

# Creation and Investigation of Protein-Core Mimetics with Parallel and Antiparallel Aligned Amino Acids

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Mimetic protein cores were created that align a set of L-Phe, D-Phe, or L-Leu residues in a parallel or an antiparallel arrangement in chloroform. Not all cores show a single conformation at room temperature. Stable structures require a synergistic relationship between the H-bonding groups and the residues within the core. The spatial arrangement of the side chains dictates whether a zippered or a crossed pattern of H-bonds is observed for these cores. Variable-temperature <sup>1</sup>H NMR experiments were used to determine the strengths of the H-bonds. The existence of H-bonds was verified through FTIR spectroscopic analysis. Large temperature coefficients exist for some protons of aromatic rings that are held in a T-shaped arrangement. A comparison of these temperature coefficients shows that a more stable core is obtained by combining benzenoid and nitrobenzenoid rings as compared to benzenoid rings. Structures were determined using a combination of 2D NMR analysis and molecular modeling.

## Introduction

Creating compounds with protein-like secondary structures remains a challenging endeavor. Impressive results have been obtained using nonnatural oligomers that fold into well-defined three-dimensional structures referred to as foldamers<sup>1</sup> and  $\beta$ -turn mimetics<sup>2</sup> that align peptide or peptidomimetic chains to form  $\beta$ -sheet structures. Our approach in creating compounds with protein-like secondary structures is to use an aromatic or aliphatic assembly to stabilize the interactions between amino acids or peptides. These protein-core mimetics (PCMs) were inspired by Kelly's isoquinoline-based  $\beta$ -sheet mimetics.<sup>3</sup> In this system, hydrophobic side chains fold back onto the isoquinoline ring, providing additional stabilization energy for  $\beta$ -sheet formation. We postulated that a more stabilized core would be obtained by using a synthetic scaffold to align aromatic rings in a T-shaped arrangement (Figure 1). Formation of the core would theoretically not require a large entropic penalty for folding. A T-shaped (or edge to face) arrangement of aromatic rings has been observed in the X-ray structures

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**FIGURE 1.** The number of aromatic rings held in a T-shaped arrangement determines whether a zippered or a crossed pattern of H-bonds is formed. A nonsynergistic relationship between the core and H-bonding residues, however, results in multiple structures.

of aromatic compounds,  $^4$  observed in proteins,  $^5$  studied using model systems,  $^6$  and investigated through theoretical calculations.  $^{6\mathrm{e},7}$ 

The design of the PCM is based on the small hydrophobic cores (typically containing Tyr, Phe, and Leu) found in the zinc finger peptides, which are often used as a model system for protein stability studies. Although small, these cores provide for a substantial amount of structural stability. Imperiali showed that modified fingers without a metal binding site can give similar structures as the native finger.<sup>8</sup> Mutating the highly conserved Phe of zinc fingers to Leu in a model of the Xfin-31 finger peptide<sup>9</sup> and in a model finger of the human Y-encoded protein ZFY<sup>10</sup> reduces their stability. Interestingly, in the latter study, the instability is caused by an increase in the finger's dynamics.

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**FIGURE 2.** (A) The original PCM that displays interactions between both aromatic side chains and one of the scaffold's aromatic rings and two isoenergetic H-bonds. (B) The new PCM also contains a scaffold and H-bonding plane, but its internal amines are not methylated.

In the initial study of our PCMs,<sup>11</sup> we also observed the importance of aromatic rings in the formation of core structures. Aromatic rings provide for more structure and H-bonds between Phe residues, when positioned in a T-shaped arrangement, as compared to scaffolds linked to Leu or to a scaffold without an aromatic core. The internal amides of these compounds were methylated (Figure 2A) to ensure that a 7-membered H-bonded ring would not form between one of these amides and a carbonyl group. We were concerned that this H-bonded ring would control structure formation and not the interactions between aromatic or aliphatic groups. In this report, we describe the properties of PCMs without these Me groups. We wanted to determine whether a set of core residues could work synergistically with H-bonding residues to form a single stable structure. Nitrated compounds were created and investigated to determine whether a change in the electrostatic forces of a core could enhance the strength of aromatic-aromatic interactions. We found that the nature of the side chain and its chirality determine core structures with either a zippered or crossed pattern of H-bonded rings (Figure 1). In some cases, however, strong aromatic-aromatic interactions can work against the H-bonds, resulting in multiple structures. Investigation of these flexible PCMs will lead to a better understanding of the dynamic instability observed in some peptides, such as in the mutated zinc finger peptides.

