Daryamides A−C, Weakly Cytotoxic Polyketides from a Marine-Derived Actinomycete of the Genus *Streptomyces* Strain CNQ-085

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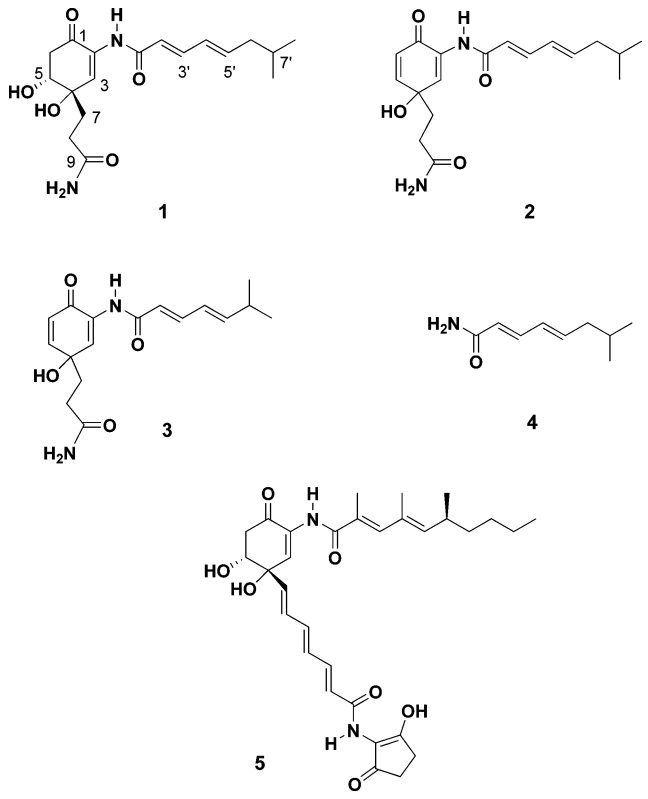
In the course of our continuing search for new antitumor-antibiotics from marine-derived actinomycete bacteria, four new cytotoxic compounds, designated as daryamides A (**1**), B (**2**), and C (**3**) and (2*E*,4*E*)-7-methylocta-2,4-dienoic acid amide (**4**), were isolated from the culture broth of a marine-derived *Streptomyces* strain CNQ-085. The structures of these new compounds were assigned by detailed interpretation of spectroscopic data. The relative configuration of **1** was determined by comprehensive NMR analysis, while the absolute configuration of **1** was determined as 4*S*,5*R* using the modified Mosher method. The daryamides show weak to moderate cytotoxic activity against the human colon carcinoma cell line HCT-116 and very weak antifungal activities against *Candida albicans*.

The discovery of actinomycete bacteria adapted to life in the sea has encouraged us to continue to explore marine sediments for these chemically prolific bacteria.[1,2] In the course of these studies, we cultivated an actinomycete strain (CNQ-085) that, on the basis of 16S rDNA sequence analysis, is a member of the phylogenetically diverse genus *Streptomyces*. Culture extracts of strain CNQ-085 were active in our initial cytotoxicity assays, and subsequent bioassay-guided fractionation led to the isolation of four new moderately cytotoxic compounds, designated as daryamides[3] A (**1**), B (**2**), and C (**3**) and (2*E*,4*E*)-7-methylocta-2,4-dienoic acid amide (**4**). In this paper we report the isolation and structure assignments of these compounds, along with the absolute configuration of daryamide A (**1**).

Results and Discussion

Streptomyces strain CNQ-085 was cultured at 27 °C by rotary shaking in multiple 2.8 L Fernbach flasks containing 1 L of a saltwater-based nutrient medium. After 6 days, Amberlite XAD-7 resin was added, and the resin was stirred for several hours, filtered, and extracted with acetone. Following solvent removal, the crude extract was subjected to repeated reversed-phase (C18) chromatography to yield compounds **1**−**4**.

