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Progress toward the synthesis of Urukthapelstatin A and two analogues

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ABSTRACT

We report our progress toward the synthesis of Urukthapelstatin A (Ustat A) and two analogues. Our retrosynthetic strategy involved the synthesis of three fragments: a tri-heteroaromatic moiety, a phenyl oxazole fragment, and a dipeptide. Described are the syntheses of three unique tri-heteroaromatic moieties. In addition, the corresponding linear precursors of Ustat A and two analogues are presented.

Natural products provide original pharmacophores, offering new scaffolds with novel mechanisms of action. Urukthapelstatin A (Ustat A) is one such natural product (Fig. 1), with cytotoxicity against cancer cells in the desirable low nanomolar range (average GI_{50} = 15.5 nM).[1] It does not share the structural homology with other classes of marketed cancer therapeutics, and it has a distinct activity profile compared to structurally similar compounds, indicating that it likely has a unique mechanism of action.[1] Given that

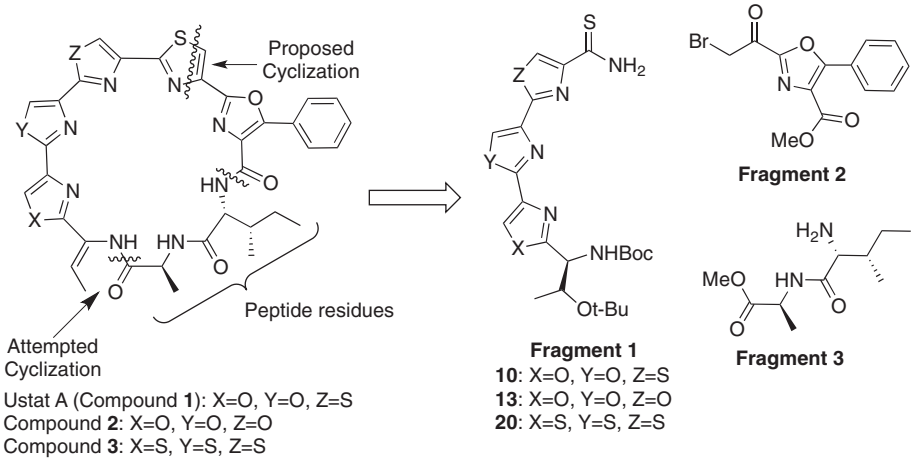

Figure 1. Retrosynthetic approach for Ustat A and analogues.

