Synthesis of Oligosaccharides on Polymer Supports. Part II.[1] Synthesis of β-D-Gentiobiose Derivatives on Soluble Support Copolymers of Styrene and 6-*O*-(*p*-Vinylbenzoyl) or 6-*O*-(*p*-Vinylphenylsulphonyl) Derivatives of D-Glucopyranose

By **Roy D. Guthrie,*† Aubrey D. Jenkins,** and **George A. F. Roberts,** School of Molecular Sciences, University of Sussex, Brighton BN1 9QJ

Styrene-based copolymers incorporating 1,2,3,4-tetra-*O*-acetyl-6-*O*-(*p*-vinylbenzoyl)-β-D-glucopyranose and the 6-*O*-(*p*-vinylphenylsulphonyl) analogue have been prepared and used as soluble support polymers for the synthesis of β-D-gentiobiose derivatives.

OLIGOSACCHARIDES of strictly defined structure are materials of considerable interest as substrates for the study of enzyme mechanisms. The methods of preparation in current use (sequential synthesis in a homogeneous phase or fractionation of partially hydrolysed natural polysaccharides) have several disadvantages and it is considered that synthesis on a support polymer might constitute a better route.

The technique of synthesis on support polymers was pioneered in the polypeptide field [2] and has also been used in the synthesis of polynucleotides.[3,4] Soluble support polymers have also been used [5,6] and each type has certain advantages and disadvantages with respect to the other. We have examined the use of soluble polymers as these increase the likelihood of achieving a high conversion at each step and enable the reactions to be more easily followed. The polymers were prepared by copolymerizing styrene with a derivative of the first monosaccharide unit of the proposed oligosaccharide which incorporates a suitable unsaturated group.[1] The use of insoluble cross-linked support polymers for the synthesis of oligosaccharides has been recently investigated by Schuerch and Frechet,[7] and by Gagnaire and his group.[8]

As described in Part I,[1] the first monosaccharide unit is attached to the polymer through the C-6 hydroxy-group, leaving the C-1 group free for formation of a glycosidic link with a second, suitably substituted, monosaccharide by means of the orthoester route developed by Kochetkov and his co-workers.[9-12]

RESULTS AND DISCUSSION

The monosaccharide derivative used initially for the synthesis of a suitable copolymer was 1,2,3,4-tetra-*O*-acetyl-6-*O*-(*p*-vinylbenzoyl)-β-D-glucopyranose.[1] This was copolymerized with styrene in dioxan, using αα'-azobisisobutyronitrile (AIBN) as initiator, to give a polymer containing *ca.* 0·15 mole fraction of the monosaccharide monomer. The composition of the copolymer was calculated from its optical activity by comparison with that of the homopolymer of the monosaccharide monomer.

The copolymer (1) was treated with hydrogen bromide in glacial acetic acid to convert the monosaccharide residue into the α-D-glucopyranosyl bromide (2). This was converted into the orthoester (3) by reaction with methyl 2,3,4-tri-*O*-benzoyl-α-D-glucopyranoside in the presence of *sym*-collidine and tetra-n-butylammonium bromide.[13] That an orthoester had been formed was shown by the extreme sensitivity of (3) to hydrolysis. Rearrangement of (3) to the isomeric disaccharide derivative (4) was brought about by refluxing in 1,2-dichloroethane in the presence of lutidinium perchlorate, toluene-*p*-sulphonic acid monohydrate, and catalytic amounts of methyl 2,3,4-tri-*O*-benzoyl-α-D-glucopyranoside.[12] The disaccharide was cleaved from the polymer by methanolysis in dioxan and the crude dried product benzoylated. The benzoylated mixture was separated by preparative t.l.c., and the methyl hepta-*O*-benzoyl-α-D-gentiobioside (5) obtained was recrystallised from ethanol. The yield was 7·1% based on the starting polymer (1) and 23·0% based on the amount of polymer (4) available for the methanolysis step.

In view of the drastic conditions required for cleavage of the disaccharide from the support polymer, other methods of linking the first monosaccharide unit to the polymer were considered. It was decided to examine the use of a benzenesulphonate group as this appeared to have three main advantages: (a) the sulphonate ester link should be cleaved under relatively mild conditions

† *Present address:* School of Science, Griffith University, Toowong, Queensland, 4066, Australia.

