Pergamon

Tetrahedron Letters, Vol. 37, No. 34, pp. 6185-6188, 1996

0040-4039/96 $15.00 + 0.00

PII: S0040-4039(96)01319-6

POEPOP and POEPS: Inert Polyethylene Glycol Crosslinked Polymeric Supports for Solid Synthesis

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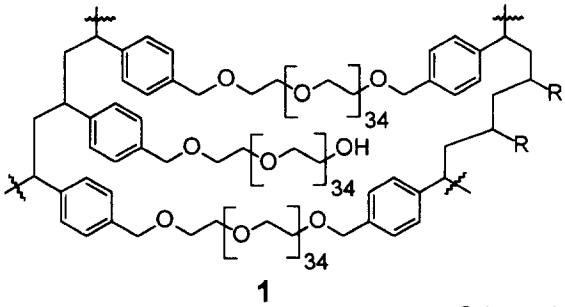
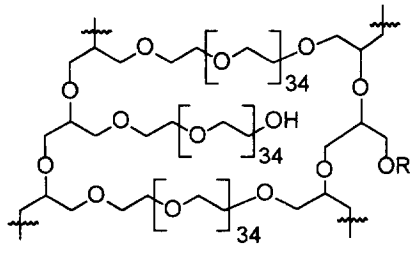
Abstract: *Two types of macromonomers were synthesized by reacting mono/di sodium derivatives of polyethylene glycol$_{1500}$ with vinylbenzyl chloride and epichlorohydrin. These monomers were bulk homo-polymerized by free-radical or anionic polymerization, respectively, to afford two different types of crosslinked polymeric supports in high yield. The resins were tested for solid-phase peptide synthesis by standard Fmoc/OPfp/Dhbt method, using a Rink amide linker. The peptides were cleaved from the support using 95% TFA-H_2O in high yield and purity. The pure peptides were characterized by MALDI-TOF mass spectrometry and amino acid analysis.*

Polyethylene glycol polyacrylamide (PEGA) was introduced[1] as a versatile hydrophilic and flexible polymer support for solid phase peptide synthesis.[2,3] The PEGA polymer matrix consists of long chain amino-polyethylene glycol (PEG) connected through polyamide linkages. Even though these supports were found to be excellent for peptide synthesis, it would be preferable to eliminate the amide backbone from the polymer matrix, for carrying out a larger diversity organic reactions.[4-7] Polystyrene based supports are being widely used in polymer supported reactions, but because of its hydrophobic nature polar reagents may fail to enter the polymer matrix and they have been found not to be suitable for bio-molecular reactions.[8] Also tetraethylene glycol crosslinked polystyrene has previously been employed as a flexible support in peptide synthesis.[9] Polyethylene glycol derivatives, due to their inert nature have a wide range of applications in polymer chemistry and biomedical sciences. End group modified PEG´s are used as carriers in peptide and combinatorial synthesis.[10] Polystyrene containing grafted PEG was found to be an efficient support in peptide synthesis.[11,12] The anionic copolymerization of CH_3O-PEG-epoxide with low molecular weight epoxide[13] or lactone[14] has been reported. However, crosslinked PEG made by homo-polymerization of epoxy or vinylbenzyl ether terminated PEG macromonomer as supports for solid phase synthesis has not previously been described. The present work describes the development of inert PEG crosslinked resins *viz* polyoxyethylene-polystyrene (POEPS) **1** and polyoxyethylene-polyoxypropylene (POEPOP) **2** where all the amide linkages present in PEGA are

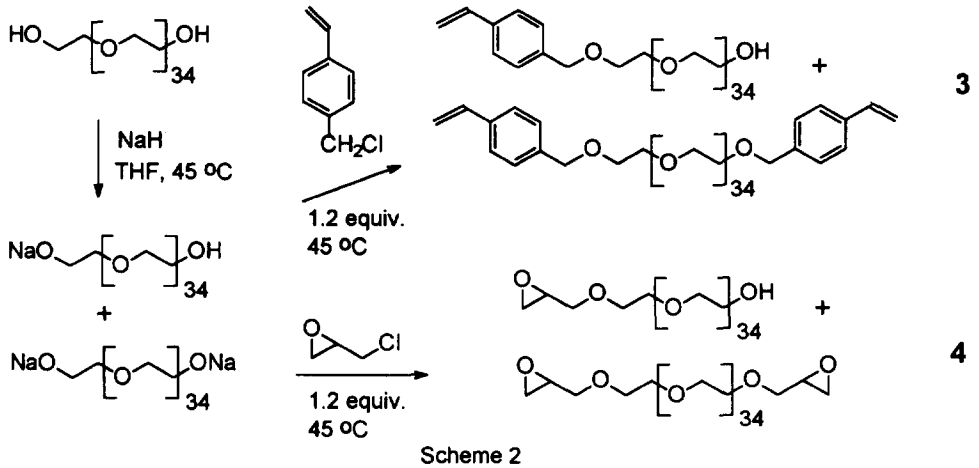


1



2

Scheme 1



Scheme 2

replaced by fairly stable ether linkages, yet retaining the optimized balance of hydrophilic-hydrophobic character. The new polymer support can be efficiently used for carrying out polymer analogues reactions e. g. carbenium or carbanion reactions in organic medium as well as biochemical reactions in aqueous medium.[15-17]

