The amphipathic helix hypothesis for the lipid-associating domains of exchangeable plasma apolipoproteins has been further studied by analysis of the structure of the complexes formed between four synthetic peptide analogs of the amphipathic helix and dimyristoyl phosphatidylcholine (DMPC). Density gradient ultracentrifugation, negative stain electron microscopy, nondenaturing gradient gel electrophoresis, $^1$H NMR, high sensitivity differential scanning calorimetry, and circular dichroism were the techniques used in these studies. The two analygs Asp-Trp-Leu-Lys-Ala-Phe-Tyr-Asp-Lys-Val-Ala-Glu-Lys-Leu-Lys-Glu-Ala-Phe (18A) and 18A-Pro-18A whose sequences most strongly mimic native amphipathic sequences were found also most strongly to mimic apolipoprotein A-I in DMPC complex structure. The covalently linked dimer of the prototype amphipathic analog 18A, 18A-Pro-18A, appears to have greater lipid affinity than 18A. This presumably is the result of the cooperativity provided by two covalently linked lipid-associating domains in 18A-Pro-18A. The studies further suggest that the charge-reversed analog of the prototype 18A, reverse-18A, has the lowest lipid affinity of the four analogs studied and forms only marginally stable discoidal DMPC complexes. We postulate that this low lipid affinity is due predominantly, but not necessarily exclusively, to the lack of a hydrophobic contribution of lysine residues at the polar-nonpolar interface of reverse-18A versus 18A. The intermediate lipid affinity of des-Val$^{10-18}$A, the fourth analog peptide, to produce a rank order of 18A-Pro-18A > 18A > des-Val$^{10-18}$A > reverse-18A, supports this interpretation. Des-Val$^{10-18}$A which has Val deleted from 18A has an amphipathic helical structure partially disrupted by the shift of 2 lysine residues away from the polar-nonpolar interface.

The amphipathic helix has been generally presumed to be the structural form of the lipid-associating domains of the exchangeable apolipoprotein classes A, C, and E from the plasma lipoproteins. The amphipathic helix model defines a general $\alpha$-helical domain containing opposing polar and nonpolar "faces." Furthermore, the polar face contains a specific distribution of the charged residues with the positive occurring along the interface between the polar and nonpolar faces, and the negative along the center of the polar face (Fig. 1A).

The theory has been supported by a reasonable body of experimental evidence (1-15). In previous studies from this laboratory (1, 2), peptide analogs of the amphipathic helix were synthesized and found to mimic the exchangeable apolipoproteins in their interaction with phospholipids. The present study focuses on the structural properties of the complexes formed between dimyristoyl phosphatidylcholine (DMPC) and four systematically different peptide analogs of the amphipathic helix, designated 18A, reverse-18A, des-Val$^{10-18}$A, and 18A-Pro-18A. Peptide 18A, an 18-residue amphipathic helix analog, serves as the prototype peptide against which the other three are compared (Fig. 1A). This peptide, containing additional Ser or Ala extensions at the NH$_2$ and COOH termini, has been studied previously (1, 2). Peptide reverse-18A is an analog of 18A in which all charged residues on the polar face have been reversed (Fig. 1B). Peptide des-Val$^{10-18}$A is an analog of 18A in which a single Val has been deleted from the center of the helix; this deletion produces a rotation of the polar-nonpolar faces at opposite ends by 100° relative to one another. Fig. 1C makes it clear that, while this rotation has markedly altered the characteristics of the resulting helical wheel representation, some amphipathic helical characteristics remain. The nonpolar face is now narrower; 2 of the Lys residues have been displaced away from the polar-nonpolar interface, and 1 Lys has been displaced nearer the interface. Finally, peptide 18A-Pro-18A is an analog consisting of an 18A dimer covalently linked by a proline and was designed to study the effects of multiple amphipathic helical domains on lipid association and apolipoprotein mimicry.

MATERIALS AND METHODS

Synthesis of Peptides—Peptides were synthesized by the solid-phase peptide synthesis procedure, using a benzhydrylamine resin support.

