



Supporting Information

Regioselektive Oxidative Phenolkupplung durch eine Unspezifische Peroxygenase aus Ständerpilzen

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Supporting Information

Regioselective Oxidative Phenol Coupling by a Mushroom Unspecific Peroxygenase

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Experimental procedures

1. Microbiological methods

Strains and cultivation: Fruiting bodies (basidiomata) of *Cortinarius odorifer* (classified as *Calonarius odorifer*,^[1] NCBI Taxonomy ID: 36062) were collected in the Black Forest near Röttenbach (Germany) at the following location 47° 52' 48", 8° 18' 22". The specimens were determined by phenotypic features as well as genetically by sequencing of the internal transcribed spacer (ITS) sequence. The fruiting bodies were frozen at -20 °C after harvest. For extraction of chromosomal DNA and RNA, the mushroom was ground to a fine powder under liquid nitrogen. Genomic DNA as well as RNA of *C. odorifer* was isolated following an adapted protocol by adjusting the pH for improved stability of the isolated nucleic acids.^[2]

Aspergillus niger strain ATNT16ΔpyrGx24^[3] served as heterologous expression platform. Genotypes of individual transformants are given in Table S4. All strains were routinely cultured at 30 °C on *Aspergillus* minimal medium (AMM)^[4] supplemented with 100 mM D-glucose and 20 mM L-glutamine (AMMG100Gln20). Conidia suspensions were obtained from solid 2 % (w/v) agar plate cultures of AMMG100Gln20. The plates were overlaid with 5 mL of sterilized ddH₂O. Conidia were harvested by swabbing the culture surface with a Drigalski spatula. The conidia suspensions were then filtered using a 40 µm Cell Strainer (Greiner Bio-One) and stored at 4 °C.

For routine cultivation and secondary metabolite analysis, liquid cultures of AMMG100Gln20 were inoculated with 0.5 mL conidia suspension and incubated at 30 °C and 150 rpm, for 48 h. Transformants were supplemented with 20 µg mL⁻¹ doxycycline to induce transgene expression. For negative controls with the parental strain *A. niger* ATNT16ΔpyrGx24 10 mM uridine was added.

For routine cloning and plasmid propagation, *Escherichia coli* StellarTM (Takara Bio Europe) was grown on solid LB medium (per liter: 5 g yeast extract, 10 g tryptone, 10 g NaCl, 15 g agar) supplemented with 50 µg mL⁻¹ ampicillin or 50 µg mL⁻¹ kanamycin sulfate, if required. The plasmids were isolated from 5 mL *E. coli* StellarTM cultures using the GeneJET[®] Plasmid Miniprep Kit (Thermo Fisher Scientific), following the provided protocol. The sequences of the inserted genes were confirmed by Sanger sequencing.

2. Molecular biology methods

Amplification of unspecific peroxygenase coding cDNA: Intron-free genes were isolated from total RNA extractions of ground fruiting bodies. 100 mg of fine powder was submerged in 200 µL RNA or DNA extraction buffer (69 mM SDS, 500 µM EDTA, 100 µM Tris-HCl, 0.1 % (v/v) DEPC at pH 5 or 8, respectively) and shaken thoroughly. 200 µL phenol:chloroform:isoamylalcohol (25:24:1) was added slowly and the suspension was incubated at 65 °C for 5 min. The mixture was cooled to room temperature and afterwards centrifuged at 10000 × g and 4 °C for 5 min. The aqueous phase (upper phase) was transferred to a new tube without disturbing the interphase. The RNA or DNA was routinely precipitated by 1 volume ice-cold 2-propanol and washed multiple times with 1 volume of 70 % ethanol. The pellet was dried at ambient temperature and resuspended in DEPC-treated ddH₂O. The cDNA was prepared according to the protocol provided by the SuperScriptTM IV First-Strand Synthesis System (Fisher Scientific).

Construction of expression plasmids: For heterologous production of native unspecific peroxygenase (UPO) in *A. niger* ATNT16ΔpyrGx24, the intron-free *C. odorifer* gene *coupo1* was amplified from cDNA using oligonucleotides listed in Table S5. All reactions were performed in 20 µL total volume using 0.4 U Phusion DNA polymerase (NEB). The reaction mixture included 0.2 mM (each) deoxynucleoside triphosphate, 0.5 µM (each) oligonucleotide primer (Table S5), supplied with the buffer. PCR parameters (condition I) are given in Table S6.

The resulting amplicon was electrophoretically purified on an agarose gel and ligated to *Nco*I-linearized phis_{SMX}press vector^[3] using the In-Fusion[®] Snap Assembly Master Mix (Takara Bio Europe, France). The constructed expression plasmid (Table S7) was pLP001.

For heterologous production of native O-methyltransferase CoOMT1 in *E. coli* BL21 Gold (DE3), *C. odorifer* *coomt1* was used as a synthesized, intron-free and codon-optimized gene and subsequently amplified using oligonucleotides listed in Table S5. PCR parameters (condition II) are given in Table S6. The resulting amplicon was ligated to *Nde*I-linearized pET28a vector using the In-Fusion[®] Snap Assembly Master Mix (Takara Bio Europe) to yield expression plasmid pLP002 (Table S7).

For all expression plasmids (listed in Table S7), accurate amplification, and ligation of inserts with vectors was verified by DNA sequencing.

Transformation of *A. niger* and genetic analysis of transformants: Polyethylene glycol-mediated transformation of *A. niger* protoplasts followed a described protocol.^[5] The transformants were selected for uracil prototrophy on AMMG100Gln20 agar plates, supplemented with 1.2 M sorbitol. In case of tLP001, transformants were additionally selected on plates containing 200 µg mL⁻¹ hygromycin B. The genomic DNA of individual transformants was isolated as described above. Integration of the respective expression cassettes (*terA*-promoter/gene of interest/*trpC*-terminator) was then confirmed by diagnostic PCR using oligonucleotides oLP56/oLP57 (condition II, Table S6, Figure S18). At least three PCR-confirmed transformants per construct were subsequently used as biological replicates for metabolic analyses. The transformants are shown in Table S4.

3. Analytical and preparative methods

Liquid chromatography and mass spectrometry: To analyze natural products, the culture filtrates of *A. niger* transformants were extracted with ethyl acetate. The organic phase was collected, dried over anhydrous sodium sulfate, and evaporated to dryness. The crude extracts were then dissolved in methanol and subjected to HPLC–MS analysis using a InfinityLab Poroshell 120 EC-C18 column (4.6 × 150 mm, 4 µm particle size, at ambient temperature) on an Agilent 1100 series instrument, interfaced to an SCIEX 4500 triple quadrupole mass detector, operated in negative ionization mode, and applying gradient I (Table S8). Extracted ion chromatograms were recorded at the respective monomer or dimer masses.

To purify torosachryson (3) from fungal cultures for NMR spectroscopy and subsequent feeding assays, the cultivation time was extended to 72 h and 30 µg mL⁻¹ doxycycline was added to induce transgene expression. For compound purification, crude extracts of *A. niger* tNAL015 were subjected to reversed phase semi-preparative HPLC using a LiChrosorb®-RP18 (4.0 × 250 mm, 5 µm particle size) column on an Agilent 1290 HPLC system, applying gradient II (Table S8), equipped with a diode-array detector (DAD). Selected fractions were pooled and lyophilized.

For analysis of the stereochemistry of 3 and asperflavin (5), *in vitro* assays of atrochryson (1) conversion with heterologously produced CoOMT1 were extracted with ethyl acetate and dried under reduced pressure. The solvent was dissolved in 100 µL methanol and subjected to HPLC–DAD analysis using a Daicel Chiralpak-AD column (4.6 × 250 mm, 10 µm particle size, at ambient temperature) on an Agilent 1100 series instrument, applying gradient III (Table S8), with a detection wavelength at 280 nm.

To purify phlegmacin (2) for NMR and CD spectroscopy, freeze-dried fruiting bodies of *C. odorifer* were ground to a fine powder under liquid nitrogen, followed by metabolite extraction with methanol until the solvent remained clear after filtration. Subsequently, the solvent was evaporated under reduced pressure. The residue was dissolved in 50 mL methanol partitioned between ethyl acetate and water (4:1) and the organic layer was separated in a separating funnel. Again, the solvent was evaporated under reduced pressure. The residue was dissolved in ethyl acetate and fractionated by semi-preparative scale HPLC using a LiChrosorb®-RP18 (4.0 × 250 mm, 5 µm particle size) column on an Agilent 1290 HPLC system, applying gradient II, equipped with a DAD. Selected fractions were pooled and lyophilized. 2 was isolated as two peaks and separately fractionated. Enantiomerically near-pure 2a and 2b were obtained after semi-preparative reversed-phase HPLC. Both products were subjected to CD analysis (Figure S17).

Nuclear magnetic resonance spectroscopy: NMR spectra were recorded on a Bruker Avance DRX 400 spectrometer operating at 400 MHz (for ¹H NMR) and 100.6 MHz (for ¹³C NMR). The chemical shifts are given in ppm with a solvent resonance as internal standard (¹H NMR: CDCl₃ 7.26 ppm; ¹³C NMR CDCl₃ 77.16 ppm).

Circular dichroism spectroscopy: Measurements were conducted with a JASCO J 810 spectrometer; the scan speed was 500 nm × min⁻¹ in a range of 200–500 nm. Samples of 2 were dissolved in methanol (HPLC-MS grade) with an estimated final concentration of 1 mM. The measurement was performed in a 1 mm cuvette and repeated five times. The spectrum of background measurement with methanol was subtracted from the sample spectrum.

4. Biochemical methods

In vivo product formation assay: Product formation assays for 2 were carried out in triplicates in 250 µL reactions containing 1 mM of 3 and 100 mg of mycelium from expression cultures after 48 h of transgene expression in 50 mM Tris-HCl buffer at pH 7.5. The reactions were incubated at 25 °C for 24 h and subsequently extracted using 4 volumes of ethyl acetate. The organic layer was dried under reduced pressure, dissolved in methanol, and analyzed by HPLC-MS using gradient I (Table S8).

Product formation assays for 3 and 5 were carried out in triplicates in 250 µL reactions containing 12.5 µM of 1, a three equimolar excess of *S*-adenosyl-L-methionine (SAM), and 100 µL of cell-free supernatant or purified protein from *E. coli* heterologously expressing the *O*-methyltransferases *ShwM1*, *ShwM2*, *SpoM*, and *CoOMT1* in 50 mM Tris-HCl buffer pH 7.5. The reactions were incubated at 25 °C for 24 h and subsequently extracted using 4 volumes of ethyl acetate. The organic layer was dried under reduced pressure, dissolved in methanol, and analyzed by HPLC-MS using gradient I or Chiral HPLC-DAD using gradient III (Table S8).

