Aurisides A and B, Cytotoxic Macrolide Glycosides from the Japanese Sea Hare *Dolabella auricularia*

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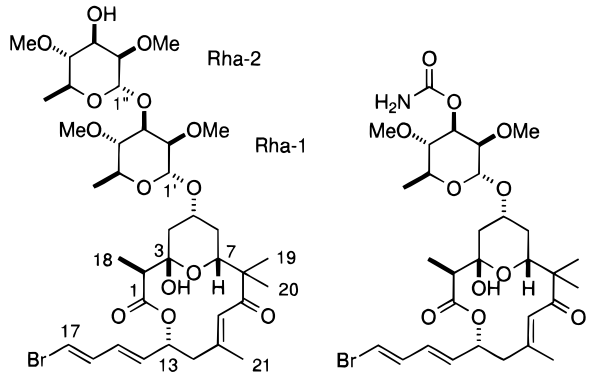
Bioassay-guided fractionation of the cytotoxic constituents of the Japanese sea hare *Dolabella auricularia* led to the isolation of two novel cytotoxic compounds, aurisides A (**1**) and B (**2**). Their gross structures were established by spectroscopic analysis including the 2D NMR technique. On the basis of the NOESY spectral analysis and the degradation experiments, their absolute stereostructures were determined to be 14-membered macrolide glycosides that contain a bromine-substituted conjugated diene structure, a cyclic hemiacetal moiety, and a 2,4-di-*O*-methyl-L-rhamnopyranoside part. Aurisides A (**1**) and B (**2**) show cytotoxicity against HeLa S_3 cells with IC_{50} values of 0.17 and 1.2 μg/mL, respectively.

The sea hare *Dolabella auricularia* is known to be a rich source of bioactive and structurally unique organic compounds such as dolastatins.[1] Recently, we have examined the constituents of Japanese specimens of this animal, resulting in the isolation of new cytotoxic depsipeptides,[2] peptides,[3] macrolides,[4] and other unique metabolites.[5] We report herein the isolation and structural elucidation of two new cytotoxic macrolide glycosides, aurisides A (**1**) and B (**2**), from the Japanese sea hare *D. auricularia*.



The MeOH extract of the internal organs (138 kg, wet wt) of the sea hare *D. auricularia* (278 kg, wet wt), collected in Mie Prefecture, Japan, was partitioned between EtOAc and water. The EtOAc-soluble material was further partitioned between 90% aqueous MeOH and hexane. The material obtained from the aqueous MeOH portion was subjected to bioassay-guided fractionation using silica gel (i. toluene/EtOAc, EtOAc, and then EtOAc/MeOH; ii. hexane/acetone and then acetone/MeOH), ODS silica gel (70% MeOH to MeOH), alumina ($CHCl_3$/acetone), silica gel (benzene/acetone), and ODS silica gel (70% MeCN), successively, to afford two cytotoxic fractions (fraction 1, IC_{50} = 0.021 μg/mL; fraction 2, IC_{50} = 0.04 μg/mL). One fraction (fraction 1)[6] was further separated by reversed-phase HPLC (ODS silica gel, 65% aqueous MeCN), silica gel TLC (1:3 hexane/EtOAc), and reversed-phase HPLC (ODS silica gel, 60% aqueous MeCN and ODS silica gel, 70% aqueous MeOH) to afford auriside A (**1**) (0.8 mg, amorphous solid). The other fraction (fraction 2) was separated by repeating HPLC (ODS, 60% MeCN; 65% MeOH; 60% MeOH; 50% MeCN; 55% MeCN) to give auriside B (**2**) (0.7 mg, amorphous solid). Aurisides A (**1**) and B (**2**) show cytotoxicity against HeLa S_3 cells with IC_{50} values of 0.17 and 1.2 μg/mL, respectively.

Compounds **1** ($[\alpha]^{31}_D$ −43 (*c* 0.050, MeOH)) and **2** ($[\alpha]^{33}_D$ −30 (*c* 0.090, MeOH)) had identical UV spectra (λ_{max} 245 nm) and very similar ^{1}H and ^{13}C NMR spectra except for the signals due to the sugar moieties.

