Peptide Conformations. 15.[1] One- and Two-Dimensional ^{1}H, ^{13}C, and ^{15}N NMR Studies of *cyclo*(Pro-Phe-Gly-Phe-Gly)$_n$ (n = 1, 2): Selective Complexation of Lithium Ions (n = 1) and Potassium Ions (n = 2)

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Abstract: The cyclic pentapeptide *cyclo*(Pro-Phe-Gly-Phe-Gly) (**1**), the cyclic decapeptide with dimer sequence **2**, and four specifically deuterated derivatives were synthesized and studied by NMR spectroscopy. Two-dimensional correlation techniques, such as SECSY spectroscopy to determine proton connectivities, and ^{1}H–^{13}C as well as ^{1}H–^{15}N shift correlation were applied. Carbon chemical shifts and $^1J_{NH}$ coupling constants measured by the INEPT technique prove the all-trans conformation of the amide bonds. A conformation containing two intramolecular hydrogen bonds (βI about the amino acids Gly^5-Pro^1-Phe^2-Gly^3 and γ^i about Gly^3-Phe^4-Gly^5) was derived for **1**, whereas a conformation with four internally oriented hydrogen bonds similar to a β-pleated sheet fits best the experimental data for **2**. Complexation of lithium and sodium ions by **1** yields drastic changes in CD and ^{1}H NMR spectra. The conformation of the peptide within the lithium complex was derived from the ^{1}H NMR data. Contrary to the preferred binding of Li^+ by **1**, only potassium forms a weak complex with the cyclic decapeptide **2**.

Cyclic pentapeptides are very suitable to conformational analysis by NMR spectroscopy due to their structural restrictions.[2–5] We report here our conformational studies of the cyclic pentapeptide *cyclo*(Pro^1-Phe^2-Gly^3-Phe^4-Gly^5) (**1**),[4] the cyclic decapeptide *cyclo*(Pro^{1,6}-Phe^{2,7}-Gly^{3,8}-Phe^{4,9}-Gly^{5,10}) (**2**), and their complexation with alkali ions.

The Cyclic Pentapeptide 1

Conformation in Solution by means of ^{1}H, ^{13}C, and ^{15}N One- and Two-Dimensional NMR Spectroscopy. The 270-MHz ^{1}H NMR spectra of **1** in the solvents Me_2SO, $CDCl_3$, CD_3CN, and their binary mixtures were recorded at different temperatures. The connectivities of the protons within their spin systems in the different amino acids have been determined by a two-dimensional SECSY spectrum,[6] which is presented in Figure 1. The most interesting feature of the SECSY spectrum is the clear detection of the long-range coupling of the Phe β-protons with the aromatic ring protons. In order to establish an unequivocal assignment of the doubled amino acids within the sequence, we measured the ^{1}H NMR of selectively α-deuterated derivatives *cyclo*(Pro-[2-H^2]Phe-Gly-Phe-Gly) and *cyclo*(Pro-Phe-Gly-Phe-[2-H^2]$_2$Gly). An independent confirmation results from NOE difference measurements: saturating a signal of an NH proton yields a NOE effect at the α-proton of the preceding amino acid.[7] The results of the ^{1}H NMR analysis are shown in Tables I–IV.

At the beginning of each interpretation of NMR spectra there is the question of conformational homogeneity. The big chemical shift differences of the diastereotopic glycine protons, $\Delta\delta_\alpha$ (Me_2SO: Gly^3 0.86, Gly^5 0.59; $CDCl_3$: 1.25 and 0.85, respectively; CD_3CN: 1.05 and 0.90, respectively, ppm), can be interpreted as a hint to the preference of one conformation. This value ($\Delta\delta_\alpha$) is found to be particularly large[8] in the case of a cyclic oligopeptide with two to six amino acids when glycine is followed by a chiral amino acid with an aromatic side chain (in this case Gly^3-Phe^4) and/or when there is a β turn with Gly in position i (in this case Gly^5, vide infra).

Table I. 270-MHz ^{1}H NMR Data of **1** in Me_2SO-d_6 at 298 K (32 K Data Points)

	Pro^1	Phe^2	Gly^3	Phe^4	Gly^5
			δ		
NH		7.66	7.80	8.34	7.74
$C_\alpha H$	4.004	4.461	A 4.034	4.514	A 4.139
			B 3.169		B 3.548
$C_\beta H$	1.987	A 3.232		A 3.086	
	1.485	B 2.810		B 2.848	
$C_\gamma H$	1.845				
$C_\delta H$	3.717				
	3.498				
			J_{ij}, Hz		
$^3J_{HNC_\alpha H}$		8.42	A 7.28	8.58	A 5.87
			B 3.09		B 4.15
$^3J_{HC_\alpha C_\beta H}$		A 5.42		A 5.97	
		B 9.78		B 9.35	
$^2J_{HC_\alpha H}$			13.18		15.96
$^2J_{HC_\beta H}$		13.91		14.22	
			$\Delta\delta/T$,[a] ppb/K		
NH		4.5	1.9	7.8	1.3

[a] Temperature coefficient; see text.

Likewise the big difference in temperature gradients of the chemical shifts of the NH protons $\Delta\delta_{NH}/T$ in Me_2SO confirm the previous considerations.[2–4] Beyond this the similarity of the spectra in the three different solvents is an indication that the peptide's conformation remains unchanged. So it seems to be justified to combine the results in the different solvents.

The carbon chemical shifts of the β and γ carbon of proline (δ (Me_2SO) 28.8 and 24.4; ($CDCl_3$) 29.6 and 24.6; (CD_3CN) 30.1 and 25.5) are consistent only with the trans conformation of the amide bond.[9] The assignment of the high-field region (0–70 ppm) was done by a two-dimensional ^{1}H–^{13}C shift correlation[10] (Figure 2). This method is particularly advantageous for molecules with diastereotopic protons (Gly) and doubled amino acids.

