

Article

Isolation of C₁₁ Cyclopentenones from Two Didemnid Species, *Lissoclinum* sp. and *Diplosoma* sp.

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Abstract: A series of new C₁₁ cyclopentenones **1–7** was isolated, together with four known metabolites **9/10**, **12** and **13**, from the extract of the didemnid ascidian *Lissoclinum* sp. The other didemnid ascidian *Diplosoma* sp. contained didemnenones **1**, **2** and **5**, and five known metabolites **8–12**. The structures of **1–7** were elucidated by spectroscopic analyses. Cytotoxicity of the isolated compounds was evaluated against three human cancer cell lines (HCT116, A431 and A549).

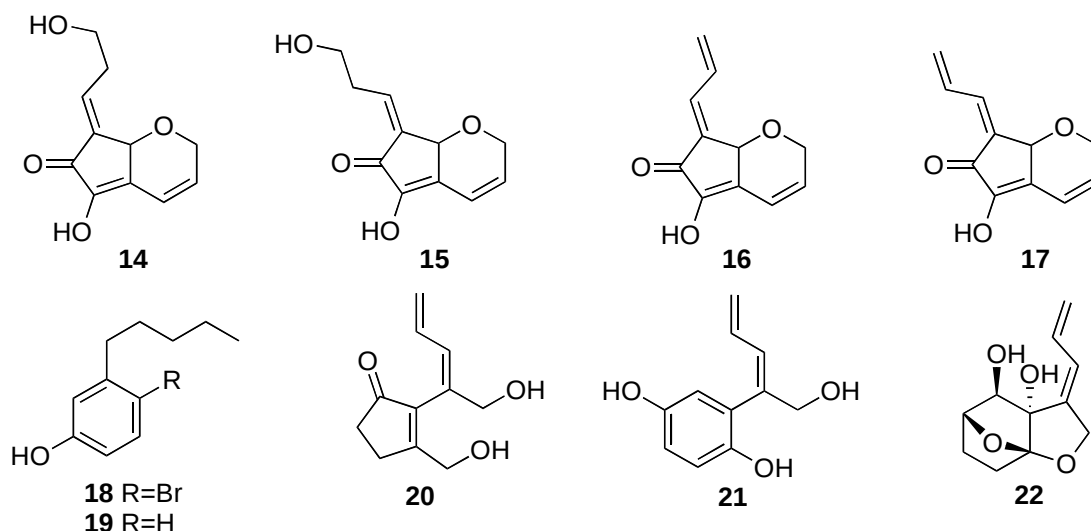
Keywords: *Lissoclinum*; *Diplosoma*; cyclopentenone; didemnenone; cytotoxicity

1. Introduction

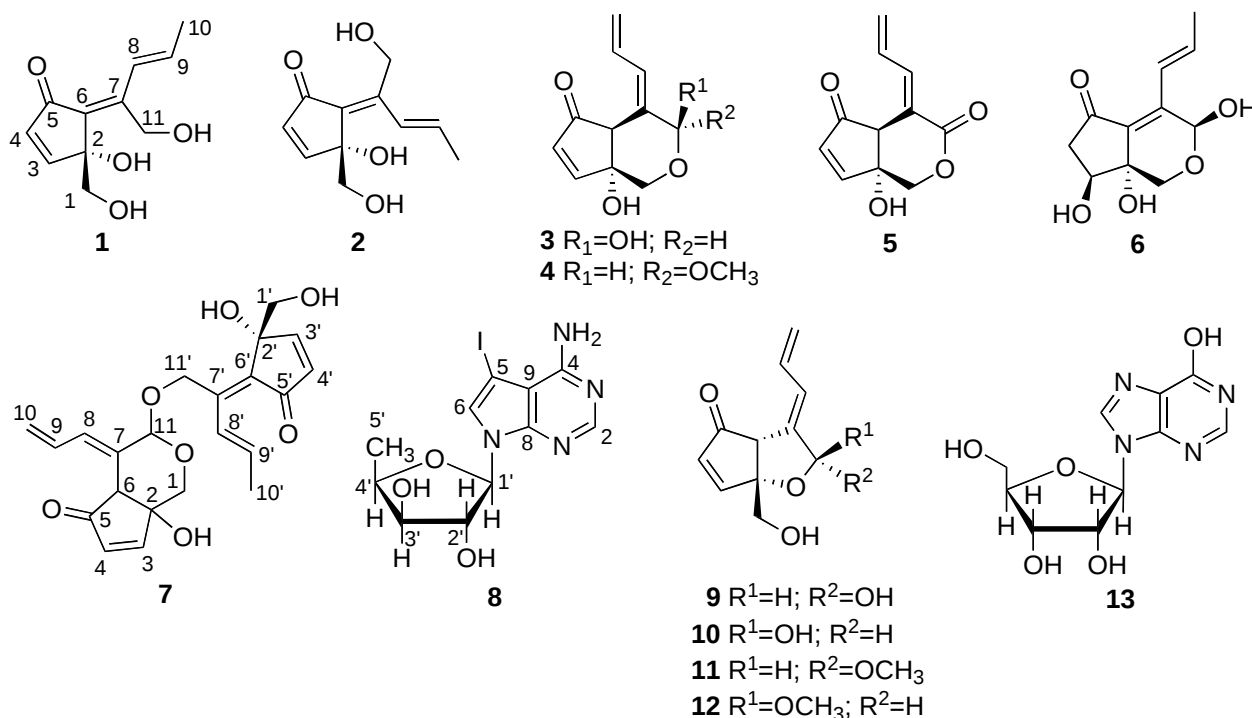
It has been amply demonstrated that ascidians are a prolific source of novel bioactive secondary metabolites [1–4]. Ascidians belonging to the family Didemnidae, e.g., *Lissoclinum* spp. and

Diplosoma spp., harbor obligate cyanobacterial symbionts of the genus *Prochloron* [5–7], and have yielded structurally unique and pharmacologically interesting compounds such as patellazoles, varacin, virenamides, haterumalide and haterumaimides [8–15]. A series of C₁₁ compounds having the distinctive exo-allylidene-lactone named didemnenone was isolated from didemnid ascidians, *Trididemnum cyanophorum* (didemnenones A and B) and *Didemnum voeltzkowi* (didemnenones C and D) [16]. They showed a wide range of biological activities, including cytotoxicity against leukemia cells as well as antimicrobial and antifungal activities [16–18]. Their structures were determined based on an X-ray investigation of the methylacetal of didemnenone A and from synthetic results [16–18]. As described previously, as part of our ongoing research aiming at the isolation of biologically active metabolites from marine organisms living in the tidal zone, we have isolated several C₁₁ compounds, dinemnenone congeners **14–17** [19–22] and pentylphenols **18** and **19** [22] from ascidian *Diplosoma* spp. (Figure 1).

Figure 1. Some C₁₁ metabolites isolated from marine organisms.



Recently, we examined the constituents of ascidians, *Lissoclinum* sp. collected on the coast of Tarama island and *Diplosoma* sp. from dead corals of Hateruma island. From the *Lissoclinum* sp. we identified the new metabolites **1–7**, along with the known metabolites, didemnenones A (**9**) and B (**10**) as an inseparable mixture, a methylacetal of didemnenone B (**12**) [16], and inosine (**13**) (Figure 2). The *Diplosoma* sp. contained didemnenones **1**, **2** and **5** along with five known metabolites **8–12** (Figure 2) [16,23–34]. In this report, we describe the isolation, structure elucidation and bioactivity of these metabolites, and we also discuss the biosynthesis of didemnenones and the related compounds.

