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Synthesis and structure–activity relationships for cytotoxicity and apoptosis-inducing activity of (+)-halichonine B

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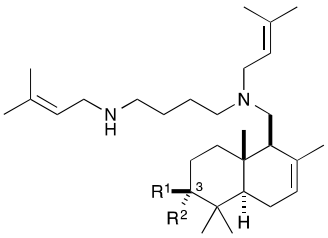
Halichonine B is a sesquiterpene alkaloid isolated from the marine sponge *Halichondria okadai* Kadota. Halichonine B has exhibited cytotoxicity against mammalian cancer cells and induced apoptosis in the human leukemia cell line HL60. Here we established a practical route for the synthesis of halichonine B and its analogues, and we evaluated their biological activities. It was revealed that the secondary amino groups in the side chain portion are important for the strong cytotoxicity of halichonine B and that the N^{11}-prenyl group is unimportant. Halichonine B and its analogues were also observed to induce apoptosis in HL60 cells.

Introduction

Halichonines A (**1**) – C (**3**), sesquiterpene alkaloids, were isolated from the marine sponge *Halichondria okadai* Kadota by Uemura and co-workers, and they exhibited cytotoxicity against mammalian cancer cells (Fig. 1).[1] Among them, halichonine B (**2**) showed the strongest cytotoxicity against the human leukemia cell line HL60 (IC_{50} = 0.60 μg/mL). Halichonine B (**2**) exhibited apoptosis-inducing activity against HL60 cells in a DNA ladder analysis.[1] Because halichonine B (**2**) showed unique and potent biological activities, we planned its asymmetric synthesis for the determination of its absolute stereochemistry and for a structure–activity relationship study of halichonine B (**2**). Uemura and co-workers reported the synthesis of (±)-halichonine A (**1**).[1]



halichonine A (**1**) R_1 = OH, R_2 = H
halichonine B (**2**) R_1 = H, R_2 = H
halichonine C (**3**) R_1 = R_2 = O

Fig. 1 Structure of halichonines A (**1**) – C (**3**).

Results and discussion

Retrosynthetic pathway of (+)-halichonine B

The retrosynthetic pathway of halichonine B (**2**) is shown in Scheme 1. Our strategy involved a key segment coupling between 2-nitorobenzenesulfonamide **5** (the decaline portion) and alcohol **4** (the side chain portion) using Fukuyama's Nosyl chemistry.[2] Ns-amide **5** would be synthesized from a known alcohol **6**.[3,4] Optically active alcohol **6** can be prepared from commercially available (3a*R*)-(+)-sclareolide (**8**).[5]

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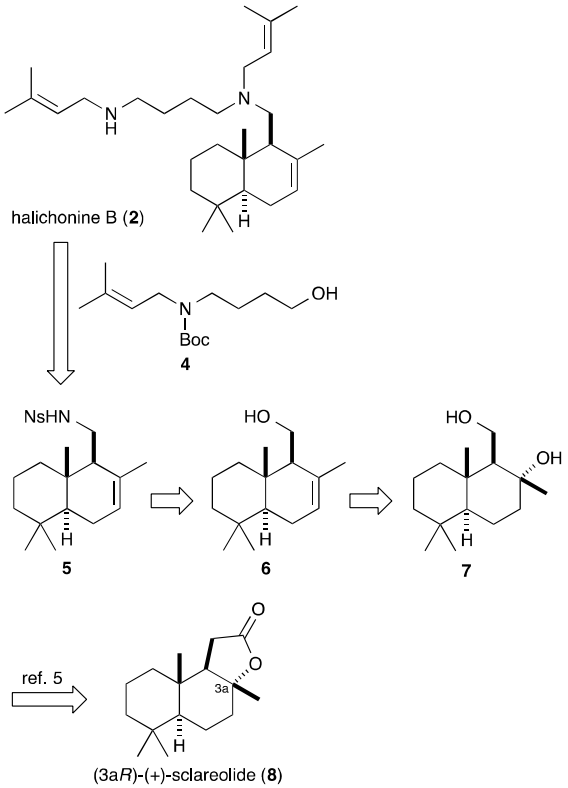
† Electronic Supplementary Information (ESI) available: Panel of 39 human cancer cell lines for halichonine B, experimental protocols and characterization data of artificial analogues, and NMR spectra of all new compounds. See DOI: 10.1039/x0xx00000x

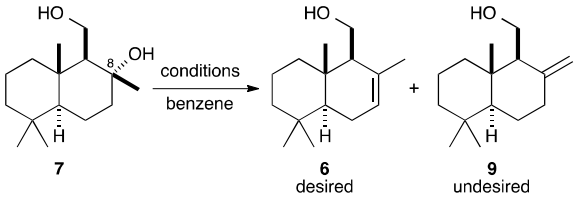


Scheme 1 Retrosynthetic pathway of halichonine B (**2**)

Synthesis of (+)-halichonine B

To convert (3a*R*)-(+)-sclareolide (**8**) into the known diol **7**, we followed the reported procedure (69% yield in four steps).[5] We attempted to prepare alcohol **6** from diol **7** by the regioselective dehydration of the C8 tertiary hydroxy group (Table 1). Alvarez-Manzaneda et al. reported the same reaction by using the Mitsunobu conditions (92% yield).[3,6] We followed their reported procedure (entry 1). However, the reaction was not completed under their conditions and gave the desired alcohol **6** along with the undesired exomethylene **9**. Because of this irreproducible result, we attempted the reaction at a higher temperature (entry 2). As a result, the reaction was completed to give the desired alcohol **6** (44%) accompanied by the undesired exomethylene **9** (52%). In entries 3 and 4, the Mitsunobu reactions with other azodicarboxylate reagents, DIAD (diisopropyl azodicarboxylate) and DMAD (dimethyl azodicarboxylate), were used. However, the yields of desired alcohol **6** were low. The result of entry 2 suggested that the reaction at a high temperature gave priority to exomethylene **9**. Thus, we next examined the Tsunoda–Itô modified Mitsunobu reaction conditions (entry 5),[7] where the reaction is known to proceed at lower temperature than that in standard Mitsunobu conditions. However, these modified reaction conditions were not effective in this case. From these results, we could not improve the yield or the regioselectivity of dehydration of diol **7**.

