

1 **Coralmycin derivatives with potent anti-Gram negative activity produced by**
2 **the myxobacteria *Corallococcus coralloides* M23**

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13 **Fermentation.** Fermentation was carried out in CYS medium containing 0.5% casitone, 0.1%
14 yeast extract, 0.3% soluble starch, 0.1% MgSO₄·7H₂O, 0.05% CaCl₂, 50 mM 4-(2-hydroxyethyl)-
15 1-piperazineethanesulfonic acid (HEPES), 0.4% trace element solution, and 0.5 µg/mL
16 cyanocobalamin. The trace element solution contained 100 mg/L MnCl₂·4H₂O, 20 mg/L CoCl₂,
17 10 mg/L CuSO₄, 10 mg/L Na₂MoO₄·2H₂O, 20 mg/L ZnCl₂, 5 mg/L LiCl, 5 mg/L SnCl₂·2H₂O, 10
18 mg/L H₃BO₃, 20 mg/L KBr, 20 mg/L KI, and 8 g/L EDTA Na-Fe³⁺ salt (trihydratepiece of agar
19 from t). The mature plate culture of the producing strain was inoculated into a 500-mL Erlenmeyer
20 flask containing 100 mL of sterile seed liquid medium with the above composition and cultured
21 on a rotary shaker (150 rpm) at 28 °C for 3 days. The 300 mL of the seed culture was transferred
22 into a 5-L Jar fermenter containing 2.5 L of the above medium containing 100 g of Amberlite
23 XAD16 (Sigma, USA), and then cultivated at 28 °C for 2 days with an aeration rate of 0.4 v/min
24 and agitation rate of 350 rpm. The resultant 3 L culture was transferred into a 50-L fermenter
25 containing 30 L of the above medium containing 1.2 kg of Amberlite XAD16, and then cultivated
26 at 28 °C for 3 days with an aeration rate of 0.4 v/min and agitation rate of 200 rpm. The resultant
27 30 L culture was transferred into a 500-L fermenter containing 300 L of the above medium
28 containing 12 kg of Amberlite XAD16, and then cultivated at 28 °C for 3 days with an aeration
29 rate of 0.4 v/min and agitation rate of 100 rpm. The resultant 300L culture was transferred into a
30 5000-L fermenter containing 3000 L of the above medium containing 120 kg of Amberlite XAD16,
31 and then cultivated at 28 °C for 6 days with an aeration rate of 0.4 v/min and agitation rate of 70
32 rpm.

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42 Table S1. Antibacterial activities of **4–7** and related compounds.

Test organisms	MIC ($\mu\text{g/mL}$)					
	4	5	6	7	10	Cip*
<i>Staphylococcus aureus</i> RN 4220	>32	>32	>32	>32	>32	0.125
MRSA CCARM 3167	>32	>32	>32	>32	>32	4
MRSA CCARM 3506	>32	>32	>32	>32	>32	2
QRSA CCARM 3505	>32	>32	>32	>32	>32	128
QRSA CCARM 3519	>32	>32	>32	>32	>32	128
<i>Streptococcus pneumoniae</i> KCTC 5412	>32	>32	>32	>32	>32	0.25
<i>Enterococcus faecalis</i> KCTC 5191	>32	>32	>32	>32	>32	0.5
<i>Acinetobacter baumannii</i> KCTC 2508	>32	>32	>32	>32	>32	0.25
<i>E. coli</i> CCARM 1356	>32	>32	>32	>32	>32	64
<i>E. coli</i> KCTC 1682	>32	>32	>32	>32	>32	0.06
<i>Pseudomonas aeruginosa</i> KCTC 2004	>32	>32	>32	>32	>32	0.03
<i>Klebsiella pneumoniae</i> KCTC 22057	>32	>32	>32	>32	>32	0.015

43 *Ciprofloxacin

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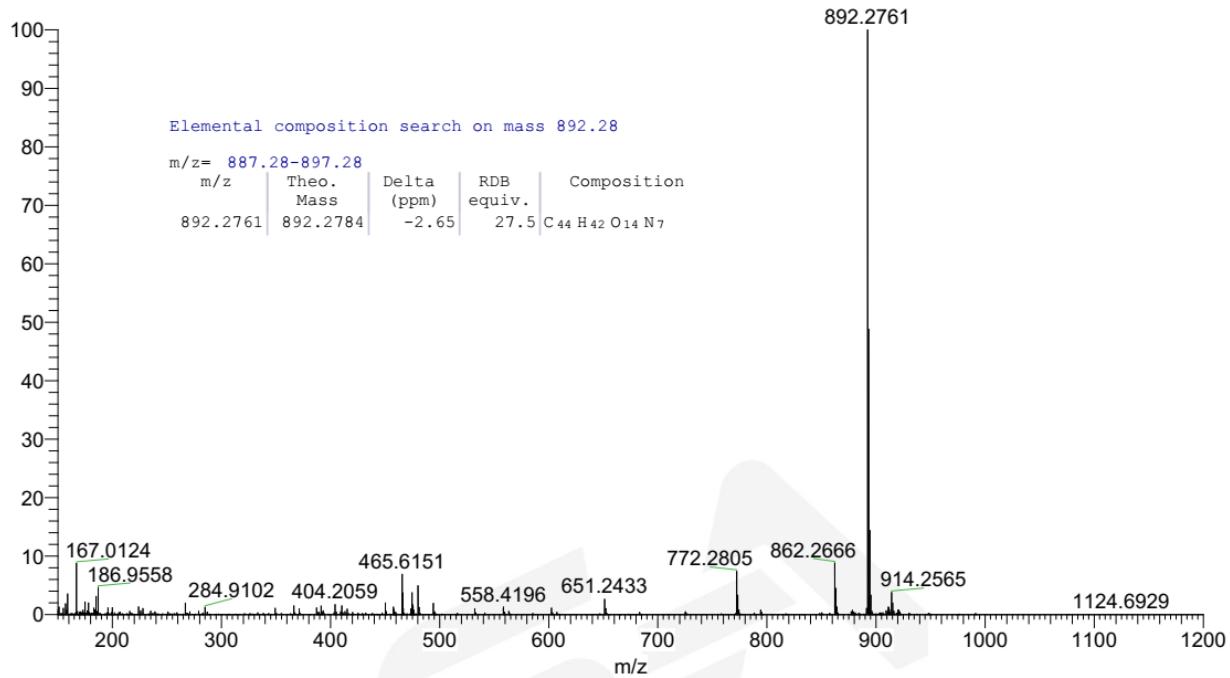
47 **Legends for Figures**

48 Figure S1. Positive HRESIMS spectrum of cystobactamid 891-2 (8).	7
49 Figure S2. ^1H -NMR spectrum of cystobactamid 891-2 (8) measured in DMSO-d ₆ at 500 MHz.	8
50 Figure S3. HMQC spectrum of cystobactamid 891-2 (8) measured in DMSO-d ₆ at 500 MHz.	9
51 Figure S4. HMBC spectrum of cystobactamid 891-2 (8) measured in DMSO-d ₆ at 500 MHz.	10
52 Figure S5. Positive HRESIMS spectrum of cystobactamid 905-2 (9).	11
53 Figure S6. ^1H -NMR spectrum of cystobactamid 905-2 (9) measured in DMSO-d ₆ at 500 MHz.	12
54 Figure S7. HMQC spectrum of cystobactamid 905-2 (9) measured in DMSO-d ₆ at 500 MHz.	13
55 Figure S8. HMBC spectrum of cystobactamid 905-2 (9) measured in DMSO-d ₆ at 500 MHz.	14
56 Figure S9. CD spectra of coralmycins C (1), D (2), and E (3), and cystobactamids 891-2 (8) and 905-2 (9).	15
57 Figure S10. HPLC profiles of coralmycins C (1) and B (13). A) compound 1 ; B) compound 13 ; C) mixture of 1 and 13 at ratio of 1:1.	16
59 Figure S11. Positive HRESIMS spectrum of coralmycin C (1).	17
60 Figure S12. ^1H -NMR spectrum of coralmycin C (1) measured in DMSO-d ₆ at 500 MHz.	18
61 Figure S13. COSY spectrum of coralmycin C (1) measured in DMSO-d ₆ at 500 MHz.	19
62 Figure S14. HMQC spectrum of coralmycin C (1) measured in DMSO-d ₆ at 500 MHz.	20
63 Figure S15. HMBC spectrum of coralmycin C (1) measured in DMSO-d ₆ at 500 MHz.	21
64 Figure S16. HECADE spectrum of coralmycin C (1) measured in DMSO-d ₆ at 700MHz.	23
65 Figure S17. NOESY spectrum of coralmycin C (1) measured in DMSO-d ₆ at 500 MHz.	233
66 Figure S18. The NOE differential spectra of coralmycin C (1) measured in DMSO-d ₆ at 700MHz.	24
67 Figure S19. NOE correlations of coralmycin C (1) in comparison with cystobactamid 919-2 and coralmycin B	25
68 Figure S20. Positive HRESIMS spectrum of coralmycin D (2).	266
69 Figure S21. ^1H -NMR spectrum of coralmycin D (2) measured in DMSO-d ₆ at 500 MHz.	277
70 Figure S22. COSY spectrum of coralmycin D (2) measured in DMSO-d ₆ at 500 MHz.	288
71 Figure S23. HMQC spectrum of coralmycin D (2) measured in DMSO-d ₆ at 500 MHz.	299
72 Figure S24. HMBC spectrum of coralmycin D (2) measured in DMSO-d ₆ at 500 MHz.	30
73 Figure S25. HETLOC spectrum of coralmycin D (2) measured in DMSO-d6 at 900MHz.	31
74 Figure S26. NOESY spectrum of coralmycin D (2) measured in DMSO-d ₆ at 500 MHz.	32
75 Figure S27. The NOE differential spectra of coralmycin D (2) measured in DMSO-d6 at 800MHz.	33
76 Figure S28. Positive HRESIMS spectrum of coralmycin E (3).	34
77 Figure S29. ^1H -NMR spectrum of coralmycin E (3) measured in DMSO-d ₆ at 500 MHz.	35
78 Figure S30. ^{13}C -NMR spectrum of coralmycin E (3) measured in DMSO-d ₆ at 500 MHz.	366

79	Figure S31. COSY spectrum of coralmycin E (3) measured in DMSO-d ₆ at 500 MHz.	37
80	Figure S32. HMQC spectrum of coralmycin E (3) measured in DMSO-d ₆ at 500 MHz.	38
81	Figure S33. HMBC spectrum of coralmycin E (3) measured in DMSO-d ₆ at 500 MHz.	39
82	Figure S34. HECADE spectrum of coralmycin E (3) measured in DMSO-d ₆ at 700 MHz.	40
83	Figure S35. NOESY spectrum of coralmycin E (3) measured in DMSO-d ₆ at 500 MHz.	41
84	Figure S36. The NOE differential spectra of coralmycin E (3) measured in DMSO-d6 at 800MHz.	42
85	Figure S37. Positive and negative ESIMS spectrum of cystobactamid 507 (10).	43
86	Figure S38. ¹ H NMR spectrum of cystobactamid 507 (10) measured in CD3OD at 700 MHz.	44
87	Figure S39. ¹³ C-NMR spectrum of cystobactamid 507 (10) measured in CD3OD at 700 MHz.	45
88	Figure S40. HMBC spectrum of cystobactamid 507 (10) measured in CD3OD at 900 MHz.	46
89	Figure S41. Positive HRESIMS spectrum of coralmycin F (4).	47
90	Figure S42. ¹ H NMR spectrum of coralmycin F (4) measured in CD ₃ OD at 800 MHz.	48
91	Figure S43. HMQC spectrum of coralmycin F (4) measured in CD ₃ OD at 500 MHz.	49
92	Figure S44. HMBC spectrum of coralmycin F (4) measured in CD ₃ OD at 800 MHz.	50
93	Figure S45. Positive HRESIMS spectrum of coralmycin G (5).	51
94	Figure S46. ¹ H NMR spectrum of coralmycin G (5) measured in DMSO-d ₆ at 800 MHz.	52
95	Figure S47. HMQC spectrum of coralmycin G (5) measured in DMSO-d ₆ at 800 MHz.	53
96	Figure S48. HMBC spectrum of coralmycin G (5) measured in DMSO-d ₆ at 800 MHz.	54
97	Figure S49. Positive HRESIMS spectrum of coralmycin H (6).	55
98	Figure S50. ¹ H NMR spectrum of coralmycin H (6) measured in DMSO-d ₆ at 800 MHz.	56
99	Figure S51. HMQC spectrum of coralmycin H (6) measured in DMSO-d ₆ at 800 MHz.	57
100	Figure S52. HMBC spectrum of coralmycin H (6) measured in DMSO-d ₆ at 800 MHz.	58
101	Figure S53. Positive HRESIMS spectrum of coralmycin I (7).	59
102	Figure S54. ¹ H NMR spectrum of coralmycin I (7) measured in DMSO-d ₆ at 500 MHz.	60
103	Figure S55. HMQC spectrum of coralmycin I (7) measured in DMSO-d ₆ at 500 MHz.	61
104	Figure S56. Expansion of HMQC spectrum of coralmycin I (7).	62
105	Figure S57. HMBC spectrum of coralmycin I (7) measured in DMSO-d ₆ at 500 MHz.	63
106	Figure S58. Expansion of HMBC spectrum of coralmycin I (7).	64
107	Figure S59. Agarose gels of <i>E. coli</i> gyrase supercoiling reactions inhibited by coralmycins (C (1) , D (2) , E (3) , F (4) , A (12) , and B (13)) and cystobactamids (891-2 (8) , 905-2 (9) , 919-2 (11) , and 507 (10)). (-), reaction without <i>E. coli</i> gyrase; (+), standard reaction in presence of 5% DMSO; rel, relaxed plasmid; SC, supercoiled plasmid.	65
110	Figure S60. HPLC profile of coralmycins (C (1) , D (2) , E (3) , A (12) , and B (13)) and cystobactamids (891-2 (8) , 905-2 (9) , and 919-2 (11)).	66

112 **Figure S61.** HPLC profile of coralmycins (**4**), **5**, **6**, and **7**) and cystobactamid **507** (**10**). 67
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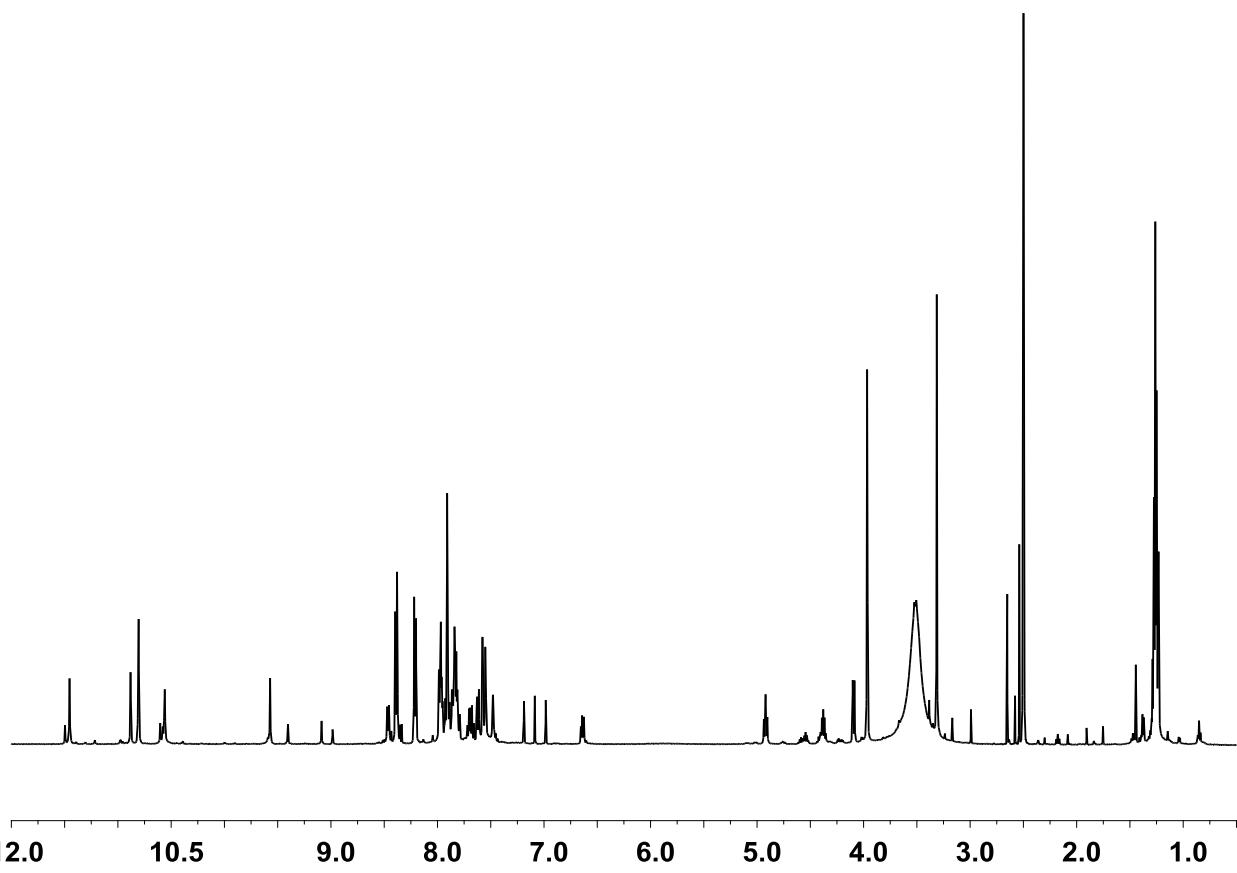
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116 **Figure S1.** Positive HRESIMS spectrum of cystobactamid 891-2 (**8**).

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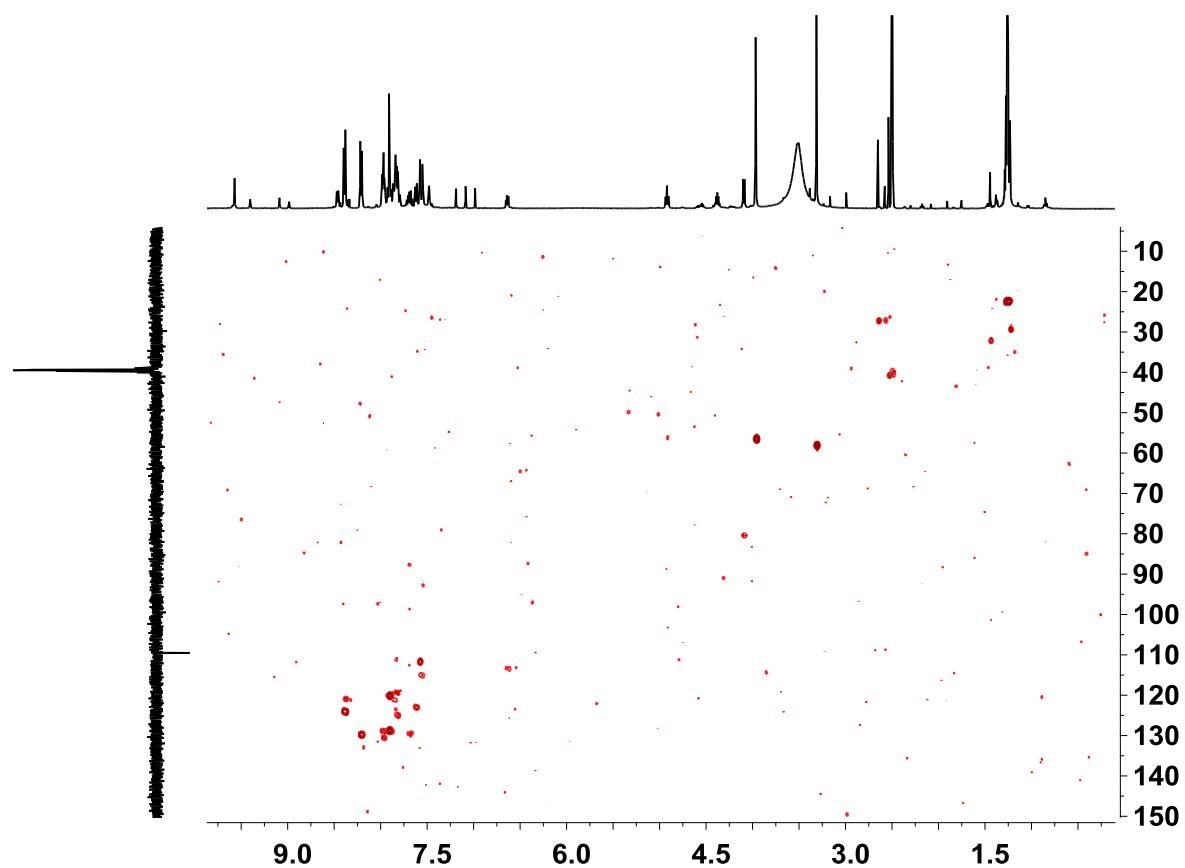


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119 **Figure S2.** ¹H-NMR spectrum of cystobactamid 891-2 (**8**) measured in DMSO-*d*₆ at 500 MHz.

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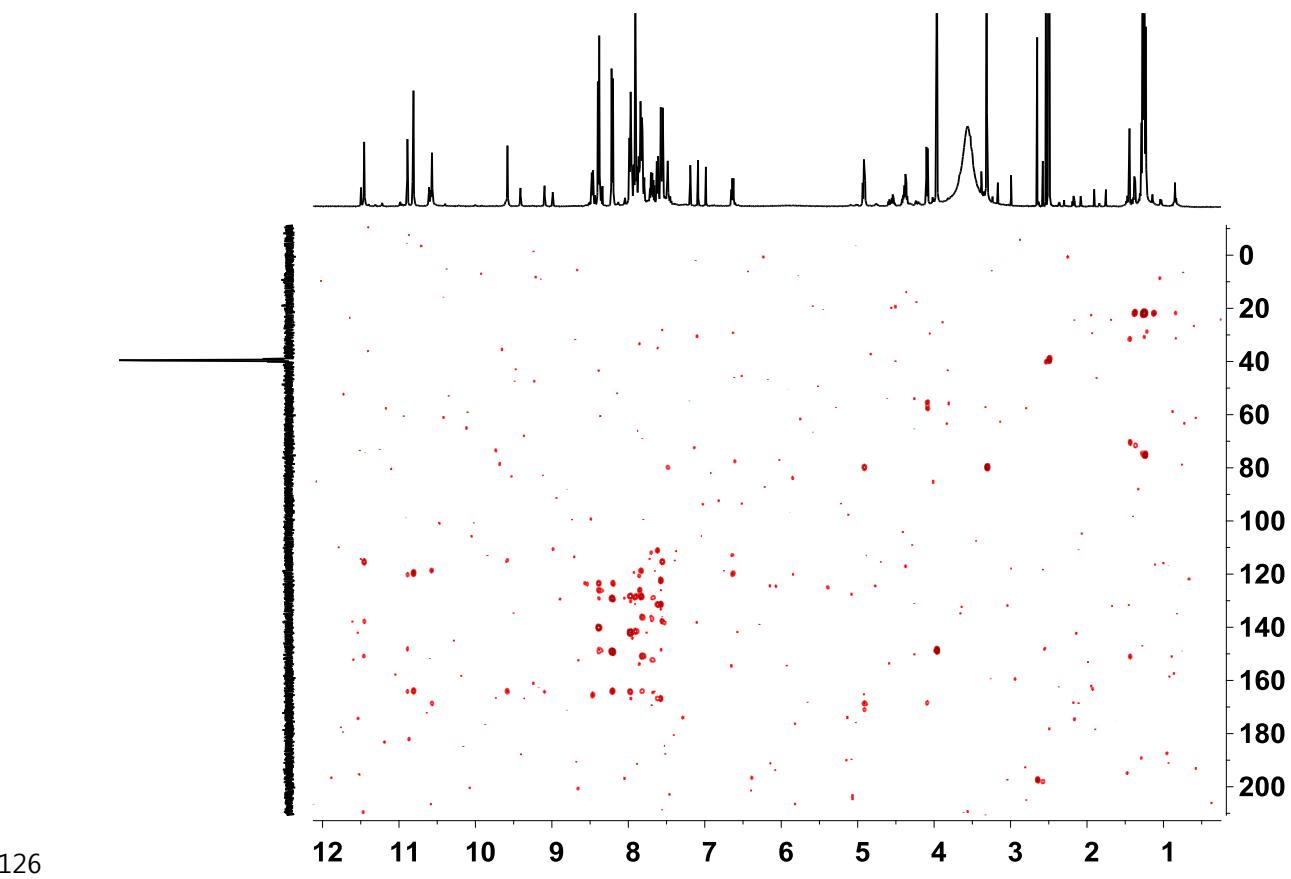


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123 **Figure S3.** HMQC spectrum of cystobactamid 891-2 (**8**) measured in $\text{DMSO}-d_6$ at 500 MHz.

