Enantioselective and Diastereoselective Molecular Recognition of Cyclic Dipeptides by a C_2 Macrolactam Host

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Abstract: Two synthetic, optically active, C_2 symmetric macrolactam hosts **11b** and **11c** have been synthesized from L-leucine, and other simple starting materials, in an 11-step, convergent synthesis. These new macrocycles, which were conceived with the aid of current molecular modeling software, were designed to complex neutral amides in organic solvents through complementary intermolecular hydrogen bonding. The hosts, which are made up of two short peptide strands alternately interlinked with two rigid aromatic spacer units to form a large macrocyclic structure, feature a binding site that consists of a convergent, ordered array of amide functions. ^{1}H NMR and solution FT-IR spectroscopy demonstrated that in CDCl₃ **11b** and **11c** each bound the five various stereoisomers of the cyclic dipeptides *cyclo*-Gly-Leu and *cyclo*-Leu-Leu, with association constants ranging from 70 to 2260 M⁻¹ (±15%). **11b** was capable of moderately high enantioselective and diastereoselective molecular recognition ($\Delta\Delta G_{enan}$ = 0.97–1.23 kcal/mol, $\Delta\Delta G_{dia}$ = 0.28–0.95 kcal/mol) of the various chiral diketopiperazines and preferred guests bearing one or more side chains of the L configuration. In contrast, the diastereomeric host **11c** showed very little enantioselectivity ($\Delta\Delta G_{enan}$ = 0.09–0.17 kcal/mol), but did show diastereoselectivity ($\Delta\Delta G_{dia}$ = 0.44–0.54 kcal/mol) and showed a modest preference for guests with side chains of the D configuration. Molecular modeling studies, as well as ^{1}H NMR data, suggest that these hosts are conformationally flexible and bind to the guests with an induced-fit mechanism. The host–guest complexes are stabilized through the formation of three to four intermolecular amide–amide hydrogen bonds in the binding cavity of the macrocycle as well as favorable van der Waals contacts between the hydrocarbon surfaces of the host and guest. The stereoselective binding observed is most likely due to the slight energetic differences in the intermolecular hydrogen bonding patterns that stabilize the host–guest complexes, which are in turn due to the varying degrees of steric interactions that occur between the side chains of the guest and of the host. It is anticipated that the strength, and the degree, of the selectivity in binding of the guests by hosts **11b,c** could be improved upon through additional structural modifications.

The study of the intermolecular binding interactions that occur in synthetic host–guest complexes has been demonstrated to be one effective way to gain insight into the origins of the highly specific and efficient binding processes utilized by enzymes, receptors, and other proteins, as well as a guide to learn how to design new supramolecular structures.[1] One possible way to attain a better understanding of the highly enantioselective recognition processes that take place when chiral ligands interact with their corresponding receptors in biological systems is to design, synthesize, and characterize model compounds that are capable of enantioselective complexation. Practical applications stemming from the rational design of enantioselective host molecules include improved chromatographic techniques for the nondestructive, efficient resolution of optical isomers,[2] as well as the development of novel catalysts for asymmetric synthesis.[3]

Since the pioneering studies twenty years ago by Wudl[4] on chiral crown ethers, and Cram and co-workers[5] on the asymmetric complexation of zwitterionic amino acids by chiral crown ethers,

workers in the field of molecular recognition have synthesized a wide variety of chiral organic compounds capable of enantioselectively binding charged, or zwitterionic, organic molecules.[6] Despite such progress, there has been a relatively small number of reports describing optically active synthetic hosts that are capable of the enantioselective recognition of neutral (uncharged) organic substrates, and most of these have appeared within the last five years or so.[7] Moreover, and perhaps of greater significance, there are even fewer accounts of research to date that have documented the enantioselective complexation of neutral peptides and/or peptide derivatives by fully synthetic hosts.[7b,e–g,l,n,o] The careful studies of small models of peptide–peptide host–guest complexes are of particular interest because

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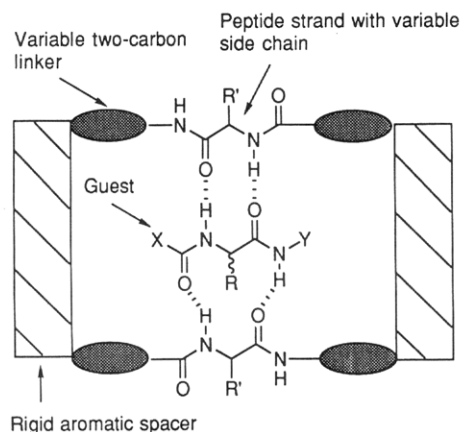* Abstract published in *Advance ACS Abstracts*, May 1, 1994.
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Figure 1. Modular design strategy for building peptide hosts.

they may provide insight into the structural basis of the high size/shape specificities and high enantioselectivities exhibited by the analogous peptide–protein recognition processes which occur in biology.[8] In this paper we report on the design, synthesis, and host–guest binding studies of a new class of optically active macrocycles, which bind chiral lactams in chloroform with enantioselectivities and diastereoselectivities of up to 1.23 and 0.95 kcal/mol, respectively.

Our general goal was to investigate some relatively unexplored issues relevant to the topic of peptide complexation by studying a new class of fully synthetic hosts. Specifically, we wished to (i) investigate the degree of conformational rigidity (i.e., pre-organization), which a host structure needs to possess in order to be able to bind flexible peptides, by incrementally increasing the conformational rigidity in a series of macrocyclic hosts, (ii) investigate potential strategies for the design of a size- and shape-selective binding site that juxtaposes hydrogen-bonding sites in an ordered manner within a well-defined nonpolar binding cavity, and (iii) develop a rational approach for the design of enantio-selective and diastereoselective hosts using a general synthetic plan that incorporates asymmetric carbon atoms (in the form of amino acids) into the host skeleton to create chiral binding sites. This paper details the efforts undertaken in an attempt to achieve the above mentioned goals and reports on the degree of success in these endeavors, as well as describes some of the shortcomings in the design, i.e., the synthetic and practical obstacles encountered that prevented us from completely achieving our original goal of binding flexible peptides.

Design of a Synthetic Host for Peptides: General Consider-ations. A possible strategy for designing a host capable of achieving molecular recognition of amides and peptides occurred to us after we had examined numerous X-ray structures of various peptides and proteins. We reasoned that a host constructed from a combination of peptidic and conformationally rigid nonpeptidic structural fragments could be connected in a C_2 symmetrical, macrocyclic fashion, to generate to preorganized conformation in the host (Figure 1). Specifically, two rigid aromatic units could be incorporated into the ring skeleton along with two peptide strands that ran in an all-extended antiparallel direction relative to each other, the net result being that a preorganized binding site complementary to a third peptide strand, i.e., the guest, is created. Thus, the amide or peptide guest then has the opportunity to hydrogen bond with the host in an intermolecular manner similar to the way three peptide chains do in an antiparallel β-sheet arrangement, which is a hydrogen-bonded structural motif commonly observed in the secondary structure of proteins. The essential feature of the host design is the binding site in which a series of functional groups (amides) are precisely positioned so

as to converge into the otherwise nonpolar pocket provided by the two aromatic spacers and thus act as hydrogen bonding donor/acceptor sites. Overall, the desired binding interactions of peptides and similar substrates with the host are envisioned occurring through a unique combination of hydrogen-bonding and van der Waals interactions. This design offers the potential to construct hosts that are capable of selectively binding peptides or peptide derivatives on the basis of their respective side-chain identity and stereochemistry, as well as the length of the peptide (number of residues).

Results

Computer Modeling. Ultimately, our success in producing a synthetic host capable of binding peptides depends on our ability to forge the three-dimensional shape and topology of the host that is complementary to the guests. Computer modeling techniques and CPK space-filling models were used extensively as tools to help us choose a suitable molecular framework to serve as a starting point for host construction. Molecular mechanics and molecular dynamics using the CHARMm force field, as implemented in the Quanta software package distributed by Polygen (see Experimental Section), were used for examining the conformational space available to a variety of structural subunits, e.g., the spacer, linker, and peptide units depicted in Figure 1. In particular, we were interested in designing these three types of building blocks so that they would control the dimensions and shape of the binding cavity, as well as enforce the convergence of the amide groups into the binding site, by virtue of their conformational preferences and their geometric dimen-sions. Various combinations of the three types of modular subunits were then constructed to generate a number of possible candidate host structures. These hybrid structures were then each energy minimized and subjected to molecular dynamics simulations. Further energy minimizations were then conducted on selected structures generated in the dynamics simulation; however, we did not put a high priority on determining the absolute minimum energy conformations but, instead, viewed the results of the calculations in a qualitative sense in order to get an idea for the amount of conformational preorganization that was present in the host structures. These refined structures were then tested for their adherence to the above mentioned criteria of convergence and well-defined structure by molecular docking simulations in which small peptides were docked in the binding site of the candidate host and the resulting complex energy was minimized. If at least two intermolecular hydrogen bonds were formed in the simulation, then the structure was considered a potential target and its feasibiilty of synthesis was considered.

Attempted Synthesis of Host 11a. The docking simulations and inspection of CPK models suggested that macrolactam **11a** (Figure 2), which is derived from *para*-terphenyl aromatic spacers and L-alanine peptide fragments, possessed a structure suitable for generating host–guest interactions with peptides. A ret-rosynthetic analysis of **11a** suggested that a macrolactamization reaction of the corresponding acyclic amino acid would effect the desired ring formation. We thus embarked on a plan to synthesize **11a**. Starting from commercially available materials, we syn-thesized the prerequisite linear C- and N-protected macrolac-tamization precursor (not shown) in 10 steps and characterized it by ^{1}H NMR, ^{13}C NMR, and high-resolution mass spectrometry. Unfortunately, this precursor to **11a** was only sparingly soluble in chloroform, DMF, DMSO, and THF. We were concerned that the target macrolactam **11a** would also probably be plagued by a similar lack of solubility, which would make the complexation studies of host **11a** with peptides difficult or impossible to carry out. Instead of attempting to complete the synthesis, we considered modifying our original host design scheme so as to assure that the hosts would be soluble enough in organic solvents.

