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Tricine as a convenient scaffold for the synthesis of *C*-terminally branched collagen-model peptides

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ABSTRACT

A novel and convenient method for the synthesis of *C*-terminally branched collagen-model peptides has been achieved using tricine (*N*-[tris(hydroxymethyl)methyl]glycine) as a branching scaffold and 1,2-diaminoethane or 1,4-diaminobutane as a linker. The peptide sequence was incorporated directly onto the linker and scaffold during solid-phase synthesis without additional manipulations. The resulting branched triple-helical peptides exhibited comparable thermal stabilities to the parent, unbranched sequence, and served as substrates for matrix metalloproteinase-1 (MMP-1). The tricine-based branch reported herein represents the simplest synthetic scaffold for the convenient synthesis of covalently linked homomeric collagen-model triple-helical peptides.

Collagen is the most abundant structural protein in all animals.[1] In humans it is the most prevalent component of the extracellular matrix (ECM).[1] The defining feature of collagen is an elegant structural motif in which three parallel polypeptide strands in a left-handed polyproline II-type helical conformation coil about each other with a one-residue staggered to form a right-handed triple-helix.[1,2]

Synthetic triple-helical peptides (THPs) that mimic the structure of native collagens have been used to investigate collagen-protein interactions as well as collagen structure and stability.[3] Our laboratory has been particularly interested in utilizing triple-helical peptides as model substrates for studying the mechanism of collagenolysis.[4–8]

Numerous scaffolds (templates) have been used to create stable THPs.[9] The main categories of template strategies involve (a) a scaffold to which the peptides are covalently attached or (b) the use of metal ions for non-covalent association. The covalent templates include Lys-Lys or Glu-Glu branching,[10–21] double disulfide knots,[22–29] *cis,cis*-1,3,5-trimethyl-cyclohexane-1,3,5-tricarboxylic acid (Kemp triacid),[30–38] tris(carboxyethoxymethyl)aminomethane (TRIS),[39–41] tris(2-aminoethyl)amine (TREN),[42,43] cyclotriverarylene (CTV),[44] and a macrocyclic scaffold.[45] Alternatively, metal ions such as Ca^{2+}, Ru^{2+}, Ni^{2+}, Fe^{3+}, Cd^{2+}, Hg^{2+}, Cu^{2+}, Zn^{2+}, or Ln^{3+} have been used in conjugation with amino acids, hydroxamic acid, catechol, or pyridyl or bipyridyl functionalities to create or stabilize multistrand linkages.[40,41,46–52]

An *N*- or *C*-terminal template is expected to align and entropically stabilize the THP and thus enhance triple-helical thermal stability.[10] Templated triple-helices have been utilized to enhance the binding of triple-helices to surfaces and for developing triple-helices as affinity chromatography ligands.[53,54] Such utilization of template triple-helices can allow for the creation of novel biomaterials and the isolation of receptors that bind to collagen. Herein, we present a convenient strategy for the synthesis of *C*-terminally branched, homotrimeric THPs compatible with 9-fluorenylmethoxycarbonyl (Fmoc) solid-phase chemistry.

While constructing peptoid-containing THPs using solid-phase methodology,[55] we considered incorporating tricine, *N*-[tris(hydroxymethyl)methyl]glycine, as a building block using a simple method that is compatible with existing Fmoc chemistry. Tricine, commonly used as a buffering agent,[56] is scarcely soluble in organic solvents. The addition of protecting groups that would make tricine suitable for Fmoc solid-phase peptide synthesis would require several synthetic manipulations. This potential inconvenience was bypassed by the direct, on-resin synthesis of a tricine residue using the submonomeric method typically applied for solid-phase peptoid synthesis (Scheme 1).[57,58]

The synthesis of the THP started with the coupling of a *C*-terminal Tyr residue to serve as a chromophore for eventual peptide concentration determination. Subsequently, after coupling of