1. The abbreviations used are: DMPC, dimyristoyl phosphatidylcholine; Boc, tert-butyloxycarbonyl; DCC, dicyclohexylcarbodiimide; PC, phosphatidyicholine; $T_m$, transition temperature; EM, electron microscopy; DMF, dimethylformamide; TFA, trifluoroacetic acid; HOBt, 1-hydroxybenzotriazole; TLC, thin layer chromatography; TFE, trifluoroethanol; GC/MS, gas chromatography/mass spectrometry; NBD, 4-nitro-2,1,3-benzoxadiazole; DSC, differential scanning calorimetry; DMSO, dimethyl sulfoxide.

2. Portions of this paper (including part of "Materials and Methods," Tables 1S-111S, Figs. 1S and 2S, and additional references) are presented in miniprint at the end of this paper. In the Miniprint, the following designations are used: $^{37}$P for 18A-Pro-18A, $^{38}$P for reverse-18A, and $^{17}$de$^3$A for des-Val$^{10-18}$A. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, MD 20814. Request Document No. 84M-2934, cite the authors, and include a check or money order for $4.80 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.
Structure of Amphipathic Helix - DMPC Complexes

Fig. 1. Helical wheel representations of the amphipathic helical structure of 18A (A), reverse-18A (18R) (B), and des-Val1°-18A (17desA) (C). The sequences of the four peptides studied are: 18A, Asp-Trp-Leu-Lys-Ala-Phe-Tyr; Asp-Trp-Leu-Ala-Glu-Lys-Leu-Lys-Glu-Ala-Phe; reverse-18A, Lys-Trp-Leu-Asp-Ala-Phe-Tyr; Lys-Ala-Glu-Lys-Leu-Lys-Glu-Ala-Phe; des-Val1°-18A, Asp-Trp-Leu-Ala-Phe-Tyr; and des-Val1°-18A, 18A-Pro-18A, (18A)-Pro-18A.

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Density Gradient Ultracentrifugation — The ability of the prototype amphipathic peptide analog 18A to make a stable complex with DMPC was studied by single vertical ultracentrifugation under conditions routinely used in this laboratory (16). The 18A was mixed with DMPC at a 1:1 weight ratio. A 1.5-ml sample at a density of 1.30 g/ml (KBr) was overlaid with 3.5 ml of normal saline and subjected to a 45-min centrifugation at 65,000 rpm. The sample then was subjected to downward drop fractionation. The results are shown in Fig. 2A. The 18A-DMPC complex bands at an intermediate position between DMPC and 18A alone. Apolipoprotein A-I/DMPC at the same 1:1 weight ratio bands at an identical position to the 18A-DMPC complex (Fig. 2B).

Electron Microscopy — 18A-DMPC and apo-A-I-DMPC complexes at 1:1 weight ratios were subjected to negative stain electron microscopy and, as shown in Fig. 3, form similar-sized protein annulus-bilayer disc structures, at this ratio, of approximately 90-Å diameter and 45-Å thickness.

Detailed EM studies have been performed on the complexes formed between DMPC and the four analog peptides, 18A, 18A-Pro-18A, des-Val1°-18A, and reverse-18A (Fig. 4). Four peptide/DMPC weight ratios were examined (1:1, 1:2.5, 1:5, 1:7.5). At all ratios, 18A and 18A-Pro-18A formed smaller discoidal particles than des-Val1°-18A and reverse-18A, and in general, 18A-Pro-18A formed smaller discs than 18A. At the two higher protein concentrations, des-Val1°-18A appears to make smaller discoidal complexes than reverse-18A, e.g. at 1:1 the des-Val1°-18A/DMPC discs have a mean diameter of 152 ± 25 Å compared to 198 ± 25 Å for reverse-18A.

Nondenaturing Gradient Gel Electrophoresis — When examined by nondenaturing gradient gel electrophoresis (Fig. 5), the 18A and 18A-Pro-18A complexes with DMPC, stained with Coomassie Blue, gave sizes that are generally compatible with those measured from electron micrographs (Table I). This is reasonable evidence that the DMPC complexes formed...
with these two peptides are stable. On the other hand, the des-Val\textsuperscript{18}-18A-DMPC complex barely enters the gel even at the 1:1 ratio and the reverse-18A-DMPC complex fails to enter the gel at any ratio. These results, when compared to the EM studies, are most compatible with a relatively unstable structure for des-Val\textsuperscript{18}-18A/DMPC and a completely unstable one for 18R/DMPC (see “Discussion”).