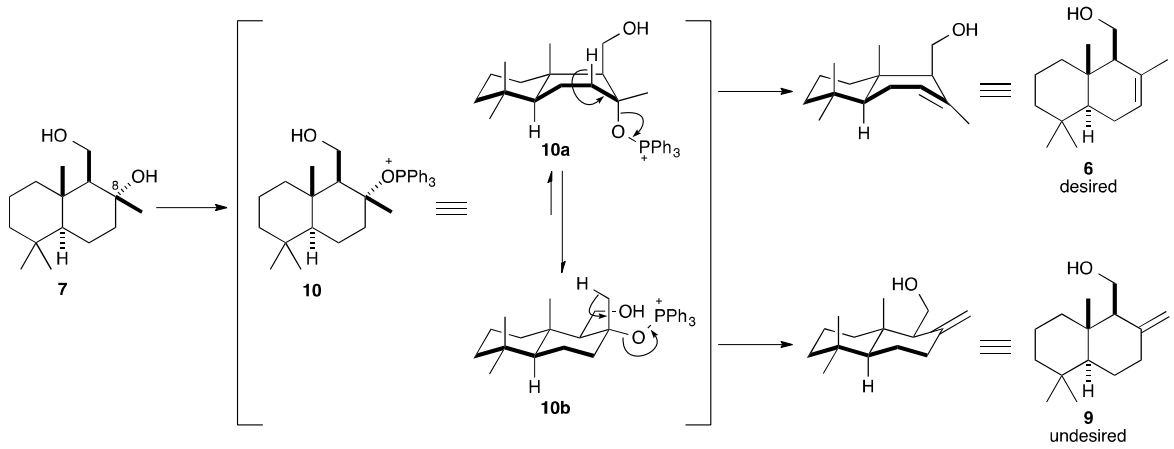
Table 1 Study of regioselective dehydration of diol **7**



	conditions			
entry	reagents (equiv.)	temp.	**6** (%)	**9** (%)
1	DEAD (2.0), PPh_3 (2.0)	rt	23	19
2	DEAD (2.0), PPh_3 (2.0)	60 °C	44	52
3	DIAD (7.0), PPh_3 (7.0)	rt	7	24
4	DMAD (2.1), PPh_3 (2.2)	rt to 60 °C	trace	trace
5	TMAD* (3.0), P(^{n}Bu$_3$) (3.0)	rt	14	9

* TMAD: *N,N,N',N'*-tetramethylazodicarboxamide

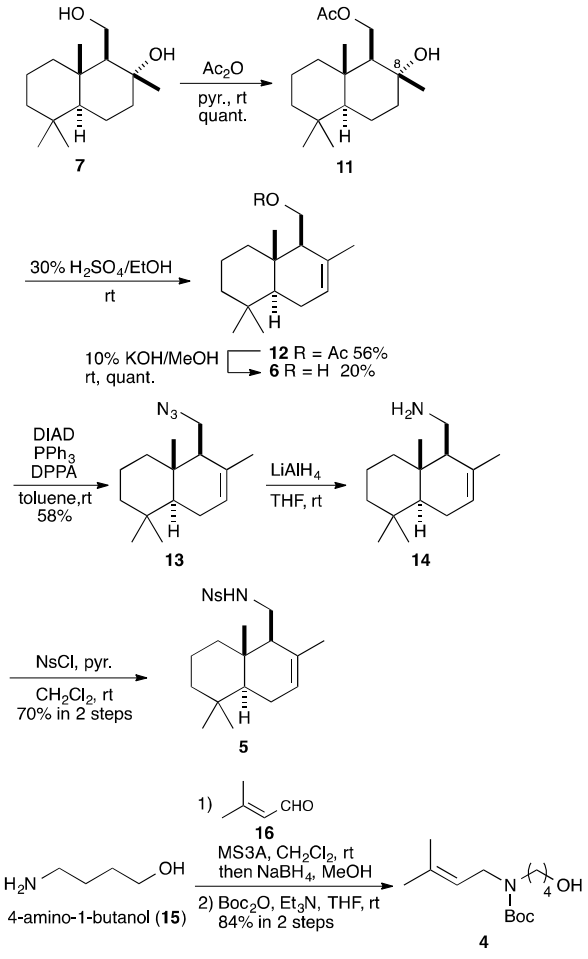
The undesired regioselectivity of the dehydration at C8 under the Mitsunobu conditions can be explained as follows (Scheme 2). Diol **7** was transformed into alkoxy phosphonium salt **10**. In the E2 elimination of **10**, the more stable chair-chair form **10b** led to the undesired alcohol **9**. Therefore, we concluded that the E2-type dehydration of **7** is unsuitable to obtain the desired alcohol **6**.



Scheme 2 Reaction pathway of dehydration of **7** under the Mitsunobu conditions

Therefore, we next attempted the synthesis of alcohol **6** from diol **7** by using E1 elimination (Scheme 3). The acetylation of the primary hydroxy group in diol **7** afforded acetate **11**. The treatment of acetate **11** with 30% H_2SO_4/EtOH afforded acetate **12** (56% yield) along with the desired alcohol **6** (20% yield).[4] The hydrolysis of acetate **12** gave alcohol **6**,[8] which was transformed into azide **13** under the Mitsunobu conditions.[9] The reduction of azide **13** with $LiAlH_4$ afforded amine **14**, which was converted into Ns-amide **5**.[2]

