Conformational Energy Study of Tuftsin[1]

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ABSTRACT: The conformational energy space of tuftsin, a tetrapeptide which functions in the immune system, has been explored. Selection strategies which ensure an economical but representative sampling of the lower regions of the energy space are discussed. The global minimum area of the tuftsin energy surface is characterized by many individual conformations, but all of these have certain common structural features. The characteristics of the average molecular structure of tuftsin are derived from the group of computed minimum-energy conformations. All low-energy conformers of tuftsin have the same general structure, which is described as "a hairpin with two split ends" where each of the splits is composed of one terminus of the backbone and a Lys or Arg side chain. Qualitative agreement between the computed data and experimental studies on tuftsin and its analogues is demonstrated.

I. Introduction

Experimental investigations of small biologically active oligopeptides are being carried out currently in many laboratories, and it is of interest to perform conformational energy calculations on these same molecules to provide an understanding of their conformational preferences. Of course, the conformation of the isolated oligopeptide is probably somewhat different from that which obtains when it forms a complex with its receptor. Information about the biologically active conformation can be deduced only from studies of such complexes. Nevertheless, experimental and theoretical studies of isolated oligopeptides may provide clues about the nature of the peptide–receptor interaction in cases where steric hindrance limits the available conformations of the isolated peptide molecule.

The computational methods described in the previous paper[2] have already been used in a study of the biologically active oligopeptide enkephalin,[3] and they are applied here to tuftsin and, in the following paper,[4] to an analogue (sequence variant) of tuftsin that has contraceptive activity. An experimental study of the conformation of this molecule is in progress[5] but, as is discussed below, previous experimental work can be compared with the computational results reported here. Tuftsin, a tetrapeptide with the sequence H-Thr-Lys-Pro-Arg-OH, functions in the immune system. It is the active segment of leucokinin, which is that portion of the γ-globulin molecule which stimulated phagocytosis by the autologous polymorphonuclear leucocyte.[6,7] Leucokinin carries the tuftsin moiety to the cell surface, where the tetrapeptide is cleaved by the enzyme leucokinase and is utilized by the cell. The tuftsin fragment has been shown to be responsible for the full activity of leucokinin.[8–10]

II. Computational Methods

A description of the computational method, of the parameters describing geometry and interaction energies, and of minimization methods is given elsewhere.[11,12] The amino acids were in the L configuration. The amino and carboxyl end groups, and the lysyl and arginyl side chains, were taken to be uncharged, a choice that has been justified elsewhere.[13,14] Bond lengths and bond angles were maintained fixed. The total energy, E, of any conformation was taken to be the sum of the intramolecular nonbonded, electrostatic, hydrogen bonding, torsional, and proline internal energies. Solvent effects were not included in the calculations. The effective dielectric constant in the electrostatic term was set equal to 2.[11,15] Energies are reported here as $\Delta E = E - E_0$, where E is the energy of the conformation under consideration and E_0 is that of the minimum-energy conformation, taken as the reference state for tuftsin.

III. Selection Strategies for Starting Conformations and Results of Energy Minimization

Problems associated with the selection of starting conformations for energy minimization are discussed in the preceding paper.[2] For tuftsin, these problems were particularly acute: preliminary studies had indicated that the lowest region of the energy surface was characterized by many minima, but the possibility of stabilization of certain backbone conformations by interactions involving the side chains had not been explored fully. Therefore, it was very important to choose a small number of starting conformations which would provide an adequate sampling of the area of the global minimum and some idea of the probability of substantial stabilization by means of side-chain interactions.

The selection strategy consisted of three main steps: (1) the determination of low-energy conformations of fragments of the tetrapeptide and their combination to form low-energy starting points for tuftsin, (2) systematic variation of the side-chain conformations, and (3) the generation of allowed "standard" chain-reversal conformations.

Since information was available on the minimum-energy conformations of terminally blocked X-Pro and Pro-X dipeptides,[16] it was decided to search for low-energy conformations of the Lys-Pro-Arg tripeptide and then combine them with the relatively few single-residue minimum-energy conformations of Thr[17] to obtain a reasonable number of starting conformations for tuftsin. Although Lys and Arg were not among the amino acids, X, in the X-Pro and Pro-X dipeptides studied previously,[16] the similarity of backbone dihedral angles ϕ and ψ and relative energies of the various conformations for the other X-Pro and Pro-X dipeptides considered[16] suggested that the combined minimum-energy backbone conformations of X-Pro and Pro-X could be used as starting conformations for Lys-Pro-Arg.

Combination of the minimum-energy conformations of Lys-Pro and Pro-Arg gave 85 starting points for Lys-Pro-Arg, with all peptide bonds in the trans conformation. Similarly, 27 starting points were obtained with a cis Lys-Pro peptide bond,[16] the other peptide bonds being trans. Of the 85 trans conformations, 18 were eliminated from further consideration because they involved backbone–backbone nonbonded interatomic distances of less than 2.0 Å (overlaps) and were not stabilized by hydrogen bonds. The Lys and Arg side chains were then attached to the remaining 67 backbone conformations. Because both Lys and Arg have a large number of minimum-energy side-chain conformations, a small number of generalized side-chain conformations was selected to keep the number of tripeptide starting points reasonably low. Each Lys and Arg residue was classified according to its values of ϕ, ψ, χ^1, and χ^2, since examination of the single-residue min-

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Table I. Conformations of Thr and Lys-Pro-Arg Used to Generate Members of Set A[a]

Thr (with Trans tripeptide)					Thr (with Cis tripeptide)				
Conformation[b]	ϕ	ψ	χ^1	$\chi^{2,1}$	Conformation[b]	ϕ	ψ	χ^1	$\chi^{2,1}$
C(I)	-54.3	83.4	61.6	63.7	C(I)	-52.1	84.4	61.0	63.8
C(II)	-70.9	79.7	-55.1	64.9	C(II)	-70.3	79.3	-57.2	68.4
C(III)	-67.8	80.6	-57.2	164.4	A	-35.6	-60.7	46.4	163.9
F(I)	-76.0	143.1	-53.8	65.4	B	-86.8	32.0	177.5	-61.8
F(II)	-71.1	142.6	-54.6	165.7	F	-99.5	158.3	-177.3	165.5
F(III)	-93.6	136.2	179.7	167.6					
F(IV)	-93.6	136.0	180.0	82.4					
G	-142.4	-47.5	-58.0	73.2					
A	-32.7	-54.7	45.4	162.0					