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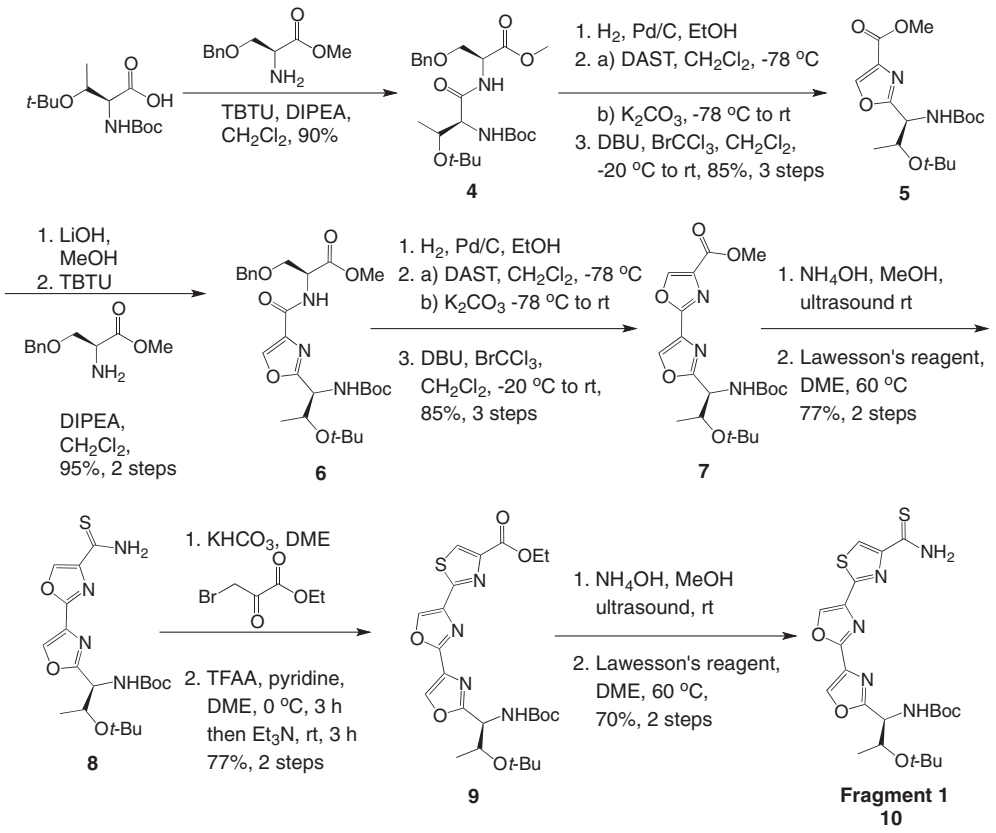
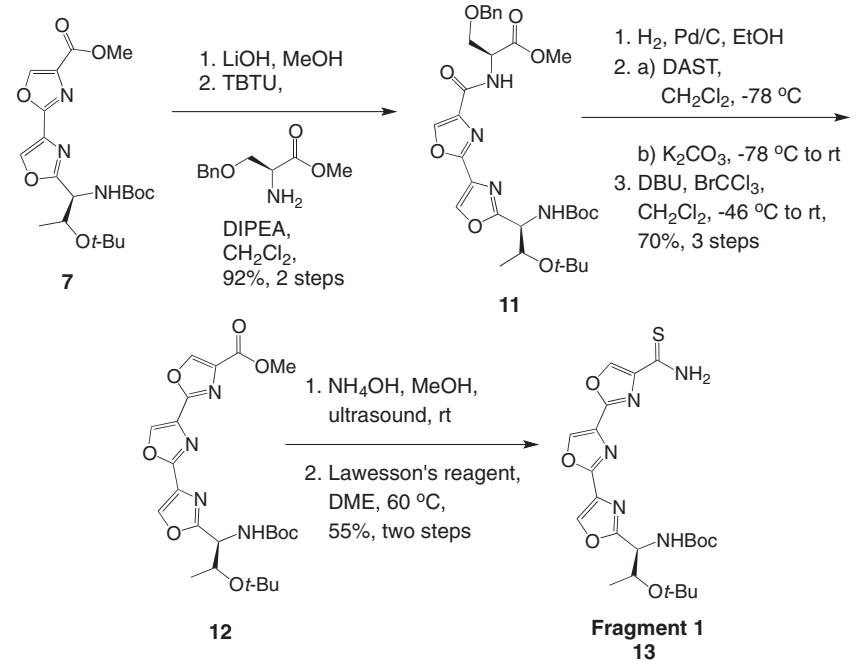
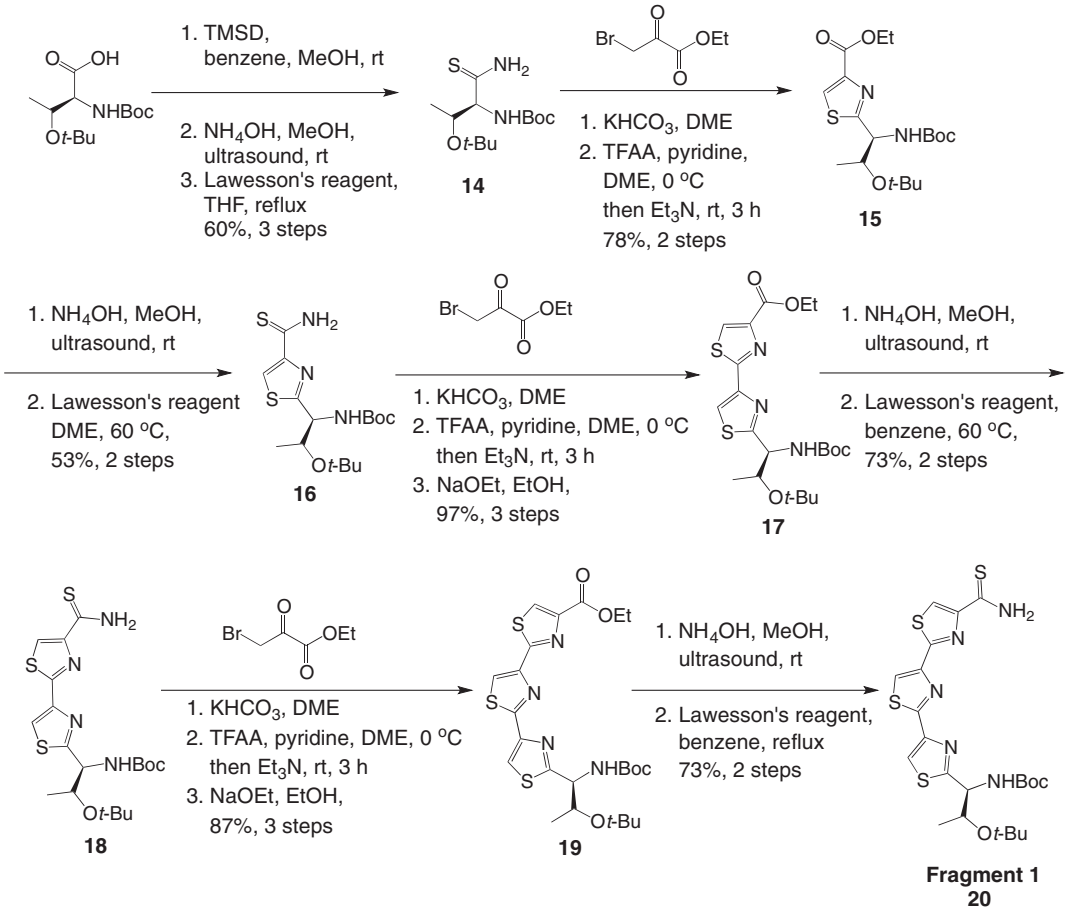
there are considerable on-going efforts to expand the arsenal of drugs that target cancer, the search for new compounds that inhibit novel cell-survival pathways is of paramount importance to the evolution of cancer medicine. Herein, we report our synthetic strategy aimed at completing the first synthesis of the natural product Ustat A and two analogues (Fig. 1). Our efficient, modular approach



