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Synthesis of Novel a PEG-Lysine Polymer

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Summary: A novel PEG functionalized-*L*-lysine-α-amino-*N*-carboxy anhydride monomer (*L*-Lys(PEG-(COOBzl))-NCA) is prepared for the synthesis of carboxylic acid terminated, PEG-sheathed poly-lysine biopolymers.

Keywords: biocompatible; biomaterial; carboxylic acid; PEG; Poly-lysine

Introduction

Poly-amino acids are often used in the field of biopolymers. Examples include poly-amino acids in block copolymers with synthetic materials, either in a linear fashion[1] or grafted[2] to the poly-amino acid backbone.

Polymers without reactive functional groups are limited in their applications to the mechanical and physical properties they present. Functional groups offer extra dimensions to polymer applications and properties in terms of solubility, crosslinking, further attachment of active molecules.

Presented here is the synthesis of a novel three-component PEG-lysine monomer for the synthesis of bio-polymers with a lysine backbone with short pendant, carboxylic acid functionalised PEGs.

The following monomer design aspects were taken into account: 1) Polymerization is to occur yielding peptide bonds through the α-amino-*N*-carboxy-anhydride lysine (Lys-NCA) moiety. The lysine would make up the backbone of the polymer. 2) Coupled to the ε-amino group of the lysine, through an amide bond, is a short carboxylic acid terminated PEG. This would constitute the PEG sheath of the polymer and contribute to its non-ionic solubility. Yu, *et al.* synthesised ethyleneglycol- and diethyleneglycol methyl ether functionalized poly-lysine. These non-ionic polymers were soluble in water and adopted an α-helix structure in solution.[3] 3) The distal carboxylic acid function is protected as a benzyl ester during monomer synthesis and polymerization, after which the ester may be hydrolysed or hydrogenated to deprotect the carboxylic acid terminus. This acid group may then be exploited for the attachment of biological molecules or peptides and ionic- (e.g. di-valent metal cations and diamines) or covalent crosslinking (diamines and diols).

Monomer Synthesis

The synthesis of the monomer involves a three step process. Firstly a short α–ω-*bis*(carboxymethyl) functionalized poly-ethylene glycol (PEG) (PEG di-acid) was asymmetrically protected through the formation of a benzyl ester. This was done by the condensation of benzyl alcohol to the carboxylic acid groups on the PEG (See Figure 2).

Secondly, once the mono-benzylated PEG di-acid was separated from the reaction mixture the remaining carboxylic acid was reacted with *N*-hydroxysuccinamide. This activated ester was, in turn, reacted with the ε-amino group of an appropriately

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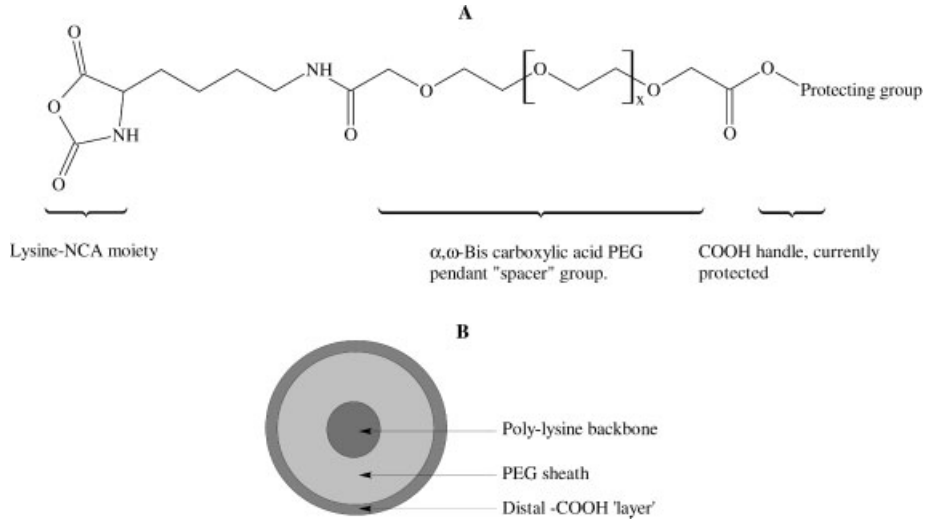

Figure 1.
A) The *L*-Lys(PEG-(COOBzl)-NCA monomer illustrating in general the three components of the monomer and B) a diagrammatic lay-out ('head-on' view of polymer) of the envisaged final polymer after the protecting groups have been removed and assuming an α-helix secondary structure.

