Electronic Supplementary Material (ESI) for Chemical Communications

Halichonines A, B, and C, Novel Sesquiterpene Alkaloids from the Marine Sponge *Halichondria okadai* Kadota

Supplementary Information

(48 pages)

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Experimental procedures

General

Chemicals and solvents were the best grade available and were used as received from commercial sources. Optical rotations were measured on a JASCO DIP-360 polarimeter using a micro-cell (light path 50 mm). CD spectrum was measured with a JASCO J-720 W spectropolarimeter. IR spectra were recorded on a JASCO FT/IR-410 instrument and are reported in wavenumbers (cm^{-1}). NMR spectra were recorded with JEOL JNM-A400 (400 MHz for ^{1}H, 100 MHz for ^{13}C), JEOL JNM-GX400 (400 MHz for ^{1}H, 100 MHz for ^{13}C), JEOL JNM-A600 (600 MHz for ^{1}H, 150 MHz for ^{13}C), or JEOL JNM-ECP800 (800 MHz for ^{1}H, 201 MHz for ^{13}C) spectrometers. ESI mass spectra were recorded on a LCT premier EX spectrometer (Waters, Milford, MA). Column chromatography was performed with silica gel FL-60D (Fuji Silysia Chem. Ltd., Aichi, Japan), TSK G3000S polystyrene gel (Tosoh Co.), or alumina (Merck, Darmstadt, Germany, alminium oxide 90 standardized). Preparative TLC was performed with glass TLC plates (Merck, 0.5 mm coated silica gel, 60 F_{254}).

Biological material

The black sponge *Halichondria okadai* Kadota was collected around Anorisaki and Daiozaki (Shima) and Toshijima (Toba) in Mie Prefecture, Japan (about 300 km southwest of Kanagawa Prefecture), in 2006. An 80 kg (wet weight) amount of the sponge, which is common in this area, was collected and did not result in any environmental damage. A voucher specimen (YH-0609) has been stored at Keio University.

X-ray crystal structure determination

The crystals for X-ray structural analysis were obtained by recrystallization from MeOH. Although several trials of recrystallization gave only tiny and twin crystals, the tiny and twin crystal was coated with oil (Immersion Oil, type B, Cargille Laboratories, Inc) and mounted on a loop. Intensity data were collected at 123 K on a Rigaku Single Crystal CCD X-ray Diffractometer (Saturn 70 with MicroMax-007) with Mo Kα radiation (λ = 0.71070 Å). A total of 12434 reflections were measured at

the maximum 2θ angle of 50.0˚, of which 7432 were independent reflections (R_{int} = 0.0932). The structure was solved by direct methods (SHELXS-97[S1]) and refined by the full-matrix least-squares on F^2 (SHELXL-97[S1]). The crystal contained two independent molecules, *i.e.*, (C1–C29, N1–N2, and Cl1–Cl2) and (C30–C58, N3–N4, and Cl3–Cl4), and water molecules (O1–O5). One 3-methyl-2-buten-1-amine moiety, *i.e.*, (C48–C50, C57, C58 and N4) of the latter molecule was disordered and was solved using appropriate models. Thus, two sets of these moieties, *i.e.*, (C48A–C50A, C57A, C58A and N4A) and (C48B–C50B, C57B, C58B and N4B) were placed and their occupancies were refined to be 0.76 and 0.24, respectively. The disordered moieties were restrained by DFIX instruction during refinement. The nitrogen atoms of the disordered moieties (N4A and N4B) and the oxygen atoms of the solvent water molecules (O4 and O5) were restrained by ISOR and SIMU instruction during refinement. All non-hydrogen atoms, except for the minor part of the disordered moieties, were refined anisotropically. All hydrogen atoms except for the solvent water molecules were placed using AFIX instructions. The crystal data are summarized in Table S5. CCDC 842822 contains the supplementary crystallographic data. The data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Cell growth analysis

L1210 cells and PC13 cells were cultured in RPMI1640 (Nissui, Tokyo, Japan) supplemented with 10% heat-inactivated FBS, 100 μg ml^{-1} kanamycin, 100 units/ml penicillin G, 300 μg ml^{-1} L-glutamine, and 2.25 mg ml^{-1} $NaHCO_3$. Cells were seeded at 4 x 10^3 cells/well in 96-well plates (Iwaki, Tokyo, Japan) and cultured overnight. Then, various concentrations of compounds were added, and incubation was continued for 72 hours. The cell proliferation was measured by using the MTT assay. 2,5,6-Tribromogramine[S2] is a known alkaloid isolated from the same marine sponge.

Trypan blue dye exclusion

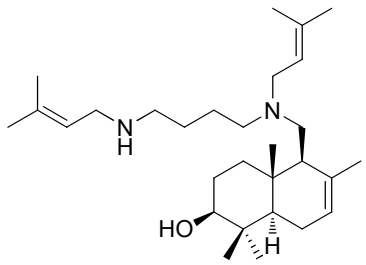
HL60 cells were obtained from RIKEN cell bank. HL60 cells were cultured in RPMI1640 supplemented with 10% heat-inactivated FBS, 100 μg ml^{-1} kanamycin, 100 units/ml penicillin G, 300 μg ml^{-1} L-glutamine, and 2.25 mg ml^{-1} $NaHCO_3$. Cells were seeded at 6 x 10^4 cells/well in 24-well plates (Iwaki) and preincubated or not

with 50 μM Z-VAD-FMK (Promega, Madison, WI) for 30 minutes. Then, the cells were treated with several concentrations of compounds for 24 hours. They were then stained with trypan blue, and the number of stained cells was counted. Tunicamycin (Sigma–Aldrich Co., St. Louis, MO) was used as a positive control (Fig. S32A).

DNA ladder analysis

HL60 cells treated with the compounds were washed with PBS. Then, cells were resuspended in lysis buffer (10 mM Tris-HCl, 10 mM EDTA, 0.5% Triton X-100, pH 7.4) at 4 °C for 10 minutes, and centrifuged at 17,700 g for 5 minutes. The supernatant was then treated with 0.2 mg ml^{-1} RNase at 37 °C for 1 hour, followed by treatment with 0.2 mg ml^{-1} protease K at 50 °C for 30 minutes. The lysates were added of same volume of 2-propanol and treated at -20 °C overnight. After centrifugation at 17,700 g for 15 minutes, the pellet was resuspended in TE buffer (10 mM Tris-HCl, 1 mM EDTA, 0.5%, pH 7.4). The DNA was then electrophoresed in a 2% agarose gel and stained with ethidium bromide. The gel was visualized and photographed under ultraviolet light. Tunicamycin was used as a positive control (Fig. S32B).

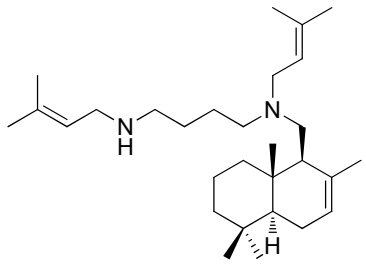
Spectral data for the isolated compounds.



Halichonine A (**1**)

Halichonine A (**1**): pale yellow amorphous powder; R_f 0.19 ($CHCl_3$/MeOH = 9/1); $[\alpha]_D^{25}$ +35.5 (*c* 0.55, $CHCl_3$); IR (neat) 3460, 3332 (br), 2928, 2856, 1725, 1669, 1443, 1366 cm^{-1}; HR-ESITOFMS *m/z* 445.4166 $[M+H]^+$, Δ = +0.8 mmu, calcd for $C_{29}H_{53}N_2O$, 445.4158; ^1H NMR (600 MHz, CD_3OD) δ_H 5.38 (brs, 1 H, H7), 5.25 (m, 1 H, H13), 5.24 (m, 1 H, H20), 3.26 (d, *J* = 6.6 Hz, 2 H, H19), 3.17 (dd, *J* = 11.0, 4.4 Hz, 1 H, H3), 3.11 (dd, *J* = 14.0, 5.9 Hz, 1 H, H12b), 2.90 (dd, *J* = 14.0, 7.3 Hz, 1 H,

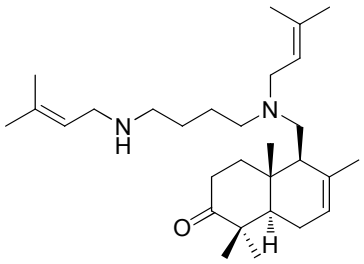
H12a), 2.63 (m, 2 H, H18), 2.53 (m, 1 H, H15b), 2.33 (m, 2 H, H11), 2.26 (m, 1 H, H15a), 2.08 (dt, *J* = 13.2, 3.7 Hz, 1 H, H1b), 1.96 (brs, 2 H, H6), 1.84 (brs, 1 H, H9), 1.73 (s, 3 H, H28), 1.73 (s, 3 H, H25), 1.71 (s, 3 H, H26), 1.67 (s, 3 H, H29), 1.63 (s, 3 H, H27), 1.59 (m, 2 H, H2), 1.52 (m, 2 H, H17), 1.48 (m, 2 H, H16), 1.17 (m, 1 H, H1a), 1.17 (m, 1 H, H5), 0.94 (s, 3 H, H23), 0.83 (s, 3 H, H22), 0.74 (s, 3 H, H24); ^{13}C NMR (150 MHz, CD_3OD) δ_C 137.5 (C21), 136.8 (C8), 135.4 (C14), 123.2 (C13), 123.2 (C7), 123.1 (C20), 79.9 (C3), 55.0 (C11), 54.6 (C15), 53.1 (C9), 52.4 (C12), 51.5 (C5), 49.9 (C18), 47.6 (C19), 40.0 (C4), 38.9 (C1), 37.4 (C10), 28.8 (C23), 28.4 (C2), 28.3 (C17), 26.2 (C28), 26.1 (C26), 25.9 (C16), 24.6 (C6), 22.9 (C25), 18.2 (C29), 18.1 (C27), 16.0 (C22), 14.3 (C24).



Halichonine B (**2**)

Halichonine B (**2**): pale yellow amorphous powder; R_f 0.25 ($CHCl_3$/MeOH = 9/1); $[\alpha]_D^{28}$ +13.8 (*c* 0.2, $CHCl_3$); IR (neat) 2923, 2851, 1733, 1669, 1559, 1444 cm^{-1}; HR-ESITOFMS *m/z* 429.4220 $[M+H]^+$, Δ = +1.1 mmu, calcd for $C_{29}H_{53}N_2$, 429.4209; ^{1}H NMR (800 MHz, CD_3OD) δ_H 5.34 (s, 1H, H7), 5.22 (m, 1H, H13), 5.22 (m, 1 H, H20), 3.18 (d, *J* = 6.5 Hz, 2 H, H19), 3.11 (dd, *J* = 13.9, 5.7 Hz, 1 H, H12b), 2.86 (dd, *J* = 13.9, 7.8 Hz, 1 H, H12a), 2.55 (m, 2 H, H18), 2.52 (m, 1 H, H15b), 2.31 (m, 2 H, H11), 2.22 (m, 1 H, H15a), 2.03 (m, 1 H, H1b), 1.95 (m, 2 H, H6), 1.84 (m, 1 H, H9), 1.74 (s, 3 H, H28), 1.74 (s, 3 H, H25), 1.70 (s, 3 H, H26), 1.68 (s, 3 H, H29), 1.63 (s, 3 H, H27), 1.55 (m, 1 H, H2b), 1.45 (m, 2 H, H17), 1.43 (m, 2 H, H16), 1.39 (m, 1 H, H2a), 1.38 (m, 1 H, H3b), 1.18 (m, 2 H, H5), 1.16 (m, 1 H, H3a), 1.01 (m, 1 H, H1a), 0.88 (s, 3 H, H22), 0.85 (s, 3 H, H23), 0.74 (s, 3 H, H24); ^{13}C NMR (100 MHz, CD_3OD) δ_C 137.4 (C21), 135.4 (C8), 134.0 (C14), 121.7 (C7), 121.5 (C13), 119.0 (C20), 53.6 (C11), 53.0 (C15), 51.7 (C9), 51.0 (C12), 50.4 (C5), 48.0 (C18), 45.8 (C19), 42.2 (C3), 39.2 (C1), 32.6 (C23), 32.5 (C4), 26.2 (C17), 24.7 (C26), 24.6 (C28), 24.3 (C16), 23.5 (C6), 21.6 (C25), 21.1 (C22), 18.5 (C2), 16.7

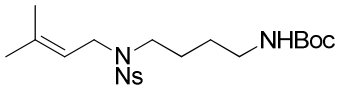
(C29), 16.7 (C27), 12.8 (C24).



Halichonine C (**3**)

Halichonine C (**3**): pale yellow amorphous powder; R_f 0.33 ($CHCl_3$/MeOH = 9/1); $[\alpha]_D^{25}$ +8.42 (*c* 0.19, $CHCl_3$); EIMS data *m/z* 442 ($C_{29}H_{50}ON_2$); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 5.44 (m, 1 H, H7), 5.36 (brt, *J* = 7.2 Hz, 1 H, H20), 5.19 (brt, *J* = 7.6 Hz, 1 H, H13), 3.47 (d, *J* = 7.2 Hz, 2 H, H19), 3.05 (dd, *J* = 5.6, 14.4 Hz, 1 H, H12b), 2.89 (dd, *J* = 7.2, 14.4 Hz, 1 H, H12a), 2.79 (brt, *J* = 7.6 Hz, 2 H, H18), 2.76 (ddd, *J* = 5.2, 5.2, 14.8 Hz, 1 H, H2b), 2.45 (m, 1 H, H15b), 2.43 (m, 1 H, H1b), 2.36 (d, *J* = 5.2 Hz, 2 H, H11), 2.26 (m, 1 H, H15a), 2.24 (m, 1 H, H2a), 2.07 (brt, *J* = 9.2 Hz, 1 H, H6b), 1.93 (m, 1 H, H6a), 1.92 (m, 1 H, H9), 1.77 (s, 3 H, H28), 1.74 (s, 3 H, H25), 1.71 (s, 3 H, H26), 1.67 (s, 3 H, H29), 1.63 (m, 1 H, H5), 1.62 (s, 3 H, H27), 1.50 (m, 1 H, H1a), 1.48 (m, 2 H, H17), 1.48 (m, 2 H, H16), 1.09 (s, 3 H, H22), 1.07 (s, 3 H, H23), 0.98 (s, 3 H, H24); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 216.7 (C3), 140.6 (C21), 135.5 (C8), 134.6 (C14), 122.0 (C13), 121.3 (C7), 115.9 (C20), 53.6 (C11), 53.3 (C15), 51.6 (C5), 50.9 (C12), 50.6 (C9), 47.5 (C18), 46.4 (C19), 44.8 (C4), 37.8 (C1), 36.0 (C10), 34.6 (C2), 25.9 (C26), 25.8 (C28), 25.2 (C23), 24.9 (C17), 24.5 (C16), 23.9 (C6), 22.7 (C25), 22.3 (C22), 18.2 (C29), 18.0 (C27), 13.4 (C24).

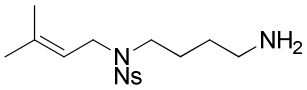
Synthesis of (±)-halichonine A (1)



5

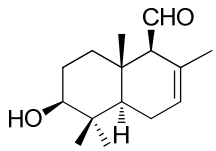
Amide 5: To a solution of *N*-(4-aminobutyl)-2-nitrobenzenesulfonamide (**4**) (100 mg, 0.366 mmol) in CH_2Cl_2 (1.3 ml) was added Boc_2O (95.9 mg, 0.439 mmol) and Et_3N (0.03 ml. 0.439 mmol) at room temperature under an argon atmosphere, and the

reaction mixture was stirred for 45 minutes. The reaction mixture was partitioned with 1 M HCl and ethyl acetate. The organic layer was washed with brine, dried over Na_2SO_4, and then evaporated. The residue was purified by silicagel column chromatography (Silicagel FL60D, Fuji Silysia Chemical, Aichi, Japan, 3.5 x 13.5 cm) with hexane/EtOAc (2:1), $CHCl_3$/MeOH (20:1), and EtOAc as eluents. After evaporation of the solvent, the residue was dissolved in DMF (1.1 ml) with 1-bromo-3-methyl-2-butene (0.108 ml, 0.936 mmol) and K_2CO_3 (107.8 mg, 0.78 mmol) at room temperature, and the reaction mixture was stirred for 1 hour. The reaction mixture was partitioned with 10% citric acid *aq* and ethyl acetate. The organic layer was washed with brine, dried over Na_2SO_4, and then evaporated. Purification of the residue by silicagel column chromatography (Silicagel FL60D, Fuji Silysia Chemical, Aichi, Japan, 3.5 x 13.5 cm) with hexane/EtOAc (5:2) as an eluent yielded amide **5** (140.3 mg, 0.285 mmol, 77% over 3 steps) as a yellow oil. **5**: R_f 0.55 (hexane/EtOAc = 1/1); IR (neat) 2932, 1700, 1545, 1367, 1253, 1162 cm^{-1}; HR-ESITOFMS *m/z* 464.1826 $[M+Na]^+$, Δ = -0.5 mmu, calcd for $C_{20}H_{31}N_3O_6SNa$, 464.1831; ^1H NMR (400 MHz, $CDCl_3$) δ 8.00 (m, 1 H), 7.64 (m, 3 H), 5.02 (t, *J* = 7.2 Hz, 1 H), 3.88 (d, *J* = 7.2 Hz, 2 H), 3.26 (t, 2 H), 3.08 (brs, 2 H), 1.65 (s, 3 H), 1.61 (s, 3 H), 1.54 (m, 2 H), 1.45 (m, 2 H), 1.42 (s, 6 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 156.0, 148.1, 137.6, 134.0, 133.4, 131.6, 130.9, 124.2, 118.8, 77.3, 46.8, 45.0, 40.1, 28.5, 27.3, 25.9, 25.5, 17.9.



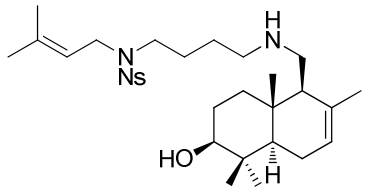
6

Amine 6: To a solution of **5** (39.1 mg, 0.089 mmol) in CH_2Cl_2 (1.0 ml) was added TFA (0.2 ml) at 0 °C under a nitrogen atmosphere, and the mixture was continuously stirred for 30 minutes. The reaction mixture was evaporated and purified by short column chromatography (alumina) with $CHCl_3$/MeOH (20:1) as an eluent yielded crude amine **6** (14.8 mg, 0.043 mmol, 54%) as a yellow oil, which was used for the next reaction without further purification.



8

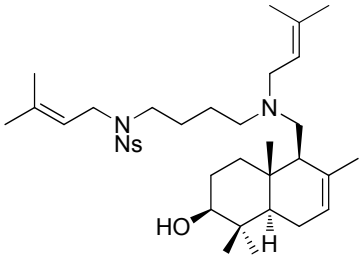
Aldehyde 8: To a solution of (±)-3β-hydroxydrimenol (**7**) (7.0 mg, 0.029 mmol) in CH_2Cl_2 (0.2 ml) was added BAIB (11.3 mg, 0.035 mmol) and TEMPO (0.45 mg, 0.0029 mmol) at room temperature under a nitrogen atmosphere, and the mixture was continuously stirred for 2 hours. The reaction mixture was extracted with ethyl acetate. The organic layer was washed with saturated $NaHCO_3$*aq*. followed by brine, dried over Na_2SO_4, and then evaporated. Purification of the residue by preparative thin layer chromatography (Silicagel $60F_{254}$ 0.5mm, Merck, Darmstadt, Germany, hexane/EtOAc: 2/1) yielded aldehyde **8** (4.8 mg, 0.020 mmol, 69%) as a colorless oil: R_f 0.46 (hexane/EtOAc = 2/1); HR-ESITOFMS *m/z* 235.1721 $[M+Na]^+$, Δ = +4.7 mmu, calcd for $C_{13}H_{24}O_2Na$, 235.1674; ^1H NMR (400 MHz, $CDCl_3$) δ 9.69 (d, *J* = 5.2 Hz, 1 H), 5.70 (brs, 1 H), 3.27 (dd, *J* = 11.0, 5.2 Hz, 1 H), 2.55 (brs, 1 H), 2.07 (m, 2 H), 1.99 (dt, *J* = 13.5, 3.1 Hz, 1 H), 1.68 (m, 2 H), 1.62 (s, 3 H), 1.43 (dt, *J* = 12.8, 4.5 Hz, 1 H), 1.14 (m, dd, *J* = 10.6, 6.3 Hz, 1 H), 1.05 (s, 3 H), 0.99 (s, 3 H), 0.89 (s, 3 H).



