Organic & Biomolecular Chemistry

PAPER



Cite this: DOI: 10.1039/c5ob01488c

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

Ichiro Hayakawa,*^a Tomomi Nakamura,^b Osamu Ohno,^c Kiyotake Suenaga^d and Hideo Kigoshi*^b

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.

Received 19th July 2015, Accepted 11th August 2015 DOI: 10.1039/c5ob01488c

www.rsc.org/obc

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 ($IC_{50} = 0.60 \ \mu g \ mL^{-1}$). 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



View Article Online

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-nitrobenzenesulfonamide 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

Synthesis of (+)-halichonine B

To convert (3aR)-(+)-sclareolide (8) into the known diol 7, we followed a 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 hydroxyl group (Table 1). Alvarez-Manzaneda *et al.* reported the same reaction by using

^aDivision of Applied Chemistry, Graduate School of Natural Science and Technology, Okayama University, 3-1-1 Tsushima-naka, Kita-ku, Okayama 700-8530, Japan. E-mail: ichiro.hayakawa@okayama-u.ac.jp; Fax: +81-86-251-8215

^bDepartment of Chemistry, Graduate School of Pure and Applied Sciences, University of Tsukuba, 1-1-1 Tennodai, Tsukuba 305-8571, Japan.

E-mail: kigoshi@chem.tsukuba.ac.jp; Fax: +81-29-853-4313

^cDepartment of Chemistry and Life Science, School of Advanced Engineering,

Kogakuin University, 2665-1 Nakano, Hachioji 192-0015, Japan

^dDepartment of Chemistry, Faculty of Science and Technology, Keio University, 3-14-1 Hiyoshi, Yokohama 223-8522, Japan

[†]Electronic supplementary information (ESI) available: A 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/c5ob01488c



Scheme 1 Retrosynthetic pathway of halichonine B (2).

Table 1 Study of regioselective dehydration of diol 7

5	HO B B B C OH conditions benzene	HO ++ (HO	//
	7 Conditions	6 desired	9 undesired	
Entry	Reagents (equiv.)	Temp.	6 (%)	9 (%)
1 2 3 4 5	DEAD (2.0), PPh ₃ (2.0) DEAD (2.0), PPh ₃ (2.0) DIAD (7.0), PPh ₃ (7.0) DMAD (2.1), PPh ₃ (2.2) TMAD ^a (3.0), P(ⁿ Bu ₃) (3.0)	rt 60 °C rt rt to 60 °C rt	23 44 7 Trace 14	19 52 24 Trace 9

^{*a*} TMAD: *N*,*N*,*N*',*N*'-tetramethylazodicarboxamide.

the Mitsunobu conditions $(92\% \text{ yield}).^{3,6}$ 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 the 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 a lower temperature than that under 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**.

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 for obtaining the desired alcohol **6**.

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 LiAlH₄ 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.

With Ns-amide 5 and alcohol 4 in hand, we next examined the segment coupling reaction. The coupling between Nsamide 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 N¹⁸-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 CHCl₃/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 the structural formula of 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 towards the panel of cell lines. On the basis of the COMPARE









Scheme 4 Synthesis of (+)-hauchonine B (

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 \dagger).

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

Paper

artificial analogues of halichonine B against HL60 cells (Table 2). The cytotoxic activity of synthetic halichonine B (2) HCl salt against HL60 cells with the same IC₅₀ value as that of the natural halichonine B (2) HCl salt was determined. In addition, the synthetic halichonine B (2) HCl salt and the synthetic halichonine B (2) free amine showed cytotoxicity with IC50 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 IC₅₀ 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 N¹¹, 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 the side chain portion, including ana-

 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) $(natural)^a$	<i>n</i> = 4	0.30	
(+)-Halichonine B (2) (synthetic) ^a	<i>n</i> = 4	0.25	1.50
(+)-Halichonine B (2) (synthetic)	<i>n</i> = 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 ^{<i>a</i>}	n = 4	0.21	1.19
21	<i>n</i> = 2	1.40	
22	<i>n</i> = 3	0.67	
23	<i>n</i> = 6	0.26	1.78
24	<i>n</i> = 8	0.45	
25 ^{<i>a</i>}	<i>n</i> = 2	1.90	
26 ^{<i>a</i>}	<i>n</i> = 3	0.33	2.00
27^a	<i>n</i> = 6	0.29	1.79
28 ^{<i>a</i>}	<i>n</i> = 8	0.29	1.40

^a HCl salt.





