Ammosamides, Biological Chemistry

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The Ammosamides: Structures of Cell Cycle Modulators from a Marine-Derived *Streptomyces* Species**

*Chambers C. Hughes, John B. MacMillan, Susana P. Gaudêncio, Paul R. Jensen, and William Fenical**

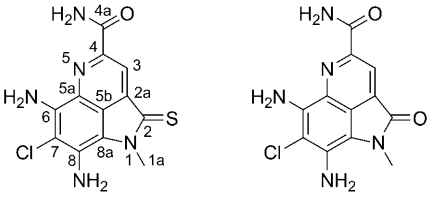
Marine-derived actinomycete bacteria are emerging as a valuable resource for bioactive natural products encompassing a variety of unique structural classes.[1] In our hands, early detection of cell growth inhibitors using in vitro cytotoxicity assays against the colon carcinoma cancer cell line HCT-116, followed by extensive mechanism of action studies, has proven to be an effective approach. As such, the HCT-116 assay has been instrumental in the identification of potentially important anticancer agents.[2]

In the course of our continued studies, *Streptomyces* strain CNR-698[3] was isolated from bottom sediments collected at a depth of 1618 meters in the Bahamas Islands in 2003. Cytotoxicity-guided (HCT-116) fractionation by C_{18} flash chromatography and RP-HPLC of crude extract led to the isolation of ammosamides A (**1**) and B (**2**) as blue and red solids, respectively (3 and 4 mg L^{-1}). Structure assignments for **1** and **2** proved to be particularly difficult due to their inherent insolubility (soluble only in dimethyl sulfoxide (DMSO)) and a lack of descriptive NMR signals, ultimately requiring the integration of NMR spectral analysis, mass spectrometry data, and single crystal X-ray diffraction studies.

High-resolution (ESI) mass spectrometric analysis of ammosamide A (**1**) indicated a molecular formula $C_{12}H_{10}{}^{35}ClN_5OS$ (*m*/*z* $[M+H]^+$: 308.0303]. The molecular weight of ammosamide B (**2**) was found to be 16 amu lower (*m*/*z* $[M+H]^+$: 292.0604) consistent with the molecular formula $C_{12}H_{10}{}^{35}ClN_5O_2$. The UV/Vis spectrum of **1** was indicative of an unusually highly conjugated structure with absorptions at $\lambda_{max} = 580$, 430, 350, and 290 nm.

Inspection of the ^{1}H NMR spectrum of **1** in [D_6]DMSO revealed six singlets between $\delta = 6.0$ and 9.0 ppm and one methyl singlet at $\delta = 4.03$ ppm, while the ^{13}C NMR spectra revealed the presence of eleven sp^2 hybridized carbon atoms and a single sp^3 hybridized carbon atom at $\delta_c = 33.3$ ppm (Table 1). The addition of D_2O (20 μL) to the sample in [D_6]DMSO resulted in the immediate disappearance of

Table 1: NMR spectral data for **1** and **2** ([D_6]DMSO).



1 **2**

Position	Ammosamide A (**1**) δ_C[a,c]	δ_H[d]	HMBC	Ammosamide B (**2**) δ_C[b,c]	δ_H[d]
1a	33.3	4.03 (s)	C-2, C-8a	28.5	3.59 (s)
2	177.2			164.0	
3	116.5	8.47 (s)	C-2, C-4 C-5b, C-4a	115.3	8.34 (s)
4	144.6			144.7	
4a	166.0			166.2	
5b	119.7			119.0	
7	103.1			104.5	
8a	110.5			106.3	
$CONH_2$		7.68 (brs)	C-4		7.66 (brs)
		8.92 (brs)	C-4a		8.91 (brs)
NH_2 (C-6)		7.16 (brs)[e]	C-7		6.73 (brs)[f]
		6.63 (s)[e]			
NH_2 (C-8)		6.89 (s)	C-7, C-8a		6.18 (brs)[f]

[a] 75 MHz. [b] 125 MHz. [c] C-2a, C-5a, C-6, and C-8 (**1**: $\delta_C = 132.6$, 134.7, 136.8, 142.7 ppm; **2**: $\delta_C = 130.6$, 130.8, 132.4, 140.5 ppm) could not be unambiguously assigned. [d] 600 MHz. [e] At certain concentrations, these two one-proton signals coalesce to a two-proton signal at $\delta = 7.09$ ppm. [f] Assigned by analogy to **1**.