High Field $^1$H NMR—We previously showed (17) that high field (400 MHz) $^1$H NMR of apolipoprotein A-I-phosphatidylcholine discoidal complexes produces a single $-N(CH_3)_2$ resonance (as compared to a bimodal resonance for sonicated PC vesicles) whose chemical shift is a function of the apo-A-I/PC ratio and thus of the diameter of the discoidal complex. Fig. 6 is a $^1$H-NMR spectrum of 18A/DMPC (1:1 weight ratio), showing a single $-N(CH_3)_2$ resonance. Fig. 7 is a plot of chemical shift versus discoidal diameter (measured by EM) for three separate 18A/DMPC weight ratios (1:1, 1:2.5, and 1:5), for two apo-A-I/egg PC weight ratios (1:1 and 1:2), and for two apo-A-I/DMPC weight ratios (1:2.5 and 1:17.5). We

![Fig. 3](image3.png)

**Fig. 3.** Negative stain electron microscopy of apo-A-I (A) and 18A (B) complexes with DMPC at 1:1 weight ratios.

![Fig. 4](image4.png)

**Fig. 4.** Morphographic analysis of diameters of discoidal complexes of analog peptides with DMPC at different weight ratios. A, 18A-DMPC; B, 18A-Pro-18A-DMPC; C, reverse 18A-DMPC; D, des-Val\textsuperscript{18}-18A-DMPC. The DMPC/peptide weight ratio for each histogram is indicated on the plot. Insets show electron micrographs of select complexes at 138,000 x.

![Table 1](image1.png)

**Table 1**

Size of DMPC-peptide complexes assessed by EM and gradient gel

| DMPC/peptide ratio | Analytical method | Diameter (Å) |
|--------------------|------------------|--------------|
|                    |                  | 18A | 18A-Pro-18A | Des-Val\textsuperscript{18}-18A | Reverse-18A |
| 1                  | EM               | 85 ± 9 | 91 ± 10 | 152 ± 20 | 198 ± 25 |
|                    | GG\textsuperscript{a} | 97 (75-140) | 103 (85-149) | 219 | >225 |
| 2.5                | EM               | 122 ± 20 | 117 ± 13 | 180 ± 34 | 233 ± 24 |
|                    | GG               | 127 (80-180) | 112 (84-154) | 222 | >225 |
| 5                  | EM               | 206 ± 30 | 148 ± 19 | 244 ± 42 | 237 ± 26 |
|                    | GG               | 179 (106-222) | 140 (88-165) | 224 | >225 |
| 7.5                | EM               | 239 ± 36 | 202 ± 29 | 244 ± 37 | 264 ± 45 |
|                    | GG               | 192 (131-222) | 152 (114-177) | 225 | >225 |

\textsuperscript{a} GG, gradient gel.

\textsuperscript{b} Range of diameter.
postulated previously (17) that the chemical shift of discoidal apo-A-I-PC complexes is the result of two different chemical shifts for the choline head groups located at the disc edge and in the disc interior in rapid equilibrium on the NMR time scale. On the right-hand axis of Fig. 7, we have plotted the fraction of the disc PC calculated to be in the disc interior for a given disc diameter (solid line). Even though the scale of the theoretical curve is forced to fit the extremes of the chemical shifts, the general shape of the predicted fit corresponds nicely to the intermediate data points, thus supporting a single two-state fast-exchange model for all three types of complexes, where one state is lipid-associated with the peptide.