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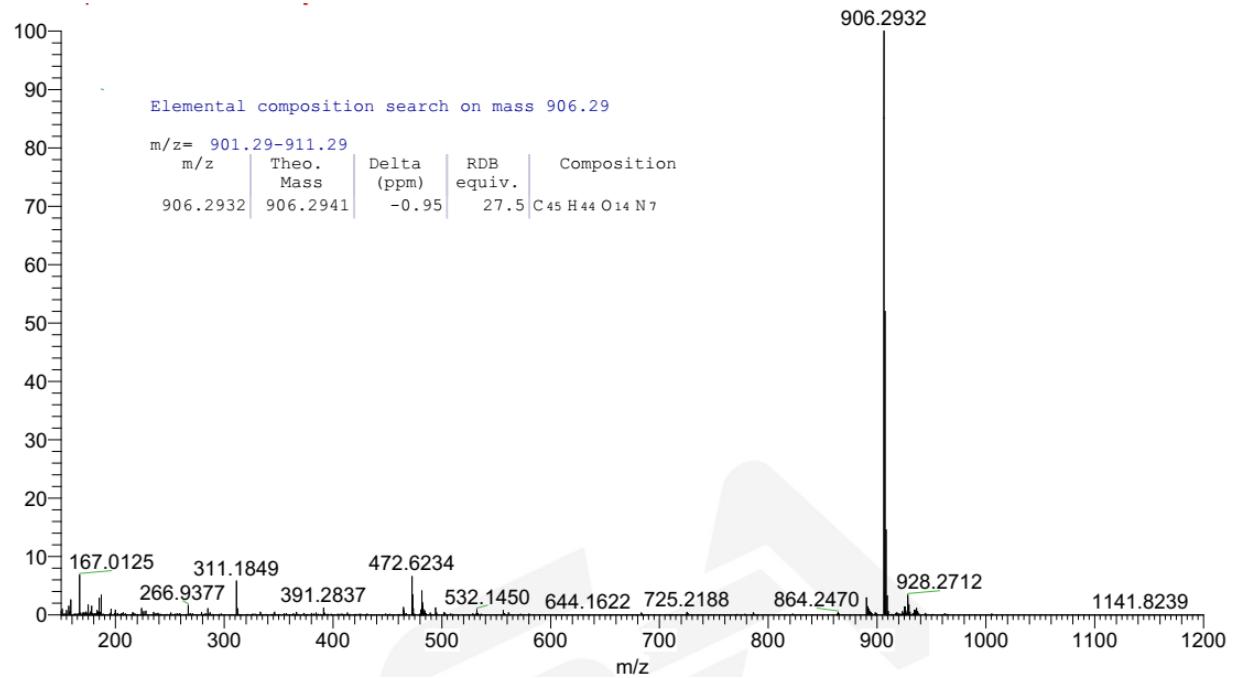
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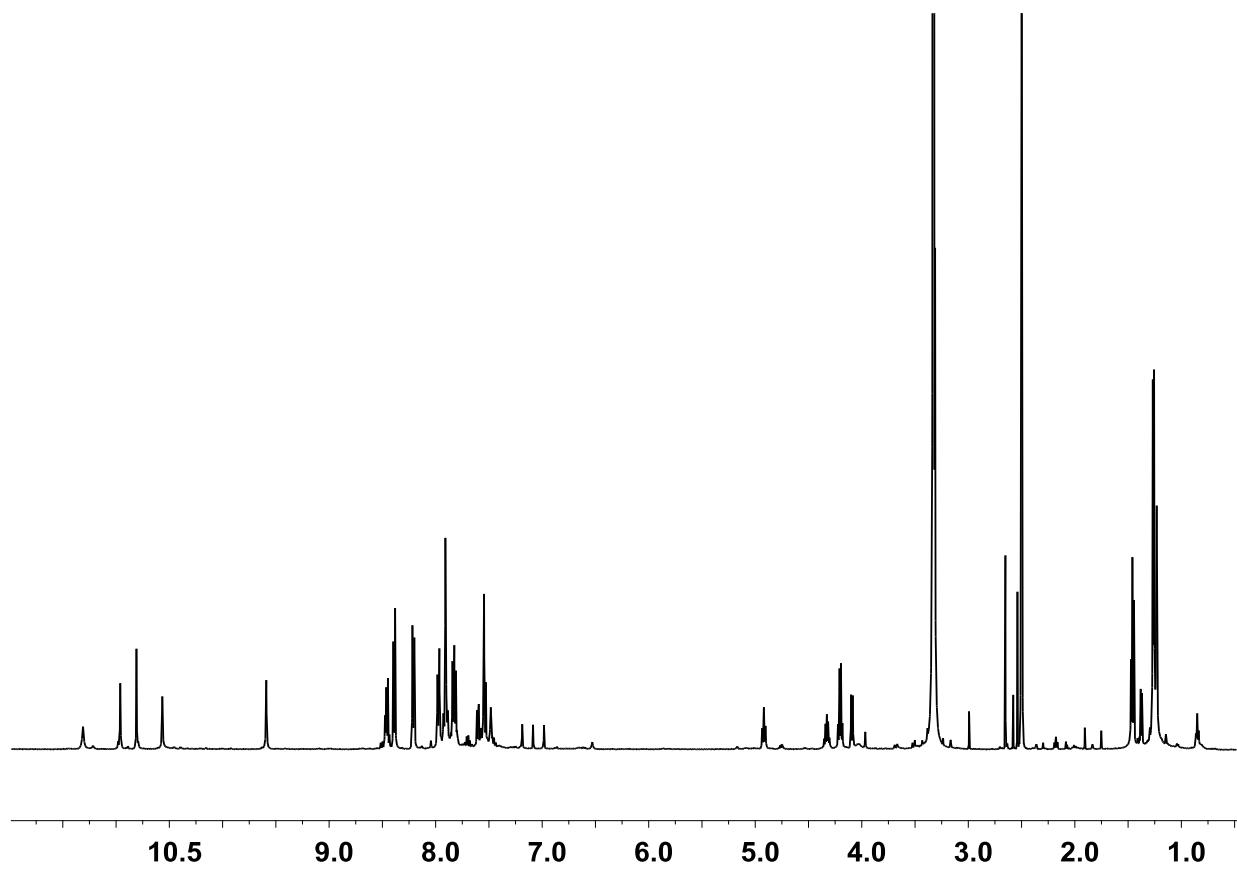


127 **Figure S4.** HMBC spectrum of cystobactamid 891-2 (**8**) measured in $\text{DMSO}-d_6$ at 500 MHz.

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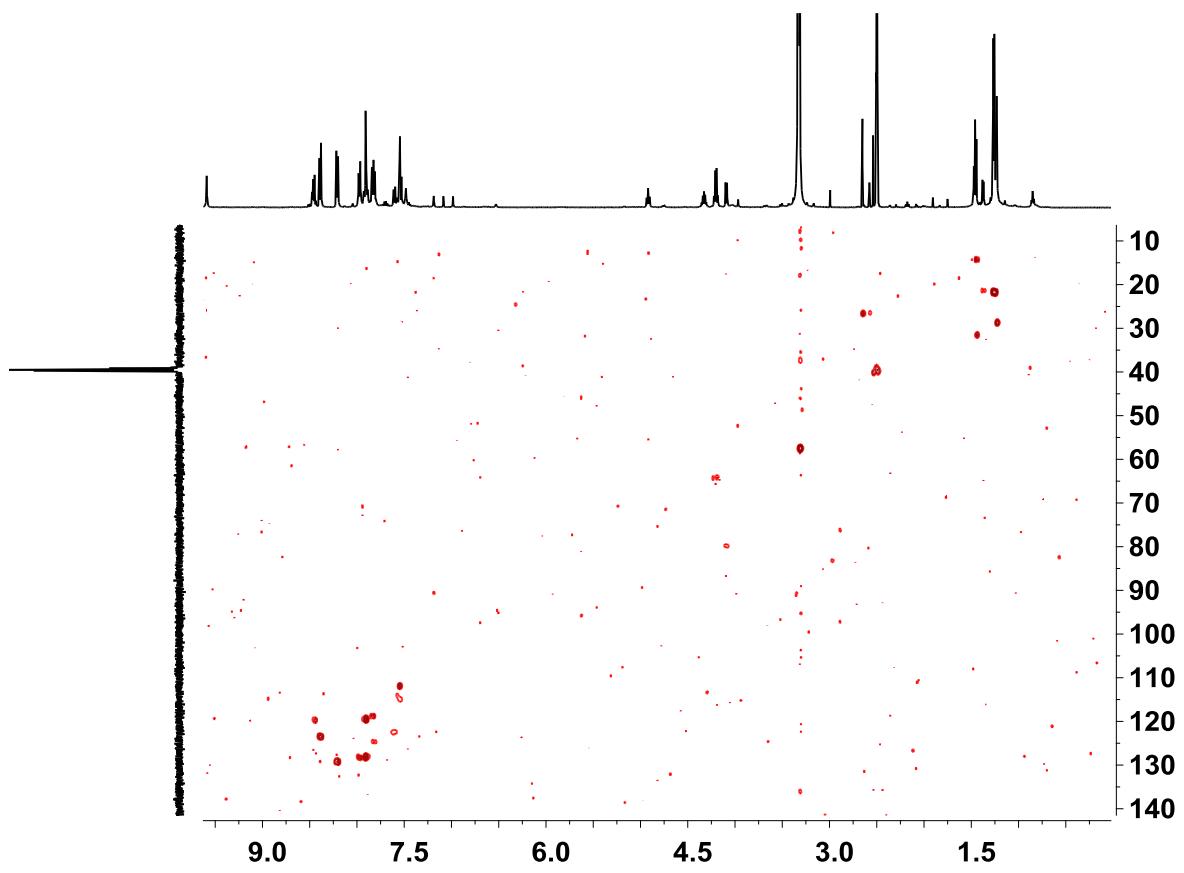


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134 **Figure S6.** ¹H-NMR spectrum of cystobactamid 905-2 (**9**) measured in DMSO-*d*₆ at 500 MHz.

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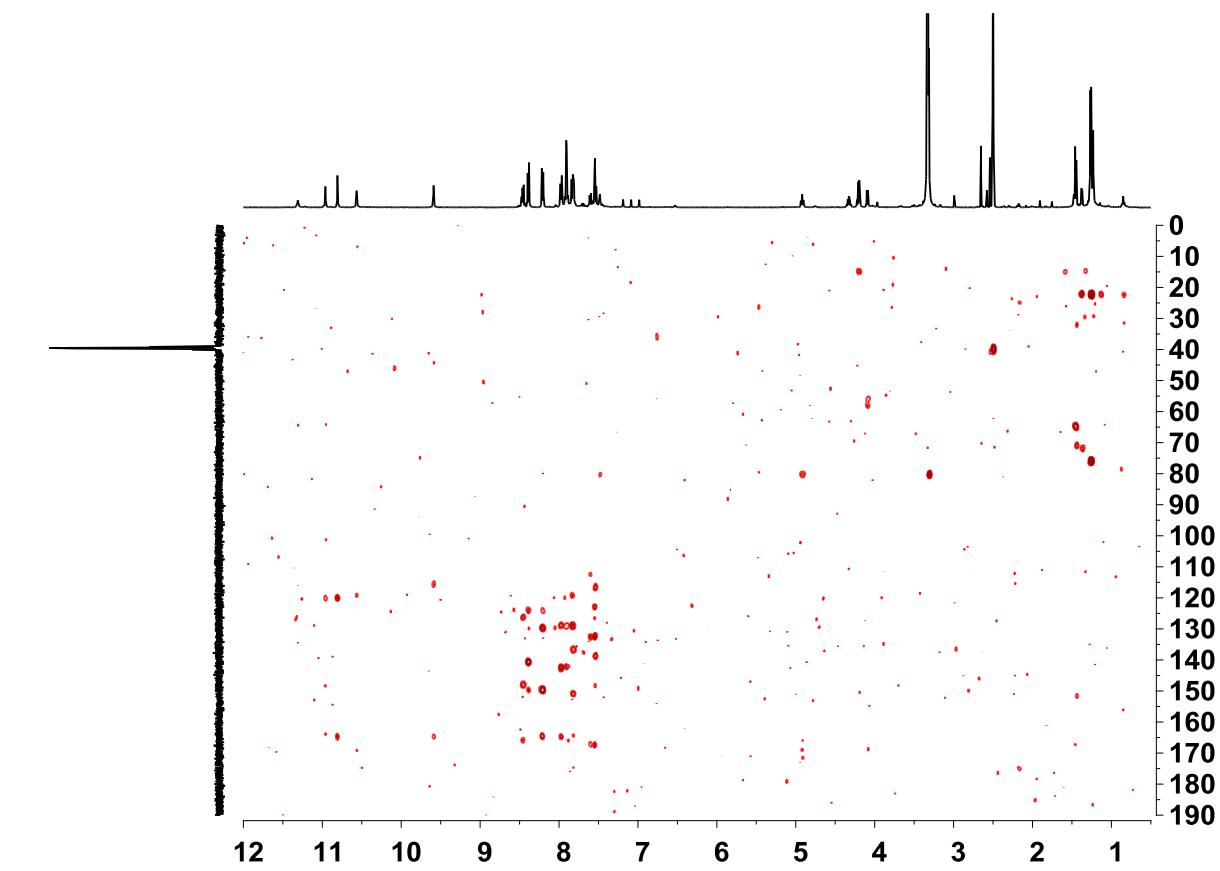


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138 **Figure S7.** HMQC spectrum of cystobactamid 905-2 (**9**) measured in DMSO-*d*₆ at 500 MHz.

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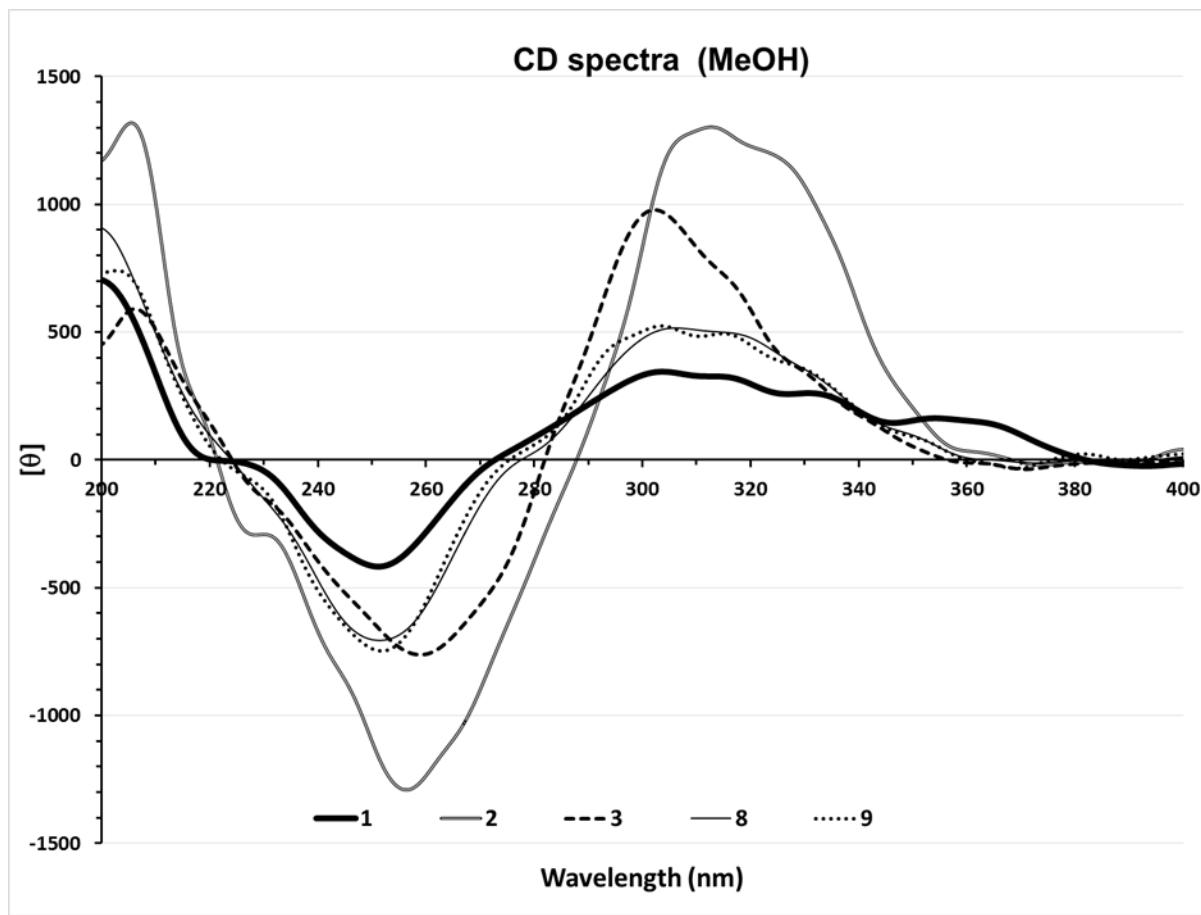


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142 **Figure S8.** HMBC spectrum of cystobactamid 905-2 (**9**) measured in $\text{DMSO}-d_6$ at 500 MHz.

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Figure S9. CD spectra of coralmycins C (**1**), D (**2**), and E (**3**), and cystobactamids 891-2 (**8**) and
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905-2 (**9**).

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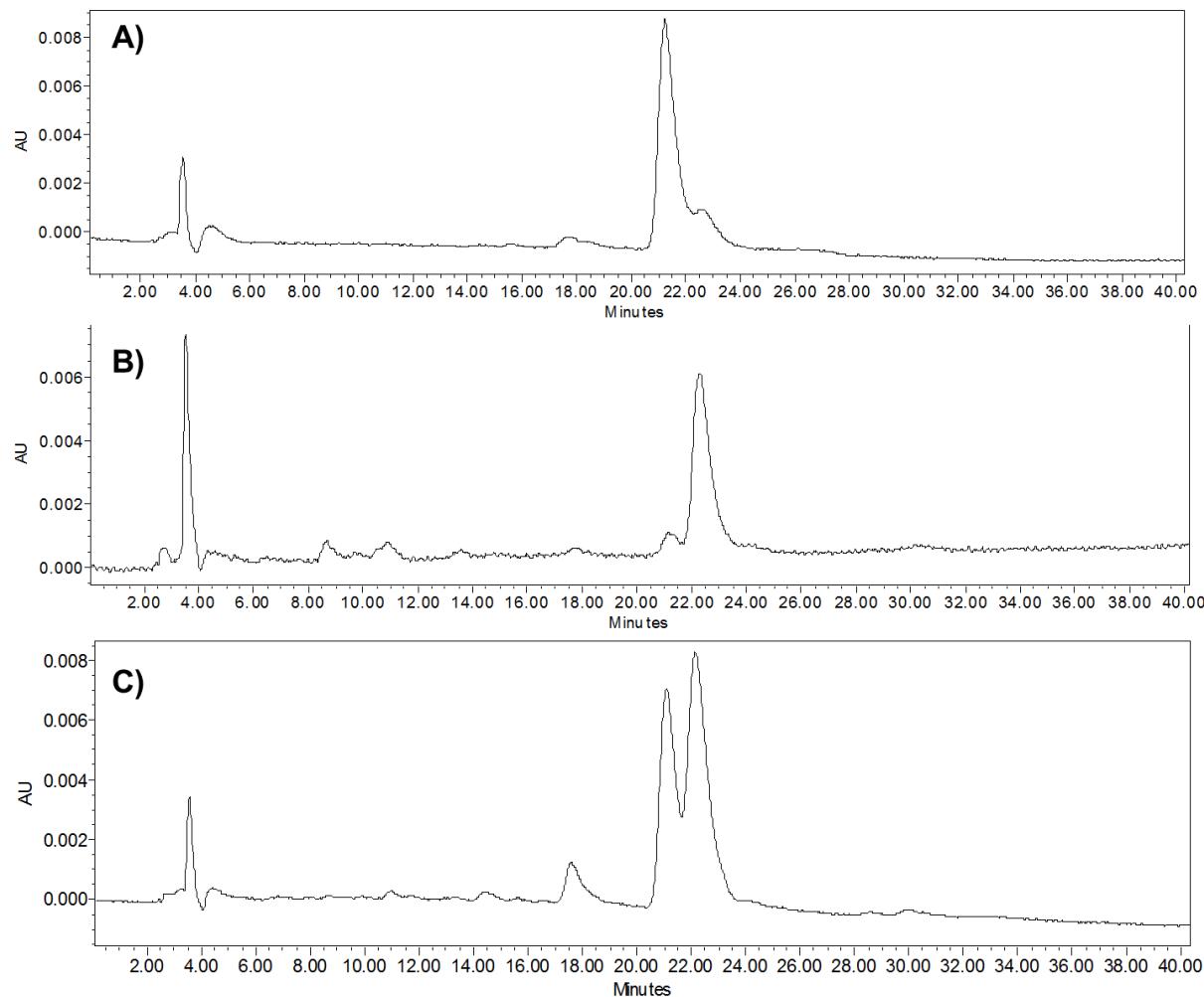
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159 **Figure S10.** HPLC profiles of coralmycins C (**1**) and B (**13**). HPLC condition: column YMC C18,
160 S-4 μ m, 4.6 x 250 mm; eluent: isocratic 50% CH₃CN in H₂O (v/v), containing 0.01% TFA at flow
161 rate of 0.8 mL/min with PDA detector Waters 996. A) compound **1**; B) compound **13**; C) mixture
162 of **1** and **13** at ratio of 1:1.

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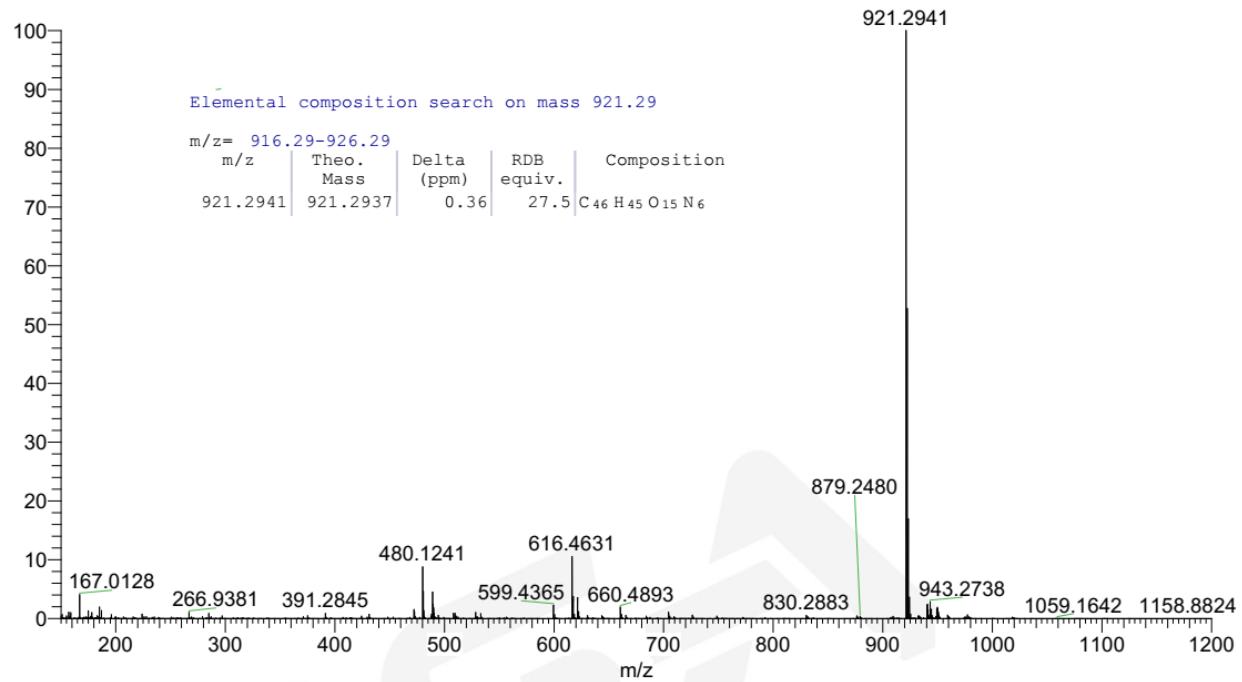
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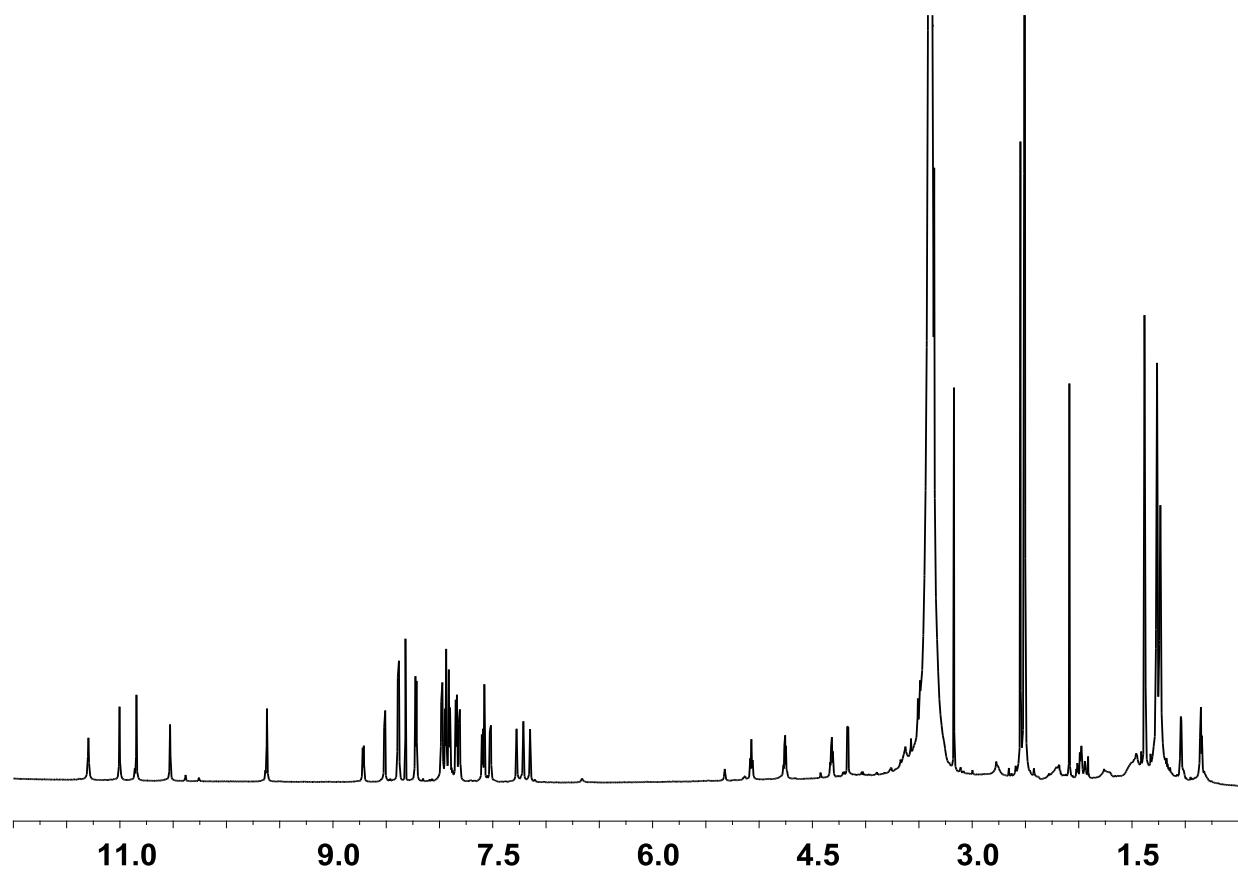
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171 **Figure S11.** Positive HRESIMS spectrum of coralmycin C (**1**).

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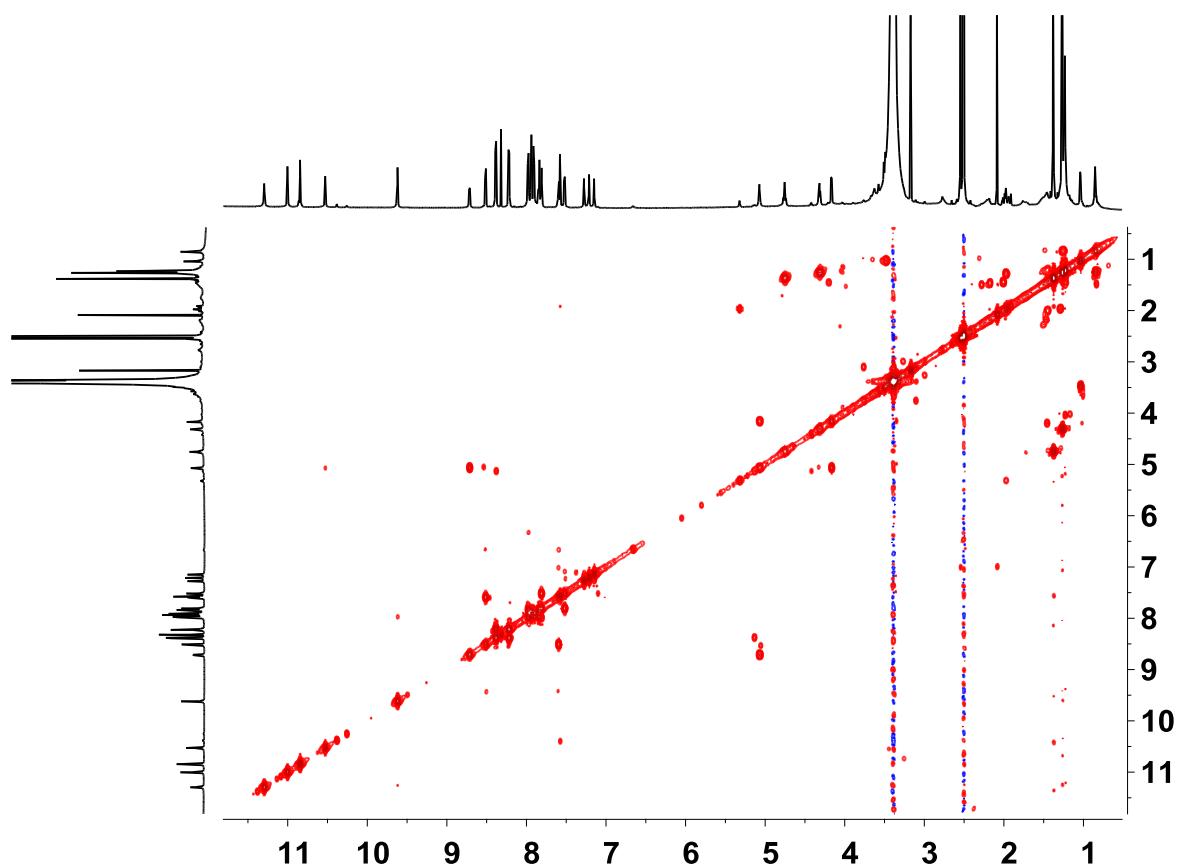


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174 **Figure S12.** ¹H-NMR spectrum of coralmycin C (**1**) measured in DMSO-d₆ at 500 MHz.