Gross Structure of Auriside A (1). The molecular formula of **1** was determined to be $C_{37}H_{57}BrO_{14}$ by HRFABMS (MNa^+, *m/z* 827.2846, Δ +1.7 mmu), which required nine degrees of unsaturation; because six signals due to olefinic carbons and two signals arising from carbonyl carbons were observed in the ^{13}C NMR spectrum (Table 1), **1** was shown to contain four rings. The IR spectrum of **1** suggested the presence of hydroxyl (3560 and 3460 cm^{-1}), ester (1715 cm^{-1}), and conjugated enone (1680 and 1620 cm^{-1}) groups, which was confirmed by the observation of two exchangeable proton signals (δ 2.21 and 4.93) in the ^{1}H NMR spectrum and the signals (δ 176.4 and 203.3) in the ^{13}C NMR spectrum (units E and F in Figure 2).

The gross structure of auriside A (**1**) was deduced from analysis of one- and two-dimensional NMR spectra (Table 1). The presence of two 2,4-di-*O*-methylrhamnopyrano-

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(1) Pettit, G. R.; Kamano, Y.; Herald, C. L.; Fujii, Y.; Kizu, H.; Boyd, M. R.; Boettner, F. E.; Doubek, D. L.; Schmidt, J. M.; Chapuis, J.-C. *Tetrahedron* **1993**, *41*, 9151−9170.

(2) (a) Sone, H.; Nemoto, T.; Ojika, M.; Yamada, K. *Tetrahedron Lett.* **1993**, *34*, 8445−8448. (b) Sone, H.; Nemoto, T.; Ishiwata, H.; Ojika, M.; Yamada, K. *Tetrahedron Lett.* **1993**, *34*, 8449−8452. (c) Ishiwata, H.; Nemoto, T.; Ojika, M.; Yamada, K. *J. Org. Chem.* **1994**, *59*, 4710−4711.

(3) (a) Ojika, M.; Nemoto, T.; Nakamura, M.; Yamada, K. *Tetrahedron Lett.* **1995**, *36*, 5057−5058. (b) Nakamura, M.; Shibata, T.; Nakane, K.; Nemoto, T.; Yamada, K. *Tetrahedron Lett.* **1995**, *36*, 5059−5062. (c) Sone, H.; Shibata, T.; Fujita, T.; Ojika, M.; Yamada, K. *J. Am. Chem. Soc.* **1996**, *118*, 1874−1880.

(4) Ojika, M.; Nagoya, T.; Yamada, K. *Tetrahedron Lett.* **1995**, *36*, 7491−7494.

(5) (a) Ojika, M.; Nemoto, T.; Yamada, K. *Tetrahedron Lett.* **1993**, *34*, 3461−3462. (b) Sone, H.; Kondo, T.; Kiryu, M.; Ishiwata, H.; Ojika, M.; Yamada, K. *J. Org. Chem.* **1995**, *60*, 4774−4781.

(6) This fraction contained crystalline dolastatin D[2b] as a major constituent, most of which was removed by crystallization: see Experimental Section. Because dolastatin D has cytotoxicity against HeLa S_3 cells with an IC_{50} value of 2.2 μg/mL,[2b] dolastatin D is not responsible for the strong cytotoxicity of this fraction (IC_{50} = 0.021 μg/mL).

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Table 1. ^{1}H and ^{13}C NMR Data and the Selected HMBC Correlations for Auriside A (1) and Auriside B (2) in C_6D_6

