Ammosamides

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Treasures from the Sea: Discovery and Total Synthesis of Ammosamides

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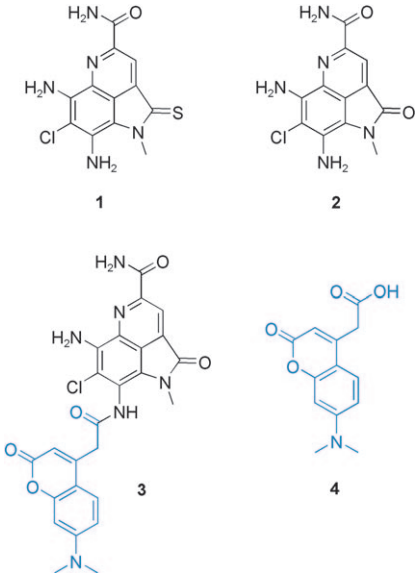
Marine organisms are highly prolific sources of biologically active metabolites, many of which have assumed key roles in the elucidation of cellular mechanisms or as lead structures for drug discovery.[1,2] Such marine natural products are often characterized by highly complex, unique molecular architectures with a multitude of stereocenters; however, potent biological activity is also found for compounds that are based on simpler, and sometimes even achiral, scaffolds. A prime example of this latter group of marine metabolites are ammosamides A (**1**) and B (**2**), whose first total synthesis was reported very recently by Hughes and Fenical.[3] The research group of Fenical is also to be credited with the discovery of **1** and **2**, which were obtained from the marine streptomyces strain CNR-698 that was isolated from oceanic bottom sediments collected at a depth of more than 1600 m.[4] Ammosamides belong to the pyrroloiminoquinone class of natural products,[5] and it is worth noting that ammosamide A (**1**) is the first natural product ever isolated to contain a thio-γ-lactam ring.[4,6] Although the structures of **1** and **2** are less complex than what one generally tends to associate with marine natural products, they still pose a formidable challenge for total synthesis, because of the dense functionalization of the aromatic core, and also because of their very poor solubility in organic solvents.[3] Hughes and Fenical have been able to meet these challenges, and the key features of their approach will be highlighted below. Before doing so, however, we shall briefly discuss the biological effects associated with **1** and **2**, as these have provided a major impetus for the total synthesis work as a first step towards structure–activity relationship (SAR) studies and the design of improved analogues.

Both **1** and **2** have been reported to be potent inhibitors of human cancer cell growth in vitro, with IC_{50} values between 20 nM and 1 μM, although specific data are only available for



the HCT-116 colon carcinoma cell line (IC_{50} = 320 nM for both **1** and **2**).[7] In the search of the molecular target(s) underlying the antiproliferative activity of ammosamides, La Clair, Fenical, and co-workers have prepared the fluorescent ammosamide B derivative **3**,[7] whose dimethylaminocoumarin fluorophore not only serves as a spectroscopic marker, but also as an epitope for binding to a monoclonal antibody (mAb). To highlight this duality, **3** and related conjugates have been referred to as "immunoaffinity fluorescence" (IAF) probes.[8] La Clair and co-workers had previously shown that the use of such IAF probes enabled the isolation of biologically active natural products from complex extract mixtures and also the subsequent determination of their molecular target by using co-immunoprecipitation (co-IP) techniques.[8] Although the IAF probe **3** showed a 50-fold reduced antiproliferative activity relative to **2** (**3**: IC_{50} (HCT-116) = 17 μM), it was still deemed to be a relevant "bait" for target fishing, especially as the compound showed pronounced arresting effects on the cell cycle in HCT-116 cells.[7] Remarkably, treatment of HeLa, PC-3, or HCT-116 cells with 1 mL of a 50 μM solution of **3** resulted in complete cellular uptake of the fluorescence within minutes; initially **3** was localized in the cytoplasm, while it was vesiculated in lysosomes after 12 h.

