Supplementary data:

Kurahyne, an acetylene-containing lipopeptide from a marine cyanobacterial assemblage of *Lyngbya* sp.

Arihiro Iwasaki [a], Osamu Ohno [a], Shinpei Sumimoto [b], Shoichiro Suda [b], Kiyotake Suenaga [a],

[a] *Department of Chemistry, Keio University, 3-14-1, Hiyoshi, Kohoku-ku, Yokohama, Kanagawa 223-8522, Japan*

[b] *Faculty of Science, University of Ryukyus, 1 Senbaru, Nishihara, Okinawa 903-0213, Japan*

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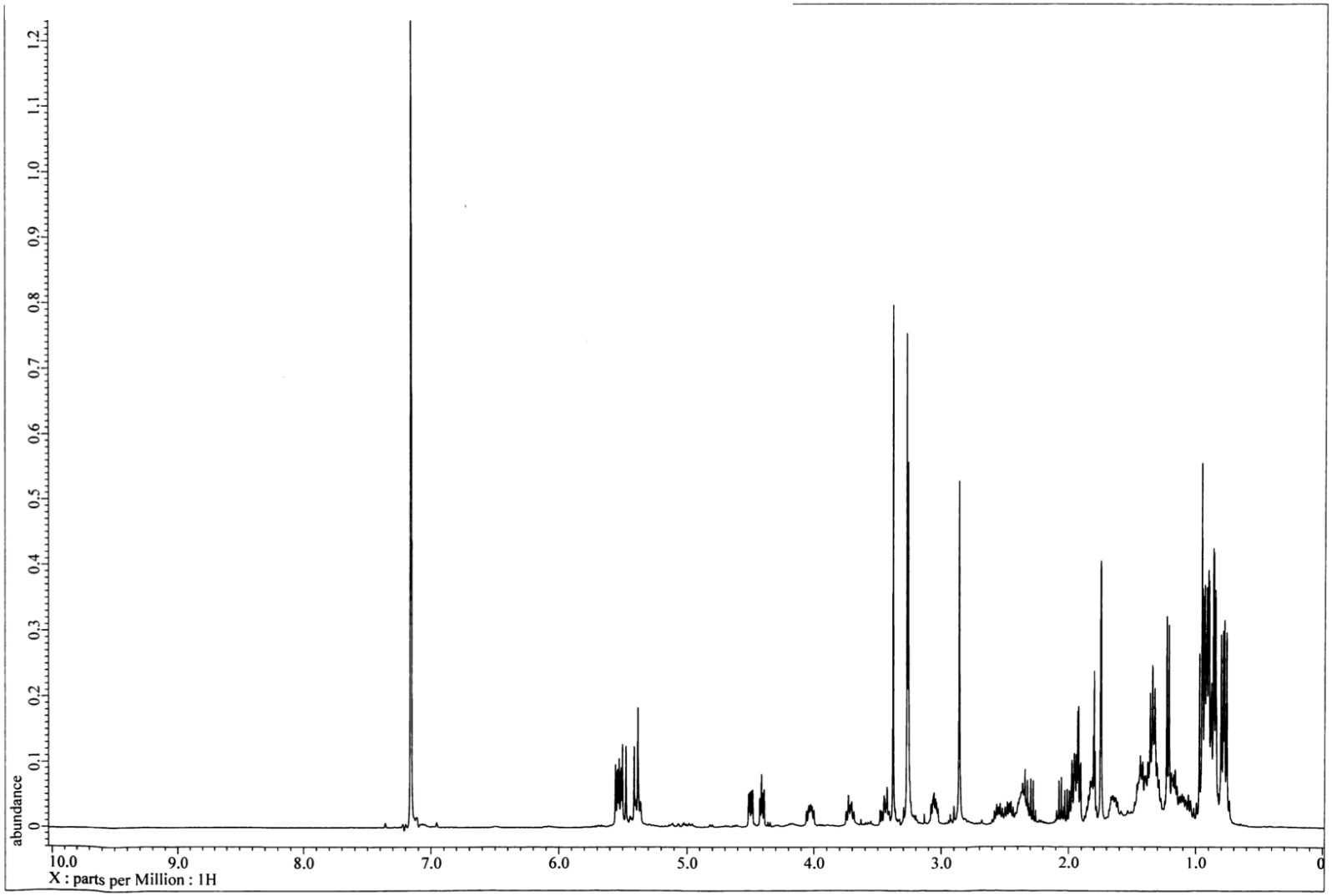
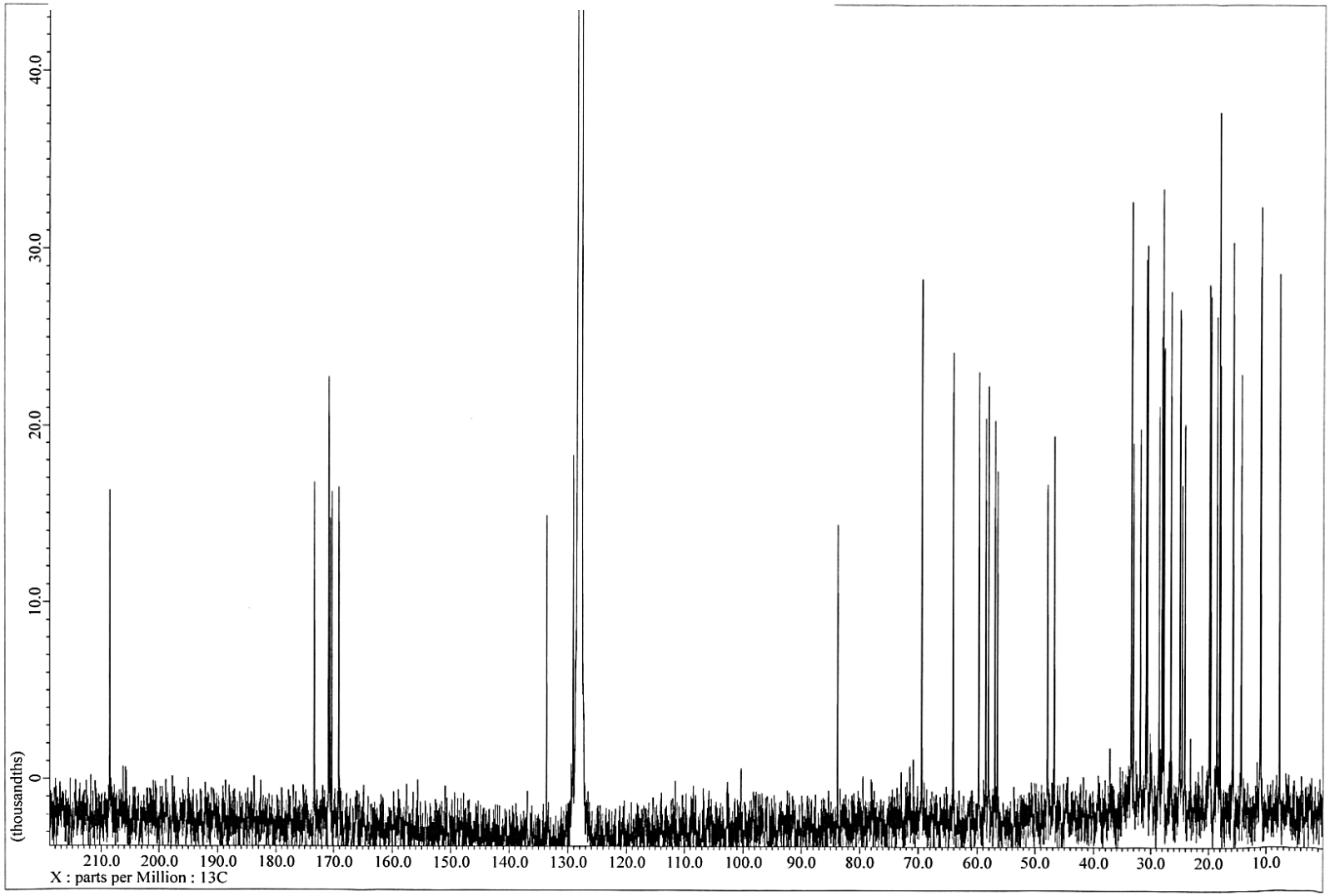
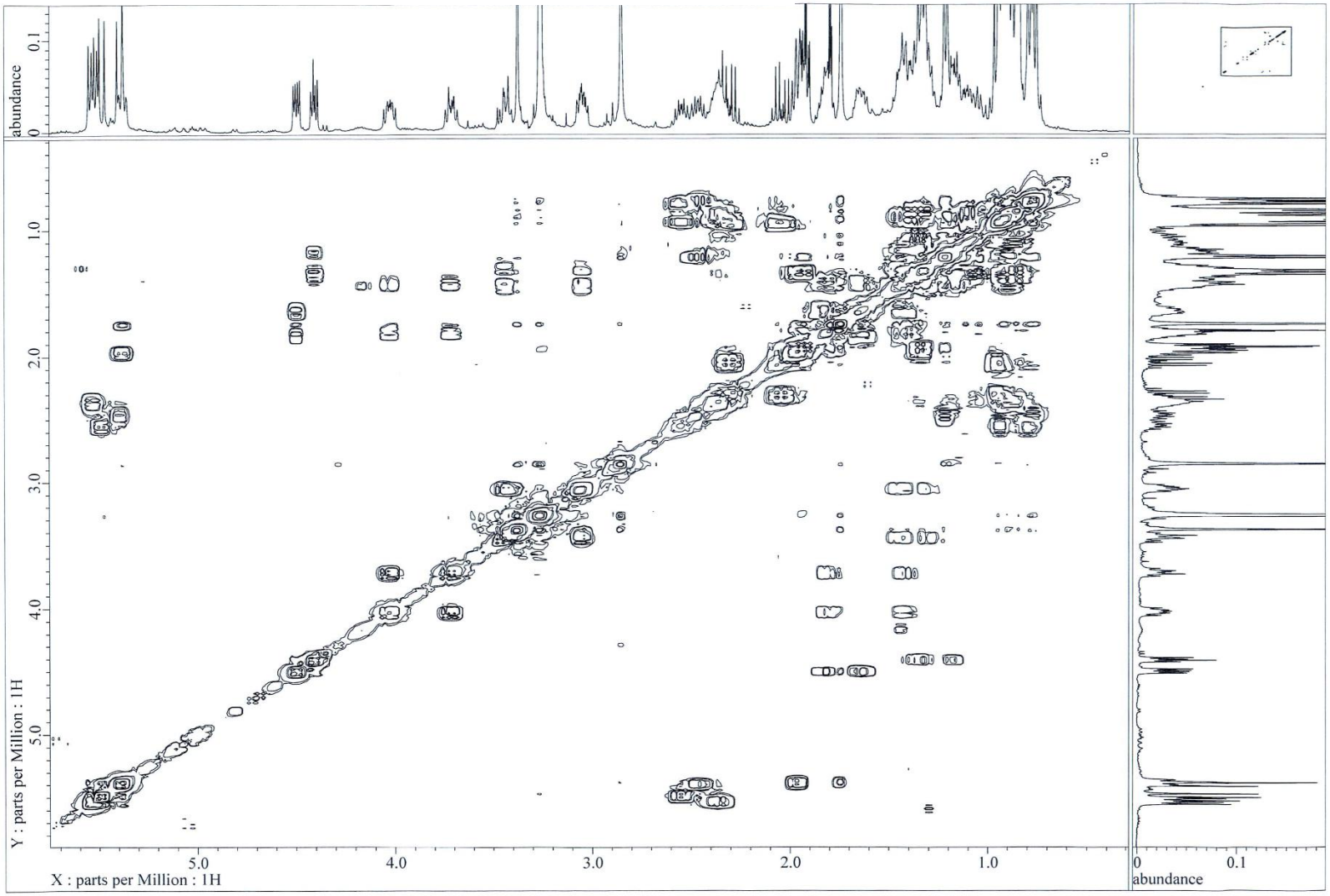


