Supporting Information

Merochlorins A–D, cyclic meroterpenoid antibiotics biosynthesized in divergent pathways with vanadium-dependent chloroperoxidases

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Table of Contents

Experimental Section

General methods

Optical rotations were measured using a Rudolph Research Analytical Autopol III polarimeter (Hackettstown, NJ, USA) with a 10-cm cell. UV spectra were acquired with an Varian Cary UV-visible spectrophotometer (Agilent Technologies, Santa Clara, CA, USA) using a path length of 1 cm. IR spectra were acquired with a Perkin-Elmer PE 1600 FTIR spectrophotometer (Perkin-Elmer, Waltham, MA, USA). NMR spectra were acquired with 500 or 600 MHz Varian Inova spectrometers using dimethyl sulfoxide (DMSO)-d_6 or chloroform-d_3 ($CDCl_3$) containing tetramethylsilane (Me_4Si) as an internal standard. HRMS analysis was conducted with time-of-flight detection. Low-resolution MS data were collected with a Agilent 1100 LC-MS instrument (Agilent Technologies), and the analytes were separated with a reversed-phase C_{18} column (Phenomenex Luna 5μ C18(2), 4.6 mm × 100 mm) using a flow rate of 0.7 mL/min. DNA isolation and manipulations were performed using standard methods for *Escherichia coli* and *Streptomyces*.[1,2]

Culture conditions

To isolate merochlorins **1** and **2**, 60 x 1 L of CNH-189 was cultured in 2.8-L Fernbach flasks with seawater-based medium (10 g/L starch, 4 g/L yeast extract, 2 g/L peptone, 1 g/L $CaCO_3$, 40 mg/L $Fe_2(SO_4)_3 \cdot 4H_2O$, and 100 mg/L KBr) at 27 °C. After 7 days of cultivation, sterilized XAD16 resin (20 g/L) was added as an adsorbent. To isolate merochlorins **3** and **4**, 40 L of CNH-189 was cultured in 2.8-L Fernbach flasks each containing 1 L of a deionized water-based medium (M1: 10 g/L glucose, 20 g/L Grandma's molasses, 5 g/L peptone, 2 g/L $CaCO_3$, 40

mg/L $Mg(SO_4)\cdot 4H_2O$, 200 mg/L KCl, 200 mg/L KBr, and 40 mg/L $Fe_2(SO_4)_3\cdot 4H_2O$) at 27 °C. After 24 h of cultivation, sterilized XAD7HP resin (20 g/L) was added, and the culture was incubated for an additional 5 days at 27 °C.

Isolation of merochlorins A (1) and B (2)

Briefly, the cell culture medium was incubated with XAD16 resin (20 g/L) for 2 h at 215 rpm at room temperature, and the resin was collected by filtering through cheesecloth. The resin was then washed with deionized water and eluted with acetone. The acetone was removed under reduced pressure, and the remaining aqueous layer was extracted with ethyl acetate (EtOAc) (3 × 500 mL). The ethyl acetate-soluble fraction was dried in vacuo to yield 4.5 g of crude extract containing **1** and **2.** The crude extract was fractionated by silica gel column chromatography (25 g) by elution with a step gradient of dichloromethane and methanol. The 100:1 dichloromethane:methanol fraction contained a mixture of the merochlorins and was purified further by reversed-phase HPLC (Phenomenex Luna 5μ C18(2), 250 × 100 mm, 2.0 mL/min, 100 Å, 210 nm detection) using isocratic conditions, 85% MeCN in water. HPLC separation afforded **1** (21 mg) and **2** (7 mg). Merochlorin A: Table S1 and reference.[3] Merochlorin B (**2**): $[\alpha]_D^{21}$ = +23 (c = 0.27, MeOH); IR (KBr) ν_{max} = 3199, 2928, 1645, 1457, 1329, 1279, 1162, 1065, and 841 cm^{-1}; UV (MeOH) λ_{max} (log ε) = 242 (3.1), 261 (4.2), 255 (4.2), 272 (4.3), and 349 (4.3) nm; ^1H and ^{13}C NMR (500 MHz, DMSO-d_6) (Table S2); HR-ESI-MS ($C_{25}H_{30}{}^{35}ClO_4$): *m/z* 429.1818 $[M+H]^+$, calculated *m/z* 429.1827 $[M+H]^+$.

Isolation of merochlorins C (3) and D (4)

After culture in the presence of XAD7HP resin, the resin was collected on cheesecloth, washed with deionized water, and eluted with acetone. The acetone was removed under reduced pressure, and the remaining aqueous layer was extracted with EtOAc (3 × 500 mL). The EtOAc-soluble fraction was dried in vacuo to yield 4.7 g of crude extract containing **3** and **4**. The crude extract was fractionated and purified as described above. HPLC separation afforded **4** (15 mg) and **3** (40 mg) as pale-yellow oils. Merochlorin C (**3**): $[\alpha]_D^{21}$ = +34 (c = 0.25, MeOH); UV (MeOH) λ_{max} (log ε) = 224 (4.6), 239 (4.7), 296 (4.3), and 334 (4.3) nm; IR (KBr) ν_{max} = 3380, 2931, 1704, 1629, 1373, 1271, and 860 cm^{-1}; ^1H and ^{13}C NMR (500 MHz, DMSO-d_6) (Table S3); HR-ESI-MS ($C_{26}H_{32}{}^{35}Cl_2O_5Na$): found *m/z* 517.1518 $[M+Na]^+$, calculated *m/z* 517.1519 $[M+Na]^+$. Merochlorin D (**4**): $[\alpha]_D^{21}$ = +28 (c = 0.2, MeOH); UV (MeOH) λ_{max} (log ε) = 245 (4.7), 301 (4.2), and 360 (4.3) nm; IR (KBr) ν_{max} = 3380, 2930, 1705, 1628, 1373, 1271, and 860 cm^{-1}; ^1H and ^{13}C NMR (500 MHz, DMSO-d_6) (Table S3); HR-ESI-MS ($C_{26}H_{33}{}^{35}ClO_5Na$): found *m/z* 483.1907 $[M+Na]^+$, calculated *m/z* 483.1909 $[M+Na]^+$.

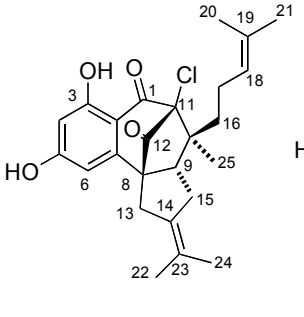
Acetylation and methylation of merochlorin A

Merochlorin A (**1**, 4.0 mg) was dissolved in freshly distilled pyridine (2 mL). Several dry crystals of dimethylaminopyridine were added, and the mixture was stirred for 15 min at room temperature. Acetic anhydride (3 mL) was added to the solution, and the reaction mixture was stirred for 12 h. The reaction was quenched with water, and the resulting mixture was extracted with EtOAc. The solvent was removed in vacuo and the residual material was dissolved in dry acetone (2 mL). An excess of K_2CO_3 and methyl iodide was added (1.5 mL), and after stirring for 6 h, the reaction mixture was filtered and evaporated, and the residual material was subjected

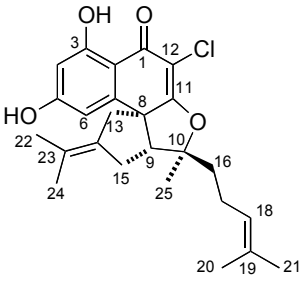
to reversed-phase HPLC (Phenomenex Luna 5μ C18(2), 250 × 100 mm, 2.0 mL/min, 100 Å, 210 nm detection) using isocratic conditions (95% MeCN in water). HPLC separation afforded 3-acetyl-5-methyl-merochlorin A (3.5 mg). The crystal structure is shown in Fig. 1. ^{1}H NMR (600 MHz, chloroform-d_3): δ (ppm) 6.80 (1H, d, J = 1.0 Hz), 6.54 (1H, d, J = 1.0 Hz), 4.96 (1H, t, J = 7.0 Hz), 3.87 (3H, s), 3.17 (1H, d, J = 15.8 Hz), 2.74 (1H, d, J = 15.8 Hz), 2.39 (3H, s), 2.38-2.36 (3H, m), 2.10 (1H, m), 1.80 (1H, m), 1.75 (3H, s), 1.62 (3H, s), 1.58 (1H, m), 1.57 (3H, s), 1.55 (3H, s), 1.18 (1H, m), 0.93 (3H, s).

Structure elucidation of merochlorins A–D (1-4)

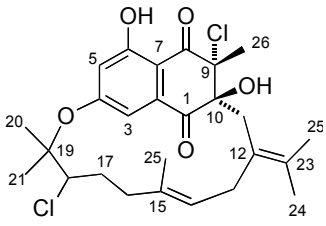
The assignment, structure, and bioactivity of merochlorin A (**1**) were recently published.[3] The crystal structure is reported in this manuscript, confirming the structure of merochlorin A reported by Saukoulas *et al*. What follows is a detailed description of the structure elucidation of merochlorins A–D (**1**–**4**). Throughout the structure elucidation section, the carbon atoms were numbered according to the following convention:



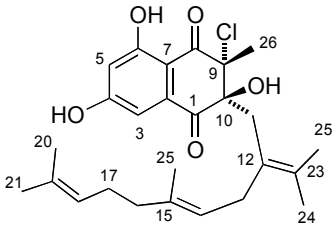
merochlorin A (**1**)



merochlorin B (**2**)

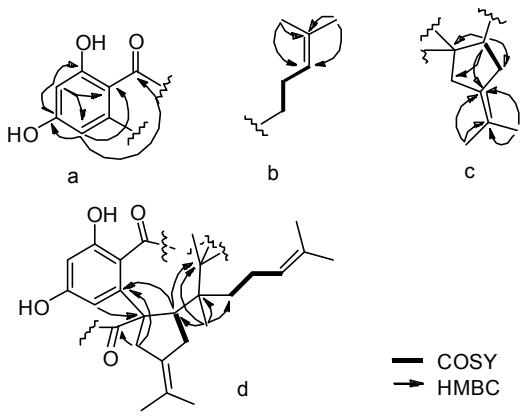


merochlorin C (**3**)



merochlorin D (**4**)

Merochlorin A (**1**) was isolated as a pale-yellow oil with a molecular formula of $C_{25}H_{29}{}^{35}ClO_4$ based on HR-ESI-MS (a pseudo-molecular ion peak at *m/z* 429.1821 $[M+H]^+$) and ^{13}C NMR data. Strong UV absorption of **1** was observed at 240, 296, and 330 nm corresponding to conjugated, aromatic, or phenolic functional groups. The IR spectrum of **1** showed broad absorptions of multiple hydroxyl groups (3380 cm^{-1}) and carbonyl groups (1704 cm^{-1}). The ^1H NMR spectrum of **1** displayed a pair of *meta*-coupled aromatic protons [H-4 (δ_H 6.16), H-6 (δ_H 6.38)], one olefin proton [H-18 (δ_H 4.92)], five methyl singlet protons [H-21 (δ_H 1.53), H-20 (δ_H 1.45), H-24 (δ_H 1.65), H-22 (δ_H 1.56), H-25 (δ_H 0.81)], and an exchangeable proton [3-OH (δ_H 11.9)]. The ^{13}C NMR and HSQC data revealed two carbonyl carbons, ten quaternary carbons, four methine carbons, four methylene carbons, and five methyl carbons.



Two-dimensional NMR spectral analysis allowed assembly of three fragments (**a**-**c**) shown to the left. The first fragment (**a**) was assembled by analyzing the ^1H-^1H coupling constants. The *meta*-coupling of two aromatic protons [H-4 (δ_H 6.16, d, J = 2.0 Hz), H-6 (δ_H 6.38, d, J = 2.0 Hz)] indicated the presence of a 1,2,3,5-tetrasubstituted benzene moiety. The long-range HMBC correlation from the aromatic proton H-4 to carbon atoms C-3 (δ_C 165.4) and C-5 (δ_C 166.5) suggested that C-3 and C-5 had hydroxyl group substituents. The presence of two hydroxyl groups was confirmed by the methylation of 5-OH followed by the acetylation of 3-OH. The carbon chemical shift of C-1 (δ_C 193.2), and the presence of a chelated hydroxyl proton [3-OH (δ_H 11.59)], strongly suggested a ketone group at C-1. The second fragment (**b**), the C-6 isoprene

side chain of the molecule, was established by COSY cross-peaks and HMBC correlations. The COSY cross-peaks of protons H-16 through H-18 [H-16 (δ_H 1.40, 1.14), H-17 (δ_H 2.03, 1.75), H-18 (δ_H 4.92)], and the long-range HMBC correlations from olefinic proton H-18 to carbons C-19 (δ_C 131.6), C-20 (δ_C 18.1), and C-21 (δ_C 26.1), allowed the assignment of the C-16 to C-21 side-chain fragment. The third fragment (**c**), the propan-2-ylidenecyclopentane moiety, was assigned by analysis of COSY and HMBC data. COSY cross-peaks between H-9 and H-15, and long-range HMBC correlations (from H-15 to C-8, C-9, C-13, C-14, and C-23; from H-22 to C-14 and C-23; and from H-24 to C-14 and C-23), allowed the assignment of the propan-2-ylidenecyclopentane moiety.

