Elucidation of Final Steps of the Marineosins Biosynthetic Pathway through Identification and Characterization of the Corresponding Gene Cluster

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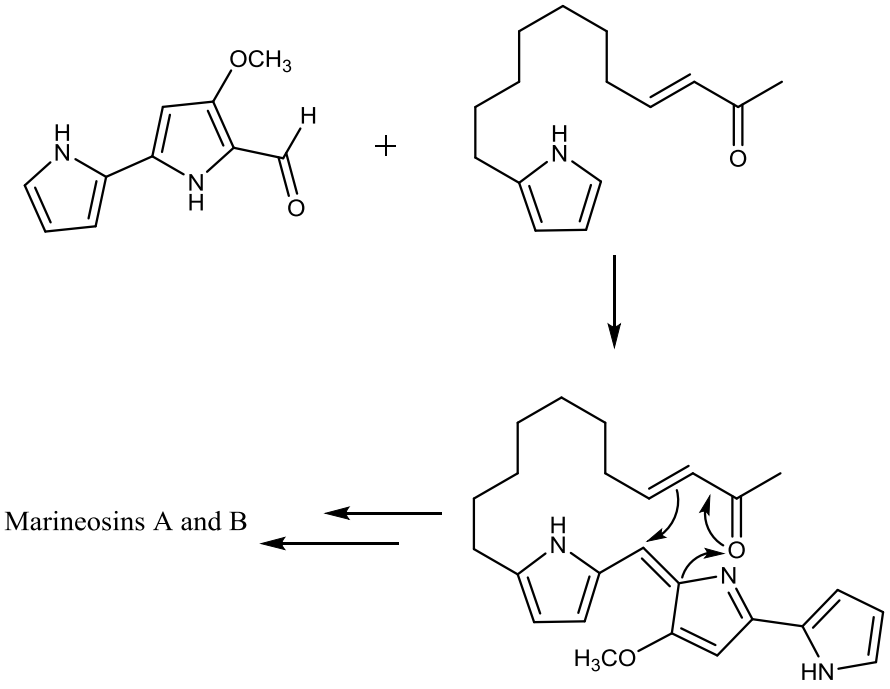


Figure S1: Fenical's Hypothesis for the Biosynthesis of Marineosin. Fenical and co-workers postulates the formation of enone-undecylprodiginine intermediate.[1]

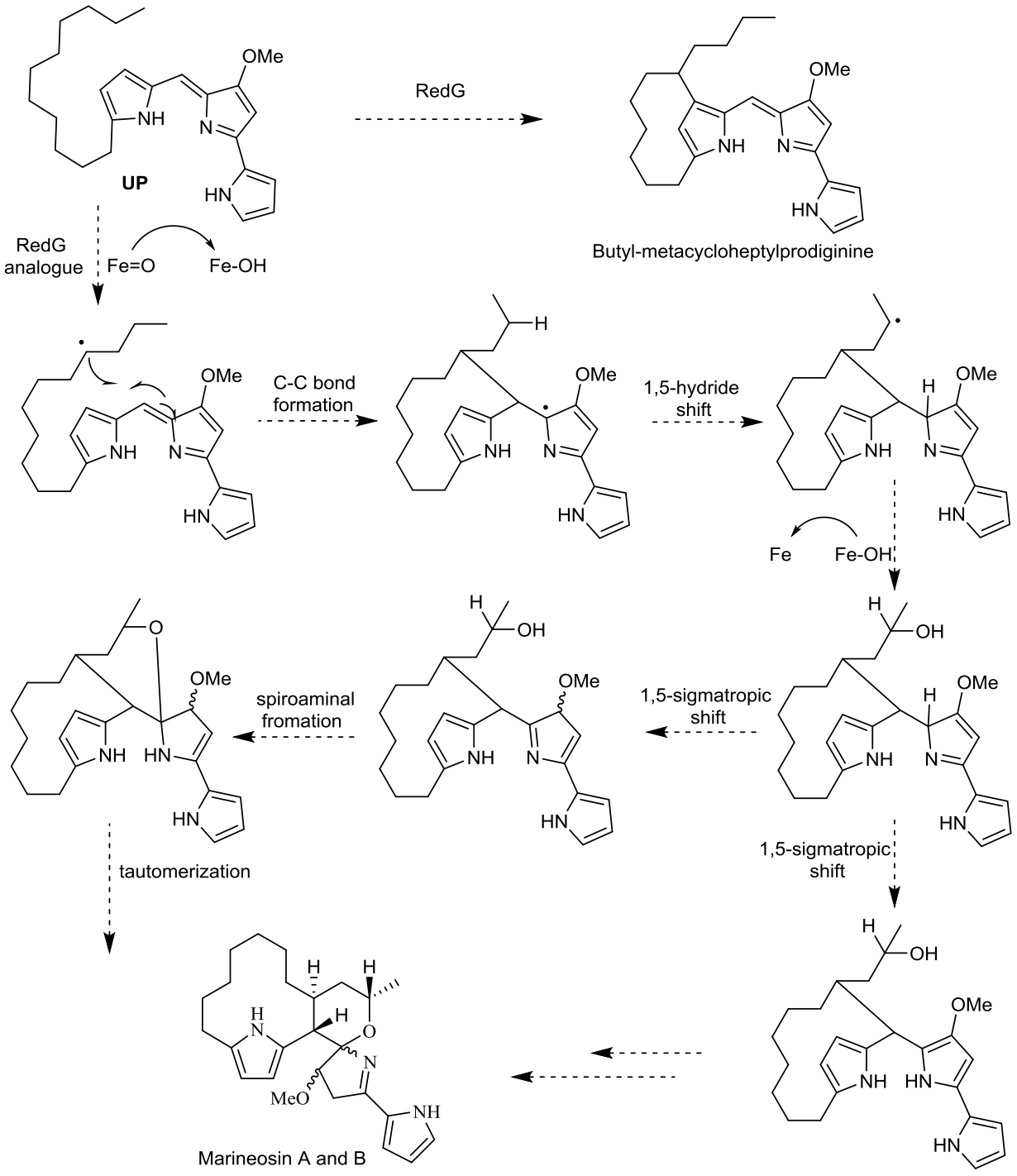


Figure S2: Snider's Hypothesis for the Biosynthesis of Marineosin. Snider and co-workers postulates the formation of a macrocyclic free radical that undergoes a number of hydride shifts mediated by a RedG homolog.[2]

Table S1. List of major cosmids generated throughout the study

Name of cosmid	Description
8A7 cosmid	A SuperCos1 cosmid carrying the *mar* gene cluster together with 6 kbp of primary metabolic genes
pMAR cosmid	8A7 cosmid in which the *bla* gene was replaced with *oriT, ΦC31 int*, and *aac(3)IV*.
pMARΔG cosmid	8A7 cosmid in which *marG* gene was replaced with *aadA* gene conferring resistance to spectinomycin
pMARΔA cosmid	8A7 cosmid in which *marA* gene was replaced with *aadA* gene conferring resistance to spectinomycin

Table S2. List and description of major strains generated throughout the study

Name of strain	Description
E. coli ET12537/pUZ8002/pMAR	Donor *E. coli* strain harboring pMAR cosmid and used for conjugation with *S. venezuelae*
S. venezuelae JND2	*S. venezuelae* expressing the *mar* gene cluster. It produces marineosin and other metabolites.
E. coli ET12537/pUZ8002/pMARΔA	Donor *E. coli* strain harboring pMARΔA cosmid and used for conjugation with *S. venezuelae*
S. venezuelae JND2ΔG	*S. venezuelae* JND2 strain in which *marG* is replaced with *aadA* gene. This strain produces 23-hydroxy- and 23-ketoundecylprodiginine
S. venezuelae JND2ΔA	*S. venezuelae* JND2 strain in which *marA* is replaced with *aadA* gene. This strain produces premarineosin and 16-ketopremarineosin

Table S3: List of primers

Degenerate primers used for PCR amplification of *marG* probe	
*marG*_FWD	5'- TCSCACAGYTSCATCG -3'
*marG*_REV	5'- YRTARCGGGTGTARTKS -3'
Degenerate primers used for PCR amplification of *marW* probe	
*marW*_FWD	5'- GCCATCACCGAGCCGGAGGCCGGTTCNGAY -3'
*marW*_REV	5'- YTGNACGGCCGCCTCACTGACCGCGATCTTCGC -3'
Primers used for PCR amplification of *aac(3)IV*, *oriT*, and *ΦC31int* from pSET1520	
pSET-FWD	5'- TGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGGTGCAATACGA ATGGCGAAA -3'
pSET-REV	5'- AATCTAAAGTATATATGAGTAAACTTGGTCTGACAGTTACTACGCCGCTAC GTCTTC C -3'
Primers for amplification of *aadA* gene	
*aadA*_FWD	5'- *GTATAC*GGCTGACGCCGTTGGATA -3'
*aadA*_REV	5'- *GTATAC*TTATTTGCCGACTACCTTGGT -3'
Primers used for *marG* disruption	
*marG*_KO_FWD	5'- GTGCTCGGCGGGAAACTCGAAAGAGAGAGGGACTGCATG*GTATACGGCT* GACGCCGTTG -3'
*marG*_KO_REV	5'- GCCGTACCATGCGTCTTCGTCCGGGACGCCGCCGC GTCAGTATACTTATTTGCCGACT -3'
Primers used for *marA* disruption	
*marA*_KO_FWD	5'- TTGTACGCCCACGCTCCGGTGCACAGAATCGTGGCCATG*GTATACGGC* TGACGCCGTTG -3'
*marA*_KO_REV	5'- AGAGGCGACGGAGATCATGACGGGATACTTCTGGCGTCAG*TATACTTATTT* GCCGACT -3'
Outer primers used to verify gene deletion	
*marG*_Out_FWD	5'- ACACCGACGCGACATGGACC -3'
*marG*_Out_REV	5'- CGGGCAGCTCCTCGTCCT -3'
*marA*_Out_FWD	5'- GTCGGCCGGTGACGGTGC -3'
*marA*_Out_REV	5'- GGAACGCGACGACAGCTAT -3'

Construction of genomic library of *Streptomyces* CNQ-617 and PCR-based screening for marineosin gene cluster. A cosmid library was constructed using the Supercos1 Cosmid Vector Kit (Agilent, CA). Purified total genomic DNA of *Streptomyces* CNQ617 was partially digested with *Sau3A*, ligated with Supercos1 and packaged using Gigapack II Gold (Agilent, CA). Colony hybridization was carried out and library was screened using ^{32}P labeled *marG* and *marW* DNA fragments as probes. Prime-It II Random Primer Labeling Kit (Agilent, CA) was used to label *marW* 617 bp DNA

fragment. A selected cosmid 8A7 was shotgun cloned, sequenced, and the gaps were closed by primer walking by MWG, Eurofins. The sequences were assembled, analyzed and annotated using Staden package (Gap4), MacVector (Oxford Molecular Group), BLAST searches on NCBI and Welcome Trust Sanger Institute (StrepDB) servers.

Table S4. HPLC gradient used in first purification of marineosin

Time (min)	Flow (ml/min)	% A[1]	% B[2]
0	10.0	90.0	10.0
2.0	10.0	90.0	10.0
40.0	10.0	0.0	100.0
50.0	10.0	0.0	100.0
51.0	10.0	90.0	10.0
60.0	10.0	90.0	10.0

[1]Buffer A: water/acetonitrile/methanol (5:1:4), [2]Buffer B: 100% methanol
Peak eluting at 45 min was collected and subjected to a second HPLC using table S5

Table S5. HPLC gradient used in second purification of marineosin

Time (min)	Flow (ml/min)	% A[1]	% B[2]
0.0	1.0	75.0	25.0
7.5	1.0	75.0	25.0
8.0	1.0	50.0	50.0
13.0	1.0	50.0	50.0
13.5	1.0	25.0	75.0
21.0	1.0	25.0	75.0
21.5	1.0	0.0	100.0
25.0	1.0	0.0	100.0
25.5	1.0	75.0	25.0
30.0	1.0	75.0	25.0

[1]Buffer A: water acidified with 0.05 % formic acid, [2]Buffer B: acetonitrile acidified with 0.05 % formic acid
Peak with retention time of 12 minutes was collected and dried under vacuum to yield pure marineosin

Table S6. HPLC conditions for purification of 9 and 10

Time (min)	Flow (ml/min)	% A[1]	% B[2]
0	4.0	95.0	5.0
30.0	4.0	15.0	85.0
35.0	4.0	15.0	85.0
36.0	4.0	95.0	5.0
46.0	4.0	95.0	5.0

[1]Buffer A: water/acetonitrile/methanol (5:1:4), [2]Buffer B: 100% methanol
Column used: 10.0 × 250.0 mm, 5 µm, reverse phase ^{18}C- Phenomenex
Peaks with retention times 15 and 21 minutes, correspond to 16-ketopremarineosin A (10) and premarineosin A (9) respectively

Table S7a. HPLC conditions for purification of 7 and 8 (First round)

Time (min)	Flow (ml/min)	% A[1]	% B[2]
0	10.0	90.0	10.0
2.0	10.0	90.0	10.0
40.0	10.0	0.0	100.0
50.0	10.0	0.0	100.0
51.0	10.0	90.0	10.0
60.0	10.0	90.0	10.0

[1]Buffer A: water/acetonitrile/methanol (5:1:4), [2]Buffer B: 100% methanol
Column used: 20.0 × 250.0 mm, 10 µm, reverse phase Ascentis ^{18}C-column (Supelco)
Peak eluting at 25 min was collected and subjected to a second HPLC using table S7b

Table S7b. HPLC conditions for purification of 7 and 8 (Second round)

Time (min)	Flow (ml/min)	% A[1]	% B[2]
0	4.0	95.0	5.0
25.0	4.0	0.0	100.0
30.0	4.0	0.0	100.0
31.0	4.0	95.0	5.0
40.0	4.0	95.0	5.0

[1]Buffer A: water/acetonitrile/methanol (5:1:4), [2]Buffer B: 100% methanol
Column used: 10.0 × 250.0 mm, 5 µm, reverse phase ^{18}C- Phenomenex

Table S8: LC/MS analysis of *S. venezuelae* JND2

Time (min)	Flow (ml/min)	% A[1]	% B[2]
0	0.2	90.0	10.0
5.0	0.2	90.0	10.0
70.0	0.2	0.0	100.0
80.0	0.2	0.0	100.0
81.0	0.2	90.0	10.0
90.0	0.2	90.0	10.0

[1]Buffer A: Water acidified with 0.05 % formic acid, [2]Buffer B: Methanol acidified with 0.05 % formic acid

Table S9. LC-UV/MS analysis of *S. venezuelae* JND2ΔG

Time (min)	Flow (ml/min)	% A[1]	% B[2]
0	0.2	90.0	10.0
5.0	0.2	90.0	10.0
40.0	0.2	0.0	100.0
50.0	0.2	0.0	100.0
51.0	0.2	90.0	10.0
60.0	0.2	90.0	10.0

[1]Buffer A: 5% ACN/H_2O acidified with 0.05 % formic acid, [2]Buffer B: acetonitrile acidified with 0.05 % formic acid

Table S10. LC-UV/MS analysis of *S. venezuelae* JND2ΔA

Time (min)	Flow (ml/min)	% A[1]	% B[2]
0	0.2	95.0	5.0
2.0	0.2	95.0	5.0
25.0	0.2	0.0	100.0
30.0	0.2	0.0	100.0
31.0	0.2	95.0	5.0
36.0	0.2	95.0	5.0

[1]Buffer A: 5% ACN/H_2O acidified with 0.05 % formic acid, [2]Buffer B: acetonitrile acidified with 0.05 % formic acid

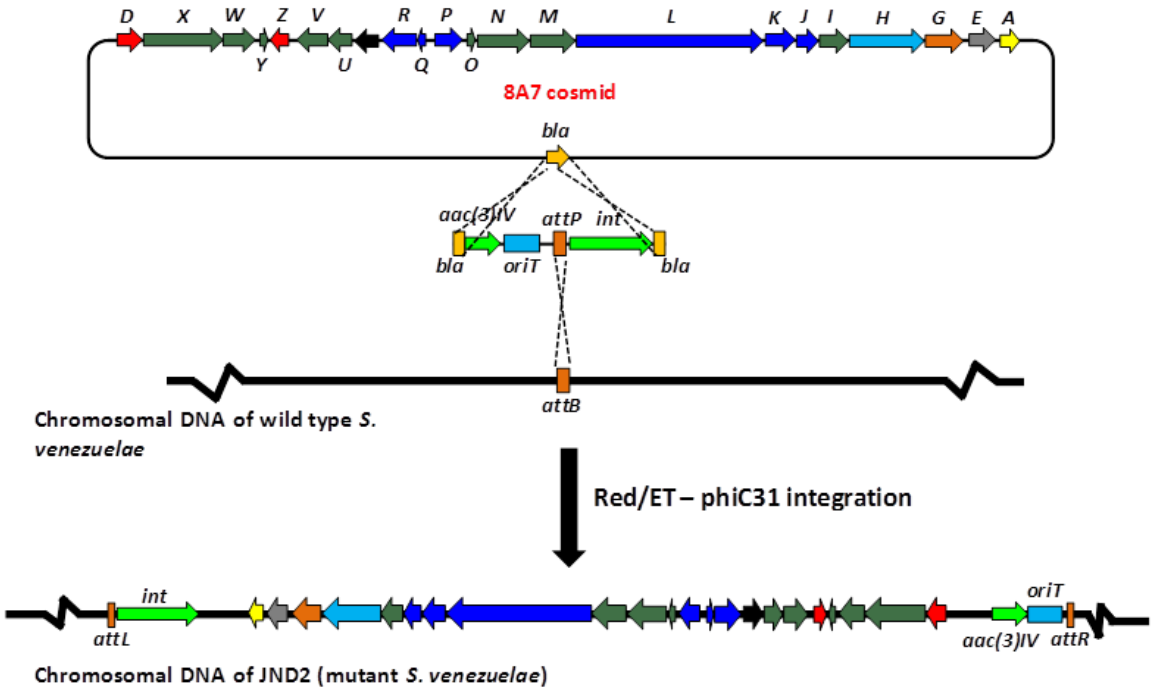


Figure S3. Recombination strategy employed to generate an integrative *mar* gene cluster. pSET 1520 plasmid was used as a template for PCR amplification of *oriT*, *phiC31* integrase and *aac(3)IV*. The PCR product was used to replace the *bla* gene in cosmid 8A7 via PCR targeted recombination followed by site-specific integration of the modified 8A7 cosmid into *S. venezuelae*. *bla* = β-lactamase; *aac(3)IV* = apramycin resistance gene; *oriT* = origin of transfer; *attP/aatB* = phiC31 attachment site of phage/bacteria; *int* = phiC31 integrase

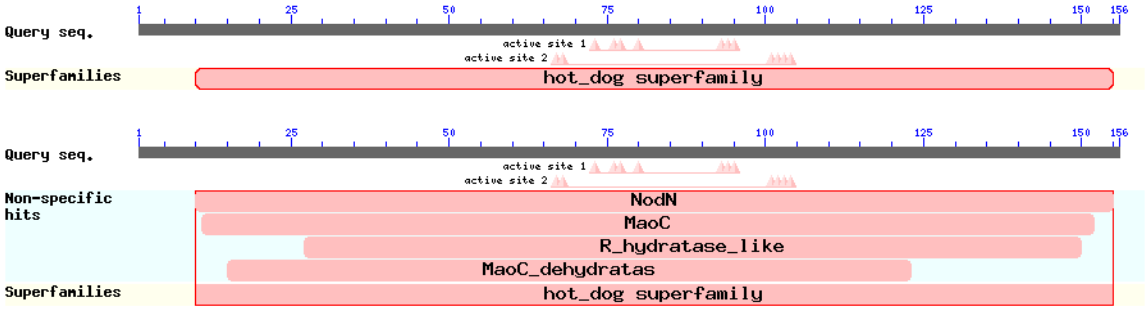


Figure S4: Graphical summary of conserved domain hits obtained after NCBI CDD search of MarA primary amino acid sequence (Query). The figure indicates that MarA belong to the HotDog superfamily of enzymes.