Daryamide A (**1**) was isolated as a pale yellow, amorphous powder, soluble in methanol, ethyl acetate, and dimethyl sulfoxide, but insoluble in chloroform and *n*-hexane. The IR spectrum of **1** indicated the presence of hydroxyl (3401 cm^{-1}), amide (1668 cm^{-1}), and conjugated diene groups (1615 cm^{-1}). The UV spectrum showed absorption maxima at 285 and 271 nm (CH_3OH), which indicated the presence of a conjugated diene-containing chromophore. The ESIMS gave a quasi-molecular ion peak at *m*/*z* 373 $[M + Na]^+$, which, when analyzed by positive ion HRESITOFMS methods, indicated a molecular formula of $C_{18}H_{26}N_2O_5$ (observed 373.1735, calcd for $C_{18}H_{26}N_2O_5Na$ 373.1734). This molecular formula was also supported by ^1H and ^{13}C NMR spectral data. The ^{13}C NMR spectra, with DEPT analysis, displayed 18 signals including two methyls, four methylenes, seven methines, and five quaternary carbons (Table 1). The ^1H NMR spectrum showed signals corresponding to 26 protons, including exchangeable protons assigned to one NH, two hydroxyl (aliphatic), and one NH_2 group. Analysis of $^1H-^1H$ COSY and gHSQC spectral data allowed three spin systems to be observed, a diene chain $-CH{=}CH-CH{=}CH-CH_2-CH(CH_3)_2$, a two-carbon hydroxyl-bearing fragment $-CH(OH)-CH_2-$, and an ethylene unit $-CH_2-CH_2-$. HMBC NMR experiments established the connectivities of three additional fragments. Long-range proton−carbon correlations from H-3 (δ 7.52) to C-1 (δ 193.6), C-2 (δ 132.9), and C-5 (δ 70.4), from H-6 (δ 2.63) to C-1 (δ 193.6) and C-4 (δ 72.0), and from H-5 (δ 3.81) to C-1 (δ 193.6) and C-3 (δ 132.4) showed the presence of a cyclohexenone unit (C-1 to C-6). Further HMBC correlations from H_2-7 (δ 1.88, 1.86 m) to C-4 (δ 72.0), C-8 (δ 30.3), and C-9 (δ 174.9) and from H_2-8 (δ 2.23, 2.17) to C-9 (δ 174.9), C-7 (δ 34.0), and C-4 (δ 72.0) indicated that this unit was attached to the aliphatic chain. A carbon signal at δ 174.9 was assigned to the $-CO-NH_2$ group, which led to substructure I (Figure 1).



Analysis of ^1H NMR COSY data and key HMBC correlations from H-2′ (δ 6.42) to C-1′ (δ 165.4) and C-4′ (δ 130.8), from the NH proton (δ 8.98) to C-1′ (δ 165.4), from H-4′ (δ 6.16) to C-5′ (δ 142.5), C-3′ (δ 141.8), and C-6′ (δ 42.0), and from H_2-6′ (δ

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Table 1. NMR Spectral Data for Daryamide A (**1**) in DMSO-d_6

