# Preparation, Characterization, and Application of Poly(vinyl alcohol)-graft-Poly(ethylene glycol) Resins: Novel Polymer Matrices for Solid-Phase Synthesis

Juntao Luo, Christophe Pardin, X. X. Zhu*, and William D. Lubell

*Département de Chimie, Université de Montréal, C.P. 6128, Succursale Centre-ville, Montréal, QC, H3C 3J7 Canada*

*Received September 25, 2006*

Spherical crosslinked poly(vinyl alcohol) (PVA) beads with good mechanical stability were prepared by reverse-suspension polymerization, using dimethyl sulfoxide (DMSO) as a cosolvent in an aqueous phase. Poly(ethylene glycol)s with varying chain lengths were grafted onto the PVA beads by anionic polymerization of ethylene oxide. The thermal behavior, morphology, and swelling were evaluated for each of the new polymer matrices. High loading and good swelling in water and organic solvents were characteristic of the PEG-grafted PVA beads. The polymer beads also exhibited good mechanical and chemical stability and were unaffected by treatment with 6 N HCl and with 6 N NaOH. The hydroxyl groups of the PVA-PEG beads were converted into aldehyde, carboxylic acid, and isocyanate functions to provide scavenger resins and were extended by way of a benzyl alcohol in a Wang linker. The transglutaminase substrates dipeptides (Z-Gln-Gly) and heptapeptides (Pro-Asn-Pro-Gln-Leu-Pro-Phe) were synthesized on PVA-PEG_5, PVA-PEG_20, and the Wang linker-derivatized PVA-PEG resins. The cleavage of the peptides from the resins using MeOH/$NH_3$ mixture at different temperatures (0 °C and room temp) and 50% TFA/DCM provided, respectively, peptide methyl esters, amides, and acids in good yields and purity as assessed by LC-MS analysis.

## Introduction

New solid supports having higher functional group loading and good swelling in a variety of solvents have been pursued to accomplish a wider range of solid-phase and solution-phase chemistry.[1] Although crosslinked polystyrene−divinylbenzene (PS-DVB) matrices[2] have been effective in solid-phase chemistry to produce peptides and small molecules, the hydrophobic polystyrene backbone has poor compatibility with aqueous and polar solvents, potential reactivity under electrophilic chemical conditions, and is generally unsuitable for on-bead magic-angle spinning (MAS) NMR analysis.[3,4] Polyethylene glycol (PEG) has been used to prepare resins that overcome the shortcomings of the PS-DVB resins. For example, PEG-grafted PS-DVB resins[5] and PEG-cross-linked resins[6] have exhibited improved swelling in water and polar solvents and are compatible for on-bead MAS NMR analysis, one-bead one-compound (OBOC) library synthesis,[7] and on-bead high-throughput screening of large compound libraries.[8]

High-loading resins are effective scavengers[9] and offer promise for a variety of chemistry. High loading on a resin with high swelling in a wide range of media has, however, not yet been achieved, in part, because of the restrictions of the hydrophobic PS-DVB core. For example, most contemporary PEG-grafted PS-DVB and PEG-cross-linked resins have normally low (0.2−0.5 mmol/g) functional group loading. As an alternative to PEG, polyglycerol[10] was grafted onto PS-DVB resin to increase loading (up to 4.3 mmol/g) and improve swelling in water (up to 2.6 mL/g); however, drawbacks to this strategy include a relatively low grafting yield and the presence of both primary and secondary alcohols that may exhibit different reactivities. Although dendrimer-based solid-phase supports have been prepared with higher functional-group loading,[11] their separation from the reaction media necessitates size-exclusion chromatography. Moreover, the presence of amide and ester bonds within the dendrimer structure limits the chemistry that can be performed on these supports.[12]

In a novel approach to address the need for a high-loading resin having optimal swelling and stability, we have grafted PEG chains onto a hydrophilic core. Cross-linked poly(vinyl alcohol) (PVA) resins are hydrophilic solid supports that have been successfully used as scavengers in organic synthesis.[9c] By grafting short PEG chains onto cross-linked poly(vinyl alcohol) resin, we have developed a novel polymer matrix that exhibits remarkable swelling in a range of solvents and high loading. The new PEG-grafted PVA resins were initially applied in the solid-phase synthesis of a dipeptide substrate and a resin-supported TEMPO catalyst for alcohol oxidation in a biphasic medium.[13] In addition, the PEG-grafted PVA resins proved suitable for the on-bead MAS NMR analysis.

In this paper, details are now relayed on the preparation and physical characterization of the PEG-grafted PVA resins with different crosslinking degrees and various chain lengths of PEG. Application of these resins in solid-phase peptide

* To whom correspondence should be addressed. E-mail: julian.zhu@umontreal.ca. Tel: (514)340-5172. Fax: (514)340-5290.

10.1021/cc060132+ CCC: $37.00 
Published on Web 06/23/2007

synthesis has been studied in the construction of peptide esters, amides, and acids. The hydroxyl groups on the PVA-PEG resin have also been oxidized into aldehyde and carboxylic acid groups to provide potential scavenger resins. These studies have demonstrated the merits of this novel high-loading resin that exhibits good swelling in both aqueous and organic media.

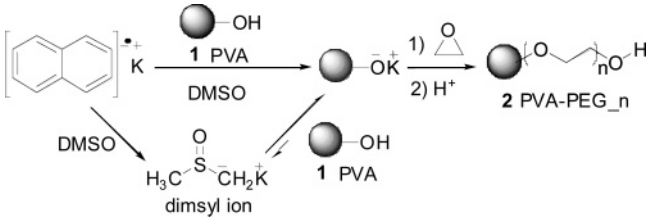
## Results and Discussions

**Preparation of PVA Beads.** Cross-linked PVA beads have previously been prepared (method A)[14] by a pre-cross-linking step involving mixing an aqueous solution of alkalized linear PVA with the cross-linker epichlorohydrin, before the addition of paraffin oil. Although spherical PVA beads were isolated from this process, the yields were relatively low because of the low solubility of epichlorohydrin in the aqueous PVA solution and the sharp increase in viscosity and gelation of the aqueous solution at the later stage of the pre-cross-linking step, which inhibited effective dispersion into the paraffin oil. By employing dimethylsulfoxide as a co-solvant (method B), a miscible solution containing epichlorohydrin and alkalized PVA was obtained that dispersed more effectively into paraffin oil to yield spherical cross-linked PVA beads in a 93% yield. The cross-linked PVA beads had a mean size of 87 $\mu$m as ascertained by particle sizing a suspension of the resin in water. The PVA beads that were prepared by method B exhibited better mechanical stability than those made by method A, likely because of their higher degree of crosslinking.

**Preparation of PVA-PEG Resin.** Cross-linked PVA beads have a relatively high loading of hydroxyl groups (15−17 mmol/g) and swell well in aqueous media. The introduction of short PEG chains onto the cross-linked PVA core was thus explored to improve swelling in organic solvents. Two pathways have been commonly used to attach PEG onto PS-DVB matrices: grafting of linear PEG onto Merrifield resin [5c,15] and anionic polymerization of ethylene oxide onto hydroxyl-terminated resin.[5a,5b,16,17] Initially, a few different strategies were attempted to graft linear PEG onto PVA resin (see Supporting Information), including ether bond formation by displacement of toluenesulfonate-activated PVA resin with alkylized PEG, alkylation of alkylized PVA resin with mono PEG chloride, as well as sequential acylations of PVA and PEG with a diisocyanate. Low-coupling yields as indicated by little mass gain was obtained in the attempted Williamson ether syntheses on the cross-linked PVA core. Although carbamate-linked PEG-grafted PVA could be made using isophorone diisocyanate (IPDI), the pronounced hydrophobic contribution of IPDI made the PVA-IPDI-PEG resin unsuitable as an amphiphilic support.

The polymerization of ethylene oxide was next explored on the cross-linked PVA core to make PEG-grafted PVA resin. Polymerization of ethylene oxide onto 2-(1-methyl)-hydroxyethyl polystyrene at high temperature under high-pressure had been reported for the preparation of PEG-grafted PS-DVB beads.[17] When ring-opening polymerization of ethylene oxide onto the cross-linked PVA beads was examined using similar high-pressure and high-temperature (110 °C) conditions in dioxane, the poor swelling of the PVA beads in dioxane inhibited polymerization on the resin. Instead, considerable amounts of linear PEG were produced. The beads obtained from this treatment exhibited relatively poor stability upon treatment with strong base at high temperature.

