New Halogenated Marine Prostanoids with Cytotoxic Activity from the Okinawan Soft Coral *Clavularia viridis*

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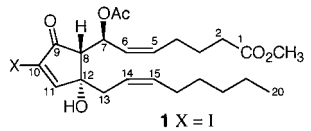
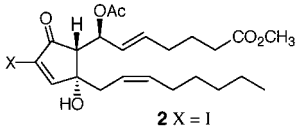
Five new halogenated prostanoids **1**−**4** and **6** were isolated from the Okinawan soft coral *Clavularia viridis*. The gross structure of **1** was elucidated mainly on the basis of NMR spectral data. The relative and absolute configurations were determined by analysis of NOESY and CD data, chemical conversion, and the modified Mosher's method. The structures of **2**−**4** and **6** were deduced by comparison of their spectral data with those of **1**. Compound **1** demonstrated cytotoxic activity.

Our continuous investigation on chemical constituents of the Okinawan soft coral *Clavularia viridis* Quoy and Gaimard (class Anthozoa, subclass Octocorallia, order Stolonifera, family Clavulariidae) resulted in isolation of a number of prostanoids[1] including clavulones,[2] steroids,[3] and a pyrazine congener.[4] Among them, halogenated prostanoids, e.g., chlorovulones,[5] received much attention because of their strong cytotoxic and antitumor activities.[5a,6] During our further efforts on studies aimed at the discovery of bioactive compounds from the soft coral *C. viridis*, six iodinated, brominated, and chlorinated prostanoids, **1**−**6** (**5** was known, but the others are new) as well as clavulones, were isolated. This paper describes the isolation and structures of these halogenated prostanoids.

The hexane extract (6.83 g out of 14.5 g) of the freeze-dried soft coral (470 g) was chromatographed on a silica gel column eluted with hexane, hexane−AcOEt (3:1 and 1:1), AcOEt, and MeOH, in turn, to obtain five fractions. The second fraction [eluted with hexane−AcOEt (3:1)] was further subjected to separation and purification by MPLC and HPLC on normal- and reversed-phase columns to obtain compounds **1** (29.8 mg), **2** (1.1 mg), **3** (2.6 mg), **4** (0.3 mg), **5** (0.6 mg), and **6** (0.1 mg).



1 X = I
3 X = Br
5 X = Cl



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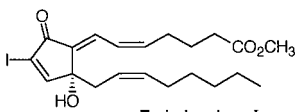
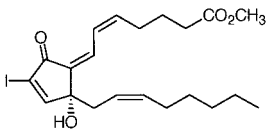


7 : iodovulone I



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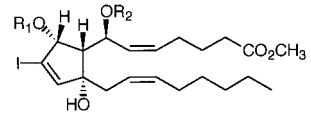

9 R_1 = H, R_2 = Ac
10 R_1 = MOM, R_2 = Ac
11 R_1 = MOM, R_2 = H
12 R_1 = MOM, R_2 = (*R*)-2NMA
13 R_1 = MOM, R_2 = (*S*)-2NMA

Results and Discussion

The HREIMS and ^{13}C NMR data of iodinated compound **1** [$[\alpha]_D$ +22.7 °C (*c* 0.67, $CHCl_3$)] indicated this molecule to possess an iodine-containing molecular formula of $C_{23}H_{33}O_6I$ [472.1109 [M − CH_3CO_2H]$^+$ (calcd for $C_{21}H_{29}O_4I$, 472.1111). In the UV and IR spectra, the presence of an α,*β*-unsaturated carbonyl group [λ_{max} 251 mn (ϵ 3500)], an acetate ester (IR ν_{max} 1732, 1240 cm^{-1}), and a hydroxyl group (3470 cm^{-1}) was suggested. The ^{13}C NMR spectrum exhibited 23 carbon signals for three methyls, eight methylenes, seven methines, and five quaternary carbons (Table 1), whose chemical shift values indicated the presence of the two ester carbonyls [174.0 (C, C-1) and 170.6 (C, CH_3CO)], two disubstituted double bonds [133.5 (CH, C-5), 126.6 (CH, C-6), 121.3 (CH, C-14), and 136.0 (CH, C-15)], and two oxygen-bearing carbons [68.0 (CH, C-7) and 81.0 (C, C-12)]. By comparison of the chemical shift values of the remaining three carbon atoms [δ_C 198.9 (C, C-9), 103.4 (C, C-10), and 170.7 (CH, C-11)] with those of 2-iodo-2-cyclopentenone,[7] the presence of an α-iodo-α,*β*-unsaturated cyclopentenone moiety in this molecule was indicated. The ^1H NMR spectrum of **1** (Table 1) showed signals due to the acetyl methyl [δ_H 1.99 (3H, s)], a carbomethoxy [3.68 (3H, s)], a terminal methyl [0.88 (3H, t, J = 7.0 Hz, H-20)], and five olefinic protons [5.59 (1H, dt, J = 10.7, 7.6 Hz, H-5), 5.84 (1H, dd, J = 10.7, 9.7 Hz, H-6), 7.77 (1H, s, H-11), 5.33 (1H, br ddd, J = 10.9, 8.1, 6.7 Hz, H-14), 5.64 (1H, br dt, J = 10.9, 7.4 Hz, H-15)]. The ^1H signal at δ_H 5.92 (1H, dd, J = 9.7, 3.1 Hz, H-7) was assignable to the methine proton bearing the secondary acetoxyl group at an allylic position from HMBC data (see below). The remaining quaternary carbon at δ_C 81.0 (C, C-12) was thus concluded to be attributed to the carbon bearing a tertiary hydroxyl group. The analysis of $^1H-^1H$ COSY spectrum (Figure 1) revealed a sequence of the correlations starting from a triplet at δ_H 2.32 (2H, t, J = 7.4 Hz, H-2) to a doublet at δ_H 2.67 (1H, d, J = 3.1 Hz, H-8), indicating the partial structure from H-2 to -8 on the α-side chain shown with the bold line in Figure 1. The connectivity from H-13 to -17 on the *ω*-side chain was also indicated by the correlations in the $^1H-^1H$ COSY spectrum starting from two broad signals at δ_H 2.51 (1H, br dd, J = 14.1, 8.1 Hz, H-13) and 2.37 (1H, br dd, J = 14.1, 6.7 Hz, H-13) and ending with the methylene proton at δ_H 1.34 (2H, m, H-17).