Heterologous expression and purification of *O*-methyltransferases: Precultures (5 mL, LB medium) were inoculated with a single colony of transformed *E. coli* BL21 Gold (DE3) cells harboring plasmid pET19b::*spoM* or pLP002 and incubated overnight at 37 °C, 180 rpm. Expression cultures (400 mL, LB medium) were inoculated with 1 mL of the preculture and incubated at 37 °C, 170 rpm in baffled shake flasks. Cells were induced with IPTG (1 mM final concentration) after cell density had reached 0.6 (OD 600 nm). The cultures were then incubated for 24 h at 24 °C. Expression cultures were subsequently harvested by centrifugation (8000 × g, 30 min, 4 °C) with an Avanti J-26S XP centrifuge (JLA–10.500 rotor; Beckmann Coulter, Brea, USA). The cell pellet was resuspended by vortexing in resuspension buffer (Tris 50 mM pH 7.5; 10 % glycerol, 150 mM NaCl) in a concentration of 0.1 g mL⁻¹ and transferred to a steel cup stored on ice. Cell disruption was performed by sonication with a Sonifier® II W–250 (Branson, Danbury, USA). Consequently, cell debris was separated by centrifugation and the supernatant was subjected to Ni-NTA affinity chromatography. The cell-free lysate was filtered (Puradisc 25, 0.45 µm, GE Healthcare, Chicago, USA) and applied to Ni-NTA resin. The resin was washed with 10 mM imidazole buffer and the protein of interest was eluted with 500 mM imidazole. After IMAC purification the volume of the protein fraction was reduced to 2.5 mL by centrifugation over 30 kDa spin filters (Macrosep Advance Centrifugal Device, Pall Corporation, Port Washington, USA). The concentrated protein

solution was loaded onto an equilibrated PD-10 column (Sephadex G 25 M, Cytiva, USA) and eluted with 3.5 mL buffer. The purified proteins were analyzed by SDS-PAGE (Figure S19).

5. Bioinformatic methods

For structural comparison of the isolated CoUPO1 with characterized UPOs, a 3D structural model of CoUPO1 was generated using AlphaFold Colab,^[6] which is a simplified version of AlphaFold v2.1.0.^[7] Other UPO structures, i.e., MroUPO (PDB 5FUJ) and AaeUPO (PDB 2YOR), and the CoUPO1 model were superimposed, analyzed and visualized by using PyMOL (version 2.0.7).^[8]

Phylogenetic analyses were based on the sequences obtained by Basic Local Alignment Search Tool (BLAST) against CoUPO1 or CoOMT1 as query with BLOSUM80 as substitution matrix.^[9] The evolutionary history was inferred by using the Maximum Likelihood method and JTT matrix-based model.^[10] Initial trees for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the JTT model, and then selecting the topology with superior log likelihood value. The trees were drawn to scale, with branch lengths measured in the number of substitutions per site. Evolutionary analyses were conducted in MEGA11.^[11]

¹H (400 MHz) and ¹³C (100.6 MHz) NMR of torosachryson (3), phlegmacin A₁ (2b), and B₁ (2a)

Table S1. ¹H and ¹³C NMR spectroscopy data for torosachryson (3) in CDCl₃.

Position	δ_{H} (mult. J [Hz])	δ_{C}	HMBC	COSY
1	-	201.54	-	-
2	2.84 (d, J = 3.78 Hz, 2H)	50.94	1	4
3	-	70.99	-	-
3-OH	1.62 (s, br, -OH)	-	-	-
4	3.05 (d, J = 2.40 Hz, 2H)	43.26	1, 3, 10, 10b	2, 10
5	6.55 (d, J = 2.36 Hz, 1H)	99.73	7, 9a, 9b, 10	7, 10
6	-	163.58	-	-
6-OCH ₃	3.89 (s, 3H)	55.42	6	-
7	6.49 (d, J = 2.35 Hz, 1H)	100.98	9a, 9b	5
8	-	159.77	-	-
8-OH	9.81 d, J = 0.84 Hz, -OH)	-	7	-
9	-	166.12	-	-
9-OH	16.12 (d, J = 0.85 Hz, -OH)	-	-	-
9a	-	108.02	-	-
9b	-	108.02	-	-
10	6.88 (t, J = 1.09 Hz, 1H)	117.6	4, 5, 9a, 9b	4, 5
10a	-	140.98	-	-
10b	-	135.13	-	-
11	1.46 (s, 3H)	28.92	2, 3, 4	-

Table S2. ¹H and ¹³C NMR spectroscopy data for phlegmacin A₁ (2a) in CDCl₃.

Position	δ_{H} (mult. J [Hz])	δ_{C}	HMBC	COSY
1	-	202.16	-	-
2	2.90-2.71 (m, 2H)	51.16	1, 3, 4, 9a, 9b, 11	4
3	2.00 (s, br, -OH)	71.34	-	-
4	3.12 (m, 2H)	43.42	3, 10, 10b	2, 10
5	6.72 (s, 1H)	98.54	6, 7, 9a, 10	6-OCH ₃ , 10
6	-	161.87	-	-
6-OCH ₃	3.78 (s, 3H)	56.07	6	5, 10
7	-	111.45	-	-
8-OH	10.01 (s, br, -OH)	156.15	7, 8, 9a	-
9-OH	16.16 (s, br, -OH)	165.99	-	-
9a	-	108.68/108.43	-	-
9b	-	108.68/108.43	-	-
10	7.02 (s, 1H)	117.82	4, 5, 9a, 9b, 10a, 10b	4, 5
10a	-	140.79/140.72	-	-
10b	-	135.99	-	-
11	1.50 (s, 3H)	29.27	2, 3, 4	-
1'	-	201.92	-	-
2'	2.90-2.71 (m)	50.59	1', 3', 4', 9'a, 9'b, 10', 10'b, 11'	4'
3'	2.00 (s, br, -OH)	70.73	-	-
4'	2.66 (dd, J = 1.34 Hz, 15.54 Hz)	41.57	2', 3', 9'a, 9'b, 10', 10'b	2'
5'	6.11 (d, J = 2.36 Hz)	99.46	7', 9'a, 9'b, 10'	7'
6'	-	163.79	-	-
6'-OCH ₃	3.65 (s, 3H)	55.42	6'	7'
7'	6.50 (d, J = 2.39 Hz)	100.44	5', 6', 8', 9'a, 9'b,	5', 6'-OCH ₃
8'-OH	10.19 (s, br, -OH)	160.79	7', 8'	-
9'-OH	16.64 (s, br, -OH)	167.02	-	-
9'a	-	108.37/108.28	-	-
9'b	-	108.37/108.28	-	-
10'	-	120.38	-	-
10'a	-	140.79/140.72	-	-
10'b	-	134.26	-	-
11'	1.32 (s, 3H)	29.06	2', 3', 4'	-

Table S3. ¹H and ¹³C NMR spectroscopy data for phlegmacin B₁ (**2b**) in CDCl₃.

Position	δ_{H} (mult. J[Hz])	δ_{C}	HMBC	COSY
1	-	202.18	-	-
2	2.90-2.71 (m, 2H)	51.16	1, 3, 4, 9a, 9b, 11	4
3	2.00 (s, br, -OH)	71.34	-	-
4	3.12 (m, 2H)	43.42	3, 10, 10b	2, 10
5	6.72 (s, 1H)	98.57	6, 7, 9a, 10	6-OCH ₃ , 10
6	-	161.54	-	-
6-OCH ₃	3.78 (s, 3H)	56.19	6	5, 10
7	-	111.45	-	-
8-OH	9.95 (s, br, -OH)	156.83	7, 8, 9a	-
9-OH	16.16 s, br, -OH)	166.02	-	-
9a	-	108.47	-	-
9b	-	108.68	-	-
10	7.02 (s, 1H)	117.87	4, 5, 9a, 9b, 10a, 10b	4, 5, 6-OCH ₃ ,
10a	-	140.80/140.72	-	-
10b	-	135.93	-	-
11	1.51 (s, 3H)	29.30	2, 3, 4	-
1'	-	201.92	-	-
2'	2.90-2.71 (m, 2H)	50.57	1', 3', 4', 9'b, 10', 10'b, 11'	4'
3'	2.00 (s, br, -OH)	70.89	-	-
4'	2.66 (dd, $J = 1.34$ Hz, 15.54 Hz, 2H)	41.40	2', 3', 9'a, 9'b, 10', 10'b	2'
5'	6.17 (d, $J = 2.38$ Hz, 1H)	99.87	6', 7', 9'a, 10'	6'-OCH ₃ , 7'
6'	-	163.79	-	-
6'-OCH ₃	3.68 (s, 3H)	55.42	6'	5', 7'
7'	6.51 (d, $J = 2.38$ Hz, 1H)	100.05	5', 6', 8', 9'a	5', 6'-OCH ₃
8'-OH	10.22 (s, br, -OH)	160.93	7', 8'	-
9'-OH	16.66 (s, br, -OH)	167.10	-	-
9'a	-	108.33/108.28	-	-
9'b	-	108.33/108.28	-	-
10'	-	120.51	-	-
10a'	-	140.80/140.72	-	-
10'b	-	134.17	-	-
11'	1.32 (s, 3H)	29.12	2', 3', 4'	-

Table S4. Fungal and bacterial strains used in this study.

Strain	Genotype	Reference
<i>Cortinarius odorifer</i>	Wild type	
<i>Aspergillus niger</i> ATNT16 Δ pyrGx24	TetOn::terR_ble; Δ pyrG::ptrA	[3]
<i>Aspergillus niger</i> tNAL015	TetOn::terR_ble; Δ pyrG::ptrA; PterA:copks4_pyrG; PterA:spoM_hph	This study
<i>Aspergillus niger</i> tLP001	TetOn::terR_ble; Δ pyrG::ptrA; PterA:copks4_pyrG; PterA:spoM_hph; PterA:coupo1_pyrG	This study
<i>Aspergillus niger</i> tLP002	TetOn::terR_ble; Δ pyrG::ptrA; PterA:coupo1_pyrG	This study
<i>Escherichia coli</i> BL21-Gold(DE3)	<i>E. coli</i> B F ⁻ ompT hsdS(r _B ⁻ m _B ⁻) dcm ⁺ Tet ^r gal λ (DE3) endA Hte	Agilent
<i>Escherichia coli</i> tLP003	Harboring pLP002	This study
<i>Escherichia coli</i>	Harboring pET19b::spoM	[12]
<i>Escherichia coli</i>	Harboring pET19b::shwM1	[13]
<i>Escherichia coli</i>	Harboring pET19b::shwM2	[13]

Table S5. Oligonucleotides used in this study.

