Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Supporting Information

Phenalenones: Insight into the biosynthesis of polyketides from the marine alga-derived fungus *Coniothyrium cereale*

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Extraction and isolation scheme

Scheme **S1**: Isolation scheme for [1-^{13}C] labeled *C. cereale* metabolites **1**, **3**, **5**, **6**, **9**, **12**, **14**-**16**

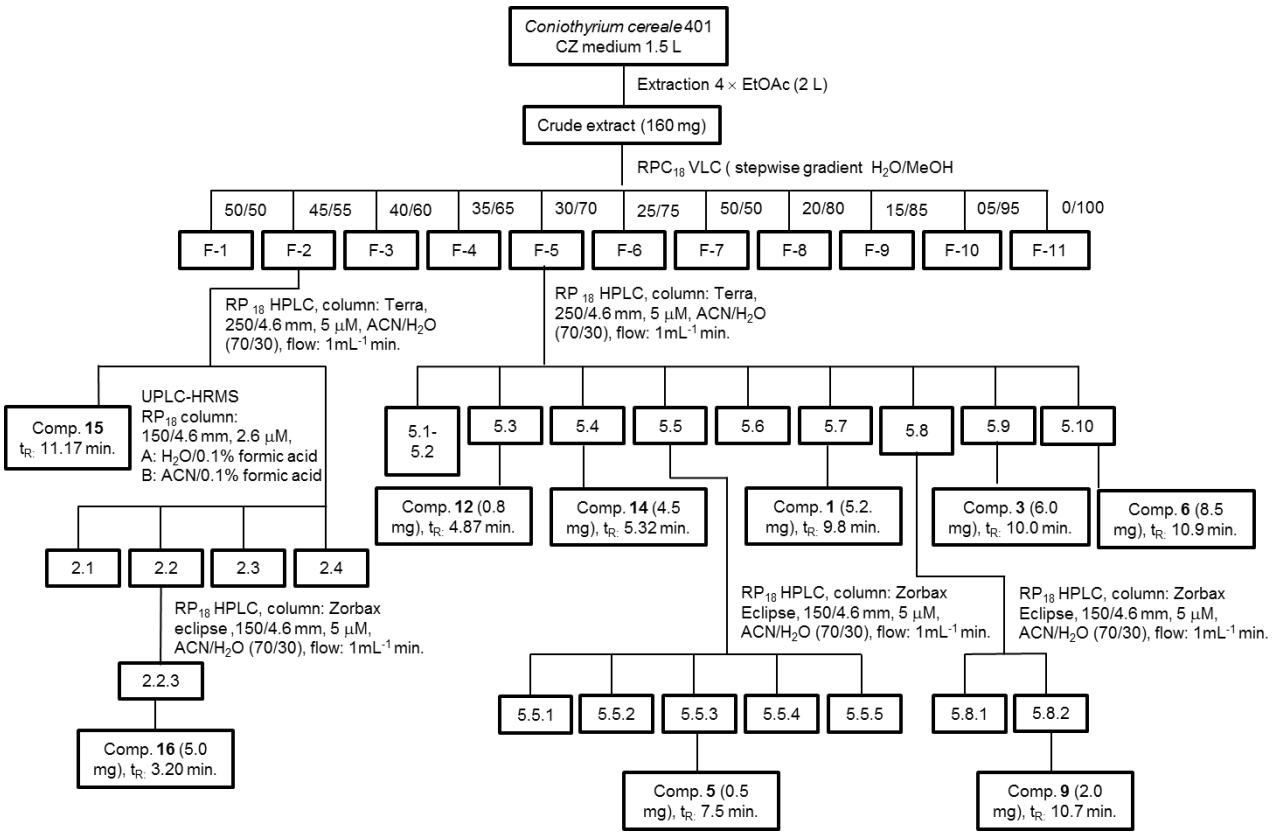


Figure **S1.1**: ^{1}H NMR spectrum of [1-^{13}C] labeled compound **1** in acetone-d_6

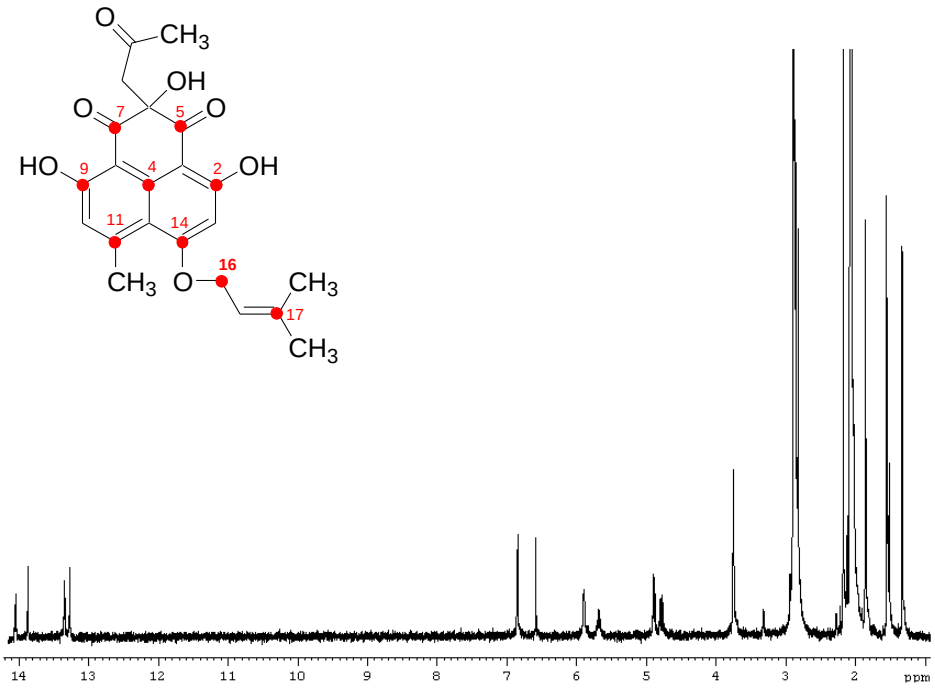


Figure **S1.2**: ^{13}C NMR spectrum of [1-^{13}C] labeled compound **1** in acetone-d_6 (x: impurity)

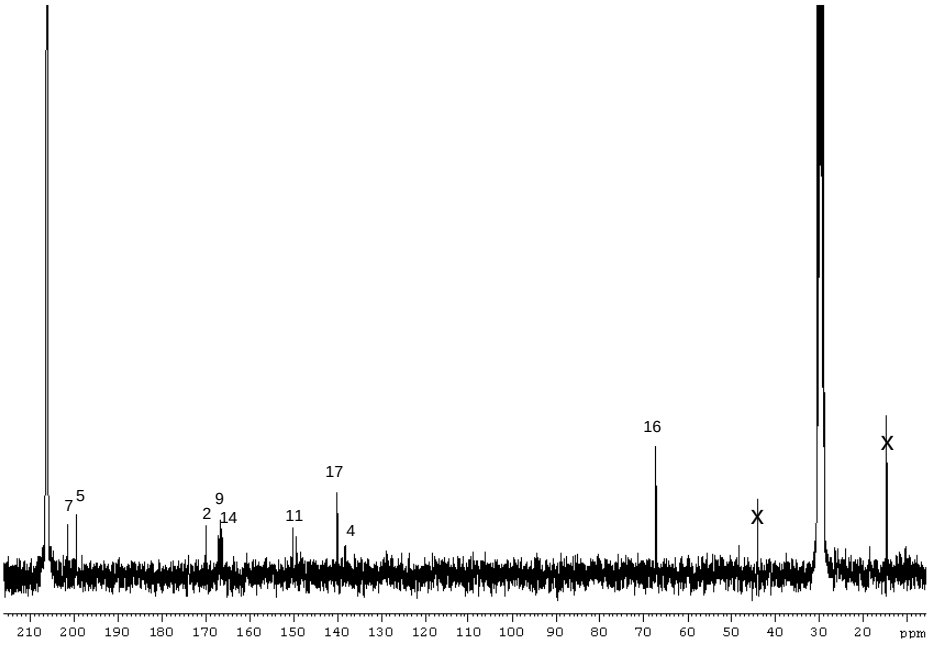


Figure **S1.3**: LC-ESIMS chromatogram (extracted ion mode; left) and LC-PDA chromatogram (total wavelength mode; right) as well as ESI mass spectrum of [1-^{13}C] labeled compound **1**

