Conformationally Restricted Cyclic Nonapeptides Derived from L-Cysteine and LL-3-Amino-2-piperidone-6-carboxylic Acid (LL-Acp), a Potent β-Turn-Inducing Dipeptide Analogue

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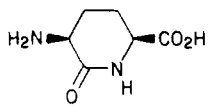
Received March 16, 1984

Syntheses of the cyclic nonapeptides *cyclo*-(Gly-L-Cys(Bzl)-Gly)$_3$, *cyclo*-(L-Pro-Gly-L-Cys(Meb))$_3$, and *cyclo*-(L-Cys(Meb)-LL-Acp)$_3$ are described, where Meb = *p*-methoxybenzyl and Acp = 3-amino-2-piperidone-6-carboxylic acid, a β-turn-inducing dipeptide analogue. Removal of the S-blocking groups from each is described, followed by ring-forming reaction with 1,3,5-tris(bromomethylene)benzene to form an unusual series of relatively rigid vase-shaped cage compounds. Strong binding of three water molecules to the Acp-derived cage is reported.

The folding of amino acid sequences into the tight tertiary structures of globular proteins is most likely directed by locally ordered regions of secondary structure,[1] and among these, the 1 → 4 or β-turn is the simplest.[2] Algorithms have been developed for predicting the probability of turns in tetrapeptide segments composed of the 20 natural amino acid residues,[3] and much recent interest has attended the development of highly potent analogues of peptide hormones in which local regions of the hormone analogue are restricted conformationally to shapes resembling one of the several types of β-turns.[4]

Recently we have reported synthesis of LL-3-amino-2-piperidone-6-carboxylic acid (**1**; *LL*-Acp),[5] which can be viewed as a rigid analogue of the dipeptide L-alaninyl-L-alanine (**2**) in which the amide function is constrained to



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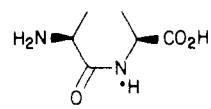

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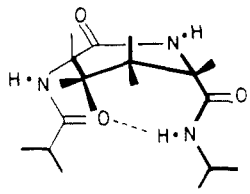
assume the unnatural s-cis orientation. When incorporated into simple peptides the LL-Acp moiety has been shown to adopt conformation **3** in which the 3-acylamino sub-



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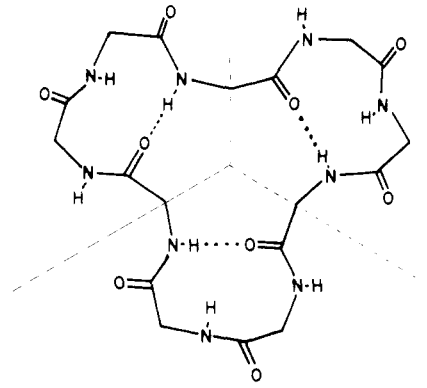
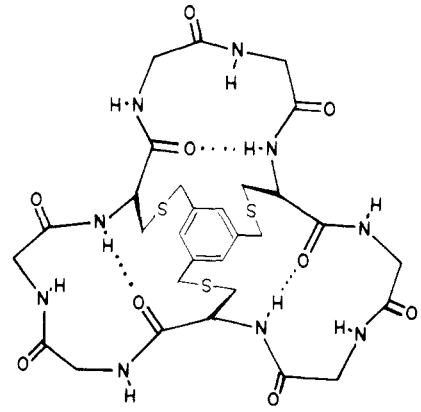
stituent assumes a pseudoequatorial and the 6-carboxamide substituent assumes a pseudoaxial orientation.[6] Thus, a polypeptide containing an LL-Acp residue is expected to adopt a β-turn structure of the rare s-cis type, hitherto observed primarily with cyclic peptides derived from tertiary amides.[7]

Conformationally restricted cyclic peptides provide valuable models for the local secondary structures of proteins such as β-turns,[8] and cyclic hexapeptides containing proline residues at sites 1 and 4 have been studied extensively.[9] Only isolated cases of cyclic nonapeptides bearing three turn regions have been reported,[10] although these are an interesting class of structures, since cooperative internal rotation should be possible, allowing the structure **4** to open and close like a threefold symmetric flower.



We have previously reported synthesis of **5**, *cyclo*-(Gly-L-Cys-Gly)$_3$, which is conformationally restricted by a triply thioether-linked 1,3,5-trimethylenebenzene cap.[11]



We were particularly intrigued by structure **6** that in models appears as a rigid vaselike structure with a potential binding region for three hydroxyl functions as

