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Efficient Total Synthesis of Ammosamide B

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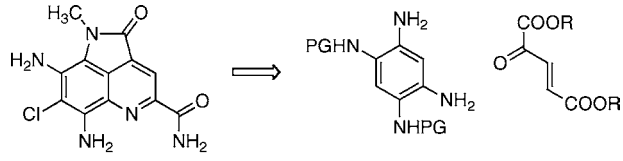
ABSTRACT



Ammosamide B

A total synthesis of ammosamide B, a metabolite of the marine-derived *Streptomyces* strain CNR-698, has been executed in nine steps and 6.9% overall yield. The key step involves the condensation of a 4,6-diBoc-protected 1,3,4,6-tetraaminobenzene derivative with dimethyl 2-ketoglutaconate, which effectively constructs the pyrrolidinone ring and the quinoline ring in a single step. This contributes a unique approach to the synthesis of pyrroloquinoline alkaloids that offers the advantages of brevity and relatively high overall yield.

The ammosamides A−C (**1**−**3**, Figure 1) are pyrroloquinoline natural products isolated from the marine *Streptomyces* strain CNR-698.[1−3] Interest in the ammosamides has been stimulated by their cytotoxicities in cancer cell cultures, as well as their abilities to influence tubulin and actin dynamics through myosin targeting.[2,4] More specifically, microtubule depolymerization and an increase in actin filaments were observed after administration of a fluorescent ammosamide B conjugate to HCT-116 cells, and histological staining suggested that the conjugate bound to several myosin families.[4]

The ammosamides and structurally related alkaloids such as lymphostin (**4**)[5] and the related pyrroloiminoquinone alkaloids,[6] including isobatzelline D (**5**),[7] present synthetically challenging, densely packed arrays of functional groups.

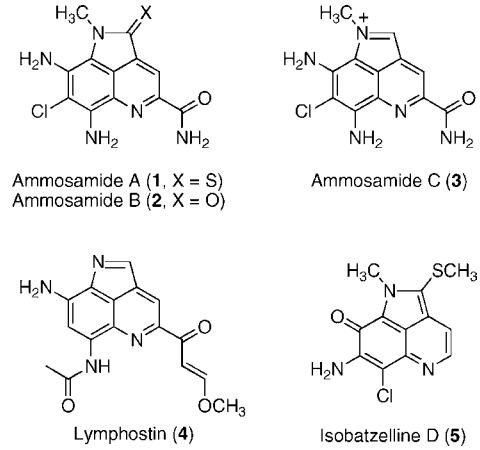


Figure 1. Ammosamides A−C and related pyrroloquinolines.

Syntheses of these compounds have generally involved first construction of quinoline systems followed by elaboration of the pyrrole derivative,[8,9] or they have started from indole derivatives followed by construction of the quinoline

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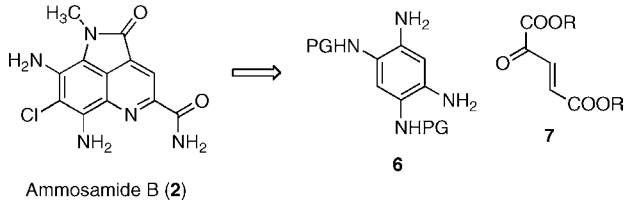
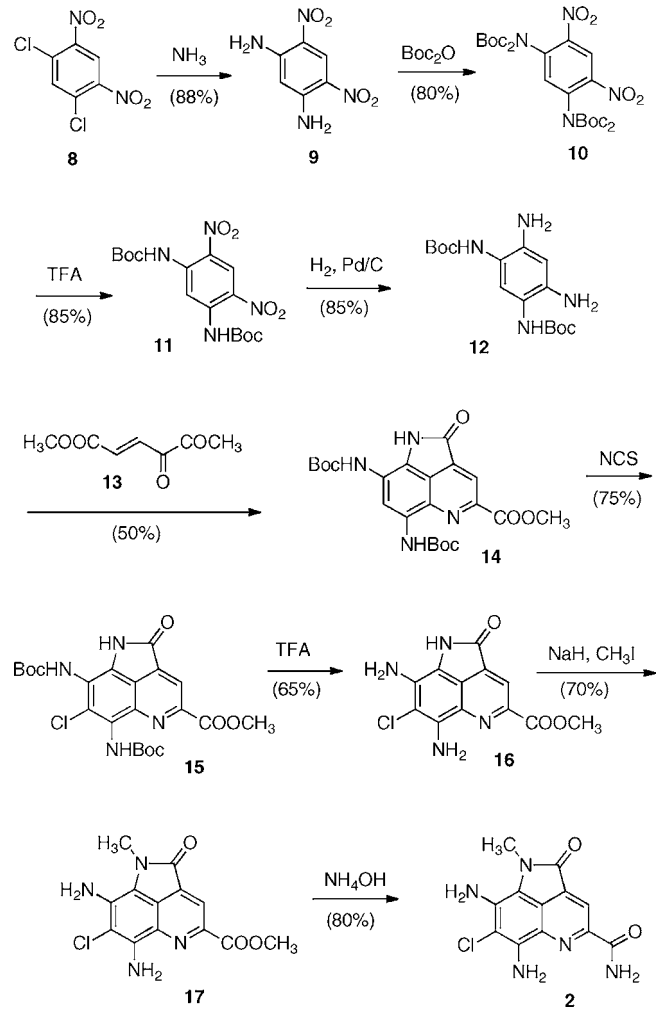
Scheme 1. Retrosynthetic Analysis of Ammosamide B



