# LETTERS

# Total Synthesis of Elastin Peptide Using High Pressure—Liquid Phase Synthesis Assisted by a Soluble Tag Strategy

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**Supporting Information** 

**ABSTRACT:** A highly aggregating elastin peptide was prepared efficiently using a high pressure—liquid phase synthesis approach assisted by a soluble tag strategy. Two standard syringes were connected to each other to construct a reactor. This simple reactor was used to apply high pressure to the highly viscous reaction mixture thereby maintaining its fluidity. The reactions were completely inhibited due to aggregation when conducted in a standard flask reactor, whereas our high pressure approach accelerated the couplings to realize complete conversion within 5–7 min. All steps were conducted at 0.10 M concentration, affording grams of the desired product.

T he high modularity and diversity of peptides result in their strong affinity for a wide range of targets, ranging from small metal ions to large biomacromolecules such as proteins and nucleic acids, depending on the precise sequence of the peptide. Peptides can recognize and interact specifically with themselves, called self-assembly, to form supramolecular networks which cause the gelation of aqueous solvents.<sup>1</sup> Such peptide-based hydrogelators are attractive tools for developing soft biomaterials because the properties of the gelator can be tuned by altering the amino acid sequences including both proteinogenic and nonproteinogenic.<sup>2</sup> Strategies that allow the rational design and control of gelation properties have recently been proposed to allow novel bioapplications of peptide gelators.<sup>3</sup>

However, the unique gelation property of peptides often makes their chemical synthesis difficult. Although gelation can seem like a technical issue, it can cause severe problems and limit the utility of the peptides, especially hydrophobic peptides. Indeed, peptide aggregation can occur even on solid phase resin, making the reactive end of the peptide inaccessible and inhibiting the peptide's elongation. Microwave-assisted methods can overcome this obstacle,<sup>4</sup> but a synthetic method applicable to large scale production of aggregating peptides, regardless of sequence, remains elusive.

We have developed a soluble tag-assisted liquid phase peptide synthesis method using hydrophobic benzyl alcohol as a support.<sup>5</sup> Both coupling and deprotection are conducted in the liquid phase using a less polar organic solvent (typically dichloromethane or tetrahydrofuran). The desired tagged



peptide is recovered as a precipitate following dilution of the reaction mixture with excess polar organic solvent (typically methanol or acetonitrile). Our approach is a promising alternative for synthesizing aggregating peptides because intermolecular hydrophobic interactions are minimized in less polar organic solvents compared with the polar organic solvents (typically *N*,*N*-dimethylformamide or *N*-methylpyrrolidone) commonly used in solid phase peptide synthesis.

Elastin is an extracellular matrix protein that provides elasticity to several human tissues, including arteries, lungs, and skin.<sup>6</sup> Tropoelastin, a water-soluble precursor, is highly cross-linked to form native insoluble elastin. The elastic properties of elastin make it an intriguing scaffold for functional biomaterials that could have medical, clinical, therapeutic, and cosmetic applications.<sup>7</sup> For example, inadequate elastin in the skin causes wrinkles. However, these physical properties of elastin are accompanied by a synthetic challenge: namely, the gelation and aggregation of the protein. The chemical structure of elastin comprises a simple repeating sequence, generally of the nonpolar amino acids glycine (Gly), valine (Val), and proline (Pro) (Figure 1). Described herein is the highly efficient large scale production of elastin peptide using high pressure-liquid phase peptide synthesis assisted by a soluble tag strategy.

The tag-assisted liquid phase peptide synthesis approach was based on our routinely used procedure. The first coupling of

Received: November 12, 2014



Figure 1. Chemical structure of the typical repeat sequence of elastin peptide, and of the soluble tag.

