VOLUME 115, NUMBER 9
MAY 5, 1993

JOURNAL
OF THE
AMERICAN
CHEMICAL
SOCIETY

Conformations of the Dermenkephalin Backbone in DMSO Solution by a New Approach to the Solution Conformations of Flexible Peptides

G. V. Nikiforovich,† O. Prakash, C. A. Gehrig, and Victor J. Hruby*

Contribution from the Department of Chemistry, University of Arizona, Tucson, Arizona 85721

Received May 18, 1992

Abstract: Dermenkephalin (DRE, H-Tyr-D-Met-Phe-His-Leu-Met-Asp-NH_2), a natural peptide found in frog skin, has high potency and receptor selectivity for δ opioid receptors and has potent in vivo analgesic activity. Structurally the compound is related to both the μ opioid receptor selective dermorphin and the δ opioid receptor selective deltorphins. Interestingly, the N-terminal tetrapeptide of DRE is potent and selective for the μ opioid receptor. Efforts to understand the conformational properties of DRE and their relationships to biological activity are of great importance. We report here a novel approach to analyze conformations of short linear peptides in solution to determine the possible solution conformations of DRE. We have combined extensive NMR studies with comprehensive conformational energy calculations, including extensive Monte Carlo sampling and statistical evaluations of the results, to obtain the statistical weight estimations for DRE low-energy backbone conformations that are consistent with all of the NMR data. A random search of conformer statistical weights was performed to satisfy the condition of statistical indistinguishability between the experimental values and the weighted sum of calculated values for each measured parameter. From these studies, two low-energy conformers were found to be essential for matching the energy calculation results with the NMR data. At least one of them should be present among the DRE solution conformers with a significant statistical weight. Except for the rotamers of the side chain groups of the Tyr^1 and Phe^3 residues, the conformations of the N-terminal tripeptide fragment match in detail a previously suggested topographical model for the conformation responsible for interaction with the δ opioid receptor. This suggests that the δ selectivity of DRE, which is a linear flexible peptide, might be due to pre-existence in solution of a specific conformer for its N-terminal tripeptide. The combined approach employed in this study offers a useful methodology to aid in conformational analysis of linear, conformationally flexible peptides that are active at receptors and other biological important acceptors.

Introduction

Dermenkephalin (DRE) is a natural peptide isolated from frog skin[1,2] that possesses very high selectivity and potency at the δ-selective opioid receptor. The DRE sequence is as follows: Tyr^2-D-Met^2-Phe^3-His^4-Leu^5-Met^6-Asp^7-NH_2. DRE belongs to a family of opioid peptides from frog skin that includes the μ-selective dermorphin and δ-selective deltorphins I and II;[3,4] some authors also refer to dermenkephalin as a deltorphin (see ref 5).

* Author to whom requests for reprints should be sent.
† Present address: Center for Molecular Design, Washington University, St. Louis, MO 63130.

(1) Amiche, M.; Sagan, S.; Mor, A.; Delfour, A.; Nicolas, P. *Molecular Pharmacology* **1989**, *35*, 774–779.
(2) Sagan, S.; Amiche, M.; Delfour, A.; Mor, A.; Camus, A.; Nicolas, P. *J. Biol. Chem.* **1989**, *29*, 17100–17106.
(3) Sagan, S.; Amiche, M.; Delfour, A.; Camus, A.; Mor, A.; Nicolas, P. *Biochem. Biophys.Res. Commun.* **1989**, *163*, 726–732.
(4) Lazarus, L. H.; Salvadori, S.; Santagada, V.; Tomatis, R.; Wilson, W. E. *J. Med. Chem.* **1991**, *34*, 1350–1355.
(5) Kreil, G.; Barra, D.; Simmaco, M.; Erspamer, V.; Erspamer, G. F.; Negri, L.; Severini, C.; Corsi, R.; Melchiorri, P. *Eur. J. Pharmacol.* **1989**, *162*, 123–128.

0002-7863/93/1515-3399$04.00/0

One aspect of understanding conformation–activity relationships for dermenkephalin includes knowledge of its possible solution conformations. Unfortunately, being a short linear peptide, dermenkephalin most likely exists in solution as a mixture of different interconverting conformers. This view is strongly supported by several papers on the NMR spectra of dermankephalin and the deltorphins in DMSO and in cryoprotective solution.[6-9] Nevertheless, other authors have derived a single dermenkephalin conformation which is presumably in good agreement with the NMR data, in particular with the NOE measurements.[9] On the other hand, we earlier proposed a model for the δ-receptor-bound conformation of DRE based on energy calculations.[10,11] The model was supported by the synthesis and biological testing of an active cyclic analog of deltorphin I.[12] This model has little resemblance to the DRE solution structure proposed earlier.[9]

The main objective of this paper is to examine possible DRE solution conformation(s) employing an approach elaborated earlier,[13] which combines NMR measurements and energy calculations. This approach does not demand that just one single conformer meet all experimental requirements (which was the basic assumption in ref 9) but rather it allows for the possible existence of several conformers in solution from the very beginning. All low-energy conformers for the peptide backbone of a particular molecule are regarded as possible solution conformations. Knowledge of these possible low-energy conformers is obtained by energy calculations, such as those performed for DRE in ref 11. This is followed by Monte Carlo sampling (or other kinds of sampling) in the vicinity of the low-energy conformers obtained to explore the shape of the potential energy surface near the various corresponding local minima. This allows us to calculate mean values, $\langle A^{calc} \rangle$, and their standard deviations, D^{calc}, for each low-energy backbone conformer and for any structure-related parameter A which can be measured by experimental means. Examples of parameters A include vicinal coupling constants $J(NHC^{\alpha}H)$, NOEs, the efficiencies of singlet–singlet energy transfer between aromatic moieties, etc. On the other hand, the experimental data can provide us with the mean experimental values $\langle A^{exp} \rangle$ and their standard deviations D^{exp} for every measured structural parameter. Finally, we can require that the $\langle A^{exp} \rangle$ values and the weighted sum of $\langle A^{calc} \rangle$ values averaged over all conformers be statistically indistinguishable (or, in other words, "in good agreement") at the chosen confidence level with the Student's coefficient t. In other words, the following conditions should be satisfied:

$$\frac{|\sum_{i=1}^{N} w_i \langle A^{calc} \rangle ik - \langle A^{exp} \rangle k|}{(\sum_{i=1}^{N} (w_i D^{calc}{}_{ik})^2 + (D^{exp}{}_k)^2)^{1/2}} < t_k \qquad (1)$$

where i and k are indexes related to the number of conformers and to the number of measured parameters, respectively. Thus, if we have N conformers and M measured parameters, the last step would consist of selecting all sets $\{w_i\}$ of N statistical weight values, each set $\{w_i\}$ satisfying to M inequalities of type (1), being at the same time limited by the obvious condition of $w_i > 0$ and $\sum_{i=1}^{N} w_i = 1$.

