Organic Letters

Letter

pubs.acs.org/OrgLett

Divergent Approach for the Synthesis of Gombamide A and Derivatives

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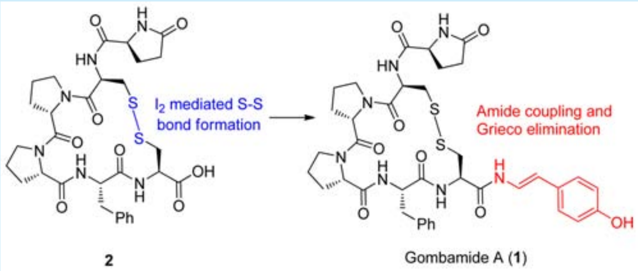
Ⓢ *Supporting Information*

ABSTRACT: A synthesis of gombamide A (**1**) using N-terminal peptide extension, oxidative disulfide bond formation, and late-stage 4-hydroxystyrylamide installation has been achieved. This divergent method was also utilized to synthesize several gombamide A derivatives with modification to the 4-hydroxystyrylamide via cyclic peptide **2**. The natural product and four derivatives were found to be devoid of Na^+/K^+-ATPase activity at 10 μM. In addition, the compounds were not cytotoxic at 10 μM against a panel of cancer cells.



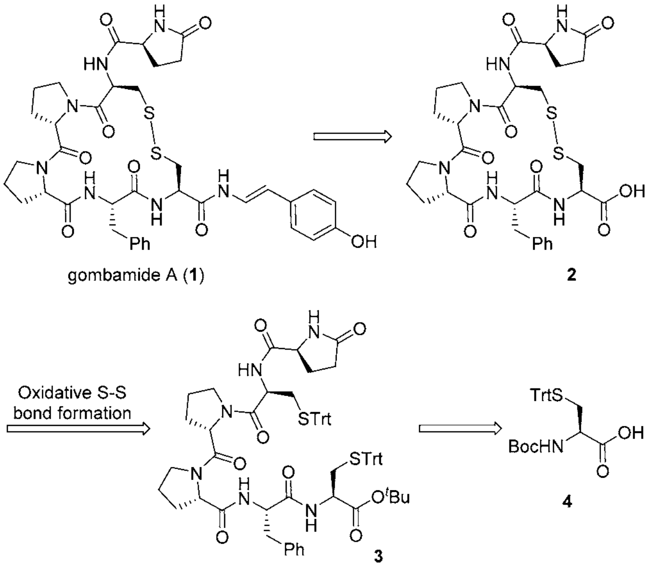
Disulfide-containing natural products possess an array of biological activities.[1] In addition, disulfide-containing cyclic peptides constitute a broad structural motif found in natural products,[1a,2a] endogenous compounds (e.g., oxytocin and calcitonin), and pharmacological agents (e.g., linaclotide and eptifibatide). The disulfide functional group provides structural rigidity that can enhance binding affinity to biomolecular targets and potentially improve oral bioavailability.[2b] Our laboratory has become interested in synthesizing and evaluating disulfide-containing cyclic peptide natural products and derivatives as potential pharmacological probes. For example, we recently reported the synthesis and antiproliferative activity evaluation of thiochondrilline C and several derivatives.[3]

Gombamide A (**1**) is a disulfide-containing cyclic peptide recently isolated from the marine sponge *Calthria gombawuiensis*.[4] In addition, one of the cysteine residues that comprise the disulfide is linked to pyroglutamic acid while the other is attached to a styrylamide moiety, an uncommon functional group among isolated natural products.[5] Gombamide A was also reported to have moderate Na^+/K^+-ATPase inhibitory activity and weak cytotoxicity against human A549 nonsmall cell lung cancer and K562 leukemia cell lines.[4]

Gombamide A has attracted interest from the organic chemistry community with a synthesis using a convergent approach recently reported by Garcia-Barrantes and Lindsley.[6] Herein, we describe a complementary synthesis of gombamide A and several derivatives that utilizes a divergent strategy that was exploited to generate several derivatives with modifications to the 4-hydroxystyrylamide. In addition, we report the evaluation of these compounds for Na^+/K^+-ATPase and antiproliferative activities.

