It is also informative to examine the distribution of the various species as a function of conversion. At zero conversion, only the species w_1 exists, in addition to the oligomer chain ends. As conversion increases, the concentration of w_1 decreases, while the concentrations of u_i, v_i, w_i ($i > 1$), R_i, and Q_i first increase, reach a maximum, and then decrease. The concentration of P_i continually increases with conversion. These results are to be expected. On the other hand, it is observed that the ratio of concentration of each species to that containing one unit less was essentially constant and independent of the species type. Stated mathematically

$$u_{i+1}/u_i \simeq v_{i+1}/v_i \simeq w_{i+1}/w_i \simeq R_{i+1}/R_i \simeq Q_{i+1}/Q_i \simeq P_{i+1}/P_i \simeq p(c)$$

where $p(c)$ is a function of conversion but is independent of i if conversion is greater than 10%. Below 10%, p slowly decreases with increasing i. Therefore, the sequence length distribution of extender units follows a geometric distribution similar to eq 8 for all of the species present at high conversion. One method of testing the results of the calculations would be to selectively hydrolyze the ester groups of a polyester macrodiol while leaving the urethane groups unchanged.[5] The hard block distribution of diisocyanate residues can be determined by gel permeation chromatography.

Acknowledgment. This work was supported by the Office of Naval Research, and the Department of Chemical Engineering, M.I.T.

Supplementary Material Available: program for computing the various distributions as a function of conversion (5 pages). Ordering information is given on any current masthead page.

References and Notes

(1) R. W. Lenz, "Organic Chemistry of Synthetic High Polymers", Interscience, New York, N.Y., 1967.
(2) L. H. Peebles, Jr., *Macromolecules*, 7, 872 (1974).
(3) A distinction is made between the present case and ref 1. Here the sequence containing i extender units contains $i - 1$ monomer units left over from the first-stage reaction plus two monomer units attached to the macrounits; the counting is different in the two treatments.
(4) Equation A56 of ref 1 should contain the term $2(\mu - 1)X_1h(s - 1)^{**}$.
(5) C. E. Wilkes, private communication.

Conformation of *cyclo*-(L-Leu-L-Tyr-δ-Avaler-δ-Avaler), a Synthetic Inhibitor of Chymotrypsin, by X-Ray Analysis

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ABSTRACT: The synthetic cyclic tetrapeptide (L-Leu-L-Tyr-δ-Avaler-δ-Avaler) is an effective inhibitor of chymotrypsin, competitive with linear peptides like Ac-L-Leu-L-Tyr-OMe. An x-ray diffraction analysis of the crystal structure of the cyclic peptide shows that the conformation of the 18-membered ring is very similar to that of one of the four conformers of cyclic hexaglycyl. There is no internal hydrogen bonding. Side chains are located on two "corners" of the approximately rectangular ring. The χ_{i1} angles for Leu and Tyr are −74 and −48°, respectively. The Leu side chain is extended away from the polypeptide ring while the Tyr side chain is folded under an adjacent carbonyl bond. The cell parameters for the space group $P2_1$ are: a = 9.361 (3) Å, b = 19.039 (10) Å, c = 9.603 (3), Å, and β = 116.54 (3)°. A molecule of $(CH_3)_2SO$ (disordered) and a molecule of H_2O cocrystallized with the cyclic peptide.

Chymotrypsin is active in the duodenum, cleaving further the partially digested food (chyme) passing from the stomach. The major sites of action on the substrates are on the peptide bonds of residues containing aromatic or large aliphatic side chains such as Trp, Phe, Tyr, and Leu. Models of effective, irreversible synthetic inhibitors like acetyl-L-Ala-L-Ala-L-Phe-chloromethyl ketone bound to the substrate binding site in chymotrypsin have been proposed by Segal et al.,[1] based on difference maps from x-ray diffraction data. More recently Tsetlin et al.[2,3] have shown that the synthetic *cyclo*-(L-Leu-L-Tyr-δ-Avaler-δ-Avaler), where Avaler ≡ aminovalerate, containing an 18-membered ring is a very effective inhibitor, competitive with linear substrates like Ac-L-Leu-L-Tyr-OMe. The inhibitor does not undergo hydrolysis. The purpose of this investigation was to determine the conformation of *cyclo*-(L-Leu-L-Tyr-δ-Avaler-δ-Avaler) in order to compare the models of the linear inhibitors used by Segal et al.[1] and to compare the known conformations of cyclic hexapeptides.[4,5]

Experimental Section

Intensity data from colorless, well-formed crystals, derived from $(CH_3)_2SO{\cdot}H_2O$ solution, were collected with the θ–2θ scan technique on a four-circle automatic diffractometer using a scan of $2.0° + 2\theta_{(\alpha_2)} - 2\theta_{(\alpha_1)}$ and a scan speed of 2°/min. The intensities of three reflections, monitored every hour, remained constant during the experiment, thus indicating that there was no deterioration of the crystal upon exposure to x rays. Data, collected to $2\theta_{max} = 126°$, were corrected for Lorentz and polarization factors. Normalized structure factors, $|E_h|$, were obtained with the use of a K curve. Cell parameters and other pertinent data are listed in Table I.

