phosphate and the hydroxyl group of a serine residue (for a review, see ref 20).

The free energy of hydrolysis of a phosphotriesters in water does not appear to have been determined experimentally. A large contribution to the negative free energy of hydrolysis, amounting to approximately −30 kJ/mol at pH 7, must arise from the ionization of the product phosphodiesters, a strong acid with a pK_a typically in the neighborhood of 1.4.[21] The contribution of product ionization is presumably enought to raise the triester to a group-transfer potential comparable with or exceeding that of ATP. This unusual phosphorylating capacity, often exploited in synthetic chemistry, has evidently not proven useful in the evolution of living organisms. Steric hindrance may limit the usefulness of triesters as biosynthetic intermediates, and they would presumably be difficult to synthesize.

The distribution behavior of methyl ethylene phosphate was found to be similar to that of trimethyl phosphate, suggesting that solvation makes no unusual contribution to the thermodynamic properties of cyclic esters of this kind.

When comparing trimethyl phosphate with triethylphosphine oxide, in which ester oxygens are replaced by methylene groups, only a small difference in apparent water affinity is observed (somewhat surprisingly triethylphosphine oxide is slightly *more* hydrophilic than trimethyl phosphate). Phosphonic acids should accordingly serve as reasonable analogues for phosphate esters in the design of enzyme antagonists, in terms of their solvation properties. A number of such inhibitors have been developed, of which the best known is probably the herbicide glyphosate, an inhibitor of the biosynthesis of anthranilic acid.[22]

Acknowledgment. This work was supported by Grant PCM-7823016 from the National Science Foundation.

Registry No. $(PrO)_3PO$, 513-08-6; $(EtO)_3PO$, 78-40-0; $(MeO)_3PO$, 512-56-1; $(BuO)_2PO(OH)$, 107-66-4; $(PrO)_2PO(OH)$, 1804-93-9; $(EtO)_2PO(OH)$, 598-02-7; $(MeO)_2PO(OH)$, 813-78-5; $PrOPO(OH)_2$, 1623-06-9; $(BuO)_2PON(CH_3)_2$, 84108-32-7; $(BuO)_2PONHCH_3$, 2014-81-5; $(BuO)_2PONHz$, 870-52-0; $(C_2H_5)_3PO$, 597-50-2; methyl ethylene phosphate, 2196-04-5.

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Cyclopeptide Alkaloids. Conformational Analysis of the Dihydro-*p*-phencyclopeptine Nucleus

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Abstract: A combined NMR spectroscopic and X-ray crystallographic approach to the analysis of the conformations of two synthetic dihydro-*p*-phencyclopeptines, (5*S*)-5,6-trimethylene-8-deamino-1,2-dihydro-*p*-phencyclopeptine (**2d**) and its *N*-methyl derivative, (5*S*)-3-methyl-5,6-trimethylene-8-deamino-1,2-dihydro-*p*-phencyclopeptine (**2a**), is described. The one-dimensional ^1H NMR spectra of both cyclopeptides are assigned by means of two-dimensional homonuclear (^1H) *J*-spectral analysis, homonuclear decoupling experiments, computer-generated spectral simulations, and steady-state ^1H NOE measurements at 270 and 360 MHz. On the basis of Karplus relationships derived from these experimental results, together with ^{13}C NMR spectrscopic analysis, predictions for the solution conformations of both cyclopeptides are deduced. The solution conformation of the *N*-methyl cyclopeptide **2a** is compared with its solid-state conformation determined by X-ray crystallography, and the two are found to be in good agreement. Comparative NMR studies of the two *p*-phencyclopeptines reveal only subtle conformational differences between the two cyclopeptides. These differences occur in the region near the N3–C4 amide where the structural difference between the two cyclopeptides, the *N*-methyl group, is localized. Of the eight possible conformations of the *p*-phencyclopeptine nucleus, both cyclopeptides adopt the same overall geometry with both amides trans. The small conformational differences that do exist between **2a** and **2d** reflect the ability of NH cyclopeptide **2d** to form an intramolecular hydrogen bond similar to those observed in γ turns in proteins. Conformational implications with respect to ion binding of the *p*-phencyclopeptines nucleus are considered.

The physical properties and biological activity of proteins are primarily dependent on levels of structure other than just the primary amino acid sequence. The analysis of peptide backbone conformations therefore provides an important route to understanding the three-dimensional arrangement of amino acid residues in proteins. In recent years, a great deal of attention has been focused on conformational studies of small cyclic peptides both natural and synthetic.[2] These substances, in addition to being interesting because of their biological activity, have been used as models for larger proteins because of the simplified analysis that is possible.

Of the spectroscopic techniques that are applicable to conformational analysis, nuclear magnetic resonance (NMR) has found increasing use.[3-5] NMR also has been one of the important techniques used for the elucidation of the structures of the cy-

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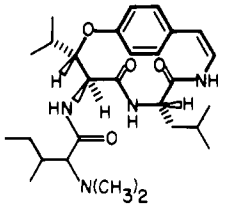
clopeptides alkaloids,[6-8] a large class of polyamide plant bases widespread in the Rhamnaceae family.[9] Its use for the conformational analysis of this unusual class of natural product has only recently been explored.[10,11] The conformational analysis of the cyclopeptide alkaloids is of interest because of the large number of configurational isomers involving the peptide bonds that are theoretically possible. Conformational studies are also an important first step to understanding the mechanism, structure, and dynamics of cyclopeptide–metal ion binding that have been implicated by ionophore experiments[12,13] and by metal-induced conformational changes observed by circular dichroism (CD) spectroscopy.[11,14]

The largest subclass of the cyclopeptide alkaloids, the *p*-phencyclopeptines,[15] is based on a 14-membered ring containing a β-hydroxyamino acid group linked through an aryl ether bond to a *p*-hydroxystyrylamine moiety as illustrated by frangulanine (**1**). In an earlier study, we have described the synthesis of five



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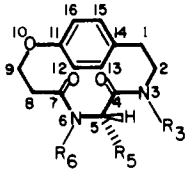
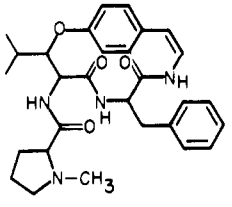


2a. $R_3 = CH_3$; $R_5, R_6 = (CH_2)_3$
2b. $R_3 = CH_3$; $R_5 = (CH_3)_2CHCH_2$; $R_6 = H$
2c. $R_3 = R_6 = CH_3$; $R_5 = (CH_3)_2CHCH_2$
2d. $R_3 = H$; $R_5, R_6 = (CH_2)_3$
2e. $R_3 = R_6 = H$; $R_5 = (CH_3)_2CHCH_2$



3

p-phencyclopeptine model compounds **2a–e** bearing different substituents on the amide nitrogens.[14] In that study, we demonstrated that the synthetic *p*-phencyclopeptine **2d** exhibited ion-induced conformational changes similar to those of ceanothine B (**3**), a natural *p*-phencyclopeptine, despite pronounced structural differences between the two compounds. Both synthetic and natural *p*-phencyclopeptines interacted preferentially with small divalent cations (Mg^{2+}, Ca^{2+}). The nature of this ion–*p*-phencyclopeptine adduct(s) is an interesting enigma since the synthetic *p*-phencyclopeptine nucleus contains only two amide groups that can act as ligands for metal ion binding.

The present study was undertaken to elucidate the solution conformations of two synthetic *p*-phencyclopeptines, **2a** and **2d**, in order to provide insight regarding the conformational requirements for ion binding. Our approach employs the acquisition of two-dimensional (2-D) ^1H NMR *J* spectra[16-18] and the generation of suitable projections to extract shifts and couplings, followed by computer simulation of one-dimensional spin subsystems to generate accurate coupling constants for bond angle calculations. These 2-D NMR studies, together with steady-state ^1H nuclear Overhauser enhancement (NOE) measurements, ^{13}C NMR studies, and X-ray crystallography of **2a**, have allowed us to assign one of eight possible conformational isomers as the structure of the *p*-phencyclopeptines **2a** and **2d**.

Table I. Single-Crystal X-ray Crystallographic Analysis of **2a**

A. Crystal Parameters	
formula	$C_{17}H_{22}N_2O_3$ (302.4)
crystallization medium	methanol/water
crystal size, mm	0.38 × 0.42 × 0.41
cell dimensions, Å	$a = 9.021$ (2)
	$b = 10.405$ (2)
	$c = 16.514$ (5)
space group	$P2_12_12_1$
molecules/unit cell	4
density obsd, g/cm³	1.28
density calcd, g/cm³	1.296
B. Refinement Parameters	
number of reflections	953
nonzero reflections	891
final *R* index, $R = \Sigma \|\|F_o\| - \|F_c\|\| / \Sigma \|F_o\|$	0.053
scale factor	0.826 (4)
secondary extinction coefficient	$4.6\ (7) \times 10^{-6}$

Experimental Section

Materials. The synthesis and characterization of the two *p*-phencyclopeptines, (5*S*)-5,6-trimethylene-8-deamino-1,2-dihydro-*p*-phencyclopeptine (**2d**) and (5*S*)-3-methyl-5,6-trimethylene-8-deamino-1,2-dihydro-*p*-phencyclopeptine (**2a**), were described in an earlier report.[14] Both cyclopeptines **2a** and **2d** were sublimed at 100 °C (10 μm) before NMR measurements.