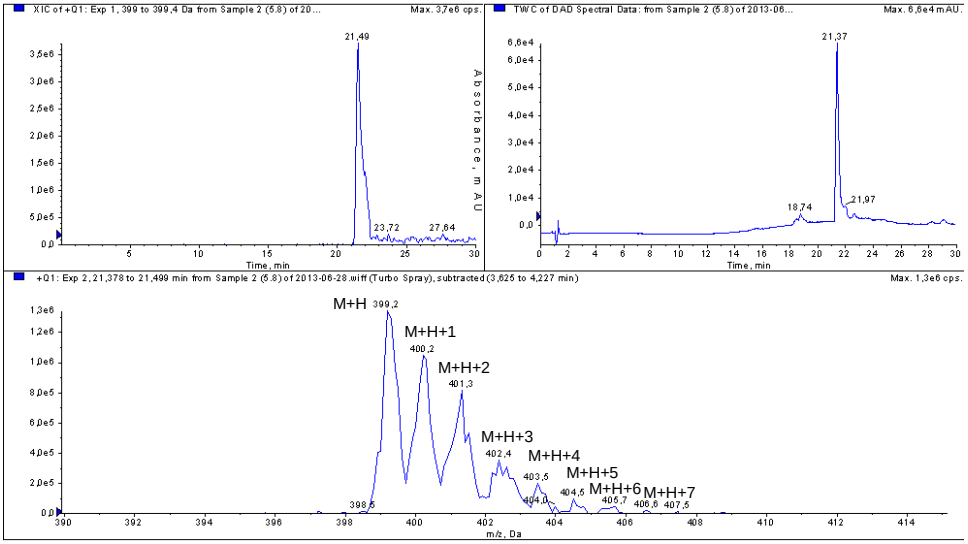


Figure **S2.1**: ^{1}H NMR spectrum of [1-^{13}C] labeled compound **3** in chloroform-d_1

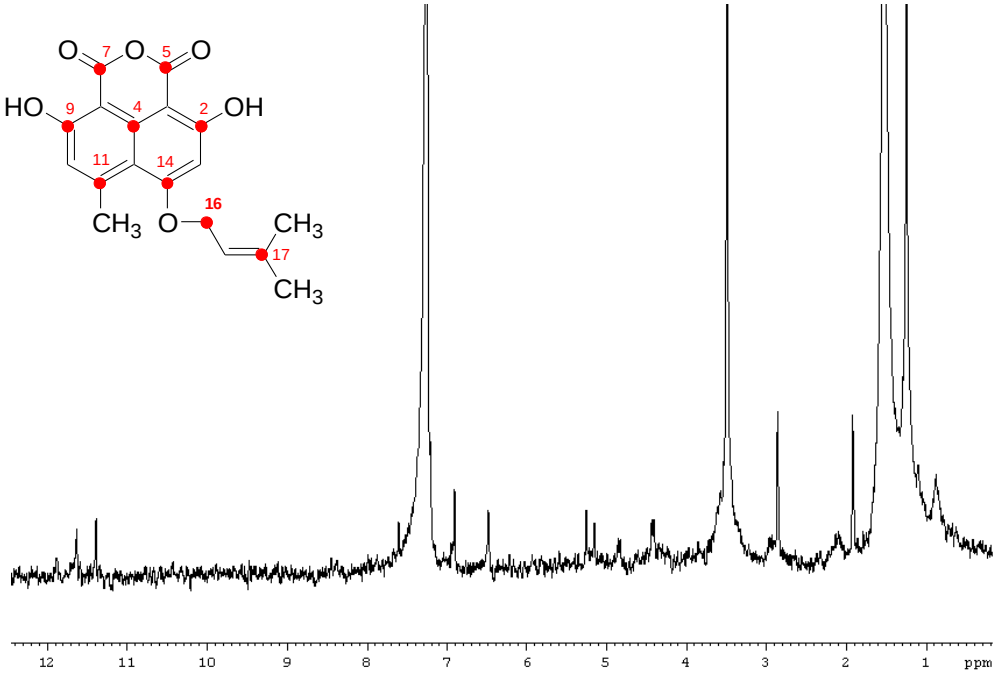


Figure **S2.2**: ^{13}C NMR spectrum of [1-^{13}C] labeled compound **3** in chloroform-d_1 (x: impurity)

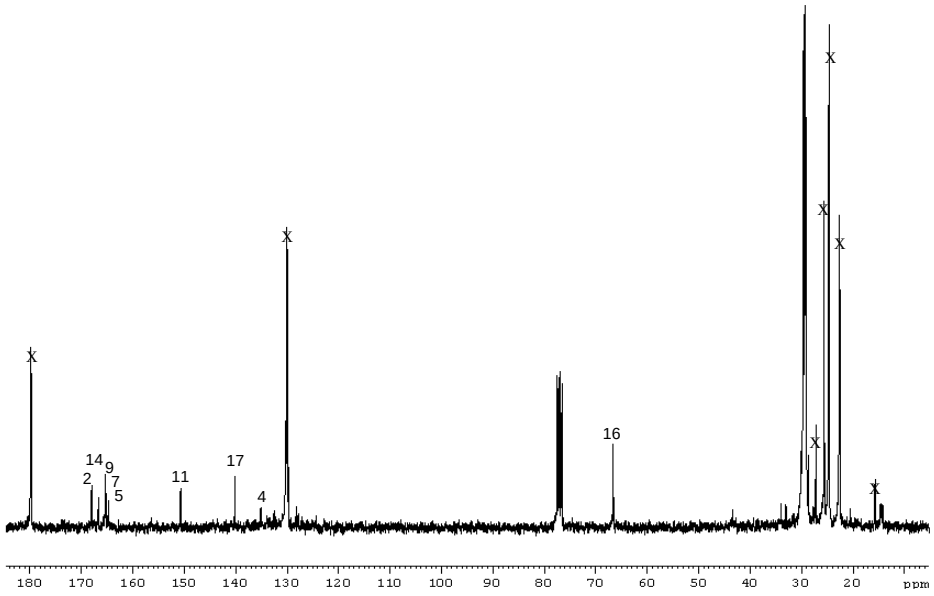


Figure **S2.3**: LC-ESIMS chromatogram (extracted ion mode; left) and LC-PDA chromatogram (total wavelength mode; right) as well as ESI mass spectrum of [1-^{13}C] labeled compound **3**

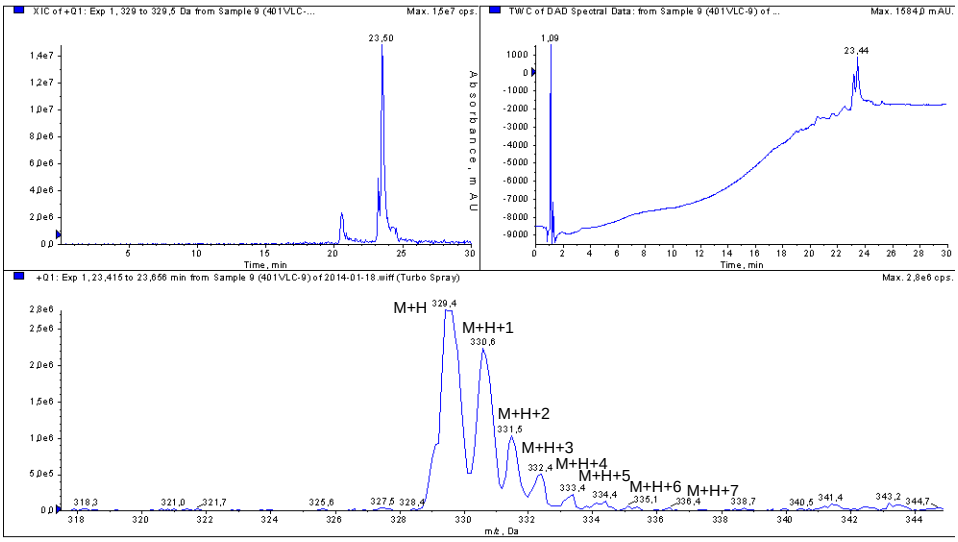


Figure **S3.1**: ^{1}H NMR spectrum of [1-^{13}C] labeled compound **6** in chloroform-d_1

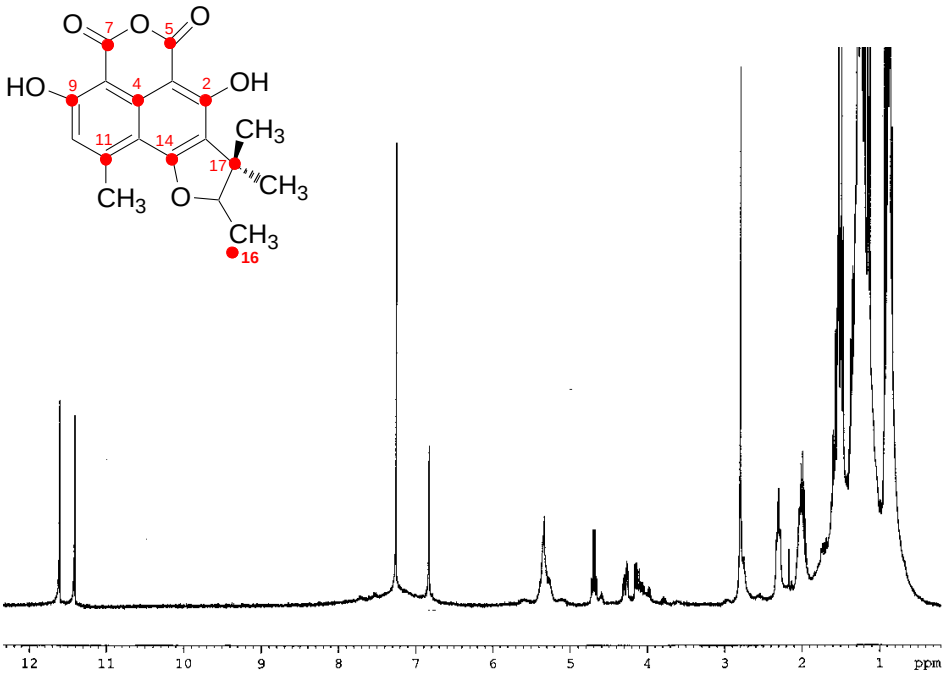


Figure **S3.2**: ^{13}C NMR spectrum of [1-^{13}C] labeled compound **6** chloroform-d_1 (x: impurity)