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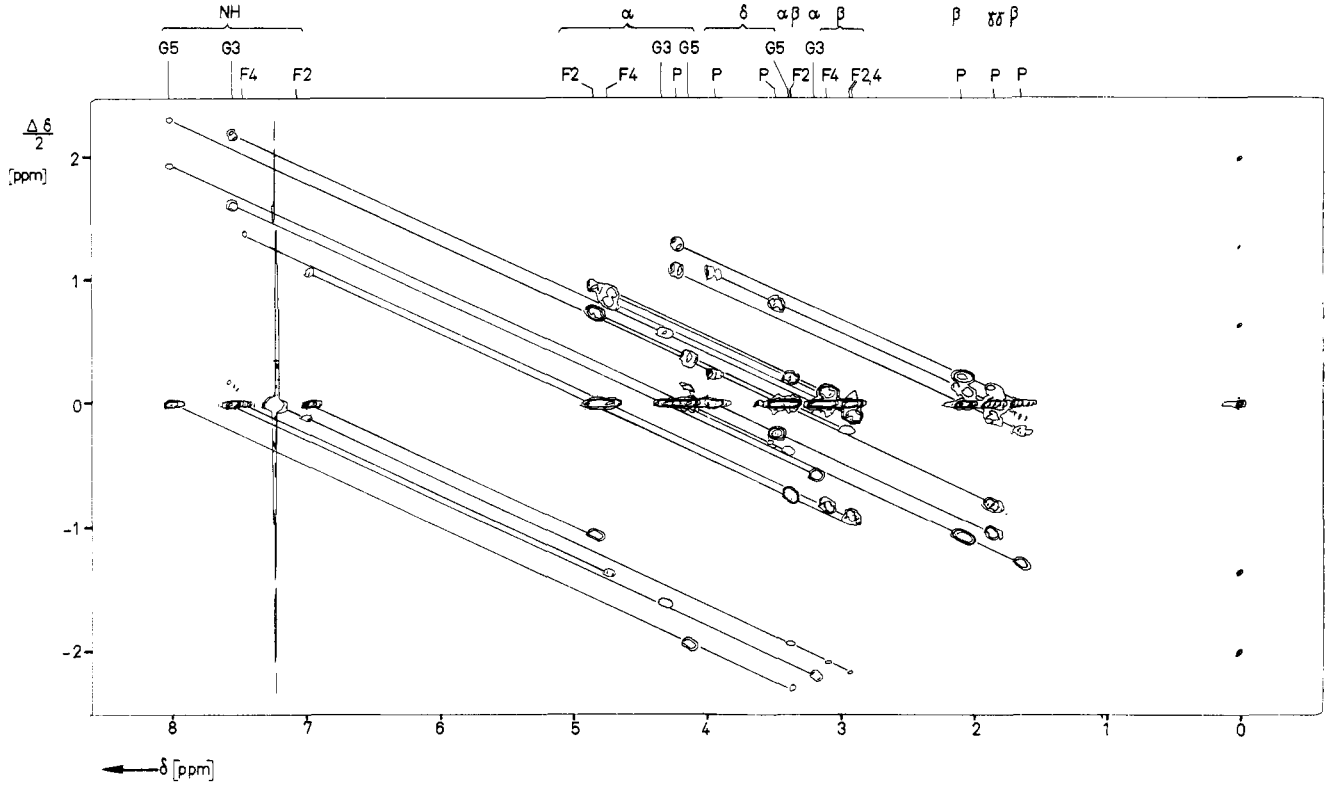

Figure 1. 270-MHz SECSY spectrum of *cyclo*(Pro-Phe-Gly-Phe-Gly) (150 mM in $CDCl_3$ at 298 K; see Experimental Section for details).

Table II. 270-MHz ^{1}H NMR Data of **1** in $CDCl_3$ at 298 K (32 K Data Points)

	Pro[1]	Phe[2]	Gly[3]	Phe[4]	Gly[5]
			δ		
NH		6.85	7.49	7.32	7.90
$C_\alpha H$	4.267	4.851	A 4.408	4.755	A 4.163
			B 3.150		B 3.309
$C_\beta H$	2.096	A 3.391		A 3.092	
	1.675	B 2.978		B 2.899	
$C_\gamma H$	1.860				
$C_\delta H$	3.956				
	3.487				
			J_{ij}, Hz		
$^3J_{HNC_\alpha H}$		8.58	A 8.58	8.75	A 5.94
			B 3.30		B 4.62
$^3J_{HC_\alpha C_\beta H}$		A 6.59		A 8.26	
		B 8.58		B 7.58	
$^2J_{HC_\alpha H}$			13.53		14.84
$^2J_{HC_\beta H}$		13.52		13.86	
			$\Delta\delta/T$,[a] ppb/K		
NH		5.9	4.1	10.0	7.4

[a] Temperature coefficient; see text.

To prove the assumed trans conformation of the other amide bonds, we measured the coupled and decoupled ^{15}N NMR spectra with the INEPT technique.[11] It is immanent that only protonated nitrogens are detected because with the chosen conditions polarization was transferred only from directly bonded protons.[11] The assignment of the individual resonances was performed by a ^{1}H–^{15}N shift correlation[10] (Figure 3).

The advantage of this two-dimensional procedure compared to conventional selective decoupling experiments lies mainly in the much higher sensitivity due to polarization transfer and in its reliance on the proton relaxation times rather than from long nitrogen T_1's. The size of the coupling constants (Table V) shows

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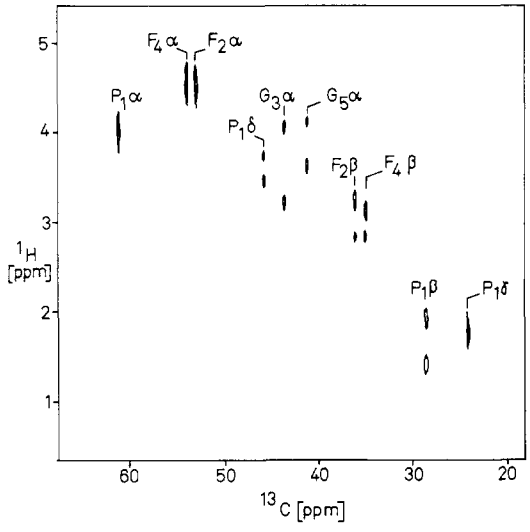

Figure 2. ^{1}H–^{13}C shift correlation of the high-field region of a 330 mM solution of *cyclo*(Pro-Phe-Gly-Phe-Gly) in Me_2SO-d_6 at 298 K; see Experimental Section for details.

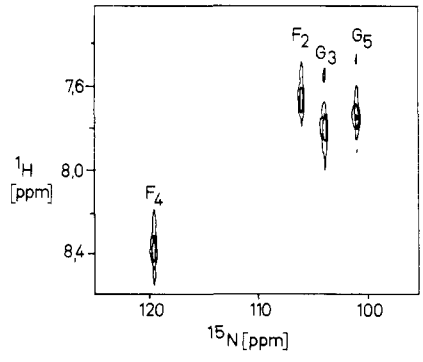

Figure 3. ^{1}H–^{15}N shift correlation of a 330 mM solution of *cyclo*(Pro-Phe-Gly-Phe-Gly) in Me_2SO-d_6 at 298 K; see Experimental Section for details.

