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Synthesis and Conformational Studies on 3-*o*-Tolylhydantoins by NMR and Molecular Modeling: Dipole-π Attractions in Peptides and Proteins

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5-Pentafluorobenzyl-3-*o*-tolylhydantoin (**2**) was synthesized for reducing this π-electron density, and NMR studies have shown that we have succeeded in changing the conformation of **2** into an extended system instead of the folded conformation for the non-fluorinated compound, 5-benzyl-3-*o*-tolylhydantoin (**1**). Molecular modeling has also confirmed the extended structure for **2**. An approach has been found for possibly modulating the physiological activity of peptides containing aromatic amino acids.

When the conformation of molecules is studied, one usually considers steric repulsion resulting in a less crowded conformation. For example, in the case of butane, the anti-conformer is favored over the eclipsed conformer due to steric and torsional strains. But when studying the conformation of small peptides and other polar compounds derived from aromatic amino acids, one should consider non-bonded attraction, which can result in a more crowded conformation. The interaction between a dipole and the dipole induced by it in a π-electron cloud constitutes a force of attraction.[1] Such attraction at the intramolecular level could influence the conformation of peptides and proteins. Recently, several reports describing cation-π attractions in peptides and proteins have appeared in the literature.[2] This type of non-bonded attraction could be an important factor governing the biological characteristics of these molecules. The conformation of these molecules and indications of the non-bonded attraction between the aromatic ring and polar group in another part of the molecule were therefore studied here with the help of nuclear magnetic resonance (NMR) and molecular modeling.

Over the past several decades, NMR spectroscopy has become firmly established as the most powerful method for structure analysis in solution in the field of biochemistry as well as organic chemistry. The parameters normally obtained from a study of the NMR spectra are the chemical shift,[3] nuclear spin-spin coupling[4] via electrons and nuclear relaxation time[5] (temperature dependent).

Monte Carlo (MC) and molecular dynamics are the primary methods used for the free-energy simulation of molecular systems. The application of molecular dynamics to molecules that have multiple conformations separated by energy barriers of more than 3 kcal/mol is problematic because of slow rates of convergence.[6] Monte Carlo sampling does not efficiently sample the bottom of the potential energy wells, thereby causing slow rates of convergence. Guarnieri and Still introduced a hybrid simulation method termed MC-SD,[6] which mixes Monte Carlo (MC) and stochastic dynamics (SD) sampling. The new method generates a canonical ensemble by altering MC and SD steps and combines the local exploration strengths of dynamics with the barrier-crossing ability of large-step Monte Carlo.[6] MC-SD simulations converge faster than either MC or SD alone and generate ensembles which are equivalent to those created by MC or SD alone.[6]

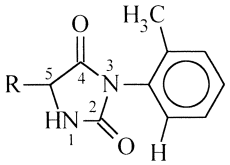
Results and Discussion

In order to obtain detailed information about the dipole-π attraction in aromatic amino acid derivatives, (±)-5-substituted 3-*o*-tolylhydantoins **1–3** and 3-*o*-tolylhydantoin (**4**) were prepared in our laboratory from aromatic and aliphatic amino acids because of their easy preparation and conformation determination from NMR spectra. Conformational studies on the hydantoins were conducted by both ^{1}H and ^{13}C NMR (nuclear magnetic resonance) and molecular modeling. Conformational information was derived from several aspects of NMR studies of some hydantoins. The aspects studied were proton chemical shifts of the *o*-methyl group, the coupling constants between the benzylic protons and C-5 proton from A_2X pattern, ^{13}C NMR, and the temperature dependence of the *o*-methyl group in 3-*o*-tolylhydantoins.

Proton Chemical Shifts of *o*-Methyl and *o*-Proton in 3-*o*-Tolylhydantoins. In view of the well-known restricted rotation of amide derivatives, it appeared to be of interest to study 3-*o*-tolylhydantoins. The structures of rotamers **A** and **B** resulting from restricted rotation about the *N*-aryl bond are shown in Fig. 1. The chemical shifts of the *o*-methyl group in 3-*o*-tolylhydantoins derived from aromatic and aliphatic amino acids are listed in Table 1. The methyl resonance of the *o*-tolyl group gives two peaks for compounds **1**, **2**, and **3** due to the restricted rotation about the *N*-aryl single bond.

The separation of the two peaks in compound **1** is larger

Table 1. ^{1}H NMR Chemical Shifts of *o*-Methyl and *o*-Proton in 3-*o*-Tolylhydantoins



Compd	R	Solvent	Chemical shift/ppm				Population ratio
			CH_{3d}[a)]	CH_3[a)]	H_d[b)]	H_u[b)]	$CH_{3d}(H_u):CH_{3u}(H_d)$
1	$C_6H_5CH_2$	$CDCl_3$	2.20	1.76	7.12	6.63	1:1
		DMSO-d_6	2.06	1.34	7.10	6.23	4:6
2	$C_6F_5CH_2$	DMSO-d_6	2.10	1.93	7.16	7.10	4:6
3	isobutyl	$CDCl_3$	2.16	2.13	7.10	7.06	
4	H	$CDCl_3$	2.23[c)]				

a) Subscript d and u stand for the downfield and upfield *o*-methyl resonances, respectively.
b) Subscript d and u stand for the downfield and upfield *o*-proton resonances, respectively.
c) The value indicates no separation for the proton resonance.

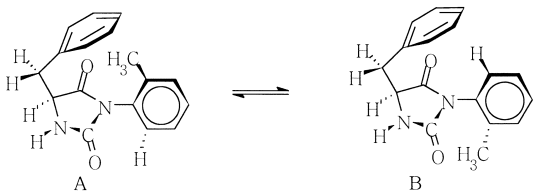


Fig. 1. The folded conformation of 5-benzyl-3-*o*-tolylhydantoin (**1**).

