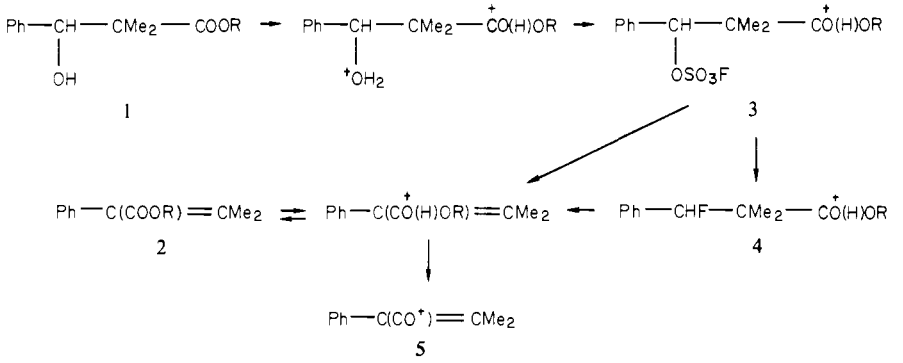
Scheme I[a]



[a] a, R = H; b, R = Me; c, R = Et.

of nearly pure, crystallized rearrangement product **2a** were isolated as the only carboxylic product: mp 149–150 °C (lit.[6] 151 °C); ^{1}H NMR ($CDCl_3$) δ 7.26 (m, 5 H), 2.22 (s, 3 H), 1.70 (s, 3 H); ^{13}C NMR ($CDCl_3$) 173.6 (C(1)), 150.7 (C(3)), 138.2–127.1 (Ph), 129.1 (C(2)), 24.4–22.8

In order to establish the migration of the HOOC group, we prepared **1a** labeled with ^{13}C at C(3) by condensation of benzaldehyde-1-^{13}C with isobutyric acid. When **1a**-3-^{13}C was submitted to treatment with HSO_3F/SO_2ClF at 0 °C, the ^{13}C label appeared in the 2 position (δ 122.1) of protonated **2a** as well as in that of **3** (δ 94.2); it was equally visible in the ^{1}H spectra by coupling of ^{13}C with the protons of the methyl groups of protonated **2a** (δ 2.62, ^3J = 4.6 Hz, and δ 2.07, ^3J = 5.0 Hz) and of **3** (δ 2.85 and 2.47, ^3J = 6.0 Hz); similar values had been found for labeled **2c**.[9]

Definite proof for the HOOC group migration in the superacid comes from use of **1a** doubly ^{13}C labeled at C(1) (90% ^{13}C) and C(3) (69% ^{13}C).[9] In the rearranged (protonated) product **2a** the signals of the H_2OOC^+ group at 180.2 ppm and of C(2) at 122.1 ppm (both increased by enrichment) are split into two doublets by direct ^{13}C,^{13}C coupling ($^1J_{CC}$ = 68.7 Hz). Furthermore, in quenching experiments starting with **1a**-$^{13}C_2$, the label appeared only in the positions 1 and 2 of (nonprotonated) **2a**, isolated and purified; ^{13}C NMR (acetone-d_6) δ 170.4 (d, $^1J_{CC}$ = 71.4, C(1)), 132.0 (d, $^1J_{CC}$ = 70.5, C(2)). We conclude that the only reaction path available for the transformation **1a** → **2a** is a 1,2 shift of the HOOC group.[17]

In order to test whether the reaction is *inter*molecular (for instance via a decarbonylation–carbonylation process[18]), we conducted a cross experiment using a 1:1 mixture of doubly labeled and unlabeled **1a**. There was no increase of monolabeled product in either the ^{13}C NMR spectra (judged by the amount of ^{13}C,^{13}C-coupling between C(1) and C(2)) or in the mass spectra of isolated **2a**; this confirms the *intra*molecular character of the HOOC migration.

