Chondropsins A and B: Novel Tumor Cell Growth-Inhibitory Macrolide Lactams from the Marine Sponge *Chondropsis* sp

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Abstract: Antiproliferative bioassay-guided fractionation of an aqueous extract of the marine sponge *Chondropsis* sp. provided two novel macrolides, chondropsins A (**1**) and B (**2**). The structures were elucidated by a combination of spectroscopic analysis and chemical modification. HMBC, TOCSY, NOESY, and HSQC-TOCSY experiments were particularly useful for the structural assignments of these polyketide-derived metabolites. The chondropsins define an unprecedented class of polyunsaturated, polyhydroxylated, 35-membered macrocycles which incorporate both lactone and lactam functionalities. An additional unique feature of these compounds is a complex, amide-linked, polyketide side chain. Testing of chondropsin A (**1**) in the NCI 60-cell screen revealed a mean-graph profile that did not correlate significantly with the profile of any compound class represented in the NCI standard agents database. The chondropsins therefore represent an interesting new lead for cancer therapeutics research.

Marine sponges have yielded a wide variety of biologically active secondary metabolites.[1] A prominent class of sponge-derived compounds is the polyketide-based macrocyclic lactones. These macrolides typically possess a pattern of oxygenation, alkylation, and dehydration along the primary aliphatic chain that is indicative of a polyketide biosynthetic origin.[2] While it is not unusual for aliphatic sponge macrolides to incorporate nitrogenous constituents, these often occur as oxazole or formamide functionalities.[3] Macrolides from sponges exhibit tremendous structural diversity, with a wide range of lactone ring sizes. Additionally, many of these compounds reportedly have cellular antiproliferative or cytotoxic activities.[4] Our studies were initiated on the basis of a distinctive pattern of differential cellular growth inhibition by an aqueous extract of an Australian collection of the sponge *Chondropsis* sp. in the National Cancer Institute's 60-cell antitumor screen.[5,6] The only previously reported investigation of sponges in the genus *Chondropsis* had resulted in the isolation of several cerebroside derivatives.[7,8] Antiproliferative bioassay-guided fractionation of the *Chondropsis* extract provided two structurally novel mac-rolides, chondropsins A (**1**) and B (**2**). Here we describe the isolation, structure elucidation, and biological activity of compounds **1** and **2**.

Results and Discussion

Samples of the sponge *Chondropsis* sp., collected off Bass Island near Wollongong, Australia, were extracted with H_2O, and the concentrated extract was fractionated on wide-pore reversed-phase C_4 media. The fraction which eluted with MeOH−H_2O (2:1) was further separated on an LH-20 column using a MeOH−H_2O (7:3) solvent system. The early eluting material from this column was ultimately purified by reversed-phase C_{18} HPLC using a linear MeOH−H_2O gradient to give chondropsins A (**1**) (17 mg) and B (**2**) (7 mg) as colorless powders.

Negative-ion FABMS analysis of chondropsin A (**1**) showed a strong pseudomolecular ion at *m/z* 1587.0, corresponding to

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Table 1. ^{1}H and ^{13}C NMR Data for Chondropsin A (**1**) in DMF-d_7