^{1}H NMR signals at $\delta = 7.16$ (1H), 6.63 (1H), 6.89 ppm (2H) and the slower disappearance of singlets at $\delta = 8.92$ (1H), and 7.68 ppm (1H) (less than 10 min). The exchangeable protons at $\delta = 7.16$, 6.63 and 6.89 ppm were assigned as aromatic amines at C-6 and C-8 (based on HMBC correlations), while the slowly exchanging protons at $\delta = 8.92$ and 7.68 ppm were assigned to a primary amide on the basis of COSY and HMBC correlations. The only non-exchangeable hydrogen atoms were the methyl singlet resonance at $\delta = 4.03$ ppm and a one-proton singlet at $\delta = 8.47$ ppm. The ^{13}C NMR spectrum of **1** indicated the presence of two carbonyl groups ($\delta_c = 177.2$ and 166.0 ppm), as well as two upfield sp^2 carbon atoms ($\delta_c =$ 103.1 and 110.5 ppm). HMBC correlations between the downfield carbonyl ($\delta_c = 177.2$ ppm) and the proton methyl

[*] Dr. C. C. Hughes, Dr. J. B. MacMillan, Dr. S. P. Gaudêncio, Dr. P. R. Jensen, Prof. W. Fenical
Center for Marine Biotechnology and Biomedicine, Scripps Institution of Oceanography, University of California at San Diego
9500 Gilman Drive, La Jolla, CA 92093-0204 (USA)
Fax: (+1) 858-534-1318
E-mail: wfenical@ucsd.edu

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singlet at $\delta = 4.03$ ppm, we thought, defined an *N*-methyl amide, although a carbon chemical shift so far downfield would not be expected. In addition to correlations from the aromatic $\delta = 8.47$ ppm singlet, the only other HMBC correlations were from the exchangeable protons at $\delta = 7.16/6.63$ ppm to C-7 ($\delta_c = 103.1$ ppm) and from $\delta = 6.89$ ppm to C-7 and C-8a ($\delta_c = 110.5$ ppm).

The spectral data for **1** suggested a highly unsaturated aza-aromatic metabolite possessing three rings. However, the lack of definitive NMR assignments that could be used to link these features forced us to concentrate efforts toward obtaining an X-ray crystal structure. We were fortunate to obtain small crystals of **1** by the slow diffusion of H_2O into a saturated solution in DMSO.[4] The X-ray assignment of ammosamide A (**1**) is shown in Figure 1. Once X-ray data became clear, the spectral data for **1** could be assigned.

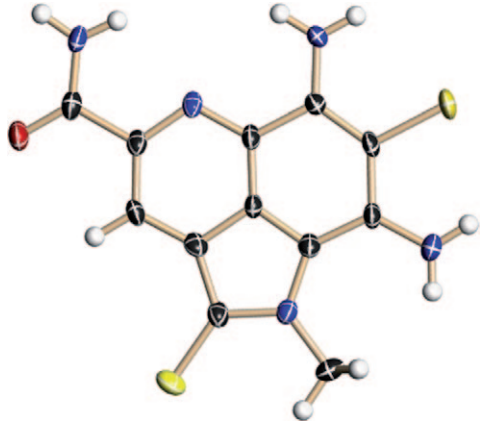
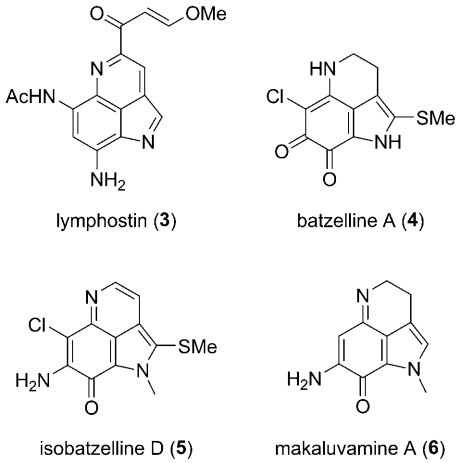


Figure 1. X-ray crystal structure of ammosamide A (**1**). Red O, blue N, yellow Cl, black C, white H.

