Tripodal Peptides with Chiral Conformations Stabilized by Interstrand Hydrogen Bonds

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Abstract: C_3 symmetric trispeptides are described that form chiral conformations and are therefore eminently suited to provide a new family of chiral receptor molecules when extended by appropriate binding sites. These trispeptides are composed of C_3 symmetric trisamines as anchors and three symmetrically extending chiral amino acid residues. Their conformations in apolar solvents fall into two main classes. One is comprised of propeller-like conformations of preferred chiral sense that are stabilized by a belt of intramolecular H-bonds (hydrogen bonds) between adjacent strands. The other class has two of its strands connected by two H-bonds to form a 10-membered ring, while the third strand may hydrogen-bond to one of the other two. The effect of the anchors and amino acids on the relative stability of the H-bonded, chiral conformations has been established by a combination of spectroscopic and theoretical means. Trispeptides derived from more lipophilic α-amino acids show a higher population of the chiral conformations. Moreover, trispeptides that are based on tris(2-aminoethyl)amine (TREN) as anchor form stronger H-bonds than those relying on 1,3,5-tris(aminomethyl)benzene (TRAM).

1. Introduction

One of the outstanding characteristics of natural ionophores is their capability of combining high binding selectivity with high ion exchange rates.[1,2] These features are believed to derive from their interconversion from ion-binding to ion-releasing conformations at the membrane-water interface.[1] Artificial ion carriers, on the other hand, have until now relied mainly on either rigid structures to achieve high selectivity[3-6] or conformationally relaxed structures to guarantee fast ion exchange rates.[7,8] In an attempt to provide entrance to an alternative class of artificial ion carriers that would mimic the properties of the natural molecules, we sought to design and synthesize molecules that will exhibit solvent-dependent conformations.

In this article we describe the synthesis and structural properties of novel families of tripodal compounds where three peptide strands are attached to a common C_3 symmetric anchor and their conformational freedom is restricted by a belt of H-bonds (hydrogen bonds) that connect each of the peptide strands with its neighbors.[9] The interlinked H-bonds generate a propeller-like structure which defines an inner space. This type of "H-bonding belt" is reminiscent of the belt of H-bonds that occurs in the macrocyclic ionophore valinomycin[10] and in some synthetic macrocyclic polypeptides.[11] This type of arrangement may have either a right-handed or a left-handed helical sense, depending on the directionality of the H-bonds. In the presence of chiral centers the two alternatives become diastereoisomeric and therefore one of the two arrangements is energetically preferred.

The structures described here were designed (i) to be readily assembled in a modular fashion and (ii) to accommodate chiral centers and generate chiral recognition sites. Furthermore, they were designed to be extendable to provide binders that form polyhedral (e.g., octahedral) binding cavities.

Two families of tripodal peptides derived from chiral α-amino acids were synthesized and examined. One family was based on linking α-amino acids to 1,3,5-tris(aminomethyl)benzene (TRAM),[12] the other on linking α-amino acids to tris(2-aminoethyl)amine (TREN).[12]

We had initiated our study with achiral α, β-, and γ-amino acids when bound to 1,3,5-tris(aminomethyl)benzene (TRAM) as anchor (Figure 1). A combination of IR and NMR spectroscopy, including temperature and solvent effects, indicated the superiority of the α- and γ- over β-amino acids in forming stable H-bonded conformations.[13] Since α-amino acids have less degrees of freedom and since there is a broad arsenal of naturally occurring, optically pure α-amino acids available, we concentrated in our present work on trispeptides derived from chiral α-amino acids.

2. Synthesis

The synthesis of the trisamine-based tripodal peptides was accomplished according to Figure 2. The TRAM and TREN based trispeptides **1** and **4** and the ester analog **3** were prepared by activating Boc-amino acids (NHS/DCC),[12] followed by coupling with 1,3,5-tris(aminomethyl)benzene (TRAM), tris(2-aminoethyl)amine (TREN), or 1,3,5-tris(hydroxymethyl)-benzene,[14] respectively. Their corresponding single strand analogs **2** and **5** were prepared by replacing the trisamines by benzylamine and *n*-propylamine, respectively. For details see Experimental Section.

3. Methods

3.1. Spectroscopic Measurements. Infrared spectroscopy established the involvement of the amide NH groups in H-bonding by the occurrence of a red (bathochromic) shift of the amide NH stretching frequency.[15,16] IR measurements down to very low concentrations (5×10^{-4} M) indicated the importance of intra-

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(12) Abbreviations used: Boc, *tert*-butoxycarbonyl; NHS, *N*-hydroxysuccinimide; DCC, dicyclohexylcarbodiimide; THF, tetrahydrofuran; TRAM 1,3,5-tris(aminomethyl)benzene; TREN, tris(2-aminoethyl)amine; and VCD, vibrational circular dichroism.
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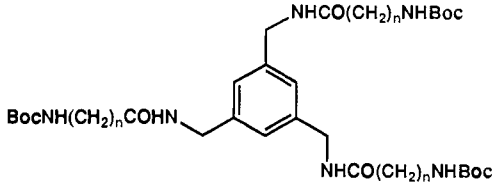

n = 1, 2, 3

Figure 1. TRAM-based trispeptides containing glycyl (n = 1), β-alanyl (n = 2), and γ-aminobutyryl (n = 3) residues.

molecular H-bonding. High-resolution ^{1}H NMR established the occurrence of conformational restrictions by the appearance of pronounced nonequivalence of diastereotopic protons such as ArCH_2NH in TRAM or NCH_2CH_2NH in TREN derivatives.[17,18] Temperature and solvent effects on the NMR shifts of the amide protons N*H*CO established the thermal lability of the H-bonds, when present, and the extent to which they are exposed to the solvent.[19] Comparison between the triple stranded trispeptides and their corresponding single stranded monopeptides helped us to distinguish between intrastrand and interstrand H-bond. We used chloroform, methanol, and DMSO as solvents of different polarities.

3.2. Theoretical Calculations. Experimental and theoretical studies have been linked throughout all stages of the research. Preliminary calculations yielded the conformations and energies of molecules considered for synthesis. Further calculations yielded, through a thorough conformational search, the molecular equilibrium conformations of lowest energy as well as other metastable conformations. These supplemented the IR, NMR, and other measurements with conformational and energetic data not obtainable in other ways.

The empirical force field (EFF) method employed in the present study was the same as used previously,[20] with the same energy functions and parameters. Calculations were made for single molecules, thus ignoring intermolecular and solvent effects. Such calculations yield often reliable predictions of some of the properties of molecules in dilute nonpolar solutions as well as in crystals.

4. Results and Discussion

4.1. Structural Properties of TRAM-Based Trispeptides. Each strand in a trispeptide molecule contains two amide groups, the TRAM amide, TRAM-NHCO, and the Boc carbamate, NHCO-Boc. Therefore intrastrand H-bonds may in principle be formed in two ways (Figure 3) and interstrand H-bonds in many ways. The interstrand H-bonding linkages may form either C_3 symmetric or nonsymmetric conformations. In the C_3 symmetric conformations the three interstrand NH···OC H-bonds may either occur between the TRAM-NH and O=C-Boc or between the Boc-NH and O=C-TRAM and may follow each other with either a clockwise or anticlockwise sense. For trispeptides with L-amino acid residues the two senses are diastereoisomers, and one may be preferred energetically (Figure 4). We will try to establish in what follows which among the possible conformations predominate in reality.

