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Synthesis of the Natural Product Marthiapeptide A

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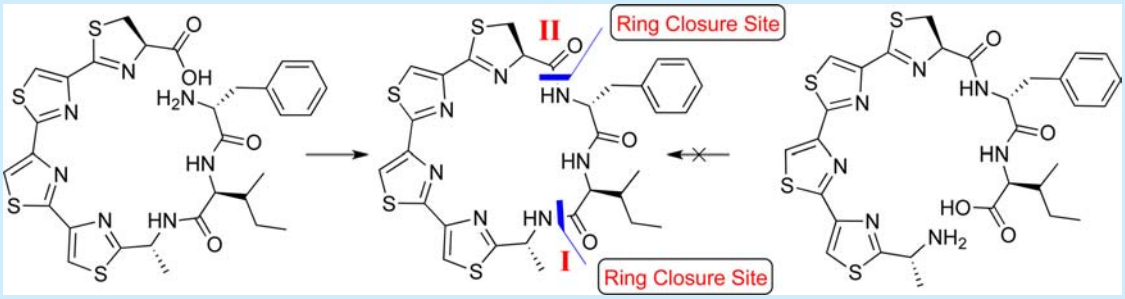
Supporting Information



ABSTRACT: The first total synthesis of marthiapeptide A is reported. Two synthetic procedures are described: the first, which was unsuccessful, attempts to close the ring at position I, and the second, which was successful, closes the ring at position II. It appears that the first route was unsuccessful because it required cyclization next to the rigid thiazole moiety, whereas the second route closed next to the more flexible thiazoline ring.

Linked azoles are unique substructures that are present in many natural products. Over the past two decades, there has been a surge in the discovery of natural product compounds that contain linked azoles, where many of these compounds are now candidates for drug development.[1] Urukthapelstatin A (**Ustat A**) (Figure 1) is a natural product isolated from marine

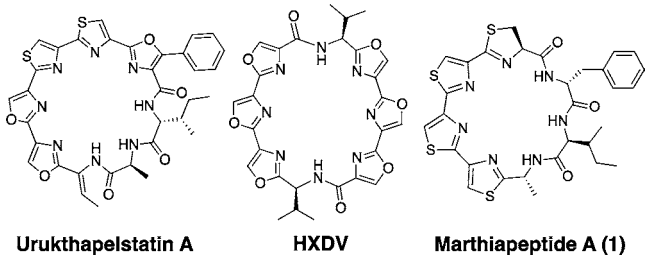


Figure 1. Structures of Ustat A, HXDV, and MTA (**1**).

bacteria, and it has a bisoxazole and a bisthiazole moiety located within the macrocycle. It shows potent anticancer activity against a panel of human cancer cell lines, with an average IC_{50} value of 12 nM.[2,3] **HXDV** (Figure 1) is a synthetic derivative of telomestatin, and it has two linked trioxazoles within its macrocyclic backbone. **HXDV** exhibits antiproliferative and apoptotic activity by stabilizing the G-quadruplex of DNA that is formed during replication.[4,5] Marthiapeptide A (**MTA**) (Figure 1) is another potent natural product that contains a linked trithiazole−thiazoline system and cytotoxicity with an IC_{50} = ∼380−520 nM against a panel of cancer cell lines.[6] We previously reported the first synthesis of **Ustat A**[3] and the synthesis of trioxazole fragments of **HXDV**.[7] Herein we report the synthesis of **MTA**.

MTA was isolated by Zhou and co-workers in 2012.[6] It is a cyclic peptide that consists of three consecutive thiazoles and thiazoline and was isolated from a south China sea derived strain *Marinactinospora thermotolerans*. This natural product exhibits significant cytotoxicity against a panel of human cancer cell lines with GI_{50} value ranges from 0.38 to 0.52 μM. D-Alanine, L-isoleucine, and D-phenylalanine connected to a trithiazole thiazoline system makes up the complex structure shown in Figure 1. Outlined is our unsuccessful initial route as well as the successful final route for completing the synthesis of **MTA**.