We designed the host structures **11b,c**, utilizing different structural components, i.e., a saturated two-carbon linker, the

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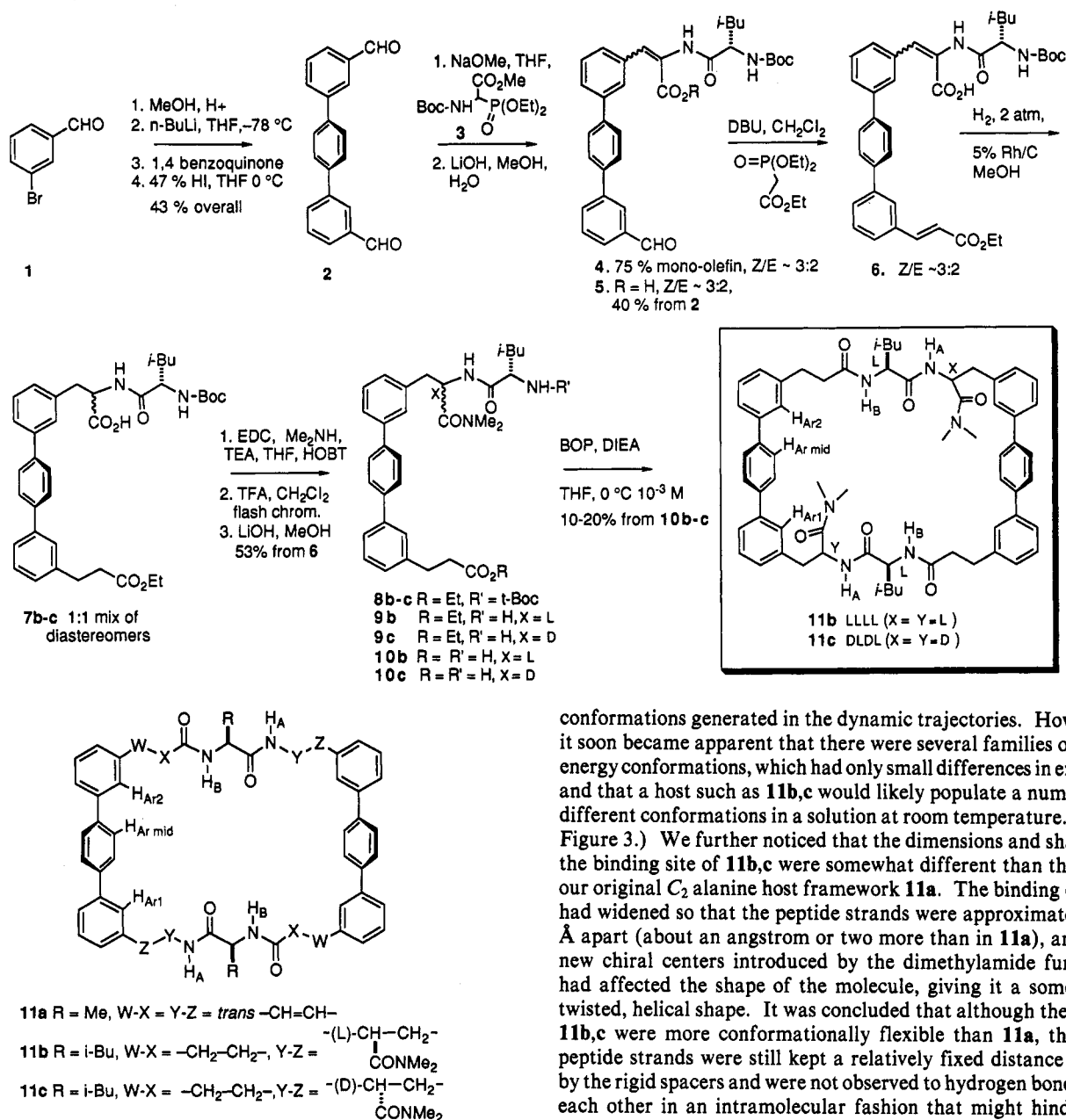
Scheme 1. Synthesis of Hosts 11b,c


Figure 2. Hosts 11a–c.

more nonpolar amino acid leucine, and an additional dimethylamide carboxyl capping function. This new design gives rise to up to four possible diasteromeric host structures because of the addition of the dimethylamide function on the two-carbon linker element, which, in the 11a host, was not present. We hoped that by incorporating the dimethyl amide into the new structures 11b,c, we could restrict bond rotations around the α-carbon (i.e., increase preorganization) and increase the solubility of the host in organic solvents. In order to simplify matters further, we focused our studies on only the two C_2 symmetrical hosts, 11b and 11c.

We used computer modeling techniques to investigate the effect that these structural changes would have on the overall conformational flexibility of the new structures. Molecular dynamic simulations of the two C_2 symmetrical diastereomers, 11b and 11c were carried at 900 K for 100 ps (after slow heating to 900 K over a 20-ps period and a further 20-ps equilibration period at this temperature before being allowed to sample conformational space freely) in an attempt to exhaustively search the conformational space available. An attempt was made to identify the lowest energy conformation by minimizing a selected number of

conformations generated in the dynamic trajectories. However, it soon became apparent that there were several families of low-energy conformations, which had only small differences in energy, and that a host such as 11b,c would likely populate a number of different conformations in a solution at room temperature. (See Figure 3.) We further noticed that the dimensions and shape of the binding site of 11b,c were somewhat different than those of our original C_2 alanine host framework 11a. The binding cavity had widened so that the peptide strands were approximately 11 Å apart (about an angstrom or two more than in 11a), and the new chiral centers introduced by the dimethylamide function had affected the shape of the molecule, giving it a somewhat twisted, helical shape. It was concluded that although the hosts 11b,c were more conformationally flexible than 11a, the two peptide strands were still kept a relatively fixed distance apart by the rigid spacers and were not observed to hydrogen bond with each other in an intramolecular fashion that might hinder its interaction with a protic peptide guest. Overall, we felt that there was still sufficient preorganization in the structures to facilitate complexation of peptide derivatives and that their total synthesis was worth pursuing.

Synthesis of Hosts 11b and 11c. The synthesis of 11b and 11c is shown in Scheme 1 and was accomplished using nearly the same retrosynthetic plan used in the effort to synthesize 11a. Proceeding in the forward direction, we synthesized the required aromatic spacer 2, by utilizing a procedure we had developed previously in our laboratory for the synthesis of 9,10-diphenylanthracene and 5,12-diphenylnaphthacene rings.[9] Thus, addition of 1,4-benzoquinone to 4 equiv of the dimethyl acetal aryllithium, which ultimately derives from the commercially available 3-bromobenzaldehyde (1) (after acetal formation with MeOH/H+ and then treatment with *n*-butyllithium in THF), at –78 °C, gave a 45% isolated yield, on a 50-g scale, of the intermediate *trans*-1,4-diaryl-1,4-dihydroxy-2,5-cyclohexadiene (not shown), as well as 25% yield of 1,4-hydroquinone. The HI reductive aromatization of the intermediate diol to cleanly provide 2 in 92% yield was noteworthy in that no phenolic by-products were detected.

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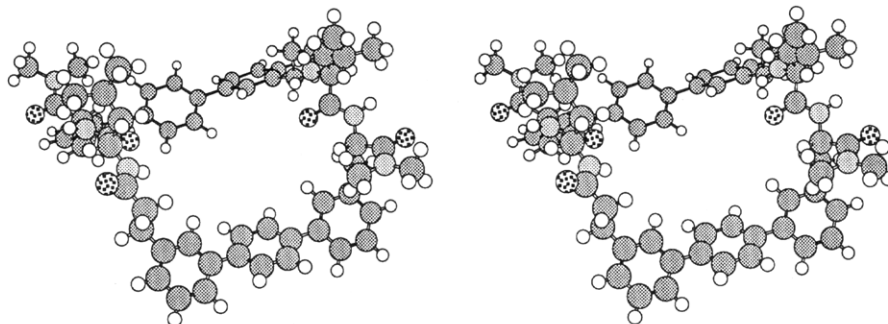
Figure 3. Stereoview of one of the calculated low-energy conformations of **11b**: oxygen = checkerboard, nitrogen = light gray, carbon = dark gray, hydrogen = white.

Phenolic by-products can result from the well-known acid-catalyzed dienone–phenol rearrangement of such aryl-diols. This method of using HI in THF to cleanly generate substituted diaryl-*p*-terphenyls from 1,4-diaryl-1,4-dihydroxy-2,5-cyclohexadienes is thus complementary to metal–metal coupling procedures for the synthesis of *para*-terphenyls.

A Horner–Emmons reaction of the bis-aldehyde **2** with the phosphonate **3**[10] formed the dehydropeptide **4** as a 3:2 *Z/E* mixture of geometrical isomers. We eventually developed an experimental method that allowed the selective monoolefination of **2** to give **4** in greater than the statistical yield expected for a reaction of 1 equiv of phosphonate with a bis-aldehyde. We observed that if the base used for the anion formation was changed from sodium hydride in THF to powdered sodium methoxide in vigorously stirring THF, the rate of reaction was slowed, but on the other hand, the isolated yields of monoolefin **4**, relative to the diolefinated product **4a** (not shown), exceeded that which was expected from the statistical distribution of 1:2:1 **2/4/4a**. There is perhaps a subtle electronic effect that renders the remaining aldehyde function of **4** less reactive toward a second olefination by the phosphonate **3**, even though the aldehyde carbonyl of **4** cannot possibly be in conjugation with the olefin of the other phenyl ring in the traditional sense depicted by electron resonance forms. Flash chromatography of the crude product mixture provided **4** as a 3:2 *Z/E* mixture of geometrical isomers, which were saponified with LiOH to afford **5** without any change in the *Z/E* ratio of isomers. A 100% *E* selective olefination of **5** with triethyl phosphonoacetate and DBU afforded **6** in good yield, again without any change in the *Z/E* ratio of the trisubstituted olefin. Hydrogenation of both of the double bonds in **6** with 5% Rh/C and 2 atm of H_2 furnished **7b,c** as a 1:1 mixture of LL and LD diastereomers.

We protected the mixture of **7b,c** with a dimethyl amide function to generate **8b,c** in moderate yield using a carbodiimide coupling protocol. The dimethyl amide function was chosen because it lacked an amide N-H proton, which we felt might induce intramolecular hydrogen bonding and possibly inhibit intermolecular hydrogen bonding to a potential guest, and also because it provided increased solubility in organic solvents. The 1:1 mixture of diastereomers **8b,c** was treated with TFA to give **9b** and **9c**, which were separated by flash chromatography, and then each diastereomer was subjected to basic conditions that cleaved the ethyl ester functionality to furnish the amino acids **10b** and **10c**.

After numerous unsuccessful attempts at macrolactamization, **10b** was successfully cyclized to **11b** by using the BOP peptide coupling reagent (3 equiv) in dilute (10^{-3} M) THF at 0 °C with Hunig's base (7 equiv) in isolated yields of 10–20%. The procedure worked equally well for the cyclization of **10c** to give **11c**. It was assumed that the insoluble solids, which were filtered off during

Scheme 2. Synthesis of Diketopiperazines **13b,c**
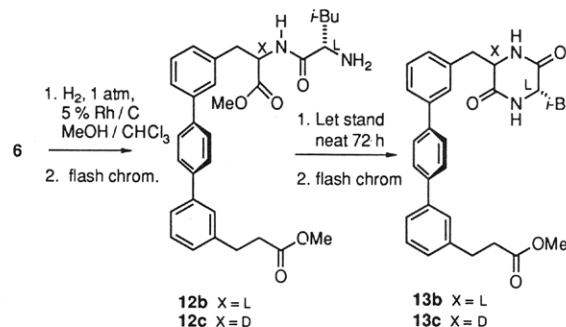
the workup procedure, were oligomeric materials because the overall mass balance was only 70–80%, and similar insoluble material also had to be removed before samples could be dissolved for purification on silica gel. The structure of the product was confirmed by ^{1}H and ^{13}C NMR, which indicated a symmetrical compound, and also analytical high-resolution FAB mass spectroscopy, which gave the exact weight of a molecular ion with the formula $C_{64}H_{74}O_6N_6$. The macrocyclization reaction is formally a cyclodimerization and takes advantage of the C_2 symmetry of the molecule.