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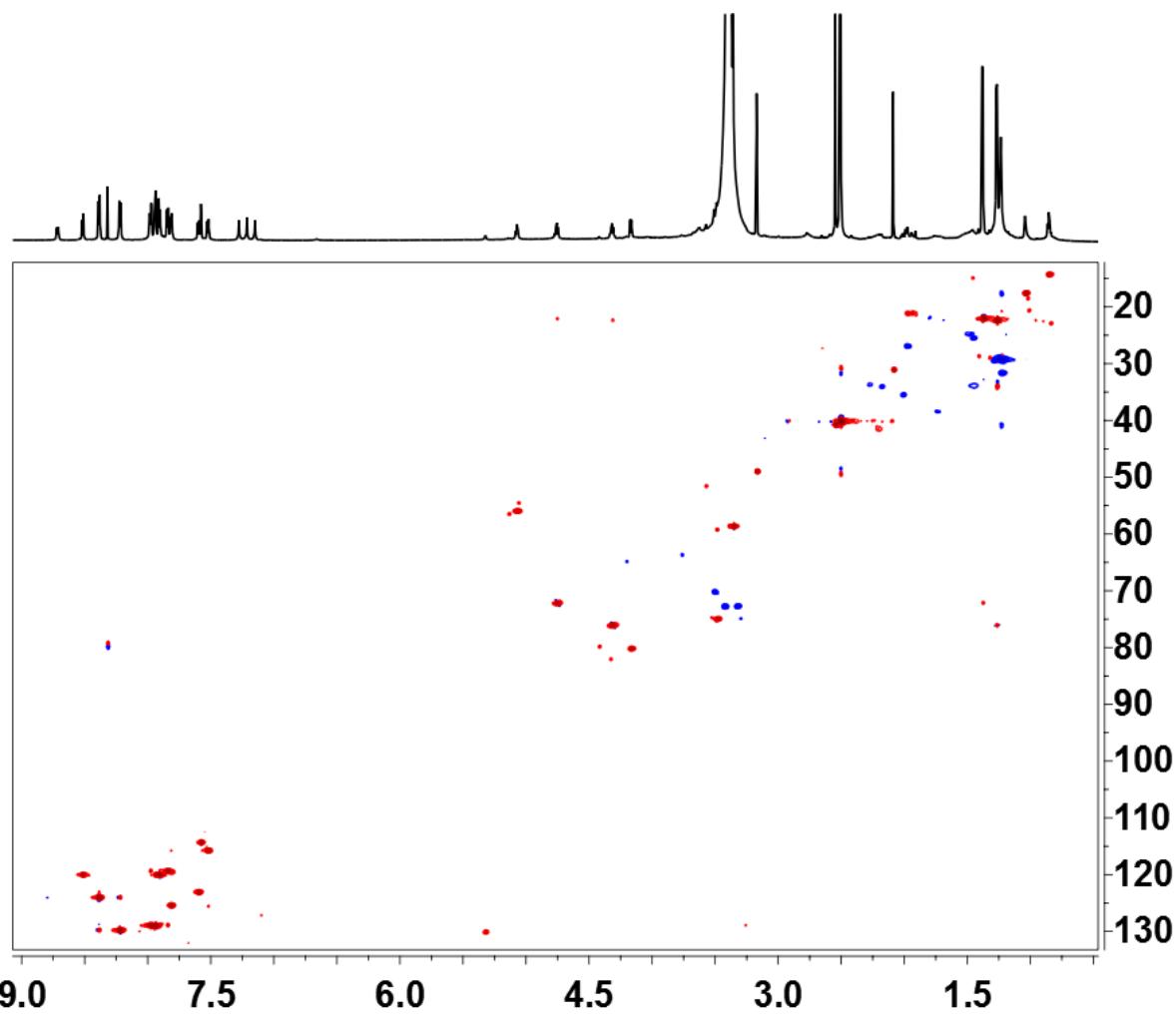


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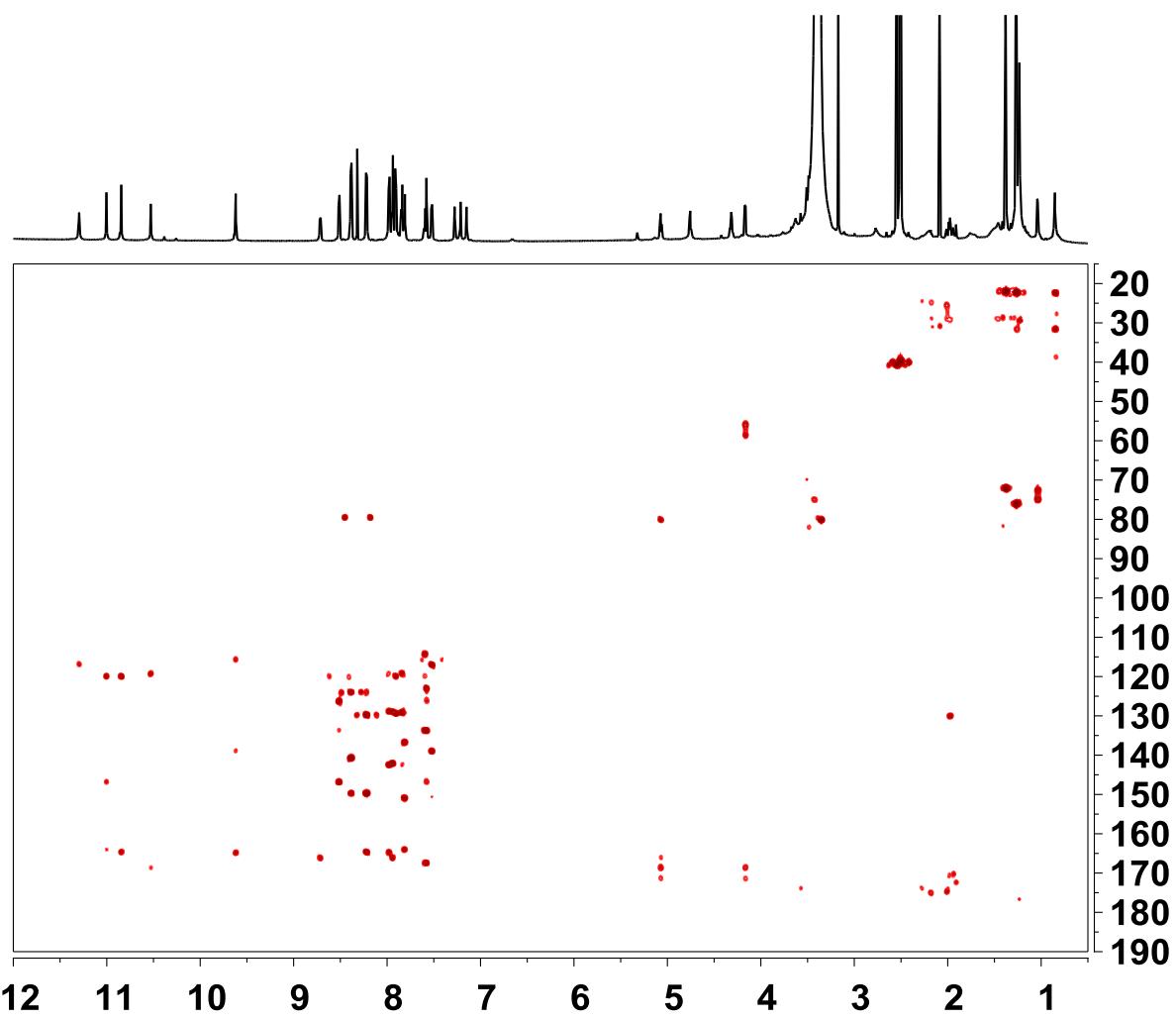
178 **Figure S13.** COSY spectrum of coralmycin C (**1**) measured in DMSO-d_6 at 500 MHz.

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181 **Figure S14.** HMQC spectrum of coralmycin C (**1**) measured in DMSO-d_6 at 500 MHz.
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185 **Figure S15.** HMBC spectrum of coralmycin C (**1**) measured in DMSO-d₆ at 500 MHz.

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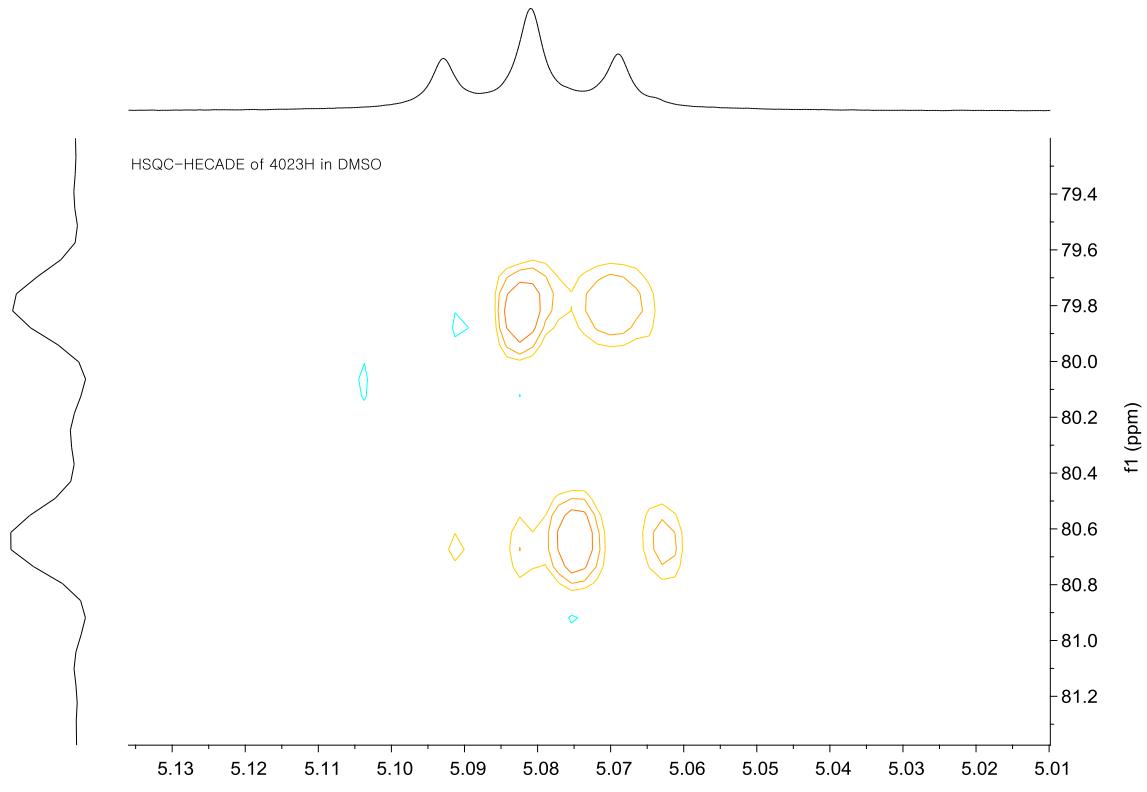
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196 **Figure S16.** HECADE spectrum of coralmycin C (**1**) measured in DMSO-*d*₆ at 700 MHz.

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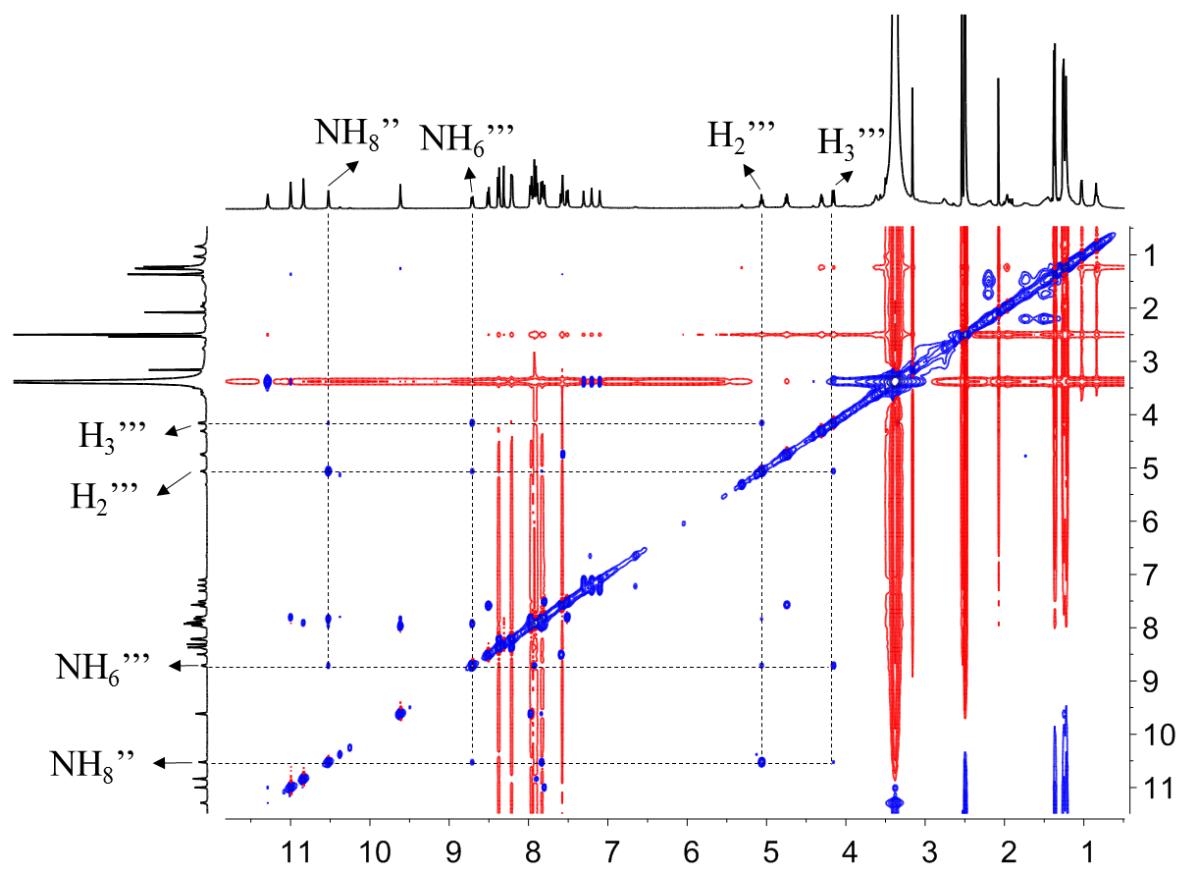
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206 **Figure S17.** NOESY spectrum of coralmycin C (**1**) measured in DMSO-d_6 at 500 MHz.

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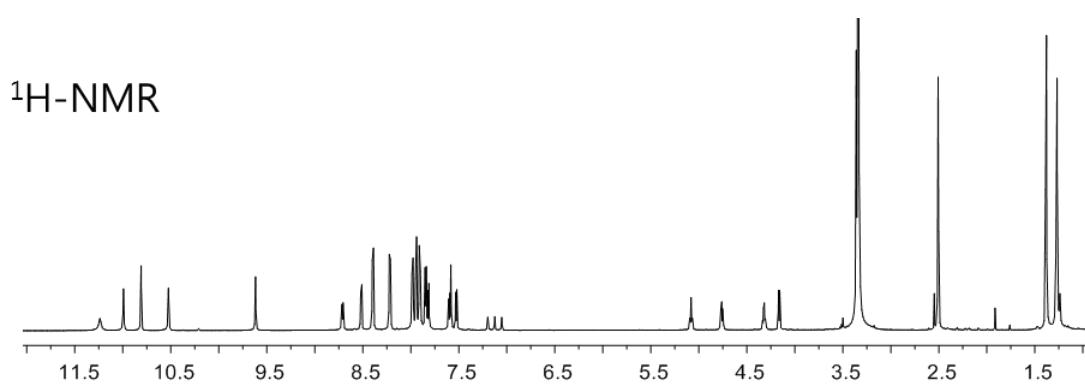
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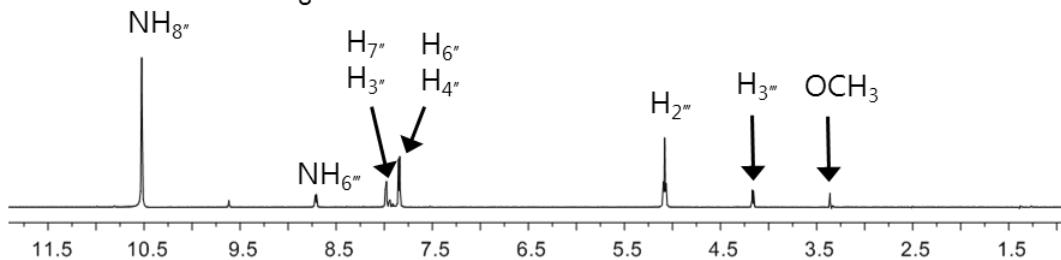
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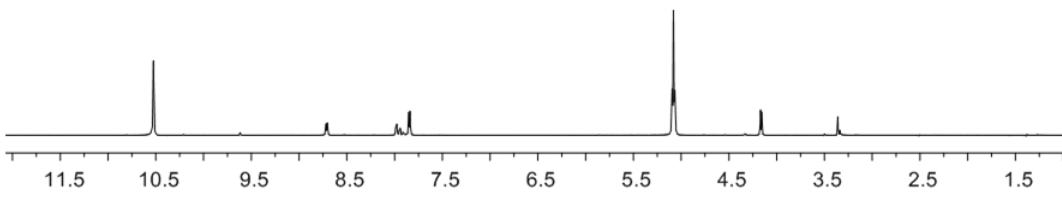
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Irradiation of NH_{8''}



Irradiation of H_{2''}



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221 **Figure S18.** The NOE differential spectra of coralmycin C (**1**) measured in DMSO-*d*₆ at 700 MHz.

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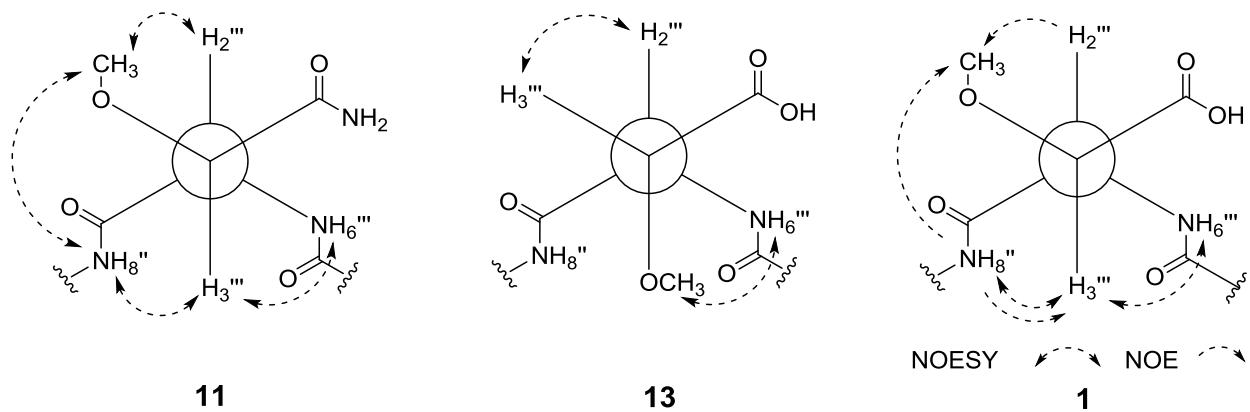
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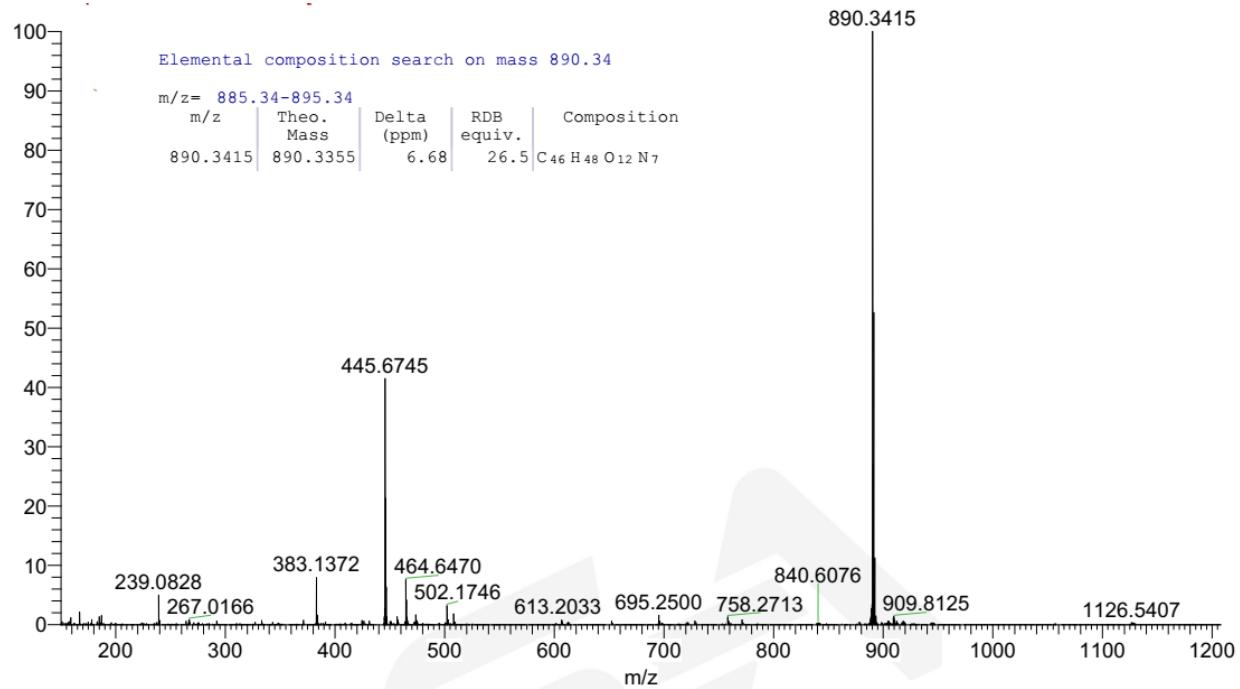


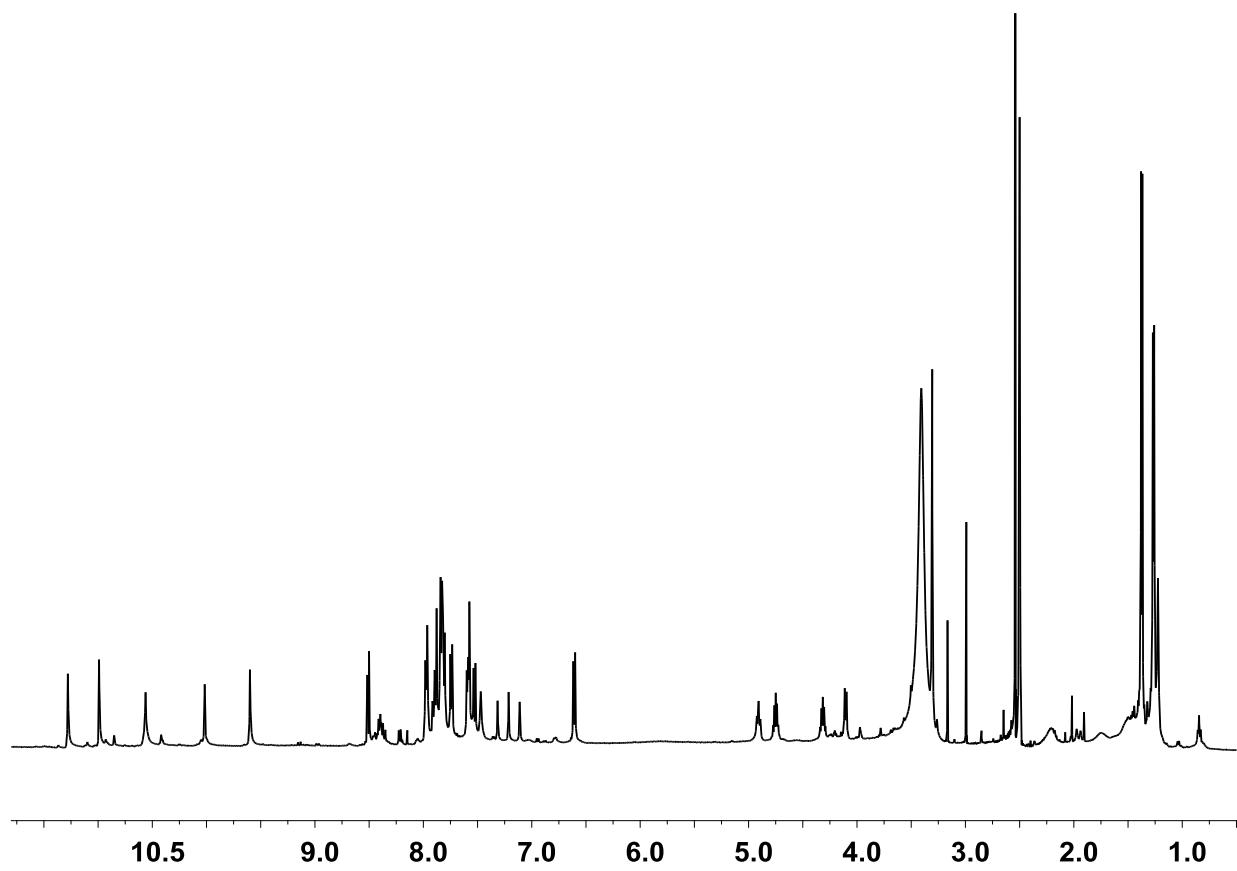
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230 **Figure S19.** NOE correlations of coralmycin C (**1**) in comparison with cystobactamid 919-2 (**11**)
231 and coralmycin B (**13**).

