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High Yielding Synthesis of 3*a*-Hydroxypyrrolo[2,3-*b*]indoline Dipeptide Methyl Esters: Synthons for Expedient Introduction of the Hydroxypyrroloindoline Moiety into Larger Peptide-Based Natural Products and for the Creation of Tryptathionine Bridges.

Jonathan P. May,[†] Pierre Fournier,[†,‡] Jonathan Pellicelli,[†] Brian O. Patrick,[†] and David M. Perrin*,[†]

Department of Chemistry, University of British Columbia, Vancouver, B.C. V6T 1Z1, Canada

dperrin@chem.ubc.ca

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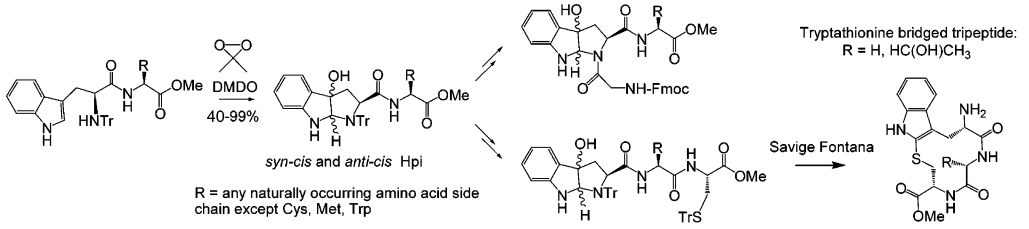

This work describes a rapid and high yielding oxidation of 14 tryptophanylated amino acid methyl esters to the corresponding 3*a*-hydroxypyrrolo[2,3-*b*]indoline (Hpi) amino acids with generally facile separation of *syn-cis* and *anti-cis* diastereomers. Structural X-ray diffraction data are presented for both diastereomers of Tr-Hpi-Gly-OMe, which allow for a putative assignment of the other 13 pairs of diastereomers reported herein, based on correlations with ^{1}H NMR chemical shifts. Selective and high yielding deprotection at either the N or C terminus is described, allowing the Hpi motif to be introduced efficiently into potential targets with minimal protecting group manipulation. Two tripeptides containing Hpi and cysteine were prepared and treated with acid in the Savige–Fontana reaction to produce a cyclic tryptathionine linkage, characteristic of both amatoxins and phallotoxins.

Introduction

The 3*a*-hydroxypyrrolo[2,3-*b*]indoline (Hpi) structural motif is of pharmaceutical interest, as it is found in a variety of natural products that include the sporidesmins,[1] brevianamides,[2] okaramines,[3,4] phakellistatins,[5] gypsetins,[6] and himastatins (Figure 1).[7–9] It is also closely related to several other pharmacophores by replacement of the 3*a*-hydroxyl with either a proton[10,11] or an alkyl group (e.g., roquefortines,[12,13] flustramines,[14] and amauromines[15]), or by insertion of an oxygen atom into the indole C2–N1 bond, as in paeciloxazine.[16] Accordingly,

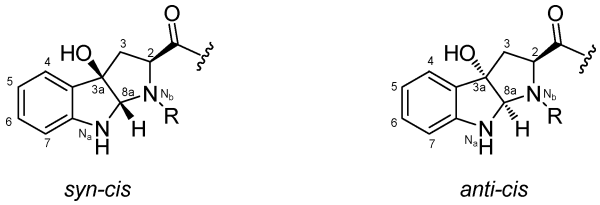

FIGURE 1. The structure of the *syn-cis* and *anti-cis* diastereomers of the 3*a*-hydroxypyrrolo[2,3-*b*]indoline (Hpi) core.

* Address correspondence to this author. Fax: +1 604 822 2847. Phone: +1 604 822 0567.

† University of British Columbia.

‡ Present address: Institute of Pharmacy and Molecular Biotechnology, University of Heidelberg, Im Neuenheimer Feld 364, 69120 Heidelberg, Germany

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there has been considerable interest in developing synthetic methods that permit facile and high yielding introduction of this motif into these structures and related analogues (vide infra).

Shioiri[17] and Christophersen[18] used the photosensitizer Rose Bengal in the presence of molecular oxygen to obtain Hpi methyl esters and dipeptides, albeit with rather low yields. In the synthesis of phakellistatin 3, Van Vranken and co-workers used this method to induce Hpi formation in a prefabricated peptide, but again they experienced both low yields and difficult separation of their final products.[5] The most direct route to 3*a*-hydroxypyrroloindoles is by a one-pot oxidation of the indole 2−3 double bond of tryptophan and intramolecular attack of the α-NH_2 at the indole 2-position. In the past this was achieved by using *m*CPBA,[19] but the yields were poor, several byproducts were produced, and purification proved troublesome.

Danishefsky and co-workers described a more successful approach en route to himastatin.[9] In their report, tryptophan, when suitably protected, was oxidized using dimethyldioxirane (DMDO). Numerous combinations of protecting groups were investigated with regards to achieving high yields and diastereomeric excess, but oxidation was most successful with tryptophan protected at the α-NH_2 and α-COOH by a trityl and a *tert*-butyl ester, respectively. With proper protection, DMDO oxidation produced exclusively a *syn-cis* stereochemistry in the resultant Hpi. Acid deprotection of the trityl moiety afforded the Hpi-*tert*-butyl ester over two steps (55% total yield). In their synthesis, selective deprotection of the *tert*-butyl ester required several more steps to avoid destruction of the acid-labile Hpi moiety. These included transient silylation of the 3*a*-OH and reprotection of N_b following detritylation, ultimately enabling incorporation of Hpi into himastatin.

Most recently, Ley and co-workers described the elegant synthesis of 3*a*-hydroxypyrroloindoles via oxidative deselenation of the corresponding 3*a*-phenylselenopyrroloindole. This was prepared by oxidative phenylselenation from fully protected tryptophan (N_α-Z, N_{indole}-Boc, C_α-OMe) with concomitant cyclization to the pyrroloindole.[20] Yields and stereocontrol depended on the N-protecting groups employed. Oxidative deselenation with *m*CPBA subsequently afforded several different N-protected Hpi methyl ester products (over 4−6 steps depending on the extent of protection).

Van Vranken and co-workers attempted a similar oxidative phenylselenation of tryptophan contained within a heptapeptide as a means of directly generating the Hpi motif in phakellistatin-like structures, but a mixture of

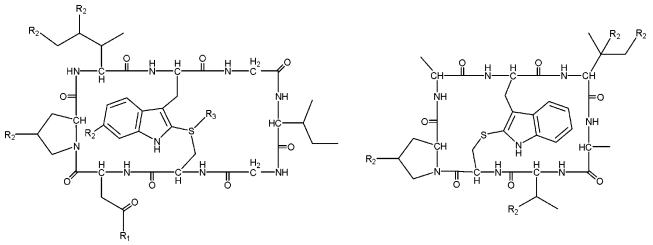

FIGURE 2. General structures of amatoxins (left) and phallotoxins (right). R_1 is either an OH or an NH_2, R_2 and R_3 represent hydroxylated and oxidized variants found in naturally occurring toxins and synthetic analogues.

