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Total Synthesis of Gombamide A

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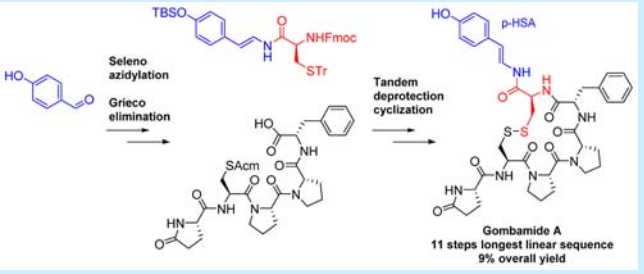
Supporting Information

ABSTRACT: The first total synthesis of Gombamide A (**1**), a cytotoxic cyclic thiopeptide from the sponge *Clathria gombawuiensis*, has been achieved. Highlights of the convergent synthesis feature a disulfide bond forming cascade to close the 17-membered macrocycle and a selenoazidylation procedure to access the unusual *para*-hydroxystyrlyamide (*p*HSA) moiety. The synthesis required 18 steps, 11 steps in its longest linear sequence, and proceeded in 9.1% overall yield. This work will facilitate the study of the biological effects of Gombamide A and provide groundwork to explore the structure−activity relationship around this rare natural product.



Gombamide A (**1**, Figure 1) was recently isolated from the red encrusting marine sponge *Clathria gombawuiensis*. The

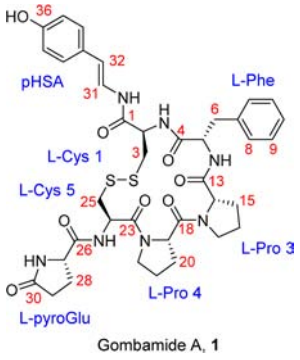


Figure 1. Gombamide A, **1**, a thiohexapeptide with unusual amino acids and a rare disulfide-containing, 17-membered macrocycle.

cyclic hexapeptide **1** displayed modest cytotoxic activity against K562 chronic myelogenous leukemia and A549 epithelial lung carcinoma cell lines (LC_{50}s of 6.9 and 7.1 μM, respectively), along with weak inhibitory activity for Na+/K+-ATPase (IC_{50} = 17.8 μM).[1]

Notably, **1** presents a number of unique chemical features in its structure. Gombamide A is a rare, 17-membered macrocyclic thiohexapeptide with two unusual exocyclic substitutions: an (*E*)-*para*-hydroxystyrylamide (*p*HSA) and an L-pyroglutamic amide (pyroGlu). The O-substituted *p*HSA feature has been observed forming part of macrocyclic peptides, primarily in 14-membered cyclopeptides in the *Z* configuration,[2] while the only *E* example has been found in the bastadins.[3] The uncapped *p*HSA has only been reported in small, linear natural products,[4] and **1** is the sole reported molecule where this moiety is present in a cyclic peptide. There are few examples of cyclic peptides with disulfide linkages originating from sponges.[5] Moreover, the 17-membered ring thiopeptide core has only been found in the eudistomide lipopeptides[6] and in the malformins,[7] metabolites from *Aspergillus niger* with antibiotic and cytotoxic activity. An homologue 18-membered macrocycle is present in antitumor antibiotics related to quinomycin, DNA polymerase, and topoisomerase inhibitors.[8] Quinomycin and malformins are bicyclic structures, and one could argue that the monocyclic Gombamide A would be a simplified pharmacophore for cytotoxic activity. In order to perform experiments to explore the biological effects and elucidate the cytotoxic mechanism of action of **1**, enough material must be available. An efficient chemical access of **1** is likely the best option, as **1** is produced in scarce amounts in *C. gombawuiensis*, making its isolation prohibitive. Also, we believed that a natural product guided synthesis would pave the way for structure−activity relationship (SAR) studies around **1** that can lead to the optimization of its biological activity. Therefore, synthetic intrigue and interest to further explore the biology of this unique thiopeptide motivated us to embark on the total synthesis of Gombamide A.

