Marine Natural Products. XXXVIII.[1] Absolute Stereostructures of Altohyrtins A, B, and C and 5-Desacetylaltohyrtin A, Potent Cytotoxic Macrolides, from the Okinawan Marine Sponge *Hyrtios altum*

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Altohyrtins A (1), B (2), and C (3) and 5-desacetylaltohyrtin A (4), extremely potent cytotoxic macrolides, have been isolated from the Okinawan marine sponge *Hyrtios altum*. The absolute stereostructures of these macrolides have been elucidated on the bases of detailed NMR analysis, application of the modified α-methoxy-α-(trifluoromethyl)phenylacetic acid (MTPA) method to the hexa-MTPA esters, and application of the circular dichroism (CD) exciton chirality method.

Key words marine sponge; macrolide; cytotoxic; altohyrtin A; *Hyrtios altum*

In 1993, we isolated four novel cytotoxic macrolides named altohyrtins A (**1**), B (**2**), and C (**3**) and 5-desacetylaltohyrtin A (**4**) from the Okinawan marine sponge *Hyrtios altum* by bioassay-guided separation.[3] Fusetani and his group have isolated a similar compound, cinachyrolide A (**5**), from a marine sponge of *Cinachyra* sp.,[4] and Pettit and his group have independently isolated spongistatins 1 (**6**)—9 from marine sponges of *Spongia* sp.[5] and *Spirastrella spinispirulifera*.[6] These macrolides have the same carbon skeleton with two spiroketals and a halogen atom and exhibit extremely potent cytotoxic activities against cultured tumor cells. We have elucidated the absolute stereostructures of altohyrtins (**1**—**4**) on the bases of detailed NMR analysis, application of the modified α-methoxy-α-(trifluoromethyl)phenylacetic acid (MTPA) method to the hexa-MTPA esters, and application of the circular dichroism (CD) exciton chirality method. The partial relative stereostructures of cinachyrolide A (**5**) and spongistatins (*e.g.* spongistatin 1 (**6**)), which have been presumed on the basis of nuclear Overhauser effect spectroscopy (NOESY) analysis, are partly in conflict with those of altohyrtin A (**1**). In this paper, we present details of the elucidation of the absolute stereostructures of altohyrtins (**1**—**4**).

The acetone extract of the title fresh sponge (112 kg, collected in July at Aragusuku-jima, Okinawa Prefecture), which exhibited cytotoxic activity (IC_{50} 0.56 μg/ml) against KB cells, was subjected to bioassay-guided separation (cytotoxicities against KB and L1210 cells). The acetone extract was partitioned into a water–AcOEt mixture to provide the cytotoxic AcOEt-soluble portion (222 g). Repeated SiO_2 column chromatography of the AcOEt-soluble portion furnished fr.B (12.5 g) [IC_{50} 0.002 μg/ml (KB)]. The fr. B was found to have potent anti-tumor activity against P388 murine leukemia (mice, *i.p.*): T/C 155% (10 mg/kg treated on days 1, 5). Further repeated chromatography (SiO_2 and ODS HPLC) of the fr. B furnished altohyrtin A (**1**) (3.4×10^{-3}% from the AcOEt-soluble portion), altohyrtin B (**2**) (2.2×10^{-4}%), altohyrtin C (**3**) (2.2×10^{-4}%), and 5-desacetylaltohyrtin A (**4**) (2.1×10^{-3}%). Altohyrtins A (**1**), B (**2**), and C (**3**) and 5-desacetylaltohyrtin A (**4**) exhibited extremely potent cytotoxicity against KB cells with IC_{50} values of 0.01, 0.02, 0.4, and 0.3 ng/ml, respectively.

The plane structures of altohyrtin A (**1**) and 5-desacetylaltohyrtin A (**4**) have been elucidated on the bases of detailed analysis of correlation spectroscopy (COSY), ^{1}H-detected heteronuclear multiple quantum coherence (HMQC), homonuclear Hartmann-Hahn (HOHAHA), and HMBC spectra of **1** and **4** taken in pyridine-d_5, DMSO-d_6 and CD_3OD.[3a] Furthermore, comparisons of chemical shifts and the observed coupling patterns in their ^{1}H-NMR spectra have led us to presume that altohyrtins B (**2**) and C (**3**) possess the same relative stereostructure as that of altohyrtin A (**1**).[3b] Acetylation of **1** and **4** with Ac_2O, pyridine, and *N*,*N*-dimethylaminopyridine (DMAP) furnished the same heptaacetate **7**.[3c] The heptaacetate **7** [δ 6.35 (d, J = 16 Hz, H-36)], having a *trans*-35-en-37-one structure, was presumably obtained by opening of the hemiketal at C-37 followed by dehydration of the 35-hydroxyl moiety. Thus, it has been confirmed that 5-desacetylaltohyrtin A (**4**) possesses the same stereostructure, including the C-5 configuration, as that of altohyrtin A (**1**).

These altohyrtins have twenty-four chiral centers. In order to elucidate the absolute stereochemistry of these chiral centers, we have collected more than 200 kg of the marine sponges. The absolute stereostructures of altohyrtins have been elucidated in the following manner. Firstly, we have examined in detail the NOESY spectra of both altohyrtin A (**1**) and 5-desacetylaltohyrtin A (**4**) (Table 1).[3c] The NOESY correlations (in DMSO-d_6) in **4** allowed us to deduce three partial relative stereostructures [C-3—C-16; C-19—C-27; C-33—C-43]. However, on this stage, we could not clarify the optical correlation of these blocks.

Part C-3—C-16: The strong NOESY correlations observed between H-3 and the 5-hydroxyl proton (H-5OH) and between H-11 and H-9OH suggested that both H-3/H-5OH and H-11/H-9OH are situated in a 1,3-diaxial relation in the respective six-membered ring. The relative stereostructure of the AB spiroketal ring (C-3—C-11) was clarified by the additional NOESY correlations between H-3 and H-11 and between H_b-6 and H_b-8. Furthermore, the relative stereostructure from C-11 to C-16 was defined by the NOESY correlations between H-5OH and H_2-53,