The advantages of the above approach are that one always considers several different conformers to fit the experimental data and in addition one must simultaneously meet all inequalities of type (1) for each parameter. The use of the Student's criterion for comparison of the calculated and experimental mean values facilitates the quantitative estimations of the closeness between these two, rather than qualitative statements such as "to be in good agreement." Also, the choice of conformers considered is limited to backbone conformations with low energy only, and in addition it takes into account their local fluctuations by the use of Monte Carlo sampling. On the other hand, the simultaneous fulfillment of all inequalities (1), together with the requirements for non-negativity and normalized statistical weight values w_i, leads to a non-unique solution of the problem, since many of sets $\{w_i\}$ for statistical weight values can satisfy these conditions. This means that in this approach, we will not obtain the "true" constant values for the statistical weights of conformers in solution, but rather their distributions, each with its mean value $\langle w_i \rangle$ and its upper, $w^{up}{}_i$, and lower, $w^{low}{}_i$, levels. So, instead of "the true" w_i values, we obtain "the most probable" w_i values depending on the accuracy of our calculations and measurements, both of which inevitably have some inaccuracy.

This approach has been successively applied to spin-labeled angiotension[13,14] and Leu-enkephalin[15] molecules. Only five low-energy conformers were found to be "indispensable" for the angiotensin backbone in aqueous solution, their lower $w^{low}{}_i$ levels being non-equal to zero. This means that none of them can be ignored when deriving possible solution structures from the available experimental data.[13] All possible angiotensin conformers in aqueous solution were shown to fall into two groups differing in the backbone conformer of the central tetrapeptide with the mean statistical weight values of 0.78 and 0.22, respectively.[14] In the case of enkephalin in DMSO solution, the approach revealed the possible existence of at least two folded conformers of the peptide backbone with mean statistical weight values of ca. 0.70 and 0.30 and with significant local conformational fluctuations.[15] The conclusions on the possible solution conformations of both peptides were confirmed also by comparison with the independent experimental measurements.[14,15]

Methods

Energy Calculations. All energy calculations for DRE were performed using the ECEPP potential field[16,17] assuming rigid valence geometry with planar *trans* peptide bonds. (Other details of the valence geometry of DRE are described in ref 11.) Electrostatic interactions were taken into account with the value of dielectric constant $\epsilon = 45$, which corresponds to the macroscopic ϵ value for DMSO. The backbone structures considered for energy calculations were those obtained by the buildup procedures with the successive "growth" of the DRE peptide chain from either the N- or C-terminus in ref 11. A total of 116 conformers were considered at the level of the entire molecule.

Monte Carlo Sampling. Monte Carlo sampling was performed in the 31-dimensional space of DRE dihedral angles in the vicinity of each of the 14 local potential energy minima found in the energy calculations. The Markov chain always was started in one of the minima. First, the "old" energy value E^{old} was calculated for the starting conformations.

(6) Temussi, P. A.; Picone, D.; Tancredi, T.; Tomatis, R.; Salvadori, S.; Marastoni, M.; Balboni, G. *FEBS Lett.* **1989**, *247*, 283–288.
(7) Balboni, G.; Marastoni, M.; Picone, D.; Salvadori, S.; Tancredi, T.; Temussi, P. A.; Tomatis, R. *Biochem. Biophys. Res. Commun.* **1990**, *169*, 617–622.
(8) Amodeo, P.; Motta, A.; Tancredi, T.; Picone, D.; Saviano, G.; Temussi, P. A.; Salvadori, S.; Tomatis, R. In *Peptides: Structure, Chemistry, Biology. Proceedings of the Twelfth American Peptide Symposium*; Smith, J. A., Rivier, J. E. Eds.; ESCOM: Leiden, 1992; pp 115–116.
(9) Tancredi, T.; Temussi, P. A.; Picone, D.; Amodeo, P.; Tomatis, R.; Salvadori, S.; Marastoni, M.; Santagada, V.; Balboni, G. *Biopolymers* **1991**, *31*, 751–760.
(10) Nikiforovich, G. V.; Hruby, V. J. *Biochim. Biophys. Res. Commun.* **1990**, *173*, 521–527.
(11) Nikiforovich, G. V.; Hruby, V. J.; Prakash, O.; Gehrig, C. A. *Biopolymers* **1991**, *31*, 941–955.
(12) Misicka, A.; Nikiforovich, G. V.; Lipkowski, A. W.; Horvath, R.; Davis, P.; Kramer, T. H.; Yamamura, H. I.; Hruby, V. J. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 547–552.
(13) Nikiforovich, G. V.; Vesterman, B.; Betins, J.; Podins, L. *J. Biomol. Struct. Dyn.* **1987**, *4*, 1119–1135.
(14) Nikiforovich, G. V.; Vesterman, B. G.; Betins, J. *Biophys. Chem.* **1988**, *31*, 101–106.
(15) Vesterman, B.; Saulitis, J.; Betins, J.; Liepins, E.; Nikiforovich, G. V. *Biochim. Biophys. Acta* **1989**, *998*, 204–209.
(16) Dunfield, L. G.; Burgess, A. W.; Scheraga, H. A. *J. Phys. Chem.* **1978**, *82*, 2609–2616.
(17) Nemethy, G.; Pottle, M. S.; Scheraga, H. A. *J. Phys. Chem.* **1983**, *87*, 1883–1887.

Table I. NMR Data of DRE in DMSO Solution

residue	J(HNC$^{\alpha}$H), Hz	NOE type	NOEs: interproton dist, Å	NOEs: this study[a]	NOEs: interproton dist limits, Å	NOEs: ref 9
D-Met[2]	8.47	αH_1–NH_2	3.01	strong/medium	2.0–3.6	+
Phe[3]	8.43	αH_2–NH_3	2.89	strong/medium	2.0–3.6	+
His[4]	8.46	αH_3–NH_4	2.68	strong/medium	2.0–3.6	+
Leu[5]	8.10	αH_4–NH_5	2.97	strong/medium	2.0–3.6	+
Met[6]	8.31	αH_5–NH_6	2.98	strong/medium	2.0–3.6	+
Asp[7]	8.00	αH_6–NH_7	3.21	medium	2.9–3.6	+
		NH_4–NH_5	4.48	medium/weak	3.5–4.8	
		NH_5–NH_6	4.52	medium/weak	3.5–4.8	+
		NH_6–NH_7	4.18	medium/weak	3.5–4.8	+
		$H^{ar}1$–$H^{ar}1$ (reference)	2.54			

[a] Cross-peak classification according to ref 26.

Then, one of the dihedral angles was randomly chosen among the dihedral angles of the molecule. If this angle Θ was a backbone angle, then it acquired the value $\Theta + \Delta\Theta$, where $\Delta\Theta$ was chosen randomly within the limits of 0 to 25°. If the angle Θ was a side chain angle, first the rotamer type for this angle was chosen randomly (i.e. g^+, t, or g^- for all χ_1's and χ_2's and χ_3's in Met residues; ±90° for χ_2's in Tyr, Phe, His, and Asp residues, and 120° for a Leu residue) and then $\Delta\Theta$ was chosen randomly within the limits of –60 to 60°. The "new" energy was calculated for the same dihedral angle set except $\Theta^{new} = \Theta + \Delta\Theta$. If the "new" energy value E^{new} was lower than E^{old}, the new conformation (the new point) was always accepted and added to the Markov chain, and E^{new} became E^{old}. If not, the new point could also be added, but the probability of acceptance in this case was proportional to $\exp((E^{new} - E^{old})/RT)$, where T is a temperature in degrees Kelvin (T = 300 K was used) and R is the universal gas constant. (In other words, we used the well-known Metropolis statistics[18] for our Monte Carlo sampling.) If the new conformation with E^{new} was not accepted, the old conformation with E^{old} was added to the Markov chain once more. In any case, after adding a point to the sample, the procedure was repeated starting from the new choice of dihedral angle to change until the given length of the Markov chain was achieved. We generated Markov chains of 200 000 steps for each starting structure, adding to the statistical samples the results of just the last one out of five subsequent steps to avoid the intrinsic correlations among conformations involved in the statistical samples. Thus, each of the statistical samples contains 40 000 conformations.