	auriside A (**1**)			auriside B (**2**)		
posotion	^{1}H[a]	^{13}C[b]	HMBC[c]	^{1}H[a]	^{13}C[b]	HMBC[c]
1		176.4 s	H-2, 13, 18		176.5 s	H-2, 13, 18
2	2.48 q (7.3)	48.1 d		2.49 q (7.3)	48.1 d	
3		98.5 s	H-2, 4b, 18, 3-OH		98.5 s	H-2, 4b, 18, 3-OH
4a	1.03 td (11.7, 2.6)	39.8 t	3-OH	0.96 td (11.7, 2.6)	39.6 t	3-OH
4b	2.33 dd (11.7, 4.4)			2.27 dd (11.7, 4.4)		
5	4.27 m	71.2 d	H-1′	4.25 m	71.4 d	H-1′
6a	1.24 q (11.7)	31.5 t		1.24 q (11.7)	31.4 t	
6b	1.82 m			1.81 m		
7	3.94 dd (11.7, 2.0)	74.6 d	H-19, 20	3.93 dd (11.7, 2.0)	74.7 d	H-19, 20
8		49.3 s	H-19, 20		49.4 s	H-19, 20
9		203.3 s	H-10, 19, 20		203.5 s	H-10, 19, 20
10	6.33 s	126.4 d	H-12a, 12b	6.35 s	126.4 d	H-12a, 12b
11		147.5 s	H-12a, 12b		147.5 s	H-12a, 12b
12a	1.81 m	47.5 t	H-10, 21	1.81 m	47.5 t	H-10, 21
12b	1.97 t (12.1)			1.98 t (12.3)		
13	5.56 m	71.3 d		5.57 m	71.3 d	
14	5.18 dd (15.4, 6.4)	131.7 d		5.20 dd (15.4, 6.6)	131.7 d	
15	5.64 dd (15.4, 11.0)	129.3 d		5.64 dd (15.4, 11.0)	129.3 d	
16	6.40 dd (13.6, 11.0)	136.5 d		6.40 dd (13.6, 11.0)	136.5 d	
17	5.92 d (13.6)	110.8 d		5.91 d (13.6)	110.8 d	
18	0.97 d (7.3)	12.4 q		0.97 d (7.3)	12.5 q	
19	1.05 s	21.6 q	H-20	1.06 s	21.6 q	H-20
20	1.14 s	18.6 q	H-19	1.16 s	18.7 q	H-19
21	2.29 s	19.4 q	H-12a, 12b	2.29 s	19.4 q	H-12a, 12b
3-OH	4.93 d (2.6)			4.92 d (2.6)		
Rha-1						
1′	5.05 d (2.0)	96.3 d	H-5	5.04 d (2.0)	96.4 d	H-5
2′	3.68 dd (3.3, 2.0)	81.5 d	2′-OMe	3.82 dd (3.3, 2.0)	80.0 d	2′-OMe
3′	4.29 dd (9.5, 3.3)	79.4 d	H-1″	5.54 dd (9.5, 3.3)	75.0 d	
4′	3.46 t (9.5)	83.3 d	4′-OMe	3.51 t (9.5)	81.4 d	4′-OMe
5′	3.90 dq (9.5, 6.2)	69.2 d	H-1′	3.99 dq (9.5, 6.2)	68.6 d	H-1′
6′	1.35 d (6.2)	18.1 q		1.37 d (6.2)	18.1 q	
2′-OMe	3.31 s	58.8 q	H-2′	3.23 s	58.7 q	H-2′
3′-$CONH_2$				3.66 br s	156.2 s	H-3′
4′-OMe	3.26 s	60.8 q	H-4′	3.36 s	60.4 q	H-4′
Rha-2						
1″	5.34 d (1.5)	98.7 d	H-3′			
2″	3.56 dd (3.7, 1.5)	81.7 d	2″-OMe			
3″	4.24 td (9.5, 3.7)	72.1 d				
4″	3.16 t (9.5)	84.5 d	4″-OMe			
5″	4.09 dq (9.5, 6.2)	68.3 d	H-1″			
6″	1.43 d (6.2)	18.4 q				
2″-OMe	3.07 s	58.0 q	H-2″			
3″-OH	2.21 d (9.5)					
4″-OMe	3.49 s	60.7 q	H-4″			

[a] Recorded at 600 MHz. Residual C_6HD_5 as internal standard (δ 7.16). Coupling constants (Hz) are in parentheses. [b] Recorded at 125 MHz. C_6D_6 as internal standard (δ 128.0). Multiplicity was based on the HSQC spectrum. [c] Protons correlated to carbon resonances in ^{13}C column. Parameters were optimized for J_{CH} = 6 and 8 Hz.

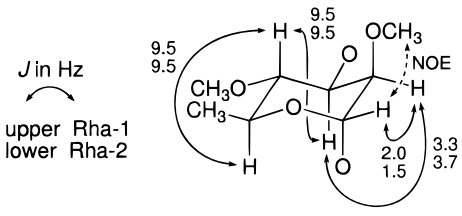


Figure 1.

side moieties in **1** was readily disclosed by the coupling constants in the ^{1}H NMR spectrum (Figure 1) and correlations in the ^{1}H−^{1}H COSY and the HMBC spectra, and further, their α-anomeric structures were determined by correlations in the NOESY spectrum (H-1′/2′-OMe and H-1″/2″-OMe in Figure 3) and $^1J_{CH}$ values at the anomeric carbons (C1′, 167 Hz; C1″, 168 Hz). Regarding the macrolide moiety, four sets of proton spin systems (units A−D in Figure 2) were detected by the ^{1}H−^{1}H COSY spectrum. It was noteworthy that one of the methylene protons at δ 1.03 (H-4a) exhibited a *W*-type coupling with 3-OH (δ 4.93). A bromine-substituted conjugated diene

