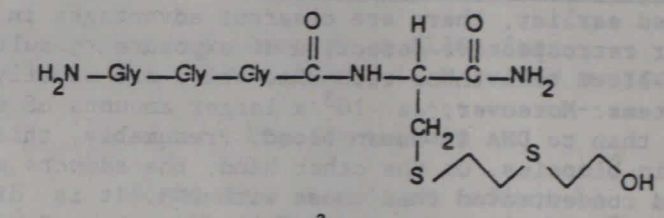
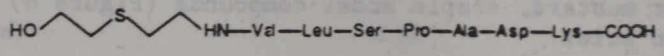
synthesis of a hapten (Figure 7), in which the cysteine adduct is connected to three glycines serving as a spacer for binding to the carrier protein.



a



b

Figure 7 Chemical structures of peptide haptens to be used for the generation of antibodies against protein adducts of sulfur mustard. The upper hapten (a) contains a mustard-reacted cystein coupled to a spacer of three glycine moieties. The lower hapten (b) represents the N -terminal heptaptide which is released from the α-chain of human hemoglobin upon tryptic digestion, after alkylation of the amino group with sulfur mustard.

Figure 7 shows also the structure of a hapten which was used already for preliminary immunization experiments. It corresponds with the amino-terminal heptapeptide which is released from the α-chain of hemoglobin upon tryptic digestion. The amino group of the N-terminal valine in this heptapeptide is rather exposed in the native structure of hemoglobin and, therefore, is alkylated in vivo by various agents, such as ethylene oxide (27). Investigation of the alkylation of the valine model compound (cf. Figure 6) in which the α-amino group was unprotected showed that an α-N-(2-hydroxyethylthioethyl) adduct was readily formed. This structure is present in the hapten derived from the heptapeptide, which was obtained by synthesis in a peptide synthesizer and subsequent N-alkylation. Recent investigations showed that the α-N-adduct of terminal valine is also formed upon in vitro incubation of hemoglobin with sulfur mustard, representing ca. 6% of the total alkylation of the protein. Since blood is a convenient ingredient to sample and since hemoglobin has a biological half life of several months, hemoglobin appeared to be a very suitable substance for biomonitoring. Therefore, it was attempted to obtain antibodies against this protein adduct. The alkylated heptapeptide was bound to a carrier protein and injected into mice. Several monoclonal antibodies were obtained having affinity for mustard adducts with amino acids. However, sofar they all belong to the IgM class, which is rather unsuitable for detection purposes. Efforts to obtain IgG antibodies are being continued.