Electronic Supplementary Material (ESI) for MedChemComm.

On the Inhibition of Capsaicin Response in Dorsal Root Ganglion Neurons by Nobilamide B and Analogues: A Structure-Activity Relationship Study

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SUPPORTING INFORMATION

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Synthesis of Fmoc-Ala-(*Z*)Dhb-OH

Dipeptide precursor Fmoc-Ala-(*Z*)Dhb-OH was synthesized as previously described.[6] H-Thr-O^{t}Bu·HCl (1 eq) was dissolved in DCM (15 mL) with triethylamine (0.9 eq) and stirred for 30 minutes. In a separate reaction, Fmoc-Ala-OH (1.2 eq) was stirred in DCM (10 mL) to which EDC·HCl (1.3 eq) was added. Each mixture was stirred for a few seconds before they were combined. The reaction mixture was left stirring for 24 hrs at room temperature under N_2, to form **Fmoc-Ala-Thr-O^{t}Bu** which is a white crystalline powder. TLC (SiO_2, 9:1 DCM/MeOH) R_f = 0.72. Percent yield = 76%.

Fmoc-Ala-Thr-O^{t}Bu (1 eq) was dissolved in 12:1 DCM/DMF (15 mL) and mixed with EDC·HCl (2 eq). A suspension containing CuCl (1.2 eq) in 5 mL 12:1 DCM/DMF was added next. The reaction mixture was allowed to stir for 48 hrs at room temperature under N_2. This step dehydrated Thr into (*Z*)Dhb, yielding **Fmoc-Ala-(*Z*)Dhb-O^{t}Bu**. The product is a yellowish white powder. TLC (SiO_2, 9:1 DCM/MeOH) R_f = 0.89. Percent yield = 54.5%.

Fmoc-Ala-(*Z*)Dhb-O^{t}Bu (1 eq) was dissolved in 95:5 TFA/DCM (2 mL) and stirred for 3 hrs at room temperature. TFA was removed by co-evaporation with DCM, followed by methanol, and finally diethylether to precipitate out and dry **Fmoc-Ala-(*Z*)Dhb-OH**. The product is a white powder. TLC (SiO_2, 9:1 DCM/MeOH) R_f = 0.30. Percent yield = 95%. Analytical HPLC (30% to 100% B over 30 minutes) t_R = 19.8 min. ESI-MS $[M+H]^+$: 395.1621 m/z ($C_{22}H_{22}N_2O_5$ = 394.42 Da). ^{1}H NMR (500 MHz, CD_3OD): 7.80 (2 H, d, $^3J_{HH}$ = 7.6 Hz, Fmoc Ph-**H**); 7.71-7.65 (2 H, m, Fmoc Ph-**H**); 7.39 (2 H, t, $^3J_{HH}$ = 7.5 Hz, Fmoc Ph-**H**); 7.31 (2 H, t, $^3J_{HH}$ = 7.4 Hz, Fmoc Ph-**H**); 6.86 (1 H, broad s, Dhb C$^\beta$**H**); 4.41-4.35 (2 H, m, Fmoc C**H**$_2$); 4.29-4.22 (2 H, m, Fmoc C**H**, Ala C$^\alpha$**H**); 1.75 (3 H, broad d, $^3J_{HH}$ = 5.5 Hz, Dhb C**H**$_3$); 1.42 (3 H, d, $^3J_{HH}$ = 7.1 Hz, Ala C**H**$_3$). ^{13}C NMR (125 MHz, $CDCl_3$): 171.35 (**C**=O Ala), 167.52 (**C**=O Dhb), 156.33 (**C**=O Fmoc), 143.70 (**C** Ph Fmoc), 143.59 (**C** Ph Fmoc), 141.25 (**C** Ph Fmoc), 136.73 (**C**H Dhb), 127.76 (**C** Ph Fmoc), 127.10 (**C** Dhb), 127.08 (**C** Ph Fmoc), 125.54 (**C** Ph Fmoc), 125.07 (**C** Ph Fmoc), 125.02 (**C** Ph Fmoc), 119.99 (**C** Ph Fmoc), 67.22 (**C**H$_2$ Fmoc), 50.62 (**C**$^\alpha$H Ala), 47.01 (**C**H Fmoc), 18.54 (**C**H$_3$ Ala), 14.71 (**C**H$_3$ Dhb).

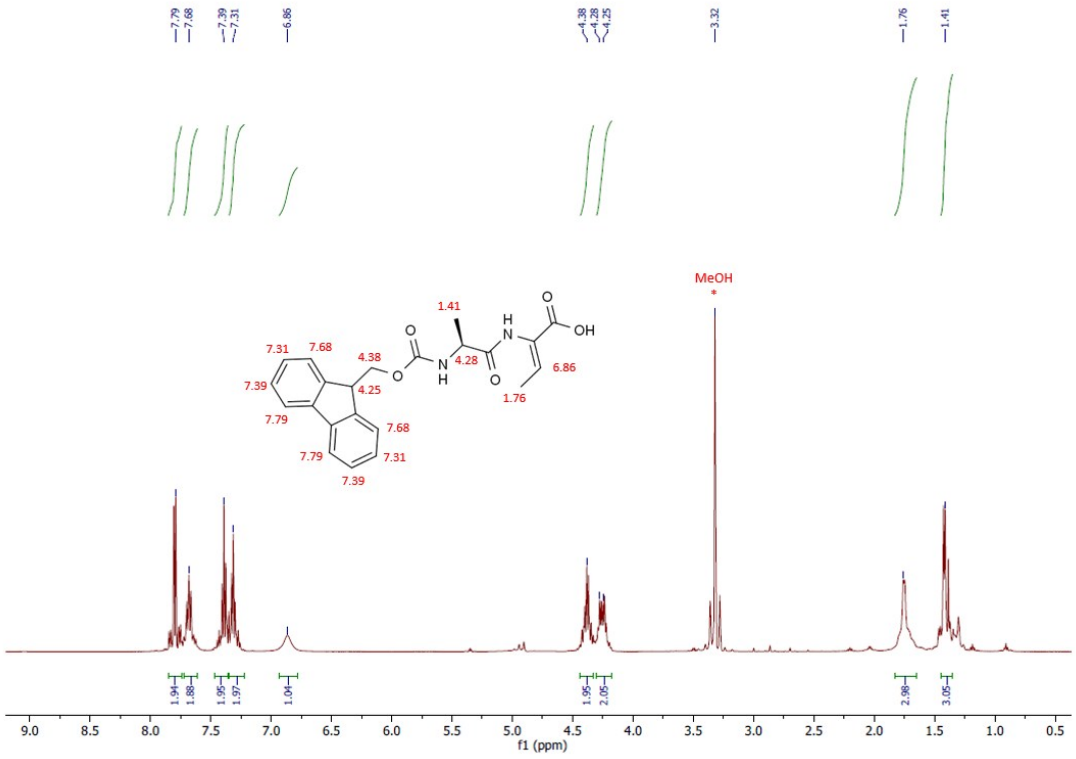

Figure S1. ^{1}H NMR of dipeptide **Fmoc-Ala-(*Z*)Dhb-OH** in CD_3OD.

