Pergamon

Tetrahedron, Vol. 52, No. 29, pp. 9823-9834, 1996

0040-4020/96 $15.00 + 0.00

PII: S0040-4020(96)00514-5

Protein Folding: The Synthesis and Conformational Studies on Cystinyl-Cystinyl-Cystine [-CSSCCSSCCSSC-] A Novel Cross Linking Motif

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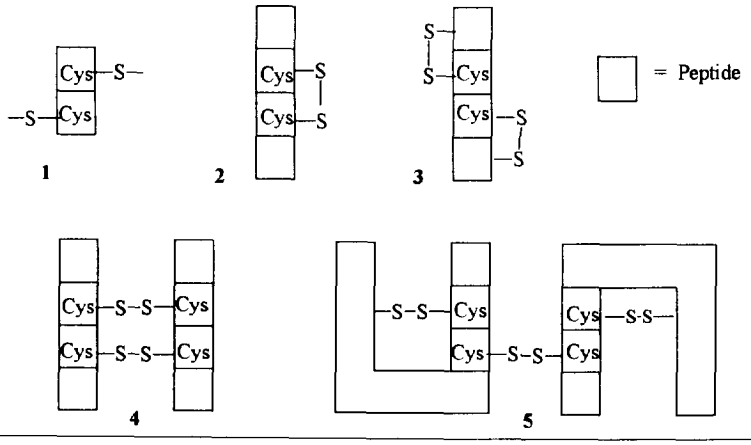
Abstract: The cysteine capped, ends protected cross linked motifs **6** and **7**, arising from pairs of proximally placed cysteines wherein within a 22 atom framework are inscribed 3 disulphide bridges and 2 peptide linkages, have been prepared and their conformations derived by detailed ^{1}H NMR studies and molecular modeling protocols, where excellent agreement was seen. Both compounds **6** and **7** possess a C_2 symmetric hydrogen bonded pair involving the peptide NH and the CO of the proximate N-protecting group (Boc or Z). The nature of the latter profoundly influences the conformation. Thus, in **6** where the unit is Boc a bend conformation was promoted by hydrophobic interactions; alternatively in **7** with a Z-protecting group the conformation was linear. Compounds **6** and **7** have much promise in diverse aspects of protein design. Copyright © 1996 Elsevier Science Ltd

The cysteinyl cysteine construct **1**, can manifest itself in the protein framework, either as a monomer or a dimer element, leading to four possible structural arrangements **[2-5]**, arising from either an 'intra' mode **[2,3]** or a 'cross linking' mode **[4, 5]** [Figure 1].

Figure 1



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As part of study to understand facets of protein folding,[1] we analysed the structural and motif profiles of 62 functional proteins, having 10000 residues, and whose 3D structures have been determined by high resolution x-ray crystallography.[2] It was of interest to note that in this broad data base, only 8 cys-cys dipeptide arrangements were seen and that half the pairs were found in one protein, namely, wheat germ agglutinin.[3] Only the 'intra mode' **3** was seen in the entire set. Although not found in the sequences examined, construct **2** is known.[4] Thus in the context of current interest in protein folding and related motifs, it was considered to be of interest to prepare systems represented by **4** and **5** and to delineate their conformational profiles. The synthesis of **4** has eluded us thus far and the present paper reports the synthesis and conformational studies of the cysteine capped and ends protected unit related to **5**, having primary structures **6** and **7**:

ZNHCH[E]CH_2*SS*CH_2CH[E]NHCOCH[NHP]CH_2*SS*CH_2CH[NHP]CONHCH[E]CH_2*SS*CH_2CH[NHZ]E

E = COOMe; P = Boc = **6**; P = Z = **7**

Compounds **6** and **7** constitute novel, and unusual structures wherein within a 22 atom framework are inscribed 3 disulfide bridges and 2 peptide linkages and provide opportunities for formation of secondary structures. The trans planar nature of the peptide bonds coupled with ~90° dihedral angles generally subtended around the disulfide bridges,[5] should make the conformational representation of **6** and **7** possible, provided that the other key variable, namely, NH-$C^{\alpha}H$ dihedral angles are determined. The possible ϕ angles around NH-$C^{\alpha}H$ determined by ^1H NMR spectra correlated well with the values arrived from molecular modeling using Biosym version 2.3.5. package on a silicon graphics IRIS Crimson Elan work station and energy minimization, using INSIGHT and DISCOVER program packages and force field data, to afford representation shown in Figure 2 and Figure 3 for **6** and **7** respectively. The so derived ϕ, ψ angles for **6** and **7** are presented in Table 1.

The presence of C_2 symmetric hydrogen bonded pair in **6** and **7**, involving Pep-NH, seen from energy minimization studies is fully supported by ^1H NMR experiments. Thus, the Pep-NH pair in **6** and **7** is not exchanged even upon prolonged keeping with D_2O, hardly show any change upon addition of increasing amounts of DMSO-d_6 to solutions in $CDCl_3$, unlike the other two NH pairs, and exhibited lowest $d\delta/dT$ values in DMSO-d_6.

As could be seen from Figure 2 and Figure 3, the shapes of the linker elements in **6** and **7** are profoundly influenced by the nature of the N-protecting group in the mid section of the molecules. In **6**, where this is a Boc group, there is a clear bend, very likely arising from close proximity of the t-butyl residues, resulting in significant hydrophobic interactions; on the other hand in **7**, having Z protecting groups, the profile is linear.