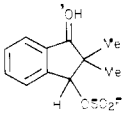
We attribute the preference for HOOC over Me migration to a difference in stability of the rearranged carbocations: if a Me group had been shifted, the positive charge would have appeared α to the carboxyl group, thus destabilizing this intermediate.

Acknowledgment. Financial support by the Swiss National Science Foundation is gratefully acknowledged.

Registry No. 1a, 23985-59-3; **2a**, 4412-08-2; **4**, 81158-98-7.

Supplementary Material Available: All ^{1}H and ^{13}C NMR spectra mentioned in the text (19 pages). Ordering information is given on any current masthead page.

(14) Other peaks observed in the reaction mixture correspond to **4** formed



4

from **1** (or from its fluorosulfate ester) by a Friedel–Crafts type cyclization and replacement of OH by by OSO_2F. The same peaks turned up upon treatment of authentic 3-hydroxy-2,2-dimethylindanone[15] with HSO_3F/SO_2ClF. This was the main product formed from **1a** with HSO_3F in the absence of a solvent when the temperature was raised rapidly.

(15) Aebi, A.; Gyurech-Vago, E.; Hofstetter, E.; Waser, P. *Pharm. Acta Helv.* **1963**, *38*, 407.

(16) By following the reaction of **1a** with the superacid from −100 to +10 °C by ^{1}H and ^{13}C NMR spectroscopy we observed spectral changes analogous to those observed[9] with **1b** and **1c** and which we interprete as the consecutive transformation into the oxonium ion, the fluorosulfate, and (partially) the fluoride $PhCHXCMe_2COOH_2^+$, X = OH_2^+, OSO_2F and F.

(17) It is not possible, however, to decide whether COOH migrates in its protonated or unprotonated form (though it might be felt that protonation would make the group too poor in electrons), nor can other transient species be excluded, e.g., a mixed anhydride (though their presence in the reaction mixture could not be detected).

(18) For example the rearrangement of cinenic acid to geronic acid: Meinwald, J.; Hwang, H. C.; Christman, D.; Wolf, A. P. *J. Am. Chem. Soc.* **1960**, *82*, 483–486.

Specific Peptide Sequences for Metal Ion Coordination. 1. Solid-Phase Synthesis of *cyclo*-(Gly-His)$_3$

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Synthetic cyclic peptides[1,2] have been used to model various aspects of protein conformation and active sites.[3-8] The advantages of cyclic peptides[3] over linear peptides are the constrained geometry and the absence of free COO^- and NH_3^+ terminals. Thus, a flexible polypeptide can be limited to a few desirable

