Nagelamides Q and R, Novel Dimeric Bromopyrrole Alkaloids from Sponges *Agelas* sp.

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Supporting Information

Experimental Section

Experimental Section

General Experimental Procedures. Optical rotation was recorded on a JASCO P-1030 polarimeter. IR and UV spectra were recorded on a Shimadzu UV-1600PC and a JASCO FT/IR-5300 spectrophotometers, respectively. ^{1}H, ^{13}C and 2D NMR spectra were measured on a JEOL ramuda-400 spectrometer and a Bruker AMX-600 spectrometer using 5 and 2.5 mm micro cells (Shigemi Co., Ltd.) for DMSO-d_6, respectively. The 2.49 and 39.5 ppm resonances of residual DMSO-d_6 and the 7.19 and 123.5 ppm resonances of residual C_5D_5N were used as internal references for ^{1}H and ^{13}C NMR spectra, respectively. Positive mode ESIMS spectra were obtained on a JEOL JMS 700-TZ spectrometer at -80 V as a focus voltage using a sample dissolved in MeOH with flow rate of 200 mL/min.

Sponge Description. The sponge (SS-1134) *Agelas* sp. (order, Agelasida, family Agelasiidae) was collected off Seragaki, Okinawa, and kept frozen until used. The sponge is open textured sponge with convoluted surface with numerous superficial depressions and occasional oscules. The Sponge has a fine ridged pattern over the surface with large apical oscules approximately 5 mm wide. The surface patterning gives a 'brain-like' appearance. The sponge has a dense internal structure and is firm and compressible. Skeleton is reticulate with fibre development, with primary fibres cored by verticillate acanthostyles, 4 spicules across, fibres are 130 μm wide and mesh spaces are approximately 500 μm wide. Secondary fibres form a tight mesh and are either cored or unispicular. Spicules are verticillate spined acanthostyles, thick, slightly curved 250 x 20 μm, some thin forms occur. The sponge (SS-956) *Agelas* sp. (order, Agelasida, family Agelasiidae) was collected off Unten-Port, Okinawa, and kept frozen until used. The sponge is medium brown mounds with smooth, faintly patterned surfaces. Mesohyl is compact with numerous pores. The sponge is firm and compressible. Skeleton is reticulate with fibre development, with primary fibres cored and echinated by verticillate spined acanthostyles. Primary fibres are 90 μm wide with brushes of spicules apically. Meshes are small and compact. Spicules are verticillate spined acanthostyles, thick, slightly curved 230 x 20 μm, some thin forms occur. The voucher specimens were deposited at Graduate School of Pharmaceutical Sciences, Hokkaido University.

Extraction and Isolation. The sponge (SS-1134, 0.70 kg, wet weight) was extracted with MeOH (2L x 2), and the extract was partitioned between BuOH (500 mL x 3) and H_2O (500 mL). Parts (7.63 g) of BuOH soluble materials (10.63 g) were subjected to a

silica gel column ($CHCl_3$/MeOH/AcOH) and then a C_{18} column (Cosmosil 140 C_{18} PREP, Nakarai Tesque Inc.; eluent, MeOH/H_2O/CF_3CO_2H, 80:20:01) to give a crude fraction of alkaloids. This fraction was separated by C_{18} MPLC (KUSANO C.I.G.; Kusano Science Corp.; eluent, CH_3OH/H_2O/CF_3CO_2H, 45:55:0.1 to 90:10:0.1 in 90 min; flow rate, 3.5 mL/min). A part of the fraction containing nagelamide Q (**1**) was purified by C_{18} HPLC [YMC Hydrospere C18, YMC Co., Inc., 10 x 250 mm; eluent, CH_3CN/H_2O/CF_3CO_2H, 25:75:0.1; flow rate, 2.5 mL/min; UV detection at 255 nm] to afford nagelamide Q (**1**, 0.0012 %, wet weight). The sponge (SS-956, 0.50 kg, wet weight) was extracted with MeOH (1.5 L x 3), and the extract was partitioned between BuOH (50 mL x 3) and H_2O (50 mL). BuOH soluble materials (4.31 g) were subjected to a silica gel column ($CHCl_3$/MeOH/AcOH) and then a C_{18} column (Cosmosil 140 C_{18} PREP, Nakarai Tesque Inc.; eluent, MeOH/H_2O/CF_3CO_2H, 80:20:01) to give a crude fraction of alkaloids. This fraction was separated by C_{18} MPLC (KUSANO C.I.G.; Kusano Science Corp.; eluent, CH_3OH/H_2O/CF_3CO_2H, 40:60:0.1 to 70:30:0.1 in 90 min; flow rate, 3.0 mL/min). A part of the fraction containing nagelamide R (**2**) was purified by C_{18} HPLC [YMC Hydrospere C18, YMC Co., Inc., 10 x 250 mm; eluent, CH_3CN/H_2O/CF_3CO_2H, 20:80:0.1 to 40:60:0.1 in 40 min; flow rate, 2.5 mL/min; UV detection at 255 nm] to afford nagelamide R (**2**, 0.0013 %).

Nagelamide Q (1): colorless amorphous solid; $[\alpha]_D^{24}$ -5 (c 0.2, MeOH); UV (MeOH) λ_{max} 276 nm (ε 18000); IR (KBr) ν_{max} 3406, 1684, 1206, and 1044 cm^{-1}; ESIMS (pos.) *m/z* 896, 898, 900, 902, and 904 [1:4:6:4:1, M^+]; HRESIMS (pos.) *m/z* 895.85868 [M^+, calcd for $C_{24}H_{26}N_{11}O_5{}^{79}Br_4S$, 895.85726].

Nagelamide R (2): colorless amorphous solid; $[\alpha]_D^{22}$ -3 (c 0.2, MeOH); UV (MeOH) λ_{max} 278 nm (ε 27000), 400 nm (ε 1600); IR (KBr) ν_{max} 3407, 1685, 1202, and 1140 cm^{-1}; ESIMS (pos.) *m/z* 773, 775, 777, 779, and 781 [1:4:6:4:1, $(M-H)^+$]; HRESIMS (pos.) *m/z* 772.85649 [$(M-H)^+$, calcd for $C_{22}H_{21}N_{10}O_2{}^{79}Br_4$, 772.85825].

