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Self-Condensation of a Thiazole-Peptide Bearing a 21-Membered Loop into a Library of Giant Macrocycles with Multiple Orthogonal Loops

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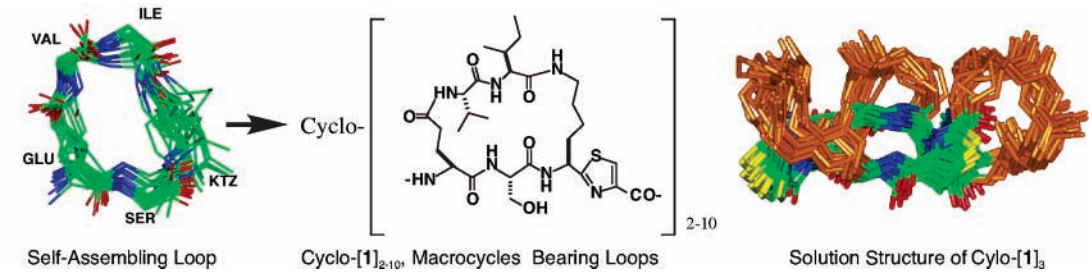
ABSTRACT



Tetrapeptide analogue H-[Glu-Ser-Lys(Thz)]-OH, containing a turn-inducing thiazole constraint, was used as a template to produce a 21-membered structurally characterized loop by linking Glu and Lys side chains with a Val-Ile dipeptide. This template was oligomerized in one pot to a library (cyclo-[1]$_n$, n = 2–10) of giant symmetrical macrocycles (up to 120-membered rings), fused to 2–10 appended loops that were carried intact through multiple oligomerization (chain extension) and cyclization (chain terminating) reactions of the template. A three-dimensional solution structure for cyclo-[1]$_3$ shows all three appended loops projecting from the same face of the macrocycle. This is a promising approach to separating peptide motifs over large distances.

Nature uses very sophisticated molecular machinery to build complex polymers by repetitive couplings of simple building blocks (amino acids, nucleotides, carbohydrates), each containing all the information necessary to yield structural diversity. On the other hand, we and others[1] are interested in more simply generating complex polymers from amino acids using oligomerization. We have reported[2] a one-pot synthesis of macrocycles from a tetrapeptide containing a turn-inducing[3] thiazole constraint. We now expand the potential of this process to the preparation of a more complex library from a self-assembling template, H-[Glu-Ser-Lys-(Thz)]-OH, which carries a structurally defined 21-membered loop on its back (Figure 1). The loop rides unchanged as a covalently incorporated passenger through multiple self-condensing (oligomerization) and chain-terminating (cyclization) steps to a library of large macrocycles with up to 10 templates and 10 loops (Figure 2).

Tetrapeptide H-[Glu-Ser-Lys(Thz)]-OH was first modified (Scheme 1, Supporting Information) to compound **1** incorporating a 21-membered passenger loop (Figure 3). The solution structure of **1** was determined from TOCSY and NOESY spectra in DMSO-d_6. Medium-range NOEs and four $^3J_{NH-CH\alpha}$ coupling constants (8.48 Hz, Lys; 8.24 Hz, Ser; 8.25 Hz, Val; 7.99 Hz, Ile) defined a novel loop, comprising two noninteracting strands connected by two aliphatic linkers. The chemical shift for Ile NH was uniquely temperature-independent ($\Delta\delta/T$ < 1 ppb/K), consistent with one possible

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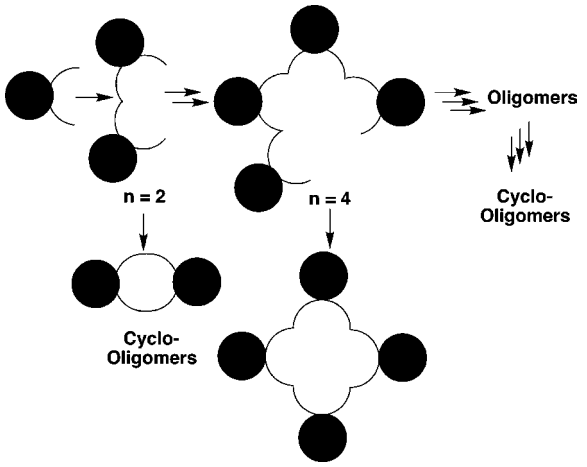

Figure 1. Competing self-condensation (oligomerization) and termination (cyclization) reactions for a template with passenger (●).

transannular hydrogen bond most likely to Glu-γCO. The three-dimensional structure of **1** was calculated from 25 NOE distance restraints (19 sequential, 6 medium-range; intraresidue NOEs were overlapped) and four ϕ angle restraints ($\phi = 120 \pm 30°$) based on coupling constants using a dynamic simulated annealing and energy minimization protocol in XPLOR. Initial structures indicated a possible H-bond (Ile NH···OC γGlu), consistent with VT-NMR data (Supporting Information Figure S2). The final 20 lowest-energy structures calculated without the H-bond restraint converged (RMSD 0.86 Å) without NOE distance (>0.2 Å) or angle (>1°) violations (Figure 3), whereas violations persisted when using the H-bond.

