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Discovery of novel antibiotics from actinomycetes by integrated metabolomics & genomics approaches

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Appendix I: Supplementary Information belonging to Chapter 4



Figure S1 Spontaneously generated streptomycin-resistant colonies of *Streptomyces* sp. MBT28 (left), *Streptomyces lividans* (middle), and wild type *Streptomyces* sp. MBT28 (right). Spores suspension of wild-type were spread on SFM agar plates containing 10 µg/mL streptomycin, and incubated at 30°C for nine days.

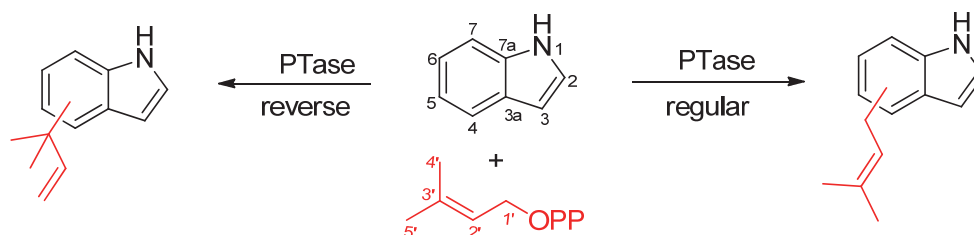


Figure S2. Schematic diagram of indole prenylation by prenyltransferases (PTase). All possible positions of the indole ring (N-1, C-2, C-3, C-4, C-5, C-6, and C-7) could be prenylated, except the two bridgeheads (C-3a, and C-7a). The prenylations may take place in a “regular” sense where the primary carbon of the allylic moiety (C-1’) adds to the indole ring, or in a “reverse” sense where the tertiary carbon (C-3’) adds.^{1,2} Some PTs responsible for prenylation of indole ring are summarized in Table S5.

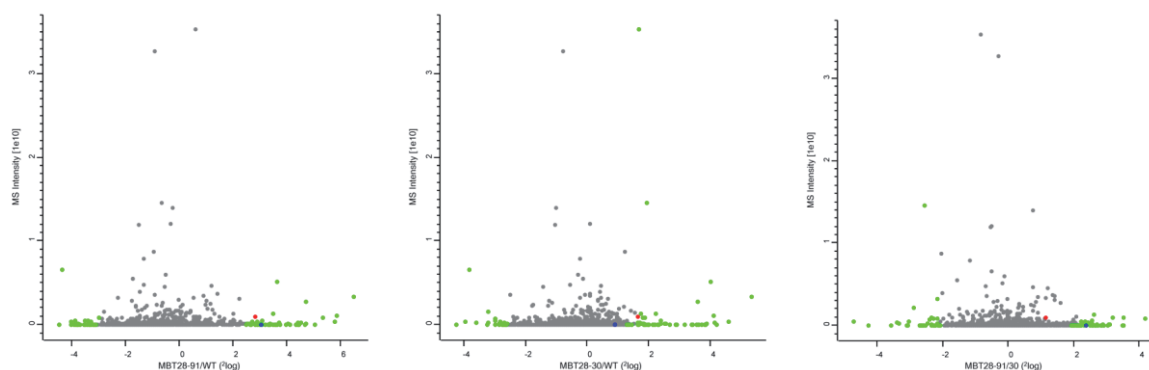


Figure S3. Volcano plot analysis of proteomics data. X axis shows the $^2\log$ ratio of protein expression, Y axis shows the MS intensity of the protein. Green dots represent proteins with the $^2\log$ expression ratio significant (p-value less than 0.05). Blue dot represent IsaA and red dot represent IsaB.

Table S1 ^1H NMR chemical shift (δ) and coupling constants (Hz) assignments for the compounds identified during the metabolomics study of *Streptomyces* sp. MBT28.

NO	Compounds	Chemical shifts
1	borrelidin	6.60 (dd, $J = 15.6, 11.4$ Hz), 6.90 (d, $J = 11.4$ Hz), and 6.31 (ddd, $J = 15.6, 10.2, 4.8$ Hz)
2	<i>p</i> -cresol	6.68 (d, $J = 8.4$ Hz); 7.07 (d, $J = 8.4$ Hz); 2.56 (s)
3	4-hydroxybenzoic acid	6.62 (d, $J = 9.0$ Hz); 7.73 (d, $J = 9.0$ Hz)
4	caffeic acid	6.27 (d, $J = 15.6$ Hz); 7.61 (d, $J = 15.6$ Hz); 6.71 (d, $J = 8.4$ Hz); 7.16 (d, $J = 2.4$ Hz); 7.09 (dd, $J = 8.4, 2.4$ Hz)
5	1 <i>H</i> -pyrrole-2-carboxylic acid	7.70 (dd, $J = 6.0, 3.0$ Hz); 7.60 (dd, $J = 6.0, 3.0$ Hz); 6.28 (t, $J = 6.0$)
6	syringic acid	7.38 (s)
7	salicylic acid	7.75 (dd, $J = 7.2, 2.4$ Hz); 7.28 (t, $J = 7.2$ Hz); 7.10 (m); 7.09 (m)
8	succinic acid	2.59 (s)
9	sucrose	5.62 (d, $J = 4.2$ Hz)
10	2,5-di- <i>tert</i> -butylphenol	7.39 (d, $J = 9.0$ Hz); 7.21 (dd, $J = 9.0, 2.4$ Hz); 7.38 (d, $J = 2.4$ Hz)
11	(3,5-di- <i>tert</i> -butyl-4-hydroxyphenyl)-propionic acid octadecyl ester	6.95 (s); 2.81 (t, $J = 7.2$ Hz); 2.64 (t, $J = 7.8$ Hz); 1.40 (s)

Table S2. Type A indole prenyltransferase gene cluster in *Streptomyces* sp. MBT28.³ This type is proposed to be involved in 7-prenylisatin biosynthesis in current study. The putative function was indicated in Figure 5 of manuscript.

orf	Protein products	Amino acid number	Putative function	Nearest homologue (enzyme, origin)	Homology	Accession number
1		986	Transcriptional regulator	<i>Streptomyces</i> sp. NRRL WC-3795	100%	WP_031020590.1
2	IsaA	341	aromatic prenyltransferase	<i>Streptomyces albogriseolus</i>	84%	AHD24368.1
3	IsaB	502	tryptophanase	<i>Streptomyces</i> sp. NRRL F-4835	99%	WP_030978677.1

Table S3. Type B indole prenyltransferase gene cluster in *Streptomyces* sp. MBT28. This type like SCO7467 in *Streptomyces coelicolor* was reported to be involved in the biosynthesis of 5-dimethylallylindole-3-acetonitrile.³

orf	Amino acid number	Putative function	Nearest homologue (enzyme, origin)	Homology	Accession number
1	276	ABC transporter ATPase	<i>Streptomyces</i> sp. NRRL WC-3795	99%	WP_031018365.1
2	228	trypsin hydrolase	<i>Streptomyces</i> sp. NRRL WC-3795	99%	WP_031018366.1
3	255	membrane protein	<i>Streptomyces</i> sp. NRRL WC-3795	99%	WP_031018369.1
4	382	histidine kinase	[<i>Streptomyces</i>]	100%	WP_029392892.1
5	133	hypothetical protein	<i>Streptomyces violaceorubidus</i>	97%	WP_030183598.1
6	125	hypothetical protein	<i>Streptomyces ambofaciens</i> ATCC 23877	94%	CAJ89643.1
7	191	ATP/GTP-binding protein	<i>Streptomyces ambofaciens</i> ATCC 23877	99%	CAJ89642.1
8	449	cytochrome P450	<i>Streptomyces</i> sp. NRRL WC-3795	99%	WP_031018371.1
9	373	indole prenyltransferase	<i>Streptomyces</i> sp. NRRL WC-3795	99%	WP_031018373.1
10	428	flavin-binding monooxygenase	<i>Streptomyces</i> sp. NRRL WC-3795	99%	WP_031018379.1

Table S4. Significantly up or down regulated (with p-value less than 0.05) quantified proteins in at least one comparison

Protein IDs	MBT28-30/WT (2log)	p-val ue	MBT28-91/WT (2log)	p-val ue	MBT28-91/30 (2log)	p-val ue	predicted function
NODE_001_gen e_001	0.2	0.604	3.1	0.020	2.8	0.008	1 4-alpha-glucan branching enzyme GlgB 2
NODE_001_gen e_012	-1.9	0.174	-3.1	0.033	-1.4	0.184	6-phosphofructokinase 2
NODE_001_gen e_037	-2.4	0.075	-3.3	0.025	-1.0	0.384	Methylmalonyl-CoA epimerase
NODE_005_gen e_007	1.1	0.077	-1.6	0.577	-2.6	0.010	Alanine--tRNA ligase
NODE_009_gen e_004	-0.3	0.994	-2.3	0.150	-2.2	0.045	Secreted protease
NODE_012_gen e_017	-1.5	0.479	-3.5	0.021	-2.3	0.029	ATP-dependent Clp protease proteolytic subunit 1
NODE_016_gen e_001	3.6	0.000	4.3	0.002	0.6	0.576	Secreted protein
NODE_016_gen e_019	-1.2	0.658	-3.8	0.010	-2.5	0.014	Ribose-phosphate pyrophosphokinase
NODE_020_gen e_023	0.2	0.581	2.8	0.030	2.4	0.026	Secreted protein
NODE_020_gen e_034	0.8	0.263	2.8	0.035	2.0	0.064	Flp pilus assembly protein CpaB
NODE_021_gen e_028	-2.6	0.068	-3.9	0.007	-1.2	0.272	Cytochrome oxidase subunit I
NODE_021_gen e_047	2.4	0.004	3.4	0.012	0.9	0.388	Secreted protein
NODE_021_gen e_048	2.5	0.003	4.7	0.001	2.7	0.012	Nuclear export factor GLE1
NODE_025_gen e_009	-2.9	0.039	-1.9	0.400	1.0	0.268	Putative PadR-like family transcriptional regulator
NODE_025_gen e_015	-1.0	0.532	-3.2	0.029	-2.4	0.029	Ectoine/hydroxyectoine ABC transporter permease EhuC
NODE_025_gen e_017	1.3	0.097	3.7	0.006	2.6	0.017	Methyltransferase
NODE_025_gen e_022	1.7	0.037	0.6	0.467	-0.9	0.439	Nucleotide-binding protein
NODE_025_gen e_023	1.7	0.033	4.5	0.001	2.5	0.021	Peptidoglycan-binding domain 1 protein
NODE_026_gen e_007	1.9	0.009	3.7	0.007	1.7	0.072	Secreted protein
NODE_027_gen e_001	2.3	0.003	3.4	0.013	1.6	0.088	Conserved hypothetical secreted protein
NODE_029_gen e_014	3.2	0.000	2.5	0.051	-0.9	0.411	Secreted protein
NODE_032_gen e_004	-1.9	0.174	-3.3	0.025	-1.2	0.254	Membrane protein
NODE_032_gen e_009	0.9	0.205	-2.5	0.117	-3.1	0.004	Starvation-induced DNA protecting protein
NODE_033_gen e_057	-2.6	0.048	-1.9	0.266	0.7	0.522	Cysteine--tRNA ligase
NODE_035_gen e_011	-2.5	0.062	-3.4	0.018	-1.0	0.368	Glycerol-3-phosphate dehydrogenase
NODE_035_gen e_012	-2.4	0.073	0.5	0.507	3.2	0.003	Glycerol kinase 1
NODE_035_gen e_015	-0.3	0.707	3.0	0.024	2.6	0.007	Methionine synthase
NODE_035_gen e_017	3.4	0.000	3.0	0.025	-0.7	0.532	Lipoprotein oligopeptide binding protein
NODE_036_gen e_009	0.1	0.429	2.2	0.073	2.1	0.030	Oxidoreductase
NODE_042_gen e_025	-1.6	0.262	-3.6	0.013	-1.8	0.089	Nucleotide sugar-1-phosphate transferase
NODE_047_gen e_002	-3.8	0.003	-4.4	0.002	-0.5	0.644	Alkylhydroperoxidase like protein AhpD family
NODE_047_gen e_007	-3.0	0.022	-2.9	0.056	-0.1	0.958	Alkylhydroperoxidase like protein AhpD family
NODE_048_gen e_013	0.9	0.111	3.5	0.011	2.2	0.028	Carboxypeptidase
NODE_048_gen e_015	3.9	0.000	3.0	0.025	-0.8	0.493	Metallopeptidase
NODE_051_gen e_011	-2.1	0.133	0.2	0.628	2.2	0.039	Monooxygenase
NODE_057_gen e_030	-3.3	0.015	-2.5	0.169	0.5	0.585	Putative oxidoreductase
NODE_057_gen e_031	-2.7	0.040	-1.6	0.388	1.7	0.105	Putative 6-phospho-3-hexuloisomerase
NODE_057_gen e_032	-2.8	0.031	-0.4	0.923	2.3	0.029	Putative triosephosphate isomerase
NODE_058_gen e_031	-3.6	0.005	-1.5	0.410	0.8	0.465	Alkylhydroperoxidase domain-containing protein AhpD family core
NODE_062_gen e_005	0.0	0.471	-2.4	0.207	-2.3	0.026	DNA-binding protein
NODE_063_gen e_013	-3.2	0.013	-2.8	0.064	0.9	0.393	Glutamine synthetase 2
NODE_063_gen e_031	2.2	0.007	2.0	0.102	-0.4	0.758	Maltose-binding protein
NODE_064_gen e_033	2.5	0.002	1.5	0.202	-1.2	0.286	Secreted protein

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NODE_065_gen e_019	-2.7	0.040	-2.7	0.078	0.3	0.745	Carboxylesterase
NODE_065_gen e_022	1.0	0.163	4.0	0.003	3.1	0.004	Cold shock domain-containing protein CspD
NODE_066_gen e_007	-4.0	0.002	-4.0	0.005	-0.2	0.872	Secreted protein
NODE_069_gen e_017	4.2	0.000	2.9	0.027	-1.1	0.323	Sugar transporter sugar binding protein
NODE_070_gen e_012	2.9	0.000	4.1	0.004	1.0	0.271	Oxidoreductase
NODE_071_gen e_024	0.2	0.347	-2.4	0.197	-2.7	0.009	Integral membrane protein
NODE_072_gen e_025	1.1	0.134	-0.8	0.798	-2.2	0.043	Succinate-semialdehyde dehydrogenase
NODE_075_gen e_003	-0.1	0.555	2.0	0.092	2.7	0.007	Putative methylesterase
NODE_078_gen e_010	-2.6	0.049	-3.8	0.008	-1.0	0.376	Putative 3-oxoacyl-ACP synthase II
NODE_079_gen e_007	1.0	0.177	-1.8	0.318	-2.3	0.035	Chloride peroxidase
NODE_079_gen e_008	0.7	0.290	-1.5	0.444	-2.4	0.029	Alpha/beta hydrolase
NODE_082_gen e_003	2.2	0.004	0.5	0.380	-1.3	0.211	Uncharacterized protein
NODE_089_gen e_031	-0.5	0.822	2.7	0.038	3.0	0.002	SPFH domain/Band 7 family protein
NODE_090_gen e_004	1.8	0.024	0.9	0.369	-1.1	0.333	Integral membrane protein
NODE_091_gen e_005	1.2	0.057	-2.2	0.257	-3.6	0.000	Hypothetical cytosolic protein
NODE_092_gen e_010	-0.1	0.526	1.9	0.095	1.9	0.046	Redoxin
NODE_092_gen e_011	-1.3	0.587	-3.4	0.024	-2.1	0.042	3-phosphoserine phosphatase
NODE_099_gen e_033	-0.8	0.665	2.3	0.066	3.1	0.004	NDP-4-keto-6-deoxy-L-hexose 2 3-reductase
NODE_102_gen e_017	2.3	0.003	2.1	0.076	-0.1	0.956	ABC-type cobalt transport system
NODE_110_gen e_015	-0.4	0.918	2.1	0.096	2.2	0.039	Pseudouridine-5'-phosphate glycosidase
NODE_110_gen e_016	0.4	0.453	2.0	0.107	2.3	0.032	Dioxygenase
NODE_118_gen e_013	1.9	0.011	4.0	0.005	2.0	0.041	Predicted protein
NODE_119_gen e_005	0.6	0.347	2.6	0.045	1.8	0.085	Putative secreted extracellular small neutral protease
NODE_126_gen e_032	1.0	0.179	-1.4	0.492	-2.3	0.030	Putative calcium binding protein
NODE_131_gen e_004	1.5	0.056	2.5	0.050	1.0	0.360	lipoprotein
NODE_131_gen e_035	-1.9	0.260	-3.2	0.044	-1.2	0.265	Uncharacterized protein
NODE_136_gen e_010	-0.9	0.880	2.5	0.047	3.5	0.000	AdpA
NODE_136_gen e_016	0.1	0.397	2.1	0.076	1.9	0.045	B-N-acetylhexosaminidase
NODE_137_gen e_015	-2.6	0.048	-1.9	0.258	0.9	0.392	Flavoprotein reductase
NODE_139_gen e_026	-1.1	0.726	0.8	0.309	2.5	0.011	Putative uncharacterized protein
NODE_143_gen e_007	-1.4	0.322	-3.3	0.025	-1.5	0.176	Succinyl-CoA ligase [ADP-forming] subunit beta-1
NODE_143_gen e_008	-4.3	0.001	-3.4	0.028	0.5	0.587	Succinyl-CoA ligase [ADP-forming] subunit alpha
NODE_144_gen e_030	2.9	0.000	3.6	0.009	0.5	0.541	Iron transport lipoprotein
NODE_146_gen e_013	0.3	0.329	-2.9	0.076	-2.6	0.011	Uncharacterized protein
NODE_146_gen e_033	1.6	0.041	-1.2	0.590	-2.6	0.014	Dehydrogenase
NODE_152_gen e_033	2.1	0.005	4.4	0.002	2.2	0.023	Amino acid transport integral membrane protein
NODE_153_gen e_002	2.7	0.001	3.3	0.014	0.4	0.595	Solute-binding lipoprotein
NODE_154_gen e_010	2.1	0.005	3.3	0.015	1.3	0.165	Putative secreted protein
NODE_154_gen e_060	NaN	NaN	2.8	0.030	2.8	0.004	Aminotransferase
NODE_154_gen e_064	-0.3	0.710	1.9	0.104	2.3	0.018	Oxidoreductase
NODE_155_gen e_014	4.6	0.000	5.8	0.000	1.2	0.244	Substrate binding protein
NODE_155_gen e_032	1.0	0.161	-1.6	0.372	-2.7	0.011	Mesaconyl-CoA hydratase
NODE_155_gen e_040	0.8	0.138	-1.7	0.530	-2.4	0.022	Secreted peptidase
NODE_155_gen e_052	-1.2	0.627	0.8	0.299	2.0	0.041	Uncharacterized protein
NODE_159_gen e_015	3.8	0.000	4.5	0.002	0.6	0.496	Putative membrane protein
NODE_168_gen e_002	0.9	0.190	-1.3	0.538	-2.2	0.045	6-pyruvoyl tetrahydropterin synthase

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NODE_171_gen e_005	-1.9	0.162	-4.0	0.004	-2.4	0.029	Sugar phosphotransferase
NODE_173_gen e_018	1.8	0.022	0.8	0.408	-1.1	0.312	Peptidoglycan-binding domain 1 protein
NODE_175_gen e_006	1.0	0.180	-1.5	0.432	-2.3	0.032	Putative lysozyme
NODE_180_gen e_024	0.9	0.116	3.0	0.023	2.4	0.016	Aromatic prenyltransferase DMATS type
NODE_180_gen e_025	1.6	0.040	2.8	0.031	1.1	0.286	Aromatic amino acid beta-eliminating lyase/threonine aldolase
NODE_188_gen e_004	2.4	0.004	-2.4	0.137	-4.7	0.000	Iron sulfur protein (Secreted protein)
NODE_191_gen e_023	0.1	0.680	2.0	0.103	2.5	0.018	3-oxoacyl-[acyl-carrier-protein] synthase 2
NODE_196_gen e_002	0.6	0.330	3.5	0.010	2.6	0.015	Secreted protein
NODE_198_gen e_010	-2.9	0.039	-2.7	0.129	0.5	0.587	Aminoacylase
NODE_207_gen e_013	0.9	0.103	3.5	0.011	2.4	0.015	Neutral zinc metalloprotease
NODE_207_gen e_015	-1.7	0.226	1.5	0.191	3.5	0.001	Phosphorylase
NODE_207_gen e_016	0.6	0.323	3.1	0.020	2.3	0.030	Alpha-amylase
NODE_211_gen e_006	3.9	0.000	2.2	0.072	-1.9	0.070	ABC transport system integral membrane protein BldKC
NODE_212_gen e_021	-0.1	0.897	-4.5	0.001	-4.3	0.000	Putative signal transduction protein with EFhand domain
NODE_226_gen e_007	-1.5	0.434	-3.7	0.012	-2.2	0.030	Exporter
NODE_227_gen e_010	1.8	0.028	1.5	0.186	-0.2	0.869	Proteinase
NODE_230_gen e_006	-3.0	0.028	-2.2	0.258	0.4	0.637	GCN5-related N-acetyltransferase
NODE_231_gen e_019	-2.9	0.030	-3.9	0.006	-0.9	0.393	Peptide transporter
NODE_231_gen e_043	-2.7	0.044	-3.1	0.035	-0.7	0.523	FAD-dependent pyridine nucleotide-disulfide oxidoreductase
NODE_244_gen e_025	-1.9	0.260	-3.3	0.033	-1.2	0.266	Phosphodiesterase
NODE_245_gen e_011	-2.2	0.143	-3.2	0.044	-0.9	0.391	Mycothioli conjugate amidase Mca
NODE_248_gen e_003	0.9	0.209	-1.5	0.441	-2.5	0.021	Peptide transport system secreted peptide-binding protein
NODE_249_gen e_004	-0.3	1.000	-3.5	0.015	-3.0	0.005	Urease subunit alpha 1
NODE_250_gen e_002	-1.3	0.558	1.3	0.188	2.6	0.009	Putative transmembrane transport protein
NODE_250_gen e_012	1.4	0.034	2.1	0.076	0.7	0.429	Cationic amino acid transporter
NODE_250_gen e_016	-2.2	0.166	-3.2	0.045	-1.1	0.312	Thioredoxin
NODE_251_gen e_002	-1.4	0.503	0.8	0.312	2.1	0.033	Sigma 54 modulation protein/SSU ribosomal protein S30P
NODE_259_gen e_010	1.7	0.035	1.3	0.242	-0.5	0.659	Subtilisin-like protease
NODE_267_gen e_023	3.5	0.000	3.6	0.009	0.1	0.872	Secreted alkaline phosphatase
NODE_271_gen e_008	-3.3	0.012	-2.1	0.208	0.1	0.886	UDP-glucose 4-epimerase
NODE_272_gen e_009	-0.5	0.854	1.3	0.197	2.0	0.043	WD40 repeat-containing protein
NODE_279_gen e_009	1.2	0.113	-1.6	0.378	-2.9	0.007	Putative lipoprotein
NODE_279_gen e_082	1.9	0.017	-0.6	0.919	-2.6	0.017	Glyceraldehyde 3-phosphate dehydrogenase
NODE_279_gen e_107	0.5	0.236	3.3	0.015	2.7	0.005	Putative cysteine desulphurases SufS
NODE_281_gen e_006	-0.1	0.825	-2.6	0.094	-2.6	0.014	3-hydroxyacyl-CoA dehydrogenase PaaC
NODE_282_gen e_002	-2.7	0.041	-2.6	0.096	0.1	0.881	Phosphocarrier protein HPr
NODE_286_gen e_026	0.7	0.302	-1.7	0.322	-2.4	0.025	Catalase
NODE_288_gen e_011	0.7	0.169	-1.6	0.580	-2.1	0.045	Uncharacterized protein
NODE_299_gen e_002	-2.8	0.049	-1.4	0.744	1.5	0.127	Bifunctional uroporphyrinogen-III synthetase/response regulator domain protein
NODE_299_gen e_014	1.9	0.011	2.2	0.073	0.4	0.616	Sugar transporter sugar-binding protein
NODE_300_gen e_002	-1.3	0.389	1.0	0.333	2.3	0.031	Adenylosuccinate synthetase
NODE_309_gen e_008	-2.1	0.177	-3.1	0.049	-0.7	0.545	Orotate phosphoribosyltransferase
NODE_316_gen e_021	1.3	0.050	-0.6	0.821	-1.4	0.172	Secreted hydrolase
NODE_320_gen e_035	0.0	0.502	2.1	0.075	2.2	0.025	3-ketosteroid-delta-1-dehydrogenase (Fragment)
NODE_321_gen e_026	-3.2	0.013	-2.7	0.081	0.5	0.647	Phosphoribosylaminoimidazole-succinocarboxamide synthase
NODE_330_gen e_015	-3.0	0.022	-3.4	0.019	-0.3	0.790	Peptidase M48

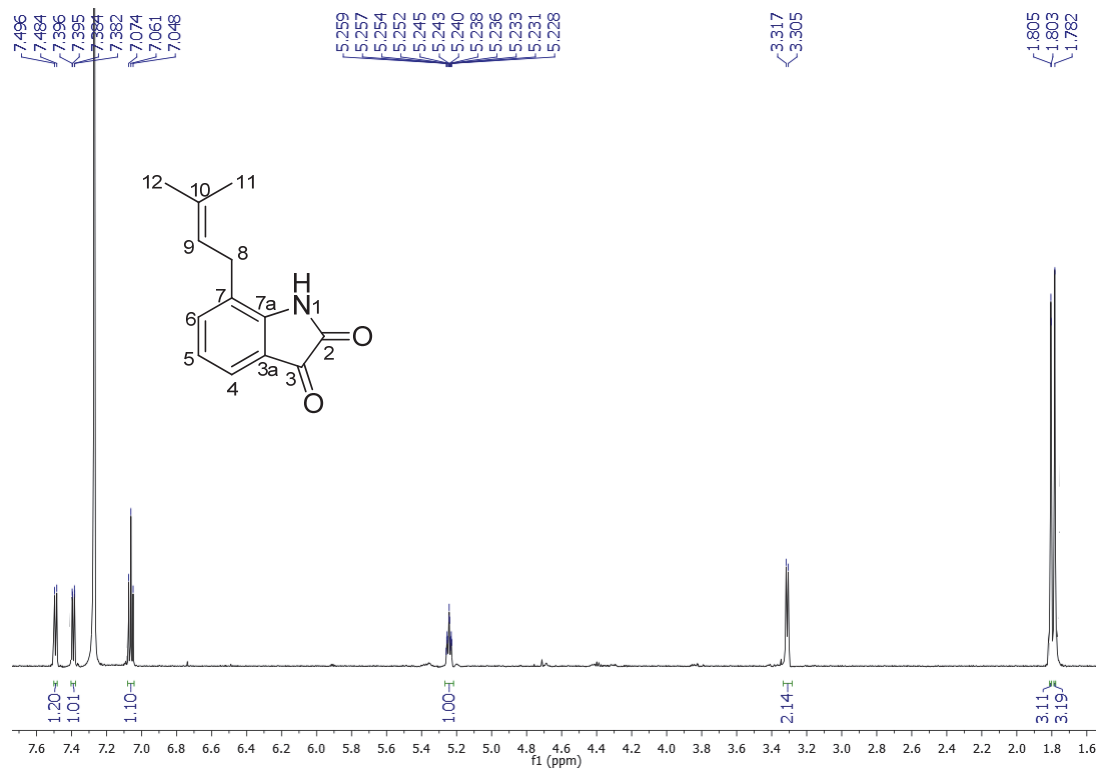
Appendix I: Supplementary Information belong to Chapter 4

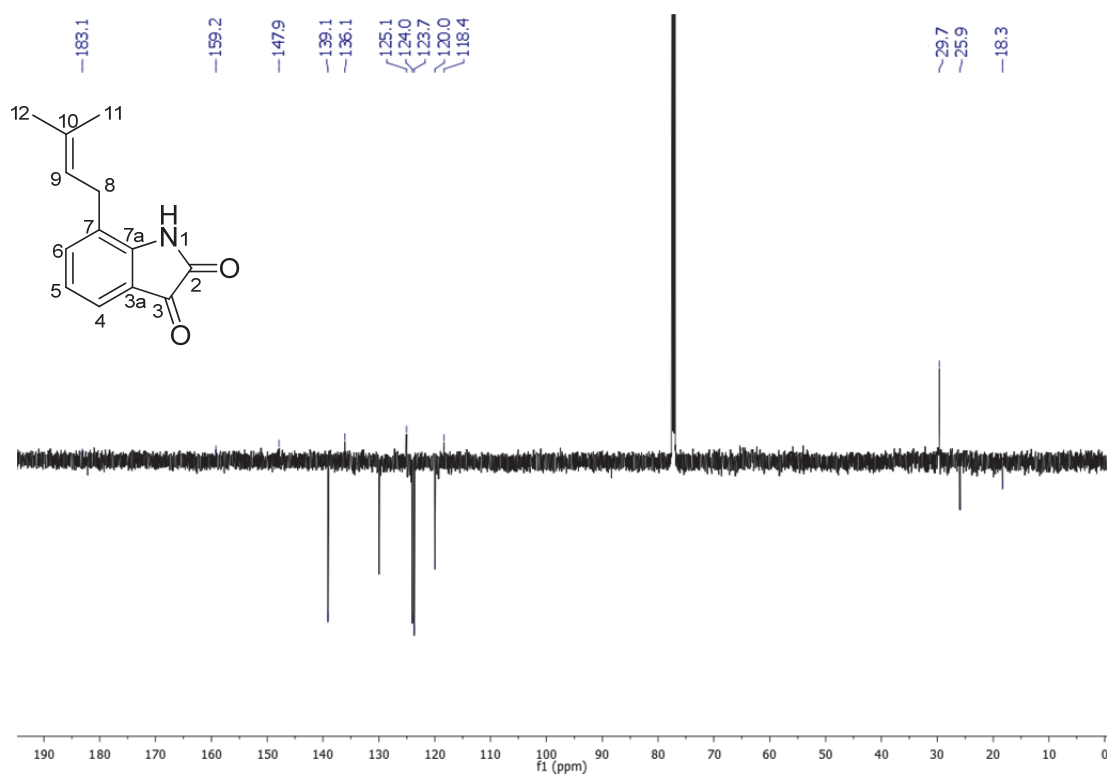
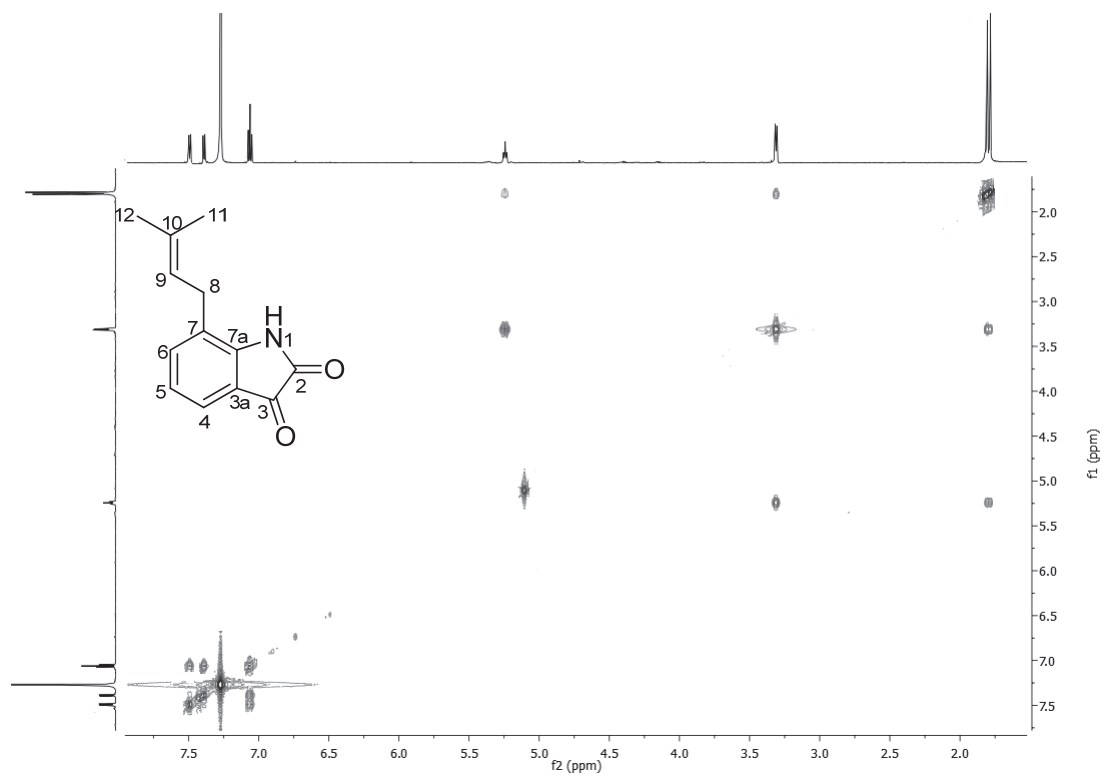
NODE_337_gen e_001	-2.5	0.093	-3.8	0.009	-0.3	0.830	Putative oxygenase
NODE_340_gen e_005	-0.8	0.937	0.9	0.272	1.9	0.049	Pyruvate phosphate dikinase
NODE_346_gen e_006	-0.6	0.899	1.1	0.238	2.0	0.038	Translation initiation factor IF-3
NODE_347_gen e_004	4.1	0.000	4.4	0.001	0.5	0.642	Lipoprotein
NODE_359_gen e_003	-0.9	0.576	-3.0	0.044	-2.0	0.066	Putative secreted protein
NODE_363_gen e_001	1.8	0.025	5.3	0.000	4.2	0.000	Putative iron transport lipoprotein
NODE_366_gen e_016	4.0	0.000	3.7	0.007	-0.2	0.882	Glutamate binding protein
NODE_366_gen e_028	-0.4	0.744	-3.1	0.056	-2.5	0.013	Predicted protein
NODE_366_gen e_037	3.4	0.000	3.6	0.010	0.3	0.714	Secreted protein
NODE_395_gen e_033	-1.8	0.311	-3.4	0.025	-1.6	0.131	HesB/YadR/YfhF family protein
NODE_395_gen e_039	-2.2	0.098	-3.8	0.008	-1.6	0.145	Uncharacterized protein
NODE_399_gen e_018	0.8	0.133	-1.7	0.554	-2.1	0.038	Uncharacterized protein
NODE_410_gen e_001	1.6	0.041	2.8	0.031	1.1	0.282	Secreted protein
NODE_416_gen e_006	1.7	0.030	1.3	0.243	-0.3	0.822	Germacradienol/geosmin synthase
NODE_431_gen e_010	0.3	0.545	2.8	0.030	2.3	0.032	Uncharacterized protein
NODE_434_gen e_008	-2.0	0.210	-3.9	0.008	-1.2	0.244	Putative polyketide cyclase
NODE_451_gen e_001	-1.1	0.474	0.9	0.361	2.4	0.026	NB-ARC domain-containing protein
NODE_453_gen e_003	-2.7	0.038	-2.8	0.060	0.4	0.705	Methylmalonyl-CoA mutase
NODE_461_gen e_007	1.7	0.038	-1.6	0.397	-3.4	0.002	Protease
NODE_463_gen e_011	2.0	0.008	3.0	0.025	1.3	0.173	ABC-type Fe3+-siderophore transporter ATP-binding protein
NODE_463_gen e_013	4.1	0.000	5.9	0.000	2.1	0.054	ABC-type Fe3+-siderophore transporter substrate-binding protein
NODE_464_gen e_004	0.4	0.282	2.6	0.039	2.1	0.032	Cobalt transport integral membrane protein
NODE_464_gen e_005	1.8	0.024	2.5	0.055	1.2	0.248	Putative ABC transporter ATP-binding protein SCO5958
NODE_487_gen e_009	1.4	0.042	4.7	0.001	3.5	0.000	Muramoyl-pentapeptide carboxypeptidase
NODE_487_gen e_101	3.6	0.000	4.7	0.001	1.6	0.135	Lipoprotein
NODE_487_gen e_102	3.7	0.000	5.1	0.001	2.0	0.041	Solute-binding lipoprotein
NODE_487_gen e_103	5.3	0.000	6.5	0.000	1.1	0.282	Secreted protein
NODE_491_gen e_002	1.8	0.013	1.4	0.168	-0.3	0.854	Secreted alkaline phosphatase
NODE_510_gen e_026	-0.3	0.664	-2.1	0.330	-2.1	0.041	Membrane protein
NODE_514_gen e_002	-2.2	0.144	-3.8	0.010	-1.6	0.127	Secreted penicillin binding protein
NODE_526_gen e_007	-2.8	0.048	-3.3	0.032	-0.3	0.865	Lipoprotein
NODE_528_gen e_003	0.8	0.135	3.2	0.018	2.5	0.010	Carrier protein membrane protein (Fragment)
NODE_532_gen e_001	1.3	0.096	2.9	0.028	1.9	0.074	Alpha-1 4-glucan:maltose-1-phosphate maltosyltransferase 1
NODE_534_gen e_001	-1.7	0.342	1.3	0.196	2.9	0.003	Alpha-1 4-glucan:maltose-1-phosphate maltosyltransferase 1
NODE_585_gen e_004	-0.9	0.607	1.6	0.172	2.4	0.023	DUF364 domain-containing protein
NODE_633_gen e_002	0.7	0.284	2.7	0.037	1.7	0.116	Nitrogen regulatory protein P-II
NODE_644_gen e_008	2.0	0.008	-1.4	0.749	-3.1	0.002	hydrolase
NODE_659_gen e_009	-0.6	0.763	2.2	0.080	2.9	0.008	Amidase
NODE_679_gen e_001	-1.9	0.169	-3.9	0.006	-2.1	0.052	Phage tail sheath protein
NODE_746_gen e_022	0.9	0.202	-2.5	0.113	-3.4	0.002	Lipoprotein
NODE_746_gen e_044	0.8	0.263	-1.3	0.543	-2.1	0.050	Putative glyoxalase/bleomycin resistance family protein
NODE_746_gen e_047	0.5	0.230	-2.7	0.119	-3.1	0.003	Phytanoyl-CoA dioxygenase

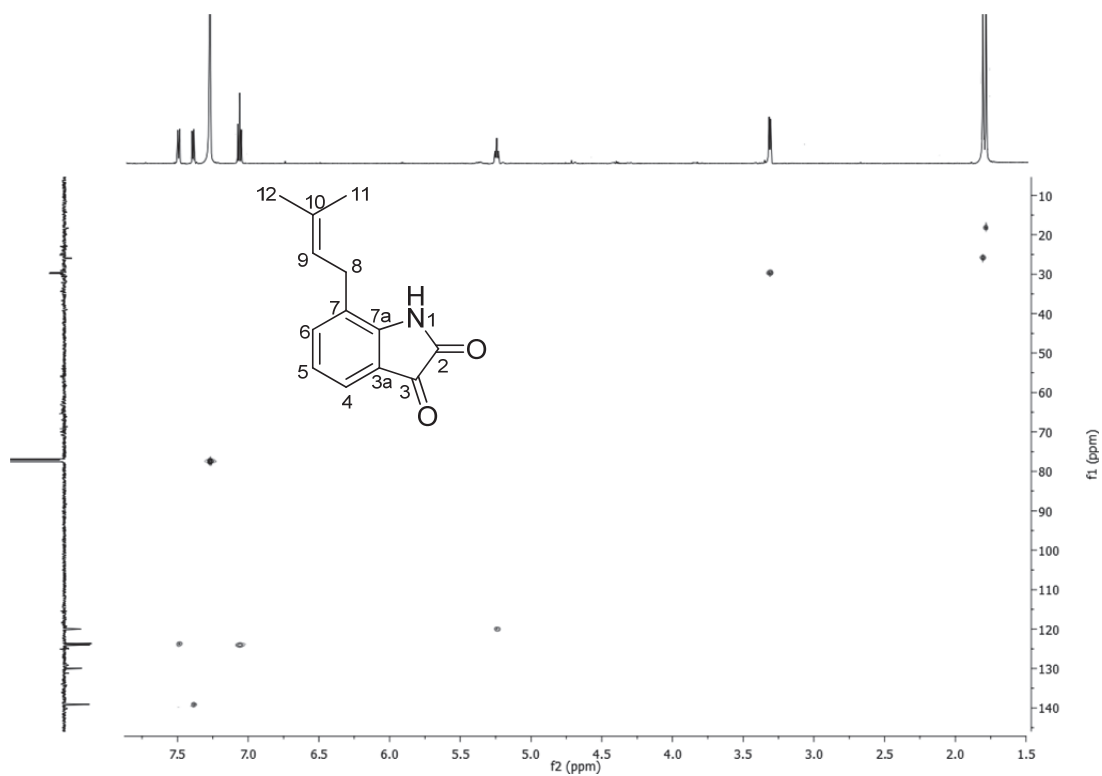
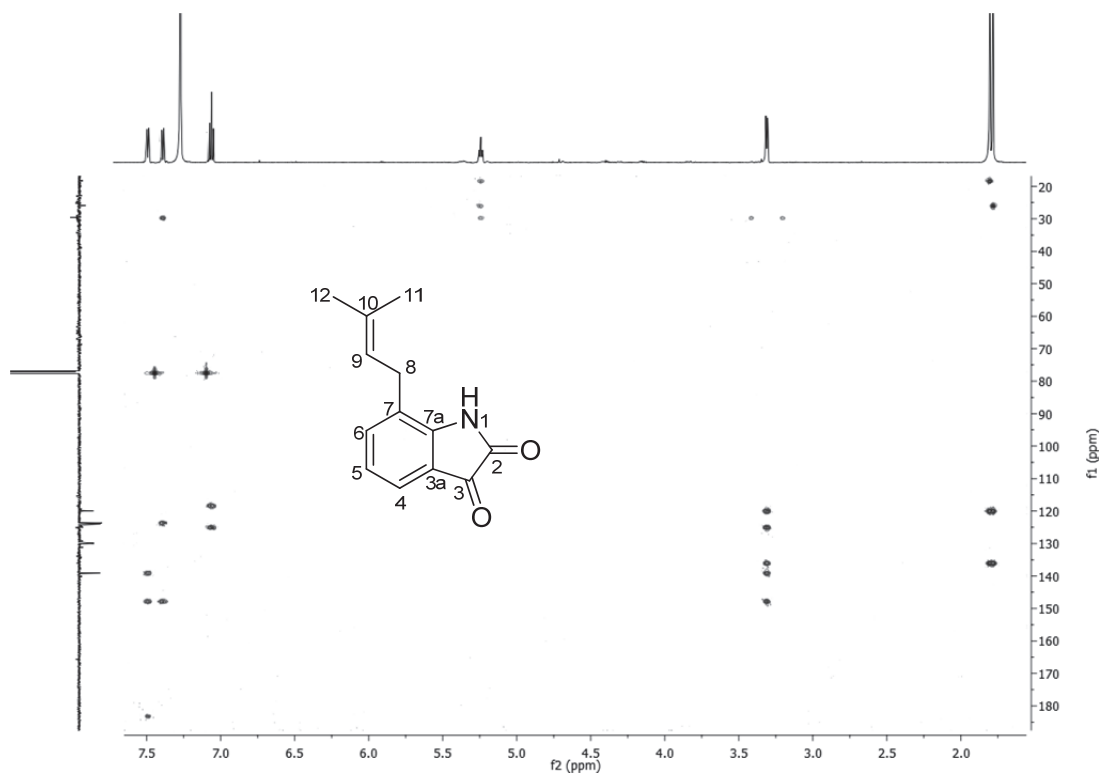
Supporting data file. Spectra of 7-prenylisatin (**1**), including NMR, MS, and UV.

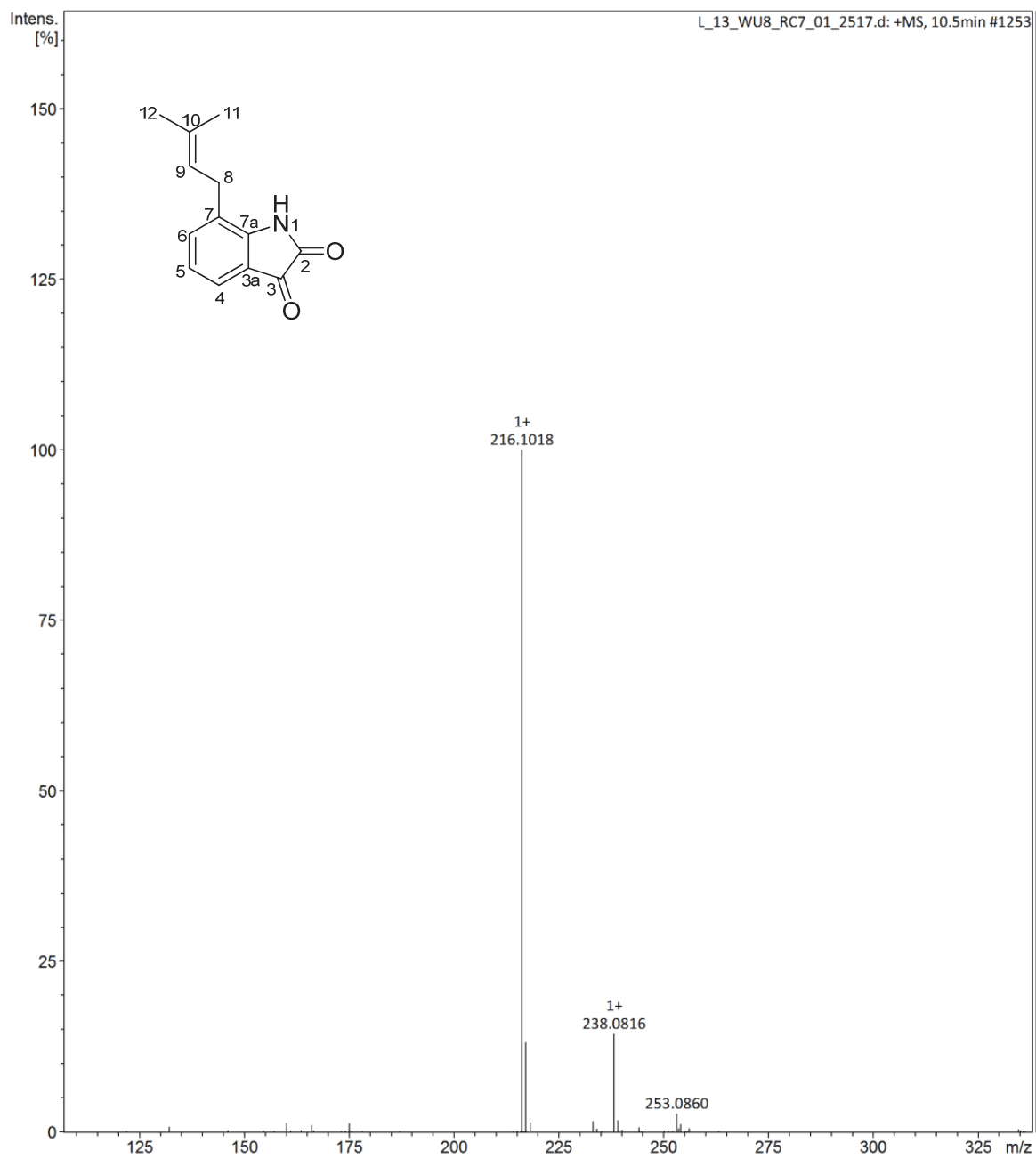
- S1. ^1H NMR spectrum of 7-prenylisatin (**1**) in CDCl_3 .
- S2. APT spectrum of 7-prenylisatin (**1**) in CDCl_3 .
- S3. ^1H - ^1H COSY spectrum of 7-prenylisatin (**1**) in CDCl_3 .
- S4. HSQC spectrum of 7-prenylisatin (**1**) in CDCl_3 .
- S5. HMBC spectrum of 7-prenylisatin (**1**) in CDCl_3 .
- S6. HRESIMS of 7-prenylisatin (**1**).
- S7. UV spectrum of 7-prenylisatin (**1**).

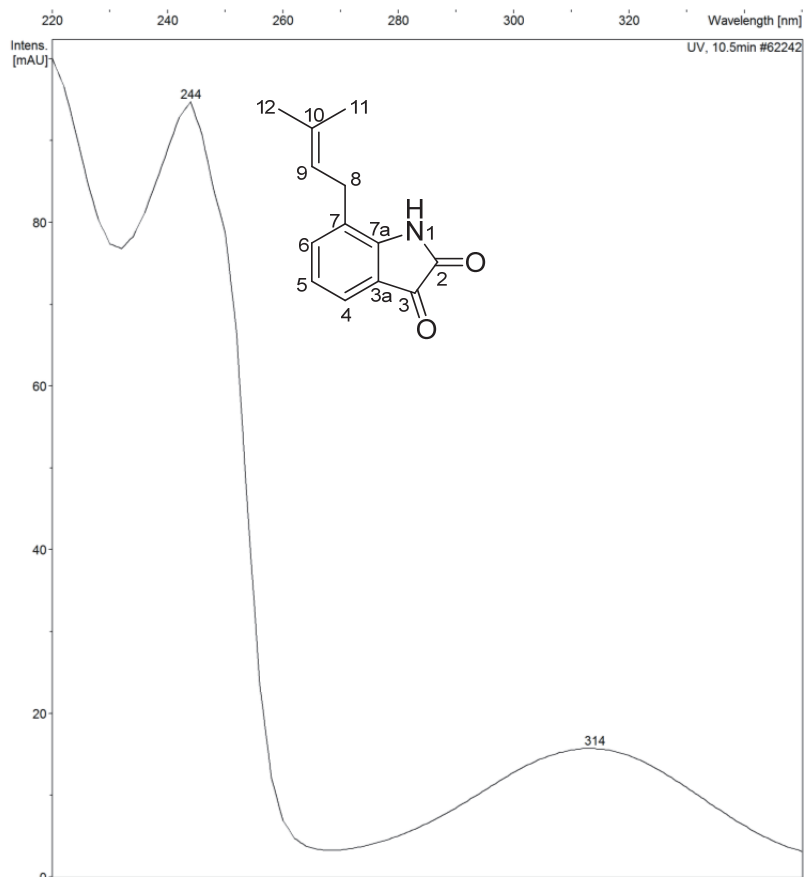
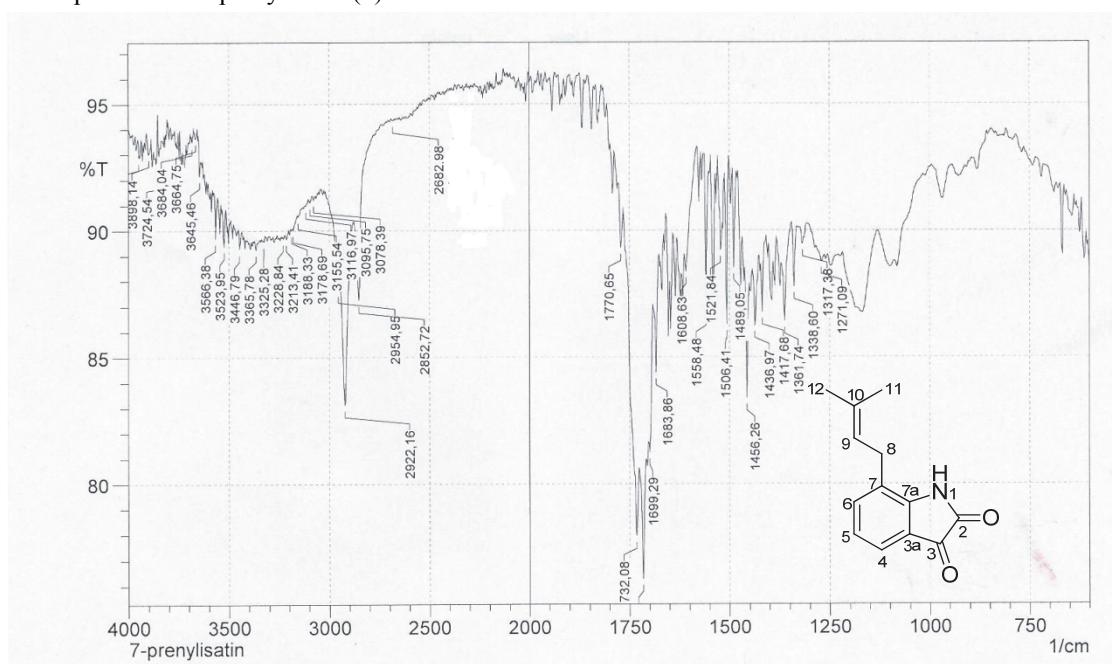
S1. ^1H NMR spectrum (600 MHz) of 7-prenylisatin (**1**) in CDCl_3 .



S2. APT spectrum (600 MHz) of 7-prenylisatin (**1**) in CDCl₃.S3. ¹H-¹H COSY spectrum (600 MHz) of 7-prenylisatin (**1**) in CDCl₃.

S4. HSQC spectrum of 7-prenylisatin (**1**) in CDCl₃.S5. HMBC spectrum of 7-prenylisatin (**1**) in CDCl₃.

S6. HRESIMS of 7-prenylisatin (**1**).

S7. UV spectrum of 7-prenylisatin (**1**).S8. IR spectrum of 7-prenylisatin (**1**).

References

- (1) Tanner, M. E. *Nat. Prod. Rep.* **2015**, 32, 88–101.
- (2) Walsh, C. T. *ACS Chem. Biol.* **2014**, 9, 2718–2728.
- (3) Ozaki, T.; Nishiyama, M.; Kuzuyama, T. *J. Biol. Chem.* **2013**, 288, 9946–56.

Appendix II: Supplementary Information belonging to Chapter 5

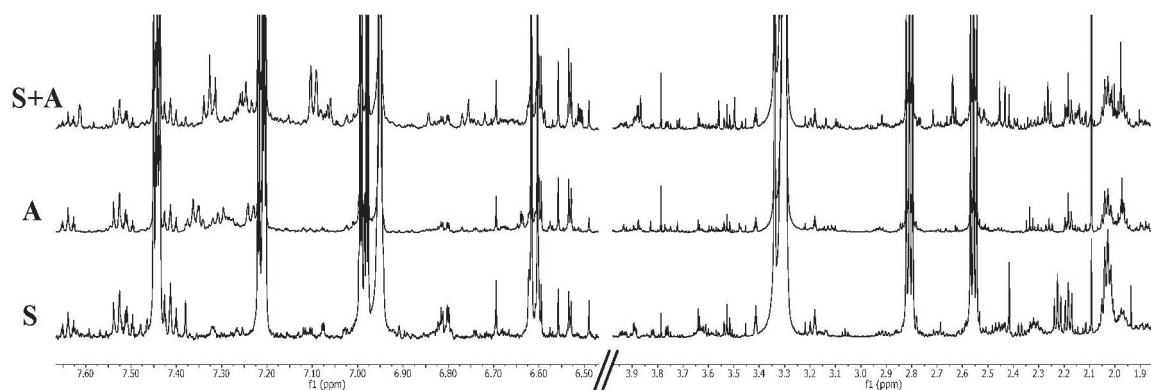


Figure S1. ¹H NMR spectra in the region of δ 6.50—7.60, and 1.90—3.90 of *Streptomyces coelicolor* single culture (S), *Aspergillus niger* single culture (A), and coculture of *Streptomyces coelicolor* with *Aspergillus niger* (S+A). Major discriminators for PCA separation (Figure 2) are summarized in Table 1.

