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Conformational Determinants of High Affinity δ Receptor Binding of Opioid Peptides

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SUMMARY

Detailed conformational analysis of linear and cyclic δ-selective opioid peptides was performed in conjunction with computer-analyzed receptor binding studies with the aim of determining conformational requirements for high affinity binding of peptides to the δ-receptor. The four linear δ-selective hexapeptides included in this study were: DSLET (Tyr-D-Ser-Gly-Phe-Leu-Thr) and its D-Thr^2 analog (DTLET) and two *t*-butyl ether analogs. In one analog an ***O*-*t*-butyl** group replaces the D-Ser^{2}OH and in the other a second ***O*-*t*-butyl** group replaces the D-Thr^{6}OH group as well. This study also includes seven cyclic pentapeptides of the type: Tyr-Cys(Pen-Gly-Phe-Cys(Pen) with various combinations of DL-cysteine and DL-penicillamine (β-dimethyl cysteine) as the second and fifth residues resulting in varying δ affinities and selectivities. Four (DPLPE, DPDPE, DPLCE, and DCLPE) have both high δ affinity and selectivity; two (DCDCE and DCLCE) have high affinity at both δ- and μ-receptors, and one (LCLCE) has low affinity for both receptors. Our investigation has shown that all analogs that have high affinity at the δ receptor have a unique common low energy conformer. This compact conformer contains intramolecular H-bonds and is very different from the β_{II}-turn-type structure associated with high affinity μ-receptor binding deduced in our previous work.

The best characterized classes of opioid receptors are known as "μ" and "δ." The μ-site was originally associated with high affinity binding of morphine and other classical fused ring opiates, while high affinity binding of the endogenous opioid peptides Leu- and Met-enkephalin was attributed to the δ-site. However, it is now well established that opioid peptides bind with high affinity to both μ- and δ-receptors, and a number of different series of peptides have been synthesized that exhibit varying degrees of affinity and selectivity for both the μ- and δ-receptor subtypes. After much effort, peptides with high selectivity at each of these receptors have now been synthesized.

In previous studies, we have identified and characterized molecular determinants of high affinity binding of opioid peptides to the μ-receptor by studying analogs of Met-enkephalin (1, 2) and of the μ-selective peptide morphiceptin (3). Theoretical characterization of conformational and electronic properties together with detailed receptor binding studies led to the identification of a β_{II}-turn-type conformer as a candidate for high affinity binding of these peptides to the μ-receptor. The present work has used similar methods to identify a conformation in which opioid peptides bind with high affinity to the δ-receptor.

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Table 1 lists the two series of enkephalin analogs that we studied. We chose both linear and cyclic peptides reported to have high δ-selectivity. The linear peptides DTLET, DSLET, and the *t*-butyl ethers of DSLET were developed by Roques *et al.* (4–6). Because hydrophobic side chains at the second position increase μ-selectivity, these investigators postulated that hydrophilic amino acids like serine or threonine would enhance δ-selectivity. A D-configuration at this position improves pharmacological activity by blocking degradation. Finally, Leu-enkephalin is more δ selective than Met-enkephalin, and structural requirements deduced from NMR studies implied that lengthening the peptide by addition of a Thr residue would improve δ-selectivity. The cyclic peptides studied were developed by Mosberg *et al.* (7, 8) and Schiller and colleagues (9, 10) to test the postulate that restricting the conformational flexibility of enkephalin analogs would increase their receptor selectivity.

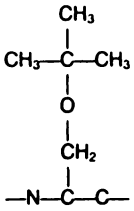
The two underlying hypotheses used in the search for conformational determinants of δ binding affinity were that all peptide analogs that have high δ-receptor affinity bind to the receptor in a similar conformation and that each opiate receptor has distinct conformational and electronic requirements for high affinity peptide binding. Thus, high affinity and selectivity of a given peptide analog for the δ-receptor should result if two conditions are satisfied: the existence of low energy conformers

ABBREVIATIONS: ECEPP, empirical conformational energy program for peptides; MOBLS, maximum overlap by least squares; RMS, root mean square; PEP, peptide energy program; MNDO, modified neglect of differential overlap; DADL, (D-Ala^2-D-Leu^5)enkephalin; EKC, ethylketocyclazocine; DHM, dihydromorphine. Other abbreviations are names of peptides defined in tables.

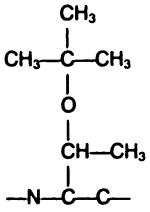
TABLE 1
Linear and cyclic peptide analogs studied

A. Linear peptides		B. Cyclic peptides	
DTLET:	Tyr-D-Thr-Gly-Phe-Leu-Thr	DPLPE:	Tyr-D-Pen-Gly-Phe-L-Pen
DSLET:	Tyr-D-Ser-Gly-Phe-Leu-Thr	DPDPE:	Tyr-D-Pen-Gly-Phe-D-Pen
DStBuLET:	Tyr-D-SertBu-Gly-Phe-Leu-Thr	DPLCE:	Tyr-D-Pen-Gly-Phe-L-Cys
DStBuLETtBu:	Tyr-D-SertBu-Gly-Phe-Leu-ThrtBu	DCLPE:	Tyr-D-Cys-Gly-Phe-L-Pen
		DCDCE:	Tyr-D-Cys-Gly-Phe-D-Cys
		DCLCE:	Tyr-D-Cys-Gly-Phe-L-Cys
		LCLCE:	Tyr-L-Cys-Gly-Phe-L-Cys

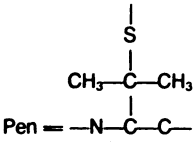
SertBu



ThrtBu



Pen =



that have optimal interaction with the δ-receptor, and the inaccessibility of conformers appropriate for high affinity binding to other receptor types. These two conditions should then exist for the linear peptide, DTLET, and the cyclic peptide, DPLPE, both of which have high δ-selectivity and affinity, making them appropriate templates to use to characterize a common conformation for high affinity binding of peptides at the δ-receptor.

In the work reported here, two independent search strategies have been used to identify low energy conformers for DTLET and DPLPE. They were used as templates to construct initial conformers for the remaining analogs, which were then optimized by energy minimizations. Among these optimized conformers, it was possible to identify a common candidate conformer for high affinity δ-receptor binding. This conformer fulfilled the two criteria of being energetically accessible to all high affinity analogs studied and retaining a high degree of overlap with the template analog.

Materials and Methods

Programs. Conformational energies were calculated using the ECEPP potential function developed by Scheraga and co-workers (11, 12). The empirical energy expression in ECEPP consists of five terms: electrostatic, repulsion, van der Waals (dispersion), hydrogen bonding, and torsion-angle energy components. We have modified this program to include atom types for nonstandard amino acids and have interfaced it to our utility programs for efficient structure generation and graphic displays (MOLECULE), and automated conformational searches (13). Conformational energies were optimized using a new energy minimization program, PEP, developed in our laboratory. The search strategies used to find low energy conformers were designed to thoroughly span the conformational space for the peptides studied. Different search strategies were employed for the linear and cyclic peptides, and the results were then combined to identify common low energy conformers.

Parameters for *t*-butyl derivatives. We used the semiempirical quantum mechanical method, MNDO (14), to obtain geometries and charges for the unusual *t*-butyl ether derivatives of Ser and Thr present in DStBuLET and DStBuLETtBu. Parameters were developed for the terminally blocked *t*-butyl ether derivatives of Ser and Thr. We replaced the amino group by an *N*-terminal acetyl group and replaced the carboxyl moiety with an *N*-methyl amide group ($CONHCH_3$). This procedure is analogous to that employed in the original development of ECEPP potential functions for the standard amino acids. The *t*-butyl ether derivatives were originally assigned standard geometries (R_{OC} = 1.34 Å, R_{CC} = 1.53 Å, R_{CH} = 1.08 Å, and θ = 109.5°), and other parameters were taken from the ECEPP standard residue libraries for Ser and Thr.