Template **1** condenses with itself[4] in BOP/DIPEA/DMF to a concentration-dependent series of novel cyclooligomers

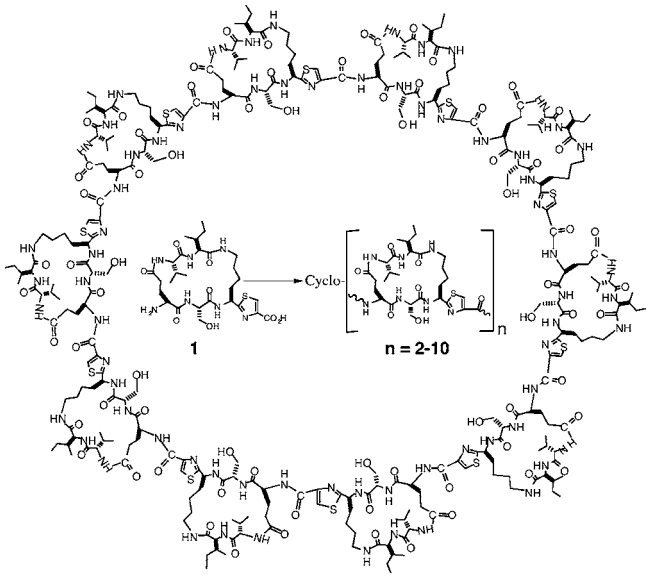

Figure 2. **1** assembles to cyclo-[**1**]$_n$, $n = 2-10$ ($n = 10$ shown).

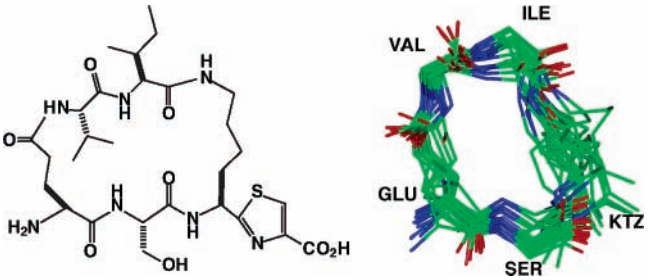

Figure 3. (Left) Structure of **1**, a template modified from H-[Glu-Ser-Lys(Thz)]-OH to create a loop. (Right) Twenty lowest-energy structures of **1** in DMSO-d_6 showing cyclic backbone. Side chains omitted for clarity, KTZ = lysine(thiazole).

2−**10** of formula cyclo-[**1**]$_n$, $n = 2-10$ (Figures 2 and 4). At 10^{-3} M **1**, cyclooligomers accounted for 83% products in relative distribution 15.5:9:4.5:1.4:0.3 for $n = 2-6$, with 50% of cyclooligomers being cyclodimer. At 0.1 M **1**, the distribution changed to 4.8:3.0:2.8:2.4:1.8:1.3:0.6:0.3:0.1 for $n = 2-10$, with 61% of the products being cyclooligomers. Cyclic products gave uniformly separated rpHPLC retention times allowing isolation and identification by tandem rpHPLC/ESMS or LC/MS.

Figure 4a shows typical electrospray mass spectra (ESMS) for products, exemplified by the expected molecular ions for

