ABSTRACT

Synthetic routes for the preparation of new polymers of different architectures were designed in the context of this dissertation. The synthesized polymers were prepared for their potential application as innovative drug delivery carriers with the emphasis on high molar mass protein/peptide active pharmaceutical ingredients. In the first part of the work, a set of three chitosan-*graft*-poly(L-glutamate) (Chi-*g*-PGlu) copolymers with different lengths of the peptide grafts was prepared. A macroinitiator – organosulfonic salt of chitosan soluble in polar organic solvent DMSO – was prepared, followed by the ring-opening polymerization of BGlu NCA monomer. The benzyl ester protected graft copolymers were deprotected by applying various deprotection procedures. The most efficient deprotection procedure in which the chitosan moiety was preserved almost intact included tetrabutylammonium hydroxide.The structure and the molar masses of the Chi-*g*-PGlu copolymers were characterized in-depth by advanced characterization techniques (NMR, SEC-MALS, FTIR). Nanoparticles (NPs) loaded to a high extent (up to 45 %) with granulocyte colony-stimulating factor (GCSF) were prepared from Chi-*g*-PGlu copolymers with the addition of trimethylchitosan (TMC). NPs were characterized by DLS to evaluate their average diameter which ranges between 240 and 320 nm, and the distribution in size which is described by the polydispersity index and was between 0.15 and 0.26. The influence of peptide graft length, together with the amount of GCSF and TMC added, on NPs' characteristics were investigated. Furthermore, evaluation of temperature, time and pH stability of thus prepared NPs revealed good stability in the temperature change between 25 and 39°C, and over time. On the other hand, NPs disintegrate at pH values above the GCSF isoelectric point (6.1) which is a desired and expected property of this NP system.

In the second part, the homopolypeptide, PGlu, was prepared and its carboxyl side groups glycosylated in a controlled manner in aqueous solution by using 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMTMM) as a coupling reagent under mild conditions. The reaction products were characterized using 1D and 2D NMR experiments, SEC-MALS and MALDI-TOF MS to determine the eventually present side products, degree of substitution and molar mass characteristics of the products.

In the third part, a model dendronized polyglutamate, P(Glu-D), was designed, synthetized and characterized. P(Glu-D) was dendronized with a second-generation dendron. Synthesized P(Glu-D) exhibits the degree of polymerization (DP_n) of 46 and the degree of dendronization of 43 %. Perfect agreement was found between the P(Glu-D)'s expected structure and the experimental data (NMR, SEC-MALS and MALDI-TOF MS). The PGlu precursor was modified by coupling with bifunctional moiety (N_3-Pr-NH_2) in the presence of DMTMM. The polyamide second-generation dendron was prepared by stepwise procedure involving the coupling of propargylamine to the L-lysine carboxyl group, followed by coupling the protected bis-MPA building block to the L-lysine amino groups. The hydroxyl functional groups of the resulting second-generation dendron were deprotected quantitatively under mild acidic conditions. The deprotected dendron with acetylene focal group was coupled to the pendant azide functional groups of the modified linear copolypeptide, P(Glu-N_3), under Cu(I) catalyzed azide-alkyne cycloaddition reaction to form 1,4-disubstituted triazole. The dendronization reaction proceeded quantitatively in 48 hours in aqueous medium as confirmed by ^{1}H NMR and FTIR spectroscopy.

In the last part of the work the synthesis of a second-generation polyester dendrimer is reported. Trifunctional aliphatic core moiety and an AB_2 type repeat unit prepared from glycolic acid and bis-MPA were applied. The carboxyl functional group of the glycolic part of the AB_2 monomer and of the second-generation dendron was protected by the benzyl ester, which was quantitatively removed using catalytic hydrogenation. The bis-MPA hydroxyl functional grups of the AB_2 monomer and the second-generation dendrimer were protected using an acetonide protection group. The acetonide groups were efficiently cleaved by using acidic ion exchange resin. The ester bonds were synthesized using DCC as a coupling reagent and DPTS as a neutral catalyst. The coupling products were purified by flash-column chromatography. The quantitative removal of acetonide protective groups was accompanied by partial hydrolysis of the dendrimer ester groups as indicated from ^{1}H NMR and SEC data. Due to the resulting G2-OH structural defects the synthesis of higher-generation dendrimers was not pursued. Namely, structural imperfections in lower generation dendrimer prevent the synthesis of dendrimers of higher generations with uniform structure and molar mass.

Copolymers of different architectures were prepared: (i) graft copolymers, (ii) linear

copolymers, (iii) a dendronized copolymer and (iv) dendrimers. Chi-*g*-PGlu graft copolymers characterized and applied for nanoparticle preparation. The nanoparticles were succesfully loaded with GCSF protein. The linear copolymers with different degrees of substitution with glucosamine (GlcN) were prepared and characterized. A dendronized copolymer was successfully prepared by using azide-alkyne click reaction to graft the second-generation dendron to the pendant azide groups of the linear precursor. A second-generation polyester dendrimer was prepared in the final part of the thesis. Due to its structural imperfection, the synthesis of the higher generations was not accomplished.

Keywords: chitosan, poly(L-glutamate), graft polymers, dendrimer, dendronized polymers, granulocyte colony-stimulating factor (GCSF), nanoparticles