**Scheme 1.** Anionic Polymerization of Ethylene Oxide onto PVA Beads




Anionic polymerization of ethylene oxide has been typically performed in THF to prevent chain transfer and yield a polymer with narrow polydispersity.[18] Multiple attempts to polymerize ethylene oxide onto the PVA beads in THF provided, however, no weight increase, and resin was recovered that exhibited the same FT-IR and NMR spectra as the starting PVA resin. The poor swelling of PVA beads in THF was concluded to have blocked the accessibility of the hydroxyl groups for deprotonation and polymerization. DMSO was found to be a good solvent for swelling PVA beads. Although under strongly basic conditions, DMSO can be deprotonated to form dimsyl ions (Scheme 1),[19] the deprotonation of DMSO was considered to occur at a rate much slower than propagation of PEG chains from alkoxides because dimsyl ions are stronger bases than alkoxides.[20] Anionic polymerizations of ethylene oxide in DMSO provided a PEG-grafted polymer of molecular weight in close agreement with the calculated values,[21] with faster polymerization rates resulting from the higher dissociation constant of paired ions.[22] The improved swelling of PVA beads in DMSO was considered to increase the accessibility of the hydroxyl groups making them easier to be deprotonated and exposed for initiating ethylene oxide polymerization.

Potassium naphthalene, dimsyl ion, and PVA-supported potassium alkoxide, all can initiate the polymerization of EO (Scheme 1). To avoid the formation of ungrafted linear PEG in solution, potassium naphthalene and the intermediate dimsyl ion were minimized by allowing the alkaline suspension to stir for an additional hour after the complete disappearance of the green color of potassium naphthalene. Although linear PEG was produced in solution and some ethylene oxide may have leaked out of the reaction mixture, the anionic polymerization of EO onto PVA beads in DMSO was reproducible as illustrated by the successful attachment of PEG segments corresponding to five EO repeating units onto the PVA beads in three consecutive reactions (Table 1, entries 2−4). By adding different amounts of ethylene oxide to the reaction, we produced a series of PVA-PEG copolymer beads **2** with different PEG chain lengths (Table 1). Hydroxyl group loading in the copolymers was measured by acetic anhydride titration and corresponded closely to the calculated loading, based on the weight increase of the beads after reaction.

**Table 1.** Conditions for Anionic Polymerization of Ethylene Oxide and Selected Properties of PVA-PEG Copolymer Resin **2**[a]

| | resins[e] | units (*n*) | EO (mL) | mass gain (%) | $L_{calcd}$[b] (mmol/g) −OH | $L_{titrd}$[c] (mmol /g) −OH | $L_{Fmoc}$[d] (mmol/g) Fmoc-gly | −OH |
|---|---|---|---|---|---|---|---|---|
| **2A**_2 | PVA-PEG_2A | 2.2 | 1.8 | 71 | 9.9 | 9.2 | 2.1 | 4.8 |
| **2A**_5 | PVA-PEG_5A | 4.3 | 3.0 | 140 | 7.3 | 6.5 | 1.9 | 4.0 |
| **2A**_5 | PVA-PEG_5A | 5.0 | 3.0 | 150 | 6.8 | 6.2 | 1.8 | 3.7 |
| **2A**_5 | PVA-PEG_5A | 5.6 | 3.0 | 165 | 6.2 | 5.8 | 1.7 | 3.2 |
| **2A**_8 | PVA-PEG_8A | 7.4 | 3.2 | 242 | 5.0 | 4.5 | 1.6 | 2.8 |
| **2A**_12 | PVA-PEG_12A | 12.1 | 4.0 | 360 | 3.7 | 3.5 | 1.4 | 2.4 |
| **2A**_15 | PVA-PEG_15A | 15.2 | 5.0 | 460 | 3.0 | 2.3 | 1.2 | 1.9 |
| **2A**_20 | PVA-PEG_20A | 19.9 | 6.0 | 598 | 2.4 | 2.1 | 1.0 | 1.4 |
| **2B**_5 | PVA-PEG_5B | 5.1 | 2.5 | 124 | 6.7 | 6.2 | 1.9 | 4.0 |
| **2B**_12 | PVA-PEG_12B | 12.5 | 4.0 | 350 | 3.6 | 3.4 | 1.6 | 2.8 |
| **2B**_20 | PVA-PEG_20B | 19.8 | 5.0 | 600 | 2.1 | 2.0 | 1.1 | 1.5 |

[a] Starting with 0.4 g PVA beads and 2.7 mmol naphthalene potassium. [b] Theoretical loadings of hydroxyl group were calculated on the basis of the mass gain onto the beads. [c] Loadings of hydroxyl group were ascertained by titrating the excess acetic acid formed upon the reaction of copolymer beads with acetic anhydride. [d] Loading was calculated by the Fmoc-UV method. [e] PVA-PEG_*n*A and *n*B resins were synthesized from PVA beads that were made by methods A and B, respectively.

**Loading of Hydroxyl Groups.** Theoretically, linear PVA has one alcohol group per repeating unit, and the loading of linear PVA with 98% hydroxylation has a maximum theoretical value of 22 mmol/g. Cross-linking PVA diminishes the number and the accessibility of the secondary hydroxyl groups. The hydroxyl group loadings of PVA beads **1** crosslinked with epichlorohydrin by both the pre-gelation (method A) and DMSO−water (method B) processes were, respectively, titrated to be 17 and 15 mmol/g. Polymerization of EO onto cross-linked PVA maintains the number of hydroxyl groups; however, the gain in mass of grafting PEG decreases the loading proportionately. Titrated loadings using $Ac_2O$ were found to be in close agreement with the calculated value, based on the weight increase of the beads (Table 1). To evaluate the accessibility of the hydroxyl groups during peptide and organic synthesis, PVA-PEG resins **2** were acylated with N-Fmoc glycine. The Fmoc loading was then determined by spectrophotometric measurement at 300 nm of the dibenzofulvene adducts formed after treatment of the Fmoc resin with piperidine. The hydroxyl group loadings were determined using this Fmoc method to be lower (1.5−4.8 mmol/g) than those measured by the $Ac_2O$ titration (2.0−9.2 mmol/g). The Fmoc method was assumed to reflect the accessibility of the OH groups because acylation with the sterically more-hindered Fmoc-glycine was expected to occur on fewer hydroxyl groups than the smaller acetic anhydride. The PVA-PEG beads **2** exhibited typically higher loading than commercial PEGylated solid supports, which have typical loadings of <0.5 mmol/g. For example, copolymers with PEG chain lengths of 15 and 20 units had hydroxyl group loadings of around 2 mmol/g. Higher loadings (2 to 5 mmol/g) were obtained by decreasing the amount of EO in the feed during the polymerization.

**Swelling of the PVA-PEG Beads.** Contingent with the growth of the PEG chain length, the graft copolymer beads **2** became softer and stickier after lyophilization, yet they dispersed well in solution. The swelling of the graft copolymer beads was thus not measured by common methods for detecting change of volume, where the dry volume and the swollen volume of resin are measured using a syringe or graduated cylinder.[23] Instead, the swelling of the PVA-PEG beads **2** was studied using a solvent−mass absorption method with a capillary to transfer the solvent to the polymer.

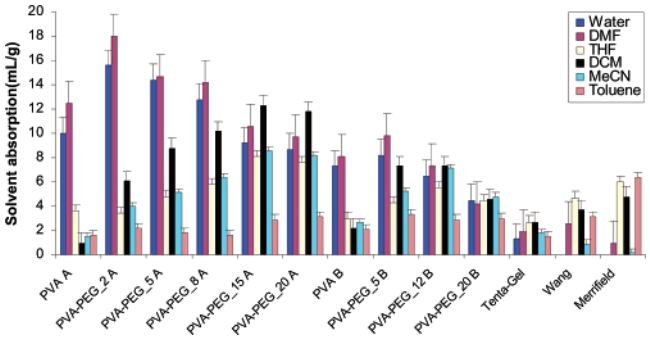



**Figure 1.** Swelling of PVA (**1A** and **1B**), PVA-PEG (**2A**_*n* and **2B**_*n*), TentaGel, Wang, and Merrifield resins.

The swelling behavior of PVA beads **1** prepared by methods A and B, PEG-grafted PVA beads **2**, Merrifield resin, Wang resin, and Tenta Gel resin was studied in six common solvents (Figure 1): water, DMF, THF, DCM, acetonitrile, and toluene. PVA beads **1B** prepared in DMSO−water according to method B were observed to swell less than beads **1A** prepared by the pre-cross-linking using method A: 7 and 10 mL/g in water and 8 and 12 mL/g in DMF, respectively. In organic solvents, such as toluene, acetonitrile, and DCM, the PVA beads **1** swelled <2 mL/g.

The copolymer beads **2** exhibited good swelling in water and in organic solvents (Figure 1). PEG-grafted PVA copolymer beads **2B** (prepared by method B) with higher PVA cross-linking swelled less in water and organic solvents relative to copolymer beads **2A** (prepared by method A) having a similar PEG chain length. Decreased swelling in DMF and water was proportional to the increase in PEG chain length for PEG-grafted PVA **2A** and **2B**. The swelling of resin **2A** in water and DMF peaked at two EO units (15 and 17 mL/g, respectively) and decreased to around 9 mL/g at longer PEG chain length (20 EO units). In the case of the polymer **2A**, an increased solubility in less-polar organic solvents was proportional to the increase in PEG chain length. On the other hand, swelling in nonpolar solvents was not proportional to PEG chain length for the PEG-grafted PVA beads **2B** that possessed higher PVA cross-linking.