Fragments **a**-**c** were connected to form fragment **d** by analysis of HMBC spectroscopic data. The linkage between C-7 and C-8 was established by three-bond HMBC correlations from H-6 to C-2, C-4, and C-8, and two-bond HMBC correlations from H-6 to C-5 and C-7. The C-16/C-10, C-10/C-11, and C-10/C-9 connections were established by long-range HMBC correlations from H-16 to C-9, C-10, and C-11; from H-15 to C-9 and C-10; and from singlet methyl proton H-25 to C-9, C-10, C-11, and C-16, respectively. The C-12/C-8 connection was established by long-range HMBC correlations from H-13 to C-8 and C-12 as well as from H-9 to C-8 and C-12.

The remaining connectivities were challenging to establish because of the absence of any proton correlations between C-1, C-11, and C-12. Thus, we carefully analyzed the chemical shifts of C-1 (δ_C 193.2), C-12 (δ_C 200.1), and C-11 (δ_C 91.1). This analysis allowed us to connect the remaining chlorine atom to C-11 and to establish the C-1/C11 and C-11/C-12 connections. The

relative configuration of **1** (8*R*, 9*R*, and 10*S*) was determined by ROESY correlations between H-16 and H-9 and between H-15 and H-25.

The molecular formula of merochlorin B (**2**) was assigned as $C_{25}H_{29}{}^{35}ClO_4$ based on a pseudo-molecular ion peak (*m/z* 429.1821 $[M+H]^+$) and ^{13}C NMR data. The ^1H NMR spectrum of **2** had similar features to that of **1**, but large chemical shift differences in the ^{13}C NMR spectrum were observed for five carbons (C-1, C-10, C-11, C-12, and C-13) as compared to the ^{13}C NMR data for **1**. Two-dimensional NMR analysis allowed the assignment of three fragments: a dihydroxynaphthalenone, a C-6 sesquiterpene side chain, and a propan-2-ylidenecyclopentane moiety. The *meta*-coupling between two aromatic protons [H-4 (δ_H 6.15, d, J = 2.0 Hz), H-6 (δ_H 6.17, d, J = 2.0 Hz)], the chelated hydroxyl proton [3-OH (δ_H 12.9)], and the upfield-shifted chemical shift of C-1 (δ_C 184.0) strongly suggested the presence of a dihydroxynaphthalenone moiety in **2**. In addition, two- or three-bond HMBC correlations from H-4 to C-2 and from 3-OH to C-2, C-3, and C-4, as well as four-bond HMBC correlations from H-4 to C-1 and from H-6 to C-1, constructed the dihydroxynaphthalenone moiety. The C-6 sesquiterpene side chain of **2** was established by COSY and HMBC correlations. The COSY cross-peaks for H-16 through H-18, and the long-range HMBC correlations from H-18 to C-19 and from C-20 to C-21 permitted the assignment of the C-16 to C-21 side chain. The propan-2-ylidenecyclopentane moiety was assigned based on COSY cross-peaks between H-9 and H-15 and long-range HMBC correlations from H-15 to C-8, C-9, C-13, C-14, and C-23 as well as from methyl singlet proton H-22 to C-14, C-23 and C-24. These three fragments were assembled on the basis of HMBC spectroscopic data. The long-range HMBC correlations from H-6 to C-2, C-4, C-6, and C-8 and from H-13 to C-7, C-8, and C-9 allowed the first and third fragments to be connected to quaternary carbon C-

8. The connection between C-8 and C-11 was also established by three-bond HMBC correlations from H-13 to C-11 and from H-9 to C-11. The second and third fragments were connected to quaternary carbon C-10 based on long-range HMBC correlations from singlet methyl proton H-25 to C-9, C-10, and C-16; from H-16 to C-9, C-10, and C-25; from H-15 to C-8, C-9, and C-10; and from H-9 to C-15, C-8, C-10, C-11, and C-25. Finally, the remaining chlorine atom was attached to C-12, and the ether linkage between C-10 and C-11 was established, based on the chemical shifts of C-11 (δ_C 174.0) and C-12 (δ_C 99.8). The relative configurations of C-8, C-9 and C-10 of **2** (8*S*, 9*R*, and 10*S*) were determined by ROESY correlations between H-25 and H-15, between H-15 and H-13, and between H-9 and H-16.

Merochlorin C (**3**) was isolated as a pale-yellow oil, and its molecular formula was assigned as $C_{26}H_{32}{}^{35}Cl_2O_5$ based on HR-ESI-MS (a pseudo-molecular ion peak at *m/z* 517.1516 $[M+Na]^+$) and ^{13}C NMR data. The ^1H NMR spectrum of **3** displayed two *meta*-coupled aromatic protons [H-3 (δ_H 7.19), H-5 (δ_H 6.91)], one olefin proton H-14 (δ_H 4.36), protons corresponding to six methyl groups [H-20 (δ_H 1.58), H-21 (δ_H 1.70), H-22 (δ_H 1.76), H-24 (δ_H 1.59), H-25 (δ_H 0.98), and H-26 (δ_H 1.74)], and one exchangeable proton [10-OH (δ_H 5.78)]. The two *meta*-coupled aromatic protons (H-3 and H-5) in the ^1H NMR spectrum of **3** as well as the two oxygenated aromatic carbons (C-4 and C-6) and the two carbonyl carbons (C-1 and C-8) in the ^{13}C NMR spectrum suggested the presence of a dihydronaphthalenedione moiety. The long-range HMBC correlations from H-3 to C-1, C-2, C-4, C-7, and C-8 and from H-5 to C-3, C-4, C-6, C-7, and C-8 also supported the presence of a dihydronaphthalenedione moiety. Analysis of 2D NMR spectroscopic data allowed the construction of the sesquiterpene bridge. The H-17/H-16 and H-17/H-18 correlations in the COSY spectrum of **3** assisted in the connection of C-16 to C-18. Two

methyl singlet protons (H-20 and H-21) attached to carbon C-19 exhibited long-range HMBC correlations to carbon C-18, and allowed the connectivity to be expanded from C-18 to C-19. The long-range HMBC correlation from methyl singlet proton H-25 to olefinic carbons C-14, C-15, and the methylene carbon C-16 allowed the expansion of the carbon unit from C-14 to C-16. The COSY cross-peaks between H-14 and H-13 and long-range HMBC correlations from H-13 to C-12, C-21, and C-11 as well as from the two methyl singlet protons H-24 and H-22 to carbon atoms C-12 and C-21 established the C-14/C-13, C-13/C-12, C-12/C-11, and C-12/C-21 connections. The four-bond HMBC correlation from singlet methyl proton H-21 to C-4 revealed the ether linkage between C-19 and C-4. The long-range HMBC correlations from the methylene proton H-11 to C-1, C-10, and C-9 as well as from the solvent-exchangeable proton 10-OH to C-1, C-9, C-10, and C-11 allowed the connection between C-10 and C-11 to be established and allowed the placement of the hydroxyl group at C-10. The long-range HMBC correlations from methyl singlet proton H-26 to C-8, C-9, and C-10 allowed the connection between H-26 and C-9. Lastly, the attachment of two chlorine atoms to C-9 and C-18 completed the assignment of the planar structure of **3**. The ROESY correlation between H-14 and H-16 established a 14*E* configuration of the olefinic bond, and the ROESY correlation between solvent-exchangeable proton 10-OH and methyl singlet proton H-26 allowed the relative configurations of C-9 and C-10 to be designated as 9*R** and 10*S**, respectively.

Merochlorin D (**4**) was isolated as a pale-yellow oil which molecular formula was assigned as $C_{26}H_{33}{}^{35}ClO_5$ based on HR-ESI-MS (pseudo-molecular ion peak at *m/z* 483.1907 $[M+Na]^+$) and ^{13}C NMR data. The ^1H NMR spectrum of **4** was nearly identical to that of **3** (see above) except for the presence of one olefinic proton [H-18 (δ_H 4.97)]. The HMBC correlations from the

olefinic proton H-18 to C-19, C-20, and C-21 suggested that **4** contained a double bond at C-19. Analysis of 2D NMR spectroscopic data allowed the planar structure of **4** to be assigned. The ROESY correlation between H-14 and H-16 established the 14*E* configuration of the double bond, and the ROESY correlation between 10-OH and H-26 suggested that C-9 and C-10 had *R** and *S** relative configurations, respectively.

Genomic DNA isolation and library construction

Mycelium of CNH-189 was inoculated in A1 medium (10 g/L starch, 4 g/L yeast extract, and 2 g/L peptone in filtered seawater). After 3 days at 28 °C, genomic DNA was isolated by phenol–chloroform extraction.[2] The isolated genomic DNA was then randomly sheared to provide short DNA fragments (200 ± 50 bp) and longer DNA fragments (40 ± 5 kb). The short DNA fragments were processed for paired-end Illumina DNA sequencing according to the manufacturer's instructions (Illumina Inc., CA, USA). The large DNA fragments were cloned into fosmid pCCFOS1, and a 500-clone fosmid library was prepared according to the manufacturer's instructions (Epicentre Biotechnologies, WI, USA).

Illumina DNA sequencing and de novo assembly

Paired-end Illumina DNA sequencing (65 nucleotide (nt)-reads, version 4) was performed with an Illumina Genome Analyzer IIx using a genomic library concentration of 50 nM. Translated-query BLAST (BLASTx) was used to query reads against a custom database of annotated proteins from the napyradiomycin biosynthetic gene cluster.[4] If both reads of a paired-end had matching expectation values $<10^{-6}$, these reads were used as seeds for a targeted local assembly using an alpha version of the Paired-Read Iterative Contig Extension (PRICE) software. PRICE

uses paired-end information to generate localized assemblies that extend existing contigs; in this case, the initial contigs were reads with the high-quality BLASTx matches described above, and the contigs were repeatedly extended through as many cycles as possible, until local drops in coverage prohibited further extension. PRICE is open-source and can be downloaded for free (http://derisilab.ucsf.edu/software/price/index.html).

Identification, sequencing, and annotation of the merochlorin gene cluster

Illumina sequencing and de novo assembly provided three contigs with approximately 30 kb of sequence homologous to known merochlorin biosynthetic genes, including *mcl17*.[5] Primers pksIII_fwd and pksIII_rev (Table S5), amplifying a 0.53-kb fragment of *mcl17*, were used for the PCR screening of the fosmid library, resulting in the isolation of fosmid 3D9. Double-stranded sequencing of the entire fosmid clone 3D9 (36,134-bp insert) was performed with the shotgun method by GenoTech (Baejeon, Korea) using 0.5–1.0 kb DNA fragments. A contiguous 36.1-kb region was assembled with an average GC content of 71.1%, characteristic of *Streptomyces* DNA. In addition, overlapping fosmids were isolated by PCR screening of the genomic library, and the sequence gaps were closed by primer walking. The complete sequence of the merochlorin biosynthetic gene cluster (57,647 bp) is available at NCBI under the accession number JX186999. In silico sequence analysis was performed using GC frame-plot,[6] BLAST,[7] and conserved protein domain searches.[8] The Geneious software package (Biomatters Ltd., Auckland, New Zealand) and Artemis (Wellcome Trust Genome Campus, Cambridge, UK) were used for sequence analysis and annotation. Sequence alignments were constructed using the ClustalX algorithm[9] and the GeneDoc alignment editor.[10]

Inactivation of *mcl17* in CNH-189

An apramycin resistance cassette was amplified from plasmid pIJ773 using primers mcl17-fwd and mcl17-rev (Table S5). The *mcl17* gene was replaced in *E. coli* BW25113/pKD20/3D9 using Red/ET-mediated recombination,[11] and the resulting fosmid merPB01 was confirmed by PCR. merPB01 was then transferred to *E. coli* S-17[12] and introduced into *Streptomyces* sp. CNH-189 by conjugation. Exconjugants resistant to apramycin were isolated and tested for the loss of chloramphenicol resistance indicating a successful double crossover. Three mutants were confirmed by PCR and designated CNH-189 *Δmcl17* 1–3.