Our initial strategy (A) involved a macrocyclization of the linear precursor via a peptide coupling reaction between the amine on the alanine residue and the carboxylic acid end of isoleucine (position **I**, Scheme 1). However, the cyclization was not successful, which was attributed to the closing point being too close to the rigid heterocyclic thiazole moiety. Our second strategy (B) involved closing between the thiazoline and peptide (position **II** Scheme 1). This approach worked, and although it was not high yielding, we believe that this successful cyclization can be attributed to the flexibility of the thiazoline, which allows a connection between the molecule's head and tail.

Cyclization for the initial strategy involved the synthesis of **2** (dipeptide) and **3** (trithiazole ester) and coupling at position **II** to give the linear molecule shown in route A. Cyclization then occurred at position **I**. The successful strategy involved coupling **2** and **3** at position **I** first and then cyclizing at position **II**. Generation of **3** was accomplished by sequentially

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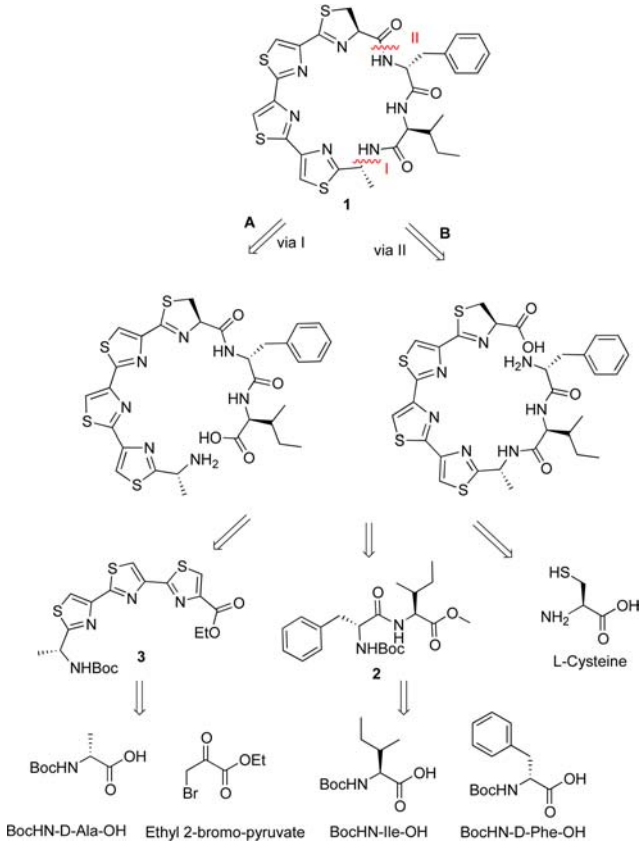
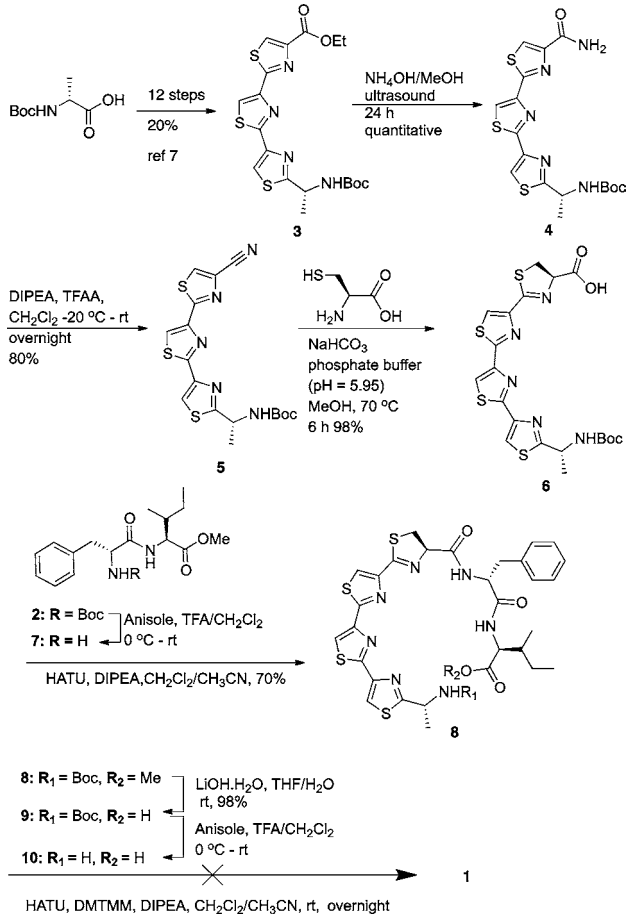
Scheme 1. Retrosynthetic Scheme for Synthesizing Marthiapeptide[a]