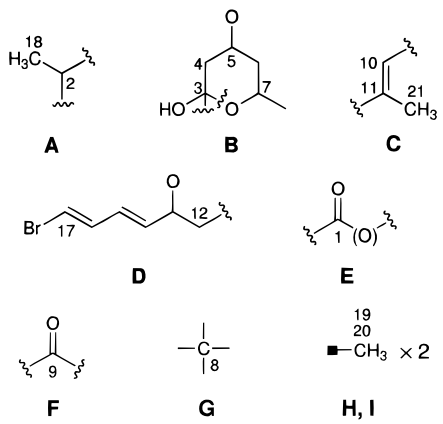


Figure 2.

structure (unit D) was shown by the chemical shifts of the four carbons [δ 131.7 (C14), 129.3 (C15), 136.5 (C16), 110.8 (C17)], which corresponded uniquely to those of the bromine-substituted conjugated diene moiety in a brominated C_{18} acetylenic acid from a marine sponge,[7] and

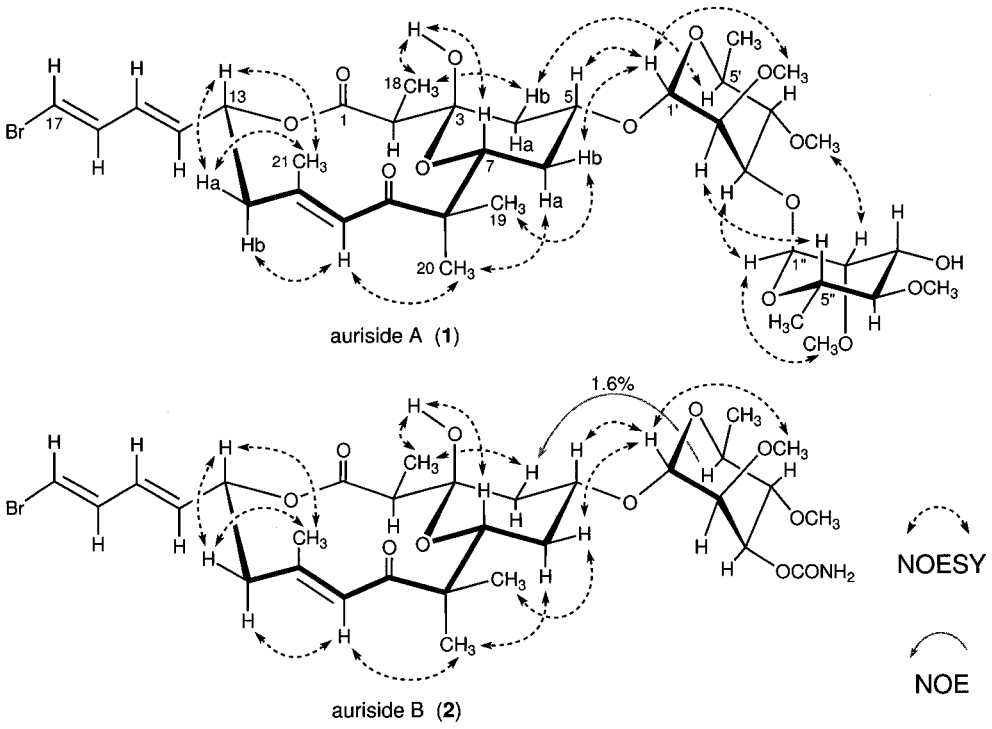


Figure 3. Plausible conformations of aurisides A (**1**) and B (**2**) in C_6D_6 with the selected correlations in the NOESY spectrum and the observed NOE in the difference NOE experiment.

the *trans,trans*-stereostructure of the conjugated diene system was easily determined by the proton−proton coupling constants (15.4 and 13.6 Hz). In addition to the partial structures (units A−F) of the aglycon of **1** described above, auriside A (**1**) had one more quaternary carbon (δ_C 49.3) and two singlet methyls (δ_H 1.05 and 1.14) (units G, H, and I in Figure 2). Connectivities among the units in Figure 2 were determined by the correlations in the HMBC spectra: H-2/C1, H-2/C3, H-4b/C3, H-10/C9, H-10/C12, H-19/C7, H-19/C8, H-19/C9, H-20/C7, H-20/C8, H-20/C9, and H-21/C12, revealing all carbon connectivities of the macrolide moiety of **1**. The presence of the 14-membered lactone structure was established by the correlation between H-13 and C1 in the HMBC spectrum. The degree of unsaturation indicated that **1** had one more ring that constitutes a cyclic hemiacetal structure, which was supported by the NOESY correlation (3-OH/H-7, Figure 3). The positions of the linkages between the macrolide and the rhamnopyranoside part (C5−C1′) and between the two rhamnopyranoside moieties (C3′−C1″) were determined by the correlations in the HMBC and the NOESY spectra, establishing the gross structure of auriside A (**1**).