9

Amine 9: To a solution of **8** (8.4 mg, 0.036 mmol) in 1,2-dichloro ethane (DCE, 0.3 ml) was added **6** (32.7mg, 0.096 mmol) and $NaBH(OAc)_3$ (38.0 mg, 0.178 mmol) at room temperature under a nitrogen atmosphere, and the mixture was continuously stirred for 12 hours. The reaction mixture was partitioned with 2 M NaOH*aq* and ethyl acetate. The organic layer was dried over Na_2SO_4, and then evaporated. Purification of the residue by preparative thin layer chromatography (Silicagel $60F_{254}$ 0.5mm, Merck, Darmstadt, Germany, $CHCl_3$/MeOH: 8/1) yielded amine **9** (8.7 mg,

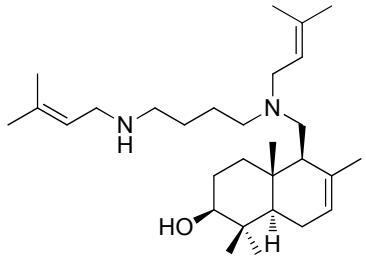
0.016 mmol, 44%) as a yellow oil: R_f 0.63 ($CHCl_3$/MeOH = 6/1); IR (neat) 3382, 2929, 2846, 1653, 1544, 1457, 1374, 1347, 1161 cm^{-1}; HR-ESITOFMS *m/z* 562.3311 $[M+H]^+$, Δ = -0.4 mmu, calcd for $C_{30}H_{47}N_3O_5SNa$, 562.3315; ^1H NMR (400 MHz, $CDCl_3$) δ 8.00 (m, 1 H), 7.65 (m, 3 H), 5.49 (brs, 1 H), 4.99 (t, *J* = 7.3 Hz, 1 H), 3.89 (d, *J* = 6.8 Hz, 2 H), 3.28 (m, 1 H), 3.28 (t, *J* = 6.8 Hz, 2 H), 2.89 (m, 2 H), 2.76 (m, 2 H), 2.05 (m, 1 H), 1.96 (m, 2 H), 1.80 (s, 3 H), 1.73 (m, 1 H), 1.65 (s, 3 H), 1.61 (s, 3 H), 1.49 (m, 2 H), 1.45 (m, 2 H), 1.38 (m, 2 H), 1.28 (m, 1 H), 1.22 (m, 1 H), 0.97 (s, 3 H), 0.85 (s, 3 H), 0.76 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 148.0, 137.9, 134.7, 133.6, 133.4, 131.6, 130.8, 124.1, 121.8, 118.2, 78.5, 53.8, 49.5, 48.8, 47.3, 46.1, 45.1, 38.6, 37.0, 36.2, 29.7, 27.7, 27.1, 25.8, 25.2, 23.3, 22.0, 17.8, 15.1, 13.9.



10

***N*-Nosyl protected halichonine A 10:** To a solution of **9** (8.7 mg, 0.016 mmol) in DCE (0.2 ml) was added 3-methyl-2-butenal (3 μl, 0.031 mmol) and $NaBH(OAc)_3$ (16.4 mg, 0.078 mmol) at room temperature under a nitrogen atmosphere, and the mixture was continuously stirred for 12 hours. The reaction mixture was partitioned with 2 M NaOH*aq* and ethyl acetate. The organic layer was dried over Na_2SO_4, and then evaporated. Purification of the residue by preparative thin layer chromatography (Silicagel $60F_{254}$ 0.5mm, Merck, Darmstadt, Germany, benzene/acetone: 3/1) yielded *N*-nosyl protected halichonine A **10** (3.2 mg, 32%) as a yellow oil: R_f 0.70 ($CHCl_3$/MeOH = 10/1); IR (neat) 3354, 2920, 2853, 1727, 1544, 1451, 1372, 1345, 1159 cm^{-1}; HR-ESITOFMS *m/z* 630.3950 $[M+H]^+$, Δ = +0.9 mmu, calcd for $C_{35}H_{55}N_3O_5SNa$, 630.3941; ^1H NMR (400 MHz, $CDCl_3$) δ 8.00 (m, 1 H), 7.63 (m, 3 H), 5.37 (m, 1 H), 5.19 (t, *J* = 7.0 Hz, 1 H), 5.03 (t, *J* = 6.5 Hz, 1 H), 3.89 (d, *J* = 7.0 Hz, 2 H), 3.28 (m, 1 H), 3.25 (t, *J* = 7.6 Hz, 2 H), 3.05 (dd, *J* = 14.8, 5.6 Hz, 1 H), 2.82 (dd, *J* = 14.6, 7.2 Hz, 1 H), 2.45 (dt, *J* = 13.0, 7.9 Hz, 1 H), 2.26 (m, 2 H), 2.19 (dd, *J* = 12.6, 6.5 Hz, 1 H), 2.05 (dt, *J* = 13.7, 3.8 Hz, 1 H), 1.96 (brs, 2 H), 1.75 (brs,

1 H), 1.72 (s, 3 H), 1.71 (s, 3 H), 1.66 (s, 3 H), 1.61 (s, 3 H), 1.55 (s, 3 H), 1.51 (m, 2 H), 1.46 (m, 2 H), 1.37 (m, 2 H), 1.29 (m, 1 H), 1.21 (m, 1 H), 0.97 (s, 3 H), 0.85 (s, 3 H), 0.73 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 148.1, 137.3, 134.3, 133.5, 133.2, 132.5, 131.5, 130.9, 124.2, 121.9, 121.8, 119.0, 79.2, 53.1, 51.5, 49.8, 49.1, 47.2, 45.7, 44.9, 38.9, 37.2, 36.1, 29.8, 27.5, 26.0, 25.9, 24.2, 23.4, 22.8, 22.4, 18.0, 17.9, 15.3, 13.8.



(±)-**1**

(±)-Halichonine A ((±)-1): To a solution of *N*-nosyl protected halichonine A **10** (2.0 mg, 0.0032 mmol) in CH_3CN (0.1 ml) was added PhSH (0.28 μl, 0.0041 mmol) and K_2CO_3 (2.0 mg, 0.0032 mmol) at room temperature under a nitrogen atmosphere, and the mixture was continuously stirred for 18 hours. The reaction mixture was partitioned with 2 M NaOH*aq* and ethyl acetate. The organic layer was dried over Na_2SO_4, and then evaporated. Purification of the residue by column chromatography (alumina) with hexane/EtOAc (1:1), $CHCl_3$, and $CHCl_3$/MeOH (50:1) as eluents yielded (±)-halichonine A ((±)-**1**) (0.2 mg, 14%) as a pale yellow oil: R_f 0.19 ($CHCl_3$/MeOH = 9/1); HR-ESITOFMS *m/z* 445.4156 $[M+H]^+$, Δ = -0.2 mmu, calcd for $C_{29}H_{53}N_2O$, 445.4158; ^{1}H NMR (400 MHz, CD_3OD) δ_H 5.37 (brs, 1 H, H7), 5.25 (m, 1 H, H13), 5.23 (m, 1 H, H20), 3.24 (m, 2 H, H19), 3.16 (dd, *J* = 10.8, 5.3 Hz, 1 H, H3), 3.10 (m, 1 H, H12b), 2.89 (dd, *J* = 14.0, 7.3 Hz, 1 H, H12a), 2.69 (t, *J* = 7.8 Hz, 2 H, H18), 2.53 (dt, *J* = 13.3, 7.6 Hz, 1 H, H15b), 2.33 (m, 2 H, H11), 2.26 (m, 1 H, H15a), 2.07 (dt, *J* = 13.8, 3.4 Hz, 1 H, H1b), 1.96 (brs, 2 H, H6), 1.83 (brs, 1 H, H9), 1.75 (s, 3 H, H28), 1.74 (s, 3 H, H25), 1.71 (s, 3 H, H26), 1.68 (s, 3 H, H29), 1.63 (s, 3 H, H27), 1.59 (m, 2 H, H2), 1.52 (m, 2 H, H17), 1.49 (m, 2 H, H16), 1.18 (m, 1 H, H1a), 1.18 (m, 1 H, H5), 0.94 (s, 3 H, H23), 0.82 (s, 3 H, H22), 0.74 (s, 3 H, H24); ^{13}C NMR (100 MHz, CD_3OD) δ_C 135.4 (C8), 135.2 (C21), 134.1 (C14), 121.8 (C7), 121.8 (C13), 121.5 (C20), 78.4 (C3), 53.6 (C11), 53.0 (C15), 51.6 (C9), 50.9

(C12), 50.0 (C5), 48.7 (C18), 45.7 (C19), 38.5 (C4), 37.4 (C1), 35.9 (C10), 29.4 (C23), 27.3 (C2), 26.8 (C17), 24.7 (C28), 24.6 (C26), 24.2 (C16), 23.1 (C6), 21.4 (C25), 16.7 (C27), 16.7 (C29), 14.5 (C22), 12.8 (C24).

References

S1. Sheldrick, G. M. *SHELX-97, Program for the Refinement of Crystal Structures*; University of Gottingen: Gottingen, Germany, 1997.

S2. A. Sato, W. Fenical, *Tetrahedron Lett*., 1983, **24**, 481.

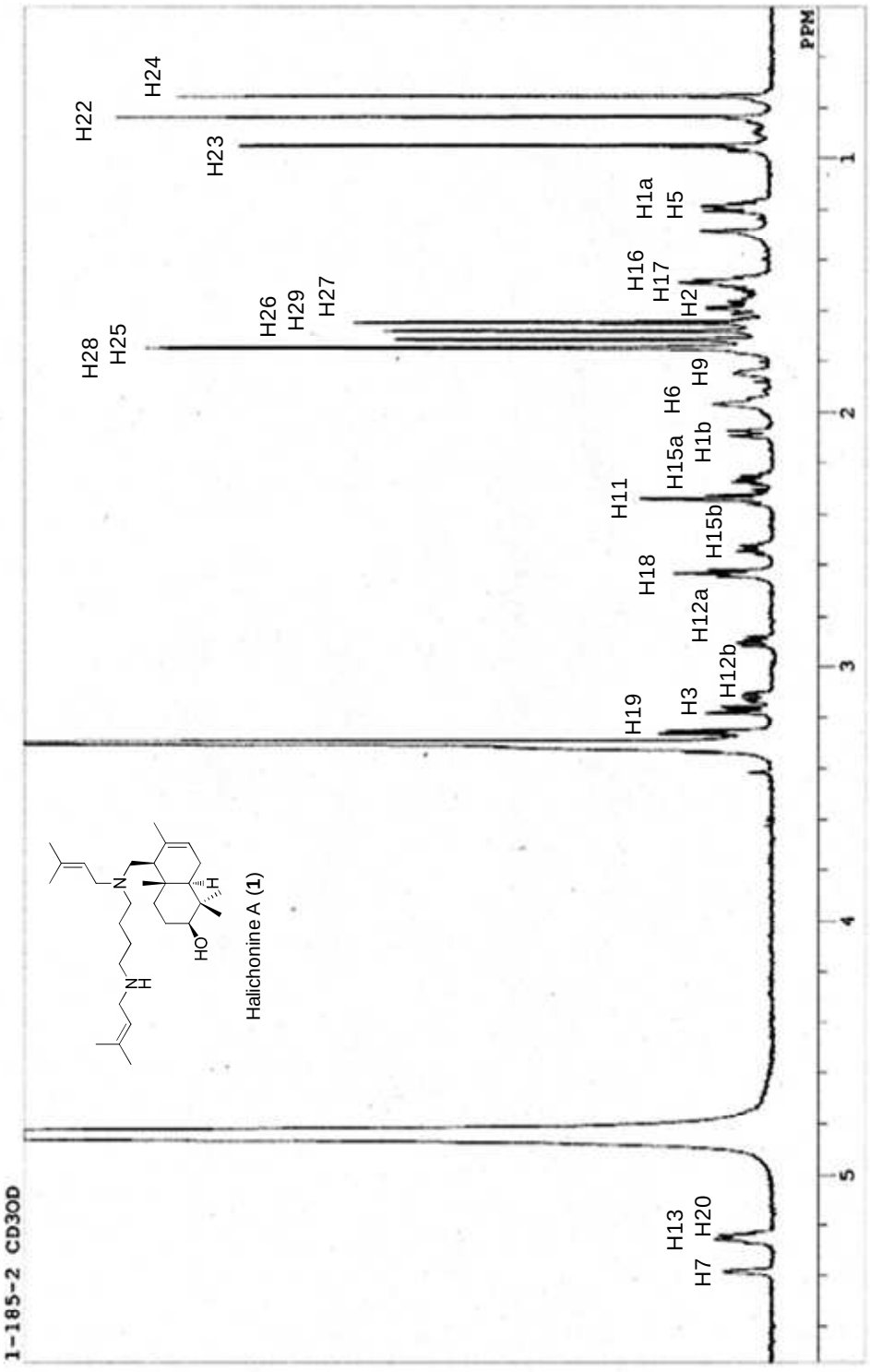


Figure S1. ^{1}H NMR spectrum of halichonine A (**1**) (600 MHz, CD_3OD).

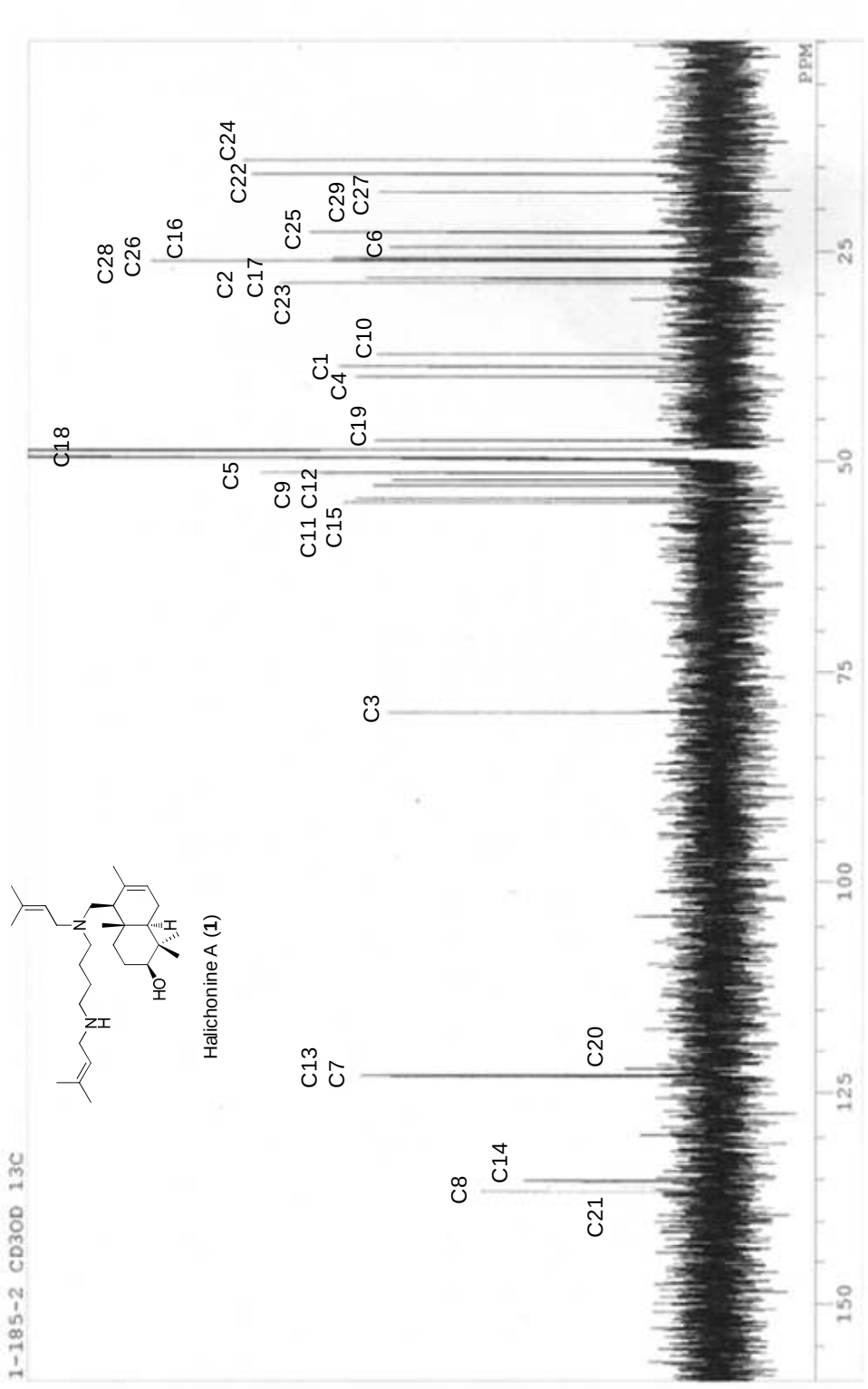


Figure S2. ^{13}C NMR spectrum of halichonine A (**1**) (150 MHz, CD_3OD).

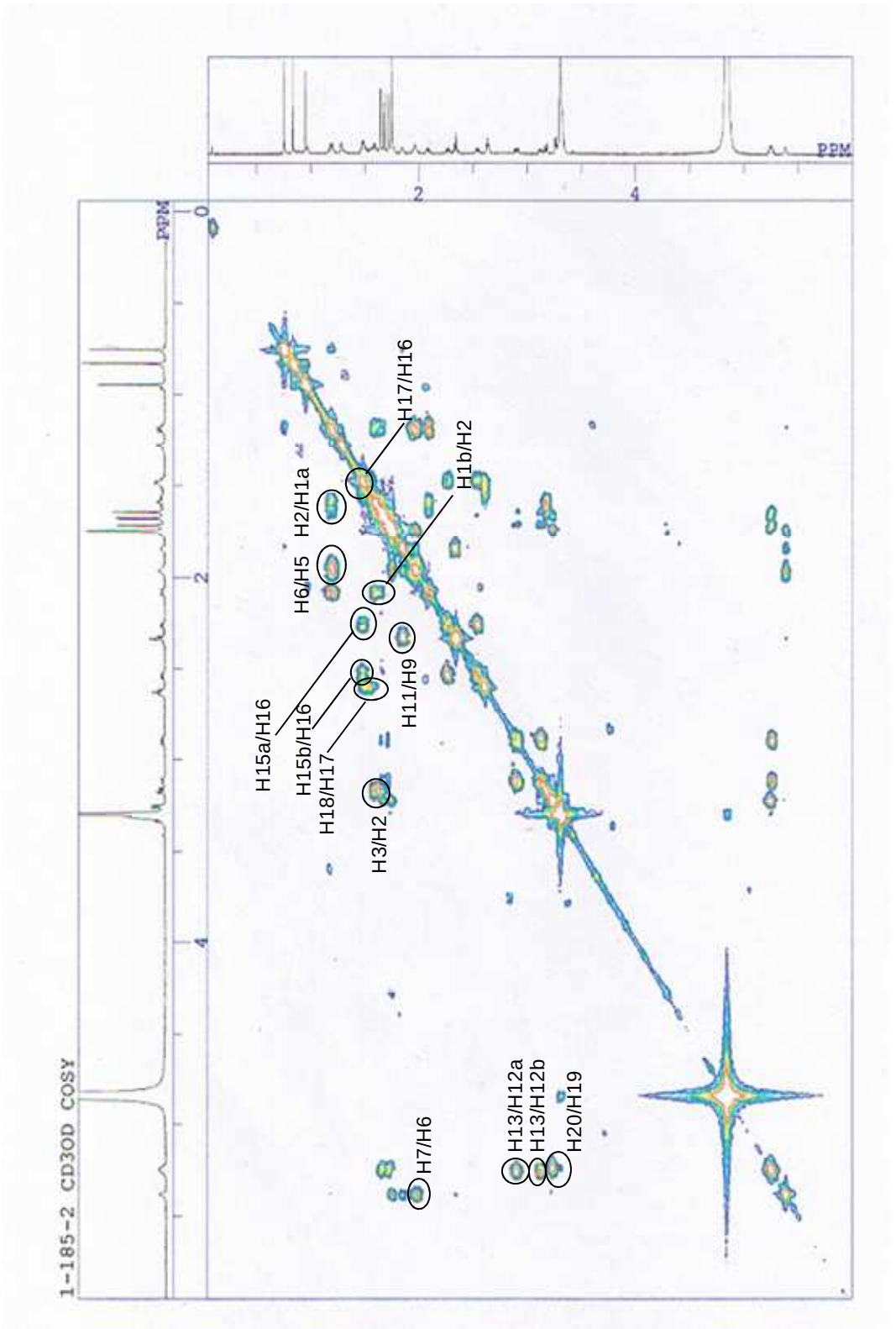


Figure S3. COSY spectrum of halichonine A (**1**) (600 MHz, CD_3OD).