With an effective method for macrolactamization in hand, we focused next on addressing the stereochemical issues raised by the incorporation of the additional asymmetric center. We needed to assign to absolute configuration of the chiral centers at the α-carbon of the dimethyl amide function in **11b** and **11c**. This could be done conveniently if the absolute configuration of the corresponding chiral centers of the diastereomers **9b** and **9c**, which resulted from the hydrogenation of the dehydroamino acid **6**, could be assigned (the absolute configuration of the leucine α-carbon is L and is ultimately derived from commercial Boc-NH-L-leucine). To these ends, we converted **6**, in one pot, into the two diastereomeric amino esters **12b** and **12c** by hydrogenation, esterification, and N-deprotection (Scheme 2). After workup, these unstable, diastereomeric, free bases of the amino esters **12b** and **12c** were immediately separated by radial chromatography, identified by ^{1}H NMR and ^{13}C DEPT analysis, concentrated in vacuo, and allowed to stand neat, whereupon they slowly converted to their respective cyclic diketopiperazines **13b** and **13c** via intramolecular lactamization over a period of a few days. The unknown configurations of the chiral centers of the diketopiperazines **13b** and **13c** (and therefore **12b** and **12c**) were then assigned using a well-established method described by Schollkopf.[11] This method, which was developed originally by Schollkopf as a way to assign the absolute configuration of the newly created chiral center that is generated by a stereoselective alkylation at the Gly α-carbon of a L-Phe-Gly derived lactim ester, takes advantage of the fact that the diastereomeric LL and LD diketopiperazine products have distinctly different ^{1}H NMR

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(11) Schollkopf, U.; Busse, U.; Kilger, R.; Lehr, P. *Synthesis* **1984**, 271–274.

spectra. The LL isomer perfers a boat conformation in which the phenyl ring is folded over the diketopiperazine nucleus and is positioned so that its anisotropic shielding region is placed directly over the protons of the opposite side chain. This causes a substantial deshielding of the side-chain protons. For example, the Leu methine proteon of **13b** has a chemical shift of 0.14 ppm. In the LD isomer **13c**, the phenyl ring is on the opposite side of the ring and the dramatic chemical shifts mentioned are not observed (the Leu methine proton is found at its normal value of 1.43 ppm, and thus the diastereomers are readily assigned their respective stereochemistries. The absolute configurations of the diastereomeric amines **9b** and **9c** were then tentatively assigned by a comparison of their ¹H NMR spectra and their relative chromatographic mobility on silica gel with those of the amines **12b** and **12c**.

¹H NMR and FT-IR Binding Studies of Hosts 11b and 11c with Peptide Guests.

After we had obtained suitable quantities of the two diastereomeric macrolactam hosts **11b** and **11c** in pure form, we set out to investigate their potential for bimolecular complexation with amides and peptides. The medium for complexation was chosen to be chloroform, since it is a relatively nonpolar solvent that favors the intermolecular hydrogen bonding between the host and guest that we were interested in studying. Hydrogen-bonding solvents, such as methanol, water, or DMSO, often interfere with complexation processes by competing for the hydrogen-bonding sites on the guest, as well as on the host, thus reducing the overall stability of the desired host–guest complex.

A simple procedure, which involved adding aliquots of the guest compound as 10-50 mM solutions in CDCl₃ to a 2–2.5 mM solution of the host, was used for the rapid screening of guests. The spectra obtained of the resulting solutions were examined for changes in the chemical shifts ($\Delta\delta > 0.1$ ppm) of either the host or guest resonances. Particular attention was paid to the amide proton resonances belonging to the host and guest because large downfield shifts of these values can indicate the presence of hydrogen-bonded complexes. To our disappointment, protected flexible peptides such as N-Ac-Leu-NHMe, Boc-Leu-Phe-OMe, and Cbz-Gly-NHMe, as well as numerous other small peptides and acyclic amides, did not show any detectable binding affinity for the host, even in cases where a large excess of guest (>10 equiv) was added. In retrospect, these results were not completely unexpected, for we had observed in the computer simulations that when we had modified our original host design of **11a** into the **11b,c** series, for the sake of the practical considerations, such as ease of synthesis and solubility in organic solvents, we had sacrificed preorganization, which had been provided by the four olefinic linkers of **11a**. A good deal of conformational rigidity is required in a host if it is to offset the unfavorable entropic penalty incurred upon complex formation with a guest, particularly in the case where the guest is a rotationally flexible peptide. Additionally, the calculated overall dimensions of the binding site were slightly larger in **11b,c** than in **11a**; the distance between the convergent hydrogen-bonding sites in the host should be highly complementary to the hydrogen-bonding array of the peptide guest. We concluded that these two factors probably accounted for the observed lack of recognition of the flexible substrates. We therefore reasoned that a guest with greater conformational rigidity, and hydrogen-bonding sites that more closely matched the spacing of the amide groups of the host binding site, should exhibit complexation with the host. We felt that the open, doughnut-like host conformations required in **11b,c** for complexation were similar in energy to possible nonproductive, closed ones and that both kinds of conformations were probably populated in solution at ambient temperature and therefore turned our attention to assaying rigid guests which had a slightly greater distance between their hydrogen bond acceptor/donor sites. Computer modeling studies suggested that *cyclo*-Gly-Leu, the cyclic diketopiperazine of the Gly-Leu peptide, fits well in the

binding site of the macrolactam hosts **11b** and **11c**. The calculations suggested that such a complex should be stabilized by at least three, if not four, intermolecular hydrogen bonds of acceptable length (2.9–3.0 Å) between the amide N-H and carbonyl oxygens of the host and guest. The modeling also predicted a close "fit"; that is, the nonpolar surface of the guest alkyl side chain was in favorable van der Waals contact with the aromatic ring and the isobutyl side chain of the host.

We were gratified to discover that the host–guest complex of macrolactam host **11b,c** and *cyclo*-Gly-Leu, which was predicted by the computer docking simulation, was confirmed by ¹H NMR experiments. For example, addition of 1 molar equiv of *cyclo*-Gly-Leu to a 2 mM solution of host **11b** in CDCl₃ caused the N-H$_B$ proton (see Figure 2 for proton nomenclature) resonances of this host to shift downfield from 5.65 to 6.95 ppm, indicating the formation of a hydrogen-bonded complex. Additional changes were observed in the chemical shifts and splitting patterns of the hosts' aromatuic protons as well as in the N-H$_A$. For example, the singlets corresponding to the aromatic protons shown as H$_{Ar1}$ and H$_{Ar2}$, which occur at 7.43 and 7.32, respectively, in the free state, both moved downfield to 7.60 and 7.55 ppm, and the AB quartet corresponding to the protons H$_{Ar,mid}$ on the middle ring of the terphenyl moiety at 7.61 coalesced into a singlet. Changes in the chemical shifts and splitting patterns of the guest proton resonances were also observed. The two N-H signals of the *cyclo*-Gly-Leu amide functions were found about 1 ppm downfield from their normal value of 6.4 ppm observed in the uncomplexed state. In addition, the AB pattern corresponding to the glycinic protons underwent a subtle, but discernible, change upon complexation with the host.

We set our to further investigate the diketopiperazine binding phenomenon by performing some experiments to discern the aggregate or lack of aggregate structure of the hosts. First, the effect of dilution on the N-H$_B$ chemical shifts of **11b,c** was probed (1–10 mM). It was found that below a host concentration of about 4–5 mM in CDCl₃ the N-H$_B$ chemical shifts remained practically invariable, indicating that these protons were not involved in any intra- or intermolecular hydrogen bonding and that the host would be essentially monomeric CDCl₃ concentrations of around 1–2 mM. In addition, ¹H NMR spectra of the hosts in CDCl₃ over the temperature range 0–55 °C were obtained. Molecular modeling suggested that, in this temperature range, both diastereomeric hosts should populate a number of conformations with closely spaced energy levels. This was reflected in the spectra, which showed little change in the resonance line widths throughout the range, thus demonstrating that the various conformations of both hosts are well on the side of fast chemical exchange on the NMR time scale, although a slight degree of line broadening did occur at 0 °C, the lowest temperature at which the host was investigated. The temperature coefficients ($\Delta\delta/\Delta T$) of the amide protons of N-H$_A$ and N-H$_B$ of **11b** were linear with temperature and were found to be -6×10^{-3} and -6.5×10^{-3} ppm/K, respectively. The $\Delta\delta/\Delta T$ values for **11c** were similar: N-H$_A$, -4.9×10^{-3}, and N-H$_B$, -5.1×10^{-3}. All of these values are indicative of N-H protons that are completely exposed to the solvent.[12] Furthermore, FT-IR analysis of a 1 mM dry CDCl₃ solution of the hosts **11b** and **11c** also indicated that there was no intra- or intermolecular hydrogen bonding occurring (Figure 4). For example, there was only one broad peak observed for the **11b** amide N–H stretch at 3421 cm⁻¹, confirming the free, non-hydrogen-bonded state. Moreover, when 1 molar equiv of *cyclo*-Gly-Leu was added to 1 equiv of **11b** and a IR spectrum recorded, it was evident that the free N-H absorbances of **11b** decreased in intensity and a new set of N-H absorbances at 3261 and 3213 cm⁻¹, which correspond to hydrogen-bonded amide N–H bond stretches, had developed. Free *cyclo*-Gly-Leu in 1 mM CDCl₃ absorbed only at 3404 cm⁻¹, indicating that its amide N–H bonds

(12) Stevens, E. S.; Sugarawa, N.; Bonora, G. M.; Toniolo, C. *J. Am. Chem. Soc.* **1980**, *102*, 7048–7050.

Table 1. Association Constants for Host–Guest Complexes of **11b,c** with Diketopiperazines

guest	host 11b			host 11c		
	$K_a{}^a1,{}^b$	$-\Delta G$	$\Delta\Delta G_{enan}{}^c$	K_a	$-\Delta G$	$\Delta\Delta G_{enan}$
cyclo-Gly-Leu	2260 ± 340	4.49	0.97	545 ± 82	3.67	−0.17
cyclo-Gly-D-Leu	420 ± 63	3.52		740 ± 110	3.84	
cyclo-Leu-Leu	606 ± 100	3.73	1.23	297 ± 45	3.31	−0.09
cyclo-D-Leu-D-Leu	74 ± 11	2.50		348 ± 52	3.40	
cyclo-Leu-D-Leu	118 ± 18	2.78	−0.28,^{d} 0.95^e	137 ± 20	2.86	−0.54,^{d} 0.44^e

^{a} In M^{-1}, determined by monitoring the chemical shift of the N-H$_B$ resonance of the host at 23 ± 3 °C, 2.0 mM host in dry $CDCl_3$ (H_2O ca. 10 mM). ^{b} Uncertainties in the values of K are estimated to be about 15%, based on, in some cases, duplicate determinations and also K values calculated from the host H_{Ar1} and H_{Ar2} chemical shift changes. ^{c} All ΔG values are in kcal/mol, enantioselecivity, $\Delta G(D) - \Delta G(L)$. ^{d} Diastereoselectivity, ΔG (*cyclo*-Leu-D-Leu) − ΔG (*cyclo*-D-Leu-D-Leu). ^{e} Diastereoselectivity, ΔG (*cyclo*-Leu-D-Leu) − ΔG (*cyclo*-Leu-Leu).
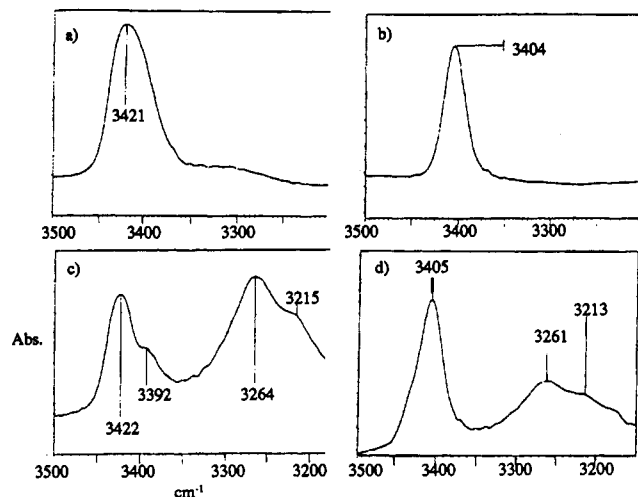
Figure 4. FT-IR showing the amide N–H stretch regions of 1 mM $CDCl_3$ solutions of (a) **11b**, free, (b) *cyclo*-Gly-Leu, free, (c) 1:1 **11b**–*cyclo*-Gly-Leu, minus *cyclo*-Gly-Leu, and (d) 1:1 **11b**–*cyclo*-Gly-Leu.

are not hydrogen bonded intermolecularly at this concentration. In addition, the IR spectrum of free *cyclo*-Gly-Leu could be subtracted mathematically from the 1:1 **11b**-*cyclo*-Gly-Leu spectrum, revealing that there were both free and bound species of the host present at this stoichiometry.