Heterologous expression of fosmids 3D9 and 6B10

A 4,443-bp region was amplified from the integrative shuttle vector pSET152[13] using primers int-fwd and int-rev (Table S5; the italicized letters represent extensions for the homologous recombination with the *cat* gene of pSET152). The PCR-amplified region contained an *attP* attachment site, the integrase gene (*int*) of phage C31, an origin of transfer (*oriT*) and apramycin resistance marker *aac(3)IV*. The PCR product was used to replace the chloramphenicol resistance marker (*cat*) in the backbone of fosmid BHXS1782.[14] The resulting construct pAEM21 was verified by restriction analysis and PCR. A 6,610-bp *Xba*I restriction fragment from pAEM21 was purified and used to replace the chloramphenicol resistance cassette in BPW1945 to generate pksLK09. A 934-bp region was amplified from SuperCos1 (Agilent Technologies, CA, USA) using primers apEXkan_fwd and apEXkan_rev (Table S5). The PCR product contained the kanamycin resistance marker (*neo*) and a 130-bp sequence upstream of the *neo* gene, a region that should include the *neo* promoter. After replacing the apramycin resistance gene and its promoter on pksLK09 using Red/ET-mediated recombination,[15] a 6,645-bp *Xba*I

restriction fragment was generated. This fragment comprised the *attP*, *int*, *oriT*, *neo*, and *neoP* components as well as a 408-bp sequence at the 5'-end and a 1,758-bp sequence at the 3'-end, homologous to the regions flanking the *cat* gene of pCC1FOS. This fragment thus represents a general cassette for the modification of pCC1FOS-based fosmids to allow stable integration of gene clusters into *Streptomyces* genomes. The cassette was named int_neo and was subsequently used to replace the *cat* gene in *E. coli* BW25113/pKD20/3D9 and *E. coli* BW25113/pKD20/6B10 via Red/ET-mediated recombineering.[11,15] The resulting fosmids merLK01 and merLK30 were verified by restriction analysis, transferred into *E. coli* ET12567,[16] and introduced into *S. coelicolor* M1152 and M1154[17] by triparental intergeneric conjugation with *E. coli* ET12567/pUB307.[18] Three kanamycin-resistant clones were selected, verified by PCR, and named *S. coelicolor* M1154/merLK01 1–3 and *S. coelicolor* M1152/merLK30 1–3, respectively.

Metabolite profiling of CNH-189 and *S. coelicolor* strains

Frozen stocks of CNH-189 (wild-type or mutant) were inoculated in A1 medium. After 3 days of incubation at 28 °C, the culture was transferred (10% final concentration) to 50 mL of M1 medium in a 250-mL Erlenmeyer flask equipped with a spring and a foam plug to prevent aggregation of the mycelium and to facilitate aeration, respectively. A spore stock of *S. coelicolor* M1154 (wild-type or fosmid-containing) was handled the same way, except that it was inoculated in tryptic soy broth (TSB) medium (17 g/L tryptone, 3 g/L soytone, 2.5 g/L glucose, 5 g/L NaCl, and 2.5 g/L K_2HPO_4 at pH 7.3) and then transferred to 50 mL of R5 medium (103 g/L sucrose, 0.25 g/L K_2SO_4, 10.12 g/L $MgCl_2 \cdot 6H_2O$, 10 g/L glucose, 0.1 g/L Casaminoacids, 5 g/L yeast extract, 5.73 g/L TES (*N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid), 80 µg/L

$ZnCl_2$, 400 µg/L $FeCl_3 \cdot 6H_2O$, 20 µg/L $CuCl_2 \cdot 2H_2O$, 20 µg/L $MnCl_2 \cdot 4H_2O$, 20 µg/L $Na_2B_4O_7 \cdot 10H_2O$, 20 µg/L $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 50 mg/L KH_2PO_4, 3 g/L L-proline, 2.94 g/L $CaCl_2$, and 280 µg/L NaOH).

After 7 days of incubation at 28 °C, 50 mL of EtOAc was added, the mixture was sonicated in a sonication bath for 5 min, and the EtOAc layer was recovered. After drying the organic layer under anhydrous $MgSO_4$, the solvent was evaporated under reduced pressure. The residue was dissolved in 1 mL of MeCN, and the solution was filtered through a C_{18} sorbent (Spice C18 Sample Preparation Cartridges, Analtech, Inc., Newark, NJ). The filtrate was evaporated under reduced pressure in a 14-mL scintillation vial, and the residue was stored at -20 °C until HPLC analysis.

The residue was dissolved in 100 µL of MeCN, and 10 µL of the dissolved residue was injected onto a reversed-phase HPLC column (Phenomenex Luna C18(2), 4.6 × 100 mm, 5 µm). The following solvent composition was used to separate the analytes: 50-100% MeCN in H_2O for 20 min, 100% MeCN in H_2O for 5 min, 100-50% MeCN in H_2O for 1.5 min, and 50% MeCN in H_2O for 2.5 min. HR-MS data were acquired using positive-mode HPLC-HR-APCI-MS.

Table S1. NMR Spectral Data for Merochlorin A (**1**) in DMSO-d_6.[a]

No.	δ_C, mult.[b]	δ_H (*J* in Hz)	COSY	HMBC
1	193.2, qC			
2	109.8, qC			
3	165.4, qC			
4	102.1, CH	6.16, d (2.0)	6	C-1, 2, 3, 5, 6
5	166.5, qC			
6	103.7, CH	6.38, d (2.0)	4	C-1, 2, 4, 5, 7, 8
7	150.5, qC			
8	61.5, qC			
9	58.8, CH	2.24, dd (9.4, 4.0)	15	C-7, 10, 11, 12, 14, 15, 16, 25
10	45.3, qC			
11	91.3, qC			
12	200.1, qC			
13	29.3, CH_2	2.87, d (13.0); 2.65, d (13.0)		C-7, 8, 9, 12, 14, 15, 23
14	132.1, qC			
15	31.9, CH_2	2.36, dd (14.0, 4.0); 2.33, dd (14.0, 9.4)	9	C-8, 9, 10, 13, 14, 23
16	39.2, CH_2	1.14, q (6.0); 1.40, dt (14.8, 4.8)	17	C-8, 9, 10, 11, 17, 25
17	22.8, CH_2	2.03, m; 1.75, m	16, 18	C-10, 16, 18, 19
18	124.2, CH	4.92, t (6.5)	17	C-16, 17, 19, 20, 21
19	131.6, qC			
20	18.1, CH_3	1.45, s		C-18, 19, 21
21	26.1, CH_3	1.53, s		C-18, 19, 20
22	20.9, CH_3	1.56, s		C-14, 23, 24
23	123.1, qC			
24	21.1, CH_3	1.65, s		C-14, 22, 23
25	16.5, CH_3	0.81, s		C-9, 10, 11, 16
3-OH		11.9, br s		

[a] ^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz). [b] The number of attached protons (multiplicity) was determined by 2D NMR data analysis.

Table S2. NMR Spectral Data for Merochlorin B (**2**) in DMSO-d_6.[a]

No.	δ_C, mult.[b]	δ_H (J in Hz)	COSY	HMBC
1	184.0, qC			
2	106.4, qC			
3	163.7, qC			
4	101.9, CH	6.17, d (2.0)	6	C-2, 3, 5, 6
5	163.0, qC			
6	104.4, CH	6.15, d (2.0)	4	C-2, 4, 5, 7, 8
7	148.4, qC			
8	60.7, qC			
9	52.5, CH	2.99, d (7.8)	15	C-7, 8, 10, 13, 14, 15, 16, 25
10	98.6, qC			
11	174.0, qC			
12	99.8, qC			
13	49.8, CH_2	2.81, d (17.0); 2.47, d (17.0)		C-7, 8, 9, 11, 13, 14, 15, 23
14	131.0, qC			
15	35.3, CH_2	2.82, d (17.8); 2.70, d (17.8)	9	C- 9, 10, 13, 14, 23
16	42.9, CH_2	1.73-1.74, m	17	C- 9, 10, 25
17	22.6, CH_2	1.99, m; 1.74, m	16, 18	C-10, 16, 18, 19
18	124.5, CH	5.05, t (7.5)	17	C-16, 17, 19, 20, 21
19	131.4, qC			
20	18.1, CH_3	1.48, s		C-18, 19, 21
21	25.6, CH_3	1.57, s		C-18, 19, 20
22	21.9, CH_3	1.70, s		C-14, 23, 24
23	126.2, qC			
24	17.0, CH_3	1.62, s		C-14, 22, 23
25	22.6, CH_3	1.41, s		C-9, 10, 16
3-OH		12.9, br s		C-1, 2, 3, 4

[a] ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz). [b] The number of attached protons (multiplicity) was determined by 2D NMR data analysis.

Table S3. NMR Spectral Data for Merochlorins C (**3**) and D (**4**) in DMSO-d_6.[a]

No.	**3**		**4**	
	δ_C, mult.[b]	δ_H (*J* in Hz)	δ_C, mult.[b]	δ_H (*J* in Hz)
1	195.9, C		196.4, C	
2	135.7, C		136.2, C	
3	108.1, CH	6.83, d (2.0)	110.6, CH	7.19, d (2.0)
4	166.0, C		159.0, C	
5	108.4, CH	6.60, d (2.0)	118.9, CH	6.91, d (2.0)
6	165.0, C		164.0, C	
7	108.6, C		118.8, C	
8	194.1, C		187.1, C	
9	77.0, C		78.0, C	
10	84.5, C		84.2, C	
11	39.0, CH_2	2.42, d (16.0); 2.24, d (16.0)	38.2, CH_2	2.88, d (16.0); 2.11, d (16.0)
12	126.5, C		124.5, C	
13	31.1, CH_2	2.82, dd (14.0, 6.0); 2.35, dd (14.0, 6.0)	30.5, CH_2	2.79, d (14.0); 1.81 d (14.0)
14	123.1, CH	4.77, t (6.0)	124.9, CH	4.36, t (6.5)
15	134.9, C		135.1, C	
16	39.6, CH_2	1.84, m	35.8, CH_2	1.96, m
17	26.7, CH_2	1.95, m	31.8, CH_2	1.61, m; 1.54, m
18	124.6, CH	4.97, t (6.0)	66.4, CH	3.84, d (10.2)
19	131.3, C		87.8, C	
20	26.8, CH_3	1.59, s	25.4, CH_3	1.58, s
21	18.8, CH_3	1.51, s	30.5, CH_3	1.70, s
22	21.0, CH_3	1.47, s	22.6, CH_3	1.76, s
23	130.3, C		132.5, C	
24	21.3, CH_3	1.08, s	21.1, CH_3	1.59, s
25	16.8, CH_3	1.42, s	16.5, CH_3	0.98, s
26	18.9, CH_3	1.77, s	19.9, CH_3	1.74, s
6-OH		11.5, s		11.2, s
10-OH		6.09, s		5.82, br s

[a] ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz). [b] The number of attached protons (multiplicity) was determined by 2D NMR data analysis.

Table S4. Representative MIC Values for Merochlorins A-D (**1**–**4**) against MRSA. Assay set up was as previously described.[3]

	MIC (μg/mL)			
	1	**2**	**3**	**4**
TCH1516 (CA)	4	4	n.d	n.d
UAMS1182 (CA)	2	4	16	n.d
Sanger 252 (HA	4	4	n.d	n.d
ATCC33591 (HA)	2	8	16	>64

Table S5. PCR Primers.

Primer name	Primer sequence, 5' → 3' [a]
pksIII_fwd	CCCATCGCSCAGCTGGGCTGCGCGGC
pksIII_rev	GGACGACGGCGCTGGCGATG
mcl17-fwd [a]	*ATAGAAGGCAGACCTAGAGAAAGTAGACCCCAGACATG*ATTCCGGGGATCCGTCGACC
mcl17-rev [a]	*GCTTCCGTCCGTGGCCGGGCGGCACGATGAGGCCAGTCA*TGTAGGCTGGAGCTCCTTC
int-fwd [a]	*GAGTTATCGAGATTTTCAGGAGCTAAGGAAGCTAAAATG*TGCTACAGAGTTCTTGAAGTG
int-rev [a]	*AGGCGTTTAAGGGCACCAATAACTGCCTTAAAAAAATTA*TCGATCAGAAACTTCTCGACA
apEXkan_fwd [b]	*CATGAGATTATCAAAAAGGATCTTCACCTGATCCTTTT*CCGGAATTGCCAGCTGGGG
apEXkan_rev [b]	*CCTTGCCCCTCCAACGTCATCTCGTTCTCCGCTCATGAG*CTCAGAAGAACTCGTCAAG

[a] The italicized letters represent 39-nt sequences that allow homologous recombination with the targeted region of a fosmid (this technique is called PCR-targeted mutagenesis).

[b] The italicized letters represent regions that overlap with the *aac(3)IV* gene.

