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Triple Helical Stabilities of Guest–Host Collagen Mimetic Structures

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Abstract—The peptoid Nleu (*N*-isobutylglycine) has been successfully incorporated into a series of collagen mimetics composed of Gly-Pro-Nleu and Gly-Nleu-Pro sequences and has been able to maintain triple helices in appropriate structures. The achiral trimeric sequence Gly-Nleu-Nleu as a guest sequence in structures such as Ac-(Gly-Pro-Hyp)$_3$-(Gly-Nleu-Nleu)$_3$-(Gly-Pro-Hyp)$_3$-NH_2 retains triple helicity. As an extension of this study, we report, in this paper, on a series of guest–host collagen mimetic structures in which Gly-Nleu-Pro sequences are employed as the host. The guest sequences for these guest–host structures include Gly-Nleu-Nleu and Gly-N_x-Pro sequences where N_x is composed of a variety of alkyl and aralkyl peptoid residues. From these guest–host collagen mimetic structures, we are able to elucidate the contributions of hydrophobic and steric effects on triple helix formation. The Gly-Nleu-Pro sequences have been shown to be effective in inducing triple helicity. Conformational characterization of the guest–host collagen mimetic structures was established by techniques such as temperature-dependent optical rotation measurements and circular dichroism (CD) spectroscopy.

Introduction

The development of collagen mimetics is crucial to understand and discover the sequences and residues that favor its triple helical structure. Such discoveries can provide insights into the preparation of novel collagen-like biomaterials. The triple helix is composed of three left-handed polyproline II-like helices which are supercoiled into a right-handed helix.[1] The primary sequence of collagen proteins often consists of repeating trimeric units of Gly-X-Y, where X and Y are most commonly Pro and Hyp, respectively. Variations of the structural units and insertion of unnatural residues can affect the structural stability of triple helices and increase biostability.[2,3] Raines and co-workers have recently reported that the presence of electron-withdrawing substituents on the pyrrolidine ring of proline residues, as in Gly-Flp-Pro (Flp = fluoroproline), strongly enhance triple helical stability.[4] In previous work, *N*-methylglycine (sarcosine, Sar) has also been used as a mimetic for the Yaa position, but the resulting high molecular weight polymers and Ac-(Gly-Pro-Sar)$_{10}$-Gly-OH do not exhibit triple helicity.[5] The incorporation of some proline and hydroxyproline analogues also has been found to destabilize the triple helical structure of collagen.[6]

In our collagen mimetic program, we discovered that the peptoid residue *N*-isobutylglycine (Nleu) (Fig. 1) can be effectively incorporated into collagen mimetic structures to form stable triple helices.[7] Peptoid residues are advantageous because of their enhanced resistance to enzymatic degradation and their usefulness in creating chemical diversity by incorporation of novel sequences into collagen-like structures.[2,8] In previous studies, single chain and template-assembled structures composed of (Gly-Pro-Nleu)$_n$[9] and (Gly-Nleu-Pro)$_n$[10] repeats were synthesized and characterized using temperature-dependent optical rotation measurements, circular dichroism (CD) spectroscopy and NMR spectroscopy.

An achiral unit Gly-Nleu-Nleu was incorporated into the design of collagen mimetics to ascertain if such simplified structures can be incorporated into triple helices.[11] A series of the single chain mimetics Ac-(Gly-Pro-Hyp)$_n$-(Gly-Nleu-Nleu)$_n$-(Gly-Pro-Hyp)$_n$-NH_2 (where $n = 1$–3) and template-assembled mimetics KTA-[Gly-(Gly-Pro-Hyp)$_n$-(Gly-Nleu-Nleu)$_n$-(Gly-Pro-Hyp)$_n$-NH_2]$_3$ (where $n = 2$) [KTA = Kemp Triacid, 1,3,5-trimethyl-1,3,5-cyclohexane-tricarboxylic acid] were synthesized and assessed for triple helical content. The acetylated molecule where $n = 3$ showed triple helicity as did the template-assembled structure where $n = 2$ (Table 1). In both cases, the triple helical stabilities are reduced as compared to the homo-oligomer Gly-Pro-Hyp sequences of equivalent length as can be seen from the thermal melting (T_m) measurements shown in Table 1. These compounds represent the first guest–host

Key words: Collagen mimetics; guest–host; triple helix; peptoid; biophysics.

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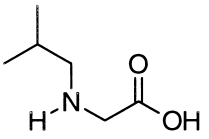


Figure 1. The structure of *N*-isobutylglycine (Nleu).

structures we prepared in order to determine the contribution to triple helicity of guest residues.

We have expanded the study of guest–host collagen mimetic structures by designing a program to explore the effect on triple helicity of various alkyl and aralkyl peptoid residues in collagen mimetics composed of Gly-Nleu-Pro sequences. Extensive studies have been reported on the triple helical nature of collagen mimetics composed of Gly-Nleu-Pro trimeric repeats. In this guest–host study, the structure Ac-(Gly-Nleu-Pro)$_8$-NH$_2$ represents the homo-oligomeric parent triple helical molecule. As an extension of the previous study, the two central tripeptide units of the parent structure were replaced by Gly-Nleu-Nleu sequences (Fig. 2A). For other guest–host collagen mimetics, the central two trimeric repeats were replaced by Gly-N$_x$-Pro sequences where N$_x$ is the *N*-alkylated glycine with side chain 'X' (Fig. 2B). The guest–host collagen mimetics were synthesized by peptide[12] and peptoid[13] solid-phase methodologies.