The structure was derived with the aid of the direct method for phase determination.[6] Some confusion arose because the strongest peaks formed several triangles sharing common sides, with distances of bonding length. It was determined later that the S atom in $(CH_3)_2SO$, a solvent molecule cocrystallized with the peptide, was disordered between two positions and that one-half S weight in two positions plus full weight for the O atom and two C atoms accounted for the strange configuration. Meanwhile, a fragment of the peptide structure, recognized in an E map from a less probable phase set, was used to derive the structure. The fragment was misplaced with respect to the origin of the cell. Therefore, the symmetry was lowered to $P1$ and the data were doubled by letting $|E_{hkl}| = |E_{\bar{h}\bar{k}l}|$ and $|E_{\bar{h}kl}| = |E_{h\bar{k}\bar{l}}|$, but with $\phi_{hkl} \neq \phi_{\bar{h}\bar{k}l}$ and $\phi_{\bar{h}kl} \neq \phi_{h\bar{k}\bar{l}}$. The fragment of the peptide was then used in the partial structure procedure[7] which yielded the positions of all the atoms in the two peptide molecules in a unit cell. From the relationship of the two

Table I
Crystallographic Data

Molecular formula	$C_{25}H_{38}N_4O_5 \cdot (CH_3)_2SO \cdot H_2O$
Molecular weight (total)	570.76
Crystal habit	Prismatic on *b*
Crystal size, mm	0.27 × 0.50 × 0.33
Call parameters	*a* = 9.361 ± 0.003 Å
	b = 19.039 ± 0.010 Å
	c = 9.603 ± 0.003 Å
	β = 116.54 ± 0.03°
Space group	$P2_1$
Volume, Å^3	1531.13
Mol/asym unit	1
Calcd density, g/cm^3	1.238
Absorption coefficient, cm^{-1}	9.21
Radiation	Cu Kα
Wavelength, Å	1.54178
No. of independent reflections	2558

molecules in the unit cell, the position of the twofold screw axis was located and the origin of the cell was shifted accordingly to correspond to space group $P2_1$. The previously unrecognized configuration of adjacent triangles mentioned earlier was now identified as the disordered $(CH_3)_2SO$ and an additional H_2O molecule was found.

Full-matrix least-squares refinement on all atoms other than H was followed by a difference map from which the coordinates of the hydrogen atoms for the peptide molecule were obtained. Hydrogen positions for the disordered $(CH_3)_2SO$ molecule were not found. Inclusion of 38 hydrogen atoms with fixed parameters in the least-squares refinement using anisotropic thermal factors for all the other atoms resulted in an agreement factor of R of 4.8% for all 2558 data. The function minimized was $R = \Sigma \omega_F(|F_o| - |F_c|)^2/|F_o|$ where $\omega_F = 1/\sigma_F^2$ and values for σ_F^2 were obtained in the manner described by Gilardi.[8]

Fractional coordinates and thermal factors for the nonhydrogen atoms are listed in Table II, bond lengths and angles are shown in Figure 1, while Table III contains the conformational angles.

Table II
Fractional Coordinates[a] and Anisotropic Thermal Parameters[b]