Fmoc-Val-OH to the tag 1 (HO-TAG) was carried out in toluene using N,N'-diisopropylcarbodiimide (DIPCI) and 4dimethylaminopyridine (DMAP), followed by dilution of the reaction mixture with an excess of acetonitrile to recover the desired Fmoc-Val-O-TAG as a precipitate. Following deprotection of the Fmoc group in tetrahydrofuran using 1,8diazabicyclo[5.4.0]undec-7-ene (DBU) and piperidine, the reaction mixture was diluted with an excess of acetonitrile to obtain H-Val-O-TAG as a precipitate.

However, although coupling of Fmoc-Gly-OH to H-Val-O-TAG proceeded smoothly in tetrahydrofuran using N-[1-(cyano-2-ethoxy-2-oxoethylideneaminooxy)dimethylamino-(morpholino)]uronium hexafluorophosphate (COMU) and N,N-diisopropylethylamine (DIPEA), during workup the addition of an excess of acetonitrile caused the immediate formation of a heterogeneous sticky precipitate containing a significant amount of COMU which was difficult to remove (Figure S1 in Supporting Information (SI)).

After investigating numerous conditions, a 1:1 (v/v) biphasic mixture of cyclohexane and acetonitrile used as the reaction *solvent* instead of tetrahydrofuran was found to dramatically affect the properties of the precipitate (Figure S2 in SI). The addition of an excess of acetonitrile caused gradual formation of a homogeneous tight precipitate readily recovered by vacuum filtration from which COMU could be effectively rinsed away (Figure S3 in SI).

The precipitate was a white powder, in contrast to the pale yellow amorphous solid obtained when tetrahydrofuran was used as the reaction solvent (Figure 2). Particle size analysis clearly showed a narrower distribution when a 1:1 (v/v) biphasic mixture of cyclohexane and acetonitrile was used compared to tetrahydrofuran as the solvent (Figure S4 in SI). The biphasic solvent mixture was effective in the deprotection of the Fmoc group using DBU and piperidine, affording H-Gly-Val-O-TAG in high yield.



Figure 2. Morphology of Fmoc-Gly-Val-O-TAG prepared in (a) a 1:1 (v/v) biphasic mixture of cyclohexane and acetonitrile and (b) tetrahydrofuran.

However, when coupling of Fmoc-Val-OH to H-Gly-Val-O-TAG was attempted in the biphasic solvent mixture using COMU and DIPEA, the reaction mixture became a highly viscous gel within 3 min and stopped the mechanical stirrer, indicating severe aggregation (Figure S5 in SI). The reaction was largely inhibited, and most of the starting H-Gly-Val-O-TAG was recovered. Although the addition of a large excess of cyclohexane and/or heating could also aid coupling, we expected that *only reestablishing the fluidity of the reaction mixture was required* since the substrate and reagents were both in "liquid" phase and their collision still potentially took place. Therefore, we connected two syringes to each other to fluidize the reaction mixture under high pressure (Figure 3).



Figure 3. Schematic illustration of the connected syringes used as a reactor.

Coupling of Fmoc-Val-OH to H-Gly-Val-O-TAG in the connected syringes proceeded smoothly in 5 min during 50 passes to provide Fmoc-Val-Gly-Val-O-TAG (Figure S6 in SI). The plungers were simply pushed manually, requiring ca. 0.10-0.20 MPa to induce sufficient fluidity at each pass. The obtained highly viscous gel was poured into an excess of acetonitrile, causing gradual formation of a homogeneous tight precipitate readily recovered by vacuum filtration. The recovered precipitate was pure Fmoc-Val-Gly-Val-O-TAG and did not contain Fmoc-Val-OH, COMU, or DIPEA. The Fmoc group was readily deprotected using DBU and piperidine by stirring in a standard flask, providing H-Val-Gly-Val-O-TAG effectively. Although further couplings caused similar highly viscous gels, use of the syringe reactor accelerated the reactions to give the elastin repeat sequence, Fmoc-Pro-Gly-Val-Gly-Val-O-TAG 2, in 68% yield over 9 steps from the tag 1 (Scheme 1). Notably, all steps were conducted at 0.10 M concentration to afford grams of the desired product (Figure S7 in SI).