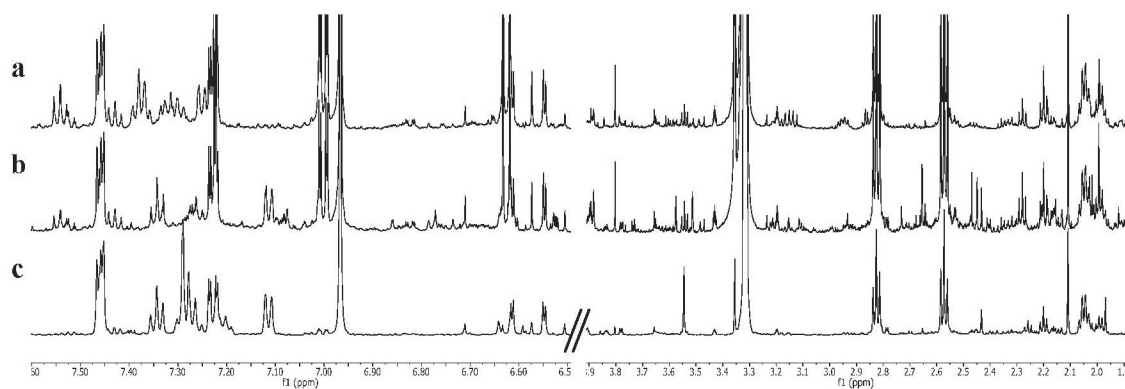


Figure S2. ¹H NMR spectra in the region of δ 6.50—7.60 and 1.90—3.90 of *Aspergillus niger* single culture (a), *Aspergillus niger* cocultured with *Streptomyces coelicolor* (b), and *Aspergillus niger* cultured in a cell-free extract of *Streptomyces coelicolor* (c). Cell-free extract of *S. coelicolor* was sufficient for eliciting *Aspergillus niger* to produce cyclo-(Phe-Phe) and phenylacetic acid

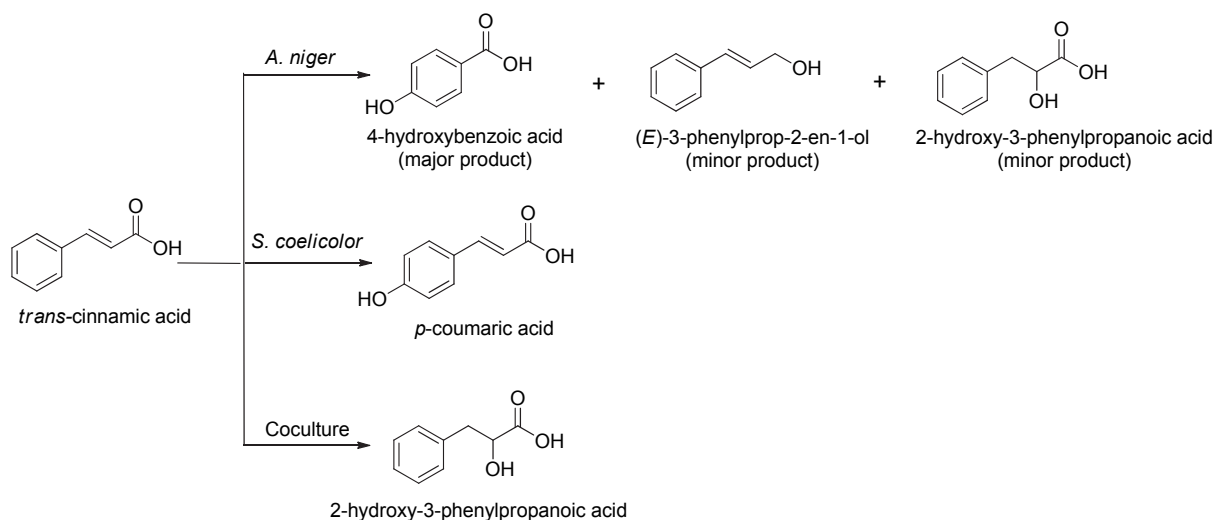


Figure S3. Biotransformation products of *trans*-cinnamic acid by *S. coelicolor* monoculture, *A. niger* monoculture and their coculture.

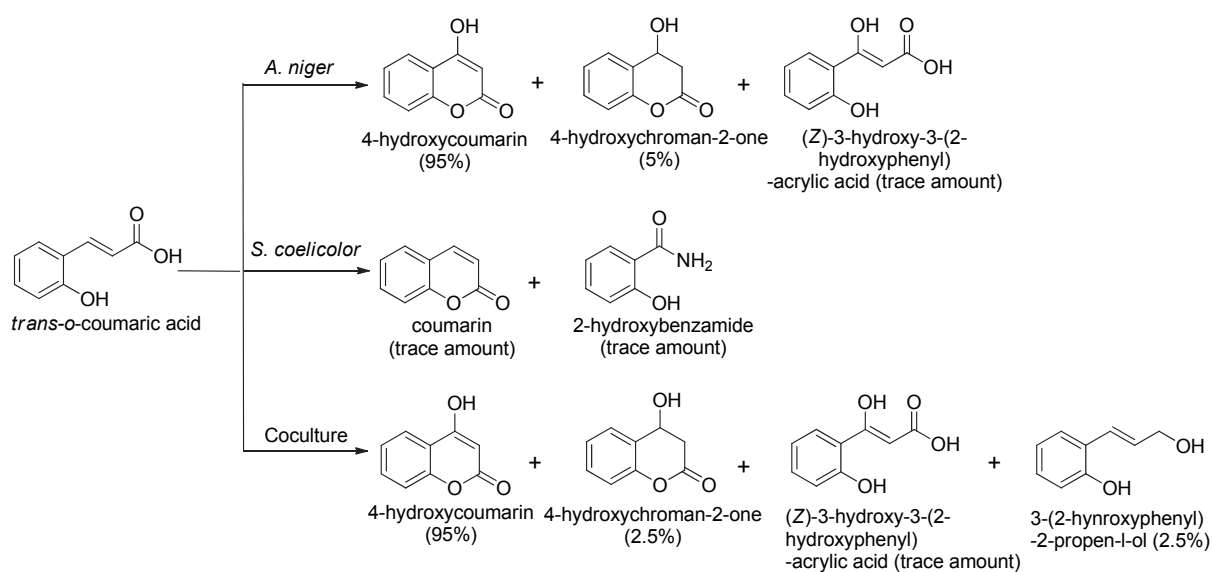


Figure S4. Biotransformation products of *trans*-*o*-coumaric acid by *S. coelicolor* monoculture, *A. niger* monoculture and their coculture.

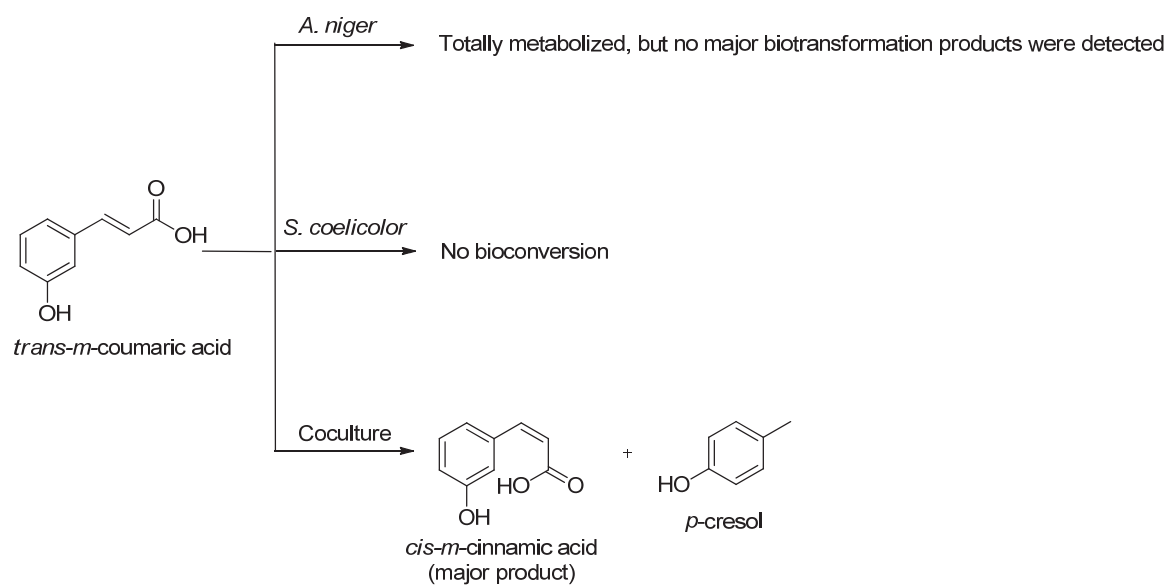


Figure S5. Biotransformation products of *trans-m-coumaric acid* by *S. coelicolor* monoculture, *A. niger* monoculture and their coculture.

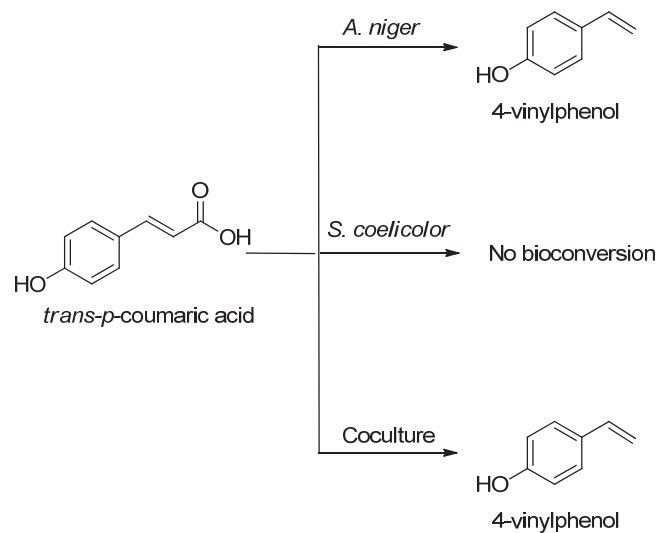


Figure S6. Biotransformation products of *trans-p-coumaric acid* by *S. coelicolor* monoculture, *A. niger* monoculture and their coculture.

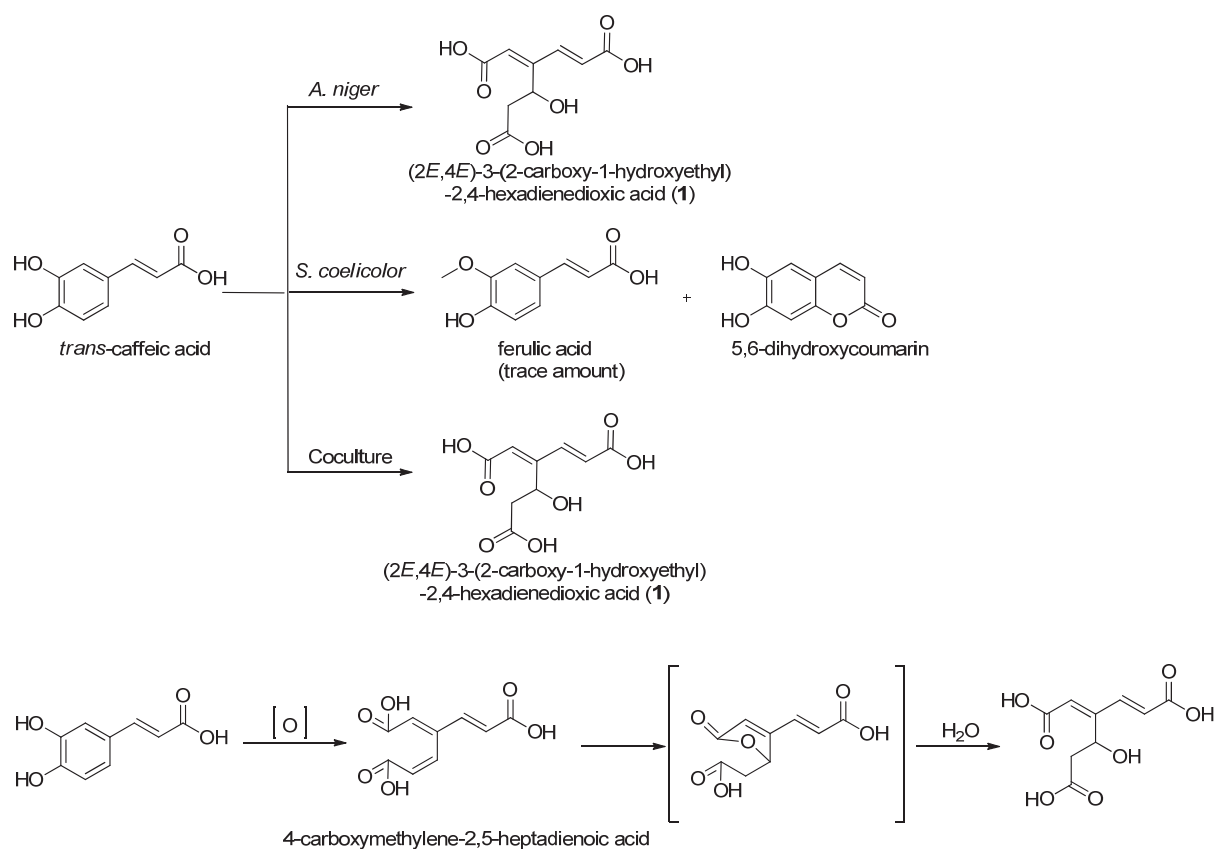


Figure S7. Biotransformation products of *trans*-caffeic acid by *S. coelicolor* monoculture, *A. niger* monoculture, and coculture.

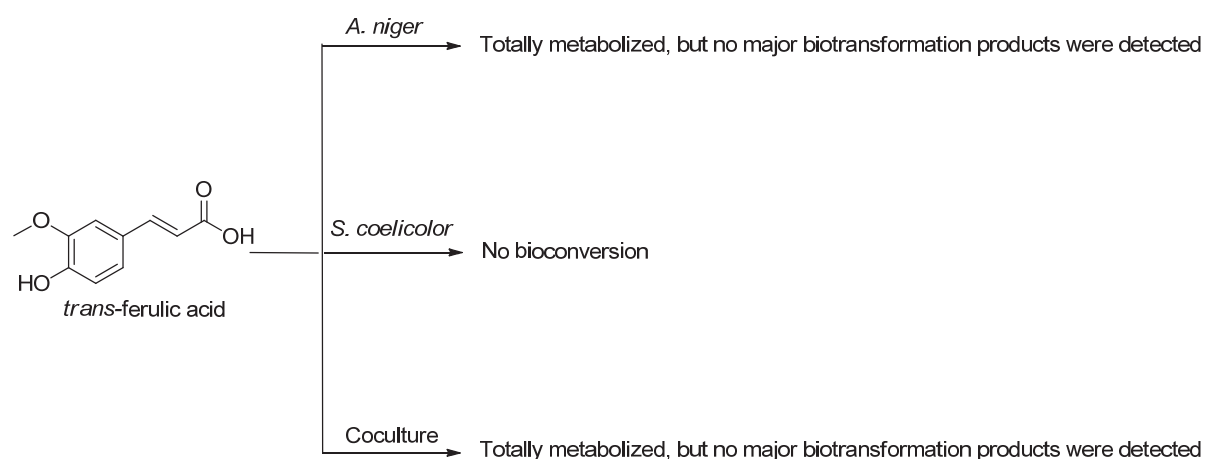


Figure S8. Biotransformation products of *trans*-ferulic acid by *S. coelicolor* monoculture, *A. niger* monoculture, and coculture.

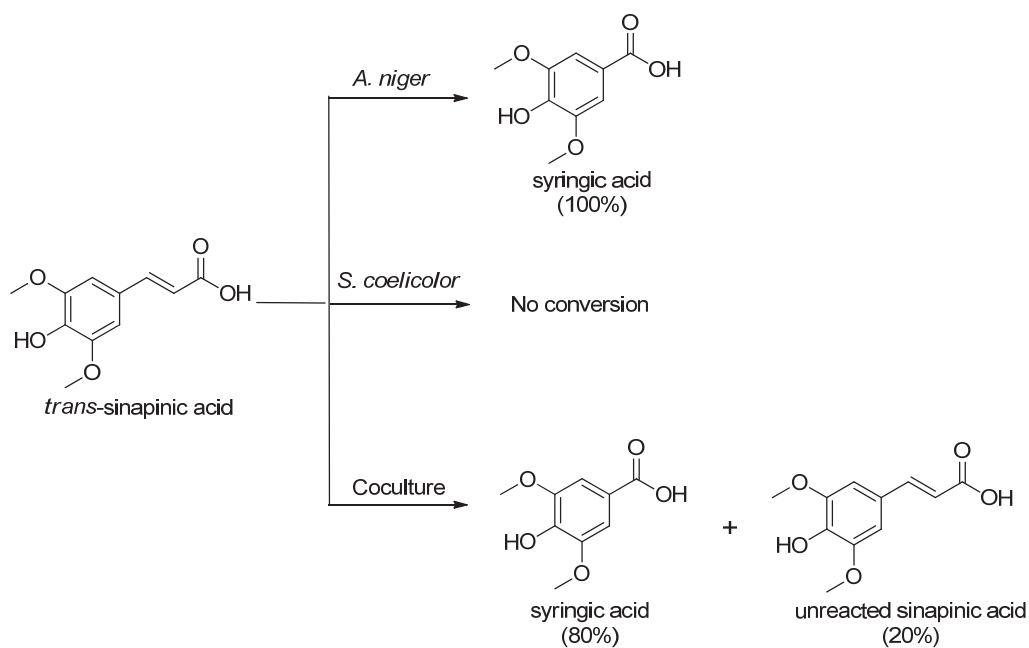


Figure S9. Biotransformation products of *trans*-sinapinic acid by *S. coelicolor* monoculture, *A. niger* monoculture and their coculture.

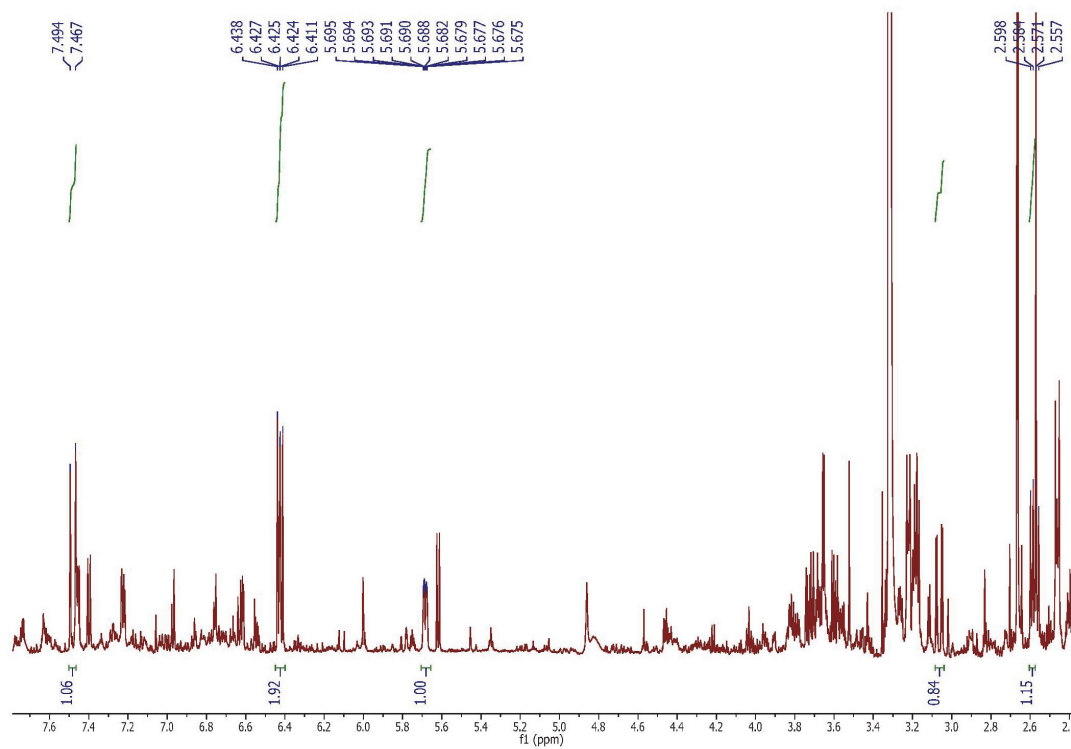


Figure S10. ^1H NMR spectrum of caffeic acid biotransformation by coculture of *S. coelicolor* with *A. niger*. Integrated signals belong to (2*E*,4*E*)-3-(2-carboxy-1-hydroxyethyl)-2,4-hexadienedioic acid (**1**)

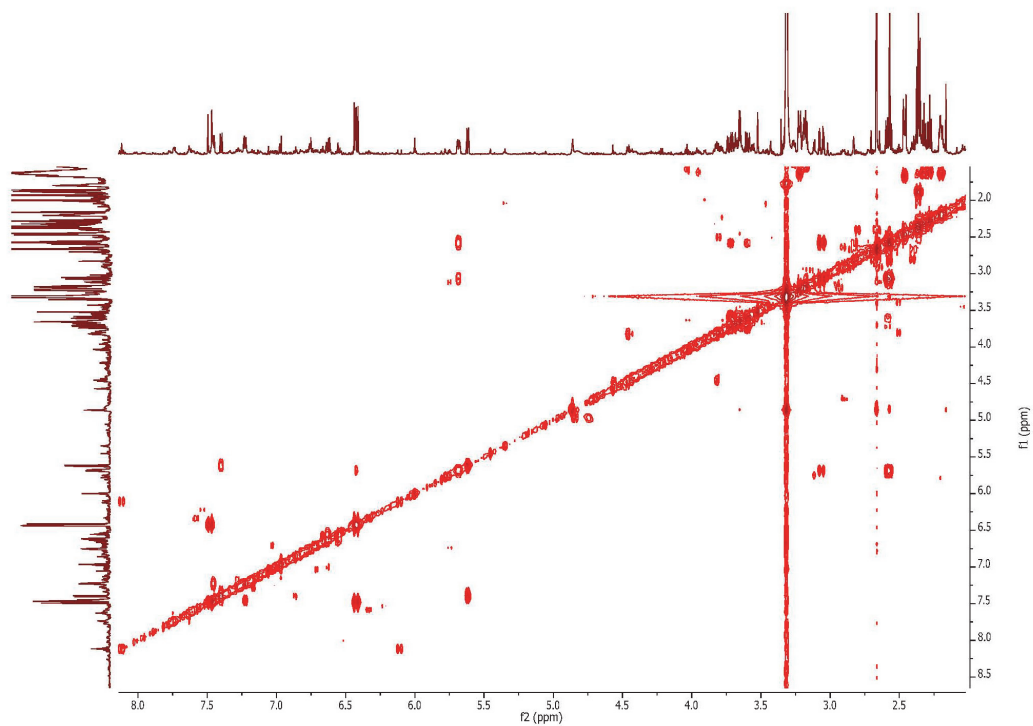


Figure S11. ^1H - ^1H COSY of caffeic acid biotransformation by coculture of *S. coelicolor* with *A. niger*.

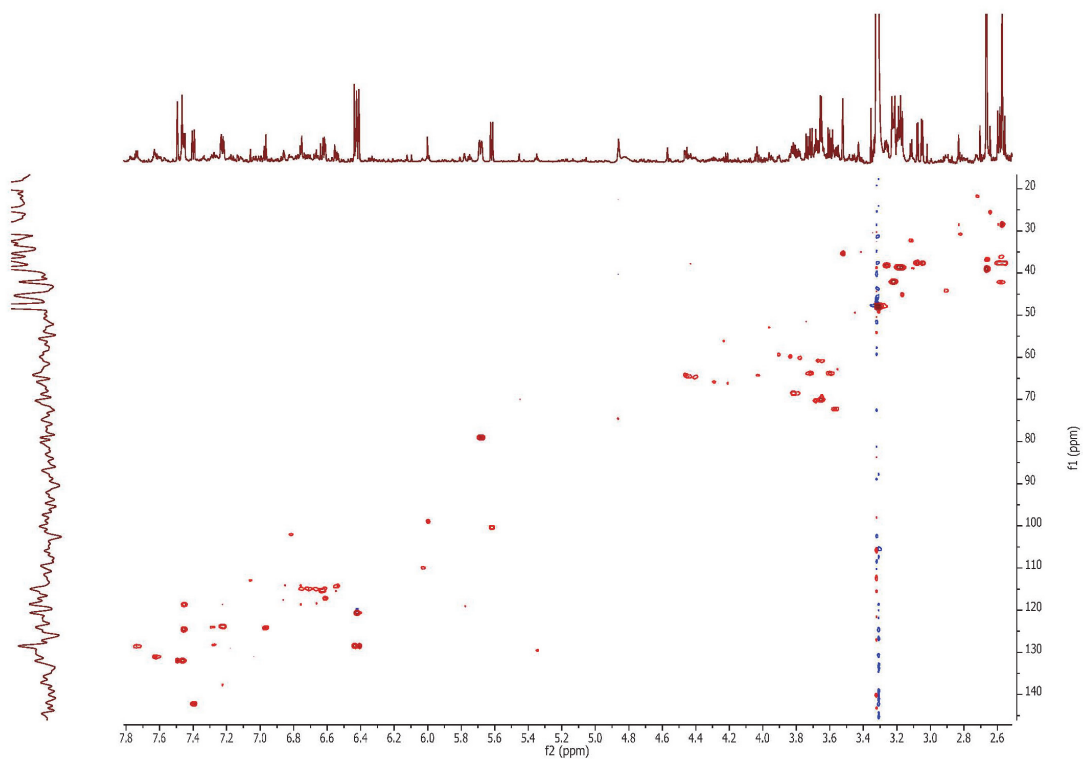


Figure S12. HSQC spectrum of caffeic acid biotransformation by coculture of *S. coelicolor* with *A. niger*.

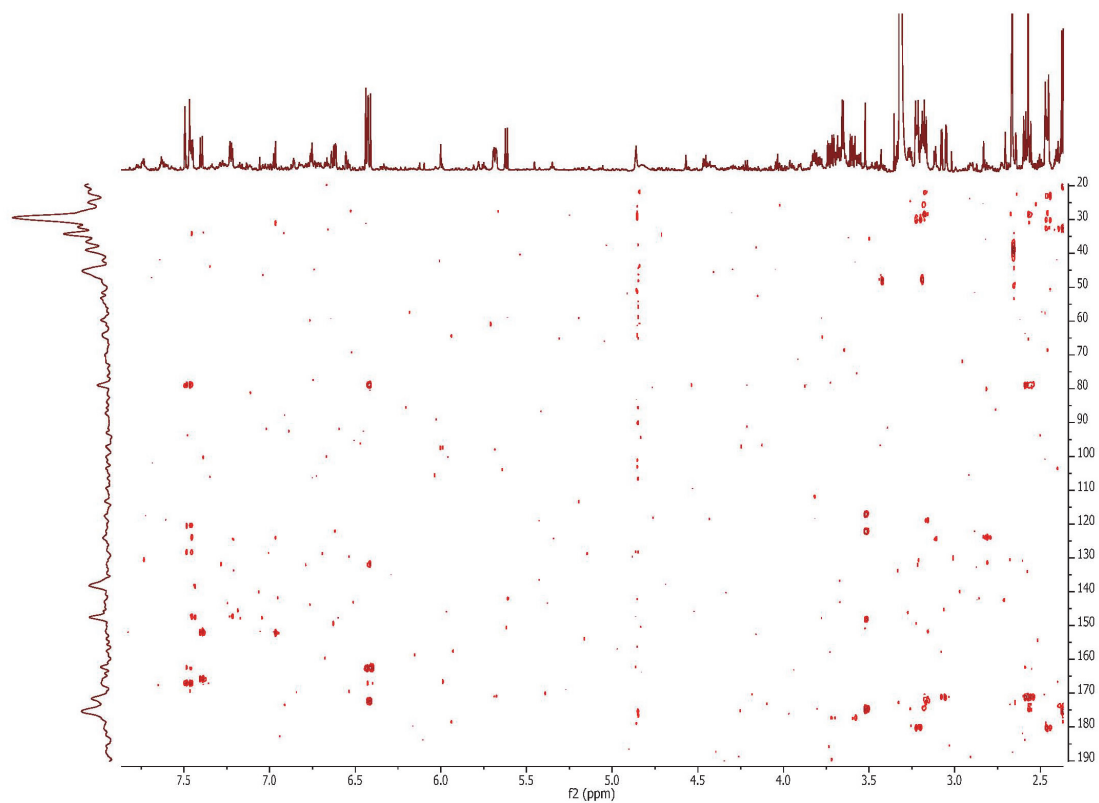


Figure S13. HMBC spectrum of caffeic acid biotransformation by coculture of *S. coelicolor* with *A. niger*.

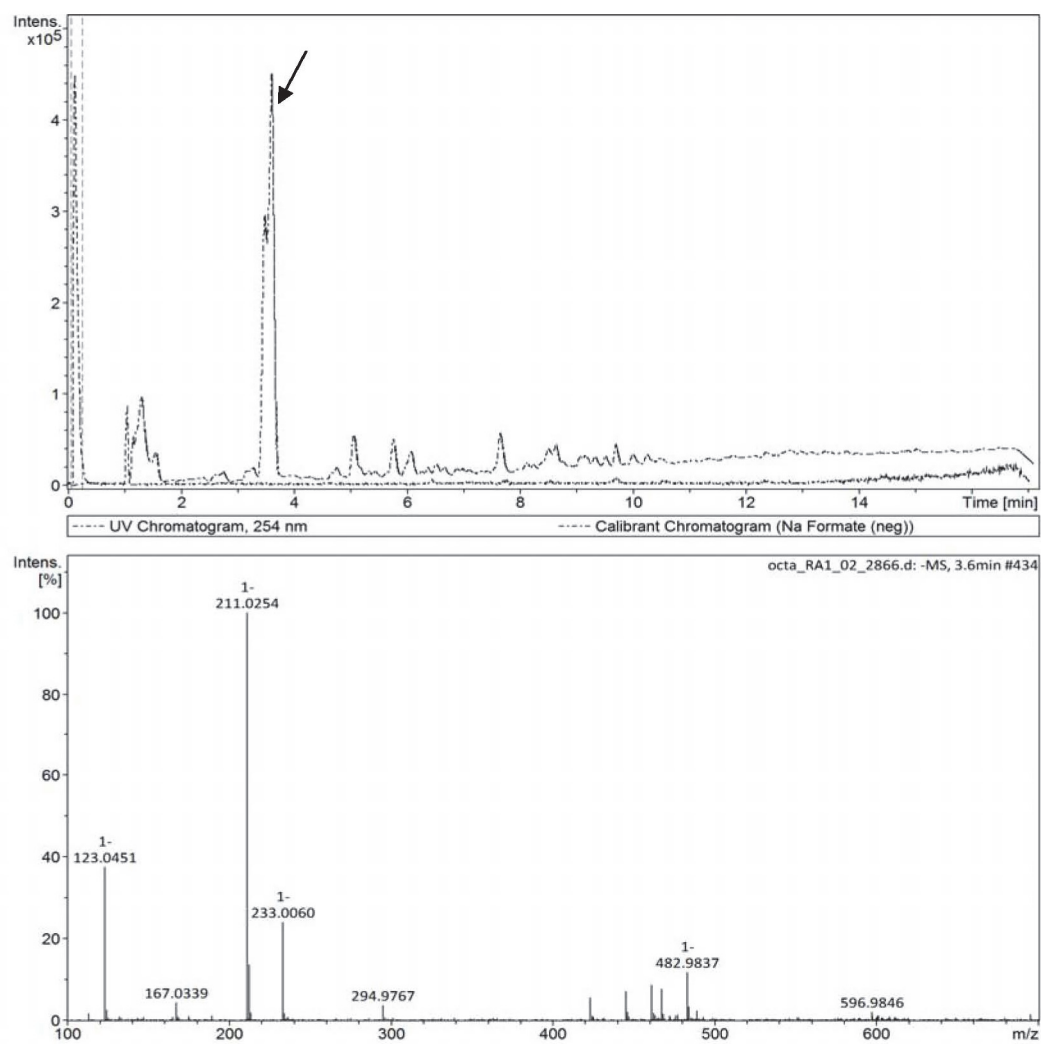


Figure S14. U(H)PLC-Q-TOF analysis of caffeic acid biotransformation by coculture of *S. coelicolor* with *A. niger*. Arrow points at the MS peak at 211.0254.

Table S1. ¹H NMR and HRMS assignment for the biotransformation products of *trans*-cinnamic acid by *S. coelicolor* monoculture, *A. niger* monoculture, and their coculture.

Origin	Compounds	Characteristic ¹ H NMR	UHPLC-Q-TOF-MS
Substrate	<i>trans</i> -cinnamic acid	7.68, d (16.2); 6.48, d (16.2); 7.59, m; 7.41, m	149.0604 [M + H] ⁺ ; 131.0507 [M – H ₂ O + H] ⁺ ; 147.0451 [M – H] ⁺
<i>S. coelicolor</i>	<i>p</i> -coumaric acid	6.28, d (6.2); 6.81, d (8.4)	165.0538 [M + H] ⁺ ; 147.0438 [M – H ₂ O + H] ⁺ ; 163.0397 [M – H ₂ O – H] ⁺
<i>A. niger</i>	4-hydroxybenzoic acid	7.88, d (9.0); 6.82, d (9.0)	139.0390 [M + H] ⁺ ; 121.0280 [M – H ₂ O + H] ⁺ ; 137.0238 [M – H] ⁺
	(<i>E</i>)-3-phenylprop-2-en-1-ol	7.41, m; 7.39, m; 6.37, dt (16.2, 6.0); 4.23, dd (6.0, 1.8)	117.0707 [M – H ₂ O + H] ⁺
	2-hydroxy-3-phenylpropanoic acid	7.27, m; 4.31, dd (7.8, 4.2); 3.10, dd (7.8, 4.2)	165.0553 [M – H] ⁺ ; 147.0461 [M – H ₂ O + H] ⁺
Coculture	2-hydroxy-3-phenylpropanoic acid	7.27, m; 4.31, dd (7.8, 4.2); 3.10, dd (7.8, 4.2)	165.0553 [M – H] ⁺ ; 147.0461 [M – H ₂ O + H] ⁺

Table S2. ¹H NMR and HRMS assignment for the biotransformation products of *trans*-*o*-coumaric acid by *S. coelicolor* monoculture, *A. niger* monoculture and their coculture.

Origin	Compounds	Characteristic ¹ H NMR	UHPLC-Q-TOF-MS
Substrate	<i>trans</i> - <i>o</i> -coumaric acid	7.97, d (16.2); 6.55, d (16.2); 7.48, dd (8.4, 1.8); 7.21, td (8.4, 1.8); 6.84, td (8.4, 1.8); 6.85, dd (8.4, 1.8)	165.0549 [M + H] ⁺ ; 187.0358 [M + Na] ⁺ ; 147.0441 [M – H ₂ O + H] ⁺ ; 163.0408 [M – H] ⁺
<i>S. coelicolor</i>	coumarin	6.43, d (9.6); 7.60, td (8.4, 1.8); 7.29, m	147.0431 [M + H] ⁺
	2-hydroxybenzamide	7.81, dd (8.4, 1.8); 7.23, td (8.4, 1.8); 6.73, dd (8.4, 1.8); 6.57, td (8.4, 1.8)	138.0548 [M + H] ⁺ ; 120.0441 [M – H ₂ O + H] ⁺ ; 136.0405 [M – H] ⁺
	(<i>E</i>)-methyl 3-(2-hydroxyphenyl)acrylate	3.60, s	179.0703 [M + H] ⁺ ; 177.0552 [M – H] ⁺
<i>A. niger</i>	4-dihydroxycoumarin	7.91, dd (7.8, 1.8); 7.64, td (8.4, 1.2); 7.35, td (8.4, 1.2); 7.34, dd (7.8, 1.8); 5.65, s	163.0375 [M + H] ⁺ ; 185.0207 [M + Na] ⁺ ; 161.0274 [M – H] ⁺
	4-hydroxychroman-2-one	7.22, dd (8.4, 2.4); 7.08, td (7.8, 1.2); 6.82, td (7.8, 1.2); 6.76, dd (8.4, 1.2); 5.39, dd (9.6, 3.6); 2.79, dd (15.6, 3.6); 2.59, dd (15.6, 9.6)	165.0547 [M + H] ⁺ ; 147.0439 [M – H ₂ O + H] ⁺ ; 163.0396 [M – H] ⁺
	(<i>Z</i>)-3-hydroxy-3-(2-hydroxyphenyl)acrylic acid	7.96, dd (7.8, 1.8); 7.52, td (8.4, 1.2); 7.27, td (8.4, 1.2); 7.26, dd (7.8, 1.8); 5.90, s	181.0490 [M + H] ⁺ ; 203.0331 [M + Na] ⁺ ; 163.0391 [M – H ₂ O + H] ⁺ ; 179.0346 [M – H] ⁺
	2-allylphenol	6.33, m; 5.15, dt (10.2, 1.8); 5.12, dt (16.8, 1.8)	135.0796 [M + H] ⁺
Coculture	4-dihydroxycoumarin	7.91, dd (7.8, 1.8); 7.64, td (8.4, 1.2); 7.35, td (8.4, 1.2); 7.34, dd (7.8, 1.8); 5.65, s	163.0375 [M + H] ⁺ ; 185.0207 [M + Na] ⁺ ; 161.0274 [M – H] ⁺
	4-hydroxychroman-2-one	7.22, dd (8.4, 2.4); 7.08, td (7.8, 1.2); 6.82, td (7.8, 1.2); 6.76, dd (8.4, 1.2); 5.39, dd (9.6, 3.6); 2.79, dd (15.6, 3.6); 2.59, dd (15.6, 9.6)	165.0547 [M + H] ⁺ ; 147.0439 [M – H ₂ O + H] ⁺ ; 163.0396 [M – H] ⁺
	3-(2-hydroxyphenyl)-2-propen-1-ol	7.36, td (7.8, 1.2); 7.04, td (7.8, 1.8); 6.76, dd (7.8, 1.2); 6.88, dt (16.2, 1.8); 6.36, dt (16.2, 6.0); 4.22, dd (6.0, 1.8)	133.0649 [M – H ₂ O + H] ⁺ ; 149.0615 [M – H] ⁺ ; 131.0502 [M – H ₂ O – H] ⁺
	(<i>Z</i>)-3-hydroxy-3-(2-hydroxyphenyl)acrylic acid	7.96, dd (7.8, 1.8); 7.52, td (8.4, 1.2); 7.27, td (8.4, 1.2); 7.26, dd (7.8, 1.8); 5.90, s	181.0490 [M + H] ⁺ ; 203.0331 [M + Na] ⁺ ; 163.0391 [M – H ₂ O + H] ⁺ ; 179.0346 [M – H] ⁺
	2-allylphenol	6.33, m; 5.15, dt (10.2, 1.8); 5.12, dt (16.8, 1.8);	135.0796 [M + H] ⁺

Table S3. ^1H NMR and HRMS assignment for the biotransformation products of *trans-m*-coumaric acid by *S. coelicolor* monoculture, *A. niger* monoculture and their coculture.

Origin	Compounds	Characteristic ^1H NMR	UHPLC-Q-TOF-MS
Substrate	<i>trans-m</i> -coumaric acid	7.59, d (16.2); 6.41, d (16.2); 7.22, t (7.8); 7.05, brd (7.2); 7.0, t (1.8); 6.84, ddd (8.4, 2.4, 1.2)	165.0547 $[\text{M} + \text{H}]^+$; 187.0361 $[\text{M} + \text{Na}]^+$; 147.0442 $[\text{M} - \text{H}_2\text{O} + \text{H}]^+$; 163.0417 $[\text{M} - \text{H}]^-$
<i>S. coelicolor</i>	No products		
<i>A. niger</i>	No major biotransformation products		
Coculture	<i>cis-m</i> -coumaric acid	6.87, d (12.6); 5.93, d (12.6); 7.15, t (7.8); 7.06, t (1.8); 7.01, ddd (8.4, 2.4, 1.2)	147.0432 $[\text{M} - \text{H}_2\text{O} + \text{H}]^+$; 163.0405 $[\text{M} - \text{H}]^-$
	<i>p</i> -cresol	6.73, d (8.4); 7.09, d (8.4)	107.0501 $[\text{M} - \text{H}]^-$

Table S4. ^1H NMR and HRMS assignment for the biotransformation products of *trans-p*-coumaric acid by *S. coelicolor* monoculture, *A. niger* monoculture and their coculture.

Origin	Compounds	Characteristic ^1H NMR	UHPLC-Q-TOF-MS
Substrate	<i>trans-p</i> -coumaric acid	7.61, d (16.2); 6.29, d (16.2); 7.45, d (8.4); 6.81, d (8.4)	165.0548 $[\text{M} + \text{H}]^+$; 187.0361 $[\text{M} + \text{Na}]^+$; 147.0446 $[\text{M} - \text{H}_2\text{O} + \text{H}]^+$; 163.0417 $[\text{M} - \text{H}]^-$
<i>S. coelicolor</i>	No products		
<i>A. niger</i>	4-vinylphenol	6.63, dd (17.4, 10.8); 5.56, dd (17.4, 1.2); 5.03, dd (10.8, 1.2); 6.73, d (8.4); 7.26, d (8.4)	121.0649 $[\text{M} + \text{H}]^+$; 119.0497 $[\text{M} - \text{H}]^-$
Coculture	4-vinylphenol	6.63, dd (17.4, 10.8); 5.56, dd (17.4, 1.2); 5.03, dd (10.8, 1.2); 6.73, d (8.4); 7.26, d (8.4)	121.0649 $[\text{M} + \text{H}]^+$; 119.0497 $[\text{M} - \text{H}]^-$

Table S5. ^1H NMR and HRMS assignment for the biotransformation products of *trans*-caffeic acid by *S. coelicolor* monoculture, *A. niger* monoculture and their coculture.

Origin	Compounds	Characteristic ^1H NMR	UHPLC-Q-TOF-MS
Substrate	<i>trans</i> -caffeic acid	7.53, d (16.2); 6.22, d (16.2); 7.04, d (1.8); 6.94, dd (8.4, 1.8); 6.79, d (8.4)	181.0492 $[\text{M} + \text{H}]^+$; 203.0312 $[\text{M} + \text{Na}]^+$; 163.0393 $[\text{M} - \text{H}_2\text{O} + \text{H}]^+$; 179.0374 $[\text{M} - \text{H}]^-$
<i>S. coelicolor</i>	<i>trans</i> -ferulic acid	7.60, d (16.2); 6.32, d (16.2); 7.18, d (1.8); 7.07, dd (8.4, 1.8); 6.82, d (8.4); 3.90, s	195.0657 $[\text{M} + \text{H}]^+$; 177.0540 $[\text{M} - \text{H}_2\text{O} + \text{H}]^+$; 193.0514 $[\text{M} - \text{H}]^-$
	5,6-dihydroxycoumarin	7.79, d (9.6); 6.19, d (9.6); 6.76, s	179.0335 $[\text{M} + \text{H}]^+$; 177.0200 $[\text{M} - \text{H}]^-$
<i>A. niger</i>	(2 <i>E</i> ,4 <i>E</i>)-3-(2-carboxy-1-hydroxyethyl)-2,4-hexadienedioic acid (1)	see Table 2	211.0254 $[\text{M} - \text{H}_2\text{O} - \text{H}]^-$
Coculture	(2 <i>E</i> ,4 <i>E</i>)-3-(2-carboxy-1-hydroxyethyl)-2,4-hexadienedioic acid (1)	see Table 2	211.0254 $[\text{M} - \text{H}_2\text{O} - \text{H}]^-$

Table S6. ^1H NMR and HRMS assignment for the biotransformation products of *trans*-ferulic acid by *S. coelicolor* monoculture, *A. niger* monoculture and their coculture.

Origin	Compounds	Characteristic ^1H NMR	UHPLC-Q-TOF-MS
Substrate	<i>trans</i> -ferulic acid	7.60, d (16.2); 6.32, d (16.2); 7.18, d (2.4); 7.07, dd (8.4, 2.4); 6.82, d (8.4); 3.90, s	195.0661 $[\text{M} + \text{H}]^+$; 217.0447 $[\text{M} + \text{Na}]^+$; 177.0563 $[\text{M} - \text{H}_2\text{O} + \text{H}]^+$; 193.0511 $[\text{M} - \text{H}]^-$
<i>S. coelicolor</i>	No products		
<i>A. niger</i>	No products		
Coculture	No products		

Table S7. ^1H NMR and HRMS assignment for the biotransformation products of *trans*-sinapinic acid by *S. coelicolor* monoculture, *A. niger* monoculture and their coculture.

Origin	Compounds	Characteristic ^1H NMR	UHPLC-Q-TOF-MS
Substrate	<i>trans</i> -sinapinic acid	7.60, d (16.2); 6.34, d (16.2); 6.90, s	225.0779 $[\text{M} + \text{H}]^+$; 247.0590 $[\text{M} + \text{Na}]^+$; 207.0683 $[\text{M} - \text{H}_2\text{O} + \text{H}]^+$; 223.0633 $[\text{M} - \text{H}]^-$
<i>S. coelicolor</i>	No conversion		
<i>A. coelicolor</i>	4-hydroxy-3,5-dimethoxybenzoic acid	7.33, s; 3.89, s	199.0608 $[\text{M} + \text{H}]^+$; 221.0423 $[\text{M} + \text{Na}]^+$; 181.0493 $[\text{M} - \text{H}_2\text{O} + \text{H}]^+$; 197.0468 $[\text{M} - \text{H}]^-$
Coculture	<i>trans</i> -sinapinic acid	7.60, d (16.2); 6.34, d (16.2); 6.90, s	225.0779 $[\text{M} + \text{H}]^+$; 247.0590 $[\text{M} + \text{Na}]^+$; 207.0683 $[\text{M} - \text{H}_2\text{O} + \text{H}]^+$; 223.0633 $[\text{M} - \text{H}]^-$
	4-hydroxy-3,5-dimethoxybenzoic acid	7.33, s; 3.89, s	199.0608 $[\text{M} + \text{H}]^+$; 221.0423 $[\text{M} + \text{Na}]^+$; 181.0493 $[\text{M} - \text{H}_2\text{O} + \text{H}]^+$; 197.0468 $[\text{M} - \text{H}]^-$

Appendix III: Supplementary Information belonging to Chapter 6

Table S1. Gene organization of type I iterative non-reducing PKS gene cluster (*icm*) for isocoumarins biosynthesis in *Streptomyces* sp. MBT76.

ORF	Product	Accession in GeneBank	Length	Putative function	Homologue in <i>Streptomyces roseovirentillatus</i>	Homology
1	<i>icmA</i>	WP_058043254.1	943	LuxR family transcriptional regulator	WP_030365549.1	84%
2	<i>icmB</i>	WP_058043255.1	224	TetR family transcriptional regulator	WP_043188202.1	92%
3	<i>icmC</i>	WP_058043256.1	496	MFS transporter	WP_043188355.1	84%
4	<i>icmD</i>	WP_058043257.1	200	NADPH-dependent FMN reductase	WP_030365552.1	88%
5	<i>icmE</i>	WP_058043258.1	246	ToxA protein	WP_030365553.1	87%
6	<i>icmF</i>	WP_058043259.1	416	oxidoreductase	WP_052392495.1	71%
7	<i>icmG</i>	WP_058043260.1	376	diaminohydroxyphosphoribosylaminopyrimidine deaminase/5-amino-6-(5-phosphoribosylamino)uracil reductase	WP_030365554.1	88%
8	<i>icmH</i>	WP_058043261.1	330	Serine/threonine kinase	WP_030365555.1	90%
9	<i>icmI</i>	WP_058043262.1	251	methyltransferase type 12	WP_043188204.1	81%
10	<i>icmJ</i>	WP_058043263.1	570	WD repeat-containing protein	WP_030365558.1	95%
11	<i>icmK</i>	WP_058043264.1	208	GTP cyclohydrolase II	WP_043188207.1	98%
12	<i>icmL</i>	WP_058043265.1	130	Hypothetical protein	WP_030365560.1	88%
13	<i>icmM</i>	WP_058043266.1	1239	iterative type I polyketide synthase	WP_030365561.1	91%
14	<i>icmN</i>	WP_058043267.1	397	AMP-dependent synthetase/ligase	WP_030365562.1	95%
15	<i>icmO</i>	WP_058043268.1	434	MFS transporter	WP_053688342.1	81%
16	<i>icmP</i>	WP_058043269.1	381	hypothetical protein	WP_030365564.1	93%
17	<i>icmQ</i>	WP_058043270.1	388	acyl-protein synthase	WP_030365565.1	96%
18	<i>icmR</i>	WP_058043271.1	834	hypothetical protein	WP_030365566.1	94%
19	<i>icmS</i>	WP_058043272.1	303	phosphotriesterase	WP_052392497.1	88%
20	<i>icmT</i>	WP_058043273.1	188	Zn-ribbon-like motif containing protein	WP_030365568.1	88%

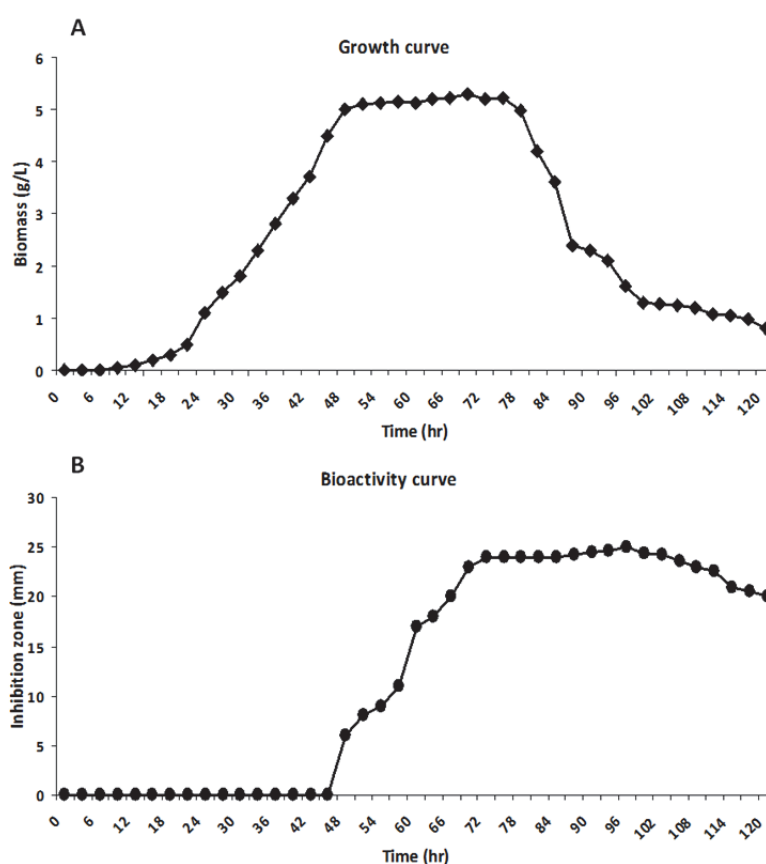


Figure S1. Growth curve of *Streptomyces* sp. MBT76. (A) and corresponding antimicrobial activity against *Bacillus subtilis* (B). Some 1.5 ml culture was collected every two hours during the daytime from the first to the fifth inoculation day. Separated supernatant from the pellet by centrifuging and kept them independently. The pellet was heated at 70 °C for overnight to determine the dry weight, while the supernatant was accessed for antimicrobial activity against *Bacillus subtilis* 168. The data were means of three replicates.

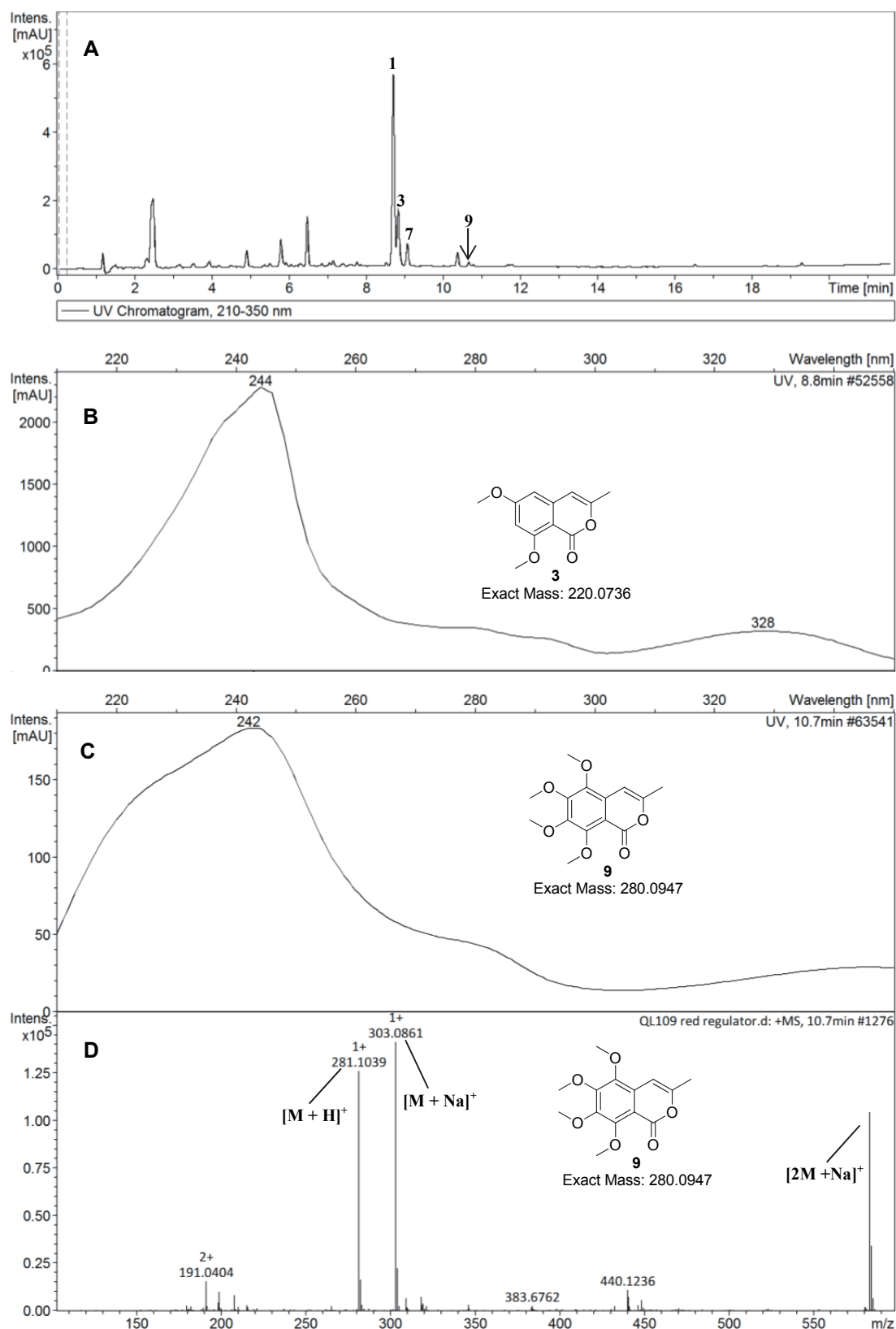


Figure S2. U(H)PLC-UV-ToF-HRMS analysis of *Streptomyces* sp. MBT76 crude extract. A) UV chromatogram of *Streptomyces* sp. MBT76 crude extract, whereby the isocoumarins 1, 3, 7, 9 were labelled; B) UV spectrum of 6,8-dimethoxy-3-methyl-isocoumarin (3); C) UV spectrum of 5,6,7,8-tetramethoxy-3-methyl-isocoumarin (9); D) HRESIMS spectrum of 5,6,7,8-tetramethoxy-3-methyl-isocoumarin (9); Electrospray (ESI) detection was achieved in positive ion mode.

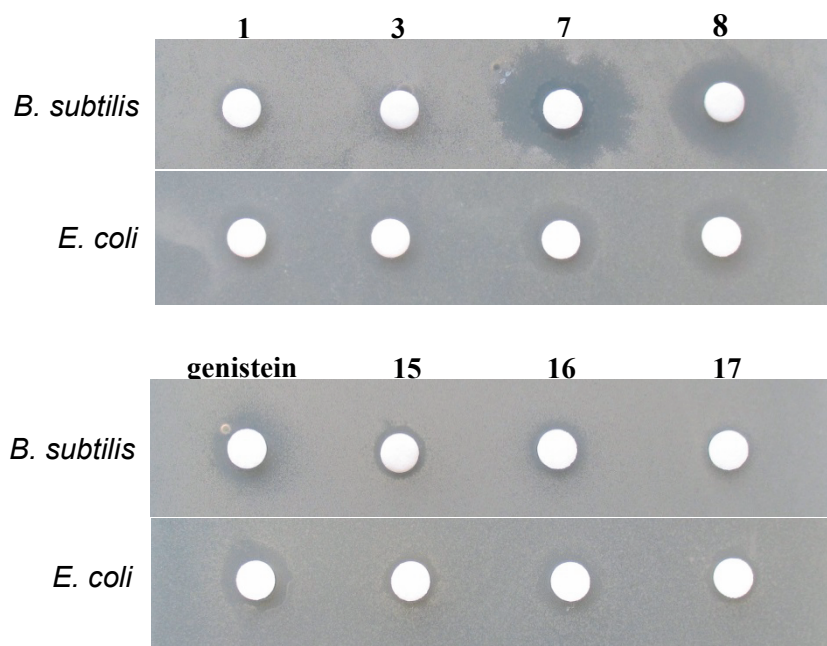


Figure S3. Antimicrobial activity of selected isocoumarins and isoflavones. The methoxylation enhanced the antimicrobial potency of isocoumarins against both Gram-positive *Bacillus subtilis* 168 and Gram-negative *Escherichia coli* K12, which in turn validated the metabolomics OPLS analysis in Figure 4. On the contrary, methylation decreased the antimicrobial activity of isoflavones.

