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Monanchocidins B−E: Polycyclic Guanidine Alkaloids with Potent Antileukemic Activities from the Sponge *Monanchora pulchra*

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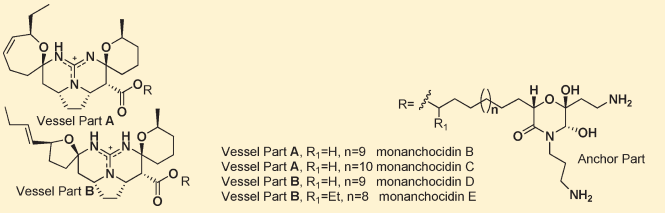
Supporting Information

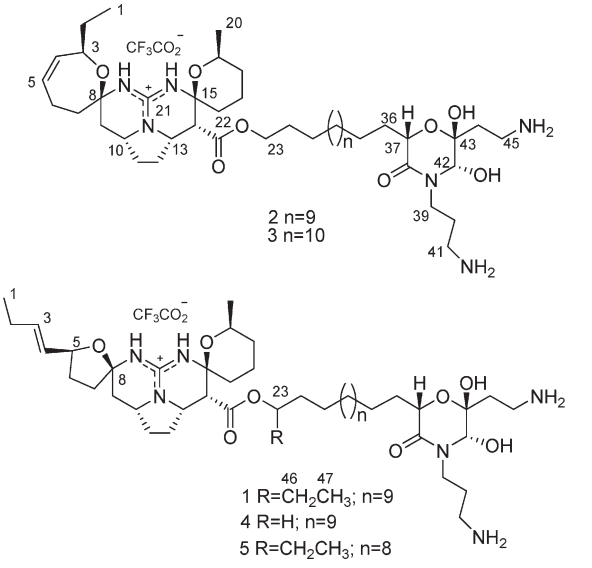
ABSTRACT: New unusual polycyclic guanidine alkaloids monanchocidins B−E (**2**−**5**) along with monanchocidin A (**1**), which we recently described, were isolated from the Far-Eastern marine sponge *Monanchora pulchra*. Their structures were established using spectroscopic data and chemical transformations. Compounds **1**−**5** show potent cytotoxic activities against HL-60 human leukemia cells with IC_{50} values of 540, 200, 110, 830, and 650 nM, respectively.



Pentacyclic guanidine alkaloids, bearing a common [5,6,8b]-triazaperhydroacenaphthalene fragment in their molecules, represent a well-known group of marine secondary metabolites.[1−5] The first representative of this group, ptilomycalin A, was isolated from the Caribbean sponge *Ptilocaulis spiculifera* and from a sponge *Hemimycale* sp. collected from the Red Sea in 1989.[1] Other related alkaloids including series of pentacyclic,[1,6−16] tricyclic,[7,16−20] and bicyclic[13,21,22] natural products were found in several marine sponges and two starfish species. Several metabolites of ptilomycalin A class were isolated from different *Monanchora* species including *M. arbuscula*,[16,17] *M. unguiculata*,[16] *M.* sp.,[13] *M. dianchora*,[9] and *M. unguifera*.[12,20] These metabolites showed a broad range of biological activities including cytotoxic,[1,6−12,17,23,24] antifungal,[1,25] antiviral,[1,6,11,13,18,19] antimicrobial,[20] antiprotozoal,[20,25] and antimalarial[17,25] properties.

As a result of our preliminary study on natural products from the Far-Eastern marine sponge *M. pulchra*, we recently published a short report concerning the isolation and structure of a new apoptosis-inducing polycyclic guanidine alkaloid with an unprecedented skeleton system named monanchocidin A (**1**).[26] Monanchocidin A possesses a collection of unusual structural features, including a new combination of two contiguous 1-oxa-6-azaspiro[4,5]decane and 1-oxa-7-azaspiro[5,5]undecane spiro-ring systems in its pentacyclic moiety (Kashman[1] referred to the polycyclic moiety of this type of alkaloids as the "vessel"). Moreover, **1** contains a new type of so-called[1] "anchor" with a vicinal hemiketal in a substituted morpholinone ring. A long hydrocarbon "chain" is attached to the bis-spiro-polycyclic moiety ("vessel") by an ester linkage through ω-3, but not the ω-position, in contrast with connections earlier encountered in all other related marine guanidine alkaloids.