#### Results

**Physical Properties of Mimetics with Parallel Aligned Amino Acids.** Attaching L-Phe, D-Phe, or L-Leu to an aromatic scaffold gives a series of PCMs that align the amino acids in a parallel fashion (Figure 2B). Parallel means that both amino acids are attached to the scaffold through their amino terminus. The physical properties of the compounds were investigated using 1D NMR analysis to determine H-bond stabilities and 2D NMR analysis for structural information. To verify the existence of H-bonds, the FTIR spectrum of each compound was examined for a H-bonded N–H stretching band,<sup>12</sup> which is generally observed between 3300 and 3360 cm<sup>-1</sup>. A free N–H band is observed between 3410 and 3450 cm<sup>-1</sup>.

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**FIGURE 3.** An overlay of the <sup>1</sup>H NMR spectra of (L,D)-Phe<sub>parallel</sub> and (L,L)-Phe<sub>parallel</sub>, showing that a correct match ing of core with H-bonding residues will lead to a stable structure.

The first compound constructed contains one L-Phe and one D-Phe. This combination of amino acids, when methylated and attached to the original scaffold (Figure 2A), produces a stable, unique PCM that displays two stable isoenergetic H-bonds. Both aromatic side chains make contact with the scaffold's aromatic ring. The addition of one L-and one D-Phe to the new scaffold gave diastereomers, which were separated by HPLC. 2D NMR analysis of one diastereomer revealed the predicted multiple NOE cross-peaks between the scaffold's aromatic ring and the aromatic side chains and a cross-peak between the protons of the external Me groups. The FTIR spectrum of the (L,D)-Pheparallel compound contains absorbance bands that are consistent with H-bonded N-H groups (data not shown). This compound, however, exists as multiple conformers at room temperature (Figure 3), confirming our concerns that the formation of a 7-membered H-bonded ring would disrupt structure formation. H-bond stability was not determined because multiple conformations exist from room temperature to 240 K.

In an attempt to obtain stable and thus useful PCMs, only L-amino acids were attached to the scaffold. The (L,L)-Phe<sub>parallel</sub> compound shows a single conformation on the NMR time scale from 240 to 300 K (300 K spectrum is shown in Figure 3). Plotting the chemical shifts of the amide protons against the temperature of the experiments produced straight lines. Temperature coefficients (TCs) derived from the slope of these lines provide a measure of H-bond stability in CDCl<sub>3</sub>.<sup>12a,b,f,13</sup> According to the literature, two N-H's of (L,L)-Phe<sub>parallel</sub> (TC = -1.3and -2.2 ppb/K, Figure 4) would be considered as being either ring-locked (H-bonds in cyclic peptides) or sheltered from the solvent. The other two coefficients of -3.3and -3.7 ppb/K are in the range found for non-H-bonded N-H's. Supporting evidence for the existence of H-bonds is the observation of an intense H-bonded N-H band in the FTIR spectrum of (L,L)-Phe<sub>parallel</sub> (Figure 5). N-Ac-Phe-CONHMe, dissolved in CHCl<sub>3</sub> to give the same concentration of 3 mM, shows only a single non-H-bonded N-H band.



**FIGURE 4.** Schematic drawings of the compounds showing key NOE cross-peaks (double-headed arrows). Temperature coefficients (standard deviations are less than  $\pm 0.1$ ) for amide N–H and aromatic Ar–H protons are given. Highlighted in (L,L)-PheNO<sub>2parallel</sub> are the observed diastereomeric differences.

