Three of the adducts were supposed to be formed according to early investigations (30): the N7-guanine monoadduct with sulfur mustard (adduct 1), the corresponding di-adduct (adduct 2), and the N3-adenine monoadduct (adduct 3). Co-chromatography with the radioactive peaks (Figure 5) and other evidence, e.g., HPLC combined with thermospray-MS detection or UV spectroscopy, learned that the N7-guanine monoadduct was the major product, whereas the di-adduct and N3-adenine monoadduct were the minor products. Evidence for formation of the O6-guanine monoadduct (adduct 4) was not found (31).

b. Detection of DNA adducts in nucleated blood cells

Since the N7-guanine monoadduct is obviously the major adduct, a hapten based on this adduct (Figure 5) was synthesized. With reference to the structure of DNA, it was intended to synthesize this adduct containing a deoxyribose-5'-phosphate moiety. However, it

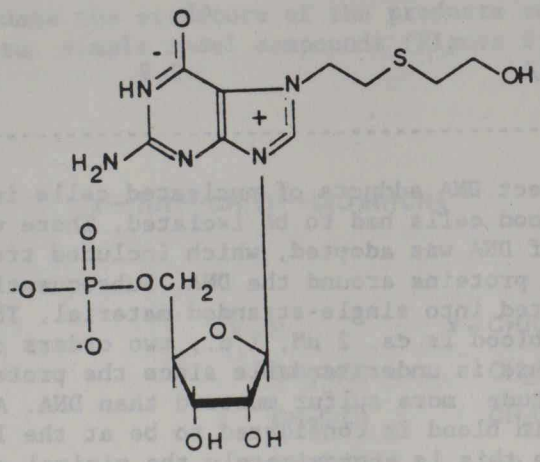

Figure 5 Chemical structure of the hapten used to generate monoclonal antibodies against the N7-guanine monoadduct of sulfur mustard. Before injection into mice, the hapten was bound to a protein via an activated ester of the phosphate moiety.

appeared that the glycosidic bond in such a hapten is too unstable during synthesis. Therefore, the deoxyribose was replaced by a ribose unit. Alkylation at N7 of guanine of this guanosine-5'-phosphate with sulfur mustard gave the hapten in sufficient quantity, in which the phosphate group is the handle to bind the hapten to a carrier protein. This product was linked to the carrier protein by means of an activated ester of the phosphate. Fortunately, the first attempts after injection in mice gave not less than ten hybridoma cell lines that produced monoclonal antibodies of the preferred class, i.e., IgG. Four of these were very specific for the major adduct in DNA. In fact, the sensitivity reached with these four monoclonals was approximately the same as that of the polyclonal antiserum that had been raised against calf-thymus DNA alkylated with sulfur mustard.

Optimization of the ELISA was labor-intensive. This was done with adducts induced in purified DNA. The major problem appeared to be the unwinding of the double-stranded DNA, which is also crosslinked by the sulfur mustard, into single-stranded DNA, without destruction of the N7-guanine adducts. Unwinding appeared to be essential for a good recognition of the adduct. Finally, a