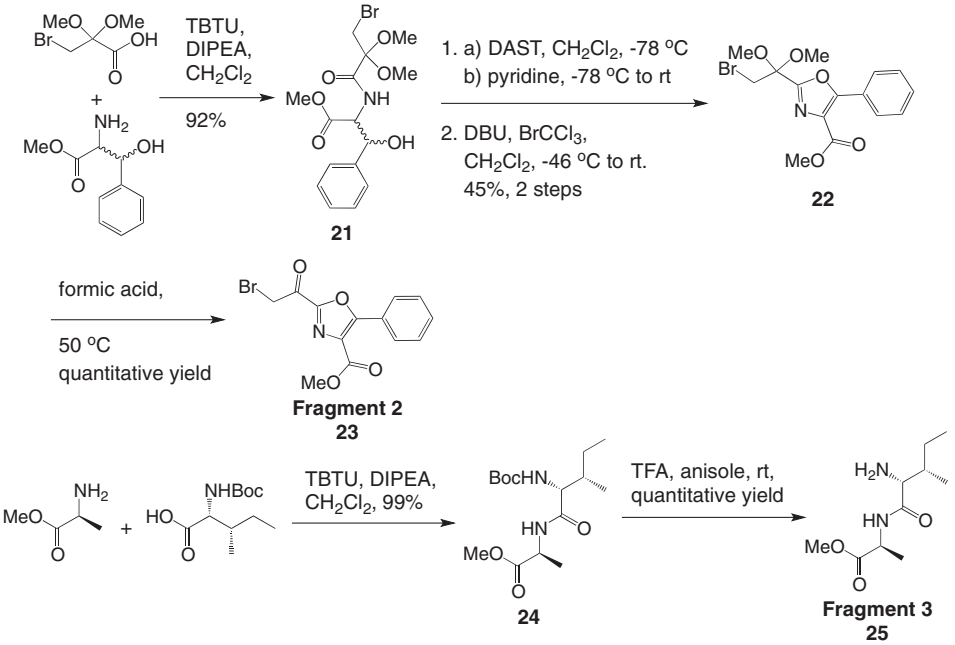
Scheme 1. Synthetic approach to Ustat A, fragment **1**.



