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PEPTIDES CONTAINING β-Turns I-- CYCLO-(GLY-L-CYS-GLY)$_3$ TRIPLY BRIDGED BY 1,3,5-TRIS-(THIOMETHYL)BENZENE

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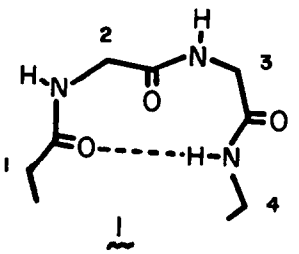
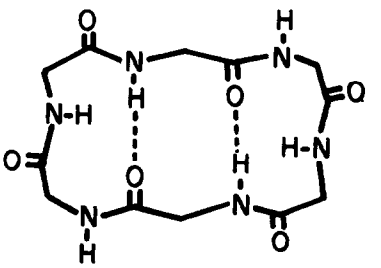
Abstract: Reaction of cyclo-(Gly-L-Cys-Gly)$_3$ with 1,3,5-tris-bromomethylbenzene yields a tris thioether of the cyclic nonapeptide in 28% yield. Both ^{1}H and ^{13}C NMR spectra are consistent with a molecule of 3-fold symmetry; the temperature dependences of chemical shifts of the amide hydrogens are consistent with a structure composed of three β-turns.

Although the β-turn[1] attracted interest much later than the α-helix and the β-sheet, it appears to be at least as prevalent in protein structures[2] and accumulating evidence suggests that it may be assumed by many peptide hormones in the hormone-receptor complex.[3] Recently Chou and Fasman have reported a simple analysis for predicting the probability that a given sequence of four amino acids will assume a β-turn conformation in a protein structure.[4] The β-turn or certain closely related structures partially define the orientation of a number of cage-type ionophores such as valinomycin.[5]

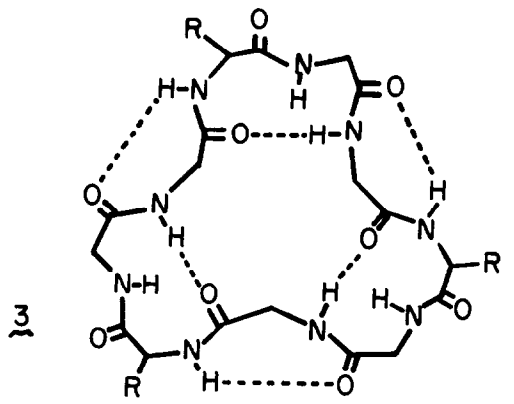


1



2

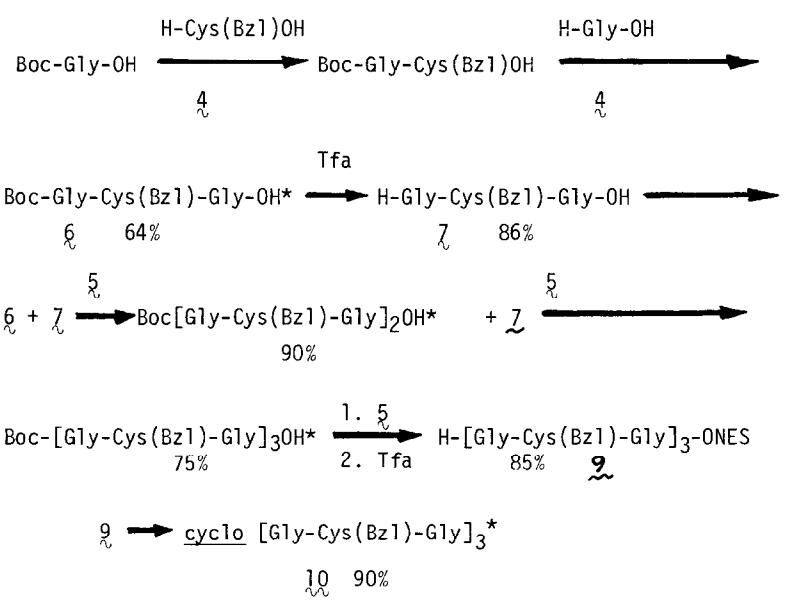
As seen in 1, the β-turn is a relatively rigid structure that is defined by only four amino acid residues. In principle rigid subunits can be linked to form larger arrays with defined structures, and as part of a general program directed toward unusual cage-type structures that can be formed from polypeptide precursors, we were led to ask, what simple structures might be formed by linkages of subunits consisting of tetrapeptides that assume the β-turn conformations. The simplest of these is a cyclic hexapeptide consisting of two β-turns, 2.[6] One example of cyclic nonapeptide 3 that contains three β-turns, has been reported,[7] in which R is a leucine side chain.



