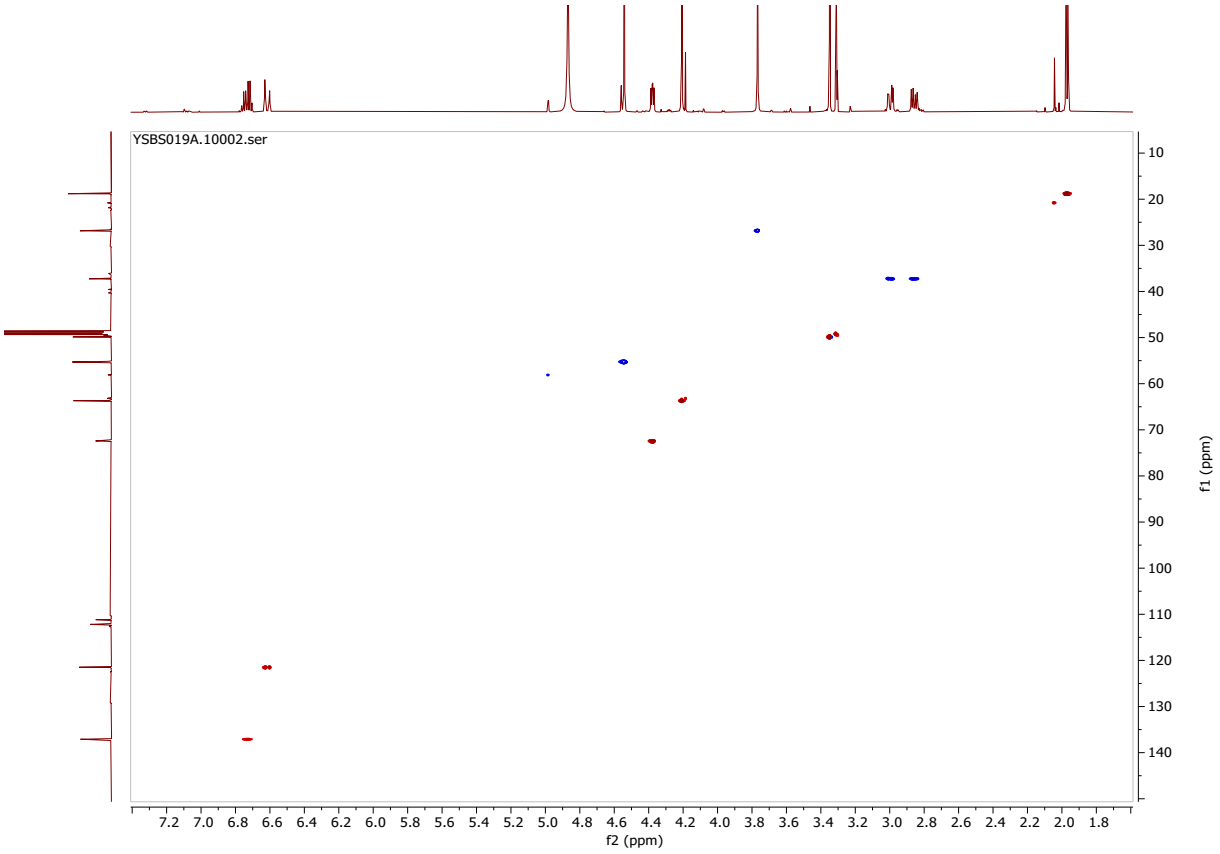


Figure S2.75 HSQC-spectrum of **13** recorded at 600, 150 MHz in CD_3OD.

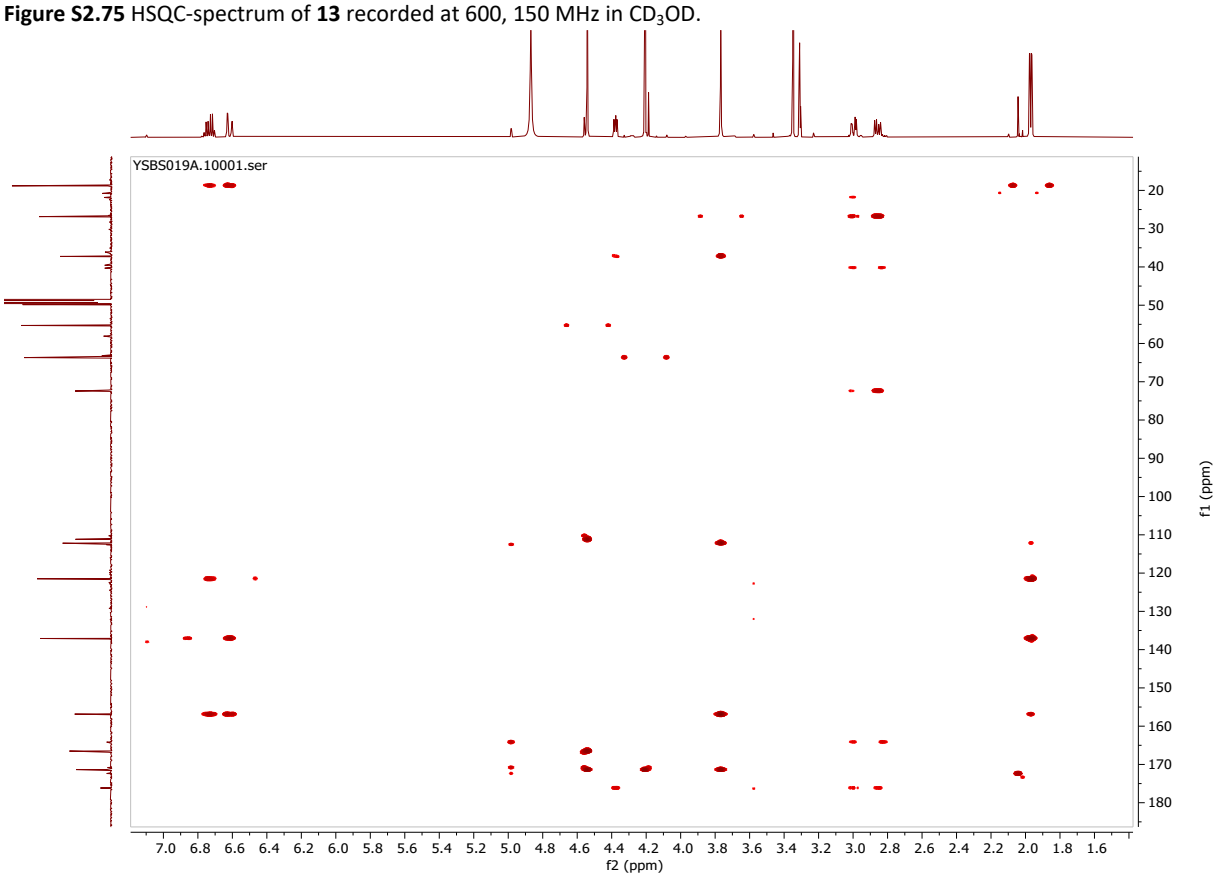


Figure S2.76 HMBC-spectrum of **13** recorded at 600, 150 MHz in CD_3OD.

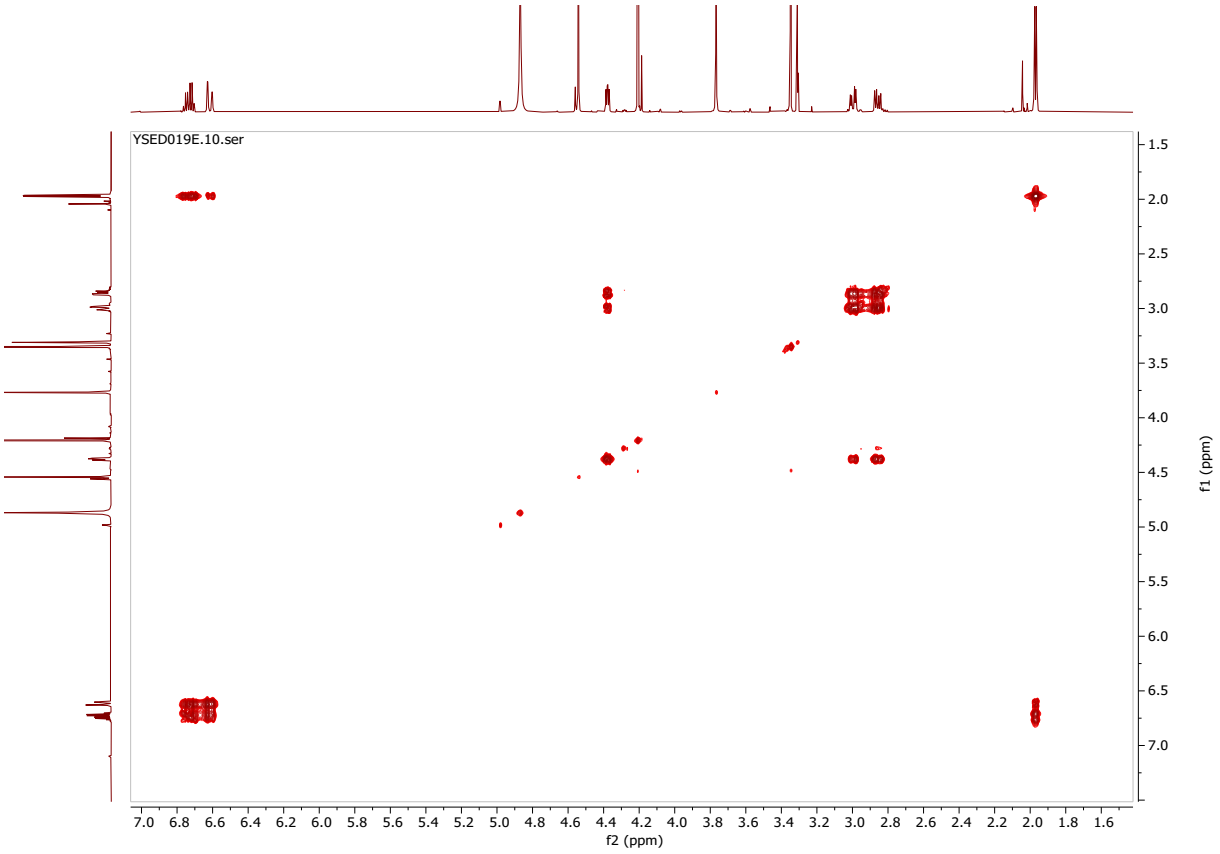
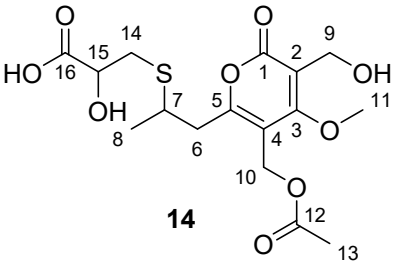


Figure S2.77 ^{1}H, ^{1}H-COSY-spectrum of **13** recorded at 600 MHz in CD_3OD.