Trans Lys-Pro-Arg

Conformation[b]	Lys				Pro		Arg			Type[c]
	ϕ	ψ	ω	χ^1	ϕ	ω	ϕ	ψ	χ^1	
DCF(I)	-149.8	82.9	177.0	-173.5	92.7	178.5	-68.9	137.1	-69.6	I
DCA(I)	-146.8	82.9	176.4	-174.9	91.6	178.9	-67.9	-42.7	-68.9	I
DCF(II)	-147.3	86.4	172.8	-173.3	73.6	-176.1	-75.8	130.6	-166.7	I
DGG(I)	-148.4	84.5	175.5	-174.0	87.0	-179.1	-150.1	-44.4	-167.3	IV
DCE(I)	-145.6	84.0	174.8	-173.7	86.5	-178.7	-149.5	132.9	-169.0	IV
ECA(I)	-134.0	151.0	168.1	-72.4	74.4	-176.7	-82.8	-46.2	-66.2	I
DAC(I)	-143.9	83.5	169.0	-171.6	-15.6	-178.2	-72.0	130.1	-66.9	VI
DFE(I)	-138.4	86.7	174.9	-171.2	160.3	-176.8	-137.3	143.9	-63.7	II
DFE(II)	-147.8	82.8	179.7	-171.0	130.2	-178.3	-166.5	163.1	56.0	VII
ECF(I)	-134.8	151.9	168.1	-73.0	74.8	-178.5	-83.8	140.1	-65.3	I
ECF(II)	-152.1	153.0	174.7	59.2	89.5	-179.5	-75.0	137.1	-67.0	I
DAG(I)	-141.7	87.5	172.9	-174.7	-20.8	177.6	-136.5	-43.1	-170.3	II
DAE(I)	-140.5	88.1	176.4	-172.9	-20.6	-177.6	-162.0	161.0	57.1	II
A*CF(I)	57.2	79.6	178.3	-171.8	93.4	177.6	-68.8	139.1	-69.2	I
DCE(I)	-141.9	85.8	179.2	-171.2	129.3	-177.4	-166.1	163.3	57.8	VII
DAE(II)	-142.0	86.3	176.3	-173.7	-24.7	-176.4	-136.8	137.5	-66.5	I
DAC(II)	-140.0	88.2	168.0	-175.2	-15.1	178.5	-69.3	126.1	-67.8	VI
FCE(I)	-75.4	151.2	169.3	-71.6	79.4	-177.4	-162.0	162.7	63.5	VII

Cis Lys-Pro-Arg

Conformation[b]	Lys				Pro		Arg			Type[c]
	ϕ	ψ	ω	χ^1	ϕ	ω	ϕ	ψ	χ^1	
EFF(I)	-153.7	151.1	-3.5	61.4	152.6	-177.4	-92.1	135.9	-66.5	I
EFA(I)	-156.4	150.9	-5.9	59.1	151.5	-176.6	-88.1	-45.5	-66.0	I
EAC(I)	-132.1	148.2	-2.5	-72.1	-28.4	177.2	-82.2	119.0	-66.2	I
EAF(I)	-151.6	146.0	-4.8	61.2	-21.5	178.3	-80.7	130.8	-66.1	I
EAC(II)	-129.7	147.9	-2.3	-70.8	-37.5	174.2	-74.8	123.2	-172.0	I
EFE(I)	-134.9	151.6	-4.7	-73.2	153.4	179.3	-162.7	136.4	-173.4	III
EAC(III)	-152.1	146.4	-3.0	59.2	-22.6	177.9	-81.2	125.8	-66.6	I
FAE(I)	-82.5	144.6	-2.5	-68.4	-40.0	175.3	-137.4	125.3	-66.2	I
EAC(IV)	-150.9	148.1	-3.7	60.0	-23.0	174.8	-74.8	123.2	-171.9	I
EFE(II)	-151.1	149.6	-4.3	59.6	153.1	-176.6	-161.1	136.3	-172.1	III
EAC(V)	-126.2	147.9	-2.4	-69.8	-34.5	178.4	-82.5	126.8	-65.6	I
DFF(I)	-159.1	90.8	14.9	-73.5	157.4	178.4	-73.4	134.8	-179.5	I

[a] The set of representative conformations (with dihedral angles in degrees) from which the conformation of tuftsin was determined. [b] The designations are according to the conformational letter codes of Ref. 17. The tripeptides are named according to the conformations of Lys, Pro, and Arg. The Roman numerals distinguish between different conformations which are described by the same letters. [c] "Type" refers to the conformation of the Arg side chain. ω and $\chi^{2,2}$ of Thr were set to 180° and 60°, respectively; χ^3 of Lys was set to 180° and χ^2 to χ^4 were all close to 180° (±5°) for all conformations. For Arg, $\chi^{6,1}$ and $\chi^{6,2}$ were set to 180°, χ^2 and χ^3 were close (±5°) to 180° and 0°, respectively, and the approximate values of χ^4 and χ^5 are denoted by one of I to VII. The values of χ^4, χ^5 for I to VII (±5°) were: I: 180°, 82°; II: 180°, -82°; III: 70°, 180°; IV: 70°, 80°; V: -70°, 180°; VI: -70°, -80°; VII: 180°, 180°.

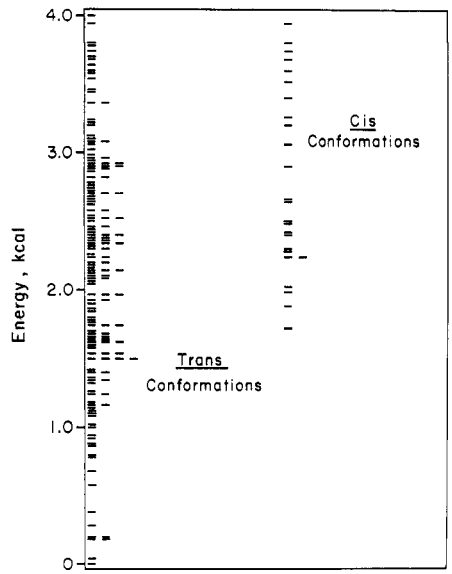

Figure 1. The relative energies ($\Delta E = E - E_0$) of the members of set B. The energy distributions of the cis and trans conformation subsets are shown separately.