As reported earlier, a combination of thermal denaturation studies and CD spectroscopy is used by us to detect the presence of triple helical conformations and assess triple helical stability. Collagen-like structures exhibit cooperative melting transitions. From optical rotation measurements, thermal melting curves are constructed. The CD spectral characteristics typical for collagen can be used as a reference to determine the triple helical content of guest–host collagen mimetics in solution. Natural collagen possesses a unique CD spectrum with a small positive peak around 220 nm, a crossover at or near 213 nm and a large trough around 200 nm.[18] The CD spectrum of the parent compound Ac-(Gly-Nleu-Pro)$_8$-NH$_2$ is typical of triple helical structures.

Table 1. Triple helical melting transitions for guest–host systems composed of Gly-Pro-Hyp and Gly-Nleu-Nleu sequences.[11] The single chain and template-assembled homo-oligomeric parent structures are included for comparison

Compound	T_m (°C)
Ac-(Gly-Pro-Hyp)$_2$-(Gly-Nleu-Nleu)$_2$-(Gly-Pro-Hyp)$_2$-NH$_2$	No transition
Ac-(Gly-Pro-Hyp)$_6$-NH$_2$	36
Ac-(Gly-Pro-Hyp)$_3$-(Gly-Nleu-Nleu)$_3$-(Gly-Pro-Hyp)$_3$-NH$_2$	25
Ac-(Gly-Pro-Hyp)$_9$-NH$_2$	67
KTA-[Gly-(Gly-Pro-Hyp)$_2$-(Gly-Nleu-Nleu)$_2$-(Gly-Pro-Hyp)$_2$-NH$_2$]$_3$[a]	20
KTA-[Gly-(Gly-Pro-Hyp)$_6$-NH$_2$]$_3$[a]	81

[a]KTA = the Kemp triacid [1,3,5-trimethyl-1,3,5-cyclohexane-tricarboxylic acid].

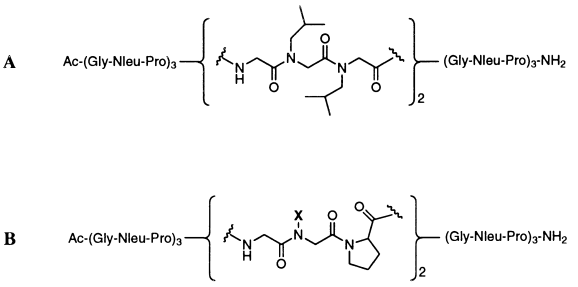


Figure 2. The guest–host collagen mimetic structures composed of Gly-Nleu-Pro sequences. The two central trimeric sequences contain the Gly-Nleu-Nleu sequences (A) or the guest peptoid Gly-N$_x$-Pro sequences (B), where 'X' is the side chain.

Results and Discussion

The parent structure

The parent structure, Ac-(Gly-Nleu-Pro)$_8$-NH$_2$, was synthesized using standard FastMoc coupling protocols on an Applied Biosystems Peptide Synthesizer Model 433A.[14] The residues Fmoc-Gly-OH, Fmoc-Nleu-OH, and Fmoc-Pro-OH were coupled in an iterative and sequential manner using *O*-benzotriazole-*N,N,N′,N′*-tetramethyluronium hexafluorophosphate (HBTU) and *N*-hydroxybenzotriazole (HOBt) activation.[12] The removal of the Fmoc protecting group was achieved by 20% piperidine in *N*-methylpyrrolidone (NMP). The single chain collagen mimetic Ac-(Gly-Nleu-Pro)$_8$-NH$_2$ was purified to >97% homogeneity by preparative reversed-phase high pressure liquid chromatography (RP-HPLC). The thermal stability (T_m) for the triple helical Ac-(Gly-Nleu-Pro)$_8$-NH$_2$ structure is 47 °C, at 0.2 mg/mL in H$_2$O, as demonstrated from temperature-dependent optical rotation measurements. In addition, the CD spectrum is indicative of its triple helix (Fig. 3). We employed the homo-oligomer Ac-(Gly-Nleu-Pro)$_8$-NH$_2$ as the benchmark structure to assess triple helicity.

Gly-Nleu-Nleu guest–host structure

The guest–host collagen mimetic Ac-(Gly-Nleu-Pro)$_3$-(Gly-Nleu-Nleu)$_2$-(Gly-Nleu-Pro)$_3$-NH$_2$ (Fig. 2A) was synthesized by Boc chemistry solid-phase protocols. We have already reported the synthesis of the building blocks Boc-Gly-Nleu-Pro-OH and Boc-Gly-Nleu-Nleu-OH.[7,11] The peptide was cleaved from the resin by anhydrous HF and purified to >95% homogeneity by RP-HPLC. The observed cooperative melting transition of Ac-(Gly-Nleu-Pro)$_3$-(Gly-Nleu-Nleu)$_2$-(Gly-Nleu-Pro)$_3$-NH$_2$ indicates the unfolding of the ordered triple helical conformation, with a melting temperature at 29 °C (Fig. 3A). Once again, the CD spectrum confirms the presence of triple helicity (Fig. 3B). The achiral unit Gly-Nleu-Nleu does lower the thermal stability, but can still be incorporated into the triple helix. The sequence Gly-Nleu-Nleu has no intrinsic chirality, but the outer Gly-Nleu-Pro sequences induce the achiral sequences into a helix which can then associate to form the collagen-like structures. The guest–host mimetic Ac-(Gly-Nleu-Pro)$_3$-(Gly-Nleu-Nleu)$_2$-(Gly-Nleu-Pro)$_3$-NH$_2$ demonstrates that Gly-Nleu-Pro