Figure 2. Compounds isolated from two didemnid ascidians.

2. Results and Discussion

Specimens of colonial ascidian *Lissoclinum* sp. were collected off the coast of Tarama island, Okinawa, Japan. The specimens were extracted with acetone and the extract was partitioned between EtOAc and H₂O. The aqueous layer was further partitioned between 1-BuOH and H₂O. The H₂O-soluble part was fractionated using RP-MPLC (reversed-phase MPLC) and eluted with a combination of H₂O and MeOH. Further purification of the obtained fractions using RP-HPLC led to isolation of **1** (0.0059% of wet weight) and **13** (0.00067%). The EtOAc extract was suspended in aqueous MeOH and then successively extracted with hexane, CHCl₃ and 1-BuOH. The resultant BuOH-soluble material was separated by a series of chromatographic steps [RP-OCC (reversed-phase open column chromatography), RP-MPLC and RP-HPLC] to afford **3** (0.0020%), **4** (0.00058%), **6** (0.0029%), **2** (0.011%), **1** (0.0049%), **12** (0.000014%), **5** (0.00030%), **7** (0.000019%) and an inseparable mixture of didemnenones A/B (**9/10**, 1:1, 0.00033%).

The colonial ascidian *Diplosoma* sp. was collected off the coast of Hateruma island, Okinawa, Japan. The specimens were extracted with acetone and the extract was partitioned between EtOAc and H₂O. The EtOAc extract was suspended in aqueous MeOH and then successively extracted with hexane, CHCl₃ and 1-BuOH. The CHCl₃-soluble material was subjected to RP-OCC and eluted with H₂O/MeOH, MeOH and MeOH/EtOAc. Further separation of the H₂O/MeOH fraction by RP-HPLC eluted with a combination of H₂O, MeOH and MeCN, led to the isolation of **1** (0.0021%), **2** (0.00066%), **5** (0.00047%), **8** (0.018%), **9/10** (1:1 mixture, 0.00069%), **11** (0.00098%) and **12** (0.00033%).

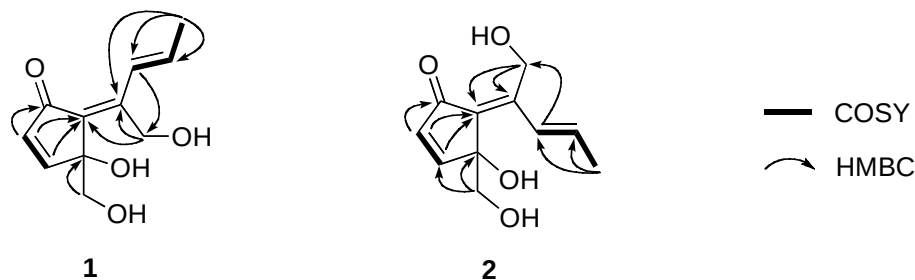
Analysis of **1** by NMR (Tables 1 and 2) and HR-ESIMS [m/z 233.0786 ($M + Na$)⁺, calcd. for C₁₁H₁₄O₄Na, 233.0784] provided a molecular formula of C₁₁H₁₄O₄. The carbon resonating at δ_C 197.1 (s) suggested the presence of a carbonyl carbon in **1** and the IR absorption band at

$\nu_{\max}$ 1675 cm^{-1} further supported the presence of the carbonyl group. Extensive analysis of ^1H - and ^{13}C -NMR data (Tables 1 and 2) supported by ^1H - ^1H COSY data indicated the presence of a *cis* double bond [δ_{C} 161.4 (d), 134.3 (d); δ_{H} 7.35 (d), 6.25 (d)], a *trans* double bond [δ_{C} 127.2 (d), 134.8 (d); δ_{H} 7.73 (dd, $J = 1.6, 16.0$ Hz), 6.43 (dq, $J = 16.0, 6.8$ Hz)], a tetrasubstituted double bond [δ_{C} 133.5 (s), 144.5 (s)], a methyl group [δ_{C} 19.1 (t); δ_{H} 1.83 (dd)], two oxygenated methylenes [δ_{C} 66.8 (t); δ_{H} 3.74 (dd), 3.51 (dd) and δ_{C} 56.6 (t); δ_{H} 4.64 (dd), 4.36 (dd)] and an oxygenated quaternary carbon [δ_{C} 80.5 (s)] in **1**. Degrees of unsaturation for these partial structures amount to four. Thus, **1** must be monocyclic to account for the five degrees of unsaturation required by the molecular formula. The connectivity of the aforementioned partial structures was established from the HMBC correlations of $\text{H}_2\text{-1/C-2}$, H-3/C-6 , H-4/C-5 , H-4/C-6 , H-8/C-11 , $\text{H}_3\text{-10/C-7}$, $\text{H}_3\text{-10/C-8}$, $\text{H}_3\text{-10/C-9}$, $\text{H}_2\text{-11/C-6}$ and $\text{H}_2\text{-11/C-7}$, as shown in Figure 3, to describe the entire carbon framework of **1**. Geometric configuration of two olefins in **1** at C-6/C-7 and C-8/C-9 were assigned to be *E* by NOEDS experiments (Figure 4), in which irradiation of H-9 caused enhancement of H-11 and irradiation of H-1 resulted in enhancement of the H-3 and OH-11 proton signals. Therefore, the planar structure of **1** was established as a class of didemnenone as shown in **1**.

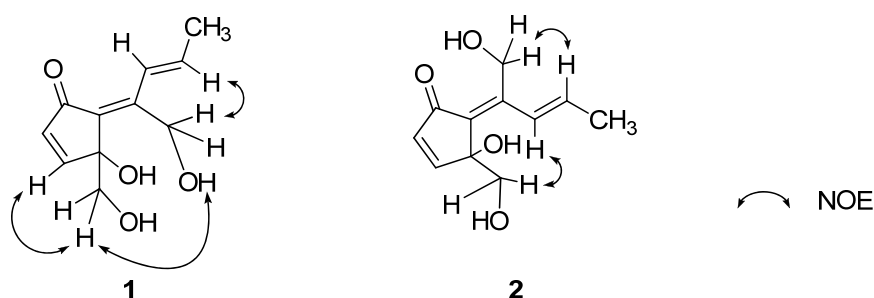
Table 1. ^1H -NMR data for compounds **1–6**.