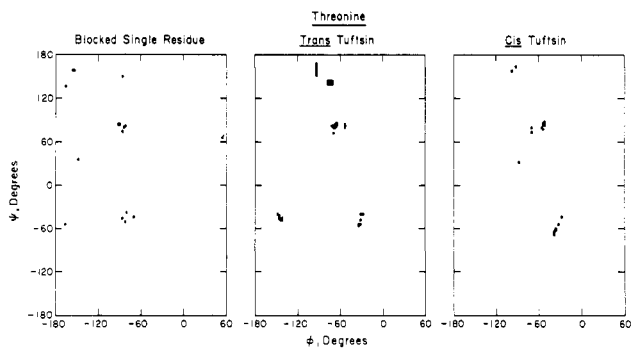

Figure 2. ϕ,ψ maps for threonine, showing the values of ϕ,ψ calculated for the blocked single residue (ref 17) and for the residue in trans and cis tuftsin. (Blocked single residues are shown for $\Delta E \leq 4$ kcal.)

ima[17] revealed that changes in dihedral angles beyond χ^2 had almost no effect on the energy of a given backbone conformation. Combination of the Lys and Arg conformations produced nearly 500 trans (and 150 cis) tripeptide starting points. Each of these was checked for overlaps, and any conformation with overlaps and no stabilizing hydrogen bonds was dropped from further consideration. While some of the eliminated conformations might have minimized to fairly low energies, subsequent calculations showed that minimization moved the nonoverlap starting points to conformations whose energies were lower than those of conformations obtained from overlap starting points. Therefore, given the broad sampling of conformational space accomplished in the generation of the tripeptide starting points and the fact that no conformational class was eliminated from consideration by the discard procedure, the dropping of overlap conformations seemed reasonable.

After discarding the overlap conformations, 360 trans and 71 cis starting points were left. The energies of all of these conformations were computed using ECEPP,[11,12] and all conformations with energies of more than 5 kcal above the lowest energy were discarded. Examination of the remaining conformations showed that the values of χ^2 of Lys or Arg had very little effect on the energy of the backbone conformation. Hence, the generalized conformations of Lys and Arg were reselected on the basis of ϕ, ψ, and χ^1 only. This reduction and the elimination by the 5 kcal energy criterion decreased the number of starting points for minimization to 109 trans and 27 cis.

All starting conformations were subjected to one cycle of the Powell minimizer,[18] allowing only the backbone dihedral angles to vary (ϕ's, ψ's; ϕ of Pro was fixed at −75°). The energies of these partially minimized conformations formed a continuum, less heavily populated at the bottom than at the top, but with no substantial gaps between the groups of conformations. Of the 109 trans conformations, 90 were within 5 kcal and 30 within 3 kcal of the minimum while, of the 27 cis conformations, 22 were within 5 kcal of the minimum. At this point, the lowest energy conformations (defined below) were chosen for combination with minimum-energy conformations of Thr to give the starting points for tuftsin.

These combinations were selected to satisfy the condition that the sum of the energies of Thr and the tripeptide was ≤3 kcal for the trans and ≤5 kcal for the cis conformations. The use of the 3 kcal cutoff has been justified elsewhere.[2] The higher cutoff was taken for cis because these conformations seemed to be more likely candidates for stabilization by hydrogen bonding involving the Lys and Arg side chains than were the trans conformations. The 107 trans and 65 cis tuftsin starting conformations selected by this criterion were each subjected to one or two cycles of Powell minimization, allowing only the backbone dihedral angles to vary (for trans, ϕ, ψ of Thr, ϕ, ψ of Lys, ψ of Pro, and ϕ, ψ of Arg; for cis, the trans dihedral angles plus ω of Lys). At the end of this step, 88 conformations were within 3 kcal of the minimum. As before, their energies formed a continuum, more heavily populated in the upper regions than in the lower ones, but without sig-

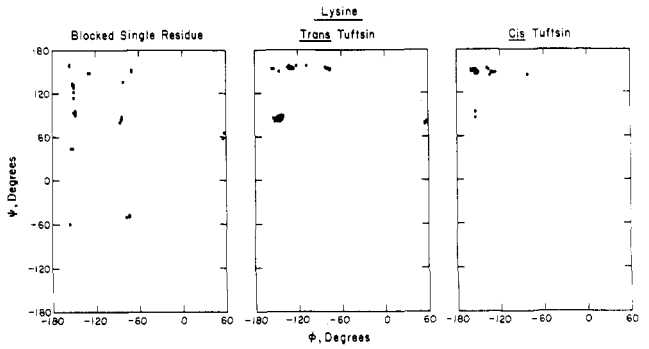

Figure 3. Similar ϕ,ψ maps for lysine.

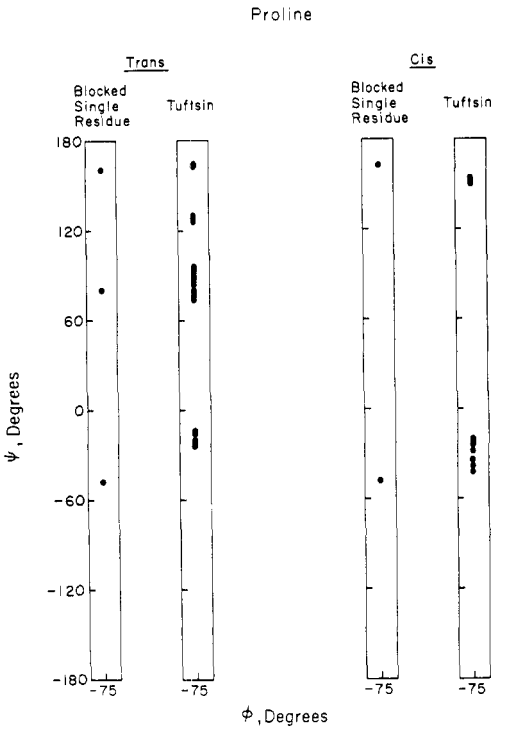

Figure 4. Similar ϕ,ψ maps for proline.