$CDCl_3$ (99.5% D, Aldrich Chemical) was distilled from P_2O_5 shortly before use. Concentrations of 1–50 mM of **2a** or **2d** in $CDCl_3$ were used for the ^1H NMR spectral measurements. NMR samples were prepared in a Vacuum Atmospheres argon-filled glovebox and were filtered through glass wool into 5-mm sample tubes (Wilmad 528-PP).

Nuclear Magnetic Resonance. The 270-MHz ^1H NMR spectra and 2-D ^1H homonuclear *J* spectra at 270 MHz were obtained with a homemade spectrometer based on a Bruker 63-kG magnet with a Nicolet 1180 data system.[19] Prior to acquisition of a 2-D file, the 90° pulse length was measured. This was found to vary from 4.95 to 6.00 μs. Two dimensional homonuclear *J* spectra were acquired with a waiting time between the 90° and 180° pulses incremented by 10 ms (t_1), which corresponded to an f_1 (*J*) frequency range of ±25 Hz; 128 different values of t_1 were utilized for these experiments. The f_2 frequency range spanned the normal spectral region (±1400 Hz and ±2500 Hz with quadrature detection) and employed 2048 complex point transforms.

A Nicolet 360-MHz spectrometer at the UCD NMR facility was utilized for the acquisition of 360-MHz ^1H NMR spectra. A tip angle of 42° (5.0 μs) with a 3.0-s delay between pulses and an acquired spectral width of ±1500 Hz (quadrature detection) were used. The number of scans ranged between 40 and 100 and the FID's were signal averaged into a 16K memory block with an acquisition time of 2.73 s.

^1H NOE measurements at 360 MHz were obtained by difference of the Fourier transformed spectra (40 scans) collected on and off resonance. For these measurements an 80° pulse (10.0 μs) with a 3.0-s recycle delay and a 1400 spectral width (QPD) were used.[20] To effect

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(20) The effects of different pulse widths on the magnitude and sign of NOE values has been described recently (Schaublin, S.; Hohener, A.; Ernst, R. R. *J. Magn. Reson.* **1974**, *13*, 196). In the present study, no attempt was made to correlate the effect of pulse length on the measured NOE values.

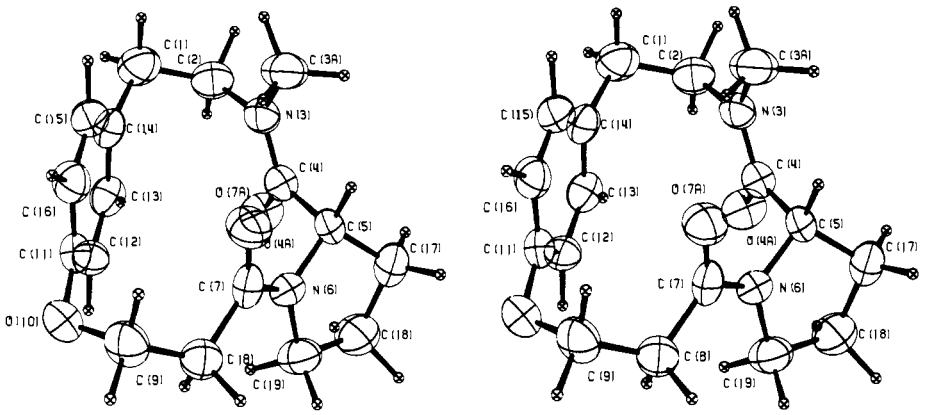

Figure 1. ORTEP stereo drawing of **2a** obtained from the X-ray crystallographic analysis.

the NOE, a saturating field was applied for 3 s between acquisitions to maintain the steady-state saturation of the dipolar-coupled proton resonance in question.

^{13}C NMR spectra of cyclopeptides **2a** and **2d** at 50.3 MHz were obtained in $CDCl_3$ at concentrations of 46 and 77 mM, respectively, using a Nicolet 200 spectrometer equipped with an Oxford Instruments wide-bore magnet. Free induction decays (320 scans) were acquired by use of the following spectrometer parameters: a 90° pulse width of 18 s, spectral width ±7000 Hz using quadrature detection, and a 3.0-s recycle delay. The FID's were sampled in 16K of memory.

Single-Crystal X-ray Analysis of 2a. A representative crystal was surveyed and a 1-Å data set (maximum $\sin\theta/\lambda = 0.5$) was collected on a Syntex P$\bar{1}$ diffractometer. The diffractometer was equipped with a graphite monochromator and copper radiation (λ = 1.5418 Å). All diffractometer data were collected at room temperature. Pertinent crystal, data collection, and refinement parameters are summarized in Table I.

A trial structure was obtained by direct methods using the MULTAN program.[21] This trial structure refined routinely. Hydrogen positions were calculated wherever possible. The methyl hydrogens were located by difference Fourier techniques. The hydrogen parameters were added to the structure factor calculations but were not refined. The final cycles of full matrix least-squares refinement contained the scale factor, secondary extinction coefficient, coordinates, and anisotropic temperature factors in a single matrix. The shifts calculated in the final cycle of refinement were all less than one-tenth of their corresponding standard deviation. The final R index was 0.053. A final difference Fourier revealed no missing or misplaced electron density.

The refined structure was plotted by using the ORTEP computer program of Johnson[22] (Figure 1). Coordinates, anisotropic temperature factors, distances, angles, and structure factor tables are available as supplementary material (Tables SI-SVI).

Results

^{1}H NMR Spectral Assignments. The 360-MHz ^{1}H NMR spectra of the p-phencyclopeptines **2a** and **2d** in $CDCl_3$ are shown in Figure 2 (lower and upper spectra, respectively). Due to the complexity of the spectra the only lines that could be readily assigned were the aromatic four-spin multiplets of both cyclopeptides and the N-methyl group of **2a**.

The traditional approach to making assignments involves exhaustive homonuclear decoupling experiments. The time-consuming part of this task is determining the exact H_2 irradiation frequency in complex regions such as 2.7–3.1 ppm in the spectrum of **2d** (Figure 2, upper spectrum) where integration indicates the presence of four protons. When lines are this closely spaced, the careful application of the correct level of decoupler power is also required. Too high a power level would lead to the collapse of multiplets coupled to adjacent lines, thus making assignments difficult. For this reason both exact frequency information and the appropriate decoupler power level are important.

The 2-D J-spectral technique[16] was used to simplify the assignment procedure. The 2-D spectrum of the N-methyl cyclopeptide **2d** at 270 MHz is shown along with the normal spectrum in Figure 3, and that of the NH cyclopeptide **2d** at 270 MHz is similarly shown in Figure 4. Two visualization schemes were used to simplify the complexity of 2-D data sets: (a) the enlarged 2-D of the δ 2.6–3.1 region of the NH cyclopeptide **2d** and (b) a contour map of exactly the same area (supplementary material, Figures S1 and S2). Contour plots of the other spectral regions of **2a** and **2d** were similarly obtained, and preliminary assignments were on this basis.

The full assignment of all lines in both spectra was facilitated with a series of homonuclear decoupling experiments that made use of the 2-D 45° projections (i.e., proton-decoupled proton spectra).[23,24] By use of the irradiation frequencies obtained from

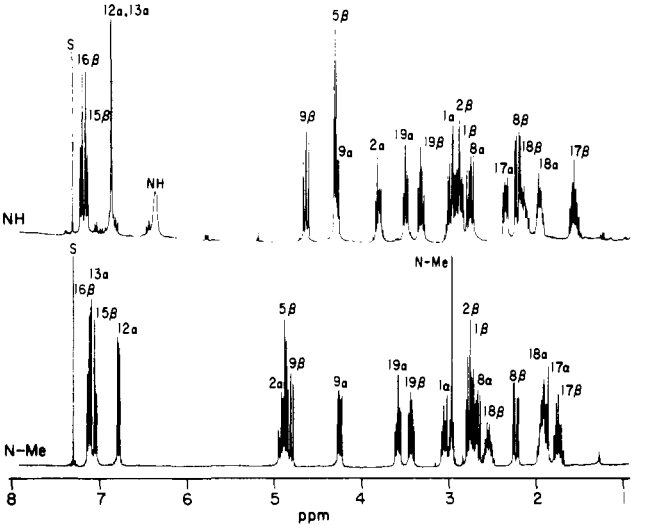

Figure 2. 360-MHz ^{1}H NMR spectra of **2a** (lower) and **2d** (upper).