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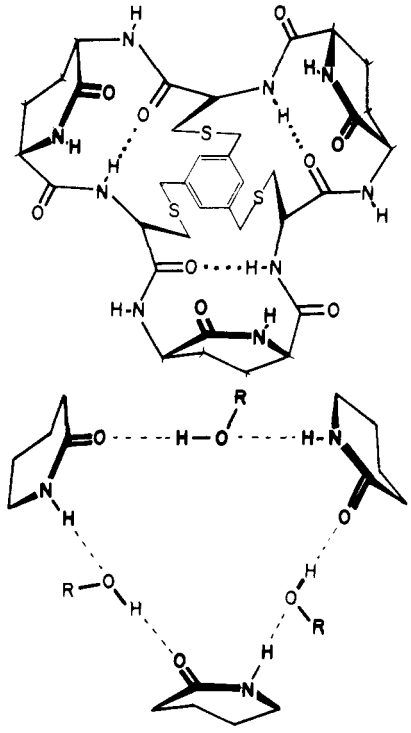
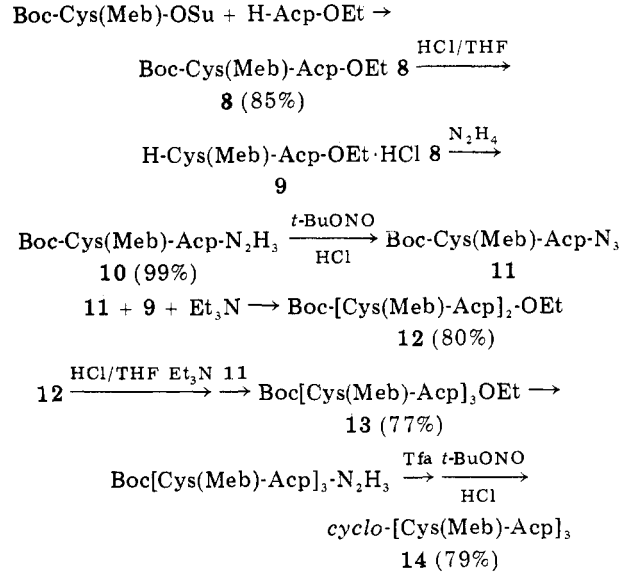


shown in 7. In this paper we report synthesis of 6, spectroscopic results pertinent to its conformation, yield results for the formation of related cyclic nonapeptides, and evidence for the tight binding of three molecules of water to 6.

Synthesis of 6. The Acp function bears structural features unlike those of the 20 common amino acids, and its compatibility with the reagents of conventional peptide synthesis had to be examined. The lactam ring was found to be rapidly cleaved by water or alcohols containing mineral acids, even at 25 °C. On the other hand, no cleavage was observed during reactions of Acp esters with hydrazine or aqueous sodium hydroxide, or by treatment of Acp derivatives with trifluoroacetic acid or anhydrous hydrogen chloride in dioxane. High yields are seen for acylation of the Acp amino function with mixed anhydrides or a variety of active esters. The carboxyl function of Acp appears to be more constrained, perhaps because of its axial orientation, and only acyl azides and mixed anhydrides proved useful amide-forming reagents in our hands.

The H-LL-Acp-OEt used in the sequence of Scheme I was generated by a modification of our original synthesis in which an intramolecular Schöllkopf alkylation[12] was used in the formation of the new C–C bond at a chiral center.[13] Since considerable epimerization at cysteine was observed during saponification of 8, hydrazinolysis and acyl azide couplings were used throughout the synthesis. Hydrazinolysis of the ester function of the nonapeptide 13 was sluggish and proceeded incompletely when attempted in methanol but proceeded satisfactorily in a mixture of butanol and DMF.[14] Cyclization of the N-deblocked acyl azide derived from 13 proceeded smoothly at 0 °C in dry pyridine at 0.001 M to give 14 in 79% yield after chromatography on a Sephadex LH-20. The homogeneity of 14 was established by TLC and HPLC as well as by the

Scheme I[a]



[a] All amino acid configurations are L; Meb = *p*-methoxybenzyl;[15] Boc = *tert*-butoxycarbonyl; OSu = 1-acyloxysuccinimide.

striking simplicity of its 270-MHz ^{1}H NMR spectrum. Elemental analysis was consistent with a trihydrate of the cyclic nonapeptide.

Formation of Cyclo Nonapeptides. In addition to formation of 14 two other cyclic nonapeptides were prepared from conventional amino acids—*cyclo*-(Gly-L-Cys-(Bzl)-Gly)$_3$ (15) and *cyclo*-(Gly-L-Cys(Meb)-L-Pro)$_3$ (16). Since the closure of the ring of cyclic peptides can be difficult, we compare results for each of these cases as a possible guide for future work with this structural class.

Not surprisingly, the free nonapeptide H-(Gly-L-Cys-(Bzl)-Gly)$_3$-OH is a rather insoluble substance, and perhaps for this reason, no reaction could be observed with diphenylphosphoryl azide[16] in DMF in the presence of triethylamine. As reported earlier, ring closure was achieved by a three-step procedure in which the *N*-*tert*-butoxycarbonyl-protected nonapeptide is first converted into its *N*-ethylsalicylamide ester by reaction with the *N*-ethylbenzisoxazolium cation.[17] N-deblocking by trifluoroacetic acid was followed by cyclization at 0.001 M in dry pyridine to yield *cyclo*-(Gly-L-Cys(Bzl)-Gly)$_3$ in 90% yield, after purification by precipitation from Me_2SO by water.