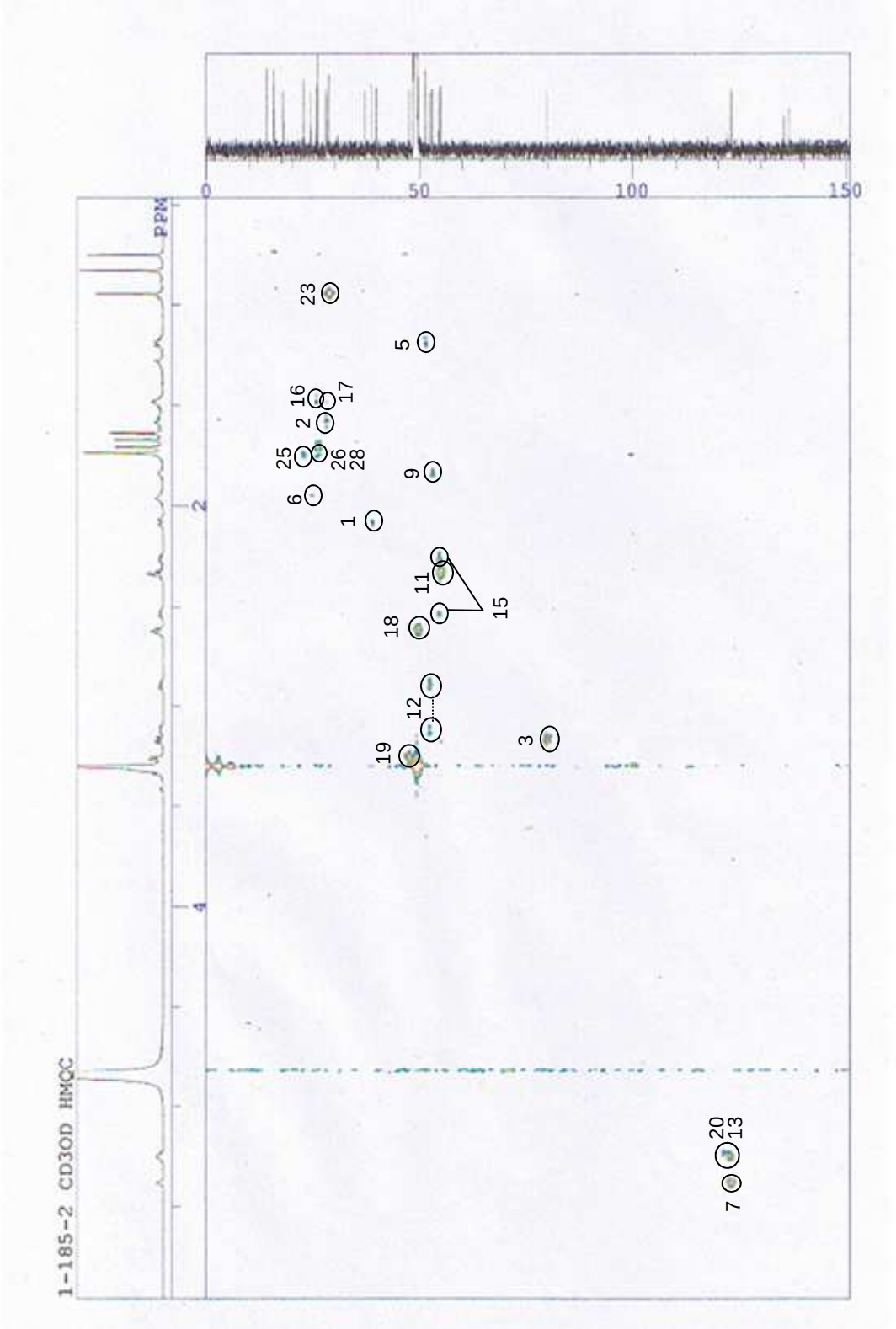


Figure S4. HMQC spectrum of halichonine A (**1**) (600 MHz, CD_3OD).

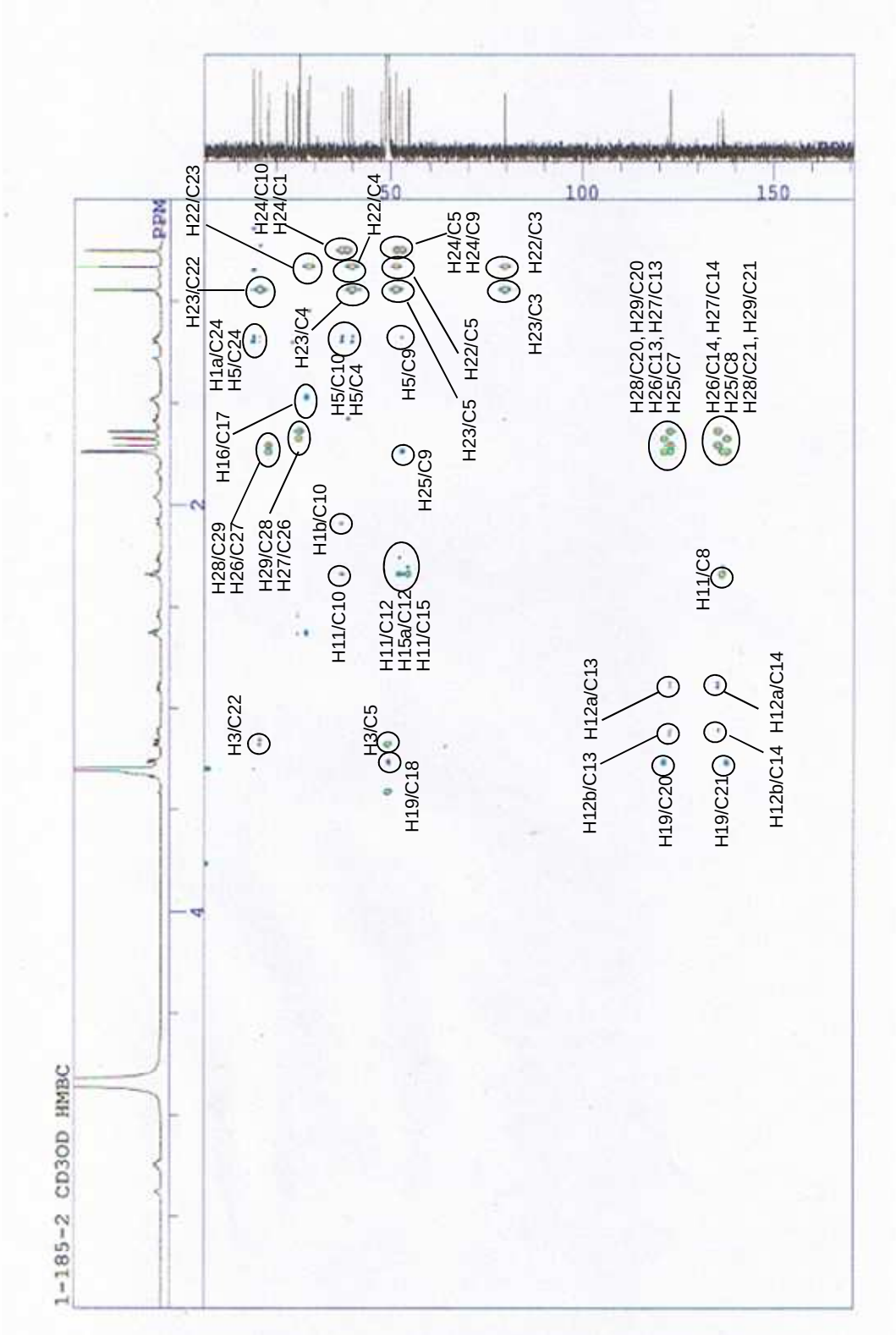


Figure S5. HMBC spectrum of halichonine A (**1**) (600 MHz, CD_3OD).

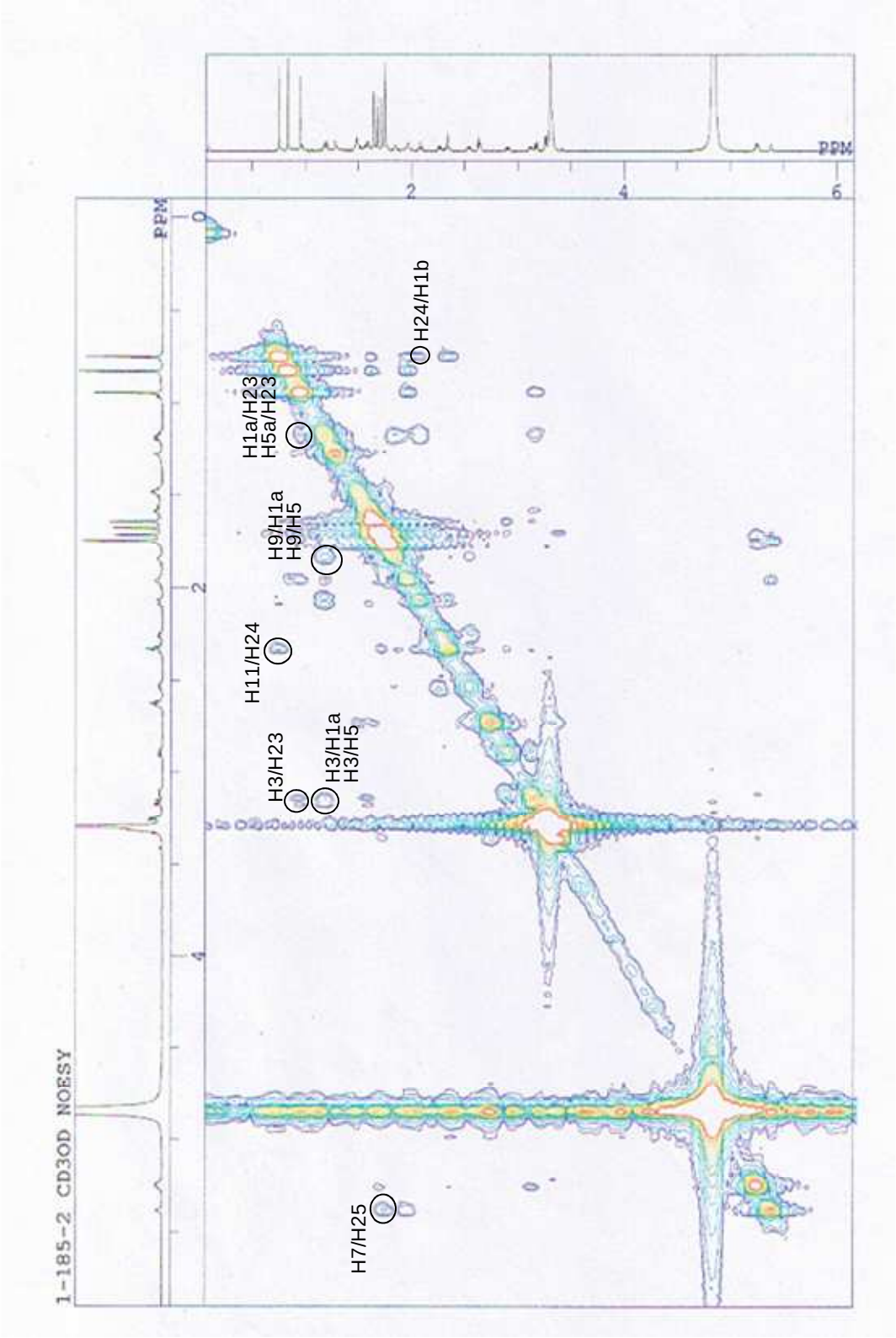


Figure S6. NOESY spectrum of halichonine A (**1**) (600 MHz, CD_3OD).

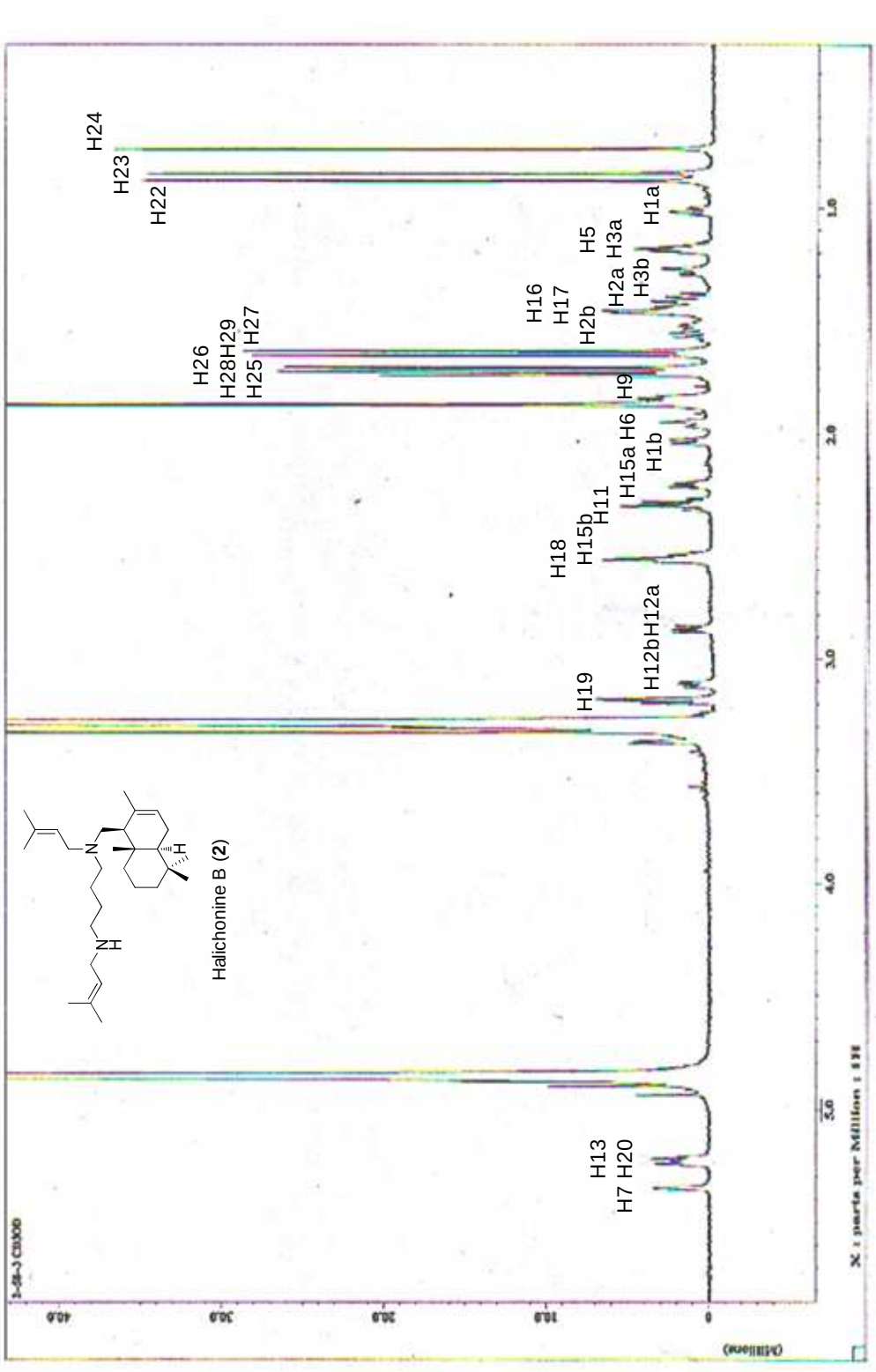


Figure S7. ^{1}H NMR spectrum of halichonine B (**2**) (800 MHz, CD_3OD).

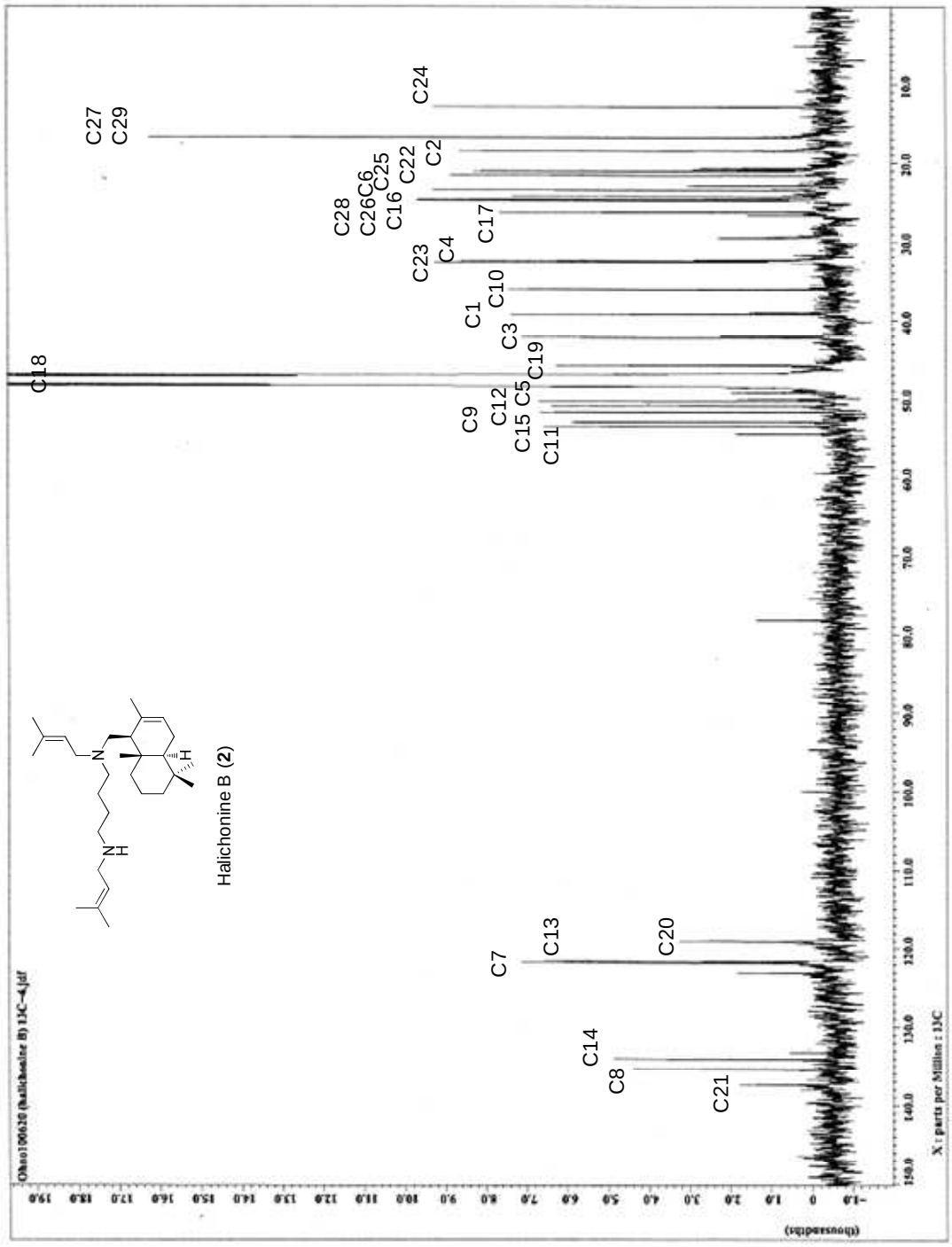


Figure S8. ^{13}C NMR spectrum of halichonine B (**2**) (100 MHz, CD_3OD).