Table S6. Gene Table. Homologous genes and proposed function of genes in the merochlorin gene cluster

Gene	AA	Protein homolog	Accession number	ID/sim. [a]	Proposed function
mcl1	285	Lct19, *S. rishiriensis*	ABX71102	81/88	methylenetetrahydrofolate reductase
mcl2	357	Fnq23, *S. cinnamonensis*	CAL34101	73/82	trans-isoprenyl diphosphate synthase
mcl3	325	Fur15, *Streptomyces* sp. KO-3988	BAD86806	74/85	ketoacyl-ACP synthase III
mcl4	391	LpmA, *Streptomyces* sp. SN-1061M	ADC96649	83/91	HMG-CoA synthase
mcl5	353	Hmgr, *S. cinnamonensis*	ADQ43377	90/95	HMG-CoA reductase
mcl6	364	IPPiso, *S. cinnamonensis*	ADQ43376	81/89	isopentenyldiphosphate isomerase
mcl7	370	Pmk, *S. cinnamonensis*	ADQ43375	69/77	phosphomevalonate kinase
mcl8	346	Mdpd, *S. cinnamonensis*	ADQ43374	76/83	mevalonate diphosphate decarboxylase
mcl9	345	Mk, *S. cinnamonensis*	ADQ43373	70/81	mevalonate kinase
mcl10	62	SACTE_6317, *Streptomyces* sp. SirexAA-E	YP_004806629	47/56	MerR-like transcriptional regulator
mcl11	209	NapU1, *S. aculeolatus*	ABS50449	58/72	hypothetical protein
mcl12	673	Fur16, *Streptomyces* sp. KO-3988	BAE78984	55/63	NAD(P)-binding oxidoreductase
mcl13	479	Fur17, *Streptomyces* sp. KO-3988	BAE78985	61/70	3-carboxymuconate cycloisomerase
mcl14	513	Fnq24, *S. cinnamonensis*	CAL34102	65/75	FAD-dependent oxidoreductase
mcl15	566	Fnq25, *S. cinnamonensis*	CAL34103	73/87	cytochrome B
mcl16	119	SrosN1_05685, *S. roseosporus* NRRL11379	ZP_04707445	36/49	hypothetical protein
mcl17	358	NapB1, *S. aculeolatus*	ABS50451	71/83	type III polyketide (THN) synthase
mcl18	186	NapB2, *S. aculeolatus*	ABS50452	71/83	THN monooxygenase
mcl19	388	Fur3, *Streptomyces* sp. KO-3988	BAE78971	77/87	aminotransferase
mcl20	528	NapB4, *S. aculeolatus*	ABS50453	81/89	acyl-CoA synthetase
mcl21	377	Fur6, *Streptomyces* sp. KO-3988	BAE78974	79/88	C-methyltransferase

mcl22	217	Orf55, *S. fradiae*	AAZ23097	25/47	undecaprenyl diphosphate synthase
mcl23	305	NapT9, *S. aculeolatus*	ABS50462	41/62	prenyltransferase
mcl24	517	NapH1, *S. aculeolatus*	ABS50458	53/69	vanadium-dependent haloperoxidase
mcl25	520	Pur8, *S. anulatus*	CAA54186	42/60	MFS-like transporter
mcl26	317	SsfY1, *Streptomyces* sp. CSF2575	ADE34490	38/57	aromatase
mcl27	240	GrhD, *Streptomyces* sp. JP95	AAM33656	41/50	thioesterase
mcl28	273	FarR3, *S. lavendulae*	BAG74713	79/88	SARP-like transcriptional regulator
mcl29	222	TylQ, *S. fradiae*	AAD40803	52/65	TetR-like transcriptional regulator
mcl30	772	SSFG_03404, *S. ghanaensis*	ZP_06577698	84/89	iron-sulfur-binding oxidoreductase
mcl31	289	FarR4, *S. lavendulae*	BAG74714	69/76	SARP-like transcriptional regulator
mcl32	215	Lct13, *S. rishiriensis*	ABX71096	53/65	TetR-like transcriptional regulator
mcl33	485	AlpM, *S. ambofaciens*	AAR30157	51/60	NRPS condensation domain
mcl34	313	VmsS, *S. virginiae*	BAF50715	55/64	SARP-like transcriptional regulator
mcl35	271	Fnq4, *S. cinnamonensis*	CAL34082	57/70	SARP-like transcriptional regulator
mcl36	280	FarR4, *S. lavendulae*	BAG74714	67/77	SARP-like transcriptional regulator
mcl37	330	SagA, *S. aurefaciens*	ADM72848	50/59	AfsA-like gamma-butyrolactone synthase
mcl38	254	JadW3, *S. venezuelae*	AAL23836	65/69	NAD(P)-binding reductase
mcl39	531	NapR5, *S. aculeolatus*	ABS50467	66/79	MFS-like transporter
mcl40	531	NapH4, *S. aculeolatus*	ABS50464	66/80	vanadium-dependent haloperoxidase
mcl41	240	SCO1940, *S. coelicolor* A3(2)	NP_626205	54/64	thioredoxin-like oxidoreductase
orf1[b]	161	SSNG_06381, *Streptomyces* sp. C	ZP_07290760	48/59	SARP-like transcriptional regulator

[a] Overall sequence homology [%]