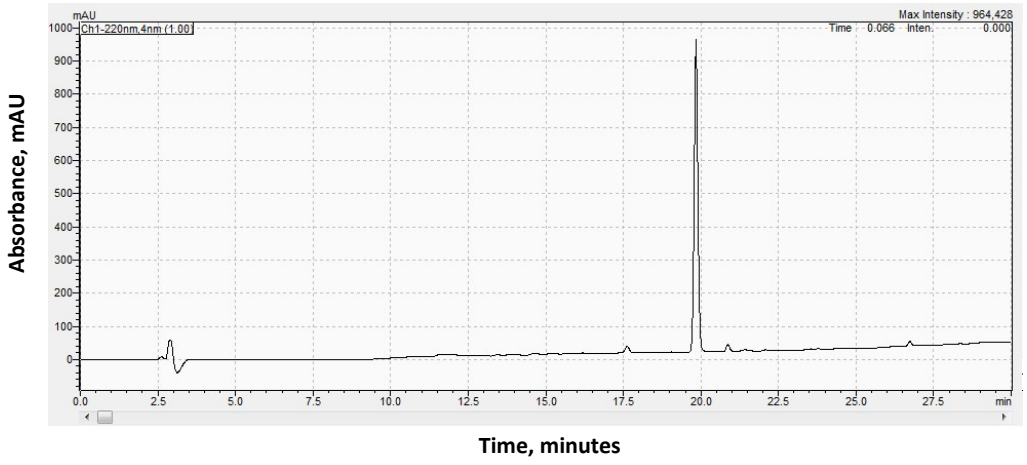

Figure S2. Analytical RP-HPLC profile of dipeptide **Fmoc-Ala-(*Z*)Dhb-OH**. Compound elutes at t_R = 19.8 min. A linear gradient of 30% to 100% MeCN over 30 minutes was used with 1 mL/min flow rate. Absorbance was detected at 220 nm.

^{1}H NMR data of nobilamide B and analogues

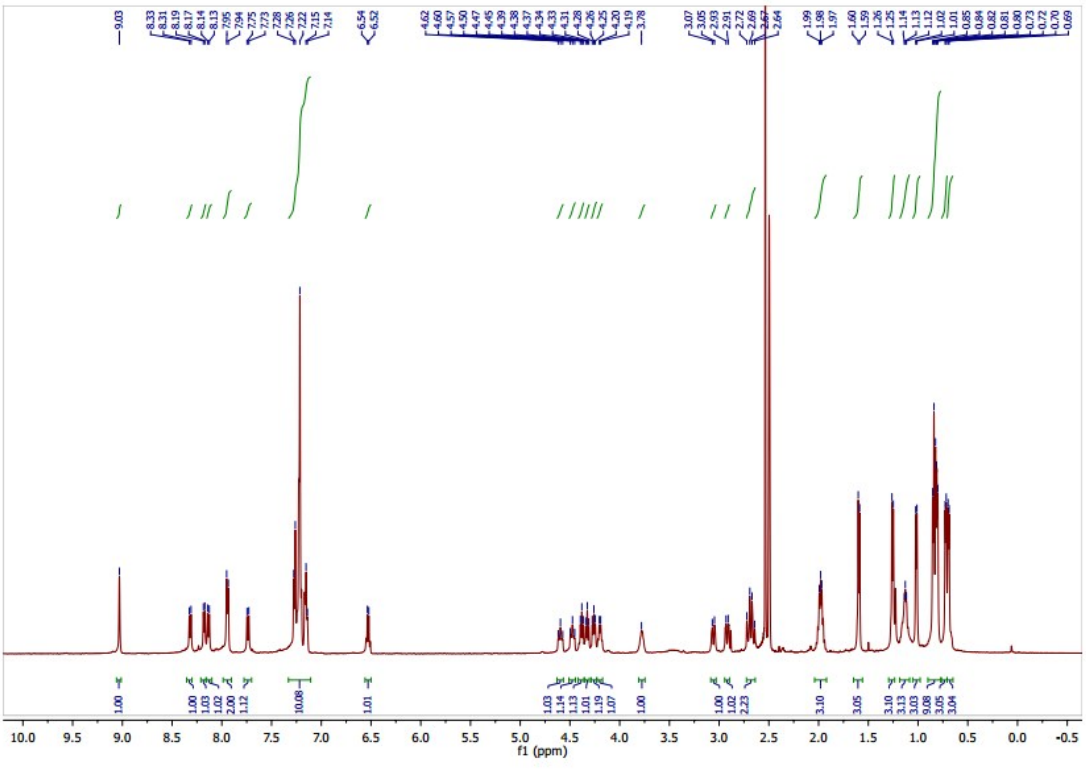

Figure S3. ^{1}H NMR of nobilamide B (**1**) in DMSO-d_6.

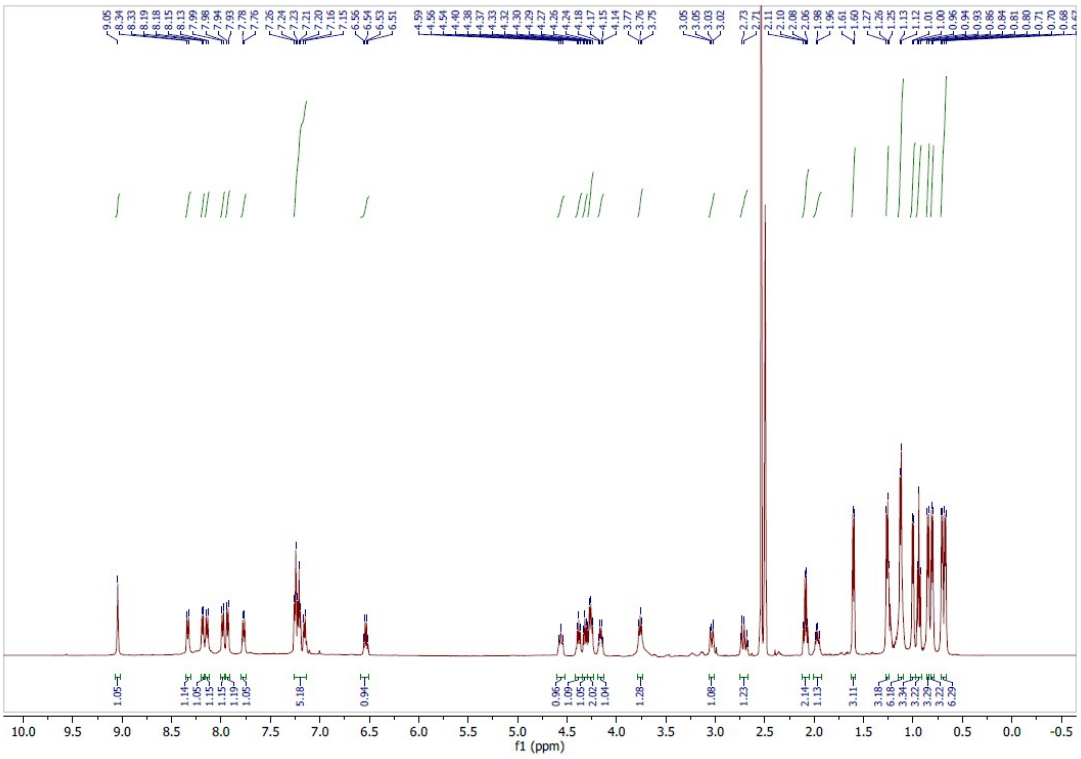

Figure S4. ^{1}H NMR of [Ala1]-nobilamide B (**2**) in DMSO-d_6.