^{1}H NMR titrations of hosts **11b** and **11c** with the various guests in dry (H_2O ca. 10 mM)[13] $CDCl_3$ solutions were carried out at a 2 mM host concentration. The association constants were calculated using a nonlinear least-squares curve fit analysis[14] assuming a 1:1 binding event and are shown in Table 1 along with their corresponding free energies of complexation and binding stereoselectivities. We estimate the uncertainties in the association constants to be approximately 10–15% of the corresponding K_a value of the complex. A typical calculated binding isotherm is shown in Figure 5.

Discussion

The binding constants range from a rather low value of 74 M^{-1} for the **11b**-*cyclo*-D-Leu-D-Leu complex to a fairly strong binding constant of 2260 M^{-1} for the **11b**-*cyclo*-Gly-Leu complex. The guests possessing only one isobutyl side chain, i.e., the *cyclo*-Gly-Leu series, show the strongest binding to **11b** (LLLL) and **11c** (DLDL). Addition of a second isobutyl chain to a guest, i.e., as in the *cyclo*-Leu-Leu guests, leads to a reduction of binding by about 5-fold with **11b** and 2-fold with **11c**. Of greater significance is that **11b** shows enantiomeric and diastereomeric selectivity amongst the guests, while the **11c** host shows very little. The highest degree of enantioselectivity ($\Delta\Delta G_{enan}$) was 1.23 kcal/mol

(13) The residual water present in the chloroform titration solutions has an effect on thermodynamics of host–guest association. For a leading discussion of this phenomena, see: Wilcox, C. S.; et al. *J. Am. Chem. Soc.* **1991**, *113*, 676; **1992**, *114*, 1398.

(14) (a) Macomber, R. S. *J. Chem. Ed.* **1992**, *69*, 375. (b) Wilcox, C. S. *Frontiers in Supramolecular Organic Chemistry and Photochemistry*; VCH: Weinheim, 1991; pp 123–143.

Host 11b-cyclo-Leu-Leu complex

Eq. of cyclo-Leu-Leu added
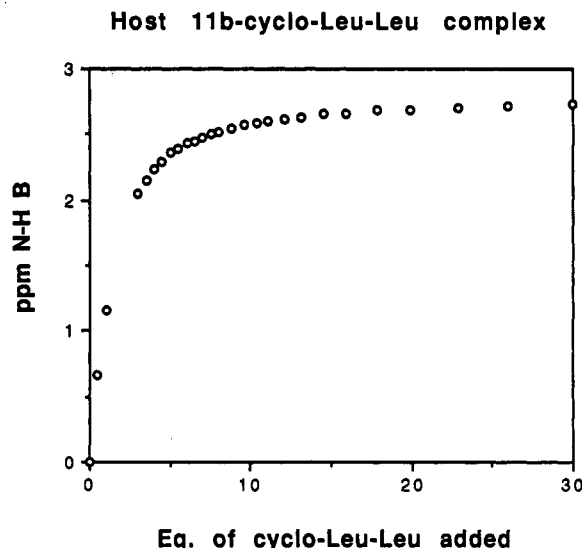
Figure 5. Typical binding isotherm calculated for hosts **11b,c** with diketopiperazine guests. Shown here is the titration of **11b** with *cyclo*-Leu-Leu.

by **11b** for *cyclo*-Leu-Leu over *cyclo*-D-Leu-D-Leu. The degree of diastereoselective binding by **11b** was highest ($\Delta\Delta G_{dia}$ = 0.95 kcal/mol) in the case of *cyclo*-Leu-Leu over *cyclo*-Leu-D-Leu. In contrast, **11c** binds the enantiomeric *cyclo*-Leu-Leu and *cyclo*-D-Leu-D-Leu equally well and also shows nearly the same affinity for the enantiomeric *cyclo*-Gly-Leu, *cyclo*-Gly-D-Leu pair. One more trend is notable, **11c** is essentially selective, relative to **11b**, for diketopiperazines possessing one or more isobutyl side chains with a D cofiguration, where **11b** prefers guests with side chains of the L configuration. The only exception to this trend is the slightly higher K_a for the **11c**-*cyclo*-Leu-Leu complex relative to the **11c**-*cyclo*-D-Leu-Leu complex.

A 2D phase-sensitive NOESY experiment was performed on a mixture of host **11b** and 5 equiv of *cyclo*-Gly-Leu in $CDCl_3$ using a mixing time of 200 ms. Unfortunately, although a well-defined spectral diagonal was obtained in the processed 2D spectrum, no inter- or intramolecular NOEs between species were observed. This was not completely unexpected, however, because the molecular weights of the host (MW 1022) and the corresponding host–guest complex (MW 1192) probably give rise to molecular correlation times that preclude the observation of NOE effects. In the absence of an X-ray structural proof, but on the basis of the computer modeling studies, ^{1}H NMR, FT-IR, and inspection of CPK space-filling models, it is believed that host complexation with the diketopiperazines is driven by hydrogen bonding between the amide functions of the host and guest. We believe that the relative binding affinities amongst the series of guests is dependent on the geometry of, and the number of, the hydrogen bonds formed in the complex. From inspection of the computer-modeled complexes, up to four mutual hydrogen bonds with O⋯H–N bond lengths of less than 3.0 Å and O⋯H–N bond angles between 150 and 180° can occur. However, amongst a
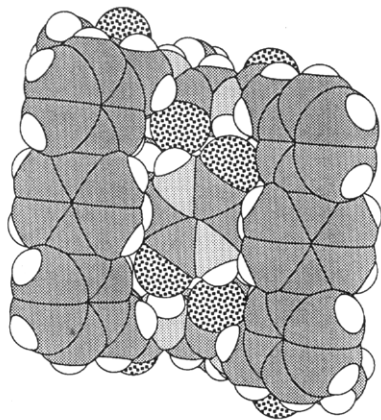
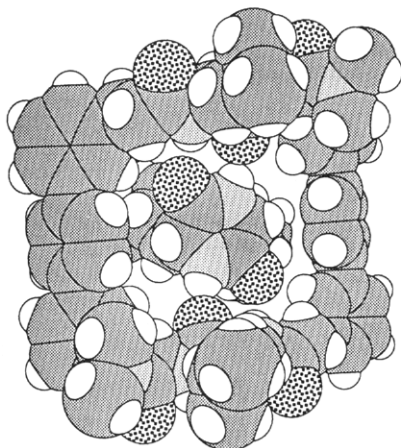**Figure 6.** Calculated complex of **11b** and *cyclo*-Leu-Leu.

Figure 7. Calculated complex of **11b** and *cyclo*-D-Leu-D-Leu.

series of diketopiperazine–host complexes it can be seen that, in some cases, the hydrogen bonds formed have less than optimal C=O⋯H–N bond lengths and angles. Thus, in the more stable complexes, there are little or no unfavorable steric contacts between the host and guest that prevent the four strong hydrogen bonds from occurring, whereas in the less stable complexes, unfavorable steric interactions between the host framework and the guest side chains only allow complexes with only two or three strong hydrogen bonds (complexes with only two hydrogen bonds are probably not formed because neither valerolactam or 1,4-cyclohexadione were observed to bind to the hosts). This is probably the most reasonable way to rationalize the observed enantio- and diastereoselectivity in the binding of **11b** to the five diketopiperazines. Figure 6 shows one of the low-energy conformations calculated for the complex of **11b** with *cyclo*-Leu-Leu. Four hydrogen bonds of about 2.9–3.2 Å are present between the amides of the host and guest. In the corresponding calculated complex of **11b** with the enantiomeric guest *cyclo*-D-Leu-D-Leu in Figure 7, it is readily seen that only two strong mutual hydrogen bonds (along with two longer, weaker ones) can form because the side chains of the *cyclo*-D-Leu-D-Leu cannot "pack" into the host binding site as well as the side chains of *cyclo*-Leu-Leu can. These secondary steric interactions, albeit relatively weak intermolecular forces in chloroform, are nevertheless very important here, because they provide a stereochemically dependent third binding-point interaction, which has to be providing the basis for the enantiomeric recognition of the chiral guests by **11b**.[2]

Conclusion

Although **11b** and **11c** did not show any detectable binding to protected acyclic amino acids and dipeptides as originally hoped for, our efforts did not go entirely unrewarded. In particular, the degree of enantioselective and diastereoselective binding of the

cyclic dipeptide *cyclo*-Leu-Leu stereoisomers by the host **11b** is relatively high ($\Delta\Delta G_{enan}$ = 1.23 kcal/mol, $\Delta\Delta G_{dia}$ = 0.95 kcal/mol, ±0.15 kcal/mol) and is noteworthy considering that it is a conformationally flexible receptor. Indeed, only two other groups have reported on synthetic hosts that bind neutral peptide substrates with an order of magnitude or so higher enantioselection ($\Delta\Delta G_{enan}$) than what is reported here. Rebek and co-workers have recently described $\Delta\Delta G_{enan}$ > 2.5 kcal/mol enantioselective binding of a diketopiperazine via an elegant acyclic host.[7g,k] Still and his research team[7e,f,lo] have reported on an impressive series of macrocyclic receptors with very high enantioselective binding affinities for flexible peptides ($\Delta\Delta G_{enan}$ > 3 kcal/mol). The higher enantioselectivity seen in those reported hosts is most likely due to the fact that they are quite rigid structurally, with well-defined binding cavities, and hence almost completely conformationally homogeneous, whereas there is a fair deal of conformational flexibility present in the less enantioselective **11b,c** hosts. It is likely that the mode of binding of the rigid diketopiperazine guests to the flexible **11b,c** hosts is of an induced-fit type, where the guest freezes out a conformation (or a group of conformations) by forming a hydrogen-bonded host–guest complex. The conformational homogeneity amongst a series of diastereomeric host–guest *complexes* is also an important factor in influencing binding selectivities (higher homogeneity means higher selectivity).[15] It is probable that there is more than one structural mode of association between **11b,c** and each of the various diketopiperazines. Each diastereomeric complex has differing intermolecular hydrogen bonding geometries due to the presence or lack of steric contacts between the host and guest hydrocarbon side chains and therefore different free energies of binding. Thus, there is conformational heterogeneity in the free host and the host–guest complexes, and this is reflected in the moderate association constants (10^2–10^3 M⁻¹) and the modest (2- to 10-fold) enantio- and diastereoselectivities observed. The binding selectivities and the strength of the associations of **11b** and **11c** with peptide guests could probably be improved upon through judicious synthetic modifications that allow for a more conformationally homogeneous host structure.

