ORGANIC LETTERS

XXXX
Vol. XX, No. XX
000–000

Total Synthesis and Biological Activity of Natural Product Urukthapelstatin A

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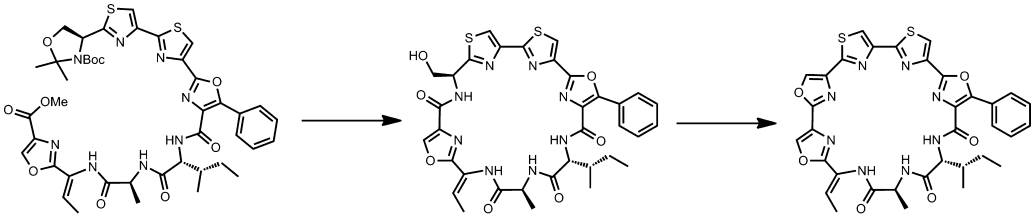
Received May 18, 2013

ABSTRACT



Herein we report the first total synthesis of the natural product Urkuthaplestatin A (Ustat A) utilizing a convergent synthetic strategy. The characterization and biological activity match those of the previously published natural product. Interestingly, several intermediates, including the linear and serine cyclized precursors, show a 100-fold decrease in cytotoxicity, with IC_{50}'s in the low micromolar range. These data indicate that the rigidity and the consecutive aromatic heterocyclic system are responsible for the biological activity.

Reports on oxazole and thiazole containing natural products have stimulated interest in this class of molecules for decades.[1] A large number of these natural products come from marine creatures, and they possess promising biological activity,[2] with many becoming drug candidates.[3] A series of biologically relevant compounds that contain a mixture of oxazole and thiazoles within their backbone include merchercharmycin A (IB-01211), ascidiacyclamide, patellamide D, and telomestatin (Figure 1). These four compounds have all shown significant anticancer activity. Merchercharmycin A shows an average IC_{50} = 43 nM[4] against multiple cancer cell lines, while ascidiacyclamide has an average of 29 μM.[5,6] Patellamide D is clinically used in combination with vinblastine,[7] and telomestatin is currently in clinical trials (average GI_{50} = 5 nM).[8,9] These four molecules resemble the structure of a novel natural product, urukthapelstatin A (Ustat A), which contains a sequential series of heterocycles. Importantly, Ustat A has an IC_{50} value of 15.5 nM[10] (average over 39 cancer cells). Despite its highly cytotoxic properties, and the similarity to current biologically active drugs,

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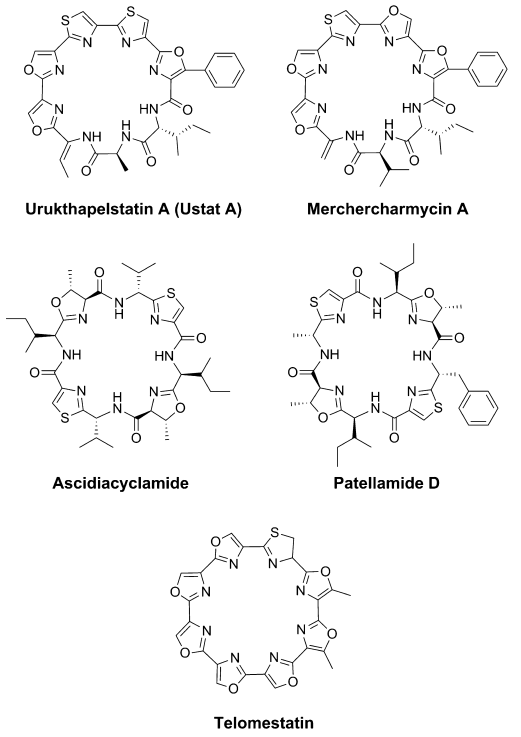

Figure 1. Ustat A and related structures: merchercharmycin A (IB-01211)[1] ascidiacyclamide,[4] patellamide D, and telomestatin.[12]

mechanistic studies have not been successful in identifying how Ustat A functions within the cell. Further, the synthetically challenging aspects of the molecule have made extensive mechanistic studies unfeasible. Indeed, several mechanisms have been investigated for Ustat A, but to date there has been no success in identifying its biological pathway.[9] Thus, this interesting molecule is an ideal synthetic target and an outstanding medicinal chemistry lead.

Herein, we report the first total synthesis of Ustat A using a convergent synthetic strategy (Figure 2). This report details the only successful route for synthesizing this class of compounds. We have published several other attempts at forming this macrocycle[13] including a Hantzsch cyclization ring closure, cyclization between the threonine and alanine, and solid phase synthesis whereupon the oxazoles and thiazoles were installed after macrocyclization. None of these approaches were successful. We believe the downfall of these strategies was the attempt to cyclize a rigid structure that was unable to connect the two ends with all heterocycles intact. Thus, this strategy maintained a single open oxazole as a serine until macrocyclization was complete (**2**, Figure 2).