Calculation of Mean Values for J(NHC$_{\alpha}$H)s and Interproton Distances. The mean energy value $\langle E \rangle$ was calculated cumulatively along with the lengthening of the Markov chain for every hundred points in each statistical sample. Typically, during the first 100 groups of 100 points, the $\langle E \rangle$ value was equilibrated and then began to oscillate around some stable value. The conformations associated with the $\langle E \rangle$ equilibration process were removed from each statistical sample. Then the vicinal coupling constants of J(NHC$_{\alpha}$H) type and the distances between all protons in the peptide backbone (except protons in the N-terminal α-amino group and C-terminal carboxamide) were calculated for every conformation of the remaining parts of each statistical sample. Several relationships of J(NHC$_A$H) vs ϕ angles have been proposed.[19-21] All of them are essentially equally valid based on experimental data. (Interestingly, the most recent dependence of this kind[22] appeared to be very close to one proposed previously.[20]) Therefore, we have considered all dependencies[19-21] as possibilities for J(NHC$_{\alpha}$H) calculations. The statistical samples were divided into 25 subgroups, each containing 1000 conformations, and all values of J(NHC$_{\alpha}$H)s and interproton distances were averaged over each subgroup. Then these averaged values were used to calculate the final mean values and standard deviations of J(NHC$_{\alpha}$H)s and the interproton distances. The goal of these procedures was to ensure a Gaussian shape for the distribution of averaged values of J(NHC$_{\alpha}$H)s and interproton distances.

Selection of Statistical Weights. The sets of conformer statistical weights $\{w_i\}$ were selected as follows. $N - 1$ random numbers were generated, each being between 0 and 1. Then they were arranged in the order of their values, and each value was considered as a point of division for an interval from 0 to 1. In this way, the interval from 0 to 1 became divided into N pieces, the length of every piece then being used as w_i values. It is obvious that the w_i values obtained in this manner will satisfy the conditions of $\sum_{i=1}^{N} w_i = 1$ and $w_i > 0$. Each w_i value was assigned to one of the N conformers, creating an initial $\{w_i\}$ set. To ensure simulations of a more uniform distribution for each w_i, the $\{w_i\}$ sets were generated also by exchanging w_i values from the initial $\{w_i\}$ sets for several pairs of conformers. Each generated $\{w_i\}$ set was then checked as to the fulfillment of all conditions (1). The procedure was continued until the number of selected $\{w_i\}$ sets was equal to the value chosen in advance (typically from 100 000 to 500 000).

NMR Measurements. The high-resolution 1D and 2D ^{1}H NMR measurements was carried out at 500.13 MHz with a Brucker AM 500 FT NMR spectrometer equipped with an ASPECT 3000 computer using a specific 5-mm ^{1}H probe head for a ca. 4 mM solution in DMSO-d_6. The techniques used in the present studies for the assignment of all of the proton assignments included two-dimensional double-quantum filtered proton correlated spectroscopy (DQCOSY),[23] relayed COSY,[24] and nuclear Overhauser enhancement spectroscopy (NOESY).[25] The pulse sequence used in the relayed COSY experiment was D_1–90–t_1–90°–D_2–180°–D_2–90°–t_2 with a D_2 value of 30 ms. The pulse sequence used in the NOESY experiments was the conventional D_1–90°–t_1–90°–t_m–90°–t_2, where t_m is the mixing time (values used were 200, 300, 400, and 500 ms). The relaxation delay (D_1) in all the experiments was fixed as 2 s. The two-dimensional spectra were obtained from 512 measurement in DMSO-d_6 solution along the t_1 axis. For each value of t_1, 1024 data points were collected along the t_2 axis. To enhance the digital resolution, the time domain data matrix was expanded by zero filling to 1024 data points along the t_1 axis and data were multiplied by sine square bell and sine bell window functions along the t_2 and t_1 directions prior to their respective Fourier transformations. All of the chemical shifts are expressed as δ (ppm) downfield from external reference tetramethylsilane (TMS).

Results

The NMR NOE data obtained for dermenkephalin are listed in Table I together with the experimental data of other authors (see Table III in ref 9). Table I includes six values of the J(HNC$^{\alpha}$H) coupling constants and nine intrabackbone NOE's observed for the DRE backbone in DMSO solution (intraresidue NOE's are omitted). The interproton distances were calculated as $r_{ij} = ((\sigma_{ref}/\sigma_{ij})r_{ref}^6)^{1/6}$, where σ_{ij} are the volumes of the corresponding cross-peaks, and the reference values of σ_{ref} (assumed as 100%) and r_{ref} = 2.54 Å are related as the distance between two aromatic protons in the Tyr[1] residue (see the last line of Table I). To estimate the NOEs (or interproton distances related to them) more quantitatively, we have used histograms of the distributions for near-neighboring interresidue αH_i–NH_{i+1} and NH_i–NH_{i+1} distances calculated[26] using the X-ray data on

(18) Metropolis, N.; Rosenbluth, A. W.; Rosenbluth, M. N.; Teller, A. H.; Teller, E. *J. Chem. Phys.* **1953**, *21*, 1087–1091.
(19) Bystrov, V. F.; Ivanov, V. T.; Portnova, S. L.; Balashova, T. A.; Ovchinnikov, Y. F. *Tetrahedron* **1973**, *29*, 873–877.
(20) Pardi, A.; Billeter, M.; Wüthrich, K. *J. Mol. Biol.* **1984**, *180*, 741–751.
(21) Demarco, A.; Llinas, M.; Wüthrich, K. *Biopolymers* **1978**, *17*, 637–650.
(22) Ludvigsen, S.; Andersen, K. V.; Poulsen, F. M. *J. Mol. Biol.* **1991**, *217*, 731–736.
(23) Piantini, U.; Sorensen, O. W.; Ernst, R. R. *J. Am. Chem. Soc.* **1982**, *104*, 6800–6801.
(24) Bolton, P. H.; Bodenhausen, G. *Chem. Phys. Lett.* **1982**, *89*, 139–144.
(25) (a) *SYBYL 5.4 Theory Manual*; Tripos Associates Inc.: St. Louis, 1991; pp 2445–2446. (b) Jeener, J.; Meier, B. H.; Bachmann, P.; Ernst, R. R. *J. Chem. Phys.* **1979**, *71*, 4546–4553.
(26) Shenderovich, M. D.; Nikiforovich, G. V.; Saulitis, J. B.; Chipens, G. I. *Biophys. Chem.* **1988**, *31*, 163–173.