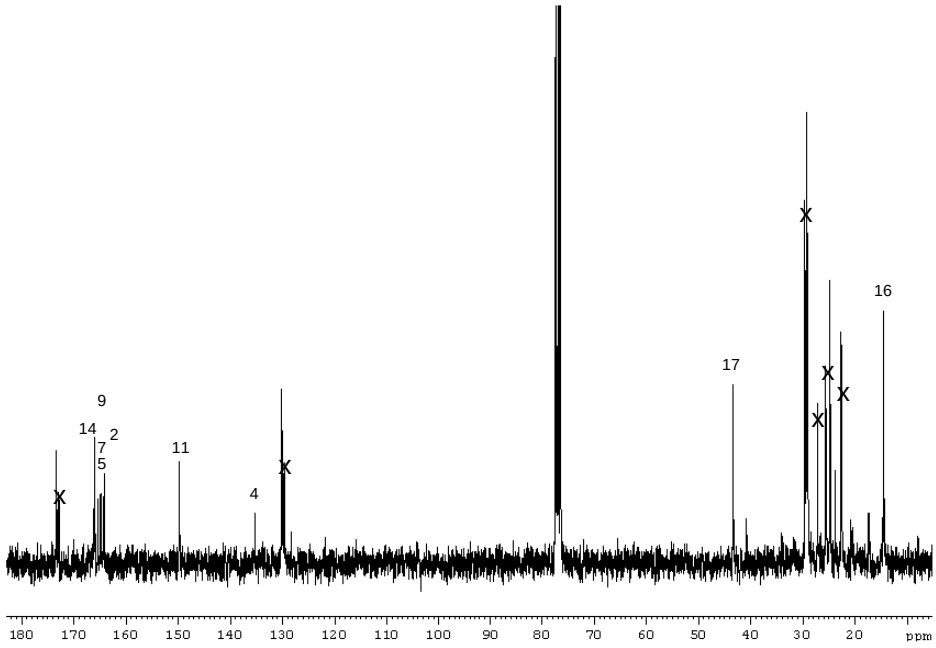


Figure **S3.3**: LC-ESIMS chromatogram (extracted ion mode; left) and LC-PDA chromatogram (total wavelength mode; right) as well as ESI mass spectrum of [1-^{13}C] labeled compound **6**

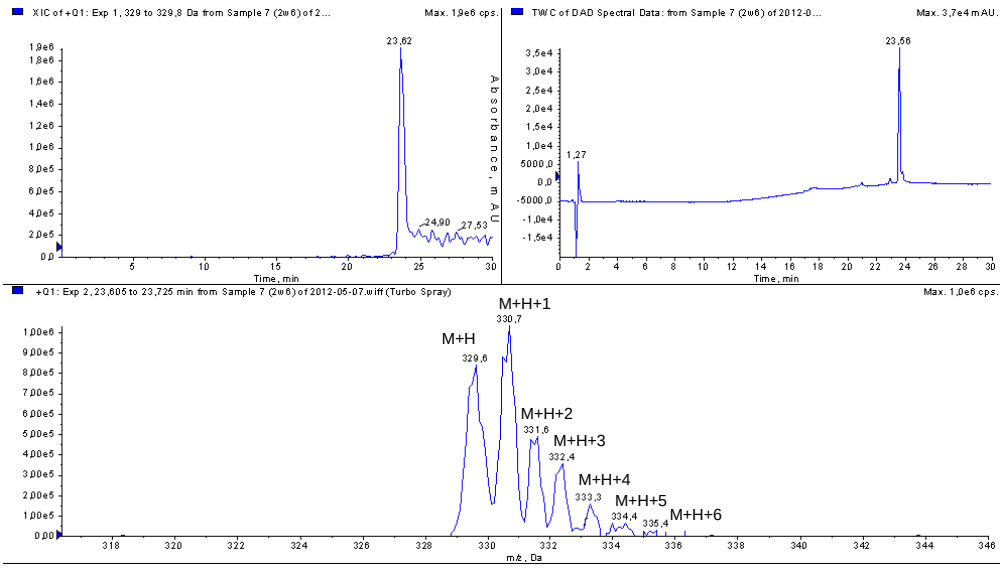


Figure **S4.1**: ^{1}H NMR spectrum of [1-^{13}C] labeled compound **9** in methanol-d_4

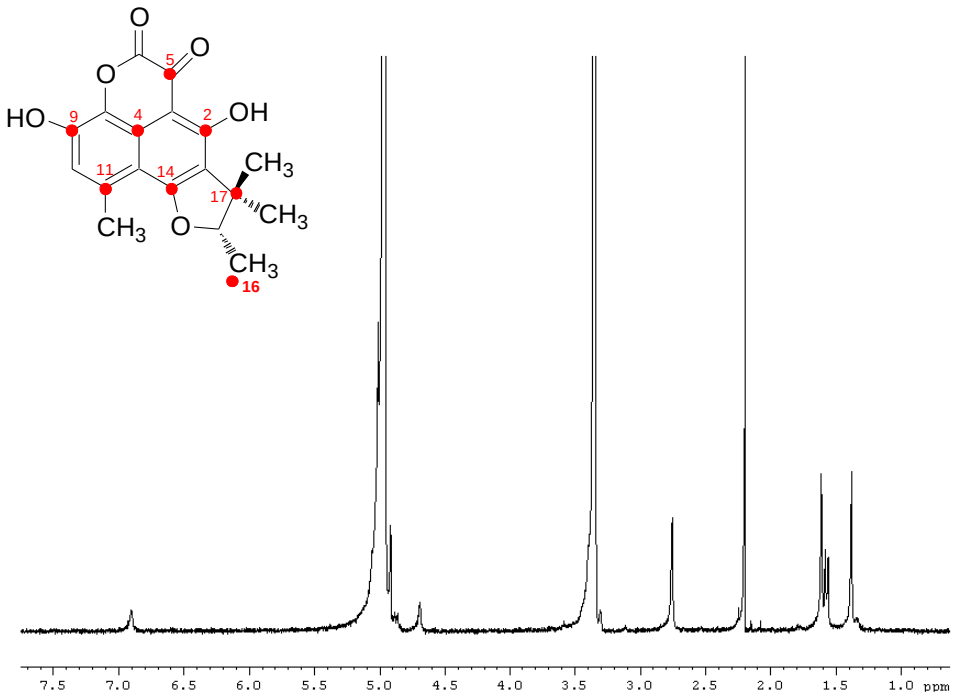


Figure **S4.2**: ^{13}C NMR spectrum of [1-^{13}C] labeled compound **9** in methanol-d_4

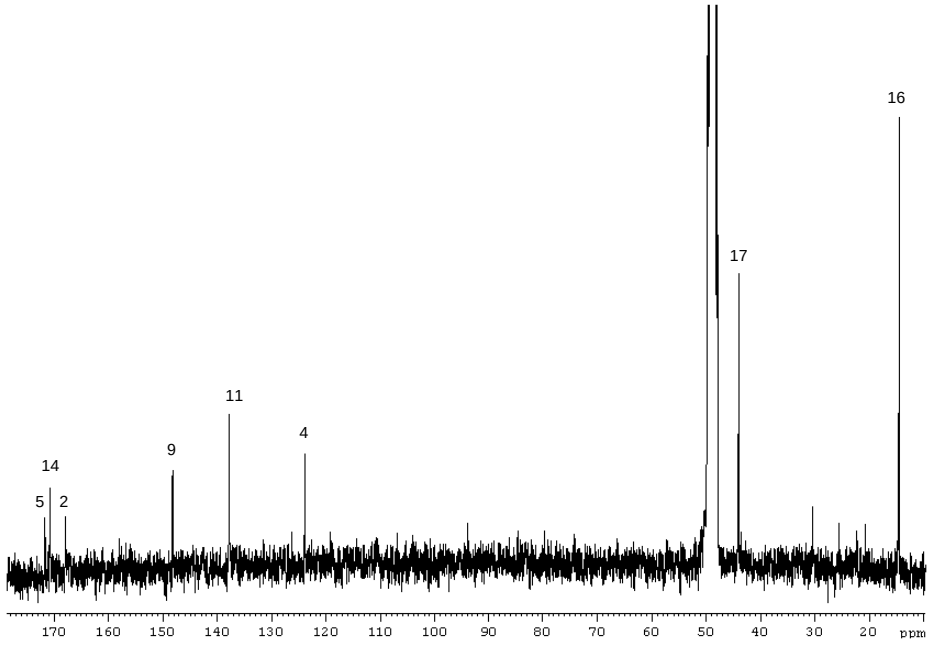


Figure **S4.3**: LC-ESIMS chromatogram (extracted ion mode; left) and LC-PDA chromatogram (total wavelength mode; right) as well as ESI mass spectrum of [1-^{13}C] labeled compound **9**