4.1.1. IR Spectroscopic Examination. The IR spectra of four TRAM-based α-amino L-trispeptides **1**, with leucyl **1a**, isoleucyl **1b**, valyl **1c**, and alanyl **1d**, have been measured in dilute chloroform solutions (5×10^{-4} M). Figure 5 presents them in the range of 3200–3600 cm^{-1}. They all exhibit a narrow absorption band with maxima in the range of 3436–3438 cm^{-1}, typical of a free amide NH stretching frequency in a chloroform solution. They also all exhibit lower frequency absorption bands that peak roughly in the range of 3320–3360 cm^{-1}, typical of H-bonded NH stretching frequencies. These bands are rather broad and vary considerably in form and size among the difference compounds of **1**, indicating a variety of H-bonding patterns. The IR spectrum of the trispeptide **1a** at 100-fold higher concentration,[21] namely 5×10^{-2} M, showed a similar trace, with a comparable intensity around 3350 cm^{-1}, but enhanced intensity around 3300 cm^{-1}. This implies that the 3350-cm^{-1} absorption belongs to intramolecular H-bonds, while the 3300-cm^{-1} absorption is an overlap of intra- and intermolecular H-bonds. Vibrational circular dichroism (VCD) confirmed and extended this analysis, by resolving the two absorptions into a highly dichroic one for the higher frequency band and a weakly dichroic one for the lower band.[21] The IR absorption of **2**, the single strand analog of **1**, exhibited an essentially free NH at 3437 cm^{-1} with only a trace of H-bonded NH at 3350 cm^{-1} in the same solvent. This indicates that the observed H-bonds in chloroform solutions of trispeptides **1** are mainly between the strands.

The IR spectrum of **3** in dilute $CDCl_3$ solution shows a strong free NH adsorption at 3444 cm^{-1}, with a very weak adsorption at 3270 cm^{-1}. The negligible presence of H-bonded structures in the triester suggests (i) that the TRAM amide is involved in the H-bond network of the trispeptides **1** and (ii) that H-bonding between Boc-amides is unfavorable.

The extent of H-bonding of the different trispeptides **1** in chloroform increased regularly with the bulkiness of the amino acid side chains. This was estimated by the ratios of the absorption intensities, I, of the H-bonded and free NH frequency bands. (The ratios $I(NH_{bonded})/I(NH_{free})$ given in Figure 6 are the ratios of the IR absorbance intensities of the highest frequency bonded NH, assigned to intramolecular H-bonds, over that of the nonbonded NH.) These ratios are proportional to the ratios between the bonded and free states, the proportionality factor being the ratio between their extinction coefficients. Figure 6 represents the correlation between $I(NH_{bonded})/I(NH_{free})$ and two hydrophobicity scales, which are parallel to the side chains bulkiness. One is due to Eisenberg and the other due to Tanford.[22] It clearly reveals that the H-bonding increases with increasing bulkiness of the R group of the α-amino acid residue.

4.1.2. NMR Spectroscopy. Conformational restriction by interstrand H-bonds should manifest itself by enhanced nonequivalence of the diastereotopic protons in the NMR spectrum.[17] Indeed, the NMR spectrum of the trispeptide **1a** (Figure 7) showed a pronounced difference in the chemical shifts of the diastereotopic ArCH_1H_2 protons, which by far exceeded that commonly encountered for diastereotopic protons of free rotating amino acid derivatives. In contrast, the single strand reference molecule **2** and the triester **3** show practically identical chemical shifts for the same diastereotopic protons under the same conditions. The anisotropy of the diastereotopic ArCH_1H_2 protons in **1a** as expressed by the chemical shift difference of the diastereotopic protons, $\Delta\delta_{1,2}$, was not only preserved upon dilution but also even increased from $\Delta\delta_{1,2}$ = 0.29 ppm at 10^{-2} M through $\Delta\delta_{1,2}$ = 0.383 ppm at 10^{-3} M to $\Delta\delta_{1,2}$ = 0.446 ppm at 10^{-4} M. This observation demonstrates that intramolecular interactions are the cause for the molecules' restricted conformation. The H-bonds observed in the IR spectra of the trispeptides **1** and the restricted conformational freedom manifested in their NMR spectra thus appear to be interrelated. Further support for this view was obtained when the observed nonequivalence of the diastereotopic protons in the trispeptide **1a** collapsed by replacing $CDCl_3$ by more polar CD_3OD or DMSO-d_6, which are known to destabilize intramolecular H-bonds. The single strand reference

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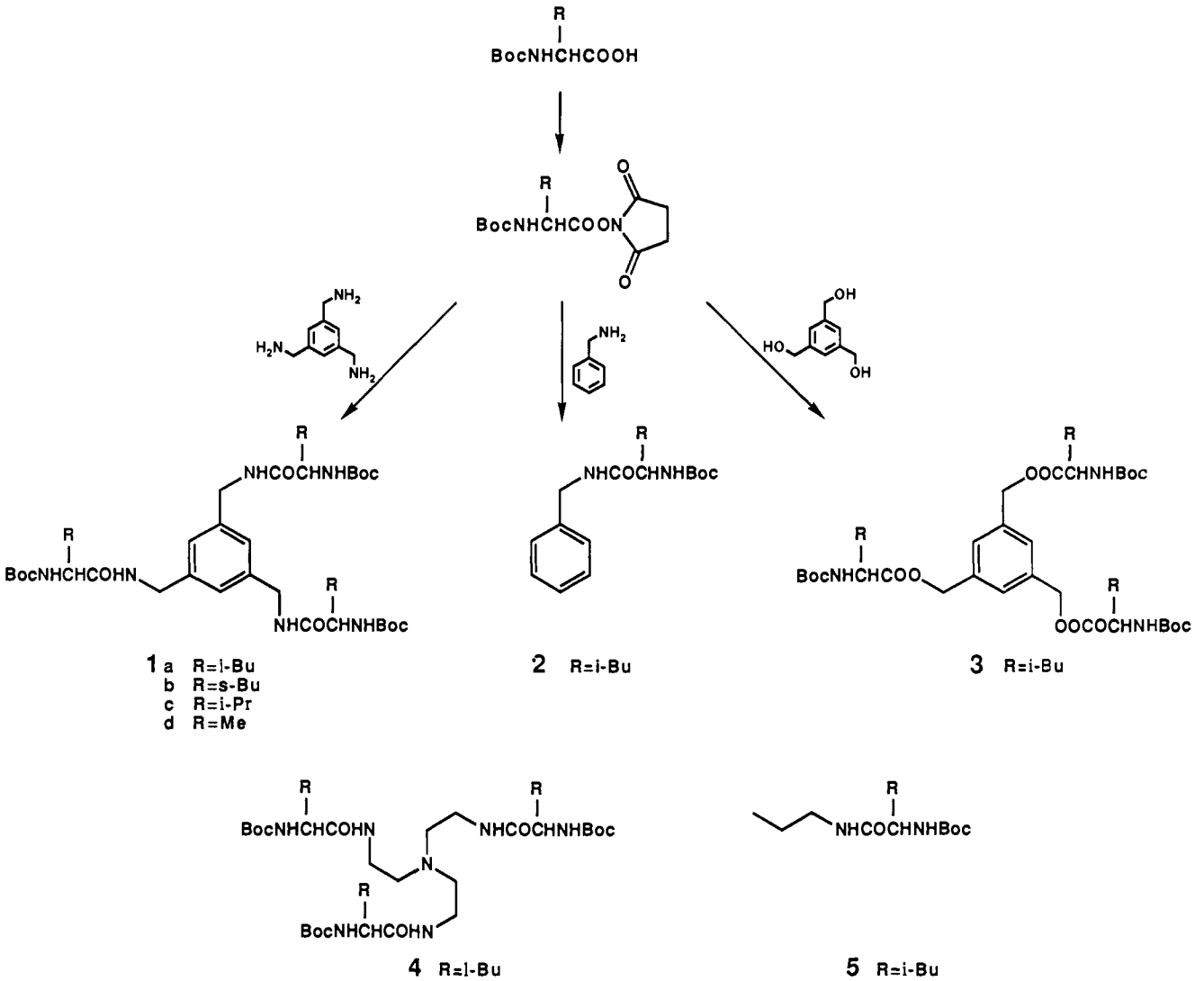

Figure 2. Synthesis of trispeptides and their single strand analogs.