Atom	*x*	*y*	*z*	B_{11}	B_{22}	B_{33}	B_{12}	B_{13}	B_{23}
N_1	0.4497	0.0257	0.2274	2.90	2.67	2.59	0.19	1.27	−0.17
$C_1\alpha$	0.5116	−0.0440	0.2885	3.14	2.74	2.90	0.14	1.33	−0.12
C_1'	0.5939	−0.0482	0.4659	3.04	2.66	3.12	0.01	1.68	−0.08
O_1	0.6406	−0.1057	0.5298	5.23	2.78	3.35	0.61	1.95	0.35
$C_1\beta$	0.6290	−0.0673	0.2272	4.94	3.71	3.68	1.14	2.32	0.01
$C_1\gamma$	0.5514	−0.0895	0.0543	7.68	4.35	3.10	1.58	2.90	0.40
$C_{11}\delta$	0.6820	−0.1048	0.0085	12.07	6.58	6.89	1.01	6.78	−0.41
$C_{12}\delta$	0.4457	−0.1546	0.0291	9.12	6.33	5.10	−1.46	2.58	−2.03
N_2	0.6116	0.0118	0.5437	2.74	2.74	2.49	0.04	1.34	−0.15
$C_2\alpha$	0.7089	0.0168	0.7118	2.77	3.32	2.39	0.05	1.23	−0.04
C_2'	0.6228	0.0593	0.7878	3.19	4.25	2.55	−0.02	1.51	−0.04
O_2	0.6989	0.0956	0.9017	3.78	9.04	4.03	−1.12	1.99	−3.60
$C_2\beta$	0.8742	0.0488	0.7553	2.91	4.07	3.62	−0.51	1.64	−0.77
$C_2\gamma$	0.9583	0.0163	0.6692	2.32	3.05	3.75	−0.47	1.40	−0.33
$C_{21}\delta$	1.0546	−0.0423	0.7259	4.14	3.89	4.61	0.19	2.56	0.82
$C_{22}\delta$	0.9428	0.0451	0.5279	2.82	3.41	3.71	0.20	1.50	0.08
$C_{21}\epsilon$	1.1343	−0.0717	0.6482	4.84	3.65	5.38	1.13	3.18	1.15
$C_{22}\epsilon$	1.0206	0.0165	0.4487	3.19	3.66	3.77	−0.30	1.83	0.07
$C_2\zeta$	1.1173	−0.0415	0.5081	3.87	3.59	5.38	0.10	2.96	−0.33
$O_2\eta$	1.2034	−0.0708	0.4386	7.09	5.17	7.17	2.12	5.72	1.43
N_3	0.4643	0.0526	0.7235	2.68	4.35	3.22	−0.26	1.49	−0.85
$C_3\alpha$	0.1278	0.2670	0.5844	4.63	3.45	4.90	−0.72	2.16	−1.09
C_3'	0.0356	0.2487	0.4129	4.03	3.46	4.63	0.40	2.19	−0.40
O_3	−0.0754	0.2067	0.3681	4.56	5.70	5.37	−2.20	1.77	−1.13
$C_3\beta$	0.1814	0.2034	0.6903	4.03	3.84	4.29	−0.76	2.56	−1.05
$C_3\gamma$	0.2993	0.1589	0.6592	4.15	4.20	5.24	−0.46	3.09	−0.38
$C_3\delta$	0.3621	0.0962	0.7675	3.32	4.90	3.44	0.34	2.05	−0.12
N_4	0.0817	0.2814	0.3166	4.98	3.37	4.56	0.35	2.62	−0.21
$C_4\alpha$	0.2508	0.1150	0.1092	3.32	3.45	3.50	0.82	1.70	0.31
C_4'	0.2972	0.0437	0.1832	3.24	3.37	2.98	−0.04	1.63	−0.23
O_4	0.2010	0.0052	0.2005	3.84	4.27	6.85	0.18	3.38	1.18
$C_4\beta$	0.1405	0.1546	0.1609	4.36	3.83	3.77	0.90	2.34	0.44
$C_4\gamma$	0.1029	0.2277	0.0911	5.10	3.96	4.63	1.47	2.80	0.83
$C_4\delta$	0.0023	0.2705	0.1481	5.52	5.03	4.71	2.08	2.47	0.40
S_D[c]	0.5467	0.2292	0.5003	3.85	2.83	5.03	0.06	2.83	0.85
S_D'[c]	0.6772	0.2138	0.5305	5.79	4.86	6.51	0.88	3.91	1.17
O_D[c]	0.5644	0.1568	0.4853	8.98	2.77	7.23	−0.84	3.80	0.14
C_D[c]	0.5854	0.2782	0.3669	7.41	4.76	6.36	−0.30	4.10	1.69
C_D[c]	0.6965	0.2627	0.6736	13.37	6.44	4.58	−3.32	1.88	−0.19
O_W[d]	0.6566	0.1247	0.1652	5.13	6.51	3.77	−1.91	2.57	−1.21

[a] Standard deviations:

O	0.0003	0.0002	0.0003
N	0.0003	0.0002	0.0003
C (ring)	0.0004_5	0.0002	0.0004_5
C (side chain)	0.0006	0.0002_5	0.0006
S	0.0003	0.0000	0.0003

[b] Thermal parameters are of the form $T = \exp[-\frac{1}{4}(B_{11}h^2a^{*2} + B_{22}k^2b^{*2} + B_{33}l^2c^{*2} + 2B_{12}hka^*b^* + 2B_{13}hla^*c^* + 2B_{23}klb^*c^*)]$. [c] Atom belongs to $(CH_3)_2SO$. [d] Oxygen atom from water molecule.