Figure 2 6: (a) Energy minimizd view (b) Structural representation

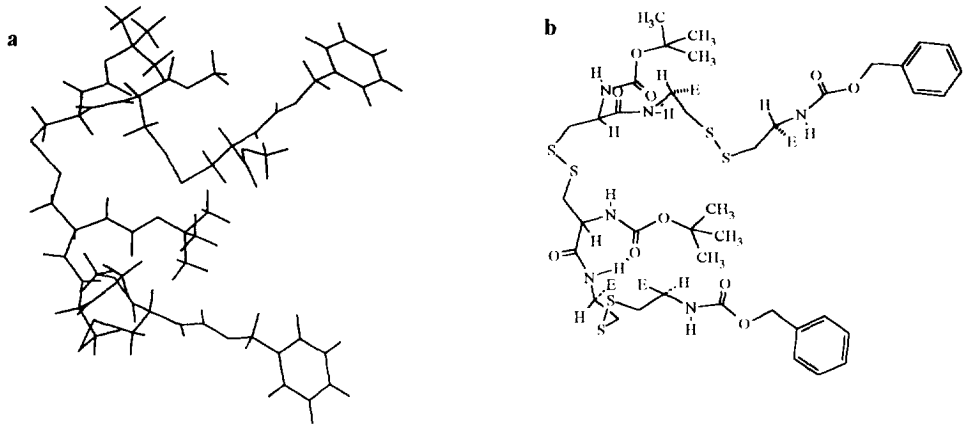


Figure 3 7: (a) Energy minimizd view (b) Structural representation

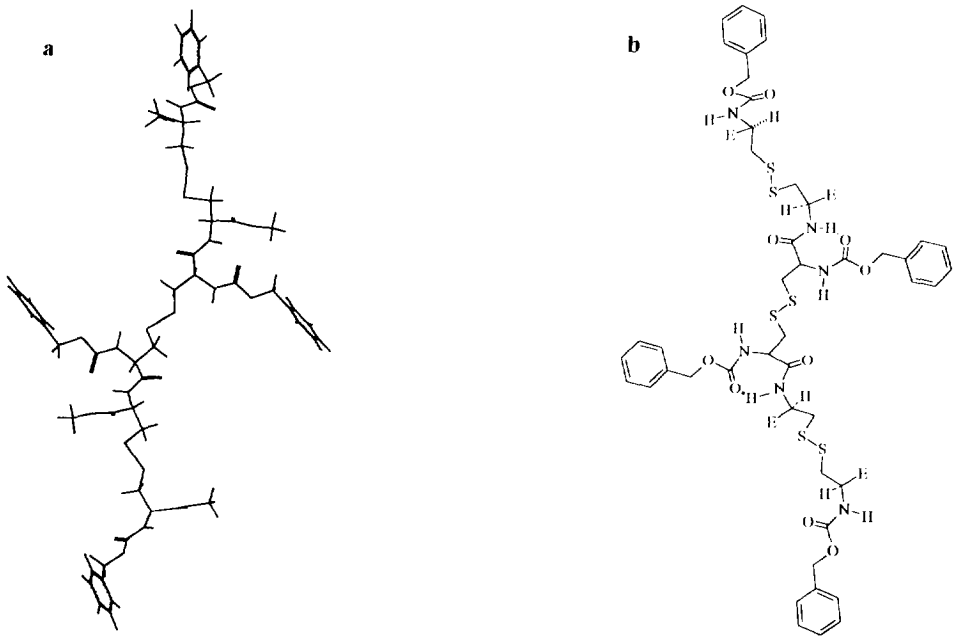


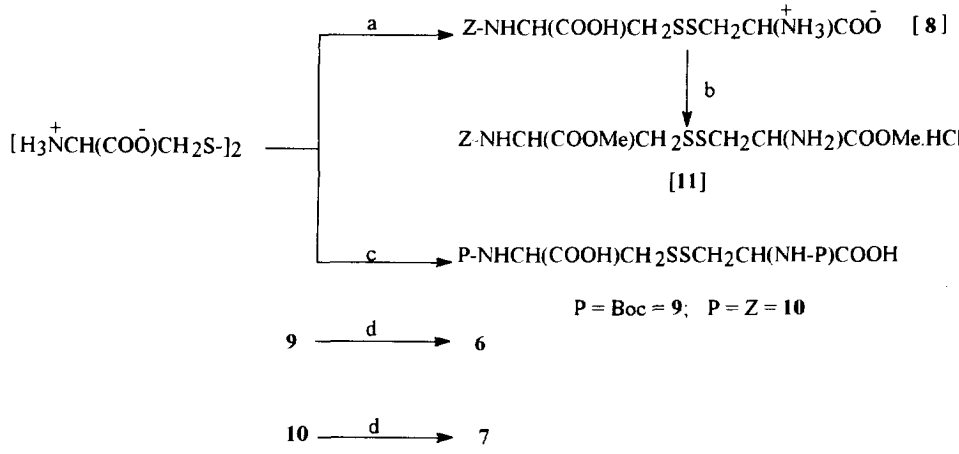
Table 1 Dihedral angles (deg) for 6 and 7 deduced from molecular modeling studies

	ϕ_1	ψ_1	ϕ_2	ψ_2	ϕ_3	ψ_3
6	-153.7	143.9	123.1	38.1	-75.4	58.7
7	-155.3	104.9	74.1	49.9	-72.8	62.4

Synthesis of 6 and 7:

Mono Z-protection of cystine was achieved by treatment with limited Z-Cl in presence of aqueous NaOH, and followed by adjustment of the pH to 3.2 when the desired mono-Z protected cystine **8** precipitates out.[6] The mono protection was clearly demonstrated by NMR and FAB mass spectra. Bis-N-protection of cystine was readily achieved using either Boc carbonate and NaOH or Z-Cl and NaOH, to afford, respectively, compounds **9** and **10**. Treatment of **8** with methanolic HCl afforded mono-Z-protected cystine.diOMe.HCl (**11**). DCC-HOBt condensation of one unit of **9** with two units of **11** led to smooth bis peptidation leading to N'-Z-cystinyl(OMe)$_2$-N"N"[(bis-Boc)cystinyl]-cystine(N'Z)-diOMe (**6**). In a similar manner peptidation of one unit of **10** with two units of **11** afforded N'Z-cystinyl(OMe)$_2$ -N"N"[(bis-Z)-cystinyl]-cystine(N'Z)-diOMe (**7**) (Figure-4).