3

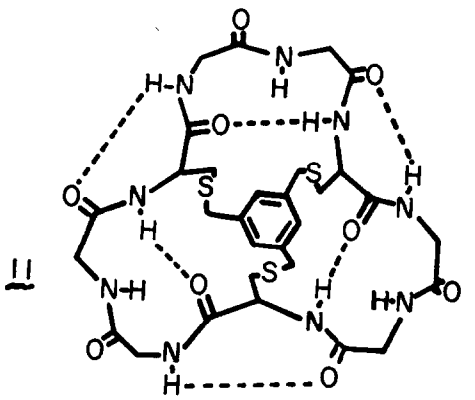
In this paper we report synthesis of a cyclononapeptide (Gly-L-Cys-Gly)$_3$ as its tri S-benzyl derivative, which was prepared by the reaction sequence of Scheme 1, using 2-ethyl-7-hydroxybenzisoxazolium fluoroborate[7] 4 and 2-ethylbenzisoxazolium fluoroborate[8] 5 as the amide-forming reagents. The cyclization of the active ester of the linear nonapeptide 9 is noteworthy for the high yield that was observed. (Structures marked with asterisks were characterized by TLC, HPLC, ^{1}H NMR, and satisfactory elemental analysis.)

Scheme 1



The 250 MHz ^{1}H NMR spectrum of 10 at 25°C in DMSO-D^6 showed three amide resonances at 7.99δ(t,3H), 8.18δ(d,3H), and 8.73δ(t,3H). The temperature dependences of these resonances are 2.4, 5.0, and 8.0 x 10^{-3} ppm/°C, respectively. Temperature dependences of less than 3 x 10^{-3} ppm/°C are characteristic of internally hydrogen bonded amide NH groups, and

values of greater than 6 x 10^{-3} ppm/°C are expected for amide NH groups that are hydrogen bonded intermolecularly with solvent.[10] Since the triplet resonances must be assigned to NH residues of glycines, we interpret the NMR evidence as consistent with structure 3 in which glycine residues appear at site 4 of the turn structure 1. Models suggest that bent hydrogen bonds can exist (dashed lines) in a stable conformation of 3. Taken together with the intermediary value seen for the temperature dependence of the cysteine NH supports structure 3 with R = CH_2-S-Bzl for the peptide 10.



11

Structure 11 is a more rigid analog of 3 in which cysteine residues appear at β-turn sites 1 and 4 (structure 1), and the sulfurs are bound as thioethers by a 1,3,5-tris-(methylene)benzene residue. When 10 was reduced with sodium in liquid ammonia and then caused to react with 1,3,5-tris(bromomethyl)benzene, a product was isolated by preparative HPLC (Whatman Magnum 9 ODS-2 C-18 reverse phase column; methanol-water eluant) which was characterized having the molecular formula of 11 by elemental analysis[11] and by field desorption MS (M^+,766). Although 11 is an insoluble substance that has thus far defied attempts at macro crystallization, important aspects of its structure can be deduced from its spectroscopic behavior. At 62.8 MHz, the ^{13}C NMR spectrum of 11 in DMSO-d^6 exhibits resonances at δ 172.6(Cys C=O,3C), 169.8(Gly C=O,3C), 169.3(Gly C=O,3C), 136.9(Ar,3C), 128.9 (Ar,3C) and 52.5 (Cys α-C,3C). The remaining four carbon resonances are obscured by solvent. The amide NH resonances at 250 MHz, 25°C, DMSO D^6, appear at 8.95δ(t,3H), 7.82δ(t,3H), and 7.29δ(d,3H), with respective temperature dependencies of -4.5,-1.4, and -1.7 ppb/°C. These results are consistent with a structure with three-fold symmetry in which the NH groups of the cysteine and one glycine residue in each Gly-Cys-Gly subunit are involved in intramolecular hydrogen bonds. The available data are thus consistent with structure 11 for this substance.

A Chou-Fasman analysis[4] of the probability of β-turn formation by the sequences Cys-Gly-Gly-Cys (as in 11) and Gly-Cys-Gly-Gly (as in 3) give values of 3.1 x 10^{-4} and 1.6 x 10^{-4}, respectively. Since turns are predicted by this model for values larger than 7.5 x 10^{-5}, both structures are predicted to have turn conformations.

Space-filling models for 11 suggest a rigid flowerpot-like structure that may exhibit interesting binding properties. Syntheses and study of analogs of 11 that should exhibit more favorable solubility properties are in process and will be reported subsequently.

Acknowledgement

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11. Calcd for $C_{30}H_{39}O_9N_9S_3 \cdot H_2O$: C 45.96, H 5.27, N 16.08, S 12.27. Found: C 46.00, H 5.32, N 16.06, S 11.91.

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