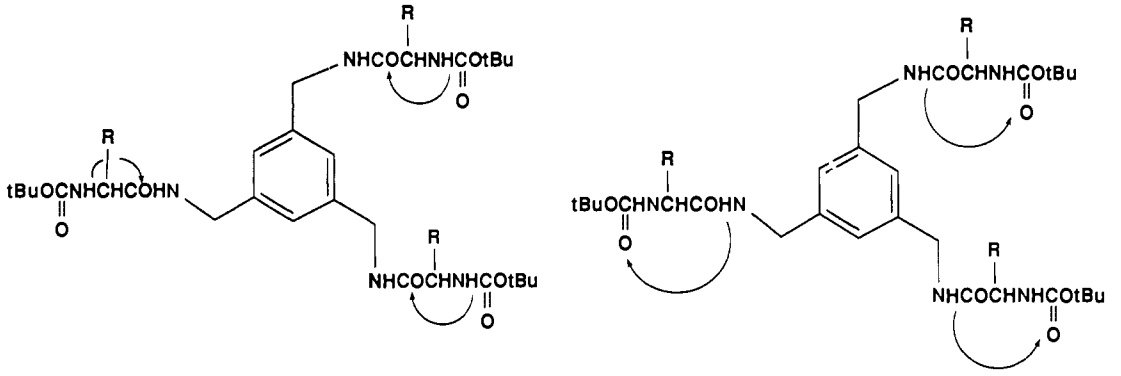

Figure 3. Schematic representation of five-membered (left) and seven-membered (right) intrastrand H-bonds.

Table I. ^{1}H NMR Chemical Shifts for Trispeptide **1a** and Monopeptide **2** in Various Solvents[a]

	trispeptide **1a**			monopeptide **2**		
proton	$CDCl_3$	CD_3OD	DMSO-d_6	$CDCl_3$	CD_3OD	DMSO-d_6
CH_2NH	4.32[b] (4.24)[c] 4.00[b] (3.97)	4.34	4.20	4.43	4.37	4.26
$C_\alpha H$	4.43[b] (4.46)	4.12	3.99	4.12	4.09	3.97
BzN*H*	7.64 (7.85)	*d*	8.25	6.41	*d*	8.31
BocN*H*	5.43 (5.58)	*d*	6.84	4.82	*d*	6.89

[a] NMR spectra were recorded at 270 MHz, 5×10^{-3} M, 298 K, unless otherwise stated. [b] 293 K. [c] Values in parenthesis are for 2×10^{-2} M. [d] Not observed due to H/D exchange.

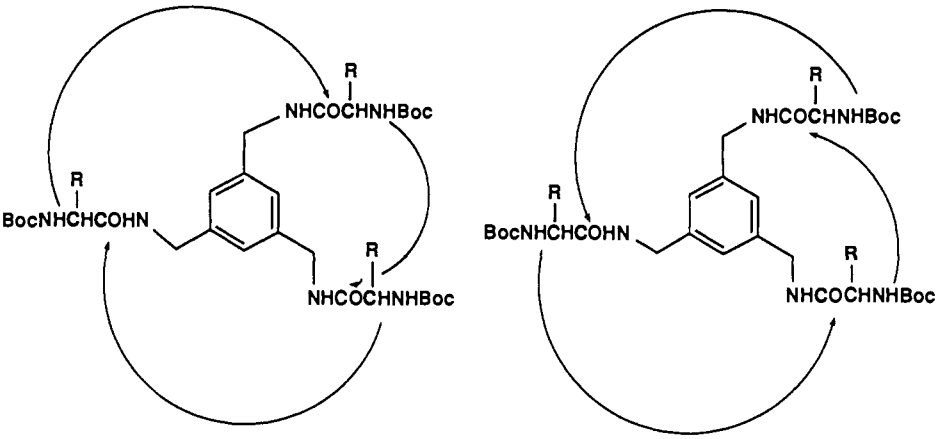

Figure 4. Schematic representation of alternative interstrand H-bonds.

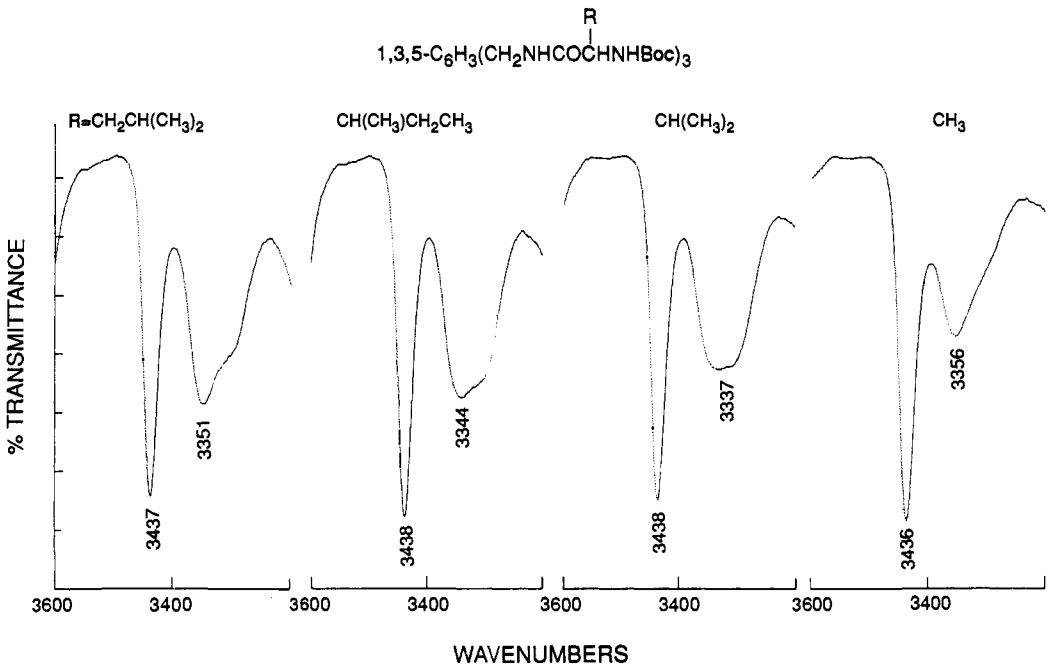

Figure 5. IR spectra of TRAM-based trispeptides in chloroform (5×10^{-4} M).

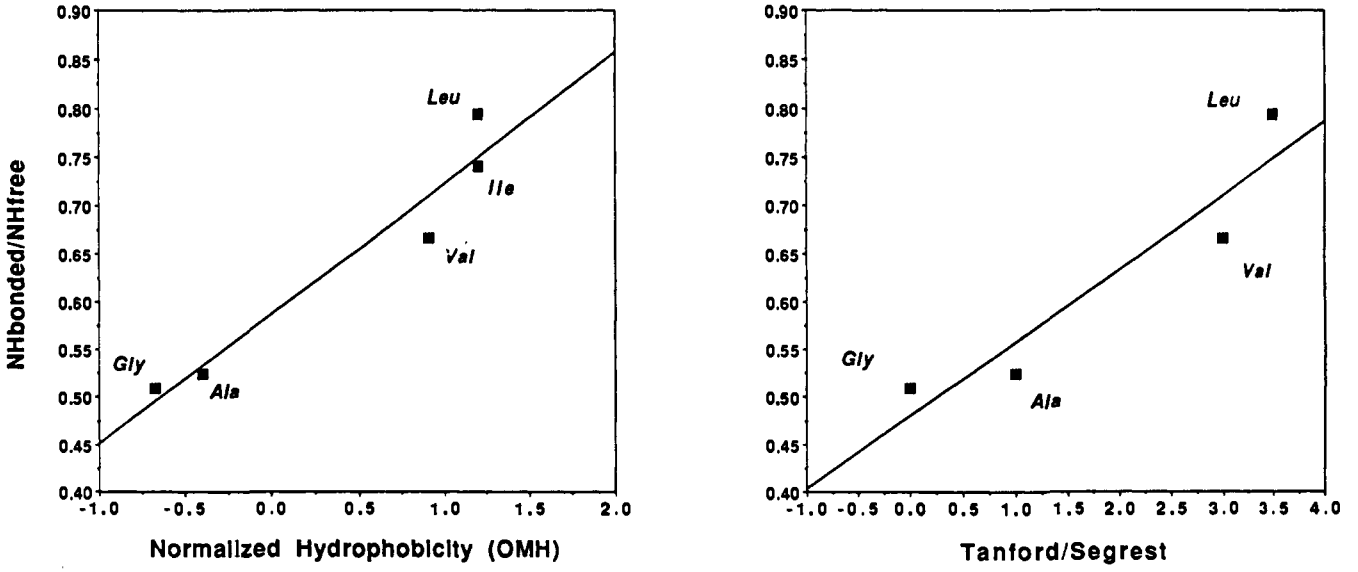

Figure 6. Correlation between $(I(NH_{bonded})/I(NH_{free})$ absorbance ratios and hydrophobicity scales for trispeptides 1.