An entirely analogous reaction sequence was used to generate the *N*-ethylsalicylamide ester of H-(Gly-L-Cys-(Meb)-L-Pro)$_3$-OH and achieve its cyclization in 50% yield, after medium-pressure liquid chromatography on silica. A Chou–Fasman analysis[3] of the tetrapeptide sequence Cys-Pro-Gly-Cys gives a probability of β-turn formation of 8.7×10^{-4}, which is more than 1 order of magnitude larger than the cutoff probability of 7.5×10^{-4}, which is roughly the probability calculated for the alternative

(12) Schöllkopf, U.; Hortwig, W.; Groth, V. *Angew. Chem., Int. Ed. Engl.* **1979**, *18*, 863–864.
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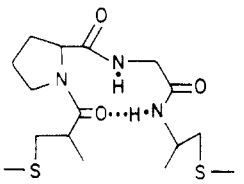
Table I. NH Chemical Shifts of 14 and 6[a]

subst	δ	assgnt	temp depndnc (°C × 10^3 ppm)
14	7.77	piperidone NH	6.3
14	8.37	Cys NH	2.5
14	8.79	Acp 3-NH	3.1
6	7.92	piperidone NH	3.1
6	8.09	Cys NH	0.3
6	9.01	Acp 3NH	2.9

[a] Thus far the six cyclic nonapeptide derivatives have failed in our hands to yield crystals suitable for X-ray structure determination. Although the ease of synthesis of **6** strongly supports the proposed vaselike conformation that appears from models to be essentially strain-free, definitive structural evidence awaits X-ray analysis.

turn-forming sequence Gly-Cys-Pro-Gly. The 270-MHz ^{1}H NMR spectrum of *cyclo*-(Gly-L-Cys(Meb)-L-Pro)$_3$ at 25 °C in Me_2SO-d_6 shows two amide NH resonances at δ 8.60 (triplet) and 7.71 (doublet) that can be assigned respectively to the Gly and Cys residues from the observed multiplicities. The temperature dependences of these resonances are −6.7 and −2.7 × 10^{-3} ppm/°C. Since temperature dependences between 0 and −3 × 10^{-3} ppm/°C are characteristic of internally H-bonded NH groups, these data are consistent with assignment to **16** of a conformation in which three β-turns **17** are present. It is interesting that an energy minimization calculation for **16**, with a starting conformation defined by formation of the peptide ring, yielded a trisymmetric structure containing three type I β-turns.[19]



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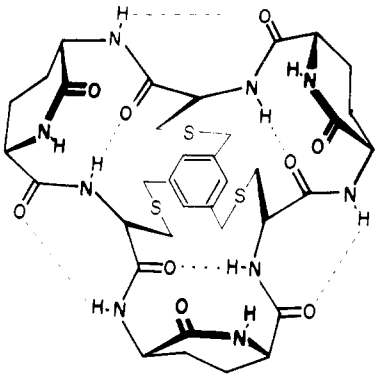
We interpret the smooth cyclization reactions observed for the three cyclic nonapeptides prepared in this study as reflections of the underlying conformational determinism of linear nonapeptides that had been selected for their high turn-forming potential.

Preparation and Properties of *cyclo*-(L-Cys-LL-Acp)$_3$, Triply Bridged by 1,3,5-Tris(thiomethyl)benzene (6). The cyclo nonapeptide **14** was converted to the conformationally restricted thioether-bridged **6** by reaction with mercuric acetate, followed by hydrogen sulfide to cleave the methoxybenzyl protective groups and liberate the three free thiol functions. Alkylation with 1,3,5-tris(bromomethyl)benzene[20] was then carried out in aqueous methanolic alkali. Isolation by Sephadex (H-20) chromatography generated **6** in 33% yield as a microcrystalline white powder. Competing thiol oxidation and polymer formation are possible causes of the low yield that was comparable to those seen for the analogous reaction sequence generating **5** and its *cyclo*-(Pro-Gly-Cys)$_3$ analogue.

Substance **6** was homogeneous in HPLC and TLC systems. The molecular formula was confirmed by elemental analysis and the field desorption mass spectrum, and ^{13}C and ^{1}H NMR spectra of **6** were consistent with a structure that has time-averaged threefold rotational symmetry. As seen in Table I the conversion of **14** to the more rigid **6** results in a marked decrease in the temperature coefficients of the chemical shifts of the amide NH resonances, indicative of a tighter, internally hydrogen-bonded structure and suggestive of γ- as well as β-turns as indicated in structure **18**.



Models indicate that triols such as 1,3,5-tris(hydroxymethylene)benzene, *cis,cis*-1,3,5-tris(hydroxymethylene)cyclohexane, and triethanolamine may bind strongly through hydrogen bonding to the piperidone amide functions. Preliminary experiments to demonstrate complex formation through changes in NMR chemical shifts or HPLC retention times have been unsuccessful. A possible reason is the strong affinity of **6** for three molecules of water. The elemental analysis for this substance reveals it as a trihydrate. Although the field desorption mass spectrum reveals only the expected molecular ion, prolonged drying under vacuum at 120 °C resulted in no water loss. Interestingly the presence of hydrogen-bonding water could not be detected in the ^{1}H NMR spectrum.