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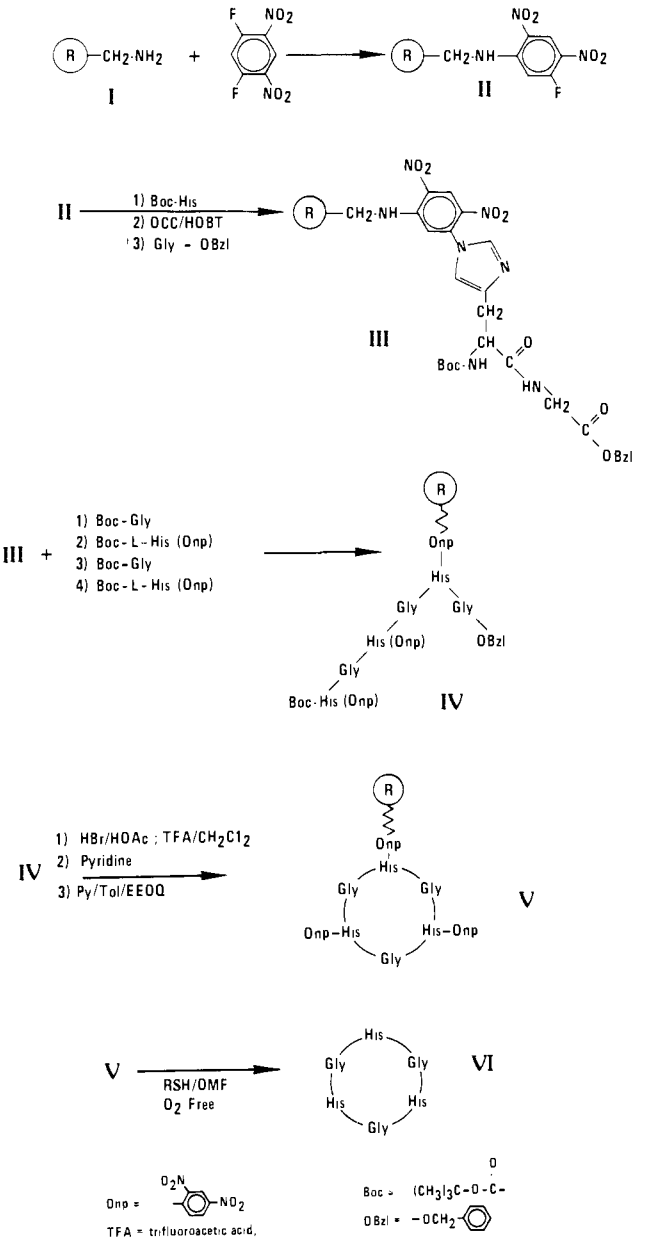
conformations upon cyclization. Cyclic peptides with specific amino acid residues such as histidine, glutamic acid, aspartic acid, methionine, tyrosine, and lysine have side chains that can bind to transition-metal ions. These cyclic peptides can be designed to have close resemblance to the active site of nonheme metalloproteins.

Most cyclic peptides have been synthesized by classical solution methods[1] or by synthesizing the corresponding linear peptide by solid-phase methods, removing it from the solid support and then cyclizing it in solution.[1,2] Only in a very few cases has a peptide been cyclized directly on a solid support.[9-11] In this communication a general approach to the solid-phase synthesis and cyclization of polypeptides suitable for metal ion coordination is described and used for the synthesis of *cyclo*-(Gly-His)$_3$. We chose to synthesize *cyclo*-(Gly-His)$_3$ as the first member of this series because the histidine side chain is one of the side chains most frequently encountered in metal ion coordination in metalloenzymes. For example, three histidine side chains are known to occupy three coordination sites on zinc in carbonic anhydrase[8]. Four histidine side chains are known to ligate copper in bovine superoxide dismutase (with one deprotonated histidine bridging the copper and the zinc ions). Also a number of other nonheme copper and iron metalloproteins have active sites in which two or more histidine side chains are involved. The cyclic hexapeptide backbone was chosen because of the biological precedence for this structure in the specific chelation of metal ions (e.g., the ferrichrome class of iron chelators.[8]).

The strategy for the synthesis of *cyclo*-(Gly-His)$_3$ is shown in Scheme I. (Aminomethyl)copoly(styrene–1%-divinylbenzene) resin(I) (0.20 mmol/g of resin) was synthesized by the method of Mitchell et al.[12] The amine content of the polymer was determined by nitrogen analysis and by picric acid titration.[13] The aminomethyl resin was added to excess 1,5-difluoro-2,4-dinitrobenzene to give II.[14] Reaction of II with Boc-His was carried out according to the method of Glass et al.[14] The amount of histidine on the resin was determined by removing it from a known weight of resin with 1.1 M deoxygenated dithiothreitol in DMF or 1.5 M deoxygenated thiophenol in DMF,[16] followed by amino acid analysis. The resin was converted to the active ester by using dicyclohexylcarbodiimide (DCC) and *N*-hydroxybenzotriazole (HOBT).[17] Glycine benzyl ester was then added to this activated resin derivative, resulting in III.[18] Four synthetic cycles[19] consisting of deprotection, neutralization, amino acid activation and coupling for Boc-Gly, Boc-His(Dnp), Boc-Gly, and Boc-His(Dnp) were carried out sequentially on compound III. The resulting resin IV contained the linear hexapeptide protected at the C terminus with a benzyl ester group, at the N terminus with a Boc group, and with dinitrophenyl groups on each of the three imidazole rings.[15]