pos	δ_H mult (J, Hz)	δ_C mult[a]	HMBC[b]	HSQC−TOCSY[c]	pos	δ_H mult (J, Hz)	δ_C mult[a]	HMBC[b]	HSQC−TOCSY[c]
1		171.7 s			44	7.55 d (9.9)		45	43
2	5.14 dd (2.2, 9.2)	55.6 d	4, 35, 36	35	45		176.8 s		
3	7.85 d (9.2)		2, 4	2	46	2.54 m	47.2 d	45, 47, 69	47, 69
4		167.4 s			47	3.53 m	73.8 d	45	46, 48, 49
5	6.29 d (15.2)	124.6 d[d]	4, 7	6, 7, 8	48	1.48 m	33.1 t	47	47
6	7.14 dd (11.0, 15.2)	140.5 d	4, 8	5, 7, 8	49	1.21 m, 1.37 m	29.6 t	47	
7	6.29 dd (11.0, 15.0)	129.9 d		5, 6, 8, 9	50	1.57 m	36.2 d		48, 49, 51
8	6.10 m	141.9 d	6	5, 6, 7	51	3.56 d (8.1)	83.1 d	49, 50, 52, 53, 70	
9	2.30 m	34.5 t[e]	7, 8, 10	6, 7, 8, 10, 12	52		137.2 s		
10	2.15 m	32.9 t	9	7, 8, 9, 12, 13	53	5.49 d (9.5)	129.5 d	51, 55, 71	54, 72
11	5.69 m	131.2 d	13	12, 13, 14	54	2.68 m	35.4 d		53, 72
12	6.17 d (14.9)	131.8 d	10	10, 11, 13, 14, 15	55	3.76 m	74.8 d	53, 73	56
13	6.17 d (14.9)	132.2 d[f]	12, 15	10, 11, 12, 14, 15, 16	56	4.08 m	53.6 d	55, 58	55
14	5.69 m	132.1 d	13	11, 12, 13, 15, 16	57	7.58 d (10.3)		58	55, 56
15	2.03 m, 2.79 m	34.6 t[e]	16	12, 13, 14, 16	58		165.9 s		
16	4.01 m	72.3 d		14, 15	59	6.36 d (15.4)	124.7 d[d]	58, 61	60
17	Hβ 1.27 m, Hα 1.48 m	38.0 t	15, 16		60	6.88 d (15.4)	146.7 d	58, 61, 62, 77, 78	59
18	1.84 m	26.0 d		17, 19, 20, 37	61		51.3 s		
19	Hβ 0.80 m, Hα 1.52 m	41.7 t	17, 20	17, 18, 20, 21, 37	62		214.7 s		
20	3.69 m	65.9 d		19, 21	63	3.20 dq (7.0, 10.0)	44.7 d	62, 64, 65, 79	64, 79
21	1.46 m, 1.23 m	43.0 t	19, 20, 22	20, 22	64	4.05 d (10.0)	77.2 d	63, 65, 80	63, 79
22	4.25 m	66.1 d		20, 21	65		46.7 s		
23	1.44 m	41.9 d	24, 38	22, 24	66		178.0 s		
24	3.87 d (8.8)	80.2 d	22, 23, 25, 26, 38, 39	23, 38	67	3.78 m	69.2 d		68
25		138.2 s			68	1.09 d (6.2)	21.6 q	43, 67	43, 67
26	5.13 d (2.2)	132.2 d[f]	24, 27, 39, 40	27, 28	69	1.14 d (6.6)	15.7 q	45, 47	46, 47
27	2.51 m	36.5 d	28, 40	26, 28, 40	70	0.95 d (5.5)	15.9 q	49, 50, 51	49, 50, 51
28	3.53 d (8.1)	82.4 d	26, 29, 30, 41	27, 40	71	1.48 s	11.9 q	51, 52, 53	53
29		138.6 s			72	1.01 d (6.9)	18.1 q	53, 54, 55	53, 54
30	5.25 dd (6.6, 6.6)	123.6 d	28, 31, 41	31, 32	73	3.61 m	75.7 d	55, 74, 76	74, 75, 76
31	2.05 m, 2.45 m	32.1 t	29, 30, 32	30, 32	74	1.48 m	31.5 d	73	73
32	4.90 m	73.1 d	42, 1′	30, 31	75	0.87 d (6.6)	19.6 q	73, 74, 76	73, 74
33	2.03 m	38.9 d	35, 42	34	76	0.94 d (6.2)	20.1 q	73, 75	73, 74
34	5.11 dd (3.3, 9.5)	76.9 d	1, 32, 33	33, 42	77	1.21 s	23.8 q[g]	60, 61, 62, 78	
35	4.83 br s	72.2 d	1, 2, 36	2	78	1.27 s	23.9 q[g]	60, 61, 62, 77	
36		171.8 s			79	0.76 d (6.6)	15.4 q	62, 63, 64	63, 64
37	0.87 d (6.6)	22.8 q	17, 18, 19	17, 19	80	1.11 s	17.7 q	64, 65, 66, 81	
38	0.63 d (7.0)	9.8 q	22, 23, 24	23, 24	81	1.18 s	25.3 q	64, 65, 66, 80	
39	1.60 s	11.2 q	24, 25, 26	26	1′		172.6 s		
40	0.67 d (6.6)	18.1 q	26, 27, 28	26, 27, 28	2′	4.53 dd (4.0, 8.4)	68.8 d	1′, 3′, 4′	3′
41	1.56 s	11.3 q	28, 29, 30	30	3′	2.60 dd (8.4, 15.8) 2.76 m	40.2 t	1′, 2′, 4′	2′
42	1.03 d (7.0)	9.7 q	32, 33, 34	33, 34	4′		172.8 s		
43	4.15 m	53.4 d	45	67	OCH₃	3.63 s	51.7 q	66	