In our retrosynthetic analysis (Scheme 1), we proposed to install the disulfide bond from two protected cysteine residues within an acyclic peptide; enabling a deprotection/disulfide bond forming cascade to close the 17-membered macrocycle. Further amide cleavage at the L-Cys 1 and L-Phe bond liberates Fragment A (**2**) and Fragment B (**3**), enabling a convergent synthetic approach. Fragment A (**2**) would be readily constructed from a linear series of amide couplings employing protected variants of L-pyroglutamic acid, L-phenylalanine, L-cysteine, and L-proline. We envisioned multiple strategies to access Fragment B (**3**) and form the enamide in *p*HSA, including dehydration of octopamine derived amide **4**, copper-catalyzed amidation of **7** with **8** and Grieco elimination on substrate **9**. In the latter, **9** will be derived from the amide coupling of a protected L-cysteine **6** and the α-

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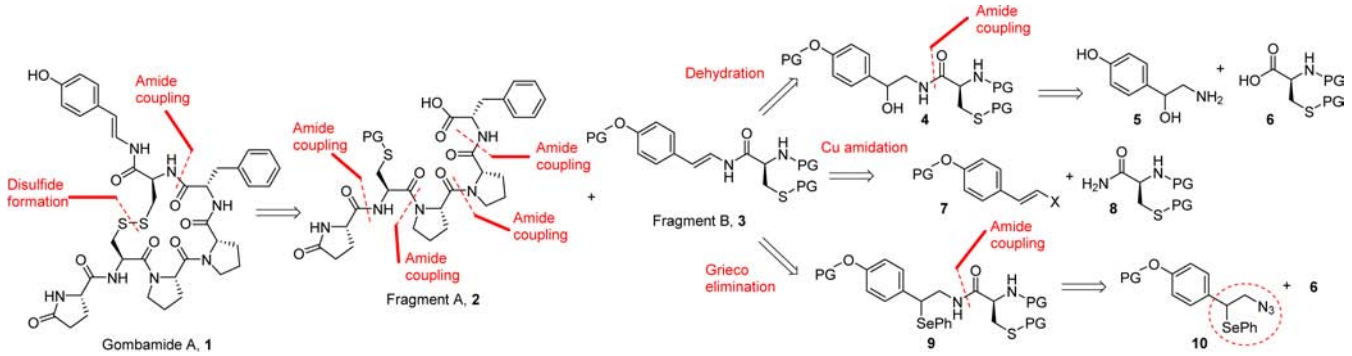
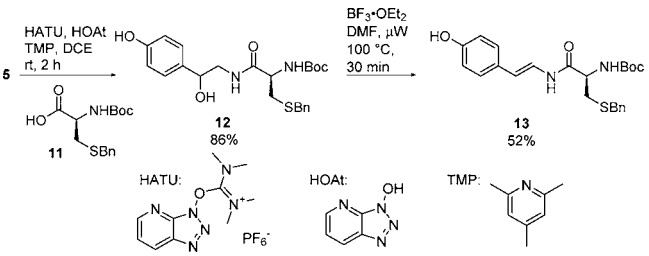
Scheme 1. Retrosynthetic Analysis of Gombamide A, 1



seleno amine produced from **10**, an intermediate generated from a selenoazidylation of a protected *p*-hydroxystyrene.