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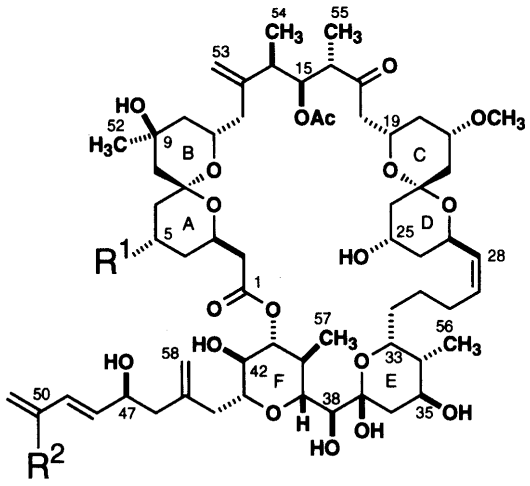
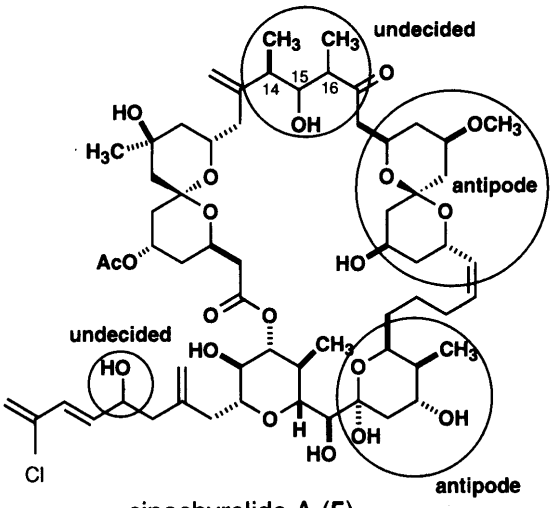
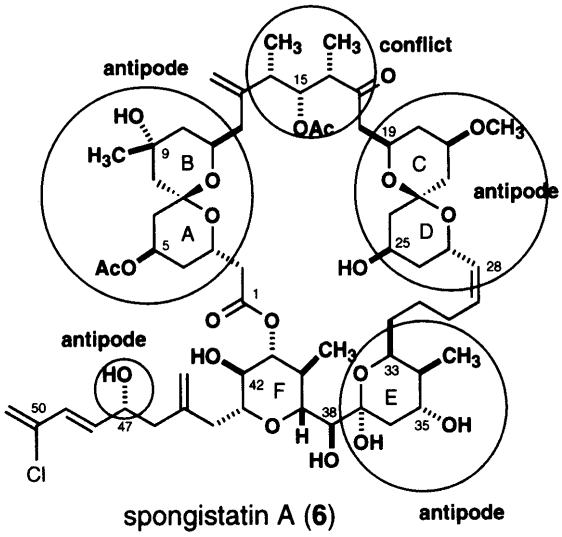


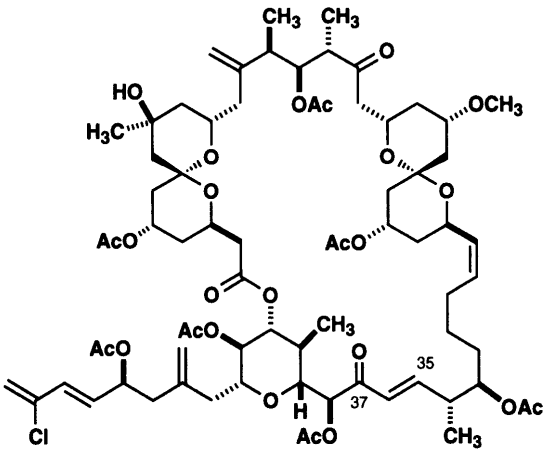
	R^1	R^2
altohyrtin A (**1**)	OAc	Cl
altohyrtin B (**2**)	OAc	Br
altohyrtin C (**3**)	OAc	H
5-desacetyl-altohyrtin A (**4**)	OH	Cl



cinachyrolide A (**5**)



spongistatin A (**6**)



7

	R^1	R^2	R^3
8	*R*-(+)-MTPA	*R*-(+)-MTPA	*R*-(+)-MTPA
9	*S*-(-)-MTPA	*S*-(-)-MTPA	*S*-(-)-MTPA
10	Ac	H	-CO-C$_6$H$_4$-Br

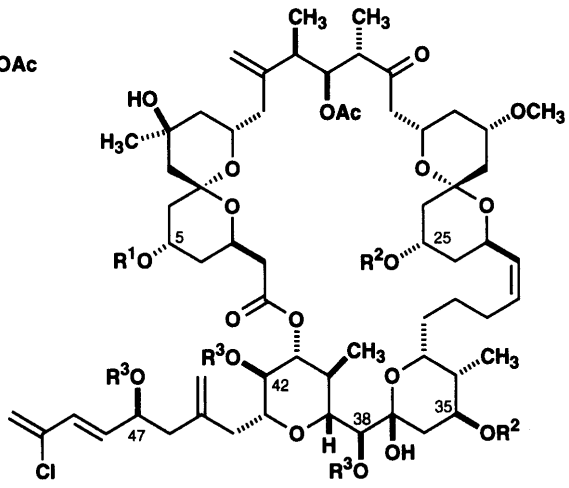


Chart 1

between H_2-10 and H_{ab}-12, between H_a-12 and H-14 and H-15, between H_b-12 and H_2-53, between H-14 and H-15 and 55-methyl protons, between H-15 and 55-methyl protons, and between 54-methyl protons and H-16 and H_2-53, as well as the coupling constants ($J_{14,15}=1.0$ Hz, $J_{15,16}=10.5$ Hz), as depicted in Fig. 1a.

Part C-19—C-27: The strong NOESY correlations observed between H-19 and H-21 and between H-25OH and H-27 indicated that both H-19 and H-21, and H-25OH and H-27 are situated in a 1,3-diaxial relation in the

Table 1. ^{1}H-NMR Data for Altohyrtins A (**1**) and 5-Desacetylaltohyrtin A (**4**) (at 500 MHz in d_6-DMSO, J Values in Hz)

Proton(s) at	1	4	Proton(s) at	1	4
2	2.50 (m)	2.58 (d-like, 10.5)	34	1.43 (m)	1.43 (m)
	2.70 (m)	2.67 (m)	35	3.60 (m)	3.60 (m)
3	4.17 (t-like, 11.5)	4.08 (m)	36	1.52 (m)	1.53 (d-like, 10.5)
4	1.53 (m)	1.47 (m)		1.83 (m)	1.83 (dd, 10.5, 4.5)
	1.65 (m)	1.56 (m)	38	3.28 (d, 7)	3.25 (d, 8)
5	4.90 (m)	3.85 (m)	39	3.66 (d, 10.5)	3.67 (d, 10)
6	1.62 (m) (a)	1.53 (m) (a)	40	1.84 (m)	1.87 (m)
	1.78 (d-like, 10.5) (b)	1.65 (m) (b)	41	4.68 (t-like, 10)	4.71 (m)
8	1.52 (s) (a)	1.52 (s) (a)	42	3.05 (ddd, 10.5, 10, 6)	3.05 (ddd, 10, 9, 6)
	1.55 (s) (b)	1.55 (s) (b)	43	3.37 (t-like, 10.5)	3.39 (t-like, 9)
10	1.21 (m)	1.32 (t-like, 12.5)	44	2.05 (m) (a)	2.00 (m) (a)
11	4.55 (t-like, 11)	4.57 (t-like, 11.5)		2.73 (m) (b)	2.72 (dd, 14, 3) (b)
12	2.05 (m) (b)	1.97 (m) (b)	46	2.12 (m)	2.12 (dd, 14, 6.5)
	2.25 (m) (a)	2.36 (d-like, 13.5) (a)		2.24 (d-like, 13)	2.25 (d-like, 14)
14	2.83 (q-like, 6.5)	2.83 (q-like, 7)	47	4.26 (dd-like, 13, 6)	4.25 (m)
15	5.17 (d-like, 11)	5.21 (d-like, 12)	48	6.07 (dd, 15, 6)	6.07 (dd, 15, 5.5)
16	2.98 (dq, 11, 7)	2.98 (dq, 10.5, 7)	49	6.40 (d, 15)	6.40 (dd, 15, 1)
18	2.67 (d-like, 18) (b)	2.67 (d-like, 18) (b)	51	5.36 (s)	5.36 (s)
	2.76 (dd, 18, 10) (a)	2.79 (dd, 18, 10) (a)		5.54 (s)	5.54 (s)
19	3.96 (t-like, 11.5)	3.95 (t-like, 11.5)	52	1.03 (s)	1.03 (s)
20	0.84 (m) (b)	0.87 (d-like, 11) (b)	53	4.75 (s) (b)	4.72 (s)
	2.00 (m) (a)	2.00 (m) (a)		4.80 (s) (a)	4.72 (s)
21	3.50 (m, Wh/2=24)	3.49 (m)	54	0.93 (d, 6.5)	0.91 (d, 7)
22	1.04 (m) (a)	1.04 (m) (a)	55	1.09 (d, 7)	1.09 (d, 7)
	1.98 (m) (b)	1.96 (dd, 11.5, 7.5) (b)	56	0.81 (d, 7)	0.81 (d, 7)
24	1.55 (m) (a)	1.55 (m) (a)	57	0.73 (d, 6.5)	0.73 (d, 6.5)
	2.23 (m) (b)	2.23 (m) (b)	58	4.82 (s) (a)	4.82 (s) (a)
25	3.87 (m, Wh/2=13)	3.85 (m)		4.85 (s) (b)	4.85 (s) (b)
26	1.43 (m)	1.42 (m)	5-Ac	1.94 (s)	—
	1.53 (m)	1.53 (m)	15-Ac	1.82 (s)	1.81 (s)
27	4.90 (m)	4.89 (td-like, 7, 4)	21-OMe	3.21 (s)	3.21 (s)
28	5.35 (d-like, 11)	5.34 (d-like, 11)	5-OH	—	3.72 (d, 10.5)
29	5.36 (m)	5.36 (m)	9-OH	3.93 (s)	3.89 (s)
30	2.05 (m)	2.02 (m)	25-OH	4.28 (d, 10.5)	4.25 (d, 10)
	2.08 (m)	2.05 (m)	35-OH	4.10 (d, 7)	4.10 (d, 7)
31	1.12 (m) (a)	1.12 (m) (a)	37-OH	4.74 (s)	4.74 (d, 2)
	1.65 (m) (b)	1.65 (m) (b)	38-OH	4.63 (d, 7)	4.63 (d, 8)
32	1.12 (m)	1.12 (m)	42-OH	5.25 (d, 6)	5.26 (d, 6)
	1.28 (m)	1.26 (m)	47-OH	4.94 (d, 6)	4.94 (d, 5.5)
33	4.05 (d-like, 10.5)	4.05 (d-like, 10)			