In continuation of our studies on natural products from Far-Eastern sponges,[27] we herein report the isolation and structures of four new monanchocidins, B−E (**2**−**5**), as well as the cytotoxic activities of monanchocidin A (**1**) and the new alkaloids **2**−**5** against human tumor HL-60 cells.



RESULTS AND DISCUSSION

The EtOH extract of the frozen sponge *M. pulchra* was concentrated and partitioned between H_2O and *n*-BuOH. After evaporation of the solvent, the BuOH-soluble materials were partitioned between *n*-hexane and aqueous EtOH. The aqueous EtOH-soluble materials were further subjected to repeated

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Table 1. NMR Data for Monanchocidin B (2) (DMSO-d_6)

position	δ_C[a]	δ_H mult (J in Hz)[b]	COSY	HMBC[c]
1	10.0	0.75, t (7.3)	H2a, H2b	2, 3
2a	28.6	1.34, m	H1, H3	1, 3
2b		1.52, m	H1	1, 4
3	70.2	4.35, brd (10.8)	H2a, H2b, H4, H5, 6b	
4	133.3	5.49, dt (10.8, 2.2)	H3, H5, H6b	3
5	130.3	5.65, brt (9.3)	H4, H3, H6a,	
6a	23.2	2.09, m	H5, H6b, H7a	4
6b		2.35, m	H6a, H4, H3	5, 7, 8
7a	35.8	1.85, m	H6a, H7b	6, 8
7b		2.37, m	H7a	5, 6, 8
8	83.5			
9a	36.1	1.28, m	H9a, H10	8, 10, 11
9b		2.63, dd (13.1, 4.7)	H9a, H10, 21NHb	8, 10
10	53.6	3.92, m	H9a, H9b, H11a, H11b	
11a		1.45, m	H10, H11b, H12b	10
11b	30.1	2.22, m	H10, H11a, H12b	10, 12
12a		1.63, m	H12b, H13	10, 11, 13, 14, 21
12b	26.2	2.29, m	H11a, H12b, H13	10, 11, 13, 14
13	52.0	4.20, dt (9.5, 5.9)	H14, H12b, H12a	12, 14, 21, 15
14	49.2	3.03, d (5.0)	H13, 21NHa	13, 15, 22, 16
15	80.3			
16	31.4	1.64, m		15, 17, 18, 21, 14
17a		1.68, m	H17b	
17b	17.6	1.95, m	H16, H18a	
18a	31.2	1.18, m	H19, H18b, H17b	
18b		1.63 m	H18a, H17b	
19	66.4	3.72, ddd (2.2, 6.0, 11.9)	H18a, H20, H18b	
20	21.3	1.02, d (6.2)	H19	19, 18
21	148.3			
21-NHa		9.34, s	H14	14, 15, 21, 16
21-NHb		9.68, s	H9b	8, 9, 21
22	168.3			
23	64.9	4.06, dt (6.4, 12.6)	H24	22
24	28.0	1.55, m	H23	23
25−35	28.9−29.3	1.20−1.25, brs		
36	31.9	1.73, m	H37	38, 37
37	70.8	4.08, dd (3.6, 7.8)	H36	36, 38
38	169.2			
39a	41.7	3.25, dt (6.4, 14.0)	H39b, H40	38, 40, 41, 42
39b		3.45, dt (6.4, 14.0)	H39a, H40	38, 40, 41, 42
40	25.5	1.81, m	H39a, H39b, H41	39, 41
41	36.7	2.77, m	H40, $NH_2$41	
41-NH_2		7.70, brs	H40	
42	80.9	4.41, d (5.3)	OH42	38, 39
42-OH		6.49, d (5.3)	H42	
43	94.4			
43-OH		6.59, s		
44a	34.7	1.94, m	H45, H44b	43, 45, 42
44b		2.04, m	H45	43, 42, 45
45	34.3	2.93, m	H44a, H44b, $NH_2$45	43
45-NH_2		7.73, brs		45

[a] 175 MHz. [b] 700 MHz. [c] HMBC correlations, optimized for 5 Hz, are from proton(s) stated to the indicated carbon.

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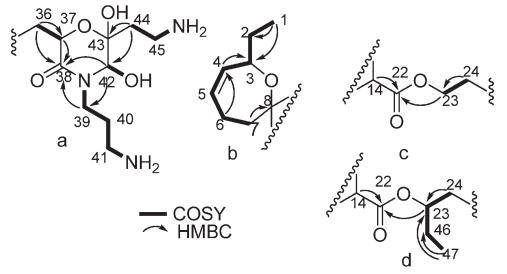

Figure 1. Substructures found in **1**−**5**.

