Antitumor Polyether Macrolides: New and Hemisynthetic Halichondrins from the New Zealand Deep-Water Sponge *Lissodendoryx* sp.

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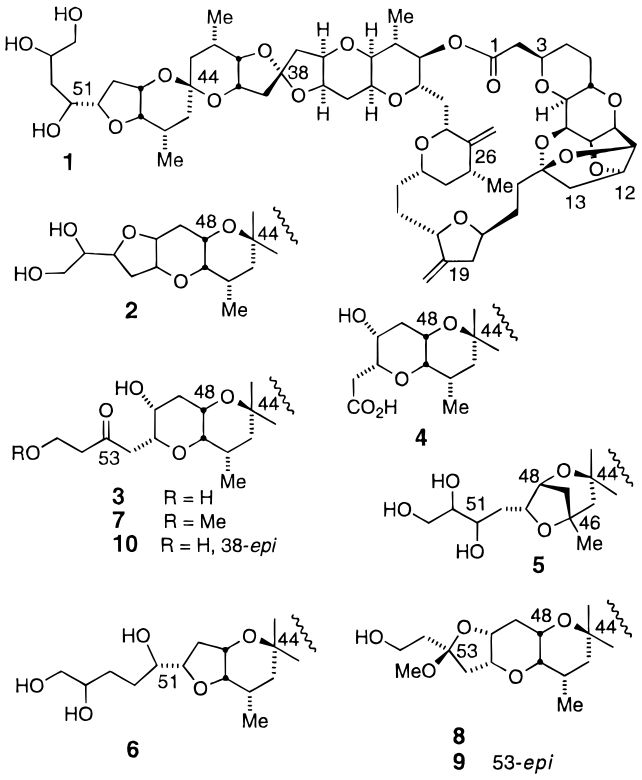
During our investigation of the biological potential of the New Zealand marine biota, attention was drawn to a bright yellow sponge *Lissodendoryx* sp. (family Myxillidae, order Poecilosclerida) collected by dredging from deep water (>100 m) off the Kaikoura Peninsula in 1983. Extracts from this sponge were strongly inhibitory against the murine leukemia cell line P388, a DNA virus (*Herpes simplex* Type I), and an RNA virus (*Polio* vaccine virus). Initial studies on the sponge in 1985 established that the active components were stable, and furthermore, crude extracts of this sponge offered significant extensions of life-span in an *in vivo* murine P388 model (T/C ~250%).[1] One strongly bioactive compound, obtained in the initial studies, gave data very reminiscent of those published for the halichondrin series of compounds,[2,3] although differences were noted which suggested that a new halichondrin had been obtained. Subsequent re-extraction of a larger quantity of the sponge (5 kg) gave a crude organic extract (3.8 g) which was subjected to bioassay-directed chromatography to yield three biologically active components, the known halichondrin B (**1**), homohalichondrin B (**2**), and a new isomer isohomohalichondrin B (**3**), as well as several minor components. The characterization of **3** was reported in a preliminary communication.[4] In order to obtain more of these halichondrins and the minor components, a further 200 kg of the *Lissodendoryx* sp. sponge was obtained by trawling off Kaikoura. This collection was freeze-dried and ground (47 kg) and the extract from a portion (20 kg) subjected to chromatography to yield the three compounds described previously, halichondrin B (**1**), homohalichondrin B (**2**), and isohomohalichondrin B (**3**), together with norhalichondrin B (**4**) and the new derivatives **5**−**9**.