Table II. Low-Energy Backbone Conformers of the DRE Molecule

	Tyr 1	D-Met[2]		Phe[3]		His[4]		Leu[5]		Met[6]		Asp[7]		
conformer	ψ^c	ϕ^c	ψ^c	ϕ^c	ψ^c	ϕ^c	ψ^c	ϕ^c	ψ^c	ϕ^c	ψ^c	ϕ^c	ψ^c	energy, kcal/mol
1[a]	157	92	41	−90	−8	62	38	−127	51	56	27	−89	−29	−41.3
	165	86	38	−79	−13	68	29	−116	57	55	27	−94	−34	−42.4
	158	90	45	−89	−6	63	38	−124	51	55	29	−93	−26	−34.6[b]
	7	9	7	7	4	4	7	8	8	5	7	9	11	
2	158	91	36	−88	−16	58	50	−137	−60	−129	20	−80	−33	−42.3
	149	99	24	−94	−14	62	34	−159	−61	−111	48	−71	−41	−39.4
	154	93	24	−95	−15	58	41	−151	−58	−102	21	−75	−37	−32.5
	8	10	15	11	9	5	9	12	5	13	17	11	12	
3	136	96	29	−95	−36	−94	−7	66	21	−162	33	−88	148	−42.7
	150	85	38	−83	−45	−95	−1	67	19	−162	30	−76	141	−42.3
	146	91	43	−86	−48	−91	−5	66	21	−160	33	−83	125	−34.3
	9	10	10	10	5	7	3	5	6	6	6	11	24	
4	143	86	39	−96	−29	−143	−65	−161	97	47	58	−78	87	−41.8
	153	85	44	−80	−23	−145	−73	−164	114	47	59	−155	150	−43.6
	154	83	41	−81	−36	−140	−71	−159	110	47	59	−159	152	−37.4
	9	9	7	9	9	11	5	6	9	6	8	8	10	
5	162	115	−164	−95	−20	−142	−63	−164	92	48	59	−73	−33	−41.9
	155	115	−164	−70	−26	−141	−70	−163	112	46	59	−88	−32	−37.1
	145	115	−164	−84	−28	−137	−65	−163	97	48	58	−82	−34	−30.6
	22	1	0	9	9	9	5	8	15	6	13	11	13	
6	−46	104	−149	−101	−25	−140	−60	−168	95	45	58	−70	150	−45.0
	−33	104	−149	−100	−34	−137	−54	−163	77	45	48	−102	106	−40.6
	−42	104	−149	−100	−27	−144	−62	−156	83	49	57	−128	137	−32.7
	12	1	2	7	14	14	5	7	11	5	13	32	29	
7	156	97	33	−93	−16	−140	−151	−50	−45	−112	67	−89	150	−40.5
	156	96	41	−95	−45	−93	165	−61	−33	−136	65	−68	128	−40.8
	154	95	28	−85	−32	−90	165	−74	−38	−133	84	−110	130	−32.4
	6	10	13	12	13	13	8	10	9	16	32	44	20	
8	155	90	40	−90	−25	−152	171	−63	139	51	61	−81	150	−43.2
	163	84	39	−80	−40	−139	181	−75	142	56	47	−78	140	−45.4
	159	86	39	−83	−37	−142	179	−74	142	57	46	−77	135	−37.6
	9	11	7	7	7	10	10	6	8	8	11	12	15	
9	165	85	−108	−69	−27	−81	−43	−151	116	−73	−33	−72	149	−45.8
	168	85	−108	−53	−30	−78	−55	−151	138	−91	−32	−77	133	−45.4
	153	85	−39	−75	−29	−75	−41	−135	110	−82	−35	−86	133	−32.4
	14	1	74	12	10	9	12	23	16	15	13	30	27	
10	148	63	−119	−96	−10	−75	−46	54	49	−79	−30	−76	−30	−45.4
	162	63	−119	−95	−9	−81	−48	52	47	−77	−29	−87	−28	−41.4
	162	63	−119	−98	−12	−74	−46	55	51	−79	−33	−76	−32	−32.9
	10	0	2	6	9	7	7	5	12	13	13	10	10	
11	155	86	−112	−83	−17	−76	−44	−95	−6	54	51	−73	−36	−44.3
	167	99	−112	−92	−5	−76	−44	−105	4	56	46	−85	−31	−42.1
	153	95	−112	−89	−11	−76	−44	−100	5	49	55	−81	−35	−33.9
	13	12	1	6	5	0	0	6	6	5	11	9	11	
12	152	80	−96	−75	−24	−89	−31	−152	112	−75	143	−72	−30	−43.5
	160	76	−96	−78	−25	−89	−31	−145	104	−75	146	−84	−28	−44.5
	155	89	−96	−74	−26	−89	−31	−144	102	−75	147	−78	−36	−38.1
	6	9	1	5	2	0	0	6	6	1	22	10	11	
13	160	81	−90	−80	−31	−82	−31	−145	127	−78	82	−74	−34	−42.5
	156	90	−90	−75	−36	−82	−18	−147	88	−78	121	−81	−29	−42.9
	152	87	−90	−79	−34	−82	−16	−154	102	−78	121	−76	−36	−35.4
	16	11	0	5	3	0	3	8	9	0	19	11	11	
14	−56	74	−102	−64	−37	−68	−33	−163	131	−76	72	−83	−29	−38.9
	−17	148	−102	−85	−25	−79	−18	−164	123	−109	2	−66	−31	−32.9
	−45	32	−102	−94	−16	−68	−34	−152	119	−88	104	−79	−35	−26.3
	14	101	0	12	10	9	11	12	23	29	42	11	12	
structure I	150	50	−101	−52	−48	−90	108	−64	−77	−47	−45	−41		−34.5
from ref 9	156	173	55	−72	−30	−84	60	−166	−54	−90	−29	−140		−40.0
	154	160	54	−70	−34	−85	64	−153	−58	−88	−32	−115		−31.9
	8	10	5	9	9	8	14	19	7	11	12	26		

[a] The first line corresponds to the starting conformation of a sample, and the second line corresponds to a conformation with the minimal energy in the sample (i.e., in the remaining part of 25 000 conformations). The third and fourth lines represent the mean values of dihedral angles and their standard deviations, respectively. [b] The value averaged over the 25 000 conformations of the sample. [c] Angles (ϕ and ψ) in degrees.

19 proteins, which have been analyzed in ref 27 (see Figure 2 in ref 26). The histograms in question are distinctly 2-fold, with the peak limits corresponding to ca. 2.0–2.8 and 2.8–3.6 Å for the αH_i–NH_{i+1} distance (i.e., to "strong" and "medium" NOEs) and to ca. 2.0–3.5 and 3.5–4.8 Å for the NH_i–NH_{i+1} distance (i.e., to "strong" and "medium/weak" NOEs).[24] These limits can be used as semiquantitative estimations for an assignment of a particular interproton distance to "strong", "medium", or "weak" NOE's. Since the observed values of αH_i–NH_{i+1} NOEs correspond mostly to interproton distances around 3 Å, they can be regarded as belonging to both regions in the histogram, which allows one to classify these NOEs as the "strong/medium" ones. The observed NH_i–NH_{i+1} NOEs can be classified in the same way as the "medium/weak" ones. Accordingly, we can define the upper and lower limits of the experimental interproton distances, which also are listed in Table I. Assuming that NOE^{up}

(27) Billeter, M.; Braun, W.; Wüthrich, K. *J. Mol. Biol.* **1982**, *155*, 312–346.