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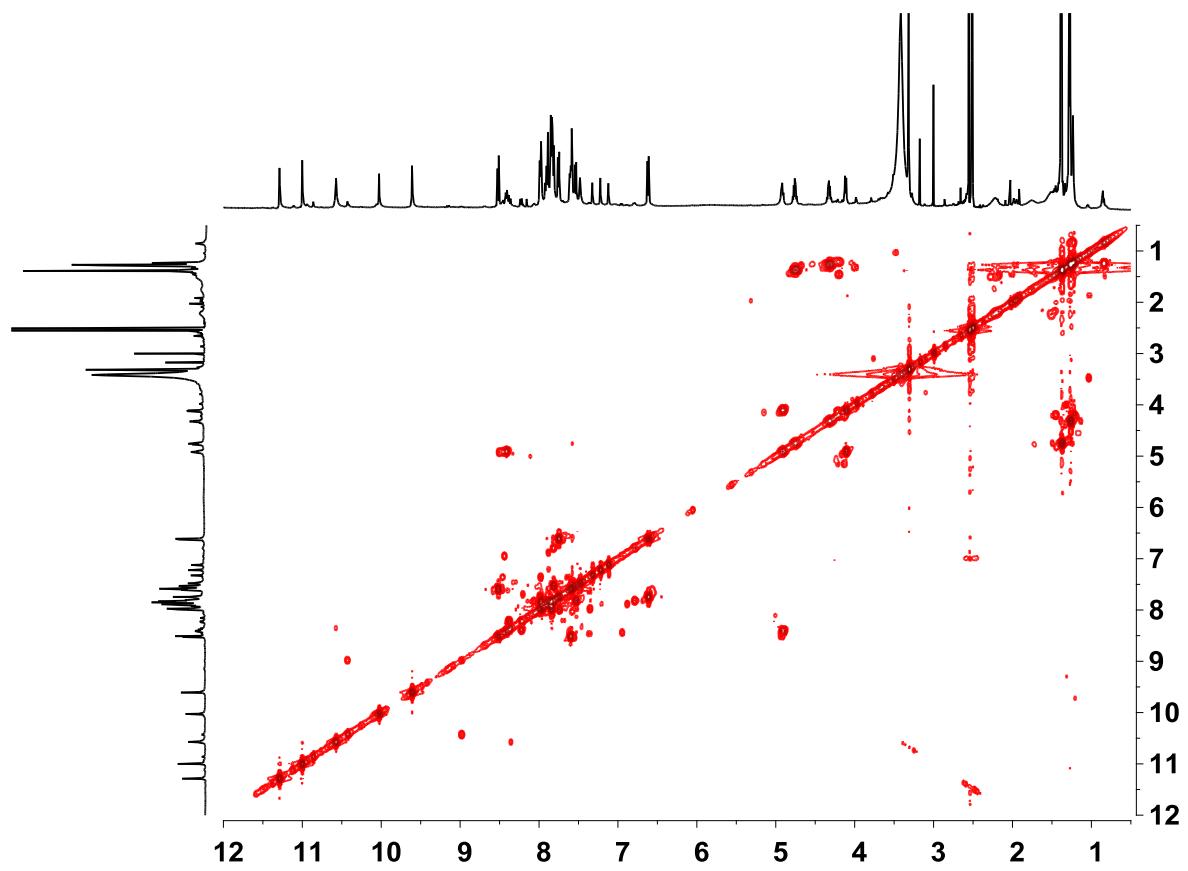


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238 **Figure S21.** ¹H-NMR spectrum of coralmycin D (**2**) measured in DMSO-*d*₆ at 500 MHz.

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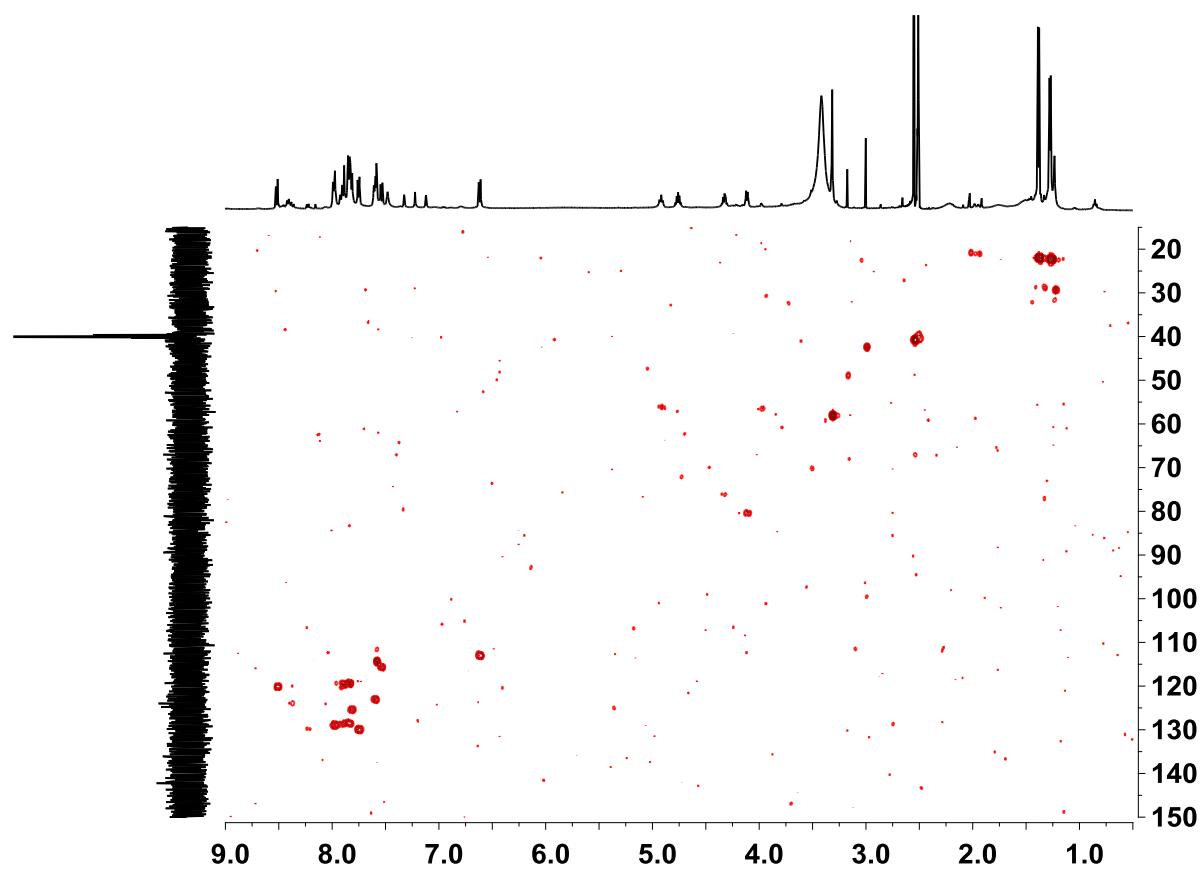


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242 **Figure S22.** COSY spectrum of coralmycin D (2) measured in $\text{DMSO}-d_6$ at 500 MHz.

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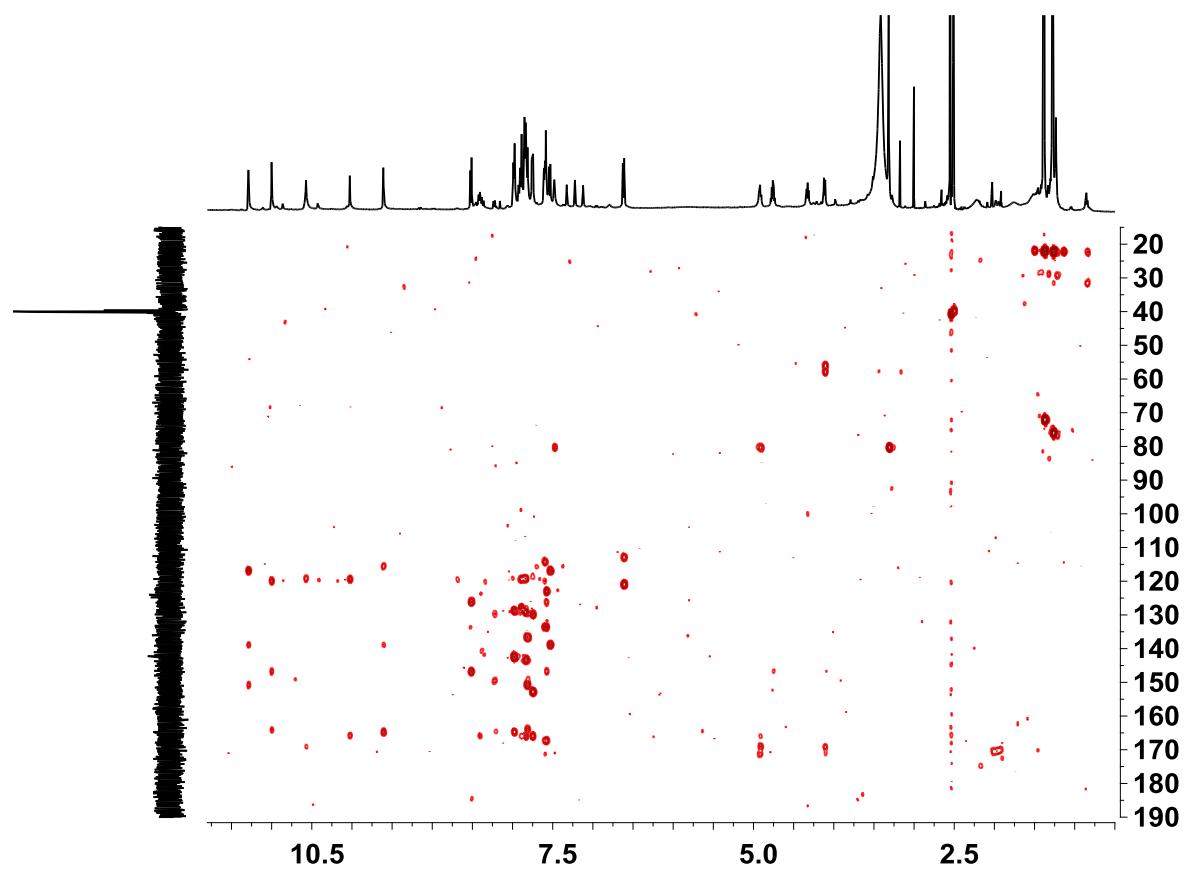


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246 **Figure S23.** HMQC spectrum of coralmycin D (**2**) measured in $\text{DMSO}-d_6$ at 500 MHz.

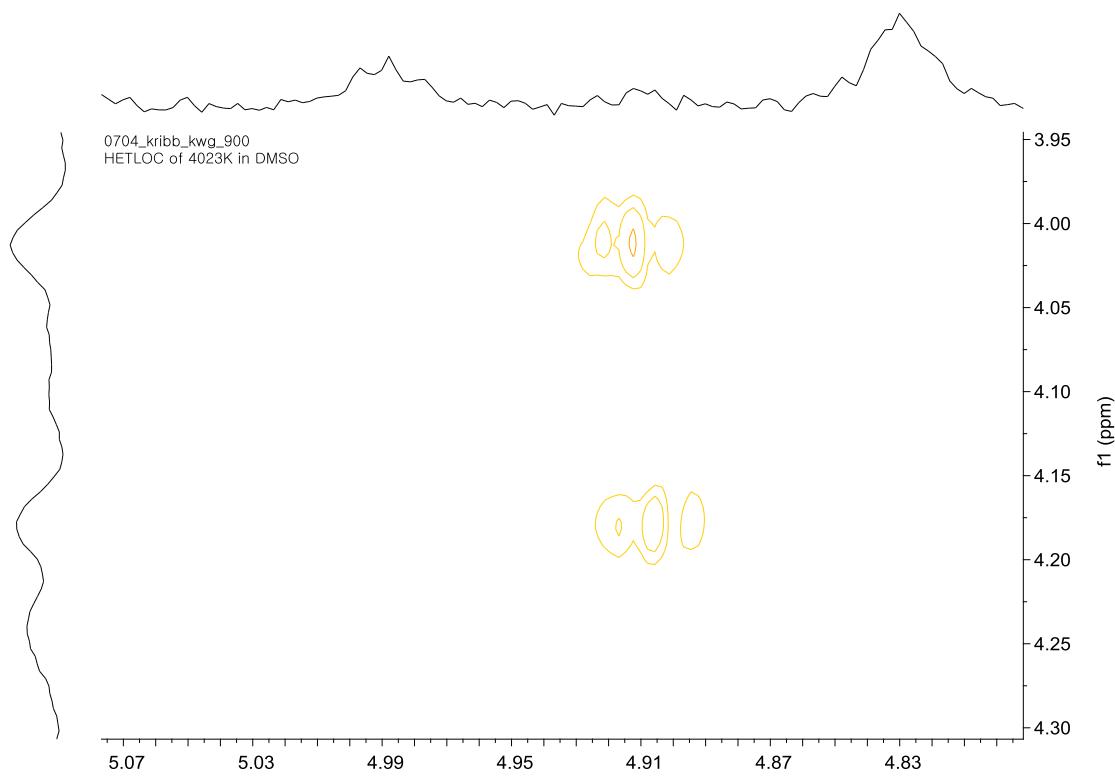
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250 **Figure S24.** HMBC spectrum of coralmycin D (**2**) measured in $\text{DMSO}-d_6$ at 500 MHz.



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253 **Figure S25.** HETLOC spectrum of coralmycin D (**2**) measured in DMSO-*d*₆ at 900 MHz.

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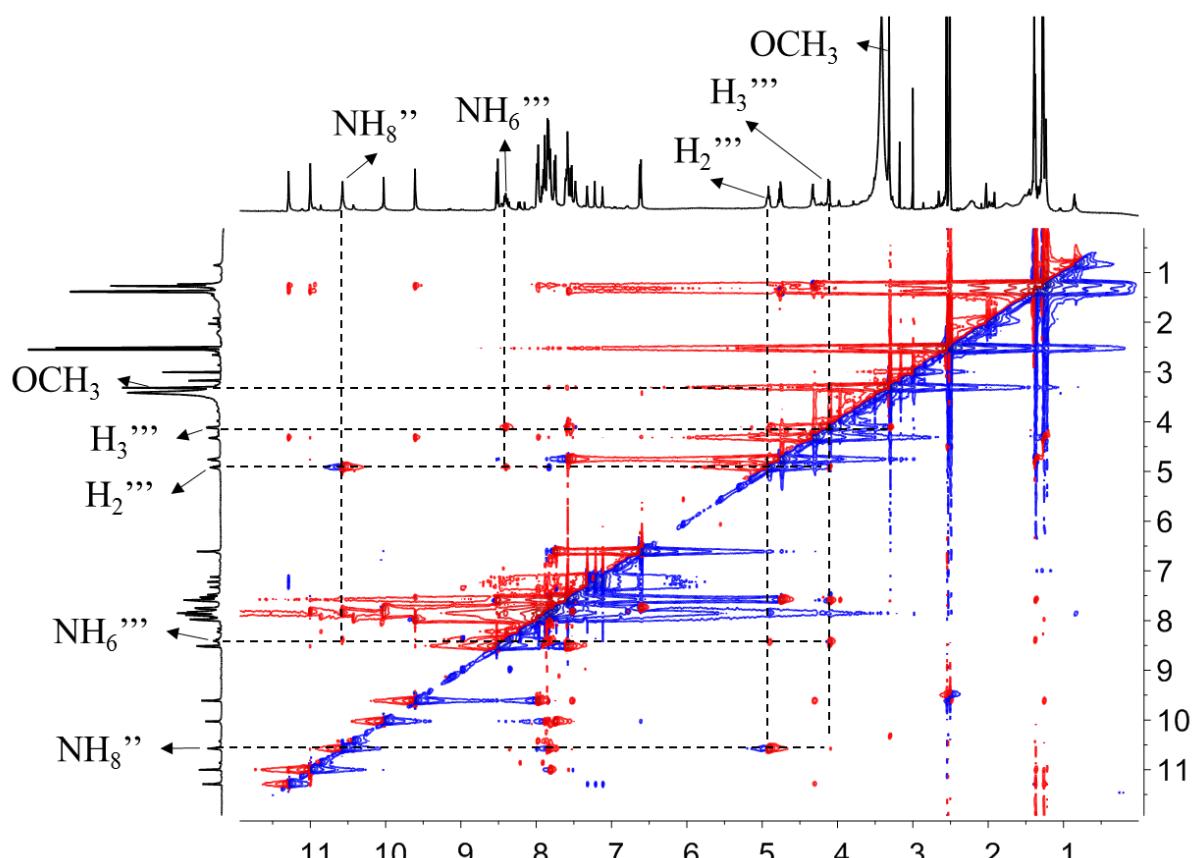
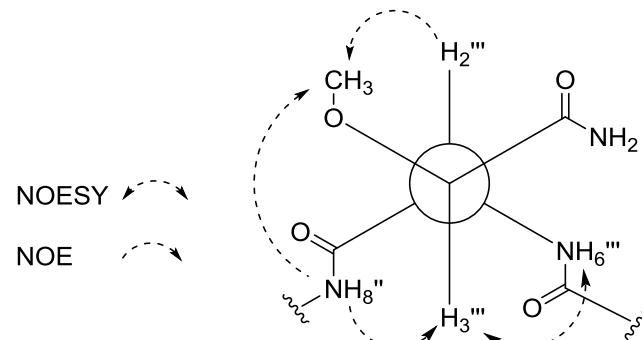
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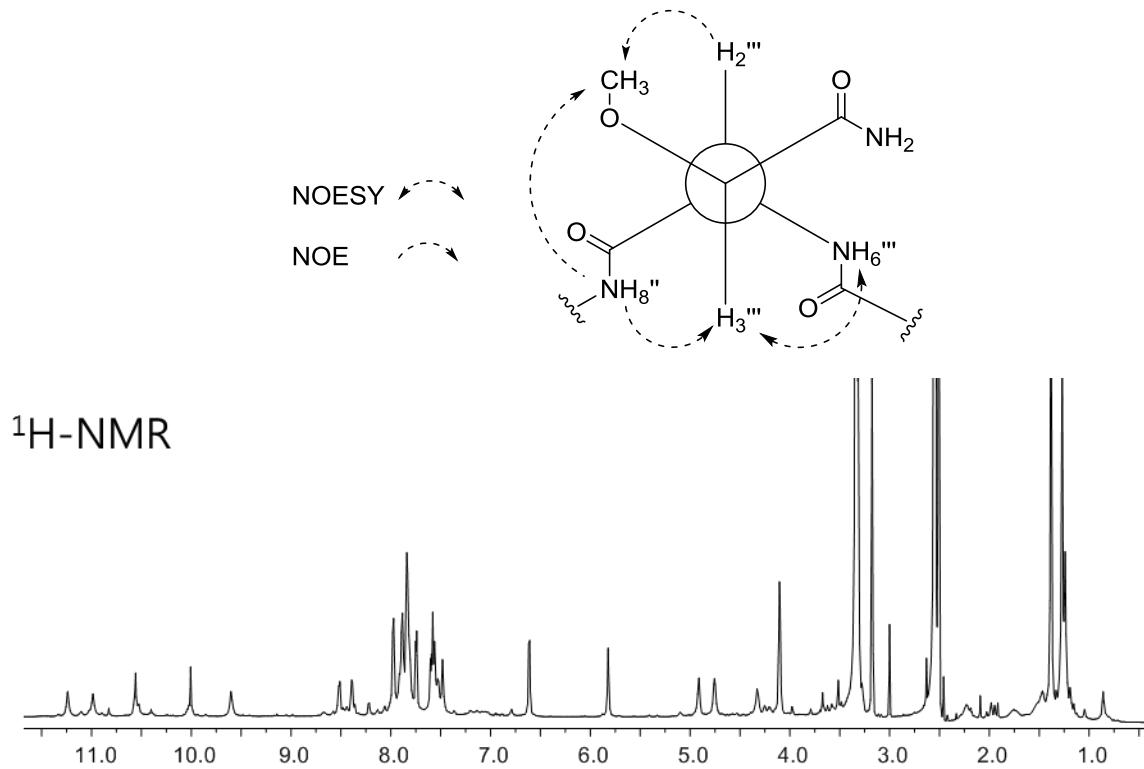
262 **Figure S26.** NOESY spectrum of coralmycin D (**2**) measured in $\text{DMSO}-d_6$ at 500 MHz.

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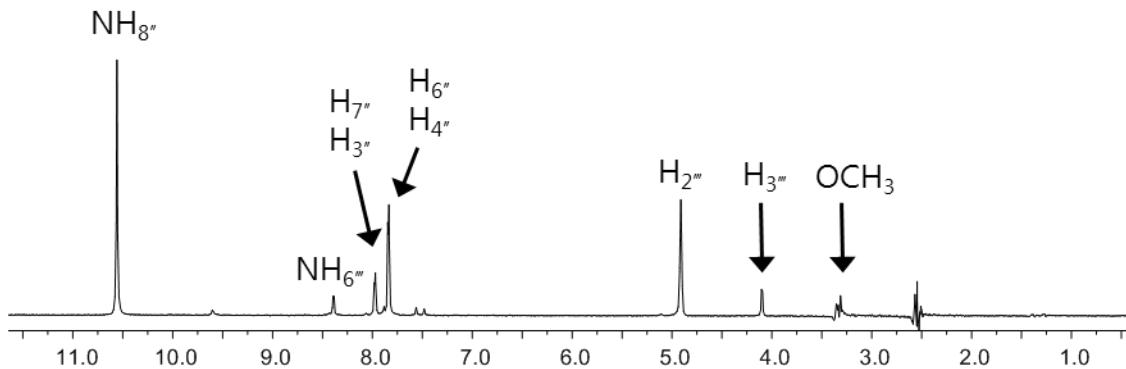
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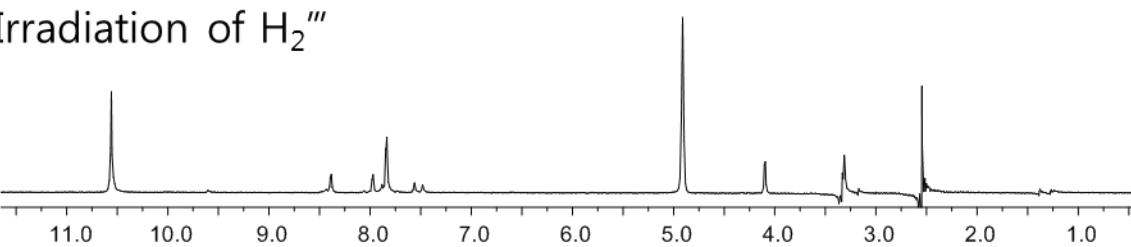
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Irradiation of NH₈''



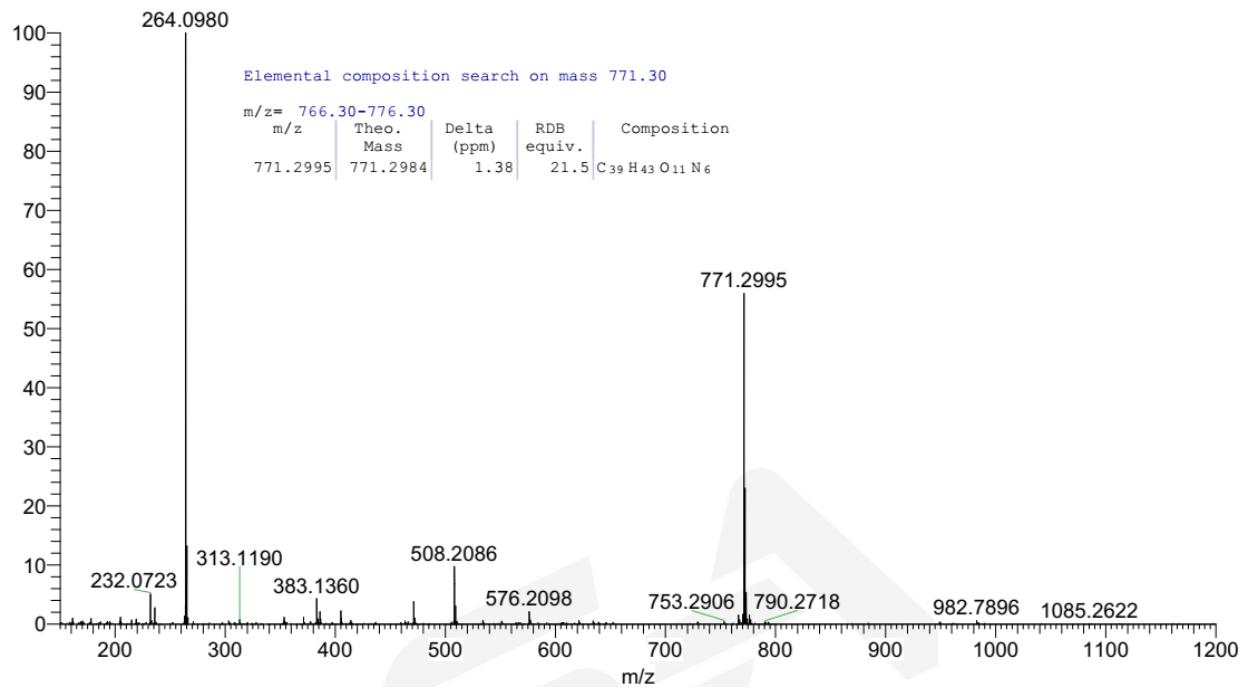
Irradiation of H₂'''



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268 **Figure S27.** The NOE differential spectra of coralmycin D (**2**) measured in DMSO-d₆ at 800 MHz.

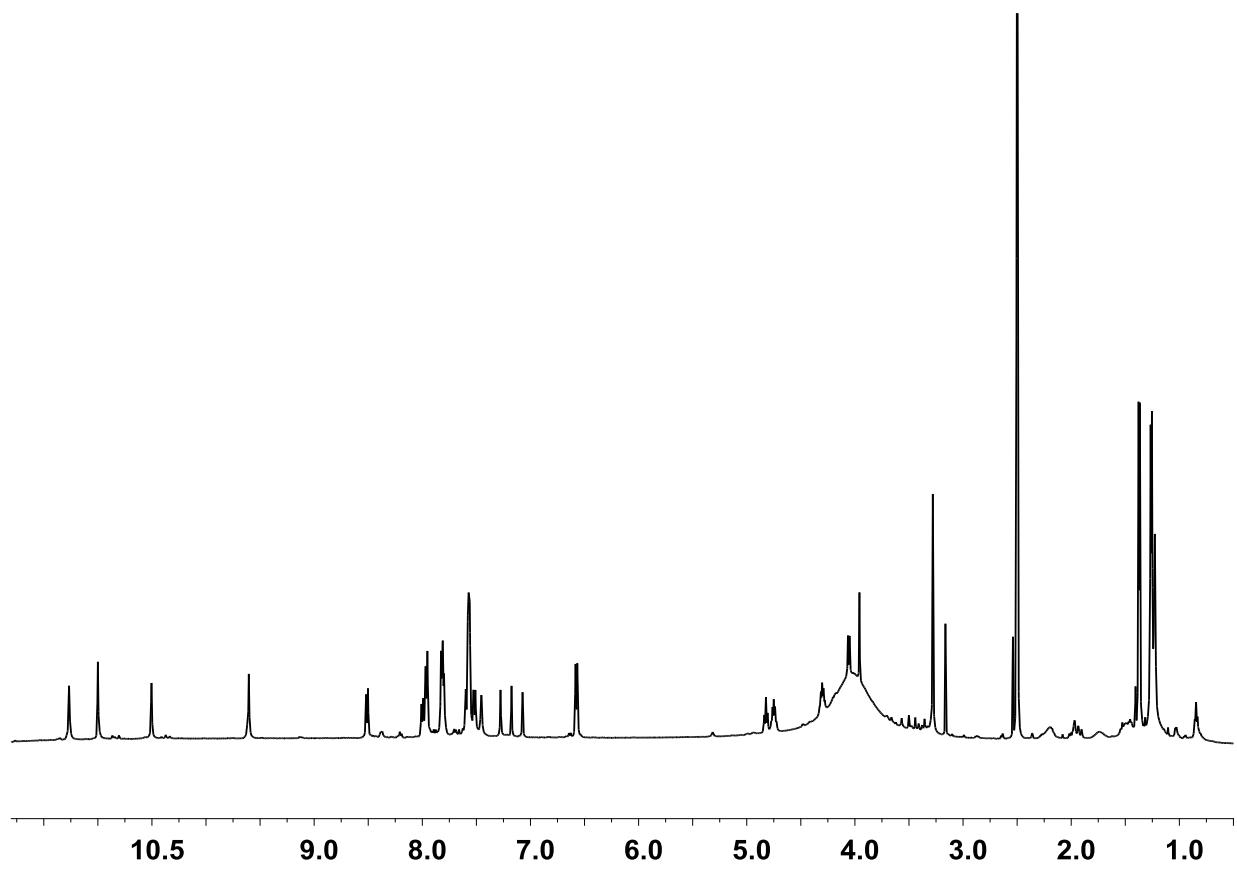
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271 **Figure S28.** Positive HRESIMS spectrum of coralmycin E (3).