Results and Discussion

The previously identified halichondrins **1**−**4** were characterized from their HRFAB mass spectra and 1D and 2D NMR spectra. The NMR data for halichondrin B (**1**) is shown in Table 1, as the complete assignments for this compound have not previously been reported. Of the new derivatives, compound **5** (0.9 mg) was the most polar and was found to have the formula $C_{59}H_{84}O_{19}$ (HRFABMS). Data from ^{1}H NMR, TOCSY, HMQC, and HMBC spectra showed that this compound was identical to halichondrin B **1** up to C44 (Table 1). There were, however, some unusual features associated with the ^{1}H and ^{13}C NMR resonances for the remainder of the molecule. In particular, the doublet signal normally seen for 46Me had been replaced by a singlet 3-proton resonance at δ 1.35, suggesting attachment of this methyl to a quaternary oxygenated carbon. Having located the terminal carbon C53 as a CH_2OH moeity, use of 2D TOCSY data then established the presence of the fragment $HOCH_2CHOHCHOHCH_2CHOCHOCH_2$. Results from HMBC and NOE difference spectra permitted the construction of the C46−C53 region as the novel (for the halichondrins) bicyclo-system as shown in Figure 1. The unusually large chemical shift for C45 (52.4 ppm) can be accounted for by the additional β substituent (O−C46) for this carbon. The large NOE effect (4.8%) between H48/H49 and between H49/H47 supports the assignment of the relative stereochemistry as shown. Molecular modeling of this fragment gave a structure with inter-proton distances consistent with the observed NOE effects. The absolute stereochemistry at C48 is kept the same as that found in all other halichondrins, although there is no direct evidence for this in compound **5**. The name neonorhalichondrin B is suggested for **5**.

Another new compound, neohomohalichondrin B **6**, with polarity similar to that of halichondrin B was found to have the formula $C_{61}H_{88}O_{19}$ (HRFABMS), which corresponds to halichondrin B with an additional CH_2. The ^{1}H and ^{13}C NMR spectral data for neohomohalichondrin B (**6**) were identical to those for halichondrin B (**1**), except for resonances for carbons and protons at positions 47 and higher (Table 1). This suggested that the additional CH_2 unit in neohomohalichondrin B was located in, or near, the side chain attached to C50. Analysis of COSY and 2D TOCSY spectra revealed a spin system involving H47−H51 the same as in halichondrin B, and also a $CHOHCH_2OH$ fragment. These data suggested that the additional CH_2 group be inserted at a position corresponding to between C51 and C53 in halichondrin B. The two CH_2 groups at C52 and C53 in neohomohalichondrin

(1) Munro, M. H. G.; Blunt, J. W.; Barns, G.; Battershill, C. N.; Lake, R. J.; Perry, N. B. *Pure Appl. Chem.* **1989**, *61*, 529.
(2) Hirata, Y.; Uemura, D. *Pure Appl. Chem.* **1986**, *58*, 701.
(3) Uemura, D.; Takahashi, K.; Yamamoto, T.; Katayama, C.; Tanaka, J.; Okumura, Y.; Hirata, Y. *J. Am. Chem. Soc.* **1985**, *107*, 4796.
(4) Litaudon, M.; Hart, J. B.; Blunt, J. W.; Lake R. J.; Munro, M. H. G. *Tetrahedron Lett.* **1994**, *35*, 9435.

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Table 1. ^{1}H (300 MHz) and ^{13}C (75 MHz) NMR chemical shift data for compounds 1, 3, 5−8, 10[a]

