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Unusual cyclo-tetra and hexa peptidation of bis-boc-cystine with cystine-di-OMe: one step preparation of the novel 32- and 48-membered cyclotetracystine and cyclohexacystine

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The unprecedented formation of 32- and 48- membered macrocycles that inscribe 4 and 6 cystine units, in the peptidation of bis-Boc-cystine with cystine di-OMe is reported.

The 16-membered 1:1 cyclic motif, that could result from the cross-linking of a cysteinyl cysteine unit [CC] with another, generating a [CCCC] box, has neither been prepared nor seen in proteins.[1] Our continuing efforts[2] to prepare such a construct has resulted in the isolation of macrocyclic systems **1** and **2**, analysis of whose formation tends to suggest structural difficulties in the formation of the 1:1 adduct.

Several rational approaches to this failed. Finally, the condensation of bis-Boc-cystine (bis-Boc-Cyst) with cystine-di-OMe (Cyst-di-OMe) (DCC, HOSu) afforded the 32 (2:2) and the 48- (3:3) membered cyclic products **1** and **2**. The desired 16 (1:1)-membered cyclic compound was absent.[3]

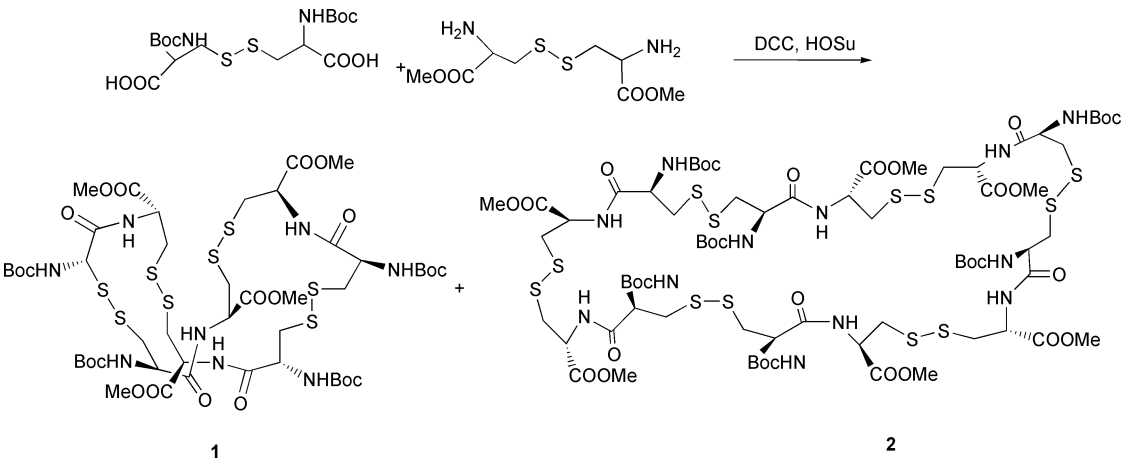
The structural assignments for **1** and **2** are supported by elemental analysis, IR, NMR and FAB MS. The mass spectra of the compounds were quite revealing, with practically every fragmentation mode accountable.[4] ^{1}H NMR studies at 500 MHz (TOCSY, NOESY and VT)[5] clearly showed that at 30 °C, both **1** and **2** existed as conformational isomers (I, II and III) in the ratios 57:28:15 and 77:15:8, respectively. The presence of significant cross peaks Cyst^{1}NH–Cyst^2 NH, Cyst^{1}H$_\alpha$-Cyst^{2}NH in ROESY of both **1** and **2** suggests that the compounds populate both the α_R and β region of the φ, ψ conformational space. The flexible nature of **1** and **2**, most likely arising from the M or P helical arrangement, with tilt in favour of P for L-cystine, around the disulfide bond, is further supported by the ~8 Hz values for $^3J_{NH\text{-}CH\alpha}$. The presence of cross peaks of the same sign as diagonals in the ROESY indicate slow isomer exchange. In the case of **1** such exchange was seen between I and II, I and III but not between II and III. Conformations P_3M, P_4 and P_2M_2, for I, II and III, respectively, would account for this, since the non-observed exchange between II and III would require two conformational flips. Molecular modeling studies also show P_3M as the minimum energy structure. With **2**, in the absence of such information, conformers I, II and III are assigned, respectively, P_3M_3, PM_5 and P_4M_2, based on modeling studies. The observed $\Delta\delta/\Delta T > 5$ in VT studies rules out the presence of intramoleculer hydrogen bonding in either **1** or **2**.