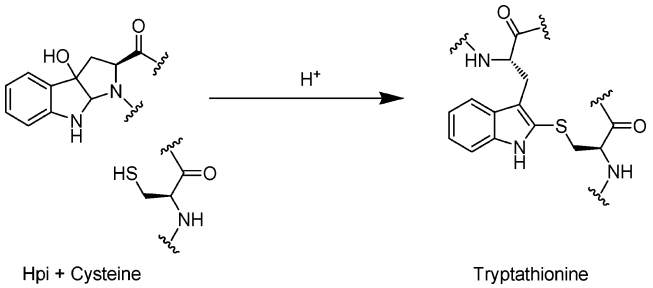

FIGURE 3. The acid-catalyzed Savige−Fontana reaction affording a tryptathionine linkage.

10 products was obtained.[5] In summary, the generation of Hpi often has required a rather elaborate and extensive use of protecting groups.

Whereas considerable effort has been devoted to methods for preparing and manipulating the Hpi core en route to the synthesis of the aforementioned highly bioactive compounds, it is worth noting that the Hpi core also provides an entrée into two other classes of highly potent natural products: the amatoxins[21,22] and phallotoxins (Figure 2). These are chemically similar bicyclic octa- and heptapeptides that bind extremely tightly to unrelated protein folds of RNA polymerase and actin, respectively.[23,24] A significant degree of this extraordinary potency is derived from a pre-ordered rigidity imparted by the characteristic tryptathionine cross-link.

This linkage may be created under acidic conditions by intramolecular condensation of a thiol (e.g., Cys) with the Hpi motif via the Savige−Fontana reaction (Figure 3).[19,25] Interest in such peptides is underscored in a recent publication from the Guy lab that described a synthesis of phallotoxins. Instead of employing the Savige−Fontana condensation following introduction of the Hpi core, they used Wieland's electrophilic condensation of tryptophan with cysteine sulfenyl chloride to create the desired tryptathionine. This necessitated four-way orthogonal protection for directional introduction in peptide synthesis.[24] Previously, Zanotti and co-workers have discussed the solution phase peptide synthesis of aman-

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itin and phalloidin derivatives by means of Savige–Fontana chemistry.[22,26]

Our interest in these bicyclic peptide structures, particularly with regards to a combinatorial array of such, led us to consider the synthesis of Hpi in the context of functionalized peptide structures and its ultimate use in the Savige–Fontana condensation. Herein we report a robust synthesis of 3*a*-hydroxypyrrolo[2,3-*b*]indoline derivatives prepared from Tr-Trp-Xaa-OMe dipeptides, by oxidation with DMDO. This reaction was shown to be compatible with dipeptides where Xaa could be any one of a number of amino acids, which were cleanly oxidized to the desired tricyclic products in high yield. Characterization of *syn-cis* and *anti-cis* diastereomers for each product was possible with X-ray crystallography and NMR. Efficient deprotection and subsequent coupling at both the N and C termini provides a reliable method for introducing the Hpi moiety into various pharmaceutical targets, either in solution phase or via linear solid-phase synthesis. Finally, Savige–Fontana cyclizations were performed on Tr-Hpi-Xaa-Cys(Tr)-OMe tripeptides to yield a cyclic tryptathionine, demonstrating the use of a tryptathionine bridge as a general rigidification strategy.[27] In contrast to the aforementioned investigations, our method avoids the extensive protecting group manipulations that are needed to install either an Hpi or a tryptathionine linkage.

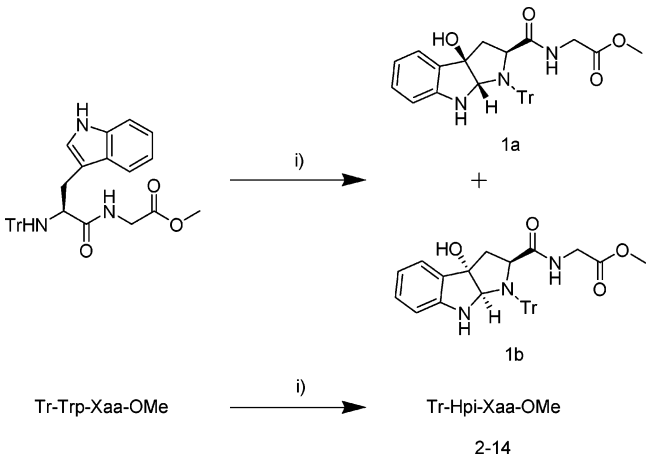
Results and Discussion

Based on the results of Danishefsky's work[6–9] we opted for DMDO oxidation to prepare the Hpi moiety from Tr-Trp-O^{t}Bu because it was found to provide the highest chemical yields. Nevertheless, to render the Hpi moiety compatible with standard peptide synthesis protocols, deprotection of the trityl and *tert*-butyl groups would be required. We were not inclined to reprotect the detritylated N_b of the pyrroloindoline in order to cleanly reveal the α-COOH. In accord with Kamenecka's extensive study of different protecting groups,[9] it was apparent that several additional steps (i.e., reprotection of N_b and transient silylation of the 3*a*-OH) would be required to afford a properly protected Hpi synthon suitable for peptide synthesis.

With the goal being to introduce the Hpi into a linear peptide fragment, ultimately for the creation of amatoxin and phallotoxin derivatives, we examined whether a tryptophanylglycine methyl ester (Tr-Trp-Gly-OMe) would cleanly undergo DMDO oxidation to afford the corresponding Tr-Hpi-Gly-OMe, thus obviating the need for α-COOH protection of tryptophan. Since glycine is found at the adjacent position in amanitin, DMDO oxidation of the dipeptide would presumably conform to a linear peptide synthesis strategy. As anticipated, DMDO treatment of Tr-Trp-Gly-OMe at low temperature (−78 °C) afforded clean conversion to Tr-Hpi-Gly-OMe in 70% yield for the two diastereomers **1a** and **1b** (Scheme 1).

Given precedence for diketopiperazines and N_α-Boc-Trp to undergo cyclization with attack of the Boc-NH to form the pyrrolidine ring,[6] we were concerned that the glycinamide NH might have competed with the more sterically hindered NH-trityl to give a six-membered ring. In such a scenario, the two observed products would have been constitutional isomers rather than diastereomers. X-ray diffraction data showed that only diastereomeric Hpi products were observed. Thus for the reaction herein, the nucleophilicity of an NH-trityl predominates over its considerable steric bulk.

SCHEME 1[a]



[a] Reagents and conditions: (i) DMDO/acetone, DCM, −78 °C, ~1 h. Where Xaa = Ala, Asn(Tr), Glu(^{t}Bu), His(Tr), Ile, Leu, Lys(Boc), Phe, Pro, Ser(^{t}Bu), Thr(^{t}Bu), Tyr(^{t}Bu), and Val.