Page S 3. ^{13}C NMR (100 MHz, C_6D_6) spectrum of kurahyne (**1**)



Page S 4. COSY (400 MHz, C_6D_6) spectrum of kurahyne (**1**)



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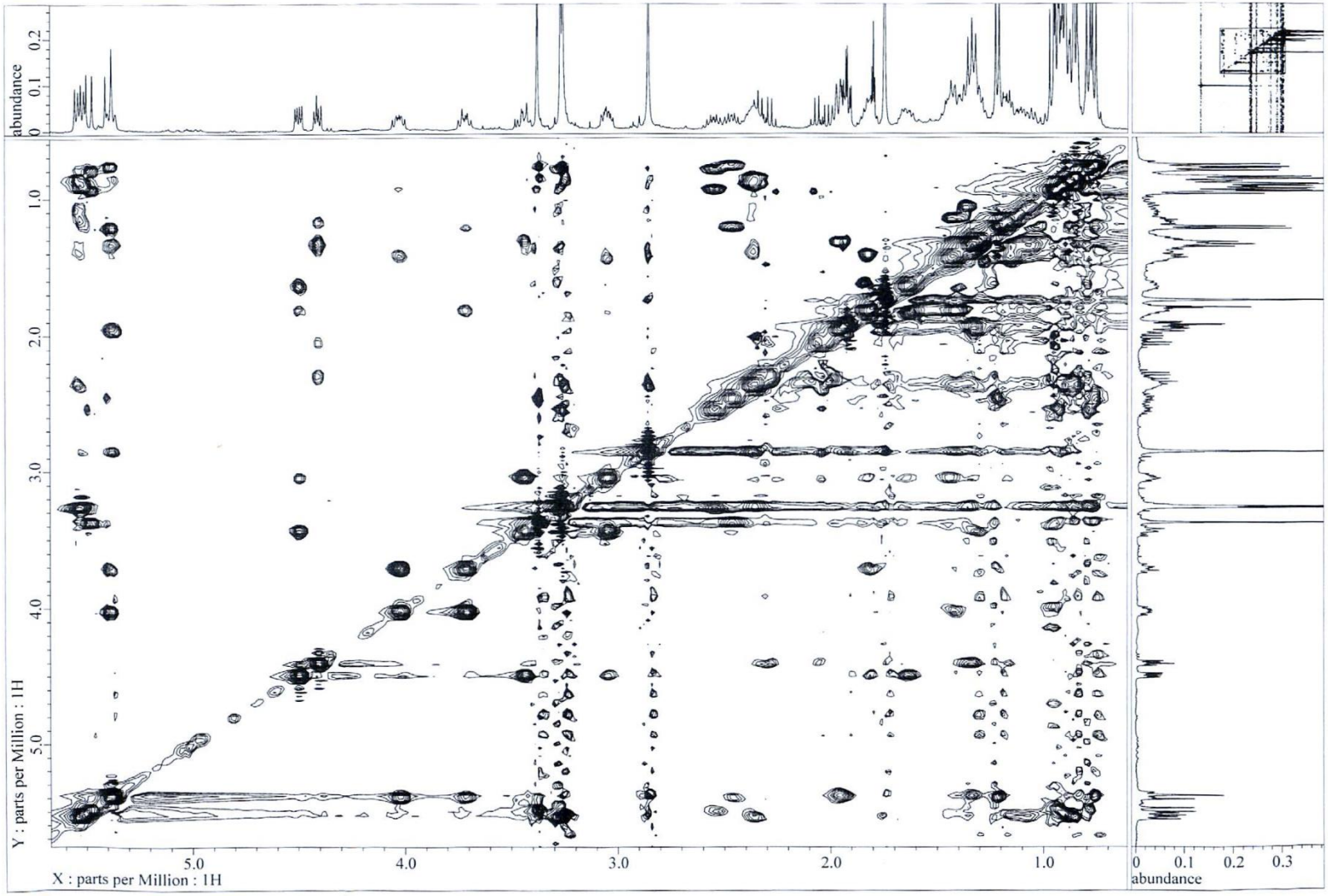