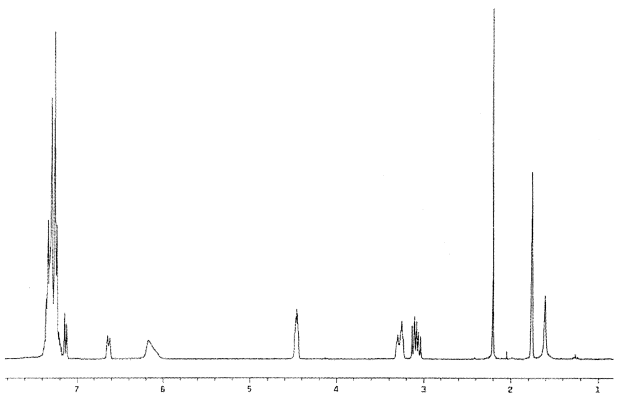


Fig. 2. ^{1}H NMR spectrum of 5-benzyl-3-*o*-tolylhydantoin (**1**).

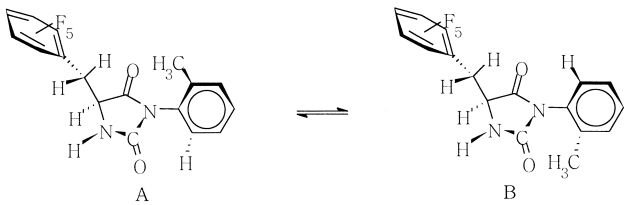


Fig. 3. The extended conformation of 5-pentafluorobenzyl-3-*o*-tolylhydantoin (**2**).

than that in **2** and **3**. This is an indication of a folded conformation in compound **1**. The lower NMR peak of the *o*-methyl group shows up around the normal position of an aryl *o*-methyl. The other peak of the methyl group is 0.44 ppm upfield from the normal peak. Obviously, the upper peak is diamagnetically shielded by the C-5 benzyl group (Fig. 1). On the other hand, the methyl group in the *o*-tolyl is not shielded when the *N*-aryl bond rotates through 180 degrees (Fig. 1). Therefore, there are two kinds of *o*-methyl peaks in compound **1**. The conformer population of forms **A** and **B** in Fig. 1 is easily determined by integration of the downfield and upfield *o*-methyl and *o*-proton in C-3 *o*-tolyl group. The ^{1}H NMR spectrum of **1** is given in Fig. 2. It is obvious that the integration ratio of the downfield *o*-methyl (I_{CH3d}) and the upfield *o*-methyl (I_{CH3u}) must be equal to the ratio of the upfield *o*-proton (I_{Hu}) and the downfield *o*-proton (I_{Hd}), ($I_{CH3d}/I_{CH3u} = I_{Hu}/I_{Hd}$). When the C-5 position in the 3-*o*-tolylhydantoin ring in **1** is substituted by the pentafluorobenzyl group, the separation of the two peaks of the *o*-methyl in compound **2** is smaller than that in **1**.

This is an indication of a conformation extended away from the hydantoin ring in compound **2** (Fig. 3). This change is due to a reduced π-dipole interaction in **2**. The lower NMR peak of the *o*-methyl group shows up around the normal position of an aryl *o*-methyl. The other peak of the methyl is at 0.17 ppm upfield from the normal peak. One can easily understand that the upper peak is not diamagnetically shielded by the C-5 pentafluorobenzyl group. The methyl group in *o*-tolyl is just chemically nonequivalent when the *N*-aryl bond rotates by 180 degrees. Therefore, there are two kinds of *o*-methyl peaks in compound **2**. The conformer population of forms **A** and **B** in Fig. 3 is also easily determined by the integration of the downfield and upfield *o*-methyl and *o*-proton in C-3 *o*-tolyl group as shown before. ^{1}H NMR spectrum of **2** is given in Fig. 4. When the C-5 position in the 3-*o*-tolylhydantoin ring in **1** is substituted by an isobutyl group, two slightly separated methyl peaks are observed. The separation of the two peaks of the *o*-methyl in compound **3** was much smaller than that in **1**. This is an indication of a conformation extended away from the hydantoin ring in compound **3** (Fig. 5).

There is no π-dipole interaction in compound **3**. The lower NMR peak of the *o*-methyl group shows up around the normal position of an aryl *o*-methyl. The other peak of the methyl is at 0.03 ppm upfield from the normal peak. It would appear that the upper and lower peaks are just chemically nonequivalent

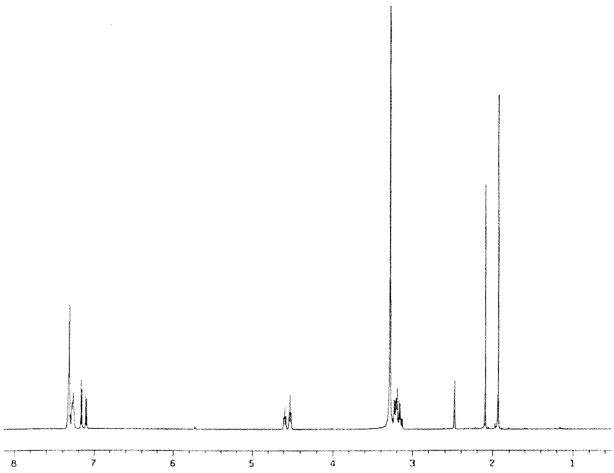


Fig. 4. ^{1}H NMR spectrum of 5-pentafluorobenzyl-3-*o*-tolylhydantoin (**2**).

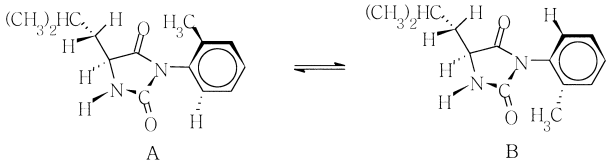


Fig. 5. The conformation of 5-isobutyl-3-*o*-tolylhydantoin (**3**).