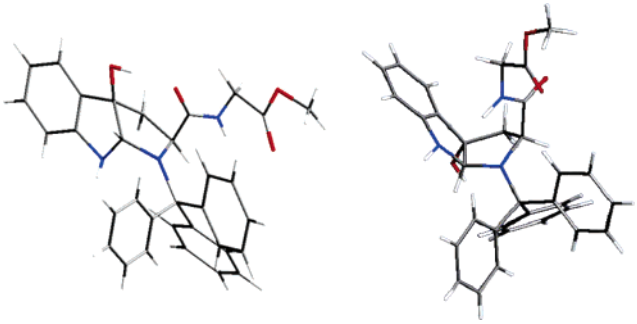


FIGURE 4. Crystal structures of the Tr-Hpi-Gly-OMe **1a** *syn-cis* (left) and **1b** *anti-cis* (right) diastereomers. Crystals were grown from EtOAc/hexanes.

In the case of Tr-Trp-Gly-OMe the oxidation proceeded in good yield, but with poor diastereoselectivity, giving both *syn-cis* (**1a**) and *anti-cis* (**1b**) diastereomers. Although separation of these diastereomers by silica gel chromatography proved rather difficult (ΔR_f < 0.05, EtOAc/hexane 1:1), pure samples of each were obtained and crystal structures were acquired (Figure 4). Both diastereomers could be used without resolution for the Savige–Fontana reaction because the stereochemistry is abolished during the synthesis of a tryptathionine linkage. Nevertheless, in light of the high yields we obtained along with feasible separation, we characterized these diastereomers further, given the general interest in preparing various Hpi-containing targets.

These structures show the contrasting conformations of each diastereomer, both **1a** and **1b** have cis geometry about the C_{3a}–C_{8a} fused bond, but the orientation of the indole moiety appears to be considerably more sterically congested in the *anti-cis* conformation. In the *syn-cis*

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TABLE 1. Tr-Hpi-Xaa-OMe Dipeptides[a]

no.	name	yield (%)	H_{8a}	NHCO
1a	Tr-Hpi-Gly-OMe	35	5.54	5.95
1b		35	5.36	9.21
2a	Tr-Hpi-Ala-OMe	28	5.52	5.64
2b		25	5.39	9.12
3a	Tr-Hpi-Asn(Tr)-OMe	59	5.54	6.65
3b		38	5.35	9.51
4a	Tr-Hpi-Glu(tBu)-OMe	20	5.53	6.09
4b		25	5.40	9.18
5a	Tr-Hpi-His(Tr)-OMe	60	5.42	9.82
5b		0	-	-
6a	Tr-Hpi-Ile-OMe	29	5.64	5.74
6b		61	5.30	8.92
7a	Tr-Hpi-Leu-OMe	40	5.60	5.60
7b		30	5.35	9.00
8a	Tr-Hpi-Lys(Boc)-OMe	35	5.55	6.11
8b		40	5.43	9.12
9a	Tr-Hpi-Phe-OMe	11	5.66	5.62
9b		32	5.31	9.11
10a	Tr-Hpi-Pro-OMe	84	5.49	N/A
10b		0	-	-
11a	Tr-Hpi-Ser(tBu)-OMe	42	5.68	6.04
11b		40	5.28	9.12
12a	Tr-Hpi-Thr(tBu)-OMe	30	5.68	5.95
12b		45	5.43	9.09
13a	Tr-Hpi-Tyr(tBu)-OMe	26	4.92	5.45
13b		21	5.29	7.91
14a	Tr-Hpi-Val-OMe	48	5.62	5.85
14b		44	5.33	8.97

[a] Isolated yields of cyclization and characteristic ^{1}H chemical shifts for the *syn-cis* (**a**) and *anti-cis* (**b**) diastereomers.

conformation there is a hydrogen bond between the 3*a*-hydroxyl and the carbonyl oxygen of the amide bond, whereas the orientation of the *anti-cis* amide is twisted through 180° away from the pyrroloindole core. These dramatic differences may in part account for the apparent trends of the amide NH shift in the ^{1}H NMR (vide infra).

We also investigated the generality of this reaction by examining a variety of tryptophanylated amino acids. Appropriately protected dipeptides (Tr-Trp-Xaa-OMe) were synthesized and each was exposed to the DMDO reaction conditions described. We observed good conversion for all dipeptides studied (compounds **2**−**14**, 40−99% for two diastereomers). For the majority of the dipeptides, a mixture of diastereomers was observed, but a clear preference for a single diastereomer was found in two instances when Xaa = Pro or His(N_τTr) (Table 1). Separation of all diastereomers was considerably easier than previously with **1a/1b**, and all products were well resolved during purification. Although this approach appears to be fairly general, we would assume DMDO oxidation is incompatible in cases where Xaa = Trp, Cys(Tr), and Met, as these side chains are readily oxidized by DMDO. Not investigated in this study were three other dipeptides where Xaa = Arg, Asp, and Gln, due to acknowledged difficulty with the solubility of the former, and a generally recognized functional group redundancy of the two latter amino acids as Glu (**4**) and Asn (**3**) were investigated.

Assignment of each dipeptide as either the *syn-cis* or the *anti-cis* diastereomer was possible by comparison of the R_f, ^{1}H NMR chemical shift, and crystal structures of the Tr-Hpi-Gly-OMe dipeptides. In the case of Tr-Trp-Pro-OMe the *syn-cis* diastereomer was given exclusively (**10a**). Some indication of steric congestion was found in the crystal structures of this dipeptide, suggesting that large groups at the C-terminus of Hpi will favor the less constrained *syn-cis* diastereomer (see the Supporting Information for this structure). In this case, the chemical shifts and R_f values were consistent with the observed trend that guided the assignment. The single diastereomer of Tr-Hpi-His(Tr)-OMe (**5a**) formed was also thought to have *syn-cis* geometry, but this cannot be undeniably confirmed without X-ray crystallography data. Possible ways to control the stereospecificity of this reaction were briefly considered, but attempts at using chiral equivalents of DMDO, such as those described by Shi et al., were unsuccessful (data not shown).[28,29] Further studies were not pursued because both diastereomers were equally valuable for the Savige−Fontana reaction.