Oligonucleotide name	Sequence	Target sequence	Purpose
oLP054	CATCACCATCACCATGGCCGTAAGTCTG TTCTCCTCA	<i>coupo1</i>	Cloning of pLP001
oLP055	CACTGCTGTTATCCATGTGACACTGGAC CGTAAGGGA	<i>coupo1</i>	Cloning of pLP001
oNAL072	TCATCATCACCATCACCATGGAACAGTTA CAAACCTCCCAAAAAGC	<i>spoM</i>	Cloning of pNAL017
oNAL073	GGTTCAGATTGAAATCACTGCTGTTAGG ACTTGACATACAACC	<i>spoM</i>	Cloning of pNAL017
oLP206	CGCGCGGCAGCCATACGATGCCTGCAT GTCCGC	<i>coomt1</i>	Cloning of pLP002
oLP207	CAGTCATGCTAGCCATATTACATTGCGGT ATACAGAAAGCT	<i>coomt1</i>	Cloning of pLP002
oLP056	CCGACAAGCAGTACTTTTCCC	phis_SM-Xpress	Proof of transgene integration
oLP057	TTGGAGGTGAAGCCAATCCC	phis_SM-Xpress	Proof of transgene integration

Table S6. PCR parameters.

Condition	Initial denaturation	Thermal cycling	Final elongation
I	98 °C for 5 min	30 cycles of 98 °C for 20 s, 60 °C for 30 s, 72 °C for 45 s	72 °C for 90 s
II	98 °C for 5 min	30 cycles of 98 °C for 20 s, 60 °C for 30 s, 72 °C for 60 s	72 °C for 120 s

Table S7. Plasmids used in this study.

Plasmid name	Vector backbone	Gene	Reference
phis_SM-Xpress	pUC19	-	[3,14]
pNAL006	phis SM-Xpress	<i>copks4</i> (gDNA)	[15,16]
pMG04	pUC19	-	[16]
pET19b:: <i>spoM</i>	pET19b	<i>spoM</i> (cDNA)	[12]
pET19b:: <i>shwM1</i>	pET19b	<i>shwM1</i> (cDNA)	[13]
pET19b:: <i>shwM2</i>	pET19b	<i>shwM2</i> (cDNA)	[13]
pNAL017	pMG04	<i>spoM</i> (cDNA)	This study
pLP001	phis SM-Xpress	<i>coupo1</i> (cDNA)	This study
pLP002	pET28a	<i>coomt1</i> (cDNA)	This study

Table S8. HPLC parameters. Eluents were: 0.1% formic acid in water (eluent A) and acetonitrile (eluent B) (condition I and II) and 100% methanol (eluent B) (condition III).

Gradient	Flow [mL min ⁻¹]	Time [min]	Eluent B [%]	Column
I	0.150	0.0	40	InfinityLab Poroshell 120 EC-C18 (4.6 × 150 mm, 4 µm particle size)
	0.225	1.5	40	
	0.300	21.5	85	
	0.225	26.0	98	
	0.225	32.0	40	
	0.150	36.0	40	
II	1.75	0.0	40	LiChrosorb® RP 18-5µ (4.0 × 250 mm, 5 µm particle size)
	2.50	2.0	40	
	2.50	7.0	60	
	2.50	22.0	85	
	2.50	25.0	98	
	2.50	30.0	98	
	2.50	35.0	40	
III	1.75	38.0	40	Daicel Chiralpak® AD (4.6 × 250 mm, 10 µm particle size)
	0.50	0.0	100	
	0.50	30.0	100	

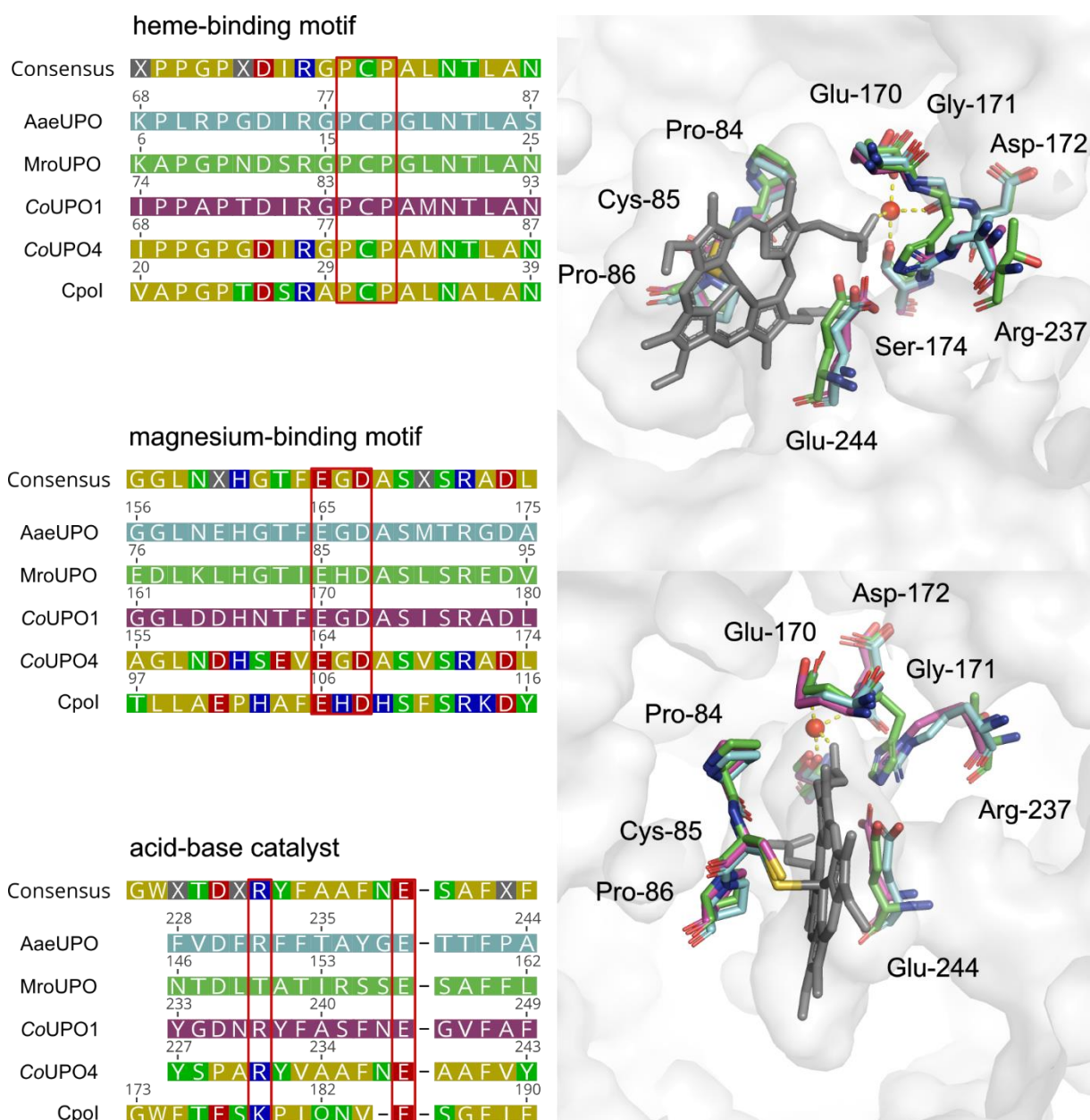


Figure S3. Sequence and structural comparison. Amino acid sequence alignment of conserved regions (heme-binding, magnesium-binding, and acid-base catalyst motif) of characterized representatives of family II AaeUPO (cyan), family I MroUPO (green) unspecific peroxygenases, and chloroperoxidase (Cpol) with putative UPOs CoUPO1 (magenta) and CoUPO4 from *C. odorifer*. CoUPO4 is a homolog of CoUPO1 adjacently encoded to the CoPKS4 on the *C. odorifer* genome. UPO structures of crystallized MroUPO (PDB 5FUJ) (green), AaeUPO (PDB 2YOR) (cyan) are superimposed on AlphaFold2 prediction of CoUPO1 (magenta) with the conserved regions depicted as sticks indicated with the residue number of CoUPO1. The polar interactions (yellow dotted line) of the magnesium (red sphere) and heme (grey sticks) of structure 2YOR are shown.

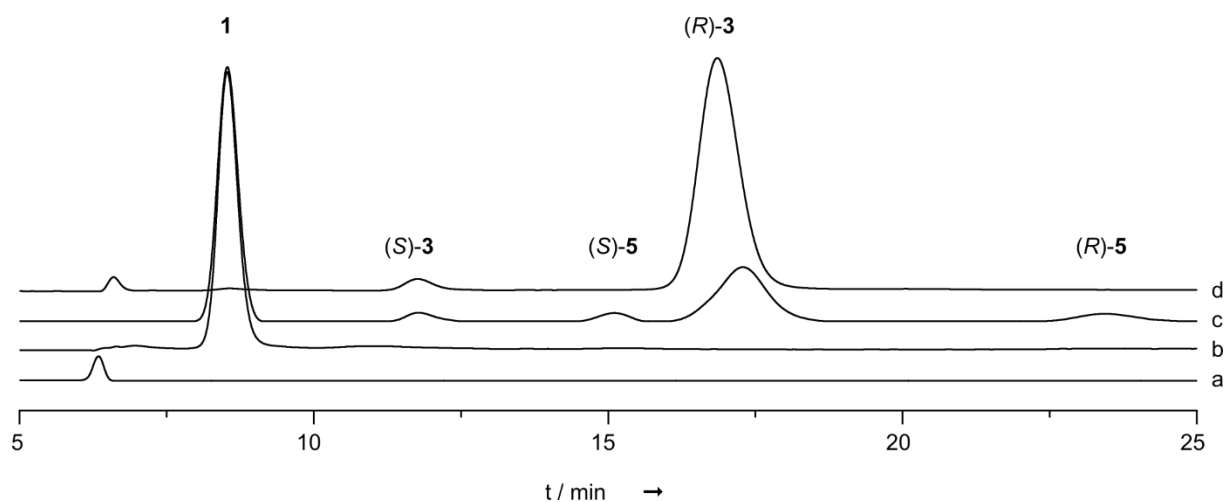


Figure S4. *In vitro* activity assays with SpoM and CoOMT1 heterologously produced in *E. coli*. Chromatograms of ethyl acetate extracts were recorded by HPLC–DAD (400 nm). All reactions were run in the presence of **1**. Lane a: methanol (negative control). b: Reaction without enzyme. c: Reaction with CoOMT. d Reaction with SpoM.