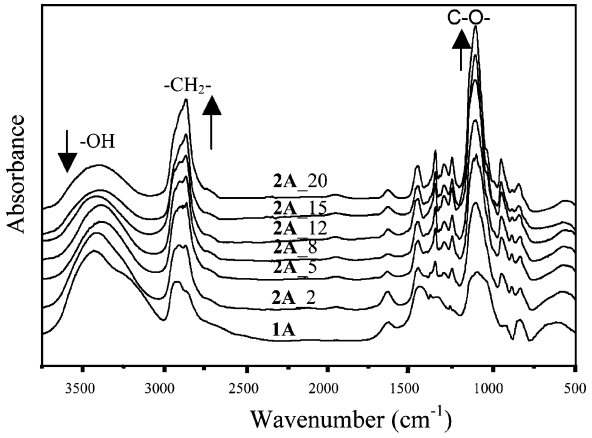



**Figure 2.** FT-IR spectra of PVA resin **1A** and PVA-PEG copolymers **2A**_*n* of different PEG lengths.

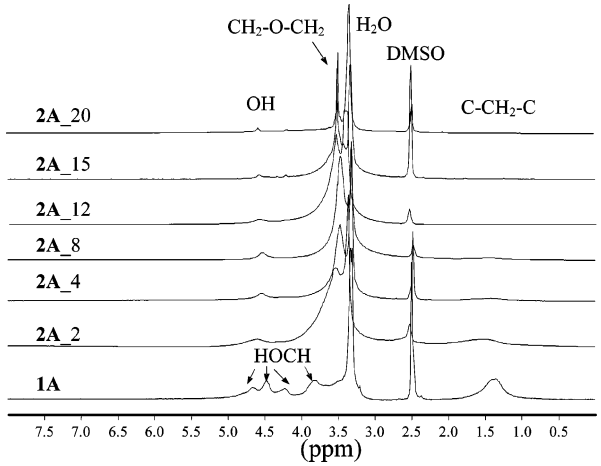



**Figure 3.** $^1$H NMR spectra of PVA resin **1A** and PVA-PEG copolymers **2A**_*n* of different PEG lengths.

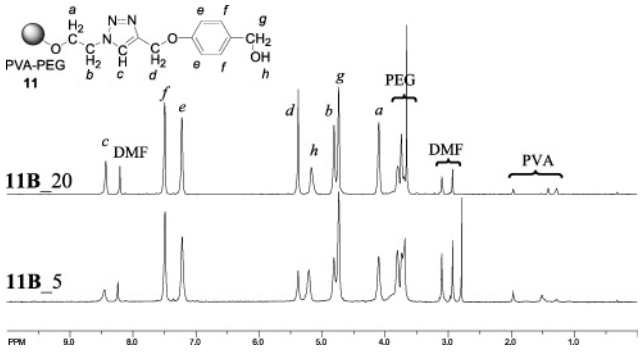



**Figure 4.** HR-MAS $^1$H NMR (600 MHz, spin rate of 6 kHz, DMF-$d_7$) spectra of PVA-PEG Wang linker resins **11B**_5 **and 11B**_20.

Relative to the PVA beads **1**, the swelling of PVA-PEG beads **2** in organic solvents was significantly improved and usually increased with PEG chain length. For example, the low swelling of 1 mL/g for PVA beads **1** in DCM was increased to 12 mL/g for **2A**_20 upon attachment of around 20 EO units (8 mL/g for densely crosslinked **2B**_20). In THF and acetonitrile, swelling was increased from 2−3 (PVA beads) to about 6−8 mL/g at longer PEG chain lengths. In toluene, the swelling increased by a factor of 2 upon going from 0 to 20 units of EO grafted polymer. In organic solvents, compared with Merrifield and Wang resin,[24] PVA-PEG copolymer resin **2** had similar swelling properties except in toluene. The PVA-PEG resin **2** exhibited consistently higher swelling and swelled faster than Tenta Gel resin in all solvents. The PVA-PEG resins may thus be useful for packing in columns for continuous-flow peptide synthesis because their consistently high swelling should result in slight volume changes in the pack material upon application of different solvents during synthesis.

**Stability of PVA-PEG Beads.** After the mixture was magnetically stirred for 48 h during the anionic polymerization of EO, the resulting copolymer beads were observed under the microscope. The densely crosslinked PVA-PEG beads **2B** exhibited a more consistent spherical form relative to **2A**, indicative of higher mechanical stability. To examine chemical stability, PVA-PEG beads **2B**_5 were exposed overnight to 6 N NaOH and 6 N HCl, respectively. The beads were observed to remain spherical under the microscope, and their FT-IR spectra were unchanged. After treatment, the PVA-PEG beads **2B**_5 were acylated with Fmoc-glycine, and the loadings of polymer-supported Fmoc-glycine were found to be 1.71 and 1.85 mmol/g by UV spectrophotometry after Fmoc cleavage for resins exposed to 6 N NaOH and to 6 N HCl, respectively, which compared favorably with the loading of original **2B**_5 supported Fmoc-glycine (1.83 mmol/g).

**FT-IR Study of the PVA-PEG Beads.** Infrared spectroscopy was employed to characterize beads before and after the anionic polymerization. In the IR spectrum of PVA beads, a strong absorption for the hydroxyl group was observed at 3380 cm$^{-1}$ (Figure 2). Although no hydroxyl groups were consumed during synthesis, the copolymer beads **2** exhibited lower hydroxyl group absorption as the mass increased from the grafting of 2−20 PEG chain lengths. Concurrently, the ether band at 1106 cm$^{-1}$ and the methylene band at 2870 cm$^{-1}$ became stronger with increasing PEG chain length.

**NMR Study of the PVA-PEG Beads.** PVA-PEG resins **2A** were initially characterized by liquid gel-phase proton NMR spectroscopy using DMSO-$d_6$ to swell and suspend the resin in a 20 cm NMR tube. The $^1$H NMR spectra showed that very distinct changes occurred to the PVA beads after the anionic polymerization (Figure 3). The chemical shifts of the diastereotopic hydroxyl resonances of the beaded PVA polymer in DMSO-$d_6$ were observed at 4.2, 4.4, and 4.6 ppm, respectively, for the different stereochemical configurations of the PVA polymeric chains.[25] In contrast, a single signal for the hydroxyl groups of the PEG-grafted PVA copolymer was observed at 4.5 ppm, and the intensity decreased proportionally with increasing PEG length. The relative intensity of the proton signals at 1.4 ppm for the PVA backbone diminished sharply on the elongation of the PEG chains. The line widths of the proton signals of the PEG methylene groups at 3.8 ppm became sharper as the PEG chains grew longer and more mobile.

MAS $^1$H NMR spectroscopy with a $^1$H MAS nanoprobe with a spinning frequency of around 6 kHz was also used to on-bead analyze resin-bound compounds. On-bead structure analysis of Wang linker resin **11B** using HR-MAS $^1$H NMR spectrometry was performed (Figure 4) and two-dimensional HMQC and TOCSY spectra (see Supporting Information) were recorded to identify the structure. Resin **11B**_20 with PEG chains of 20 units provided a better resolution and

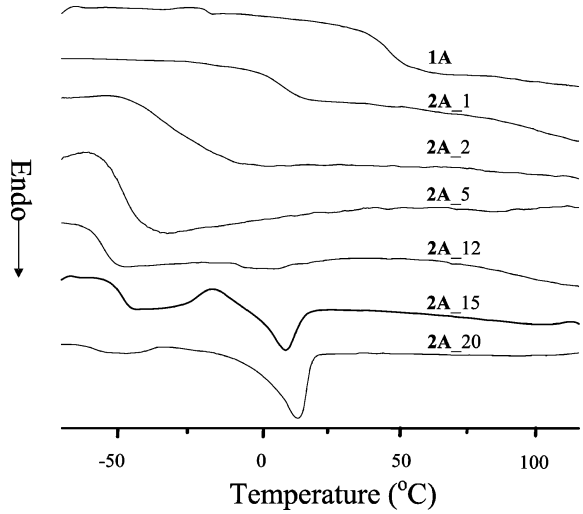



**Figure 5.** DSC thermograms of PVA-PEG resins **2A**_*n* possessing different PEG chain lengths at the first heating.

narrower line widths in DMF-$d_7$ than **11B**_5. Splittings of aromatic proton doublets were observed to be 6.5 Hz at 7.50 and 7.22 ppm in the spectrum of Wang resin **11B**_20; in contrast, no splittings were observed in the spectrum of **11B**_5.