Scheme 2. Synthetic approach to compound **2**, fragment **1**.



Scheme 3. Synthetic approach to compound **3**, fragment **1**.



Scheme 4. Synthetic approach to fragments **2** and **3**.

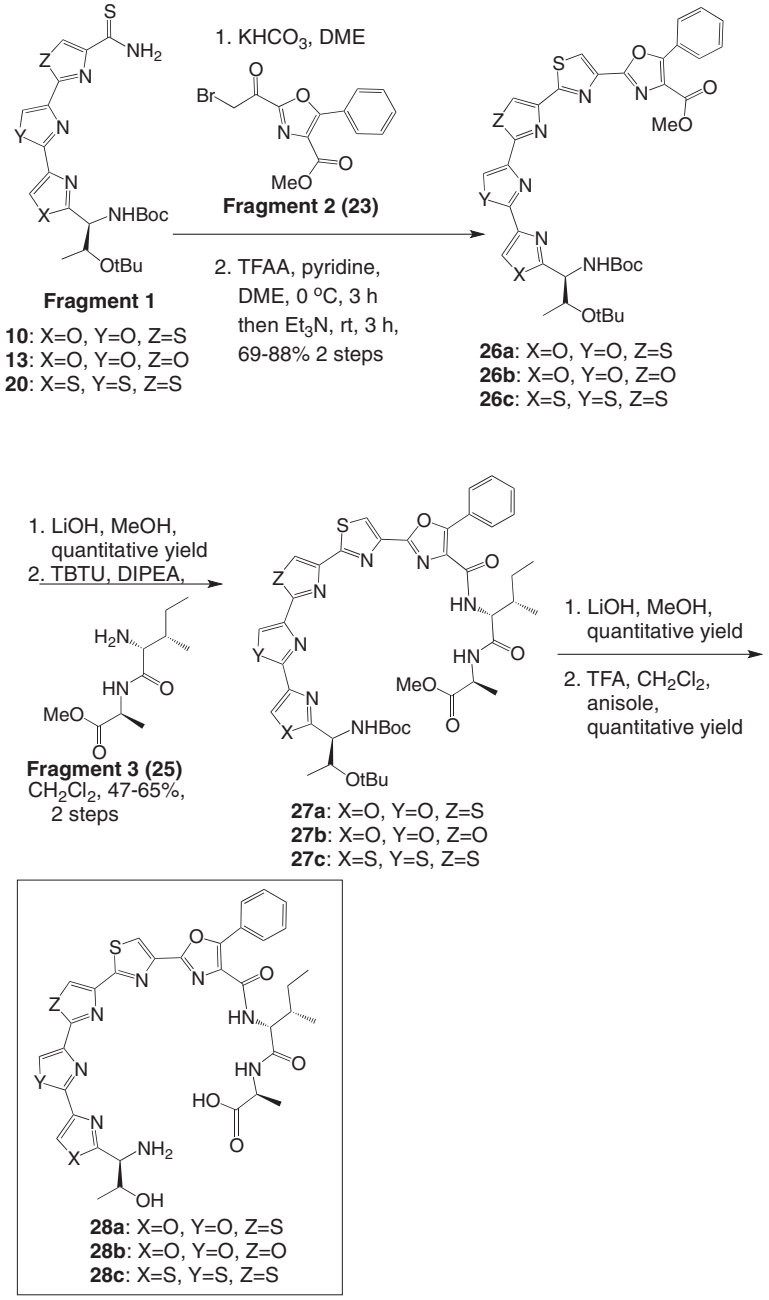
is the first report that targets this unique pharmacophore, or that outlines a method for analogue development. Our approach is a flexible method intended for building Ustat A derivatives, thereby providing access to a series of unique molecular scaffolds.