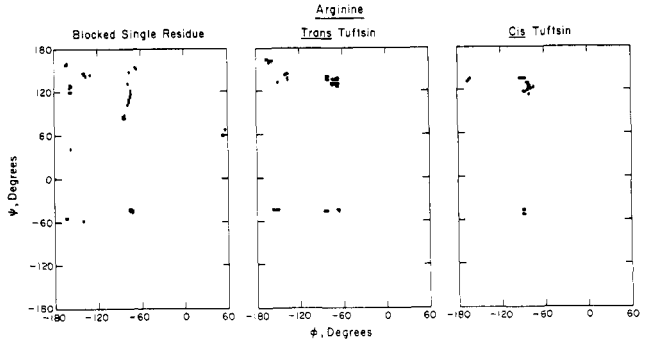

Figure 5. Similar ϕ,ψ maps for arginine.

nificant unpopulated gaps. The energies of these 88 conformations were minimized further with respect to ϕ, ψ, χ^1, $\chi^{2,1}$ of Thr, ϕ, ψ, ω, χ^1 of Lys, ψ, ω of Pro, and ϕ, ψ, χ^1 of Arg, using the minimizer MINOP.[19] Except for χ^1, the side chains of Lys and Arg were kept fixed in the extended conformation throughout all minimizations up to this point. Once again, the energies of the minima formed a continuum. The 88 minimum-energy conformations showed which combinations of low-energy Thr and tripeptide conformations gave low-energy

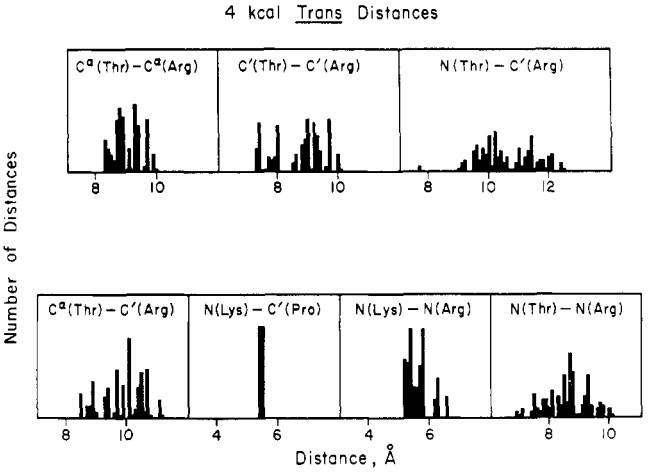

Figure 6. The backbone–backbone distance histograms for trans tuftsin, including all minimum-energy conformations for which ΔE is 4 kcal or less.

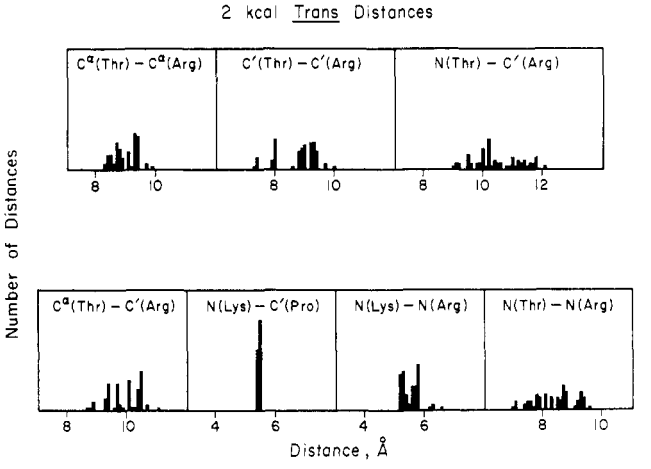

Figure 7. The backbone–backbone distance histograms for trans tuftsin, including all minimum-energy conformations for which ΔE is 2 kcal or less.

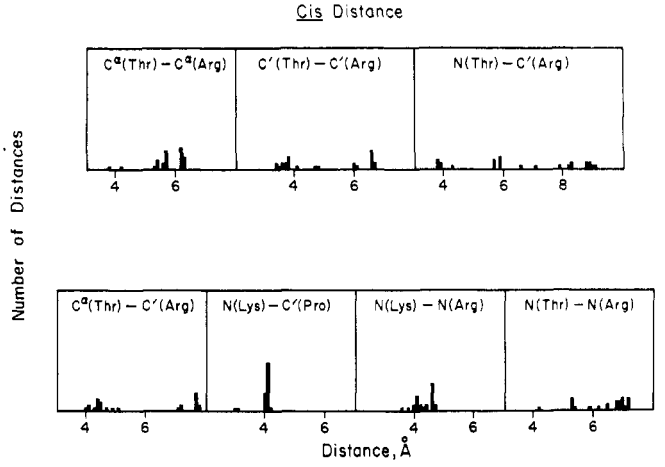

Figure 8. The backbone–backbone distance histograms for cis tuftsin, including all minimum-energy conformations for which ΔE is 4 kcal or less.

tuftsin conformations and were used as a basis for the derivation of generalized low-energy conformations for Thr and the tripeptide. These were combined to give a more complete set of low-energy starting points of tuftsin, once the optimum