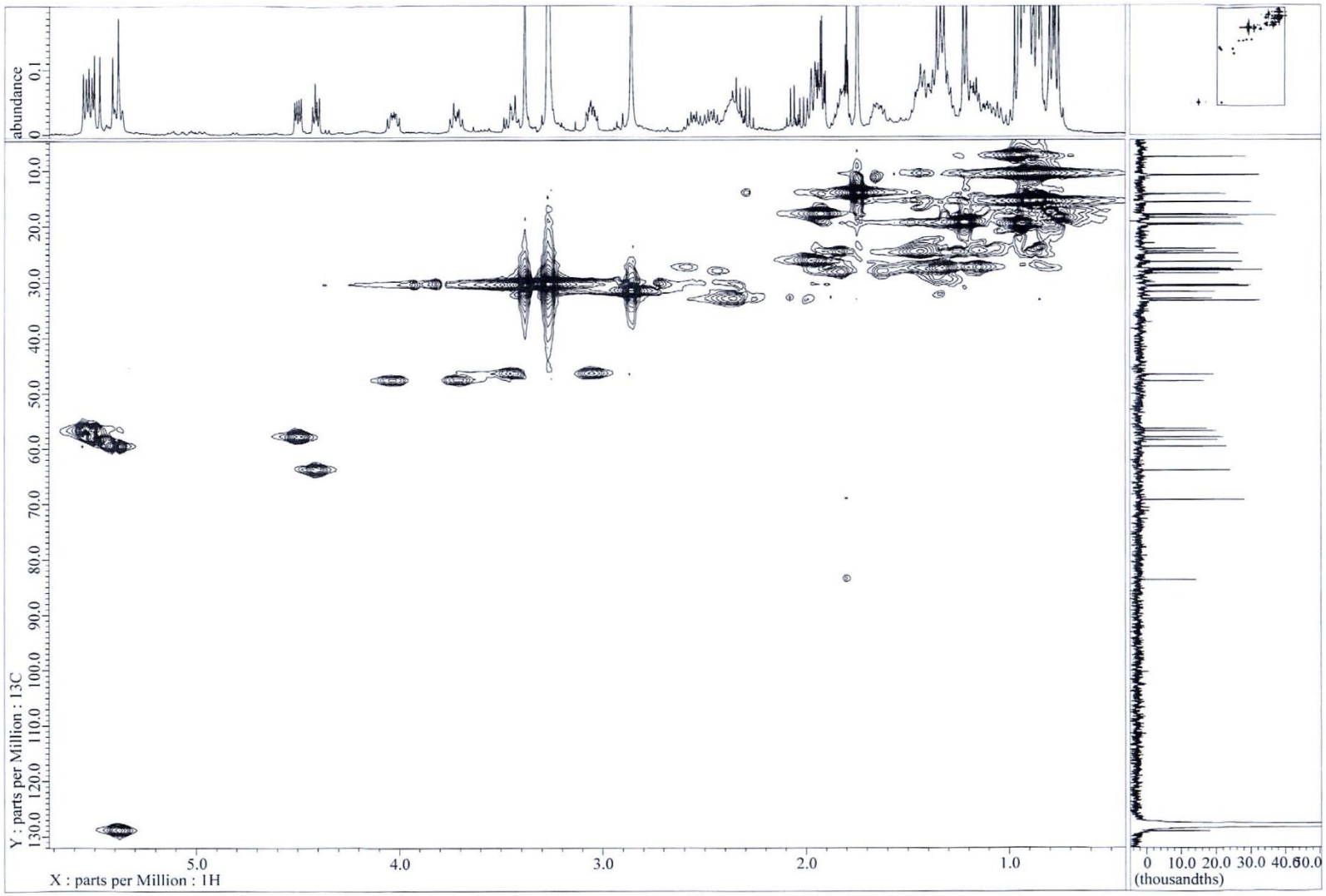
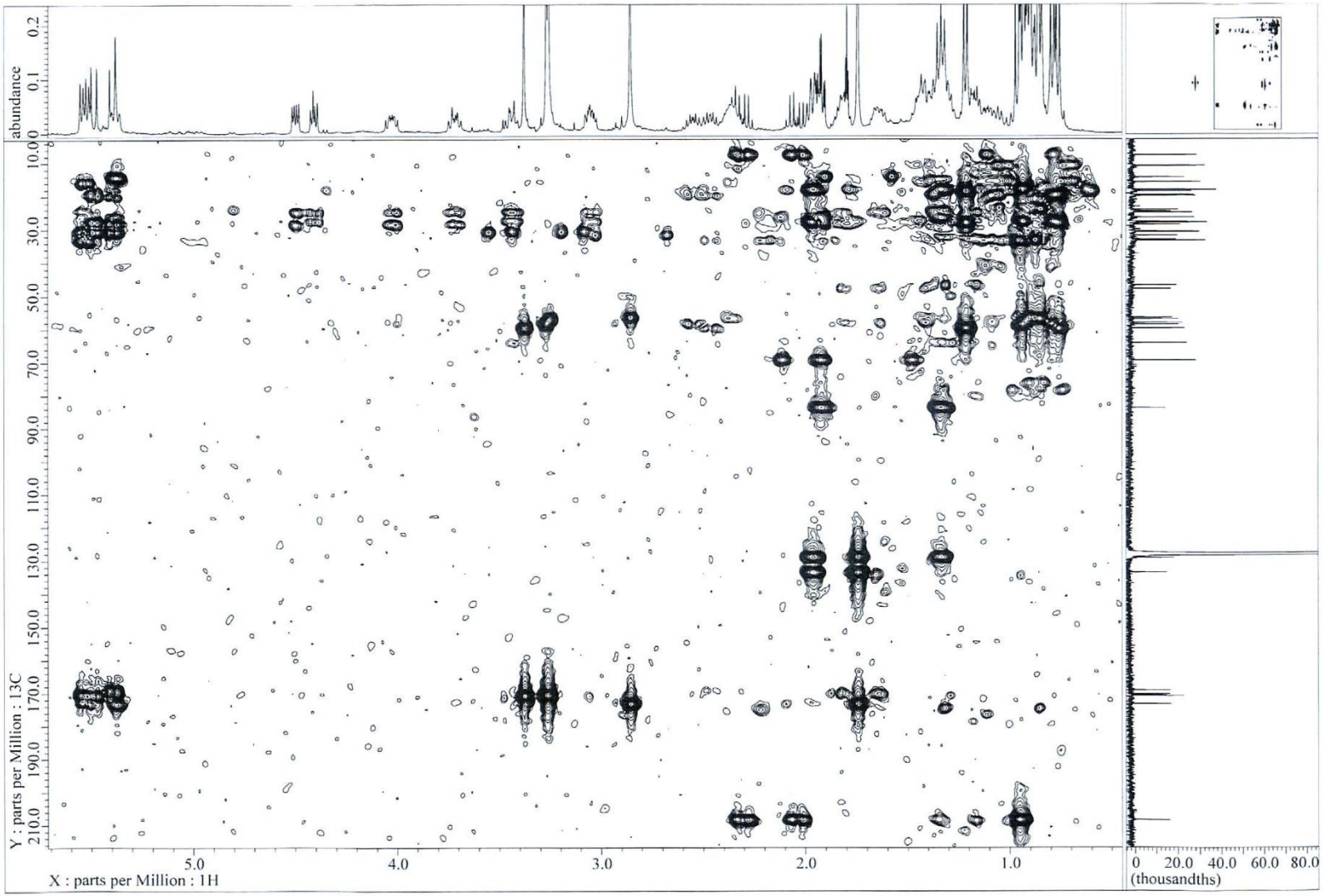


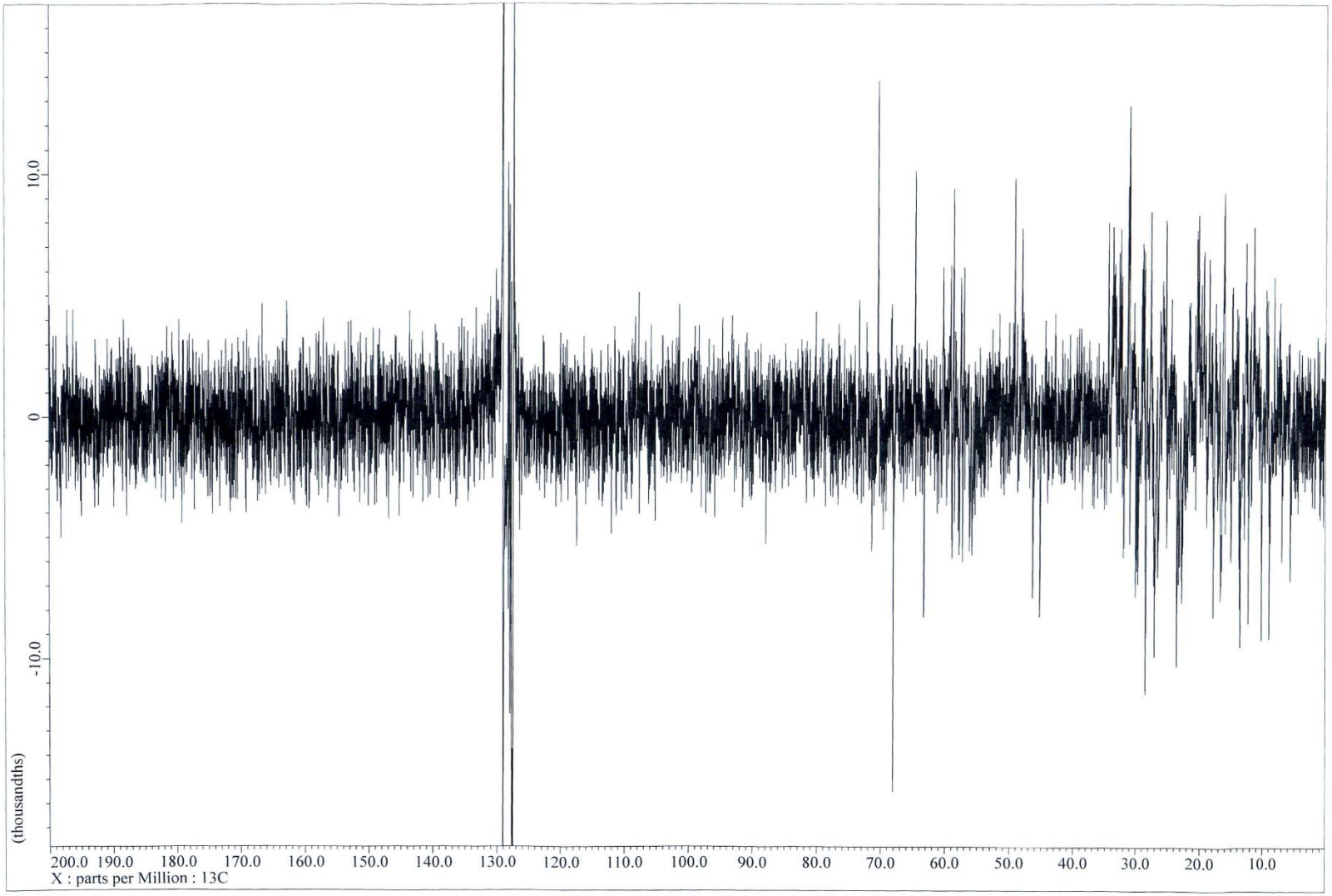
Page S 6. HMQC (400 MHz, C_6D_6) spectrum of kurahyne (**1**)

