Novel Cylindrical, Conical, and Macrocyclic Peptides from the Cyclooligomerization of Functionalized Thiazole Amino Acids

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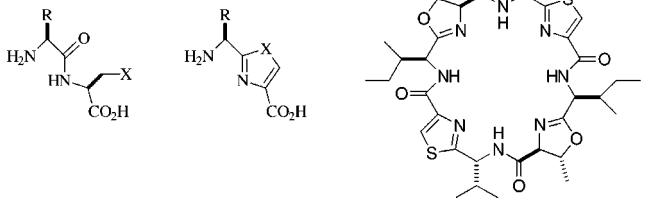
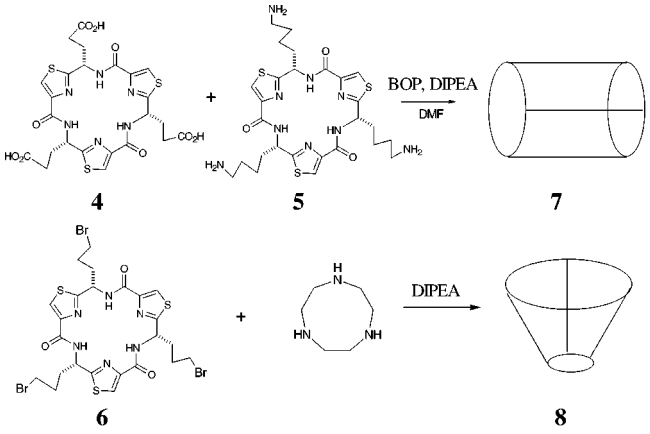
Intramolecular condensation of cysteine, serine, or threonine side chains of dipeptides Aaa-Cys, Aaa-Ser, or Aaa-Thr (**1**) results in dipeptide surrogates (**2**) that incorporate five-membered heterocyclic ring constraints such as thiazole (X = S), oxazole (X = O) or their reduced analogues. Such dipeptide surrogates have been found in many natural products, where they often profoundly influence three-dimensional structures and bioactivities.[1−3] The number and oxidation state of heterocyclic rings can enforce macro-chair or macro-boat conformations on macrocycles such as **3**,[4] and this has been shown to result in very different affinities for metal ions such as Cu^{2+},[5a] Zn^{2+} ,[5b] and Ca^{2+}.[5c] Dipeptide surrogates such as **2** have been exploited as β-turn-inducing constraints to control cyclooligomerizations[6] used to form macrocycles such as [Ile-Ser-D-Val(Thz)]$_{2-19}$, with up to 76 amino acids in the cycle.[6b]



1 **2** **3**

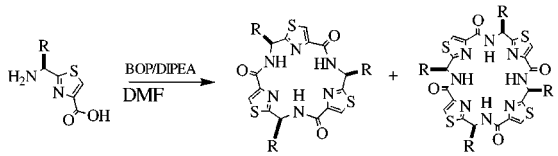
We now report high-yielding cyclotrimerization reactions of functionalized dipeptide surrogates L-Glu(Thz) (**2a**, X= S, R = $-(CH_2)_2CO_2H$) and L-Lys(Thz) (**2b**, X = S, R = $-(CH_2)_4NH_2$) into cyclic hexapeptides (**4**−**6**), and their subsequent elaboration to two new types of highly constrained chiral cavitands, the peptidic cylinder (**7**) and cone (**8**). Both the cylinder and cone are pentacyclic molecules, featuring two 18-membered macrocycles joined by three intercyclic linkages that create three additional 30-membered rings. These unique peptide cavitands illustrate new classes of supramolecular peptides for conceivable further development to catalysts and artificial proteins.



4 **5** **7**

6 **8**

Cyclic hexapeptide **4** was prepared by converting the protected amino acid BocGlu(OcHx)-OH to the thiazole dipeptide BocGlu-(OcHx)Thz-OtBu in 86% overall yield by an established method.[7] This involved derivatization of BocGlu(OcHx)-OH to the primary amide, conversion to the corresponding thioamide with Lawesson's reagent, followed by a modified Hantzch synthesis using *tert*-butyl bromopyruvate to give the protected thiazole amino acid. Treatment of the protected thiazole with TFA yielded **9** (R = $-(CH_2)_2CO_2cHx$). Cyclooligomerization of **9** (2×10^{-3} M) with BOP using DIPEA as base and DMF as solvent gave high isolated yields of cyclic hexapeptide **10a** (85%) and cyclic octapeptide **11a** (10%) where R = $-(CH_2)_2CO_2cHx$), which were separated by column chromatography.[8] This contrasts with poor cyclooligomerization yields reported for similar oxazole-amino acids.[9] Removal of the cyclohexyl-protecting group from **10a** with HF produced the cyclic hexapeptide **4**. Cyclooligomerization proceeds equally well at higher concentrations of **9** (85% **10a** at 4×10^{-2} M; 83% at 0.1 M).