A retrosynthetic analysis of **1** is outlined in Scheme 1. This divergent approach introduces the 4-hydroxystyrylamide or other amides late in the synthesis via carboxylic acid **2**, providing flexibility for modification of this exocyclic portion of the molecule. Cyclic intermediate **2** would be prepared by oxidative disulfide bond formation of **3**, which would be generated by an *N*-terminal peptide extension starting from **4**.

Scheme 1. Retrosynthetic Analysis of Gombamide A (1)

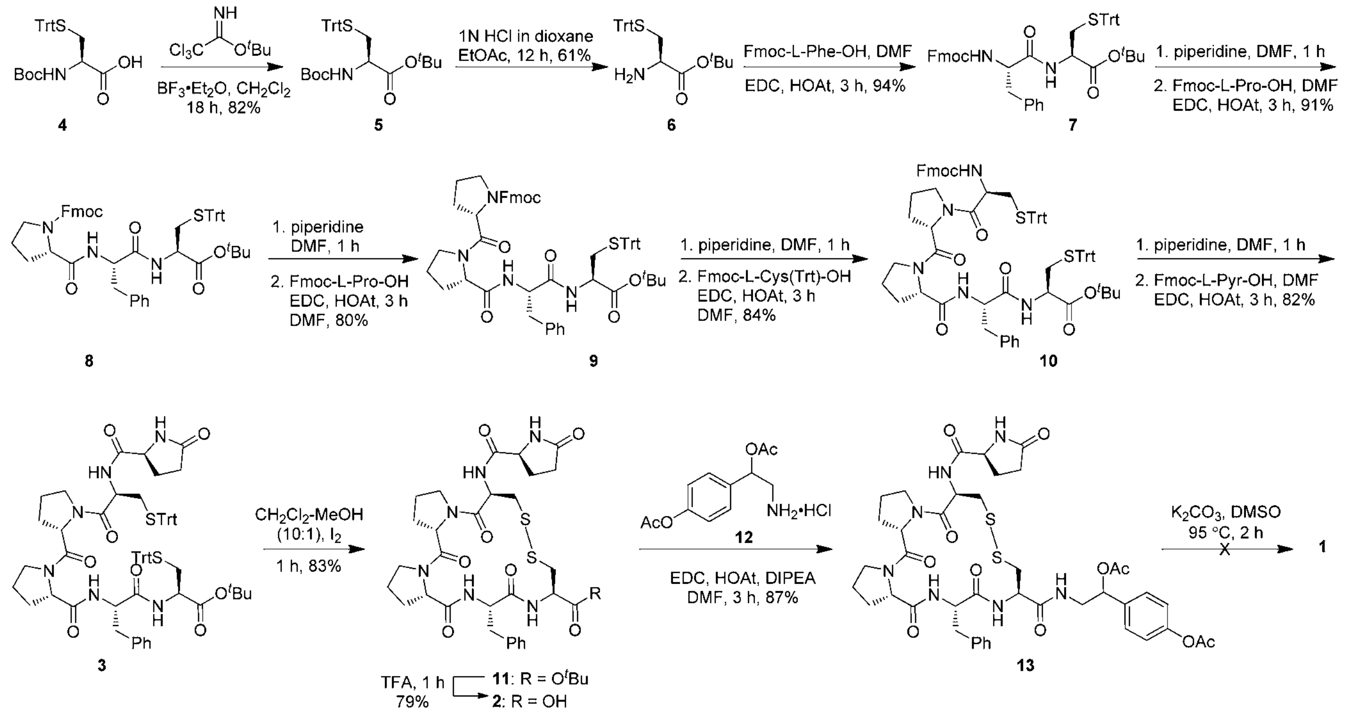


The synthesis of gombamide A and several derivatives began with esterification of *N*-Boc-*S*-tritylcysteine, **4**, in the presence of *tert*-butyl 2,2,2-trichloroacetamidate to furnish the corresponding *tert*-butyl ester **5** in 82% yield (Scheme 2). Selective removal of the Boc in the presence of the trityl group was accomplished with 1 N HCl to provide **6** in 61% yield.[7] Amine

