J|A|C|S COMMUNICATIONS

Published on Web 02/04/2010

Total Synthesis of the Ammosamides

Chambers C. Hughes and William Fenical*

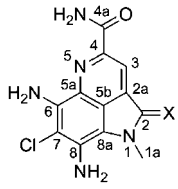
Center for Marine Biotechnology and Biomedicine, Scripps Institution of Oceanography, University of California, San Diego, La Jolla, California 92093-0204

Received December 17, 2009; E-mail: wfenical@ucsd.edu

The secondary metabolites of actinomycetes often display exceptional biological activities.[1] These genetically encoded small molecules, which were subjected to evolutionary pressures over millions of years, generally possess high affinities for their protein targets. Our studies of marine actinomycetes have focused on those strains isolated from deep-ocean sediments.[2]

The ammosamides A−B (**1**−**2**) are chlorinated alkaloids characterized by a dense array of heteroatoms (N,O,S) and a relative paucity of hydrogen atoms (Chart 1). The molecules are related to the pyrroloiminoquinone class of natural products,[3] although they exhibit several distinct features. The isolation and structural elucidation of ammosamides A and B (**1**, **2**) from a marine sediment-derived *Streptomyces* strain CNR-698 was described in a previous communication by our group.[4] In a back to back communication, we also showed that the metabolites target the cellular cytokinetic protein myosin.[5] Few natural products have been demonstrated to target this protein.[6]

Chart 1. Ammosamides A (**1**) and B (**2**)



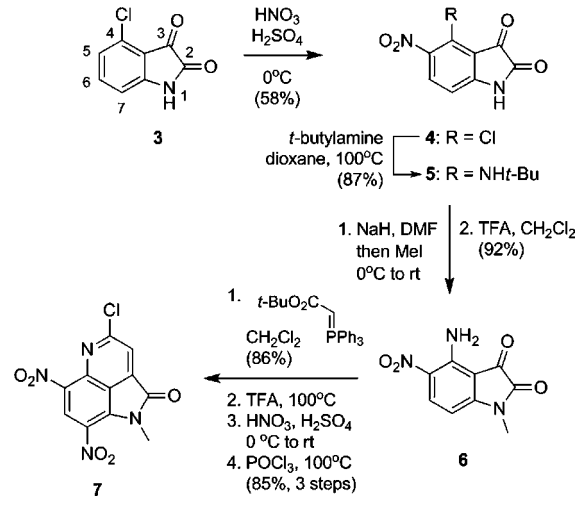
1: ammosamide A (X = S)
2: ammosamide B (X = O)

The pronounced bioactivity of the ammosamides, derived from a disruption in cellular cytokinesis, encouraged us to undertake a total synthesis of this unique class of metabolites. An examination of synthetic analogues with different functional groups on the pyrroloquinoline core, we thought, might provide a molecule with increased potency and/or overall improved properties. Although interest in related pyrroloiminoquinones culminated in several total syntheses,[7] none of the reported routes was directly amenable to the ammosamide class.

We envisioned a total synthesis commencing from 4-chloroisatin (**3**), itself derived from reaction of 3-chloroaniline with chloral hydrate (Scheme 1).[8] The synthesis was designed with several key principles in mind, namely that (1) the aromatic amines, to which we ascribed much of the natural products' hydrophilicity and lack of solubility, would be disguised as nitro groups; (2) the C-4 chlorine substituent in **3** would be displaced by ammonia (or its equivalent) in a nucleophilic aromatic substitution reaction; (3) the C-3 carbonyl of **3** would be elongated at a later stage through Wittig olefination; (4) the C-7 chlorine substituent would be installed late in the synthesis. A late-stage chlorination would mean that the synthesis could be adapted to provide deschloro derivatives or derivatives with bromine or iodine at C-7. Since comparable activity was observed for ammosamides A (**1**) and B (**2**), we pursued a synthesis specifically of **2** to avoid manipulations with the thiolactam in **1**. We had shown earlier that **2** could be converted to **1** using Lawesson's reagent.[4]

Isatin **3** was first mononitrated at 0 °C to give **4**. The position of the nitro group at C-5 was determined by X-ray crystallographic analysis of *N*-methyl **4**.[9] Nitroisatin **4** was treated with *t*-butylamine in dioxane at elevated temperatures to produce **5** in 87% yield. *N*-methylation of this material was followed by acidic deprotection of the C-4 amine to give **6**. The quinoline ring was then constructed via olefination with *t*-butyl (triphenylphosphoranylidene) acetate and acidic deprotection/ condensation. The Wittig reagent was added over a 3 h period using a syringe pump, minimizing the formation of phosphorus-containing byproducts that are derived from conjugate addition of excess reagent to the intermediate enone.[10,11] The quinoline scaffold was selectively nitrated at C-7, and the C-2 alcohol was then converted to the chloride to give **7** in 34% yield from **3**.