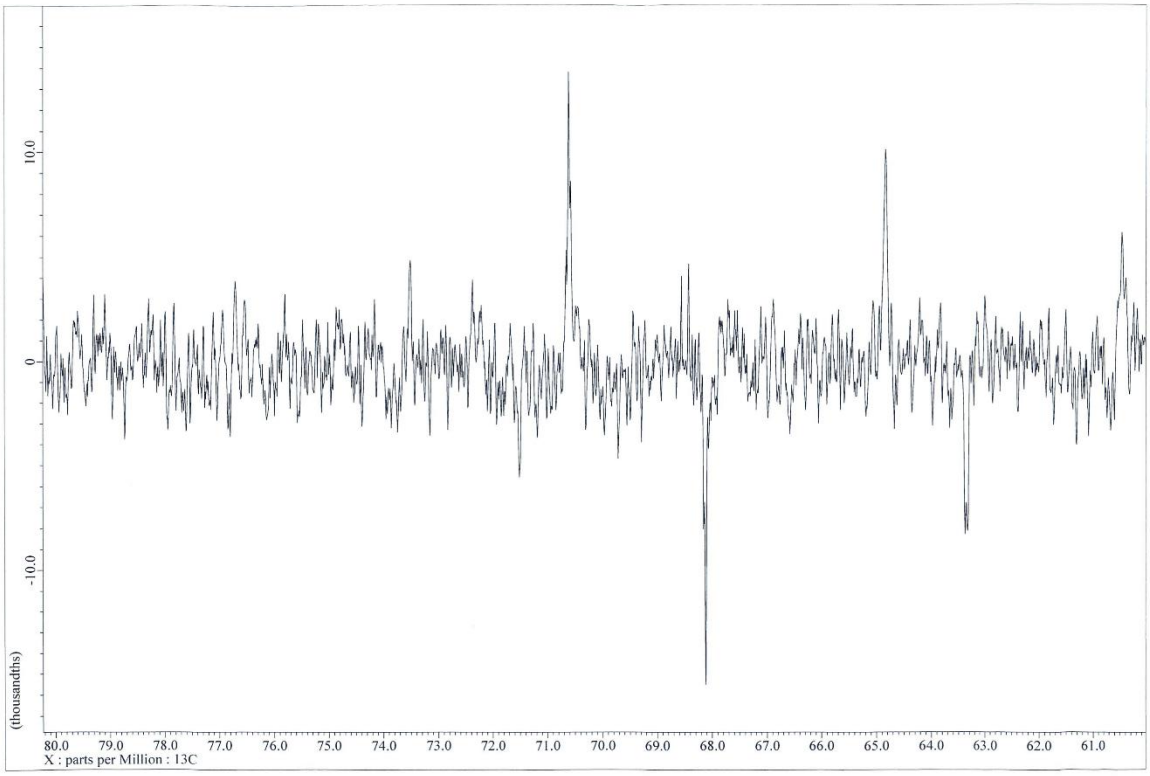


Page S 7. HMBC (400 MHz, C_6D_6) spectrum of kurahyne (**1**)



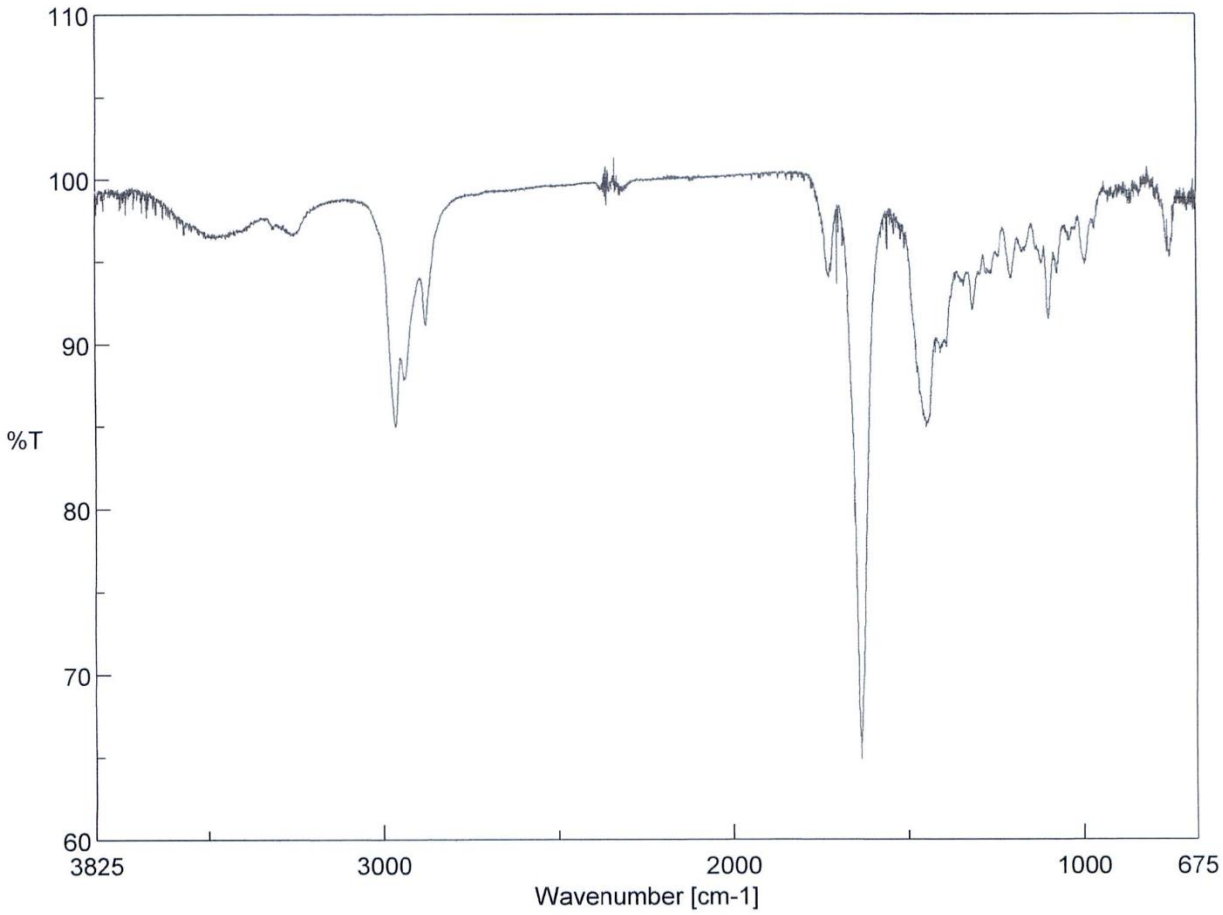
Page S 8. INEPT (100 MHz, C_6D_6) spectrum of kurahyne (**1**)





Zoomed INEPT spectrum of kurahyne (**1**), [60-80 ppm]

Regarding the ^{13}C signal at 69.4 ppm, the magnitude of the coupling constant ($^{1}J_{C\text{-}H}$ = 253 Hz) indicated that the present carbon was a terminal acetylene carbon.



IR spectrum of kurahyne (**1**)

Experimental

1. General

Chemicals and solvents were the best grade available and used as received from commercial sources. All NMR spectral data were recorded on a JEOL ECX-400 spectrometer for ^{1}H (400 MHz) and ^{13}C (100 MHz). ^{1}H NMR chemical shifts (referenced to residual C_6HD_5 observed at δ 7.16) were assigned using a combination of data from COSY and HMQC experiments. Similarly, ^{13}C NMR chemical shifts (referenced to C_6D_6 observed at δ 128.06) were assigned based on HMBC and HMQC experiments. ESI mass spectra were obtained on an LCT premier EX spectrometer (Waters). Optical rotations were measured with a JASCO DIP-1000 polarimeter. IR spectra were recorded on a JASCO RT/IR-4200 instrument. Fluorescence micrographs were taken with Nikon Eclipse Ti-S.