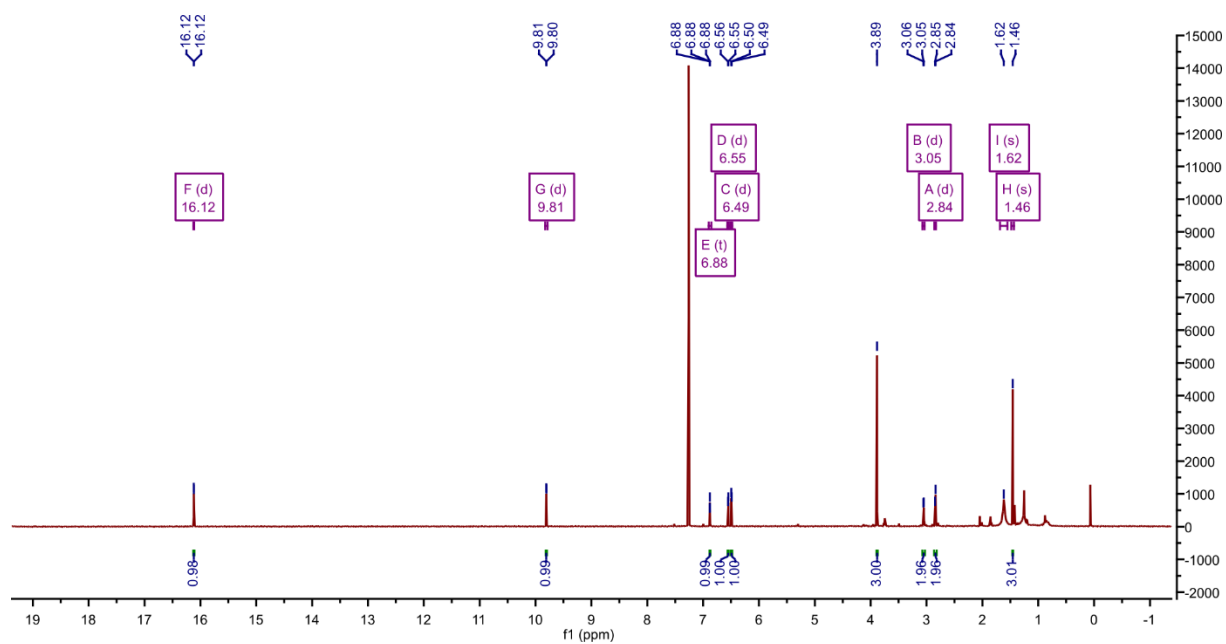


Figure S5. ¹H NMR spectrum of a fractionated *A. niger* tNAL015 extract with torosachryson (3) as main metabolite (400 MHz, CD₃Cl).

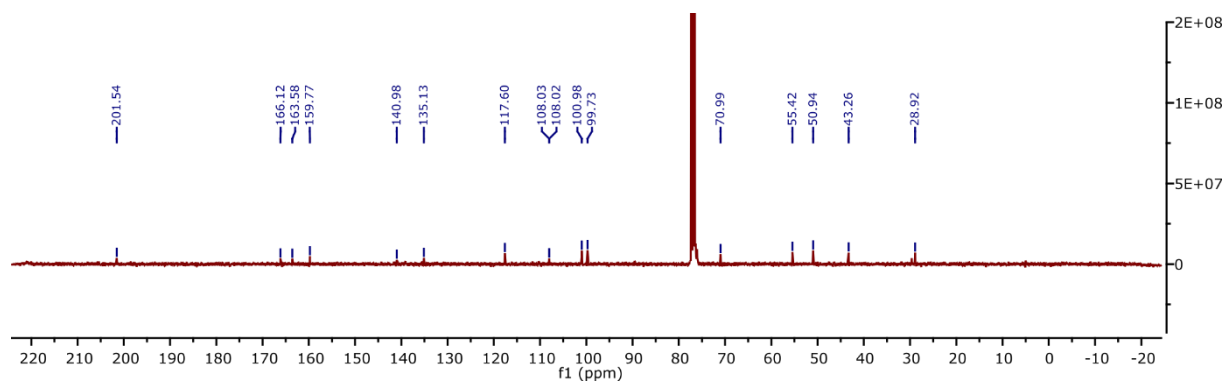


Figure S6. ¹³C NMR spectrum of a fractionated *A. niger* tNAL015 extract with torosachryson (3) as main metabolite (100.6 MHz, CD₃Cl).

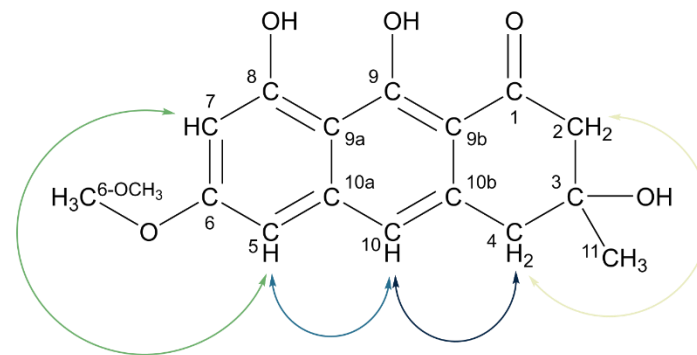
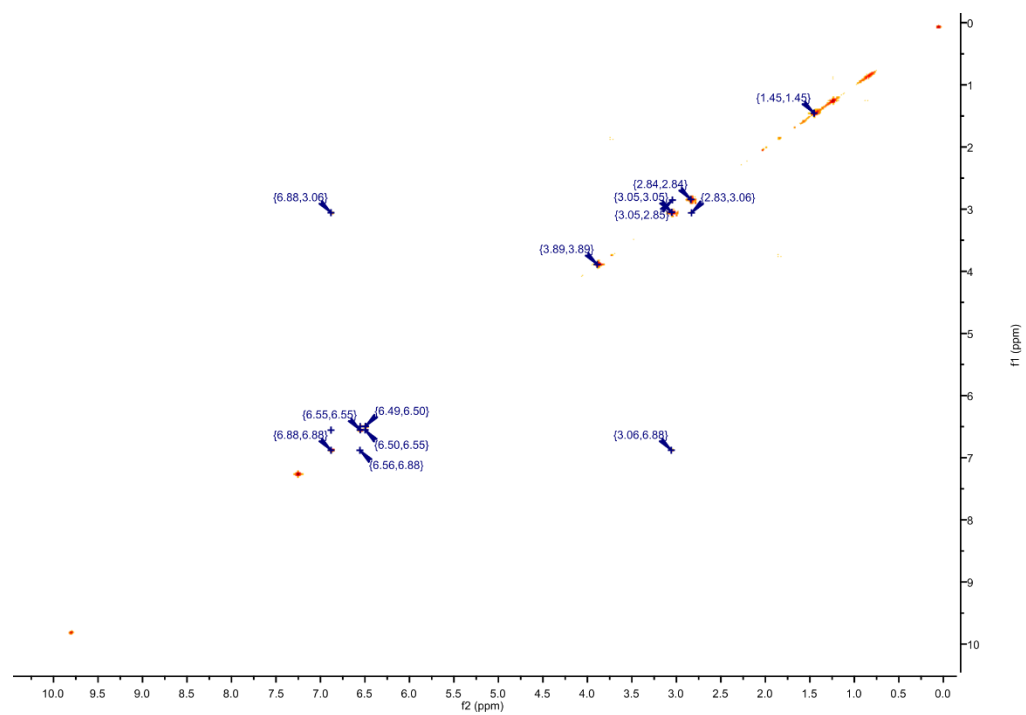


Figure S7. ^1H - ^1H COSY spectrum of a fractionated *A. niger* tNAL015 extract with torosachryson (**3**) as main metabolite.

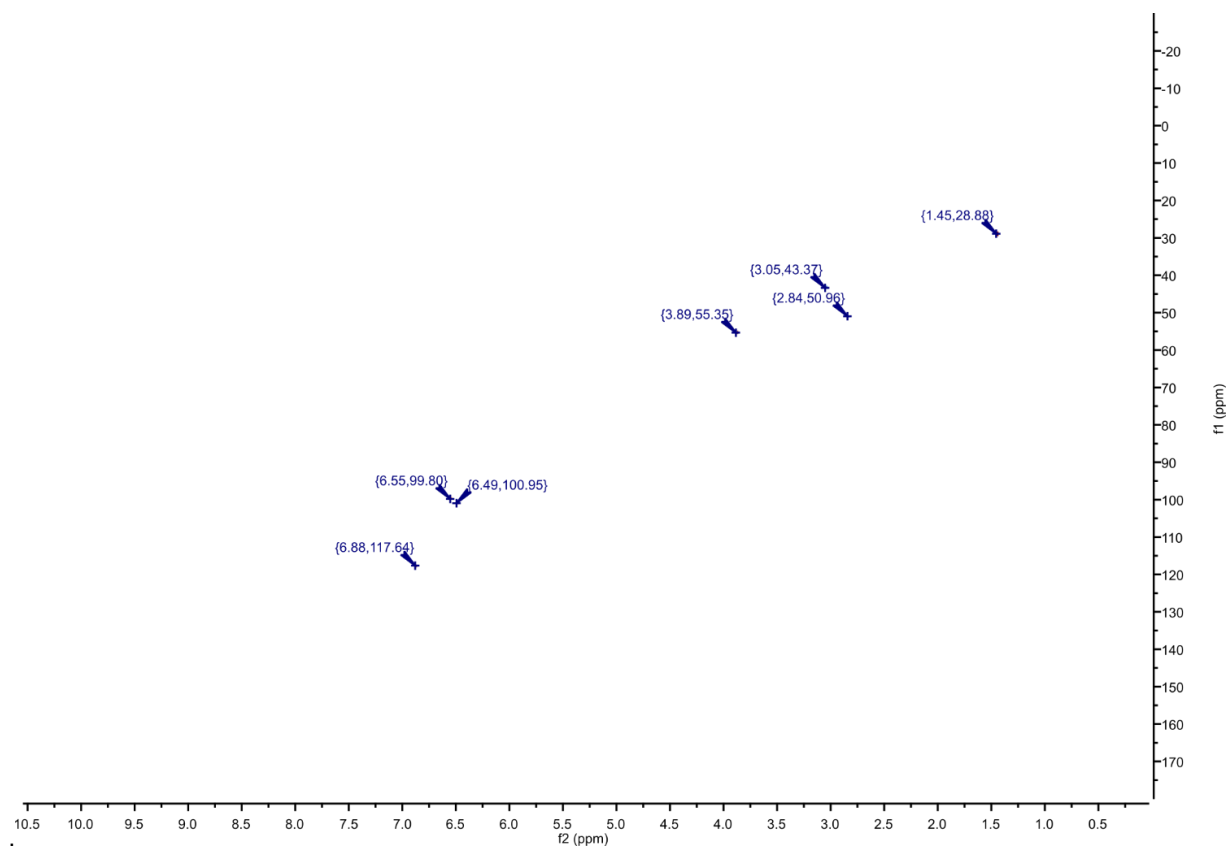


Figure S8. HSQC spectrum of a fractionated *A. niger* tNAL015 extract with torosachryson (**3**) as main metabolite.

