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Nonterpenoid C_{15} Acetogenins from *Laurencia marilzae*

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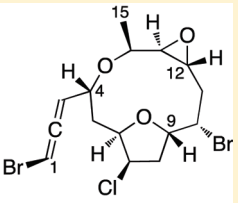
Supporting Information

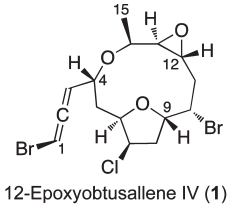
ABSTRACT: Eight new halogenated C_{15} acetogenins, **1**−**8**, were isolated from the organic extract of the red alga *Laurencia marilzae*. The structure elucidation and the assignments of the relative configurations were established by extensive use of spectroscopic studies, particularly 1D and 2D NMR data, while the absolute configurations of compounds **1** and **5** were determined by single-crystal X-ray diffraction analysis. Compounds **1**, **2**, **4**, **5**, and **7**, along with the previously reported related cyclic ether obtusallene IV (**9**), were evaluated against six human solid tumor cell lines. All compounds were found to be essentially inactive (GI_{50} > 10 μg/mL).

Laurencia marilzae

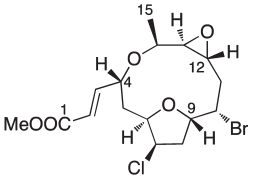


12-Epoxyobtusallene IV

Halogenated nonterpenoid C_{15} compounds constitute an interesting group of secondary metabolites. They are found only in red algae of the genus *Laurencia* (Rhodomelaceae, Ceramiales) as well as in herbivorous opisthobranch mollusks, which obtain these compounds through their diet.[1] These C_{15} acetogenins, which are generally accepted to arise from fatty acid metabolism, show remarkable structural variations that include unusual cyclic ethers with diverse ring sizes, typically a five- to nine-membered central acetogenic oxygen, an enyne or allene side chain, and at least one bromine atom.[2] Given their fascinating structures and their potentially important biological activities,[3−5] the interest in these halogenated cyclic ethers continues to stimulate organic chemists beyond the natural products field, with a focus on the development of new methodologies for their synthesis[6,7] or on providing new biosynthetic hypotheses.[8−11]



12-Epoxyobtusallene IV (**1**)



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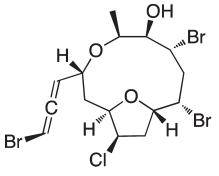
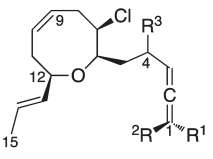


Obtusallene X (**3**)

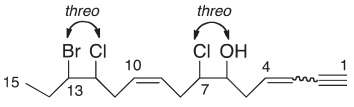


Marilzallene; R^1=Br, R^2=H, R^3= (4*R*)-OH (**4**)
(+)-4-Acetoxymarilzallene, R^1=Br, R^2=H, R^3= (4*R*)-OAc (**5**)
(−)-4-Acetoxymarilzallene, R^1=H, R^2=Br, R^3=OAc (**6**)

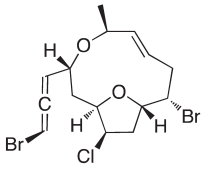
As part of our ongoing studies of the biologically active and structurally unique natural products isolated from *Laurencia* species,[12−14] we now report the structures of eight new halogen-containing acetogenins, **1**−**8**, from a newly described species, *Laurencia marilzae* Gil-Rodríguez, Sentíes et M.T. Funji sp. nov.,[15] collected from the Canary Islands. Details of the isolation, structure elucidation, and *in vitro* cytotoxicity of these metabolites are described herein.

RESULTS AND DISCUSSION

The fresh alga *L. marilzae* was extracted with CH_2Cl_2/MeOH (1:1, v/v) and subjected to purification using a series of chromatographic steps over Sephadex LH-20, silica gel, and normal-phase HPLC to yield compounds **1**−**8**.



Z-Adrienyne (**7**)
E-Adrienyne (**8**)



Obtusallene IV (**9**)

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Table 1. NMR Spectroscopic Data for 12-Epoxyobtusallene IV (1) and Compound 2 (in $CDCl_3$, 298 K)

	12-epoxyobtusallene IV (**1**)[a]		compound **2**[b]	
position	δ_C, mult.	δ_H (J in Hz)	δ_C, mult.	δ_H (J in Hz)
1	74.1, CH	6.04, dd (1.7, 5.7)	167.0, C	
2	201.1, C		120.2, CH	6.10, dd (1.8, 15.5)
3	103.4, CH	5.37, dd (5.7, 6.4)	150.2, CH	6.94, dd (4.0, 15.5)
4	66.4, CH	4.45, dddd (1.7, 1.8, 6.4, 11.0)	67.9, CH	4.45, dddd (1.8, 1.8, 4.0, 11.2)
5	37.7, CH_2	β 1.89, ddd (1.8, 10.8, 13.8)	37.3, CH	β 1.89, ddd (1.8, 10.8, 14.0)
		α 1.74, ddd (1.9, 11.0, 13.8)		α 1.58, ddd (1.8, 11.2, 14.0)
6	79.2, CH	4.27, ddd (1.8, 1.9, 10.8)	79.0, CH	4.30, ddd (1.8, 1.8, 10.8)
7	62.1, CH	4.41, br dd (1.8, 4.4)	62.0, CH	4.42, br dd (1.8, 4.5)
8	39.0, CH_2	β 2.50, dd (6.5, 14.4)	39.0, CH_2	β 2.52, dd (6.8, 14.4)
		α 2.43, ddd (4.4, 9.5, 14.4)		α 2.45, ddd (4.5, 9.7, 14.4)
9	77.8, CH	4.76, ddd (5.9, 6.5, 9.5)	77.3, CH	4.78, ddd (5.8, 6.8, 9.7)
10	46.9, CH	4.50, ddd (2.9, 5.9, 12.7)	46.8, CH	4.51, ddd (2.9, 5.8, 12.6)
11	35.8, CH_2	β 2.64, dd (2.9, 15.0)	35.7, CH_2	β 2.65, dd (2.9, 14.9)
		α 1.89, ddd (8.7, 12.7, 15.0)		α 1.91, ddd (8.7, 12.6, 14.9)
12	53.9, CH	3.18, dd (2.1, 8.7)	54.0, CH	3.17, dd (2.1, 8.7)
13	61.6, CH	3.03, dd (2.1, 3.4)	61.6, CH	3.08, dd (2.1, 3.5)
14	72.2, CH	4.54, dd (3.4, 7.1)	72.0, CH	4.59, dd (3.5, 7.1)
15	10.2, CH_3	1.08, d (7.1)	10.0, CH_3	0.99, d (7.1)
OCH_3			51.8, CH_3	3.75, s

[a] Data recorded at 500/125 MHz ($^1H/^{13}C$ nuclei). [b] Data recorded at 600/150 MHz ($^1H/^{13}C$ nuclei).

