First Soluble Multipolymer MPEG-Supported Liquid-Phase Convergent Synthesis of Tripeptide Fmoc-GlyVal-GlyOH

Chuan-Xin Zhang,* Hai-Bo Tong, and Chao-Guo Yan

College of Chemistry and Chemical Engineering, Yangzhou University, Yangzhou 225002, Jiangsu, P.R.China

Polymer supported solid-phase organic synthesis has been widely used in combinatorial chemistry.[1] The preparation of peptides was realized only after the application of the technique to solid-phase peptide synthesis (SPPS),[2] which was first developed by Merrifield.[3] However, the heterogeneous nature of such synthesis presents many problems associated with insoluble polymers, including nonlinear kinetic behavior, unequal distribution or access to the chemical reaction, solvent problems associated with the nature of the support, and synthetic difficulties in transferring the standard organic to the solid phase. Moreover the characterization of all intermediates in polymer-supported reactions has been a long-standing problem.

In recent years, the soluble polymer-supported liquid-phase organic synthesis (LPOS) has received significant attention[4] because it possesses many advantages over solid-phase synthesis such as homogeneous reaction conditions, easy-to-monitor reaction by NMR, more normal reaction kinetics, easier compound characterization, isolation, and purification through precipitation and filtration.

The cornerstone of soluble polymer-supported organic synthesis is a soluble, linear polyethylene glycol monomethyl ether (MPEG) that also serves as terminal protecting group for the library of compounds synthesized. MPEG exhibits solubility in a wide range of solvents including DMF, dichloromethane, toluene, acetonitrile, and water. Because of MPEG's poor solubility in diethyl ether, hexane, *tert*-butyl methyl ether, and isopropyl alcohol, these solvents have been used to induce MPEG precipitation for purification.[5] The solubility of MPEG permits homogeneous reaction conditions, in addition to allowing individual reaction steps to be monitored without requiring cleavage from the polymer support in synthesis and LPOS.[6] The characterization of the MPEG-immobilized organic moieties is often identical to solution-phase small-molecule characterization because the polymer does not interfere with spectroscopic or the chemical methods of analysis. MPEG also has the single methoxy group (3.38 ppm; ethyl protons of PEG backbone 3.64 ppm in CDCl$_3$), and we can use those as an internal integration standard to easily monitor the chemical reaction and identify the product chemical structure and polymer loading by ^{1}H NMR.

The use of MPEG-supported stepwise liquid-phase synthesis (LPPS) was first reported by Bayer in 1972.[7] More recently, others have reported using this technique.

The convergent solid-phase peptide synthesis (C-SPPS) is more efficient than the stepwise SPPS. By limitation of the number of coupling steps between fragments, C-SPPS can help to increase the yield and purity of the target peptide and avoid closely related deletion sequences.[13] For fully C-SPPS to be truly convergent, at least one of the molecular fragments must be synthesized on a soluble polymer so that it can be coupled to a fragment attached to either another soluble polymer carrier or an insoluble, heterogeneous one. There have been limited reports of such reactions that involve multiple polymers. The first example involves the use of the soluble polymer polyethylene glycol (PEG) to deliver individual amino acids in polystyrene-supported SPPS.[14] Another example uses PEG as the carrier for a ligand that is used to asymmetrically dihydroxylate alkene substrates that are bound to an insoluble polymer.[15] In a related system, a soluble polystyrene-supported trialkylamine reagent was used to deprotonate insoluble polystyrene-supported substrates in the product cleavage reaction during the synthesis of a tertiary amine library.[16] Finally, and perhaps most significantly, the first report of truly convergent fully liquid-phase organic synthesis (LPOS) has appeared.[11] In this research, a Pd(II)-catalyzed MPEG-supported liquid-phase convergent Suzuki coupling reaction is used to generate bisaryl-linked hexapeptides. To the best our knowledge, the backbone amide linkage (BAL) and hydroxymethylphoxy ester linkage (HMP) are herein reported in MPEG-supported liquid-phase peptide synthesis for the first time.

Treatment of soluble polymer MPEG-OMs (**1**) with 4-formyl-3,5-dimethoxyphenol or 4-hydroxybenzyl alcohol in anhydrous DMF in presence of Cs$_2$CO$_3$ results in **2** (BAL linker) and **3** (HMP linker), respectively (Scheme 1). The first residue Fmoc-Gly-OH was attached to alcohol polymer **3** by DIC/DMAP-mediated esterification to get polymer **4**. The Fmoc group was removed by treatment with 20% peperidine in DMF to obtain free amine fragment **A** (Scheme 2). Allyl Val-NH$_2$ was then loaded to MPEG by reductive amination empolying NaBH(OAc)$_3$ to form secondary amine **7**. The next stepwise introduction of Fmoc-Gly OH in synthesis of fragment **B** involves in situ carboxyl activation of the acylation to completion, and an excess of activated amino acid derivative is used. The general coupling activated