respective six-membered ring. Furthermore, the relative stereostructure from C-19 to C-27 (CD spiroketal ring) was defined by the NOESY correlations between H-19 and H_b-24 and H-25OH, and between H_b-22 and H_a-24 (Fig. 1b).

Part C-33—C-43: The strong NOESY correlations observed between H-33 and H-35OH and H-37OH, and between H-35OH and H-37OH indicated that H-33, H-35OH, and H-37OH are situated in 1,3-diaxial positions with respect to each other and the relative stereostructure from C-33 to C-37 (E ether ring) was clarified by the additional NOESY correlations between the 56-methyl protons and H-35 and H_a-36, and between H-33 and H-34 (Fig. 1c). The relative stereostructure from C-39 to C-43 (F ether ring) was similarly clarified based on the strong NOESY correlations between H-39 and H-41 and H-43, between H-41 and H-43, and between H-40 and H-42 (Fig. 1d). Finally, the correlation between part C-33—C-37 and part C-38—C-43 was presumed on the basis of the additional NOESY correlations between H-38 and H_{ab}-36, H-40 and the 57-methyl protons. Furthermore, NOESY correlations between H_a-18 and H_a-20, between H-27 and H_{ab}-30, between H_a-31 and H-33, between H-42 and H_{ab}-44, and between H_a-44 and H_a-58 were very useful to construct the total ring structures of 5-desacetylaltohyrtin A (**4**), as shown in Fig. 2.

Secondly, we applied the modified MTPA method[7] to determine the absolute stereostructures of altohyrtins (**1**—**4**). Treatment of **4** with (*R*)-(+)-2-methoxy-2-(trifluoromethyl)phenylacetic acid ((*R*)-(+)-MTPA) or (*S*)-(−)-MTPA, dicyclohexylcarbodiimide (DCC) and DMAP in CH_2Cl_2 at room temperature furnished the hexa-MTPA ester (**8** or **9**, respectively). The proton signals of both **8** and **9** were assigned as given in Table 2 on the bases of COSY, HOHAHA, and HMQC experiments. Detailed comparisons of the chemical shifts of all the proton signals in **8** and **9** gave the $\Delta\delta$ values (δ_S–δ_R) depicted in Fig. 3 (Table 2). The absolute configuration of the C-5 secondary hydroxyl group is defined as *S* from the correlation between the A block and the B block. In the same way, the absolute configurations of secondary hydroxyl groups [C-25 (B block and C block), C-35 (D block and E block), C-38 (D′ block and E block), C-42 (A block and F block), and C-47 (G block and H block)] have been clarified to be 25*S*, 35*S*, 38*S*, 42*R*, and 47*S*, respectively. In this experiment, significant $\Delta\delta$ values were

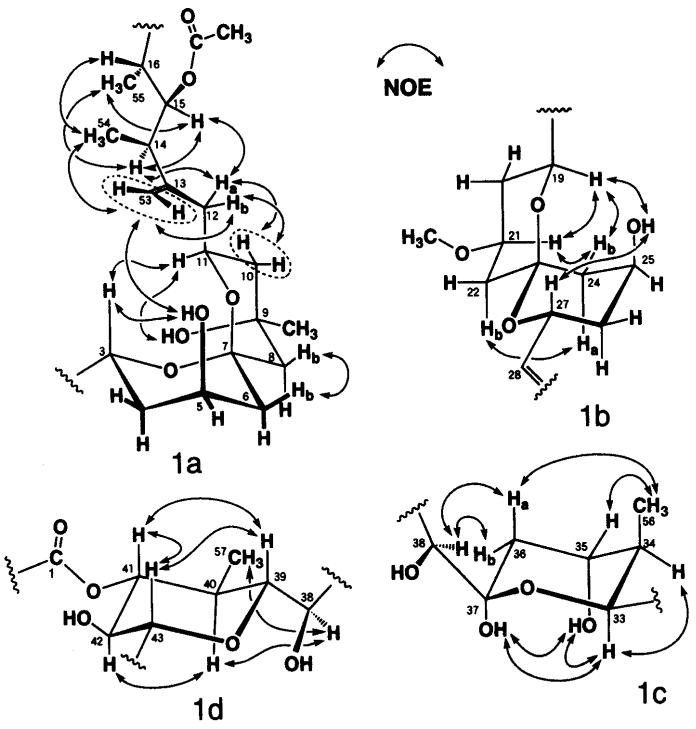


Fig. 1. NOESY Data for the Partial Structures of 5-Desacetylaltohyrtin A (**4**)