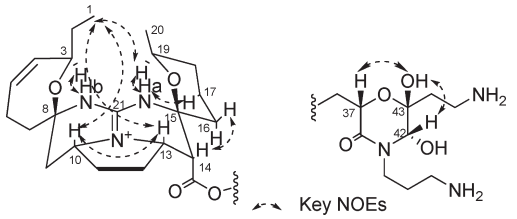

Figure 2. Key NOE correlations in the NMR spectra of **2**.

column chromatography on Sephadex LH-20 (elution with EtOH) to obtain a crude mixture of monanchocidins. Separation and purification of these alkaloids were carried out by repeated HPLC (YMC-ODS-A column) to provide pure monanchocidin A (**1**) (0.15%)[26] and monanchocidins B (**2**), C (**3**), D (**4**), and E (**5**) (0.067%, 0.008%, 0.002%, and 0.01%, respectively, based on the dry weight of the sponge).

The molecular formula of monanchocidin B (**2**), $C_{45}H_{78}N_6O_8$, was established from a high-resolution mass measurement of the $[M + H]^+$ ion peak in the HR MALDI-TOF-MS spectrum. ^1H and ^{13}C NMR data in DMSO-d_6 of **2** revealed the presence of a guanidine group (δ_C 148.3; δ_H 9.34, 9.68), two methyl groups (δ_H 0.75, 1.02; δ_C 10.0, 21.3), one disubstituted double bond (δ_H 5.49, 5.65; δ_C 133.3, 130.3), two N-substituted CH carbons (δ_H 3.92, 4.20; δ_C 53.6, 52.0), four oxymethines (δ_H 4.35, 3.72, 4.08, 4.41; δ_C 70.2, 66.4, 70.8, 80.9), one oxymethylene group (δ_H 4.06; δ_C 64.9), two carbonyl groups (δ_C 168.3, 169.2), one carbonyl-linked methine (δ_H 3.03; δ_C 49.2), three quaternary carbons (δ_C 83.5, 80.3, 94.4), and an aliphatic long hydrocarbon chain (δ_H 1.20−1.25; δ_C 28.9−29.3) (Table 1). Interpretation of the COSY, HSQC, and HMBC data established that compound **2** contained the 2-aminoethyl-3-aminopropylmorpholinone ring (Figure 1, substructure **a**), the same as was found in monanchocidin A (**1**).[26] The ^1H and ^{13}C NMR data of **2** (Table 1) also showed that, in contrast with **1**, the guanidine pentacyclic moiety of monanchocidin B contained a 7-ethyl-2,3,4,7-tetrahydrooxepine fragment (Figure 1, substructure **b**), also present in ptilomycalin A and other previously known pentacyclic guanidine alkaloids.[1,6−16] This was evidenced by chemical shifts of the C-1 methyl group and the methine groups CH-3, CH-4, and CH-5, which were very similar to those in the spectrum of ptilomycalin A. Finally, a long hydrocarbon chain substituted by a 2-morpholinone unit in **2** is attached to the "vessel" moiety by an ester linkage through the ω-position, but not the ω-3 position, as was encountered in monanchocidin A.[26] Therefore, interpretation of the NMR data established that compound **2** contained substructure **c** (Figure 1). These substructures and the connection between them were confirmed by analysis of the 2D NMR data, including HSQC, COSY, and HMBC experiments. As a result, monanchocidin B (**2**) was formulated as a new guanidine alkaloid, modified in the "anchor" part in comparison with all other previously known pentacyclic alkaloids from sponges, except monanchocidin A. In contrast to **1**, it contains a different core bis-spiro-polycyclic fragment ("vessel") and an oxypolymethylene spacer derived from a C_{16} ω-hydroxy fatty acid.

Comparison of the ^1H and ^{13}C NMR data for monanchocidin B (**2**) with those for ptilomycalin A[1] and other related compounds[6−16] suggests the same relative configuration for all of the asymmetric centers in these compounds. This was then confirmed by analysis of the ROESY spectra of **2** (in DMSO-d_6). Diagnostic NOE correlations between the resonances of 21-NHb (δ_H 9.68) and H-3 and NOEs from H_3-1 to H-19 and from H_3-1 to H-10 and H-13 (Figure 2) were indicative of the (3*R**,8*R**) relative configuration in substructure **b** (Figure 1). The (15*S**,19*S**) relative configuration was established by NOE correlations between 21-NHa (δ_H 9.34) and H-19, H-17. Correlations between H-10, H-13, and H-16 in the ROESY spectrum, together with the observed coupling constants between H-13 and H-14 (J = 5.0 Hz), located all these protons on the same side of the molecule. In addition, NOEs between OH-43 (δ_H 6.59) and H-37 and between OH-43 and H-42 were observed, confirming the *trans*-position of hydroxy groups and a *cis*-position for H-37 and OH-43 in the "anchor" part (Figure 2).

