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Facile and efficient assembly of collagen-like triple helices on a TRIS scaffold

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Abstract

The TRIS scaffold, Boc-β-Ala-TRIS-$(OH)_3$, was utilized to assemble triple helices composed of the Gly-Nleu-Pro sequence (Nleu denotes *N*-isobutylglycine). The scaffold assembly can be achieved efficiently through direct coupling between long peptide chains and the TRIS scaffold using DEPBT, a recently developed peptide coupling reagent. CD spectroscopy and thermal denaturation studies demonstrated that Boc-β-Ala-TRIS-$[(Gly\text{-}Nleu\text{-}Pro)_n\text{-}OMe]_3$ exhibits triple helicity in H_2O when n equals 5, 6, and 8, while the shorter analogs (where $n = 1$ and 4) do not. TRIS-assembled structures possess several advantages over the KTA- and TREN-assembled structures previously reported from our laboratory (where KTA and TREN denotes cis-1,3,5-trimethyl cyclohexane-1,3,5-tricarboxylic acid and tris(2-aminoethyl)amine, respectively). The protecting groups on the scaffold and at the C-terminus of the TRIS-assembled peptides can be readily removed to synthesize collagen mimetic dendrimers and metal-complexing collagen-like peptides respectively, both of which can lead to further enhanced thermal stability.

Keywords: Scaffold-assembled collagen mimetics; Triple helix; Collagen; Circular dichroism; DEPBT

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1. Introduction

Collagen is the most abundant protein in mammals, constituting a quarter of their total body weight. It is the major fibrous element of the skin, bone, tendon, cartilage, blood vessels, and teeth [1–3]. Collagen is composed of the characteristic Gly-Xaa-Yaa trimer repeats and proline and hydroxyproline (Hyp) occur most often in the Xaa and Yaa positions [3,4]. The three polypeptide strands in collagen, each of which forms a left-handed helix, wind around each other to form a right-handed triple helix [5,6].

In synthetic collagen mimetic structures, the primary sequence of the peptide can affect the folding and conformational stability significantly [7–14]. The folding of peptides into their native secondary or tertiary structures is the essential requirement to induce the proper biological response or activity. Much research has been carried out on attaching peptides to cofactors (such as scaffolds and metal ions) which can lead to native-like folding [15–19]. In all these cases, *de novo* design mediated by scaffolds is an effective technique for the construction of peptidomimetics.

The incorporation of a scaffold into the synthesis of collagen-like structures favors intramolecular triple helix folding by minimizing the entropic effects. An appropriate collagen mimetic scaffold should have at lease three functional groups to attach peptide chains and it should also be flexible enough to accommodate the one amino acid register shift between adjacent chains. The utilization of various scaffolds for the assembly of triple helical collagen-like structures has been reported [20–26]. In our design of scaffold-assembled collagen mimetics, we have incorporated both cis-1,3,5-trimethyl cyclohexane-1,3,5-tricarboxylic acid (KTA) and tris(2-aminoethyl)amine (TREN) into collagen mimetic peptides. Spacers were used for both scaffolds to reduce steric hindrance, glycine for KTA and succinic acid for TREN, respectively [27–30].

The KTA and TREN scaffolds facilitate and enhance the formation of collagen-like triple helices as compared to the corresponding non-scaffold-assembled analogs [3,14,27–33]. In addition, these studies demonstrated that structures composed of the Gly-Nleu-Pro sequence, where Nleu denotes *N*-isobutylglycine, have higher triple helical propensities than structures composed of the isomeric Gly-Pro-Nleu sequence. Functional modification for further development of the KTA- and TREN-assembled arrays can not be achieved readily since they both lack an orthogonal reactive site. Therefore, another scaffold which contains an extra amino group, the TRIS scaffold, has been prepared for triple helical assembly. The protecting groups on the scaffold and at the C-termini of the peptide chains can be easily removed for further modification to afford more complex structures.

2. Materials and methods

All amino acids were purchased from Novabiochem. EDC was purchased from Chem-Impex. Reagent grade and HPLC-grade solvents were purchased from Fisher Scientific (solvents were distilled before use when appropriate). Deuterated solvents were purchased from Cambridge Isotope Labs and Isotech Inc. All other reagents were purchased either from Aldrich or Acros.