Conversion of Nagelamide R (2) to Nagelamide L: Nageamide R (**2**, 100 μg) was dissolved in the mixture of DMSO and TFA (99:1) and stand at room temperature. After 24 h, DMSO and TFA in the reaction mixture were removed by lyophilization to afford nagelamide L (85 μg), whose spectrum data were identical with those of natural nagelamide L.

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

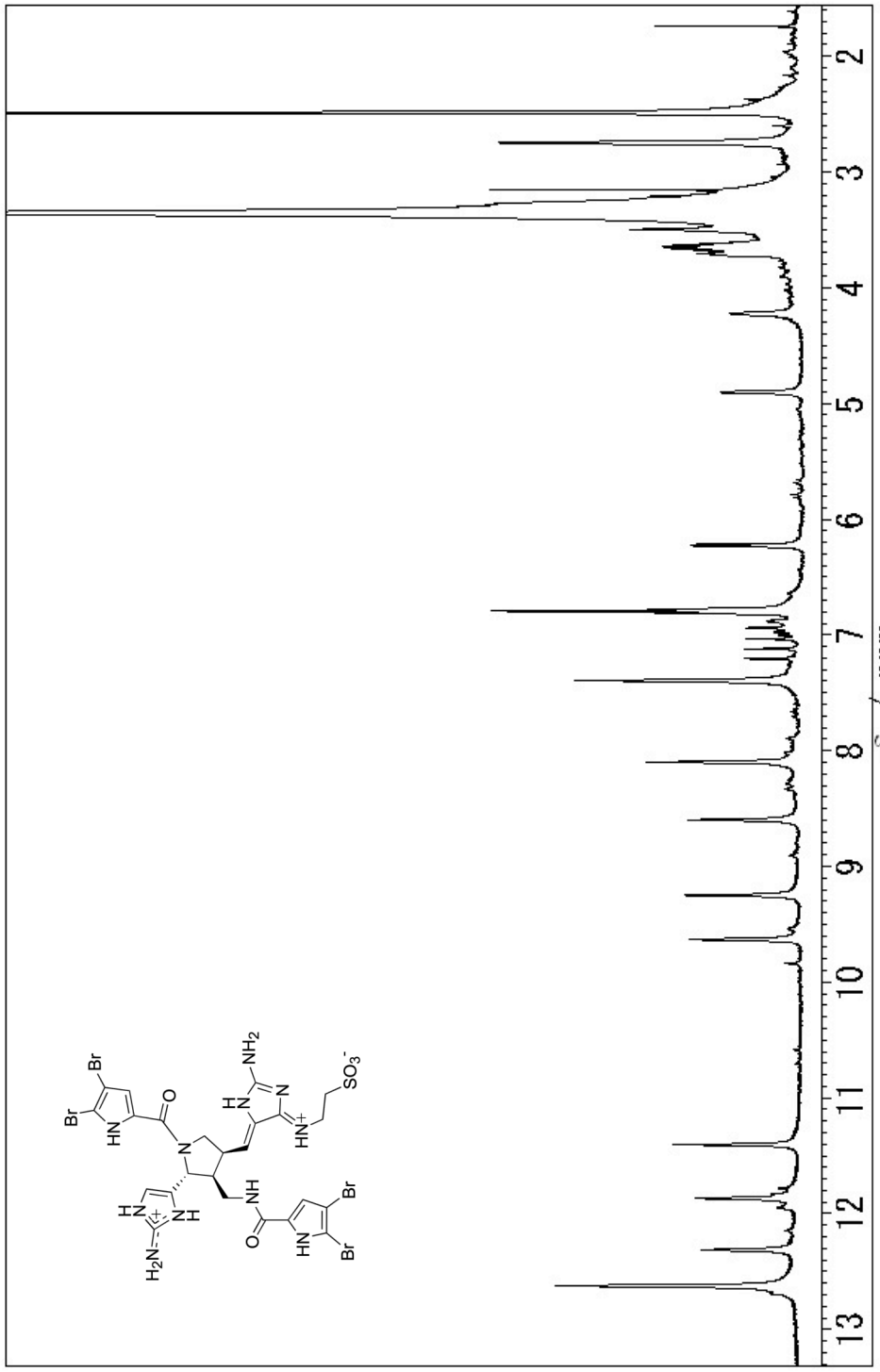