Pos.	δ_{H} (mult., J/Hz) ^a					
	1	2	3	4	5	6
1a	3.74 (dd, 6.0, 11.2)	3.68 (dd, 6.2, 10.8)	3.53 (d, 11.4)	3.49 (d, 11.2)	4.26 (d, 11.2)	4.27 (d, 11.4)
1b	3.51 (dd, 6.0, 11.2)	3.49 (dd, 6.2, 10.8)	3.42 (d, 11.4)	3.43 (d, 11.2)	4.13 (d, 11.2)	3.50 (d, 11.4)
3	7.35 (d, 6.0)	7.35 (d, 6.2)	7.52 (d, 5.6)	7.52 (d, 5.6)	7.71 (d, 5.6)	3.99 (brt, 4.8)
4a	6.25 (d, 6.0)	6.27 (d, 6.2)	6.28 (d, 5.6)	6.30 (d, 5.6)	6.40 (d, 5.6)	2.74 (dd, 4.8, 18.4)
4b						1.98 (brd, 18.4)
6			3.45 (s)	3.48 (s)	3.74 (s)	
8	7.73 (dd, 1.6, 16.0)	6.91 (d, 16.0)	6.48 (d, 11.2)	6.45 (brd, 10.8)	7.22 (d, 11.6)	7.27 (dd, 1.4, 16.0)
9	6.43 (dq, 16.0, 6.8)	6.43 (dq, 16.0, 5.8)	6.58 (ddd, 11.2, 11.6, 16.4)	6.59 (ddd, 10.4, 10.8, 16.4)	6.86 (ddd, 10.0, 11.6, 16.8)	6.31 (dq, 16.0, 6.8)
10a	1.83 (dd, 1.6, 6.8)	1.85 (d, 5.8)	5.38 (d, 16.4)	5.40 (brd, 2.0, 16.4)	5.85 (dd, 1.6, 10.0)	1.82 (dd, 1.4, 6.8)
10b			5.23 (d, 11.6)	5.26 (brd, 2.0, 10.0)	5.67 (dd, 1.6, 16.8)	
11a	4.64 (dd, 6.8, 13.4)	4.82 (dd, 5.6, 11.2)	5.10 (brd, 5.8)	4.82 (s)		5.51 (d, 5.4)
11b	4.36 (dd, 6.8, 13.4)	4.59 (dd, 5.6, 11.2)				
OH-1	4.89 (t, 6.0)	4.84 (t, 6.2)				
OH-2	5.72 (s)	5.73 (s)	5.87 (s)	5.94 (s)	6.33 (brs)	5.24 (s)
OH-3						5.14 (brd, 4.8)
OH-11	4.66 (brs)	4.53 (t, 5.6)	6.73 (d, 5.8)			6.59 (d, 5.4)
OCH ₃				3.31 (s)		

^a Recorded at 400 MHz in DMSO- d_6 .

Figure 3. Partial structures of **1** and **2** based on COSY (bold line) and some important HMBC correlations (arrows).**Table 2.** ^{13}C -NMR data for compounds **1–6**.

C no.	δ_{C} (mult.) ^a					
	1	2	3	4	5	6
1	66.8 (CH ₂)	66.3 (CH ₂)	63.2 (CH ₂)	63.1 (CH ₂)	69.7 (CH ₂)	63.1 (CH ₂)
2	80.5 (qC)	80.4 (qC)	79.7 (qC)	79.2 (qC)	75.8 (qC)	73.0 (qC)
3	161.4 (CH)	161.4 (CH)	165.1 (CH)	165.1 (CH)	164.9 (CH)	70.5 (CH)
4	134.3 (CH)	135.1 (CH)	132.8 (CH)	132.8 (CH)	134.2 (CH)	46.3 (CH ₂)
5	197.1 (qC)	196.9 (qC)	203.8 (qC)	203.6 (qC)	202.9 (qC)	206.1 (qC)
6	133.5 (qC)	134.0 (qC)	55.5 (CH)	55.1 (CH)	53.5 (CH)	130.4 (qC)
7	144.5 (qC)	144.9 (qC)	135.0 (qC)	132.4 (qC)	122.7 (qC)	142.3 (qC)
8	127.2 (CH)	128.6 (CH)	131.7 (CH)	132.4 (CH)	143.1 (CH)	124.4 (CH)
9	134.8 (CH)	134.7 (CH)	132.3 (CH)	132.1 (CH)	132.0 (CH)	135.3 (CH)
10	19.1 (CH ₃)	19.4 (CH ₃)	119.9 (CH ₂)	120.5 (CH ₂)	128.0 (CH ₂)	19.1 (CH ₃)
11	56.6 (CH ₂)	54.0 (CH ₂)	91.7 (CH)	98.7 (CH)	166.9 (qC)	86.6 (CH)
OCH ₃				54.9 (CH ₃)		

^a Recorded at 100 MHz in DMSO-*d*₆.**Figure 4.** Selected NOEs for **1** and **2**.

Compound **2** had the same molecular formula as **1**, C₁₁H₁₄O₄, as established by HR-ESIMS [*m/z* 233.0786 (M + Na)⁺, calcd. for C₁₁H₁₄O₄Na, 233.0784]. The IR absorption bands at ν_{max} 1695 and 3360 cm^{−1} indicated the presence of carbonyl and hydroxyl groups. The NMR data (Tables 1 and 2) of **2** showed close similarity to those of **1**, except for chemical shifts of H-8. The chemical shift of H-8 in **2** (δ_{H} 6.91) was at higher field than in **1** (δ_{H} 7.73) due to the magnetic anisotropy effect of the carbonyl group, suggesting a *Z* configuration for the C-6 olefin in **2**. This was confirmed by NOES

experiments (Figure 4), in which irradiation of H-9 caused enhancement of H-11 and irradiation of H-1 resulted in enhancement of H-8.

Analysis of the ^{13}C -NMR and HR-ESIMS [m/z 231.0632, $(\text{M} + \text{Na})^+$, calcd for $\text{C}_{11}\text{H}_{12}\text{O}_4\text{Na}$, 231.0628] for **3** provided a molecular formula $\text{C}_{11}\text{H}_{12}\text{O}_4$, which suggested six degrees of unsaturation. The carbon resonating at δ_{C} 203.8 (s) suggested the presence of a carbonyl carbon in **3** and the IR absorption band at ν_{max} 1710 cm^{-1} further supported the presence of the carbonyl group. The ^1H - and ^{13}C -NMR analyses (Tables 1 and 2) coupled with ^1H - ^1H COSY data indicated the presence of an α,β -unsaturated ketone moiety [δ_{C} 203.8 (s), 132.8 (d), 165.1 (d); δ_{H} 6.28 (d), 7.52 (d)], a conjugated diene moiety [δ_{C} 135.0 (s), 131.7 (d); δ_{H} 6.48 (d) and δ_{C} 132.3 (d), 119.9 (t); δ_{H} 6.58 (ddd), 5.23 (d), 5.38 (d)], an acetal [δ_{C} 91.7 (d); δ_{H} 5.10 (brd)], a methine [δ_{C} 55.5 (d); δ_{H} 3.45 (s)], an oxygenated methylene [δ_{C} 63.2 (t); δ_{H} 3.53 (d), 3.42 (d)] and an oxygenated quaternary carbon [δ_{C} 79.7 (s)] in **3**. Extensive analysis of ^1H - ^1H COSY demonstrated two isolated spin systems, C-3–C-4 and C-8–C-10. The connectivity of the aforementioned partial structures was established from the HMBC correlations of H₂-1/C-2, H₂-1/C-3, H₂-1/C-6, H₂-1/C-11, H-3/C-2, H-3/C-4, H-3/C-5, H-3/C-6, H-6/C-2, H-6/C-4, H-6/C-5, H-6/C-7, H-6/C-11, H-8/C-6, H-8/C-11, H-9/C-7, H-11/C-1, H-11/C-6 and H-11/C-7, to describe the entire carbon framework of **3**. Geometric configuration of the olefins in **3** at C-7/C-8 was assigned to be *E* by NOEDS experiments, in which irradiation of H-6 caused enhancement of H-9 and irradiation of H-8 caused enhancement of H-11 and H-10. Therefore, the planar structure of **3** was concluded to be a class of didemnenone, as depicted in **3**. The NOE observed between a hydroxyl proton (OH-2) and H-6 revealed a *cis* fusion of two rings. The NOEs; OH-2/H-11; H-1b/H-11 allowed the assignment of the H-11 as α .