C/H #	δ_H (*J* Hz)	δ_C		COSY	HMBC	NOESY
1		193.6	C			
2		132.9	C			
3	7.52 d (1.2)	132.4	CH		C-1, C-2, C-5	
4		72.0	C			
5	3.81 dd (6.2, 4.3)	70.4	CH	H-6ax, H-6eq	C-1, C-3	H-7a
6ax	2.63 dd (16.8, 4.3)	42.4	CH_2	H-5, H-6eq	C-1, C-4	H-7a
6eq	2.69 dd (16.8, 6.2)			H-5, H-6ax	C-1, C-4	OH-4
7a	1.88 m	34.0	CH_2	H-8a, H-8b, H-7b	C-3, C-4, C-5, C-8	
7b	1.86 m			H-8a, H-8b, H-7a	C-3, C-4, C-5, C-8	
8a	2.23 m	30.3	CH_2	H-7a, H-7b, H-8b	C-4, C-7, C-9	
8b	2.17 m			H-7a, H-7b, H-8a	C-4, C-7, C-9	
9		174.9	C			
1′		165.4	C			
2′	6.42 d (15.4)	123.8	CH	H-3′	C-1′, C-4′	H-4′
3′	7.08 dd (15.3, 10.3)	141.8	CH	H-2′, H-4′	C-1′, C-5′	H-5′
4′	6.16 dd (15.3, 10.3)	130.8	CH	H-3′, H-5′	C-6′, C-3′	H-2′
5′	6.15 m	142.5	CH	H-4′, H-6′	C-3′, C-6′	H-3′
6′	2.02 t (6.0)	42.0	CH_2	H-5′, H-7′	C-4′, C-5′, C-7′, C-8′, C-9′	
7′	1.69 m	28.3	CH	H-6′, H-8′, H-9′	C-5′, C-6′, C-8′, C-9′	
8′	0.86 d (6.8)	22.9	CH_3	H-7′	C-6′, C-7′	
9′	0.86 d (6.8)	22.9	CH_3	H-7′	C-6′, C-7′	
NH-2	8.98 s				C-1, C-2, C-3, C-1′	
OH-4	6.76 br s				C-7	OH-5
OH-5	7.32 br s					OH-4, H-6eq
NH_2-9	5.04 br s					

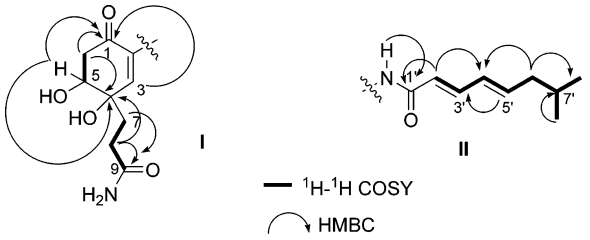

Figure 1. Key ^{1}H−^{1}H COSY and HMBC correlations observed in substructures I and II of daryamide A (**1**).

2.02) to C-5′ (δ 142.5), C-4′ (δ 130.8), C-7′ (δ 28.3), C-8′ and C-9′ (δ 22.9) led to substructure II (Figure 1).

Finally, the NH proton signal at δ 8.98 from substructure II showed key HMBC correlations to C-1 and C-2 of the cyclohexanone ring (substructure I), which allowed these two substructures to be connected to provide structure **1**. The configurations of the two side-chain double bonds in daryamide A (**1**) were assigned as *E* on the basis of 15.3 Hz coupling constants (H-2′ to H-3′ and H-3′ to H-4′) and also on the basis of observed NOEs between H-2′ and H-4′ and H-3′ and H-5′. The relative stereochemistry of the cyclohexenone moiety of **1**, which has two chiral centers at the C-4 and C-5 positions, was defined by analysis of coupling constants and from the results of NOE NMR experiments. The small proton coupling constants, $^3J_{H5,H6ax} = 4.3$ and $^3J_{H5,H6eq} = 6.2$ Hz, indicated that the H-5 proton was in a quasi-equatorial position.[4] This assignment was also supported by the observation of a long-range "*W*" coupling between H-3 and H-5 ($^4J_{H3,H5} = 1.2$ Hz). The observation of an NOE correlation between H-6ax and H-7a and between H-5 and H-7a of the aliphatic chain indicated that the aliphatic chain was positioned in a quasi-axial position. An observed NOE correlation between the C-5-O*H* and C-4-O*H* protons and between H-6eq and the C-4-O*H* proton indicated that both of the hydroxyl groups must be on the same face of the ring. Thus, from these observations, the cyclohexene ring of daryamide A (**1**) was concluded to have a half-chair conformation, and the relative stereochemistry of C-4 and C-5 was assigned as *cis*, leading to the assignment of the 4*S*,5*R* configuration, as shown in Figure 2.