Starting structures were totally optimized in MNDO using the AM1 parameters (15) and the symmetry option. Charges obtained for the *t*-butyl group were averaged to ensure that all equivalent carbon or hydrogen atoms had equal charges. The resulting structures and charges were then inserted into our version of ECEPP for refinement by rotation of dihedral angles. The optimized conformer of D-Ser-*O*-*t*-butyl showed no significant change in side chain conformation from the MNDO-optimized version, so its side chain geometry and conformation were used for studies of the complete peptides, DStBuLET and DStBuLETtBu.

For D-Thr-*O*-*t*-butyl a number of iterations between the MNDO and ECEPP program were needed to obtain a mutually consistent geometry. The first MNDO optimization yielded a distorted C-O-C bond angle of 127°, which we attributed to steric hindrance from the β-methyl group. We accordingly used ECEPP to determine the C_α-C_β-O_γ-C_δ dihedral angle which minimized steric interactions between the *t*-butyl methyl groups and the β-methyl group, and reoptimized the resulting conformer in MNDO. This procedure was repeated until charges became self-consistent. Diagonalization of the MNDO force constant matrix gave all positive eigenvalues, showing that a local energy minimum was reached. The resulting geometry and charges were reentered into MOLECULE in a fashion similar to that used for D-Ser-*O*-*t*-butyl. The C-O-C bond angle was a more reasonable 117°. Further optimization of this structure using PEP showed no significant change in the side chain conformation, and the resulting conformation was used for studies of the complete peptide, DStBuLETtBu.

Molecular overlaps. The extent of overlap between any two conformers was determined using a program called MOBLS. This program determines the minimum RMS of the distance between user-selected, matched atoms in two molecules, without allowing conformational change of either molecule. In this study, when comparing two conformers of the same analog, all atoms in the two molecules were included in the overlap calculations. When two different analogs were being compared, only analogous atoms present in both analogs were included in determining RMS values. For RMS comparison of all analogs versus DTLET, all the atoms in the Tyr^1, Gly^3, and Phe^4 residues were included. For each analog only those atoms in the second and fifth residues which could be directly compared with atoms in Thr^2 and Leu^5 were included (Table 2). For DSLET and DStBuLET, all the atoms in the Thr^6 residues were included. For DStBuLETtBu, all atoms in the sixth residues were included except the hydrogen in the hydroxyl group of DTLET and the *t*-butyl group of DStBuLETtBu. Based on graphical comparison, an RMS value of greater than 1.5 Å was taken to indicate a significant difference between two conformations.

Conformational search procedures. Fig. 1 illustrates the "buildup" strategy that we used to identify low energy conformers of

TABLE 2

Atoms in second and fifth residues of each analog which were included in RMS comparison with DTLET[a]

A. 2nd Residue: Atoms included

DTLET[b] Thr[2]	DSLET Ser[2]	DStBuLET DStBuLETtBu SertBu[2]	DPLPE, DPDPE DPLCE Pen[2]	DCLPE, DCDCE DCLCE, LCLCE Cys[2]
NH	NH	NH	NH	NH
$C_\alpha H$	$C_\alpha H$	$C_\alpha H$	C_α	C_α
C=O	C=O	C=O	C=O	C=O
C_β	C_β	C_β	C_β	C_β
H_β	H_β	H_β	H_β	H_β
$C_\gamma H_3$			$C_\gamma H_3$	
O	O	O		
H	H			

B. 5th Residues: Atoms included

DTLET[b], DStBuLET DSLET, DStBuLETtBu Leu[5]	DPLPE, DPDPE DCLPE Pen[5]	DPLCE, DCLCE DCDCE, LCLCE Cys[5]
NH	NH	NH
C_α	C_α	C_α
C=O	C=O	C=O
$C_\beta H_2$	C_β	$C_\beta H_2$
$C_\gamma H$		
$C_\delta H_3$		
$C_\delta H_3$		

[a] In the comparisons of all analogs with DTLET, all atoms of Tyr[1], Gly[3] and Phe[4] residues were included.
[b] Template.

the template linear peptide, DTLET. We began with low energy side chain conformations of single amino acids (16) and assembled these to generate starting conformers of both dipeptides and tripeptides. The latter were then optimized by energy minimization varying dihedral angles only. Pairs of resulting low energy tripeptides were joined to create hexapeptides that subsequently were refined by dihedral angle energy minimization.

Because of the constraint of the backbone ring structure, an entirely different search strategy was used for the cyclic peptides. This strategy is outlined in Fig. 2. DPLPE was used as the template for the cyclic peptides, and a systematic search was performed to determine sets of backbone dihedral values that would allow closure of the ring. This search combines systematic backbone dihedral angle generation with a ring-closing algorithm developed by Go and Scheraga (17). This program accepts bond distances and bond angles for a ring containing N dihedral angles, systematically varies a user-specified set of N-6 dihedral angles in the ring, and then determines all possible sets of the remaining six dihedral angles that will complete the closed ring.

All conformers which allowed ring closing were then further screened by examining distances between ring and C_β atoms. If the distance between any two of these atoms was less than a selected cutoff of 1.8 Å, the particular cyclic conformer was discarded. In the next step, the energies of the ring structures that satisfied the distance criterion were calculated without optimization. This energy calculation included atoms in or adjacent to the ring and a variable set of appropriate user-specified side chain and backbone atoms outside the ring. Conformations were then ranked in order of increasing energy and only those within a specified relative energy range, selected as 10 kcal/mol in this study, were retained as possible candidate conformers.

The search strategy described is applied to DPLPE in the following manner: DPLPE has a cyclic structure which contains 14 bonds (Fig. 3). Therefore, sets of eight independent angles must be specified to determine allowed cyclic structures. For this purpose, as shown in Fig.

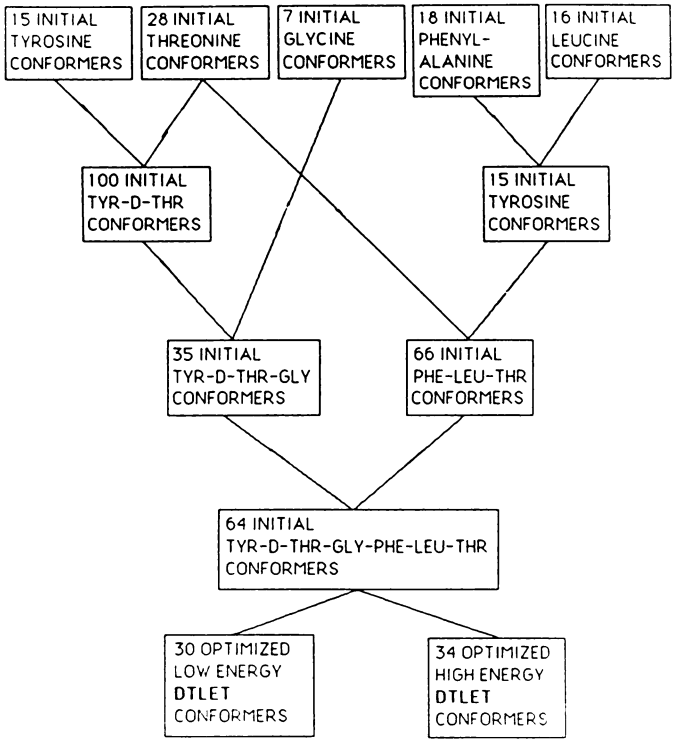


Fig. 1. Schematic diagram of the buildup search strategy used for identifying low energy conformers of the linear peptide DTLET.