	1		3		5		6		7	8		10	
position	C	H	C	H	C[b]	H	C	H	H	C	H	C	H
1	171.2		171.1		*c*		171.2			171.2		170.9	
2	40.4	2.35, 2.60	40.4	2.36, 2.61	40.4	2.35, 2.62	40.4	2.35, 2.60	2.35, 2.60	40.4	2.35, 2.59	40.4	2.37, 2.59
3	73.7	3.86	73.6	3.89	73.7	3.88	73.7	3.88	3.88	73.7	3.87	73.7	3.88
4	30.7	1.75, 1.37	30.6	1.75, 1.38	30.7	1.74, 1.40	30.7	1.75, 1.38	1.75, 1.37	30.7	1.72, 1.37	30.7	1.76, 1.39
5	30.0	1.35, 2.08	30.0	1.41, 2.12	30.0	1.38, 2.08	30.0	1.41, 2.11	1.40, 2.11	30.0	1.39, 2.10	30.1	*c*, 2.11
6	68.2	4.34	68.2	4.35	68.2	4.33	68.2	4.33	4.34	68.2	4.32	68.2	4.34
7	77.7	2.94	77.6	2.95	77.7	2.95	77.7	2.95	2.94	77.7	2.94	77.7	2.94
8	74.3	4.33	74.3	4.33	74.3	4.33	74.3	4.33	4.32	74.3	4.32	74.3	4.31
9	73.8	4.04	73.8	4.06	73.8	4.05	73.8	4.05	4.05	73.8	4.04	73.8	4.06
10	76.5	4.20	76.5	4.22	76.6	4.20	76.6	4.20	4.21	76.5	4.19	76.5	4.19
11	82.1	4.60	82.1	4.60	82.1	4.60	82.1	4.60	4.59	82.1	4.58	82.0	4.60
12	81.1	4.68	81.0	4.70	81.1	4.69	81.1	4.69	4.69	81.1	4.69	81.0	4.68
13	48.3	1.94, 2.15	48.3	1.95, 2.16	48.3	1.93, 2.15	48.3	1.95, 2.17	1.96, 2.15	48.3	1.96, 2.15	48.4	1.95, 2.13
14	110.1		110.0		110.1		110.1			110.1		110.0	
15	34.4	2.18, 1.62	34.4	2.18, 1.62	34.4	2.16, 1.60	34.4	2.18, 1.62	2.18, 1.62	34.4	2.18, 1.62	34.4	*c*, 1.62
16	28.2	2.16, 1.42	28.1	2.18, 1.42	28.2	2.15, 1.38	28.2	2.16, 1.42	2.16, 1.42	28.1	2.16, 1.42	28.2	2.17, 1.41
17	75.5	4.10	75.3	4.10	75.5	4.10	75.5	4.10	4.09	75.4	4.09	75.2	4.09
18	38.7	2.26, 2.80	38.7	2.27, 2.80	38.7	2.24, 2.80	38.7	2.27, 2.80	2.26, 2.80	38.7	2.26, 2.80	38.7	2.26, 2.80
19	151.8		151.7		*c*		151.8			151.6		151.3	
19 =CH_2	104.5	4.98, 4.92	104.4	5.01, 4.93	104.0	5.00, 4.92	104.5	5.00, 4.92	5.01, 4.92	104.5	4.99, 4.91	104.3	4.99, 4.92
20	75.4	4.37	75.3	4.39	75.4	4.39	75.4	4.39	4.39	75.3	4.37	75.2	4.38
21	29.5	1.40, 1.88	29.3	1.42, 1.90	29.5	1.41, 1.88	29.5	1.41, 1.90	1.42, 1.90	29.4	1.42, 1.90	29.4	1.40, 1.87
22	32.0	1.60, 1.60	32.0	1.62, 1.62	32.0	1.60, 1.60	32.0	1.62, 1.62	1.61, 1.61	32.0	1.61, 1.61	32.1	1.62, 1.62
23	74.9	3.53	74.8	3.55	74.9	3.52	74.9	3.54	3.54	74.8	3.54	74.9	3.51
24	43.4	1.04, 1.70	43.3	1.05, 1.72	43.4	1.04, 1.68	43.4	1.04, 1.72	1.05, 1.72	43.4	1.05, 1.69	43.3	1.05, 1.74
25	35.9	2.20	35.9	2.23	35.9	2.20	35.9	2.23	2.22	35.9	2.22	36.0	2.20
25-Me	18.0	1.07	18.0	1.07	18.0	1.06	18.0	1.06	1.07	18.0	1.07	18.1	1.07
26	151.6		151.5		153.0		151.6			151.7		151.7	
26 =CH_2	104.2	4.81, 4.77	104.1	4.83, 4.78	103.8	4.82, 4.78	104.2	4.80, 4.75	4.83, 4.78	104.2	4.80, 4.75	103.9	4.81, 4.76
