Nagelamides X–Z, Dimeric Bromopyrrole Alkaloids from a Marine Sponge *Agelas* sp.

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Supporting Information

Experimental Section

1. General Experimental Procedures. Optical rotations and IR spectra were recorded on a JASCO P-1030 digital polarimeter and a JASCO FT/IR-230 spectrophotometer, respectively. UV spectra were recorded using a Shimazu UV-1600PC spectrophotometer. NMR spectra were measured by a Bruker AMX-600 spectrometer and a JEOL ECA 500 spectrometer. The resonances of CHD_2SOCD_3 (δ_H 2.49) and DMSO-d_6 (δ_C 39.5) were used as internal references for ^1H and ^{13}C NMR chemical shifts, respectively. HRESIMS spectra were recorded on a Thermo Scientific Exactive spectrometer.

2. Sponge Description. The sponge *Agelas* sp. (Order Agelasida, Family Agelasidae) (SS-162) collected at Kerama islands, Okinawa, was kept frozen until used. The sponge has a smooth surface. Sponges are firm, springy and compressible. Skeleton is dense reticulate fibre skeleton with grainy texture. Primary fibres cored and echinated by verticillate spined acanthostyles. Spicules are verticillate, regularly spined acanthostyles, 210x12 μm, some oxeote modifications and thin forms occur. The voucher specimen is deposited at the Graduate School of Pharmaceutical Sciences, Hokkaido University.

3. Extraction and Isolation. The sponge *Agelas* sp. (3.9 kg, wet weight) was extracted with MeOH (5 L x 3) to give the extracts (256 g). A part of the extracts (135 g) were partitioned successively with EtOAc (750 mL x 3), *n*-BuOH (750 mL x 3), and water (750 mL). The EtOAc-soluble materials were partitioned between *n*-hexane (500 mL x 3) and 10% MeOH aq. (500 mL) to yield 10 % MeOH aq.-soluble materials (34.49 g). The 10% MeOH aq.-soluble materials were subjected to silica gel column ($CHCl_3$/MeOH/AcOH, 80:20:2→0:100:2) to give seven fractions (frs. 1–7). Fractionation of fr. 4 on ODS column (MeOH/H_2O/TFA, 30:70:0.1→ 100:0:0.1) gave nine fractions (frs. 4.1–9), and fr. 4.5 was separated by Sephadex LH-20 column (MeOH/H_2O/TFA, 60:40:0.1→80:20:0.1) chromatographies to give seven fractions (frs. 4.5.1–7). Fr. 4.5.4 was loaded on ODS HPLC (YMC ODS-AQ, 20 x 250 mm; flow rate 7.0 mL/min; UV detection at 254 nm; eluent MeCN/H_2O/TFA, 35:65:0.1) to give eight fractions (frs. 4.5.4.1–8). Purification of fr. 4.5.4.2 on C_{30} HPLC (Develosil RPAQUEOUS-AR-5, 10 x 250 mm; 3.0 mL/min; 254 nm; MeCN/H_2O/TFA, 35:65:0.1) gave nagelamide X (**1**, 2.2 mg, 0.000057%, wet weight). Fr. 4.5.4.3 was purified by ODS HPLC (YMC ODS-AQ, 20 x 250 mm; 6.0 mL/min; 254 nm; MeCN/H_2O/TFA, 30:70:0.1, and then YMC Hydrosphere C18, 10 x

250 mm; 3.0 mL/min; 254 nm; MeCN/H_2O/TFA, 25:75:0.1) to afford nagelamide Y (**2**, 2.9 mg, 0.000074 mg). Separation of fr. 4.5.5 by ODS HPLC (YMC ODS-AQ, 20 x 250 mm; 5.0 mL/min; 254 nm; MeCN/H_2O/TFA, 30:70:0.1, and then YMC Hydrosphere C18, 10 x 250 mm; 2.5 mL/min; 254 nm; MeCN/H_2O/TFA, 30:70:0.1) yielded nagelamide Z (**3**, 13.7 mg, 0.000035%).

3.1. Nagelamide X (**1**): colorless amorphous solid; $[\alpha]^{21}_{D}$ ≈0 (*c* 0.25, MeOH); UV (MeOH) λ_{max} 212 (ε 18800, sh) and 278 (16300) nm; IR (KBr) ν_{max} 3581, 1684, 1206, and 1138 cm^{-1}; ^{1}H and ^{13}C NMR (Table 1); ESIMS: *m/z* 914, 916, 918, 920, and 922 (1:4:6:4:1) $[M]^+$; HRESIMS: *m/z* 913.86795 $[M]^+$ (calcd for $C_{24}H_{28}N_{11}O_6{}^{79}Br_4S$, 913.86728).

3.2. Nagelamide Y (**2**): colorless amorphous solid; $[\alpha]^{22}_{D}$ ≈0 (*c* 0.25, MeOH); UV (MeOH) λ_{max} 212 (ε 22900, sh) and 278 (18400) nm; IR (KBr) ν_{max} 3390, 1684, 1205, and 1136 cm^{-1}; ^{1}H and ^{13}C NMR (Table 1); ESIMS: *m/z* 898, 900, 902, 904, and 906 (1:4:6:4:1) $[M]^+$; HRESIMS: *m/z* 897.87359 $[M]^+$ (calcd for $C_{24}H_{28}N_{11}O_5{}^{79}Br_4S$, 897.87236).

3.3 Nagelamide Z (**3**): pale yellow amorphous solid; $[\alpha]^{22}_{D}$ –3.0 (*c* 0.25, MeOH); UV (MeOH) λ_{max} 280 (ε 21400) nm; IR (KBr) ν_{max} 3175, 1685, 1203, and 1138 cm^{-1}; ^{1}H and ^{13}C NMR (Table 1); ESIMS: *m/z* 773, 775, 777, 779, and 781 (1:4:6:4:1) $[M–H]^+$; HRESIMS: *m/z* 772.86011 $[M–H]^+$ (calcd for $C_{22}H_{21}N_{10}O_2{}^{79}Br_4$, 772.85770).

Figure S1. ^{1}H NMR spectrum of nagelamide X (**1**) in DMSO-d_6 (600 MHz).

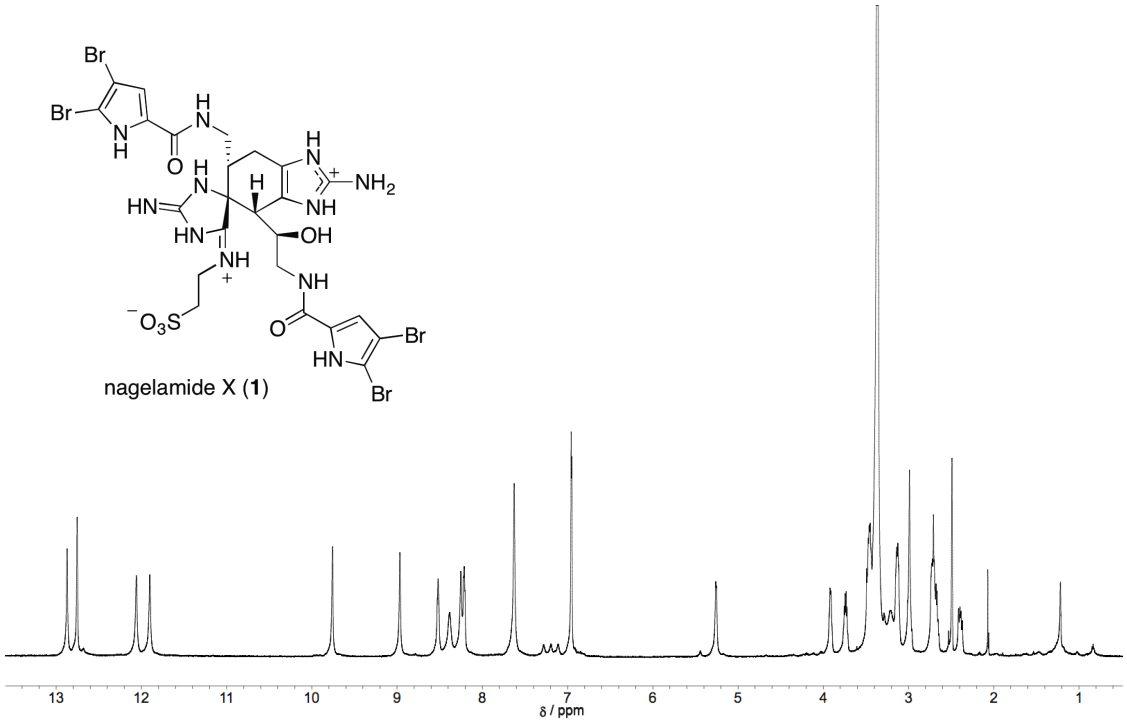


Figure S2. ^{13}C NMR spectrum of nagelamide X (**1**) in DMSO-d_6 (150 MHz).