The molecular formula of **4** was deduced to be $\text{C}_{12}\text{H}_{14}\text{O}_4$ based on HR-ESIMS [m/z 245.0794, $(\text{M} + \text{Na})^+$, calcd for $\text{C}_{12}\text{H}_{14}\text{O}_4\text{Na}$, 245.0784]. The ^1H - and ^{13}C -NMR spectral data (Tables 1 and 2) of **4** resembled those of **3**, except for the presence of a proton signal at δ_{H} 3.31 (s) and a carbon signal at δ_{C} 54.9 (q) in **4**. Geometric configuration of the double bond at C-7/C-8 in **4** was assigned to be *E* by NOEDS experiments, in which irradiation of H-6 caused enhancement of H-9 and irradiation of H-8 caused enhancement of H-11 and H-10. Therefore, the planar structure of **4** was elucidated to be a methylacetal of **3**. The NOE between a hydroxyl proton (OH-2) and H-6 also indicated the ring junction to have the *cis*-geometry. The NOEs; OH-2/ H-1b; H-1a /H-11 allowed the assignment of the H-11 as β . We cannot affirm that **4** is a natural product, because it is conceivable it arises from **3** in the isolation process.

The molecular formula of **5** was determined to be $\text{C}_{11}\text{H}_{10}\text{O}_4$, based on HR-ESIMS [m/z 207.0654 $(\text{M} + \text{H})^+$, calcd for $\text{C}_{11}\text{H}_{11}\text{O}_4$, 207.0652]. The carbons resonating at δ_{C} 202.9 (s) and 166.9 (s) suggested the presence of a carbonyl carbon and an ester carbonyl carbon, respectively in **5** (Table 2) and the IR absorption band at ν_{max} 1717 cm^{-1} further supported the presence of the carbonyl groups. The ^1H - and ^{13}C -NMR (Tables 1 and 2) and 2D NMR spectral data of **5** are similar to those of γ -lactone didemnenone [16], except for the HMBC correlation observed between an oxymethylene proton H₂-1 at δ_{H} 4.13 and a carbonyl carbon C-11 at δ_{C} 166.9. Geometric configuration of the double bond at C-7/C-8 in **5** was assigned to be *E* by NOEDS experiments, in which irradiation of H-6 caused enhancement of H-9. Therefore, the planar structure of **5** was concluded to be a class of didemnenone, as depicted in **5**. The NOE between a hydroxyl proton (OH-2) and H-6 allowed the ring junction to be assigned as *cis*.

Analysis of the ^{13}C -NMR and HR-ESIMS [m/z 249.0742, $(\text{M} + \text{Na})^+$, calcd for $\text{C}_{11}\text{H}_{14}\text{O}_5\text{Na}$, 249.0733] for **6** provided a molecular formula $\text{C}_{11}\text{H}_{14}\text{O}_5$. The carbon resonating at δ_{C} 206.1 (s) suggested the presence of a carbonyl carbon in **6** (Table 2) and the IR absorption band at ν_{max} 1720 cm^{-1} further supported the presence of the carbonyl group. Extensive analysis of ^1H - and ^{13}C -NMR data (Tables 1 and 2) supported with ^1H - ^1H COSY data indicated the presence of a *trans* double bond [δ_{C} 124.4 (d), 135.3 (d); δ_{H} 7.27 (dd, $J = 1.4, 16.0\text{ Hz}$), 6.31 (dq, $J = 16.0, 6.8\text{ Hz}$)], a tetrasubstituted double bond [δ_{C} 130.4 (s), 142.3 (s)], an oxygenated methylene [δ_{C} 63.1 (t); δ_{H} 4.27 (d), 3.50 (d)], two oxygenated methines [δ_{C} 86.6 (d); δ_{H} 5.51 (d) and δ_{C} 70.5 (d); δ_{H} 3.99 (brt)], an oxygenated quaternary carbon [δ_{C} 73.0 (s)], a methylene [δ_{C} 46.3 (t); δ_{H} 2.74 (dd), 1.98 (brd)] and a methyl [δ_{C} 19.1 (q); δ_{H} 1.82 (dd)] in **6**. These functionalities accounted for three of the five degrees of unsaturation, therefore **6** is bicyclic. The connectivity of the aforementioned partial structures was established from the HMBC correlations of H-1/C-2, H-1/C-6, H-1/C-11, H-4/C-5, H-4/C-6, H-8/C-11 and H-11/C-6, to describe the entire carbon framework of **6**. The NOE between H-9 and H-11 also indicated the double bond at C-8 to have the *trans*-geometry, and the NOEs; OH-2/H-1a; OH-2/H-3; H-1a/H-11 allowed the assignment of the 3-OH and the 11-OH both as β .

Table 3. NMR data ($\text{DMSO}-d_6$) for compound **7**.

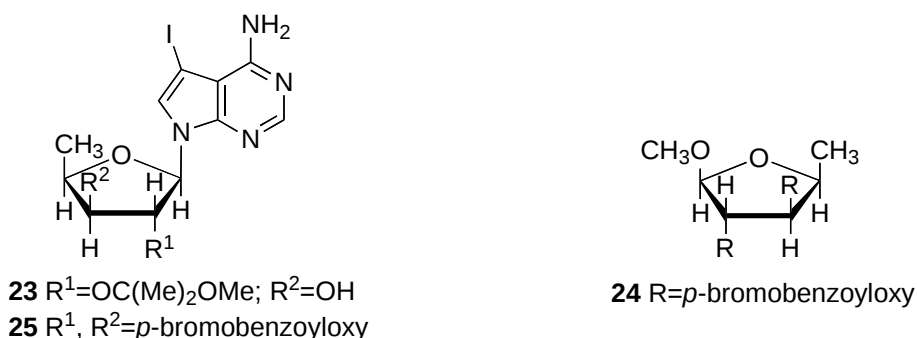
7							
C no.	δ_{C} (mult.) ^a	δ_{H} (mult., J/Hz) ^b	HMBC	C no.	δ_{C} (mult.) ^a	δ_{H} (mult., J/Hz) ^b	HMBC
1	63.3 (CH_2)	3.53 (brd, 8.0)	2, 3, 6, 11	1'a	66.6 (CH_2)	3.70 (dd, 6.0, 10.8)	6', 3', 2'
2	79.1 (qC)			1'b		3.46 (dd, 6.0, 10.8)	6', 3', 2'
3	165.1 (CH)	7.55 (d, 5.6)	1, 2, 4, 5, 6,	2'	80.4 (qC)		
4	132.8 (CH)	6.31 (d, 5.6)	2, 3, 5, 6	3'	161.6 (CH)	7.37 (d, 6.0)	6', 5', 3', 2'
5	203.6 (qC)			4'	134.9 (CH)	6.28 (d, 6.0)	6', 5', 3', 2'
6	55.1 (CH)	3.48 (s)	2, 3, 4, 5, 7, 8	5'	196.7 (qC)		
7	132.6 (qC)			6'	135.2 (qC)		
8	132.8 (CH)	6.37 (d, 10.0)	6, 7, 9, 10, 11	7'	140.7 (qC)		
9	132.2 (CH)	6.59 (ddd, 10.0, 10.0, 17.2)	8	8'	126.9 (CH)	7.78 (dd, 1.4, 16.0)	11', 7', 10'
10a	120.3 (CH_2)	5.30 (d, 17.2)	8, 9	9'	134.7 (CH)	6.41 (m)	8', 10'
10b		5.26 (d, 10.0)	8, 9	10'	19.1 (CH_3)	1.88 (dd, 1.4, 6.8)	8', 9'
11	97.9 (CH)	5.08 (s)	1, 7, 8, 11'	11'a	62.7 (CH_2)	4.86 (d, 10.4)	11, 7', 6', 8'
OH-2		5.95 (s)	1, 2, 3, 6	11'b		4.49 (d, 10.4)	11, 7', 6', 8'
				OH-1'		4.93 (t, 6.0)	1', 2'
				OH-2'		5.80 (s)	6', 2', 1'

^a Recorded at 100 MHz; ^b Recorded at 400 MHz.