[b] Gene is considered incomplete on fosmid PB6B10

Figure S1. ^{1}H NMR Spectrum (600 MHz) of Merochlorin A (**1**) in DMSO-d_6

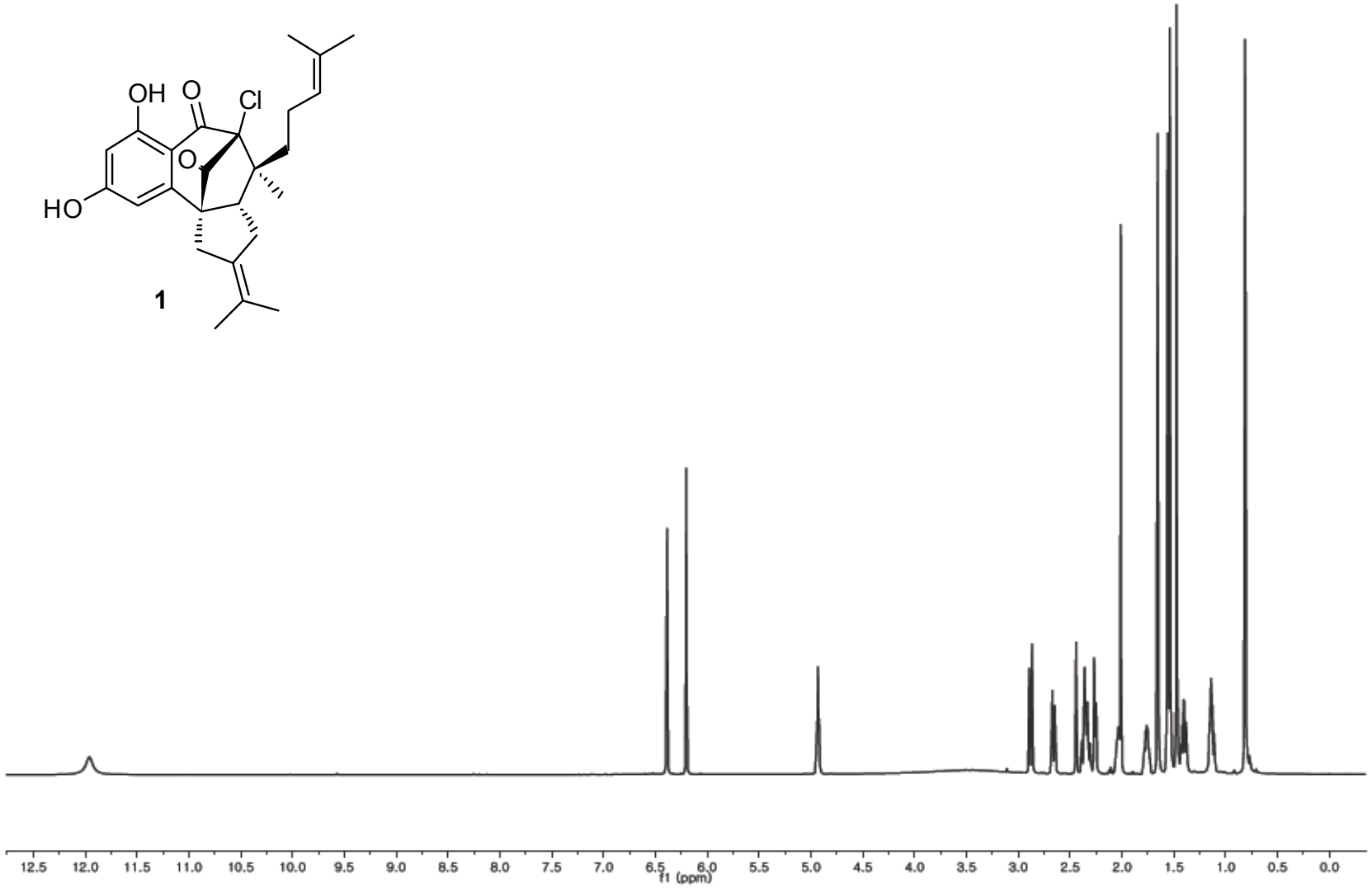


Figure S2. ^{13}C NMR Spectrum (150 MHz) of Merochlorin A (**1**) in DMSO-d_6

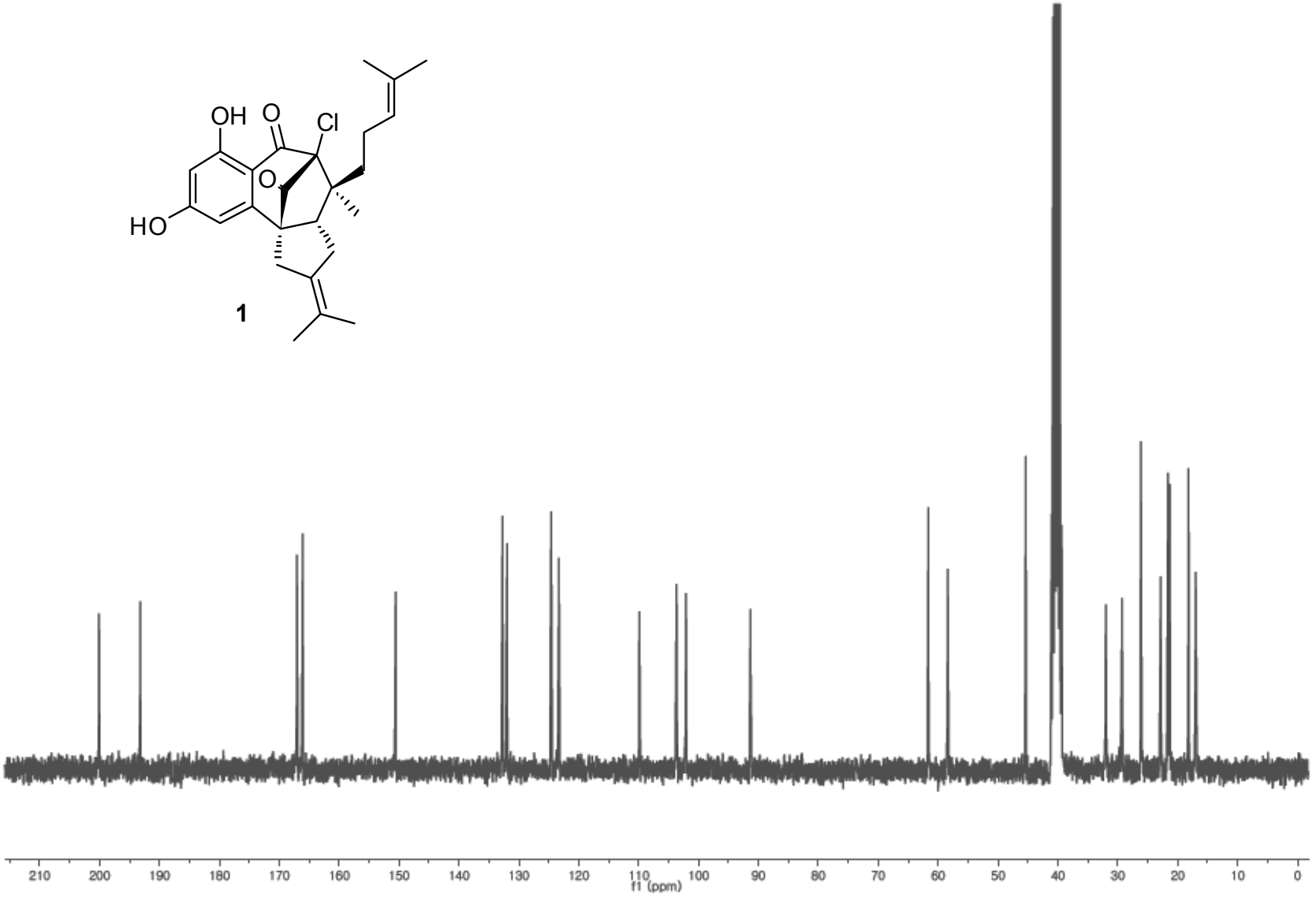


Figure S3. COSY Spectrum (600 MHz) of Merochlorin A (**1**) in DMSO-d_6

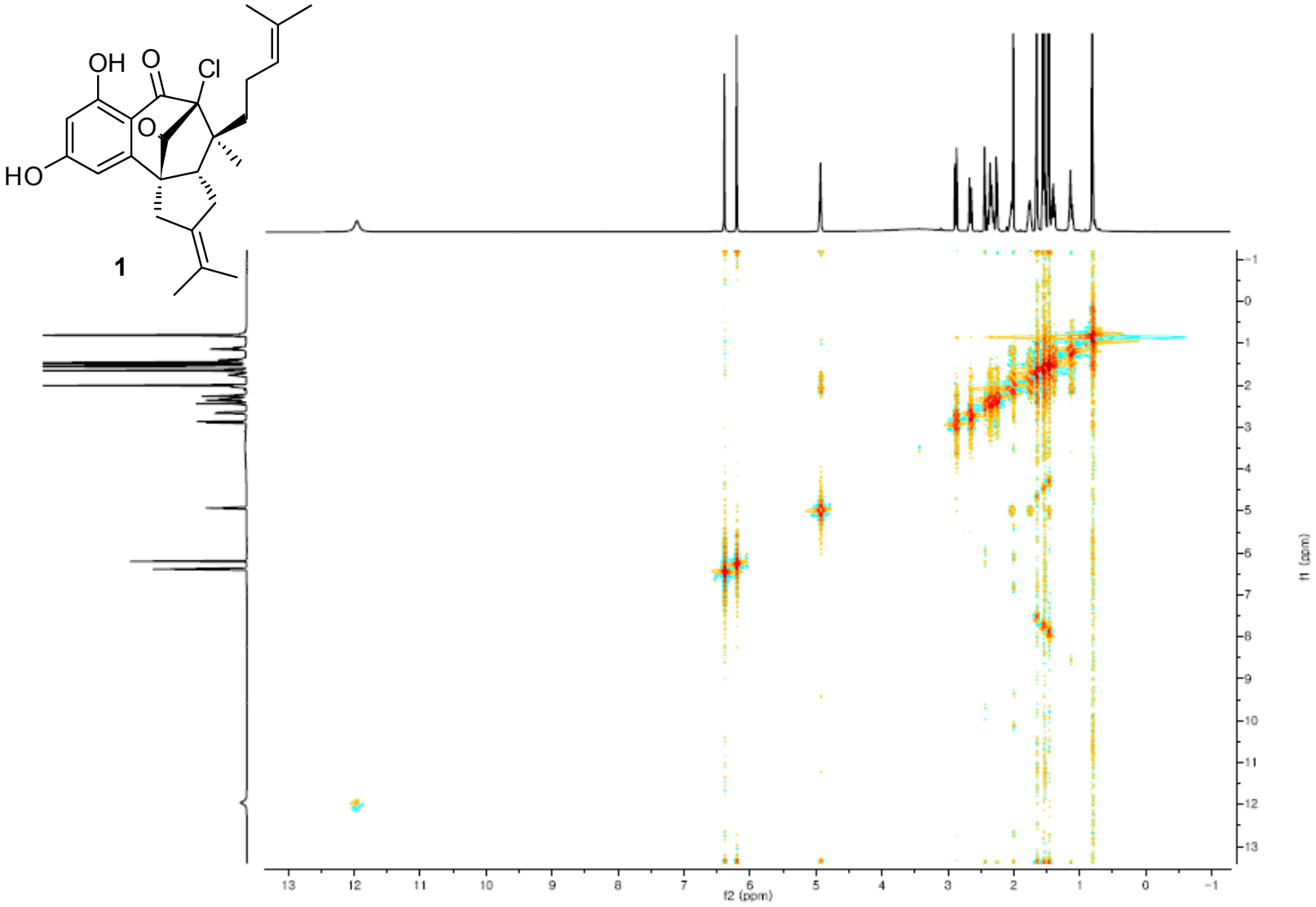


