

Supplementary Information for

Enhancing Microbial Metabolic Capacity through High-Energy Electron Beam-Induced Intense Structural Variations

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Figure S1. Agarose gel electrophoresis images of plasmid (pIJ773) irradiated with UV, MW, X-ray, γ -ray, ARTP or HEPE. M: marker. C: control plasmid without any treatment. C-HindIII: control plasmid digested with HindIII. M30-180: plasmid irradiated with MW at 30, 60, 90 and 180s, respectively. X20-100: plasmid irradiated with X-ray at 20, 50, 80, and 100 Gy, respectively. γ 0.2-3.0: plasmid irradiated with γ -ray at 0.2, 0.5, 1 and 3 KGy, respectively. U30-U600: plasmid irradiated with UV at 30, 90, 180 and 600s, respectively. A15-A300: plasmid irradiated with ARTP at 15, 45, 90 and 300s, respectively. H1-H20: plasmid irradiated with HEPE at 1, 3, 6 and 20 kGy, respectively.

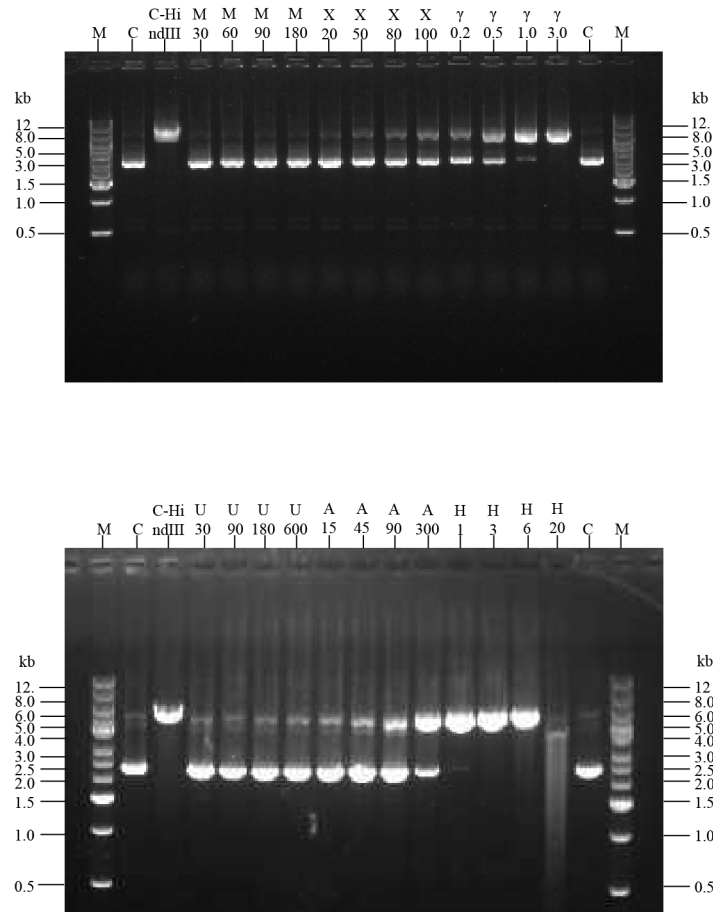


Figure S2. Subgeneration plates of pigmented mutants irradiated with UV (a), ARTP (b) or HEPE (c).

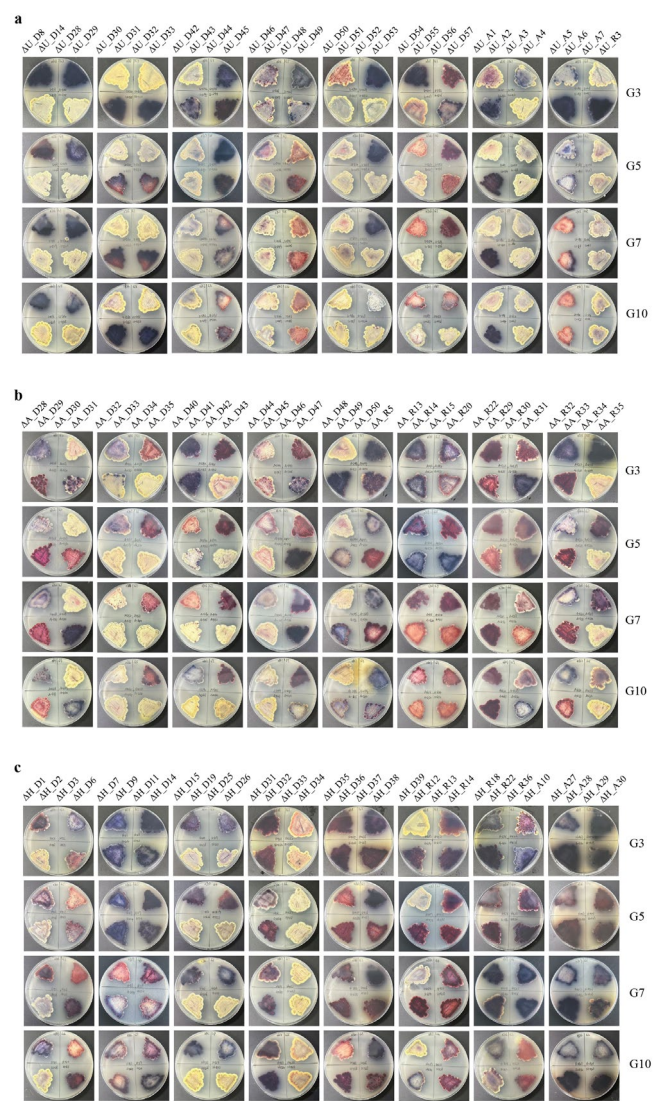
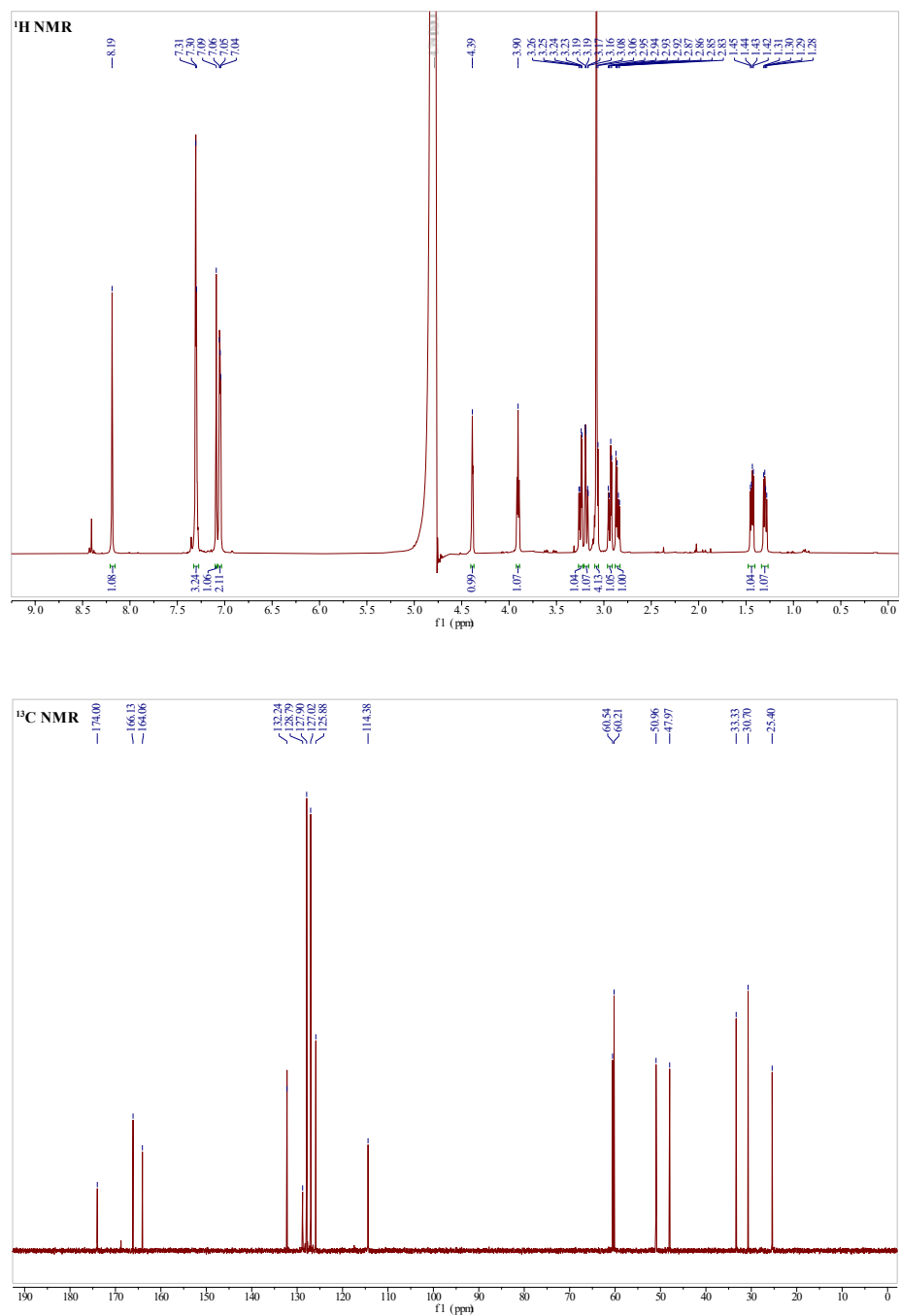
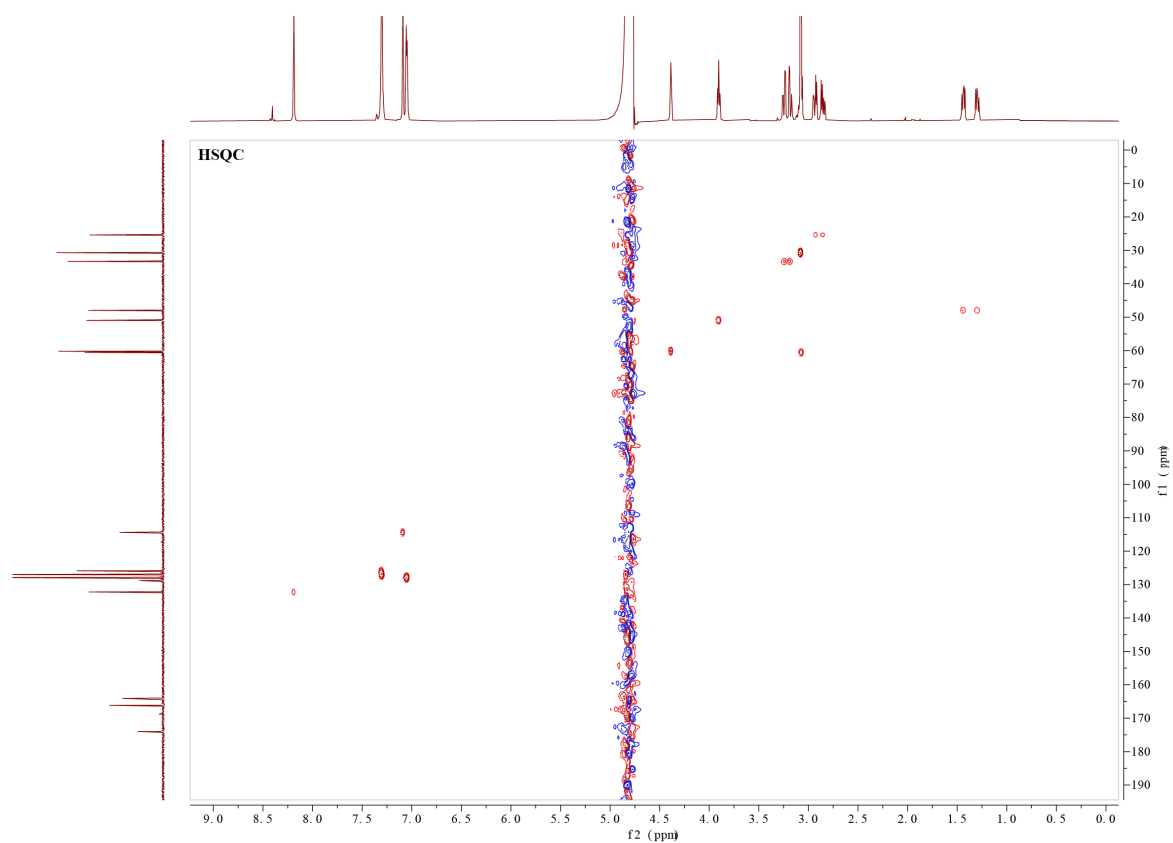
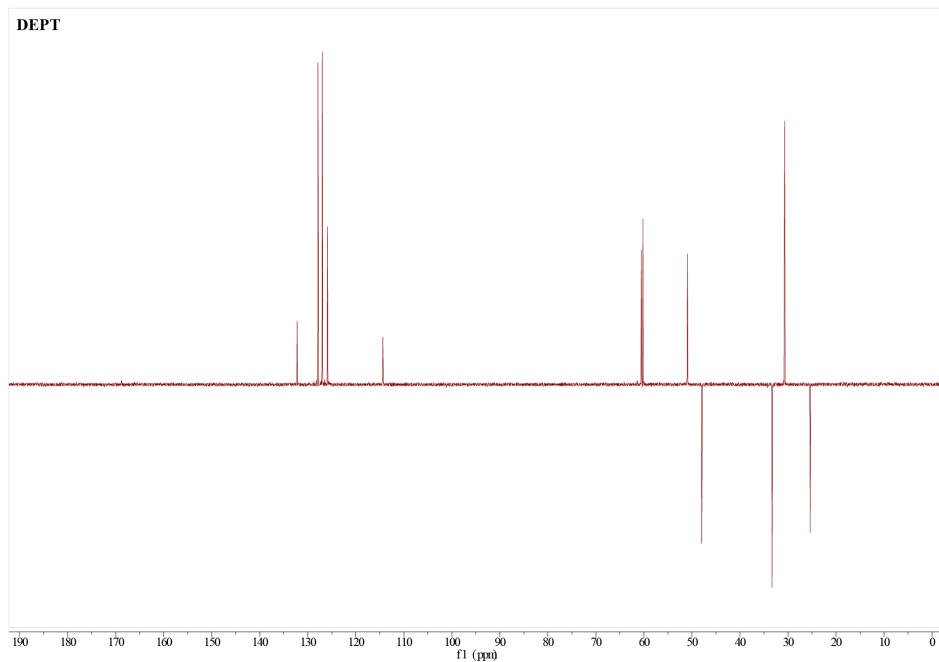