With the repeat sequence of the elastin peptide in hand, we focused on fragment coupling to prepare a longer variant (Scheme 2). Thus, Fmoc-Pro-Gly-Val-Gly-Val-O-TAG **2** was divided into two aliquots and the tag was removed from one aliquot in a 5:1 (v/v) mixture of dichloromethane and trifluoroethanol using trifluoroacetic acid to give Fmoc-Pro-Gly-Val-Gly-Val-OH **3** in 89% yield (see SI for experimental details). The reaction mixture was diluted with an excess of methanol to precipitate the removed tag, and then the crude peptide was reprecipitated from tetrahydrofuran using isoproyl ether to give pure Fmoc-Pro-Gly-Val-Gly-Val-OH **3**. Fmoc group deprotection of Fmoc-Pro-Gly-Val-Gly-Val-O-TAG **2** was achieved using a standard 1:1 (v/v) mixture of cyclohexane and acetonitrile to give H-Pro-Gly-Val-Gly-Val-O-TAG **4**. However, fragment coupling of Fmoc-Pro-Gly-Val-Gly-Val-Gly-Val-

Scheme 1. Preparation of the Repeat Sequence of Elastin Peptide



OH 3 and H-Pro-Gly-Val-Gly-Val-O-TAG 4 in a 1:1 (v/v) biphasic mixture of cyclohexane and acetonitrile was unsuccessful, as Fmoc-Pro-Gly-Val-Gly-Val-OH 3 was not soluble in either cyclohexane or acetonitrile. This problem was solved by use of acetone instead of acetonitrile (Figure S8 in SI); the coupling proceeded smoothly in a 1:1 (v/v) mixture of cyclohexane and acetone to give Fmoc-(-Pro-Gly-Val-Gly-Val-)<sub>2</sub>-O-TAG 5 in 77% yield over 2 steps (Figure S9 in SI). Finally, the Fmoc group was deprotected and the tag was removed using standard conditions to give the desired elastin

peptide H-(-Pro-Gly-Val-Gly-Val-)<sub>2</sub>-OH **6** in 88% yield over 2 steps (see SI for experimental details). After precipitating the released tag by adding an excess of methanol, the crude peptide was washed with acetonitrile to give pure H-(-Pro-Gly-Val-Gly-Val-)<sub>2</sub>-OH **6** (Figure S10 in SI).

In conclusion, we have demonstrated the efficient preparation of a highly aggregating elastin peptide using a high pressure-liquid phase synthesis approach assisted by a soluble tag strategy. Two syringes were connected to construct a reactor that could pressurize the highly viscous reaction mixture and maintain its fluidity. Using this approach, each coupling was completed during 50 passes within 5-7 min, even at 0.10 M concentration to afford grams of the desired product. After coupling, the obtained highly viscous reaction mixture was simply poured into excess acetonitrile, resulting in the gradual formation of a homogeneous tight precipitate which was easily recovered by vacuum filtration and showed excellent purity. Although the reaction mixtures were highly viscous gels, all substrates and reagents were maintained in a "liquid" phase in the syringe reactor which provided sufficient fluidity to realize effective collisions between the reactant molecules. It should be noted that the procedure required only standard equipment found in any laboratory. We believe that the approach described herein could be applied to an automated pressurized peptide synthesizer to realize the preparation of aggregating peptides. A description of such a synthesizer will be presented in due course.

Scheme 2. Preparation of Longer Elastin Peptide through Fragment Coupling



#### **Organic Letters**

ASSOCIATED CONTENT

#### **Supporting Information**

Additional figures, general, and experimental information. This material is available free of charge via the Internet at http://pubs.acs.org.

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### Notes

The authors declare no competing financial interest.

#### ACKNOWLEDGMENTS

This work was partially supported by Grant-in-Aid for Scientific Research from Japan Society for the Promotion of Science.

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