Table III. 270-MHz ^{1}H NMR Data of 1 in CD_3CN (32 K Data Points)[a]

	Pro[1]	Phe[2]	Gly[3]	Phe[4]	Gly[5]
			δ		
NH		6.94	7.25	7.07	7.11
$C_\alpha H$	3.989	4.627	A 4.250	4.627	A 4.381
			B 3.196		B 3.478
$C_\beta H$	1.329	A 3.432		A 3.153	
	2.000	B 2.845		B 2.864	
$C_\gamma H$	1.82				
$C_\delta H$	3.71				
	3.48				
			J_{ij}, Hz		
$^3J_{HNC_\alpha H}$		9.95	A 8.58	9.86	A 7.20
			B 1.20		B 2.23
$^3J_{HC_\alpha C_\beta H}$	b	b		A 5.62	
				B 9.98	
$^2J_{HC_\alpha H}$			13.72		16.11
$^2J_{HC_\beta H}$	b	13.89		14.40	
			$\Delta\delta/T$,[c] ppb/K		
NH		4.8	2.6	7.1	2.5

[a] Positions of signals covered by other signals were determined by difference decoupling techniques. [b] Not analyzed. [c] Temperature coefficient; see text.

Table IV. Results of the NOE Difference Experiments of 1 in CD_2Cl_2 (270 MHz, 32 K Data Points)[a]

irradiated signal	observed NOE	%
Phe[2] NH	Phe[2] $C_\alpha H$	3.5
	Pro $C_\alpha H$	1.1
(Gly[3] + Phe[4] NH)	Phe[2] + Phe[4] $C_\alpha H$	6.0
	Gly[3] $C_\alpha H$ (L)	5.5
	Gly[3] $C_\alpha H$ (H)	5.0
Gly[5] NH	Gly[5] $C_\alpha H$ (L)	1.1
	Phe[4] $C_\alpha H$	11.5
Gly[3] $C_\alpha H$ (L)	Gly[3] + Phe[4] NH	8.3
	Gly[3] $C_\alpha H$ (H)	9.1
Gly[3] $C_\alpha H$ (H)	Gly[3] NH	2.1
	Gly[3] $C_\alpha H$ (L)	8.6
Gly[5] $C_\alpha H$ (L)	Gly[5] NH	2.8
	Gly[5] $C_\alpha H$ (H)	7.9
Gly[5] $C_\alpha H$ (H)	Gly[5] NH	4.3
	Gly[5] $C_\alpha H$ (L)	6.8
(Phe[2] + Phe[4] $C_\alpha H$)	Phe[2] NH	1.8
	Gly[3] + Phe[4] NH	2.7
	Gly[5] NH	3.0

[a] H = signals at high field, L = signals at low field.

Table V. 20.28-MHz ^{15}N Chemical Shifts and Coupling Constants $^1J_{NH}$ of a 330 mM Solution of 1 in Me_2SO-d_6

	δ[a]	$^1J_{HN}$, Hz
Phe-4	119.5	92.7
Phe-2	106.1	93.2[b]
Gly-3	103.9	93.7
Gly-5	101.1	93.2[b]

[a] The chemical shifts were measured relative to external 90% formamide in acetone-d_6 and converted to a standard of liquid NH_3, conversion constant 108.5 ppm. [b] The inner lines of these two doublets overlap and, due to the "up-down pattern" of a doublet measured by INEPT,[11] cancel each other partially so that wrong values for the coupling constant are found. These $^1J_{NH}$ are calculated by doubling the difference between the other undisturbed line and the chemical shift in the decoupled spectrum measured under exactly the same conditions.

clearly that all four peptide bonds are trans oriented.[12]

The ^{15}N resonance of Phe-4 is shifted downfield by 13.4 ppm compared to Phe-2. This could possibly be explained by the intramolecular hydrogen bond to the adjacent CO group of Gly[3]

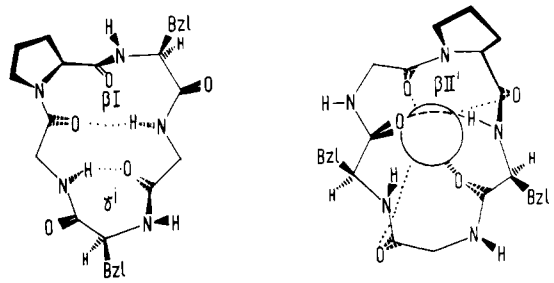

Figure 4. $\beta I\gamma^i$ conformation of *cyclo*(Pro-Phe-Gly-Phe-Gly) (left) and conformation of the lithium complex (right).

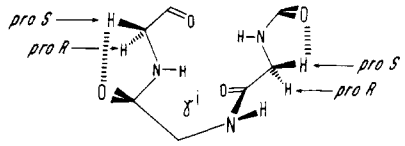

Figure 5. Assignment of the Gly protons (see text).

(see below). Similar effects have been observed in other cyclic peptides.[13]

On the basis of the temperature coefficients of the NH protons in Me_2SO (see Table I), one can assume the internal orientation of the Gly[3] NH and Gly[5] NH protons, whereas the Phe NH protons are solvent exposed; this is confirmed by the titration of the solution in Me_2SO with $CDCl_3$ ($\Delta\delta_{Me_2SO\rightarrow CDCl_3}$: −0.81 (Phe[2] NH), −1.02 (Phe[4] NH), −0.30 (Gly[3] NH), and +0.24 ppm (Gly[5] NH)). We concluded already in 1976 from these facts the existence of a γ loop[14] Gly[3]-CO←HN-Gly[5].[4] A second hydrogen bond can build up either a β loop (Gly[5]-CO←HN-Gly[3]) or a second γ loop (Pro[1]-CO←NH-Gly[3]). A determination of this alternative is done by titration with trifluoroethanol,[3-5] whereby the shift of the Phe[2] NH signal to low field (7.7 Hz/10% TFE) shows that there is no participation of the Pro[1] carbonyl in an intramolecular hydrogen bond. Therefore a $\gamma\gamma$ structure[3,16,17] is eliminated. Also, a detailed discussion of the coupling constants $^3J_{HNC\alpha H}$ excludes all possible γ,γ conformations.[15] There are four possible structures for non-N-alkylated amino acids (βI, βI′, βII, βII′).[18,19] βI′ and βII′ can be eliminated since the position i + 2 of the β loop requires angles ϕ_L of 90° for βI′ and −80° for βII′, which are not compatible with the coupling constant[20] (Phe[2]: $^3J_{HNC\alpha H}$ = 8.4 Hz (Me_2SO)). Beyond that the particular large value for the just mentioned coupling in the solvent CD_3CN (9.95 Hz) does not agree with a βII turn. Another confirmation of the βI turn is delivered by the small NOE effect of 1.1% found between Phe[2] NH and Pro $C_\alpha H$ (see Table IV).[21] Additionally, a strong point is that a β turn of type I was always observed in X-ray structures of β turns when the amino acids in positions i + 1 and i + 2 possess the L configuration.[19]