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[12] A. F. Bochkov, V. I. Snyatkova, and N. K. Kochetkov, *Izvest. Akad. Nauk S.S.S.R., Ser khim.*, 1967, 2684.
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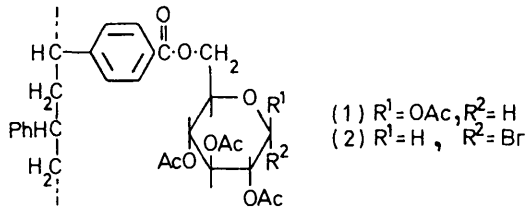
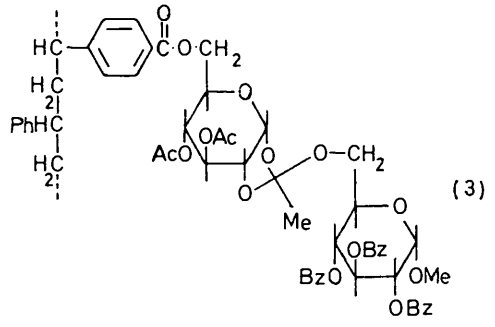
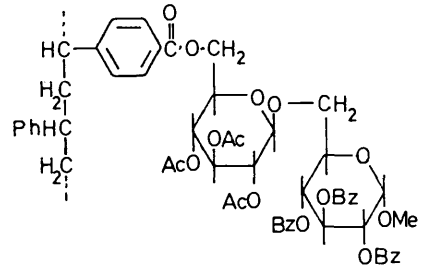
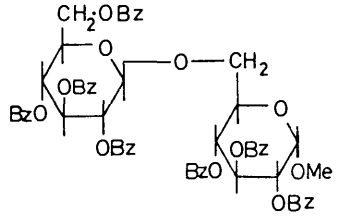
by reaction with potassium acetate; (b) vinylbenzenesulphonates should copolymerise readily with styrene; (c) the required *p*-vinylbenzenesulphonyl chloride is described in the literature.[14,15]