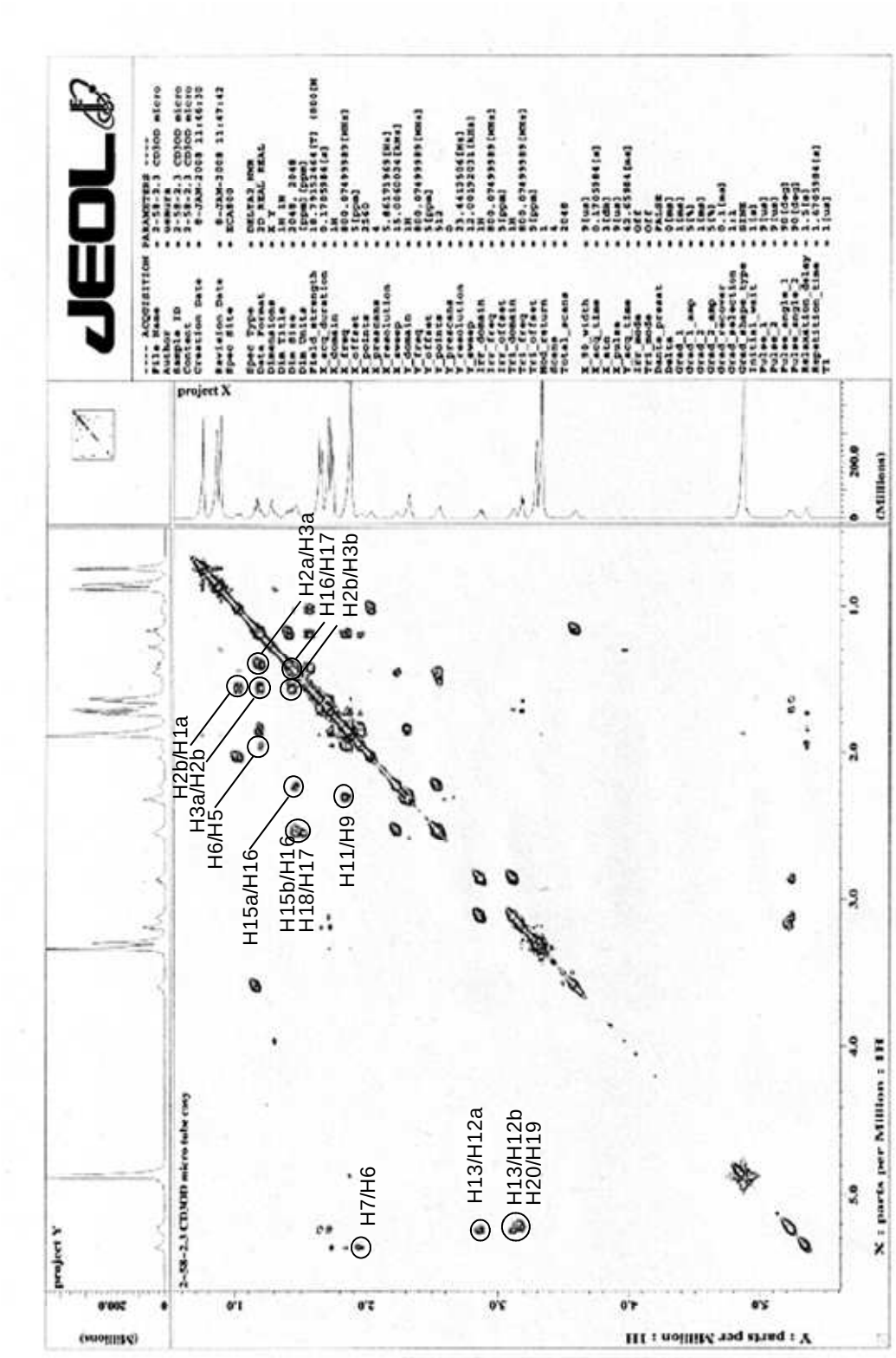


Figure S9. COSY spectrum of halichonine B (**2**) (800 MHz, CD_3OD).

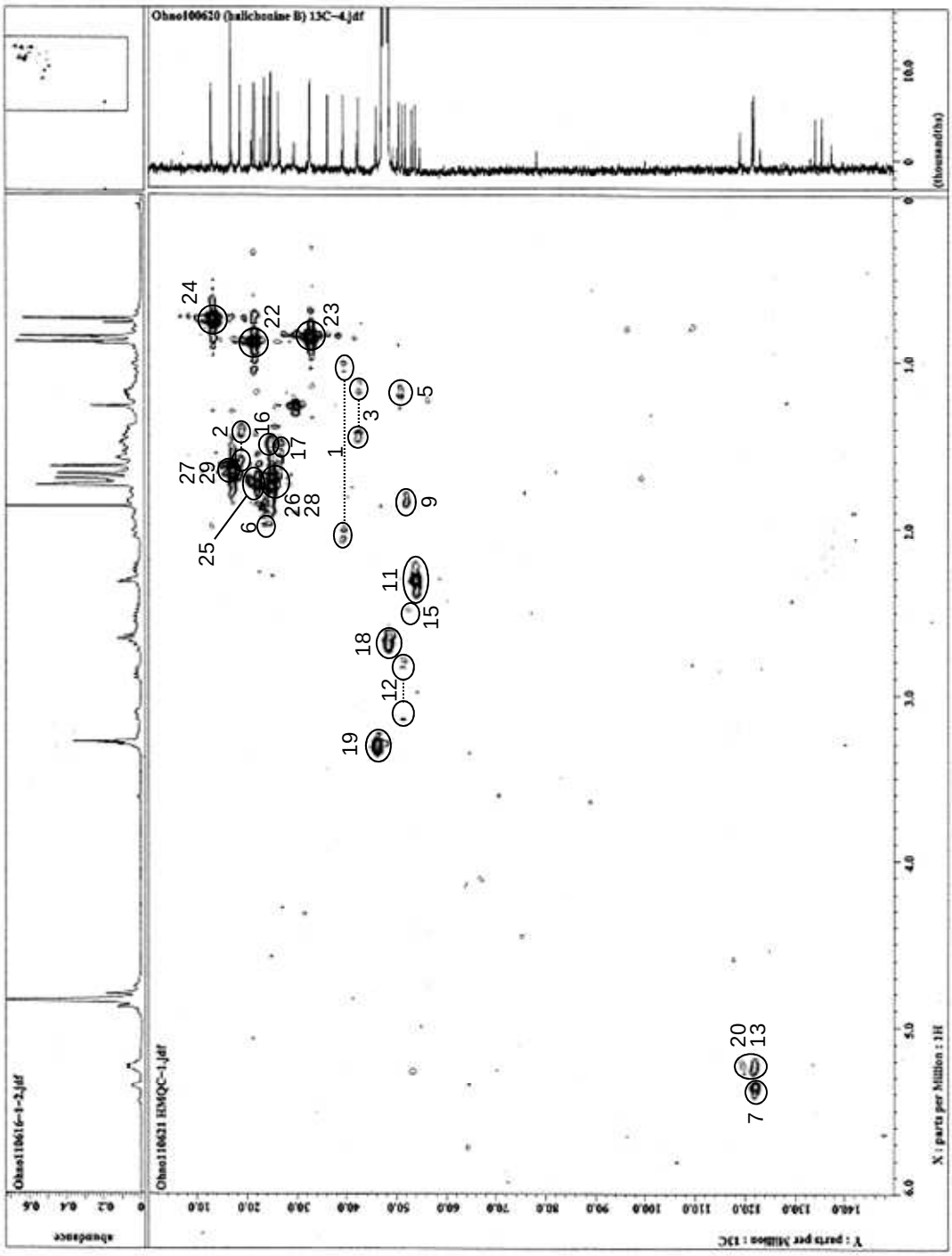


Figure S10. HMQC spectrum of halichonine B (**2**) (400 MHz, CD_3OD).

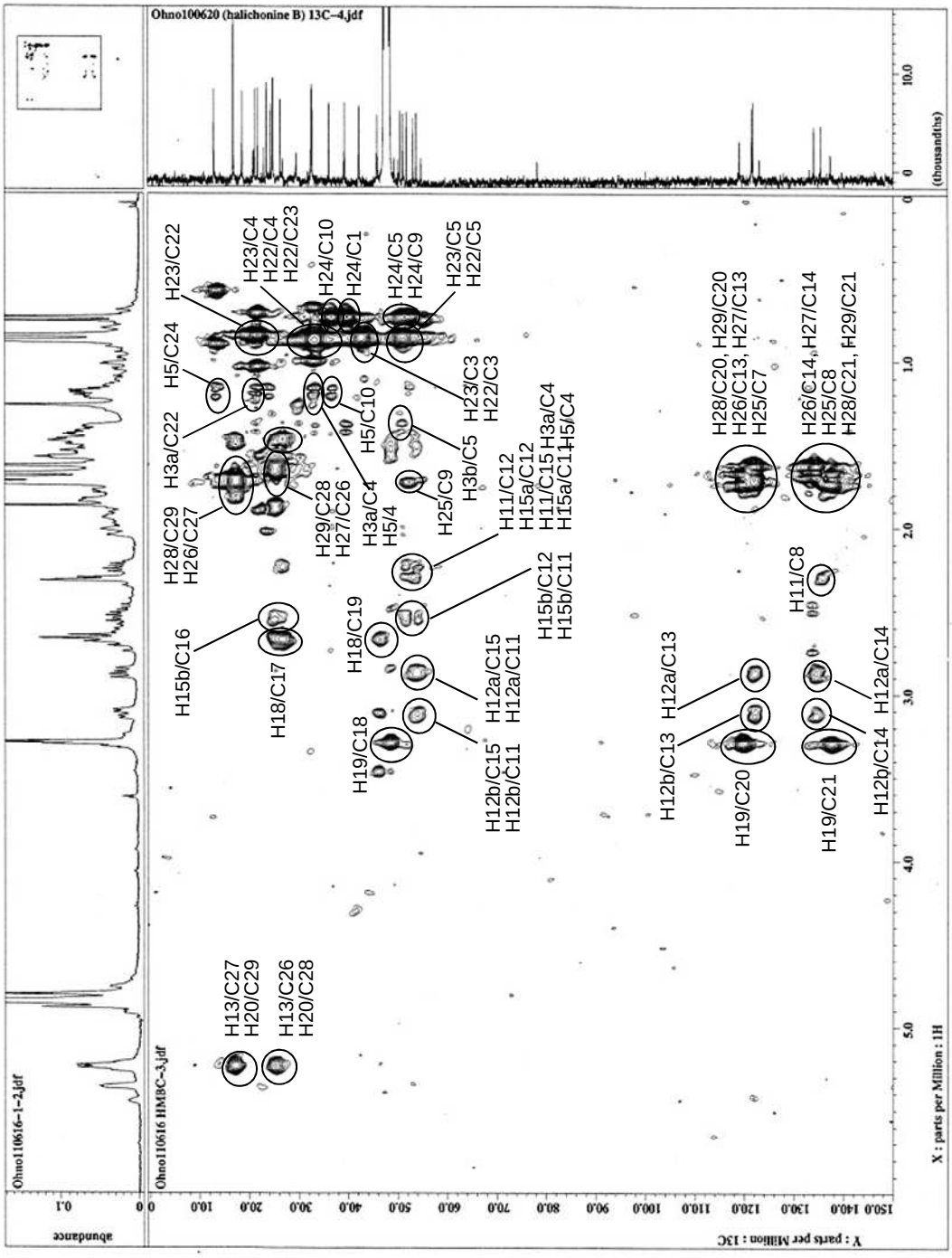


Figure S11. HMBC spectrum of halichonine B (**2**) (400 MHz, CD_3OD).

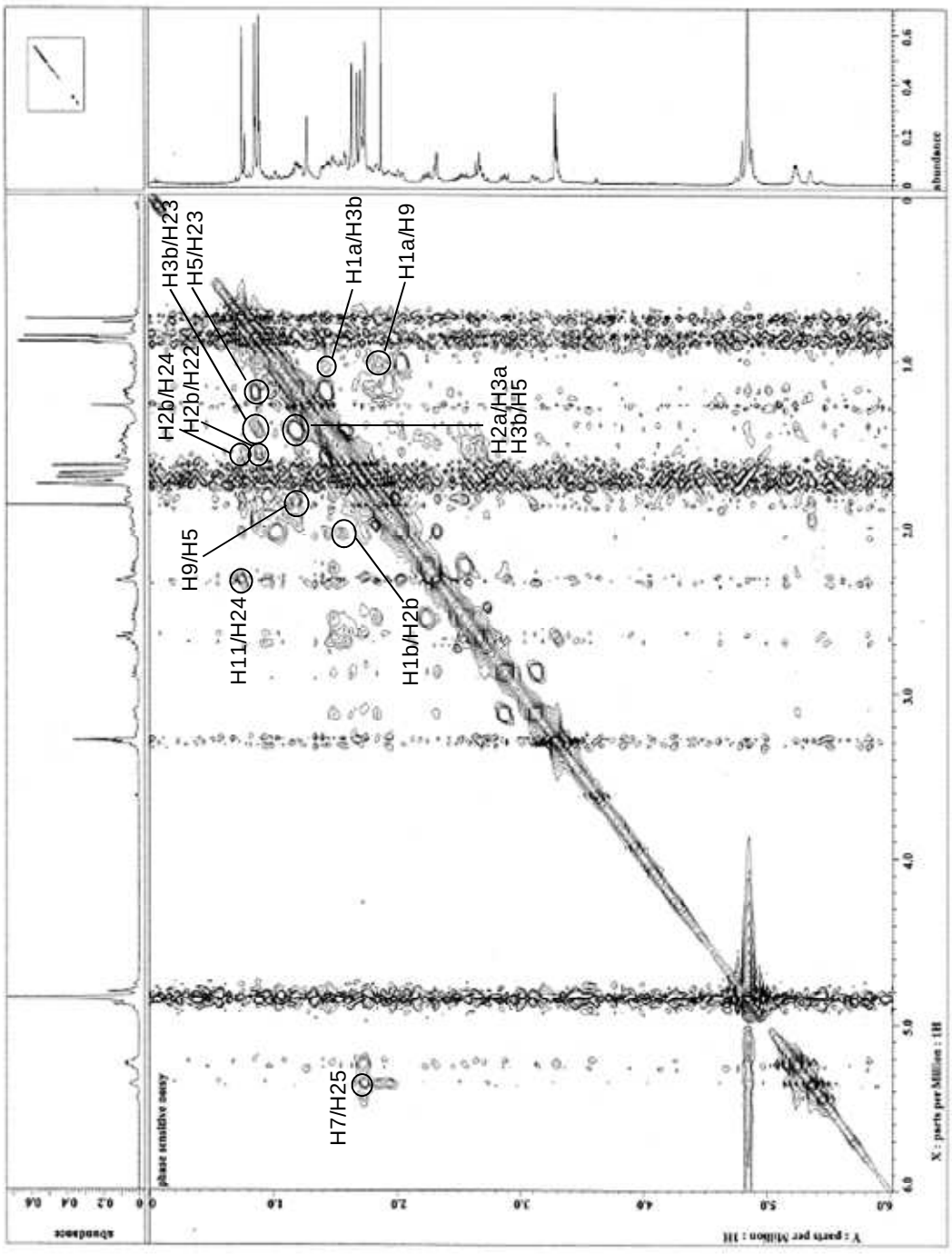


Figure S12. NOESY spectrum of halichonine B (**2**) (400 MHz, CD_3OD).

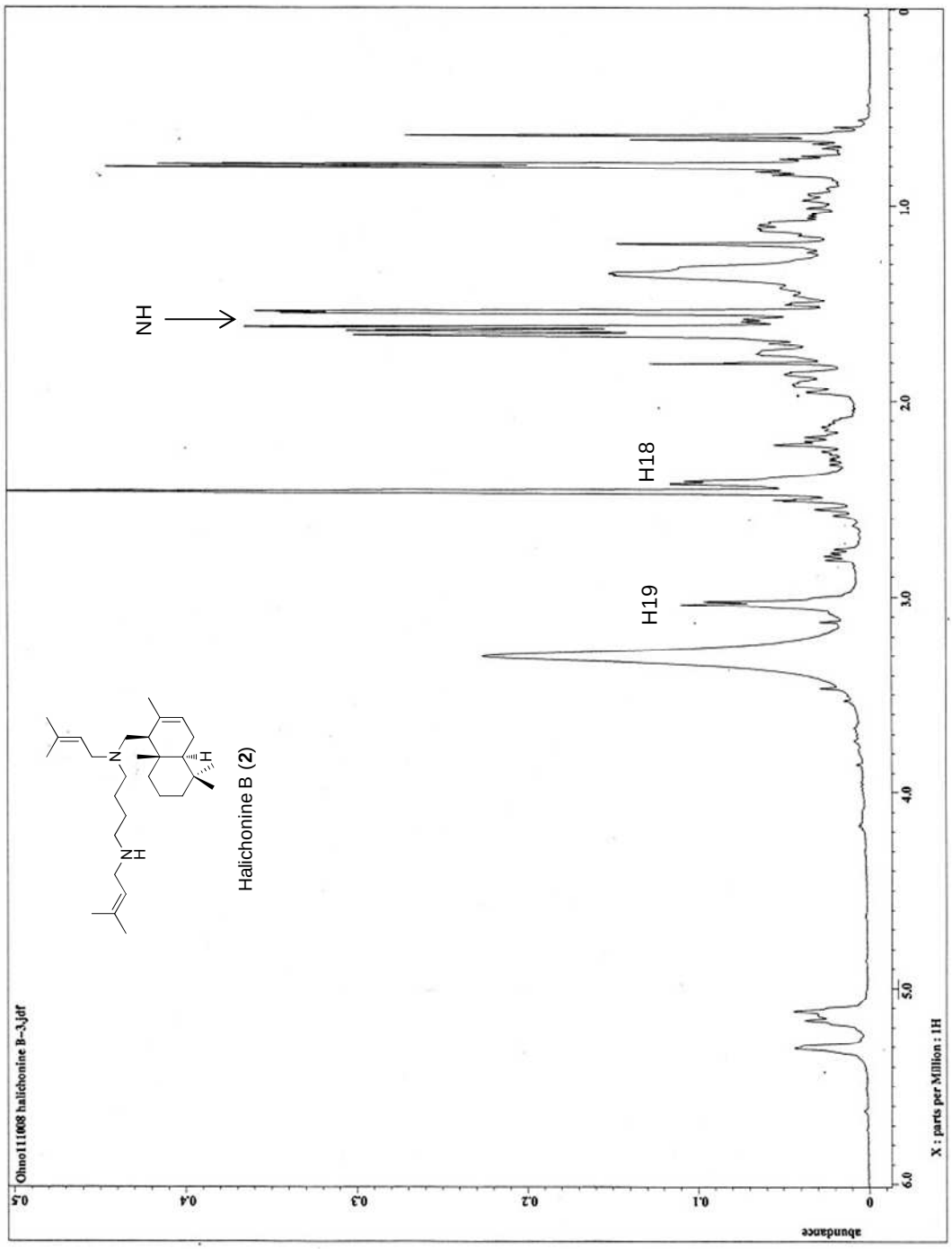


Figure S13. ^{1}H NMR spectrum of halichonine B (**2**) (400 MHz, DMSO-d_6).

