Supporting Information

Penilumamide, a novel lumazine peptide isolated from the marine-derived fungus *Penicillium* sp. CNL-338

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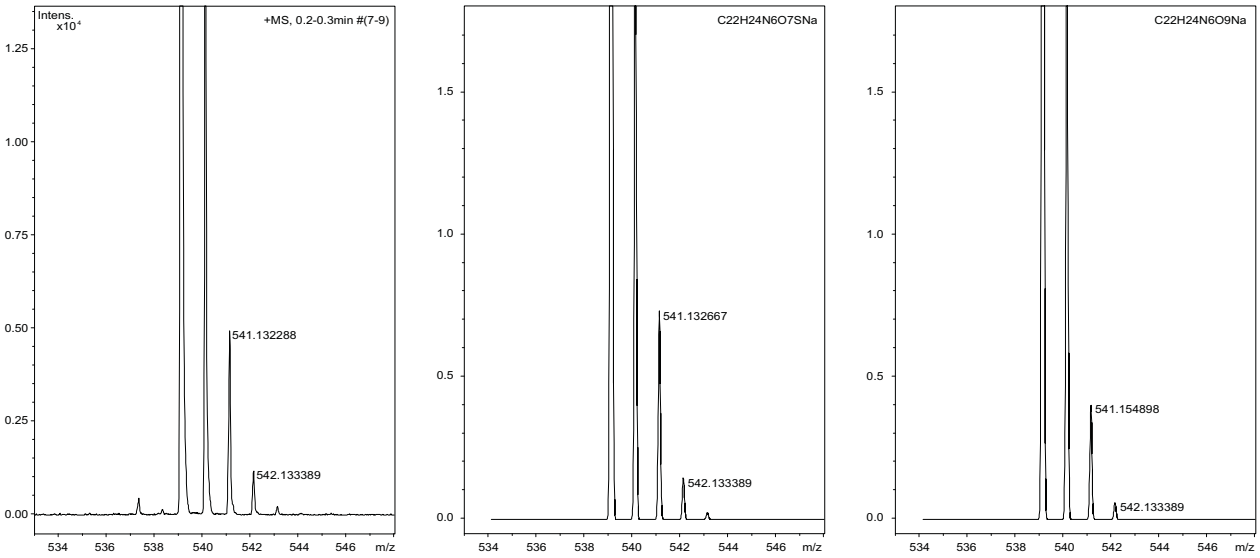


Figure S1. Comparison of the isotopic pattern of the molecular ion of penilumamide (**1**, left) with simulated mass spectra of two different alternative molecular formulae: $[C_{22}H_{24}N_6O_7SNa]^+$ (center) and $[C_{22}H_{24}N_6O_9Na]^+$ (right). The latter one was originally proposed from the HR FAB-MS data.

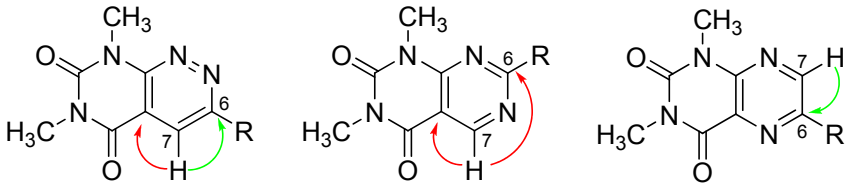


Figure S2. Structure elucidation of the heterocyclic moiety ($C_8H_7N_4O_2$) of penilumamide (**1**). The green arrows indicate correlations which are observed in the experiments whereas the red arrows indicate correlations which should be observed but were not observed experimentally (compare to Figure S7). The 1,4-aza variant (**2b**) is the constitution proposed for **1**.