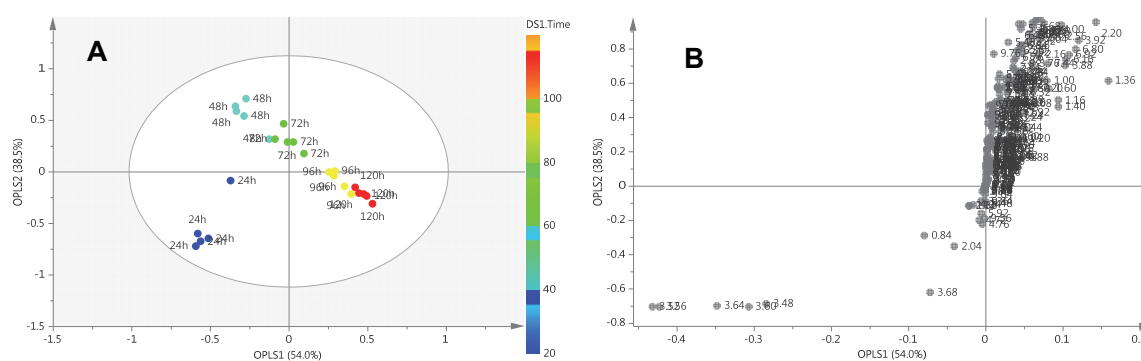


Figure S4. OPLS analysis of the NMR spectra by using time as the Y-variable. (A) score plot, and (B) the corresponding loading S-plot.

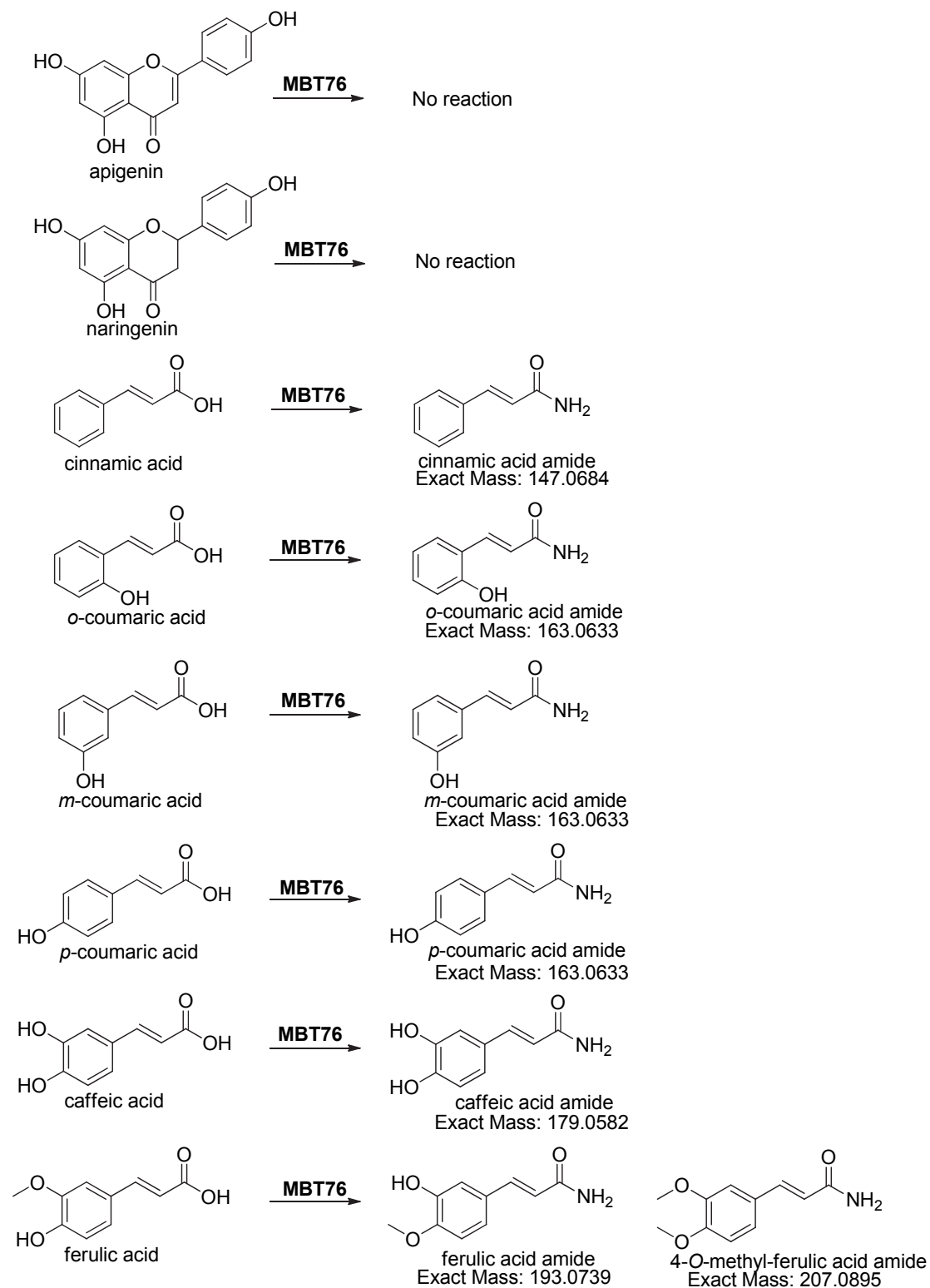
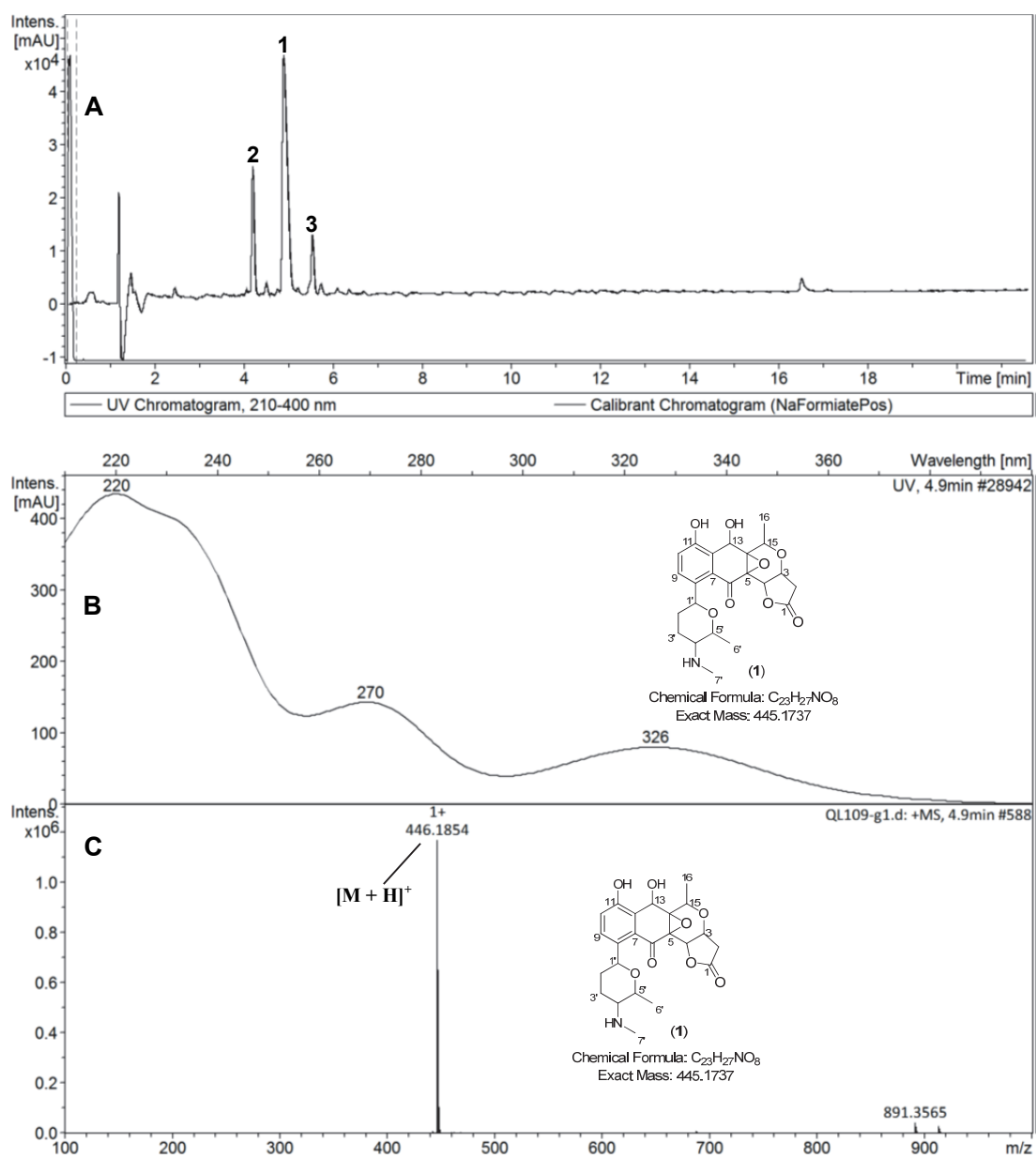


Figure S5. Biotransformation study in *Streptomyces* sp. MBT76 by using apigenin, naringenin, and (hydroxyl)cinnamic acids as substrates. Products were identified on the basis of ^1H NMR and/or UHPLC-TOF-MS high resolution mass.

Appendix IV: Supplementary Information belonging to Chapter 7

Table S1. Spectral data assignments for the compounds **4**—**15** shown in Figure 2. Compound identification was based on ^1H NMR, high resolution mass spectrometry, and UV absorption spectra. Proton coupling constants (J in Hz) are given in parentheses.

NO.	Name	Chemical shifts (ppm)	HRMS (Da)	UV (λ_{max} nm)	Ref
4	BE-54238A	7.90, d (8.4); 6.47, d (8.4); 5.26, m; 4.51, m; 4.31, m; 3.32, m; 3.18, dd (10.8, 9.0); 2.96, dd (16.2, 10.8); 2.77, dd (8.4, 4.2); 2.73, dd (15.6, 4.2); 2.62, dd (15.6, 8.4); 1.63, d (6.6); 1.32, d (6.6)	398.1630 $[\text{M} + \text{H}]^+$	276, 414	¹
5	BE-54238B	8.01, d (9.0); 6.56, d (9.0)	396.1459 $[\text{M} + \text{H}]^+$	278, 423	¹
6	kalafungfin		301.0710 $[\text{M} + \text{H}]^+$	216, 256, 425	²
7	dihydrokalafungfin		303.0856 $[\text{M} + \text{H}]^+$	222, 278	³
8	11- <i>O</i> -methyl-aloesaponarin II	7.80, dd (7.8, 1.2); 7.70, t (7.8); 7.50, brd (8.4); 7.43, d (1.8); 7.43, d (1.8); 6.99, d (1.8); 3.98, s; 2.71, s	269.0825 $[\text{M} + \text{H}]^+$; 291.0638 $[\text{M} + \text{Na}]^+$	218, 274, 390	⁴
9	3,8-dihydroxy-1-methyl-anthraquinone-carboxylic acid	7.70, d (7.8); 7.64, t (7.8); 7.27, d (7.8); 7.60, s	299.0555 $[\text{M} + \text{H}]^+$	222, 280, 410	⁴
10	GTTRI-02	6.62, s; 4.24, m; 3.15, dd (16.2, 3.6); 2.91, dd (16.2, 7.2); 2.85, dd (16.2, 3.6); 2.60, dd (16.2, 7.2); 2.45, s; 2.42, s	235.0956 $[\text{M} + \text{H}]^+$; 257.0776 $[\text{M} + \text{Na}]^+$; 217.0850 $[\text{M} - \text{H}_2\text{O} + \text{H}]^+$	216, 278	⁵
11	2-acetyl-3-hydroxy-furan	7.92, d (6.0); 6.38, d (6.0); 2.34, s	127.0428 $[\text{M} + \text{H}]^+$	214, 276	⁶
12	2-acetyl-3-methoxy-furan	7.95, d (6.0); 6.39, d (6.0); 3.78, s; 2.35, s	141.0599 $[\text{M} + \text{H}]^+$; 163.0416 $[\text{M} + \text{Na}]^+$	212, 260	⁶
13	6,8-dihydroxy-3-methyl-isocoumarin	6.29, d (1.2); 6.24, d (2.4); 6.27, d (2.4); 2.21, d (1.2)	193.0511 $[\text{M} + \text{H}]^+$; 215.0312 $[\text{M} + \text{Na}]^+$	244, 328	⁷
14	phenylacetamide	7.29, m; 7.23, m; 3.50, s	136.0759 $[\text{M} + \text{H}]^+$	218, 260	⁸



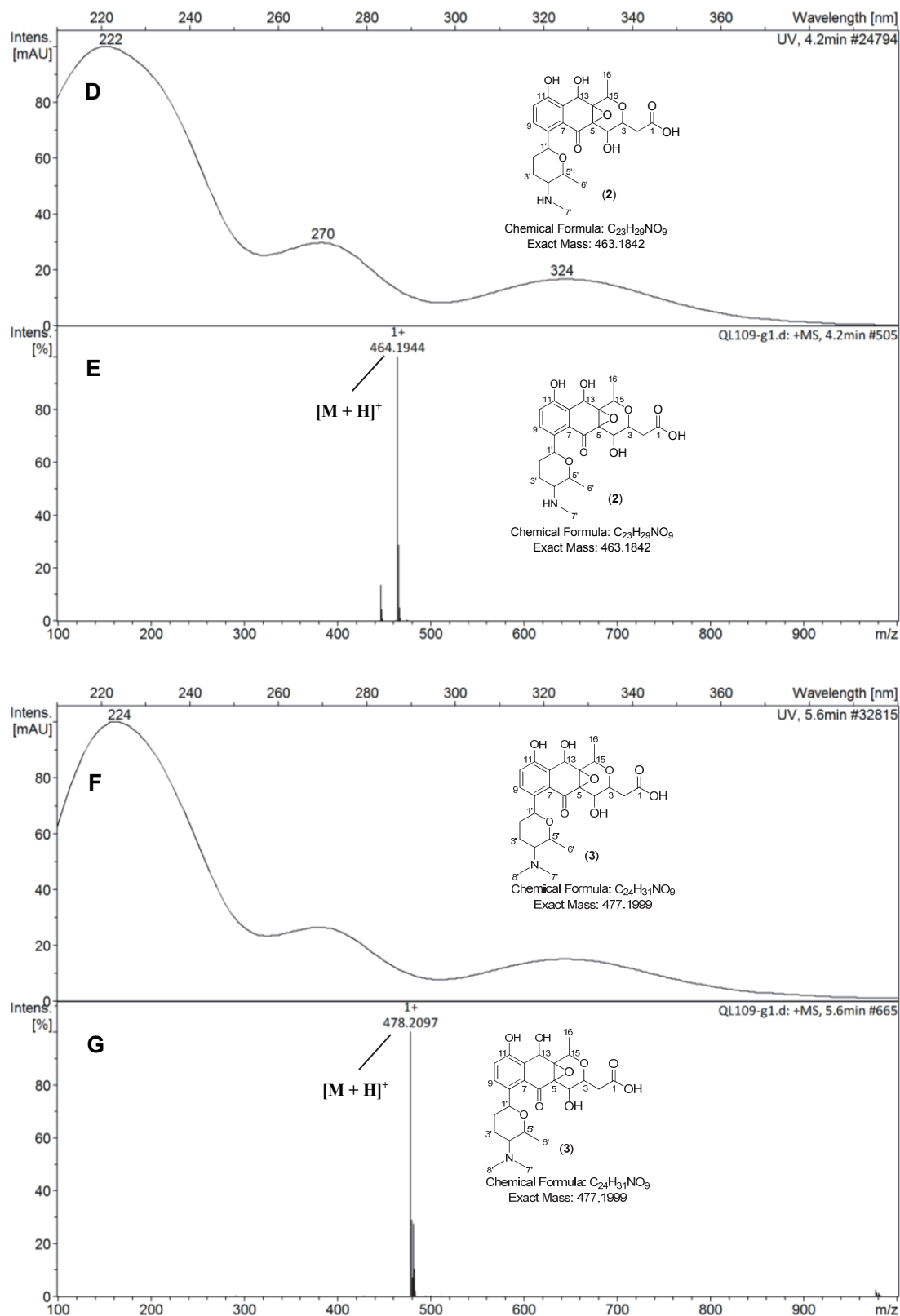


Figure S1. U(H)PLC-UV-TOF-HRMS analysis of Fr1. A) UV chromatogram (detected at 210 nm) of Fr1 presented three major compounds 1–3 with quantity ratio around 6:2:1; B) UV spectrum of compound 1; C) HRESIMS spectrum of compound 1; D) UV spectrum of compound 2; E) HRESIMS spectrum of compound 2; F) UV spectrum of compound 3; G) HRESIMS spectrum of compound 3. Electrospray (ESI) detection was achieved in positive ion mode. The respective inserts are structures of compounds 1–3 with chemical formula and theoretical mass calculated by software ChemDraw Ultra 12.0.

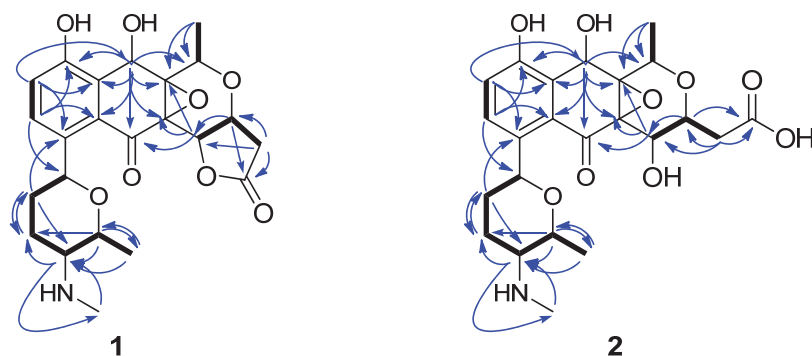
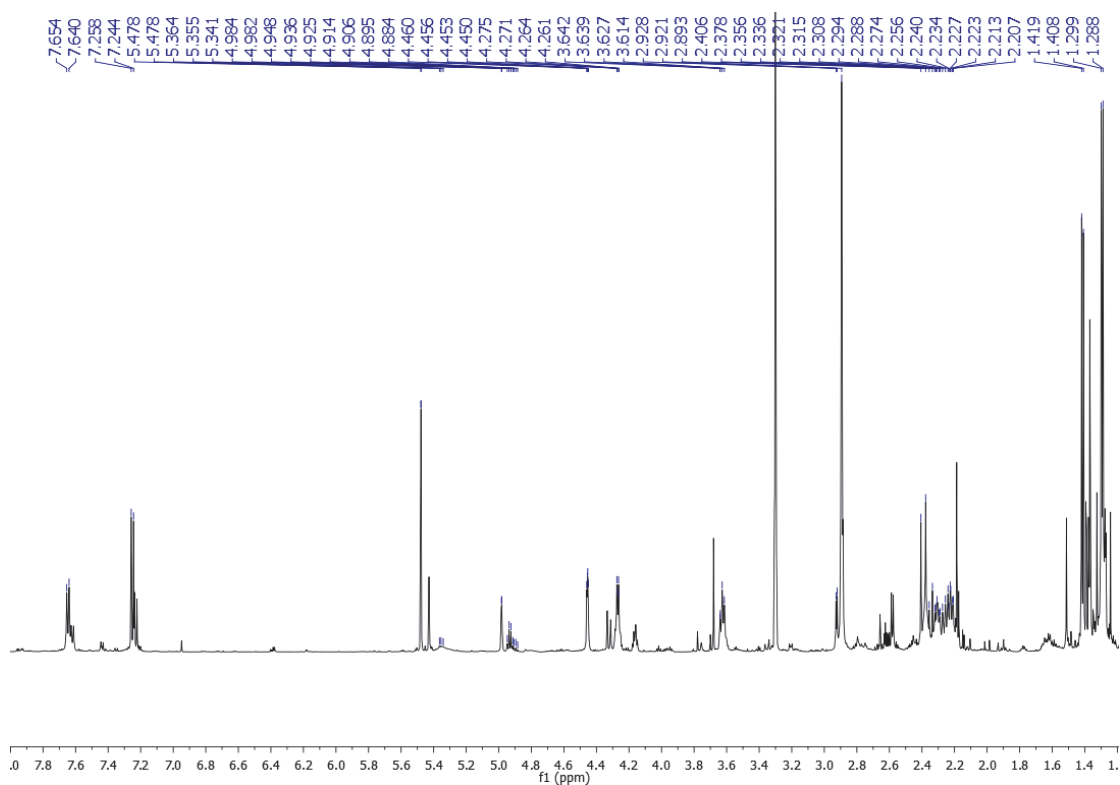


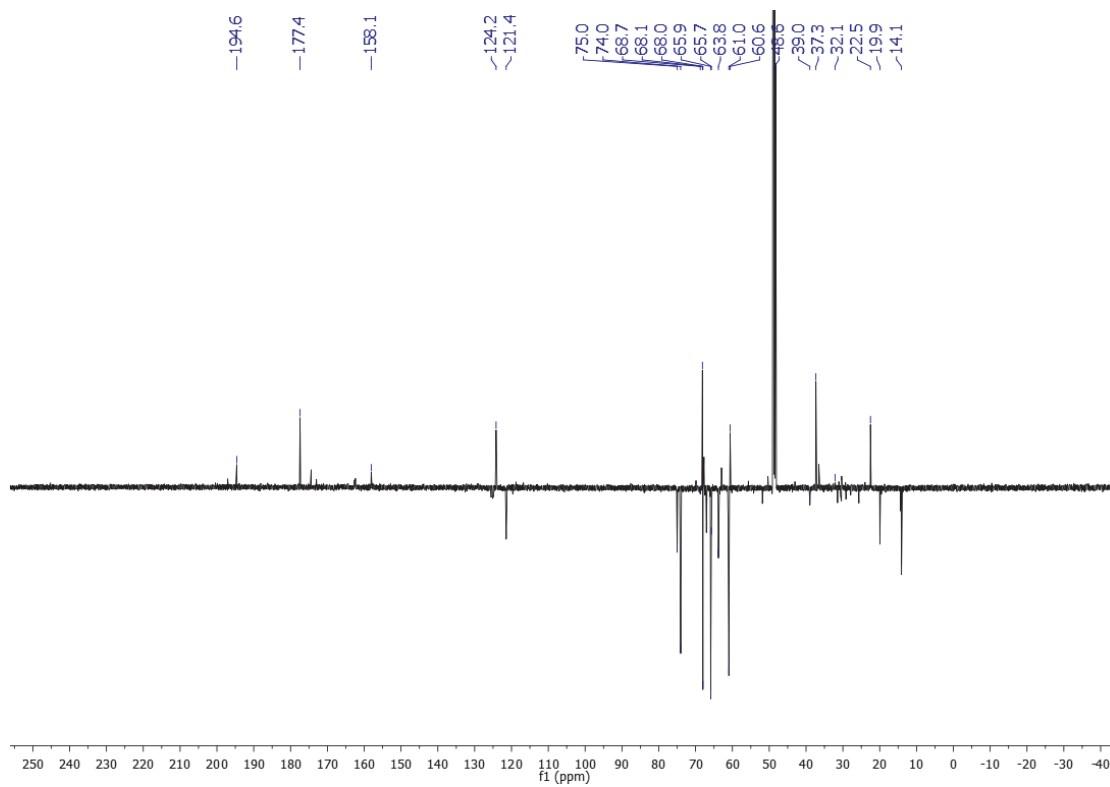
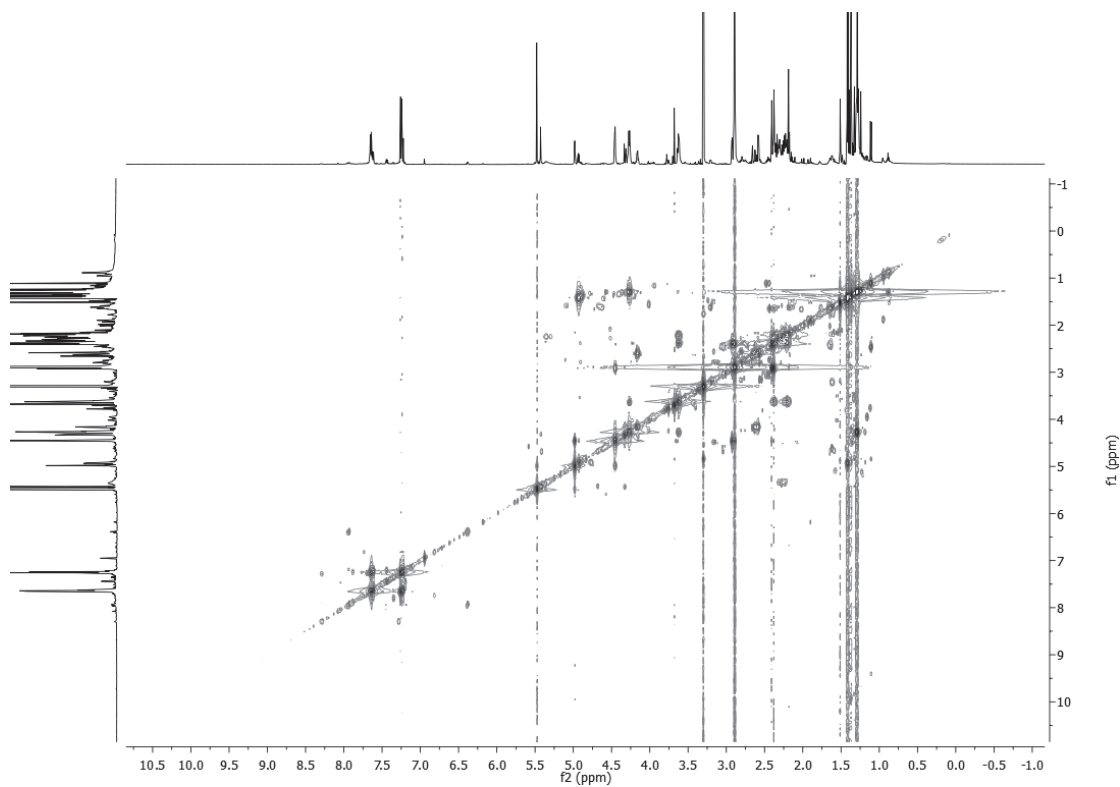
Figure S2. Observed HMBC (↷) and COSY (—) correlations of compounds 1 and 2.

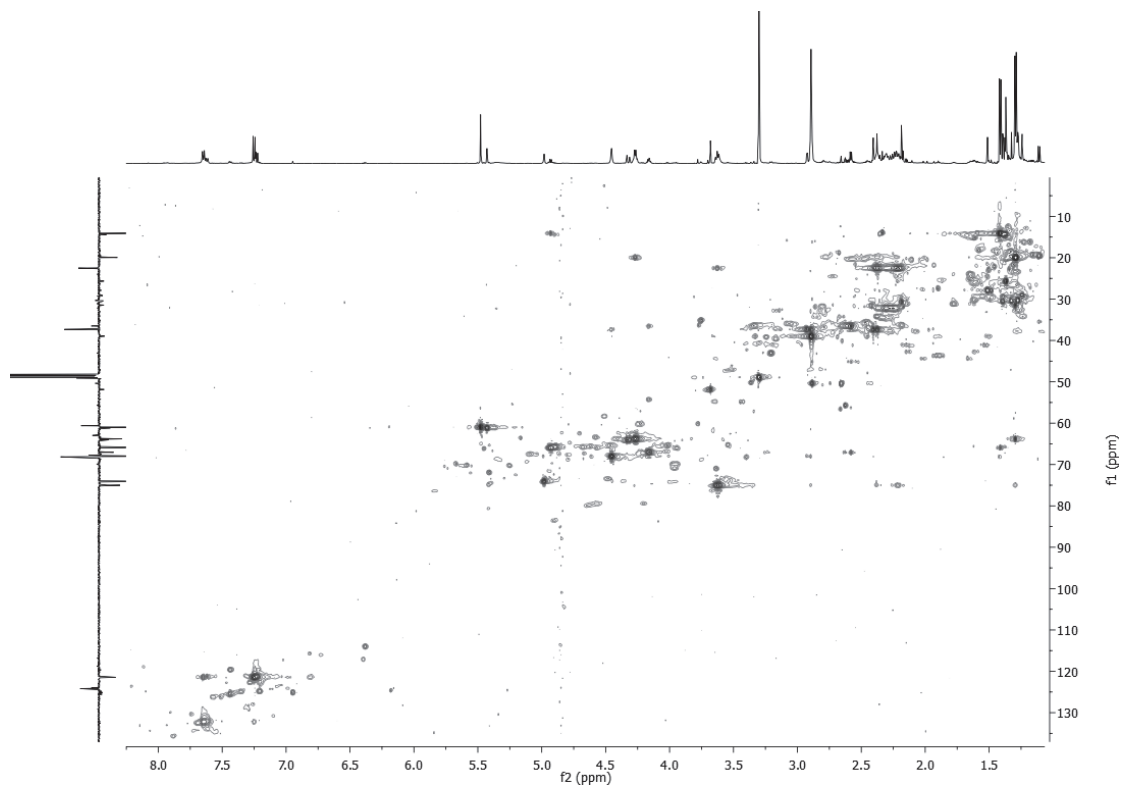
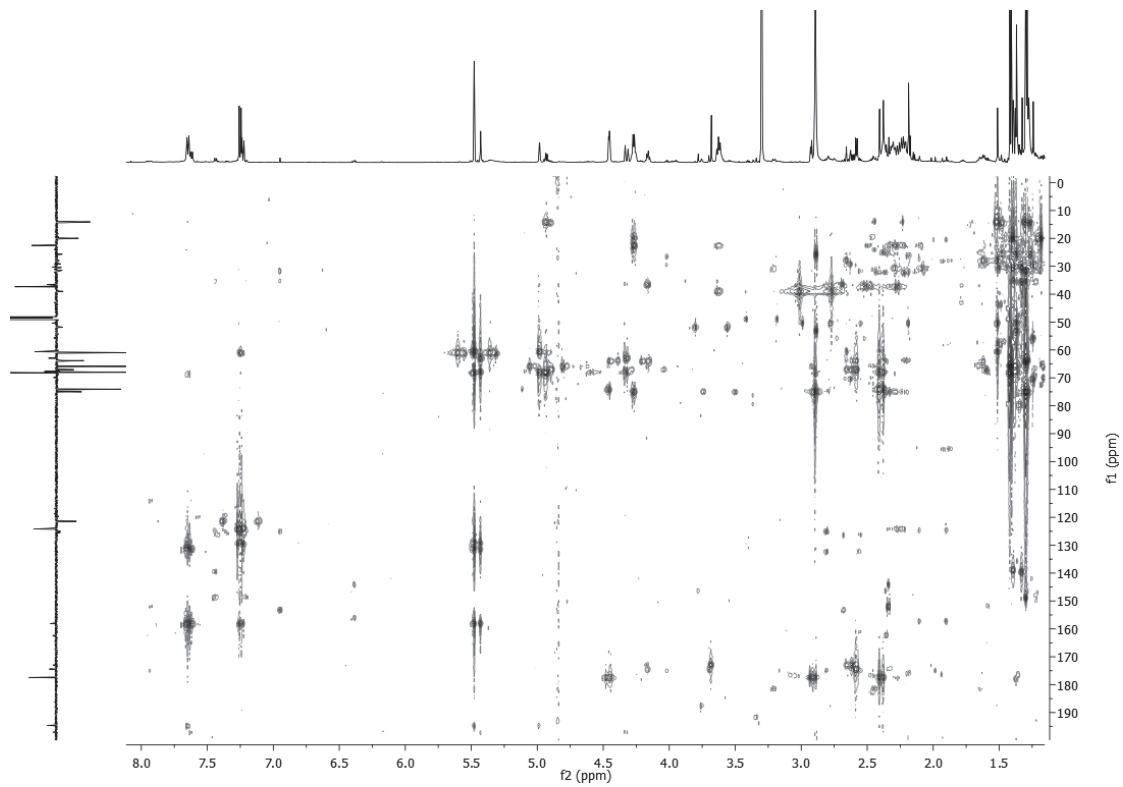
Supporting data file. NMR Spectra of Fr1 mainly consisting of compounds 1—3.

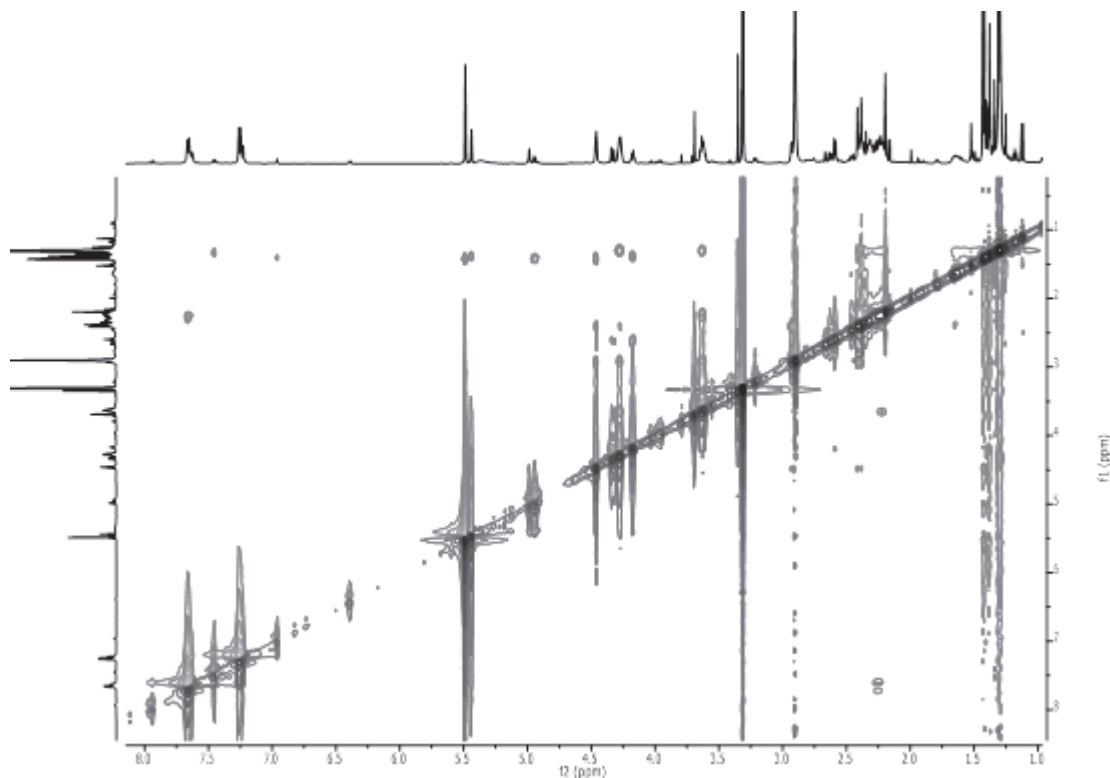
- S1. ^1H NMR spectrum of Fr1 in methanol- d_4 .
- S2. APT spectrum of Fr1 in methanol- d_4 .
- S3. ^1H - ^1H COSY spectrum of Fr1 in methanol- d_4 .
- S4. HSQC spectrum of Fr1 in methanol- d_4 .
- S5. HMBC spectrum of Fr1 in methanol- d_4 .
- S6. NOESY spectrum of Fr1 in methanol- d_4 .

S1. ^1H NMR spectrum of Fr1 in methanol- d_4 .



S2. APT spectrum of Fr1 in methanol- d_4 .S3. ^1H - ^1H COSY spectrum of Fr1 in methanol- d_4 .

S4. HSQC spectrum of Fr1 in methanol- d_4 .S5. HMBC spectrum of Fr1 in methanol- d_4 .

S6. NOESY spectrum of Fr1 in methanol- d_4 .

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Appendix V: Supplementary Information belonging to Chapter 8

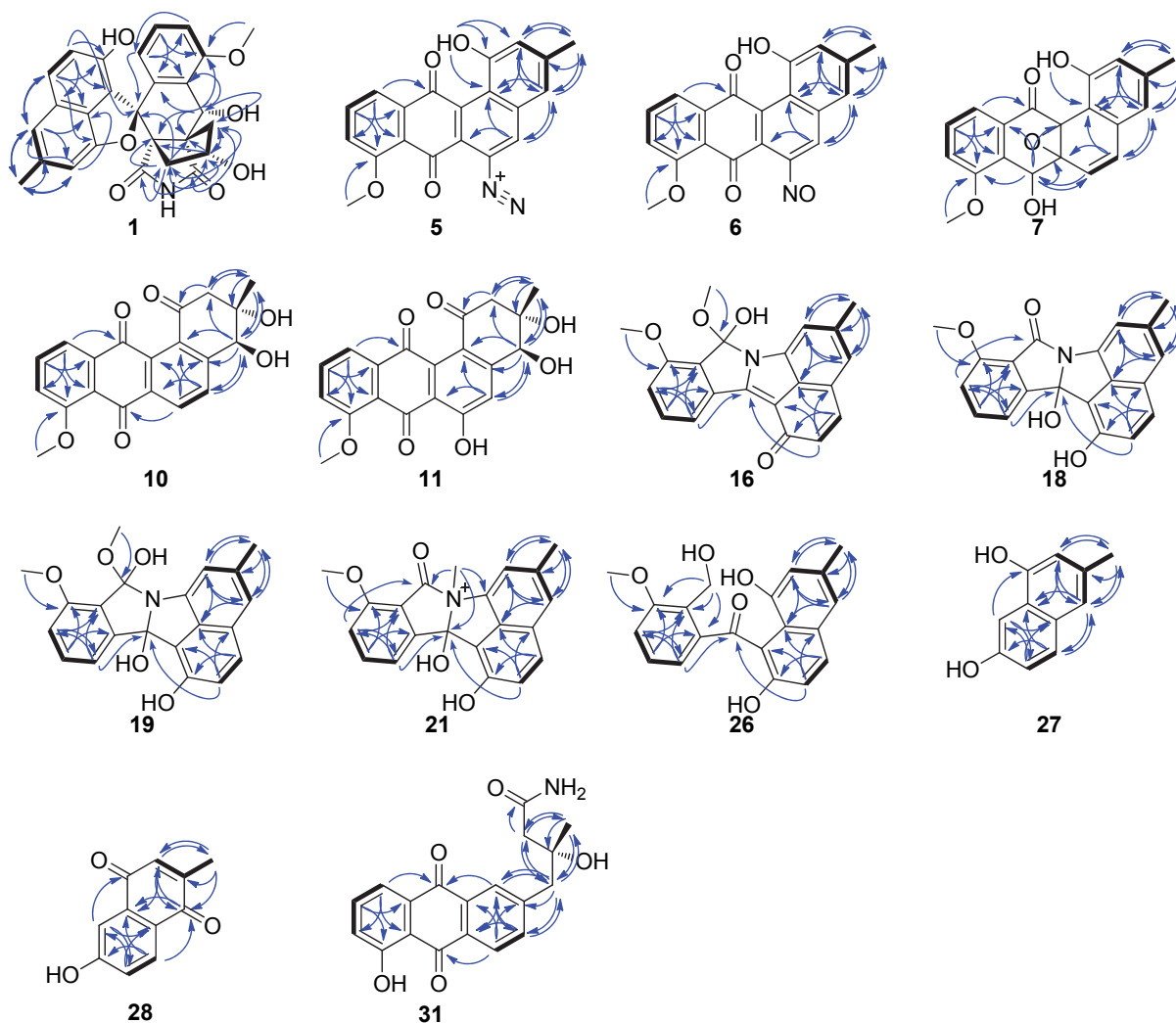


Figure S1. 2D NMR correlations of lugdunomycin (1), and selected new angucyclines 5–7, 10, 11, 16, 18, 19, 22, 27, and 31. Displayed are HMBC ($\curvearrowright$) and COSY (—) correlations for determination of planar structure.

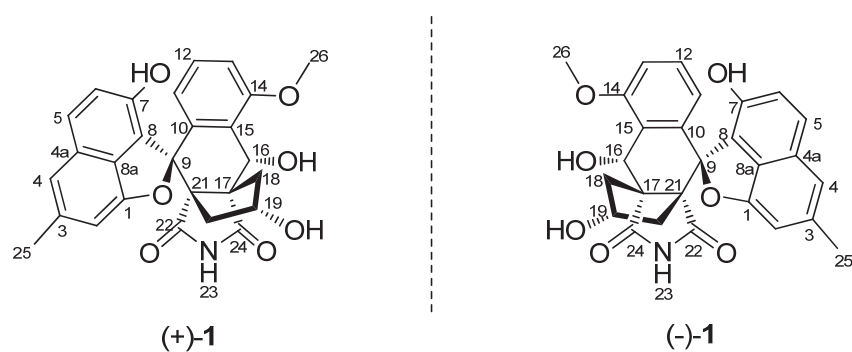


Figure S2. Structure of lugdunomycin (+)-1 and (-)-1

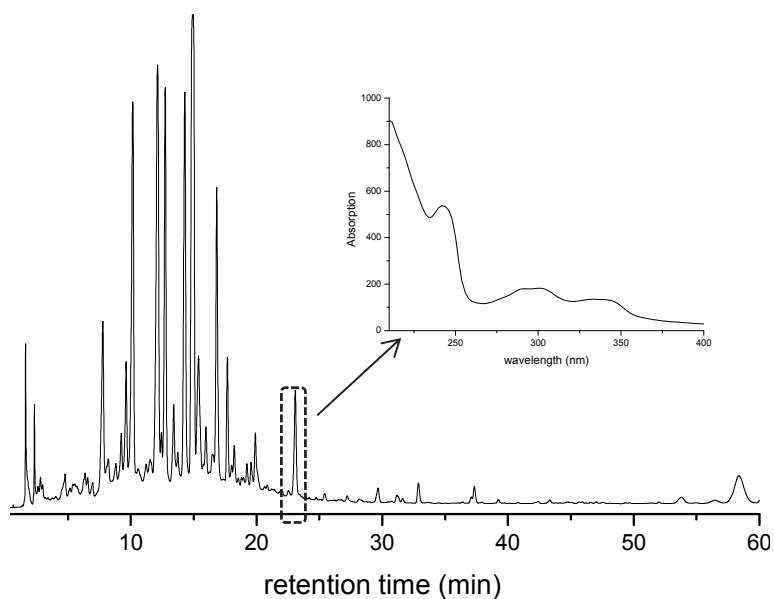


Figure S3. HPLC-DAD analysis (254 nm) of *Streptomyces sp.* QL37 grown on R5 + 0.8% peptone + 1% mannitol. The compound in dashed box gives a UV spectrum comparable to that of lugdunomycin (see S9).

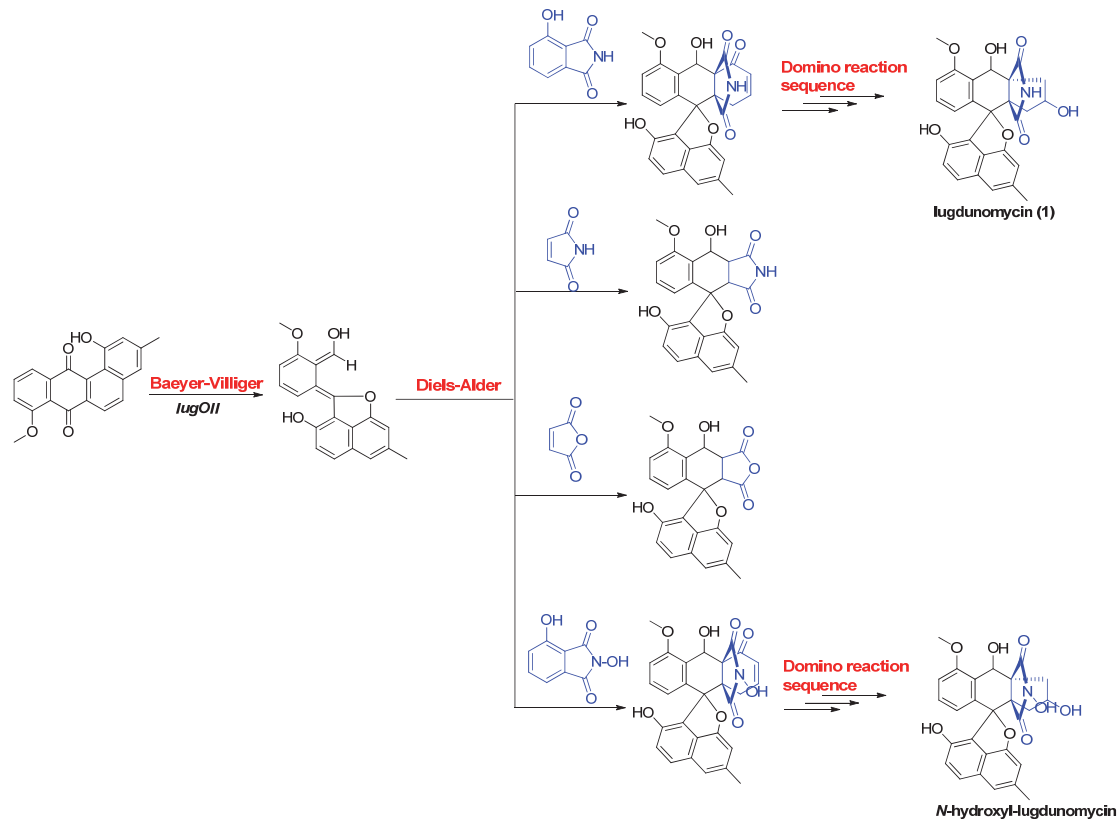


Figure S4. Schematic illumination of potential chemoenzymatic approach to synthesize lugdunomycin variants.

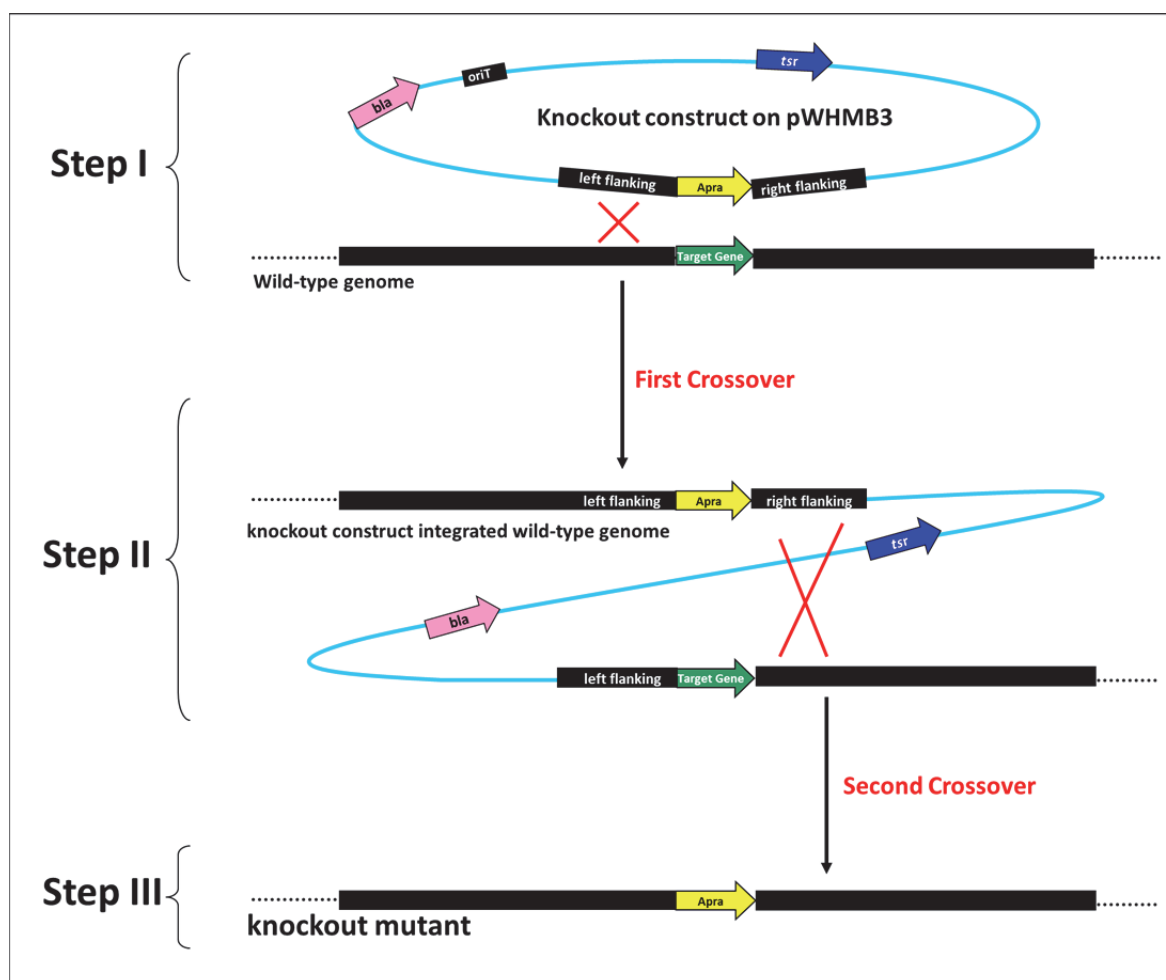


Figure S5. Schematic explanation of deletion mutants for genes *lugA*–*lugC*, *lugOII*, *lugOIV*, and *lugS*. Plasmid pWHM3 engineered with *oriT* fragment was used for conjugation .

Table S1. Crystal Data for lugdunomycin (1)

Identification code	Lugdunomycin	F(000)	1984
Empirical formula	C ₂₇ H ₂₃ NO ₇	Crystal size	0.7 x 0.5 x 0.5 mm ³
Formula weight	473.46	Theta range for data collection	1.241 to 25.340°
Temperature	100(2) K	Index ranges	-27<=h<=27, -27<=k<=27, -9<=l<=9
Wavelength	0.71073 Å	Reflections collected	31098
Crystal system	Tetragonal	Independent reflections	4053 [<i>R</i> _{int} = 0.1666]
Space group	P 42/n	Completeness to theta = 25.242°	100.0 %
Unit cell dimensions	a = 23.204(2) Å, α = 90°.	Refinement method	Full-matrix least-squares on F ²
	b = 23.204(2) Å, β = 90°.	Data/restraints/parameters	4053 / 330 / 321
	c = 8.2073(9) Å, γ = 90°.	Goodness-of-fit on F²	1.036
Volume	4419.1(10) Å ³	Final R indices [<i>I</i>>2σ(<i>I</i>)]	<i>R</i> ₁ = 0.0615, w <i>R</i> ₂ = 0.1134
Z	8	R indices (all data)	<i>R</i> ₁ = 0.1245, w <i>R</i> ₂ = 0.1379
Density (calculated)	1.423 Mg/m ³	Extinction coefficient	n/a
Absorption coefficient	0.104 mm ⁻¹	Largest diff. peak and hole	0.287 and -0.236 e.Å ⁻³

Table S2. Culture media with different components combination for lugdunomycin production.