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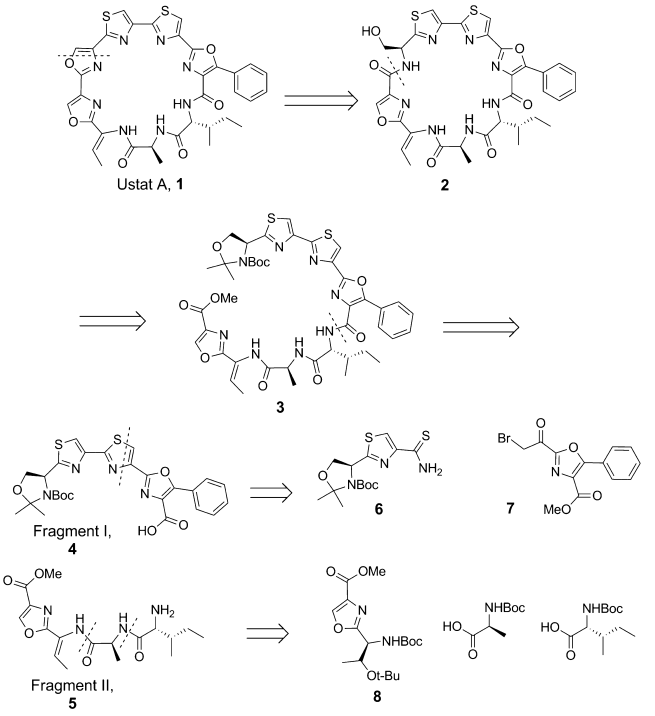


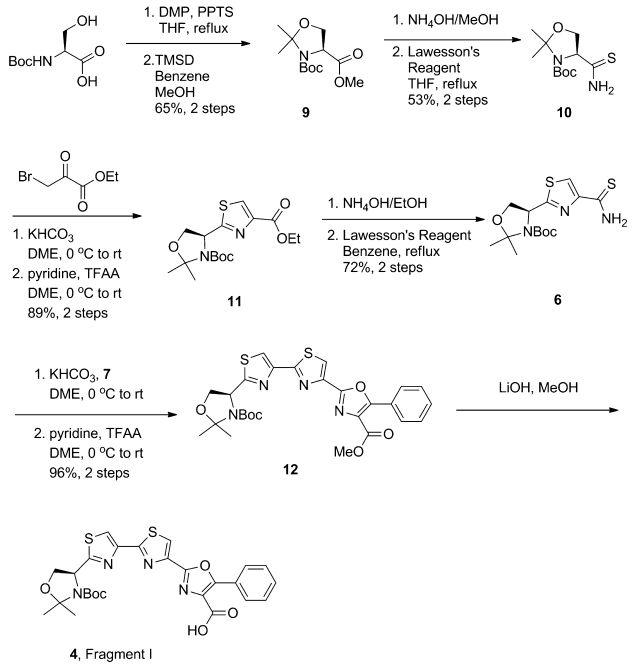
Figure 2. Retrosynthetic strategy for Ustat A.

Formation of Ustat A (**1**) involved intramolecular cyclization of cyclic precursor **2** and subsequent oxidation of the oxazoline intermediate. Structure **2** was formed via cyclization of the linear precursor **3** between the serine N-terminus and the free acid on the oxazole moiety. Compound **3** was synthesized by coupling Fragment I (**4**) and Fragment II (**5**). Fragment I was obtained by condensing the thioamide **6** and the bromoketone oxazole **7** via the Hantzsch thiazole synthesis, followed by an acid deprotection. The thiazole in **6** was also generated by the Hantzsch thiazole synthesis, and the phenyloxazole of **7** was obtained from the cyclization and oxidation of a β-hydroxyl Phe residue. Fragment II was derived from peptide coupling of the oxazole **8** to Ala, followed by the installation of the enamide moiety. Subsequent N-terminal extension of the oxazole-enamide pseudopeptide by peptide coupling to D-*allo*-Ile delivered Fragment II.