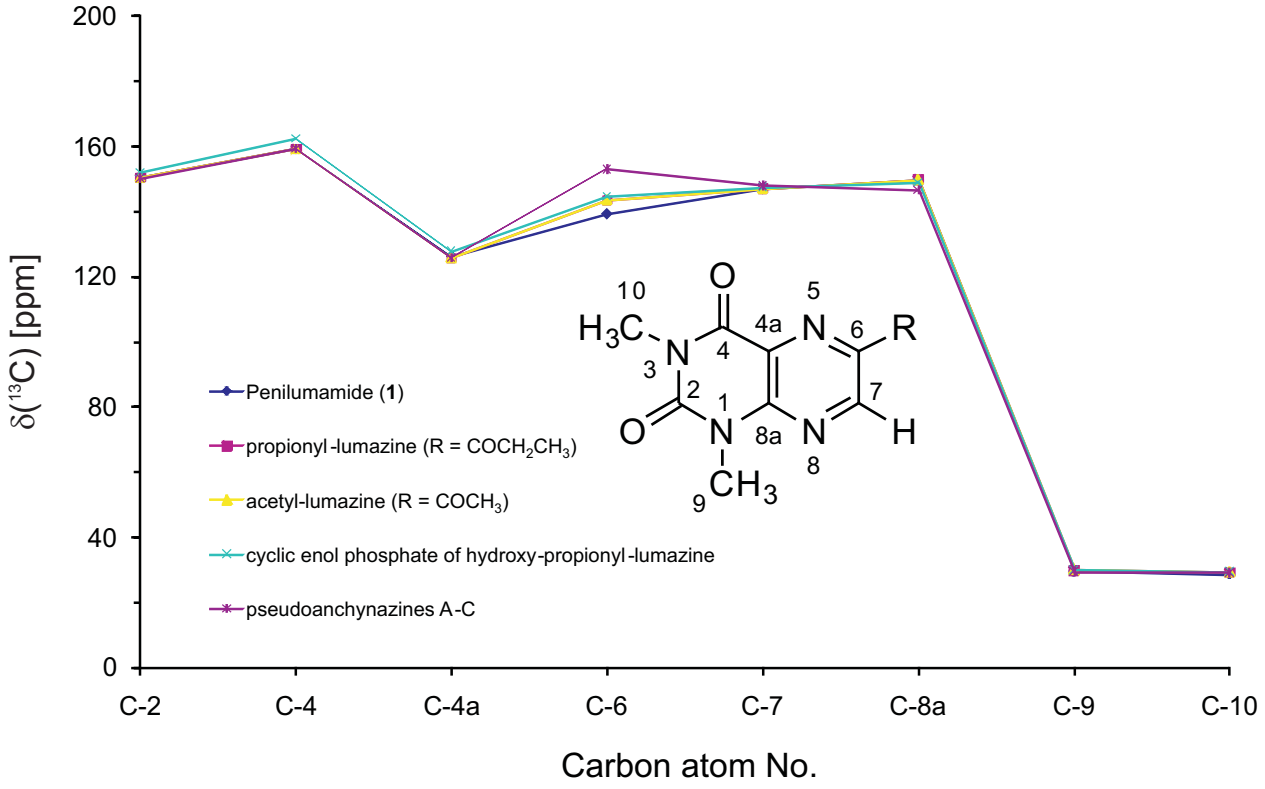
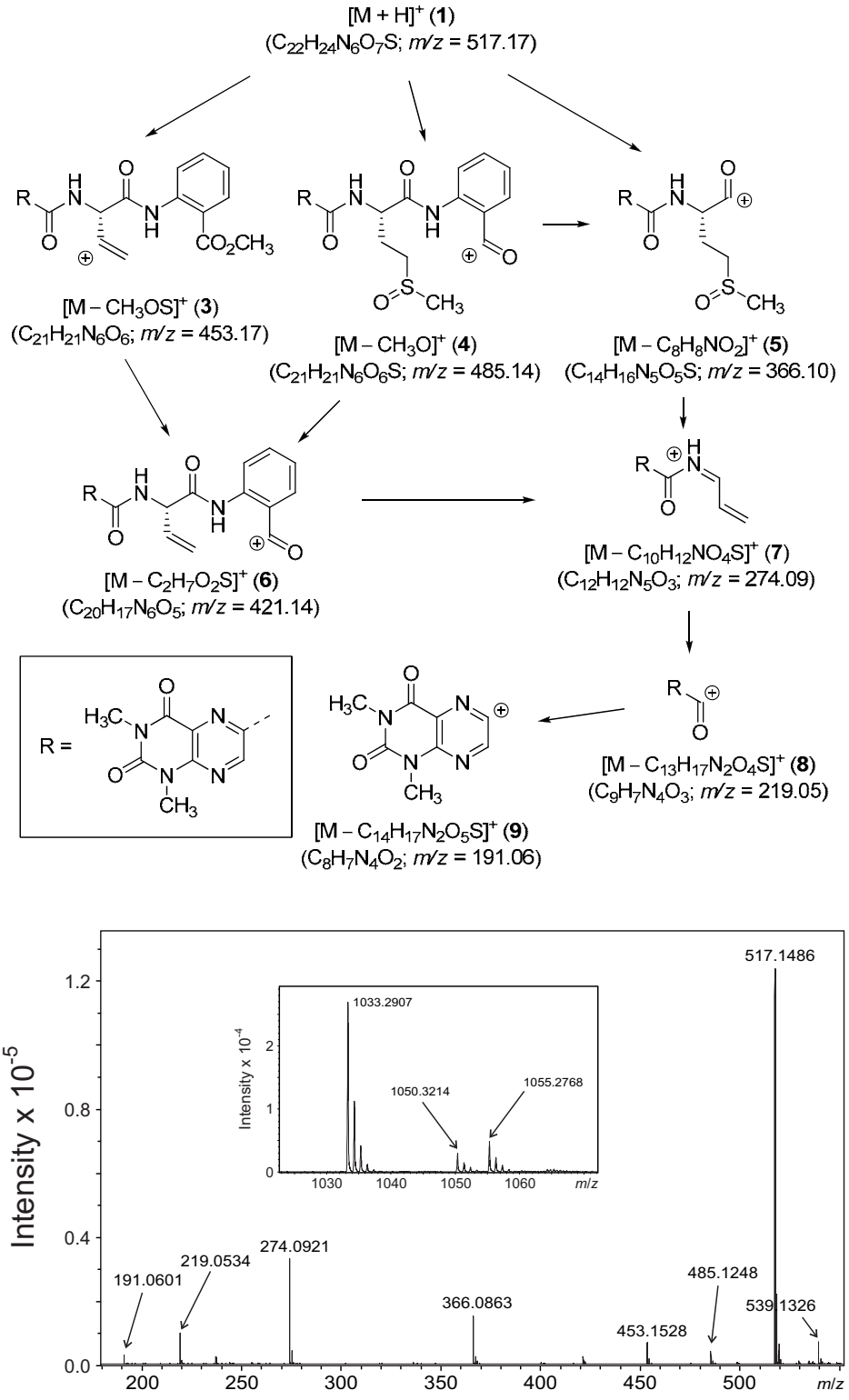