Analysis of NOESY and ROESY spectra of (L,L)-Pheparallel revealed key NOE cross-peaks: two between one aromatic side chain and the scaffold's aromatic ring and one between the methyl groups of the external amides. The chemical shift of the ortho proton of the scaffold that is correlated to the side chain is shielded  $(\delta_{Ar-H} = 5.48 \text{ ppm})$  and greatly affected by temperature (TC = 5.7 ppb/K). These results are consistent with a T-shaped arrangement of aromatic rings. As the solution was cooled, the population of compounds with a scaffold proton in the shielding cone of the side chain's aromatic ring increased. To further demonstrate the importance of the aromatic-aromatic interactions for structure, compound 10 (Figure 4; for formation see Supporting Information), which does not have an aromatic ring, was constructed and investigated. In chloroform, a single H-bonded ring possibly forms (TC = -1.9 ppb/K), and the other N-H's are not H-bonded. Only a few NOE cross-peaks exist in its ROESY and NOESY spectra and none occur between the amino acid protons. Although a single conformer exists, this scaffold does not promote an alignment of the amino acids or extend the H-bonded network.

Although these findings suggest that strong aromaticaromatic interactions in (L,L)-Phe<sub>parallel</sub> contribute to structural stability, another possibility is that the aromatic side chain is in a T-shaped arrangement because of restricted conformational freedom. In this case, stability would be a result of steric constraints. To explore this possibility, the electronic property of the scaffold was changed by the addition of a nitro group. Favorable aromatic–aromatic interactions should increase<sup>7b</sup> with

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**FIGURE 5.** FTIR spectral data from the N–H stretching region of the PCMs and *N*-Ac-phenylalanine methyl amide. All samples were 3 mM in CHCl<sub>3</sub> at 298 K. Each spectrum had its baseline corrected, and the absorbance of  $CHCl_3$  was subtracted.

(L,L)-PheNO<sub>2parallel</sub>. The nitro group pulls electron density out of the ring, making its Ar-H more acidic and attracted to the electron-rich face of a stacked aromatic ring. (L,L)-PheNO<sub>2parallel</sub> was isolated and investigated as an approximately 50:50 diastereomeric mixture. Each diastereomer exists as a single conformer on the NMR time scale from 240 to 300 K. Both external amides are strongly H-bonded (TC = -6.2 and -6.6 ppb/K, Figure 4). One internal amide forms a moderately stable H-bond, and the other internal amide is ring-locked or shielded (TC = -4.6 and -1.0 ppb/K, respectively). As seen with the (L,L)-Phe<sub>parallel</sub> compound, one Ar-H of the scaffold is significantly shielded ( $\delta_{Ar-H} = 5.62$  ppm) at room temperature. The chemical shift of this proton, however, is more temperature-sensitive (TC = 7.1 ppb/K) than the one observed for the (L,L)-Phe<sub>parallel</sub> compound (TC = 5.7ppb/K;  $\Delta TC = 1.4$  ppb/K). This larger temperature coefficient suggests that the nitrated benzenoid ring forms a more favorable aromatic-aromatic interaction than the benzenoid ring. Further evidence for the existence of more favorable aromatic-aromatic interactions was the detection of other temperature-sensitive chemical shifts for more than one scaffold Ar-H (TC = 2.6, 1.4, and 0.5 ppb/K) and NOE cross-peaks between the scaffold's aromatic ring and both aromatic side chains. Another different feature observed for (L,L)-PheNO<sub>2parallel</sub> as compared to (L,L)-Phe<sub>parallel</sub> is the existence of an NOE cross-peak between each external Me group and the  $\alpha$ -proton of the opposite amino acid (Figure 4).

To further demonstrate the importance of aromaticaromatic interactions, the properties of the compounds that contain phenylalanine residues are compared to a compound that contains leucine residues. In our previous study,<sup>11</sup> we found that aromatic-aromatic interactions provided for a different structure and H-bond stability of PCMs when compared to aromatic-aliphatic interactions. (L,L)-Leu<sub>parallel</sub> (Figure 4) shows a single conformer on the NMR time scale from 240 to 300 K. Three strong H-bonds exist (TC = -8.0, -6.4, and -5.1 ppb/K), and the fourth N-H is not H-bonded (TC = -2.9 ppb/K). The properties of (L,L)-Leu<sub>parallel</sub> are remarkably similar to the properties observed for (L,L)-PheNO<sub>2parallel</sub>. Both compounds show NOE cross-peaks between the external Me groups and between each external Me group and the  $\alpha$ -C–H of the opposite amino acid. One major difference is that only one side chain of (L,L)-Leu\_{parallel} is positioned close to the scaffold's aromatic ring. There are also differences in the stability of the H-bonded rings. (L,L)-Leu\_{parallel} contains a very stable 7-membered H-bonded ring (TC = -8.0 ppb/K), and a close to zero temperature coefficient is not observed.