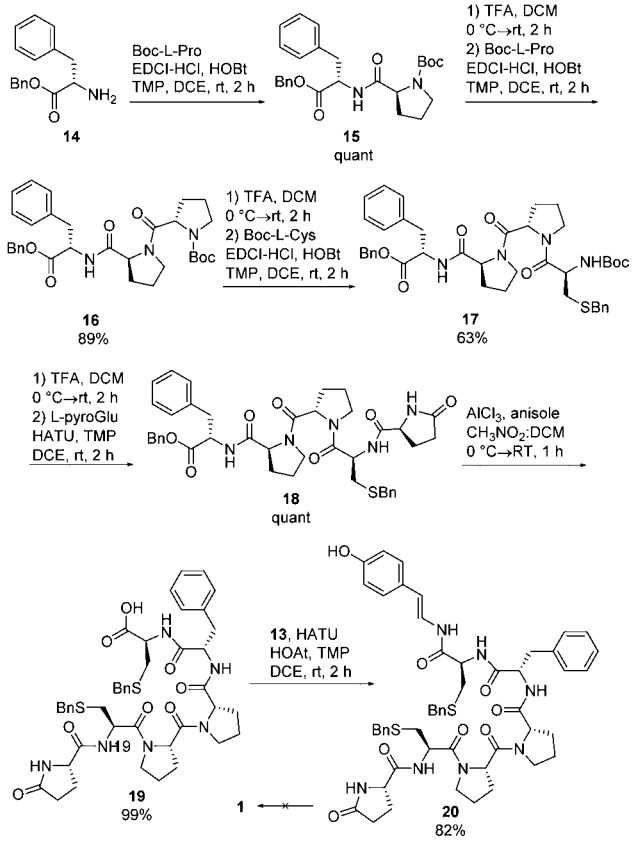
Initially, we pursued the dehydration approach for the synthesis of **3** (Scheme 2). Here, HATU-coupling of (±)-octopamine **5** with **11**, led to the isolation of **12** in 86% yield, without the need for protecting group manipulation of the alcohols. The stability of the benzylthioether protecting group allowed us to explore a wide range of dehydration conditions. Burgess reagent and Martin sulfurane did not provide desired dehydration product **13**, using different equivalents and temperatures. Numerous conditions such as trifluoroacetic acid (TFA), trifluoroacetic anhydride, *p*-toluensulfonic acid, *p*-toluensulfonic anhydride, and *p*-toluensulfonyl chloride in pyridine or thionyl chloride in pyridine led to either no conversion or decomposition of the starting material. Finally, we were able to effectively achieve this transformation by the use of $BF_3{\cdot}OEt_2$ in *N*,*N*-dimethylformamide (DMF), a procedure previously used in the synthesis of mekaluvamine E.[9] In our case, the reported conditions (entry 1, Table S1) affected the desired dehydration with concomitant Boc-deprotection of **12**, but only in trace amounts. In an effort to optimize this conversion, we sought more mild conditions varying temperature and Lewis acid equivalents. The use of 5 equiv of $BF_3{\cdot}OEt_2$ allowed the isolation of **13** in 27% yield (entry 2, Table S1); while lowering the temperature led to trace amounts of the product. A great improvement was observed when heating the reaction in the microwave (entry 6, Table S1), as the yield increased almost 2-fold to 52% after only 30 min, allowing an efficient way to access **13**.

Scheme 2. Synthesis of 13



With amide **13** in hand, attention now focused on assembling fragment A. The route to compound **19** began with an EDCI/HOBt-mediated coupling between L-Phe-OBn **14** and Boc-L-Pro to provide **15** in quantitative yield (Scheme 3). TFA deprotection of the Boc group and a second EDCI/HOBt-mediated coupling with another Boc-L-Pro delivered tripeptide **16** in 89% yield over two steps. Subsequent TFA deprotection of the Boc group and an EDCI/HOBt-mediated coupling with Boc-L-Cys-SBn **11** gave tetrapeptide **17** in 63% over two steps. Repetition of the same two-step sequence with **17** but employing L-pyroglutamic acid afforded the key pentapeptide **18** in quantitative yield for the two-step deprotection/coupling sequence. Benzyl deprotection to give the desired fragment **19** proved challenging, as the majority of standard conditions were not compatible with the sulfur containing starting material, due to its ability to poison palladium-based deprotection conditions and loss of optical purity during basic hydrolysis. Ultimately, we found that Lewis acid conditions ($AlCl_3$, anisole, CH_3NO_2/DCM) were effective, generating key fragment **19** in 99% isolated yield.