Not only did we wish for a high yielding synthesis of the Hpi moiety, but we also sought an efficient incorporation of this motif into linear peptides with a view to coupling the Hpi-Xaa dipeptide at either terminus. The protecting groups we chose allow for high yielding and orthogonal deprotection of the Tr-Hpi-Xaa-OMe dipeptide. Efficient deprotection of the trityl moiety with HFIP (hexafluoro-2-propanol) in DCM (1:4) for 15 min (Scheme 2) yielded the diastereomers **15a** (40%) and **15b** (30%), which were easily separated by silica gel chromatography. Unfortunately, the yield was a little lower than what is normally expected for a deprotection due to the inherent acid sensitivity of the Hpi moiety. This mild deprotection removed the trityl protection on the Hpi selectively over other acid labile groups, even when other trityl protected amino acid side chains are present in the molecule (e.g., Cys(Tr) and Asn(Tr), data not shown). These H-Hpi-Xaa-OMe dipeptides could then be extended by coupling amino acids at N_b. Although similar couplings have been reported to be problematic,[20] coupling of Fmoc-Gly-OH with PyBOP yielded a clean tripeptide product **16** in good yield from **15a**. Nevertheless, the amide bond introduced at this position displayed strong cis−trans atropisomerism, and hence ^{1}H NMR spectra in $CDCl_3$ displayed severe line-broadening, consistent with similar structures previously reported by Ley et al.[20]

Saponification of the C-terminal methyl ester is rapid and efficient with minimal decomposition (Scheme 3); compounds **1a/b** and **12a** were saponified with LiOH in quantitative yield to give the free acids, which were purified as triethylammonium salts (**17a/b** and **18a**). Coupling of *S*-trityl cysteine methyl ester to the C-terminus of **17a/b** and **18a** with DCC/HOBt afforded the two Tr-Hpi-Xaa-Cys(Tr)-OMe tripeptides with Xaa = Gly (conformationally flexible) and Thr(^{t}Bu) (sterically encumbered) as the variable amino acid (**19a/b** and **20**). This demonstrated the feasibility for functionalizing the C-terminus of the Tr-Hpi-Xaa-OH, while also forming a precursor for the Savige−Fontana cyclization. Subsequent treatment with TFA at room temperature removed trityl protecting groups and produced the corresponding cyclic products (**21**, **22**), both of which displayed a λ_{max} of 292 nm characteristic of the tryptathionine linkage. The yields for these cyclic structures were low (16−54%), but are similar to previous reports on cyclic tryptathionine

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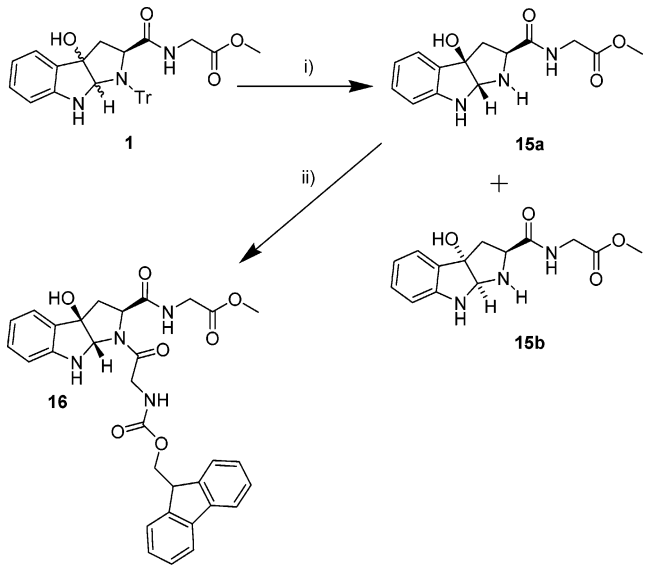
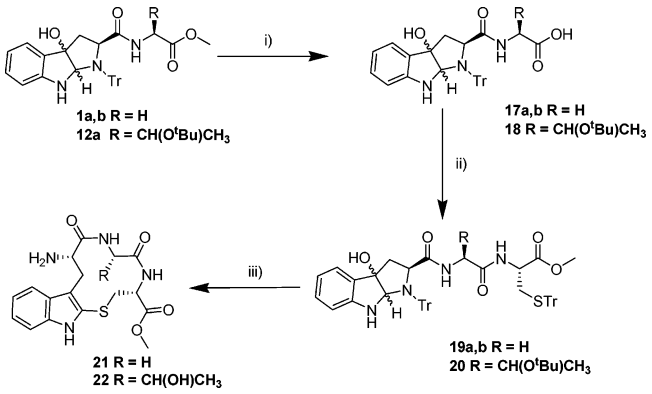
SCHEME 2[a]



[a] Reagents and conditions: (i) HFIP/CH_2Cl_2 (1:4), rt, 30 min (**15a**: 40%, plus other diastereomer; **15b**: 30%); (ii) Fmoc-Gly-OH, PyBOP, HOBt, DIPEA, DMF, rt, 4 h (44%).

SCHEME 3[a]



[a] Reagents and conditions: (i) LiOH, dioxane/H_2O (2:1), rt, 1 h; (ii) H-Cys(Tr)-OMe, DCC, HOBt, NEt_3, DCM, 0 °C → rt, 15 h; (iii) TFA, rt, 5 h.

compounds and comparable with other non-main chain cyclizations.[27,30]

Conclusion

An efficient, high yielding, and versatile synthesis of Tr-Hpi-Xaa-OMe dipeptides is described where Xaa = 14 different L-amino acids. In certain cases, high diastereomeric excess is observed in the oxidation with DMDO, but the majority form readily separable mixtures of the *syn-cis* and *anti-cis* diastereomers in relatively equal amounts. These studies have demonstrated that this oxidation is compatible with a large range of natural amino acids, excepting those with indoles and sulfur atoms, and highlights the generality of this reaction for the synthesis of an array of Hpi containing peptides. Direct oxidation of the Trp-Xaa dipeptide obviates multiple protections and deprotections needed to afford an Hpi. In just three steps from unprotected tryptophan, good to excellent yields of both the *syn-cis* and *anti-cis* diastereomers of Hpi can be isolated in pure form. Although stereocontrol was not investigated, protecting group use is minimal, yields for each Hpi-Xaa diastereomer are relatively high, and products are easily separated. Thus, the approach herein represents a simple synthesis of Hpi synthons suitable for incorporation into larger peptides or natural products. Efficient, orthogonal deprotections are described for both the N- and C-termini of the Hpi containing dipeptide. Subsequent peptide coupling of these Hpi-Xaa dipeptides is described for both N- and C-termini, yielding protected tripeptides. Two Tr-Hpi-Xaa-Cys(Tr)-OMe tripeptides were also prepared and Savige−Fontana cyclizations were performed to yield cyclic tryptathionine containing structures. The apparent generality of the Savige−Fontana reaction would suggest utility for this biomimetic rigidification strategy in the generation of a combinatorial array of bicyclic peptides, inspired by two of Nature's extremely potent toxins: amanitin and phalloidin.

Experimental Section

General Method for Preparation of Hpi Derivatives. To a solution of the appropriate side chain protected Tr-Trp-Xaa-OMe (0.10 mmol) in dry CH_2Cl_2 (1 mL) at −78 °C was added a solution of DMDO in acetone (0.11 mmol, 1.1 equiv).[31] After 15 min the mixture was concentrated to dryness under reduced pressure at room temperature. The crude material was purified by column chromatography on pretreated (NEt_3) silica gel (hexanes/EtOAc/NEt_3 80:20:1 to 60:40:1) to give fast-running product **a** (*syn-cis*) and a slow running product **b** (*anti-cis*).