Table II. ^{1}H NMR Spectral Assignments for p-Phencyclopeptines 2a and 2d in $CDCl_3$[a]

chemical shift		proton	chemical shift		proton
2a	2d	assignment	2a	2d	assignment
3.03	2.98[b]	1α	6.77	6.85[b]	12α
2.71	2.85[b]	1β	7.11	6.85[b]	13α
4.89	3.80	2α	7.03	7.13[b]	15β
2.75	2.91	2β	7.09	7.19[b]	16β
2.95	6.36	NMe/NH	1.89	2.34	17α
4.85	4.29	5β	1.72	1.56	17β
2.66	2.74	8α	1.92	1.93	18α
2.21	2.20	8β	2.53	2.14	18β
4.23	4.27	9α	3.57	3.50	19α
4.80	4.62	9β	3.41	3.31	19β

[a] Chemical shifts in ppm are relative to internal tetramethylsilane (Me_4Si). [b] On the basis of the present spectral analysis these resonances have been reassigned from those reported previously in ref 10.

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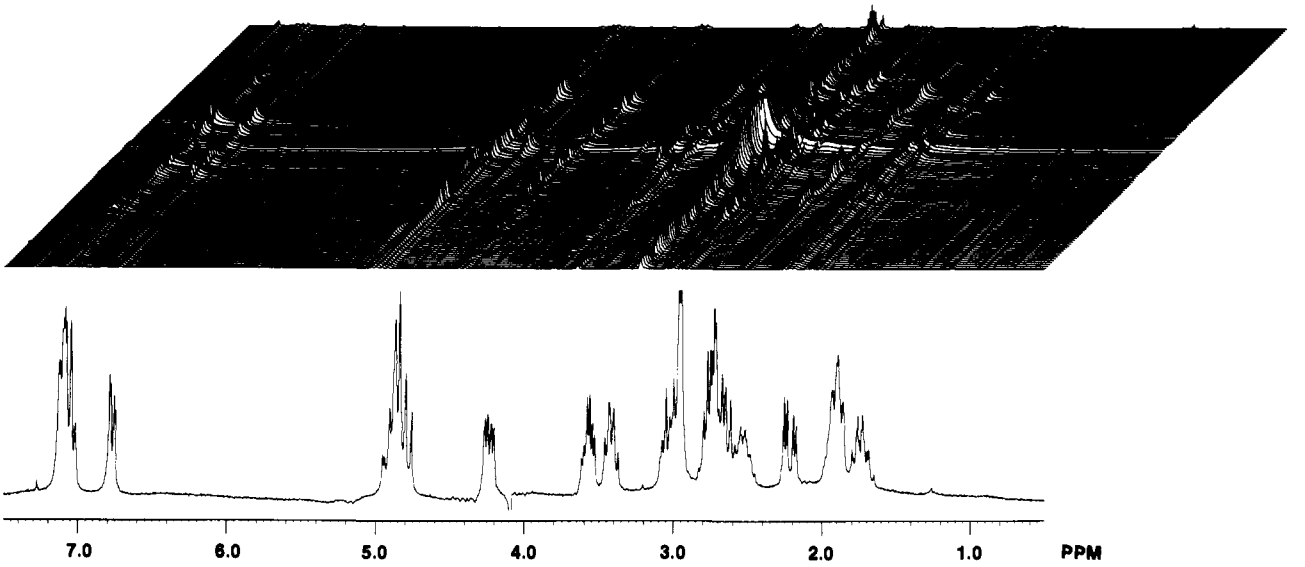

Figure 3. 270-MHz ^{1}H 2-D homonuclear J spectrum of **2a** (upper) shown with the normal spectrum (lower).

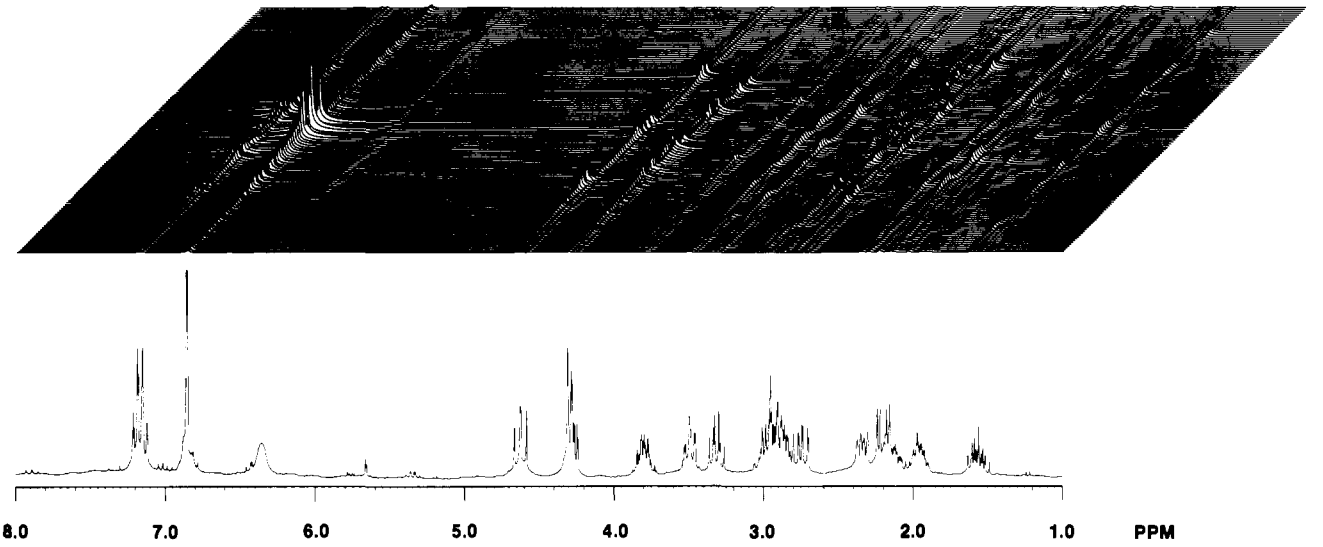

Figure 4. 270 MHz ^{1}H 2-D homonuclear J spectrum of **2d** (upper) shown with the normal spectrum (lower).

these 45° projections (supplementary material, Figures S3 and S4), homonuclear decoupling experiments led to the assignments of most of the lines in the spectra of **2a** and **2d**. In combination with the calculated spectrum simulations and with steady-state NOE measurements (see below), the complete spectral assignments for both cyclopeptides, shown in Figure 2 and summarized in Table II, were made.

Spectrum Simulations. Proline Seven-Spin System. The proline 5β–17α–17β–18α–18β–19α–19β seven-spin systems were assigned with the aid of the published data on the *cyclo*-tri-L-prolyl system.[25] By use of chemical-shift frequencies derived from the 2-D 45° projections and published coupling constants,[25] a simulated spectrum was obtained for the proline seven-spin system of cyclopeptide **2a** that compared well with the experimental spectrum (see supplmentary material, Figure S5). The similarity between the calculated and experimental spectra therefore allowed a straightforward assignment of the proline seven-spin system, presumably owing to the rigidity of the five-membered proline ring. For this reason an iterative solution was deemed not worthwhile because of the large number of transition assignments that would have been necessary. A similar analysis of the NH cyclopeptide **2d** led to the assignment of its proline ring system.

C8–C9 Four-Spin System. The assignment of the 8α–8β–9α–9β four-spin systems has been based on chemical shift arguments and confirmed by spectral simulations. Because of the deshielding effect of the adjacent ether oxygen atom the two lower field resonances have been assigned to the C9 hydrogens. The as-

Table III. Comparison of Bond Angles for *N*-Methyl-*p*-phencyclopeptine **2a** (X-ray Data and Calculated Solution Data) and NH *p*-Phencyclopeptine **2d** (Calculated Solution Data)

dihedral angle	2a, deg		2d, deg
	X-ray	calcd[a]	calcd[a]
Hα–8–9–Hα	84.3	74.8	76.6
Hα–8–9–Hβ	−157.6	−165.2	−163.4
Hβ–8–9–Hα	33.8	45.2	43.4
Hβ–8–9–Hβ	84.4	74.8	76.6
Hα–1–2–Hα	42.5	45.1	42.1
Hα–1–2–Hβ	76.0	74.9	162.1
Hβ–1–2–Hα	160.7	165.1	77.9
Hβ–1–2–Hβ	42.5	45.1	42.1

[a] These angles were derived from the experimental coupling constants and the minimization method described in the text (see Table VII).