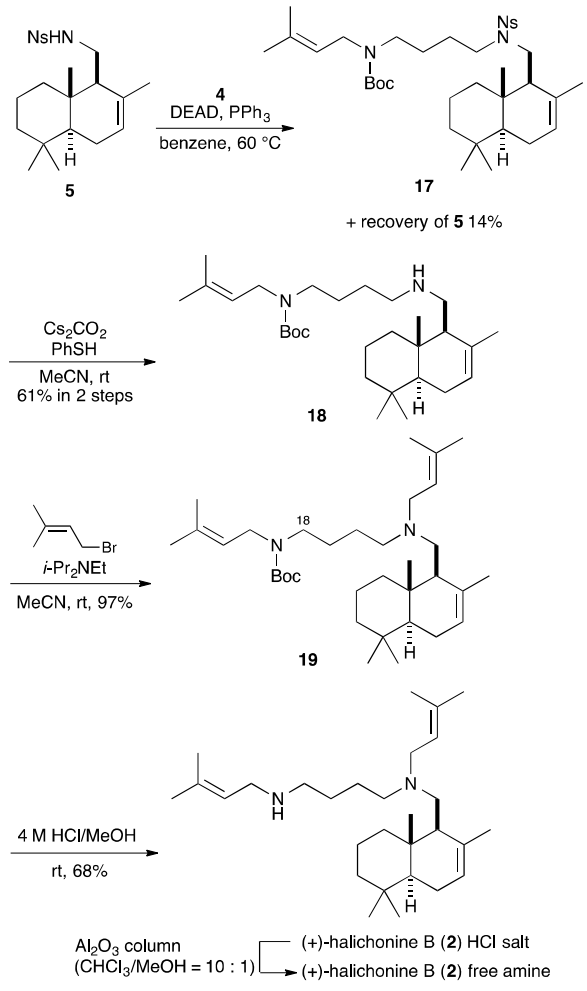
On the other hand, alcohol **4** was synthesized from 4-amino-1-butanol (**15**) by reductive amination with 3-methyl-2-butenal (**16**) and protection of the resulting amino group with the Boc group.



Scheme 3 Synthesis of Ns-amide **5** and alcohol **4**

With Ns-amide **5** and alcohol **4** in hand, we next examined the segment coupling reaction. The coupling between Ns-amide **5** and alcohol **4** under the Mitsunobu conditions afforded the

desired coupling compound **17** (Scheme 4). The removal of the nosyl group in **17** and the alkylation of the resultant amino group with prenyl bromide gave N^{18}-Boc halichonine B (**19**). Finally, the removal of the Boc group of **19** with 4 M HCl/MeOH yielded halichonine B (**2**) as a HCl salt, the NMR data of which were in good agreement with those of the natural product.[1,10] The purification of (+)-halichonine B (**2**) HCl salt by using Al_2O_3 column chromatography with $CHCl_3$/MeOH (10:1) as an eluent gave (+)-halichonine B (**2**) free amine. The sign of optical rotation of synthetic (+)-halichonine B (**2**) is consistent with the natural sign.[11] Therefore, this synthesis established the absolute configuration of (+)-halichonine B (**2**) as depicted in structural formula **2**.



Scheme 4 Synthesis of (+)-halichonine B (**2**)

Structure–cytotoxicity relationships of (+)-halichonine B

The cytotoxicities of synthetic halichonine B (**2**) HCl salt and free amine were evaluated against a panel of 39 human cancer cell lines (termed JFCR39) at the Japanese Foundation for Cancer Research (see Table S1 in the ESI†). Both halichonine B (**2**) HCl salt and free amine showed broad cytotoxicity in the panel. On the basis of the COMPARE analysis,[12] the patterns of the differential cytotoxicities of halichonine B (**2**) HCl salt and free amine suggested that they inhibited cancer cell proliferation through an unknown mechanism (see Table S2 in the ESI†).

To investigate the structure–cytotoxicity relationships of halichonine B (**2**), we evaluated the cell growth inhibitory effects of halichonine B, its synthetic intermediates, and the artificial analogues of halichonine B against HL60 cells (Table 2). The cytotoxic activity of synthetic halichonine B (**2**) HCl salt against HL60 cells had the same IC_{50} value as that of natural halichonine B (**2**) HCl salt. In addition, synthetic halichonine B (**2**) HCl salt and synthetic halichonine B (**2**) free amine showed cytotoxicity with IC_{50} values of 0.25 and 0.26 μM, respectively. From these results, the chloride ion of halichonine B (**2**) HCl salt was shown to be unimportant for the strong cytotoxicity of halichonine B (**2**). Azide **13** and amine **14**, which lack the whole side chain portion of halichonine B (**2**), showed very weak cytotoxicity, whereas Ns-amide **5** exhibited cytotoxicity with the IC_{50} value of 1.70 μM. Although synthetic intermediates **17** and **19**, which have the Boc-protected side chain portion, exhibited almost no cytotoxicity, intermediate **18** and diamine analogue **20**,[13] which lack the prenyl group at N^{11}, showed considerable cytotoxicity. In particular, the cytotoxicity of diamine **20** was at the same level as that of halichonine B (**2**).

To investigate the structure–cytotoxicity relationships of halichonine B (**2**) and diamine **20**, we prepared analogues with different lengths of side chain portion, including analogues **21**–**24** possessing the prenyl group at N^{11} (natural product-type) and analogues **25**–**28** lacking the prenyl group at N^{11} (diamine-type).[14] The cytotoxic activity of natural product-type analogue **21** (n = 2) was weaker than that of halichonine B (**2**) (n = 4). In contrast, natural product-type analogues **22**–**24** (n = 3–8) indicated cytotoxicity that was similar to that of halichonine B (**2**) (see Figure S1 in the ESI†). The cytotoxicities of diamine-type analogues **25**–**28** showed a pattern that was similar to those of the natural-type analogues **21**–**24**.

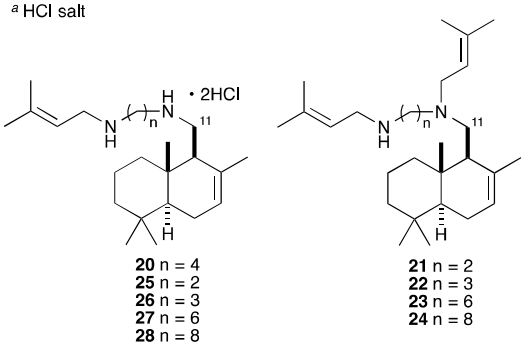
These results indicated that natural product-type and diamine-type analogues containing n = 3 or more carbon chains at the side chain portion have potent cytotoxicity against HL60 cells. In our comparison with the natural product-type and diamine-type analogues with the same length of side chain portion, the diamine-type analogues showed slightly stronger cytotoxicity. These results also indicated that the presence of the secondary amino group at N^{18} in the side chain portion is responsible for the strong cytotoxicity. In addition, the secondary amino group at N^{11} was substitutable for N^{18} secondary amino group (Fig. 2).