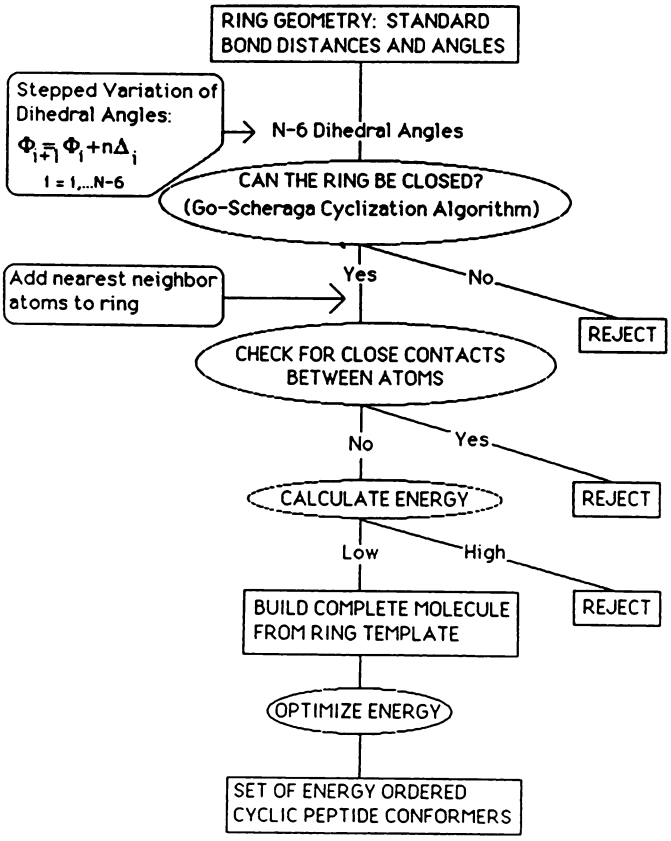


Fig. 2. Schematic diagram of the systematic cyclization search strategy used for identifying low energy conformers of the cyclic peptide DPLPE.

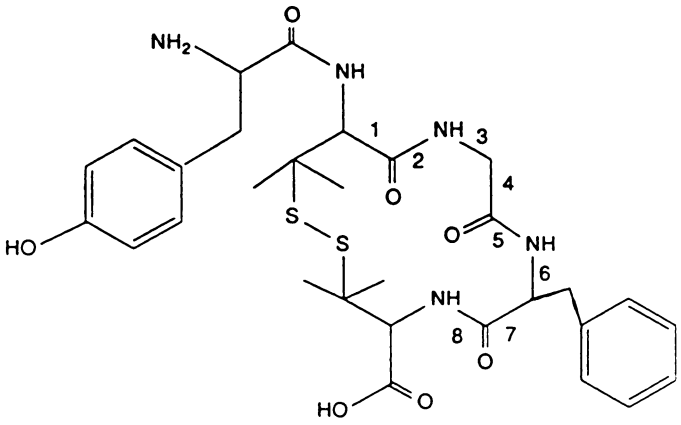


Fig. 3. Structure of the cyclic peptide, DPLPE, indicating the eight independent backbone angles varied for ring closure.

3, the eight angles chosen were $\phi_2(1)\psi_2(2)$; $\phi_3(3)\psi_3(4)\omega_3(5)$; and $\phi_4(6)\psi_4(7)\omega_4(8)$. All values of ω were held fixed at 180° and ϕ and ψ angles varied systematically by 30°. For the single-point energy calculations, the atoms comprising the ring were augmented by adding an amino group to the α carbon of Pen[2], a carboxyl group to the α carbon of Pen [5], and the two methyl groups to the β carbons of both Pen[2] and Pen[5].

All ring conformations produced by this process with energies within 10 kcal/mol of the lowest energy were then used to form conformers of the complete DPLPE pentapeptide. The complete peptides were then optimized using a ring-closing option which allows all dihedral angles to vary but holds the ring closed using a penalty function to maintain the proper bond distances and bond angles for the β carbons and sulfurs of the penicillamine side chains consistent with the presence of the disulfide bond.

Template conformers. Overlap analysis was used to identify classes of similar conformers. Specifically, conformers were considered to be similar if the RMS value for their overlap was ≤0.5. Whereas an RMS value cutoff of 1.5 is generally used to indicate significant difference between conformations, the cutoff of 0.5 was used to ensure that all conformers in a group had very similar conformations. The lowest energy conformers from each group were then selected as a set of unique conformers to be used in further study.

The unique low energy conformers of DTLET and DPLPE produced by their respective search strategies were then used as templates for each other in an attempt to identify common low energy conformers for the linear and cyclic peptides. All conformers within 7.5 kcal/mol of the lowest energy conformer for both DTLET and DPLPE were considered as candidates for the bioactive form which binds at the receptor. This cutoff, although arbitrary, is a reasonable estimate of the possible gain in energy from additional receptor interactions such as H-bonding (~3–5 kcal/mol each), which would compensate for the energy required if a higher energy conformer were to bind. Each candidate conformer thus selected was used as a template conformer for the remaining linear and cyclic analogs. The energy required to achieve this conformer for each analog, as well as the RMS value for comparison with the template analog in the candidate conformer, was used as a possible indicator of the relative affinity of each analog. A conformer which was low energy (<7.5 kcal/mol) for all high affinity analogs and high energy (>7.5 kcal/mol) for the low affinity analog was identified as a candidate conformer in which these analogs bind to the δ-receptor.

Receptor binding studies. Opiate receptor binding assays were performed essentially as described by Pasternak *et al.* (18). Briefly, rat (Sprague-Dawley) whole brain homogenates were prepared, preincubated at 37° for 1 hr, and resuspended in Tris, pH 7.7, at 6.7 mg of tissue/ml. Receptor binding incubations contained 1.8 ml of tissue suspension, 0.1 ml of labeled ligand, and unlabeled drugs in a total volume of 2.0 ml. The tubes at each drug concentration were incubated in triplicate at 25° for 1 hr before filtration.

In the present studies, self- and cross-competition experiments were conducted using two different concentrations of the five labeled ligands: tritiated naloxone, DADL, DSLET, EKC, and DHM. In addition to the resulting five-by-five "matrix" of competitive inhibition behavior, inhibition of binding of all five labeled ligands with four cyclic peptide analogs and the linear analog DTLET was performed, again at two labeled ligand concentrations.

Data obtained were analyzed by a modified version of the program LIGAND (19). This program uses a weighted, nonlinear, least squares regression analysis algorithm to fit a set of self-consistent receptor-binding affinities and capacities to a postulated model that comprises *N* different receptor sites.

In the procedure used, all self- and cross-competition studies involving the five labeled ligands were analyzed together assuming one-, two-, three-, four-, five-, and six-site models of receptor binding, and results were compared for statistical significance and other indications of reliability. Inhibition data for the five δ-specific analogs were then added to the matrix obtained for the labeled and unlabeled ligands and the data were reanalyzed simultaneously for self-consistent receptor-binding affinities and capacities. Three-, four-, and five-site models for receptor binding were systematically explored.

Materials. [^{3}H]Naloxone, [^{3}H]DADL, [^{3}H]DSLET, and [^{3}H]EKC were from New England Nuclear, and [^{3}H]DHM was from Amersham. Unlabeled DADL and DSLET were from Sigma, EKC was from Sterling Winthrop, DHM was from the National Institute on Drug Abuse, and naloxone was from Endo Labs. The cyclic peptides were the kind gift of Dr. H. I. Mosberg.