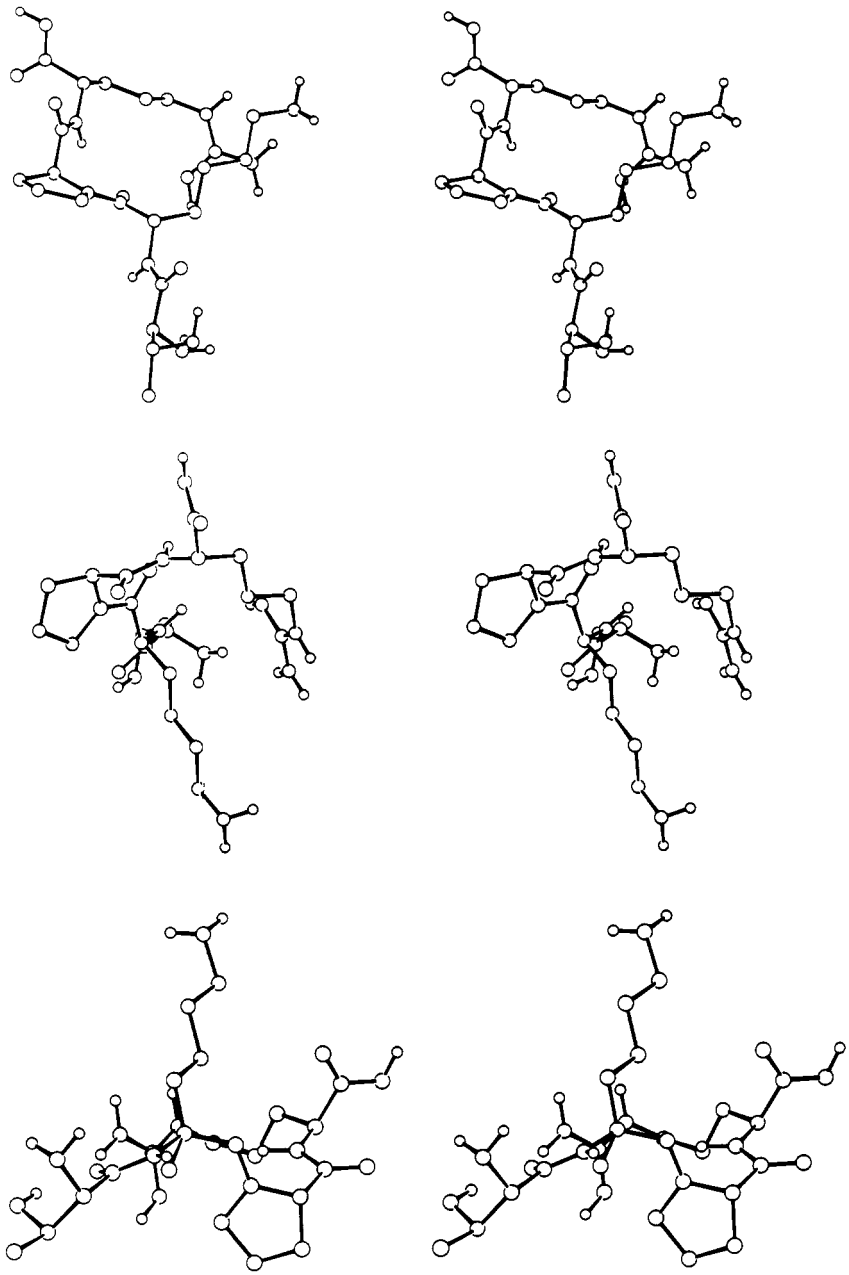

Figure 9. Stereo views of representative tuftsin conformations: from top to bottom, (trans) C(I)DCF, ΔE = 0.0 kcal; (trans) C(I)ECF, ΔE = 0.042 kcal; and (trans) C(I)DAC, ΔE = 0.195 kcal.

conformations of the side chains of Lys and Arg had been determined, as described below.

Variation of χ^2 to χ^5 of Lys did not produce any conformations with lower energy than those with this portion of the side chain in the extended conformation. Inspection of molecular models showed that the probability of the existence of stabilizing interactions between the Lys and Arg side chains was very low. Variations of the Arg side-chain conformation were then carried out on the tripeptide Lys-Pro-Arg. Having already varied χ^1 (and also finding[17] that $\chi^2 \simeq 180°$ for Arg single-residue minima), starting conformations were selected by taking seven possible combinations of χ^3 and χ^4 (with χ^5, $\chi^{6,1}$, and $\chi^{6,2}$ fixed at values for the single-residue minima). These were combined with the general backbone conformations (18 trans and 12 cis) of the Lys-Pro-Arg tripeptide derived from the 88 low-energy conformations of tuftsin, described above, to provide 210 tripeptide starting conformations. Their energies were then minimized with respect to ψ, ω of Pro and ϕ, ψ, χ^1, χ^2, χ^3, χ^4, χ^5 of Arg, with the Lys side chain held fixed in the extended conformation. After minimization, the lowest energy member of each of the 30 tripeptide backbone conformational classes was selected for combination with various conformations of Thr to form a set (designated as set A) of initial conformations of tuftsin whose energies were then minimized to form set B (see below). The conformations of Thr that were chosen were those which had been obtained previously in low-energy conformations of tuftsin; nine and five conformations of Thr were picked for combination with the trans and cis tripeptide conformations, respectively. The conformations of Thr and of the tripeptide used to generate set A are shown in Table I. The energies of the derived conformations ($9 \times 18 + 5 \times 12 = 222$) were minimized with respect to ϕ, ψ, χ^1, $\chi^{2,1}$ of Thr, ϕ, ψ, ω, χ^1 of Lys, ψ, ω of Pro, and ϕ, ψ, χ^1 of Arg. Of these, 189 of the resulting energies were within 4 kcal of the minimum. These 189 conformations form set B, which will be discussed below.

Additional starting conformations were obtained by generating various types of bends[16] not already included in set A (with ϕ, ψ of Lys and ψ of Pro defining the bend, and Thr and Arg kept in the extended form). The energies were mini-