Compound **1** was isolated as optically active colorless crystals. ESI-FTICR mass spectrometry established a molecular formula for **1** of $C_{15}H_{19}Br_2ClO_3$. Signals observed in its ^1H and ^{13}C NMR spectra (Table 1) accounted for the presence in the structure of a bromoallene functionality [δ_C 201.1 (C), 103.4 (CH), 74.1 (CH); δ_H 6.04 (dd, J = 1.7, 5.7 Hz), 5.37 (dd, J = 5.7, 6.4 Hz), confirmed by a characteristic band at 1959 cm^{-1} in the IR spectrum]; eight methines bonded with either a halogen or oxygen atom; three methylenes; and one secondary methyl. The correlations between all geminal and vicinal protons observed in the $^1H-^1H$ COSY NMR spectrum, as well as the HSQC NMR correlations, revealed the presence of only one large spin system, comprising C-3→C-15, a concurrent disubstituted epoxide situated at C-12−C-13, and the rest of the heteroatoms located on carbons C-4, C-6, C-7, C-9, C-10, and C-14. These data enabled all of the proton and carbon resonances to be connected, leading to the assignments summarized in Table 1. On the basis of long-range HMBC NMR correlations observed between H-4 (δ_H 4.45) and C-14 (δ_C 72.2), as well as between H-9 (δ_H 4.76) and C-6 (δ_C 79.2), two ether linkages were established between the respective positions, in agreement with the remaining two degrees of unsaturation required by the molecular formula and by the absence of hydroxy groups in the IR spectrum. Compound **1**, therefore, contains a 12-membered *O*-heterocycle and, as a result, becomes a new member of the obtusallene subset of the *Laurencia* acetogenin family.[16] The relative configuration of **1** was determined by a combination of NOESY data and *J*-based configuration analysis. NOESY enhancements were observed between H_3-15 (δ_H 1.08) and both H-4 and H-12 (δ_H 3.18), between H-10 (δ_H 4.50) and H-9/H-12, and between H-4 and one of the diastereotopic H-5 methylene proton resonances (δ_H 1.89), indicating the *syn* orientation for H-4, H-5β, H-9, H-10, H-12, and H_3-15. In addition, a large coupling constant, $^3J_{H\text{-}5\beta,H\text{-}6}$ = 10.8 Hz, indicated their *anti* arrangement. Accordingly, the observed NOE enhancements between H-6 (δ_H 4.27) and H-7 (δ_H 4.41)/H-13 (δ_H 3.03) and between H-13 and H-14 (δ_H 4.54) located all of these protons on the opposite face of the molecule relative to H-5β. This contention was supported by the relative upfield shift of C-4, due to a γ-*gauche* effect of C-15 on C-4 in **1**, consistent with the *cisoid* H-4/H_3-15 orientation previously observed in only one reported member of the obtusallene family, obtusallene IV (**9**).[17,18] This compound was reisolated in this study; it displays the bromoallene unit with an *S* configuration.[19,20] All structural features of compound **1**, including the absolute configuration, were clarified by a single-crystal X-ray diffraction study on crystals obtained from a CH_2Cl_2/*n*-hexane solvent mixture. The results are shown in Figure 1. The absolute configuration for **1** was therefore determined to be 4*R*, 6*R*, 7*R*, 9*S*, 10*S*, 12*R*, 13*R*, and 14*S* at the eight stereogenic centers and *S* at the bromoallene residue, allowing the structure to be identified as 12-epoxyobtusallene IV.

Compound **2** was isolated as a colorless, amorphous solid. The molecular formula was deduced to be $C_{16}H_{22}BrClO_5$ by ESI-HRMS and ^{13}C NMR data. Comparison of the ^1H and ^{13}C NMR data of **2** with those of **1** (Table 1) revealed very close similarity in the structures of both compounds, sharing the same C-4→C-15 core system. Differences in the NMR spectra between **1** and **2** corresponded to those resonances assigned to C-1→C-3 [δ_C 167.0 (C), 120.2 (CH), 150.2 (CH); δ_H 6.10 (dd, J = 1.8, 15.5 Hz), 6.94 (dd, J = 4.0, 15.5 Hz) in **2** vs δ_C 74.1 (CH), 201.1 (C), 103.4 (CH); δ_H 6.04 (dd, J = 1.7, 5.7 Hz), 5.37 (dd, J = 5.7, 6.4 Hz) in **1**]. These data together with signals corresponding to an extra *O*-methyl group [δ_C 51.8 and δ_H 3.75 (s)] in **2**, located at C-1 on the basis of HMBC correlations such as those from the protons of the methoxy to C-1 and C-2, suggested that the bromoallene function in **1** had been exchanged for an α,β-unsaturated methyl ester in **2**. The geometry of the double bond Δ^2 was assigned as *E* on the basis of the large coupling constant

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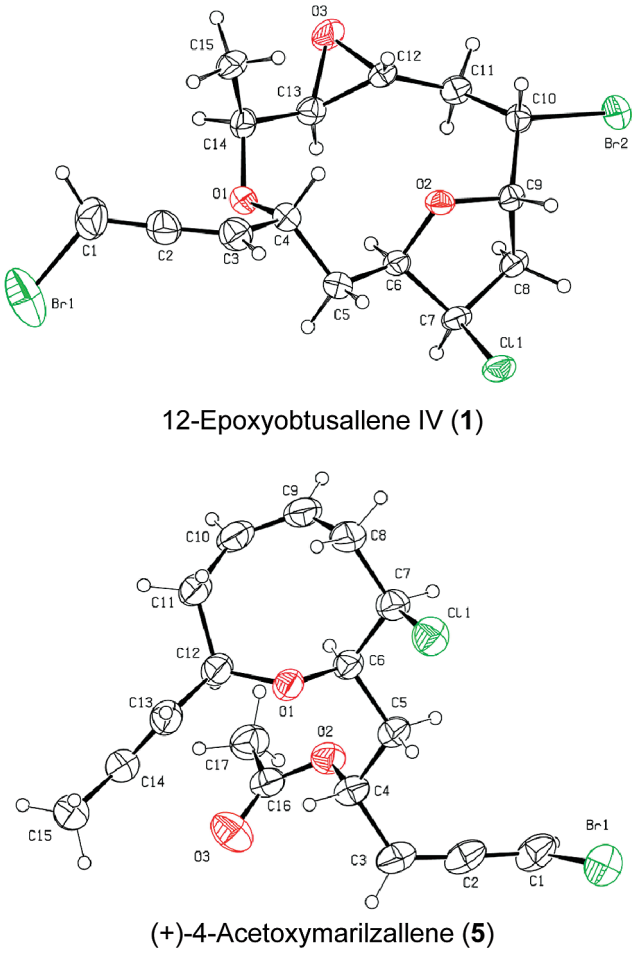

