Conformational Studies on Peptides. X-Ray Structure Determinations of Six N-Methylated Cyclic Dipeptides Derived from Alanine, Valine, and Phenylalanine

Ettore Benedetti, Richard E. Marsh, and Murray Goodman*

Contribution from the Department of Chemistry, University of California, San Diego, La Jolla, California 92039, and the A. A. Noyes Laboratory of Chemical Physics, California Institute of Technology, Pasadena, California 91125.
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Abstract: We have solved the structures of six N-methylated diketopiperazines derived from alanine, valine, and phenylalanine. The optically active isomer of cyclo(*N*-methyl-L-alanyl)$_2$, its racemate, cyclo(*N*-methyl-L-valyl)$_2$, and cyclo(*N*-methyl-L-phenylalanyl)$_2$ assume boat conformations with axial substituents on the C$^\alpha$ carbon atoms. The meso isomers, cyclo(*N*-methyl-L-valyl-*N*-methyl-D-valyl) and cyclo(*N*-methyl-L-phenylalanyl-*N*-methyl-D-phenylalanyl), assume chair structures with axial dispositions of the substituents on the C$^\alpha$ carbon atoms. The parts of the side chains extending beyond C$^\beta$ can fold over the diketopiperazine ring or be forced away from the ring system by steric effects.

For many years our laboratories have been engaged in the study of the conformations of peptides and polypeptides in their own right and as model systems for the elucidation of protein structure.[1] We have investigated the conformations of linear and cyclic peptides in solution and in the solid state by a variety of spectroscopic techniques and by x-ray diffraction methods.

Cyclic peptides represent an enticing class of compounds to study since they do not contain the terminal residues present in linear molecules. As a result, the interpretation of spectra and structure may be simplified, and allowed conformations may be reduced because of the cyclic character of the molecule.

Cyclic dipeptides (diketopiperazines or 2,5-piperazinediones) are the simplest cyclic peptides. Many natural products contain elements of this structural system.[2-12] Even though the peptide bonds in diketopiperazines must be cis, they are important peptides to study by x-ray and spectral means because so many elements of molecular structure can be established and explained. The conformational patterns found for these molecules can be extrapolated to answer general questions about peptide and protein structure.

Numerous diketopiperazines have been studied in solution.[13-16] Some of their structures have been determined by x-ray diffraction.[17-27] In this paper we report the crystal structure determinations of six N-methylated diketopiperazines derived from alanine, valine, and phenylalanine. These molecules were chosen to investigate the effect of steric hindrance on the conformation of the diketopiperazine ring, the planarity of the amide bonds, and the orientations of the side chains. In a subsequent paper,[28] we will compare our findings in the solid state with spectroscopic studies of the same molecules in solution.

Experimental Section

The synthesis of the six compounds examined by x-ray diffraction analysis in this paper will be reported in the following paper.[28] Crystals of the various compounds were obtained either by slow sublimation under reduced pressure or by slow evaporation of aqueous or methanolic solutions. Preliminary oscillation and Weissenberg photographs were taken in each case to establish the crystal symmetry and the space groups. Values of cell dimensions for each crystal were obtained by a least-squares fitting of the 2θ, χ, and ϕ values for a minimum of 12 reflections accurately centered on an automated diffractometer. For one of the crystals, cyclo(*N*-methyl-L-phenylalanyl-D-phenylalanyl), the values of the cell dimensions obtained by this method were compared with those obtained from Weissenberg photographs prepared with a Straumanis-type camera. Density measurements were carried out by flotation methods in mixtures of chloroform–*n*-hexane. A complete list of crystal data for the six compounds studied is given in Table I.

Intensity data were all collected on a Datex-automated General Electric XRD-5 diffractometer using nickel-filtered Cu Kα radiation and a scintillation counter in all cases except for cyclo(*N*-Me-L-Val)$_2$ where zirconium-filtered Mo Kα radiation was used. At least one set of independent reflections was collected for each crystal with the θ–2θ scan mode in the range 4–130° for 2θ (Cu Kα) and 4–50° for Mo Kα radiations. Background counts were collected at each scan extremum. Three check reflections were monitored every 25 reflections. The number of reflections measured in each case is reported in Table II together with the number of reflections whose intensities were less than one esd above background and to whom zero weight was assigned in the refinement process; observational variances, $\sigma^2(I)$, for the observed reflections were based on counting statistics for scan and background plus an additional term $(0.02S)^2$ where S is the scan count. Intensities and standard deviations were corrected in the usual way for Lorentz and polarization factors.

Structure Determinations and Refinements. All six structures were solved by direct methods, by the application of Multan,[29] the phase-permutation computer program in its latest version, which includes fast Fourier, molecular fragment recognition, and structure factor calculation programs. A brief summary of the number of E's used, their minimum value, the sets of phases generated, the number of atoms located in the E map (corresponding in all cases to the set of phases having the highest combined figure of merit), and the conventional R value at the beginning of the isotopic refinement for the six structure determinations is reported in Table III. The approximate positions of the hydrogen atoms were usually derived from difference Fourier maps. However, before the final least-squares cycles, adjustments were usually made in order to have a stereochemically reasonable structure as far as bond lengths and bond angles, including hydrogen atoms, were concerned.

Refinements were carried out by full-matrix least-squares procedures. Heavy atoms (C, N, O) were refined with anisotropic temperature factors, while isotropic temperature factors were used for the hydrogen atoms. Refinements were ended when the shifts in the adjusted parameters were less than one-third of their standard deviations. [With the exception of the two alanine compounds, a secondary extinction factor (see ref 30) was refined in the last two cycles. For the two alanine diketopiperazine structures, the isotropic temperature factors of the hydrogen atoms were kept fixed to the values of the isotropic temperature factor of the atom to which they are bonded.] Table II gives a summary of the number of reflections used in the refinement for all the crystal structures, the parameters adjusted, the final conventional R values, the "goodness of fit" $[\Sigma\omega(F_o^2 - F_c^2)^2/m - s]^{1/2}$, and the total number of least-squares cycles necessary to achieve convergence. Calculations were carried out using an IBM 370/158 computer under the CRYM system.[31] Atomic form factors for C, O, and N were from the International Tables for X-Ray

Table I. Crystal Data

Compound	Mol formula	Mol wt, amu	Crystal system	a, Å	b, Å	c, Å	β, deg	Space group	Z, molecule/unit cell	d_{expl}, g/cm^3	d_{calcd}, g/cm^3
Cyclo(*N*-Me-L-Ala)$_2$	$C_8H_{14}N_2O_2$	170.21	Orthorhombic	10.516 (3)	8.899 (1)	10.073 (4)		$P2_12_12_1$	4	1.20	1.199
rac-Cyclo(*N*-Me-Ala)$_2$	$C_8H_{14}N_2O_2$	170.21	Monoclinic	10.314 (2)	9.037 (1)	10.988 (2)	118.10 (5)	$P2_1/n$	4	1.25	1.251
Cyclo(*N*-Me-L-Val)$_2$	$C_{12}H_{22}N_2O_2$	226.32	Monoclinic	11.148 (2)	9.118 (1)	6.482 (1)	91.86 (5)	$P2_1$	2	1.14	1.142
Cyclo(*N*-Me-L-Val-*N*-Me-D-Val)	$C_{12}H_{22}N_2O_2$	226.32	Monoclinic	8.900 (1)	7.440 (1)	11.953 (2)	124.68 (5)	$P2_1/c$	2	1.15	1.154
Cyclo(*N*-Me-L-Phe)$_2$	$C_{20}H_{22}N_2O_2$	322.41	Orthorhombic	24.445 (7)	8.566 (2)	8.226 (2)		$P2_12_12_1$	4	1.24	1.243
Cyclo(*N*-Me-L-Phe-*N*-Me-D-Phe)	$C_{20}H_{22}N_2O_2$	322.41	Monoclinic	6.134 (1)	16.215 (2)	9.000 (2)	102.76 (5)	$P2_1/c$	2	1.22	1.226

Table II. Summary of the Refinements

Compound	Measd reflections	Zero-weight reflections	Refined parameters	Final conventional R	Goodness of fit	Total least-squares cycles
Cyclo(*N*-Me-L-Ala)$_2$	944	13	165	0.066	3.6	8
rac-Cyclo(*N*-Me-Ala)$_2$	1370	119	165	0.074	2.7	6
Cyclo(*N*-Me-L-Val)$_2$	1102	134	233	0.088	1.5	6
Cyclo(*N*-Me-L-Val-*N*-Me-D-Val)	1109	74	118	0.050	2.1	5
Cyclo(*N*-Me-L-Phe)$_2$	1708	46	306	0.049	2.2	7
Cyclo(*N*-Me-L-Phe-*N*-Me-D-Phe)	1622	83	154	0.054	3.5	6

Table III. Summary of Structure Determinations

Compound	No. of E's used	Min value of E	E's in starting set	Σ_1	Total sets of phases	Atoms located in E map	R value at start of refinement
Cyclo(*N*-Me-L-Ala)$_2$	135	1.30	3	0	32	12	0.38
rac-Cyclo(*N*-Me-AIa)$_2$	130	1.65	4	2	16	12	0.32
Cyclo(*N*-Me-L-Val)$_2$	150	1.50	3	0	32	14	0.37
Cyclo(*N*-Me-L-Val-*N*-Me-D-Val)	100	1.70	4	0	16	8	0.32
Cyclo(*N*-Me-L-Phe)$_2$	230	1.41	3	0	32	24	0.25
Cyclo(*N*-Me-L-Phe-*N*-Me-D-Phe)	130	1.88	4	2	16	12	0.24

Crystallography[32] and those for H from Stewart et al.[33] The final parameters, fractional coordinates, and temperature factors are given in Table IV. The standard deviations in the parameters were calculated from the diagonal elements of the inverse matrices (see paragraph at end of paper regarding supplementary material).