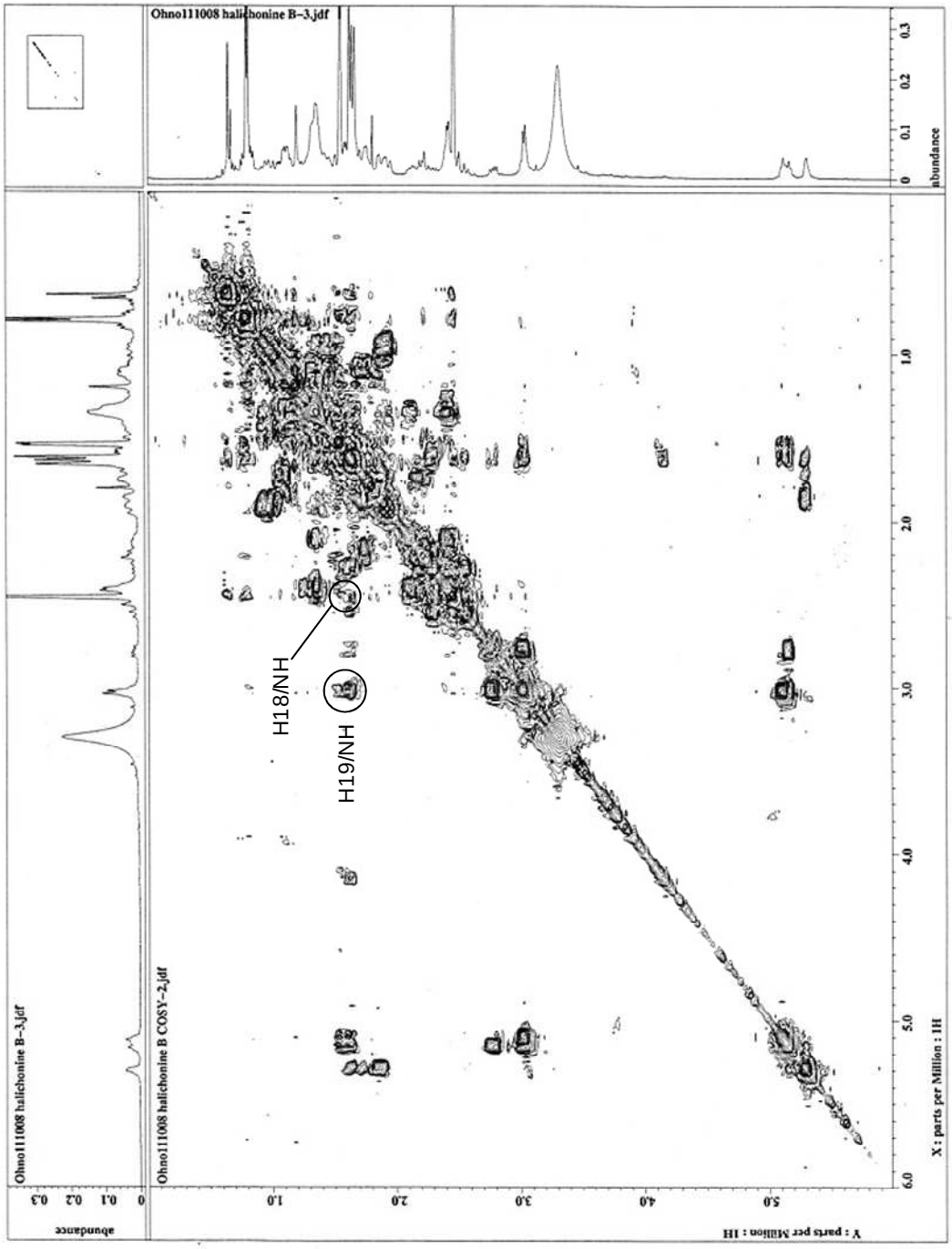


Figure S14. COSY spectrum of halichonine B (**2**) (400 MHz, DMSO-d_6).

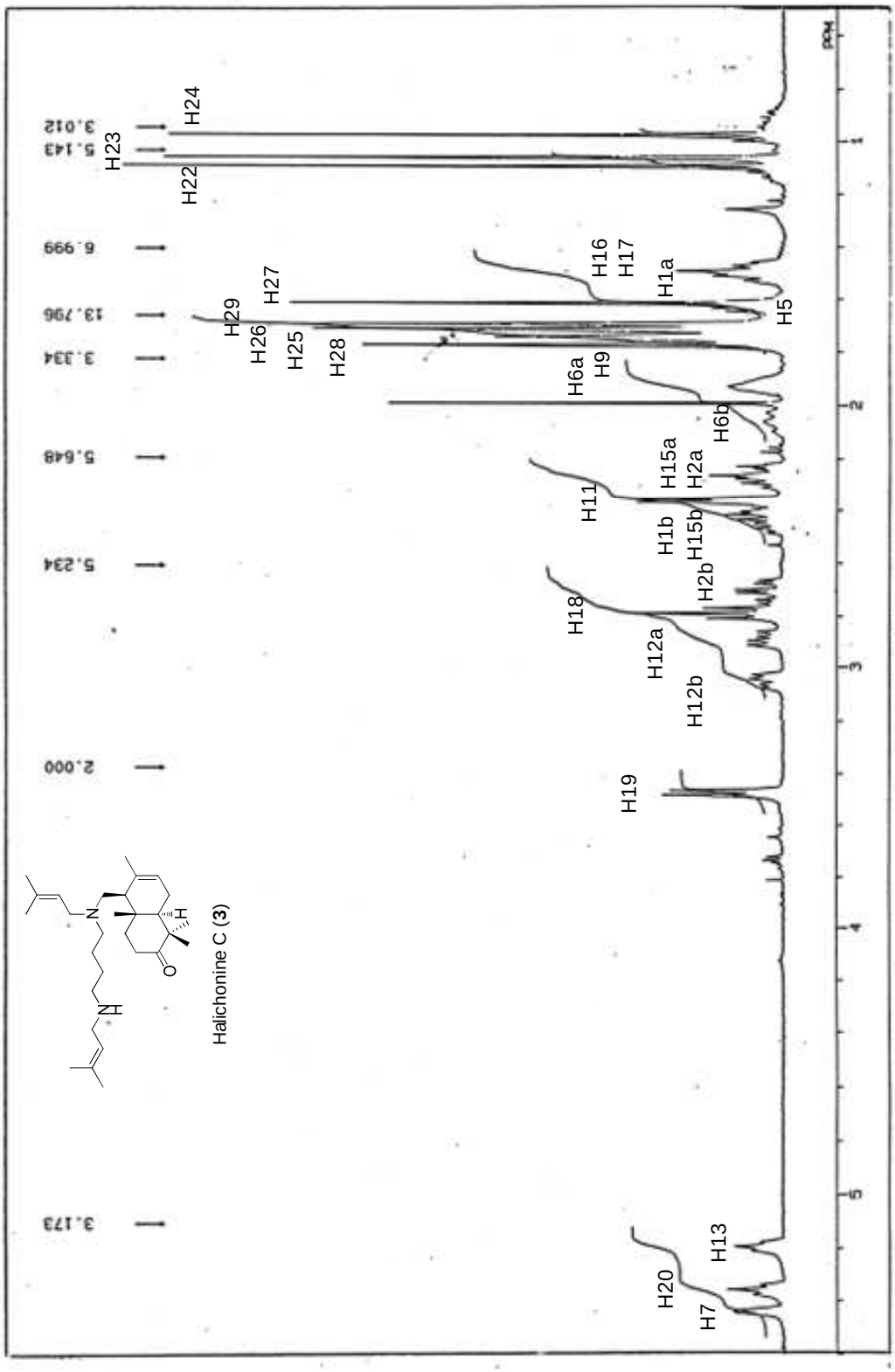


Figure S15. ^{1}H NMR spectrum of halichonine C (**3**) (400 MHz, $CDCl_3$).

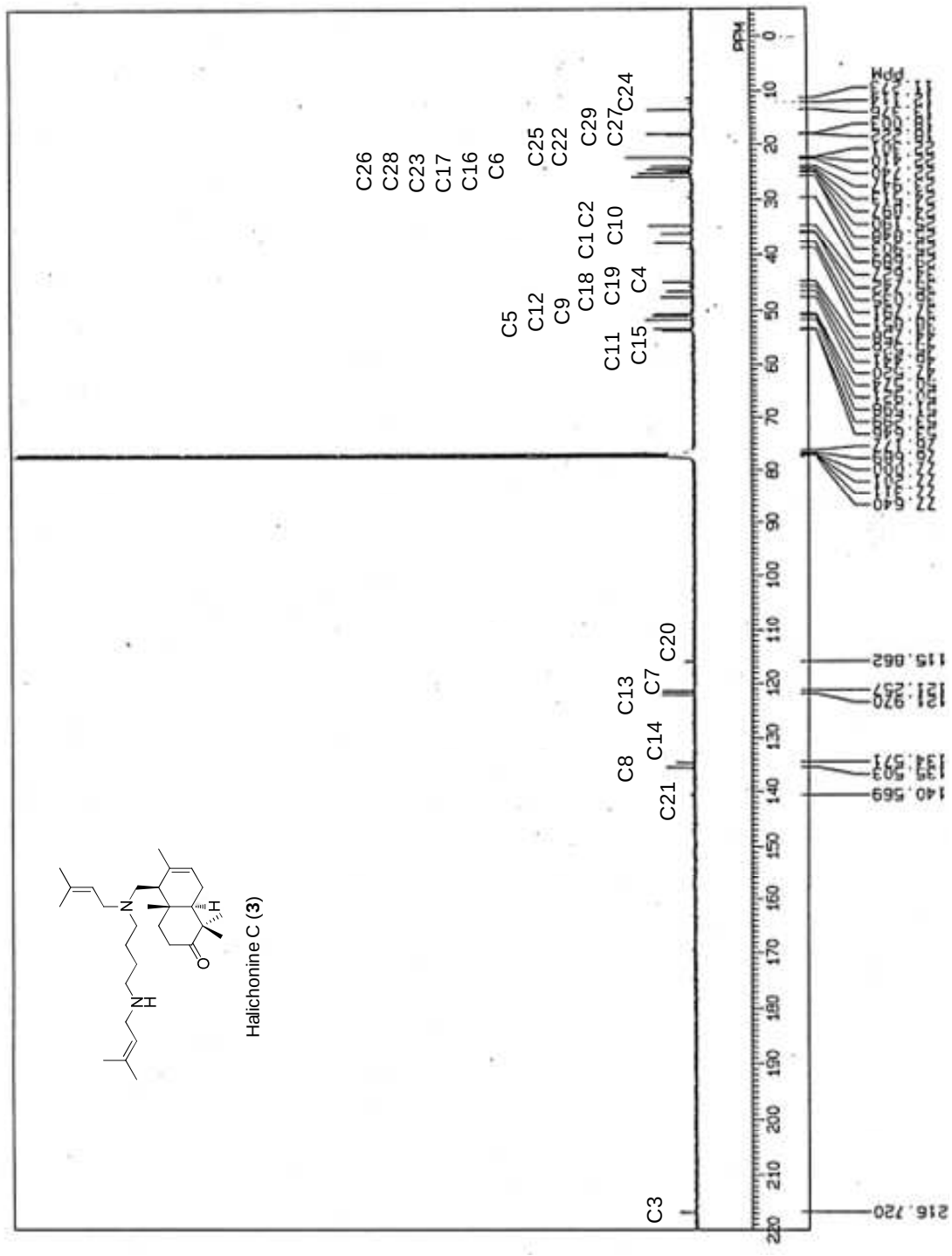


Figure S16. ^{13}C NMR spectrum of halichonine C (**3**) (100 MHz, CDCl3).

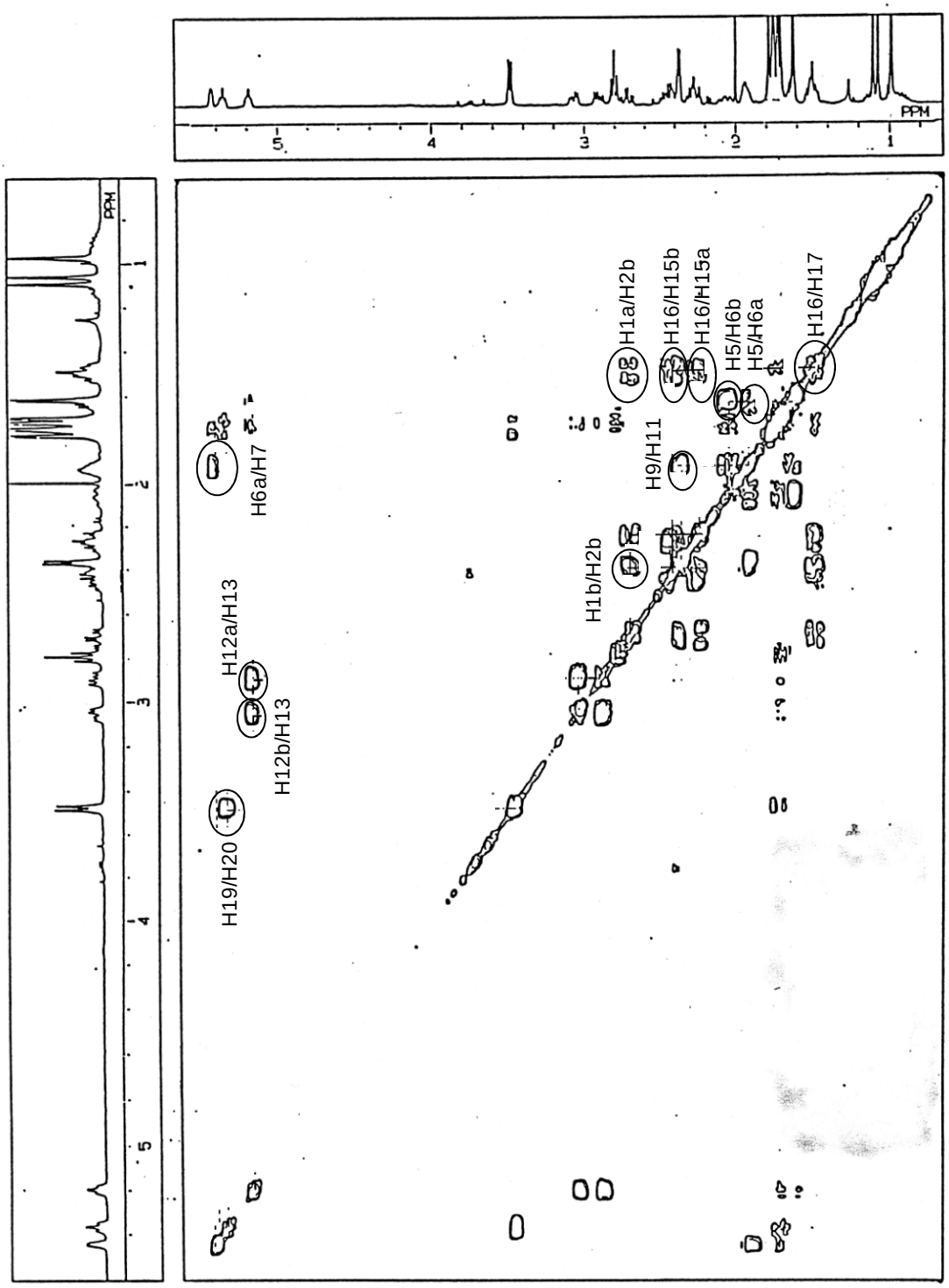


Figure S17. COSY spectrum of halichonine C (**3**) (400 MHz, $CDCl_3$).

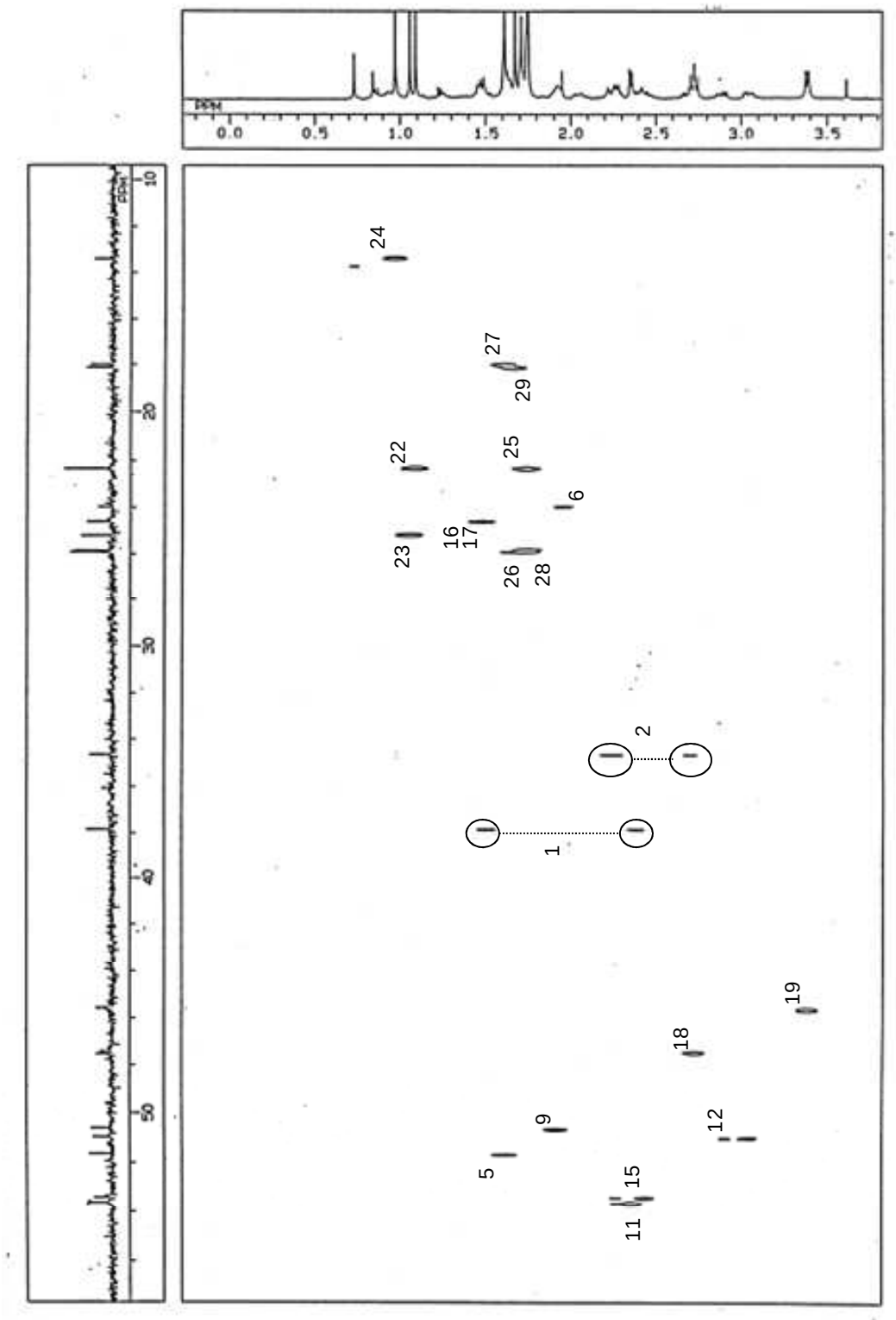


Figure S18. CH-COSY spectrum of halichonine C (**3**) (100 MHz, $CDCl_3$).