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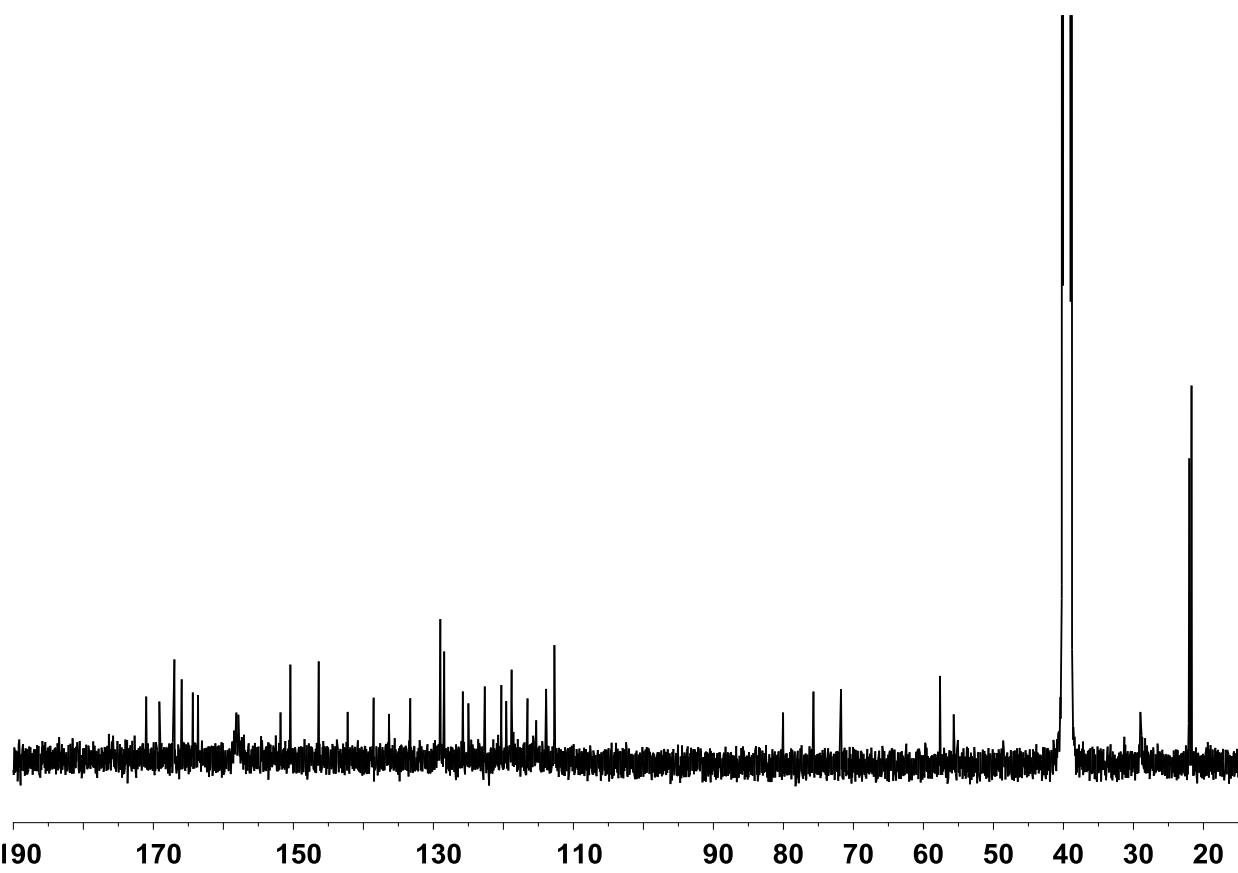


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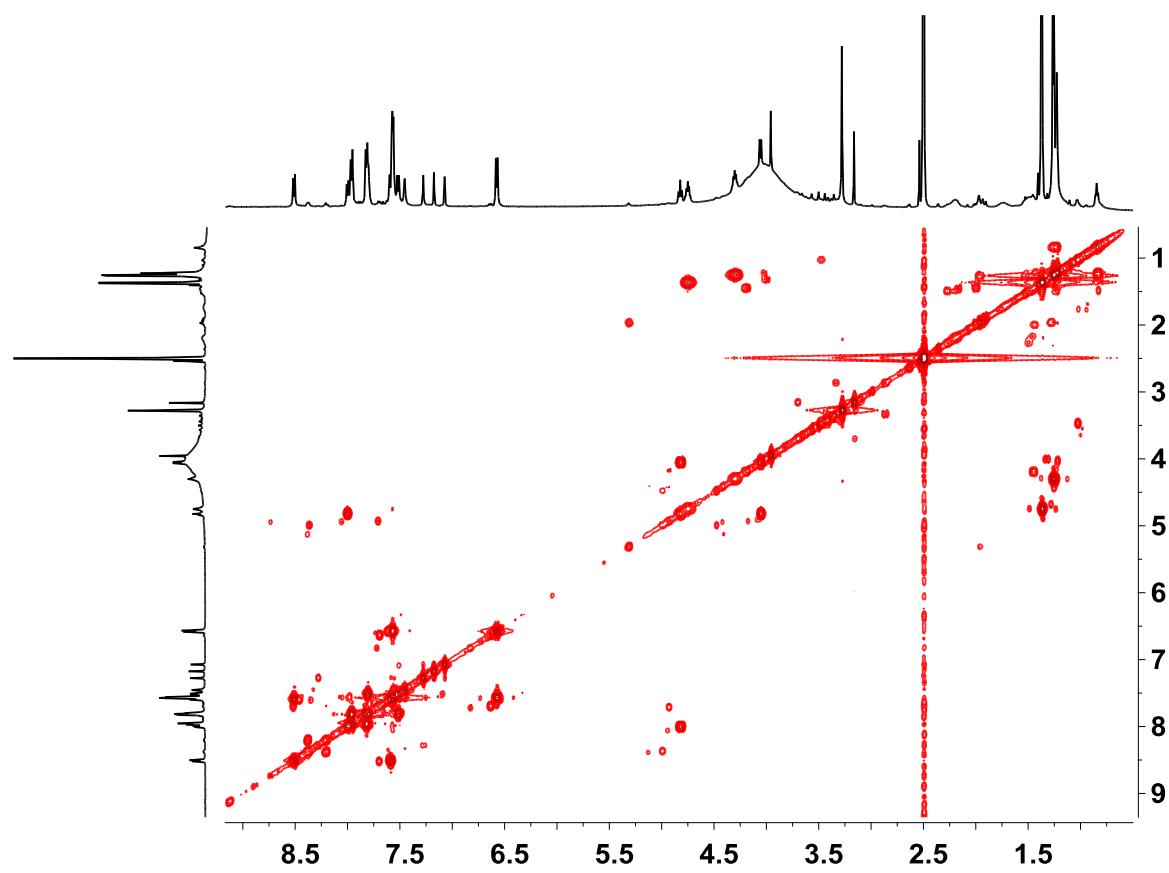
274 **Figure S29.** ¹H-NMR spectrum of coralmycin E (3) measured in DMSO-*d*₆ at 500 MHz.

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277 **Figure S30.** ¹³C-NMR spectrum of coralmycin E (**3**) measured in DMSO-*d*₆ at 500 MHz.
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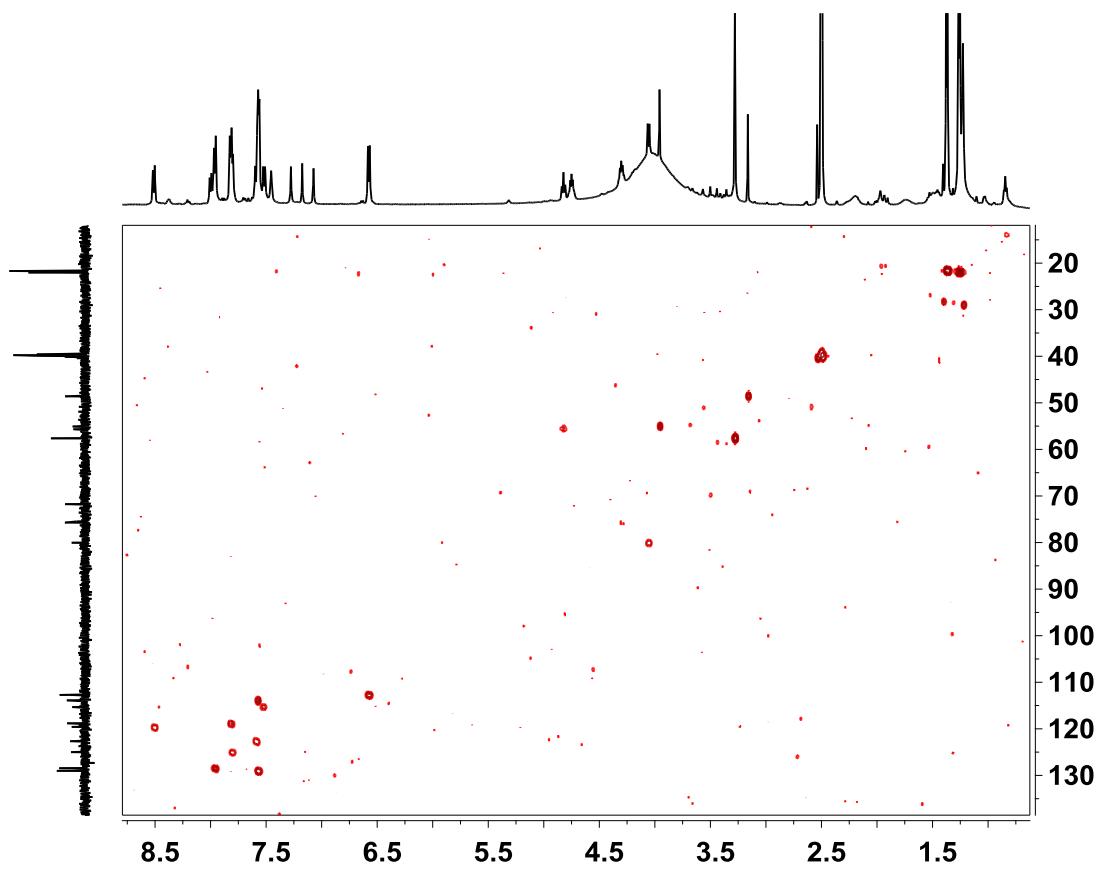


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281 **Figure S31.** COSY spectrum of coralmycin E (**3**) measured in DMSO-*d*₆ at 500 MHz.

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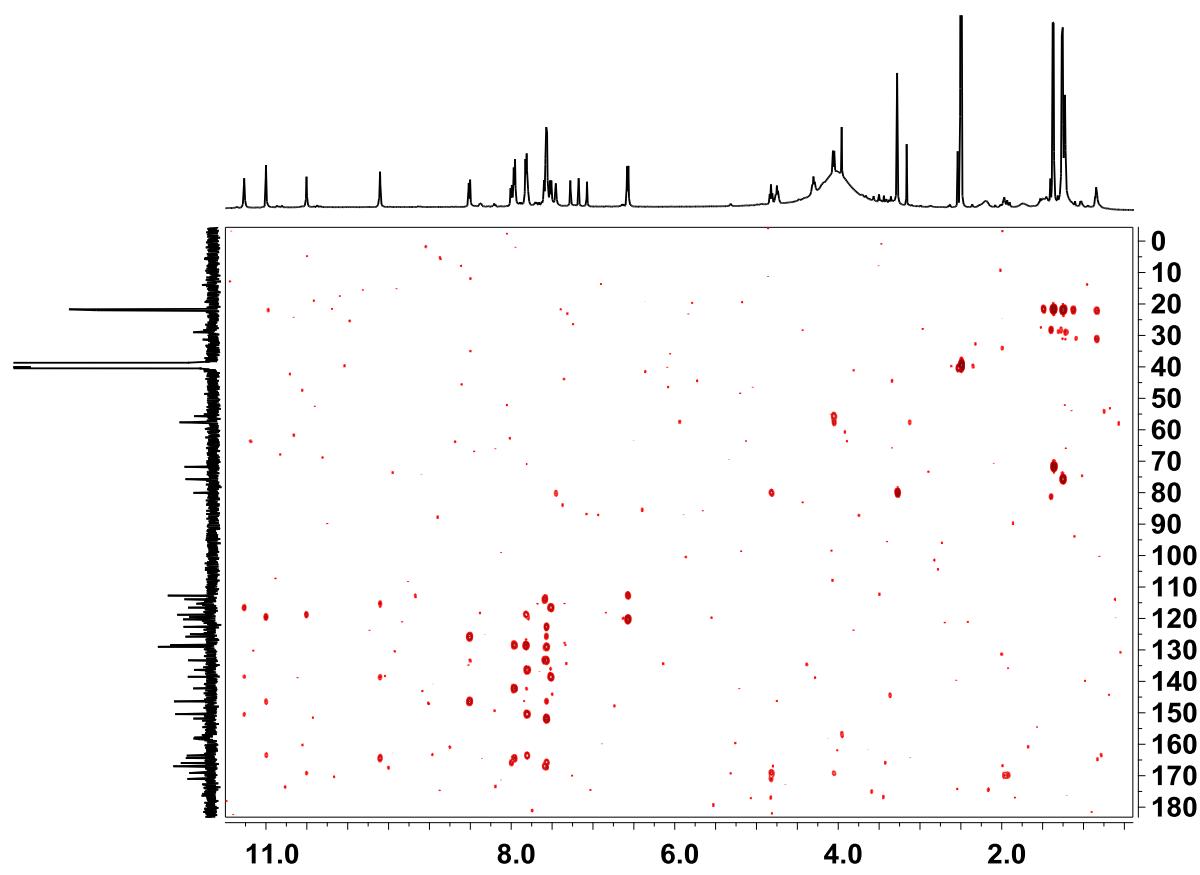


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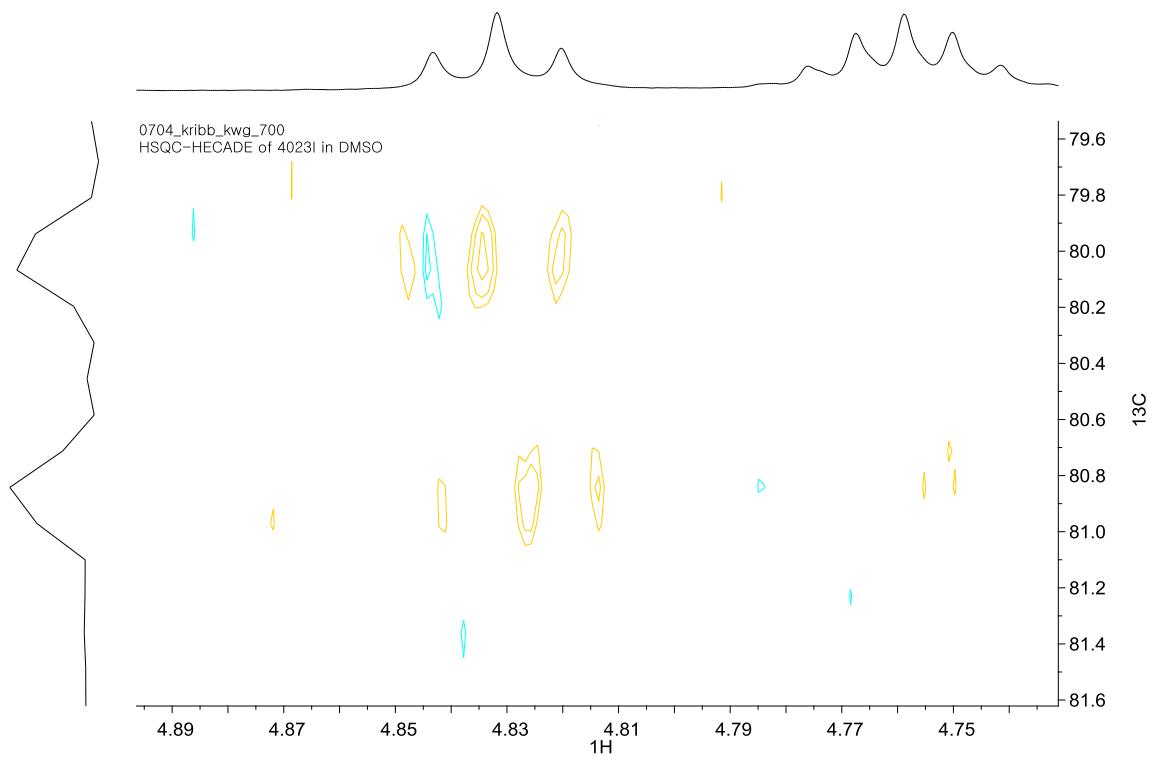
285 **Figure S32.** HMQC spectrum of coralmycin E (**3**) measured in $\text{DMSO}-d_6$ at 500 MHz.

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289 **Figure S33.** HMBC spectrum of coralmycin E (**3**) measured in $\text{DMSO}-d_6$ at 500 MHz.
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301 **Figure S34.** HECADE spectrum of coralmycin E (**3**) measured in $\text{DMSO}-d_6$ at 700 MHz.

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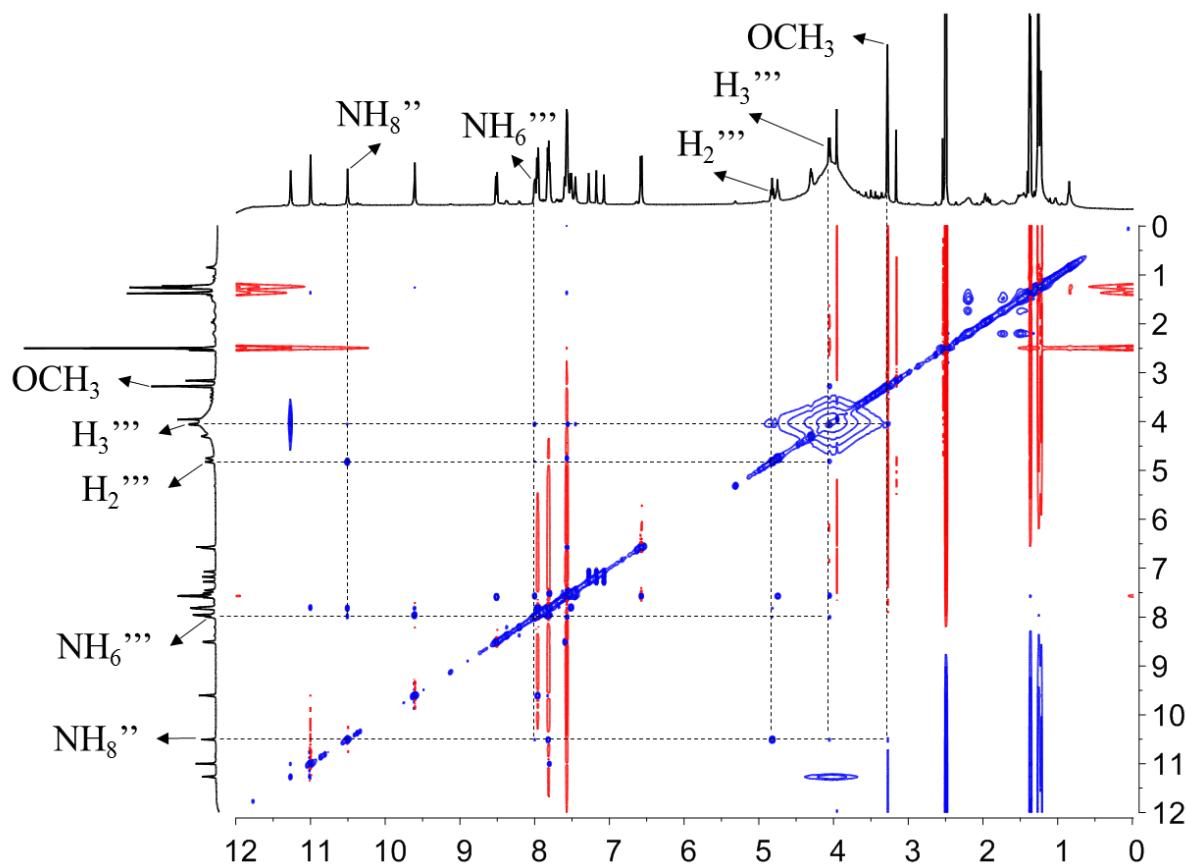
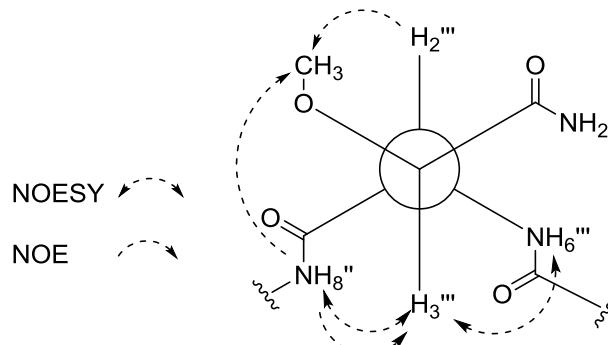
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314 **Figure S35.** NOESY spectrum of coralmycin E (**3**) measured in $\text{DMSO}-d_6$ at 500 MHz.

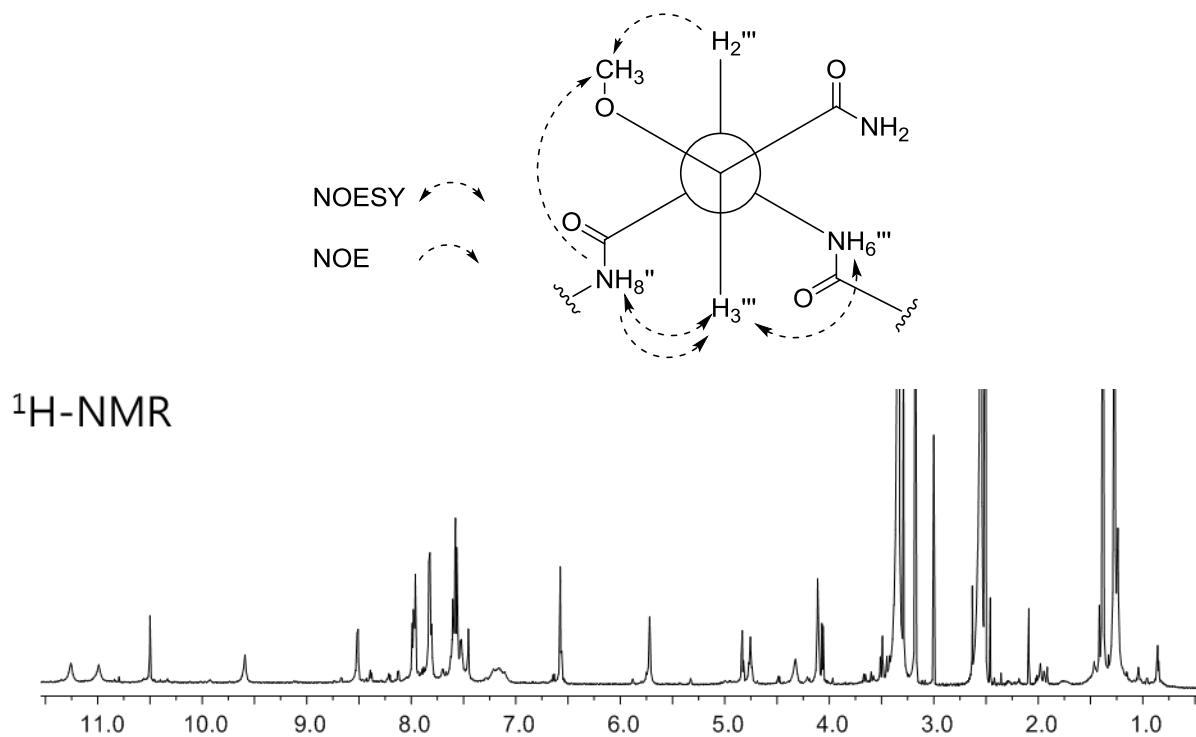
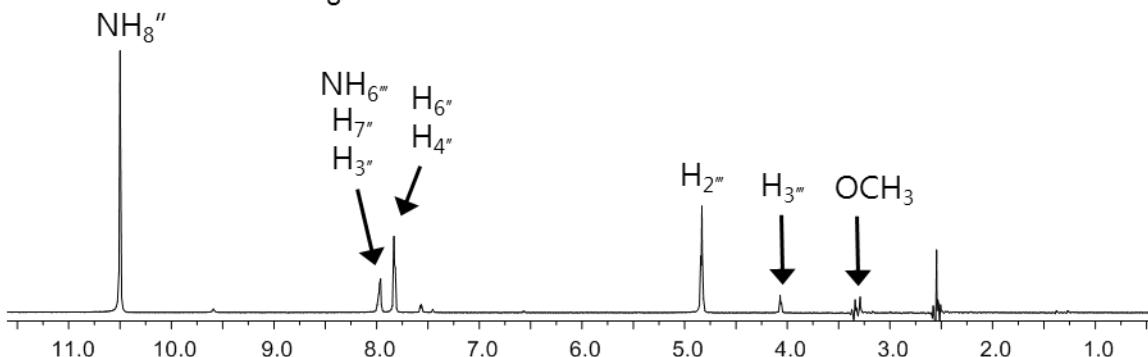
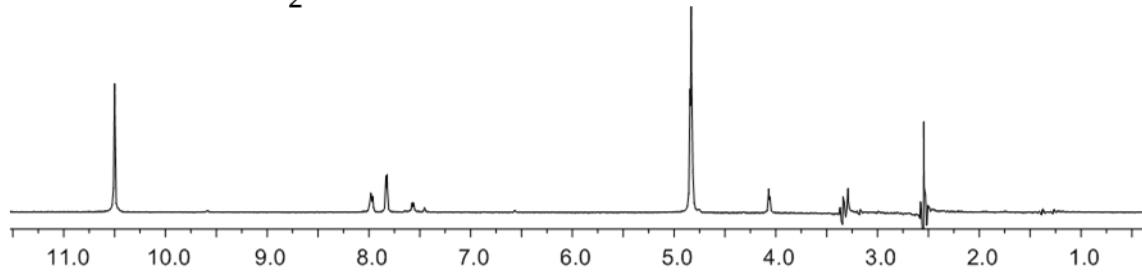
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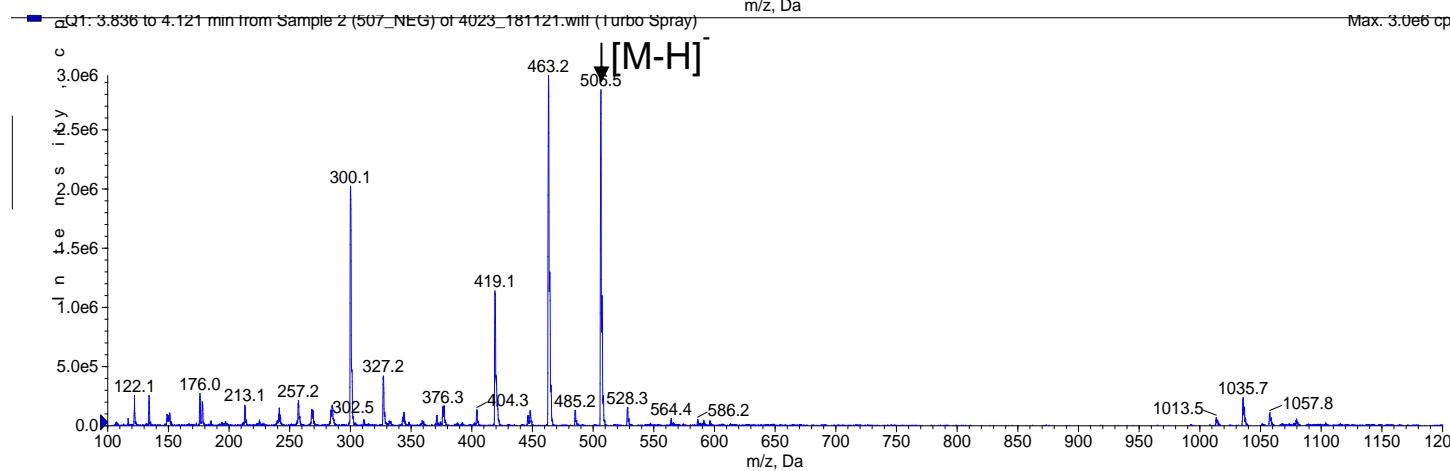
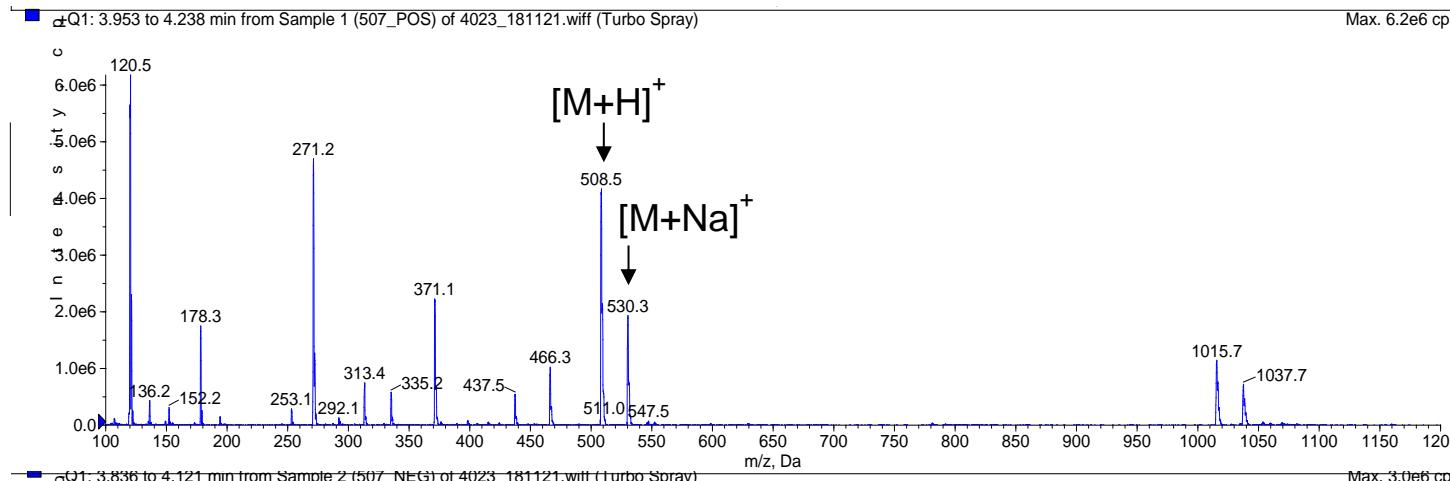
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Irradiation of NH₈''Irradiation of H₂'''

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321 **Figure S36.** The NOE differential spectra of coralmycin E (**3**) measured in DMSO-*d*₆ at 800 MHz.