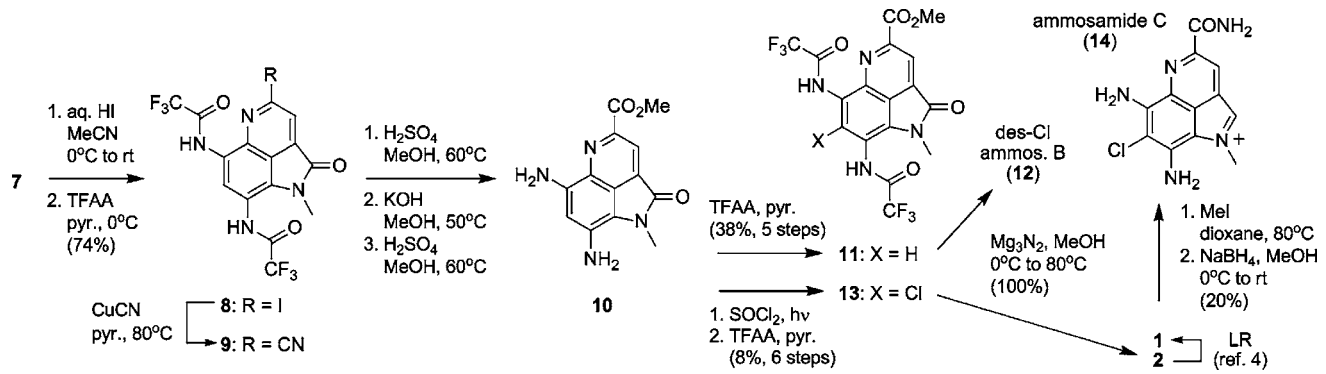
Scheme 1. Synthesis of the Ammosamide Core



Treatment of **7** with hydroiodic acid led to reduction of the nitro groups to amines and substitution at C-4 with iodide (Scheme 2). This compound (not shown), and subsequent diamino intermediates, adopted physical characteristics similar to the ammosamides. Its solubility in organic solvents was severely limited, purification with silica gel chromatography became unmanageable, and the material assumed a dark purple color (λ_{max} = 520 nm). Thus, the iodide was directly converted to **8** upon treatment with trifluoroacetic anhydride. Iodide **8** was then transformed into the corresponding C-4 nitrile employing copper(I) cyanide. Conversion of the aromatic amines to trifluoroacetamides improved yields and allowed for purification by silica gel chromatography. The amines were subsequently deprotected under acidic conditions. Ester **10** was

10.1021/ja9106572

Scheme 2. Total Synthesis of Ammosamides A (**1**), B (**2**), C (**14**) and Deschloro Ammosamide B (**12**)



obtained by conversion of the nitrile to the imidate with methanolic KOH and subsequent acidic esterification.

Deschloro ammosamide B (**12**) was prepared after installation of the final nitrogen atom. First, ester **10** was converted to **11** to facilitate silica gel purification. Deprotection and conversion to the C-4 amide resulted from treatment with magnesium nitride.[12] The ^{1}H NMR spectrum of **12** was similar to that of ammosamide B (**2**) except for the additional C-7 signal at 6.16 ppm.

Ammosamide B (**2**) was also synthesized from ester **10** after chlorination in neat thionyl chloride under UV light (254 nm).[13] The reaction represents the first example of the chlorination of an unsubstituted 1,3-dianiline. Synthetic **2**, indistinguishable from natural ammosamide B (LC/MS, NMR, HRMS), was obtained after trifluoroacetylation to **13** and amidation.

Though their biological properties are comparable, there is a dramatic difference in the stability of natural ammosamide B (**2**) and synthetic deschloro ammosamide B (**12**). Exposed to air and light, **12** readily degraded within a week via a nitroso intermediate.[14] Natural **2**, however, could be stored in this way for much longer (degradation $t_{1/2} \approx 1$ month). The exact role of the biosynthetic chlorination may be to confer stability toward oxidative degradation.

In an effort to generate additional analogues for structure−activity studies, several semisynthetic derivatives of the ammosamides were prepared. In one case, conversion of ammosamide A (**1**) to quinolinium **14** was accomplished via *S*-methylation and reduction with sodium borohydride (see Scheme 1). The conspicuous UV profile of this derivative was recognized later, in very low titer, in the LC/MS chromatogram of the crude CNR-698 extract. The structure of a third member of the ammosamide class, ammosamide C (**14**), was thus elucidated. Metabolite **14** is likely a biosynthetic precursor to **1** and **2**, the latter species arising from nucleophilic oxidation or sulfuration at C-2.