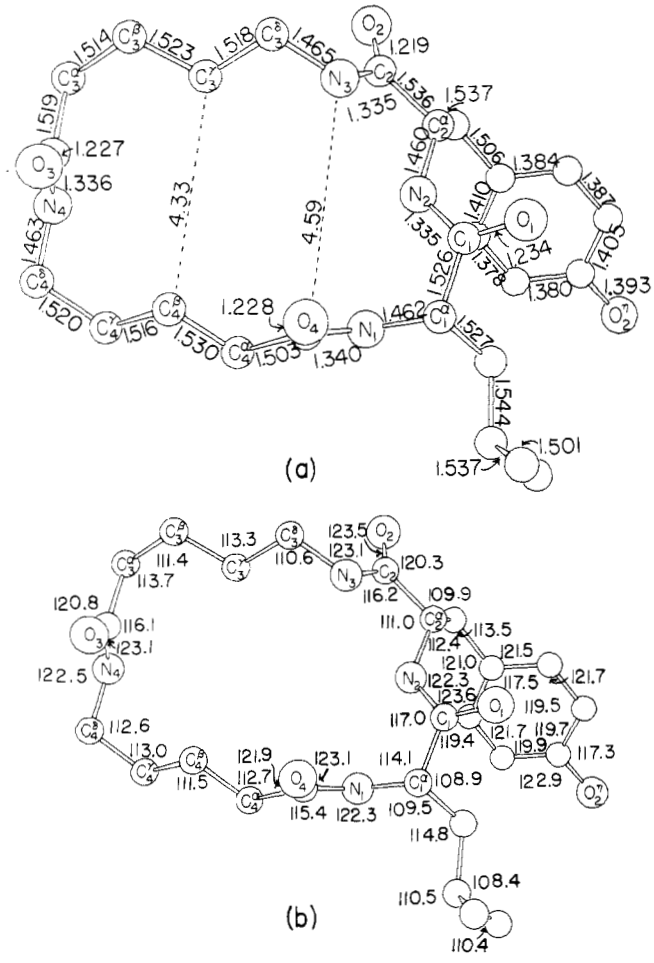

Figure 1. (a) Bond lengths in Å units. The standard deviations based on the least-squares fit alone are 0.006 Å or less in the peptide ring and ~0.0075 Å in the side groups. (b) Bond angles in degrees. The standard deviations are near 0.4°.

Results and Discussion

The Ring. The conformation of the peptide molecule is shown in the stereodiagram in Figure 2. The 18-membered ring is approximately rectangular in shape. There is no internal hydrogen bonding across the ring. The N_3 and O_4 atoms, which could be expected to form a hydrogen bond of the type found in β turns, are actually separated by 4.59 Å and there is a sizable hole inside the ring. One of the interesting features is that the proton on N_3 does not participate in any kind of hydrogen bonding.

The trimethylene moieties in each δ-Avaler unit mimic peptide units in size and conformation, so that the ring as a whole mimics a cyclic hexapeptide. Five conformations

Table III
Conformational Angles (degrees)

cyclo-(L-Leu-L-Tyr-δ-Avaler$_2$)		*cyclo*-(Gly$_6$), conformer b		Δ
		Ring		
ϕ_1	−96	ϕ_1	−95	−1
ψ_1	−5	ψ_1	−2	−3
ω_1	−171	ω_1	−172	+1
ϕ_2	−136	ϕ_2	−130	−6
ψ_2	35	ψ_2	24	−11
ω_2	−171	ω_2	−174	+3
ϕ_3	94	ϕ_3	109	−15
μ_{31}[a]	175	ψ_3	137	+38
μ_{32}[a]	178	ω_3	−176	−6
μ_{33}[a]	64	ϕ_4	90	−26
ψ_3'	−132	ψ_4	−149	+18
ω_3	−179	ω_4	−170	−9
ϕ_4'	−108	ϕ_5	−82	−26
μ_{41}[a]	61	ψ_5	−17	+78
μ_{42}[a]	−176	ω_5	−174	−2
μ_{43}[a]	−183	ϕ_6	−105	−78
ψ_4'	−140	ψ_6	−136	−4
ω_4	−174	ω_6	−167	−7
		Side Chains		
χ_{11}	−74			
χ_{12}^1	175			
χ_{12}^2	−64			
χ_{21}	−48			
χ_{22}^1	−88			
χ_{22}^2	+92			

[a] Torsional angles about the C–C bonds in the two trimethylene moieties in the peptide ring.

have been found thus far for cyclic hexapeptides in the crystalline state, four for cyclic hexaglycyl[4,9] and one for the 18-membered ring in Ferrichrome A.[5] The ring in the latter molecule contains one intramolecular hydrogen bond and therefore cannot be compared directly with the conformation of the present molecule. Three of the four conformers of *cyclo*-(Gly$_6$), Figure 3, do not contain intramolecular hydrogen bonds and from among them, conformer b and the ring in the present molecule have very similar conformations.