Received: August 9, 2016
Published: September 7, 2016

DOI: 10.1021/acs.orglett.6b02379
Org. Lett. 2016, 18, 4726–4729

Scheme 2. Synthesis of Disulfide-Containing Cyclic Peptide Intermediate 2



6 was coupled with Fmoc-L-Phe-OH using EDC and HOAt to provide dipeptide **7** in 94% yield. The dipeptide was treated with piperidine to remove the Fmoc protecting group followed by coupling with Fmoc-L-Pro-OH in the presence of EDC and HOAt to generate tripeptide **8** in 91% yield. The tripeptide was again treated with piperidine to remove the Fmoc protecting group followed by coupling with Fmoc-L-Pro-OH using EDC and HOAt to generate the tetrapeptide **9** in 80% yield. Fmoc deprotection of the tetrapeptide with piperidine followed by coupling with Fmoc-L-Cys(Trt)-OH using EDC and HOAt gave pentapeptide **10** in 84% yield. This material was treated with piperidine and then coupled with Fmoc-L-Pyr-OH using EDC and HOAt to generate **3** in 82% yield. The hexapeptide was then exposed to iodine to mediate oxidative disulfide bond formation that provided the cyclic peptide **11** in 83% yield.[3,8] Deprotection of the *tert*-butyl ester was carried out with trifluoroacetic acid to generate the corresponding carboxylic acid **2** in 79% yield.

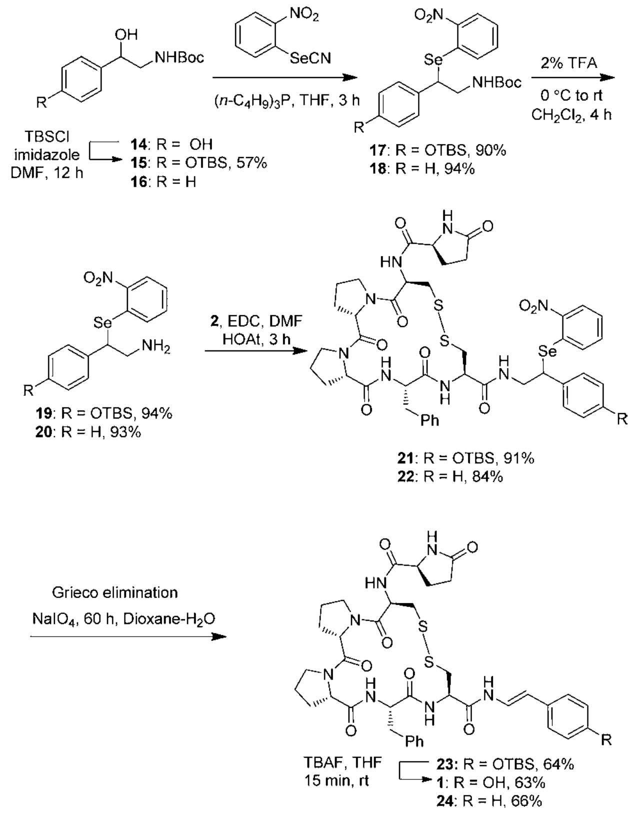
Our initial plan for generating the 4-hydroxystyrylamide was via an intermolecular copper-mediated coupling of *p*-(*tert*-butyldimethylsiloxy)-*β*-bromostyrene with the corresponding primary amide of **2**[9a,b] since intramolecular versions of this type of process have been applied to the synthesis of cyclopeptide alkaloids.[9c−f] However, a model reaction of this styrene derivative with *N*-acetyl-DL-phenylalaninamide was not successful.

An alternative strategy for introducing the 4-hydroxystyrylamide that we selected was elimination of the corresponding *β*-acetoxyphenethylamide.[10] In pursuit of this approach, **12** was prepared from (±)-octopamine hydrochloride via *N*-Boc protection followed by treatment with acetic anhydride in the presence of pyridine and subsequent removal of the Boc group using 1 N HCl. Coupling of **12** with the model substrate *N*-Boc-Cys(Trt)-OH, followed by treatment with K_2CO_3 in DMF at 95 °C, furnished the corresponding 4-hydroxystyrylamide product. Encouraged by this result, we attempted to employ this method to introduce 4-hydroxystyrylamide in the synthesis of gombamide A. Coupling of acid **2** with amine **12** using EDC and HOAt generated **13** in 87% yield. However, treatment of **13** with K_2CO_3 in DMF at 95 °C resulted in decomposition.