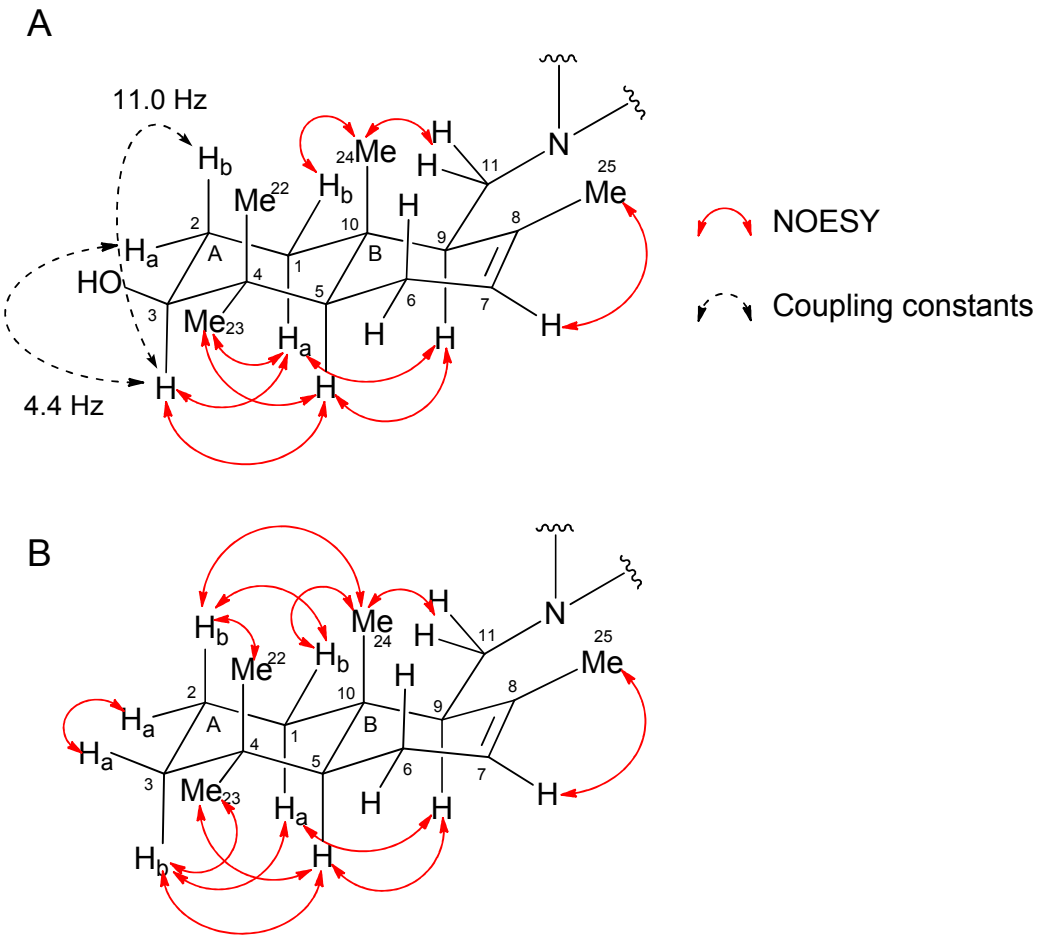


Figure S19. Relative configuration of the 6,6-bicyclic ring system in **1** (A) and **2** (B).

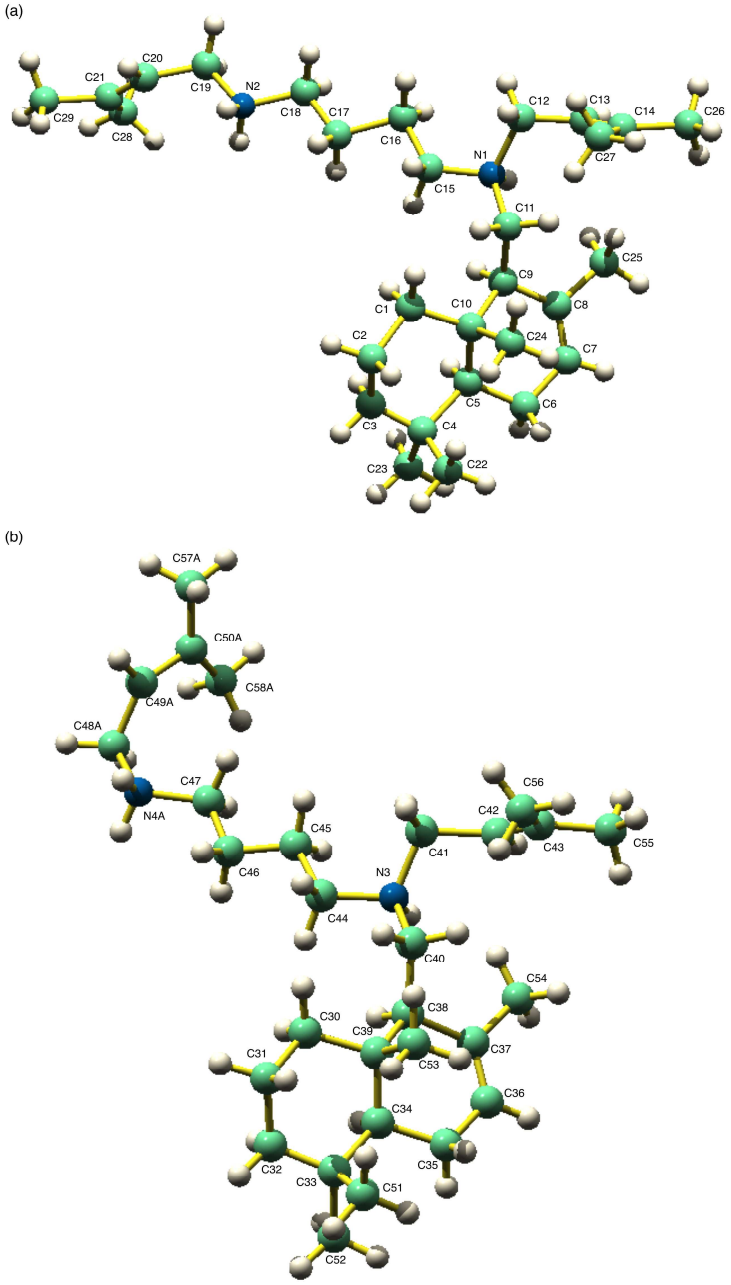


Figure S20. Crystal structures of HCl complex of halichonine B (**2**). The two independent molecules are shown; (a) the molecule composed of C1–C29 and N1–N2 and (b) the molecule of C30–C58 and N2–N3. The counter anions of Cl^- and the solvent water molecules are omitted for clarity.

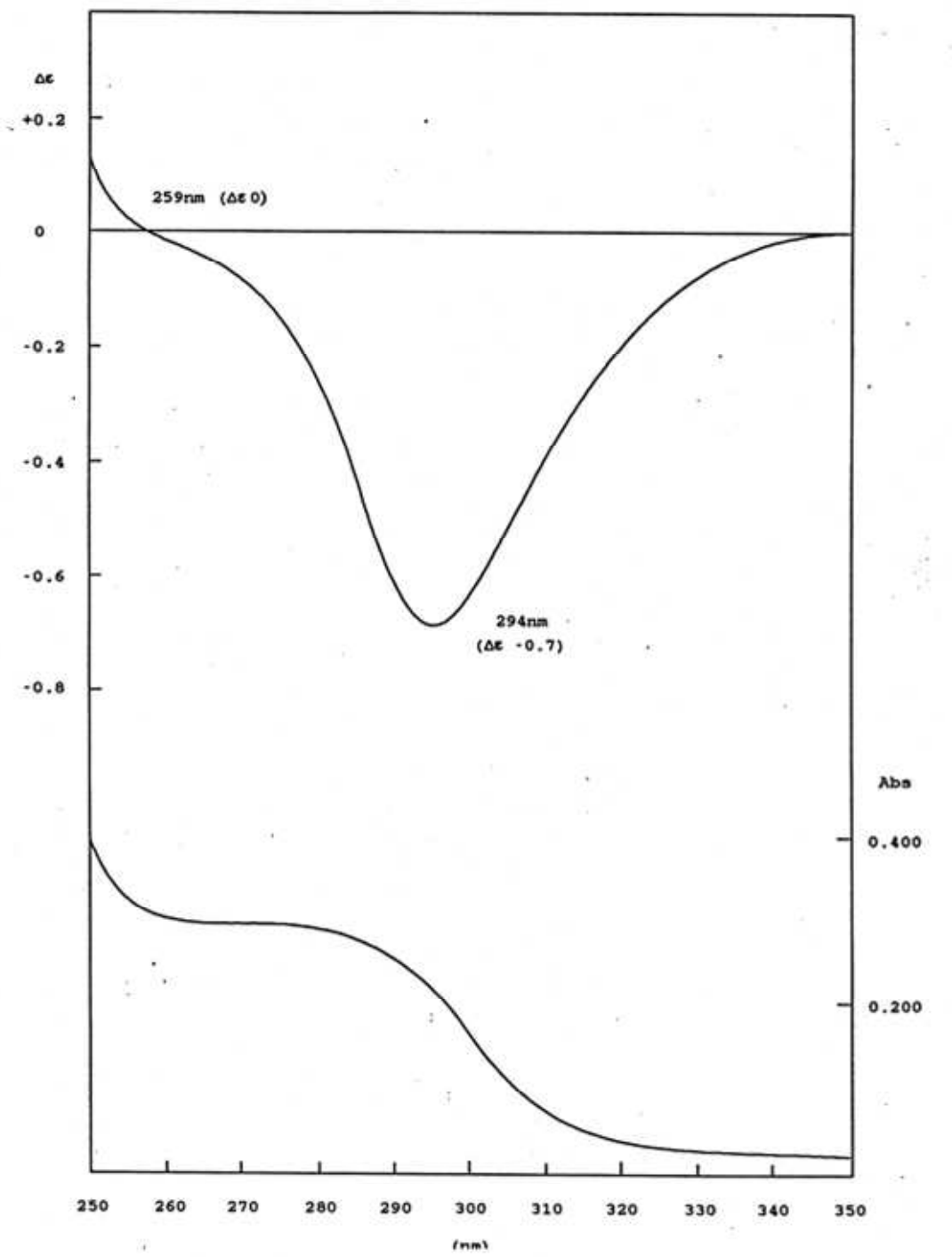


Figure S21. UV and CD spectra of halichonine C (**3**).

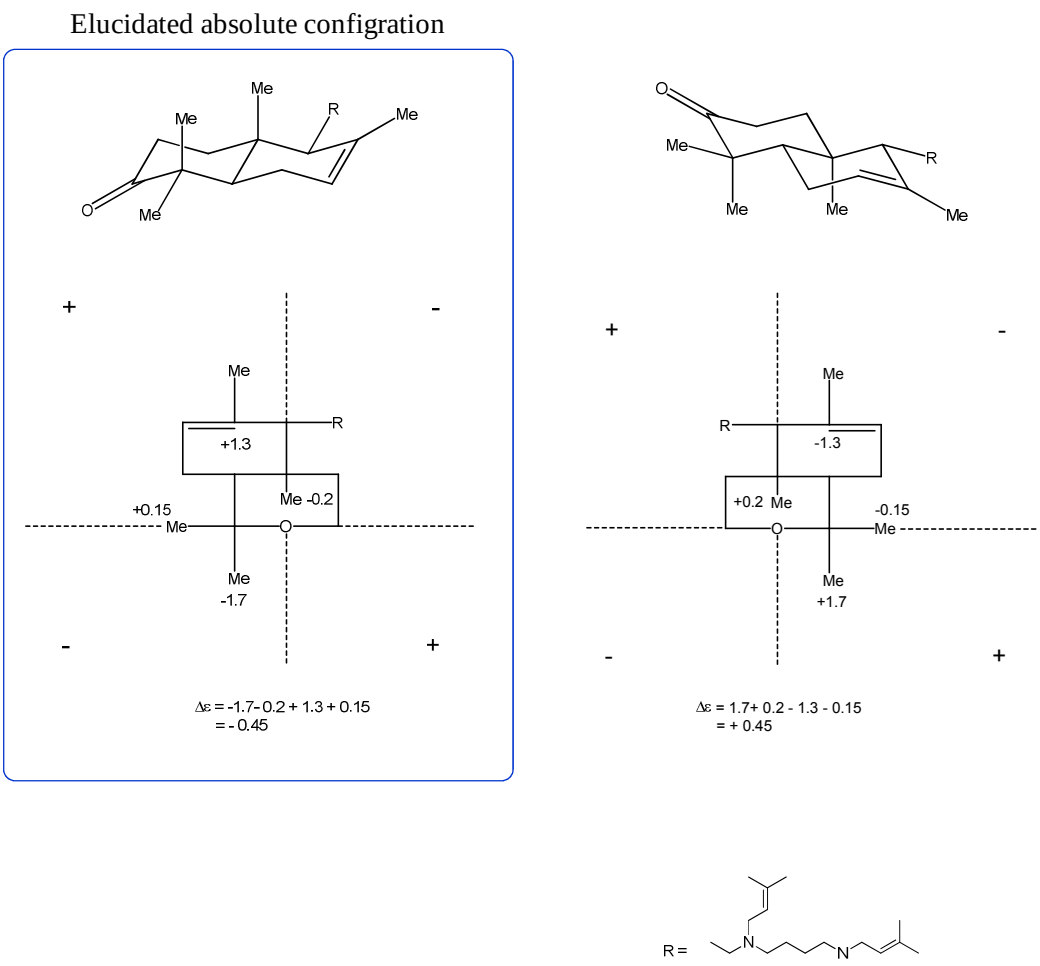


Figure S22. Octant diagram of halichonine C (**3**).

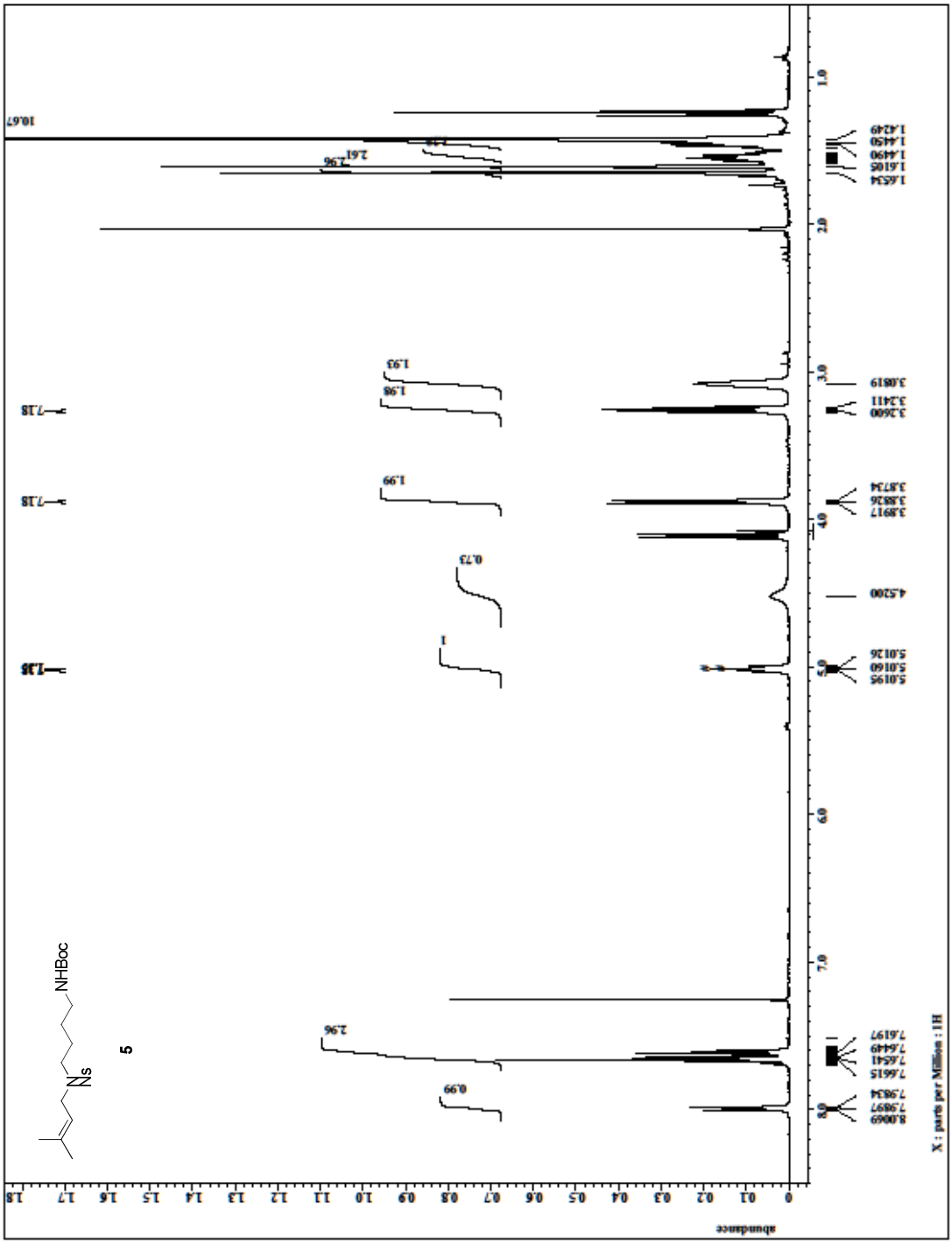


Figure S23. ^{1}H NMR spectrum of **5** (400 MHz, $CDCl_3$).

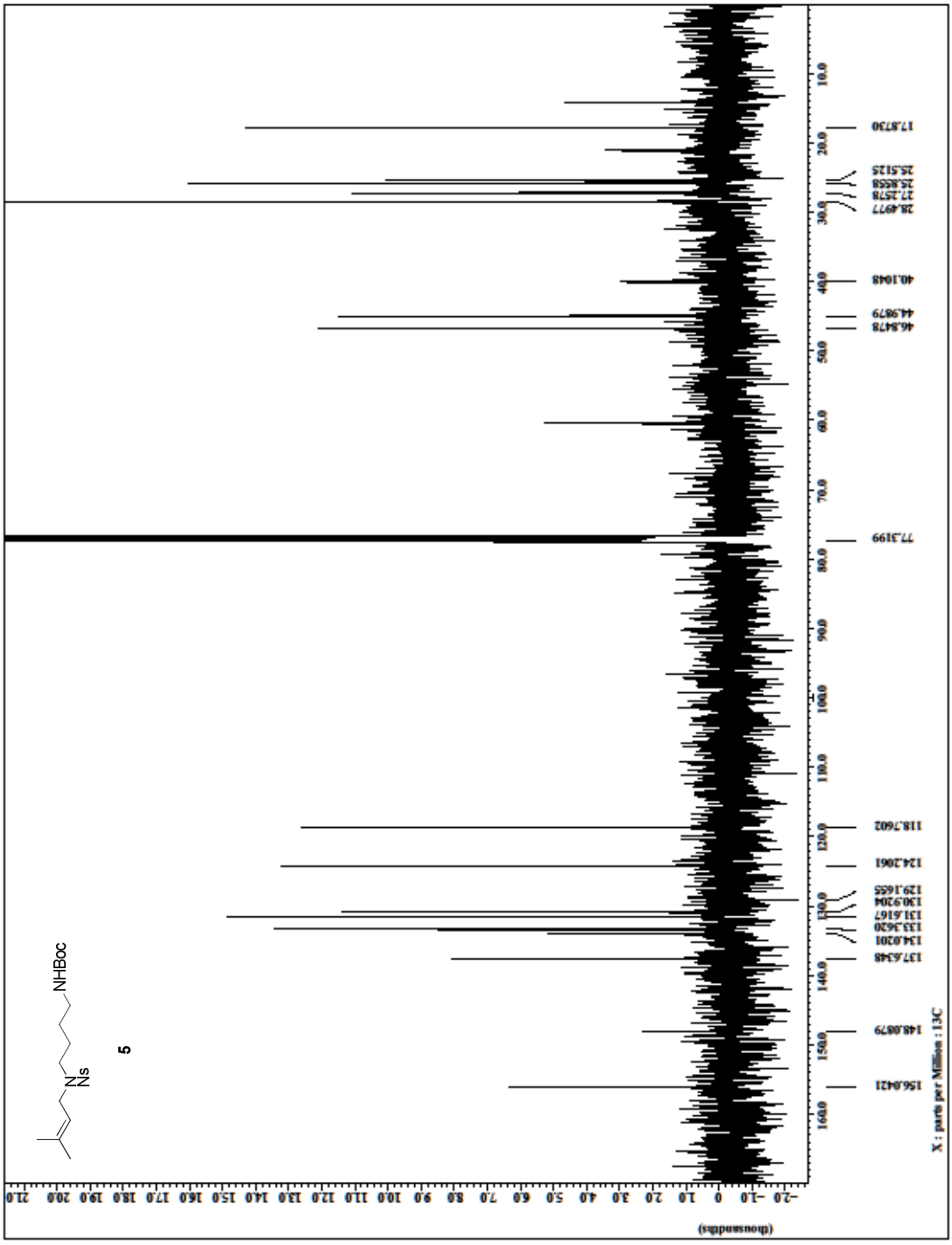


Figure S24. ^{13}C NMR spectrum of **5** (100 MHz, $CDCl_3$).