9 **10** **11**

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(8) Typically: TFA·H-Glu(OcHX)Thz-OH (3.5 g, 8.2 mmol) and BOP (5.5 g, 12.4 mmol) were dissolved in DMF (200 mL), cooled to 0 °C, DIPEA (10 mL, 57.4 mmol) added, and stirred (0 °C, 5 h). Solvent was evaporated in vacuo, and the residue was dissolved (150 mL EtOAc), washed with 20% aqueous citric acid (1 × 50 mL), saturated $NaHCO_3$ (4 × 60 mL), water (1 × 80 mL), and brine (50 mL), dried (anhydrous $MgSO_4$), and concentrated. Chromatography on Si gel with petroleum ether/EtOAc gave **10a** cyclo-[Glu-(OcHx)Thz]$_3$ (2.0 g, 85%; HRMS: M + H exptl 1177.4253, calcd 1177.4230) and **11a** cyclo-[Glu(OcHx)Thz]$_4$ (241 mg, 10%; HRMS: M + H exptl 883.3201, calcd 883.3192).

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Cyclic peptide **5** was similarly prepared by sequential conversion of FmocLys(Boc)-OH via FmocLys(Boc)-NH_2 to FmocLys-(Boc)Thz-OEt (96% overall yield), followed by selective deprotection of the ethyl ester and Fmoc groups. Surprisingly the Fmoc- and ethyl ester protecting groups were not easily removed with LiOH or KOH. These reagents cleanly removed the Fmoc group, while the ethyl ester was resistant to hydrolysis. Tetramethylammonium hydroxide however, did remove both protecting groups. Subsequent cyclooligomerization at 4×10^{-2} M and deprotection of the side chain with TFA yielded 95% cyclic products, as cyclic hexapeptide **5** (83%) and cyclic octapeptide **11b** (R = $-(CH_2)_4$-NH_2) (12%), which were readily separated by rpHPLC.

To investigate the generality and efficiency of this reaction, the alternatively protected dipeptide analogue H-Lys(Z)Thz-OH (**2c**, X = S, R = $-(CH_2)_4NHCOCH_2C_6H_5$) was cyclooligomerized at differing concentrations. Lower combined isolated yields of cyclic trimer **10b** plus tetramer **11b** (R = $-(CH_2)_4$NH-Z) were obtained (71% at 1×10^{-2} M, 56% at 0.1 M) due to competition from polymerization. H-Lys(Z)Thz-OH was similarly obtained from BocLys(Z)-OH as described above for H-Lys(Boc)Thz-OH. When an oxazole ring constraint (Ox) was present instead of thiazole, cyclooligomerization of H-Glu(OcHx)Ox-OH (**2d**, X = O, R = $-(CH_2)_2CO_2C_6H_{11}$) at 4×10^{-2} M gave respectable isolated yields of the oxazole analogues of **10a** and **11a**, namely cyclo-[Glu(OcHx)Ox]$_3$ (48%) and cyclo-[Glu(OcHx)Ox]$_4$ (16%), under conditions used for H-Glu(OcHx)Thz-OH (**9**).[8]

Cyclic peptides **4** and **5** are planar, by analogy with the crystal structure of c[Ala(Ox)D-Val(Thz)Gly(Thz)],[10] and are rigid templates that direct their three L-Lys or L-Glu side chains from the same face of the macrocycle. ^{1}H NMR spectra for cyclic hexapeptides **4**, **5**, and **10** [R = $-(CH_2)_2CO_2$cHx, $-(CH_2)_2CO_2$-tBu or $-(CH_2)_4$NHBoc] gave only one set of resonances for their three dipeptide units, indicating high C_3 symmetry. In addition, the chemical shift (δ 8.43) of the amide-NH resonance of **10** [R = $-(CH_2)_2CO_2$tBu] is independent of temperature ($CDCl_3$, $\Delta\delta$/T = 0.6 ppb/K) and does not significantly exchange in CD_3OD after 1 h. This is consistent with the amide-NH protons being involved in intramolecular H-bonding which would fix a planar conformation. The ^{1}H NMR spectrum for cyclic octapeptide **11** [R = $-(CH_2)_2CO_2$tBu] similarly indicates high C_4 symmetry. However, the chemical shift (δ 7.91 ppm) for the single amide-NH resonance of **11** is more temperature-dependent ($CDCl_3$, $\Delta\delta$/T = 3 ppb/K) than for **10** [R = $-(CH_2)_2CO_2$tBu] and exchanges instantly in CD_3OD. Here the symmetry is due to conformational averaging not rigidity.

Cylinder **7** was synthesized by coupling cycle **4** with a stoichiometric equivalent of cycle **5** at 1×10^{-3} M using BOP, DIPEA, and DMF.[11] Some HMPA and hydroxybenzotriazole (degradation from BOP) were trapped by **7**, shown by their co-elution from rpHPLC columns and by ^{1}H NMR spectra, suggesting potential host−guest properties for **7**. These were removed slowly by stirring with $NaHCO_3$ in aqueous DMF. The cylinder was isolated pure in 47% yield, its ^{1}H NMR spectrum (Figure 1) is simple due to C_3 symmetry, and resonances for both sets of hexapeptide rings are distinct.

