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Application of High-Field NMR and Cryogenic Probe Technologies in the Structural Elucidation of Poecillastrin A, a New Antitumor Macrolide Lactam from the Sponge *Poecillastra* Species

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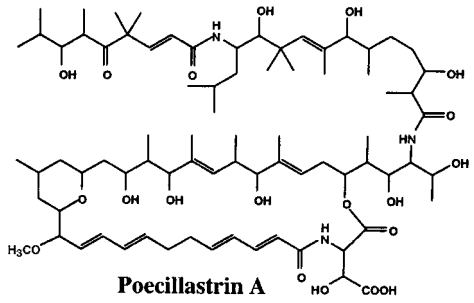
ABSTRACT



Poecillastrin A

Poecillastrin A (1), a new polyketide-derived macrolide lactam, was isolated from a deep-water collection of the marine sponge *Poecillastra* species. The structure of poecillastrin A (1) was assigned using NMR data acquired at 500 MHz with an inverse-detection cryogenic probe and at 800 MHz with a room-temperature probe.

A significant increase in the sensitivity of nuclear magnetic resonance (NMR) experiments can be achieved using cryogenic probes in which the preamplifier and radio frequency coils of the NMR probe are cooled to very low temperatures.[1,2] The utility of super-cooled probes for studying proteins in solution[3] and the application of this technology for high-throughput screening[4] and protein structure determination[5] have recently been described. Published accounts of cryogenic probe NMR studies with smaller molecules have generally focused on sensitivity determinations and signal-

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to-noise comparisons using known compounds[6,7] or *J*-based stereochemical assignments.[8] We describe herein the structural elucidation of poecillastrin A (**1**), a new complex marine macrolide, using NMR data acquired at 500 MHz with an inverse-detection cryogenic probe and at 800 MHz with a roomtemperature probe. All of the structural studies of poecillastrin A (**1**), which has a nominal molecular weight of 1441.9 Da, were performed on a limited mass sample of approximately 800 μg (0.55 μmol).

The aqueous extract of a deep-water (−359 m) Caribbean collection of the marine sponge *Poecillastra* sp.[9] produced a differential pattern of antiproliferative and cytotoxic activity in the NCI's 60-cell antitumor screen.[10] COMPARE-algorithm analysis of the resulting mean-graph profiles[11] of the extract showed that they were similar to the mean-graph profiles of the chondropsins.[12−14] The chondropsins comprise a new class of polyketide-derived macrolide lactams that were recently isolated from two different genera of marine sponges: *Chondropsis* and *Ircinia*. They have potent in vitro cytotoxic activity and their 60-cell mean-graph profiles do not correlate with any mean-graph profiles in the NCI's standard agents database, thus it appears that their mechanism of tumor cell inhibition is different from that of current antitumor agents. Since there were few previous reports describing the chemistry of *Poecillastra* sponges,[15] we initiated a bioassay-guided study to isolate and identify the cytotoxic constituents from the extract.

A multistep fractionation of the *Poecillastra* sp. extract (20 g) ultimately provided approximately 800 μg of HPLC purified poecillastrin A (**1**) as the principle active agent. Since a timely recollection of the deep-water sponge to supply additional compound was problematic, spectral characterization of the 800 μg sample was initiated. The molecular formula of **1** was established to be $C_{79}H_{131}N_3O_{20}$ by HRFAB MS measurements of a CsI-doped sample ($[M - H + 2Cs]^+$ *m*/*z* 1706.7375, calcd for $C_{79}H_{130}N_3O_{20}Cs_2$, 1706.7350). A 180 μL sample of **1** in DMF-d_7 was prepared, and ^{1}H, COSY, TOCSY, ROESY, and HSQC NMR data were obtained at 800 MHz using a 3 mm inverse-detection room-temperature probe. Heteronuclear multiple bond correlation (HMBC) data, which were required for the structural elucidation of **1**, were acquired at 500 MHz using a 5 mm HCN inverse-detection Varian Chili-Probe in which the radio frequency coils and preamplifier were cooled to approximately 25 and 60 K, respectively. The combination of high spectral dispersion generated at 800 MHz along with enhanced sensitivity achieved with the cryogenic probe allowed complete assignment of the ^{1}H and ^{13}C resonances in **1** (Table 1). Since a ^{13}C NMR spectrum was never recorded, the carbon assignments for **1** were made indirectly from HSQC and HMBC correlations. The NMR data clearly established that poecillastrin A (**1**) was a new, highly functionalized macrolide, structurally related to the chondropsins.[12−14]