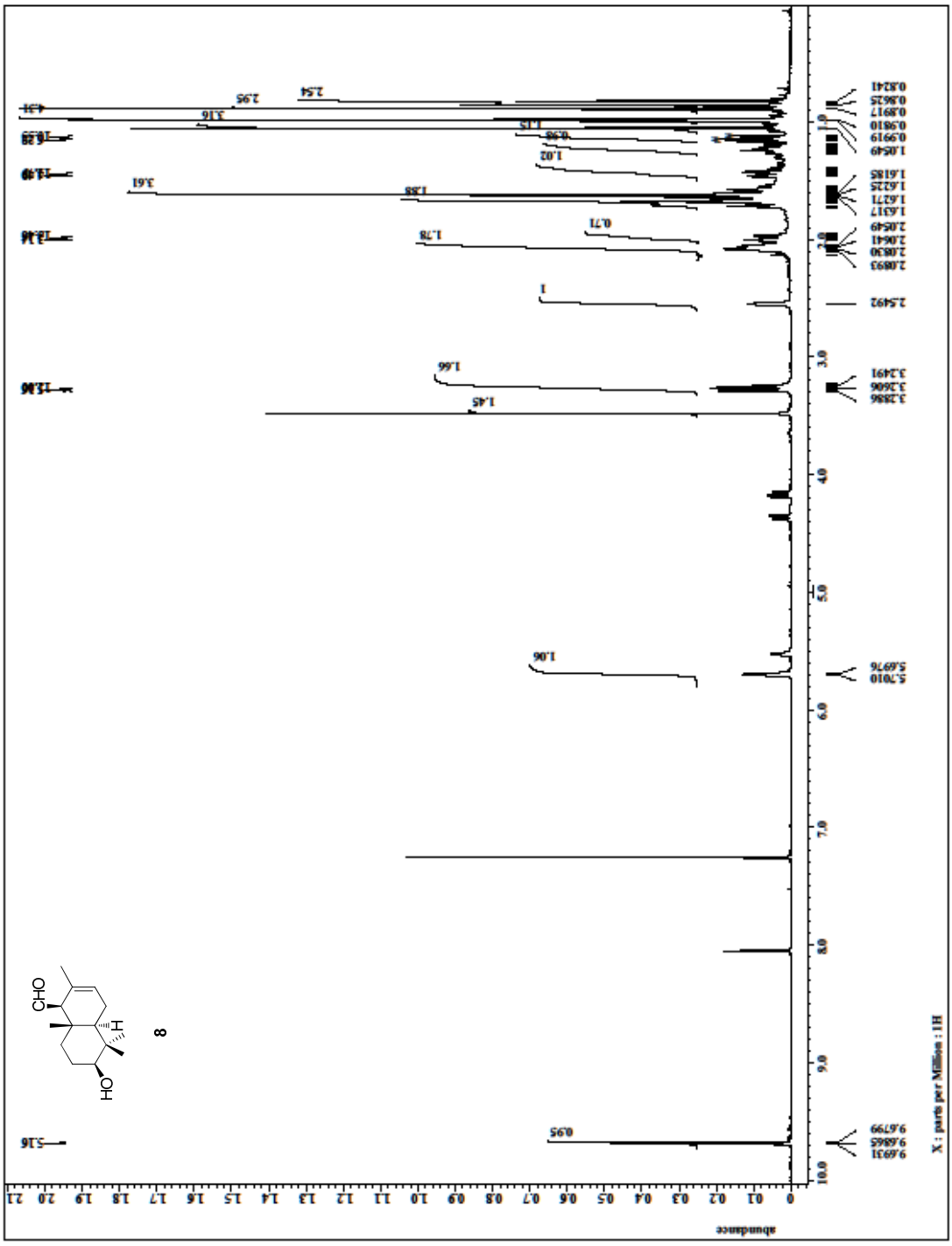


Figure S25. ^{1}H NMR spectrum of **8** (400 MHz, $CDCl_3$).

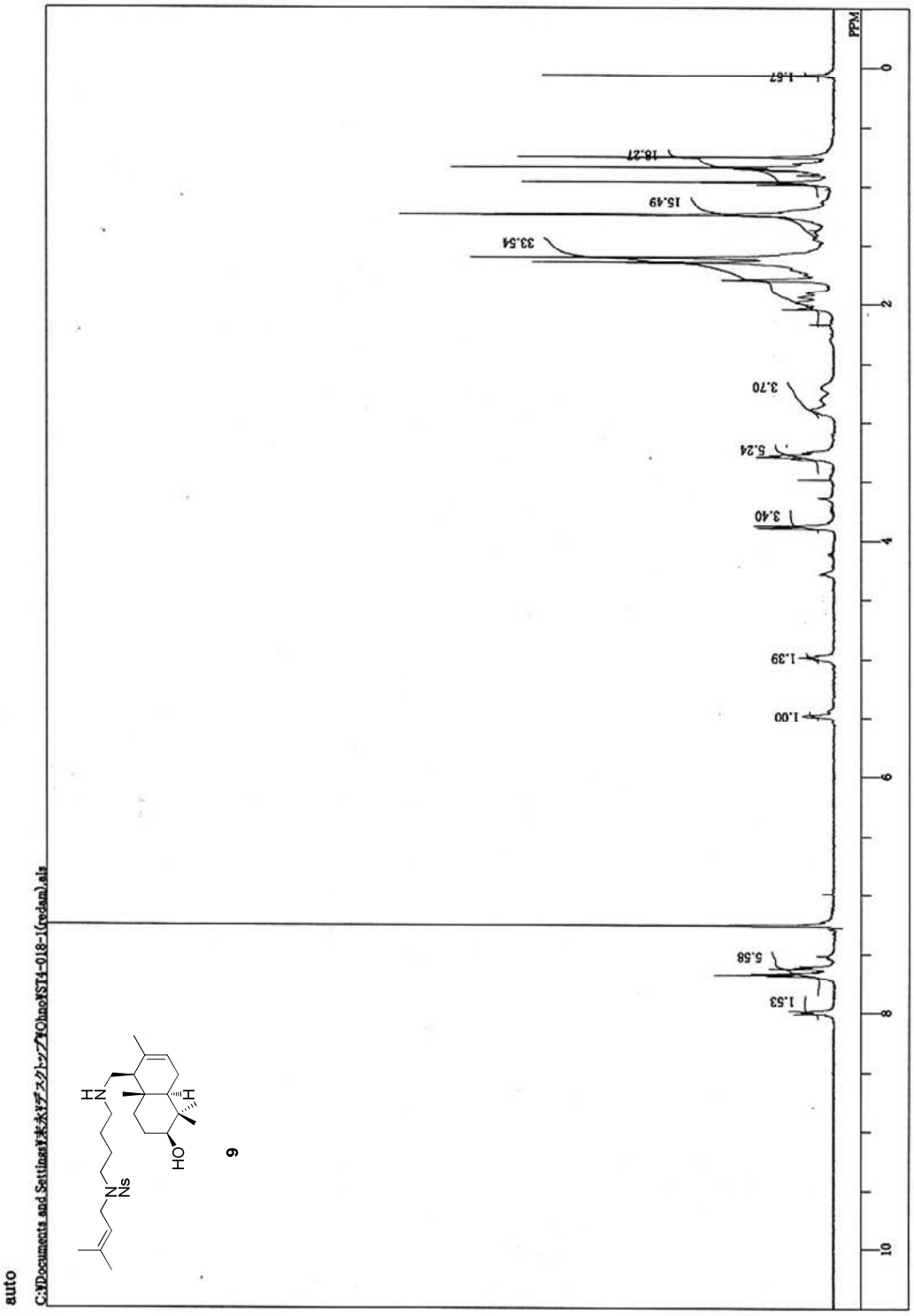


Figure S26. ^{1}H NMR spectrum of **9** (400 MHz, $CDCl_3$).

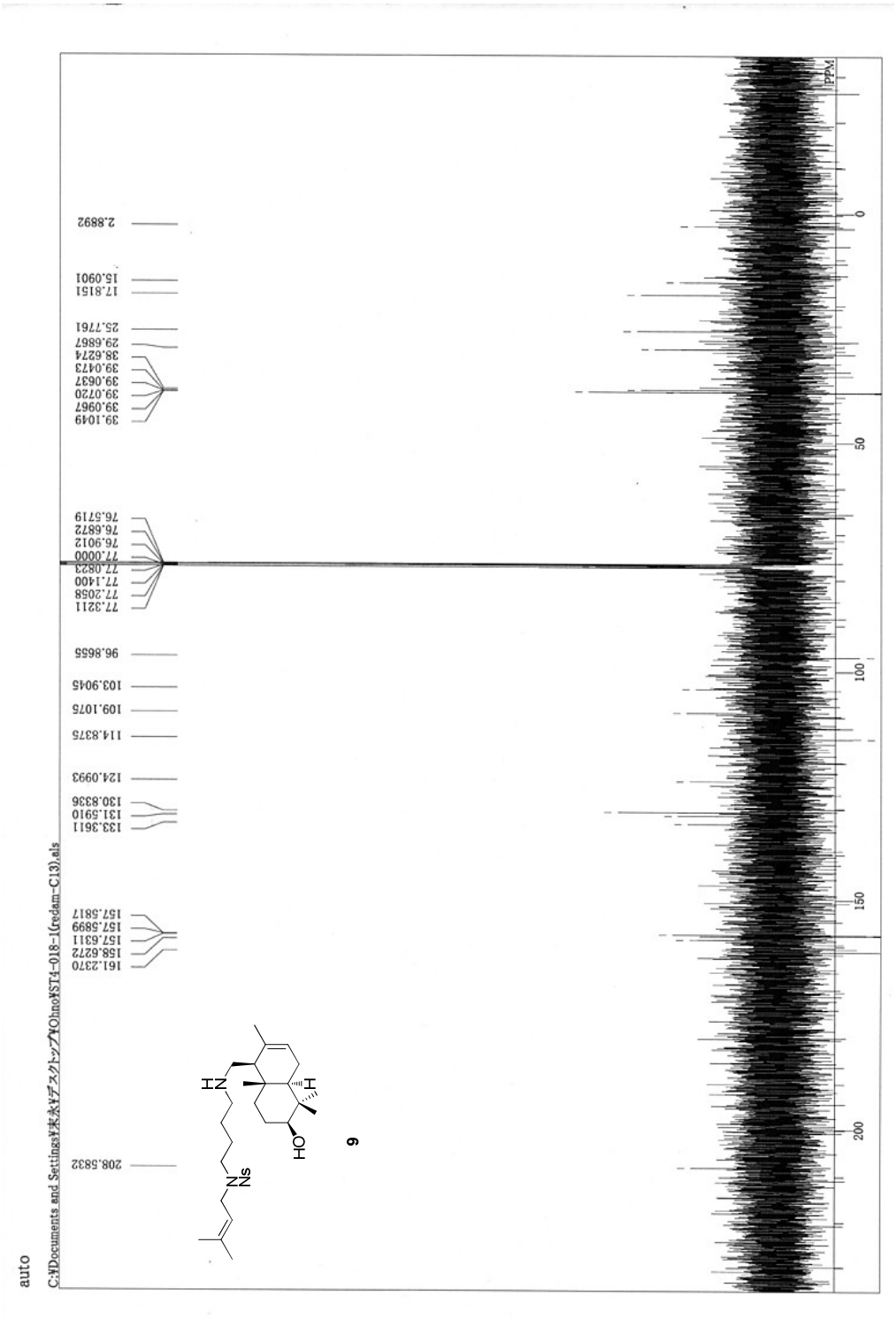


Figure S27. ^{13}C NMR spectrum of **9** (100 MHz, $CDCl_3$).

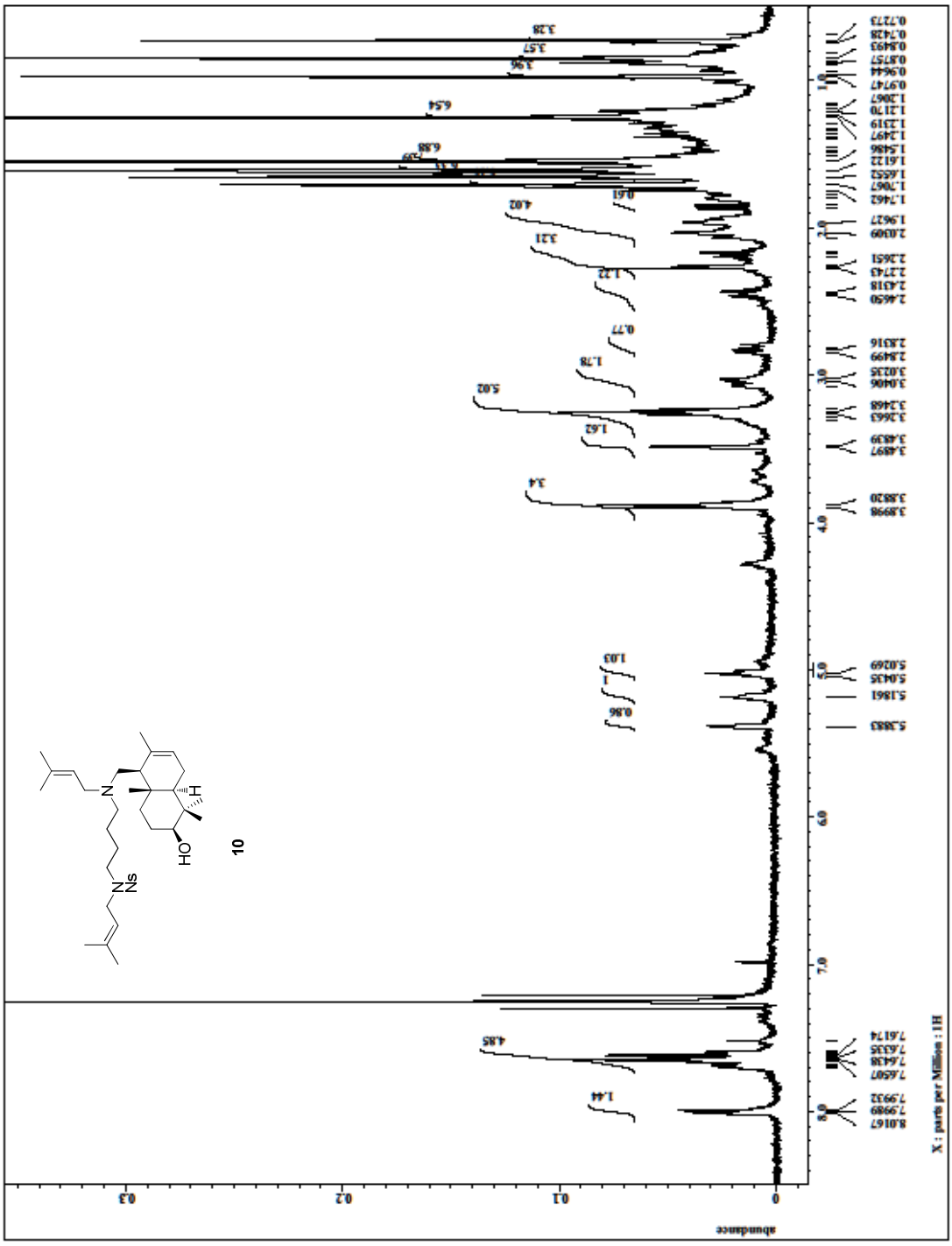


Figure S28. ^{1}H NMR spectrum of **10** (400 MHz, $CDCl_3$).

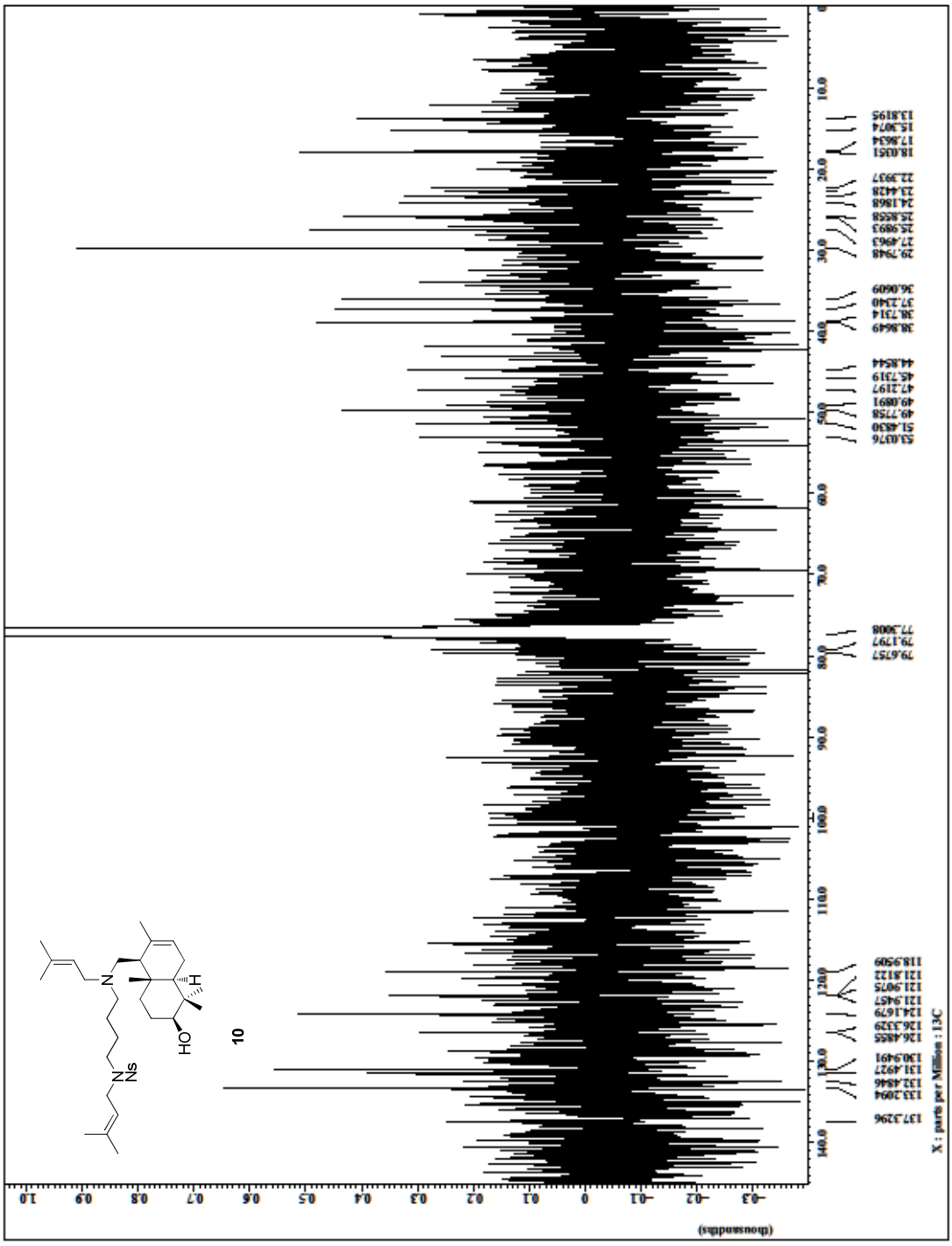


Figure S29. ^{13}C NMR spectrum of **10** (100 MHz, $CDCl_3$).