Experimental Section

Proton NMR spectra were obtained on Bruker Model WM-250 or WM-270 instruments by J. Owens. Optical rotations were measured in a 1.0-dm thermostated cell in a Perkin-Elmer Model 141 polarimeter. Elemental analyses were performed by Galbraith Laboratories, Knoxville, TN. Field desorption mass spectra were obtained courtesy of Dr. C. Costello in the laboratory of Prof. K. Biemann.

TLC was carried out on aluminum-backed silica gel 60 plates (F-254, EM Reagents) in the following solvent systems: A, chloroform–methanol (9:1); B, chloroform–methanol (4:1); C, butanol–acetic acid–ethyl acetate–water (1:1:1:1).

HPLC was performed on a Waters Associates 6000A system equipped with a Model 660 solvent programmer and using a Whatman Partisil-10 ODS-3 column.

Boc-L-Cys(Meb)-LL-Acp-NH-NH$_2$ (10). Hydrazine hydrate (0.97 mL, 20 mmol) was added to an ice-cold solution of Boc-Cys(Meb)-Acp-OEt (**8**)[21] (2.04 g, 4.0 mmol) in 20 mL of methanol. After standing for 18 h at 0 °C, the solution was evaporated. The residue was dissolved in 3 mL of chloroform–methanol (9:1), loaded onto 20 g of silica gel in a short column, and eluted with the same solvent mixture; 15-mL fractions were collected and examined by TLC (K, R_f 0.46). Appropriate fractions were pooled and evaporated. The residue was dissolved in ethyl acetate, filtered, and precipitated with 10 volumes of petroleum ether to yield 1.97 g (99%) of an amorphous white powder: TLC R_f 0.46 (B); HPLC (MeOH–H_2O (1:1)) 1.5 mL/min, 50.1 mL; ^{1}H NMR (Me_2SO-d_6) δ 1.68 (9 H, s), 1.94–2.40 (4 H, m), 3.06 (1 H, dd, 4.3 and 13.8 Hz), 3.99 (2 H, s), 4.02 (3 H, s), 4.13 (1 H, m), 4.34–4.51 (2 H, m), 4.56–4.85 (2 H, brs), 7.14 (2 H, d, 8.6 Hz), 7.25 (1 H, d, 9.5 Hz), 7.52 (2 H, d, 8.6 Hz), 8.00 (1 H, s), 8.37 (1 H, d, 8.6 Hz), 9.38 (1 H, s).

HCl·H-L-Cys(Meb)-LL-Acp-OEt (9). Boc-L-Cys(Meb)-LL-Acp-OEt (**8**)[21] (1.53 g, 3.0 mmol) was dissolved in 15 mL of 5.5 N HCl in tetrahydrofuran and 1.5 mL of anisole and kept at 0 °C for 1 h. The solvent was evaporated, leaving an oil. A white powder was obtained by rubbing the oil with ether–petroleum

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(21) Prepared as described in ref 13; mp 109–111 °C.

ether (1:2). The powder was collected by filtration, washed with petroleum ether, and dried in a vacuum desiccator over NaOH to yield 1.35 g (100%) of the salt.

Boc-(L-Cys(Meb)-LL-Acp)$_2$-OEt (12). A solution of **10** (1.33 g, 2.69 mmol) in 5 mL of DMF was cooled to −20 °C; hydrogen chloride (3.36 mL of a 2.38 N solution in dioxane; 8.00 mmol) and *tert*-butyl nitrite (346 μL, 2.96 mmol) were added, and the solution was stirred for 15 min. The solution gave a negative hydrazide test (HPLC). A cold (−20 °C) solution of **9** (1.20 g, 2.70 mmol) in 2 mL of DMF was added; DMF (2 × 0.5 mL) was used as a rinse. The solution was cooled to −30 °C, and triethylamine (1.49 mL, 10.70 mmol) was added. The mixture was stirred at −15 °C for 3 h. The pH was maintained at 7.5–8.0 (moist pH paper) by addition of triethylamine. The mixture was stirred at 5 °C for 12 h and then was partitioned between 50 mL of ethyl acetate and 50 mL of water. The layers were separated, and the aqueous layer was extracted with ethyl acetate (2 × 25 mL). The organic layers were pooled, washed with water (1 × 10 mL) and brine (1 × 10 mL), dried ($MgSO_4$), and evaporated. The crude product, an oil, was dissolved in 4 mL of methanol and applied to a 2.5 × 56 cm Sephadex LH-20 column, packed, and eluted with methanol at a flow rate of 10 mL/h; 6-mL fractions were collected and examined by TLC (B, R_f 0.70). Fractions 29–37 were pooled and evaporated to yield 1.88 g (80%) of a white solid: TLC R_f 0.34 (H), 0.70 (K); HPLC (B, MeOH–H_2O (7:3)) 1.5 mL/min, 8.4 mL; ^{1}H NMR (Me_2SO-d_6) δ 1.21 (3 H, t, 7.3 Hz), 1.39 (9 H, s), 1.71–2.25 (8 H, m), 2.58–2.88 (4 H, m), 3.65–3.73 (10 H, m), 3.96–4.28 (7 H, m), 4.58 (1 H, m), 6.83 (4 H, m), 6.93 (1 H, d, 9.5 Hz), 7.22 (4 H, m), 7.77 (2 H, m), 8.08 (1 H, d, 7.3 Hz), 8.15 (1 H, d, 7.9 Hz), 8.31 (1 H, d, 8.5 Hz). Anal. Calcd for $C_{41}H_{56}N_6O_{11}S_2$: C, 56.40; H, 6.47; N, 9.63; S, 7.34. Found: C, 56.23; H, 6.35; N, 9.54; S, 7.58.