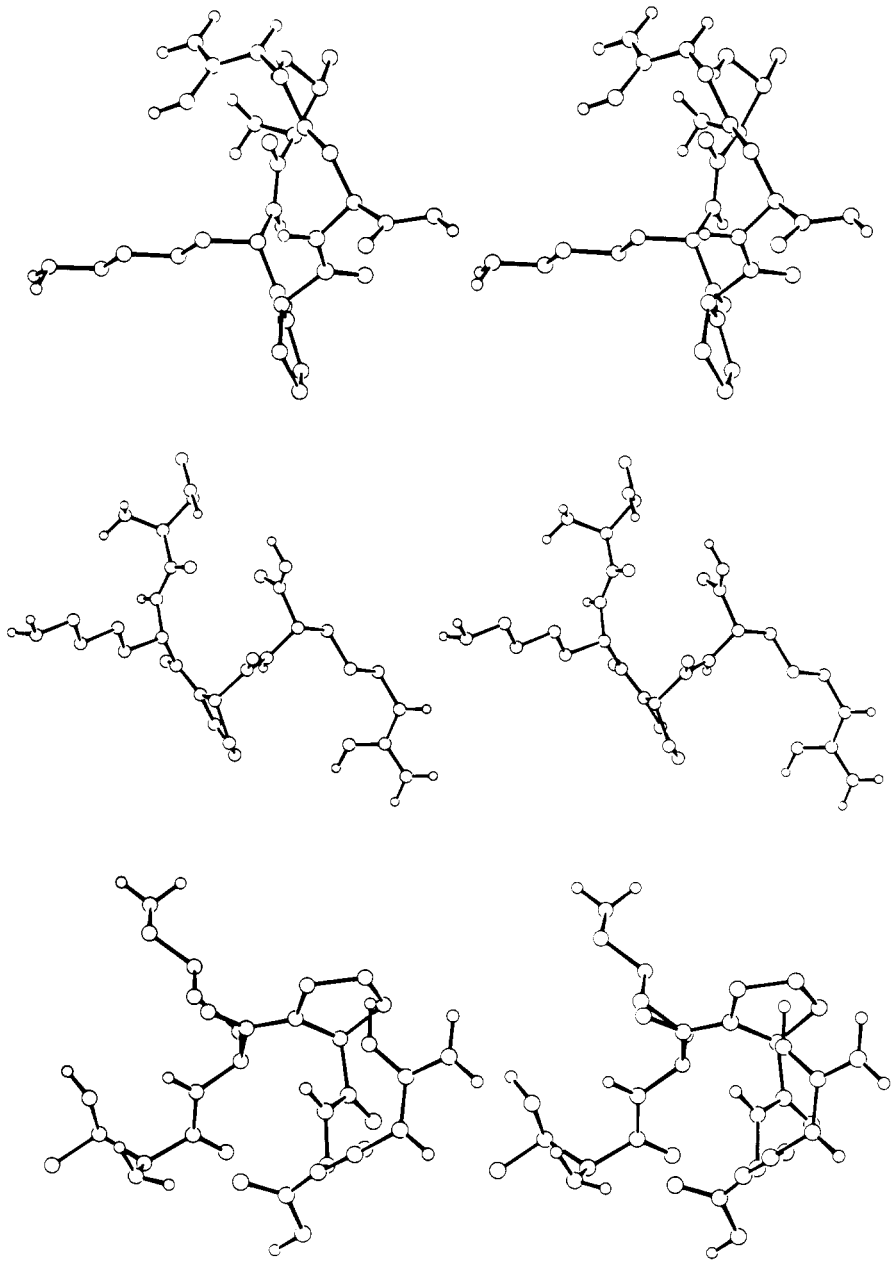

Figure 10. Stereo views of representative tuftsin conformations: from top to bottom, (cis) C(I)EFF, ΔE = 1.718 kcal; (cis) C(I)EAC, ΔE = 1.972 kcal; and (cis) AEAC, ΔE = 2.290 kcal.

mized with respect to ϕ, ψ, χ^1, χ^2 of Thr, ϕ, ψ, ω, χ^1 of Lys, ψ, ω of Pro, and ϕ, ψ, χ^1 of Arg, giving the molecule the required freedom (during the minimization[20]) to assume low-energy bend conformations. All minimized energies were several kcal above the minimum. Apparently, there are no low-energy bend conformations of tuftsin other than those included in set B.

In summary, it can be seen that a broad search of conformational space has shown that the region of the global minimum is characterized by many structures whose energies are quite similar. A group of conformations which provides a fairly complete representation of the low-energy region (set B) has been generated. The energies of the members of set B are shown in Figure 1. It is not to be supposed that the set contains all conformations which are within 4 kcal of the minimum, since variation of the side-chain conformations would increase the size of the set to cumbersome proportions. However, the selection strategies employed and the results obtained suggest that the great majority of the backbone conformations which are within 4 kcal of the minimum are represented. At any rate, the 189 conformations of tuftsin of set B form the basis for the discussion of the results presented below.

IV. Discussion

From Figure 1, it is clear that there is no single unique conformation of tuftsin. Rather, this molecule must exist as an ensemble of conformations, appropriately (Boltzmann) weighted by the energies shown in Figure 1.

First of all, it can be seen from Figure 1 that the ensemble is dominated by trans conformers. Second, the Lys-Pro segment occurs in only a few conformations, viz., DA, DC, DF, EA, EC, EF, FC, DA, DF, and A*C, while the conformations of Thr and Arg are less restricted, as may be seen in Table I.

More quantitative information about the conformational restrictions on the single residues is presented in the ϕ,ψ plots for the terminally blocked single residues and for the corresponding residues in trans and cis tuftsin shown in Figures 2 to 5. These plots contain no information about the frequency of occurrence of a given ϕ,ψ pair; a point at a certain location

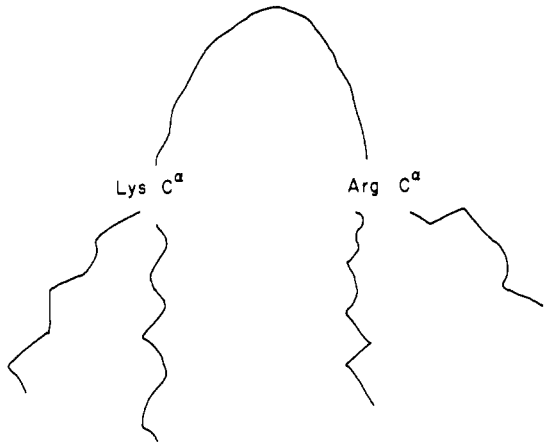

Figure 11. Schematic drawing of the average structure of tuftsin: the hairpin with two split ends.

indicates only that this pair was found at least once. Also, in considering the plots for Thr and Arg, it must be remembered that these residues in tuftsin each have one unblocked terminus, whereas the plots for the terminally blocked residues pertain to structures with no unblocked termini.