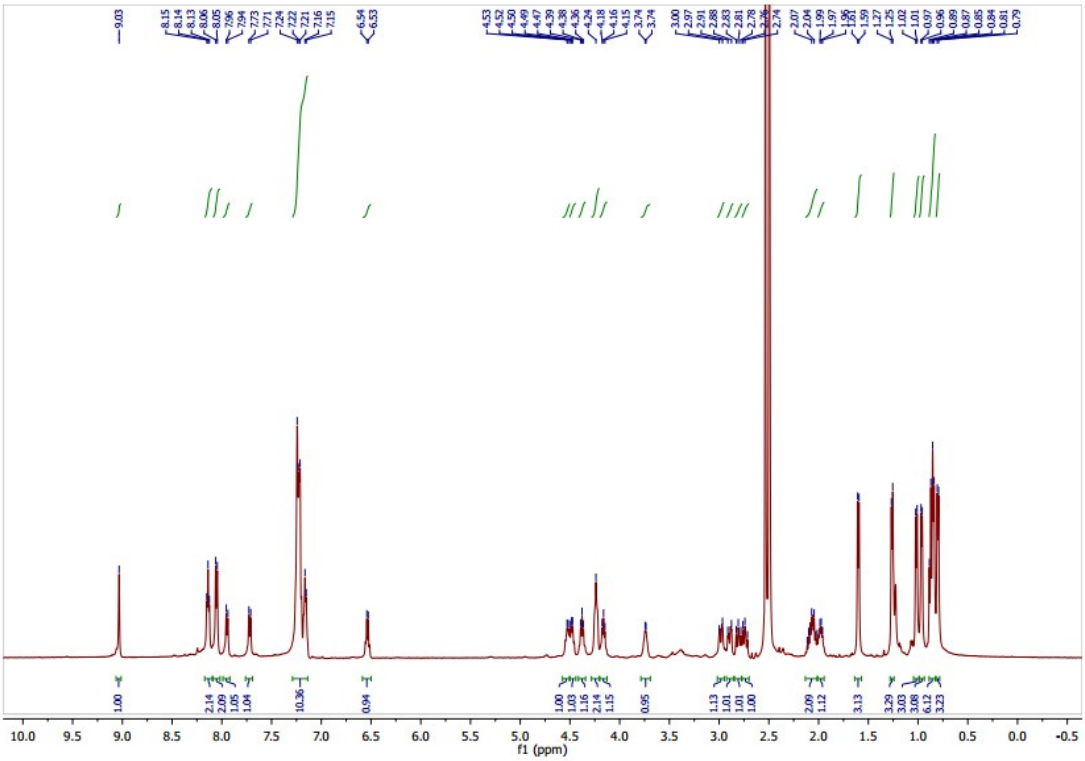

Figure S5. ^{1}H NMR of [Ala2]-nobilamide B (**3**) in DMSO-d_6.

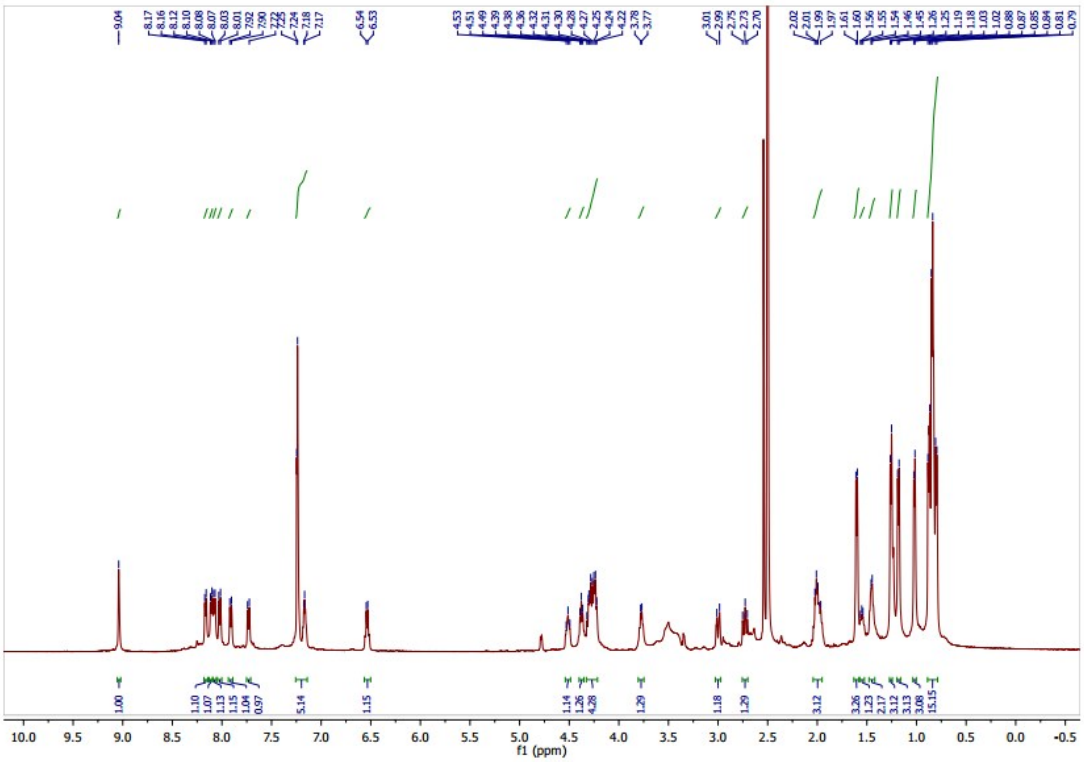

Figure S6. ^{1}H NMR of [Ala3]-nobilamide B (**4**) in DMSO-d_6.

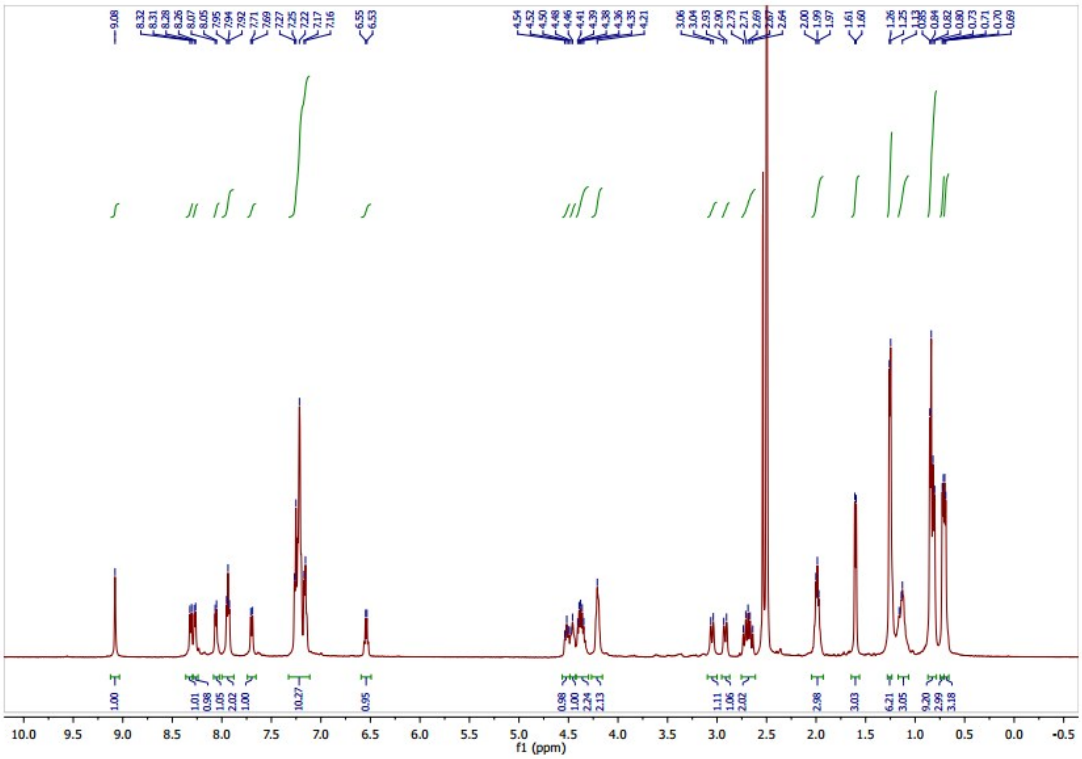

Figure S7. ^{1}H NMR of [Ala4]-nobilamide B (**5**) in DMSO-d_6.