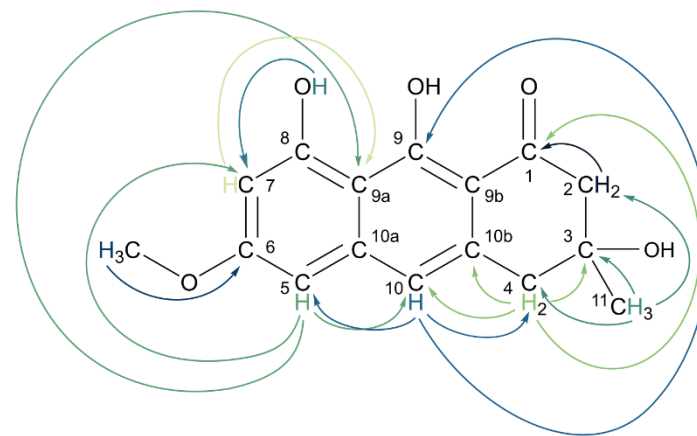
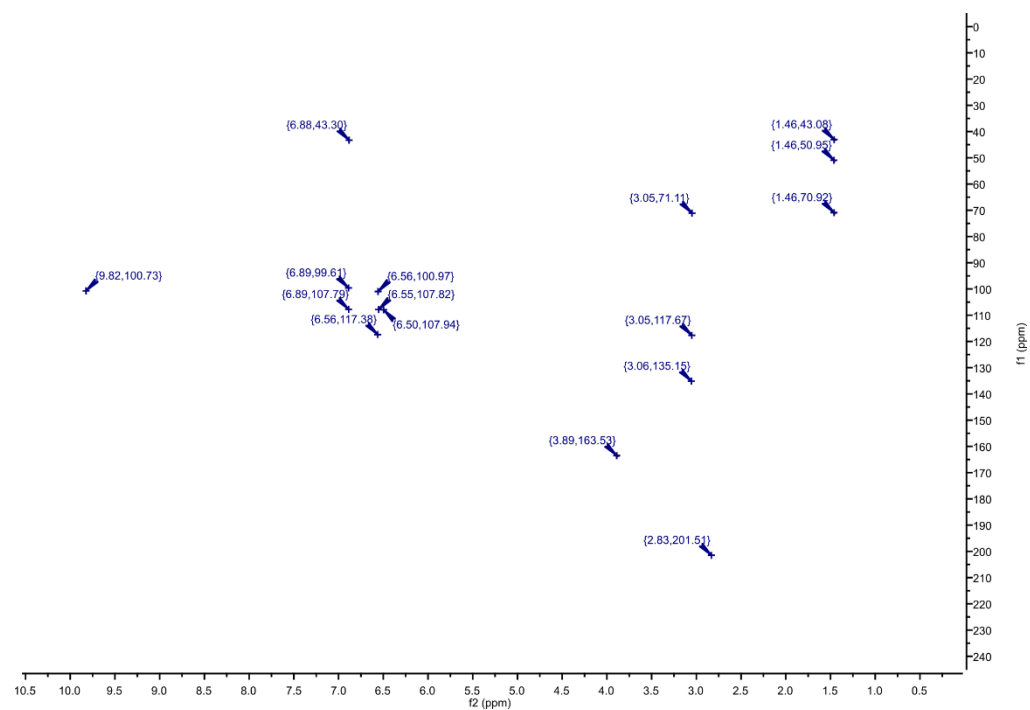


Figure S9. HMBC spectrum of a fractionated *A. niger* tNAL015 extract with torosachryson (**3**) as main metabolite.

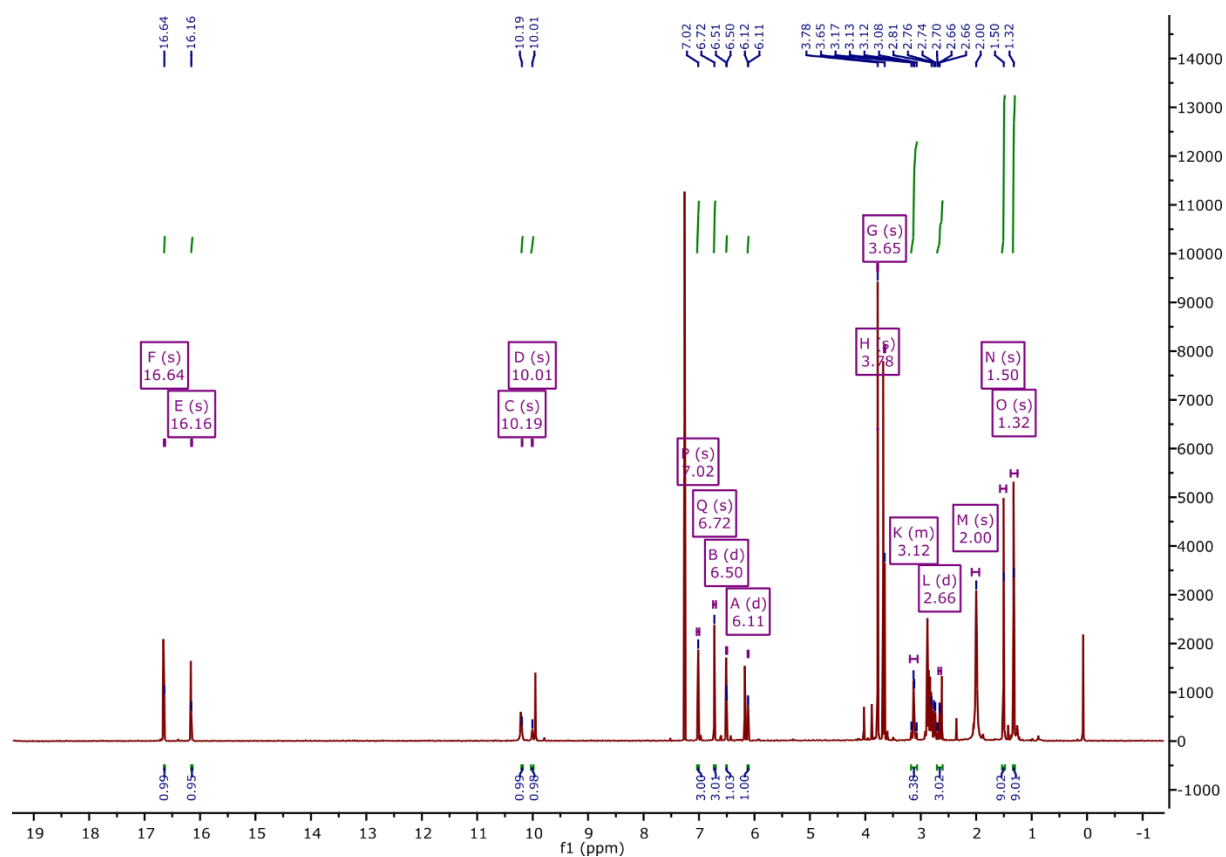


Figure S10. ^1H NMR spectrum of a fractionated *C. odorifer* extract with phlegmacin A_1 (**2a**) as main metabolite (400 MHz, CD_3Cl).

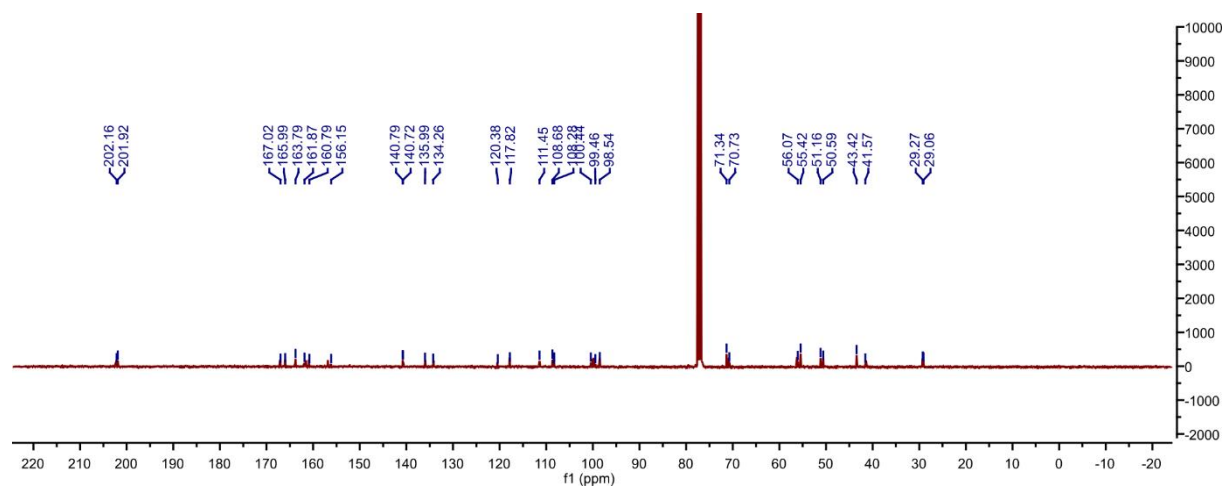


Figure S11. ^{13}C NMR spectrum of a fractionated *C. odorifer* extract with phlegmacin A_1 (**2a**) as main metabolite (100.6 MHz, CD_3Cl).