2. Collection, extraction and isolation

Marine cyanobacterial samples were collected at Kuraha, Okinawa Prefecture, Japan, at a depth of 0-1 m in March 2013. The collected cyanobacterium (2.3 kg) was extracted with methanol (4 L) for one week. The extract was filtered, and the filtrate was concentrated. The residue was partitioned between ethyl acetate (3 × 0.4 L) and water (0.4 L). The material obtained from the organic layer was partitioned between 90% aqueous methanol (0.4 L) and hexane (3 × 0.4 L). The aqueous methanol fraction (1.3 g) was first separated by column chromatography on ODS (13 g) eluted with 40% methanol, 60% methanol, 80% methanol, and methanol. The fraction (439 mg) eluted with 80% methanol was subjected to HPLC [Cosmosil Cholester (φ20 × 250 mm); flow rate 5mL/min; detection, UV 215 nm; solvent 50% MeCN] in eight batches to give a fraction that contained kurahyne (312 mg, t_R = after 42.0 min, last collected fraction). This fraction was further separated by HPLC [Cosmosil $5C_{18}$MS-II (φ20 × 250 mm); flow rate 5mL/min; detection, UV 215 nm; solvent 80% MeOH] in six batches to give kurahyne (**1**) (29.9 mg, t_R = 57.5 min, total yield 0.0013% based on wet weight).

Kurahyne (**1**): colorless oil; $[\alpha]^{29}_{D}$ -258 (*c* 0.20, CH_3OH); IR (film) λmax 3474, 2963, 2875, 1723, 1635, 1448 cm^{-1}; ^{1}H NMR, ^{13}C NMR, COSY, HMBC and NOESY data, see Table 1; HRESIMS m/z 839.5991 $[M+H]^+$ (calcd for $C_{47}H_{79}N_6O_7$, 839.6010).

3. Reduction-acid hydrolysis of 1

To a mixture of kurahyne (**1**) (2.4 mg, 2.9 μmol) in MeOH (0.6 mL) at room temperature was added sodium borohydride (30 mg, 790 μmol). After the mixture was stirred at room temperature for 1.5 h, it was diluted with EtOAc (5 mL) and H_2O (5 mL). The organic layer was separated, and the aqueous layer was extracted with EtOAc (3 × 5 mL). The organic layer and the extracts were combined, washed with saturated aqueous NaCl (10 mL), dried with Na_2SO_4, and concentrated to give a colorless oil (1.8 mg). An aliquot of the residual oil (0.8 mg) and 6 M HCl (0.1 mL) were charged in a reaction tube, sealed under reduced pressure, and heated at 110 °C for 45 h. The mixture was evaporated to dryness and could be separated into each component. [Conditions for HPLC separation of amino acids: column, Cosmosil $5C_{18}$-PAQ (φ20 × 250 mm); flow rate, 5.0 mL/min; detection at 215 nm; solvent H_2O. Retention times of components: Pro (t_R = 11.6 min), *N*-Me-Val (t_R = 13.5 min), *N*-Me-Ile (t_R = 20.2 min)], [Conditions for HPLC separation of 2-(1-hydroxypropyl)-pyrrolidine (Opp derivatives): column, Cosmosil $5C_{18}$-PAQ (φ4.6 × 250 mm); flow rate, 1.0 mL/min; detection at 215 nm; solvent MeOH/H_2O/TFA = 1/99/0.1. Retention times: 7.8, 8.9 min]

4. HPLC analysis of amino acid components

With regard to *N*-Me-Ile, the fraction dissolved in H_2O (50 μL) was analyzed by reversed-phase HPLC, and the retention times were compared to those of *N*-Me-Ile and *N*-Me-*allo*-Ile. [Cosmosil $5C_{18}$-PAQ (φ4.6 × 250 mm); flow rate, 1.0 mL/min; detection at 215 nm; solvent H_2O] The retention time of *N*-Me-Ile in the hydrolysate matched that of *N*-Me-Ile (t_R = 5.3 min), but not *N*-Me-*allo*-Ile (t_R = 5.1 min).

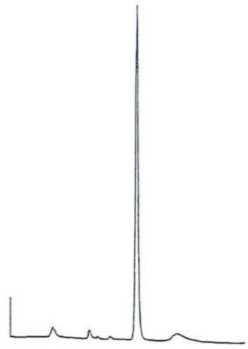
Further, each fraction that contained amino acids was dissolved in H_2O (50 μL) and analyzed by chiral HPLC, and the retention times were compared to those of authentic standards. [DAICEL CHIRALPAK (MA+) (φ4.6 × 50 mm); flow rate, 1.0 mL/min; detection at 254 nm; solvent 2.0 mM $CuSO_4$] The retention times of the amino acids in the hydrolysate matched those of L-Pro (t_R = 5.1 min), *N*-Me-L-Val (t_R = 5.2 min) and *N*-Me-L-Ile (t_R = 12.9 min), but not D-Pro (t_R = 3.0 min), *N*-Me-D-Val (t_R = 3.0 min) or *N*-Me-D-Ile (t_R = 6.6 min).

<u>*N*-Me-Ile</u>:

Condition 1: column, Cosmosil $5C_{18}$-PAQ (φ4.6 × 250 mm); flow rate, 1.0 mL/min; detection at 215 nm; solvent, H_2O

t_R (min): Authentic samples: *N*-Me-Ile (5.3), *N*-Me-*allo*-Ile (5.1).

N-Me-Ile from natural **1** (5.3).



N-Me-Ile from natural **1**

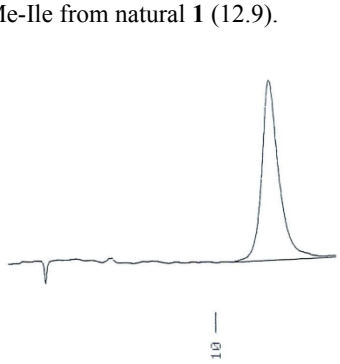
N-Me-Ile from natural **1** with *N*-Me-Ile

N-Me-Ile from natural **1** with *N*-Me-*allo*-Ile

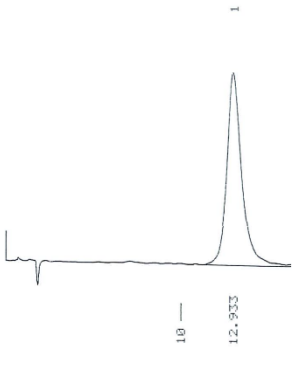
Condition 2: column, DAICEL CHIRALPAK (MA+) (φ4.6 × 50 mm); flow rate, 1.0 mL/min; detection at 254 nm; solvent, 2.0 mM $CuSO_4$

t_R (min): Authentic samples: *N*-Me-L-Ile (12.9), *N*-Me-D-Ile (6.6).

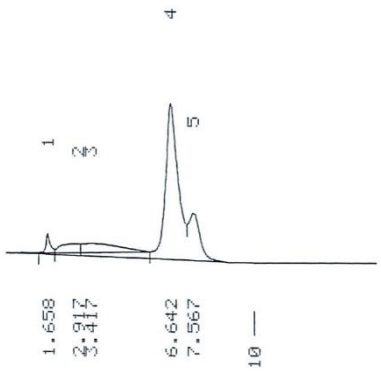
N-Me-Ile from natural **1** (12.9).



N-Me-Ile from natural **1**



N-Me-Ile from natural **1** with *N*-Me-L-Ile

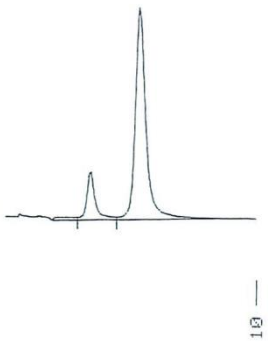


N-Me-D-Ile

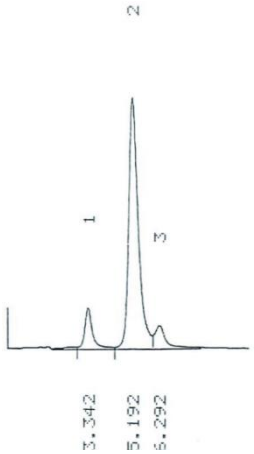
N-Me-Val: column, DAICEL CHIRALPAK (MA+) (φ4.6 × 50 mm); flow rate, 1.0 mL/min; detection at 254 nm; solvent, 2.0 mM $CuSO_4$

t_R (min): Authentic samples: *N*-Me-L-Val (5.2), *N*-Me-D-Val (3.0)

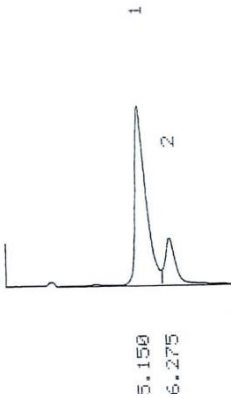
N-Me-Val from natural **1** (5.2).