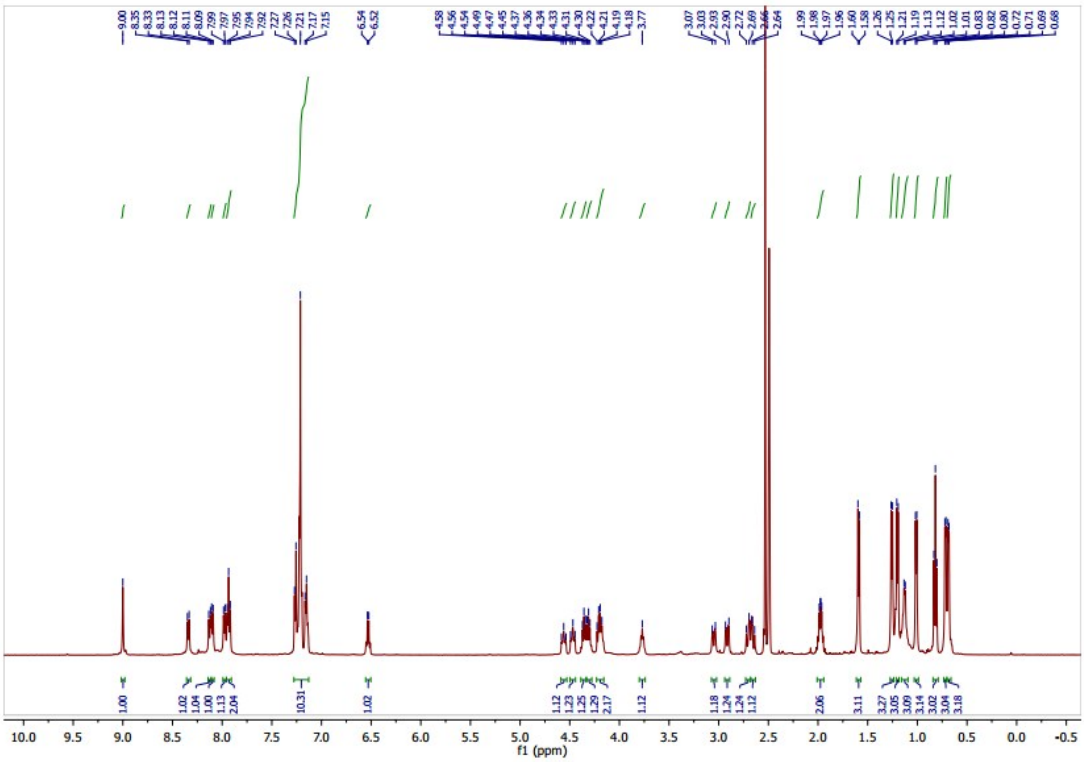

Figure S8. ^{1}H NMR of [Ala5]-nobilamide B (**6**) in DMSO-d_6.

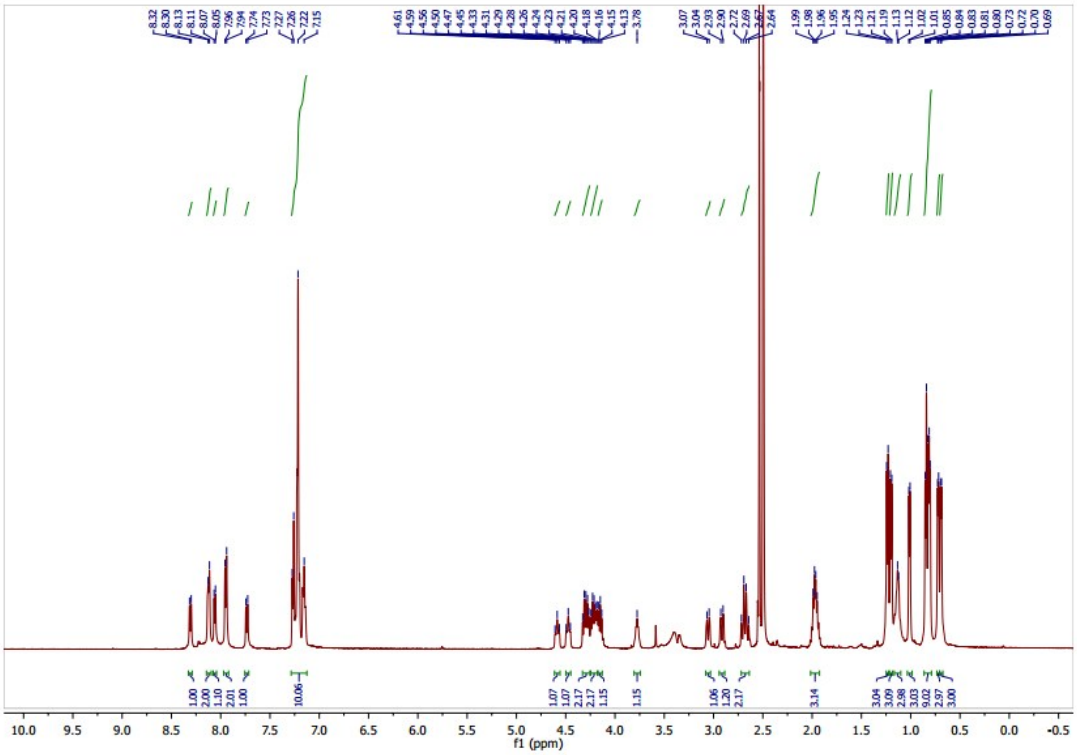

Figure S9. ^{1}H NMR of [Ala7]-nobilamide B (**7**) in DMSO-d_6.