Stereostructure of Auriside A (1). The stereochemistry of auriside A (**1**) was elucidated as follows. The relative stereochemistry and conformation of the macrolide moiety of **1** in C_6D_6 was determined by analysis of the vicinal spin−spin coupling constants (Table 1) and the NOESY spectrum (Figure 3). The proton−proton coupling constants ($J_{4a,5} = J_{5,6a} = J_{6a,7} = 11.7$ Hz) indicated that the six-membered hemiacetal ring (unit B) had a chair conformation and that the substituents at C5 and C7 were equatorial. It was found that the 3-hydroxyl group was axial and the 3-hydroxyl and C18-methyl groups were on the same side of the 14-membered ring on the basis of the correlations in the NOESY spectrum (H-7/3-OH and H-18/3-OH). The axial assignment of one (H-6a) of the methylene protons at C6 was based on the large coupling constants with H-5 and H-7, and further, the correlations in the NOESY spectrum (H-6a/H-20, H-20/H-10, H-10/H-12b) indicated that these protons were on the same side of the 14-membered ring, that the trisubstituted olefin at C10−C11 was *trans*, and that the conjugated enone system had an *s-cis*-conformation, which was supported by the fact that the absorption band due to $\nu_{C=C}$ at 1620 cm^{-1} was stronger than that due to $\nu_{C=O}$ at 1680 cm^{-1} in the IR spectrum. The correlations in the NOESY spectrum (H-12a/H-13, H-12a/H-21, H-13/H-21) revealed that the bromine-substituted conjugated diene group at C13 was oriented as illustrated in Figure 3. Thus, the relative stereostructure and the plausible conformation of the macrolide moiety of auriside A (**1**) were elucidated as depicted in Figure 3.

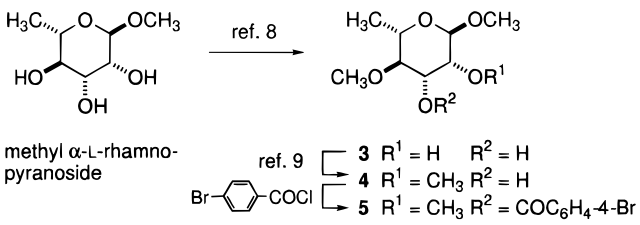
The relative stereochemistry between the macrolide and the rhamnopyranoside part and between the two rhamnopyranoside moieties was established by the correlations among these three moieties in the NOESY spectrum (H-4b/H-5′, H-6b/H-1′, H-2′/H-5″, and 4′-OMe/H-2″), revealing the relative stereochemistry of **1** (Figure 3). Further, acid hydrolysis of **1** with 0.9 M HCl/MeOH and subsequent 4-bromobenzoylation (4-BrC_6H_4COCl, DMAP, pyridine) afforded methyl 3-*O*-(4-bromobenzoyl)-2,4-di-*O*-methyl-α-L-rhamnopyranoside (**5**), which was identified by chiral HPLC (CHIRALCEL OD, hexane−2-propanol 50:1) analysis. The authentic samples of both enantiomers of **5** were prepared as follows (Scheme 1). Using the procedures reported,[8,9] methyl α-L-rhamnopyranoside was converted into diol **3**, which was methylated to give alcohol **4**. Alcohol **4** was acylated (4-BrC_6H_4COCl,

(7) Fusetani, N.; Li, H.-y.; Tamura, K.; Matsunaga, S. *Tetrahedron* **1993**, *49*, 1203−1210.

(8) Butler, K.; Lloyd, P. F.; Stacey, M. *J. Chem. Soc.* **1955**, 1531−1536.

(9) Toman, R.; Karacsonyi, S.; Palovcik, R. *Carbohydr. Res.* **1977**, *56*, 191−194.

Scheme 1



DMAP, pyridine) to afford **5**. Starting from methyl α-D-rhamnopyranoside,[10] *ent*-**5** was also prepared.

These results indicated that the absolute stereochemistry of the macrolide moiety of **1** is 2*S*,3*S*,5*R*,7*R*,13*R* and that of the two rhamnopyranoside moieties is L.