The molecular formula of monanchocidin C (**3**), $C_{46}H_{80}N_6O_8$, was obtained from a high-resolution mass measurement of the $[M + H]^+$ ion peak in the HR MALDI-TOF-MS spectrum. The ^1H NMR data (Table 2) of **3** closely coincided with those of monanchocidin B (**2**), but MS data showed the molecular mass of **3** to be 14 amu higher than that of **2**, suggesting that the hydrocarbon chain is extended by one additional CH_2 group in **3** (derived from C_{17}-ω-hydroxy fatty acid). Comparison of the ^1H and ^{13}C NMR data of monanchocidin B (**2**) with the data for monanchocidin C (**3**) and their complete coincidence suggest the same relative configuration as for **2**.

The molecular formula of monanchocidin D (**4**), $C_{45}H_{78}N_6O_8$, was established by HR MALDI-TOF-MS. The MS data showed the molecular mass of **4** to be 28 amu less than that of **1**, suggesting that the hydrocarbon chain was shortened by two CH_2 groups in comparison with **1**.

The ^1H (Table 2) and ^{13}C NMR (Table 3) data of **4** were similar to those of monanchocidin A, except for the absence of signals for the CH_3-47 and CH-23 oxymethine groups, which are characteristic of the branching hydrocarbon chain in **1**.[26] However, the NMR spectrum of **4** revealed signals for an oxymethylene group (−O$\underline{CH_2}$−: δ_H 4.13, m, δ_C 67.2). The structure of this moiety (Figure 1, substructure **c**) was confirmed by the ^{13}C NMR data (Table 3). Comparison of the ^1H and ^{13}C NMR data for monanchocidin D (**4**) with those for monanchocidin A (**1**) argues that the same relative configurations in the "vessel" and "anchor" parts are present. Therefore, monanchocidin D (**4**) was formulated as an analogue of **1** with a modification of the spacer moiety.

The molecular formula of monanchocidin E (**5**), $C_{46}H_{80}N_6O_8$, was obtained from a high-resolution mass measurement. The MS data showed the molecular mass for **5** is 14 amu less than of **1**, suggesting that the hydrocarbon chain connecting the "anchor" and "vessel" parts in **5** was shortened by one CH_2 group in comparison with **1**. In fact, the ^1H and ^{13}C NMR data of **5** (Tables 2, 3) closely coincided with those for monanchocidin. The signals for the CH_3-47 and CH-23 groups, like those in the spectra

Table 2. ^{1}H NMR Data for Monanchocidins 2, 3, 4, and 5 (CD_3OD, TMS)