After direct $^1H-^{13}C$ correlations were established from the HMQC spectrum, the gross structure of **1** was elucidated on the basis of the analysis of the HMBC spectrum (Figure 1). The location of the acetoxyl group was determined by correlations from the acetyl methyl at δ_H 1.99 and the oxygen-bearing methine proton at δ_H 5.92 (H-7) to the acetyl carbonyl carbon at δ_C 170.6. The correlations

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Table 1. ^{1}H and ^{13}C NMR Spectral Data for Compounds **1**−**4**

	1		**2**		**3**		4
position	$^{13}C^a$	$^1H^b$	$^{13}C^a$	$^1H^b$	$^{13}C^a$	$^1H^b$	$^1H^b$
1(C)	174.0		173.9		174.0		
2(CH_2)	33.4	2.32(t, 7.4)	33.3	2.30(t, 7.4)	33.4	2.33(t, 7.3)	2.30(t, 7.5)
3(CH_2)	24.4	1.72(quintet, 7.4)	23.9	1.72(quintet, 7.4)	24.4	1.72(m)	1.72(quintet, 7.5)
4(CH_2)	27.0	2.20(m)	31.4	2.09(m)	27.0	2.21(m)	2.09(m)
5(CH)	133.5	5.59(dt, 10.7, 7.6)	134.8	5.73(dt, 15.5, 6.5)	133.6	5.60(dt, 10.7, 7.6)	5.73(dt, 15.5, 6.5)
6(CH)	126.6	5.84(dd, 10.7, 9.7)	126.8	5.82(dd, 15.5, 7.8)	126.6	5.84(dd, 10.7, 9.7)	5.82(dd, 15.5, 7.8)
7(CH)	68.0	5.92(dd, 9.7, 3.1)	73.1	5.60 (dd, 7.8, 3.1)	68.0	5.94 (dd, 9.7, 3.1)	5.63(dd, 7.7, 3.1)
8(CH)	55.7	2.67(d, 3.1)	55.6	2.72(d, 3.1)	56.7	2.68 (d 3.1)	2.73(d, 3.1)
9(C)	198.9		198.8		196.9		
10(C)	103.4		103.4		126.6		
11(CH)	170.7	7.77(s)	170.74	7.77(s)	162.7	7.53(s)	7.52(s)
12(C)	81.0		81.0		78.6		
13(CH_2)	39.2	2.51(br dd, 14.1, 8.1) 2.37(br dd, 14.1, 6.7)	39.1	2.51 (br dd, 14.3, 8.3) 2.36(br dd, 14.3, 6.8)	39.3	2.54(br dd, 14.2, 8.2) 2.39(br dd, 14.2, 7.1)	2.53(br dd, 14.3, 8.2) 2.38(br dd, 14.3, 7.2)
14(CH)	121.3	5.33(br ddd, 10.9, 8.1, 6.7)	121.2	5.31(br ddd, 11.0, 8.3, 6.8)	121.2	5.34(br ddd, 11.0, 8.2, 7.1)	5.33(br ddd, 11.0, 8.2, 7.2)
15(CH)	136.0	5.64 (br dt, 10.9, 7.4)	136.2	5.65(br dt, 11.0, 7.4)	136.1	5.65(br dt, 11.0, 7.4)	5.66(br dt, 11.0, 7.4)
16(CH_2)	27.5	1.98(m)	27.5	2.02(m)	27.5	2.00(m)	2.00(m)
17(CH_2)	29.1	1.34 (m)	29.1	1.33 (m)	29.1	1.34(m)	1.35(m)
18(CH_2)	31.5	1.26 (m)	31.5	1.27(m)	31.5	1.28 (m)	1.28(m)
19(CH_2)	22.5	1.28(m)	22.5	1.30(m)	22.5	1.30(m)	1.30(m)
20(CH_3)	14.0	0.88(t, 7.0)	14.0	0.89(t, 7.0)	14.0	0.88(t, 7.0)	0.89(t, 6.9)
OCH_3	51.6	3.68(s)	51.5	3.68(s)	51.6	3.67(s)	3.67(s)
CH_3CO	170.6		170.67		170.5		
CH_3CO	21.2	1.99(s)	21.3	2.01(s)	21.1	1.99(s)	2.01(s)
OH		3.38(s)				3.27(s)	

[a] Multiplicities of ^{13}C resonances were achieved by DEPT experiments. [b] Multiplicities and *J* (Hz) values are presented in parentheses.