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

logues **21–24** possessing the prenyl group at N¹¹ (natural product-type) and analogues **25–28** lacking the prenyl group at N¹¹ (diamine-type).¹⁴ The cytotoxic activity of the 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 halichonine B (2) (see Fig. 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 the side chain portion, the diamine-type analogues showed slightly stronger cytotoxicity. These results also indicated that the presence of the secondary amino group at N¹⁸ in the side chain portion is responsible for the strong cytotoxicity. In addition, the secondary amino group at N¹¹ was substitutable for the N¹⁸ 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.

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.

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 evalu-



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 μ M concentrations for 24 h. Cellular DNA was then extracted and electrophoresed on an agarose gel. M: 100 bp DNA ladder marker.

ated 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 in 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, CH2Cl2, 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 was 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 ¹³C NMR spectra were recorded on a Bruker AVANCE 400, a Bruker DPX 400 spectrometer, or a Bruker AVANCE 600 spectrometer. The ¹H and ¹³C chemical shifts were referenced to the solvent peaks, $\delta_{\rm H}$ 7.26 (residual CHCl₃) and $\delta_{\rm C}$ 77.0 ppm

(*C*DCl₃), or $\delta_{\rm H}$ 3.30 (residual *CHD*₂OD) and $\delta_{\rm C}$ 49.0 ppm (*C*D₃OD), 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.

((1S,2R,4aS,8aS)-2-Hydroxy-2,5,5,8a-tetramethyldecahydronaphthalen-1-vl)methyl acetate (11). To a stirred solution of diol 7 (552 mg, 2.30 mmol) in pyridine (2.3 mL) was added Ac₂O (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 NaHCO₃ (4.0 mL) and extracted with EtOAc (10 mL \times 3). The combined extracts were washed with brine (10 mL), dried over Na₂SO₄, 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-Et₂O 2:1); $[\alpha]_{D}^{24}$ -7.7 (c 0.77, CHCl₃); IR (CHCl₃) 3591, 3480, 3000, 2930, 2870, 1727, 1467, 1389, 1370, 1246, 1030, 938, 839 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) 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); ¹³C NMR (100 MHz, CDCl₃) 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 C₁₇H₃₀NaO₃ [M + Na]⁺ 305.2093.

((15,4a5,8a5)-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% H₂SO₄/EtOH (3.2 mL) at 0 °C. After being stirred at room temperature for 15 min, the reaction mixture was diluted with saturated aqueous NaHCO₃ (30 mL) and extracted with Et₂O (20 mL × 3). The combined extracts were washed with brine (10 mL), dried over MgSO₄, filtered, and concentrated. The crude product was purified by column chromatography on silica gel (5.5 g, *n*-hexane-Et₂O 12:1 \rightarrow 9:1) to give acetate 12 (169 mg, 56%) and alcohol 6 (50.7 mg, 20%) as colorless oils, respectively.

Acetate **12**: $R_{\rm f} = 0.22$ (*n*-hexane–Et₂O 2:1); $[\alpha]_{\rm D}^{24}$ +12.7 (*c* 0.52, CHCl₃); IR (CHCl₃) 3026, 2960, 2927, 2850, 1730, 1457, 1442, 1388, 1366, 1260, 1034, 969, 861 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) 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); ¹³C NMR (100 MHz, CDCl₃) 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 C₁₇H₂₈NaO₂ [M + Na]⁺ 287.1987.

((15,4a5,8a5)-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 H₂O (5 mL) and extracted with Et₂O (10 mL × 3). The combined extracts were washed with brine (10 mL), dried over MgSO₄, filtered, and concentrated. The crude product was purified by column chromatography on silica gel (3.0 g, *n*-hexane–Et₂O 9:1) to give alcohol **6** (148 mg, quant) as a colorless oil: $R_{\rm f} = 0.45$ (*n*-hexane–Et₂O 2 : 1); $[\alpha]_{\rm D}^{24}$ –14.6 (*c* 0.63, CHCl₃); IR (CHCl₃) 3625, 3461, 2926, 2849, 1458, 1443, 1389, 1366, 1032, 962, 841 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) 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); ¹³C NMR (100 MHz, CDCl₃) 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 C₁₅H₂₆NaO [M + Na]⁺ 245.1881.