**Physical Properties of Mimetics with Antiparal**lel Aligned Amino Acids. PCMs with antiparallel aligned L-Phe or D-Phe were constructed (Figure 6) to further test the ability of core interactions to control structure. Antiparallel means that one phenylalanine is attached through its carboxylate and the other is attached through its amine. The N-H's are more directly aligned with the carbonyl oxygen atom of the opposite amino acid, which could have produced more stable H-bonded rings as compared to the parallel compounds. Separation of diastereomers 6a and 6b (for formation see Supporting Information) led to two diastereomeric sets called 1 and 2 (Figure 7). The addition of a L-Phe or D-Phe to each diastereomer produced four antiparallel aligned PCMs, which will be referred to as (L,L)1-Phe<sub>antiparallel</sub>, (L,D)1-Phe<sub>antiparallel</sub>, (L,L)2-Phe<sub>antiparallel</sub>, and (L,D)2-Phe<sub>antiparallel</sub>.

One compound from each diastereomeric set (1 and 2) shows a single conformation on the NMR time scale from 240 to 300 K. The other two compounds display multiple conformations. For the structurally stable (L,L)-Phe<sub>antiparallel</sub> compound (Figure 6), a stable H-bond and a very stable H-bond exist for one external N-H (TC = -6.3 ppb/K) and the internal N-H of the other phenylalanine residue (TC = -7.6 ppb/K), respectively. For the structurally stable (L,D)-Phe<sub>antiparallel</sub> compound, both external amides are involved in moderately stable H-bonds (TC = -4.5 and -4.3 ppb/K) and one internal N–H forms a very stable H-bond (TC = -7.0 ppb/K). For both compounds, the chemical shift of one internal N-H is temperature-insensitive, which suggests that it was shielded or existed in a ring-locked H-bond. The FTIR spectra for the anitparallel compounds (Figure 6) are



**FIGURE 6.** Key NOE cross-peaks (double-headed arrows), temperature coefficients for amide N-H and aromatic Ar-H protons, and FTIR spectral data from the N-H stretching region are given for the antiparallel compounds.



**FIGURE 7.** The diastereomeric sets of the antiparallel compounds, a cartoon depiction of their calculated stable structures highlighting the orientation of the aromatic rings (side chain rings are given as rectangles), and whether a single conformer is observed.

significantly different than those observed for the parallel compounds. Multiple peaks are observed for these compounds at a lower frequency than normally found for H-bonded amides (low-frequency bands have been observed for  $\beta$ -sheet mimetics).<sup>12d</sup> The same IR cell was

used for all spectra shown in Figures 5 and 6, and the maximum absorbances were within 0.07-0.1 absorbance units. Therefore, the low-frequency bands observed for the antiparallel cores are not an artifact of the IR cell or the experiment. Multiple NOE cross-peaks between both



**FIGURE 8.** A zippered (dashed double-headed arrow) or a crossed (solid double-headed arrow) pattern of H-bonds exists between the amino acids of the parallel aligned compounds and the antiparallel aligned compounds. The numbers indicate the length of a H-bonded ring. R is a set of L-Phe, D-Phe, or L-Leu and X is H or NO<sub>2</sub>.

aromatic side chains and the scaffold's aromatic ring are observed for both compounds. For (L,D)-Phe<sub>antiparallel</sub>, a unique cross-peak exists between its  $\alpha$ -protons and no cross-peak is found between its external Me groups. For (L,L)-Phe<sub>antiparallel</sub>, a NOE cross-peak is observed between the external Me groups. As seen with the parallel compounds, the spatial arrangement of the aromatic side chains of the antiparallel compounds has a pronounced affect on their structures.