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The incubation of cell lysates of HCT-116 cells with **3** followed by co-IP with Affigel Hz containing the anti-dye XRI-TF35 mAb, extensive washing of the resin, elution of bound protein by treatment of the resin with 7-dimethylaminocoumarin acetic acid (**4**), and LC/MS/MS analysis of the eluted material revealed a protein similar to those of the myosin family (Figure 1). Myosin as a cellular target of **3** was

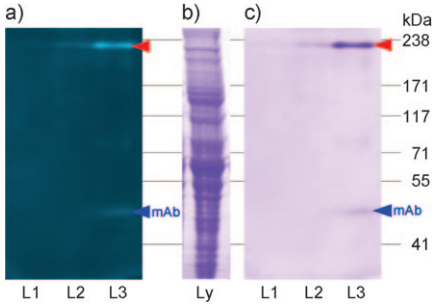


Figure 1. Co-immunoprecipitation with IAF probe **3**. a) SDS-PAGE gel showing the fluorescent bands arising from co-IP of an HCT-116 lysate with **3** and Affigel Hz resin containing XRI-TF35 mAb. After incubation for 12 h and multiple washing with phosphate-buffered saline (PBS) at 4 °C, the bound protein was eluted from the XRI-TF35-Affigel Hz resin with 0.1 M Tris-Cl pH 6.8 (L1), 5 μM **4** in 0.1 M Tris-Cl pH 6.8 (L2), or 50 μM **4** in 0.1 M Tris-Cl pH 6.8 (L3) at 23 °C. b) HCT-116 lysate stained with GelCode blue. c) GelCode blue staining of the gel in (a). Reproduced from Ref. [7]. SDS-PAGE = sodium dodecylsulfate polyacrylamide gel electrophoresis, Tris = tris(hydroxymethyl)aminomethane.

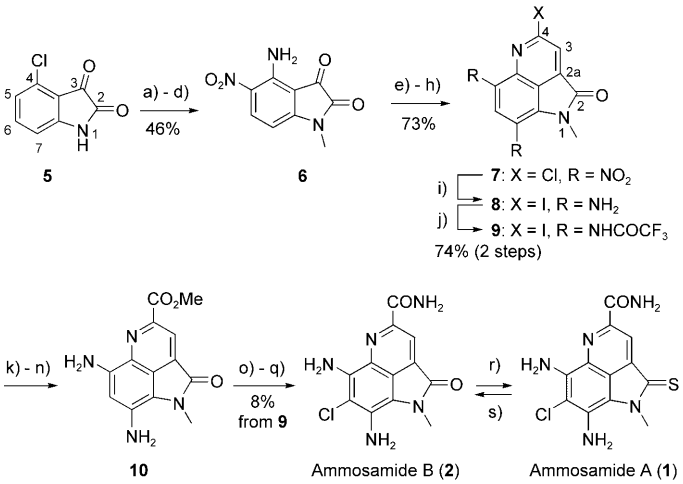
then confirmed by co-immunoprecipitation experiments with rabbit skeletal muscle myosin, by assessing the effects of **3** on actin and microtubule restructuring in HCT-116 cells, and through histological staining of various tissues that myosin was a cellular target of **3**. Importantly, rabbit skeletal muscle myosin could also be stained with **1** or **2**, thus indicating that **3** does indeed reproduce the cellular effects of unmodified ammosamides. The staining of myosin with **3** appeared to be irreversible, which is in line with the observation that **3** could not be displaced from cells by the subsequent addition of excess ammosamides; in contrast, the cellular uptake of **3** could be suppressed almost completely by pretreatment of cells with unmodified **1** or **2.**

Myosins are an important class of motor proteins that are involved in muscle contraction and a variety of cellular processes, including cytokinesis and cell migration. Few small molecules have so far been described that inhibit myosin in a selective fashion. One such inhibitor is (*S*)-(−)-blebbistatin (a synthetic small molecule identified in a high-throughput screen),[9] which has served as an important compound in the dissection of the cellular role(s) of myosin proteins.[9,10] The structure of the complex between myosin II from *Dictyostelium discoideum* and blebbistatin has been solved at 2.0 Å resolution;[10] IAF probe **3** could be successfully modeled into the blebbistatin binding site of this structure,[7] thus providing a structural rationale for the interactions of ammosamides with myosin.