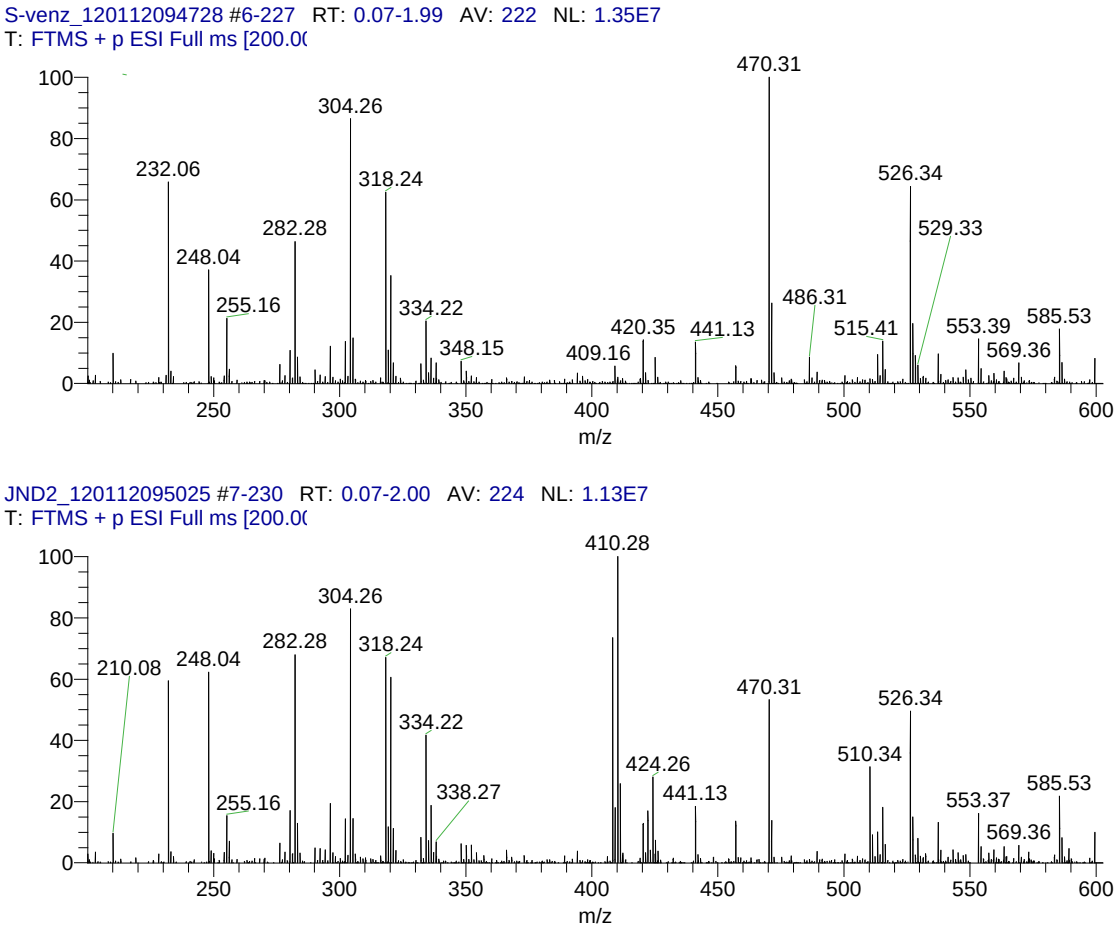


Figure S5. Comparison between the total ion spectrum of crude extract of wild type *S. venezuelae* (top panel) and *S. venezuelae* JND2 (bottom panel). The MS profile of the wild type *S. venezuelae* (top panel) shows the presence of methymycin and pikromycin antibiotics with $[M + H]^+$ of *m/z* 470.31 and 526.34 respectively, while the MS profile of *S. venezuelae* JND2 shows the presence of marineosin with $[M + H]^+$ of *m/z* 410.28 in addition to methymycin, pikromycin and other new compounds.

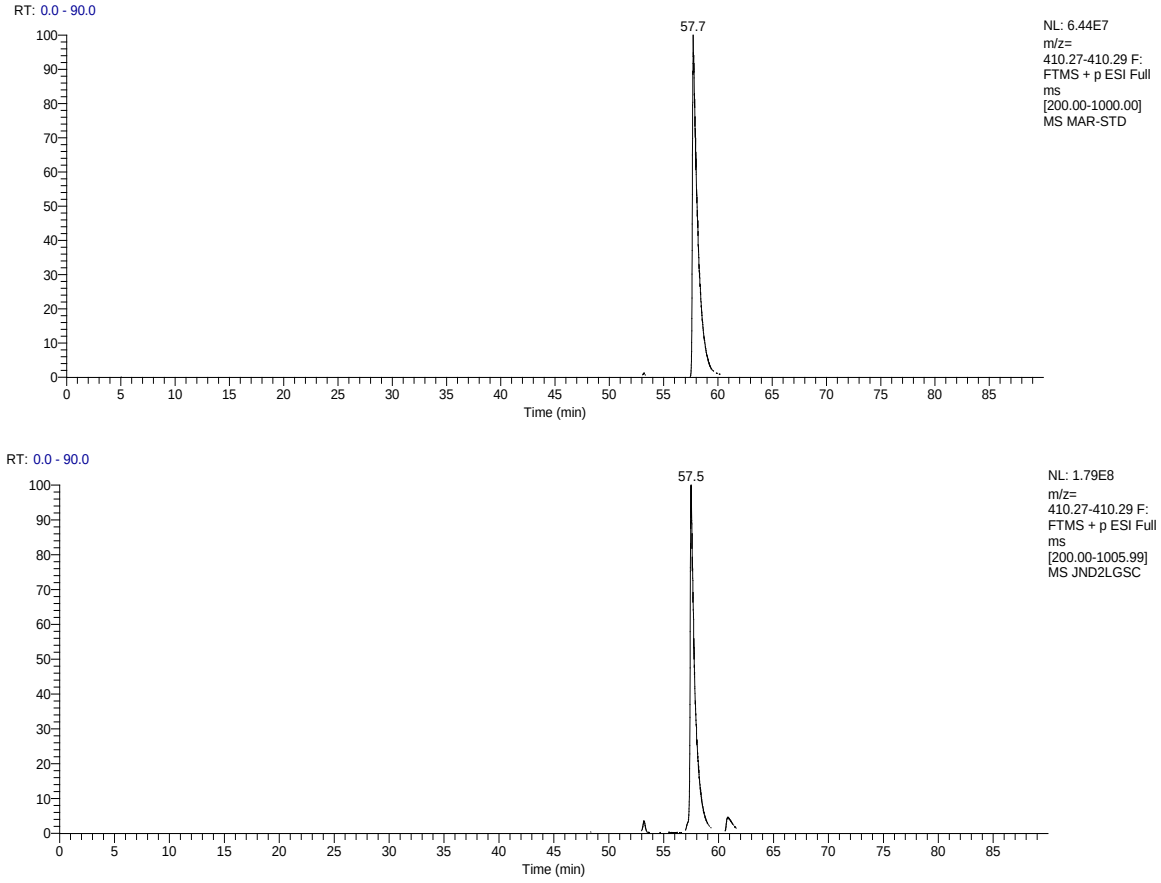


Figure S6. LC/MS comparison between marineosin standard and JND2 extract. An extracted ion chromatogram (EIC) *of m/z* 410.28 for marineosin standard (top panel) and JND2 extract (bottom panel). For LC gradient used, please refer to Table S8.

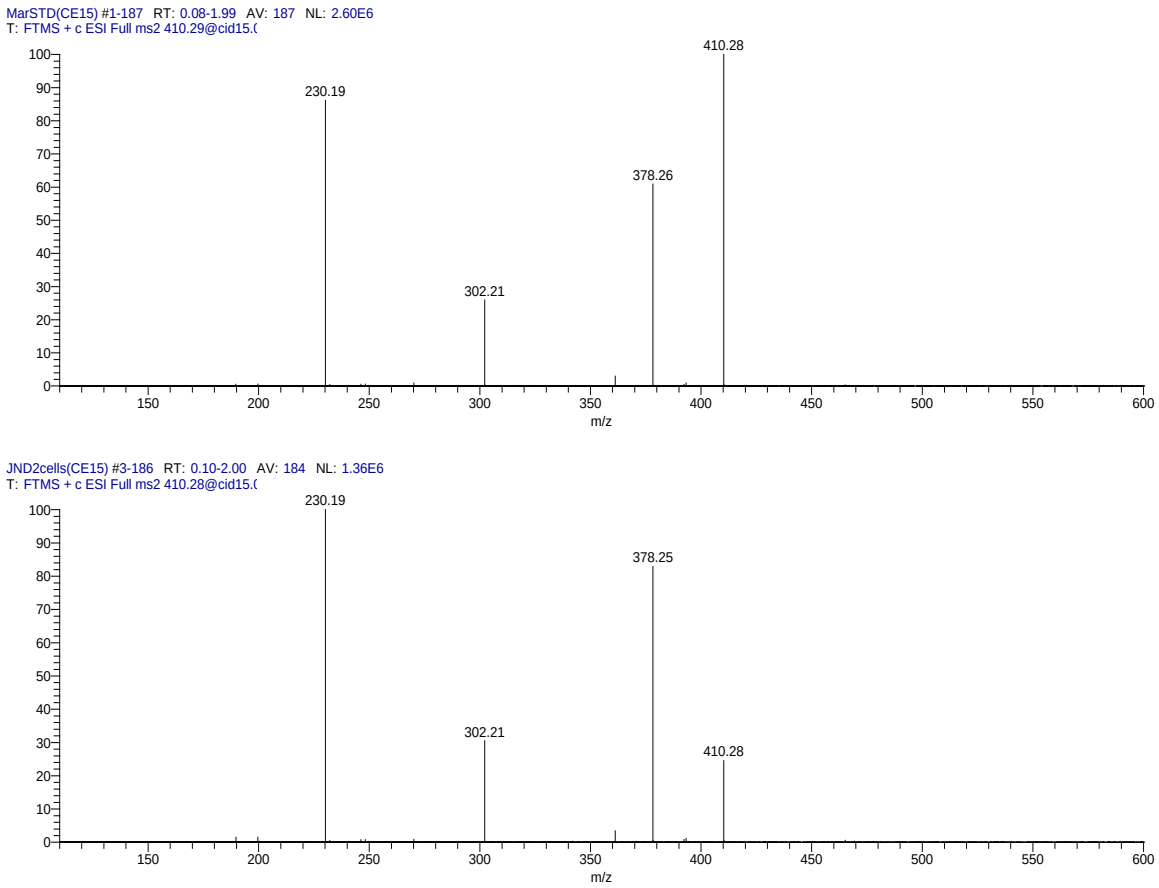


Figure S7. Comparison between the MS/MS profile of marineosin standard (top panel) and the compound with m/z 410.28 in *S. venezuelae* JND2 extract (bottom panel). Compounds in both top and bottom panels show the presence of identical fragment ions *m/z* 230.19, *m/z* 302.21 and *m/z* 378.25.

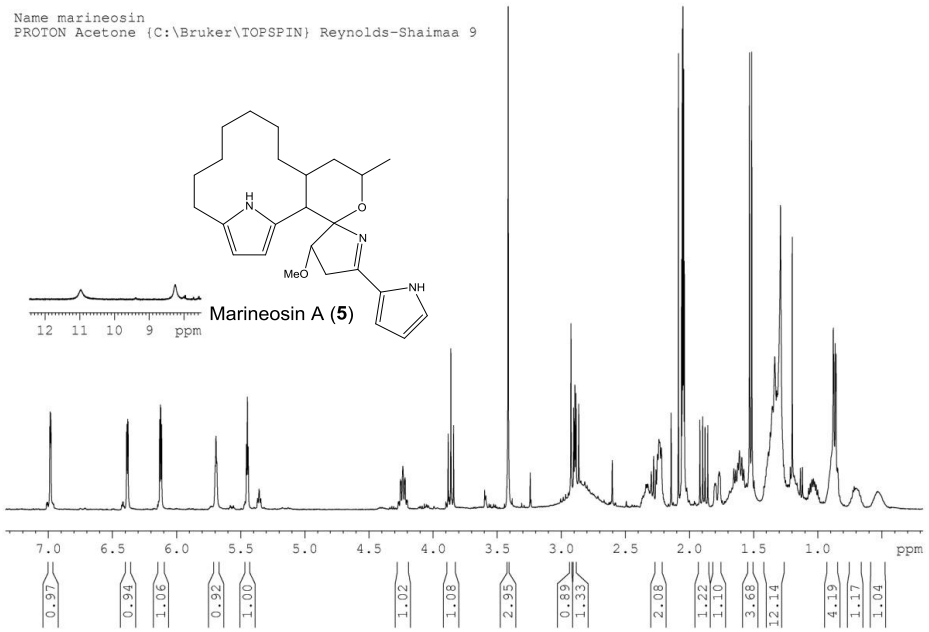


Figure S8. ^{1}H NMR spectrum of marineosin A (5) isolated from *S. venezuelae* JND2 (600 MHz, Acetone-d6)

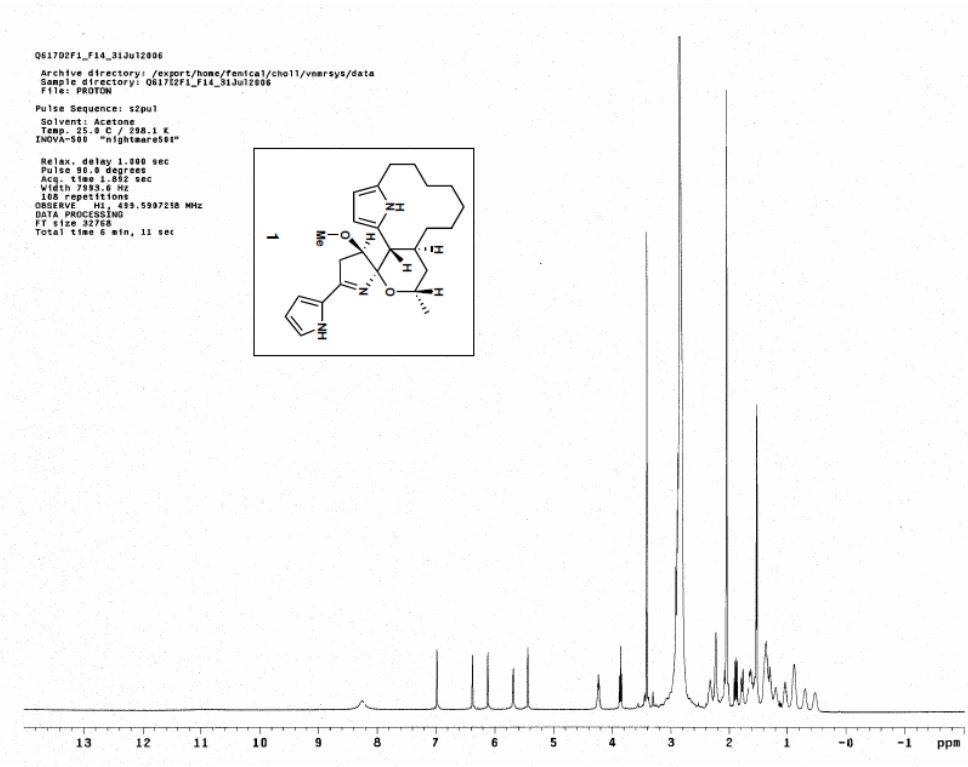


Figure S9. ^{1}H NMR spectrum of marineosin A (5) standard (500 MHz, Acetone-d6). Copied from supporting information of reference 1

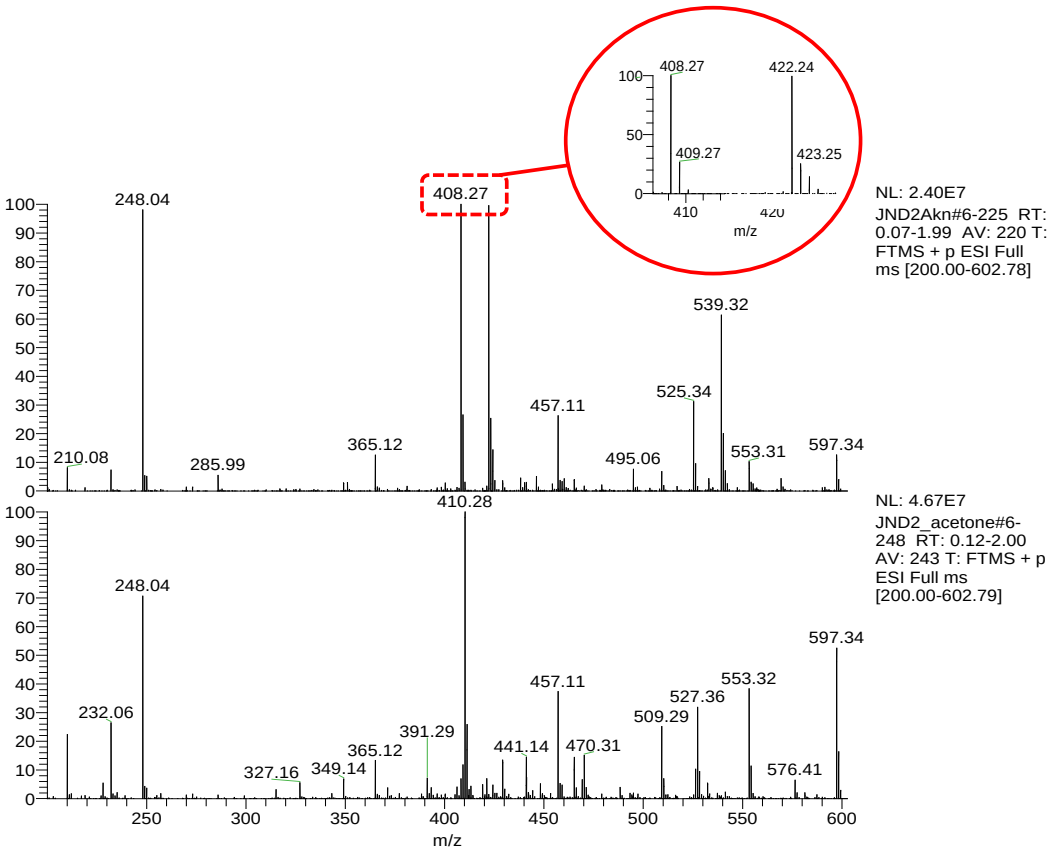


Figure S10a. Comparison between the MS profiles of crude extracts of *S. venezuelae* JND2ΔA (top panel), and JND2 strain (bottom panel). MS profile of JNDΔA strain (top panel) shows two main compounds with *m/z* 408.26 and 422.24 (for illustration purpose, the two peaks are highlighted and magnified) while the MS profile of JND2 strain shows the same two compounds, to a much lower extent relative to marineosin, *m/z* 410.28.