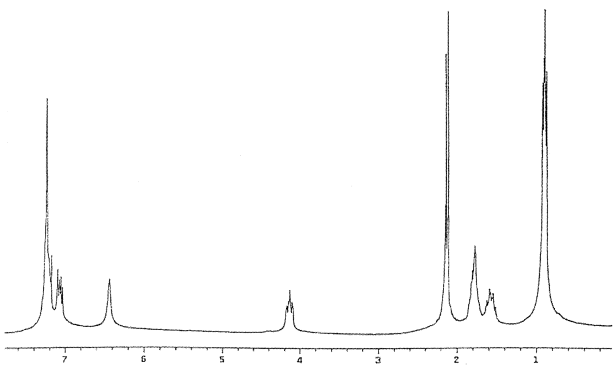


Fig. 6. ^{1}H NMR spectrum of 5-isobutyl-3-*o*-tolylhydantoin (**3**).

when the *N*-aryl bond rotates by 180 degrees (Fig. 5). Therefore, there are two kinds of *o*-methyl peaks in compound **3**. The conformer population of forms **A** and **B** in Fig. 5 is also easily determined by the integration of the downfield and upfield *o*-methyl and *o*-proton in C-3 *o*-tolyl group, as shown before. The ^{1}H NMR spectrum of **3** is given in Fig. 6. When the C-5 position in the 3-*o*-tolylhydantoin ring in **1** is not substituted, no two separated methyl peaks are observed for compound **4**.

As expected from a consideration of symmetry, the ^{1}H NMR spectrum of **4** shows a single peak for the *o*-methyl in tolyl group (Figs. 7 and 8).

In summary, **4** shows a single peak for the methyl protons of *o*-tolyl group due to symmetry of the hydantoin ring. In **2** and **3**, two slightly separated methyl signals are observed. This ac-

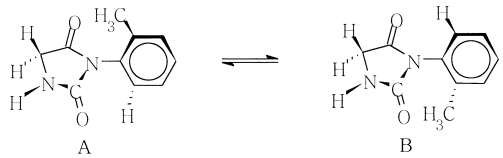


Fig. 7. The conformation of 3-*o*-tolylhydantoin (**3**).

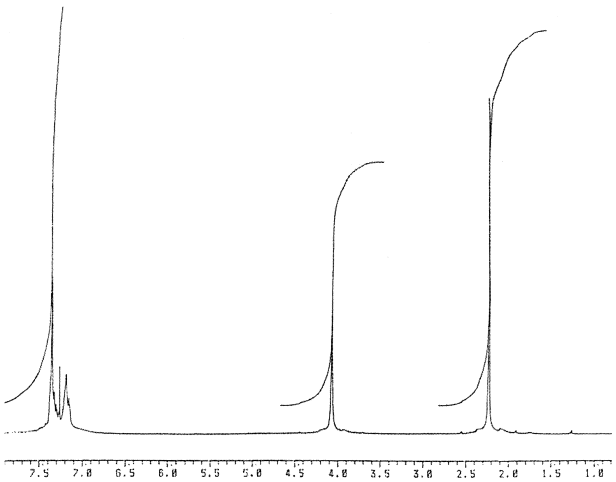
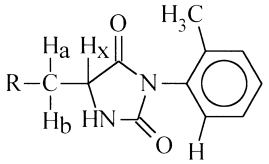


Fig. 8. ^{1}H NMR spectrum of 3-*o*-tolylhydatoin (**4**).

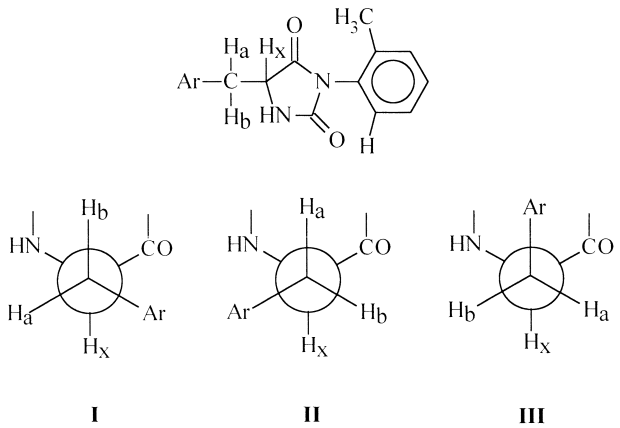
counts for a reduced non-bonded attraction and no non-bonded attraction, respectively. But in **1**, there is clear evidence for a shielding of the methyl group due to the aromatic ring at C-5, resulting from the non-bonded attraction. The downfield methyl signal can be ascribed to the rotamer **A** in Fig. 1, while the upfield signal is due to rotamer **B** in Fig. 1.

The Coupling Constants of the Benzylic Protons and the C-5 Proton from A_2X Patterns in 3-*o*-Tolylhydantoins. The two benzylic protons (H_a, H_b) couple with the C-5 proton (H_x) in **1** and **2** to a coupling pattern, depending upon the solvent. In general, the ABX pattern is obtained in a $CDCl_3$ solution while a DMSO-d_6 solution gives an A_2X pattern. The coupling constants of **1** and **2** are listed in Table 2. The rotational isomerism of hydantoin derivatives in solution is usually represented in terms of the equilibrium mixture of the three staggered rotamers[7,8] illustrated in Fig. 9. Since the internal rotation is sufficiently rapid, the observed coupling constants are weighted averages over those corresponding to the individual rotamers (Eq. 1):

Table 2. The Coupling Constants between Benzylic Protons and C-5 Proton from A_2X Pattern in 3-*o*-Tolylhydantoins



Compd	R	Solvent	J_{avg}/Hz	Population ratio
				Extended : Folded
1	C_6H_5	DMSO-d_6	4.5	3 : 7
2	C_6F_5	DMSO-d_6	7.0	8 : 2



I **II** **III**

Fig. 9. Conformers of 3-*o*-tolylhydantoins.

$$J_{ax} = X_{I}J^{I}_{ax} + X_{II}J^{II}_{ax} + X_{III}J^{III}_{ax},$$
$$J_{bx} = X_{I}J^{I}_{bx} + X_{II}J^{II}_{bx} + X_{III}J^{III}_{bx},$$
$$J_{ab} = X_{I}J^{I}_{ab} + X_{II}J^{II}_{ab} + X_{III}J^{III}_{ab},$$
$$X_{I} + X_{II} + X_{III} = 1. \quad \text{(Eq. 1)}$$

where J_{ax}, J_{bx}, and J_{ab} are accurately calculated experimental vicinal and geminal coupling constants, and J^{I}_{ax} means the coupling constant between nuclei a and x in the rotamer **I**. X_{I}, X_{II}, and X_{III} are also defined as the rotamer populations for forms **I**, **II**, and **III**, respectively. The dependence on coupling constants on the dihedral angle in accordance with the Karplus equation[9] (Eq. 2) is well documented:

$$J_{av} = K_1 \cos^2 \varphi - 0.28 \text{ for } 0° < \varphi < 90°,$$
$$J_{av} = K_2 \cos^2 \varphi - 0.28 \text{ for } 90° < \varphi < 180°. \quad \text{(Eq. 2)}$$