The torsional angles for the two molecules are compared in Table III. A labeling scheme has been improvised for some of the torsional angles[10,11] since peptide units other than α amino had not been considered in the IUPAC-IUB convention.[12] Torsional angles for the ring bonds C_i^α–C_i^β, C_i^β–C_i^γ, and C_i^γ–C_i^δ are designated by μ_{i3}, μ_{i2}, and μ_{i1}, respectively, and N_i–C_i^δ is designated by ϕ_i'. As can be seen in Table III, the agreement between the peptide units in the present molecule and conformer b of *cyclo*-(Gly$_6$) is excellent and the largest differences occur where the trimethylene chains in one molecule are compared to peptide units in the other. The similarity in conformation between the

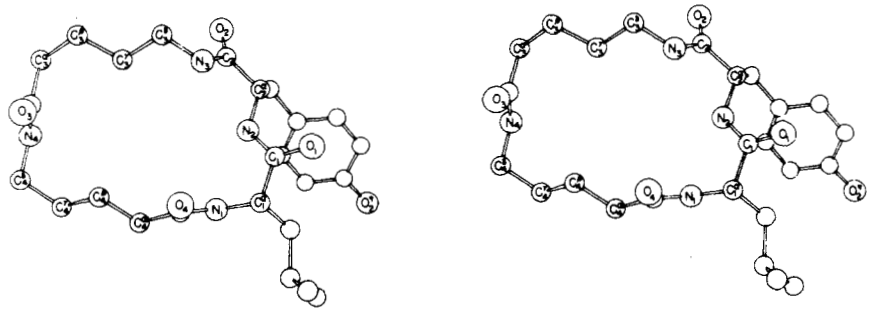

Figure 2. Stereodiagram of *cyclo*-(L-Leu-L-Tyr-δ-Avaler-δ-Avaler) drawn from experimentally determined coordinates by a computer program prepared by Johnson.[20]

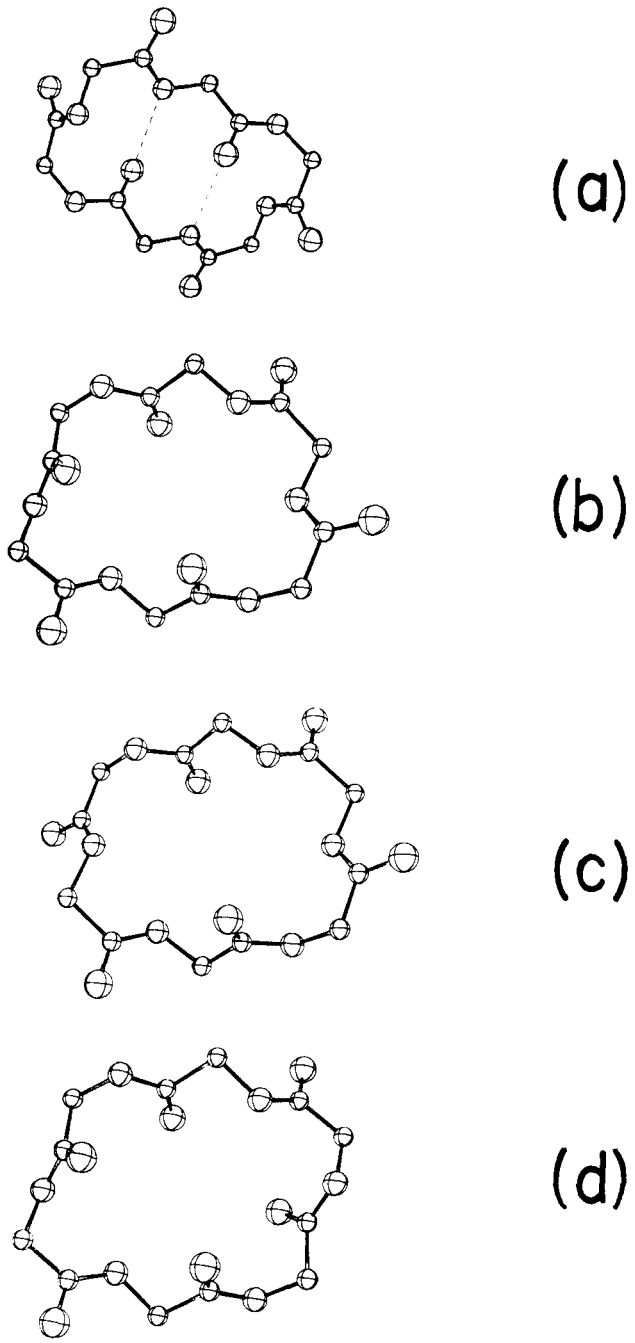

Figure 3. Four conformers of cyclic hexaglycyl cocrystallized in the same unit cell.[4]