Scheme 3. Synthesis of Acyclic Intermediate 20



The amide coupling between fragments **13** and **19** proceeded smoothly to yield intermediate **20** in 82%, which, once deprotected, should cyclize to give **1**. Unfortunately, attempts for deprotection/cyclization using iodine,[10] CH_3SiCl_3/Ph_2SO in

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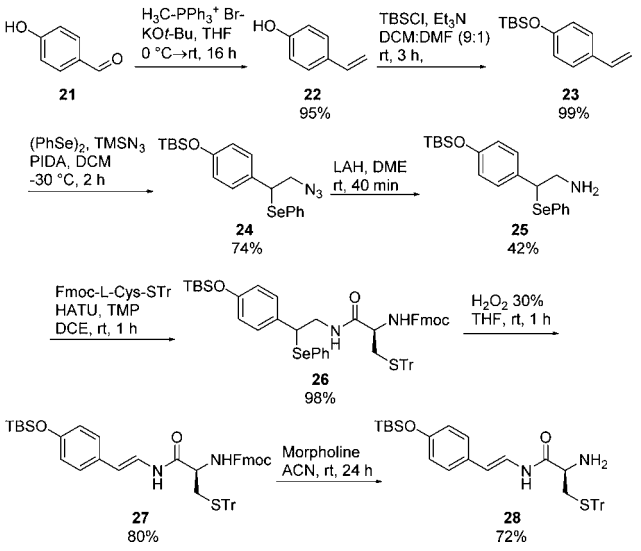
TFA,[11] or sodium in liquid ammonia[12] failed to provide the natural product. Due to the difficulties found in the deprotection of the benzylthioether, we tried different more labile protecting groups, including acetamidomethyl, *p*-methylbenzyl, *p*-methoxybenzyl, and trityl groups; however, none of these were compatible with the dehydration conditions previously employed to obtain **13**, thus impeding the evaluation of the new protecting groups in the cyclization reaction.

Exploring other routes outlined in our retrosynthesis (Scheme 1), we tried the copper-catalyzed amidation to obtain Fragment B in similar fashion to Porco's synthesis of antitumor macrolide lobatamide C.[13] To assess this alternative, a S-Acm protected carboxamide **8** was prepared and the amidation of *E*-4-(2-halidevynyl)phenol **7** attempted. The reaction was performed with the bromide and iodide vinyl halides and also their TBS phenoxysilane protected versions; however, none of the substrates yielded the desired product after using different copper sources and ethylendiamine ligands.

Efforts then shifted toward the preparation of the required intermediates to pursue the Grieco elimination procedure to access the unusual *p*HSA moiety of **1** (Scheme 4). First, using the