Experimental Section

General. Unless otherwise mentioned, starting materials were obtained from commercial sources and used without further purification. When a solvent is specified as being dry, it has been distilled from calcium hydride or benzophenone ketyl. When air-water-sensitive reagents were used, an inert atmosphere was maintained by using dry nitrogen with an on-line calcium sulfate drying tube, and all water-sensitive reactions were performed in flame-dried glassware, in dry solvents under dry nitrogen. Analytically pure samples were obtained by radial chromatography equipped with Kieselgel 60 PF₂₅₄ silica gel and flash chromatography with Kieselgel 60 silica gel. Thin-layer chromatography (TLC) was performed on 0.25-mm Merck percoated silica gel plates (60 F-254). Optical rotations were determined using a Jasco Model DIP-370 polarimeter. Infrared (IR) spectra were recorded on a Perkin-Elmer Model 1600 series. FT-IR binding studies were done on a Mattson Galaxy 5000 series. Nuclear magnetic resonance (NMR) spectra were recorded on a General Electric GN-500 MHz spectrometer with Nicolet software. High-resolution mass spectrometry was performed in-house with a Finnegan spectrometer. Elemental analyses were performed by Desert Analytical, Tucson, AZ. Flash chromatography was performed according to the method described by Still, and radial chromatography was performed with the Harrison Chromatron. Workup A: The reaction solvent was removed in vacuo at 10–20 mmHg on a rotary evaporator, and the residue was treated with EtOAc and 5% aqueous KHSO₄ solution. The aqueous solution was extracted twice with EtOAc, and the organic extracts were combined. The organic extract was washed twice with 1 M aqueous Na₂CO₃ and twice with saturated aqueous NaCl and dried over Na₂SO₄

(15) (a) Li, G.; Still, W. C. *J. Org. Chem.* **1991**, *56*, 6964–6966. (b) Wang, X.; Erickson, S. D.; Iimori, T.; Still, W. C. *J. Am. Chem. Soc.* **1992**, *114*, 4128–4137. (c) Liu, R.; Still, W. C. *Tetrahedron Lett.* **1993**, *34*, 2573–2576.

or MgSO$_4$. After filtration, the solvent was removed on a rotary evaporator, as described before, and placed on high vacuum (0.05 mm) overnight.

3,3″-Diformyl-*p*-terphenyl (2). To a solution of 3-bromobenzaldehyde dimethyl acetal (0.35 mol, 80.7 g, obtained by stirring 3-bromobenzaldehyde (1) with MeOH/catalytic H$_2$SO$_4$ for 24 h) in 350 mL of THF at −78 °C stirring under N$_2$ was slowly added *n*-BuLi (0.34 mol, 184 mL, 1.85 M in cyclohexane) via an addition funnel. After the addition was complete (45 min), the orange solution was stirred an additional 1 h at this temperature. 1,4-Benzoquinone (0.10 mol, 10.8 g) in 50 mL of THF was then slowly added to the reaction mixture over a 1-h period. As the reaction was allowed to warm to −40 °C over a 4-h period, the intense blue color of the solution gradually turned green. The reaction was then quenched with excess MeOH. The resulting red solution was then treated with enough NH$_4$Cl to bring the pH to about 10 and the brown mixture transferred to a separatory funnel. The layers were separated, and the aqueous phase was extracted with Et$_2$O 3×. The brown organic layers were combined and washed with saturated aqueous NaCl and 1 M Na$_2$-CO$_3$ and then dried over Na$_2$SO$_4$. Evaporation of the solvent in vacuo furnished 57 g of a brown oil. The oil was immediately purified by flash chromatography on a 130-mm-diameter column using a 1:1 mixture of EtOAc/CHCl$_3$ as the eluant (100–200-mL fractions). The product was collected, providing 19 g of a thick clear oil, which was judged to be ≥95% pure 1,4-bis(3-formylphenyl)-1,4-dihydroxy-2,5-cyclohexadiene by ^{1}H NMR. The product decomposes rapidly in CDCl$_3$, and slowly if left standing neat or in ether solution at room temperature, so it was used without further purification in the next step: ^{1}H NMR (DMSO-d_6) δ 7.81 (s, 2H), 7.72 (d, *J* = 9.1 Hz, 2 H), 7.65 (d, *J* = 7.4 Hz, 2 H), 7.40 (t, *J* = 7.7 Hz, 5.99 (s, 4 H), 5.52 (s, 2 H, O-H), 3.20 (s, 12 H); IR (film) 3392, 3052, 2936, 2830, 1697, 1105, 1054, 993, 908, 872 cm^{-1}. To a 500-mL THF solution of the diol stirring at −5 °C was added 80 mL of 47% HI (excess) from a newly opened bottle (Fisher brand) over 30 min via an addition funnel. The dark red solution was allowed to warm to 10 °C over a 2.5-h period, at which time a precipitate was observed. The reaction mixture was carefully quenched with 750 mL of 3 M NaOH, followed by 750 mL of 20% Na$_2$SO$_3$. The solid was filtered via a sintered glass funnel and washed thoroughly with distilled water. The beige solid was dried at 0.1 mmHg overnight and then recrystallized from EtOAc to give 12.1 g of analytically pure **2** (92%): mp 140–142 °C, ^{1}H NMR (CDCl$_3$) δ 10.12 (s, 2 H, C*H*O), 8.17 (s, 2 H, H$_{Ar1}$), 7.93 (d, 2 H, *J* − 8.2 Hz, H$_{Ar}$), 7.90 (d, 2 H, *J* = 7.8 Hz, H$_{Ar3}$), 7.76 (s, 4 H, H$_{Ar,mid}$), 7.66 (t, *J* = 8 Hz, 2 H, H$_{Ar4}$); IR (Nujol mull) 3050, 2730, 1691, 1463, 1376, 1179, 792 cm^{-1}; HRMS, CI$^+$, calcd for C$_{20}$H$_{14}$O$_2$ 286.0994, found 286.0982 (M$^+$, 33), 287.1072 (M$^+$ + H, 100). Anal. Calcd for C$_{20}$H$_{14}$O$_2$: C, 83.90; H, 4.93. Found: C, 83.86; H, 4.77.

(*E*)- and (*Z*)-*N*-(*tert*-Butoxycarbonyl)-(*S*)-leucyl-3-[4-(3′-formylbiphenyl)yl]dehydrophenylalanine Methyl Ester (4). The aldehyde **2** (12.0 g, 41.9 mmol) and methyl *N*-(*tert*-butoxycarbonyl)-*S*-leucyl-1-diethylphosphonoglycinate **3** (16.1 g, 36.5 mmol) were dissolved in 300 mL of dry THF. Finely powdered NaOMe (5.9 g, 109.5 mmol) was added to this mixture via a powder addition funnel with vigorous stirring over a 45-min period. The resulting orange suspension was allowed to stir 12 h, at which point the reaction mixture was concentrated and worked up according to method A. A yellow foam was obtained and was purified by flash chromatography (130-mm diameter 3–10% EtOAc/CHCl$_3$), affording 11.2 g (72% based on recovered starting material) of a 3:2 *Z*/*E* mixture of **4**: mp 74–76 °C; ^{1}H NMR (CDCl$_3$) δ 10.10 (s, 1 H, C*H*O), 8.44 (br s, 1 H, *E* isomer dehydro N-H), 8.16 (s, 1 H, H$_{Ar1}$), 7.90 (m, 2 H, H$_{Ar3}$, H$_{Ar7}$), 7.4–7.7 (m, 10 H, H$_{Ar}$, olefin C-H, *Z* dehydro N-H), 4.9 (m, 1 H, Boc N-H), 4.35 (m, 1 H, Leu α-H), (3.8, 3H, *E* isomer OCH$_3$), 3.85 (s, 3 H, *Z* isomer OCH$_3$), 1.7 (m, 3 H, Leu CH$_2$C*H*), 1.48 (s, 9 H, *E* O(CH$_3$)$_3$), 1.44 (s, 9 H *Z* O(CH$_3$)$_3$), 0.98 (t, *J* = 7 Hz, 6 H, *E* CH$_3$), 0.91 (t, *J* = 7 Hz, 6 H *Z* CH$_3$); IR (KBr) 3284.4, 2953.4, 2780, 1701.4, 1250, 950, 900, 841.2 cm^{-1}; [α]^{20}D −23° (*c* = 1, 100 mm, CHCl$_3$); HRMS, CI$^+$, calcd for C$_{34}$H$_{38}$O$_6$N$_2$ 570.2732, found 570.2681 (M$^+$, 7), 571.2776 (M$^+$ + H, 6), 515.2169 (M$^+$ − C$_4$H$_8$$^+$, 100). Anal. Calcd for C$_{34}$H$_{38}$O$_6$N$_2$: C, 71.56; H, 6.71; N 4.91. Found: C, 71.74; H, 6.68; N, 4.84.

(*E*)- and (*Z*)-*N*-(*tert*)-Butoxycarbonyl)-(*S*)-leucyl-3-[4-(3′-formylbiphenyl)yl]dehydrophenylalanine (5). The ester **4** (*Z*/*E* 3:2 mixture) (9.0 g, 16.2 mmol) was dissolved in 13 mL of MeOH. One equivalent (0.39 g, 16.2 mmol) of LiOH·H$_2$O was added to the solution with stirring. After 4 h, 1 equiv more of LiOH·H$_2$O was added along with 3 mL of MeOH and 0.2 mL of H$_2$O. Eighteen hours later another 1 equiv was added (total: 1.2 g, 48.6 mmol). After an additional 10 h the reaction was subjected to workup A. Eight grams of a yellow foam was obtained,

which resisted crystallization from a variety of solvents and could not be purified satisfactorily by flash chromatography. ^{1}H NMR showed the crude product to be a 3:2 *Z*/*E* mixture of **5** and an impurity (about 10%), which could not be separated away from the desired product. Therefore, the reaction mixture was carried on directly to the next step: mp 123–125 °C; ^{H}NMR (CDCl$_3$) δ 10.08 (s, 1 H, C*H*O), 8.27 (br s, 1 H, *Z* isomer dehydro N-H), 8.1 (s, 1 H, H$_{Ar1}$), 7.85–7.87 (m, 2 H, H$_{Ar3}$, H$_{Ar7}$) 7.3–7.6 (m, 10 H, H$_{Ar}$, olefin C-H and *D* dehydro N-H) (br s, 1 H, *D* Boc N-H), 5.18 (br s, 1 H, *Z* Boc N-H), 4.45 (m, 1 H, Leu α-H), 1.3–1.8 (m, 3 H, Leu C*H$_2$C*H*), 1.42 (s, 9 H, *E* O(CH$_3$)$_3$), 1.42 (s, 9 H, *Z* O(CH$_3$)$_3$), (t, *J* = 7.1 Hz, 3 H, C*H$_3$*CH$_2$), 0.8–0.9 (m, 6 H, *Z* and *E* CH(CH$_3$)$_2$); IR (KBr) 3298.1, 2958.6, 1697.1, 1575, 1245, 1046.5, 841.4, 790.6 cm^{-1}; [α]^{20}D −35° (*c* = 1, 100 mm, CHCl$_3$).