(4aS,5S,8aS)-5-(Azidomethyl)-1,1,4a,6-tetramethyl-1,2,3,4,4a,5,8,8aoctahydronaphthalene (13). To a stirred solution of alcohol 6 (500 mg, 2.25 mmol) and PPh₃ (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 the crude product, which was purified by column chromatography on silica gel (40 g, pentane \rightarrow *n*-hexane–Et₂O 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, CHCl₃); IR (CHCl₃) 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); ¹³C NMR (100 MHz, CDCl₃) 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,5,6,7,8,8aoctahydronaphthalen-1-yl)methyl)benzenesulfonamide (5). To a stirred solution of azide 13 (100 mg, 405 μ mol) in THF (2.7 mL) was added LiAlH₄ (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 to H₂O (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 CH₂Cl₂ (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 being cooled to 0 °C, 1.0 M aqueous HCl (0.1 mL) and H₂O (10 mL) were added. The resultant mixture was extracted with EtOAc (10 mL \times 3). The combined extracts were washed with brine (10 mL), dried over Na₂SO₄, filtered, and concentrated. The crude product was purified by column chromatography on silica gel (3.0 g, n-hexane-Et₂O 5:1/1% Et₃N) to give Ns-amide 5 (116 mg, 70% in 2 steps) as a colorless oil: $R_f = 0.37$ (*n*-hexane-Et₂O 2:1); $[\alpha]_{D}^{25}$ +24.5 (c 0.25, CHCl₃); IR (CHCl₃) 3382, 3032, 2926, 2850, 1716, 1542, 1457, 1441, 1416, 1363, 1170, 1061, 855 cm⁻¹; ¹H NMR (600 MHz, CDCl₃) 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); ¹³C NMR (150 MHz, CDCl₃) 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 C₂₁H₃₀N₂NaO₄S [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 μ L, 1.19 mmol) in CH₂Cl₂ (7.1 mL) containing molecular sieves of 3 Å (239 mg) was added 3-methyl-2-butenal (16, 130 μ L, 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 CH₂Cl₂ (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 NaBH₄ (90.0 mg, 2.38 mmol) at 0 °C. After being stirred at room temperature for 30 min, the reaction mixture was diluted with H₂O (5 mL) and extracted with CH₂Cl₂ (5 mL × 5). The combined extracts were washed with brine (5 mL), dried over Na₂SO₄, 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 Boc₂O (0.30 mL, 1.3 mmol) and Et₃N (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 NH4Cl (10 mL) and extracted with EtOAc (10 mL \times 3). The combined extracts were washed with brine (10 mL), dried over MgSO₄, filtered, and concentrated. The crude product was purified by column chromatography on silica gel (7.0 g, *n*-hexane–EtOAc $4: 1 \rightarrow 1: 1$) to give alcohol 4 (264 mg, 86% in 2 steps) as a colorless oil: $R_f = 0.54$ (*n*-hexane-EtOAc 1:1); IR (CHCl₃) 3629, 3420, 3009, 2979, 2935, 1668, 1455, 1419, 1252, 1167, 1028, 880 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) 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); ¹³C NMR (100 MHz, CDCl₃) & 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-((((15,4aS,8aS)-2,5,5,8atetramethyl-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 PPh₃ (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 the crude product, which was purified by column chromatography on silica gel (40 g, *n*-hexane–Et₂O 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 μ L, 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-Et₂O 5:1 \rightarrow 1:1) to give amine **18** (69 mg, 61% in 2 steps) as a yellow oil: $R_{\rm f} = 0.43$ (CHCl₃-MeOH 9:1); $[\alpha]_{\rm D}^{25}$ +2.3 (c 0.50, CHCl₃); IR (CHCl₃) 3359, 3018, 2928, 2851, 1682, 1456, 1418, 1366, 1252, 1167, 1137, 881 cm⁻¹; ¹H NMR (600 MHz, CDCl₃) 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; ¹³C NMR (150 MHz, CDCl₃) 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)-(((1S,4aS,8aS)-2,5,5,8a-tetramethyl-1,4,4a,5,6,7,8,8a-octahydronaphthalen-1-yl)methyl)amino)butyl)carbamate (19). To а stirred solution of amine 18 (33.0 mg, 71.6 µmol) in MeCN (0.30 mL) were added i-Pr2NEt (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 \times 3). The combined extracts were washed with brine (5 mL), dried over Na₂SO₄, 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: $R_{\rm f} = 0.75$ (*n*-hexane-Et₂O 2:1); $[\alpha]_{\rm D}^{25}$ +41.6 (c 0.25, CHCl₃); IR (CHCl₃) 2927, 2859, 1681, 1455, 1418, 1378, 1366, 1268, 1251, 1168, 1144, 880 cm⁻¹; ¹H NMR (600 MHz, CDCl₃) 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); ¹³C NMR (150 MHz, CDCl₃) 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 Al₂O₃ (0.7 g, CHCl₃–MeOH 10 : 1) to give halichonine B (2) free amine (20.3 mg, 74%) as a yellow oil: $R_{\rm f} = 0.34$ (CHCl₃–MeOH 9 : 1); $[\alpha]_{\rm D}^{25}$ +68.0 (*c* 0.16, CHCl₃); IR (CHCl₃) 3367, 2964, 2851, 1675, 1459, 1367, 1241, 1051, 983 cm⁻¹; ¹H NMR (600 MHz, CD₃OD) 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; ¹³C NMR (150 MHz, CD₃OD) 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 C₂₉H₅₃N₂ [M + H]⁺ 429.4209.