The ^{1}H NMR spectra were obtained on a Varian HG-400 (400 MHz) spectrometer. Chemical shifts (δ) are reported in parts per million (ppm) relative to residual undeuterated solvent as an internal reference. The following abbreviations were used to explain the

multiplicities: s, singlet; d, doublet; t, triplet; q, quartet; dd, doublet of doublets; m, multiplet; b, broad.

Reactions carried out in solution were monitored either by TLC or HPLC. Developed TLC plates were visualized using either UV light (254 nm), ninhydrin, or *o*-tolidine. Silica gel 60 (230–400 mesh, EM Science) was used for column chromatography. Both preparative and analytical HPLC were carried out on Waters HPLC systems [34,35]. The solvents used were water with 0.1% TFA (A) and acetonitrile with 0.1% TFA (B). The flow rate was 10 mL/min (Vydac, C-18 and C-4, 25×2.2 cm) for preparative purification and 1 mL/min for analytical runs (Vydac, C-18 and C-4, 25×0.46 cm).

Circular dichroism (CD) measurements were carried out as reported earlier [34,35]. System automation, multiple scan signal averaging, and base line subtraction were carried out with computer control. The CD spectra were obtained using a 0.02–0.5 cm path length cell by signal averaging 10 scans from 190 to 300 nm at a scan speed of 0.8 nm/s.

Thermal denaturation studies were performed on a Perkin-Elmer 241 Polarimeter equipped with a Model 900 isotemp refrigerator circulator (Fisher Scientific). Data were collected at 365 nm (Hg lamp). The solutions were stored in a refrigerator (4 °C) for at least 24 h for triple helix formation. Before recording an optical rotation, the sample was equilibrated at least 30 min at the initial temperature. At each subsequent temperature point (increment of 2–7 °C each time), the samples were allowed to equilibrate for 30 min.

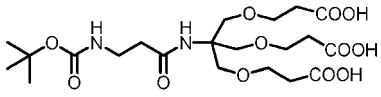
Mass spectra were obtained at UC San Diego or the Scripps Research Institute. Fast atom bombardment (FAB), electrospray ionization (ESI) or matrix-assisted laser desorption ionization (MALDI) methods were used to verify product mass.

2.1. The modified-TRIS scaffold

A scaffold developed by Newkome and coworkers was chosen for our collagen mimetic assembly [36]. The originally published synthesis of the scaffold was modified to fit our requirement for the assembly of collagen mimetic peptides [34]. A β-alanine linker was introduced because we encountered low reactivity of the amino group on the scaffold which was attributed to steric hindrance. The structure of the modified TRIS scaffold, Boc-β-Ala-TRIS-$(OH)_3$, is shown in Fig. 1. It has three carboxylic acid groups for attaching peptide chains, and a protected amino group which can be used for further modification.

2.2. Synthesis of TRIS-assembled collagen mimetic peptides

Boc-β-Ala-TRIS-$[(Gly\text{-}Nleu\text{-}Pro)_1\text{-}OMe]_3$ was synthesized using EDC coupling with excellent yield (93.6%). However, attaching longer peptide chains (more than 3 trimer repeats) to the TRIS scaffold using EDC did not give the product in acceptable yield.



Boc-β-Ala-TRIS-$(OH)_3$

Fig. 1. Chemical structure of the modified TRIS scaffold Boc-β-Ala-TRIS-$(OH)_3$.

Several other coupling reagents (DCC, HBTU, HATU, etc.) gave similar poor results. We believe the low yields are due to the large size of the reactant. Once the first chain is attached to the scaffold, the steric bulk inhibits attachment of additional chains.

DEPBT (3-(diethoxyphosphoryloxy)-1,2,3-benzotriazin-4(3*H*)-one, Fig. 2) [37,38], afforded the TRIS-assembled product in good yield through direct peptide coupling. The peptide chains HCl·H-(Gly-Nleu-Pro)$_n$-OMe (where $n = 4$, 5, 6, and 8) were attached to the Boc-β-Ala-TRIS-(OH)$_3$ scaffold directly (Fig. 2). The single chain oligomers can be purified readily by silica gel column chromatography and only the final products require HPLC purification. Thus, they can be prepared on a relatively large scale for further modification. The significantly improved yields over other coupling reagents are most probably attributed to a proposed intramolecular rearrangement step that generates the HOOBT active esters, thus greatly reducing the entropy effect [37,38]. A representative MALDI-TOF mass spectrum of the TRIS-assembled peptide Boc-β-Ala-TRIS-[(Gly-Nleu-Pro)$_8$-OMe]$_3$ shows the expected mass of 6988 (Fig. 3).