Figure S3. Full NMR spectra (600 MHz) of griseobrucins A in H₂O:D₂O(9:1).





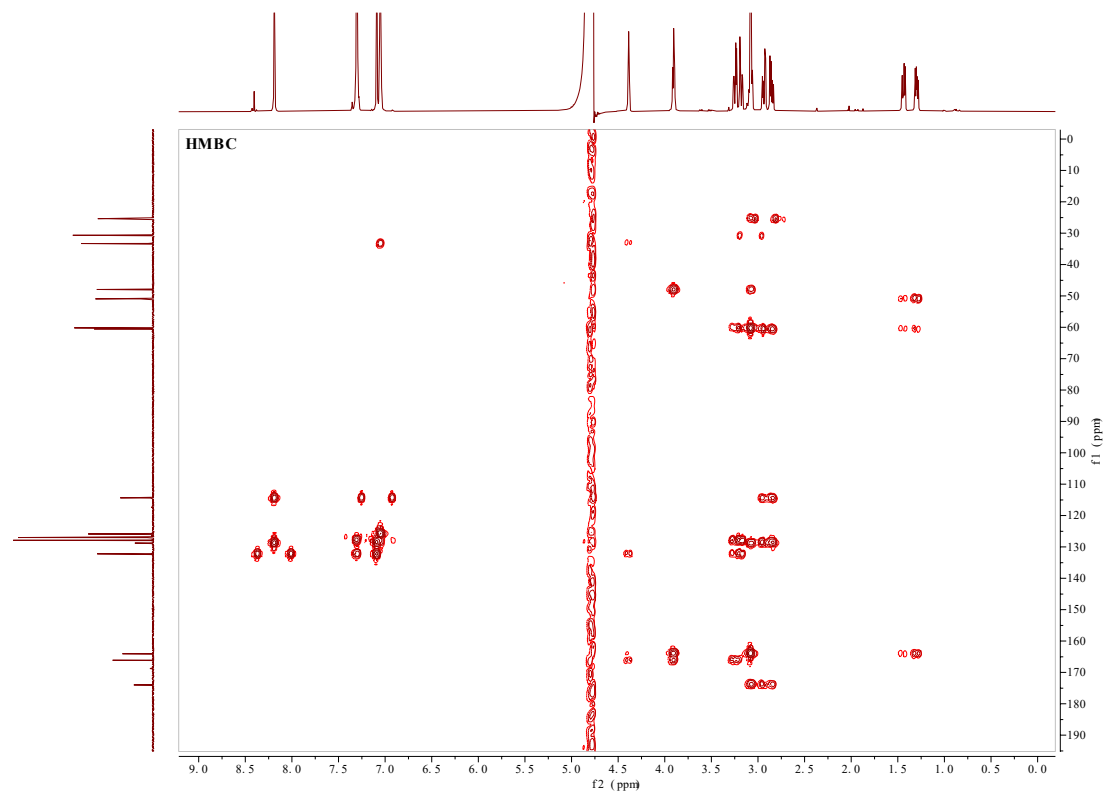
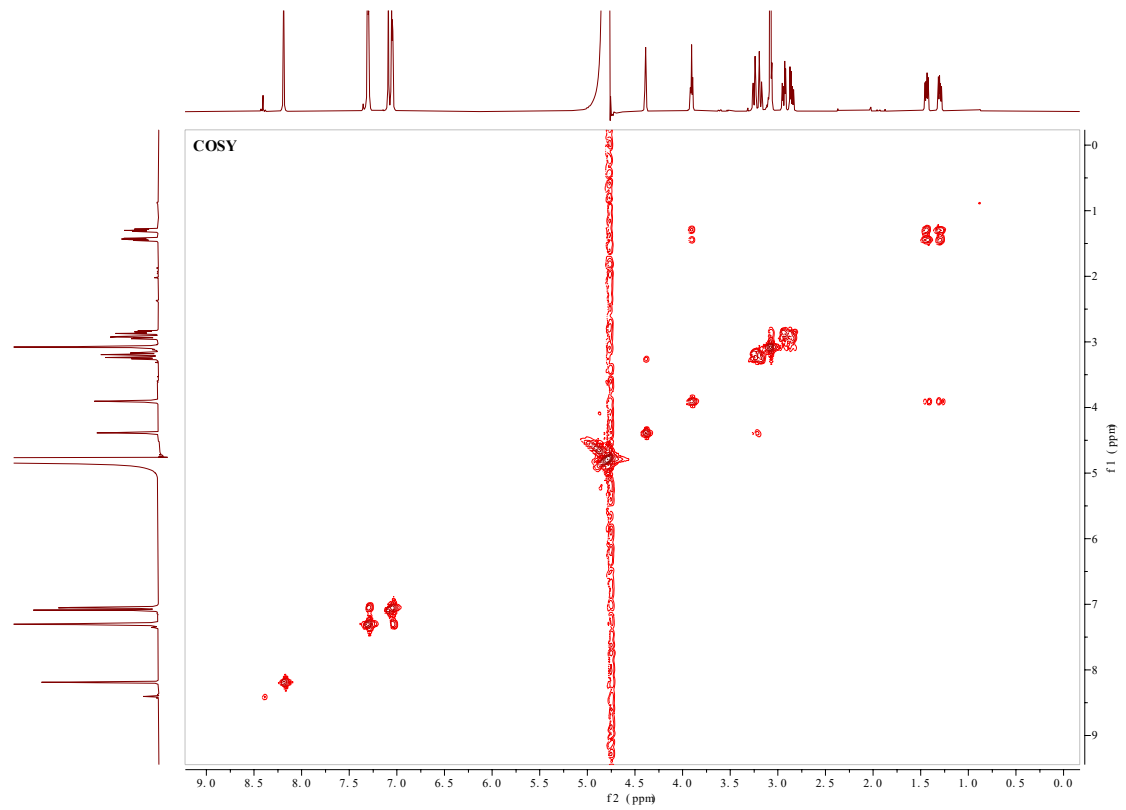
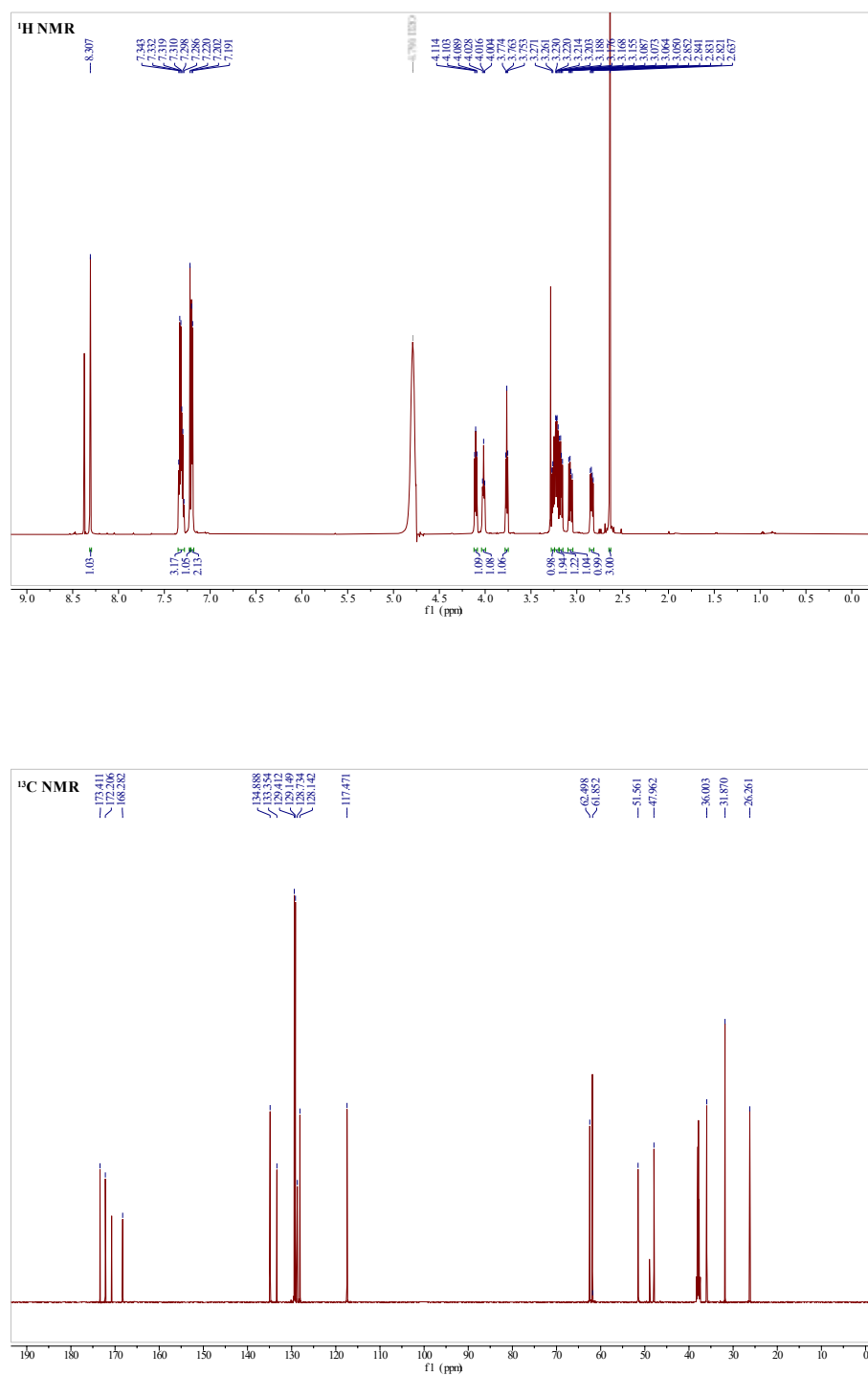
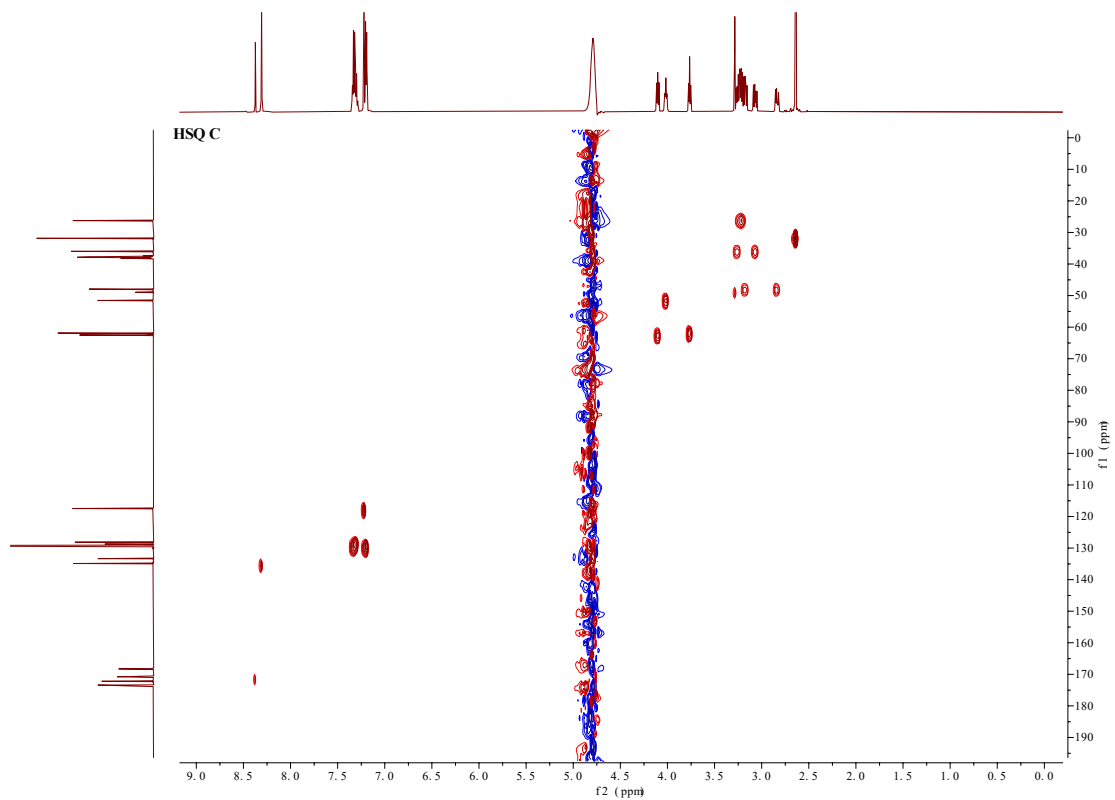
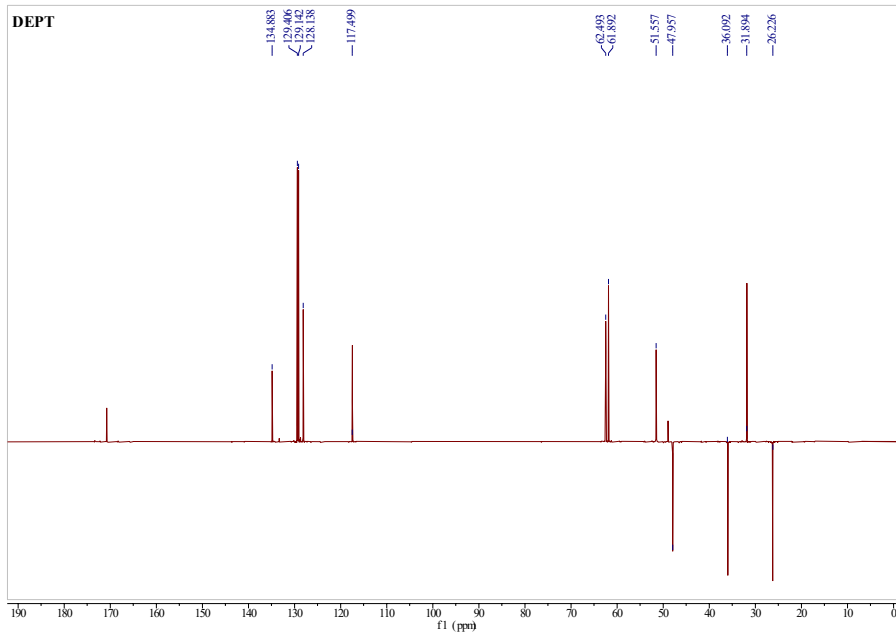


