Electronic Supplementary Material (ESI) for MedChemComm.

Supporting Information for

Nonomuraea sp. ATCC 55076 harbours the largest actinomycete chromosome to date and the kistamicin biosynthetic gene cluster

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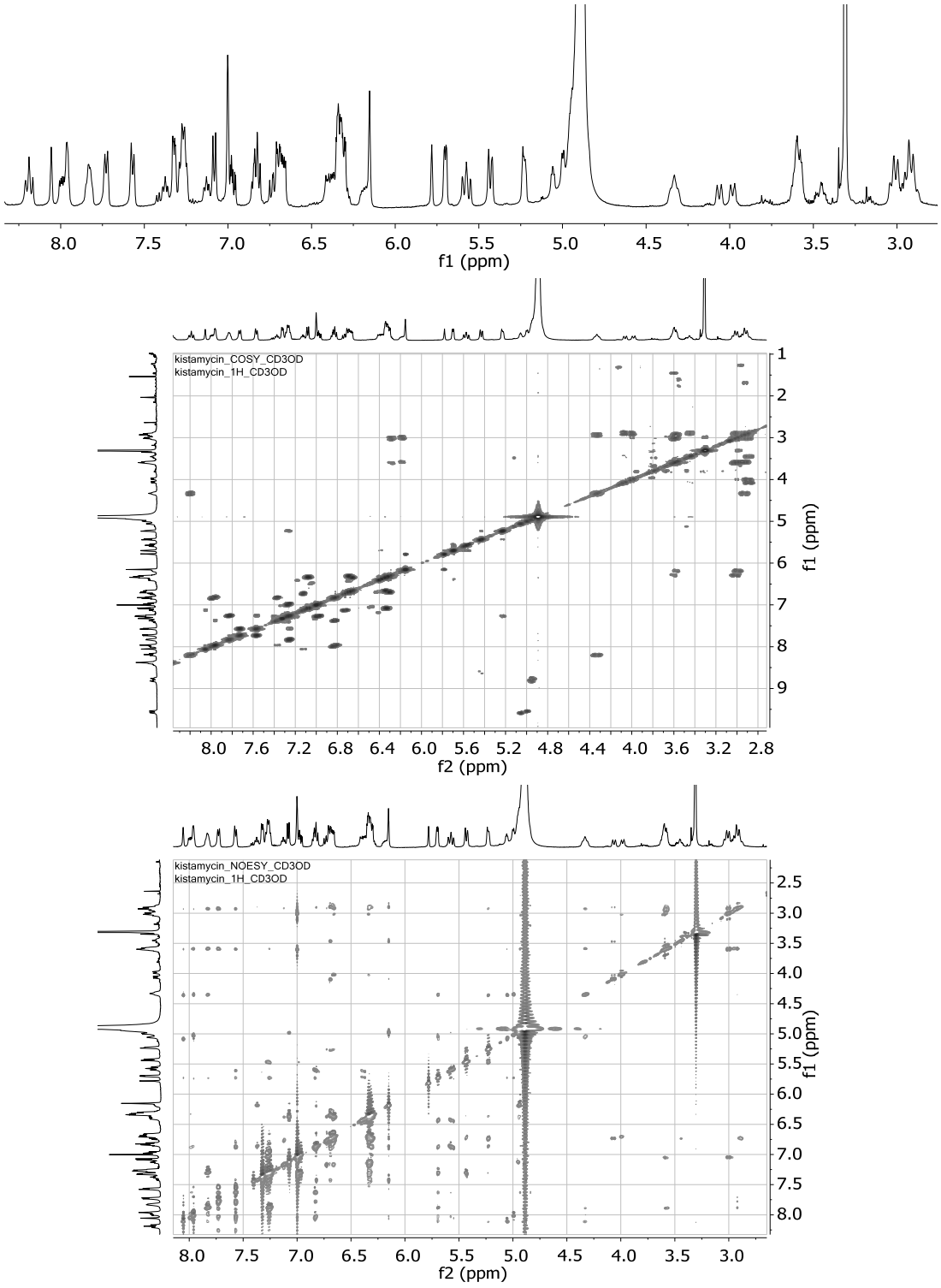

Figure S1. NMR spectra for kistamicin A in CD_3OD. Shown are ^1H (top), gCOSY (middle) and NOESY (bottom) spectra recorded at 500 MHz. See Table S1 for peak assignments.