Figure S2. ^{13}C NMR spectrum of nagelamide Q (**1**) in DMSO-d_6

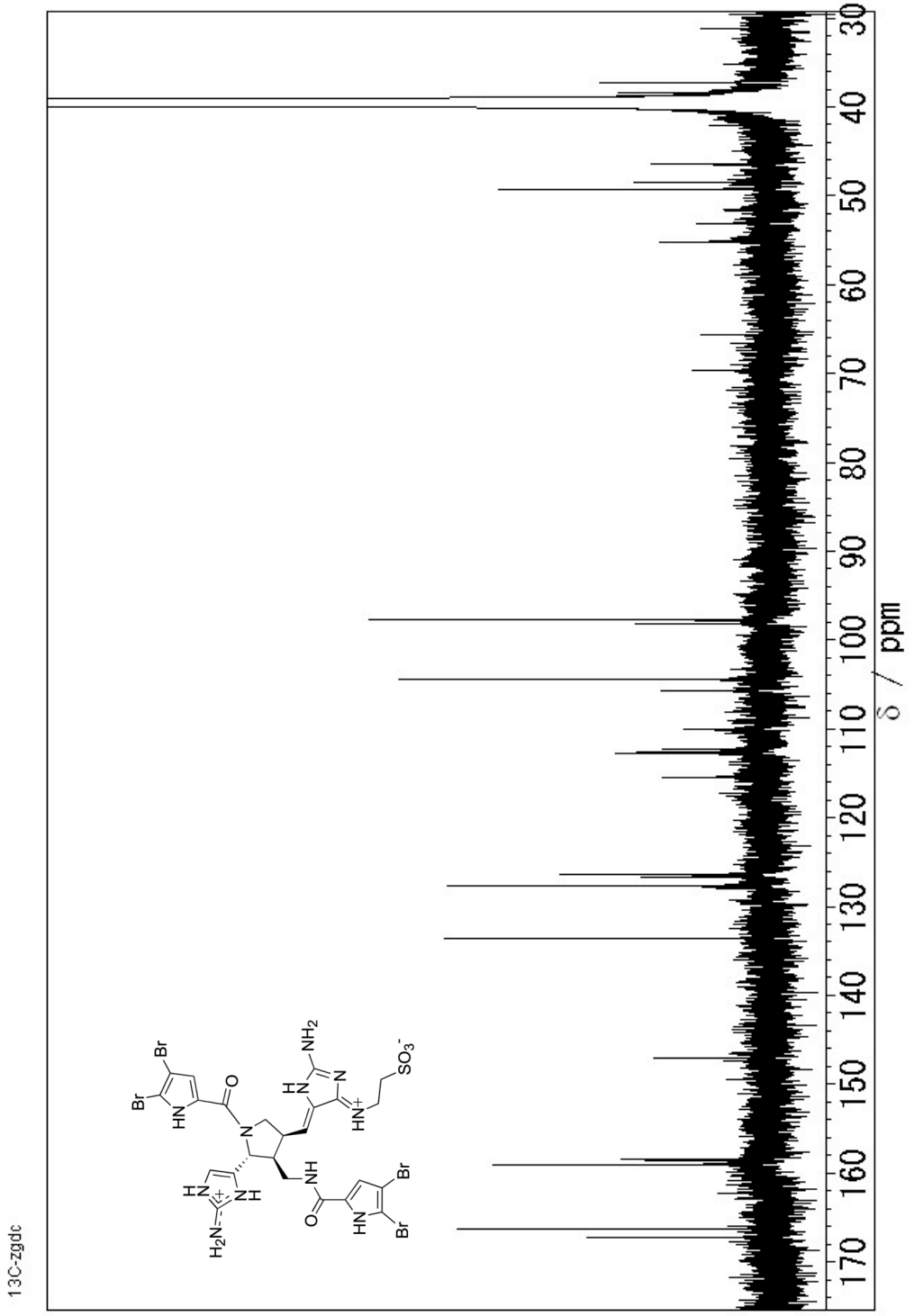

Figure S3. ^{1}H-^{1}H COSY spectrum of nagelamide Q (**1**) in DMSO-d_6

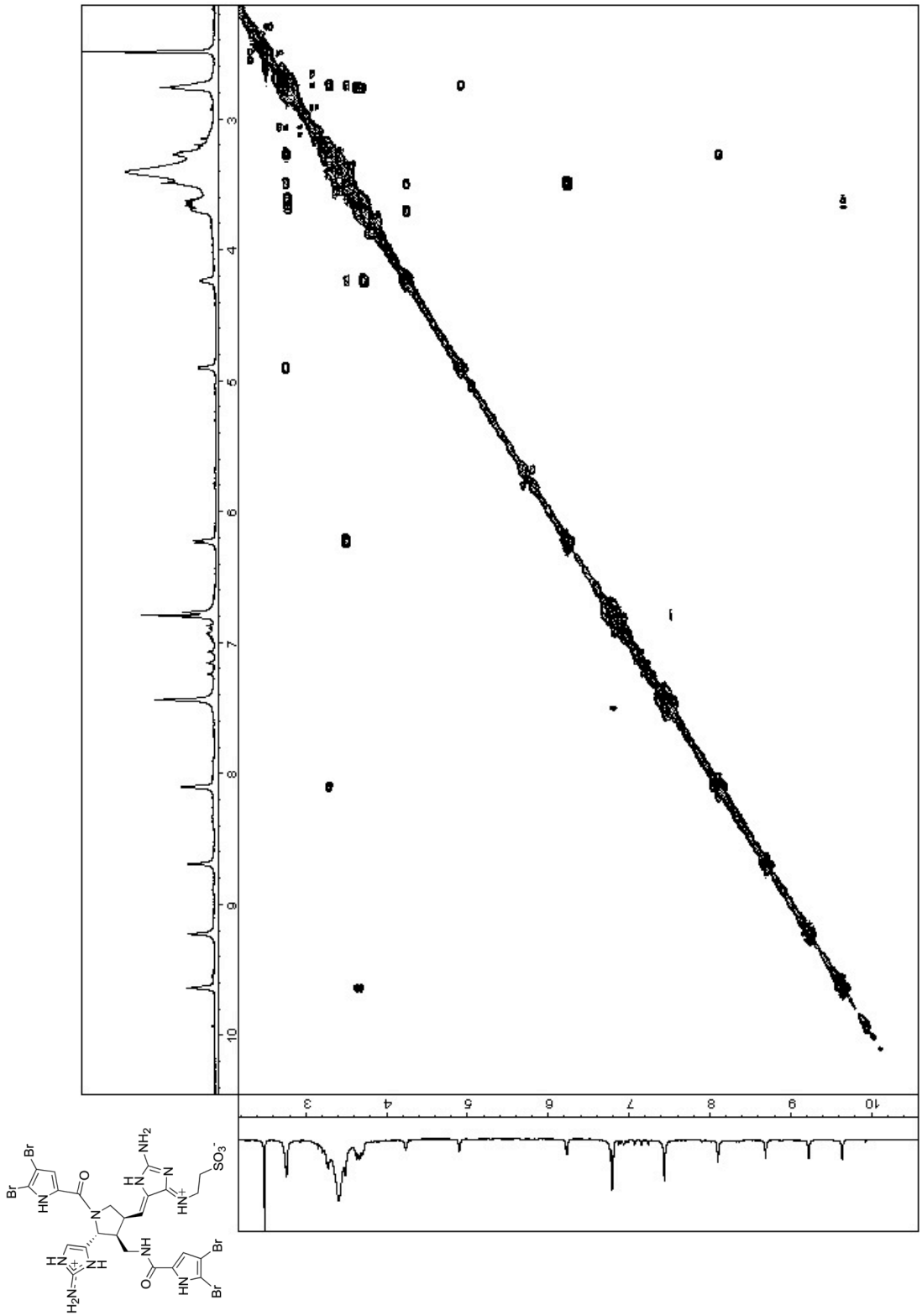