	2	3	4	5
position	δ_H mult (J Hz)[a]	δ_H mult (J Hz)[a]	δ_H mult (J Hz)[a]	δ_H mult (J Hz)[a]
1	0.84, t (7.3)	0.84, t (7.3)	0.99, t (7.5)	0.99, t (7.5)
2a	1.46, m	1.46, m	2.05, m	2.05, m
2b	1.54, m	1.54, m		
3	4.40, brd (10.3)	4.40, brd (10.3)	5.77, dt (6.4, 15.3)	5.77, dt (6.4, 15.3)
4	5.50, dt (2.2, 11.0)	5.50, dt (2.2, 11.0)	5.45, ddt (1.5, 7.2, 15.3)	5.45, ddt (1.5, 7.2, 15.3)
5	5.71, brt (10.3)	5.71, brt (10.3)	4.57, brq (7.2)	4.57, brq (7.2)
6a	2.16, m	2.16, m	1.81, m	1.81, m
6b	2.42, m	2.42, m	2.23, m	2.23, m
7a	1.99, m	1.99, m	2.16, m	2.16, m
7b	2.37, m	2.37, m	2.23, m	2.23, m
8				
9a	1.42, m	1.42, m	1.69, m	1.69, m
9b	2.63, dd (4.7, 13.0)	2.63, dd (4.7, 13.0)	2.27, dd (4.0, 13.0)	2.27, dd (4.0, 13.0)
10	4.05, m	4.05, m	4.03, m	4.03, m
11a	1.60, m	1.60, m	1.64, m	1.64, m
11b	2.30, m	2.30, m	2.29, m	2.29, m
12a	1.82, m	1.82, m	1.77, m	1.77, m
12b	2.33, m	2.33, m	2.29, m	2.29, m
13	4.34, m	4.34, m	4.32, m	4.32, m
14	3.07, d (5.0)	3.07, d (5.0)	3.07, d (5.0)	3.05, d (5.0)
15				
16	1.66, m	1.66, m	1.69, m	1.69, m
17	1.73, m	1.73, m	1.82, m	1.82, m
	1.82, m	1.82, m		
18a	1.28, m	1.28, m	1.27, m	1.27, m
18b	1.69, m	1.69, m	1.69, m	1.69, m
19	3.83, m	3.83, m	3.86, m	3.86, m
20	1.09, d (6.3)	1.09, d (6.3)	1.13, d (6.4)	1.13, d (6.4)
21				
22				
23	4.13, m	4.13, m	4.13, m	4.82, m
24	1.65, m	1.65, m	1.66, m	1.58, m
35	1.45, m	1.45, m	1.45, m	1.45, m
36	1.79, m	1.79, m	1.79, m	1.79, m
	1.86, m	1.86, m	1.86, m	1.86, m
37	4.27, dd (3.6, 8.2)	4.27, dd (3.6, 8.2)	4.27, dd (3.6, 8.2)	4.27, dd (3.6, 8.2)
38				
39	3.46, dt (6.0, 14.2)	3.46, dt (6.0, 14.2)	3.46, dt (6.0, 14.2)	3.46, dt (6.0, 14.2)
	3.64, m	3.64, m	3.66, m	3.66, m
40	2.00, m	2.00, m	2.00, m	2.00, m
41	2.95, m	2.95, m	2.95, m	2.95, m
42	4.59, brs	4.59, brs	4.59, brs	4.59, brs
43				
44	2.12, m	2.12, m	2.12, m	2.12, m
	2.22, m	2.22, m	2.22, m	2.22, m
45	3.18, m	3.18, m	3.18, m	3.18, m
46				1.60, m
47				0.90, t (7.4)

[a] 700 MHz.

of **1**, were also easily recognized.[26] The corresponding substructure (**d**) (Figure 1) in **5** was confirmed by 2D NMR data, including HMBC experiments. Comparison the ^{1}H and ^{13}C NMR data for monanchocidin E (**5**) with those for monanchocidin A (**1**) also

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Table 3. ^{13}C NMR Data for Monanchocidins 2−5 (CD_3OD)

	2	3	4	5
position	δ_C[a]	δ_C[a]	δ_C[a]	δ_C[a]
1	11.4	11.4	14.3	14.3
2	30.6	30.6	26.8	26.8
3	72.9	72.9	136.9	136.9
4	134.9	134.9	130.7	130.7
5	131.9	131.9	82.4	82.4
6	25.1	25.1	33.5	33.5
7	38.8	38.8	38.4	38.4
8	85.7	85.7	90.6	90.6
9	38.4	38.4	39.9	39.9
10	56.2	56.2	55.4	55.47
11	31.3	31.3	31.7	31.7
12	28.2	28.2	28.2	28.2
13	54.8	54.8	55.3	55.45
14	51.4	51.4	51.3	51.5
15	82.8	82.8	83.2	83.2
16	33.2	33.2	33.2	33.2
17	20.0	20.0	20.0	20.0
18	33.2	33.2	33.2	33.2
19	69.0	69.0	69.1	69.1
20	22.4	22.4	22.5	22.5
21	150.8	150.8	151.0	151.0
22	170.8	170.8	170.8	170.8
23	67.2	67.2	67.2	79.2
24	30.2	30.2	30.2	34.8
35	27.1	27.1	27.4	27.5
36	34.0	34.0	34.0	34.0
37	73.5	73.5	73.5	73.5
38	173.5	173.5	173.5	173.5
39	43.5	43.5	43.5	43.3
40	27.4	27.4	27.4	27.4
41	38.7	38.7	38.6	38.6
42	83.4	83.4	83.4	83.3
43	96.7	96.7	96.7	96.7
44	36.5	36.5	36.5	36.5
45	36.7	36.7	36.7	36.7
46				28.3
47				10.6

[a] 175 MHz.

indicates the same relative configurations in the "vessel" and "anchor" of both compounds. Therefore, monanchocidin E (**5**) was formulated as an analogue of **1** with a modified hydrocarbon fragment that is derived from a C_{17}-ω-3-hydroxy fatty acid.