N¹-(3-Methylbut-2-en-1-yl)-N⁴-(((15,4aS,8aS)-2,5,5,8a-tetramethyl-1,4,4a,5,6,7,8,8a-octahydronaphthalen-1-yl)methyl)butane-1,4diamine (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: $R_{\rm f} = 0.21$ (CHCl₃-MeOH 9:1); $[\alpha]_{\rm D}^{24}$ -5.5 (*c* 0.38, CHCl₃); IR (CHCl₃) 3378, 2964, 2849, 2771, 1588, 1457, 1386, 1241, 982 cm⁻¹; ¹H NMR (600 MHz, CD₃OD) 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 protons (NH) were not observed; ¹³C NMR (150 MHz, CDCl₃) 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% CO₂ in RPMI (Nissui) supplemented with 10% heat-inactivated FBS (FBS; Cell Culture Bioscience), 100 units per mL penicillin, 100 µg mL⁻¹ streptomycin, 0.25 µg mL⁻¹ amphotericin B, 2 mM L-glutamine, and 2.25 mg mL⁻¹ NaHCO₃. HeLa cells were cultured at 37 °C with 5% CO₂ in DMEM (Nissui) supplemented with 10% heat-inactivated fetal bovine serum, 100 units per mL penicillin, 100 µg mL⁻¹ streptomycin, 0.25 µg mL⁻¹ amphotericin B, 2 mM L-glutamine, and 2.25 mg mL⁻¹ NaHCO₃. HL60 cells were seeded at 1 × 10⁴ cells per well in 96-well plates (Iwaki). HeLa cells were seeded at 4 × 10³ cells per 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^{-1} NaCl, 200 mg L^{-1} KCl, 1.15 g L^{-1} Na₂HPO₄·2H₂O, 200 mg L^{-1} KH₂PO₄). 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 17700g at 4 °C for 5 min, the supernatant was treated with 0.2 mg mL⁻¹ RNase A at 37 °C for 1 h. The samples were treated with 0.2 mg mL⁻¹ 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

Published on 11 August 2015. Downloaded by University of Prince Edward Island on 20/08/2015 18:27:05.

mixtures were cooled overnight at -20 °C, DNA was collected by centrifugation at 17 700g 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⁻¹ ethidium bromide. The gel was visualized and photographed under UV light.

Acknowledgements

The authors thank Drs Hiroyuki Seimiya, Takao Yamori, and Shingo Dan (Screening Committee of Anticancer Drugs supported by Grant-in-Aid for Scientific Research on Innovative Areas, Scientific Support Programs for Cancer Research, from The Ministry of Education, Culture, Sports, Science and Technology, Japan) for screening in the human cancer cell line panel. We also thank Dr Masaya Imoto and Mr Shun Saito (Keio University) for their valuable suggestions. This work was supported by Grants-in-Aid for Scientific Research on Innovative Areas 'Chemical Biology of Natural Products' and for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology (MEXT)/Japanese Society for the Promotion of Science (JSPS). I.H. thanks the Kurata Grant awarded by the Kurata Memorial Hitachi Science and Technology Foundation, the NOVARTIS Foundation (Japan) for the Promotion of Science, the Naito Foundation, and the Suzuken Memorial Foundation for their financial support.