The structure assignment of ammosamide B (**2**) followed from analysis of spectral data and chemical interconversion. Comparison of the C-2 carbonyl chemical shifts in **1** ($\delta_C = 177.2$ ppm) and **2** ($\delta_C = 164.0$ ppm) revealed a difference of 13 ppm, consistent with the typical ^{13}C chemical shift difference between a carbonyl and a thiocarbonyl (ca. 20 ppm).[5] In order to chemically confirm the presence of the thiolactam functionality, we used Lawesson's reagent [2,4-bis(*p*-methoxyphenyl)-1,3-dithiadiphosphetane-2,4-disulfide] to convert lactam **2** into thiolactam **1**.[6] The low yield of this reaction is likely attributable the nucleophilic amines in **2**.[7] Exposed to air during storage, **1** was gradually converted to ammosamide B (**2**). Notably, the transformation could also be accomplished in 10 min, upon treatment of **1** with hydrogen peroxide in aqueous methanol.[8] This reactivity has been previously observed in other thioamide-containing compounds.[9]

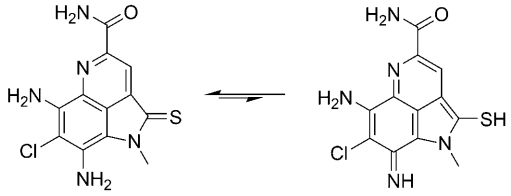
The structural similarities between the ammosamides and the microbial product lymphostin (**3**) are clear,[10] as is the relationship of the ammosamides to several sponge-derived pyrroloiminoquinone natural products, including batzelline A (**4**),[11a] isobatzelline D (**5**),[11b] and makaluvamine A (**6**)[11c] (Scheme 1). The sponge metabolites **4**–**6** possess different



Scheme 1. Related metabolites from bacteria and sponges.

patterns of Cl and NH_2 substitution and assume *p*-iminoquinone and *o*-quinone structures. The presence of an amino group at C-8 in the ammosamides results in a fundamentally different structure type in which the quinoline tautomer predominates. The pyrrole moiety in **3**–**6** is uniquely oxidized to the pyrrolidinone in ammosamide B (**2**). Finally, though methyl sulfides are present in **4** and **5**, ammosamide A (**1**) is the first natural product to contain a thio-γ-lactam functionality.[12]

The fact that the ammosamides are highly colored (**1**: $\lambda_{max} = 580$ nm; **2**: $\lambda_{max} = 530$ nm), yet lack quinone or iminoquinone functionalities, leads to speculation about the electronic character and reactivity of these metabolites. The intense colors of these compounds could reflect a strong charge separation between the two six-membered aromatic rings due to the effects of electron-donating groups on the chlorine-containing ring and electron-withdrawing substituents on the pyridine ring. It is, conceptually, also explained by the potential for ammosamide A to exist in an equilibrium with its bis-iminoquinone tautomer (Scheme 2). Furthermore,



Scheme 2. Possible tautomeric form of ammosamide A (**1**).

in **1** and **2** the chlorine atom at C-7 is poised to engage in nucleophilic aromatic substitution with a suitable nucleophile, particularly when the molecule exists as its bis-iminoquinone tautomer.[13] This reactivity may be relevant to the molecule's interaction with its protein target.[14]

Ammosamides A (**1**) and B (**2**) exhibited significant in vitro cytotoxicity against HCT-116 colon carcinoma, each with $IC_{50} = 320$ nM. These compounds also demonstrated

pronounced selectivity in a diversity of cancer cell lines with values ranging from 20 nM to 1 μM, indicating a specific target mechanism of action. To explore the intracellular target of the ammosamides, ammosamide B (**2**) was converted to a highly fluorescent molecule by conjugation.[14] Treatment of HCT-116 colon carcinoma or HeLa cells with this fluorescent molecule produced immediate and irreversible labeling of a specific protein in the cellular cytosol. Using a cell and molecular biology approach, the target of the ammosamides was identified as a member of the myosin family, important cellular proteins that are involved in numerous cell processes, including cell cycle regulation, cytokinesis, and cell migration.

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[4] X-ray measurements were made on a SMART CCD area detector with graphite monochromated $Mo_{K\alpha}$ radiation ($\lambda = 1.54178$ Å). **1** ($C_{12}H_{10}ClN_5OS$): crystal dimensions $0.30 \times 0.20 \times 0.10$ mm, monoclinic, space group $P2_1/c$, $a = 9.3766(8)$, $b = 18.2930(12)$, $c = 7.0462(6)$ Å, $V = 1208.55(17)$ Å^3, $Z = 4$, $\rho_{calcd} = 1.691$ mg m^{-3}, $\mu = 4.456$ mm^{-1}, $T = 100(2)$ K, $2\theta_{max} = 136.3°$, 7060 measured reflections, 1872 independent reflections ($R_{int} = 0.0399$), 183 parameters refined, $R = 0.0541$ (for 4205 reflections with $I > 2.00\sigma(I)$), $R_w = 0.1523$, max/min residual peaks in the final difference map 0.495/−0.817 e Å^{-3}. CCDC 694293 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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