We also evaluated the cytotoxicity of halichonine B (**2**) and artificial analogues (**20**, **23**, **26**, **27**, and **28**), which exhibited strong activity against HL60 cells, using HeLa cells. Each compound showed cytotoxicity against HeLa cells, with the same tendency as the cytotoxicity against HL60 cells.

Table 2 Cytotoxicity of (+)-halichonine B (**2**), its synthetic intermediates, and the artificial analogues against HL60 cells and HeLa cells

compound	length of the diamine chain	HL60 cells IC_{50} (μM)	HeLa cells IC_{50} (μM)
(+)-halichonine B (**2**) (natural)[a]	n = 4	0.30	
(+)-halichonine B (**2**) (synthetic)[a]	n = 4	0.25	1.50
(+)-halichonine B (**2**) (synthetic)	n = 4	0.26	1.52
5		1.70	
13		16.6	
14		13.0	
17	n = 4	23.7	
18	n = 4	1.40	
19	n = 4	20.1	
20[a]	n = 4	0.21	1.19
21	n = 2	1.40	
22	n = 3	0.67	
23	n = 6	0.26	1.78
24	n = 8	0.45	
25[a]	n = 2	1.90	
26[a]	n = 3	0.33	2.00
27[a]	n = 6	0.29	1.79
28[a]	n = 8	0.29	1.40

[a] HCl salt



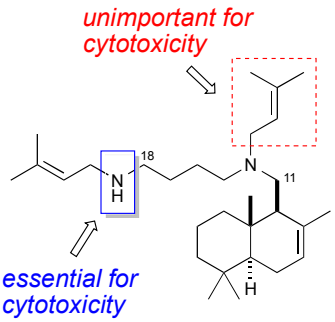


Fig. 2 Summary of the structure–cytotoxicity relationships of (+)-halichonine B (**2**)

DNA ladder analysis of halichonine B and artificial analogues

To confirm whether halichonine B (**2**) and artificial analogues (**20**, **23**, **26**, **27**, and **28**) can induce apoptosis, we incubated HL60 cells with them for 24 h, and DNA fragmentation was detected using a DNA ladder analysis. As shown in Fig. 3, these compounds significantly induced apoptotic DNA fragmentation in the HL60 cells, indicating that the compounds induced apoptosis in HL60 cells. Because their mechanisms of action are still unknown, further studies are warranted.

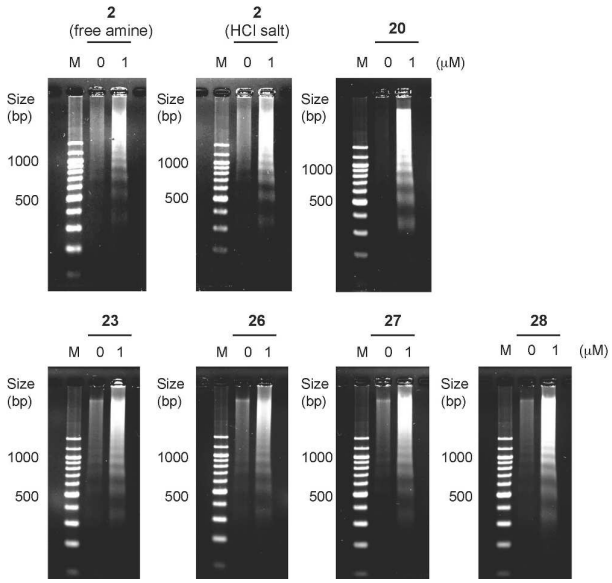


Fig. 3 Induction of DNA fragmentation in HL60 cells by (+)-halichonine B (**2**) and its artificial analogues. HL60 cells were incubated with the compounds at 0 and 1 μM concentrations for 24 h. Cellular DNA was then extracted and electrophoresed on an agarose gel. M: 100 bp DNA ladder marker.

Conclusions

We have established the practical synthesis of halichonine B (**2**) and its analogues. From this synthetic work, we determined the absolute configuration of halichonine B (**2**). We also evaluated the cytotoxicity of halichonine B (**2**) and its analogues, and the results indicate that the secondary amino groups in the side chain portion are important for the strong cytotoxicity of halichonine B (**2**) and that the prenyl group at the side chain portion is unimportant. In addition, halichonine B (**2**) and its analogues were found to induce apoptosis in HL60 cells.

Experimental

Chemistry

General: All moisture-sensitive reactions were performed under an atmosphere of argon or nitrogen, and the starting materials were azeotropically dried with benzene before use. Anhydrous MeOH, EtOH, CH_2Cl_2, THF, toluene, benzene, MeCN, and pyridine were purchased from Kanto Chemical Co., Inc., or Wako Pure Chemical Industries Ltd., and used without further drying. TLC analysis were conducted on E. Merck precoated silica gel 60 F_{254} (0.25 mm layer thickness). Fuji Silysia silica gels BW-820MH (75–200 μm), FL-60D (45–75 μm), and E. Merck aluminium oxide 90 (activity II–III) were used for column chromatography. Optical rotations were measured

with a JASCO DIP-370 polarimeter. Infrared (IR) spectra were recorded on a JASCO FT/IR-300 instrument and only selected peaks are reported in wavenumbers (cm^{-1}). ^1H and ^{13}C NMR spectra were recorded on a Bruker AVANCE 400, a Bruker DPX 400 spectrometer, or a Bruker AVANCE 600 spectrometer. The ^1H and ^{13}C chemical shifts were referenced to the solvent peaks, δ_H 7.26 (residual $C\underline{H}Cl_3$) and δ_C 77.0 ppm ($\underline{C}DCl_3$), or δ_H 3.30 (residual $C\underline{H}D_2OD$) and δ_C 49.0 ppm ($\underline{C}D_3OD$), respectively. *J* values are given in Hz. The following abbreviations are used for spin multiplicity: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, and br = broad. High resolution ESI/TOF mass spectra were recorded on a JEOL AccuTOFCS JMS-T100CS spectrometer.