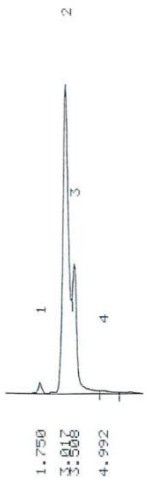
N-Me-Val from natural **1**



N-Me-Val from natural **1** with *N*-Me-L-Val



N-Me-L-Val

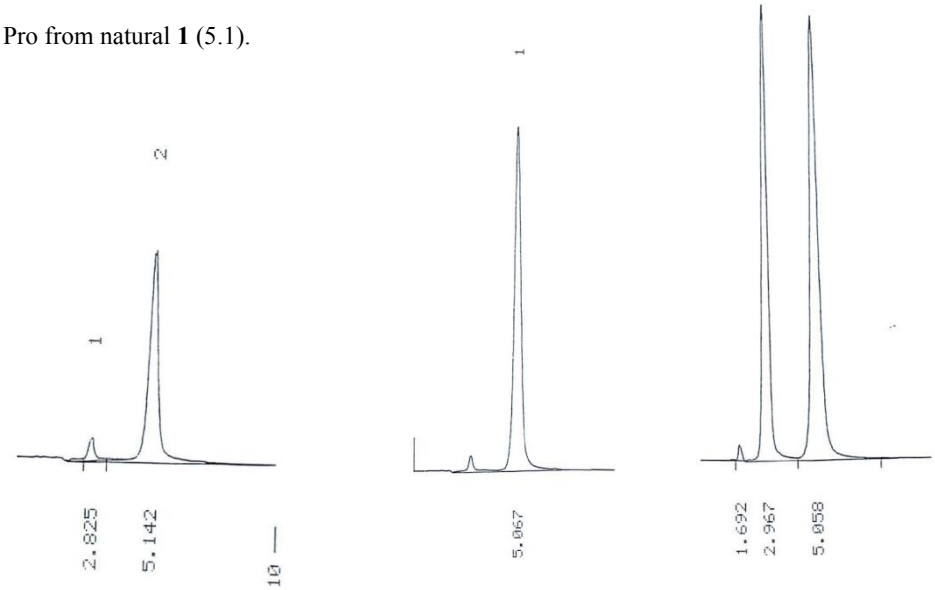


N-Me-D-Val

Pro: column, DAICEL CHIRALPAK (MA+) (φ4.6 × 50 mm); flow rate, 1.0 mL/min; detection at 254 nm; solvent, 2.0 mM $CuSO_4$

t_R (min): Authentic samples: L-Pro (5.1), D-Pro (3.0).

Pro from natural **1** (5.1).



Pro from natural **1** Pro from natural **1** with L-Pro D,L-Pro

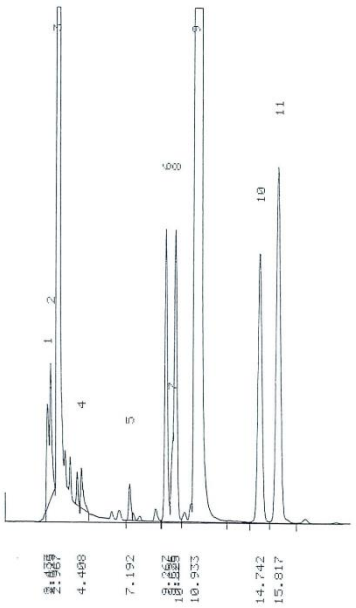
5. Marfey's analysis of Opp derivatives

The 2-(1-hydroxypropyl)-pyrrolidine-containing fraction was dissolved in H_2O (50 μL). A 1.0% 1-fluoro-2,4-dinitro-phenyl-5-L-alaninamide (Marfey's reagent) solution in acetone (100 μL) and 25 μL of 1 M $NaHCO_3$ were added, and the mixture was heated at 80 °C for 3 min. The solution was cooled to room temperature, neutralized with 1 M HCl, and evaporated to dryness. The residue was resuspended in 100 μL of MeCN/H_2O (1:1), and the solution was analyzed by reversed-phase HPLC. [Cosmosil 5C_{18}-MS-II (φ4.6 × 250 mm); flow rate 1mL/min; detection, UV 340 nm; solvent 0.02 M NaOAc - MeCN (53:47)] The retention times of the derivatized Opp in the hydrolysate matched those of Marfey derivatives of 2(*S*)-(1-hydroxypropyl)-pyrrolidine authentic samples (t_R = 14.8 min, 15.8 min), but not Marfey derivatives of 2(*R*)-(1-hydroxypropyl)-pyrrolidine authentic samples (t_R = 16.4 min, 17.4 min).

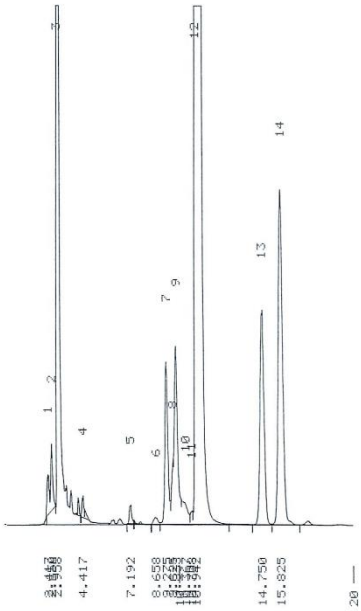
2-(1-hydroxypropyl)-pyrrolidine: column, Cosmosil 5C_{18}-MS-II (φ4.6 × 250 mm); flow rate 1mL/min; detection, UV 340 nm; solvent, 0.02 M NaOAc - MeCN (53:47)

t_R (min): Marfey derivatives of authentic samples: 2(*R*)-(1-hydroxypropyl)-pyrrolidine [6*S*, 6*R*] (16.4, 17.4), 2(*S*)-(1-hydroxypropyl)-pyrrolidine [6*S*, 6*R*] (14.8, 15.8).

Marfey derivatives of 2-(1-hydroxypropyl)-pyrrolidine from natural **1** (14.8, 15.8).

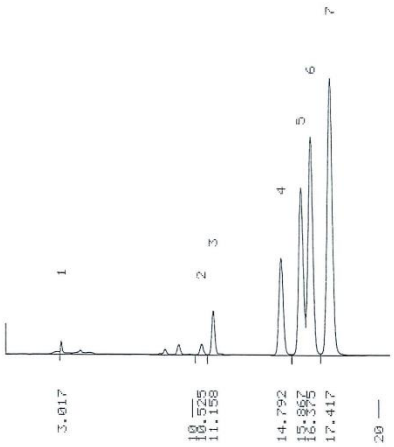


2-(1-hydroxypropyl)-pyrrolidine [6*S*, 6*R*]
from natural **1**
(Other peaks are derived from reagents)



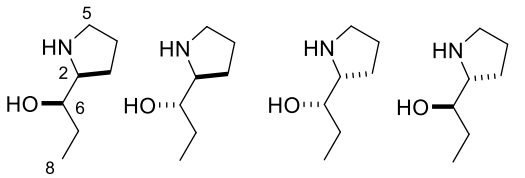
2(*S*)-(1-hydroxypropyl)-pyrrolidine [6*S*, 6*R*]

with 2-(1-hydroxypropyl)-pyrrolidine [6*S*, 6*R*]
from natural **1**



2(*R*)-(1-hydroxypropyl)-pyrrolidine [6*S*, 6*R*]

2(*S*)-(1-hydroxypropyl)-pyrrolidine [6*S*, 6*R*]



2-(1-hydroxypropyl)-pyrrolidine

6. Gene Sequencing

A cyanobacterial filament was isolated under a microscope. Genomic DNA was extracted using the DNeasy Plant Mini kit (Qiagen) following the manufacturer's specifications. The 16S rRNA genes were PCR-amplified from isolated DNA using the primer set 16S 27F, a universal primer, and 23S 30R, a cyanobacterial-specific primer. The PCR reaction contained DNA derived from a cyanobacterial filament, 12.5 µL of GoTaq (Promega), 1.0 µL of each primer (10 pM) and 10.5 µL H_2O for a total volume of 25 µL. The PCR reaction was performed as follows: initial denaturation for 10 min at 95 °C, amplification by 35 cycles of 1 min at 94 °C, 1 min at 60 °C and 1 min at 72 °C, and final elongation for 7 min at 72 °C. PCR products were analyzed on agarose gel (1%) in TAE buffer and visualized by ethidium bromide staining. The obtained DNA was sequenced with 16S 27F, 16S 1494R and 23S 30R primers. The 16S rRNA gene sequence is available in the DDBJ/EMBL/GenBank databases under accession number AB857842.