Figure S4. Full NMR spectra (600 MHz) of griseobrucins B in H₂O:D₂O(9:1).





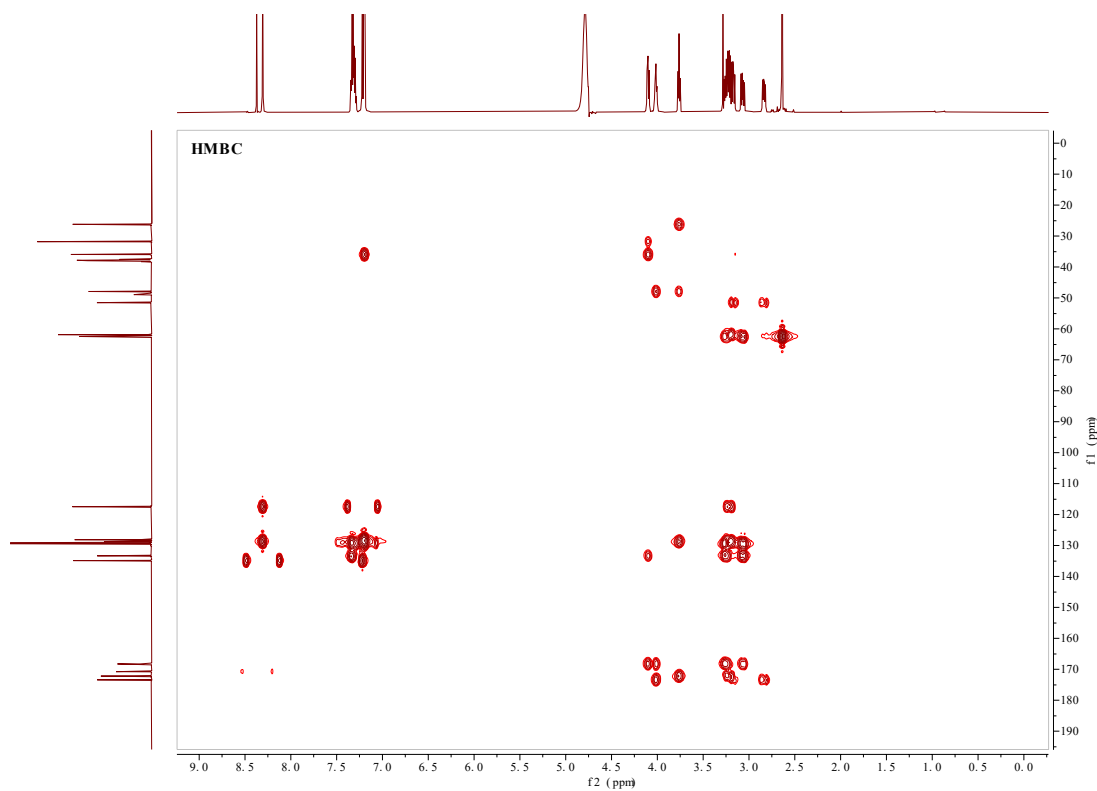
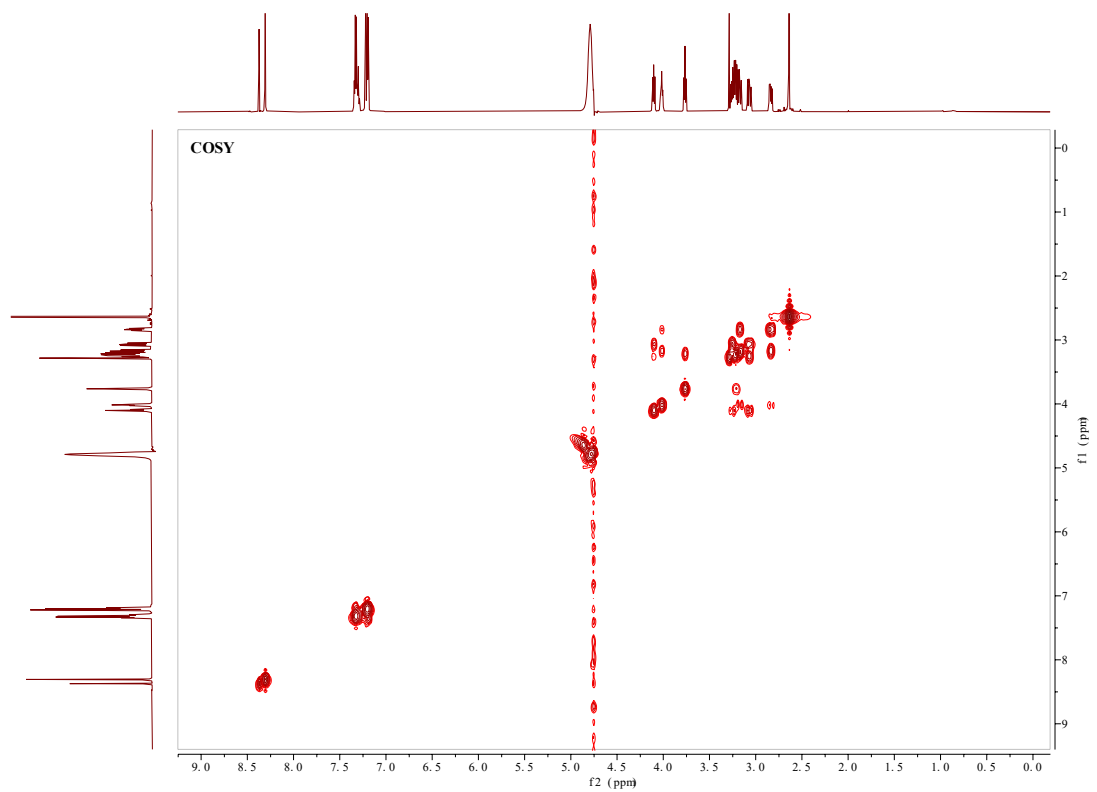
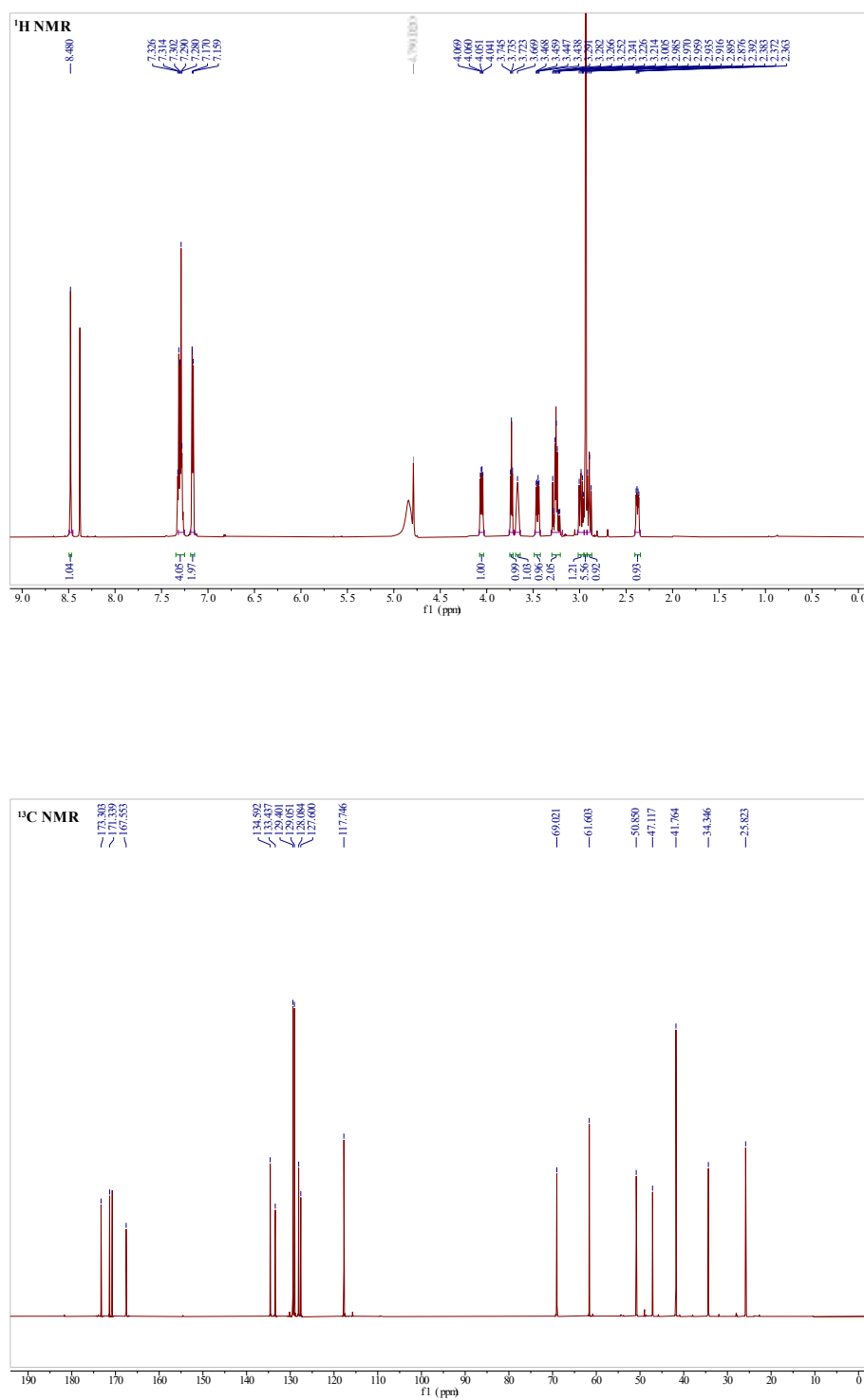
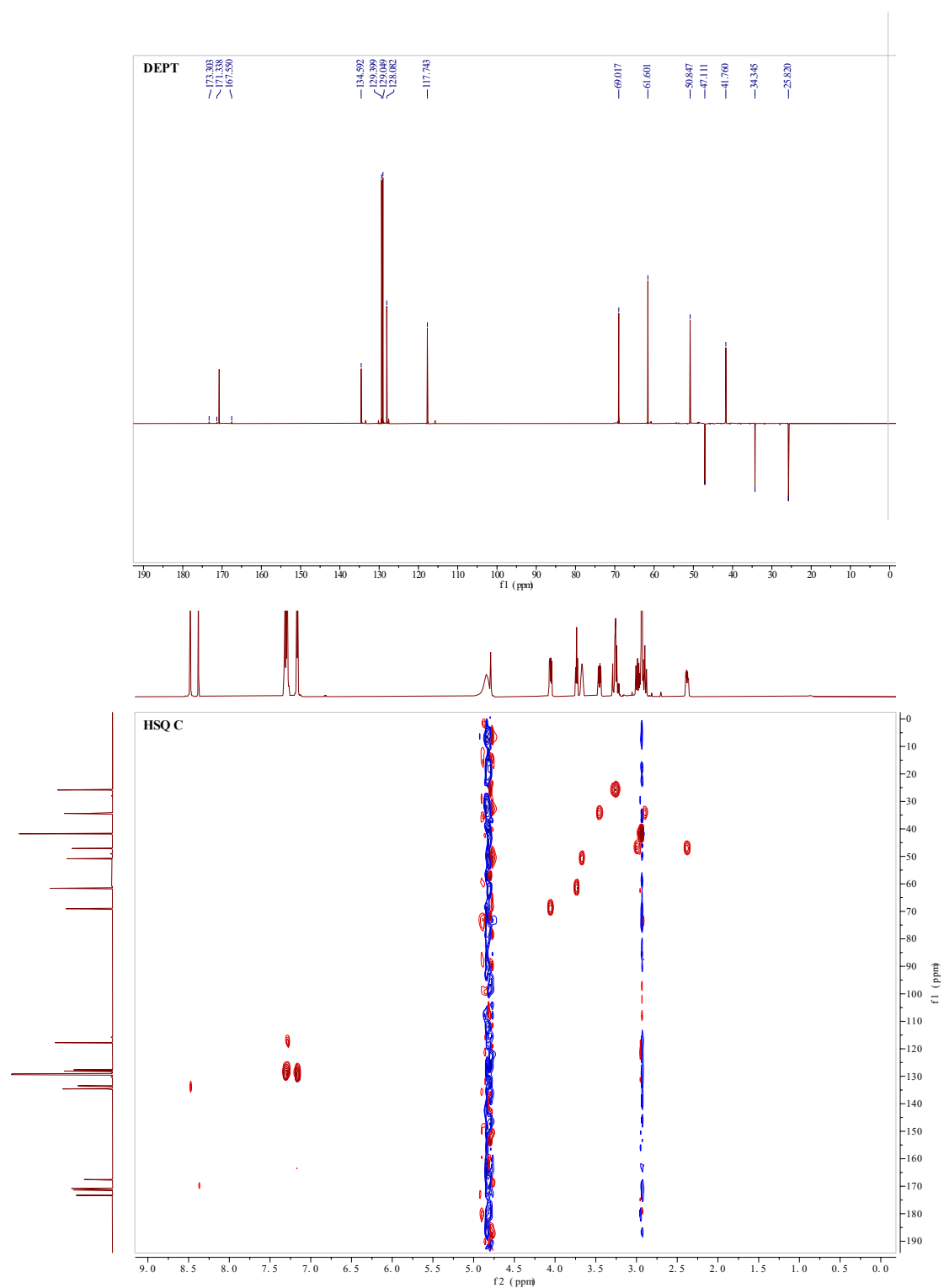


Figure S5. Full NMR spectra (600 MHz) of griseobrucins C in H₂O:D₂O(9:1).