***N*1-Trityl-3*a*-hydroxypyrrolo[2,3-*b*]indolylglycine Methyl Ester (*syn-cis*) (1a).** R_f (hexane/EtOAc, 1:1) 0.4, white solid, 0.02 g, 35% yield. ^{1}H NMR ($CDCl_3$) δ: 7.50 (d, 6H, J = 6.8 Hz, Ar**H**^{Tr}), 7.48−7.18 (m, 10H, Ar**H**), 7.07 (t, 1H, J = 7.6 Hz, C**H**^{6}), 6.73 (t, 1H, J = 7.6 Hz, C**H**^{5}), 6.38 (d, 1H, J = 7.8 Hz, C**H**^{7}), 5.95 (dd, 1H, J = 5.0, 4.9 Hz, N**H**CO), 5.54 (d, 1H, J = 3.6 Hz, C**H**^{8a}), 5.49 (s, 1H, O**H**), 3.81 (d, 1H, J = 9.3 Hz, C**H**^{2}), 3.65 (s, 3H, C**H**$_3^{OMe}$), 3.59 (dd, 1H, J = 18.5, 5.0 Hz, C**H**$^{\alpha}$), 3.52 (dd, 1H, J = 18.5, 4.9 Hz, C**H**$^{\alpha}$), 3.01 (d, 1H, J = 3.6 Hz, N**H**), 2.48 (dd, 1H, J = 13.9, 9.3 Hz, C**H**^{3}), 2.28 (d, 1H, J = 13.9 Hz, C**H**^{3}). ^{13}C NMR ($CDCl_3$) δ: 176.6 (**C**), 169.8 (**C**), 146.9 (**C**), 143.7 (**C**), 131.1 (**C**H), 130.1 (**C**), 128.8 (**C**H), 127.5 (**C**H), 127.1 (**C**H), 122.7 (**C**H), 118.8 (**C**H), 110.0 (**C**H), 92.3 (**C**H), 86.8 (**C**), 75.6 (**C**), 64.5 (**C**H), 52.3 (**C**H$_3$), 42.8 (**C**H$_2$), 41.0 (**C**H$_2$). ES$^+$/MS: 556.2 (M + Na)$^+$. HRMS (ES$^+$) for $C_{33}H_{31}N_3O_4$ (M + H)$^+$: calculated 534.2393, found 534.2390. UV/vis (MeOH): 239 (1.5 × 10^6 cm^2 mol^{-1}), 297 nm (0.3 × 10^6 cm^2 mol^{-1}).

***N*1-Trityl-3*a*-hydroxypyrrolo[2,3-*b*]indolylglycine Methyl Ester (*anti-cis*) (1b).** R_f (hexane/EtOAc, 1:1) 0.4, white solid, 0.02 g, 35% yield. ^{1}H NMR ($CDCl_3$) δ: 9.25−9.19 (br, 1H, N**H**CO), 7.71 (d, 6H, J = 7.6 Hz, Ar**H**^{Tr}), 7.48−7.25 (m, 9H, Ar**H**), 7.13−6.98 (m, 2H, C**H**^{6,4}), 6.73 (t, 1H, J = 7.4 Hz, C**H**^{5}), 6.62 (t, 1H, J = 7.4 Hz, C**H**^{7}), 5.36 (d, 1H, J = 5.5 Hz, C**H**^{8a}), 4.89 (d, 1H, J = 5.5 Hz, N**H**), 4.25−4.19 (m, 2H, C**H**^{2}, C**H**$^{\alpha}$), 3.80 (s, 3H, C**H**$_3^{OMe}$), 3.18 (dd, 1H, J = 18.3, 3.2 Hz, C**H**$^{\alpha}$), 2.35 (d, 1H, J = 13.4 Hz, C**H**^{3}), 1.08 (dd, 1H, J = 13.4, 9.8 Hz, C**H**^{3}). ^{13}C NMR ($CDCl_3$) δ: 174.3 (**C**), 169.8 (**C**), 148.5 (**C**), 144.8 (**C**), 130.1 (**C**), 129.3 (**C**H), 128.8 (**C**H), 128.3 (**C**H), 126.8 (**C**H), 124.5 (**C**H), 120.7 (**C**H), 110.3 (**C**H), 90.7 (**C**), 89.6 (**C**H), 78.6 (**C**), 66.7 (**C**H), 52.2 (**C**H$_3$), 44.9 (**C**H$_2$), 41.3 (**C**H$_2$).

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ES^+/MS: 556.1 $(M + Na)^+$. HRMS (ES^+) for $C_{33}H_{31}N_3O_4$ $(M + H)^+$: calculated 534.2393, found 534.2396. UV/vis (MeOH): 235 (1.8×10^6 cm^2 mol^{-1}), 292 nm (0.2×10^6 cm^2 mol^{-1}).

3*a*-Hydroxypyrrolo[2,3-*b*]indolylglycine Methyl Ester (15a, 15b). A 1:1 diastereomeric mixture of Tr-Hpi-Gly-OMe (0.20 g, 0.3 mmol) was dissolved in CH_2Cl_2 (20 mL) and hexafluoro-2-propanol (5 mL) was added slowly, with stirring at room temperature. The solution turned red/orange and TLC (CH_2Cl_2/MeOH, 9:1) showed disappearance of starting material after 15 min. The solution was evaporated to dryness and the crude material was purified by silica gel column chromatography (CH_2Cl_2/MeOH, 95:5) to yield the two diastereomers, each as a pale orange foam: upper 0.04 g (40%); lower 0.03 g (30%).

15a: R_f (CH_2Cl_2/MeOH, 9:1) 0.3. ^{1}H NMR (d_4-MeOH) δ: 7.23 (d, 1H, J = 7.6 Hz, C**H**^{4}), 7.02 (t, 1H, J = 7.6 Hz, C**H**^{6}), 6.71 (t, 1H, J = 7.6 Hz, C**H**^{5}), 6.38 (d, 1H, J = 7.6 Hz, C**H**^{7}), 4.92 (s, 1H, C**H**^{8a}), 3.91 (s, 2H, C**H**$^{\alpha}$), 3.70 (s, 3H, C**H**$_3^{OMe}$), 3.64 (dd, 1H, J = 10.6, 6.1 Hz, C**H**^{2}), 2.55 (dd, 1H, J = 12.2, 6.1 Hz, C**H**^{3}), 2.28 (dd, 1H, J = 12.2, 10.6 Hz, C**H**^{3}). ^{13}C NMR (d_4-MeOH) δ: 173.8 (**C**), 170.4 (**C**), 149.4 (**C**), 130.0 (**C**H), 129.9 (**C**), 124.1 (**C**H), 119.9 (**C**H), 110.5 (**C**H), 88.5 (**C**), 84.8 (**C**H), 60.4 (**C**H), 52.3 (**C**H$_3$), 44.3 (**C**H$_2$), 40.7 (**C**H$_2$). ES^+/MS: 292.1 $(M + H)^+$. HRMS (ES^+) for $C_{14}H_{18}N_3O_4$ $(M + H)^+$: calculated 292.1297, found 292.1300.