Differential Scanning Calorimetry—DMPC complexes at the indicated peptide/lipid weight ratios were studied by DSC for 18A (1:2.5 and 1:5), 18A-Pro-18A (1:5), reverse-18A (1:5), and des-Val^10^-18A (1:5). The DSC transition curves are shown in Fig. 8, and the transition parameters calculated from these curves are listed in Table II. Several trends are apparent. (i) The lipid-phase transition for each complex studied can be deconvoluted into two distinct transitions: one occurring at or slightly below (T_m-1) and one occurring well above (T_m-2) the T_m for pure DMPC multilamellar vesicles (23.6 °C). (ii) When heated to temperatures just above the lipid-phase transition, the h-modal transition appears reversible. This fact, coupled with the lack of a change in the CD spectrum in this temperature region, supports the assumption that these two transitions are due to phase changes in the lipid of the complexes. (iii) There is a clear trend in the half-height width of transition 1 at the 1:5 peptide/lipid ratios as follows: 18A-Pro-18A > 18A > des-Val^10^-18A > reverse-18A. There is considerably less variation in the half-height width of transition 2. (iv) The smaller 18A-DMPC complex (1:2.5 ratio) has a broader transition 1 than the larger 18A-DMPC complex (1.5 ratio). (v) The two smallest complexes (18A at the 1:2.5 ratio and 18A-Pro-18A) have the lowest total enthelpies, 3.1 and 2.6 kcal/mol, respectively, compared to 3.9 ± 0.1 kcal/mol for the three remaining complexes. (The enthalpy for pure DMPC multilamellar vesicles is about 5.5 kcal/mol.)

Circular Dichroism—One of the hallmarks of the interaction of apolipoprotein A-I with phospholipid is an increase in α-helicity as measured by CD. Fig. 9 shows CD spectra of the four amphipathic peptide analogs in the presence (1:2.5, peptide/lipid weight ratio) and absence of DMPC, and in 80% trifluoroethanol (TFE), a helix promoter. Table III lists the per cent α-helicity of each peptide under the three conditions. Peptide 18A shows a doubling in α-helicity, from 15 to 30%, upon DMPC interaction, with an additional increase to 39% in TFE. Peptide 18A-Pro-18A is quite helical in the presence or absence of DMPC (53 and 49%, respectively); there is a decrease in α-helicity in TFE to 41%. Peptide reverse-18A shows no significant α-helicity in either the presence or absence of DMPC. Peptide des-Val^10^-18A shows an increase in α-helicity, from 12 to 20%, upon interaction with DMPC with an additional increase to 33% in TFE.

**DISCUSSION**

The density gradient ultracentrifugation, negative stain electron microscopy, and nondenaturing gradient gel electrophoresis studies indicate that 18A and 18A-Pro-18A produce stable discoidal complexes with DMPC that are similar in density and diameter to equal weight/weight complexes of apolipoprotein A-I-DMPC. However, while gradient gel electrophoresis suggests that the 18A and 18A-Pro-18A complexes with DMPC are heterogeneous in size, there is no evidence for size quantitation as was observed for apolipoprotein A-I-DMPC complexes (17). This is not unexpected since we postulated that the size quantitation of apo-A-I-DMPC complexes requires multiple, cooperative amphipathic helical lipid-associating domains within a single polypeptide chain.

The electron microscopy studies suggest that des-Val^10^-18A and 18R form large discoidal complexes with DMPC. While the correspondence between size as measured by electron microscopy and gradient gel electrophoresis is good for 18A and 18A-Pro-18A complexes, this is not true for des-Val^10^-18A-DMPC complexes at the lower lipid ratios (1:1 and 1:2.5) nor for reverse-18A at the lowest lipid ratio (1:1). Since size measured by gradient gel electrophoresis is greater than size measured by electron microscopy for the latter three complexes, we consider this evidence for relatively unstable structures for des-Val^10^-18A and reverse-18A-DMPC discoidal complexes. Furthermore, since the reverse-18A-DMPC complexes at all ratios stain poorly with Coomassie Blue, we suggest that essentially all of the reverse-18A has dissociated itself from the DMPC on the gel. If this is true, then the reverse-18A-DMPC complex must be the least stable of the four complexes studied.

The 1H NMR data are compatible with a peptide annulus-bilayer disc structure for the 18A-DMPC particles and, by analogy, for the des-Val^10^-18A-DMPC particles. The interpretation of the NMR data is that the peptide annulus produces physical chemical changes in the DMPC molecules at the edge of the bilayer disc (17).

It is also tempting to interpret the DSC data in light of a peptide annulus-bilayer disc edge effect. The assumption in such a model would be that the two distinct phase transitions
Structure of Amphipathic Helix·DMPC Complexes

Fig. 8. Heat capacity profiles of DMPC-analog peptide complexes. The weight ratio is 5:1 where not indicated. Curves are identified as follows: —, raw data; — — —, theoretical curve; — — — —, individual transitions from which the composite theoretical curve is derived. Heat capacity axis is in kcal deg⁻¹ mol⁻¹, and temperature axis is in °C. 17desA, des-Val'0-18A; 37pA, 18A-Pro-18A; 18R, reverse-18A.