Analysis of **7** by ^{13}C -NMR (Table 3) and HR-ESIMS [m/z 401.1602 $(\text{M} + \text{H})^+$, calcd for $\text{C}_{22}\text{H}_{25}\text{O}_7$, 401.1595] provided a molecular formula of $\text{C}_{22}\text{H}_{24}\text{O}_7$. Extensive analysis of ^1H - (Table 3) and ^{13}C -NMR data, supported with ^1H - ^1H COSY data, indicated the presence of two α,β -unsaturated ketone moieties [δ_{C} 203.6 (s), 132.8 (d), 165.1 (d); δ_{H} 6.31 (d), 7.55 (d) and δ_{C} 196.7 (s), 134.9 (d), 161.6 (d); δ_{H} 6.28 (d), 7.37 (d)], a conjugated diene moiety [δ_{C} 132.6 (s), 132.8 (d); δ_{H} 6.37 (d) and δ_{C} 132.2 (d), 120.3 (t); δ_{H} 6.59 (ddd), 5.30 (d), 5.26 (d)], a *trans* double bond [δ_{C} 126.9 (d), 134.7 (d); δ_{H} 7.78 (dd, $J = 1.4, 16.0\text{ Hz}$), 6.41 (m)], a tetrasubstituted double bond [δ_{C} 135.2 (s), 140.7 (s)], an acetal

$[\delta_C 97.9$ (d); $\delta_H 5.08$ (s)], three oxygenated methylenes [$\delta_C 63.3$ (t); $\delta_H 3.53$ (brd), $\delta_C 66.6$ (t); $\delta_H 3.70$ (dd), 3.46 (dd) and $\delta_C 62.7$ (t); $\delta_H 4.86$ (d), 4.49 (d)], two oxygenated quaternary carbons [$\delta_C 79.1$ (s), 80.4 (s)], a methine [$\delta_C 55.1$ (d); $\delta_H 3.48$ (s)] and a methyl [$\delta_C 19.1$ (q); $\delta_H 1.88$ (dd)] in **7**. The structure of **7** was elucidated to be a dimeric didemnenone composed of **1** and **3**, from the molecular formula, the NMR data and the HMBC correlations of H-11/C-11' and H₂-11'/C-11. Geometric configuration of three olefins in **7** at C-7/C-8, C-6'/C-7' and C-8'/C-9' was assigned to be *E* by NOEDS experiments, in which irradiation of H-6 caused enhancement of H-9, irradiation of H-8 caused enhancement of H-11 and H-10, irradiation of H-9' caused enhancement of H-11' and irradiation of H-3' resulted in enhancement of the H-1' proton signal. Therefore, the structure of **7** was established as a didemnenone dimer as shown in **7**. We could not determine the C-2/C-6 ring junction stereochemistry owing to decomposition of **7**.

Figure 5. Compounds used for determining the absolute structure of **8**.



The structure of marine metabolite **8** was determined to be 4-amino-7-(5'-deoxy- β -D-xylofuranosyl) -5-iodopyrrolo[2,3-*d*]pyrimidine by 1D and 2D NMR spectra for **8** and **23**, and by CD spectra of compounds **8**, **24** and **25** (Figure 5), as previously described [23]. The absolute stereochemistry of the new compounds was tentatively deduced to be as depicted in **1–7** based on the assumption that there is a similar biogenetic relationship between these compounds and (+)-didemnenone A. (+)Didemnenones **9–12** and inosine (**13**) were unambiguously identified by comparison of their spectral data with those described in the literature [16].

Compounds **1–13** were tested *in vitro* for their cytotoxic activities against the HCT116, A431 and A549 cancer cell lines (Table 4). Compounds **1**, **2** and **8** were significantly cytotoxic against the HCT116, A431 and A549 cancer cell lines, and compounds **3**, **4**, **7**, **9/10** and **12** were significantly cytotoxic against two cell lines, HCT116 and A431. In contrast to **12** (a β -anomer), its isomer **11** (an α -anomer) was not cytotoxic against any of the three cell lines. Among the isolated compounds tested, the iodinated nucleoside **8** showed the strongest cytotoxic activity against the HCT116, A431 and A549 cancer cell lines, with IC₅₀ values of 1.8, 3.1 and 3.5 $\mu\text{g/mL}$, respectively.

Table 4. Cytotoxic activity of compounds **1**–**13**.

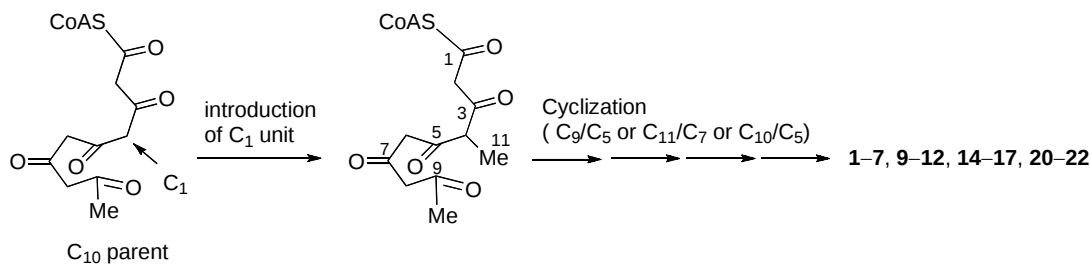
Compounds	Cell Line IC ₅₀ (ppm)		
	HCT116 ^a	A431 ^b	A549 ^c
1	3.2	6.1	6.4
2	3.0	6.4	4.8
3	5.2	7.5	>20
4	4.7	6.6	11.1
5	2.3	3.9	>20
6	6.8	15.3	15.9
7	2.8	9.4	12.8
8	1.8	3.1	3.5
9/10	3.1	3.6	>20
11	>20	>20	>20
12	2.4	3.3	>20
13	7.2	>20	>20

^a HCT116: human colorectal carcinoma; ^b A431: human epidermal carcinoma; ^c A549: human lung cancer.

To date, a variety of C₁₁ compounds have been isolated from ascidians (compounds **9** and **10**), cyanobacteria (compounds **20** and **21**) and a sponge (compound **22**) (Figures 1 and 2) [16,35,36]. Compounds **16** and **17** have been isolated from the ascidian *Diplosoma virens* and a sponge *Ulosa* sp. (Figure 2) [19,20]. Isolation of a series of the C₁₁ compounds including compounds **18** and **19** (Figure 2) from unrelated marine organisms supports the potential microbial origin of these compounds. From this viewpoint, we assume that the ascidian *Diplosoma* sp. might not be the actual producer of the C₁₁ compounds, but suggest a possible microorganism source such as *Prochloron* spp. We conducted, therefore, the following experiments. The *Prochloron* spp., which are obligatory symbionts of ascidians, were separated from the body of the ascidians *Lissoclinum* sp. and *Diplosoma* spp. by squeezing through the plankton net. ¹H-NMR spectra of the acetone extracts of the separated *Prochloron* spp. showed the presence of the same peaks as present in those of didemnenones. This confirms our assumption that *Prochloron* spp. are the actual producers of didemnenones.