Figure-4



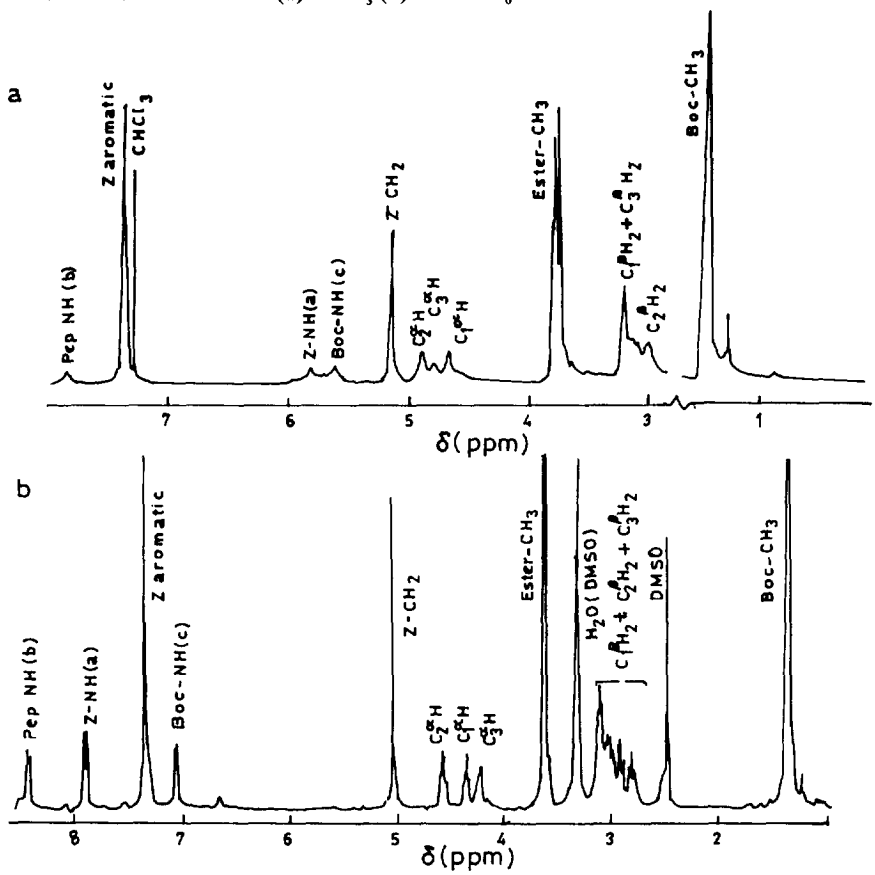
a. Z-Cl, NaOH, pH 3.2 b. MeOH-HCl c. Boc-carbonate, NaOH (**9**) or Z-Cl, NaOH (**10**)
d. **11**, NEt3/DCC-HOBt

The structures of **6** and **7** are supported by spectral and analytical data. Extensive NMR studies *(vide infra)* enabled most probable conformations of these in $CDCl_3$ and DMSO-d_6.

Conformations of 6 and 7:

A wealth of information was secured by detailed NMR studies involving both **6** and **7**. These tend to unequivocally support structures shown in Figure 2 and Figure 3. The well separated nature of the three pairs of NH protons, particularly in DMSO-d_6 [Figure 5 and Figure 6] enabled, by decoupling experiments, the location of the C^{α}-protons, whose decoupling, in turn, led to assignments of the C^{β}-protons as well. The 400 MHz ^{1}H NMR spectra of **6** in $CDCl_3$ and DMSO-d_6 are presented in Figure 5.

Figure 5 6: 400 MHz ^{1}H NMR in (a) $CDCl_3$ (b) DMSO-d_6



^{1}H NMR Studies on 6:

1 2 3

(ZNHCH[E]CH_2*SS*CH_2CH[E]NHCOCH[NHBoc]CH_2*S*-)$_2$ [6]

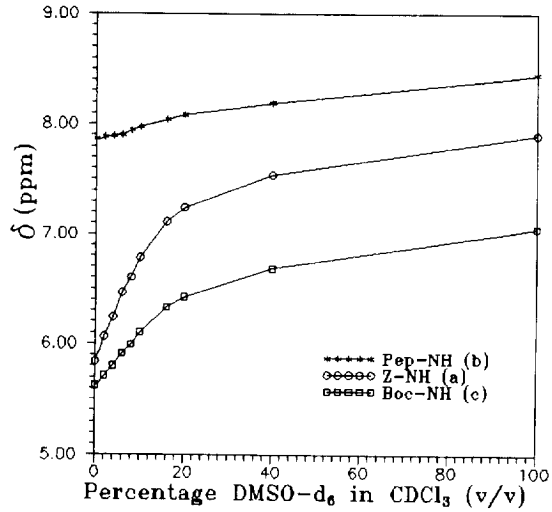
a b c

[a = Z-NH; b = Pep-NH; c = Boc-NH]; [1, 2, 3 = C^{α}]