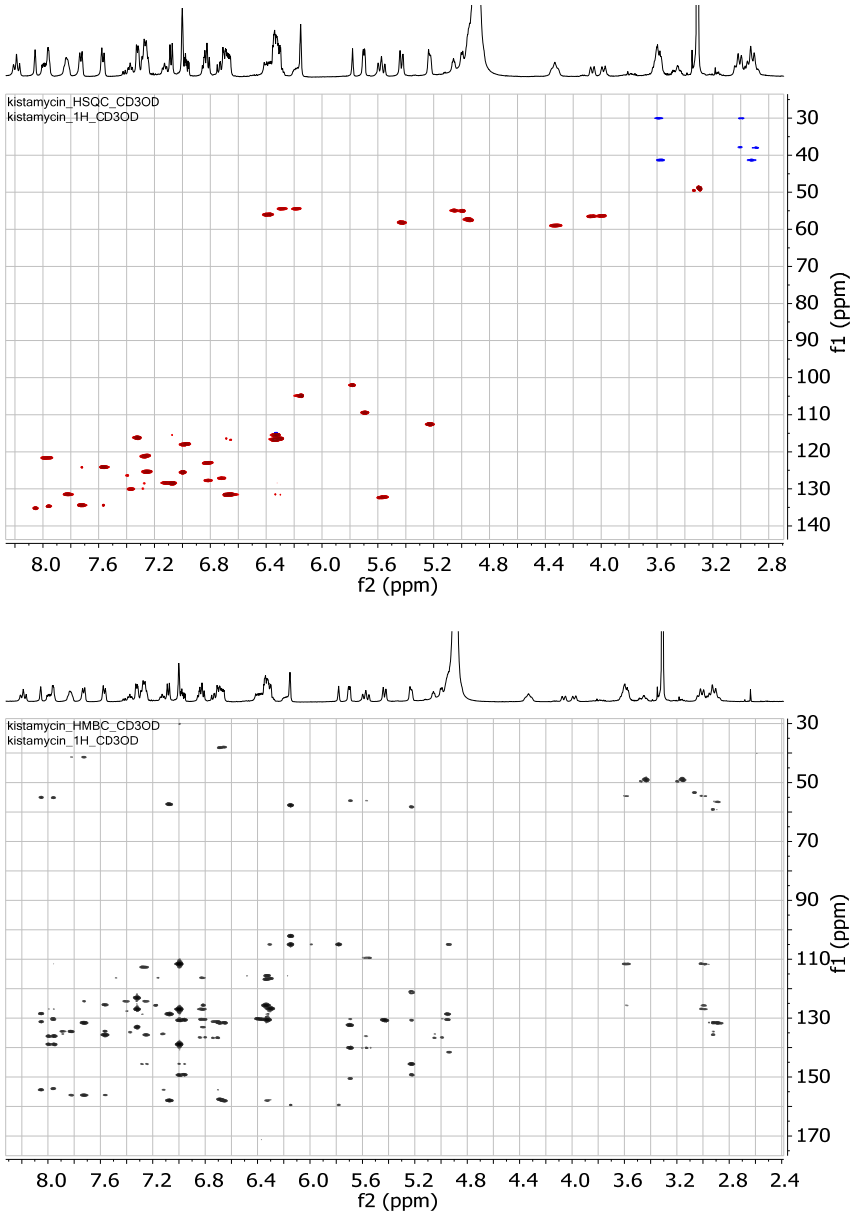

Figure S2. H-C Correlation NMR spectra for kistamicin A in CD_3OD. Shown are gHSQC (top) and gHMBC (bottom) spectra recorded at 500 MHz. See Table S1 for peak assignments.