A chemical transformation was used to confirm the structures. Treatment of monanchocidin B (**2**) with $NaBH_4$ in EtOH at 50 °C for 20 h resulted in the loss of the C-42−C-45 fragment due to cleavage of the hemiaminal group at C-42 and the hemiacetal group at C-43.[28] The hemiaminal groups at C-8 and C-15 were also cleaved.[29] The structure of product **6** (Figure 3) was established using NMR, HR-MALDI-TOF-MS, and MALDI-TOF/TOF-MS data (see Experimental Section). The ^{1}H NMR spectrum of **6** (DMSO-d_6) indicated the presence of three new hydroxy groups at C-3, C-19, and C-37 (δ_H 4.52, 4.39, and 5.45),

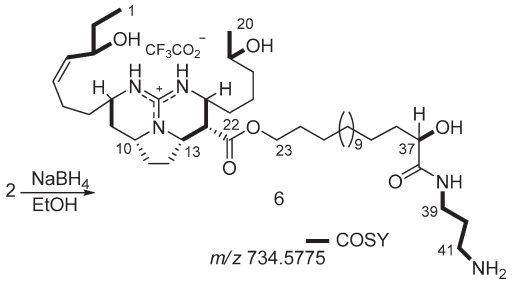


Figure 3. Reductive degradation of **2**.

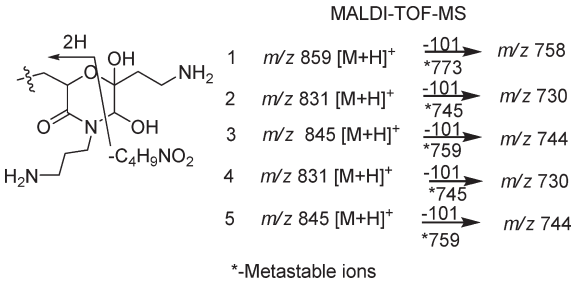


Figure 4. Hypothetical scheme of metastable ion formation during MALDI-TOF-MS of **1**−**5**.

respectively. Similar transformations were earlier described during the structure elucidation of monanchocidin A (**1**).[26]

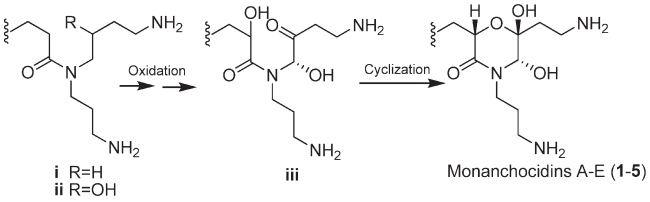
Interestingly, the loss of the same C-42−C-45 fragment by **1**−**5** probably takes place during MALDI-TOF mass spectrometry analysis of these compounds. Intense peaks at *m/z* 773, 745, 759, 745, and 759 in the reflectron spectra of **1**−**5** corresponded to metastable ions, confirming the removal of 101 Da fragments from the corresponding $[M + H]^+$ ions (Figure 4). This was established by the MALDI-TOF/TOF-MS (LIFT) of the parent $[M + H]^+$ ions of **1**−**5**. Indeed, the $(M + H - 101)^+$ peaks at *m/z* 758 (MS/MS of **1**), 730 (MS/MS of **2**), 744 (MS/MS of **3**), 730 (MS/MS of **4**), and 744 (MS/MS of **5**) instead of metastable ion peaks were obtained in these cases. Another confirmation of the metastable nature of ions with *m/z* 773, 745, 759, 745, and 759 was obtained using linear mode MALDI-MS. Metastable ion peaks were also not detected in these spectra. We consider the above-mentioned metastable ion peaks in the reflectron mode of MALDI-TOF-MS as a consequence of cleavages at the O−C(43) and N−C(42) bonds with transfer of 2H to the charge-bearing moieties. It shows that care should be taken when interpreting the MALDI-TOF-MS data of this group of natural products.

We suggest that monanchocidins with a morpholinone ring such as in **1**−**5** are biosynthesized from precursors having a spermidine (**i**) or oxyspermidine (**ii**) unit. This biosynthesis could be realized through additional oxidations into an unknown biosynthetic intermediate (**iii**) followed by cyclohemiacetalization, as occurs in sugars (Scheme 1).