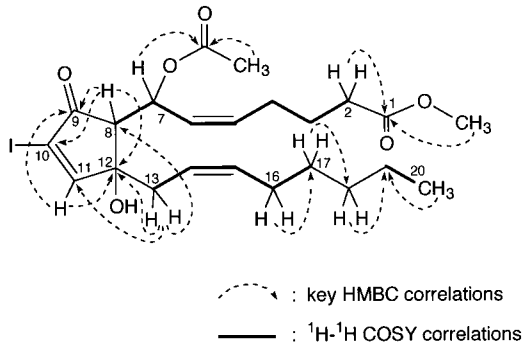

Figure 1. ^{1}H−^{1}H COSY and key HMBC correlations of **1**.

from the methine proton at δ_H 2.67 (H-8) to the carbonyl carbon at δ_C 198.9 (C-9), olefinic carbon at δ_C 103.4 (C-10) bearing the iodine atom, and quaternary carbon at δ_C 81.0 (C-12) bearing the tertiary hydroxyl group showed that C-8 is connected between the carbonyl carbon (C-9) and oxygen bearing quaternary carbon (C-12) on the cyclopentenone ring. The correlations from the signals at δ_H 2.51 and 2.37 (H-13) to the quaternary carbon at δ_C 81.0 (C-12), methine carbon at δ_C 55.7 (C-8), and olefinic carbon at δ_C 170.7 (C-11) revealed that the ω-side chain was connected to the C-12 carbon on the cyclopentenone ring. The correlations from the C-2 methylene at δ_H 2.32 and ester methyl at δ_H 3.68 to the ester carbonyl carbon at δ_C 174.0 (C-1) indicated the connectivity from the C-2 methylene to the methyl ester group. Finally the remaining two methylene groups were embedded between C-17 and the terminal methyl group to complete the prostanoid structure for **1**.

The (5*Z*,14*Z*) configurations of two disubstituted double bonds were determined on the basis of the ^{1}H coupling constants between the olefinic protons, H-5 and -6 (10.7 Hz), and H-14 and -15 (10.9 Hz), respectively, and the ^{13}C chemical shifts of allylic carbons of C-4 and -16 (δ_C 27.0 and 27.5, respectively).

The trans orientation of the α- and ω-side chains on the cyclopentenone ring was concluded from the NOE correlations between H-8 and H-13, and H-7 and the hydroxyl proton at C-12 [δ_H 3.38 (1H, s)], in the NOESY spectrum. The absolute configuration at C-12 was determined by the chemical conversion of **1** to iodovulone I (**7**).[8] The absolute stereochemistry of **7** was previously determined by comparison of its CD data with those of chlorovulone I, whose absolute stereochemistry was established by the total synthesis.[5b] Treatment of **1** with K_2CO_3 in methanol gave **7** and its 7*Z* isomer **8**. The spectral data of **7** including the CD spectrum were identical with those of iodovulone I, which confirmed the 12*R* configuration. Therefore, the configuration at C-8 in **1** was also *R*.

The absolute configuration at C-7 bearing the secondary acetoxyl group was independently clarified by the modified Mosher's method.[9,11] Compound **1** was converted to the diol **11** through reduction by $NaBH_4$ with $CeCl_3$ in methanol to give **9**, protection of the resulting hydroxyl group on C-9 by a methoxymethyl (MOM) group to give **10**, and saponification of the acetate ester on C-7 by treatment with K_2CO_3 in methanol to give **11**. The secondary alcohol **11**[10] was reacted with a racemic mixture of methoxy(2-naphthyl)-acetic acid (2NMA)[11] in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) and 4-(dimethylamino)pyridine (DMAP) in $CHCl_3$ to give a diastereomeric mixture of the corresponding 2NMA esters. The (*R*)-2NMA ester (**12**) and (*S*)-2NMA ester (**13**) were separated by normal-phase HPLC, and the configuration of the chiral center in 2NMA was assigned on the basis of the CD spectrum of each compound.[12] The $\Delta\delta$ values ($\Delta\delta = \delta_{R\ \text{ester}} - \delta_{S\ \text{ester}}$) for each proton as shown in Figure 2 were consistent with the *S* configuration at C-7.[13] Thus the 7*S*,8*R*,12*R* configurations in **1** were established. The compound **1** was named 7-acetoxy-7,8-dihydroiodovulone I.

The molecular formula of the iodinated compound **2** [$[\alpha]_D$ +38.7° (*c* 0.075, $CHCl_3$)] was assigned as $C_{23}H_{33}O_6I$ by HREIMS [472.1118 [M − CH_3CO_2H]$^+$ (calcd for $C_{21}H_{29}O_4I$, 472.1111)] and the ^{13}C NMR data. The ^{1}H and ^{13}C NMR spectra of **2** were very similar to those of **1** except for the

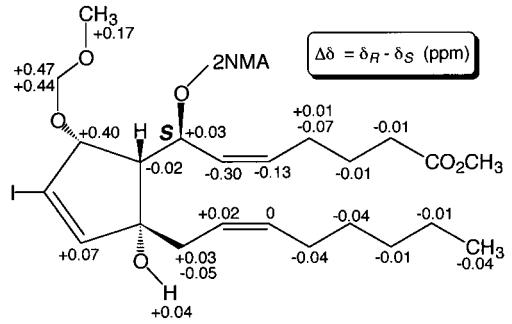

Figure 2. $\Delta\delta$ values of the 2NMA esters of **11**.