Results and Discussion

The complete molecular geometries for the following six compounds studied are reported in Figure 1: (A) cyclo(*N*-Me-L-Ala)$_2$, (B) *rac*-cyclo(*N*-Me-Ala)$_2$,[40] (C) cyclo(*N*-Me-L-Val)$_2$, (D) cyclo(*N*-Me-L-Val-*N*-Me-D-Val), (E) cyclo(*N*-Me-L-Phe)$_2$, (F) cyclo(*N*-Me-L-Phe-*N*-Me-D-Phe). The conformations of these molecules (Figure 2) are shown in the stereodiagrams prepared from the observed coordinates of the atoms by the computer program ORTEP. In the solid state, none of the LL isomer molecules maintain the C_2 symmetry as a crystallographic symmetry. The only molecules which can somehow be described as approaching the C_2 symmetry are cyclo(*N*-Me-L-Ala)$_2$ and *rac*-cyclo(*N*-Me-Ala)$_2$. Both LD isomers of *N*-methylvaline, cyclo(*N*-Me-L-Val-*N*-Me-D-Val), and *N*-methylphenylalanine, cyclo(*N*-Me-L-Phe-*N*-Me-D-Phe), retain centers of inversion as their crystallographic symmetry in the solid state, so that the asymmetric unit for these molecules consists of only half the molecule.

As expected bond lengths do not differ appreciably in this series. However, some differences do occur with respect to the values of the recommended standard peptide unit, calculated by Ramachandran[34] using potential energy functions which also take into account the possible variation of bond angles and internal rotation angles about the peptide bond. Larger differences are noted for bond angles. In particular, for cyclo(*N*-Me-L-Val)$_2$ the folding of the diketopiperazine ring occurs to an unusual extent with a resulting significant distortion of some bond angles. As we have pointed out recently,[18] small bond angle deformations coupled with large changes in the internal rotation angles may provide easy kinetic paths for conformational changes as well as relieving intramolecular interactions which are otherwise too severe.

We are aware of only two previously published crystal structure determinations of N-methylated diketopiperazines: cyclo(Sar)$_2$,[27a] the cyclic dipeptide of *N*-methylglycine, and

Table IV. Final Atomic Parameters and Their Standard Deviations (in Parentheses)[a]

A. Cyclo(*N*-Me-L-Ala)$_2$

	X	*Y*	*Z*	*B*(11)	*B*(22)	*B*(33)	*B*(12)	*B*(13)	*B*(23)
O_1	−1 935 (25)	−9 845 (32)	49 572 (41)	421 (14)	549 (15)	1247 (30)	−48 (12)	56 (15)	81 (18)
N_1	23 242 (29)	16 089 (33)	42 008 (39)	474 (16)	354 (14)	822 (22)	−59 (13)	8 (17)	−11 (16)
C_1'	8 742 (32)	−5 338 (38)	46 455 (44)	378 (16)	418 (16)	692 (22)	−21 (14)	−24 (16)	−4 (18)
C_1^α	10 168 (34)	10 453 (40)	41 431 (47)	416 (17)	413 (17)	729 (23)	27 (15)	−17 (17)	13 (19)
C_1^β	4 302 (60)	11 985 (59)	27 713 (61)	824 (31)	691 (29)	855 (34)	−10 (28)	−245 (26)	176 (28)
C_{N_1}	24 698 (57)	32 396 (46)	42 513 (78)	750 (29)	363 (18)	1309 (49)	−72 (21)	−28 (38)	10 (25)
O_2	44 361 (27)	12 031 (35)	42 254 (50)	444 (15)	644 (18)	1501 (34)	−138 (14)	59 (19)	−63 (24)
N_2	19 056 (26)	−13 953 (29)	47 362 (35)	416 (14)	356 (14)	713 (19)	−10 (12)	−15 (14)	31 (14)
C_2'	33 441 (34)	7 212 (43)	42 112 (48)	429 (18)	507 (20)	810 (25)	−50 (16)	0 (19)	−27 (20)
C_2^α	31 384 (33)	−9 642 (38)	41 669 (44)	402 (16)	444 (18)	674 (21)	8 (15)	−6 (17)	−2 (19)
C_2^β	33 261 (59)	−15 647 (61)	27 698 (56)	829 (32)	696 (27)	774 (31)	115 (31)	82 (26)	−135 (26)
C_{N_2}	17 771 (51)	−29 198 (44)	52 489 (74)	593 (24)	422 (19)	1216 (43)	−18 (18)	−42 (28)	181 (26)

	X	*Y*	*Z*	*B*		*X*	*Y*	*Z*	*B*
HC_1^α	563 (44)	1673 (47)	4754 (47)	4.89	HC_2^α	3813 (44)	−1334 (49)	4815 (45)	4.64
$H_1C_1^\beta$	334 (45)	2348 (61)	2619 (55)	6.56	$H_1C_2^\beta$	3382 (45)	−2659 (65)	2898 (54)	5.94
$H_2C_1^\beta$	832 (58)	577 (61)	2229 (61)	6.56	$H_2C_2^\beta$	2654 (48)	−1173 (59)	2261 (50)	5.94
$H_3C_1^\beta$	−522 (52)	908 (57)	2953 (54)	6.56	$H_3C_2^\beta$	4192 (54)	−1319 (55)	2583 (49)	5.94
$H_1C_{N_1}$	3275 (60)	3415 (56)	4232 (66)	7.06	$H_1C_{N_2}$	1178 (53)	−3114 (59)	5817 (56)	6.63
$H_2C_{N_1}$	2061 (56)	3760 (74)	3715 (62)	7.06	$H_2C_{N_2}$	1974 (55)	−3487 (61)	4403 (65)	6.63
$H_3C_{N_1}$	1882 (56)	3642 (64)	5114 (64)	7.06	$H_3C_{N_2}$	2523 (52)	−3347 (61)	5619 (56)	6.63

B. *rac*-Cyclo(*N*-Me-Ala)$_2$

	X	*Y*	*Z*	*B*(11)	*B*(22)	*B*(33)	*B*(12)	*B*(13)	*B*(23)
O_1	63 822 (24)	112 216 (30)	48 657 (22)	732 (15)	667 (19)	483 (14)	−109 (14)	−103 (12)	77 (12)
N_1	46 282 (28)	86 615 (33)	76 427 (24)	631 (16)	359 (20)	502 (15)	−3 (14)	−134 (13)	13 (12)
C_1'	56 470 (32)	107 870 (40)	60 525 (31)	537 (18)	528 (26)	471 (17)	−73 (17)	−145 (15)	−12 (15)
C_1^α	58 936 (35)	92 603 (39)	64 333 (31)	603 (19)	452 (23)	455 (17)	4 (16)	−102 (15)	−22 (14)
C_1^β	72 507 (44)	92 376 (55)	66 101 (42)	645 (23)	708 (33)	724 (26)	77 (21)	−196 (19)	−6 (20)
C_{N_1}	45 233 (58)	70 610 (57)	77 538 (48)	951 (33)	551 (36)	696 (29)	−25 (26)	−173 (26)	8 (23)
O_2	27 001 (25)	90 060 (29)	97 477 (22)	673 (15)	637 (19)	541 (14)	−76 (14)	−91 (12)	69 (12)
N_2	46 631 (28)	116 427 (33)	70 519 (24)	637 (16)	408 (21)	483 (15)	21 (13)	−170 (13)	17 (12)
C_2'	36 734 (32)	95 092 (39)	86 654 (30)	513 (18)	559 (24)	445 (17)	−9 (17)	−156 (15)	5 (15)
C_2^α	37 918 (35)	111 633 (40)	84 819 (28)	510 (17)	502 (23)	436 (16)	1 (16)	−149 (14)	−6 (14)
C_2^β	43 346 (51)	118 438 (51)	94 144 (40)	875 (28)	583 (26)	629 (22)	−32 (22)	−347 (22)	−74 (18)
C_{N_2}	44 892 (49)	131 838 (47)	67 460 (42)	822 (28)	477 (24)	724 (25)	64 (21)	−260 (22)	83 (18)

	X	*Y*	*Z*	*B*		*X*	*Y*	*Z*	*B*
HC_1^α	5957 (30)	8 558 (38)	5 614 (28)	4.52	HC_2^α	2835 (32)	11 441 (35)	8 767 (27)	4.10
$H_1C_1^\beta$	8129 (36)	9 534 (42)	5 734 (33)	6.05	$H_1C_2^\beta$	3717 (36)	11 630 (41)	10 263 (35)	6.09
$H_2C_1^\beta$	7501 (35)	8 322 (46)	6 858 (31)	6.05	$H_2C_2^\beta$	4382 (36)	12 915 (47)	9 379 (30)	6.09
$H_3C_1^\beta$	7122 (36)	9 846 (46)	7 383 (34)	6.05	$H_3C_2^\beta$	5315 (37)	11 590 (41)	9 219 (31)	6.09
$H_1C_{N_1}$	5166 (40)	6 696 (49)	6 960 (36)	7.57	$H_1C_{N_2}$	3541 (37)	13 512 (44)	7 483 (33)	6.10
$H_2C_{N_1}$	3577 (46)	6 784 (50)	8 145 (37)	7.57	$H_2C_{N_2}$	4369 (37)	13 163 (41)	5 948 (32)	6.10
$H_3C_{N_1}$	4639 (40)	6 804 (50)	8 547 (40)	7.57	$H_3C_{N_2}$	5325 (36)	13 614 (46)	6 411 (34)	6.10