Figure 1. ORTEP diagrams of 12-epoxyobtusallene IV (**1**) and (+)-4-acetoxymarilzallene (**5**). The ellipsoids are drawn at the 30% probability level, and H-atoms are shown as spheres of arbitrary radii.

($^3J_{H\text{-}2,H\text{-}3}$ = 15.5 Hz). The relative configuration of **2** was established through key ROE correlations and was found to be identical to **1**. The co-isolation of **1** and **2** raises the possibility that the latter compound is biogenetically derived from **1**.

Obtusallene X (**3**) was isolated as a white, amorphous solid. The ESI-HRMS spectrum of **3** had three pseudomolecular $[M + Na]^+$ ions at m/z 544.8540, 546.8513, and 548.8574, corresponding to the molecular formula $C_{15}H_{20}Br_3ClO_3$. IR as well as ^1H and ^{13}C NMR data (Table 2) suggested the presence of a bromoallene function as described above. Moreover, extensive assessment of 1D and 2D NMR data obtained for **3** and compared with those for **1** indicated that compound **3** is closely related to 12-epoxyobtusallene IV (**1**). The main difference is the replacement of the epoxy group between C-12 and C-13 of **1** by a bromohydrin in **3**, C-12 (bromide) and C-13 (hydroxy), as evident from the chemical shifts observed for these positions (Tables 1 and 2). Nevertheless, several peculiarities emerged for compound **3**. Its NMR spectrum at room temperature, in sharp contrast to compound **1**, showed resonances attributable to two equilibrating conformers (1:1 ratio), more evident in the C-10 to C-15 fragment of the macrocycle, similar to the slow conformational motions described for other macrocyclic obtusallenes such as obtusallenes II and IV.[16,17] Therefore, the assignment of the relative configurations in **3** proved troublesome because of the more complex NMR spectra and the unavoidable superimposition of ^1H NMR signals. This problem was resolved by reacquiring NMR spectra in C_6D_6 and CD_3OD (see Supporting Information). Thus, the relative configuration at centers C-4, C-6, C-7, C-9, C-10, C-12, C-13, and C-14 was based on the results of 2D TROESY NMR experiments run in these deuterated solvents, which showed that H-4, H-9, H-10, H-12, and H_3-15 were oriented *syn*, leaving H-6, H-7, H-13, and H-14 in *anti* positions. On this basis, compounds **1** and **3** share the relative configuration determined to be 4*R**, 6*R**, 7*R**, 9*S**, 10*S**, 12*R**, 13*R**, and 14*S**, as well as displaying the bromoallene unit with an *S* configuration.

Compound **4** was shown to have the molecular formula $C_{15}H_{20}BrClO_2$ by ESI-HRMS. From its ^{13}C NMR data (Table 3), the presence of a bromoallene moiety was suggested [δ_C 200.2 (C), 105.1 (CH), and 75.3 (CH)], as were eight other carbon signals bearing withdrawing substituents, four olefinic carbons [δ_C 132.2 (CH), 131.2 (CH), 128.5 (CH), and 126.5 (CH)] and four heteroatom-bearing methines [δ_C 81.6 (CH), 75.3 (CH), 66.5 (CH), and 65.6 (CH)]. The oxygen functionalities within **4** were present as ether and hydroxy groups because the IR spectrum showed strong bands at 3421, 1190, and 1093 cm^{-1} and the lack of a carbonyl absorption. In addition, the presence of a 1-propenyl moiety was evident in the ^1H NMR spectrum by the appearance of a vinyl methyl signal at δ_H 1.68 (dd, J = 1.8, 6.4 Hz) and the signals at δ_H 5.56 (ddd, J = 1.8, 6.6, 15.4 Hz) and 5.71 (dd, J = 6.4, 15.4 Hz). The value of the coupling constant between H-13 and H-14 ($^3J_{H\text{-}13,H\text{-}14}$ = 15.4 Hz) indicated the *E* geometry for the double bond. Furthermore, an additional double bond was established on the basis of the signals at δ_H 5.69 (ddd, J = 6.3, 9.7, 11.2 Hz) and 5.93 (ddd, J = 7.1, 8.4, 9.7 Hz). Careful examination of the homonuclear and heteronuclear NMR correlations exhibited in the COSY, HSQC, and HMBC NMR spectra revealed the presence of a continuous chain of carbons from C-1 to C-15: CHBr=C=CHCH(X)-CH_2-CH(X)-CH(Cl)-CH_2-CH=CH-CH_2-CH(X)-CH=CH-CH_3. Moreover, HMBC NMR cross-peaks from H-6 (δ_H 4.25) to C-12 (δ_C 81.6) established the ether linkage between these positions, indicating the presence of an oxocene ring in the molecule, and therefore placed the remaining hydroxy group at C-4. The relative configuration of this metabolite was assigned on the basis of ROE enhancements. Because the ^1H NMR signals for H-7 and H-12 in $CDCl_3$ overlapped, the experiments were carried out in pyridine-d_5 (see Supporting Information). In this spectrum, H-6 was centered at δ_H 4.65 (ddd, J = 2.1, 2.1, 10.4 Hz), H-7 at δ_H 4.18 (ddd, J = 2.1, 4.7, 11.4 Hz), and H-12 at δ_H 4.27 (br dd, J = 5.6, 9.3 Hz). The ROE enhancement observed for H-6/H-12, as well as a small coupling constant of $^3J_{H\text{-}6,H\text{-}7}$ = 2.1 Hz, provided evidence that H-6, H-7, and H-12 were on the same side of the molecule. Comparison of the above data with those for laurenynes[21] revealed that **4** is an interesting variation on the laurenyne structure. The terminal bromoallene unit in **4** may be biosynthetically produced by a nucleophilic attack of H_2O in response to electrophilic bromination on the enyne at C_1. For compound **4**, the trivial name marilzallene is proposed.