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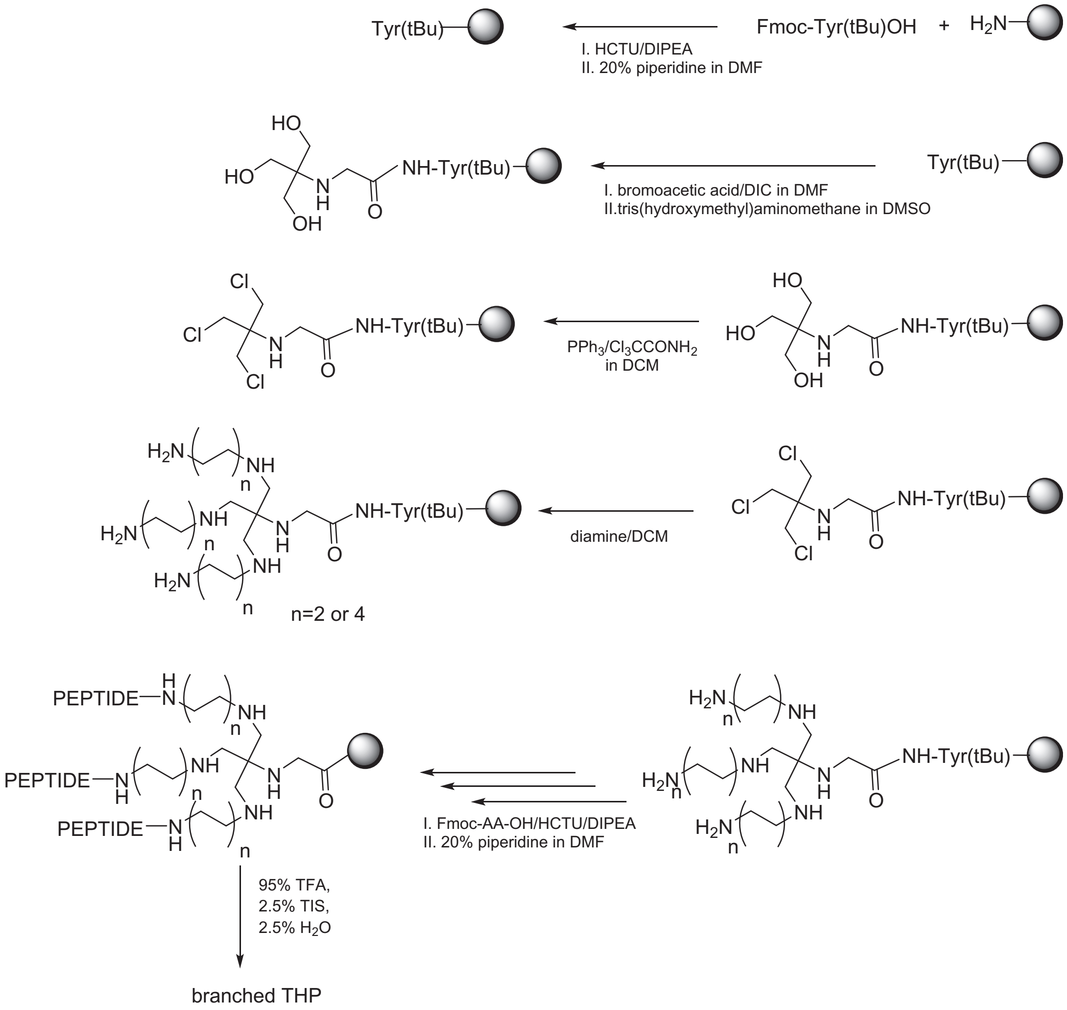

Scheme 1. Synthetic route for on-resin incorporation of the tricine residue and branching strategy for DAE α1(I)772–786 THP and DAB α1(I)772–786 THP syntheses. Conditions were as follows: step 1, (I) Fmoc-Tyr(*t*Bu), HCTU, DIPEA (3 equiv., 60 min) in DMF, (II) 20% piperidine in DMF for 2 × 5 min; step 2, (I) bromoacetic acid, DIC (5 equiv., 45 min) in DMF, (II) tris(hydroxymethyl)aminomethane (40 equiv.) in DMSO (18 h); step 3, PPh_3 (20 equiv.), Cl_3CCONH_2 (20 equiv.) in DCM (60 min); step 4, diamine (50 equiv.) in DCM (4 h); step 5, peptide synthesis as previously described;[55] step 6, peptide cleavage and side-chain deprotection as previously described.[55]

bromoacetic acid using *N*,*N*-diisopropylcarbodiimide, the bromine was substituted with TRIS to afford a tricine molecule (Scheme 1).

During the course of our investigations, we found that the resin-bound terminal hydroxyl groups could be easily converted into chlorides using triphenylphosphine and trichloroacetamide *via* previously reported in-solution conditions.[59] This method was found to be mild and suitable for solid-phase synthesis on acid-labile resins. The resin-bound tricine was treated with triphenylphosphine and trichloroacetamide in CH_2Cl_2 in order to convert the terminal hydroxyl groups to chlorides (Scheme 1).

Terminal chloride moieties undergo substitution with diamine linkers to afford amino groups ready for further synthesis. In general, the linkers that support branched collagen-like peptides play a crucial role in alignment of the three strands.[30,61] For this purpose, 1,2-diaminoethane (DAE) and 1,4-diaminobutane (DAB) were tested as suitable linkers between the tricine branch and the collagen-model peptide sequence. DAE or DAB was incorporated onto the resin-bound tricine, displacing the chlorides (Scheme 1). The resin substitution level was then quantified using a ninhydrin test or, following installation of an Fmoc protecting group, by spectrophotometric analysis of the fulvene-piperidine adduct.[60]

The α1(I)772–786 collagen sequence, commonly used as matrix metalloproteinase (MMP) model substrate,[62,63] was then incorporated onto the tricine scaffold (Scheme 1 and Fig. 1). With the exception of the manual incorporation of tricine, the synthetic route proceeded using standard Fmoc chemistry on a microwave peptide synthesizer (Liberty Blue, CEM Corporation) according to previously published protocols.[55]

The obtained THPs were compared to the unbranched α1(I) 772–786 THP and the Lys-Lys branched α1(I)772–786 THP (Fig. 1) reported previously.[53] The triple-helical conformations of DAE α1(I)772–786 THP, DAB α1(I)772–786 THP, and unbranched α1(I)772–786 THP were evaluated by circular dichroism (CD) spectroscopy. At room temperature, all peptides exhibited typical features of a collagen-like conformation,[2] such as a large negative molar ellipticity ([Θ]) at $\lambda = 200$ nm and a positive [Θ] at $\lambda = 225$ nm (data not shown). The thermal stabilities of the peptides were evaluated by measuring [Θ] at $\lambda = 225$ nm as a function of temperature (Fig. 2). When the temperature was increased from 5 to 70 °C, DAE α1(I)772–786 THP and DAB α1(I)772–786 THP showed structural transitions (triple-helix → unfolded species) with midpoints (T_m values) of 34 and 35 °C, respectively, compared with 38 °C for the unbranched α1(I)772–786 THP (Fig. 3). While both DAE α1(I)772–786 THP and DAB α1(I)772–786 THP formed relatively stable triple-helices, it was surprising that the tricine branch did not enhance the thermal stability of the α1(I)772–786