Compound 14



14

Chemical Formula: $C_{16}H_{22}O_9S$
Exact Mass: 390.0985

Compound 14				
Pos.	**δ_C / ppm**	**δ_H / ppm (J/Hz)**	**^{1}H-^{1}H COSY**	**HMBC (H-C)**
1	166.8			
2	110.2			
3	170.8			
4	112.6			
5	164.2			
6	39.6	2.83, 1H, dd (14.7, 7.2) 3.01, 1H, dd (14.7, 7.7)	6, 7 6, 7	4, 5, 7 4, 5, 7
7	40.3	3.36, 1H, m	6, 8, 14	5, 6, 14
8	21.8	1.34, 3H, d (6.8)	7	7
9	55.2	4.56, 2H, s		1, 2, 3
10	58.1	4.99, 2H, d (1.8)	13	3, 4, 5, 12
11	63.2	4.19, 3H, s		3
12	172.4			
13	20.8	2.04, 3H, s		10, 12
14	36.1	2.85, 1H, m 2.97, 1H, m	7, 15 7, 15	7, 15, 16 7, 15, 16
15	72.2	4.28, 1H, dd (6.7, 4.0)	14	14, 16
16	176.2			

Table S2.12 Summarized NMR signals for ^{13}C, ^{1}H, ^{1}H-^{1}H COSY, HMBC for **14** recorded in CD_3OD.

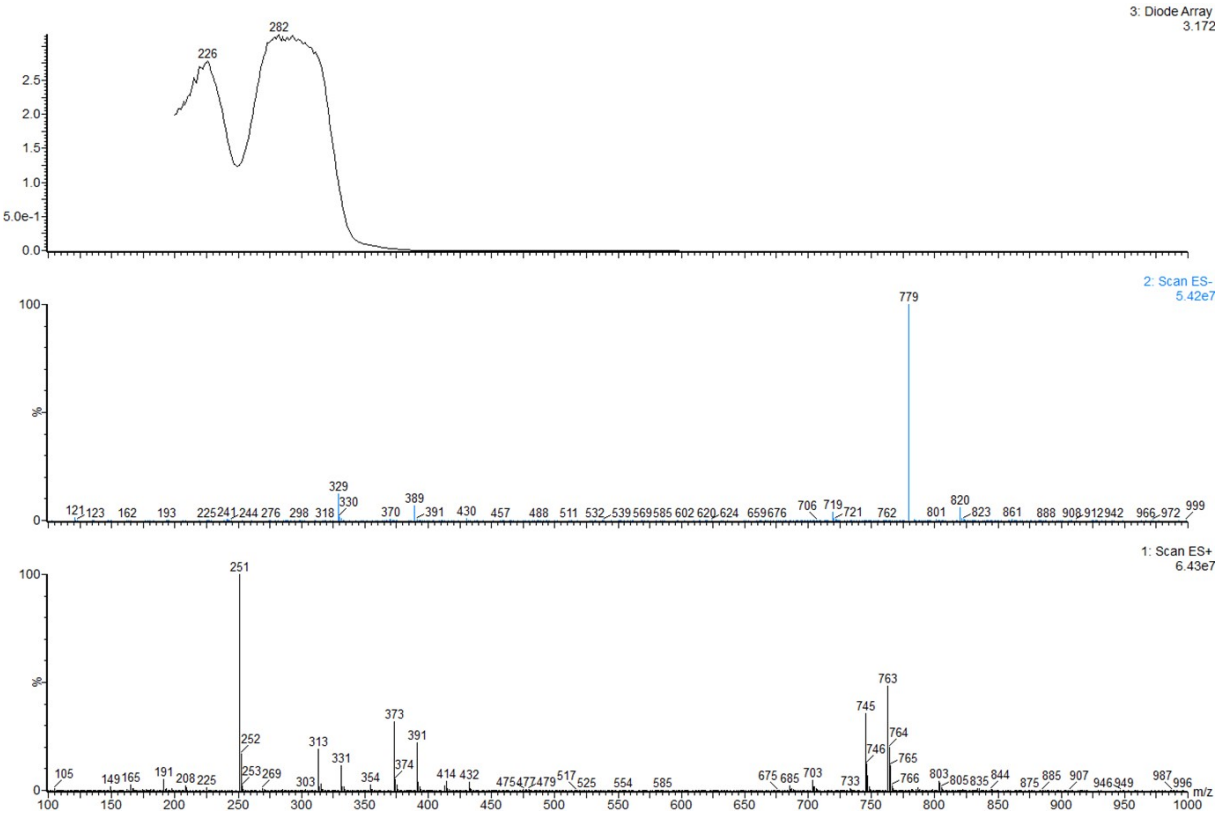


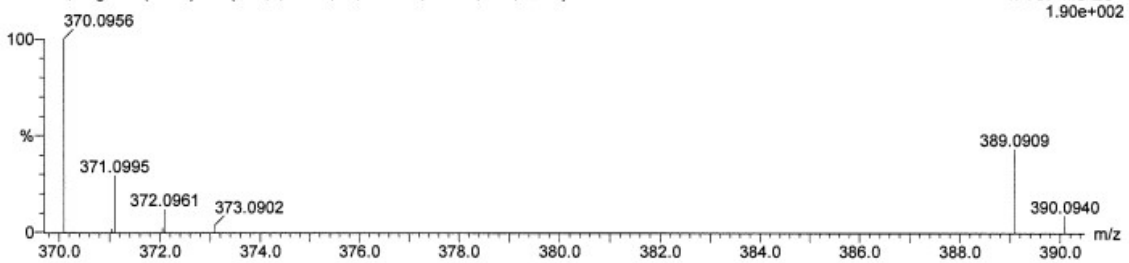
Figure S2.78 UV-absorption (top) and fragmentation pattern of **14** in ES^- (middle) and ES^+ TIC (bottom) by LR-LCMS.

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 20.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
130 formula(e) evaluated with 6 results within limits (up to 30 closest results for each mass)
Elements Used:
C: 0-85 H: 0-110 O: 0-9 S: 0-2

Sun QTof Premier HAB321
YS 020b, neg 416 (4.249) AM (Cen,5, 85.00, Ht,10000.0,554.26,0.70,LS 10)



Minimum:				-1.5						
Maximum:		5.0	20.0	50.0						
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula			
389.0909	389.0906	0.3	0.8	6.5	12.0	2.4	C16	H21	O9	S
	389.0881	2.8	7.2	10.5	13.7	4.1	C20	H21	O4	S2
	389.0940	-3.1	-8.0	1.5	13.9	4.3	C13	H25	O9	S2
	389.0873	3.6	9.3	11.5	9.8	0.2	C19	H17	O9	
	389.0966	-5.7	-14.6	24.5	12.2	2.6	C30	H13	O	
	389.0848	6.1	15.7	15.5	13.6	4.0	C23	H17	O4	S

Figure S2.79 HRMS data for **14**; *m/z* $(M-H)^-$ calc. mass is 389.0906, 389.0909 was found.

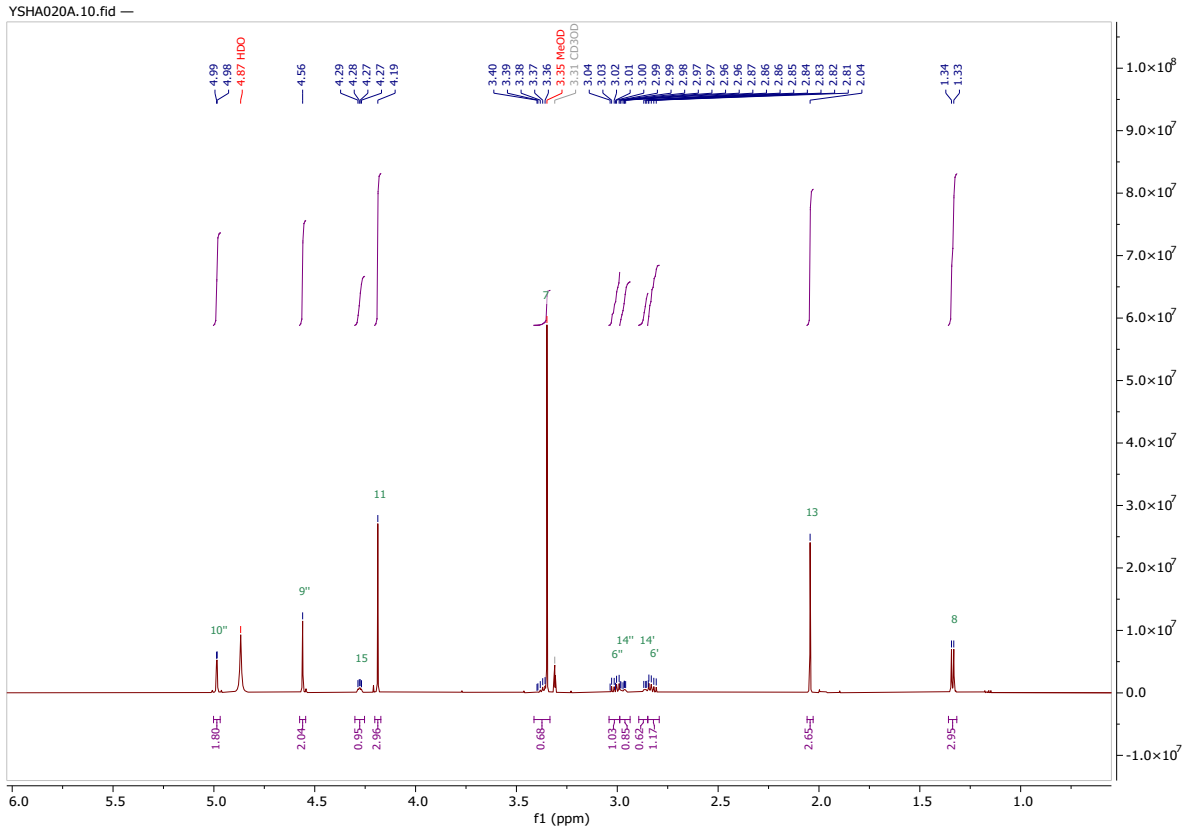


Figure S2.80 ^{1}H-NMR of **14** recorded at 600 MHz in CD_3OD.