$J^{I,\,II,\,III}_{ax}$ in Eq. 1 may be replaced by J_g and J_t for vicinal proton *gauch* and *trans* to one another. Then Eq. 1 becomes Eq. 3:

$$J_{ax} = (1 - X_{II})\,J_g + X_{II}J_t,$$
$$J_{bx} = (1 - X_{I})\,J_g + X_{I}J_t. \quad \text{(Eq. 3)}$$

If J_g and J_t were known, the populations of the three rotamers could be evaluated from the Eq. 3. According to the values of K_1 and K_2 of the Karplus equation by Pachler.[10] J_g and J_t calculated to be 2.60 and 13.56 for α-amino acid, respectively. The average coupling constant, J_{av}, can also be calculated from the electronegativity values[11] for the atom directly attached to the C–C fragment[12] (Eq. 4):

$$J_{av} = 18.0 - 0.80\Sigma E_i$$
$$= 18.0 - 0.80 \times 14.56 = 6.35 \text{ (Hz)}. \quad \text{(Eq. 4)}$$

Unfortunately the elctronegativity of the hydantoin ring has not yet been reported. However, in a simple comparison of the vicinal coupling constants of the C-2 methyl protons and the C-5 methyl protons between DL-alanine (**1**) and 5-methylhydantoin (**2**), there is not much difference (Fig. 10).

The calculated average coupling constant from Eq. 4 for DL-alanine is 6.7 Hz and for phenylalanine is 6.25 Hz. The observed and estimated coupling constants of 3-ethyl-5-phenethylhydatoin are 6.9 Hz and 6.6 Hz, respectively. Therefore, it can be assumed that the values of J_g and J_t for the hydantoin derivative are very close to that for an α-amino acid (J_g = 2.60, J_t = 13.56). Since it is not possible to distinguish which is the H_a and H_b in the benzyl protons without a labeling study, the population of rotamer **I** and **II** in Fig. 9 is not distinguishable. The total population of rotamer **I** and **II** is independent of the assignment of Ha and H_b. The average vicinal coupling constant (J_{av}) can be measured directly from the ^{1}H NMR spectra. The average coupling constant was derived from Eq. 3:

$$J_{av} = (J_{ax} + J_{bx})/2 = \{2 - (X_{I} + X_{II})J_g + (X_{I} + X_{II})J_t\}/2$$
$$= \{(1 + X_{III})J_g + (1 - X_{III})J_t\}/2.$$

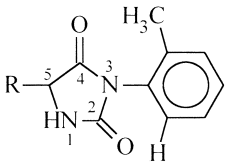
The rotamer populations of **1** and **2** are listed in Table 2.

^{13}C NMR Spectra of 3-*o*-Tolylhydantoins. The ^{13}C NMR spectra are considerably more sensitive to the stereochemistry of the molecule. The naturally abundant ^{13}C NMR spectra of *o*-tolylhydantoins were measured in $CDCl_3$ and DMSO-d_6. The chemical shifts are listed in Table 3. The

1 **2**

Fig. 10. DL-Alanine (1) and 5-methylhydantoin (2).

Table 3. ^{13}C NMR Chemical Shifts of 3-*o*-Tolylhydantoins

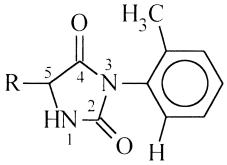


Compd	R	Chemical shifts/ppm			
		CH_3	C-5	CO(2)	CO(4)
1	$C_6H_5CH_2$	17.2, 17.9	58.5, 58.7	172.1	156.6
2	$C_6F_5CH_2$	17.1, 17.9	56.6, 56.8	172.2	156.0
3	isobutyl	17.8, 18.0	41.1, 41.7	173.3	156.7
4	H	17.7	46.7	170.5	157.8

chemical shifts of aromatic carbon from the spectra are difficult to assign due to the complex pattern, but the signals of the *o*-methyl carbon, benzylic carbon, and hydantoin ring carbons are relatively easy to identify. Compounds **1**, **2**, and **3** lose symmetry. The ^{13}C NMR spectra of *o*-methyl carbon show two distinguishable peaks due in part to the asymmetric center at C-5. Compound **4** has symmetry, and the ^{13}C NMR spectrum of *o*-methyl carbon displays a single peak. This ^{13}C NMR result strongly supports the existence of conformational isomers. Since compounds **1**, **2**, and **3** are asymmetric at the C-5, the ^{13}C NMR spectra for C-5 also give two peaks. The carbonyl resonance is split and appears downfield with C-2 at a relatively higher field than C-4. This agrees with the carbonyls in 3-hydroxyethyl-5-dimethylhydantoin[13] and in substituted uracils.[14] The down-field carbonyl peaks are moved downfield 2–3 ppm by substitution at the 5-position. Similar effects in alkyl substitution on acetone are also reported by Jackman and Kelly.[15] The difference in the chemical shifts between two resonances of the *o*-methyl increases considerably when the substituent at C-5 is a benzyl group, indicating a substantial contribution from the folded conformation of the hydantoin. The split for *o*-methyl peaks in compound **1** is largest (0.73 ppm) in Table 3, and supports the folded conformation between the 5-benzyl and the hydantoin ring.