15b: R_f (CH_2Cl_2/MeOH, 9:1) 0.2. ^{1}H NMR ($CDCl_3$) δ: 7.79 (s, 1H, N**H**), 7.15 (d, 1H, J = 7.6 Hz, C**H**^{4}), 7.03 (t, 1H, J = 7.6 Hz, C**H**^{6}), 6.68 (t, 1H, J = 7.6 Hz, C**H**^{5}), 6.49 (d, 1H, J = 7.6 Hz, C**H**^{7}), 4.99 (s, 1H, C**H**^{8a}), 4.02 (dd, 1H, J = 8.5, 5.3 Hz, C**H**^{2}), 3.81 (dd, 1H, J = 18.4, 6.0 Hz, C**H**$^{\alpha}$), 3.70 (s, 3H, C**H**$_3^{OMe}$), 3.43 (dd, 1H, J = 18.4, 4.7 Hz, C**H**$^{\alpha}$), 2.55 (dd, 1H, J = 13.3, 8.5 Hz, C**H**^{3}), 2.28 (dd, 1H, J = 13.3, 5.3 Hz, C**H**^{3}). ^{13}C NMR ($CDCl_3$) δ: 173.9 (**C**), 170.6 (**C**), 149.9 (**C**), 129.9 (**C**H), 128.6 (**C**), 123.9 (**C**H), 119.6 (**C**H), 110.2 (**C**H), 89.5 (**C**), 86.2 (**C**H), 61.6 (**C**H), 52.2 (**C**H$_3$), 42.9 (**C**H$_2$), 40.7 (**C**H). ES^+/MS: 292.1 $(M + H)^+$. HRMS (ES^+) for $C_{14}H_{17}N_3O_4Na$ $(M + Na)^+$: calculated 314.1117, found 314.1114.

***N*-(9*H*-Fluoren-9-ylmethoxy)carbonylglycyl(3*a*-hydroxypyrrolo[2,3-*b*]indolyl)glycine Methyl Ester (16).** To a solution of Fmoc-Gly-OH (0.60 g, 0.2 mmol) in dry DMF (8 mL) was added PyBOP (0.11 g, 0.2 mmol), H-Hpi-Gly-OMe (0.06 g, 0.2 mmol), and DIPEA (20 μL, 0.2 mmol). The mixture was stirred for a period of 2 h at room temperature before the solvent was evaporated to dryness. The mixture was purified by flash chromatography on silica gel (hexane:EtOAc 1:1) to yield product as a white solid, 0.05 g (44%). R_f (CH_2Cl_2/MeOH, 9:1) 0.5. ^{1}H NMR ($CDCl_3$) δ: 7.75 (d, 2H, J = 7.4 Hz, Ar**H**^{Fmoc}), 7.55 (d, 2H, J = 7.4 Hz, Ar**H**^{Fmoc}), 7.35 (t, 2H, J = 7.4 Hz, Ar**H**^{Fmoc}), 7.30−7.20 (m, 3H, Ar**H**^{Fmoc}), 7.12−6.95 (m, $^5/_3$H, N**H**CO′ + Ar**H**^{5}), 6.85 (br, $^1/_3$H, N**H**CO′), 6.80−6.69 (m, 1H, Ar**H**^{6}), 6.57−6.48 (m, 1H, Ar**H**^{7}), 5.59−5.72 (br, 1H, N**H**CO), 5.63 (br, $^2/_3$H, J = 2.8 Hz, C**H**^{8a}), 5.55 (br, $^1/_3$H, N**H**), 5.42 (br, $^2/_3$H, N**H**), 5.25 (s, $^1/_3$H, C**H**^{8a}), 4.85 (d, $^1/_3$H, J = 7.0 Hz, C**H**^{2}), 4.54 (d, $^2/_3$H, J = 8.7 Hz, C**H**^{2}), 4.35−4.06 (m, $^{11}/_3$H, C**H**^{Fmoc}, C**H**$^{\alpha}$), 4.00 (dd, $^2/_3$H, J = 18.1, 6.5 Hz, C**H**$^{\alpha}$), 3.88−3.60 (m, $^5/_3$H, C**H**$^{\alpha}$, C**H**$^{\alpha'}$), 3.57−3.51 (m, 3H, C**H**$_3^{OMe}$), 3.49−3.38 (m, $^2/_3$H, C**H**$^{\alpha'}$), 3.24 (dd, $^1/_3$H, J = 18.1, 4.0 Hz, C**H**$^{\alpha'}$), 3.09 (dd, $^2/_3$H, J = 18.1, 4.0 Hz, C**H**$^{\alpha}$), 2.86 (dd, $^1/_3$H, J = 13.4, 9.6 Hz, C**H**^{3}), 2.80−2.71 (m, 1H, C**H**^{3}), 2.59 (dd, $^2/_3$H, J = 13.4, 9.6 Hz, C**H**^{3}). ^{13}C NMR ($CDCl_3$) δ: 172.1, 172.0 (**C**), 171.4, 171.3 (**C**), 170.7 (**C**), 158.4 (**C**), 149.8, 149.6 (**C**), 145.2, 145.1, 142.7 (**C**), 131.5 (**C**), 132.0, 131.8 (**C**H), 129.2, 128.5, 126.5 (**C**H), 125.7, 125.5 (**C**H), 122.4, 122.0 (**C**H), 121.4 (**C**H), 113.0, 112.5 (**C**H), 87.7 (**C**), 84.7, 84.6 (**C**), 68.9, 68.7 (**C**H$_2$), 62.7 (**C**H), 54.9 (**C**H$_3$), 53.7, 53.6 (**C**H), 48.4 (**C**H), 43.8 (**C**H$_2$), 42.6, 42.4 (**C**H$_3$). ES^+/MS: 593.0 $(M + Na)^+$. HRMS (ES^+) for $C_{31}H_{30}N_4O_7Na$ $(M + Na)^+$: calculated 593.2012, found 593.2023.

***N*1-Trityl-3*a*-hydroxypyrrolo[2,3-*b*]indolylglycine Triethylammonium Salt (17a, 17b).** To a solution of Tr-Hpi-Gly-OMe (mixture of diastereomers) (0.13 g, 0.2 mmol) in dioxane/water (15 mL, v:v 2:1) was added LiOH (0.05 g, 10 equiv) and the reaction was stirred for 1 h at room temperature. Following consumption of the starting material by TLC (CH_2Cl_2/MeOH, 9:1), the reaction mixture was evaporated to dryness and the residue was purified by a short silica plug, eluting with CH_2Cl_2/MeOH/NEt_3 (90:10:1). Fractions were combined to yield a white solid as the triethylamine salt of the two diastereomers, 0.13 g (85%). Due to the acid sensitivity of the Tr-Hpi-Gly-OH, purification and comprehensive characterization was avoided and the mixture was reacted further to form the tripeptide **19**.