[a] Multiplicity inferred using the DEPT pulse sequence. [b] Optimized for J = 3.5 and 8.5 Hz. Carbons correlated to the proton resonance(s) in the ^{1}H column. [c] Carbons correlated to the proton resonance(s) in the ^{1}H column. [d−g] Assignments may be interchanged.

[M − H]⁻. Subsequent positive-ion HRFABMS analysis of a CsI-doped sample of **1** detected an adduct containing two Cs atoms [M − H + Cs₂]⁺, m/z 1852.7305 (calcd for $C_{83}H_{132}N_3O_{26}$-Cs₂, 1852.7208, Δ + 9.7 mmu). The facile incorporation of two Cs ions suggested that **1** contained two carboxylic acid functionalities. Treatment of **1** with diazomethane provided the bis methylated derivative **3** (HRFABMS, CsI-doped, m/z 1748.8524 [M + Cs]⁺), confirming the two carboxylic acid groups in **1**. The molecular formula of **1**, which was assigned as $C_{83}H_{133}N_3O_{26}$ on the basis of the HRFABMS measurements, was consistent with a detailed analysis of the ^{1}H and ^{13}C NMR data (Table 1). Despite the relatively large number of protons and carbons in **1**, the NMR spectra it provided were generally well-resolved and rich in structural information. However, ambiguities that did arise due to overlapping resonances required the analysis of complete NMR data sets in different solvents, including DMF-d_7, DMSO-d_6, and CD₃OH. NMR spectral analyses revealed 19 methyl groups in **1**, comprising one downfield methyl ester, three downfield vinyl methyls, 11 aliphatic methyl doublets, and four methyl singlets. Fifteen oxymethine groups, eight olefins, one ketone, and eight ester, amide, or carboxylic acid carbonyls were also defined. Extensive 2-D NMR analysis of **1**, particularly based on data from COSY,

TOCSY,[9] HSQC,[10] and HMBC[11] pulse sequences, resulted in the elucidation of five (**a−e**) structural fragments (Figure 1).

Partial structure **a** was established as a linear, four-carbon fragment which consisted of a carboxylic acid, an ester carbonyl, a nitrogen-substituted methine (δ 55.6, C-2), and an oxymethine group (δ 72.2, C-35). The H-2 resonance (δ 5.14) showed COSY correlations both to H-35 (δ 4.83) and to a well-resolved amide proton at δ 7.85 (H-3). HMBC correlations from H-2 and H-3 to a carbonyl at δ 167.4 (C-4) confirmed the attachment of **a** to substructure **b** via an amide bond at N-3. At this point, it was not possible to distinguish which carbonyl in fragment **a** existed as a free carboxylic acid and which was part of an ester link. Three-bond heteronuclear correlations from either H-3 or the hydroxyl proton on C-35 might have facilitated assignment of these two carbonyls; however, no definitive HMBC correlations were observed, even when a variety of different NMR parameters and experimental conditions were explored. Similarly, no diagnostic NOE interactions were observed.