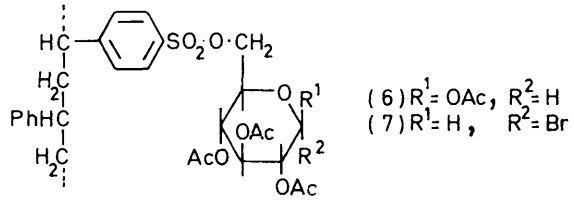


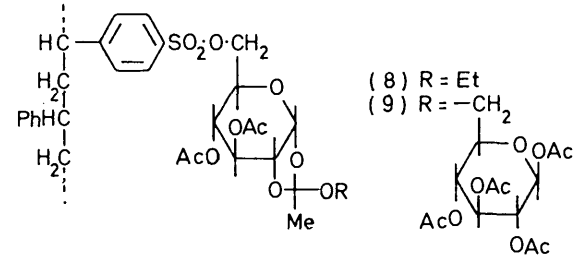


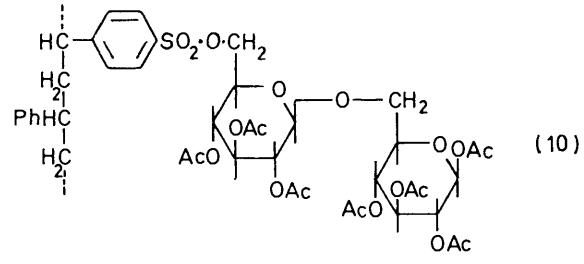


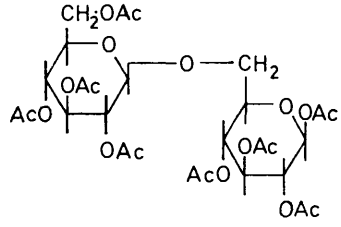
The requisite monomer, 1,2,3,4-tetra-*O*-acetyl-6-*O*-(*p*-vinylphenylsulphonyl)-β-D-glucopyranose was synthesised from 1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranose and *p*-vinylbenzenesulphonyl chloride, which was prepared from (2-bromoethyl)benzene, *via* *p*-(2-bromoethyl)-benzenesulphonyl chloride and potassium *p*-vinylbenzenesulphonate.[14,15] The formation of the sulphonate ester was carried out in 1,2-dichloroethane using triethylamine as an acid scavenger. The crystalline product had $[\alpha]_D^{20}$ +23·8° ($CHCl_3$); *cf.* +24° for 1,2,3,4-tetra-*O*-acetyl-6-*O*-tosyl-β-D-glucopyranose. It readily underwent homopolymerisation and copolymerisation with styrene in the presence of a free radical initiator. The homopolymer has $[\alpha]_D^{20}$ +25° ($CHCl_3$).

The reactivity ratios for copolymerisation of this monomer with styrene in dioxan at 60° were determined by the method of intersecting slopes.[15] The copolymer compositions were calculated from their optical rotations relative to the homopolymer (Table). The values obtained were $r_1 = 0{\cdot}35 \pm 0{\cdot}15$ and $r_2 = 1{\cdot}07 \pm 0{\cdot}02$, where the suffix 1 refers to the sugar-containing monomer and 2 to styrene. These values show that styrene is more reactive than the sugar-containing monomer which is readily understandable on the basis of steric hindrance between the chain radical and the bulky glucose derivative. However, they are at variance with the results









obtained earlier with three 6-*O*-(*p*-vinylbenzoyl)glucopyranose derivatives, for which the styrene reactivity ratio was much smaller than that of the glucose monomer in each case.[1]

The *p*-vinylbenzenesulphonate monomer and styrene

[14] N. Yoda and C. S. Marvel, *J. Polymer Sci., A.*, 1965, **3**, 2229,
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were copolymerised together giving a polymer having *ca.* 0·06 mole fraction of the glucose derivative (6). This was converted into the C-1 bromo-derivative (7) by treatment with hydrogen bromide in glacial acetic acid. By use of the methods of Kochetkov,[12] copolymer (7) was converted first into the ethyl orthoacetate (8) before transorthoesterification to give the orthoester (9) and this was followed by rearrangement to the isomeric disaccharide derivative (10). Cleavage from the support polymer was carried out by reaction with potassium acetate in refluxing *NN*-dimethylformamide to give β-D-gentiobiose octa-acetate (11), identical with a sample prepared by a standard procedure.[16] The yield was 42% based on the weight of the starting polymer (6) and 64·3% based on the amount of polymer (10) available for the final step.

These results show that the synthesis of oligosaccharides on soluble support polymers is a practical proposition. However, there is considerable loss of the support polymer during the filtration step after each reaction stage. This could be avoided by using insoluble cross-linked polymer beads, as in the original Merrifield technique.[2] This latter technique [2] has a number of drawbacks such as the difficulty of obtaining quantitative yields in the coupling reactions and a reduced choice as regards solvents, protective groups, and coupling method.[6] These drawbacks are more than compensated for in polypeptide synthesis as the technique permits automated syntheses to be carried out. Because of the more prolonged and complex reactions necessary for the formation of glycosidic linkages the development of an automated process is fraught with difficulty, and thus the advantages attained by the use of a soluble support polymer, in terms of accessibility and reaction control, therefore become correspondingly more important. The loss of soluble support polymer might be avoided by use of a technique recently proposed by Bayer *et al.*[6] in which purification of the polymer-supported product is carried out after each step by ultrafiltration (membrane filtration), so avoiding the necessity of frequent precipitation and filtration steps with accompanying losses.

EXPERIMENTAL

Drying of polymers and evaporation of solvents were carried out under reduced pressure. All rotations are quoted for 1% solutions in chloroform unless otherwise stated.