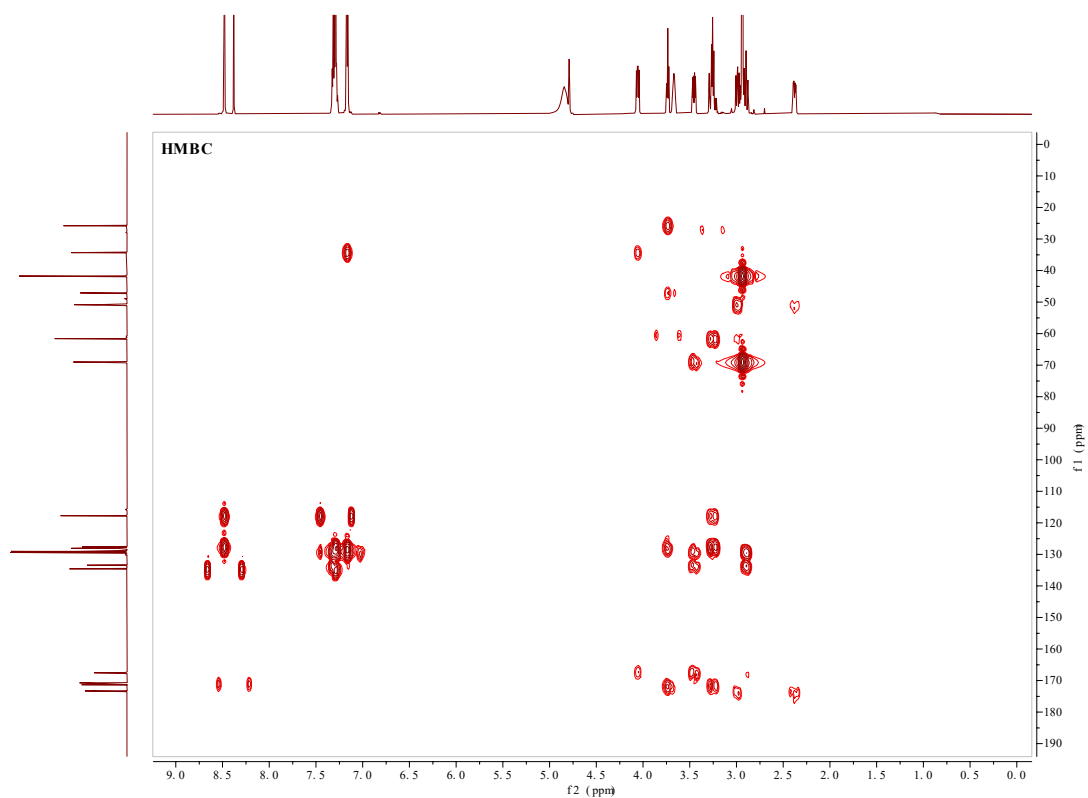
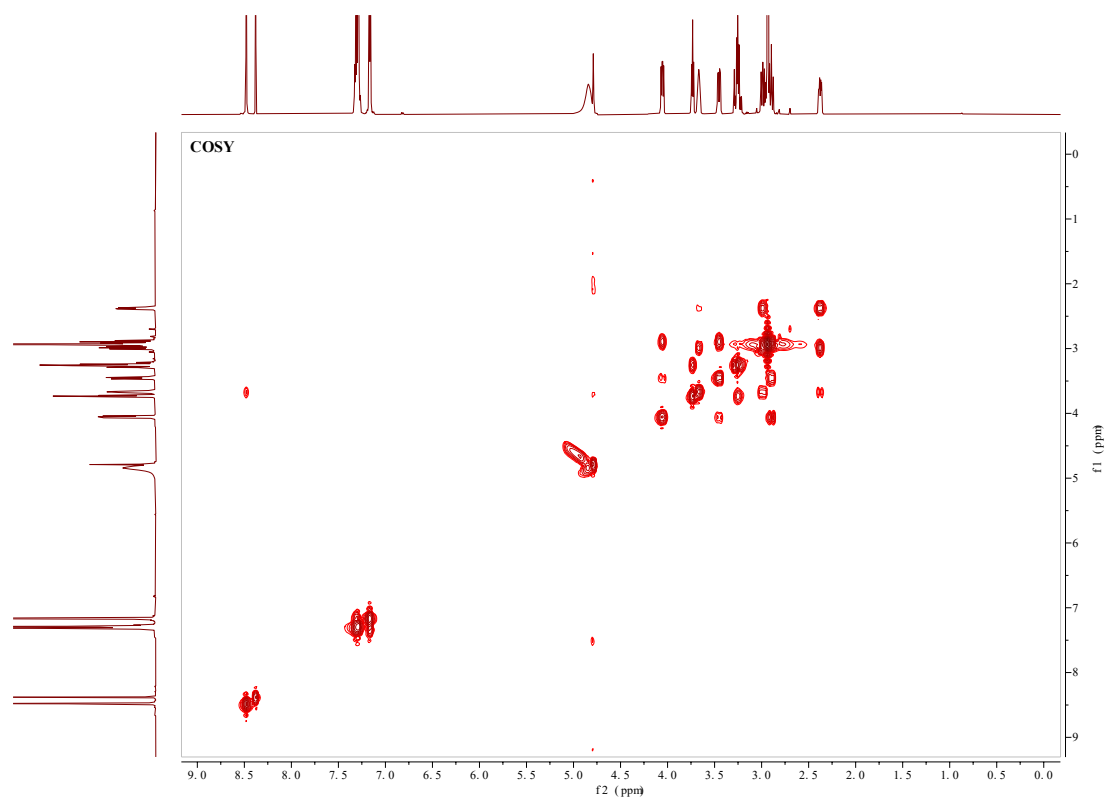


Figure S6. Proposed biosynthetic pathway of griseobrucins.