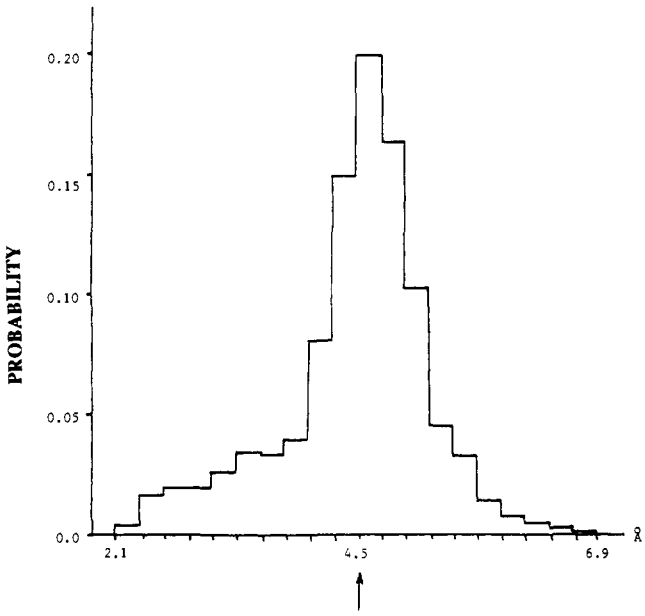

Figure 1. Histogram of the distribution for the intramolecular distance $C^{\alpha}{}_1$–$C^{\alpha}{}_7$ in the statistical sample corresponding to conformer **8** in Table II. An arrow corresponds to the mean distance value.

$= \langle NOE \rangle^{exp} + D^{exp}$ and $NOE^{low} = \langle NOE \rangle^{exp} - D^{exp}$, we can find the values of $\langle NOE \rangle^{exp}$'s and D^{exp}'s for use in inequalities (1). (The interproton limits selected in Table I are also in agreement with a calibration of NOE values vs interproton distances in a recent study.[28])

The temperature coefficients of the chemical shifts for the DRE amide protons were found in our study to be in the range of −5 to −11 ppb/deg K (in the range of −4 to −7 ppb/deg K in ref 6). Generally the values in this range correspond to amide protons more or less exposed to the solvent and do not provide any definite structural information.

Then the statistical samples for 14 low-energy coformers of the DRE peptide backbone (see Table II) were calculated as described in the Methods section. Generally, the quality of statistical samples depends primarily on the numerical values of the Monte Carlo sampling parameters, like the length of the $\Delta\theta$ step or the acceptance rate, etc. Typically, this quality should be checked for at each particular sampling. One of the best means of checking is to calculate the distribution for the end-to-end intramolecular distance and to compare its shape to the Gaussian distribution (see e.g., ref 29). As an example, Figure 1 contains the histogram of the distribution for $C^{\alpha}{}_1$–$C^{\alpha}{}_7$ distance in the statistical sample corresponding to DRE conformer **8** in Table II. The histogram shows that the shape approximates a Gaussian distribution.

Table II also contains the values of backbone dihedral angles for the lowest energy conformation found for each statistical sample (second lines in the entries corresponding to each conformer). One can see that sometimes the Monte Carlo sampling yields conformers with energies that are lower than those for the starting conformers of a sample despite the preliminary minimization of their energies. This discrepancy reflects the inherent inaccuracy of the numerical methods of energy minimization and Monte Carlo sampling used in any study employing molecular mechanics. In our view it is one more reason not to confine ourselves to a single low-energy DRE structure, but to consider simultaneously several conformers possessing relative energies within a certain energy gap. The third and fourth lines in the entries related to each conformer in Table II describe the mean values and standard deviations of the backbone dihedral angles averaged over a corresponding statistical sample, as well as the average energies. The values of standard deviations for dihedral angles represent the levels of internal lability for each DRE conformer; with few exceptions all DRE conformers appear to be similar in this respect. In two cases ($\Delta\psi_2$ for conformer **9** and $\Delta\phi_2$ for conformer **14**) the large values of the standard deviations indicate that these particular statistic samplings covered not one but two connected local energy minima for the D-Met[2] residue.

Comparison of Experimental and Calculated Structural Parameters. In total, we have derived 15 experimental parameters (6 and 9 interproton distances) to match statistical samples for DRE conformers obtained by energy calculations. Then the mean values and standard deviations of the parameters were calculated for each of the statistical samples. In the case of the $J(NHC_{\alpha}H)$s, we actually have calculated several mean values and standard deviations for each statistical sample because we employed various $J(\phi)$ dependencies[19-21] (see Methods). Accordingly, we have used as conditions in eq 1 both the largest ($\langle J^{max} \rangle_{ik}$) and the smallest ($\langle J^{min} \rangle_{ik}$) calculated values. This means that we have reformulated the conditions in (1) into two separate inequalities:

$$\frac{\sum_{i=1}^{N} w_i \langle J^{max} \rangle_{ik} - \langle J^{exp} \rangle_k}{\left(\sum_{i=1}^{N} (w_i D^{max}{}_{ik})^2 + 0.25\right)^{1/2}} < t_k \qquad (1')$$

and

$$\frac{\langle J^{exp} \rangle_k - \sum_{i=1}^{N} w_i \langle J^{min} \rangle_{ik}}{\left(\sum_{i=1}^{N} (w_i D^{min}{}_{ik})^2 + 0.25\right)^{1/2}} < t_k \qquad (1'')$$

The values of the upper (A_{up}) and lower (A_{low}) limits were then calculated as $(A^{calcd}{}_{up})_{ik} = \langle A^{calcd}{}_{max} \rangle_{ik} + (D^{calcd}{}_{max})_{ik}$ and $(A^{calcd}{}_{low})_{ik} = \langle A^{calcd}{}_{min} \rangle_{ik} - (D^{calcd}{}_{min})_{ik}$ for the calculated values of $J(HNC^{\alpha}H)$s and the interproton distances and as $(A^{exp}{}_{up})_k = A^{exp}{}_k + D^{exp}{}_k$ and $(A^{exp}{}_{low})_k = A^{exp}{}_k - D^{exp}{}_k$ for their measured values. These limits for every parameter and for each low-energy DRE conformer are listed in Table III. Then they were used in conditions (1), (1′), and (1″) for selection of statistical weight $\{w_i\}$ sets for the 14 DRE conformers (performed according to the Methods section). The values of t_k here and in all cases mentioned below were equal to 1.96, which corresponds to the 95% level of the confidence interval. The selection procedure was stopped when 500 000 $\{w_i\}$ sets satisfying the conditions (1) were obtained, which allowed us to estimate the distributions of statistical weight values for 14 DRE conformers. It is obvious that only 500 000 $\{w_i\}$ sets (each of them being a point in the 14-dimensional space) are not enough to cover the 14-dimensional volume with the density sufficient to result in realistic statistical weight distributions. Indeed, the only result obtained upon which we can rely was that the lower limits of statistical weights w_i can be close to zero for all DRE conformers. The highest mean values among all 14 conformers were ca. 0.14 and 0.11 for conformers **7** and **8**, respectively. The results of the selection procedure showed also that even for this estimation, the distributions of statistical weights for these two conformers possessed a very tight correlation (the correlation coefficient was −0.91). The upper and lower limits of the calculated structural parameters in question (i.e., $\langle A^{calcd}{}_{up} \rangle_k = \sum_{i=1}^{N} w_i (A^{calcd}{}_{ik} + D^{calcd}{}_{ik})$ and $\langle A^{calcd}{}_{low} \rangle_k = \sum_{i=1}^{N} w_i (A^{calcd}{}_{ik} - D^{calcd}{}_{ik})$), averaged over all 500 000 $\{w_i\}$ sets, are listed in the first column in Table IV.