The portion of **1** from C-5 to C-24 contained a chain of 20 contiguous protonated carbons, and it was possible using the 800 MHz NMR data to assemble this structural fragment along with the associated methyl and oxygenated substituents. The section of **1** that encompassed C-25 to C-29 was assigned by proton−proton spin system analysis and HMBC data (Figure 1). The spin system that extended from C-30

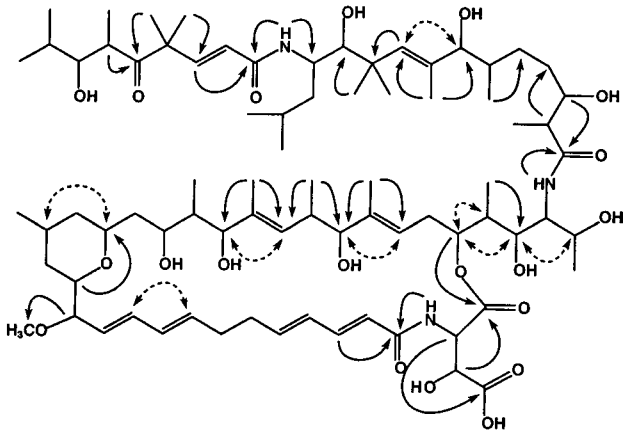

Figure 1. Key HMBC correlations (solid arrow) and ROESY correlations (dashed arrow) for **1**.

through to C-68 was assigned in a similar manner. However, no vicinal coupling was observed between the protons on C-41 and C-42, and the HMBC data did not reveal appropriate correlations to confirm that these carbons were adjacent to each other. The connection between C-41 and C-42 was ultimately assigned based on strong ROESY interactions observed between H-41 and H-67 (Figure 1). The C-32 oxymethine proton had a downfield chemical shift (δ_H 5.26) which indicated that the oxygen at position 32 was esterified. This was confirmed by an HMBC correlation from H-32 to an ester carbonyl at δ_C 172.0. COSY and HMBC correlations established that the ester carbonyl was part of an amino malic acid moiety that was attached to C-4 (δ_C 166.6) via an amide bond. Thus, poecillastrin A (**1**) contains a 33-membered macrocyclic ring that incorporates an amide bond between

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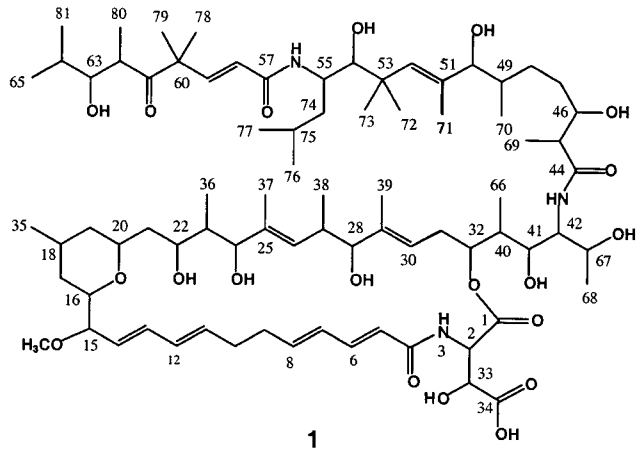
Table 1. ^{1}H and ^{13}C NMR Data for Poecillastrin A (**1**) in DMF-d_7[a]