**DSC study of the PVA-PEG beads 2A.** Differential scanning calorimetry (DSC) was used to study the thermal behavior of the PVA-PEG beads **2A**. The first DSC heating curves of the copolymers **2A** with different lengths of PEG were recorded at a heating rate of 10 °C/min after they were cooled with liquid nitrogen from room temperature to −100 °C at a speed of −10 °C/min. The pure PVA beads have a glass transition at about 50 °C (Figure 5). For the copolymers with short PEG chains, the glass transition temperature decreased from 50 to to −25 °C and finally shifted to the glass transition of PEG (about −50 °C) at the PEG chain lengths of ≥8. The absence of multiple $T_g$'s suggests that grafting was homogeneous. The DSC curves for copolymers **2A** with PEG chain lengths of 15 and 20 exhibited melting peaks of the PEG chain at about 10 and 20 °C, respectively, suggesting that the PEG chains were long enough to align themselves into a crystalline phase. The melting point of linear PEG has been reported[26] to depend on PEG molecular weight: 400−500 and 600 g/mol PEG melted at 10 and 20−25 °C, respectively. The grafted PEG also showed a similar relationship between melting points and molecular weight. Beads with PEG chain lengths of 15 and 20 were calculated to have PEG molecular weights of 660 and 880 g/mol, and exhibited melting points that were 10 and 20 °C, respectively, lower than that of the corresponding linear PEG because of the inhibition of PEG-chain crystallization by the crosslinked PVA polymer matrix.

**Morphology Study.** Cross-linked PVA beads **1B** have been shown to have porous structures by scanning electron microscopy (SEM),[14] and the pore size decreased with the degree of cross-linking. The SEM spectra of the cross-linked PEG-derivatized PVA beads **2B** suggested that the pores were filled in by flexible PEG (Figure 6). Finally, PVA-PEG copolymer **2B**_5 and **2B**_20 with PEG chains of 5 and 20 units have a gel-like structure. Beads **2B**_20 had a

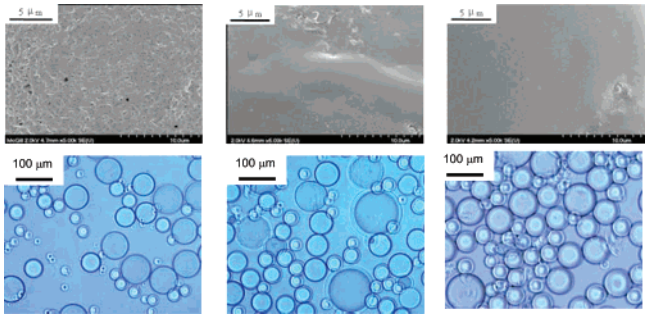
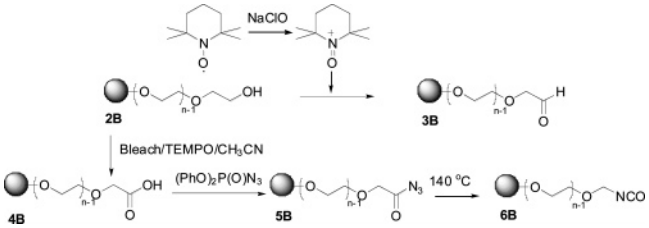



**Figure 6.** Scanning electron micrographs (top) and optical micrographs (bottom) of PVA beads **1B** (left), PVA-PEG beads **2B**_5 (middle), and **2B**_20 beads (right).

**Scheme 2.** Preparation of Aldehyde, Carboxylic Acid, and Isocyanate Resins **3B**, **4B**, and **6B** from PVA-PEG Resin **2B**

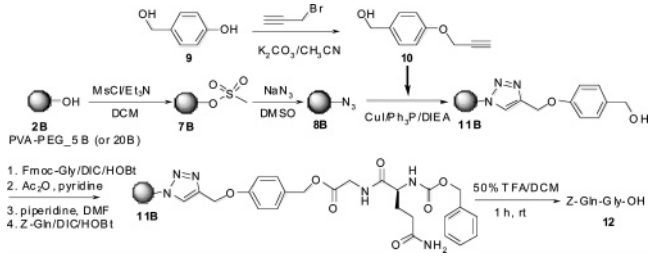



smoother surface than **2B**_5. Optical microscopy showed that PVA beads and PVA-PEG beads were both spherically shaped with bead sizes varying from 30 to 130 $\mu$m in water.

**Modification of PVA-PEG resin 2B.** Densely cross-linked PVA beads **2B** had better mechanical properties than PVA beads **2A**,[14] which broke when stirred magnetically. Beads **2B** also swelled well in aqueous and organic media. These mechanical properties suggested application of resins **2B** in solid-phase chemistry.

High functional-group density is particularly desired for scavenger resins.[9] In the interest of providing potential scavenger resins, the hydroxyl groups on the PVA-PEG resin **2B**_12 were oxidized into aldehyde and carboxylic acid groups and further converted into an isocyanate function. To prepare aldehyde resin **3B**_12, PVA-PEG resin **2B**_12 was initially exposed to Swern oxidation conditions at −60 °C; however, no carbonyl band was observed by FT-IR spectroscopy, most likely, because of the poor diffusion of the oxidant into the rigidified backbone of PEG at low temperature ($T_g$ of PEG = −50 °C). The bleach/TEMPO/DCM procedure was next employed to convert the hydroxyl groups on the PVA-PEG resin into aldehyde groups (Scheme 2). When excess bleach (≥2.5 equiv) and a long reaction time (overnight) were used to push conversion, over-oxidation of the OH groups occurred; the corresponding acid was observed by FT-IR spectroscopy (1735 $cm^{-1}$), and no aldehyde groups were detected by a dansylhydrazine-flourescence titration. Presumably, aldehydes were being oxidized by bleach into acids. Aldehyde resin **3B**_12 was produced by a two-step TEMPO oxidation process. First, TEMPO in DCM was oxidized with bleach. The phases were separated, and the organic phase was in turn applied to oxidize PVA-PEG resin **2B**_12. After this oxidation process was employed, no carboxylic acid was observed by FT-IR, and the aldehyde loading was ascertained to be 0.8 mmol/g using rapid fluorescence titration[27] for resin **3B**_12.

**Scheme 3.** Preparation of PVA-PEG Resin **11B** Possessing a Wang Linker and Dipeptide Synthesis




To prepare carboxylic acid resin **4B**_5, acetonitrile was used to solvate both TEMPO and PVA-PEG resin **2B**_5 in the aqueous bleach phase during the oxidation (Scheme 2). The resulting resin **4B**_5 exhibited a strong carbonyl band (1737 $cm^{-1}$) in the FT-IR spectrum indicative of the formation of carboxylic acid. Carboxylic acid resin **4B**_5 was reacted with diphenyl phosphoryl azide in 1-methyl-2-pyrrolidinone to yield the carbonyl azide resin **5B**_5. In the FT-IR spectrum of **5B**_5, the carbonyl band shifted to 1718 $cm^{-1}$, and a characteristic azide band appeared at 2162 $cm^{-1}$. Nitrogen elemental analysis of resin **5B**_5 indicated an azide loading of 2.27 mmol/g. Curtius rearrangement by heating carbonyl azide resin **5B**_5 in THF at 140 °C provided isocyanate resin **6B**_5. The shift of the $-CON_3$ band (2162 $cm^{-1}$) to a $-NCO$ band (2266 $cm^{-1}$) in the FT-IR was indicative of isocyanate formation. The application of PVA-PEG aldehyde, carboxylic acid, and isocyanate resins **3B**, **4B**, and **6B** as scavengers and supports is presently under investigation.

Wang linker resins **11B** were synthesized[13] to compare reactivity with the popular PS-DVB counterpart in peptide and small-molecule synthesis. Immobilization of Wang linker onto the PVA-PEG resin was accomplished using the Cu-(I)-catalyzed [2 + 3] dipolar cycloaddition reaction between an organic azide and a terminal alkyne (Scheme 3) because such chemistry is usually high yielding and tolerant of oxygen and water.[28] Azide resins **8B**_5 and **8B**_20, having chain lengths of 5 and 20 units, respectively, were first synthesized in two steps from **2B**. Methanesulfonation of resins **2B**, with methanesulfonyl chloride in DCM at 0 °C, gave methanesulfonate resins **7B**, the FT-IR spectrum (Figure 7) of which exhibited two strong sulfonyl stretch bands at 1352 and 1174 $cm^{-1}$, as well as a significantly decreased hydroxyl absorption around 3400 $cm^{-1}$ relative to the starting alcohol resin **2B**. After exposure of methanesulfonate resin **7B** to sodium azide, the FT-IR spectrum indicated the complete disappearance of the sulfonyl bands and the appearance of a new strong azide absorption at 2106 $cm^{-1}$. Nitrogen elemental analysis indicated azide loadings of 2.42 and 1.25 mmol/g for azido resin **8B**_5 and **8B**_20 with PEG chain lengths of 5 and 20 units, respectively. 4-Propargyloxybenzyl alcohol **10** was prepared by alkylation of the phenoxide ion of *p*-hydroxy benzyl alcohol **9** with propargyl bromide in a 96% yield. Propargyl ether **10** was then reacted with azide resins **8B**_5 and **8B**_20 in the presence of catalytic CuI to give the triazole-linked PVA-PEG Wang resins **11B**_5 and **11B**_20, respectively. The loading of the benzyl alcohol group tethered by the triazole was determined by nitrogen elemental analysis to be 1.63 and 1.08 mmol/g, respectively,