Compounds **5** and **6** displayed a close structural relationship with **4**. The molecular formulas of both compounds were determined to be $C_{17}H_{22}BrClO_3$ on the basis of ESI-HRMS data. The pseudomolecular $[M + Na]^+$ and fragment $[M + Na - Ac]^+$ ions at m/z 413 and 368, respectively, in the mass spectrum, in conjunction with the absorption band at 1743 (in **5**)/1731 (in **6**) cm^{-1} in the IR spectrum, supported the presence of an acetoxy group. Therefore, the ^1H NMR spectrum

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Table 2. NMR Spectroscopic Data for Observed Conformations of Obtusallene X (3) (in $CDCl_3$, 298 K)

	obtusallene X (**3a**)[b]		obtusallene X (**3b**)[b]	
position	δ_C, mult.	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	73.5, CH	6.03, br d (5.6)	73.5	[a]
2	202.2, C		202.1	
3	99.9, CH	5.74, dd (5.6, 8.9)	100.2	5.70, dd (5.6, 8.7)
4	70.4, CH	4.55, m	70.2	[a]
5	35.3, CH_2	β 2.37, ddd (1.8, 12.0, 14.4)	35.4	β 2.33, ddd (2.0, 12.3, 14.3)
		α 1.72, ddd (3.8, 7.3, 14.4)		[a]
6	76.4, CH	4.39, ddd (3.8, 4.8, 12.0)	[a]	4.37, br dd (3.5, 12.3)
7	59.7, CH	4.58, m	59.9	[a]
8	41.1, CH_2	α 2.54, m	41.3	[a]
		β 2.26, br dd (5.7, 13.7)		β 2.25, br dd (5.6, 13.6)
9	74.2, CH	4.49, m	74.6	[a]
10	48.6, CH	4.46, m	48.2	4.44, m
11	43.6, CH_2	α 2.87, br dd (12.1, 15.5)	43.0	α 2.75, br dd (11.8, 15.3)
		β 2.65, ddd (2.3, 9.3, 15.5)		β 2.50, ddd (2.0, 8.9, 15.3)
12	58.1, CH	4.59, m	63.5	4.55, m
13	76.5, CH	3.69, dd (3.3, 6.1)	[a]	3.72, dd (3.7, 6.1)
14	73.0, CH	4.18, dd (3.3, 6.5)	72.6	4.16, dd (3.7, 6.6)
15	13.1, CH_3	1.36, d (6.5)	12.7	1.34, d (6.6)

[a] Signal overlapped by the resonance of the conformer **3a**. [b] Data recorded at 600/150 MHz ($^1H/^{13}C$ nuclei).

Table 3. NMR Spectroscopic Data for Compounds 4−6 (in $CDCl_3$, 298 K)

	marilzallene (**4**)[a]		(+)-4-acetoxymarilzallene (**5**)[b]		(−)-4-acetoxymarilzallene (**6**)[a]	
position	δ_C, mult.	δ_H (J in Hz)	δ_C, mult.	δ_H (J in Hz)	δ_C, mult.	δ_H (J in Hz)
1	75.3, CH	6.15, dd (2.2, 5.4)	75.0, CH	6.09, dd (2.0, 5.3)	74.9, CH	6.10, dd (1.9, 5.6)
2	200.2, C		201.4, C		202.4, C	
3	105.1, CH	5.51, dd (5.3, 5.4)	100.6, CH	5.46, m	99.5, CH	5.58, dd (5.5, 5.6)
4	65.6, CH	4.47, dddd (2.2, 2.9, 5.3, 9.8)	67.4, CH	5.45, dddd (2.0, 2.0, 4.7, 9.3)	67.7, CH	5.40, dddd (1.9, 4.6, 5.5, 8.8)
5	41.6, CH_2	β 2.14, ddd (2.9, 10.6, 14.5)	39.1, CH_2	β 2.18, ddd (2.0, 10.2, 15.0)	38.3, CH_2	β 2.20, ddd (4.6, 9.3, 14.1)
		α 1.47, ddd (2.3, 9.8, 14.5)		α 1.71, ddd (2.3, 9.3, 15.0)		α 1.87, ddd (3.5, 8.8, 14.1)
6	75.3, CH	4.25, ddd (2.3, 2.5, 10.6)	75.1, CH	3.97, ddd (2.3, 2.3, 10.2)	73.8, CH	3.96, ddd (2.5, 3.5, 9.3)
7	66.5, CH	3.95, ddd (2.5, 5.2, 11.3)	65.9, CH	3.94, ddd (2.3, 4.9, 11.9)	65.6, CH	3.99, ddd (2.5, 4.8, 11.3)
8	34.6, CH_2	β 2.96, ddd (11.2, 11.3, 11.9)	34.5, CH_2	β 2.97, ddd (9.9, 11.9, 12.7)	34.4, CH_2	β 2.96, ddd (9.9, 11.3, 12.3)
		α 2.53, ddd (5.2, 6.3, 11.9)		α 2.54, ddd (4.9, 6.9, 12.7)		α 2.53, ddd (4.8, 6.5, 12.3)
9	128.5, CH	5.69, ddd (6.3, 9.7, 11.2)	128.5, CH	5.69, ddd (6.9, 9.9, 10.4)	128.6, CH	5.69, ddd (6.5, 9.9, 10.3)
10	131.2, CH	5.93, ddd (7.1, 8.4, 9.7)	131.2, CH	5.90, ddd (7.0, 8.3, 10.4)	131.0, CH	5.91, ddd (7.1, 8.3, 10.3)
11	35.0, CH_2	β 2.48, ddd (7.1, 8.9, 14.4)	34.8, CH_2	β 2.48, ddd (7.0, 9.6, 14.4)	34.8, CH_2	β 2.48, ddd (7.1, 8.9, 14.3)
		α 2.16, ddd (1.7, 8.4, 14.4)		α 2.15, ddd (1.8, 8.3, 14.4)		α 2.17, ddd (1.6, 8.3, 14.3)
12	81.6, CH	3.97, ddd (1.7, 6.6, 8.9)	82.1, CH	3.63, dddd (1.1, 1.8, 6.9, 9.6)	81.7, CH	3.76, dddd (1.6, 1.6, 6.3, 8.9)
13	132.2, CH	5.56, ddd (1.8, 6.6, 15.4)	131.6, CH	5.54, ddd (1.6, 6.9, 15.4)	131.7, CH	5.54, ddd (2.3, 6.3, 15.4)
14	126.5, CH	5.71, dd (6.4, 15.4)	127.3, CH	5.72, ddd (1.1, 6.5, 15.4)	126.8, CH	5.72, ddd (1.6, 6.5, 15.4)
15	17.8, CH_3	1.68, dd (1.8, 6.4)	17.9, CH_3	1.69, dd (1.6, 6.5)	17.8, CH_3	1.70, dd (2.3, 6.5)
CO(Ac)			170.0, C		170.0, C	
CH_3(Ac)			21.1, CH_3	2.09, s	21.0, CH_3	2.06, s