We have evaluated the cytotoxicities of monanchocidin A (**1**) and the new compounds (**2**−**5**) against HL-60 human leukemia cells. Monanchocidin A (**1**), monanchocidin B (**2**), monanchocidin C (**3**), monanchocidin D (**4**), and monanchocidin E (**5**) exhibited potent inhibitory activities with IC_{50} values of 540, 200, 110, 830, and 650 nM, respectively.

The new alkaloids monanchocidins B and C may be considered as unexpected hybrid structures, containing the same

Scheme 1. Hypothetical Pathways for the Biosynthesis of "Anchor" Part in Monanchocidins A−E



pentacyclic guanidinium ring systems (the "vessel" part) as previously found in some tropical sponges[1,6−10,12−16] or starfish[11] and the morpholinone ring unit (the "anchor" part), previously found only in monanchocidin A from the same Far-Eastern sponge *Monanchora pulchra*.[26] The new alkaloids monanchocidins D (**4**) and E (**5**) contain both the "vessel" and the "anchor" identical to those of monanchocidin A,[26] but the internal lipid linkage is changed, being derived from ω-hydroxyhexadecanoic acid in **4** and ω-3-hydroxyheptadecanoic acid in **5** instead of being derived from ω-3-hydroxyoctadecanoic acid, which is the spacer found in monanchocidin A itself.[26]

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured using a Perkin-Elmer 343 polarimeter. The ^{1}H and ^{13}C NMR spectra were recorded on Bruker DRX-500 and Avance III-700 spectrometers at 500, 700, and 125, and 175 MHz, respectively, with Me_4Si as an internal standard. MALDI-TOF mass spectra were obtained on a Bruker Ultraflex III TOF/TOF laser desorption spectrometer coupled with delayed extraction using a Smartbeam MALDI 200 laser with 2,5-dihydroxybenzoic acid as the matrix. Low-pressure column liquid chromatography was performed using Sephadex LH-20 (Sigma Chemical Co.). Si gel plates (4.5 × 6.0 cm, 5−17 μm, Sorbfil) were used for thin-layer chromatography. HPLC was performed using an Agilent Series 1100 Instrument equipped with the differential refractometer RID-DE14901810 and a YMC-ODS-A (250 × 10 mm) column.

Animal Material. The sponge *Monanchora pulchra* (Lambe, 1894) was collected by dredging during the 36th scientific cruise of *R/V Academic Oparin*, in August 2008, near Urup Island (45°57,9 N; 150°44,9 E; depth 66 m). A voucher specimen is kept under the registration number PIBOC #O36-152 in the marine invertebrates collection of the Pacific Institute of Bioorganic Chemistry (Vladivostok, Russia).

Extraction and Isolation. The fresh collection of the sponge *M. pulchra* was immediately frozen and kept at −20 °C. The biological material (49.9 g, dry weight) was extracted with EtOH (200 mL × 3). The combined EtOH extract was concentrated and partitioned between *n*-BuOH and H_2O. The butanol layer was separated and concentrated *in vacuo*. The butanol-soluble materials were further partitioned between *n*-hexane and aqueous EtOH. The aqueous EtOH-soluble materials were concentrated and further separated by column chromatography on Sephadex LH-20 (elution with EtOH) to obtain a crude mixture of monanchocidins, which were subjected to preparative HPLC (YMC ODS-A column, 65% EtOH/0.1% TFA) to give pure monanchocidin A (**1**, 54 mg, 0.15%)[26] and monanchocidins B (**2**), C (**3**), D (**4**), and E (**5**) (0.067%, 0.008%, 0.0032%, and 0.01%, respectively, based on dry weight of the sponge).

Monanchocidin B. TFA salt **2**: 24.1 mg, colorless oil; $[\alpha]_D^{20}$ −10.4 (*c* 0.52, EtOH); ^{1}H and ^{13}C NMR data (DMSO-d_6), see Table 1, (CD_3OD) see Tables 2 and 3; HRMALDI-TOF-MS *m/z* 831.5978 $[M + H]^+$ calcd for $C_{45}H_{79}N_6O_8$ 831.5954; MALDI-TOF-MS data see Supporting Information.