Figure S4. HSQC Spectrum (600 MHz) of Merochlorin A (**1**) in DMSO-d_6

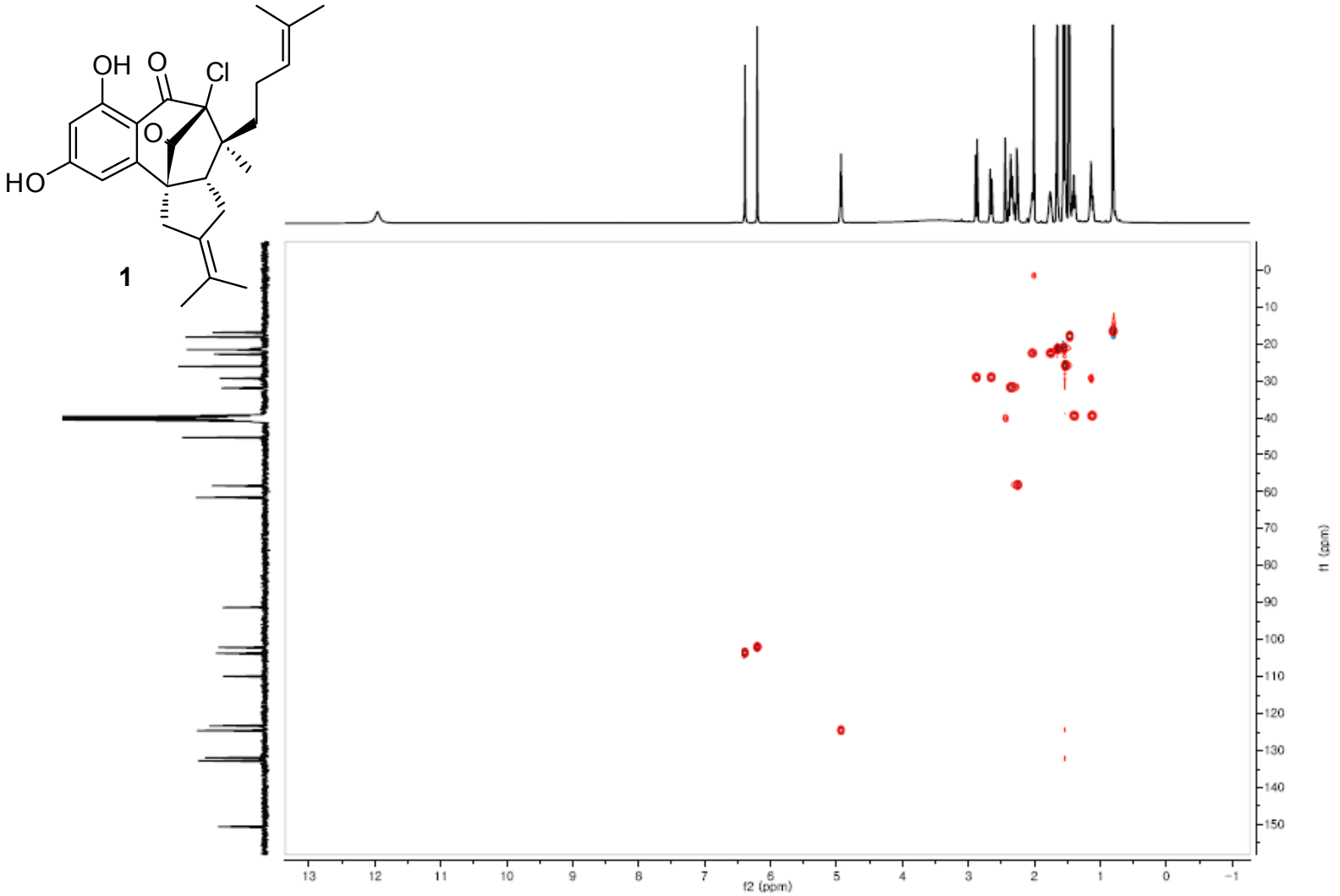


Figure S5. HMBC Spectrum (600 MHz) of Merochlorin A (**1**) in DMSO-d_6

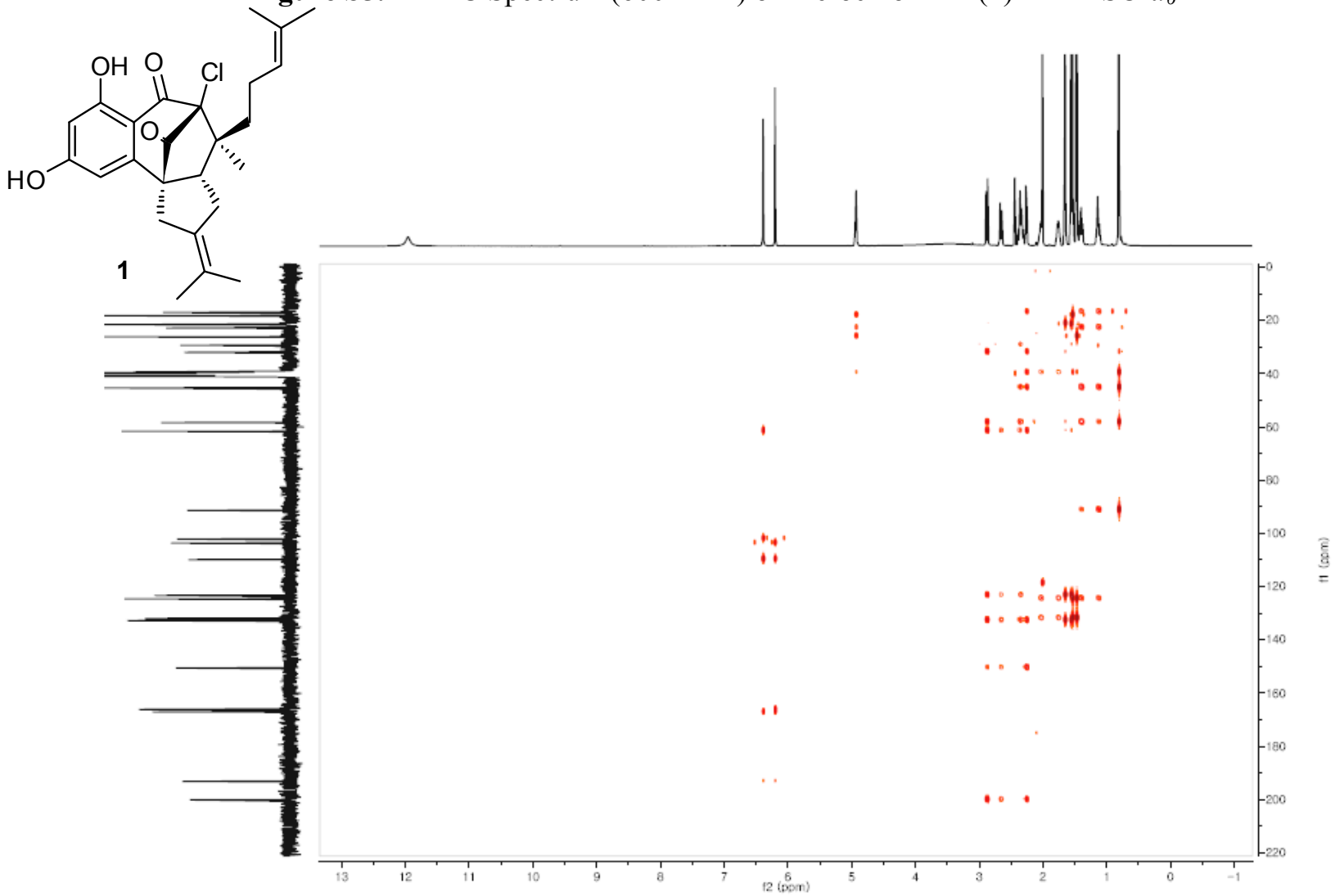


Figure S6. ROESY Spectrum (600 MHz) of Merochlorin A (**1**) in DMSO-d_6

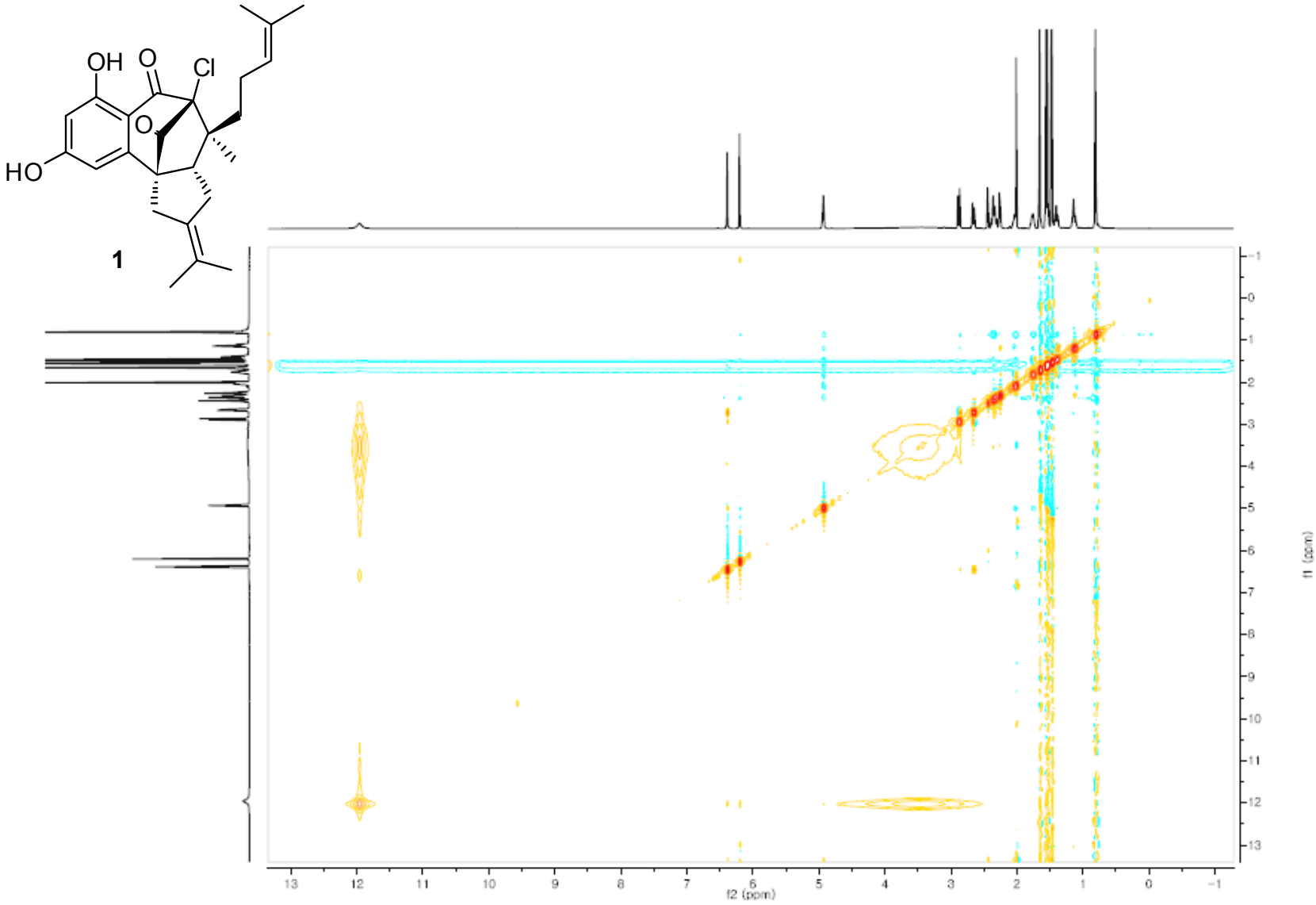
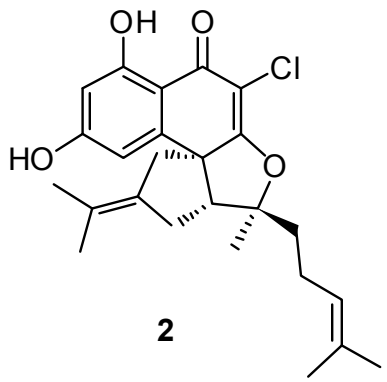
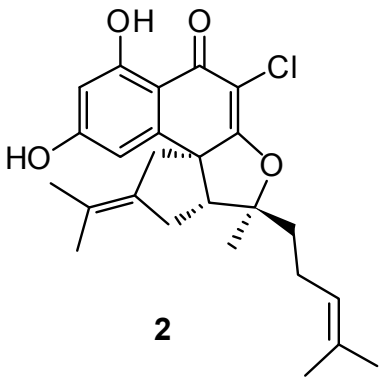