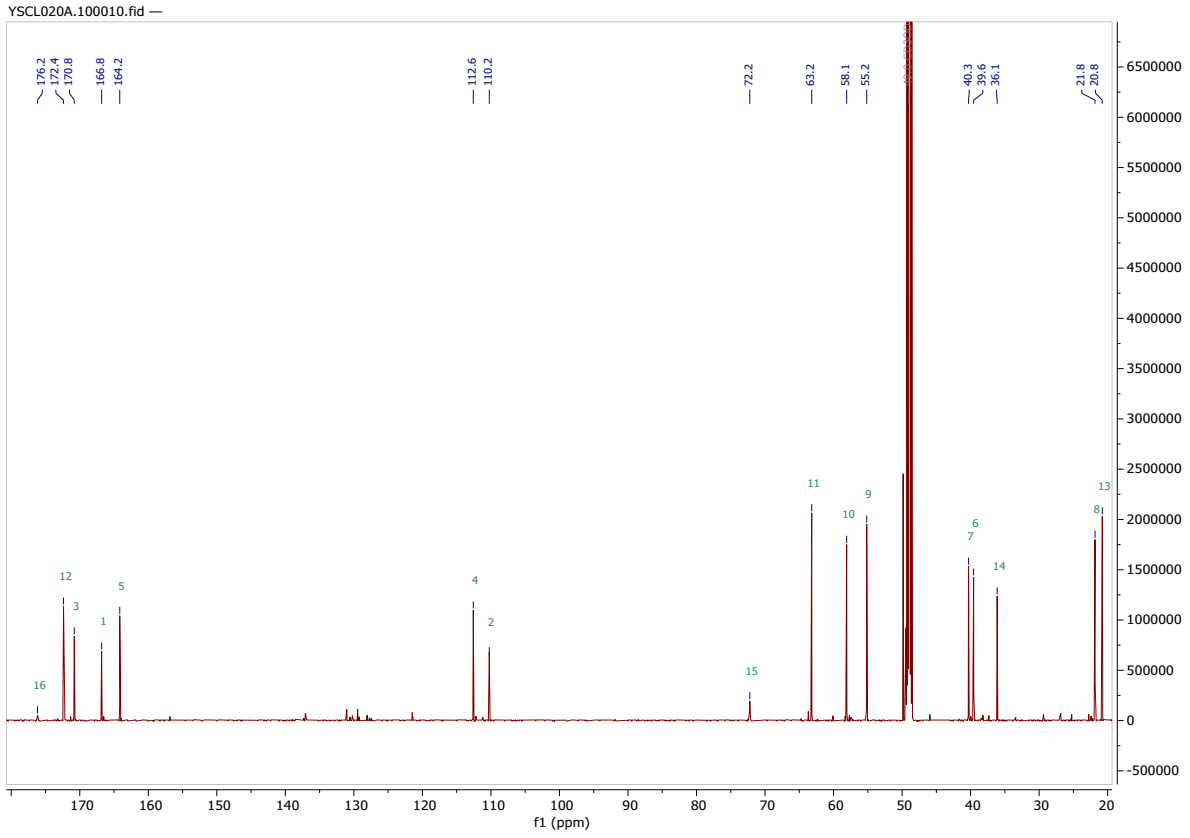


Figure S2.81 ^{13}C-NMR of **14** recorded at 150 MHz in CD_3OD.

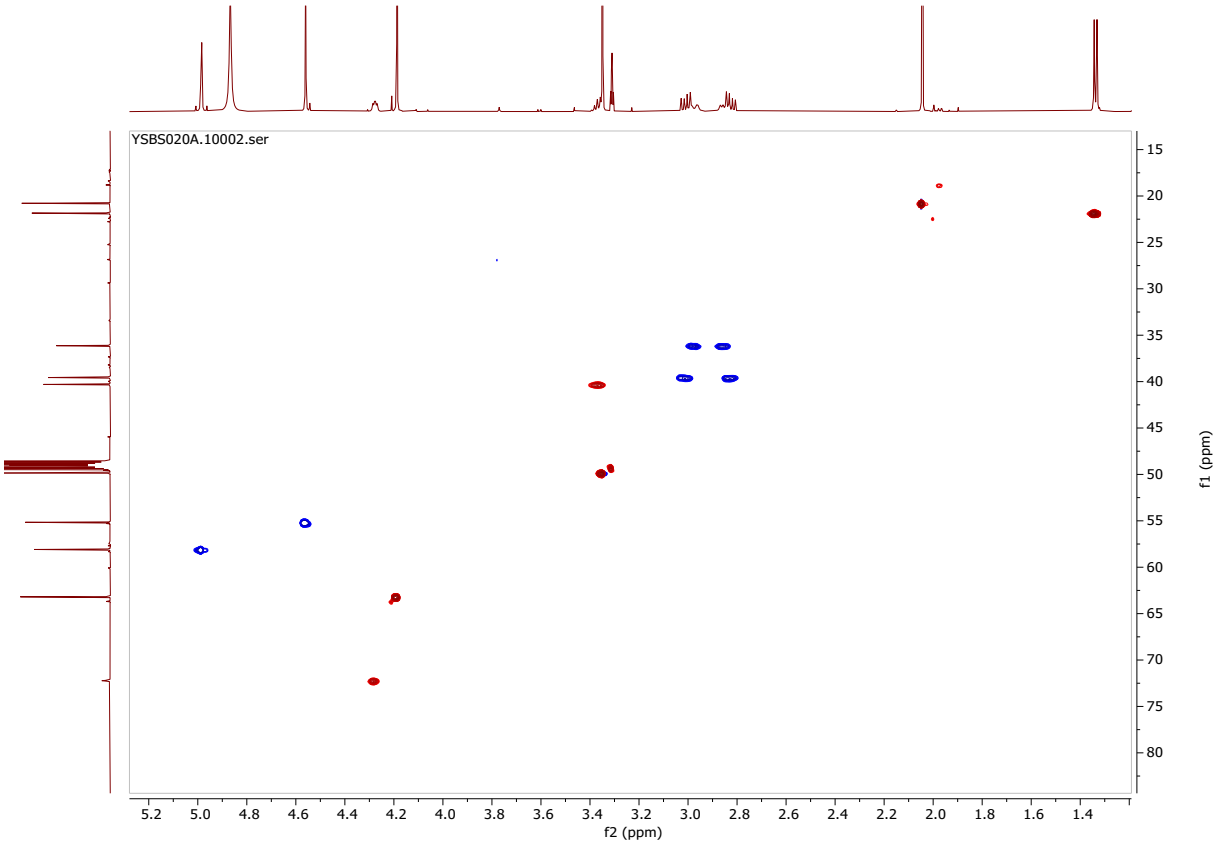


Figure S2.82 HSQC-spectrum of **14** recorded at 600, 150 MHz in CD_3OD.

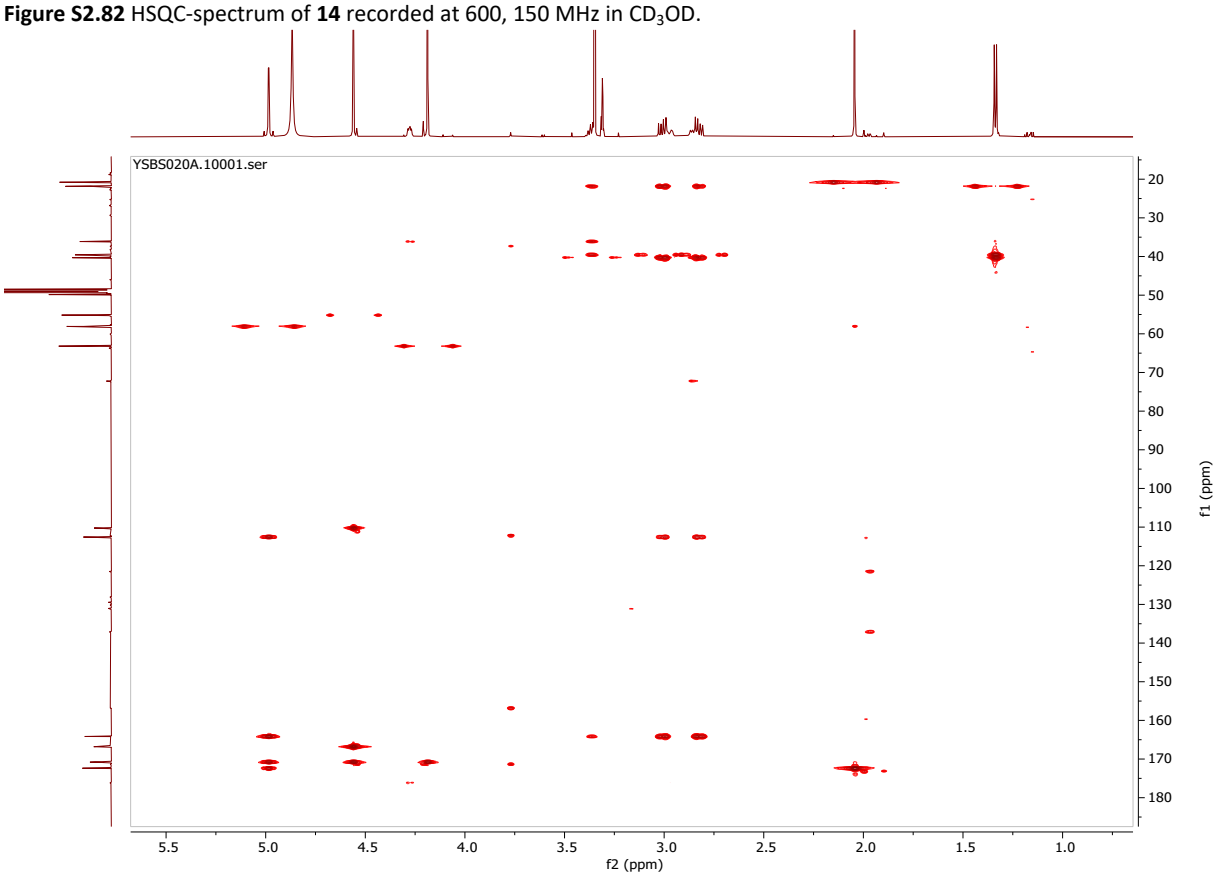


Figure S2.83 HMBC-spectrum of **14** recorded at 600, 150 MHz in CD_3OD.