This leaves the two possibilities $\beta I\gamma$ and $\beta I\gamma^i$, which are both in agreement with the coupling constants. These two structures differ from each other distinctly in the distance between the protons Gly[5] NH and Phe[4] $C_\alpha H$: for the γ^i orientation the distance is 2.9 Å, for the γ orientation on the other hand it is 3.6 Å. The actual measured NOE effect of the Phe[4] $C_\alpha H$ proton when saturating the Gly[5] NH is 11.5%, which is the largest value found

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Table VI. Assignment of Pro-*S*- and Pro-*R* C_α Protons of the Gly Residues in **1**[a]

residue	$^3J_{NHC_\alpha H}$, Hz	θ angle, deg: from coupling constants	θ angle, deg: from model
Gly[3]	7.32	16–28	
	(H^S)	*135–142*	130
	3.11	51–58	
	(H^R)	*113–120*	110
Gly[5]	5.86	30–38	
	(H^S)	*127–135*	130
	4.15	41–49	
	(H^R)	*117–125*	110

[a] Agreements between angles obtained via the Karplus relationship and those obtained from Dreiding models are in italic type. Solvent Me_2SO-d_6. Similar results are found in $CDCl_3$ and in CD_3CN.

in this molecule (Table IV). This confirms the $\beta I\gamma^l$ conformation (Figure 4) as the most stable conformation in solution.

The assignment of the diastereotopic glycine protons is of NMR spectroscopic interest. As a rule, the vicinal glycine coupling constants are analyzed only as a sum with respect to the angle ϕ.[12] In this case, an assignment of the α-protons seems to be meaningful because of the high differences in chemical shifts $\Delta\delta_\alpha$ and the different coupling constants $^3J_{HNC_\alpha H}$. The partial structure (Figure 5) shows that a downfield shift has to be expected for the pro-*S* protons of both glycines caused by the carbonyl groups of the preceding amino acid. In agreement with the previous conclusion, a correlation of coupling constants with angles can be obtained from the Dreiding model (Table VI). A connection between chemical shift and coupling constant (low-field proton = pro-*S* = large coupling) has previously been pointed out by Wyssbrod et al.[22]

Complexation. Studies of complexation were possible only in CD_3CN, as the peptide is not soluble in other common solvents, e.g., methanol or water. The complexation at addition of alkali perchlorates is recognized by strong changes in the CD spectrum[23] and can be quantified by analyzing the ellipticity at 230 nm in the usual way.[24] The stability constant is 160 mol^{-1} for lithium and 50 mol^{-1} for sodium. Addition of potassium does not cause visible spectral changes. Although the complex stabilities are relatively small, they deserve special attention because of the preference of lithium.[5,25]

Further information about the conformational changes shown by CD spectroscopy can be obtained from ^1H NMR spectra of the lithium–peptide complex. The NH signal of Phe[4] and most of the α-proton signals change continuously with successive addition of $LiClO_4$ in CD_3CN to the solution of the peptide. As there are no separate sets of signals for complexed and uncomplexed peptide, the complexation has to be fast related to the NMR time scale. After addition of more than 1 mol of Li^+ per mol of peptide, only small spectral changes take place. The 270-MHz ^1H NMR spectrum of **1** in the presence of 1 molar equiv of $LiClO_4$ has been assigned and analyzed as described above for pure **1** (see Table VII). Although the $\Delta\delta_\alpha$ values of the glycine protons are distinctly smaller (Gly[3] 0.51 and Gly[5] 0.20 ppm) than in pure **1**, their size allows the conclusion at a uniform conformation. A discussion of the NH temperature coefficients in other solvents than Me_2SO is not without problems,[26] but the $\Delta\delta_{NH}/T$ values

Table VII. 270-MHz ^1H NMR Data of the Lithium Complex (1 mol of Cyclopentapeptid 1 and 1 mol of $LiClO_4$) at 298 K in CD_3CN

	Pro[1]	Phe[2]	Gly[3]	Phe[4]	Gly[5]
		δ			
NH		6.94	7.60	7.45	7.39
$C_\alpha H$	4.05	4.58	A 3.95	4.46	A 4.02
			B 3.44		B 3.82
$C_\beta H$	2.05	A 3.17		A 3.12	
	1.52	B 2.90		B 2.92	
$C_\gamma H$	*a*				
$C_\delta H$	3.81				
	3.56				
		J_{ij}, Hz			
$^3J_{HNC_\alpha H}$		9.16	A 6.16	7.93	A 4.84
			B 4.50		B 6.74
$^3J_{HC_\alpha C_\beta H}$		A 5.13		A 5.42	
		B 10.73		B 9.84	
$^2J_{HC_\alpha H}$			14.35		15.87
$^2J_{HC_\alpha H}$		13.43		13.73	
		$\Delta\delta/T$, ppb/K[b]			
		1.8	7.4	4.8	6.0

[a] Covered by the solvent. [b] Temperature coefficient; see text.

show the same tendency for pure **1** in Me_2SO and CD_3CN. Therefore we interpret the $\Delta\delta_{NH}/T$ values of the complex in acetonitrile in the same manner. Yet, these results have to be used with care, as there is another uncertainty introduced by the temperature dependence of the complexation.

An internal orientation is found in the complex only for the NH proton of Phe[2] because of its small temperature coefficient (1.8 ppb/K). As the ^{13}C NMR spectrum of the complex shows a trans amide bond Gly[5]–Pro (proline: C_β 29.2, C_γ 24.6 ppm), the observed coupling constant $^3J_{HNC_\alpha H}$ of Phe[2] (9.16 Hz), according to a ϕ range of −148° to −128° among others is only in agreement with a βII′ turn. The Dreiding model of a γ loop requires different ϕ angles;[15] on the other hand, a βI′ or a βII loop is not consistent with proline in the $i + 2$ position.