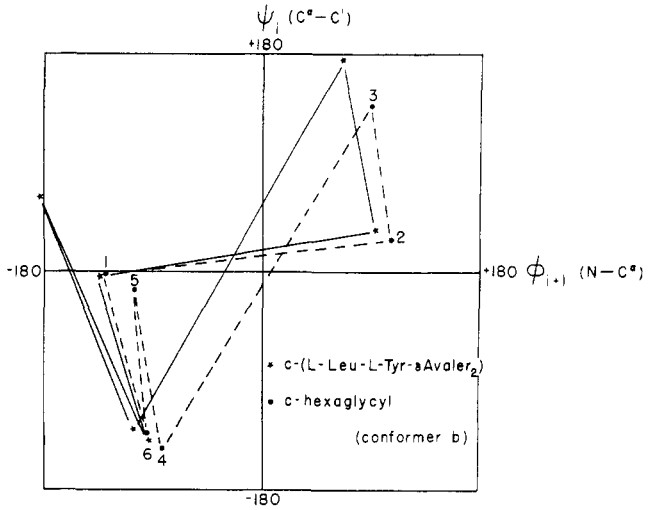

Figure 4. A comparison of the conformations of *cyclo*-(L-Leu-L-Tyr-δ-Avaler-δ-Avaler) and cyclic hexaglycyl (conformer b) on a ψ_i, ϕ_{i+1} plot.

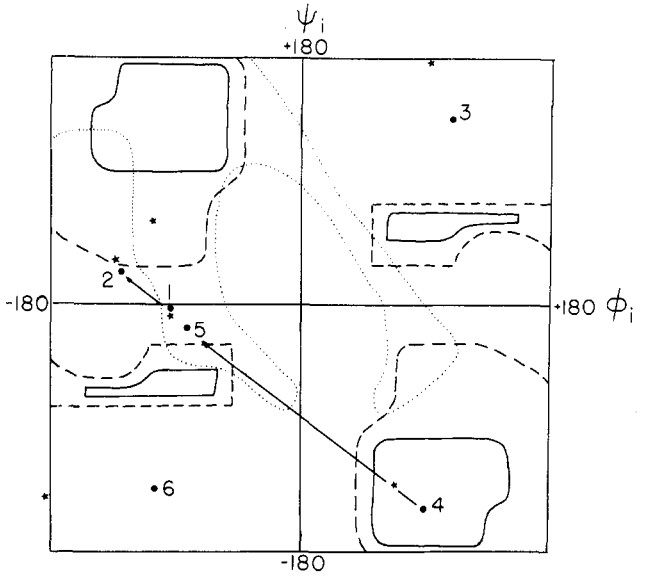

Figure 5. A comparison of the experimental conformational angles ϕ_i, ψ_i for *cyclo*-(L-Leu-L-Tyr-δ-Avaler-δ-Avaler) represented by ★ and for cyclic hexaglycyl (conformer b) represented by ●. Solid lines contain the regions of fully allowed conformations and dashed lines contain the regions of partially allowed conformations as calculated for dipeptide models by Ramachandran and Sasisekharen,[14] while the dotted line represents the allowed conformational regions (4 kcal/mol above the minimum energy) as calculated by Pullman[15] using quantum-mechanical considerations. The two arrows connect the C_i^α atoms involved in each of the U-turns of the peptide rings.

two molecules and the asymmetric nature of the molecular rings is shown in the ψ_i, ϕ_{i+1} plot in Figure 4 (after Go and Scheraga[13]).

The more conventional ϕ_i, ψ_i plot for both molecules is illustrated in Figure 5. Also shown are the "allowed" conformational regions as calculated by Ramachandran and Sasisekharan[14] based on interatomic contacts of a hard-sphere dipeptide model with C^β present. Solid lines contain the regions of fully allowed conformations while the dashed lines outline the regions of partially allowed conformations. The results of quantum-mechanical calculations by Pullman[15] for the same dipeptide are represented by the dotted line which encloses an allowed region with 4 kcal/mol above the minimum. The experimental points for both molecules, *cyclo*-(Gly_6) without any side chains and the present molecule with bulky side chains at i = 1 and 2, fall outside the allowed regions, for the most part. Residues with i = 3 and 6 in *cyclo*-(Gly_6) and their equivalents (marked by an asterisk) in the present molecule have ϕ, ψ values especially far from the calculated "allowed" regions.