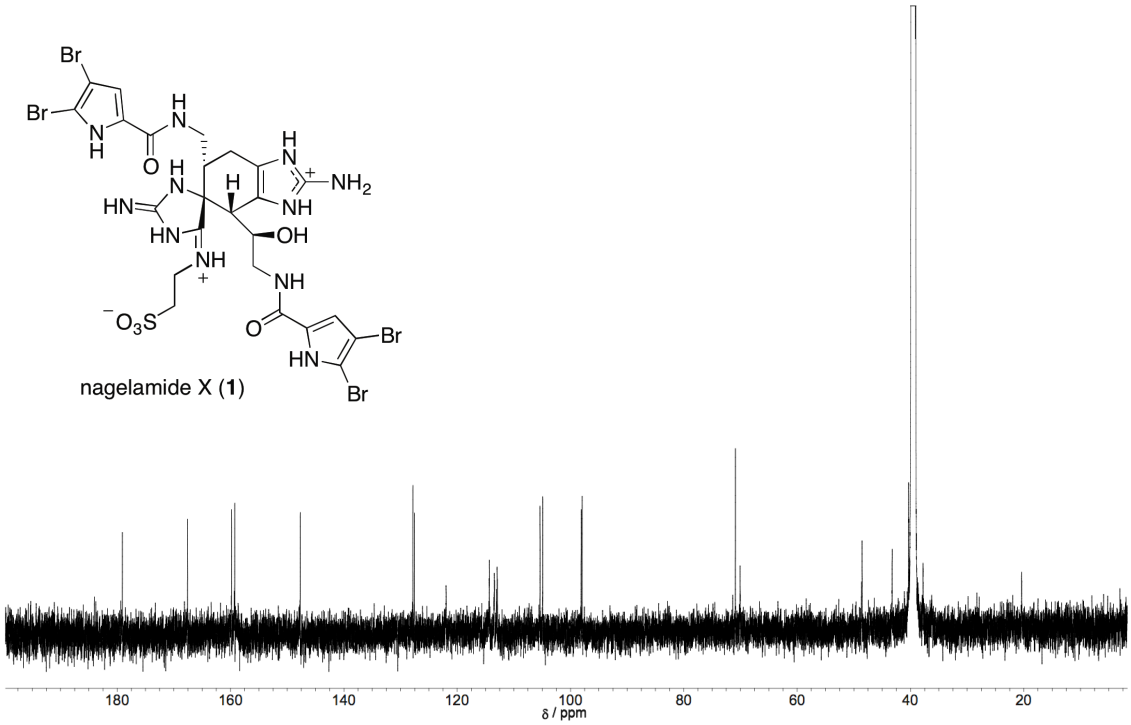


Figure S3. ^{1}H-^{1}H COSY spectrum of nagelamide X (**1**) in DMSO-d_6 (600 MHz).

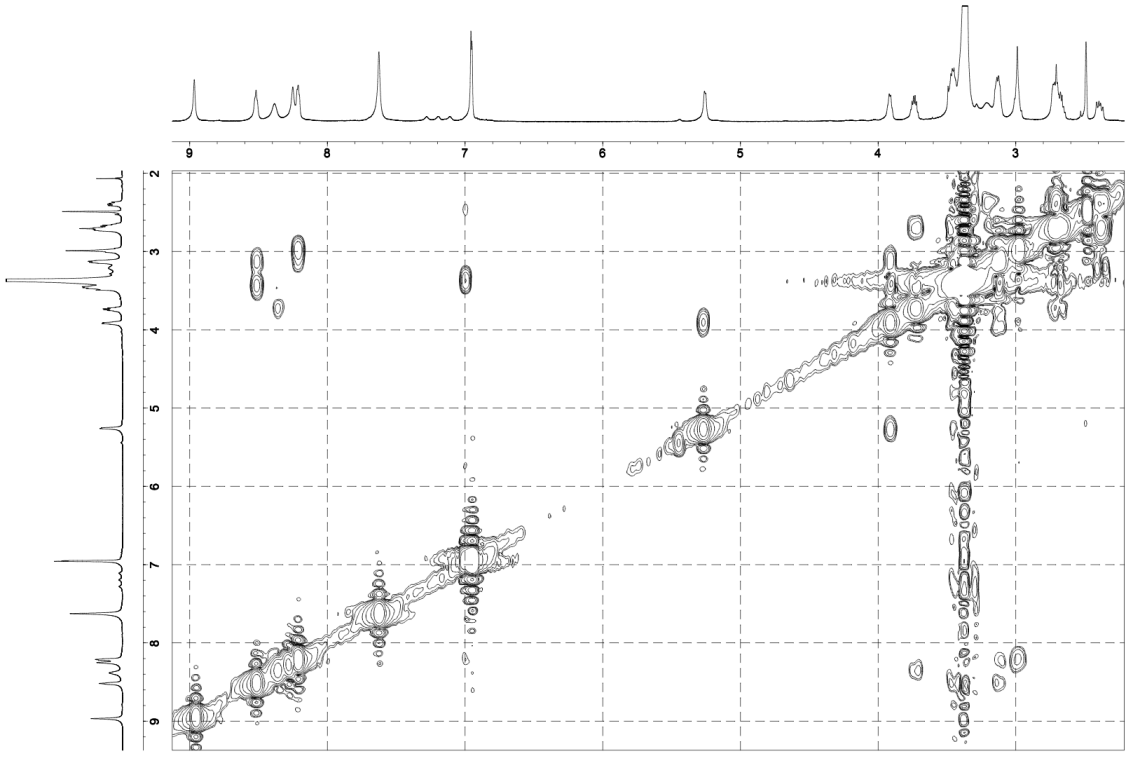


Figure S4. TOCSY spectrum of nagelamide X (**1**) in DMSO-d_6 (600 MHz).

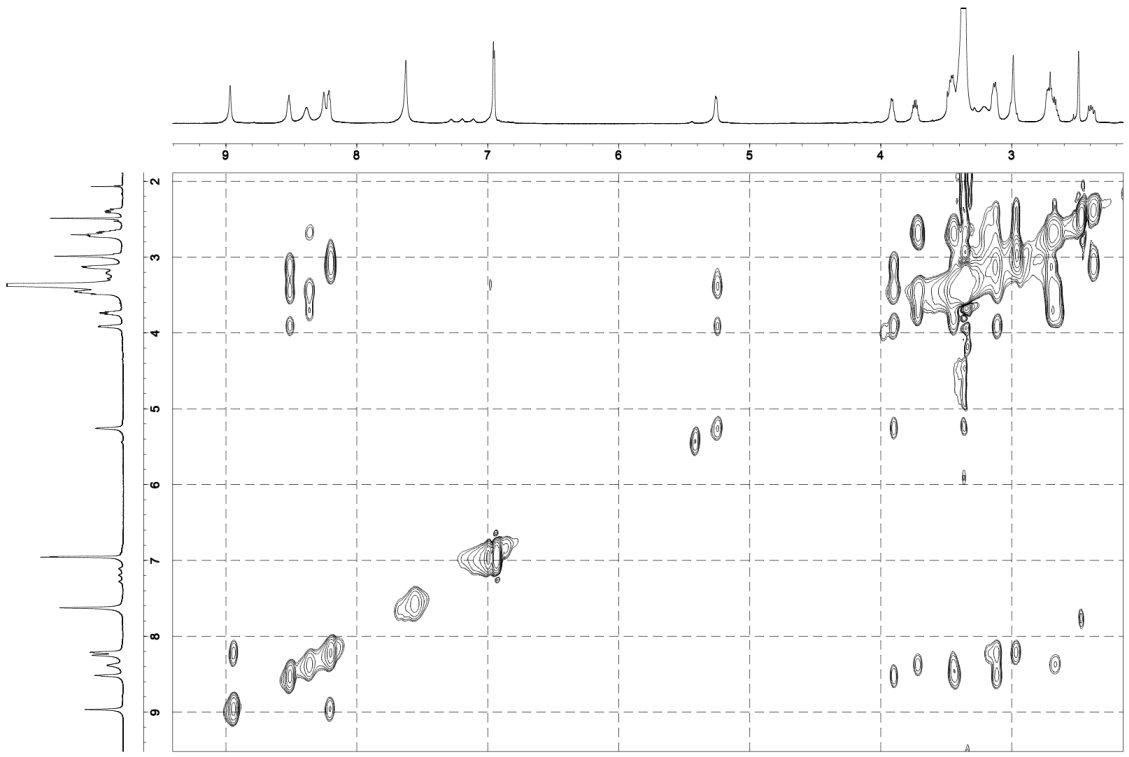


Figure S5. HMQC spectrum of nagelamide X (**1**) in DMSO-d_6 (600 MHz).