Due to difficulties encountered with the acetoxy elimination strategy, Grieco's method for elimination of *O*-nitrobenzeneselenenic acid was pursued in order to introduce the styrylamide.[11,12] *N*-Boc-(±)-octopamine **14** was treated with TBSCl in the presence of imidazole to furnish **15** in 57% yield (Scheme 3). Compound **15** was allowed to react with *O*-nitroselenocyanide and tributylphosphine to furnish **17** in 90% yield. This compound was treated with 2% trifluoroacetic acid in DCM to remove the Boc group, generating **19** in 94% yield. Amine **19** was coupled with **2** using EDC and HOAt to generate **21** in 91% yield. Treatment of **21** with $NaIO_4$ (6 equiv) under standard Grieco elimination conditions resulted in the formation of the *O*-TBS-protected gombamide A derivative **23** along with a byproduct likely due to mono-oxidation at one of the sulfurs (monitored by LCMS).[11b,12d] In order to enhance formation of the desired product and to suppress unwanted *S*-oxidation, **21** was treated with only 1 equiv of $NaIO_4$, which resulted in the formation of **23** in 64% isolated yield. Removal of the TBS group was carried out using TBAF (1 equiv) providing gombamide A (**1**) in 63% yield. The spectral data of the synthetic material are consistent with the previously reported data.[4,6]

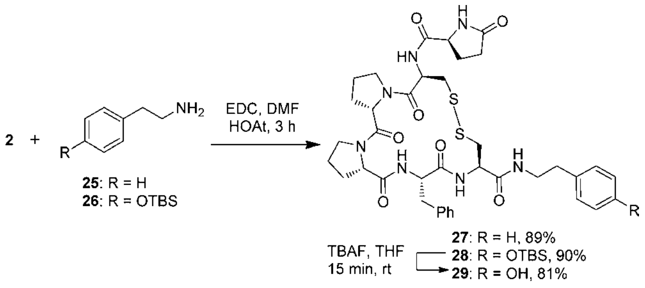
In order to highlight the versatility of this divergent approach, several gombamide A derivatives were prepared using the intermediate carboxylic acid **2**. First, another styrylamide derivative was synthesized (Scheme 3). Des-hydroxyl derivative **16** was treated with *O*-nitroselenocyanide and tributylphosphine to furnish **18** in 94% yield. Again, removal of the Boc group using 2% TFA in DCM gave **20** in 93% yield. This material was coupled with **2** using EDC and HOAt to give **22** in 84% yield. Treatment of **22** with $NaIO_4$ (1 equiv) furnished derivative **24** in 66% yield. Next, two

Scheme 3. Synthesis of Gombamide A (1) and Derivative 24



phenethylamine derivatives were prepared (Scheme 4). Compound **27** was obtained by coupling carboxylic acid **2** with **25** using EDC and HOAt in 89% yield. Similarly, **2** was coupled with **26** to furnish **28** in 90% yield. Removal of the TBS group with TBAF gave **29** in 81% yield.

Scheme 4. Synthesis of Gombamide A Derivatives 27 and 29



Gombamide A (**1**) and four derivatives (**2**, **24**, **27**, and **29**) were evaluated for activity against Na$^+$/K$^+$-ATPase at 10 μM in duplicate.[13] However, all of the compounds were found to be inactive (<2% inhibition). In addition, this set of compounds was profiled for antiproliferative activity utilizing the National Cancer Institute (NCI) 60 cancer cell line assay, which included both A549 and K562 cell lines. All of the compounds were found to be noncytotoxic at 10 μM as determined by their effect on cell growth (mean inhibition of each compound against 60 cell lines was <5%).[14]

In summary, a divergent approach was developed for the synthesis of gombamide A providing the natural product in 5.7% overall yield in 16 steps starting from *N*-Boc-L-Cys(Trt)-OH (**4**). Two key features of the synthesis were iodine-mediated oxidative disulfide bond formation to generate the versatile cyclic peptide carboxylic acid **2** and introduction of the 4-hydroxystyrylamide late in the synthesis via a Grieco elimination procedure. The utility of this methodology was further illustrated with the synthesis of several gombamide A derivatives. The natural product and four analogs (**2**, **24**, **27**, and **29**) were shown to be devoid of Na$^+$/K$^+$-ATPase activity at 10 μM. Finally, these five compounds were not cytotoxic against a panel of cancer cell lines at 10 μM, which should encourage exploration of this chemotype for nononcology pharmacological applications.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b02379.

Description of the detailed experimental procedures and NMR spectral data (PDF)

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank the University of Houston for financial support and the National Cancer Institute for testing the compounds in the 60 cell line assays.

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