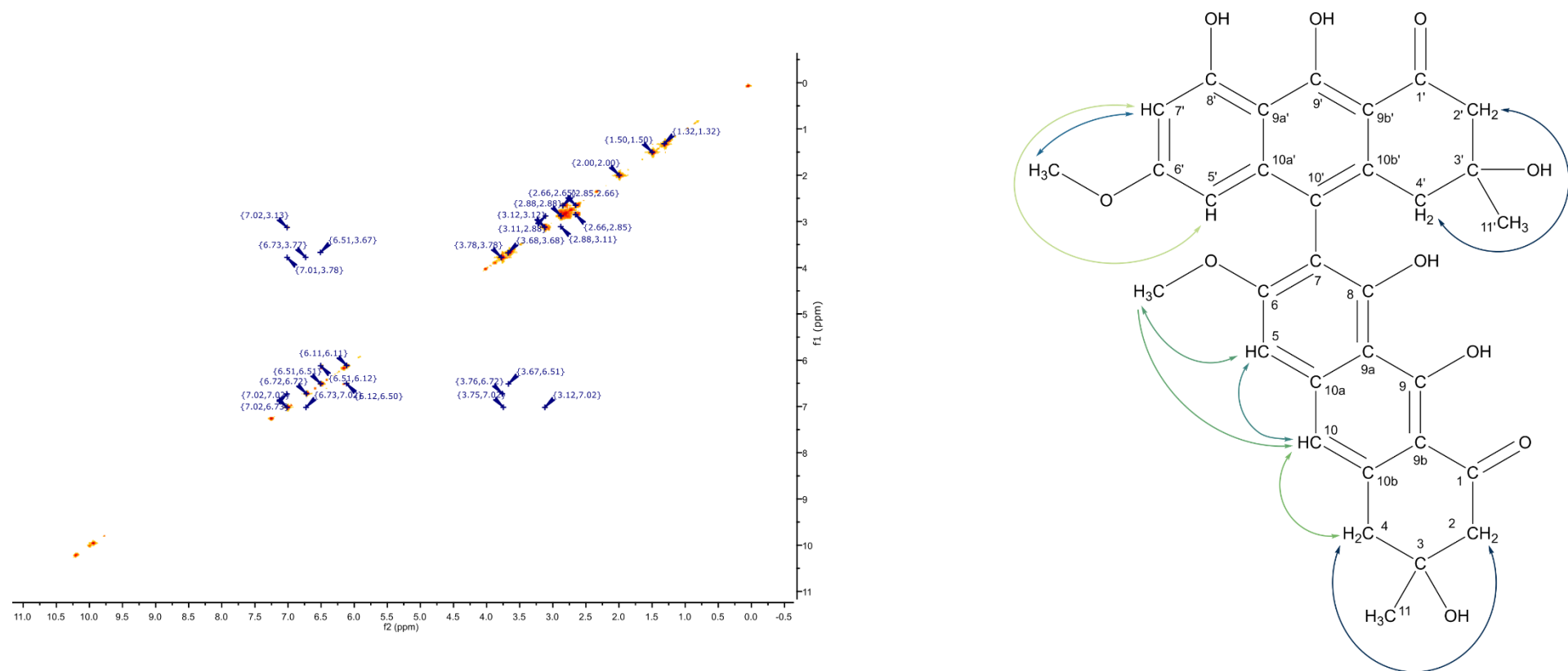


Figure S12. ^1H - ^1H COSY spectrum of a fractionated *C. odorifer* extract with phlegmacin A₁ (**2a**) as main metabolite.

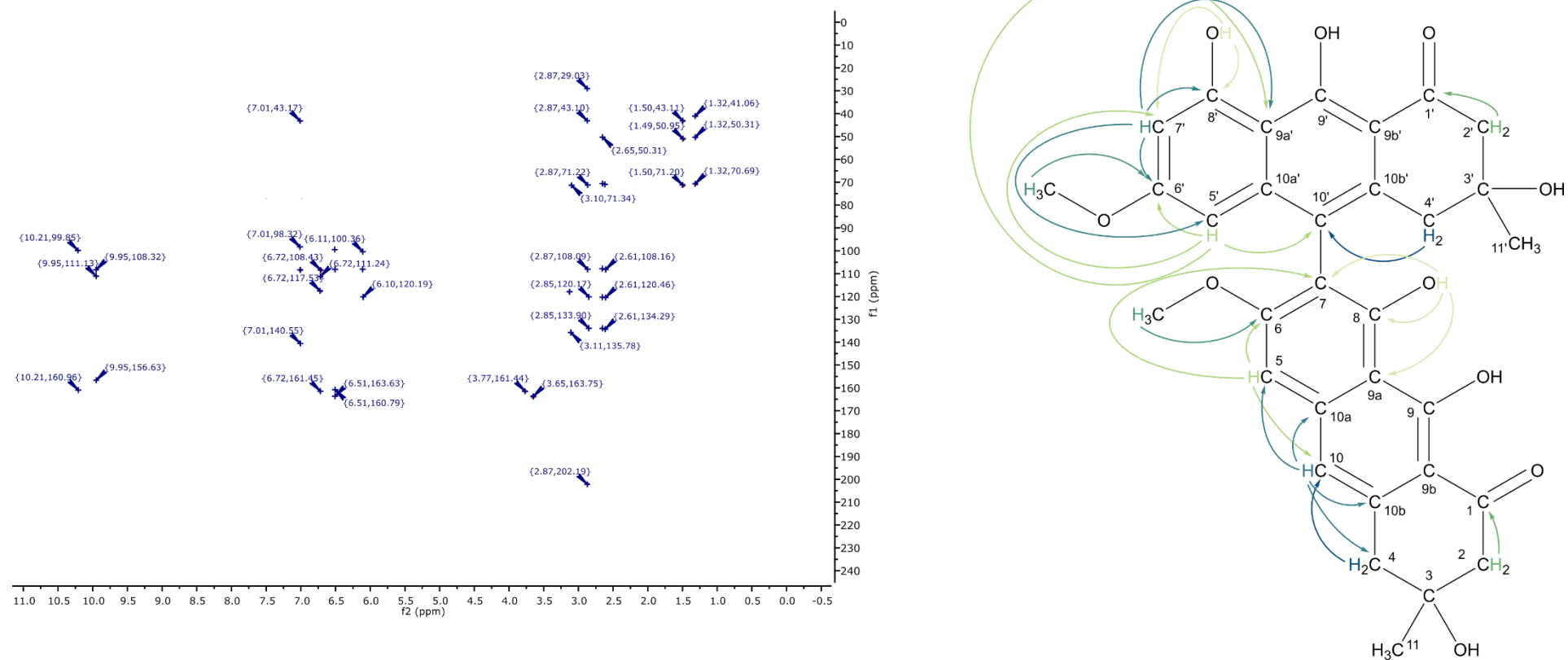


Figure S13. HMBC spectrum of a fractionated *C. odorifer* extract with phlegmacin A₁ (**2a**) as main metabolite. For structure elucidation, only the relevant couplings are visualized by colored arrows.

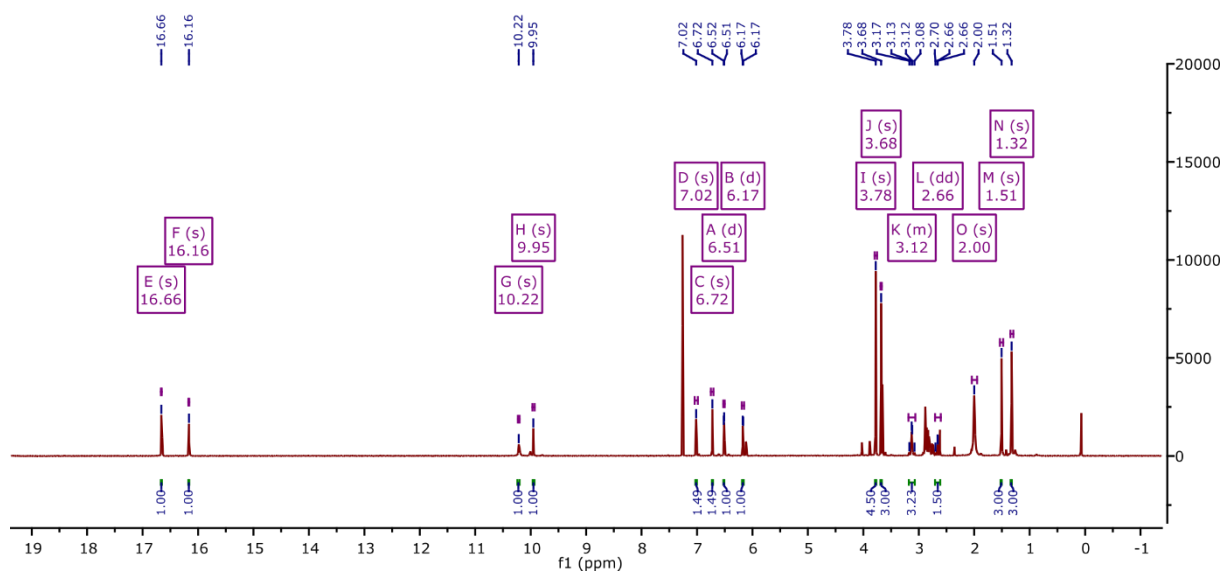


Figure S14. ^1H NMR spectrum of a fractionated *C. odorifer* extract with phlegmacin B_1 (**2b**) as main metabolite (400 MHz, CD_3Cl).

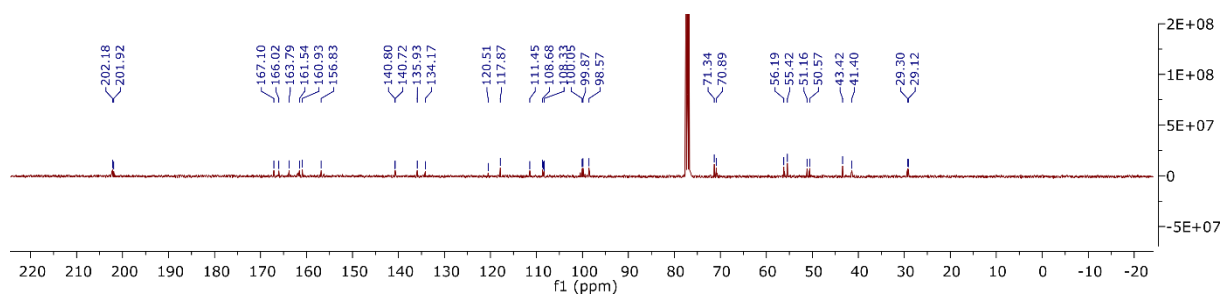


Figure S15. ^{13}C NMR spectrum of a fractionated *C. odorifer* extract with phlegmacin B_1 (**2b**) as main metabolite (100.6 MHz, CD_3Cl).

