Formation of Constrained, Fluorescent Peptides via Tryptophan Dimerization and Oxidation

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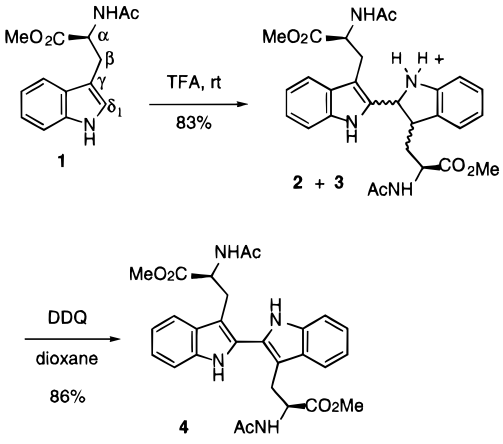
Tryptophan derivatives can undergo three types of undesired side reactions in anhydrous trifluoroacetic acid (TFA): pyrrolidino[2,3-*b*]indoline formation, Bischler−Napieralski cyclization, and δ_1,δ_1' dimerization.[1] Here, we report that acid-promoted δ_1,δ_1' dimerization of tryptophan-containing peptides, followed by oxidation, is a useful way to form highly fluorescent chromophores. For peptides containing multiple tryptophan residues, cyclization/oxidation produces heterodetic[2] cyclic peptides with conformational and fluorescent properties that are sequence dependent.

Although dimerization of tryptophan-containing peptides in TFA has been previously reported, the utility of this process is limited by lack of stereochemical control. For example, the dimerization of tryptophan derivative **1** in TFA gives two products, **2** and **3**, which are reported to be cis and trans on the basis of NMR coupling constants (eq 1).[3] Such assignments

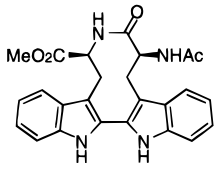
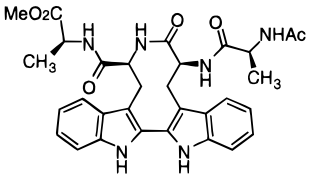
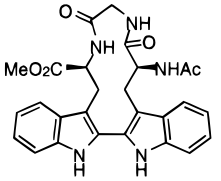
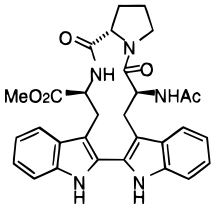
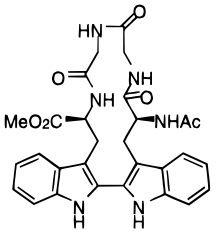


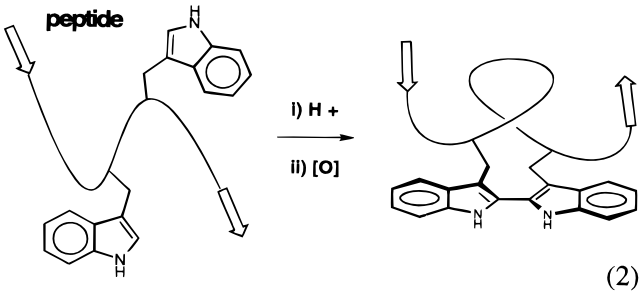
(1)

can be unreliable, and when the two tryptophans are not identically substituted, the stereochemical assignment becomes even more precarious because a total of eight stereoisomers are possible.[4] This problem may be overcome by dehydrogenation of the indoline ring, which results in removal of the new stereogenic centers.[5] This transformation is easily accomplished by treatment of the dimerization products **2** and **3** with DDQ to afford the fully aromatic δ_1,δ_1'-ditryptophan **4** in 86% yield.

The acid-promoted cyclization of Ac-Trp-Trp-OMe by indole dimerization has been reported, but there have been no further examples of this cyclization reaction, possibly due to the problem of isomer formation.[6] We have extended the simple two-step sequence of cyclization and oxidation to polypeptides containing multiple tryptophan residues as a useful way to form novel heterodetic cyclic peptides (eq 2). The results are summarized in Table 1.

Table 1. Formation of δ_1,δ_1'-Ditryptophan Cross-Links

	PRODUCT	STRUCTURE	YIELD
5	Ac-WW-OMe		29%
6	Ac-AWWA-OMe		33%
7	Ac-WGW-OMe		25%
8	Ac-WPW-OMe		60%
9	Ac-WGGW-OMe		43%



(2)

The peptide substrates were synthesized using solution-phase BOC chemistry.[7] To favor indole dimerization, the amino termini were acetylated, and the carboxy termini were protected as the methyl esters. It has been shown that pyrrolidino[2,3-*b*]indoline formation predominates when the tryptophan α-amino

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(2) In peptides, the term heterodetic refers to non-peptide linkages.