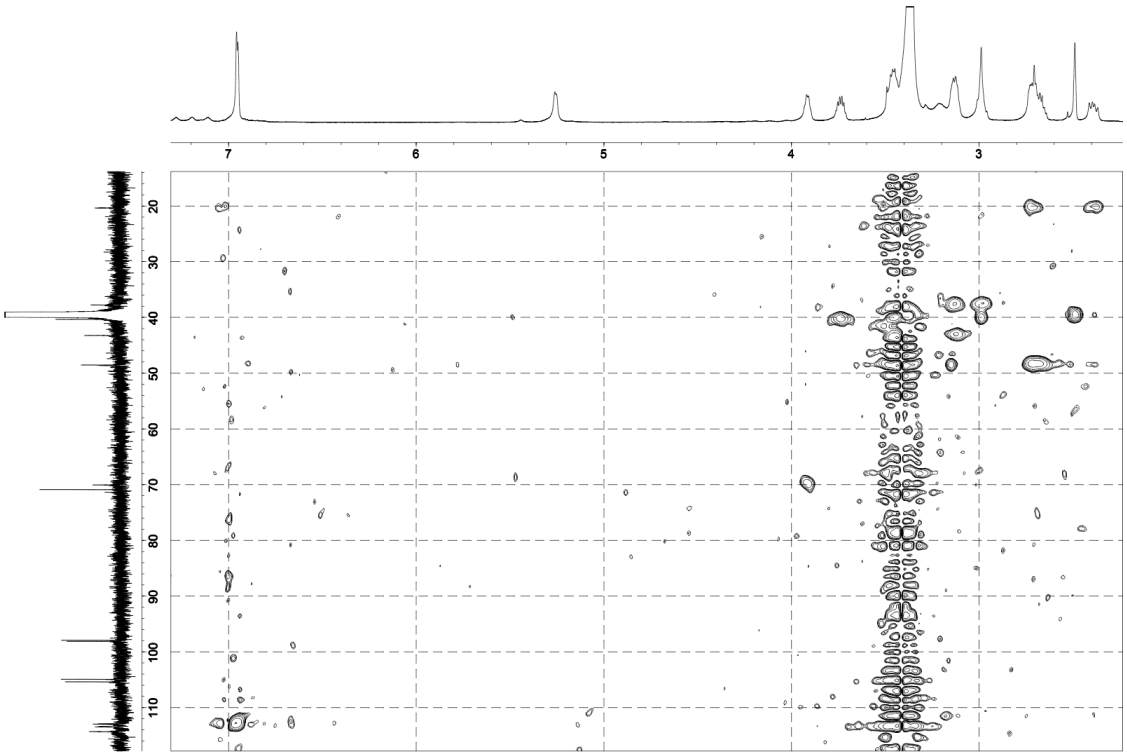


Figure S6. HMBC spectrum of nagelamide X (**1**) in DMSO-d_6 (500 MHz).

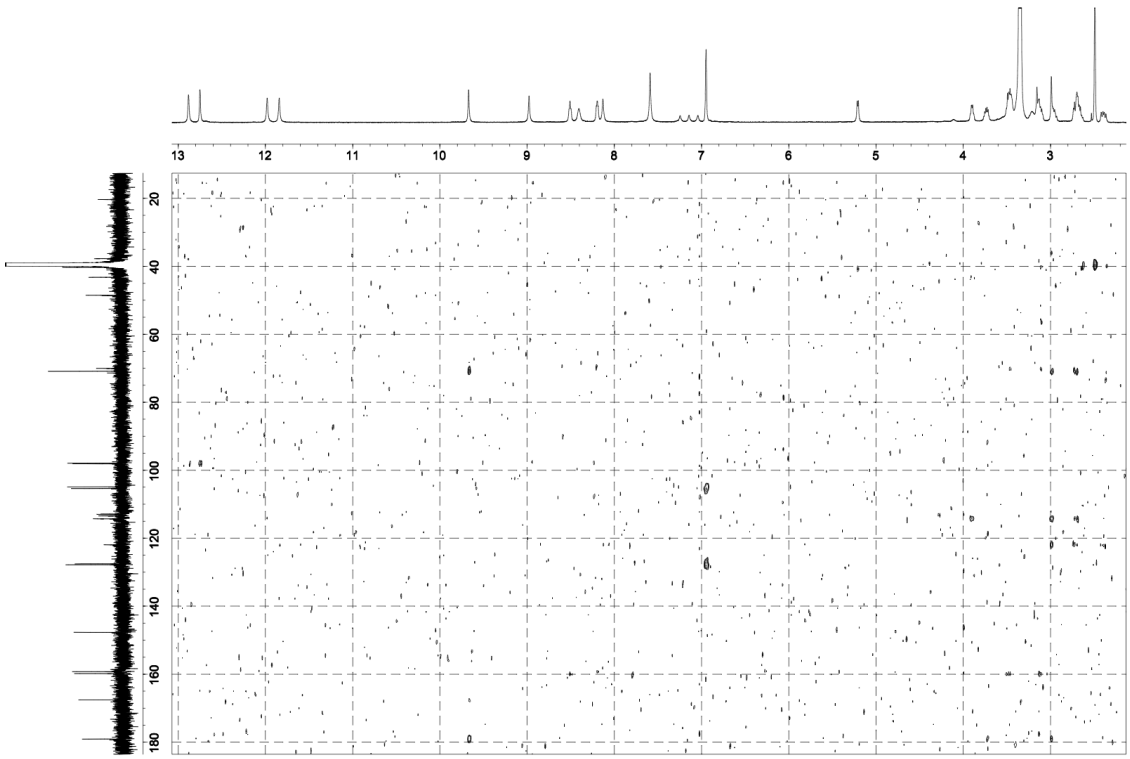


Figure S7. ROESY spectrum of nagelamide X (**1**) in DMSO-d_6 (500 MHz).