7. Cell growth analysis

HeLa cells were cultured at 37 °C with 5% CO_2 in DMEM (Nissui, Japan) supplemented with 10% heat-inactivated FBS, 100 units/mL penicillin, 100 µg/mL streptomycin, 0.25 µg/mL amphotericin, 300 µg/mL L-glutamine, and 2.25 mg/mL $NaHCO_3$. HL60 cells were cultured at 37 °C with 5% CO_2 in RPMI (Nissui, Japan) supplemented with 10% heat-inactivated FBS, 100 units/mL penicillin, 100 µg/mL streptomycin, 0.25 µg/mL amphotericin, 300 µg/mL L-glutamine, and 2.25 mg/mL $NaHCO_3$. HeLa cells were seeded at 2×10^4 cells/well in 96-well plates (Iwaki, Japan) and cultured overnight. HL60 cells were seeded at 1×10^5 cells/well in 96-well plates. Various concentrations of compounds were then added, and cells were incubated for 72 h. Cell proliferation was measured by the MTT assay. IC_{50} values were calculated based on three parallel experiments, and data represent the mean ± standard error.

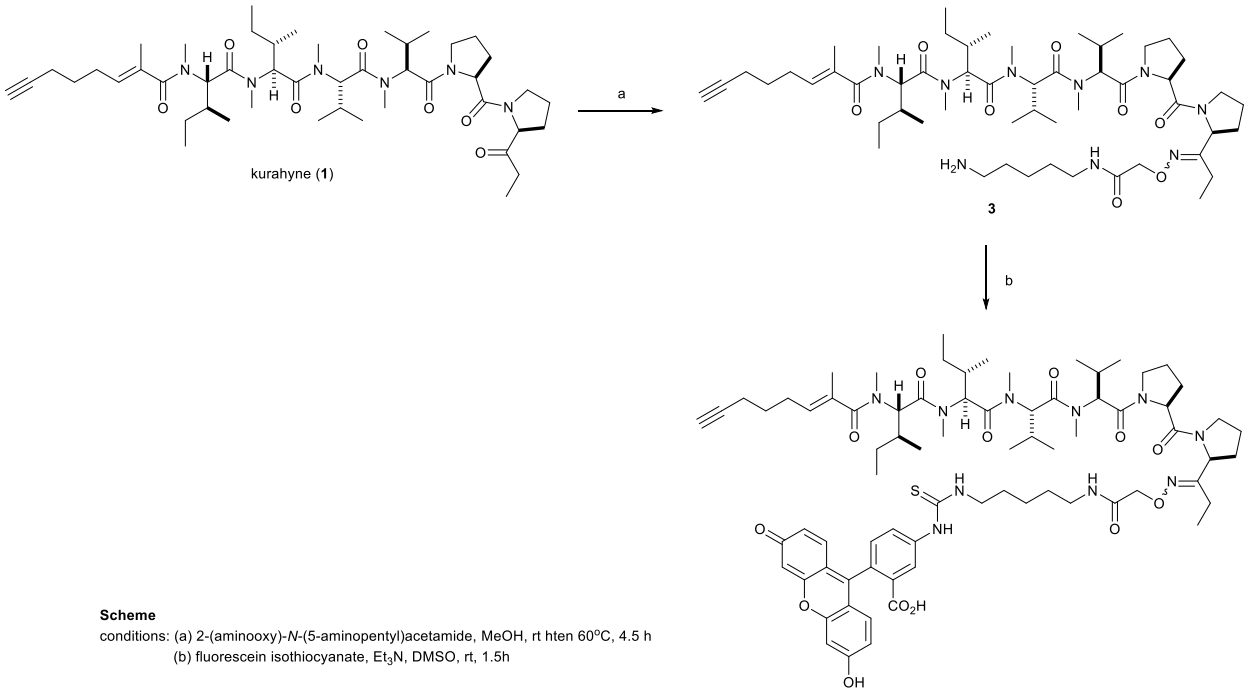
8. Trypan blue dye exclusion assay

HeLa cells were seeded at 4×10^4 cells/well in 24-well plates (Iwaki, Japan), cultured overnight, and then preincubated with or without 50 µM Z-VAD-FMK (Promega, Madison, WI) for 30 min. The cells were then treated with various concentrations of compounds for 48 h. They were then stained with 0.8 mg/mL trypan blue (Sigma-Aldrich, St. Louis, MO), and the cell viability was determined by counting the number of stained (killed) cells.

9. Analysis of DNA fragmentation

HeLa cells, treated with various concentrations of compounds for 36 h, were washed with phosphate-buffered saline (PBS; 8 g/L NaCl, 200 mg/L KCl, 1.15 g/L $Na_2HPO_4 \cdot 2H_2O$, 200 mg/L KH_2PO_4). The cells were then resuspended in lysis buffer (10 mM Tris-HCl [pH 7.4], 10 mM EDTA, 0.5% Triton X-100) at 4 °C for 10 min. After centrifugation at 17,700 g at 4 °C for 5 min, the supernatant was treated with 0.2 mg/mL RNase A at 37 °C for 1 h. The samples were treated with 0.2 mg/mL proteinase K at 50 °C for 30 min, and to the lysates were added 5 M NaCl (0.5 M of total) and isopropyl alcohol (50% of total). After the mixtures were cooled overnight at -20 °C, DNA was collected by centrifugation at 17,700 g for 15 min. The pellet was finally dissolved in TE buffer (10 mM Tris-HCl [pH 7.4], 1 mM EDTA). The DNA was then electrophoresed on 2% agarose gel and stained with 1.0 µg/mL ethidium bromide. The gel was visualized and photographed under UV light.

10. Synthesis of kurahyne-fluorescein conjugate (2)



Scheme
conditions: (a) 2-(aminooxy)-*N*-(5-aminopentyl)acetamide, MeOH, rt hten 60°C, 4.5 h
(b) fluorescein isothiocyanate, Et_3N, DMSO, rt, 1.5h

Synthesis of 3

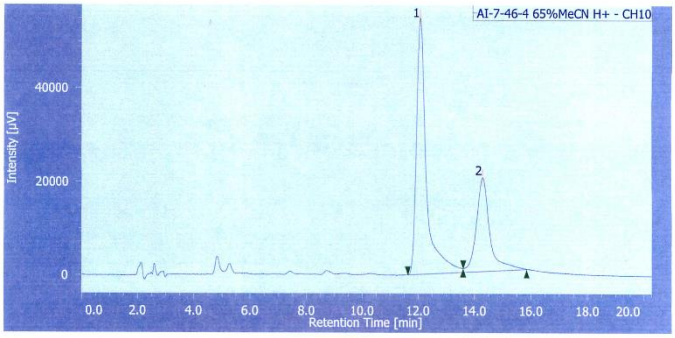
To a solution of kurahyne (**1**) (0.7 mg, 0.8 μmol) in MeOH (3 drops) was added 2-(aminooxy)-*N*-(5-aminopentyl)acetamide* (8.1 mg, 46 μmol). The reaction mixture was stirred at room temperature for 4 h, and then at 60 °C for 0.5 h. The mixture was separated by preparative TLC (chloroform-MeOH 9:1) to afford amine **3** (1.6 mg) as a crude product, which was used for the next reaction without further purification.