Figure S4. ROESY spectrum of nagelamide Q (**1**) in DMSO-d_6

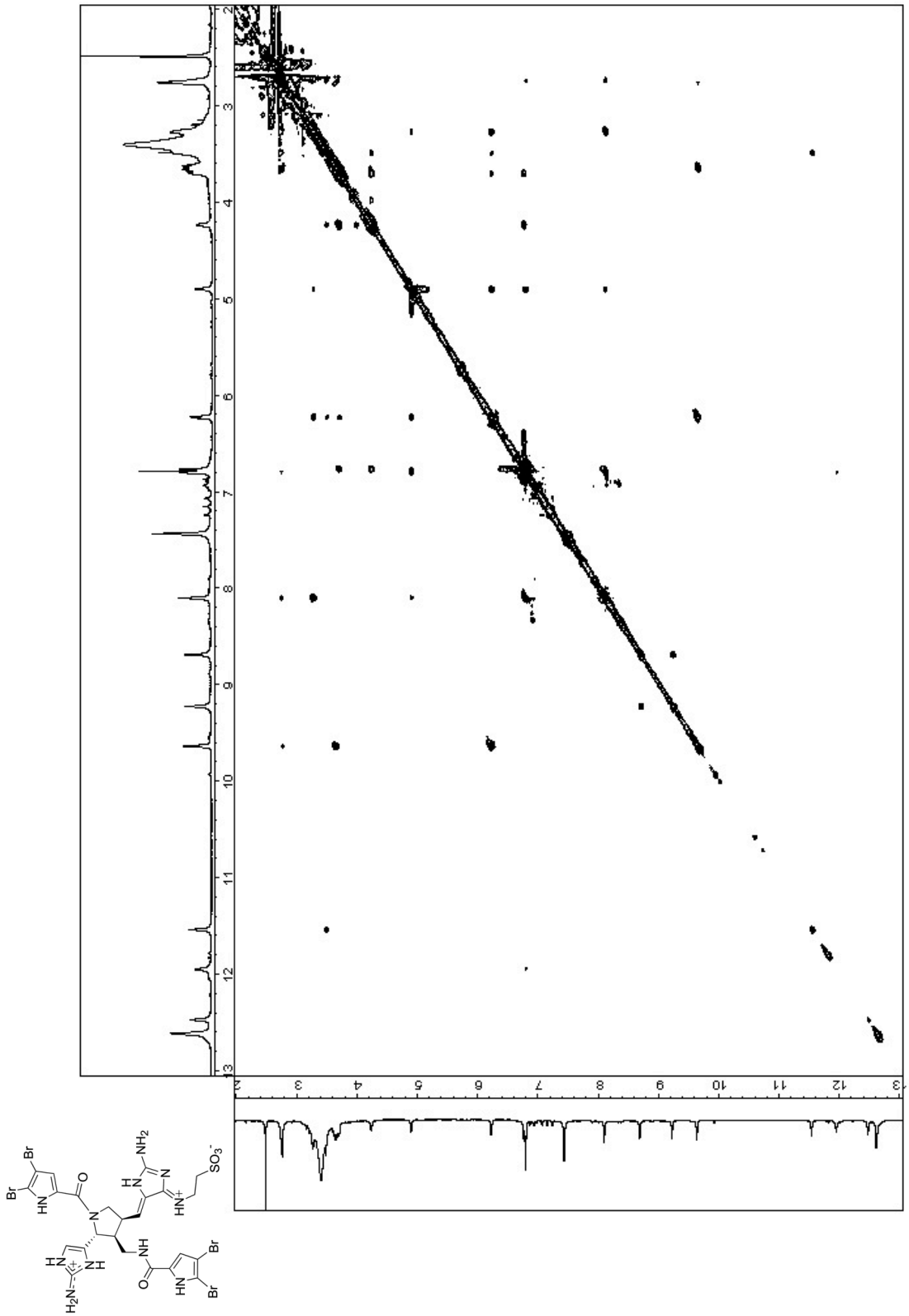

Figure S5. HMQC spectrum of nagelamide Q (**1**) in DMSO-d_6

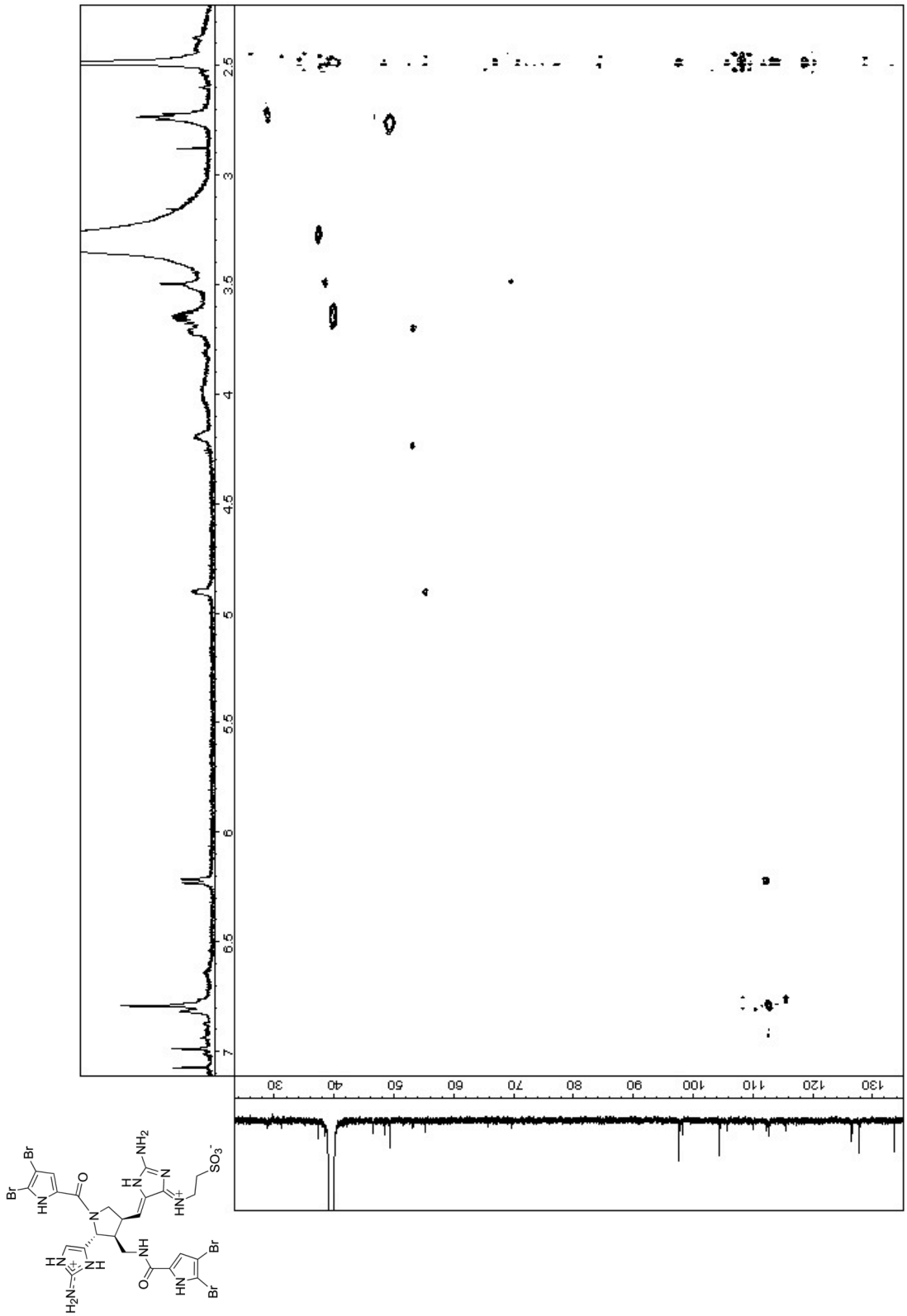