17a/b: R_f (CH_2Cl_2/MeOH, 9:1) 0.2. **17a/b**: mixture of diastereomers, but ^{1}H NMR was possible to be assigned separately.

17a: ^{1}H NMR (d_4-MeOH) δ: 9.59−9.53 (br, 1H), 7.51 (d, 6H, J = 7.8 Hz), 7.27−7.11 (m, 10H), 7.01−6.45 (m, 2H), 6.25 (t, 1H, J = 7.9 Hz), 5.49 (s, 1H), 4.21 (d, 1H, J = 9.5 Hz), 3.14−3.09 (m, 2H), 2.25 (dd, 1H, J = 13.8, 9.5 Hz), 2.18 (d, 1H, J = 13.8 Hz).

17b: ^{1}H NMR (d_4-MeOH) δ: 9.55−9.48 (br, 1H), 7.75 (d, 6H, J = 7.8 Hz), 7.40−7.25 (m, 10H), 7.01−6.45 (m, 3H), 5.26 (s, 1H), 3.98 (d, 1H, J = 10.1 Hz), 3.77 (d, 1H, J = 17.6 Hz), 2.81 (d, 1H, J = 17.6 Hz), 2.07 (d, 1H, J = 13.0 Hz), 1.21 (dd, 1H, J = 13.0, 10.1 Hz).

17a/b: mixture of diastereomers. ^{13}C NMR (d_4-MeOH) δ: 180.0, 176.0 (**C**), 175.7 (**C**), 156.3, 151.3 (**C**), 146.4, 145.4 (**C**), 130.9, 129.9 (**C**H), 130.3 (**C**), 129.4 (**C**H), 129.2, 128.8 (**C**H), 125.1 (**C**H), 120.0, 119.8 (**C**H), 111.0 (**C**H), 94.8, 93.6 (**C**H), 90.7, 90.2 (**C**), 80.1 (**C**), 68.0, 66.0 (**C**H), 56.4, 54.9 (**C**H$_2$), 46.6 (NEt$_3$), 44.8, 44.4 (**C**H$_2$), 7.8 (NEt$_3$). **ES$^-$/MS**: 518.4 $(M - H)^-$. HRMS (ES^-) for $C_{32}H_{28}N_3O_4$ $(M - H)^-$: calculated 518.2080, found 518.2062.

Compound **18** was prepared using the same method as **17a/b**, but crude product was reacted directly to form the tripeptide **20**, characterized below.

General Method for the Synthesis of Hpi Tripeptides (19a/b, 20). Tr-Hpi-Xaa-OH·NEt_3 (1.0 mmol), DCC (1.1 mmol), and HOBt (1.1 mmol) were dissolved in dry CH_2Cl_2 and stirred under inert atmosphere at 0 °C for 10 min. A mixture of H-Cys(Tr)-OMe (1.1 mmol) and triethylamine (1.2 mmol) was added to this and the vessel was allowed to warm slowly to room temperature. Stirring was continued for 15 h and followed by TLC (CH_2Cl_2/MeOH, 9:1). On consumption of starting material the mixture was evaporated to dryness and redissolved in EtOAc and the white precipitate was filtered off. The filtrate was washed with solutions of citric acid (5%), $NaHCO_3$ (sat.), and NaCl (sat.), then dried over Na_2SO_4 (anhydrous). Evaporation in vacuo gave the crude tripeptides as pale yellow oils. These were purified by silica gel column chromatography (hexane/EtOAc, 2:1 to 1:1) to afford the pure tripeptides.

***N*1-Trityl-3*a*-hydroxypyrrolo[2,3-*b*]indolylglycyl-*S*-trityl-L-cysteine Methyl Ester (19a and 19b) (mixture of diastereomers).** R_f (hexane/EtOAc, 1:1) 0.2. White solid, 0.06 g, 24% yield. ^{1}H NMR ($CDCl_3$) δ: 9.23−9.14 (br, 1H), 7.69 (d, 6H, J = 7.8 Hz), 7.60−6.92 (m, 24H), 6.74−6.67 (m, 1H), 6.65−6.58 (m, 1H), 6.33−6.19 (m, 1H), 5.35−5.28 (m, 1H), 4.91−4.80 (m, 1H), 4.63−4.48 (m, 1H), 4.12−4.07 (m, 1H), 3.89−3.78 (m, 1H), 3.73−3.65 (m, 3H), 3.64−3.53 (m, 2H), 3.11−2.88 (m, 1H), 2.72−2.54 (m, 2H), 2.36−0.77 (m, 2H). ^{13}C NMR ($CDCl_3$) δ: 174.8, 170.2 (**C**), 169.0 (**C**), 148.2 (**C**), 144.7, 144.1 (**C**), 129.9 (**C**), 129.8 (**C**H), 129.4, 129.2, 128.2, 127.9, 126.8 (**C**H), 126.8 (**C**H), 120.4 (**C**H), 110.2 (**C**H), 90.5 (**C**H), 86.9 (**C**), 78.6, 77.5 (**C**), 66.7 (**C**H), 52.6 (**C**H), 51.3 (**C**H$_3$), 47.2 (**C**H), 43.6 (**C**H$_2$), 33.6 (**C**H$_2$). ES^+/MS: 901.5 $(M + Na)^+$. HRMS (ES^+) for $C_{55}H_{50}N_4O_5S$ $(M + Na)^+$: calculated 901.3400, found 901.3405.

***N*1-Trityl-3*a*-hydroxypyrrolo[2,3-*b*]indolyl-*O*-^{t}butyl-L-threonyl-*S*-trityl-L-cysteine Methyl Ester (20).** R_f (hexane/EtOAc, 1:1) 0.3. White solid, 0.03 g, 25% yield (2 steps). ^{1}H NMR ($CDCl_3$) δ: 7.65 (d, 1H, J = 8.0 Hz), 7.55 (d, 6H, J = 7.3 Hz), 7.48−7.11 (m, 24H), 6.98 (t, 1H, J = 7.5 Hz), 6.71 (t, 1H, J = 7.3 Hz), 6.36−6.28 (m, 2H), 6.18 (d, 1H, J = 5.2 Hz), 5.74 (d, 1H, J = 2.7 Hz), 4.53−4.48 (m, 1H), 4.14−4.06 (m, 1H), 3.87−3.82 (m, 1H), 3.67 (s, 3H), 3.42 (d, 1H, J = 9.0 Hz), 2.82

