Synthesis of Antigenic Carbohydrate Polymers Recognized by Lectins and Antibodies

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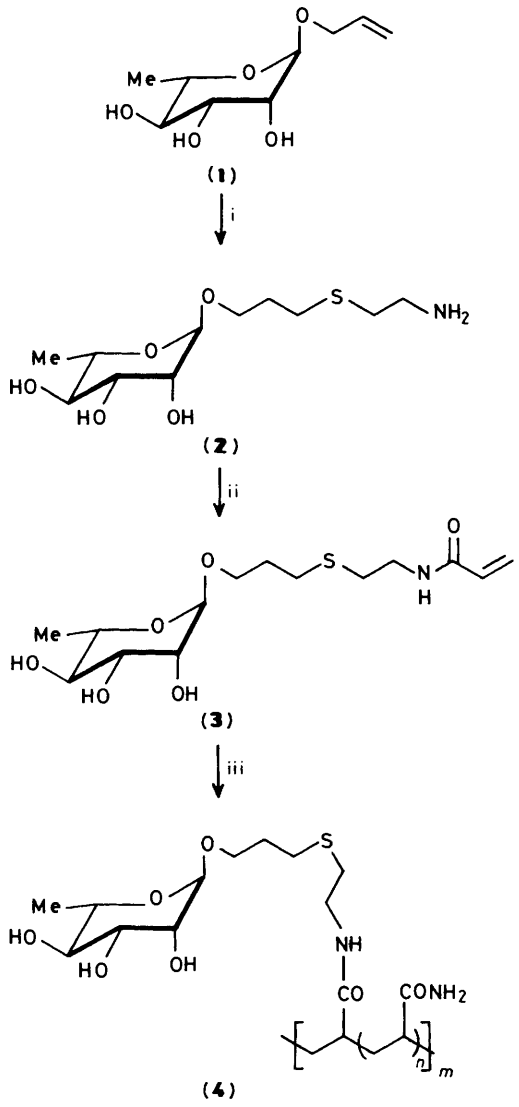
The alkene group of allyl α-L-rhamnopyranoside has been converted to an *N*-acryloyl spacer arm which was copolymerized with acrylamide to afford water soluble carbohydrate copolymers; these have antigenic properties demonstrated by their binding to *Ricinus communis* lectin and to the antisera to the capsular polysaccharide of *Streptococcus pneumoniae* type 23.

In spite of the ubiquitous examples of immobilized carbohydrates utilized as solid matrices for affinity chromatography,[1] there have been few examples of multivalent non-protein carbohydrates used as water soluble antigens.[2,3] The values of these soluble carbohydrate antigens are particularly important in light of the recent progress made with monoclonal antibodies of narrow specificities. The increasing needs for such defined pseudopolysaccharides could be attributed to the recent recognition that the carbohydrate chains of glycolipids and glycoproteins serve as



Scheme 1. *Reagents and conditions*: i, $HSCH_2CH_2NH_2.HCl$, u.v. (254 nm), 1.5 h, 25 °C; ii, acryloyl chloride, $CHCl_3$/MeOH, resin (OH^-), 0.5 h, 0 °C; iii, acrylamide, $(NH_4)_2S_2O_8$, 0.25 h, 100 °C, H_2O.

onco-developmental antigens and as receptors for infective agents.[4] In order to develop new carbohydrate antigens useful for the screening and for the binding studies of monoclonal antibodies by radioimmunoassays and by enzyme linked immunosorbent assays (ELISA), we describe here an example of the synthesis of pseudopolysaccharides containing rhamnose. These synthetic antigens were shown to express affinities for the plant lectin *Ricinus communis* and the rabbit antisera raised against the capsular polysaccharide of *Streptococcus pneumoniae* type 23 possessing branched α-L-rhamnose residues linked to a trisaccharide repeating unit.