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Chondropsins A and B
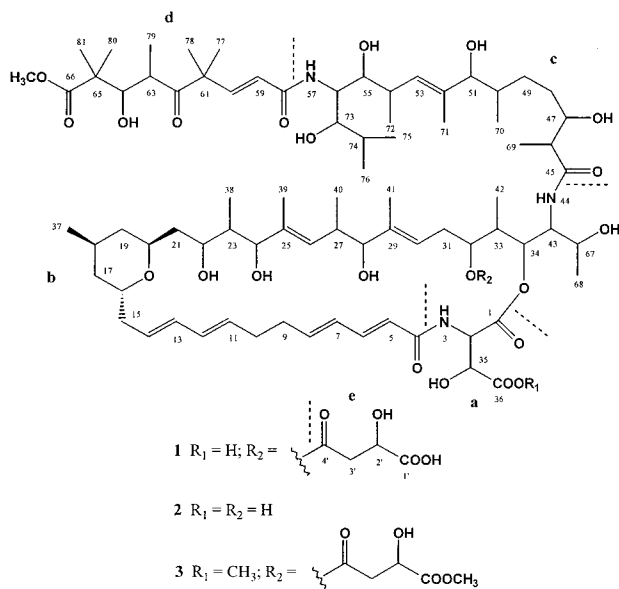
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Figure 1. Chondropsins A (**1**) and B (**2**) and methylated derivative **3**. Dotted lines delineate partial structures elucidated for compound **1**.

The structure of fragment **b** could be inferred largely from COSY and TOCSY correlation data. A proton-spin system which contained two pairs of conjugated dienes separated by two allylic methylenes was apparent, and its proximity to partial structure **a** was defined by HMBC correlations from H-5 (δ 6.29) and H-6 (δ 7.14) to the C-4 amide carbonyl. It was also possible to establish in **b** the presence of a trisubstituted tetrahydropyran ring in which one oxymethine (δ 4.01, H-16) was coupled to the C-15 allylic methylene protons. The other oxygenated methine (δ 3.69, H-20) was coupled to the C-21 aliphatic methylene group, and a methyl group was substituted at C-18. Extension of the proton-spin system through the highly overlapped C-17, C-19, and C-21 methylene protons to H-22 (δ 4.26) was aided by a combination of HMBC and HSQC-TOCSY[12] correlations (Table 1).

Assignment of the remainder of partial structure **b** employed COSY and TOCSY data to establish the proton-spin systems, HSQC correlations to confirm the sites of oxygenation or nitrogen substitution, and HMBC data to define the locations of the nonprotonated olefinic carbons (C-25 and C-29). An HMBC correlation from the C-42 methyl protons to C-34 helped to establish the C-33−C-34 connectivity, because no vicinal coupling was observed between H-33 and H-34. An HMBC correlation between H-34 and a carbonyl resonance (δ 172.6) in substructure **a** revealed that chondropsin A (**1**) incorporated a macrocyclic ring which resulted from esterification between a carbonyl in fragment **a** and the C-34 oxygen substituent. The downfield chemical shift of H-34 (δ 5.26) supported the assignment of an ester linkage at this position. The substitution of a nitrogen atom on C-43 was indicated by its ^{13}C NMR chemical shift (δ 53.7) and by proton−proton coupling between H-43 (δ 4.15) and an amide NH (δ 7.55, H-44). HMBC correlations from H-43 and H-44 to the C-45 carbonyl (δ 176.8) confirmed the presence of an amide at this position. Thus, partial structure **b** was joined by amide bonds to fragments **a** and **c**, and it formed a macrocycle via esterification to a carbonyl in **a**.

Partial structure **c** consisted of a 15-carbon chain that contained one olefin, one nitrogen substituent, and numerous
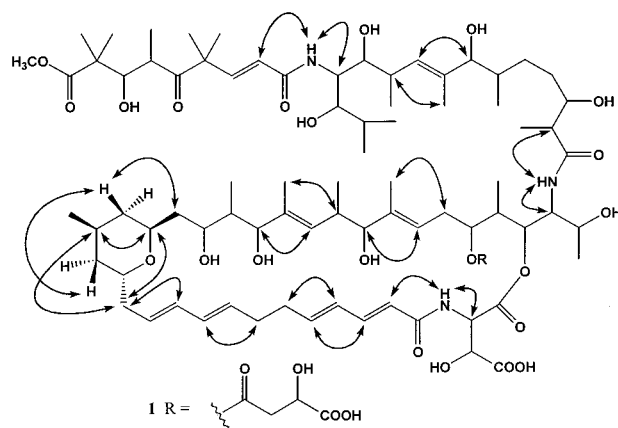
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Figure 2. Key NOESY correlations in chondropsin A (**1**).