Most C₁₁ compounds are derived from polyketides (six acetates–C₁) or polyketides (five acetates + C₁) [37]. Pentylphenols such as **18** and **19**, and some compounds which have a carbon skeleton of 5-methyldecane are known to be derived from the former with a loss of CO₂ from C₁₂ parent (six acetates). Some C₁₁ metabolites are ascertained to be derived from a polyketide precursor (five acetates + C₁) which has a carbon skeleton of 4-methyldecane [37]. We found that didemnenone-related compounds **1**–**7**, **9**–**12**, **14**–**17** and **20**–**22** have a common carbon skeleton of 4-methyldecane from a consideration of the carbon skeleton of these compounds. Consequently, We propose that these compounds should be derived from the polyketides (five acetates + C₁) via cyclization between C-9/C-5, between C-11/C-7 or between C-10/C-5 (Scheme 1).

Scheme 1. Plausible biosynthesis of the carbon skeletons for didemnenones and related compounds.



3. Experimental Section

3.1. General experimental procedures

Optical rotations were measured on either a JASCO P-1020 or JASCO DIP-1000 polarimeter. Ultraviolet-visible spectra were obtained in methanol on a JASCO V-550 spectrophotometer. Infrared spectra were recorded as dry films on either JASCO FT/IR-300 or Spectrum 2000 Explorer (PERKIN ELMER). CD spectra were recorded on a JASCO J-720W Circular Dichroism Spectrometer. ¹H- and ¹³C-NMR spectra were recorded on a JEOL JNM α-500 FT-NMR spectrometer or a JEOL JNM lambda 400 FT-NMR spectrometer, and chemical shifts were referenced to the solvent signals [δ_{H} 7.24 and δ_{C} 77.0 in chloroform-*d*, δ_{H} 2.49 and δ_{C} 39.5 in DMSO-*d*₆, δ_{H} 3.30 and δ_{C} 49.0 in methanol-*d*₄]. Inversed-detected heteronuclear correlations were measured using HMQC and HMBC pulse sequences with a pulse field gradient. HR-ESIMS data were obtained on a LTQ ORBITRAP (ThermoFisher Scientific, Germany), and HR-FABMS data were obtained on a JEOL JMS-700 mass spectrometer. LR-ESIMS data were measured on a Waters Quattro micro API triple quadrupole mass analyzer. RP-OCC and RP-MPLC were performed on COSMOSIL[®] 140C₁₈-OPN. Preparative RP-HPLC was run on a Waters 600 multi solvent system using ODS columns (YMC-Pack ODS-A, 150 × 20 mm I.D., YMC-Pack ODS-A, 250 × 20 mm I.D., YMC-Pack ODS-C8, 250 × 20 mm I.D., YMC-Pack ODS-AQ, 250 × 20 mm I.D., Develosil ODS-HG-5, 250 × 20 mm I.D. and COSMOSIL[®] -packed C₁₈, 250 × 10 mm I.D.). All solvents used were reagent grade.

3.2. Animal material

The colonial brown ascidian was collected by hand at the tidal zone of Tarama island, Okinawa, Japan, and the colonial green ascidian was collected by hand from the coast of Hateruma island, Okinawa, Japan. The ascidians were stored at −15 °C until extraction. The brown ascidian and the green ascidian were identified as *Lissoclinum* sp. and *Diplosoma* sp., respectively, by Euichi Hirose, University of the Ryukyus, Japan. The voucher specimens were deposited at the University of the Ryukyus (Specimen no. URKU-801 for the brown ascidian and URKU-802).

3.3. Extraction and isolation

The ascidian *Lissoclinum* sp. (5.7 kg, wet weight) was initially extracted with acetone (18 L) and filtered to remove debris. The filtrate was concentrated *in vacuo* to remove acetone and the resultant

mixture was partitioned between EtOAc (3.5 L) and H₂O (3.5 L). The H₂O layer was extracted with 1-BuOH to give the 1-BuOH extract (18.3 g) and the H₂O extract (141.2 g). An aliquot of 27.8 g of the H₂O-soluble material was subject to RP-MPLC on ODS (COSMOSIL[®] 140C₁₈-OPN, 140 µm, 50 × 3 cm I.D.) with H₂O (500 mL), H₂O/MeOH (9:1, 500 mL; 7:3, 500 mL; 5:5, 500 mL, 3:7, 500 mL; 1:9, 500 mL) and MeOH (500 mL) to give seven fractions. The second fraction (860 mg) was subjected to HPLC on ODS [YMC-Pack ODS-AQ, 250 × 20 mm I.D.; linear gradient elution, H₂O/MeOH (3:1)–MeOH] to give 18 fractions. The 18th fraction contained pure **1** (66.0 mg). The 14th fraction (84.0 mg) was subjected to HPLC on ODS [YMC-Pack ODS-AQ, 250 × 20 mm I.D.; linear gradient elution, 1% HCOOH /H₂O (1:9)–1% HCOOH/MeCN (1:9)] to give **13** (7.6 mg). The EtOAc layer was concentrated *in vacuo* to give a brown material (29.3 g). The material was subjected to a modified Kupchan's partitioning procedure as follows. The material was suspended in a mixture of H₂O/MeOH (1:1) and then successively extracted with hexane and CHCl₃. The resultant aqueous phase was concentrated to remove MeOH and then extracted with 1-BuOH. An aliquot of 0.58 g of the BuOH-soluble material (3.93 g) was separated by RP-MPLC (COSMOSIL[®] 140C₁₈-OPN, 140 µm, 50 × 3 cm I.D.) using H₂O (500 mL), H₂O/MeOH (9:1, 500 mL; 7:3, 500 mL; 5:5, 500 mL, 3:7, 500 mL; 1:9, 500 mL) and MeOH (500 mL) to give seven fractions (fr. 1–7). The first fraction (fr. 1, 436 mg) was purified by RP-HPLC [YMC-Pack ODS-A, 250 × 20 mm I.D.; linear gradient elution, H₂O/MeOH (4:1)–MeOH] to afford **3** (165 mg) and **4** (3.2 mg). The second fraction (fr. 2, 272 mg) was separated by RP-HPLC [YMC-Pack ODS-A, 150 × 20 mm I.D.; linear gradient elution, H₂O/MeOH (4:1)–MeOH] to give 18 fractions (fr. 2-1–2-18). Purification of the second fraction (fr. 2-2, 170 mg) by RP-HPLC (Develosil ODS-HG-5, 250 × 20 mm I.D.) using H₂O/MeOH (9:1) led to the isolation of **6** (24.6 mg), **2** (92.3 mg), **1** (92.7 mg) and an inseparable mixture of didemnenones A/B (**9/10**, 2.8 mg). HPLC separation [Develosil ODS-HG-5, 250 × 20 mm I.D., H₂O/MeOH (9:1)] of seventh fraction (fr. 2-7, 6.8 mg) led to the isolation of **12** (0.8 mg). An aliquot of 3.35 g of the 1-BuOH extract (3.93 g) was chromatographed on ODS (COSMOSIL[®] 140C₁₈-OPN, 140 µm, 100 g) with H₂O/MeOH (3:7, 500 mL; 2:8, 100 mL; 1:9, 100 mL) and MeOH (100 mL) to give nine fractions. The second fraction (1.1 g) was purified by HPLC on ODS [YMC-Pack C8, 250 × 20 mm I.D.; linear gradient elution, H₂O/MeCN (8:2–1:9)], to give **1** (186.8 mg), **5** (14.6 mg), **4** (29.7 mg) and **7** (0.9 mg).