Results and Discussion

Receptor selectivity. In the receptor binding studies, a model that has five receptor sites gave the best fit to the experimental data. Table 3 lists the affinities of the compounds studied as well as the maximum binding capacity of each receptor site determined using these ligands. The sites are labeled according to the following convention. First, μ_1 is labeled as the common high affinity site for all ligands. Second, μ_2 is the sole remaining site that has high affinity for DHM, δ is a high affinity site for DSLET, and κ is the remaining high affinity site for EKC. The site we are labeling κ might represent more than a single binding site. Recent studies indicate a very small number of κ sites in rat brain, and another site to which EKC binds with moderate affinity.[1] The fifth site has very high capacity but low affinities for all ligands and is most likely unrelated to opioid activity. DSLET, DTLET, DPLPE, DPDPE, DPLCE, and DCLPE all have high affinity at the δ site, although DPDPE has a 10-fold lower affinity for this site than the others. These analogs exhibit varying degrees of specificity for the δ site compared to the μ_1 or μ_2 sites. DPDPE and DPLPE are the most δ selective, having the lowest affinities at both μ_1 and μ_2. Pasternak and co-workers (20) also reported a low affinity for DPDPE at μ-receptors. These results are consistent with the evidence presented by Porreca *et al.* (21), that DPDPE exerts its analgesic effects through the δ-receptor.

Conformational analysis. In the search for low energy conformers for the linear peptide DTLET, the "buildup" procedure produced 64 initial hexapeptide conformers. After total optimization, 30 conformers were found to have energies within 7.5 kcal/mol of the lowest energy conformer. For the cyclic peptide DPLPE, systematic variation of the five independent dihedral

[1] L. Toll *et al.*, submitted for publication.

TABLE 3
Receptor affinities and maximum binding capacities for a five-receptor site model

	K_d				
	Site 1 (μ_1)	Site 2 (μ_2)	Site 3 (δ)	Site 4 (κ)	Site 5
Naloxone[a]	0.57 ± 0.12	4.44 ± 0.62	32.7 ± 2.8	0.84 ± 0.11	136 ± 41
DADL[a]	1.30 ± 0.28	23.1 ± 3.5	2.59 ± 0.29	653 ± 192	8237 ± 2848
EKC[a]	0.81 ± 0.18	6.38 ± 0.83	26.0 ± 2.3	0.15 ± 0.05	0.00[b]
DSLET[a]	6.64 ± 1.34	45.4 ± 0.64	1.24 ± 0.14	1302 ± 343	3439 ± 1201
DHM[a]	0.30 ± 0.06	12.6 ± 2.4	178 ± 16	678 ± 203	79.5 ± 24.6
DPLPE	102.5 ± 31	520 ± 145	1.33 ± 0.29	4826 ± 3229	0.00[b]
DPDPE	338.0 ± 84	2754 ± 680	13.1 ± 2.6	3535 ± 2988	0.00[b]
DPLCE	14.4 ± 3.2	79.4 ± 14.0	0.80 ± 0.16	3140 ± 1098	655 ± 213
DCLPE	5.02 ± 1.08	7.94 ± 1.89	1.89 ± 0.29	3136 ± 887	0.00[b]
DTLET	24.9 ± 3.4	4.78 ± 1.74	1.02 ± 0.25	5988 ± 3135	7033 ± 4005
B_{max} (pmol/g)	2.1	23.3	7.3	9.3	112

[a] ^{3}H-ligand used for binding experiments.
[b] Value held equal to zero.

angles in the ring gave 248,832 sets of independent angles. Of the resulting structures which allowed ring closure, 22,670 fulfilled the distance criterion. After the single-point energy calculations, 104 of these conformers had energies within 10 kcal/mol of the lowest energy conformer. After total optimization of these conformers of the complete pentapeptide, 31 unique conformers had energies within 7.5 kcal/mol of the lowest energy conformer. Thus, although the cyclization of the pentapeptide DPLPE was originally intended to produce a conformationally restricted analog, energy-conformational studies of DPLPE and the linear peptide, DTLET, produced approximately the same number of low energy conformers. In this instance, then, there is little difference in the degree of conformational flexibility for these linear and cyclic peptides.

The 31 unique conformers of the cyclic peptide DPLPE were used as templates to construct 31 corresponding DTLET conformers and conformers of three other cyclic analogs, DPDPE, DCLPE and DPLCE. These initial conformers were then optimized by energy minimization. This procedure resulted in only one DTLET conformer within 7.5 kcal/mol of the lowest energy conformer found by the buildup search for DTLET. For the three cyclic congeners the DPLPE template set generated 1 DPDPE, 2 DCLPE, and 10 DPLCE conformers within 7.5 kcal/mol of the lowest energy conformer for each compound using either of the two search strategies employed. Most significantly, this procedure failed to produce a low energy conformer common to all five analogs.

In a complementary approach, the 30 low energy DTLET conformers obtained by the buildup search were used as templates to construct 30 corresponding DPLPE conformers. Each of these DPLPE conformers was optimized by energy minimization that incorporated the ring-closing penalty function. One of the resulting conformers was found to be 1.6 kcal lower in energy than the lowest energy conformer found using the systematic cyclization search strategy. Fifteen other low energy ($\Delta E < 7.5$ kcal/mol) DPLPE conformers were found using DTLET conformers as templates which were not among those identified by the systematic cyclization search strategy. These same 30 DTLET conformations or their corresponding DPLPE conformations were then used as template conformers for all the remaining analogs, both linear and cyclic.

The RMS value for the overlap of each of the 16 optimized low energy DPLPE conformers with its corresponding DTLET conformer was determined. Eight of the 16 DPLPE conformers showed a significant similarity to those of DTLET as indicated by an RMS value less than or equal to 1.5 and were considered to be common low energy conformers. Table 4 lists the dihedral angles for DTLET in each of these eight conformers.

Selection of template. The RMS values for the overlap of each of these eight DTLET conformers with the eight corresponding optimized conformers of the remaining nine analogs were also determined. These RMS values for overlap of the eight candidate conformers for each analog are summarized in Table 5.

As can be seen from Table 5, only one of these conformers, **5**, was found to have both a low relative energy (<7.5 kcal/mol) and a low RMS (<1.5) for all analogs showing high affinity at the δ-receptor site. This conformer is the lowest energy form for 5 of the 10 high affinity analogs and has a relative energy of less than 3 kcal/mol for the other 5. This conformer also has a very high energy and RMS value for the low activity analog LCLCE. Conformer **5** thus appears to satisfy the stringent requirements of being a common low energy form of both linear and cyclic high δ affinity analogs and being inaccessible to an inactive analog. It can thus be unambiguously selected as the most likely candidate conformer for high affinity binding of these opioid peptides to the δ-receptor. Table 6 lists the dihedral angles for each analog in this conformer.

Fig. 4 shows DPLPE and DTLET superimposed in the candidate δ-binding conformer. Apparent from this figure is the compact nature of the DTLET structure that makes it such a good template for the cyclic analog. The three internal hydrogen bonds shown appear to stabilize this particular conformer for DTLET. Both DPLPE and DTLET have a strong "head-to-tail" hydrogen bond between the carbonyl oxygen of the fifth residue and the hydrogen of the Tyr hydroxyl group. In addition, in the DTLET conformer both Ser and Thr hydroxyl groups are involved in strong hydrogen bonds.