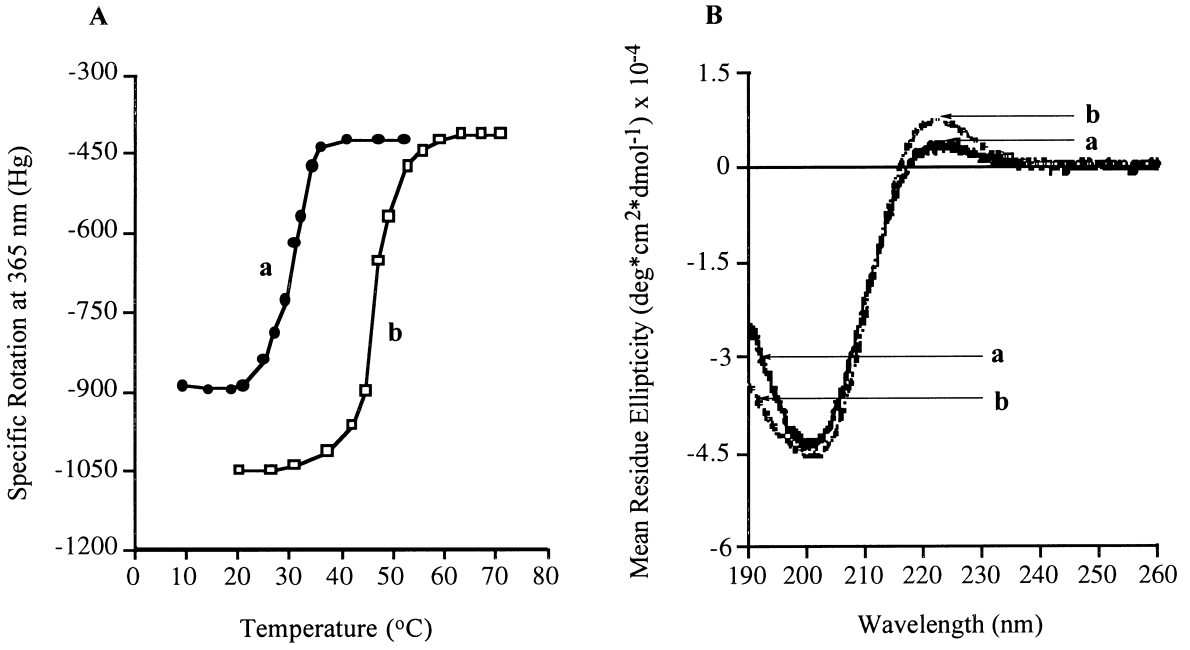


Figure 3. (A) Thermal melting curves of the guest–host collagen mimetic Ac-(Gly-Nleu-Pro)$_3$-(Gly-Nleu-Nleu)$_2$-(Gly-Nleu-Pro)$_3$-NH_2 [●](a) and the parent structure Ac-(Gly-Nleu-Pro)$_8$-NH_2 [□](b), observed by optical rotation measurements (0.2 mg/mL in H_2O). (B) CD spectra of the guest–host collagen mimetic Ac-(Gly-Nleu-Pro)$_3$-(Gly-Nleu-Nleu)$_2$-(Gly-Nleu-Pro)$_3$-NH_2 (a) and the parent structure Ac-(Gly-Nleu-Pro)$_8$-NH_2 (b). The CD measurements were carried out at 20 °C.

not only possesses a triple helical-inducing ability, but also is an effective collagen mimetic building block.

Gly-N_x-Pro guest–host structures

The general formula of the Gly-N_x-Pro containing guest–host collagen mimetics is presented in Figure 2B. In all cases, the N-terminus is acetylated and the C-terminus is amidated to eliminate charge effects at the two termini. The segment Fmoc-Pro-(Gly-Nleu-Pro)$_3$-resin of the guest–host collagen mimetic sequence was synthesized using standard FastMoc coupling protocols on an Applied Biosystems Peptide Synthesizer Model 433A. The peptide-bound resin was separated into individual solid-phase reaction vessels or sealed polypropylene 'tea-bags',[15] where the various peptoid residues were introduced by parallel solid-phase peptoid oligomer syntheses.[13] The syntheses were completed by coupling protected Gly, Nleu, and Pro amino acids as noted above.

All peptides were cleaved from the resin with 95% trifluoroacetic acid (TFA). Analytical RP-HPLC determined the crude material to be 50–75% pure. Prior to biophysical characterization, all the compounds were purified to >95% homogeneity by preparative RP-HPLC. Molecular weights of the guest–host collagen mimetic peptides were confirmed by MALDI mass spectrometry (see supplementary material).

In Figure 4A(a) and B(a), we show the transition and CD spectrum for the parent homo-oligomer Ac-(Gly-Nleu-Pro)$_8$-NH_2 which clearly establishes the criteria for triple helicity. Figure 4A(b) and B(b) contain results for the non-triple helical guest–host collagen mimetic Ac-(Gly-Nleu-Pro)$_3$-(Gly-Nval-Pro)$_2$-(Gly-Nleu-Pro)$_3$-NH_2, where *N*-isopropylglycine is Nval. In Figure 4A(b), no melting transition is observed and in Figure 4B(b), the CD spectrum is consistent with the absence of triple helicity. It appears that the isopropyl group of Nval sterically interferes with the helical backbone thus preventing triple helix formation.