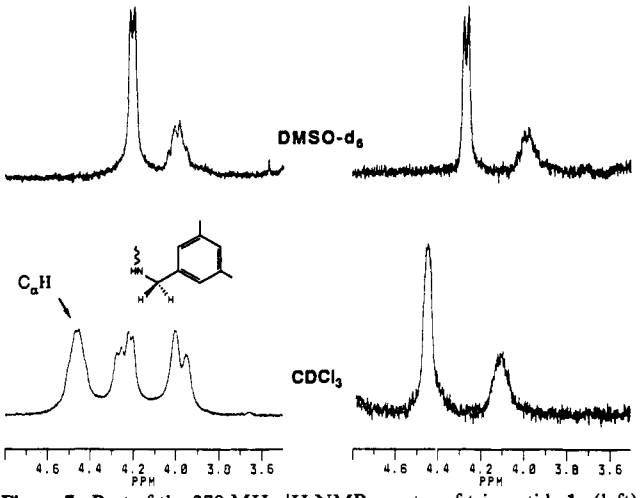

Figure 7. Part of the 270 MHz ^1H NMR spectra of trispeptide **1a** (left) and monopeptide **2** (right) in $CDCl_3$ and DMSO-d_6 (298 K; 1 in $CDCl_3$ is 2×10^{-2} M, all the rest 5×10^{-3} M).

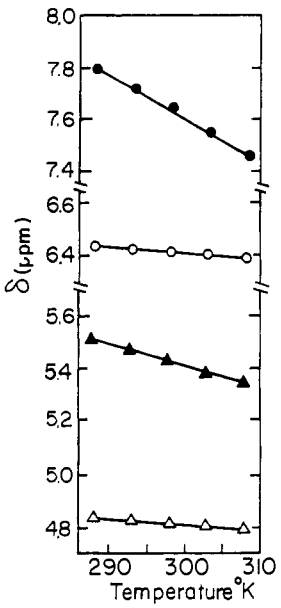

Figure 8. Temperature dependence of the NH NMR chemical shifts in $CDCl_3$ at 5×10^{-3} M for trispeptide **1a** (● = BzlNH, ▲ = BocNH) and single strand analog **2** (○ = BzlNH, △ = BocNH).

molecule **2**, on the other hand, showed only negligible solvent dependence of its NMR pattern (Table I and Figure 7).

The ^1H NMR anisotropy between the two diastereotopic methylene protons decreases with decreasing bulkiness of the side chains ($\Delta\delta$ = 0.34, 0.26, 0.24 ppm at 2.5×10^{-3} M concentrations for the leucyl, valyl, and alanyl trispeptide derivatives respectively). This trend suggests that the trispeptides with bulkier amino acids are conformationally more restricted and is consistent with the trend observed in the IR spectra, where the intensity of bonded NH decreases with decreasing bulkiness.

The temperature dependence of the amide NH chemical shifts of the trispeptide **1a** and its single chain analog **2** were measured in chloroform (Figure 8). The higher temperature effects in the trispeptide **1a** ($\Delta\delta/\Delta T$ = −0.0170 and −0.0086 ppm/K for the TRAM-NH and Boc-NH protons, respectively) than in the monopeptide **2** (−0.0026 and −0.0023 ppm/K, respectively) are suggestive of weakly bonded H-bonded conformations in equilibrium with nonbonded conformations.[19,23] At higher temperatures the chemical shifts for the TRAM-NH and Boc-NH protons in the trispeptide **1a** approach those in the monopeptide, in agreement with increasing populations of nonbonded conformations.

Addition of small increments of DMSO-d_6 to a $CDCl_3$ solution of **1a** was found to cause a smaller shift of the Boc-NH ($\Delta\delta$/%-DMSO-d_6 = + 0.00968) than of the TRAM-NH signal ($\Delta\delta$/%-DMSO-d_6 = −0.02488). These results imply that the Boc-NH forms stronger H-bonds than the TRAM-NH. Consequently, it is reasonable to suggest that the Boc-NH is the predominant H-donor, while the TRAM-NH is at least partially nonbonded. Since we have seen that the interstrand H-bonds stabilize the trispeptides, we may conclude that these bonds occur preferentially between the Boc-NH and the O=C-TRAM groups.

Attention should be drawn to the fact that the ^1H NMR spectra represent superpositions of the individual molecular conformations, as the NMR time scale does not permit resolution of the equilibrium mixtures into their components. A comprehensive picture of the conformational properties of the molecules is obtained, however, by combining the NMR results with the other experimental data as well as the EFF calculations. The latter provide also an estimate of the relative stability of the individual conformations, while confirming the major conclusions derived from experiment, as discussed below.

4.1.3. Theoretical Studies. Empirical force field (EFF) calculations helped to correlate the observed spectroscopic properties of the trispeptide family with specific molecular conformations and energies and to suggest structural details that could not be discerned experimentally. Two chiral TRAM trispeptides were calculated in detail, the modified leucyl **1a'** and the modified alanyl **1d'**, where the strands ended with a methyl instead of a butoxy group. We had to replace the terminal carbamates by amide groups because our force field represents faithfully neither the partial atomic charges nor the torsion potentials of the carbamate group RNHCOOR but has been proven reliable for amides. A detailed examination of the calculated results will be seen to supply ample evidence that the interstrand H-bonds of the calculated amidated trispeptides correspond closely to those of the synthesized trispeptides with terminal carbamate groups.

A systematic, exhaustive scanning of the conformational space of these two molecules yielded many low- and medium-energy conformations. All the low-energy conformations (within 2 kcal/mol of the absolute mininum) as well as most of the other conformations (up to 7 kcal/mol) were found to fall within three distinct classes. The first class, to be denoted by RH, comprises structures that are characterized by a right-handed helical twist of the three strands, stabilized by a ring of hydrogen bonds. The second class, denoted by r10, comprises conformations where two juxtaposed strands in antiparallel orientation to each other form two hydrogen bonds that close a 10-membered ring, while the third strand may hydrogen bond to one of the other strands. The third class is characterized by conformations that form a left-handed helical twist, LH, which are diastereoisomers of RH. The low-energy conformations of the TRAM-based leucyl trispeptide **1a'** that are within 2 kcal/mol of the lowest energy conformation are comprised of seven conformations of class RH, seven of class r10, and one of class LH. The corresponding numbers for the alanyl trispeptide **1d'** are 2, 6, and 1, respectively. Table II presents the most stable conformations of the three classes for these two molecules, **1a'** and **1d'**, as well as their relative energies and their patterns of hydrogen bonds. The more stable conformations RH and r10 included in Table II are visualized by stereoviews, Figures 9–12. All stereoviews are oriented as defined in the footnotes to Table II, with the strand S1 pointing upwards from the phenyl plane and S2 and S3 following counterclockwise. More detailed information of all the low-energy conformations up to 2 kcal/mol is available as supplementary material to this paper.

Two main conclusions may be deduced from the calculations. One deals with the common features of **1a'** and **1d'** and the other with the differences between them.