[a] Data recorded at 600/150 MHz ($^1H/^{13}C$ nuclei). [b] Data recorded at 500/125 MHz ($^1H/^{13}C$ nuclei).

included a signal for an acetate methyl group [δ_H 2.09 (s) (in **5**)/2.06 (s) (in **6**)] and a deshielded oxygenated methine [δ_H 5.45 (dddd, J = 2.0, 2.0, 4.7, 9.3 Hz) (in **5**)/5.40 (dddd, J = 1.9, 4.6, 5.5, 8.8 Hz) (in **6**)]. Analysis of the 2D NMR spectra (HSQC, HMBC, and COSY) for **5** and **6** suggested the same planar structure. The acetate functionality present in the molecule was placed at C-4 for both compounds on the basis of HMBC NMR cross-peaks observed between the methine and methylene protons, H-4 and H_2-5, and the corresponding carbonyl ester carbon (Table 4). The absolute configuration of **5** was established by means of a single-crystal X-ray analysis. A computer-generated perspective drawing of the X-ray model of **5** is shown in Figure 1.

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Table 4. NMR Spectroscopic Data for *Z* and *E* Adrienynes (in $CDCl_3$, 298 K)

	Z-adrienyne (**7**)[a]		*E*-adrienyne (**8**)[a]	
position	δ_C, mult.	δ_H (*J* in Hz)	δ_C, mult.	δ_H (*J* in Hz)
1	82.6, CH	3.18, dd (0.7, 2.3)	77.1, CH	2.84, br d (2.0)
2	80.0, C		81.8, C	
3	111.2, CH	5.65, dd (2.3, 10.8)	112.2, CH	5.62, dd (2.0, 15.4)
4	140.2, CH	6.14, dddd (0.7, 7.3, 7.3, 10.8)	140.9, CH	6.22, ddd (7.5, 7.5, 15.4)
5	35.6, CH_2	2.73, m	38.5, CH_2	2.45 (2H), m
		2.66, m		
6	72.4, CH	3.84, ddd (3.6, 5.1, 7.8)	72.0, CH	3.76, m
7	67.1, CH	3.99, ddd (3.6, 5.6, 9.0)	66.3, CH	3.95, ddd (2.9, 6.0, 8.7)
8	33.1, CH_2	2.74, m	33.2, CH_2	2.71, ddd (6.0, 6.6, 14.7)
		2.66, m		2.63, m
9	128.4, CH	5.68, m[b]	128.3, CH	5.63, m
10	127.9, CH	5.62, m[b]	128.0, CH	5.60, m
11	34.1, CH_2	2.84, ddd (5.6, 7.7, 15.1)	34.0, CH_2	2.80, ddd (5.5, 6.3, 14.6)
		2.62, m		2.60, ddd (6.0, 8.8, 14.6)
12	64.3, CH	4.06, ddd (2.6, 5.6, 8.3)	64.3, CH	4.03, ddd (2.5, 5.5, 8.8)
13	61.0, CH	4.15, ddd (2.6, 4.4, 9.5)	61.0, CH	4.12, ddd (2.5, 4.5, 9.0)
14	28.8, CH_2	2.06, ddd (4.4, 7.3, 14.3)	28.8, CH_2	2.04, ddd (4.5, 7.3, 14.6)
		1.98, ddd (7.3, 9.5, 14.3)		1.95, ddd (7.5, 9.0, 14.6)
15	12.6, CH_3	1.10, dd (7.3, 7.3)	12.6, CH_3	1.08, dd (7.3, 7.5)

[a] Data recorded at 500/125 MHz ($^1H/^{13}C$ nuclei). [b] The $^3J_{H\text{-}9,H\text{-}10}$ = 11.5 Hz determined by DQF-COSY.

On this basis, the absolute configuration at centers C-4, C-6, C-7, and C-12 was assigned as 4*R*, 6*R*, 7*R*, and 12*R*, as well as an *S* configuration for the bromoallene unit. The relative configuration of **6** at centers C-6, C-7, and C-12 was established upon analysis of the TROESY NMR experiment and by comparison with NMR data of **5**, with an identical relative configuration at those centers. The observation of large-magnitude opposite signs for the specific rotations for both compounds, +89 for **5** and −58 for **6**, as well as the NMR differences centered on the chemical shift of H-3 [δ_H 5.46 (m) in **5** vs δ_H 5.58 (dd, *J* = 5.5, 5.6 Hz) in **6**], suggested that (+)-4-acetoxymarilzallene (**5**) displays the bromoallene unit with an *S* configuration, while (−)-4-acetoxymarilzallene (**6**) is in fact *R*. However, due to the inability to establish the configuration at C-4 through spectroscopic analysis, it does remain possible that the variation in configuration is instead at C-4. Additionally, in order to complete the stereochemical assignment of marilzallene (**4**), and taking in account that it shares the same sign of specific rotation for (+)-**5**, we performed the hydrolysis of the latter. A product that resulted identical to **4** in all respects was obtained (see Experimental Section), indicating that both compounds shared the same absolute configurations at C-4, C-6, C-7, C-12, and the bromoallene unit.