From earlier studies, collagen mimetics composed of Gly-Pro-Sar sequences did not exhibit triple helicity.[5] However, we examined the effect on triple helicity of sarcosine in Gly-Sar-Pro as a guest sequence. Interestingly, Ac-(Gly-Nleu-Pro)$_3$-(Gly-Sar-Pro)$_2$-(Gly-Nleu-Pro)$_3$-NH_2 (Table 2, entry 6) does exhibit triple helicity as seen by the cooperative melting transition, with a T_m at 37 °C (Figure 4A(c)). In contrast, no melting transition was observed for Ac-(Gly-Pro-Sar)$_{10}$-Gly (Fig. 4A(d)).[5] The establishment of triple helicity for the guest–host structure is supported by comparing the CD spectra at low and high temperatures. Figure 4B(c) and 4B(c′) show the CD spectra of Ac-(Gly-Nleu-Pro)$_3$-(Gly-Sar-Pro)$_2$-(Gly-Nleu-Pro)$_3$-NH_2 at 10 °C and at 44 °C, respectively. Clearly, the difference in the two CD spectra indicates the transition from ordered triple helicity to a disordered structure. As demonstrated with Ac-(Gly-Nleu-Pro)$_3$-(Gly-Nleu-Nleu)$_2$-(Gly-Nleu-Pro)$_3$-NH_2, the triple helical-inducing ability of Gly-Nleu-Pro sequences is also seen in Ac-(Gly-Nleu-Pro)$_3$-(Gly-Sar-Pro)$_2$-(Gly-Nleu-Pro)$_3$-NH_2.

The significance of the effect of steric bulk is demonstrated by comparing guest–host collagen mimetics incorporating guest peptoid residues with branched side chains to that of guest–host structures incorporating peptoid residues with linear side chains. In Figure 5, the

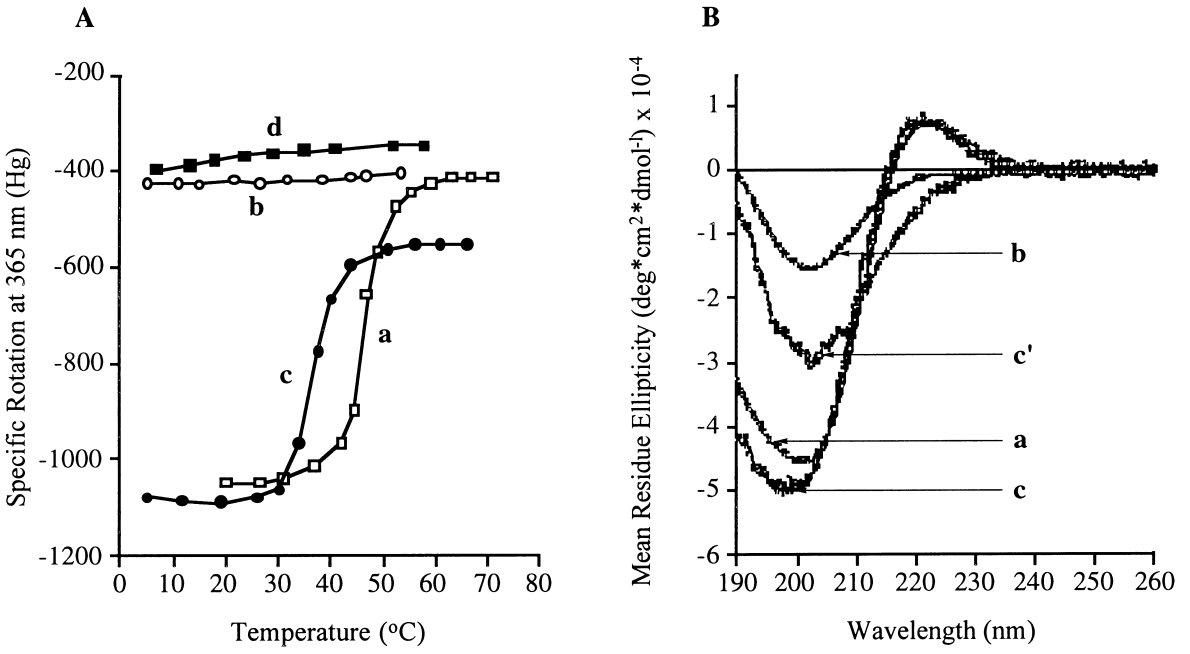
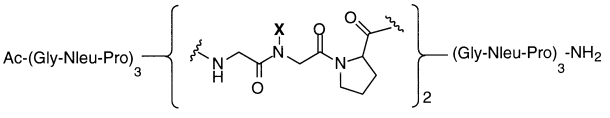


Figure 4. (A) Thermal melting curves of the parent structure Ac-(Gly-Nleu-Pro)$_8$-NH$_2$ [□](a), the guest–host collagen mimetics Ac-(Gly-Nleu-Pro)$_3$-(Gly-Nval-Pro)$_2$-(Gly-Nleu-Pro)$_3$-NH$_2$ [○](b), where Nval is *N*-isopropylglcine, and the Ac-(Gly-Nleu-Pro)$_3$-(Gly-Sar-Pro)$_2$-(Gly-Nleu-Pro)$_3$-NH$_2$ [●](c) and the homo-oligomer Ac-(Gly-Pro-Sar)$_{10}$-Gly,[5] [■](d), observed by optical rotation measurements (0.2 mg/mL in H_2O). (B) CD spectra of Ac-(Gly-Nleu-Pro)$_8$-NH$_2$ (a) at 20 °C, Ac-(Gly-Nleu-Pro)$_3$-(Gly-Nval-Pro)$_2$-(Gly-Nleu-Pro)$_3$-NH$_2$ (b) at 10 °C and Ac(Gly-Nleu-Pro)$_3$-(Gly-Sar-Pro)$_2$-(Gly-Nleu-Pro)$_3$-NH$_2$ (c) at 10 °C and (c′) at 44 °C (0.2 mg/mL in H_2O).