The formation of cyclic peptides in the polymerization of amino acids is unprecedented. Detailed molecular modeling studies[6] tend to show the key involvement of the orthogonally disposed disulfide linkage in directing the cyclization.

Primary peptidation involving bis-Boc-Cyst and Cyst-di-OMe would lead to a construct that has an option to form either the 1:1 adduct or oligomers such as **1** and **2**. The relative energies of the most stable conformations of the 1:1 adduct and the 2:2 adduct (**1**) are −51.32 and −59.37 kJ mol^{-1} respectively. Further, the transition from open precursors to these are separated by 15.0 and 12.43 kJ mol^{-1}, based on the carbonyl C-atom and amine N-atom distance of < 4.0 Å, from their respective 'global' minima. Both these factors should promote formation of the 2:2 adduct (**1**), as has been experimentally observed. Conformations **1** and **2**, shown in Scheme 1, represent minimum energy states, as derived from calculations,[6] and are in agreement with NMR observations which also suggest a P_3M conformation for **1**.

Gram quantities of both **1** and **2** can be prepared in a single operation. The 32-membered **1** has a near perfect cleft with a highly polar opening by the proximate alignment of the C_2 oriented peptide bonds, augmented by pairs of carboxyl and nitrogen protecting groups. The clustering of the eight sulfur centres around a central core constitutes another unique feature. The 48-membered **2**, projects a rectangular array, where two parallel 16-membered cystinyl cystines, resembling β sheets, are tethered at the ends by single cystine units.

Compounds **1** and **2**, heavily endowed with amide and sulfur centres, appeared attractive for the formation of silver com-



Scheme 1

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plexes, especially since the overwhelming preference as a donor centre for sulfur has been recognized.[7] Such complexes have potential in ^{111}Ag-based radioimmunotherapy.[8] Compound **1** readily afforded $[(Cyst)_4M]^+X^-$ complexes on treatment with silver nitrate, silver picrate and cupric chloride. Interestingly, compound **2** gave the bis complex, $[(Cyst)_6Ag_2](NO_3)_2$. The structural assignments for the complexes are supported by, elemental analysis, FT-IR, 500 MHz NMR and Mass spectroscopy (MALDI-TOF).[9] A binding constant of $\sim 10^3$ mol^{-1} has been secured for the interaction of silver picrate with **2**.

In Nature, sulfur clusters on a peptide scaffold, play pivotal roles, as could be exemplified with, iron–sulfur clusters, which rank with heme and flavin in pervasive occurrence and multiplicity of functions,[10] transferins that control the levels of iron,[11] metallothionein involved in metal detoxification and highly specific regulatory processes,[12] neurotoxins,[13] heat stable enterotoxins[14] and several others. Compounds **1** and **2** are excellent precursors for the crafting of metal sulfur clusters.

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Notes and references

1 An examination of structural motifs present in 63 functional proteins, having ~10,000 residues, and whose 3 dimensional structures have been established by high resolution X-ray, failed to show the 16-membered cyclic motif.

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3 To an ice cooled and stirred solution of bis-Boc-cystine (4.73 g, 10.7 mmol) in dry CH_2Cl_2–DMF (95:5, 300 ml) was added *N*-hydroxysuccinimide (2.46 g, 21.4 mmol) and DCC (4.85 g, 23.54 mmol) followed by, in drops, over 8 h, cystine-di-OMe (generated *in situ* by the dropwise addition of triethylamine (3.6 mL, 25.8 mmol) to an ice cooled and stirred solution of cystine dimethyl ester dihydrochloride (4.4 g, 12.9 mmol) in dry CH_2Cl_2 (500 mL). The mixture was left stirring at rt for 5 d, filtered, washed with $CHCl_3$ (3 × 25 mL), the filtrates washed successively with cold satd. $NaHCO_3$ (3 × 25 mL), 2 N H_2SO_4 (3 × 25 mL), distilled water (1 × 25 mL), dried ($MgSO_4$) and evaporated *in vacuo*. The residue was chromatographed on silica gel (~400 g) using $CHCl_3$–MeOH as eluent (gradient 99.8:0.2 to 97:3; fraction volume: 20 mL). Fractions 42–61 ($CHCl_3$–MeOH = 98:2) contained pure **1** (2.26 g, 31%) and fractions 73–85 pure **2** (1.1 g, 16%).