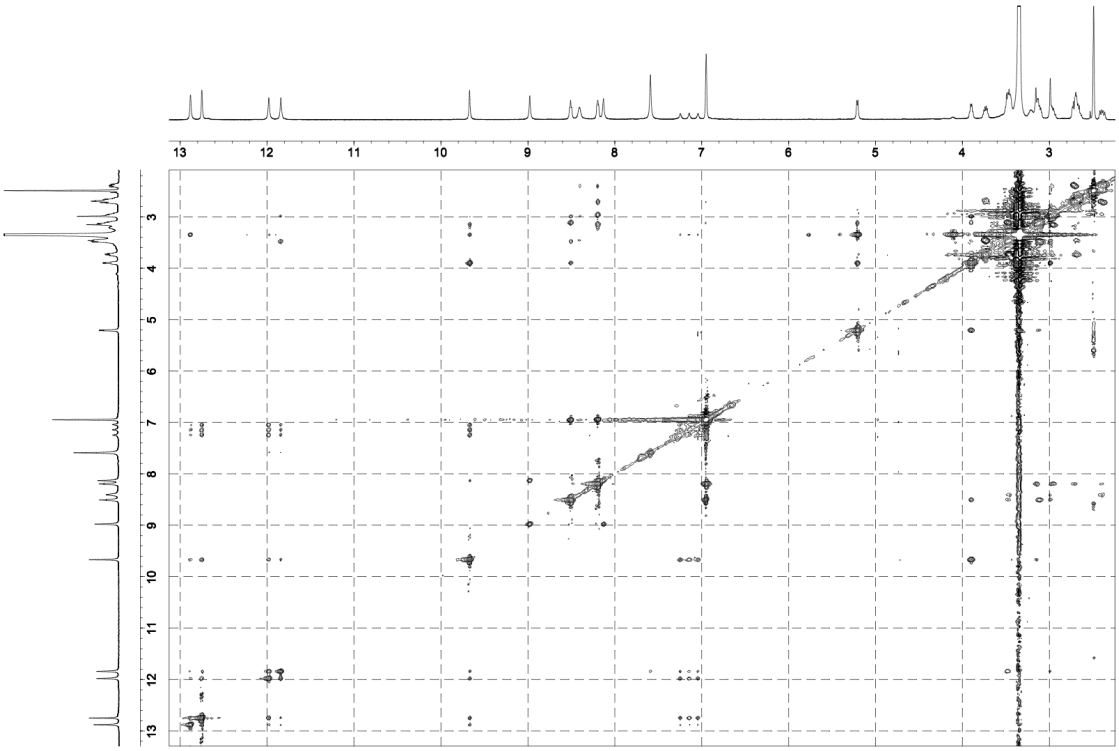


Figure S8. ^{1}H NMR spectrum of nagelamide Y (**2**) in DMSO-d_6 (600 MHz).

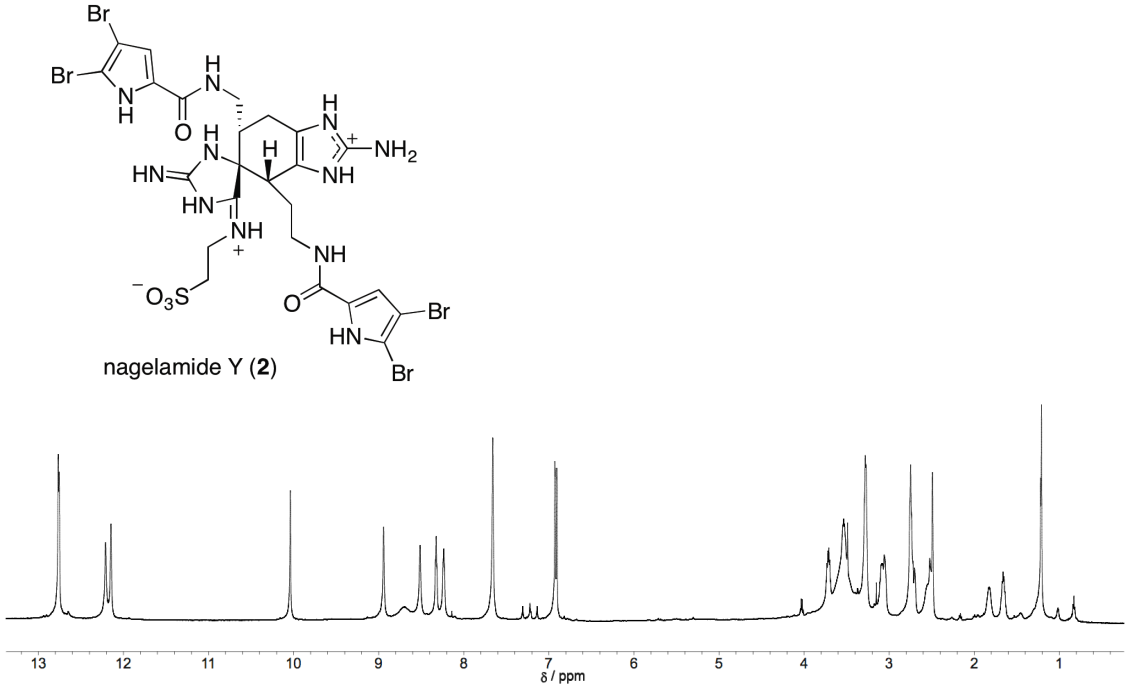


Figure S9. ^{13}C NMR spectrum of nagelamide Y (**2**) in DMSO-d_6 (150 MHz).

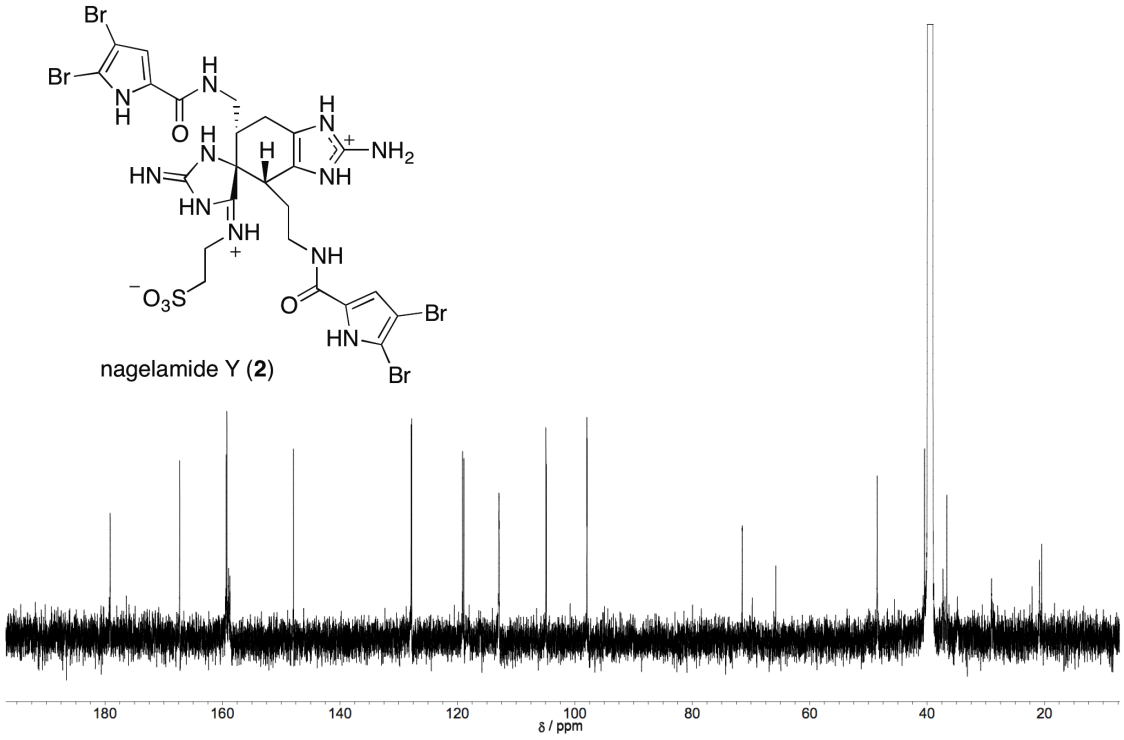


Figure S10. ^{1}H-^{1}H COSY spectrum of nagelamide Y (**2**) in DMSO-d_6 (600 MHz).