thermal melting data illustrate the structural difference between the guest–host collagen mimetic incorporating *N*-2′-(*R*)-hydroxypropylglycine (Table 2, entry 4) and the guest–host structure incorporating the linear *N*-2′-hydroxyethylglycine. It is interesting that functionalized alkyl linear peptoid residues such as *N*-2′-hydroxyethylglycine and *N*-2′-aminoethylglycine (not shown) disrupt the collagen mimetic triple helical structure. The side chains of these peptoid residues seem to lack the appropriate steric and hydrophobic character which favors intermolecular side-chain–side-chain interaction so as to promote triple helical structures in aqueous solvents.[10b] Additionally, the solvation of the side chain hydroxyl groups and the charge on the side chain amino groups sufficiently destabilize so as to disrupt triple helix formation. At this stage, we cannot evaluate the effect of chirality on the triple helix stability but this question will be addressed in future endeavors from our laboratories.

We investigated alkyl and aralkyl peptoid residues, in which the side chains of the peptoid residue possess an additional methylene group before the side chain branching, similar to that of Nleu. The *N*-3′,3′-dimethylbutylglycine and *N*-2′-(*S*)-methylbutylglycine residues (Table 2, entry 2 and 3, respectively) can be incorporated into the collagen mimetic triple helix, as observed by the cooperative melting transition and appropriate CD spectra (Fig. 6A and B). The series of benzyl and substituted benzyl substituents gave melting transitions between 41 and 47 °C (Table 3, Fig. 7). The CD spectra of these compounds also are indicative of triple helical conformations (Fig. 7B). The melting transition temperatures of these guest–host

Table 2. Triple helical melting transitions for guest–host collagen mimetics with alkyl guest residues



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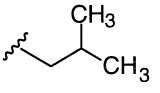
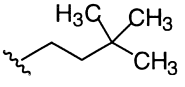
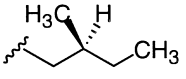
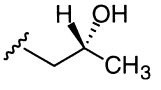
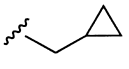
1		47
2		44
3		49
4		42
5		38
6		37

[a]Sample concentration was 0.2 mg/mL in H_2O. Optical rotations were measured at 365 nm (Hg).

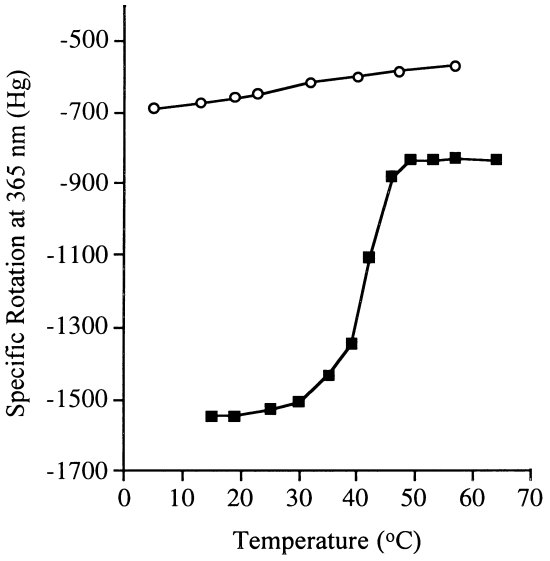


Figure 5. Thermal melting curves of the guest–host collagen mimetics incorporating guest peptoid residues with side chains 2′-hydroxyethyl (○) and 2′-(*R*)-hydroxypropyl (■) (0.2 mg/mL in H_2O). Optical rotations were measured at 365 nm (Hg).

collagen mimetics are similar to that of the parent collagen mimetic Ac-(Gly-Nleu-Pro)$_8$-NH_2, which reveal that these alkyl and aralkyl guest peptoid residues possess the necessary steric constraints and hydrophobicity for triple helicity and can be accommodated in the triple helical structure of Gly-Nleu-Pro oligomers without adversely affecting thermal stability.

We could not observe melting transitions for the naphthalenemethyl-containing guest–host collagen mimetics because its aqueous solution becomes cloudy at 40 °C, which is below its thermal melting temperature.[10a] Similar behavior had been observed with Ac-(Gly-Nleu-Pro)$_{10}$-NH_2 in a previous study.[19] In these cases, the triple helix most likely desolvates and aggregates.

We synthesized another block co-oligomer Ac-(Gly-Nleu-Pro)$_2$-(Gly-Nphe-Pro)$_4$-(Gly-Nleu-Pro)$_2$-NH_2, to investigate the effect of the increase in the number of guest residues on triple helicity. The *N*-benzylglycine (Nphe) was selected because of the similar melting temperature of Ac-(Gly-Nleu-Pro)$_3$-(Gly-Nphe-Pro)$_2$-(Gly-Nleu-Pro)$_3$-NH_2 to that of the parent Ac-(Gly-Nleu-Pro)$_8$-NH_2 collagen mimetic. Once again, we found that we could not obtain a melting transition for this co-oligomeric structure, where the number of Gly-Nphe-Pro sequences equals the number of Gly-Nleu-Pro sequences, because this collagen mimetic precipitates from solution at 40 °C. However, the CD spectrum at room temperature is fully indicative of triple helical conformations. We also synthesized the homo-oligomeric sequence Ac-(Gly-Nphe-Pro)$_8$-NH_2 which is insoluble in H_2O and all common organic solvents. Future biophysical characterizations will be carried out using solid state NMR and IR spectroscopies.