(*E*)- and (*Z*)-*N*-(*tert*-Butoxycarbonyl)-(*S*)-leucyl-3-[4-[3′-[*E*]-(ethoxycarbonyl)ethenyl]biphenyl]yl]dehydrophenylalanine (6). DBU (12.0 mL, 31.1 mmol) was added dropwise over 5 min to a suspension of the 3:2 *Z*/*E* mixture of **5** (8.04 g, 14.8 mmol) and triethyl phosphonoacetate (3.2 g, 14.5 mmol) in 30 mL of 1:1 THF/CH$_2$Cl$_2$. Solution gradually occurred, and the mixture turned orange and warmed slightly. The reaction was allowed to stir for 1.25 h at room temperature. The reaction solvent was concentrated in vacuo and the residue subjected to workup A, except that the resulting organic layer containing the product was diluted with hexane and cooled in an ice–salt bath to −5 °C. The solution became cloudy, and the mixture was allowed to stand overnight. The precipitate was filtered off and the filtrate concentrated to a yellow foam. This foam was dissolved in hot 3:1 EtOAc/MeOH (30 mL), allowed to cool to room temperature, and then placed in an ice–salt bath for 1 h. A fine white solid was filtered off. The mother liquor was concentratd in vacuo and redissolved in hot 1:1 EtOAc/hexane. Cooling the solution to 0 °C afforded white fluffy crystals (3.6 g), which were shown by ^{1}H NNR to be enriched in the *Z* isomer. Repeated crystallizations yielded 2.2 g of crystals containing a 1:1 *Z*/*E* mixture, and a final concentration of the mother liquor resulted in 2.6 g of solid which was a 1:2 *Z*/*E* mixture. The overall combined yield of the last three crops was 3.2 g of **6** as a 3:2 *Z*/*E* mixture, 88% yield: mp 108–110 °C; ^{1}H NMR (CDCl$_3$) δ 8.2 (br s, 1 H, *Z* isomer dehydro N-H), 7.75 (d, *J* − 15.7 Hz, 1 H, H$_{Ar2}$), 7.25–7.7 (m, 12 H, H$_{Ar}$, olefin C-H, and *D* dehydro N-H) 6.52 (d, *J* = 14.9 Hz, 1 H, olefin C-H), 5.3 (br s, 1 H, *E* isomer Boc N-H), 5.16 (br s, 1 H, *Z* isomer Boc N-H), 4.7 (m, 1 H, *E* isomer α-C-H), 4,5 (m, 1 H, *Z* isomer Leu α-C-H), 4.27 (q, *J* = 7 Hz, 2 H, OCH$_2$CH$_3$), 1.3–1.7 (br, m, 3 H, Leu C*H$_2$CH*), 1.44 (s, 9 H, *E* isomer O$_2$C(CH$_3$)$_3$), 1.40 (s, 9 H, *Z* isomer O$_2$C(CH$_3$)$_3$), 1.35 (t, *J* = 7 Hz, 3H, CH$_3$CH$_2$), 0.97 (m, 6 H, *E* isomer HC(CH$_3$)$_2$), 0.89 (d, *J* = 6 Hz, 3 H, *Z* isomer Hc(CH$_3$)$_2$), 0.84 (d, *J* = 6 Hz, 3 H, *Z* isomer HC(CH$_3$)$_2$); IR (KBr) 3300, 3250, 2956.9, 1700.1, 1200, 1026.1, 800, 750 cm^{-1}; [α]^{20}D =33° (*c* = 1, 100 mm, CHCl$_3$); HRMS, FAB$^+$, calcd for C$_{37}$H$_{42}$O$_7$N$_2$ 626.2992, found 626.3005 (M$^+$, 26), 627.3109 (M$^+$ + H, 100).

N-(*tert*-Butoxycarbonyl)-(*S*)-leucyl-3-[4-[3′-[(ethoxycarbonyl)ethyl]-biphenyl]yl]-(*S*)-phenylalanine (7b) and *N*-(*tert*-Butoxycarbonyl)-(*S*)-leucyl-3-[4-[3′-(ethoxycarbonyl)ethyl]biphenyl]yl]-(*R*)-phenylalanine (7c). A 3:2 *Z*/*E* mixture of **6** (4.56 g, 7.3 mmol) was hydrogenated using 5% Rh/C in 20 mL of EtOH at 30 psi using a Parr apparatus. After 12 h the mixture was filtered through a pad of Celite. The Celite was washed with a copious amount of MeOH and the filtrate concentrated in vacuo. The product was a white foam (4.5 g) which could not be crystallized from a variety of solvents. NMR showed that the crude reaction mixture consisted of a 1:1 mixture of diastereomers **7b,c**, which was carried on to the next step: mp 66–68 °C; ^{1}H NMR (CDCl$_3$ δ 7.61 (s, 4 H, H$_{Ar}$), 7.1–7.55 (m, 8 H, H$_{Ar}$), 6.86 (br s, 1 H, Leu N-H), 5.24 (br s, 1 H, *E* Boc N-H), 5.24 (br s, 1 H, *Z* Boc N-H), 4.84 (m, 1 H, *E* α-C-*H*), 4.76 (m, 1 H, *Z* α-C-*H*), 4.45 (m, 1 H, *E* Leu α-C-*H*), 4.31 (m, 1 H, *Z* Leu α-C-*H*), 4.14 (q, *J* = 7 Hz, 2 H, CO$_2$CH$_2$CH$_3$), 3.32 (m, 2 H, *E* α-CHC*H$_2$*), 3.16 (m, 2 H, *Z* α-CHC*H$_2$*), 3.02 (t, *J* = 8.9 Hz, 2 H, ArCH$_2$CH$_2$CO$_2$Et), 2.67 (t, *J* = 8.6 Hz, 2 H, ArCH$_2$C*H$_2$*CO$_2$Et)), 1.3–1.8 (m, 3 H, Leu C*H$_2$CH*(CH$_3$)$_2$), 1.40 (s, 9 H, diastereo O$_2$C(CH$_3$)$_3$), 1.36 (s, 9 H, diastereo O$_2$C(CH$_3$)$_3$), 1.24 (t, *J* = 7.1 Hz, 3 H, cH$_3$CH$_2$), 0.85 (m, 6 H, diastereo CH(CH$_3$)$_2$), 0.76 (d, *J* = 6.4 Hz, 3 H, diastereo CH(CH$_3$)$_2$), 0.71 (d, *J* = 6.4 Hz, 3 H, diastereo CH(CH$_3$)$_2$); IR (KBr) 3330.4, 2930.7, 1716.0, 1225, 1168.8, 1045.0, 800 cm^{0l}; [α]^{20}D −6° (*c* = 1, 100 mm, CHCl$_3$); HRMS, FAB$^+$, calcd for C$_{37}$H$_{46}$O$_7$N$_2$ 630.3305, found 630,3315 (M$^+$, 73), 631.3389 (M$^+$ + H, 100).

N-(*tert*-Butoxycarbonyl)-(*S*)-leucyl-3-[4-[3′-[(ethoxycarbonyl)ethyl]-biphenyl]yl]-(*S*)phenylalanine *N*,*N*-Dimethylcarboxamide (8b) and *N*-(*tert*-Butoxycarbonyl)-(*S*)-leucyl-3-[4-[3′-[(ethoxycarbonyl)ethyl]biphenyl]yl]-(*R*)-phenylalanine *N*,*N*-Dimethylcarboxamide (8c). To a slurry of dimethylamine HCl (0.557 g, 6.97 mmol), EDC (0.668 g, 3.48 mmol),

HOBT (0.354 g, 2.32 mmol), and the 1:1 mixture of **7b,c**, 1.63 g, 2.32 mmol) stirring in 8 mL of CH₂Cl₂ at 0 °C was added 1.0 mL of Et₃N (6.96 mmol). The reaction mixture was allowed to warm to room temperature overnight. The reaction mixture was subjected to workup A to yield 1.5 g of a white foam. The crude product was purified by radial chromatography (4 mm, 20–40% EtOAc/CH₂CL₂) to provide 0.95 g (59%) of **8b,c** as a white foam. NMR analysis showed that this material was a 1:1 mixture of diastereomers: mp 53–55 °C; ¹H NMR (CDCl₃) δ 7.65 (s, 4 H, H$_{Ar}$), 7.1–7.5 (m, 8 H, H$_{Ar}$), 6.91 (br s, 1 H, Leu N-H), 5.15 (m, 1 H, Boc N-H), 4.82 (m, 1 H, α-C-*H*), 4.13 (m, 1 H, Leu α-C-*H*), 4.13 (q, *J* = 7.3 Hz, 2 H, CO₂C*H*₂CH₃), 3.07 (s, 3 H, NC*H*₃), 3.04 (t, *J* = 7.9 Hz, 2 H, α CHC*H*₂), 2.87 (d, *J* = 6.4 Hz, 2. H, ArC*H*₂CO₂Et), 2.69 (t, *J* = 7.9 Hz, 2 H, ArCH₂C*H*₂CO₂Et), 2.63 (s, 3 H, NC*H*₃), 1.44 (s, 9 H, O₂C(C*H*₃)₃), 1.3–1.8 (m, 3 H, Leu C*H*₂CH(CH₃)₂), 1.24 (t, *J* = 7.2 Hz, 3 H, C*H*₃CH₂), 0.88 (m, 6 H, Leu HC(C*H*₃)₂); IR (KBr) 3301.0, 2928.0, 1715.6, 1636.2, 1250.7, 1168.0, 1125, 1044.7, 842.5 cm⁻¹; [α]²⁰$_D$ –13° (*c* = 0.5, 100 mm, CHCl₃); HRMS, Cl⁺, calcd for C₃₉H₅₁O₆N₃ 657.3778, found 657.3734 (M⁺, 6.5), 558.2869 (MH⁺ – CO₂ – C₄H₈⁺, 100). Anal. Calcd for C₃₉H₅₁O₆N₃: C, 71.21; H, 7.81; N, 6.39. Found: C, 70.68; H, 8.23; N, 6.58.