27	73.5	3.54	73.5	3.56	73.5	3.53	73.5	3.53	3.56	73.5	3.52	73.4	3.52
28	36.9	2.02, 1.94	36.9	2.01, 1.95	36.9	2.02, 1.95	36.9	2.00, 1.95	2.00, 1.95	36.9	2.00, 1.92	36.9	*c*, 1.94
29	71.2	4.21	71.1	4.22	71.2	4.21	71.2	4.21	4.22	71.2	4.19	71.2	4.20
30	76.9	4.63	77.2	4.66	77.2	4.66	77.2	4.66	4.65	77.2	4.65	77.1	4.66
31	36.6	2.04	36.5	2.03	36.6	2.03	36.6	2.03	2.03	36.5	2.03	36.3	2.04
31-Me	15.1	0.99	15.0	1.00	15.1	1.00	15.1	1.00	0.99	15.0	0.99	14.9	0.99
32	77.5	3.18	77.5	3.20	77.4	3.19	77.4	3.19	3.20	77.5	3.18	77.3	3.12
33	66.3	3.80	66.4	3.84	66.3	3.81	66.3	3.81	3.84	66.4	3.79	67.2	3.77
34	29.1	1.79, 2.13	29.0	1.81, 2.16	29.1	1.76, 2.13	29.1	1.80, 2.13	1.80, 2.15	29.0	1.78, 2.14	29.7	2.06, 2.25
35	75.0	4.10	75.1	4.12	75.1	4.10	75.1	4.10	4.12	75.0	4.11	77.2	4.07
36	76.2	4.10	76.2	4.12	76.2	4.10	76.2	4.10	4.12	76.3	4.11	76.8	4.14
37	43.5	2.35, 1.92	43.3	2.37, 1.92	43.5	2.35, 1.92	43.5	2.35, 1.90	2.37, 1.92	43.4	2.35, 1.90	44.0	2.24, 2.24
38	112.5		112.4		112.5		112.5			112.4		113.9	
39	42.7	2.24, 2.24	42.5	2.22, 2.22	42.7	2.18, 2.18	42.7	2.24, 2.24	2.22, 2.22	42.6	2.20, 2.20	44.6	2.03, 2.30
40	71.7	4.00	71.2	3.94	70.9	4.18	71.6	4.03	3.93	70.9	3.88	71.8	3.93
41	79.0	3.63	79.0	3.64	78.6	3.59	79.0	3.64	3.64	79.4	3.58	78.3	3.84
42	25.6	2.23	25.7	2.29	26.1	2.21	25.5	2.24	2.29	25.6	2.26	25.4	2.25
42-Me	17.6	0.94	17.5	0.95	17.6	0.94	17.6	0.94	0.95	17.6	0.93	17.8	0.99
43	36.6	1.52, 1.29	36.9	1.55, 1.33	38.6	1.48, 1.30	36.6	1.53, 1.30	1.55, 1.33	35.5	1.63, 1.35	36.9	1.48, 1.37
44	97.5		97.2		96.4		97.4			97.6		97.0	
45	36.9	1.50, 1.42	37.2	1.49, 1.52	52.4	1.88, 2.11	36.9	1.49, 1.42	1.49, 1.52	37.1	1.72, 1.40	37.4	1.49, 1.37
46	25.7	2.35	28.6	2.18	80.2		25.5	2.36	2.18	28.5	2.20	28.7	2.10
46-Me	17.8	0.99	16.8	0.90	25.5	1.35	17.8	1.00	0.90	17.0	0.91	17.0	0.91
47	80.2	3.61	75.9	3.25	36.8	2.82, 1.50	79.4	3.52	3.23	74.2	3.08	75.7	3.23
48	71.7	4.05	66.4	3.75	75.9	4.28	71.7	4.05	3.73	63.3	3.59	66.3	3.71
49	35.9	2.32, 1.90	34.4	2.13, 1.84	81.7	4.01	32.0	2.10, 1.95	2.12, 1.84	29.7	2.24, 1.96	34.4	2.13, 1.84
50	79.8	4.08	66.4	3.52	35.0	1.74, 1.84	80.8	4.05	3.52	73.0	3.87	66.3	3.51
51	73.0	3.80	76.4	3.82	71.6	3.90	72.6	3.82	3.82	77.7	3.96	76.4	3.81
52	37.2	1.62, 1.79	45.2	2.93, 2.62	73.6	3.56	30.6	1.40	2.90, 2.64	45.3	2.24	45.2	2.93, 2.62
53	70.4	4.02	209.5		65.0	3.73	31.0	1.60		109.3		210.4	
53-OMe										48.6	3.23		
54	66.9	3.54, 3.61	46.1	2.74			72.2	3.70	2.75	35.5	1.72, 2.30	46.0	2.73
55			57.8	3.86			67.0	3.45, 3.63	3.64	58.3	3.79, 3.68	57.9	3.83
55-OMe									3.34				