Figure S3. Comparison of the ^{13}C chemical shifts of penilumamide (**1**) with four similar substituted natural products.

Table S1. Comparison of the ^{13}C chemical shifts of penilumamide (**1**) with four similar substituted natural products.

Compound	C-2	C-4	C-4a	C-6	C-7	C-8a	C-9	C-10
Penilumamide (**1**)	150.4	159.2	126.1	139.3	146.8	149.5	29.4	28.6
Propionyl-lumazine (R = $COCH_2CH_3$)[a]	150.4	159.3	125.7	143.4	147.0	149.7	29.8	29.2
Acetyl-lumazine (R = $COCH_3$)[a]	150.4	159.2	125.8	143.6	147.1	149.8	29.8	29.2
Cyclic enol phosphate of hydroxy-propionyl-lumazine[a]	152.0	162.3	127.7	144.6	147.4	148.9	29.8	29.3
Pseudoanchynazines A-C	150.2	159.3	125.7	153.0	147.9	146.6	29.3	29.0

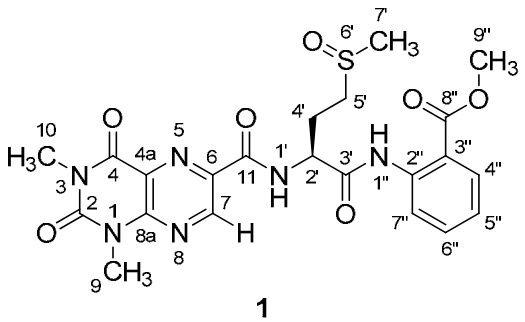
[a] These compounds were published without assignment of the carbon atoms. The assignment was carried out by us according to the given signal multiplicity.



Scheme S1. MS spectrum (bottom) and MS fragmentation pattern (top) of penilumamide (**1**): $[M+H]^+$ (*m/z* 517.1486), $[M+Na]^+$ (*m/z* 539.1326), $[2M+H]^+$ (*m/z* 1033.2907), $[2M+Na]^+$ (*m/z* 1055.2768).

Table S2. NMR correlations of penilumamide (**1**, 400 MHz, DMSO-d_6) in ppm. Underlined correlations are ^4J.