### Discussion

The experimental results show that not all PCMs have a single conformation in chloroform. The structural stable compounds show a different number of core interactions and H-bonding patterns depending on whether they contained phenylalanine residues, leucine residues, or a substituted aromatic ring. To understand the synergistic relationship between the interactions, a Monte Carlo method was used to search the conformational space of the compounds (MMFF94 force field, package procedure of SpartanPro).<sup>14</sup> A series of low-energy conformers were obtained that contained a zippered or a crossed pattern of H-bonds (Figures 1 and 8). One or the other pattern is consistent with the observed properties of the PCMs. For the parallel compounds, the zippered pattern contains 7- and 12-membered H-bonded rings, and the crossed pattern contains one 7-membered and two 10-membered H-bonded rings. For the antiparallel compounds, the zippered pattern contains 8- and 12-membered H-bonded rings, and the crossed pattern contains 8-, 11-, and 12membered H-bonded rings. For the antiparallel compounds, the length of each H-bonded ring was considered independent of the other rings. Both patterns of H-bonds require the internal carbonyls to form an up-down pattern.

(14) Wavefunction, Inc.: Irvine, CA.

The necessity of this pattern explains the lack of an observed single conformer for (L,D)-Phe<sub>parallel</sub> (Figure 3). One low-energy conformer observed in the model study shows a zippered pattern of H-bonds. As seen in this structure (Figure 9), one aromatic ring forms a T-shaped arrangement with the scaffold's aromatic ring and the other aromatic ring is positioned to also interact with the core. For one conformer, we observed NOE cross-peaks between the scaffold aromatic ring and an aromatic side chain and the existence of a shielded scaffold aromatic proton (5.82 ppm, Figure 3), which are consistent with T-shaped aromatic rings. We speculate that the formation of the second T-shaped arrangement gives the observed multiple structures. According to the molecular modeling results, when the second aromatic side chain forms a T-shaped arrangement with the scaffold's aromatic ring, its internal carbonyl shifts up and away from the face of this aromatic ring. Both internal carbonyls are now positioned up, and the 7-membered H-bonded ring breaks. Apparently, the stabilizing energies provided by the 7-membered H-bonded ring and the second T-shaped aromatic interaction are similar. Because these two interactions promote different structures, a nonsynergistic relationship exists for structure formation.

The position of the amino acid side chains relative to the scaffold's aromatic ring dictates which pattern of H-bonds forms. This statement is readily evident when comparing the properties of (L,L)-Phe<sub>parallel</sub> to (L,L)-PheNO<sub>2parallel</sub>. In the latter compound, both aromatic side chains are positioned close to the scaffold's aromatic ring. According to the molecular modeling studies, when the second aromatic ring is placed near the scaffold's ring the external amide of this amino acid rotates toward the internal carbonyls (Figure 9). This rotation gives (L,L)-PheNO<sub>2parallel</sub> a crossed pattern of H-bonds, which is consistent with its three stable H-bonds and the NOE cross-peaks between each external methyl group to the opposite amino acid's  $\alpha$ -proton. The lack of this crosspeak and the existence of an NOE cross-peak between the external methyl groups suggests that the (L,L)-Phe<sub>parallel</sub> compound forms a zippered pattern. Although the temperature coefficients of the possible H-bonded N-H's observed for (L,L)-Phe<sub>parallel</sub> are surprisingly close to zero, the observation of a strong H-bonded N-H stretching band in the FTIR spectrum supports the existence of a H-bond. Apparently, the 7- and 12membered H-bonded rings are the preferred pattern when one set of benzenoid rings interacts. The less stable crossed pattern is forced to form when both aromatic side chains interact with the scaffold's aromatic ring. This result shows that nitrobenzenoid-benzeniod interactions are more stabilizing than benzenzoid-benzenoid interactions. The enhanced stability is most likely a result of a more stable H-bond<sup>15</sup> or a greater  $\pi - \sigma$  interaction<sup>16</sup> and consistent with the results obtained by Sherrill,7b who performed a computation analysis of the interaction energies between substituted aromatic rings. Any possible steric hindrance imposed by the NO<sub>2</sub> group would have only lessened the favorable aromatic interaction energies.