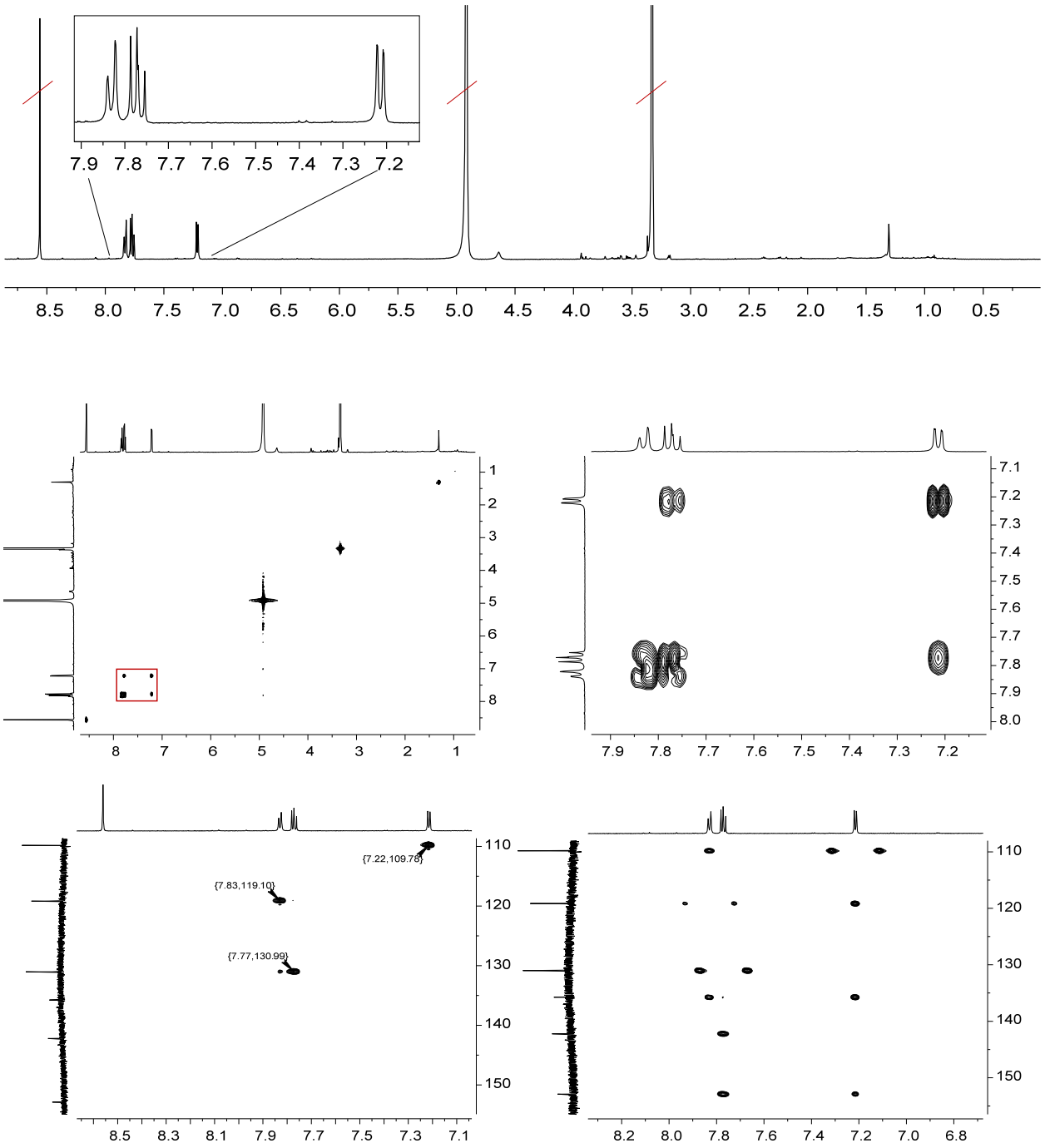

Figure S3. NMR spectra for 1,6-dihydroxyphenazine in CD_3OD. Shown are ^1H (top), gCOSY (middle, left), a magnified view of the gCOSY spectrum (middle, right), gHSQC (bottom, left), and gHMBC (bottom, right) spectra recorded at 800 MHz. See Table S2 for peak assignments.