1. The TRAM trispeptides with L-amino acid residues adopt two main classes of conformations of roughly equal distribution. One class is characterized by conformations that form a right handed helix stabilized by a ring of hydrogen bonds. Its most

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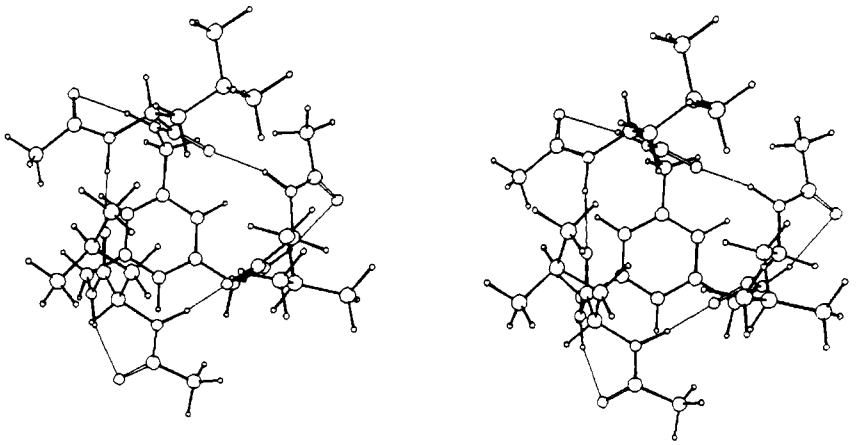


Figure 9. Stereoview of the lowest energy C_3 symmetric, RH conformation of trispeptide **1a'**. The hydrogen bonds are drawn thin. The intrastrand ones are seen to be unfavorably oriented. The NH···OC interstrand hydrogen bonds form a counterclockwise ring. The strands are bent counterclockwise away from the benzyl ring, as in a right handed helix.

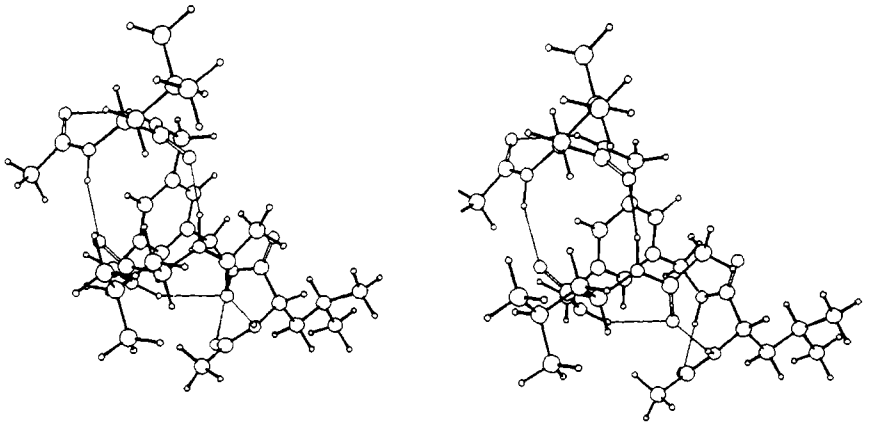
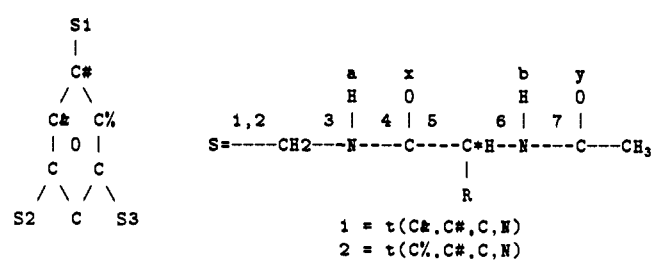


Figure 10. Stereoview of the lowest energy r10 conformation of **1a'**. The strands S1 and S2 are arranged antiparallel to each other to form two hydrogen bonds that close a 10-atom ring. The molecular axis is somewhat tilted to facilitate viewing.

Table II. Calculated Conformations of L-Leucyl and L-Alanyl Trispeptide **1a'** and **1d'**

												Hydrogen Bonds[e]			
		torsional angles[c]							ΔE[d]			interstrand		intrastrand	
class[a]	str.[b]	1	2	3	4	5	6	7	**1a'**	**1d'**		**1a'**	**1d'**	**1a'**	**1d'**
RH	S1	55	−121	60	−165	64	−88	176	0.0	0.2	b1−x2	1.93	2.02	2.14	2.10
r10	S1	−7	176	79	−172	77	−88	175	0.4	0.0	b1−x2	2.07	2.16	2.24	2.20
	S2	102	−74	94	−156	73	−91	171			b2−x1	1.93	1.98	2.30	2.26
	S3	114	−63	−67	168	−66	74	−176			b3−y2	2.10	2.11	1.95	1.95
LH	S1	104	−71	−80	161	−89	−102	179	1.1	1.5	b1−x3	2.36	2.32		
	S2	118	−60	−69	178	−62	71	174			b2−y1	2.05	2.05	1.91	1.90
	S3	126	−51	−56	166	−64	76	−176			b3−x2	2.11	2.14	1.96	1.95

[a] RH denotes a conformation with a right handed twist of the strands and a counterclockwise sense of the interstrand NH → OC hydrogen bonds, as seen in the stereoviews. LH has an opposite sense to RH. r10 is a conformation with two strands linked by two hydrogen bonds that form a 10-atom ring. [b] Strands, S1, S2, and S3 are numbered counterclockwise when viewed as extending above the plane of the mesitylene ring. The C_3 symmetric conformation RH is represented by its first line S1. [c] Angles in degrees, for the alanyl trispeptide **1d'**, defined according to the scheme below. The torsions for the corresponding leucyl **1a'** conformations are almost the same ±4°, except for LH, where it is ±6°. Detailed data for both **1a'** and **1d'** are included in the supplementary material. [d] Calculated EFF energy, kcal/mol, relative to the most stable conformation. [e] The interstrand hydrogen bonds are defined in a code in which the hydrogens of each strand are labeled as a and b, and the oxygens as x and y, respectively, as shown in the scheme below. The number following each letter identifies to which strand the atom belongs. This code is followed by the hydrogen bond length in angstroms. All intrastrand hydrogen bonds are a−y (seven-membered rings).



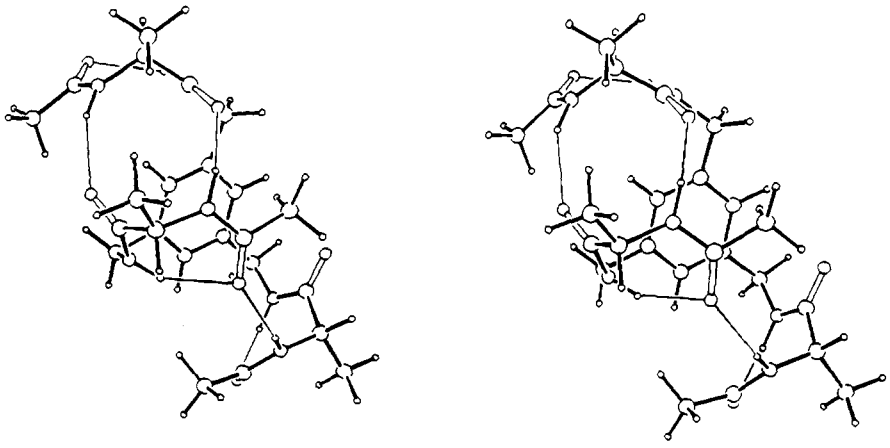


Figure 11. Stereoview of the lowest energy r10 conformation of trispeptide **1d'**. The H-bonds are drawn thin. The 10-atom ring stands out on top.