The ascidian *Diplosoma* sp. (900 g, wet weight) was initially extracted with acetone (2.2 L). After filtration, the extracts were concentrated *in vacuo* to give an acetone extract. The acetone extract was partitioned between EtOAc and H₂O. The H₂O layer was further extracted with 1-BuOH. The 1-BuOH layer was concentrated *in vacuo* to give a BuOH-soluble material (2.14 g). The EtOAc layer was concentrated *in vacuo* to give an EtOAc extract (7 g). The EtOAc extract was suspended in H₂O/MeOH (1:1, 400 mL) and then successively extracted with hexane and CHCl₃ to give a hexane-soluble material (4.0 g), a CHCl₃-soluble material (2.0 g) and an aqueous material (2.1 g). The aqueous material and the BuOH-soluble material (2.14 g) were combined. The combined polar fraction (4.24 g) was chromatographed on ODS (COSMOSIL[®] 140C₁₈-OPN) with H₂O/MeOH (1:1, 2:1), MeOH and MeOH/EtOAc (9:1) as eluent. The first fraction (2.0 g) contained more than 95% of **1** from its ¹H NMR data. The CHCl₃-soluble material (2.0 g) was subjected to OCC on ODS [COSMOSIL[®] 140C₁₈-OPN, H₂O/MeOH (1:4), MeOH and MeOH/EtOAc (7:3)]. The first fraction (fr. 1, 1.0 g) was further separated by OCC on ODS (COSMOSIL[®] 140C₁₈-OPN) using H₂O/MeOH (1:1, 2:1), MeOH

and MeOH/EtOAc (9:1). The first aqueous methanol fraction (fr. 1-1, 902 mg) was subjected to HPLC on ODS (COSMOSIL[®]-packed C₁₈, 250 × 10 mm I.D.) using H₂O/MeOH (1:1) to give six fractions (fr. 1-1-1–fr. 1-1-6). The first fraction (fr. 1-1-1, 92.9 mg) was separated by HPLC on ODS [COSMOSIL[®]-packed C₁₈, 250 × 10 mm I.D., H₂O/MeCN (1:1)] first, and then by RP-HPLC [COSMOSIL[®]-packed C₁₈, 250 × 10 mm I.D., H₂O/MeOH/MeCN (5:2:2)] to yield **1** (18.7 mg) and **2** (5.9 mg). Further separation of the second fraction (fr. 1-1-2, 61.6 mg) by repeated HPLC [COSMOSIL[®]-packed C₁₈, 250 × 10 mm I.D., H₂O/MeOH/MeCN (50:31:19) and then H₂O/MeCN (1:1)] to give **5** (4.2 mg). An inseparable mixture (6.2 mg) of didemnenones A and B (**9** and **10**) was obtained along with their methylacetals **11** (8.8 mg) and **12** (3.0 mg) from the fourth fraction (fr. 1-1-4, 70.4 mg) by repeated ODS HPLC [COSMOSIL[®]-packed C₁₈, 250 × 10 mm I.D., H₂O/MeOH/MeCN (50:31:19) and then H₂O/MeCN (1:1)]. The fifth fraction (fr. 1-1-5, 40.0 mg) was purified by RP-HPLC (COSMOSIL[®]-packed C₁₈, 250 × 10 mm I.D.) with H₂O/MeOH/MeCN (50:31:19) to afford **8** (9.9 mg). The sixth fraction (fr. 1-1-6, 210.7 mg) was purified by HPLC on ODS (COSMOSIL[®]-packed C₁₈, 250 × 10 mm I.D.) using MeOH/EtOAc (19:1) to yield **8** (164.4 mg).

3.3.1. Compound 1

Pale yellowish oil; $[\alpha]_D^{27} +21.0^\circ$ (c 0.66, MeOH); UV (MeOH) $\lambda_{\max}$ (log ϵ) 246 (3.39), 308 (3.69) nm; FT/IR (film) $\nu_{\max}$ 3355, 1675, 1035 cm⁻¹; ¹H-NMR and ¹³C-NMR (DMSO-*d*₆) see Tables 1 and 2; LR-ESIMS *m/z* 211 (M + H)⁺, 209 (M – H)[–]; HR-ESIMS *m/z* (M + Na)⁺ 233.0786 (calcd. for C₁₁H₁₄O₄Na, 233.0784).

3.3.2. Compound 2

Pale yellowish oil; $[\alpha]_D^{27} +22.5^\circ$ (c 0.43, MeOH); UV (MeOH) $\lambda_{\max}$ (log ϵ) 245 (3.38), 307 (3.74) nm; FT/IR (film) $\nu_{\max}$ 3359, 1712, 1379 cm⁻¹; ¹H-NMR and ¹³C-NMR (DMSO-*d*₆) see Tables 1 and 2; LR-ESIMS *m/z* 233 (M + Na)⁺, 211 (M + H)⁺, 209 (M – H)[–]; HR-ESIMS *m/z* (M + Na)⁺ 233.0786 (calcd. for C₁₁H₁₄O₄Na, 233.0784).

3.3.3. Compound 3

Pale yellowish oil; $[\alpha]_D^{24} +147^\circ$ (c 0.62, H₂O); UV (H₂O) $\lambda_{\max}$ (log ϵ) 246 (3.51), 274 (3.54); FT/IR (film) $\nu_{\max}$ 3400, 1710, 1050 cm⁻¹; ¹H-NMR and ¹³C-NMR (DMSO-*d*₆) see Tables 1 and 2; LR-ESIMS *m/z* 231 (M + Na)⁺, 207 (M – H)[–]; HR-ESIMS *m/z* (M + Na)⁺ 231.0632 (calcd. for C₁₁H₁₂O₄Na, 231.0628).

3.3.4. Compound 4

Pale yellowish oil; $[\alpha]_D^{24} +459^\circ$ (c 0.015, MeOH); UV (MeOH) $\lambda_{\max}$ (log ϵ) 249 (3.65); FT/IR (film) $\nu_{\max}$ 3410, 1700, 1045 cm⁻¹; ¹H-NMR and ¹³C-NMR (DMSO-*d*₆) see Tables 1 and 2; LR-ESIMS *m/z* 223 (M + H)⁺; HR-ESIMS *m/z* (M + Na)⁺ 245.0794 (calcd. for C₁₂H₁₄O₄Na, 245.0784).

3.3.5. Compound 5

Pale yellowish oil; $[\alpha]_D^{27} + 112^\circ$ (*c* 0.26, MeOH); UV (MeOH) $\lambda_{\max}$ ($\log \epsilon$) 273 (3.83); FT/IR (film) $\nu_{\max}$ 3419, 1717, 1049 cm^{-1} ; ^1H -NMR and ^{13}C -NMR (DMSO- d_6) see Tables 1 and 2; LR-ESIMS m/z 207 ($\text{M} + \text{H}$) $^+$, 205 ($\text{M} - \text{H}$) $^-$; HR-ESIMS m/z ($\text{M} + \text{H}$) $^+$ 207.0654 (calcd. for $\text{C}_{11}\text{H}_{11}\text{O}_4$, 207.0652).