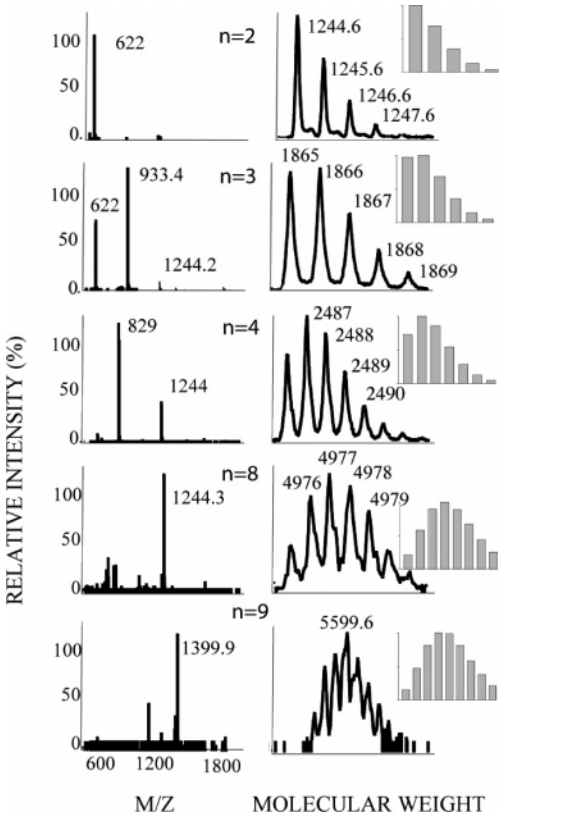

Figure 4. ESMS. (A, left panel) m/z ions for cyclo-[**1**]$_n$, $n = 2$, 3, 4, 8, 9. (B, right panel) Experimental masses and isotope distributions (theoretical distributions, inset).

compounds cyclo-[**1**]$_n$, n = 2 (m/2 622), n = 3 (m/2 933, m/3 622, 2m/3 1244), n = 4 (m/2 1244, m/3 829), n = 8 (m/4 1244), and n = 9 (m/4 1400). Isotope distributions within peaks confirmed formulations (e.g., m/2z peaks varied by 0.5 mass unit, m/3z by 0.33, etc.). Figure 4b shows reconstructed m/z molecular ions and isotopic distributions, exactly matching calculated isotope distributions (Figure 4b, insets), confirming molecular weights. Incremental differences in mass units of cyclo-[**1**]$_n$ and unique isotope patterns established macrocycle identity, supported by single-peak ESMS (Figure S1) and MALDI-TOF MS (not shown).

These cyclooligomers are complex. Even one of the smaller condensation products, cyclo-[**1**]$_4$, consists of a 48-membered macrocycle (with 12 endocyclic amide bonds, 4 thiazoles, and 4 serine side chains) joined to four 21-membered rings. The largest member of the library, cyclo-[**1**]$_{10}$ (Figure 2), has MW 6210 Da and is a 120-membered macrocycle (with 30 endocyclic amide bonds, 10 thiazoles, and 10 serine side chains) fused to ten 21-membered rings.

The ^{1}H NMR spectra for cyclo-[**1**]$_n$, n = 2−4 in d_6-DMSO (Figure 5) are simple, each compound displaying only six

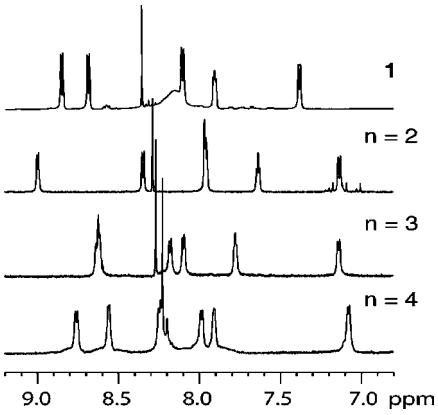

Figure 5. ^{1}H NMR spectra (amide NH region) for **1** and cyclo-[**1**]$_n$, n = 2, 3, 4 in DMSO-d_6 indicating high symmetry.

amide NH resonances such as **1**. This is indicative of high symmetry in the macrocycles even with appended loops. The ^{13}C NMR spectrum (Figure S9) of cyclo-[**1**]$_n$ also indicated high symmetry. Variable-temperature experiments (Supporting Information) for cyclo-[**1**]$_2$ and cyclo-[**1**]$_3$ indicated that the Ile NH proton was uniquely temperature-independent as in **1** and that the loop amide coupling constants $^3J_{NH-CH\alpha}$ 8−9 Hz (Supporting Information) are atypical of random coil values (∼6−7 Hz). We conclude that high symmetry is a consequence of the loop structure in **1** being transferred into the larger molecules, since asymmetry or conformational averaging would otherwise be expected.