Table II

DSC transition parameters for peptide·DMPC complexes

| Transition parameter | 18A       | 37pA, 17desA, 18R, 1:5 |
|----------------------|-----------|------------------------|
| ΔH/T°                | 3.1       | 4.5                    |
| ΔH/1ΔH·T°            | 0.48      | 0.36                   |
| HHW (Tm-1)°          | 10.2 °C   | 7.3 °C                 |
| HHW (Tm-2)°          | 6.6 °C    | 7.3 °C                 |
| Tm-1                 | 21.7 °C   | 23.7 °C                |
| Tm-2                 | 26.7 °C   | 26.1 °C                |

Kilocalories/mole.

Table II

| Transition parameter | 18A (1:5) | 37pA, 17desA, 18R, 1:5 |
|----------------------|-----------|------------------------|
| ΔH/T°                | 3.1       | 4.5                    |
| ΔH/1ΔH·T°            | 0.48      | 0.36                   |
| HHW (Tm-1)°          | 10.2 °C   | 7.3 °C                 |
| HHW (Tm-2)°          | 6.6 °C    | 7.3 °C                 |
| Tm-1                 | 21.7 °C   | 23.7 °C                |
| Tm-2                 | 26.7 °C   | 26.1 °C                |

Ratio of enthalpy of transition 1 to total enthalpy.

Hhw, half-height width.

observed for the peptide·DMPC complexes (Fig. 10A) represent separate disc edge (Tm-2) and disc core (Tm-1) lipid transitions. This model predicts that the core DMPC (associated with transition 1) should have a phase transition temperature very close to that of pure DMPC, which is the case (mean Tm-1 = 22.9 ± 0.8 °C versus 23.6 °C, respectively). A second prediction of the model is that transition 1 should be more cooperative in the larger discoidal complexes. This also is the case as shown by an increase in sharpness of Tm-1 with increasing disc size. At peptide/DMPC ratios of 1:5, the rank order of size is 18A-Pro-18A < 18A < des-Val'0-18A < reverse-18A (Table III) and the rank order of half-height width of transition 1 is 18A-Pro-18A > 18A > des-Val'0-18A > reverse-18A (Table II); 18A·DMPC complexes at two different peptide/lipid ratios show the same trend. A third prediction of the model in Fig. 10A is that the fraction of the total enthalpy represented by transition 1 should vary directly with the disc diameter. As indicated in Table II this is not the case; there is no consistent trend in the fractional enthalpy of transition 1. A modification of the edge-core model of Fig. 10A, in which the peptide associates with the core DMPC with varying concentrations that depend upon the lipid affinity of the peptide and affect the size of the DMPC core (Fig. 10B), could be invoked as an alternative model to explain the DSC data. However, because the smallest two complexes have the lowest total enthalpies, the possibility must be considered that a third zone of lipid should be added to the models in Fig. 10, immediately adjacent to the protein annulus, that represents boundary lipid for which no phase transition occurs (9). In support of the general two-lipid domain model for discoidal complexes schematically illustrated in Figure 10 are the DSC data on glucagon·DMPC complexes reported by Epand and Sturtevant (19). These complexes exhibit both a discoidal
morphology and two distinct calorimetric phase transitions, one near the phase transition of pure DMPC (23 °C) and one above it (26.1 °C). At this time, however, we can not rule out the simplest (although, in our view, least likely) explanation for the low temperature transition, that is, it represents the presence of small amounts of unreacted lipid vesicles in the mixtures examined by DSC, which were not detected by either electron microscopy or gradient gel electrophoresis (as material too large to enter the resolving gel).

The CD studies clearly show that the rank order per cent α-helicity of the peptide in its DMPC complex is 18A-Pro-18A > 18A > des-Val10-18A > reverse-18A, an order reverse to both the complex diameter (18A-Pro-18A < 18A < des-Val10-18A < reverse-18A) and cooperativity of the DSC transition 1 (18A-Pro-18A < 18A < des-Val10-18A < reverse-18A). Consistent with the CD studies is the intrinsic tryptophan fluorescence of the peptides which shows a blue shift for only the 18A-Pro-18A and 18A peptides from the λmax in solution to the DMPC-bound form (data not shown). These results suggest a lipid-associating affinity of the four peptides as follows: 18A-Pro-18A > 18A > des-Val10-18A > reverse-18A.