	δ(^{1}H), $CDCl_3$	δ(^{1}H)	δ(^{13}C)	δ(^{15}N)	COSY	HMBC	1,1-ADEQ
1	-	-	-	134	-	-	-
2	-	-	150.4	-	-	-	-
3	-	-	-	163	-	-	-
4	-	-	159.2	-	-	-	-
4a	-	-	126.1	-	-	-	-
5	-	-	-	333	-	-	-
6	-	-	139.3	-	-	-	-
7	9.45 (d, *J* = 9.2 Hz)	9.32 (s)	146.8	-	-	4a, 5, 8, 8a	6
8	-	-	-	304	-	-	-
8a	-	-	149.5	-	-	-	-
9	3.79 (s)	3.60 (s)	29.4	-	-	1, 2, 8	-
10	3.58 (s)	3.37 (s)	28.6	-	-	2, 3, 4	-
11	-	-	163.0	-	-	-	-
1’	9.18 (s)	9.31 (s)	-	121	-	13, 2’, 3’, 4’	-
2’	5.00 (ddd, *J* = 13.2, 8.9, 5.2 Hz)	4.85 (ddd, *J* = 13.2, 9.2, 4.8 Hz)	53.3	-	1’, 4’	13, 3’, 4’, 5’	-
3’	-	-	169.4	-	-	-	-
4’	2.47 - 2.58 (m) 2.62 - 2.72 (m)	2.30 - 2.39 (m) 2.42 - 2.49 (m)	23.8	-	2’, 5’	2’, 3’, 5’	-
5’	2.89 - 2.96 (m) 2.96 - 3.03 (m)	2.73 - 2.81 (m) 2.88 - 2.97 (m)	49.7	-	4’	2’, 4’, 7’	-
7’	2.64 (s)	2.57 (s)	37.9	-	-	5’	-
1’’	11.61 (s)	11.06 (s)	-	133	-	3’, 7’’	-
2’’	-	-	139.0	-	-	-	-
3’’	8.67 (d, *J* = 8.4 Hz)	8.33 (d, *J* = 7.9 Hz)	121.1	-	4’’	2’’, 5’’, 7’’	2’’, 4’’
4’’	7.56 (ddd, *J* = 8.0, 7.5, 1.2 Hz)	7.63 (ddd, *J* = 7.9, 7.5, 1.5 Hz)	134.0	-	3’’, 5’’, 6’’	2’’, 6’’, 3’’, 7’’	3’’, 5’’
5’’	7.13 (dd, *J* = 8.0, 7.5 Hz)	7.21 (dd, *J* = 7.5, 7.0 Hz)	123.6	-	4’’, 6’’	3’’, 4’’, 6’’, 7’’	4’’, 6’’
6’’	8.01 (dd, *J* = 8.0, 1.2 Hz)	7.90 (dd, *J* = 7.0, 1.5 Hz)	130.6	-	4’’, 5’’	2’’, 4’’, 8’’	5’’, 7’’
7’’	-	-	117.8	-	-	-	-
8’’	-	-	167.3	-	-	-	-
9’’	3.79 (s)	3.68 (s)	52.3	-	-	8’’	-



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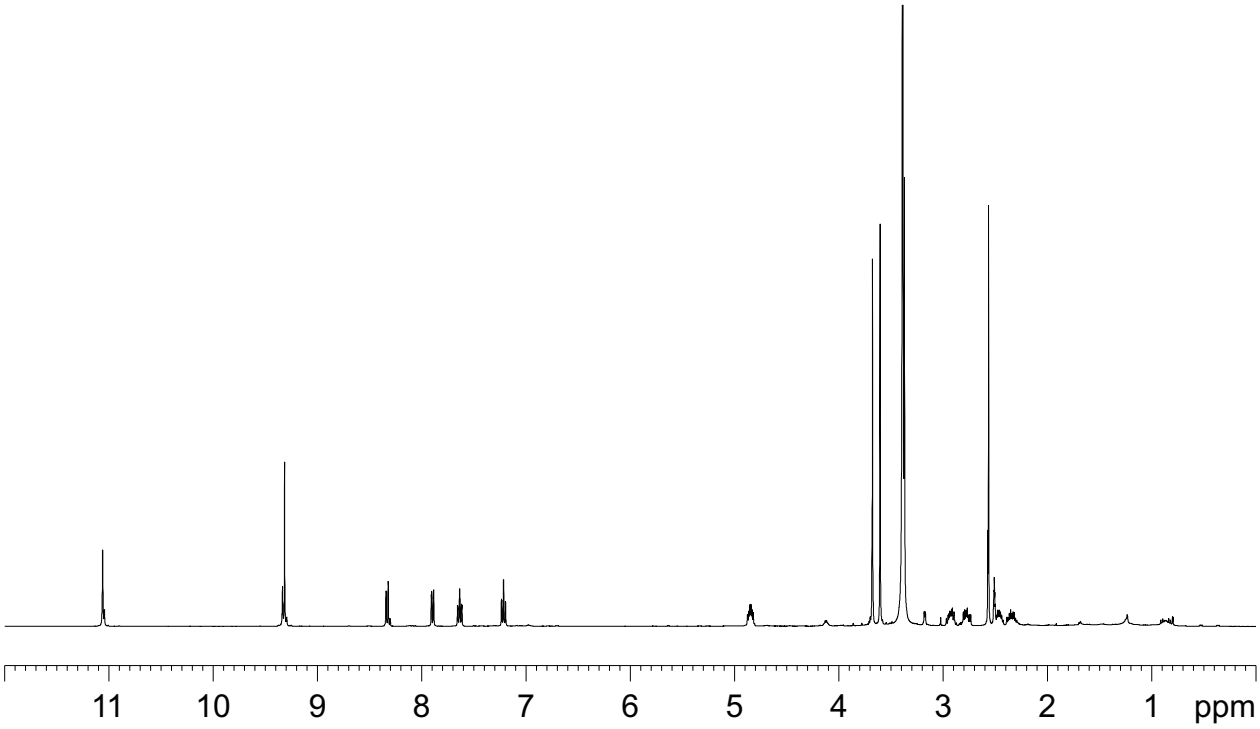