(23) 2-D plots do not always appear to deploy multiplets along 45° diagonals because of the difference in plot scaling ($4z$/cm) that is usually used.
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signment of the C8–C9 four-spin system of the *N*-methyl cyclopeptide **2a** was simplified by utilizing the vicinal dihedral angles (θ) obtained by X-ray crystallography (Table III) and the generalized Karplus relationships[26,27] of the form

$$^3J(\theta) = 8.5 \cos^2 \theta - 0.28 \qquad 0° \leq \theta \leq 90° \qquad (1)$$

$$^3J(\theta) = 9.5 \cos^2 \theta - 0.28 \qquad 90° \leq \theta \leq 180° \qquad (2)$$

In this first guess approximation, geminal couplings were chosen to vary between −12 and −14 Hz. By use of the values obtained from the Karplus relationship for the vicinal coupling constants and the chemical shift values obtained from the 2-D 45° projections, an NMR spectrum of this four-spin system was generated. The spectrum thus obtained closely matched the experimental spectrum when the assignments shown in Table II were taken. After a least-squares iterative fit using a version of the LAOCN3 program was performed[28] the calculated shifts and coupling constants produced a simulated spectrum that is in excellent agreement with the experimental spectrum (RMS error of fit <0.2 Hz; see supplementary material, Figure S5).

The 8α–8β–9α–9β four-spin system of the NH cyclopeptide **2d** was analyzed in a similar fashion. Since the molecular geometry of this four-spin system of **2d** was unavailable by X-ray crystallography, the same vicinal and geminal angles as in **2a** were used as a starting point in iterative calculations. The calculated best-fit shifts and coupling constants led to a simulated spectrum (Figure S7, supplementary material) that again gave an excellent match with the experimental spectrum (RMS error of fit <0.2 Hz).

C1–C2 Four-Spin System of 2a and C1–C2–N3–H. Five-Spin System of 2d. At 270 MHz the NMR spectrum of this spin system for both cyclopeptides **2a** and **2d** is highly non first order, and its assignment was particularly difficult. With the aid of 360-MHz ^{1}H NMR spectra this task was somewhat simplified. The assignment of each of the four resonances to their respective hydrogen atoms was not intuitively obvious from first principles, however, as both the aromatic ring and N3–C4 amide moieties can impart considerable diamagnetic anisotropic shifts to nearby protons, depending on their distance and relative orientation. Overlapping lines attributed to other protons in the cyclopeptide also made our task considerably more onerous.

The assignments of the *N*-methyl **2a** spectrum were made by utilizing homonuclear spin decoupling experiments and by estimating vicinal coupling constants with the Karplus relationships of eq 1 and 2 and the X-ray bond angles (Table III). Double irradiation experiments permitted the assignment of the two pairs of geminal protons owing to the generally larger geminal coupling constants vs. those of vicinal protons. The assignment of the lower field multiplet at 4.89 ppm to one of the C2–H's was evident from a long-range coupling (0.6 Hz) from the methyl group on N3. As shown in Figure 5, double irradiation of the *N*-methyl resonance at 2.95 ppm removes this small splitting evident in the resonance at the 4.89-ppm position assigned to the 2α proton. That this irradiation produces no changes in the multiplicity of the resonances attributed to the other three protons of this spin system demonstrates that the observed change in the resonance at 4.89 ppm is not due to partial saturation of the nearby 1α resonance (data not shown). This small five-bond coupling is also manifested in the *N*-methyl resonance. Resolution enhancement of the methyl line shows it to be a doublet with a small splitting, which is removed upon double irradiation of the 4.89-ppm multiplet. When the low-field resonance at 4.89 ppm was assigned to one of the C2 protons, the assignment of the resonance at 3.03 ppm to one of the C1 protons was therefore possible from the results of the homonuclear decoupling experiments.

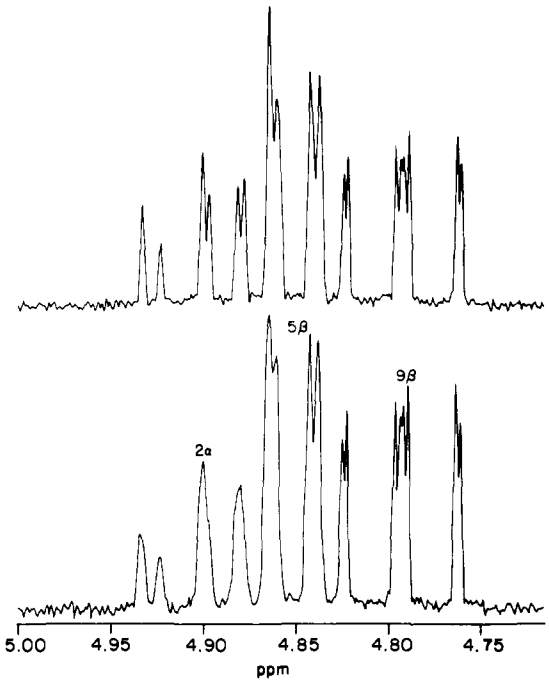


Figure 5. Partial 360-MHz ^{1}H NMR spectrum of **2a**: after decoupling the *N*-methyl group at 2.95 ppm (upper); normal spectrum (lower).

The complete assignment of this four-spin system of **2a** was provided from the X-ray data. By use of these vicinal dihedral angles (Table III) and the Karplus relationships of eq 1 and 2, a reasonably close simulation of the spectrum was obtained only when the relative chemical assignments of Table II were taken. Subsequently, an iterative best fit of the experimental spectrum, utilizing the chemical shifts and coupling constants derived from the 2-D 45° projection, has afforded a simulated spectrum (Figure 6) that is in excellent agreement with the experimental spectrum (RMS error of fit 0.15 Hz).

A similar analysis of the NH cyclopeptide **2d** was made. Initially (to simplify the spectrum to that of a four-spin system) the N proton was doubly irradiated. By use of the vicinal and geminal coupling constants obtained above for the *N*-methylcyclopeptide **2a** as a starting point, a simulated spectrum (Figure 7) that closely matched the experimental NH-decoupled spectrum was iteratively obtained (RMS error of fit 0.13 Hz). With these results and two new vicinal NH–CH coupling constants, the iterative solution of the five-spin system of the NH cyclopeptide was simplified. The coupling constants from the C2–H's to the NH thus derived by the iterative procedure generated the simulation spectrum of Figure 8, which agrees well with the experimental spectrum (RMS error of fit 0.15 Hz).

Aromatic Four-Spin System. By use of the chemical shift positions obtained from the 2-D *J* spectra and the iterative best fit procedure, simulated spectra for the 12α–13α–15β–16β four-spin system of the *N*-methyl cyclopeptine **2a** and the NH cyclopeptide **2d**, which closely match the experimental spectra (RMS error of fit <0.2 Hz), were obtained (see supplmentary material, Figures S8 and S9).

Due to the lack of a center of symmetry of the peptide macrocycle, the 12- and 16-protons and 13- and 15-protons are magnetically nonequivalent. For this reason and owing to the close proximity of transannular substituents that appear to alter the magnetic environment of the aromatic protons, the assignment of the aromatic four-spin system of the *p*-phencyclopeptine nucleus is particularly difficult. Although spin-decoupling experiments provide evidence to distinguish between the two pairs of adjacent protons (12α,13α and 15β,16β, which are ortho to each other), because of their larger *J* values, it is not possible to assign any one resonance on first principles owing to the above considerations. The complete assignments of the aromatic systems of **2a** and **2d**, however, has been accomplished by means of steady-state NOE

(26) (a) Karplus, M. *J. Chem. Phys.* **1959**, *30*, 11. (b) Karplus, M. *J. Am. Chem. Soc.* **1963**, *85*, 2870.

(27) The more recent detailed analysis of K. G. R. Pachler (*J. Chem. Soc., Perkin Trans. 2* **1972**, 1936) using modified Karplus equations with empirically derived parameters offered the same general result as those of Karplus (e.g., ref 26) with considerably more complexity.

(28) Castellano, S.; Bothner-By, A. A. *J. Chem. Phys.* **1964**, *41*, 3863.