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**FIGURE 9.** Low-energy structures obtained from molecular modeling studies that are consistent with the observed properties of the compounds. For (L,D)-Phe<sub>parallel</sub>, moving the second aromatic side chain to interact with the scaffold's aromatic ring forces the internal carbonyl to rotate up (indicated by arrows), which breaks the 7-membered H-bonded ring. The  $\alpha$ -proton is shown to indicate that the aromatic interactions do not require bond rotation around the amide bond. For (L,L)-PheNO<sub>2parallel</sub> as compared to (L,L)-Phe<sub>parallel</sub>, the more favorable aromatic interactions drives the second aromatic side chain to the scaffolds ring, which rotates the amide bond (indicated by arrows), giving the crossed pattern of H-bonds. This phenomenon also occurs for (L,D)2-Phe<sub>antiparallel</sub>. T indicates a T-shaped arrangement of rings, and H-atoms not involved in H-bonds are removed for clarity.

Differences in aromatic-aromatic interactions as compared to aromatic-aliphatic interactions are readily apparent when comparing the properties of (L,L)-Phe<sub>parallel</sub> and (L,L)-Leuparallel. As predicted, there is a closer association of the aromatic core residues as evident by the greater number of NOE cross-peaks for (L,L)-Phe<sub>parallel</sub> as compared to (L,L)-Leu<sub>parallel</sub>. The patterns of H-bonds are also different. Surprisingly, the structures and properties of (L,L)-Leuparallel are very similar to those of (L,L)-PheNO<sub>2parallel</sub>. Both compounds display the required three stable H-bonds and the key NOE cross-peaks between each external methyl group to the opposite amino acid's  $\alpha$ -proton. Does this mean that benzenoid-aliphatic interactions are more stable than the interactions between benzenoid rings and equivalent in energy to interactions between nitrobenzenoid-benzenoid rings? The answer is probably no. Only a single NOE cross-peak is observed between one isobutyl side chain and the scaffold's aromatic ring for (L,L)-Leu<sub>parallel</sub>, which suggests that extensive aliphatic-aromatic interactions do not occur. Most likely, the very stable 7-membered H-bonded ring of (L,L)- $Leu_{parallel}$  forced the crossed pattern of H-bonds. The N-H in this ring has a temperature coefficient (-8.0 ppb/K) that is substantially smaller than the ones observed for the other parallel compounds and even smaller than the ones observed for the antiparallel compounds. Additionally, steric interactions between the side chains most likely kept them from both residing above the H-bonding plane.

To determine whether the observed structures of the PCMs with antiparallel aligned amino acids depend on the spatial arrangement of the side chains, we had to assign the stable diastereomers. One diastereomer contains two L-Phe and the other contains one L-Phe and one D-Phe. According to the synthetic route, all compounds contain a L-Phe that is attached to the scaffold through its amine.

Because these diastereomers were separated before the attachment of the second amino acid, we know that a compound belongs to one of the two sets of diastereomers, which are called set 1 and 2 (Figure 7). For example, one compound containing two (L)-Phe's could either be (L,L)1-Phe<sub>antiparallel</sub> or (L,L)2-Phe<sub>antiparallel</sub>. Results obtained from the 2D NMR experiments and molecular modeling stud-

ies were used to assign the conformationally stable antiparallel compounds. Molecular modeling results show that a stable zippered pattern of H-bonds should be observed for one diastereomer of the (L,L)1-Pheantiparallel and (L,L)2-Phe<sub>antiparallel</sub> pair with both aromatic side chains in contact with the scaffold's aromatic ring (Figure 9 and diastereomer A of Figure 7). The other compound (diastereomer C) would have had both side chains above the H-bonding plane. (L,L)1-Phe<sub>antiparallel</sub> displays a zippered pattern and NOE cross-peaks between both aromatic side chains and the scaffold's aromatic ring and thus is assigned as the stable diastereomer A. The assignment of (L,L)1-Phe<sub>antiparallel</sub> removes the possibility that (L,D)1-Pheantiparallel is a stable PCM because only one compound of this diastereomic set displays a single conformer. Thus, the other stable diastereomer has to be the other compound that contains one L-Phe and one D-Phe, which we call (L,D)2-Pheantiparallel (diastereomer D). For this compound, we observe multiple NOE cross-peaks between both aromatic side chains and the scaffold's aromatic ring, a cross-peak between the  $\alpha$ -protons, and no crosspeak between the external Me groups. A low-energy structure consistent with these results was obtained in a molecular modeling study (Figure 9) by constraining the aromatic rings at a distance observed for T-shaped aromatic interactions (5 Å).<sup>5b</sup> This structure nicely shows both external amides being H-bonded, a close proximity of the  $\alpha$ -protons, and far-separated external Me groups. The modeling results also demonstrates that when the second aromatic ring is positioned near the scaffold's aromatic ring, its external carbonyl rotates back toward the scaffold, giving the crossed pattern of H-bonds. This phenomenon also occurs for (L,L)-PheNO<sub>2parallel</sub>.