C. Cyclo(*N*-Me-L-Val)$_2$

	X	*Y*	*Z*	*B*(11)	*B*(22)	*B*(33)	*B*(12)	*B*(13)	*B*(23)
O_1	46 006 (34)	49 450 (0)	41 468 (71)	407 (25)	611 (34)	1339 (42)	−140 (24)	−196 (24)	−122 (29)
N_1	22 695 (41)	23 389 (70)	45 340 (74)	480 (31)	433 (33)	699 (35)	−78 (26)	62 (25)	124 (29)
C_1'	36 051 (53)	44 002 (89)	37 765 (90)	482 (41)	485 (43)	596 (44)	4 (36)	23 (29)	−42 (36)
C_1^α	34 372 (56)	27 658 (86)	36 939 (99)	415 (35)	503 (46)	502 (41)	106 (31)	−45 (29)	118 (34)
C_1^β	37 165 (54)	21 053 (93)	16 208 (104)	553 (39)	648 (50)	555 (45)	84 (36)	3 (34)	159 (39)
C_{N_1}	21 606 (81)	10 460 (111)	58 167 (152)	757 (58)	661 (56)	1087 (78)	−22 (53)	107 (54)	291 (60)
$C_1^{\gamma 1}$	34 049 (77)	4 725 (100)	14 792 (139)	703 (52)	611 (55)	742 (59)	77 (41)	−23 (47)	−179 (47)
$C_1^{\gamma 2}$	50 039 (91)	22 865 (141)	10 790 (241)	790 (72)	840 (77)	1619 (121)	−60 (64)	559 (75)	−109 (84)
O_2	2 800 (33)	28 153 (71)	47 727 (72)	474 (26)	782 (33)	1009 (33)	−125 (25)	294 (23)	43 (30)
N_2	26 142 (40)	52 190 (76)	34 079 (74)	408 (30)	358 (34)	763 (38)	−63 (25)	−14 (25)	−27 (28)
C_2'	12 665 (55)	31 577 (88)	41 165 (90)	579 (43)	489 (45)	644 (43)	−77 (35)	145 (34)	−109 (36)
C_2^α	14 226 (48)	45 582 (86)	29 021 (94)	349 (30)	522 (45)	581 (44)	−52 (31)	29 (28)	−30 (37)
C_2^β	11 459 (50)	43 021 (97)	5 966 (97)	427 (34)	607 (45)	586 (44)	−20 (35)	−63 (29)	18 (39)
C_{N_2}	26 639 (102)	68 037 (104)	35 713 (179)	675 (68)	462 (52)	1076 (81)	−27 (46)	−165 (61)	−169 (46)
$C_2^{\gamma 1}$	18 218 (118)	52 754 (151)	−8 198 (150)	1094 (88)	1276 (96)	671 (61)	139 (88)	−1 (61)	−38 (68)
$C_2^{\gamma 2}$	−2 032 (75)	43 313 (190)	1 038 (177)	619 (49)	1498 (118)	775 (69)	47 (67)	−150 (45)	−162 (87)

Table IV (*Continued*)

	X	*Y*	*Z*	*B*		*X*	*Y*	*Z*	*B*
HC_1^{α}	3922 (33)	2405 (44)	4440 (57)	1.11 (0.82)	HC_2^{α}	895 (52)	5141 (77)	3755 (85)	6.97 (1.70)
HC_1^{β}	3203 (43)	2418 (57)	388 (76)	3.88 (1.27)	HC_2^{β}	1247 (48)	3135 (76)	293 (82)	6.37 (1.56)
$H_1C_{N_1}$	2873 (67)	523 (96)	6334 (107)	9.78 (2.40)	$H_1C_{N_2}$	2006 (72)	7254 (87)	3339 (109)	6.52 (2.37)
$H_2C_{N_1}$	1622 (58)	446 (80)	5186 (96)	6.55 (2.08)	$H_2C_{N_2}$	3003 (68)	7301 (94)	1748 (130)	12.72 (2.36)
$H_3C_{N_1}$	1804 (54)	1363 (74)	7394 (98)	6.63 (1.80)	$H_3C_{N_2}$	3204 (69)	7039 (88)	4202 (115)	1.05 (2.55)
$H_1C_1^{\gamma 1}$	3635 (57)	52 (93)	127 (100)	7.38 (1.89)	$H_1C_2^{\gamma 1}$	1405 (51)	5004 (70)	−2257 (95)	5.91 (1.52)
$H_2C_1^{\gamma 1}$	2620 (67)	176 (92)	2139 (103)	8.84 (2.24)	$H_2C_2^{\gamma 1}$	2487 (88)	4947 (156)	−1183 (147)	10.11 (4.30)
$H_3C_1^{\gamma 1}$	3924 (53)	36 (77)	2646 (98)	6.39 (1.78)	$H_3C_2^{\gamma 1}$	1334 (66)	6179 (90)	−453 (120)	7.51 (2.31)
$H_1C_1^{\gamma 2}$	5240 (61)	1951 (80)	−581 (123)	7.89 (1.85)	$H_1C_2^{\gamma 2}$	−219 (70)	3939 (94)	−1485 (120)	10.14 (2.49)
$H_2C_1^{\gamma 2}$	5556 (53)	1807 (69)	2026 (81)	4.25 (1.81)	$H_2C_2^{\gamma 2}$	−262 (86)	5382 (119)	252 (154)	10.39 (3.93)
$H_3C_1^{\gamma 2}$	5273 (60)	3034 (81)	1219 (103)	5.28 (1.94)	$H_3C_2^{\gamma 2}$	−587 (56)	4035 (73)	1294 (97)	5.26 (1.88)

D. Cyclo(*N*-Me-L-Val-*N*-Me-D-Val)

	X	*Y*	*Z*	*B*(11)	*B*(22)	*B*(33)	*B*(12)	*B*(13)	*B*(23)
O_1	8 107 (17)	71 606 (17)	66 104 (13)	580 (8)	491 (8)	573 (8)	157 (7)	392 (7)	209 (7)
N_1	8 532 (18)	84 786 (19)	49 368 (14)	441 (8)	322 (8)	451 (8)	37 (7)	306 (7)	23 (7)
C_1'	3 980 (22)	84 391 (24)	58 320 (17)	363 (9)	399 (10)	373 (9)	8 (8)	214 (8)	31 (8)
C_1^{α}	7 774 (23)	100 780 (24)	42 010 (18)	408 (10)	378 (10)	339 (9)	10 (8)	238 (8)	20 (8)
C_1^{β}	27 376 (24)	107 732 (28)	47 872 (19)	401 (10)	435 (11)	494 (11)	40 (8)	286 (9)	57 (9)
C_{N_1}	17 513 (32)	68 857 (28)	48 556 (25)	576 (13)	414 (12)	638 (14)	93 (10)	416 (12)	21 (10)
$C_1^{\gamma 1}$	36 512 (35)	115 217 (46)	62 126 (26)	500 (13)	914 (20)	588 (14)	−172 (14)	240 (11)	−96 (15)
$C_1^{\gamma 2}$	27 890 (36)	120 934 (39)	38 429 (31)	560 (14)	699 (17)	866 (18)	27 (13)	499 (14)	226 (15)

	X	*Y*	*Z*	*B*		*X*	*Y*	*Z*	*B*
HC_1^{α}	255 (22)	9 706 (24)	3277 (18)	3.08 (0.37)	$H_2C_1^{\gamma 1}$	3590 (32)	10 534 (35)	6806 (24)	7.12 (0.64)
HC_1^{β}	3352 (23)	9 670 (26)	4821 (18)	3.39 (0.40)	$H_3C_1^{\gamma 1}$	3024 (38)	12 737 (40)	6180 (27)	9.33 (0.78)
$H_1C_{N_1}$	1806 (30)	6 945 (31)	4058 (24)	6.03 (0.56)	$H_1C_1^{\gamma 2}$	4021 (34)	12 833 (32)	4216 (22)	6.75 (0.58)
$H_2C_{N_1}$	988 (29)	5 682 (31)	4701 (21)	6.18 (0.53)	$H_2C_1^{\gamma 2}$	2189 (35)	13 334 (35)	3783 (24)	6.93 (0.62)
$H_3C_{N_1}$	2950 (35)	6 696 (32)	5640 (25)	6.32 (0.59)	$H_3C_1^{\gamma 2}$	2234 (38)	11 458 (36)	2937 (31)	9.65 (0.79)
$H_1C_1^{\gamma 1}$	4862 (39)	11 755 (35)	6557 (26)	8.00 (0.68)					