Figure S4. ^{1}H-NMR spectra of penilumamide (**1**) in DMSO-d_6.

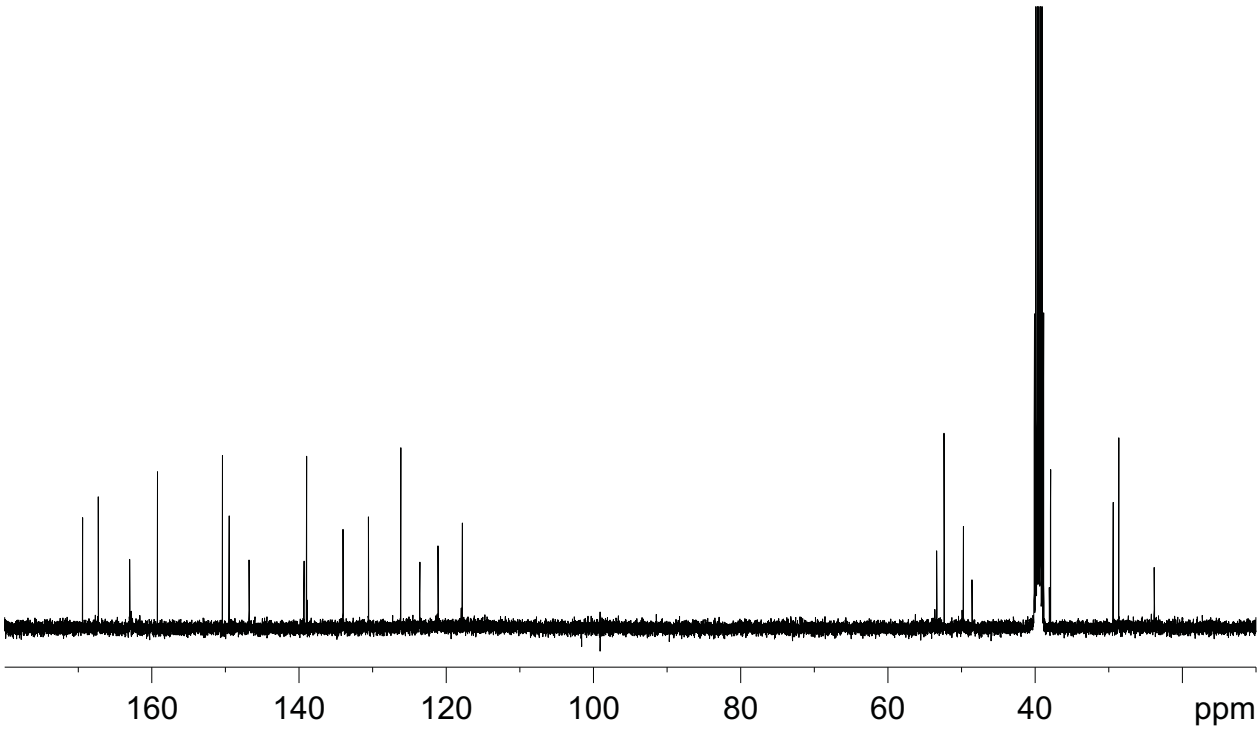


Figure S5. ^{13}C-NMR spectra of penilumamide (**1**) in DMSO-d_6.