Notes and references

- O. Ohno, T. Chiba, S. Todoroki, H. Yoshimura, N. Maru, K. Maekawa, H. Imagawa, K. Yamada, A. Wakamiya, K. Suenaga and D. Uemura, *Chem. Commun.*, 2011, 47, 12453.
- 2 T. Fukuyama, C.-K. Jow and M. Cheung, *Tetrahedron Lett.*, 1995, **36**, 6373; Review: T. Kan and T. Fukuyama, *Chem. Commun.*, 2004, 353.
- 3 E. J. Alvarez-Manzaneda, R. Chahboun, I. Barranco Pérez, E. Cabrera, E. Alvarez and R. Alvarez-Manzaneda, *Org. Lett.*, 2005, 7, 1477.
- 4 K. I. Kuchkova, A. N. Aricu, I. P. Dragalin and P. F. Vlad, *Russ. Chem. Bull. Int. Ed.*, 2004, **53**, 2745.

- 5 (a) S. Chackalamannil, Y. Wang, Y. Xia and M. Czarniecki, *Tetrahedron Lett.*, 1995, 36, 5315; (b) I. Margaros, T. Montagnon, M. Tofi, E. Pavlakos and G. Vassilikogiannakis, *Tetrahedron*, 2006, 62, 5308.
- 6 Review: O. Mitsunobu, Synthesis, 1981, 1.
- 7 T. Tsunoda, J. Otsuka, Y. Yamamiya and S. Itô, *Chem. Lett.*, 1994, 539; S. Itô and T. Tsunoda, *Pure Appl. Chem.*, 1999, 71, 1053.
- 8 Katoh and co-workers recently reported the synthesis of alcohol **6** from diol 7 by using *p*-TsOH·H₂O in CH₂Cl₂ (79% yield). These reaction conditions can transform diol 7 into alcohol **6** in one step; T. Kamishima, T. Kikuchi, K. Narita and T. Katoh, *Eur. J. Org. Chem.*, 2014, 3443.
- 9 B. Lal, B. N. Pramanik, M. S. Manhas and A. K. Bose, *Tetrahedron Lett.*, 1977, 18, 1977.
- 10 There was a printing error in ref. 1. The NMR frequency marked with "b" in the footnote of Table 2 should have been presented as "800 MHz" (not "600 MHz").
- 11 Although the sign of optical rotation of synthetic (+)-halichonine B { $[\alpha]_D^{25}$ +68.0 (*c* 0.16, CHCl₃)} is consistent with the natural one { $[\alpha]_D^{28}$ +13.8 (*c* 0.2, CHCl₃)},¹ the absolute values were different. The plausible reason for this difference is an error in the concentration of the analytical sample solution, may be because a sufficient amount of the natural product was not available for analysis.
- 12 (a) COMPARE analysis is an information-intensive approach to identify the molecular target and/or the mode of action of a test compound by comparison of the pattern of sensitivity against human cancer cell lines between known drugs and a test compound; (b) K. D. Paull, R. H. Shoemaker, L. Hodes, A. Monks, D. A. Scudiero, L. Rubinstein, J. Plowman and M. R. Boyd, *J. Natl. Cancer Inst.*, 1989, **81**, 1088; (c) T. Yamori, A. Matsunaga, S. Sato, K. Yamazaki, A. Komi, K. Ishizu, I. Mita, H. Edatsugi, Y. Matsuba, K. Takezawa, O. Nakanishi, H. Kohno, Y. Nakajima, H. Komatsu, T. Andoh and T. Tsuruo, *Cancer Res.*, 1999, **59**, 4042.
- 13 Diamine 20 was prepared from compound 18 (see the Experimental section).
- 14 The artificial analogues **21–28** were synthesized by a strategy similar to that used for halichonine B (2) and diamine **20** (see the ESI†).