E. Cyclo(*N*-Me-L-Phe)$_2$

	X	*Y*	*Z*	*B*(11)	*B*(22)	*B*(33)	*B*(12)	*B*(13)	*B*(23)
O_1	98 226 (9)	53 931 (34)	−32 922 (38)	544 (14)	980 (20)	1451 (29)	49 (15)	194 (16)	−143 (24)
N_1	84 022 (10)	44 162 (32)	−30 787 (35)	596 (18)	514 (15)	617 (18)	−73 (13)	13 (15)	−50 (16)
C_1'	93 384 (13)	54 728 (43)	−28 904 (49)	551 (19)	569 (20)	897 (28)	7 (17)	59 (20)	−212 (23)
C_1^{α}	89 234 (13)	48 025 (38)	−38 805 (42)	678 (20)	456 (17)	620 (22)	68 (17)	119 (18)	−54 (18)
C_1^{β}	88 564 (16)	53 482 (43)	−56 051 (46)	982 (25)	578 (21)	604 (22)	120 (20)	149 (21)	−136 (20)
C_{N_1}	80 401 (15)	32 059 (43)	−37 151 (49)	868 (23)	679 (22)	795 (26)	−242 (20)	−127 (21)	−64 (22)
C_1^{γ}	89 773 (13)	42 870 (37)	−70 031 (41)	647 (19)	469 (17)	575 (20)	72 (16)	113 (17)	−141 (18)
$C_1^{\delta 1}$	95 108 (13)	38 436 (45)	−73 270 (45)	557 (19)	753 (24)	767 (24)	97 (17)	67 (19)	−53 (22)
$C_1^{\epsilon 1}$	96 289 (17)	28 880 (53)	−86 361 (59)	848 (27)	846 (29)	980 (32)	241 (24)	304 (26)	−101 (29)
C_1^{ζ}	92 169 (21)	23 664 (50)	−96 180 (54)	1172 (34)	702 (26)	811 (30)	75 (27)	278 (30)	−48 (26)
$C_1^{\epsilon 2}$	86 952 (18)	27 585 (50)	−93 088 (50)	1048 (30)	711 (26)	627 (23)	−154 (24)	−58 (24)	−99 (24)
O_2	77 442 (9)	52 941 (34)	−14 031 (34)	584 (13)	1065 (20)	987 (20)	65 (15)	196 (15)	−69 (20)
N_2	91 742 (11)	62 834 (33)	−15 867 (37)	662 (17)	528 (16)	690 (19)	−64 (14)	−111 (16)	−76 (16)
C_2'	82 158 (13)	53 635 (40)	−19 296 (44)	596 (19)	571 (19)	636 (22)	33 (17)	24 (18)	−119 (21)
C_2^{α}	86 023 (14)	65 178 (38)	−11 750 (42)	727 (20)	556 (19)	560 (20)	42 (18)	24 (18)	−27 (19)
C_2^{β}	84 085 (15)	82 175 (42)	−14 453 (46)	941 (25)	564 (19)	654 (23)	77 (20)	77 (21)	79 (21)
C_{N_2}	95 838 (16)	69 940 (50)	−5 436 (58)	999 (27)	844 (27)	1053 (32)	−253 (23)	−443 (26)	−137 (27)
C_2^{γ}	84 228 (14)	87 200 (36)	−31 929 (46)	688 (21)	407 (16)	710 (24)	67 (16)	−47 (20)	−22 (18)
$C_2^{\delta 1}$	88 863 (15)	93 457 (42)	−38 780 (49)	745 (22)	505 (19)	788 (27)	−8 (18)	−133 (21)	−74 (20)
$C_2^{\epsilon 1}$	88 942 (18)	97 866 (45)	−54 850 (60)	1017 (30)	543 (23)	995 (33)	−107 (23)	120 (28)	−134 (25)
C_2^{ζ}	84 319 (24)	96 143 (48)	−64 144 (54)	1587 (45)	558 (22)	778 (28)	−19 (30)	−236 (33)	−116 (24)
$C_2^{\epsilon 2}$	79 598 (20)	90 162 (48)	−57 819 (63)	1159 (33)	636 (25)	1085 (38)	−34 (24)	−411 (31)	−76 (28)
$C_2^{\delta 2}$	79 611 (15)	85 588 (45)	−41 258 (60)	739 (23)	587 (22)	1107 (35)	24 (19)	−86 (24)	−102 (25)

	X	*Y*	*Z*	*B*		*X*	*Y*	*Z*	*B*
HC_1^{α}	9 065 (10)	3 555 (36)	−4 108 (35)	5.30 (0.71)	HC_2^{α}	8 575 (11)	6 339 (39)	18 (38)	6.94 (0.83)
$H_1C_1^{\beta}$	9 114 (13)	6 207 (41)	−5 684 (43)	8.08 (0.89	$H_1C_2^{\beta}$	8 043 (13)	8 311 (41)	−913 (43)	8.08 (0.89)
$H_2C_1^{\beta}$	8 451 (12)	5 753 (41)	−5 776 (43)	7.98 (0.89)	$H_2C_2^{\beta}$	8 665 (12)	8 846 (37)	−800 (38)	6.01 (0.80)
$H_1C_{N_1}$	9 435 (14)	7 634 (52)	396 (48)	10.34 (1.05)	$H_1C_{N_2}$	8 208 (15)	2 613 (51)	−4 458 (47)	10.47 (1.05)
$H_2C_{N_1}$	9 844 (14)	6 237 (48)	−186 (50)	10.40 (1.05)	$H_2C_{N_2}$	7 877 (16)	2 654 (56)	−2 772 (56)	14.64 (1.24)
$H_3C_{N_1}$	9 806 (15)	7 797 (54)	−1 275 (52)	11.88 (1.15)	$H_3C_{N_2}$	7 710 (15)	3 611 (49)	−4 254 (51)	11.54 (1.12)
$HC_1^{\delta 1}$	9 813 (11)	4 297 (38)	−6 695 (39)	7.54 (0.84)	$HC_2^{\delta 1}$	9 222 (12)	9 458 (40)	−3 177 (38)	6.74 (0.81)
$HC_1^{\epsilon 1}$	10 011 (13)	2 661 (48)	−8 957 (45)	8.60 (0.96)	$HC_2^{\epsilon 1}$	9 224 (13)	10 162 (42)	−5 923 (42)	6.57 (0.89)
HC_1^{ζ}	9 298 (13)	1 618 (47)	−10 522 (45)	9.94 (1.01)	HC_2^{ζ}	8 430 (13)	9 878 (46)	−7 638 (45)	8.93 (1.02)
$HC_1^{\epsilon 2}$	8 381 (12)	2 318 (43)	−10 009 (42)	7.05 (0.86)	$HC_2^{\epsilon 2}$	7 647 (15)	8 883 (49)	−6 407 (52)	11.57 (1.19)
$HC_1^{\delta 2}$	8 185 (12)	4 079 (37)	−7 762 (40)	7.64 (0.85)	$HC_2^{\delta 2}$	7 622 (13)	8 148 (43)	−3 616 (45)	8.40 (0.97)

Table IV (*Continued*)

	F. Cyclo(*N*-Me-L-Phe-*N*-Me-D-Phe)								
	X	*Y*	*Z*	*B*(11)	*B*(22)	*B*(33)	*B*(12)	*B*(13)	*B*(23)
O_1	75 619 (21)	93 752 (9)	−67 576 (14)	523 (8)	844 (9)	752 (9)	48 (6)	237 (7)	−182 (7)
N_1	42 712 (23)	93 833 (9)	−60 616 (15)	448 (9)	580 (9)	592 (9)	−34 (7)	144 (7)	−134 (7)
C_1'	63 393 (29)	96 565 (10)	−59 700 (19)	440 (10)	594 (10)	561 (9)	40 (8)	152 (8)	−39 (8)
C_1^α	28 626 (31)	96 118 (11)	−50 250 (21)	405 (10)	630 (11)	637 (10)	−12 (8)	138 (9)	−75 (8)
C_1^β	25 670 (36)	88 879 (14)	−39 805 (27)	573 (12)	717 (13)	897 (14)	−108 (10)	307 (11)	−38 (11)
C_{N_1}	33 566 (47)	87 795 (19)	−72 376 (33)	695 (16)	934 (19)	857 (16)	−174 (14)	166 (13)	−390 (14)
C_1^γ	47 097 (34)	86 496 (11)	−29 009 (21)	711 (13)	539 (10)	735 (12)	−33 (9)	330 (11)	83 (9)
$C_1^{\delta 1}$	53 152 (50)	89 988 (15)	−14 743 (25)	1104 (20)	816 (15)	682 (13)	257 (14)	335 (14)	78 (11)
$C_1^{\epsilon 1}$	73 202 (57)	88 135 (16)	−5 109 (30)	1344 (25)	968 (18)	631 (13)	294 (17)	168 (16)	58 (13)
C_1^ζ	87 773 (53)	82 736 (16)	−9 590 (28)	1031 (20)	883 (16)	737 (14)	210 (15)	198 (14)	186 (12)
$C_1^{\epsilon 2}$	81 880 (48)	79 143 (15)	−23 514 (28)	987 (19)	736 (14)	865 (16)	234 (13)	332 (15)	126 (12)
$C_1^{\delta 2}$	61 783 (40)	80 984 (12)	−33 246 (27)	865 (16)	589 (12)	796 (14)	47 (11)	263 (12)	1 (10)