Difference NOE experiments in DMSO-d_6 have provided significant information pertaining to the spatial proximity of several key bonds. Irradiation of the Pep-NH (b) resulted NOE connections with $C_2^{\alpha}H$, Boc-NH (c), $C_1^{\alpha}H$ and $C_1^{\beta}H$. Irradiation of Z-NH (a) did not exhibit any connection. Irradiation of Boc-NH (c) resulted in the connections of Pep-NH (b), $C_3^{\alpha}H$, $C_2^{\alpha}H$, $C_1^{\alpha}H$ and $C_3^{\beta}H$. These experiments clearly show that unlike the Z-NH (a) located at the terminal positions both Pep-NH (b) and Boc-NH (c) are in close proximity. When coupled with the fact that the latter two are in vicinity of all the three $C^{\alpha}H$ linkages, the conformation for **6** is in excellent agreement with that derived from molecular modeling (Figure 2). The NOESY spectrum of **6** in $CDCl_3$ revealed connectivity between $C_1^{\alpha}H$ --->$C_1^{\beta}H$, $C_1^{\alpha}H$--->Z-NH (a) and $C_2^{\alpha}H$ --->Pep-NH (b).

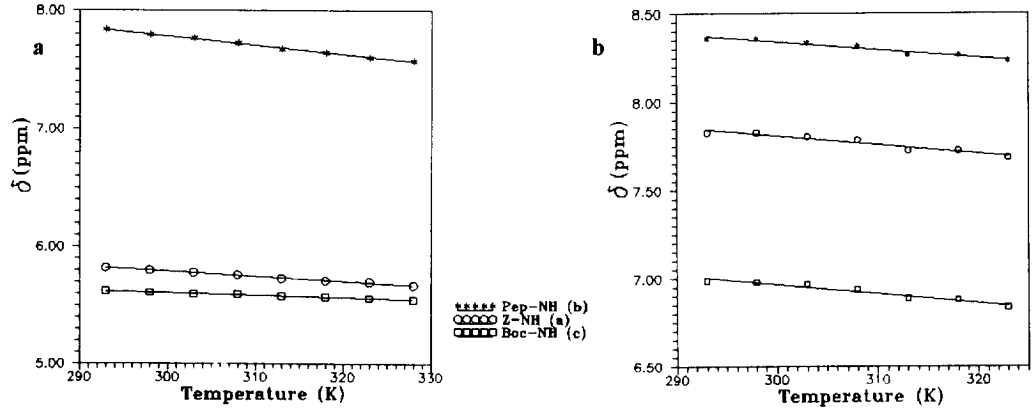
Solvent titration studies and variable temperature (VT) studies were complementary. Chemical shift measurements with **6**, as a function of increasing amounts of DMSO-d_6 in $CDCl_3$, are presented in Chart 1. As could be seen from Chart 1, the shift of 2.06 ppm for the Z-NH (a) is noteworthy and clearly shows that it is normally solvent exposed. An intermediate value of 1.43 ppm for the Boc-NH (c) indicates it is perhaps involved in weak association. The very low value of 0.58 ppm shift for the Pep-NH (b) shows that it is intramolecularly hydrogen bonded as illustrated in Figure 2.

Chart 1 **6**: Chemical shifts of NH protons as a function of varying concentrations of DMSO-d_6 in $CDCl_3$



^{1}H NMR variable temperature studies with **6** in $CDCl_3$ and DMSO-d_6 are presented in Chart 2. The most useful result is the low dδ/dT value of -3.99 ppb/K seen for the Pep-NH (b) in DMSO-d_6, which further confirmed the involvement of this proton in intramolecular hydrogen bonding as seen in Figure 2.

Chart 2 **6**: Temperature dependence NH proton chemical shifts in (a) $CDCl_3$ (b) DMSO-d_6



^{1}H NMR Studies on 7:

1 2 3

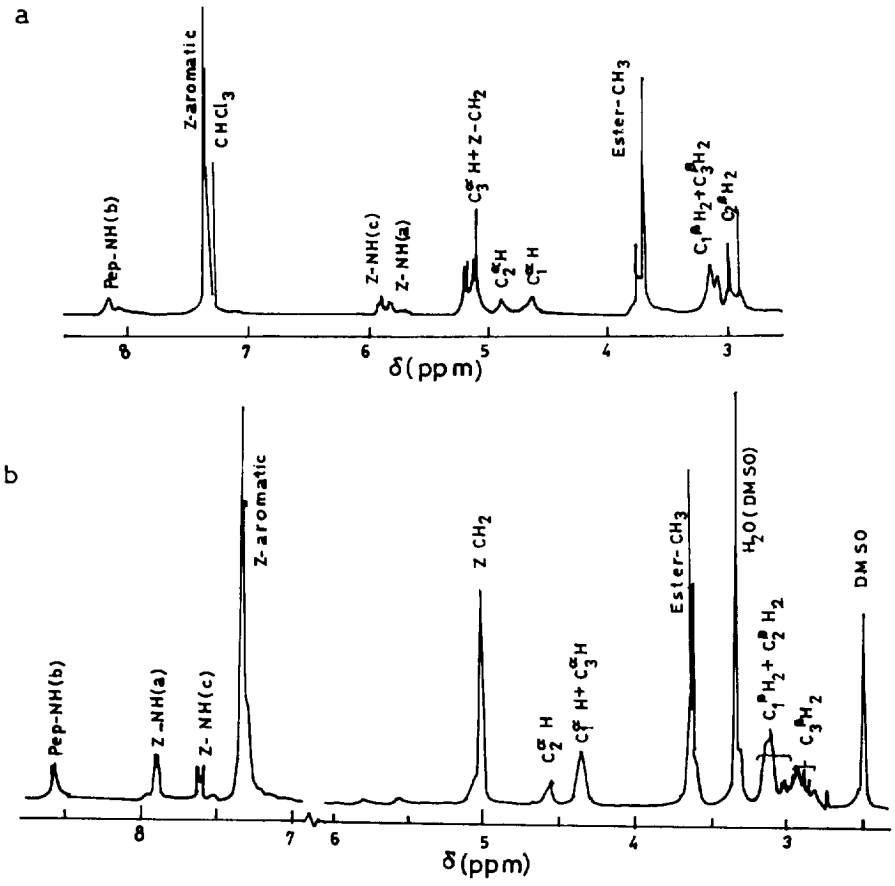
$(ZNHCH[E]CH_2SSCH_2CH[E]NHCOCH[NHZ]CH_2S-)_2$