Synthesis of Fragment I (Scheme 1) started with protection of serine as a dimethyloxazolidine and was accomplished using 2,2-dimethoxypropane (DMP) and pyridinium *p*-toluenesulfonate (PPTS) in THF. The free acid of Ser was then converted to a methyl ester using trimethylsilyl diazomethane (TMSD) in a mixture of methanol and benzene, affording the ester **9**. Amide conversion of **9** was completed using ammonium hydroxide and methanol. The amide was subjected to Lawesson's reagent to furnish the thioamide **10**. Next, modified Hantzsch thiazole conditions were applied to the thioamide **10** and ethyl bromopyruvate. Potassium bicarbonate ($KHCO_3$) was used in the first step to generate a hydroxyl thiazoline intermediate, which was subsequently dehydrated using trifluoroacetic anhydride (TFAA) and

pyridine, generating the desired thiazole **11**. Conversion of the ethyl ester into a thioamide using ammonium hydroxide and Lawesson's reagent resulted in the formation of thioamide **6**. The bromoketone oxazole **7** was prepared using previously reported conditions.[13a15a] Reacting the thioamide **6** with the oxazole **7** under the presence of $KHCO_3$ gave a thiazoline intermediate, whereupon subsequent dehydration was performed to give triazole **12** in an excellent yield. Finally, methyl ester hydrolysis of **12** was accomplished using lithium hydroxide (LiOH) in methanol and furnished the desired Fragment I (**4**).

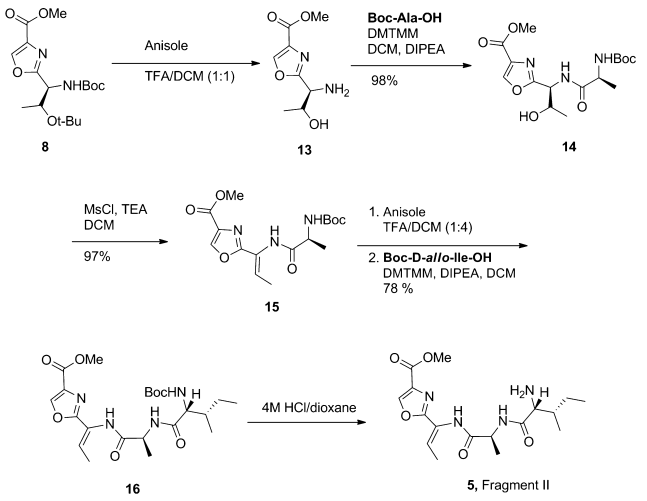
Scheme 1. Synthesis of Fragment I



Fragment II (**5**) (Scheme 2) was generated by starting with oxazole **8**, which we have previously reported.[13a15a] Simultaneous alcohol/amine deprotection of **8** using 50% trifluoroacetic acid (TFA) in dichloromethane generated H-Thr-oxazole-OMe (**13**). Coupling Boc-Ala-OH to the free amine **13** with 4-(4,6-dimethoxy-1,3, 5-triazin-2-yl)-4-methylmorpholinium chloride (DMTMM) and *N,N*-diisopropylethylamine (DIPEA) produced the pseudopeptide **14** with a free Thr residue. Methanesulfonyl chloride (MsCl) was used to convert the free alcohol of **14** into a leaving group, whereupon E2 elimination using triethylamine (TEA) installed only the desired *Z*-enamide (as determined by NOESY)[14] moiety of **15**. The Boc protecting group of **15** was removed using 25% TFA in dichloromethane; the resulting amine was coupled to Boc-D-*allo*-Ile-OH using DMTMM and DIPEA in dichloromethane to yield the pseudopeptide **16**. Fragment II (**5**) was then generated utilizing 4 M hydrochloric acid solution in dioxane to remove the Boc group of **16**.

(14) See Supporting Information for synthetic details

Scheme 2. Synthesis of Fragment II



The macrocyclization of Ustat A is summarized in Scheme 3. Peptide coupling of the free acid Fragment I (**4**) and the free amine Fragment II (**5**) was carried out with 2-(7-aza-1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU), DMTMM, bromo-tris-pyrrolidino-phosphonium hexafluorophosphate (PyBroP), and DIPEA to furnish the protected linear precursor **3**. Subsequently acid and base deprotection prepared the linear precursor **17** ready for cyclization. Macrocyclization of **17** was performed using HATU, DMTMM, 2,4,6-tripropyl-1,3,5,2,4,6-trioxatriphosphorinane-2,4,6-trioxide (T3P), and DIPEA in a dilute condition to afford the cyclic precursor **2**.