The coupling constant $^3J_{HNC_\alpha H}$ of Phe[4] (7.93 Hz) and model considerations agree only with a ϕ range of −157° to −143°;[15] the Phe[4] amide proton points consequently in the same direction as the side chains of the phenylalanines ("above" the ring); the adjacent carbonyl group of Gly[3] is thus "below" the ring. Since the lithium ion cannot be in the ring plane for sterical reasons, the NMR analysis suggests the Li^+ to be "below" the ring plane (Figure 5). The interpretation of the coupling constants shows that four of the five carbonyl groups are "rotated downwards" during complexaton. The upper side is shielded by the lipophilic benzyl and pyrrolidine side chains and the hydrogen bond.

The occurrence of a βII′ turn with proline in the $i + 2$ position in the complex needs some comment. This arrangement has not yet been found in a cyclic pentapeptide,[27] although it is possible in a model and was found for a cyclic hexapeptide.[28] It has to be considered that all previous conformational studies of cyclic pentapeptides in solution with one exception[29] always found two intramolecular NH orientations with $\beta\gamma$[4,5,14,27] or $\gamma\gamma$ structure.[3,16,18] In these conformations there is always a glycine or a D-amino acid, respectively in position $i + 3$ of the $\beta\gamma$ structure and in the corresponding position between the two γ loops of the $\gamma\gamma$ structure.[3] If a second D-amino acid (or Gly) is introduced in the $i + 1$ position (as required by βII′ turns) the molecule would adopt a conformation with the two D-amino acids (or Gly's) in the i and $i + 3$ position of the β turn (the LL sequence in $i + 1$ and $i + 2$ leads to the β type I conformation). Furthermore the

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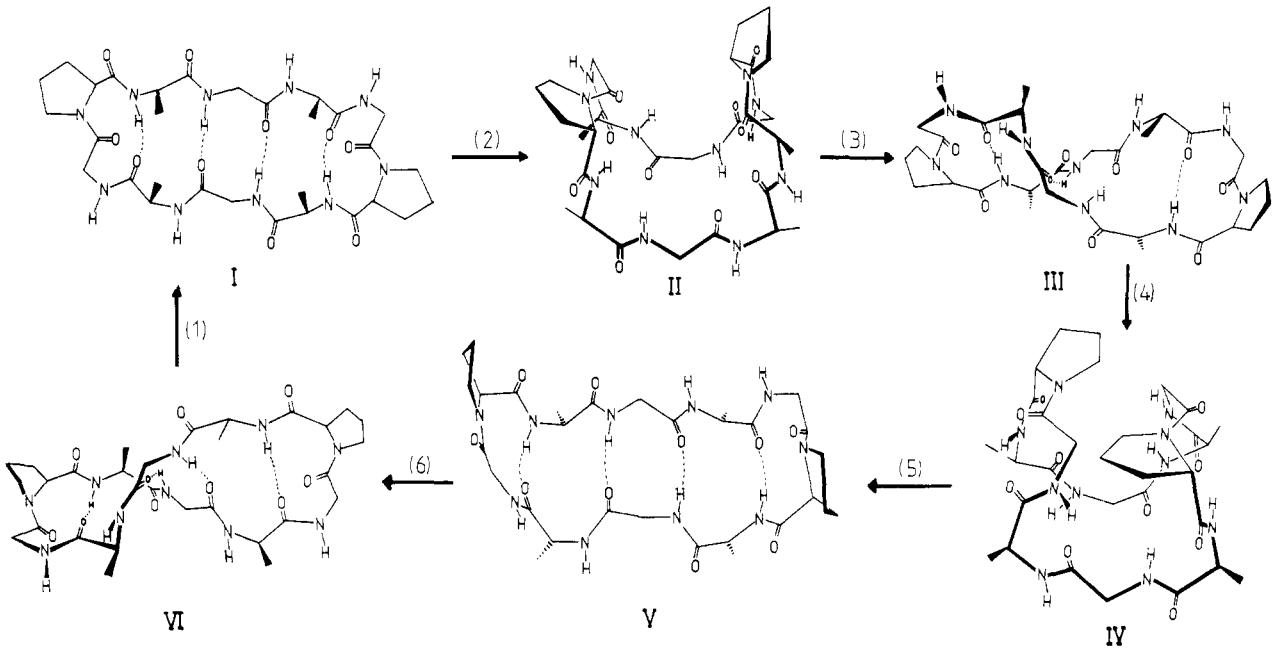

Figure 6. Conformations of **2** and their interconversion via the following operations: (1) inward torsion of the Phe^{4,9} side chains, (2) torsion of the Gly^{3,8} $C_\alpha H_2$ units, (3) outward torsion of the Phe^{2,7} side chains, (4) outward torsion of Phe^{4,9} side chains, (5) Torsion of the Gly^{3,8} $C_\alpha H_2$ units, and (6) inward torsion of the Phe^{2,7} side chains. The side chains of the Phe's are only indicated as dashed lines or wedges.

proline then occupies the $j + 1$ position of the γ turn, which is a preferred position.[5]

The Cyclic Decapeptide 2

Conformation in Solution. The larger flexibility of cyclic decapeptides makes a conformational analysis much more difficult. Thus we confine ourselves to the NMR spectroscopic conformational analysis in Me_2SO. In $CDCl_3$ and CD_3CN the diastereotopic protons of glycine and phenylalanine are not splitted, which can be taken as a hint to a rapid equilibrium between several conformations.[15] On the other hand one observes in Me_2SO $\Delta\delta_\alpha$ values of 0.40 and 0.25 ppm for the Gly^{3,8} and the Gly^{5,10} protons, respectively (see Table VIII). There are only five different amino acid signals in all solvents, which means the conformation is C_2 symmetric on the NMR time scale, which is taken into account at the notation of the amino acids.