2.3. Detailed synthesis of TRIS-assembled collagen mimetics

2.3.1. Single-chain peptide synthesis

The single-chain collagen mimetics HCl·H-(Gly-Nleu-Pro)$_n$-OMe and (where $n = 1$, 4, 5, 6, and 8) were synthesized by a series of segment condensation. They were purified in gram quantities and characterized by TLC, HPLC and mass spectrometry as we have reported earlier [27,34]. The syntheses of Boc-β-Ala-TRIS-(OEt)$_3$, Boc-β-Ala-TRIS-(OH)$_3$, and Boc-β-Ala-TRIS-[(Gly-Nleu-Pro)$_6$-OMe]$_3$ have been reported previously [34].

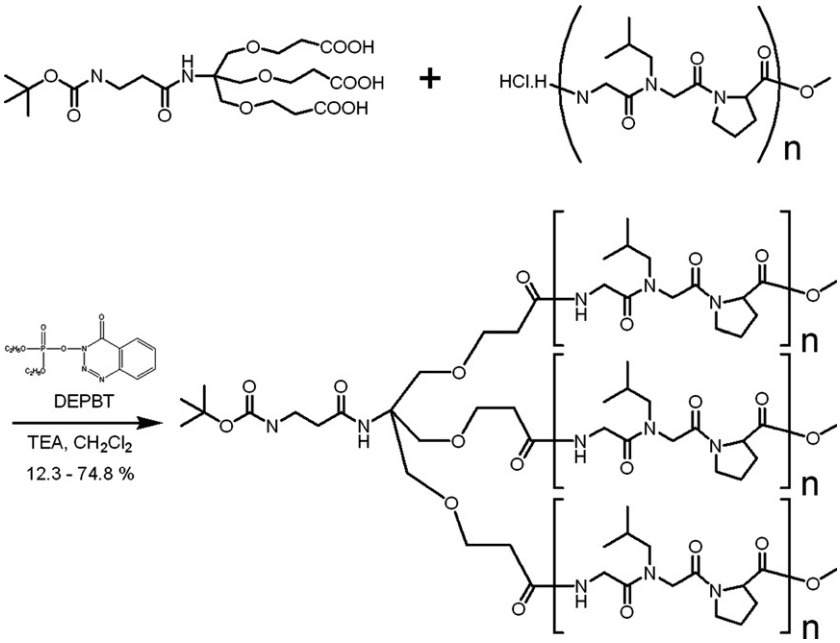


Fig. 2. Attachment of peptide chains directly to Boc-β-Ala-TRIS-(OH)$_3$ using DEPBT ($n = 4$, 5, 6, and 8). The reaction yield ranges from 12.3% to 74.8%.

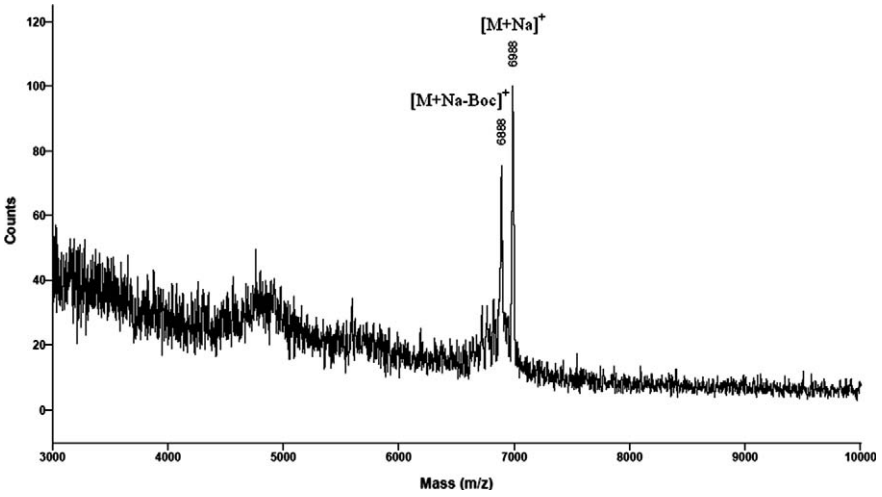


Fig. 3. MALDI-TOF mass spectra of Boc-β-Ala-TRIS-[(Gly-Nleu-Pro)$_8$-OMe]$_3$. $[M+Na]^+$ calculated for $C_{336}H_{546}N_{74}O_{84}$ 6989; found 6988 $[M+Na]^+$, 6888 $[M+Na–Boc]^+$.