Both of the above calculations used models of peptide units based on bond lengths and angles quoted by Pauling[16] in 1960. Table IV contains a tabulation of the averaged values for bond lengths and angles for trans peptide units found in a number of crystalline cyclic peptides containing four to twelve peptide units. Based on these results, it would seem advisable to adjust the values in the model for an "average" peptide unit by 0.01–0.02 Å and 1–2°. Although the averaged values for most bond lengths and angles are quite consistent from molecule to molecule, it ap-

Table IV
Averaged Values from Cyclicpeptides for Trans Peptide Units[a]

	Pauling[16]	Tetra (A)	Tetra (B)	Hexa (C)	Hexa (D)	Deca (E)	Dodeca (F)
N–Cα	1.47	1.477	1.449	1.460	1.463	1.465	1.465
Cα–C′	1.53	1.536	1.503	1.515	1.521	1.542	1.525
C′–O	1.24	1.229	1.237	1.232	1.227	1.226	1.235
C′–N	1.32	1.344	1.341	1.338	1.336	1.344	1.355
C′–N–Cα, deg	123	124	120.5	122.5	122.5	121	118
N–Cα–C′, deg	110	105	114.5	113		111	108
Cα–C′–O, deg	121	120	121	121	120.5	120	*b*
O–C′–N, deg	125	123	122	123	123	123	122.5
Cα–C′–N, deg	114	116.5	116.5	115.5	116	117	119.5

[a] (A) Chlamydocin;[17] (B) *cyclo*-(L-Ser(O-*t*-Bu)-β-Ala-Gly-L-β-Asp(OMe));[11] (C) *cyclo*-(Gly-Gly-Gly-Gly-D-Ala-D-Ala);[9] (D) *cyclo*-(L-Leu-L-Tyr-δ-Avaler$_2$); (E) Li$^+$ antamanide;[18] (F) Valinomycin.[19] [b] Affected by O atoms in depsipeptide.

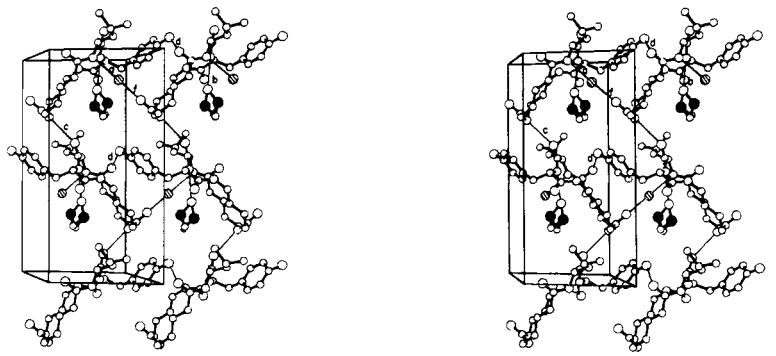

Figure 6. Stereodiagram of the crystal packing of the cyclic tetrapeptide and the cocrystallized H_2O, denoted by ⊗, and $(CH_3)_2SO$, with the disordered S atom denoted by ●. Hydrogen bonds are labeled by the letters a–f. Axial directions are a →, b ↓, and c directed into the page.

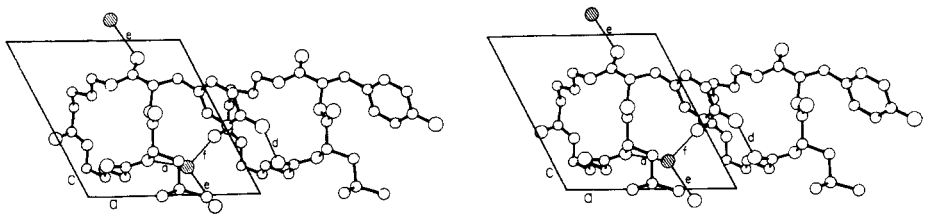

Figure 7. Stereodiagram of two adjacent peptide molecules in the crystal drawn at right angles to the diagram in Figure 6. Hydrogen bonds are labeled by the letters a–f. The H_2O molecule is denoted by ⊗.

pears that both the C′NCα angle and the NCαC′ angle can undergo considerable adjustment, with differences ranging up to 9°, from molecule to molecule.

Side Chains. The ring in the present molecule, as well as in conformer b of *cyclo*-(Gly$_6$), has a rectangular shape. Side chains are attached to two of the "corner" atoms, C_1^α and C_2^α, along the short side of the rectangle. Angles χ_{11} and χ_{21} have values of −74 and −48°, respectively. The isobutyl chain on C_1^α is extended away from the polypeptide ring, while the *p*-hydroxybenzyl moiety on C_2^α is tucked under the $C_1'{=}O_1$ bond. The intramolecular separations between the carbonyl group and the *p*-hydroxytoluyl group are all greater than 3.9 Å so that there is no particular attraction between the two groups.