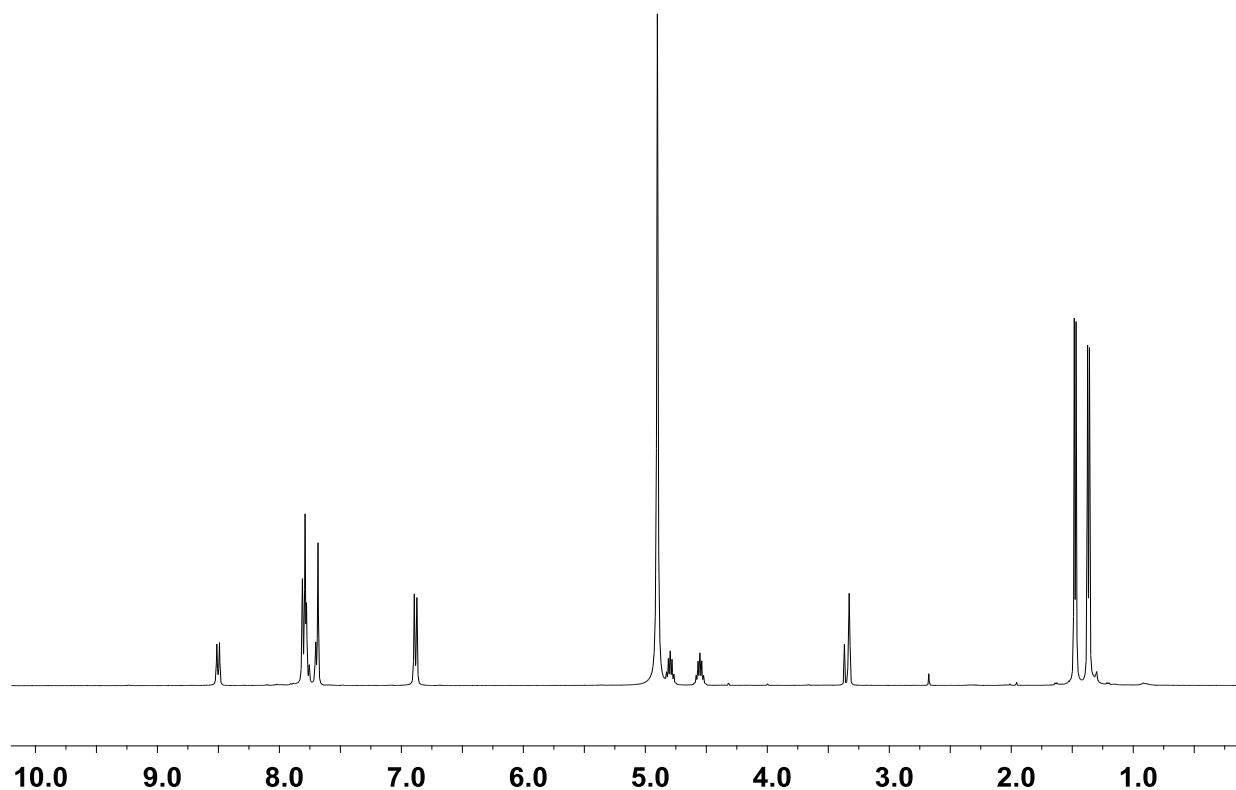
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324 **Figure S37.** Positive and negative ESIMS spectrum of cystobactamid 507 (**10**).

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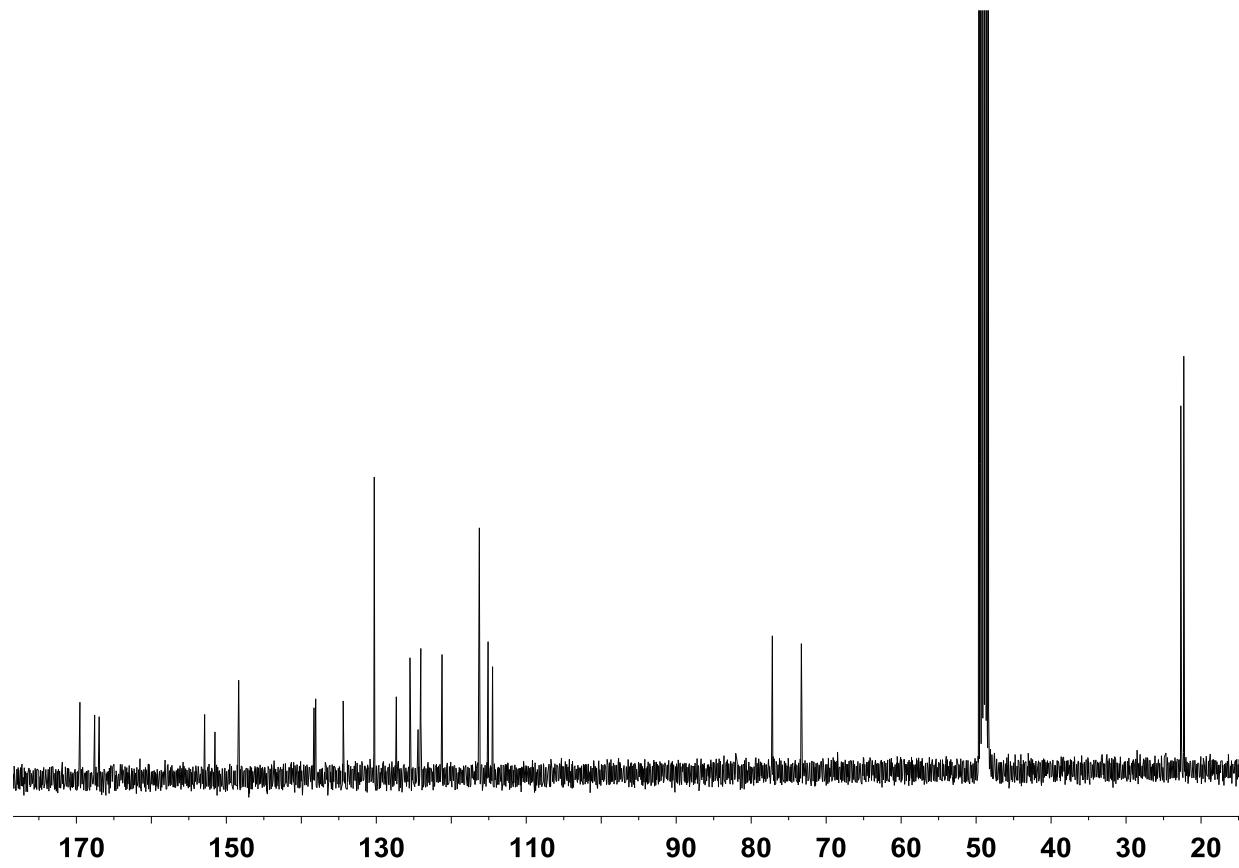
327 **Figure S38.** ¹H NMR spectrum of cystobactamid 507 (**10**) measured in CD₃OD at 700 MHz.

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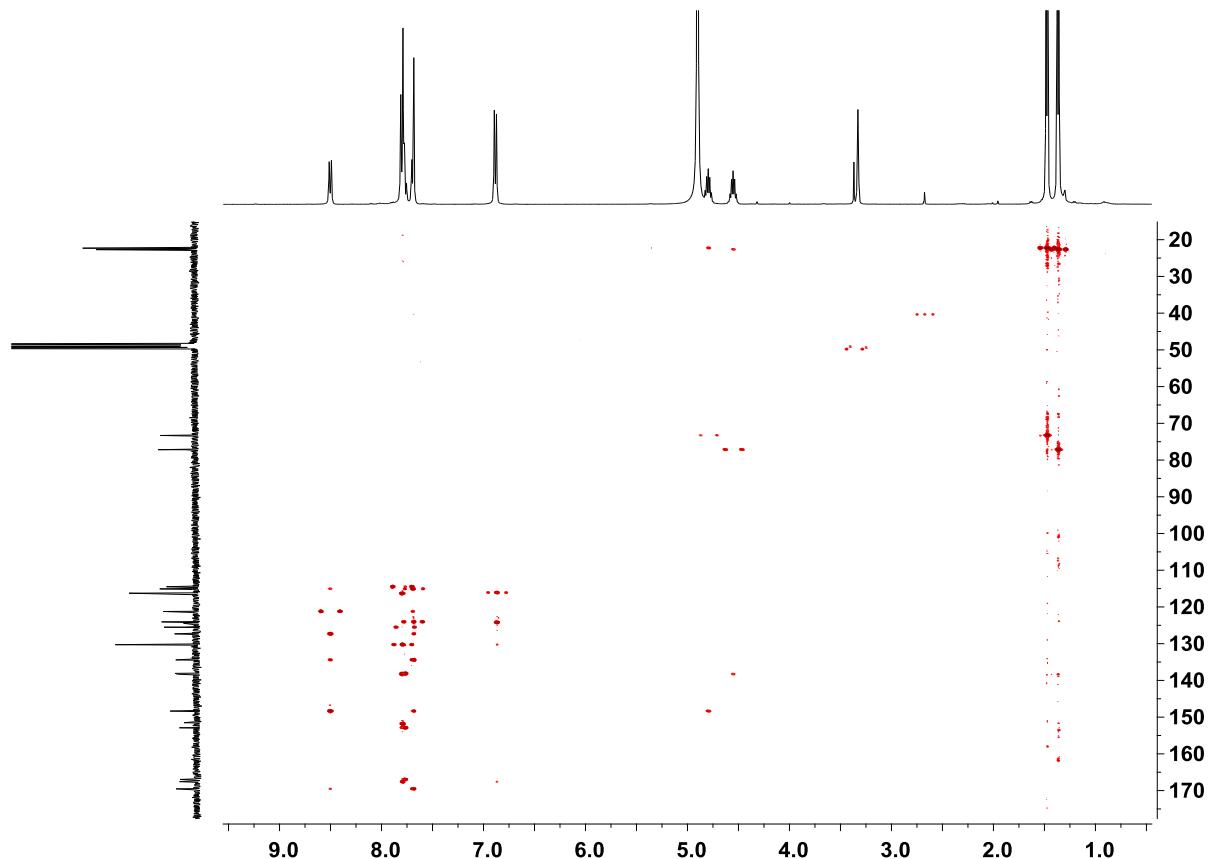
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333 **Figure S39.** ¹²C-NMR spectrum of cystobactamid 507 (**10**) measured in CD₃OD at 700 MHz.
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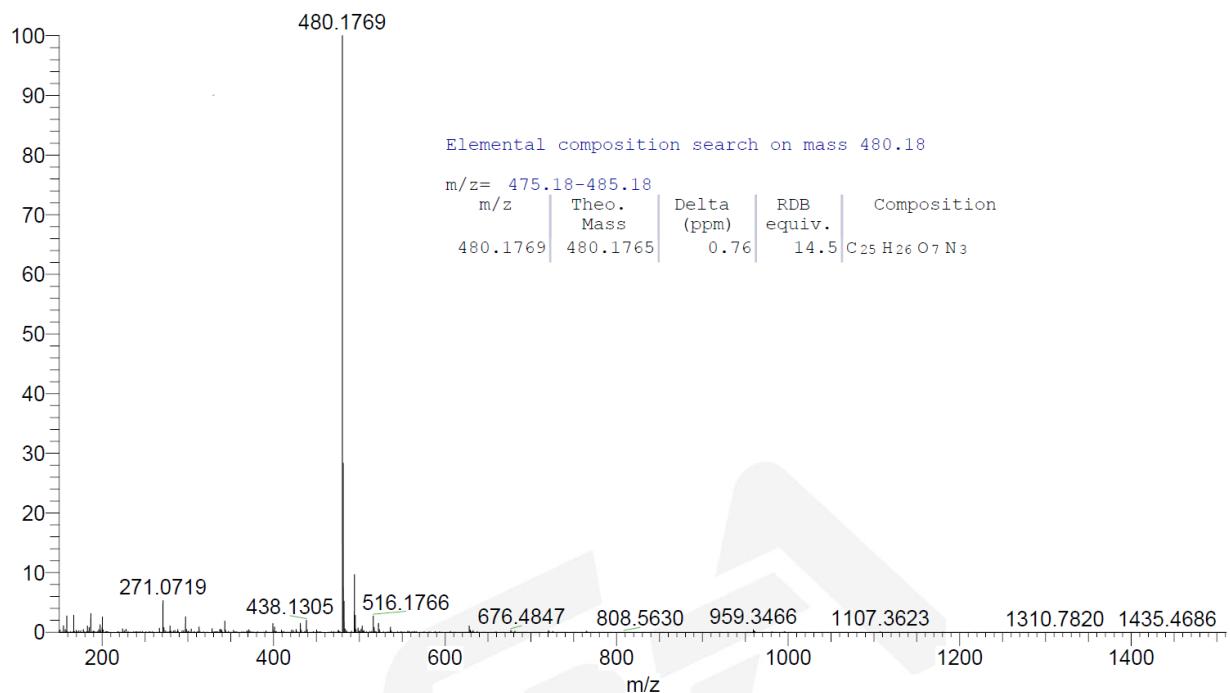


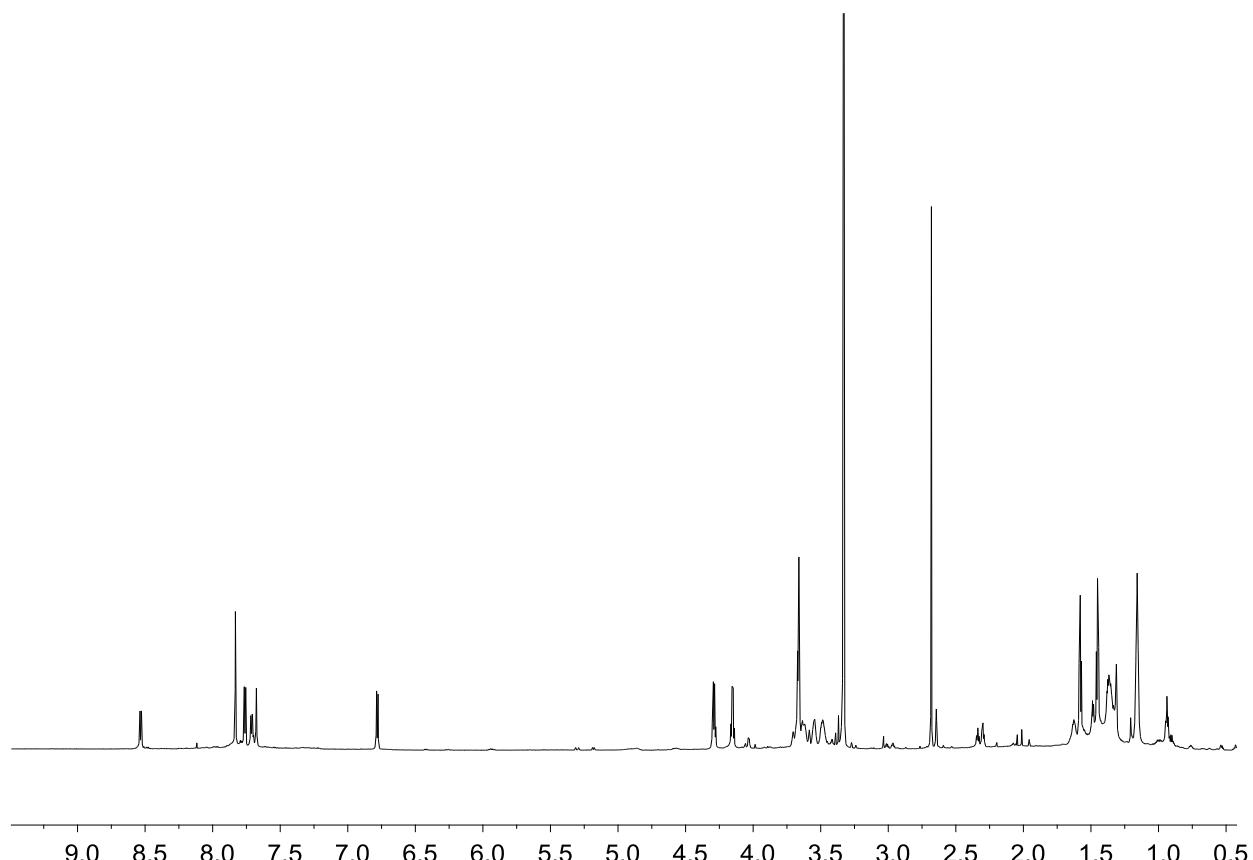
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347 **Figure S40.** HMBC spectrum of cystobactamid 507 (**10**) measured in CD_3OD at 800 MHz.

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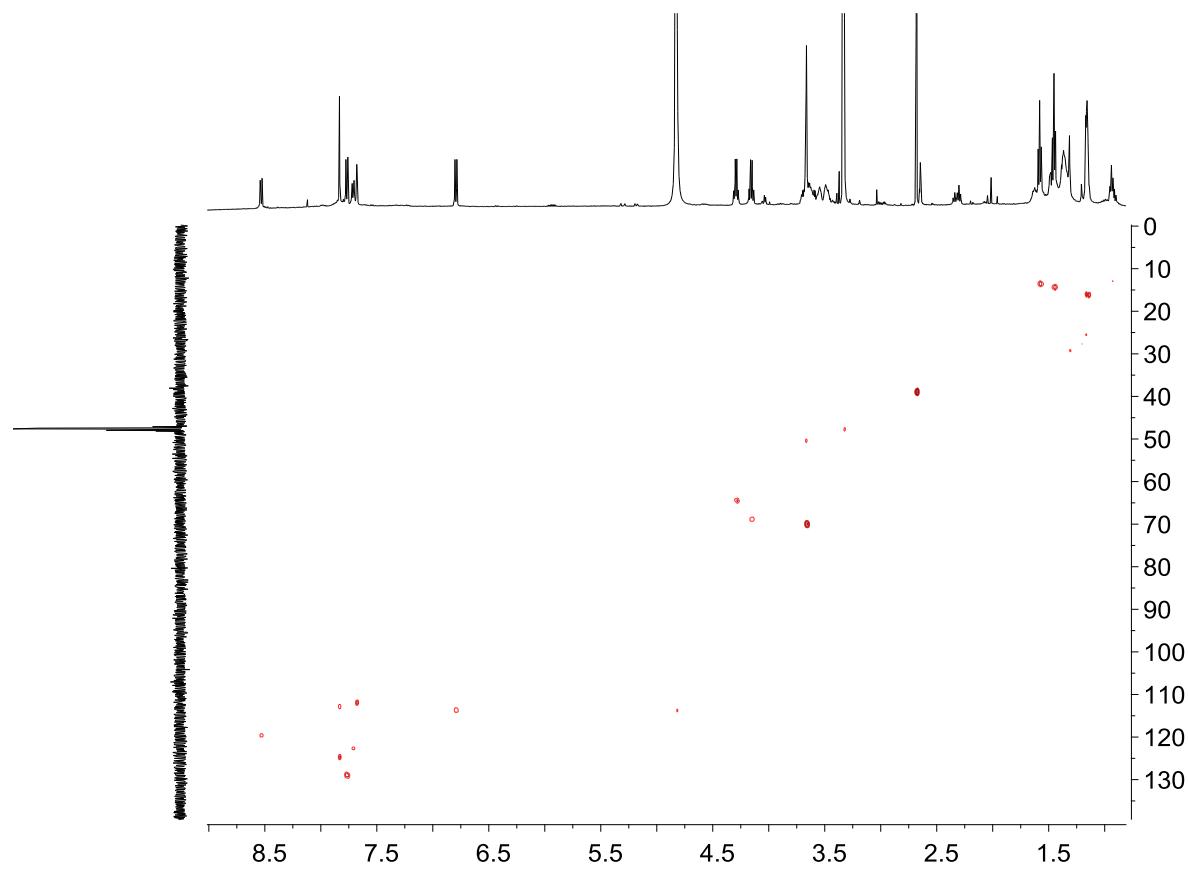


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354 **Figure S42.** ¹H NMR spectrum of coralmycin F (4) measured in CD₃OD at 800 MHz.

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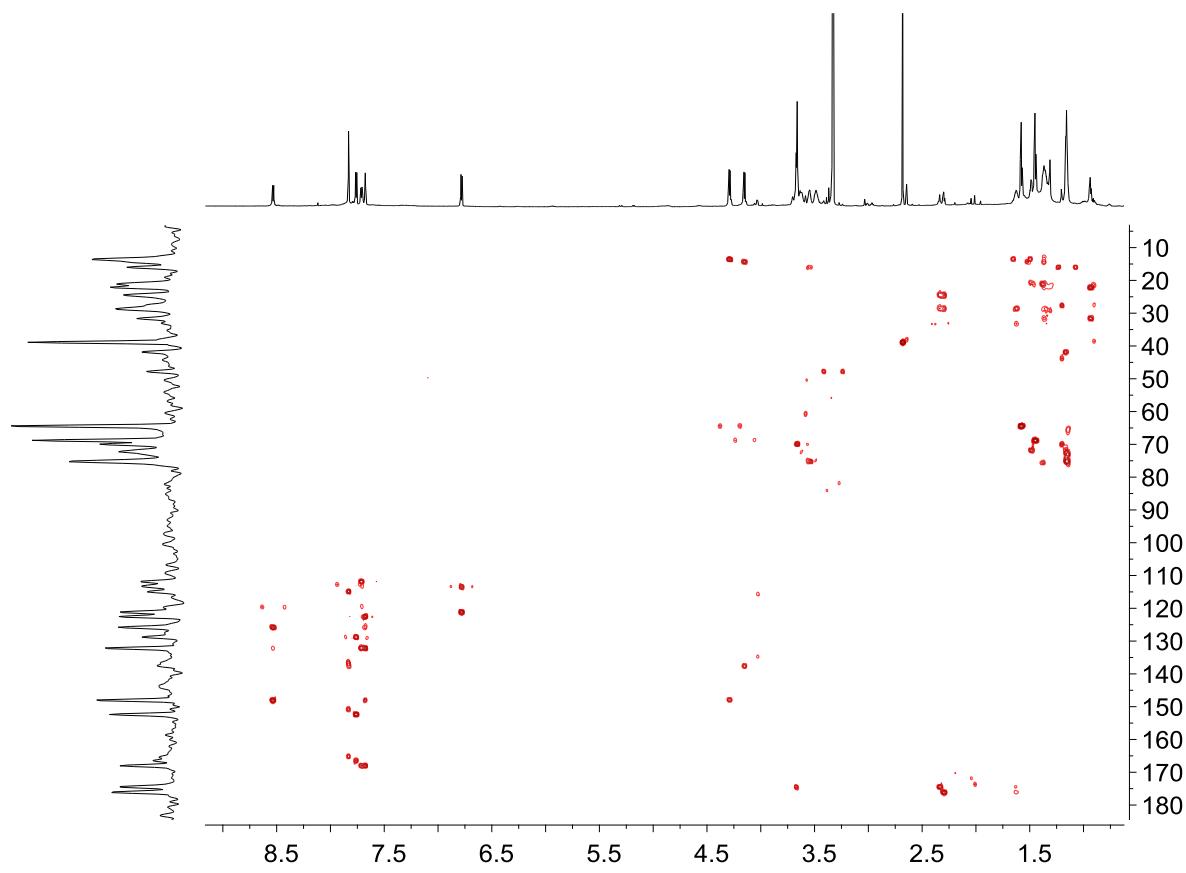
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358 **Figure S43.** HMQC spectrum of coralmycin F (**4**) measured in CD_3OD at 500 MHz.