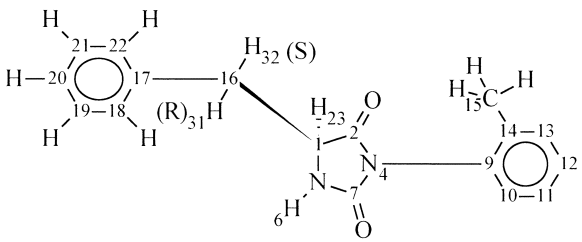
Temperature Dependence of ^{1}H NMR Spectra of 3-*o*-Tolylhydantoins. The chemical shifts and the coupling constants of hydantoin derivatives change with temperature.[1] Temperature studies on 5-benzyl-3-*o*-tolylhydantoin (**1**) in DMSO-d_6 solution show that the *o*-methyl and *o*-proton coalesce at 150 °C (Table 4). The *o*-tolyl group can rotate freely above this temperature. The downfield *o*-methyl peak moves further upfield, while the upfield methyl peak moves further downfield. As the temperature increases, the 5-benzyl group also starts to rotate and the diamagnetically shielded peak moves rapidly downfield. The coupling constant of the benzylic protons with the adjacent C-5 proton and the chemical shift of *o*-methyl are changed when the temperature decreases in acetone-d_6.[1] The downfield *o*-methyl peak is independent of the temperature. In contrast, the upfield methyl peak moves further upfield, and at the same time the coupling constant (J_{avg}) decreases, indicating the stability of the folded conformation at low temperature. Temperature studies on 5-pentafluorobenzyl-3-*o*-tolylhydantoin (**2**) in DMSO-d_6 solution show that the *o*-methyl and *o*-proton coalesce at 120 °C (Table 4). The *o*-tolyl group can rotate freely above this temperature. The coupling constant of the benzylic protons with the adjacent C-5 proton and the chemical shift of *o*-methyl are changed when the temperature increases in DMSO-d_6. The downfield *o*-methyl peak moves further upfield, while the upfield methyl peak moves further downfield (Table 4).

Table 4. Coalescence Temperatures for 3-*o*-Tolylhydantoins

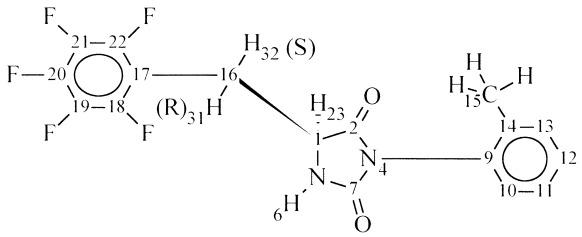


Compd	R	Coalescence temp./°C
1	$C_6H_5CH_2$	150
2	$C_6H_5CH_2$	120

Molecular Modeling Studies on 3-*o*-Tolylhydantoins. We were interested in predicting the relative populations of folded and extended conformations of the hydantoin system **1** and **2** described earlier. This can be done efficiently using modern computational techniques. A technique was needed which would generate a canonical ensemble for the benzyl and pentafluorobenzyl hydantoin system in a high dielectric solvent, since the NMR experiment was performed in a DMSO-d_6 solution. To this end, a hybrid Monte Carlo (MC)–stochastic dynamics (SD) calculation[6] was performed using a continuum water solvent mode[16] implemented in Macro Model[17] version 5.0. The simulation method MC–SD was chosen because it combines the local exploration strengths of dynamics and the barrier-crossing ability of large-step Monte Carlo.[6] The simulation was done in water (dielectric constant = 78.7), as being the most representative dielectric available which is similar to DMSO (dielectric constant = 45). After a period of 100 ps of stochastic dynamics equilibrium, long simulations (5000 ps) were carried out at 300 K. The simulations were to be converged by the obtained constant temperature and average enthalpies. All three ratable bonds were permitted to vary during the simulations. During the simulations, 1000 snapshots were taken at equal intervals. From this sampling, the populations of the folded versus extended forms were obtained, as well as the populations of the *o*-tolyl groups in the two conformations-above and below the plane of the hydantoin ring. The torsions T1 and T2 defined by H23–C1–C16–H32 (Fig. 11) and H6–C1–C16–H31 (Fig. 11) were used to quantitate to the groups



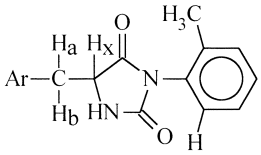
5-Benzyl-3-*o*-tolylhydantoin (**1**)

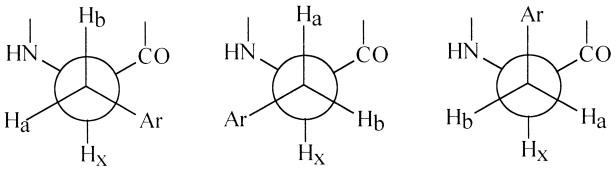


5-Pentafluorobenzyl-3-*o*-tolylhydantoin (**2**)

Fig. 11. The torsions T1 and T2 defined by H23–C1–C16–H32 and H6–C1–C16–H31.

Table 5. Torsion Angle Constraints to Determine Populations of States I, II, and III





I **II** **III**

Torsion (T)	Atoms defining T	State I	State II	State III
T1	H23–C1–C16–H32	−60 ± 60	180 ± 60	60 ± 60
T2	H6–C1–C16–H31	180 ± 60	60 ± 60	−60 ± 60

Table 6. Populations of Each Conformational State Based on the 1000 Snapshot Sampling for the Folded versus Extended Form

	Compound **1**	Compound **1**	Compound **2**	Compound **2**
State	# of conformers	% of conformers	# of conformers	% of conformers
I	293	29.3	557	55.7
II	105	10.5	409	40.9
III	601	60.1	32	3.2

Table 7. Populations of Each Conformational State Based on the 1000 Snapshot Sampling of the *o*-Tolyl Group (Torsion: C7–C4–C9–C14)

	B (90 ± 45°)	**B** (90 ± 45°)	**A** (−90 ± 45°)	**A** (−90 ± 45°)
Compound	# of conformers	% of conformers	# of conformers	% of conformers
1	373	37.3	616	61.6
2	423	42.3	568	56.8

previously defined as conformer I, II and III described earlier (Fig. 11).

The torsion defined by C7–C4–C9–C14 (Fig. 11) was used to determine the rotamer states of the *o*-tolyl groups. The results are summarized in the Tables 5–7 below.

The folded form is defined as the conformers where the benzyl group is over the hydantoin ring, state III. The extended form is defined by states I and II (Figs. 12 and 13). For compound **1**, the population ratio of the extended form to the folded form was calculated to be 4:6. For compound **2**, the population ratio of extended form to folded form was found to be 97:3.

