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CONVENIENT SYNTHESIS OF A CYCLIC PEPTIDE DISULFIDE: A TYPE II β-TURN STRUCTURAL MODEL

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Summary: Type II β-turn peptides can be stabilized by a disulfide bond. This bond is conveniently formed while the peptide remains attached to a polymeric support.

Disulfide bridges (1) and β-turn conformations (2) are each important elements of peptide and protein structures. Disulfide bonds between cysteine residues located at different points in the linear sequence stabilize the three-dimensional structure. Recent studies by Balaram's group (3) of peptides of the general structure Cys-X-Y-Cys suggest that the disulfide bond forces the 14-membered ring system to adopt a favorable 4 $\longrightarrow$ 1 intramolecularly hydrogen bonded β-turn conformation.

In this paper, we describe a novel synthesis, as well as the conformational analysis, of Ac-L Cys-L Pro-D Val-L Cys-NH_2. Proline was used because of the high frequency with which this residue is found in β-turns (2, 4). Valine was chosen in order to enhance solubility in organic solvents and to facilitate ^{1}H-NMR assignments; it was further reasoned that the D-configuration might specifically induce type II β-turns (2).

Preparation of the title peptide hinges on reliable chemistry for formation of the disulfide bond. Usually, a linear sequence is assembled by solid-phase or solution methods, and then protecting groups (as well as the anchoring linkage in the solid-phase case) are cleaved. There follows non-specific oxidation in dilute solution in order to minimize unwanted dimerization and oligomerization. The alternative of carrying out deprotection and oxidation of the cysteines while the peptide chain remains anchored to the support has been little studied (5). Such an approach takes advantage of a solid-phase *pseudo-dilution* phenomenon which favors intramolecular disulfide bond formation (6). In the present work, oxidation of the resin-bound peptide was carried out using two different reagents: (i) thallium (III) trifluoroacetate, a novel reagent introduced by Yajima's group (7); and (ii) iodine under acidic conditions (8).

The linear peptide synthesis was performed by a conventional strategy (9) on a commercially available benzhydrylamine (BHA)-resin (0.65 mmol/g), using the *tert*-butyloxycarbonyl (Boc) group for temporary protection of N^{α}-amino groups and acetamidomethyl (Acm) functions for protection of the β-thiols of cysteines. Once chain assembly was completed,

the fully protected peptide-resin in trifluoroacetic acid--anisole (21:1) was treated with $(CF_3COO^-)_3Tl^{3+}$ (1.2 equiv.) for 1 h at 0 °C. The oxidized peptide-resin was then cleaved with HF--anisole (9:1), 1 h, 0 °C, to release 91% of the chains from the resin; the crude peptide product included 94% of the desired cyclized product (10). The corresponding experiment in which the fully protected peptide-resin was oxidized with iodine (10 equiv.) in acetic acid--water (4:1, v/v), 2 h, 25 °C, gave an overall crude yield for the desired target peptide of only 52%.

Oxidation yields appear to be sequence dependent (e.g., I_2 oxidation of a resin-bound di-Acm-oxytocin gave only a 6% yield, ref 5a). For the title problem, the validity of a solid-phase approach is clearly indicated by a comparison to oxidation yields in dilute solution for tetrapeptides of similar structure. The reported solution yields ranged from 15-25% (3), whereas the polymer-supported experiments gave 3- to 5-fold better yields.

In a second phase of this work, we have used high-field two-dimensional nuclear magnetic resonance to evaluate the conformation of the title peptide. ^{1}H-NMR data (200 MHz) were acquired in d_6-DMSO (Table). Signals were assigned by a combination of double quantum filtered (DQF) H,H-COSY (11) and rotating frame nuclear Overhauser enhancement spectroscopy ROESY (12). The latter experiment was applied instead of the more common NOESY because of unfavorable correlation times for peptides of this size.