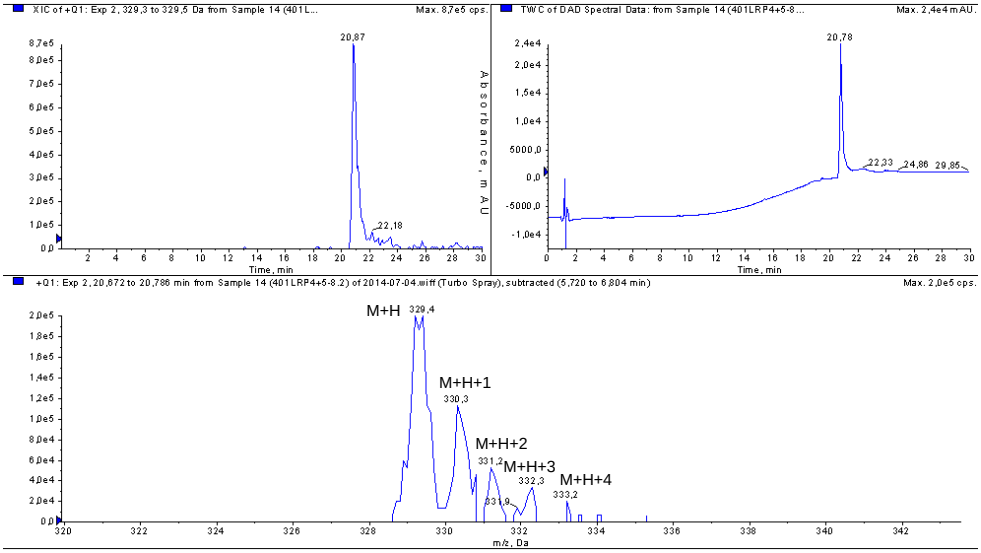


Figure **S5.1**: ^{1}H NMR spectrum of [1-^{13}C] labeled compound **12** in methanol-d_4

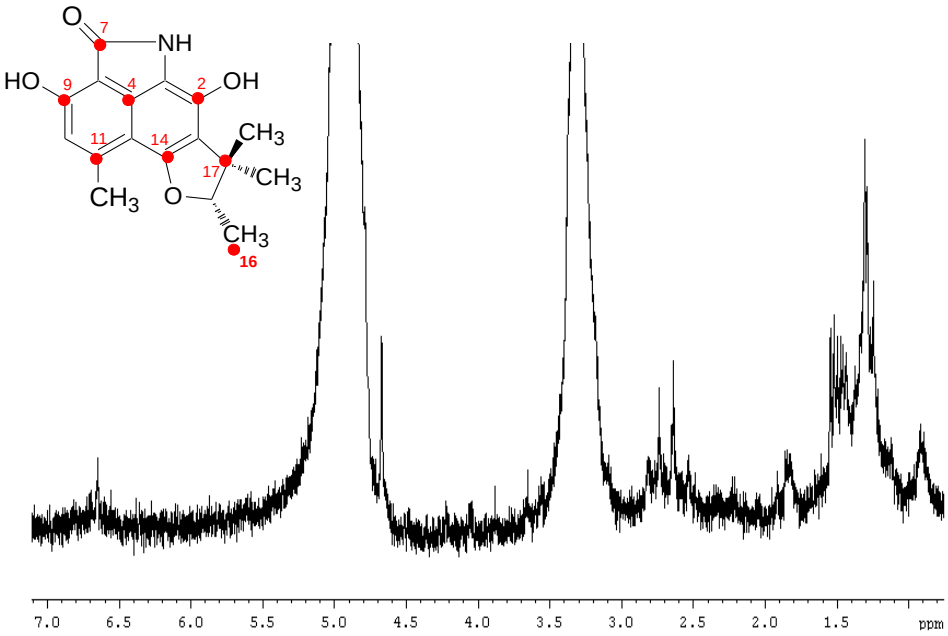


Figure **S5.2**: ^{13}C NMR spectrum of [1-^{13}C] labeled compound **12** in acetone-d_6 (x: impurity)

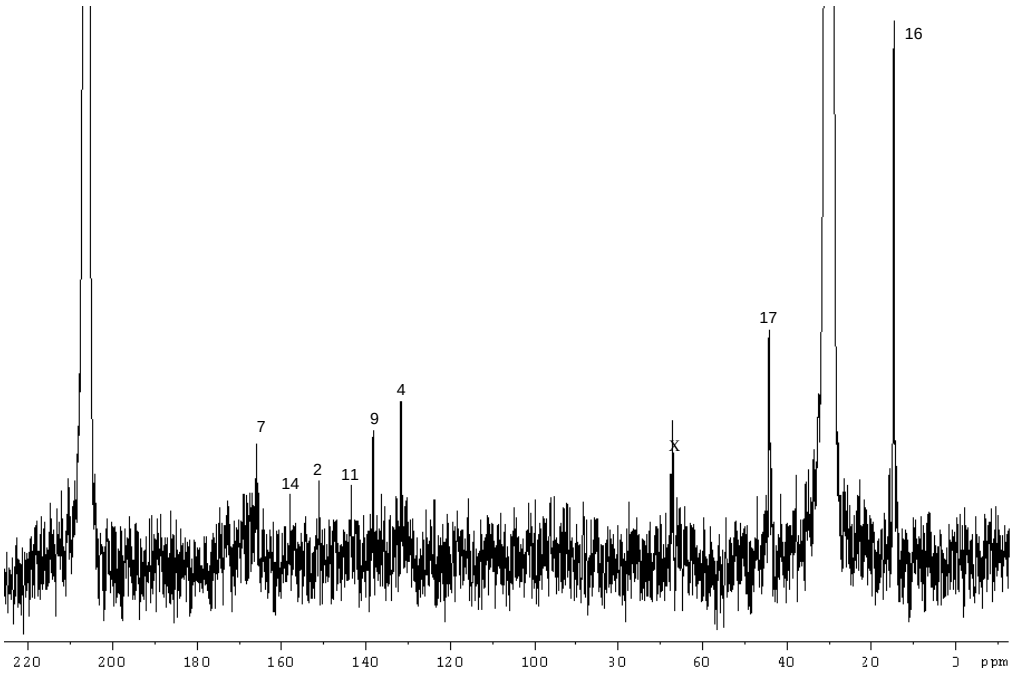


Figure **S5.3**: UPLC-HRMS spectrum of [1-^{13}C] labeled compound **12**

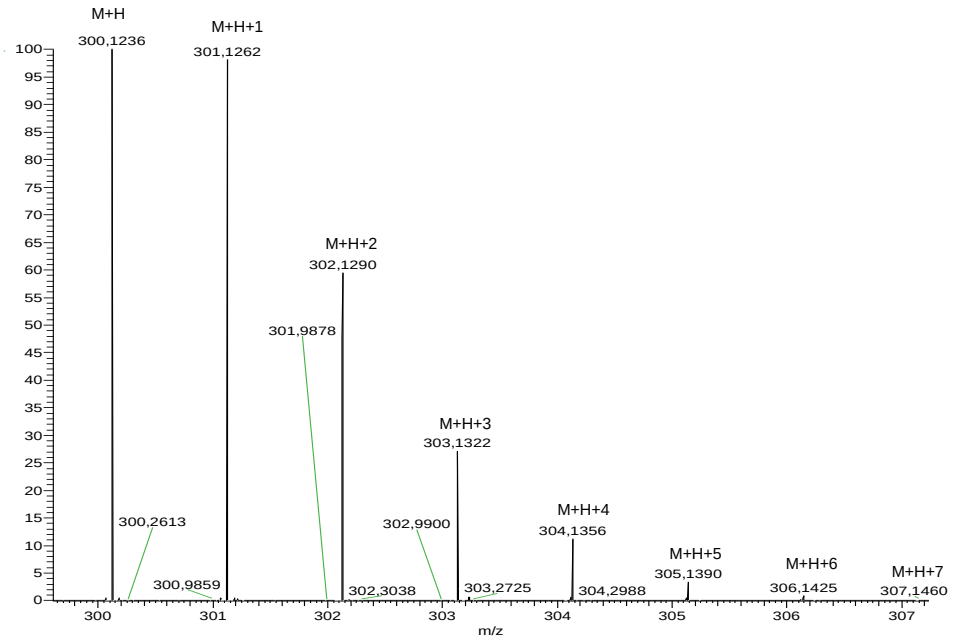


Figure **S6.1**: ^{1}H NMR spectrum of [1-^{13}C] labeled compound **14** in acetone-d_6