The assignments have been made by double resonance and with the aid of the partially deuterated derivatives *cyclo*(Pro-[2-^{2}H]-Phe-Gly-Phe-Gly)$_2$ and *cyclo*(Pro-Phe-[2-$^2H_2$]Gly-Phe-Gly)$_2$. The chemical shifts of the β and γ carbons of proline again indicate the trans conformation of the amide bond Gly–Pro (C_β, 28.5; C_γ, 24.6 ppm in Me_2SO-d_6). The NH chemical shifts change linearly in the range from 300 to 350 K; above this temperature they bend slightly.[30] From the low-temperature range one can deduct the existence of only one conformation with Phe^{2,7} and Gly^{3,8} NH protons internally, Phe^{4,9} NH protons externally oriented. This result is confirmed by the solvent titration $Me_2SO \rightarrow CDCl_3$. Here, the Phe^{2,7} and Gly^{3,8} NH protons are shifted to low field, all other amide protons to high field. Thus, the Gly^{5,10} NH protons are probably externally oriented, especially since one often finds relatively small coefficients for Gly NH protons even when they are oriented toward the solvent.[2] The four internal orientations must include two turns with Phe^{2,7} NH protons. The formation of a β-pleated sheet structure, commonly found for cyclic peptides with $(4n + 2)$ amino acids (n = 1,2 ...),[31] implies a β turn Phe^{4,9}-CO←HN-Phe^{2,7}. A γ turn, which would include Pro^{1,5}, can be excluded by reason of the ^{13}C spectrum, since in this case a strong high-field shift for the β-carbon of proline would be expected.[5,27] The second hydrogen bond can only exist between the Gly^{3,8} amino acids (Gly^{3,8}-CO←HN-Gly^{8,3}).

Table VIII. 270-MHz ^{1}H NMR Data of **2** at 323 K in Me_2SO-d_6 (32 K Data Points)

	Pro^{1,6}	Phe^{2,7}	Gly^{3,8}	Phe^{4,9}	Gly^{5,10}
		δ			
NH		8.60	7.68	8.07	7.77
$C_\alpha H$	4.09	4.16	A 4.11	4.60	A 4.05
			B 3.71		B 3.80
$C_\beta H$	1.58	A 3.37		A 3.01	
		B 2.99		B 2.90	
$C_\gamma H$	~2.0				
$C_\delta H$	~3.5				
		J_{ij}, Hz			
$^3J_{HNC_\alpha H}$		7.09	A 8.93	7.58	A 5.84
			B 3.43		B 1.92
$^3J_{HC_\alpha C_\beta H}$	*a*	A 3.92		A 4.80	
		B 10.44		B 8.72	
$^2J_{HC_\alpha H}$			16.99		17.82
$^2J_{HC_\beta H}$	*a*	14.02		13.86	
		$\Delta\delta/T$,[b] ppb/K			
		1.9	0.4	6.0	3.8

[a] Not analyzed. [b] Temperature coefficients of the NH protons between 300 and 350 K.

The coupling constant $^2J_{HC_\alpha H}$ (Gly^{5,10} = 17.8 Hz) is suitable for the deduction of the β turn geometry. According to Chandrasekaran[32] the ψ angle ranges of the different turns from −30° (βI), +30° (βI′), +120° (βII), −120° (βII′). Only βI and βI′ are in agreement with the coupling constant.[20] The rigid prolyl pyrrolidine ring excludes in addition the βI′ conformation.

There are six possible conformers with C_2 symmetry (see Fig. 6), which can be converted into each other by rotation of the Phe side chains or inversion of Gly residues on the molecule plane. Unfavorable sterical interactions between prolyl and phenylalanyl side chains exclude conformations I, II, and IV (see Figure 6). For the remaining conformations it was investigated whether the angles found in the model agree with the experimental values. The coupling constant $^2J_{HC_\alpha H}$ of Gly^{3,8} (17.0 Hz) does not agree with the ϕ angle of +110° found in the model of structure V. The

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helical isomers III and VI are consistent with all experimentally found angles, which means a decision between them is not possible.

Complexation. The CD spectrum of the peptide in acetonitrile changes very much when $KClO_4$ is added, while $NaClO_4$ or $LiClO_4$ do not cause any effect. The quantification yields a stability constant of approximately 300 mol^{-1} for the potassium complex. This value is not remarkable compared to the constants of other cyclic peptides. The ^1H NMR studies of the potassium complex of **2** were done with 50 molar equiv of KSCN, as spectral changes were found up to this concentration. SCN^- was chosen as negative ion because of the improved solubility in CD_3CN; CD spectra with KSCN cannot be recorded because of its own absorption. The assignment of the spin systems was done with the above-mentioned methods.

The spectral changes caused by complexation prove the conversion of the peptide into another conformation with C_2 symmetry. In our opinion, the experimental material is not sufficient to draw more definitive conclusions about the conformation of the complex.

Measurement Conditions

The one-dimensional ^1H NMR spectra were measured on a WH 270 spectrometer (Bruker AG) with an observing pulse of 60° ($\pi/2$ = 6.4 μs for ^1H) and data processing as reported. Internal Me_4Si was used as standard for all spectra.

SECSY. The two-dimensional spin–echo-correlated ^1H NMR experiment was also performed on the WH 270. The applied pulse sequence was $(\pi/2)-(t_1/2)-(\pi/4)-(t_1/2)$ acquisition with phase cycling according to ref 6b to yield frequency discrimination in t_1. The spectral width in F_1 was 1400 Hz, in F_2 2500 Hz; the number of data points in F_2 was 1024, and 256 increments were recorded. Before Fourier transformation the data were multiplied with an unshifted sine bell. Zero filling was applied in each dimension. Total acquisition time was 12 h. The $\pi/2$ pulse is 6.4 μs.

^1H–^{13}C Shift Correlation. The experiment was performed on a XL-200 spectrometer (Varian AG). The applied pulse sequence was $(\pi/2, {}^1H)-(t_1/2)-(\pi, {}^{13}C)-(t_1/2)-\Delta_1(\pi/2, {}^1H; \pi/2, {}^{13}C)-(\Delta_2)$ acquisition with Δ_1 = 4 ms and Δ_2 = 2.66 ms. All pulses were phase cycled according to ref 10b. The spectral width in F_1 was 1000 Hz, in F_2 2500 Hz; the number of data points in F_2 was 1024, and 64 increments were recorded. Before the Fourier transformation the data were weighted with decreasing exponential functions to yield a line broadening of 3 Hz in F_2 and 8 Hz in F_1. Zero filling was applied to double the number of data points in each dimension. Total acquisition time was 11.3 h. The $\pi/2$ pulse of the machine is 15 μs for ^{13}C, and the decoupler $\pi/2$ pulse for ^1H is 31 μs.