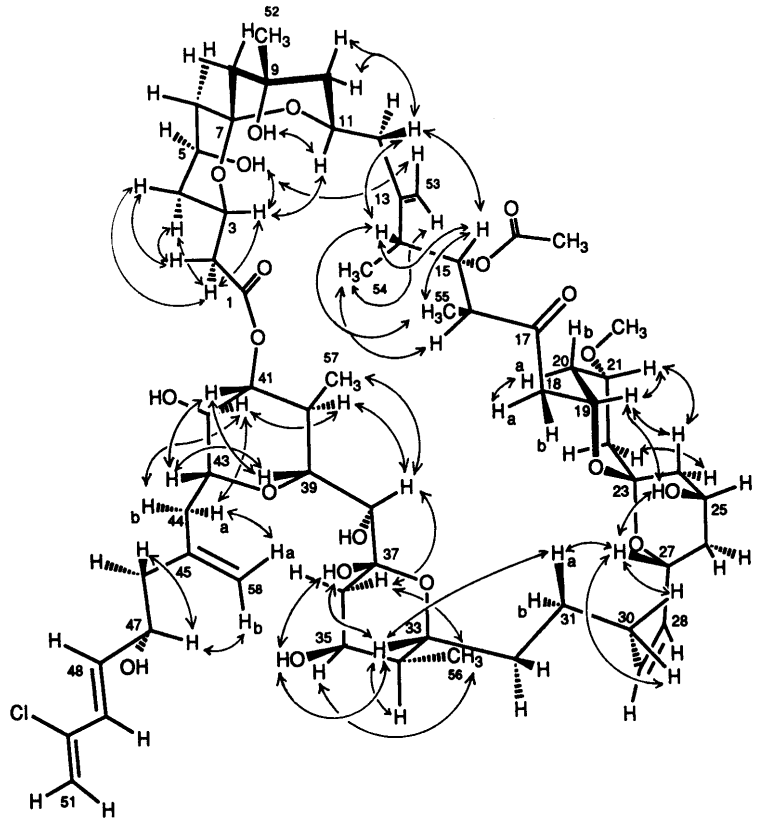


Fig. 2. NOESY Data for 5-Desacetylaltohyrtin A (**4**)

observed for the signals of the 56-methyl protons ($\Delta\delta = +0.46$ ppm) and H-40 ($\Delta\delta = -0.44$ ppm). These significant $\Delta\delta$ values indicated that the chemical shift of the 56-methyl protons was strongly affected by both the C-35 and C-38 MTPA residues and that of H-40 was also affected by both the C-38 and C-42 MTPA residues. Furthermore, the $\Delta\delta$ values of the signals of H-15, H-16, H_{ab}-18 and the 54-methyl protons showed the opposite sign compared with those of neighboring protons. From conformational analysis using a molecular model, it is presumed that these reversed shifts of $\Delta\delta$ values are caused by the spatial locations with respect to the benzene ring of the *R*-(+)-MTPA residue at C-25 and these protons are deshielded owing to the paramagnetic anisotropy of

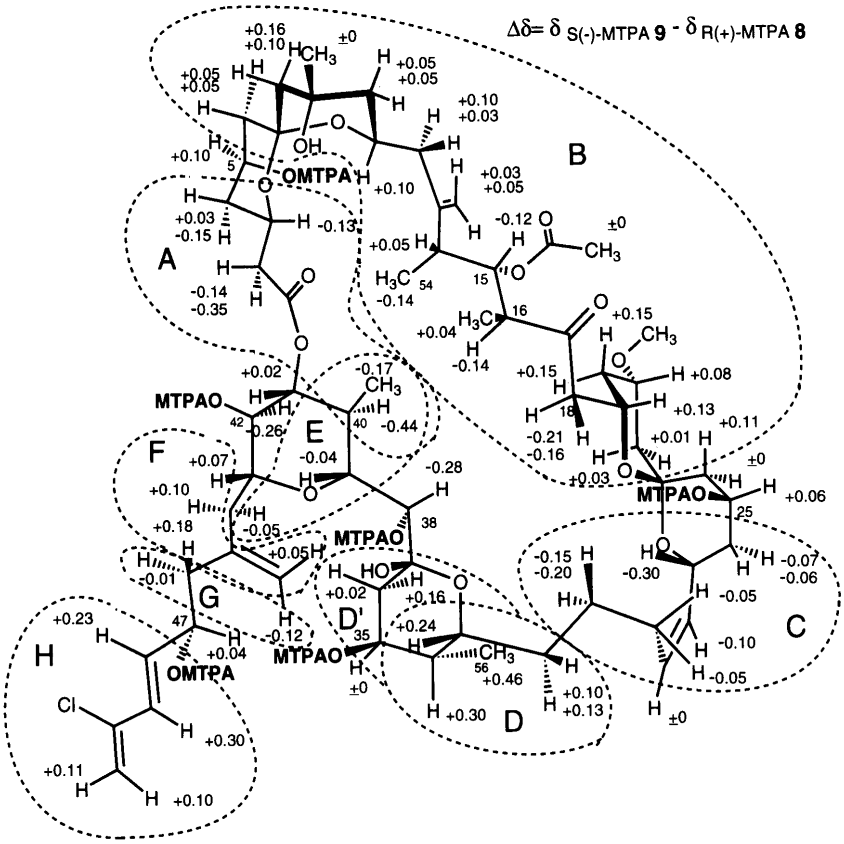


Fig. 3. Application of Modified Mosher's Method to **8** and **9**

Table 2. ^{1}H-NMR Data for the (*R*)-(+)-Hexa-MTPA Ester (**8**) and (*S*)-(−)-Hexa-MTPA Ester (**9**) (at 500 MHz in d_6-DMSO, *J* Values in Hz)

Proton(s) at	8	9	Proton(s) at	8	9
2	2.50 (m), 2.61 (m)	2.15 (m), 2.47 (m)	33	3.88 (t-like, 9)	4.12 (t-like, 10)
3	4.15 (m)	4.02 (m)	34	1.40 (m)	1.70 (m)
4	1.58 (m), 2.00 (m)	1.61 (m), 1.85 (m)	35	4.92 (m)	4.92 (m)
5	5.28 (m)	5.38 (m)	36	1.73 (m), 1.74 (m)	1.75 (m), 1.90 (m)
6	1.80 (m), 1.85 (m)	1.85 (m), 1.90 (m)	38	4.98 (s)	4.70 (s)
8	1.45 (s), 1.48 (s)	1.55 (s), 1.64 (s)	39	3.94 (d, 11)	3.90 (d, 11)
10	1.10 (m), 1.60 (m)	1.15 (m), 1.65 (m)	40	1.94 (m)	1.50 (m)
11	3.95 (m)	4.05 (m)	41	4.90 (m)	4.92 (m)
12	1.69 (m), 2.00 (m)	1.72 (m), 2.10 (m)	42	4.96 (m)	4.70 (m)
14	2.40 (q-like, 7)	2.45 (m)	43	3.73 (t-like, 10)	3.80 (td, 10.5, 2)
15	4.97 (m)	4.85 (t-like, 5)	44	1.83 (m), 2.02 (m)	1.93 (m), 1.97 (m)
16	2.94 (m)	2.80 (t-like, 5)	46	2.05 (m), 2.24 (m)	2.23 (m), 2.23 (m)
18	2.61 (m), 2.71 (m)	2.45 (m), 2.50 (m)	47	5.59 (dd-like, 12, 6)	5.63 (dd-like, 12, 6)
19	3.57 (t-like, 11)	3.70 (m)	48	5.78 (dd, 15, 6)	6.01 (dd, 15, 6)
20	0.63 (m), 1.91 (m)	0.78 (m), 2.06 (m)	49	6.20 (d, 15)	6.50 (d, 15)
21	3.42 (m)	3.50 (m)	51	5.45 (s), 5.50 (s)	5.55 (s), 5.61 (s)
22	1.02 (m), 1.97 (m)	1.05 (m), 1.98 (m)	52	1.05 (s)	1.05 (s)
24	1.75 (m), 2.27 (m)	1.75 (m), 2.38 (m)	53	4.70 (s), 4.75 (s)	4.75 (s), 4.78 (s)
25	5.28 (m)	5.34 (m)	54	0.89 (d, 7)	0.75 (d, 7)
26	1.67 (m), 1.79 (m)	1.60 (m), 1.73 (m)	55	0.91 (d, 7)	0.95 (d, 7)
27	5.05 (td-like, 10, 4)	4.75 (m)	56	0.24 (d, 7)	0.70 (d, 7)
28	5.45 (m)	5.35 (m)	57	0.95 (d, 6.5)	0.78 (d, 7)
29	5.40 (m)	5.40 (m)	58	4.65 (s), 4.82 (s)	4.70 (s), 4.70 (s)
30	1.90 (m), 1.90 (m)	1.85 (m), 1.85 (m)	15-Ac	1.78 (s)	1.78 (s)
31	2.00 (m), 2.00 (m)	1.80 (m), 1.85 (m)	21-OMe	3.29 (s)	3.29 (s)
32	1.05 (m), 1.20 (m)	1.18 (m), 1.30 (m)			

the benzene ring. These significant and/or characteristic values of $\Delta\delta$ are useful to construct three-dimensional structures.