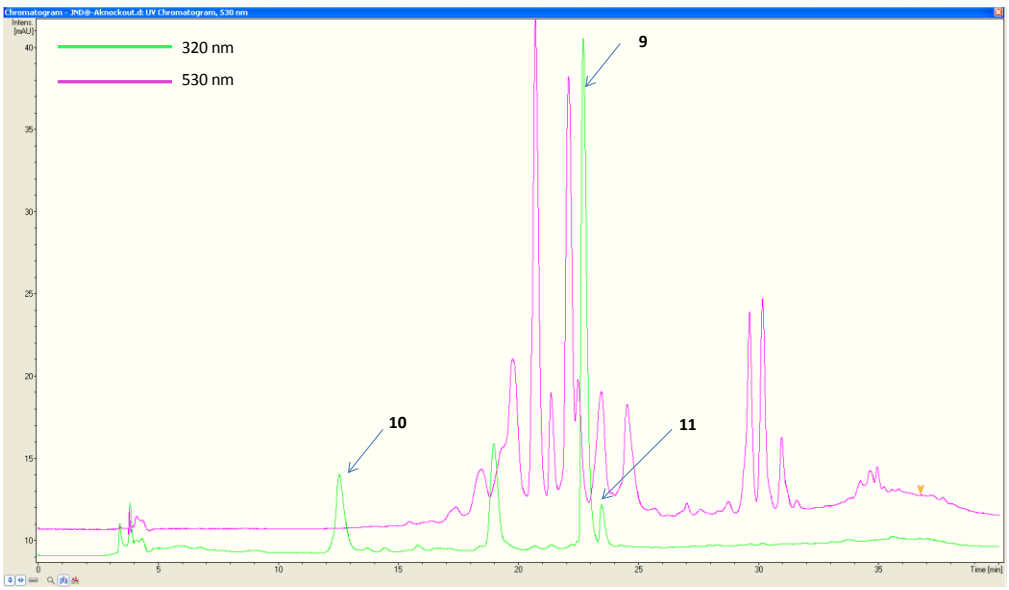
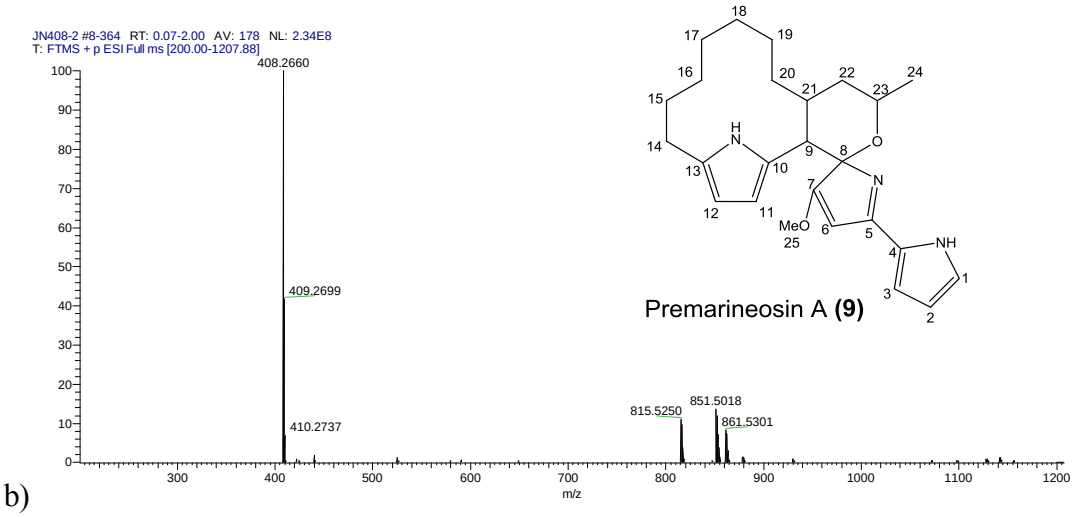


Figure S10b. LC-UV/MS profile of *S. venezuelae* JND2ΔA. For LC gradient used, please refer to Table S10.

a)



b)

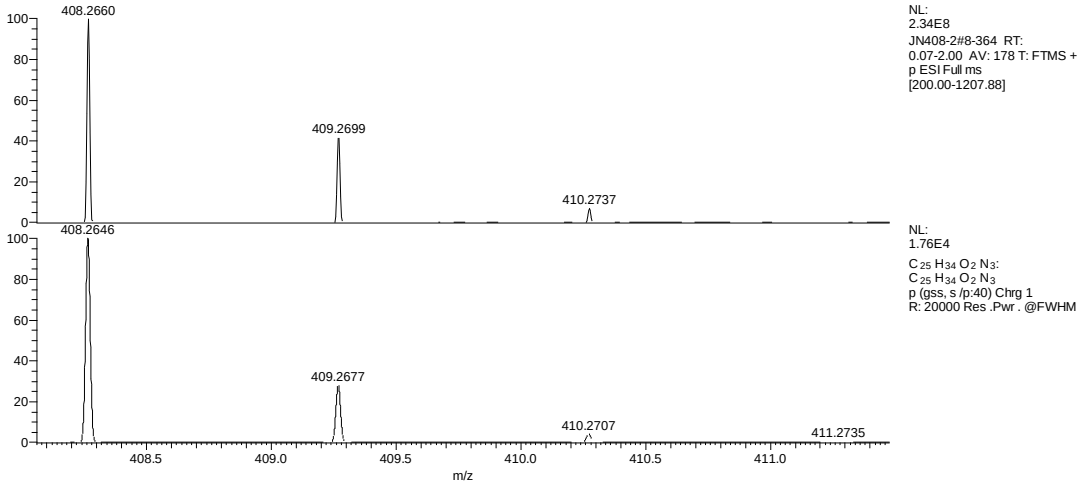
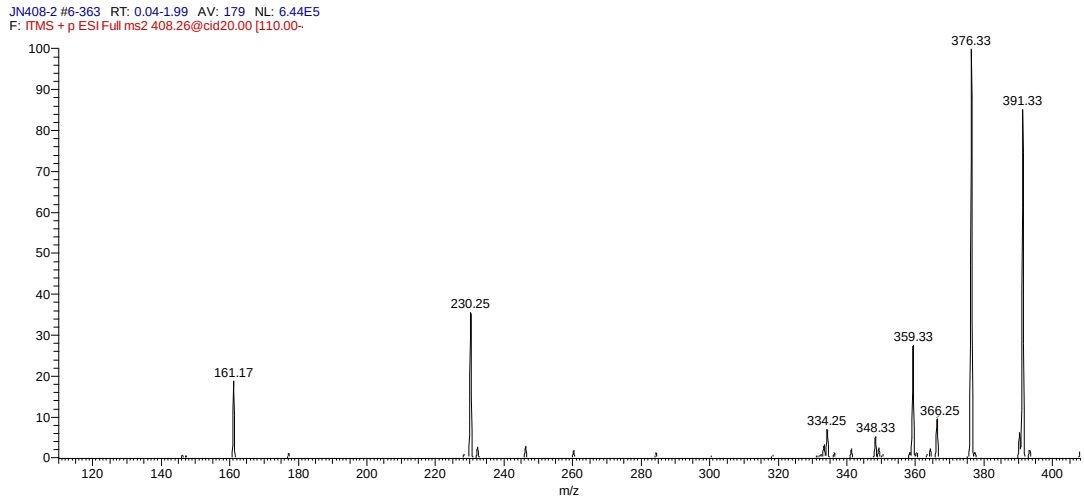


Figure S11. a) HRMS (ESI) of Premarineosin A (9) b) Comparison between the acquired (top panel) and predicted (bottom panel) mass for premarineosin A (9). There is a difference of *m/z* 0.0014 between acquired and predicted mass.

a)



b)

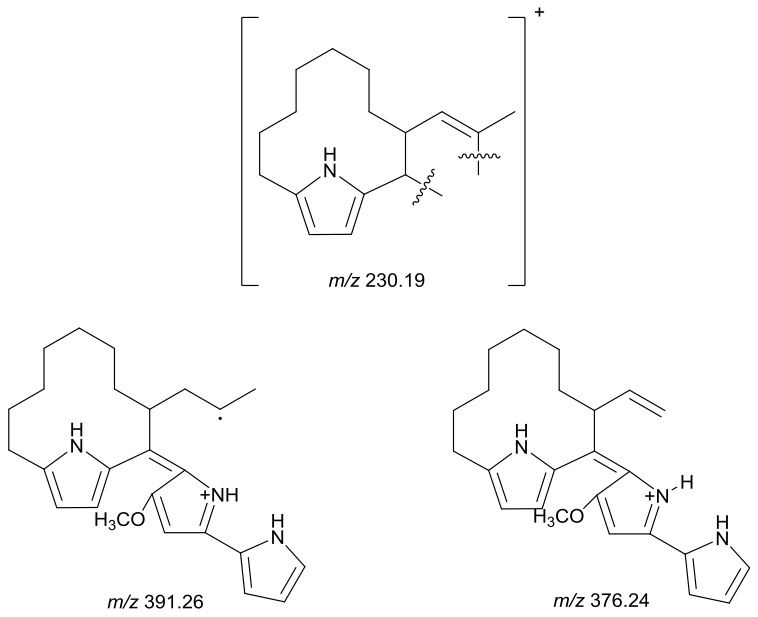
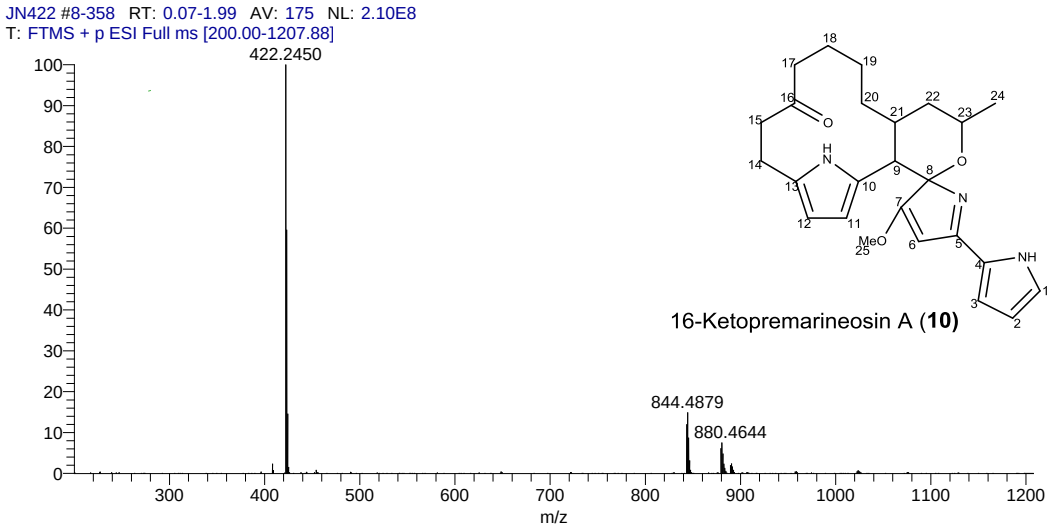


Figure S12. a) ESI-CID-MS^n profile of premarineosin A (9). The MS/MS profile of Premarineosin A (9) is characterized by the presence of *m/z* 230.25, 376.33 and 391.33 daughter ion fragments. Parent ion of *m/z* 408.26 is not shown. **b) Proposed structures of ion fragments shown in ESI-CID-MS^n profile of premarineosin A (9).**

a)



b)

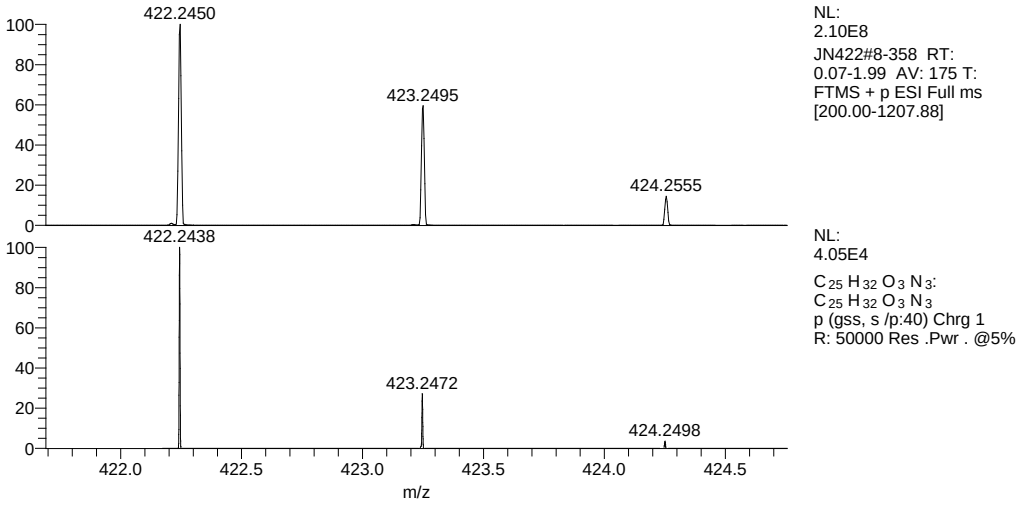
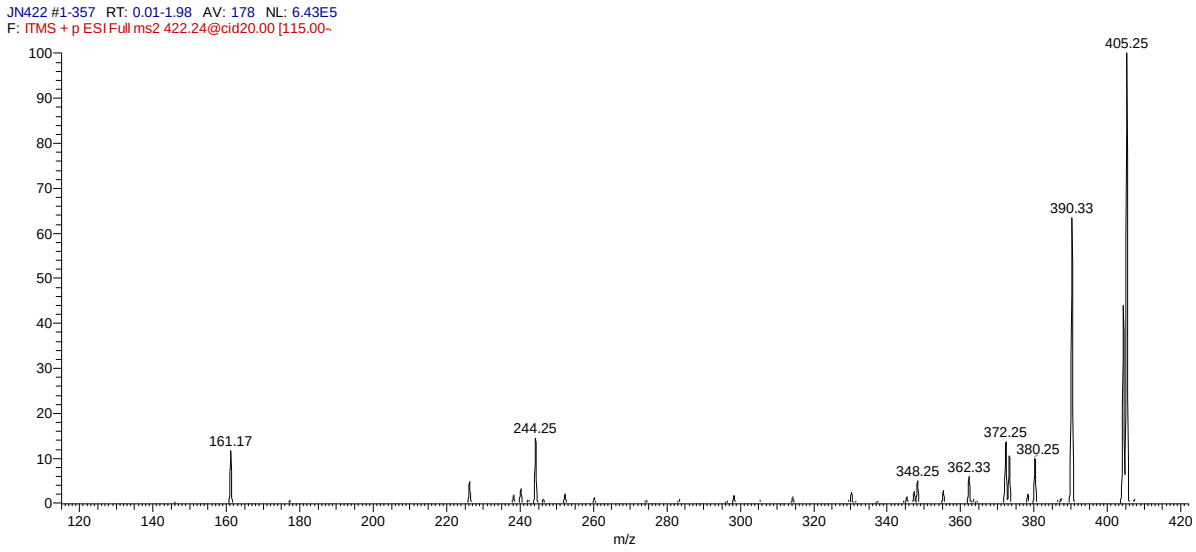


Figure S13. a) HRMS (ESI) of 16-ketopremarineosin A (10) b) Comparison between the acquired (top panel) and predicted (bottom panel) mass for 16-ketopremarineosin A (10). There is a difference of *m/z* 0.0012 between acquired and predicted mass.

a)



b)

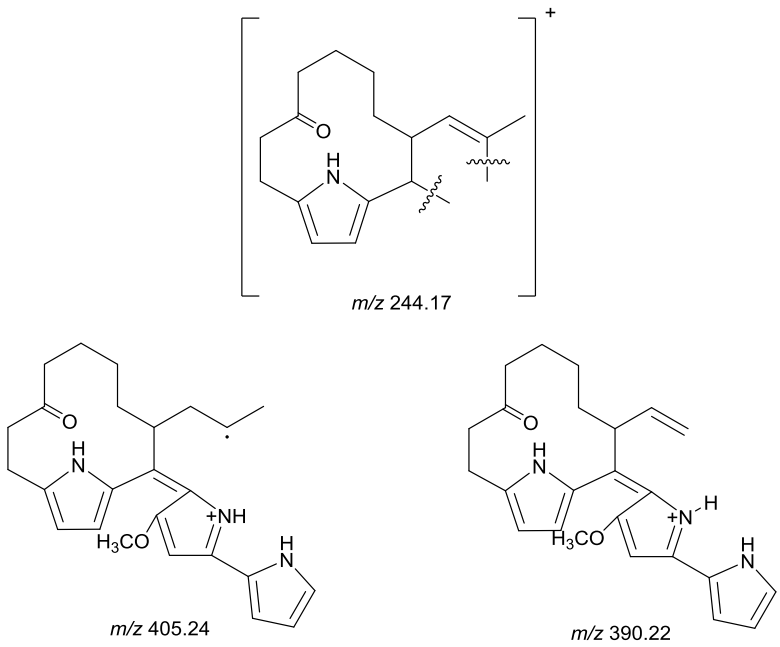


Figure S14. a) ESI-CID-MS^n profile of 16-ketopremarineosin A (10). The MS/MS profile of 10 is characterized by the presence of m/z 244.25, 390.33 and 405.25 daughter ion fragments. Parent ion of *m/z* 422.24 is not shown. **b) Proposed structures of ion fragments shown in ESI-CID-MS^n profile of 16-ketopremarineosin A (10).**

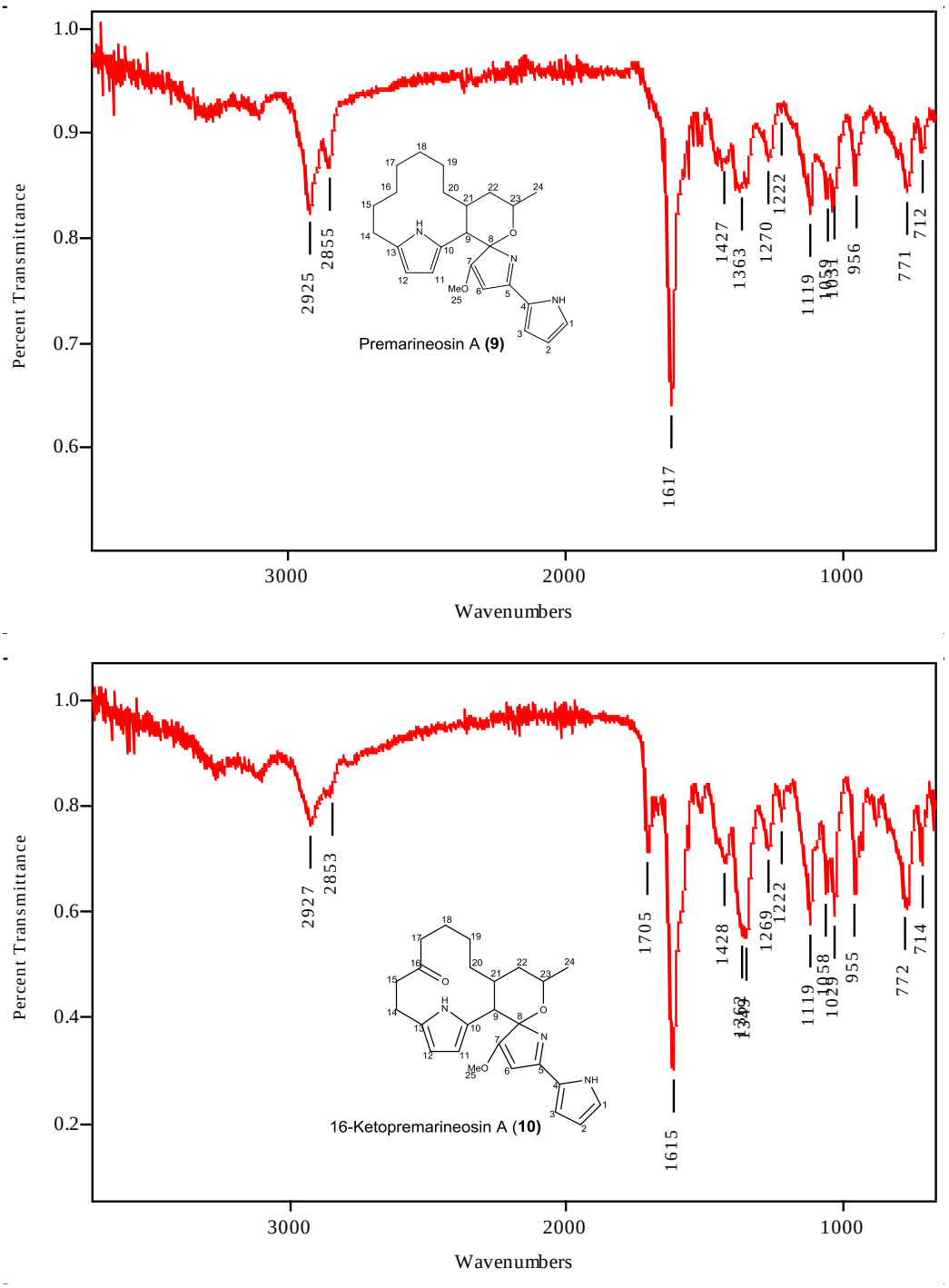


Figure S15. IR spectrum of 9 (top panel) and 10 (bottom panel)