Copoly{styrene–[1,2,3,4-tetra-O-acetyl-6-O-(p-vinylbenzoyl)-β-D-glucopyranose]} (1).—Styrene (0·87 g) and 1,2,3,4-tetra-*O*-acetyl-6-*O*-(*p*-vinylbenzoyl)-β-D-glucopyranose (1·0 g) were dissolved in dioxan (8 ml), transferred to a polymerisation tube containing AIBN (0·17 g), and washed down with a portion of dioxan (2 ml). The tube was sealed under nitrogen, and heated at 60° for 168 h. The polymer was isolated by precipitation on addition of the mixture to methanol. After drying, it was redissolved in dioxan and reprecipitated by dropwise addition to well-stirred, chilled methanol. The collected polymer was dried to give a white amorphous powder (1·65 g), $[\alpha]_D^{21}$ +15·9°.

Copoly{styrene–[2,3,4-tri-O-acetyl-6-O-(p-vinylbenzoyl)-α-D-glucopyranosyl bromide]} (2).—The polymer (1) (1·5 g) in chloroform (15 ml) was cooled to 0° and hydrogen bromide (45% w/v) in glacial acetic acid (6 ml) was added. The solution was kept at 0° for 16 h, and the polymer was then precipitated by dropwise addition of the chloroform solution to well-stirred methanol cooled to 0°. After filtration, the precipitate was washed with methanol at 0°, then dried at room temperature. The product was a white amorphous powder (1·48 g), $[\alpha]_D^{20}$ +82·5°.

Copoly{styrene–[3,4-di-O-acetyl-6-O-(p-vinylbenzoyl)-α-D-glucopyranose methyl 2′,3′,4′-tri-O-benzoyl-α-D-glucopyranoside 1,2,6′-orthoacetate]} (3).—The polymer (2) (1·45 g) was dissolved in 1,2-dichloroethane (10 ml) containing *sym*-collidine (1·0 ml) and methyl 2,3,4-tri-*O*-benzoyl-α-D-glucopyranoside (1·0 g). Tetra-n-butylammonium bromide (0·1 g) was added, and the mixture was heated at 50° for 96 h. The polymer was precipitated by addition to ice-cold methanol, but was of such fine particle size that it passed through the sintered glass filter. The suspension was evaporated under reduced pressure, the residue was taken up in 1,2-dichloroethane (3·5 ml) and the polymer was reprecipitated by dropwise addition to ice-cold methanol. The product was filtered off and dried at room temperature, giving a white powder (1·05 g), $[\alpha]_D^{21}$ +57·6°. To the solution used for measurement of the optical rotation was added a drop of 1·0N-hydrochloric acid. The mixture was shaken and then left for 30 min at room temperature. After drying ($MgSO_4$) the optical rotation was +38·3°.

Copoly{styrene–[methyl O-2,3,4-tri-O-acetyl-6-O-p-vinylbenzoyl-β-D-glucopyranosyl-(1 ⟶ 6)-2,3,4-tri-O-benzoyl-α-D-glucopyranoside]} (4).—The polymer (3) (1·02 g) was dissolved in 1,2-dichloroethane (35 ml) and the solution boiled until *ca.* 7·5 ml had distilled over. Then lutidinium perchlorate (5 mg) was added and the mixture was refluxed for 1 h. After the addition of toluene-*p*-sulphonic acid monohydrate (5 mg) and methyl 2,3,4-tri-*O*-benzoyl-α-D-glucopyranoside (75 mg), the mixture was refluxed for a further 2 h, then evaporated. The residue was taken up in chloroform (5 ml) and added dropwise to well-stirred, ice-cold methanol. The precipitated polymer was isolated by filtration, washed with methanol, and dried; yield 0·78 g, $[\alpha]_D^{21}$ +46·2°.

Methyl Hepta-O-benzoyl-α-D-gentiobioside (5).—The polymer (4) (0·75 g) was dissolved in dioxan (15 ml) and aqueous N-sodium methoxide (5 ml) was added. More dioxan was added to redissolve some polymer that had precipitated out. The mixture was stirred at room temperature for 24 h, acetic acid was added to neutralise any excess of methoxide, and the solution was evaporated. Benzene (5 ml) was added to the residue and the mixture was evaporated under pressure; this process was repeated. The residue was dissolved in pyridine and cooled to 0°, benzoyl chloride was added, and the solution was left at room temperature for 24 h. The presence of methyl hepta-*O*-benzoyl-α-D-gentiobioside was shown by t.l.c. (silica). This component was separated by preparative layer chromatography, and the product was recrystallised from absolute ethanol to give compound (5), $[\alpha]_D^{21}$ +49·4°, m.p. and mixed m.p.[17] 187—188·5°; yield 113 mg.

p-*Vinylbenzenesulphonyl Chloride*.[14]—Potassium *p*-vinyl-

[16] D. D. Reynolds and W. L. Evans, *J. Amer. Chem. Soc.*, 1938, **60**, 2559.