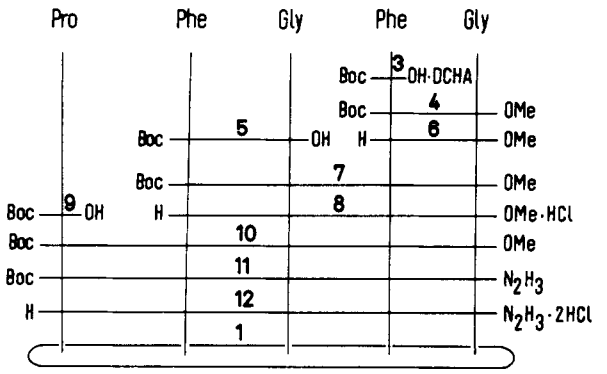
INEPT. The experiment was also performed on the XL-200 spectrometer. The pulse sequences described in ref 11b was used. The delay τ was 2.78 ms for both the decoupled and the coupled spectrum; the delay Δ was 5.55 ms for the decoupled and 0 for the coupled spectrum. The spectral width was 1000 Hz, 1024 data points were used for acquisition, and FIDs were Fourier transformed after appropriate Lorentz-to-Gauss conversion and zero filling. The $\pi/2$ pulse was 27.5 μs for ^{15}N and the decoupler $\pi/2$ pulse 31 μs for ^1H. Total acquisition time was 1 h for the decoupled spectrum and 2 h for the coupled spectrum.

^1H–^{15}N Shift Correlation. The experiment was performed on the XL-200 spectrometer. The pulse sequence was $(\pi/2, {}^1H)-(t_1/2)-(\pi, {}^{15}N)-(t_1/2)-\Delta_1(\pi/2, {}^1H; \pi/2, {}^{15}N)-\Delta_2$ acquisition (with ^1H broad band decoupling) with $\Delta_1 = \Delta_2$ = 5.55 ms; all pulses were phase cycled according to ref 10b. The spectral width in F_1 was 500 Hz and in F_2 1000 Hz, the number of data points in F_2 was 1024, and 64 increments were recorded. The data were weighted before Fourier transformation with decreasing exponential functions to yield a line broadening of 3 Hz in F_2 and of 8 Hz in F_1. Zero filling was applied to double the number of data points in each dimension. Total acquisition time was 15.2 h.

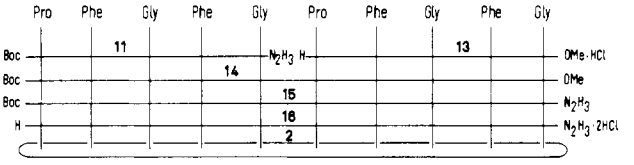
Experimental Section

Abbreviations: Boc = *tert*-butoxycarbonyl; DCC = dicyclohexycarbodiimide; DCHA = dicyclohexylamine; HOBt = *N*-hydroxybenzotriazole; NMM = *N*-methylmorpholine.

Scheme I



Scheme II



Melting points were determined with a Kofler apparatus and are uncorrected.

TLC: eluant A, 1-butanol/acetic acid/water, 3:1:1; B, chloroform/methanol/acetic acid, 95:5:3; C, ethyl acetate/1-butanol/water/pyridine, 20:10:5:3. Development with *o*-tolidine after chlorination. Optical rotation (Perkin-Elmer, Model 141); 589 nm at 25 °C.

The synthesis of **1** is represented in Scheme I. The following compounds were prepared as previously described in the literature: Boc-Phe-OH·DCHA (**3**),[28] Boc-Phe-Gly-OCH_3 (**4**),[29,33] Boc-Phe-Gly-OH (**5**),[35] H-Phe-Gly-OCH_3·HCl (**6**),[34] Boc-Phe-Gly-Phe-Gly-OCH_3 (**7**),[36] H-Phe-Gly-Phe-Gly-OCH_3·HCl (**8**),[36] and Boc-Pro-OH (**9**).[28,33]

Boc-Pro-Phe-Gly-Phe-Gly-OCH_3 (10). The coupling was performed as usual with DCC[36] from 10.0 g (20.9 mmol) of **8** and 5.62 g (26.1 mmol) of **9** under addition of HOBt and NMM as base. The product was purified via gel chromatography (Sephadex LH-20, DMF) to yield 9.20 g (49%): mp 184–187 °C; R_f A 0.65, B 0.31, C 0.74; $[\alpha]^{25}_D$ −40.9° (*c* 1, DMF). Anal. Calcd for $C_{33}H_{43}N_5O_8$ (637.73): C, 62.15; H, 6.80; N, 10.98. Found: C, 62.02; H, 6.66; N, 10.82.

Boc-Pro-Phe-Gly-Phe-Gly-N_2H_3 (11).[36] To a solution of 4.46 g (7.0 mmol) of **10** in 30 mL methanol was added 14 mL (280 mmol) of hydrazine monohydrate, and the mixture was stirred for 2 h at 40 °C. The solvent was evaporated and, to remove the excess of hydrazine, again evaporated 3 times after addition of 20 mL of methanol. Purification via precipitation from methanol/ether yielded 3.60 g (81%): mp 197–200 °C; R_f A 0.54, B 0.05, C 0.60; $[\alpha]^{25}_D$ −40.8° (*c* 1, DMF).

H-Pro-Phe-Gly-Phe-Gly-N_2H_3·2HCl (12).[36] **11** (8.90 g, 14 mmol) was stirred for 2 h with 140 mL of 2 N HCl in dioxane. The solvent was removed and the product mixed 3 times with methanol and evaporated to remove HCl. Recrystallization from methanol/ether yielded 6.40 g (80%): mp 154–156 °C; R_f A 0.39, B 0, C 0.18. $[\alpha]^{25}_D$ −49.5° (*c* 1, DMF).

***cyclo*(Pro-Phe-Gly-Phe-Gly) (1).** **12** (1.10 g, 1.91 mmol) was dissolved in 10 mL of DMF and chilled to −15 °C, and 0.76 mL (9.5 mmol) of concentrated HCl as well as 1.87 mL (3.8 mmol) of 14% sodium nitrite in water were added.[37] After 30 min the mixture was diluted with 640 mL of cold DMF (3×10^{-3} mol/L) and neutralized with 1.48 mL (13.3 mmol) of NMM. After standing 4 days at room temperature, the solvent was removed under vacuum and the product treated with 10 g of mixed-bed ion exchanger in 100 mL of 2:1 methanol/water for 2 h. The filtrate was evaporated and the residue purified by gel chromatography (Sephadex LH 20, DMF). The first fraction, which shows optical ac-

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tivity, contains 10 mg of cyclodecapeptide **2**. The next fraction was chromatographed again over Sephadex LH 60 to yield 378 mg (39%) of **1**: mp 266–267 °C; R_f A 0.44, B 0.09, C 0.48; $[\alpha]^{25}_D$ −149.5° (*c* 0.5, DMF). The FD mass spectrum exhibits a peak at m/e = 505. Anal. Calcd for $C_{27}H_{31}N_5O_5$ (505.58): C, 64.14; H, 6.18; N, 13.85; O, 15.82. Found: C, 63.5; H, 6.0; N, 13.8; O, 15.5.