[a]Ring closing was successful only at position II via route B.

Scheme 2. Initial Synthetic Route (A) Was Unsuccessful at Producing the Desired Natural Product 1



synthesizing thiazoles using a modified Hantzsch synthesis, which condensed ethyl 2-bromopyruvate and a thioamide. The synthesis of dipeptide **2** was accomplished via standard peptide-coupling conditions between Boc-NH-D-Phe-OH carboxylic acid with H_2N-Ile-OMe amine.

The synthesis of **3** was completed using our previously reported procedure.[7] Specifically, BocHN-D-Ala-OH underwent 12 steps, via sequential thiazole Hantzsch synthesis, to generate **3** in an overall yield of 20%. The first strategy started from **3**, where the trithiazole ester was sonicated with a solution of ammonium hydroxide (Scheme 2) generating amide **4**. Dehydration of the amide to the nitrile using trifluoroacetic anhydride (TFAA) in the presence of *N*,*N*-diisopropylethylamine (DIPEA) and CH_2Cl_2 produced the nitrile **5**. The crude material underwent flash column chromatography to afford pure **5** in an 80% yield. Condensation of the nitrile with L-cysteine was then carried out using $NaHCO_3$ in the presence of phosphate buffer (pH = 5.95) at 70 °C to furnish thiazoline **6**. Amine deprotection of **2** was achieved using trifluoroacetic acid (TFA) generating **7**. Coupling crude **7** to free acid thiazoline **6** using coupling agent 1-bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxide hexafluorophosphate (HATU) in the presence of DIPEA generated **8**. Ester hydrolysis of **8** produced crude free acid **9**, where the crude was treated with TFA to remove the Boc protecting group generating **10**. Subsequent cyclization of the double deprotected linear precursor **10** using HATU and 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMTMM) in the presence of DIPEA failed to generate compound **1**.

We hypothesized that the ring failed to close at position I because the ring-closure site was too close to the rigid thiazole moieties. The proximity of the four relatively rigid structures to the ring-closing site likely made it impossible to connect the head and tail end of the compound.

Similar to the initial route, the final route (B) was also initiated starting with **3**, whereupon Boc removal produced **11** (Scheme 3). Conversion of **2** to the free acid using $LiOH{\cdot}H_2O$ produced **12** in preparation for coupling to **11**. Using HATU in the presence of DIPEA, **11** was coupled to **12** generating **13** in a 72% yield. Subsequent conversion to the amide **14** using ammonium hydroxide was accomplished using sonication over 48 h (Scheme 3). The larger structure **13** converted more slowly to the amide **14** than the smaller structure (**3** to amide **4**, Scheme 2). Dehydration of the amide **14** to the nitrile **15** was produced in a lower yield than the corresponding dehydration of the smaller molecule used in the initial route (Scheme 2, structure **4** to **5**). Indeed, the conditions for nitrile **15** formation required modification, whereupon the optimal yield was produced when using molecular sieves. Condensing L-cysteine with **15** in the presence of sodium bicarbonate in a phosphate buffer generated linear precursor **16**. Amine deprotection using TFA produced the linear precursor. Cyclization was accomplished using a syringe pump, which added the compound into a cocktail of three coupling agents: HATU, DMTMM, and *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TBTU) in the presence of DIPEA.