a b c

[a = Z-NH; b = Pep-NH; c = Z-NH]; [1, 2, 3 = C^α]

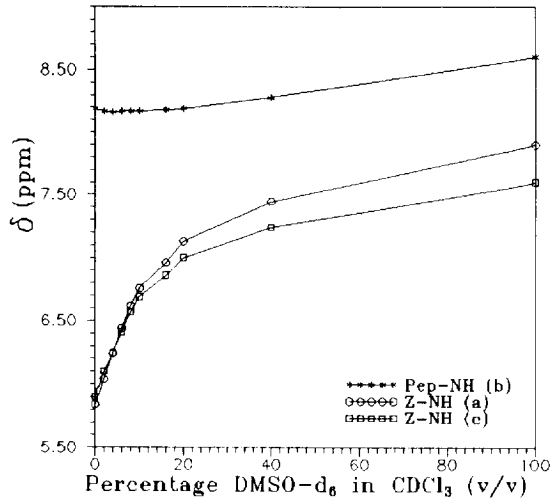
The 400 MHz ^{1}H NMR spectra of 7 in $CDCl_3$ and DMSO-d_6 are presented in Figure 6. Difference NOE studies of 7 in DMSO-d_6 showed spatial connectivity of Pep-NH (b) with $C_2{}^\alpha H$, $C_1{}^\alpha H$ and Z-NH (c). Irradiation of Z-NH (a) showed connection with $C_1{}^\alpha H$.

Figure 6 7: 400 MHz ^{1}H NMR in (a) $CDCl_3$ (b) DMSO-d_6



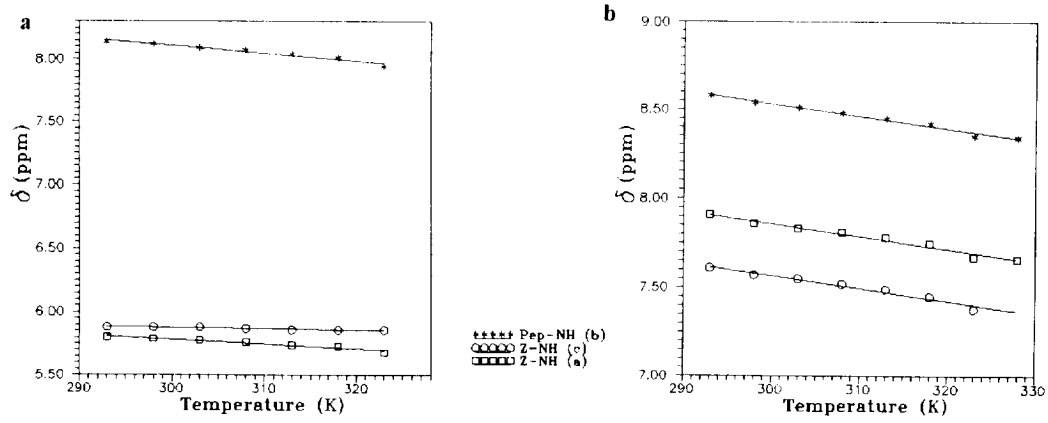
The result of the solvent titration experiment is presented in Chart 3.

Chart 3 7: Chemical shifts of NH protons as a function of varying concentrations of DMSO-d_6 in $CDCl_3$



The large chemical shift observed for Z-NH (a) [2.07 ppm] is similar to that of **6** [2.06 ppm], demonstrating that the proton is solvent exposed. Again an intermediate value of 1.7 ppm [cf. **6**: 1.43 ppm] for Z-NH (c) indicates it is perhaps involved in weak association. Finally, the very low value of 0.41 ppm shift seen for Pep-NH (b) [cf. **6**: 0.58 ppm] shows that it is intramolecularly hydrogen bonded as shown in Figure 3. ^1H NMR variable temperature studies with **6** in $CDCl_3$ and DMSO-d_6 are presented in Chart 4.

Chart 4 7: Temperature dependence NH proton chemical shifts in (a) $CDCl_3$ (b) DMSO-d_6



Here also the most useful information is that the lowest dδ/dT value was found for Pep-NH (b) in DMSO-d_6 as was the case with **6**.

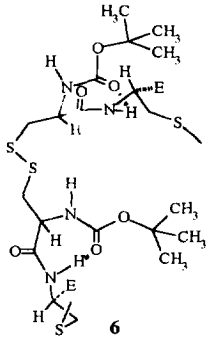
Thus, all NMR observations are in excellent agreement with structures presented in Figure 2 and Figure 3 for **6** and **7** respectively. Whilst the C_2 hydrogen bonded motif centred around Pep-NH (b) seen here seems

secure, as well as the exposed nature of the terminal NH (a), the intermediate profile seen for the mid-NH (c), very likely arises from being part of the seven membered ring hydrogen bonded unit.