3.3.6. Compound 6

Pale yellowish oil; $[\alpha]_D^{24} + 16.3^\circ$ (*c* 0.17, MeOH); UV (MeOH) $\lambda_{\max}$ ($\log \epsilon$) 294 (3.91); FT/IR (film) $\nu_{\max}$ 3390, 1720, 1050 cm^{-1} ; ^1H -NMR and ^{13}C -NMR (DMSO- d_6) see Tables 1 and 2; LR-ESIMS m/z 249 ($\text{M} + \text{H}$) $^+$, 223 ($\text{M} + \text{H}$) $^+$; HR-ESIMS m/z ($\text{M} + \text{Na}$) $^+$ 249.0742 (calcd. for $\text{C}_{11}\text{H}_{14}\text{O}_5\text{Na}$, 249.0733).

3.3.7. Compound 7

Pale yellowish oil; $[\alpha]_D^{24} + 11.7^\circ$ (*c* 0.12, MeOH); UV (MeOH) $\lambda_{\max}$ ($\log \epsilon$) 248 (3.78), 308 (3.83); FT/IR (film) $\nu_{\max}$ 3400, 1580, 1050 cm^{-1} ; ^1H -NMR and ^{13}C -NMR (DMSO- d_6) see Table 3; LR-ESIMS m/z 423 ($\text{M} + \text{Na}$) $^+$, 401 ($\text{M} + \text{H}$) $^+$, 399 ($\text{M} - \text{H}$) $^-$; HR-ESIMS m/z ($\text{M} + \text{Na}$) $^+$ 423.1423 (calcd for $\text{C}_{22}\text{H}_{24}\text{O}_7\text{Na}$, 423.1414), ($\text{M} + \text{H}$) $^+$ 401.1602 (calcd. for $\text{C}_{22}\text{H}_{24}\text{O}_7$, 401.1595).

3.3.8. Compound 8

Pare yellowish oil: $[\alpha]_D^{26} - 69^\circ$ (*c* 0.1, MeOH); UV (MeOH) $\lambda_{\max}$ ($\log \epsilon$) 283 nm (3.59); FT/IR (film) $\nu_{\max}$ 3461, 3317, 3132, 1633, 1584, 1474, 1084, 755 cm^{-1} ; NMR data were described in the previous paper [13]. LR-EIMS m/z (rel.%) 376 (M^+ , 7), 303 (3), 289 (13), 261 (33), 260 (100), 233 (18). HR-FABMS m/z (M) $^+$ 376.0016 (calcd. for $\text{C}_{11}\text{H}_{13}\text{IN}_4\text{O}_3$, 376.0027).

3.3.9. Acetal 23

To a solution of iodinated nucleoside **8**, (10.0 mg, 26.6 μmol) in 2,2-dimethoxypropane (1 mL) and acetone (2 mL) was added a catalytic amount of camphorsulfonic acid. The mixture was stirred at rt for 24 h and at 45 $^\circ\text{C}$ for 4 h. The reaction mixture was diluted with ether, washed with saturated aqueous Na_2CO_3 and brine. The organic phase was dried (MgSO_4) and concentrated *in vacuo*. The residual oil was purified by preparative TLC [CHCl_3 -MeOH (3:0.2)] to give the acetal as colorless oil (**23**, 3.0 mg, 25%). HR-FABMS and ^1H NMR data for compound **23** were described in the earlier paper [23].

3.3.10. Methyl 5-deoxy 2.3-di-O-(4-bromobenzoyl)- β -L-xylofuranoside (24)

The mixture of methyl 5-deoxy- β -L-xylofuranoside [38,39] (56.5 mg, 0.11 mmol), 4-bromobenzoyl chloride (250.1 mg, 0.87 mmol), and DMAP (2.2 mg, 0.018 mmol), in pyridine (0.8 mL) was stirred at ambient temperature for 24 h, and water (0.5 mL) was added to the mixture. After being stirred at ambient temperature for 30 min, the mixture was concentrated. The residual solid was purified by HPLC [Develosil ODS-HG-5 (250 $\times$ 20 mm I.D.); flow rate 5 mL/min; detection UV 256 nm; solvent

85% MeOH] to give methyl 5-deoxy 2,3-di-*O*-(4-bromobenzoyl)- β -L-xylofuranoside (**24**, 17.2 mg, 30%) and its corresponding α -anomer (15.3 mg, 27%), respectively. Spectral data for compound **24** were described in the previous paper [23].

3.3.11. Dibenzoate **25**

The mixture of iodinated nucleoside **8** (5.0 mg, 0.013 mmol), 4-bromobenzoyl chloride (25.2 mg, 0.115 mmol), and DMAP (2.2 mg, 0.018 mmol), in pyridine (0.5 mL) was stirred at ambient temperature for 24 h, and water (0.5 mL) was added to the mixture. After being stirred at ambient temperature for 30 min, the mixture was concentrated. The residual solid was purified by HPLC [Develosil ODS-HG-5 (250 $\times$ 20 mm I.D.); flow rate 5 mL/min; detection UV 256 nm; solvent 85% MeOH] to give dibenzoate **25** (2.0 mg, 21%). Spectral data for compound **25** were described in the earlier paper [23].

3.3.12. Condition of cell cultures

Human colorectal carcinoma (HCT116), human epidermal carcinoma (A431) and human lung cancer (A549) cells were cultured in DMEM medium (including 10% FBS, 100 U/mL penicillin and 100 ng/mL streptomycin) at 37 °C in a 5% CO₂ atmosphere.

3.3.13. Determination of cytotoxicity

Growth inhibition experiments were carried out in quadruplicate on 96-well flat-bottomed microplates, and the amount of viable cells at the end of incubation was determined with the MTT [3-(4,5-dimethylthiazol-2-yl) 2,5-diphenyltetrazolium bromide] dye reduction assay. Test compounds were dissolved in DMSO (4.0 mg/mL) and diluted with H₂O such that the final DMSO concentration was 0.5%. Viable cells (HCT116, A431 and A549) in the growth medium were seeded on 96-well microplates (1.0 $\times$ 10⁴ cells/well) and incubated at 37 °C in a 5% CO₂ atmosphere, and continuously cultured without or with five concentrations (20, 10, 5, 2.5, 1.25 μ g/mL, final concentration) of test compounds for 48 h from the next day. After incubation, 10 μ L of MTT (5 mg/mL in phosphate-buffer saline) was added each well, the samples were again incubated. After standing for 3 h, the medium was removed, and the resulting formazan crystals were dissolved with DMSO (100 μ L). The optical density (O.D.) was measured at 570 nm, provided the reference for reading at 655 nm with a microplate reader (Model 550, BIO-RAD, USA).

4. Conclusions

We have isolated seven new didemnenone derivatives **1–7** together with four known metabolites **9/10**, **12** and **13** from *Lissoclinum* sp. We have also isolated didemnenones **1**, **2** and **5** and five known metabolites **8–12** from the other didemnid ascidian *Diplosoma* sp. Compounds **1–4**, **7–10** and **12** were significantly cytotoxic against the HCT116, A431 and/or A549 cancer cell lines. Didemnenone-related compounds **1–7**, **9–12**, **14–17** and **20–22**, which have been ever isolated from marine organisms [19–22,35,36], were found to have a common carbon skeleton of 4-methyldecane. From consideration of the carbon skeleton of these compounds, a biosynthesis of the carbon skeletons for didemnenones

and related compounds was proposed (Scheme 1). ¹H-NMR spectra of the extracts of the separated *Prochloron* spp. from the body of the ascidians *Lissoclinum* sp. and *Diplosoma* spp. showed the presence of the same peaks as present in those of didemnenones, which suggested that *Prochloron* spp. are the actual producers of didemnenones.

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Sample Availability: Samples of the stable compounds are available from the authors.

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