The two main states relative to the hydantoin ring were defined as **A**, *o*-methyl group on the same side of the hydantoin ring as the benzyl substituent (torsion C7–C4–C9–C14; −90 ± 45°) and **B**, *o*-methyl group on the opposite side as the benzyl substituent (torsion C7–C4–C9–C14; 90 ± 45°). For compound **1**, the population ratio of **B** to **A** is 37.3%:61.6%. For compound **2**, the population ratio of **B** to **A** is 42.3%:56.8%. This result corresponds to the result of a ^{1}H NMR study (see Table 1).

Conclusions

It is necessary to take into consideration the dipole-π attraction as well as the steric repulsion in studying the conformation of peptides and enzyme-substrate binding. Basic hydrolysis of 5-benzyl-3-*o*-tolylhydantoin (**1**) gives phenylalanine.[18] Similarly, pentafluorophenylalanine could be obtained by the hydrolysis of 5-pentafluorobenzyl-3-*o*-tolylhydantoin (**2**). Phenylalanine is often a constituent of peptides and other polar compounds derived from aromatic amino acids. The biological characteristics of these molecules could be changed by substituting pentafluorophenylalanine or less fluorinated phenylalanine for phenylalanine. Thus, an approach has been found for possibly modulating the physiological activity of peptides containing aromatic amino acids.

Experimental

^{1}H and ^{13}C NMR Studies on 3-*o*-Tolylhydantoins. One-Dimensional ^{1}H and ^{13}C NMR. ^{1}H NMR spectra were taken using a 30° pulse angle by either a 500 MHz Varian Unity spectrometer equipped with a 3-mm triple-resonance gradiant probe (Nalo-

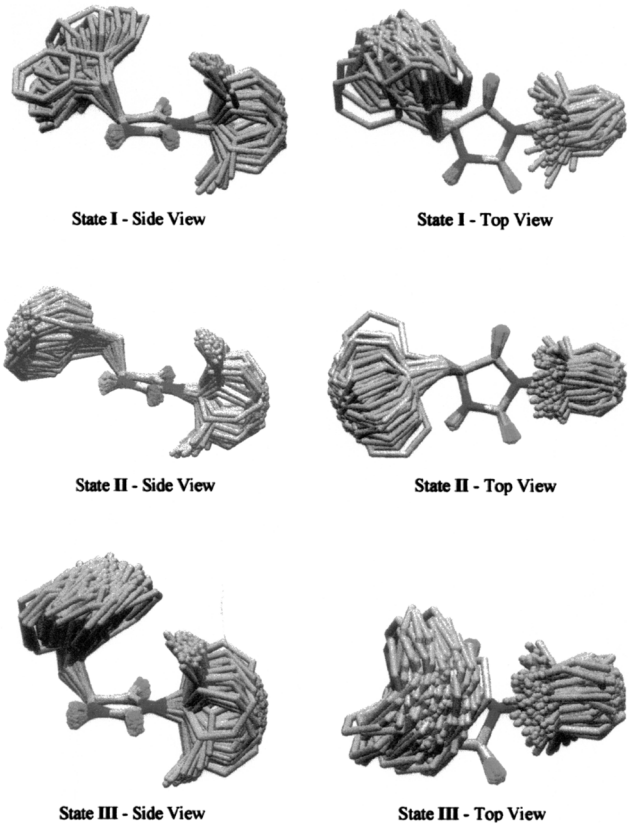


Fig. 12. Supereimposed conformers of state I, II, and III of **1** from the MC/SD Simulation.

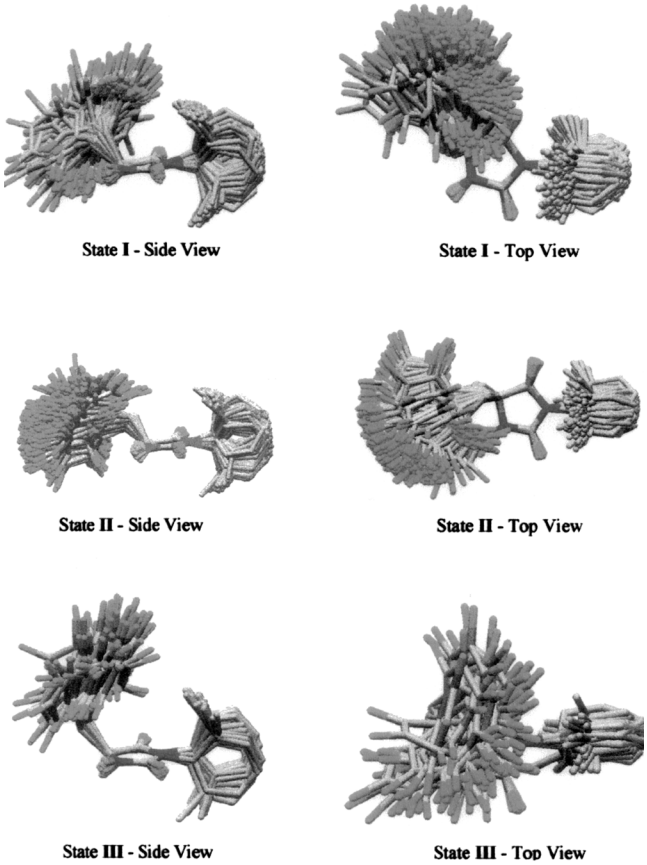


Fig. 13. Supereimposed conformers of state I, II, and III of **2** from the MC/SD Simulation.

rac) or a 300 MHz Varian Unity spectrometer equipped with a 5-mm four-nuclei (^{1}H, ^{13}C, ^{19}F, and ^{31}P) probe. Each spectrum was obtained by a Sun "Spark" Data Station using the in-house MNMR software. Each sample was dissolved in either $CDCl_3$ or DMSO-d_6.

Temperature Study. ^{1}H NMR spectra at various temperatures were recorded by a 300 MHz Varian Unity spectrometer equipped with a 5-mm two-nuclei (^{1}H and ^{19}F) probe. The calibration of the probe temperature was made immediately after the sample was analyzed at the temperature using an ethylene glycol calibration sample. Each sample was dissolved in DMSO-d_6.