Monanchocidin C. TFA salt **3**: 2.9 mg, colorless oil; $[\alpha]_D^{20}$ −23 (*c* 0.13, EtOH); ^{1}H and ^{13}C NMR data (CD_3OD), see Tables 2 and 3; HRMALDI-TOF-MS *m/z* 845.6150 $[M + H]^+$ calcd for $C_{46}H_{81}N_6O_8$ 845.6110; MALDI-TOF-MS data see Supporting Information.

Monanchocidin D. TFA salt **4**: 0.8 mg, colorless oil; $[\alpha]_D^{20}$ −10 (*c* 0.08, EtOH); ^{1}H and ^{13}C NMR data (CD_3OD), see Tables 2 and 3; HRMALDI-TOF-MS *m/z* 831.5920 $[M + H]^+$ calcd for $C_{45}H_{79}N_6O_8$ 831.5954; MALDI-TOF-MS data see Supporting Information.

Monanchocidin E. TFA salt **5**: 3.6 mg, colorless oil; $[\alpha]_D^{20}$ −10 (*c* 0.09, EtOH); ^{1}H and ^{13}C NMR data (CD_3OD), see Tables 2 and 3; HRMALDI-TOF-MS *m/z* 845.6120 $[M + H]^+$ calcd for $C_{46}H_{81}N_6O_8$ 845.6110; MALDI-TOF-MS data see Supporting Information.

$NaBH_4$ Reductive Degradation of 2. A solution of **2** (4.2 mg) in EtOH (0.5 mL) was reduced with $NaBH_4$ at 50 °C for 20 h. After cooling, the solution was neutralized with AcOH, and the solvent was then removed under reduced pressure. The residue was purified by HPLC (YMC-ODS-A column, 65% EtOH/0.1% TFA) to provide **6** (1.0 mg). Selected ^{1}H NMR data (700 MHz, DMSO-d_6): 7.92 (t, *J* = 6.1, NH-39), 7.63 (m, NH_2-41), 5.45 (d, *J* = 5.2, OH-37), 5.33 (m, H-4, H-5), 4.52 (d, *J* = 4.8, OH-3), 4.39 (d, *J* = 4.4, OH-19), 4.15 (m, H-3), 4.12 (m, H-23), 3.82 (m, H-37), 3.76 (m, H-13), 3.70 (m, H-8*), 3.58 (m, H-19), 3.50 (m, H-15), 3.37 (m, H-10*), 3.14 (m, H-39), 2.75 (m, H-41), 2.45 (t, *J* = 10.5, H-14), 1.68 (m, H-40), 1.45 (m, H-2b), 1.33 (m, H-2a), 1.26−1.30 (brs, H-25−H-34), 1.05 (d, *J* = 6.5, H-20), 0.82 (t, *J* = 7.2, H-1), * signals may be interchanged; HRMALDI-TOF-MS *m/z* 734.5775 $[M]^+$ (calcd for $C_{41}H_{76}N_5O_6$ 734.5790); MALDI-TOF/TOF-MS of the ion $[M]^+$ at *m/z* 734.5775, 408.006 $[M - C_{19}H_{39}N_2O_2 + H]^+$, 390.017 $[M - C_{19}H_{39}N_2O_2 - H_2O + H]^+$.

Bioassay. The action of **1**−**5** on the viability of the HL-60 human leukemia cell line was evaluated using MTS reduction into its formazan product as described previously.[30,31] Briefly, the corresponding cells were cultured for 12 h in 96-well plates (6000/50 μL of media per well). Then monanchocidins (**1**−**5**) at various concentrations in a volume of 50 μL of media were added, and the cells were incubated for 72 h. Finally, 20 μL of the MTS reagent was added into each well, and MTS reduction was measured 2 h later spectrophotometrically at 492 nm and at 690 nm as background using the μQuant microplate reader (Bio-Tek Instruments, Inc.). In this assay, Taxol was used as a positive control (IC_{50} 3.4 nM).

ASSOCIATED CONTENT

Supporting Information. This material is available free of charge via the Internet at http://pubs.acs.org.

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(29) The formation of only one (compound **6**) of four possible products was indicated by HPLC and NMR data after the reduction. That is why we suggest a direct reductive pathway for transformation of the "vessel" part, but not via hydrolysis followed by reduction of the intermediate imine. See for direct hydrogenolysis of hemiaminal groups: Berdini, V.; Cesta, C.; Curti, R.; D'Anniballe, G.; Di Bello, N.; Nano, G.; Nicolini, L.; Topai, A.; Allegretti, M. *Tetrahedron* **2002**, *58*, 5669–5674.
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