The macromonomers **3** and **4** were synthesized (Scheme 2) by dissolving PEG_{1500} (5 g, 6.6 mmol) in dry THF (5 mL) and sodium hydride (55% in oil) (0.15 g 3.9 mmol) was added to this with stirring. After 6 h vinylbenzyl chloride (0.57 mL, 4 mmol) to give **3** or epichlorohydrin[18] (0.32 mL, 3.9 mmol) to give **4** was added to the reaction mixture at 45 °C and allowed to stir for another 16 h. The products were precipitated using diethyl ether and filtered off. They were purified by dissolving in CH_2Cl_2 and filtered to remove any insoluble impurities. The products were precipitated using cold diethyl ether, filtered and dried under high vacuum (4.8 g, 95% **3** and 4.5g, 90% **4** respectively). The pure products were characterized using ^{13}C NMR spectroscopy.[19] There was no polymerization reaction during macro monomer preparation (ie.at 45°C) as indicated by nature of the product (**3** and **4**) and NMR data. The macromonomer **3** (2.5 g) was bulk homo-polymerized (Scheme 2) in a pyrex tube under argon at 100°C using a free-radical initiator (organic peroxides 0.10 g, Rohm GmbH, Germany) for 12 h to afford the PEG crosslinked polymer **1**. It was washed with methanol and granulated through 1 mm sieves, further washed with CH_2Cl_2, methanol and ether and dried under high vacuum (1.5 g, 60%). The anionic polymerization of macromonomer **4** (2.6 g) catalyzed by *t*-BuOK (0.10 g) was carried out at 100°C under argon atmosphere and the sticky point was reached after 30 min. The polymerization was continued for 12 h. The reaction was quenched by the addition of methanol and the product **2** was granulated through 1 mm sieves. The polymer was stirred in 4 M HCl for 2 h to remove potassium salts and then washed with water and ethanol and dried under high vacuum (2.5 g 96%). In the case of polymer **1** the yield is only 60% since only 1.2 equiv vinylbenzyl chloride were added to obtain 50-60% mono- or di-vinylbenzyl modified PEG along with unmodified PEG as reported earlier in the case of acrylamido PEG derivatives.[3] Polymer **2** was obtained in a nearly quantitative yield, which can be explained since the product is formed by ring opening anionic polymerization[14]and thereby increasing the amount PEG end group modification during macropolymer formation. The polymers **1** and **2** were characterized[3] by gel phase ^{13}C NMR spectroscopy

(Fig. 1). The hydroxyl group capacity of the resin **1** and **2** were determined by esterification with Fmoc-Gly (3 equiv.) activated by 1-(2-mesitylenesulfonyl)-3-nitro-1,2,4 triazole (MSNT) [20] (3 equiv.) dissolved in dry CH_2Cl_2 in presence of a base N-methyl imidazole (2.25 equiv.) and measuring the UV-absorbance of the adduct of dibenzofulvene and piperidine formed by treatment of weighed polymer sample with 20% piperidine/DMF. The OH group capacities of these supports can be easily varied from 0.1 to 0.6/g by adjusting the monomer composition. The swelling capacity of the new supports **1** and **2** were determined by the syringe method as reported.[3] The swelling volume of resin **1** and **2** were 8.6 and 9.2 mL/g in DMF; 10.7 and 12.2 mL/g in CH_2Cl_2 and 8.6 and 9.2 mL/g in H_2O respectively.