Scheme I. Solid-Phase Synthesis and Cyclization: Preparation of *cyclo*-(Gly-His)$_3$



Cyclization of this linear hexapeptide was carried out on the solid support by (a) deprotecting the C- and N-terminals by treatment with a mixture of equal volumes of 40% HBr/acetic acid and 50% TFA/CH_2Cl_2 twice for 0.5 h, followed by thorough washing with CH_2Cl_2 and pyridine and (b) activating the C-terminal residue of the peptide with 3 equiv of *N*-(ethoxycarbonyl)-2-ethoxy-1,2-dihydroquinoline (EDDQ)[20] in a 1:1 by volume mixture of pyridine and toluene at room temperature and allowing 24 h for coupling (Scheme I). The EDDQ treatment was repeated one more time. At the end of the reaction no free amino group could be detected by a ninhydrin reagent.[21b] After the resin was washed with pyridine and CH_2Cl_2, the resulting cyclic peptide was removed from the resin in 85% yield (as determined by amino acid analysis) by using thiophenol in DMF,[22] and the solution was evaporated to dryness. The residue was dissolved

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(22) The resin was treated with deoxygenated 1.5 M thiophenol in DMF solution for 6 h at 25 °C.

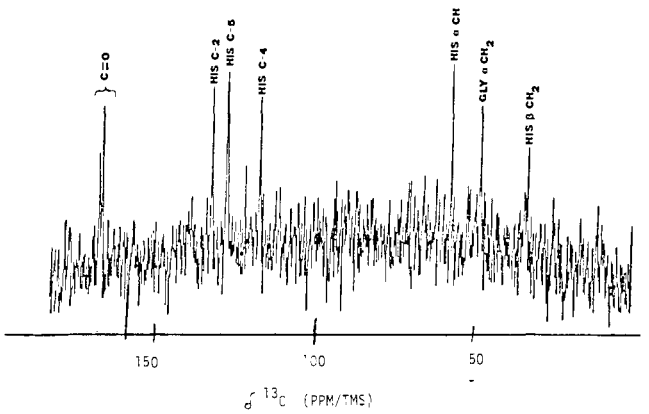

Figure 1. ^{13}C NMR spectrum of *cyclo*-(Gly-His)$_3$ in D_2O

in 2% HOAc and extracted with ethyl acetate to remove the free dinitrophenyl derivatives. The aqueous layer was then concentrated, and the yellowish residual Dnp peptides (incompletely deprotected) were separated from the colorless free peptide by using reverse-phase chromatography (μ-Bondapak C_{18}, Waters) and eluting with 2% aqueous HOAc. The yield of crude hexapeptide after chromatography was 42% (as determined by amino acid analysis of several samples and calculated based on the loading of the (aminomethyl)polystyrene resin). The crude peptide was further purified by ion-exchange chromatography (Chelex 100, 200–400 mesh). The cyclic hexapeptide was eluted with 0.1 M trifluoroacetic acid and precipitated as a white hydrochloride salt (or a trifluoroacetate salt).