^{1}H coupling constant between two olefinic protons at H-5 and -6 (15.5 Hz; 10.7 Hz in **1**) and the ^{13}C chemical shift at C-4 (δ_C 31.4; 27.0 in **1**). Compound **2** was thus assigned as a 5*E* isomer of **1**. The absolute stereochemistry of **2** was determined by a CD spectrum, which exhibited the same Cotton effect as **1**, indicating the 7*S*,8*R*,12*R* configurations in **2** the same as those for **1**. Compound **2** was named 7-acetoxy-7,8-dihydroiodovulone II.

The molecular formula ($C_{23}H_{33}O_6Br$) of the brominated compound **3** [$[\alpha]_D$ +37.0° (*c* 0.17, $CHCl_3$)] was obtained on the basis of HREIMS [425.1251 [M − CH_3CO_2H]$^+$ (calcd for $C_{21}H_{29}O_4{}^{79}Br$, 424.1249)] and ^{13}C NMR data. The ^{1}H and ^{13}C NMR spectra of **3** were quite similar to those of **1**, but low-field shift of the C-10 carbon signal (δ_C 126.6; 103.4 in **1**) and high-field shift of the C-11 carbon signal (δ_C 162.7; 170.7 in **1**) clearly indicated that the iodine atom at C-10 in **1** was replaced with a bromine atom in **3**. The comparison of the CD spectrum of **3** with that of **1** demonstrated the 7*S*,8*R*,12*R* configurations for **3**. Compound **3** was named 7-acetoxy-7,8-dihydrobromovulone I.

The molecular formula of $C_{23}H_{33}O_6Br$ for **4** [$[\alpha]_D$ +40.0° (*c* 0.025, $CHCl_3$)] was deduced from HREIMS [425.1243 [M − CH_3CO_2H]$^+$ (calcd for $C_{21}H_{29}O_4{}^{79}Br$, 424.1249)]. The ^{1}H NMR spectrum of **4** was very similar to that of **3** except for the coupling constant between two olefinic protons at H-5 and -6 (15.5 Hz; 10.7 Hz in **3**), which indicates that **4** is a 5*E* isomer of **3**. The 7*S*,8*R*,12*R* configurations were determined by comparison of the CD spectrum of **4** with that of **3**. Compound **4** was named 7-acetoxy-7,8-dihydrobromovulone II.

The structure of the chlorinated compound **5** [$[\alpha]_D$ +43.9° (*c* 0.04, $CHCl_3$)], which possesses the molecular formula $C_{23}H_{33}O_6Cl$, determined by HREIMS [380.1770 [M − CH_3CO_2H]$^+$ (calcd for $C_{21}H_{29}O_4{}^{35}Cl$, 384.1754)] and ^{13}C NMR data was found to be the same as that of punaglandin 8,[14] whose ^{1}H NMR spectral data coincided with those of **5**. However, neither the specific rotation nor CD spectral data of punaglandin 8 were reported in the literature, and the absolute configuration was not determined.[14] Therefore, the stereostructure of **5** including absolute configuration was independently assigned as mentioned below. The ^{1}H and ^{13}C NMR spectra of **5** were quite similar with those of **1** and **3**, indicating the same relative stereochmistry of **5** as that of **1** and **3**. The comparison of the CD spectrum of **5** with that of **1** demonstrated that **5** possesses 7*S*,8*R*,12*R* configurations, the same as **1**, **2**, **3**, and **4**.

The molecular formula of $C_{23}H_{33}O_6Cl$ for compound **6** [$[\alpha]_D$ +45.5° (*c* 0.011, $CHCl_3$)] was deduced from HREIMS [380.1749 [M − CH_3CO_2H]$^+$ (calcd for $C_{21}H_{29}O_4{}^{35}Cl$, 384.1754)]. By comparison of the ^{1}H NMR spectra with that of **5**, the structure of **6** was concluded to be a 5*E* isomer of **5**. The absolute configuration of **6** was confirmed by comparison of the CD spectrum with that of **5**, indicating 7*S*,8*R*,12*R* configurations. Compound **6** was named 7-acetoxy-7,8-dihydrochrolovulone II.

Compound **1** showed cytotoxic activity[15] against MOLT-4 (human T lymphocyte leukemia), DLD-1 (human colorectal adenocarcinoma), and IMR-90 (human diploid lung fibroblast) cells at IC_{50} 0.52, 0.6, and 4.5 μg/mL, respectively.