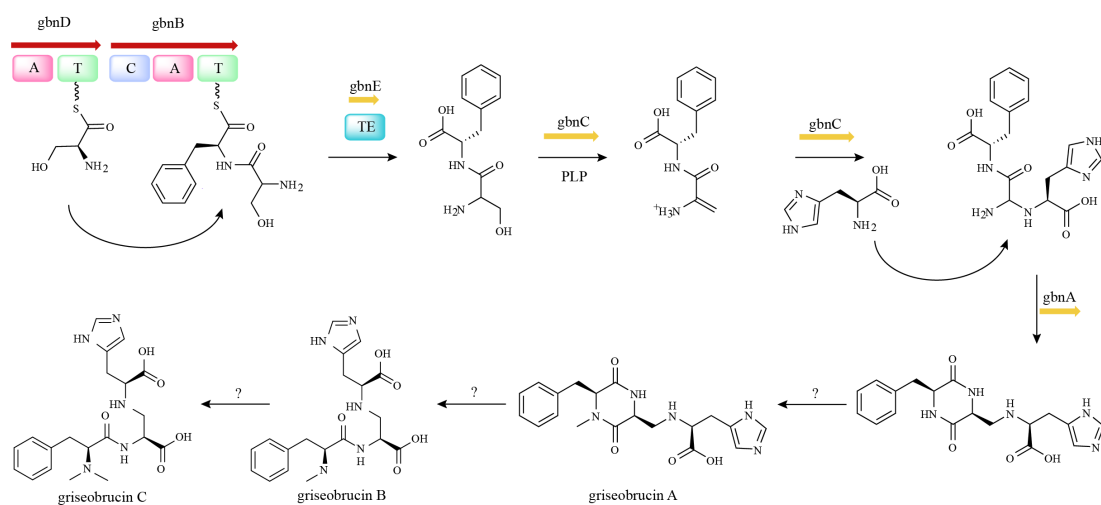
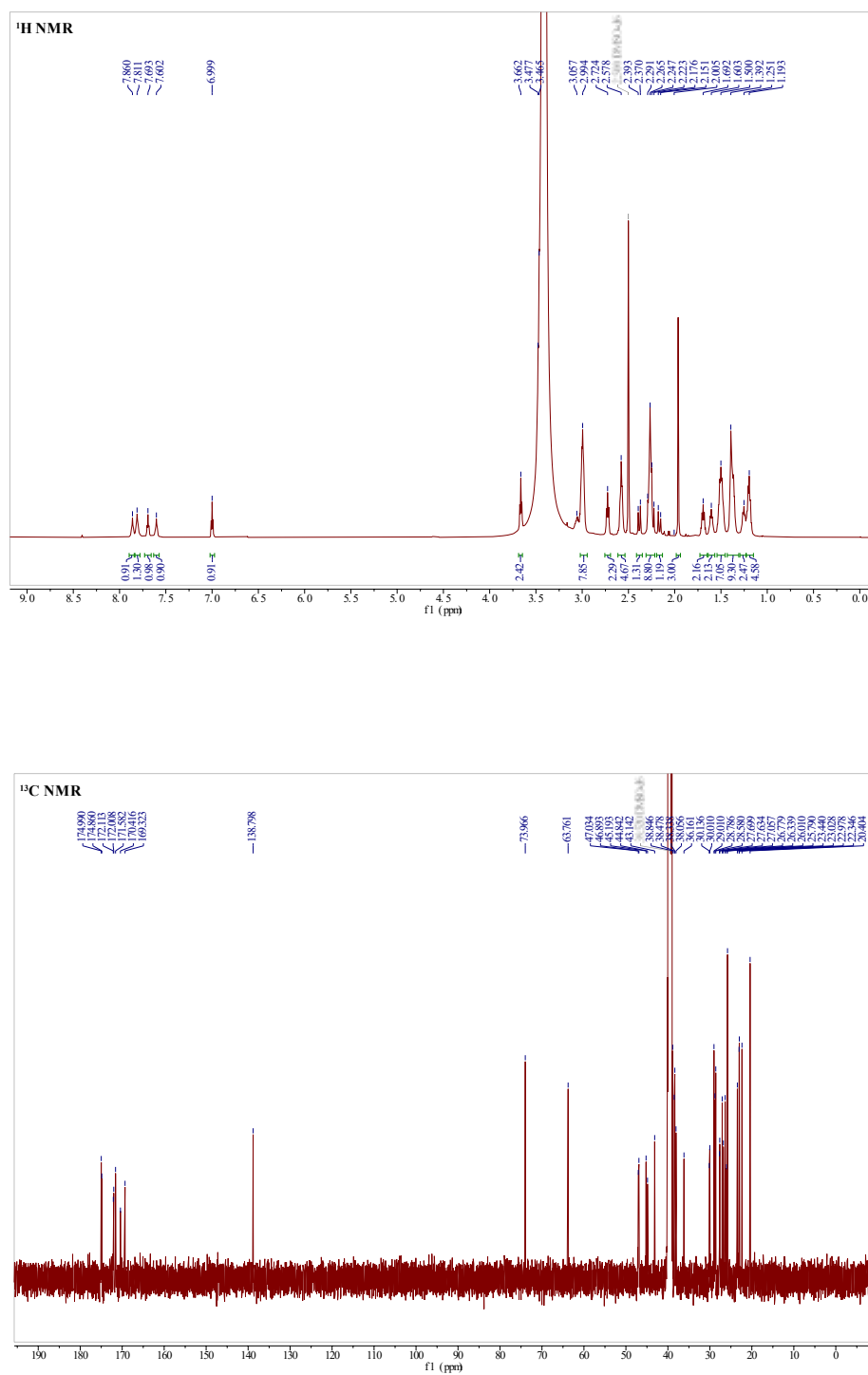
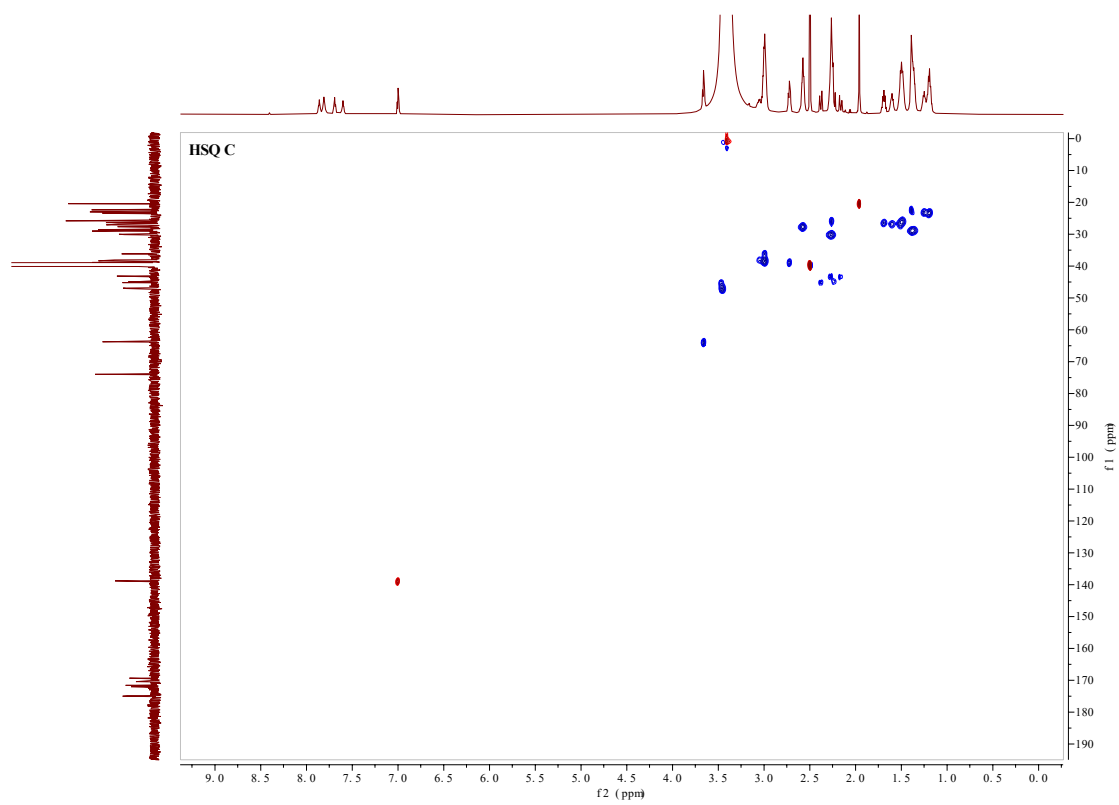
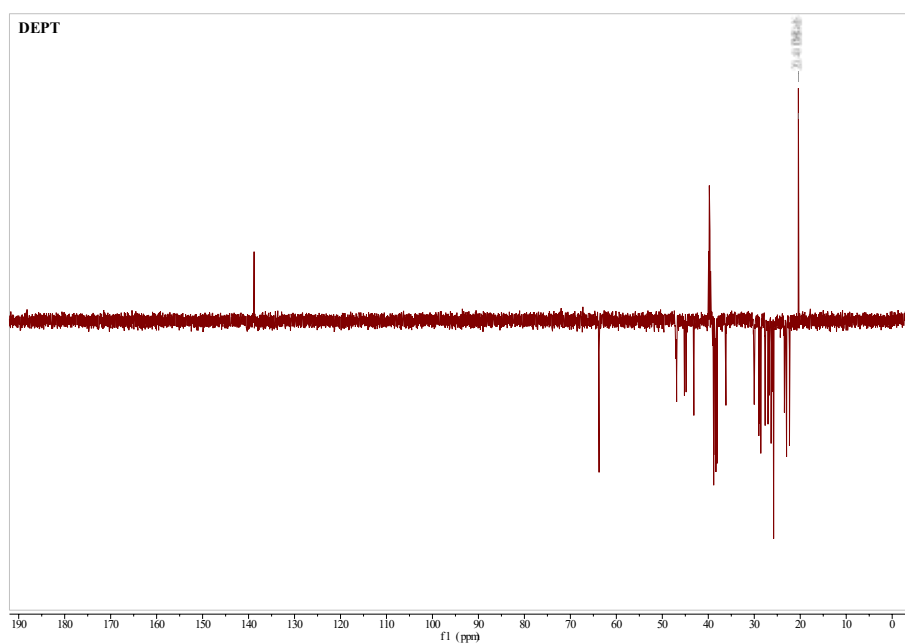