Structure of Auriside B (2). The molecular formula of auriside B (**2**) was determined to be $C_{30}H_{44}BrNO_{11}$ by HRFABMS (MNa^+, *m*/*z* 696.2001, Δ +0.6 mmu). Comparison of the spectral data for auriside B (**2**) with those for auriside A (**1**) revealed that **2** was an analogue of **1**, which had a carbamoyl group instead of one of the two rhamnopyranoside moieties in **1** [IR 3510, 3440, 1740, and 1580 cm^{-1}; ^1H NMR δ 3.66 (s, 2 H); ^{13}C NMR δ 156.2 s] (Table 1). Analysis of the vicinal spin−spin coupling constants, the NOESY spectrum, and the difference NOE experiments indicated that the relative stereostructure of **2** was identical with that of **1** (Figure 3). The absolute stereochemistry of **2** was determined by identification of rhamnopyranoside derivative **5** obtained from **2** (1. $LiAlH_4$ reduction of **2**; 2. acid hydrolysis; 3. 4-bromobenzoylation). Thus, auriside B (**2**) was shown to consist of the same macrolide and rhamnopyranoside moieties as **1**, as depicted in formula **2**.

Conclusions. Aurisides A (**1**) and B (**2**), isolated from *D. auricularia*, are cytotoxic macrolide glycosides that have unique structures: the aglycon possesses a new type of carbon backbone, 5,7,13-trihydroxy-3,9-dioxoheptadecanoic acid, and contains a bromine-substituted conjugated diene moiety, a 14-membered lactone, and a cyclic hemiacetal part. Natural products containing a bromine-substituted conjugated diene moiety are very rare: three antifungal brominated C_{18} acetylenic acids were isolated from the marine sponge.[7] Structurally related to **1** and **2** are acutiphycins,[11] cytotoxic agents from the freshwater blue-green alga, and polycavernosides,[12] toxins from the edible red alga that are responsible for fatal human intoxication.

Experimental Section

General Methods. Unless otherwise noted, materials were obtained from commercial suppliers and used without further purification. All solvents were purified by a standard procedure before use. Fuji silysia silica gel BW-820 MH was used for column chromatography unless otherwise noted. Merck precoated silica gel 60 F_{254} plates were used for thin-layer chromatography (TLC). Melting points are uncorrected. NMR spectra were measured at 270 or 600 MHz for ^1H and 125 MHz for ^{13}C. *J* values are given in Hz.

Isolation of Aurisides A (1) and B (2). *D. auricularia* (278 kg, wet wt) were collected at a depth of 0−1 m off the coast of the Shima Peninsula, Mie Prefecture, Japan, in 1992 and 1993. The internal organs (138 kg, wet wt) of the specimens were extracted with MeOH (291 L). The methanolic extract was evaporated and partitioned between EtOAc and H_2O. The EtOAc portion (910 g) was further partitioned between 90% MeOH and hexane. The aqueous MeOH portion (335 g) was chromatographed on silica gel, eluting with 1:1 toluene/EtOAc and EtOAc, successively. The fraction (22.7 g) eluted with EtOAc was chromatographed on silica gel, eluting with 2:1 hexane/acetone and then 9:1 acetone/MeOH. The fraction (9.88 g) eluted with 9:1 acetone/MeOH was subjected to ODS MPLC with increasing amounts (70% → 100%) of MeOH in H_2O to give a fraction (1.07 g) eluted with 89−98% MeOH. The fraction was chromatographed on alumina (E. Merck, Aluminum oxide 90, activity II−III), eluting with 4:1 $CHCl_3$/acetone. The fraction (287 mg) eluted with 4:1 $CHCl_3$/acetone was further separated by MPLC (silica gel FL60D, step gradient eluting with 20:1, 10:1, 5:1, and 0:1 benzene/acetone). The fraction (150 mg) eluted between 5:1 benzene/acetone and acetone was separated by preparative HPLC (Develosil ODS-HG-5, 7:3 MeCN/H_2O) to afford a fraction (fraction 1) (52.1 mg, IC_{50} against HeLa S_3 cells = 0.021 μg/mL) containing auriside A (**1**) and a fraction (fraction 2) (19.8 mg, IC_{50} = 0.04 μg/mL) containing auriside B (**2**).