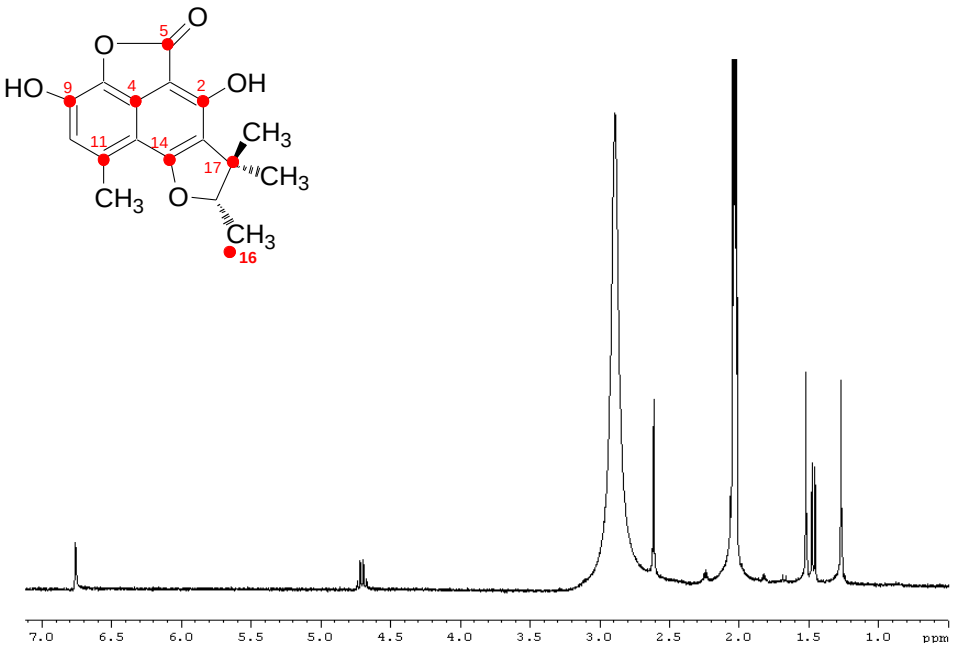


Figure **S6.2:** ^{13}C NMR spectrum of [1-^{13}C] labeled compound **14** in acetone-d_6

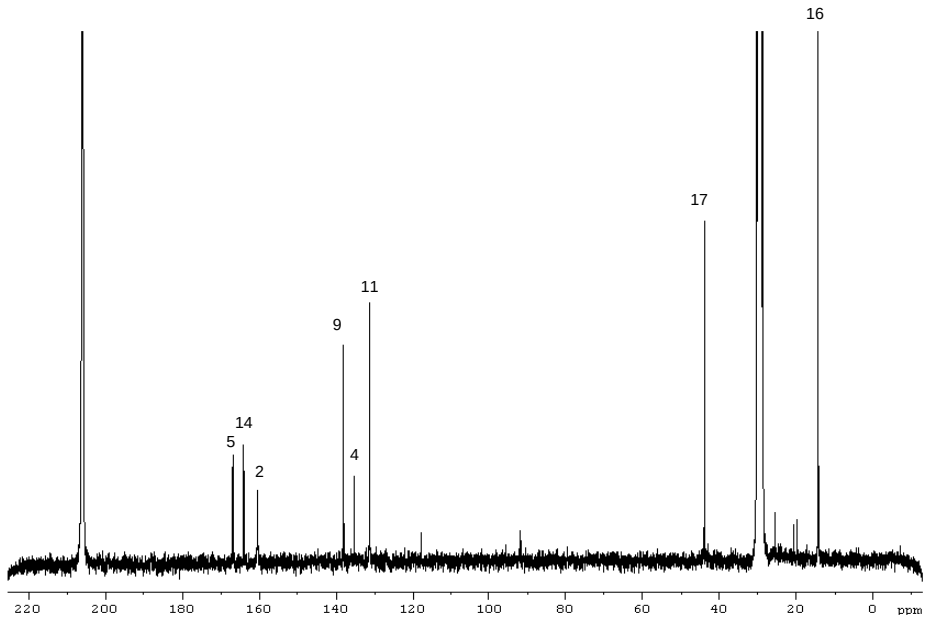


Figure **S6.3:** LC-ESIMS chromatogram (extracted ion mode; left) and LC-PDA chromatogram (total wavelength mode; right) as well as ESI mass spectrum of [1-^{13}C] labeled compound **14**

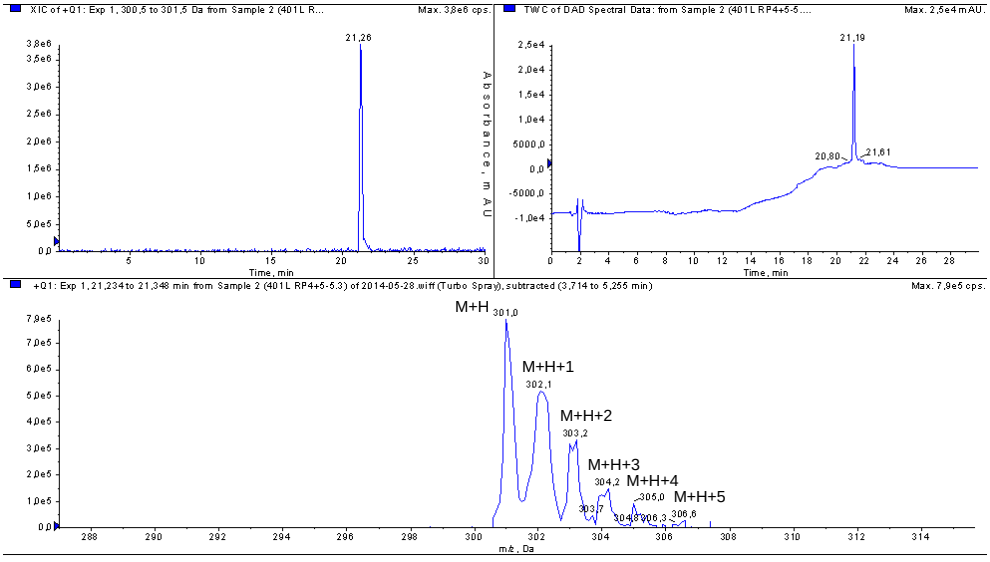


Figure **S7.1:** UPLC-HRMS spectrum of [1-^{13}C] labeled compound **15**

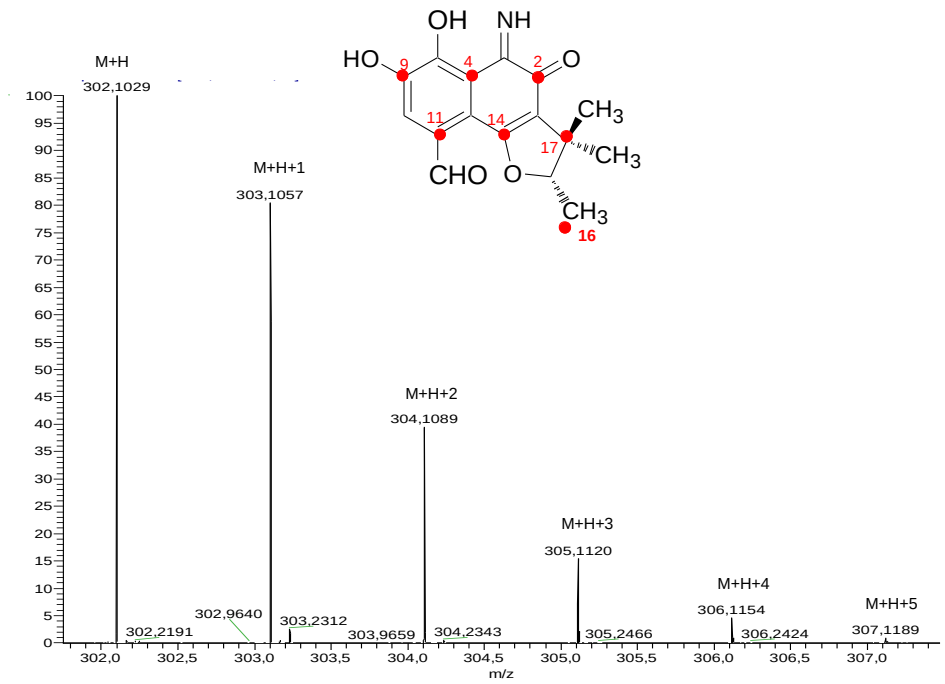


Figure **S8.1**: ^{1}H NMR spectrum of [1-^{13}C] labeled compound **16** in methanol-d_4