One consistent pattern observed for the Phe-containing compounds is that at least one aromatic side chain has to interact with the scaffold's aromatic ring to obtain a single, stable structure. In the cases of (L,L)-PheNO<sub>2parallel</sub> and (L,D)-Phe2antiparallel, the second aromatic side chain also makes contact with the scaffold's aromatic ring, resulting in a crossed pattern of H-bonds. In the case of (L,L)-Phe1<sub>antiparallel</sub>, both aromatic side chains interact with the scaffold's aromatic ring, but a zippered pattern is formed. The lack of a single conformer for (L,D)-Phe<sub>parallel</sub> (Figure 3) demonstrates that a synergistic relationship will not always occur. Another nonsynergistic relationship appears to occur for (L,D)-Phe1<sub>antiparallel</sub> (diastereomer B). Its instability was unexpected. A comparison of the cartoon structures of diastereomer B with D as drawn in Figure 7 suggests that both compounds should have had a stable structure with one pair of interacting rings. Molecular modeling results bolstered that expectation by showing both diastereomers existing in a stable zippered pattern of H-bonds with one T-shaped, aromatic arrangement. Although both aromatic side chains of diastereomer D interact with the scaffold's aromatic ring, a stable structure is formed. Most likely, two sets of T-shaped arrangements of rings is formed for diastereomer B as well. Unlike with diastereomer D, molecular modeling results show that favorable H-bonds are disrupted when the second aromatic side chain is placed near the scaffold's aromatic ring of diastereomer B. One major difference between diastereomers B and D is the nature of the H-bonding atoms in the 7-membered ring for the amino acid side chain that is drawn close to the scaffold in Figure 7. For diastereomer D, its carbonyl is involved in the 7-membered ring. For diastereomer B, its N-H would be involved in the 7-membered ring. Apparently, this subtle difference is enough to disrupt a synergistic relationship between the core and H-bonding residues for diastereomer B.

## Conclusion

A series of protein-core mimetics were constructed to investigate the synergistic relationship between an assemblage of aliphatic or aromatic side chains and H-bond forming groups of the amide backbone that is required to produce stable protein cores. Although H-bonds are very stable in chloroform (used to represent the core environment) and could have dominated the PCM properties, interactions between aromatic or aliphatic groups either stabilize the H-bonds, alter the pattern of H-bonds, or disrupt the H-bonds. A greater number of NOE crosspeaks are observed between core residues for a PCM with a more acidic Ar–H, which is most likely caused by more favorable T-shaped aromatic interactions. Besides the observation of NOE cross-peaks, experimental evidence for aromatic interactions is obtained through the observation of temperature-sensitive chemical shifts of aromatic protons. Our PCMs could potentially provide an experimental measure of T-shaped aromatic interaction energies. Aromatic interactions are not required to obtain a stable core. The structural stability of the Leu-containing compound, however, most likely arose through a very stable H-bond and steric constraints and not through core interactions. The study of our PCMs demonstrates that subtle differences in the spatial arrangement of the interacting functional groups of amino acids have a great impact on the properties of the mimetics. Considering that protein cores are much more complex than our model systems, it is not surprising that the a priori creation of proteins remains a challenging endeavor.

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**Supporting Information Available:** 1-Dimensional <sup>1</sup>H NMR, ROESY and TOCSY spectra of the compounds and synthetic procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

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