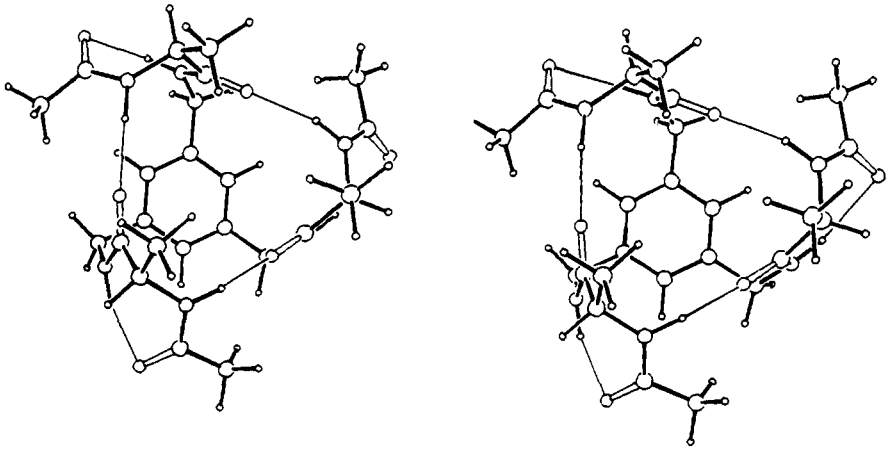


Figure 12. Stereoview of the lowest energy C_3 symmetric RH conformation of trispeptide **1d'**.

stable member adopts a C_3 symmetric chiral structure, while in the other, less stable members, the perfect symmetry is lost. The second class is characterized by a 10-membered ring formed by two of the three strands. An inspection of Table II and the corresponding stereoviews in Figures 10 and 11 shows that the 10-membered ring also possesses a chiral sense imposed by the chirality of the amino acid residues. The third class of LH type conformations has been calculated as the least stable of the three classes in both the leucyl and alanyl trispeptides.

The torsional angles representing the typical conformations of the three classes are presented in Table II only for the alanyl trispeptide, since those of the leucyl are very similar, varying at most within 4° in RH and r10, 6° in LH. An inspection of the stereoviews shows that in all cases the methyl groups of the alanyl residues are located on the surface of the molecule, such that their substitution by isobutyl groups can be accommodated without interfering with the pattern of hydrogen bonds.

In both RH and r10 conformations the strands are folded to form an apparent H-bonded seven-membered ring, with the β-carbon of the amino acid in an equatorial position. These apparent H-bonds are indicated in the figures, and their O···H distances are indicated in Table II. However, closer inspection of the stereoviews indicates that the C=O and NH bonds in these seven-membered rings are mostly unfavorably oriented for H-bonds to be formed. Similarly, inspection of Table II indicates that the interstrand H-bonds are mostly rather long. Therefore, such H-bonds are probably weak, if at all occurring. Consequently, the dominance of interstrand H-bonds in the calculated **1a'** and **1d'** are consistent with the experimentally observed H-bond patterns of the trispeptides.

2. In spite of their essential conformational similarities, **1a'** and **1d'** seem to differ to some extent. In the leucyl trispeptide the C_3 symmetric RH conformation is more stable by 0.4 kcal/mol than the asymmetric r10 conformation, while in the alanyl trispeptide r10 is more stable than RH by 0.2 kcal/mol. These differences are, admittedly, within the range of inaccuracy of the EFF, but they are supplemented by the trend of the interstrand hydrogen bond distances being systematically somewhat shorter in **1a'** than in **1d'** (Table II). Van der Waals attractions between the bulky isobutyl groups in **1a'** may be the cause of these small changes, as can be seen by observing the close contacts between all three isobutyls in Figure 9 and between those belonging to the two strands that form the 10-membered ring in Figure 10.

The above conclusions are corroborated by independent experimental results[21] and are consistent with the experimental results reported here. Although approximate, these calculations yield a comprehensive conformational description that supplements the experimental data and provides a coherent picture.

First, vibrational circular dichroism (VCD)[21] experiments demonstrated the existence of two bands, one strongly and one weakly dichroic, that were attributed to the IR spectra of the right handed helix (RH) and the 10-membered ring (r10) conformations, respectively, in accordance with our conclusion 1.

Second, the predicted interstrand hydrogen bonds between all the strands are consistent with the NMR results that indicate restriction of conformational freedom. Furthermore, the pronounced difference in the chemical shift of diastereotopic protons implies that RH and LH are not equally populated, in agreement with the calculated preference of RH.

Third, our conclusion 2 that the interstrand H-bonds are stronger in the leucyl derivative **1a** than in the alanyl derivative **1d** is corroborated by VCD,[21] IR, and NMR data. VCD spectroscopy yielded higher dichroism for the leucyl trispeptide than for the alanyl derivative and showed that the NH frequency of the C_3 conformation is by 20 cm^{-1} lower in the leucyl derivative **1a** than in the alanyl derivative **1d** and its dichroism 3-fold higher. The dichroism of the NH frequencies of the r10 conformations in the leucyl derivative **1a** is considerably weaker, and in the alanyl

derivative **1d** it is too weak to be detected. The same trend is also expressed in the IR data given in Figures 5 and 6, which show a larger ratio of nonbonded over bonded NH absorptions in the leucyl derivative **1a** than in the alanyl trispeptide **1d**. Furthermore, the more pronounced nonequivalence of the diastereotopic protons in the leucyl derivative **1a** than in the alanyl derivative **1d** in the NMR spectra indicates a higher population of interstrand H-bonded conformations with preferred chiral sense in the former.

4.2. Structural Properties of TREN-Based Trispeptide. The successful generation of C_3 symmetric, H-bonded conformations with TRAM-based trispeptides led us to consider other anchors that would impose less strain on the H-bonded polycyclic structures and thereby result in a higher population of the desired conformers. Toward this end TRAM was replaced by TREN. This replacement created trispeptide strands with one more conformational degree of freedom (of rotation around a single bond) than the strands of the TRAM trispeptides.

The IR spectrum of the TREN trispeptide **4** in dilute chloroform solution shows a weak free NH absorption (3440 cm^{-1}) and a strong bonded NH absorption (3332 cm^{-1}), suggesting stronger H-bonds than those of trispeptide **1a**. Little changes of the IR traces were observed in C_2Cl_4 within a concentration range of 5×10^{-4}–5×10^{-5} M, with retention of the amide-NH absorptions around 3300 cm^{-1}. The latter observation demonstrates the intramolecular nature of the H-bonds. The ^1H NMR spectrum of the trispeptide **4** in $CDCl_3$ shows a very high anisotropy for the diastereotopic methylene protons of the anchor ($CONHCH_1H_2$ $\Delta\delta_{1,2}$ = 0.54 ppm), indicating as before conformational restriction. This anisotropy is retained in the ^1H NMR spectrum with CD_3OD although its extent is significantly reduced ($\Delta\delta_{1,2}$ = 0.2 ppm). Furthermore, the Boc-NH proton does not undergo instantaneous H/D exchange in CD_3OD and is observed in a relatively low field (6.74 ppm). This is in marked contrast to the TRAM-based trispeptides **1**, where the Boc-NH's were never observed in CD_3OD due to a very fast H/D exchange. The temperature coefficients of the Boc-NH proton in $CDCl_3$ was found to be small (−0.0028 ppm/K) compared to the TREN-NH (−0.0055 ppm/K) and both were found to be smaller by a factor of 3 than the corresponding values of the TRAM-based trispeptide, which were −0.0086 and −0.0170 ppm/K, respectively. These observations support the conclusion that trispeptide **4** forms stronger H-bonds than the TRAM derivative **1a**. The single strand analogue of **4**, namely **5**, showed similar NMR and IR properties to that of the single strand analog **2**.

5. Conclusions

A new family of compounds was introduced where three peptide strands are attached to a common anchor of C_3 symmetry. These trispeptides exist as an equilibrium mixture of mainly two classes of conformations, each with a unique pattern of interstrand H-bonds. The first class comprises propeller-like conformations of preferred chiral sense that are stabilized by a belt of intramolecular H-bonds between adjacent strands. The second class is characterized by the presence of two hydrogen bonds between a pair of strands arranged antiparallel to form a 10-membered ring, while the third strand may form H-bonds to one of the other two. The first class is preferred in the leucyl trispeptide **1a**, the second in the alanyl trispeptide **1d**. In conformations of the first class, the tilted strands of the propeller together with the ring of H-bonds create a polycyclic surface which defines an inner space. The stability of this polycyclic surface is believed to depend on the size of each of the rings as in covalent polycyclic structures.