2.3.2. *Boc-β-Ala-TRIS-[(Gly-Nleu-Pro)$_1$-OMe]$_3$*

A solution of HCl·H-Gly-Nleu-Pro-OMe (73 mg, 0.22 mmol), Boc-β-Ala-TRIS-(OH)$_3$ (31.3 mg, 0.062 mmol) and HOBT (29.2 mg, 0.22 mmol) in DMF (6 mL) was stirred under N_2 until homogeneous. After 5 min, TEA (86 μL, 0.62 mmol) was added followed by EDC (47 mg, 0.25 mmol). The reaction mixture was stirred under N_2 for 16 h and the solvent was removed under reduced pressure. The remaining residue was dissolved in 50 mL $CHCl_3$ and washed with 1 N HCl (3× 10 mL), saturated $NaHCO_3$ (3× 10 mL) and brine (10 mL). The organic layer was dried over anhydrous Na_2SO_4. After filtration, the solvent was again removed under reduced pressure. The resulting white solid was purified by silica gel column chromatography (0–8% MeOH/$CHCl_3$) to obtain a white powder (78 mg, 93.6%). MS-MALDI-TOF (*m/z,* $[M+Na]^+$) calculated for $C_{63}H_{105}N_{11}O_{21}$ 1374; found 1374 $[M+Na]^+$, 1352 $[M+H]^+$. $R_f = 0.10$ (90:9:1 CH_2Cl_2/MeOH/AcOH).

2.3.3. *Boc-β-Ala-TRIS-[(Gly-Nleu-Pro)$_4$-OMe]$_3$*

A solution of HCl·H-(Gly-Nleu-Pro)$_4$-OMe (72 mg, 0.063 mmol) and Boc-β-Ala-TRIS-(OH)$_3$ (6.4 mg, 0.013 mmol) in freshly distilled DCM (6 mL) was stirred under N_2 until homogeneous. After 5 min, TEA (21 μL, 0.15 mmol) was added followed by DEPBT (23 mg, 0.076 mmol). The reaction mixture was stirred under N_2 for 16 h and the solvent was removed under reduced pressure. The remaining residue was dissolved in 4 mL $CHCl_3$ and precipitated with 10 mL Et_2O. The suspension was centrifuged and the supernatant was discarded. This precipitation/centrifugation procedure was repeated three times. The remaining residue was dissolved in 100 mL $CHCl_3$ and washed with 1 N HCl (2× 10 mL), saturated $NaHCO_3$ (3× 10 mL) and brine (10 mL). The organic layer was dried over anhydrous Na_2SO_4. After filtration, the solvent was removed under reduced pressure and the resulting slightly yellow solid was dialyzed over molecular weight cutoff (MWCO) 3500 membrane in H_2O for 24 h. After lyophilization, a white solid was obtained and purified by preparative RP-HPLC to obtain a white powder (5.8 mg, 12.3%).

MS-MALDI-TOF (*m/z*, $[M+Na]^+$) calculated for $C_{180}H_{294}N_{38}O_{48}$ 3780, found 3779 $[M+Na]^+$, 3679 $[M+Na–Boc]^+$. Analytical RP-HPLC $R_t = 22.0$ min (20–90% solvent B over 30 min).

2.3.4. Boc-β-Ala-TRIS-[(Gly-Nleu-Pro)$_5$-OMe]$_3$

A solution of HCl·H-(Gly-Nleu-Pro)$_5$-OMe (219 mg, 0.16 mmol) and Boc-β-Ala-TRIS-$(OH)_3$ (16 mg, 0.031 mmol) in freshly distilled DCM (6 mL) was stirred under N_2 until homogeneous. After 5 min, TEA (52 μL, 0.37 mmol) was added followed by DEPBT (56 mg, 0.19 mmol). The reaction mixture was stirred under N_2 for 16 h and the solvent was removed under reduced pressure. The remaining residue was dialyzed over MWCO 3500 membrane in 1:1 MeOH/H_2O for 24 h. After lyophilization, a white solid was obtained and purified by preparative RP-HPLC to obtain a white powder (69 mg, 48.5%). MS-MALDI-TOF (*m/z*, $[M+H]^+$) calculated for $C_{219}H_{357}N_{47}O_{57}$ 4561; found 4561 $[M+H]^+$. Preparative RP-HPLC $R_t = 12.2$ min (45–80% solvent B over 30 min).