Figure S6. HMBC spectrum of nagelamide Q (**1**) in DMSO-d_6

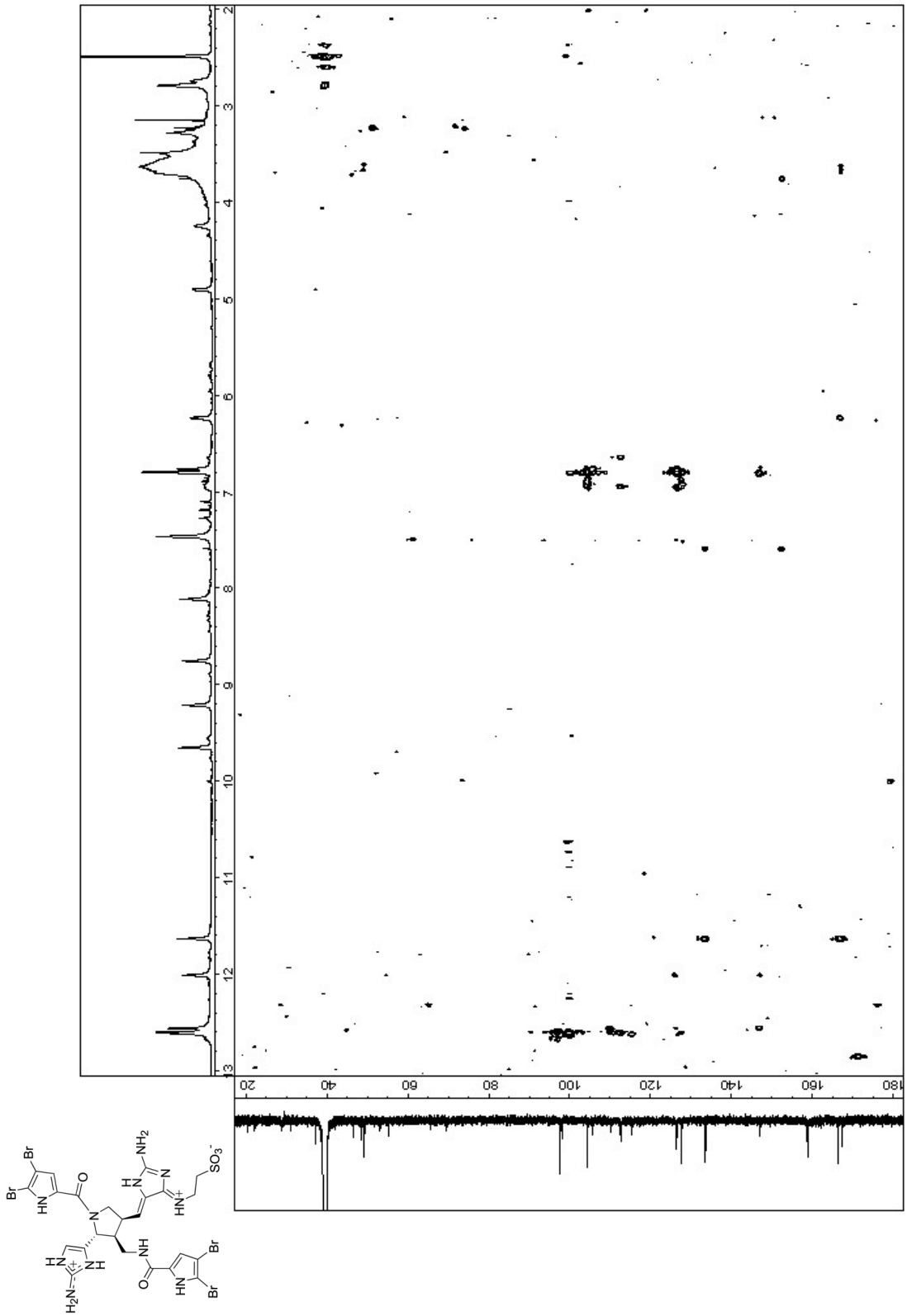

Figure S7. ^{1}H NMR spectrum of nagelamide R (**2**) in C_5D_5N

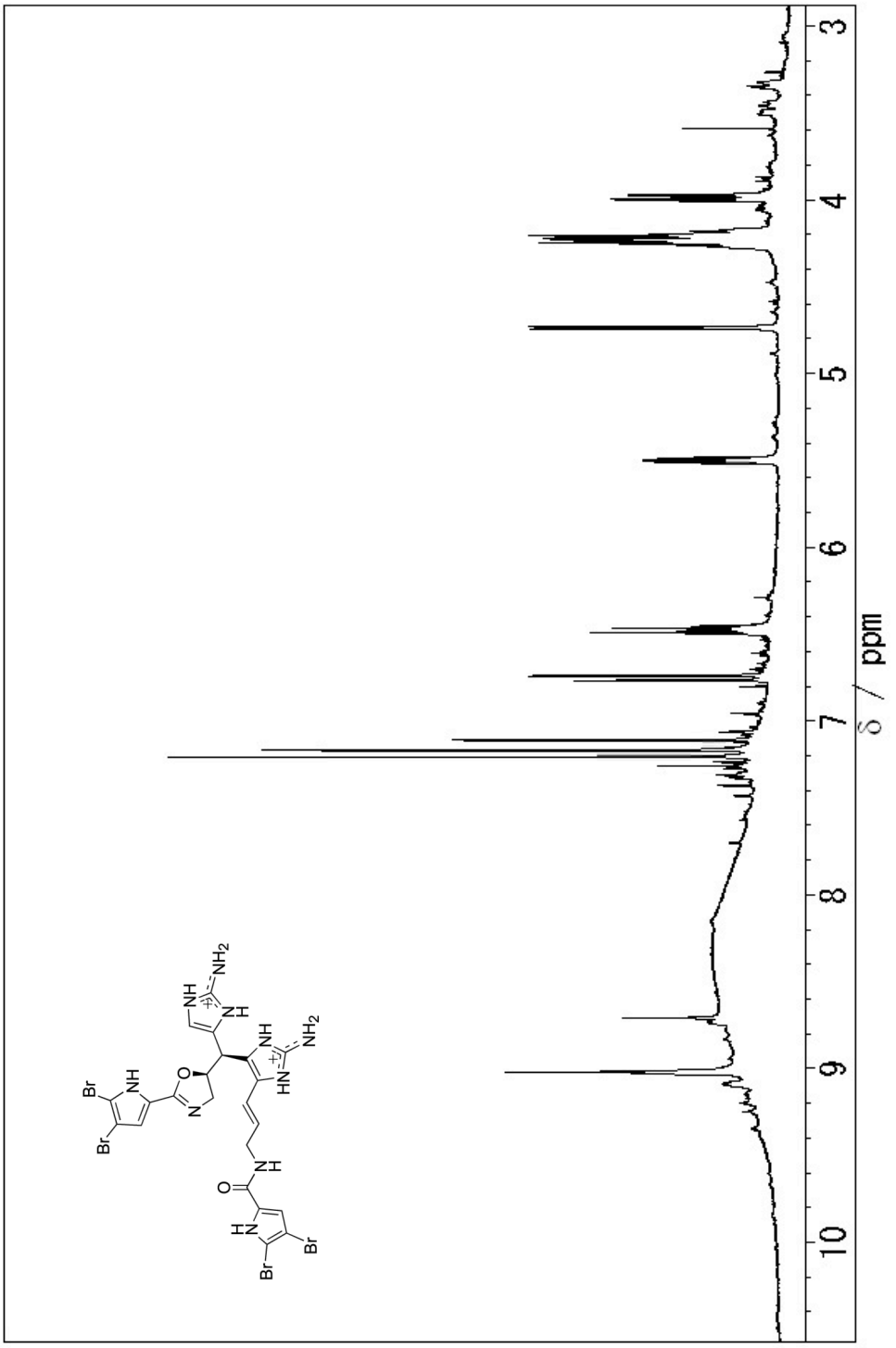

Figure S8. ^{13}C NMR spectrum of nagelamide R (**2**) in C_5D_5N