	X	*Y*	*Z*	*B*		*X*	*Y*	*Z*	*B*
HC_1^α	1 439 (39)	9762 (11)	−5708 (22)	5.70 (0.45)	$HC_1^{\delta 1}$	4 252 (43)	9372 (16)	−1153 (25)	8.12 (0.60)
$H_1C_1^\beta$	1 463 (41)	9050 (12)	−3406 (23)	6.65 (0.50)	$HC_1^{\epsilon 1}$	7 734 (51)	9048 (16)	516 (34)	10.11 (0.69)
$H_2C_1^\beta$	1 945 (40)	8431 (15)	−4737 (26)	7.38 (0.54)	HC_1^ζ	10 234 (50)	8145 (16)	−239 (31)	9.68 (0.69)
$H_1C_{N_1}$	1 659 (57)	8762 (16)	−7417 (30)	9.97 (0.69)	$HC_1^{\epsilon 2}$	9 157 (45)	7555 (16)	−2669 (27)	8.63 (0.63)
$H_2C_{N_1}$	3 828 (55)	8216 (21)	−6918 (34)	12.21 (0.90)	$HC_1^{\delta 2}$	5 755 (37)	7842 (13)	−4378 (25)	6.49 (0.49)
$H_3C_{N_1}$	3 833 (48)	8883 (16)	−8160 (34)	9.58 (0.70)					

[a] The atomic coordinates of the heavy atoms have been multiplied by 10^5 and those for the hydrogen atoms by 10^4. The anisotropic temperature factor is expressed in the form $\exp[-\frac{1}{4}[h^2a^{*2}B(11) + \ldots + 2klb^*c^*B(23)]]$; values of B_{ij} are multiplied by 10^2.

Table V. Relevant Conformation Parameters

	A. Diketopiperazine Rings			
	ω	ψ	ϕ	β
Cyclo(*N*-Me-L-Ala)$_2$	0, −10	−18, −27	24, 33	−25
rac-Cyclo(*N*-Me-Ala)$_2$	1, −8	−17, −26	20, 29	−12
Cyclo(*N*-Me-L-Val)$_2$	−1, −4	−33, −36	36, 40	−41
Cyclo(*N*-Me-L-Val-*N*-Me-D-Val)	19	−17	18	
Cyclo(*N*-Me-L-Phe)$_2$	−7, −12	−11, −15	21, 25	−19
Cyclo(*N*-Me-L-Phe-*N*-Me-D-Phe)	14	−13	14	

	B. Side Chains[a]							
	$\chi_1^{2,1}$	$\chi_1^{2,2}$	$\chi_2^{2,1}$	$\chi_2^{2,2}$	$\chi_1^{3,1}$	$\chi_1^{3,2}$	$\chi_2^{3,1}$	$\chi_2^{3,2}$
Cyclo(*N*-Me-L-Val)$_2$	−44	193	−24	207				
Cyclo(*N*-Me-L-Val-*N*-Me-D-Val)	−68	164						
Cyclo(*N*-Me-L-Phe)$_2$	111		−64		71	−108	87	−93
Cyclo(*N*-Me-L-Phe-*N*-Me-D-Phe)	65		−65		90	−86		

[a] Torsion angles in the side chains are denoted by χ according to the recommendation of the IUPAC–IUB Commission on Biochemical Nomenclature. The notation $\chi_i^{j,k}$ specifies the torsion angle for the side chain in residue i where j indicates the bond about which the angle is measured ($j = 2$ for rotation about the C^α–C^β bond, $j = 3$ for rotation about the C^β–C^γ bond, etc.) and k indicates that the angle is measured relative to atom k of the branched chain. (In valine, for example, the torsion angles are $\chi_i^{2,1}$ and $\chi_i^{2,2}$.)

cyclo(Sar-L-Val),[27b] the cyclic dipeptide of *N*-methylglycine and L-valine. The diketopiperazine ring of cyclo(Sar)$_2$ is essentially planar, with perhaps some slight distortion toward the chair conformation. In cyclo(Sar-L-Val), the N-methylated peptide group is essentially planar but the unmethylated peptide group is appreciably nonplanar, leading to a twist-boat conformation with the valyl side chain in the flagpole position; the methyl groups of this side chain are apparently disordered. The conformations assumed by the N-methylated diketopiperazines of alanine, valine, and phenylalanine in the solid state are best described by the ω, ϕ, and ψ values reported in Table V.

In both the racemate and the optically active forms of N-methylated alanine diketopiperazines, the diketopiperazine ring is folded in the boat conformation with the methyl substituents on the C_α carbon atoms in the axial position directed toward each other across the ring (cf. Figure 2). The conformational parameters ψ and ϕ are $\psi_1 = -26°$, $\phi_1 = 29°$, $\psi_2 = -17°$, $\phi_2 = 20°$ for the racemate and $\psi_1 = -18°$, $\phi_1 = 24°$, $\psi_2 = -27°$, $\phi_2 = 33°$ for the optically active isomer (see Table V). In both structures, one of the two amide groups deviates from planarity, exhibiting values of −10 and −8° for the LL isomer and the racemate, respectively. The other amide group in both solid-state conformations is essentially planar.

The dihedral angle between the two amide planes is a direct measure of the folding of the diketopiperazine ring, exhibiting values of 155 and 168° for the optically active and the racemate crystals, respectively (β values in the Hooker notation[35] −25 and −12°). This result shows that the molecule must be considered rather flexible and that crystal packing forces are effective in fixing the conformation. Similar results were found in the case of the unmethylated cyclic dimer of alanine[19-21]

having both residues of the same configuration, for which energy calculations have shown a considerable conformational flexibility.[36] Upon methylation of the nitrogen atom, we believe that a similar pattern is maintained with a flat minimum in the conformation energy map which is unsymmetrical with respect to the planar conformation and shifted toward a folded form of the ring with negative values of β in the Hooker notation.[35]

The LL isomer of N-methylated valine diketopiperazine in the solid state exists with the ring in the boat conformation and the isopropyl groups in the axial positions. The two amide groups are much closer to planarity (the ω values being -1 and $-4°$ for the two residues) than in the case of the N-methylated alanine and phenylalanine diketopiperazine molecules. The values of ϕ and ψ are extremely high for diketopiperazine ring systems ($\phi_1 = 36°, \psi_1 = -33°, \phi_2 = 40°, \psi_2 = -36°$). In absolute terms these conformational parameters are greater than those recently reported by us[37] for the diketopiperazine of L-proline, cyclo(L-Pro)$_2$ ($\phi_1 = -38°, \psi_1 = 37°, \phi_2 = -37°, \psi_2 = 36°$). In the L-proline diketopiperazine ring, the substituents are in the equatorial position while in the case of N-methylated L-valine diketopiperazine they are in the axial position (note the signs of ϕ and ψ are inverted for the two compounds). The dihedral angle between the two amide planes in cyclo(*N*-Me-L-Val)$_2$ is 139° ($\beta = -41°$) while it is 142° ($\beta = 38°$) for cyclo(L-Pro)$_2$. The angle β for cyclo(*N*-Me-L-Val)$_2$ represents the largest distortion from planarity ever found or predicted for such a ring system with or without substitution on the nitrogen atoms. As a matter of fact, even if methylation of the nitrogen atoms does not disturb the planarity of the amide groups in cyclo(*N*-Me-L-Val)$_2$, it certainly affects the overall conformation of the molecule. In particular, the conformation of the isopropyl side chains which face each other across the ring is such that the substituents on the C^α and C^β carbon atoms deviate from a staggered conformation in order to relieve the intramolecular contacts between (a) the two C^β hydrogen atoms and (b) the methyl groups of the isopropyl moieties and either the methyl groups on the nitrogen atoms or the oxygen atoms. These close contacts are energetically most unfavorable.

In the experimentally observed conformation, the relief of these intramolecular interactions is achieved by (a) a slight decrease in the bond angles within the ring system which is more marked at the C^1 and N atoms; (b) an increase of the C^β-C^α-N angle, and (c) slight rotation around the C^α-C^β bonds in such a way that the internal rotation angles, $\chi_1^{2,1}$, $\chi_1^{2,2}$, $\chi_2^{2,1}$, and $\chi_2^{2,2}$, vary with respect to the values of the perfectly staggered conformation (i.e., 60, 180, $-60°$). The values for $\chi_1^{2,1}$, $\chi_2^{2,1}$, $\chi_1^{2,2}$, and $\chi_2^{2,2}$ are -44, -24, 193, and 207°, respectively. The resulting intramolecular contact distances between atoms four or more bonds apart are for $CH_3\cdots CH_3$, $C_1{}^{\gamma 2}\cdots C_{N_1}$ = 3.22 Å, $C_2{}^{\gamma 1}\cdots C_{N_2}$ = 3.28 Å; for $CH_3\cdots O$, $C_1{}^{\gamma 1}\cdots O_1$ = 3.17 Å, $C_1{}^{\gamma 2}\cdots O_2$ = 3.35 Å; and for $H\cdots H$, $HC_1{}^\beta\cdots HC_2{}^\beta$ = 2.27 Å.

Packing forces should have very little effect on this experimentally found conformation since no hydrogen bonds are present and only van der Waals interactions should make appreciable contributions to the packing.