2.3.5. Boc-β-Ala-TRIS-[(Gly-Nleu-Pro)$_8$-OMe]$_3$

A solution of HCl·H-(Gly-Nleu-Pro)$_8$-OMe (171 mg, 0.078 mmol) and Boc-β-Ala-TRIS-$(OH)_3$ (9.4 mg, 0.018 mmol) in freshly distilled DCM (10 mL) was stirred under N_2 until homogeneous. After 5 min, TEA (39 μL, 0.28 mmol) was added followed by DEPBT (44 mg, 0.15 mmol). The reaction mixture was stirred under N_2 for 16 h and the solvent was removed under reduced pressure. The remaining residue was dissolved in 4 mL $CHCl_3$ and precipitated with Et_2O. The suspension was centrifuged and the supernatant was discarded. This precipitation/centrifugation procedure was repeated twice. The remaining residue was dissolved in 100 mL $CHCl_3$ and washed with 1 N HCl (2× 10 mL) and saturated $NaHCO_3$ (2× 10 mL). The organic layer was dried over anhydrous Na_2SO_4. After filtration, the solvent was removed under reduced pressure and the resulting white solid was purified by preparative RP-HPLC to obtain a white powder (44 mg, 34.5%). MS-MALDI-TOF (*m/z*, $[M+Na]^+$) calculated for $C_{336}H_{546}N_{74}O_{84}$ 6989; found 6988 $[M+Na]^+$, 6888 $[M+Na–Boc]^+$. Analytical RP-HPLC $R_t = 15.3$ min (40–90% solvent B over 30 min).

3. Results and discussion

3.1. CD spectroscopy

Natural collagen has a characteristic CD spectrum which has a small positive peak at about 220 nm, a crossover near 213 nm, and a large trough at approximately 197 nm [39–41]. These features have been used as a reference to determine the presence of synthetic collagen-like triple helices in solution. The CD spectra (obtained at 6 °C) of the TRIS-assembled collagen mimetic structures in H_2O at 0.2 mg/mL concentration are shown in Fig. 4a.

The CD spectra of collagen mimetic peptides composed of the Gly-Nleu-Pro sequence exhibit the positive peak only when it is triple helical [27]. The CD spectra for Boc-β-Ala-TRIS-[(Gly-Nleu-Pro)$_n$-OMe]$_3$ (where $n = 1$ and 4) are typical of non-triple helical arrays as evidenced by the shallow troughs and lack of a positive peak. However, the CD spectra for the longer analogs Boc-β-Ala-TRIS-[(Gly-Nleu-Pro)$_n$-OMe]$_3$ ($n = 5$, 6, and 8) are indicative of triple helical structures, with crossovers near 216 nm, peaks at 222 nm, and troughs at around 201 nm. These spectral absorbances and Rpn values (the ratio of the