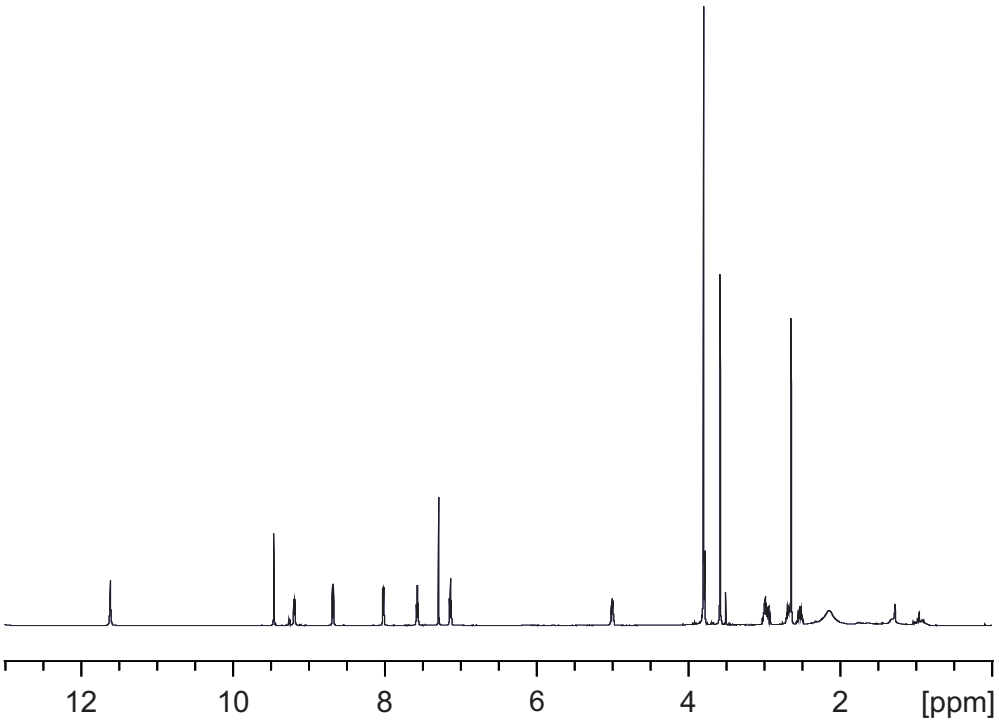


Figure S6. ^{1}H-NMR spectra of penilumamide (**1**) in $CDCl_3$.

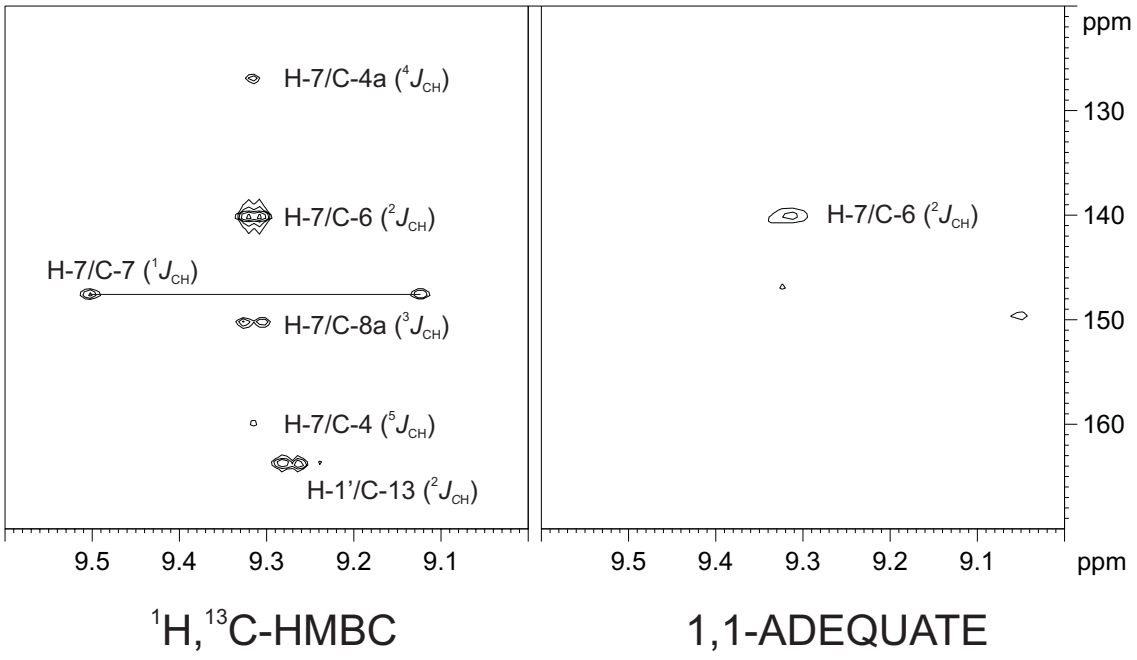


Figure S7. Comparison of a ^{1}H,^{13}C-HMBC and a 1,1-ADEQUATE spectrum of penilumamide (**1**). The fact that only one $^2J_{CH}$ correlation (H-7/C-6) can be observed in the 1,1-ADEQUATE spectrum proves the 1,4-aza constitution of the lumazine moiety of **1**.