Thirdly, we have applied the CD exciton chirality method[8] to the *p*-bromobenzoate (**10**), which was prepared from **1** by treatment with *p*-bromobenzoic acid, DCC, and DMAP. Detailed analysis of the COSY and HOHAHA spectra of **10** clarified that **10** was the 38, 42,

47-tri-*p*-bromobenzoate. Among four chromophores, the 47-*p*-bromobenzoate and the 48,50-diene chromophores are close to each other and strong exciton coupling between these two chromophores is expected. Compound **8** showed characteristic split CD maxima ($\Delta\varepsilon+44.5$ at 244 nm, $\Delta\varepsilon-15.5$ at 230 nm). In the case of the acyclic allylic benzoate group, the large J value of 5.2—9.2 Hz between the olefinic proton and carbinyl proton is necessary for application of this method. The coupling constant between H-47 and H-48 was 6.5 Hz in CD_3OD in **10**. Thus, the absolute configuration at C-47 of **10** and consequently of **1** have been determined as *S*. The result thus obtained is consistent with that obtained by the above MTPA method.

The above-mentioned evidence led us to propose the absolute stereostructures of altohyrtins (**1**—**4**) as shown. The plane structures of spongistatins 1 (**6**), 2, and 3 were identical with those of our altohyrtins A (**1**) and C (**3**), and 5-desacetylaltohyrtin A (**4**), respectively, and cinachyrolide A (**5**) seems to a 15-desacetyl analogue of **1**. The NMR data of cinachyrolide A (**5**) and spongistatins are closely similar to those of altohyrtins. So, the absolute stereostructures of these compounds (**5** and **6**) are presumed to be the same as those of altohyrtins. The relative stereostructures of cinachyrolide A (**5**)[4] and spongistatin 1 (**6**)[6c] have been partly presumed from the analysis of NOESY data. However, the optical correlation of the blocks in these stereostructures was not defined. Furthermore, the relative stereochemistry of C-14, -15, and -16 in **5** is undetermined and that in **6** is in conflict with that in **1**. In order to accumulate more evidence concerned with the absolute stereostructure of altohyrtins, a molecular modeling study of 5-desacetylaltohyrtin A (**4**) using restrained molecular dynamics calculation[9] was performed. The NOESY spectrum of 5-desacetylaltohyrtin A (**4**) was measured in DMSO at 20 °C. The intensities of NOE cross peaks were classified into five classes and translated into upper bounds for distance restraints; 322 distance restraints were used for the simulated annealing calculation. Simulated annealing calculations were carried out with the program Discover NMRchitect (Biosym). So far, the average RMSD of the backbone (C-1—C-43) for the ten lowest energy structures was 0.55 Å. This RMSD value strongly supports the correctness of our proposed stereostructure.[10]

In order to explain the reversed shifts observed in the hexa-MTPA ester of 5-desacetylaltohyrtin A (**4**) using the modified MTPA method, we have added an *R*-MTPA residue to the 25-hydroxyl moiety in the graphical drawing of the lowest energy structure obtained by restrained molecular dynamics calculation. It was found that each of H-15, H-16, H_2-18, and the 54-methyl protons, which showed the reversed shifts, was situated near the end of the benzene ring at the 25-MTPA residue. This strongly supports the presumption that these protons were subject to the deshielding effect of the paramagnetic anisotropy of the benzene ring.

As for the mechanism of cytotoxicity of these macrolides, Pettit and his group reported that spongistatin 1 (**6**) inhibited mitosis by binding to the *Vinca* alkaloid domain of tubulin.[11]

As described above, altohyrtins and related compounds have been isolated from several different genera (and/or subclasses) of marine sponge. By bioassay-guided separation (cytotoxicities against KB and L1210 cells), we have recently isolated altohyrtins A (**1**) and C (**3**) from a marine sponge of *Haliclona* sp., which was collected at Amami Island, Kagoshima Prefecture, each in 3.1×10^{-4}% yield from the AcOEt extract. Most marine sponges live as a "miniature conglomerate" which usually comprises several kinds of microorganisms, such as cyanobacteria, fungi, and/or bacteria. Thus, symbiotic or parasitic microorganism(s) may be responsible for the production of these macrolides. During our attempts to find a microorganism(s) producing altohyrtins, we have isolated a bacterium of *Vibrio* sp. from the fresh marine sponge *Hyrtios altum*, and have isolated a new antibiotic indole trimer named trisindoline from the culture.[12]

Experimental

The IR spectra were obtained with a Hitachi 260-30 IR spectrometer or a JASCO FT-IR 5300 spectrometer. Optical rotations were measured with a JASCO DIP-370 digital polarimeter. The ^{1}H- and ^{13}C-NMR spectra were measured with a JEOL GX-500 (500 MHz) spectrometer using Me_4Si (0 ppm), pyridine-d_5 (135.0 ppm), DMSO-d_6 (39.5 ppm) and CD_3OD (49.8 ppm) signals as internal standards. 2D-NMR spectra were recorded on a JEOL GX-500 (500 MHz), a Bruker AMX500 NMR (500 MHz), and/or a Varian UNITY-600 (600 MHz) spectrometer. The UV spectra were obtained with a Hitachi 330 spectrometer. The FAB MS were recorded on a JEOL JMS SX-102 mass spectrometer.

Isolation from the Marine Sponge *Hyrtios altum* The frozen sponge (112 kg, wet weight) was extracted with acetone at room temperature 3 times for 8 h each. The residue obtained by evaporation of the solvent under reduced pressure was partitioned into an ethyl acetate–water mixture (1 : 1), and the ethyl acetate layer was evaporated to give the ethyl acetate-soluble portion (222 g). The ethyl acetate-soluble portion was subjected to bioassay-guided separation (cytotoxicities against KB and L1210 cells). The SiO_2 column eluted with AcOEt→acetone→MeOH gave three fractions [fr. A (88.0 g), fr. B (12.5 g), fr. C (111.4 g)]. The fr. B was further separated by SiO_2 column ($CHCl_3$: MeOH = 30 : 1→10 : 1→MeOH) and MPLC (SiO_2, $CHCl_3$: MeOH = 25 : 1) to give cytotoxic fractions B-2-1 (1.3 g) and B-2-2 (0.8 g). The fraction B-2-1 was further separated by HPLC (ODS, Cosmosil 5C_{18}-AR, MeOH : H_2O = 5 : 2) to give altohyrtin A (**1**, 7.6 mg, 3.4×10^{-3}% from the AcOEt extract) and altohyrtin B (**2**, 0.5 mg, 2.2×10^{-4}%). The fraction B-2-2 was also purified by HPLC (ODS, Cosmosil 5C_{18}-AR, MeOH : H_2O = 2 : 1) to give altohyrtin C (**3**, 0.5 mg, 2.2×10^{-4}%) and 5-desacetylaltohyrtin A (**4**, 4.7 mg, 2.1×10^{-3}%).