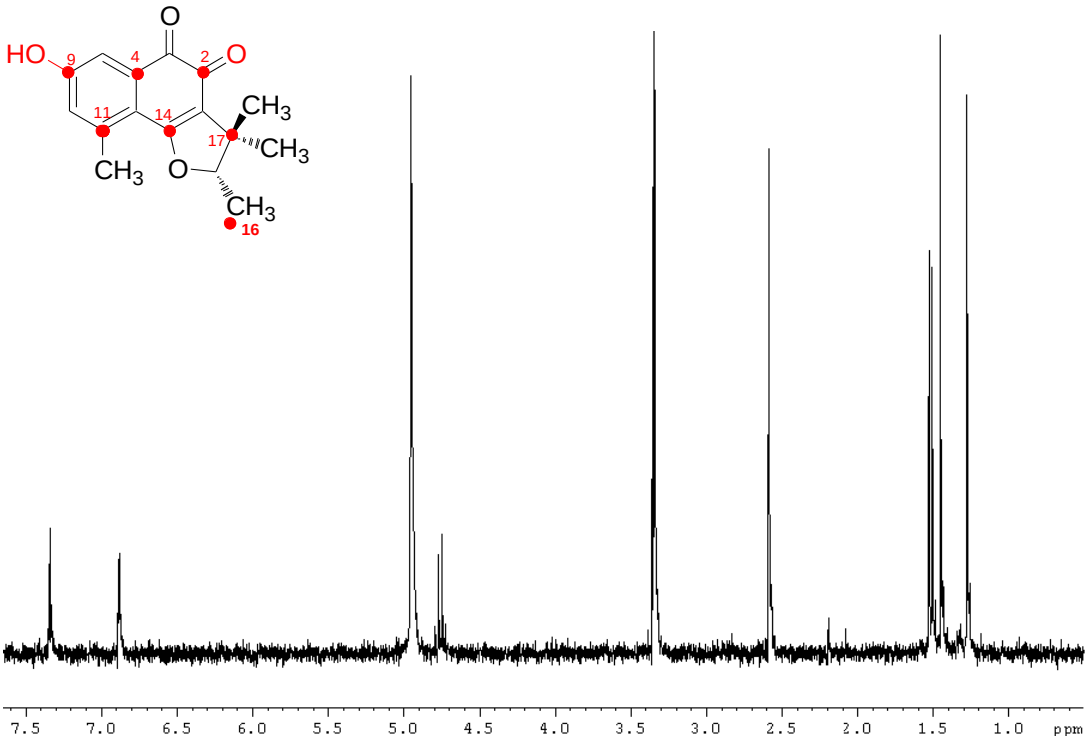


Figure **S8.2**: ^{13}C NMR spectrum of [1-^{13}C] labeled compound **16** in methanol-d_4