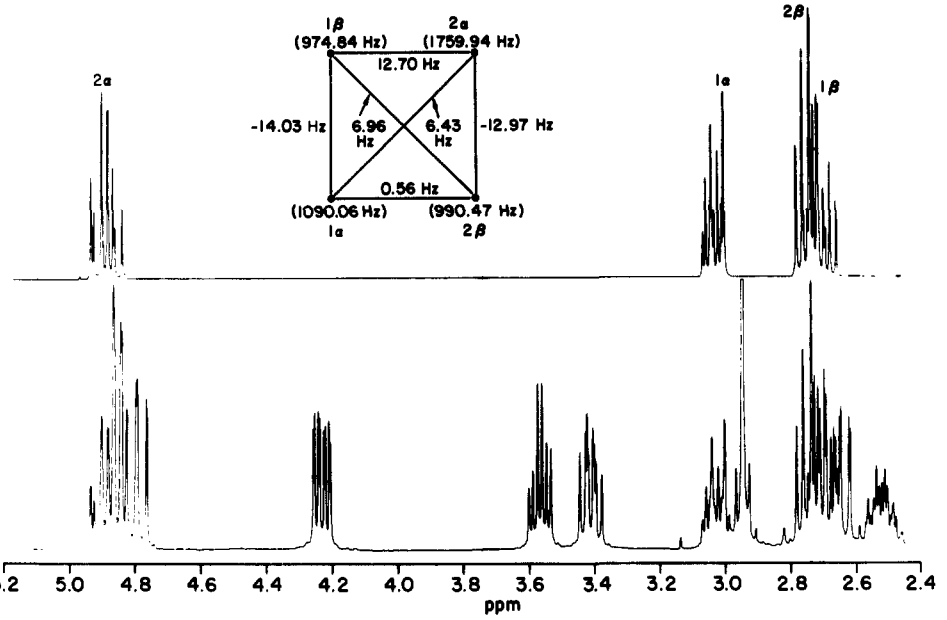


Figure 6. Calculated 360-MHz ^{1}H NMR spectrum for the 1α–1β–2α–2β four-spin system of **2a** (upper) shown with the normal spectrum (lower). Line width assumed in simulation, 2.0 Hz.

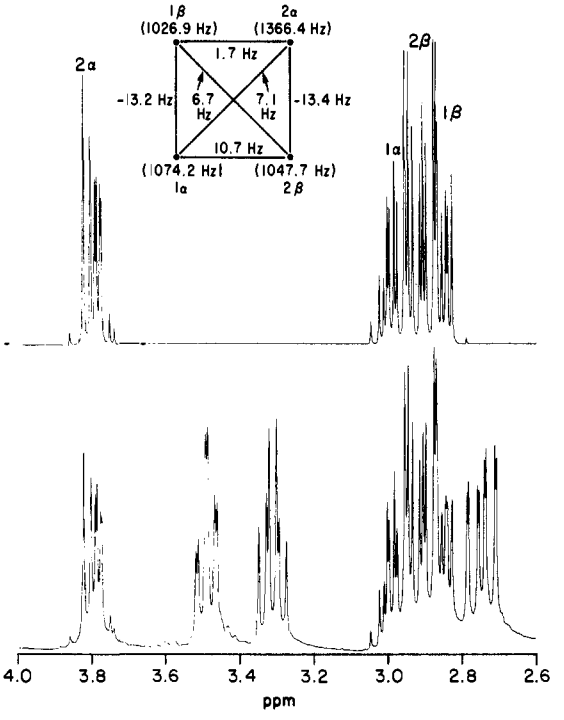


Figure 7. Calculated 360-MHz ^{1}H NMR spectrum for the 1α–1β–2α–2β four-spin system of **2d** (upper) shown with the NH decoupled spectrum (lower). Line width assumed in simulation, 2.0 Hz.

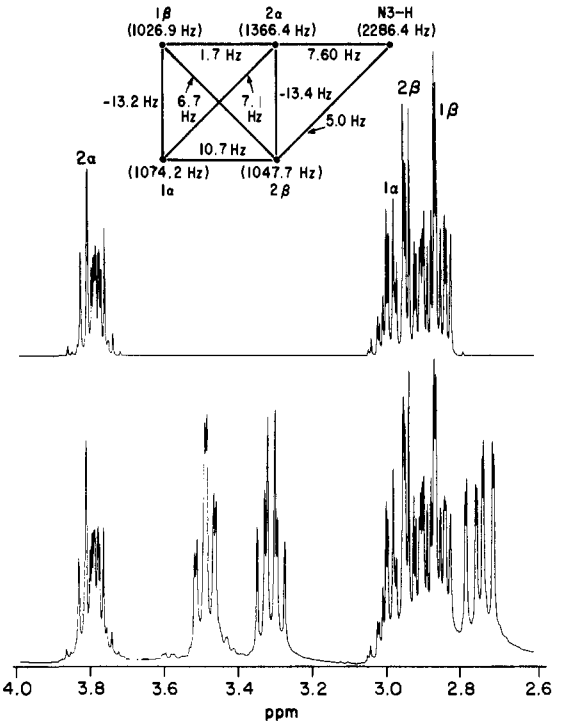


Figure 8. Calculated 360-MHz ^{1}H NMR spectrum for the 1α–1β–2α–2β–N3–H five-spin system of **2d** (upper) shown with the normal spectrum (lower). Line width assumed in simulation, 2.0 Hz.

measurements described below.

Nuclear Overhauser Enhancement (NOE) Measurements.[29] Because of the small ring size and rigidity, hence the potential nearness of transannular substituents on the peptide macrocycle, steady-state NOE measurements have been a particularly useful tool for the assignment of the solution conformation of the *p*-phencyclopeptine nucleus.[10a] The present experiments, summarized in Table IV, have been undertaken to address two major conformational objectives: (1) to determine the geometries of both N3–C4 and N6–C7 amides and their relative orientation to the peptide macrocycle and (2) to assign the NMR spectra of the four aromatic protons. In Figure 9, a representative NOE difference spectrum used for the conformational analysis of cyclopeptide **2d** is illustrated.

***N*-Methyl *p*-Phencyclopeptine 20.** To determine in which direction the N3–C4 amide carbonyl points relative to the cyclopeptide macrocycle, NOEs from the N^3-methyl group of **2a** were measured. Steady-state saturation of the N^3-methyl group at 2.95 ppm provided enhancements of resonances at 4.85 (14%

(29) Due to the presence of closely spaced lines in the ^{1}H NMR spectra of both cyclopeptides **2a** and **2d**, the selective saturation of a single resonance was often impossible to realize. This complicates the interpretation of such steady-state ^{1}H NOE measurements because even a small "spillover" of saturation to an adjacent resonance could lead to an artifactual enhancement of other resonances. Based on a complete steady-state NOE investigation of **2a** and **2d** (not shown in Table IV), these cross-saturation effects could be identified. The NOE values reported in Table IV are therefore truly attributable to the irradiated proton in question. In addition, the NOE's reported in this study (Table IV) are strictly qualitative, and their comparison with theoretical values according to the method of Glickson et al. (Glickson, J. D.; Gordon, S. L.; Pitner, T. P.; Agresti, D. G.; Walter, R. *Biochemistry* **1976**, *15*, 5721) is impractical.

Table IV. Steady-State ^{1}H NOE Measurements for *p*-Phencyclopeptines **2a** and **2d**[29]

irradiated proton		enhanced proton		
chemical shift, ppm	assignment	NOE, %[a]	chemical shift, ppm	assignment
		2a		
2.95	N3-Me	14.0	4.85	5β
		0.8	7.03	15β
4.23	9α	19.5	4.80	9β
		3.2	2.21	8β
		1.0	6.77	12α
		−1.1	7.09	16β
6.77	12α	6.8	7.11	13α
		1.4	2.66	8α
		0.9	3.57	19α
		0.7	4.23	9α
		−0.4	2.21	8β
2.21	8β	24	2.66	8α
		3.3	4.23	9α
		1.5	4.80	9β
		2d		
6.36	N3-H	5.5	4.29	5β
		2.5	2.91	2β
		1.6	7.13	15β
4.62	9β-H	23.1	4.27	9α
		4.7	7.19	16β
6.85	12α-H, 13α-H	4.3	2.98	1α
		2.6	3.50	19α
		2.0	2.74	8α
		−0.6	3.31	19β
		−0.8	2.20	8β

[a] NOE values were obtained by a peak ratio method. If the enhanced resonance was a multiplet, the average ratio for each peak of the multiplet was reported.

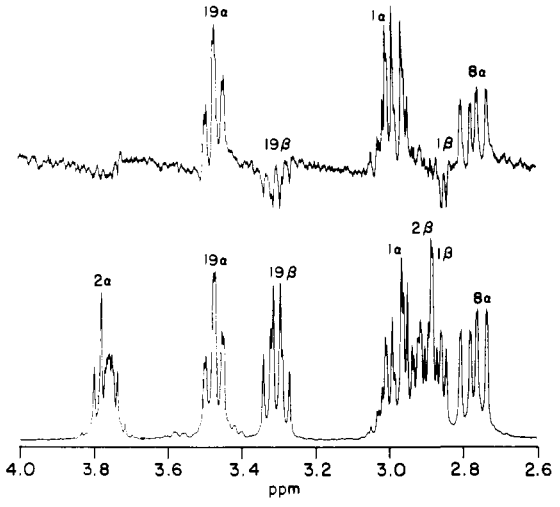


Figure 9. NOE difference spectrum for **2d** at 360 MHz obtained by steady-state saturation of the 12α and 13α proton resonances at 6.85 ppm (upper) shown with the normal spectrum (lower).