In the DStBuLET and DStBuLETtBu analogs, the tertiary butyl ether groups preclude hydrogen bonds to the Ser and Thr hydroxyl groups. Nonetheless, as seen in Table 5, the lowest energy-optimized conformer of each *t*-butyl derivative is the candidate conformer, **5**. These conformers retain good overlap with the DTLET template conformer. Fig. 5 shows DPLPE and DStBuLETtBu overlapped in the candidate δ-binding conformer. As can be seen in this figure, the bulky *t*-butyl side chains rotate to a position which does not interfere with the compact structure. The maintenance of a compact structure

TABLE 4

Dihedral angle values for the eight candidate conformers of DTLET for high affinity binding to the δ-opioid receptor

Conformer	Tyr	D-Tyr	Gly	Phe	Leu	Thr
2	$\phi = 34\ \chi_1 = 177$ $\gamma = 155\ \chi_2 = 68$ $\omega = 179\ \chi_3 = 194$	$\phi = 171\ \chi_1 = 94$ $\gamma = 50\ \chi_2 = 289$ $\omega = 179\ \chi_3 = 311$	$\phi = 264$ $\chi = 304$ $\omega = 176$	$\phi = 211\ \chi_1 = 173$ $\lambda = 146\ \chi_2 = 66$ $\omega = 179$	$\phi = 280\ \chi_1 = 204$ $\gamma = 101\ \chi_2 = 171$ $\omega = 176\ \chi_3 = 301$ $\chi_4 = 312$	$\phi = 281\ \chi_1 = 48$ $\gamma = 101\ \chi_2 = 64$ $\omega = 181\ \chi_3 = 61$
3	$\phi = 35\ \chi_1 = 176$ $\gamma = 153\ \chi_2 = 68$ $\omega = 179\ \chi_3 = 201$	$\phi = 170\ \chi_1 = 94$ $\gamma = 50\ \chi_2 = 290$ $\omega = 179\ \chi_3 = 311$	$\phi = 262$ $\gamma = 309$ $\omega = 177$	$\phi = 213\ \chi_1 = 182$ $\gamma = 156\ \chi_2 = 66$ $\omega = 179$	$\phi = 290\ \chi_1 = 203$ $\gamma = 96\ \chi_2 = 170$ $\omega = 175\ \chi_3 = 301$ $\chi_4 = 311$	$\phi = 197\ \chi_1 = 267$ $\gamma = 137\ \chi_2 = 64$ $\omega = 186\ \chi_3 = 51$
5	$\phi = 35\ \chi_{11} = 184$ $\gamma = 155\ \chi_{12} = 71$ $\omega = 182\ \chi_{13} = 185$	$\phi = 166\ \chi_1 = 93$ $\gamma = 216\ \chi_2 = 293$ $\omega = 186\ \chi_3 = 307$	$\phi = 63$ $\gamma = 255$ $\omega = 169$	$\phi = 274\ \chi_1 = 184$ $\gamma = 116\ \chi_2 = 67$ $\omega = 187$	$\phi = 286\ \chi_1 = 206$ $\gamma = 303\ \chi_2 = 167$ $\omega = 180\ \chi_3 = 301$ $\chi_4 = 312$	$\phi = 194\ \chi_1 = 267$ $\gamma = 324\ \chi_2 = 65$ $\omega = 180\ \chi_3 = 52$
6	$\phi = 34\ \chi_{11} = 177$ $\gamma = 155\ \chi_{12} = 68$ $\omega = 178\ \chi_{13} = 195$	$\phi = 171\ \chi_1 = 94$ $\gamma = 50\ \chi_2 = 289$ $\omega = 179\ \chi_3 = 311$	$\phi = 265$ $\gamma = 304$ $\omega = 176$	$\phi = 210\ \chi_1 = 179$ $\gamma = 149\ \chi_2 = 63$ $\omega = 183$	$\phi = 282\ \chi_1 = 204$ $\gamma = 98\ \chi_2 = 171$ $\omega = 175\ \chi_3 = 301$ $\chi_4 = 311$	$\phi = 205\ \chi_1 = 180$ $\gamma = 20\ \chi_2 = 302$ $\omega = 180\ \chi_3 = 56$
8	$\phi = 34\ \chi_{11} = 177$ $\gamma = 155\ \chi_{12} = 67$ $\omega = 177\ \chi_{13} = 188$	$\phi = 172\ \chi_1 = 95$ $\gamma = 50\ \chi_2 = 288$ $\omega = 177\ \chi_3 = 311$	$\phi = 264$ $\gamma = 302$ $\omega = 172$	$\phi = 220\ \chi_1 = 184$ $\gamma = 125\ \chi_2 = 67$ $\omega = 188$	$\phi = 276\ \chi_1 = 209$ $\gamma = 303\ \chi_2 = 171$ $\omega = 181\ \chi_3 = 301$ $\chi_4 = 311$	$\phi = 193\ \chi_1 = 267$ $\gamma = 328\ \chi_2 = 66$ $\omega = 180\ \chi_3 = 52$
13	$\phi = 33\ \chi_{11} = 179$ $\gamma = 161\ \chi_{12} = 67$ $\omega = 181\ \chi_{13} = 162$	$\phi = 170\ \chi_1 = 94$ $\gamma = 50\ \chi_2 = 288$ $\omega = 189\ \chi_3 = 312$	$\phi = 252$ $\gamma = 268$ $\omega = 192$	$\phi = 285\ \chi_1 = 189$ $\gamma = 322\ \chi_2 = 277$ $\omega = 183$	$\phi = 268\ \chi_1 = 298$ $\gamma = 66\ \chi_2 = 163$ $\omega = 179\ \chi_3 = 299$ $\chi_4 = 303$	$\phi = 262\ \chi_1 = 297$ $\gamma = 304\ \chi_2 = 97$ $\omega = 180\ \chi_3 = 56$
15	$\phi = 36\ \chi_{11} = 170$ $\gamma = 146\ \chi_{12} = 64$ $\chi = 179\ \chi_{13} = 171$	$\phi = 167\ \chi_1 = 93$ $\gamma = 216\ \chi_2 = 294$ $\omega = 183\ \chi_3 = 307$	$\phi = 77$ $\gamma = 288$ $\omega = 175$	$\phi = 216\ \chi_1 = 181$ $\gamma = 151\ \chi_2 = 64$ $\omega = 182$	$\phi = 285\ \chi_1 = 204$ $\gamma = 103\ \chi_2 = 170$ $\omega = 175\ \chi_3 = 301$ $\chi_4 = 312$	$\phi = 197\ \chi_1 = 287$ $\gamma = 130\ \chi_2 = 63$ $\omega = 180\ \chi_3 = 50$
16	$\phi = 34\ \chi_{11} = 181$ $\gamma = 156\ \chi_{12} = 68$ $\omega = 181\ \chi_{13} = 166$	$\phi = 167\ \chi_1 = 94$ $\gamma = 221\ \chi_2 = 292$ $\omega = 181\ \chi_3 = 308$	$\phi = 81$ $\gamma = 289$ $\omega = 175$	$\phi = 216\ \chi_1 = 179$ $\gamma = 148\ \chi_2 = 64$ $\omega = 183$	$\phi = 282\ \chi_1 = 204$ $\gamma = 98\ \chi_2 = 171$ $\omega = 175\ \chi_3 = 301$ $\chi_4 = 311$	$\phi = 206\ \chi_1 = 182$ $\gamma = 12\ \chi_2 = 305$ $\omega = 171\ \chi_3 = 58$

TABLE 5

Relative energies and RMS values of possible candidate conformers[a] for high affinity δ-receptor binding of linear and cyclic opioid peptides