From the various studies described the following may be concluded: 1. The trispeptides form interstrand H-bonds which restrict their conformational freedom. 2. These H-bonds occur in apolar solvents but collapse or are significantly weakened in polar solvents. 3. The interstrand H-bonds formed are between nonidentical amides where the Boc-amide is the H-donor and the anchor's amide is the acceptor. 4. The interstrand H-bonds generate propeller-like structures of preferred right handedness, RH, when the strands are built from L-amino acids. 5. The trispeptides derived from TREN form stronger H-bonds than the TRAM-based trispeptides, as evident from lower frequency NH absorptions, slower NH–ND exchange rates, and retention of the NMR anisotropy even in polar solvents.

6. Experimental Section

General. Unless otherwise stated, all NMR spectra were recorded on a 270-MHz Bruker WH-270 instrument. Chemical shifts are reported in ppm relative to internal Me_4Si. Infrared spectra were measured using a Nicolet MX-1 FTIR spectrophotometer. Optical rotations were measured with a Perkin-Elmer Model 141 polarimeter. Elemental analyses were performed by the Microanalysis Laboratory at the Hebrew University, Jerusalem, Israel. Low resolution FAB MS were performed at the University of Texas at Austin, using a Finnigan-MAT TSQ-70 instrument. Melting points are uncorrected.

Synthesis of Trispeptides. General Procedure. To a cold THF solution of Boc-amino acid (1.0 equiv) and *N*-hydroxysuccinimide (1.1 equiv) was added DCC (1.1 equiv) followed by a catalytic amount of DMAP (0.1 equiv). The reaction mixture was kept at 0–4 °C for 8–12 h. The dicyclohexylurea (DCU) was filtered and washed with THF, and the active ester solution was reacted with 1,3,5-tris(aminomethyl)benzene ($^1/_3$ equiv) at room temperature for ca. 24 h. The THF was evaporated, and the residue was dissolved in chloroform, washed twice with water, dried ($MgSO_4$ or Na_2SO_4), and evaporated to dryness. Purification of the products was performed by flash chromatography using mixtures of methanol in dichloromethane as eluents. The corresponding TREN derivative was prepared according to the same procedure by replacing 1,3,5-tris(aminomethyl)benzene by tris(2-aminoethyl)amine. The single chain analogs were prepared according to the same procedure by replacing the trifunctional amines by benzylamine or *n*-propylamine. The spectral data of each of the products are given under optimal conditions in terms of the concentrations and solvents used. For comparison purposes, all NMR and IR spectra have been recorded in $CDCl_3$ at identical concentrations, as indicated in the text.

Tris(Boc-L-Leu) 1a.[24] Activation of Boc-L-leucine monohydrate according to the general procedure and treatment of the resulting active ester with 1,3,5-tris(aminomethyl)benzene afforded the product (58% yield) after purification by flash chromatography (3% $MeOH/CH_2Cl_2$), as a white solid: mp 230 °C; $[\alpha]^{32}_D$ = +24 ($CHCl_3$, *c* 0.7). IR ($CHCl_3$) 3437, 3351 cm^{-1} (N–H), 1700 (shoulder) 1679 cm^{-1} (C=O); ^1H NMR ($CDCl_3$) δ 7.58 (br, 3 H, CH_2NH), 7.03 (s, 3 H, ArH), 5.39 (br d, 3 H, BocN*H*), 4.40 (m, 3 H, $C_\alpha H$), 4.36, 4.02 (ABq, 6 H, $ArCH_2N$), 1.7–1.5 (m, 9 H, CH and CH_2[*i*-Bu]), 1.36 (s, 27 H, *t*-Bu), 0.93 (m, 18 H, CH_3); $^{13}C\{^1H\}$ NMR (300 MHz, $CDCl_3$) δ 173.65 (*C*ON), 156.10 (*C*O-Boc), 138.44 (1,3,5-aromatic), 126.45 (2,4,6-ArH), 79.47 (CMe_3), 52.84 (C_α), 43.26 ($ArCH_2$), 42.24 (CH_2[*i*-Bu]), 28.35 (CH_3[*t*-Bu]), 24.77 ($CHMe_2$), 23.13, 21.71 (CH_3[i-Bu]). Anal. Calcd for $C_{42}H_{72}N_6O_9$: C, 62.66; H, 9.01. Found: C, 62.88; H, 8.61.

Tris(Boc-L-Ile) 1b. Similar procedure afforded the product (37% yield) after flash chromatography (3% $MeOH/CH_2Cl_2$), as a white solid: mp 280–2 °C dec; $[\alpha]^{30}_D$ = −6 (TFE, *c* 0.15); IR ($CHCl_3$) 3438, 3344 cm^{-1} (NH); ^1H NMR (DMSO-d_6) δ 8.31 (br, 3 H, CH_2NH), 7.02 (s, 3 H, ArH), 6.70 (br d, 3 H, BocN*H*), 4.20 (br, 6 H, $ArCH_2N$), 3.83 (m, 3 H, $C_\alpha H$), 1.38 (s, 27 H, *t*-Bu), ~1.7–1.4 (m, 9 H, CH and CH_2), 0.85 (m, 18 H, CH_3).

Tris(Boc-L-Val) 1c. Similar procedure using Boc-L-valine afforded the product (38% yield) after flash chromatography (3.5% $MeOH/CH_2Cl_2$) as a white solid: mp 232–3 °C dec; $[\alpha]^{30}_D$ = −10.5 (MeOH, *c* 0.2). IR ($CHCl_3$) 3438, 3337 (N–H), 1700, 1678 cm^{-1} (C=O). ^1H NMR ($CDCl_3/CD_3OD$) δ 7.11 (s, 3 H, ArH), 4.35 (br d, 6 H, $PhCH_2N$), 3.90 (br d, J = 6.3 Hz, 3 H, $C_\alpha H$), 2.05 (m, 3 H, $CHMe_2$), 1.44 (s, 27 H, *t*-Bu), 0.93 (m, 18 H, CH_3). Anal. Calcd for $C_{39}H_{66}N_6O_9$: C, 61.39; H, 8.72. Found: C, 61.08 H, 8.35.

Tris(Boc-L-Ala) 1d. Similar procedure using Boc-L-alanine afforded the trispeptide (55% yield) after flash chromatography (3–5% $MeOH/CH_2Cl_2$) as a white solid: mp 165–8 °C; $[\alpha]^{30}_D$ = −15.6 (MeOH, *c* 0.5); IR ($CHCl_3$) 3436, 3356 cm^{-1} (N–H), 1700 (sh), 1681 cm^{-1} (C=O). ^1H NMR ($CDCl_3$) δ 7.15 (br t, 3 H, CH_2NH), 7.03 (s, 3 H, ArH), 5.35 (br d, 3 H, BocN*H*), 4.31 (m, 3 H, $C_\alpha H$), 4.41, 4.17 (Abq, 6 H, $ArCH_2$), 1.37 (s + shoulder, 36 H, *t*-Bu and CH_3). Anal. Calcd for $C_{33}H_{54}N_6O_9$: C, 58.39; H, 8.01. Found: C, 58.07; H, 7.95.