The purified cyclic peptide showed a negative ninhydrin test, indicating the absence of free amino groups, and therefore contained no linear structures. It also gave a positive Pauli test,[21b] indicating the presence of unsubstituted histidine side chains. Amino acid analysis of the purified peptide showed a ratio of 1.02:1.00 of Gly to His. Field desorption mass spectral analysis of *cyclo*-(Gly-His)$_3$ showed peaks at $(MH)^+$ 583, $(MH - H_2O)^+$ 565, and $(M + Na)^+$ 605, as expected for a molecular ion of 582. There was no indication of higher molecular weight cyclic or linear peptides. High-pressure liquid chromatographic analysis of the purified peptide on an amino column (Altex Ultrasphere amino, 4.6 mm × 25 cm) using 65% aqueous acetonitrile containing 4 × 10^{-3} M KH_2PO_4 buffer showed a single peak (with a retention time of 25 min at a flow rate of 1.5 mL/min). The ^{13}C NMR spectrum[23] of the cyclic hexapeptide (as a trifluoroacetate salt) in D_2O shows signals from eight different carbons, which have been assigned as shown in Figure 1.[24] This simple carbon-13 pattern is consistent with the C_3-type symmetry expected for the symmetrical cyclic hexapeptide. The proton NMR spectrum in D_2O at 100 and 360 MHz also showed a simple pattern consistent with the cyclic structure, with the carbon protons assigned as follows: His CH-2, 905 ppm (s, 1 H); His CH-4, 7.74 ppm (s, 1 H); His α-CH, 5.22 ppm (s, 1 H); Gly α-CH_2, 4.37 ppm (m, br, 2 H); His β-CH_2, 3.67 (m, br, 2 H). The UV spectrum of the cyclic hexapeptide (in water) showed a shoulder at 214 nm followed by strong end absorption.

The solid-phase cyclization method used here (with a resin loading of 0.2 mmol NH_2/g of resin) resulted in a high yield of ring closure with no measurable amount of linear peptides remaining. Neither the crude product obtained directly after thiolytic cleavage nor the purified cyclic hexapeptide contained detectable amounts of linear or oligomeric cyclic peptides. This was checked by several techiques, including gel filtration, high-voltage electrophoresis, high-pressure liquid chromatography, and mass spectral analysis of both the unpurified and the final purified peptide products.

(23) The ^{13}C NMR spectrum was obtained on a Varian XL-100-Nicholet TT-100 system (100 MHz) with a ^{13}C microprobe (Varian).

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The synthetic scheme described here has provided convenient access to *cyclo*-(Gly-His)$_3$, making use of the bidirectional peptide synthesis method.[14c] By variation of the amino acids, this scheme can lead to cyclic hexapeptides with different coordination environments. Such cyclic peptides can be designed to incorporate amino acid side chains that are important for the function of various enzymes.

The cyclic hexapeptide, *cyclo*-(Gly-His)$_3$, described here provides only three coordination positions around the metal ion and the fourth coordination position to the metal ion can be varied to include oxygen-, nitrogen-, or sulfur-donor ligands such as water, imidazole, dimethyl sulfide, or mercaptoethanol. The 1:1 complexes of *cyclo*-(Gly-His)$_3$ with Cu(II) and Zn(II) have been formed in solution and their spectral, physical, and electrochemical properties are currently under study in our laboratory.

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Registry No. VI, 80954-37-6; Boc-His, 17791-52-5; Gly-OBzl, 1738-68-7; Boc-Gly, 4530-20-5; Boc-His(Dnp), 25024-53-7.

Liquid Crystalline Catalysis. 1. Reactivity Induced by Smectic Solvents

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Reported results of catalytic and stereochemical effects of liquid crystalline solvents in thermal[1-3] and photochemical reactions[4] seem to discourage expectations of notable developments; the use of thermotropic liquid crystalline solvents as a tool in the elucidation of reaction mechanisms, as well emphasized by Nerbonne and Weiss,[3] might seem to be the only really encouraging development to be expected in this field.

However, apart from one exception[4] (where the rate of a photochemical reaction is enhanced only by a cholesteric and not a smectic or a nematic solvent) these results have been all obtained by using only nematic or cholesteric liquid crystalline solvents whose "microscopic matrix effect", determined by the short-range orientational order, is very similar to that of isotropic solvents.[5,6] By use of more tightly ordered smectic solvents and selection of a reaction whose energy of activation is expected to be mainly determined by the entropy term (i.e., with severe orientational demands in the transition state), relevant catalytic effects due to solvent ordering have been displayed and are presented in this paper.

The guest–host intermolecular interactions resulting from dissolving an organic substance in a liquid crystal induce modifications in the orientational correlation of the molecules of the mesomorphic solvent and anisotropy in both the diffusion and

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