Z-Adrienyne (**7**) was isolated as an amorphous, white solid. The molecular formula was established as $C_{15}H_{21}BrCl_2O$ on the basis of ESI-HRMS data. The presence of a *Z*-2-penten-4-ynyl side chain was evident from the UV [λ_{max} (log ε) 225 (3.59) nm], IR [ν_{max} 3298 and 2300 cm^{-1}], and ^1H NMR [δ_H 3.18 (1H, dd, *J* = 0.7, 2.3 Hz), 5.65 (1H, dd, *J* = 2.3, 10.8 Hz), and 6.14 (1H, dddd, *J* = 0.7, 7.3, 7.3, 10.8 Hz)] spectra. Furthermore, the ^1H NMR spectrum revealed the presence of a methyl group, four diastereotopic methylenes, four methine groups flanked by two bromines, one chloride or an oxygen atom, and one additional double bond (Table 4). Correlations in the COSY and HSQC NMR spectra allowed for the assignment of the carbon skeleton

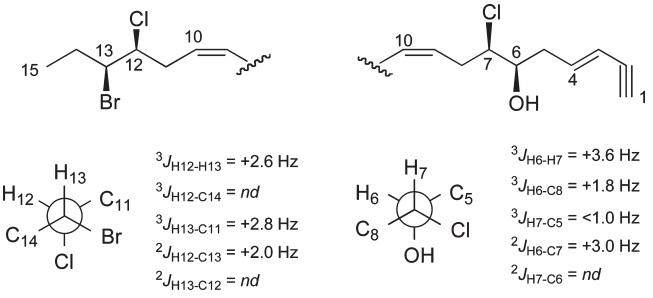

Figure 2. Relative configurations proposed for C-6, C-7, C-12, and C-13 of *Z*-adrienyne (**7**).

as a unique spin system, the heteroatoms being placed at C-6 (hydroxy), C-7 (chloride), C-12 (chloride), and C-13 (bromide) and the double bond between C-9 and C-10. The geometry of double bond Δ^9, due to overlapping signals, was assigned as *Z* from the measured H-10 coupling constant ($^3J_{H\text{-}9,H\text{-}10}$ = 11.5 Hz) from the ^1H DQF-COSY NMR experiment and by the chemical shifts of allylic carbons C-8 (δ_C 33.1) and C-11 (δ_C 34.1).[22] HMBC NMR correlations from protons H-1, H-3, and H-4 to the quaternary carbon C-2 (δ_C 80.0) completed the structure. Thus, the chemical structure of **7** was determined to be (3*Z*,9*Z*)-13-bromo-7,12-dichloropentadeca-3,9-dien-1-yn-6-ol. The relative configurations of the stereogenic centers C-6, C-7, C-12, and C-13 were determined by using the configuration analysis based on coupling constants (Figure 2).[23] The heteronuclear coupling constants were accurately measured from the HSQC-HECADE NMR experiment. The protons H-6 and H-7 displayed a small coupling constant ($^3J_{H\text{-}6,H\text{-}7}$ = 3.6 Hz), which was consistent with a *gauche* conformation and produced four possible relative conformations. $^3J_{C-H}$ values of <1.0 Hz for H-7/C-5 and 1.8 Hz for H-6/C-8, as well as a $^2J_{C-H}$ value of 3.0 Hz for H-6/C-7,

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indicated an *anti* orientation between C-5 and C-8. On the basis of these data, the relative configuration between stereocenters C-6 and C-7 was assigned as *threo*. Likewise, the *gauche* orientation between H-12 and H-13 was deduced from their small $^3J_{H-H}$ coupling constant (2.6 Hz). The values for $^2J_{H\text{-}12,C\text{-}13}$ and $^3J_{H\text{-}13,C\text{-}11}$ were determined to be 2.0 and 2.8 Hz, respectively, also consistent with a *threo* configuration for these stereocenters (Figure 2).

Comparison of the spectroscopic and spectrometric data of *E*-adrienyne (**8**) with those observed for **7** (see Table 4 and Experimental Section) clearly showed that compounds **7** and **8** were *Z* and *E* isomers in the terminal enyne moiety. Thus, the ^{1}H NMR spectrum of **8** showed the characteristic shift signals at δ_H 2.84 (1H, br d, J = 2.0 Hz), 5.62 (1H, dd, J = 2.0, 15.4 Hz), and 6.22 (1H, ddd, J = 7.5, 7.5, 15.4 Hz) of the *E*-enyne unit.

The *Laurencia* acetogenins are considered to be biogenetically derived from C_{15} linear hydroxy, halohydroxy, or epoxypolyenynes that, in turn, trace their origin to hexadeca-4,7,10,13-tetraenoic acid.[8] In this way, the two new acyclic acetogenins **7** and **8** isolated in this study, *Z*- and *E*-adrienynes, might be considered the linear biosynthetic precursors of compounds **1**−**6**. According to this hypothesis, to correctly predict the framework and relative configurations of the new macrocyclic or lauroxocene derivatives, the relative configuration of carbons C-6 and C-7 in compounds **7** and **8** would be 6*R** and 7*R**. This result is consistent only with the *threo* rotamer determined above.

Metabolites **1**, **2**, **4**, **5**, **7**, and obtusallene IV (**9**) were evaluated for their *in vitro* antiproliferative activity in a representative panel of human solid tumor cell lines: A2780 (ovarian), HBL-100 (breast), HeLa (cervix), SW1573 (non-small-cell lung), T-47D (breast), and WiDr (colon).[24] After 48 h of incubation none of the metabolites showed significant activity against the above-mentioned cancer cell lines (GI_{50} > 10 μg/mL).

EXPERIMENTAL SECTION

General Experimental Procedures. Melting points were determined on a Büchi 535 melting point apparatus and are uncorrected. Optical rotations were measured in $CHCl_3$ on a Perkin-Elmer 241 polarimeter by using a Na lamp. Ultraviolet−visible spectra were run as EtOH solutions on a Jasco Inc. V-560 spectrophotometer. IR spectra were recorded on a Bruker IFS55 spectrometer. NMR spectra were recorded on either a Bruker Avance 600 instrument equipped with a 5 mm TCI inverse detection cryo-probe operating at 600/150 MHz (^{1}H/^{13}C nuclei) or a Bruker Avance 500 spectrometer operating at 500/125 MHz (^{1}H/^{13}C nuclei). Chemical shifts were reported in ppm referenced to solvent signals ($CDCl_3$: δ_H 7.26, δ_C 77.0; CD_3OD: δ_H 3.31, δ_C 49.1; py-d_5: δ_H 8.74 δ_C 150.3; C_6D_6: δ_H 7.16, δ_C 128.4), and coupling constants were calculated in Hz. Standard Bruker NMR pulse sequences were utilized. EI-MS and HRMS data were performed on a Micromass Autospec spectrometer. Single-crystal X-ray diffraction analysis was measured on an Oxford Diffraction Supernova Dual diffractometer equipped with an Atlas CCD, using Cu Kα radiation. HPLC separations were carried out with a LKB 2248 system equipped with a photodiode array detector. Gel filtration flash chromatography was carried out using Sephadex LH-20 (Sigma-Aldrich). TLC were performed on AL Si gel Merck 60 F254, visualized by spraying with phosphomolybdic acid reagent (10% in MeOH) and heating. Cytotoxicity against the human solid tumor cell lines (A2780, HBL-100, HeLa, SW1573, T-47D, and WiDr) was measured by using the NCI protocol with minor modifications.[24]

Biological Material. Specimens of *Laurencia marilzae* were collected by hand in the intertidal zone at Paraíso Floral (Tenerife, Canary Islands). A voucher specimen was deposited at Departamento de Biología Vegetal, Botánica, Universidad de La Laguna, Tenerife (TFC Phyc 9860).