Because of limited computer resources, it would be helpful to decrease the number of DRE conformers considered. We have used the data of Table III for rough estimates of the relative agreement of calculated and experimental data for each particular

(28) Hyberts, S. G.; Goldberg, M. S.; Havel, T. F.; Wagner, G. *Protein Science* **1992**, *1*, 736–751.
(29) Premilat, S.; Maigret, B. *J. Chem. Phys.* **1977**, *66*, 3418–3425.

Table III. Calculated and Measured Structural Parameters for DRE Low-Energy Structures[a]

	J(HNC$^{\alpha}$H), Hz for residues 2–7						interproton distances, Å								
							αH_i–NH_{i+1}						NH_i–NH_{i+1}		
conformer	2	3	4	5	6	7	12	23	34	45	56	67	45	56	67
1	6.30	6.61	5.56	8.29	5.53	6.85	2.40	3.61	3.38	2.63	2.86	2.95	2.70	2.76	2.98
	8.45	7.85	7.92	9.82	7.87	8.37	2.40	3.61	3.38	2.63	2.86	2.99	2.70	2.76	2.98
2	6.90	6.88	5.55	6.17	7.31	4.87	2.36	3.48	4.65	2.73	2.81	2.18	3.63	2.61	3.03
	8.43	8.76	7.90	8.19	9.43	6.66	2.36	3.50	4.65	2.75	2.83	2.18	3.63	2.71	3.09
3	6.18	5.87	6.54	5.50	5.62	5.49	2.30	3.60	3.62	2.32	3.35	2.56	3.07	2.07	2.93
	8.80	8.19	8.46	7.84	7.15	7.93	2.30	3.60	3.62	2.34	3.35	2.56	3.07	2.07	2.93
4	5.82	5.43	7.19	5.69	5.29	5.77	2.36	3.60	3.58	2.47	3.64	3.80	2.20	3.07	2.61
	7.57	7.51	9.54	7.25	7.60	7.08	2.36	3.60	3.58	2.49	3.64	3.84	2.20	3.09	2.61
5	8.39	5.83	7.57	5.06	5.34	5.45	2.33	2.49	3.54	2.31	3.64	3.49	2.26	3.05	2.61
	9.93	7.84	9.53	6.83	7.58	7.67	2.35	2.49	3.54	2.33	3.64	3.57	2.26	3.09	2.65
6	7.98	7.48	6.29	5.95	5.39	5.94	3.60	2.35	3.51	2.26	3.64	3.28	2.34	3.03	2.62
	9.31	9.16	9.45	7.61	7.66	8.77	3.60	2.35	3.53	2.28	3.64	3.36	2.36	3.05	2.66
7	6.85	5.50	5.73	4.12	7.24	4.46	2.36	3.52	3.55	4.57	2.49	2.45	3.57	3.34	2.41
	8.88	8.46	9.12	7.03	10.12	7.35	2.36	3.52	3.55	4.57	2.51	2.51	3.57	3.78	2.49
8	5.47	5.81	7.02	4.48	5.57	4.35	2.41	3.59	3.58	4.38	2.66	4.62	2.27	2.82	2.76
	8.70	7.53	9.12	6.84	7.93	7.83	2.41	3.59	3.58	4.38	2.66	4.62	2.27	2.84	2.78
9	6.41	4.55	5.04	6.92	5.42	5.03	2.36	2.42	3.54	2.49	3.59	3.99	2.22	2.41	3.56
	7.34	6.85	6.48	8.53	7.62	6.57	2.38	3.28	3.54	2.51	3.59	4.03	2.22	2.43	3.56
10	3.47	7.46	4.83	5.53	5.38	5.32	2.44	2.20	3.43	2.53	3.61	2.84	2.69	2.45	3.55
	4.91	8.83	6.50	7.86	6.87	6.44	2.44	2.20	3.43	2.55	3.61	2.86	2.71	2.47	3.55
11	6.83	6.62	5.44	7.60	5.38	5.69	2.37	2.21	3.42	2.48	3.61	2.33	3.25	3.00	2.65
	8.63	7.93	6.30	8.98	7.66	7.05	2.37	2.21	3.42	2.48	3.61	2.33	3.25	3.02	2.67
12	6.84	6.52	5.44	7.54	5.39	5.70	2.37	2.26	3.53	2.22	3.55	3.83	2.21	4.57	2.38
	8.63	7.99	6.30	9.04	7.67	7.08	2.37	2.26	3.53	2.22	3.55	3.83	2.21	4.59	2.40
13	5.81	5.60	6.10	6.04	5.66	4.83	2.36	2.30	3.58	2.36	3.45	3.72	2.22	4.46	2.26
	8.35	6.70	6.98	7.94	6.51	6.79	2.38	2.30	3.58	2.36	3.45	3.74	2.22	4.48	2.26
14	5.11	6.34	2.85	6.25	4.30	5.52	3.60	2.23	3.45	2.54	3.55	3.89	2.25	3.38	2.33
	6.61	9.17	6.18	7.84	8.70	6.80	3.60	2.23	3.45	2.58	3.55	4.03	2.25	4.72	2.49
exp	7.97	7.93	7.96	7.60	7.81	7.50	2.00	2.00	2.00	3.50	2.00	3.50	2.00	3.50	2.90
	8.97	8.93	8.96	8.60	8.81	8.50	3.60	3.60	3.60	4.80	3.60	4.80	3.60	4.80	3.60

[a] The first line contains the values of $A^{up} = A^{max} + D^{max}$, and the second line contains the values of $A^{low} = A^{min} - D^{min}$.

Table IV. Comparison of Calculated and Experimental NMR Parameters

	averaged over conformers given				
residue	all conformers	1, 4, 7, 8	7, 8	structure I, ref 9	exptl
		J(HNC$^{\alpha}$H), Hz			
2	5.9–8.6	5.7–8.7	5.6–8.8	0.5–5.1	8.0–9.0
3	5.9–8.3	5.5–8.1	5.6–8.3	0.6–5.3	7.9–8.9
4	5.4–8.3	5.8–9.4	6.0–9.1	5.3–9.4	8.0–9.0
5	5.6–8.2	4.4–8.8	4.2–7.0	1.6–6.6	7.6–8.6
6	5.4–8.5	5.4–9.3	5.7–9.7	0.4–4.8	7.8–8.8
7	5.1–7.6	4.5–8.0	4.4–7.8	0.3–4.2	7.5–8.5
		αH_i–NH_{i+1} distances, Å			
$i, i+1$					
12	2.4–2.7	2.4–2.4	2.4–2.4	2.3	2.0–3.6
23	2.8–3.2	3.5–3.6	3.5–3.6	2.2	2.0–3.6
34	3.5–3.7	3.5–3.6	3.5–3.6	3.6	2.0–3.6
45	3.1–3.3	2.6–3.2	2.5–2.6	2.2	2.0–3.6
56	2.5–3.0	2.3–3.1	2.4–3.3	3.6	2.0–3.6
67	2.6–2.9	2.5–2.8	2.5–2.8	3.6	2.9–3.6
		NH_i–NH_{i+1} distances, Å			
45	2.9–3.2	2.9–4.4	4.4–4.5	4.3	3.5–4.8
56	2.9–3.7	2.9–4.2	2.9–4.5	3.1	3.5–4.8
67	2.9–3.5	2.9–3.5	2.9–3.6	2.9	3.5–4.8