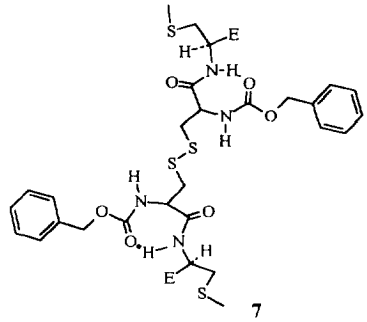
In the context of the present findings, we are hopeful of seeing motifs represented by **5** in naturally occurring proteins, wherein the necessary six cysteines residues are propitiously aligned.

The linker termini in **6** and **7** (Figure 7) show a markedly differential separation, arising from the nature of the N-protection in the mid-section. This aspect would be of practical interest not only in protein design, but also in the crafting of peptidomimetics, with restricted conformational mobility,[7] by modulation of the relevant N-protecting groups to secure the correct spacing of the linker element.

Figure 7 The linker profile of **6** and **7**



6



7

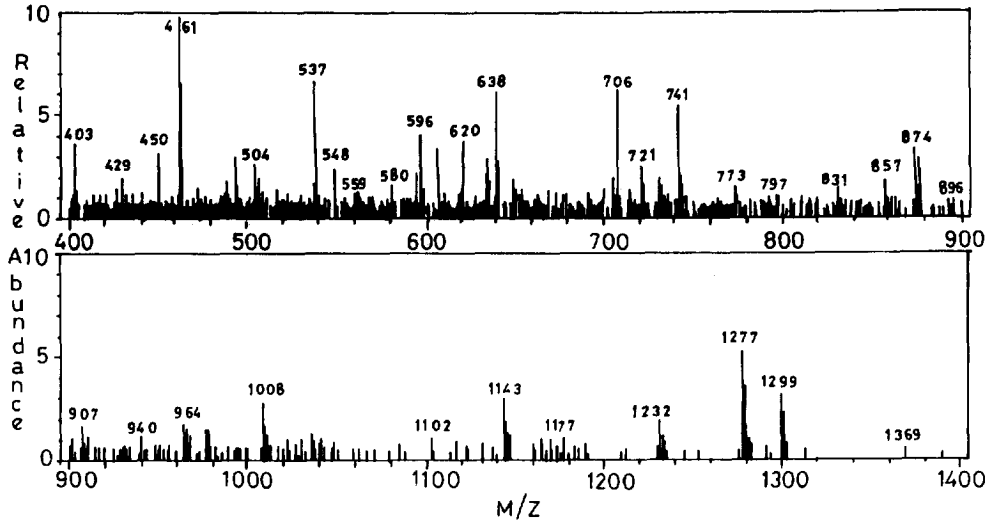
Endowed with four each of ester groups and N-protected amino functions, compounds **6** and **7** offer infinite opportunities in the protein domain. Our immediate objective is to attach peptide segments at the termini of **6** and **7**, either by preferential hydrolysis of the vastly less hindered terminal esters or by attachment of alternate N-terminal ends whose deprotection can easily be done without affecting the disulfide bridges. Other appealing prospects with **6** and **7** may include their uses as core motifs in the crafting of peptide dendrimers.[8]

Of relevance is the recent synthesis of a 28 membered cyclic peptide based on two interchain disulfide bonds- inspired by valinomycin- which binds alkali and alkaline earth metal ions, wherein the pairs of disulfide play an important role.[9] Thus it was not surprising to see that **7** binds strongly to Na^+ ions as seen from its FAB mass spectrum (Figure 8). Based on this, detailed studies on the ion binding properties of **6** and **7** are planned.

EXPERIMENTAL

General: The amino acid cystine used was of L-configuration. The chromatography was performed using silica gel (Merck) The organic extracts were invariably dried over anhydrous $MgSO_4$ and solvents

Figure 8 FAB mass spectrum of 7



evaporated *in vacuo*. The solvents used during experiments were purified by standard literature procedures. IR spectra were recorded on PE 580 / 1600 FT instruments. ^{1}H NMR spectra were recorded on WP 80 / WM 400 Bruker instruments. The chemical shifts are given in ppm using tetramethyl silane as an internal or external standard. Electronic spectra were obtained using PE Lambda-2 UV-VIS spectrophotometer. FAB mass spectra were recorded on a JEOL SX-120 instrument. Elemental analyses were performed using automatic analyser.

Monobenzyloxycarbonyl-L-Cystine [Mono-Z-Cystine] (8):

To an ice-cold and vigorously stirred solution of L-cystine (10 g, 41.7 mmol) in 1.65N NaOH (100 ml) was added benzyloxycarbonyl chloride (95%) (2.63 mL, 19 mmol) over a period of 0.5 h. After an additional 20 min, the reaction mixture was carefully adjusted to pH 6 with 6N HCl and the stirring and cooling continued for 20 min longer. The precipitate of excess of L-cystine was filtered and washed with water (10 mL). The combined filtrates and washings were adjusted to pH 3.2 with 6N HCl and after cooling to 4° C for 10 h was filtered and washed with alcohol (5 x 10 mL) then ether (3 x 10 mL). Recrystallization could be effected by suspending the material in water (100 mL), bringing the pH to 6.5 by the addition of 2N NaOH with stirring to effect solution, filtering off any undesired material, and then adjusting the pH to 3.2 by the addition of 6N HCl. After cooling, the precipitate was filtered and washed successively with cold water (2 x 10 mL), ethanol (3 x 10 mL) and ether (3 x 10 mL). The product on drying weighed 3.24g (46%). mp: 196-200° C; ir(KBr): 3360 (br), 3060, 1704, 1678, 1523, 1448, 1399; FAB ms: 375 $(MH)^+$.