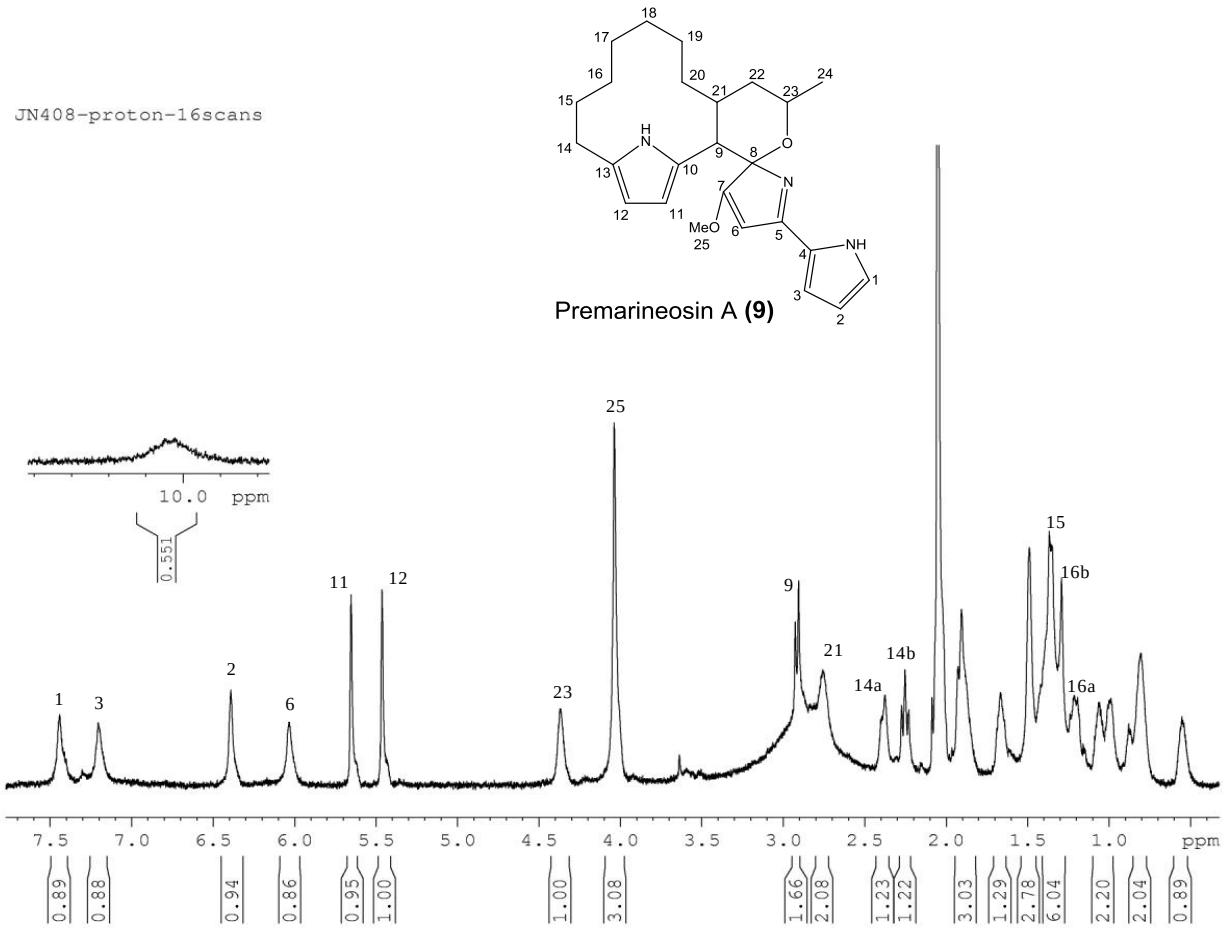


Figure S16. ^{1}H NMR spectrum of premarineosin A (9) (600 MHz, Acetone-d6)

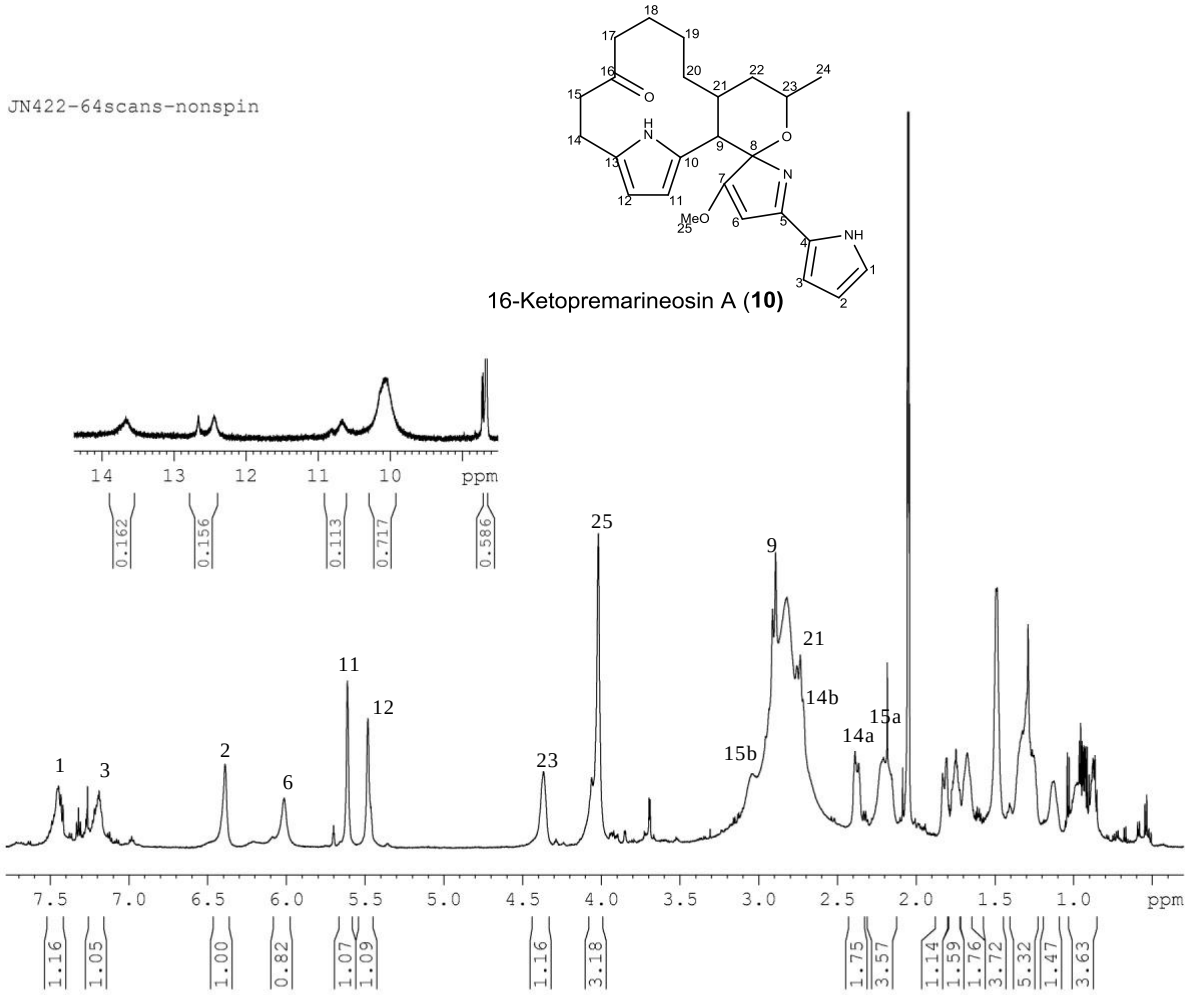


Figure S17. ^{1}H NMR spectrum of 16-ketopremarineosin A (10) (600 MHz, Acetone-d6)

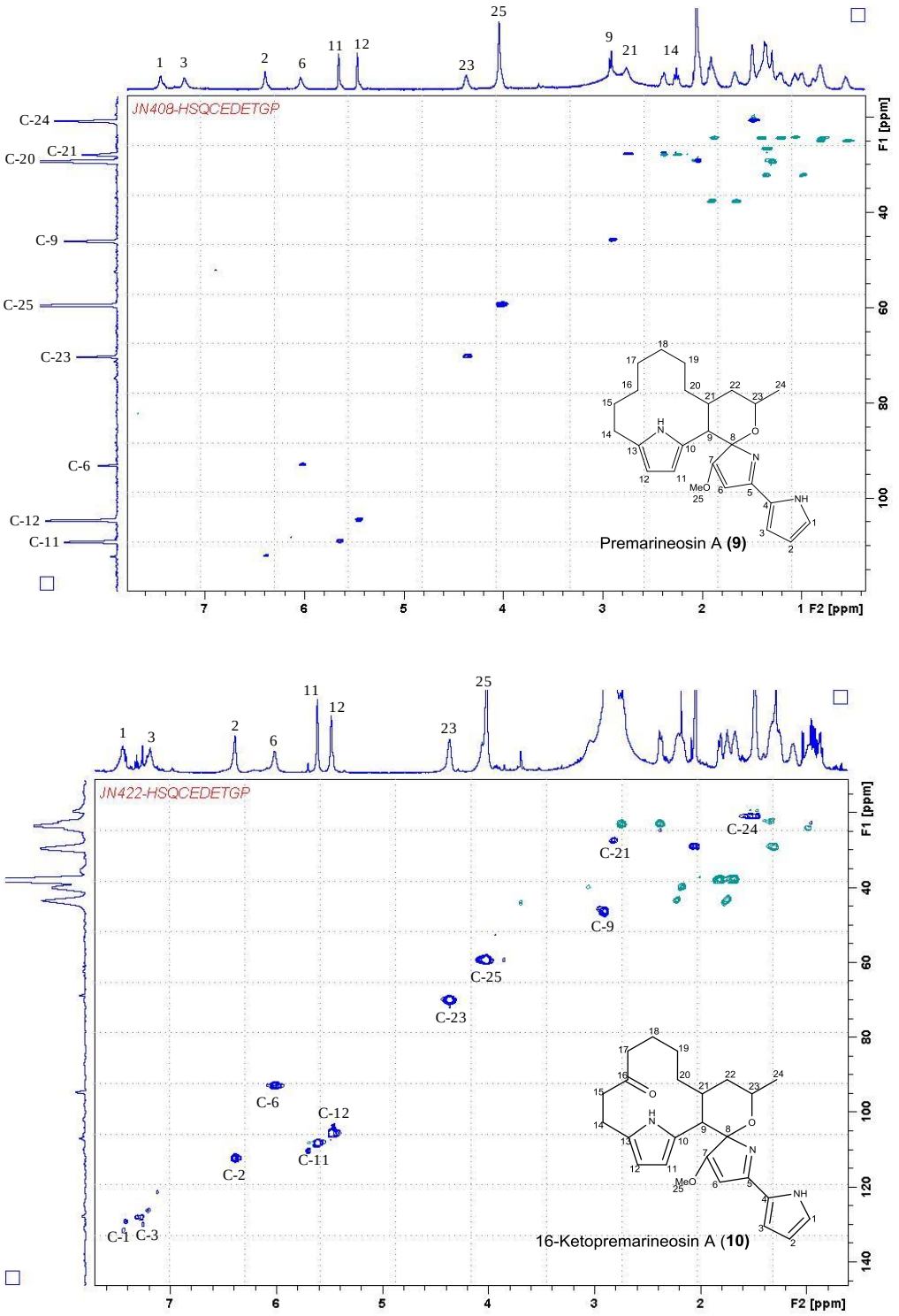


Figure S18. 2D HSQC ($^{1}H \rightarrow ^{13}C$) spectrum of 9 (top panel) and 10 (bottom panel). Key peak assignments are indicated on spectra

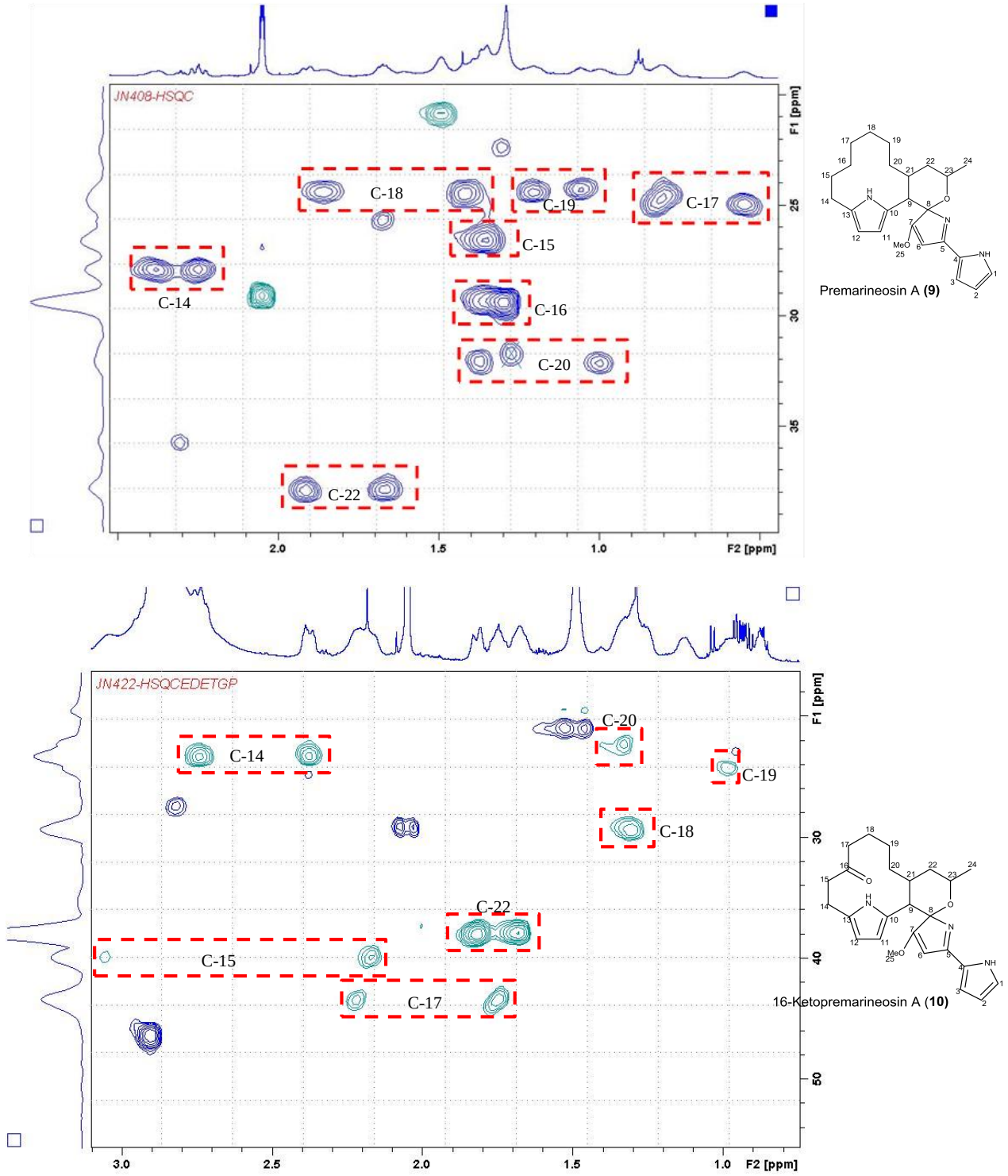


Figure S19. 2D HSQC ($^{1}H \rightarrow ^{13}C$) spectrum of 9 (top panel) and 10 (bottom panel) - aliphatic region expanded

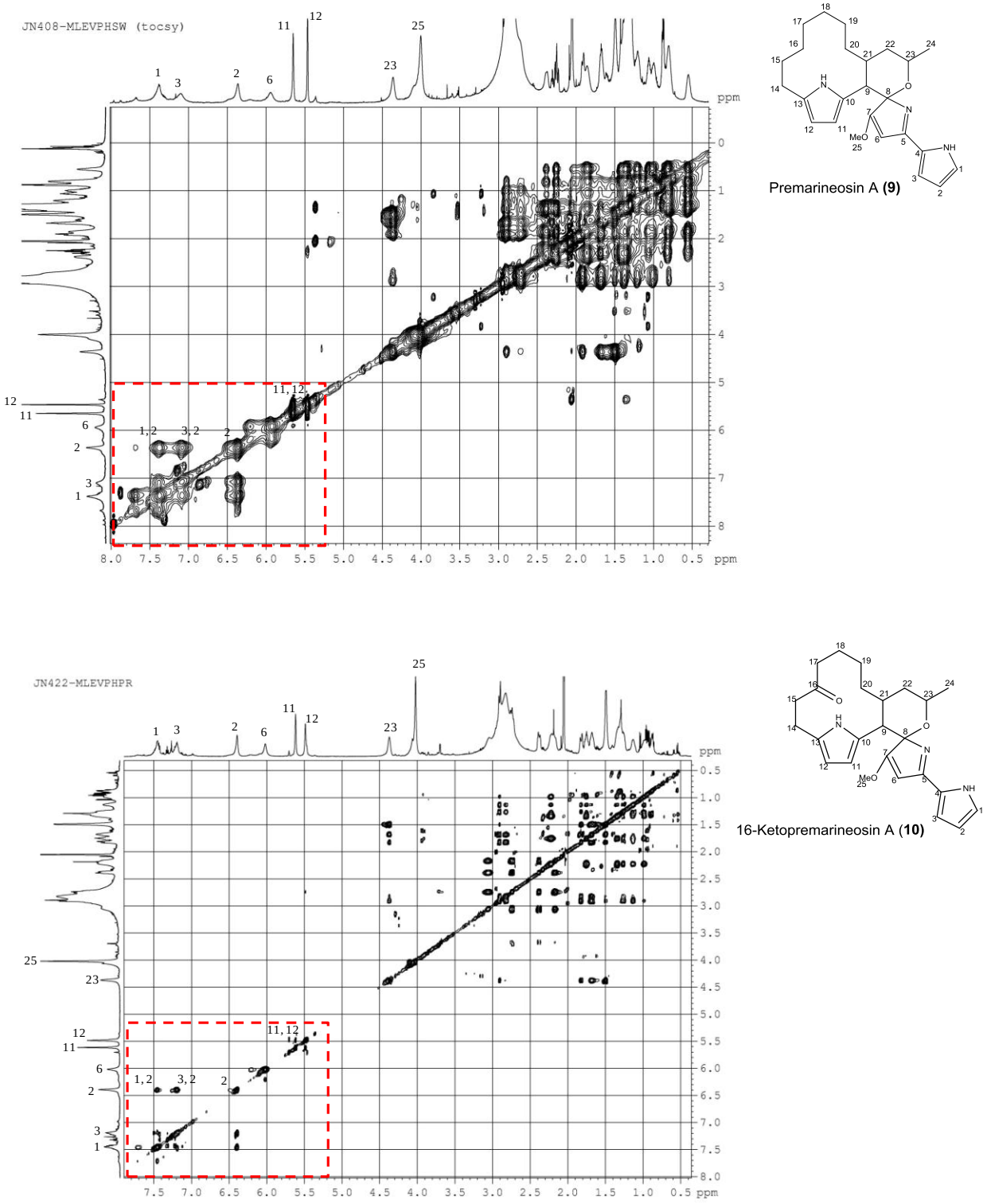


Figure S20. 2D TOCSY (^{1}H↔^{1}H) spectrum of 9 (top panel) and 10 (bottom panel)