^{13}C NMR data of nobilamide B and analogues

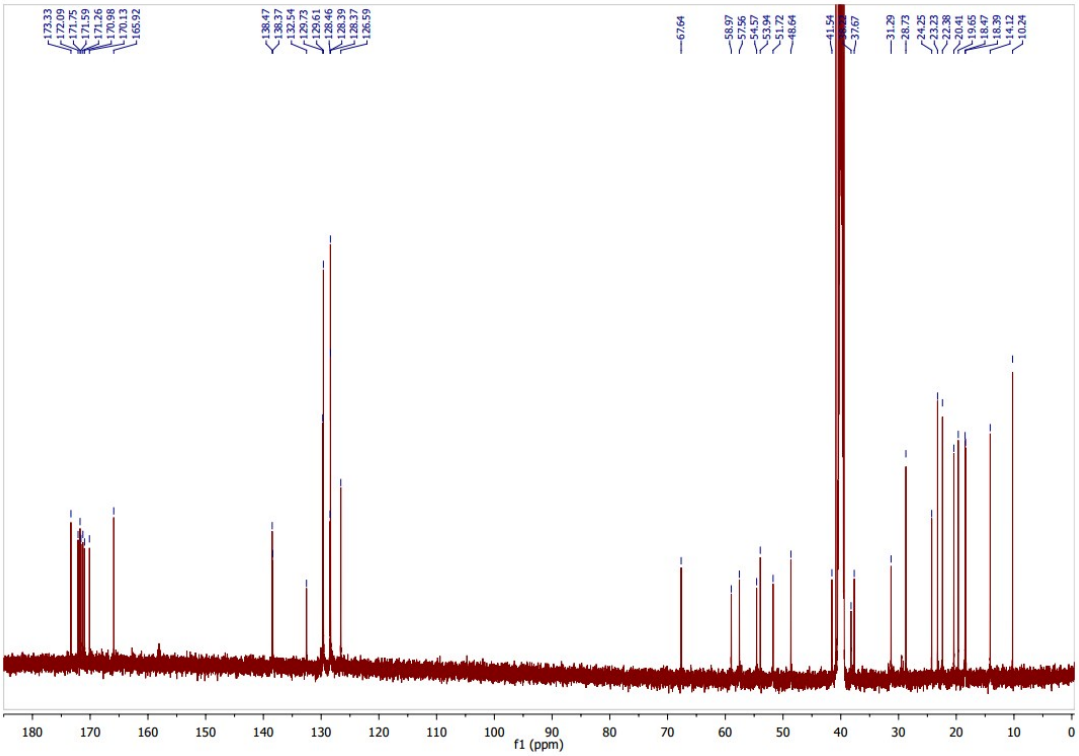

Figure S10. ^{13}C NMR of nobilamide B (**1**) in DMSO-d_6.

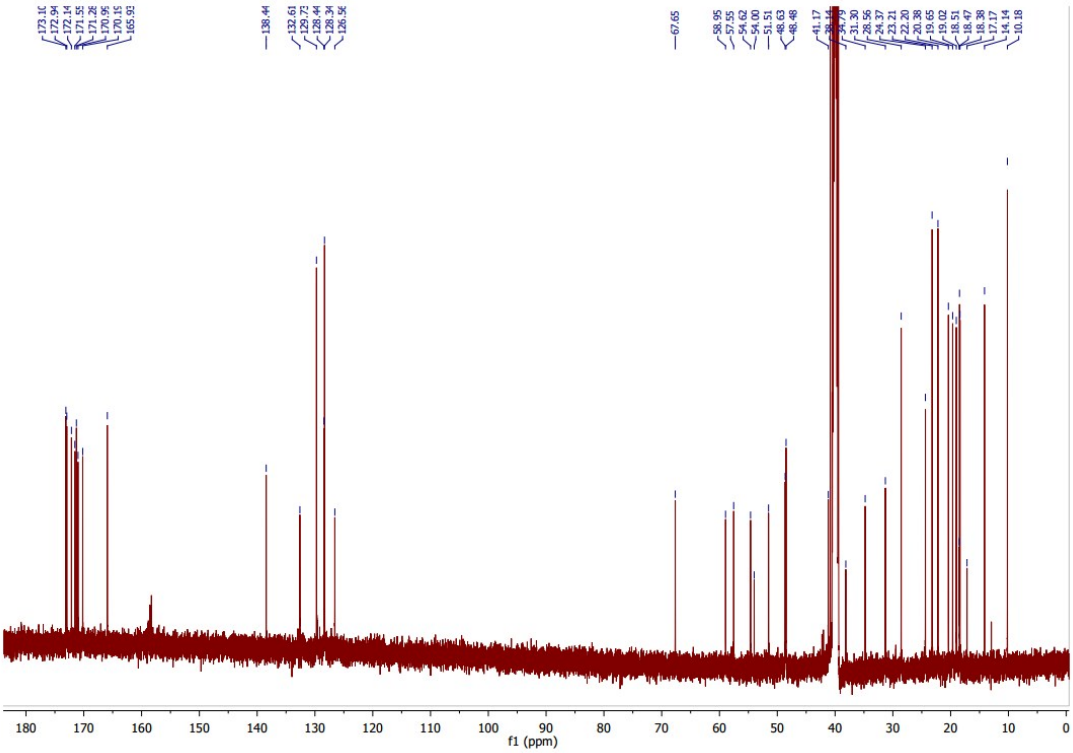

Figure S11. ^{13}C NMR of [Ala1]-nobilamide B (**2**) in DMSO-d_6.

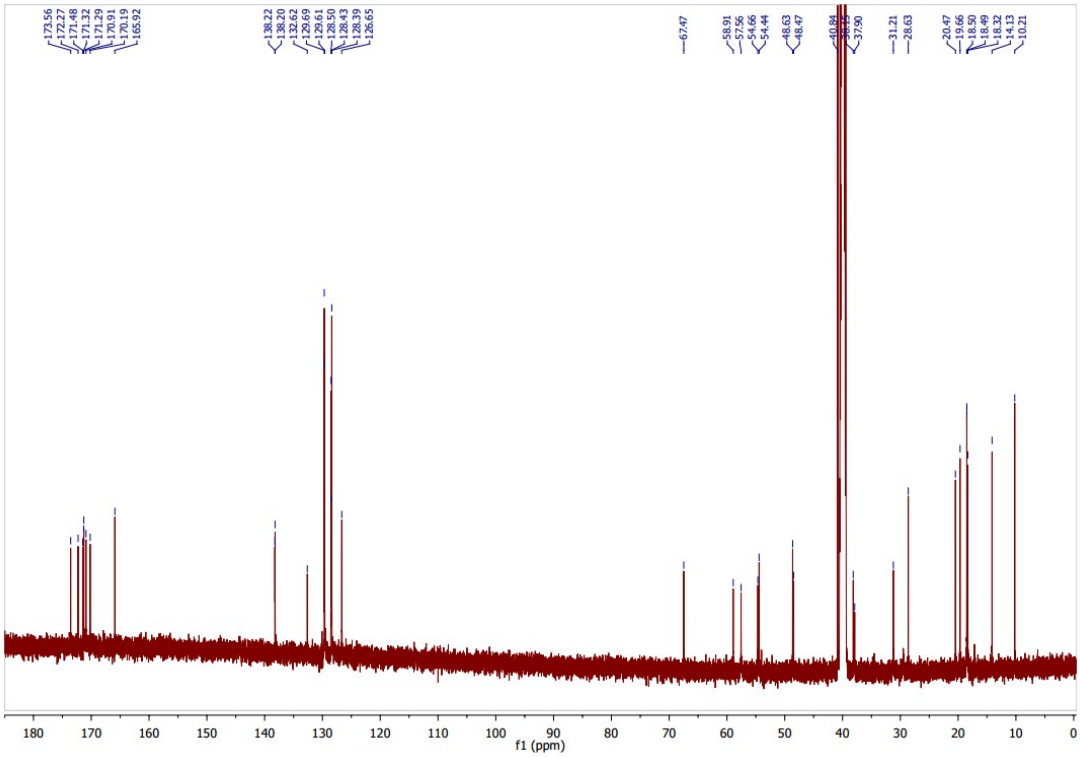

Figure S12. ^{13}C NMR of [Ala2]-nobilamide B (**3**) in DMSO-d_6.