HCl·H-(L-Cys(Meb)-LL-Acp)$_2$-OEt. A solution of **12** (1.88 g, 2.15 mmol) in 10 mL of 5.5 N HCl in tetrahydrofuran and 1 mL of anisole was kept at 0 °C for 45 min. The solvent was evaporated, and the resulting gum was triturated with ether–petroleum ether (1:2). The powder was collected by filtration, washed with ether–petroleum ether, and dried in a vacuum desiccator over NaOH to yield 1.71 g (98%) of the salt.

Boc(L-Cys(Meb)-LL-Acp)$_3$-OEt (13). A solution of **10** (870 mg, 1.75 mmol) in 3.5 mL of DMF was cooled to −20 °C and treated with hydrogen chloride (2.20 mL of a 2.38 N dioxane solution; 5.24 mmol) and *tert*-butyl nitrite (226 μL, 1.93 mmol), and the solution was stirred for 20 min. A cold (−20 °C) solution of HCl·H-L-Cys(Meb)-LL-Acp)$_2$-OEt (1.42 g, 1.75 mmol) in 2.5 mL of DMF was added; DMF (2 × 0.5 mL) was used as a rinse. The solution was cooled to −30 °C, and triethylamine (0.976 mL, 7.0 mmol) was added. The mixture was stirred at −15 °C for 3.5 h. The pH was kept at 7.5–8.0 by periodic addition of triethylamine. The mixture was stirred at 5 °C for 2 days. The reaction was partitioned between 50 mL of ethyl acetate and 50 mL of water. The layers were separated, and the aqueous layer was extracted with ethyl acetate (2 × 25 mL). The organic layers were pooled, washed with water (1 × 10 mL) and brine (1 × 10 mL), dried (Na_2SO_4), and evaporated. The crude product, an oil, was dissolved in 4 mL of methanol and applied to a 2.5 × 56 cm column of Sephadex LH-20. The column was eluted with methanol at a flow rate of 10 mL/h; 6-mL fractions were collected and examined by TLC (B, R_f 0.54). Fractions 23–29 were pooled and evaporated to yield 1.07 g of pure product. Fractions 30–39 were pooled and evaporated. This residue was rechromatographed on the same column, yielding 0.46 g of pure product. Impure fractions from the second column were pooled and evaporated. The residue was purified by preparative TLC (B) to yield 140 mg of pure product. Overall, 1.67 g (77%) of a white powder was obtained: TLC R_f 0.15 (A), 0.54 (B); HPLC (MeOH–H_2O (75:25)) 1.5 mL/min, 31.9 mL; ^{1}H NMR (Me_2SO-d_6) δ 1.21 (3 H, t, 7.3 Hz), 1.39 (9 H, s), 3.63–3.75 (15 H, m), 3.93 (2 H, brs), 3.97–4.30 (7 H, m), 4.58 (2 H, m), 6.82 (6 H, m), 6.97 (1 H, d, 9.7 Hz), 7.22 (6 H, m), 7.77 (3 H, s), 8.08 (2 H, m), 8.19 (1 H, d, 8.1 Hz), 8.33 (2 H, m). Anal. Calcd for $C_{58}H_{77}O_{15}N_9S_3$: C, 56.34; H, 6.28; N, 10.19; S, 7.78. Found: C, 56.18; H, 6.31; N, 10.09; S, 7.70.

Boc-(L-Cys(Meb)-LL-Acp)$_3$-N_2H_3. Hydrazine hydrate (417 μL, 8.6 mmol) was added to an ice-cold solution of **13** (1.07 g, 0.86 mmol) in 4.3 mL of DMF and 4.3 mL of butanol. The solution was kept at 0 °C for 1 h and at 5 °C for 16 h. The solution was evaporated, leaving the product as an oil. A solid was obtained when the oil was rubbed with 25 mL of ether. The mixture was kept in the freezer for 2 h, and the product was collected by filtration, washed with ether, and dried, yielding 1.05 g (100%) of a white powder: HPLC (MeOH–H_2O (75:25)) 1.5 mL/min, 20.0 mL.