methyl and hydroxyl substituents. Data from COSY and TOCSY experiments established the connectivities of the two major proton-spin systems in **c**. Proton resonances associated with the adjacent C-48−C-49 methylene pair were in a heavily overlapped region of the NMR spectrum, and thus, difficult to interpret. However, HMBC and HSQC−TOCSY correlations unambiguously defined the location of these methylene groups. The position of the Δ^{52} olefin was established by HMBC correlations from H-51 to the C-52 and C-53 olefinic carbons and by coupling between H-53 and H-54. The presence of a nitrogen attached to C-56 was revealed by the ^{13}C chemical shift (δ 53.6), and coupling between H-56 (δ 4.08) and the amide proton H-57 (δ 7.58).

Characteristic ^{1}H and ^{13}C NMR signals indicated that fragment **d** contained an α,β-unsaturated amide, a ketone, a methyl ester, and two gem dimethyl groups. The structural assignment of **d** was facilitated by analysis of proton−proton couplings and heteronuclear correlation data. HMBC correlations from H-56, H-57, H-59, and H-60 to the C-58 carbonyl (δ 165.9) established that **d** was joined to substructure **c** via an unsaturated amide linkage. Placement of a gem dimethyl substituent at C-61 followed from HMBC correlations between the two methyl groups and C-60 and the C-62 ketone resonance (δ 214.7). Additional HMBC correlations from both H-63 and the C-79 methyl protons to C-62 established that the C-63 methine was also situated α to the ketone. The position of the second gem dimethyl group was defined by HMBC correlations from the C-80 and C-81 methyl protons to C-64 (δ 77.2), C-65 (δ 46.7), and C-66 (δ 178.0), but the methyl ester was defined by a correlation from the singlet methyl protons (δ 3.63) to the C-66 ester carbonyl.

The only remaining unassigned NMR resonances, which consisted of a methylene, an oxymethine group, an ester carbonyl, and a carboxylic acid moiety, were assigned to a malic acid residue (substructure **e**); however, it was not possible to define the relative position of the ester and carboxylic acid moieties within **e** on the basis of HMBC or NOE correlation data. While the orientation of attachment of **e** remained ambiguous, its position within chondropsin A (**1**) was clearly established. An HMBC correlation between H-32 (δ 4.90) and the ester carbonyl (δ 172.8) in **e** revealed that the malic acid residue was esterified to the C-32 oxygen substituent in substructure **b**.

The geometries of the olefinic bonds in **1** were assigned, as all which are trans-based, on a combination of proton coupling constant analyses and observed NOE interactions (Figure 2). The $J_{5,6}$, $J_{7,8}$, $J_{11,12}$, $J_{13,14}$, and $J_{59,60}$ vicinal coupling constants of 15.2, 15.0, 14.9, 14.9, and 15.4 Hz, respectively, were

indicative of *E* double bonds. A trans configuration for Δ^{25} was established by an NOE observed between H-26 and H-24, and one between the C-39 vinyl methyl protons and the H-27 allylic methine proton. Similar analyses of NOE interactions about the Δ^{29} and Δ^{52} double bonds (Figure 2) also revealed trans geometries. The relative stereochemistry of the tetrahydropyran ring substituents in **1** was also deduced from NOE data. One of the H-15 protons (δ 2.79) exhibited strong NOE interactions with both H-18 and H-20. This indicated that C-15, H-18, and H-20 shared a common 1,3-diaxial orientation about the tetrahydropyran ring. All other NOE interactions detected among the ring substituents, including a correlation between H-18 and H-20, supported this assignment.

Our efforts to define the relative position of the ester and carboxylic acid functionalities in substructures **a** and **e** in chondropsin A (**1**) were inconclusive. Thus, extensive spectral characterization and the complete assignment of NMR resonances for the bis methylated derivative **3** were undertaken. The specific HMBC correlations that were required for assignment of the carbonyls in substructures **a** and **e** were still lacking in **3**; however, NOE data proved somewhat helpful. The ^{1}H NMR spectrum of compound **3** provided two new O-methyl singlets (δ 3.71 and 3.67) in addition to the C-66 methyl ester (δ 3.63). The methyl resonance at δ 3.71 showed a strong NOE interaction with the H-35 oxymethine proton in **a**, which indicated that this new O−Me group was attached to C-36. Thus, in chondropsin A (**1**), C-36 exists as a free carboxylic acid and C-1 is the ester carbonyl linked to the oxygen on C-34. Similar NOE studies failed to differentiate the carbonyl groups in fragment **e**.