((1*S*,2*R*,4a*S*,8a*S*)-2-hydroxy-2,5,5,8a-tetramethyldecahydronaphthalen-1-yl)methyl acetate (11)

To a stirred solution of diol **7** (552 mg, 2.30 mmol) in pyridine (2.3 mL) was added Ac_2O (0.65 mL, 6.9 mmol) at 0 °C. After being stirred at room temperature for 3 h, the reaction mixture was diluted with saturated aqueous $NaHCO_3$ (4.0 mL) and extracted with EtOAc (10 mL × 3). The combined extracts were washed with brine (10 mL), dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by column chromatography on silica gel (15 g, *n*-hexane–Et_2O 2 : 1) to give acetate **11** (651 mg, quant.) as a yellow oil: R_f = 0.45 (*n*-hexane–Et_2O 2 : 1); $[\alpha]_D^{24}$ –7.7 (*c* 0.77, $CHCl_3$); IR ($CHCl_3$) 3591, 3480, 3000, 2930, 2870, 1727, 1467, 1389, 1370, 1246, 1030, 938, 839 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 4.35 (dd, *J* = 11.8, 4.4 Hz, 1H), 4.24 (dd, *J* = 11.8, 5.3 Hz, 1H), 2.38 (br s, 1H), 2.03 (s, 3H), 1.89 (ddd, *J* = 12.7, 3.2, 3.2 Hz, 1H), 1.70–0.89 (m, 11H), 1.18 (s, 3H), 0.88 (s, 3H), 0.86 (s, 3H), 0.82 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.3, 72.6, 62.6, 59.9, 55.7, 43.9, 41.7, 39.7, 38.1, 33.4, 33.2, 24.6, 21.5, 21.3, 20.3, 18.4, 15.8; HRMS (ESI) *m/z* 305.2089, calcd for $C_{17}H_{30}NaO_3$ $[M+Na]^+$ 305.2093.

((1*S*,4a*S*,8a*S*)-2,5,5,8a-tetramethyl-1,4,4a,5,6,7,8,8a-octahydronaphthalen-1-yl)methyl acetate (12)

The acetate **11** (324 mg, 1.15 mmol) was treated with 30% H_2SO_4/EtOH (3.2 mL) at 0 °C. After being stirred at room temperature for 15 min., the reaction mixture was diluted with saturated aqueous $NaHCO_3$ (30 mL) and extracted with Et_2O (20 mL × 3). The combined extracts were washed with brine (10 mL), dried over $MgSO_4$, filtered, and concentrated. The crude product was purified by column chromatography on silica gel (5.5 g, *n*-hexane–Et_2O 12 : 1 → 9 : 1) to give acetate **12** (169 mg, 56%) and alcohol **6** (50.7 mg, 20%) as colorless oils, respectively.

acetate **12**: R_f = 0.22 (*n*-hexane–Et_2O 2 : 1); $[\alpha]_D^{24}$ +12.7 (*c* 0.52, $CHCl_3$); IR ($CHCl_3$) 3026, 2960, 2927, 2850, 1730, 1457, 1442, 1388, 1366, 1260, 1034, 969, 861 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 5.49 (m, 1H), 4.24 (dd, *J* = 11.6, 3.3 Hz, 1H), 4.08 (dd, *J* = 11.6, 5.4 Hz, 1H), 2.06–1.80 (m, 4H), 2.03 (s, 3H), 1.67 (br s, 3H), 1.59–1.04 (m, 6H), 0.89 (s, 3H), 0.86 (s, 3H), 0.81 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.9, 132.3, 123.4, 63.2, 53.4, 49.9, 42.1, 39.5, 35.8, 33.3, 33.0, 23.6, 21.9, 21.7, 21.2, 18.7, 14.5; HRMS (ESI) *m/z* 287.1958, calcd for $C_{17}H_{28}NaO_2$ $[M+Na]^+$ 287.1987.

((1*S*,4a*S*,8a*S*)-2,5,5,8a-tetramethyl-1,4,4a,5,6,7,8,8a-octahydronaphthalen-1-yl)methanol (6)

The acetate **12** (169 mg, 0.641 mmol) was treated with 10% KOH/MeOH (3.1 mL) at 0 °C. After being stirred at room temperature for 25 min., the reaction mixture was diluted with H_2O (5 mL) and extracted with Et_2O (10 mL × 3). The combined extracts were washed with brine (10 mL), dried over $MgSO_4$, filtered, and concentrated. The crude product was purified by column chromatography on silica gel (3.0 g, *n*-hexane–Et_2O 9 : 1) to give alcohol **6** (148 mg, quant) as a colorless oil: R_f = 0.45 (*n*-hexane–Et_2O 2 : 1); $[\alpha]_D^{24}$ –14.6 (*c* 0.63, $CHCl_3$); IR ($CHCl_3$) 3625, 3461, 2926, 2849, 1458, 1443, 1389, 1366, 1032, 962, 841 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 5.54 (m, 1H), 3.86 (dd, *J* = 11.3, 3.2 Hz, 1H), 3.74 (dd, *J* = 11.3, 4.8 Hz, 1H), 2.05–1.80 (m, 4H), 1.78 (s, 3H), 1.63–0.99 (m, 7H), 0.89 (s, 3H), 0.86 (s, 3H), 0.86 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 132.8, 124.1, 60.9, 57.3, 49.9, 42.1, 39.9, 36.0, 33.3, 32.9, 23.6, 22.0, 21.9, 18.7, 14.9; HRMS (ESI) *m/z* 245.1906, calcd for $C_{15}H_{26}NaO$ $[M+Na]^+$ 245.1881.