We believe that this molecule should have restricted freedom of motion in the solid state. The diketopiperazine ring should be forced into the boat conformation with axial substituents on the C^α carbon atom, which is probably the only possible form without prohibited intramolecular interactions. The boat conformation with the isopropyl groups in equatorial positions ($\beta > 0$) would result in unacceptable values for the C^γ-methyls$\cdots C_N$-methyls and C^γ-methyls$\cdots$O interactions. In solution, however, even if we expect the same conformation of the ring system and axial position of the isopropyl groups, we cannot rule out possible rotation around the C^α-C^β bonds coupled with slight deformations in bond angles at the C^α and C^β carbon atoms, since the energy barrier to free rotation about C^α-C^β bonds in such an overcrowded molecule should be quite small.

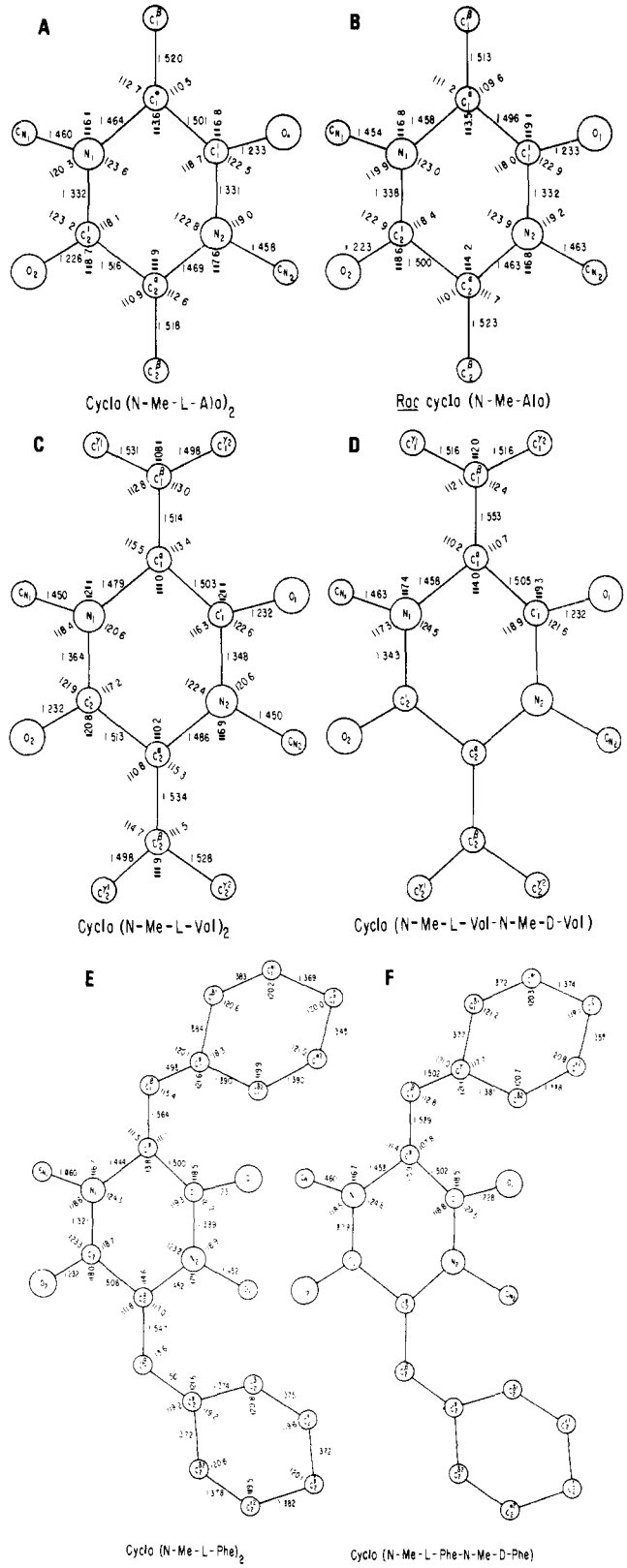

Figure 1. Final molecular geometry of the N-methylated diketopiperazine derived from (A and B) alanine, (C and D) valine, and (E and F) phenylalanine. Bond lengths are in ångströms, and bond angles are reported in degrees. Their average estimated standard deviations are 0.004 Å and 0.2°, respectively.

In contrast to the above structures, cyclo(*N*-Me-L-Val-*N*-Me-D-Val) has a nearly regular chair conformation (cy-

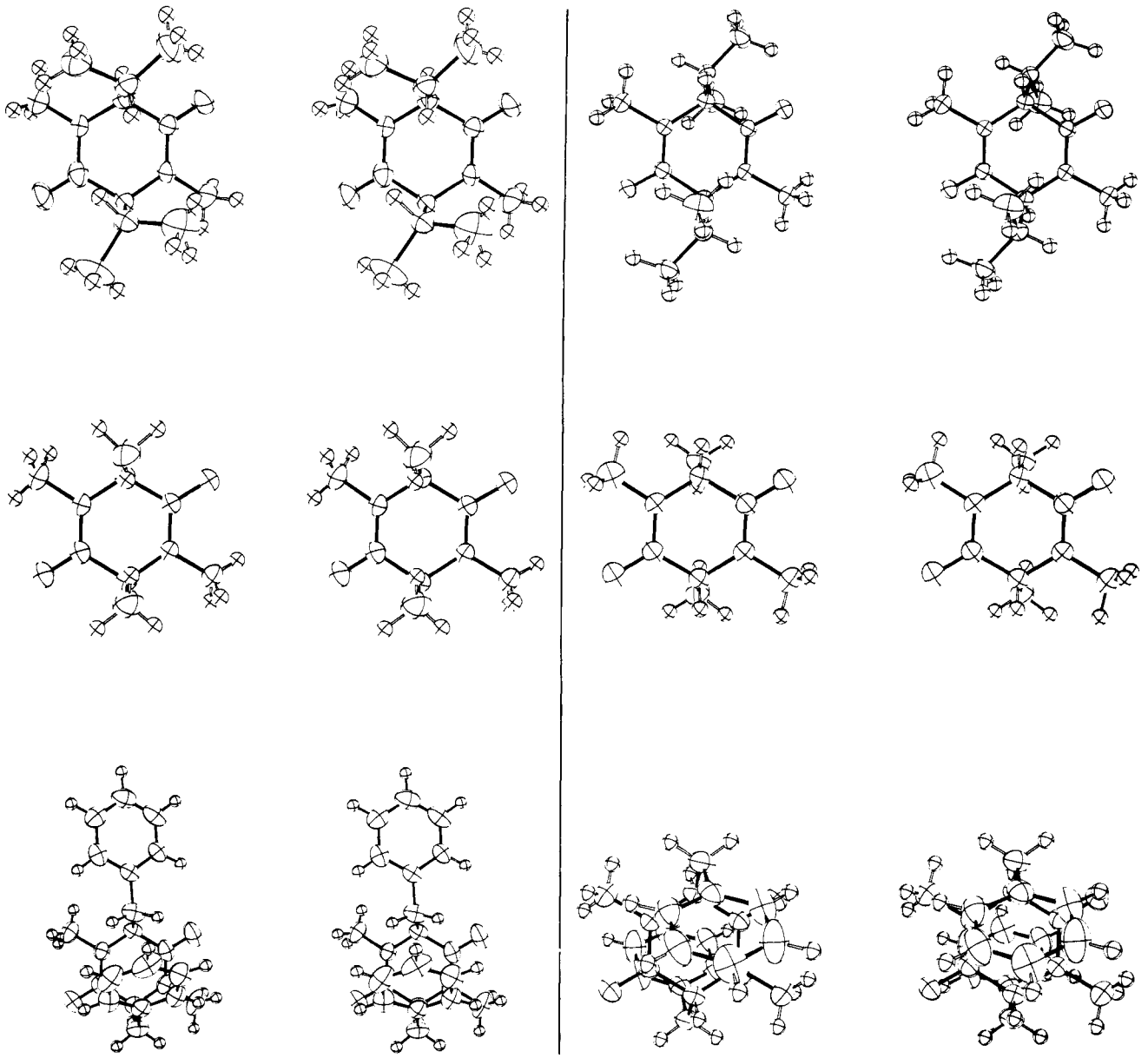

Figure 2. Stereoscopic drawings of the N-methylated diketopiperazines as seen along the axis perpendicular to the mean plane of the diketopiperazine ring system. Ellipsoids were drawn at the 50% probability level with ORTEP. For the sake of clarity, the isotropic temperature factors of the hydrogen atoms were given a value of 2.0 in producing the drawings.

clohexane-like) with a succession of internal rotation angles in the ring having essentially the same absolute value but alternating positive and negative signs ($\omega_1 = 19°$, $\psi_1 = -17°$, $\phi_1 = 18°$; $\omega_2 = -19°$, $\psi_2 = 17°$, and $\phi_2 = -18°$). The last three angles are simply centrosymmetric values since the molecule retains a center of symmetry in the solid state. As far as we know, this is the first time such a conformation has been found in the solid state for diketopiperazine ring systems. Of course, the methylation at the nitrogen atoms must contribute to such a conformation because of the ease of deformation about the tertiary amide bond compared with a secondary amide bond. The ω values in this case assume the unusually large value of $\pm 19°$ The conformation of the isopropyl side chains is slightly different from that found in cyclo(*N*-Me-L-Val)$_2$, with one methyl folded toward the center of the ring and the other close to the oxygen atom, $\chi_1^{2,1} = -68°$, $\chi_1^{2,2} = 164°$.

