Supporting Information

Agesamides A and B, Bromopyrrole Alkaloids from Sponge *Agelas* Species: Application of DOSY for Chemical Screening of New Metabolites

Masashi Tsuda,[†] Tetsuro Yasuda,[†] Eri Fukushi,[‡] Jun Kawabata,[‡] Mitsuhiro
Sekiguchi,[§] Jane Fromont,^{||} and Jun'ichi Kobayashi^{†,*}

Graduate School of Pharmaceutical Sciences, Hokkaido University, Sapporo 060-0812,
Japan, Graduate School of Agriculture, Hokkaido University, Sapporo 060-8589, Japan,
Astellas Pharma Inc., Tsukuba 350-8585, Japan, and Western Australian Museum, Perth
WA 6000, Australia

Experimental Section

page S2 ~ S3

Figures S1 ~ S8

page S4 ~ S11

Experimental Section

Sponge Material. SS1056 The sponge *Agelas* sp. (order Agelasida, family Agelasiidae) was collected off Gessashi, Okinawa, and kept frozen until used. The sponge was firm but somewhat compressible, and its colour was medium orange-brown throughout porous surface and interior. It had some adherent membrane around cavities and irregular surface pitted with large cavities. Its fibres were cored and echinated by verticillate spined styles. Reticulate fibre skeleton was neat oval meshes, primary fibres were 150 μm , and secondary fibres 90 μm . Verticillate acanthostyles were 160 x 25 μm . The voucher specimen (SS-1056) was deposited at Graduate School of Pharmaceutical Sciences, Hokkaido University.

Extraction and Isolation. The sponge (1.35 kg, wet weight) was extracted with MeOH (1L x 2), and the extract was partitioned between EtOAc (600 mL x 3) and 1N NaCl aq (500 mL). Parts (3.00 g) of the *n*-BuOH soluble materials (14.13 g) were subjected to a silica gel column ($\text{CHCl}_3/n\text{-BuOH}/\text{AcOH}/\text{H}_2\text{O}$, 1.5:6:1:1) and then a C_{18} column (SepPak C_{18} cartridge, Waters.; eluent, $\text{CH}_3\text{CN}/\text{H}_2\text{O}/\text{CF}_3\text{CO}_2\text{H}$, 40:60:0.1) to give a crude fraction of bromotyrosine alkaloids. The crude fraction was purified by C_{18} HPLC (YMC Pack AM323, 5 μm , YMC Inc Co., 10 x 250 mm; eluent, $\text{CH}_3\text{CN}/\text{H}_2\text{O}/\text{CF}_3\text{CO}_2\text{H}$, 25:75:0.1; flow rate, 2.0 mL/min; UV detection at 255 nm) to afford a mixture (3.5 mg, 0.00012 %, t_R 12 min) of agesamides A (**1**) and B (**2**) together with oroidin, longamide, and dispacamide A. A part (0.5 mg) of the mixture was separated using C_{18} HPLC (LUNA Phenylhexyl, 5 μm , Phenomenex[®], 10 x 250 mm; eluent, $\text{CH}_3\text{CN}/\text{H}_2\text{O}$, 25:75; flow rate, 2.0 mL/min; UV detection at 255 nm) to afford agesamides A (**1**, 0.2 mg, t_R 26 min) and B (**2**, 0.2 mg, t_R 28 min)

DOSY Measurement. The DOSY spectrum was recorded with a Bruker AMX 500

spectrometer (^1H =500 MHz) equipped with a 2.5 mm ϕ TXI probehead. Pulse sequence used was bipolar pulse longitudinal eddy current delay (BPPLIED).¹⁰ The duration of the magnetic field pulse gradients (δ) and diffusion time (Δ) were 0.1 and 500 ms, respectively. The pulsed field gradients were incremented from 1 to 32 G cm⁻¹. A series of 32 BPPLIED spectra on 16K data points were collected with 2816 transients, total measuring time was ca. 64 h. After Fourier transformation and baseline correction, the diffusion dimension was processed with the Bruker Xwinnmr software package (version 2.6).

Figure S1 ^1H NMR spectrum of agesamide A (1) in $\text{DMSO}-d_6$.

Figure S2. ^1H NMR spectrum of agesamide B (**2**) in $\text{DMSO}-d_6$.

Figure S3. ^{13}C NMR spectrum of mixture of agesamides A (1) and B (2) in $\text{DMSO-}d_6$.

Figure S4. ^1H - ^1H COSY spectrum of mixture of agesamides A (1) and B (2) in $\text{DMSO}-d_6$.

Figure S5. HSQC spectrum of mixture of agesamides A (1) and B (2) in DMSO- d_6 .

Figure S6. HMBC spectrum of mixture of agesamides A (1) and B (2) in DMSO- d_6 .

Figure S7. ROESY spectrum of mixture of agsamides A (1) and B (2) in DMSO- d_6 .

Figure S8. DOSY slice spectra for logD (a) -10.25, (b) -9.92, and (c) -9.87, and (d) ^1H NMR spectrum of bromopyrrole-containing fraction **A** from a sponge *Agelas* species.