One fraction (fraction 1), which contained crystalline dolastatin D[2b] as a major constituent, was recrystallized from hexane/CH_2Cl_2. The concentrated mother liquor (36.3 mg) was separated by preparative HPLC (Develosil ODS-HG-5, 65:35 MeCN/H_2O). A fraction (18.4 mg), which still contained dolastatin D, was further recrystallized from hexane/benzene. The concentrated mother liquor (11.7 mg) was separated by preparative TLC (1:3 hexane/EtOAc) followed by repetition of HPLC (Develosil ODS-HG-5 column, 6:4 MeCN/H_2O; 7:3 MeOH/H_2O) to give pure **1** (0.8 mg from the wet animals in a yield of 3×10^{-7}%) as a colorless amorphous solid: t_R = 17.6 min [Develosil ODS-HG-5 column (4.6 × 250 mm), 8:2 MeOH/H_2O, flow rate 1.0 mL/min, detection at 215 nm]; R_f = 0.5 (2:1 benzene/acetone), 0.3 (1:3 hexane/EtOAc); $[\alpha]^{31}_D$ −43 (*c* 0.050, MeOH); UV (MeCN) λ_{max} 245 (ε 31 000) nm; IR (CCl_4) 3560, 3460, 1715, 1680, 1620 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; FABMS *m*/*z* (relative intensity) 829/827 (MNa^+, 30:31), 323 (33), 173 (100); HRFABMS calcd for $C_{37}H_{57}{}^{79}BrNaO_{14}$ *m*/*z* 827.2829 (MNa^+), found 827.2846.

The other fraction (fraction 2) (19.8 mg) described above was separated by repeated HPLC (Develosil ODS-HG-5 column, 6:4 MeCN/H_2O; 65:35 MeOH/H_2O; 6:4 MeOH/H_2O; 5:5 MeCN/H_2O; 55:45 MeCN/H_2O) to give pure **2** (0.7 mg from the wet animals in a yield of 3×10^{-7}%) as a colorless amorphous solid: t_R = 12.9 min [Develosil ODS-HG-5 column (4.6 × 250 mm), 8:2 MeOH/H_2O, flow rate 1.0 mL/min, detection at 215 nm]; R_f = 0.6 (2:1 benzene/acetone), 0.5 (1:3 hexane/EtOAc) $[\alpha]^{33}_D$ −30 (*c* 0.090, MeOH); UV (MeCN) λ_{max} 245 (ε 35 000) nm; IR (CCl_4) 3560, 3510, 3460, 3440, 1740, 1715, 1680, 1620, 1580 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; FABMS *m*/*z* (relative intensity) 698/696 (MNa^+, 100:93), 413 (29); HRFABMS calcd for $C_{30}H_{44}{}^{79}BrNNaO_{11}$ *m*/*z* 696.1995 (MNa^+), found 696.2001.

Methyl 4-*O*-Methyl-α-D-rhamnopyranoside (*ent*-3). Using the same procedure for the preparation[8] of **3**, methyl α-D-rhamnopyranoside[10] was converted to *ent*-**3** in 54% yield: colorless oil; R_f = 0.1 (1:2 hexane/ether); $[\alpha]^{30}_D$ +98 (*c* 0.57, $CHCl_3$) [lit.[8] for L-form $[\alpha]^{16}_D$ −87.3 (*c* 1.1, MeOH)]; HRFABMS calcd for $C_8H_{16}NaO_5$ *m*/*z* 215.0896 (MNa^+), found 215.0909.

Methyl 2,4-Di-*O*-methyl-α-D-rhamnopyranoside (*ent*-4). Using the same procedure for the preparation[9] of **4**, methyl 4-*O*-methyl-α-D-rhamnopyranoside (*ent*-**3**) was converted into *ent*-**4** in 43% yield: colorless oil; R_f = 0.2 (1:3 hexane/ether); $[\alpha]^{30}_D$ +68 (*c* 0.12, $CHCl_3$) [lit.[8] for L-form $[\alpha]^{22}_D$ −68 (*c* 4.1, MeOH)]; HRFABMS calcd for $C_9H_{18}NaO_5$ *m*/*z* 229.1052 (MNa^+), found 229.1038.

Methyl 3-*O*-(4-Bromobenzoyl)-2,4-di-*O*-methyl-α-L-rhamnopyranoside (5). The mixture of methyl 2,4-di-*O*-methyl-α-L-rhamnopyranoside (**4**)[9] (3.2 mg, 0.016 mmol), 4-bromobenzoyl chloride (25.2 mg, 0.115 mmol), and DMAP (2.2 mg, 0.018 mmol) in pyridine (0.50 mL) was stirred at ambient temperature for 13 h, and water (0.05 mL) was added to the mixture. After being stirred at ambient temperature for 30 min, the

(10) Haskins, W. T.; Hann, R. M.; Hudson, C. S. *J. Am. Chem. Soc.* **1946**, *68*, 628−632.
(11) Barchi, J. J., Jr.; Moore, R. E.; Patterson, G. M. L. *J. Am. Chem. Soc.* **1984**, *106*, 8193−8197.
(12) (a) Yamashita, M. Y.; Haddock, R. L.; Yasumoto, T. *J. Am. Chem. Soc.* **1993**, *115*, 1147−1148. (b) Yamashita, M. Y.; Seki, T.; Paul, V. J.; Naoki, H.; Yasumoto, T. *Tetrahedron Lett.* **1995**, *36*, 5565−5566.