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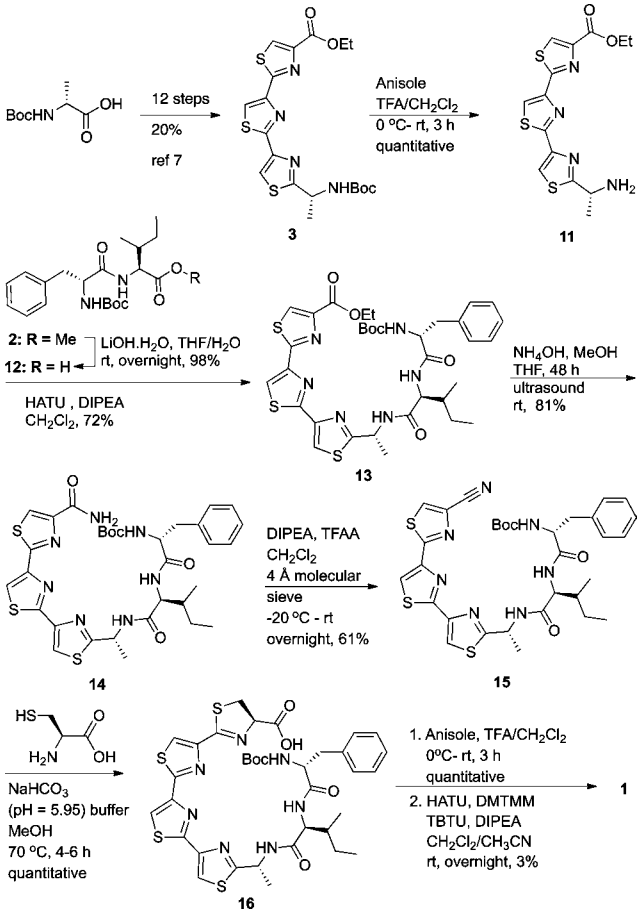
Scheme 3. Final Synthetic Route (B) Successfully Produced the Desired Natural Product 1



Upon disappearance of the starting material, as observed when monitoring by LCMS, the reaction was washed with an acid and then base in order to remove coupling agent side products. The resulting crude material was purified via flash column chromatography on silica gel. The relatively product was isolated from the column, and a second purification was done using reverse phase HPLC. Final purity was verified by evaluating the compound using two different conditions on the LCMS (Supporting Information) and co-injecting it with the natural product kindly provided by the original isolation team[6] using these two systems. The LCMS coinjection data show that the synthetic product was identical in retention time and mass to the natural product. The HRMS also indicated that our sample was pure, where the calculated exact mass and observed mass were 688.1269 and 688.1260 respectively, (Supporting Information). ^{1}H and 2-D NMR were assigned (Supporting Information), and by use of 2-D NMR a carbon spectral shift difference was completed between the synthetic and the natural product (Supporting Information). The carbon shifts were essentially identical between the natural product and the synthetic compound **1** (with a maximum of 1 ppm difference between assigned carbons).

In conclusion, we have described two synthetic routes where one produced the natural product marthiapeptide A. The successful ring closing occurred next to the more flexible thiazoline versus the unsuccessful ring closing attempted next to the more rigid thiazole. Future analogues might be generated most effectively by cyclizing between the phenylalanine and the isoleucine residue because this is likely the most flexible position around the macrocyclic backbone.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.5b02574.

Synthetic and biological procedures, characterization data, and biological data (PDF)

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Notes

The authors declare no competing financial interest.

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