NOE) and 7.03 ppm (0.8% NOE). The significant NOE to the C5–H resonance at 4.85 ppm is consistent with the X-ray crystallographic results, which show the *N*-methyl group to be on the same side (β face) of the 14-membered ring and therefore quite close to the 5β-H.[30] On the basis of this NOE result we can place the conformational constraint that the 5β-H and the N^3-methyl groups lie on the same face of the peptide macrocycle of **2a**. Furthermore, these NOE results indicate that the N3–C4 amide adopts a trans configuration, since a cis geometry would orient the N^3-methyl group particularly distant from the 5β-H.

A smaller but significant NOE was also observed from the N^3-methyl group to one of the aromatic protons at 7.03 ppm. Because of the steric restrictions to free rotation of the benzene ring (which is evident from a CPK model of **2a**) and the close proximity of the "β" facing the *N*3-methyl group and the 15β-H of the aromatic ring, we have assigned the resonance at 7.03 ppm to the 15β-H. From the spectrum simulation (see supplementary material, Figure S8), this assignment therefore necessitates the assignment of the 7.09-ppm resonance to the 16β-H.

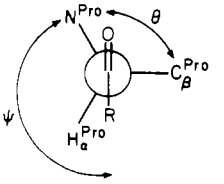
The resonances attributed to the 12α- and 13α-H were distinguished by NOE experiments as well. Examination of molecular models reveals the close orientation of the 9α and 9β hydrogen atoms to 12α-H and 16β-H, respectively. Hence, when irradiation of the 9α-H at 4.23 ppm led to a 1.0% NOE to the aromatic resonance at 6.77 ppm, this multiplet was assigned to the 12α-H. Similarly, steady-state saturation of the 9β resonance at 4.80 ppm effected a significant NOE of the aroamtic group at 7.09 ppm, consistent with the 16β assignment. These assignments of the aromatic protons were further supported by NOE's from 12α-H (6.77 ppm) to both 8α-H (2.66 ppm, 1.4% NOE) and 19α-H (0.9% NOE). The observed NOE from 12α-H to the proline 19α-H provides strong evidence for the assignment of the trans geometry for the N6–C7 amide (see later discussion). Additional supporting evidence for the assignment of the amide geometries is presented below.

NH *p*-Phencyclopeptine 2d. As in the case of the *N*-methylcyclopeptide **2a**, the trans conformation of the N3–C4 amide was ascertained by NOE measurements. This was demonstrated by the significant NOE (5.5%) from the N3 amide proton to the 5B proton. Furthermore, a pronounced NOE to the resonance at 2.91 ppm attributable to the 2β proton also confirms the trans configuration of the N3–C4 amide where the N3 amide proton points toward the same side (β face) of the macrocyclic ring of **2d**.

The assignment of the aromatic protons was again made possible by NOE measurements. A 4.75% NOE from the 9β-H to the aromatic residue at 7.19 ppm confirms the 16β assignment to this resonance. The assignments of resonances at 7.13 ppm to 15β-H and the two proton resonances at 6.85 ppm to 12α-H and 13α-H follow from the spectrum simulation (see supplmentary material, Figure S9). These assignments received support from the dipolar coupling observed between the 12α,13α pair at 6.85 ppm and 1α-H (4.3% NOE), 19α-H (2.6% NOE), and 8α-H (2.0% NOE). As in the case of the *N*-methyl cyclopeptide, this NOE from the aromatic protons at 6.85 ppm to the proline 19α′-H supports the trans β geometry of the N6–C7 amide (see later discussion).

It is interesting to note that small negative NOE's to 8β and 19β protons are observed upon irradiation of the 12α,13α proton pair. Such negative enhancements are characteristic of indirect dipole–dipole interactions involving a linear orientation of three spins.[31] Consistent with this interpretation is the observation of large positive NOE's to 8α- and 19α-H originating from direct dipole–dipole interactions between irradiated (12α,13α) and observed (8α,19α) protons.

^{13}C NMR Spectra of *p*-Phencyclopeptines 2a and 2d.[32] Owing to extensive NMR conformational and comparative X-ray studies on cyclic peptides containing L-proline, a correlation between the X–Pro amide geometry and the ^{13}C NMR chemical shifts of the proline Cβ and Cγ resonances has been drawn.[33-37] A proposed



$$\Delta\delta_{\beta\gamma} = 0.081|\theta| + 2.47 \quad \text{For cis X-Pro} \quad (3)$$

$$\Delta\delta_{\beta\gamma} = 0.036|\theta| + 0.73 \quad \text{For trans X-Pro} \quad (4)$$

(30) The definition of β and α face of the peptide macrocycle is provided in Table V.

(31) Noggle, J. H.; Schirmer, R. E. "The Nuclear Overhauser Effect. Chemical Applications"; Academic: New York, 1971; pp 57–64.

(32) ^{13}C NMR spectroscopy has been used in a previous study of the solvent-induced effects on the conformation of frangulanine, a natural *p*-phencyclopeptine.[10a]

Table V. [^{13}C]Proline C$^{\beta}$ and C$^{\gamma}$ Chemical Shifts in $CDCl_3$[a]

peptide	δ-(C$^{\beta}$≡C17)	δ-(C$^{\gamma}$≡C18)	$\Delta\delta_{\beta\gamma}$	predicted θ(ψ)[b] trans	cis
2a	28.45	24.71	3.74	84° (144°)	16° (76°)
2d	26.44	24.93	1.51	23° (83°)	c

[a] Chemical shifts are reported in ppm downfield from Me_4Si. Assignments are based on heteronuclear decoupling experiments. [b] According to the method of Siemion et al.;[32] see eq 3 and 4. [c] For this small value of $\Delta\delta_{\beta\gamma}$, the cis (eq 4) gives a nonreal solution.

semiempirical relationship between the proline angle and the chemical shift difference between the proline Cβ and Cγ resonances[34,38] has received additional support from X-ray, circular dichroism, and ^{1}H NOE measurements.[37,39] According to this interpretation,[34] the difference in the ^{13}C chemical shift of the β and γ proline carbons ($\Delta\delta_{\beta\gamma}$) is proportional to the dihedral angle θ between the proline carbonyl (in this case C4) and the Cα–Cβ (C5–C17) bond by the relationships of eq 3 and 4. With this method, information regarding the orientation of the X–Pro amide bond, cis or trans, can be obtained.

For this reason, the ^{13}C NMR spectra of *p*-phencyclopeptines **2a** and **2d** were obtained. By use of eq 3 and 4, dihedral angles θ and ψ for the L-proline residues of *p*-phencyclopeptines **2a** and **2d** were calculated from the ^{13}C data shown in Table V. From this analysis, only a trans N6–C7 amide configuration is possible for the NH cyclopeptide **2d** because the smallest allowed value for $\Delta\beta_{\beta\gamma}$(cis) is 2.47 ppm. The experimental value for $\Delta\delta_{\beta\gamma}$ is somewhat smaller, at 1.51 ppm. For a trans N6–C7 amide bond, eq 4 predicts dihedral angles θ of 84° and 23° for **2a** and **2d**, respectively. By use of eq 3, a 16° dihedral angle θ is predicted for a cis N6–C7 amide bond if present in cyclopeptide **2a**. As we shall now show, the trans N6–C7 assignment for both cyclopeptides is more consistent with the experimental results.

Conformational Analysis and Discussion

X-ray Structure vs. Solution Conformation of 2a. Examination of CPK molecular models of cyclopeptide **2a** reveals that the *p*-phencyclopeptine nucleus can adopt eight possible conformations that depend on the geometry (cis or trans) of the two amides. These isomeric forms are summarized in Table VI. The following analysis will describe the evidence that the *p*-phencyclopeptine **2a** adopts only one of these conformations (isomer 1, Table VI), identical with the geometry predicted by X-ray crystallography.

The X-ray structure of **2a** (Figure 1) shows that the *N*-methylcyclopeptide adopts the trans,trans configuration in the crystal state (isomer 1, with the carbonyls pointing toward opposite sides of the peptide macrocycle). A very similar conformation of N-methylated frangulanine methiodide, the permethylated derivative of the natural product frangulanine (**1**), has been demonstrated by X-ray crystallography.[6,7]

That the solution conformation of **2a** is nearly identical with the crystal structure is strongly supported by these NMR investigations. First, with respect to the geometry of the two amides, the trans,trans assignment has been based mainly on steady-state ^{1}H NOE and ^{13}C NMR measurements. The pronounced NOE from the *N*-methyl group to the proline 5β-H (14% NOE, Table IV) supports the trans (α) geometry of the N3–C4 amide since the interproton distances for the H–C5–C4(0)–N3–H system would be considerably larger for trans (β) and cis (α or β) geometries of the N3–C4 amide. Consistent with the trans (α) assignment for the N3–C4 amide is the small coupling between the N^3-methyl group and the 2α-H, suggesting the near planarity and trans orientation of the H_3C–N3–C2–Hα system. Additionally, steady-state saturation of the N^3-methyl and 1α-H resonances gives NOE's to different aromatic resonances at 7.03 and 6.77 ppm, respectively. This result agrees with the assignment of the trans (α) geometry for the N3–C4 amide where the *N*-methyl group is directed toward the β face of the cyclopeptide **2a**.