Experimental Section

General Experimental Procedure. Optical rotations were measured in $CHCl_3$ solution on a JASCO DIP-370 automatic polarimeter. UV spectra were recorded with a JASCO V-520 spectrophotometer. IR spectra were recorded with a Perkin-Elmer FT-IR 1600 spectrophotometer. High-resolution electron-impact mass spectra (HREIMS) were obtained by electron impact on a Micromass Auto Spec spectrometer. ^{1}H and ^{13}C NMR spectra were recorded at 500 and 125 MHz, respectively, with a Bruker DRX-500 spectrometer using $CDCl_3$ as a solvent. Proton chemical shifts were referenced to the residual $CHCl_3$ signal at δ_H 7.26 ppm, and ^{13}C NMR spectra were referenced to the central peak of $CDCl_3$ at δ_C 77.0 ppm. Two-dimensional (2D) spectra, such as ^{1}H−^{1}H COSY, NOESY, HMQC, and HMBC, were measured on the basis of standard Bruker pulse sequences. CD spectra were measured on a JASCO J-720 circular dichrometer. Liquid column chromatography (LCC) and flash column chromatography (FCC) were carried out on a Merck Si gel 60 (particle size: 0.063−0.200 mm and 0.040−0.063 mm, respectively) column. Medium-pressure liquid chromatography (MPLC) was carried out with a Kusano CIG prepack column CPS-HS-221-05 (Si gel) and CPO-HS-221-20 (ODS Si gel) for normal and reversed phase, respectively. High-performance liquid chromatography (HPLC) was conducted with a YMC-Pack SIL-06 (Si gel, SH-043-5-06) and a YMC-Pack ODS-AM (ODS Si gel, SH-343-5) for normal and reversed phase, respectively. The HPLC system was equipped with a recycle loop, which was used depending on need.

Collection, Extraction, and Isolation. Animal Material. The soft coral, *Clavularia viridis* Quoy and Gaimard, was collected from the coral reef of Ishigaki Island, Okinawa Prefecture, Japan, in March 1988, at a depth of 1−2 m. A voucher specimen (No. MRS-63318-3) is on deposit at the Tokyo University of Pharmacy and Life Science, Tokyo, Japan.

Isolation. Freeze-dried specimens (470 g) were extracted successively with hexane (2 L × 2), AcOEt (2 L × 2), and MeOH (2 L × 2). After filtration, each extract was concentrated under reduced pressure to give hexane (14.5 g), AcOEt (3.7 g), and MeOH (33.4 g) extracts. A part of the hexane extract (6.83 g) was chromatographed over Si gel eluted with hexane (1 L), hexane−AcOEt (3:1, 850 mL and 1:1, 700 mL), AcOEt (700 mL), and MeOH (700 mL), in turn.

The second fraction [4.54 g, eluted with hexane−AcOEt (3:1)] was separated by normal-phase LCC eluted with hexane−AcOEt (9:1, 8:2, and 7:3) and normal-phase MPLC eluted with hexane−AcOEt (8:2) to obtain crude halogeneted prostanoid fractions. Further separation and purification of these fractions by normal-phase [eluent: hexane−AcOEt (8:2 and 7:3)] and reversed-phase [eluent: CH_3CN−H_2O (8:2)] recycling HPLC afforded compounds **1** (29.8 mg), **2** (1.1 mg), **3** (2.6 mg), **4** (0.3 mg), **5** (0.6 mg), and **6** (0.1 mg).

The third fraction [0.77 g, eluted with hexane−AcOEt (1:1)] containing mainly clavulones I, II, and III was separated by reversed-phase HPLC [eluted with CH_3CN−H_2O (8:2)] to afford clavulone II (281 mg) and a mixture of clavulones I and III (325 mg).

7-Acetoxy-7,8-dihydroiodovulone I (1): colorless oil; $[\alpha]_D$ +22.7° (*c* 0.67, $CHCl_3$); CD (MeOH) λ_{ext} nm ($\Delta\epsilon$) 332.5 (+2.97), 266.4 (−3.95), 249.0 (−3.99), 215.8 (−2.63); IR ν_{max} (cm^{-1}) 3470, 2928, 1732, 1240; UV (MeOH) λ_{max} nm (ϵ) 251 (3500); ^{1}H and ^{13}C NMR, see Table 1; EIMS (*m*/*z*) [M]$^+$ 532; HREIMS (*m*/*z*) 472.1109 [M − CH_3CO_2H]$^+$ (calcd for $C_{21}H_{29}O_4I$, 472.1111).

7-Acetoxy-7,8-dihydroiodovulone II (2): colorless oil; $[\alpha]_D$ +38.7° (*c* 0.075, $CHCl_3$); CD (MeOH) λ_{ext} nm ($\Delta\epsilon$) 332.5 (+2.40),