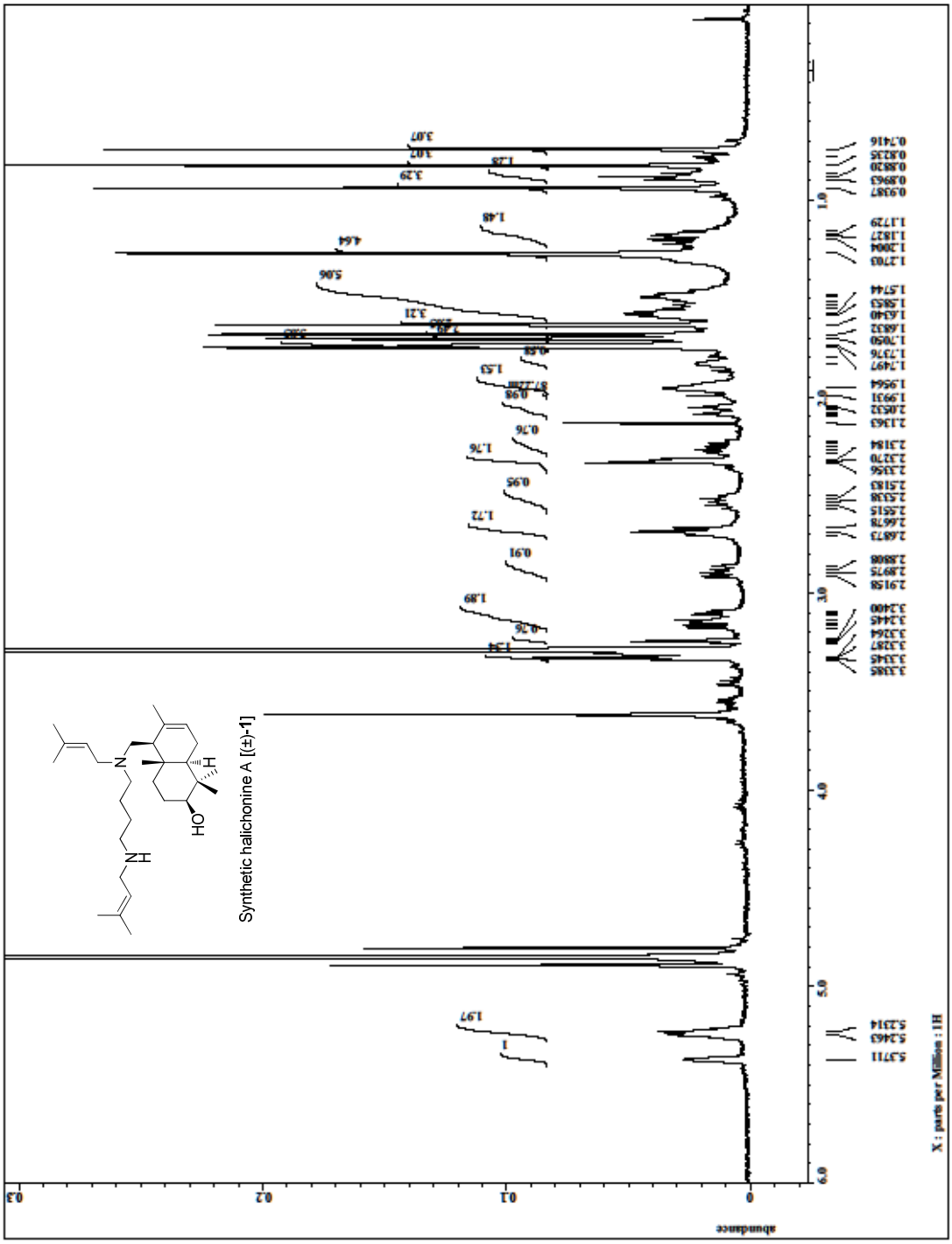


Figure S30. ^{1}H NMR spectrum of synthetic halichonine A [(±)-**1**] (400 MHz, CD_3OD).

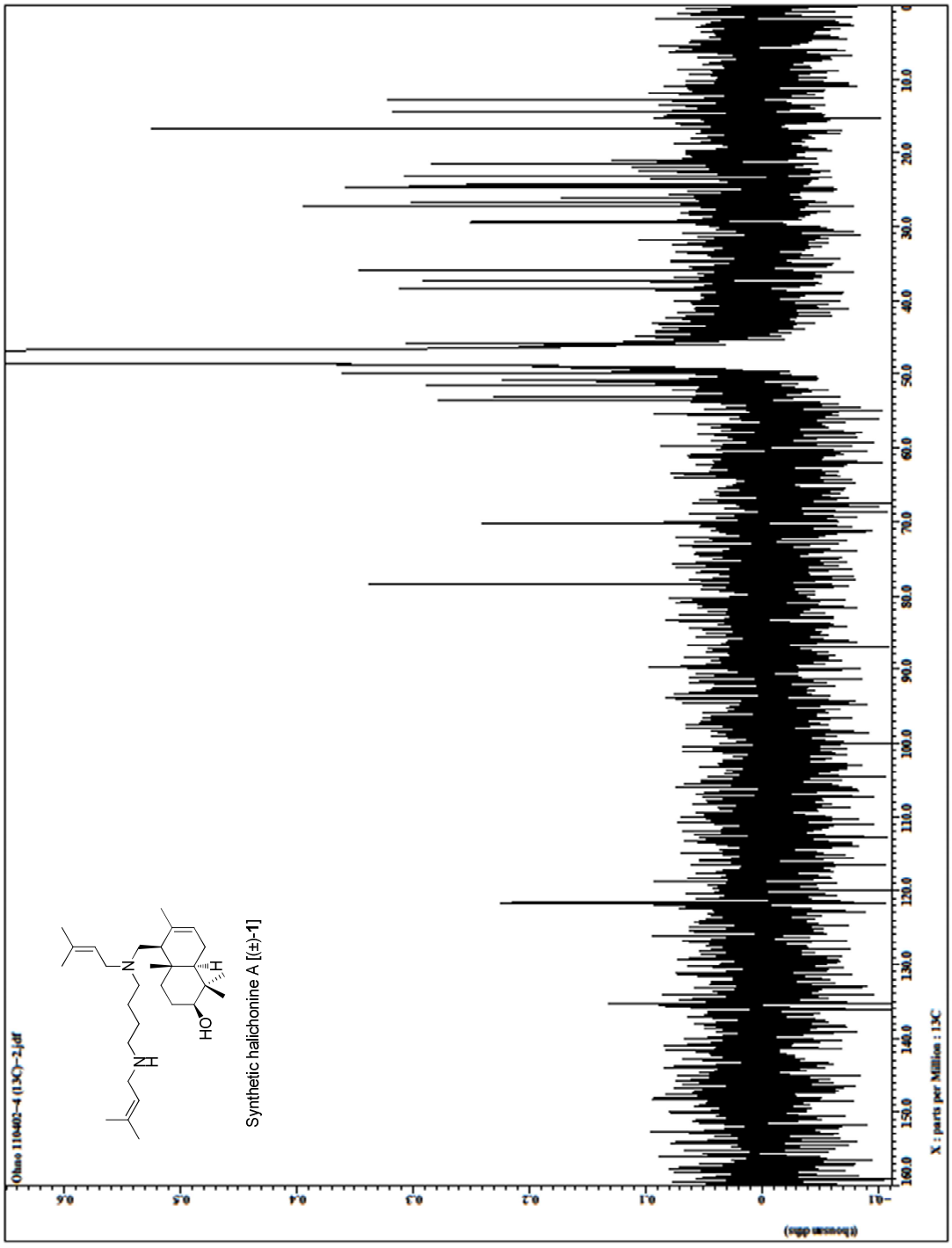


Figure S31. ^{13}C NMR spectrum of synthetic halichonine A [(±)-**1**] (100 MHz, CD_3OD).

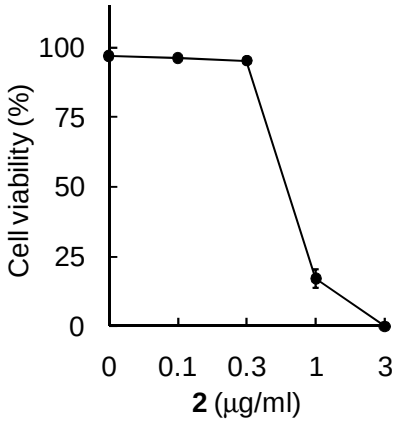


Figure S32. Cytotoxicity of **2** against HL60 cells. HL60 cells were incubated with the indicated concentrations of **2** for 24 h. Cell viability was then determined using trypan blue dye exclusion. Values are the means ± SD of quadruplicate determinations.

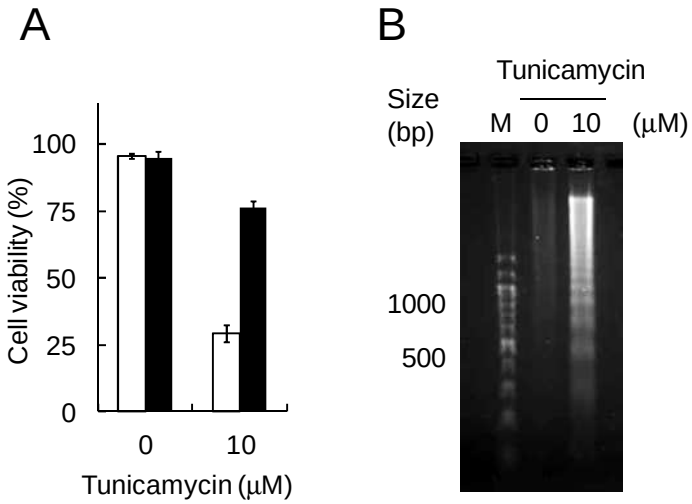


Figure S33. Induction of apoptosis in HL60 cells by tunicamycin. (A) HL60 cells were preincubated (solid column) or not (open column) with 25 μM Z-VAD-FMK, and then treated with 0 or 10 μM tunicamycin. After 24 h, cell viability was determined. Values are the means ± SD of quadruplicate determinations. (B) HL60 cells were incubated with 0 or 10 μM tunicamycin for 24 h. Cellular DNA was then extracted and electrophoresed on an agarose gel.

Table S1. NMR data for for halichonine A (**1**) in CD_3OD.

position		Halichonine A (**1**)			
		δ_C (mult.)[a]	δ_H (mult., *J* in Hz)[b]	COSY[b]	HMBC ($^1H \rightarrow ^{13}C$)[b]
1	a	38.9 (t)	1.17 (m)	H1b, 2	C24
	b		2.08 (dt, 3.7, 13.2)	H1a, 2	C10
2		28.4 (t)	1.59 (m, 2H)	H1a, 1b, 3	C1, 3
3		79.9 (d)	3.17 (dd, 4.4, 11.0)	H2	C5, 22
4		40.0 (s)			
5		51.5 (d)	1.17 (m)	H6	C4, 6, 9, 10, 24
6		24.6 (t)	1.96 (brs, 2H)	H5, 7	
7		123.2 (d)	5.38 (brs)	H6	
8		136.8 (s)			
9		53.1 (d)	1.84 (brs)	H11	
10		37.4 (s)			
11		55.0 (t)	2.33 (m, 2H)	H9	C8, 10, 12, 15
12	a	52.4 (t)	2.90 (dd, 7.3, 14.0)	H12b, 13	C13, 14
	b		3.11 (dd, 5.9, 14.0)	H12a, 13	C13, 14
13		123.2 (d)	5.25 (m)	H12a, 12b	
14		135.4 (s)			
15	a	54.6 (t)	2.26 (m)	H15b, 16	C12
	b		2.53 (m)	H15a, 16	C16
16		25.9 (t)	1.48 (m, 2H)	H15a, 15b, 17	C17
17		28.3 (t)	1.52 (m, 2H)	H16, 18	
18		49.9 (t)	2.63 (m, 2H)	H17	C16, 17
19		47.6 (t)	3.26 (d, 6.6, 2H)	H20	C18, 20, 21
20		123.1 (d)	5.24 (m)	H19	
21		137.5 (s)			
22		16.0 (q)	0.83 (s, 3H)		C3, 4, 5, 23
23		28.8 (q)	0.94 (s, 3H)		C3, 4, 5, 22
24		14.3 (q)	0.74 (s, 3H)		C1, 5, 9, 10
25		22.9 (q)	1.73 (s, 3H)		C7, 8, 9
26		26.1 (q)	1.71 (s, 3H)		C13, 14, 27
27		18.1 (q)	1.63 (s, 3H)		C13, 14, 26
28		26.2 (q)	1.73 (s, 3H)		C20, 21, 29
29		18.2 (q)	1.67 (s, 3H)		C20, 21, 28

[a]Recorded at 150 MHz. [b]Recorded at 600 MHz.

Table S2. NMR data for for halichonine B (**2**) in CD_3OD.

position		Halichonine B (**2**)			
		δ_C (mult.)[a]	δ_H (mult., *J* in Hz)[b]	COSY[b]	HMBC ($^1H \rightarrow ^{13}C$)[c]
1	a	39.2 (t)	1.01 (m)	H1b, 2b	C2
	b		2.03 (m)	H1a	
2	a	18.5 (t)	1.39 (m)	H3a	C1, 10
	b		1.55 (m)	H1b, 3a, 3b	
3	a	42.2 (t)	1.16 (m)	H2a, 2b	C4, 22
	b		1.38 (m)	H2b	C5, 22, 23
4		32.5 (s)			
5		50.4 (d)	1.18 (m)	H6	C4, 6, 9, 10, 24
6		23.5 (t)	1.95 (m, 2H)	H5, 7	
7		121.7 (d)	5.34 (s)	H6	C25
8		135.4 (s)			
9		51.7 (d)	1.84 (m)	H11	
10		36.1 (s)			
11		53.6 (t)	2.31 (m, 2H)	H9	C8, 12, 15
12	a	51.0 (t)	2.86 (dd, 7.8, 13.9)	H12b, 13	C11, 13, 14, 15
	b		3.11 (dd, 5.7, 13.9)	H12a, 13	C11, 13, 14, 15
13		121.5 (d)	5.22 (m)	H12a, 12b	C26, 27
14		134.0 (s)			
15	a	53.0 (t)	2.22 (m)	H15b, 16	C11, 12, 17
	b		2.52 (m)	H15a, 16	C11, 12, 16
16		24.3 (t)	1.43 (m, 2H)	H15a, 15b, 17	C17
17		26.2 (t)	1.45 (m, 2H)	H16, 18	C15
18		48.0 (t)	2.55 (m, 2H)	H17	C17, 19
19		45.8 (t)	3.18 (d, 6.5, 2H)	H20	C18, 20, 21
20		119.0 (d)	5.22 (m)	H19	C28, 29
21		137.4 (s)			
22		21.1 (q)	0.88 (s, 3H)		C3, 4, 5, 23
23		32.6 (q)	0.85 (s, 3H)		C3, 4, 5, 22
24		12.8 (q)	0.74 (s, 3H)		C1, 5, 9, 10
25		21.6 (q)	1.74 (s, 3H)		C9, 7, 8
26		24.7 (q)	1.70 (s, 3H)		C13, 14, 27
27		16.7 (q)	1.63 (s, 3H)		C13, 14, 26
28		24.6 (q)	1.74 (s, 3H)		C20, 21, 29
29		16.7 (q)	1.68 (s, 3H)		C20, 21, 28

[a]Recorded at 100 MHz. [b]Recorded at 600 MHz. [c]Recorded at 400 MHz.

Table S3. NMR data for for halichonine C (**3**) in $CDCl_3$.

position		Halichonine C (**3**)		
		δ_C (mult.)[a]	δ_H (mult., *J* in Hz)[b]	COSY[b]
1	a	37.8 (t)	1.50 (m)	H1b, 2b
	b		2.43 (m)	H1a, 2b
2	a	34.6 (t)	2.24 (m)	H2b
	b		2.76 (ddd, 5.2, 5.2, 14.8)	H1a, 1b, 2a
3		216.7 (s)		
4		44.8 (s)		
5		51.6 (d)	1.63 (m)	H6a, 6b
6	a	23.9 (t)	1.93 (m)	H5, 6b, 7
	b		2.07 (brt, 9.2)	H5, 6a
7		121.3 (d)	5.44 (m)	H6a
8		135.5 (s)		
9		50.6 (d)	1.92 (m)	H11
10		36.0 (s)		
11		53.6 (t)	2.36 (d, 5.2, 2H)	H9
12	a	50.9 (t)	2.89 (dd, 7.2, 14.4)	H12b, 13
	b		3.05 (dd, 5.6, 14.4)	H12a, 13
13		122.0 (d)	5.19 (brt, 7.6)	H12a, 12b
14		134.6 (s)		
15	a	53.3 (t)	2.26 (m)	H15b, 16
	b		2.45 (m)	H15a, 16
16		24.5 (t)	1.48 (m, 2H)	H15a, 15b, 17
17		24.9 (t)	1.48 (m, 2H)	H16
18		47.5 (t)	2.79 (brt, 7.6, 2H)	
19		45.5 (t)	3.47 (d, 7.2, 2H)	H20
20		115.9 (d)	5.36 (brt, 7.2)	H19
21		140.6 (s)		
22		22.3 (q)	1.09 (s, 3H)	
23		25.2 (q)	1.07 (s, 3H)	
24		13.4 (q)	0.98 (s, 3H)	
25		22.7 (q)	1.74 (s, 3H)	
26		25.9 (q)	1.71 (s, 3H)	
27		18.0 (q)	1.62 (s, 3H)	
28		25.8 (q)	1.77 (s, 3H)	
29		18.2 (q)	1.67 (s, 3H)	

[a]Recorded at 100 MHz. [b]Recorded at 400 MHz.

Table S4. Growth-inhibitory activities of **1**-**3** toward mammalian cancer cells

Compounds	IC_{50} (μM)	
	L1210 Mouse leukemia	PC13 Human lung cancer
1	7.2	11.5
2	10.5	12.6
3	5.2	7.7
2,5,6-Tribromogramine	-	9.3

Table S5. Crystal data and structure refinement for HCl complex of halichonine B (**2**)

Identification code	halichob	
Empirical formula	$C_{58}\ H_{108}\ Cl_4\ N_4\ O_5$	
Formula weight	1083.28	
Temperature	173(2) K	
Wavelength	0.71069 Å	
Crystal system	Monoclinic	
Space group	$P2_1$	
Unit cell dimensions	a = 18.130(8) Å	α = 90°
	b = 6.686(5) Å	β = 98.098(17)°
	c = 27.098(17) Å	γ = 90°
Volume	3252(3) $Å^3$	
Z	2	
Density (calculated)	1.106 Mg/m^3	
Absorption coefficient	0.227 mm^{-1}	
F(000)	1184	
Crystal size	0.10 x 0.05 x 0.01 mm^3	
Theta range for data collection	1.13 to 25.00°	
Index ranges	-20<=h<=19, -6<=k<=7, -32<=l<=31	
Reflections collected	12434	
Independent reflections	7432 [R(int) = 0.0932]	
Completeness to theta = 25.00°	80.0%	
Max. and min. transmission	0.9977 and 0.9777	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	7432 / 60 / 663	
Goodness-of-fit on F^2	1.128	
Final R indices [I>2sigma(I)]	R1 = 0.1352, wR2 = 0.3374	
R indices (all data)	R1 = 0.2588, wR2 = 0.4198	
Absolute structure parameter	0.1(2)	
Largest diff. peak and hole	0.398 and -0.277 $e.Å^{-3}$	