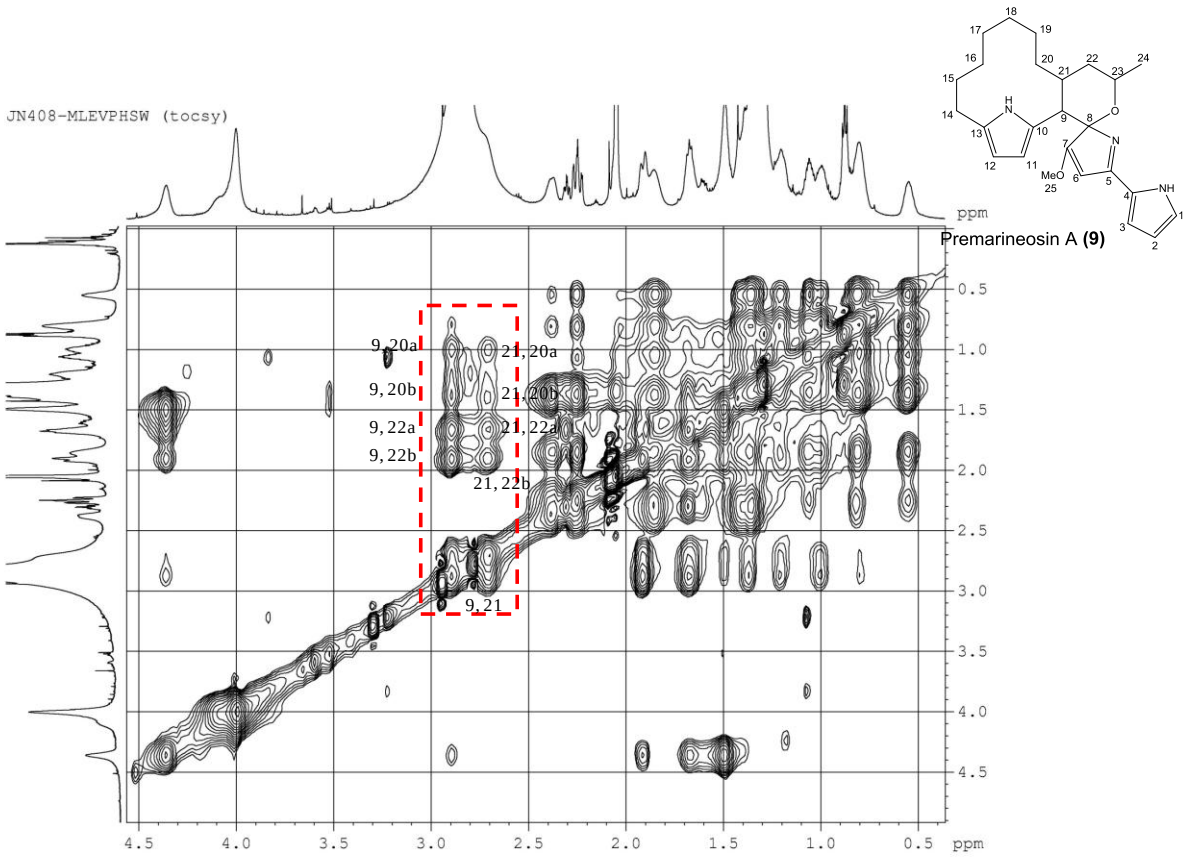


Figure S21. Selected 2D TOCSY (^{1}H↔^{1}H) spectrum of 9 (aliphatic region expanded)

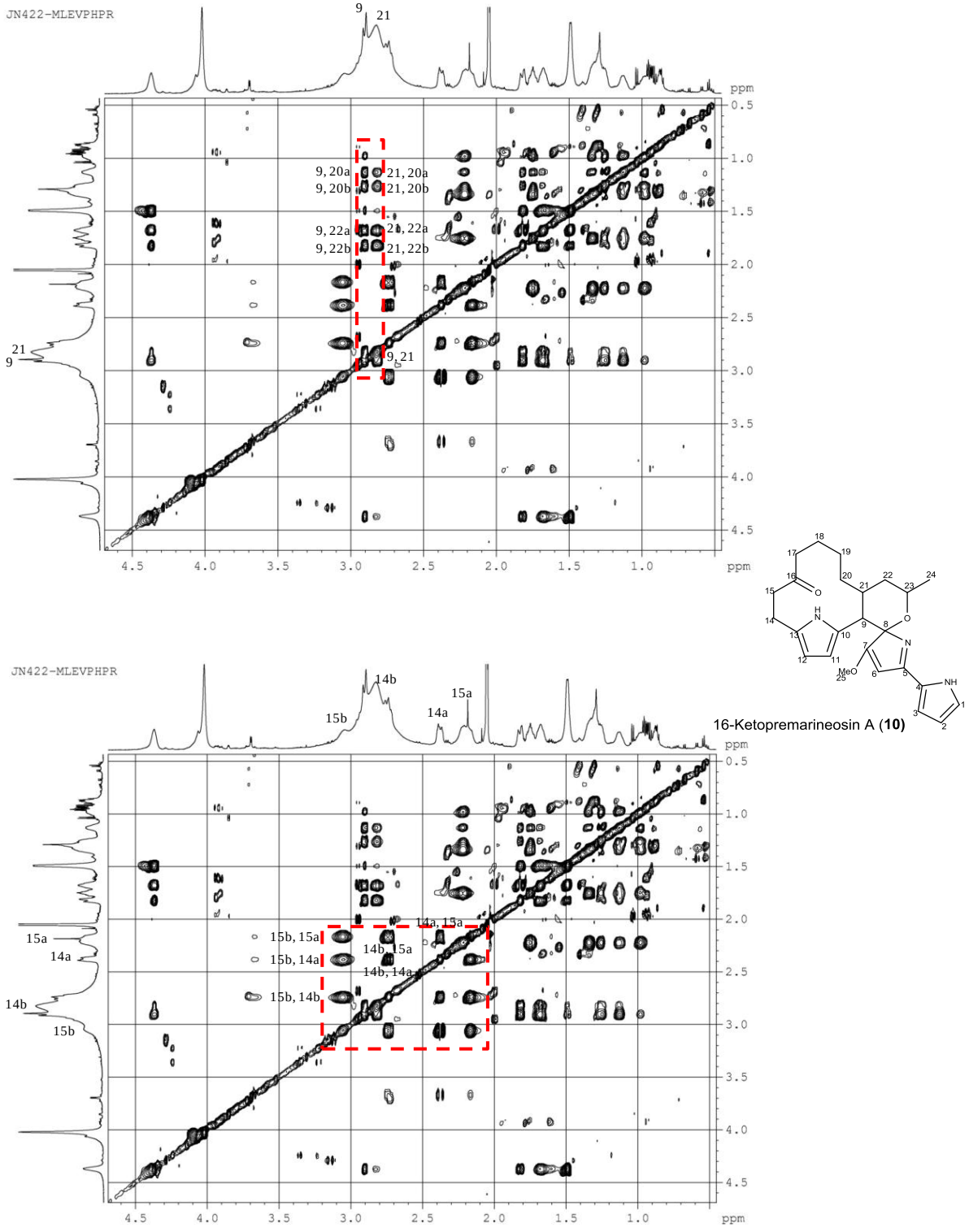


Figure S22. Selected 2D TOCSY (^{1}H↔^{1}H) spectrum of 10 (aliphatic region expanded)

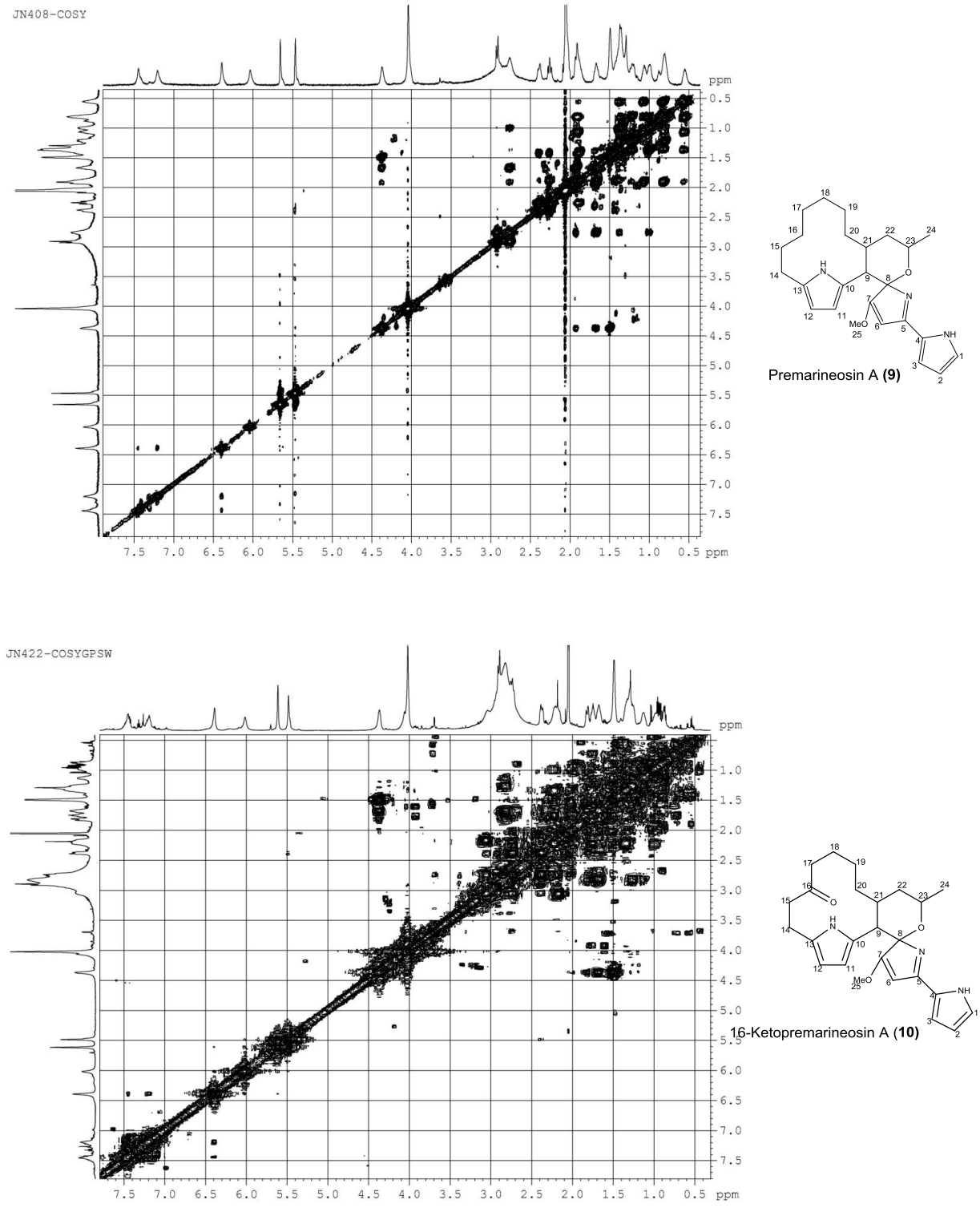


Figure S23. 2D COSY (^{1}H↔^{1}H) spectrum of 9 (top panel) and 10 (bottom panel)

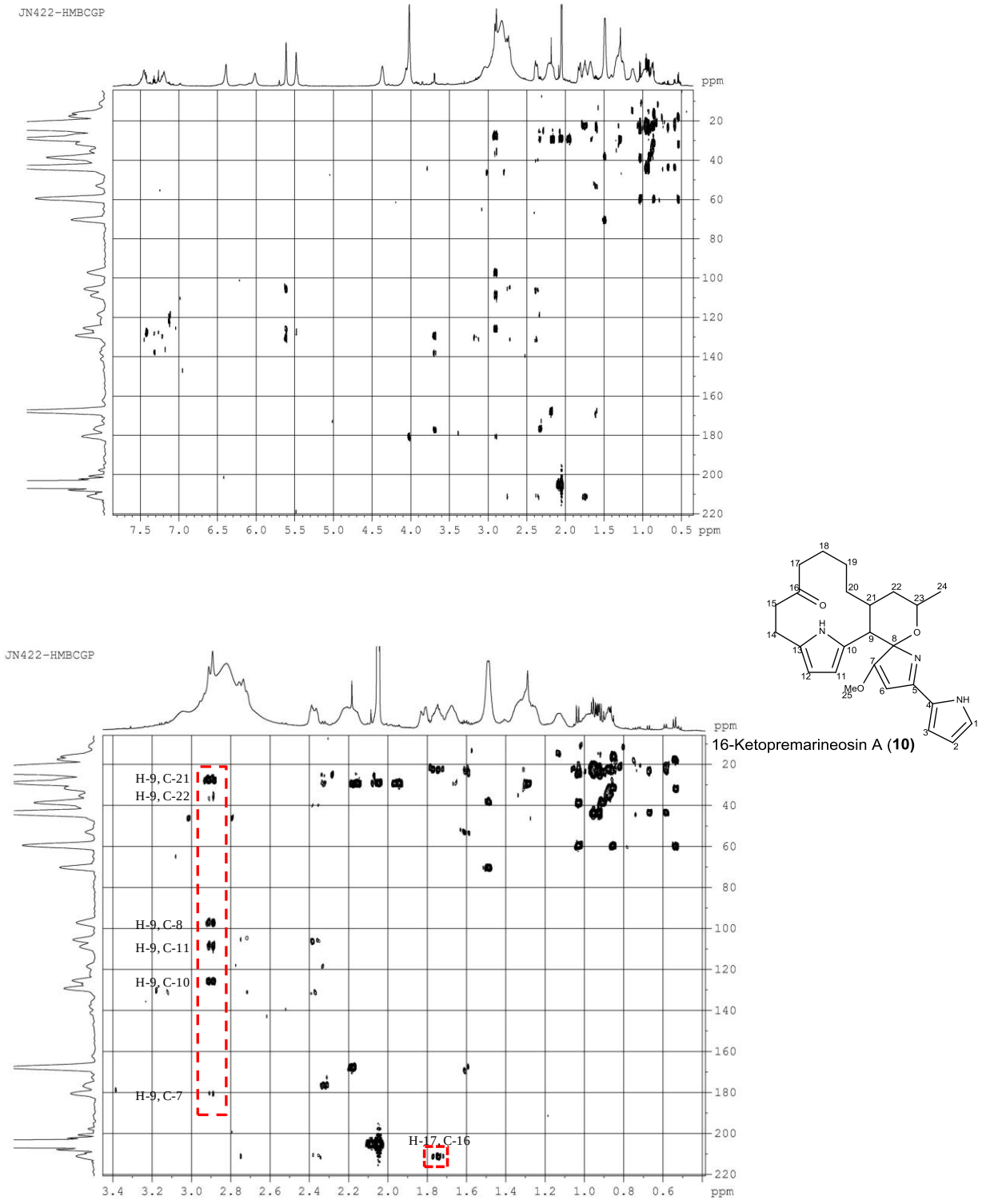
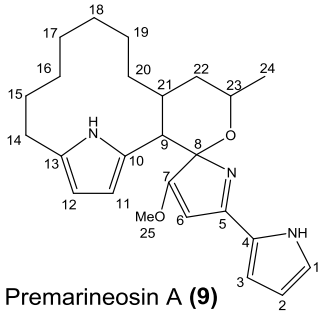


Figure S24. 2D HMBC ($^{1}H \rightarrow ^{13}C$) spectrum of 10 (top panel), aliphatic region expanded (bottom panel)

Table S11. Selected COSY (^{1}H↔^{1}H) and TOCSY (^{1}H↔^{1}H) NMR correlations for 9

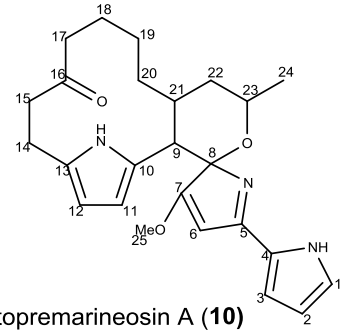


Premarineosin A **(9)**

Position	δ_H (J in Hz)[a]	COSY	TOCSY
1	7.37, brs	2, 3	2, 3
2	6.37, brs	1, 3	1, 3
3	7.08, brs	1, 2	1, 2
4			
5			
6	5.93, s		
7			
8			
9	2.89, d (6.9)	21	20, 21, 22, 23, 24
10			
11	5.64, brs	12	12
12	5.46, brs	11	11
13			
14	2.24, 2.37, m	15	15, 16, 17
15	1.37, m	14, 17	14, 16, 17, 18
16	1.34, 1.37, m	15, 18	14, 15, 17, 18, 19
17	0.54, 0.80, m	16, 18	15, 16, 18, 19, 20
18	1.42, 1.86, m	17, 19	15, 16, 17, 19, 20
19	1.05, 1.20, m	18, 20	16, 17, 18, 20, 21
20	1.00, 1.37, m	19, 21	9, 17, 18, 19, 21, 22
21	2.71, m	9, 20, 22	9, 19, 20, 22, 23, 24
22	1.67, 1.91, m	21, 23	9, 20, 21, 23, 24
23	4.36, m	22, 24	9, 20, 21, 22, 24
24	1.50, d (6.5)	23	9, 21, 22, 23
25	3.99, s		

[a] Coupling constants were not determined for aromatic protons due to brs

Table S12. Selected COSY (^{1}H↔^{1}H) and TOCSY (^{1}H↔^{1}H) NMR correlations for 10

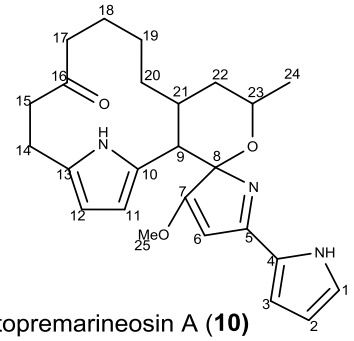


16-Ketopremarineosin A (**10**)

Position	δ_H (J in Hz)[a]	COSY	TOCSY
1	7.45, brs	2, 3	2, 3
2	6.39, brs	1, 3	1, 3
3	7.20, brs	1, 2	1, 2
4			
5			
6	6.01, s		
7			
8			
9	2.90, d (7.1)	21	20, 21, 22, 23, 24
10			
11	5.61, brs	12	12
12	5.48, brs	11	11
13			
14	2.37, 2.73, m	15	15
15	2.16, 3.05, m	14	14
16			
17	1.71, 2.21, m	18	18, 19, 20
18	1.29, 1.31, m	17, 19	17, 19, 20
19	0.94, 1.33, m	18, 20	17, 18, 20, 21
20	1.12, 1.26, m	19, 21	9, 17, 18, 19, 21, 22
21	2.80, m	9, 20, 22	9, 19, 20, 22, 23, 24
22	1.67, 1.82, m	21, 23	9, 20, 21, 23, 24
23	4.36, m	22, 24	9, 20, 21, 22, 24
24	1.50, d (6.7)	23	9, 21, 22, 23
25	4.02, s		

[a] Coupling constants were not determined for aromatic protons due to brs

Table S13. NMR Spectral data for 10 (600 MHz, Acetone-d6) and Selected HMBC correlations

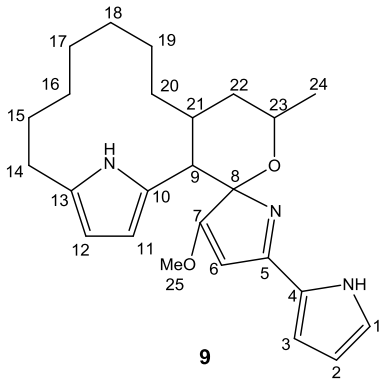


16-Ketopremarineosin A (**10**)

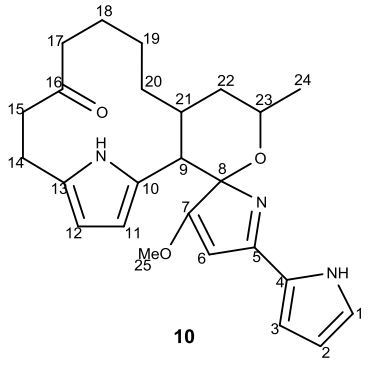
Position	δ_H (*J* in Hz)	δ_C [a]		Selected HMBC correlations
1	7.45, brs	131.5	CH	
2	6.39, brs	112.4	CH	
3	7.20, brs	125.9	CH	
4		N.D.	C	
5		N.D.	C	
6	6.01, s	93.1	CH	
7		180.6	C	
8		97.4	C	
9	2.90, d (7.1)	46.3	CH	7, 8, 10, 11, 20, 21, 22
10		125.7	C	
11	5.61, brs	108.3	CH	10, 12, 13
12	5.48, brs	105.5	CH	10, 13
13		130.1	C	
14	2.37, 2.73, m	23.6	CH_2	12, 13, 15, 16
15	2.16, 3.05, m	39.9	CH_2	
16		211.3	C=O	
17	1.71, 2.21, m	43.6	CH_2	16, 19
18	1.29, 1.31, m	29.4	CH_2	19, 20
19	0.94, 1.33, m	22.1	CH_2	18
20	1.12, 1.26, m	34.7	CH_2	9
21	2.80, m	27.3	CH	
22	1.67, 1.82, m	37.9	CH_2	
23	4.36, m	70.0	CH	
24	1.50, d (6.7)	20.6	CH_3	22, 23
25	4.02, s	59.4	CH_3	7

[a] Assignment by 1D (^{1}H), and 2D (COSY, TOCSY, HSQC) NMR spectroscopy; N.D.: not determined.