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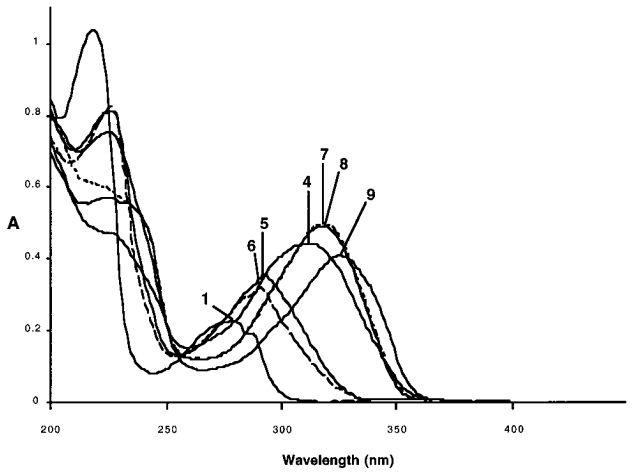

Figure 1. Absorbance spectra of tryptophan (4.2×10^{-5} M) and ditryptophan (2.1×10^{-5} M) derivatives.

group is protected as a carbamate instead of an amide—an effect attributable to the electronic differences between the amides and carbamates.[8] The peptides (0.1−0.05 M) were stirred in trifluoroacetic acid until starting material was consumed (10−30 h). Trifluoroacetic acid was removed, and the crude product mixture was treated with 1.2 equiv of DDQ in dioxane, resulting in a rapid oxidation. The products were purified by silica gel chromatography or reversed-phase (C_{18}) HPLC. Chromatography on silica gel (methanol/chloroform) led to some loss of material, but this has not been quantified.

Using this macrocyclization protocol, rings of up to 16 atoms have been made in yields ranging from 25 to 60% (unoptimized). While the yields for this process are modest, they are comparable to those for acylation reactions which generate cyclic peptides.[9] The formation of 10−16-membered rings is noteworthy since it proceeds via carbon−carbon bond formation.

Cross-linking of tryptophan side chains at the δ_1 positions produces conformational constraints which are apparent in the ^{1}H NMR spectra (DMSO-d_6). While Ac-Trp-Trp-OMe gives a single set of ^{1}H NMR signals at room temperature, the product of indole dimerization/oxidation, **5**, gives two sets of signals at room temperature in a ratio of 2.6:1. A similar ratio has been observed for the cis and trans amide isomers of a cyclic Cys-Cys diad.[10] The two sets of signals in the ^{1}H NMR spectrum of **5** coalesce at 70 °C, suggesting a process with an activation energy barrier of about 18 kcal/mol. In contrast, while the sequence Ac-Trp-Pro-Trp-OMe exists as a 1:2.3 mixture of cis and trans proline amide isomers at room temperature, cross-linking constrains the Trp-Pro amide bond in **8** to a single geometry. Other methods for constraining peptide conformation can entail fairly lengthy synthetic sequences.[11] There is no evidence for conformational constraints in **9**, since it shows only one set of ^{1}H NMR signals at room temperature before and after cyclization.

The δ_1,δ_1'-ditryptophans **4**−**9** are superior to analogous tryptophans as fluorescent chromophores; this is seen in the UV−vis absorbance and fluorescence emission spectra shown in Figures 1 and 2, respectively. The absorbance maximum for **1** is only 279 nm, but it is shifted toward longer wavelengths for ditryptophans **4**−**9**. The increased fluorescence emission intensity observed upon δ_1,δ_1'-ditryptophan cross-linking is also accompanied by a shift in the excitation and emission maxima

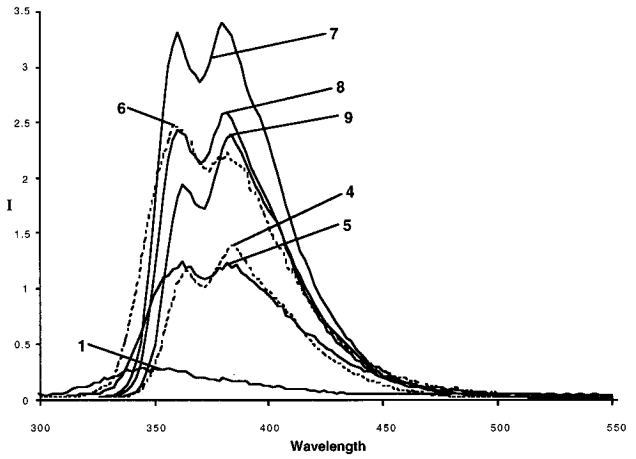

Figure 2. Fluorescence emission spectra in water.[13]

to longer wavelengths. The emission maximum is 353 nm for **1**, but it is shifted to 370−383 nm for **4**, **7**, **8**, and **9**, with a substantial amount of light emitted in the visible region. The excitation and emission spectra lack a mirror image relationship, consistent with a different conformational arrangement in the excited state as compared to the ground state. This effect has been noted for similar systems such as biphenyl.[12] The differing spectral properties of the heterodetic cyclic peptides **5**−**9** may correlate with differences in conformation about the aryl−aryl bond and, if so, might constitute a useful way to detect coupled changes in peptide conformation.[14]

The quantum yields in degassed aqueous solutions for these cyclic and acyclic ditryptophans range between 0.22 and 0.52.[15] For the analogous monomeric tryptophan derivative **1**, the quantum yield is only 0.14.[16] The extinction coefficient at the absorbance maxima is only 5400 cm^{-1} M^{-1} for **1**, but it ranges from 15 100 to 23 600 cm^{-1} M^{-1} for **4**−**9**.

In summary, we have demonstrated the acid-promoted cyclization and oxidation of peptides containing two tryptophans. These macrocyclization reactions afford peptides with unique fluorescent chromophores and, in some cases, introduce conformational constraints which are apparent in the ^{1}H NMR spectra. Tryptophan dimerization is topologically related to cystine disulfide formation and 3,3′-dityrosine formation and may prove to be a useful posttranslational modification for proteins.[17] We are now extending this work to biologically relevant peptides and proteins.

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Supporting Information Available: Experimental procedures and characterization data (8 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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