[17] T. Alfrey, J. J. Bohrer, and H. Mark, 'Copolymerization,' Interscience, New York, 1952.

benzenesulphonate [18] (15·0 g) was placed in a flask wrapped in aluminium foil and cooled in ice–water. It was stirred while finely powdered phosphorus pentachloride (16·0 g) was added during 30 min. After a further 2 h stirring the mixture was warmed to room temperature and stirring was continued for a further 3 h. The mixture was left overnight at room temperature, and then poured onto vigorously agitated crushed ice (800 g). The mixture was extracted with 1,2-dichloroethane (200 ml). The organic layer was separated, washed twice with ice–water and once with sodium hydrogen carbonate solution (2·5%; 200 ml), dried ($MgSO_4$), filtered, and evaporated to yield a viscous oil (11·3 g). Although purification of *p*-vinylbenzenesulphonyl chloride by vacuum distillation is reported [16] two attempts at this resulted in polymerisation of the monomer. Purification was therefore carried out by two treatments with activated charcoal in 1,2-dichloroethane solution to give an almost colourless oil.

1,2,3,4-*Tetra*-O-*acetyl*-6-O-(p-*vinylphenylsulphonyl*)-β-D-*glucopyranose*.— 1,2,3,4-Tetra-*O*-acetyl-β-D-glucopyranose (3·5 g) was dissolved in 1,2-dichloroethane (20 ml) and triethylamine (1·45 ml) was added. The solution was cooled to 0°, and *p*-vinylbenzenesulphonyl chloride (2·1 g) in 1,2-dichloroethane (10 ml) was added dropwise with stirring. After 1 h at 0—5° the solution was warmed to room temperature and left overnight. Hydrochloric acid (0·2N; 50 ml) was then added, the mixture was shaken, and the organic layer separated. This was washed twice with water, once with aqueous sodium hydrogen carbonate (2·5%), then again with water, dried ($MgSO_4$), and evaporated after the addition of *o*-benzoquinone (0·1 g). The residue was recrystallised from ethanol to give the *product* (3·7 g, 76%), $[\alpha]_D^{20}$ +23·8° (Found: C, 51·2; H, 5·1. $C_{22}H_{26}O_{12}S$ requires C, 51·35; H, 5·1%). The compound polymerised on heating.

Poly-[1,2,3,4-*tetra*-O-*acetyl*-6-O-(p-*vinylphenylsulphonyl*)-β-D-*glucopyranose*].—The monomer (0·5 g) and AIBN (0·0017 g) were dissolved in dioxan (5 ml) in a polymerisation tube, which was sealed under nitrogen, and then placed in a water bath at 60° for 120 h. The mixture was then added dropwise to well-stirred water in order to precipitate the polymer. After drying, the product was dissolved in the minimum of chloroform and the polymer was reprecipitated by dropwise addition to ether–methanol (4 : 1), filtered off, and dried at room temperature to give a white amorphous powder (0·36 g), $[\alpha]_D^{20}$ +25°.

Copoly{*styrene*–[1,2,3,4-*tetra*-O-*acetyl*-6-O-(p-*vinylphenylsulphonyl*)-β-D-*glucopyranose*]} (6).—1,2,3,4-Tetra-*O*-acetyl-6-*O*-(*p*-vinylphenylsulphonyl)-β-D-glucopyranose (1·0 g) and AIBN (0·036 g) were dissolved in dioxan (5 ml) and styrene (2·24 ml) was added. The solution was transferred to a polymerisation tube and rinsed in with dioxan (1·5 ml). The tube was sealed under nitrogen and placed in a water-bath at 60° for 120 h. The copolymer was then precipitated by dropwise addition to well-stirred methanol cooled to 0°, filtered off, air-dried, then dissolved in chloroform and reprecipitated in the same way. The product was dried at room temperature to give a white amorphous powder (2·45 g), $[\alpha]_D^{20}$ +5·6°.