The solution structure of cyclo-[**1**]$_3$ was determined from TOCSY and NOESY spectra in DMSO-d_6. Medium-range NOEs and nine $^3J_{NH-CH\alpha}$ coupling constants (8.8 Hz: Glu1, Glu4, Glu7; 9.2 Hz: Lys3, Lys6, Lys9; 8.3 Hz: Ile11, Ile12, Ile15) defined a novel bowl-shaped macrocyclic scaffold (Figure 6, green) supporting three identical dipeptide loops

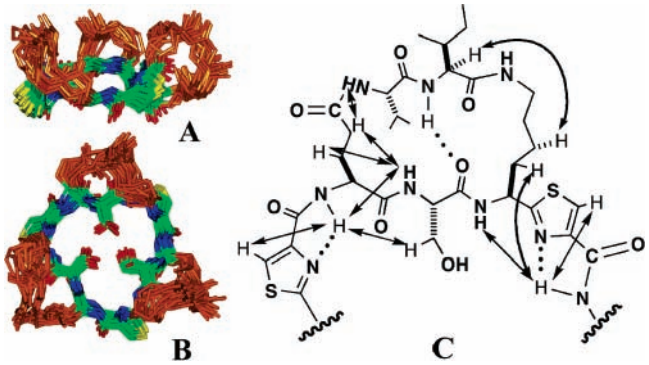

Figure 6. Twenty lowest-energy NOESY-derived structures of cyclo-[**1**]$_3$ in DMSO-d_6 show macrocyclic trimer (green) and ancillary loops (orange). (A) Side view, loops project orthogonally from same face of macrocycle. (B) Top view, Ser side chains project into center of cycle and H-bond to one another; other side chains omitted for clarity. (C) Observed NOEs shown as arrows.

(Figure 6, orange) that project from the same face of the scaffold (Figure 6A). The low temperature dependence of chemical shifts for Glu NH, Ile NH, and Ser OH ($\Delta\delta/T$ 1.4, 1.3, 6.0 ppb/K, respectively) and slow exchange in D_2O were consistent with intramolecular hydrogen bonds.[5] The sharp triplet (J = 4.6 Hz) for Ser γOH suggested H-bonding.[5b] Corresponding H-bond acceptors, determined by NOE and dihedral angle violations and lowest-energy structures, were thiazole-N (for Glu NH), Ser O (for Ile NH), and Ser side chain O (for another Ser OH), the latter supporting projection of the Ser side chains into the interior of the macrocyclic scaffold (Figure 6B).

The three-dimensional structure of cyclo-[**1**]$_3$ was calculated from 115 NOE distance restraints (26 sequential, 89 medium range) and nine ϕ angle restraints (ϕ = 120 ± 30° from coupling constants) using a dynamic simulated annealing and energy minimization protocol in XPLOR. The 20 lowest-energy structures converged (RMSD 0.89 Å) without NOE distance (>0.2 Å) or angle (>1°) violations (Figure 6) and featured weak NOEs between scaffold and loop residues (Figure 6C) that defined the loop orientation relative to scaffold. The loops do not interact with one another and are directed orthogonally from the pseudo-planar scaffold by the chirality of the Glu-Lys side chains and the constrained bowl shape of the scaffold (Figure 6A).

(4) Self-condensation of **1** (0.1 M in DMF). DIPEA (12 μL, 6.89 × 10^{-5} mol) was added to a solution of **1** (20 mg, 2.66 × 10^{-5} mol) and BOP (15.3 mg, 3.46 × 10^{-5} mol) in DMF (0.27 mL) before stirring at 20 °C for 3 h. Solvent was removed in vacuo, and the residue was dissolved in MeCN/H_2O (1:1, 20 mL) and lyophilized. The crude mixture was analyzed by LC/MS, and cyclic peptides were isolated by rpHPLC using MeCN/H_2O eluant gradients. Isolated compounds (n = 2, 3, 4) were characterized by negative ninhydrin assay, rpHPLC retention times, MS, and 2D ^{1}H NMR spectra.

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Self-condensation of a structurally defined template has been used to produce a complex macrocyclic library that was tolerant of appended carrier motifs. Such one-pot syntheses have scope for increasing molecular complexity through combinatorial and heterogeneous variations of templates and passenger motifs. Such chemistry could conceivably be used to append other components of protein structure (helices, turns, strands, sheets, and combinations) to constrained templates, which, as shown here, can be converted into huge conformationally restricted scaffolds that span vast distances yet can project attached peptide motifs from the same face. These macrocyclic scaffolds approach the sizes (10−25 Å) of cyclic peptide domains of proteins. This cyclooligomerization[2,6,7] approach may be useful for separating peptide structural motifs over large surface areas in new nanomaterials such as macrocyclic peptidomimetics,[8] protein surface mimics,[9] antibiotics,[10] or multimetal sequestrants.[11]

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Supporting Information Available: Synthesis and characterization data (rpHPLC, NMR (^{1}H, ^{13}C,VT), MS) for isolated **1** and cyclo[**1**]$_{2-4}$. Solution structures for **1** and cyclo[**1**]$_{3}$. This material is available free of charge via the Internet at http://pubs.acs.org.

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