Figures 2 to 5 show that the occupied areas of ϕ,ψ are fairly well localized. This indicates that, while there are many minima for tuftsin, they are well defined. The cis and trans plots are similar for all residues. The plots for Thr, Pro, and Arg in tuftsin are similar to those for the blocked single residues, the locations in the tuftsin plots being shifted somewhat and some of the single-residue regions not appearing. The Lys plots, however, are different, since the Pro residue restricts the space of the preceding residue; in general, values of $\psi_X <$ 70° (in X-Pro dipeptides) cause atomic overlaps and hence are not observed.[16] However, as is true for other X-Pro dipeptides,[16] the values of ψ_{Lys} in tuftsin are much more severely restricted than this, the observed ϕ,ψ pairs being confined to two narrow bands between ψ = 80 and 90° and ψ = 150 and 160°. Only the D, E, F, and A* conformations of Lys are observed, the majority of the low-energy conformations being D. While the conformation of Lys depends (primarily) on intraresidue interactions and interactions with Pro, the similarity of the tuftsin and single-residue plots for Thr, Pro, and Arg indicates that the conformations of these residues are determined largely by intraresidue interactions. Further, the central Lys-Pro portion of tuftsin is similar in most of the low-energy conformations.

A comparison of the various minimum-energy structures of set B can be obtained from an examination of the distance histograms for various backbone atoms, shown in Figures 6–8. In the case of the trans conformers, there were enough low-energy minima to justify considering both a 4 and a 2 kcal cutoff (Figures 6 and 7, respectively). A comparison of Figures 6 and 7 provides information as to how the inclusion of higher energy conformations (which occur only infrequently) affects the average structure of the ensemble; the spread in the distances appears to depend only weakly on the range of conformational energies. The 4-kcal cutoff was used for the cis distance histograms. The observed limits on the cis and trans distance distributions imply that both subensembles have fairly well-defined average structures; however, the average structures for cis and trans appear to be different. All trans conformers have a very sharp N(Lys)···C′(Pro) distribution at 5.4 Å, indicating that the Lys-Pro portion is similar in all the trans conformers. The same is true for the cis conformers, but the distance is shorter (4.1 Å). The N(Lys)···N(Arg) histograms lead to the same conclusion. The average cis structure is a bend, while the average trans structure is not, as may be

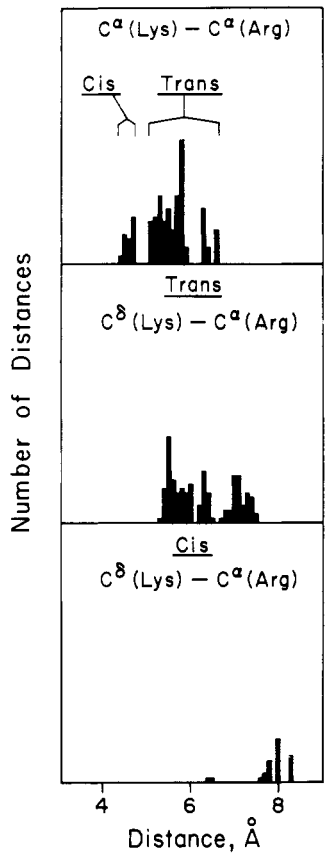

Figure 12. The C^{α}(Lys)···C^{α}(Arg) and C^{δ}(Lys)···C^{α}(Arg) distance histograms.

seen from the C^{α}(Thr)–C^{α}(Arg) distance plots (bends being defined[20] as structures in which this distance is ≤7 Å). The distributions of C^{α}(Thr)···C^{α}(Arg) distances, though broader than those mentioned above, are sharper than those involving the end atoms [N(Thr)···C′(Arg)]. It thus appears that there are essentially two average structures (one for trans and one for cis), each ensemble having its own characteristic structure between C^{α}(Thr) and C^{α}(Arg), with a number of different conformations of the end atoms.

The distance histograms have shown that the average molecular structures are well-defined; therefore, these structures may be pictured accurately from stereo drawings of only a few individual conformations. Stereo drawings of six representative conformations of tuftsin, 3 trans and 3 cis, are shown in Figures 9 and 10. These drawings indicate that there is only one (not two) general average structure for the molecule, which is shown in Figure 11. It may be described as a hairpin with two split ends. In the trans conformations, the outer portions of the splits are formed by the backbone, and the inner portions by the side chains, while the reverse is true for the cis conformations. In gross terms, the cis and trans structures are the same.

The stereo drawings suggest that the average C^{α}(Lys)··· C^{α}(Arg) distances should be roughly the same for the cis and trans structures. Also the average cis and trans C^{δ}(Lys)··· C^{α}(Arg) distances should be close to the average trans and cis C^{α}(Thr)···C^{α}(Arg) distances, respectively. The C^{α}(Lys)··· C^{α}(Arg) and C^{δ}(Lys)···(Arg) distance histograms are shown in Figure 12, and the C^{α}(Thr)···C^{α}(Arg) distance histograms are shown in Figures 6–8. The average cis C^{α}(Lys)···C^{α}(Arg) distance is slightly shorter than the corresponding trans distance; similarly, the cis C^{δ}(Lys)···C^{α}(Arg) distance is a bit shorter than the trans C^{α}(Thr)···C^{α}(Arg) distance, while the trans C^{δ}(Lys)···C^{α}(Arg) distance is marginally longer than the

Table II
Phagocytic Activity of Certain Tuftsin Analogues[a]

Peptide	Phagocytic index[b]	Peptide	Phagocytic index[b]
Thr-Lys-Pro-Arg (Tuftsin)	19	Thr-Gly-Gly-Lys	1
Thr-Lys-Pro-Ala	12	Thr-Lys-Ala-Ala	2–3
Thr-Ala-Val-Arg	14	Thr-Lys-Lys-Ala	9–10
Thr-Ala-Arg-Lys	13		

[a] Reference 22. [b] The "phagocytic index" is the number of cells (above the control level) per 100 cells which have ingested at least one phage. Tuftsin increases the number of phagocytizing cells by a factor of 2–2.5 above the control level.

cis C^{α}(Thr)···C^{α} distance. These comparisons reflect the slightly shorter N(Lys)···C′(Pro) distance in the cis conformers. However, the distance histograms in Figure 12 not only support the picture of the general average structure suggested by the stereo drawings but also help to characterize it.