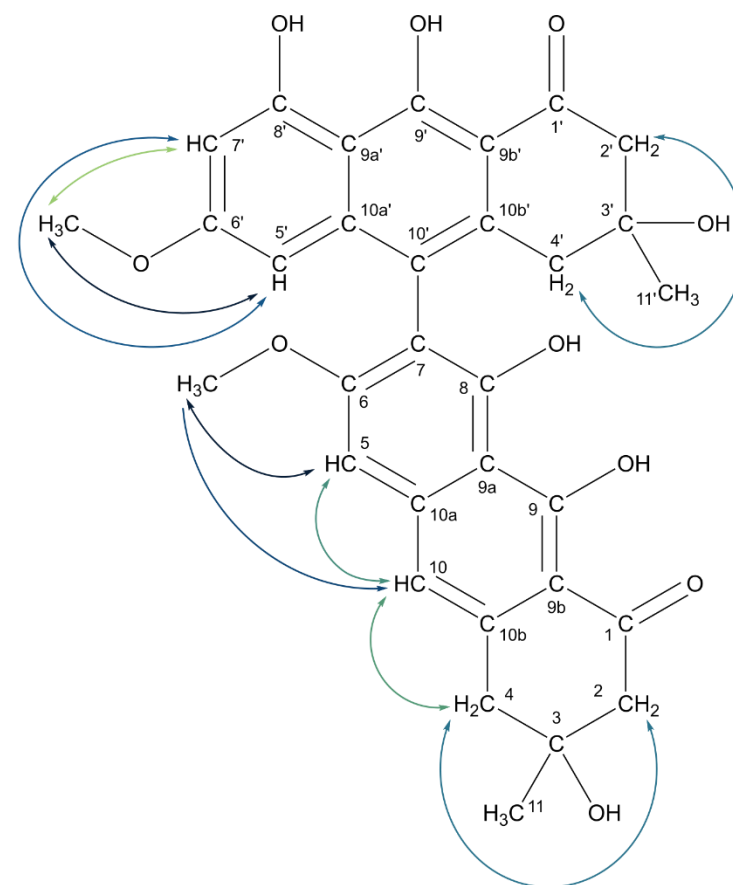
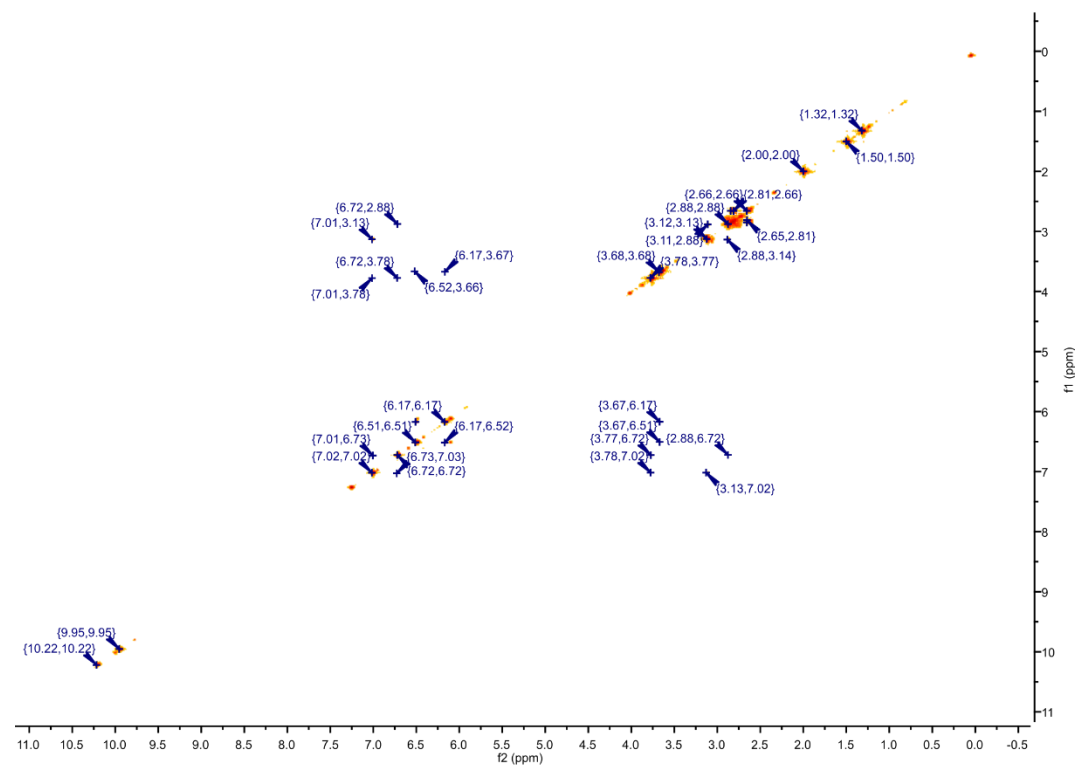


Figure S16. ^1H - ^1H COSY spectrum of a fractionated *C. odorifer* extract with phlegmacin B₁ (**2b**) as main metabolite.

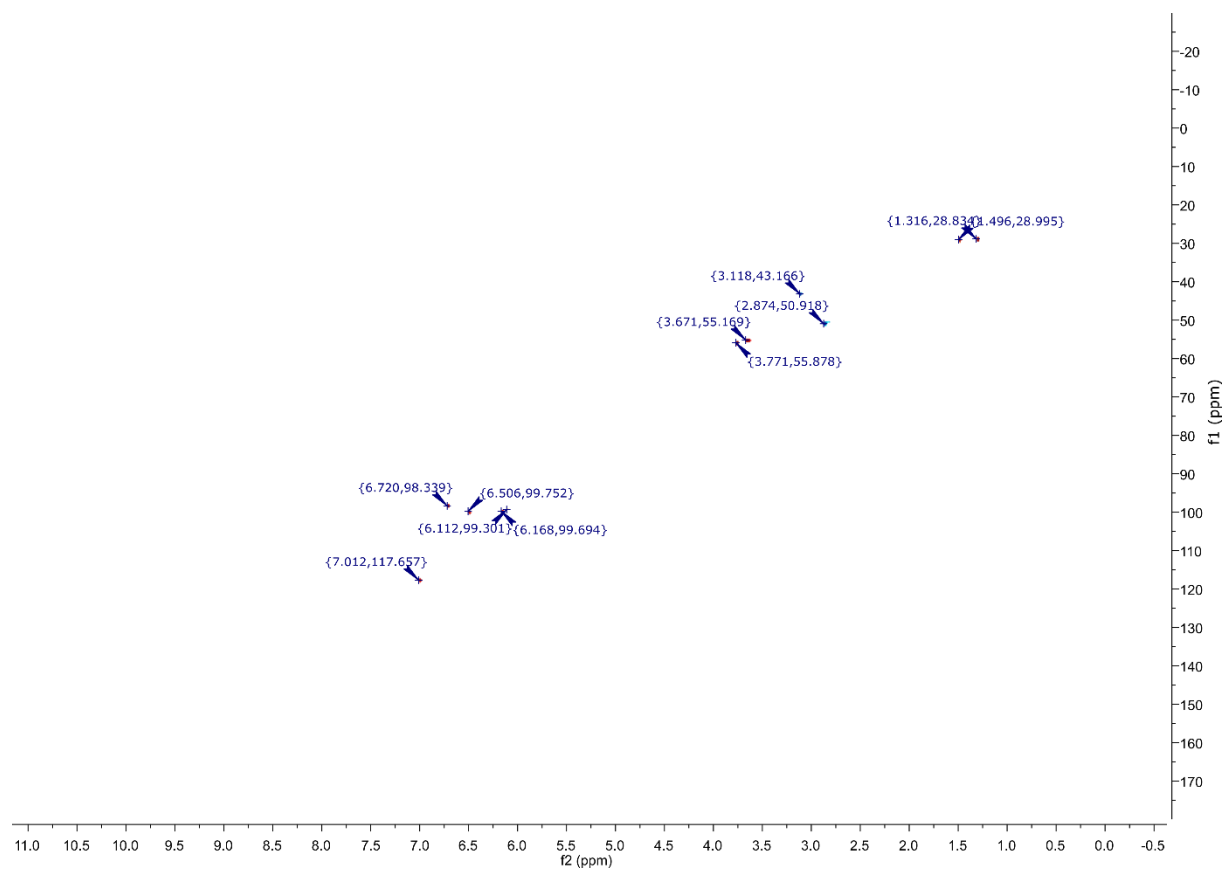


Figure S17. HSQC spectrum of a fractionated *C. odorifer* extract with phlegmacin A₁ (**2a**) and B₁ (**2b**) as main metabolites.

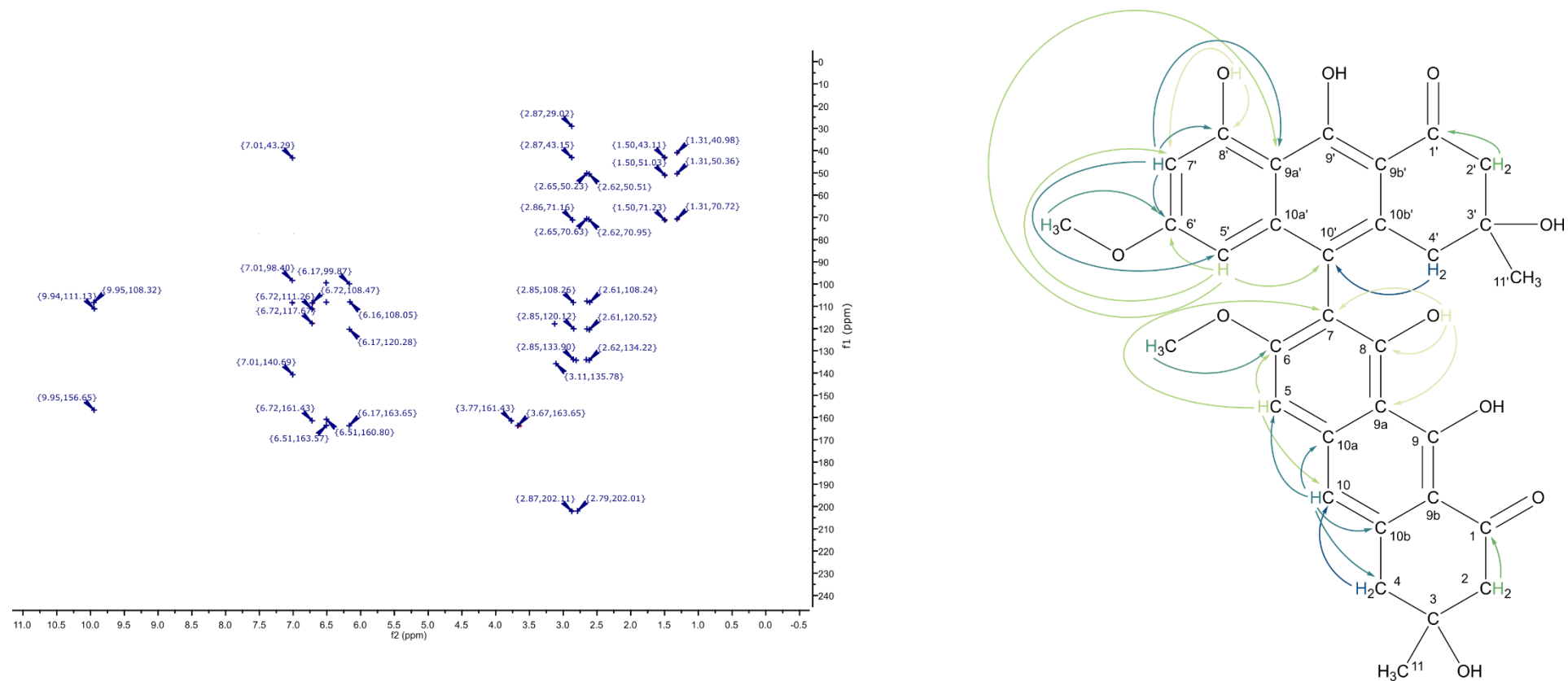


Figure S18. HMBC spectrum of a fractionated *C. odorifer* extract with phlegmacin B₁ (**2b**) as main metabolite. For structure elucidation, only the relevant couplings are visualized by colored arrows.