The ammosamides are one of the first classes of natural products known to target myosin.[6] Our synthesis of the ammosamides has provided new analogues with which to assess the role of each functional group on the pyrroloquinoline core. Based on observations of the deschloro compound, halogenation of the 1,3-diamino moiety helps protect the natural product from oxidative degradation. Studies detailing the bioactivity of each synthetic compound will soon be disclosed.

Acknowledgment. Financial support was provided by the National Cancer Institute, NIH (under grant R37 CA44848). We thank Phil S. Baran (The Scripps Research Institute) for indispensable advice, and Drs. Arnold L. Rheingold and Antonio G. D'Pasquale (UCSD) for assistance with X-ray diffraction experiments.

Supporting Information Available: Details of the isolation and spectral data for **14**. Crystallographic data for *N*-methyl **4** (CCDC 756061) in CIF format. HRMS data, proton, and carbon NMR spectra for selected intermediates. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Cragg, G. M.; Grothaus, P. G.; Newman, D. J. *Chem. Rev.* **2009**, *109*, 3012–3043.

(2) (a) Fenical, W.; Jensen, P. R. *Nat. Chem. Biol.* **2006**, *2*, 666–673, and references therein. (b) Hughes, C. C.; Prieto-Davó, A.; Jensen, P. R.; Fenical, W. *Org. Lett.* **2008**, *10*, 629–631. (c) Kwon, H. C.; Kauffman, C. A.; Jensen, P. R.; Fenical, W. *J. Org. Chem.* **2009**, *74*, 675–684.

(3) For a review: Antunes, E. M.; Copp, B. R.; Davies-Coleman, M. T.; Samaai, T. *Nat. Prod. Rep.* **2005**, *22*, 62–72.

(4) Hughes, C. C.; MacMillan, J. B.; Gaudêncio, S. P.; Jensen, P. R.; Fenical, W. *Angew. Chem., Int. Ed.* **2009**, *48*, 725–727.

(5) Hughes, C. C.; MacMillan, J. B.; Gaudêncio, S. P.; Fenical, W.; La Clair, J. J. *Angew. Chem., Int. Ed.* **2009**, *48*, 728–732.

(6) The marine natural product pentabromopseudilin also inhibits myosin motor activity: Fedorov, R.; Böhl, M.; Tsiavaliaris, G.; Hartmann, F. K.; Taft, M. H.; Baruch, P.; Brenner, B.; Martin, R.; Knölker, H.-J.; Gutzeit, H. O.; Manstein, D. *J. Nat. Struct. Biol.* **2009**, *16*, 80–88.

(7) For example: (a) Peat, A. J.; Buchwald, S. L. *J. Am. Chem. Soc.* **1996**, *118*, 1028–1030. (b) Roberts, D.; Joule, J. A.; Bros, M. A.; Alvarez, M. *J. Org. Chem.* **1997**, *62*, 568–577. For the first and only synthesis of lymphostin: (c) Tatsuta, K.; Imamura, K.; Itoh, S.; Kasai, S. *Tetrahedron Lett.* **2004**, *45*, 2847–2850.

(8) Sandmeyer, T. *Helv. Chim. Acta* **1919**, *2*, 234–242.

(9) Material for X-ray analysis was prepared via nitration of *N*-methyl-4-chloroisatin. This isatin derivative was identical (^{1}H NMR, LC/MS) to the product resulting from methylation of **4**.

(10) This reactivity has been observed with other electron-deficient enones: (a) Faber, K.; Stueckler, H.; Kappe, T. *J. Heterocycl. Chem.* **1984**, *21*, 1177–1181. (b) Augustin, M.; Jeschke, P. *J. Prakt. Chem.* **1987**, *329*, 637–648.

(11) With 4-amino-1-methyl-5,7-dinitroisatin, formation of the undesirable phosphocycle [LRESIMS: *m/z* (MH^+) = 741] was predominant.

(12) Veitch, G. E.; Bridgwood, K. L.; Ley, S. V. *Org. Lett.* **2008**, *10*, 3623–3625. Concerning the potential for magnesium nitride to explode: Crane, S. *Chem. and Engineer. News* **2009**, *87*, 2–4.

(13) Thionyl chloride is reduced to sulfur monoxide. For similar examples: (a) Kim, D. H.; Santilli, A. A. *Tetrahedron Lett.* **1971**, *12*, 2441–2442. (b) Soidinsalo, O.; Wähälä, K. *Phosphorus, Sulfur, Silicon* **2007**, *182*, 2761–2767.

(14) The difference in stability between a 2-halo-1,3-nitrosoanilines and their nonhalogenated analogues has been noted: Frumkin, A. E.; Churakov, A. M.; Strelenko, Y. A.; Smirnov; Tartakovsky, V. A. *Russ. Chem. Bull.* **1999**, *48*, 2103–2107.

JA9106572