	Conformer 2		Conformer 3		Conformer 5		Conformer 6	
	ΔE	RMS	ΔE	RMS	ΔE	RMS	ΔE	RMS
	kcal/mol	*Å*	*kcal/mol*	*Å*	*kcal/mol*	*Å*	*kcal/mol*	*Å*
DTLET	0.98	0.00	1.04	0.00	1.94	0.00	2.06	0.00
DPLPE	2.66	1.33	3.04	1.34	0.00	0.45	3.50	1.38
DPDPE	2.84	2.10	0.00	3.03	1.22	1.23	0.04	3.08
DPLCE	3.68	1.45	4.29	1.66	0.00	0.40	8.21	2.43
DCLPE	6.24	0.80	9.60	1.63	2.35	0.50	4.35	0.79
DSLET	0.05	0.17	0.27	0.08	0.00	0.69	1.15	0.08
DCDCE	7.79	1.00	6.72	0.99	1.25	1.33	0.00	0.57
DCLCE	0.66	0.73	9.25	1.73	0.51	0.54	3.70	0.81
DStBuLET	5.52	0.22	1.45	0.43	0.00	1.13	1.95	0.07
DStBuLETtBu	4.50	0.42	1.34	0.48	0.00	1.15	259.23	0.27
LCLCE	3.88	2.68	8.85	2.91	22.88	2.45	3.84	2.67
	Conformer 8		**Conformer 13**		**Conformer 15**		**Conformer 16**	
	ΔE	RMS	ΔE	RMS	ΔE	RMS	ΔE	RMS
	kcal/mol	*Å*	*kcal/mol*	*Å*	*kcal/mol*	*Å*	*kcal/mol*	*Å*
DTLET	2.43	0.00	4.81	0.00	5.26	0.00	5.51	0.00
DPLPE	0.83	0.92	4.62	1.27	1.72	0.64	2.07	0.86
DPDPE	6.38	2.86	17.36	1.28	7.44	1.47	5.67	1.44
DPLCE	1.59	0.78	11.38	2.70	1.47	0.68	1.43	0.68
DCLPE	5.59	1.00	13.34	2.66	0.00	1.46	0.64	1.01
DSLET	1.91	0.26	2.49	0.46	3.92	0.13	4.59	0.15
DCDCE	9.27	0.96	4.79	0.70	8.75	2.13	6.08	1.48
DCLCE	5.45	1.00	14.48	2.63	0.00	1.50	0.00	1.38
DStBuLET	3.46	0.55	9.19	0.52	3.99	0.15	4.87	0.12
DStBuLETtBu	4.30	0.59	17.02	0.59	3.83	0.27	235.03	0.34
LCLCE	7.61	2.74	32.33	3.39	32.84	2.28	5.24	2.40

[a] All conformers had values for $\Delta E \leq 7.5$ kcal/mol and RMS ≤ 1.5 for DSLET and DPLPE.

TABLE 6

Comparison of the selected candidate conformer for high affinity binding at the δ-opioid receptor for eleven peptides

Analog			
DTLET	$\phi_1 = 35$ $\chi_{11} = 184$ $\phi_2 = 166$ $\chi_{21} = 93$ $\gamma_1 = 155$ $\chi_{12} = 71$ $\gamma_2 = 216$ $\chi_{22} = 293$ $\omega_1 = 182$ $\chi_{13} = 185$ $\omega_2 = 186$ $\chi_{23} = 307$	$\phi_3 = 63$ $\phi_4 = 274$ $\chi_{41} = 184$ $\phi_4 = 286$ $\chi_{51} = 206$ $\phi_6 = 194$ $\chi_{61} = 267$ $\gamma_3 = 255$ $\gamma_4 = 116$ $\chi_{42} = 67$ $\gamma_5 = 303$ $\chi_{52} = 167$ $\gamma_6 = 324$ $\chi_{62} = 65$ $\omega_3 = 169$ $\omega_4 = 187$ $\omega_5 = 180$ $\chi_{53} = 301$ $\omega_6 = 180$ $\chi_{63} = 52$ $\chi_{54} = 312$
DPLPE	$\phi_1 = 29$ $\chi_{11} = 204$ $\phi_2 = 139$ $\chi_{21} = 308$ $\gamma_1 = 174$ $\chi_{12} = 55$ $\gamma_2 = 214$ $\chi_{22} = 65$ $\omega_1 = 187$ $\chi_{13} = 199$ $\omega_2 = 185$ $\chi_{23} = 55$ $\chi_{24} = 235$	$\phi_3 = 52$ $\phi_4 = 274$ $\chi_{41} = 178$ $\phi_5 = 300$ $\chi_{51} = 303$ s-s = 161 $\gamma_3 = 249$ $\chi_4 = 97$ $\chi_{42} = 64$ $\gamma_5 = 315$ $\chi_{52} = 63$ $\omega_3 = 168$ $\omega_4 = 174$ $\omega_5 = 178$ $\chi_{53} = 62$ $\chi_{54} = 305$
DPDPE	$\phi_1 = 30$ $\chi_{11} = 197$ $\phi_2 = 137$ $\chi_{21} = 292$ $\gamma_1 = 172$ $\chi_{12} = 58$ $\gamma_2 = 208$ $\chi_{22} = 66$ $\omega_1 = 187$ $\chi_{13} = 232$ $\omega_2 = 187$ $\chi_{23} = 57$ $\chi_{24} = 235$	$\phi_3 = 84$ $\phi_4 = 278$ $\chi_{41} = 181$ $\phi_5 = 65$ $\chi_{51} = 307$ s-s = 173 $\gamma_3 = 214$ $\gamma_4 = 84$ $\chi_{42} = 63$ $\gamma_5 = 27$ $\chi_{52} = 59$ $\omega_3 = 175$ $\omega_4 = 197$ $\omega_5 = 181$ $\chi_{53} = 68$ $\chi_{54} = 285$
DPLCE	$\phi_1 = 35$ $\chi_{11} = 186$ $\phi_2 = 134$ $\chi_{21} = 308$ $\gamma_1 = 154$ $\chi_{12} = 57$ $\gamma_2 = 220$ $\chi_{22} = 63$ $\omega_1 = 186$ $\chi_{13} = 183$ $\omega_2 = 177$ $\chi_{23} = 57$ $\chi_{24} = 277$	$\phi_3 = 58$ $\phi_4 = 277$ $\chi_{41} = 180$ $\phi_5 = 207$ $\chi_{51} = 52$ s-s = 141 $\gamma_3 = 228$ $\gamma_4 = 105$ $\chi_{42} = 65$ $\gamma_5 = 325$ $\chi_{52} = 174$ $\omega_3 = 174$ $\omega_4 = 186$ $\omega_5 = 180$
DCLPE	$\phi_1 = 37$ $\chi_{11} = 198$ $\phi_2 = 160$ $\chi_{21} = 307$ $\gamma_1 = 156$ $\chi_{12} = 55$ $\gamma_2 = 196$ $\chi_{22} = 233$ $\omega_1 = 187$ $\chi_{13} = 192$ $\omega_2 = 184$	$\phi_3 = 63$ $\phi_4 = 277$ $\chi_{41} = 179$ $\phi_5 = 297$ $\chi_{51} = 305$ s-s = 167 $\gamma_3 = 254$ $\gamma_4 = 89$ $\chi_{42} = 63$ $\gamma_5 = 314$ $\chi_{52} = 65$ $\omega_3 = 171$ $\omega_4 = 175$ $\omega_5 = 178$ $\chi_{53} = 61$ $\chi_{54} = 307$
DSLET	$\phi_1 = 39$ $\chi_{11} = 182$ $\phi_2 = 171$ $\chi_{21} = 86$ $\gamma_1 = 144$ $\chi_{12} = 70$ $\gamma_2 = 204$ $\chi_{22} = 308$ $\omega_1 = 182$ $\chi_{13} = 185$ $\omega_2 = 188$	$\phi_3 = 68$ $\phi_4 = 279$ $\chi_{41} = 178$ $\phi_5 = 288$ $\chi_{51} = 196$ $\phi_6 = 196$ $\chi_{61} = 267$ $\gamma_3 = 262$ $\gamma_4 = 94$ $\chi_{42} = 64$ $\gamma_5 = 309$ $\chi_{52} = 149$ $\gamma_6 = 327$ $\chi_{62} = 64$ $\omega_3 = 167$ $\omega_4 = 181$ $\omega_5 = 176$ $\chi_{53} = 299$ $\omega_6 = 180$ $\chi_{63} = 51$ $\chi_{54} = 305$
DCDCE	$\phi_1 = 35$ $\chi_{11} = 192$ $\phi_2 = 157$ $\chi_{21} = 288$ $\gamma_1 = 159$ $\chi_{12} = 76$ $\gamma_2 = 209$ $\chi_{22} = 239$ $\omega_1 = 184$ $\chi_{13} = 217$ $\omega_2 = 186$	$\phi_3 = 90$ $\phi_4 = 282$ $\chi_{41} = 182$ $\phi_5 = 66$ $\chi_{51} = 309$ s-s = 176 $\chi_3 = 207$ $\gamma_4 = 80$ $\chi_{42} = 63$ $\gamma_5 = 22$ $\chi_{52} = 278$ $\omega_3 = 172$ $\omega_4 = 198$ $\omega_5 = 172$
DCLCE	$\phi_1 = 35$ $\chi_{11} = 197$ $\phi_2 = 159$ $\chi_{21} = 300$ $\gamma_1 = 158$ $\chi_{12} = 60$ $\gamma_2 = 195$ $\chi_{22} = 235$ $\omega_1 = 185$ $\chi_{13} = 193$ $\omega_2 = 183$	$\phi_3 = 65$ $\phi_4 = 280$ $\chi_{41} = 179$ $\phi_5 = 283$ $\chi_{51} = 299$ s-s = 175 $\gamma_3 = 255$ $\gamma_4 = 95$ $\chi_{42} = 65$ $\gamma_5 = 295$ $\chi_{52} = 308$ $\omega_3 = 172$ $\omega_4 = 183$ $\omega_5 = 179$
DStBuLET	$\phi_1 = 39$ $\chi_{11} = 189$ $\phi_2 = 160$ $\chi_{21} = 163$ $\chi_{24} = 169$ $\gamma_1 = 148$ $\chi_{12} = 76$ $\gamma_2 = 180$ $\chi_{22} = 165$ $\chi_{25} = 299$ $\omega_1 = 180$ $\chi_{13} = 171$ $\omega_2 = 188$ $\chi_{23} = 42$ $\chi_{26} = 307$	$\phi_3 = 73$ $\phi_4 = 285$ $\chi_{41} = 175$ $\phi_5 = 269$ $\chi_{51} = 209$ $\phi_6 = 193$ $\chi_{61} = 267$ $\gamma_3 = 282$ $\gamma_4 = 123$ $\chi_{42} = 64$ $\gamma_5 = 299$ $\chi_{52} = 172$ $\gamma_6 = 328$ $\chi_{62} = 66$ $\omega_3 = 180$ $\omega_4 = 181$ $\chi_{41} = 301$ $\omega_5 = 180$ $\chi_{51} = 52$ $\omega_6 = 180$ $\chi_{63} = 52$ $\chi_{54} = 310$
DStBu-LETtBu	$\phi_1 = 38$ $\chi_{11} = 190$ $\phi_2 = 160$ $\chi_{21} = 163$ $\chi_{24} = 169$ $\gamma_1 = 148$ $\chi_{12} = 76$ $\gamma_2 = 181$ $\chi_{22} = 165$ $\chi_{25} = 300$ $\omega_1 = 180$ $\chi_{13} = 172$ $\omega_2 = 188$ $\chi_{23} = 42$ $\chi_{26} = 307$	$\phi_3 = 73$ $\phi_4 = 285$ $\chi_{41} = 176$ $\phi_5 = 270$ $\chi_{51} = 209$ $\phi_6 = 199$ $\chi_{61} = 186$ $\chi_{65} = 283$ $\gamma_3 = 282$ $\gamma_4 = 122$ $\chi_{42} = 66$ $\gamma_5 = 299$ $\chi_{52} = 172$ $\gamma_6 = 341$ $\chi_{62} = 116$ $\chi_{66} = 59$ $\omega_3 = 169$ $\omega_4 = 180$ $\omega_5 = 183$ $\chi_{53} = 301$ $\omega_6 = 180$ $\chi_{63} = 66$ $\chi_{67} = 71$ $\chi_{54} = 310$ $\chi_{64} = 62$
LCLCE	$\phi_1 = 34$ $\chi_{11} = 303$ $\phi_2 = 227$ $\chi_{21} = 39$ $\gamma_1 = 166$ $\chi_{12} = 276$ $\chi_2 = 146$ $\chi_{22} = 74$ $\omega_1 = 309$ $\chi_{13} = 181$ $\omega_2 = 172$	$\phi_3 = 283$ $\phi_4 = 274$ $\chi_{41} = 181$ $\phi_5 = 284$ $\chi_{51} = 302$ s-s = 111 $\gamma_3 = 291$ $\gamma_4 = 82$ $\chi_{42} = 63$ $\gamma_5 = 310$ $\chi_{52} = 285$ $\omega_3 = 171$ $\omega_4 = 182$ $\omega_5 = 179$