Hydrogen Bonding. The peptide ring does not contain any internal hydrogen bonds. Atom N_3, which could be expected to be the donor for O_4, if a hydrogen bond across a β turn were to form, is at a distance of 4.59 Å from O_4. The proton on N_3 is directed toward the O of the Tyr side chain on a neighboring molecule (related by $-1 + x$); however, the $N_3\cdots O_2^{\eta}$ separation is 3.60 Å. Three of the four carbonyl oxygens, acceptors for hydrogen bonds with neighboring or solvent molecules, are located on one side of the average plane of the peptide ring while O_2 is on the other side of the average plane. All the active donors, N_1H, N_2H, N_4H, and OH, are also on the other side of the average plane of the peptide ring. The peptide molecules are linked in all three directions by hydrogen bonds directly between peptide molecules, (c) $N_4H\cdots O_1$ and (d) $O_2H\cdots O_4$, or indirectly with the H_2O molecule as an intermediary, (a) $N_1H\cdots O_w$, (e) $O_wH\cdots O_2$, and (f) $O_wH\cdots O_3$. The hydrogen bonds are shown in the packing diagrams, Figures 6 and 7, and the lengths are listed in Table V.

The peptide molecules are inclined to the *b* axis by ~45° and stacked in layers parallel to the (010) planes. Large cavities are formed between the planes of peptide molecules which contain the disordered $(CH_3)_2SO$ molecules. A hydrogen bond is formed between N_2H, a nitrogen atom located in the middle position of one of the U-turns in the molecule, and the O atom in the $(CH_3)_2SO$.

Chymotrypsin Activity

The elucidation of the conformation of this cyclic inhibitor does not yield any particular clue to the mode of binding with chymotrypsin. If the aromatic group is positioned in the same manner in the chymotrypsin as the aromatic group in the model of the linear inhibitor, Figure 8, the next few adjacent atoms have similar conformations in both inhibitors. There is the possibility of a hydrogen bond to the carbonyl of Ser-214 and the possibility for an ester

Table V
Hydrogen Bonds

Donor	Acceptor	Length, A	Symmetry position of acceptor	Label in Figures 6 and 7
N_1	O_W	2.948	x, y, z	a
N_2	O^a	2.812	x, y, z	b
N_4	O_1	3.183	$1-x, \frac{1}{2}+y, 1-z$	c
O_W	O_2	2.787	$x, y, -1+z$	e
O_W	O_3	2.857	$1+x, y, z$	f
$O_2\eta$	O_4	2.697	$1+x, y, z$	d

[a] O in $(CH_3)_2SO$.

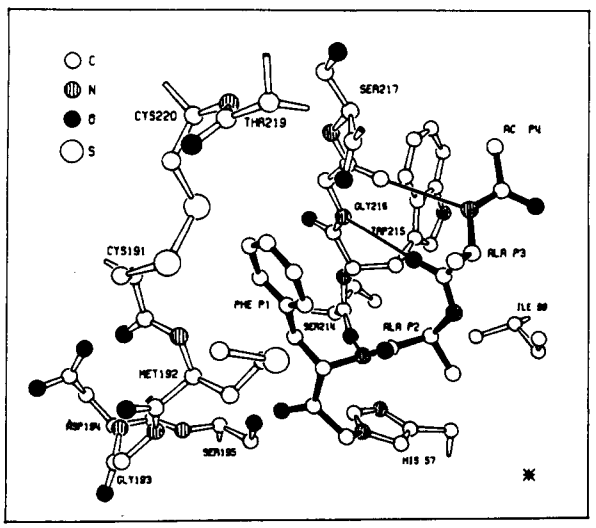

Figure 8. Proposed binding of the inhibitor moiety *N*-acetyl-L-Ala-L-Ala-L-Phe-methylene to chymotrypsin A_γ. Only pertinent portions of the protease molecule are shown (from Segal et al.[1]).

linkage with O_γ of Ser-195. However, in that case, the Leu side chain in the cyclic inhibitor would occupy the space used by the remainder of the peptide backbone in the linear inhibitor, and the peptide ring in the cyclic inhibitor would hang downward. This kind of orientation of the cyclic peptide appears unlikely. Another possibility is to insert the Leu side chain into the aromatic binding site. Again there is a possibility for a hydrogen bond to Ser-214 and an ester linkage to Ser-195, while the peptide ring would extend sideways to the right. Of course, in the binding process the conformation of the chymotrypsin may be locally altered near the binding site or the cyclic inhibitor may assume a different conformation. Whether any of these possibilities has any substance can probably be answered only by examining the crystal structure of chymotrypsin with the cyclic inhibitor bound to it.

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Supplementary Material Available: approximate coordinates of hydrogen atoms as determined from difference map (1 page). Ordering information is given on any current masthead page.

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