conformer. We have checked possible overlappings of the intervals $[A^{calcd}_{low}, A^{calcd}_{up}]_k$ and $[A^{exp}_{low}, A^{exp}_{up}]_k$ for every conformer. If these intervals were not overlapping, the values $s_{ik} = (a^{exp}_{up})_k - (A^{calcd}_{low})_{ik}$ (if $A^{calcd}_{low} > A^{exp}_{up}$) or $s_{ik} = (A^{exp}_{low})_k - (A^{calcd}_{up})_{ik}$ (if $A^{calcd}_{up} < A^{exp}_{low}$) have been calculated. These values can provide a kind of measure of disagreement between the calculated and measured structural parameters. The s_{ik} values are described in Table V, where the "–" signs signify the cases when overlapping occurs, i.e., agreement exists. Table V shows that no single DRE conformer alone can satisfy all experimental data. It is noteworthy also that the s_{ik} values for different conformers related to the same parameter in all cases are of the same sign. This means that no conformer possessing a significant s_{ik} value could be "balanced" by another conformer also possessing a significant s_{ik} value for the same parameter, but with the opposite sign. The special significance of conformers **7** and **8** also could be seen in the data in Table V, since they are the only ones displaying overlappings in the NH_4–NH_5 distance.

It is reasonable to assume that the conformers with the highest possible statistical weights would be those with the smallest $|s_{ik}|$ values. Supposing the arbitrary limits for $|s_{ik}|$ values of 1.0 Hz in the case of the J(HNC$^{\alpha}$H)'s and 1.0 Å in the case of interproton distances, we can pick conformers **1**, **4**, **7**, and **8** as the most probable ones. The same selection procedure has found 500 000 $\{w_i\}$ points satisfying the conditions (1) for these four conformers. The estimates for the $\langle w_i \rangle$, w^{low}_i, and w^{up}_i values were about 0.20–0.29, 0.00, and 0.55–0.63, respectively, for all four conformers. Interestingly, the estimates for $\langle w_i \rangle$, w^{up}_i, and w^{low}_i were virtually

Table V. Overlappings between Intervals of Calculated and Measured Structural Parameters for DRE Low-Energy Structures[a]

	J(HNC$^{\alpha}$H), Hz for residues 2–7						interproton distances, Å								
							αH_i–NH_{i+1}						NH_i–NH_{i+1}		
conformer	2	3	4	5	6	7	12	23	34	45	56	67	45	56	67
1	–	0.1	–	–	–	–	–	–	–	–	–	–	0.9	0.5	0.7
2	–	–	0.1	–	–	0.8	–	–	–1.1	–	–	–	0.8	1.3	0.8
3	–	–	–	–	0.7	–	–	–	–	–	–	–	1.2	0.9	1.4
4	0.4	0.4	–	0.4	0.2	0.4	–	–	–	–	–	0.3	1.0	–	0.4
5	–	0.1	–	0.8	0.2	–	–	–	–	–	–	0.3	1.2	–	0.4
6	–	–	–	–	0.2	–	–	–	–	–	–	0.2	1.2	0.1	0.5
7	–	–	–	0.6	–	0.2	–	–	–	–	–	0.4	–	1.0	–
8	–	0.4	–	0.8	–	–	–	–	–	–	–	0.1	–	–	0.7
9	0.6	1.1	1.5	–	0.2	0.9	–	–	–	–	–	–	1.0	–	1.0
10	3.0	–	1.5	–	1.0	1.1	–	–	–	–	–	–	1.0	0.6	1.0
11	–	–	1.7	–	0.2	0.4	–	–	–	–	–	0.2	1.0	1.2	0.5
12	–	–	1.7	–	0.1	0.4	–	–	–	–	–	0.5	1.3	–	–
13	–	1.2	1.0	–	1.3	0.7	–	–	–	–	–	0.6	1.1	–	–
14	1.3	–	1.8	–	–	0.7	–	–	–	–	–	0.4	0.9	–	–

[a] The sign "–" marks the existence of an overlap.

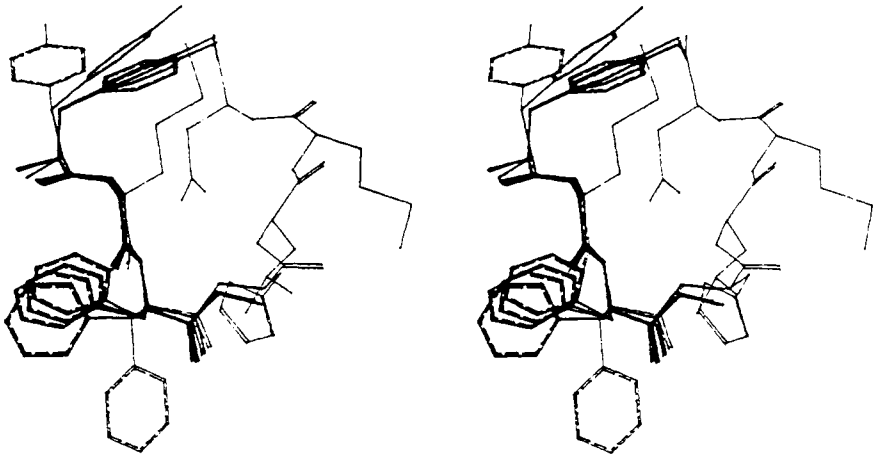

Figure 2. Stereoview of overlapped models for DRE proposed[10] δ-receptor-bound conformation (thin line) and the N-terminal tripeptide moieties of conformers **1**, **4**, **7**, and **8** (bold lines). All hydrogens as well as the D-Met side chain in the N-terminal tripeptides of conformers **1**, **4**, **7**, and **8** are omitted.

the same when just 100 000 $\{w_i\}$ points were found, which indicates their reliability. The values of $\langle A^{\text{calcd}}{}_{\text{up}}\rangle_k$ and $\langle A^{\text{calcd}}{}_{\text{low}}\rangle_k$, averaged over 500 000 $\{w_i\}$ points for conformers **1**, **4**, **7**, and **8**, are listed in the second column in Table IV. Interestingly, there is not much difference between the data in the first and second columns in Table IV. On the other hand, the selection procedure failed to find even a single $\{w_i\}$ point satisfying the conditions (1) for the ten remaining DRE conformers (i.e., for 14 original ones without conformers **1**, **4**, **7**, and **8**), despite employing more than 10^7 trial points.

Since the data of Table V show that no single DRE conformer could satisfy all of the experimental data, the "minimal" set of low-energy DRE conformers matching the experimental data in DMSO solution should consist of at least two structures. All six possible pairs of conformers **1**, **4**, **7**, and **8** were checked for the selection of statistical weight values that will satisfy the conditions (1). Four out of six pairs were found to match the experimental data, namely the pairs of conformers **1** and **7**, **4** and **7**, **4** and **8**, and **7** and **8**. Thus, the DRE conformers **7** and **8** seem to be the "indispensable" ones in that the experimental data could not be matched simultaneously by any combination of DRE low-energy conformers without taking into account either conformer **7** or conformer **8**. To estimate the $w^{\text{up}}{}_i$ values for these conformers, the selection of 100 000 $\{w_i\}$ points was performed for the pair of conformers **7** and **8**, which gave the $w^{\text{up}}{}_i$ values of 0.81 and 0.92 for conformers **7** and **8**, respectively. On the contrary, to obtain the estimation of their $w^{\text{low}}{}_i$ values, the selection procedures were performed for all DRE conformers, but without either conformer **7** or conformer **8**. The $w^{\text{low}}{}_i$ values were 0.17 for conformer **7** and 0.16 for conformer **8**.