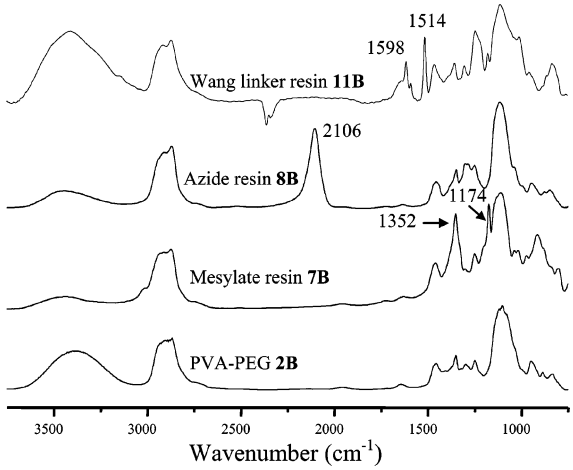
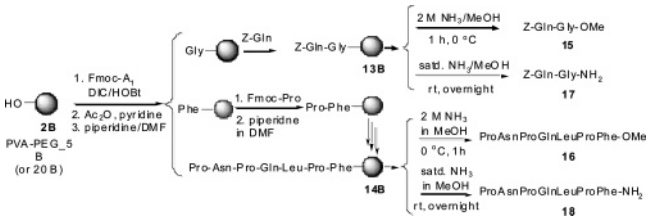



**Figure 7.** FT-IR spectra of PVA-PEG Wang linker resin **11B** and intermediate resins.

**Scheme 4.** Dipeptide and Heptapeptide Synthesis on PVA-PEG Resin




for **11B**_5 and **11B**_20. Similar loadings of hydroxyl groups were ascertained for resins **11B**_5 and **11B**_20 using the assay featuring Fmoc-glycine coupling followed by UV detection of the dibenzofulvene adduct after the cleavage with piperidine (1.74 and 1.08 mmol/g, respectively). On-bead HR-MAS $^1H$ NMR spectra were recorded to confirm the structure of Wang linker resin **11B** as discussed above (Figure 4). Three characteristic absorptions for the aromatic ring were observed at 1598, 1514, and 1465 $cm^{-1}$ in the FT-IR spectrum of PVA-PEG Wang resins **11B**_5 and **11B**_20 (Figure 7).

**Peptide Synthesis on the PVA-PEG Resins.** The transglutaminase substrates dipeptide Z-Gln-Gly[29] and heptapeptide Pro-Asn-Pro-Gln-Leu-Pro-Phe[30] were synthesized on PVA-PEG resins **2B**_5 and **2B**_20 and Wang resins **11B**_5 and **11B**_20 using standard Fmoc chemistry[31] and DIC and HOBt for coupling. The peptides were cleaved from the resins using three different conditions (Scheme 4). The quality of product from cleavage by hydrolysis and aminolysis of the peptide residue from the resin was observed to be sensitive to the concentration of the alkaline solution and temperature. The dipeptide was cleaved from resins **13B**_5 and **13B**_20 using a 2.0 M solution of ammonia in methanol at 0 °C for 1 h to furnish Z-Gln-GlyOMe **15**[32] **(**75 and 84% yields and 98 and 96% LC-MS purities). Heptapeptide methyl ester **16** was obtained from **14B**_5 and **14B**_20 using the same conditions in 53 and 18% isolated yields and 75 and 50% LC-MS purities. Dipeptide amide **17**[13] was obtained from resin **13B**_5 using a saturated solution of ammonia in methanol at room temperature overnight in a 98% isolated yield and a 92% LC-MS purity. Heptapeptide amide **18** was isolated from treatment of resin **14B**_5 with the same conditions by precipitation from

acetone with diethyl ether in a 99% isolated yield and a 70% LC-MS purity. Z-Gln-Gly[33] was synthesized on Wang resins **11B**_5 and **11B**_20 and cleaved using a 50% solution of TFA in DCM (v/v) at room temperature for 1 h which gave 100 and 65% yields and 90 and 91% LC-MS purities, respectively.

## Conclusion

High-loading resins with remarkable swelling in organic and aqueous media were developed by grafting short PEG chains onto a cross-linked PVA core by anionic polymerization of ethylene oxide. PEG-grafted PVA copolymer beads **2** were shown by high-resolution scanning electron microscopy to be a typical gel phase with a smooth surface. Relatively homogeneously shaped and functionalized beads **2** were characterized by NMR and FT-IR spectroscopy as well as DSC. Moreover, PEG-grafted PVA Wang resin **11B**_20 exhibited a well-resolved on-bead MAS NMR spectrum. Modification of PVA-PEG resins **2B** by way of alcohol activation and displacement, as well as by oxidation, gave resins with aldehyde **3**, carboxylic acid **4,** acyl azide **5**, isocyanate **6**, and azide **8** functions. In addition, dipolar cycloaddition of a propargylated Wang linker on azide resin **8** gave Wang resin **11**. Peptide synthesis was successfully accomplished on grafted copolymer resins **2** and the Wang resins **11** derivatized with different PEG chain lengths. In summary, their observed physical and spectral properties, as well as their utility in synthesis, all indicate that PEG-grafted PVA resins should find many practical applications for solid-phase chemistry and solid-supported catalysis.

## Experimental Section

**Materials and Instruments.** Linear PVA (98% hydrolyzed, $M_w$ = 13 000−23 000), epichlorohydrin (EP, 99%), sorbitan monoleate (Span 80), ethylene oxide, *n*-butyl lithium solution in hexane (1.6 mol/L), Z-Gln-OH, 1-hydroxybenzotriazole hydrate (HOBt), 1,3-diisopropylcarbodiimide (DIC), 4-dimethylaminopyridine (DMAP), ammonia (2.0 M solution in methanol), and piperidine were purchased from Sigma-Aldrich. Paraffin oil was purchased from American Chemicals; Fmoc-Gln-OH, Fmoc-Gly-OH, Fmoc-Pro-OH, Fmoc-Asn-OH, Fmoc-Leu-OH, and Fmoc-Phe-OH were purchased from Novabiochem. The chemicals mentioned above were used without further purification. Acetic anhydride (99% from Aldrich) was dried and distilled from sodium and stored under argon, tightly sealed. Pyridine was dried and distilled from sodium hydroxide. Dimethyl sulfoxide (DMSO) and benzene were dried and distilled from calcium hydride. Tetrahydrofuran (THF) was dried and distilled from sodium in the presence of benzophenone after the solution turned dark blue. Potassium naphthalene was prepared in dry THF from naphthalene and potassium at a concentration of 0.45 M (titrated with a standard hydrochloric acid solution using phenolphthalein as indicator). Merrifield resin (2% DVB crosslinking, 100−200 mesh, loading of 2.0 mmol/g) and Wang resin (1% DVB crosslinking, 100−200 mesh, loading of 1.0 mmol/g) were purchased from Fluka, and Tenta Gel S OH resin (90 $\mu$m, 0.2−0.3 mmol/g) was obtained from Rapp Polymere GmbH.

Magic-angle spinning (MAS) $^1$H NMR experiments were performed on a Bruker Avance 600 NMR spectrometer (600 MHz) equipped with a 4 mm HRMAS $^1$H probe with a spin rate of ∼6 kHz. Approximately 5 mg of beads was transferred into a Nano NMR tube and swollen in 40 $\mu$L of DMF-$d_7$. The spectra were recorded at room temperature with a presaturation at 3.65 ppm. $^1$H and $^{13}$C NMR spectra of PVA-PEG resins and small molecular-weight products were recorded on a Bruker Avance 400 spectrometer operating at 400 MHz for $^1$H and 100 MHz for $^{13}$C. FT-IR spectra of the PVA beads, PVA-PEG beads, and their functionalized resins were recorded on a Bomen MB-100 FT-IR spectrometer at room-temperature using potassium bromide pellets made with ground polymer beads. The thermal properties of the polymer beads were analyzed on a differential scanning calorimeter (TA instrument DSC2910). The optical images of the beads were recorded under an Axioskop 2 Plus (Zeiss) optical microscope. The morphology of the polymer beads was examined by electron microscopy using a field-emission scanning electron microscope (FE-SEM, from S-4700) after the sample was coated with gold. Liquid chromatography/mass spectrometry (LC/MS) traces were obtained on a coupled Gilson LC-ThermoFinnigan MSQ instrument equipped with a Prevail Allteck C18 (5 micron 250 × 46 mm) column using a gradient consisting of a mixture of A (0.01% TFA in $H_2O$) and B (0.01% TFA in $CH_3CN$) over 20 min with 80% A/20% B, then over 5 min with 20% A/80% B, then over 5 min with 10% A/90% B, and finally with 80% A/20% B; the MS conditions were as follows: scan, 100−500; cone voltage, 30 kV; temperature, 400 °C; mode (polarity), positive. UV−vis data were obtained on a UV−vis spectrometer (Cary 300 Bio, Varian). Bead size was analyzed on beads suspended in water using a Horiba LA 950 particle sizer.