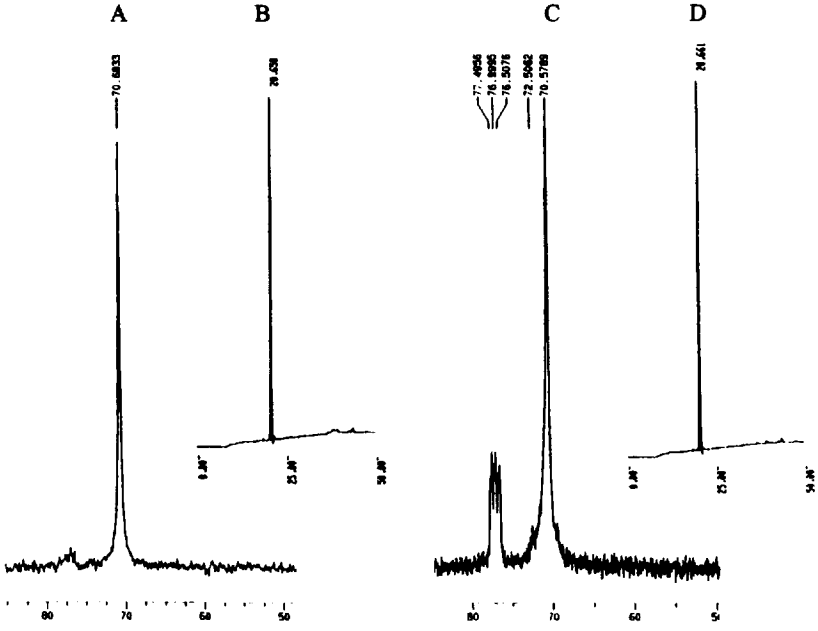


Figure 1. ^{13}C NMR of polymers and HPLC of crude synthetic peptides. A) Polymer **1**, B) HPLC of peptide **5** synthesized on **1**, C) Polymer **2**, D) HPLC of peptide **6** synthesized on **2**.

Rink amide handle[21] was attached to support **1** and **2** by the MSNT esterification method[20] as decribed above for use in peptide synthesis. The support **1** (200 mg, 0.3 mmol/g) was employed in the synthesis of pentapeptide G-F-S-F-G-NH_2 by a standard Fmoc-AA-OPfp ester coupling in presence of Dhbt-OH catalyst.[22,23] Single coupling reactions with 2.5 equiv. of Pfp ester were employed and the reactions were complete in 10-15 min as indicated by the disappearance of the yellow color of Dhbt-OH ionized by resin bound amino groups. After the synthesis the peptide was cleaved from the resin using 95% aqueous TFA concentrated and precipitated using diethyl ether. Peptide resin, 51 mg, yielded 17 mg crude peptide (Fig. 1) which on HPLC purification afforded 7.6 mg pure peptide[24] (**5**) (93% yield based on initial loading by Fmoc monitoring). The same peptide was prepared using support **2** (0.15 g, 0.5 mmol/g) according to the procedure decribed above. Penta-peptide resin, 30 mg, yielded 13 mg crude product (Fig. 1) which on HPLC purification

gave 6 mg pure peptide[24] (6) (85.5% based on initial loading). Thus, approximately similar practical yields were obtained from the two polymers.

These novel supports will be investigated further for efficiency in the synthesis of large peptides as well as general polymer supported organic synthesis. The two polymers POEPOP and POEPS can be expected to have different properties e. g. under hydrogenolytic or strongly acidic conditions and may complement each other. The main advantages of these classes of polymer supports are the complete lack of functional groups like amides in the polymer backbone, high capacity, optimum hydrophilic-hydrophobic balance and high mechanical and chemical stability. These supports are cost effective since they are prepared by an easy process using readily available low cost bio-compatible polyethyleneglycols. The hydroxyl groups of the polymer support are amenable to a wide range functional group transformations without effecting the polymer back bone. The structure of the polymer provides excellent flow properties and reagent or solvent accessibility under organic reaction conditions. Studies are in progress to develop a new technique for making PEG crosslinked support in beaded form.

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18. Caution! Suspected carcinogen
19. ^{13}C NMR of compound **3** dC (250 MHz $CDCl_3$) 61.6 (HO-$\underline{C}H_2$-CH_2-); 72.4 (HO-CH_2-$\underline{C}H_2$-); 69.4 (-CH_2-$\underline{C}H_2$-O-CH_2-Ø); 72.8 (-$\underline{C}H_2$-CH_2-O-CH_2-Ø); 73.0 (CH_2-CH_2-O-$\underline{C}H_2$-Ø); 136.6, 127.4, 125.4, (phenyl ring); 113.7,(-CH_2-=$\underline{C}H_2$). 136.4 (-$\underline{C}H_2$==CH_2). Compound **4** 61.5 (HO-$\underline{C}H_2$-CH_2-); 72.3 (HO-CH_2-$\underline{C}H_2$-); 50.6 (-$\underline{C}H_2$-CH_2-O-); 44.04 (-CH_2-$\underline{C}H_2$-O-); 70.5 (-$\underline{C}H_2$-CH_2-O-CH_2-epoxide); 70.2 (-CH_2-$\underline{C}H_2$-O-CH_2-epoxide);71.8 (-CH_2-CH_2-O-$\underline{C}H_2$-epoxide).
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24. Data for compound **5**: Mass, m/z 513.58 (C_{25} H_{31} N_6 O_6 required M+H=513.57) ; Amino Acid (AA) analysis Gly:1. 92 (2), Ser:1.05 (1), Phe:2.03 (2). Compound **6**: Mass, m/z 513.19 (C_{25} H_{31} N_6 O_6 required M+H=513.57) ; AA analysis Gly:1.95 (2), Ser:1.1 (1), Phe:1.91(2)

(*Received in UK* 17 *May* 1996; *revised* 3 *July* 1996; *accepted* 4 *July* 1996)