The strategy described here has numerous advantages over the few existing examples.[2,3] For instance, the acryloylated rhamnoside (**3**) possesses a better reactivity ratio with acrylamide than the allyl α-L-rhamnopyranoside (**1**). Moreover, it permits the introduction of a spacer arm (*ca.* 11 Å) which renders the rhamnose residues more accessible to the macromolecules and gives access to flexibilities by allowing the incorporation of other co-monomers for specific needs. Furthermore, it gives non-biodegradable materials for which the molecular weight and sugar contents could be controlled at will.

The synthetic pathway is depicted in Scheme 1. In a typical reaction sequence, allyl α-L-rhamnopyranoside (**1**), prepared by the Fischer glycosidation of rhamnose with allyl alcohol and hydrogen chloride, was subjected to u.v. catalysed addition of 2-aminoethanethiol for 1.5 h to give a quantitative yield of (**2**).† The addition[5] of the thiol group to the double bond occurred in an essentially anti-Markovnikov manner as shown by ^1H n.m.r. spectroscopy. The transformation of the aminoglycoside (**2**) into the suitable *N*-acryloylated co-monomer (**3**) was efficiently performed with acryloyl chloride ($CHCl_3$/MeOH, 0 °C) in the presence of Amberlite resin IRA-400 (OH^-) as proton scavenger (quantitative yield). The 3-(2-*N*-acryloylaminoethylthio)propyl α-L-rhamnopyranoside (**3**) was then subjected to an aqueous electron-transfer copolymerization with acrylamide in the presence of ammonium persulphate. The reactions could equally well be performed for 15 min at 100 °C or overnight at room temperature in the presence of *N,N,N',N'*-tetramethylethylenediamine (TEMED). Dialysis followed by freeze-drying gave the water soluble copolymers (**4**) in 50—60% yield (by weight). The sugar contents (22 mole%) were estimated by ^1H n.m.r. spectroscopy and by the colorimetric method of Dubois *et al.*[6] Copolymers with a 1:4.5 molar ratio and molecular weight of 40 000–60 000 were found to be serologically active. The molecular weights can be controlled by the amount of persulphate used and were determined by comparison of their relative mobilities with similar copolymers in agarose gels.[3]

The complete utility of the water soluble copolymer (**4**) was clearly established in serological experiments using the lectin *Ricinus communis*[7] and a rabbit antisera against the pneumococcal type 23 capsular polysaccharide[8] (Statens Serum Institut, Denmark). Indeed, double radial immunodiffusion in agarose gel at a concentration of 2 mg/ml in phosphate buffer saline (PBS) gave sharp precipitin bands with the natural polysaccharide. This indicated the importance of the immunodominant rhamnose side-chains within the polysaccharide[9] and that an antibody population in the sera clearly had an affinity for the rhamnose residues in the polymers. Sensitization of ELISA plates (Linbro, Titertek, VA, U.S.A.) with (**4**) at the usual concentration in PBS was also useful in the sandwich mode using peroxidase labelled goat anti-rabbit IgG and protein A.

To further substantiate the usefulness of the above intermediates, compound (**2**) was coupled to carboxylated latex particles (Estapor, Rhône-Poulenc, 0.667 μm) in borate buffer pH 8.2 using a water soluble carbodiimide (EDC). The incorporation of rhamnose was 2.8% by weight. The beads gave a positive latex agglutination test with the above lectin. Furthermore, compound (**2**) could be directly coupled to Eupergit C for affinity chromatography. It is interesting to note that compound (**2**) could also be attached to an activated water soluble polyacrylamide. However, this approach would lack the versatility of the present approach.

The success of these cell mimic carbohydrate antigens in immunochemical experiments has been extended to other mono- and oligo-saccharides of known cross-reactivity with available antibodies.

† All intermediate sugar derivatives were fully characterized by ^1H and ^{13}C n.m.r. spectroscopy, m.p., optical rotation, and fast-atom bombardment mass spectrometry.

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