No.	Media Combination	No.	Media Combination
1	NMMP + 0.2% L-asparagine + 50 mM TES buffer + 2% glycerol	40	MM + 0.5% yeast extract + 50mM GluNAc
2	NMMP + 0.2% L-asparagine + 50 mM TES buffer + 1% glucose	41	MM + 0.8% peptone + 1% mannitol
3	NMMP + 0.2% L-asparagine + 50 mM TES buffer + 1% mannitol	42	MM + 0.8% peptone + 1% glucose
4	NMMP + 0.2% L-asparagine + 50 mM TES buffer + 1% xylose	43	MM + 0.8% peptone + 1% glycerol
5	NMMP + 0.2% L-asparagine + 50 mM TES buffer + 1% GluNAc	44	MM + 0.8% peptone + 50mM GluNAc
6	NMMP + 0.2% (NH ₄) ₂ SO ₄ + 50 mM TES buffer + 1% glycerol	45	MM + 1% mannitol
7	NMMP + 0.2% (NH ₄) ₂ SO ₄ + 50 mM TES buffer + 1% glucose	46	MM + 1% glucose
8	NMMP + 0.2% (NH ₄) ₂ SO ₄ + 50 mM TES buffer + 1% mannitol	47	MM + 1% glycerol
9	NMMP + 0.2% (NH ₄) ₂ SO ₄ + 50 mM TES buffer + 1% xylose	48	MM + 50mM GluNAc
10	NMMP + 0.2% (NH ₄) ₂ SO ₄ + 50 mM TES buffer + 1% GluNAc	49	MM + 25mM NaBu + 1% mannitol
11	NMMP + 0.2% L-glutamine + 50 mM TES buffer + 1% glycerol	50	MM + 25mM NaBu + 1% glucose
12	NMMP + 0.2% L-glutamine + 50 mM TES buffer + 1% glucose	51	MM + 25mM NaBu + 1% glycerol
13	NMMP + 0.2% L-glutamine + 50 mM TES buffer + 1% mannitol	52	MM + 25mM NaBu + 50mM GluNAc
14	NMMP + 0.2% L-glutamine + 50 mM TES buffer + 1% xylose	53	R5+ proline + NaOH + CaCl ₂ + 1% glucose
15	NMMP + 0.2% L-glutamine + 50 mM TES buffer + 1% GluNAc	54	R5+ proline + NaOH + CaCl ₂ + 1% glycerol
16	NMMP + 0.2% L-glutamine + 50 mM TES buffer + 1% fructose	55	R5+ proline + NaOH + CaCl ₂ + 1% mannitol
17	NMMP + 0.2% L-glutamine + 50 mM TES buffer + 1% maltose	56	R5+ proline + NaOH + CaCl ₂ + 1% 50mM GluNAc
18	NMMP + 0.2% L-arginine + 50 mM TES buffer + 1% glycerol	57	R5+ proline + NaOH + CaCl ₂ + without sugar
19	NMMP + 0.2% L-arginine + 50 mM TES buffer + 1% glucose	58	R5 + 1% glucose
20	NMMP + 0.2% L-arginine + 50 mM TES buffer + 1% mannitol	59	R5+ 1% glycerol
21	NMMP + 0.2% L-arginine + 50 mM TES buffer + 1% xylose	60	R5+ 1% mannitol
22	NMMP + 0.2% L-arginine + 50 mM TES buffer + 1% GluNAc	61	R5 + 50mM GluNAc
23	NMMP + 0.2% L-arginine + 50 mM TES buffer + 1% fructose	62	R5 + without sugar
24	NMMP + 0.2% L-arginine + 50 mM TES buffer + 1% maltose	63	R5 + 200mM NaCl+ 1% glucose
25	NMMP + 0.2% L-proline + 50 mM TES buffer + 1% glycerol	64	R5 + 200mM NaCl+ 1% glycerol
26	NMMP + 0.2% L-proline + 50 mM TES buffer + 1% glucose	65	R5 + 200mM NaCl+ 1% mannitol
27	NMMP + 0.2% L-proline + 50 mM TES buffer + 1% mannitol	66	R5 + 200mM NaCl + 50mM GluNAc
28	NMMP + 0.2% L-proline + 50 mM TES buffer + 1% xylose	67	R5 + 200mM NaCl + without sugar
29	NMMP + 0.2% L-proline + 50 mM TES buffer + 1% GluNAc	68	R5 + 0.8% peptone+ 1% glucose
30	NMMP + 0.2% L-proline + 50 mM TES buffer + 1% fructose	69	R5 + 0.8% peptone + 1% glycerol
31	NMMP + 0.2% L-proline + 50 mM TES buffer + 1% maltose	70	R5 + 0.8% peptone + 1% mannitol
32	NMMP + 0.2% (NH ₄) ₂ SO ₄ + phosphate buffer + 1% glycerol + 0.5% mannitol	71	R5 + 0.8% peptone + 50mM GluNAc
33	NMMP + 0.2% (NH ₄) ₂ SO ₄ + phosphate buffer + 1% glycerol + 0.5% mannitol + 1% SFM	72	R5 + 0.8% peptone + without sugar
34	NMMP + 0.2% (NH ₄) ₂ SO ₄ + phosphate buffer + 1% glycerol + 0.5% mannitol + 0.5% yeast extract	73	R5 + 0.5% Yeast extract+ 1% glucose
35	NMMP + 0.2% (NH ₄) ₂ SO ₄ + phosphate buffer + 1% glycerol + 0.5% mannitol + 1% peptone	74	R5 + 0.5% Yeast extract + 1% glycerol
36	NMMP + 0.2% (NH ₄) ₂ SO ₄ + phosphate buffer + 1% glycerol + 0.5% mannitol + pH 10	75	R5 + 0.5% Yeast extract + 1% mannitol
37	MM + 0.5% yeast extract + 1% mannitol	76	R5 + 0.5% Yeast extract + 50mM GluNAc
38	MM + 0.5% yeast extract + 1% glucose	77	R5 + 0.5% Yeast extract + without sugar
39	MM + 0.5% yeast extract + 1% glycerol		

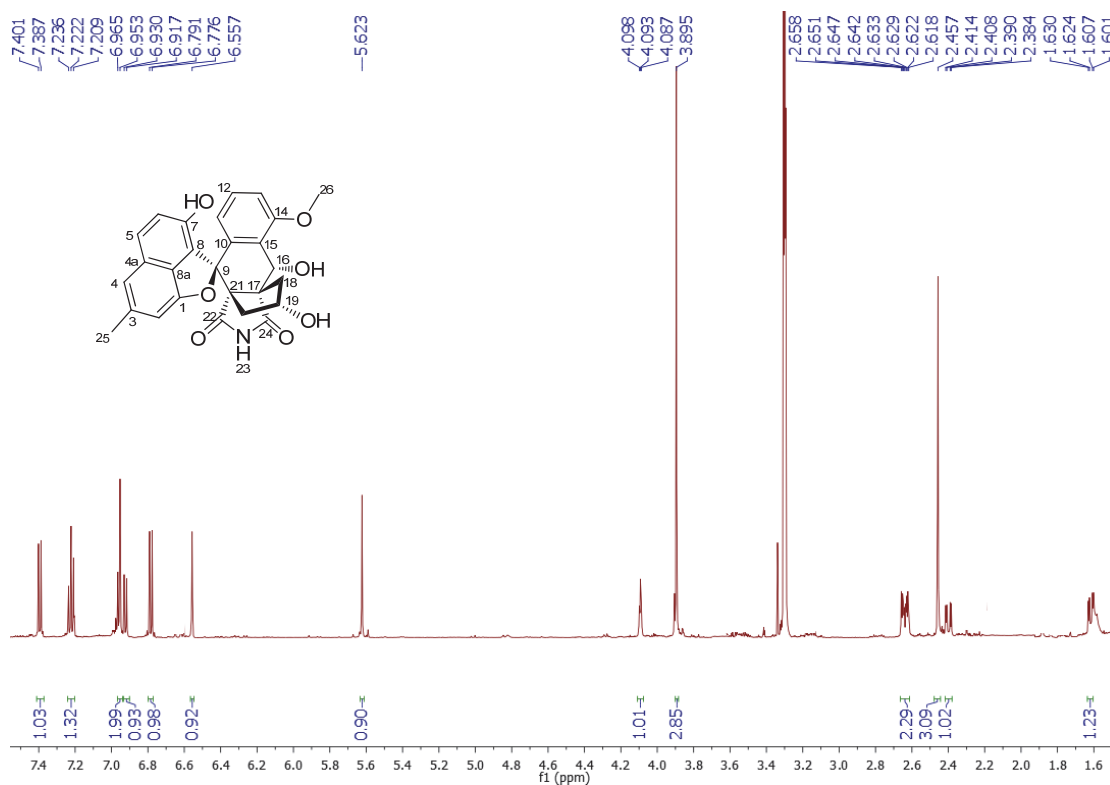
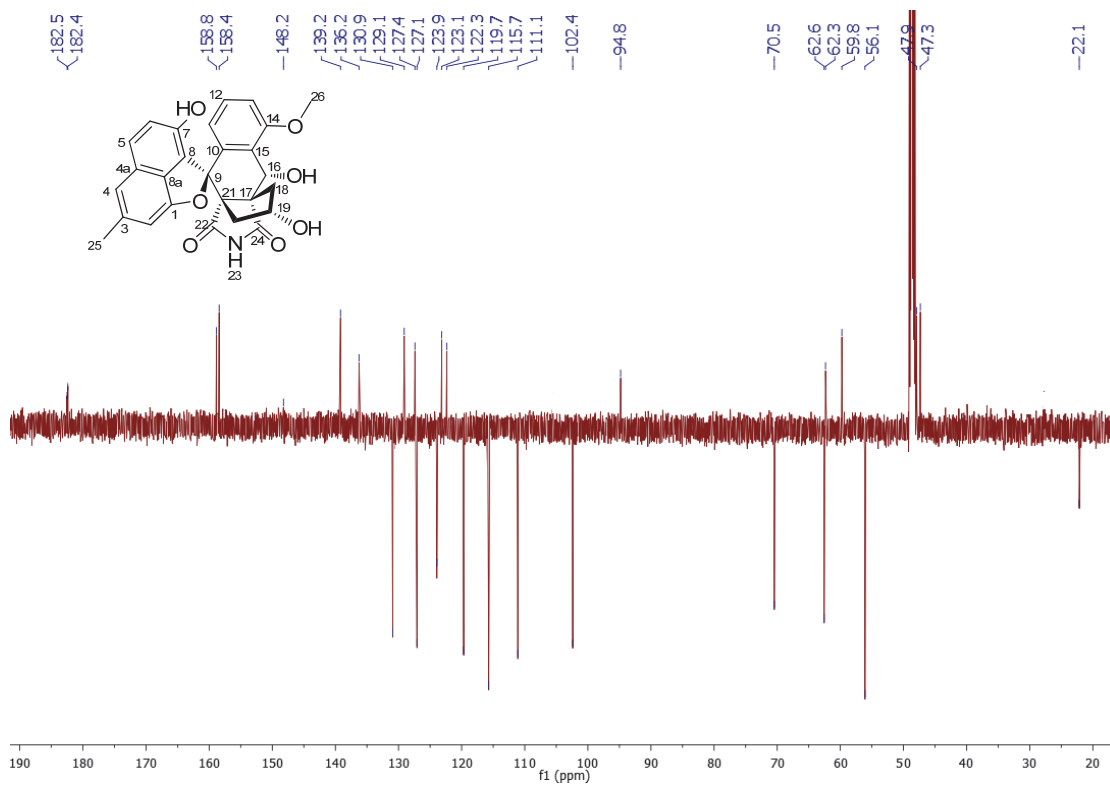
Table S3. Primers used for gene knockout of genes *lugA*–*lugC*, *lugOII*, *lugOIV*, and *lugS*

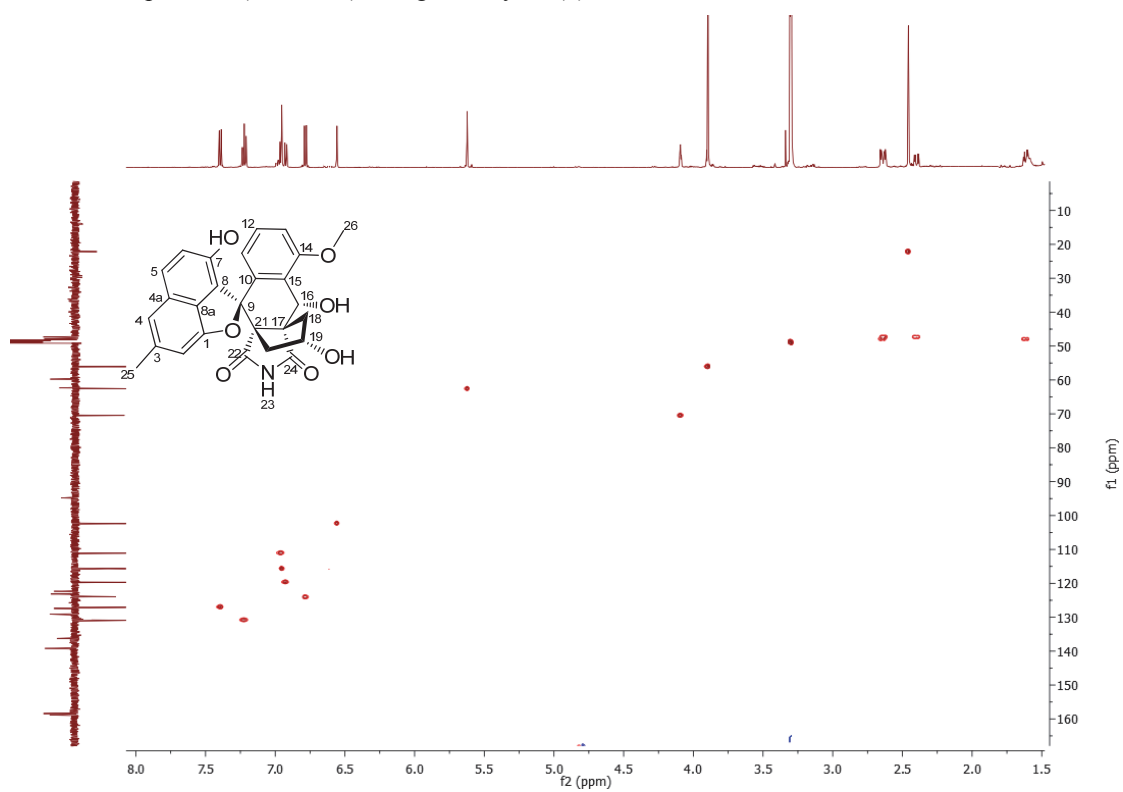
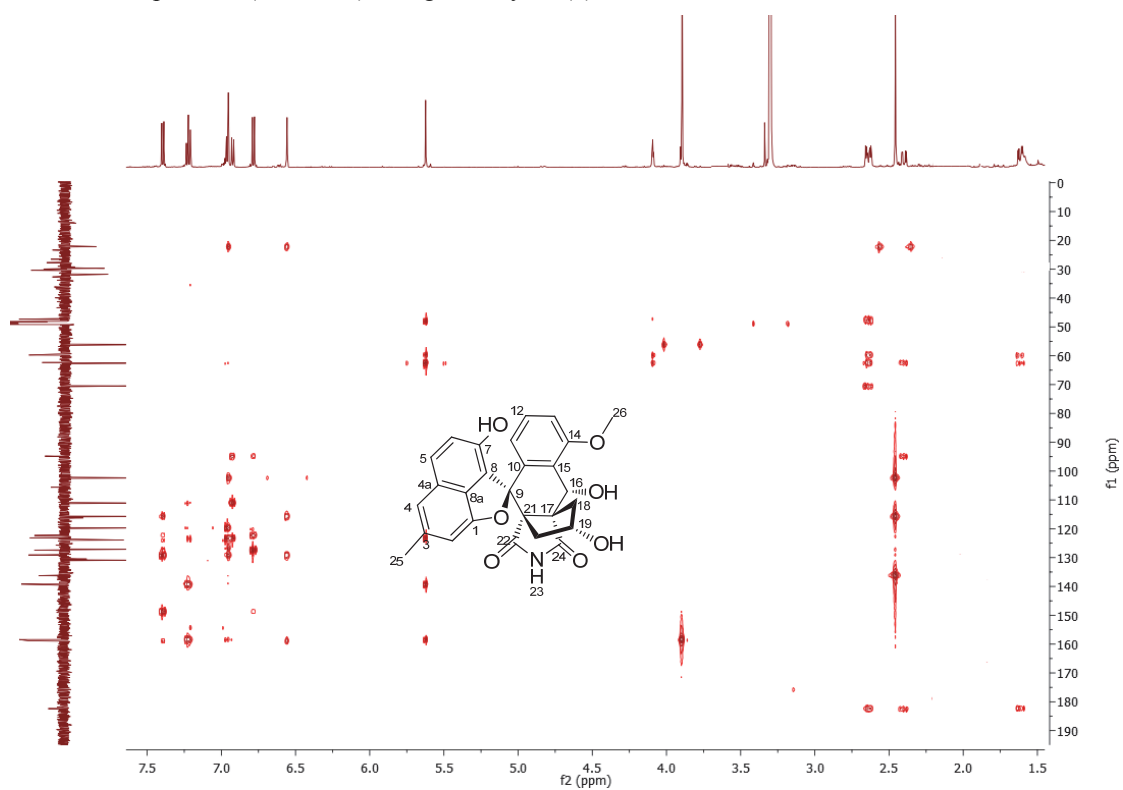
primer	sequence (5'→3') [#]	Function
MinPKS_LF_Fw	ctag <u>GAATTC</u> CCCGCCACCACCGAGCTCTTC	Forward primer for PCR of left flanking region of <i>lugA-C</i>
MinPKS_LF_Rv	GAAGTTATCCATCACCT <u>TCTAGA</u> GATACCGGTGATGACGACCC	Reverse primer for PCR of left flanking region of <i>lugA-C</i>
MinPKS_RF_Fw	GAAGTTATCGCGCATCT <u>TCTAGA</u> GCCGAGCAGCTCGACCGTTAC	Forward primer for PCR of right flanking region of <i>lugA-C</i>
MinPKS_RF_Rv	ctag <u>GGATCC</u> CTGCCCTTGTCTGAGAAGCAGTG	Reverse primer for PCR of right flanking region of <i>lugA-C</i>
LugOII_LF_Fw	cgat <u>AAGCTT</u> GGTACCGAGCTGTGGCTGGACGTGATCAAC	Forward primer for PCR of left flanking region of <i>lugOII</i>
LugOII_LF_Rv	cgat <u>TCTAGA</u> CGTGGTGCCACGGGTCAGC	Reverse primer for PCR of left flanking region of <i>lugOII</i>
LugOII_RF_Fw	cgat <u>TCTAGA</u> GGCGGGACGCTCCTCGGATG	Forward primer for PCR of right flanking region of <i>lugOII</i>
LugOII_RF_Rv	cgat <u>GAATTC</u> GGTGAGGGCCGGCGAGTAGG	Reverse primer for PCR of right flanking region of <i>lugOII</i>
LugOII_Chk_Fw	TCGACCCACACCGGAATC	Forward primer for PCR check of <i>lugOII</i> knockout mutant
LugOII_Chk_Rv	TTCCTCGCCGATGTGCTTGG	Reverse primer for PCR check of <i>lugOII</i> knockout mutant
LugOIV_LF_Fw	ctag <u>GAATTC</u> CACCGGGTGCCGAGGTAGC	Forward primer for PCR of left flanking region of <i>lugOIV</i>
LugOIV_LF_Rv	ctag <u>TCTAGA</u> TCGACGAGCTGACCCGAGAAC	Reverse primer for PCR of left flanking region of <i>lugOIV</i>
LugOIV_RF_Fw	ctag <u>TCTAGA</u> TTCCGACGCGGTGCGATGAG	Forward primer for PCR of right flanking region of <i>lugOIV</i>
LugOIV_RF_Rv	ctag <u>GGATCC</u> GGCCGCCAGATGAAGGGTG	Reverse primer for PCR of right flanking region of <i>lugOIV</i>
LugOIV_Chk_Fw	GTATCCGGCCCGCATGCCTC	Forward primer for PCR check of <i>lugOIV</i> knockout mutant
LugOIV_Chk_Rv	CCCTCGCCGACGCACACTTC	Reverse primer for PCR check of <i>lugOIV</i> knockout mutant
LugS_LF_Fw	cgatAAGCTTGAATTCCCGCTACATGGACCACTAC	Forward primer for PCR of left flanking region of <i>lugS</i>
LugS_LF_Rv	cgat <u>TCTAGA</u> CATGCGGGTGCTCCATGTTT	Reverse primer for PCR of left flanking region of <i>lugS</i>
LugS_RF_Fw	cgat <u>TCTAGA</u> CGCGTCTGATCCGCTCTCC	Forward primer for PCR of right flanking region of <i>lugS</i>
LugS_RF_Rv	cgat <u>GAATTC</u> CCGCGGTACCAAGTTGGACGAC	Reverse primer for PCR of right flanking region of <i>lugS</i>
LugS_Chk_Fw	GAAGATCTCCGGCATGGAGG	Forward primer for PCR check of <i>lugS</i> knockout mutant
LugS_Chk_Rv	GGGAGAGGTCTGGATCTGGG	Reverse primer for PCR check of <i>lugS</i> knockout mutant

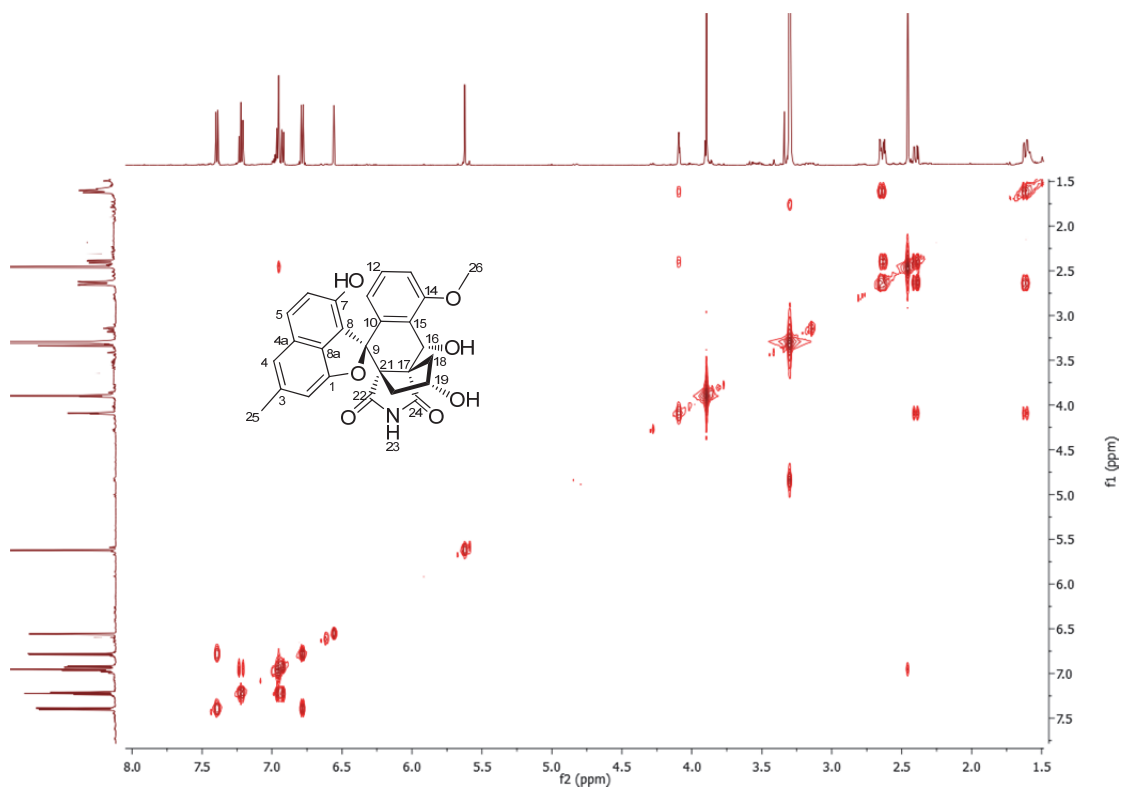
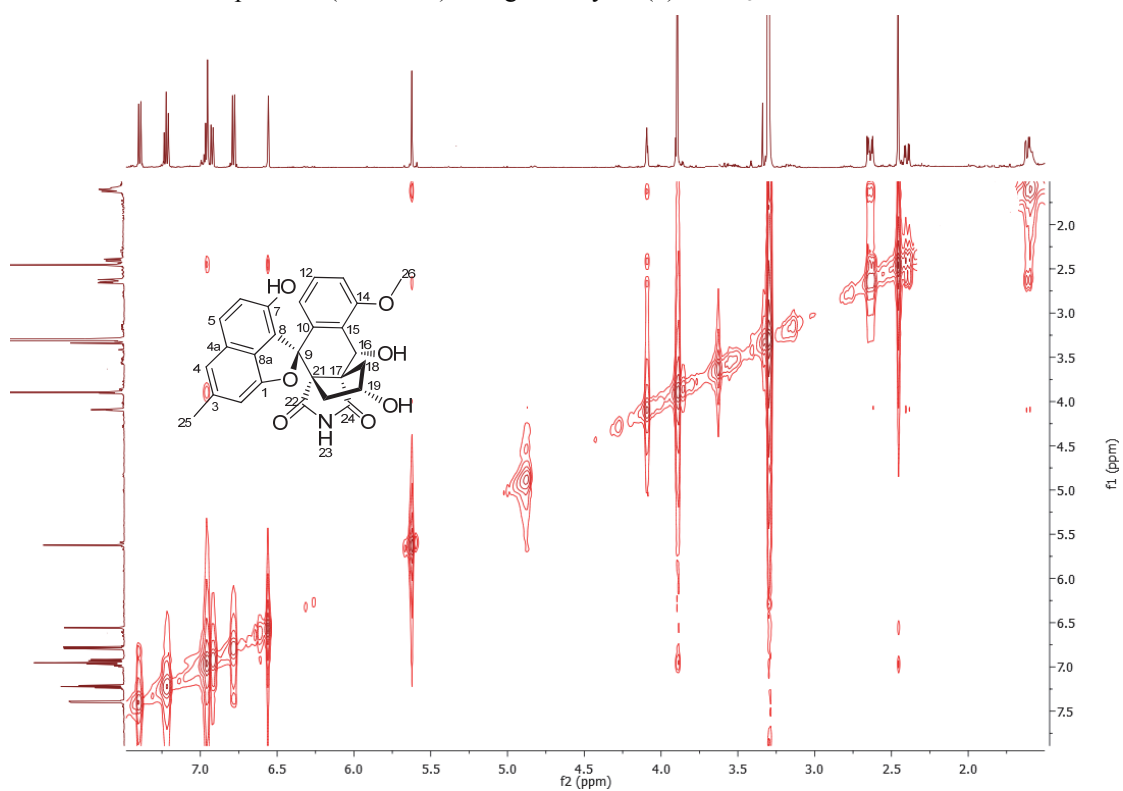
[#]Restriction sites used for cloning are underlined and in bold face. GAATTC, EcoRI; TCTAGA, XbaI; AAGCTT, HindIII; GGATCC, BamHI.

Spectra list of lugdunomycin (1).

- S1. ¹H NMR spectrum (600 MHz) of lugdunomycin (1) in CD₃OD.
- S2. APT spectrum (600 MHz) of lugdunomycin (1) in CD₃OD.
- S3. HSQC spectrum (600 MHz) of lugdunomycin (1) in CD₃OD.
- S4. HMBC spectrum (600 MHz) of lugdunomycin (1) in CD₃OD.
- S5. ¹H-¹H COSY spectrum (600 MHz) of lugdunomycin (1) in CD₃OD.
- S6. ¹H-¹H NOSEY spectrum (600 MHz) of lugdunomycin (1) in CD₃OD.
- S7. HRESIMS spectrum of lugdunomycin (1).
- S8. IR spectrum of lugdunomycin (1).
- S9. UV spectrum of lugdunomycin (1).
- S10. CD spectrum of lugdunomycin (1).

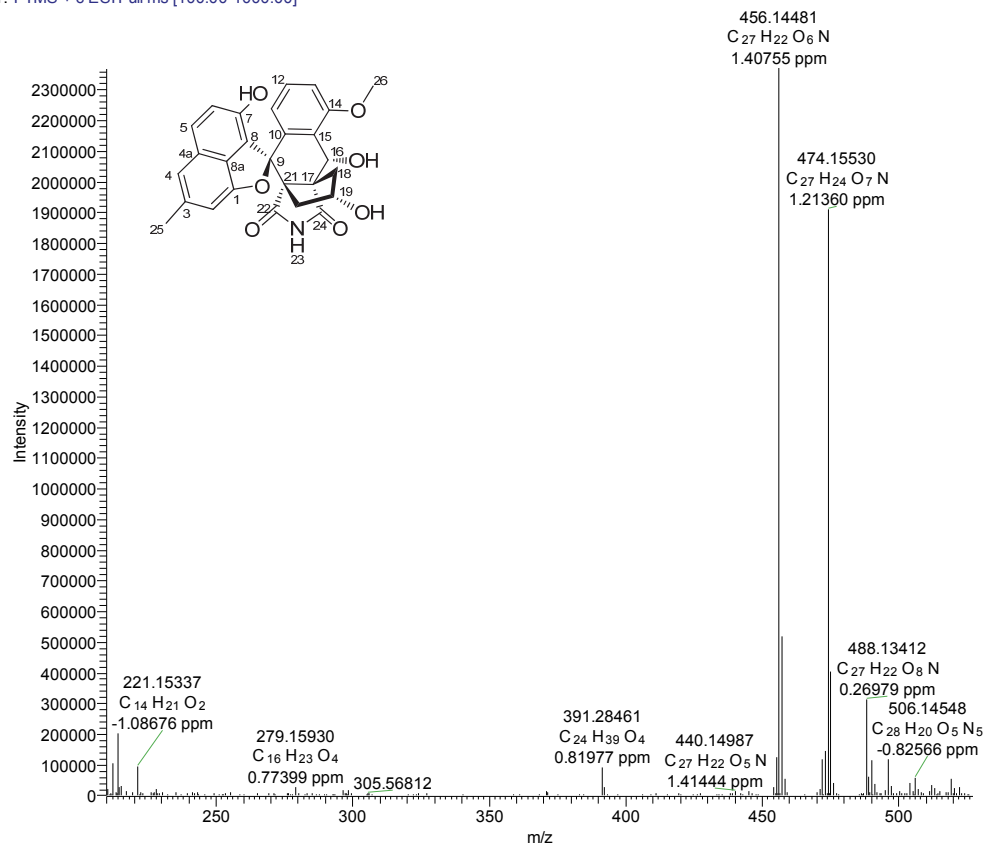
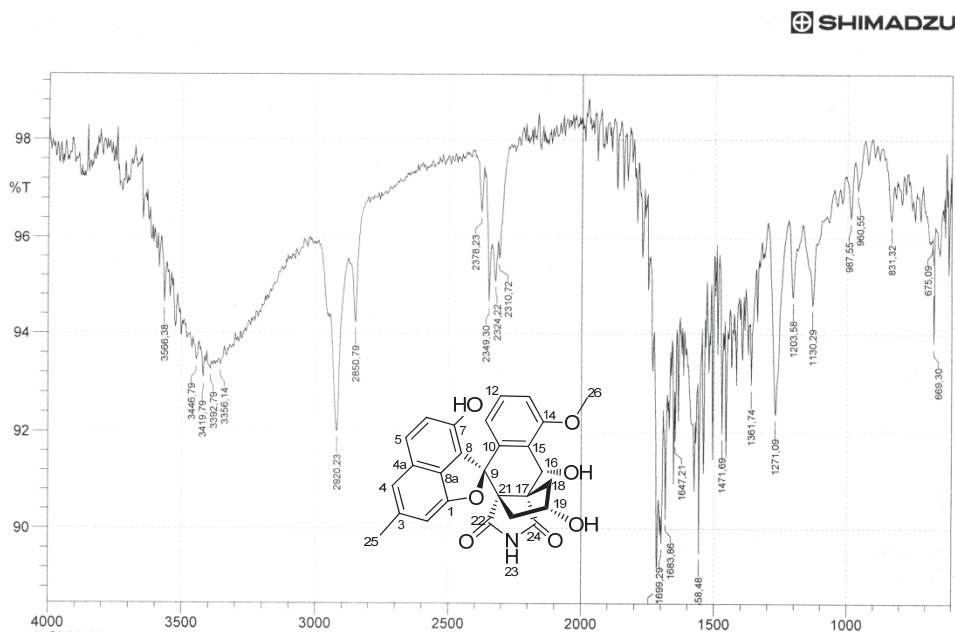
S1. ^1H NMR spectrum (600 MHz) of lugdunomycin (**1**) in CD_3OD .S2. APT spectrum (150 MHz) of lugdunomycin (**1**) in CD_3OD .

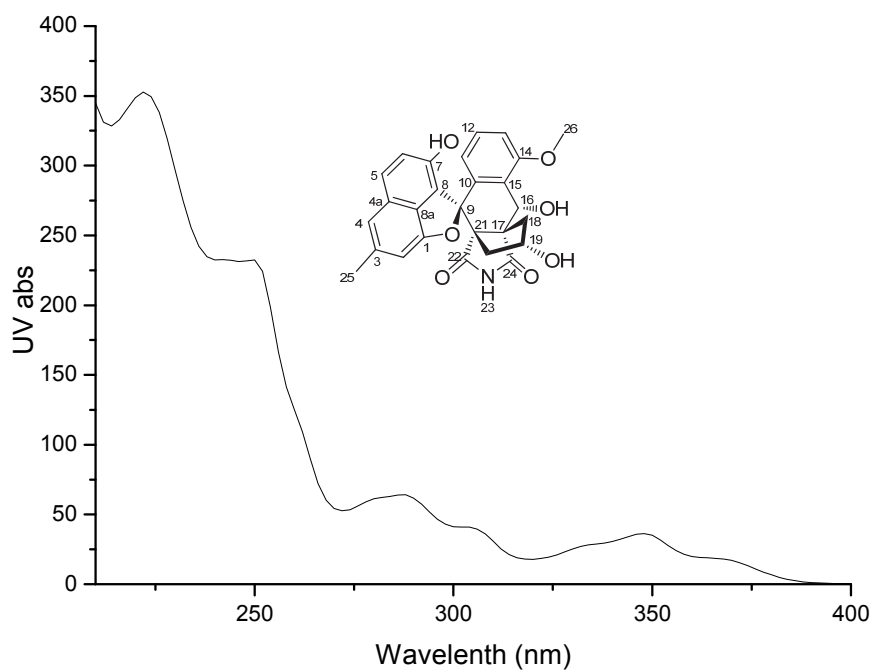
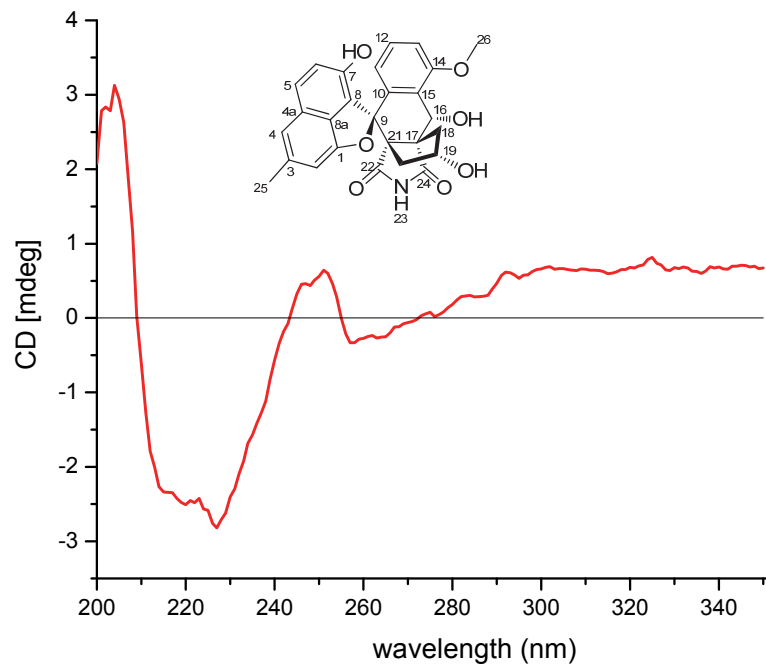
S3. HSQC spectrum (600 MHz) of lugdunomycin (**1**) in CD₃OD.S4. HMBC spectrum (600 MHz) of lugdunomycin (**1**) in CD₃OD.

S5. ^1H - ^1H COSY spectrum (600 MHz) of lugdunomycin (**1**) in CD_3OD .S6. ^1H - ^1H NOESY spectrum (600 MHz) of lugdunomycin (**1**) in CD_3OD .

S7. HRESIMS spectrum of lugdunomycin (**1**).

QL37-15 #741 RT: 12.82 AV: 1 NL: 2.37E6
T: FTMS + c ESI Full ms [100.00-1000.00]

S8. IR spectrum of lugdunomycin (**1**).

S9. UV spectrum of lugdunomycin (**1**).S10. CD spectrum of lugdunomycin (**1**).

Appendix VI: Supplementary Information belonging to Chapter 9

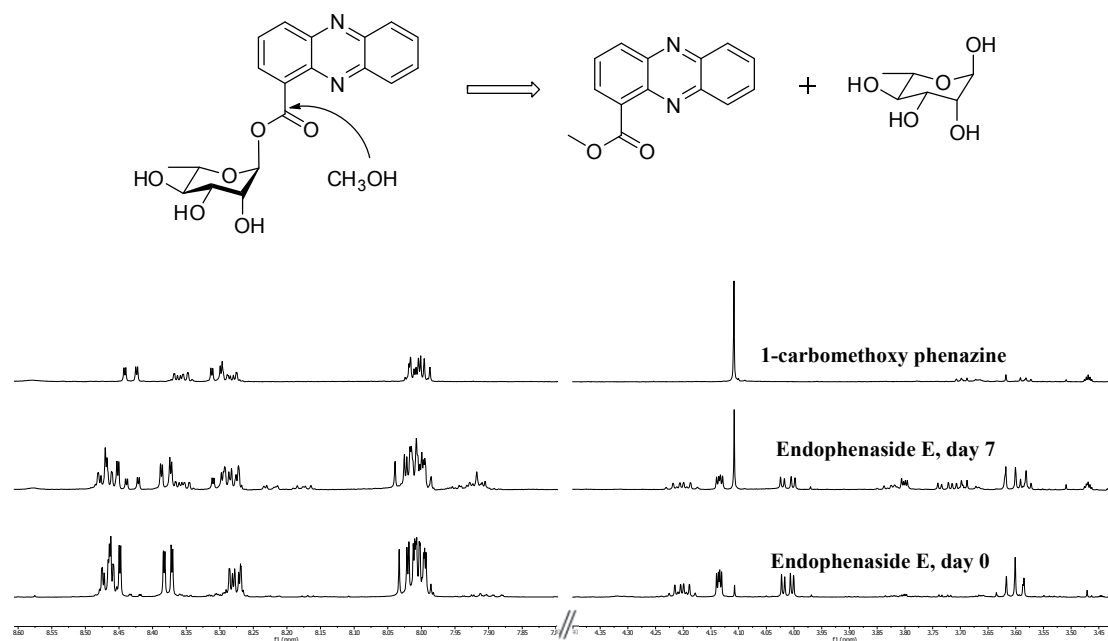


Figure S1. Instability of endophenaside E in methanol. Endophenaside E was unstable in methanol, which was prone to nucleophilic reaction (methanolysis) and degraded into 1-carbomethoxy phenazine and rhamnose. The reaction was observed in deuterated methanol when measuring NMR data in one week. The product identification was done in comparison with the ¹H NMR of purified 1-carbomethoxy phenazine.

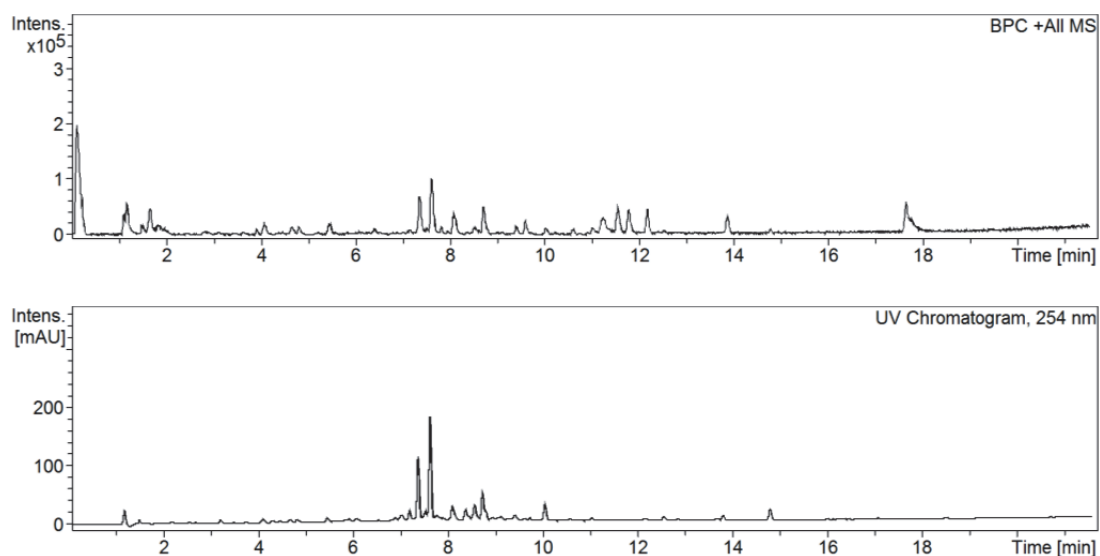


Figure S2. UHPLC-Q-TOF analysis of *Kitasatospora* sp. MBT66. UHPLC-Q-TOF profiling of secondary metabolites produced by *kitasatospora* sp. MBT66. A) mass detection, and B) UV detection at 254 nm.

Table S1. Extracted peaks and ions of UHPLC-Q-TOF chromatogram for the confirmation of previously identified endophenazines.

Retention time	Detected mass (m/z)	Deduced chemical formula	Corresponding compound described by Heine D. <i>et al</i>
8.5 min	285.0879 [M + H] ⁺	C ₁₅ H ₁₃ N ₂ O ₄	5-(2-Hydroxyacetyl)-5,10-dihydrophenazine-1-carboxylic acid (2)
8.8 min	309.1231 [M + H] ⁺	C ₁₈ H ₁₇ N ₂ O ₃	(<i>E</i>)-9-(4-Hydroxy-3-methylbut-2-en-1-yl)phenazine-1-carboxylic acid (3)
9.4 min	309.1234 [M + H] ⁺	C ₁₈ H ₁₇ N ₂ O ₃	(<i>E</i>)-6-(3-Hydroxy-3-methylbut-1-en-1-yl)phenazine-1-carboxylic acid (5)
14.1 min	377.1853 [M + H] ⁺	C ₂₃ H ₂₅ N ₂ O ₃	5,9-bis(3-Methylbut-2-en-1-yl)-7-oxo-5,7-dihydrophenazine-1-carboxylic acid (4)

Physical properties of the new compounds

Endophenaside A (**1**): yellowish, amorphous powder, UV (MeOH) $\lambda_{\max}$ (log ϵ) 251 (4.79), 366 (4.17) nm; IR 3352, 2926, 2855, 1674, 1597, 1560, 1456, 1204, 16, 1067, 958, 748 $\nu_{\max}$ cm⁻¹; ¹H and ¹³C NMR data, see Table 1 and 2; HRMS (positive mode) m/z 400.15076 [M + H]⁺ (calcd for C₂₀H₂₂N₃O₆ 400.15031), 422.13339 [M + Na]⁺ (calcd for C₂₀H₂₁N₃O₆Na 422.13281).

Endophenaside B (**2**): yellowish, amorphous powder, UV (MeOH) $\lambda_{\max}$ (log ϵ) 252 (4.72), 365 (4.10) nm; IR $\nu_{\max}$ 3350, 2920, 1732, 1672, 1531, 85, 1263, 1123, 1061, 978, 947, 760 cm⁻¹; ¹H and ¹³C NMR data, see Table 1 and 2; HRMS (positive mode) m/z 455.18187 [M + H]⁺ (calcd for C₂₄H₂₇N₂O₇ 455.18128), 477.16375 [M + Na]⁺ (calcd for C₂₄H₂₇N₂O₇Na 477.16377).

Endophenaside C (**3**): yellowish, amorphous powder, UV (MeOH) $\lambda_{\max}$ (log ϵ) 251 (4.50), 367 (3.97) nm; IR $\nu_{\max}$ 3341, 2926, 2853, 1682, 1456, 1418, 1271, 1203, 18, 1067, 841, 764 cm⁻¹; ¹H and ¹³C NMR data, see Table 1 and 2; HRMS (positive mode) m/z 469.19641 [M + H]⁺ (calcd for C₂₅H₂₉N₂O₇ 469.19693), 491.18021 [M + Na]⁺ (calcd for C₂₅H₂₈N₂O₇Na 491.17942).

Endophenaside D (**4**): yellowish, amorphous powder, UV (MeOH) $\lambda_{\max}$ (log ϵ) 251 (4.58), 368 (4.05) nm; IR $\nu_{\max}$ 3400, 2926, 2855, 1734, 1717, 1682, 1456, 77, 1277, 1126, 1069, 976, 762 cm⁻¹; ¹H and ¹³C NMR data, see Table 1 and 2; HRMS (positive mode) m/z 455.18243 [M + H]⁺ (calcd for C₂₄H₂₇N₂O₇ 455.18128), 477.16458 [M + Na]⁺ (calcd for C₂₄H₂₆N₂O₇Na 477.16377), 493.13879 [M + K]⁺ (calcd for C₂₄H₂₆N₂O₇K 493.13771).

Endophenaside E (**5**): yellowish, amorphous powder, UV (MeOH) $\lambda_{\max}$ (log ϵ) 248 (5.53), 365 (3.91) nm; IR $\nu_{\max}$ 3358, 2924, 2853, 1721, 1670, 1522, 1422, 1277, 1125, 1059, 941, 831, 750 cm⁻¹; ¹H and ¹³C NMR data, see Table 1 and 2; HRMS (positive mode) m/z 371.1233 [M + H]⁺ (calcd for C₁₉H₁₉N₂O₆ 371.1238), 393.1062 [M + Na]⁺ (calcd for C₁₉H₁₈N₂O₆Na 393.1063), 225.0657 [M + H - sugar]⁺ (calcd for C₁₃H₉N₂O₂ 225.0659).

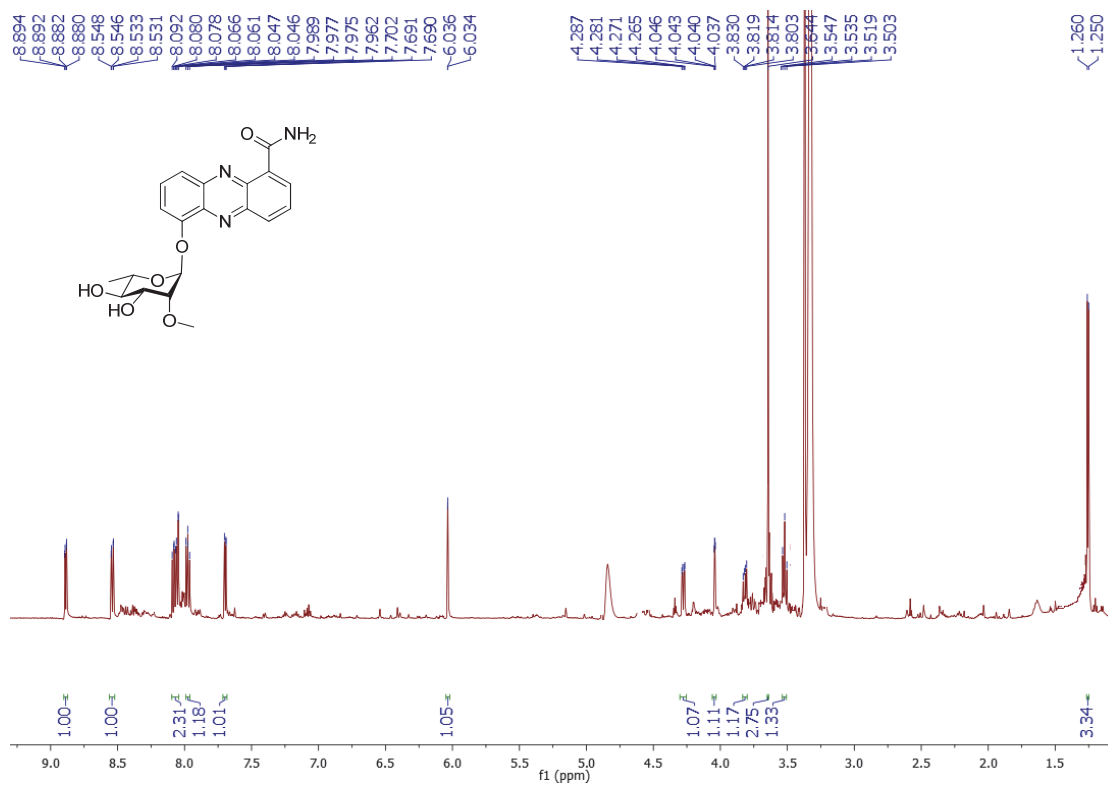
Endophenazine F1 (**6**): purple, amorphous powder, UV/vis (MeOH) $\lambda_{\max}$ (log ϵ) 283 (4.33), 376 (3.83), 518 (3.79) nm; IR $\nu_{\max}$ 3348, 2920, 2855, 1732, 1717, 1684, 1624, 1539, 1456, 96, 1233, 1206, 745 cm⁻¹; ¹H and ¹³C NMR data, see Table 1 and 2; HRMS (positive mode) m/z 393.18115 [M + H]⁺ (calcd for C₂₃H₂₅N₂O₄ 393.18088).

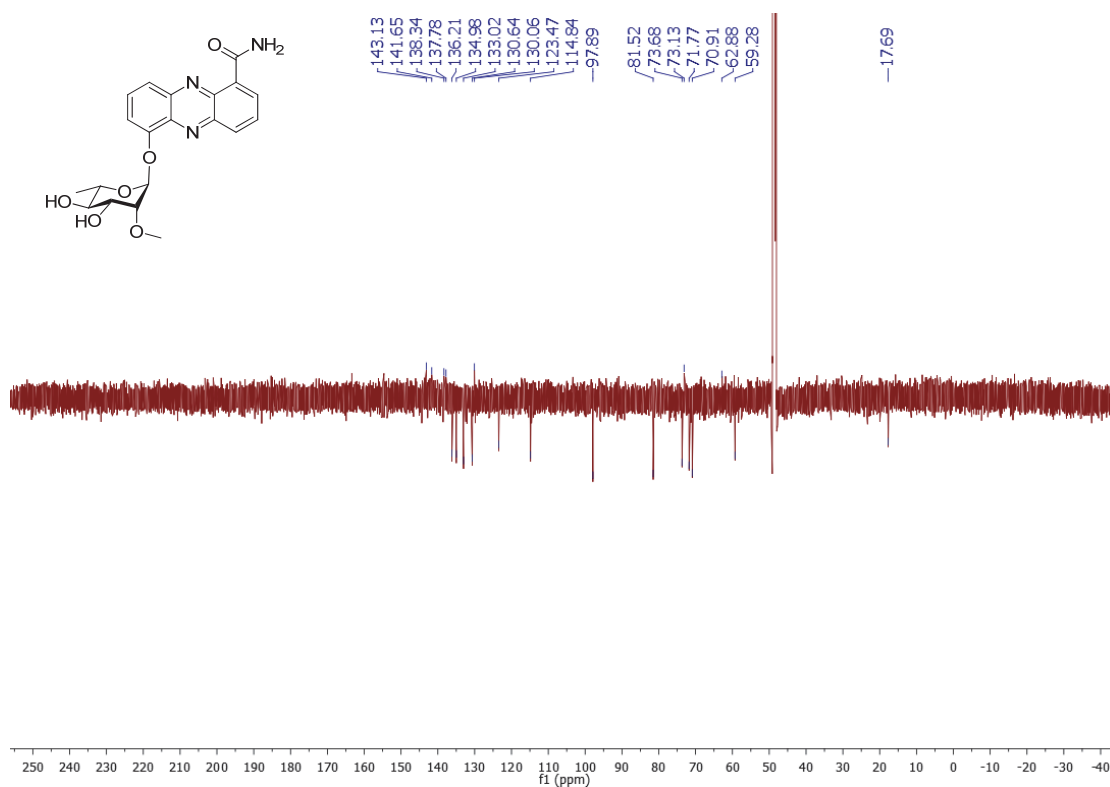
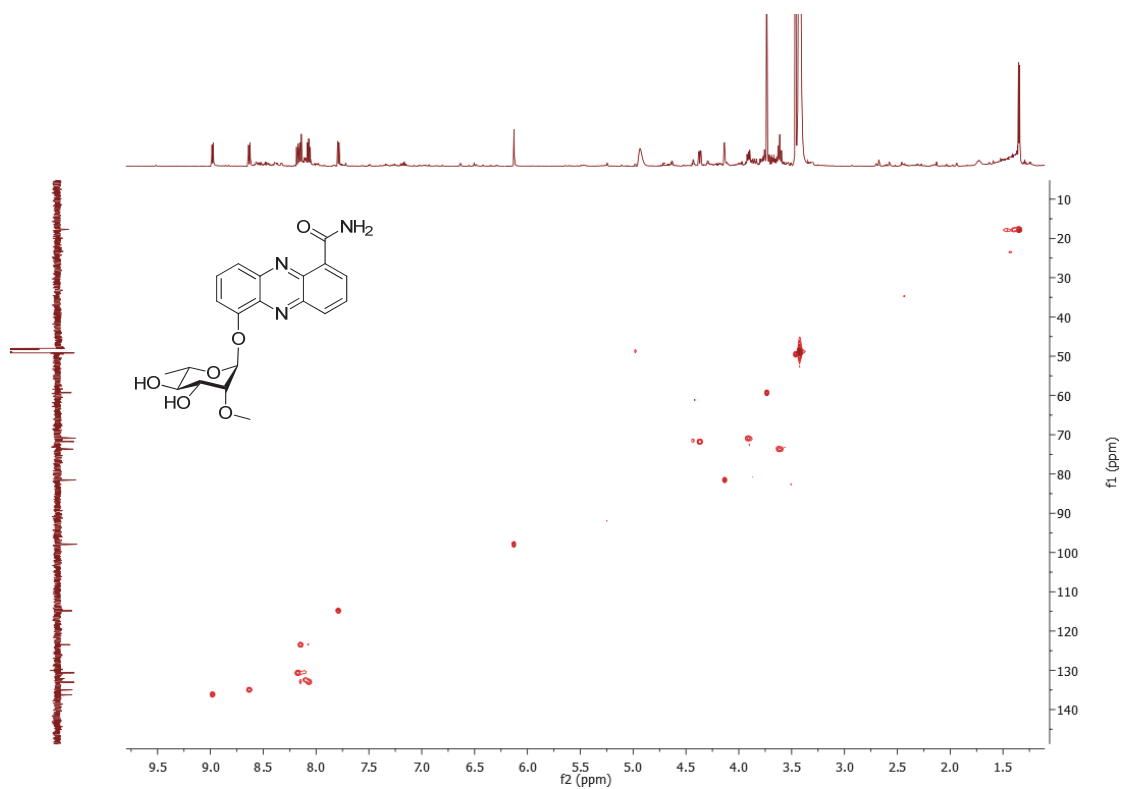
Spectral file. Spectra list of new compounds

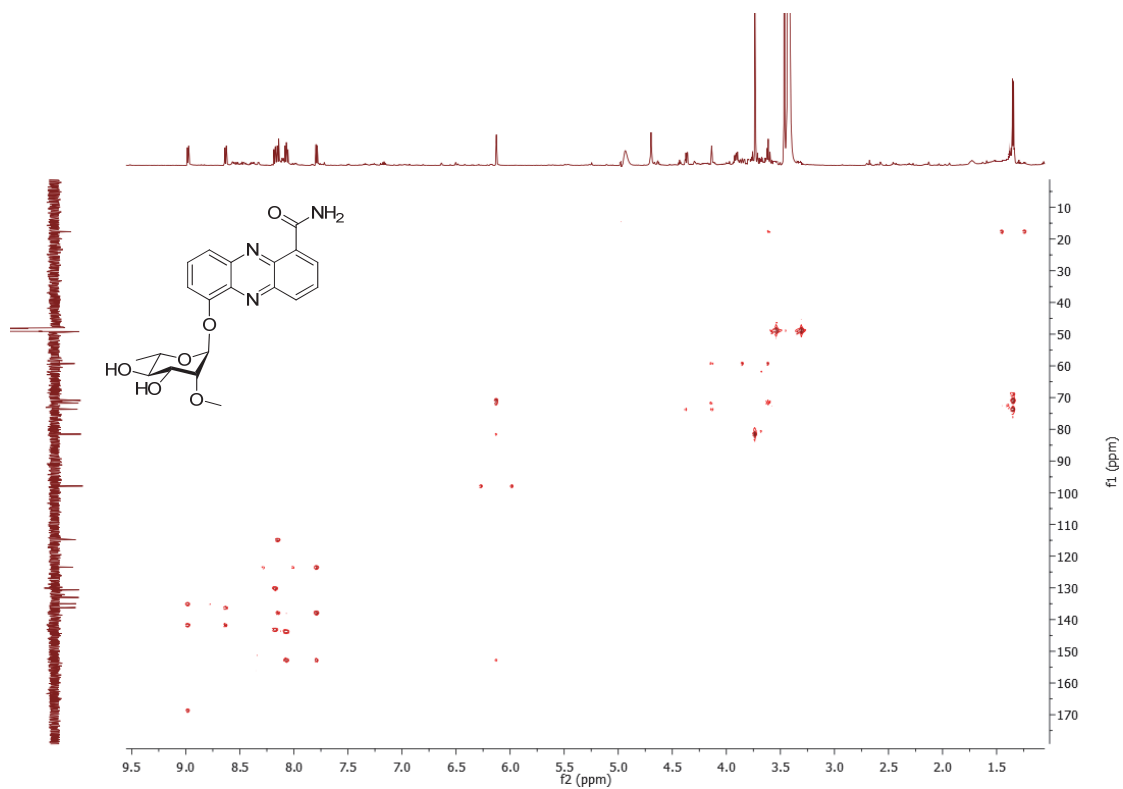
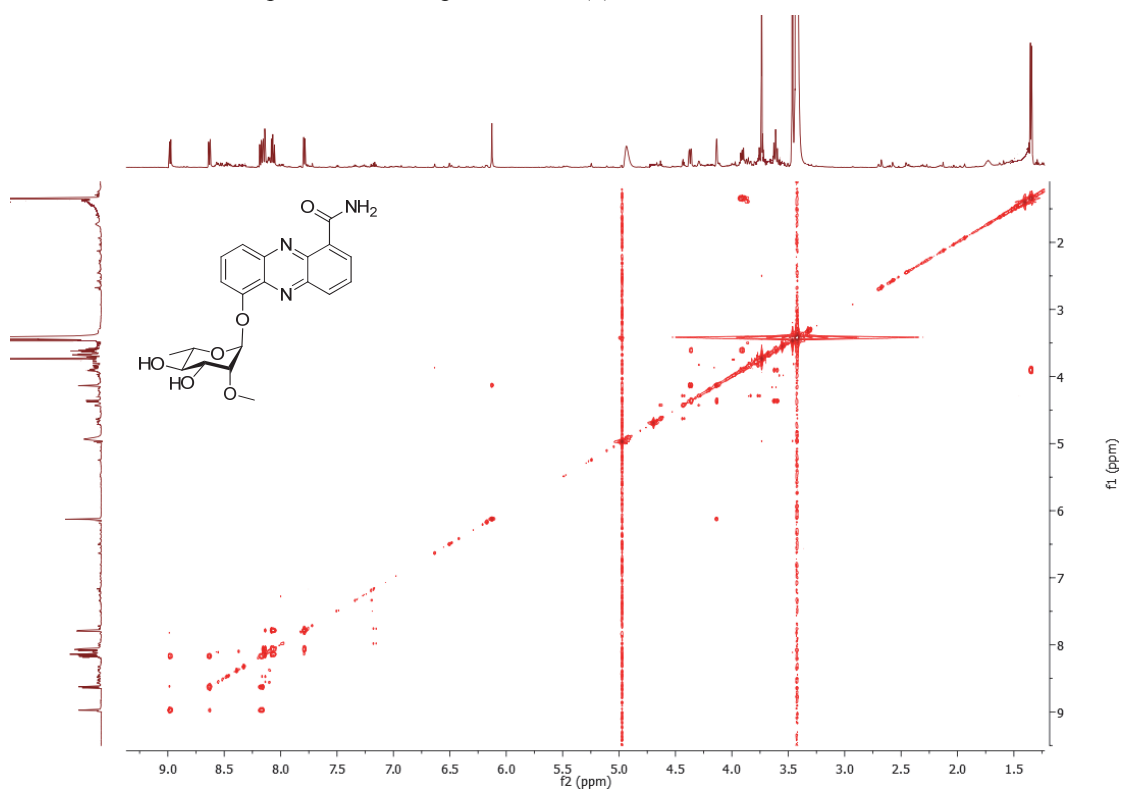
- S1. ^1H NMR spectrum (600 MHz) of endophenaside A (**1**) in CD_3OD .
- S2. APT spectrum of endophenaside A (**1**) in CD_3OD .
- S3. HSQC spectrum of endophenaside A (**1**) in CD_3OD .
- S4. HMBC spectrum of endophenaside A (**1**) in CD_3OD .
- S5. ^1H - ^1H COSY spectrum of endophenaside A (**1**) in CD_3OD .
- S6. ^1H - ^1H NOSEY spectrum of endophenaside A (**1**) in acetone- d_6 .
- S7. HRMS spectrum of endophenaside A (**1**).
- S8. IR spectrum of endophenaside A (**1**).
- S9. UV spectrum of endophenaside A (**1**).
- S10. ^1H NMR spectrum (500 MHz) of endophenaside B (**2**) in CD_3OD .
- S11. APT spectrum of endophenaside B (**2**) in CD_3OD .
- S12. HSQC spectrum of endophenaside B (**2**) in CD_3OD .
- S13. HMBC spectrum of endophenaside B (**2**) in CD_3OD .
- S14. ^1H - ^1H COSY spectrum of endophenaside B (**2**) in CD_3OD .
- S15. ^1H - ^1H NOSEY spectrum of endophenaside B (**2**) in acetone- d_6 .
- S16. HRMS spectrum of endophenaside B (**2**).
- S17. IR spectrum of endophenaside B (**2**).
- S18. UV spectrum of endophenaside B (**2**).
- S19. ^1H NMR spectrum (500 MHz) of endophenaside C (**3**) in CD_3OD .
- S20. APT spectrum of endophenaside C (**3**) in CD_3OD .
- S21. HSQC spectrum of endophenaside C (**3**) in CD_3OD .
- S22. HMBC spectrum of endophenaside C (**3**) in CD_3OD .
- S23. ^1H - ^1H COSY spectrum of endophenaside C (**3**) in CD_3OD .
- S24. HRMS spectrum of endophenaside C (**3**).
- S25. IR spectrum of endophenaside C (**3**).
- S26. UV spectrum of endophenaside C (**3**).
- S27. ^1H NMR spectrum (500 MHz) of endophenaside D (**4**) in CD_3OD .
- S28. APT spectrum of endophenaside D (**4**) in CD_3OD .
- S29. HSQC spectrum of endophenaside D (**4**) in CD_3OD .
- S30. HMBC spectrum of endophenaside D (**4**) in CD_3OD .
- S31. ^1H - ^1H COSY spectrum of endophenaside D (**4**) in CD_3OD .
- S32. HRMS spectrum of endophenaside D (**4**).
- S33. IR spectrum of endophenaside D (**4**).
- S34. UV spectrum of endophenaside D (**4**).
- S35. ^1H NMR spectrum (500 MHz) of endophenaside E (**5**) in CD_3OD .
- S36. HSQC spectrum of endophenaside E (**5**) in CD_3OD .
- S37. HMBC spectrum of endophenaside E (**5**) in CD_3OD .
- S38. ^1H - ^1H COSY spectrum of endophenaside E (**5**) in CD_3OD .
- S39. HRMS spectrum of endophenaside E (**5**).
- S40. IR spectrum of endophenaside E (**5**).
- S41. UV spectrum of endophenaside E (**5**).
- S42. ^1H NMR spectrum (600 MHz) of endophenazine F1(**6**) in CD_3OD .
- S43. APT spectrum of endophenazine F1(**6**) in CD_3OD .
- S44. HSQC spectrum of endophenazine F1(**6**) in CD_3OD .