**Preparation of PVA Beads.** Crosslinked PVA beads **1A** were first prepared as previously reported (method A)[14] using epichlorohydrin as a cross-linker. Subsequently, PVA beads **1B**, having a higher degree of crosslinking, were prepared by method B as described below. Sodium hydroxide (4 g) was dissolved into a 15 wt % solution of PVA (40 g) in DMSO and $H_2O$ (1:1 v/v) and filtered into a 500 mL round-bottom flask equipped with mechanical stirrer. The mixture was stirred and heated to 70 °C to obtain a clear solution. Epichlorohydrin (7 mL) was then added to the PVA solution with gentle mechanical stirring at 70 °C for pre-crosslinking for 1−2 min. Paraffin oil (200 mL, preheated to 70 °C) was added to the viscous solution, and mechanical stirring was increased to a speed of 800 rpm; then Span 80 (0.4 mL) was added to the reaction mixture. Beads were formed in the reverse suspension and solidified at 70 °C for 24 h. The PVA beads were filtered and rinsed with petroleum ether, THF, and water and were transferred into a Soxhlet extractor. The beads were extracted successively with THF, acetone, and water, each for 24 h. The beads were transferred to a lyophilizer and freeze-dried for 48 h to provide PVA beads **1B** (5.6 g, 93% yield) with the swollen mean bead size of 87 $\mu$m in water. The hydroxyl group loading of PVA beads **1B** was analyzed to be 15 mmol/g by back-titration of the excess of acetic acid after acetylation of the hydroxyl groups

with acetic anhydride in pyridine. The FT-IR spectrum of the PVA beads **1B** exhibited a strong OH stretching band around 3400 $cm^{-1}$.

**Anionic Polymerization of Ethylene Oxide onto the PVA Beads.** PVA beads **1**, prepared by either method A or B, were employed in PEG grafting. All glassware and needles were flame-dried under vacuum and purged 3 times with nitrogen. PVA beads **1** (0.4 g) were placed into a 100 mL flask, equipped with a Dean−Stark trap, containing 20 mL of dry benzene and heated at a reflux overnight to remove the water−benzene azeotrope. Benzene was distilled off under vacuum, and 25 mL of dry DMSO was charged into the flask to swell the PVA resin. A calibrated amount of potassium naphthalene in THF (0.45 M, 6 mL, 0.45 equiv) was introduced dropwise into the flask using a double-tip needle and argon pressure. The mixture was stirred for 2 h to allow the green color from the naphthalene potassium to completely disappear. Ethylene oxide of a known volume was passed through a calcium hydride drying column and condensed into a dry flask containing 2 mL of *n*-butyl lithium (1.6 mol/L) solution in hexane at −78 °C. The dry ice/acetone bath was removed, and as the flask warmed, the ethylene oxide was allowed to distill into the resin suspension which was cooled to −78 °C. After ethylene oxide transfer was completed, the polymerization mixture was heated and magnetically stirred at 40 °C for 48 h. The resin was filtered and rinsed with water and THF and subsequently extracted successively with THF and water using a Soxhlet extractor for 24 h per solvent. The beads were finally freeze-dried for 48 h to give copolymer resin **2**, which was characterized by FT-IR, NMR, DSC, and SEC (Figures 2−6) and stored below 5 °C under nitrogen.

**Loading Determination by Fmoc-Fluorescence Measurement.**[34] Swollen PVA-PEG resin **2** (20 mg) was acylated with *N*-(Fmoc)glycine (∼500 mol % relative to the titration loading of the resin) by stirring overnight at room temperature in 0.5 mL of DMF in the presence of DIC (∼500 mol % relative to the titration loading of the resin) and 2.5 mg of DMAP (∼10 mol % relative to the titration loading of the resin). The resin was successively rinsed with 15 mL of DMF, 25 mL of DCM, and 10 mL of diethyl ether and was dried to a constant weight. A 5 mg aliquot of the coupled resin was treated with 1 mL of piperidine solution in DMF (1:4) for 1 h and diluted to 50 mL with ethanol. The loading of the Fmoc-glycine was determined by UV measurement at 301 nm of the liberated dibenzofulvene adduct generated by treating the resin with the piperidine/DMF solution. The loading of OH group was calculated from the Fmoc loading.

**Swelling.** A glass capillary was used to transfer solvent to the beads (roughly 3 mg), which were weighed on a poly(tetrafluoroethylene) slice to avoid excess solvent adhering to the matrix. The beads absorbed solvent from the capillary until equilibrium between the solvent in capillary and in the swollen bead was reached. This procedure was repeated several times over a period of 10 min to allow full swelling of the beads. The beads saturated with solvent were then weighed using a microbalance, and the weight gain was converted into the volume of solvent retained per gram of polymer.

**PVA-PEG Aldehyde Resin 3B_12.** A stoppered plastic tube-shaped reactor fitted with a Teflon filter was charged with TEMPO (0.45 mmol, 70 mg) in 2 mL of DCM and 1.25 mL of bleach (0.4 M), buffered with $KHCO_3$ at pH 9.1, treated with aqueous KBr (0.5 M, 0.08 mL), and shaken at room temperature for 30 min. The phases were separated. The deep red DCM phase was transferred into another plastic tube-shaped reactor containing copolymer **2B**_12 resin (50 mg, 3.0 mmol/g), and the mixture was shaken for 2 h. The resin **3B**_12 was filtered and washed with DCM, THF, and diethyl ether, and then it was dried under vacuum. A strong absorption was observed at 1610 $cm^{-1}$ in the FT-IR spectrum of the oxidized PVA-PEG beads. The aldehyde loading was ascertained to be 0.66 mmol/g by a rapid fluorescence determination[27] of the excess dansylhydrazine remaining after treatment of aldehyde resin **3B**_12 with approximately 200 mol % (or 300 mol % relative to aldehyde loading) of dansylhydrazine.

**PVA-PEG Carboxylic Acid Resin 4B_5.** PEG-grafted PVA **2B**_5 (0.1 g, 4.0 mmol/g) was agitated with 3 mL of bleach (0.4 M, 3 eq.) and 0.12 mL of KBr (0.5 M, 10 mol %) at pH 9.1 (buffered with $NaH_2CO_3$). A TEMPO (6.3 mg, 0.04 mmol) solution in acetonitrile was added to the mixture, which was agitated overnight at room temperature. The resin was filtered and washed with water, 0.1 M HCl, water, THF, and diethyl ether and dried under vacuum (FT-IR 1747 $cm^{-1}$). The carboxylic acid loading of resin **4B**_5 was measured to be 2.3 mmol/g by nitrogen elemental analysis after the conversion of the carboxylic acid groups into the corresponding acyl azides[35] upon treatment with diphenyl phosphoryl azide in THF overnight. The FT-IR spectrum of acyl azide resin **5B**_5 showed a strong band at 2155 $cm^{-1}$ for the azide absorption. Isocyanate resin **6B**_5 was prepared from acyl azide resin **5B**_5 by Curtius rearrangement on heating to 140 °C in THF for 30 min. The FT-IR spectrum of resin **6B**_5 exhibited an isocyanate band at 2255 $cm^{-1}$.

**Methanesulfonate PVA-PEG Resin 5B.** To a suspension of PVA-PEG resin **2** (0.5 g, 6.2 mmol/g for PVA-PEG_5B and 1.55 g, 2.0 mmol/g for PVA-PEG_20B, respectively) and $Et_3N$ (3.3 mL, ∼1000 mol %) in DCM at 0 °C, methanesulfonyl chloride (3.48 g, ∼1000 mol %) was added dropwise. The ice bath was removed, and the reaction mixture was stirred overnight at room temperature. The resin was filtered, washed with DCM, THF, and diethyl ether three times each, respectively, and dried under vacuum, to yield a light yellow methanesulfonate resin **7B**. FT-IR: 1352, 1174 $cm^{-1}$.

**Azide PVA-PEG Resin 8B.** A suspension of methanesulfonate resin **7B** (∼3 mmol) and $NaN_3$ (2.0 g, ∼1000 mol %) in DMSO (40 mL) was heated and stirred overnight at 70 °C, filtered, washed with DMF, DCM, THF, and diethyl ether, 3 times each, and dried under vacuum to yield yellow azide resin **8B**. The FT-IR spectrum showed a strong absorption at 2106 $cm^{-1}$ for $N_3$. Azide loadings of resin **8B** were determined to be 2.42 and 1.25 mmol/g by nitrogen elemental analysis for the resins with PEG chains of 5 and 20 units, respectively.