Altohyrtin A (**1**): amorphous solid. $[\alpha]_D$ +21.7° (c = 1.2, MeOH). UV λ_{max}^{MeOH}: 227 nm (ε 19000). IR $\nu_{max}^{CHCl_3}$: 3423, 1733 cm^{-1}. FAB-MS: m/z 1229 $(M+Li)^+$. HR-FAB MS m/z: Calcd for $C_{63}H_{95}O_{21}{}^{35}ClLi$: 1229.6214. Found: 1229.6100. ^{1}H-NMR (500 MHz, DMSO-d_6) δ: as shown in Table 1. ^{1}H-NMR (500 MHz, CD_3OD) δ: 2.62 (m, H_a-2), 2.60 (d-like, J = 9.5 Hz, H_b-2), 4.35 (m, H-3), 1.69 (m, H_a-4), 1.61 (m, H_b-4), 5.04 (br s, H-5), 1.90 (d-like, J = 15 Hz, H_a-6), 1.69 (m, H_b-6), 1.68 (d, J = 14 Hz, H_a-8), 1.49 (d, J = 14 Hz, H_b-8), 1.49 (m, H_a-10), 1.37 (m, H_b-10), 4.58 (t-like, J = 11.5 Hz, H-11), 2.35 (m, H_a-12), 2.17 (m, H_b-12), 2.98 (d-like, J = 7 Hz, H-14), 5.32 (d, J = 10.5 Hz, H-15), 3.10 (dd, J = 10.5, 7 Hz, H-16), 2.92 (dd, J = 18, 10 Hz, H_a-18), 2.74 (m, H_b-18), 4.08 (m, H-19), 2.04 (m, H_a-20), 1.05 (m, H_b-20), 3.57 (m, H-21), 2.04 (m, H_a-22), 1.18 (m, H_b-22), 2.35 (m, H_a-24), 1.61 (m, H_b-24), 4.01 (br s, H-25), 1.61 (m, H_2-26), 5.04 (m, H-27), 5.39 (t-like, J = 10 Hz, H-28), 5.49 (m, H-29), 2.35 (m, H_a-30), 2.17 (m, H_b-30), 1.69 (m, H_a-31), 1.60 (m, H_b-31), 1.44 (m, H_a-32), 1.29 (m, H_b-32), 4.21 (m, H-33), 1.61 (m, H-34), 3.77 (m, H-35), 2.04 (m, H_a-36), 1.69 (m, H_b-36), 3.40 (s, H-38), 3.81 (d, J = 10.5 Hz, H-39), 1.97 (m, H-40), 4.85 (br s, H-41), 3.16 (t-like, J = 9 Hz, H-42), 3.40 (m, H-43), 2.79 (t-like, J = 15.5 Hz, H_a-44), 2.17 (m, H_b-44), 2.35 (m, H_a-46), 2.26 (m, H_b-46), 4.39 (m, H-47), 6.15 (dd, J = 14.5, 6.5 Hz, H-48), 6.42 (d, J = 14.5 Hz, H-50), 5.44 (s, H_a-51), 5.34 (s, H_b-51), 1.13 (s, H-52), 4.92 (s, H_a-53), 4.85 (s, H_b-53), 1.05 (d, J = 7 Hz, H-54), 1.22 (d, J = 7 Hz, H-55), 0.91 (d, J = 7.5 Hz, H-56), 0.84 (d, J = 6.5 Hz,

H-57), 4.97 (s, H_2-58), 2.01 (s, H-5Ac), 1.86 (s, H-15Ac), 3.33 (s, H-21OMe).

^{13}C-NMR (125 MHz, CD_3OD) δ_C: 174.2 (s, C-1), 40.9 (t, C-2), 63.5 (d, C-3), 35.5 (t, C-4), 68.6 (d, C-5), 39.2 (t, C-6), 100.3 (s, C-7), 47.7 (t, C-8), 71.1 (s, C-9), 46.2 (t, C-10), 65.8 (d, C-11), 45.2 (t, C-12), 149.4 (s, C-13), 37.7 (d, C-14), 76.1 (d, C-15), 48.9 (d, C-16), 214.0 (s, C-17), 52.2 (t, C-18), 67.3 (d, C-19), 38.7 (t, C-20), 75.4 (d, C-21), 44.9 (t, C-22), 101.0 (s, C-23), 35.7 (t, C-24), 65.9 (d, C-25), 39.8 (t, C-26), 62.5 (d, C-27), 132.2 (d, C-28), 134.9 (d, C-29), 28.9 (t, C-30), 28.4 (t, C-31), 34.0 (t, C-32), 68.7 (d, C-33), 40.7 (d, C-34), 72.8 (d, C-35), 34.9 (t, C-36), 100.0 (s, C-37), 74.2 (d, C-38), 82.6 (d, C-39), 38.5 (d, C-40), 81.4 (d, C-41), 74.4 (d, C-42), 80.7 (d, C-43), 41.5 (t, C-44), 144.6 (s, C-45), 45.1 (t, C-46), 71.8 (d, C-47), 139.5 (d, C-48), 128.7 (d, C-49), 140.3 (s, C-50), 117.1 (t, C-51), 30.9 (q, C-52), 115.6 (t, C-53), 12.9 (q, C-54), 15.0 (q, C-55), 12.7 (q, C-56), 13.7 (q, C-57), 117.2 (t, C-58), 173.5 (s, C-5Ac), 22.4 (q, C-5Ac), 172.0 (s, C-15Ac), 21.6 (q, C-15Ac), 56.7 (q, 21-OMe).

Altohyrtin B (**2**): Amorphous solid. $[\alpha]_D$ +44.7° (c=0.2, MeOH). UV λ_{max}^{MeOH}: 228 nm (ε 20000). IR ν_{max}^{KBr}: 3418, 1734 cm^{-1}. FAB-MS: m/z 1291 $(M+Na)^+$. HR-FAB MS m/z: Calcd for $C_{63}H_{95}O_{21}{}^{81}BrNa$: 1291.5449. Found: 1291.5426.

Altohyrtin C (**3**): Amorphous solid. $[\alpha]_D$ +31.2° (c=0.2, MeOH). UV λ_{max}^{MeOH}: 226 nm (ε 17000). IR ν_{max}^{KBr}: 3420, 1736 cm^{-1}. FAB-MS: m/z 1195 $(M+Li)^+$. HR-FAB MS m/z: Calcd for $C_{63}H_{96}O_{21}Li$: 1195.6604. Found: 1195.6720.

5-Desacetylaltohyrtin A (**4**): Amorphous solid. $[\alpha]_D$ +18.6° (c=1.1, MeOH). UV λ_{max}^{MeOH}: 228 nm (ε 20000). IR $\nu_{max}^{CHCl_3}$: 3416, 1736 cm^{-1}. FAB-MS m/z: 1203 $(M+Na)^+$. HR-FAB MS m/z: Calcd for $C_{61}H_{93}O_{20}{}^{35}ClNa$: 1203.5819. Found: 1203.5872. ^{1}H-NMR (500 MHz, DMSO-d_6) δ: as shown in Table 1.