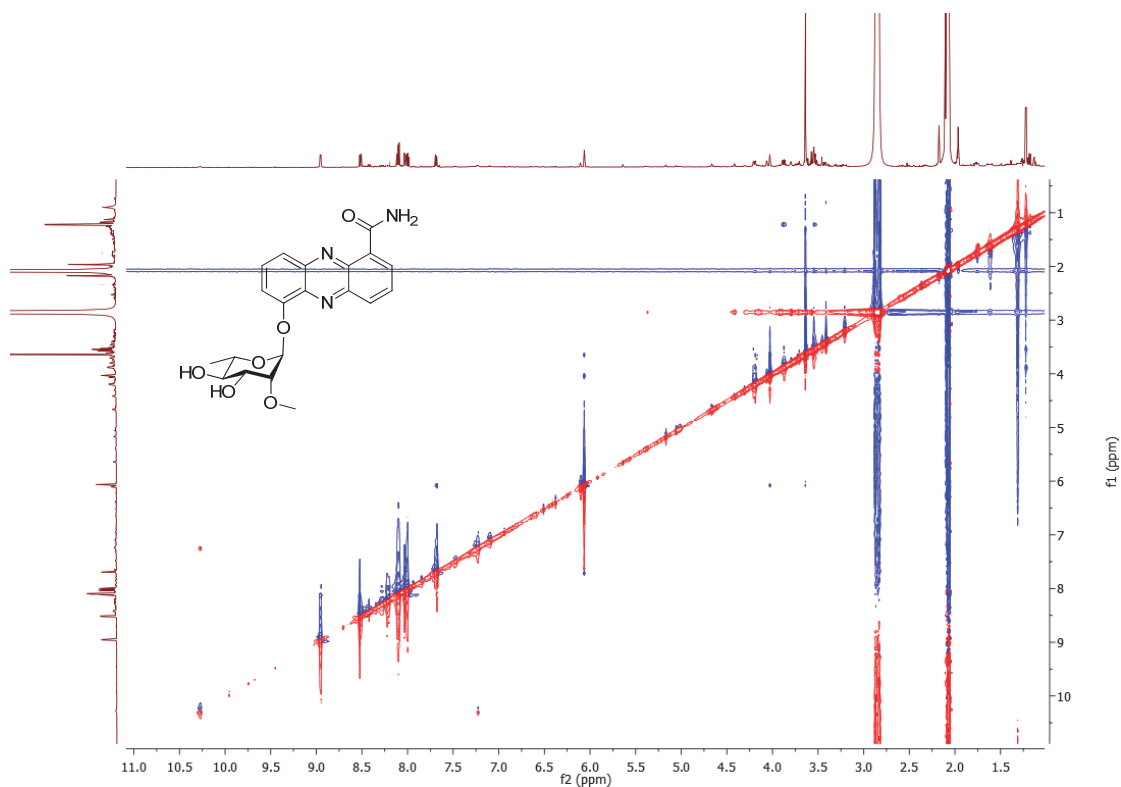
- S45. HMBC spectrum of endophenazine F1(**6**) in CD₃OD.
S46. ¹H-¹H COSY spectrum of endophenazine F1 (**6**) in CD₃OD.
S47. ¹H-¹H NOSEY spectrum of endophenazine F1 (**6**) acetone-*d*₆..
S48. HRMS spectrum of endophenazine F1(**6**).
S49. IR spectrum of endophenazine F1(**6**).
S50. UV spectrum of endophenazine F1(**6**).

S1. ¹H NMR spectrum (600 MHz) of endophenaside A (**1**) in CD₃OD.

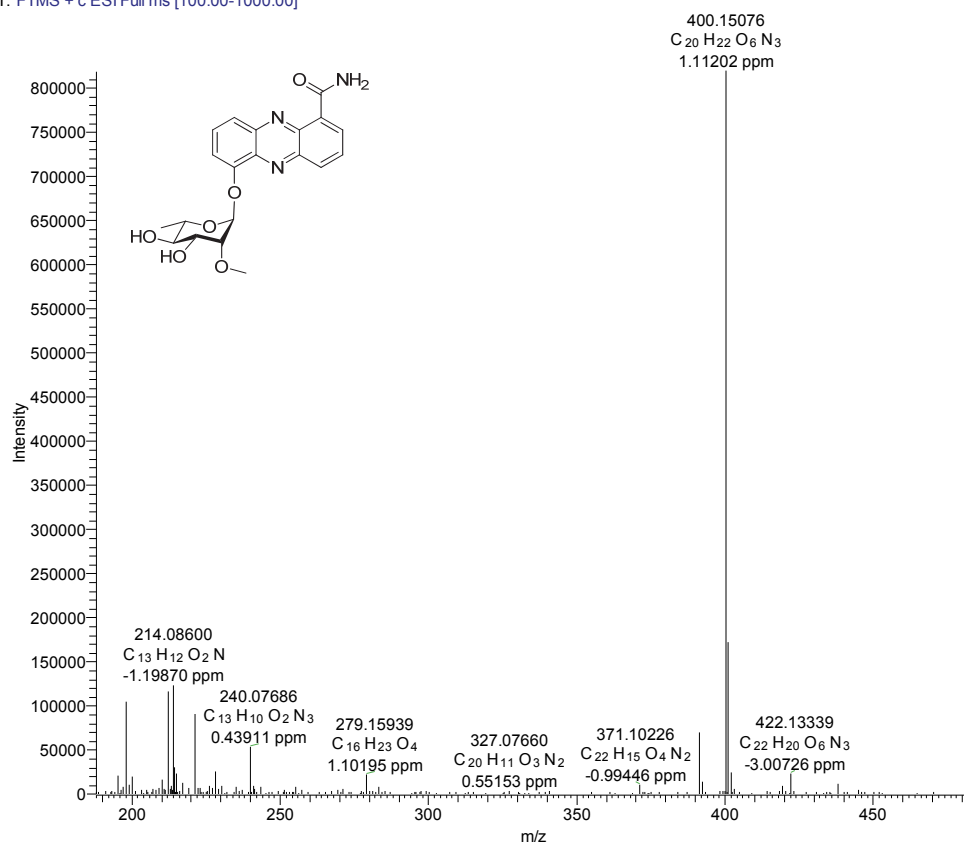


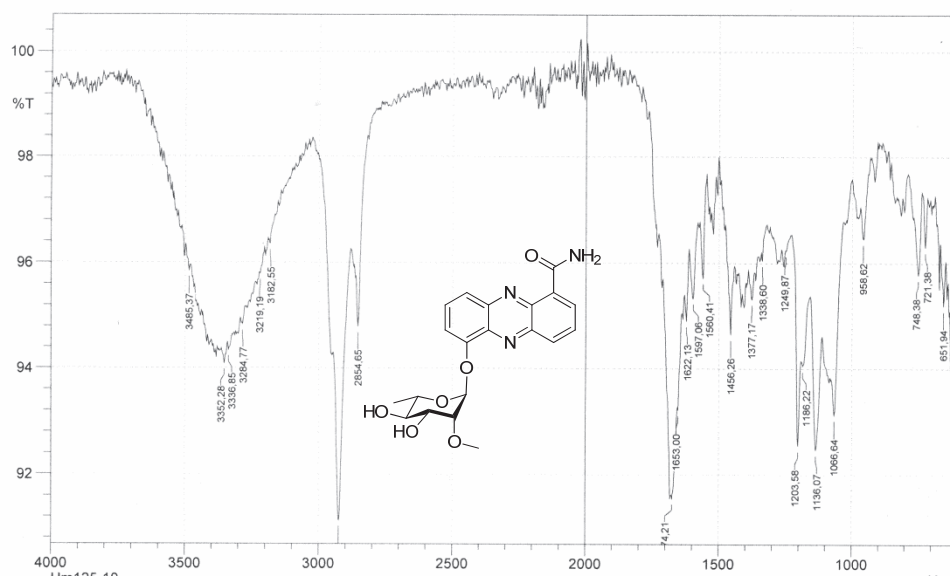
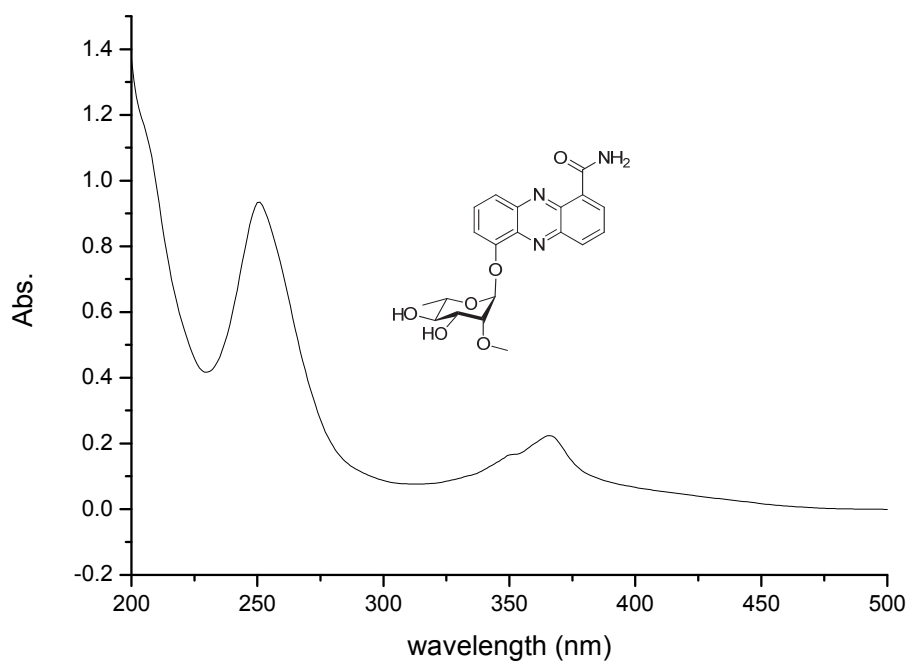
S2. APT spectrum of endophenaside A (**1**) in CD₃OD.S3. HSQC spectrum of endophenaside A (**1**) in CD₃OD.

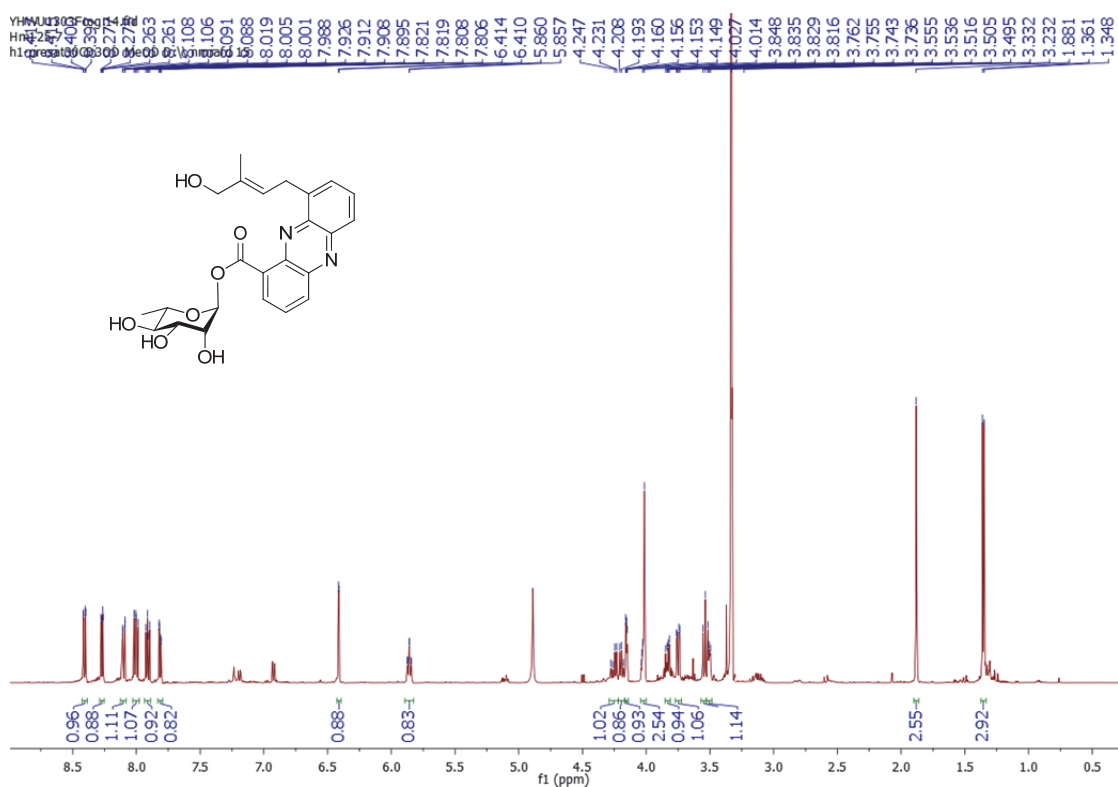
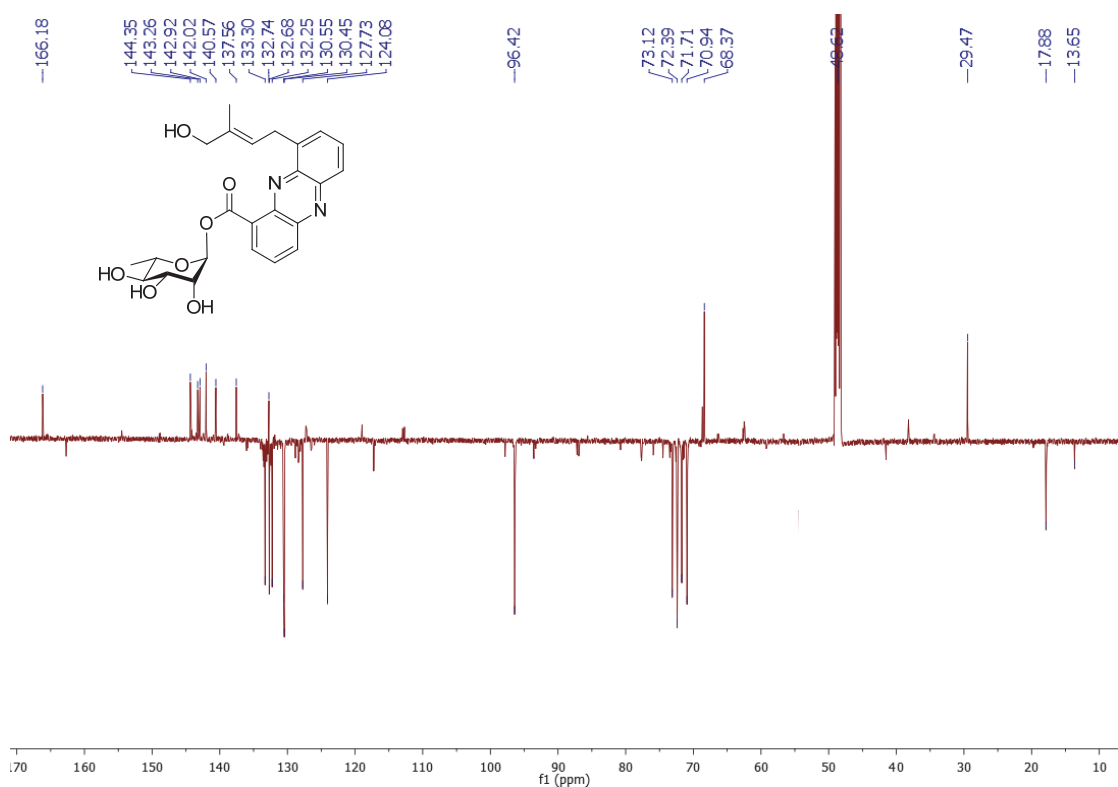
S4. HMBC spectrum of endophenaside A (**1**) in CD₃OD.S5. ¹H-¹H COSY spectrum of endophenaside A (**1**) in CD₃OD.

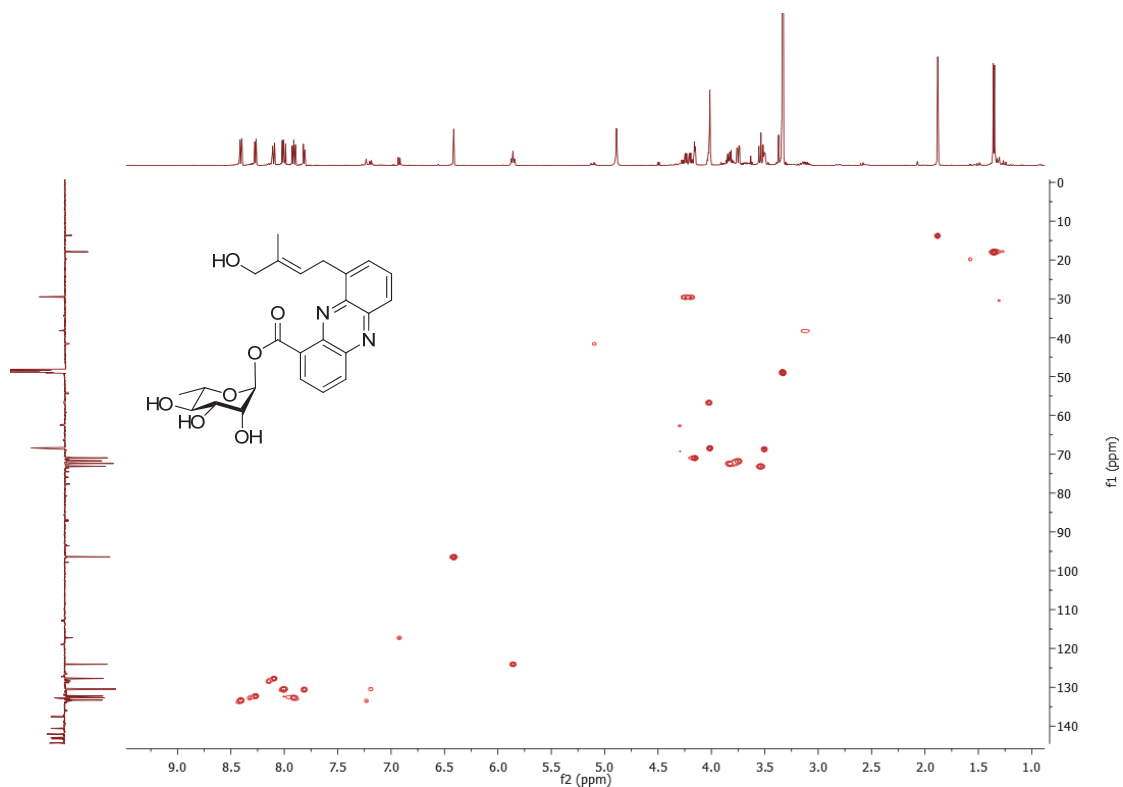
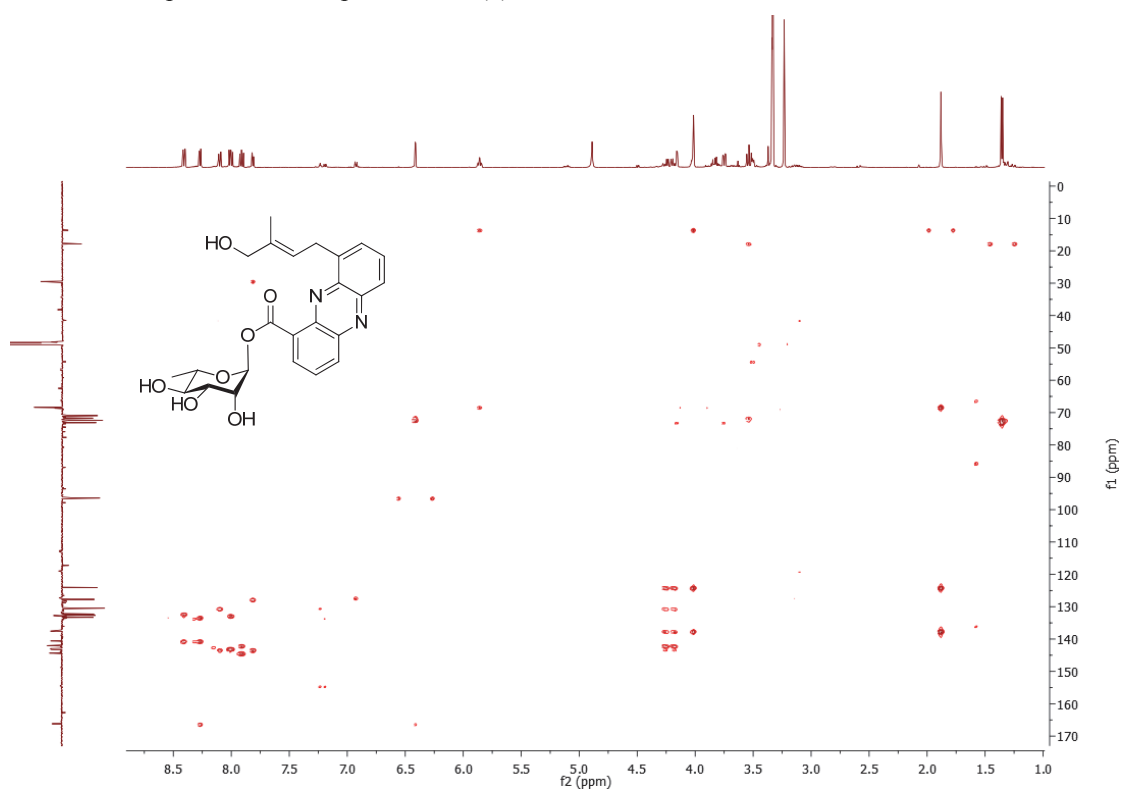
S6. ^1H - ^1H NOSEY spectrum of endophenaside A (**1**) in acetone- d_6 .S7. HRMS spectrum of endophenaside A (**1**).

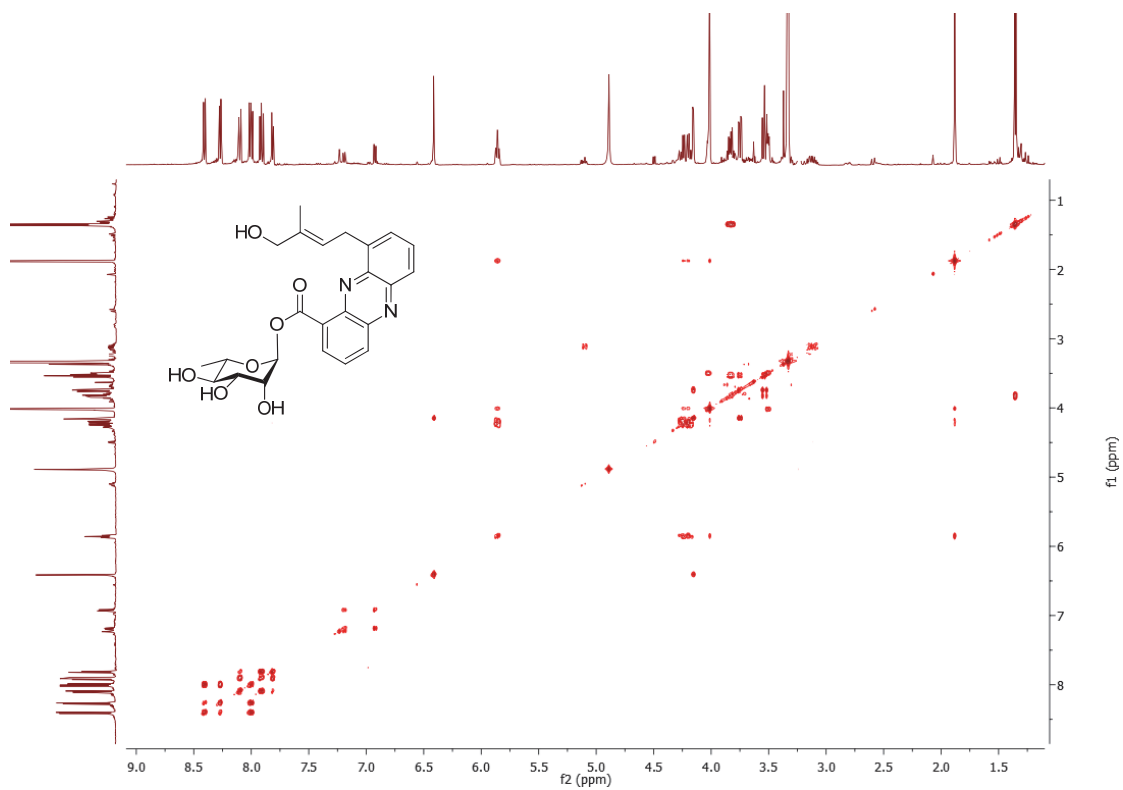
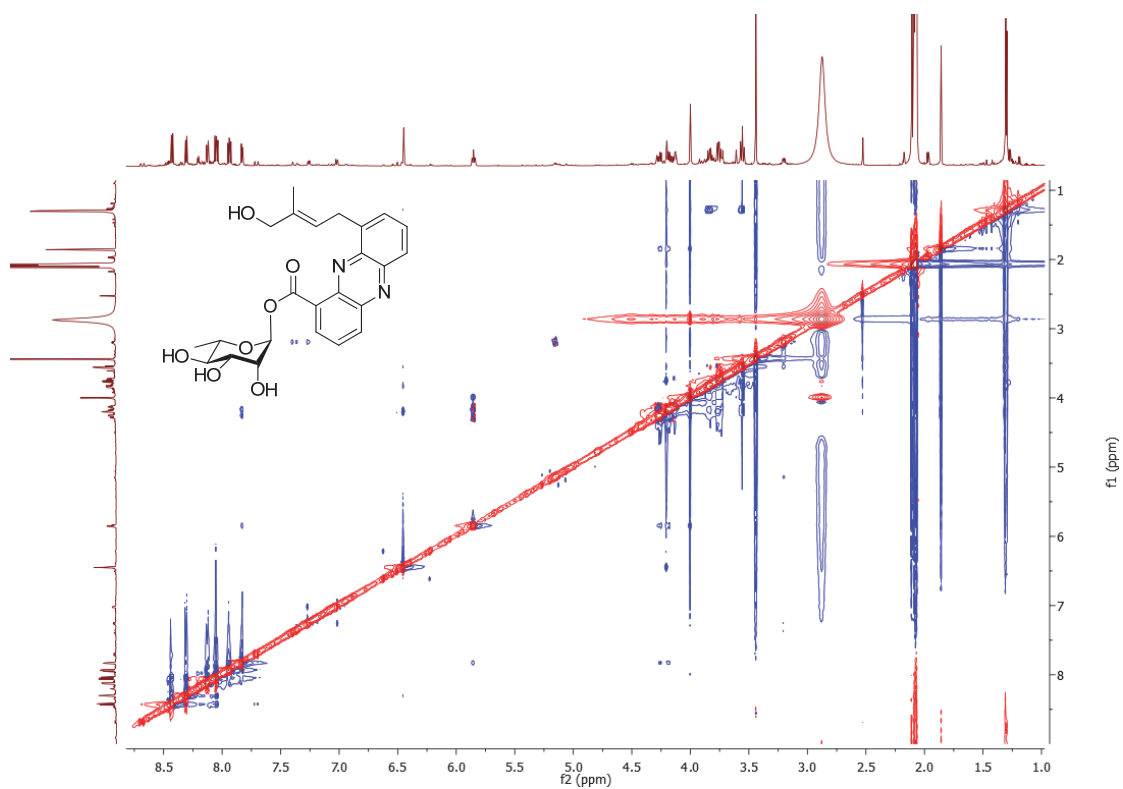
Hm125-10 #464 RT: 8.02 AV: 1 NL: 8.18E5
T: FTMS + c ESI Full ms [100.00-1000.00]



S8. IR spectrum of endophenaside A (**1**).S9. UV spectrum of endophenaside A (**1**).

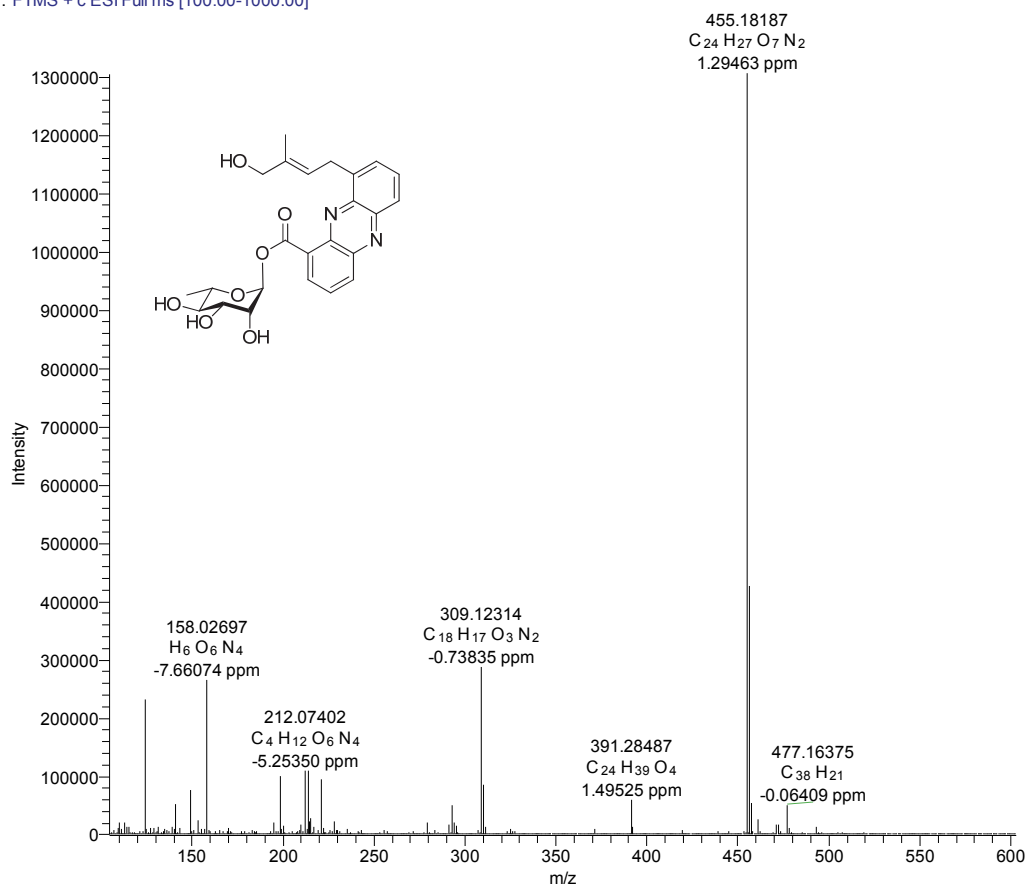
S10. ^1H NMR spectrum (500 MHz) of endophenaside B (**2**) in CD_3OD .S11. APT spectrum of endophenaside B (**2**) in CD_3OD .

S12. HSQC spectrum of endophenaside B (**2**) in CD₃OD.S13. HMBC spectrum of endophenaside B (**2**) in CD₃OD.

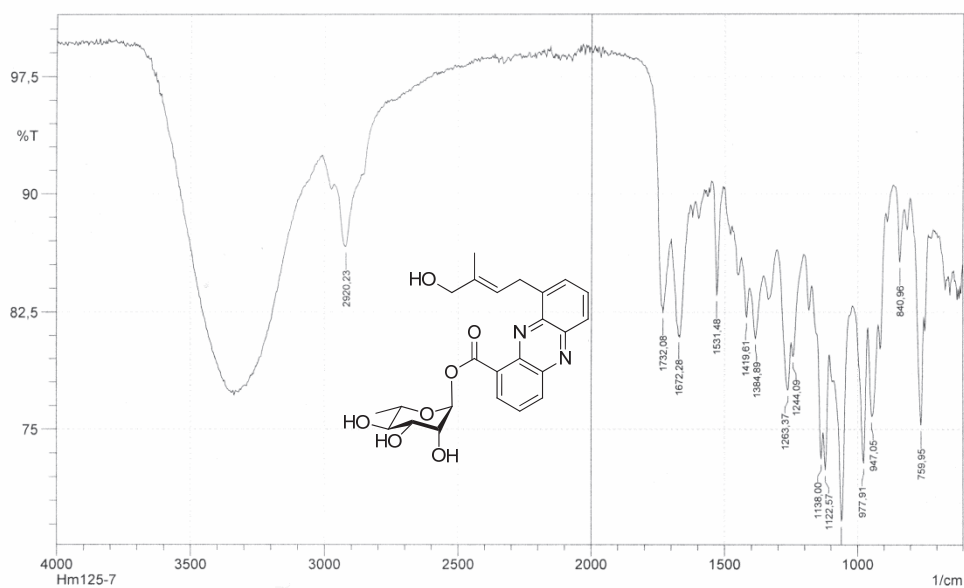
S14. ^1H - ^1H COSY spectrum of endophenaside B (**2**) in CD_3OD .S15. ^1H - ^1H NOSEY spectrum of endophenaside B (**2**) in acetone- d_6 .

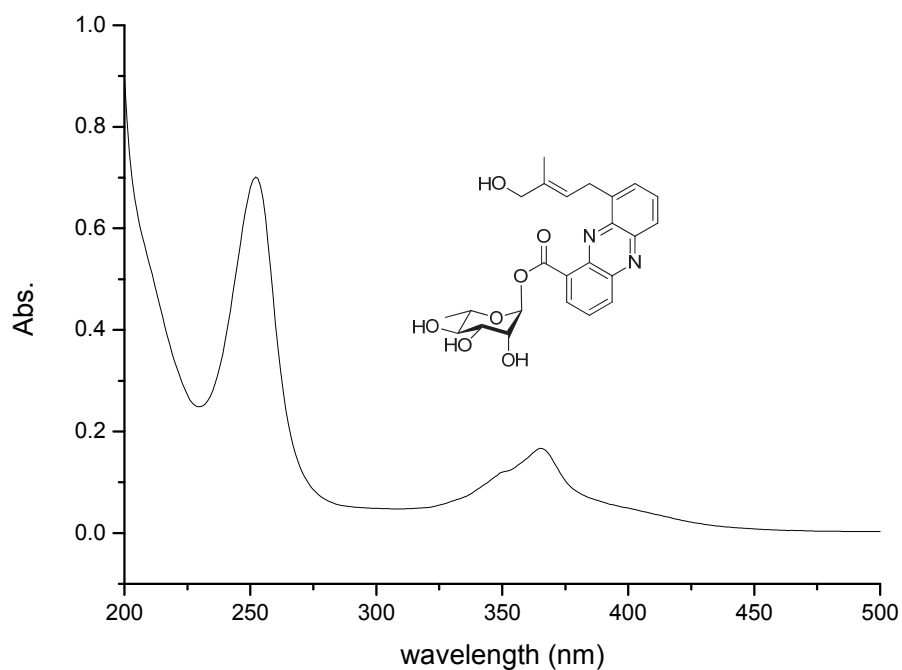
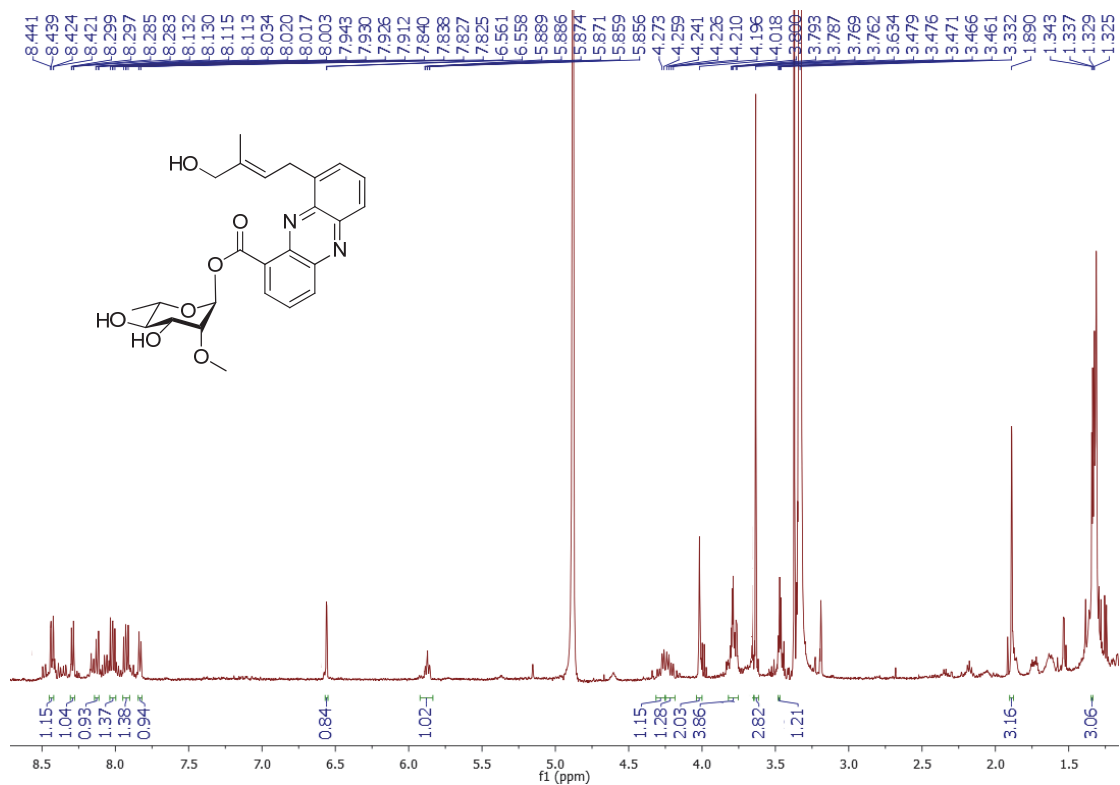
S16. HRMS spectrum of endophenaside B (2).

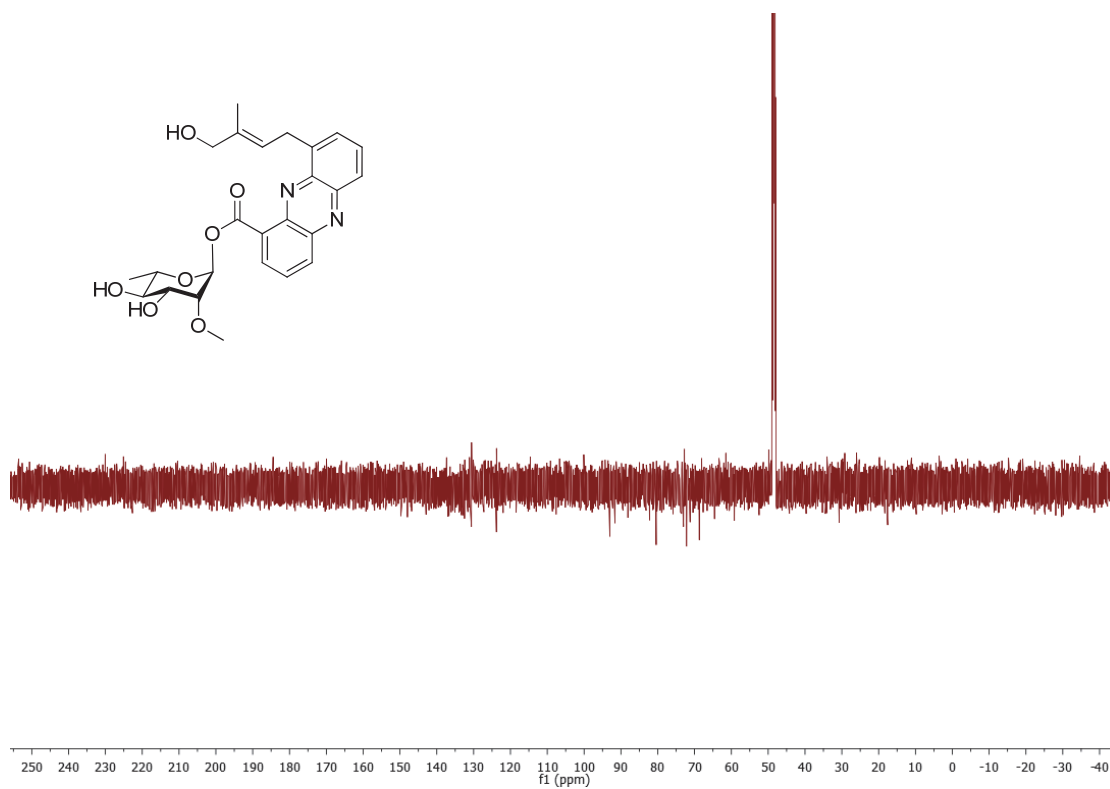
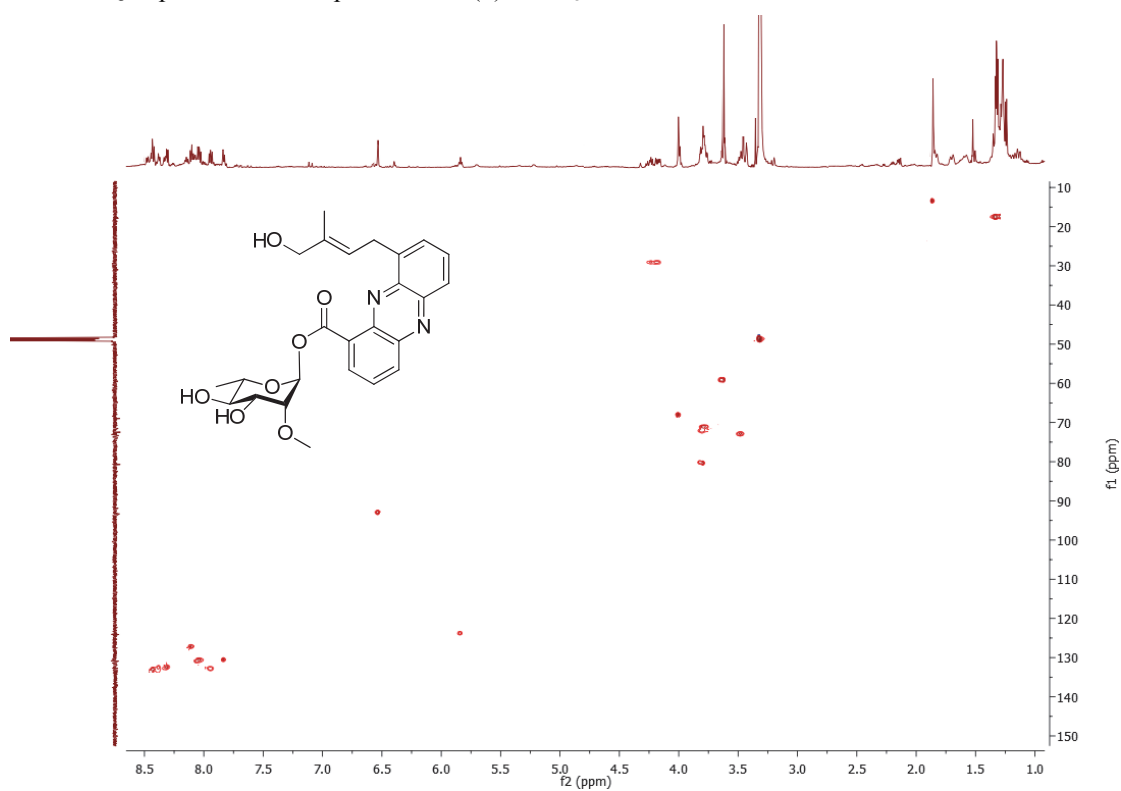
Hm125-7 #555-592 RT: 9.61-10.24 AV: 38 NL: 1.30E6
T: FTMS + c ESI Full ms [100.00-1000.00]

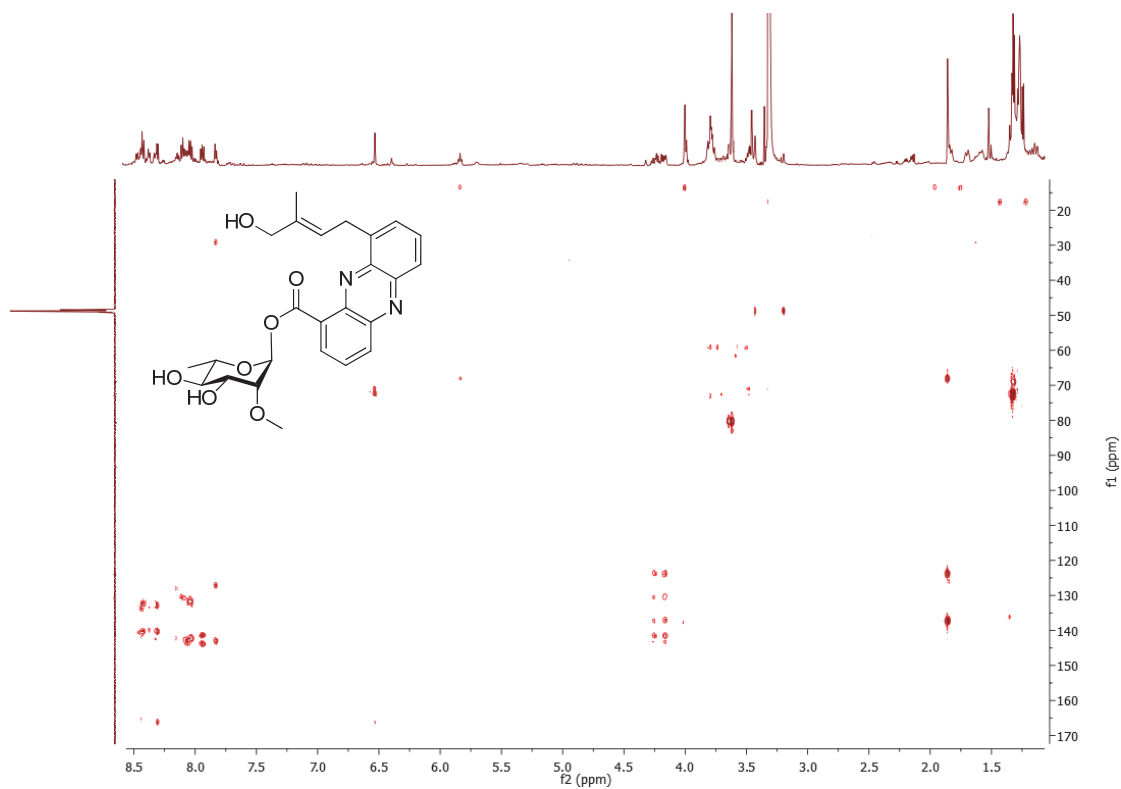
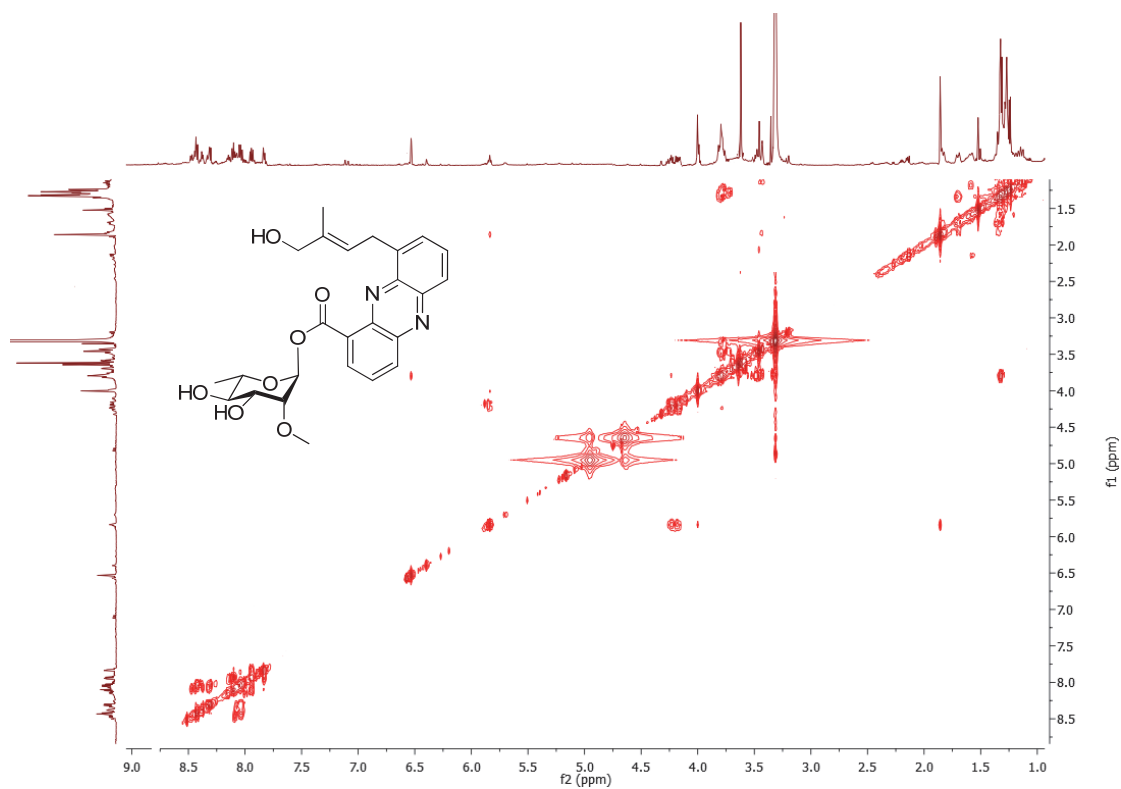


S17. IR spectrum of endophenaside B (2).



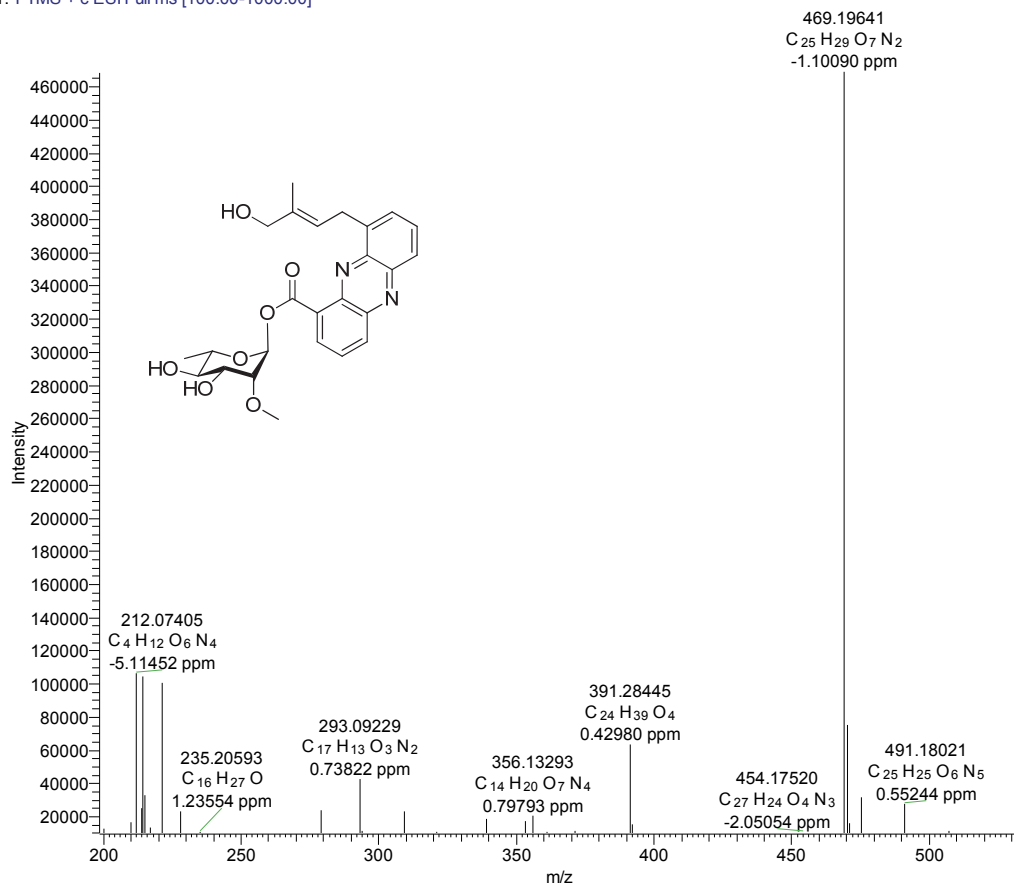
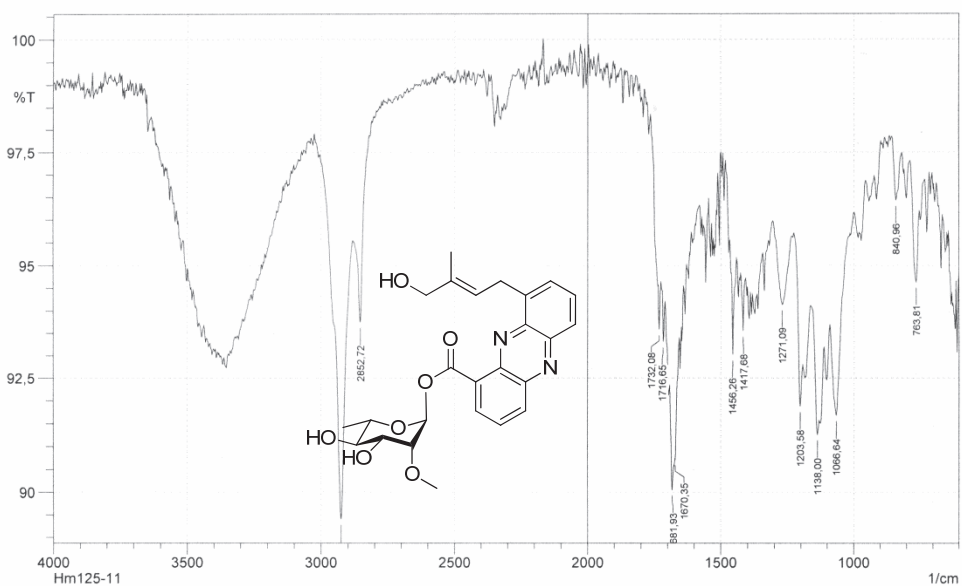
S18. UV spectrum of endophenaside B (**2**).S19. ^1H NMR spectrum (500 MHz) of endophenaside C (**3**) in CD_3OD .