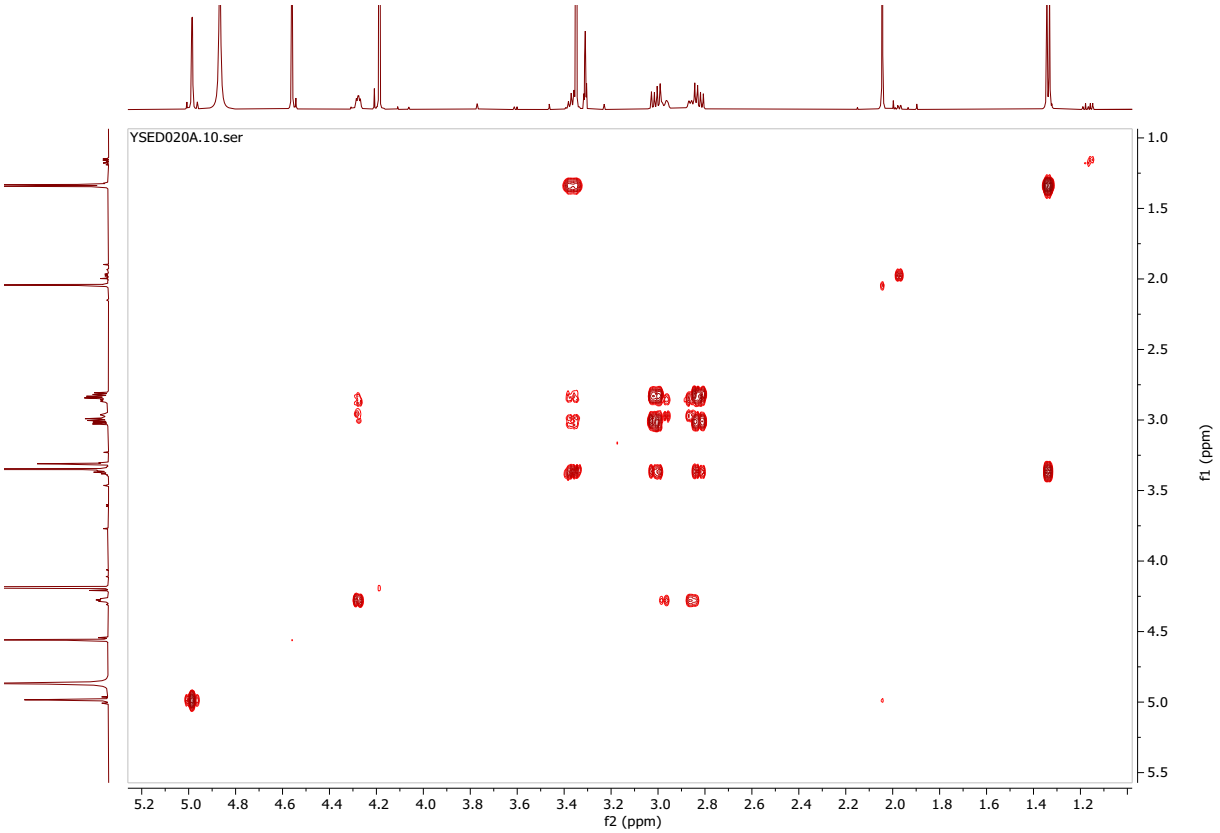
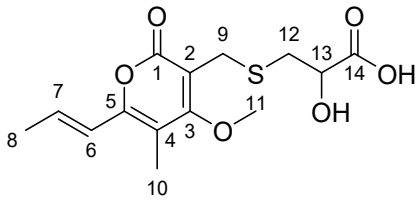


Figure S2.84 ^{1}H, ^{1}H-COSY-spectrum of **14** recorded at 600 MHz in CD_3OD.

Compound 15



15

Chemical Formula: $C_{14}H_{18}O_6S$
Exact Mass: 314.0824

Compound 15				
Pos.	**δ_C / ppm**	**δ_H / ppm (*J*/Hz)**	**^{1}H-^{1}H COSY**	**HMBC (H-C)**
1	162.7			
2	111.6			
3	168.5			
4	108.7			
5	153.1			
6	120.5	6.5, 1H, m	7, 8	5, 7, 8
7	133.8	6.5, 1H, m	6, 8	5, 6, 8
8	18.4	1.91, 3H, m	6, 7	6, 7
9	26.2	3.61, 2H, s		1, 2, 3, 12
10	9.4	1.97, 3H, s		3, 4, 5
11	61.2	3.87, 3H, s		3
12	36.6	2.77, 1H, dd (13.5, 6.8) 2.89, 1H, dd (13.5, 4.8)	12, 13 12, 13	9, 13, 14 9, 13, 14
13	70.4	4.14, 1H, m	12	12, 14
14	174.1			

Table S2.13 Summarized NMR signals for ^{13}C, ^{1}H, ^{1}H-^{1}H COSY, HMBC for **15** recorded in DMSO-d_6.

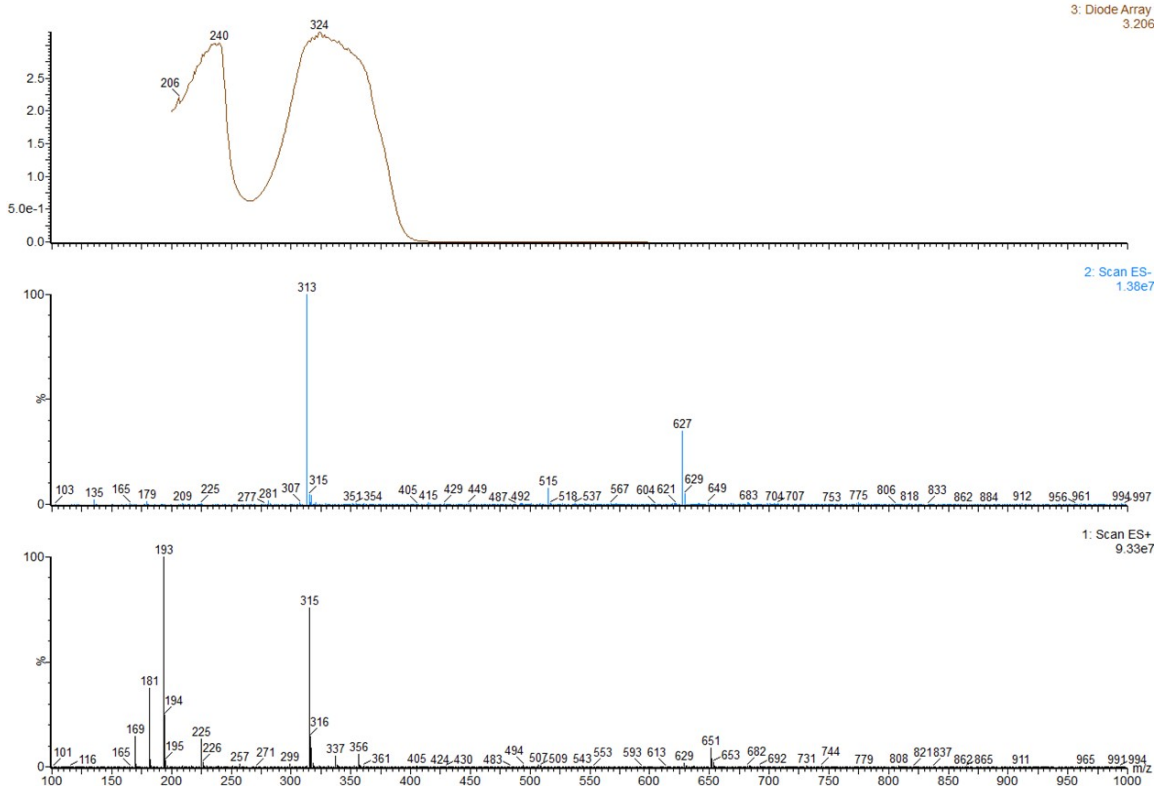


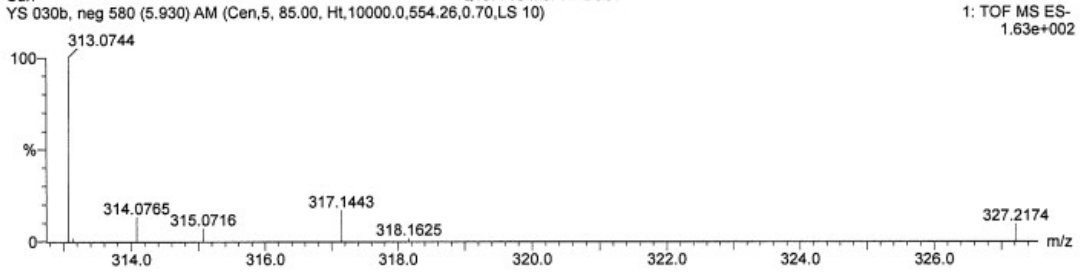
Figure S2.85 UV-absorption (top) and fragmentation pattern of **15** in ES^- (middle) and ES^+ TIC (bottom) by LR-LCMS..

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 20.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
103 formula(e) evaluated with 5 results within limits (up to 30 closest results for each mass)
Elements Used:
C: 0-85 H: 0-110 O: 0-9 S: 0-2

Sun QTof Premier HAB321
YS 030b, neg 580 (5.930) AM (Cen,5, 85.00, Ht,10000.0,554.26,0.70,LS 10)



Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
Minimum:				-1.5			
Maximum:		5.0	20.0	50.0			
313.0744	313.0746	-0.2	-0.6	6.5	7.6	0.3	C14 H17 O6 S
	313.0721	2.3	7.3	10.5	9.9	2.6	C18 H17 O S2
	313.0712	3.2	10.2	11.5	10.8	3.5	C17 H13 O6
	313.0780	-3.6	-11.5	1.5	9.3	2.0	C11 H21 O6 S2
	313.0687	5.7	18.2	15.5	12.1	4.8	C21 H13 O S

Figure S2.86 HRMS data for **15**; *m/z* $(M-H)^-$ calc. mass is 313.0746, 313.0744 was found.