The orientation of substructure **e** was ultimately assigned by comparing the NMR spectral data of this residue in **1** with spectral data from synthetic monomethyl esters of L-malic acid.[13] The NMR data for **1** showed significantly closer correspondence to the C-4 methyl ester derivative of L-malic acid than to the C-1 derivative. In particular, the chemical shifts of H-2N (δ 4.53) and H-3N (δ 2.60 and 2.76) in **1** were consistent with those of the oxymethine (δ 4.52) and methylene (δ 2.63 and 2.77) protons of the C-4 monomethyl ester derivative of L-malic acid. Resonances recorded for the oxymethine and methylene protons of the C-1 methyl ester derivative were δ 4.49 and δ 2.65 and 2.83, respectively. Thus, attachment of the malic acid residue **e** was assigned as in **1**.

Negative-ion FABMS analysis of chondropsin B (**2**) provided a pseudomolecular ion at *m/z* 1471.0 which corresponded to [M − H]⁻. Positive-ion HRFABMS of a CsI-doped sample showed an adduct [M + Cs]⁺ which contained one Cs atom (*m/z* 1604.8199), accountable to $C_{79}H_{129}N_3O_{22}Cs$. The molecular formula of **2** thus differed from that of chondropsin A (**1**) by a lack of $C_4H_4O_4$. The NMR data for **2** were very similar to those of **1**, with a few notable exceptions. Resonances assigned to the H-2N oxymethine and H-3N methylene protons in **1** were absent in the ^{1}H NMR spectra of **2**. In addition, the signal for H-32 appeared upfield at δ 3.52 for **2**, in contrast to the corresponding δ 4.90 for **1**. In the ^{13}C NMR spectrum of **2**, signals previously assigned to C-1N−C-4N of the malic acid residue were also missing. An independent assignment of the NMR spectral data (Table 2) and a complete structural elucidation confirmed that chondropsin B (**2**) is identical to **1**, except for the lack of the malic acid side chain at C-32 in **1**.

Chondropsins A (**1**) and B (**2**) are unique metabolites having a number of uncommon structural features. The three principal

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Table 2. ^{1}H and ^{13}C NMR Data for Chondropsin B (**2**) in DMF-d_7

pos	δ_H mult (*J*, Hz)	δ_C mult[a]	position	δ_H mult (*J*, Hz)	δ_C mult[a]
1		172.6 s	42	0.92 d (8.0)	9.4 q
2	5.03 m	55.4 d	43	4.18 m	53.7 d
3	7.99 m		44	7.61 m	
4		167.0 s	45		176.9 s
5	6.27 d (14.5)	124.1 d	46	2.58 m	46.9 d
6	7.16 dd (11.5, 14.5)	141.2 d	47	3.52 m	73.9 d
7	6.30 dd (11.5, 14.5)	129.9 d	48	1.47 m	33.3 t
8	6.15 m	142.3 d	49	1.21 m	29.9 t
9	2.31 m	34.4 t[b]	50	1.55 m	36.3 d
10	2.16 m	32.9 t	51	3.54 m	83.2 d
11	5.69 m	131.4 d	52		137.2 s
12	6.17 d (14.9)	131.8 d	53	5.50 d (9.5)	129.5 d
13	6.17 m (14.9)	132.1 d	54	2.68 m	35.4 d
14	5.69 m	132.1 d	55	3.77 m	74.7 d
15	2.01 m, 2.79 m	34.5 t[b]	56	4.09 m	53.8 d
16	4.00 m	72.3 d	57	7.61 m	
17	Hβ 1.26 m, Hα 1.47 m	38.0 t	58		165.9 s
18	1.86 m	26.0 d	59	6.38 d (15.5)	124.6 d
19	Hβ 0.80 m, Hα 1.51 m	41.7 t	60	6.88 d (15.5)	146.7 d
20	3.69 m	65.9 d	61		51.3 s
21	1.24 m, 1.46 m	43.1 t	62		214.7 s
22	4.26 m	66.0 d	63	3.22 m	44.7 d
23	1.46 m	41.8 d	64	4.05 m	77.2 d
24	3.87 d (9.0)	80.2 d	65		46.7 s
25		138.4 s	66		178.0 s
26	5.13 m	132.5 d	67	3.78 m	69.2 d
27	2.53 m	36.6 d	68	1.12 m	21.6 q
28	3.55 m	82.8 d	69	1.14 d (7.0)	15.7 q
29		137.4 s	70	0.95 d (7.0)	16.0 q
30	5.34 m	126.5 d	71	1.48 s	11.8 q
31	2.23 m, 2.30 m	32.4 t	72	1.01 d (6.5)	18.1 q
32	3.52 m	69.2 d	73	3.61 m	75.7 d
33	1.80 m	38.9 d	74	1.48 m	31.5 d
34	5.26 m	77.6 d	75	0.87 d (6.0)	19.6 q
35	4.87 br s	72.1 d	76	0.94 d (7.0)	20.2 q
36		171.8 s	77	1.21 s	23.8 q[c]
37	0.87 d (6.0)	22.8 q	78	1.27 s	23.9 q[c]
38	0.62 d (7.0)	9.7 q	79	0.77 d (6.5)	15.4 q
39	1.62 s	11.2 q	80	1.11 s	17.7 q
40	0.71 d (7.0)	17.9 q	81	1.18 s	25.3 q
41	1.57 s	11.4 q	OCH$_3$	3.63 s	51.8 q