Table 2. ^{1}H and ^{13}C NMR Spectral Data for Compounds **5** and **6**

	5		6
position	^{13}C[a]	^{1}H[b]	^{1}H[b]
1(C)	174.0		
2(CH_2)	33.4	2.33(t, 7.4)	2.30(t, 7.5)
3(CH_2)	24.4	1.72(m)	1.72(quintet, 7.5)
4(CH_2)	27.0	2.21(m)	2.09(m)
5(CH)	133.7	5.59(dt, 10.8, 7.6)	5.74(dt, 15.5, 6.5)
6(CH)	126.5	5.83(dd, 10.8, 9.6)	5.82(dd, 15.5, 7.7)
7(CH)	68.0	5.96(dd, 9.6, 3.4)	5.64 (dd, 7.7, 3.3)
8(CH)	57.0	2.68(d, 3.4)	2.73(d, 3.3)
9(C)	196.5		
10(C)	136.4		
11(CH)	158.1	7.33(s)	7.32(s)
12(C)	77.0[c]		
13(CH_2)	39.4	2.55(br dd, 14.3, 8.7) 2.40(br dd, 14.3, 7.2)	2.54 (br dd, 14.5, 8.2) 2.39(br dd, 14.5, 7.5)
14(CH)	121.3	5.34(br ddd, 11.0, 8.7, 7.2)	5.33(br ddd, 11.0, 8.2, 7.5)
15(CH)	136.2	5.66(br dt, 11.0, 7.4)	5.67(br dt, 11.0, 7.3)
16(CH_2)	27.5	2.00(m)	2.00(m)
17(CH_2)	29.1	1.20−1.35(m)	1.20−1.35(m)
18(CH_2)	31.5	1.20−1.35(m)	1.20−1.35(m)
19(CH_2)	22.5	1.20−1.35(m)	1.20−1.35(m)
20(CH_3)	14.0	0.89(t, 7, 0)	0.89(t, 6, 9)
*CH_3*O	51.6	3.68 (s)	3.67(s)
CH_3*C*O	170.3		
*CH_3*CO	21.1	1.99(s)	2.01(s)
OH		3.16(s)	3.09(s)

[a] Multplicities of ^{13}C resonances were achieved by DEPT experiments. [b] Multiplicities and *J* (Hz) values are presented in parentheses. [c] The chemical shift was measured in C_6D_6 because this signal was overlapped on the chloroform signal. Chemical shift was referenced to the central peak of C_6D_6 at δ_C 128.0 ppm.

258.5 (−3.30), 216.0 (−2.36); IR ν_{max} (cm^{-1}) 3458, 2925, 1731, 1238; UV (MeOH) λ_{max} nm (ϵ) 245 (4500); ^{1}H and ^{13}C NMR, see Table 1; EIMS (*m*/*z*) $[M]^+$ 532; HREIMS (*m*/*z*) 472.1118 $[M - CH_3CO_2H]^+$ (calcd for $C_{21}H_{29}O_4I$, 472.1111).

7-Acetoxy-7,8-dihydrobromovulone I (3): colorless oil; $[\alpha]_D$ +37.0° (*c* 0.17, $CHCl_3$); CD (MeOH) λ_{ext} nm ($\Delta\epsilon$) 331.7 (+2.76), 254.9 (−6.21); IR ν_{max} (cm^{-1}) 3459, 2926, 1732, 1239; UV (MeOH) λ_{max} nm (ϵ) 236 (5900); ^{1}H and ^{13}C NMR, see Table 1; EIMS (*m*/*z*) $[M - CH_3CO_2H]^+$ 424, 426 (1:1); HREIMS (*m*/*z*) 424.1251 $[M - CH_3CO_2H]^+$ (calcd for $C_{21}H_{29}O_4{}^{79}Br$, 424.1249).

7-Acetoxy-7,8-dihydrobromovulone II (4): colorless oil; $[\alpha]_D$ +40.0° (*c* 0.025, $CHCl_3$); CD (MeOH) λ_{ext} nm ($\Delta\epsilon$) 331.5 (+1.82), 245.5 (−4.67); IR ν_{max} (cm^{-1}) 3442, 2923, 1732, 1238; UV (MeOH) λ_{max} nm (ϵ) 233 (6300); ^{1}H NMR, see Table 1; EIMS (*m*/*z*) $[M - CH_3CO_2H]^+$ 424, 426 (1:1); HREIMS (*m*/*z*) 424.1243 $[M - CH_3CO_2H]^+$ (calcd for $C_{21}H_{29}O_4{}^{79}Br$, 424.1249).

Compound 5 (punaglandin 8):[14] colorless oil; $[\alpha]_D$ +43.9° (*c* 0.04, $CHCl_3$); CD (MeOH) λ_{ext} nm ($\Delta\epsilon$) 331.2 (+2.56), 245.5 (−6.09); IR ν_{max} (cm^{-1}) 3443, 2919, 1732, 1216; UV (MeOH) λ_{max} nm (ϵ) 227 (6700); ^{1}H and ^{13}C NMR, see Table 2; EIMS (*m*/*z*) $[M - CH_3CO_2H]^+$ 380, 382 (2:1); HREIMS (*m*/*z*) 380.1770 $[M - CH_3CO_2H]^+$ (calcd for $C_{21}H_{29}O_4{}^{35}Cl$, 380.1754).

7-Acetoxy-7,8-dihydrochrolovulone II (6): colorless oil; $[\alpha]_D$ +45.5° (*c* 0.011, $CHCl_3$); CD (MeOH) λ_{ext} nm ($\Delta\epsilon$) 331.0 (+1.82), 237.0 (−4.04); IR ν_{max} (cm^{-1}) 3440, 2921, 1732, 1238; ^{1}H NMR, see Table 2; EIMS (*m*/*z*) $[M - CH_3CO_2H]^+$ 380, 382 (2:1); HREIMS (*m*/*z*) 380.1749 $[M - CH_3CO_2H]^+$ (calcd for $C_{21}H_{29}O_4{}^{35}Cl$, 380.1754); UV (MeOH) λ_{max} nm (ϵ) 226 (5700).