The absolute configurations at C-4 and C-5 were assigned by application of the modified Mosher method.[5] Mosher acylation of **1** with both *S*- and *R*-MTPA chloride yielded the C-5 *R*- and *S*-α-methoxy-α-(trifluoromethyl)phenyl acetyl (MTPA) esters. During

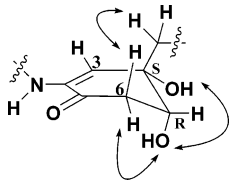

Figure 2. Half-chair conformation for daryamide A, which accommodates observed NOE correlations

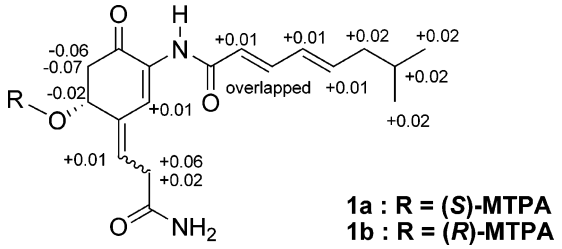

Figure 3. $\Delta\delta_{S-R}$ values for the C-5 Mosher esters **1a** and **1b** of daryamide A (**1**).

preparation of the Mosher esters, the tertiary hydroxyl in daryamide A (**1**) was lost to give the corresponding Mosher esters **1a** and **1b**, respectively. The ^{1}H NMR chemical shift differences of the H-6ax and H-6eq protons of **1a** and **1b** ($\Delta\delta_{S-R} = \delta(S) - \delta(R)$) were −0.06 and −0.07, respectively. These results clearly confirmed the 4*S*,5*R* configuration for compound **1** (Figure 3).

These results were also consistent with the results of a CD measurement. Daryamide A (**1**) showed a positive Cotton effect at 320 nm and negative Cotton effect at 265 nm, indicating an *S* configuration at C-4. These results were analogous to those derived from the related cyclohexenones asukamycin and manumycin D, both of which showed a positive and negative Cotton effect with an *S* configuration at C-4.[6]

Daryamide B (**2**) was isolated as a yellow oil that analyzed for the molecular formula $C_{18}H_{24}N_2O_4$ by HRESITOFMS analysis (*m*/*z* $[M + Na]^+$ (obsd) 355.1631). This formula required 8 degrees of unsaturation, one more than daryamide A (**1**). The MS data also showed the presence of at least one hydroxyl functionality on the basis of the observation of a fragment ion at *m*/*z* 314 $[M - H_2O]^+$. This was corroborated by IR absorptions characteristic of hydroxyl, amide, and conjugated ketone functionalities at 3410, 1660, and

Table 2. NMR Spectral Data for Daryamides B (**2**) and C (**3**) in DMSO-d_6

	daryamide B (**2**)		daryamide C (**3**)	
C/H #	δ_H (*J* Hz)	δ_C	δ_H (*J* Hz)	δ_C
1		180.4 C		180.5 C
2		130.9 C		130.9 C
3	7.62 d (2.9)	132.4 CH	7.61 d (2.9)	132.4 CH
4		69.1 C		69.8 C
5	6.93 dd (10.1, 2.9)	154.5 CH	6.93 dd (10.1, 2.9)	154.8 CH
6	6.18 d (10.1)	125.2 CH	6.14 d (10.1)	125.1 CH
7	1.88 m	35.9 CH_2	1.88 m	35.8 CH_2
8	1.96 m	29.7 CH_2	2.01 m	29.6 CH_2
9		173.4 C		173.3 C
1′		164.9 C		164.8 C
2′	6.45 d (15.3)	123.1 CH	6.46 d (15.2)	123.1 CH
3′	7.13 dd (15.3, 10.3)	141.1 CH	7.09 dd (15.3, 10.1)	141.1 CH
4′	6.16 dd (15.3, 10.3)	129.5 CH	6.16 dd (15.3, 10.1)	125.1 CH
5′	6.15 m	142.0 CH	6.13 m	149.3 CH
6′	2.03 m	41.6 CH_2	2.41 m	30.8 CH
7′	1.66 m	27.8 CH	0.99 d (7.0)	21.7 CH_3
8′	0.87 d (6.6)	22.2 CH_3	0.99 d (7.0)	21.7 CH_3
9′	0.87 d (6.6)	22.2 CH_3		
NH-2	9.11 s		9.11 s	
OH-4	5.75 s		5.73 s	
NH_2-9	6.72 br s		6.70 br s	