Molecular Modeling Studies on 3-*o*-Tolylhydantoins. MC–SD simulations were applied to 3-*o*-tolylhydantoins to determine their conformational populations. A hybrid Monte Carlo (MC)–stochastic dynamics (SD) calculation was performed using a continuum water solvent mode[16] implemented in Macro Model[17] version 5.0. The simulation was conducted in water (dielectric constant = 78.7), as being the most representative dielectric available which is similar to DMSO (dielectric constant = 45). After a period of 100 ps of stochastic dynamics equilibrium, long simulations (5000 ps) were carried out at 300 K. The simulations were to be converged by the obtained constant temperature and average enthalpies. All three rotatable bonds were permitted to vary during the simulations. During the simulations, 1000 snapshots were taken at equal intervals. From this sampling, the populations of the folded versus extended forms were obtained, as well as the populations of the *o*-tolyl group being up, versus down. The torsions were defined by H23–C1–C16–H32 and H6–C1–C16–H31 to quantitate to the groups previously defined as conformer I, II, and III described earlier (Figs. 9 and 11). The torsions were defined by C7–C4–C9–C14 to determine the rotamer states of the *o*-tolyl groups.

General Procedure for the Synthesis of 3-*o*-Tolylhydantoins (1–4). The melting points were determined on a Mel-Temp (50/60 cycles, 110–120 volts, 250 watts) apparatus and are uncorrected. Infrared spectra were recorded on either a Perkin–Elmer 1310 or a Perkin-Elmer 1760 FTIR spectrophotometer. NMR spectra were recorded on a Bruker 200-MHz FTNMR spectrometer, a 500 MHz Varian Unity spectrometer equipped with a 3-mm triple-resonance gradiant probe (Nalorac) or a 300 MHz Varian Unity spectrometer equipped with a 5-mm four-nuclei (^{1}H, ^{13}C, ^{19}F, and ^{31}P) probe. ^{1}H NMR and ^{13}C NMR chemical shifts are reported in δ value vs TMS and $CHCl_3$, respectively. Mass spectra were obtained on a Scientific Research Instruments Biospect mass spectrometer. Flash column chromatography was performed on silicagel 60 (230–400 mesh, Merck). All chromatographic separations were monitored by TLC analyses, performed using glass plates precoated with 0.25-mm 230–400-mesh silica gel impregnated with a fluorescent indicator (254 nm).

Preparation of 5-Isobutyl-3-*o*-tolylhydantoin (3). DL-Leucine (1.00 g, 7.6 mmol) was dissolved in 15 mL of water containing 0.51 g (9.1 mmol) of potassium hydroxide at 0–5 °C. *o*-Tolyl isocyanate (1.21 g, 9.1 mmol) was added dropwise into the amino acid solution over a period of 5 min. The reaction mixture was warmed to 60–70 °C and kept at that temperature for 1 h. The mixture was filtered and the filtrate was acidified with conc. hydrochloric acid in order to precipitate the hydantoic acid, which was then separated by filtration. A solution of 4 mL of conc. hy-

drochloric acid and 4 mL of water was added to the hydantoic acid derivative and refluxed until the precipitate of the hydantoic acid dissolved in the acidic solution (1–2 h), effecting cyclization to the hydantoin. After cooling the solution, the hydantoin precipitated out and was filtered. Recrystallization from absolute ethanol gave 0.40 g (23% yield) of the title compound as a white solid: mp 123–125 °C (Ref. 1, 182–183 °C); IR ($CHCl_3$) 1710 cm^{-1} (–NHCO–); CIMS (CH_4, 160 °C) *m/z* 233 $[M+H]^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 0.90–0.95 (m, 6H, 2 CH_3), 1.53–1.61 (m, 1H, CH), 1.76–1.83 (m, 2 H, CH_2), 2.13 and 2.16 (2s, total 3H, *o*-Me), 4.12–4.18 (m, 1H, C5 H), 6.44 (s, 1H, NH), 7.05 and 7.10 (2d, J = 7.5 Hz, total 1H, *o*-H), 7.10–7.29 (m, 3H, Ar); ^{13}C NMR (200 MHz, $CDCl_3$) δ 17.6 17.8, 21.7, 21.8, 23.0, 23.1, 25.0, 25.2, 25.3, 41.1, 41.7, 56.1, 56.2, 126.8, 126.9, 128.4, 128.6, 129.4, 130.4, 130.5, 131.1, 131.2, 136.2, 136.4, 156.7, 173.3.

Preparation of *o*-Tolylhydantoic Acid. *o*-Tolylhydantoic acid was prepared by a modification of the method used by Finkbeiner.[19] Glycine (1.5 g, 20 mmol) was dissolved in 8 mL of potassium hydroxide solution (68 g of potassium hydroxide in 400 mL of water). The solution was stirred on a magnetic stirrer while *o*-tolyl isocyanate (2.9 mL, 22 mmol) was added dropwise over a period of 20 min (during the addition of the *o*-tolyl isocyanate, the mixture first became cloudy and then a solid precipitate of 1,3-di-*o*-tolylurea separated). The reaction mixture was kept at room temperature overnight. The solid urea was removed by centrifuging (5000 rpm, 15 min) and a cloudy supernatant layer was filtered. The precipitate was washed three times with a small amount of the potassium hydroxide stock solution. The combined filtrate and washes were acidified with conc. hydrochloric acid when a white precipitate was formed. After cooling in an ice bath for 1–2 h to complete precipitation, the precipitated *o*-tolylhydantoic acid was filtered and dried. The product was purified by recrystallization from hot methanol to give 3.00 g (73% yield) of the title compound as a white solid: mp 200–201 °C; IR ($CHCl_3$) 1710 cm^{-1} (–NHCO–); CIMS (CH_4, 160 °C) *m/z* 209 $[M+H]^+$.