***cyclo*-(L-Cys(Meb)-LL-Acp)$_3$ (14).** The above nonapeptide hydrazide (917 mg, 0.75 mmol) was dissolved in 9 mL of Tfa and 1 mL of anisole and kept at 0 °C for 1 h. The solvent was evaporated, leaving an oil. A white powder was obtained by rubbing the oil with 20 mL of Et_2O. The powder was collected, washed with Et_2O, and dried in a vacuum desiccator.

The crude hydrazide salt was dissolved in 5 mL of DMF and cooled to −20 °C, and hydrogen chloride (0.9 mL of 2.38 N dioxane solution; 2.14 mmol) and *tert*-butyl nitrite (0.92 μL, 0.79 mmol) were added. After 20 min, no hydrazide was present. The solution was added dropwise to 750 mL of magnetically stirred dry pyridine cooled to −25 °C. Addition required 2 min; DMF (2 × 1 mL) was used as a rinse. The solution was stirred for 5 h at −20 °C and then stored at 0 °C for 6 days. After solvent removal, the residue was dissolved in 4 mL of DMF and applied to a 2.5 × 56 cm column of Sephadex LH-20 equilibrated with DMF. The column was eluted with DMF at a flow rate of 12 mL/h; 6-mL fractions were collected and examined by TLC (C, R_f 0.58). Fractions 25–29 were pooled and evaporated. The residue was dissolved in 3 mL of DMF, and the product was precipitated by addition of 25 mL of ether. The powder was washed with ether and dried to afford 646 mg of an amorphous white solid, 79% based on nonapeptide hydrazide.

HPLC (MeOH–H_2O (75:25); 1.5 mL/min, 18.7 mL; ^{1}H NMR (Me_2SO-d_6) δ 1.66–2.06 (12 H, m), 2.23–2.43 (3 H, m), 2.58–2.78 (3 H, m), 3.63–3.82 (18 H, m), 3.91 (3 H, m), 4.71 (3 H, m), 681 (6 H, d, 8.5 Hz), 7.21 (6 H, d, 8.5 Hz), 7.80 (3 H, s), 8.38 (3 H, d, 8.5 Hz), 8.81 (3 H, brs). Anal. Calcd for $C_{51}H_{63}O_{12}N_9S_3$: C, 56.18; H, 5.82; N, 11.56; S, 8.82. Calcd for $C_{51}H_{63}O_{12}N_9S_3 \cdot 3H_2O$: C, 53.52; H, 6.08; N, 11.02; S, 8.41. Found: C, 53.71; H, 6.08; N, 11.02; S, 8.51.

***cyclo*-(L-Cys-LL-Acp)$_3$ Triply Bridged with 1,3,5-Tris(thiomethyl)benzene.** To an ice-cold solution of **14** (218 mg, 0.2 mmol) in 5 mL of trifluoroacetic acid was added mercuric acetate (223 mg, 0.7 mmol). After 1 h at 0 °C, the deep purple solution was evaporated. The residue was kept under vacuum over NaOH for 1 h, 15 mL of oxygen-free 80% aqueous acetic acid was added, and hydrogen sulfide was passed into the mixture for 15 min. The mixture was stirred under N_2 for 45 min and then filtered through Celite using a positive nitrogen pressure. The solvent was evaporated, 10 mL of oxygen-free methanol was added, and the mixture was taken to dryness. The residue, a white solid, was kept under vacuum over NaOH for 10 h.

Oxygen-free MeOH (10 mL) was added, and the mixture was cooled in ice as sodium hydroxide (0.6 mL of 1 N aqueous solution; 0.6 mmol) and a solution of 1,3,5-tris(bromomethyl)benzene[20] (71 mg, 0.2 mmol) in 2.5 mL of methanol were added with 2 × 0.5 mL. The mixture was stirred at 0 °C for 4 h and at 5 °C overnight. The pH was maintained at ca. 8 by periodic addition of 1 N base. The solvent was evaporated, the residue was extracted with hot DMF (3 × 5 mL), and the extracts were pooled and evaporated. The residue was dissolved in 3 mL of hot DMF, cooled to room temperature, and applied to a 2.5 × 56 cm column of Sephadex LH-20 equilibrated with DMF. The column was eluted with DMF at a flow rate of 8 mL/h; 4-mL fractions were collected and examined by TLC (C, R_f 0.41). Fractions 43–45 were collected, pooled, and evaporated. The residue was precipitated from DMF with ether to afford 56 mg (33% based on **14**) of a powder: TLC R_f 0.41 (C); HPLC (MeOH–H_2O (6:4)) 1.5 mL/min, 17.4 mL; ^{1}H NMR (Me_2SO-d_6) δ 1.60–1.72 (3 H, m), 1.82–1.95 (9 H, m), 2.13 (3 H, m), 2.43 (3 H, m), 3.40–3.67 (6 H, m), 3.80 (3 H, m), 4.67 (3 H, m), 6.88 (3 H, s), 7.92 (3 H, d, 4.9 Hz), 8.09 (3 H, d, 9.8 Hz), 9.01 (3 H, d, 6.7 Hz); ^{13}C NMR (Me_2SO-d_6) δ 23.3, 24.3, 35.3, 38.6, 50.3, 50.8, 55.8, 127.6, 138.1, 167.4, 167.9, 171.7. Anal. Calcd for $C_{36}H_{45}O_9N_9S_3$: C, 51.23; H, 5.37; N, 14.94; S, 11.40. Calcd for $C_{36}H_{45}O_9N_9S_3 \cdot 3H_2O$: C, 48.14; H, 5.72; N, 14.04; S, 10.71. Found: C, 48.37; H, 5.61; N, 13.99; S, 10.67.