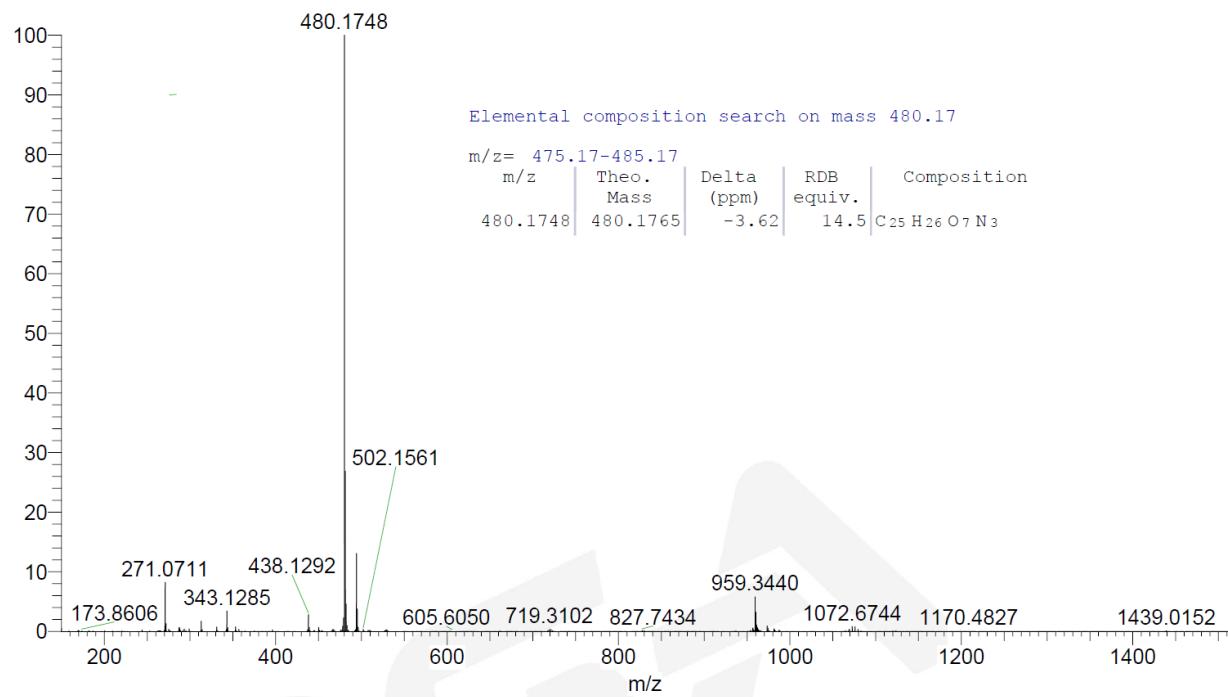
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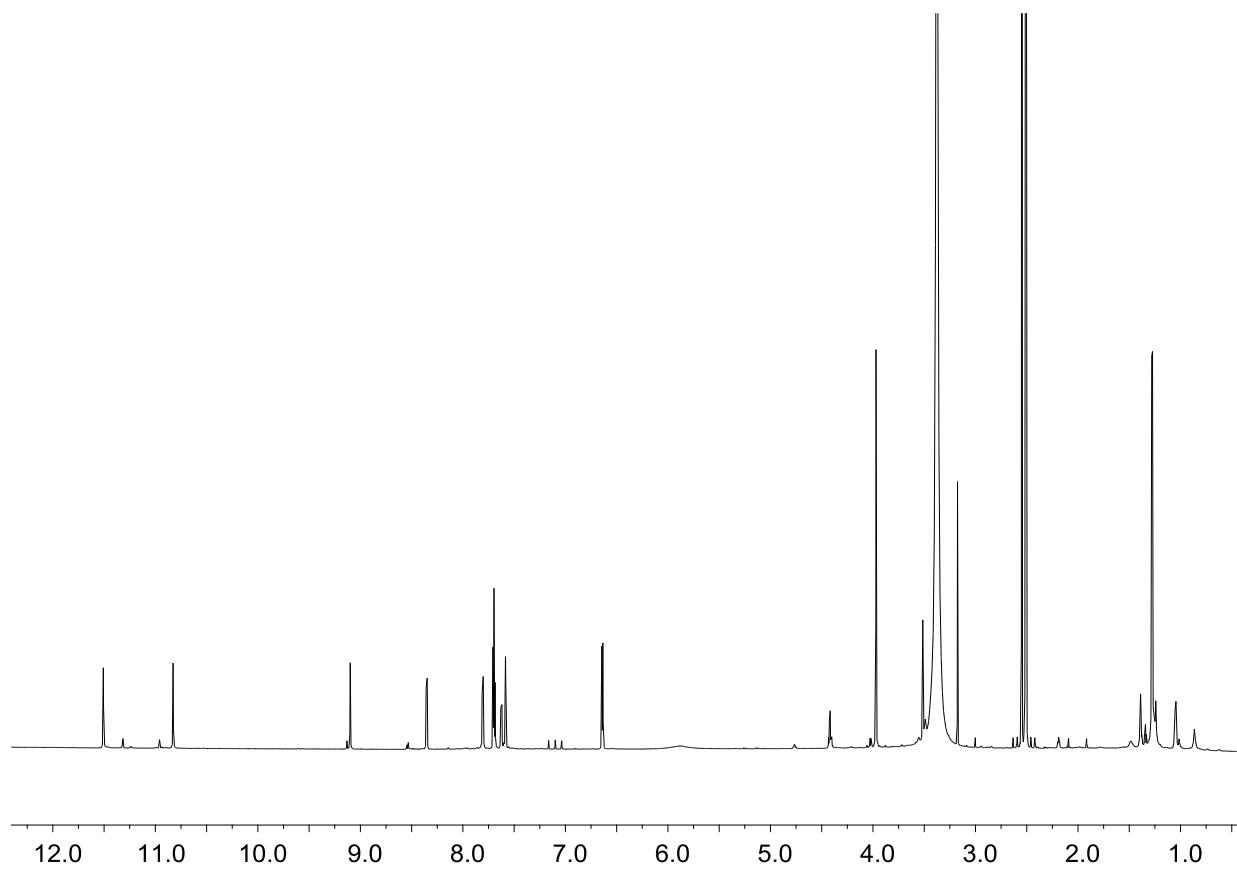


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361 **Figure S44.** HMBC spectrum of coralmycin F (**4**) measured in CD_3OD at 800 MHz.

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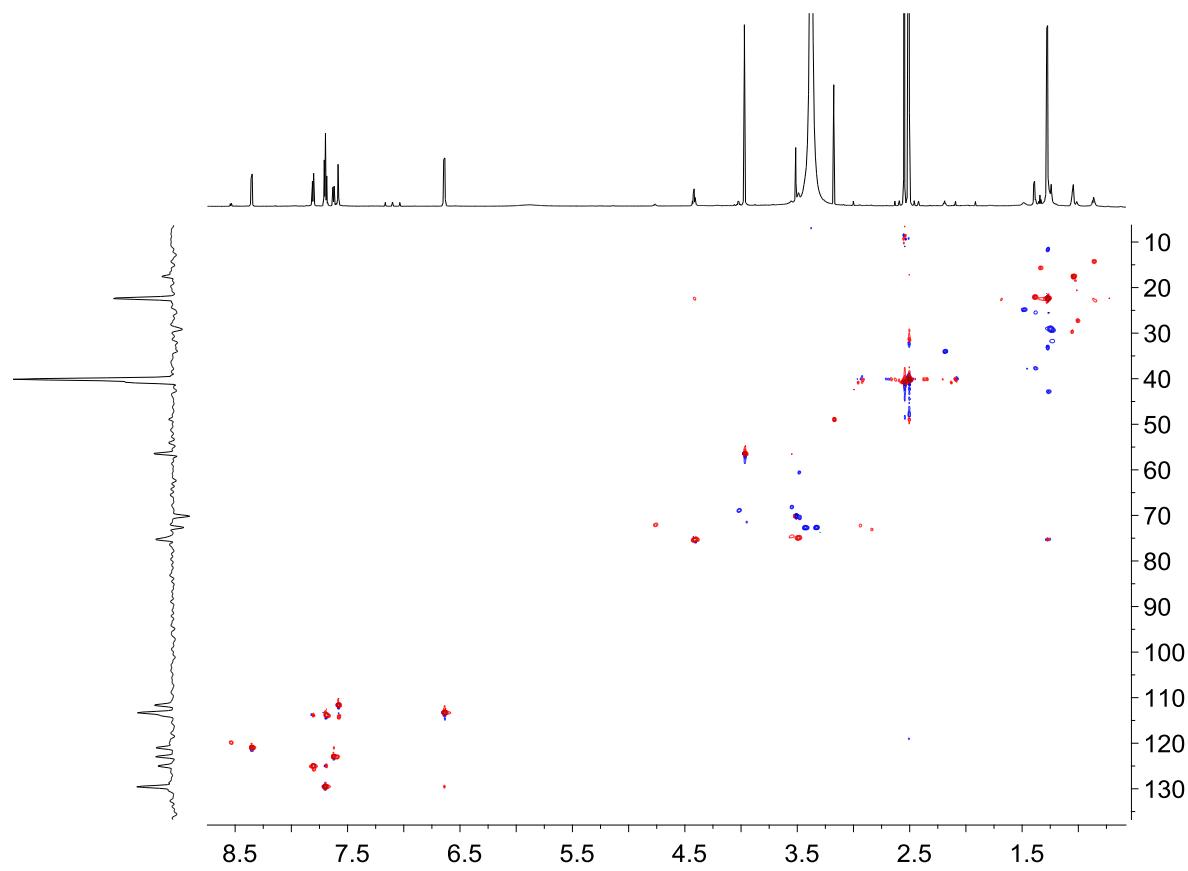




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367 **Figure S46.** ¹H NMR spectrum of coralmycin G (5) measured in DMSO-*d*₆ at 800 MHz.

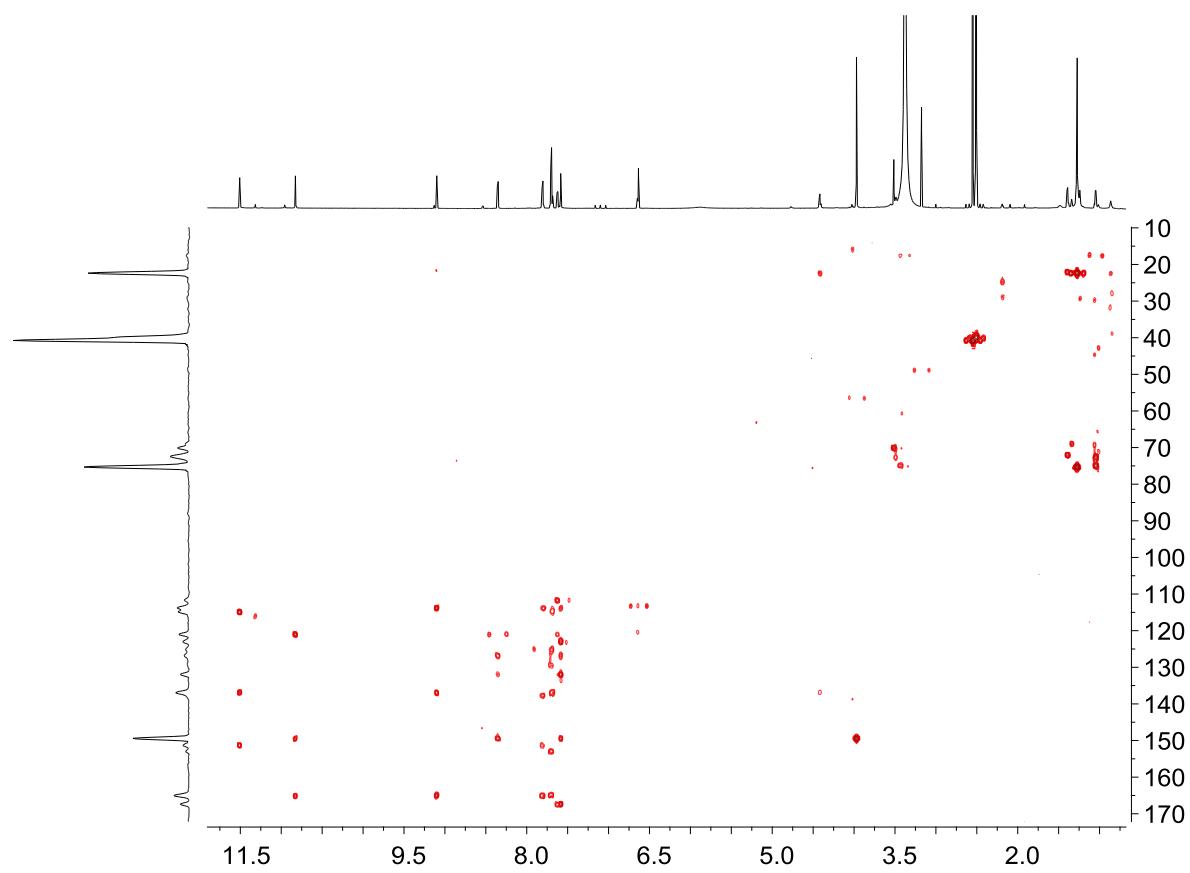
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370 **Figure S47.** HMQC spectrum of coralmycin G (**5**) measured in $\text{DMSO}-d_6$ at 800 MHz.

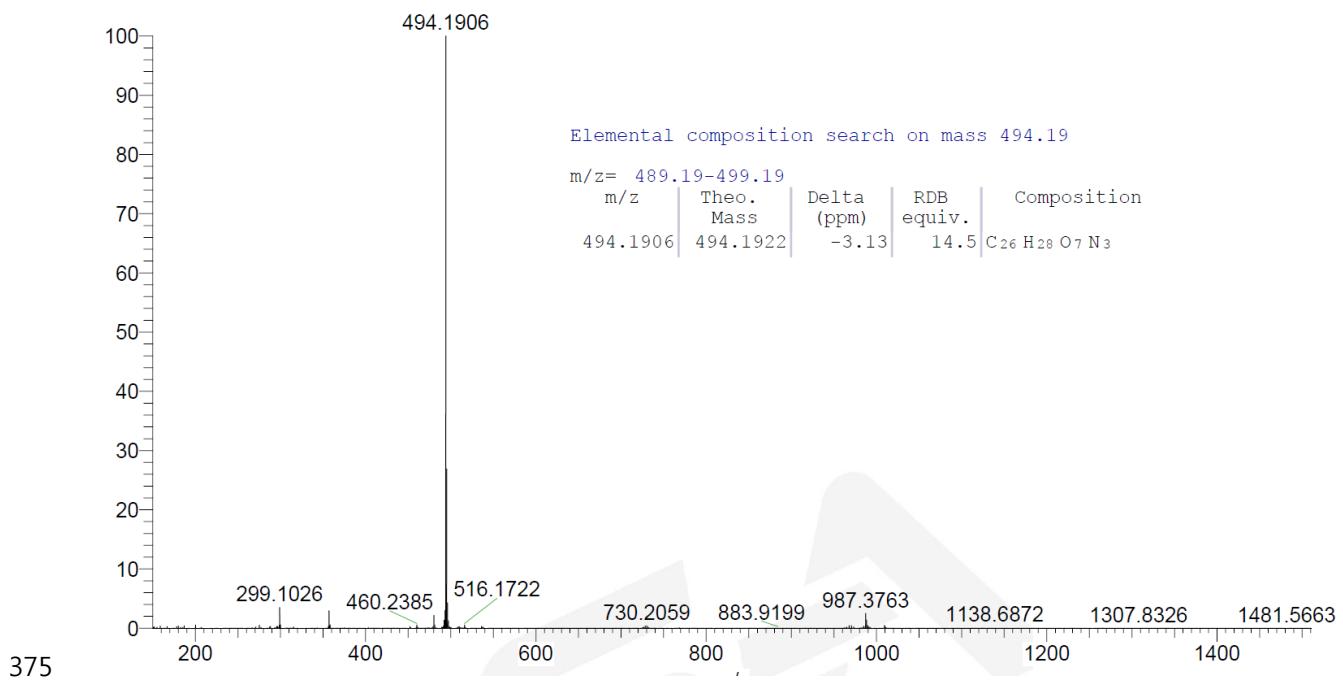
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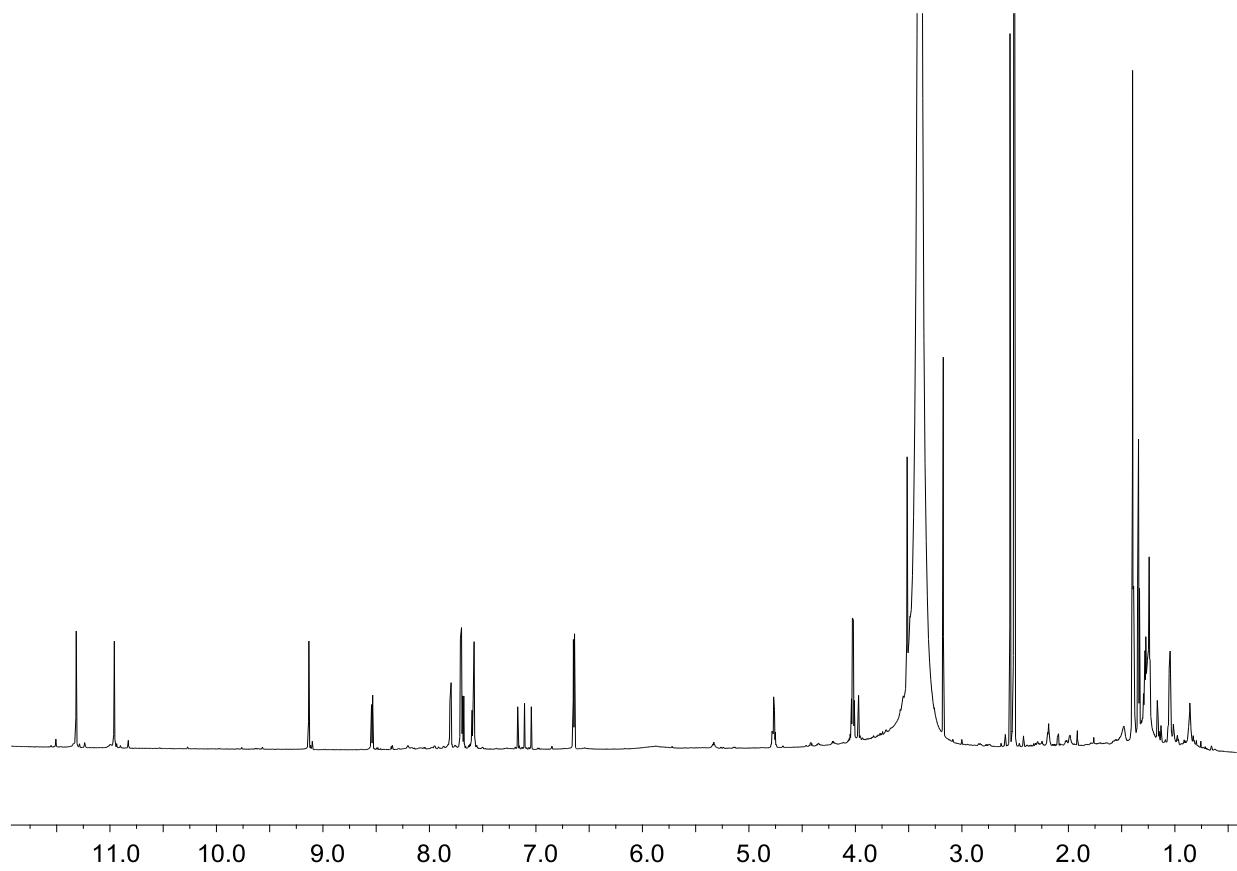


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373 **Figure S48.** HMBC spectrum of coralmycin G (**5**) measured in DMSO-*d*₆ at 800 MHz.

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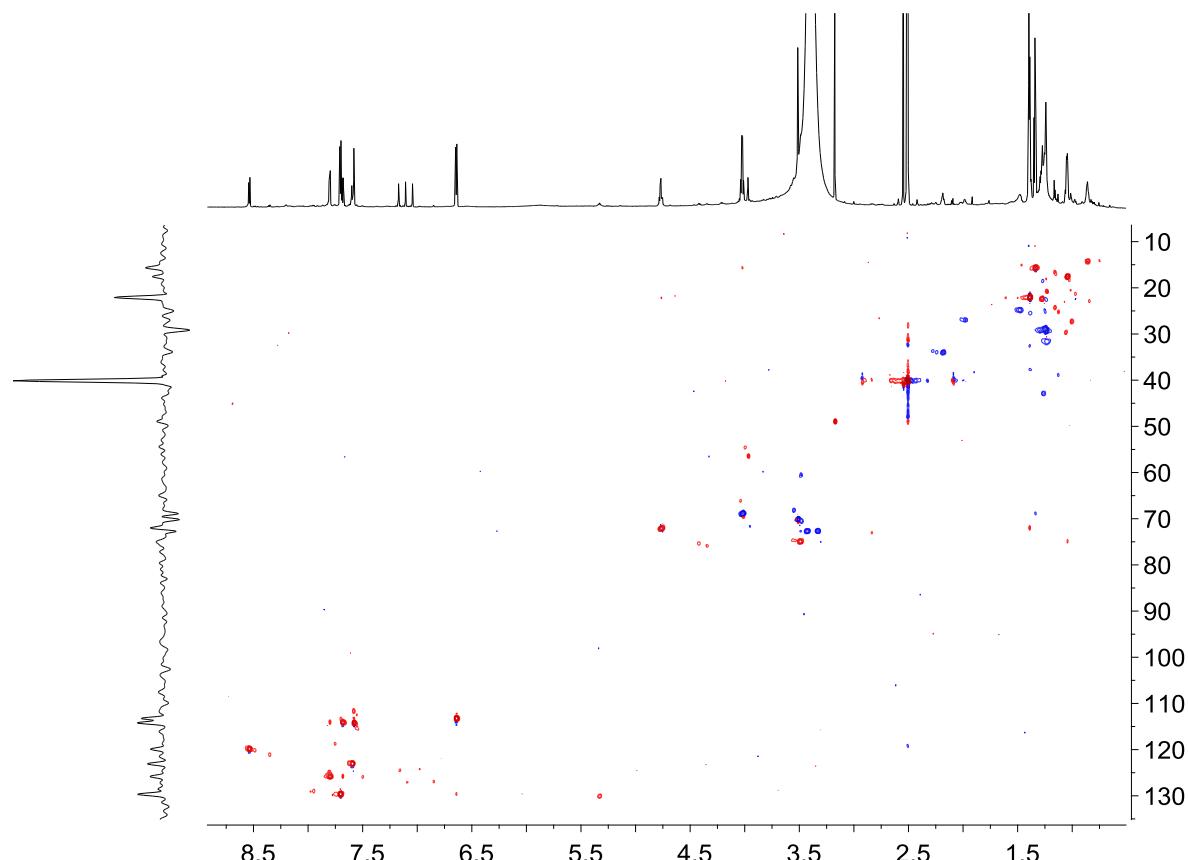




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379 **Figure S50.** ^1H NMR spectrum of coralmycin H (**6**) measured in $\text{DMSO}-d_6$ at 800 MHz.

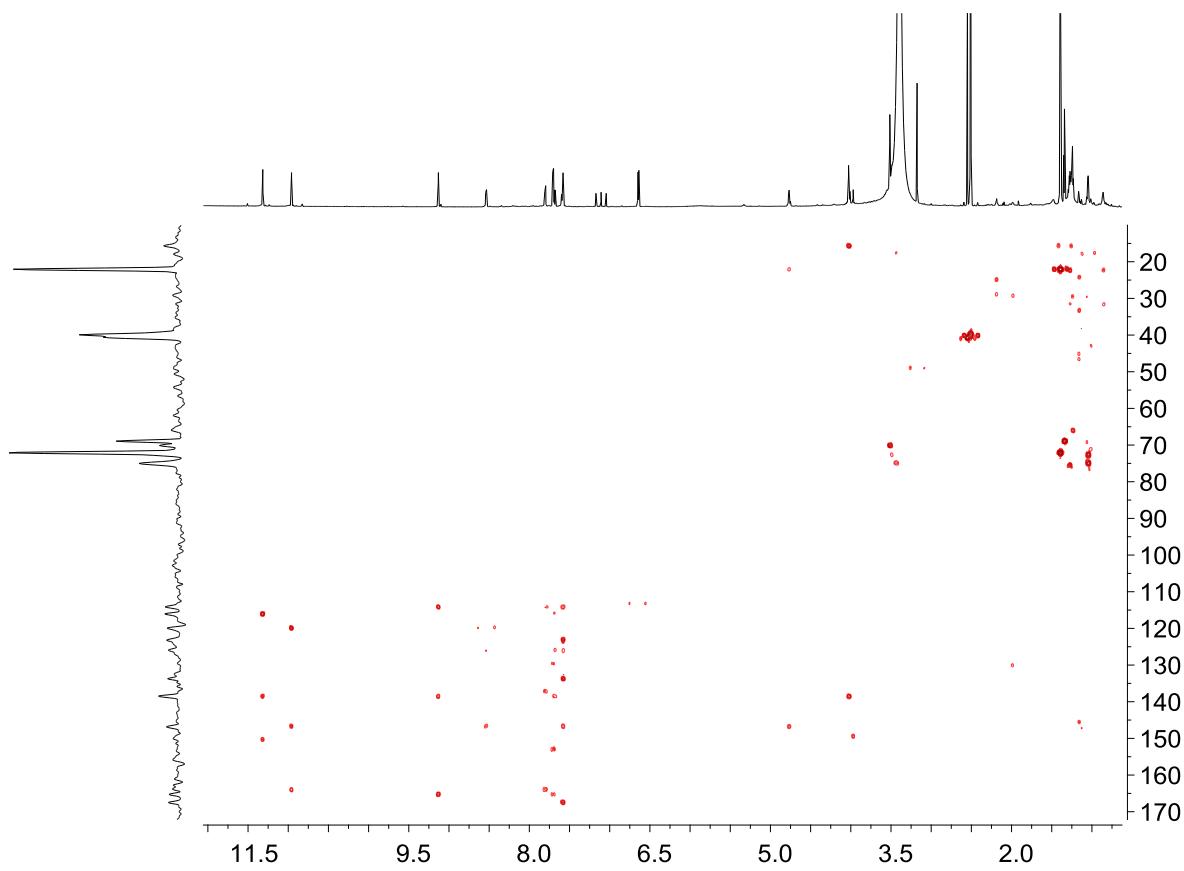
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382 **Figure S51.** HMQC spectrum of coralmycin H (**6**) measured in DMSO-*d*₆ at 800 MHz.