4 Compound **1**: mp 185–187 °C; ^{1}H NMR ($CDCl_3$–DMSO-d_6) δ 1.5 (s, 36H), 2.8–3.2 (m, 16H), 3.75 (s, 12H), 4.35 (m, 4H), 4.7 (m, 4H), 6.75 (br s, 4H), 8.1–8.3 (m, 4H); FAB MS (*m*/*z*) (%): 1367 (44) $(M + Na)^+$, 1245 (21) $(M - 1\ Boc + H)^+$, 1045 (11) $(M - 3Boc + H)^+$, 945 (100) $(M - 4Boc + H)^+$; $[\alpha]_D^{29}$: −230.29. (1c, DMF); Anal. Calcd for: $C_{48}H_{80}N_8O_{20}S_8$; C, 42.85; H, 5.95; N, 8.33; found: C, 42.69; H, 6.04; N, 7.90%. Compound **2**: mp 170–172 °C; ^{1}H NMR ($CDCl_3$) δ 1.40 (s, 27 H), 1.60 (s, 27H) 2.95–3.30 (m, 24H), 3.75 (s, 18 H), 4.75 (m, 6H), 4.9 (m, 6H), 5.75 (br s, 6H), 7.8 (m, 6H); FAB MS (*m*/*z*) (%): 2039 (11) $(M + Na)^+$, 1918 (5) $(M - 1Boc + H)^+$, 1417 (8) $(M - 6Boc + H)^+$. $[\alpha]_D^{29}$: −185.92 (1c, DMF). Anal. Calcd for: $C_{72}H_{120}N_{12}O_{30}S_{12}$.$CHCl_3$: C, 40.45; H, 5.62; N, 7.86; found: C, 41.12; H, 5.80; N, 7.77%.

5 500 MHz NMR spectra were recorded on a ~6–8 mM solution in $CDCl_3$–DMSO-d6. TOCSY and ROESY were used to assign the resonances. Variable temperature (VT) studies were carried out in the range, 30–70 °C at 10 °C intervals.

6 The conformation analysis of cyclic adducts 1:1 (16), 2:2 (32) (**1**), 3:3 (48) (**2**) have been carried out using simulated annealing molecular dynamics.

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9 *Silver nitrate complexes*: a solution of **1** or **2** (0.002 mmol) in dry THF–DMSO was admixed with $AgNO_3$ (0.0406 mmol), left stirring for 1 h at rt, solvents were evaporated *in vacuo*, the residue was triturated with water and dried to afford the complexes in near quantitative yeilds. $[(Cyst)_4Ag]NO_3$: MALDI TOF MS (*m*/*z*) (%): 1452 $[(Cyst)_4Ag]^+$ (49) $[(Cyst)_6Ag_2](NO_3)_2$: MALDI TOF MS (*m*/*z*) (%): 1116 $[(Cyst)_6Ag_2]^{++}$ (100) *Silver picrate complex*: a solution of **1** (0.002 mmol) in dry MeOH–DMSO and silver picrate (0.02 mmol) was processed as above. $[(Cyst)_4Ag][C_6H_2N_3O_7]$: MALDI TOF MS (*m*/*z*) (%): 1452 $[(Cyst)_4Ag]^+$ (100) $[(Cyst)_6Ag_2][C_6H_2N_3O_7]_2$:^{1}H NMR ($CDCl_3$) δ 1.40 (s, 27 H), 1.60 (s, 27H), 2.95–3.35 (m, 24H), 3.75 (s, 18 H), 4.75 (m, 6H), 4.9 (m, 6H), 5.75 (br s, 6H), 7.8 (m, 6H); *Copper chloride complex*: Prepared from **1** (0.0007 mmol) and $CuCl_2{\cdot}2H_2O$ (0.008 mmol) in DMSO–THF as described above. $[(Cyst)_4Cu]NO_3$: MALDI TOF MS (*m*/*z*) (%): 1408 $[(Cyst)_4Cu]^+$ (30).

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