[a] Values in ppm relative to C*H*Cl$_3$ (δ 7.25) and *C*DCl$_3$ (δ 77.0). [b] Values obtained from HMQC and HMBC spectra. [c] Values not detected.

B **6** had broad ^{1}H signals at δ 1.4 and 1.6 respectively, with connectivities, revealed in COSY and 2D TOCSY spectra, appropriate to these assignments.

A third, lower-polarity compound, 55-methoxyisohomohalichondrin B (**7**), was obtained in very low yield (0.7 mg) and found to have the formula $C_{62}H_{88}O_{19}$ (HR-FABMS) (corresponding to isohomohalichondrin B with an additional CH_2). The ^{1}H NMR and 2D TOCSY spectra of this compound were very similar to those of isohomohalichondrin B (**3**) except for the appearance of a 3-proton singlet resonance at 3.34 ppm, and a shift of the triplet resonance for 55H$_2$ from δ 3.85 to 3.62 ppm. These data implied that the terminal hydroxyl group of **3** had been methylated. This was supported by the observation of an NOE effect between 55H$_2$ and the terminal methoxyl protons. As a consequence of the small amount of

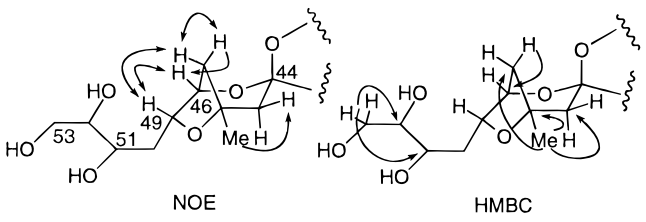

Figure 1. NOE and HMBC correlations for the C44−C53 region of compound **5**.

material isolated, no ^{13}C NMR data for this compound were obtained.

The remaining two compounds isolated, **8** and **9**, were both lower-polarity derivatives and are most probably artefacts produced during the extraction process. HR-FABMS data suggested a formula for **8** of $C_{62}H_{88}O_{19}$, which is equivalent to that for isohomohalichondrin B (**3**) with an additional CH_2. The ^1H NMR spectrum of **8** differed from that of **3** by the absence of the two triplets at δ 2.74 and 3.86 for $54CH_2$ and $55CH_2$, respectively, in **3**, and the appearance of a sharp 3-proton singlet at δ 3.23. In the ^{13}C NMR spectrum, there was no carbonyl resonance at δ 209.5 as found for **3**, but there was a new resonance at δ 109.3. These data, together with correlations observed in the HMQC and HMBC spectra, suggested the formulation of **8** as the methyl ketal derived from isohomohalichondrin B (**3**) and methanol. As a new homohalichondrin skeleton results from this transformation, the name 53-methoxyneoisohomohalichondrin B is used. The observation of NOE effects from H47 to H46, $46CH_3$, H48, and H51, from H51 to $52CH_2$ and H47, and from H50 to H51, H49, and $53CH_3O$ established the relative stereochemistries at C47, C48, C50, and C51 as being the same as in isohomohalichondrin B (**3**), and the stereochemistry at C53 as being *S*.

During the initial investigations on **8**, it was noted that the ^1H NMR spectrum of a sample of **8** kept in a $CDCl_3$ solution (without added C_5D_5N) slowly reverted to the spectrum characteristic of **3**. Subsequently, treatment of **8** in CH_3CN/H_2O/0.001% $HClO_4$ was shown to convert **8** quantitatively to **3** within 1 min. The reversibility of this process was demonstrated by placing a sample of isohomohalichondrin B **3** in MeOH-d_4. Over a period of time, the ^1H NMR spectrum changed from that of **3** to one characteristic of **8** (except for the absence of the signal for $53CH_3O$ since this would now be $53CD_3O$). These observations suggest that the ketal **8** is not a naturally occurring derivative, but is rather an artefact of the isolation, where the methanol used during extraction and chromatography caused the conversion of **3** to **8**. Further evidence for this supposition is obtained from the isolation of a second relatively nonpolar compound **9** (1.5 mg), which was isobaric with **8**. Like **8**, this compound was unstable in $CDCl_3$ solution (without added C_5D_5N), giving eventually a ^1H NMR spectrum identical to that of **3**. However, before its conversion to **3**, the spectrum of **9** was identical to that of **8** except for different resonance positions for $53CH_3O$ (δ 3.40, *cf.* δ 3.23 for ketal **8**) and for H51 (δ 3.91 *cf.* 3.96 for ketal **8**). The instability of this compound precluded the collection of a full set of spectroscopic data and structural elucidation. The structure of **9** is presumed to be 53-*epi*-53-methoxyneoisohomohalichondrin B.