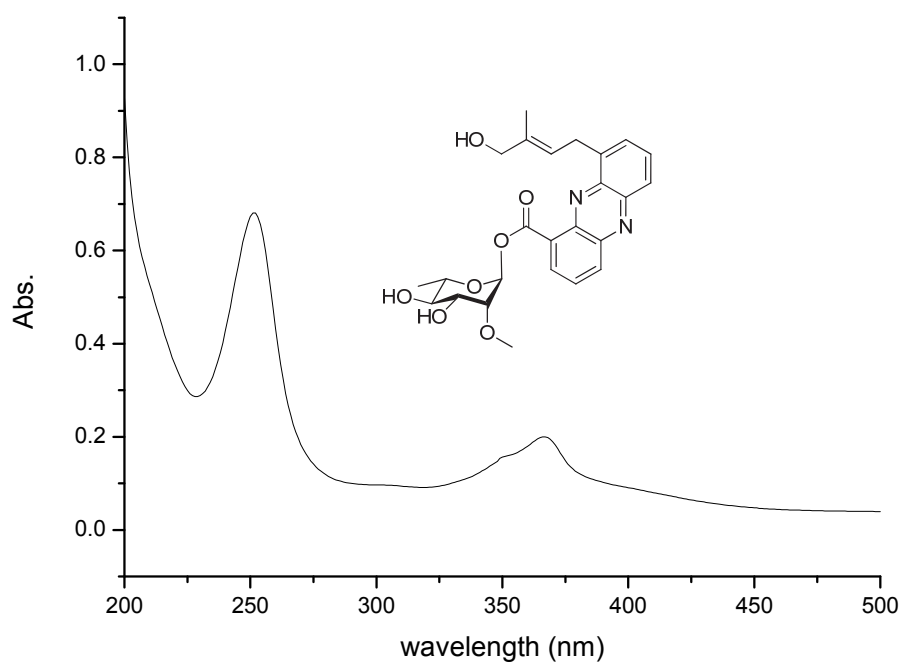
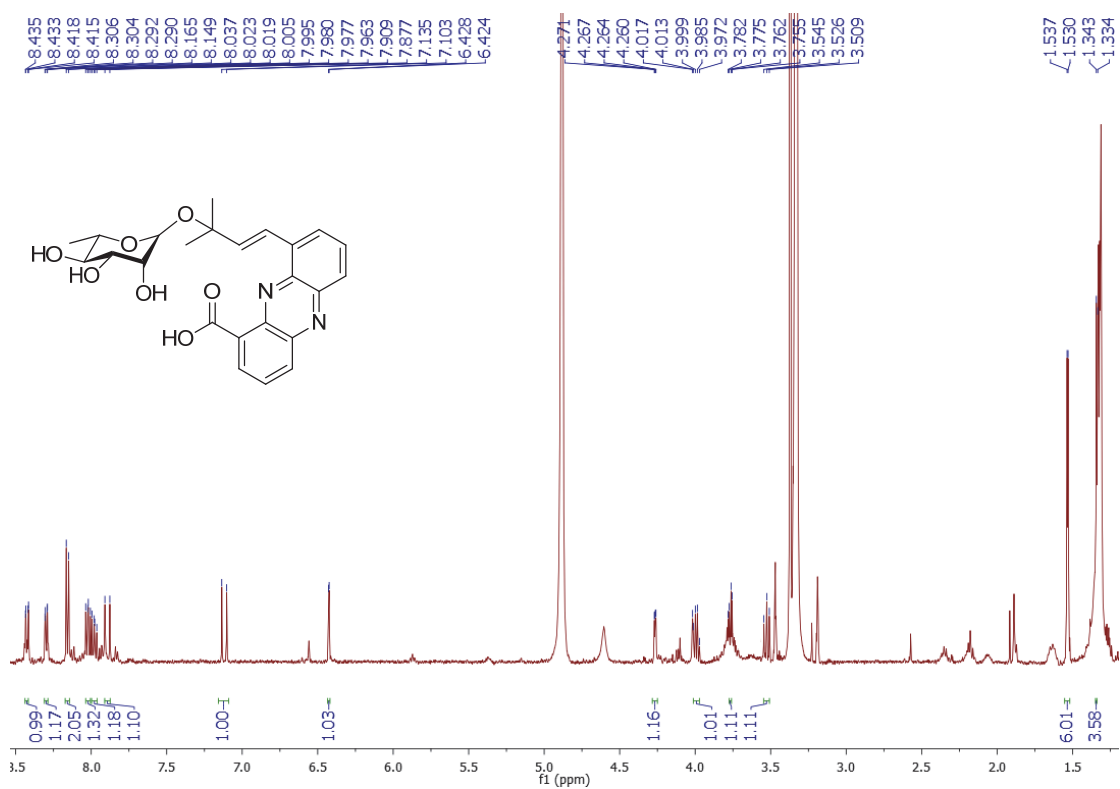
S20. APT spectrum of endophenaside C (**3**) in CD₃OD.S21. HSQC spectrum of endophenaside C (**3**) in CD₃OD.

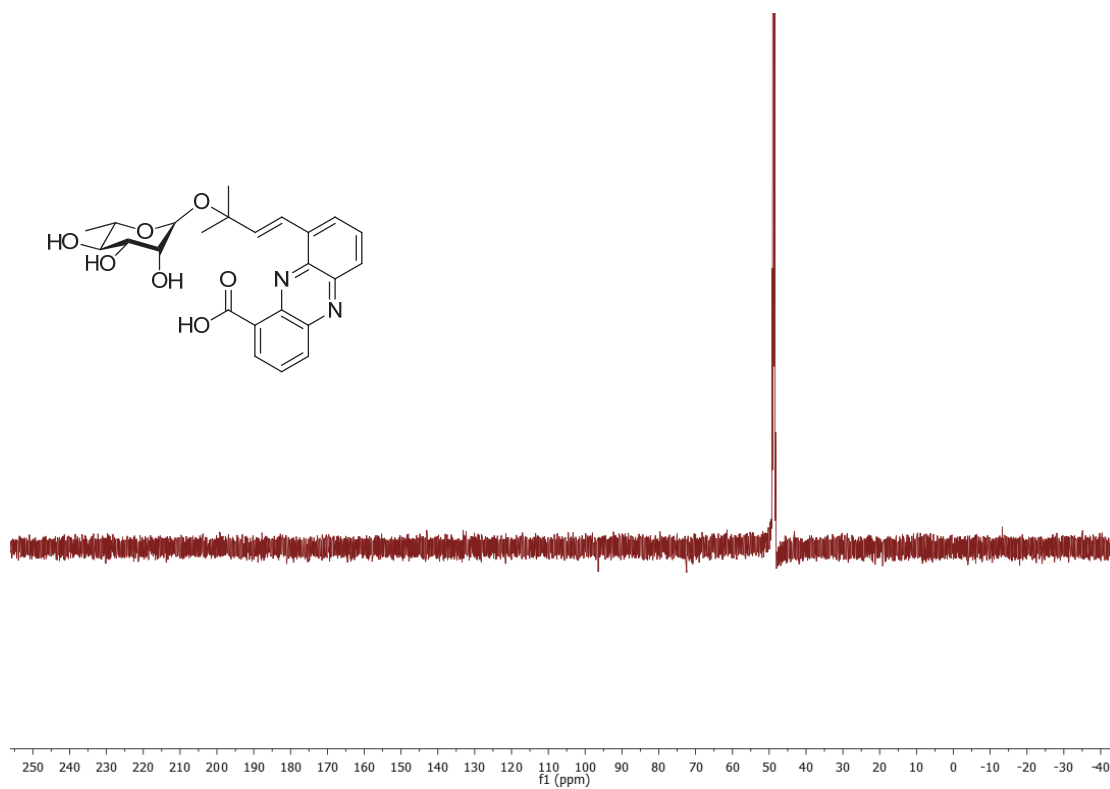
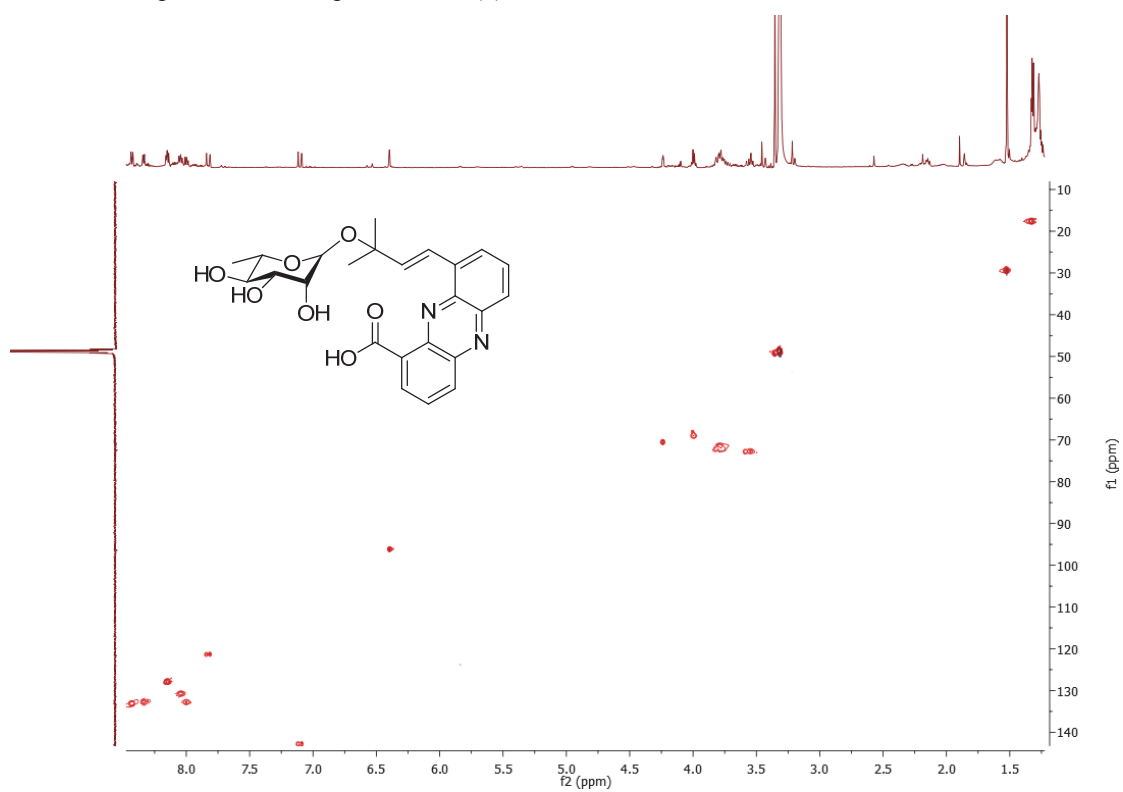
S22. HMBC spectrum of endophenaside C (**3**) in CD₃OD.S23. ¹H-¹H COSY spectrum of endophenaside C (**3**) in CD₃OD.

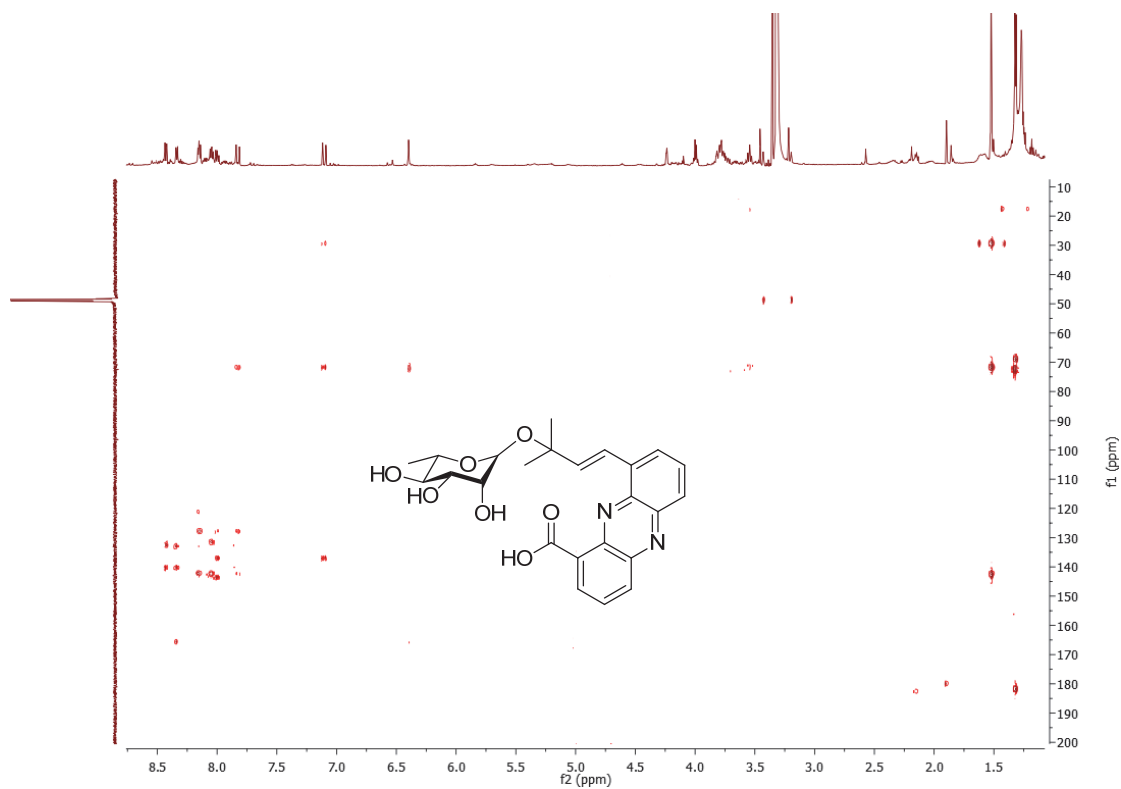
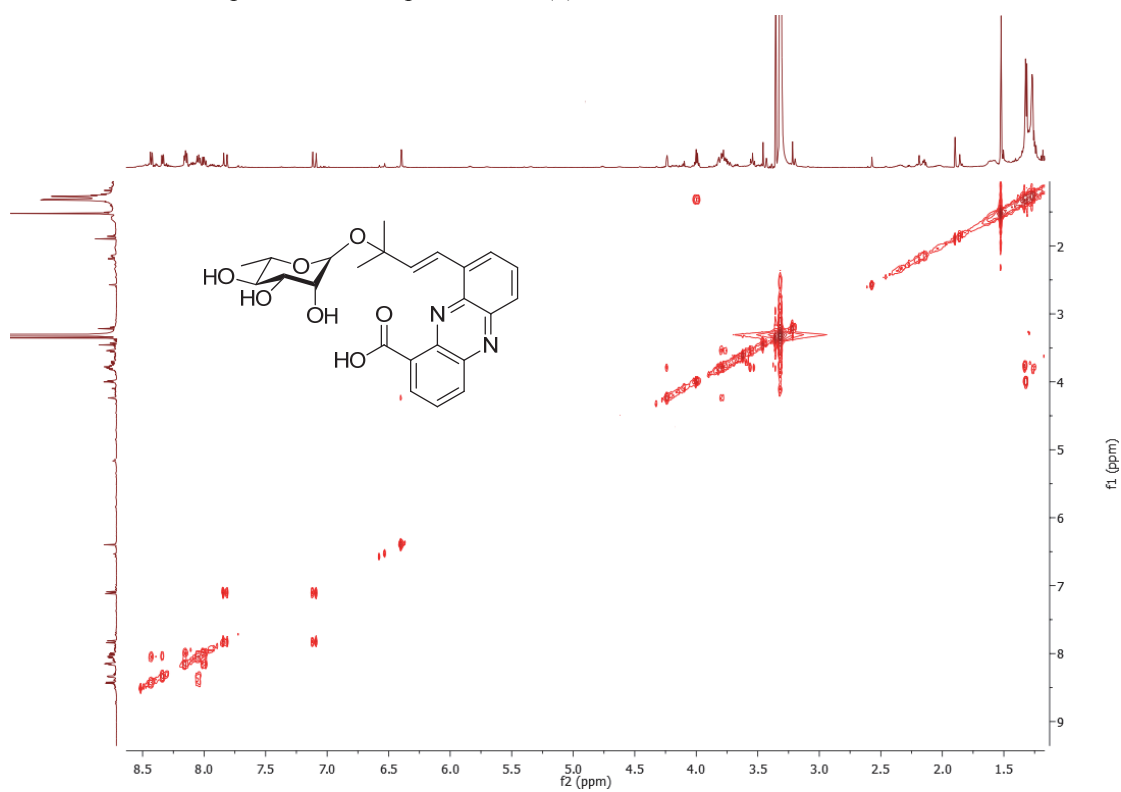
S24. HRMS spectrum of endophenaside C (**3**).

Hm125-11 #588 RT: 10.17 AV: 1 NL: 4.68E5
T: FTMS + c ESI Full ms [100.00-1000.00]

S25. IR spectrum of endophenaside C (**3**).

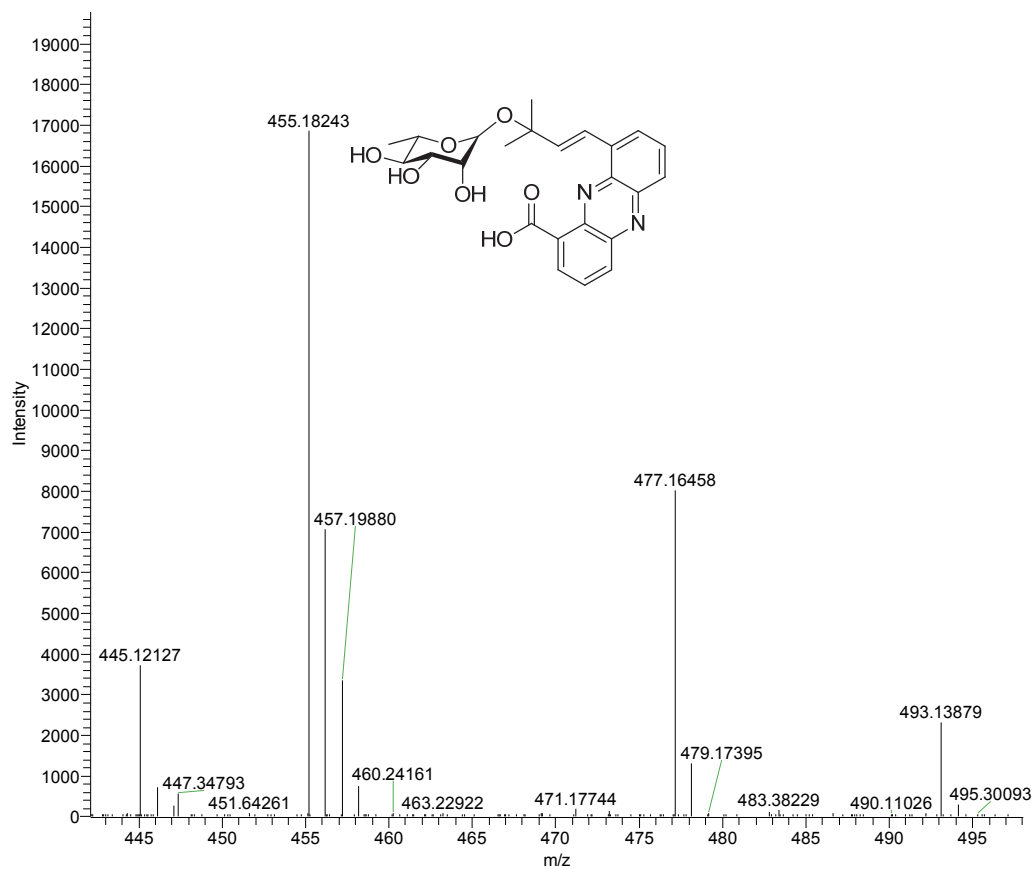
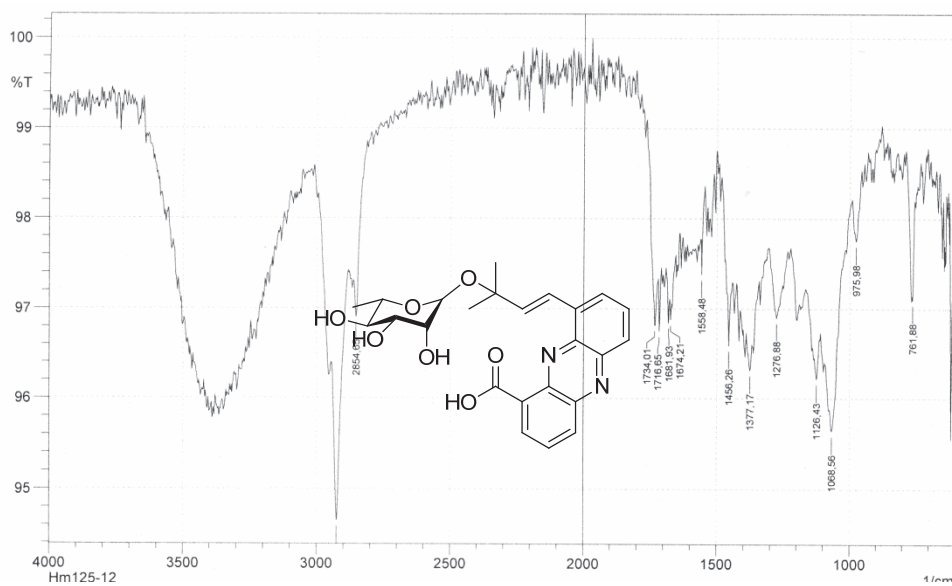
S26. UV spectrum of endophenaside C (**3**).S27. ^1H NMR spectrum (500 MHz) of endophenaside D (**4**) in CD_3OD .

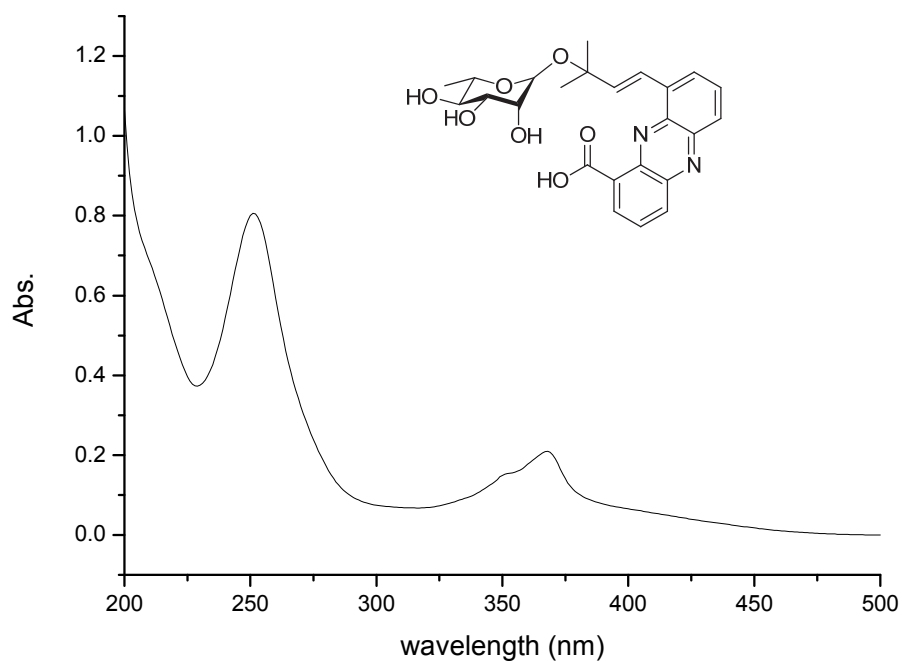
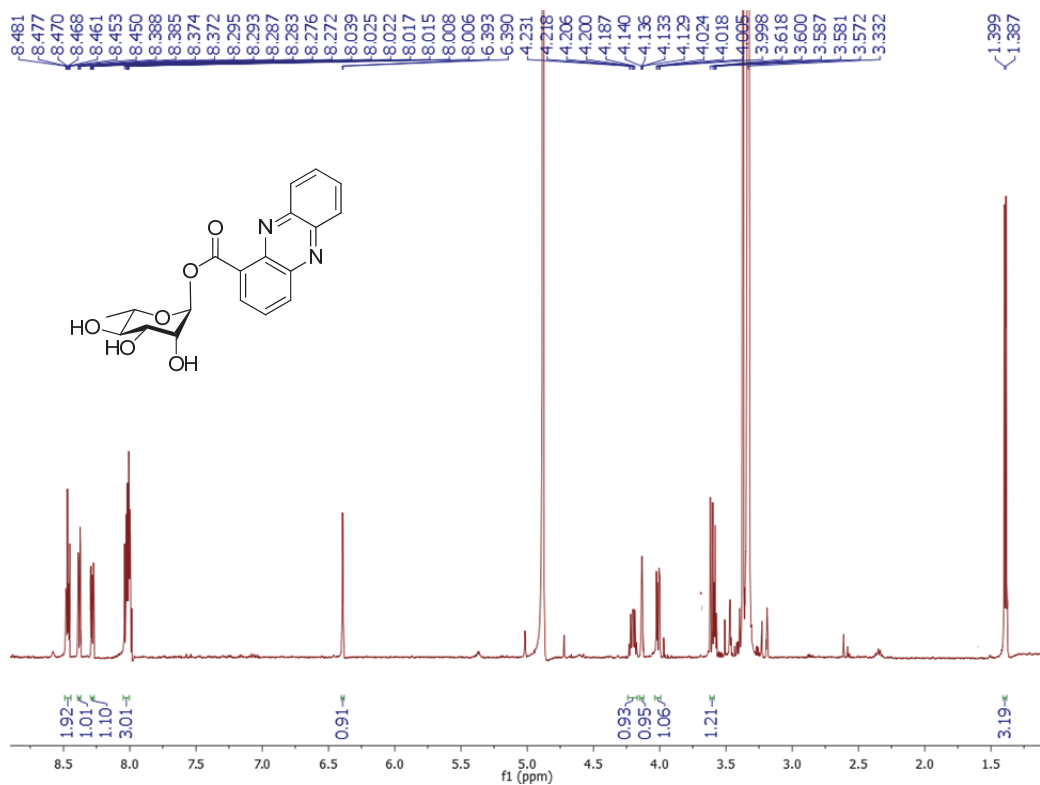
S28. APT spectrum of endophenaside D (**4**) in CD₃OD.S29. HSQC spectrum of endophenaside D (**4**) in CD₃OD.

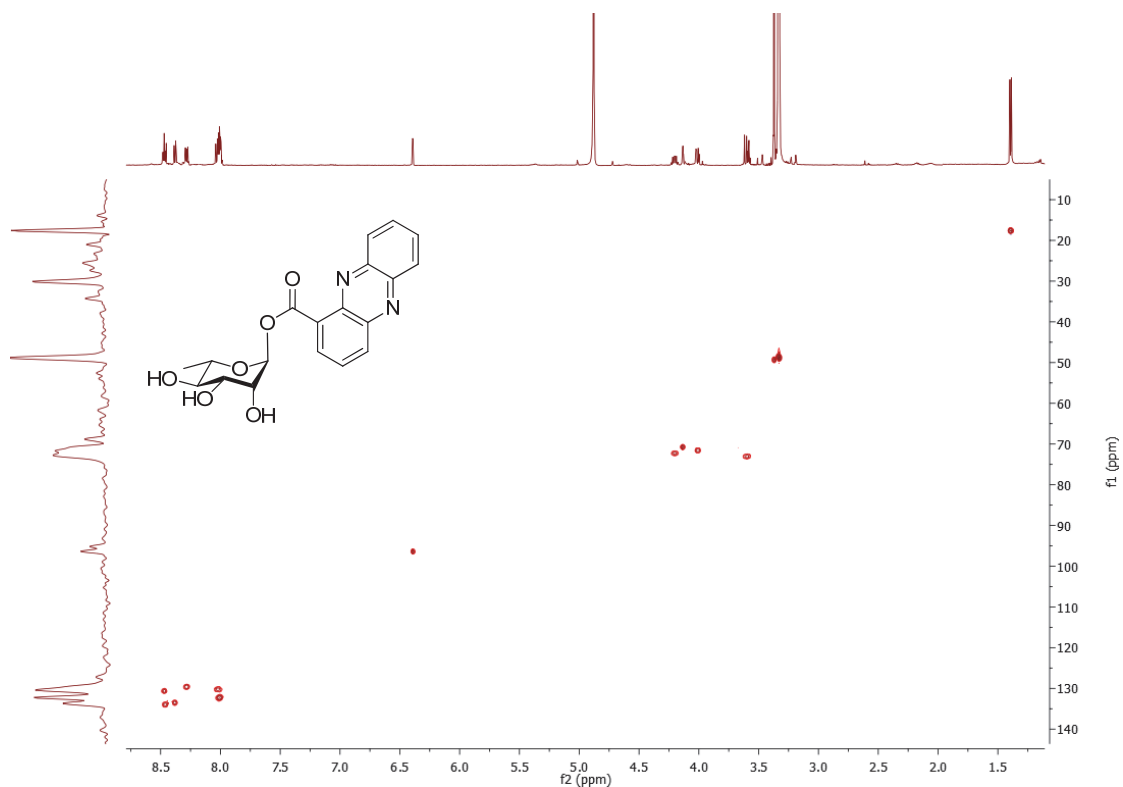
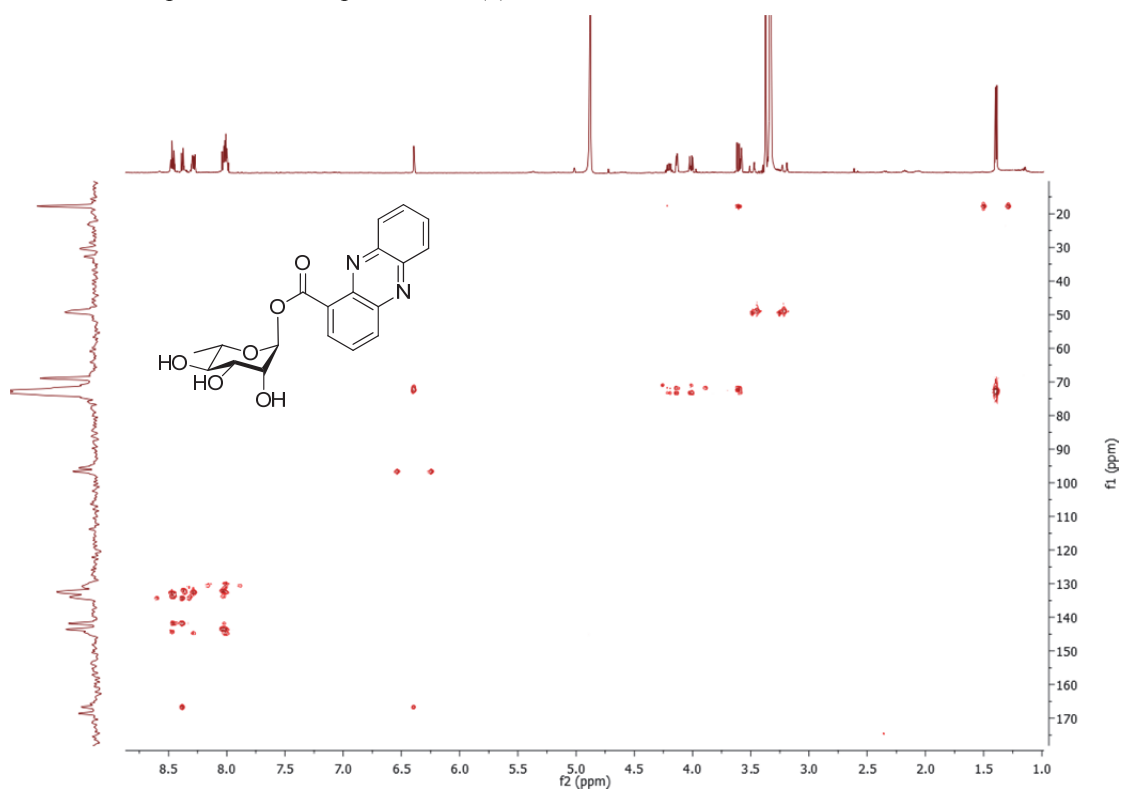
S30. HMBC spectrum of endophenaside D (**4**) in CD₃OD.S31. ¹H-¹H COSY spectrum of endophenaside D (**4**) in CD₃OD.

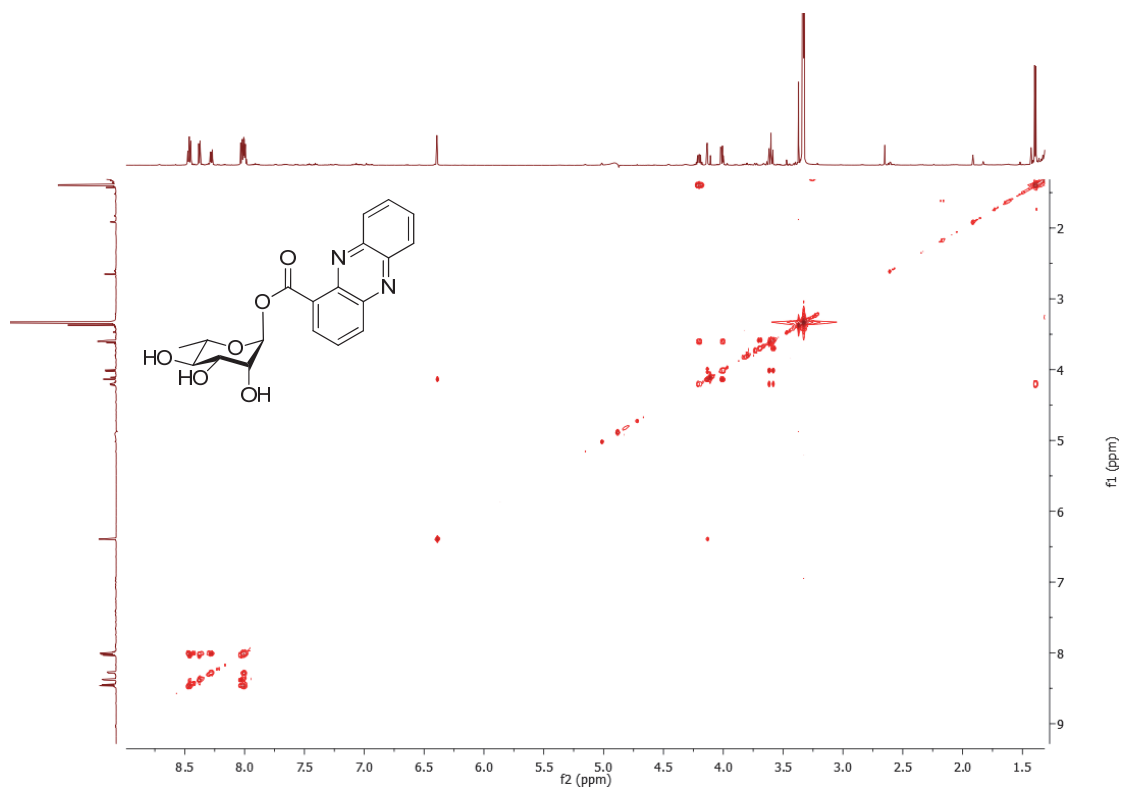
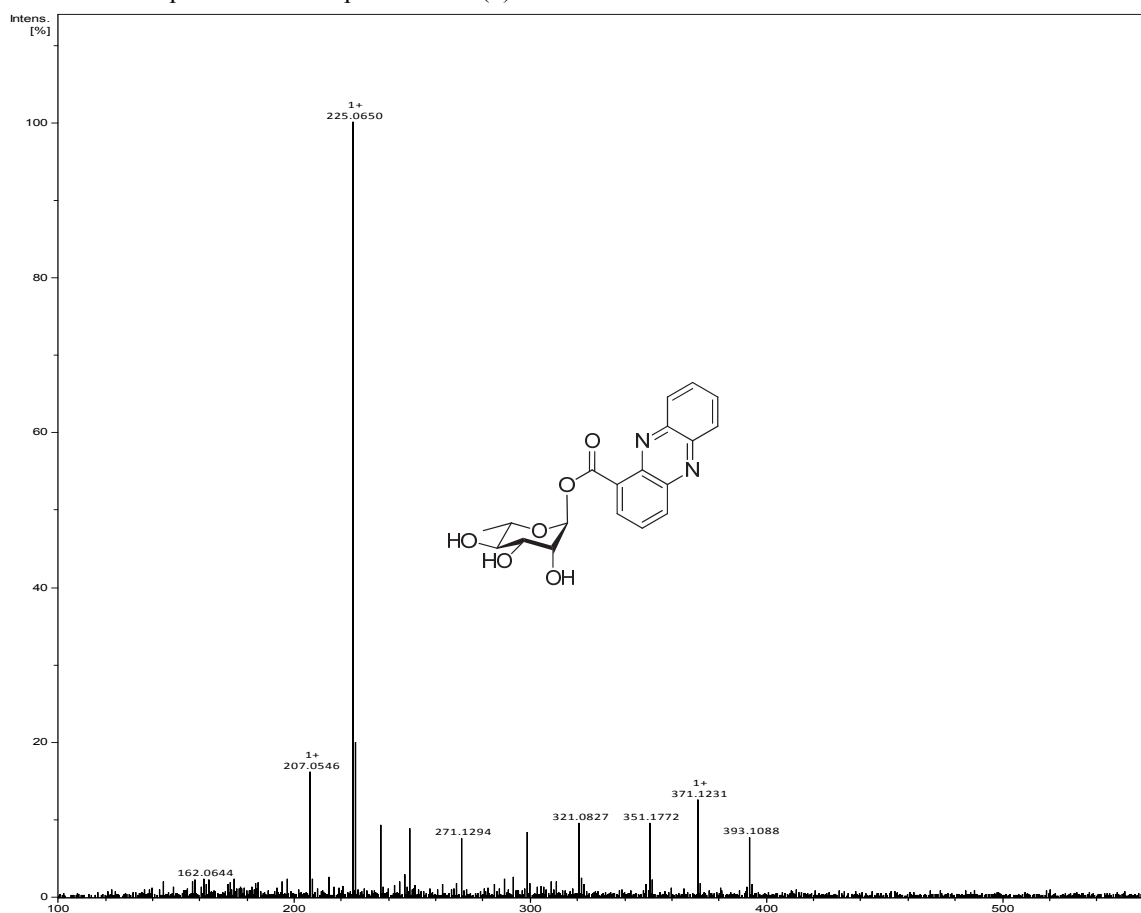
S32. HRMS spectrum of endophenaside D (**4**).

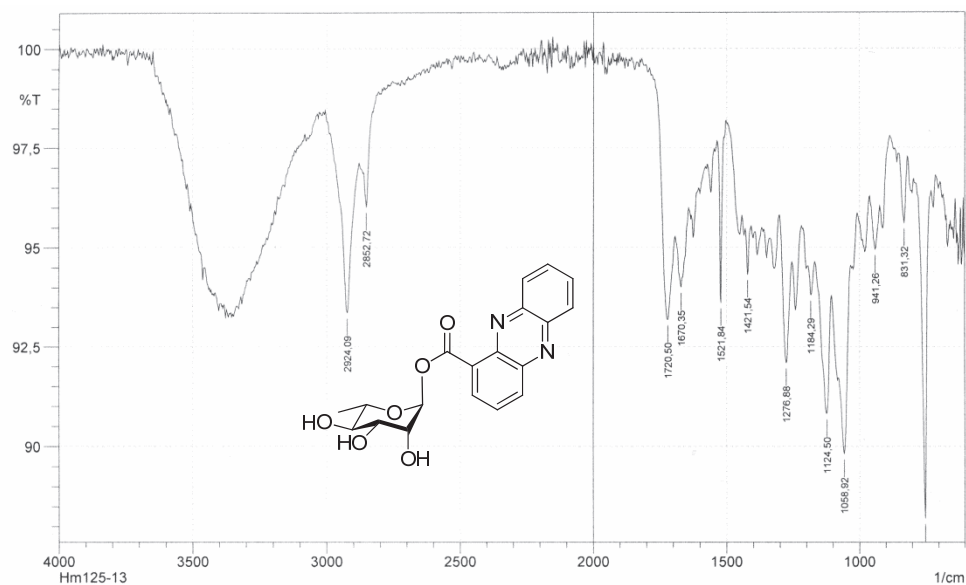
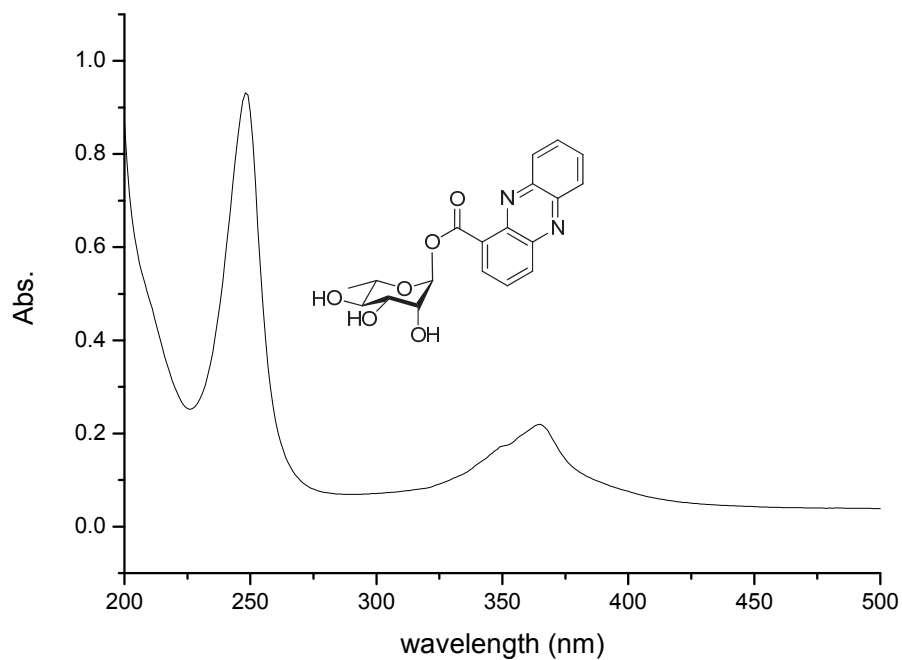
Hm125-12 #554-578 RT: 9.62-10.03 AV: 25 NL: 1.68E4
T: FTMS + c ESI Full ms [100.00-1000.00]

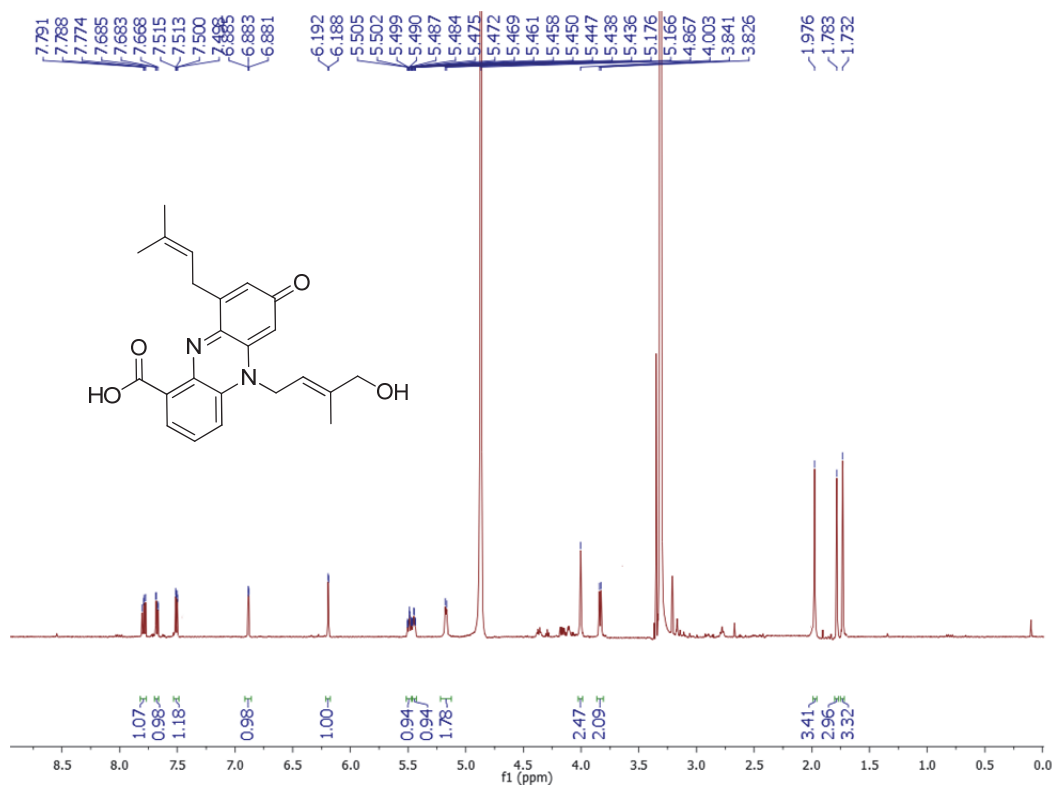
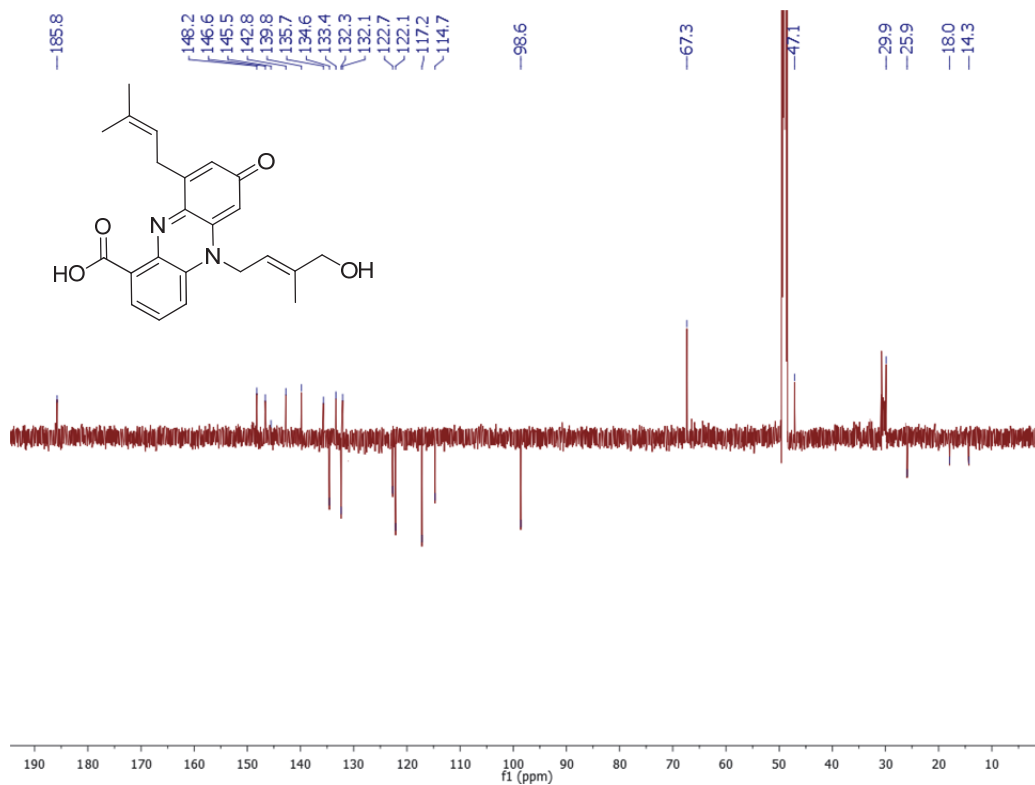
S33. IR spectrum of endophenaside D (**4**).

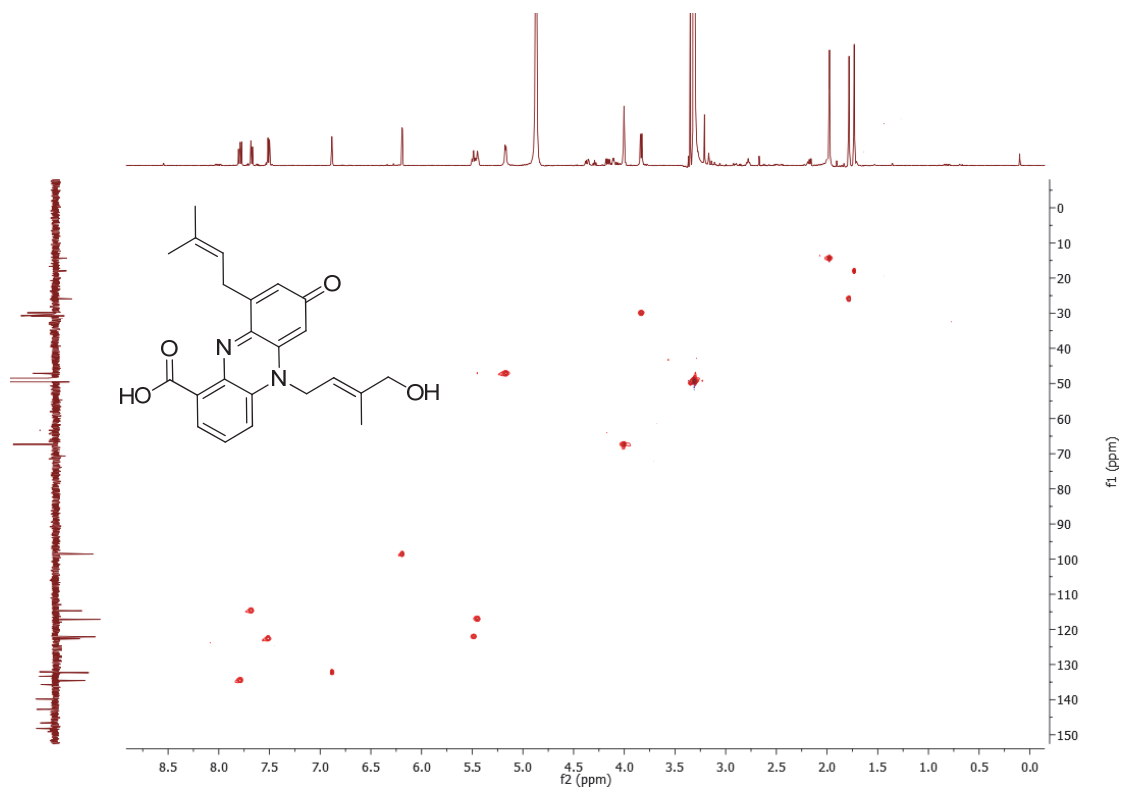
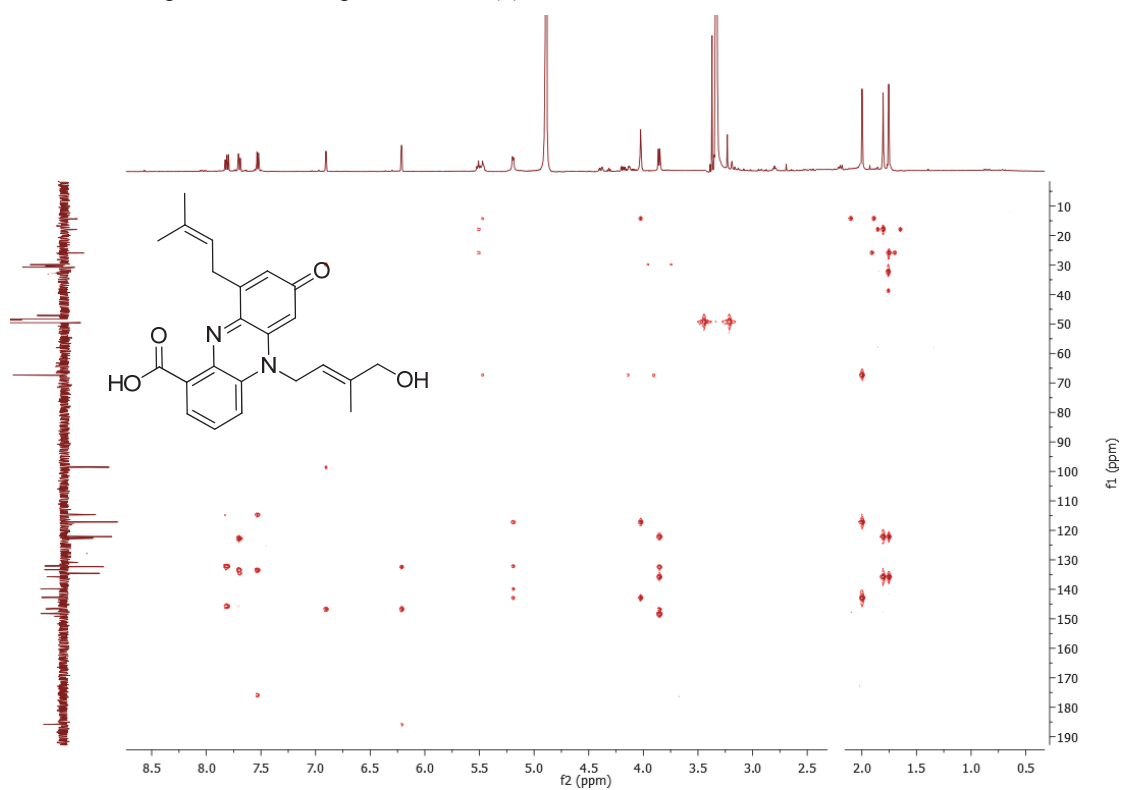
S34. UV spectrum of endophenaside D (**4**).S35. ^1H NMR spectrum (500 MHz) of endophenaside E (**5**) in CD_3OD .

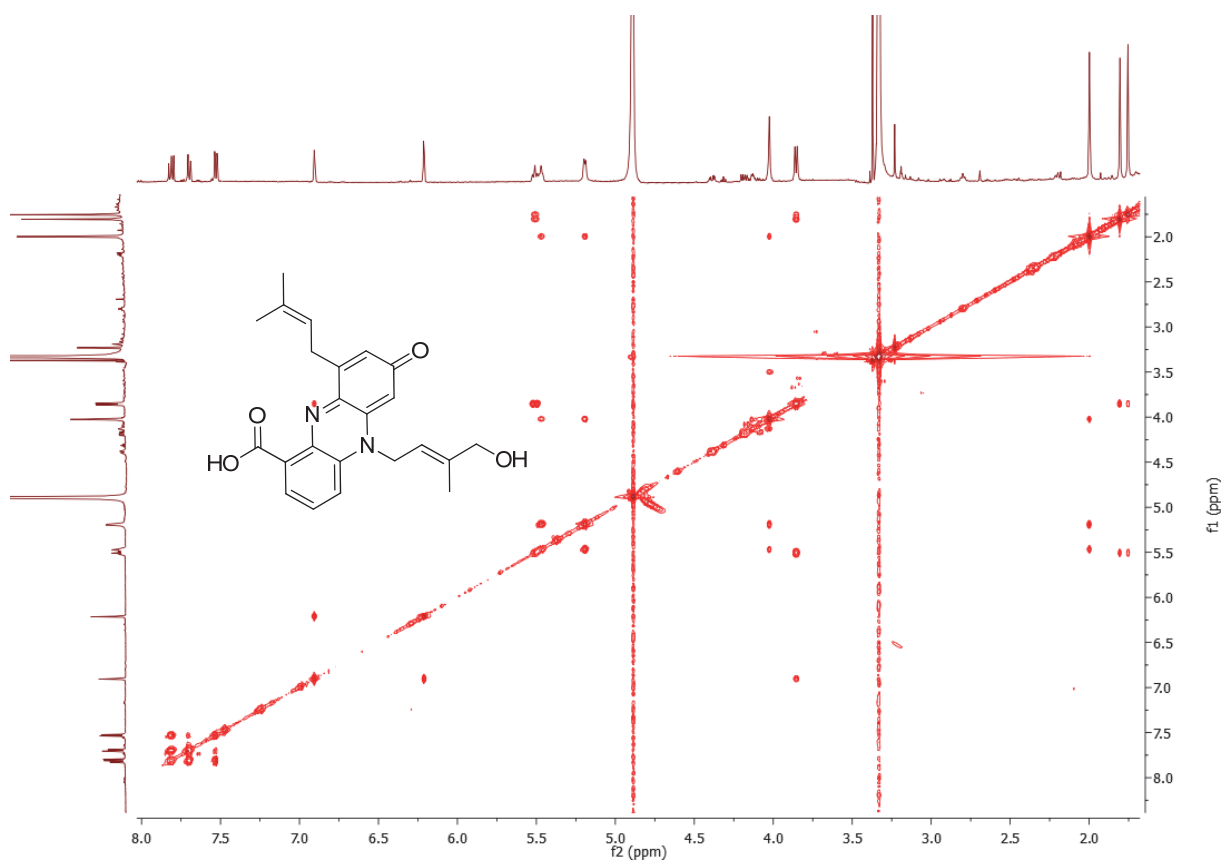
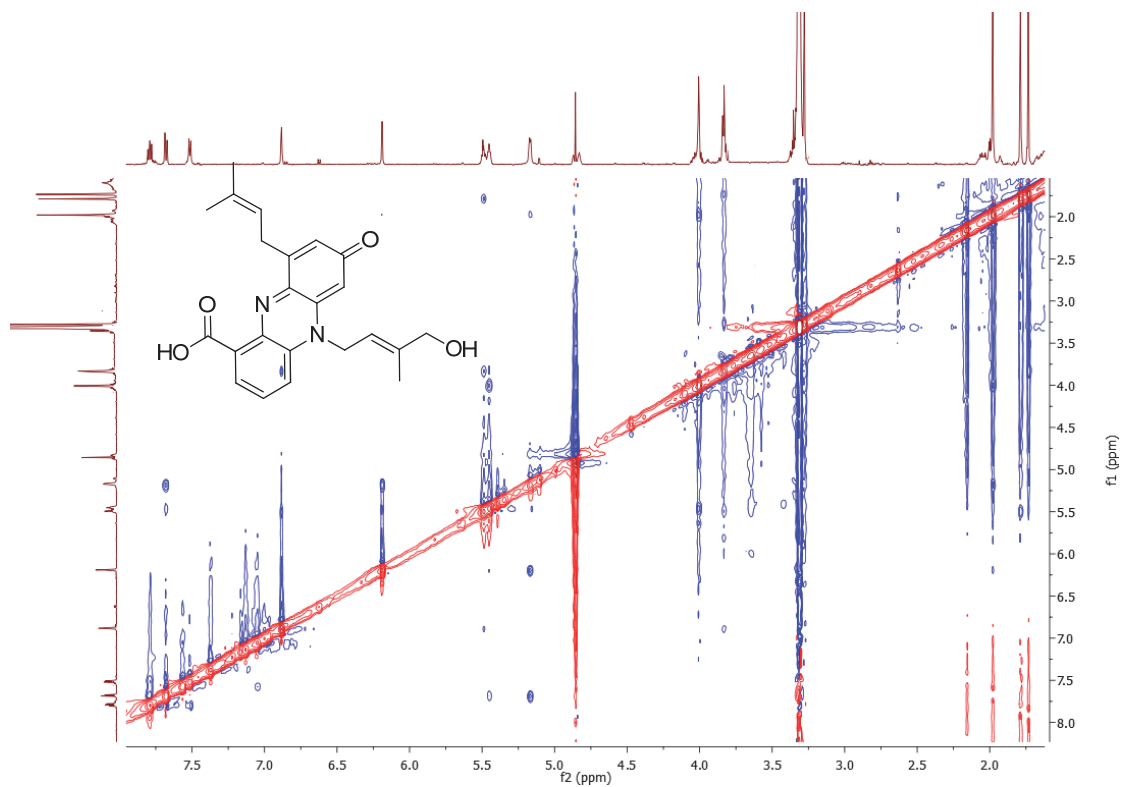
S36. HSQC spectrum of endophenaside E (**5**) in CD₃OD.S37. HMBC spectrum of endophenaside E (**5**) in CD₃OD.