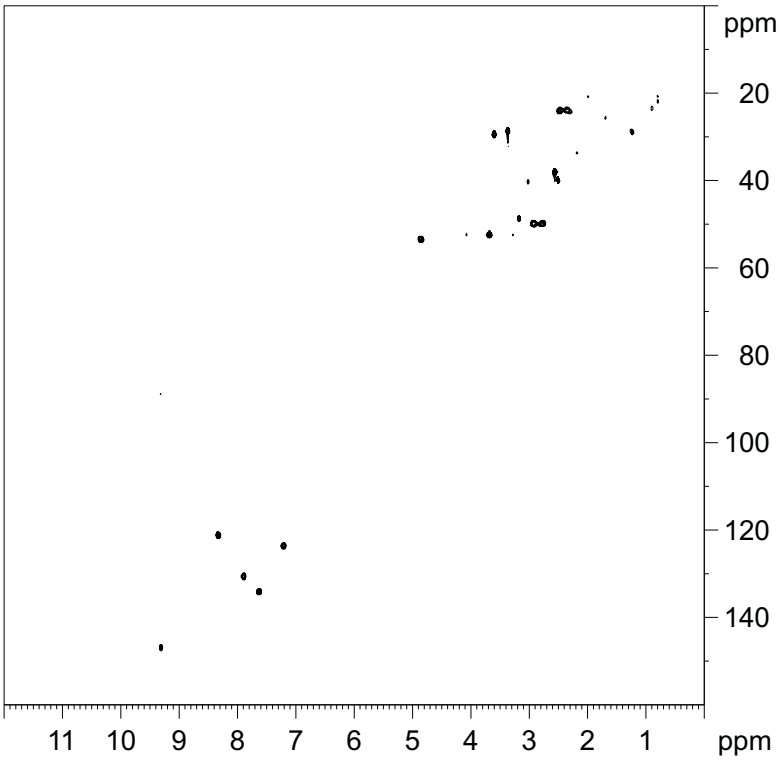


Figure S8. ^{1}H, ^{13}C-HSQC spectrum of penilumamide (**1**).