Conclusions

The present studies establish two significant effects for our guest–host collagen mimetic systems. First, a range of peptoid residues can be incorporated into the design for collagen mimetics which maintain triple helical conformations. These peptoid residues contribute steric and

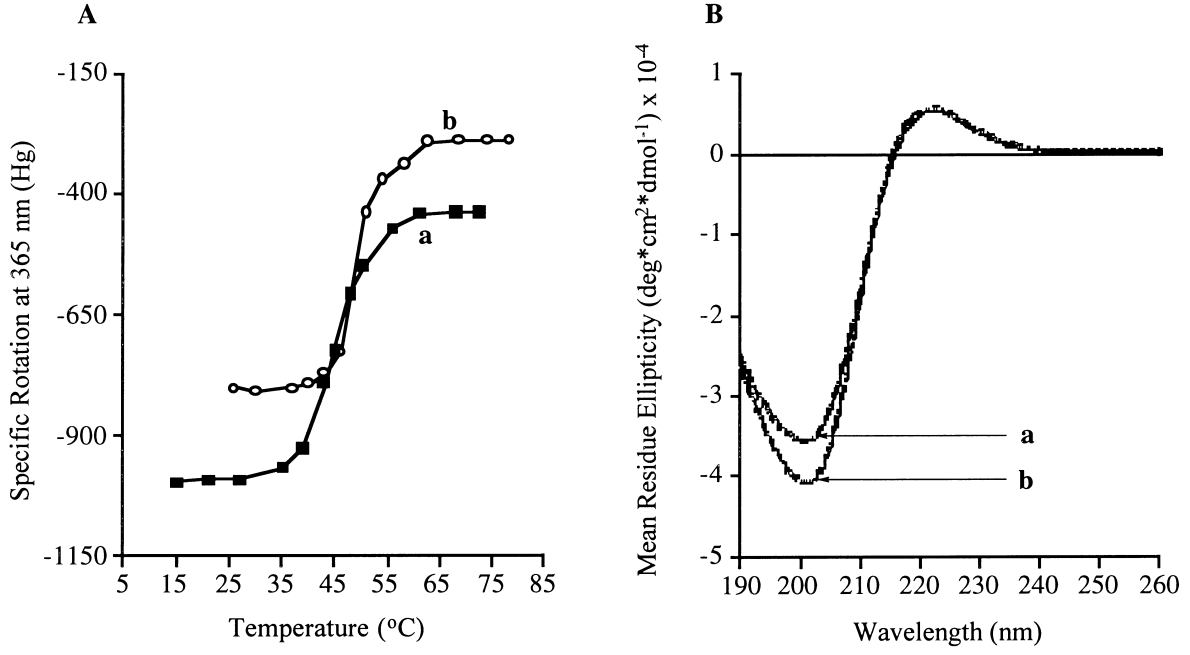
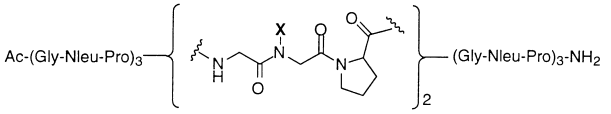


Figure 6. (A) Thermal melting curves of representative guest–host collagen mimetics incorporating guest peptiod residues with alkyl side chains dimethylbutyl [■](a) and 2′-(*S*)-methylbutyl [○](b) (0.2 mg/mL in H_2O). Optical rotations were measured at 365 nm (Hg). (B) CD spectra of the same representative guest–host collagen mimetics incorporating guest peptoid residues with alkyl side chains dimethylbutyl (a) and 2′-(*S*)-methylbutyl (b). The CD measurements were carried out at 20 °C.

Table 3. Triple helical melting transitions for guest–host collagen mimetics with aralkyl guest residues



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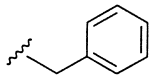
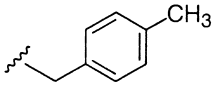
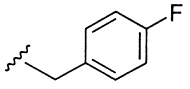
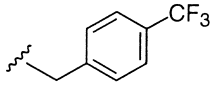
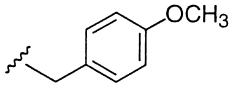
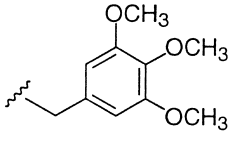
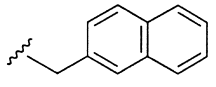
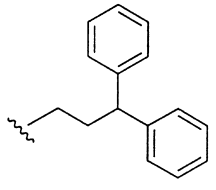
1		45
2		43
3		44
4		41
5		47
6		46
7		>40[b]
8		24

[a]Sample concentration was 0.2 mg/mL in H_2O. Optical rotations were measured at 365 nm (Hg).
[b]The solution became cloudy at 40 °C which prevented a T_m determination.

hydrophobic effects that are important for the inter- and intra-chain interactions in aqueous solvents. Second, the collagen mimetic building block Gly-Nleu-Pro possesses strong triple helical-inducing ability, as seen in Ac-(Gly-Nleu-Pro)$_3$-(Gly-Nleu-Nleu)$_2$-(Gly-Nleu-Pro)$_3$-NH_2 and Ac-(Gly-Nleu-Pro)$_3$-(Gly-Sar-Pro)$_2$-(Gly-Nleu-Pro)$_3$-NH_2. These results reinforce our findings that the sequence Gly-Nleu-Pro is an appropriate and effective collagen mimetic building block. We plan to extend our studies of peptoid residues and triple helicity in order to develop novel collagen-like biomaterials.