Conversion of 1 to Iodovulone I (7) and Its Isomer (8). To a solution of **1** (2.6 mg) in MeOH (1 mL) was added potassium carbonate (0.4 mg), and the reaction mixture was stirred for 10 min at room temperature. After one drop of saturated ammonium chloride solution was added, the mixture was concentrated under reduced pressure to yield a residue, which was passed through a Si gel short column eluted with AcOEt. The crude products were separated and purified by reversed-phase HPLC [eluent: CH_3CN-H_2O (7:3)] to give iodovulone I (**7**, 0.3 mg) and its isomer (**8**, 2.1 mg). Compound **8** was a new compound and very unstable, easily isomerized to a 5*E* isomer.

Compound 8: colorless oil; HREIMS (*m*/*z*) 472.1091 $[M]^+$ (calcd for $C_{21}H_{29}O_4I$, 472.1111); ^{1}H NMR δ ppm ($CDCl_3$) 7.65 (1H, s, H-11), 7.32 (1H, ddt, *J* = 12.1, 10.9, 1.5 Hz, H-6), 6.99 (1H, dd, *J* = 12.1, 1.0 Hz, H-7), 6.01 (1H, dtd, *J* = 10.9, 8.0, 1.0 Hz, H-5), 5.57 (1H, dt, *J* = 11.0, 7.4 Hz, H-15), 5.31 (1H, dtt, *J* = 11.0, 7.6 1.6 Hz, H-14), 3.68 (3H, s, CO_2CH_3), 2.64 (1H, br dd, *J* = 14.6, 7.6 Hz, H-13), 2.55 (1H, br dd, *J* = 14.6, 7.6 Hz, H-13), 2.38 (2H, m, H-4), 2.34 (2H, t, *J* = 7.3 Hz, H-2), 1.98 (2H, m, H-16), 1.79 (2H, m, H-3), 1.32 (2H, m, H-17), 1.29 (2H, m, H-19), 1.26 (2H, m, H-18), 0.88 (3H, t, *J* = 7.1 Hz, H-20).

Conversion of 1 to 11. To a solution of **1** (7.0 mg) in MeOH (1 mL) was added $CeCl_3$ heptahydrate (5.2 mg) and sodium borohydride (0.2 mg), and the reaction mixture was stirred for 1.5 h at 0 °C. After one drop of saturated ammonium chloride solution was added, the mixture was concentrated under reduced pressure to yield a residue, which was passed through a Si gel short column eluted with AcOEt. The crude products were purified by normal-phase HPLC [eluent: hexanes−ether (7:3)] to give **9** (4.2 mg).

To a solution of **9** (4.2 mg) in $CHCl_3$ (1 mL) was added *N*-ethyl diisopropylamine (50 μL) and chloromethyl methyl ether (2 drops), and the reaction mixture was stirred for 1 h at room temperature. After one drop of saturated ammonium chloride solution was added, the mixture was concentrated under reduced pressure to yield a residue, which was passed through a Si gel short column eluted with AcOEt. The crude product was purified by normal-phase HPLC [eluent: hexanes−ether (7:3)] to give **10** (2.7 mg).

To a solution of **10** (2.7 mg) in MeOH (1 mL) was added potassium carbonate (0.5 mg), and the reaction mixture was stirred for 1.5 h at room temperature. After one drop of saturated ammonium chloride solution was added, the mixture was concentrated under reduced pressure to yield a residue, which was passed through a Si gel short column eluted with AcOEt. The crude products were separated and purified by normal-phase HPLC [eluent: hexane−ether (3:7)] to give **11** (2.2 mg).

Compound 11: colorless oil; $[\alpha]_D$ +61.8° (*c* 0.11, $CHCl_3$); ^{1}H NMR δ ppm ($CDCl_3$) 6.42 (1H, s, H-11), 5.63 (1H, dd, *J* = 10.7, 9.1 Hz, H-5), 5.56 (1H, m, H-15), 5.56 (1H, m, H-6), 5.31 (1H, m, H-14), 4.96 (1H, d, *J* = 6.5 Hz, MOM), 4.92 (1H, d, *J* = 6.5 Hz, MOM), 4.86 (1H, br ddd, *J* = 7.1, 6.7, 5.9 Hz, H-7), 4.63 (1H, d, *J* = 6.7 Hz, H-9), 3.68 (3H, s, CO_2CH_3), 3.58 (1H, d, *J* = 5.9 Hz, C7-O*H*), 3.53 (3H, s, MOM), 2.95 (1H, s, 12C-O*H*), 2.48 (br dd, *J* = 14.7, 6.7 Hz, H-13), 2.41 (1H, br dd, *J* = 14.7, 7.9 Hz, H-13), 2.36 (2H, t, *J* = 7.4 Hz, H-2), 2.30 (1H, t, *J* = 6.7 Hz, H-8), 2.27 (1H, m, H-4), 2.20 (1H, m, H-4), 2.03 (2H, m, H-16), 1.78 (1H, m, H-3), 1.75 (1H, m, H-3), 1.35 (2H, m, H-17), 1.32 (2H, m, H-18), 1.30 (2H, m, H-19), 0.91 (3H, t, *J* = 7.0, H-20); ^{13}C NMR δ ppm ($CDCl_3$) 174.0 (C, C-1), 150.5 (CH, C-11), 133.9 (CH, C-15), 131.64 (CH, C-5 or -6), 131.62 (CH, C-5 or -6), 123.2 (CH, C-14), 98.9 (C, C-10), 98.6 (CH_2, MOM), 90.0 (CH, C-9), 84.0 (C, C-12), 65.0 (CH, C-7), 56.8 (CH_3, MOM), 54.4 (CH, C-8), 51.5 (CH_3, $-CO_2CH_3$), 36.2 (CH_2, C-13), 33.5 (CH_2, C-2), 31.6 (CH_2, C-18), 29.2 (CH_2, C-17), 27.5 (CH_2, C-16), 27.1 (CH_2, C-4), 24.6 (CH_2, C-3), 22.6 (CH_2, C-19), 14.1 (CH_3, C-20).