Table S14. Comparison of NMR Spectral data for 9 and 10 (600 MHz, Acetone-d6)



9



10

Position	**9** δ_H[a]	**10** δ_H	**9** δ_C[a]	**10** δ_C
1	7.37	7.45	131.3	131.5
2	6.37	6.39	111.6	112.4
3	7.08	7.20	127.5	125.9
4			N.D.	N.D.
5			N.D.	N.D.
6	5.93	6.01	93.1	93.1
7			N.D.	180.6
8			N.D.	97.4
9	2.89	2.90	46.1	46.3
10			N.D.	125.7
11	5.64	5.61	109.0	108.3
12	5.46	5.48	104.5	105.5
13			N.D.	130.1
14	2.24, 2.37	2.37, 2.73	28.0	23.6
15	1.37	2.16, 3.05	26.5	39.9
16	1.34, 1.37		29.3	211.3
17	0.54, 0.80	1.71, 2.21	24.7	43.6
18	1.42, 1.86	1.29, 1.31	24.4	29.4
19	1.05, 1.20	0.94, 1.33	24.3	22.1
20	1.00, 1.37	1.12, 1.26	31.9	34.7
21	2.71	2.80	28.1	27.3
22	1.67, 1.91	1.67, 1.82	37.8	37.9
23	4.36	4.36	70.1	70.0
24	1.50	1.50	20.9	20.6
25	3.99	4.02	59.1	59.4

[a] Assignment by 1D (^{1}H), and 2D (COSY, TOCSY, HSQC) NMR spectroscopy; N.D.: not determined.

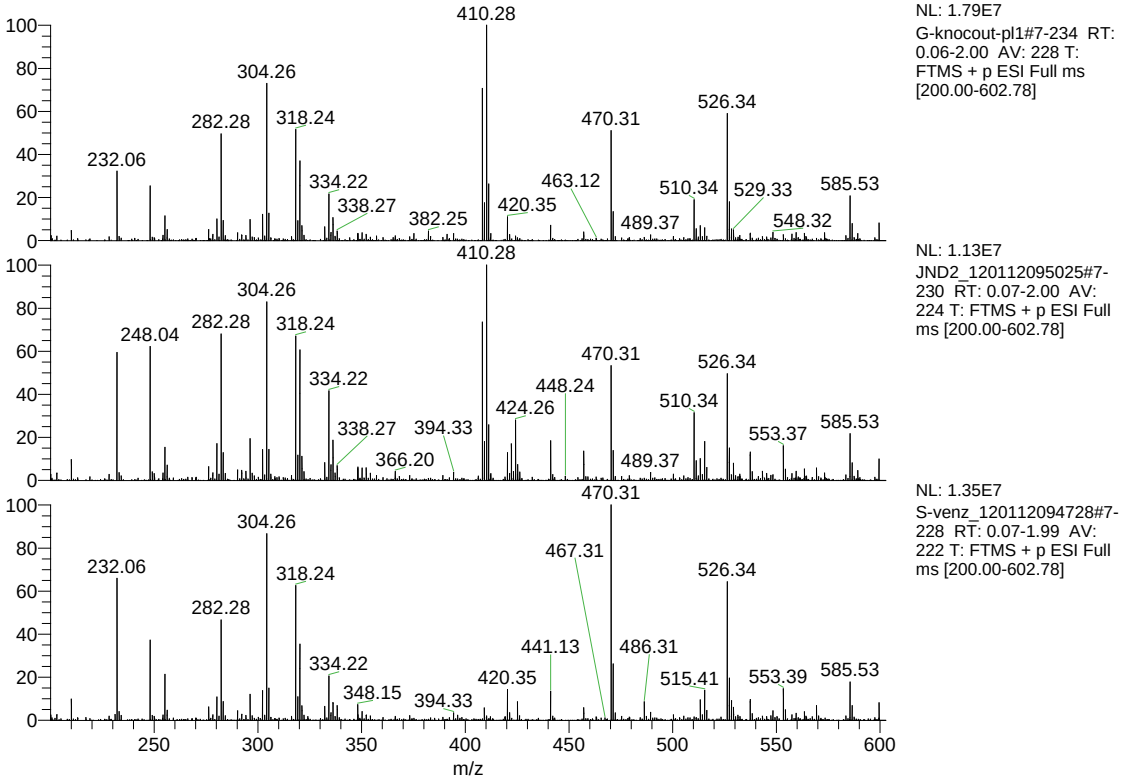


Figure S25. Comparison of the MS profiles of crude extract of *S. venezuelae* JND2ΔG (top panel), JND2 strain (middle) and wild type *S. venezuelae* (bottom). JND2ΔG (top panel) and JND2 (middle panel) strains have the same MS profile where both strains show a compound of *m/z* 410.28 in addition to the metabolites normally produced by the wild type *S. venezuelae* (bottom) such as methymycin and pikromycin, *m/z* 470.31 and 526.34 respectively.

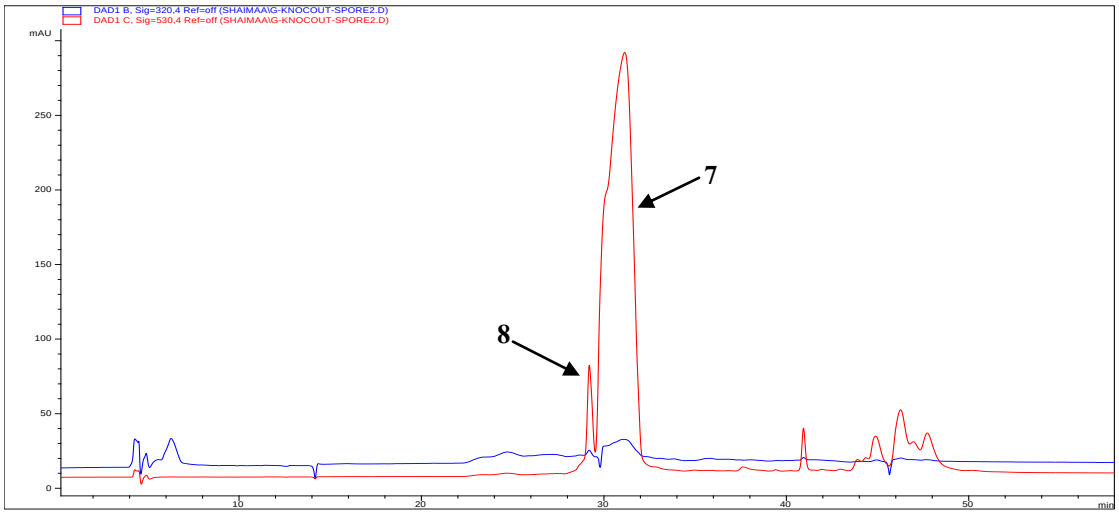
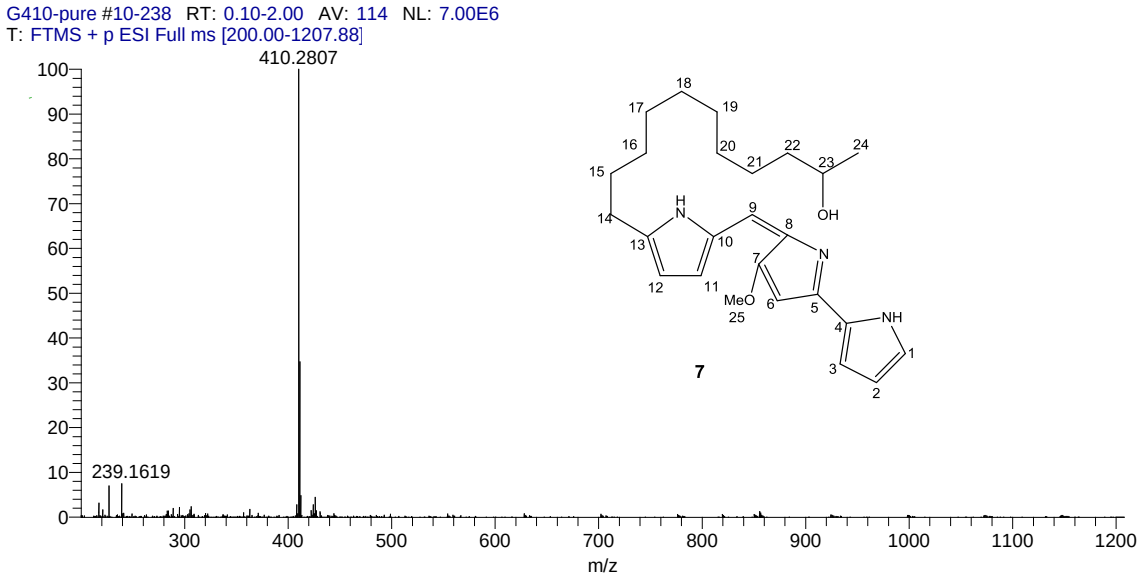


Figure S26. LC-UV/MS profile of *S. venezuelae* JND2ΔG. The red chromatogram represents absorption at 530 nm while blue chromatogram represents absorption at 320 nm. For LC method used, please refer to Table S8.

a)



b)

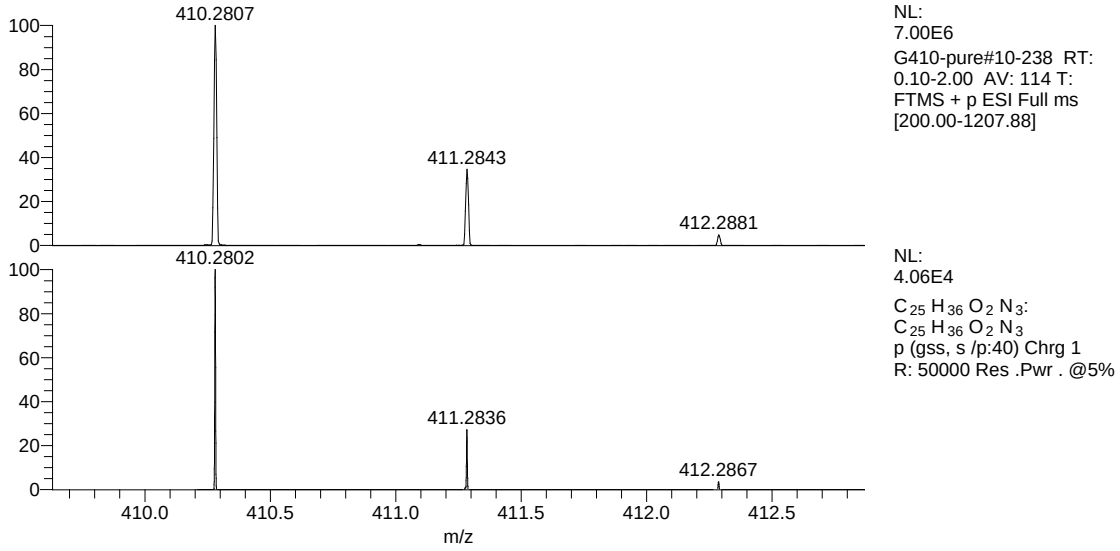
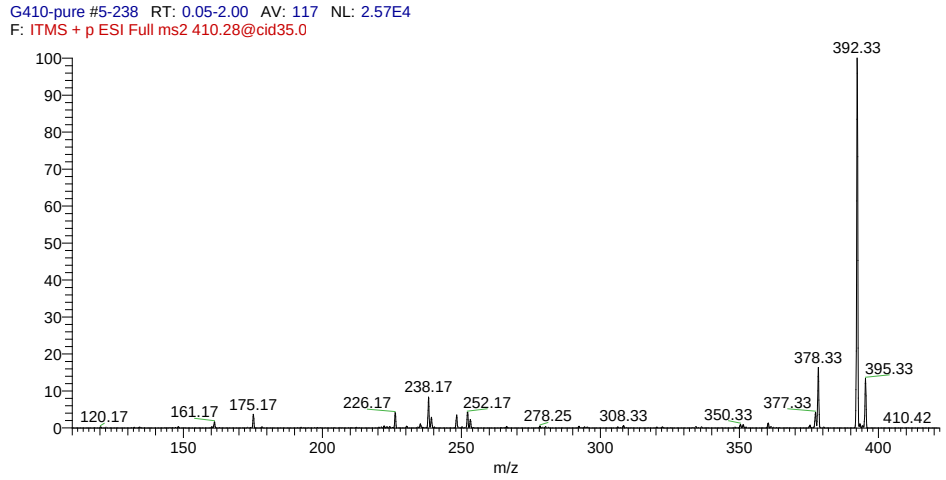


Figure S27. a) HRMS (ESI) 23-hydroxyundecylprodiginine (7). b) Comparison between the acquired (top panel) and predicted (bottom panel) mass for 23-hydroxyundecylprodiginine (7). There is a difference of *m*/*z* 0.0005 between acquired and predicted mass.

a)



b)

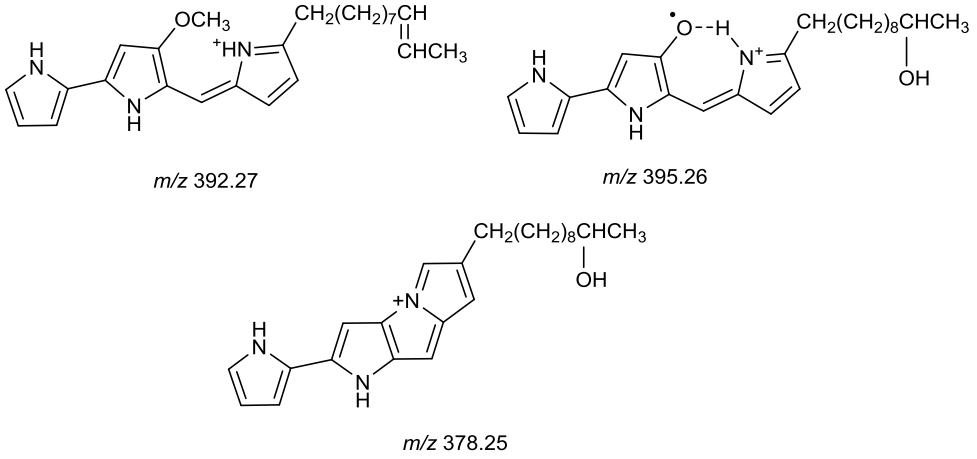


Figure S28. a) ESI-CID-MS^n profile of 23-hydroxyundecylprodiginine (7). The MS/MS profile of 7 is characterized by the presence of *m/z* 395.33, 392.33, and 378.33 fragments equivalent to the loss of *m/z* 18, 15 and 32 respectively. Parent ion of *m/z* 410.28 is not shown. **b) Proposed structures of ion fragments shown in ESI-CID-MS^n profile of 23-hydroxyundecylprodiginine (7).**

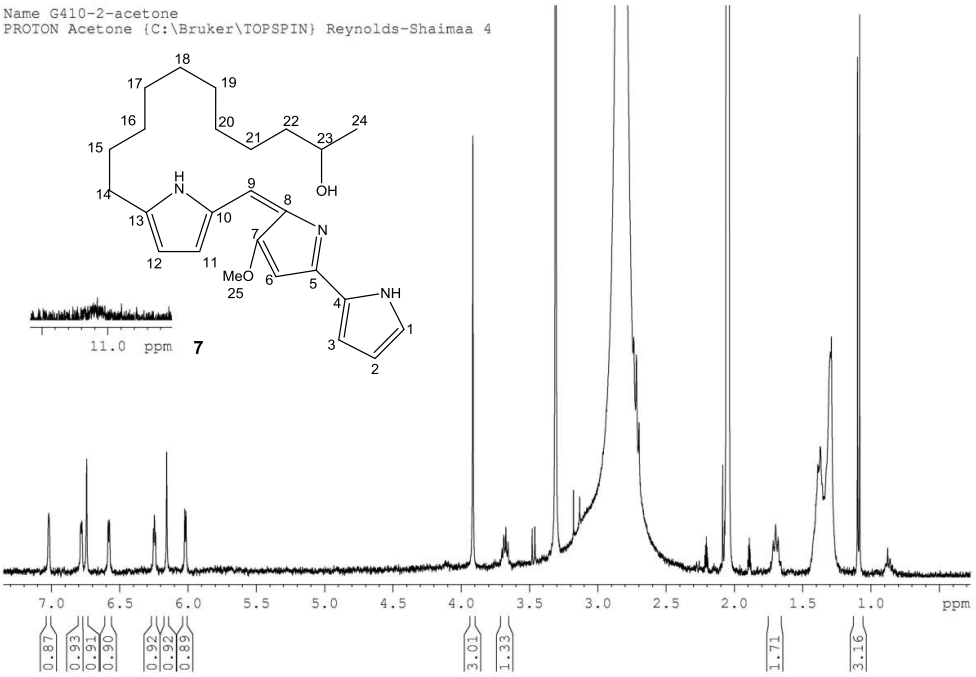


Figure S29. ^{1}H NMR spectrum of 23-hydroxyundecylprodiginine (7) (600 MHz, Acetone-d6)

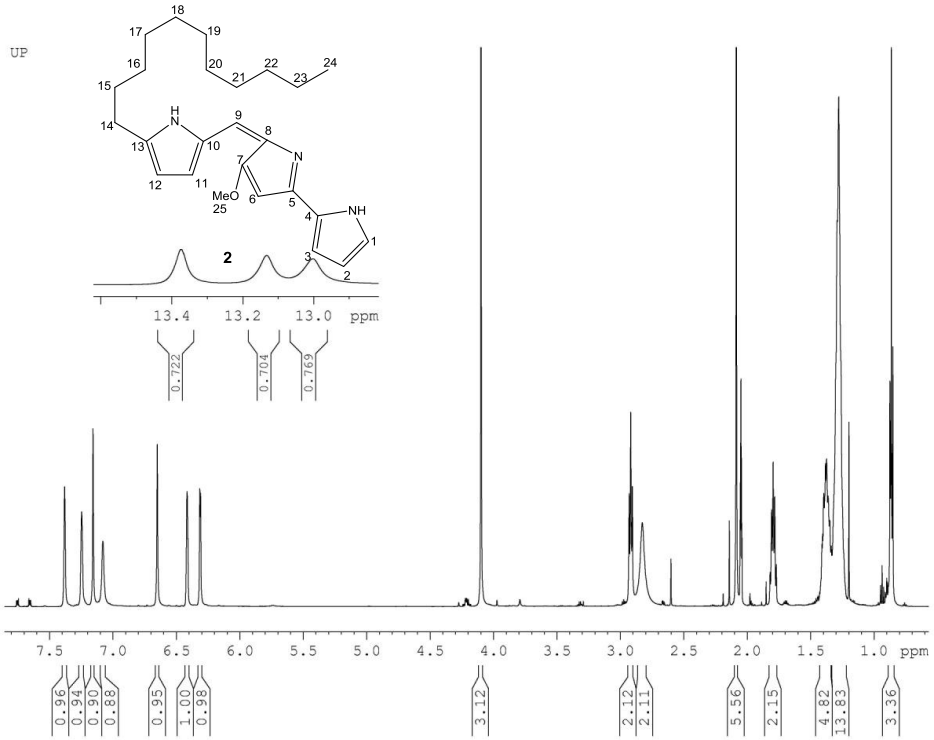


Figure S30. ^{1}H NMR spectrum of undecylprodiginine HCl (2) standard (600 MHz, Acetone-d6)