The cyclodecapeptide **2** was prepared via fragment condensation (Scheme II) as well as via cyclodimerization.[38-40]

H-Pro-Phe-Gly-Phe-Gly-OCH$_3$ (13). Deprotection of the N terminus as described above (see **12**) yields a hygroscopic product: yield, 3.28 g (91%); mp 210–212 °C. R_f A 0.45, B 0.02, C 0.27; $[\alpha]^{25}_D$ −64.5° (*c* 1, DMF).

Boc-Pro-Phe-Gly-Phe-Gly-Pro-Phe-Gly-Phe-Gly-OMe (14).[5] **11** (222 mg, 0.35 mmol) was dissolved in 20 mL of DMF and treated at 0 °C under stirring with 0.18 mL (0.35 mmol) of 14% sodium nitrite in water and 0.06 mL of concentrated HCl. After 30 min it was neutralized with 0.08 mL of NMM. A solution of 200 mg (0.35 mmol) of **14** in 10 mL of DMF, neutralized with 0.04 mL of NMM, was added within 1 h at 0 °C and stirred for 24 h at room temperature. The solvent was evaporated, and the residue was stirred for 2 h with 5 g of mixed-bed ion exchanger in about 50 mL of methanol/water. The filtrate was evaporated and the residue chromatographed (Sephadex LH 20, DMF) to yield 200 mg (50%): mp 135–138 °C; R_f A 0.59, B 0.04, C 0.77; $[\alpha]^{25}_D$ −33.2° (*c* 1, DMF). Anal. Calcd for $C_{60}H_{74}N_{10}O_{13}$ (1143.31): C, 63.04; H, 6.52; N, 12.25. Found: C, 63.33; H, 6.60; N, 12.06.

Boc-Pro-Phe-Gly-Phe-Gly-Pro-Phe-Gly-Phe-Gly-N$_2$H$_3$ (15). The hydrazinolysis was performed as described under **11**. From 115 mg (0.1 mmol) of **14** was obtained 85 mg (74%) of **15**: mp 149–153 °C; R_f A 0.47, B 0, C 0.60; $[\alpha]^{25}_D$ −53.0° (*c* 1, DMF).

H-Pro-Phe-Gly-Phe-Gly-Pro-Phe-Gly-Phe-Gly-N$_2$H$_3$·2HCl (16). N-Deprotection was performed as described under **12** from 286 mg (0.25 mmol) of **15**. During the reaction some absolute methanol was added to dissolve the precipitate of the product: yield, 266 mg (96%); mp 180 °C; R_f A 0.34, B 0.03, C 0.24.

***cyclo*(Pro-Phe-Gly-Phe-Gly)$_2$ (2). Via Fragment Condensation.** **17** (245 mg, 0.22 mmol) was cyclized, worked up, and purified as described under **1**; yield: 82 mg (37%); mp 174–175 °C; R_f *a* 0.03, B 0.48, C 0.59; $[\alpha]^{25}_D$ −122.8° (*c* 0.5, DMF). Anal. Calcd for $C_{54}H_{62}N_{10}O_{10}$ (M_r 1011.16): C, 64.14; H, 6.18; N, 13.85; O, 15.82. Found: C, 62.4; H, 6.2; N, 13.5; O, 15.8. The deviation in the elemental analysis is caused by a small amount of an impurity, which could not be separated via chromatography. The field desorption mass spectrum shows a peak at m/e = 1011 but a further very small peak at m/e = 976.

Via Cyclodimerization. **12** (3.45 g, 6.0 mmol) was dissolved in 35 mL of DMF (0.1 mol/L) and cyclized as described under **1** but without further dilution by DMF. The gel chromatographic fraction (Sephadex LH 20, DMF) was chromatographed again separately (Sephadex LH 60, DMF) to yield 229 mg (7.6%) of **1** and 156 mg (5.2%) of **2**. **2** is identical in all physical properties with **2** obtained via fragment condensation. Also, the impurity mentioned above was present.

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Registry No. 1, 61898-89-3; **2**, 82247-64-1; **3**, 4192-12-5; **4**, 7625-57-2; **5**, 25616-33-5; **6**, 7625-59-4; **7**, 66876-50-4; **8**, 66876-51-5; **9**, 15761-39-4; **10**, 82265-53-0; **11**, 82265-54-1; **12**, 82247-65-2; **13**, 82247-66-3; **14**, 82247-67-4; **15**, 82247-68-5; **16**, 82247-69-6.

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The H_3O^+ Cation: Molecular Structure of an Oxonium–Macrocyclic Polyether Complex

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Abstract: The oxonium ion has been isolated as a discrete entity by inclusion into a macrocyclic cavity. The structure of the complex formed by a tetracarboxylic 18-crown-6 ligand with H_3O^+ has been determined by X-ray crystallography ($P2_12_12_1$; a = 10.526 (2), b = 14.325 (2), and c = 15.234 (2) Å). The structure was solved by direct methods and refined to R = 0.048 for 1957 ($I \geq 2\sigma(I)$) reflections. Hydrogen atoms were located on a Fourier difference map. The overall shape of the ligand is similar to that found in related complexes, as two carboxylic acid groups extend on both sides of the pseudoplanar macrocycle. The H_3O^+ cation is anchored in the center of the cavity by three OH^+···O hydrogen bonds (2.67, 2.73, and 2.74 Å) whereas the chloride counterion is H bonded to three carboxylic acid groups 5.5 Å away from the cation. The pyramidal geometry found for the oxonium cation in the present molecular complex shows this conformation to be the most stable in an ion-solvating environment.

The oxonium ion, H_3O^+, has remained a hypothetical molecule for many years. Its existence, first postulated in 1907,[1] was better accepted with the development of the acid–base theory of Brønsted and Lowry.[2] Nevertheless, its occurrence was the subject of many discussions until various physical methods allowed it to be identified unambiguously.[3] The first structural information on H_3O^+ came from spectroscopic studies. ^{1}H NMR spectroscopy brought the proof of the equivalence of the three protons in oxonium perchlorate,[4] and infrared spectroscopy of monohydrated strong

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