with low energy indicates that the hydrogen bonds formed by the Thr hydroxyl groups are not the primary factors stabilizing the candidate conformation. To further investigate the importance of all hydrogen bonds in maintaining the candidate conformer structure, the candidate conformers for the template peptides DTLET and DPLPE were both reoptimized with the value of the hydrogen bond potential set to 0. Comparing the resulting conformer with that previously obtained with hydrogen bonding for each analog gave an RMS value of 1.4 Å for DTLET and 0.1 Å for DPLPE. DTLET shows a higher degree of conformational change than DPLPE when the hydrogen bond energies are removed, but both compounds essentially retain the candidate conformation.

Conformational basis of selectivity. Previous studies using analogs of the μ-selective peptides, morphiceptin and D-Ala-Met-enkephalin-amide, have identified as candidate conformers for high affinity binding to the μ-receptor site two similar turns: β_{II} and $\beta_{II'}$. In order to compare the candidate δ-binding conformer with these candidate μ-binding conformers, DPLPE was conformed to a $\beta_{II'}$-turn and then energy-minimized to obtain an optimum ring-closed structure. Fig. 6 compares the resulting DPLPE "μ" conformer with the candidate δ-binding DPLPE conformer in relative orientations such that their *N*-terminal Tyr (tyramine) groups are superimposed.

As seen from this figure, when the tyramine groups believed to be important for peptides binding to all opioid receptor types are overlapped, the remainder of the peptide has very different orientations in the two conformations. This difference is consistent with the hypothesis that the μ- and δ-receptors, while both sharing a requirement for an *N*-terminal Tyr are structurally different proteins each with unique conformational requirements for binding.

Conclusions

Two extensive search strategies have been used to identify low energy conformers of 11 linear and cyclic δ-selective peptides. Low energy conformers of DPLPE generated by a systematic angle variation and ring closure strategy for cyclic peptides did not include an appropriate template for a low energy conformer common to the linear peptide, DTLET, and the remaining cyclic analogs. Surprisingly, the alternative buildup search strategy employed to identify the low energy conformers

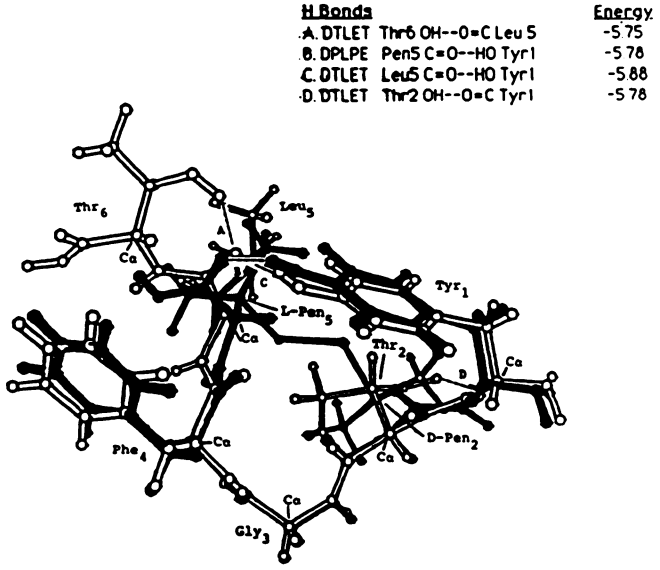


Fig. 4. Relative orientation of DPLPE (*black*) with DTLET (*white*), both in the candidate δ-binding conformer, **5**, with maximum overlap.