The cone-shaped molecule **8** was prepared by reduction of the macrocyclic triacid **4** by activation with BOP followed by reduction with $NaBH_4$ to the tri-alcohol (83%). This was converted

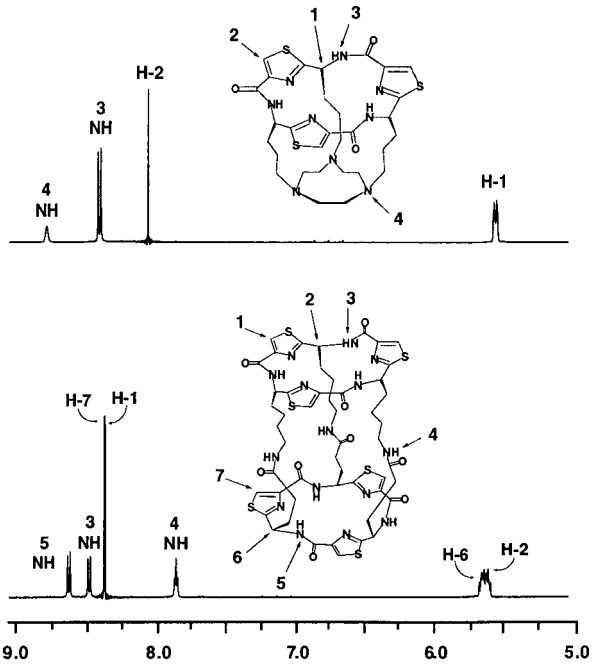

Figure 1. ^{1}H NMR spectra (5−9 ppm) for **7** (bottom) and **8** (top) in d_6-DMSO at 293 K.

with CBr_4/PPh_3 to the tribromide **6** in 57% yield. Reaction of **6** with 1,4,7-triazacyclononane (TACN) in DMF (0.002 M) with DIPEA as base gave the cone **8** (55%).[12] The ^{1}H NMR spectrum (Figure 1) is consistent with C_3 symmetry, although interestingly only one of the three TACN nitrogens appears to be protonated based on proton integration. The $CH_\alpha-CH_\beta$ coupling constants for the tribromide **6** ($J_{H\alpha-H\beta}$ = 5.7 Hz) are significantly larger than for the cone **8** ($J_{H\alpha-H\beta}$ = 3.9 Hz, $J_{H\alpha-H\beta}$ = 2.8 Hz), consistent with a smaller Hα−C−C−Hβ dihedral angle in **8** and more conformational restriction. Preliminary CD studies suggest that **8** interacts with Cu^{2+} in MeOH indicating potential host−guest properties for **8**.

This work has demonstrated that highly functionalized thiazole-amino acids can be cyclooligomerized in high yields to constrained cyclic hexa- and octa-peptides. These cycles have been used as scaffolds to construct unique conical and cylindrical peptides, which can potentially capture small molecules, metal ions, and short peptides within their interiors. In cones of this type all three nitrogen lone pairs project into the interior[13] due to the high barrier to inversion in TACN. Modified cylinders, enlarged through the use of bigger cycles and extended through insertion of amino acids or peptides between Glu and Lys side chains, could conceivably be developed as mimics of pseudocylindrical enzyme active sites. This new approach to cavitands promises to lead to novel classes of molecular receptors.

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Supporting Information Available: Syntheses and characterization (NMR, MS, HPLC) for all compounds (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(11) Cyclo-(-Glu(Thz)-)$_3$ **4** (106 mg, 0.17 mmol), cyclo-(-Lys(Thz)-)$_3$·3TFA **5** (163 mg, 0.17 mmol) and BOP reagent (320 mg, 0.72 mmol) were dissolved in DMF (160 mL), DIPEA (0.60 mL, 3.44 mmol) was added, and the mixture was stirred (65 h, 20 °C). After evaporation of the solvent in vacuo, the residue was stirred with concentrated $NaHCO_3$ for 30 h, freeze-dried, and purified by HPLC to yield **7** (95 mg, 47%; HRMS: M + H exptl 670.2437, calcd 670.2416).

(12) Cyclo-(-Glu(CH_2Br)(Thz)-)$_3$ **6** (79 mg, 0.1 mmol) was dissolved in DMF (50 mL) and DIPEA (0.18 mL, 1 mmol) to which was added 1,4,7-triazacyclononane (2 mL of 0.1 M solution in dioxane). The mixture was stirred overnight at 50 °C and evaporated to a residue that was purified by preparative HPLC to yield **8** (43 mg, 55%; HRMS: M + H exptl 1216.2912, calcd 1216.2866).

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