1690 cm^{-1}, respectively. Detailed analysis of ^1H and ^{13}C NMR data suggested that daryamide B (**2**) had the same carbon skeleton as daryamide A (**1**), but possessed a new olefinic bond at C-5−C-6. This double bond was easily assigned since the signal for H-5 and the methylene pair H_2-6 present in **1** were lost and were replaced by two olefinic protons at $\delta = 6.93$ dd ($J = 10.1, 2.9$ Hz) and $\delta = 6.18$ d ($J = 10.1$ Hz). Similarly, the ^{13}C NMR spectrum showed two new olefinic carbons for C-5 and C-6 at δ 154.5 (CH) and 125.2 (CH). As in **1**, comprehensive analysis of 2D NMR data allowed the full planar structure of **2** to be assigned (Table 2). NOESY NMR spectral data and the observation of typical coupling constants (ca. 15 Hz) confirmed the geometry of double bonds in the 7-methylocta-2,4-dienoic acid amide side chain of daryamide B as 2*E* and 4*E*. The absolute configuration at C-4 was not determined for **2**, but is expected to be identical to that in **1**.

Daryamide C (**3**) was isolated as a dark yellow oil, which analyzed for the molecular formula $C_{17}H_{22}N_2O_4$ by HRESITOFMS analysis (*m*/*z* $[M + Na]^+$ (obsd) 341.1477). In comparison with data from **2**, ^1H NMR, ^{13}C NMR, and mass spectral data revealed the lack of one methylene group in this isomer (Table 2). Furthermore, the disappearance of the multiplet methylene signal at δ 2.03 and the presence of a new multiplet methine signal at δ 2.41 suggested the loss of the methylene unit from the side chain. Our assumption was confirmed by interpretation of $^1H-^1H$ COSY data, which allowed the assembly of the structural fragment $-CH{=}CH-CH{=}CH-CH(CH_3)_2$. As in **1** and **2**, interpretation of $^1H-^1H$ COSY, gHSQC, and gHMBC NMR data allowed all the protons and carbons to be assigned and structure **3** to be defined.

The smaller amide isolated, (2*E*,4*E*)-7-methylocta-2,4-dienoic acid amide (**4**), analyzed for the molecular formula $C_9H_{15}NO$ by HRESITOF mass spectrometry (*m*/*z* 176.1044 $[M + Na]^+$). The proton NMR spectrum of **4** showed four signals for protons at sp^2 carbon atoms, as well as two methyl signals, one methylene signal, and one methine signal. Interpretation of 2D NMR spectral data ($^1H-^1H$ COSY, HSQC, HMBC, and NOESY) led to the assignment of this compound as (2*E*,4*E*)-7-methylocta-2,4-dienoic acid amide (**4**), the side-chain amide component of both **1** and **2**.

Daryamides A−C (**1**−**3**) and (2*E*,4*E*)-7-methylocta-2,4-dienoic acid amide (**4**) were subjected to cytotoxicity evaluation against the human colon carcinoma cell line, HCT-116. Daryamide (**1**) exhibited significantly more potent cancer cell cytotoxicity ($IC_{50} = 3.15$ μg/mL) compared to the other compounds. In addition, compounds **1**−**4** were examined for antimicrobial activities; however only **1** and **2** exhibited very weak antifungal activities (Table 3). None of the compounds showed significant activity against methicillin-resistant *Staphylococcus aureus*.