Table VI. Conformational Isomers of the Dihydro-*p*-phencyclopeptine Ring System[a]

isomer	N6–C7 (amide)[b]	N3–C4 (amide)	isomer	N6–C7 (amide)[b]	N3–C4 (amide)
1[c]	trans (β)	trans (α)	5	cis (α)	trans (α)
2	trans (β)	trans (β)	6	cis (α)	trans (β)
3	trans (β)	cis (α)	7	cis (α)	cis (α)
4	trans (β)	cis (β)	8	cis (α)	cis (β)

[a] The Greek symbols α and β represent the face of the macrocyclic ring (lower and upper, respectively) to which the carbonyl oxygen is pointing when the molecule is oriented as shown in Figure 1 with the L-proline α hydrogen (5-H) directed toward the upper (β) face of the macrocycle. The conformational isomers assume cis or trans planar amide geometries. [b] Owing to the small ring size of the *p*-phencyclopeptine molecule the trans (α) and cis (β) conformations of the N6–C7 amide moieties are geometric impossibilities. No such constraints exist for the N3–C4 primary amide. [c] This conformation represents that determined by X-ray crystallography as shown in Figure 1.

The assignment of the trans (β) geometry for the N6–C7 amide was based upon the observed NOE from the aromatic 12α-H resonance to one of the proline δ methylenes (C19α). Examination of molecular models shows that the intramolecular distance between the aromatic 12α-H and the proline 19α-H would be considerable (>3 Å) if the N6–C7 amide adopted a cis geometry. Supporting evidence for the assignment of the trans geometry for the N6–C7 amide has been obtained by ^{13}C NMR spectroscopy (Table V). By use of the method of Siemion et al.[34] eq 3 and 4 predict proline ψ angles of 144° and 76° for trans and cis N6–C7 amide geometries, respectively.[40] Close examination of molecular models reveals that a ψ of 76° is unfavorable owing to severe steric interaction with the transannular benzene ring of the *p*-phencyclopeptine nucleus. In fact, a CPK molecular model of the *p*-phencyclopeptine system with the ψ angle of 76° for the N6–C7 amine cannot be constructed. A trans N6–C7 amide geometry with ψ = 144° is considerably more reasonable.

The vicinal coupling constants of the two four-spin systems, 8α–8β–9α–9β and 1α–1β–2α–2β, provide additional indices of ring conformation. In the earlier discussion we have shown that by utilizing the X-ray bond angles with the generalized Karplus relationships of eq 1 and 2,[26] spectra have been simulated that closely match the experimental spectra for both four-spin systems.

The similarity of the crystalline vs. solution conformations of the *N*-methyl cyclopeptide **2a** was further demonstrated by fitting the X-ray vicinal angles to a Karplus relationship of the general form of eq 5. Regression of the experimental values of ^3J with

$$^3J(\theta) = A\cos^2\theta - B \qquad (5)$$

the vicinal θ's for the 8–9 and 1–2 four-spin systems obtained from X-ray crystallography yielded the parameters shown in Table VII parts a and c. The excellent fit (R^2 = 98.6) of the 1–2 four-spin system suggested that the geometry in the crystal was very similar to that in solution. The poorer fit (R^2 = 89.6) of the 8–9 spin system may suggest a greater mobility of this region in solution; however, this result clearly demonstrates the similarity of solution and crystal conformations.

(33) Madison, V. In "Peptides, Polypeptides and Proteins", Blout, E. R., Bovey, F. A., Goodman, M., Lotan, N., Eds.; 1974, Wiley: New York; pp 89–98.
(34) Siemion, I. Z.; Wieland, T.; Pook, K. H. *Angew. Chem., Intl. Ed. Engl.* **1975**, *14*, 702.
(35) Pease, L. G.; Watson, C. *J. Am. Chem. Soc.* **1978**, *100*, 1279.
(36) Pease, L. G.; Niu, C-H.; Zimmerman, G. *J. Am. Chem. Soc.* **1979**, *101*, 184.
(37) Gierasch, L. M.; Deber, C. M.; Madison, V.; Niu, C-H.; Blout, E. R. *Biochemistry* **1981**, *20*, 4730.
(38) A description of the conventions used for the dihedral angle nomenclature is in IUPAC–IUB Commission on Biochemical Nomenclature 1970 (*Biochemistry* **1970**, *9*, 3471–3479).
(39) Madison, V.; Kopple, K. *J. Am. Chem. Soc.* **1980**, *102*, 4855.
(40) The validity of the method of Siemion, et al. (ref 34) for N-methylated proline peptides has not yet been demonstrated and the interpretation of ψ's from the experimental $\Delta\delta_{\beta\gamma}$ values may be in error.

Table VII. Calculated Karplus Parameters for the Two Aliphatic Four-Spin Systems of 2a and 2d

spin system	$^3J(\theta) = A\cos^2\theta - B$ A	B	θ_0[a]	R^2
N-Methyl-*p*-phencyclopeptine, 2a				
1α–1β–2α–2β				
(a) regression best fit (X-ray)	14.5	−0.711	42.5°	98.6
(b) minimization method (solution)	13.9	−0.278	45.1°	
8α–8β–9α–9β				
(c) regression best fit (X-ray)	9.52	0.681	84.4°	89.7
(d) minimization method (solution)	10.8	0.169	74.8°	
NH Cyclopeptide, 2d				
1α–1β–2α–2β				
(e) minimization method (solution)	11.5	0.489	42.1°	
8α–8β–9α–9β				
(f) minimization method (solution)	11.1	0.089	76.6°	

[a] θ_0 is defined as the angle between vicinal protons on the same face of the cyclopeptide (note that the vicinal dihedral angles must obey the following geometric constants: $\angle 1\alpha\text{–}2\alpha = \angle 1\beta\text{–}2\beta$ and $\angle 8\alpha\text{–}9\alpha = \angle 8\beta\text{–}9\beta$).

Comparison of Solution Conformations of 2a and 2d. Substantial differences in the ^{1}H NMR spectra of cyclopeptides **2a** and **2d** (Figure 2, Table II) led to an earlier suggestion that the two *p*-phencyclopeptines may exhibit different ring conformations in solution.[14] As we shall show, closer examination of the present results reveals that the solution conformations of **2a** and **2d** are actually quite similar.

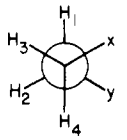
Analogous to the methodology described above, ^{1}H NOE and ^{13}C NMR spectral analyses support the trans–trans isomer (Table V) for the NH cyclopeptide **2d**. A significant NOE (5.5%) from N3–H to the proline α proton (C5–H) is consistent with the trans (α) assignment for the N3–C4 amide configuration. Indicative of the trans (β) geometry for the N6–C7 amide of **2d** is the 2.6% NOE for the aromatic protons C12α and C13β (6.85 ppm) to one of the proline δ protons (C19α–H) at 3.50 ppm. The ^{13}C NMR results summarized in Table V also support the trans N6–C7 amide geometry with a predicted ψ of 83°. Furthermore, the method of Siemion et al.[34] gives a value of 1.51 ppm for $\Delta\delta_{\beta\gamma}$ which is too small to be consistent with a cis N6–C7 amide bond. Thus the trans assignment for the N6–C7 amide moeity was made.

The H–N3–C2–Hα and H–N3–C2–H β dihedral angles θ have been calculated for **2d** by using the relationship of eq 6 derived

$$^3J_{\mathrm{HNC}\alpha\mathrm{H}}\ (\mathrm{Hz}) = 6.55\cos^2\theta + 1.55\cos\theta + 1.35 \quad (6)$$

by Ramachandran et al.[41] These predicted dihedral angles for H–N3–C2–Hα and H–N3–C2–Hβ, 150° and 29.1°, respectively, lend still further support to the trans (α) assignment for the N3–C4 amide geometry of **2d**.

Comparative examination of the 8α–8β–9α–9β four-spin systems of *p*-phencyclopeptines **2a** and **2d** was accomplished next. By use of the Karplus relationship derived from the regression best fit of the experimental data for the 8α–8β–9α–9β four-spin system (Table VII, parameters in part c) and experimental ^3J values (see supplementary material, Figure S7), newly derived angles ($\angle 8\alpha,9\alpha = 42.6°$, $\angle 8\alpha,9\beta = 44.3°$) indicated a conformation of the 8,9 four-spin system of **2d** similar to that of the *N*-methyl cyclopeptide **2a**. However, when the appropriate vicinal angles thus derived were summed, the geminal angles were found to be approximately 109° when viewed down the C–C bond axis. Since the projection of a tetrahedron should yield geminal angles of 120°, we felt a need to develop a Karplus-type equation with the additional constraints that the projection of the geminal angles be fixed at 120°. When the geminal angles are thus fixed, the vicinal angles are interrelated as follows: $\angle 2,4 = \angle 1,3 = \theta_0$, $\angle 1,4 = 120 - \theta_0$, $\angle 2,3 = 120 + \theta_0$ (where the numbers refer to the protons represented in the Newman projection shown below).