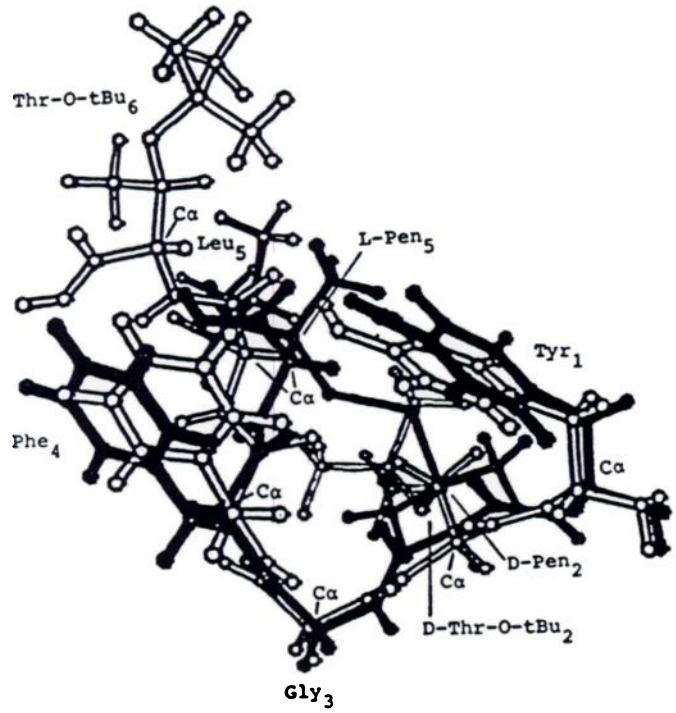


Fig. 5. Relative orientation of DPLPE (*black*) with DStBuLETtBu (*white*), both in the candidate δ-binding conformer, **5**, with maximum overlap.

of a linear peptide (DTLET) did yield a common low energy conformer for both linear and cyclic analogs. This compact conformer allowed a hydrogen bond to form between the carbonyl oxygen of the fifth residue and the hydrogen of the Tyr hydroxyl group. The systematic ring closure search strategy emphasizes backbone geometries that allow ring formation and was done with a fragment which does not include the Tyr residue which is outside of the ring. Therefore, it could not identify a conformer which has a Tyr side chain to backbone hydrogen bond. These results indicate the need for using multiple search strategies in peptide conformational analysis. It is also interesting to note that the conformational restriction

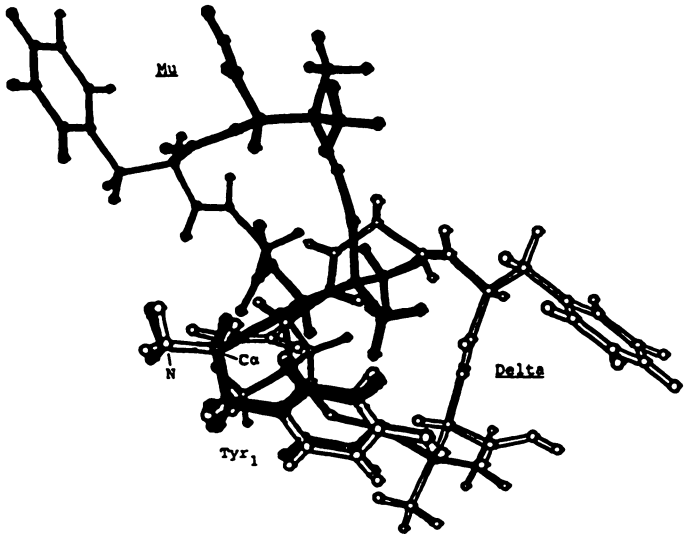


Fig. 6. DPLPE in both the candidate δ-binding conformer, **5** (*white*), and the candidate μ-binding conformer $\beta_{II'}$ (*black*), shown with Tyr overlap.

TABLE 7

Comparison of relative energy and RMS values with δ-receptor binding affinity for cyclic and linear opioid peptides

	ΔE^a	RMS vs. DTLET[b]	MVD IC_{50}[c]	δ IC_{50}[d]	δ K_d[e]
	kcal/mol		*nM*	*nM*	*nM*
DCLCE	0.51	0.54	0.80[f,g]	0.80[f,h]	
DTLET[i]	1.94	0.00	0.15[j]	1.35[j,k]	1.02
DCDCE	1.25	1.33	0.30[f,g]	1.60[f,h]	
DStBuLETtBU	0.00	1.15		4.20[g,l]	
DSLET	0.00	0.69	0.40[j]	4.80[j,k]	1.24
DStBuLET	0.00	1.13	1.07[m]	6.14[k,m]	
DCLPE	2.35	0.50		7.00[n]	1.89
DPLPE[i]	0.00	0.45	2.50[o]	10.00[o]	1.33
DPLCE	0.00	0.40	0.32[p]	12.00[p]	0.80
DPDPE	1.22	1.47	2.20[o]	16.20[o]	13.10
LCLCE	22.88	2.45	950.00[n]		

[a] ΔE values were calculated with respect to lowest energy conformer of each analog.
[b] Residual value of root mean square atom-atom displacement of each analog in candidate conformer **5** with respect to DTLET in candidate conformer **5**.
[c] IC_{50} for inhibition of electrically stimulated contractions in mouse vas deferens.
[d] Inhibition of [^{3}H]DADL binding in rat brain homogenate (except where indicated).
[e] K_d from five-receptor site fit (see Table 2).
[f] Activity was measured for amide analog.
[g] Data from Ref. 9.
[h] Data from Ref. 10 for enkephalenamide; the free acid has never been reported.
[i] Selected as templates due to high affinity and selectivity at δ.
[j] Data from Ref. 5.
[k] K_i for inhibition of [^{3}H]DSLET binding in rat brain homogenate.
[l] Data from B. P. Roques, personal communication.
[m] Data from Ref. 6.
[n] Data from our binding studies.
[o] Data from Ref. 7.
[p] Data from Ref. 8.

attributed to cyclization is not apparent in these peptides. The numbers of unique conformers found with energies within 7.5 kcal/mol of the lowest energy conformer were approximately equal for DTLET and DPLPE, the template linear and cyclic analogs.

These studies unambiguously identify a unique candidate conformer for high affinity δ-receptor binding. It is the lowest energy conformer found for five of the high affinity analogs and is within 2.5 kcal of the lowest energy conformer for the remaining five high affinity analogs. The RMS values used as

a measure of similarity between these analogs and DTLET are all within 1.5 Å, indicating that the candidate conformer does not differ significantly among analogs.

Table 7 compares two conformational parameters of each analog, namely, the relative energy required to achieve the candidate δ-binding conformer and its similarity to the DTLET template as measured by RMS, with relative δ-binding affinities at and activity in the mouse vas deferens tissue assay. Because the high affinity analogs chosen for study all show approximately the same affinity for the δ-receptor, and because consistent values of K_d from detailed binding studies are not available for all of these analogs, it is not possible to make a quantitative correlation between relative affinities and the conformational parameters used to select a candidate conformer. However, a qualitative comparison of the theoretical and experimental results given in this table validates our hypothesis that a candidate conformer for high affinity binding to the opioid δ-receptor could be found. The interesting features of this conformer are its compact structure and its difference from the candidate conformer identified for μ-selective peptides.

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