pos	δ_C mult	δ_H mult (J in Hz)	HMBC	pos	δ_C mult	δ_H mult (J in Hz)	HMBC
1	172.1 s			42	54.7 d	3.89 dd (9.0, 4.0)	C-67
2	56.3 d	5.10 dd (9.5, 2.2)	C-34	43		7.38 d (9.0)	C-44
3		8.21 d (9.5)	C-4	44	176.7 s		
4	166.6 s			45	47.2 d	2.50 pent (7.0)	C-44, C-46, C-47, C-69
5	124.0 d	6.34 d (15.2)	C-4, C-7	46	74.1 d	3.51 m	C-44, C-69
6	141.3 d	7.12 dd (15.2, 11.0)	C-4, C-5, C-7, C-8	47	33.5 t	1.51 m, 2H	
7	129.8 d	6.26 m	C-5, C-8, C-9	48	30.2 t	1.29 m, 2H	
8	142.0 dt	6.08 d (15.0, 10.4)	C-6, C-9	49	36.5 d	1.56 m	
9	33.9 t	2.23 m, 2.31 m		50	83.5 d	3.57 m	C-52, C-71
10	32.8 t	2.19 m, 2H		51	136.1 s		
11	134.5 d	5.82 dt (15.0, 8.0)		52	135.1 d	5.47 bs	C-50, C-54, C-71, C-72, C-73
12	131.2 d	6.25 m	C-10, C-14	53	40.8 s		
13	134.5 d	6.36 dd (15.2, 11.0)	C-11, C-15	54	81.7 d	3.51 m	C-52, C-55, C-74
14	131.5 d	5.54 dd (15.2, 8.3)	C-12	55	50.3 d	4.18 m	
15	80.9 d	3.98 m,	C-13, C-16, OCH_3	56		7.70 d (9.6)	C-55, C-57
16	75.5 d	3.55 m	C-15, C-18, C-20	57	164.8 s		
17	34.4 t	1.14 and 1.86 m		58	124.7 d	6.16 d (15.2)	C-57, C-60
18	26.0 d	1.85 m		59	146.8 d	6.90 d (15.2)	C-57, C-58, C-60, C-61, C-79
19	41.5 t	0.80 and 1.57 m		60	51.2 s		
20	68.5 d	3.55 m		61	215.3 s		
21	43.6 t	1.29 m, 1.43 m		62	44.8 d	3.15 dq (9.6, 7.0)	
22	66.1 d	4.18 m		63	77.7 d	3.52 m	
23	41.6 d	1.45 m		64	29.8 d	1.74 m	C-65, C-81
24	80.1 d	3.82 d (9.6)	C-22, C-23, C-26, C-37	65	14.2 q	0.82 d (7.0), 3H	C-63, C-64, C-81
25	138.1 s			66	10.1 q	0.94 d (7.0), 3H	C-32, C-40, C-41
26	136.1 d	5.17 bd (8.0)	C-24, C-37	67	70.6 d	3.99 m	
27	37.1 d	2.56 m		68	20.8 q	1.11 d (6.0), 3H	C-42, C-67
28	81.7 d	3.65 d (9.0)	C-26, C-27, C-29, C-30	69	15.9 q	1.16 d (7.0), 3H	C-44, C-45, C-46
29	138.9 s			70	15.6 q	0.90 d (7.0), 3H	C-48, C-49, C-50
30	122.0 d	5.27 m	C-28, C-39	71	13.7 q	1.65 s, 3H	C-50, C-51, C-52
31	31.1 t	2.35 m, 2H	C-29, C-30, C-32	72	25.4 q	1.10 s, 3H	C-52, C-53, C-54
32	75.3 d	5.26 m	C-1, C-41, C-66	73	26.9 q	1.18 s, 3H	C-52, C-53, C-54
33	73.3 d	4.87 d (2.2)	C-1	74	40.8 t	1.46 m, 1.54 m	
34	171.7 s			75	25.3 d	1.53 m	
35	22.9 q	0.88 d (6.5), 3H	C-17, C-18, C-19	76	24.5 q	0.84 d (7.0), 3H	C-74, C-75, C-77
36	10.0 q	0.60 d (6.8), 3H	C-22, C-23, C-24	77	21.8 q	0.86 d (7.0), 3H	C-74, C-75, C-76
37	11.4 q	1.60 s, 3H	C-24, C-25, C-26	78	23.7 q	1.21 s, 3H	C-59, C-60, C-61
38	17.8 q	0.76 d (6.8), 3H	C-26, C-27, C-28	79	23.9 q	1.27 s, 3H	C-59, C-60, C-61, C-78
39	12.3 q	1.59 s, 3H	C-28, C-29, C-30	80	15.7 q	0.83 d (7.0), 3H	C-61, C-62, C-63
40	40.3 d	1.84 m		81	20.7 q	0.92 d (7.0), 3H	C-63, C-64, C-65
41	74.3 d	3.66 m		OCH_3	56.4 q	3.23 s, 3H	C-15