Bis-N-'Butyloxycarbonyl-L-Cystine [Bis-Boc-Cystine] (9):

To a stirred solution of L-cystine (2.4 g, 10 mmol) in 1N NaOH (22 mL) was added slowly di-tertiary butyl dicarbonate (4.36 g, 20 mmol) for 2 h and left stirred overnight. The resulting milky solution was extracted with petroleum ether (4 x 15 mL), the organic layer extracted with saturated aqueous $NaHCO_3$ (4 x 15 mL), the combined aqueous layers adjusted to pH 1 by careful addition of $KHSO_4$ (8.96 g in 60 mL of

water), extracted with EtOAc (4 x 30 mL), washed with water (3 x 20 mL), dried, evaporated with bath temperature 30°C and dried thoroughly *in vacuo* to afford 2.9 g (66%) of bis-Boc-cystine. mp: 137-139°C (lit.[10] mp 143-145°C) ; ir (neat): 3368, 2988, 1743, 1718, 1680, 1509, 1410; nmr ($CDCl_3$): 1.47 (s,s, 18H, Boc-CH_3 x 2), 3.25 (m, 4H, $C^{\beta}H_2$ x 2), 4.47 (q, 2H, $C^{\alpha}H$ x 2), 5.78 (d, 2H, NH x 2), 6.28 (s, 2H, COOH x 2).

Bis-N-Benzyloxycarbonyl-L-Cystine [Bis-Z-Cystine] (10):

To an ice-cold and stirred solution of L-cystine (4.8 g, 20 mmol) in 1N NaOH (100 mL) was added, in drops, benzyloxycarbonyl chloride (95% v/v in toluene) (9 mL, 60 mmol) maintaining the pH throughout at 9-10 by addition of 1N NaOH. The reaction was left stirred for 4 h at 0° C, washed with ether (4 x 25 mL), the pH of the aqueous layer adjusted to 3 with 6N HCl, filtered and the filtrate extracted with EtOAc (6 x 15 mL). The initially precipitated material was dissolved in warm EtOAc, the combined extracts washed with 0.6N HCl (4 x 25 mL), water (4 x 25 mL), dried and evaporated to give 7.32 g (72%) of the product. mp: 118-120°C (lit.[11] mp 114°C); ir (neat): 3333, 3033, 1694, 1586, 1530, 1455.

Mono-N-Benzyloxycarbonyl-L- Cystine Bis-Methyl Ester Hydrochloride (11):

A solution of mono-benzyloxycarbonyl L-cystine (1.9 g, 5 mmol) in cold 2N methanolic HCl (15 mL) was held at room temperature for one day and then concentrated to dryness *in vacuo* at 40°C. The evaporation was repeated twice after the addition each time of methanol (25 mL). The residue thereby obtained is dissolved in cold 1N methanolic HCl (15 mL) and the entire procedure repeated. Solution of the final residual material in a little methanol followed by the addition of acetone leads to the precipitation of the product as colourless needles. yield: 1.69 g (76%); mp: 149-151°C (lit.[6] mp 159-160°C); ir (KBr): 3375, 2946, 2840, 1732, 1684, 1514; nmr ($CDCl_3$-DMSO-d_6): 3.38 (m, 7H, NH_3^+ + $C^{\beta}H_2$ x 2), 3.81 (s,s, 6H, $COOCH_3$ x 2), 4.13-4.69 (m, 2H, $C^{\alpha}H$ x 2), 5.13 (s, 2H, Z-CH_2), 7.34 (s, 6H, NH + Z-aromatic); FAB ms: 403 $(MH)^+$ - HCl.

N'-Z-Cystinyl(OMe)$_2$-N''-N'''-[(bis-Boc)Cystinyl]- Cystine(N'-Z)-diOMe (6):

1-Hydroxy benzotriazole (HOBt) (0.337 g, 2.5 mmol) followed by a solution of DCC (0.515 g, 2.5 mmol) in CH_2Cl_2 (5 mL) was added to a stirred solution of bis-Boc-cystine (9) (0.55 g, 1.25 mmol) in dry CH_2Cl_2 (20 mL). A solution of mono-Z-cystine-diOMe - freshly prepared by dropwise addition of triethylamine (0.35 mL, 2.5 mmol) to an ice-cold and stirred solution of mono-Z-cystine-diOMe.HCl (11) (1.095 g, 2.5 mmol) in dry DMF (15 mL) and leaving aside for 0.5 h- was then added. The mixture was left stirred overnight, filtered, washed with CH_2Cl_2 (15 mL), the filtrate and washings evaporated, the residue dissolved in EtOAc, washed with 2N HCl (2 x 25 mL), saturated NaCl (2 x 15 mL), dried, evaporated and recrystallized from EtOAc - hexane to give 0.94 g of the product as white powder. yield: 0.94 g (62%); mp: 93°C; ir (KBr): 3341, 2928, 1740, 1692, 1665, 1521, 1456, 1436, 1392; nmr ($CDCl_3$, 400 MHz): 1.45 (s, 18H, Boc-CH_3 x 6), 3.0 (m, 4H, $C_2^{\beta}H_2$ x 2), 3.06-3.36 (m, 8H, $C_3^{\beta}H_2$ x 2 + $C_1^{\beta}H_2$ x 2), 3.75 (m, 12H, $COOCH_3$ x 4), 4.66 (q, 2H, $C_1^{\alpha}H$ x 2), 4.79 (q, 2H, $C_3^{\alpha}H$ x 2), 4.88 (q, 2H, $C_2^{\alpha}H$ x 2), 5.12 (s,s, 4H, Z-CH_2 x 2), 5.62 (d, 2H, Boc-NH x 2), 5.86 (d, 2H, Z-NH x 2), 7.36 (s,s, 10H, aromatic), 7.85 (d, 2H, pep-NH x 2).