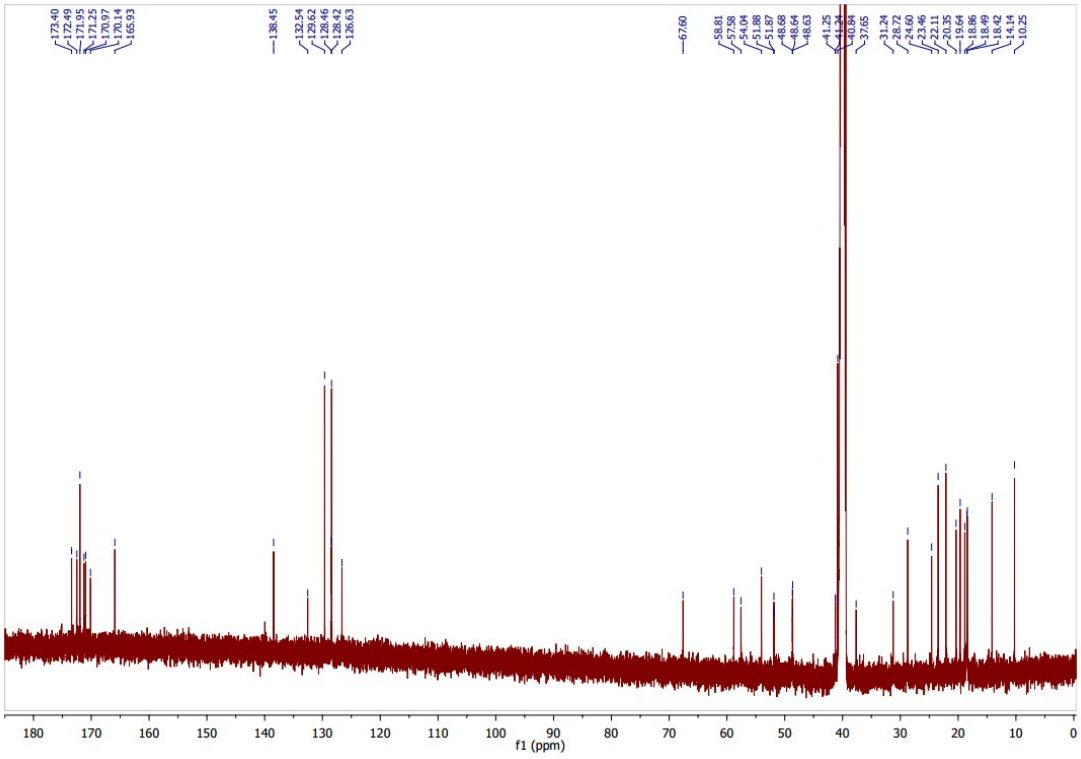

Figure S13. ^{13}C NMR of [Ala3]-nobilamide B (**4**) in DMSO-d_6.

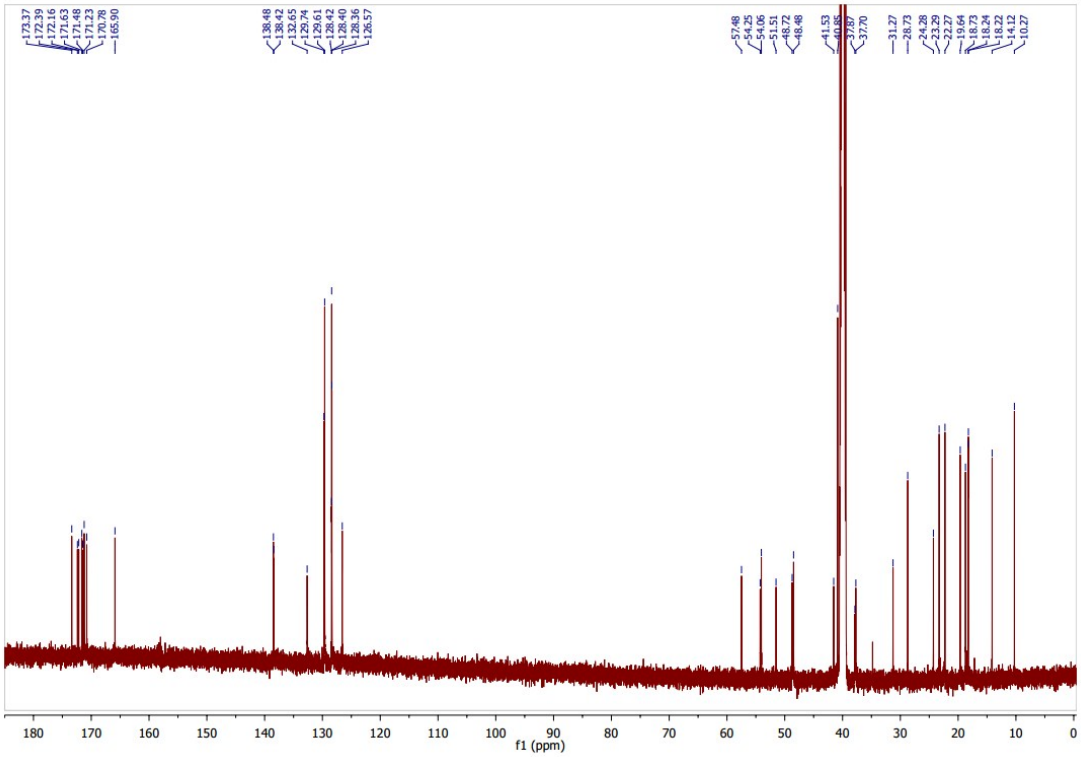

Figure S14. ^{13}C NMR of [Ala4]-nobilamide B (**5**) in DMSO-d_6.

