Supporting Material

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Manuscript Title: 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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Enclosed Materials:

NMR Data for Poecillastrin A (**1**) in DMF-*d*₇:

S1-S2	¹ H NMR (800 MHz)
S3	COSY (800 MHz)
S4	TOCSY (800 MHz)
S5	ROESY (800 MHz)
S6	HSQC (800 MHz)
S7-S8	HMBC (500 MHz, cryogenic Chili-Probe)
S9-S11	Experimental Section

S1

Pocillastatin A (1), ^1H NMR, 800 MHz, DMF-d_7

S2

Pocillastrin A (1), ^1H NMR, 800 MHz, $\text{DMF-}d_7$

Experimental Section

General Experimental Procedures. HPLC was performed on a Varian-Rainin system employing a Dynamax C₁₈ column (1 x 25 cm), using a flow rate of 3 mL/minute and UV detection at 220 nm. Optical rotations were measured with a Perkin-Elmer 241 polarimeter. Ultraviolet (UV) and infrared (IR) spectra were obtained on a Beckman DU-640 and a Perkin-Elmer 1600 FTIR spectrometer, respectively. High resolution mass spectra were acquired on a JEOL SX102 mass spectrometer.

NMR spectra were recorded at 29° C on 800 µg of poecillastrin A (**1**) dissolved in 180 µL of DMF-*d*₇ and placed in a 3 mm NMR tube. The HMBC data were collected on a Varian INOVA 500 spectrometer outfitted with a 5 mm HCN inverse-detection Chili-Probe. The radio frequency coils and preamplifier of the Chili-Probe were cooled with liquid helium to approximately 25° K and 60° K, respectively. The HMBC, which was optimized for $^1J_{CH} = 140$ Hz and $^nJ_{CH} = 6$ Hz, was acquired in approximately 32.5 hrs with 1025 (*F*2) x 256 (*F*1) points, * 256 scans per increment, and *F*2 and *F*1 spectral widths of 6000 and 27,036, respectively. The *F*1 dimension was zero-filled to 2K and the data was processed with a sinebell weighting function. All other NMR data were obtained on a Varian INOVA 800 spectrometer equipped with a 3 mm inverse-detection probe operated at room temperature. An absolute value COSY was acquired with 2K (*F*2) x 192 (*F*1) points, 16 scans per increment, and 8468 Hz spectral width in both dimensions. The TOCSY data were acquired with a 60 ms mixing time at 8.3 KHz field strength, 1K x 224 points, 16 scans per increment, and 6000 Hz spectral width in both dimensions. The ROESY was acquired with a 300 ms mixing time at 5.5 KHz field strength, 2K x 256 points, 16 scans per increment, and 8468 Hz spectral width in both dimensions. The multiplicity-edited HSQC, with $^1J_{CH} = 140$ Hz, was acquired with 1536 x 256 points, 192 scans per increment, and spectral widths of 8468 Hz in *F*2 and 28,139 Hz in *F*1. The COSY data were processed with a sinebell weighting function and gaussian weighting was used for the other data sets obtained at 800 MHz. All of these experiments

had the F_2 dimension zero-filled to 4K, except for the HSQC which was zero-filled to 2K. The F_1 dimension of the HSQC and COSY were zero-filled to 1K, while in the TOCSY and ROESY F_1 was zero-filled to 512. Two- and three-fold linear prediction was performed on the HSQC and COSY data, respectively. Chemical shifts are reported in ppm relative to the residual nondeuterated solvent. Assignments for the ^{13}C resonances were based on HSQC and HMBC correlations, and their multiplicities were inferred from the multiplicity-edited HSQC experiment. *All data points reported represent complex data points.

Animal Materials. Samples of the sponge *Poecillastra* sp. were collected by a manned submersible at a depth of -359 meters near Settlement Point, Grand Bahama Island, Bahamas, in November, 1987. The collection was made by the Harbor Branch Oceanographic Institute under contract with the NCI, and taxonomic identification was provided by Shirley A. Pomponi. A voucher specimen (# Q66B974) for this collection is maintained at the Smithsonian Institution, Washington, D.C.

Extraction and Isolation. The frozen sponge (394 g) was ground to a fine powder and extracted with H_2O to give 94 g of aqueous extract. After numerous chemical separation schemes were attempted, the following bioassay-guided isolation procedure was employed. A 20 g portion of the extract was dissolved in 120 mL of H_2O and precipitated by addition of 120 mL of EtOH and storage at -20°C for 16 h. After removal of the precipitate by centrifugation, the supernatant was evaporated to dryness and then partitioned between H_2O and *n*-BuOH. The *n*-BuOH soluble material (1.03 g) was triturated with MeOH to give a 917 mg MeOH soluble fraction. Separation of this material on Sephadex LH-20 eluted with MeOH- H_2O (7:3) provided a cytotoxic fraction (43 mg) that was chromatographed on a second Sephadex LH-20 column eluted with 100% MeOH. The cytotoxic fractions from this LH-20 column were pooled to give 12.1 mg of material that was subjected to C_{18} column chromatography eluted with MeOH- H_2O (2:3). Final purification was achieved by C_{18} HPLC eluted with a linear gradient of CH_3CN - H_2O (9:11) to

100% CH₃CN (0.1% TFA vol/vol in the eluant) over 45 min to give 800 µg of poecillastrin A (**1**).

Poecillastrin A (1): White gum; UV [MeOH] λ_{max} 229 (ϵ 10,800), 235 (ϵ 10,900), 263 (ϵ 10,200) nm; IR ν_{max} (neat on a NaCl plate) 3500-3300, 1716, 1697, 1683, 1206, 1135 cm⁻¹; ¹H and ¹³C NMR data see Table 1; FABMS (M + Na)⁺ m/z 1464.9; HRFABMS of a CsI doped sample, (M - H + 2Cs)⁺ m/z 1706.7375, calcd for C₇₉H₁₃₀N₃O₂₀Cs₂, 1706.7350. An accurate determination of the optical rotation was difficult due to the limited sample size. Forty two individual measurements were made and all of them provided negative optical rotations. The optical rotation calculated for **1** was based on the average of these forty two measurements: $[\alpha]_{\text{D}}^{27}$ -8.3° (c 0.056, MeOH).