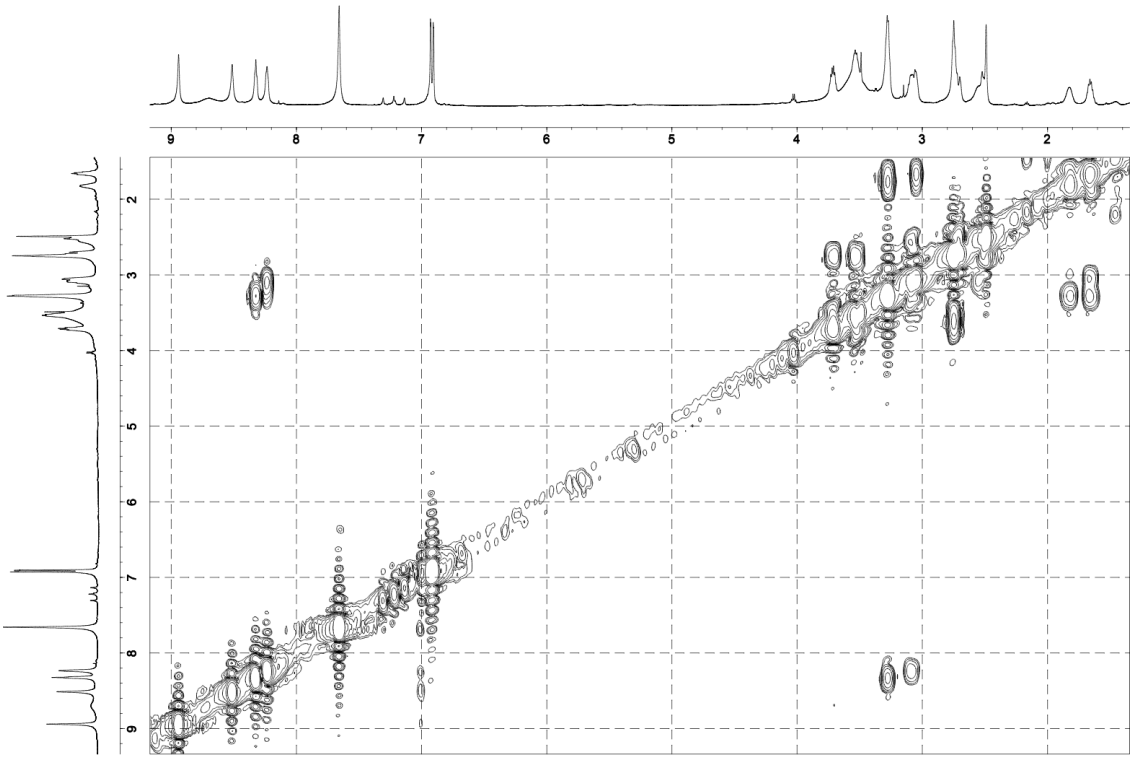


Figure S11. TOCSY spectrum of nagelamide Y (**2**) in DMSO-d_6 (600 MHz).

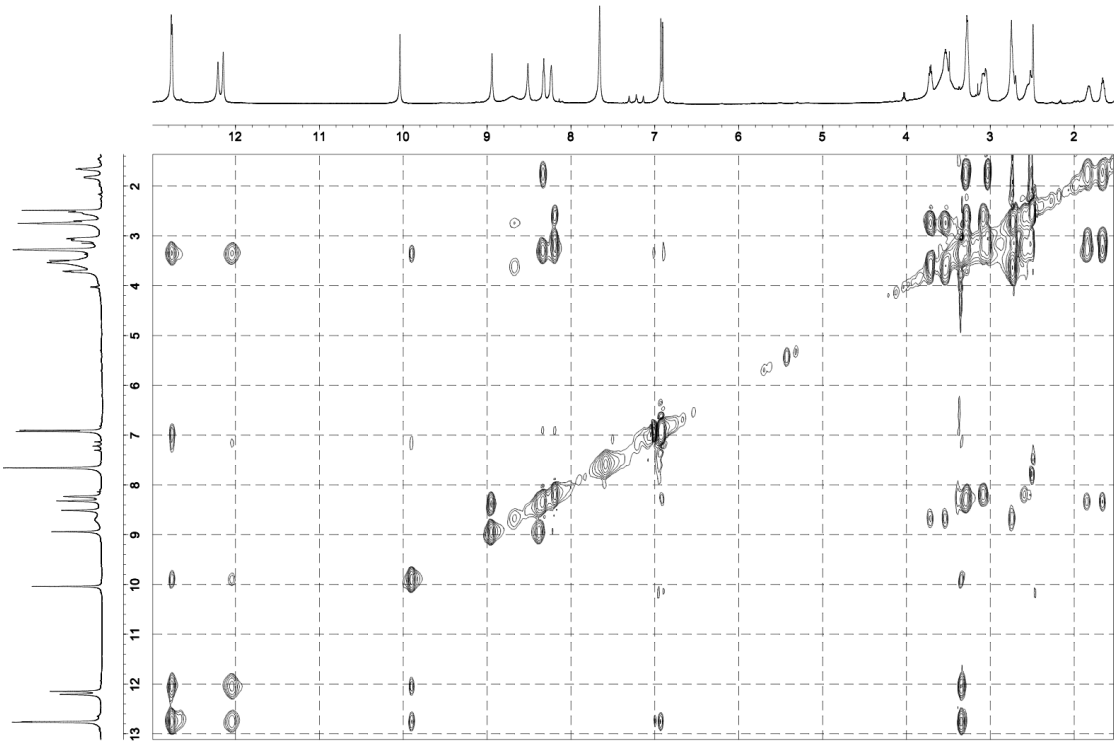


Figure S12. HMQC spectrum of nagelamide Y (**2**) in DMSO-d_6 (600 MHz).

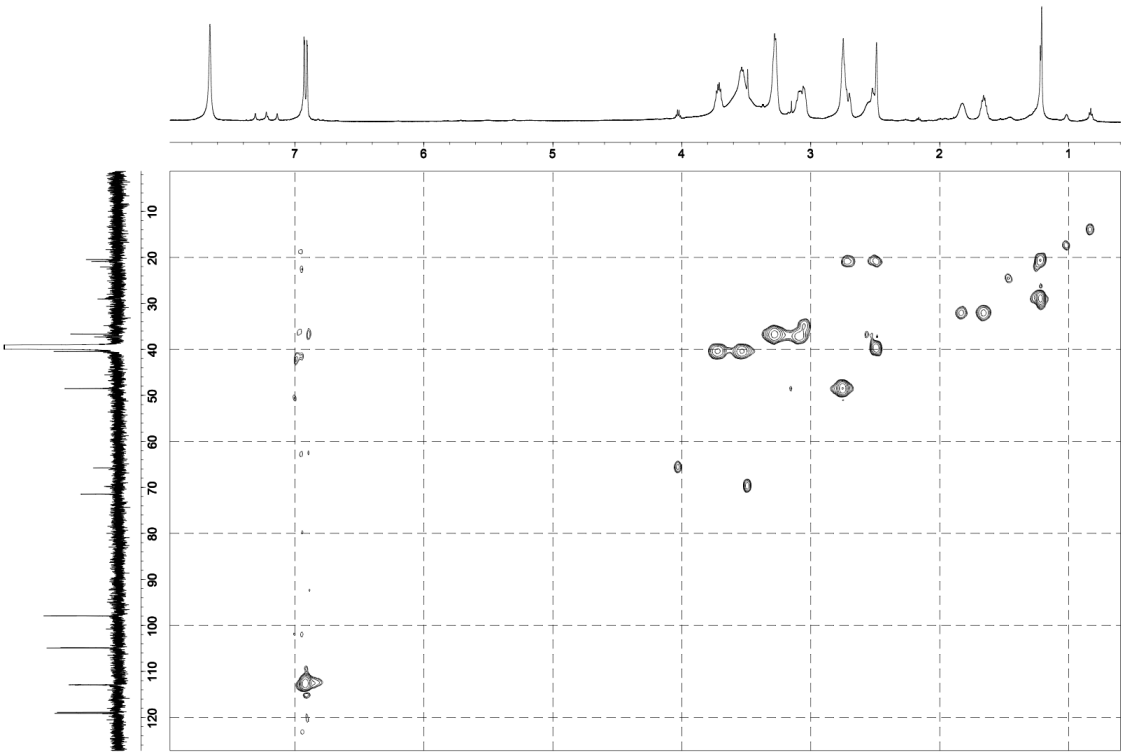


Figure S13. HMBC spectrum of nagelamide Y (**2**) in DMSO-d_6 (600 MHz).