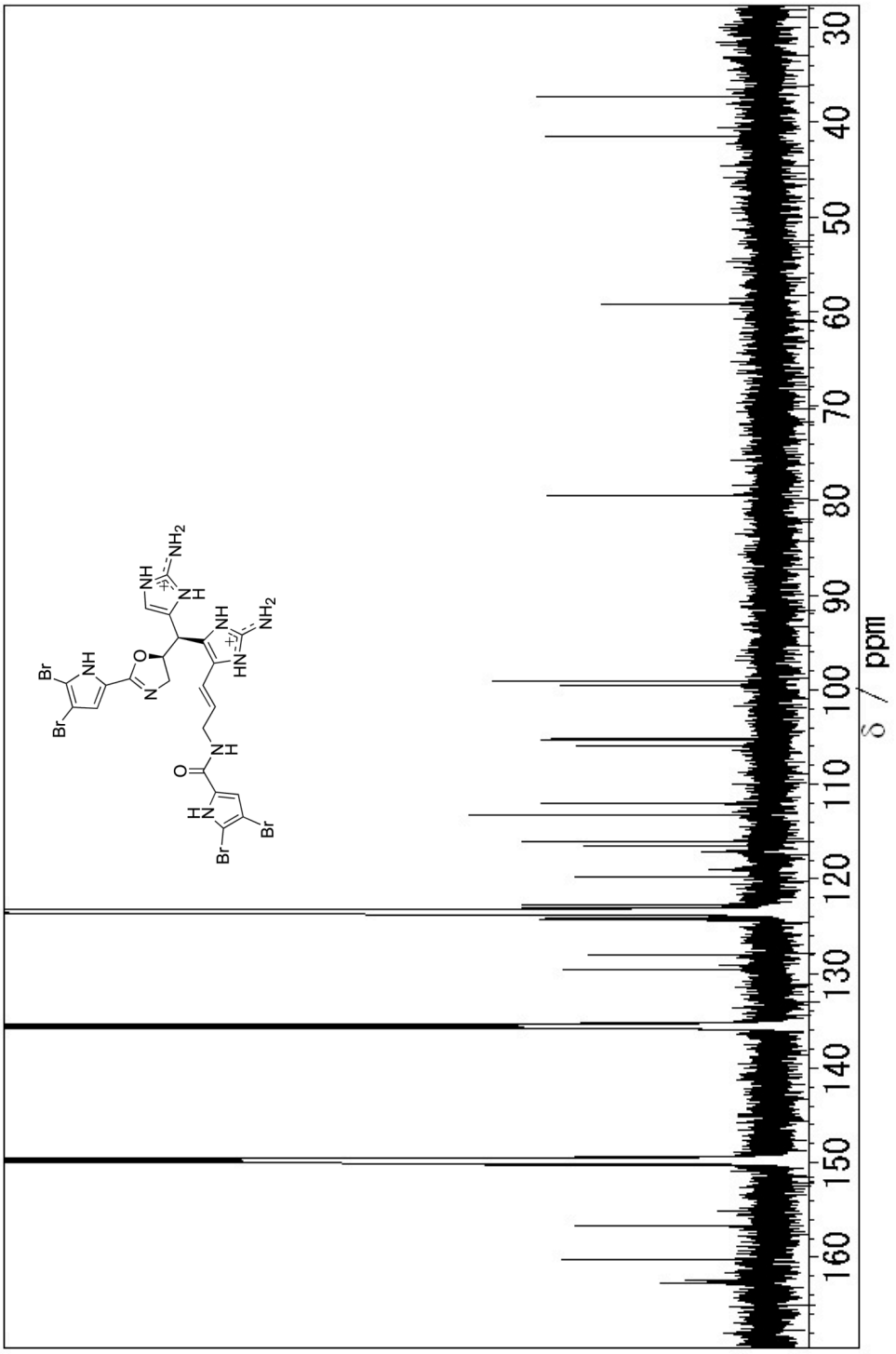

Figure S9. ^{1}H-^{1}H COSY spectrum of nagelamide R (**2**) in C_5D_5N

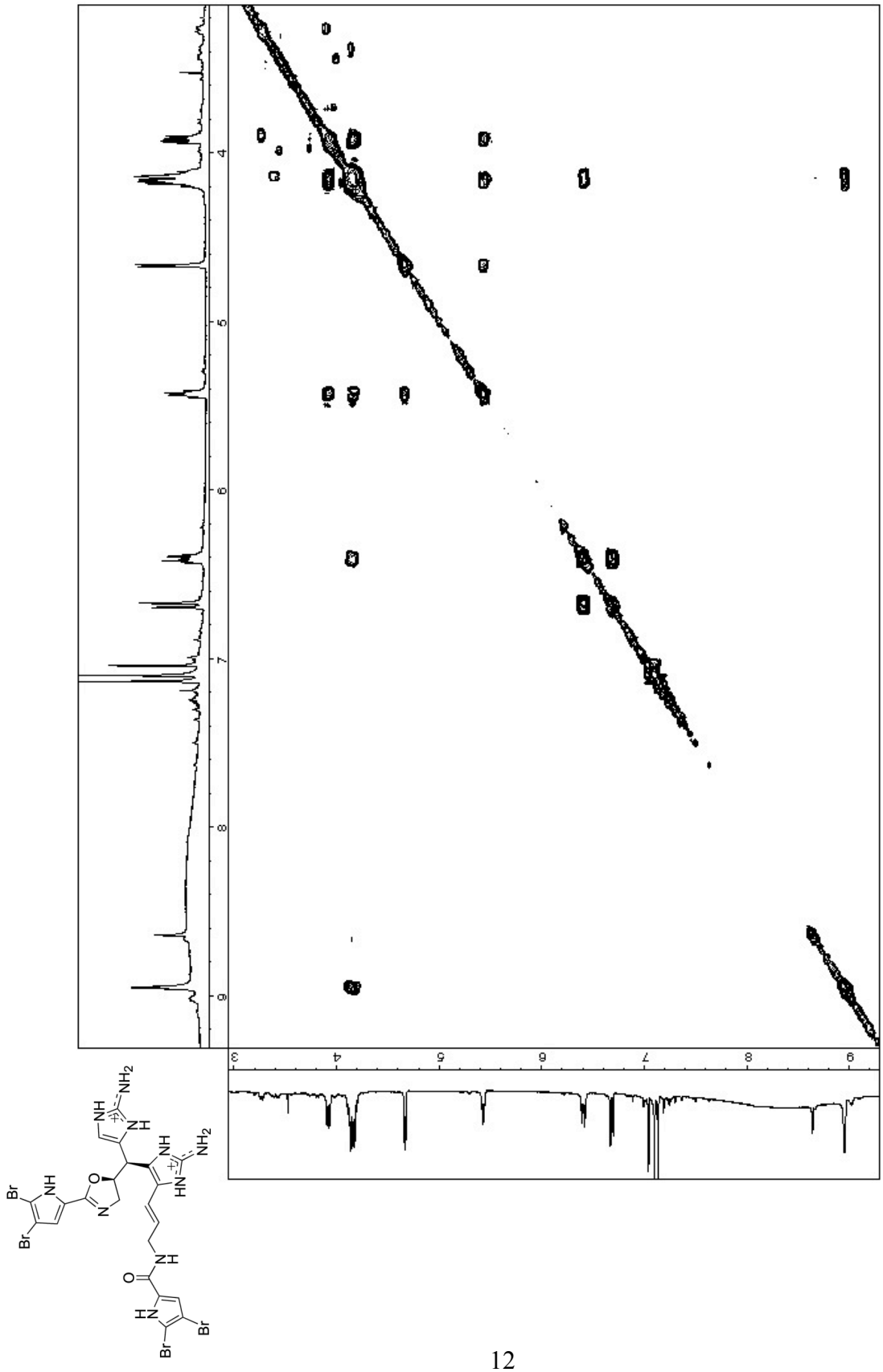

Figure S10. ROESY spectrum of nagelamide R (**2**) in C_5D_5N