Preparation of (*R*)- and (*S*)-2NMA Esters (12 and 13). To a solution of **11** (2.2 mg) in $CHCl_3$ (0.5 mL) was added 2NMA (racemic mixture, 2.4 mg), DMAP (0.9 mg), and EDC (3.6 mg), and the mixture was stirred at room temperature for 1 h. After 2 drops of H_2O were added to the mixture, the mixture was partitioned between 10% aqueous citric acid solution and AcOEt. The organic layer was separated and washed once with 10% aqueous citric acid solution, twice with saturated sodium bicarbonate solution, and once with saturated NaCl solution. The organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The crude products were separated and purified by normal-phase HPLC [eluent: hexane−AcOEt (7:3)] to afford (*R*)-2NMA ester (**12**, 0.5 mg) and (*S*)-2NMA ester (**13**, 0.4 mg). The assignment

of (*R*)- and (*S*)-2NMA ester was made on the basis of CD spectra for both compounds.[12]

(*R*)-2NMA ester (12): colorless oil; $[\alpha]_D$ −60.6° (*c* 0.033, $CHCl_3$); CD (MeOH) λ_{ext} nm ($\Delta\epsilon$) 286.8 (shoulder, −0.60), 275.4 (−1.29), 266.2 (−1.45), 258.0 (−1.36), 235.8 (−14.49); ^{1}H NMR δ ppm ($CDCl_3$) 7.84 (4H, m, 2NMA), 7.50 (3H, m, 2NMA), 6.43 (1H, s, H-11), 5.90 (1H, t, J = 10.2 Hz, H-7), 5.48 (1H, m, H-15), 5.45 (1H, m, H-5), 5.20 (1H, m, H-14), 5.03 (1H, dd, J = 10.6, 10.2 Hz, H-6), 4.88 (1H, s, 2NMA), 4.60 (1H, d, J = 7.0 Hz, MOM), 4.56 (1H, d, J = 7.0 Hz, MOM), 4.44 (1H, d, J = 5.4 Hz, H-9), 3.65 (3H, s, $-CO_2CH_3$), 3.42 (3H, s, 2NMA), 3.36 (3H, s, MOM), 2.45 (1H, br dd, J = 14.5, 6.4 Hz, H-13), 2.40 (1H, m, H-4), 2.37 (1H, dd, J = 10.6, 5.4 Hz, H-8), 2.33 (1H, s, C12-OH), 2.30 (1H, m, H-13), 2.29 (2H, t, J = 7.7 Hz, H-2), 2.23 (1H, m, H-4), 1.92 (2H, br q, J = 7.1 Hz, H-16), 1.71 (2H, m, H-3), 1.27 (2H, m, H-17), 1.25 (2H, m, H-18), 1.25 (2H, m, H-19), 0.85 (3H, t, J = 7.0 Hz, H-20).

(*S*)-2NMA ester (13): colorless oil; $[\alpha]_D$ +8.3° (*c* 0.024, $CHCl_3$); CD (MeOH) λ_{ext} nm ($\Delta\epsilon$) 287.2 (+0.61), 276.9 (+0.91), 268.6 (+0.74), 258.2 (shoulder, +0.39), 234.6 (+7.62); ^{1}H NMR δ ppm ($CDCl_3$) 7.91 (1H, br s, 2NMA), 7.85 (3H, m, 2NMA), 7.55 (1H, dd, J = 8.6, 1.6 Hz, 2NMA), 7.49 (2H, m, 2NMA), 6.36 (1H, s, H-11), 5.87 (1H, t, J = 10.1 Hz, H-7), 5.58 (1H, dt, J = 10.8, 7.1 Hz, H-5), 5.48 (1H, m, H-15), 5.33 (1H, dd, J = 10.8 10.1 Hz, H-6), 5.18 (1H, m, H-14), 4.86 (1H, s, 2NMA), 4.16 (1H, d, J = 6.8 Hz, MOM), 4.09 (1H, d, J = 6.8 Hz, MOM), 4.04 (1H, d, J = 5.4 Hz, H-9), 3.63 (3H, s, $-CO_2CH_3$), 3.42 (3H, s, 2NMA), 3.19 (3H, s, MOM), 2.42 (1H, m, H-13), 2.39 (1H, m, H-4), 2.39 (1H, dd, J = 10.1, 5.4 Hz, H-8), 2.37 (1H, s, C12-OH), 2.35 (1H, m, H-13), 2.30 (2H, t, J = 7.6 Hz, H-2), 2.30 (1H, m, H-4), 1.96 (2H, br q, J = 7.2 Hz, H-16), 1.72 (2H, m, H-3), 1.31 (2H, m, H-17), 1.26 (2H, m, H-18), 1.26 (2H, m, H-19), 0.89 (3H, t, J = 7.0 Hz, H-20).

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References and Notes

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