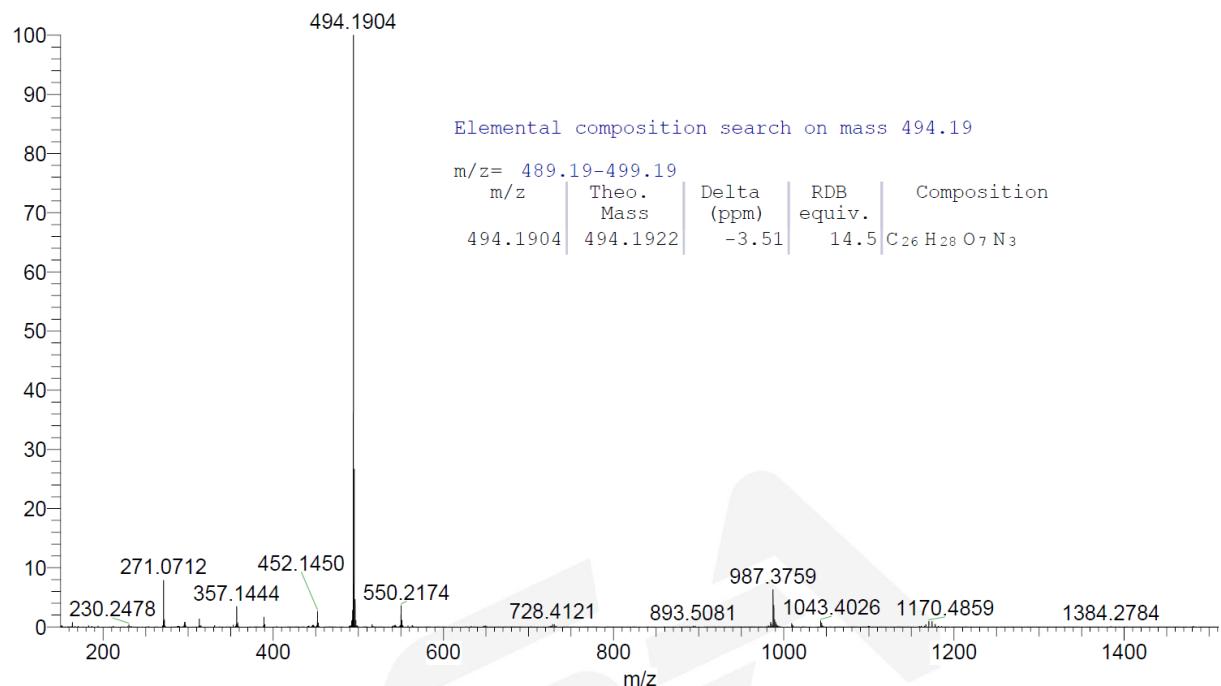
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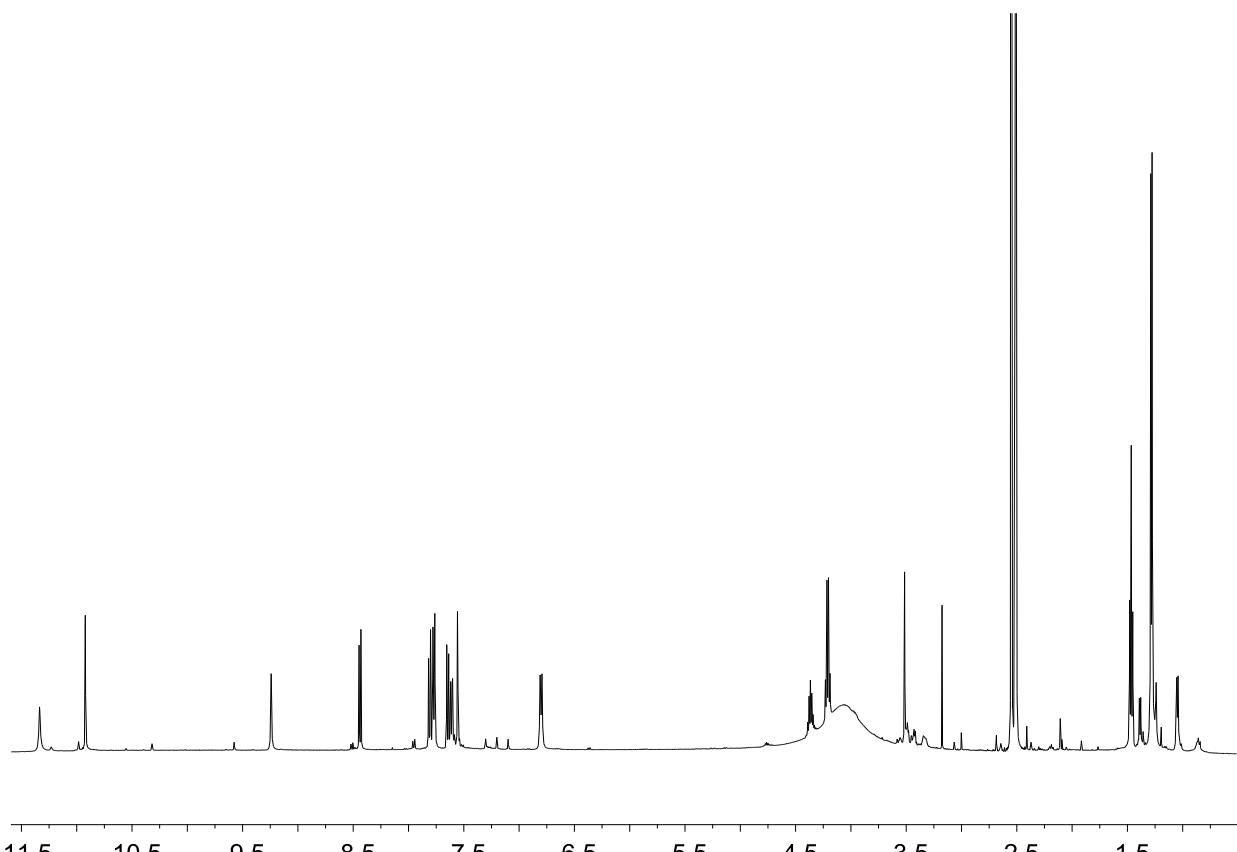


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385 **Figure S52** HMBC spectrum of coralmycin H (**6**) measured in $\text{DMSO}-d_6$ at 800 MHz.

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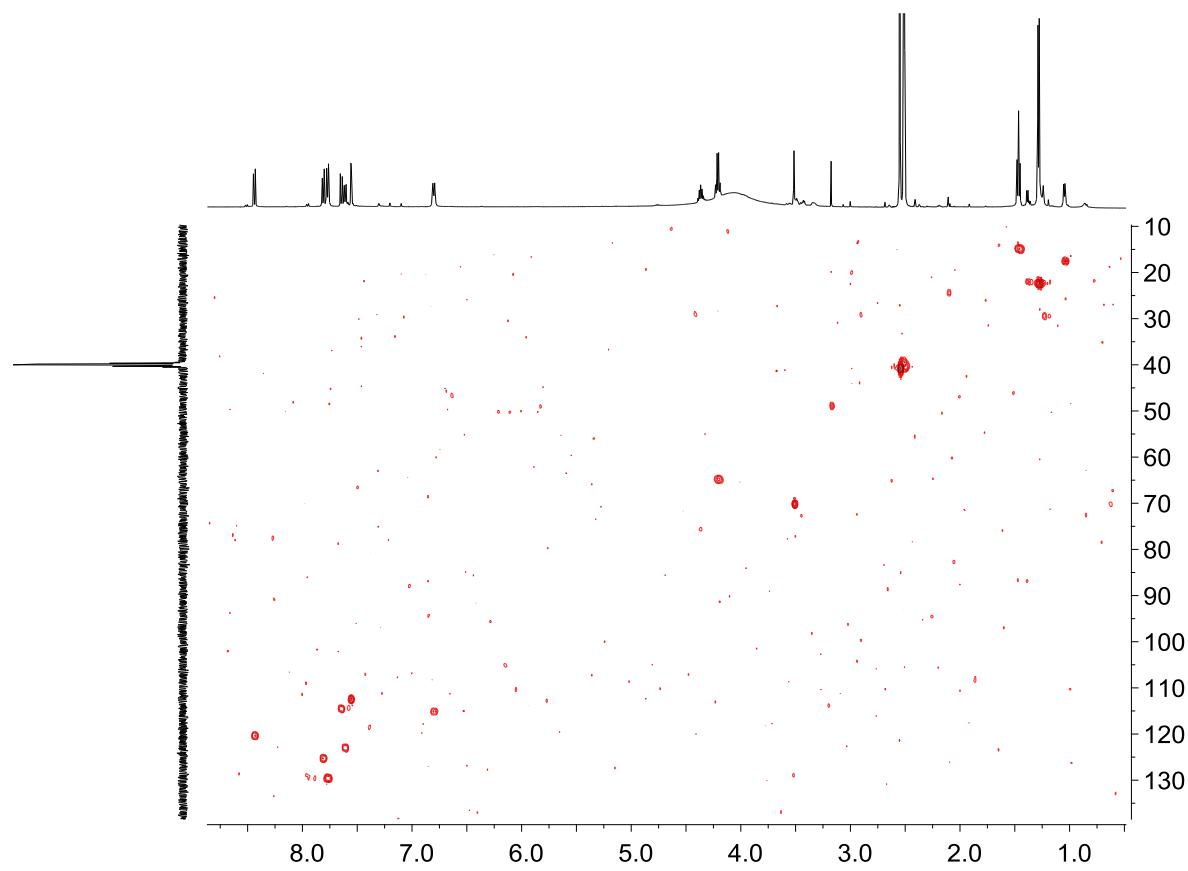




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391 **Figure S54.** ¹H NMR spectrum of coralmycin I (**7**) measured in DMSO-*d*₆ at 500 MHz.

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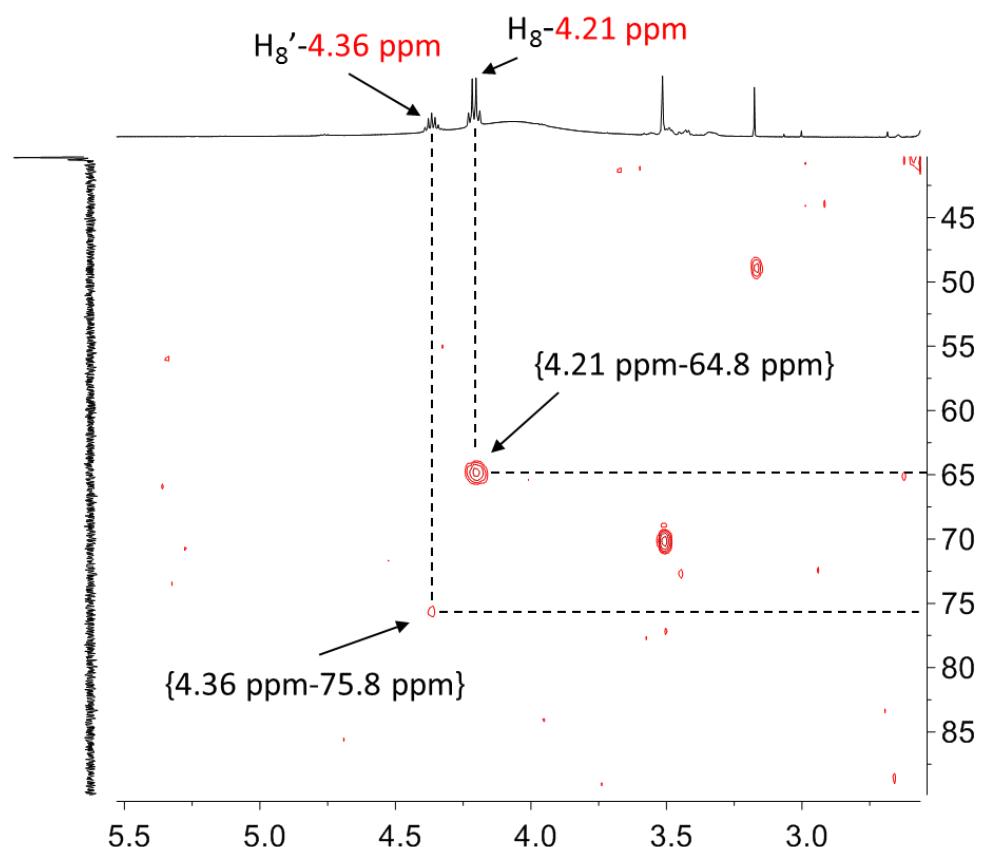


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394 **Figure S55.** HMQC spectrum of coralmycin I (**7**) measured in $\text{DMSO}-d_6$ at 500 MHz.

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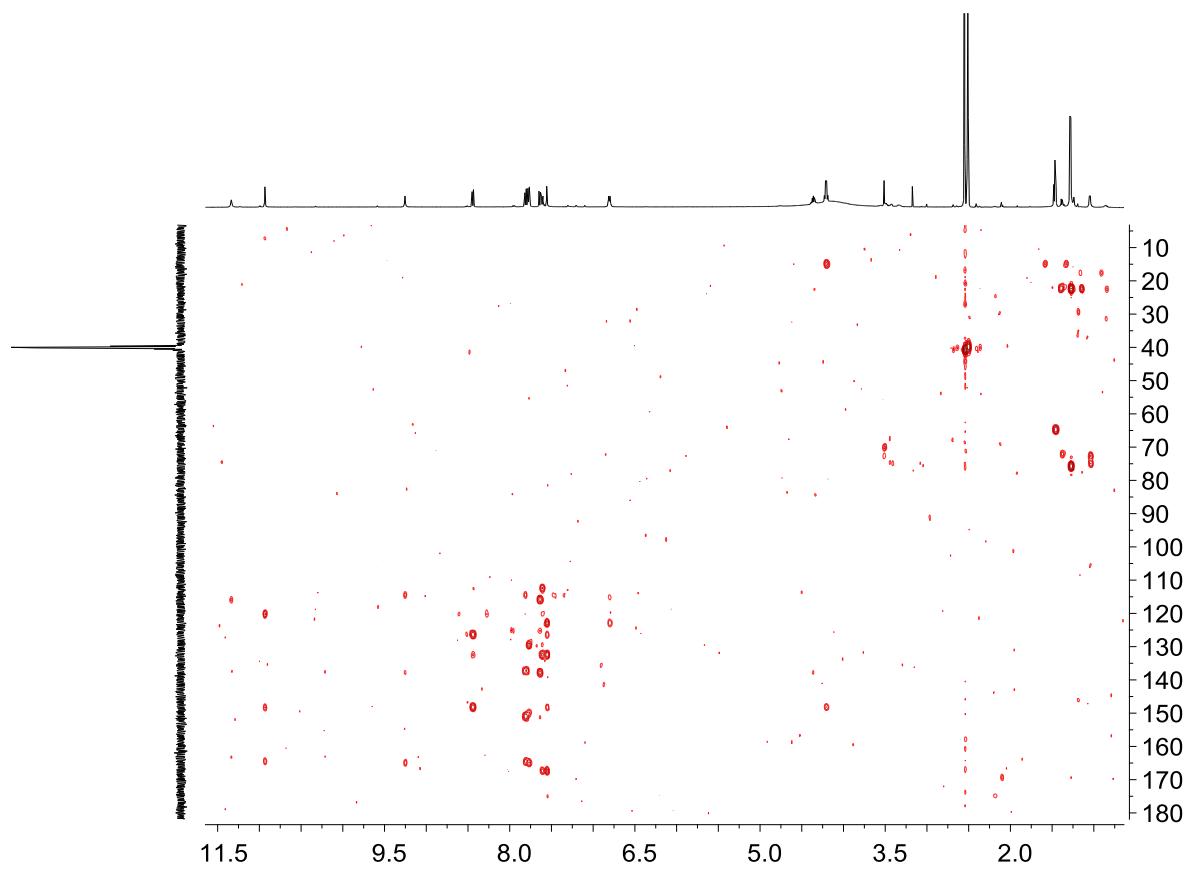
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398 **Figure S56.** Expansion of HMQC spectrum of coralmycin I (**7**).

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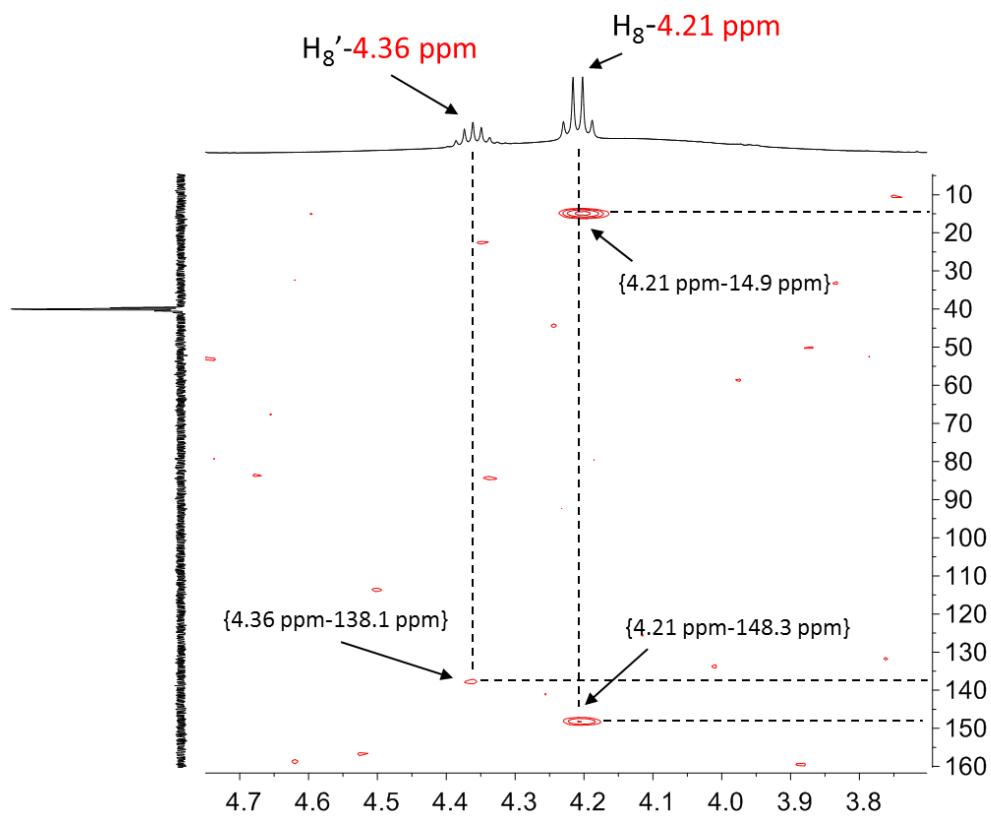


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401 **Figure S57.** HMBC spectrum of coralmycin I (**7**) measured in DMSO-*d*₆ at 500 MHz.

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Figure S58. Expansion of HMBC spectrum of coralmycin I (**7**).

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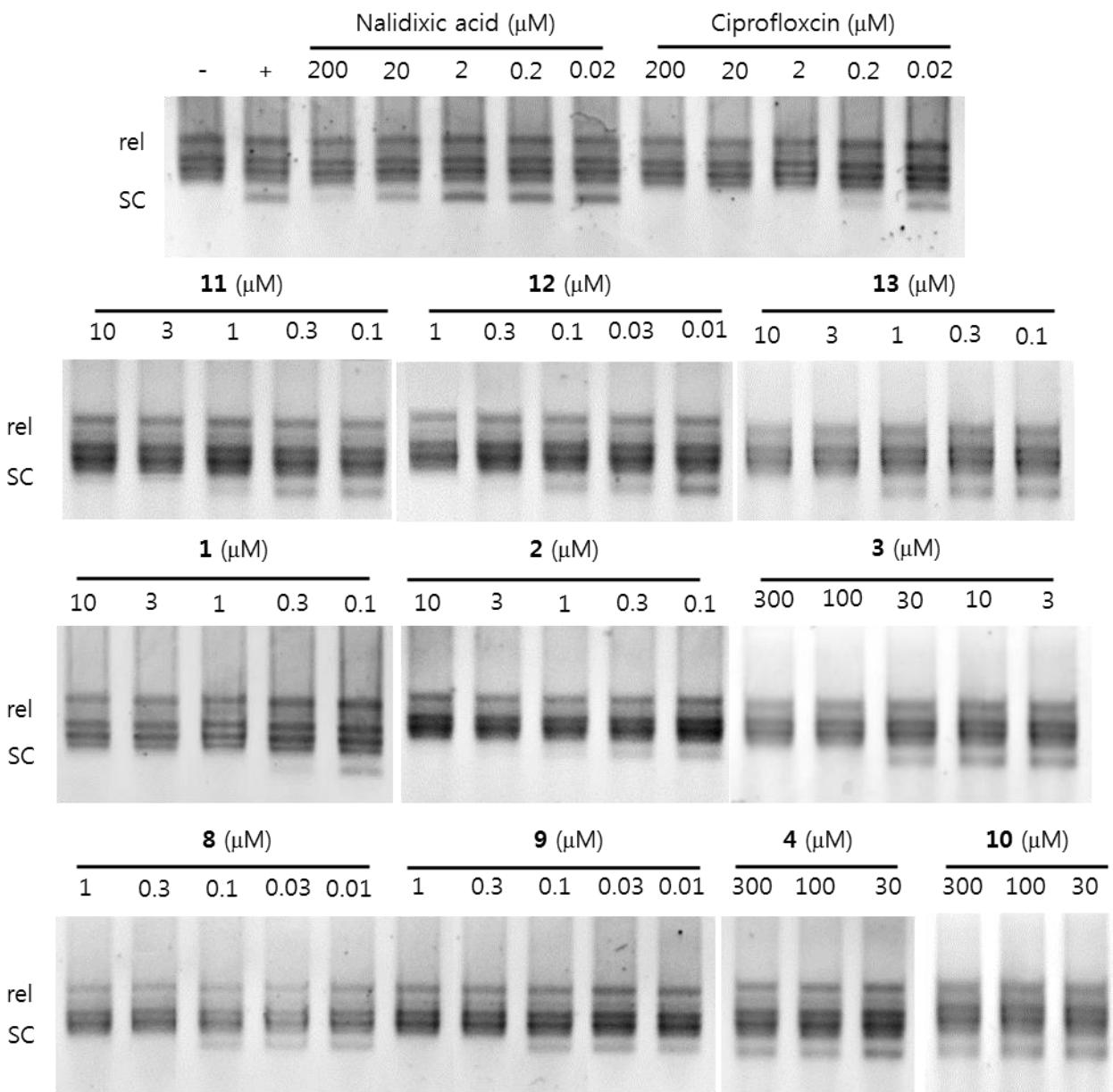
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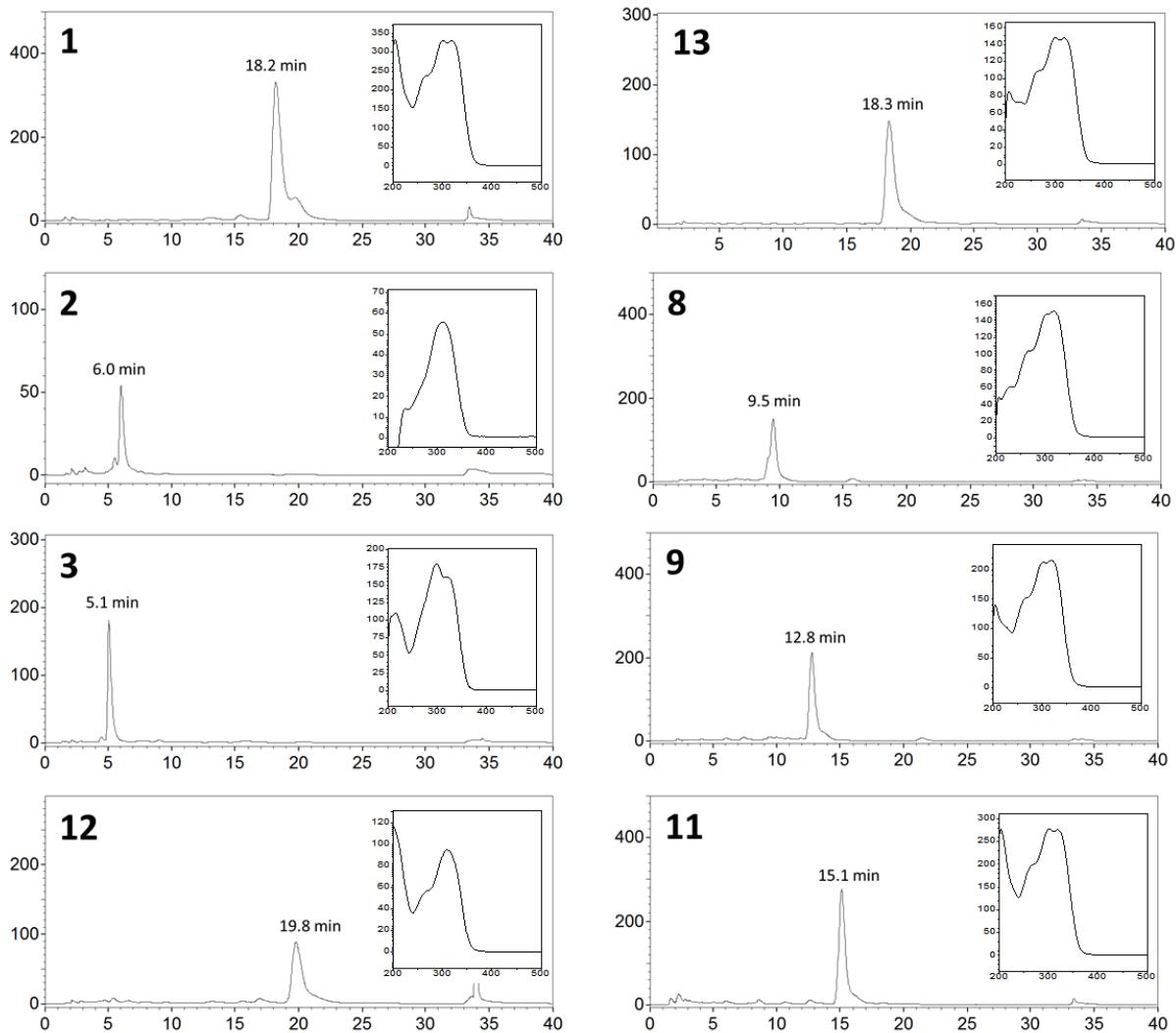
420 **Figure S59.** Agarose gels of *E. coli* gyrase supercoiling reactions inhibited by coralmycins (C (**1**),
 421 D (**2**), E (**3**), F (**4**), A (**12**), and B (**13**)) and cystobactamids (891-2 (**8**), 905-2 (**9**), 919-2 (**11**),
 422 and 507 (**10**)). (-), reaction without *E. coli* gyrase; (+), standard reaction in presence of 5% DMSO; rel,
 423 relaxed plasmid; SC, supercoiled plasmid.

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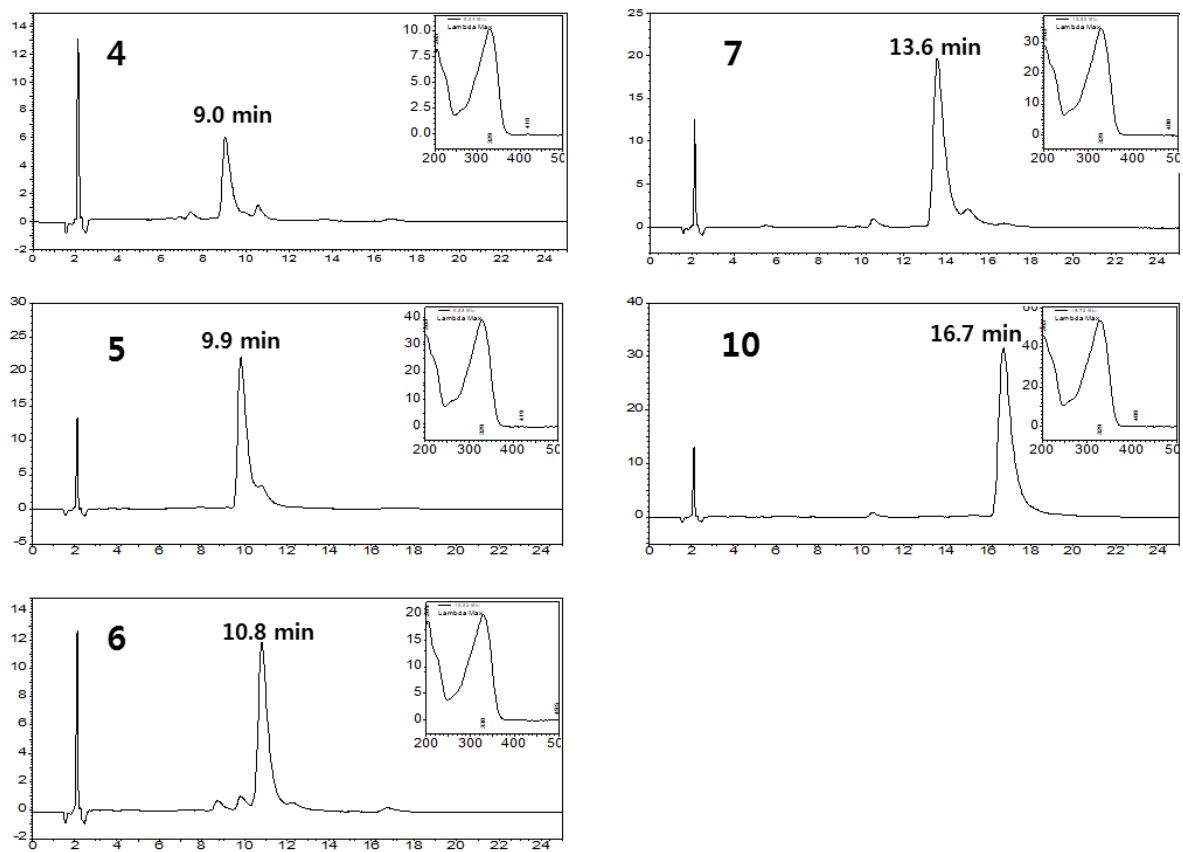
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429 **Figure S60.** HPLC profiles of coralmycins (C (1), D (2), E (3), A (12), and B (13)) and
430 cystobactamids (891-2 (8), 905-2 (9), and 919-2 (11)). J'sphere ODS-H80 (150x4.6 mm I.D.), 50%
431 ACN + 0.01% TFA, 0.8 mL/min, 25°C

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433

434 **Figure S61.** HPLC profiles of coralmycins F (4), G (5), H (6), and I (7), and cystobactamid 507
 435 (10). J'sphere ODS-H80 (150x4.6 mm I.D), 50% ACN + 0.01% TFA, 0.8 mL/min, 25°C.

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