[a] Multiplicity inferred using the DEPT pulse sequence. [b,c] Assignments may be interchanged.

structural fragments (**b**−**d**) apparently originate from polyketide biosynthesis. A fourth fragment (**a**), possibly derived from an aminated malic acid residue, is incorporated as a bridge between the two ends of fragment **b** to generate a combined lactone/lactam macrocyclic ring. This 35-membered macrocycle, which incorporates both amide and ester linkages to effect cyclization, is unprecedented. The acyclic portion of the chondropsins consists of two structurally novel fragments, **c** and **d**, which are attached through amide linkages. Formation of complex polyketides via amide coupling of multiple polyketide fragments has only rarely been described before.[14,15] The locations of all of the olefins, oxygenated carbons, and methyl substituents within chondropsins A (**1**) and B (**2**) are consistent with polyketide biosynthesis. The only apparent exception to this is the methyl substituent (C-37) within the tetrahydropyran ring. Careful reexamination of the NMR spectral data confirmed the location of this substituent; thus, it is likely the C-37 methyl group was added after the primary polyketide biosynthesis. The

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gem dimethyl groups that occur in the chondropsins could arise from a branched polyketide precursor or a subsequent methylation of the polyketide product. Although the chondropsins share some general structural features with an array of cyclic and acyclic polyhydroxy polyenes produced by symbiotic marine dinoflagellates,[16] the actual biosynthetic source of these novel macrolides has not been defined.

Chondropsin A (**1**) was tested in the U.S. National Cancer Institute (NCI)'s 60-cell antitumor screen as described.[5,6] The compound showed potent, differential growth inhibition of the 60 tumor cell lines, yielding a mean-panel GI_{50} value of 2.4×10^{-8} M and a range of $>10^3$ in relative sensitivities of the individual cell lines. Compare-algorithm analyses of the mean-graph profile[6] of **1** revealed no significant correlation to any mean-graph profiles contained in the NCI's "standard agents database". Thus, it appears that the mechanism of tumor growth inhibition by the chondropsins is different from that of conventional antitumor agents. This possibility, in addition to the very high potency of **1** (e.g., low nanomolar GI_{50}'s) against certain tumor cell lines (melanoma, colon, leukemia) indicates that the chondropsins will be an interesting new structural class for cancer therapeutics research.

Experimental Section

Animal Material. Samples of *Chondropsis* sp. were collected approximately 100 m from the shore of Bass Island off the coast of Wollongong, Australia. Samples were kept frozen prior to extraction. A voucher specimen (# Q66C1004) has been deposited with the Smithsonian Institution, Washington, D.C.