The results with 18A are similar to those we reported before for the Ser3-18A-Ser3 analog (1). One explanation for the 18-Pro-18A results is that this dimeric amphipathic analog forms a helical hairpin structure with the bend centered about the proline residue such that the two nonpolar faces associate to minimize aqueous contact and thus stabilize α-helical structure. The blue shift of 18A-Pro-18A in solution (358 nm) compared to 18A (361 nm) supports this model.

In conclusion, we present here for the first time a comprehensive study of the structure of the complexes formed between synthetic peptide analogs of the amphipathic helix and DMPC. The two analogs whose sequences most strongly mimic native amphipathic sequences also most strongly mimic apolipoprotein A-I in its interaction with DMPC. Peptide 18A-Pro-18A appears to have greater lipid-associating affinity than 18A, which presumably is the result of the cooperativity provided by two covalently linked lipid-associating domains. The studies further suggest that reverse-18A has the lowest lipid-associating affinity of the four peptides studied. We postulate that this is due predominantly, but not necessarily exclusively, to the lack of hydrophobic contribution of lysine residues at the polar-nonpolar interference of reverse-18A versus 18A. The intermediate lipid-associating affinity of des-Val10-18A, whose amphipathic helix is partially disrupted by the shift of 2 lysine residues away from the polar-nonpolar interface, supports this interpretation.

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Peptide synthesis. Peptides were synthesized by the solid-phase procedure on a Symphony \textsuperscript{\textregistered} Synthesizer (10). Boc-Thr (A2)-conjugate was loaded on the resin according to the manufacturer's instructions, and the peptides were prepared using the Fmoc method as previously described (Fig. 1A). The final peptides were purified by high-performance liquid chromatography (HPLC) on a C\textsubscript{18} silica column (5 mm, 4.6 mm, 150 mm) using a linear gradient of aqueous acetonitrile solution at a flow rate of 1 ml/min. Fractions containing the desired peptides were collected, concentrated under reduced pressure, and subjected to amino acid analysis. The purity of the final peptides was confirmed by HPLC and mass spectrometry.

Peptides containing sarcosine acid in the sequence are known to undergo side reactions both to acidic and basic conditions which are commonly used to remove the protecting groups from sarcosine acid-containing peptides and in the solid-phase procedures during removal of peptide from the resin (11). Acidic reagents also cause the destruction of tryptophan. For these reasons we decided to use transfer-hydrogenation technique to release the peptide from the resin and simultaneously to remove the protecting groups. Even transfer hydrogenation using 10\% formic acid as the hydrogen donor is known to reduce the intact ring of tryptophan if left unprotected (6). We have established that in DMF and with 5\% of the final concentration of formic acid this side reaction is minimal (Fig. 1B). Transfer hydrogenation is known to be a mild procedure (12). We have also established that this procedure, all the protecting groups have been removed (Fig. 2).

Preparation of peptide-DMPC complexes. DMPC (Avanti Polar Lipids, Inc., Birmingham, AL) (1:7.2 mol/mol phospholipid) was dried by overnight lyophilization, resuspended in phosphate buffer (10 mm in 150 mm NaCl, pH 7.4) and vortexed to give micellar solutions. Peptide solutions (concentrations determined by Assay II) in the same buffer were added to the micellar vesicle suspension to achieve the desired peptide/DMPC weight ratio. In the case of peptides TMA and TMA, the turbid DMPC suspension clarified within seconds to produce solutions containing peptide-DMPC complexes.

CD measurements. Prepared peptide-DMPC complexes were stirred with 3\% (v/v) phosphate buffer, pH 7.4, and examined with a Philips PU 4400 microscope on carbon-coated, covered grids.