(S)-Leucyl-3-[4-[3′-[(ethoxycarbonyl)ethyl]biphenyl)]yl]-(S)-phenylalanine N,N-Dimethylcarboxamide (9b). A solution of a 1:1 diastereomeric mixture of **8b,c** (3.65 g, 5.57 mmol) was dissolved in 25 mL of CH₂Cl₂ and cooled to 0 °C under N₂. Trifluoroacetic acid (10 mL, excess) was then added dropwise via an addition funnel. After stirring 1.3 h at 0 °C the yellow reaction mixture was quenched with 10 mL of absolute EtOH. The clear solution was concentrated to an oil and diluted with 100 mL of ether. Saturated aqueous NaHCO₃ was added and the mixture extracted 3× with ether. The ether layers were combined and washed 2× with saturated aqueous NaCl. The ether was dried over Na₂SO₄ and concentrated in vacuo to yield a clear oil. The oil was subjected to flash chromatography (100-mm column, 3–8% MeOH/CH₂Cl₂), which gave only partial separation of diastereomers. The fractions containing only one diastereomer, **9b**, were collected, while the fractions containing a mixture of **9b** and **9c** were concentrated and separated by radial chromatography (4-mm plate, 3–10% MeOH/CH₂Cl₂). The total yield of **9b** was 1.18 g, 76%. The HCl salt of **9b** was prepared by addition of an ethereal HCl solution to the free base of **9b** in ether. Data for the HCl salt: mp 101–103 °C; ¹H NMR (CDCl₃) δ 8.4 (br s, 3 H, NH₃), 7.63 (d, *J* = 4.4 Hz, 4 H, H$_{Ar,mid}$), 7.2–7.5 (m, 8 H, H$_{Ar}$), 5.19 (m, 1 H, α-C-*H*), 4.40 (m, 1 H, Leu α-C-*H*), 4.12 (q, *J* = 7.1 Hz, 2 H, CO₂C*H₂*-CH₃), 3.1 (d, *J* = 7.3 Hz, 2 H, α-CHC*H*₂), 3.02 (t, *J* = 7.8 Hz, 2 H, ArC*H*₂CO₂Et), 2.93 (s, 3 H, NC*H*₃), 2.89 (s, 3 H, NC*H*,, 2.67 (t, *J* – 7.7 Hz, 2 H, ArCH₂C*H*₂CO₂Et), 1.67–1.76 (m, 2 H, Leu CHC*H*₂), 1.34 (m, 1 H, Leu C*H*CH₂), 1.24 (t, *J* – 7.1 Hz, 3 H, C*H*₃CH₂), 0.85 (d, *J* = 6.4 Hz, 3 H, Leu HC(C*H*₃)₂), 0.80 (d, *J* = 6.5 Hz, 3 H, Leu HC(C*H*₃)₂); IR (KBr) 3498, 3190, 3053.9, 2956.9, 1723.4, 1691.8, 1636.2,1605.3, 1149.9, 1050, 850.0 cm⁻¹; [α]²⁰$_D$ –9° (*c* = 1, 100 mm, CHCl₃); HRMS, FAB, calcd for C₃₄H₄₄O₄N₃Cl 593.3020, calcd for M⁺ – Cl C₃₄H₄₄O₃N₃ 558.3332, found 558.3357 (M⁺ – Cl, 100). Anal. Calcd for C₃₄H₄₄O₄N₃Cl: C, 68.73; H, 7.46; N, 7.07. Calcd for C₃₄H₄₄O₄Cl·3H₂O: C, 62.99; H, 7.77; N, 6.48. Found: C, 63.14; H, 6.98, N, 6.62.

(S)-Leucyl-3-[4-[3′[(ethoxycarbonyl)ethyl]biphenyl)]yl]-(R)-phenylalanine N,N-Dimethylcarboxamide (9c). **9c** was prepared from a 1:1 **8b,c** mixture using the same procedure as for **9b**. The free base of **9c** was purified by radial chromatography, as described above. The total yield of the oil **9c** was 1.1 g (71%). However, the HCl and sulfate salts of **9c** were hygroscopic. Data for the free base **9c**: oil; ¹H NMR (CDCl₃) δ 7.81 (d, *J* = 8.4 Hz, 2 H, NH₂), 7.65 (s, 4 H, H$_{Ar}$), 7.19–7.53 (m, 8 H, H$_{Ar}$), 5.18 (m, 1 H, α-C-*H*), 4.13 (q, *J* = 7.5 Hz, 2 H, CH₃C*H*₂), 3.34 (m, 1 H, Leu α-C-*H*), 3.08 (d, *J* = 7 Hz, 1 H, α-CHC*H*₂Ar), 3.04 (t, *J* = 7.8 Hz, 2 H, ArC*H*₂CH₂CO₂Et), 2.90 (m, 1 H, α-CHC*H*₂), 2.88 (s, 3 H, NC*H*₃), 2.7 (s, 3 H, NC*H*₃), 2.68 (m, 2 H, ArCH₂C*H*₂CO₂Et), 1.3–1.6 (m, 2 H, Leu C*H*₂CH(CH₃)₂, 1.34 (m, 1 H, Leu CH₂C*H*(CH₃)₂), 1.24 (t, *J* = 7.1 Hz, 3 H, C*H*₃CH₂), 0.90 (d, *J* = 6.5 Hz, 3 H, Leu HC(C*H*₃)₂), 0.89 (d, *J* = 6.4 Hz, 3 H, Leu HC(C*H*₃)₂); IR (film) 3498, 3190, 3053.9, 2956.9, 1723.4, 1691.8, 1636.2, 1605.3, 1149.9, 1050, 850.0 cm⁻¹.

Lithium (S-Leucyl-3-[4-[3′-(carboxyethyl)biphenyl)]yl]-(S)-phenylalanine N,N-Dimethylcarboxamide (10b). Amino ester **8b** (0.8 g, 1.44 mmol) was dissolved in 3 mL of 6:3:1 THF/MeOH/H₂O. To this solution was added LiOH·H₂O (0.060 g, 1.66 mmol) and the mixture heated at a gentle reflux for 24 h. The yellow solution was cooled to room temperature, 10 mL of 1:1 MeOH/toluene was added, and the solution was evaporated in vacuo. The yellow solid was placed on a high-vacuum line (0.05 mm)

overnight. The crude yellow solid (0.78 g) was purified by radial chromatography (2-mm plate, 100:15:1 CHCl₃/MeOH/H₂O) to afford 0.65 g (78%) of **10b** as a white foam: mp 142–144 °C; ¹H NMR (CD₃-CO₂D) δ 7.68 (s, 4 H, H$_{Ar}$), 7.2–7.6 (m, 8 H, H$_{Ar}$), 5.31 (m, 1 H, α-C-*H*), 4.2 (m, 1 H, Leu α-C-*H*), 3.0–3.2 (m, 2 H, α-CHC*H*₂), 3.0 (t, *J* = 7.7 Hz, 2 H, ArC*H*₂CH₂CO₂Et), 2.85 (s, 3 H, NC*H*₃), 2.78 (s, 3 H, NC*H*₃), 2.73 (t, *J* = 7.6 Hz, 2 H, ArCH₂C*H*₂CO₂Et), 1.7 (m, 2 H, Leu CHC*H*₂), 1.25 (m, 1 H, Leu C*H*CH₂), 0.83–0.90 (m, 6 H, Leu HC(C*H*₃)₂); IR (KBr) 3300, 3100, 2926.2, 2400, 1634.0, 1590, 1250, 1000, 900, 841.0 cm⁻¹; [α]²⁰$_D$ +12° (*c* = 0.5, 100 mm, CHCl₃); HRMS, FAB, calcd for C₃₂H₃₈O₄N₃Li 535.3022, calcd for C₃₂H₄₀O₄N₃ 530.3019, found 530.3016 (M⁺ – Li⁺ + 2H⁺, 100). Anal. Calcd for C₃₂H₃₈O₄N₃Li: C, 7.176; H, 7.15; N, 7.85. Found: C, 7.127; H, 7.52; N, 7.22.

Lithium (S)-Leucyl-3-[4-(4-(carboxyethyl)biphenyl)]yl]-(R)-phenylalanine N,N-Dimethylcarboxamide (10c). **10c** was prepared from **8c** exactly as **10b**. The Li salt of **10c** was hygroscopic and could not be purified satisfactorily so it was carried directly on to the next step: mp 150–160 °C dec; ¹H NMR (CD₃CO₂D) δ 7.69 (s, 4 H, H$_{Ar}$), 7.2–7.6 (m, 8 H, H$_{Ar}$), 5.41 (m, 1 H, α-C*H*C*H*₂), 4.02 (m, 1 H, Leu α-C-*H*), 3.0–3.2 (m, 2 H, α-CHC*H*₂), 3.01 (t, *J* = 7.8 Hz, 2 H, ArC*H*₂CH₂CO₂Et), 3.11 (s, 3 H, NC*H*₃), 2.95 (s, 3 H, NC*H*₃), 2.73 (t, *J* = 7.6 Hz, 2 H, ArCH₂C*H*₂-CO₂Et), 1.25–1.4 (m, 3 H, Leu C*H*CH₂), 0.70–0.73 (m, 6 H, Leu HC-(C*H*₃)₂); IR (KBr) 3300, 3100, 2926.2, 2400, 1634.0, 1590, 1250, 1000, 900, 841.0 cm⁻¹; HRMS, FAB, calcd for C₃₂H₃₈O₄N₃Li 535.3022, calcd for C₃₂H₄₀O₄N₃ 530.3019, found 530.2995 (M⁺ – Li⁺ + 2H⁺, 100).

***cyclo*-(S)-Leucyl-3-[4-[3′-[(methoxycarbonyl)ethyl]yl]-(S)-phenylalanine (13b).** The acid **6** (4.85 g, 7.77 mmol) was hydrogenated with 5% Rh/C (1 g) at 1 atm in 50 mL of MeOH/CHCl₃ 3:1 for 3 days. The catalyst was filtered off with the aid of Celite in a sintered glass funnel. The filtrate was concentrated in vacuo and the residue partitioned between 1 M aqueous Na₂CO₃ and ether. The ether later was dried over K₂CO₃ and concentrated in vacuo to provide a clear oil. The oil was immediately fractionated into the unstable diastereomeric free amines **12b** and **12c** via radial chromatography (4-mm plate, 0–10% MeOH/CHCl₃) and characterized as rapidly as possible by ¹H NMR and ¹³C NMR DEPT spectroscopy. Upon standing 3 days, the amines partially converted into the diketopiperazines **13b,c**, which were then purified by radial chromatography (4-mm plate, 10–20% MeOH/CHCl₃). Data for **13b**: mp 194–196 °C; ¹H NMR (CDCl₃/CD₃Od, 4:1) δ 7.61 (s, 4 H, H$_{Ar}$), 7.15–7.52 (m, 8 H, H$_{Ar}$), 4.31 (t, *J* = 4.3 Hz, 1 H, Leu α-C-*H*); 3.65 (s, 3 H, C*H*₃O₂C), 3.02 (dd, *J* = 4.7 Hz, 2 H, CHC*H*₂), 2.99 (t, *J* – 7.8 Hz, 2 H, ArC*H*₂CH₂CO₂Me), 2.95 (m, 1 H, α-C*H*CH₂Ar), 2.68 (t, *J* = 7.8 Hz, 2 H, arCH₂C*H*₂CO₂Me), 1.00 (m, 2 H, LeuC*H*∞CH(CH₃)₂), 0.69 (d, *J* – 6.9 (d, *J* = 6.5 Hz, 3 H, Leu HC(C*H*₃)₂), 0.62 (d, *J* = 6.5 Hz, 3 H, Leu HC(C*H*₃)₂), 0.14 (m, 1 H, Leu *H*C(CH₃)₂); IR (KBr) 3188, 3057, 2960, 1736, 1670, 1457, 1325, 1169, 841, 788 cm⁻¹; [α]²⁰$_D$ + 21° (*c* = 0.5, 100 mm, DMF); HRMS, Cl⁺, calcd for C₃₁H₃₄O₄N₂ 498.2518, found 498.2547 (M⁺, 42), 499.2605 (M⁺ + H, 100). Anal. Calcd for C₃₁H₃₄O₄N₂: C, 74.67; H, 6.87; N, 5.62. Found: C, 73.85; H, 6.82; N, 5.46.