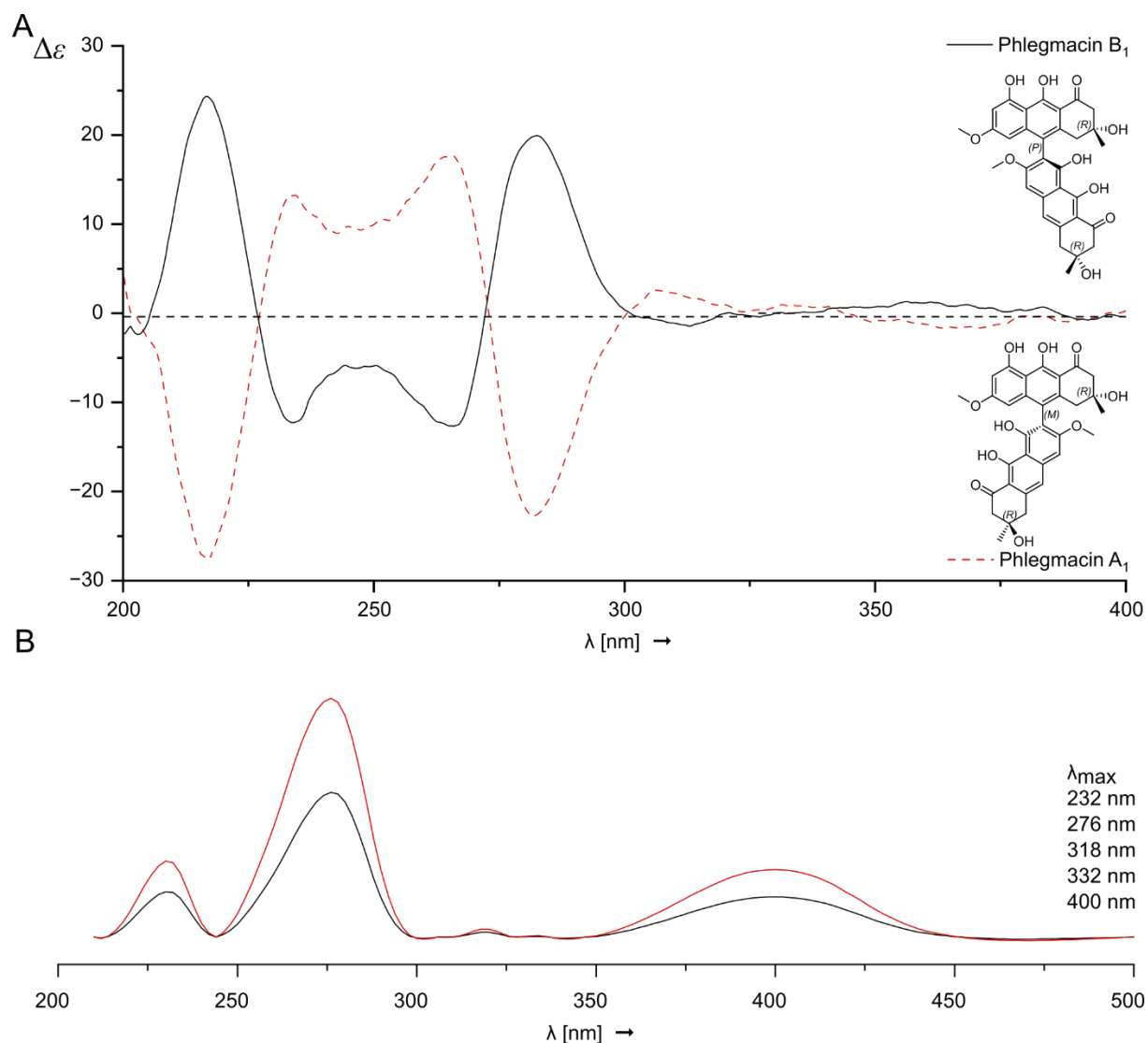


Figure S19. CD and UV spectrum of **2a** and **2b**. A) CD spectroscopy of isolated phlegmacin B₁ (black) and phlegmacin A₁ (red) in methanol. B) UV spectra of the separated atropisomers **2a** (red) and **2b** (black).

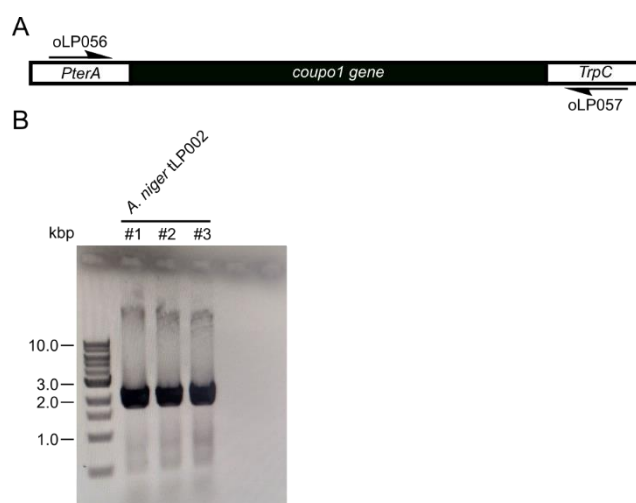


Figure S20. Agarose gel to verify *C. odorifer* UPO gene integration in the *A. niger* host genome. A) PCR strategy to amplify the entire cassette of *pterA* promoter/PKS gene/*trpC* terminator. Expected amplicon lengths in case of a full-length accurate integration is 1.9 kilobase pairs (kbp). B) tLP002: harboring the gene *coupo1*.

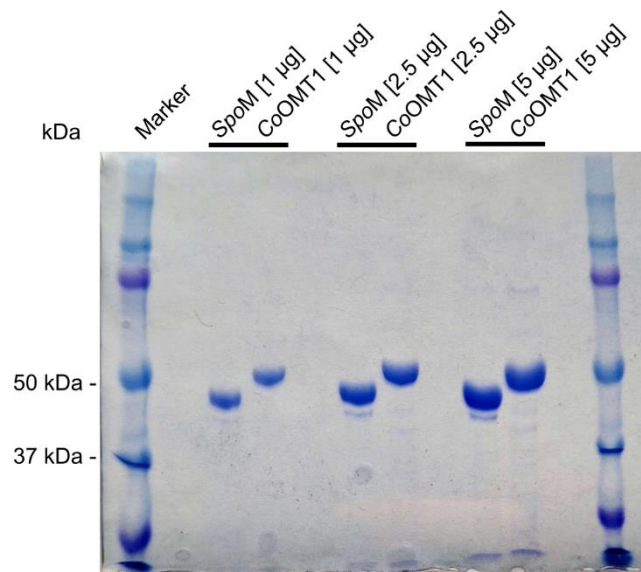


Figure S21. SDS-polyacrylamide gel electrophoresis of SpoM-N-His₁₀ (48.5 kDa) and CoOMT1-N-His₆ (52.2 kDa). Purified protein solution was analyzed on a 12 % gel to verify the purity of the respective proteins displayed at three different protein concentrations (1, 2.5, and 5 µg µL⁻¹).

Sequences

gDNA sequences*

>*coupo1* (Accession PP372684)

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>*coomt1* (Accession PP372683)

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*Introns are underlined based on AUGUSTUS^[17] gene prediction and experimental evidence.

cDNA sequences

>*coupo1*

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Amino acid sequences

>CoJPO1

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References

- [1] K. Liimatainen, J. T. Kim, L. Pokorny, P. M. Kirk, B. Dentinger, T. Niskanen, *Fungal Diversity* **2022**, 112, 89–170.
- [2] R. Argumedo-Delira, D. González-Mendoza, A. Alarcón, *Ann. Microbiol.* **2008**, 58, 761–763.
- [3] E. Geib, M. Brock, *Fungal Biol. Biotechnol.* **2017**, 4:13.
- [4] G. Pontecorvo, J. A. Roper, L. M. Hemmons, K. D. Macdonald, A. W. J. Bufton, *The genetics of Aspergillus nidulans*, Academic Press, New York, NY, **1953**.
- [5] E. Geib, M. Gressler, I. Viediarnikova, F. Hillmann, I. D. Jacobsen, S. Nietzsche, C. Hertweck, M. Brock, *Cell Chem. Biol.* **2016**, 23, 587–597.
- [6] M. Mirdita, K. Schütze, Y. Moriwaki, L. Heo, S. Ovchinnikov, M. Steinegger, *Nat. Methods* **2022**, 19, 679–682.
- [7] J. Jumper, R. Evans, A. Pritzel, T. Green, M. Figurnov, O. Ronneberger, K. Tunyasuvunakool, R. Bates, A. Žídek, A. Potapenko et al., *Nature* **2021**, 596, 583–589.
- [8] L. L. Schrödinger, *The PyMOL Molecular Graphics System, Version 2.0.7*, **2015**.
- [9] S. F. Altschul, W. Gish, W. Miller, E. W. Myers, D. J. Lipman, *J. Mol. Biol.* **1990**, 215, 403–410.
- [10] D. T. Jones, W. R. Taylor, J. M. Thornton, *Bioinformatics* **1992**, 8, 275–282.
- [11] K. Tamura, G. Stecher, S. Kumar, *Mol. Biol. Evol.* **2021**, 38, 3022–3027.
- [12] W. Thiele, S. Obermaier, M. Müller, *ACS Chem. Biol.* **2020**, 15, 844–848.
- [13] W. Thiele, R. Froede, W. Steglich, M. Müller, *ChemBioChem* **2020**, 21, 1423–1427.
- [14] E. Geib, F. Baldeweg, M. Doerfer, M. Nett, M. Brock, *Cell Chem. Biol.* **2019**, 26, 223–234.
- [15] a) N. A. Löhr, F. Eisen, W. Thiele, L. Platz, J. Motter, W. Hüttel, M. Gressler, M. Müller, D. Hoffmeister, *Angew. Chem. Int. Ed.* **2022**, 134, e202116142; b) N. A. Löhr, M. C. Urban, F. Eisen, L. Platz, W. Hüttel, M. Gressler, M. Müller, D. Hoffmeister, *ChemBioChem* **2023**, 24, e202200649.
- [16] N. A. Löhr, M. Rakhmanov, J. M. Wurlitzer, G. Lackner, M. Gressler, D. Hoffmeister, *Fungal Biol. Biotechnol.* **2023**, 10:17.
- [17] M. Stanke, M. Diekhans, R. Baertsch, D. Haussler, *Bioinformatics* **2008**, 24, 637–644.