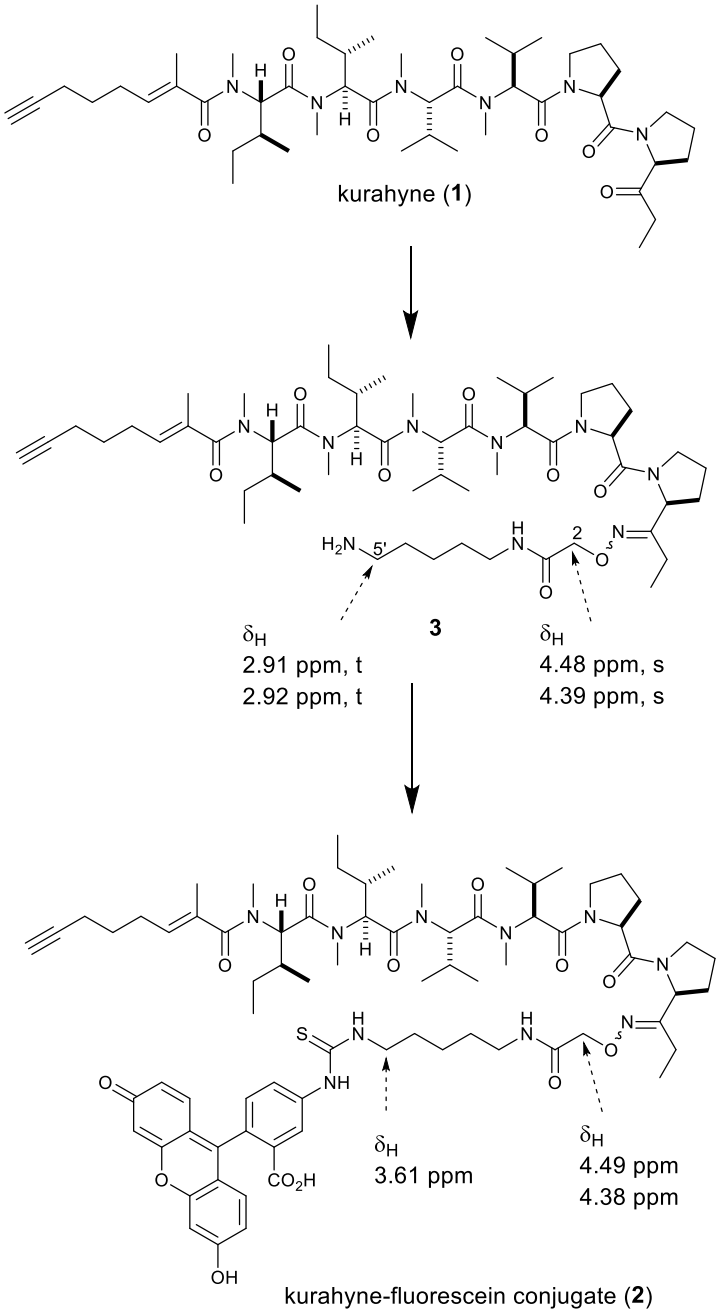
* This compound was prepared by the reported method: E. Sumiya; H. Shimogawa; H. Sasaki; M. Tsutsumi; K. Yoshita; M. Ojika; K. Suenaga and M. Uesugi, *ACS Chem. Biol.*, 2011, **6**, 425–431

Synthesis of kurahyne-fluorescein conjugate (2)

To a solution of **3** (1.6 mg) and triethylamine (0.1 mL) in DMSO (0.3 mL) was added fluoresceinisothiocyanate (1.5 mg, 3.9 μmol). The reaction mixture was stirred at room temperature for 1.5 h, and purified by reversed-phase HPLC (Cosmosil 5C_{18}AR-II, 80% MeOH in 0.1% TFA) to give **2** (0.7 mg, 0.5μmol, 63% in 2 steps) as a mixture of two stereoisomers. The identity was verified by mass spectral analysis of the isolated compound. HRESIMS m/z 1385.7524 $[M+H]^+$ (calcd for $C_{75}H_{105}N_{10}O_{13}S$, 1385.7583). In addition, a thioacylated amino group was identified based on a low field shift of the ^{1}H NMR chemical shift of the attached methylene protons (see S18). The purity of compound **2** was confirmed by reversed-phase HPLC analysis as indicated below.

kurahyne-fluorescein conjugate (**2**): column, Cosmosil 5C_{18}-AR-II (φ4.6 × 250 mm); flow rate 1mL/min; detection, UV 254 nm; solvent, 65% MeCN in 0.1% TFA

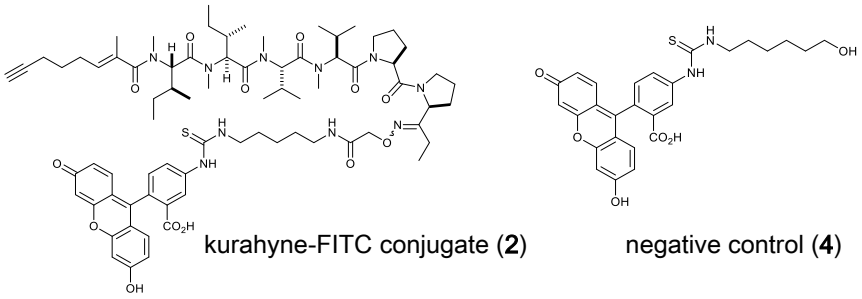


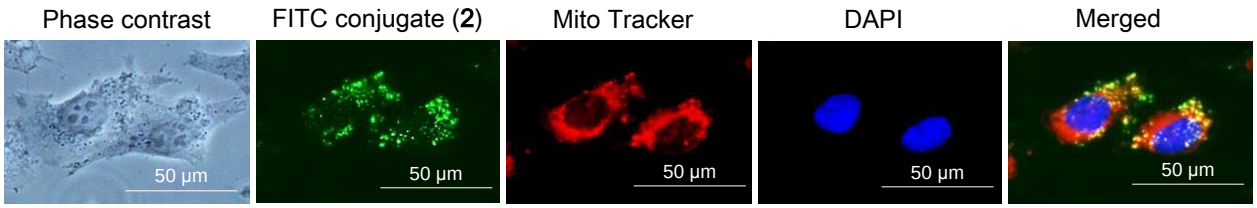


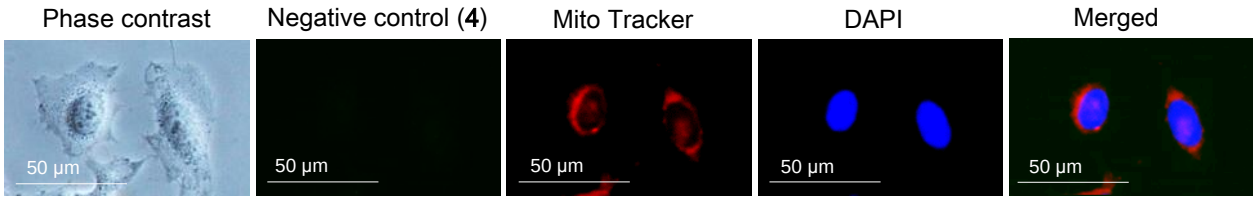
Identification of a thioacylated amino group based on chemical shifts of ^{1}H NMR spectra

11. Subcellular localization of kurahyne-FITC conjugate (2) in HeLa cells

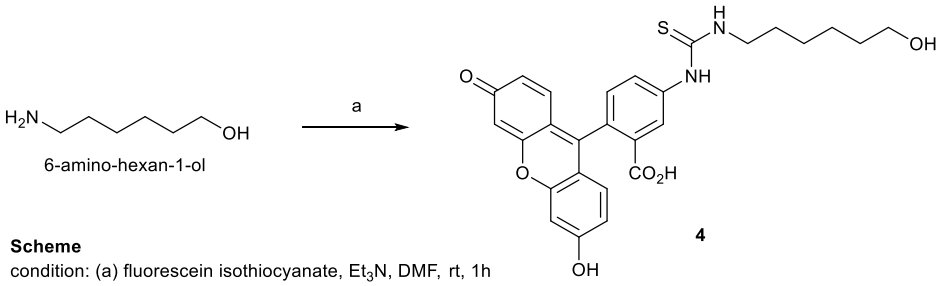
HeLa cells were treated with 10 μM of kurahyne-FITC conjugate (**2**) or 10 μM of **4** as negative control. 10 h after the treatment, the cells were stained with Mito Tracker Red CMXRos and DAPI. **4** was not localized in HeLa cells.





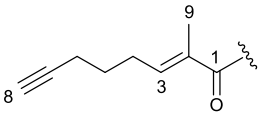


Synthesis of negative control (4)



Scheme
condition: (a) fluorescein isothiocyanate, Et_3N, DMF, rt, 1h

To a solution of 6-amino-hexan-1-ol (5.9 mg, 50 μmol) and triethylamine (0.1 mL) in DMF (0.2 mL) was added fluoresceinisothiocyanate (16.2 mg, 42 μmol). The reaction mixture was stirred at room temperature for 1 h, and extracted with EtOAc (3 × 3 mL). The extract was washed with brine and concentrated to give **4** (21.1 mg, quant.) The purity of compound **4** was confirmed by ^{1}H NMR analysis. The identity was verified by mass spectral of the isolated compound. HRESIMS m/z 529.1419 $[M+Na]^+$ (calcd for $C_{27}H_{26}N_2O_6SNa$, 529.1409); ^{1}H NMR (400 MHz, CD_3OD) δ 8.32 (1H, s), 7.92 (1H, dd, *J* = 8.4, 2.0 Hz), 7.28 (1H, d, *J* = 8.4 H, 1H), 7.17 (2H, m), 7.02 (2H, br s), 6.88 (2H, d, *J* = 8.4 Hz), 3.58 (2H, t, J = 6.4 Hz), 3.28 (2H, m), 1.70 (2H, m), 1.57 (2H, m), 1.46-1.42 (4H, m).



position	Kurahyne δ_C (C_6D_6)	Dragonamide E δ_C ($CDCl_3$)
1	173.4	173.9
2	133.6	133.0
3	129.1	129.8
4	26.6	26.6
5	28.0	27.8
6	18.2	18.2
7	83.8	84.0
8	69.4	69.0
9	14.4	14.4

Comparison of the ^{13}C chemical shifts of Fatty acid moiety between kurahyne (1) and dragonamide E