Isolation. The frozen sponge samples (357 g, wet weight) were ground in dry ice to a fine powder and extracted with H_2O at 4 °C. The aqueous extract was removed by centrifugation and subsequently lyophilized to give 44.0 g of extract. A 5-g aliquot of the aqueous extract was dissolved in 50 mL of distilled water and applied to a chromatography column (9 cm i.d.) containing 62 g of C_4 reversed-phase media (J. T. Baker, Wide-Pore Butyl). The column was eluted using increasing concentrations of MeOH in H_2O. The fraction eluting with MeOH–H_2O (2:1) was concentrated to provide 90 mg of material, which was further separated on a Sephadex LH-20 column (2.5 × 95 cm) and

eluted with MeOH–H_2O (7:3). The early eluting fractions from the LH-20 column were concentrated to provide 29 mg of residue that was subsequently dissolved in 2 mL of MeOH–H_2O (1:1) for HPLC. Reversed-phase HPLC separation (Dynamax ODS, 10 × 250 mm, 8 μm; flow rate, 4 mL/min) was performed using a linear MeOH–H_2O gradient (65:35–85:15 over 40 min.) which provided 2 mg of **1** and 0.8 mg of **2**. The remainder of the extract was processed in a manner similar this to provide a total of 17 mg of **1** (0.005% wet weight) and 7 mg of **2** (0.002% wet weight).

Chondropsin A (1): Colorless powder; $[\alpha]^{27}_D$ +7.1° (*c* 0.28, MeOH); UV [MeOH] λ_{max} 229 (ϵ 15372), 259 (ϵ 16229) nm; IR ν_{max} (KBr) 3418, 3298, 1689, 1610, 1533, 1207 cm^{-1}; ^{1}H and ^{13}C NMR see Table 1; FABMS (negative-ion; glycerol matrix) m/z 1587.0 [M − H]$^-$, HRFABMS (positive-ion; magic bullet matrix; CsI-doped) m/z 1852.7305 [M − H + Cs$_2$]$^+$, calcd for $C_{83}H_{132}N_3O_{26}Cs_2$, 1852.7208, Δ+9.7 mmu.

Chondropsin B (2): Colorless powder; $[\alpha]^{27}_D$ +30.6° (*c* 0.36, MeOH); UV [MeOH] λ_{max} 227 (ϵ 14792), 260 (ϵ 13124) nm; IR ν_{max} (KBr) 3422, 2957, 1695, 1635, 1207 cm^{-1}; ^{1}H and ^{13}C NMR see Table 2; FABMS (negative-ion; glycerol matrix) m/z 1471.0 [M − H]$^-$, HRFABMS (positive-ion; magic bullet matrix; CsI-doped) m/z 1604.8199 [M + Cs]$^+$, calcd for $C_{79}H_{129}N_3O_{22}Cs$, 1604.8122, Δ+7.7 mmu.

Methylation of 1. A 5.5-mg solution of chondropsin A (**1**) in 2.8 mL of MeOH was treated at room temperature with a solution of CH_2N_2 in diethyl ether (3 mL). Removal of the solvent under a stream of N_2 and HPLC purification of the residue as described above for **1**, provided 3.5 mg of the methylated derivative **3**: colorless powder; $[\alpha]^{27}_D$ +14.7° (*c* 0.34, MeOH); UV [MeOH] λ_{max} 228 (ϵ 20762), 261 (ϵ 20317) nm; IR ν_{max} (KBr) 3420, 3304, 1684, 1534, 1205, 1140 cm^{-1}; HRFABMS (positive-ion; magic bullet matrix, CsI-doped) m/z 1748.8524 [M + Cs]$^+$, calcd for $C_{85}H_{137}N_3O_{26}Cs$, 1748.8545, Δ−2.0 mmu. NMR spectral data for **3** are provided in the Supporting Information section.

Antiproliferative Bioassay. DMSO solutions of the chromatography fractions and aliquots of the purified chondropsins were assayed for antiproliferative properties using LOX (melanoma) and OVCAR-3 (ovarian) human tumor cell lines. Experimental details of this 2-day, in vitro assay have been described previously.[17]

Supporting Information Available: Analytical data for compounds **1**−**3**. This material is available free of charge via the Internet at http://pubs.acs.org.

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