As regards the significance of ammosamides as possible lead structures for (anticancer) drug discovery, the inhibition of myosin function has, to the best of our knowledge, not been seriously pursued as a therapeutic strategy and such an approach may be associated with serious toxicity issues. Independent of this, however, ammosamides could be highly valuable tools to investigate myosin-related cellular processes (known and unknown), even if the currently available data do not definitively exclude the involvement of molecular targets other than myosin in the antiproliferative effects of **1** and **2** (given the significant difference in the cellular potency between IAF probe **3** and natural ammosamides). Further studies will be necessary to clarify (and perhaps improve) the specificity of ammosamide action and these studies will have to include the delineation of the structural features that are required for myosin-directed, as well as general, antiproliferative activity.

The first step towards the design and synthesis of natural product analogues for SAR studies involves, in general, the total synthesis of the natural product itself. This is why the establishment of the total synthesis of ammosamides by Hughes and Fenical[3] is an important milestone on the way to a deeper understanding and the further refinement of the biological effects of these natural products, in addition to the intrinsic accomplishment represented by the first total synthesis of a difficult structure.

The synthesis (Scheme 1) starts from 4-chloroisatin (**5**), which was elaborated into intermediate **6** in 46 % overall yield through mononitration at C5, followed by chloride displacement at C4 by *tert*-butylamine, methylation of the amide nitrogen, and acid-catalyzed removal of the *N*-*tert*-butyl group. The conversion of **6** into the pyrroloquinoline core structure of ammosamides involved a C_2 extension at C3 through a Wittig olefination followed by acid-catalyzed cleavage of the ester and simultaneous ring closure. Nitration



Scheme 1. a) HNO_3, H_2SO_4, 58 %; b) *t*$BuNH_2$, 87 %; c) NaH, MeI; d) TFA, 92 % (2 steps); e) *t*$BuO_2CC(H)=PPh_3$, 86 %; f) TFA; g) HNO_3, H_2SO_4; h) $POCl_3$, 85 % (3 steps); i) aq HI; j) TFAA, 74 % (2 steps); k) CuCN; l) H_2SO_4; m) KOH, MeOH; n) H_2SO_4, MeOH; o) $SOCl_2$, UV; p) TFAA, 8 % (from **9**); q) Mg_3N_2, quant.; r) Lawesson's reagent; s) exposure to air or H_2O_2. TFA = trifluoroacetic acid, TFAA = trifluoroacetic anhydride.

at C7 (isatin numbering) and chlorination then gave **7** in 73% yield (over 4 steps). Reduction of both nitro groups and concomitant Cl→I exchange with hydroiodic acid led to intermediate **8**. As diamino intermediates such as **8** showed very limited solubility in most organic solvents (similar to natural ammosamides), the anilino groups were converted into the corresponding trifluoroacetamides, which helped to improve the yields and facilitated purification by flash chromatography in subsequent steps (for example, **10**→**2**). The advanced precursor **10** was obtained in a four-step sequence (from **9**) that included the transformation of **9** into the corresponding nitrile with CuCN, cleavage of the trifluoroacetamide moieties under acidic conditions, conversion of the cyano group into the imidate (KOH/MeOH), and hydrolysis of the imidate to an ester. Chlorination in neat thionyl chloride under UV light (254 nm) followed by trifluoroacetylation, amidation, and deprotection then led to ammosamide B (**2**), which was obtained in 8% overall yield from **9**. Finally, conversion of ammosamide B (**2**) into ammosamide A (**1**) was achieved with Lawesson's reagent, although in low yield (no yields are specified for this last transformation in Ref. [3]).

In conclusion, by employing a highly innovative approach, the research groups of Fenical and La Clair have identified myosin as a target of the marine metabolites ammosamides A and B. The recent total synthesis of ammosamides by Hughes and Fenical now represents a further enabling step on the way to a more complete understanding of the chemical biology of these fascinating natural products.

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