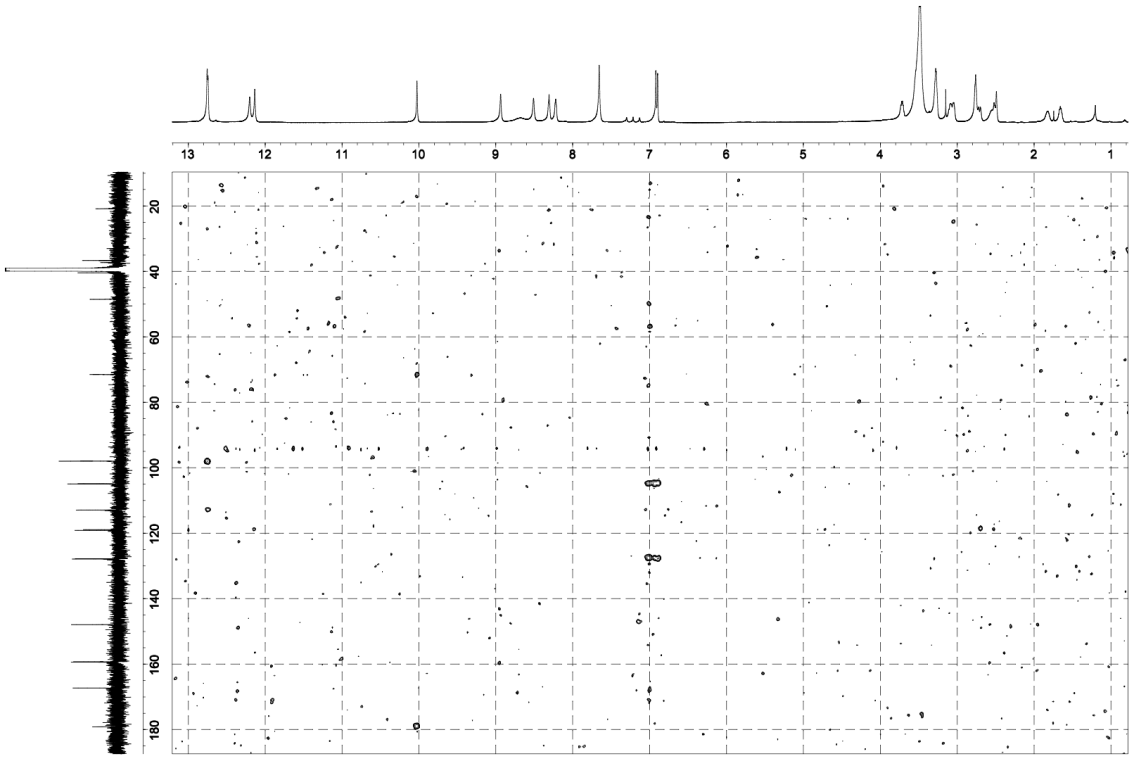


Figure S14. HMBC spectrum of nagelamide Y (**2**) in DMSO-d_6 (500 MHz).

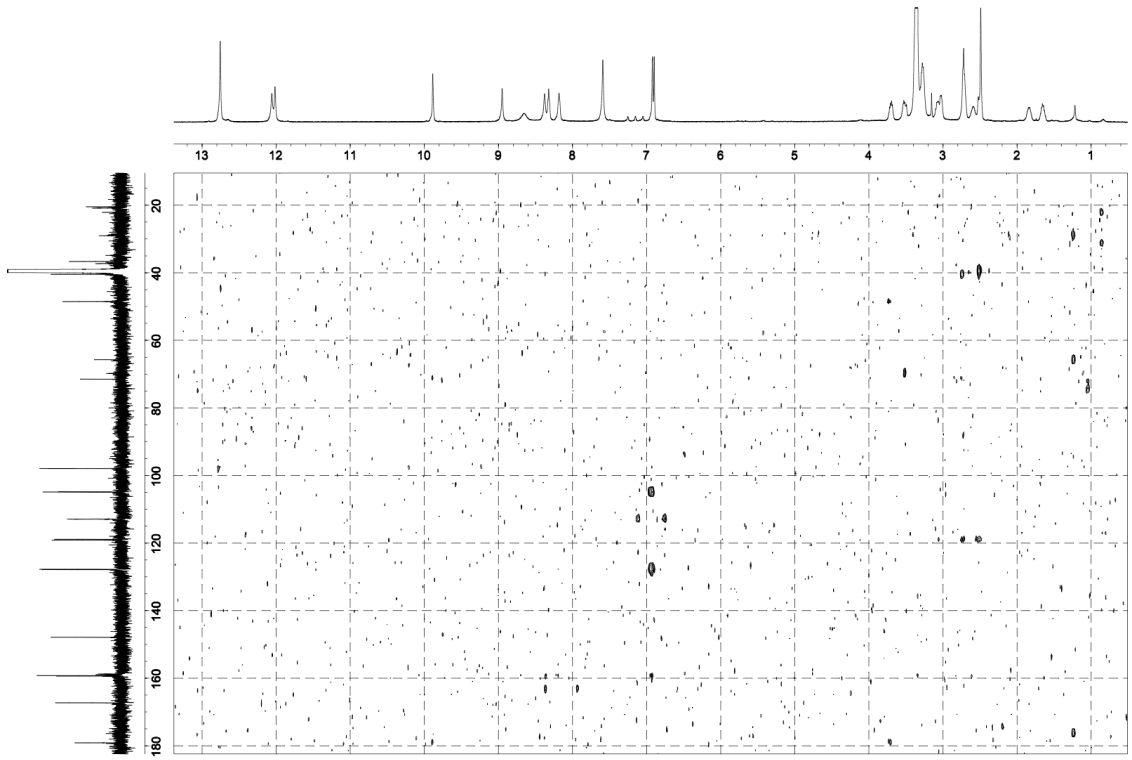


Figure S15. ROESY spectrum of nagelamide Y (**2**) in DMSO-d_6 (600 MHz).

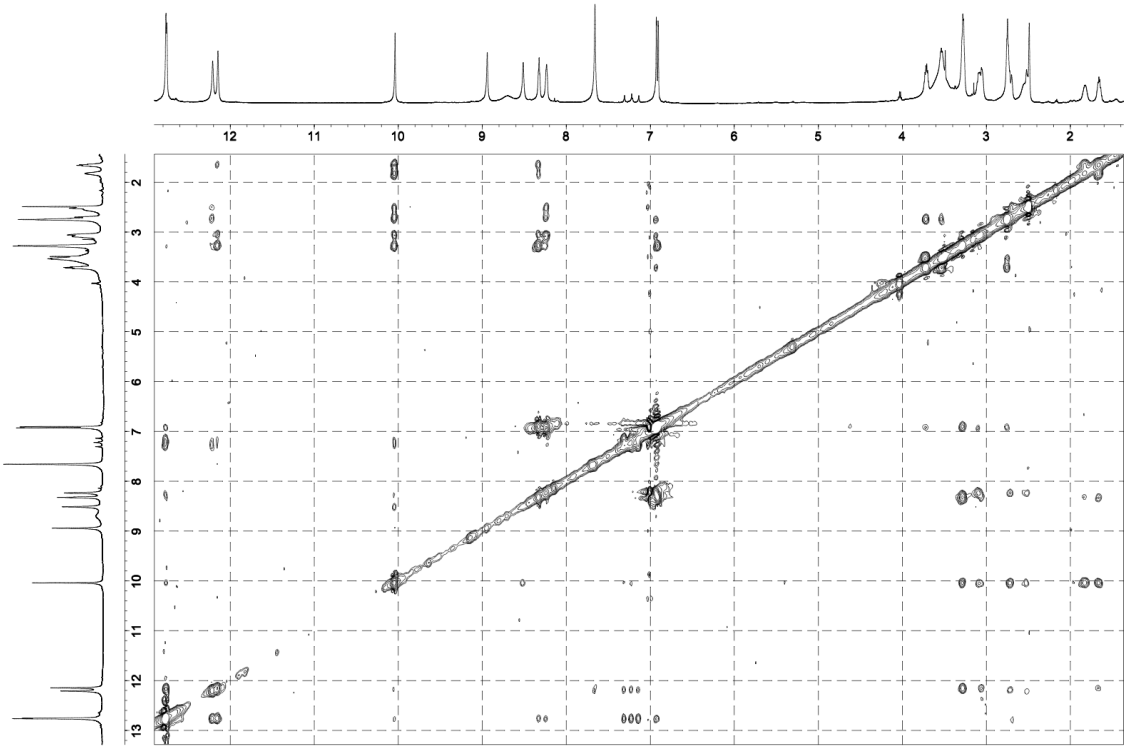
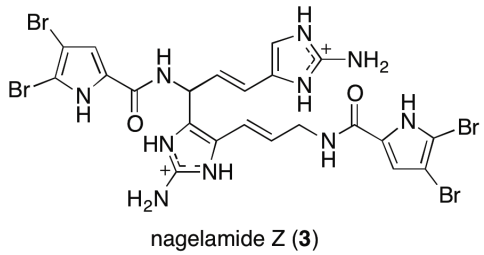


Figure S16. ^{1}H NMR spectrum of nagelamide Z (**3**) in DMSO-d_6 (600 MHz).



nagelamide Z (**3**)