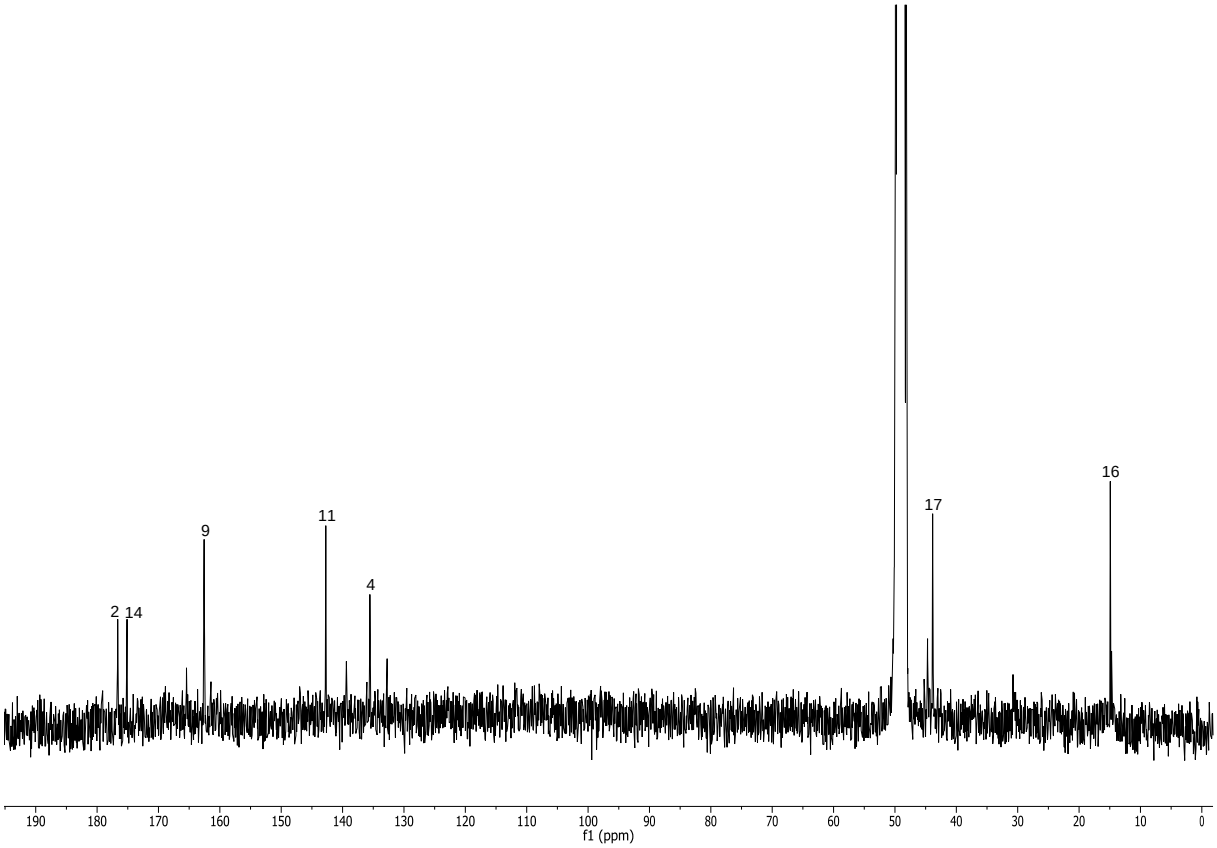


Figure **S8.3**: UPLC-HRMS spectrum of [1-^{13}C] labeled compound **16**

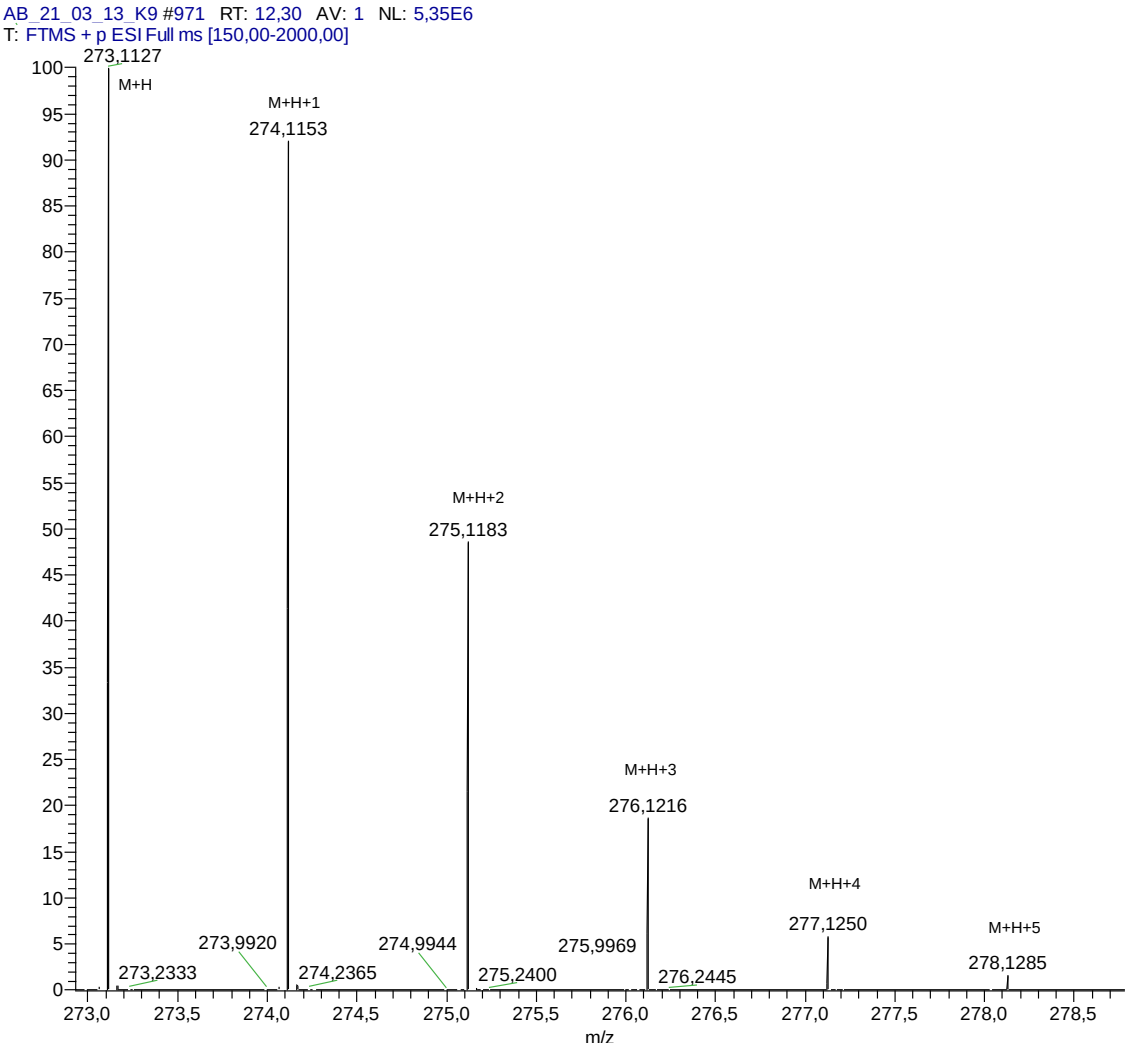


Table S1: ^{13}C NMR spectroscopic data for ^{13}C enriched compounds **1**, **3**, **6**, **9**, **12**, **14** and **16**

Pos.	Comp. 1[b]	Comp. 3[a]	Comp. 6[a]	Comp. 9[c]		Comp. 12[c]	Comp. 14[b]		Comp. 16[b]
	δ_C, mult.[d]	δ_C, mult.[d]	δ_C, mult.[d]	δ_C, mult.	enrich.[e]	δ_C, mult.[d]	δ_C, mult.[d]	enrich.[e]	δ_C, mult.[d]
2	170.03, C	167.98, C	164.20, C	168.12, C	2.2	151.18, C	160.72, C	3.8	176.64, C
4	138.27, C	135.09, C	135.31, C	124.13, C	2.4	131.73, C	135.62, C	3.2	135.55, C
5	199.46, C	164.72, C	164.84, C	171.90, C	2.1	-	167.17, C	3.5	-
7	201.45, C	165.15, C	165.41, C	-	-	165.79, C	-	-	-
9	167.23, C	165.22, C	165.93, C	148.44, C	2.3	138.33, C	138.38, C	5.9	162.56, C
11	150.21, C	150.70, C	149.72, C	138.06, C	4.0	143.50, C	131.65, C	3.2	142.74, C
14	166.67, C	166.68, C	166.13, C	170.99, C	2.6	157.75, C	164.35, C	3.4	175.12, C
16	67.27, CH_2	66.58, CH_2	14.51, CH_2	14.82, CH_3	9.2	14.66, CH_3	14.64, CH_3	12.7	14.92, CH_3
17	140.07, C	140.13, C	43.38, C	44.25, C	6.3	44.23, C	44.21, C	7.5	43.86, C
18	-	-	-	25.56, CH_3	1.0	-	25.63, CH_3	1.0	-

[a] In chloroform-d_1. [b] In acetone-d_6. [c] In methanol-d_4. [d] Implied multiplicities determined by DEPT. [e] ^{13}C enrichment ratios of labeled compounds were calculated on the basis of the relative intensity of CH_3-18 (1.0). Due to low concentrations for compounds **1**, **3**, **6**, **12**, and **16** no enrichment ratios could be calculated.

Table S2: UPLC-HRMS results (experimental and calculated mass values (*m/z*) of isotopic molecular ions) of [1-^{13}C] labeled compound **12**

Molecular formula	Isotopic molecular ions	Calculated mass values (*m/z*)	Experimental mass values (*m/z*)
$^{12}C_{17}H_{17}NO_4H$	$[M+H]^+$	300.1236	300.1236
$^{12}C_{16}+^{13}C_1+H_{17}NO_4H$	$[M+H+1]^+$	301.1270	301.1262
$^{12}C_{15}+^{13}C_2+H_{17}NO_4H$	$[M+H+2]^+$	302.1304	302.1290
$^{12}C_{14}+^{13}C_3+H_{17}NO_4H$	$[M+H+3]^+$	303.1338	303.1322
$^{12}C_{13}+^{13}C_4+H_{17}NO_4H$	$[M+H+4]^+$	304.1372	304.1356
$^{12}C_{12}+^{13}C_5+H_{17}NO_4H$	$[M+H+5]^+$	305.1406	305.1390
$^{12}C_{11}+^{13}C_6+H_{17}NO_4H$	$[M+H+6]^+$	306.1440	306.1425
$^{12}C_{10}+^{13}C_7+H_{17}NO_4H$	$[M+H+7]^+$	307.1474	307.1460
$^{12}C_9+^{13}C_8+H_{17}NO_4H$	$[M+H+8]^+$	308.1508	308.1494

Table S3: UPLC-HRMS results (experimental and calculated mass values (*m/z*) of isotopic molecular ions) of [1-^{13}C] labeled compound **15**

Molecular formula	Isotopic molecular ions	Calculated mass values (*m/z*)	Experimental mass values (*m/z*)
$^{12}C_{16}H_{15}NO_5H$	$[M+H]^+$	302.1028	302.1029
$^{12}C_{15}+^{13}C_1+H_{15}NO_5H$	$[M+H+1]^+$	303.1062	303.1057
$^{12}C_{14}+^{13}C_2+H_{15}NO_5H$	$[M+H+2]^+$	304.1096	304.1089
$^{12}C_{13}+^{13}C_3+H_{15}NO_5H$	$[M+H+3]^+$	305.1130	305.1120
$^{12}C_{12}+^{13}C_4+H_{15}NO_5H$	$[M+H+4]^+$	306.1164	306.1154
$^{12}C_{11}+^{13}C_5+H_{15}NO_5H$	$[M+H+5]^+$	307.1198	307.1189
$^{12}C_{10}+^{13}C_6+H_{15}NO_5H$	$[M+H+6]^+$	308.1232	308.1223
$^{12}C_9+^{13}C_7+H_{15}NO_5H$	$[M+H+7]^+$	309.1266	309.1252

Table S4: UPLC-HRMS results (experimental and calculated mass values (*m/z*) of isotopic molecular ions) of [1-^{13}C] labeled compound **16**

Molecular formula	Isotopic molecular ions	Calculated mass values (*m/z*)	Experimental mass values (*m/z*)
$^{12}C_{16}H_{16}O_4H$	$[M+H]^+$	273.1127	273.1127
$^{12}C_{15}+^{13}C_1+H_{16}O_4H$	$[M+H+1]^+$	274.1161	274.1153
$^{12}C_{14}+^{13}C_2+H_{16}O_4H$	$[M+H+2]^+$	275.1195	275.1183
$^{12}C_{13}+^{13}C_3+H_{16}O_4H$	$[M+H+3]^+$	276.1229	276.1216
$^{12}C_{12}+^{13}C_4+H_{16}O_4H$	$[M+H+4]^+$	277.1263	277.1250
$^{12}C_{11}+^{13}C_5+H_{16}O_4H$	$[M+H+5]^+$	278.1297	278.1285
$^{12}C_{10}+^{13}C_6+H_{16}O_4H$	$[M+H+6]^+$	279.1331	279.1322
$^{12}C_9+^{13}C_7+H_{16}O_4H$	$[M+H+7]^+$	280.1365	280.1350

Table S5: Results of time scale experiments showing the presence (+) or absence (-) of certain *C. cereale* metabolites. Measurements were performed by LC-MS.

No.	Compounds	Molecular weight	Retention times (t_R) in LCMS	Fungus *C. cereale* extracts (3-30 days)												
				3	5	7	9	11	13	15	17	19	21	23	25	30
1	rousselianone A′	398	20.70-21.55	–	–	+	+	+	+	+	+	+	+	+	+	+
2	lamellicolic anhydride	260	16.92-16.95	–	–	–	–	–	–	–	–	–	–	–	–	+
3	coniosclerodin	328	22.88-23.59	–	+	+	+	+	+	+	+	+	+	+	+	+
4	*Z*-coniosclerodinol	344	20.78-20.90	–	–	–	–	–	–	–	–	–	–	–	+	+
5	*E*-coniosclerodinol	344	20.36-20.54	–	–	–	–	–	–	–	–	–	–	–	+	+
6	(-)-sclerodin	328	22.88-23.59	–	+	+	+	+	+	+	+	+	+	+	+	+
7	*S,S*-sclerodinol	344		n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
8	coniosclerodinol	328	19.76-19.94	–	+	+	+	+	+	+	+	+	+	+	+	+
9	(-)-scleroderolide	328	19.89-20.02	–	+	+	+	+	+	+	+	+	+	+	+	+
10	coniosclerodione	312	18.97-19.15	–	+	+	+	+	+/–	+	+	+/–	+/–	+	+	+
11	(-)-sclerodione	312	19.34-19.80	–	+	+	+	+	–	+/–	+	+/–	+/–	+	+	+
12	(-)-cereolactam	299	20.96-21.97	–	–	–	–	–	–	–	–	+	+	+/–	+/–	+
13	coniolactone	300	19.28-19.46	–	–	–	–	+	+/–	+/–	+/–	+	+/–	+	+	+
14	(-)-cereolactone	300	21.17-21.40	–	–	+	+	+	+	+	+	+	+	+	+	+
15	(-)-cereoaldomine	301	17.05-17.17	–	–	–	–	–	–	–	–	–	+	+/–	+/–	+
16	(-)-trypethelone	272	19.51-19.63	–	–	–	–	–	+	+/–	+/–	+	+	+	+	+
17	conioamide	287		n.d.	n.d.	n.d.	n.d.	n.d.	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
18	cereoanhydride	306	18.63-18.87	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d

For LCMS measurements: see experimental part; n.d. = not determined; +/- = amount below the detection limit.