nmr (DMSO-d_6, 400 MHz): 1.38 (s, 18H, Boc-CH_3 x 6), 2.5 (DMSO), 2.8, 2.9 (q,q, 4H, $C_2^{\beta}H_2$ x 2), 3.0 (m, 4H, $C_3^{\beta}H_2$ x 2), 3.1 (m, 4H, $C_1^{\beta}H_2$ x 2), 3.32 (H_2O), 3.65 (s,s, 12H, $COOCH_3$ x 4), 4.21 (m, 2H, $C_3^{\alpha}H$ x 2), 4.35 (m, 2H, $C_1^{\alpha}H$ x 2), 4.55 (m, 2H, $C_2^{\alpha}H$ x 2), 5.05 (s, 4H, Z-CH_2 x 2), 7.05 (d, 2H, Boc-NH x 2), 7.35 (s, 10H, aromatic), 7.9 (d, 2H, Z-NH x 2), 8.45 (d, 2H, pep-NH x 2); FAB ms: 1009 $(MH)^+$ - 2 Boc; anal: calcd. for $C_{48}H_{68}N_6O_{18}S_6$ C, 47.68; H, 5.62; N, 6.95; found C, 47.84; H, 5.93; N, 7.24; uv-vis λ (nm) (CH_3CN): 251 (sh, 1527), 256 (1461), 262 (sh, 1223), 267 (sh, 965), 325 (sh, 78).

N'-Z-Cystinyl(OMe)$_2$-N''-N'''-[(bis-Z)Cystinyl]- Cystine(N'-Z)-diOMe (7):

1-Hydroxy benzotriazole (HOBt) (0.675 g, 5 mmol) followed by a solution of DCC (1.03 g, 5 mmol) in CH_2Cl_2 (10 mL) was added to a stirred solution of bis-Z-cystine (10) (1.27 g, 2.5 mmol) in dry CH_2Cl_2 (20 mL). A solution of mono-Z-cystine-diOMe - freshly prepared by dropwise addition of triethylamine (0.7 mL, 5 mmol) to an ice-cold and stirred solution of mono-Z-cystine-diOMe.HCl (11) (2.19 g, 5 mmol) in dry DMF (15 mL) and leaving aside for 0.5 h - was then added. The mixture was left stirred overnight, filtered, washed with CH_2Cl_2, the filtrate and washings evaporated, the residue dissolved in EtOAc, washed with 2N HCl (2 x 25 mL), saturated NaCl (2 x 15 mL), dried, evaporated and chromatographed on silica gel. Elution with PhH : EtOAc (3 : 2) gave 1.65 g (52%) of the product as white powder. mp: 127-130°C; ir (KBr): 3331, 2951, 1736, 1652, 1533; nmr ($CDCl_3$, 400 MHz): 2.9 (d, 4H, $C_2^{\beta}H_2$ x 2), 3.15 (m, 8H, $C_1^{\beta}H_2$ x 2 + $C_3^{\beta}H_2$ x 2), 3.70 (s,s, 12H, $COOCH_3$ x 4), 4.62 (q, 2H, $C_1^{\alpha}H$ x 2), 4.88 (q, 2H, $C_2^{\alpha}H$ x 2), 5.1 (s,s, 8H, Z-CH_2 x 4), 5.15 (d,d, 2H, $C_3^{\alpha}H$ x 2), 5.8 (d, 2H, Z-NH [a] x 2), 5.9 (d, 2H, Z-NH [c] x 2), 7.3 (m, 20H, aromatic), 8.15 (d, 2H, pep-NH [b] x 2).

nmr (DMSO-d_6, 400 MHz): 2.85 (d,d, 4H, $C_2^{\beta}H_2$ x 2), 2.95 (m, 4H, $C_3^{\beta}H_2$ x 2), 3.1 (m, 4H, $C_1^{\beta}H_2$ x 2), 3.65 (s,s, 12H, $COOCH_3$ x 4), 4.32 (H_2O), 4.35(m, 4H, $C_1^{\alpha}H$ x 2 + $C_3^{\alpha}H$ x 2), 4.55 (q, 2H, $C_2^{\alpha}H$ x 2), 5.05 (s, 8H, Z-CH_2 x 4), 7.3 (s, 20H, aromatic), 7.6 (d, 2H, Z-NH [c] x 2), 7.9 (d, 2H, Z-NH [a] x 2), 8.6 (d, 2H, pep-NH [b] x 2); FAB ms: 1277 $(MH)^+$; uv-vis λ (nm) (CH_3CN): 245 (sh, 2218), 251 (2158), 256 (2099), 262 (sh, 1743), 267 (sh, 1347).

Acknowledgements: We are grateful to Dr. Darshan Ranganathan for valuable discussions, and to Dr. K. P. Madhusudanan, Central Drug Research Institute, Lucknow for FAB mass. Support from Indian National Science Academy, New Delhi is warmly acknowledged.

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(*Received in UK* 2 *February* 1996; *revised* 28 *May* 1996; *accepted* 30 *May* 1996)