Initial attempts at converting the acetal **8** to isohomohalichondrin B (**3**) using acid employed CH_3CN/H_2O/0.007% $HClO_4$ for 6 min. This gave a 1:1 mixture of **3** and an isomeric compound **10**. A shortening of the reaction time to 1 min gave a **3**:**10** ratio of 3:1. Subsequent dilution of the acid to 0.001% as described above allowed the formation of **3** only. The HRFABMS of **10** gave a formula of $C_{61}H_{86}O_{19}$, the same as for **3**. A comparison of the NMR data for the isomeric compound **10** with that for isohomohalichondrin B (**3**) showed that the only differences were small changes in chemical shifts for protons and carbons around C38. This behavior was identical with what we had previously reported[5] for compounds arising from acid treatment of homohalichondrin B (**2**), in which epimerization at C38 had occurred. Compound **10** was thus assigned as 38-*epi*-isohomohalichondrin B. In the present case, the acid conditions were apparently not strong enough to cause the opening of the trioxadecalin ring system, as had been seen in the earlier work on homohalichondrin B (**2**).[5] The equilibrium character of the C38 epimerization was shown by treating the epimer **10** with CH_3CN/H_2O/0.007% $HClO_4$ for 3 h after which time a 1:1 mixture of the two epimers **3** and **10** was established.

Table 2. Activities of Halichondrin Compounds 1−3, 5−8, and 10 in the *in Vitro* P388 Assay

halichondrin compound	IC_{50} (ng/mL)
1	0.8
2	0.2
3	0.2
5	0.4
6	0.8
7	10
8	0.1
10	3.4

The biological activities of the halichondrin compounds **1**−**3**, **5**−**8**, and **10** were evaluated in an *in vitro* P388 assay, and the results are shown in Table 2. Clearly, all compounds have comparable potency in this assay, except for compounds **7** and **10**, whose activities are significantly reduced from that of the most closely related compound, isohomohalichondrin B (**3**). The result for compound **7** suggests that the presence of a terminal hydroxyl group is necessary for the expression of maximal activity in the P388 assay. In subsequent publications we shall be describing the biological activities of these compounds, and a series of other hemisynthetic compounds derived from the halichondrins **2** and **3**, in a range of other antitumor assays. The reduction in activity for the C38-*epi* compound **10** was also observed in the homohalichondrin B series.[5]

Experimental Section

All NMR spectra were recorded at 23 °C on a 300 MHz spectrometer fitted with a 3 or 5 mm indirect detection probe, using $CDCl_3$ with 0.1% C_5D_5N as the solvent. FAB mass spectra were obtained using Xe as the reagent gas, with the ion gun operating at 8 kV and 2 mA current with a *m*-nitrobenzyl alcohol (NOBA) matrix.

The *Lissodendoryx* sp. sponge (200 kg) was collected by trawling at depths of ~100 m off the Kaikoura coast of New Zealand in February 1992. Voucher specimens of the collected sponge are kept in the NIWA (Wellington, NZ) voucher collection. The sponge was frozen immediately after collection and then freeze-dried to give a powder (47 kg). Extraction of a portion of this powder (20 kg) was carried out in several batches, for which the following description provides a representative example of the procedures employed. Powder (6 kg) was extracted with CH_2Cl_2 (4 × 4 L), followed by MeOH (4 × 4 L)

(5) Hart, J. B.; Blunt, J. W.; Munro, M. H. G. *J. Org. Chem.* **1996**, *61*, 2888.