(4a*S*,5*S*,8a*S*)-5-(azidomethyl)-1,1,4a,6-tetramethyl-1,2,3,4,4a,5,8,8a-octahydronaphthalene (13)

To a stirred solution of alcohol **6** (500 mg, 2.25 mmol), PPh_3 (2.36 g, 9.00 mmol) in toluene (20 mL) were added DIAD (1.74 mL, 8.84 mmol) and DPPA (2.18 mL, 9.72 mmol) at 0 °C, and the mixture was stirred at room temperature for 3 h. Removal of the solvent afforded crude product, which was purified by column chromatography on silica gel (40 g, pentane → *n*-hexane–Et_2O 9 : 1) to give azide **13** (320 mg, 58%) as a yellow oil: R_f = 0.46 (*n*-hexane); $[\alpha]_D^{24}$ –14.2 (*c* 0.20, $CHCl_3$); IR ($CHCl_3$) 2925, 2849, 2097, 1670, 1457, 1388, 1366, 1271, 895, 876 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 5.50 (m, 1H), 3.59 (dd, *J* = 12.8, 3.1 Hz, 1H), 3.16 (dd, *J* = 12.8, 7.0 Hz, 1H), 2.05–1.79 (m, 4H), 1.72 (s, 3H), 1.60–1.01 (m, 6H), 0.89 (s, 3H), 0.87 (s, 3H), 0.80 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 132.3, 124.0, 53.8, 50.1, 49.8, 42.0, 39.5, 35.9, 33.2, 32.9, 23.6, 21.9, 21.7, 18.7, 14.2; HRMS (ESI) *m/z* 220.2056, calcd for $C_{15}H_{26}N$ $[M-N_2+H]^+$ 220.2060.

2-nitro-*N*-(((1*S*,4a*S*,8a*S*)-2,5,5,8a-tetramethyl-1,4,4a,5,6,7,8,8a-octahydronaphthalen-1-yl)methyl)benzenesulfonamide (5)

To a stirred solution of azide **13** (100 mg, 405 μmol) in THF (2.7 mL) was added $LiAlH_4$ (61.5 mg, 1.62 mmol) at 0 °C, and the mixture was stirred at room temperature for 10 min. The reaction mixture was added with H_2O (0.1 mL) and 15% aqueous NaOH (0.1 mL) and stirred at room temperature for 10 min. The mixture was filtered through a pad of Celite, and the residue was washed with EtOAc (15 mL). Removal of the solvent afforded crude amine **14** (86.6 mg), which was used for the next reaction without further purification.

To a stirred solution of crude amine **14** (86.6 mg) in CH_2Cl_2 (2.5 mL) were added pyridine (38.0 μL, 472 μmol) and NsCl (76.6 mg, 346 μmol) at 0 °C. The mixture was stirred at room temperature for 4.5 h. After cooled to 0 °C, 1.0 M aqueous HCl (0.1 mL) and H_2O (10 mL) were added. The resultant mixture

was extracted with EtOAc (10 mL × 3). The combined extracts were washed with brine (10 mL), dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by column chromatography on silica gel (3.0 g, *n*-hexane–Et_2O 5 : 1 / 1% Et_3N) to give Ns-amide **5** (116 mg, 70% in 2 steps) as a colorless oil: R_f = 0.37 (*n*-hexane–Et_2O 2 : 1); $[\alpha]_D^{25}$ +24.5 (*c* 0.25, $CHCl_3$); IR ($CHCl_3$) 3382, 3032, 2926, 2850, 1716, 1542, 1457, 1441, 1416, 1363, 1170, 1061, 855 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 8.14 (m, 1H), 7.86 (m, 1H), 7.78–7.71 (m, 2H), 5.51 (m, 1H), 5.26 (br m, 1H), 3.29 (ddd, *J* = 12.7, 6.4, 3.0 Hz, 1H), 3.03 (ddd, *J* = 12.7, 6.2, 6.2 Hz, 1H), 2.00–1.76 (m, 4H), 1.78 (s, 3H), 1.55–1.37 (m, 3H), 1.16–1.08 (m, 2H), 0.96 (ddd, *J* = 13.0, 13.0, 3.7 Hz, 1H), 0.86 (s, 3H), 0.84 (s, 3H), 0.78 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 148.1, 133.5, 133.4, 132.7, 131.5, 131.2, 125.4, 125.1, 54.6, 49.7, 41.9 (2C), 39.5, 36.3, 33.2, 32.9, 23.5, 21.9, 21.7, 18.6, 14.4; HRMS (ESI) *m/z* 429.1810, calcd for $C_{21}H_{30}N_2NaO_4S$ $[M+Na]^+$ 429.1824.

***tert*-butyl (4-hydroxybutyl)(3-methylbut-2-en-1-yl)carbamate (4)**

To a stirred solution of 4-amino-1-butanol (**15**) (110 μL, 1.19 mmol) in CH_2Cl_2 (7.1 mL) containing molecular sieves 3Å (239 mg) was added 3-methyl-2-butenal (**16**, 130 μL, 1.36 mmol) at room temperature, and the mixture was stirred at room temperature for 20 h. The mixture was filtered through a pad of Celite, and the residue was washed with CH_2Cl_2 (15 mL). Concentration of the filtrate and washings afforded the crude imine intermediate.

To a stirred solution of the crude imine intermediate in MeOH (1.2 mL) was added $NaBH_4$ (90.0 mg, 2.38 mmol) at 0 °C. After being stirred at room temperature for 30 min., the reaction mixture was diluted with H_2O (5 mL) and extracted with CH_2Cl_2 (5 mL × 5). The combined extracts were washed with brine (5 mL), dried over Na_2SO_4, and filtered. Removal of the solvent afforded the crude amine 168 mg), which was used for the next reaction without further purification.

To a stirred solution of the crude amine (168 mg) in THF (4.8 mL) were added Boc_2O (0.30 mL, 1.3 mmol) and Et_3N (0.30 mL, 2.2 mmol) at 0 °C. After being stirred at room temperature for 1.5 h, the reaction mixture was diluted with saturated aqueous NH_4Cl (10 mL) and extracted with EtOAc (10 mL × 3). The combined extracts were washed with brine (10 mL), dried over $MgSO_4$, filtered, and concentrated. The crude product was purified by column chromatography on silica gel (7.0 g, *n*-hexane–EtOAc 4 : 1 → 1 : 1) to give alcohol **4** (264 mg, 86% in 2 steps) as a colorless oil: R_f = 0.54 (*n*-hexane–EtOAc 1 : 1); IR ($CHCl_3$) 3629, 3420, 3009, 2979, 2935, 1668, 1455, 1419, 1252, 1167, 1028, 880 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 5.13 (m, 1H), 3.88–3.71 (br m, 2H), 3.64 (t, *J* = 6.0 Hz, 2H), 3.27–3.08 (br m, 2H), 1.90 (br s, 1H), 1.70 (s, 3H), 1.64 (s, 3H), 1.62–1.50 (m, 4H), 1.43 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.7, 134.6, 121.0, 79.3, 62.5, 45.8, 44.5, 29.7, 28.4 (3C), 25.7, 24.6, 17.7; HRMS (ESI) *m/z* 280.1901, calcd for $C_{14}H_{27}NNaO_3$ $[M+Na]^+$ 280.1889.