Results. Peptide agonists for the human rhodopsin receptor mediated by the 7-transmembrane domain (7TM) of the receptor were identified in a high-throughput screen of a large peptide library (8). The peptide agonists were found to stimulate the 7TM domain of the receptor by activating the G\textsubscript{q} protein, leading to the production of cyclic AMP (9). The G\textsubscript{q} protein is a member of the family of membrane-bound G-proteins that are involved in signal transduction pathways in cells (10). The G\textsubscript{q} protein is activated by the binding of a GTP analogue to the effector domain of the receptor, leading to the production of cyclic AMP (11). The cyclic AMP is a second messenger that activates a number of downstream effectors, including protein kinases and cytoplasmic kinases (12). The cyclic AMP is hydrolyzed by a phosphodiesterase enzyme, leading to the conclusion that the cyclic AMP signal is short-lived (13).

Circular Dichroism. The peptides and peptide-lipid complexes were placed in 10 mm quartz cuvettes and placed in a circular dichroism spectrometer (14). The CD spectra were measured at a wavelength range of 200-260 nm, with a scan rate of 20 nm/minute, and a bandwidth of 1 nm. The CD spectra were normalized to the peptide concentration. The peptide-lipid complexes were prepared by incubating the peptides with the lipids at a 1:10 molar ratio for 24 hours at room temperature. The CD spectra were obtained at 25°C, with a scan rate of 10 nm/minute, and a bandwidth of 1 nm. The CD spectra were normalized to the peptide concentration.

Biochemistry. The procedures and reagents used were in accordance with the methods described in the literature (15). The CD spectra were obtained at 25°C, with a scan rate of 10 nm/minute, and a bandwidth of 1 nm. The CD spectra were normalized to the peptide concentration.
Table 1. Peptide Synthesis Schedule

| STEP | REAGENTS | NO. OF CYCLES | VOLUME | TIME (MIN) |
|------|-----------|---------------|--------|------------|
| 1    | CH<sub>2</sub>C<sub>Cl</sub> | 1             | 30 ml  | 5          |
| 2    | 40 % TFA in CH<sub>2</sub>C<sub>Cl</sub> (10 % w/v triethylamine) | 1 | 30 ml | 25        |
| 3    | 5 % OEA in CH<sub>2</sub>C<sub>Cl</sub> | 2 | 30 ml | 2         |
| 4    | CH<sub>2</sub>C<sub>Cl</sub> | 2             | 30 ml  | 2         |
| 5    | 40 % TFA in CH<sub>2</sub>C<sub>Cl</sub> | 2 | 30 ml | 2         |
| 6    | TFA in CH<sub>2</sub>C<sub>Cl</sub> | 2 | 30 ml | 2         |
| 7    | 5 % OEA in CH<sub>2</sub>C<sub>Cl</sub> | 2 | 30 ml | 2         |

Table II

| PEPTIDE | Asp | Glu | Lys | Val | Tyr | Leu | Phe | Ala | Pro |
|---------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| MA      | 1.8 | 2.1 | 3.7 | 1.0 | 0.9 | 2.2 | 2.2 | 3.0 |     |
| MR      | 1.7 | 1.9 | 3.8 | 1.0 | 0.8 | 2.1 | 2.1 | 3.0 |     |
| 17MA    | 1.9 | 2.0 | 3.3 |     | 0.9 | 2.1 | 1.9 | 3.0 |     |
| 38MA    | 3.7 | 3.8 | 7.4 | 1.1 | 0.9 | 3.9 | 3.7 | 5.8 | 1.0 |

Table III*

| NMMH (%) | A | B | C |
|-----------|---|---|---|
| 90        | 45| 0.0| 0.0|
| 90       to NMMH | 90.6| 1.2| 0.2|
| 90       to NMMH | 99.6| 0.4|

*Ac-Trp-OH was subjected to transfer hydrogenation using freshly prepared Pd-black for 24 hr. at 25°C. The reaction mixture was subjected to GC/MS analysis. A: Ac-Trp-OH; B: Ac-hydroxy-Trp-OH; C: Ac-(in)dehydro-Trp-OH.

Figure 1. High performance liquid chromatography (HPLC) purification of synthetic peptides. The purification of the crude mixture from preparative HPLC was performed with a C<sub>18</sub> reverse phase column. The final purification of the purified product was performed on a C<sub>18</sub> reverse phase column. A: crude peptide mixture; B: purified peptide mixture.

Figure 2. <sup>1</sup>H NMR spectra (300 MHz) of MA in DMSO.

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