Figure S7. ^{1}H NMR Spectrum (600 MHz) of Merochlorin B (**2**) in DMSO-d_6



2

Figure S8. ^{13}C NMR Spectrum (125 MHz) of Merochlorin B (**2**) in DMSO-d_6



2

Figure S9. gCOSY Spectrum (500 MHz) of Merochlorin B (**2**) in DMSO-d_6

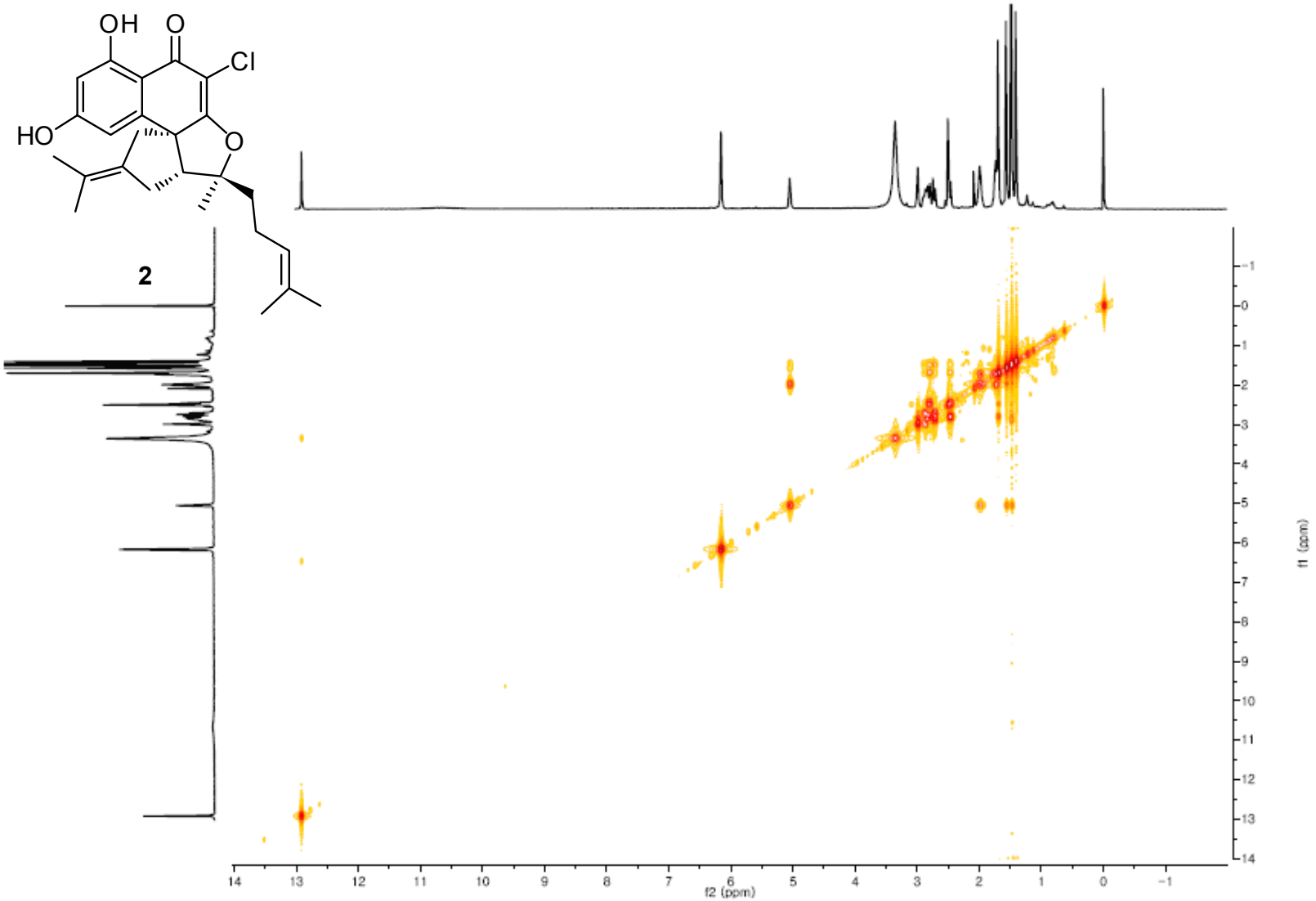


Figure S10. gHSQC Spectrum (500 MHz) of Merochlorin B (**2**) in DMSO-d_6

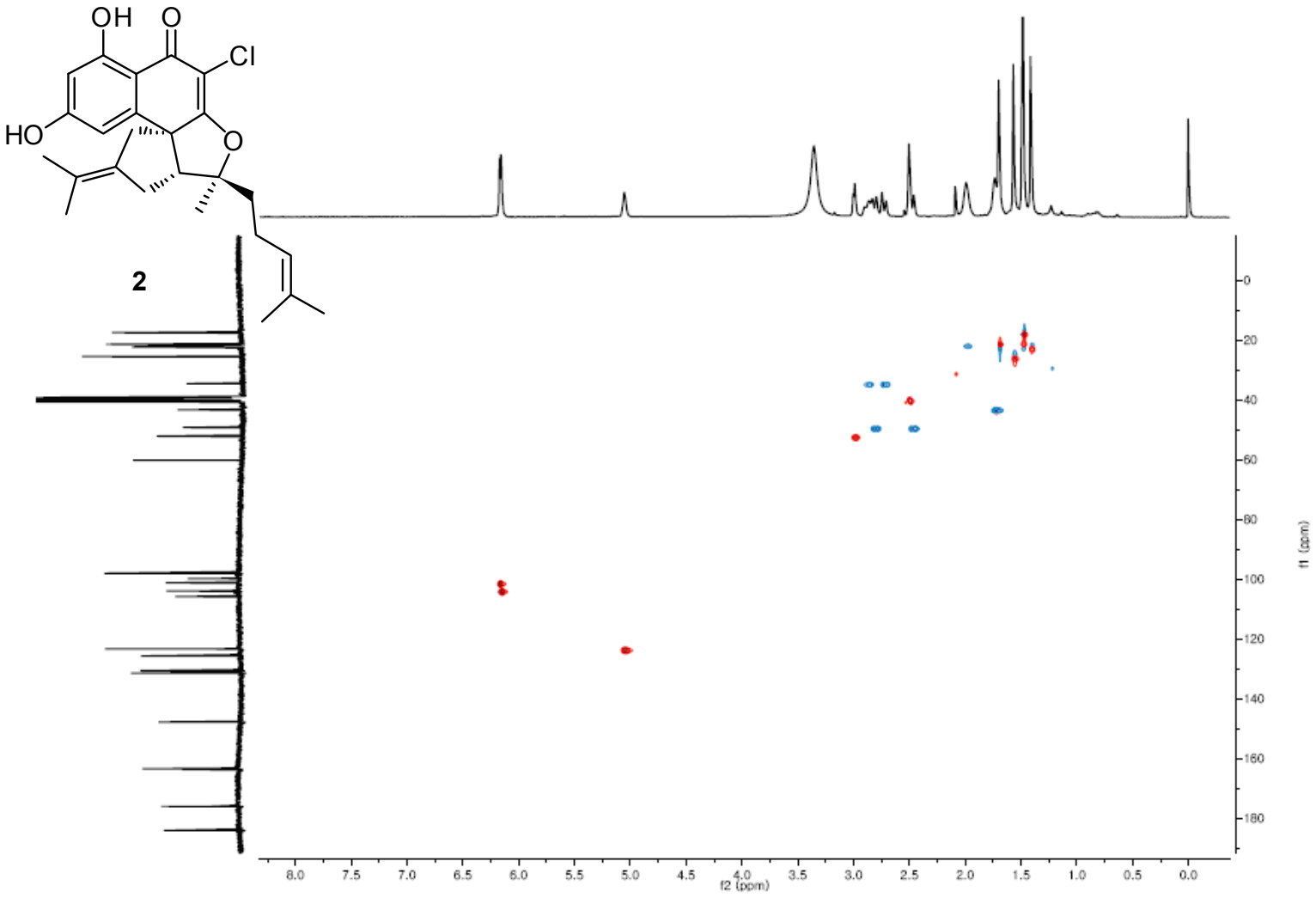


Figure S11. gHMBC Spectrum (500 MHz) of Merochlorin B (**2**) in DMSO-d_6

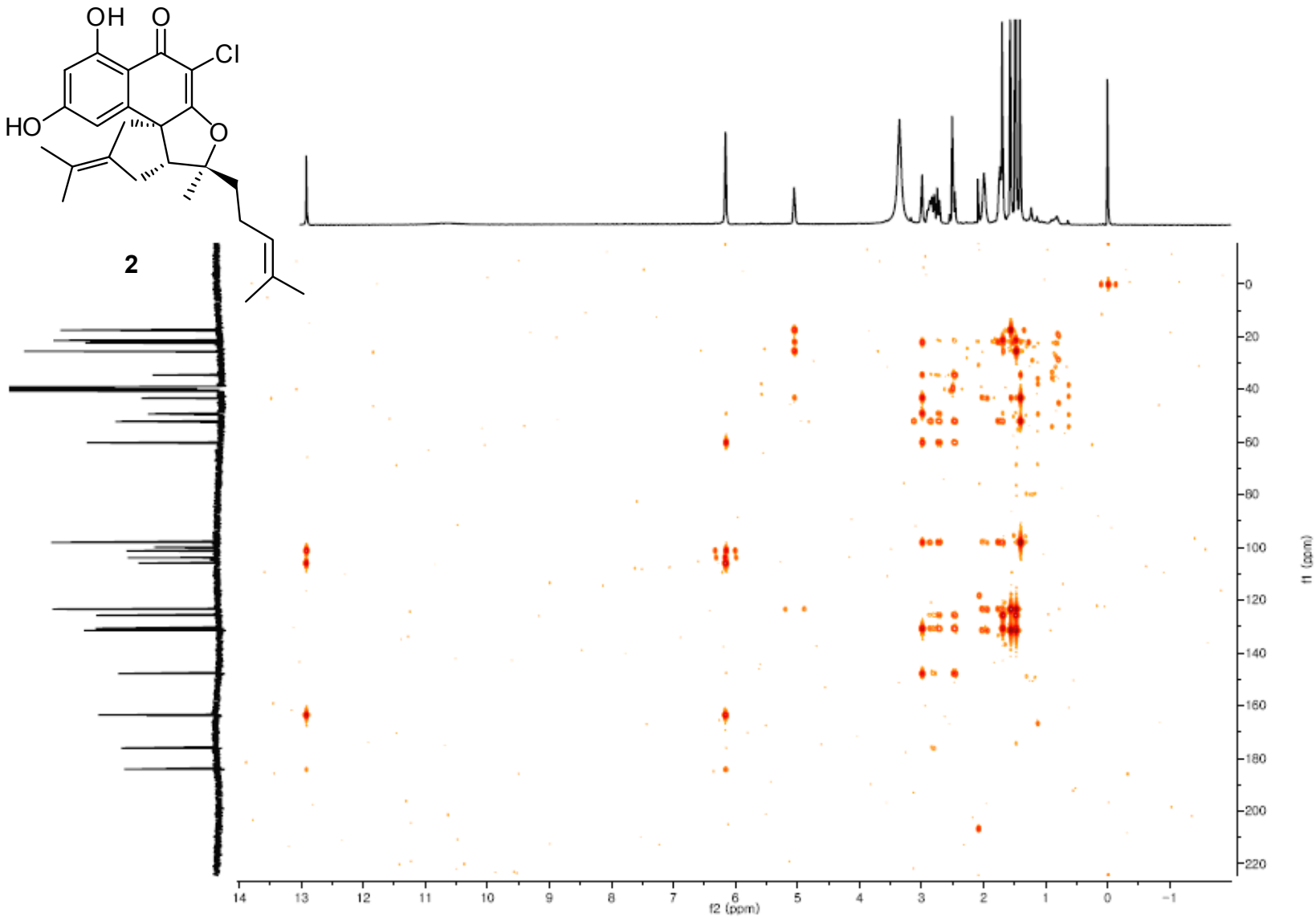


Figure S12. ROESY Spectrum (500 MHz) of Merochlorin B (**2**) in DMSO-d_6

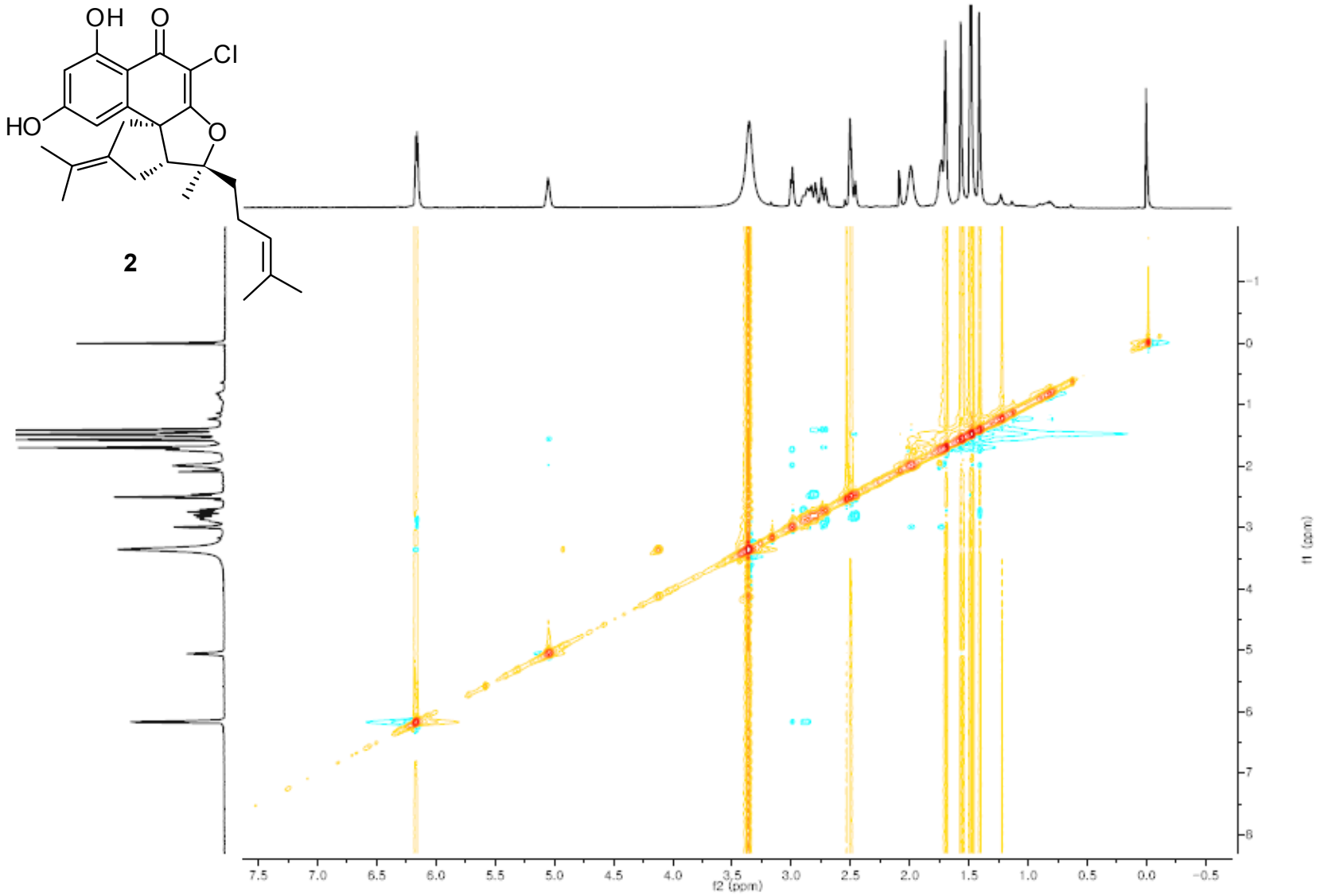


Figure S13. ^{1}H NMR Spectrum (500 MHz) of Merochlorin C (**3**) in DMSO-d_6

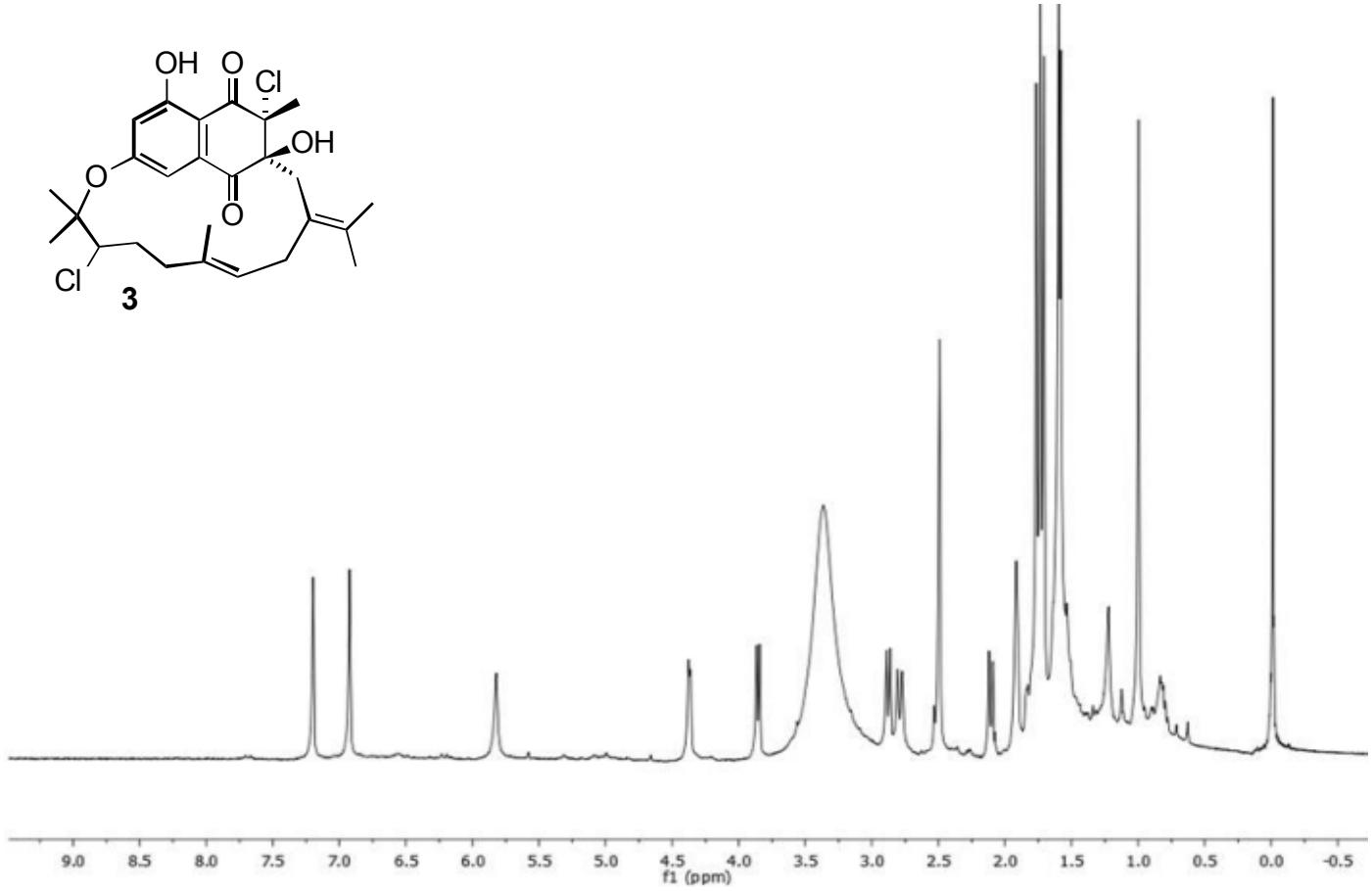


Figure S14. ^{13}C NMR Spectrum (125 MHz) of Merochlorin C (**3**) in DMSO-d_6