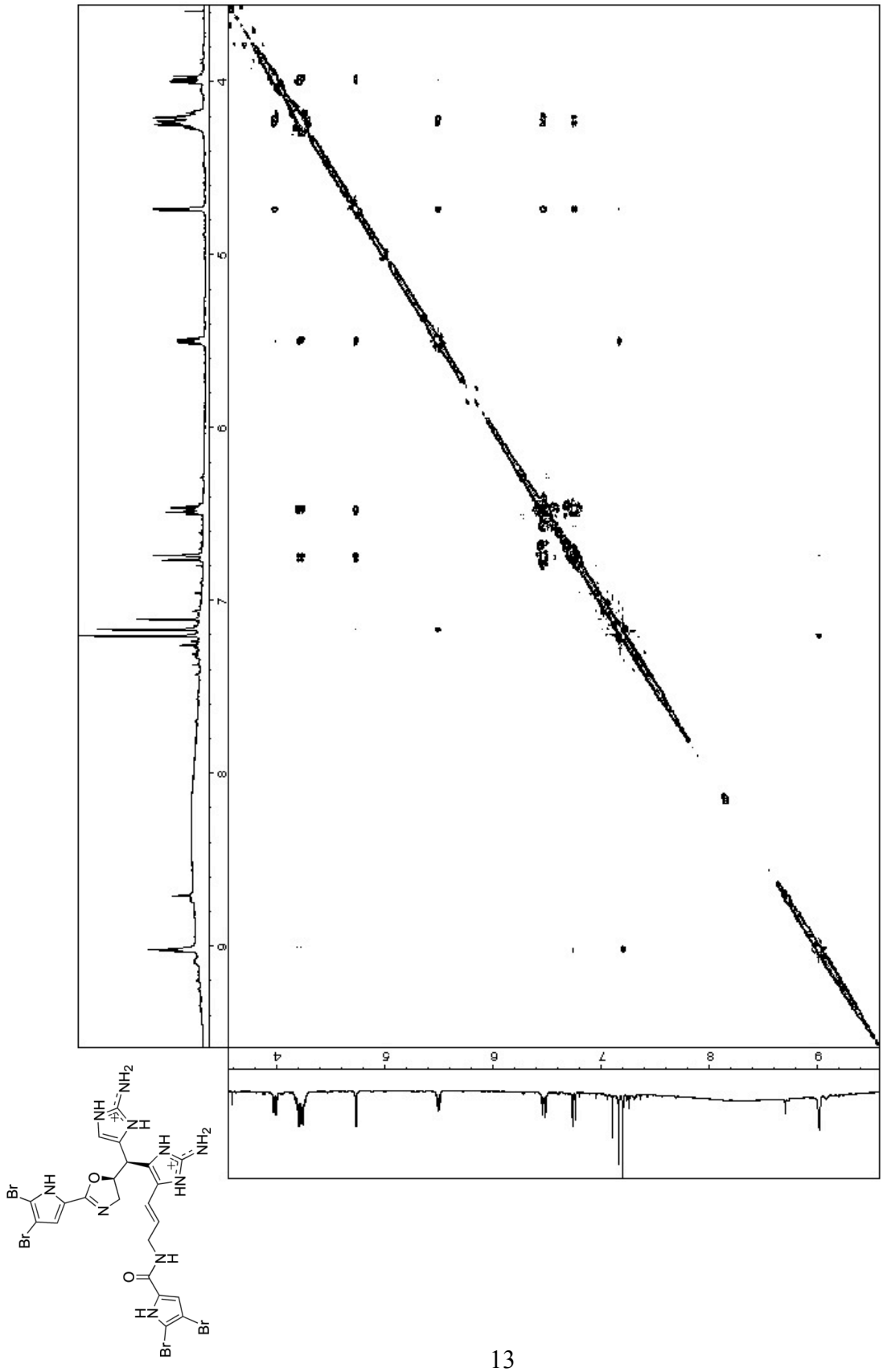

Figure S11. HMQC spectrum of nagelamide R (**2**) in C_5D_5N

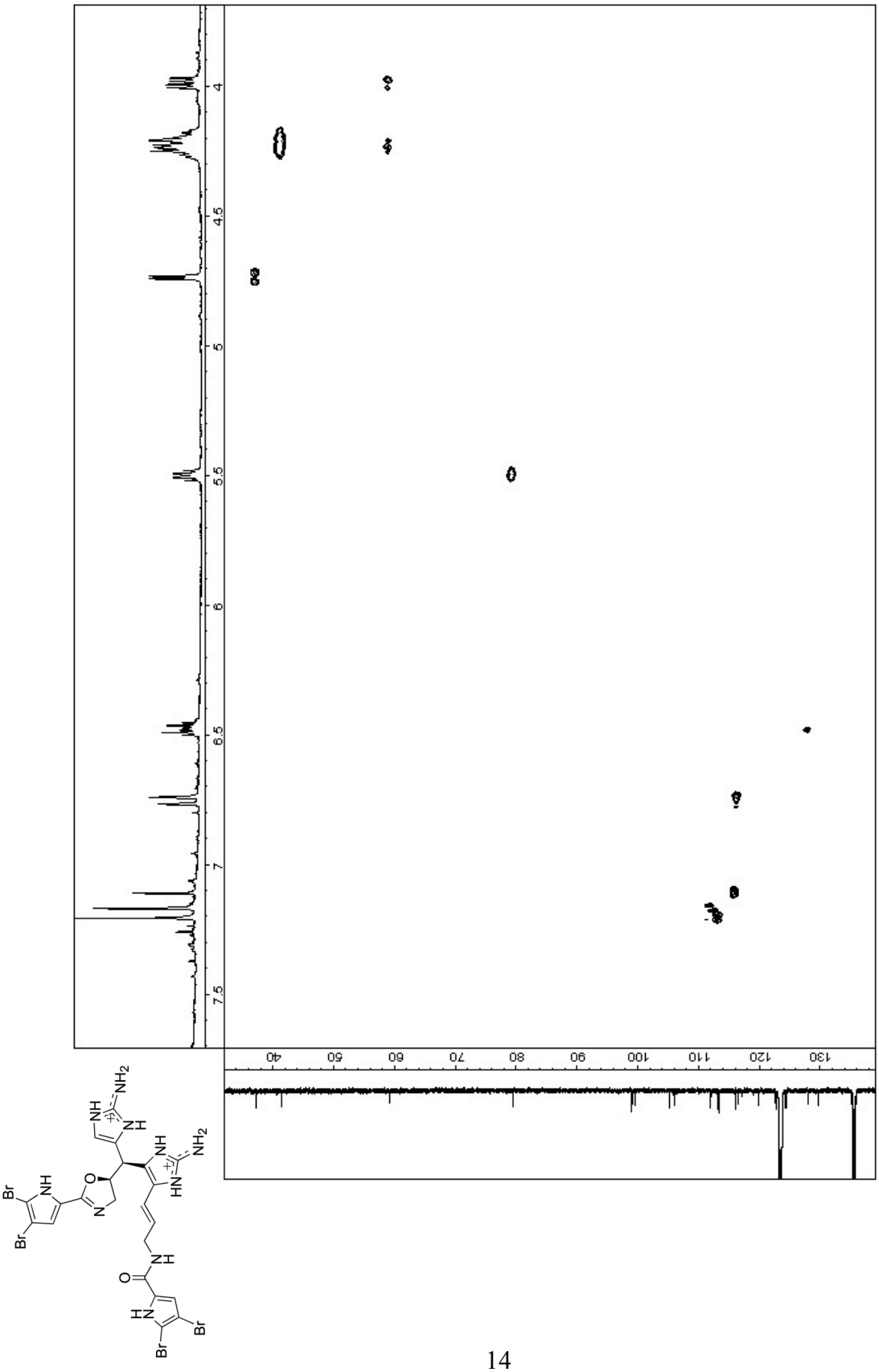

Figure S12. HMBC spectrum of nagelamide R (**2**) in C_5D_5N