**Preparation of Wang Linker Resin 11B.** Azido-PVA-PEG_20 resin **6** (400 mg, 1.25 mmol/g) was agitated with

4-propargyloxy-benzyl alcohol **10B** (405 mg, ~500 mol %), CuI (9.6 mg, 10 mol %), DIPEA (0.5 mL, 525 mol %), and $Ph_3P$ (14 mg, 10 mol %) in DMF (3 mL) at room temperature for 24 h. The suspension was filtered, and the resin was rinsed successively with pyridine (3 × 20 mL), DCM (3 × 20 mL), THF (3 × 20 mL), and diethyl ether (3 × 20 mL) and dried under vacuum to yield a brown Wang linker resin **11B**. The Wang linker loadings were determined by nitrogen elemental analysis to be 1.63 and 1.08 mmol/g, respectively, for resins **11B** with PEG chains of 5 and 20 units. The loading of hydroxyl groups were also measured to be 1.74 and 1.08 mmol/g, respectively, for **11B**_5 and **11B**_20, by acylation of the resin with Fmoc-glycine and detection of the fluorescence of the fulvalene adduct after Fmoc removal.[7] FT-IR spectrum of Wang linker resin **11B**: 1614, 1598, 1514, 1465 $cm^{-1}$. On-bead HR/MAS $^1H$ NMR in DMF-$d_7$: $\delta$ 8.43 (s, 1H), 7.50 (d, 2H, $J$ = 6.5 Hz), 7.22 (d, 2H, $J$ = 6.0 Hz), 5.38 (s, 2H), 5.17 (bs, 1H), 4.81 (s, 2H), 4.74 (s, 2H), 4.10 (s, 2H), 3.68−3.80 (m, PEG-H), 1.28−1.92 (m, PVA-H)

**General Procedure for Peptide Synthesis.** Swollen PVA-PEG resin **2B** (105 mg for **2B**_20 and 35 mg for **2B**_5) in 1.5 mL of DMF was treated with *N*-(Fmoc)glycine (309 mg, ~500 mol %), DIC (168 $\mu$L, ~500 mol %), and DMAP (2.5 mg, ~10 mol %), stirred overnight at room temperature, filtered, and successively rinsed with 15 mL of DMF, 25 mL of DCM, and 10 mL of diethyl ether. Loading was measured as described above by the Fmoc-fluorescence measurement. The swollen acylated resin was stirred for 2 h with 3 mL of acetic anhydride/pyridine solution (2:3 v/v) to acetylate any residual alcohol groups. The resin was successively washed with 15 mL of DMF and 25 mL of DCM. The loading of the Fmoc-glycine was determined by UV measurement at 301 nm of the liberated dibenzofulvene adduct.[34] Fmoc cleavage was carried out with 3 mL of a 20% piperidine solution in DMF for 1 h. The deprotected resin exhibited a positive Kaiser test.[36] The coupling of the first amino acid (Fmoc-Phe) in the heptapeptide synthesis and elongation were performed by treating the deprotected resin with *N*-(Fmoc)-amino acid (~250 mol %), HOBt (250 mol %), and DIC (250 mol %) in DMF (1.5 mL). The mixture was shaken overnight at room temperature, and the resin was filtered and washed successively with 15 mL of DMF, 50 mL of DCM, and 10 mL of diethyl ether. Coupling was monitored by the Kaiser test.

**Z-Gln-Gly 12.** The title compound was synthesized on Wang resins **11B**_5 and **11B**_20 following the methods described above. The cleavage of dipeptide from the Wang-linker resins **11B** was performed on treatment with a 50% solution of trifluoroacetic acid in dichloromethane for 1 h at room temperature. After resin filtration and dichloromethane wash, the filtrate and washings were combined and evaporated to give a white solid. The peptide Z-Gln-Gly **12** was isolated in a 72% yield and was determined, without further purification, to be of 90% purity by LC-MS analysis (3.9 mg from 25 mg of resin **11B**_5 with a loading of 0.64 mmol/g) and in an 84% yield and 91% purity by LC-MS analysis (3.1 mg from 25 mg of resin **11B**_20 with a loading of 0.43 mmol/g). $^1H$ NMR (300 MHz, $CD_3OD$): $\delta$ 7.33 (m, 5H, CH aromatic benzyl), 5.08 (s, 2H, $CH_2$ benzyl), 4.18 (m, 1H, CH Gln), 3.93 (m, 2H, $CH_2$ Gly), 2.34 (m, 2H, $CH_2$ $\gamma$ Gln), 2.08 (m, 1H, $CH_2$ $\beta$ Gln), 1.93 (m, 1H, $CH_2$ $\beta$ Gln. $^{13}C$ NMR (75 MHz, $CD_3OD$): $\delta$ 178.1, 177.6, 175.1, 160.3, 141.2, 129.7, 128.2, 128.1, 54.8, 44.9, 32.8, 30.0. LC-MS: $t_R$ 3.60 min, $m/z$ 338.0 $[M + H]^+$.

**Z-Gln-Gly-OMe 15.** The title compound was synthesized on **2B**_5 (1.39 mmol/g) and **2B**_20 resins (0.98 mmol/g) following the methods described above. In the coupling step, Z-Gln was employed instead of the Fmoc amino acid. The cleavage of peptides from the PVA-PEG resin **13B** was performed in a 2.0 M solution of ammonia in methanol for 1 h at 0 °C. After resin filtration and the methanol wash, the filtrate and washings were combined and evaporated. Z-Gln-Gly-OMe **15** was precipitated with methanol/ether and isolated in a 75% yield (14.6 mg, 98% purity by LC-MS analysis) and in an 84% yield (28.8 mg, 96% purity by LC-MS analysis) from **2B**_5 and **2B**_20, respectively. A white solid was obtained. $^1H$ NMR (300 MHz, $CD_3OD$): $\delta$ 7.33 (m, 5H, CH aromatic benzyl), 5.08 (s, 2H, $CH_2$ benzyl), 4.18 (m,1H, CH Gln), 3.93 (m, 2H, $CH_2$ Gly), 3.70 (s, 3H, methyl ester), 2.34 (m, 2H, $CH_2$ $\gamma$ Gln), 2.08 (m, 1H, $CH_2$ $\beta$ Gln), 1.93 (m, 1H, $CH_2$ $\beta$ Gln). $^{13}C$ NMR (75 MHz, $CD_3OD$): $\delta$ 177.9, 174.9, 171.7, 158.4, 138.1, 129.5, 129.1, 128.9, 67.8, 55.9, 52.6, 41.8, 32.4, 29.1. LC-MS: $t_R$ 12.95 min, $m/z$ 352.0 $[M + H]^+$.

**Pro-Asn-Pro-Gln-Leu-Pro-Phe-OMe 16.** The title compound was synthesized on resins **2B**_5 (1.2 mmol/g) and **2B**_20 (0.64 mmol/g) following the above-mentioned protocols. Cleavage of peptide ester **16** from the PEG-grafted PVA resin **14B** was performed using a 2.0 M solution in methanol of ammonia at 0 °C for 1 h. After resin filtration and the methanol wash, the filtrate and washings were combined and evaporated. Heptapeptide methyl ester **16** was obtained in a 53% yield (10.1 mg from 59 mg of resin, 75% purity) and an 18% yield (12.9 mg from 46 mg of resin, 50% purity by LC-MS analysis) from resins **2B**_5 and **2B**_20, respectively. LC-MS: $t_R$ 12.96 min, $m/z$ 826.4 $[M + H]^+$.

**Pro-Asn-Pro-Gln-Leu-Pro-Phe-$NH_2$ 18.** The Pro-Asn-Pro-Gln-Leu-Pro-Phe peptide was also cleaved from resin **14B**_5 using a saturated methanol solution of ammonia at room temp overnight. After resin filtration and the methanol wash, the filtrate and washings were combined and evaporated. Heptapeptide amide **18** was isolated by precipitation from acetone/diethyl ether at −20 °C in a 99% yield (20 mg from 35 mg of resin with a loading of 0.65 mmol/g) and 70% purity by LC-MS analysis. LC-MS: $t_R$ 3.95 min, $m/z$ 811.3 $[M + H]^+$. HR-MS: $m/z$ calcd for $C_{39}H_{59}N_{10}O_9$ $[M + H]^+$ 811.4461, found $[M + H]^+$ 811.4465.

**Acknowledgment.** Financial support from the Natural Sciences and Engineering Research Council (NSERC) of Canada, Valorisation-Recherche Québec (VRQ), and the Canada Research Chair program is gratefully acknowledged.

**Supporting Information Available.** Experimental details for the grafting of PEG onto PVA resins and 2D HR-MAS NMR spectra of resin **11B**. This material is available free of charge via the Internet at http://pubs.acs.org.