S38. ^1H - ^1H COSY spectrum of endophenaside E (**5**) in CD_3OD .S39. HRMS spectrum of endophenaside E (**5**).

S40. IR spectrum of endophenaside E (**5**).S41. UV spectrum of endophenaside E (**5**).

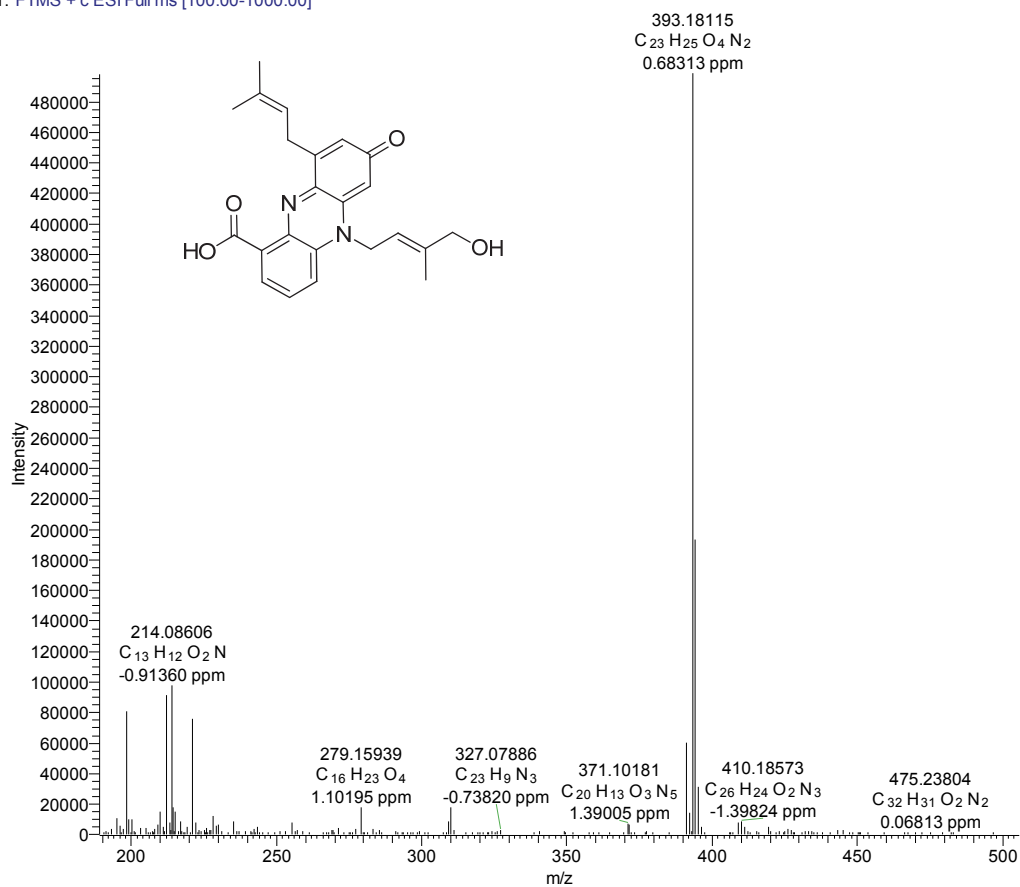
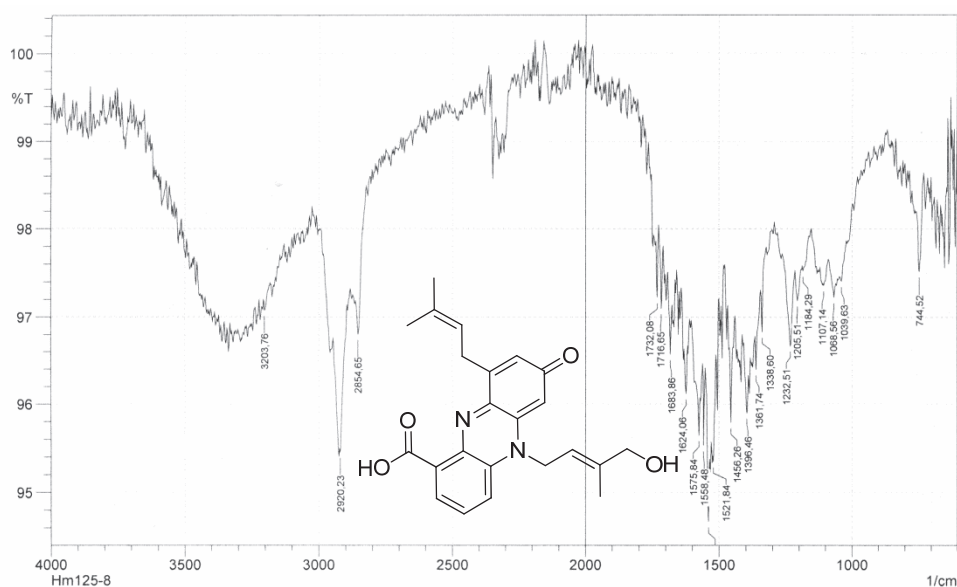
S42. ^1H NMR spectrum (600 MHz) of endophenazine F1 (**6**) in CD_3OD .S43. APT spectrum of endophenazine F1 (**6**) in CD_3OD .

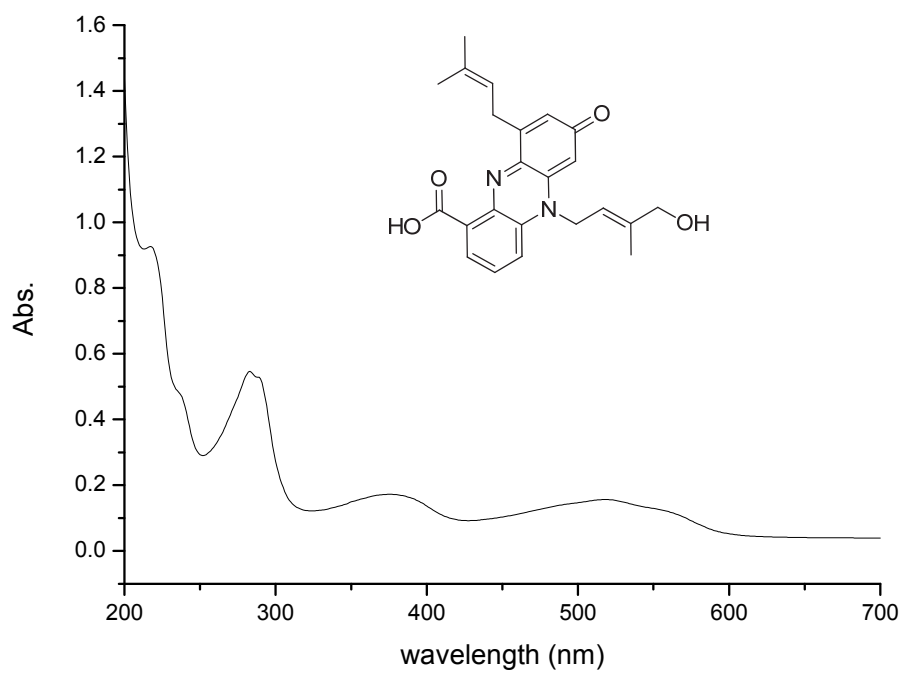
S44. HSQC spectrum of endophenazine F1 (**6**) in CD₃OD.S45. HMBC spectrum of endophenazine F1 (**6**) in CD₃OD.

S46. ^1H - ^1H COSY spectrum of endophenazine F1 (**6**) in CD_3OD .S47. ^1H - ^1H NOSEY spectrum of endophenazine F1 (**6**) in CD_3OD .

S48. HRMS spectrum of endophenazine F1 (**6**).

Hm125-8 #673 RT: 11.64 AV: 1 NL: 4.98E5
T: FTMS + c ESI Full ms [100.00-1000.00]

S49. IR spectrum of endophenazine F1 (**6**).

S50. UV spectrum of endophenazine F1 (**6**).

Appendix VII: Supplementary Information belonging to Chapter 10

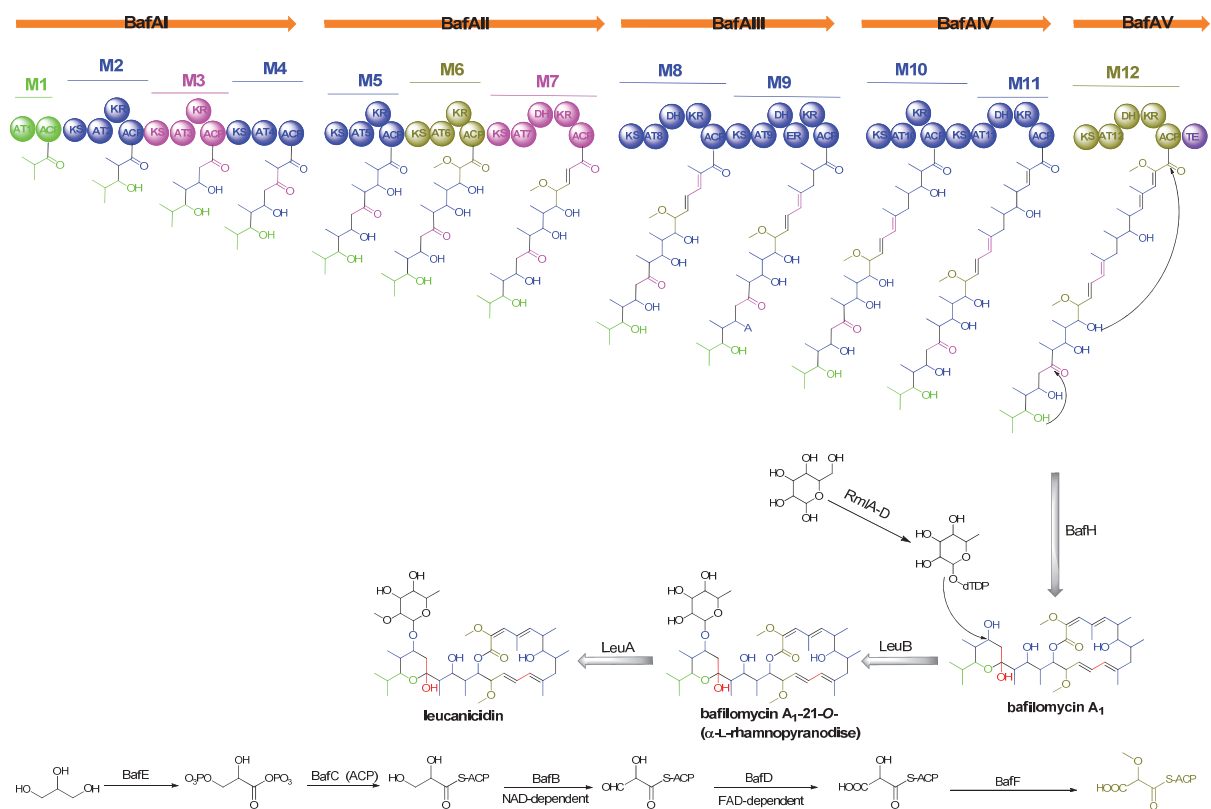


Figure S1. Biosynthetic pathway of leucanicidin. The domain organization of the PKS encoded by ORFs BafAI to BafAV is annotated in Table 1. Each circle represents an enzymatic domain in the PKS multifunctional protein. ACP, acyl carrier protein; KS, β -ketoacyl-ACP synthase; AT, acyltransferase; KR, β -ketoacyl ACP reductase; DH, β -hydroxyl-thioester dehydratase; ER, enoyl reductase; TE, thioesterase. The module with different color means the module encompass acyltransferase domain for specific building block respectively, via isobutyrate (green), malonate (red), methylmalonate (blue), and methoxymalonate (brown).

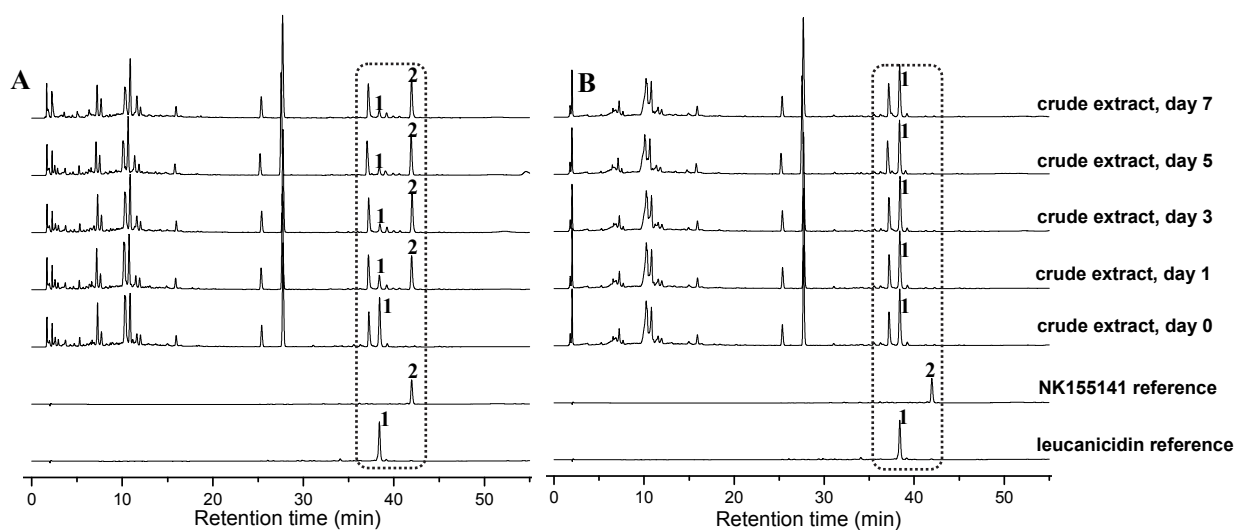


Figure S2. HPLC-UV profiling showing that NK155141 is an artifact. *Kitasatospora* sp. MBT66 crude extract was separately dissolved in methanol (A) and in acetonitrile (B). The methylation of leucanicidin in respective solvent was conducted within one week, which was examined by HPLC-UV (254 nm) in time-dependent manner.

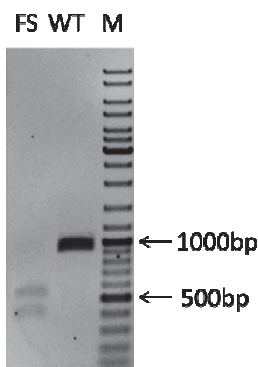


Figure S3. Verification of the frame-shift mutation in *leuB* by PCR. The -370/+584 region of *leuB* was amplified by PCR and separated electrophoretically on a 1.2% agarose gel in 1xTAE buffer. FS, PCR-amplified DNA fragment from one of ex-conjugants; WT, PCR-amplified *leuB* obtained from wild-type *Kitasatospora* sp. MBT66; M, DNA marker. 500 bp and 1000 bp bands of the DNA marker are indicated.

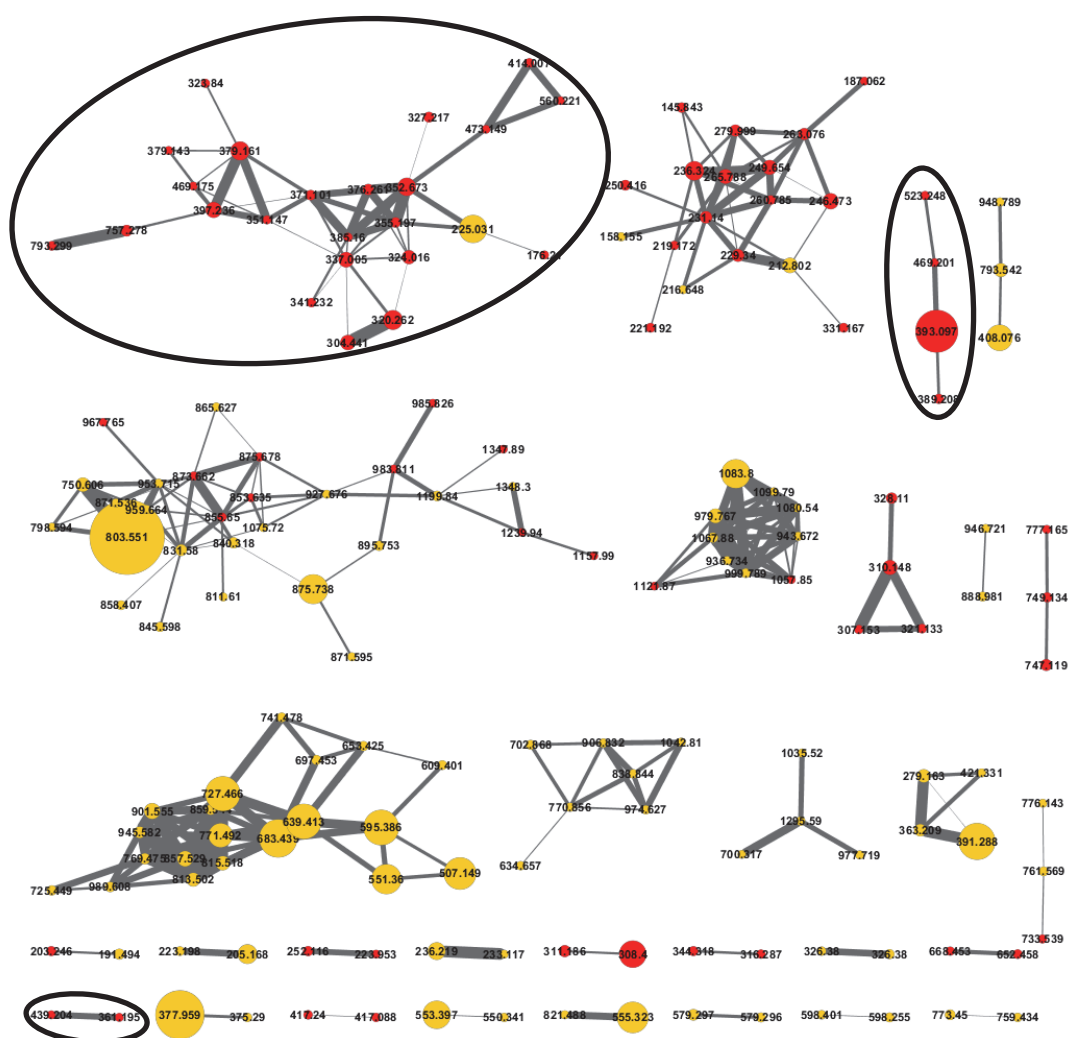


Figure S4. Molecular networking for all secondary metabolites harvested from *Kitasatospora* sp. MBT66 grown on MM + 1% glucose. Yellow nodes represent compounds that were simultaneously observed in the control experiment (blank culture medium) and bacterial extracts, while red nodes were exclusively found in the MBT66 extract. Circled are subnetworks for endophenazines and/or endophenazines.

Table S1. Oligonucleotides used in construction of *leuB* mutants using the CRISPR/Cas9 system

primer	sequence (5'→3') [#]
LeuB_spacer_For	ACGCTTCACCGACGACCGCCAGAG
LeuB_spacer_Rev	AAACCTCTGGCGGTCGTCGGTGAA
LeuB_LF-976_EX	CAGTGAATTCTCTAGATCACCTGGCACGGCATCGAG
LeuB_LR+33_H	CTGACATGAGAAAGCTTGAAGACATGGCCGTGGGCCGGGAT
LeuB_FS_RF+47_H	CTGACAGTAGAAGCTTCGTCGGTGAACTGGTACGCCG
LeuB_RR+1032_EX	CTGAGAATTCTCTAGACAGCAGGCTCACGTCGACCTCC
LeuB_F-370	CAACTGCTCGACGGCCTCAAC
LeuB_R+584	TCGTCGAAGCTCTCGCCGTAC

[#]Restriction sites used for cloning are underlined and in bold face. GAATTC, EcoRI; TCTAGA, XbaI; AAGCTT, HindIII.

Table S2. Signal intensities for the masses of the secondary metabolites harvested from *Kitasatospora* sp. MBT66 grown on MM + 1% glucose.

Mass	Intensity	Mass	Intensity	Mass	Intensity	Mass	Intensity
145.843	611720	331.167	390584	634.657	213764	865.627	204844
158.155	149132	337.005	2254280	639.413	8876750	871.536	58668
176.21	113348	341.232	281596	652.458	327564	871.595	105668
187.062	55008	344.318	458592	653.425	442716	873.662	197120
191.494	777504	351.147	131356	668.453	197540	875.678	181480
203.246	442216	352.673	3224250	683.439	9768180	875.738	6980100
205.168	3631080	355.197	638224	697.453	458052	888.981	397808
212.802	2256640	361.195	90196	700.317	48700	895.753	495132
216.648	103164	363.209	1276040	702.868	383580	901.555	2490700
219.172	265696	371.101	633928	725.449	508188	906.832	209588
221.192	383768	375.29	206656	727.466	8745590	927.676	83500
223.198	331496	376.261	912612	733.539	144124	936.734	171248
223.953	70096	377.959	1.33E+07	741.478	395956	943.672	191484
225.031	6403020	379.143	66380	747.119	1072710	945.582	1080510
229.34	1403060	379.161	3433840	749.134	518052	946.721	120304
231.14	1317040	385.16	249796	750.606	1882480	948.789	201756
233.117	24360	389.208	506440	757.278	1005690	953.715	269248
236.219	3001340	391.288	9285400	759.434	201568	959.664	150388
236.324	3589910	393.097	1.12E+07	761.569	309696	967.765	19080
246.473	2493900	397.236	2480790	769.475	869976	974.627	194080
249.654	2102040	408.076	5840700	770.856	215160	977.719	111076
250.416	690616	414.007	318992	771.492	5210540	979.767	1996310
252.116	499196	417.088	206896	773.45	644072	983.811	121616
260.785	368800	417.24	267976	776.143	65944	985.826	22652
263.076	1118620	421.331	73448	777.165	84452	989.608	596864
265.788	2099370	439.204	56852	793.299	329708	999.789	402352

279.163	1632550	469.175	65140	793.542	1721760	1035.52	62748
279.999	1153380	469.201	128208	798.594	85912	1042.81	116364
304.441	2211310	473.149	66048	803.551	2.19E+07	1057.85	38840
307.153	249204	507.149	7845160	811.61	58148	1067.88	606872
308.4	6145340	523.248	216752	813.502	1785100	1075.72	49324
310.148	2129790	550.341	160000	815.518	1924600	1080.54	24352
311.186	1143310	551.36	7095300	821.488	85840	1083.8	6809140
316.287	227768	553.397	6451200	831.58	1028170	1099.79	38856
320.262	3680420	555.323	7501280	838.844	242556	1121.87	28828
321.133	360704	560.221	45760	840.318	822044	1157.99	32684
323.84	162548	579.296	411056	845.598	470628	1199.84	579312
324.016	1797830	579.297	975100	853.635	67244	1239.94	49932
326.38	232984	595.386	8902080	855.65	309252	1295.59	211464
326.38	430364	598.255	387284	857.529	2381860	1347.89	26764
327.217	704036	598.401	261604	858.407	335136	1348.3	49268
328.11	874660	609.401	363288	859.544	616224		

Table S3. ¹H NMR data assignments for new endophenazines **6—8**, **16**, and **22—26**.

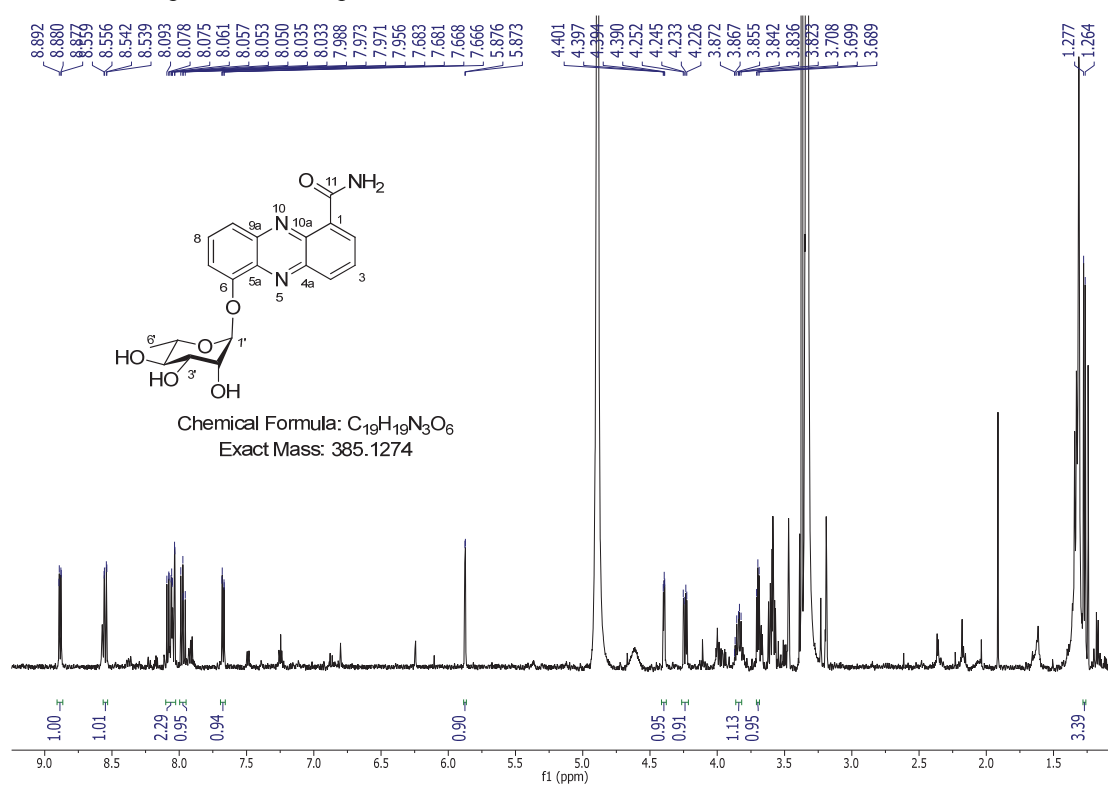
NO.	6	7	8	16	22	23	24	25	26
2	8.89, dd (7.5, 1.5)	8.26, dd (7.2, 1.2)	8.25, dd (7.2, 1.2)	8.38, dd (7.2, 1.2)	8.48, dd (9.0, 1.2)	8.38, dd (7.2, 1.2)	8.38, dd (7.2, 1.2)	7.87, dd (8.4, 1.2)	7.88, dd (7.8, 1.2)
3	8.08, dd (9.0, 7.5)	8.00, dd (9.0, 7.2)	7.99, dd (9.0, 7.2)	8.02, dd (8.4, 7.2)	8.05, dd (9.0, 7.2)	8.02, dd (8.4, 7.2)	8.02, dd (8.4, 7.2)	7.23, m	7.23, m
4	8.55, dd (9.0, 1.5)	8.41, dd (9.0, 0.6)	8.40, dd (9.0, 0.6)	8.46, dd (8.4, 1.2)	8.48, dd (7.2, 1.2)	8.46, dd (8.4, 1.2)	8.46, dd (8.4, 1.2)	7.65, brd (7.8)	7.65, dd (7.8, 1.2)
6		8.09, brd (9.0)	8.08, brd (9.0)	8.28, m	8.57, d (1.2)	8.28, m	8.28, m	7.39, brd (8.4)	7.39, brd (7.8, 1.2)
7	7.68, dd (7.5, 1.0)	7.91, dd (9.0, 7.0)	7.90, dd (9.0, 7.0)	8.02, m		8.02, m	8.02, m	7.06, m	7.06, m
8	7.97, dd (8.5, 7.5)	7.77, brd (6.6)	7.76, brd (6.6)	8.01, m	8.34, dd (9.0, 1.2)	8.01, m	8.01, m	7.07, m	7.07, m
9	8.04, dd (8.5, 1.0)			8.01, m	8.60, d (9.0)	8.01, m	8.01, m	7.05, m	7.05, m
1'	5.87, d (1.5)	6.55, d (1.8)	6.40, d (1.2)	6.54, d (1.8)	6.39, d (1.8)	4.63, d (10.8, 4.8); 4.57, d (10.8, 6.0)	3.96, d (10.8, 4.8); 3.92, d (10.8, 6.0)	6.40, d (1.8)	6.25, d (1.8)
2'	4.40, dd (3.5, 2.0)	3.78, dd (3.6, 1.8)	4.13, dd (3.6, 1.8)	3.78, dd (3.0, 1.8)	4.13, dd (3.0, 1.8)	4.12, m	5.43, m	3.64, dd (3.0, 1.8)	3.99, dd (3.0, 1.8)
3'	4.24, dd (9.5, 3.5)	3.75, dd (9.6, 3.6)	3.72, dd (9.6, 3.0)	4.08, dd (9.6, 3.0)	4.05, dd (9.6, 3.0)	3.82, d (11.4, 5.4); 3.78, d (11.4, 5.4)	3.96, d (10.8, 4.8); 3.92, d (10.8, 6.0)	3.88, dd (9.6, 3.0)	3.85, dd (9.6, 3.0)
4'	3.59, t (9.5)	3.44, t (9.6)	3.52, t (9.6)	3.54, t (9.6)	3.60, t (9.6)			3.46, t (9.6)	3.52, t (9.6)
5'	3.84, m	3.78, m	3.82, m	4.16, m	4.24, m			3.75, m	3.77, m
6'	1.27, d (6.5)	1.30~1.35 d (6.6)	1.34, d (6.6)	1.33, d (6.6)	1.38, d (6.6)			1.25~1.35, d (6.6)	1.25~1.35, d (6.6)
7'		3.62, s		3.64, s				3.57, s	
1''		4.17, brd (7.2)	4.15, brd (7.2)						
2''		5.58, m	5.57, m					4.31, s	4.31, s
3''		1.83, s	1.82, s		7.96, dd (8.4, 1.2)				
4''		1.79, s	1.78, s		7.63, t (7.8)				
5''					7.75, m				
6''					7.63, t (7.8)				
7''					7.96, dd (8.4, 1.2)				

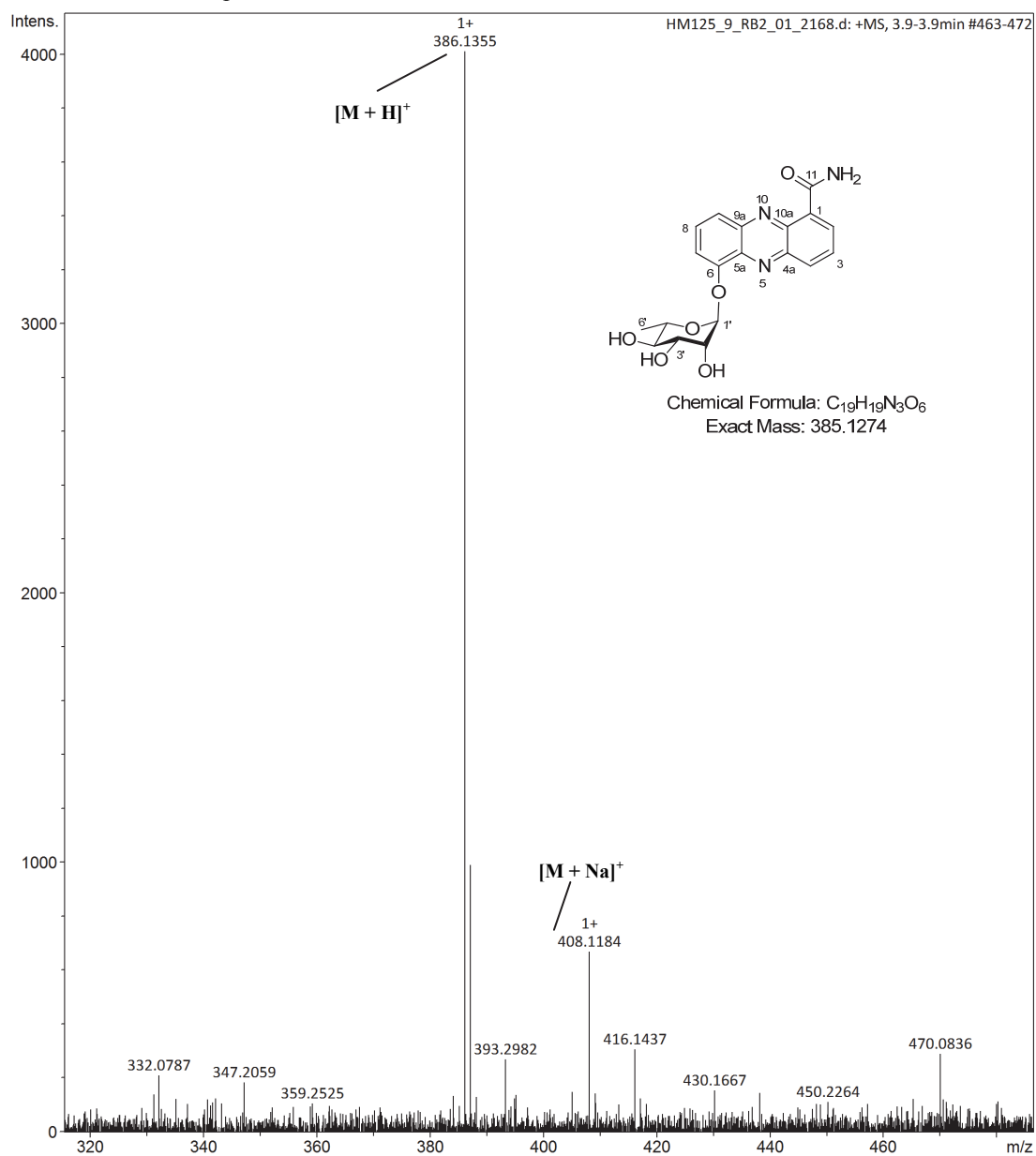
Recorded in CD₃OD. All ¹H NMR spectra were recorded at 600 MHz, except **6** at 500 MHz. Proton coupling constants (*J*) are in Hz.

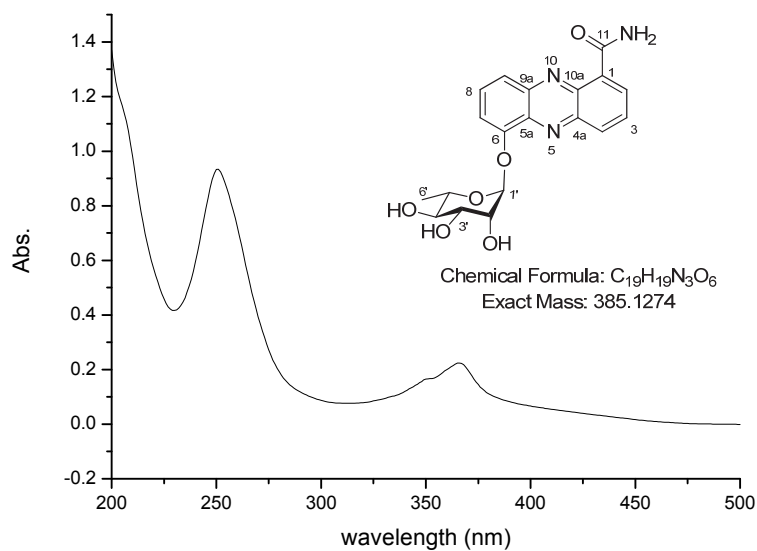
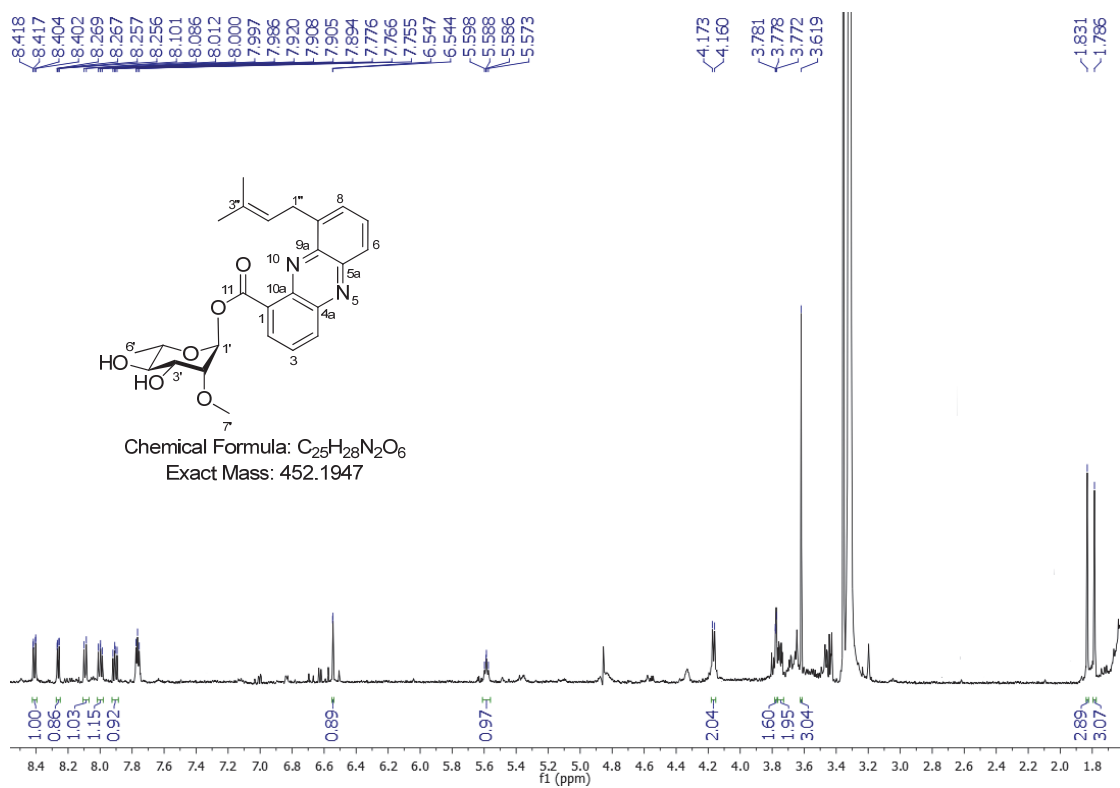
Spectral file. Spectra of nine new phenazine glycosides **6—8**, **16**, and **22—26**, including ^1H NMR, MS, and UV.

- S1. ^1H NMR spectrum of compound **6** in CD_3OD .
- S2. HRESIMS of compound **6**.
- S3. UV spectrum of compound **6**.
- S4. ^1H NMR spectrum of compound **7** in CD_3OD .
- S5. HRESIMS of compound **7**
- S6. UV spectrum of compound **7**
- S7. ^1H NMR spectrum of compound **8** in CD_3OD .
- S8. HRESIMS of compound **8**
- S9. UV spectrum of compound **8**
- S10. ^1H NMR spectrum of compound **16** in CD_3OD .
- S11. HRESIMS of compound **16**.
- S12. UV spectrum of compound **16**.
- S13. ^1H NMR spectrum of compound **22** in CD_3OD .
- S14. HRESIMS of compound **22**.
- S15. UV spectrum of compound **22**.
- S16. ^1H NMR spectrum of compounds **23** and **24** in CD_3OD .
- S17. APT spectrum of compounds **23** and **24** in CD_3OD .
- S18. ^1H - ^1H COSY spectrum of compounds **23** and **24** in CD_3OD .
- S19. HSQC spectrum of compounds **23** and **24** in CD_3OD .
- S20. HMBC spectrum of compounds **23** and **24** in CD_3OD .
- S21. HRESIMS of compounds **23** and **24**.
- S22. UV spectrum of compounds **23** and **24**.
- S23. ^1H NMR spectrum of compound **25** in CD_3OD .
- S24. HRESIMS of compound **25**.
- S25. UV spectrum of compound **25**.
- S26. ^1H NMR spectrum of compound **26** in CD_3OD .
- S27. HRESIMS of compound **26**.
- S28. UV spectrum of compound **26**.

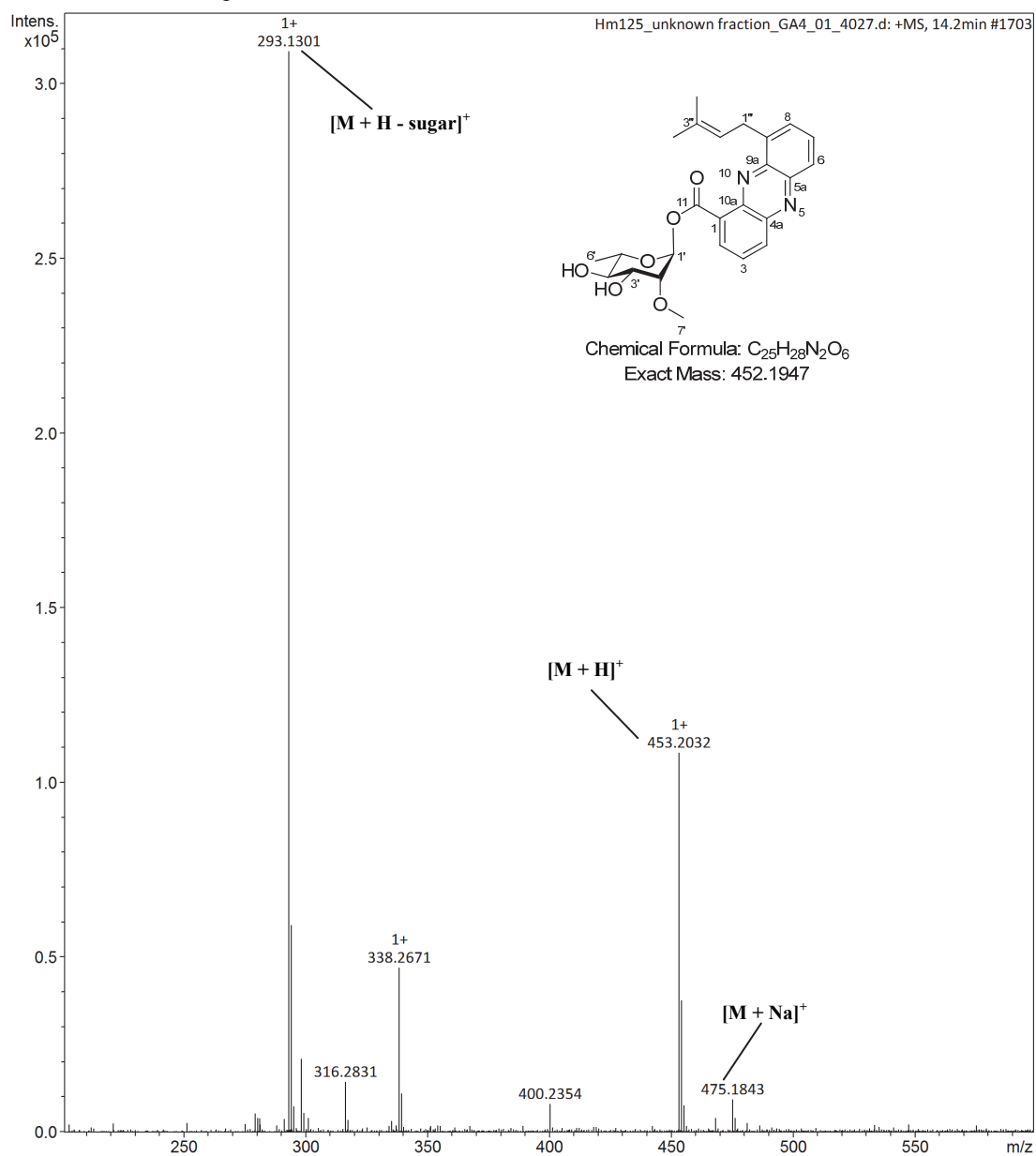
S1. ^1H NMR spectrum of compound **6** in CD_3OD .

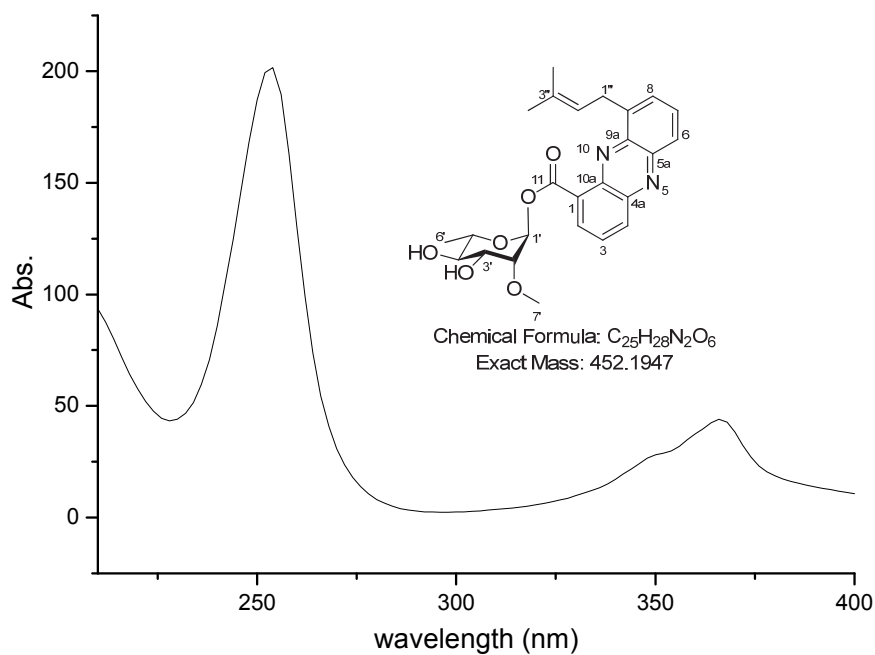
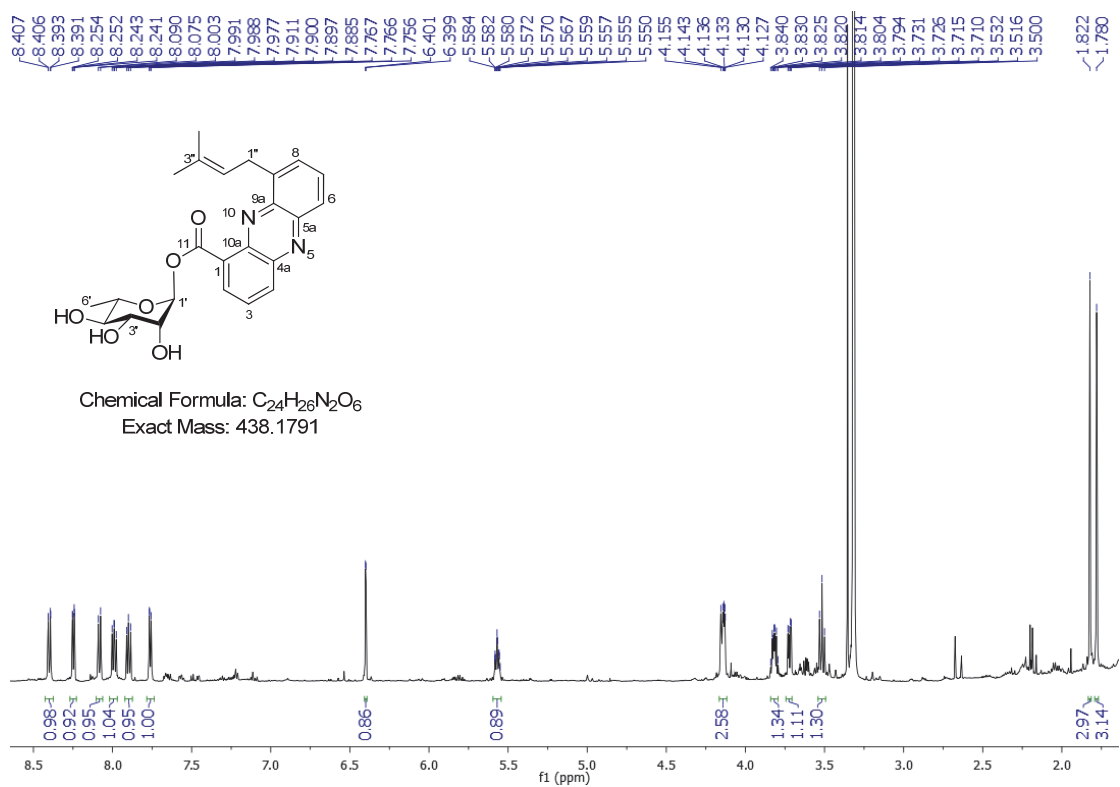


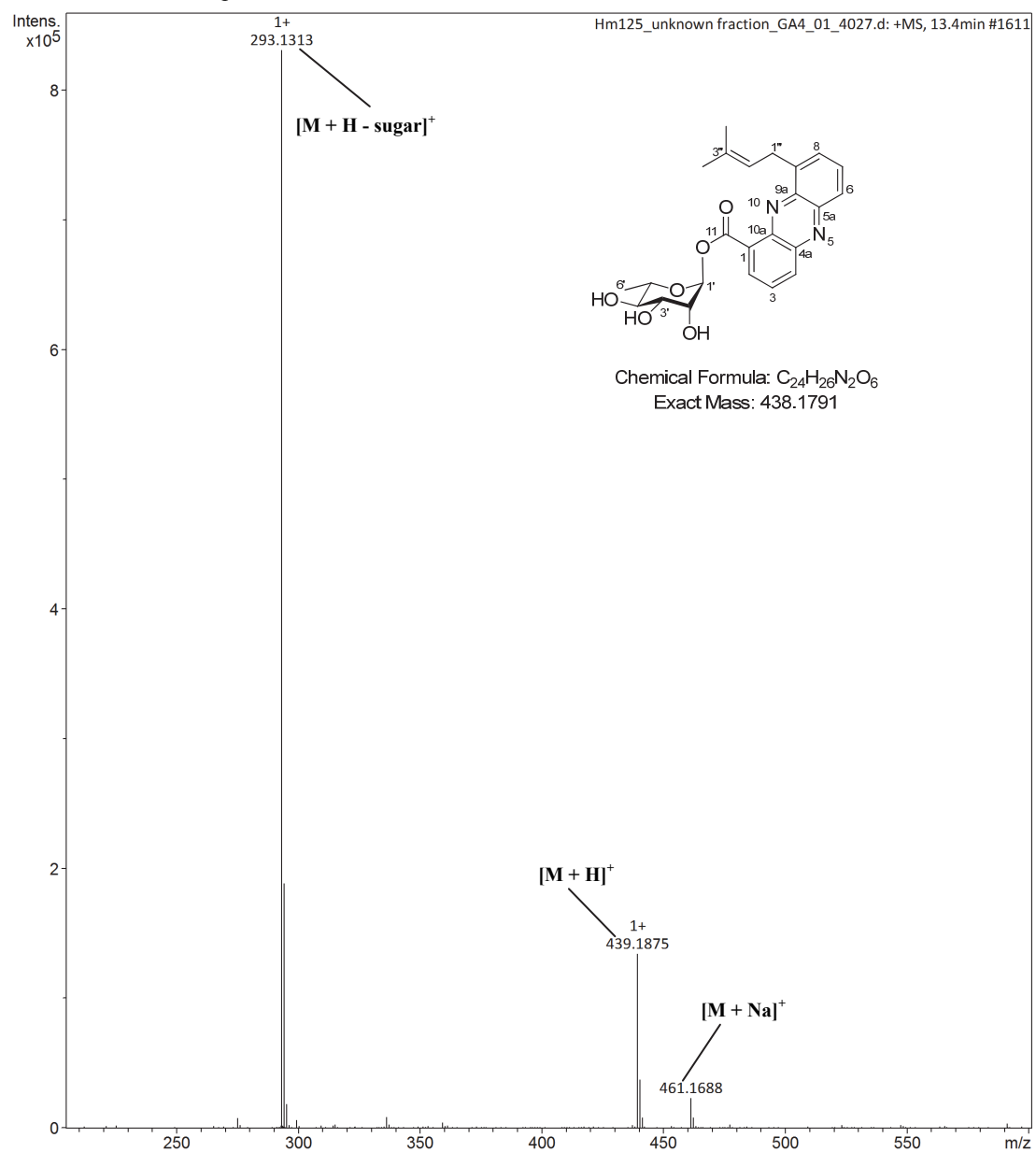
S2. HRESIMS of compound **6**.

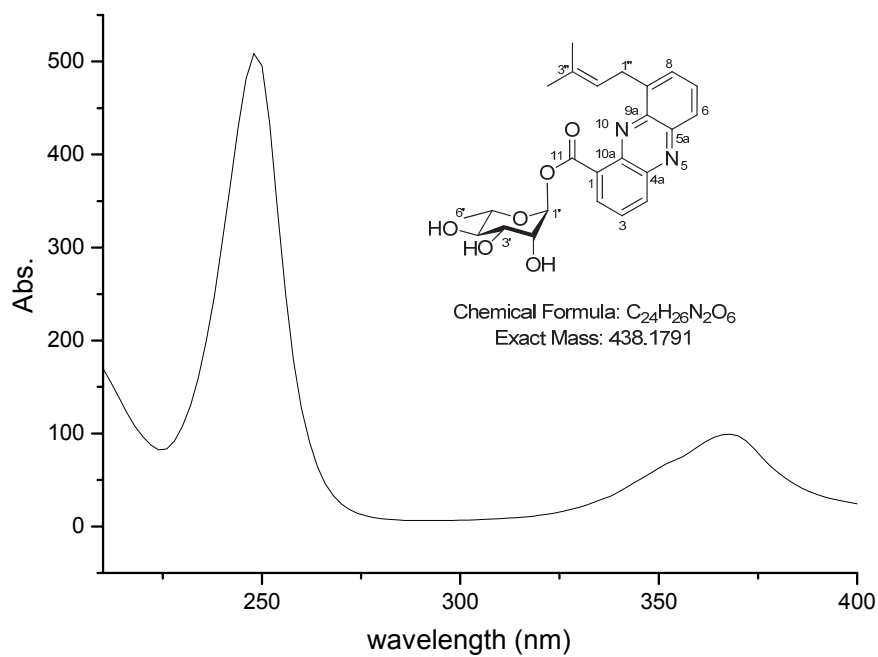
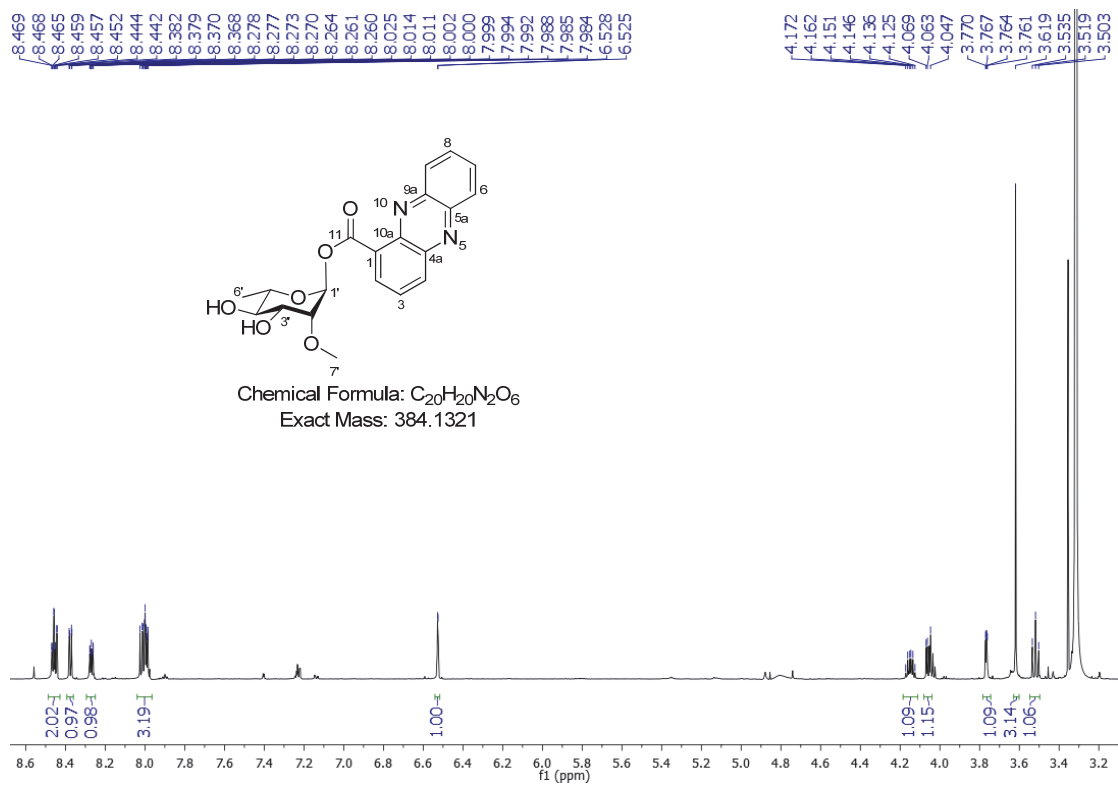
S3. UV spectrum of compound **6**.S4. 1H NMR spectrum of compound **7** in CD_3OD .