and then CH_2Cl_2 (4 × 2 L). The MeOH extract was dried down and partitioned between CH_2Cl_2 and H_2O. The CH_2Cl_2 fraction was combined with the original CH_2Cl_2 extracts and dried down to give the organic soluble material (112 g). This was partitioned between heptane (1 L) and MeOH/H_2O (2:8, 3 × 400 mL). After washing the combined MeOH/H_2O fractions with further heptane (500 mL), the MeOH/H_2O solution was extracted with CH_2Cl_2 (500 mL and 3 × 250 mL). The combined CH_2Cl_2 extracts were dried down to an oil (2.17 g). This material was applied to an LH-20 chromatography column in CH_2Cl_2. The bioactive fractions from this column were combined and rechromatographed on LH-20 to give active fractions which were combined to give an oil (60 mg). For some of the extractions, a further fractionation, by means of reverse-phase flash chromatography on ODS-silica columns using MeOH/CH_2Cl_2 eluents with compositions ranging from 1:9 to 5:95, was employed between the two LH-20 separations. The combined active fractions from all of the similar extractions from the freeze-dried sponge (20 kg) were then subjected to HPLC separations on ODS-silica semipreparative columns using a solvent system of CH_3CN/H_2O (11:9). These separations yielded the various halichondrin compounds as follows.

Halichondrin B (**1**) (43 mg; 5.1×10^{-5}%). NMR data in Table 1. Homohalichondrin B (**2**) (45 mg; 5.3×10^{-5}%). NMR data in Table 1.[5] Isohomohalichondrin B (**3**) (57 mg; 6.7×10^{-5}%). NMR data in Table 1. Norhalichondrin B (**4**) (0.6 mg; 7×10^{-7}%). Neonorhalichondrin B (**5**) as an oil (0.9 mg; 1×10^{-6}%), HRFABMS 1135.5236 (MK^+, calculated for $C_{59}H_{84}O_{19}K$ 1135.5243). NMR data in Table 1. Neohomohalichondrin B (**6**) as an oil (7 mg; 8×10^{-6}%), HRFABMS 1163.5553 (MK^+, calculated for $C_{61}H_{88}O_{19}K$ 1163.5550). NMR data in Table 1. 55-Methoxyisohomohalichondrin B (**7**) as an oil (0.7 mg; 8×10^{-7} %), HRFABMS 1175.5521 (MK^+, calculated for $C_{62}H_{88}O_{19}K$ 1175.5556). NMR data in Table 1. 53-Methoxyneoisohomohalichondrin B (**8**) as an oil (15 mg; 1.8×10^{-5}%), LRFABMS 1175.4 (MK^+) and 1159.5 (MNa^+). HRFABMS 1297.5755 (MK^+ + NOBA − OCH_3, calculated for $C_{68}H_{92}NO_{21}K$ 1297.5798). NMR data in Table 1. 53-*epi*-Methoxyneoisohomohalichondrin B (**9**) as an oil (1.5 mg).

Acid Treatment of 53-Methoxyneoisohomohalichondrin B (8). Ketal **8** (2.0 mg) in CH_3CN (0.06 mL) was treated with aqueous $HClO_4$ (0.035%, 0.015 mL) for 6 min, after which the solution was applied to a short ODS-silica column. The acid was washed through with H_2O, and the halichondrins were then stripped using CH_3CN. The mixture was analyzed by ODS-silica HPLC (CH_3CN/H_2O, 9:1), which revealed a 1:1 mixture of **3** and **10**. A further sample of ketal **8** (7.5 mg) in CH_3CN (0.3 mL) was treated with aqueous $HClO_4$ (0.035%, 0.08 mL) for 1 min. Workup and analysis similar to that used in the first reaction gave a 3:1 mixture of **3** and **10**. A pure sample of the 38-*epi*-isohomohalichondrin B (**10**) was prepared by semipreparative HPLC on ODS-silica (CH_3CN/H_2O, 9:1) as an oil. HRFABMS 1145.5653 (MNa^+, calculated for $C_{61}H_{86}O_{19}Na$ 1145.5661). NMR data in Table 1.

Acid Treatment of 38-*epi*-Isohomohalichondrin B (10). 38-*epi*-isohomohalichondrin B (**10**) (3.5 mg) in CH_3CN (0.11 mL) was treated with aqueous $HClO_4$ (0.035%, 0.03 mL). Workup and analysis of aliquots of this solution similar to that used in the previous reactions showed that a 1:1 mixture of **3** and **10** was achieved after 3 h, with no further change at longer times.

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Supporting Information Available: Copies of ^1H NMR spectra of **5**−**10** (6 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS. See any current masthead page for ordering information.

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