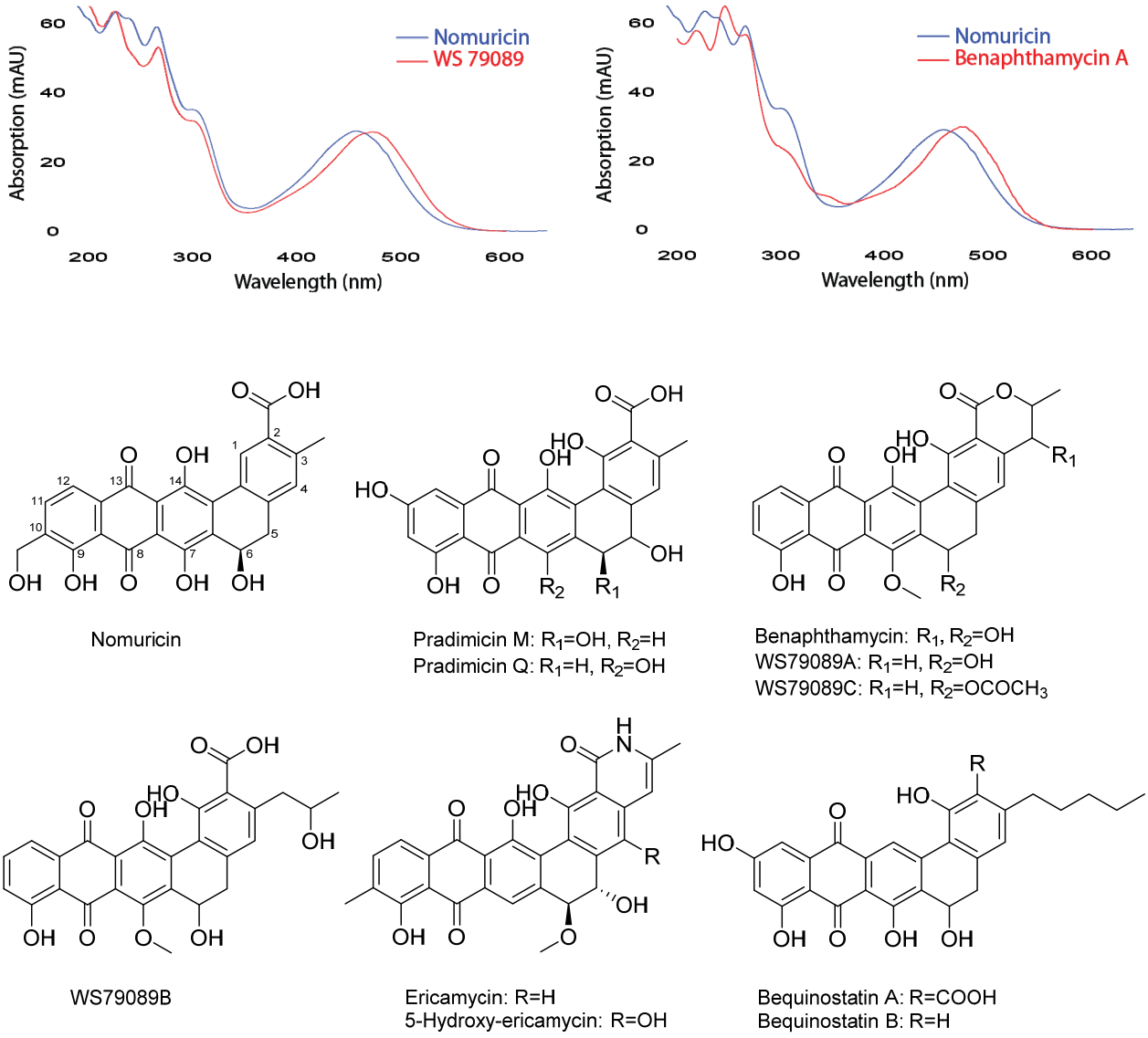

Figure S4. UV-vis spectra for nomuricin and other anthracycline natural products in MeOH. (Top) Comparison of UV-vis spectra for nomuricin (blue traces), WS 79089A and benahpthamycin (red traces), as indicated. (Bottom) Structures of various anthraquinone natural products. See Table S3 for comparison of λ_{max} values for all compounds.