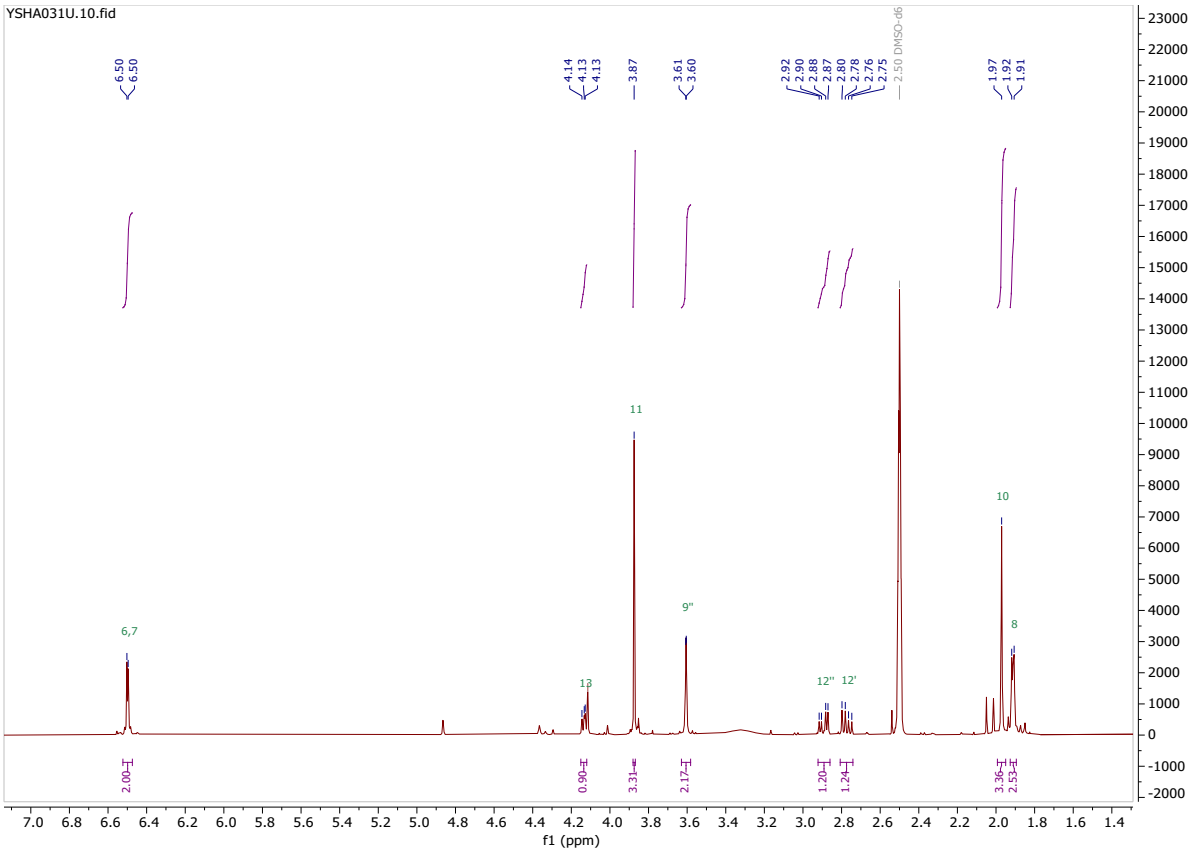


Figure S2.87 ^{1}H-NMR of **15** recorded at 400 MHz in DMSO-d_6.

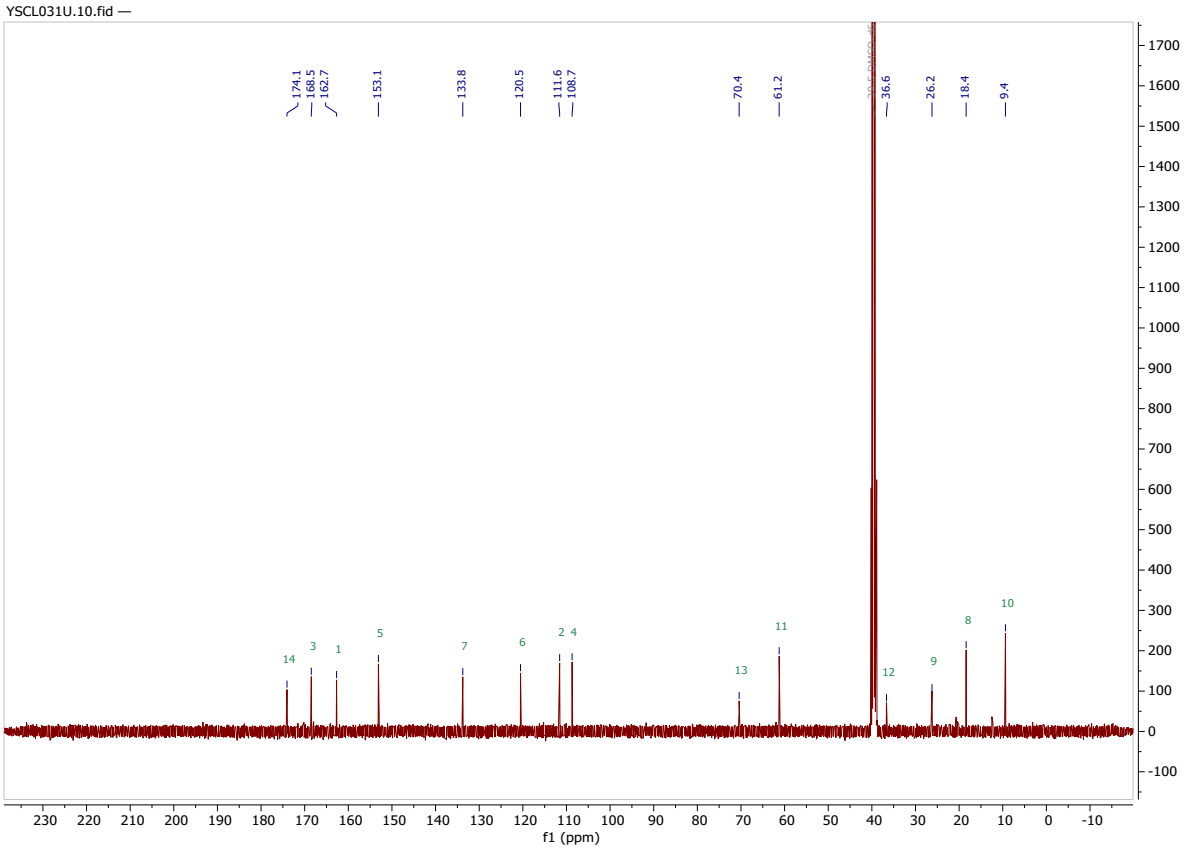


Figure S2.88 ^{13}C-NMR of **15** recorded at 100 MHz in DMSO-d_6.

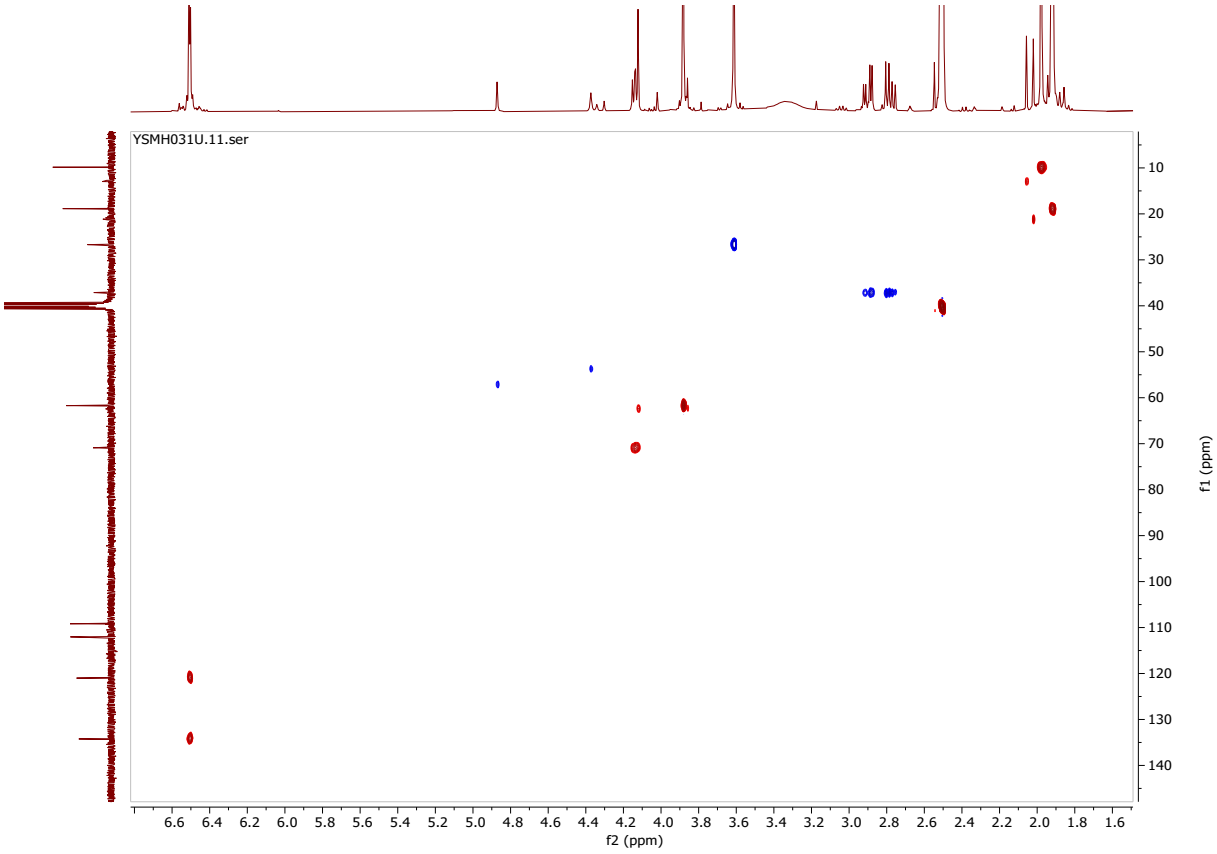


Figure S2.89 HSQC-spectrum of **15** recorded at 400, 100 MHz in DMSO-d_6.