These conformations were also examined for possible hydrogen bonds, considered to exist if the distance between a polar H and an acceptor N or O atom was less than 2.3 Å.[16] No strong hydrogen bonds between Thr and the rest of the molecule were found (only those seen in the terminally blocked single residue were found). Hydrogen bonding distances involving other residues in several conformations were near the 2.3-Å limit; for trans, this was usually associated with a C_7^{eq} bond between the C=O of Lys and the NH of Arg while, for cis, the interaction was between the backbone N and the carboxyl H of Arg. The C_7 hydrogen bond was absent in the lowest energy trans conformations. Hence, since interresidue hydrogen bonds play no role in stabilizing the conformation of tuftsin, our omission of solvent effects and choice of ionizable groups as neutral would not alter the conclusions drawn here about the average structure of the isolated tuftsin molecule.

These conclusions about the average structure are consistent with experimental evidence on analogues of tuftsin. Studies of the peptides Thr-Lys-Pro, Lys-Pro-Arg, and Thr-Lys-Pro-Pro-Arg showed that the two tripeptides do not stimulate phagocytosis, while the pentapeptide not only has no stimulatory activity but indeed blocks the enhancement of phagocytosis by tuftsin.[21] The results of another study[22] are shown in Table II. As can be seen from this table, the charge on the side chains of Lys and Arg appears to have only a minor influence on the phagocytic activity of the peptides. The major factor influencing activity appears to be a bulky side chain in position 3. Finally, Thr in position 1 is not essential for activity, since the tetrapeptide Leu-Lys-Pro-Arg stimulates phagocytosis even more strongly than does tuftsin itself.[23]

All of these observations are in accord with the notion that it is the average structure (rather than the state of ionization) which determines the activity of tuftsin. The fact that no one computed conformation is markedly more stable than the others is consistent with the observation that diverse analogues mimic the behavior of tuftsin; from the variety of active analogues, it seems that the action of tuftsin does not depend upon a narrowly defined molecular conformation. However, certain structural features do appear to be necessary for an analogue to show biological activity. The studies described in ref 21–23, when linked with the description of the average structure (determined by the pyrrolidine ring and derived from the computations), allow the formulation of a fairly complete picture of the receptor site and mode of physiological action of tuftsin. A more complete exposition of these ideas will be presented elsewhere.[24]

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Miniprint Material Available: Full size copies of Table I showing conformations of Thr and Lys-Pro-Arg used to generate members of set A (2 pages). See the ordering information for supplementary material on any current masthead page.

References and Notes

(1) This work was supported by research grants from the National Institute of Arthritis and Metabolic Diseases of the National Institutes of Health, U.S. Public Health Servide (AM-13743), and from the National Science Foundation (PCM75-08691). S.F. was a Chaim Weizmann Postdoctoral Fellow.
(2) I. Simon, G. Némethy, and H. A. Scheraga, *Macromolecules*, preceding paper in this issue.
(3) Y. Isogai, G. Némethy, and H. A. Scheraga, *Proc. Natl. Acad. Sci. U.S.A.*, **74**, 414 (1977).
(4) J. S. Anderson and H. A. Scheraga, *Macromolecules*, following paper in this issue.
(5) S. J. Leach, I. D. Rae, and H. A. Scheraga, work in progress.
(6) B. V. Fidalgo and V. A. Najjar, *Biochemistry*, **6**, 3386 (1967).
(7) V. A. Najjar, B. V. Fidalgo, and E. Stitt, *Biochemstry*, **7**, 2376 (1968).
(8) V. A. Najjar and A. Constantopoulos, *RES, J. Reticuloendothel. Soc.*, **12**, 197 (1972).
(9) K. Nishioka, A. Constantopoulos, P. S. Satoh, and V. A. Najjar, *Biochem. Biophys. Res. Commun.*, **47**, 172 (1972).
(10) K. Nishioka, A. Constantopoulos, P. S. Satoh, W. M. Mitchell, and V. A. Najjar, *Biochim. Biophys. Acta*, **310**, 217 (1973).
(11) F. A. Momany, R. F. McGuire, A. W. Burgess, and H. A. Scheraga, *J. Phys. Chem.*, **79**, 2361 (1975).
(12) A computer program named ECEPP (Empirical Conformational Energy Program for Peptides) was used.[11] The Fortran computer program for ECEPP, its description, and the associated structural and energy parameters are available on magnetic tape from the Quantum Chemistry Program Exchange, as program No. QCPE 286. See footnote 60 of ref 11 for the procedure to obtain this material.
(13) J. C. Howard, F. A. Momany, R. H. Andreatta, and H. A. Scheraga, *Macromolecules*, **6**, 535 (1973).
(14) J. C. Howard, A. Ali, H. A. Scheraga, and F. A. Momany, *Macromolecules*, **8**, 607 (1975).
(15) R. F. McGuire, G. Vanderkooi, F. A. Momany, R. T. Ingwall, G. M. Crippen, N. Lotan, R. W. Tuttle, K. S. Kashuba, and H. A. Scheraga, *Macromolecules*, **4**, 112 (1971).
(16) S. S. Zimmerman and H. A. Scheraga, *Biopolymers*, **16**, 811 (1977).
(17) S. S. Zimmerman, M. S. Pottle, G. Nemethy, and H. A. Scheraga, *Macromolecules*, **10**, 1 (1977).
(18) M. J. D. Powell, *Comput. J.*, **7**, 155 (1964).
(19) J. E. Dennis and H. H. W. Mei, Technical Report No. 75-246, 1975, Deptartment of Computer Sci., Cornell University, Ithaca, N.Y., 14853.
(20) P. N. Lewis, F. A. Momany, and H. A. Scheraga, *Biochim. Biophys. Acta*, **303**, 211 (1973).
(21) K. Nishioka, P. S. Satoh, A. Constantopoulos, and V. A. Najjar, *Biochim. Biophys. Acta*, **310**, 230 (1973).
(22) D. Konopinska, E. Nawrocka, I. Z. Simon, S. Szymaniec, and E. Klonowska, *Int. J. Pept. Protein Res.*, **9**, 71 (1977).
(23) S. Matsuura, A. Takasaki, H. Hirotami, T. Kotera, and S. Fujiwara, *Japan Kokai*, **13**, 373 (1975); *Chem. Abstr.*, **83**, 114937 (1975).
(24) S. Fitzwater, J. S. Anderson, and H. A. Scheraga, to be submitted.