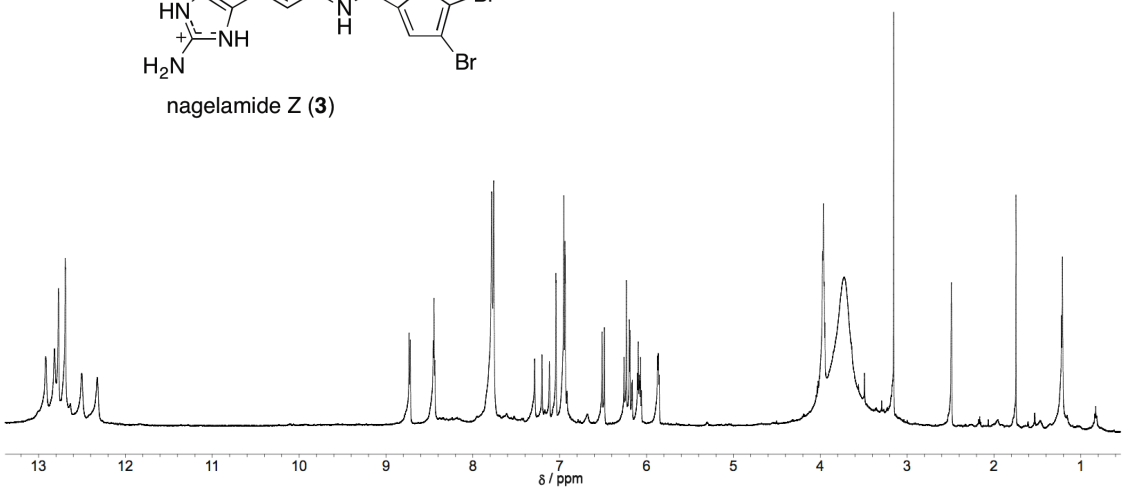


Figure S17. ^{13}C NMR spectrum of nagelamide Z (**3**) in DMSO-d_6 (150 MHz).

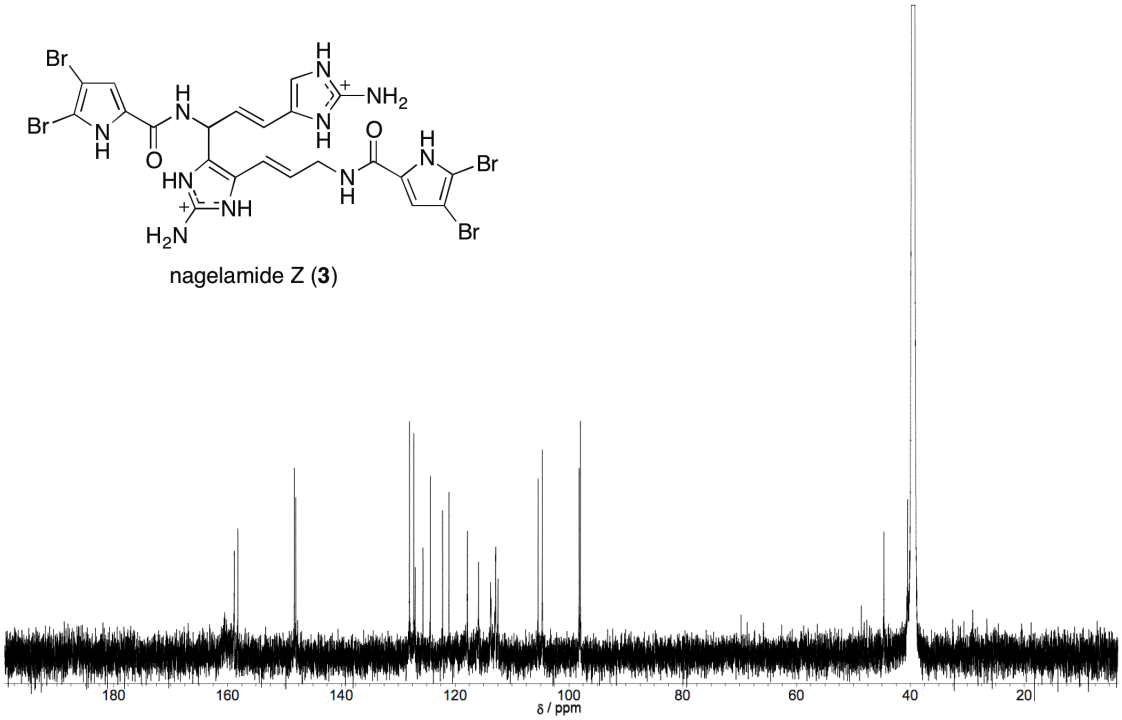


Figure S18. ^{1}H-^{1}H COSY spectrum of nagelamide Z (**3**) in DMSO-d_6 (600 MHz).

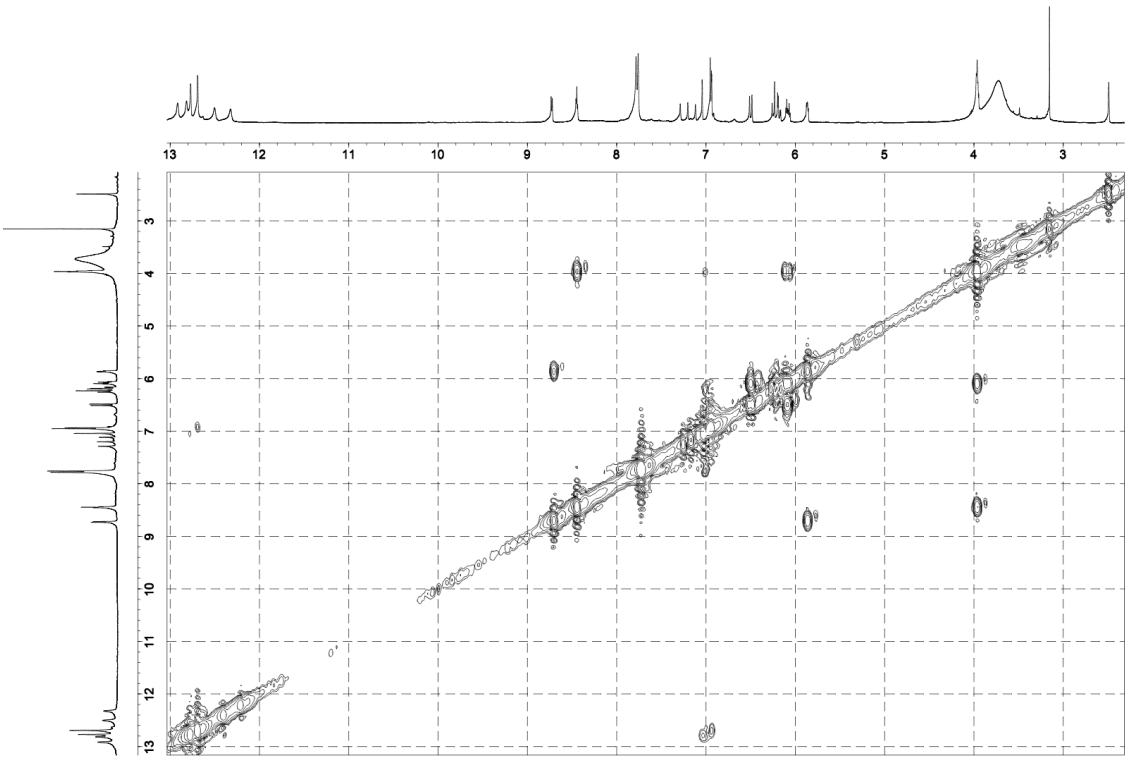


Figure S19. HMQC spectrum of nagelamide Z (**3**) in DMSO-d_6 (600 MHz).