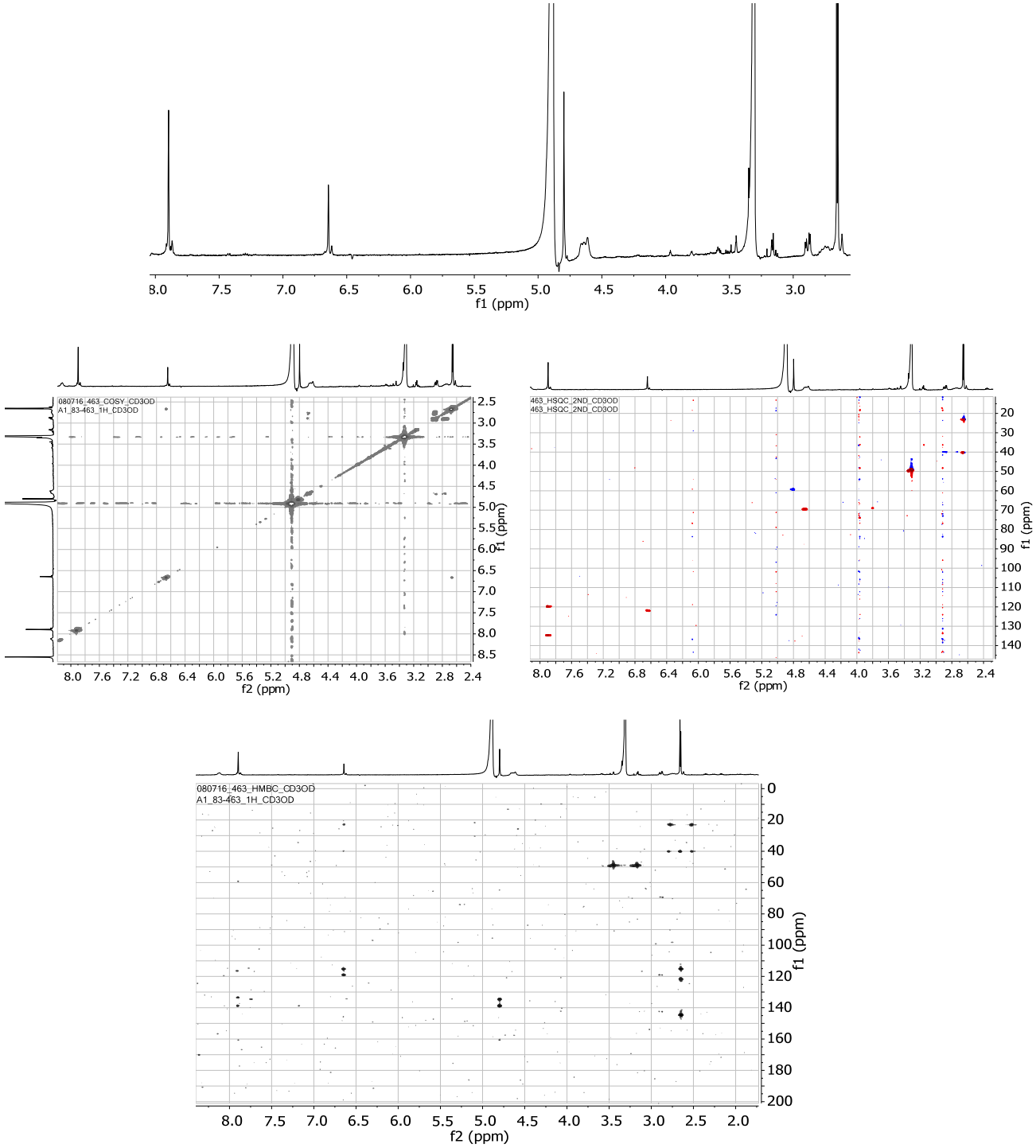

Figure S5. NMR spectra for nomuricin in CD_3OD. Shown are ^1H (top), gCOSY (middle, left), DEPT-edited gHSQC (middle, right), and gHMBC (bottom) spectra recorded at 800 MHz. See Table S4 for peak assignments.