α-amino-protected (Pr-) amino acid to yield Pr-*L*-Lys(PEG-(COOBzl))-OH (Figure 3).

Figure 4 outlines the different approaches for the third and final step in the monomer synthesis – the formation of the α-amino carboxy-anhydride (NCA) moiety. This was achieved directly, either from the benzyloxycarbonyl- (Z) or the *tert*-butyloxycarbonyl (tBoc) protected α-amino acids through the reaction with dichloromethyl methyl ether (DMME). Alternatively starting with the tBoc protected amino acid – the protecting group can be removed by reaction with trifluoro acetic acid. Reacting the deprotected product with bis(trichloromethyl) carbonate (triphosgene) also yielded the NCA product.

^{1}H NMR (400MHz) ***L*-Lys(PEG-(COOBzl)- NCA:** in DMSO-D_6/TFA-D. δ in ppm relative to TMS. 7.36–7.24 (m; 5H; Arm. Bzl), 5.12 (s; 2H; –C<u>H</u>$_2$–Ph), 4.37 (m; 1H; –NH–C<u>H</u>–CO–), 4.16 (s; 2H; –O–C<u>H</u>$_2$–COO–Bzl), 3.84 (s; 2H; –O–C<u>H</u>$_2$–CONH–CH$_2$–), 3.68–3.47 (m; 8H;

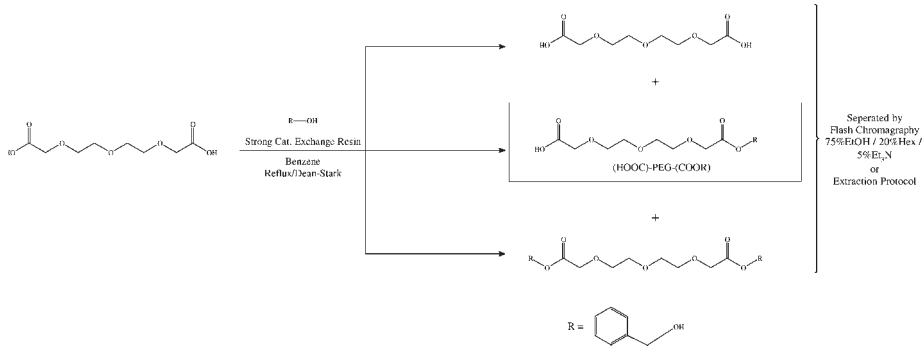

Figure 2.
Procedure for the asymmetric benzyl protection of the bis(carboxymethyl)-PEG as the first step of the monomer synthesis.

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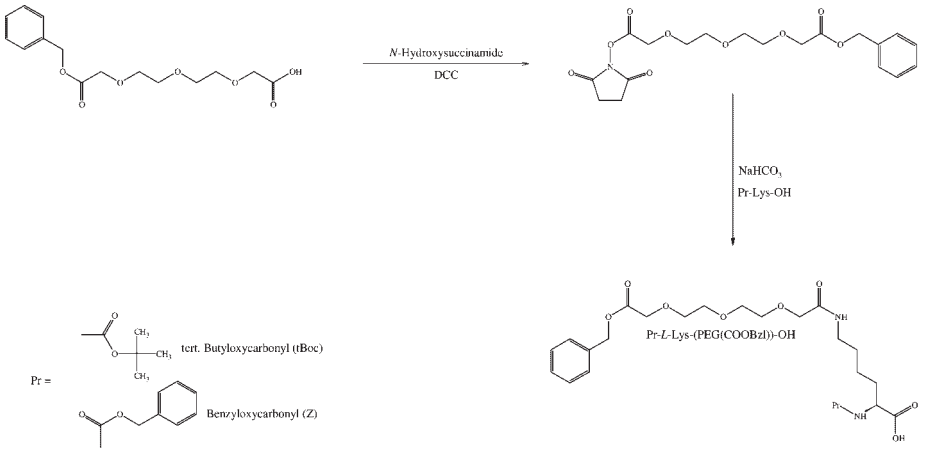

Figure 3.
Coupling of the asymmetrically protected PEG to an α-amino protected lysine as the second step in the Pr-*L*-Lys(PEG-(COOBzl))-OH monomer synthesis.