Figure S7. Full NMR spectra (600 MHz) of fradibactin A in DMSO- d_6 .





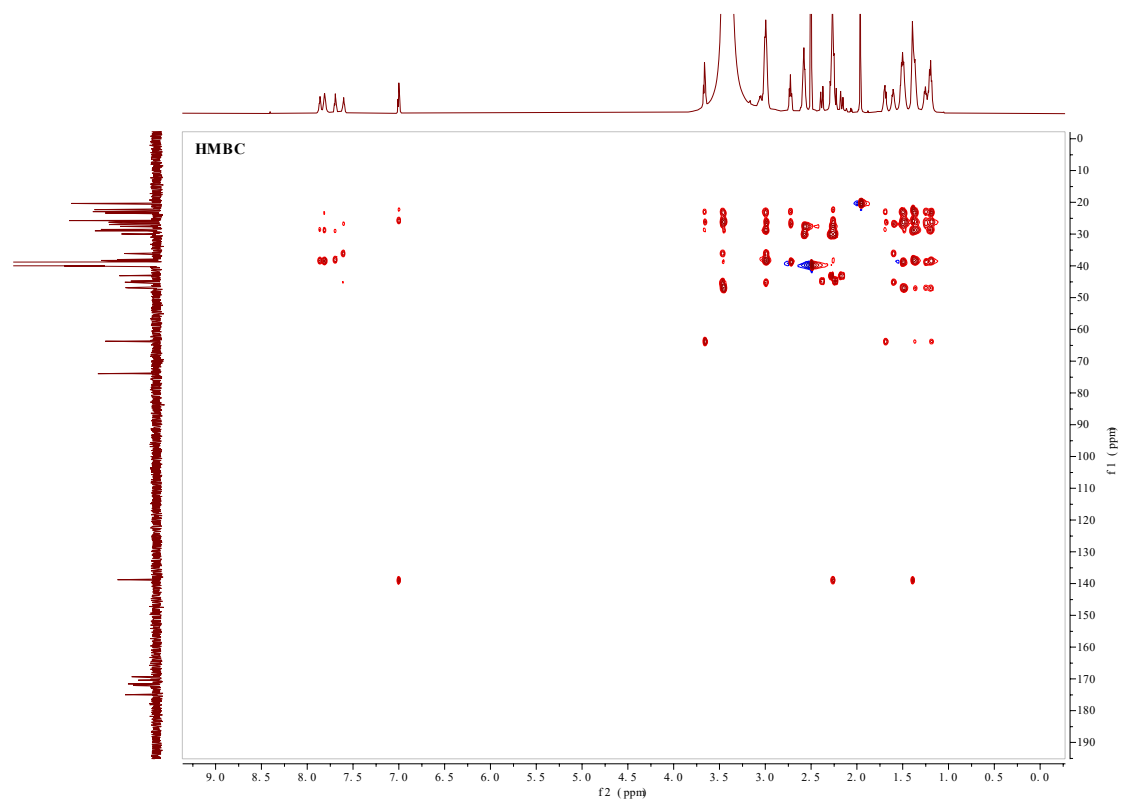
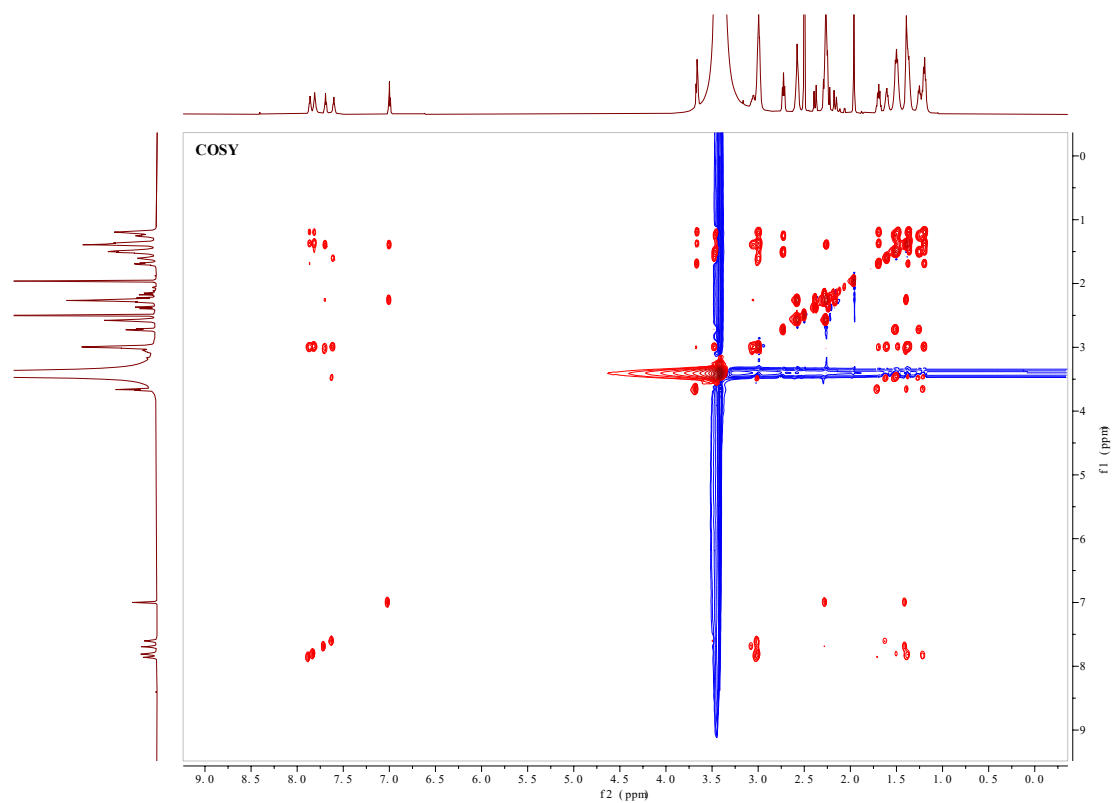


Figure S8. Two BGCs responsible for hydroxamate-type siderophores in *S. fradiae*.

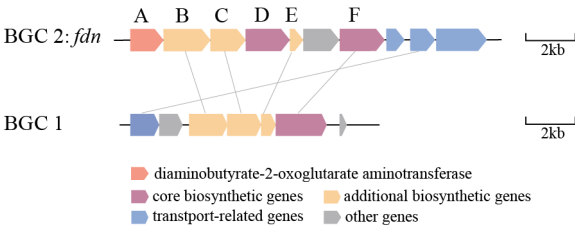


Figure S9. Gene inactivation of *fdnA* using the homologous recombination method. a, scheme of the construction of *fdnA* replacement mutant. b, PCR confirmation of the *fdnA* inactivation by insertion of an apramycin resistance-*oriT* cassette (*aac(3)IV+oriT*). d, verification of metabolite alteration in *fdnA* inactivation mutant.

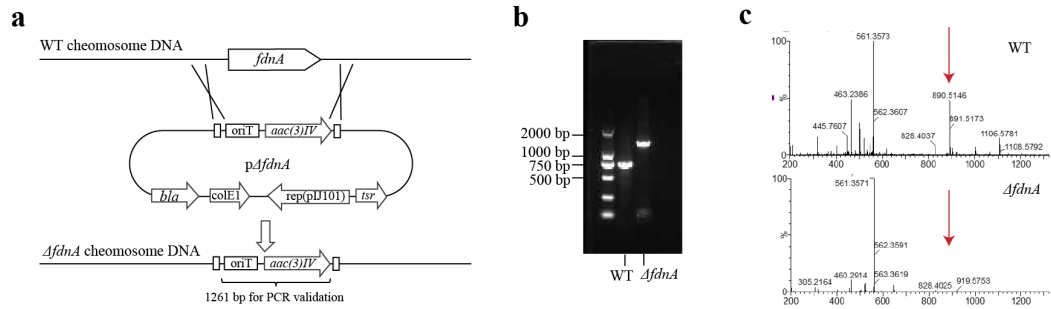


Figure S10. The high-energy linear electron accelerator, model CIAE-DZ-10/20, was developed by the China Institute of Atomic Energy. It operates at an electron energy of 10 MeV and a maximum power of 20 kW. The scanning width on the conveyor chain beneath the ultra-thin titanium window is 800 mm, with dose uniformity within 5.0%. The electron beam spot size 30 cm below the window is 40.0 mm. During experiments, the irradiation dose was controlled by adjusting the conveyor chain speed.



Table S1. The total number of variants in the UV, ARTP, and HEPE group.