Table 3. Bioactivities of Compounds **1**−**4** against HCT-116 and *Candida albicans*

compound	HCT-116, IC_{50} (μg/mL)	*C. albicans* MIC (μg/mL)
1	3.15	62.5
2	9.99	125
3	10.03	NSA
4	21.69	NSA

Analysis of the literature revealed that compounds **1**−**4** are related to the manumycins, a class of microbial natural products that show antimicrobial, cytotoxic, and other biological activities. It has been reported that manumycin and its analogues inhibit Ras farnesyl transferase and the growth of *Ki*-ras-activated murine fibrosarcoma in mice, raising the possibility that **1**−**4** have potential as antitumor agents.[7] The manumycin group is a small and discrete class of antibiotics, which includes about 26 secondary metabolites. Manumycin D (**5**) contains two unsaturated carbon chains (upper and lower chain) linked in *meta*-fashion to a unique central multifunctional six-membered ring. Daryamide A has some structural features similar to the type II manumycin antibiotics, possessing the unusual lower carbon chain. The side chains in compounds **1**−**4** appear to be typical polyketide-derived moieties, differing with respect to their combinations of starter and elongation of the acetate units. The central cyclohexene ring may be derived from the polyketide as in the case of manumycins or from some modified amino acids.

Experimental Section

General Experimental Procedures. Melting points were determined on a Mel-Temp apparatus and are uncorrected. Optical rotations were measured using a Rudolph Research Autopol III polarimeter with a 10 cm cell. UV spectra were recorded on a Perkin-Elmer Lambda 19 UV/vis spectrophotometer with a path length of 1 cm. The CD spectra were obtained on a Jasco 810 spectropolarimeter with a path length of 1 cm. IR spectra were acquired on a Perkin-Elmer 1600 series FTIR spectrometer. ^1H, ^{13}C, and 2D NMR spectral data were obtained on Varian Inova 500 MHz and Varian Inova 300 MHz NMR spectrometers. High-resolution mass spectra were recorded on a ThermoFinnigan MAT900XL with an Agilent ESI-TOF at The Scripps Research Institute, La Jolla. Low-resolution LC/MS spectra were obtained on a Hewlett-Packard HP1100 intergrated LC-MS system with a reversed-phase C18 column (Agilent, 100 mm × 4.6 mm, 5 μm) at the flow rate of 0.7 mL/min. Reversed-phase HPLC separations were performed using a semipreparative C18 Altima 5 μm, 60 Å (250 mm × 10 mm) column coupled with a Waters R401 refractive index detector. Preparative HPLC was performed on a Waters 4000 system with a UV variable-wavelength detector, monitoring at 210 nm, using a C18 Nova-Pak 6 μm, 60 Å (300 mm × 40 mm) column.

Bacterial Isolation and Identification. The bacterial strain CNQ-085 was obtained from a marine sediment collected at a depth of ca. 50 m off San Diego, CA. The strain was identified as a member of the genus *Streptomyces* on the basis of a 98.4% 16S rDNA sequence identity (799 base pairs) to the *S. macrosporus* type strain (NCBI accession number AB184617). Strain CNQ-085 also shared high sequence identity with other *Streptomyces* strains (99.1% with NCBI accession number AF492845), including 98.6% with an actinomycete isolated from a marine sponge (NCBI accession number AY944257).

Bacterial Cultivation. *Streptomyces* strain CNQ-085 was cultured at 27 °C for 6 days while shaking at 215 rpm in 9 × 1 L volumes of the liquid medium (A1Bfe) [composed of 10 g of starch, 4 g of yeast extract, 2 g of peptone, 40 mg of $Fe_2(SO_4)_3 \cdot 4H_2O$, 100 mg of KBr, per 1 L of seawater]. Amberlite XAD-7 resin (20 g/L) was added at the end of the fermentation period to adsorb extracellular secondary metabolites. The culture and resin were shaken at 215 rpm for two additional hours. The resin and cell mass were collected by filtration through cheesecloth and washed with DI water to remove salts. The resin, cell mass and cheesecloth were then soaked for 2 h in acetone,

after which the acetone was filtered and dried under vacuum to give 1.2 g of crude material from a 9 L culture.

Purification of Compounds 1−4. The crude extract was fractionated by reversed-phase C18 vacuum liquid chromatography (H_2O/CH_3OH; gradient 90:10 to 0:100%) to give eight fractions. Fraction 4 was then chromatographed by preparative reversed-phase HPLC (Prep Nova-Pak HRC 18, 6 μm, 300 mm × 40 mm) with CH_3CN/H_2O as eluent, followed by semipreparative isocratic HPLC to give daryamides A (**1**, 1.5 mg) and C (**3**, 0.9 mg). Fraction 5 was further purified by gradient reversed-phase HPLC to yield daryamides A (**1**, 2.5 mg) and B (**2**, 3.8 mg) and (2*E*,4*E*)-7-methylocta-2,4-dienoic acid amide (**4**, 3.4 mg).