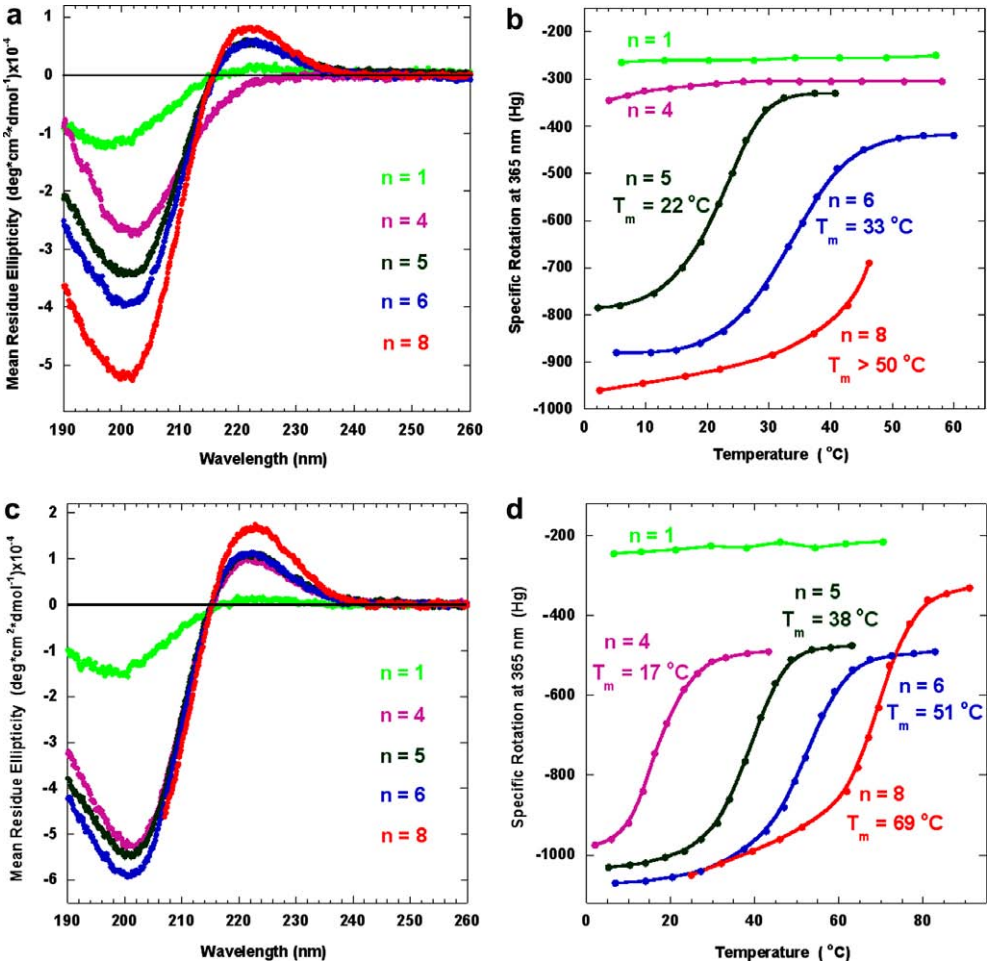


Fig. 4. (a) CD spectra at 6 °C and (b) thermal melting curves of Boc-β-Ala-TRIS-[(Gly-Nleu-Pro)$_n$-OMe]$_3$ ($n = 1$, 4, 5, 6, and 8) at 0.2 mg/mL in H_2O. Boc-β-Ala-TRIS-[(Gly-Nleu-Pro)$_8$-OMe]$_3$ precipitated when heated to above 45 °C. (c) CD spectra at 6 °C and (d) thermal melting curves of Boc-β-Ala-TRIS-[(Gly-Nleu-Pro)$_n$-OMe]$_3$ ($n = 1$, 4, 5, 6, and 8) at 0.2 mg/mL in 2:1 EG/H_2O.

positive peak intensity over the negative peak intensity) [27,29], listed in Table 1, are comparable to known collagen triple helical conformations as observed in natural collagen and (Gly-Pro-Hyp)$_{10}$-NH_2 [39,40,42]. Our previous studies have demonstrated that Rpn values above 0.10 are indicative of triple helical structures in solution [27–29].

The solvent mixture ethylene glycol–water (EG/H_2O) is known to stabilize helical structures and is useful to amplify and detect weak triple helical collagen-like propensities [39,40]. The CD spectra (obtained at 6 °C) of the TRIS-assembled collagen mimetic structures in 2:1 EG/H_2O at 0.2 mg/mL concentration are shown in Fig. 4c. There is no indication of a triple helix conformation (absence of a positive peak and shallow trough) for Boc-β-Ala-TRIS-[(Gly-Nleu-Pro)$_1$-OMe]$_3$ since the peptide chain is too short to form a triple helix. All other arrays ($n = 4$, 5, 6, and 8) are triple helical. A comparison between

Table 1
The CD parameters (0.2 mg/mL in H_2O at 6 °C) of TRIS-assembled peptides Boc-β-Ala-TRIS-[(Gly-Nleu-Pro)$_n$-OMe]$_3$ (n = 1, 4, 5, 6, and 8). "NA" denotes "not applicable"