Acetylation of Altohyrtin A (1) and 5-Desacetylaltohyrtin A (4) A solution of **1** (1.1 mg) in pyridine (0.3 ml) was treated with Ac_2O (0.3 ml) and DMAP (1.0 mg). The reaction mixture was stirred at room temperature (25 °C) for 3 h under an N_2 atmosphere. The whole was partitioned into ethyl acetate–water mixture and the organic layer was evaporated under reduced pressure to provide the crude product. The crude product was purified by HPLC (ODS, Cosmosil 5C_{18}-AR, MeOH : H_2O : CH_2Cl_2 = 85 : 15 : 1) to afford the heptaacetate **7** (1.1 mg). A solution of **4** (1.0 mg) in pyridine (0.3 ml) was also treated in the same manner to afford the heptaacetate **7** (1.0 mg).

Heptaacetate **7**: UV λ_{max}^{MeOH}: 224 (ε 21000), 230 nm (ε 22000). IR $\nu_{max}^{CHCl_3}$: 3323, 1736, 1234 cm^{-1}. FAB-MS m/z: 1437 $(M+Na)^+$, HR-FAB MS m/z: Calcd for $C_{73}H_{103}O_{25}{}^{35}ClNa$: 1437.6370. Found: 1437.6431. ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 2.74 (m, H_a-2), 2.52 (m, H_b-2), 4.18 (t-like, J=11 Hz, H-3), 1.65 (m, H_a-4), 1.50 (m, H_b-4), 4.89 (m , H-5), 1.80 (m, H_a-6), 1.65 (m, H_b-6), 1.50 (m, H_2-8), 1.48 (m, H_a-10), 1.30 (m, H_b-10), 4.38 (t-like, J=11 Hz, H-11), 2.23 (m, H_a-12), 2.12 (m, H_b-12), 2.77 (m, H-14), 5.18 (d-like, J=10 Hz, H-15), 2.98 (dq, J=10, 7 Hz, H-16), 2.82 (m, H_a-18), 2.70 (dd, J=17.5, 10 Hz, H_b-18), 3.79 (dd-like, J=11, 5.5 Hz, H-19), 1.97 (m, H_a-20), 0.95 (m, H_b-20), 3.49 (m, H-21), 2.00 (m, H_a-22), 1.07 (m, H_b-22), 2.30 (m, H_a-24), 1.58 (m, H_b-24), 4.95 (m, H-25), 1.65 (m, H_a-26), 1.58 (m, H_b-26), 4.95 (m, H-27), 5.32 (t-like J=11 Hz, H-28), 5.52 (m, H-29), 2.10 (m, H_a-30), 1.92 (m, H_b-30), 1.50 (m, H_a-31), 1.32 (m, H_b-31), 1.64 (m, H_a-32), 1.55 (m, H_b-32), 4.88 (d-like, J=10 Hz, H-33), 2.59 (m, H-34), 6.79 (dd, J=16, 8 Hz, H-35), 6.35 (d, J=16 Hz, H-36), 5.57 (d, J=2 Hz, H-38), 3.95 (dd, J=10, 2 Hz, H-39), 1.95 (m, H-40), 4.85 (m, H-41), 4.56 (dd, J=9, 9 Hz, H-42), 3.50 (m, H-43), 2.10 (m, H_a-44), 2.00 (m, H_b-44), 2.25 (m, H_2-46), 5.37 (dd-like, J=12, 6 Hz, H-47), 6.00 (dd, J=15, 6 Hz, H-48), 6.52 (d, J=15 Hz, H-49), 5.58 (s, H_a-51), 5.50 (s, H_b-51), 1.03 (s, H-52), 4.85 (s, H_a-53), 4.79 (s, H_b-53), 0.97 (d, J=6.5 Hz, H-54), 1.17 (d, J=7 Hz, H-55), 1.02 (d, J=7 Hz, H-56), 0.82 (d, J=6 Hz, H-57), 4.73 (s, H_2-58), 3.83 (s, 9-OH), 1.80—2.00 (s, Ac × 7).

Synthesis of the Hexa-MTPA Ester of 5-Desacetylaltohyrtin A (4) A solution of **4** (0.9 mg) in CH_2Cl_2 (0.2 ml) was treated with (*R*)-(+)-MTPA (1.0 mg), DCC (2.3 mg), and DMAP (1.0 mg). The reaction mixture was stirred at room temperature (25 °C) for 48 h under an N_2 atmosphere. MeOH (0.5 ml) was added, and the whole directly purified by HPLC (ODS, Cosmosil 5C_{18}-AR, MeOH : H_2O = 15 : 1) to afford the (*R*)-(+)-hexa-MTPA ester **8** (1.3 mg). A solution of **4** (0.9 mg) in CH_2Cl_2 (0.2 ml) was similarly treated with (*S*)-(−)-MTPA (1.0 mg), DCC (2.1 mg), and DMAP (1.0 mg) to afford the (*S*)-(−)-hexa-MTPA ester **9** (1.2 mg).

(*R*)-(+)-Hexa-MTPA Ester **8**: UV λ_{max}^{MeOH}: 228 nm (ε 40000). IR ν_{max}^{KBr}: 1745, 1265, 1170 cm^{-1}. FAB-MS m/z: 2499 $(M+Na)^+$, HR-FAB MS m/z: Calcd for $C_{121}H_{135}O_{32}{}^{35}ClF_{18}Na$: 2499.823. Found: 2499.822. ^{1}H-NMR (500 MHz, DMSO-d_6) δ: as shown in Table 2.

(*S*)-(−)-Hexa-MTPA Ester **9**: UV λ_{max}^{MeOH}: 228 nm (ε 42000). IR ν_{max}^{KBr}: 1747, 1244, 1172 cm^{-1}. FAB-MS m/z: 2499 $(M+Na)^+$, HR-FAB MS m/z: Calcd for $C_{121}H_{135}O_{32}{}^{35}ClF_{18}Na$: 2499.823. Found: 2499.805. ^{1}H-NMR (500 MHz, DMSO-d_6) δ: as shown in Table 2.

Synthesis of Tri-*p*-bromobenzoate (10) of Altohyrtin A (1) A solution of **1** (1.1 mg) in CH_2Cl_2 (0.3 ml) was treated with *p*-bromobenzoic acid (3.0 mg), DCC (2.0 mg), and DMAP (1.0 mg). The reaction mixture was stirred at room temperature (25 °C) for 48 h under an N_2 atmosphere. The whole was partitioned into ethyl acetate–water mixture and the organic layer was evaporated under reduced pressure to provide the crude product. The crude product was purified by HPLC (ODS, Cosmosil 5C_{18}-AR, MeOH : H_2O = 10 : 1) to afford the tri-*p*-bromobenzoate **10** (1.1 mg).