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Table. ^{1}H-NMR data for Ac-L-Cys-L-Pro-D-Val-L-Cys-NH_2 in d_6-DMSO
S----------S

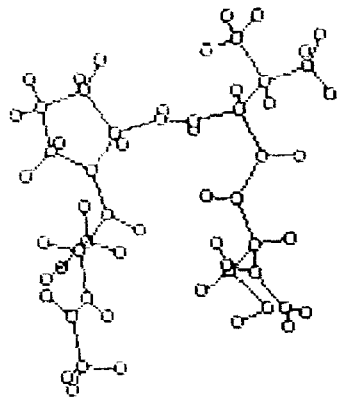
	NH	H^α	H^β	Others	$^3J(NH,H^\alpha)$	$^3J(H^\alpha,H^{\beta a})$	$^3J(H^\alpha,H^{\beta b})$	$^2J(H^{\beta a},H^{\beta b})$	$\Delta\delta/\Delta T$(ppb/°K)
L-Cys^1	8.30	4.70	3.47[a]		8.4	8.8	2.0	14.0	-5.83
			2.58[b]						
L-Pro^2	—	4.61	1.91	H^γ 2.16[a]					
			1.91	H^γ 1.84[b]					
				H^δ 3.55					
D-Val^3	8.28	3.92	2.21	H^γ 0.92	7.6	4.4			-3.54
L-Cys^4	7.38	4.14	3.28[a]		7.4	11.4	3.0	11.4	0.38
			3.05[b]						
NH_2	7.43[a]								-4.55
	7.05[b]								-3.75

Chemical shifts (δ) are expressed in ppm downfield from tetramethylsilane (TMS). The acetyl group was at 1.87 ppm. Coupling constants (J) are expressed in Hz. These data were determined at 303 °K. For the last column, the temperature coefficient of NH protons was determined over a range of 298-343 °K.

The ^{1}H-NMR (Table) and ^{13}C-NMR (not shown) spectra were found to contain only one set of signals. A conformationally rigid structure is further indicated by the large chemical shift difference between the two diastereotopic β-protons of Cys^1, and the very low temperature coefficient for the NH of Cys^4 (see Table). For both Cys^1 and Cys^4, the values of the two J(H^α,H^β) coupling constants are near the limits of pure J_{trans} (12.4 Hz) and pure J_{gauche} (3.2 Hz) respectively. These results indicate that there is a single predominant rotamer about the α–β bonds. A strong ROE observed between the NH of DVal^3 and the H^α of Pro^2 supports a type II β-turn. ROE's were observed between DVal^3 NH/DVal^3 H^α, DVal^3 NH/Cys^4 NH, and Cys^4 NH/DVal^3 H^α, and these are also in agreement with the short distances expected for a type II β-turn structure. Some distortion from the theoretical type II β-turn is suggested by the absence of an ROE between the H^α of Pro^2 and the NH of Cys^4, and by the ^{3}J(NH, H^α) of DVal^3 which is larger than expected. The amide bond between Cys^1 and Pro^2 is apparently *trans*, based on the proline C^γ = 24.9 at 50 MHz in d_6-DMSO (expected for *trans* = 25.1 ± 0.5 ppm, see ref 13).

We assume that the low-field β-proton of Cys^1 is *gauche* to the sulfur-sulfur bond, based on results with models of known geometry (14). Since we further observe ROE's between this assigned proton of Cys^1 and both β-protons of Cys^4, it becomes possible to predict the conformation of the disulfide bridge. Thus, our assignments and experimental observations, together with the constraint that the disulfide bridge is part of a cyclic system with a β-turn geometry, are consistent with a model where the disulfide has M chirality (15). This would also explain the rather low geminal coupling constant (11.4 Hz) between the two β-protons of Cys^4.

In summary, we have devised an advantageous polymer-supported route to a disulfide-bridged model peptide, and shown by NMR that this peptide assumes a type II β-turn. The excellent yields of the synthetic portion of this work may be in part related to the intrinsic ability of the sequence selected to adopt a folded conformation. In this regard, it is significant to note that ECEPP calculations (16) on the linear analogue show that the folded conformation is only 2 kcal/mol above the extended minimum energy conformer.



Computer-generated representation of the minimum energy folded conformation of linear Ac-LCys-LPro-DVal-LCys-NH_2

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9. Cycles for incorporation of Boc-amino acids comprised deprotection with CF_3COOH—CH_2Cl_2 (3:7), neutralization with iPr_2EtN—CH_2Cl_2 (1:19), and single coupling (2.5-fold) mediated by *N,N'*-dicyclohexylcarbodiimide in CH_2Cl_2; all couplings were ninhydrin negative within two hours.

10. This value was ascertained by comparison of the HPLC peak areas with those from an authentic standard of known concentration. Reversed-phase medium pressure liquid chromatography [Vydac C-18 column with a linear gradient of CH_3CN—H_2O—$CH_3CH_2CO_2H$] provided the cyclic peptide of >99 % purity in an isolated yield of 85%.

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