Scheme 2. Total Synthesis of Ammosamide B



rings.[3,10] To date, only one synthesis of the ammosamides has been reported, which produced ammosamide B in 17 steps from 4-chloroisatin in an overall yield of 2.7%.[3] In the case of lymphostin, the synthesis proceeds in 21 steps with an overall yield of 2.0%.[10]

The present ammosamide B synthesis was predicated on the hypothesis that the pyrroloquinoline ring system could proceed with construction of both the pyrrole and the quinoline rings in a single step through condensation of a symmetrical, diprotected 1,3,4,6-tetraaminobenzene **6** with a diester of 2-ketoglutaconic acid (a variation of the Skraup−Doebner−Von Miller quinoline synthesis) (Scheme 1). If successful, this would constitute a fundamentally different approach to the synthesis of pyrroloquinoline alkaloids that could conceivably offer advantages in terms of overall yield and the number of steps involved. It would also constitute a formal synthesis of ammosamides A and C, which have been prepared from ammosamide B.[3]

Investigation of the strategy outlined in Scheme 1 eventually resulted in the total synthesis of ammosamide B as outlined in Scheme 2. Subjection of the commercially available starting material **8** using ammonia in ethylene glycol at 140 °C for 3 h afforded the expected diamine **9** as previously reported.[11] Treatment of intermediate **9** with 5 equiv of Boc_2O and DMAP in DMF at room temperature for 12 h afforded the tetra-Boc compound **10** in 80% yield along with the undesired tri-Boc product in 10% yield, which were separated chromatographically and characterized. Deprotection of the tetra-Boc intermediate **10** with TFA in methylene chloride at 0 °C for 4 h provided the desired di-Boc substance **11**. Catalytic reduction of the dinitro compound **11** over Pd/C in ethyl acetate at room temperature for 12 h produced the diamine **12**. Condensation of intermediate **12** with dimethyl 2-ketoglutaconic acid in the presence of PTSA and $Cu(OAc)_2$ in refluxing chloroform for 8 h, with the mixture open to air, provided the pyrroloquinoline system **14** in the lowest yield of the synthesis (50%).[12] Chlorination of intermediate **14** with *N*-chlorosuccinimide occurred regioselectively at 60 °C in DMF over a period of 30 min to afford compound **15**. Removal of the two Boc protecting groups from **15** was carried out with TFA at room temperature for 6 h, producing the diamine **16** as a highly polar compound displaying the intense purple color of ammosamide B. Deprotonation of the lactam **16** with 1.2 equiv of sodium hydride in DMF for 30 min provided the anion, which was regioselectively alkylated using 1.5 equiv of methyl iodide to afford the methylated lactam **17**, the penultimate intermediate of the synthesis. The conversion of **17** to ammosamide B (**2**) was readily carried out in the presence of aq ammonium hydroxide in THF at room temperature for 24 h.[13] The spectroscopic and chromatographic properties of synthetic ammosamide B were identical to those of an authentic sample of the natural product that was kindly provided by Professor Fenical.

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Several aspects of the synthesis deserve further comment: (1) The condensation of intermediate **9** with dimethyl 2-ketoglutaconate (**13**) was tried without success, presumably because of the reduced nucleophilicities of the two amines resulting from the presence of the two electron-withdrawing nitro groups.[12] (2) The overall yield for formation of intermediate **11** from **9** could likely be improved by using the crude, unseparated mixture of **10** and the tri-Boc side product instead of pure **10**. (3) The di-Boc protection of **9** to yield **11** directly was tried by limiting the number of equivalents of Boc_2O, but undesired mixtures of products were always obtained. (4) The methylation of the sodium anion derived from **16** had to be carefully monitored by TLC and the reaction time limited in order to avoid undesired methylation of the amino groups. (5) Several attempts were made to prepare 1,2,4,5-tetraaminobenzene from **9** and employ it as a potential intermediate, but unfortunately, it proved to be too unstable to be of any practical value.

The ammosamide synthesis outlined in Scheme 2 proceeds in nine simple steps from a commercially available starting material, with an overall yield of 6.9%. The general approach does therefore offer certain advantages over the previous synthesis of the ammosamides[3] and related pyrroloquinoline natural products. The synthesis appears to be general, and it should allow access to the preparation of a variety of structural analogues for investigation of pharmacological structure−activity relationships. There is general interest in the design and synthesis of myosin inhibitors because of the possibility that fine-tuning their structures might allow the preparation of compounds that could selectively target only one myosin as opposed to general targeting of multiple myosin families. This could possibly provide an effective method for defining the roles of selected myosins in cytoskeletal dynamics and structuring.

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Supporting Information Available: Complete experimental section describing the preparation and characterization of all synthetic intermediates and ammosamide B, as well as a complete set of ^{1}H and ^{13}C NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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