Extraction and Isolation. Fresh alga (1.3 kg) was extracted with CH_2Cl_2/MeOH (1:1, v/v) at room temperature and the solvent removed *in vacuo* to yield a dark green, viscous oil (42.9 g). The extract was subjected to Sephadex LH-20 (*n*-hexane/CH_2Cl_2/MeOH (2:1:1)) column chromatography. Selected fractions exhibiting similar TLC profiles were rechromatographed by medium-pressure normal-phase chromatography using a Lobar LiChroprep Si 60 column with *n*-hexane/EtOAc (7:3). Final purifications were achieved on a μ-Porasil HPLC column, 10 μm, 19 × 150 mm, using *n*-hexane/EtOAc (8:2 and 1:1), yielding compounds **9** (3.2 mg), **1** (6.2 mg), **2** (3.1 mg), **3** (3.8 mg), **6** (3.9 mg), **5** (13.5 mg), **4** (5.8 mg), **7** (4.2 mg), and **8** (1.0 mg), in order of increasing polarity.

The known compound obtusallene IV (**9**) has been identified by detailed analysis of the NMR and MS spectrometric data and comparison with those reported in the literature.[17,18]

*12-Epoxyobtusallene IV (**1**)*:. colorless needles; mp 143−145 °C (CH_2Cl_2/*n*-hexane); $[\alpha]^{25}_D$ +53 (*c* 0.12, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 204 (4.43) nm; IR ($CHCl_3$) ν_{max} 3060, 2958, 2927, 2861, 1959, 1380, 1265, 1122, 1076, 1032 cm^{-1}; ^{1}H and ^{13}C NMR data ($CDCl_3$), see Table 1; ESI-FTICR *m*/*z* 462.9285, 464.9262, 466.9241 $[M + Na]^+$ (22.9:42.8:34.3) (calcd for $C_{15}H_{19}{}^{79}Br_2{}^{35}ClO_3Na$, 462.9287; $C_{15}H_{19}{}^{79}Br^{81}Br^{35}ClO_3Na$, 464.9267; $C_{15}H_{19}{}^{81}Br_2{}^{35}ClO_3Na$, 466.9246).

*Compound **2***:. white, amorphous substance; $[\alpha]^{25}_D$ +63 (*c* 0.10, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 204 (3.93) nm; IR ($CHCl_3$) ν_{max} 3475, 2953, 2925, 2855, 1721, 1658, 1446, 1382, 1278, 1168, 1094, 1027 cm^{-1}; ^{1}H and ^{13}C NMR data ($CDCl_3$), see Table 1; ESI-HRMS *m*/*z* 431.0257, 433.0280 $[M + Na]^+$ (32.7:41.6) (calcd for $C_{16}H_{22}{}^{79}Br^{35}ClO_5Na$, 431.0237; $C_{16}H_{22}{}^{79}Br^{37}ClO_5Na$, 433.0207).

*Obtusallene X (**3**)*:. white, amorphous substance; $[\alpha]^{25}_D$ +20 (*c* 0.29, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 204 (4.04) nm; IR ($CHCl_3$) ν_{max} 3455, 3057, 2926, 2854, 1957, 1380, 1261, 1186, 1145, 1073, 1030 cm^{-1}; ^{1}H and ^{13}C NMR data ($CDCl_3$), see Table 2; ESI-HRMS *m*/*z* 544.8540, 546.8513, 548.8574 $[M + Na]^+$ (34.1:42.4:23.5) (calcd for $C_{15}H_{20}{}^{79}Br_2{}^{81}Br^{35}ClO_3Na$, 544.8528; $C_{15}H_{20}{}^{79}Br_2{}^{81}Br^{37}ClO_3Na$, 546.8499; $C_{15}H_{20}{}^{79}Br^{81}Br_2{}^{37}ClO_3Na$, 548.8478).

*Marilzallene (**4**)*:. white, amorphous substance; $[\alpha]^{25}_D$ +77 (*c* 0.11, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 202 (3.97) nm; IR ($CHCl_3$) ν_{max} 3421, 3022, 2926, 2854, 1958, 1720, 1646, 1451, 1378, 1190, 1093, 1064, 1042, 1011 cm^{-1}; ^{1}H and ^{13}C NMR data ($CDCl_3$), see Table 3; ESI-HRMS *m*/*z* 369.0234, 371.0215, 373.0193 $[M + Na]^+$ (27.2:100.0:77.5) (calcd for $C_{15}H_{20}{}^{79}Br^{35}ClO_2Na$, 369.0233; $C_{15}H_{20}{}^{81}Br^{35}ClO_2Na$, 371.0212; $C_{15}H_{20}{}^{79}Br^{37}ClO_2Na$, 373.0183).

*(+)-4-Acetoxymarilzallene (**5**)*:. colorless needles; mp 94−96 °C (CH_2Cl_2/*n*-hexane); $[\alpha]^{25}_D$ +89 (*c* 0.06, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 201 (3.97) nm; IR ($CHCl_3$) ν_{max} 3023, 2928, 2855, 1962, 1743, 1641, 1450, 1370, 1232, 1106, 1057, 1019 cm^{-1}; ^{1}H and ^{13}C NMR data ($CDCl_3$), see Table 3; ESI-HRMS *m*/*z* 411.0334, 413.0298, 415.0358 $[M + Na]^+$ (85.0:100.0:38.9) (calcd for $C_{17}H_{22}{}^{79}Br^{35}ClO_3Na$, 411.0339; $C_{17}H_{22}{}^{79}Br^{37}ClO_3Na$, 413.0309; $C_{17}H_{22}{}^{81}Br^{37}ClO_3Na$, 415.0289).