[a] ^{13}C assignments were made using HSQC and HMBC data and multiplicities inferred using a multiplicity-edited HSQC pulse sequence.

N-3 and C-4, and an ester link between C-1 and the oxygen on C-32.



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An HMBC correlation from the N-43 proton (δ_H 7.38) to the C-44 carbonyl (δ_C 176.7) defined another amide linkage at this position, while homonuclear proton couplings and HMBC data allowed extension of the acyclic portion of **1** out through the *gem* dimethyl substituents on C-75. The remaining structural fragment from C-57 to C-65 was assembled using proton coupling data to define the spin systems and HMBC correlations to bridge the nonprotonated carbons. Attachment of this fragment through an α,β-unsaturated amide bond was established by HMBC correlations from both NH-56 (δ_H 7.70) and H-59 (δ_H 6.90) to C-57 (δ_C 164.8). The geometry of the olefin bonds in **1** were assigned as all *E* based on large proton−proton couplings and characteristic ROESY interactions (Figure 1), while placement of a methoxyl group at C-15 was revealed by an HMBC correlation from the OCH_3 group (δ_H 3.23) to C-15 (δ_C 80.7). Stereochemical assignments for **1** were not

attempted due to the large number of asymmetric carbons (24) in the molecule and the small sample size.

Poecillastrin A (**1**) is structurally related to the chondropsin class of macrolide lactams.[12−14] However, it is the first member of this family of polyketide derivatives with a 33-membered macrocyclic ring and it has unique patterns of methylation and oxygenation. Poecillatrian A (**1**), when tested against four different human tumor cell lines[16] and two murine mast cell lines,[17] was differentially cytotoxic and antiproliferative with EC_{50}'s that ranged from <25 nM to >10000 nM, which were comparable to those observed with the chondropsins. Thus, the new structural features found in poecillastrin A (**1**) broadens the known structural diversity of this family of potent antitumor macrolide lactams. The combination of high field (800 MHz) NMR, which provided improved spectral resolution, and cryogenic probe technologies, which allowed the acquisition of critical heteronuclear multiple bond correlation data on a mass limited sample, demonstrate the power of recent NMR advances for the structural elucidation of novel and complex organic molecules.

(16) The cytotoxicity assay, details of which have been described previously,[18] utilized melanoma (LOX), breast (A-549), ovarian (OVCAR-3), and nonsmall cell lung (SNB-19) human tumor cell lines and IC-2^{WT} and IC-2^{V814} murine mast cell lines.[17]

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Supporting Information Available: General experimental procedures and NMR data for poecillastrin A. This material is available free of charge via the Internet at http://pubs.acs.org.

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