Tri-*p*-bromobenzoate **10**: UV λ_{max}^{MeOH}: 243 nm (ε 40000). IR ν_{max}^{KBr}: 3380, 1732 cm^{-1}. FAB-MS m/z: 1769 $(M+H)^+$, HR-FAB MS m/z: Calcd for $C_{84}H_{105}O_{24}{}^{35}Cl^{79}Br_3$: 1769.423. Found: 1769.412. CD (MeOH): 244 nm ($\Delta\varepsilon$ = +44.5), 230 nm ($\Delta\varepsilon$ = −15.5). ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 2.73 (m, H_a-2), 2.51 (m, H_b-2), 4.18 (t-like, J=11 Hz, H-3), 1.65 (m, H_a-4), 1.54 (m, H_b-4), 4.92 (m , H-5), 1.80 (m, H_a-6), 1.65 (m, H_b-6), 1.56 (m, H_a-10), 1.28 (m, H_b-10), 4.51 (m, H-11), 2.25 (m, H_a-12), 2.05 (m, H_b-12), 2.72 (m, H-14), 5.18 (m, H-15), 3.07 (dq, J=10, 7 Hz, H-16), 2.89 (m, H_a-18), 2.71 (m, H_b-18), 3.96 (m, H-19), 2.00 (m, H_a-20), 0.85 (m, H_b-20), 3.50 (m, H-21), 1.99 (m, H_a-22), 1.06 (m, H_b-22), 2.23 (m, H_a-24), 1.53 (m, H_b-24), 3.87 (m, H-25), 1.53 (m, H_a-26), 1.44 (m, H_b-26), 4.91 (t-like J=11 Hz, H-27), 5.33 (m, H-28), 5.35 (m, H-29), 2.05 (m, H_2-30), 1.21 (m, H_a-32), 1.08 (m, H_b-32), 4.15 (m, H-33), 1.43 (m, H-34), 3.61 (m, H-35), 1.80 (m, H_a-36),1.63 (m, H_b-36), 4.75 (s, H-38), 4.08 (d, J=11 Hz, H-39), 1.78 (m, H-40), 5.17 (m, H-41), 4.83 (dd, J=9, 9 Hz, H-42), 4.02 (m, H-43), 2.06 (m, H_a-44), 2.05 (m, H_b-44), 2.60 (m, H_2-46), 5.67 (dd-like, J=12, 6 Hz, H-47), 6.15 (dd, J=15, 6 Hz, H-48), 6.50 (d, J=15 Hz, H-49), 5.58 (s, H_a-51), 5.43 (s, H_b-51), 1.07 (s, H-52), 4.82 (s, H_2-53), 0.93 (d, J=7 Hz, H-54), 1.15 (d, J=7 Hz, H-55), 0.87 (d, J=6.5 Hz, H-56), 0.91 (d, J=7 Hz, H-57), 4.96 (s, H_2-58), 1.93 (s, H-5Ac), 1.82 (s, H-15Ac), 3.18 (s, H-21OMe), 3.83 (s, H-9OH), 4.23 (d, J=9 Hz, H-25OH), 4.48 (d, J=6 Hz, H-35OH).

Isolation from a Marine Sponge, *Haliclona* sp. The frozen sponge (14 kg, wet weight) was extracted with acetone at room temperature 3 times for 8 h each. The residue obtained by evaporation of the solvent under reduced pressure was partitioned into an ethyl acetate–water mixture (1 : 1), and the ethyl acetate layer was evaporated to give the ethyl acetate-soluble portion (63 g). The ethyl acetate-soluble portion was subjected to bioassay-guided separation (cytotoxicities against KB and L1210 cells). The SiO_2 column eluted with a $CHCl_3$–MeOH solvent system (100 : 1 → 1 : 1) gave three fractions [fr. A′ (52.5 g), fr. B′ (3.9 g), fr. C′ (5.4 g)]. The fr. B′ was further separated on an SiO_2 column (*n*-hexane : acetone = 7 : 3 → 1 : 1 → acetone, $CHCl_3$: MeOH = 20 : 1 → 10 : 1 → MeOH) to give cytotoxic fr. B′-3-2 (65 mg). The fr. B′-3-2 was separated by HPLC (ODS, CAPCELL PAK C_{18} AG120, MeOH : H_2O = 4 : 1, CH_3CN : H_2O : CH_2Cl_2 = 40 : 60 : 1) to give altohyrtin A (**1**, 0.2 mg, 3.1 × 10^{-4}% from the AcOEt extract) and altohyrtin C (**3**, 0.2 mg, 3.1 × 10^{-4}%).

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References and Notes

1) Part XXXVI: Kobayashi M., Mahmud T., Tajima H., Wang W., Aoki S., Nakagawa S., Mayumi T., Kitagawa I., *Chem. Pharm. Bull.*, **44**, 720—724 (1996).
2) Present address: *Faculty of Pharmaceutical Sciences, Kinki University, Kowakae, Higashiosaka, Osaka 577, Japan.*
3) *a*) Kobayashi M., Aoki S., Sakai H., Kawazoe K., Kihara N., Sasaki T., Kitagawa I., *Tetrahedron Lett.*, **34**, 2795—2798 (1993); *b*) Kobayashi M., Aoki S., Sakai H., Kihara N., Sasaki T., Kitagawa, I., *Chem. Pharm. Bull.*, **41**, 989—991 (1993); *c*) Kobayashi M., Aoki S., Kitagawa, I., *Tetrahedron Lett.*, **35**, 1243—1246 (1994).
4) Fusetani N., Shinoda K., Matsunaga S., *J. Am. Chem. Soc.*, **115**,

3977—3981 (1993).
5) *a*) Pettit G. R., Cichacz Z. A., Gao F., Herald C. L., Boyd M. R., Schmidt J. M., Hooper J. N. A., *J. Org. Chem.*, **58**, 1302—1304 (1993); *b*) Pettit G. R., Cichacz Z. A., Gao F., Herald C. L., Boyd M. R., *J. Chem. Soc., Chem. Commun.*, **1993**, 1166—1168.
6) *a*) Pettit G. R., Herald C. L., Cichacz Z. A., Gao F., Schmidt J. M., Boyd M. R., Christie N. D., Boettner F. E., *J. Chem. Soc., Chem. Commun.*, **1993**, 1805—1807; *b*) Pettit G. R., Herald C. L., Cichacz Z. A., Gao F., Boyd M. R., Christie N. D, Schmidt J. M., *Nat. Prod. Lett.*, **3**, 239—244 (1993); *c*) Pettit G. R., Cichacz Z. A., Herald C. L., Gao F., Schmidt J. M., Ernest H., Bai R., *J. Chem. Soc., Chem. Commun.*, **1994**, 1605—1606.
7) *a*) Kusumi T., Ohtani I., Inoue M., Kakisawa H., *Tetrahedron Lett.*, **29**, 4731—4734 (1988); *b*) Kusumi T., *Yuki Gosei Kyokai Shi*, **51**, 462—470 (1993).
8) Gonnella N. C., Nakanishi K., Martin V. S., Sharpless K. B., *J. Am. Chem. Soc.*, **104**, 3775—3776 (1982).
9) Clore G. M., Nilges M., Skurumaran D. K., Brunger A. T., Karplus M., Gronenborn A. M., *EMBO J.*, **5**, 2729—2738 (1986).
10) The details will be reported in due course.
11) *a*) Bai R., Cichacz Z. A., Herald C. L., Pettit G. R., Hamel E., *Molecular Pharmacology*, **44**, 757—766 (1993); *b*) Bai R., Taylor G. F., Cichacz Z. A., Herald C. L., Kepler J. A., Pettit G. R., Hamel E., *Biochemistry*, **34**, 9714—9721 (1995).
12) Kobayashi M., Aoki S., Gato K., Matsunami K., Kurosu M., Kitagawa I., *Chem. Pharm. Bull.*, **42**, 2449—2451 (1994).