*(−)-4-Acetoxymarilzallene (**6**)*:. white, amorphous substance; $[\alpha]^{25}_D$ −58 (*c* 0.08, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 202 (3.99) nm; IR ($CHCl_3$) ν_{max} 2961, 2926, 2856, 1950, 1731, 1634, 1534, 1380, 1266, 1238, 1077, 1019 cm^{-1}; ^{1}H and ^{13}C NMR data ($CDCl_3$), see Table 3; ESI-HRMS *m*/*z* 413.2685 $[M + Na]^+$ (calcd for $C_{17}H_{22}{}^{81}Br^{35}ClO_3Na$, 413.0318).

*Z-Adrienyne (**7**)*:. white, amorphous substance; $[\alpha]^{25}_D$ −4 (*c* 0.11, $CHCl_3$); UV (CH_2Cl_2) λ_{max} (log ε) 225 (3.59) nm; IR ($CHCl_3$) ν_{max} 3440, 3298, 2971, 2934, 2300, 1828, 1728, 1606, 1443, 1385, 1267, 1189, 1095 cm^{-1}; ^{1}H and ^{13}C NMR data ($CDCl_3$), see Table 4; ESI-HRMS *m*/*z* 389.0082, 391.0040, 392.9998 $[M + Na]^+$ (25.7:38.3:17.9) (calcd for $C_{15}H_{21}{}^{79}Br^{35}Cl_2ONa$, 389.0051; $C_{15}H_{21}{}^{81}Br^{35}Cl_2ONa$, 391.0030; $C_{15}H_{21}{}^{79}Br^{37}Cl_2ONa$, 392.9992).

*E-Adrienyne (**8**)*:. white, amorphous substance; $[\alpha]^{25}_{D}$ −20 (*c* 0.04, $CHCl_3$); UV (CH_2Cl_2) λ_{max} (log ε) 225 (3.58) nm; IR ($CHCl_3$) ν_{max} 3411, 3302, 2959, 2925, 2854, 2341, 1725, 1641, 1461, 1379, 1261, 1094, 1030 cm^{-1}; ^1H and ^{13}C NMR data ($CDCl_3$), see Table 4; ESI-HRMS *m/z* 391.0165 $[M + Na]^+$ (calcd for $C_{15}H_{21}{}^{81}Br^{35}Cl_2ONa$, 391.0030).

*Chemical Conversion of (+)-4-Acetoxymarilzallene (**5**) to Marilzallene (**4**)*. Compound **5** (0.5 mg, 1.3 μmol) was dissolved in a suspension of K_2CO_3 (1% in MeOH) (2 mL) at 0 °C. After the mixture was stirred for 2 h, the solvent was removed *in vacuo*, and the solid washed with $CHCl_3$ (2×) and then purified by normal-phase HPLC (μ-Porasil column, 10 μm, 19 × 150 mm, *n*-hexane/EtOAc (8:2), 1 mL/min) to yield marilzallene (**4**) (0.4 mg), $[\alpha]^{25}_{D}$ +77 (*c* 0.04, $CHCl_3$).

X-ray Crystal Structure Analysis. Colorless crystals of **1** and **5** were obtained in a solvent mixture of CH_2Cl_2 and *n*-hexane. Intensity data were collected at room temperature on an Oxford Diffraction Supernova Dual diffractometer equipped with an Atlas CCD, using Cu Kα radiation. Cell refinement and data reduction were performed with CrysAlisPro.[25] The structures were solved by direct methods using SIR97.[26] Refinements were performed with SHELXL-97[27] using full-matrix least-squares, with anisotropic displacement parameters for all the non-hydrogen atoms. The H-atoms were placed in calculated positions and refined using a riding model. Corrections for absorption were performed using the multiscan facilities implemented in PLATON.[28] Calculations were mainly performed with the WinGX[29] set of programs. Molecular graphics were computed with PLATON. Crystallographic data (excluding structure factor tables) for the structures reported have been deposited with the Cambridge Crystallographic Data Center as supplementary publications no. CCDC 798406 for **1** and CCDC 798407 for **5**. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB 1EZ, UK [fax: Int. +44(0) (1223) 336 033); e-mail: deposit@ccdc.cam.ac.uk].

*Crystal Data for 12-Epoxyobtusallene IV (**1**)*:. $C_{15}H_{19}Br_2ClO_3$, M_w = 442.6, monoclinic, space group, $P2_1$, Z = 2, a = 5.176(2) Å, b = 8.594(3) Å, c = 19.502(4) Å; β = 96.68(2)°, V = 861.6(5) Å^3, μ(Cu Kα) = 7.45 mm^{-1}, ρ_{calc} = 1.71 g cm^{-3}; S = 1.07, final R indices: R_1 = 0.037 and R_w = 0.102 for 3271 observed from 3370 independent and 6298 measured reflections (θ_{max} = 73.3, $I > 2\sigma(I)$ criterion and 215 parameters); maximum and minimum residues are 0.59 and −0.71 e Å^{-3}, respectively. The Flack[30] parameter value was x = −0.04(2), indicating that the absolute structure has been determined correctly.

*Crystal Data for (+)-4-Acetoxymarilzallene (**5**)*:. $C_{17}H_{22}BrClO_3$, M_w = 389.7, monoclinic, space group, $P2_1$, Z = 2, a = 9.4049(3) Å, b = 9.0465(3) Å, c = 11.0073(4) Å; β = 98.23(1)°, V = 926.87(5) Å^3, μ(Cu Kα) = 4.42 mm^{-1}, ρ_{calc} = 1.39 g cm^{-3}; S = 1.05, final R indices: R_1 = 0.0860 and R_w = 0.2094 for 2504 observed from 2635 independent and 3366 measured reflections (θ_{max} = 73.0, $I > 2\sigma(I)$ criterion and 202 parameters); maximum and minimum residues are 1.05 and −0.67 e Å^{-3}, respectively. The Flack[30] parameter value was x = −0.06(3), indicating that the absolute structure has been determined correctly.

■ ASSOCIATED CONTENT

Supporting Information. ^1H and ^{13}C NMR spectra of compounds **1**, **2**, and **4**−**8** and the HSQC NMR spectrum of compound **3**. NMR spectroscopic data for compounds **3** (in C_6D_6 and CD_3OD) and **4** (in pyridine-d_5). This material is available free of charge via the Internet at http://pubs.acs.org.

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■ DEDICATION

Dedicated to Dr. Koji Nakanishi of Columbia University for his pioneering work on bioactive natural products.

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