–O–CH$_2$–CH$_2$–O–CH$_2$–CH$_2$–O–), 3.10 (m; 2H; –CH$_2$–CH$_2$–NH–CO–), 1.84–1.17 (m; 6H; –CH$_2$–CH$_2$–CH$_2$–). **^{13}C:** 172.08 (–C=O–O–C=O–NH, NCA), 170.58 (–O–CH$_2$–C=O–OBzl), 169.76 (–O–CH$_2$–C=O–NH–CH$_2$–), 152.14 (–C=O–O–C=O–NH, NCA), 136.41 (Arm. Ipso –C(C)=, Bzl), 128.89, 128.57, 128.55 (Arm. –C(H)=, Bzl), 70.83 (–O–CH$_2$–COO-Bzl), 70.65 (–O–CH$_2$–CO–NH–CH$_2$–) 70.42, 70.28, 70.03, 68.26 (–O–CH$_2$–CH$_2$–O–CH$_2$–CH$_2$–O–), 66.17 (–CH$_2$–Ph), 57.48 (–NH–CH–CO–), 38.16

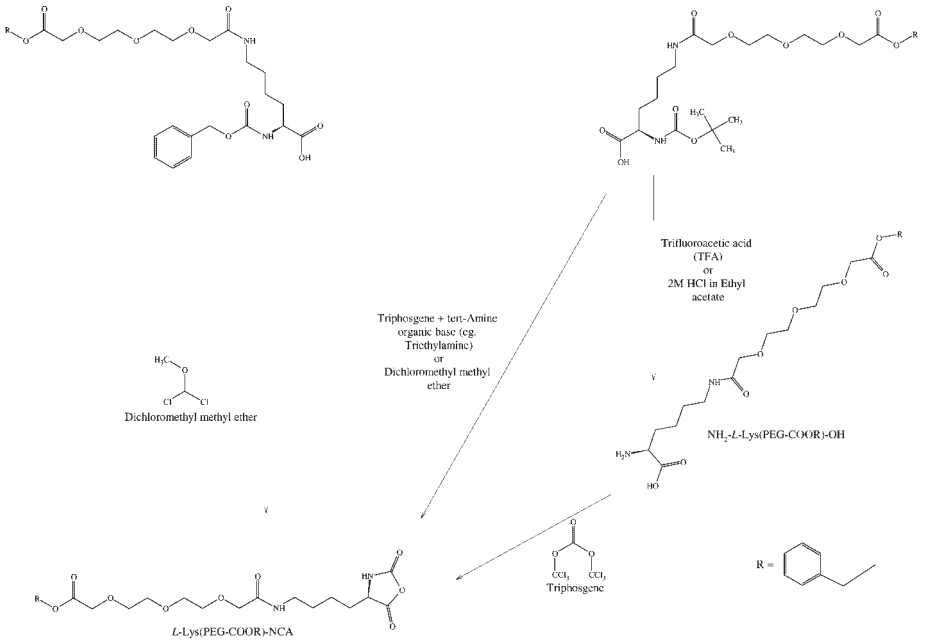

Figure 4.
Final step in the *L*-Lys(PEG-(COOBzl)-NCA synthesis – the formation of the α-amino acid-*N*-carboxy-anhydride (NCA) moiety.

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(–CH_2–$\underline{C}H_2$–NH–CO–CH_2–), 31.23, 29.07, 22.25 (–$\underline{C}H_2$–$\underline{C}H_2$–$\underline{C}H_2$–).

The final NCA product, like the PEG containing intermediates, were very viscous oils and were difficult to clean by means of conventional methods like precipitation or crystallization and due to the reactivity of the NCA moiety silica-gel chromatography was also not viable option.

Preliminary NMR evidence suggests that polymerization is effectively initiated using *n*-butyl amine where the initiator to monomer ratio is 1:5 and 1:10. Polymerizations using higher ratios like 1:15 and 1:20 were unsuccessful. This is most probably due to residual contaminants quenching the amine initiator. Preliminary *n*-butyl amine initiated copolymerization with Lys(Z)-NCA and the use of a dendritic initiator (generation 1.0 dendrimer, *N*, *N*, *N*′, *N*′ tetrakis (3-aminopropyl)-1,4-butanediamine (DAB-Am-4)) both proved to be successful according to NMR. Conditions for MALDI-ToF and GPC still need to be established for molecular mass and polydispersity determinations. An effective purification protocol, like preparative HPLC, is needed to achieve effective polymerization.

Conclusion

A novel carboxylic acid functionalized (protected as a benzyl ester) PEG modified lysine-NCA monomer, *L*-Lys(PEG-(COOBzl)-NCA, was successfully synthesized. In the absence of an effective purification protocol, impurities trapped in the 'syrup-like' final product only allowed for limited amine initiated polymerization reactions of this NCA monomer. Work is currently underway to solve this problem.

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