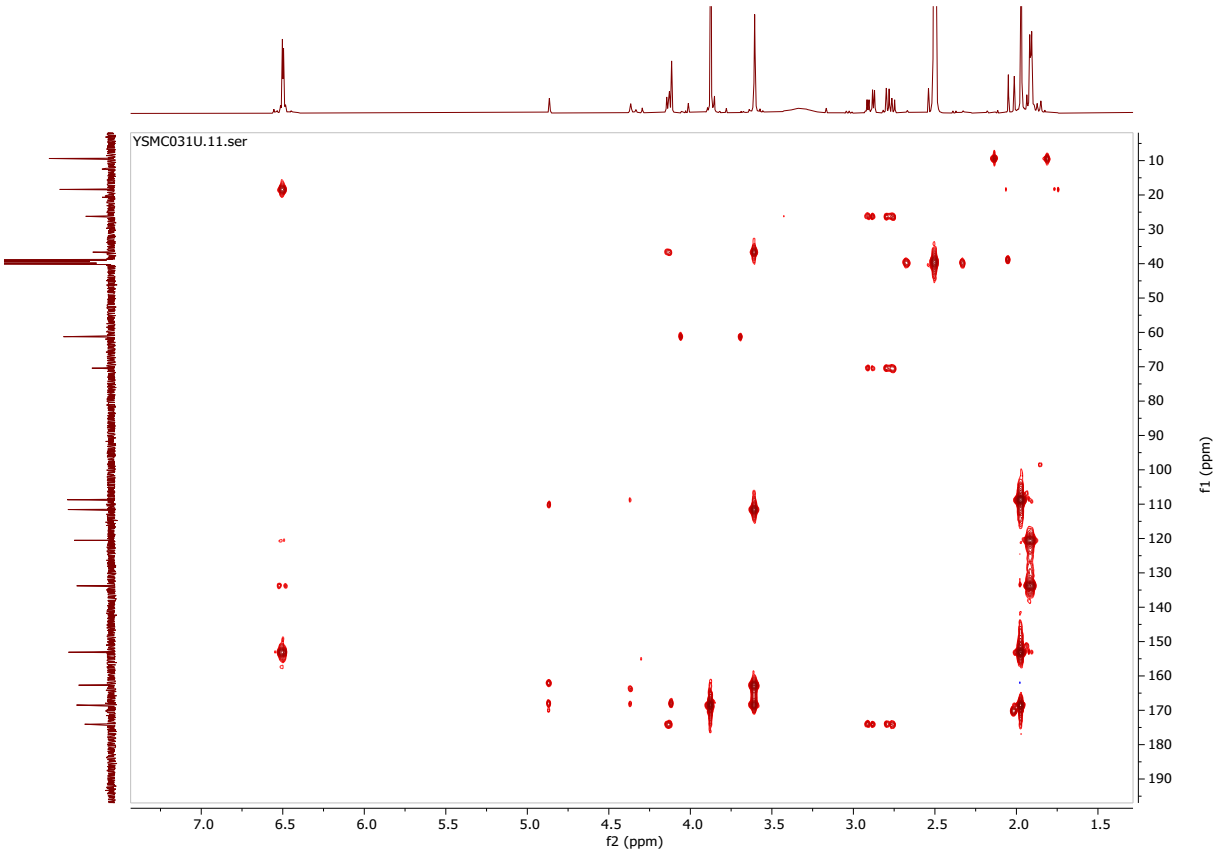


Figure S2.90 HMBC-spectrum of **15** recorded at 400, 100 MHz in DMSO-d_6.

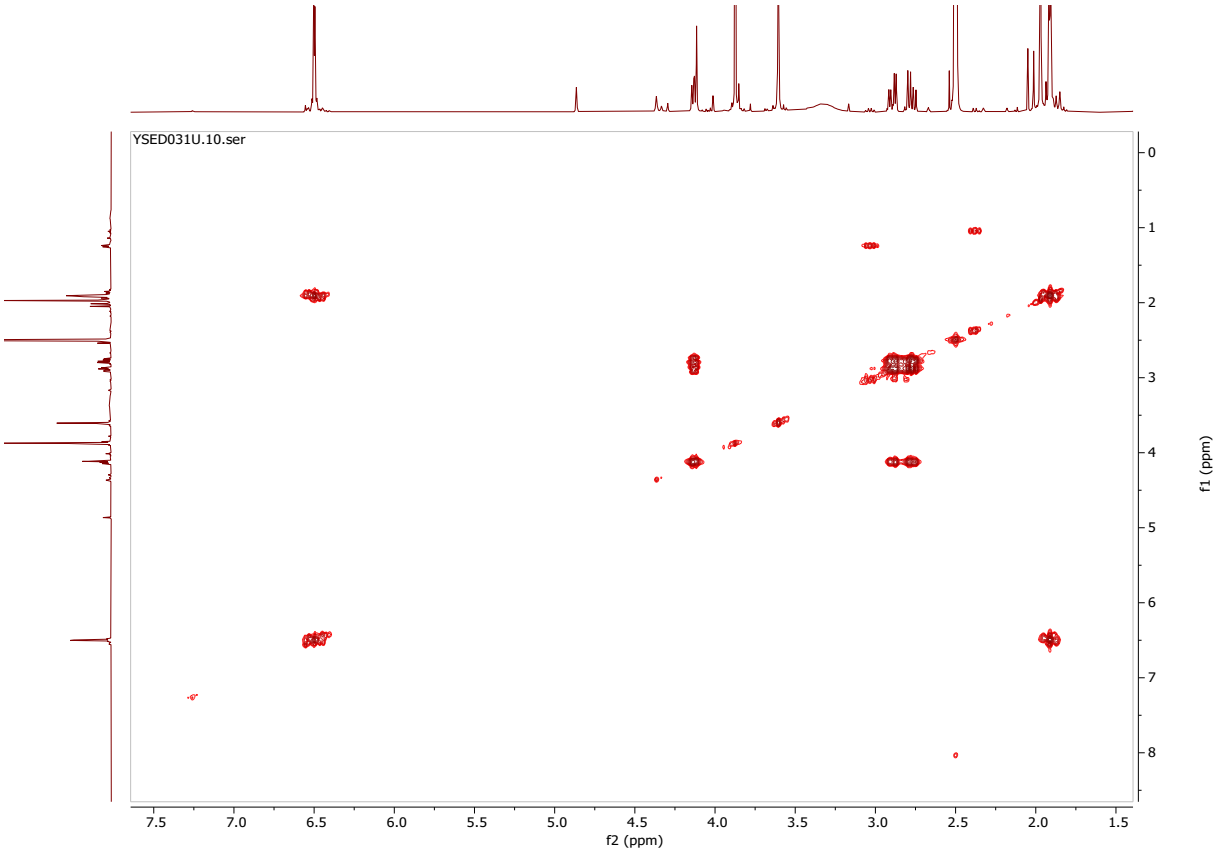
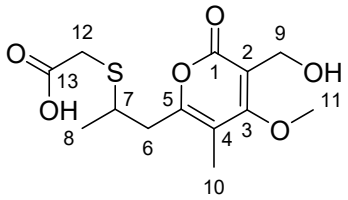


Figure S2.91 ^{1}H, ^{1}H-COSY-spectrum of **15** recorded at 400 MHz in DMSO-d_6.

Compound 16



16

Chemical Formula: $C_{13}H_{18}O_6S$
Exact Mass: 302.0824

Compound 16				
Pos.	**δ_C / ppm**	**δ_H / ppm (J/Hz)**	**^{1}H-^{1}H COSY**	**HMBC (H-C)**
1	164.1			
2	109.4			
3	168.8			
4	110.3			
5	157.1			
6	37.4	2.68, 1H, dd (14.5, 7.9) 2.84, 1H, dd (14.5, 6.9)	6, 7 6, 7	4, 5, 7, 8 4, 5, 7, 8
7	38.3	3.24, 1H, ddd (7.9, 6.9, 6.9)	6, 8	5, 6, 8, 12
8	20.5	1.24, 3H, d (6.8)	7, 8	6, 7
9	53.3	4.34, 2H, s		1, 2, 3
10	10.2	1.89, 3H, s		3, 4, 5
11	61.2	4.04, 3H, s		3
12	32.2	3.31, 2H, s		7, 13
13	171.6			

Table S2.14 Summarized NMR signals for ^{13}C, ^{1}H, ^{1}H-^{1}H COSY, HMBC for **16** recorded in DMSO-d_6.