Several natural product peptidomimetic structures that contain oxazoles and thiazoles have been isolated and synthesized.[2–8] Among these natural products, telomestatin is the only natural product whose mechanism of action has been thoroughly studied.

Although Ustat A is structurally similar to telomestatin, it does not appear to target telomerase (using the traditional TRAP assay),[1] and thus its highly cytotoxic potency is associated with an as yet unknown mechanism of action. The isolated natural product was also tested in numerous anticancer assays, which revealed that it is also unlikely to be an HDAC, farnesyl transferase, or proteasome inhibitor.[1] The activity profile of Ustat A using COMPARE analysis resembles that of the known anticancer agent cytarabine, which is an antimetabolite.[1] Indeed, the COMPARE profile suggests that this molecule acts via a unique mechanism, indicating the value of further investigation and analogue synthesis.

Herein we outline an efficient synthetic strategy for three fragments. Using a Hantzsch thiazole synthesis to couple fragments **1** and **2**, followed by a peptide coupling of fragment **3** completes the linear precursor. Although cyclization conditions are currently on-going, we anticipate completing the macrocycle by coupling between alanine and threonine. Subsequent elimination to the *Z*-enamine[9] would give the final product. In this Letter, we focus on developing linear precursors with modified heterocyclic components. Oxazoles form stronger hydrogen bonds than thiazoles, but are significantly more rigid when placed in a macrocyclic conformation.[10] Thus, syntheses of the natural product and two analogues were designed to investigate how modification of the heterocycles would alter: (a) the ability of these analogues to hydrogen bond and (b) the ability of the rings to close. The synthesis of fragment **1** differed for each of the three macrocycles, with Schemes 1–3 describing the synthetic approaches for compounds **1**, **2**, and **3**, respectively. However, the synthesis of fragments **2** and **3** were the same for all three linear precursors.

Utilizing a peptide approach to generate the heterocycles provided the fragments in the fewest number of steps (vs a cross-coupling approach-theoretical calculation) and gave high overall



Scheme 5. Synthetic approach to the linear precursors.

yields. The synthesis of fragment **1** for compound **1** began with the construction of the dipeptide **4** (Scheme 1). This dipeptide was generated by coupling free amine H_2N-Ser(Bn)-OMe and free acid Boc-Thr(O^{t}Bu)-OH using 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (TBTU) and diisopropylethylamine (DIPEA) in anhydrous methylene chloride (CH_2Cl_2) yielding **4** in 90% yield. Removal of the benzyl ether protecting group was accomplished via hydrogenolysis using Pd/C (10%) as catalyst. A two-step procedure involving an intramolecular cyclization using the fluorinating agent diethylaminosulfur trifluoride (DAST) and K_2CO_3 to yield the intermediate oxazoline, which was oxidized using bromochloroform ($BrCCl_3$) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), generated **5** in 85% overall yield over three steps. Hydrolysis of the ester **5** using LiOH and subsequent coupling between the free acid and free amine H_2N-Ser(Bn)-OMe was performed using TBTU and DIPEA. This yielded compound **6** in 95% yield. Hydrogenolysis of **6** and subsequent conversion of the free serine into an oxazole using DAST/$BrCCl_3$ resulted in **7** (85% overall yield over three steps). The formation of thioamide **8** was carried out using ammonium hydroxide in methanol followed by conversion of the resulting amide into the thioamide using Lawesson's reagent (77% yield over two steps). A base-induced Hantzsch thiazole synthesis[11] was performed using an excess of ethyl bromopyruvate and $KHCO_3$ to afford the intermediate thiazoline, which was dehydrated using trifluoroacetic anhydride (TFAA) and pyridine to give compound **9** (77% yield over two steps). Compound **10** was generated using ammonium hydroxide in methanol followed by treatment with Lawesson's reagent (70% yield over two steps).