Daryamide A (1): yellow solid; mp 153−154 °C; $[\alpha]^{25}_D$ −6.9 (0.25, CH_3OH); UV (CH_3OH) λ_{max} (log ϵ) 285 (4.23), 271 (4.22) nm; CD (*c* 4.8 μM, CH_3OH) nm $\Delta_{\epsilon 320}$ +1.45, $\Delta_{\epsilon 265}$ −2.12; IR (neat) ν_{max} 3401, 2930, 1668, 1635, 1615, 1520, 1364, 1191, 1000 801, 606 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) and ^{13}C NMR (125 MHz, DMSO-d_6), see Table 1; ESIMS *m*/*z* 333 $[M - H_2O + H]^+$, 373 $[M + Na]^+$, 723 $[2M + Na]^+$; HRESITOFMS *m*/*z* 373.1735 $[M + Na]^+$ (calcd for $C_{18}H_{26}N_2O_5Na$ 373.1734).

Preparation of Mosher Esters of Daryamide (1). One crystal of dimethylaminopyridine (DMAP) was added to 0.5 mg (1.42 μmol) of compound **1**. One milliliter of freshly distilled pyridine was added to this mixture, and the mixture was stirred at RT for 30 min. Next, 20 μL of (*R*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (5.36 μmol/mL) was added. The resultant reaction mixture was stirred at RT for 90 min under N_2. After removal of solvents under vacuum, the residue was purified by reversed-phase HPLC (Waters Prep LC 4000 system, Dynamax C18, 300 mm × 10 mm, 2.0 mL/min, UV detection at 280 nm) using a gradient solvent system (0−10 min; 40% aqueous CH_3CN, 10−20 min; 70% aqueous CH_3CN, 20−40 min; 90% aqueous CH_3CN, 40−50 min; 100% CH_3CN). The *S*-MTPA ester obtained (**1a**, 0.81 mg) eluted at 45.70 min. The identical procedure was carried out to obtain the *R*-MTPA ester (**1b**, 0.85 mg) with (*S*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride.

5-(*S*-MTPA) ester of daryamide A (1a): ^1H NMR (500 MHz, $CDCl_3$) δ 7.58 (1H, s, H-3), 6.15 (2H, m, H-4′, H-5′), 5.82 (1H, d, J = 15.2 Hz, H-2′), 5.32 (1H, m, 5-H), 4.20 (1H, m, H-7), 3.02 (1H, dd, J = 17.9, 5.7 Hz, 6-H), 2.86 (1H, dd, J = 17.9, 3.6 Hz, 6-H), 2.65 (1H, m, H-8), 2.54 (1H, m, H-8), 2.06 (2H, m, H_2-6′), 1.70 (1H, m, H-7′), 0.90 (6H, d, J = 6.9 Hz, H_3-8′, H_3-9′), 3.44 (3H, s, OCH_3 of MTPA), 7.44 (5H, phenyl-H of MTPA); ESIMS *m*/*z* 549 $[M + H]^+$, 571 $[M + Na]^+$.

5-(*R*-MTPA) ester of daryamide A (1b): ^1H NMR (500 MHz, $CDCl_3$) δ 7.57 (1H, s, H-3), 6.14 (2H, m, H-4′, H-5′), 5.81 (1H, d, J = 14.9 Hz, H-2′), 5.34 (1H, m, 5-H), 4.19 (1H, m, H-7), 3.09 (1H, dd, J = 17.6, 5.9 Hz, 6-H), 2.92 (1H, dd, J = 17.6, 3.5 Hz, 6-H), 2.59 (1H, m, H-8), 2.52 (1H, m, H-8), 2.04 (2H, m, H_2-6′), 1.69 (1H, m, H-7′), 0.88 (6H, d, J = 7.1 Hz, H_3-8′, H_3-9′) 3.43 (3H, s, OCH_3 of MTPA), 7.42 (5H, phenyl-H of MTPA); ESIMS *m*/*z* 549 $[M + H]^+$, 571 $[M + Na]^+$.