***cyclo*-S-Leuyl-3-[4-[3′-[(methoxycarbonyl)ethyl]biphenyl)]yl]-(R)-phenylalanine (13c).** mp 212–214 °C; ¹H NMR (CDCl₃/CD₃OD, 4:1) δ 7.61 (s, 4 H, H$_{Ar}$), 7.15–7.52 (m, 8 H, H$_{Ar}$), 4.26 (t, *J* = 4.5 Hz, 1 H, Leu α-C-*H*); 3.65 (s, 3 H, C*H*₃O₂C), 3.20 (m, 2 H, CHC*H*₂Ar), 2.99 (t, *J* = 7.8 Hz, 2 H, ArC*H*₂CH₂CO₂Me), 2.95 (m, 1 H, α-C*H*₂CHCH₂-Ar), 2.68 (t, *J* = 7.9 Hz, 2 H, ArCH₂C*H*₂CO₂Me), 1.59 (m, 2 H, Leu C*H*₂CH(CH₃)₂), 1.43 (m, 1 H, Leu CH₂C*H*(CH₃)₂), 0.81 (d, *J* = 6.7 Hz, 3 H, Leu HC(C*H*₃)₂), 0.71 (d, *J* = 6.3 Hz, 3 H, Leu HC(C*H*₃)₂); IR (KBr) 3188, 3057, 2960, 1736, 1670, 1457, 1325, 1169, 841, 788 cm⁻¹; [α]²⁰$_D$ –16° (*c* = 0.5, 100 mm, DMF); HRMS, FAB⁺, calcd for C₃₁H₃₄O₄N₂ 498.2518, found 498.2501 (M⁺, 33), 499.2586 (M⁺ + H, 100).

***cyclo*-[Leucyl-3-[4-(3′-propanoylbiphenyl)yl]-(R)-phenylalanine N,N-Dimethylcarboxamide]₂. Macrolactam Host (11c).** To a 4-mL solution of amino acid **10c** (0.192 g, 0.359 mmol) in dry DMF in a 3-L round-bottom flask was added 0.64 mL (1.0 mmol) of Hunig's base followed by 1.5 L of absolute THF. The clear solution was cooled to 0 °C under N₂. BOP (475 mg, 1.07 mmol) was then added as a 1-mL DMF solution. The reaction mixture was stirred for 4 h at 0 °C and then allowed to warm to room temperature gradually over 12 h. The reaction mixture was subjected to workup A. The crude product was purified by two radial chromatographies using a 1:1 CHCl₃/THF 0–10% MeOH gradient on a 2-mm plate. The product crystallized out of the test tubes collected in the second chromatography when allowed to stand overnight. The white solid was collected and dried to yield 20 mg (11%): mp 298–300 °C dec; ¹H NMR (CDCl₃) δ 7.61 (s, 8 H, H$_{Ar,mid}$), 7.49 (d, *J* = 7.4 Hz,

11c

Figure 8. Designation of the protons in host **11c**.

2 H, H_{Ar3}), 7.44 (d, $J = 7.8$ Hz, 2 H, H_{Ar4}), 7.44 (s, 4 H, H_{Ar1}, H_{Ar2}), 7.35 (m, 4 H, H_{Ar5}, H_{Ar6}), 7.18 (d, $J = 7.6$ Hz, 2 H, H_{Ar7}), 7.09 (d, $J = 7.7$ Hz, 2 H, H_{Ar8}), 6.64 (d, $J = 7.7$ Hz, 2 H, H-H_A), 5.76 (d, $J = 8.6$ Hz, 2 H, N-H_B), 5.18 (dd, $J = 7.3$ Hz, $J = 7.0$ Hz, 2 H, α-C-H_A), 4.42 (m, 2 H, α-C-H_B), 2.9–3.1 (m, 8 H, ArCH_2CHCON(CH$_3$)$_2$ and ArCH$_2$CH_2CONH), 2.92 (s, 6 H, N(CH_3)$_2$), 2.81 (s, 6 H, N(CH_3)$_2$), 2.5–2.6 (m, 4 H, ArCH_2CH$_2$CONH), 1.1–1.3 (m, 6 H, CH_2CH(CH$_3$)$_2$), 0.71 (d, $J = 6.2$ Hz, 6 H, CH$_2$CH(CH_3)$_2$), 0.64 (d, $J = 6.2$ Hz, 6 H, CH$_2$CH(CH_3)$_2$); ^{13}C NMR (CDCl$_3$) δ 171.6, 171.4, 170.8, 141.2, 140.9, 140.7, 140.1, 139.5, 136.8, 128.9, 128.8, 128.8, 128.3, 128.1, 127.5, 127.3, 127.0, 126.9, 125.6, 124.8, 51.5, 50.1, 41.9, 39.7, 38.1, 36.9, 35.8, 31.5, 24.7, 22.9, 22.0; IR (CDCl$_3$, 2 mM, 1-mm cell path) 3425, 3037, 2962, 2935, 2876, 1653, 1508 cm^{-1}; $[\alpha]^{20}_D$ $-35°$ ($c = 0.36$, 100 mm, CHCl$_3$); HRMS, FAB$^+$, calcd for C$_{64}$H$_{74}$O$_6$N$_6$ 1022.5670, found 1022.5660 (M$^+$, 25), 1023.5733 (M$^+$ + H, 100). (See Figure 8.)

cyclo-[(S)-Leucyl-[3-[4-(3′-propanoylbiphenyl)yl]-(S)-phenylalanine *N,N*-dimethylcarboxamide]$_2$. **Macrolactam Host (11b).** **11b** was prepared as above from **10b** in 14% yield: mp 286–290 °C dec; ^{1}H NMR (CDCl$_3$) δ 7.61 (AB quartet, 8 H, $H_{Ar,mid}$), 7.50 (d, $J = 7.2$ Hz, 2 H, H_{Ar3}), 7.43 (s, 2 H, H_{Ar1}), 7.39 (d, $J = 8.0$ Hz, 2 H, H_{Ar4}), 7.30–7.35 (m, 4 H, H_{Ar5}, H_{Ar6}), 7.32 (s, 2 H, H_{Ar2}), 7.16 (d, $J = 7.7$ Hz, 2 H, H_{Ar7}), 7.09 (d, $J = 7.1$ Hz, 2 H, H_{Ar8}), 6.85 (d, $J = 8.1$ Hz, 2 H, N-H_A), 5.67 (d, $J = 8.0$ Hz, 2 H, N-H_B), 5.14 (m, 2 H, α-C-H_A), 4.47 (m, 2 H, α-C-H_B), 2.9–3.0 (m, 8 H, ArCH$_2$CHCON(CH$_3$)$_2$ and ArCH_2CH$_2$CONH), 2.92 (s, 6 H, N(CH_{3U})$_2$), 2.76 (s, 6 H, N(CH_{3D})$_2$), 2.4–2.5 (m, 4 H, ArCH$_2$-CH$_2$CONH), 1.4–1.6 (m, 6 H, CH_2CH(CH$_{3Y}$)$_2$), 0.88 (d, $J = 6.3$ Hz, 6 H, CH$_2$CH(CH_{3X})$_2$), 0.87 (d, $J = 6.1$ Hz, 6 H, CH$_2$CH(CH_3)$_2$); IR (CDCl$_3$, 2 mM, 1-mm cell path) 3425, 3037, 2962, 2935, 2876, 1653, 1508 cm^{-1}; $[\alpha]^{20}_D$ $-22°$ ($c = 0.39$, 100 mm, CHCl$_3$); HRMS, FAB$^+$, calcd for C$_{64}$H$_{74}$O$_6$N$_6$ 1022.5670, found 1022.5651 (M$^+$, 16), 1023.5720 (M$^+$ + H, 100).

Molecular Modeling. All molecular modeling was performed on a Silicon Graphics Personal Iris workstation using Polygen's QUANTA software package (version 3.2.3). The Chemnote application was used to assign atom types and partial atomic charges and to construct structures. The crude structures were then energy minimized with the CHARMm

force field using the adopted-basis Newton–Raphson algorithm with the distance dependent dielectric term set at 10. Molecular dynamics were carried out by starting with a minimized structure at 0 K and slowly heating the structure to 900 K over a 10–20-ps time period. This was followed by an equilibration at this temperature over another 20-ps period. The structure was then allowed to sample conformational space freely for 50–100 ps. Selected conformations generated in the dynamics trajectory were further energy minimized until they reached convergence. The molecular docking simulations involved placing an energy-minimized guest peptide in the center cavity of the host structure so that it was in a favorable position for intermolecular hydrogen bond formation and subjected to energy minimization. The criteria for a hydrogen bond was considered to be a N–H–O bond length and dihedral angle of less than 3.1 Å and greater than 130°, respectively.

Calculation of Binding Constants. The association constants of the host–guest complexes were obtained at host concentrations of 2 mM using ^{1}H NMR in the following manner: 0.5 mL of a 2 mM CDCl$_3$ solution of the host was added to a dry NMR tube under nitrogen. Stock solutions of a diketopiperazine guest in CDCl$_3$ ranging from 20 to 50 mM were prepared and added in 10–100-uL aliquots to the host, and the spectra was recorded. In most cases, saturation was achieved after 10 or more equivalents of guest were added and the chemical shifts reached constant values. This allowed for at least 20 or more data points to be used in the calculation of the binding isotherm. The curve-fitting process gives the K_a and the ν max, which is the theoretical value of δ, the host resonance in the complex.[14] Essentially, the difference in the host N–H$_B$ chemical shift between the complexed and the free state of the host was plotted against the change in concentration of the added guest, producing a curve that shows a saturation-type behavior. The calculation is based on a one-to-one binding model, which, on the NMR time scale, is under fast exchange conditions; thus, the resonances observed represent an averaged value of δ for the free and bound species. Although changes in the chemical shifts of other protons of the hosts occurred upon titration with the guests (0.1–0.2 ppm), they were usually not nearly as large as in the case of the N–H$_B$ proton of the hosts (1–2 ppm). Thus, the association constants calculated from the small chemical shift changes of host protons other than N–H$_B$ are inherently less accurate due to the increased uncertainty associated with their measurement. However, these K_a values, in general, did agree roughly with those based solely on the N–H$_B$ chemical shifts. In this report, the K_a values presented are based exclusively on the observed changes in the host N–H$_B$ chemical shifts. We assign a value of 15% uncertainty to the K_a values. The CDCl$_3$ used in these titrations was always freshly distilled from CaH$_2$. The concentration of residual water[13] in the CDCl$_3$ titration solutions was approximately 10 mM, as assessed by the integration of the residual HDO peak at 1.59 ppm, and did not appreciably increase as the titration progressed.