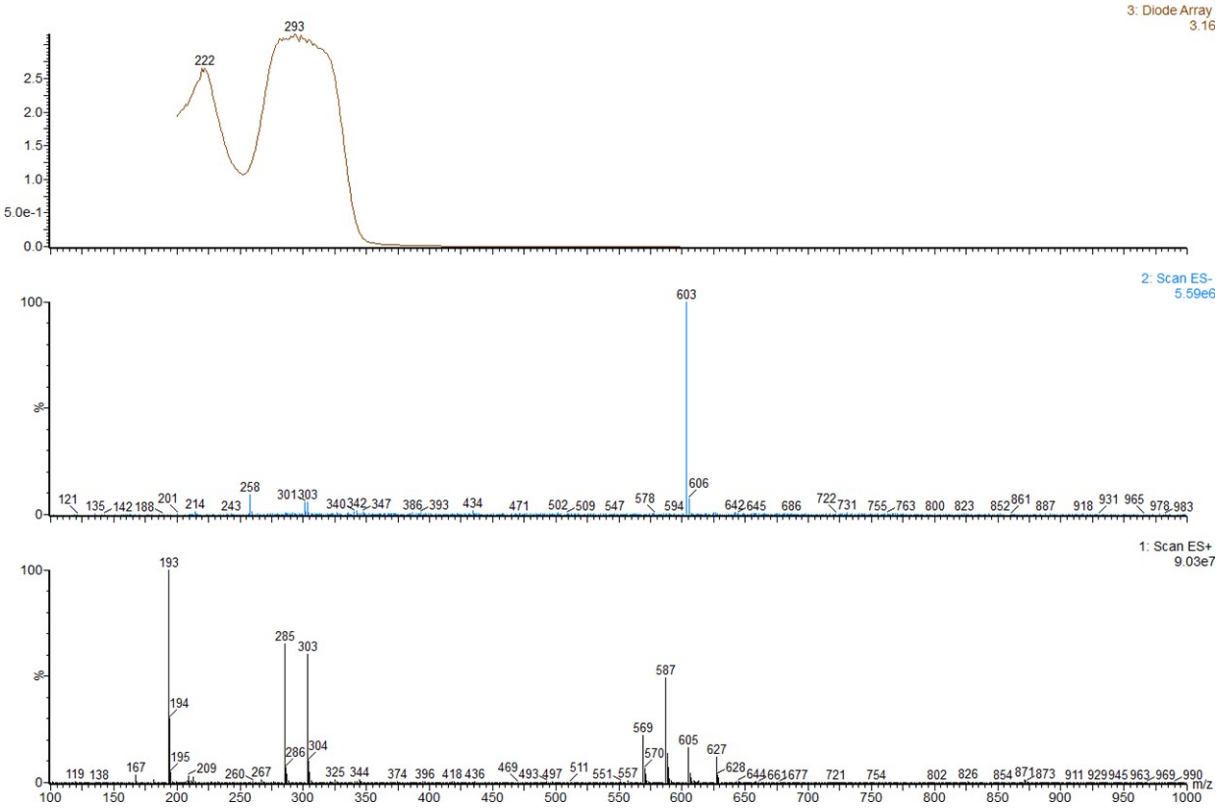


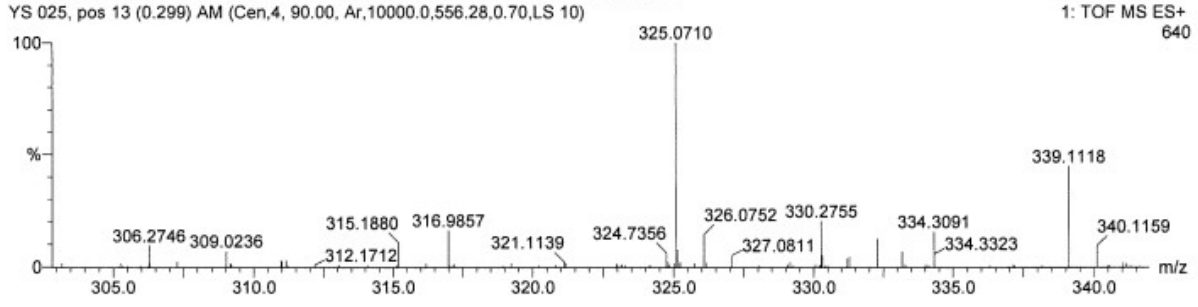
Figure S2.92 UV-absorption (top) and fragmentation pattern of **16** in ES^- (middle) and ES^+ TIC (bottom) by LR-LCMS.

Elemental Composition Report **Page 1**

Single Mass Analysis (displaying only valid results)
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Selected filters: None

Monoisotopic Mass, Even Electron Ions
218 formula(e) evaluated with 2 results within limits (up to 40 closest results for each mass)
Elements Used:
C: 0-72 H: 0-50 N: 0-1 O: 0-6 Na: 0-1 S: 0-1

Sun LCT Premier KD070
YS 025, pos 13 (0.299) AM (Cen,4, 90.00, Ar,10000.0,556.28,0.70,LS 10)



Minimum:				-1.5		
Maximum:		5.0	5.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
325.0710	325.0712	-0.2	-0.6	12.5	9.5	C18 H13 O6
	325.0722	-1.2	-3.7	4.5	2.5	C13 H18 O6 Na S

Figure S2.93 HRMS data for 1**6**; *m/z* (M + Na) calc. mass is 325.0712, 325.0710 was found.

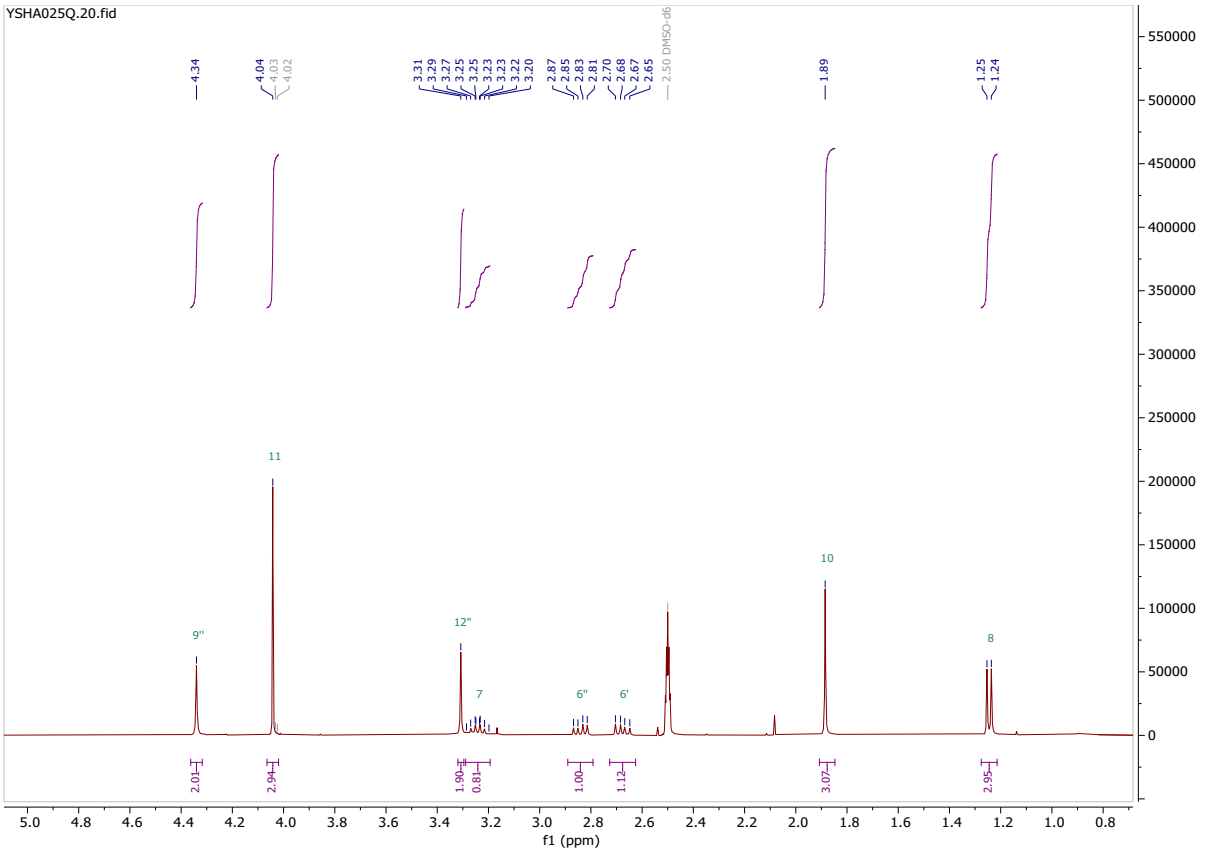


Figure S2.94 ^{1}H-NMR of **16** recorded at 400 MHz in DMSO-d_6**.**

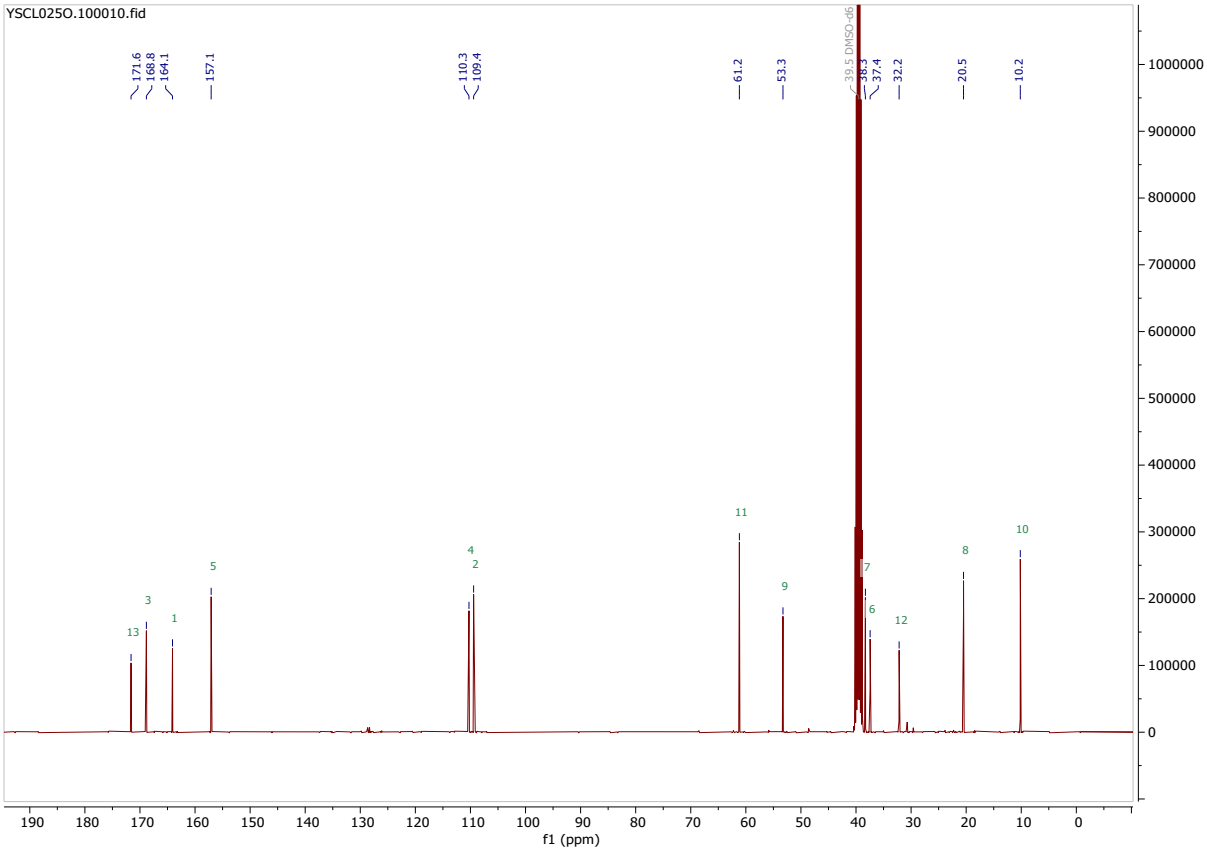


Figure S2.95 ^{13}C-NMR of **16** recorded at 100 MHz in DMSO-d_6.