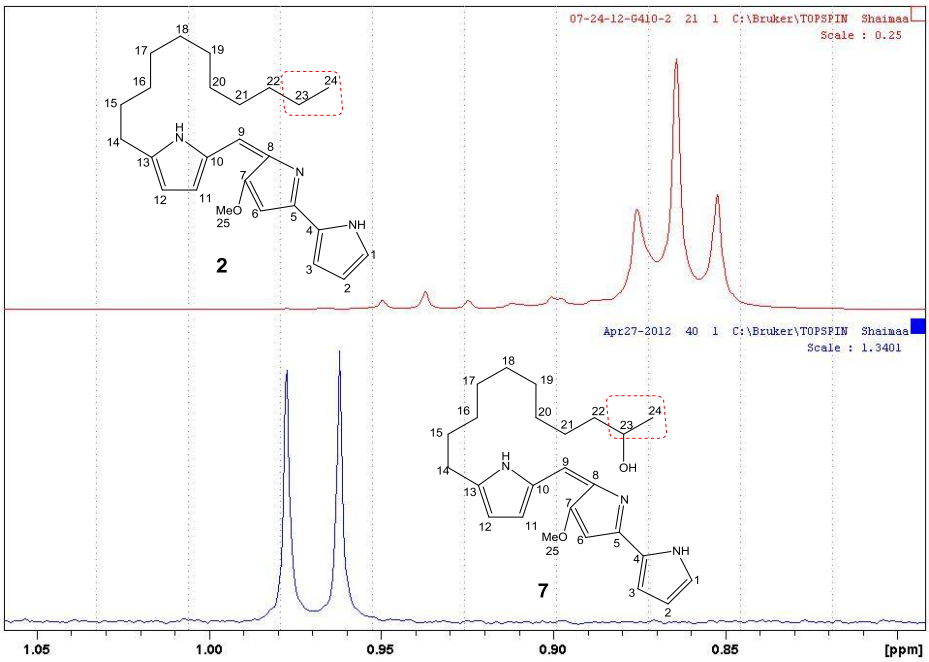


Figure S31. ^{1}H NMR spectrum showing comparison between the splitting pattern of methyl protons at C-24 in undecylprodiginine (2) and 23-hydroxyundecylprodiginine (7)

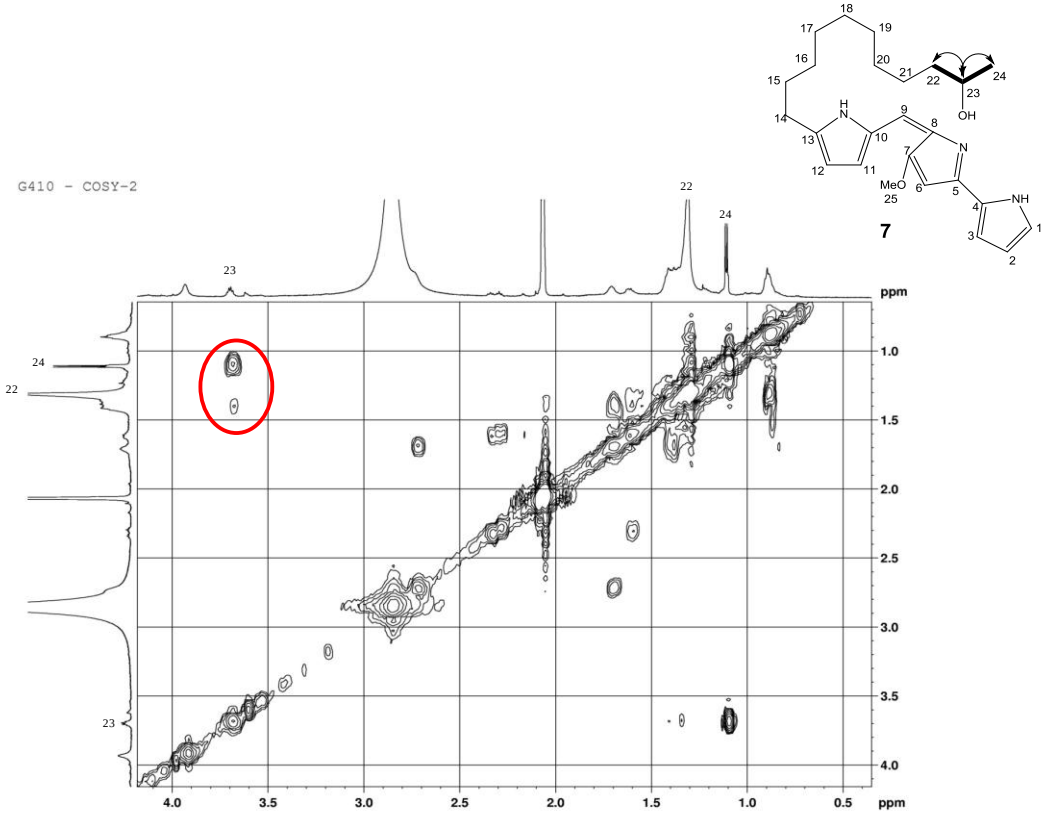


Figure S32: 2D COSY NMR (^{1}H↔^{1}H) correlations between C-22, C-23, and C-24 of 23-hydroxy-undecylprodiginine (7).

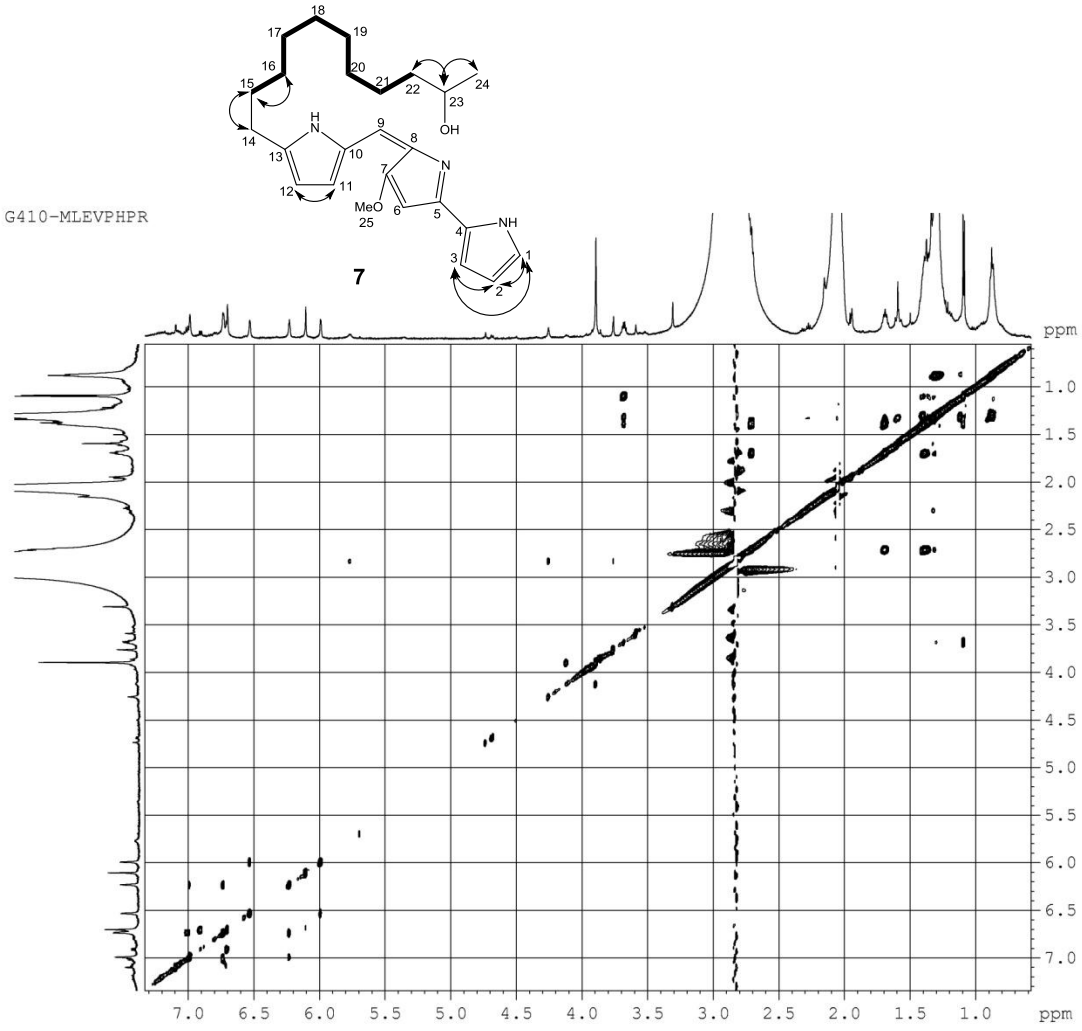
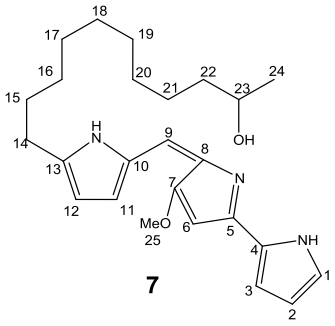
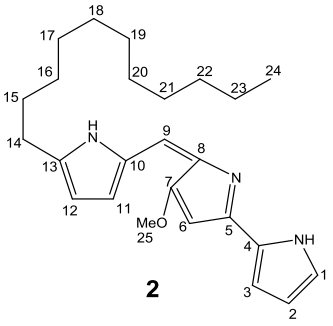


Figure S33. 2D TOCSY (^{1}H↔^{1}H) spectrum of 7. The bold bonds indicate 16H integrated into one large peak at 1.3 ppm.

Table S15: ^{1}H NMR spectral data for 23-hydroxyundecylprodiginine (7) (600 MHz, Acetone-d6) and side by side comparison of 7 and 2



7



2

Position[a]	**7** δ_H (J in Hz)[a]	**2** δ_H(J in Hz)[b]
1	7.02, dd (1.4, 2.7)	7.38, m
2	6.24, dd (2.7, 3.6)	6.41, m
3	6.78, dd (1.4, 3.6)	7.24, m
4	-	-
5	-	-
6	6.15, s	6.65, s
7	-	-
8	-	-
9	6.74, s	7.15, s
10	-	-
11	6.58, d (3.5)	7.07, d (3.6)
12	6.02, d (3.5)	6.31, d (3.6)
13	-	-
14	2.70, t (7.2)	2.92, t (7.4)
15	1.69, m	1.79, m
16	1.33, m	1.38, m
17	1.33, m	1.38, m
18	1.33, m	1.38, m
19	1.33, m	1.38, m
20	1.33, m	1.38, m
21	1.33, m	1.38, m
22	1.34, m	1.38, m
23	3.67, m	1.28, m
24	1.09, d (6.1)	0.86, m
25	3.91, s	4.09, t (7.1)

[a]Assignments are for protons bonded to the specified positions using 1D (^{1}H), and 2D (COSY, TOCSY) NMR spectroscopy in comparison to 2, [b]chemical shifts reported are for the HCl salt of **2**

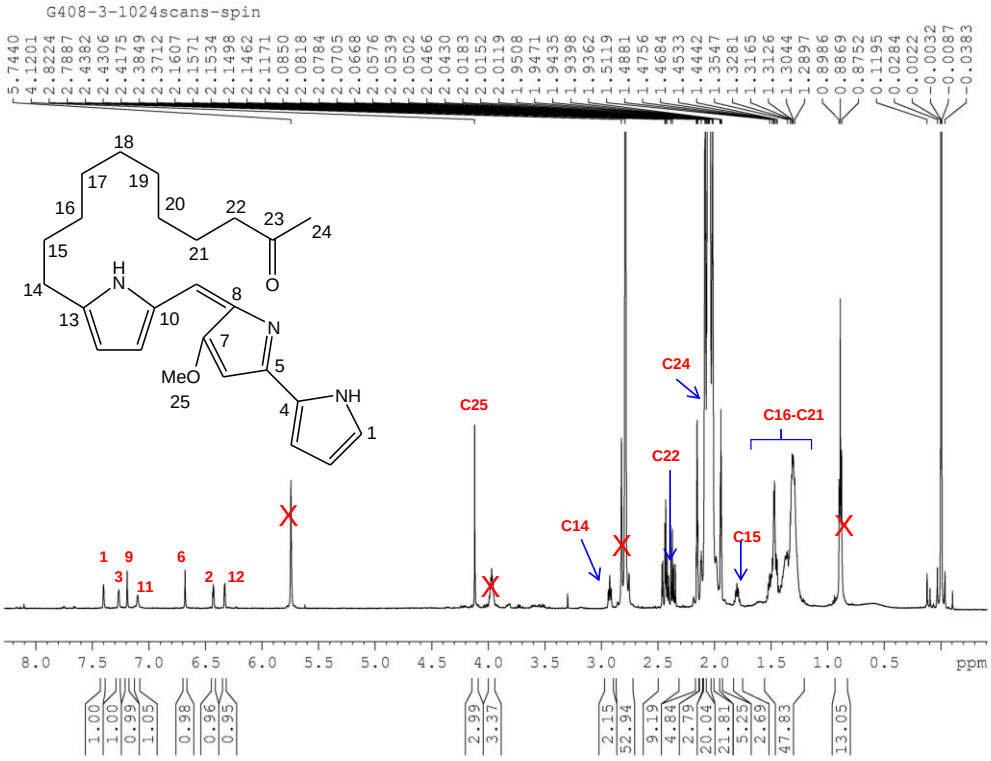


Figure S34. ^{1}H NMR spectrum of 23-ketoundecylprodiginine (8) (600 MHz, Acetone-d6)

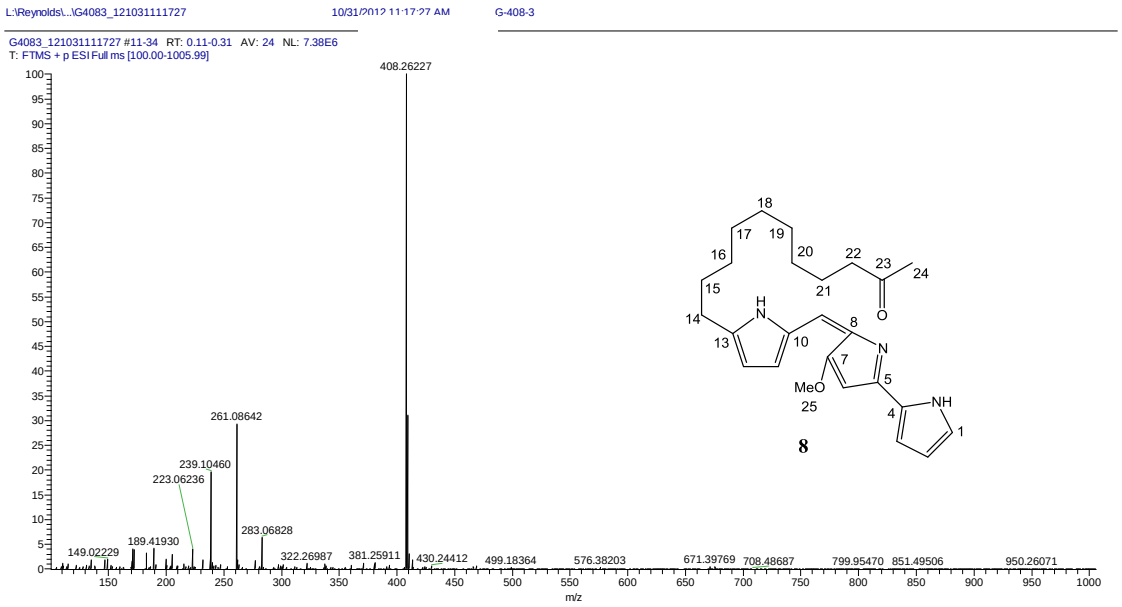


Figure S35. HRMS (ESI) of 23-ketoundecylprodiginine (8)

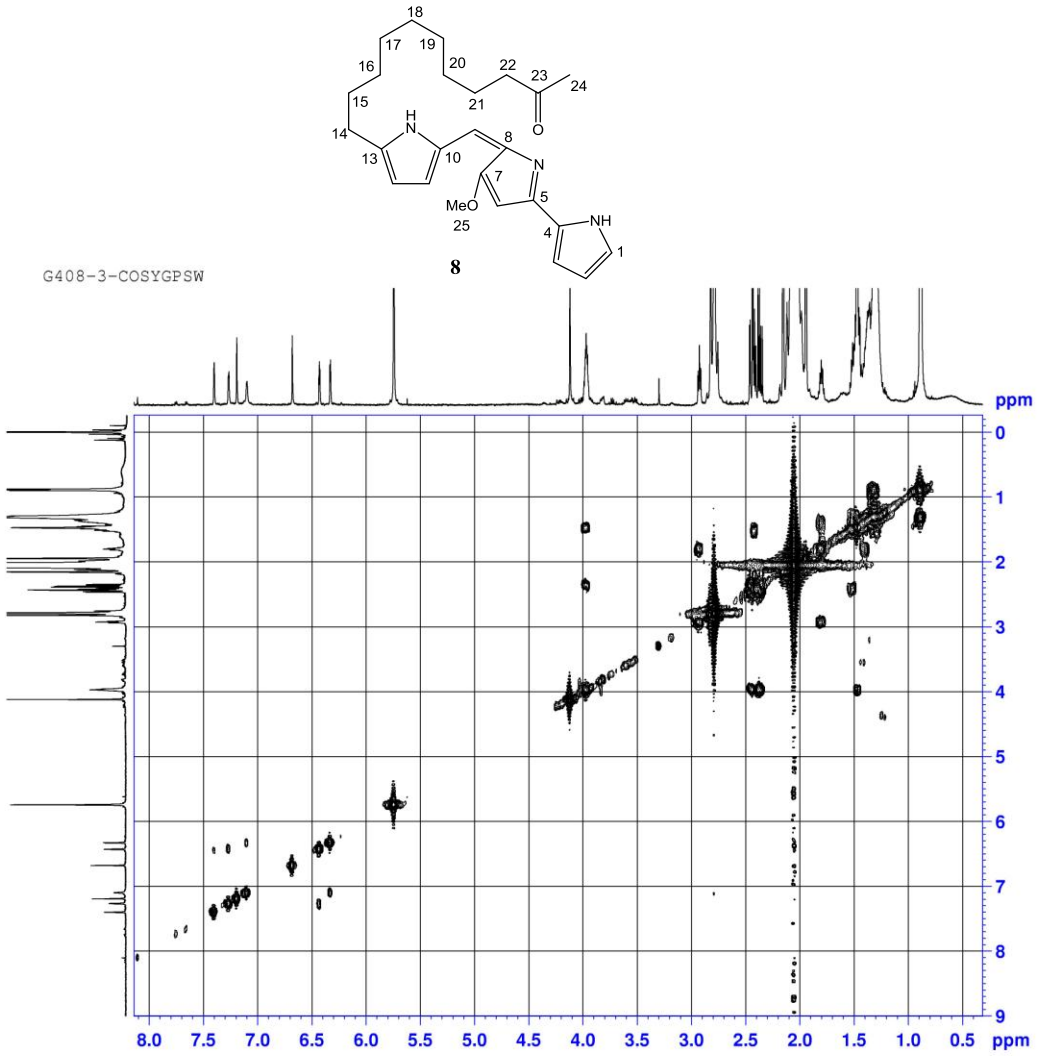


Figure S36. 2D COSY (^{1}H↔^{1}H) spectrum of 23-ketoundecylprodiginine (8)