Peptide	Max*	Cross	Min*	Rpn
Boc-β-Ala-TRIS-[(Gly-Nleu-Pro)$_1$-OMe]$_3$	NA	NA	197 nm (−1.2)	NA
Boc-β-Ala-TRIS-[(Gly-Nleu-Pro)$_4$-OMe]$_3$	NA	NA	202 nm (−2.7)	NA
Boc-β-Ala-TRIS-[(Gly-Nleu-Pro)$_5$-OMe]$_3$	222 nm (0.5)	216 nm	201 nm (−3.4)	0.15
Boc-β-Ala-TRIS-[(Gly-Nleu-Pro)$_6$-OMe]$_3$	222 nm (0.5)	216 nm	201 nm (−3.9)	0.13
Boc-β-Ala-TRIS-[(Gly-Nleu-Pro)$_8$-OMe]$_3$	222 nm (0.8)	216 nm	201 nm (−5.2)	0.15
Natural collagen[a]	220 nm (0.7)	213 nm	198 nm (−5.4)	0.13
(Gly-Pro-Hyp)$_{10}$-NH_2[b]	225 nm (0.4)	218 nm	198 nm (−3.4)	0.13

* Values in the parenthesis represent mean residue ellipticity in unit (deg cm^2 dmol^{-1}) × 10^{-4}.
[a] Refs. [39,40].
[b] Ref. [42].

the CD spectra of Boc-β-Ala-TRIS-[(Gly-Nleu-Pro)$_n$-OMe]$_3$ in H_2O (Fig. 4a) and 2:1 EG/H_2O (Fig. 4c) clearly demonstrates the enhancement of triple helicity by the latter solvent. Boc-β-Ala-TRIS-[(Gly-Nleu-Pro)$_4$-OMe]$_3$ transforms from a non-triple helical structure to a triple helical one when the solvent changes, exhibiting a previously non-existent positive peak in the CD spectrum. For the longer peptides ($n = 5$, 6, and 8), the intensity of both the positive peaks and the troughs is stronger in 2:1 EG/H_2O.

3.2. Thermal denaturation studies

The melting transitions of the TRIS-assembled collagen mimetics are consistent with the observations from CD spectroscopy. Temperature-dependent optical rotation measurements were used to monitor the transition from triple helical to non-triple helical structures. Fig. 4b shows the melting transitions for the TRIS-assembled peptides in H_2O. While Boc-β-Ala-TRIS-[(Gly-Nleu-Pro)$_n$-OMe]$_3$ (where $n = 1$ and 4) exhibits no transition, the T_m (melting temperature) values for Boc-β-Ala-TRIS-[(Gly-Nleu-Pro)$_n$-OMe]$_3$ ($n = 5$ and 6) are 22 and 33 °C, respectively. As the peptide chain length increases, the thermal stability of the TRIS-assembled structures increases. The solution of Boc-β-Ala-TRIS-[(Gly-Nleu-Pro)$_8$-OMe]$_3$ became cloudy soon after the triple helix started to melt, therefore the T_m could not be determined accurately. However, it is safe to say that the T_m of Boc-β-Ala-TRIS-[(Gly-Nleu-Pro)$_8$-OMe]$_3$ is higher than 50 °C. It is known that many triple helical structures precipitate at elevated temperature as the triple helices melt [27,28]. Previously reported molecular modeling studies of the triple helices composed of

Table 2
Melting temperatures of TRIS-assembled collagen mimetic peptides composed of the Gly-Nleu-Pro sequence

Peptide	H_2O (°C)	2:1 EG/H_2O (°C)
Boc-β-Ala-TRIS-[(Gly-Nleu-Pro)$_1$-OMe]$_3$	—	—
Boc-β-Ala-TRIS-[(Gly-Nleu-Pro)$_4$-OMe]$_3$	—	17
Boc-β-Ala-TRIS-[(Gly-Nleu-Pro)$_5$-OMe]$_3$	22	38
Boc-β-Ala-TRIS-[(Gly-Nleu-Pro)$_6$-OMe]$_3$	33	51
Boc-β-Ala-TRIS-[(Gly-Nleu-Pro)$_8$-OMe]$_3$	> 50	69

All measurements were carried out at a concentration of 0.2 mg/mL.

the Gly-Nleu-Pro sequence revealed that most of the hydrophobic side chains are buried inside the triple helix at low temperature [3,32,33]. Therefore, when the temperature increases and the triple helix starts to melt, the peptide chains unwind and all the hydrophobic side chains are exposed to the solvent. Thus, aggregation occurs and the peptide precipitates.

