

2.3.10 Solid Phase Peptide Synthesis (SPPS)

Solid phase peptide synthesis was performed using standard Fmoc-based chemistry. The peptides u7 and Gu11G were prepared in the Univ Nijmegen by Dr M. Thanou. Scrambled u7 and scrambled u11 peptides were synthesised in collaboration with Sian Owens (Welsh School of Pharmacy, Cardiff University, UK) on an automated Symphony Quartet Peptide Synthesiser (Zinsser analytic, UK). First the amino groups of the rink amide resin were deprotected with a solution of 20 % w/v piperidine in DMF, this was done three times. The resin was then washed twice with NMP and the Kaiser test performed (which should be positive, indicating free NH_2). Sequential coupling of the amino acids and cleavage of N-protecting group was then performed in the C-N direction as outlined (Fig. 2.9). After the final coupling, deprotection and cleavage from the resin was performed using trifluoroacetic acid. The peptides prepared are shown in Fig. 2.10. Mass spectroscopy was used to characterise the products (Fig. 2.11). The molecular mass found correlated with the predicted molecular masses, the predominant ion in the analysis of Gu11G was the $\frac{1}{2}$ mass peak due to ionisation producing 2 +ve charges, probably on the terminal amine and the amine of lysine.

2.3.11 Gel Permeation Chromatography (GPC)

For the GPC characterisation of PEG conjugates, PBS (0.1 M, pH 7.4) was used as mobile phase and it was first filtered (0.22 μm nylon Micropore filter) then sonicated for 30 min to remove dissolved gases. The flow rate was set to 1 ml/min, the system was purged and the baseline allowed to stabilise before injecting any samples. Samples were introduced to the system through a 20 μl sample loop. A calibration curve was made with PEG standards and samples characterised against it (Fig. 2.12) using Caliber software.

An organic GPC system was also used. The flow rate was also set to 1 ml/min with the system being purged and stabilised before injection of samples. Toluene was used as a flow rate marker. The calibration curve (Fig. 2.13) was made with polystyrene standards using Cirrus GPC software.

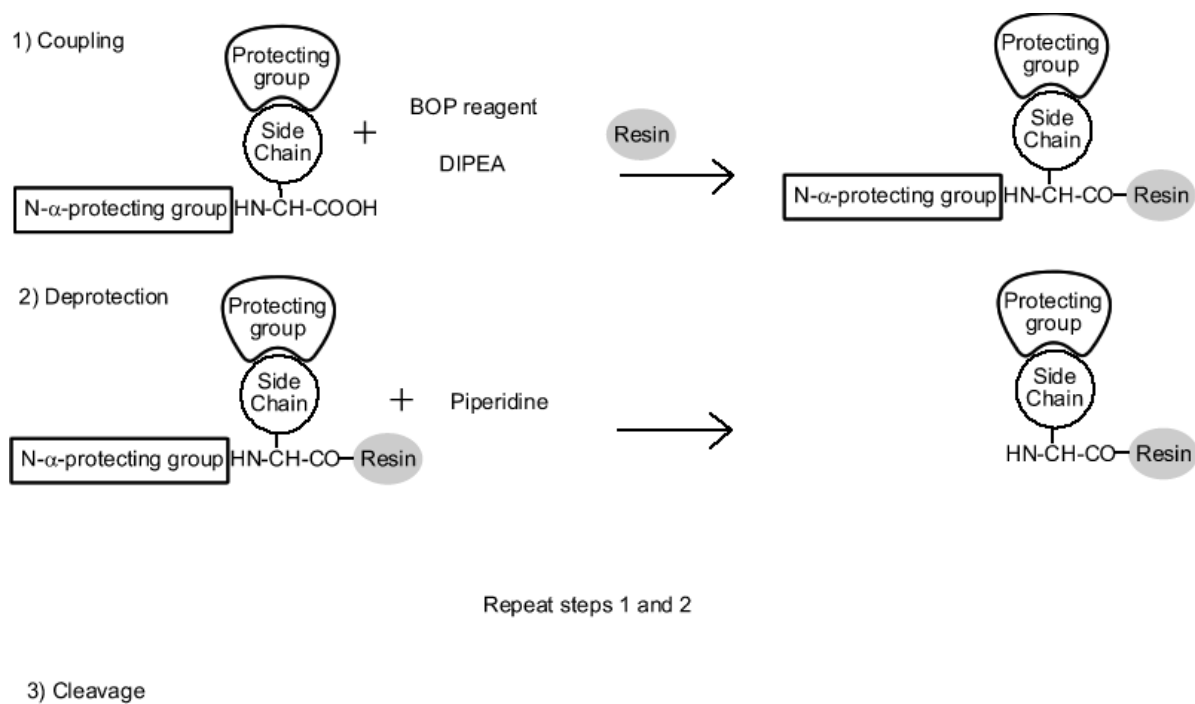


Figure 2.9 - Solid phase peptide synthesis

Peptides were synthesised by the above scheme in a sequential addition in the C-N direction.