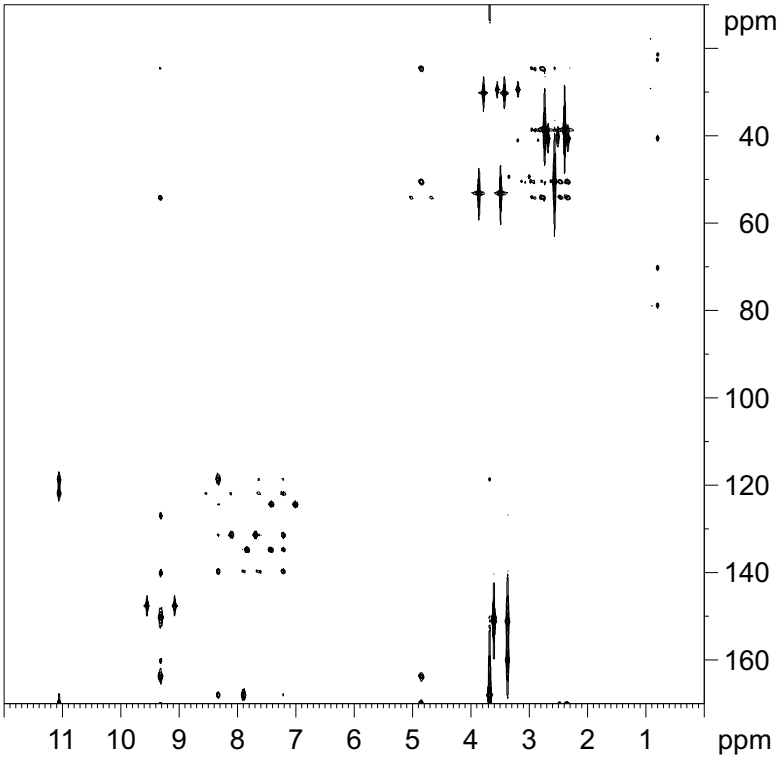


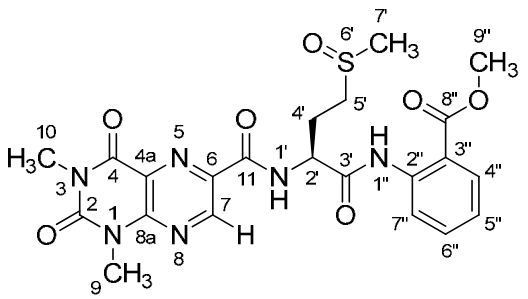
Figure S9. ^{1}H, ^{13}C-HMBC spectrum of penilumamide (**1**).

Table S3. 600 MHz NMR data and complex-induced ppm shift of penilumamide (**1**) with *R*- and *S*-MPAA in $CDCl_3$.[a]

Position (H-)	δ(^{1}H) [ppm]	Δδ [Hz] with *R*-MPAA	Δδ [Hz] with *S*-MPAA
7	9.46	−0.42	−0.70
9	3.80	−1.81[b]	−2.00[b]
10	3.44	−0.71	−1.40
1’	9.10	−7.11	−9.80
2’	4.85	± 0	−1.40
4’	2.47	−5.40	−6.60
	2.64	−2.50	−3.50
5’	2.88	+9.80	+10.60
	2.95	+0.81	+1.20
7’	2.60	+1.64	+2.40
1’’	11.58	−1.57	−2.70
3’’	8.69	−1.60	−2.40
4’’	7.58	± 0	± 0
5’’	7.14	± 0	± 0
6’’	8.02	± 0	± 0
9’’	3.80	−1.81[b]	−2.00[b]

[a] Used concentrations: a) 2.1 mg penilumamide (**1**) with 0.6 mg *R*-MPAA and b) 2.3 mg penilumamide (**1**) with 0.8 mg *S*-MPAA.

[b] Only one signal at 3.80 ppm (H-9 or H-9’’) was affected by *R*-/*S*-MPAA.



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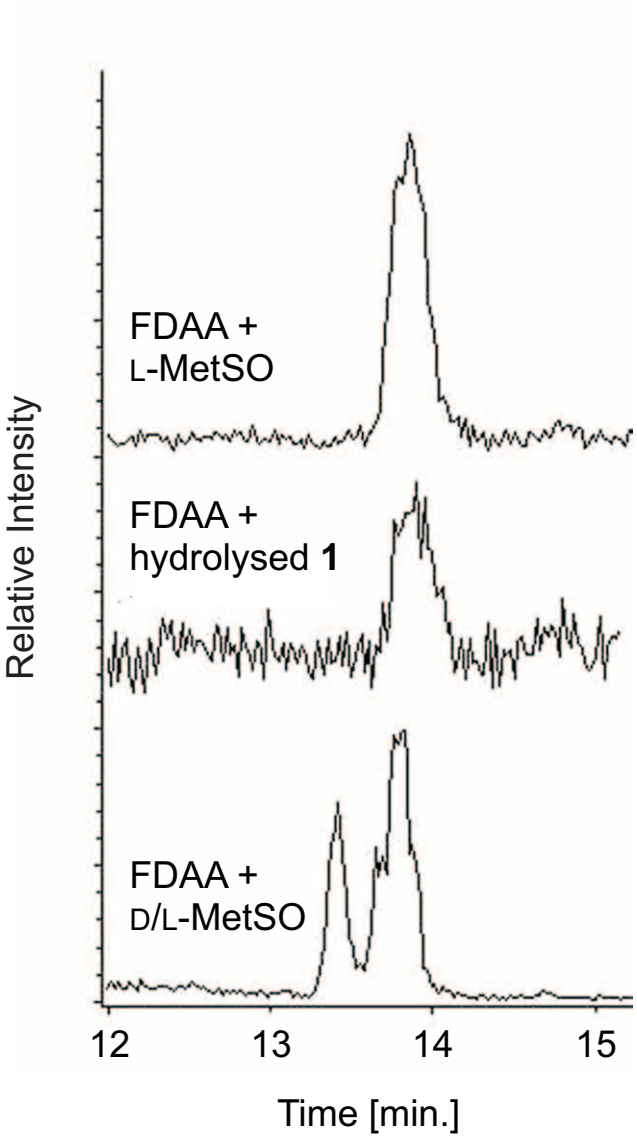

Figure S10. Retention times of FDAA-modified methionine sulfoxides and penilumamide (**1**).