In addition, melting transitions for Boc-β-Ala-TRIS-[(Gly-Nleu-Pro)$_n$-OMe]$_3$ ($n = 1$, 4, 5, 6, and 8) were assessed in 2:1 EG/H_2O solutions (Fig. 4d). As expected, the T_m values in EG/H_2O are higher than those measured in H_2O since it is known that EG/H_2O mixtures enhance the thermal stability of triple helical conformations (Table 2) [39,40]. Even with only four repeats of Gly-Nleu-Pro, the peptide is triple helical with a T_m of 17 °C. A complete thermal melting curve of Boc-β-Ala-TRIS-[(Gly-Nleu-Pro)$_8$-OMe]$_3$ was obtained in 2:1 EG/H_2O without precipitation possibly because ethylene glycol can solvate the hydrophobic side chains much better than water.

3.3. Discussion

The TRIS scaffold was selected for the present study because it contains an additional reactive site (besides the three carboxylic acid groups which were used for attaching peptide chains) which can be employed in future studies and development of biomaterials. TRIS-assembled peptides have comparable thermal stability as the KTA-assembled collagen mimetics of the same chain length, while both have lower T_m values than TREN-assembled peptides [27–30]. This is probably due to the slightly greater flexibility of the TREN scaffold over the TRIS scaffold which can better accommodate the register shift between the three peptide strands.

The TRIS scaffold has several advantages over the KTA and the TREN scaffolds. As we have reported, we prepared KTA- and TREN-assembled collagen minetics via solid phase synthesis because the various coupling reagents we used failed to give acceptable yields in solution when attaching peptide chains longer than three Gly-Xaa-Yaa repeats directly to the scaffolds [27–30]. Moreover, the KTA and the TREN scaffolds contain only three carboxylic acid groups to attach peptide chains. After the peptide chains are attached, the scaffolds cannot be easily modified. However, the TRIS scaffold contains an extra Boc-protected amino group. Recently, we reported the synthesis and characterization of collagen mimetic dendrimers in which the Boc group was removed and the TRIS-assembled structures were attached to a trimesic acid core structure [34,43,44]. To our surprise, it was found that the collagen mimetic dendrimer exhibits higher thermal stability than the component TRIS-assembled structures (37 °C vs. 33 °C in H_2O; 63 °C vs. 51 °C in 2:1 EG/H_2O). Concentration-dependent thermal melting experiments revealed that the stabilizing effect arises from an intramolecular clustering of the triple helical arrays about the core structure. This ensemble excludes solvent from the interior portion of the array, and therefore stabilizes the triple helical bundle.

TRIS-assembled peptides also contain C-terminal ester groups which can be readily removed to attach other functional groups for biomaterial purposes. We have reported the attachment of Fe^{3+}-binding groups (i.e. 2,3-dihydroxybenzoic acid via an ethylenediamine linker) to the C terminus of TRIS-assembled structures containing six repeats of the Gly-Nleu-Pro sequence [35]. When Fe^{3+} is absent, the catechol-containing peptide has a similar T_m value to the parent structure (36 and 33 °C, respectively). However, when 1/3 equiv of Fe^{3+} was added to the peptide solution in pH 10 CAPS

(3-cyclohexylamino-1-propane sulfonic acid) buffer, the 1:3 Fe^{3+}–catechol complex functions as an extra C-terminal scaffold to produce a triple helix where both the N- and the C-termini of the peptide chains are tethered. This di-scaffold-assembled triple helix has an extraordinary T_m of 58 °C.

4. Conclusions

The TRIS scaffold, Boc-β-Ala-TRIS-$(OH)_3$, was used for facile and efficient assembly of triple helical collagen-like peptides. The scaffold assembly can be achieved through direct coupling between long peptide chains and the TRIS scaffold using DEPBT with high efficiency and good yield (74.8% after HPLC purification for Boc-β-Ala-TRIS-[(Gly-Nleu-Pro)$_6$-OMe]$_3$). The TRIS-assembled structures exhibit comparable thermal stability as our previously reported KTA- or TREN-assembled peptides while possessing several additional advantages. The protecting group on the TRIS scaffold and at the C-termini of the peptide chains can be easily removed for further modification such as the making of peptide dendrimers and attachment of metal-binding groups at the C-termini, both of which benefit from the fact that the TRIS-assembly can be carried out in relatively large scale at acceptable yield because of the synthesis in solution using DEPBT.

Acknowledgments

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