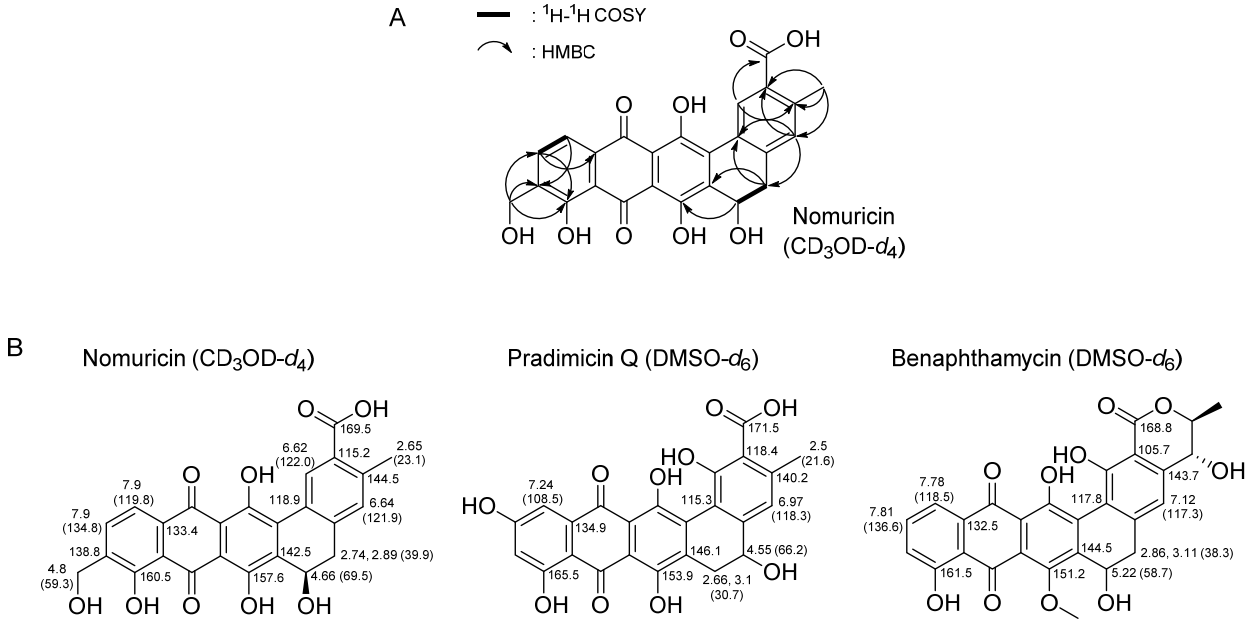

Figure S6. Relevant NMR correlations for nomuricin and comparison with other hydroxy-anthraquinone natural products. (A) Relative COSY and HMBC correlations used to solve the structure of nomuricin. (B) Comparions of the NMR data for nomuricin with those of pradimicin Q and benaphthamycin.

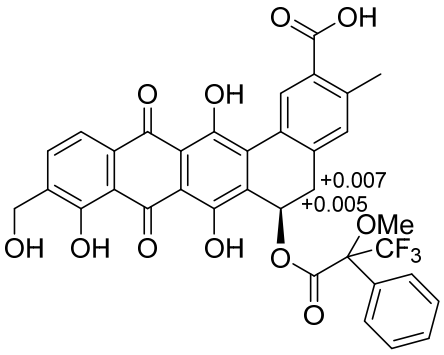

Figure S7. Structure of nomuricin MTPA ester annotated with $\Delta\delta_{S-R}$ values. The shifts indicate the *R* stereoconfiguration for the hydroxyl group on C-16.

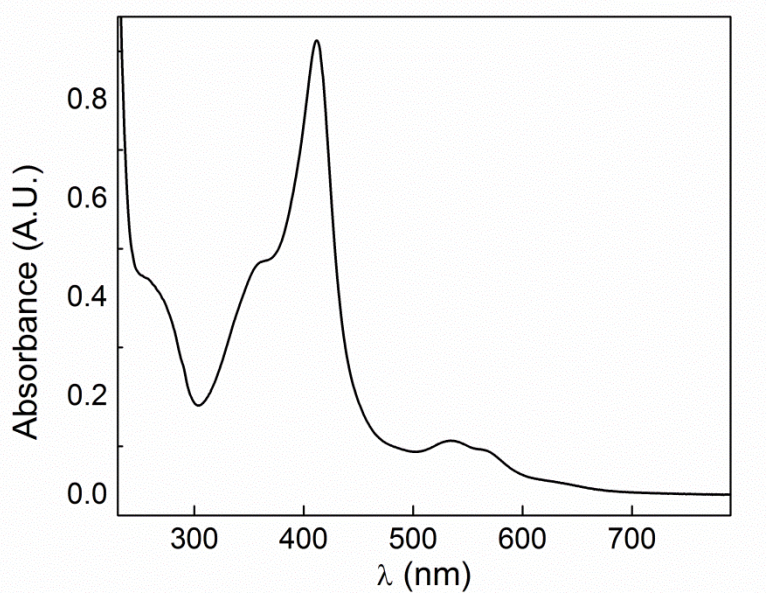

Figure S8. UV-vis spectrum of purified $OxyB_{kis}$. The Soret and Q bands are typical spectral features of low-spin Fe^{III}-heme.

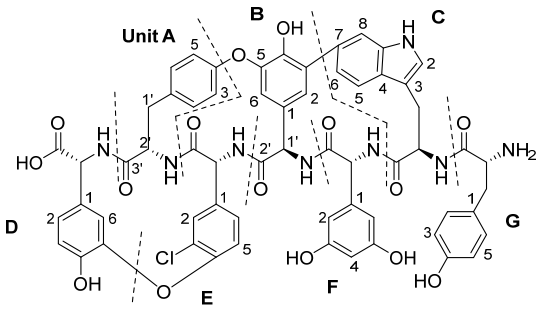

Table S1. NMR assignments for kistamicin. The structure and numbering scheme for kistamicin is shown above.