Figure **S9**: Biosynthesis of the prenyl group

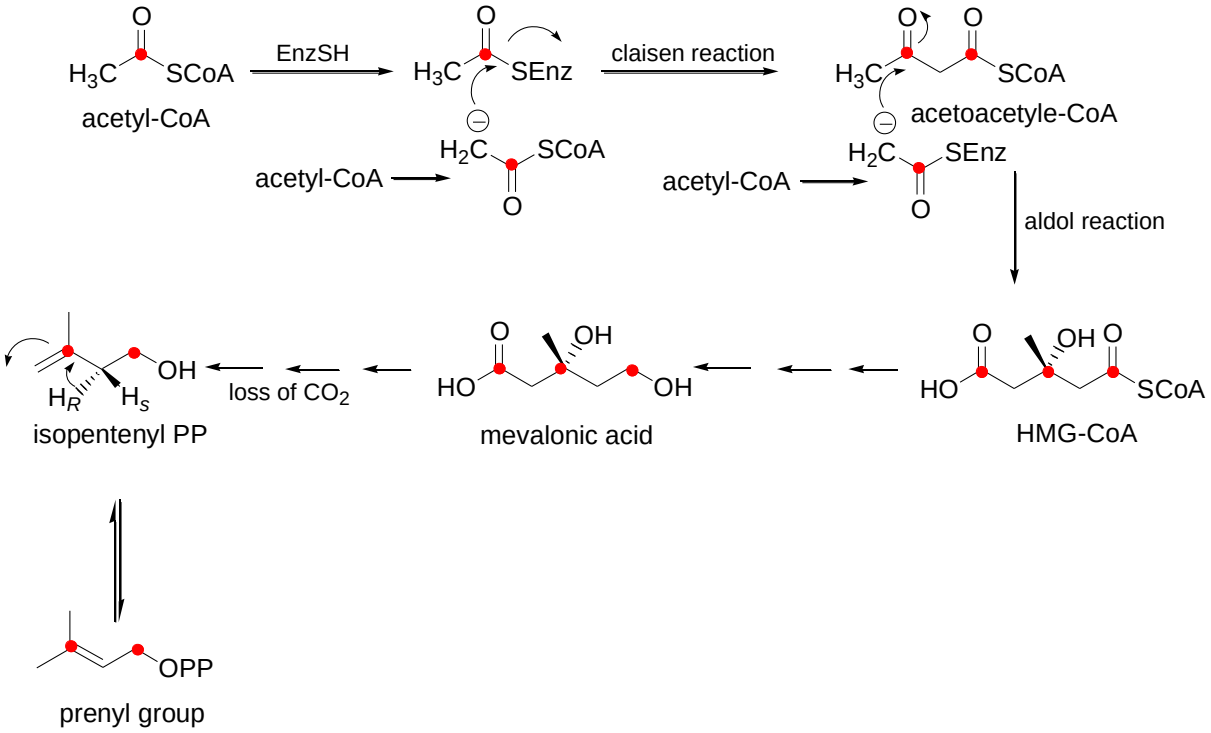


Figure **S10**: Structures and labeling pattern of sclerodin, scleroderolide, sclerodione, deoxyherqueinone and atrovenetin

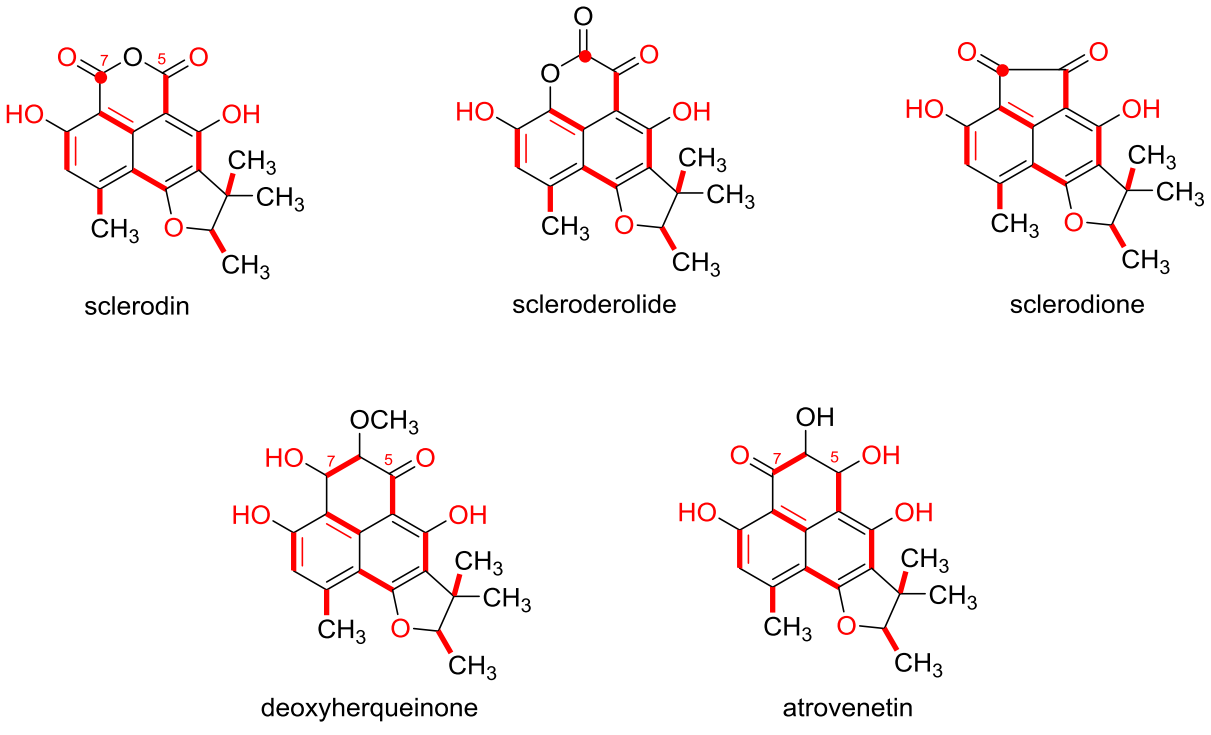
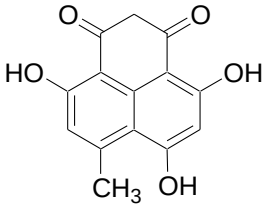


Figure **S11**: Carbon skeleta of polyketides produced by the fungus *Coniothyrium cereale*



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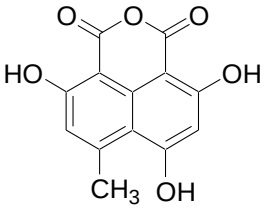

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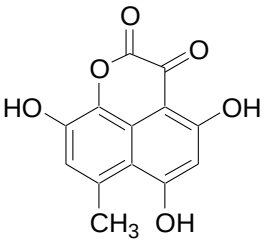
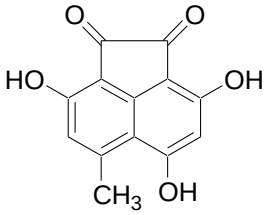


III



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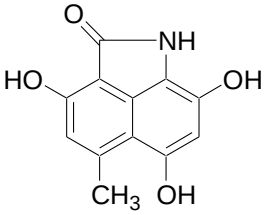

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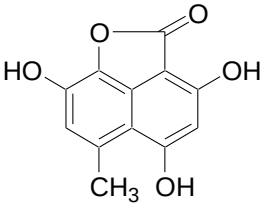

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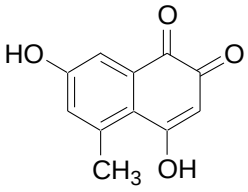

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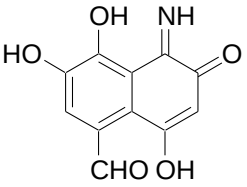

VIII