mixture was concentrated. The residual solid was purified by preparative TLC (1:2 hexane/ether) to give **5** (4.8 mg, 79%) as colorless needles: mp 131.5−132.5 °C (hexane/CH_2Cl_2); R_f = 0.7 (1:3 hexane/ether); $[\alpha]^{31}_D$ −17 (*c* 0.22, $CHCl_3$); IR ($CHCl_3$) 1720, 1590 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 1.36 (d, J = 6.3 Hz, 3 H), 3.39 (t, J = 9.6 Hz, 1 H), 3.39 (s, 3 H), 3.43 (s, 3 H), 3.48 (s, 3 H), 3.71 (dq, J = 9.6, 6.3 Hz, 1 H), 3.72 (dd, J = 3.3, 1.7 Hz, 1 H), 4.70 (d, J = 1.7 Hz, 1 H), 5.33 (dd, J = 9.6, 3.3 Hz, 1 H), 7.61 (m, 2 H), 7.97 (m, 2 H); FABMS *m/z* (relative intensity) 391/389 (MH^+, 32:37), 359/357 (53:53), 185/183 (100:98), 145 (93) 136 (66); HRFABMS calcd for $C_{16}H_{22}{}^{79}BrO_6$ *m/z* 389.0600 (MH^+), found 389.0598.

Methyl 3-*O*-(4-Bromobenzoyl)-2,4-di-*O*-methyl-α-D-rhamnopyranoside (*ent*-5). Using the same procedure as described for the preparation of **5**, *ent*-**4** was converted into *ent*-**5** in 88% yield: colorless needles; mp 131.5−132.5 °C (hexane/CH_2Cl_2); $[\alpha]^{28}_D$ +16 (*c* 0.20, $CHCl_3$); HRFABMS calcd for $C_{16}H_{22}{}^{79}BrO_6$ *m/z* 389.0600 (MH^+), found 389.0618.

Degradation of Auriside A (1). The mixture of auriside A (**1**) (0.090 mg, 0.11 nmol) in 0.9 M HCl−MeOH (1:4, 0.10 mL) was stirred at 85 °C for 27 h and concentrated. The residual solid was azeotroped with benzene (×3). To the solid were added 4-bromobenzoyl chloride (7.6 mg, 0.035 mmol), DMAP (0.5 mg, 0.004 mmol), and pyridine (0.10 mL), and the mixture was stirred at ambient temperature for 15 h. Water (0.05 mL) was added to the suspension, and the mixture was stirred at ambient temperature for 30 min and concentrated. The residual solid was purified by preparative TLC (1:2 hexane/ether) to give a trace amount of **5** that was used for chiral HPLC analysis [CHIRALCEL OD (4.6 × 250 mm), 50:1 hexane/2-propanol, flow rate 1.0 mL/min, detection at 254 nm].

Degradation of Auriside B (2). A solution of $LiAlH_4$ in THF (1.0 M, 0.020 mL) was added to auriside B (**2**) (0.195 mg, 0.289 nmol), and the mixture was stirred at ambient temperature for 1 h. The mixture was diluted with concd HCl−MeOH (1:4, 0.20 mL), stirred at 85 °C for 27 h, and concentrated. The residual solid was azeotroped with benzene (×3). To the solid were added 4-bromobenzoyl chloride (11.4 mg, 0.0519 mmol), DMAP (1.4 mg, 0.011 mmol), and pyridine (0.10 mL), and the mixture was stirred at ambient temperature for 16.5 h. Water (0.1 mL) was added to the suspension, and the mixture was stirred at ambient temperature for 30 min and concentrated. The residual solid was purified by preparative TLC (1:2 hexane/ether) to give a trace amount of **5** that was used for chiral HPLC analysis [CHIRALCEL OD (4.6 × 250 mm), 50:1 hexane/2-propanol, flow rate 1.0 mL/min, detection at 254 nm].

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Supporting Information Available: ^1H and ^{13}C NMR, $^1H-^1H$ COSY, HSQC, HMBC, and NOESY spectra of **1** and **2**; difference NOE spectrum of **2**; ^1H NMR spectra of **5** and *ent*-**5**; and charts of chiral HPLC analysis of rhamnopyranoside derivative **5** (16 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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