***tert*-butyl (3-methylbut-2-en-1-yl)(4-((((1*S*,4a*S*,8a*S*)-2,5,5,8a-tetramethyl-1,4,4a,5,6,7,8,8a-octahydronaphthalen-1-yl)methyl)amino)butyl)carbamate (18)**

To a stirred solution of Ns-amide **5** (100 mg, 0.247 mmol), alcohol **4** (190 mg, 0.740 mmol), and PPh_3 (194 mg, 0.740 mmol) in benzene (2.5 mL) was added DIAD (0.14 mL, 0.711 mmol) at room temperature. The mixture was stirred at reflux for 7 h. Removal of the solvent afforded crude product, which was purified by column chromatography on silica gel (40 g, *n*-hexane–Et_2O 5 : 1) to give coupling compound **17** (152 mg, containing a small quantity of impurity) and recovery of Ns-amide **5** (14 mg, 14%). Coupling compound **17** was used for the next reaction without further purification.

To a stirred solution of coupling compound **17** (152 mg, containing a small quantity of impurity) and Cs_2CO_3 (93.0 mg, 0.285 mmol) in MeCN (2.0 mL) was added PhSH (25 μL, 0.24 mmol) at 0 °C. After being stirred at room temperature for 17.5 h, the mixture was filtered through a pad of Celite, and the residue was washed with EtOAc. The combined filtrate and washings was concentrated to give the crude product, which was purified by column chromatography on Al_2O_3 (4 g, *n*-hexane–Et_2O 5 : 1 → 1 : 1) to give amine **18** (69 mg, 61% in 2 steps) as a yellow oil: R_f = 0.43 ($CHCl_3$–MeOH 9 : 1); $[\alpha]_D^{25}$ +2.3 (*c* 0.50, $CHCl_3$); IR ($CHCl_3$) 3359, 3018, 2928, 2851, 1682, 1456, 1418, 1366, 1252, 1167, 1137, 881 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 5.42 (m, 1H), 5.12 (m, 1H), 3.85–3.68 (br m, 2H), 3.20–3.05 (br m, 2H), 2.68 (dd, *J* = 12.2, 1.6 Hz, 1H), 2.61 (m, 1H), 2.53 (m, 1H), 2.45 (dd, *J* = 12.2, 7.3 Hz, 1H), 2.00–1.36 (m, 11H), 1.71 (s, 3H), 1.70 (s, 3H), 1.64 (s, 3H), 1.43 (s, 9H), 1.20–1.14 (m, 2H), 1.06 (ddd, *J* = 13.1, 13.1, 3.7 Hz, 1H), 0.86 (s, 3H), 0.84 (s, 3H), 0.74 (s, 3H). A signal due to one proton (NH) was not observed; ^{13}C NMR (150 MHz, $CDCl_3$) δ 155.6, 134.7, 134.3, 122.9, 121.1, 79.1, 55.3, 50.0 (2C), 48.2, 46.1, 44.6, 42.2, 39.3, 36.2, 33.2, 32.9, 28.5 (3C), 27.4, 26.4, 25.7, 23.7, 21.9 (2C), 18.8, 17.8, 14.0; HRMS (ESI) *m/z* 461.4125, calcd for $C_{29}H_{53}N_2O_2$ $[M+H]^+$ 461.4107.

***tert*-butyl (3-methylbut-2-en-1-yl)(4-((3-methylbut-2-en-1-yl)(((1*S*,4a*S*,8a*S*)-2,5,5,8a-tetramethyl-1,4,4a,5,6,7,8,8a-octahydronaphthalen-1-yl)methyl)amino)butyl)carbamate (19)**

To a stirred solution of amine **18** (33.0 mg, 71.6 μmol) in MeCN (0.30 mL) were added *i*-Pr_2NEt (12.6 μL, 72.3 μmol) and prenyl bromide (20.0 μL, 172 μmol) at 0 °C. After being stirred at room temperature for 15 h, the reaction mixture was diluted with H_2O (5 mL) and extracted with EtOAc (5 mL × 3). The combined extracts were washed with brine (5 mL), dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by column chromatography on Al_2O_3 (1.0 g, *n*-hexane–Et_2O 10 : 1) to give N^{18}-Boc halichonine B (**19**) (37.0 mg, 97%) as a yellow oil: R_f = 0.75 (*n*-hexane–Et_2O 2 : 1); $[\alpha]_D^{25}$ +41.6 (*c* 0.25, $CHCl_3$); IR ($CHCl_3$) 2927, 2859, 1681, 1455, 1418, 1378, 1366, 1268, 1251, 1168, 1144, 880 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 5.38 (m, 1H), 5.22 (m, 1H), 5.14 (m, 1H), 3.86–3.70 (br m, 2H), 3.20–3.05 (br m, 3H), 2.84 (dd, *J* = 14.1, 7.6 Hz, 1H), 2.50 (m, 1H), 2.33–2.24 (m, 2H), 2.20 (m, 1H), 2.04–1.91 (br m, 2H), 1.89–1.31 (m, 9H), 1.75 (s, 3H), 1.71 (s, 6H), 1.65 (s,