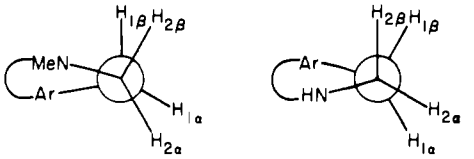


With these constraints, a new Karplus relationship of the general form of eq 5 was then determined by minimizing the function to give eq 7. The resulting best fit of the experimental ^3J values

$$F = \sum_{i=1}^{4}(J_i^{\mathrm{exptl}} - J_i^{\mathrm{calcd}})^2 = \sum_{i=1}^{4}(J_i^{\mathrm{exptl}} - A\cos^2\theta + B)^2 \quad (7)$$

of the 8,9 four-spin system for **2a** and **2d** gave the parameters for *A*, *B*, and θ_0 in Table VII, sets d and f, respectively. The striking similarity of these minimization parameters for the 8,9 four-spin system derived for both cyclopeptides demonstrates the near identity of the conformations of the two cyclopeptides in the C8–C9 region. The best fit θ's based on this analysis are shown in Table III along with the vicinal angles (θ) for **2a** obtained from the X-ray analysis. This comparison shows the similarity between the X-ray conformation of **2a** and the solution conformations at C8 and C9 for both **2a** and **2d**.

In contrast to the analysis of the 8,9 four-spin systems of **2a** and **2d**, a similar comparison of the 1α–1β–2α–2β–N3–H five-spin system of **2d** with the 1α–1β–2α–2β four-spin system of **2a** reveals significant conformational differences. With the application of the minimization procedure of eq 7 using the experimental ^3J values and the Karplus parameters shown in Table VII (b and e), the best fit bond angles (θ's) for the 1,2 spin systems of both *p*-phencyclopeptines were calculated (Table III). From this analysis it is clear the the C1–C2 bond of **2a** is twisted by approximately 90° relative to that of **2d**. Represented by Newman projections shown below, this conformational difference is analogous to boat–chair isomerism observed in cyclohexanes.



Conformer 1A For **2a** Conformer 1B For **2d**

In summary, these results show that the two cyclopeptides have very similar overall conformations, that of isomer 1, possessing two trans amide conformations (Table VI), differing only in the geometry of the C1–C2–N3 region of the molecule. In later discussions we will refer to the slightly different conformations of **2a** and **2d** conformers 1A and 1B, respectively (see Newman projections).

Examination of molecular models with these two different C1–C2–N3 geometries now reveals a reasonable explanation for the differences in the ^{1}H NMR spectra of the two cyclopeptides. The significant chemical shift difference between the resonances assigned to the 2α-H, 4.89 and 3.80 ppm for **2a** and **2d**, respectively (Figure 2), can be rationalized by the influence of the nearby C4 carbonyl group. For conformer 1A, the 2α-H and C4 carbonyl bond axes lie nearly in the same plane and therefore experience a quasi 1,3-diaxial interaction. Such an orientation agrees with the unusually low-field value for the 2α-H of **2a**, which results from deshielding by the neighboring C4 carbonyl moiety. For conformer 1B, the orientation of the 2α-H and C4 carbonyl groups is considerably different. Because the 2α-H lies significantly out of the plane of the amide carbonyl in this conformer, it is reasonable that its resonance occurs at a higher field value.

The difference in the chemical shift position of the 13α-H resonance of **2a** and **2d** is also consistent with the assignment of conformers 1A and 1B to **2a** and **2d**, respectively. Since the 13α-H

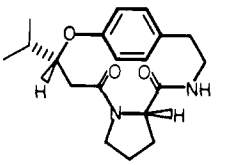
(41) Ramachandran, G. N.; Chandrasekaran, R.; Kopple, K. D. *Biopolymers* **1971**, *10*, 2113.

lies above the plane of the C4 carbonyl we might expect this resonance to be shielded by the carbonyl π system. Inspection of molecular models reveals that the distance between the C4 carbonyl and 13α-H is considerably smaller for conformer 1B of **2d**. The high-field position of the 13α-H resonance of **2d** can thus be rationalized.

The conformational differences between *N*-methyl **2a** and NH **2d** cyclopeptides that involve the C1–C2 region are also consistent with the ability of the NH cyclopeptide to form a hydrogen bond between the N3-H and *C*7-carbonyl groups. A similar type of hydrogen-bonding interaction (ie, an $i + 2 \rightarrow i$ hydrogen bond) has been observed for other cyclopeptides with γ turns.[35,36] In order to decrease the distance between N3-H and the C7 carbonyl oxygen and therefore strengthen this H-bonding interaction, the *p*-phencyclopeptine adopts the geometry of conformer 1B. With the substitution of a methyl group on N3 in **2a**, the preferred geometry is that of conformer 1A, which minimizes transannular steric interactions between the N^3-methyl group and the C7 carbonyl and diaxial 1,2 interactions between N^3-methyl and 2β-H moieties.

Consistent with this H-bonding interaction (γ turn) is the difficulty we have observed in exchanging the N3-H of **2d** with deuterium. Under conditions that readily exchange the amide proton of the acyclic *p*-phencyclopeptine precursors, exchange of N3-H of **2d** with D_2O does not occur. The chemical shift of the N3-H resonance at 6.36 ppm is at an unusually high-field position for an H-bonded amide proton. Inspection of molecular models shows that this proton lies directly above the aromatic ring of the *p*-phencyclopeptine nucleus and consequently may be shielded by the induced ring current of the benzene ring.

In conclusion, on the basis of our X-ray crystallographic analysis and NMR investigations, we have assigned one of the eight possible conformations of the *p*-phencyclopeptine nucleus (i.e., isomer 1, Table VI; Figure 1) to both cyclopeptides **2a** and **2d**. With regard to these conformational assignments, we presented NMR spectroscopic evidence that the solution conformations of the NH cyclopeptide **2d** and another NH *p*-phencyclopeptine **4**,



4

which contains the natural product substitution pattern at C9 (i.e., a C9-isopropyl group), were identical.[11] On the basis of the present conformational analysis, the original assignment[11] of the *R* absolute configuration for C9 as drawn in structure **4** has been confirmed. Hence, since all *p*-phencyclopeptines found in nature possess *R* stereochemistry at C9,[9] our methodology for the preparation of the *p*-phencyclopeptine nucleus[11,14] is applicable to the total synthesis of these natural products with the correct absolute stereochemistries.

The question of the nature of metal ion binding with the *p*-phencyclopeptine nucleus implicated in earlier investigations[14] remains to be addressed. Whether both carbonyls are involved in metal binding or possibly one nitrogen and one carbonyl will be studied in future investigations. The preference of divalent alkaline earth elements Ca^{2+} and Mg^{2+} for oxygen ligands is well-known; however, the *p*-phencyclopeptine nucleus would have to undergo a significant conformational change to allow ion complexation with both carbonyl oxygen atoms. Another possibility for ion complexation is that the metal replaces the N3-H involved in hydrogen bonding. This suggestion implies that the *N*-methyl cyclopeptide **2a** would not bind metals. Preliminary metal ion titrations of **2a** monitored by circular dichroism reveal, however, that the *N*-methyl cyclopeptide **2a** also undergoes metal-induced conformational change. Hence, the nature of the *p*-phencyclopeptine–metal complex remains an enigma. Experiments to answer these questions are in progress.

Acknowledgment. The use of a Nicolet 360-MHz NMR spectrometer was made possible from grants from the UC Davis NMR Facility (to J.C.L.) and from NSF Grant CHE 79-04832 (to the U.C.D. Chemistry Department). We are grateful for the discussions and advise of Drs. R. J. Abraham, J. Dallas, and K.-E. Falk. The research was supported in part by the Director, Office of Energy Research, Office of Basic Energy Sciences, Chemical Sciences Division of the U.S. Department of Energy, under Contract W-7405-ENG-48, and the NSF (Grant PCM 81-08090 to J.C.L.).

Registry No. 2a, 69100-23-8; **2b**, 69100-22-7.

Supplementary Material Available: Full details of the X-ray crystallographic determination including coordinates, anisotropic temperature factors, distances, angles, and structure factor tables, calculated 270-MHz ^{1}H NMR spectra for seven- and four-spin systems of **2a** and **2d**, contour map of the 2.6–3.1-ppm region of **2d**, 270-MHz ^{1}H NMR 2-D *J* spectra of **2a** and **2d**, and 270-MHz ^{1}H 45° projection sums of the 2-D *J* spectra of **2a** and **2d** (21 pages). Ordering information is given on any current masthead page.