Conversion of the chair conformation of cyclo(*N*-Me-L-Val-*N*-Me-D-Val) to either the boat or planar ring structure produces unacceptable contacts between the methyl groups of the side chain and the atoms attached to the diketopiperazine ring. We have recently solved the conformation of the unmethylated cyclo(L-Val-D-Val) in the solid state.[38] The diketopiperazine ring is slightly folded toward a boat as shown in Figure 3: $\omega_1 = -1°$, $\psi_1 = 11°$, $\phi_1 = -13°$, $\omega_2 = 3°$, $\psi_2 = 8°$, $\phi_2 = -9°$ ($\beta \geqq 0$). The side-chain methyl groups occur in the gauche-gauche positions relative to the ring nitrogen atom as predicted by Ramachandran for the side chain of cyclo(Gly-L-Val).[39] The conformation observed for the isopropyl side chains in cyclo(*N*-Me-L-Val-*N*-Me-D-Val) is different from the one observed in the unmethylated analogue. It is, however, similar to one of the preferred conformations recently calculated for cyclo(Gly-L-Val), in which the two methyl groups are in the gauche-trans position. As noted above, the deepest minimum they calculate occurs for the conformation in which the methyl groups of the isopropyl side chain are in the gauche-gauche position with respect to the nitrogen atom of the ring.

The conformation observed for the side chains of cyclo(*N*-Me-L-Val-*N*-Me-D-Val) in the solid state releases all intramolecular close contacts between the methyl groups of the side chains and the *N*-methyl groups of the ring (the shortest

$C_1^{\gamma 1}\cdots C_{N_1}$ or $C_1^{\gamma 1}\cdots C_{N_2}$ is 3.78 Å). The methyl group trans to the nitrogen atom exhibits a short contact with the oxygen of the ring ($C_1^{\gamma 2}\cdots O_1$ = 2.98 Å). The other methyl group of the isopropyl side chain which is folded over the diketopiperazine ring does not have any bad contacts ($C_1^{\gamma 1}\cdots N_2$ = 3.42 Å and $C_1^{\gamma 1}\cdots C_2^{1}$ = 3.51 Å). Rotation of the side-chain groups around the C^{α}–C^{β} bond, maintaining the ring system in the same conformation, will give rise to unacceptable $CH_3\cdots CH_3$ or $CH_3\cdots O$ interactions.

For cyclo(*N*-Me-L-Val-*N*-Me-D-Val), the chair form and the conformation of the isopropyl side chain should be similar in solution to the solid-state conformation since we believe this overall molecular structure represents the deepest potential energy minimum. It is important to note that chair conformations for diketopiperazine rings cannot be described utilizing Hooker's β parameter[35] since chair conformations will necessarily give values of zero for β. Such a value has been applied by Hooker only to describe the planar conformation of the diketopiperazine ring.

We have also solved the structure for cyclo(*N*-Me-L-Phe-*N*-Me-D-Phe) and found a chair conformation similar to the N-methylated LD isomer of valine ($\omega_1 = 14°, \psi_1 = -13°, \phi_1 = 14°, \omega_2 = -14°, \psi_2 = 13°, \phi_2 = -14°$). In this molecule a center of inversion is maintained as a crystallographic symmetry. Even the side-chain conformation in this case resembles that found for the cyclo(*N*-Me-L-Val-*N*-Me-D-Val). In fact, the phenyl ring is folded over the diketopiperazine ring so that the C^{γ} atom of the benzene ring is more or less in the same position to that found for one of the methyl groups of the isopropyl residue of the valine. The diketopiperazine ring in this case is sandwiched between the benzene rings on the opposite side and no unusually short intramolecular contacts are present.

The dihedral angle between the average plane of the diketopiperazine ring and the plane of the benzyl residue is 50°. Interactions between the stacked aromatic rings and the diketopiperazine ring have been invoked[13,14] to explain the NMR results of phenylalanine-containing cyclic dipeptides, and minimum energy calculations have confirmed the preference for such conformations of the side chain. The $\chi_1^{2,1}$, $\chi_1^{3,1}$, $\chi_1^{3,2}$ conformational parameters experimentally found in this case are 65, 90, −86°, which fit the deepest minimum of the energy map reported by Chandrasekaran et al.[39] for cyclo(Gly-L-Phe) even if in that calculation distortions of the amide bonds and chair-like conformations were not considered. (The global minimum occurs near $\chi_1^{2,1} = 60°$ and $\chi_1^{3,1} = 90°$.)

The diketopiperazine ring of cyclo(*N*-Me-L-Phe)$_2$ also exists in the boat conformation ($\beta = -19°$) in the solid state with axial dispositions for the C^{β} carbon atoms, with the following conformational parameters: $\omega_1 = -7°, \psi_1 = -15°, \phi_1 = 25°, \omega_2 = -12°, \psi_2 = -11°$, and $\phi_2 = 21°$. The LL isomers of the N-methylated alanine and valine diketopiperazines exhibit near C_2 symmetry as noted above. This symmetry element does not occur in cyclo(*N*-Me-L-Phe)$_2$ since one of the aromatic rings folds over the diketopiperazine ring while the other is forced away from the ring. It is impossible to maintain the boat structure ($\beta < 0$) with both aromatic groups folded over the ring since severe steric repulsions prevent the molecule from attaining such a structure.

In the experimental conformation, none of the carbon atoms of the two aromatic groups shows intramolecular contacts between atoms four or more bonds apart of less than 3.3 Å. The positions of the side chains are best described by the values of the internal rotation angles around the C^{α}–C^{β} and the C^{β}–C^{γ} bonds ($\chi_1^{2,1}$, $\chi_2^{2,1}$). For residue 1 ($\chi_1^{3,1}$, $\chi_1^{3,1}$, $\chi_2^{3,1}$, $\chi_3^{3,2}$), in which the aromatic group bends away from the diketopiperazine ring, the $\chi_1^{2,1}$ and $\chi_1^{3,1}$ values are 111 and 71°; for residue 2, the one eclipsing the diketopiperazine ring, the values

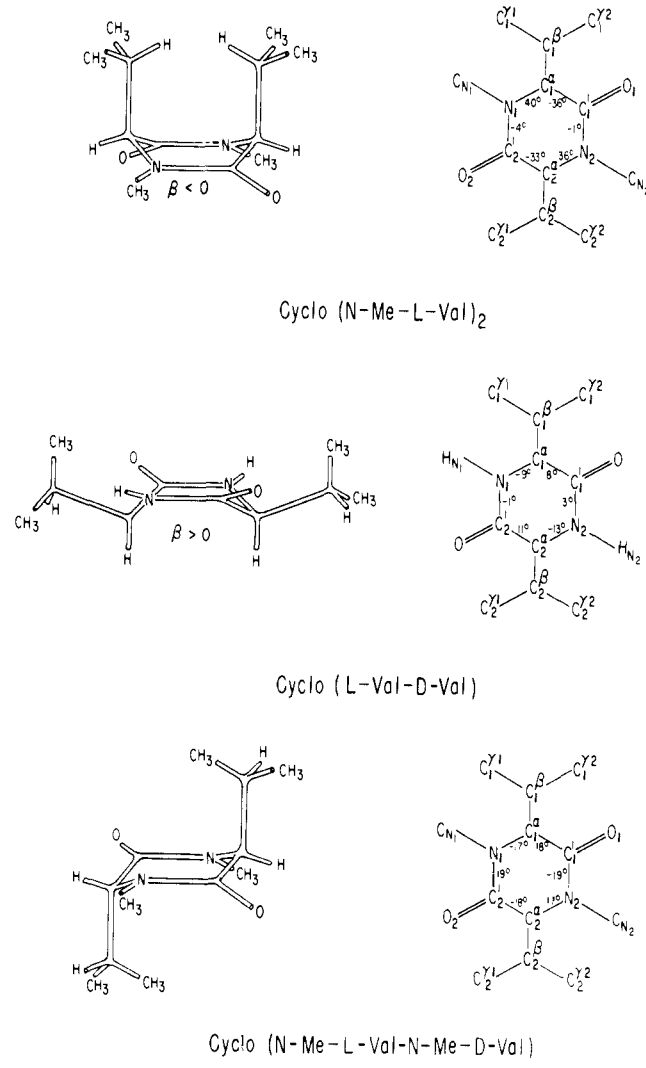

Figure 3. Schematic drawings of cyclo(*N*-Me-L-Val)$_2$, cyclo(L-Val-D-Val), and cyclo(*N*-Me-L-Val-*N*-Me-D-Val). The different conformations assumed by the molecules in the solid state are represented. The ϕ, ψ, and ω values in degrees are also reported for the three ring systems.

of $\chi_2^{2,1}$ and $\chi_2^{3,1}$ are −64 and 87°, which are close to the values found for the LD isomer of the same molecule (−65 and 90°, respectively). The dihedral angle between the average plane of the diketopiperazine ring and the planes of the two benzyl groups is 162° and 55° for residues 1 and 2, respectively.

In conclusion, we are able to make the following general comments based on the x-ray structures of the six N-methylated diketopiperazines.