The synthesis of **13** en route to the Ustat A analogue utilized dioxazole **7**, which was converted into the free acid using LiOH in methanol (Scheme 2). Subsequent peptide bond formation between the free acid Boc-Thr(O^{t}Bu)-dioxazole-OH and the free amine H_2N-Ser(Bn)-OMe using TBTU and DIPEA in anhydrous CH_2Cl_2 gave **11** (92% yield, 2 steps). The benzyl ether of **11** was removed via hydrogenolysis followed by the formation of the third oxazole moiety, **12**, using DAST/$BrCCl_3$ in 70% yield over the three steps. Conversion of the ester into a thioamide to generate **13** was achieved using ammonium hydroxide followed by sulfur installation using Lawesson's reagent (55% yield, 2 steps).

The construction of Ustat A analogue **20** is summarized in Scheme 3. Starting with protection of the free acid Boc-Thr(O^{t}Bu)-OH using trimethylsilyl diazomethane (TMSD) in methanol and then conversion into the thioamide via ammonium hydroxide and Lawesson's reagent resulted in the formation of **14** (60% yield over three steps). The Hantzsch thiazole synthesis was then performed reacting **14**, ethyl bromopyruvate, and $KHCO_3$ to yield the intermediate thiazoline, whereupon subsequent dehydration was facilitated using TFAA and pyridine at 0 °C to give **15** (78% over two steps). Generation of **16** was accomplished using a two-step procedure involving ammonium hydroxide and Lawesson's reagent (53% yield, two steps). The next two thiazole moieties were installed by repeating the Hantzsch thiazole synthesis process, whereby the thioamide was reacted with ethyl bromopyruvate and $KHCO_3$. Dehydration using TFAA and pyridine and then treatment with NaOEt in EtOH gave **17** (97% yield over three steps). This approach was repeated on **17** to generate thioamide **18** (73% yield over two steps), and then tri-thiazole **19** (87% yield over three steps). Conversion of **19** into a thioamide using ammonium hydroxide and Lawesson's reagent provided **20** (73% yield over two steps).

Fragments **2** (**23**) and **3** (**25**) were synthesized next (Scheme 4). Phenylserine **21** was generated by coupling 3-bromo-2,2-dimethoxypyruvic acid with (2*R*,3*S*)/(2*S*,3*R*)-racemic H_2N-β-hydroxy-Phe-OMe using TBTU and DIPEA in anhydrous CH_2Cl_2 (92% yield). Racemic **21** was then subjected to intramolecular cyclization induced by DAST and then pyridine in CH_2Cl_2 to afford the intermediate phenyloxazoline. This intermediate was then advanced to phenyloxazole through oxidation using $BrCCl_3$ and DBU (45% overall yield 2 steps). Deprotection of ketone was achieved using formic acid induced hydrolysis at 50 °C for 20 min yielding **23** (quantitative yield). Fragment **3** (**25**), was generated via a peptide coupling reaction between free acid Boc-d-allo-Ile-OH and free amine H_2N-Ala-OMe using TBTU and DIPEA (99% yield). The dipeptide was then amine deprotected using TFA, providing **25** (quantitative yield).

With fragments **1** and **2** in hand, we performed the Hantzsch thiazole synthesis (Scheme 5) to generate the heterocyclic hemisphere of Ustat A and analogues (overall yield for two steps **26a, b**, and **c** = 69%, 72%, and 88%, respectively). Upon gaining access to penta-heterocycle fragments **26a–c**, we performed an acid deprotection and coupled the resulting free acids to fragment **3** using TBTU and DIPEA in CH_2Cl_2, thereby generating **27a–c** (61%, 65%, and 47% yields, respectively). The ester group in compounds **27a–c** was deprotected using LiOH, and the Boc and *tert*-butyl groups were removed using TFA, generating compounds **28a–c**. NMR spectroscopic studies and high resolution mass spectroscopy confirmed the structures of the three linear precursors **28a–c**.

In summary, we have outlined an efficient and viable synthetic approach to three different rigid penta-heterocycles. We then converted these into the linear precursors in preparation for cyclization into Urukthapelstatin A (**1**) and two analogues **2** and **3**. We are currently investigating cyclization strategies,[12] which will be reported in due course. These linear analogues will be tested for their ability to inhibit colon and pancreatic cancer cell growth.[13,14]

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2012.05.105.

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