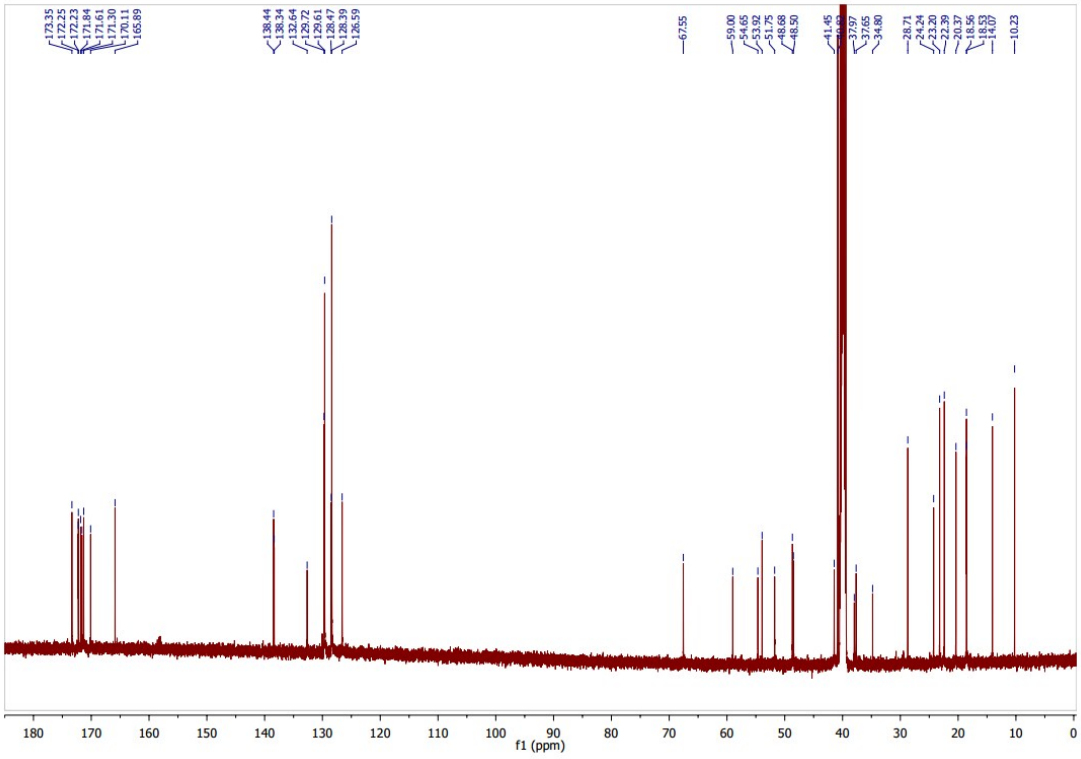

Figure S15. ^{13}C NMR of [Ala5]-nobilamide B (**6**) in DMSO-d_6.

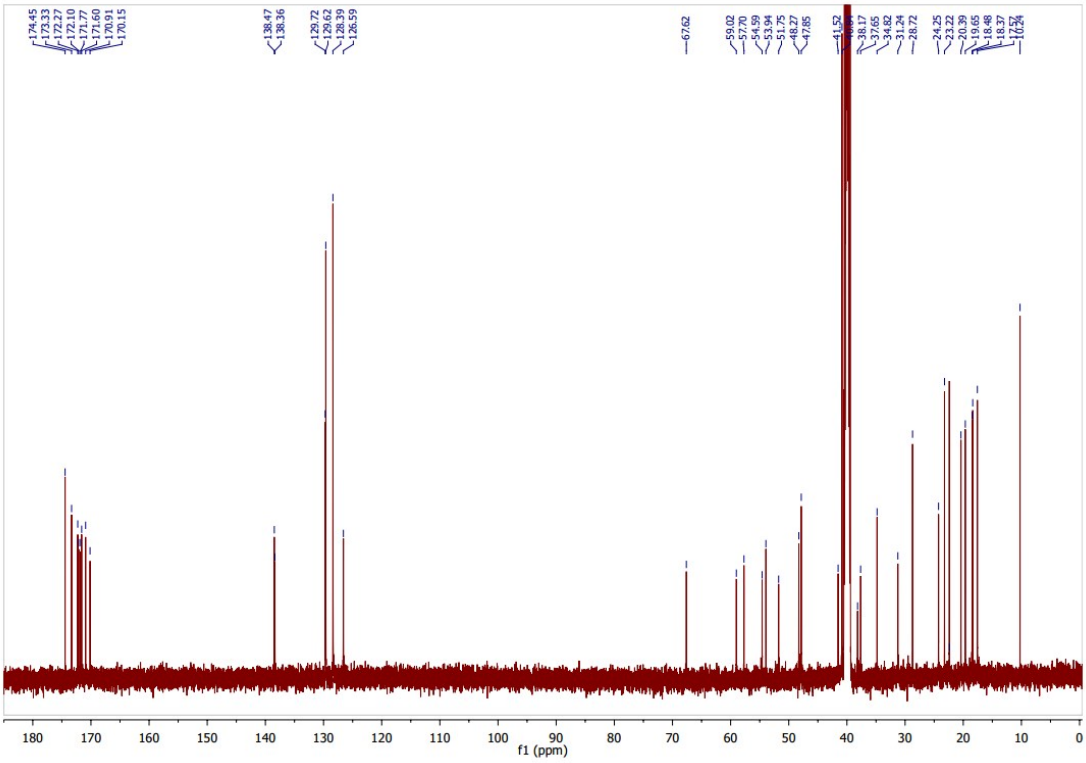

Figure S16. ^{13}C NMR of [Ala7]-nobilamide B (**7**) in DMSO-d_6.

Table S1. ^{13}C chemical shifts of peptides **1** to **7**.

		Chemical shift, ppm						
Residue	No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Z-Dhb	1	169.52 qC	165.93 qC	165.52 qC	165.93 qC	165.90 qC	165.89 qC	
	2	128.46 qC	128.44 qC	128.43 qC	128.46 qC	128.42 qC	128.47 qC	
	3	132.54 CH	132.61 CH	132.62 CH	132.54 CH	132.65 CH	132.64 CH	
	4	14.12 CH_3	14.14 CH_3	14.13 CH_3	14.14 CH_3	14.12 CH_3	14.07 CH_3	
L-Ala[6]	1	171.26 qC	171.28 qC	171.32 qC	171.25 qC	171.23 qC	171.30 qC	172.27 qC
	2	48.64 CH	48.63 CH	48.63 CH	48.63 CH	48.48 CH	48.68 CH	48.27 CH
	3	18.47 CH_3	18.47 CH_3	18.50 CH_3	18.49 CH_3	18.73 CH_3	18.56 CH_3	18.37 CH_3
L-Val	1	170.98 qC	170.99 qC	170.91 qC	170.97 qC	170.78 qC		170.91 qC
	2	57.56 CH	57.55 CH	57.56 CH	57.58 CH	57.48 CH		57.70 CH
	3	31.29 CH	31.30 CH	31.21 CH	31.24 CH	31.27 CH		31.24 CH
	4/5	18.39/19.65 CH_3	18.38/19.65 CH_3	18.32/19.66 CH_3	18.42/19.64 CH_3	18.22/19.64 CH_3		18.48/19.65 CH_3
D-a-Thr	1	170.13 qC	170.19 qC	170.19 qC	170.14 qC		170.11 qC	170.15 qC
	2	58.97 CH	58.95 CH	67.47 CH	58.51 CH		59.00 CH	59.02 CH
	3	67.64 CH	67.65 CH	58.91 CH	67.60 CH		67.55 CH	67.62 CH
	4	20.41 CH_3	20.38 CH_3	20.47 CH_3	20.35 CH_3		20.37 CH_3	20.39 CH_3

Table S1 *(cont'd)*. ^{13}C chemical shifts of peptides **1** to **7**.

Residue	No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**
L-Phe	1	171.59 qC	171.55 qC	171.29 qC		171.48 qC	171.61 qC	171.60 qC
	2	54.57 CH	54.62 CH	54.66 CH		54.25 CH	54.65 CH	54.59 CH
	3	38.22 CH_2	38.14 CH_2	37.90 CH_2		37.87 CH_2	37.97 CH_2	38.17 CH_2
	4	138.37 qC	138.44 qC	138.20 qC		138.42 qC	138.34 qC	138.36 qC
	5/9	129.73 CH	129.73 CH	129.61 CH		128.40 CH	129.72 CH	129.72 CH
	6/8	128.39 CH	128.34 CH	128.39 CH		129.74 CH	128.39 CH	128.39 CH
	7	126.59 CH	126.56 CH	126.65 CH		126.57 CH	126.59 CH	126.59 CH
D-Leu	1	171.75 qC	172.14 qC		171.95 qC	172.16 qC	172.25 qC	172.10 qC
	2	51.72 CH	48.48 CH		51.87 CH	51.51 CH	51.75 CH	51.75 CH
	3	24.25 CH_2	41.17 CH_2		24.60 CH_2	24.28 CH_2	24.24 CH_2	24.25 CH_2
	4	41.54 CH	24.37 CH		41.24 CH	41.53 CH	41.45 CH	41.52 CH
	5/6	22.38/23.23 CH_3	22.20/23.21 CH_3		22.11/23.46 CH_3	22.27/22.29 CH_3	22.39/23.20 CH_3	22.37/23.22 CH_3
D-Phe	1	172.09 qC		171.48 qC	171.95 qC	171.63 qC	171.84 qC	171.77 qC
	2	53.94 CH		54.44 CH	54.04 CH	54.06 CH	53.92 CH	53.94 CH
	3	37.67 CH_2		38.15 CH_2	37.65 CH_2	37.70 CH_2	37.65 CH_2	37.65 CH_2
	4	138.47 qC		138.20 qC	138.45 qC	138.48 qC	138.44 qC	138.47 qC

Table S1 *(cont'd)*. ^{13}C chemical shifts of peptides **1** to **7**.