Experimental

Materials and general procedures

Amino acids Fmoc-protected and Rink Amide methylbenzhydrylamine (MBHA) resin (substitution level = 0.55 mmol/g) were purchased from NovaBiochem. Benzylchloroformate was purchased from Acros. The activating agent *O*-benzotriazole-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HBTU) was purchased from Chem-Impex. The reagent *N*-hydroxybenzotriazole (HOBt) was purchased from Aldrich Chemical Co. Amines were purchased from Aldrich Chemical Co. and used without further purification. Dichloromethane (DCM) and *N*,*N*′-dimethylformamide (DMF) were dried over 4 Å molecular sieves.

The ^1H NMR spectra were recorded on a QE-300 NMR spectrometer or a home-built 360 MHz spectrometer, employing a TechMag pulse programmer and an Oxford Instruments superconducting magnet. Trimethylsilane (TMS) was used as a standard. The following abbreviations were used to explain the multiplicities: s = singlet, d = double, t = triplet, q = quartet, m = multiplet, b = broad. Analytical thin-layer chromatography was performed on precoated aluminum sheets (silica gel 60 F_{254}, 0.2 mm thickness, EM Separations Technology). Preparative thin-layer chromatography was carried out on precoated glass backed plates (silica gel GF, 20×20 cm, 1000 microns, Analtech). Infrared spectra were obtained on a Nicolet Magna-IR Spectrometer Series II.

Preparative RP-HPLC purification and analysis was carried out on a MILLENNIUM 2010 system consisting of a Waters 715 Ultra WISP sample processor, a Waters TM 996 photodiode array detector, two Waters 510 pumps and a NEC Power Mate 486/33I computer. Solvents used in HPLC purification were as follows: solvent A: 0.1% TFA/H_2O: solvent B: 0.1% TFA/CH_3CN. The flow rate was 8 mL/min for the preparatory column (Vydac C-18, 25×2.2 cm, 10 microns, 300 Å) and 1.0 mL/min for the analytical column (Vydac C-18, 25×0.46 cm, 5 microns, 300 Å).

Mass spectra were determined at the Scripps Research Institute using FAB, electrospray, and MALDI mass spectrometric techniques.

Optical rotations were measured with a Perkin–Elmer 241 Polarimeter equipped with a Model 900 isotemp refrigerator circulator (Fisher Scientific) and data were collected at 365 nm (Hg). The CD measurements were made on a modified Cary-61 Spectropolarimeter as described previously.[20] Spectra were obtained using a 0.05 cm path length cell by signal-averaging 10 scans from 190–300 nm with a scan speed of 1.0 nm/s.

Peptide synthesis, purification and characterization

The resin-bound structure, Fmoc-Pro-(Gly-Nleu-Pro)$_3$-resin, was synthesized on an Applied Biosystems Peptide Synthesizer Model 433A using the standard FastMoc protocols. The synthesis of Fmoc-Nleu-OH

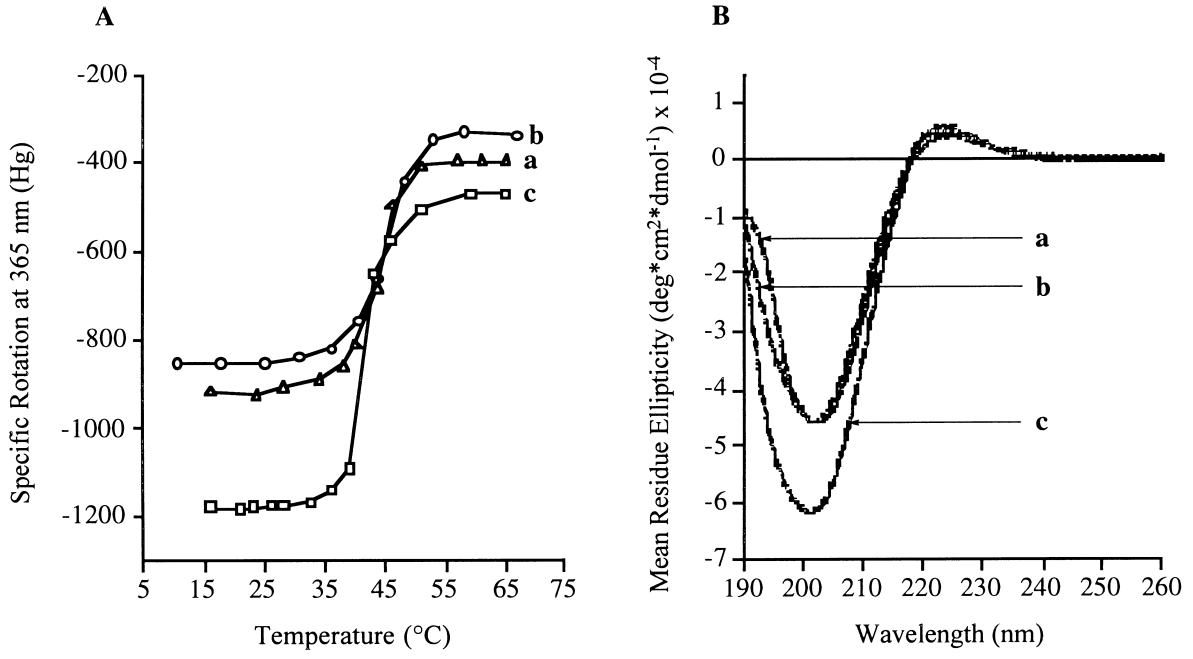


Figure 7. (A) Thermal melting curves of representative guest–host collagen mimetics incorporating guest peptoid residues with aralykl side chains *p*-methylbenzyl [△](a), benzyl [○](b), and *p*-trifluoromethylbenzyl [□](c) (0.2 mg/mL in H_2O). Optical rotations were measured at 365 nm (Hg). (B) CD spectra of the same representative guest–host collagen mimetics incorporating guest peptoid residues with aralkyl side chains *p*-methylbenzyl (a), benzyl (b) and *p*-trifluoromethylbenzyl (c). The CD measurements were carried out at 20 °C.