Variation type Irradiation type	SNV	indel	SV			Total
			DUP	DEL	TRA	
UV	2047	700	33	27	10	2817
ARTP	1748	664	43	59	10	2523
HEPE	1730	664	49	46	56	2545

Table S2. Genomic localization of TRA variants in the UV, ARTP, and HEPE group.

Group	Start	Genetic partition	End	Genetic partition
UV	30026	L	7486639	R
	7438868	R	7440442	R
	7504278	R	7650168	R
	7511792	R	7536953	R
	7541369	R	7541369	R
ARTP	1089347	L	7793456	R
	7511558	R	7551816	R
	7516299	R	7517021	R
	7631304	R	7635231	R
	7740170	R	7740742	R
HEPE	30423	L	8258415	R
	47414	L	7747360	R
	168988	L	4376188	C
	187285	L	7604619	R
	194217	L	4584420	C
	244343	L	7460822	R
	291875	L	6322270	R
	310148	L	4759199	C
	311822	L	6164156	R
	324483	L	356287	C
	326799	L	5895275	C
	506719	L	4930219	C
	914854	L	951594	L
	929365	L	968228	L
	4566639	C	6921074	R
	5694417	C	7572191	R
	6424827	R	7838175	R
	6654709	R	6809171	R
	6855663	R	7549545	R
	7491751	R	7525572	R
	7492039	R	7494987	R
	7522111	R	7548855	R
	7566778	R	7579114	R
	7646760	R	7647047	R
	7666091	R	7704319	R
	7814351	R	7928484	R
	7835954	R	7836528	R

Note: L-left arm, C-core region, R-right arm. The genetic partition was as shown in figure S4.

Table S3. The features retained in strain *S. griseobrunneus* and *S. fradiae* fermentation broth filtered by feature dereplication algorithm.

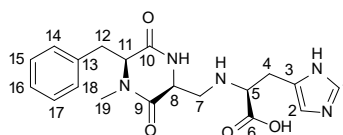
m/z	rt (s)	Exact mass (Da)	Abundance	Matching
301.1862	270.098	600.3577	174184.5	
326.2014	329.715	325.1942	17756	
328.1473	46.928	327.1407	13503	
349.2133	22.132	348.2061	17883.5	
354.1142	17.384	706.2139	10715.5	
362.103	17.384	722.1915	12331	
365.1115	17.911	342.121	433576	
375.2298	74.363	374.2226	10912	
381.0856	17.384	342.121	340464	
386.1888	72.252	385.1816	23299.5	-1
390.2174	196.763	778.4198	18894.5	
391.2248	101.797	390.2176	28647	
395.1994	32.156	394.1922	10730	
401.2458	270.098	400.2386	159433	
404.1992	23.715	403.1919	490062.5	-1
411.1046	16.328	342.121	10167	
418.2143	26.88	417.2071	10741	-1
419.2566	167.218	418.2494	16263.5	
420.1942	21.604	403.1919	51249	
421.1996	43.763	420.1924	21567	
431.2204	53.259	430.2132	38025.5	
447.2264	18.439	446.2192	11862	
502.3315	200.984	501.3243	11474	
801.225	393.441	800.2178	13073	
803.2408	358.453	802.2336	12024.5	
805.4309	187.125	402.2084	71005	
806.3798	177.961	805.3726	12669	
807.379	280.983	823.4129	11601	
808.3924	206.285	807.3852	21402.5	
811.3215	280.983	405.1579	190333.5	
813.4079	107.986	812.4007	19631	
816.3991	178.239	769.3803	24212.5	
819.3898	220.725	818.3826	12244	
820.3561	41.068	819.3489	22010	
821.4199	215.726	820.4127	11056	
825.4424	235.997	824.4352	13152.5	
826.3954	157.412	787.4334	15308.5	
828.3478	280.983	405.1579	37176	
829.2419	235.442	414.1173	350406	
831.2352	362.062	830.2288	472086	
832.3947	244.328	785.3741	13838	

835.4373	243.773	398.2249	16784	
836.3884	235.72	835.3812	17761	
839.39	125.201	838.3824	13830	
840.3995	221.558	801.442	102244	
841.3791	108.819	420.1861	166283	
846.3626	89.66	845.3554	16603	
847.4759	225.168	846.4687	23705	
848.3718	225.723	847.3646	13058	
849.1595	387.332	848.1523	12210	
850.4175	174.629	849.4103	12926	
853.4294	222.113	869.4399	19396.5	
854.3409	62.449	853.3337	12984	
856.4032	247.383	855.396	12966	
858.3731	143.806	857.3688	15473	
860.4609	155.468	859.4537	11255	
861.3596	161.577	860.3524	41641	
862.4892	186.014	861.482	52577.5	
863.3811	248.216	862.3739	100433	
868.3461	176.295	867.3389	31943	
869.4105	138.808	868.4011	900332.5	
870.4428	219.336	869.4399	13046.5	
876.5041	199.898	875.4974	187742	-1
879.3268	108.541	420.1861	18173	
881.418	328.743	880.4108	441936	
887.4252	187.403	886.418	17948	
890.52	211.561	889.5133	205465	-1
893.1491	360.674	892.1419	32365.5	
895.1479	360.674	894.1407	20963	
897.1468	360.674	896.1396	15966	
904.5357	220.725	903.5285	37432.5	-1
905.4091	117.982	904.4019	17214	
911.3904	235.442	910.3832	12219.5	
912.4406	141.862	911.4334	14766	
915.3976	160.744	876.4389	14468.5	
918.3946	108.264	917.3874	17518	
920.4394	173.518	919.4322	18157	
925.4731	188.513	462.2347	1025501	
926.4511	160.467	925.4439	12216	
941.3644	314.581	940.3572	10070.5	
949.4365	226.001	948.4293	11669	
950.3545	170.464	949.3473	12966.5	
953.5044	221.002	476.2499	553236	
956.4575	159.911	917.4836	16087	
957.4324	225.723	956.4252	11289	

963.3593	235.72	962.3521	13698
965.4786	142.973	982.4767	10488
969.4424	223.501	968.4352	15781
970.4805	184.07	969.4733	10080
981.5471	169.908	980.5399	49243

Table S4. ^1H and ^{13}C NMR data for griseobrucins A in $\text{H}_2\text{O}:\text{D}_2\text{O}$ (9:1).

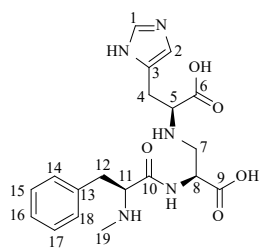
No.	δ_{C}	δ_{H} (J in Hz)
1	132.24, CH	8.19, s
2	114.38, CH	7.09, s
3	128.79, C	
4	25.40, CH_2	2.93, dd (15.4, 5.5) 2.85, dd (15.4, 7.4)
5	60.54, CH	3.06, overlap
6	174.00, C	
7	47.97, CH_2	1.44, dd (12.5, 6.4) 1.30, dd (12.4, 7.2)
8	50.96, CH	3.90, t (6.5)
9	164.06, C	
10	166.13, C	
11	60.21, CH	4.39, t (4.1)
12	33.33, CH_2	3.25, dd (14.4, 4.8) 3.18, dd (14.4, 3.7)
13	132.24, C	
14	127.90, CH	7.05, m
15	127.02, CH	7.31, overlap
16	125.88, CH	7.31, overlap
17	127.02, CH	7.31, overlap
18	127.90, CH	7.05, m
19	30.70, CH_3	3.08, overlap



griseobrucin A

Table S5. ^1H and ^{13}C NMR data for griseobrucins B in $\text{H}_2\text{O}:\text{D}_2\text{O}(9:1)$.

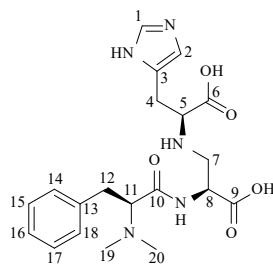
No.	δ_{C}	δ_{H} (J in Hz)
1	134.89, CH	8.31, s
2	117.47, CH	7.22, s
3	128.73, C	
4	26.26, CH_2	3.22, m
5	61.85, CH	3.76, t (6.4)
6	172.21, C	
7	47.96, CH_2	3.17, dd (12.3, 7.6) 2.84, dd (12.3, 6.4)
8	51.56, CH	4.02, t (7.1)
9	173.41, C	
10	168.28, C	
11	62.50, CH	4.10, t (7.4)
12	36.00, CH_2	3.26, m 3.07, dd (13.8, 8.4)
13	133.35, C	
14	129.41, CH	7.20, d (6.6)
15	129.15, CH	7.32, overlap
16	128.14, CH	7.32, overlap
17	129.15, CH	7.32, overlap
18	129.41, CH	7.20, d (6.6)
19	31.87, CH_3	2.64, s



griseobrucin B

Table S6. ^1H and ^{13}C NMR data for griseobrucins C in $\text{H}_2\text{O}:\text{D}_2\text{O}(9:1)$.

No.	δ_{C}	δ_{H} (J in Hz)
1	134.59, CH	8.48, s
2	117.75, CH	7.28, overlap
3	127.60, C	
4	25.82, CH_2	3.25, m
5	61.60, CH	3.73, t (6.5)
6	171.34, C	
7	47.12, CH_2	2.99, m
		2.38, dd (12.1, 5.5)
8	50.85, CH	3.67, m
9	173.30, C	
10	167.55, C	
11	69.02, CH	4.06, dd (11.1, 5.7)
12	34.35, CH_2	3.45, dd (12.6, 5.7)
		2.90, t (11.9)
13	133.44, C	
14	129.40, CH	7.16, d (6.6)
15	129.05, CH	7.30, overlap
16	128.08, CH	7.30, overlap
17	129.05, CH	7.30, overlap
18	129.40, CH	7.16, d (6.6)
19	41.76, CH_3	2.93, s
20	41.76, CH_3	2.93, s



griseobrucin C

Table S7. Crystal data and structural refinement for compound griseobrucin C.

Characteristic	Property
Empirical formula	C ₄₀ H ₅₈ N ₁₀ O ₁₃
Formula weight	886.96
Temperature/K	170.00
Crystal system	orthorhombic
Space group	P2 ₁ 2 ₁ 2
Unit cell dimensions	a = 19.1007 (12) Å, α = 90° b = 20.2554 (12) Å, β = 90° c = 5.5263 (4) Å, γ = 90°
Volume/Å ³	2138.1(2)
Z	2
Calculated density (g/m ³)	1.378
Absorption coefficient /mm ⁻¹	0.555
F (000)	944.0
Crystal size /mm ³	0.09 × 0.02 × 0.02
Radiation	GaK α (λ = 1.34139)
Theta range for data collection/°	7.594 to 121.812
Index ranges	-24 ≤ h ≤ 24, -26 ≤ k ≤ 26, -7 ≤ l ≤ 7
Reflections collected	67658
Independent reflections	4899 [R _{int} = 0.1113, R _{sigma} = 0.0513]
Data/restraints/parameters	4899/84/327
Goodness-of-fit on F ²	1.003
Final R indices [I ≥ 2σ(I)]	R ₁ = 0.0501, wR ₂ = 0.1195
Final R indices [all data]	R ₁ = 0.0725, wR ₂ = 0.1297
Largest diff. peak and hole (e Å ⁻³)	0.21/-0.28
Flack parameter	0.09(14)

Table S8. Annotation of the griseobrucins biosynthetic gene cluster (*gbn*)^a.