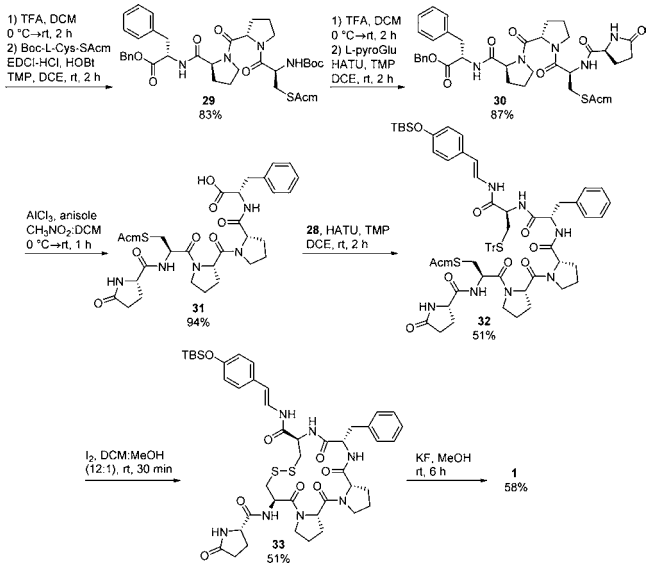
Scheme 4. Synthesis of Enamide 28



amide derived from **5** and Boc-L-Cys-S-Acm, we tried to substitute the alcohol with the selenide, which has been typically used to generate endocyclic *Z* olefins in cyclic peptides,[14] but this approach led to no satisfactory result. Therefore, we envisioned introducing the selenide prior the amide coupling. Here, a Wittig reaction using *p*-hydroxybenzaldehyde **21** provides styrene **22** in 95% yield, and a subsequent TBS protection of the phenol proceeded in quantitative yield to deliver **23**. An anti-Markovnikov regiospecific selenoazidylation, using phenyliodo-(III) diacetate (PIDA), NaN_3, and $(PhSe)_2$,[15] afforded the key α-seleno azide **24**, in low yields (30−40%) and with poor scalability. The replacement of NaN_3 with azidotrimethylsilane,[16] rendered the reaction homogeneous, more robust to scale up, and improved the yield to 74%. LAH reduction of **24** produced the corresponding α-seleno amine **25** in 42% yield, similar results were obtained with Staudinger reaction conditions. A HATU-mediated amide coupling reaction between **25** and Fmoc-L-Cys-STr generated the elaborated scaffold **26**, poised for the key Grieco elimination. Treatment of **26** with hydrogen peroxide in THF smoothly delivered the protected **27** in 80% yield, without observable production of the *Z*-enamide. The selection of the trityl group was essential for this reaction as it prevented the overoxidation of the cysteine, a feature that was observed with its Acm protected congener; also, the steric bulk of this protecting group probably contributes to the selective formation of the *E* enamide. Removal of the Fmoc group of **27** to afford **28** proved difficult as classical conditions (20% piperidine in DMF) led to complex mixtures. We found that **28** was base sensitive, and that ultimately morpholine, a weaker base ($pK_a \approx 8$), was effective in removing the Fmoc protecting group cleanly and in good isolated yield (72%).

With **28** in hand, attention now focused on assembling fragment A and producing the acyclic precursor of **1** (Scheme 5).

Scheme 5. Synthesis of 1



Starting with compound **16**, Boc deprotection followed by amide coupling with Boc-L-Cys-S-Acm afforded **29** in 83% yield in two steps. Subsequent TFA deprotection and HATU-mediated coupling with L-pyroglutamic acid yielded 30 (87%). $AlCl_3$-facilitated benzyl deprotection produced **31** in 94% yield. Finally, **28** and **31** were coupled using HATU conditions providing acyclic, bis-cysteine protected, OTBS-hexapeptide **32** in 51% yield. This set the stage for the disulfide bond forming cascade to close the 17-membered macrocycle and to deliver **1** for the first time.

We envisioned an oxidative cyclization protocol employing molecular iodine[9] wherein the S-Trityl and S-Acm protecting groups would be removed, followed by disulfide formation; as their removal conditions should be milder with respect to the benzyl thioether. The conversion of **32** to the TBS-protected congener of **1** required optimization, as both the equivalents of I_2 and concentration were key reaction parameters (Table S3). Under optimized conditions, 5.0 equiv of I_2 in DCM/MeOH (12:1) (0.0008 M) provided **33** in 51% yield (entry 2, Table S3). Deprotection of the phenolic TBS moiety also proved nontrivial, as standard conditions caused product degradation (TBAF or HCl/MeOH). Finally, HF-pyridine or KF in MeOH proved effective, the latter being faster (6 h to completion, compared to 24 h), thus providing, for the first time, Gombamide A in 58% yield.

In summary, we have completed the first total synthesis of gombamide A (**1**), employing a convergent synthetic route that

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required 18 synthetic steps, with an overall yield of 2.5% from *p*-hydroxybenzldehyde **21** (10 linear step sequence), and 9.1% from benzyl L-phenylalaninate **14** (11 linear step sequence). This synthetic route gives access to Gombamide A, allowing further characterization of its biological properties.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b01825.

Experimental procedures, characterization data, and ^{1}H and ^{13}C NMR spectra for new compounds (PDF)

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Notes

The authors declare no competing financial interest.

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