(a) Optically active N-methylated diketopiperazines (LL or DD) and racemic isomers (LL mixed with identical amounts of DD) assume a boat conformation in the solid state with the C^{β} atoms in the axial positions ($\beta < 0$). The other boat structure ($\beta > 0$) which places the C^{β} atom in the pseudoequatorial positions is not possible for these compounds because of severe steric interactions between the side chains and the adjacent substituents on the ring (*N*-methyl and oxygen). The magnitude of the folding of the boat (i.e., the actual value of β) depends on the severity of steric interactions between the side chains and the substituents on the ring systems.

(b) In N-methylated diketopiperazines the amide bonds are easily deformed, as can be seen from the ω values of all of the compounds analyzed in this paper. This effect most probably arises as a result of the flattening of the potential energy minima about the planar conformation of the amide groups.

(c) Optically inactive meso isomers (LD) of N-methylated diketopiperazines assume the chair structure as a preferred

conformation. Side chains adopt axial positions on opposite sides of the ring system (i.e., trans).

(d) For N-methylated compounds with side chains extending beyond C^{β} the side chains in axial positions can fold over the diketopiperazine or be forced away from the ring, depending upon steric interactions.

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Supplementary Material Available: Listing of structure factor calculations (31 pages). Ordering information is given on any current masthead page.

References and Notes

(1) (a) M. Goodman, F. Chen, C. Gilon, R. Ingwall, D. Nissen, and M. Palumbo in "Peptides, Polypeptides and Proteins", Proceedings of the Rehovot Symposium, E. R. Blout et al., Ed., Wiley, New York, N.Y. 1974, p 126; (b) M. Goodman, C. Toniolo, and F. Naider, in ref 1a, p 308; (c) R. E. Marsh and J. Donohue, *Adv. Protein Chem.*, **22**, 235 (1967).
(2) M. R. Bell, J. R. Johnson, B. S. Wildi, and R. B. Woodward, *J. Am. Chem. Soc.*, **80**, 1001 (1958).
(3) J. M. Ronaldson, A. Taylor, E. P. White, and R. J. Abraham, *J. Chem. Soc.*, 3172 (1963).
(4) S. Safe and A. Taylor, *J. Chem. Soc.*, 432 (1970).
(5) R. Nagarajan, L. L. Huckstep, D. H. Lively, D. C. Delong, M. M. Marsh, and N. Neuss, *J. Am. Chem. Soc.*, **90**, 2980 (1968).
(6) D. B. Cosulich, N. R. Nelson, and J. H. van den Hende, *J. Am. Chem. Soc.*, **90**, 6519 (1968).
(7) D. Hauser, H. P. Weber, and H. P. Sigg, *Helv. Chim. Acta*, **53**, 1061 (1970).
(8) H. Minato, M. Matsumoto, and T. Katayama, *Chem. Commun.*, 44 (1971).
(9) J. Fridrichsons and A. McL. Mathieson, *Acta Crystallogr.*, **23**, 439 (1967).
(10) J. W. Moncrief, *J. Am. Chem. Soc.*, **90**, 6517 (1968).
(11) J. Fridrichsons and A. McL. Mathieson, *Acta Crystallogr.*, **18**, 1043 (1965).
(12) G. Grawne, G. W. Kenner, N. H. Rogers, R. C. Sheppard, and K. Titlestad, "Peptides", E. Bricas, Ed., North-Holland Publishing Co., Amsterdam, 1968, p 28.
(13) K. D. Kopple and D. H. Marr, *J. Am. Chem. Soc.*, **89**, 6193 (1967).
(14) K. D. Kopple and M. Ohnishi, *J. Am. Chem. Soc.*, **91**, 962 (1969).
(15) I. Z. Siemion, *Justus Liebigs Ann. Chem.*, **748**, 88 (1971).
(16) Ziauddin, K. D. Kopple, and C. A. Bush, *Tetrahedron Lett.*, 483 (1972).
(17) R. Degeilh and R. E. Marsh, *Acta Crystallogr.*, **12**, 1007 (1959).
(18) E. Benedetti, P. Corradini, M. Goodman, and C. Pedone, *Proc. Natl. Acad. Sci. U.S.A.*, **62**, 650 (1969).
(19) E. Benedetti, P. Corradini, and C. Pedone, *J. Phys. Chem.*, **73**, 2891 (1969).
(20) E. Benedetti, P. Corradini, and C. Pedone, *Biopolymers*, 7, 51 (1969).
(21) E. Sletten, *J. Am. Chem. Soc.*, **92**, 172 (1970).
(22) I. L. Karle, *J. Am. Chem. Soc.*, **94**, 81 (1972).
(23) L. E. Webb and C. Lin, *J. Am. Chem. Soc.*, **93**, 3818 (1971).
(24) I. L. Karle, H. C. J. Ottenheym, and B. Witkop, *J. Am. Chem. Soc.*, **96**, 539 (1974).
(25) R. B. Von Dreele, *Acta Crystallogr., Sect. B*, **31**, 966 (1975).
(26) A. J. Morris, A. J. Geddes, and B. Sheldrick, *Cryst. Struct. Commun.*, **3**, 345 (1974).
(27) (a) P. Groth, *Acta Chem. Scand.*, **23**, 3155 (1969); (b) P. A. Timmins, *Acta Crystallogr., Sect. B*, **31**, 2561 (1975).
(28) M. Goodman, N. Ueyama, E. Benedetti, and W. Radding, unpublished results.
(29) G. Germain, P. Main, and M. M. Woolfson, *Acta Crystallogr., Sect. A*, **27**, 368 (1971).
(30) A. C. Larson, *Acta Crystallogr.*, **23**, 664 (1967).
(31) D. J. Duchamp, Abstracts, American Crystallography Association, Bozeman, Mont., paper no. 14, p 29.
(32) "International Tables for X-Ray Crystallography", Vol. III, Kynoch Press, Birmingham, 1962, p 202.
(33) R. F. Stewart, E. R. Davidson, and W. T. Simpson, *J. Chem. Phys.*, **42**, 3175 (1965).
(34) G. N. Ramachandran and V. Sasisekharan, *Adv. Protein Chem.*, **23**, 283 (1968).
(35) T. M. Hooker, Jr., P. M. Bayley, W. Radding, and J. A. Schellman, *Biopolymers*, **13**, 549 (1974).
(36) S. Karplus and S. Lifson, *Biopolymers*, **10**, 1973 (1971).
(37) E. Benedetti, M. Goodman, R. E. Marsh, H. Rapoport, and J. A. Musich, *Cryst. Struct. Commun.*, **4**, 641 (1975).
(38) E. Benedetti, M. Goodman, D. C. Easter, and R. E. Marsh, unpublished results.
(39) R. Chandrasekaran, A. V. Lakshminarayanan, P. Mohanakrishnan, and G. N. Ramachandran, *Biopolymers*, **12**, 1421 (1973).
(40) *rac*-Cyclo(*N*-Me-Ala)$_2$ refers to a racemic mixture of the two cyclic dipeptide derivatives cyclo(*N*-Me-L-Ala)$_2$ and cyclo(*N*-Me-D-Ala)$_2$.

Nature of the Amino-Enzyme Intermediate in Pepsin-Catalyzed Reactions

Marc S. Silver,* Mai Stoddard, and Michael H. Kelleher

Contribution from the Department of Chemistry, Amherst College, Amherst, Massachusetts 01002. *Received March 25, 1976*

Abstract: The most primitive version of the amino-enzyme mechanism for pepsin-catalyzed hydrolyses requires that cleavage of X-Phe-Trp at the Phe-Trp bond yields the intermediate pepsin-Trp, whose subsequent behavior must be independent of X. At pH 4.5, pepsin-Trp can undergo hydrolysis to Trp or yield Ac-Phe*-Trp from a trapping reaction with 2.5×10^{-2} M Ac-Phe* (radioactive Ac-Phe). The ratio [Ac-Phe*-Trp]/[Trp], measured under identical experimental conditions, is 0.30, 0.04, 0.04, and ≤ 0.005 for X = Ac, Ac-Gly-Gly, Z-His, and Z-Ala-His, respectively. The data unequivocally disprove the simple amino-enzyme mechanism and define some necessary attributes of expanded versions of the mechanism.

A satisfactory mechanism for pepsin-catalyzed hydrolyses has yet to be formulated. Mechanistic speculations for many years centered on the possible role of an "amino-enzyme" intermediate.[1,2] Recent experiments have suggested that pepsin shows behavior characteristically associated with the formation of both amino- and acyl-enzyme intermediates during the hydrolysis of peptides bearing a free α-amino group.[3,4] Tentative efforts have been made to accommodate both types of intermediates in one general mechanism.[3-5]

The suggestion that pepsin-catalyzed reactions give rise to an amino enzyme derives from the observation that peptic hydrolysis of simple acylated peptide derivatives at pH 4.5 or higher gives rise both to hydrolysis and to transpeptidation reactions in which the amino fragment of the cleaved substrate has been transferred to a suitable acceptor.[6] There is currently no evidence for acyl transfer in the reactions of these substrates. Equation 1 illustrates how the amino-enzyme hypothesis, in its simplest form, accounts for hydrolysis and amino transpeptidation; breakage of the Phe-Trp bond of X-Phe-Trp[7] yields the amino enzyme, pepsin-Trp, which undergoes either hydrolysis or trapping by the "acceptor", radioactive Ac-Phe (Ac-Phe*),[8] to yield the transpeptidation product, Ac-Phe*-Trp.

In an earlier study[1] we quantitatively evaluated the parti-