## References and Notes


(1) (a) Gallop, M. A.; Barrett, R. W.; Dower, W. J.; Fodor, S. P. A.; Gorden, E. M. *J. Med. Chem.* **1994**, *37*, 1233−1251. (b) Gallop, M. A.; Barrett, R. W.; Dower, W. J.; Fodor, S. P. A.; Gorden, E. M. *J. Med. Chem.* **1994**, *37*, 1385−1401. (c) Terrett, N. K.; Gardner, M.; Gorden, D. W.; Kobylecki, R. J.; Steel, J. *Tetrahedron* **1995**, *51*, 8135−8173. (d) Thompson, L. A.; Ellman, J. A. *Chem. Rev.* **1996**, *96*, 555−600. (e) Früchtel, J. S.; Jung, G. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 17−42.

(2) Merrifield, R. B. *J. Am. Chem. Soc.* **1963**, *85*, 2149−2154.

(3) Grotli, M.; Gotfredsen, C. H.; Rademann, J.; Buchardt, J.; Clark, A. J.; Duus, J. O.; Meldal, M. *J. Comb. Chem.* **2000**, *2*, 108−119.

(4) Keifer, P. A. *Drug Discovery Today* **1997**, *2*, 468−478.

(5) (a) Bayer, E.; *Angew. Chem., Int. Ed. Engl*. **1991**, *30*, 113−128. (b) Gooding, O. W.; Baudart, S.; Deegan, T. L.; Heisler, K.; Labadie, J.; Newcomb, W. S.; Porco, J. A.; Van Eikeren, P. *J. Comb. Chem.* **1999**, *1*, 113−122. (c) Adams, J. H.; Cook, R. M.; Hudson, D.; Jammalamadaka, V.; Lyttle, M. H.; Songster, M. F. A. *J. Org. Chem.* **1998**, *63*, 3706−3716.

(6) (a) Renil, M.; Ferreras, M.; Delaisse, J. M.; Foged, N. T.; Meldal, M. *J. Pept. Sci.* **1998**, *4*, 195−210. (b) Renil, M.; Meldal, M. *Tetrahedron Lett.* **1996**, *37*, 6185−6188. (c) Kempe, M.; Barany, G. *J. Am. Chem. Soc.* **1996**, *118*, 7083−7093. (d) García-Martín, F.; Quintanar-Audelo, M.; García-Ramos, Y.; Cruz, L. J.; Gravel, C.; Furic, R.; Coté, S.; Tulla-Puche, J.; Albericio, F. *J. Comb. Chem.* **2006**, *8*, 213−220. (e) Miranda, L. P.; Lubell, W. D.; Halkes, K. M.; Groth, T.; Grotli, M.; Rademann, J.; Gotfredsen, C. H.; Meldal, M. *J. Comb. Chem.* **2002**, *4*, 523−529.

(7) Lam, K. S.; Salmon, S. E.; Hersh, E. M.; Hruby, V. J.; Kazmierski, W. M.; Knapp, R. J. *Nature* **1991**, *354*, 82−84.

(8) Burger, M. T.; Bartlett, P. A. *J. Am. Chem. Soc.* **1977**, *119*, 12697−12698.

(9) (a) Lipshutz, B. H.; Blomgren, P. A. *Org. Lett.* **2001**, *3*, 1869−1871. (b) Chesney, A.; Stell, P. G.; Stonehouse, D. F. *J. Comb. Chem*. **2000**, *2*, 434−437. (c) Wang, Z.; Luo, J. T.; Zhu, X. X.; Jin, S. J.; Tomaszewski, M. J. *J. Comb. Chem.* **2004**, *6*, 961−966.

(10) Roller, S.; Türk, H.; Stumbé, J. F.; Rapp, W.; Haag, R. *J. Comb. Chem.* **2006**, *8*, 350−354.

(11) Van Heerbeek, R.; Kamer, P. C. J.; Van Leeuwen, P. W. N. M.; Reek, J. N. H. *Chem. Rev.* **2002**, *102* (10), 3717−3756.

(12) Lebreton, S.; Monaghan, S.; Bradley, M. *Aldrichim. Acta* **2001**, *34*, 75−83.

(13) (a) Luo, J. T.; Pardin, C.; Lubell, W. D.; Zhu, X. X. *Chem. Commun.* **2007**, *22*, 2136−2138. (b) Gheorghe, A.; Matsuno, A.; Reiser, O. *Adv. Synth. Catal.* **2006**, *348*, 1016−1020.

(14) (a) Wan, Y.; Huang, W-Q.; Wang, Z.; Zhu, X. X. *Polymer* **2004**, *45*, 71−77. (b) Gauthier, M. A.; Luo, J.; Calvet, D.; Ni, C.; Zhu, X. X.; Garon, M.; Buschmann, M. D. *Polymer* **2004**, *45*, 8201−8210.

(15) (a) Hellermann, H.; Lucas, H. W.; Maul, J.; Pillai, V. N. R.; Mutter, M. *Makromol. Chem.* **1983**, *184*, 2603−2617. (b) Kates, S. A.; McGuiness, B. F.; Blackburn, C.; Griffin, G. W.; Sole, N. A.; Barany, G.; Albericio, F. *Biopolymer* **1998**, *47*, 365−380.

(16) Wright, P.; Lloyd, D.; Rapp, W.; Andrus, A. *Tetrahedron Lett.* **1993**, *34*, 3373−3376.

(17) Park, B. D.; Lee, H. I.; Ryoo, S. J.; Lee, Y. S. *Tetrahedron Lett.* **1997**, *38*, 591−594.

(18) Cabasso, I.; Zilkha, A. *J. Macromol. Sci. Chem.* **1974**, *A8*, 1313−1324.

(19) Price, G. G.; Whiting, M. C. *Chem. Ind*. **1963**, 775−776.

(20) Ledwith, A.; McFarlane, N. R. *Proc. Chem. Soc*. **1964**, 108−108.

(21) Bawn, C. E. H.; Ledwith, A.; McFarlane, N. R. *Polymers* **1967**, *8*, 484−487.

(22) (a) Chang, E. P.; Kirsten, R. O.; Slagowski, E. L. *Polym. Eng. Sci*. **1978**, *18*, 932. (b) Hirata, Y.; Vemura, S.; Kotaka, T. *Rep. Prog. Phys. Jpn.* **1979**, *22*, 177.

(23) (a) Santini, R.; Griffith, M. C.; Qi, M. *Tetrahedron Lett.* **1998**, *39*, 8951. (b) Wilson, M. E.; Paech, K.; Zhou, W.-J.; Kurth, M. J. *J. Org. Chem.* **1998**, *63*, 5094.

(24) Vaino, A. T.; Goodin, D. B.; Janda, K. D. *J. Comb. Chem.* **2000**, *2*, 330−336.

(25) (a) Moriani, T.; Fujiwara, Y. *Macromolecules* **1977**, *10*, 532−535. (b) Budhlall, B. M.; Landfester, K.; Sudol, E. D.; Dimonie, V. L.; Klein, A.; El-Asser, M. S. *Macromolecules* **2003**, *36*, 9477−9484.

(26) Herclor H. *Elastomer-Ethylene Thiurea Cure System* Bulletin ORH-3F; Hercules, Inc: Wilmington, DE, 1983.

(27) Yan, B.; Li, W. B. *J. Org. Chem.* **1997**, *62*, 9354−9357.

(28) (a) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2002**, *41*, 2596. (b) Kolb, H. C.; Finn, M. G.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2001**, *40*, 2004.

(29) Gillet, S. M. F. G.; Chica, R. A.; Keillor, J. W.; Pelletier, J. N. *Protein Expression Purif.* **2004**, *33*, 256−264.

(30) (a) Keillor, J. Personnal communication. (b) Lin, C.-W.; Ting, A. Y. *J. Am. Chem. Soc.* **2006**, *128*, 4542−4543.

(31) (a) Cammish, L.E.; Kates, S. A. *Fmoc Solid Phase Peptides Synthesis: A Practical Approach*; Chan, W. C., White, P. D., Eds.; Oxford University Press: Oxford, U.K., 2000; p 277. (b) Lubell, W. D.; Blankenship, J. W.; Fridkin, G.; Kaul, R. *Peptides. Science of Synthesis 21.11, Chemistry of Amides*; Thieme: Stuttgart, Germany, 2005; pp 713−809.

(32) Tsuboi, S.; Okada, Y. *Chem. Pharm. Bull.* **1989**, *37*, 46−49.

(33) Gagnon, P.; Huang, X.; Therrien, E.; Keillor, J. W. *Tetrahedron Lett.* **2002**, *43*, 7717−7719.

(34) Atherton, E.; Sheppard, R. C. *Solid Phase Peptide Synthesis: A Pratical Approach*; IRL Press: Oxford, U.K., 1989

(35) Okaniwa, M.; Takeuchi, K.; Asai, M.; Ueda, M. *Macromolecules* **2002**, *35*, 6224−6231.

(36) Kaiser, E.; Colescott, R. L.; Bossinger, C. D.; Cook, P. I. *Anal. Biochem.* **1970**, *34*, 595.


CC060132+