Unit	δ_H	δ_C	CH_x	Unit	δ_H	δ_C	CH_x
A-1	–	135.7	C	D-1	–	131.1	C
2	7.73 (d)	134.5	CH	2	7.27 (d)	121.1	CH
3	7.56 (d)	124.3	CH	3	6.96 (d)	117.9	CH
4	–	156.2	C	4	–	149.1	C
5	7.25 (d)	125.5	CH	5	–	145.8	C
6	7.82 (d)	131.5	CH	6	5.23 (s)	112.6	CH
1’	3.58 (m)	41.4	CH_2	1’	5.43 (s)	58.3	CH
	2.91 (m)			2’	–	170.5	C
2’	4.33 (dd)	59.0	CH	E-1	–	136.5	C
3’	–	170.7	C	2	7.95 (s)	134.8	CH
B-1	–	130.2	C	3	–	127.0	C
2	5.57 (s)	132.2	CH	4	–	154.0	C
3	–	136.0	C	5	6.83 (d)	127.8	CH
4	–	140.2	C	6	7.37 (d)	130.1	CH
5	–	150.5	C	1’	5.05 (s)	54.9	CH
6	5.70 (s)	109.6	CH	2’	–	171.6	C
1’	6.39 (s)	56.4	CH	F-1	–	141.7	C
2’	–	171.2	C	2, 6	6.15 (s)	104.8	CH
C-2	7.0 (s)	125.5	CH	3, 5	–	159.6	C
3	–	111.5	C	4	5.79 (s)	101.9	CH
4	–	130.2	C	1’	4.95 (s)	57.5	CH
5	7.99 (d)	121.7	CH	2’	–	172.3	C
6	6.84 (d)	123.1	CH	G-1	–	125.5	C
7	–	138.9	C	2, 6	6.66 (d)	131.7	CH
8	7.26 (s)	118.5	CH	3, 5	6.30 (d)	116.8	CH
9	–	135.6	C	4	–	157.9	C
1’	3.59 (m)	30.2	CH_2	1’	3.43 (m)	38.7	CH_2
	3.01 (m)				2.89 (m)		
2’	6.29 (m)	54.8	CH	2’	4.03 (dd)	56.6	CH
3’	–	170.2	C	3’	–	171.0	C

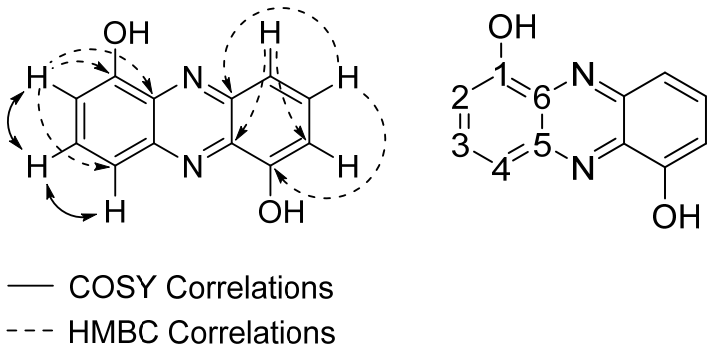

Table S2. NMR assignments for 1,6-dihydroxyphenazine. The structure and numbering scheme for the molecule, along with a summary of correlations observed, is shown above.

C/H	δ_H	Multiplicity	δ_C	COSY	HMBC
1			152.88		
2	7.22	d	109.78	H3	C1, C4, C6
3	7.77	dd	130.99	H2, H4	C1, C5
4	7.83	d	119.10	H3	C2, C6
5			142.18		
6			135.73		

Table S3. UV-vis data for nomuricin and other anthracycline natural products. See Fig. S4 for structures.

Compound	λ_{max} in MeOH (nm)
Nomuricin	227, 241, 266, 303, 457
Pradimicin M[1]	240, 301, 466
Pradimicin Q[2]	229, 288, 514
Benaphthamycin[3]	475
WS 79089A[4]	220, 245, 266, 300, 345, 477
WS 79089B	224, 245, 266, 313, 486
WS 79089C	222, 245, 266, 300, 345, 479
5-Hydroxyericamycin[5]MeCN	262, 305, 336, 370, 389, 453, 510
Bequinostatin A[6]	232, 267, 304, 317, 483
Bequinostatin B	203, 228, 267, 294, 313, 479

[1] *J. Antibiot.* 1990, **43**, 1367-1374.
[2] *J. Antibiot.* 1993, **46**, 507-510.
[3] *J. Antibiot.* 1997, **50**, 791-793.
[4] *J. Antibiot.* 1994, **47**, 619-630.
[5] These λ_{max} values were measured in MeCN: *J. Antibiot.* 2012, **65**, 623-625.
[6] *J. Antibiot.* 1993, **46**, 914-920.