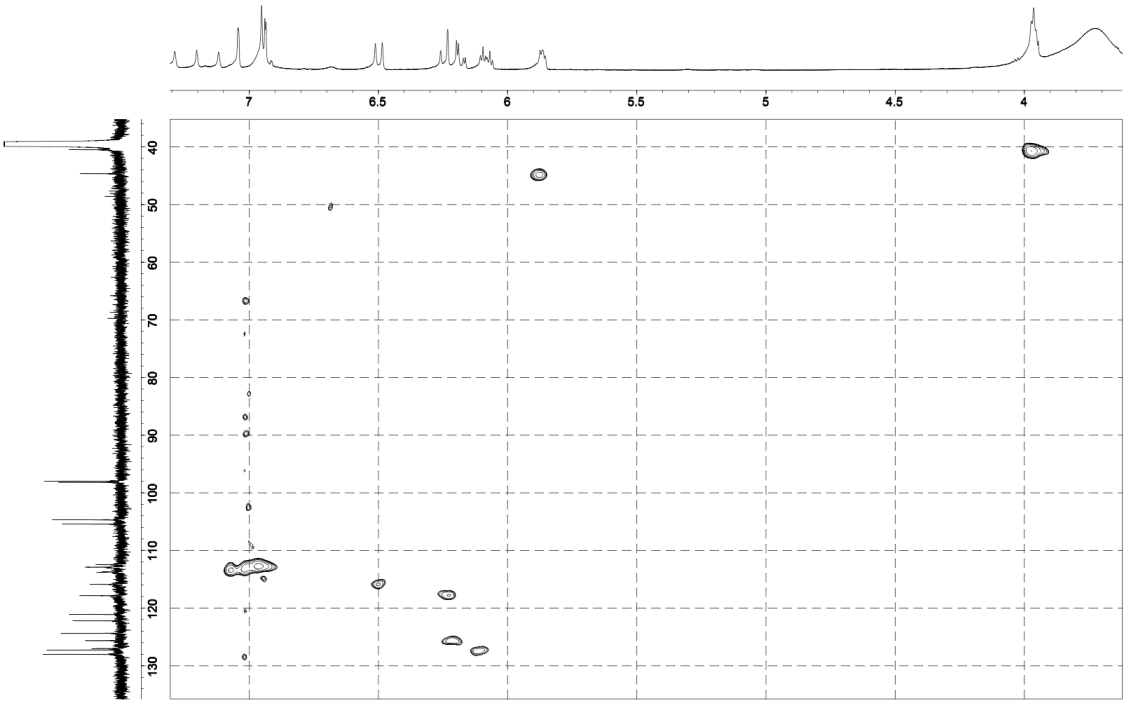


Figure S20. HMBC spectrum of nagelamide Z (**3**) in DMSO-d_6 (500 MHz).

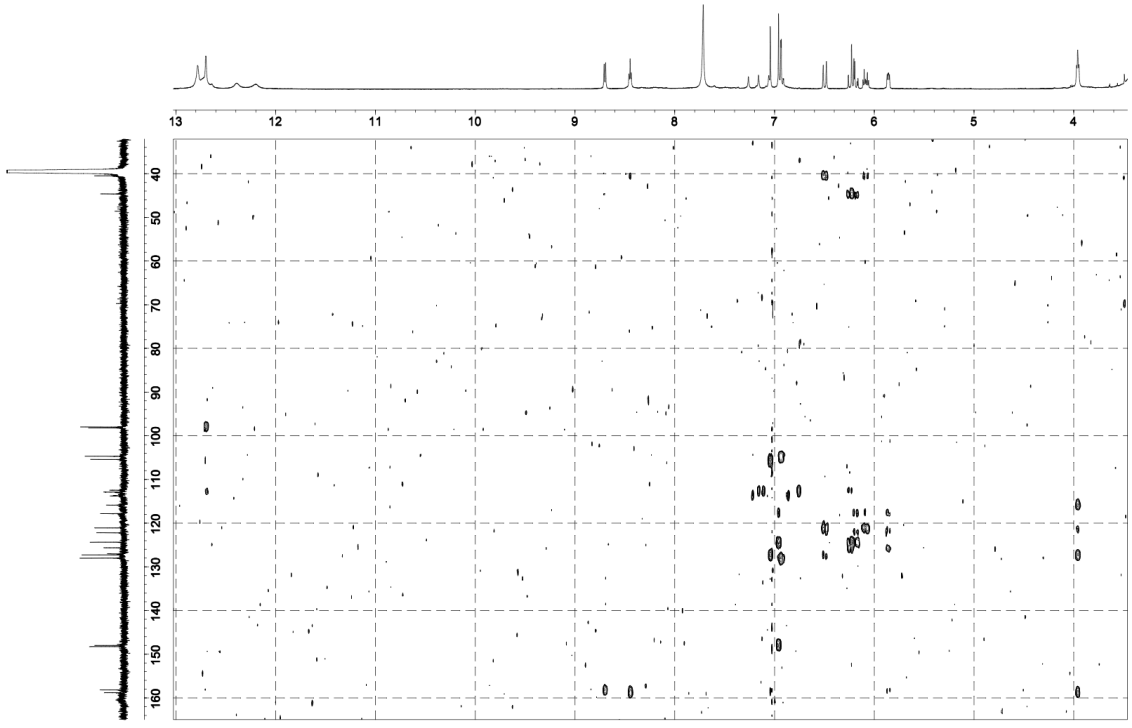


Figure S21. ROESY spectrum of nagelamide Z (**3**) in DMSO-d_6 (600 MHz).

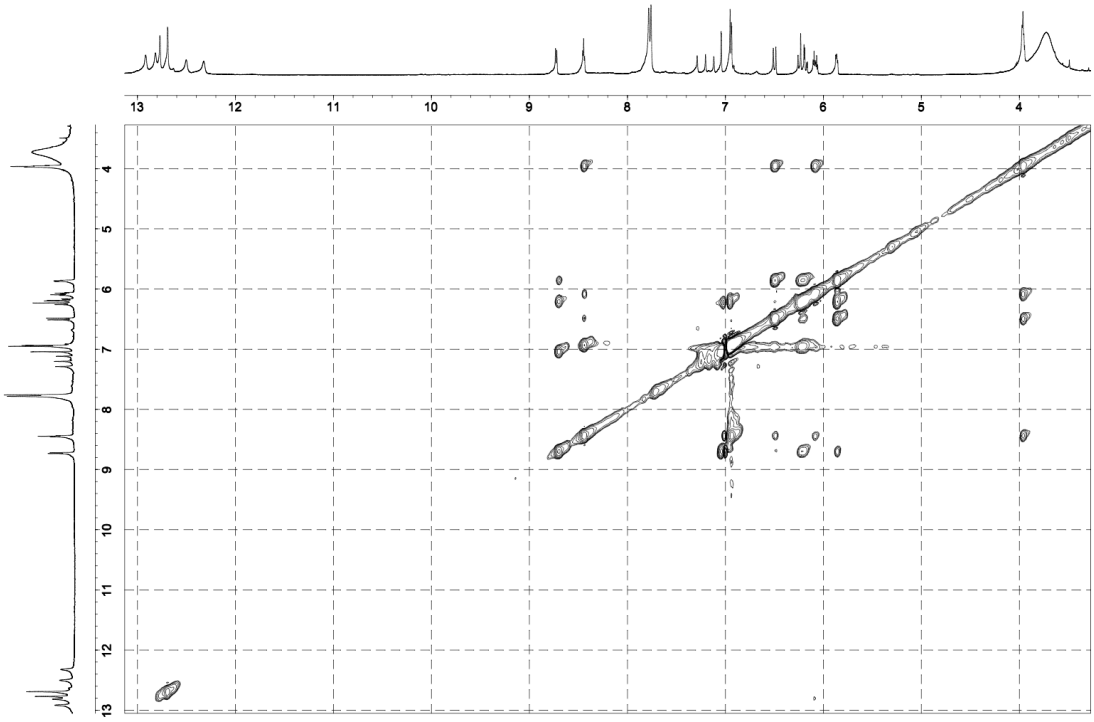


Figure S22. Selected 2D NMR correlations for nagelamide Y (**2**).

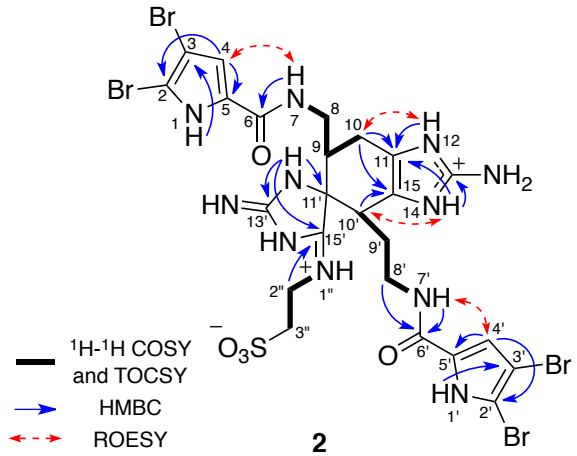


Figure S23. Selected ROESY correlations and the relative stereochemistry for nagelamide Y (**2**).