3H), 1.61 (s, 3H), 1.44 (s, 9H), 1.23–1.13 (m, 2H), 0.99 (ddd, *J* = 13.1, 13.1, 3.3 Hz, 1H), 0.87 (s, 3H), 0.85 (s, 3H), 0.73 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 155.6, 136.1, 134.7, 133.8, 122.1, 121.9, 121.2, 79.0, 53.7, 53.3, 51.5, 51.2, 50.2, 46.3, 44.6, 42.3, 39.2, 36.1, 33.3, 33.0, 28.5 (3C), 26.8, 25.9, 25.7, 24.3, 23.7, 22.4, 22.0, 18.8, 17.9, 17.8, 13.7; HRMS (ESI) *m/z* 529.4739, calcd for $C_{34}H_{61}N_2O_2$ $[M+H]^+$ 529.4733.

halichonine B (2)

The N^{18}-Boc halichonine B (**19**) (31.2 mg, 59.0 μmol) was treated with 4.0 M HCl/MeOH (0.10 mL) at 0 °C. After being stirred at room temperature for 2 h, the reaction mixture was concentrated to afford halichonine B (**2**) HCl salt. The halichonine B (**2**) HCl salt was purified by column chromatography on Al_2O_3 (0.7 g, $CHCl_3$–MeOH 10 : 1) to give halichonine B (**2**) free amine (20.3 mg, 74%) as a yellow oil: R_f = 0.34 ($CHCl_3$–MeOH 9 : 1); $[\alpha]_D^{25}$ +68.0 (*c* 0.16, $CHCl_3$); IR ($CHCl_3$) 3367, 2964, 2851, 1675, 1459, 1367, 1241, 1051, 983 cm^{-1}; ^{1}H NMR (600 MHz, CD_3OD) δ 5.36 (m, 1H), 5.28–5.20 (m, 2H), 3.18 (d, *J* = 7.0 Hz, 2H), 3.13 (dd, *J* = 14.1, 6.1 Hz, 1H), 2.88 (dd, *J* = 14.1, 7.7 Hz, 1H), 2.59–2.51 (m, 3H), 2.36–2.28 (m, 2H), 2.27–2.20 (m, 1H), 2.04 (br m, 1H), 1.96 (br m, 1H), 1.90–1.80 (m, 2H), 1.75 (s, 3H), 1.73 (s, 3H), 1.71 (s, 3H), 1.66 (s, 3H), 1.64 (s, 3H), 1.61–1.36 (m, 7H), 1.23–1.16 (m, 2H), 1.03 (ddd, *J* = 13.1, 13.1, 3.3 Hz, 1H), 0.89 (s, 3H), 0.86 (s, 3H), 0.76 (s, 3H). A signal due to one proton (NH) was not observed; ^{13}C NMR (150 MHz, CD_3OD) δ 136.8, 136.0, 135.2, 123.1, 123.0 (2C), 55.0, 54.5, 53.1, 52.4, 51.7, 49.9, 47.6, 43.5, 40.6, 37.4, 34.0, 33.9, 28.5, 26.1, 25.9, 25.8, 24.8, 22.9, 22.4, 19.9, 18.1, 18.0, 14.2; HRMS (ESI) *m/z* 429.4202, calcd for $C_{29}H_{53}N_2$ $[M+H]^+$ 429.4209.

N^1-(3-methylbut-2-en-1-yl)-N^4-(((1*S*,4a*S*,8a*S*)-2,5,5,8a-tetramethyl-1,4,4a,5,6,7,8,8a-octahydronaphthalen-1-yl)methyl)butane-1,4-diamine (20)

The amine **18** (9.4 mg, 20 μmol) was treated with 4.0 M HCl/MeOH (0.10 mL) at 0 °C. After being stirred at room temperature for 2 h, the reaction mixture was concentrated to afford diamine (**20**) HCl salt (8.1 mg, 92%) as a yellow solid: R_f = 0.21 ($CHCl_3$–MeOH 9 : 1); $[\alpha]_D^{24}$ –5.5 (*c* 0.38, $CHCl_3$); IR ($CHCl_3$) 3378, 2964, 2849, 2771, 1588, 1457, 1386, 1241, 982 cm^{-1}; ^{1}H NMR (600 MHz, CD_3OD) δ 5.59 (m, 1H), 5.33 (t, *J* = 7.2 Hz, 1H), 3.66 (d, *J* = 7.2 Hz, 2H), 3.24–3.02 (m, 6H), 2.17–1.43 (m, 11H), 1.83 (s, 3H), 1.82 (s, 3H), 1.79 (s, 3H), 1,32–1.16 (m, 3H), 0.92 (s, 3H), 0.89 (s, 3H), 0.83 (s, 3H). Signals due to two proton (NH) were not observed; ^{13}C NMR (150 MHz, $CDCl_3$) δ 144.1, 131.7, 126.2, 115.1, 53.8, 51.0, 49.8, 47.9, 47.2, 46.4, 43.0, 40.0, 37.7, 33.9, 33.5, 26.0, 24.7, 24.5, 24.0, 22.1, 21.8, 19.6, 18.3, 14.0; HRMS (ESI) *m/z* 361.3574, calcd for $C_{24}H_{45}N_2$ $[M+H]^+$ 361.3583.

Cell growth analysis

HL60 cells were cultured at 37 °C with 5% CO_2 in RPMI (Nissui) supplemented with 10% heat-inactivated FBS (FBS; Cell Culture Bioscience), 100 units/mL penicillin, 100 μg/mL streptomycin, 0.25 μg/mL amphotericin B, 2 mM L-glutamine, and 2.25 mg/mL $NaHCO_3$. HeLa cells were cultured at 37 °C with 5% CO_2 in DMEM (Nissui) supplemented with 10% heat-inactivated fetal bovine serum, 100 units/mL penicillin, 100 μg/mL streptomycin, 0.25 μg/mL amphotericin B, 2 mM L-glutamine, and 2.25 mg/mL $NaHCO_3$. HL60 cells were seeded at 1 × 10^4 cells/well in 96-well plates (Iwaki). HeLa cells were seeded at 4 × 10^3 cells/well in 96-well plates and cultured overnight. Various concentrations of compounds were then added, and cells were incubated for 72 h. Cell proliferation was measured by using the MTT assay.

Analysis of DNA fragmentation

HL60 cells, treated with compounds for 24 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 □g/mL ethidium bromide. The gel was visualized and photographed under UV light.

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