(d, 1H, J = 2.7 Hz), 2.54 (dd, 1H, J = 12.0, 5.8 Hz), 2.48 (dd, 1H, J = 12.0, 4.7 Hz), 2.23 (dd, 1H, J = 13.6, 9.0 Hz), 2.01 (d, 1H, J = 13.6 Hz), 1.28 (s, 9H), 0.48 (d, 3H, J = 6.2 Hz), 0.12 (s, 1H). ^{13}C NMR ($CDCl_3$) δ: 171.6 (**C**), 170.2 (**C**), 169.0 (**C**), 148.2 (**C**), 144.2 (**C**), 131.2 (**C**), 130.9 (**C**H), 129.4 (**C**H), 128.7 (**C**H), 127.7 (**C**H), 126.8 (**C**H), 118.7 (**C**H), 110.1 (**C**H), 92.7 (**C**H), 86.9 (**C**), 80.1 (**C**), 75.5 (**C**), 66.5 (**C**H), 65.7 (**C**H), 65.1 (**C**H), 57.2 (**C**H), 52.4 (**C**H_3), 43.3 (**C**H_2), 33.9 (**C**H_2), 28.2 (**C**H_3), 16.1 (**C**H_3). ES$^+$/MS: 1001.5 $(M + Na)^+$. HRMS (ES$^+$) for $C_{61}H_{62}N_4O_6S$ $(M + Na)^+$: calculated 1001.4288, found 1001.4283.

General Method for the Synthesis of Cyclic Tryptathionines.[27] To protected tripeptide methyl ester (0.5 mmol) was added trifluoroacetic acid (2 mL) and the yellow solution was stirred for 5 h. Methanol was added and the solvent was removed in vacuo. This was repeated twice more, and then the residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH 9.5:0.5).

2-Mercapto-L-tryptophanylglycyl-L-cysteine Cyclic Sulfide Methyl Ester (21). R_f (CH_2Cl_2/MeOH, 9:1) 0.4. White solid, 16% yield. ^{1}H NMR (d_4-MeOH) δ: 7.60 (d, 1H, J = 7.7 Hz, C**H**^{4}), 7.30 (d, 1H, J = 7.7 Hz, C**H**^{7}), 7.16 (t, 3H, J = 7.7 Hz, C**H**^{6}), 7.08 (t, 1H, J = 7.7 Hz, C**H**^{5}), 4.89−4.84 (m, 1H, C**H**$^{\alpha\ cys}$), 4.25 (d, 1H, J = 15.0 Hz, C**H**$^{\alpha\ gly}$), 4.03−3.95 (m, 1H, C**H**$^{\alpha\ trp}$), 3.82 (s, 3H, C**H**$_3^{OMe}$), 3.64 (dd, 1H, J = 14.5, 2.6 Hz, C**H**$^{\beta\ cys}$), 3.47 (dd, 1H, J = 14.5, 4.5 Hz, C**H**$^{\beta\ cys}$), 3.39 (dd, 1H, J = 13.5, 12.0 Hz, C**H**$^{\beta\ trp}$), 3.12 (d, 1H, J = 15.0 Hz, C**H**$^{\alpha\ gly}$), 3.09 (dd, 1H, J = 13.5, 2.6 Hz, C**H**$^{\beta\ trp}$). ^{13}C NMR (d_4-MeOH) δ: 178.3 (**C**), 173.0 (**C**), 172.9 (**C**), 141.0 (**C**), 130.1 (**C**), 129.9 (**C**), 125.2 (**C**H), 122.2 (**C**H), 121.0 (**C**H), 114.7 (**C**), 113.5 (**C**H), 56.5 (**C**H), 55.2 (**C**H), 54.8 (**C**H_3), 46.4 (**C**H), 39.3 (**C**H_2), 33.6 (**C**H_2). ES$^+$/MS: 377.1 $(M + H)^+$. HRMS (ES$^+$) for $C_{17}H_{21}N_4O_4S$ $(M + H)^+$: calculated 377.1284, found 377.1292. UV/vis (MeOH): 284 (6.9×10^6 cm^2 mol^{-1}), 292 (7.8×10^6 cm^2 mol^{-1}), 301 nm (7.0×10^6 cm^2 mol^{-1}).

2-Mercapto-L-tryptophanyl-L-threonyl-L-cysteine Cyclic Sulfide Methyl Ester (22). R_f (CH_2Cl_2/MeOH, 9:1) 0.3. White solid, 20% yield. ^{1}H NMR (d_4-MeOH) δ: 7.66 (d, 1H, J = 7.7 Hz, C**H**^{4}), 7.29 (d, 1H, J = 7.7 Hz, C**H**^{7}), 7.14 (t, 3H, J = 7.7 Hz, C**H**^{6}), 7.09 (t, 1H, J = 7.7 Hz, C**H**^{5}), 4.76 (t, 1H, J = 3.9 Hz, C**H**$^{\alpha\ cys}$), 4.21 (d, 1H, J = 4.6 Hz, C**H**$^{\alpha\ thr}$), 4.03−3.87 (m, 2H, C**H**$^{\beta\ thr}$, C**H**$^{\alpha\ trp}$), 3.76 (s, 3H, OC**H**$_3$), 3.61 (dd, 1H, J = 14.5, 4.5 Hz, C**H**$^{\beta\ cys}$), 3.49 (dd, 1H, J = 14.6, 3.6 Hz, C**H**$^{\beta\ cys}$), 3.43−3.31 (m, 1H, C**H**$^{\beta\ trp}$), 3.04 (dd, 1H, J = 14.2, 3.0 Hz, C**H**$^{\beta\ trp}$), 1.02 (d, 1H, J = 6.4 Hz, C**H**$^{\gamma\ thr}$). ^{13}C NMR (d_4-MeOH) δ: 178.0 (**C**), 172.9 (**C**), 171.0 (**C**), 139.6 (**C**), 128.4 (**C**), 128.2 (**C**), 123.7 (**C**H), 120.7 (**C**H), 119.5 (**C**H), 113.8 (**C**), 112.0 (**C**H), 65.3 (**C**H_2) 59.1 (**C**H), 55.2 (**C**H), 54.1 (**C**H), 53.3 (**C**H_3), 36.8 (**C**H_2), 33.2 (**C**H_2), 20.2 (**C**H_2). ES$^+$/MS: 421.1 $(M + H)^+$, 443.0 $(M + Na)^+$. HRMS (ES$^+$) for $C_{19}H_{24}N_4O_5S$ $(M + H)^+$: calculated 421.1546, found 421.1550. UV/vis (MeOH): 284 (7.1×10^6 cm^2 mol^{-1}), 292 (8.1×10^6 cm^2 mol^{-1}), 301 nm (7.5×10^6 cm^2 mol^{-1}).

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Supporting Information Available: Supporting synthetic procedures and characterization for compounds **2**−**14**, including ^{1}H and ^{13}C NMR data and ES/HRMS data; crystallography data for compounds **1a**, **1b**, and **10a**; copies of the ^{1}H and ^{13}C NMR spectra for compounds **1a**, **1b**, and **15**−**22** and ^{1}H NMR for **2**−**14**; 2D COSY spectra for compounds **1a/b**, **15a/b**, **16**, **21**, and **22**. This material is available free of charge via the Internet at http://pubs.acs.org.

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