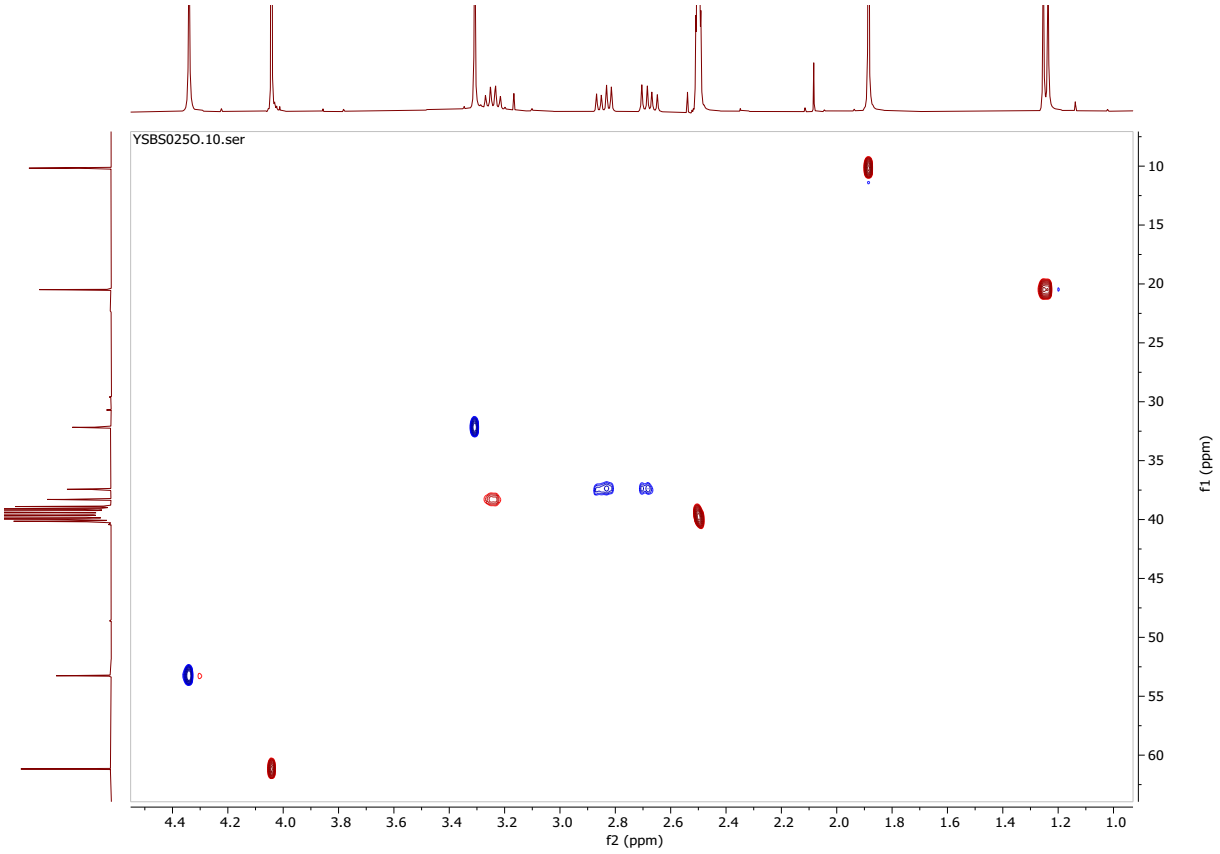


Figure S2.96 HSQC-spectrum of **16** recorded at 400, 100 MHz in DMSO-d.

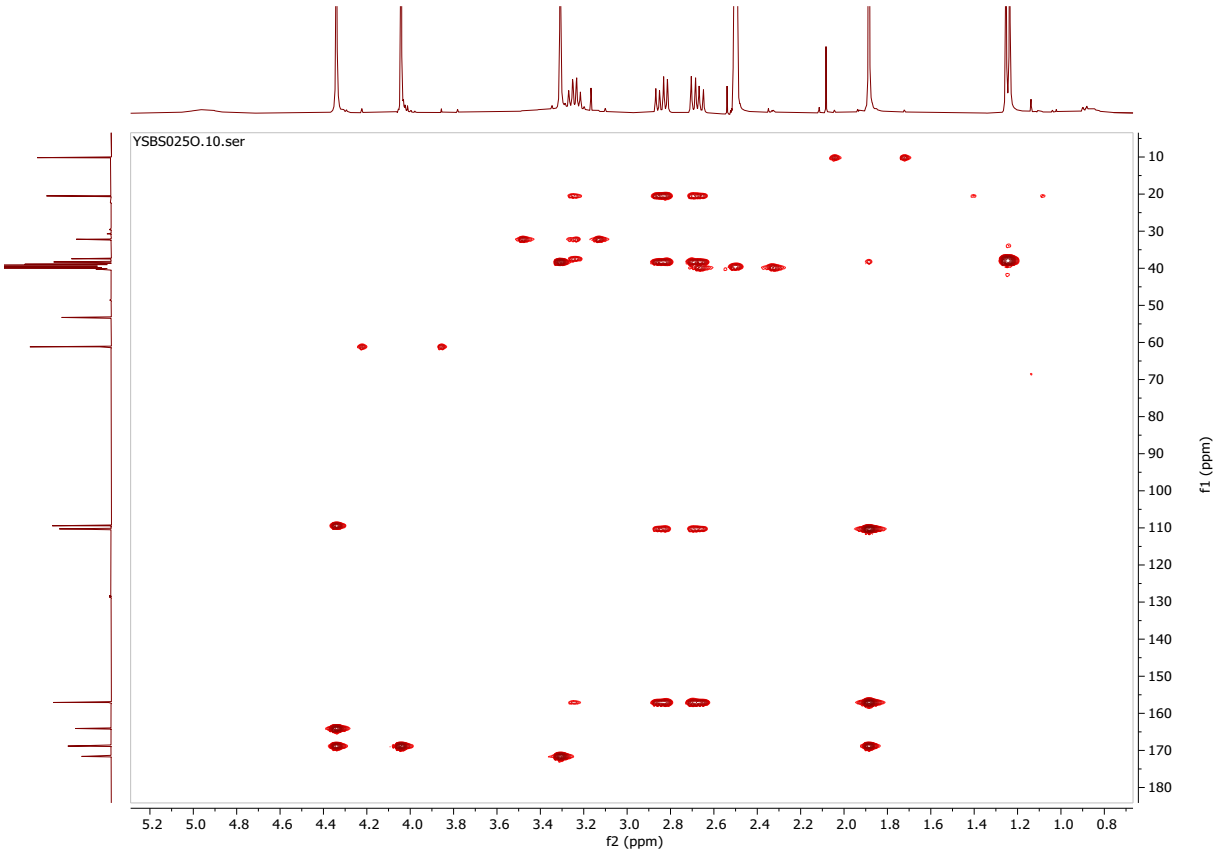


Figure S2.97 HMBC-spectrum of **16** recorded at 400, 100 MHz in DMSO-d_6.

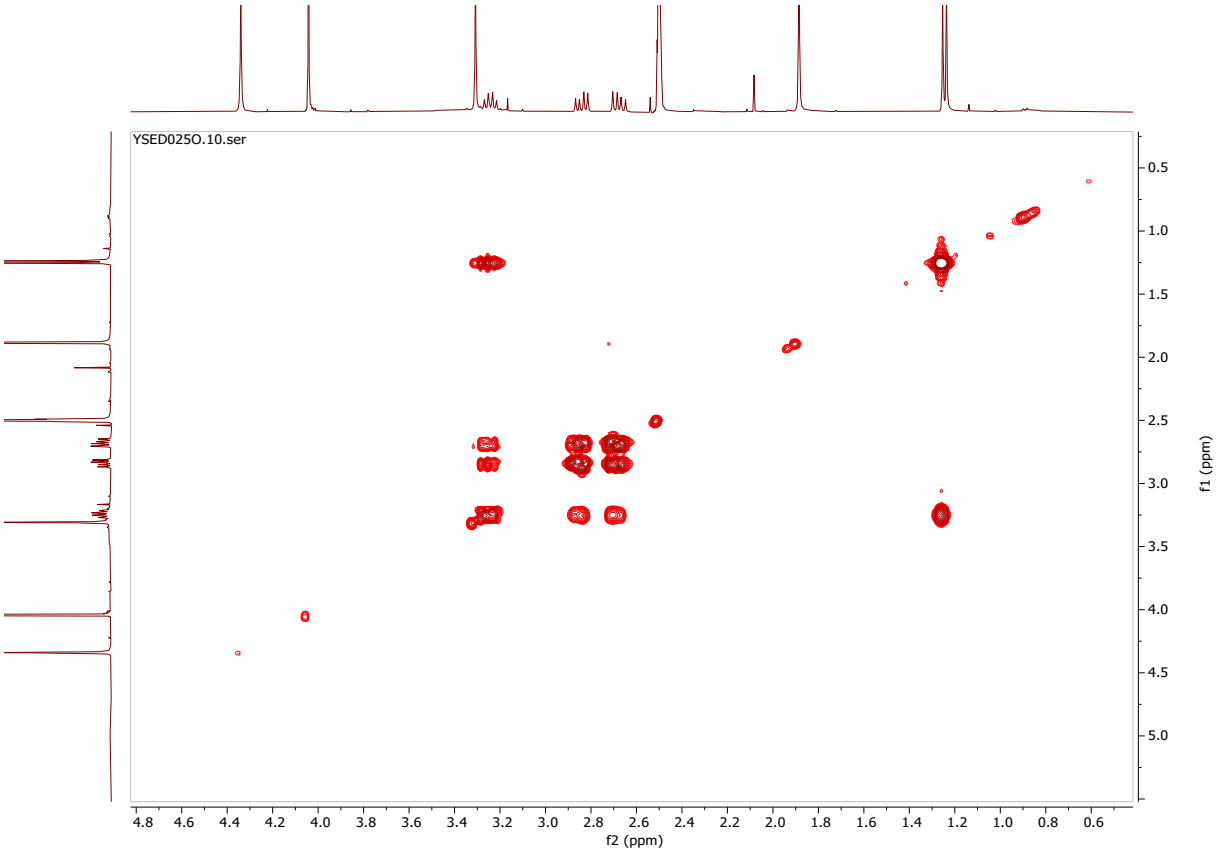


Figure S2.98 ^{1}H, ^{1}H-COSY-spectrum of **16** recorded at 400 MHz in DMSO-d_6.

3. Reference

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