***cyclo*-(Gly-L-Cys(Bzl)-Gly)$_3$ (15).** Boc-(Gly-L-Cys(Bzl)-Gly)$_3$-OH (1.70 g, 1.63 mmol) was dissolved in a mixture of 65 mL of water, 33 mL of acetonitrile, 1.63 mL of N NaOH, and 4

mL of pyridine. The solution was cooled in a vigorously stirred ice slurry. Finely ground *N*-ethylbenzisoxazolium fluoroborate (500 mg, 2.14 mmol) was added in one portion. The mixture was stirred for 45 min at 0 °C and for 15 min at room temperature; 150 mL of ether was added, the reaction mixed well, and the solid collected by filtration. The product was washed with water and ether and dried under vacuum to give 1.67 g (86%) of the active ester as an amorphous white powder: ^{1}H NMR (Me_2SO-d_6) δ 1.08 (3 H, t, 7 Hz), 1.37 (9 H, s), 7.13–7.77 (19 H, m). The ester was used without further purification although HPLC (linear gradient 50% aqueous MeOH to MeOH in 15 min, 1.5 mL/min, 20.0 mL) showed minor impurities.

The above ester (832 mg, 0.7 mmol) was dissolved in 9 mL of trifluoroacetic acid and the resultant mixture allowed to stand for 30 min. The solution was evaporated and the oil rubbed with ether to form a white solid. The product was collected on a filter, washed with ether, and dried under vacuum to give a quantitative amount of the salt (896 mg) as a white powder. This was dissolved in 18 mL of DMF and added dropwise over 4.5 h to 680 mL of dry pyridine. The solution was stirred for 65 h. The solution was concentrated to approximately 15 mL, and the peptide was precipitated by adding 10 volumes of ether. The mixture was chilled in an ice bath and the product collected, washed with ether, and dried. The peptide was dissolved in ca. 0.5 mL of Me_2SO and precipitated with 10 volumes of water. The mixture was chilled in an ice bath and the product collected, washed with water and ether, and dried. Final yield was 646 mg (90% from active ester) of a solid: HPLC (linear gradient 50% aqueous MeOH to MeOH in 15 min) 1.5 mL/min, 17.5 mL; ^{1}H NMR (Me_2SO-d_6) δ 2.56–2.83 (6 H, m) 3.51–3.96 (18 H, m) 4.38 (3 H, m), 7.30 (15 H, d), 7.99 (3 H, t, 5.7 Hz), 8.18 (3 H, d, 6.2 Hz), 8.73 (3 H, t, 5.3 Hz). Anal. Calcd for $C_{42}H_{51}O_9N_9S_3$: C, 54.70; H, 5.58; N, 13.67; S, 10.43. Found: C, 54.67; H, 5.64; N, 13.50; S, 10.25.

***cyclo*-(L-Pro-Gly-L-Cys(Meb))$_3$ (16).** The *N*-ethylsalicylamide ester of Boc(>ly-L-Cys(Meb)-L-Pro)$_3$-OH was prepared as a crude white solid in 99% yield by a procedure analogous to that described above: ^{1}H NMR (Me_2SO-d_6) δ 1.38 (9 H, s), 2.68–2.87 (3 H, m), 4.19–4.33 (3 H, m), 4.63–4.79 (3 H, m), 6.86 (6 H, d + d), 6.99 (1 H, t, 6.2 Hz), 7.24 (6 H, d + d), 7.95–8.32 (5 H, m), 12.42 (1 H, 6, s). Anal. Calcd for $C_{59}H_{79}O_{15}N_9S_3$: C, 56.66; H, 6.37; N, 10.08; S, 7.69. Found: C, 56.64; H, 6.42; N, 9.93; S, 7.81.

(The required nonapeptide acid was prepared by a ((3 + 3) + 3) synthesis entirely analogous to that reported for the glycine analogue.)