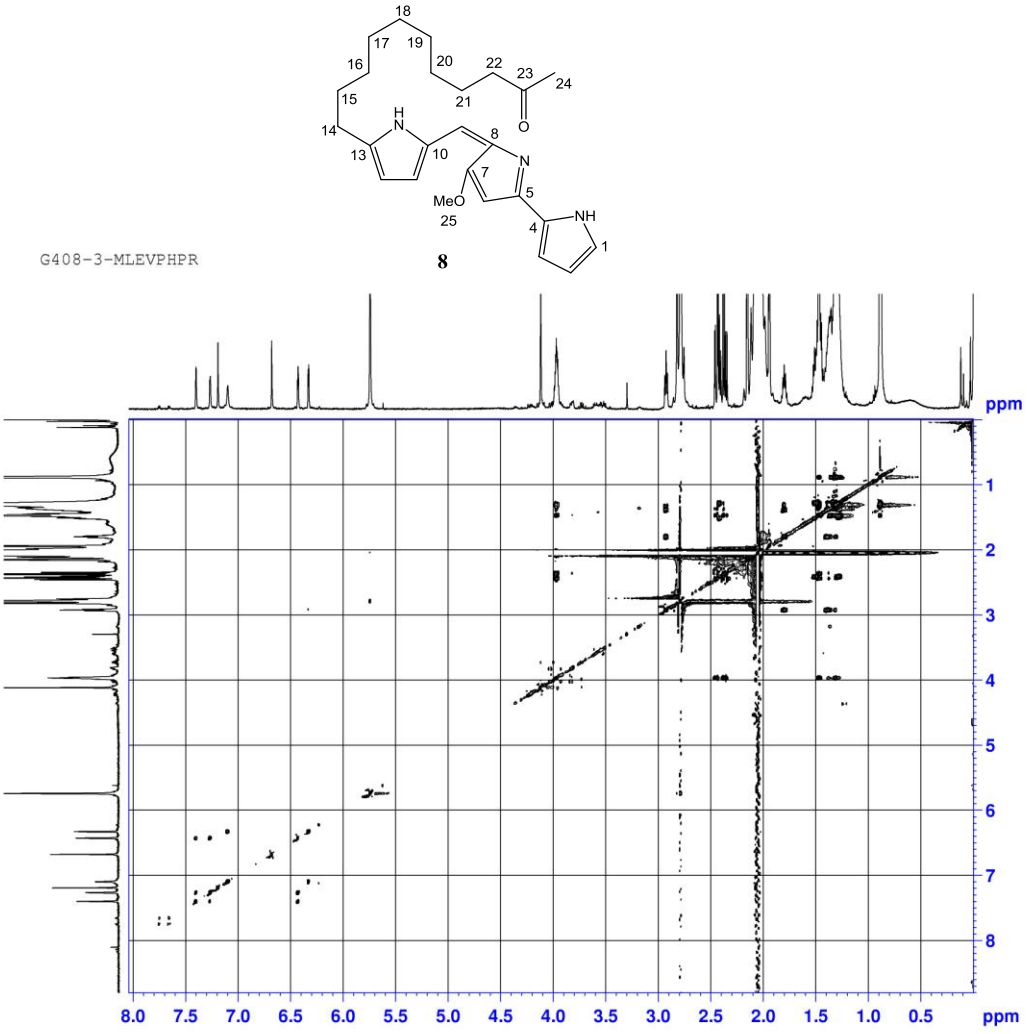


Figure S37. 2D TOCSY (^{1}H↔^{1}H) spectrum of 23-ketoundecylprodiginine (8)

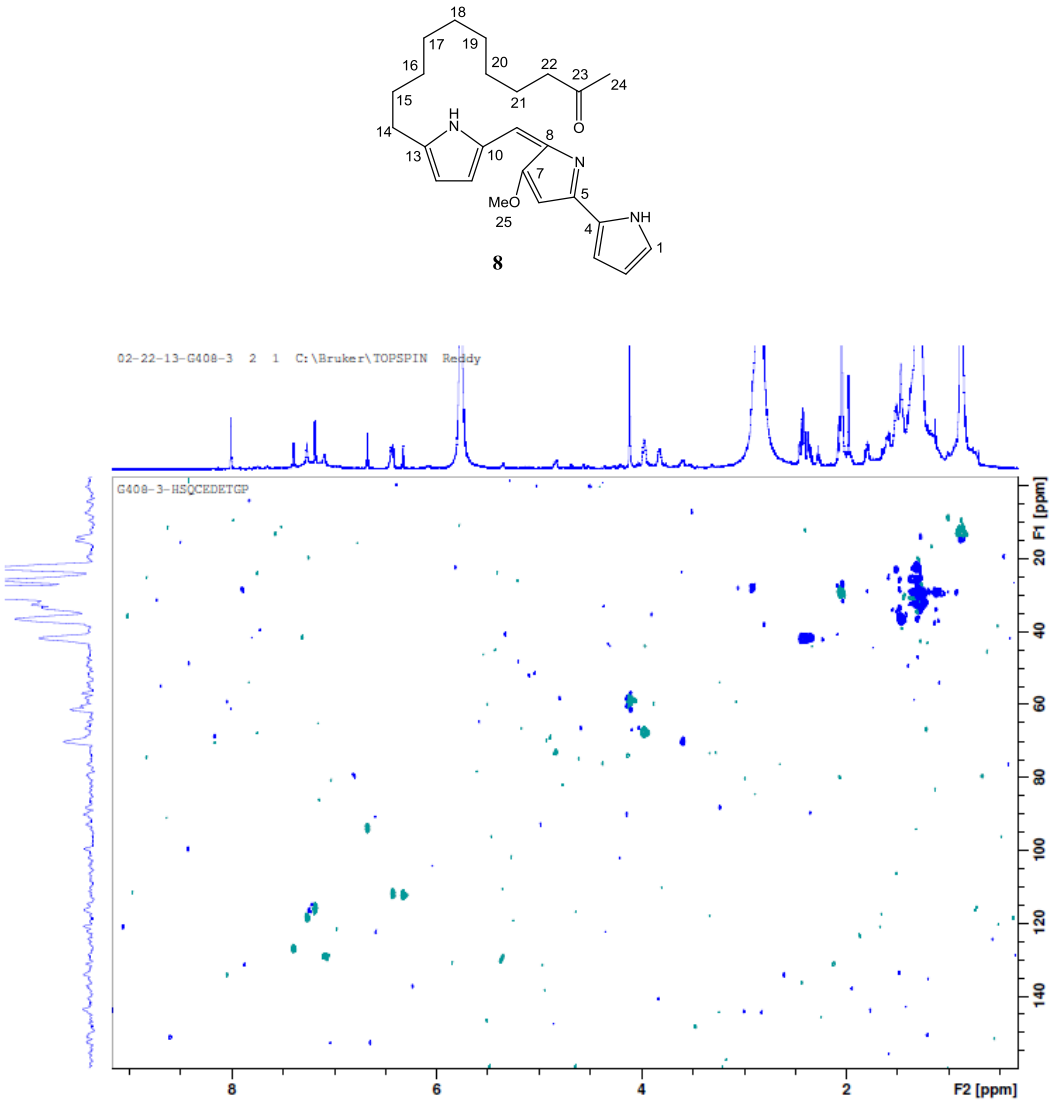
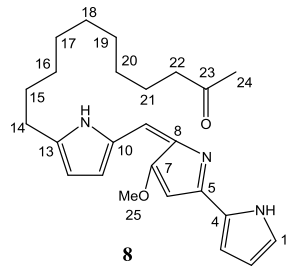


Figure S38. 2D HSQC ($^{1}H \rightarrow ^{13}C$) spectrum of 23-ketoundecylprodiginine (8)



8

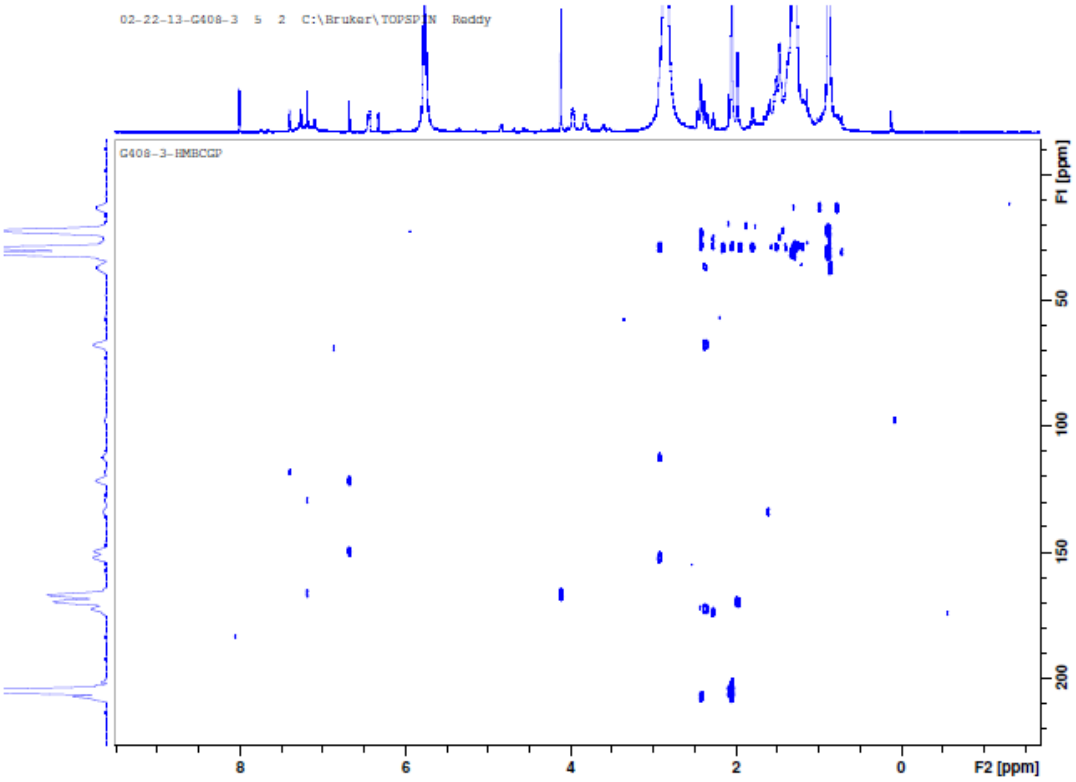
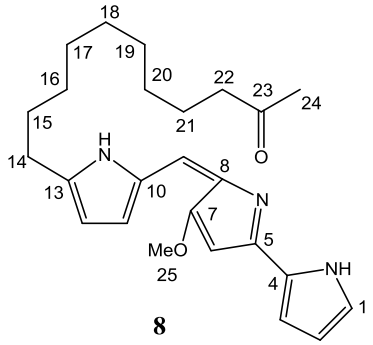


Figure S39. 2D HMBC ($^{1}H \rightarrow ^{13}C$) spectrum of 23-ketoundecylprodiginine (8)

Table S16. NMR spectral data, and selected HMBC ($^1H \rightarrow ^{13}C$ correlations) for 8 (600 MHz, Acetone-d6)



8

Position	δ_H (J in Hz)[a]	δ_C[a]		Selected HMBC correlations
1	7.40, dd (1.5, 2.1)	127.1	CH	3, 4
2	6.42, dd (2.1, 3.7)	112.1	CH	
3	7.26, dd (1.5, 3.7)	118.4	CH	
4	-	122.5	C	
5	-	N.D	C	
6	6.68, s	94.1	CH	4, 8
7	-	167.2	C	
8	-	149.4	C	
9	7.19, s	115.9	CH	7, 10
10		129.5	C	
11	7.09, d (3.4)	129.1	CH	
12	6.32, d (3.4)	112.7	CH	
13	-	N.D	C	
14	2.92, t (7.5)	28.1	CH_2	12, 15, 16
15	1.80, m	28.3	CH_2	14, 16
16-21[b]	1.51–1.28, m	N.D	CH_2	
22	2.46, t (7.2)	42.0	CH_2	21, 23, 24
23	-	207.1	C=O	
24	2.04, s	26.9	CH_3	23
25	4.12, s	59.2	CH_3	7

[a] assignment by 1D, and 2D NMR spectroscopy; [b] protons of C16-C21 are overlapped; N.D.: not determined

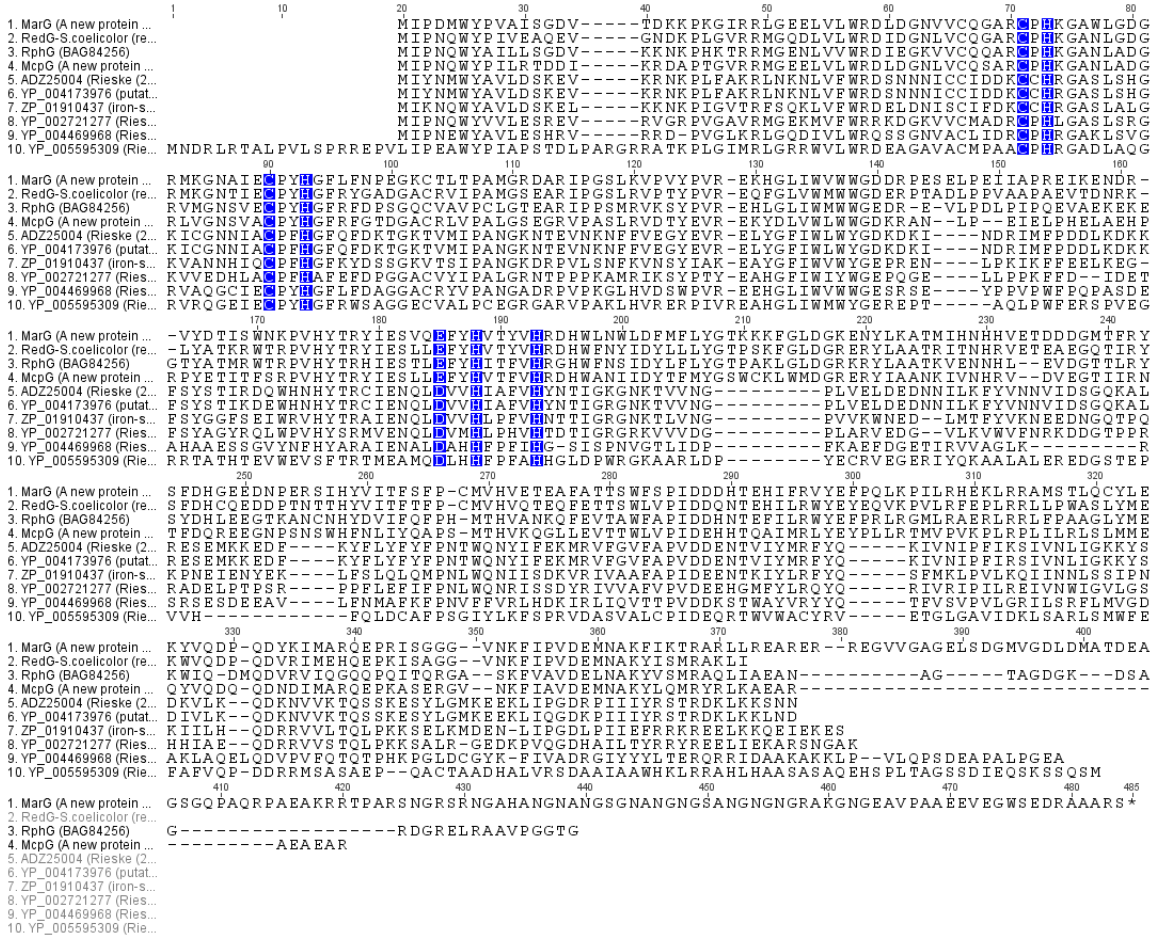


Figure S40. Sequence alignment of MarG and RedG with other Rieske-oxygenases. MarG and RedG share the CXH, CXXH motifs of other Rieske-oxygenases at their N-terminal end and DXXHX_4H motif at the C-terminal end. It should be noted that the conserved aspartate (D) is glutamate (E) in MarG and RedG.

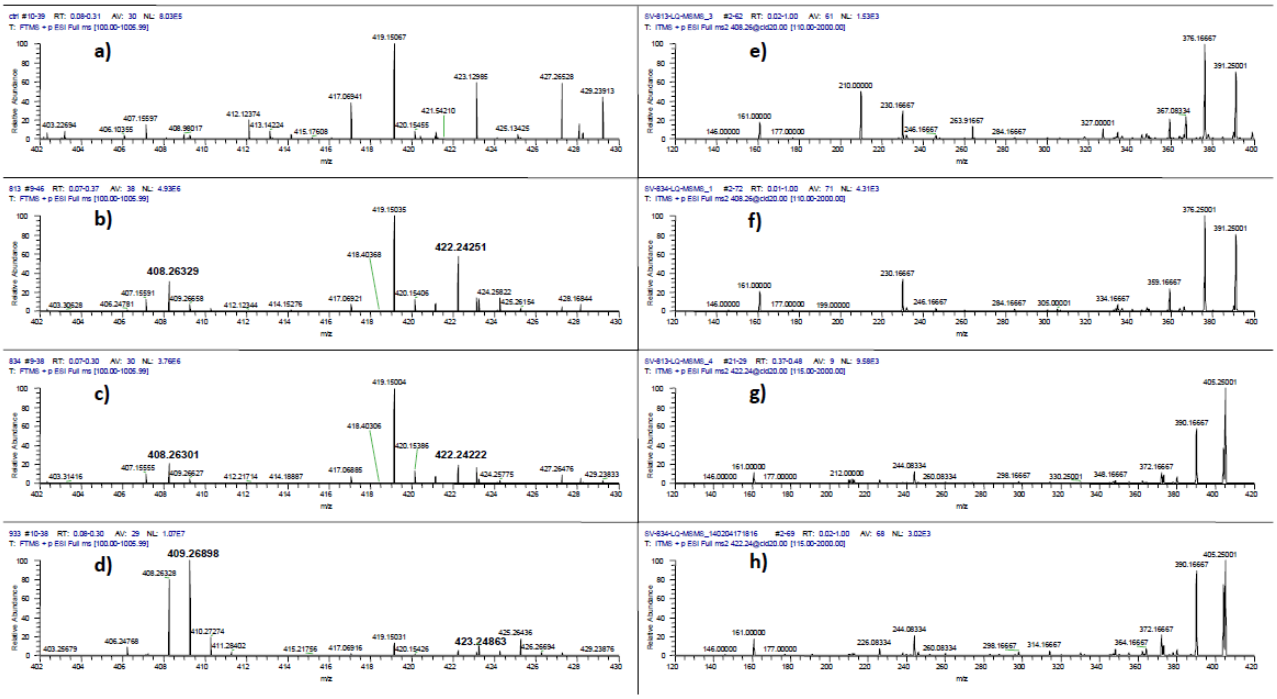


Figure S41. Total ion chromatogram (TIC) of total extract of *S. venezuelae* expressing MarG after feeding of 7, 8 and [23-d]-7. a) The MS profile of *S. venezuelae* expressing *marG*; b) The MS profile of *S. venezuelae* expressing *marG* fed with 23-hydroxyundecylprodiginine (**7**), *m/z* 408.26 and *m/z* 422.24 corresponding to $[M+H]^+$ of premarineosin (**9**) and 16-Ketopremarineosin (**10**); c) The MS profile of *S. venezuelae* expressing *marG* fed with 23-ketoundecylprodiginine (**8**), *m/z* 408.26 and *m/z* 422.24 corresponding to $[M+H]^+$ of premarineosin (**9**) and 16-Ketopremarineosin (**10**); d) The MS profile of *S. venezuelae* expressing *marG* fed with 23-deuterated-hydroxyundecylprodiginine (~90% D), *m/z* 409.27 and *m/z* 423.25 corresponding to $[M+H]^+$ of 23-deuterated-premarineosin and 23-deuterated-16-Ketopremarineosin; e)The MS/MS profile of **9** from *S. venezuelae* expressing *marG* fed with **7** (Same as in Figure S12); f) The MS/MS profile of **9** from *S. venezuelae* expressing *marG* fed with **8** (Same as in Figure S12); g) The MS/MS profile of **10** from *S. venezuelae* expressing *marG* fed with **7** (Same as in Figure S14); h)The MS/MS profile of **10** from *S. venezuelae* expressing *marG* fed with **8** (Same as in Figure S14).

References

1. Boonlarppradab, C.; Kauffman, C. A.; Jensen, P. R.; Fenical, W. *Org. Lett*. 2008, *10*, 5505–5508.
2. Cai, X. C.; Wu, X.; Snider, B. B. *Org. Lett*. 2010, *12*, 1600–1603.