S5. HRESIMS of compound 7

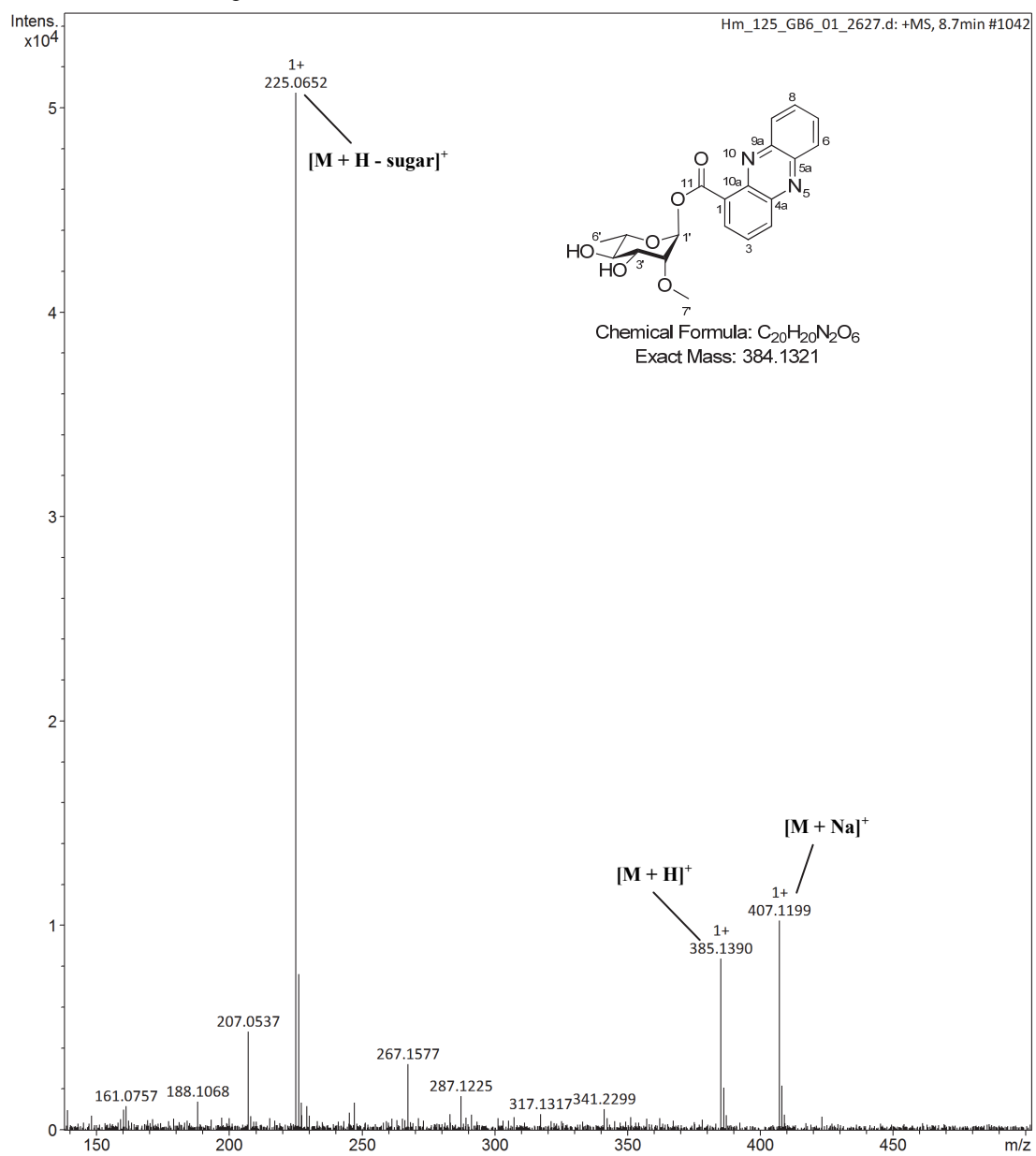


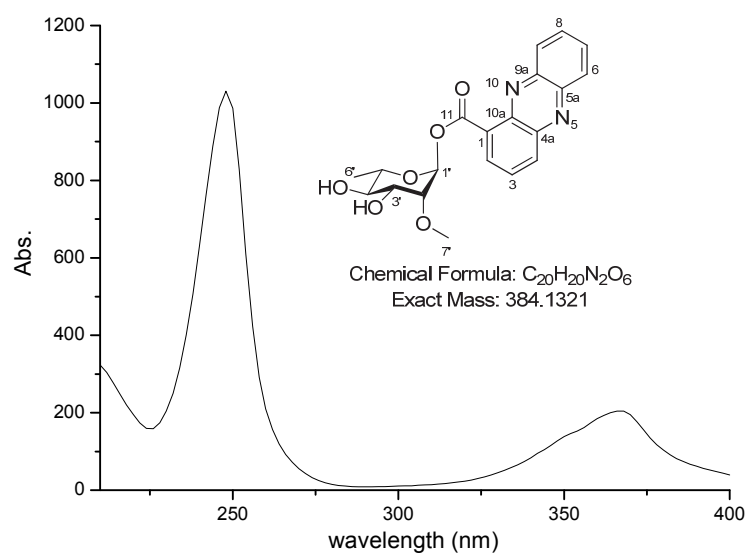
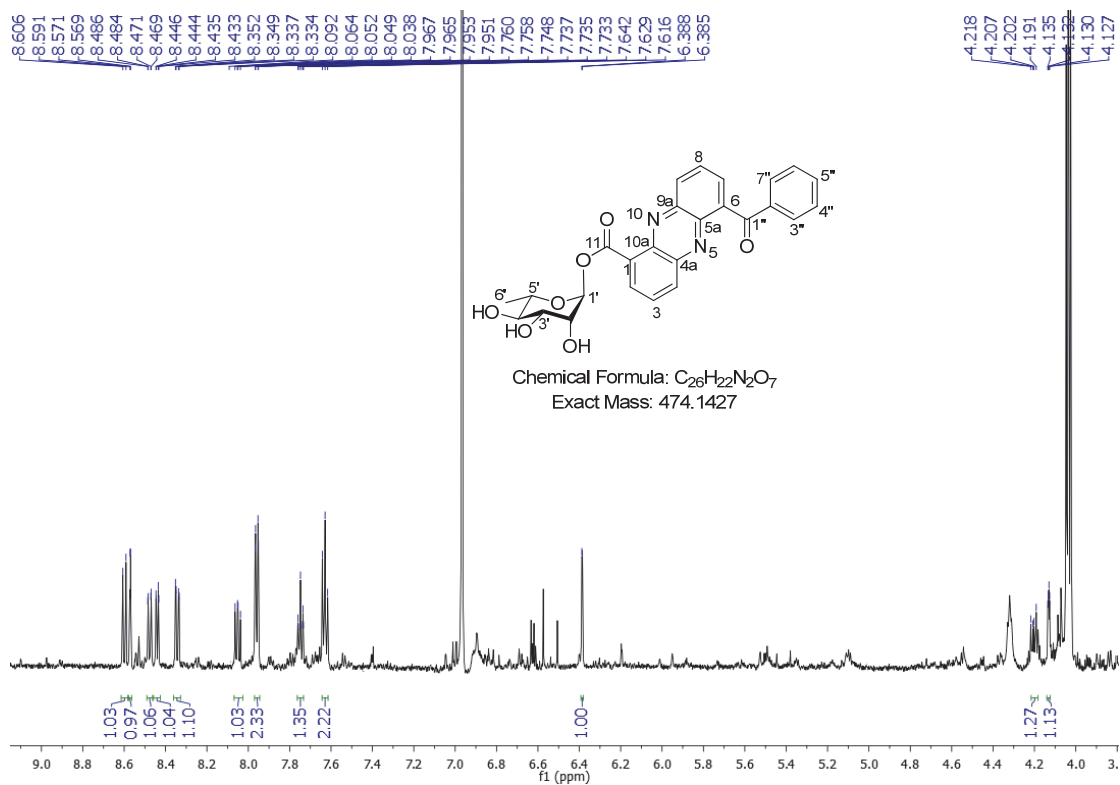
S6. UV spectrum of compound **7**S7. 1H NMR spectrum of compound **8** in CD_3OD .

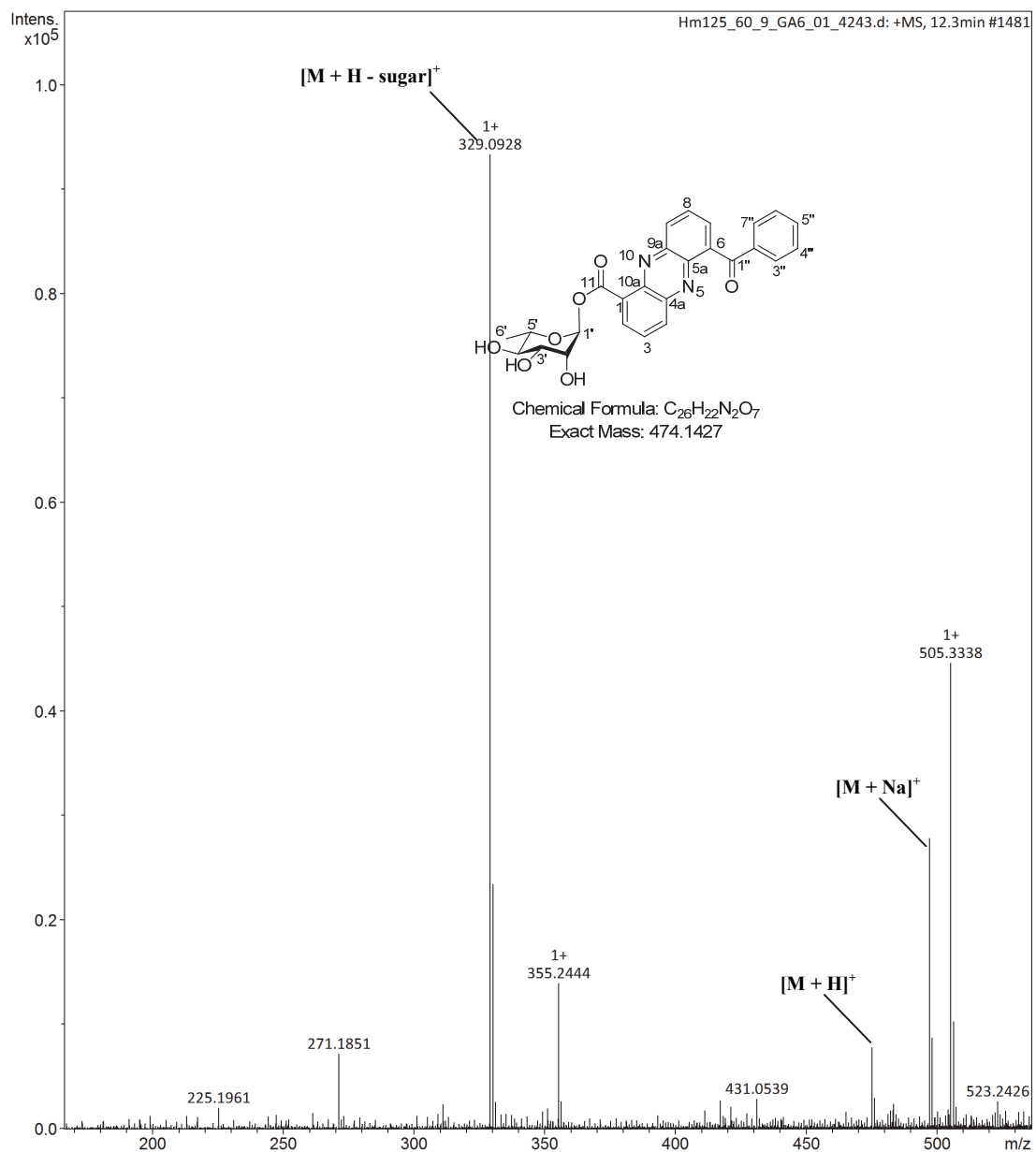
S8. HRESIMS of compound **8**

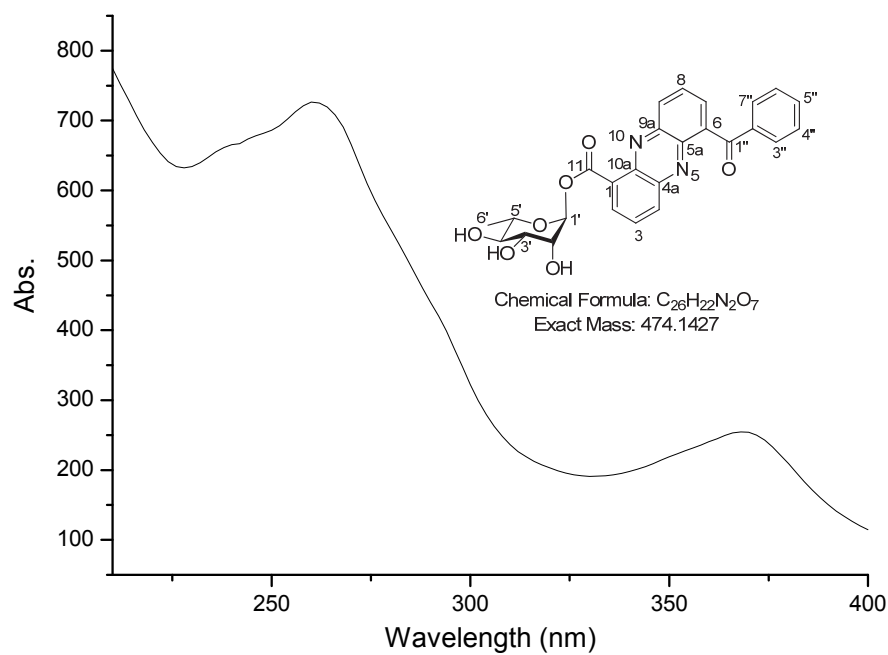
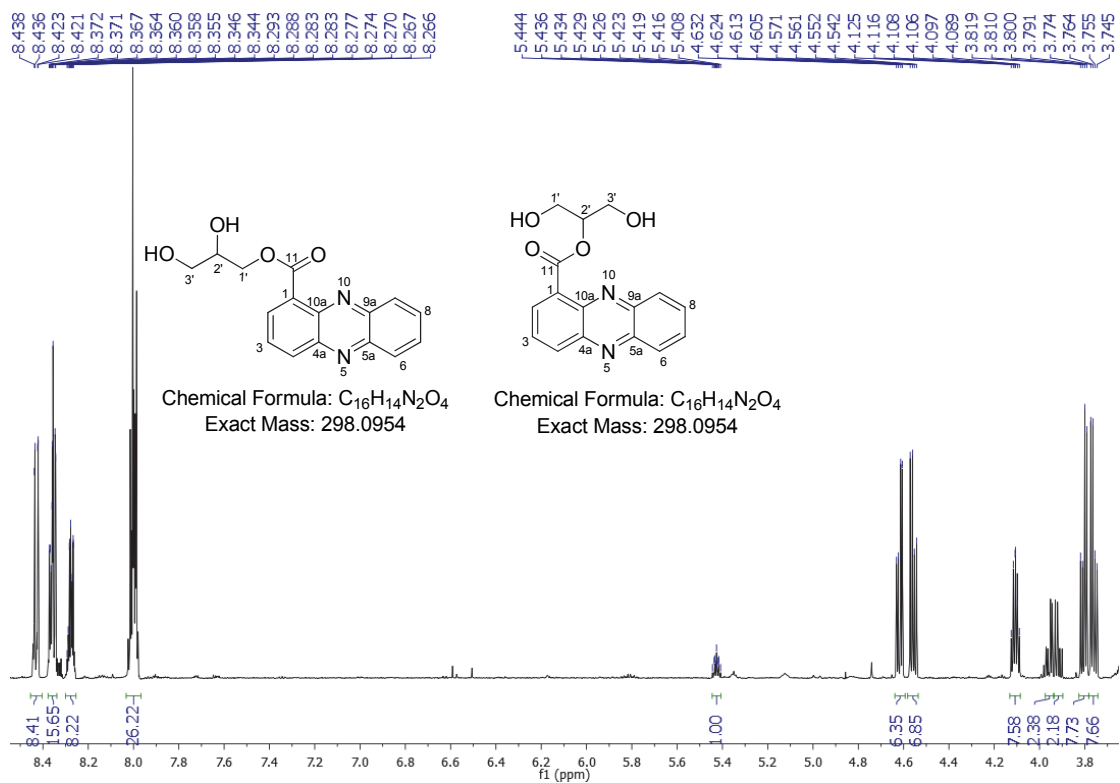
S9. UV spectrum of compound **8**S10. 1H NMR spectrum of compound **16** in CD_3OD .

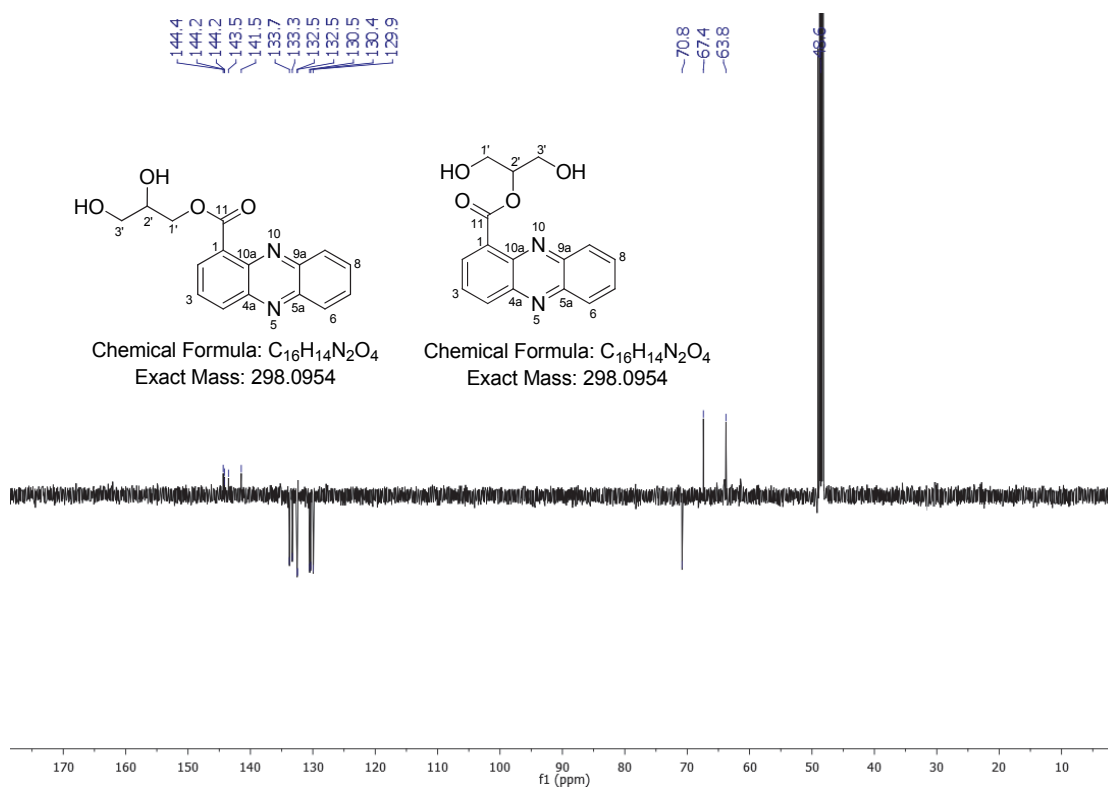
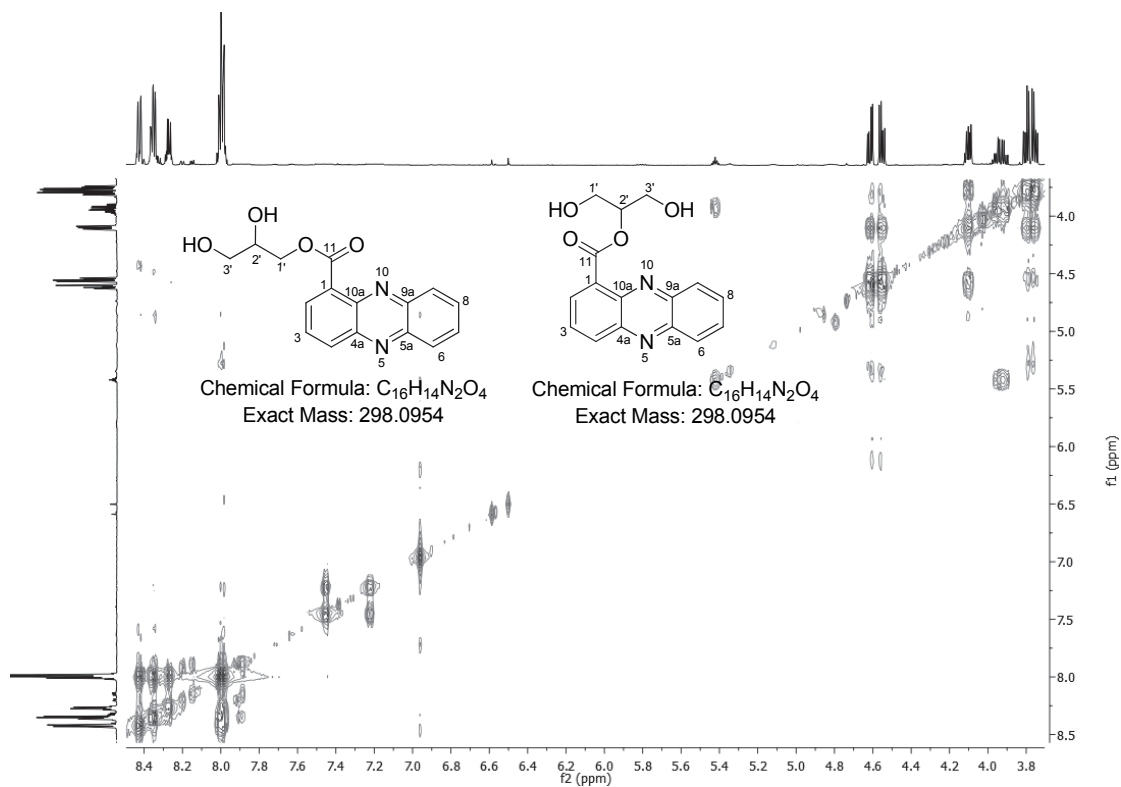
S11. HRESIMS of compound 16.

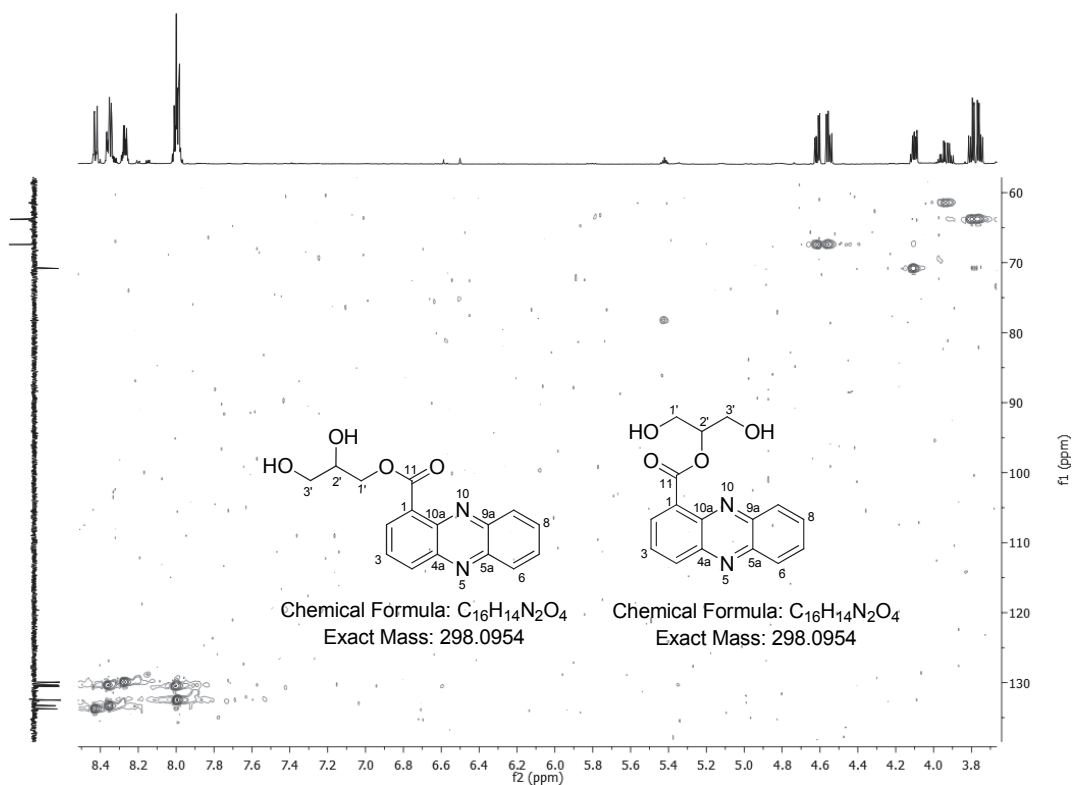
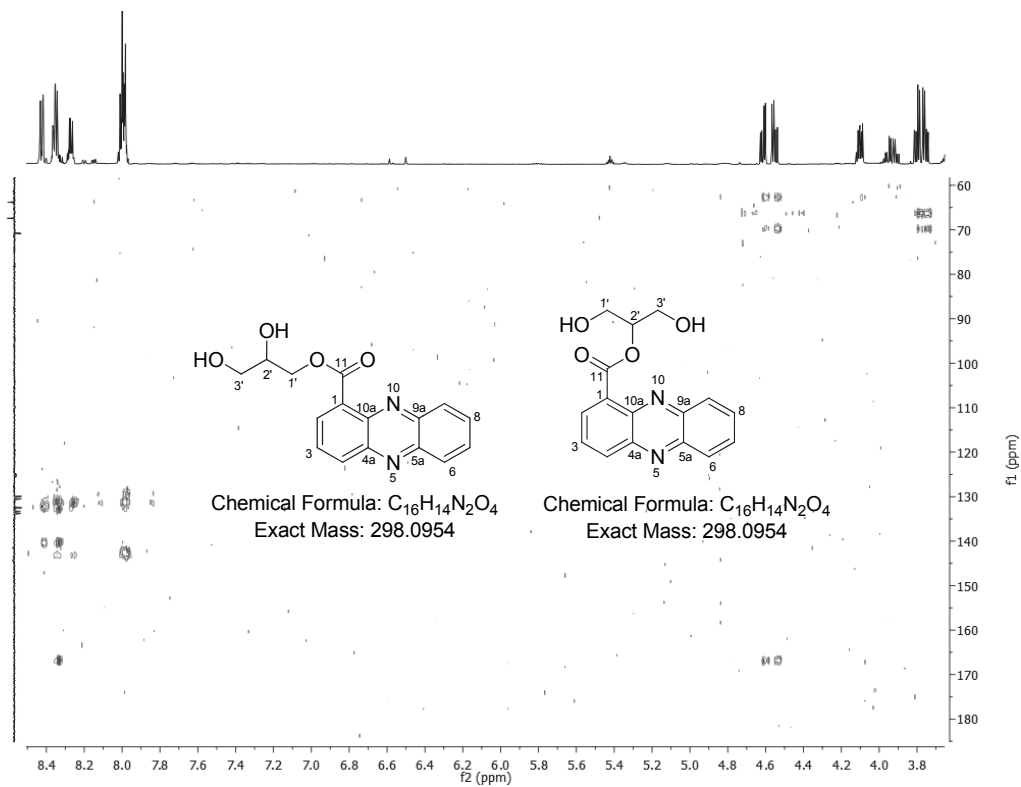


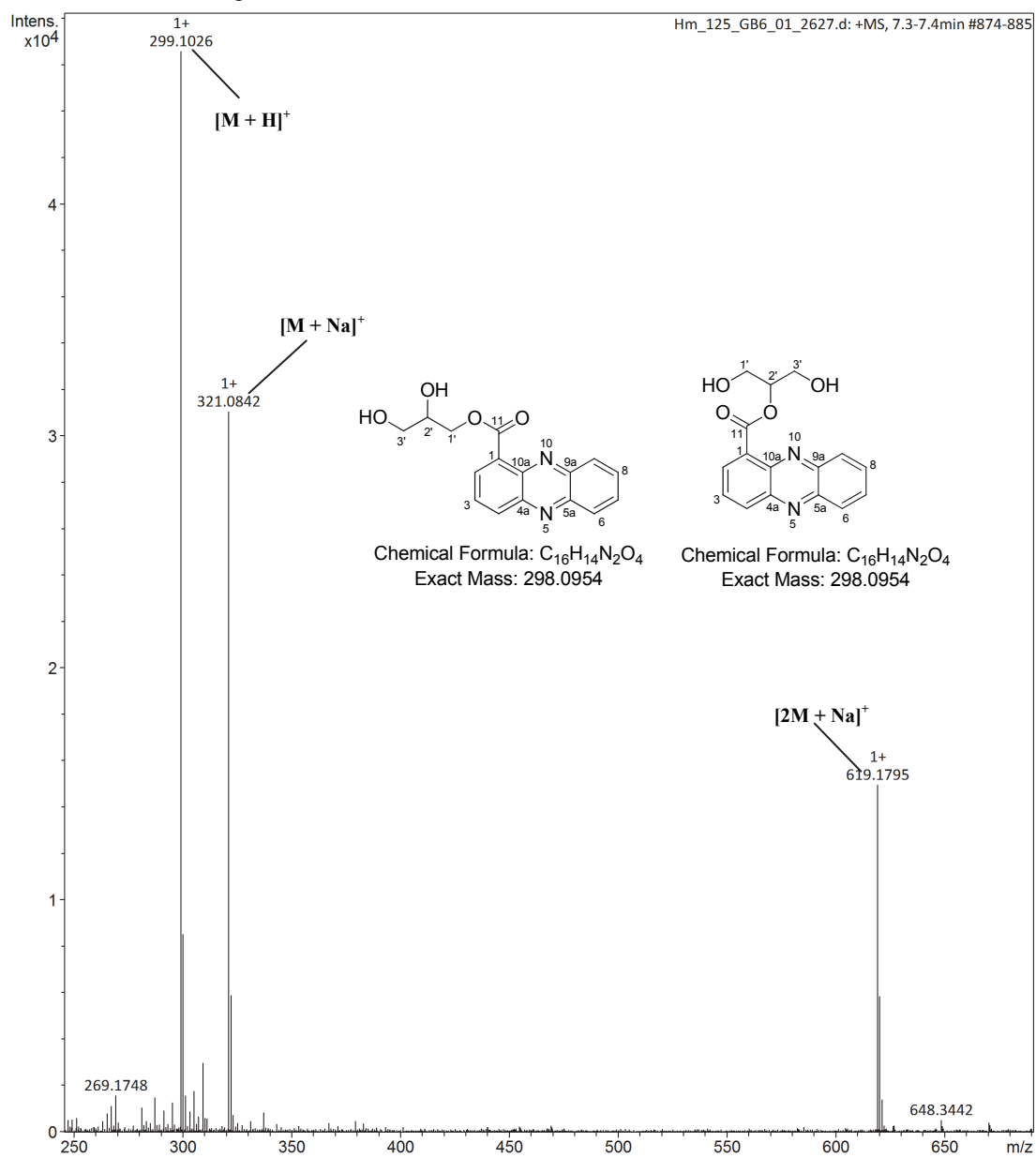
S12. UV spectrum of compound **16**.S13. 1H NMR spectrum of compound **22** in CD_3OD .

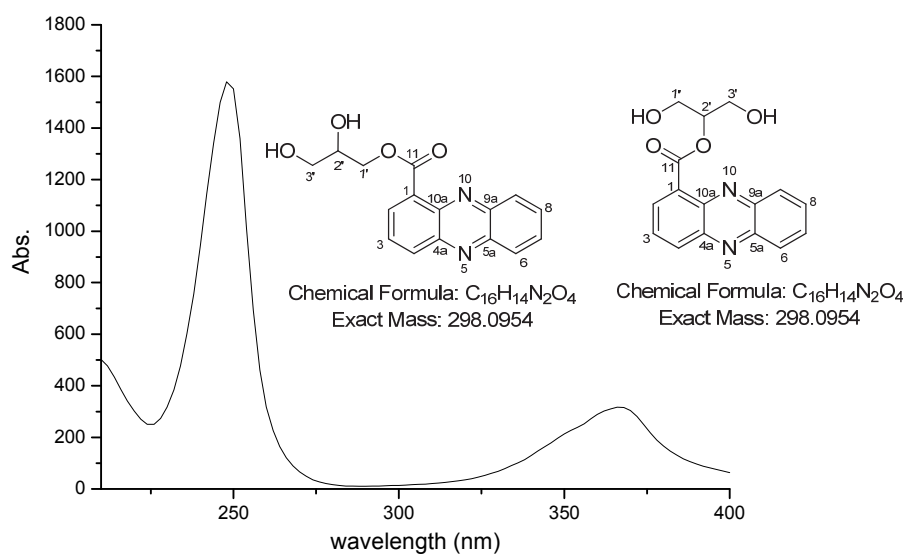
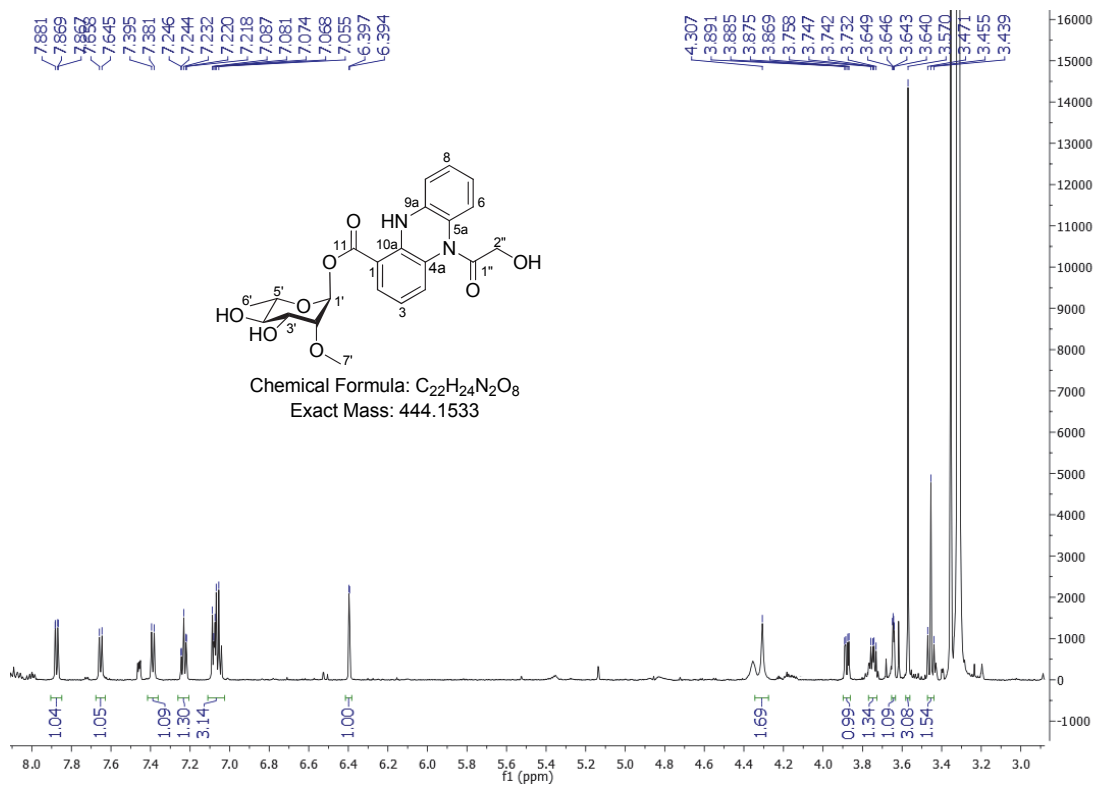
S14. HRESIMS of compound **22**.

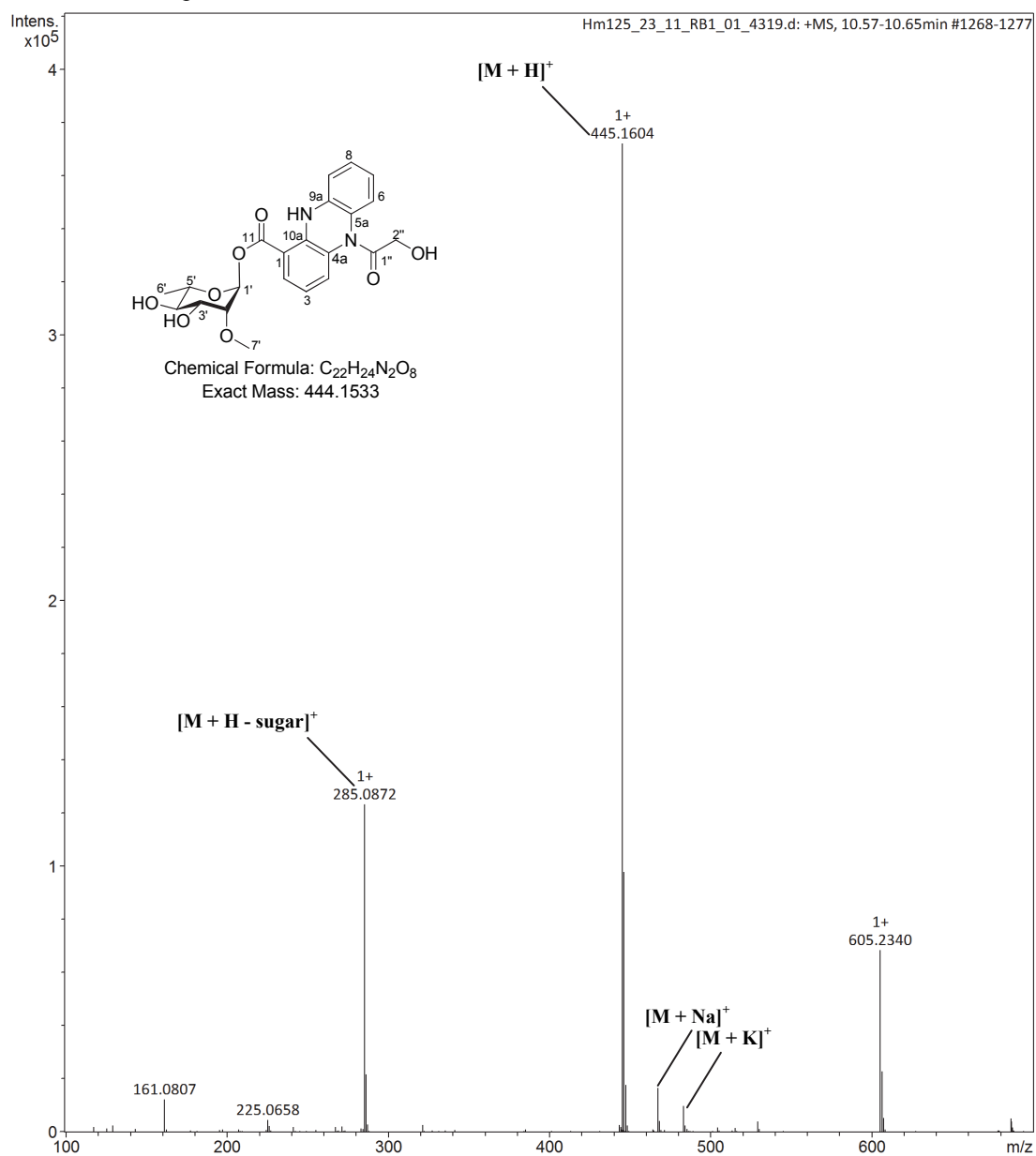
S15. UV spectrum of compound **22**.S16. 1H NMR spectrum of compounds **23** and **24** in CD_3OD .

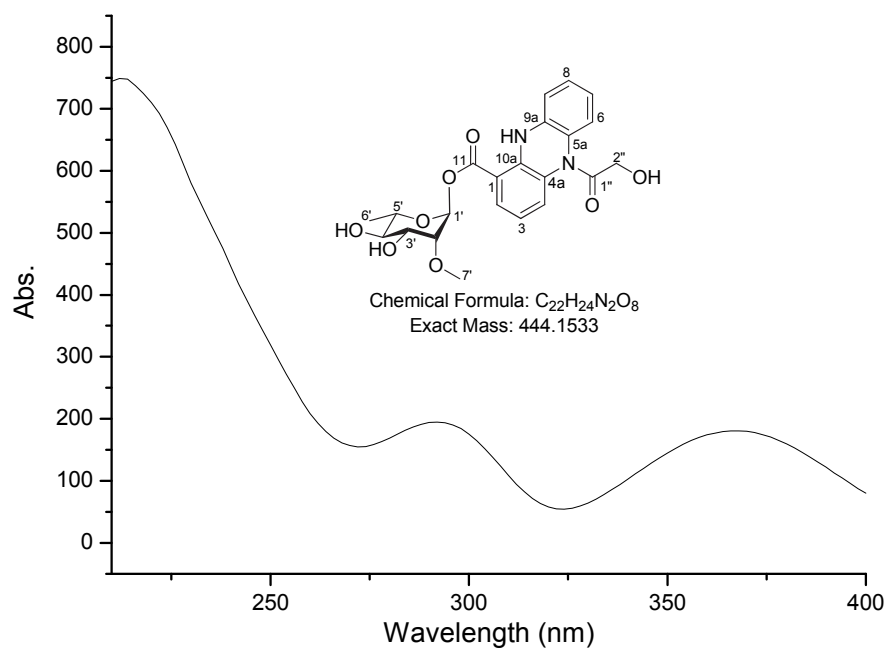
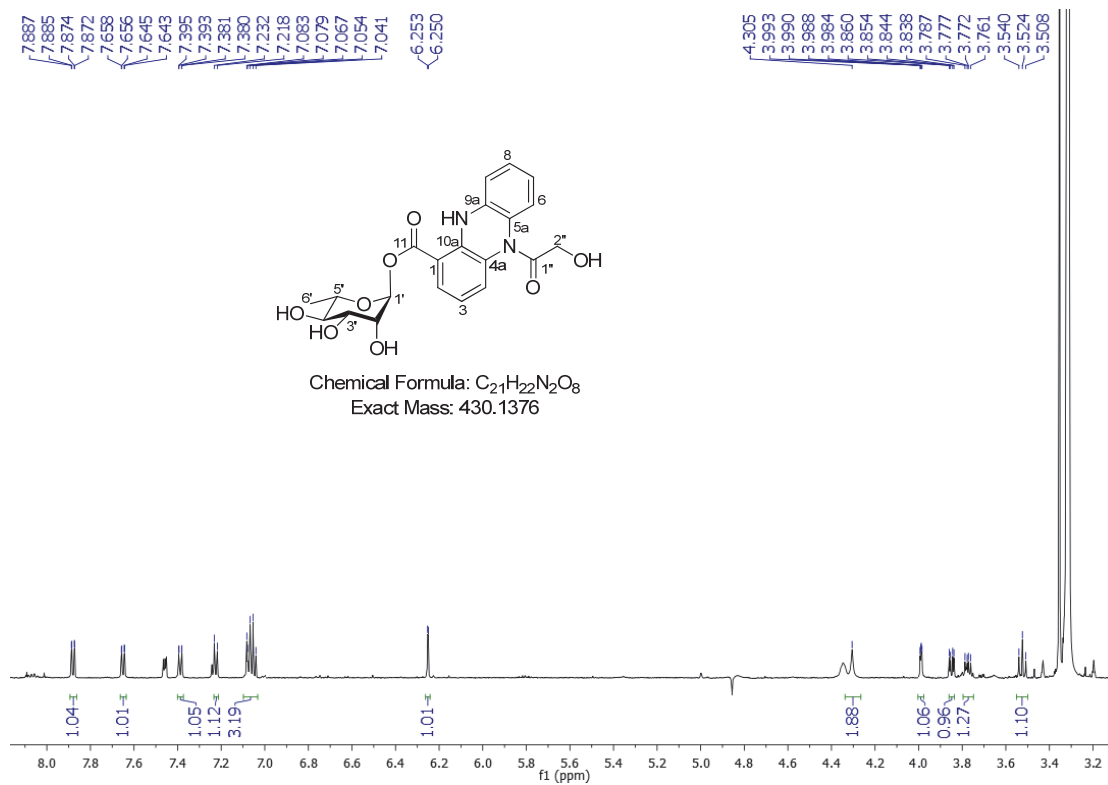
S17. APT spectrum of compounds **23** and **24** in CD₃OD.S18. ¹H-¹H COSY spectrum of compounds **23** and **24** in CD₃OD.

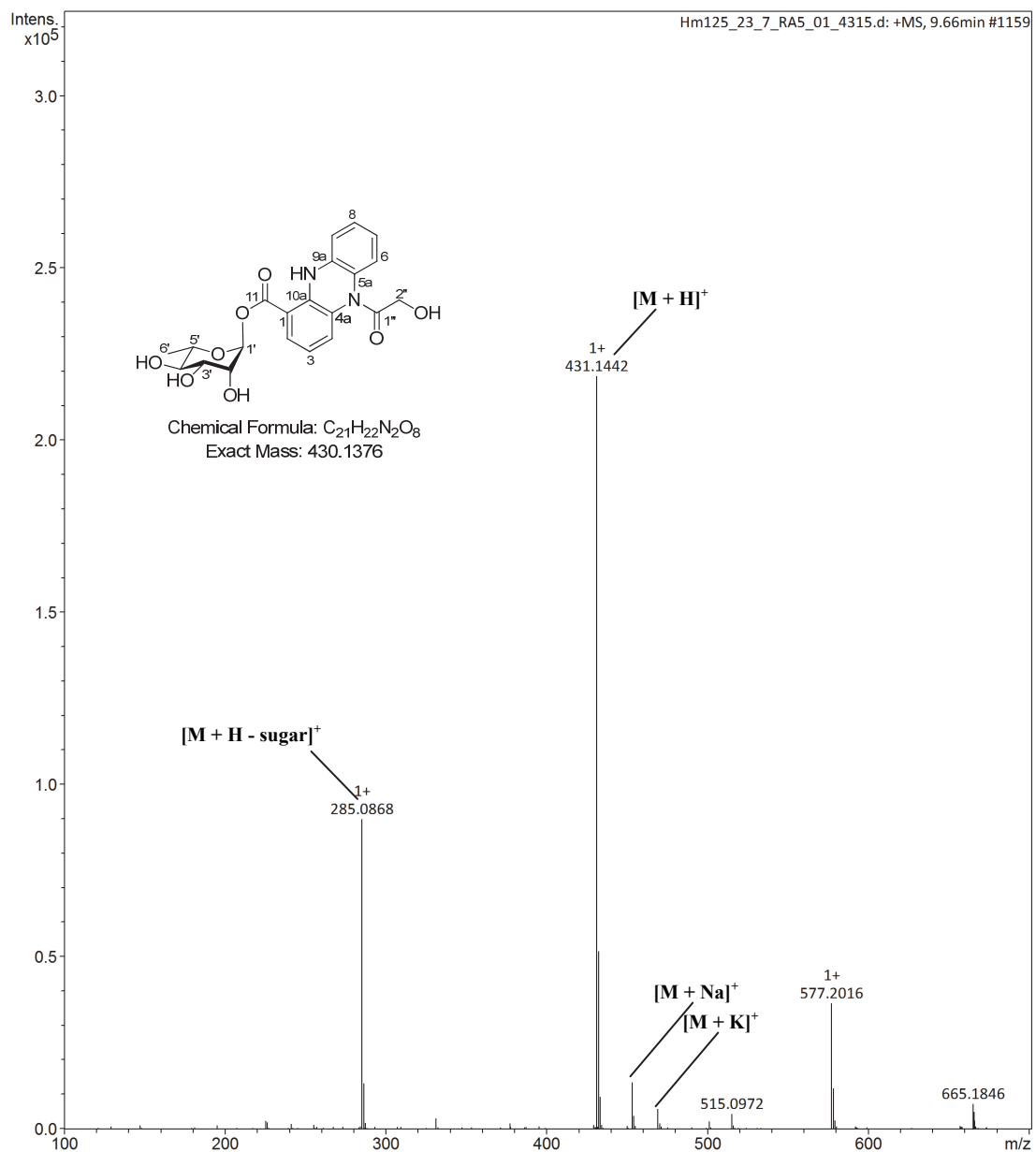
S19. HSQC spectrum of compounds **23** and **24** in CD₃OD.S20. HMBC spectrum of compounds **23** and **24** in CD₃OD.

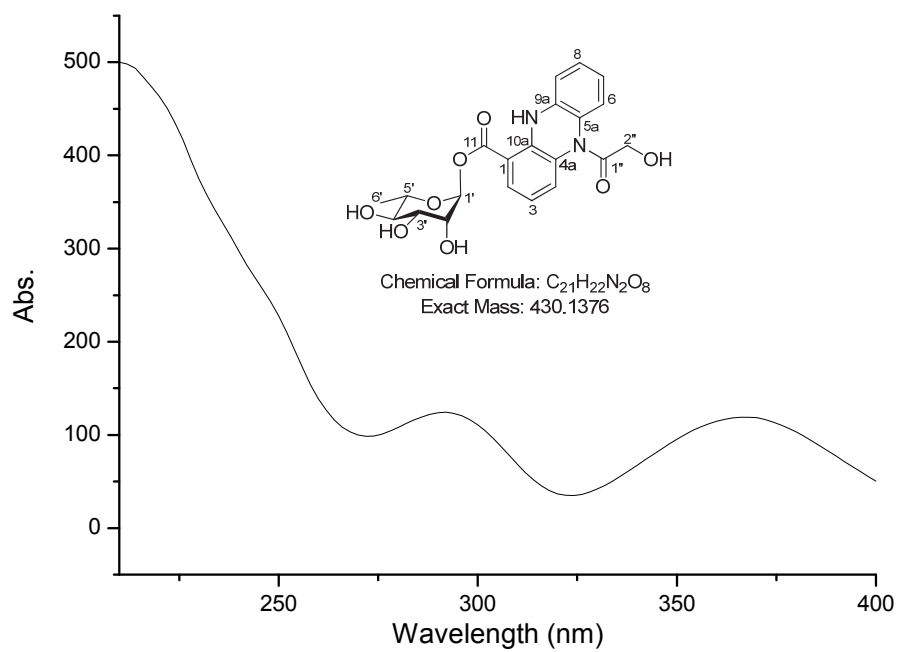
S21. HRESIMS of compounds **23** and **24**.

S22. UV spectrum of compounds **23** and **24**.S23. 1H NMR spectrum of compound **25** in CD_3OD .

S24. HRESIMS of compound **25**.

S25. UV spectrum of compound **25**.S26. 1H NMR spectrum of compound **26** in CD_3OD .

S27. HRESIMS of compound **26**

S28. UV spectrum of compound **26**.

Appendix VIII: Supplementary Information belonging to Chapter 11

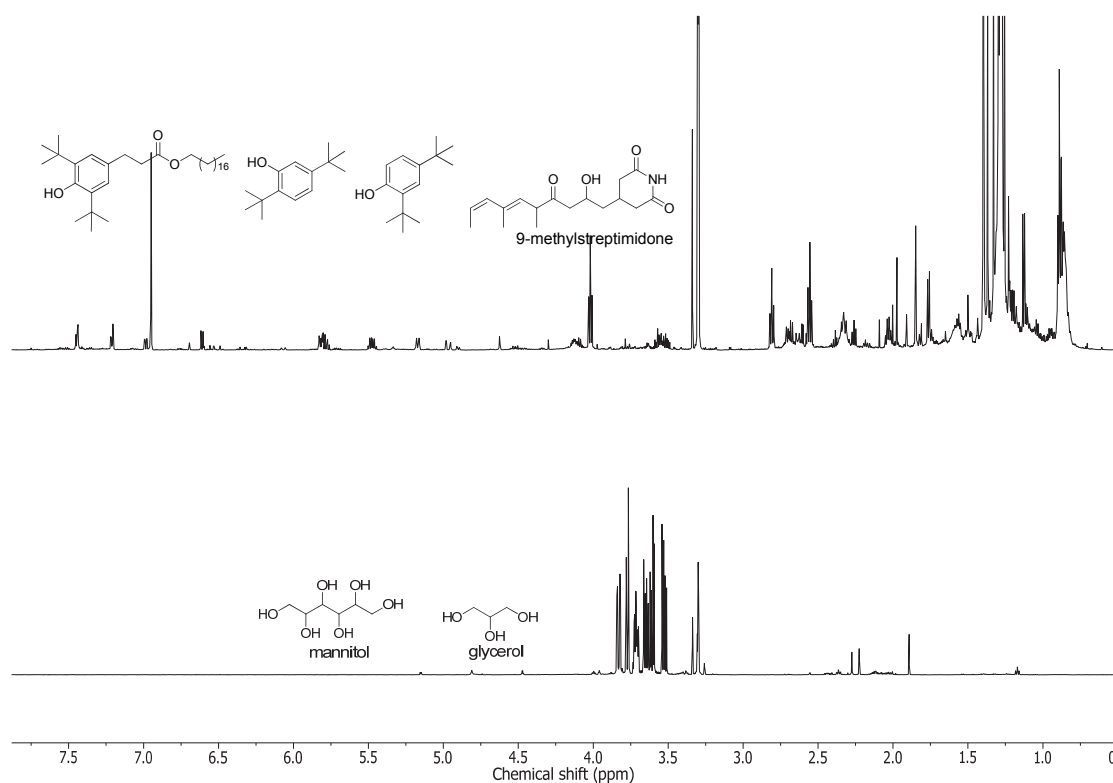


Figure S1. NMR comparison of cellulose (down) and EtOAc (up) extraction of culture broth *Streptomyces* sp. MBT73. EtOAc extracted apolar *di*-tert-butylphenols in culture medium and secondary metabolite 9-methylstreptimidone, while cellulose exclusively bonded a considerable amount of supplemented mannitol and glycerol.

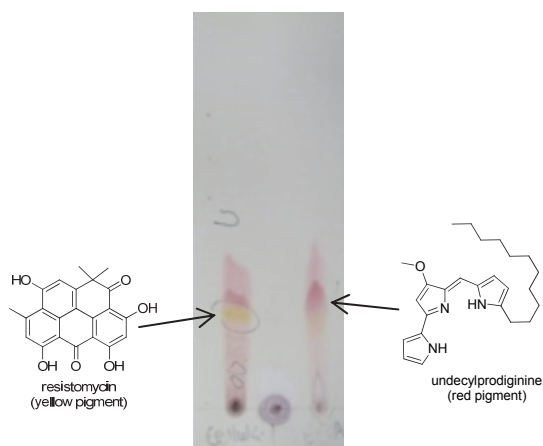


Figure S2. TLC comparison of cellulose (left) and EtOAc (right) extraction of culture broth *Streptomyces* sp. MBT73. Cellulose selectively extracted more yellow pigment (resistomycin) than the red pigment (undecylprodiginine).