Unpurified active ester (527 mg, 0.35 mmol) was dissolved in 2.7 mL of 4.8 M HCl in tetrahydrofuran and 0.3 mL of anisole. The solution was kept at room temperature for 45 min and then evaporated; 3 mL of tetrahydrofuran was added, and the solution was evaporated. A foam was obtained that was rubbed with pentane–tetrahydrofuran (4:1) to give a pale yellow powder. The powder was collected by centrifugation, washed, and dried under vacuum over P_2O_5 and KOH. The salt was dissolved in 5 mL of DMF and added dropwise, over 2 h, to 500 mL of pyridine at room temperature. The solution was stirred for 90 h. The crude product, a yellow oil, was obtained by evaporating the solvent. The oil was dissolved in 2 mL of methanol and precipitated, in a centrifuge tube, with 20 mL of water. A gum was obtained that was rubbed with 10 mL of 0.5 N HCl and then with 10 mL of water. The gum was kept under vacuum over P_2O_5 until it became a solid. The solid was dissolved in 2 mL of dichloromethane and precipitated, in a centrifuge tube, with 20 mL of hexane. The solvent was decanted, and the product was rubbed with hexane. These operations removed most of the salts and phenolic materials. The peptide was purified by medium-pressure LC. The sample was loaded onto an E. Merck Size C column in chloroform and eluted with chloroform–methanol (85:10) at a flow rate of 300 mL/h; 16-mL fractions were collected and examined by TLC. Appropriate fractions were pooled and evaporated. The product was kept under high vacuum to yield 201 mg (50% based on ester): HPLC (MeOH–H_2O (8:2)) 1.0 mL/min, 8.7 mL; ^{1}H NMR (Me_2SO-d_6) δ 2.67–2.93 (6 H, m), 3.62 (9 H, s), 4.90 (3 H, m), 6.79 (6 H, d, 8.4 Hz), 7.16 (6 H, d, 8.4 Hz), 7.71 (3 H, d, 9.3 Hz), 8.61 (3 H, t, 5.7 Hz). Anal. Calcd for $C_{54}H_{69}O_{12}N_9S_3 \cdot CH_3OH$: C, 56.73; H, 6.32; N, 10.83; S, 8.26. Found: C, 56.17; H, 6.27; N, 10.73; S, 8.94.

***cyclo*-(Gly-L-Cys-Gly)$_3$ Triply Bridged by 1,3,5-Tris(thiomethyl)benzene (5).** The experimental procedure of Stewart and Young for sodium in liquid-ammonia reactions was followed. Small pieces of sodium were added to a refluxing mixture of **15** (184 mg, 1.2 mmol) in 50 mL of ammonia, freshly distilled from sodium, until a permanent light blue color was obtained. After 3 min, 1,3,5-tris(bromomethyl)benzene[20] (72 mg, 0.2 mmol) in 1 mL of ether was added. The solution was stirred at reflux for 4 h. Ammonium chloride (130 mg) was added, and then ammonia was evaporated under a stream of nitrogen. The white residue was dissolved in a mixture of DMF (1 mL), water (0.5 mL), and methanol (0.5 mL) and purified by preparative HPLC (Whatman Magum-9 ODS-2, MeOH–H_2O (55:45), 3.0 mL/min, 84–105 mL).

The product was dissolved in DMF (4 mL) and precipitated with ether (30 mL) to give 44 mg (29% based on **15**) of a white powder: HPLC (MeOH–H_2O (45:55) 1.5 mL/min, 10.5 mL; ^{1}H NMR (Me_2SO-d_6) δ 3.37–3.91 (18 H, m), 4.20 (3 H, m), 7.14 (3 H, s), 7.30 (3 H, d, 5.3 Hz), 7.82 (3 H, t, 5.3 Hz), 8.94 (3 H, t, 3.7 Hz); ^{13}C NMR (Me_2SO-d_6) δ 52.5, 128.9, 136.9, 169.3, 169.8, 172.6. Anal. 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, 16.08; S, 12.27.

Acknowledgment. Financial support from the National Science Foundation (Grant 8116986) is gratefully acknowledged.

Generation and Chemistry of Cyclohexyloxy Radicals†

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Received June 12, 1985

In this paper we report our work on cyclohexyloxy radicals from dicyclohexyl hyponitrite ($CyON_2OCy$) and the related CyO· sources dicyclohexyl peroxydicarbonate [$(CyOCO_2)_2$] and dicyclohexyl peroxide (CyO_2Cy), including the ^{13}C and ^{1}H NMR spectra of the radical sources, the kinetics of DCHN decomposition over a wide range of temperature (followed by both UV and chemiluminescence), ESR spin-trapping studies, the kinetics of hydrogen atom abstraction from a variety of substrates by CyO·, and the effect of CyO· radical source on product composition. We have also investigated the effects of dissolved O_2 on DCHN decomposition in cyclohexane and comment on the source of the observed chemiluminescence.

The study of alkoxy radicals is a subject of continuing interest.[1-3] They can be generated by thermal or photochemical decomposition of peroxides, peresters, nitrates, nitrites, and hypohalite compounds or by the metal-catalyzed decomposition of hydroperoxides. Many of these

† Contribution No. 3797.

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0022-3263/85/1950-5838$01.50/0