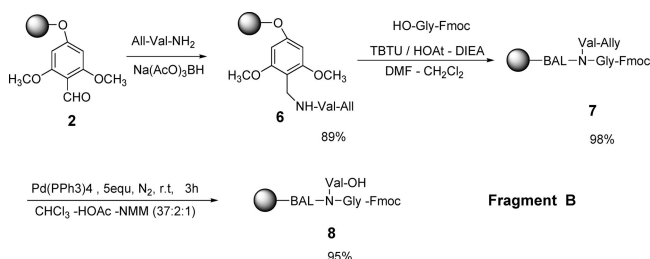**Scheme 1.** Synthesis of Intermediates **2** and **3**

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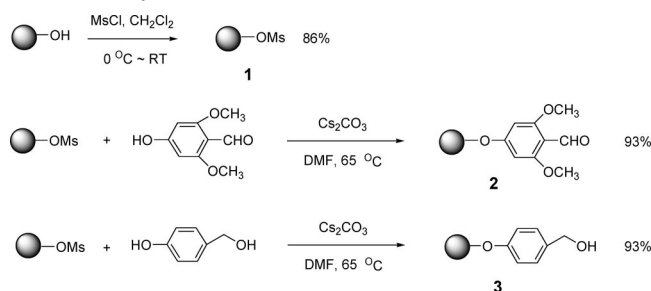
* To whom correspondence should be addressed. Telephone: +86 514 8797 5290. Fax: +86 514 8797 5244 . E-mail: cxzhang@yzu.edu.cn.
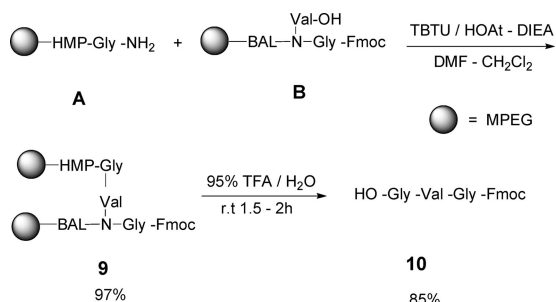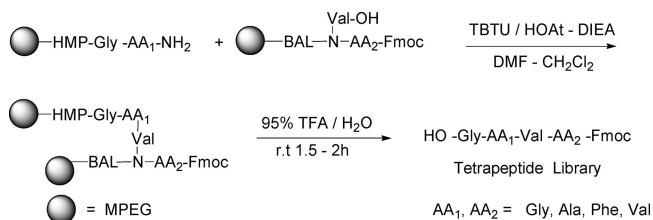
Scheme 2. Synthesis of Fragment **A**
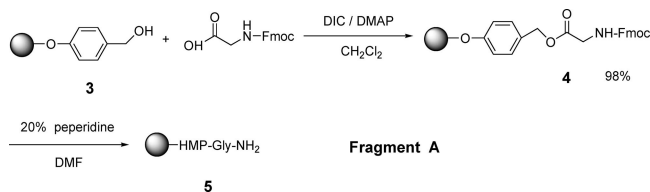
Scheme 3. Synthesis of Fragment **B**

Scheme 4. Convergent Synthesis of the Tripeptide HO-GlyVal-Gly Fmoc

Scheme 5. Reported Method used in Soluble Multi-Polymer-Supported Liquid-Phase Combinatorial Synthesis of Pure Tetrapeptide Library

reagents used were EDCI/HOAt or DIC/HOBt. Because of steric hindrance, peptide formation with the secondary amine is difficult. Some highly reactive coupling reagents, such as TBTU/DIEA, TBTU/HOAt-DIEA, HBTU/DIEA, or PyBOP/DIEA, were used to optimize the acylation reaction conditions. We found that TBTU/HOAt-DIEA yielded the best results in this case. Clean and selective removal of the C-terminal allyl ester of the fragment fragment **B** was achieved by treatment with Pd(PPh₃)₄ (5 equiv) in CHCl₃-HOAc-NMM (37:2:1) under argon at 25 °C for 3 h (Scheme 3). The C-terminal free fragment B was preactivated with TBTU/HOAt-DIEA and then coupled with N-terminal-free fragment **A** in liquid phase to convergently synthesize fragment **9**. Cleavage of the tripeptide from MPEG support by 95% TFA in water and recrystallization in anhydrous alcohol (to remove MPEG) produced the target crude HO-Gly-Val-Gly Fmoc. Further purification by silica gel chromatography (0.5% HAc in 10% CH₃OH /CH₂Cl₂) afforded the desired peptide (Scheme 4).

In summary, soluble MPEG has been used for the first time in the multipolymer-supported liquid-phase convergent synthesis of tripeptide HO-Gly-Val-Gly Fmoc. Backbone amide linkage (BAL) and hydroxymethylphoxy ester linkage (HMP) have been introduced for the MPEG-supported liquid-phase convergent synthesis of peptide. This new method possesses many advantages such as running under homogeneous reaction conditions, providing high yields of products, ease for monitoring by NMR, facile compound characterization, isolation, and purification through precipitation and filtration, and it can be used in soluble multipolymer liquid-phase combinatorial synthesis of pure peptide libraries.

Supporting Information Available. Procedures and ¹H NMR spectral data for intermediates **1**–**9** and tripeptide **10**. This material is available free of charge via the Internet at http://pubs.acs.org.

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