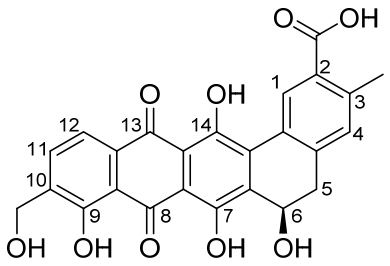

Table S4. NMR assignments for nomuricin. The structure and numbering scheme for nomuricin is shown above.

C/H	δ_H	Mult. (*J* in Hz)	δ_C	CH_x
1	6.62	s	122.0	CH
2	–		115.2	C
3	–		144.5	C
3a	2.65	s	23.1	CH_3
4	6.64	s	121.9	CH
5	2.89	dd (14.0, 5.0)	39.9	CH_2
	2.74	m		
6	4.66	m	69.5	CH
6a	–		142.5	C
7	–		157.6	C
9	–		160.5	C
10	–		138.8	C
10a	4.80	s	59.3	CH_2
11	7.90		134.8	CH
12	7.90		119.8	CH
12a	–		133.4	C
14b	–		118.9	C
15	–		169.5	C

Table S5. Functional annotation of proteins in the *kis* biosynthetic gene cluster.

Protein Name	Com homologue	Metabolic Pathway	Predicted function	Protein Length	C[1]	P[1]	A[1]
KisA	ComO (PD)	Hpg synthesis	Prephenate dehydrogenase	379	-	-	-
KisB	ComG	Regulation	DNA binding response regulator	217	-	-	-
KisC		Unknown function		165	-	-	-
KisD		Transport		341	-	-	-
KisE		Transport	ABC transporter ATP binding protein	245	-	-	-
KisF		Transport	ABC transporter permease	416	-	-	-
KisG		Regulation	Two-component sensor histidine kinase	393	-	-	-
KisH	ComL	Transport	ABC Transporter	627	-	-	-
KisI	ComA	Heptapeptide assembly	NRPS	1680	-	-	-
KisJ	ComB	Heptapeptide assembly	NRPS	1486	-	-	-
KisK	ComC	Heptapeptide assembly	NRPS	2000	-	-	-
KisL	ComD	Heptapeptide assembly	NRPS	1765	+[2]	+[2]	-
KisM	ComE	Unknown function		76	-	-	-
KisN	ComI	Oxidative cross-linking	P450	387	+	+	-
KisO	ComJ	Oxidative cross-linking	P450	426	+	+	-
KisP	ComN (HpgT)	Hpg synthesis	p-hydroxy-phenylglycine aminotransferase	440			
KisQ		Dhpg synthesis (DpgA)	PKS type III	362			
KisR		Dhpg synthesis (DpgB)	Enoyl-CoA dehydratase	220			
KisS		Dhpg synthesis (DpgC)	Oxygenase	429			
KisT		Dhpg synthesis (DpgD)	Enoyl-CoA dehydratase	271			
KisU	ComH	Halogenation	FAD-dependent halogenase	494	-	-	-
KisV		Transport	Sodium-proton exchanger	426	-	-	-
KisW	ComM (HmaS)	Hpg synthesis	p-Hydroxymandelate synthase	360			
KisX	ComL (Hmo)	Hpg synthesis	p-Hydroxymandelate oxidase	382			

[1]C = cloned, P = purified, A = assayed. [2] The X-domain within KisD was expressed and purified.

Table S6. Primers used to clone *kisN* (*oxyA*) and *kisO* (*oxyB*).

Primer name	Sequence	Location in genome	Product size
KisN-F	ATCATATGGTCGCGCCGGAGCACAGAGTC[1]	1827137..1828300	1164 bp
KisN-R	GCAAGCTTCCGTACGCGTCCACCCACTGC[2]		
KisO-F	ATCATATGACGGATGTGACGCCAGGCTTCGTGGTG[1]	1828368..1829648	1281 bp
KisO-F	TAAAGCTTCCGAGCTCATCGGGTCGCGGCTGAC[2]		

[1]NdeI site is underlined
[2]HindIII site is underlined