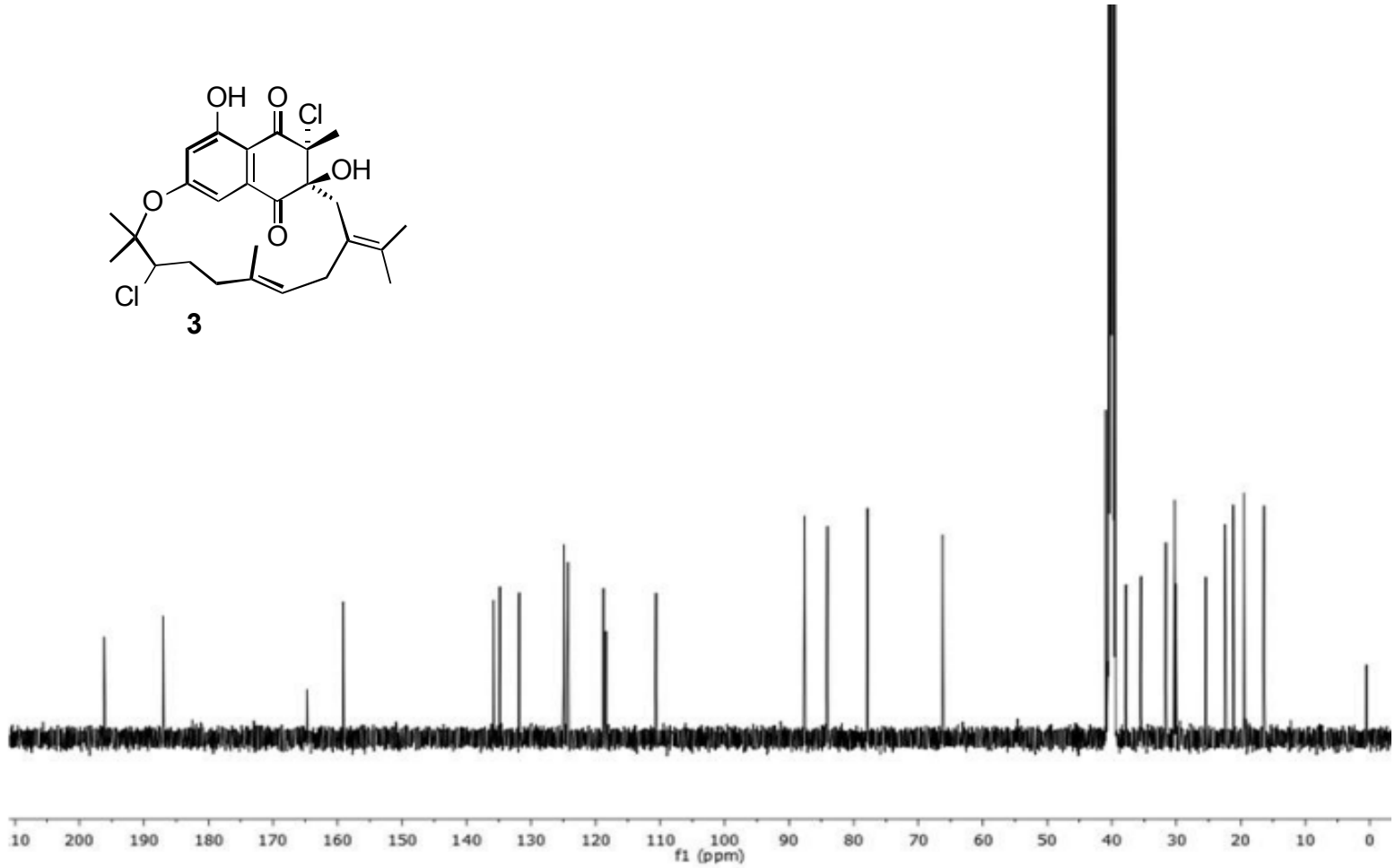


Figure S15. ^{1}H NMR Spectrum (500 MHz) of Merochlorin D (**4**) in DMSO-d_6

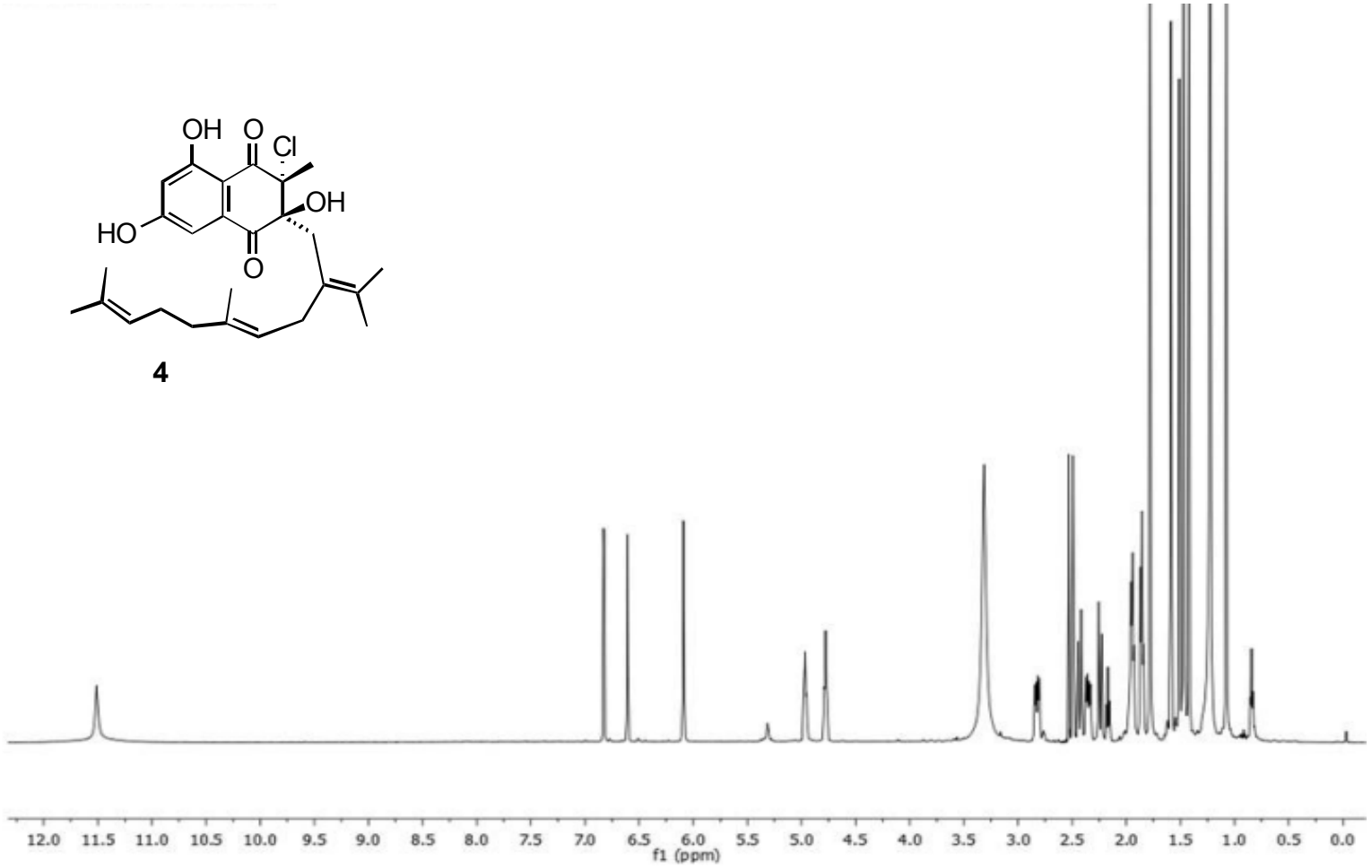


Figure S16. ^{1}H NMR Spectrum (75 MHz) of Merochlorin D (**4**) in DMSO-d_6

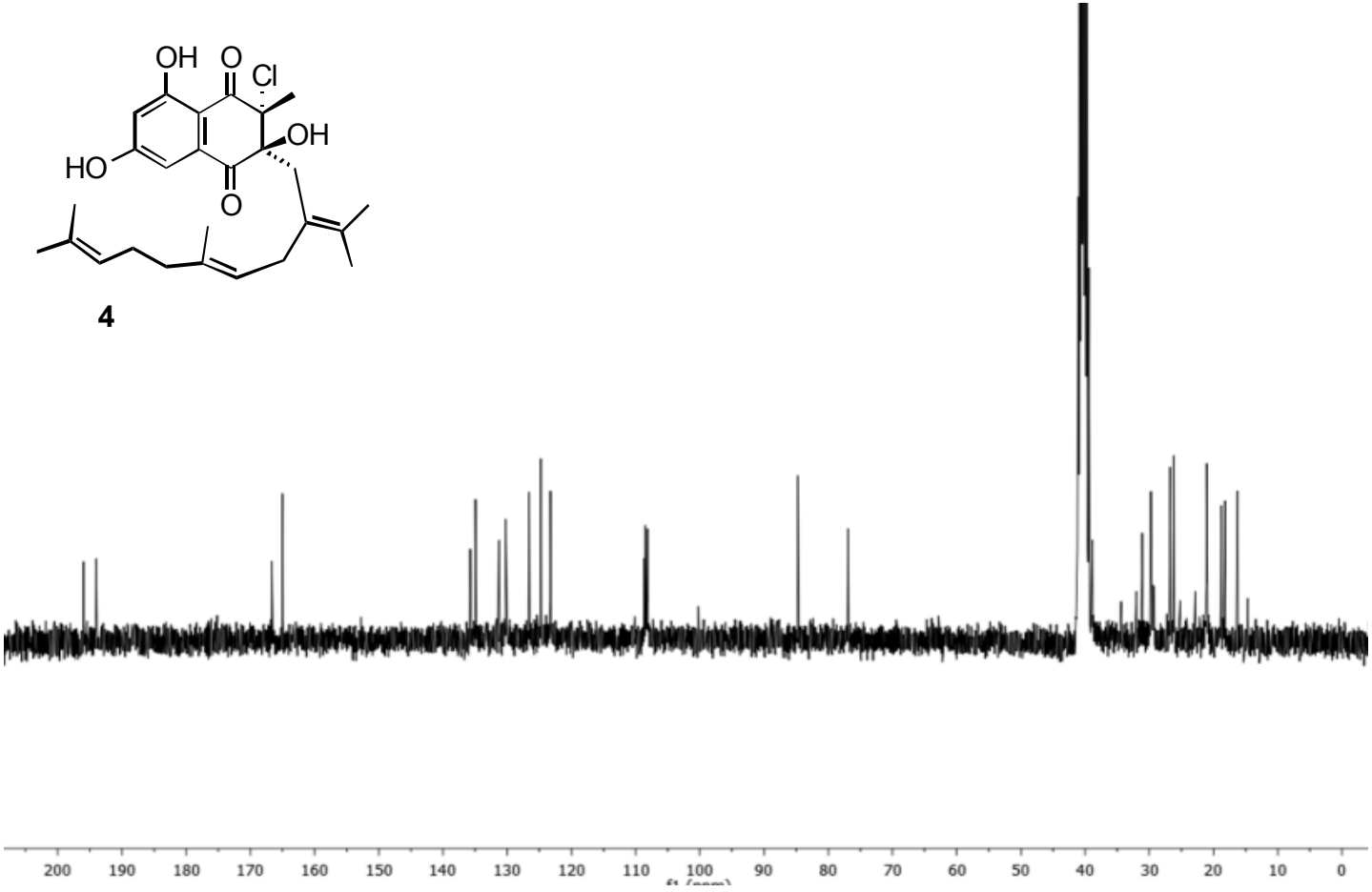


Figure S17. Contigs obtained from Illumina-sequencing of the *Streptomyces* sp. CNH-189 genome with homologous genes to the napyradiomycin biosynthetic gene cluster

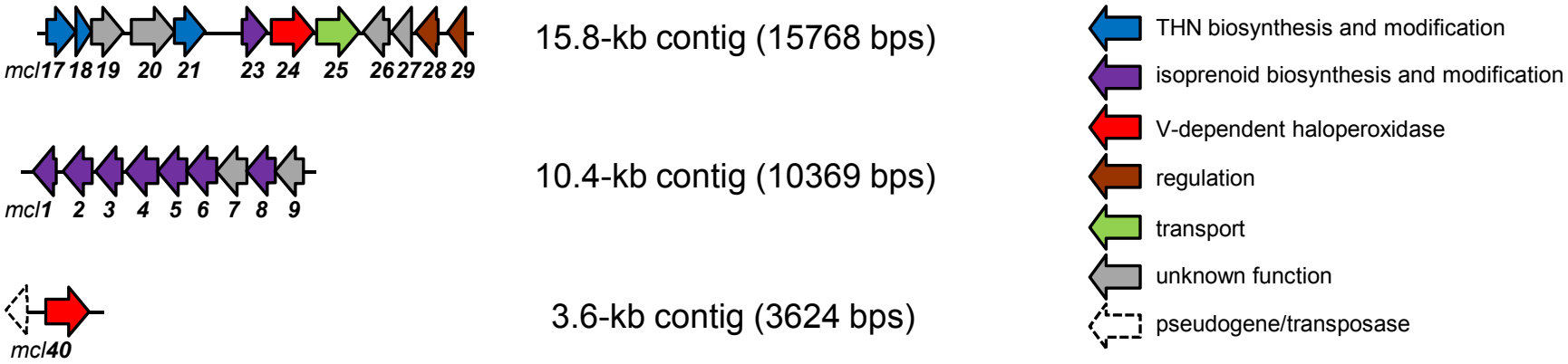
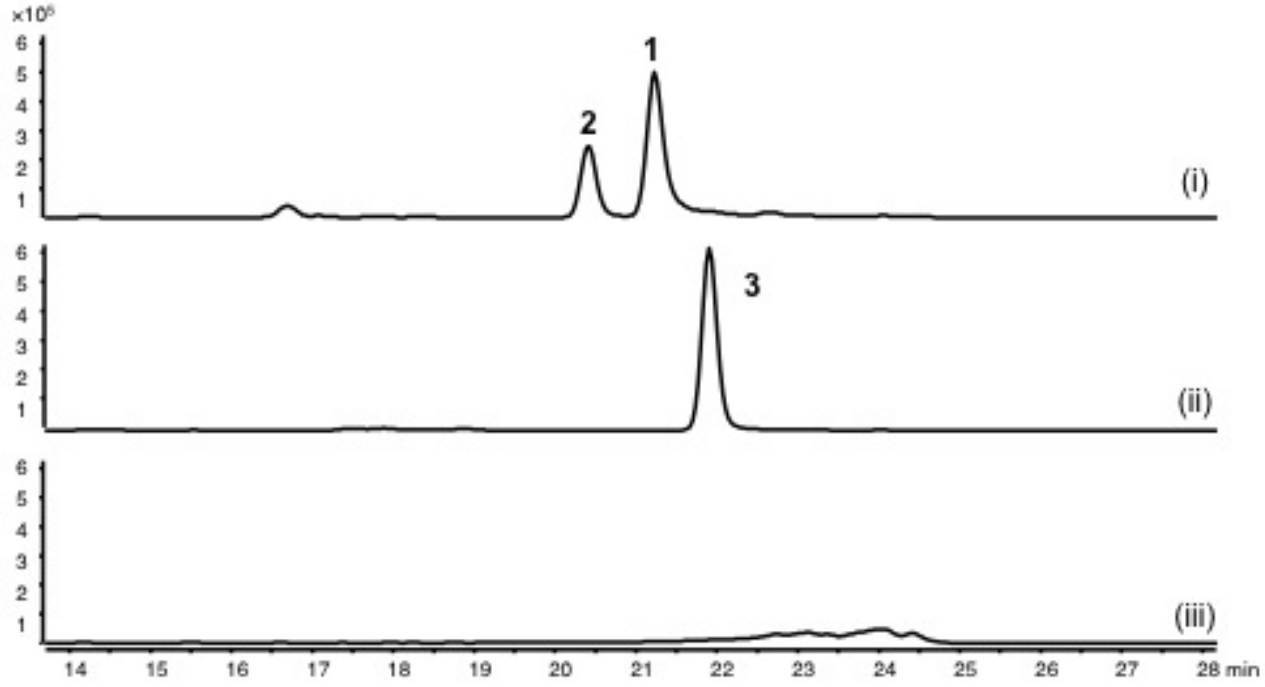


Figure S18. LC-MS analysis of extracts from *S. coelicolor* M1154/merLK01. Extracted ion chromatograms: (i), *m/z* 429 (merochlorins A **1** and B **2**); (ii), *m/z* 461 (merochlorin D **4**); (iii), *m/z* 495 (merochlorin C **3**).



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