Daryamide B (2): dark yellow oil; $[\alpha]^{25}_D$ −42.8 (0.51, CH_3OH); UV (CH_3OH) λ_{max} (log ϵ) 278 (4.78) nm; CD (*c* 1.46 μM, CH_3OH) nm $\Delta_{\epsilon 304}$ −1.67, $\Delta_{\epsilon 278}$ −1.27, $\Delta_{\epsilon 228}$ −3.32; IR (neat) ν_{max} 3410, 2913, 1690, 1660, 1635, 1614, 1533, 1380, 1265, 1205, 1002, 764, 609 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) and ^{13}C NMR (125 MHz, DMSO-d_6), see Table 2; ESIMS *m*/*z* 315 $[M - H_2O + H]^+$, 355 $[M + Na]^+$, 687 $[2M + Na]^+$; HRESITOFMS *m*/*z* 355.1631 $[M + Na]^+$ (calcd for $C_{18}H_{22}N_2O_4Na$ 355.1628).

Daryamide C (3): dark yellow oil; $[\alpha]^{25}_D$ −17.4 (0.075, CH_3OH); UV (CH_3OH) λ_{max} (log ϵ) 273 (4.19) nm; CD (*c* 2.13 μM, CH_3OH) nm $\Delta_{\epsilon 296}$ −0.87, $\Delta_{\epsilon 278}$ −0.69, $\Delta_{\epsilon 226}$ −1.04; IR (neat) ν_{max} 3405, 2920, 1665, 1635, 1615, 1525, 1360, 1190, 1025, 1000, 798, 570 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) and ^{13}C NMR (500 MHz, DMSO-d_6), see Table 2; ESIMS *m*/*z* 341 $[M + Na]^+$, 659 $[2M + Na]^+$; HRESITOFMS *m*/*z* 341.1477 $[M + Na]^+$ (calcd for $C_{17}H_{22}N_2O_4Na$ 341.1472).

(2*E*,4*E*)-7-Methylocta-2,4-dienoic acid amide (4): white solid; UV (CH_3OH) λ_{max} (log ϵ) 262 (4.12) nm; IR (neat) ν_{max} 3405, 2940, 1664, 1604, 1400, 1130, 995, 740, 585 cm^{-1}; ^1H NMR (300 MHz, CD_3CN) δ 7.1 (1H, dd, J = 15.7, 10.4 Hz, H-3), 6.20 (1H, dd, J = 15.7, 10.4 Hz, H-4), 6.12 (1H, dt, J = 15.7, 6.6 Hz, H-5), 5.85 (1H, d, J = 15.7 Hz, 2-H), 2.06 (2H, t, J = 6.6 Hz, 6-H_2), 1.72 (1H, m, H-7), 0.92 (6H, d, J = 6.8 Hz, 8-H_3, 9-H_3); ^{13}C NMR (125 MHz, CD_3CN) δ 167.7 (C, C-1), 141.8 (CH, C-5), 140.9 (CH, C-3), 130.5 (CH, C-4), 122.0 (CH, C-2), 42.0 (CH_2, C-6), 28.3 (CH, C-7), 21.5 (2 CH_3, C-8, C-9); ESIMS *m*/*z* 154 $[M + H]^+$, 176 $[M + Na]^+$; HRESITOFMS *m*/*z* 176.1044 $[M + Na]^+$ (calcd for $C_9H_{15}NONa$ 176.1046).

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Supporting Information Available: The UV, HRESITOFMS, 1D, ^1H, ^{13}C, and 2D NMR spectra of **1**, **2**, and **4** and UV, HRESITOFMS, ^1H, and 2D NMR spectra **3** are available free of charge via the Internet at http://pubs.acs.org.

References and Notes

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