Residue	No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**
D-Phe	5/9	129.61 CH		129.69 CH	129.62 CH	128.36 CH	129.61 CH	129.62 CH
	6/8	128.37 CH		128.50 CH	128.42 CH	129.61 CH	128.39 CH	128.39 CH
	7	126.59 CH		126.65 CH	126.63 CH	126.57 CH	126.59 CH	126.59 CH
Prop.	1	173.33 qC	173.10 qC	173.56 qC	173.40 qC	173.37 qC	173.35 qC	173.33 qC
	2	28.73 CH_2	28.56 CH_2	28.63 CH_2	28.72 CH_2	28.73 CH_2	28.71 CH_2	28.72 CH_2
	3	10.24 CH_3	10.18 CH_3	10.21 CH_3	10.25 CH_3	10.27 CH_3	10.23 CH_3	10.24 CH_3
L-Ala[*x*]	1		172.94 qC	172.57 qC	172.49 qC	172.39 qC	172.23 qC	174.45 qC
	2		51.51 CH	48.47 CH	48.68 CH	48.72 CH	48.50 CH	47.85 CH
	3		19.02 CH_3	18.49 CH_3	18.86 CH_3	18.24 CH_3	18.53 CH_3	17.57 CH_3

HPLC profiles of nobilamide B and analogues. Analytical RP-HPLC with a linear gradient of 5% to 100% MeCN (0.1% TFA) was used with 1 mL/min flow rate. Absorbance was detected at 220 nm.

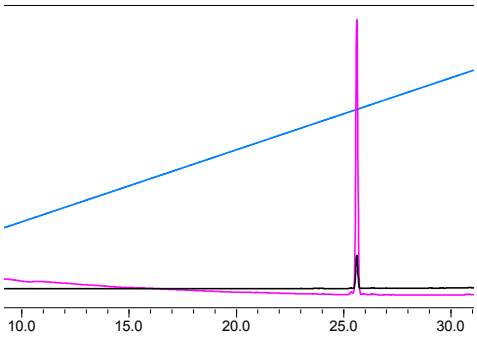

Figure S17. Analytical RP-HPLC profile of nobilamide B (**1**). Compound elutes at t_R = 25.2 min.

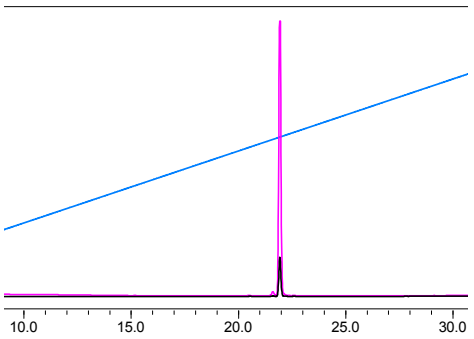

Figure S18. RP-HPLC profile of [Ala1]-nobilamide B (**2**). Compound elutes at t_R = 22.0 min.

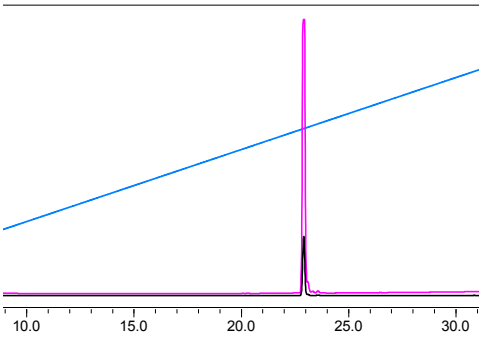

Figure S19. RP-HPLC profile of [Ala2]-nobilamide B (**3**). Compound elutes at t_R = 22.9 min.

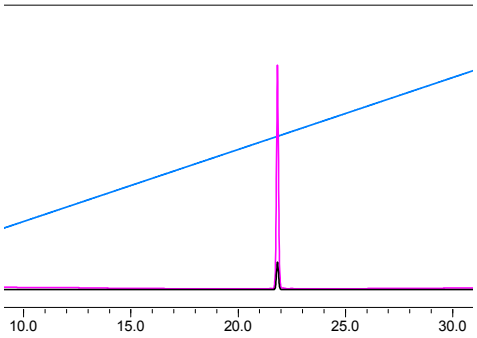

Figure S20. RP-HPLC profile of [Ala3]-nobilamide B (**4**). Compound elutes at t_R = 22.1 min.

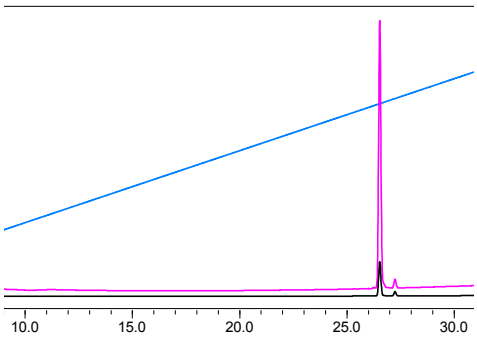

Figure S21. RP-HPLC profile of [Ala4]-nobilamide B (**5**). Compound elutes at t_R = 26.7 min.

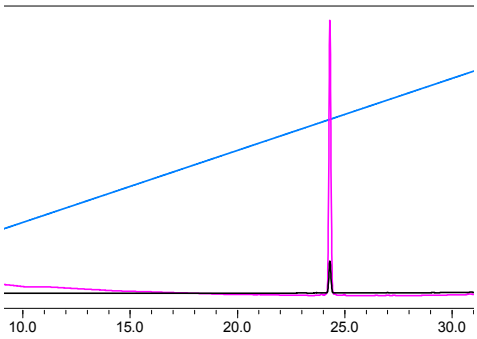

Figure S22. RP-HPLC profile of [Ala5]-nobilamide B (**6**). Compound elutes at t_R = 24.4 min.

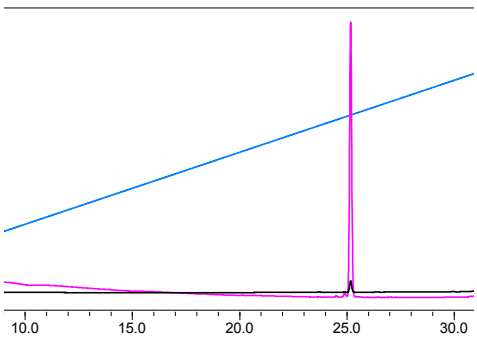

Figure S23. RP-HPLC profile of [Ala7]-nobilamide B (**7**). Compound elutes at t_R = 25.2 min.