Copoly{*styrene*–[2,3,4-*tri*-O-*acetyl*-6-O-(p-*vinylphenylsulphonyl*)-α-D-*glucopyranosyl bromide*]} (7).—The copolymer (6) (2·3 g) was dissolved in chloroform (25 ml) and cooled to −15°. Hydrogen bromide (45% w/v) in glacial acetic acid (2·5 ml) was added and the mixture was left for 20 h at 0°. The polymer was then precipitated by dropwise addition of this solution to vigorously stirred ethanol (200 ml) at 0°. After isolation and drying it was dissolved in chloroform (10 ml) and reprecipitated into cold ethanol, filtered off, and dried; yield 1·98 g, $[\alpha]_D^{20}$ +25·5°.

Copoly{*styrene*–[3,4-*di*-O-*acetyl*-1,2-O-(1-*ethoxyethylidene*)-6-O-(p-*vinylphenylsulphonyl*)-α-D-*glucopyranose*]} (8).—The polymer (7) (1·8 g) was dissolved in chloroform (20 ml) containing *sym*-collidine (0·5 ml), ethanol (1·0 ml), and tetra-n-butylammonium bromide (0·2 g).[13] The solution was heated at 50° for 85 h, after which the polymer was precipitated into ethanol at 0°. After filtering and drying the polymer was redissolved in chloroform (5 ml), reprecipitated as before, and then dried; yield 1·55 g, $[\alpha]_D^{20}$ +5°. The chloroform solution for which the rotation was measured was treated with hydrochloric acid (1·0N; 1 drop), shaken, and left for 30 min. After drying ($MgSO_4$), the optical rotation was +21°.

Copoly{*styrene*–[O-1,2,3,4-*tetra*-O-*acetyl*-β-D-*glucopyranosyl*-(1 ⟶ 6)-2,3,4-*tri*-O-*acetyl*-6-O-(p-*vinylphenylsulphonyl*)-β-D-*glucopyranose*]} (10).—The polymer (8) (1·5 g) was dissolved in 1,2-dichloroethane (39 ml) containing 1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranose (0·35 g). 1,2-Dichloroethane was distilled off slowly with continuous dropwise addition of fresh 1,2-dichloroethane to maintain the solution volume constant. After removal of *ca.* 25 ml of solvent by distillation, toluene-*p*-sulphonic acid (3·5 mg) was added and the distillation was continued for a further 1 h. Pyridine perchlorate (3·5 mg) was then added and the solution was refluxed for 1 h.[17] The polymer was isolated by evaporation, dissolving the residue in chloroform (8 ml), and precipitating into cooled ethanol (200 ml). The precipitate was stirred in a fresh portion of ethanol, filtered off, and dried at room temperature; yield 1·62 g, $[\alpha]_D^{20}$ +2°.

β-D-*Gentiobiose Octa-acetate* (11).—The polymer (10) (1·6 g) was dissolved in *NN*-dimethylformamide (50 ml) and potassium acetate (0·3 g) was added. The mixture was refluxed, and monitored by t.l.c., with authentic β-D-gentiobiose octa-acetate for comparison. After 6 h the mixture was evaporated, the residue taken up in chloroform, and the solution filtered and added dropwise to stirred ethanol. The precipitated polymer was filtered off, air-dried, and extracted with hot ethanol. The two portions of ethanol were combined and evaporated. The residue was extracted with boiling ethanol (6 ml); the solution was filtered and left at 4° overnight. The crystals deposited were collected and dried, to give the product (11) (0·285 g), m.p. and mixed m.p.[16] 190·5—191·5°, $[\alpha]_D^{20}$ −2·8 °(*c* 21·3).

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[18] N. I. Gritsai and O. A. Prib, *J. Org. Chem.* (*U.S.S.R.*), 1967 **3**, 1554.