Discussion

The approach used in this study is quite different compared to other approaches to the problem of solution conformation for short peptides (e.g. see ref 9). The point we have tried to underline is that at present any information available about the solution conformation for a relatively small peptide inevitably involves a considerable amount of uncertainty. This uncertainty starts with inaccuracies in the experimental mesurements used and in the choice of experimental conditions. A second difficulty is the approximations made to extract structural parameters from the values measured (e.g. interproton distances from NOE values or dihedral angles from J(HNC$^{\alpha}$H)s). A third problem is the intrinsic inaccuracies of the various methods of molecular modeling used to match a particular peptide model(s) with the experimental data. Thus, any conclusion about the statistical weights of different conformers for a given small peptide is always an estimate of the "true" equilibrium values. In this respect, the main difference in our approach is that we explicitly include the uncertainty in the estimation process.

Another feature of our approach is the combined use of experimental and calculation data. We assume that energy calculations are capable of finding the low-energy conformers of the peptide backbone for a given peptide. Nonetheless, a low-energy conformer set obtained by energy calculations may miss some conformers with low energies due to the intrinsic inaccuracies

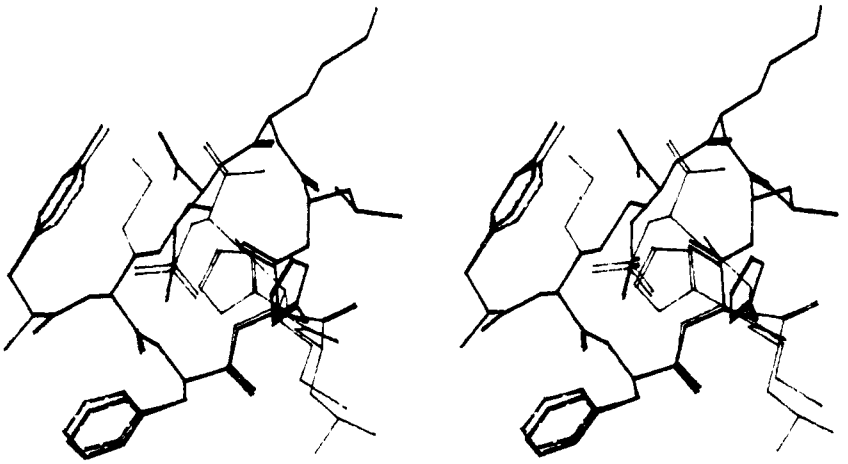

Figure 3. Stereoview of overlapped conformers **7** (bold line) and **8** (thin line). All hydrogens are omitted.

of the calculations. However, we are interested in finding not just conformers possessing low energies but the family or families of conformers representing the different geometrical possibilities of the peptide in terms of the experimental parameters. (For instance, all DRE conformers in Table II represent the same single family in terms of experimental interproton distances αH_1–NH_2, αH_3–NH_4 and αH_5–NH_6, see Tables III and V.) Typically, the number of such families is significantly less than the number of low-energy backbone conformers.[11] Thus we are less likely to omit a family than some low-energy conformers in the calculation results. Nevertheless, we cannot guarantee that no other low-energy conformers for peptide backbone can be found except those found in the study. For example, structure I previously proposed as the DRE solution conformation[9] possesses an energy higher than our conformers after energy minimization using the same force field. However, Monte Carlo sampling of the vicinity of structure I found conformations with energies comparable to those of conformers in this study (see the last entry of Table II).

Keeping the above in mind, one has to be rather cautious in conclusions that can be formulated in this study regarding the DRE solution conformation. Nonetheless, we can safely conclude that the family of low-energy conformers **1**, **4**, **7**, and **8** is in good agreement with experimental data. On the other hand, no agreement could be found not including these conformers. Moreover, we can conclude that at least one out of two conformers **7** and **8** is highly probable, with a total w^{low}_i value not less than 0.16. The agreement with the experimental data provided by this family of conformers is much better than that in the case of structure I found by other authors[9] (see the fourth column of Table IV, especially the data on the $J(HNC^{\alpha}H)$s). At the same time, these conformers as well as structure I include one close interproton contact, NH_3–NH_4, which was not observed experimentally in either our measurements or the earlier ones. A possible reason for this discrepancy might be the very close proximity of the various NH–NH diagonal peaks in the NOESY spectra, as also was noted earlier.[9]

The common feature of conformers **1**, **4**, **7**, and **8** is the similar values of dihedral angles for the peptide backbone of the N-terminal tripeptide region (see Table II). As suggested earlier,[10,11] a model of the δ-receptor-bound conformation of DRE primarily involves the conformation of its N-terminal tripeptide with specific spatial arrangement of the aromatic side chains of the Try^1 and Phe^3 residues. It is very interesting that the backbone conformation of the N-terminal tripeptide for the DRE conformers **1**, **4**, **7**, and **8** (as well as for conformers **2** and **3**) is essentially the conformation proposed[10] as a model of the δ-receptor-bound conformer of DRE (see an overlap of the discussed conformers in Figure 2). The differences occur in the values of dihedral angles for the Tyr^1 and Phe^3 side chains. However, it should be noted that this study did not deal with various side chain rotamers but with peptide backbone conformations only.

The indispensible conformers **7** and **8** are actually very similar to each other, differing mainly in a Leu^6-Met^7 peptide bond plane rotation (see Figure 3). They share the specific feature of an extended backbone conformation in the region of the His^4 residue. This helps maintain a kind of chain reversal within the central type I β-like turn in conformer **7** (conformer 8 has β-II like turn) at the Leu^5–Met^6 residues and with antiparallel extended "legs" formed by the peptide bonds Phe^3–His^4 and His^4–Leu^5 from the one side and by the peptide bond Met^6–Asp^7 and the side chain of the Asp^7 from the other side. This reversal could be further stabilized by hydrogen bonding between the NH of Phe^3 and the β-carboxyl of Asp^7.

In summary, the main conclusions of this study are as follows:

1. At least one of the low-energy backbone conformers **7** and **8** should exist among the solution conformations for DRE in DMSO with a statistical weight not less than 0.15. This does not exclude the presence of other low-energy conformers; but the presence of at least one of those two is crucial for reproducing the available NMR data for DRE in DMSO solution.

2. One cannot match the available NMR data for the peptide backbone of DRE in DMSO solution without considering conformers possessing the peptide backbone conformation of the N-terminal tripeptide, which corresponds to a model for the δ-receptor-bound DRE conformer. This suggests that the δ-receptor-bound DRE conformation pre-exists in solution with a significant statistical weight. This conformation could be matched completely by conformers **1**, **4**, **7**, and **8** just by rotating the side chains of Tyr^1 and Phe^3.

Acknowledgment. The authors are grateful to Drs. Garland R. Marshall and Denise Beusen for reading the manuscript and for valuable discussions. The research was supported by Grants from the U.S. Public Health Service (NS-19972) and a Grant from NIDA (06248).