The final oxazole formation began with fluorination of the free serine side chain on **2** (Scheme 4) using diethylaminosulfur trifluoride (DAST), followed by base-induced cyclodehydration to yield an oxazoline intermediate. Oxazoline oxidation with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) and bromotrichloromethane furnished Ustat A as an inseparable mixture of 2:1 *Z*/*E* isomers.[15] During the oxazoline formation step using DAST, HF is formed. We believe that *Z*/*E* isomerization of the enamide

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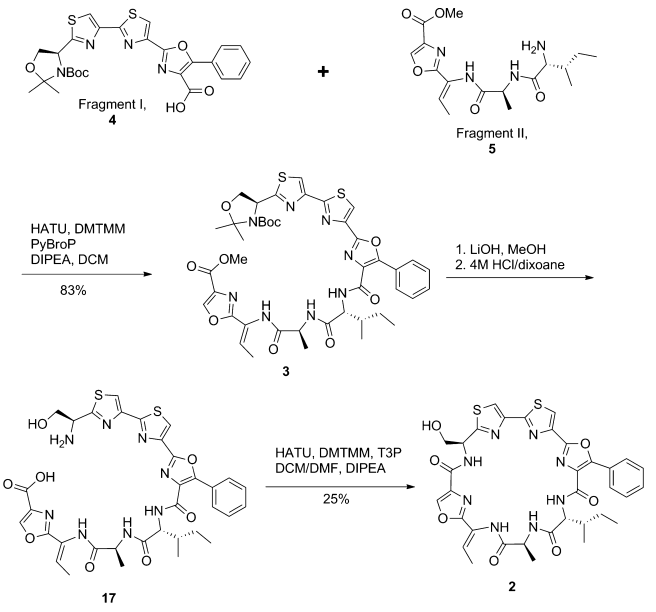
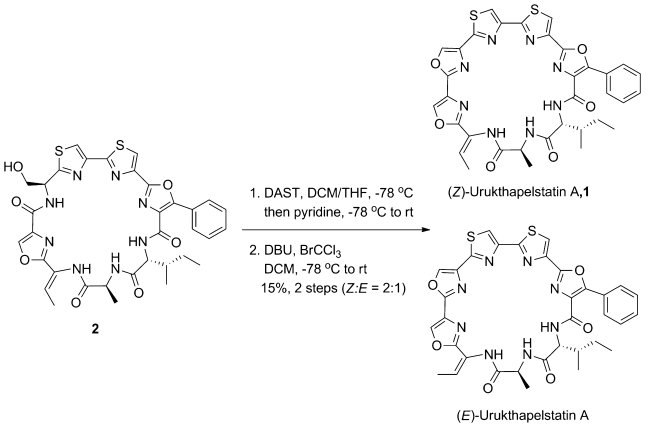
Scheme 3. Synthesis of Linear and Cyclic Precursors



Scheme 4. Synthesis of Urukthapelstatin A



was induced at this step, as compound **2** was the *Z*-enamide, while NMR confirmed that the final product after oxazole formation generated the natural product **1** with the *Z*-enamide as well as the *E*-enamide.

Compounds **12**, **16**, **3**, **2**, and **1** (Fragments I, II, linear, cyclized precursors, and Ustat A) were tested in cytotoxicity assays against the HCT-116 colon cancer cell line. GI_{50} (growth inhibition value for inhibiting 50% of cell growth) of compound **12** was 8 μM, a surprising result given that both **16** and **3** showed no biological activity (Table 1). These results suggest that the potency comes from the upper hemisphere of the molecule. However, as shown in numerous reports,[15] conformation of the macrocycle plays a key role in biological activity, where **2** is not as active as the small fragment **12**. Finally, the natural product, **1**, demonstrated a GI_{50} similar to published reports in the low nanomolar range.[4]

Table 1. Cytotoxicity Values of Compounds against HCT-116 Colon Cancer Cell Line

compd	**12**	**16**	**3**	**2**	**1**
%GI[a]	99	10	0	100	100
IC_{50}[b]	8 ± 0.7 μM	N/A	N/A	30 ± 2.0 μM	20 ± 1.7 nM

[a] % growth inhibition at 40 μM. [b] IC_{50} values were only determined for compounds that showed greater than 50% growth inhibition at 40 μM. The experiments were run in triplicates with each data point performed in quadruplicates. Each IC_{50} value is an average value of three independent cytotoxicity assays with the standard deviations representing errors.

In summary, we have described the first total synthesis of the natural product Urukthapelstatin A (**1**) and verified the structure that was proposed by Matsuo et al.[10] We have also identified the (*E*)-enamide analog of Ustat A. It appears that the configuration of the enamide has little impact on the biological activity as the GI_{50} of the mixture matches that of the published natural product. These data suggest that the upper hemisphere of the molecule is responsible for the biological activity of Ustat A. Mechanistic studies of Ustat A, analogs, and fragments used to build this natural product are currently underway in our lab.

Acknowledgment. We thank the University of New South Wales for the support of S.R.M. and C.C.L. We thank Dr. Macro Ciufolini (UBC) and Dr. David Lefranc (UBC) for their helpful discussions on this project. We also thank Dr. Donald Thomas at the NMR facility UNSW for his help in collecting NMR data.

Supporting Information Available. General experimental procedures, NMR and mass spectral data for compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.