has been previously reported.[21] The following procedures were used for the manual synthesis of the guest–host collagen mimetics (typically 0.05 mmol). The Fmoc protecting group was removed with 20% piperidine in *N*-methylpyrrolidone (NMP) (4 mL), followed by solvent washings with DMF (5×4 mL). Prior to amino acid coupling, the Fmoc-amino acid (10 equiv) was dissolved in a solution of 0.5 M HBTU/HOBt in DMF (2 mL) for 5 min. A solution of 2 M diisopropylethylamine (DIEA) in NMP (1 mL) was then added. This solution was added to the resin and the coupling reaction was monitored by the Kaiser test for primary amines[22] and the 2-chloroanil test for secondary amines.[23] The resin was then washed with DMF (5×4 mL) and the cycle repeated for the next amino acid.

The peptoid residues were synthesized according to the methodology designed by Zuckermann and co-workers.[13a] Upon completion of the guest–host sequence, the N-terminus was capped with acetic anhydride (4 mL, 0.5 M acetic anhydride, 0.125 M DIEA and 0.015 M HOBt in NMP) for 30 min, followed by DMF (5×5 mL) and DCM (5×5 mL) washings. The final product on resin was dried under vacuum before the final cleavage reaction.

In the 'tea-bag' approach, the resin was sealed in polypropylene tea-bags and placed in a single polypropylene bottle, which was used as the reaction vessel.[15] The reaction was agitated on the mechanical shaker. The amounts of reagents and solvents were adjusted to account for the multiple parallel syntheses and the reaction times were generally extended by a factor of 1.5. For peptoid oligomer synthesis, the labeled tea-bags were separated into individual polypropylene bottles for the introduction of the different amines.

The peptides were cleaved from the resin by 95% TFA/H_2O and the solvent was removed under reduced pressure. The peptide was triturated with diethyl ether, centrifuged and washed several times with fresh diethyl ether, followed by filtration and lyophilization. Analytical RP-HPLC indicated that crude yields were between 50–75%. After RP-HPLC purification, overall yields of the pure product ranged from 7 to 15%. To allow for triple helical formation, compounds were incubated at 10 mg/mL in H_2O at 4 °C for 7 days. The compounds were then diluted to 0.2 mg/mL and equilibrated at this concentration for 24 h at 4 °C, prior to biophysical characterizations.

Synthesis of protected amines

The diamine, *tert*-butyloxycarbonylethylenediamine, was prepared according to the procedure described by Krapcho and Kuell.[24] The hydroxyamine, 2-[(triisopropylsilyl)oxy]ethylamine, was prepared according to a procedure described by Zuckermann and co-workers.[13c]

Preparation of (2*R*)-[(triisopropylsilyl)oxy]-propylamine

The reactant, *N*-benzyloxycarbonyl-2-(*R*)-hydroxypropylamine, was prepared from standard procedures from (*R*)-(−)-1-amino-2-propanol. The compound exhibited an ^1H NMR spectrum similar to that reported in the literature.[25]

A cooled solution of *N*-benzyloxycarbonyl-2-(*R*)-hydroxypropylamine (7.0 g, 34 mmol), imidazole (5.0 g, 74 mmol), and DMF (60 mL) was stirred under nitrogen. Triisopropylsilyl chloride (7.9 mL, 37 mmol) was then added dropwise. After the addition, the ice bath

was removed and the reaction solution was allowed to stir overnight at room temperature. The solvent was removed on a rotary evaporator and the remaining residue was dissolved in EtOAc (200 mL). The organic phase was washed with saturated aqueous Na_2CO_3 solution (3×300 mL) and saturated aqueous NaCl solution (3×300 mL). The organic phase was dried over Mg_2SO_4, filtered, and the solvent removed under reduced pressure. The product was obtained as a liquid (10.4 g, 85% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.2–1.05 (m, 24H, isopropyl + CH_3), 3.16–3.26 (m, 2H, CH_2), 4.06–4.08 (m, 1H, CH), 5.06–5.11 (m, 3H, $PhCH_2O$ + NH), 7.31–7.37 (m, 5H, Ar-H); IR (film) ν_{max} 3453, 3346, 2960, 2866, 1708 cm^{-1}; MS-FAB calcd for $C_{20}H_{36}NO_3Si$ (M): 365, found 366 (M + H), 388(M + Na); $[\alpha]_D$ –8.48 ($CHCl_3$, *c* 0.017).

Nitrogen gas was passed through a solution of the protected amino alcohol (7 g, 19 mmol) in MeOH (140 mL). A catalytic amount of 10% Pd/C (0.56 g, 5.0 mmol) was added and the reaction mixture was stirred under hydrogen pressure for 4 h. After filtration through Celite, the solvent was removed under reduced pressure to yield (2*R*)-[(triisopropylsilyl)oxy]-propylamine as a liquid (4.24 g, 96% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.02–1.16 (m, 24H, isopropyl + CH_3), 2.21 (bs, 2H, NH_2), 2.57–2.74 (m, 2H, CH_2), 3.90–3.95 (m, 1H, CH); IR (film) 2960, 2943, 2867, 1464 cm^{-1}; MS-FAB calcd for $C_{12}N_{29}OSiN$: 231, found 232 (M + H); $[\alpha]_D$ –12.8 ($CHCl_3$, *c* 0.017).

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Supporting Information Available: Table of MALDI mass spectrometric data of the guest–host structures for Figure 2B.

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