Trisester Analog 3. Boc-L-Leu monohydrate (0.373 g, 1.5 mmol) and 1,3,5-tris(hydroxymethyl)benzene (0.084 g, 0.5 mmol, prepared according to Cochrane[14]) were dissolved in THF (10 mL), cooled to 0 °C, and treated with DCC (0.62 g, 3.3 mmol in 4 mL of THF) and DMAP (0.01 g, 0.08 mmol). The reaction mixture was kept at 0–4 °C for 34 h. The DCU was filtered, and the THF was evaporated. Purification by flash chromatography (*n*-hexane/ethyl acetate 4:1) afforded the triester in 67%

(24) The IUPAC systematic name for this derivative is [1(*S*)-[1(*R**),3-(*R**),5(*R**)]]-[1,3,5-benzenetriyltris[methyleneimino[1-(2-methylpropyl)-2-oxo-2,1-ethanediyl]]]triscarbamic acid tris(1,1-dimethylethyl)ester.

yield as a white solid: IR ($CDCl_3$) 3444, 3267 (very weak) cm^{-1} (NH), 1739 cm^{-1} ($COOCH_2$), 1711 cm^{-1} (Boc); ^1H NMR ($CDCl_3$) δ 7.29 (s, 3 H, ArH), 5.15 (br s, 6 H, CH_2O), 4.93 (br d, 3 H, BocN*H*), 4.35 (m, 3 H, $C_\alpha H$), 1.7-1.5 (m, 9 H, CH, CH_2{*i*-Bu}), 1.43 (s, 27 H, *t*-Bu), 0.93 (d, 18 H, CH_3{*i*-Bu}).

TREN Trispeptide Derivative 4. Activation of Boc-L-leucine·H_2O as described and treatment of the active ester with $^1/_3$ equiv of tris(2-aminoethyl)amine afforded a glassy solid (67% yield) after flash chromatography purification (2.5-3% $MeOH/CH_2Cl_2$): FAB MS (3-nitrobenzyl alcohol) 786 $(M + H)^+$, 686 $(M + H\text{-Boc})^+$, 586 $(M + H\text{-}2Boc)^+$, 486 $(M + H\text{-}3Boc)^+$; IR ($CHCl_3$) 3440, 3332 (N-H), 1699, 1656 cm^{-1} (C=O); ^1H NMR ($CDCl_3$) δ 7.92 (br, 3 H, CH_2NH), 5.56 (br d, 3 H, BocN*H*), 4.45 (m, 3 H, $C_\alpha H$), 3.47, 2.93 (br split AB, 6 H, NCH_2CH_2NH), 2.56 (br, 6 H, NCH_2CH2NH), ~1.6 (br m, 9 H, CH and CH_2{*i*-Bu}), 1.41 (s, 27 H, *t*-Bu), 0.94 (m, CH_3{*i*-Bu}).

Single Chain Analogs. L-$PhCH_2NHCOCH$(*i*-Bu)NHBoc (2). Treatment of activated Boc-L-leucine with benzylamine afforded the product in 93% yield after purification by flash chromatography (1% $MeOH/CH_2Cl_2$) as a white solid: mp 76-9 °C; $[\alpha]^{32}_D = -28$ ($CHCl_3$, *c* 0.6); IR ($CHCl_3$) 3437 cm^{-1} (N-H), 1698, 1680 cm^{-1} (C=O); ^1H NMR ($CDCl_3$) δ 7.34-7.23 (m, 5 H, Ph), 6.51 (br, 1 H, CH_2NH), 4.82 (br, 1 H, BocN*H*), 4.43 (d, J = 5.7 Hz, 2 H, $PhCH_2$), 4.11 (m, 1 H, $C_\alpha H$), 1.41 (s, 9 H, *t*-Bu), 1.7-1.5 (m, 3 H, CH and CH_2{*i*-Bu}), 0.94 (m, 6 H, CH_3); $^{13}C\{^1H\}$ NMR (300 MHz, $CDCl_3$) δ 172.6 (*C*ON), 155.8 (*C*O-Boc), 138.1 (1-Ph), 127.5 (2-Ph), 128.6 (3-Ph), 127.3 (4-Ph), 80.0 (CMe_3), 53.2 (C_α), 43.3 ($ArCH_2$), 41.30 (CH_2{*i*-Bu}), 28.30 (CH_3{*t*-Bu}), 24.8 ($CHMe_2$), 22.9, 22.0 (CH_3{*i*-Bu}). Anal. Calcd for $C_{18}H_{28}N_2O_3$: C, 67.47; H, 8.81; N, 8.74. Found: C, 67.76; H, 8.78; N, 8.48.

L-$CH_3CH_2CH_2NHCOCH$(*i*-Bu)NHBoc (5). Activation of Boc-L-Leu·H_2O according to the general procedure and coupling with *n*-propylamine, followed by flash chromatography (ether/*n*-hexane 1:1) afforded the aliphatic single chain analog (87% yield) as a white crystalline material: mp 102-105 °C; $[\alpha]^{30}_D = -28$ (MeOH, *c* 0.15); IR ($CHCl_3$) 3440 cm^{-1} (N-H), 1703 cm^{-1} (BocC=O), 1675 cm^{-1} (CONH); ^1H NMR ($CDCl_3$) δ 6.07 (br t, 1 H, CH_2NH), 4.84 (br d, 1 H, BocN*H*), 4.40 (m, 1 H, $C_\alpha H$), 3.21 (m, 2 H, CH_2N), 1.65 (m, 3 H, CH and CH_2{*i*-Bu}), 1.52 (m, 2 H, CH_2CH_2N), 1.44 (s, 9 H, *t*-Bu), 0.93 (m, 6 H, CH_3{*i*-Bu}), 0.92 (m, 3 H, CH_3{*n*-Pr}).

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Supplementary Material Available: Two tables providing full lists of the calculated conformations of the L-alanyl and the L-leucyl trispeptides (6 pages). Ordering information is given on any current masthead page.

Chiral Siderophore Analogs: Enterobactin

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Abstract: Two families of chiral enterobactin analogs, based on 1,3,5-tris(aminomethyl)benzene (TRAM) and on tris(2-aminoethyl)amine (TREN) as anchors and amino acids linking the anchor to catechol residues, have been prepared and their structures and iron(III) binding properties examined. The TRAM catechoylamides with L-leucyl (**5a**) and L-alanyl (**5b**) were found to adopt random conformations in protic solvents, while the corresponding TREN catechoylamides with L-leucyl (**8a**) and L-alanyl (**8b**) form H-bonded structures under analogous conditions. All ligands bind Fe^{3+} in a 1:1 stoichiometry, and most of them adopt preferentially Δ-cis configurations when L-amino acids are used, similar to enterobactin. The TREN derivative **8a** was shown to be the most efficient Fe^{3+} ion binder so far prepared, approaching enterobactin's binding constant within less than three orders of magnitude. The superiority of the TREN derivative **8a** is discussed in the light of experimental and theoretical (EFF calculations) results. Spectroscopic data include mainly the following: (i) NMR data of the protected ligands in relation to their H-bonded conformations and (ii) CD data of the Fe^{3+} complexes in relation to their optical purity.

Introduction

Biomimetic chemistry is a rapidly advancing area at the interface between chemistry and biology.[1] Its purpose is to identify the essential structural features that are responsible for the performance of natural compounds and to reproduce these features with the simplest possible synthetic molecules. Such molecules provide biological probes to elucidate biological processes and to reproduce specific functions of the biological machinery inside and outside living systems. In this article we describe synthetic enterobactin analogs that reproduce the essential structural features of natural enterobactin: its capability to adopt an organized conformation in the free state and to form complexes of high chiral purity that are stabilized by intramolecular H-bonding.

Enterobactin (**1**) (Figure 1) is a siderophore produced and excreted by bacteria in iron deficient media in order to bind and assimilate extracellular iron.[2-8] Enterobactin binds ferric ions with a very high formation constant ($\log K_f = 49$)[5] to give a charged octahedral triscatecholate complex with a Δ-cis configuration (Figure 1).[4] It is now well-established that after extracellular iron complexation the complex interacts with a specific receptor in the outer cell membrane and is then taken into the cell.[7] Recognition and membrane transport of the ferric complex by the ferric–enterobactin receptor has been shown to be stereoselective, such that enantioenterobactin which forms the Λ-cis complex lacks biological activity.[9]

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