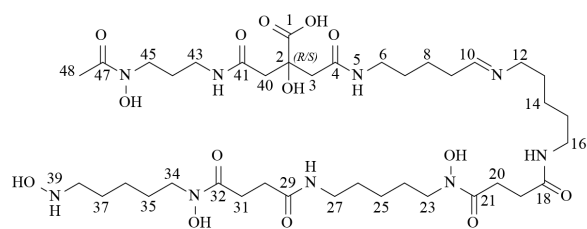
Orf name	Length (AA)	homology-predicted function
orf 1	485	bromoperoxidase-catalase
orf 2	348	hypothetical protein
orf 3	230	hypothetical protein
orf 4	324	diaminopimelate epimerase
<i>gbn</i> A	379	aminotransferase
<i>gbn</i> B	1059	NRPS (C-A)
<i>gbn</i> C	467	hydroxymethyltransferase
<i>gbn</i> D	803	NRPS (A)
<i>gbn</i> E	271	NRPS (TE)
orf 5	439	hypothetical protein
orf 6	564	regulatory protein AfsR

^a Gene name, the corresponding protein amino acid length, and a homology-predicted function are listed.

Table S9. ^1H and ^{13}C NMR data for fradibactin A in $\text{DMSO-}d_6$.

Position	δ_{C}	δ_{H} (J in Hz)
1	174.9, C	
2	73.97, C	
3	43.1, CH_2	2.27, m 2.16, d (15.2)
4	174.99, C	
5	NH	7.69, t (6.1)
6	38.1, CH_2	2.99, p (6.3) 1.38, m
7	28.8, CH_2	1.38, m 1.19, m
8	22.3, CH_2	1.38, m
9	25.8, CH_2	2.25, m
10	138.8, CH	7.00, t (5.8)
11	N	
12	63.8, CH_2	3.66, t (6.7)
13	26.3, CH_2	1.69, p (7.0)
14	28.7, CH_2	1.19, p (7.7)
15	28.6, CH_2	1.38, m
16	38.5, CH_2	2.99, p (6.3)
17	NH	7.81, t (5.7)
18	171.56, C	
19	30.1, CH_2	2.25, m
20	27.7, CH_2	2.58, m
21	172.0, C	
22	N	
23	47.0, CH_2	3.43, m
24	27.1, CH_2	1.50, m
25	23.4, CH_2	1.19, p (7.7)
26	29.0, CH_2	1.38, m
27	38.3, CH_2	2.99, p (6.3)
28	NH	7.86, t (5.8)
29	171.58, C	
30	30.0, CH_2	2.25, m
31	27.6, CH_2	2.58, m
32	172.1, C	
33	N	
34	46.9, CH_2	3.43, m
35	27.1, CH_2	1.50, m
36	22.98, CH_2	1.26, q (7.8)
37	26.0, CH_2	1.50, m
38	38.8, CH_2	2.72, t (7.5)
39	NH	

40	44.8, CH ₂	2.23, m 2.38, d (14.0)
41	169.3, C	
42	NH	7.6, t (5.9)
43	36.1, CH ₂	2.99, p (6.3)
44	26.7, CH ₂	1.60, p (7.1)
45	45.2, CH ₂	3.43, m
46	N	
47	170.4, C	
48	20.4, CH ₃	1.96, s



fradibactin A

Table S10. Annotation of the fradibactin A biosynthetic gene cluster (*fdn*)^a.

Orf name	Length (AA)	homology-predicted function
<i>fdn</i> A	446	aminotransferase
<i>fdn</i> B	630	L-2,4-diaminobutyrate decarboxylase
<i>fdn</i> C	473	N-monooxygenase
<i>fdn</i> D	593	NI-siderophore: IucA_IucC
<i>fdn</i> E	182	O-acetyltransferase
orf 1	483	hypothetical protein
<i>fdn</i> F	610	NI-siderophore: IucA_IucC
orf 2	267	ABC transporter
orf 3	334	ABC transporter
orf 4	695	transport system permease protein

^a Gene name, the corresponding protein amino acid length, and a homology-predicted function are listed.

Table S11. Primers for plasmids construction used in genetic inactivation.

NO.	Sequence
1	gcggtggcggccgctctgatgctcggctacctgttc
2	cctacacatcgaattggagtaactaccggagaagg
3	atccccggaatatcacaagaacccggtcgtcatcc
4	gggcgaattgggtacgatgtccaccttgctcatcc
5	tgtgtaggctggagctgcttcg
6	caggtgcgaataaggacagtgc
7	atgtacctgacggaggtgtc
8	cgaagtccttcaggaacagc

Table S12. Clavulanic acid yield changes of mutants irradiated by HEPE compared to start strain.

Sample	Fold change	Sample	Fold change	Sample	Fold change
1	0.39	38	0.72	75	0.58
2	0.74	39	0.89	76	0.28
3	1.02	40	0.55	77	1.048
4	1.31	41	0.92	78	0.278
5	1.57	42	0.9	79	1.058
6	0.74	43	0.41	80	0.24
7	0.11	44	0.41	81	1.28
8	0.39	45	1.16	82	0.35
9	0.01	46	08	83	0.91
10	1.25	47	0.73	84	0.55
11	0.80	48	0.26	85	1.14
12	0.03	49	1.06	86	0.49
13	1.53	50	0.29	87	1.32
14	1.37	51	0.26	88	0.37
15	1.50	52	0.99	89	0.74
16	0.08	53	0.54	90	0.46
17	1.35	54	0.99	91	0.72
18	0.09	55	0.87	92	0.25
19	0.49	56	0.71	93	0.29
20	1.49	57	0.32	94	0.85
21	1.50	58	0.17	95	0.54
22	1.41	59	1.18	96	0.77
23	1.32	60	1.57	97	0.21
24	0.04	61	0.69	98	1.18
25	0.14	62	0.48	99	0.93
26	0.01	63	1.23	100	0.17
27	1.25	64	0.19	101	0.76
28	1.31	65	1.07	102	0.47
29	0.80	66	0.31	103	0.97
30	0.03	67	0.98	104	0.33
31	1.53	68	1.35	105	0.76
32	1.37	69	0.18	106	0.78
33	1.50	70	1.14	107	0.01
34	0.08	71	1.08	108	0.81
35	0.58	72	0.22	109	0.01
36	0.72	73	0.51	110	0.79
37	0.01	74	1.42		

Table S13. MccJ25 yield changes of mutants compared to start strain in the UV, ARTP and HEPE group.

Group	UV				ARTP				HEPE			
Dose	60	120	180	240	30	60	90	120	1KGy	2	3	4
Fold change	1.25	1.29	1.07	1.12	1.54	1.36	0.77	0.76	1.19	3.53	1.29	1.43
	0.95	0.90	0.97	1.03	1.97	1.00	0.78	0.85	1.33	1.81	1.31	1.37
	1.15	1.49	1.05	1.03	1.66	1.11	0.67	0.70	0.73	1.74	1.49	0.81
	0.98	0.88	1.09	1.19	2.05	1.06	0.86	0.72	0.94	1.51	1.26	1.70
	1.11	1.08	0.99	0.85	1.74	1.91	0.62	0.66	1.22	1.39	1.31	1.28
	1.11	0.43	0.89	0.96	1.75	1.73	0.94	0.74	1.11	3.21	1.55	1.02
	1.45	1.19	0.94	1.04	1.83	1.71	0.82	0.77	1.29	2.49	1.45	1.39
	0.85	0.56	0.83	0.76	1.66	0.94	0.72	0.82	0.86	2.25	1.33	0.96
	1.03	1.22	0.96	1.02	1.59	1.79	0.56	0.69	1.33	2.38	1.50	1.79
	1.26	0.99	0.86	0.99	1.77	1.14	0.57	0.76	0.93	1.95	1.26	1.75
	1.24	0.90	0.84	1.17	1.73	1.67	0.59	0.85	0.73	1.33	1.56	1.67
	0.79	1.19	1.09	0.92	1.66	1.56	0.72	0.90	0.84	3.13	1.53	1.34
	1.40	1.32	1.08	0.91	2.01	1.63	0.64	0.78	0.69	1.69	1.49	1.27
	1.10	1.12	1.18	0.85	1.98	1.91	0.74	0.80	0.74	2.58	1.78	0.76
	1.68	1.29	1.17	0.81	1.61	1.97	0.71	0.89	0.87	2.88	1.85	2.18
	0.72	1.35	1.09	0.97	1.79	1.43	0.59	0.73	0.91	3.05	1.46	1.07
	1.25	1.35	1.08	0.86	1.93	1.54	0.69	0.69	1.24	1.71	1.34	0.73
	1.04	1.65	1.03	0.62	1.92	1.68	0.56	0.66	1.12	1.78	1.53	1.40
	0.85	1.01	1.25	0.50	1.60	0.87	0.59	0.76	1.06	2.46	1.44	1.39
	1.12	1.63	0.99	0.61	1.75	1.38	0.68	0.74	0.80	2.69	1.59	2.05

Table S14. Lovastatin yield change of mutants compared to start strain in the UV, ARTP and HEPE group.

Group	UV				ARTP				HEPE			
Dose	20	30	40	50	25	50	70	90	1	2	3	4
Fold	0.10	1.03	0.50	0.84	0.08	0.08	0.69	0.83	1.01	4.22	0.62	0.51
change	0.00	1.10	0.61	0.00	0.09	0.48	0.85	0.53	1.91	6.12	0.46	0.83
	0.06	0.06	0.17	0.07	0.00	0.79	0.76	1.33	2.30	3.67	0.80	0.67
	0.00	1.04	1.27	0.53	0.15	0.43	2.24	0.78	1.68	2.13	0.74	0.52
	0.00	1.05	1.21	0.70	0.17	0.40	1.89	0.87	2.04	2.13	0.69	0.01
	1.23	0.91	1.12	0.86	0.15	0.39	1.70	0.77	1.90	1.83	0.80	0.42
	0.09	0.79	0.89	0.44	0.16	0.50	0.28	0.81	1.29	2.38	0.95	0.70
	0.33	0.98	1.35	0.12	0.25	0.87	0.80	0.84	1.43	1.85	1.05	1.07
	1.34	1.46	1.14	1.07	0.19	0.75	0.54	0.77	1.45	1.59	1.18	1.06
	0.22	1.05	1.36	1.47	0.35	1.04	1.03	0.85	1.21	1.12	0.91	1.24
	0.01	0.95	1.78	0.51	0.38	0.64	0.94	0.85	0.88	1.16	0.71	1.10
	0.01	2.02	1.02	0.81	0.35	0.63	0.98	1.09	1.00	1.05	0.97	0.90
	0.16	0.07	0.79	0.05	1.47	1.42	1.14	0.93	0.63	1.37	0.92	0.87
	0.39	0.21	0.04	0.01	1.35	0.86	1.20	0.82	0.40	1.03	0.87	0.66
	0.00	1.04	0.13	0.08	1.35	1.92	1.19	0.91	0.64	1.11	0.75	0.70