Preparation of 3-*o*-Tolylhydantoin (4). 3-*o*-Tolylhydantoin was prepared by a modification of the method used by Finkbeiner.[19] The *o*-tolylhydantoic acid (3.00 g, 14.4 mmol) obtained previously was added to a solution of 6 mL of conc. hydrochloric acid and 1.5 mL of water. Upon heating to reflux, the mixture became a clear solution. After refluxing for 2 h, the reaction mixture was allowed to cool to room temperature and stand overnight to complete precipitation. The precipitated 3-*o*-tolylhydantoin was filtered and dried. The product was purified by recrystallization from absolute methanol to give 2.53 g (92% yield) of the title compound as a white solid: mp 152–153 °C (Ref. 10, 153 °C); IR ($CHCl_3$) 1710 cm^{-1} (–NHCO–); CIMS (CH_4, 160 °C) *m/z* 191 $[M+H]^+$; ^1H NMR (200 MHz, $CDCl_3$) δ 2.23 (s, 3H, *o*-Me), 4.07 (s, 2H, CH_2), 7.20 (s, 1H, NH), 7.05–7.40 (m, 4H, Arm).

Preparation of Magnesium Methyl Carbonate (MMC). MMC in DMF–A 2 M solution of MMC in *N*,*N*-dimethylformamide (DMF) was prepared as described by Finkbeiner and Stiles.[21] A stock solution of magnesium methyl carbonate in *N*,*N*-dimethylformamide was prepared as follows and used within one month. Over a period of 3 h, magnesium turnings (6 g) were added to dry methanol (100 mL) in a 250-mL round-bottom flask equipped with a condenser, a stirrer and a gas inlet tube. After the magnesium had reacted completely, methanol was removed under reduced pressure at a bath temperature of 50 °C. *N*,*N*-Dimethylformamide (commercial untreated) was added to give a total volume of 125 mL and carbon dioxide was passed to the stirred solution as rapidly as it was absorbed. After the solid had dissolved, a claisen head was attached to the flask and residual methanol was distilled under a slow stream of carbon dioxide until the temperature of the head reached 110 °C. Then, a 5-plate bubble-cap column was attached and distillation continued until the head temperature reached 152 °C. The solution was then cooled to room temperature and stirred under carbon dioxide for 1–3 h.

Preparation of 5-Benzyl-3-*o*-tolylhydantoin (1) (adapted from that of Finkbeiner[19]). 2 M MMC in DMF (2 mL) was placed into a 50-mL round-bottom flask equipped to maintain a nitrogen atmosphere. The solution was stirred with a magnetic stirring bar for 0.5 h under carbon dioxide and then heated to 80 °C. The carbon dioxide was replaced with nitrogen and the nitrogen atmosphere was maintained throughout the reaction. To the reaction mixture was added **4** (380 mg, 2 mmol), and the solution was heated at 80 °C and kept at that temperature for 1 to 1.5 h. Then, benzyl bromide (376 mg, 2.2 mmol) was added and the reaction mixture was allowed to be stirred for 5 h at 110 °C. It was then cooled and poured onto a mixture of 2 g of ice and 0.5 mL of conc. hydrochloric acid. A gummy solid precipitated, which became hard upon standing. The mixture was allowed to stand overnight to complete precipitation. Once precipitation was complete, the solid was filtered. The crude product was then dried. Column chromatography on silica gel (hexane/ethyl acetate: 7/3) gave 3.60 g (64% yield) of the title compound as a white solid: mp 134–135 °C (Ref. 20, 134–135 °C); IR ($CHCl_3$) 1700 cm^{-1} (–NHCO–); ^1H NMR (300 MHz, $CDCl_3$) δ 1.76 and 2.20 (2s, total 3H, *o*-Me), 3.04–3.30 (m, 2H, benzylic), 4.44–4.48 (m, 1H, CH), 6.18 (s, 1H, NH), 6.63 and 7.12 (2 dd, J = 7.5 Hz, total 1H, *o*-H), 7.23–7.37 (m, 8H, Arm); ^{13}C NMR (300 MHz, $CDCl_3$) δ 17.2 and 17.9 (CH_3), 37.8 and 38.0 ($PhCH_2$), 58.5 and 58.7 (C5), 127.0, 127.7, 127.8, 128.3, 128.5, 129.0, 129.7, 129.8, 130.0, 130.1, 131.2, 131.3, 134.7, 136.4, 156.6, 172.0, 172.1; CIMS (CH_4, 160 °C) *m/z* 281 $[M+H]^+$; Anal. Calcd for $C_{17}H_{16}N_2O_2$: C, 72.89; H, 5.62; N, 10.05%. Found: C, 72.92; H, 5.62; N, 10.09%.

Preparation of 5-Pentafluorobenzyl-3-*o*-tolylhydantoin (2) (adapted from that of Finkbeiner[19]). Following the procedure described above for the preparation of **1** using benzyl bromide, α-bromo-2,3,4,5,6-pentafluorotoluene (1.73 g, 6.6 mmol) was used. Column chromatography on silica gel (hexane/ethyl acetate: 7/3) gave 1.10 g (50% yield) of the title compound as a white solid: mp 189–191 °C (EtOAc); IR ($CHCl_3$) 1685 cm^{-1} (–NHCO–); ^1H NMR (500 MHz, DMSO-d_6) δ 1.93 and 2.10 (2s, total 3H, *o*-Me), 3.13 and 3.16, 3.20 and 3.23 (2 dd, J = 7.0 Hz, total 1H, benzylic), 4.52 and 4.53, 4.58 and 4.59 (2 dd, J = 7.0 Hz, total 1H, CH), 7.10 and 7.16 (2d, J = 7.5 Hz, total 1H, *o*-H), 7.25–7.34 (m, 3 H, Arm), 8.53 (s, a H, NH); ^{13}C NMR (300 MHz, DMSO-d_6) δ 17.1 and 17.9 (*o*-CH_3), 25.5, 45.2, 56.0 and 56.2 (C5), 127.2, 129.3, 129.6, 131.1, 156.0, 172.2; CIMS (CH_4, 170 °C) *m/z* 372 $[M+2]^+$, 371 $[M+H]^+$, 189 $[M-181]^+$; Anal. Calcd for $C_{17}H_{16}N_2O_2$: C, 55.14; H, 2.99; N, 7.56%. Found: C, 55.06; H, 2.82; N, 7.60%.

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