Carbohydrate Research, 39 (1975) 151–155

Note

Solid-phase synthesis of oligosaccharides.
V. Preparation of an inorganic support

RONALD EBY AND CONRAD SCHUERCH

Department of Chemistry, College of Environmental Science and Forestry, State University of New York, Syracuse, New York 13210 (U. S. A.)

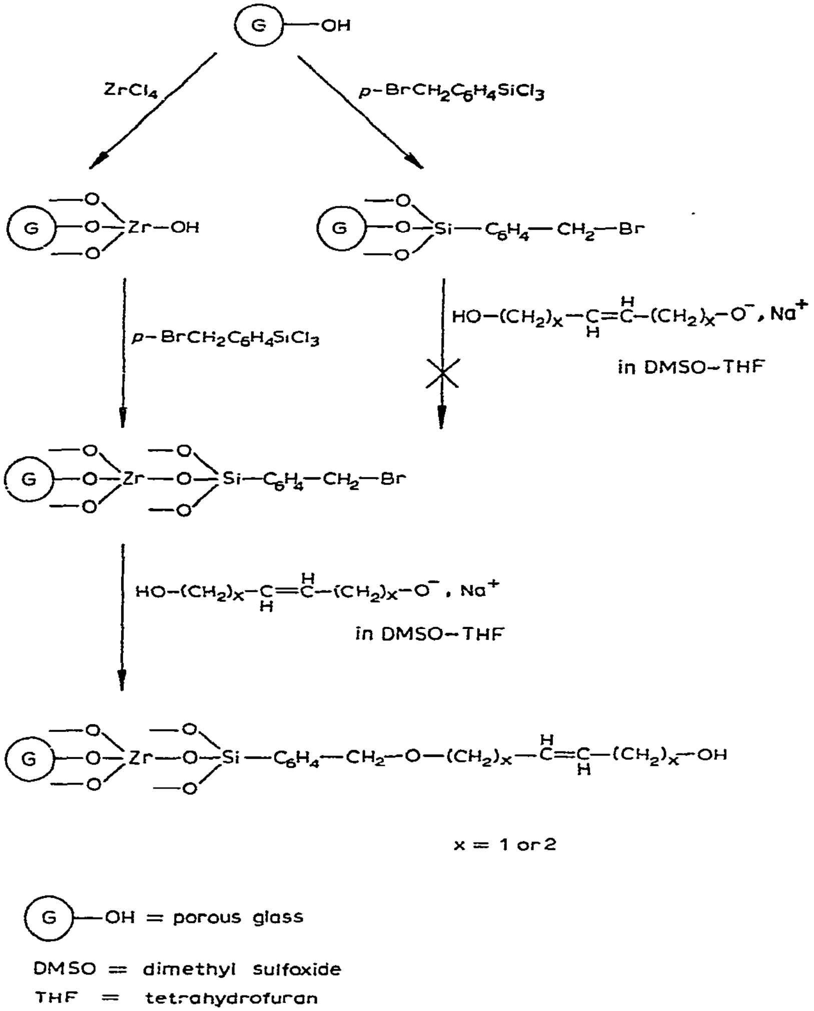
(Received July 15th, 1974; accepted in revised form, August 7th, 1974)

The development of the solid-phase synthesis of polypeptides by Merrifield[1] has led to the use of organic supports for the synthesis of polynucleotides[2–4] and oligosaccharides[5,6]. The main advantage of the solid-phase method is that purification of intermediates is effected merely by filtering and washing the support. The organic polymer supports have a major drawback. Different solvents swell the polymers to various degrees and, thus, have a large and variable effect on the accessibility of the polymers, the rates of reaction, the yield of coupling, and the removal of impurities[7]. Also, since the functional groups are distributed throughout the polymer matrix, the reactions may be diffusion-controlled. Some of these problems can be eliminated by the use of a soluble polymer[6]. However, isolation of the polymer by precipitation usually results in losses.

Most of the disadvantages can in principle be overcome by the use of an inorganic support, such as silica gel[8] or porous glass[9,10], which are rigid solids, usually with large pores of fixed diameter. Thus, the reactions can be performed in a variety of solvents. This report discusses the preparation of a coated, porous glass, and its attempted use in the synthesis of oligosaccharides.

As the functional moiety attached to the glass surface, an unsaturated alcohol group provided a hydroxyl group for the coupling of the saccharide unit and an olefinic bond that could be cleaved by ozonolysis to remove the synthesized oligosaccharide from the glass support (Scheme 1). A similar group was linked to a polystyrene support for the synthesis of oligosaccharides[5,11]. The porous glass was treated with (*p*-bromomethylphenyl)trichlorosilane in toluene. The capacity of the glass, determined by alkaline hydrolysis and titration of the soluble halogen, ranged from 0.07 to 0.90 mmol of bromine derivative per g of glass.

The reaction of 2-butene-1,4-diol or 3-hexene-1,6-diol with one equivalent of sodium in 1:1 (v/v) dimethyl sulfoxide–tetrahydrofuran gave the monosodium salt. Although the salt reacted rapidly with benzyl bromide to give the 4- or 6-benzyloxy derivatives of the diols in model reactions, reaction on the benzyl bromide-bound glass did not proceed as expected. All the bromine was eliminated, but the glass



Scheme 1. Reaction scheme for the preparation of porous glass-bound functional groups.

matrix was partially dissolved and left a fine powder with no olefinic groups, as a result of the hydrolysis of the silicon–oxygen–silicon bonds.

The sensitivity of the glass to base was reduced by treatment of the glass with zirconium tetrachloride at 350°, and then by the hydrolysis of the residual zirconium chloride bonds with water[12]. Thus the silicon–oxygen–silicon bonds were replaced with the more base-stable silicon–oxygen–zirconium bonds. Electron micrographs showed that the porous structure was still present, and energy-dispersive X-ray analysis that the zirconium atoms were evenly distributed throughout the glass matrix.

The coated glass was treated with (*p*-bromomethylphenyl)trichlorosilane to give the benzyl bromide-bound glass having 0.04–0.08 mmol of bromide derivative per g.

The volumetric capacity was no less than that of the non-zirconium coated glass, because the coating increased the density of the glass. The coated glass was treated with hexamethyldisilazane in dry hexane to block completely any unreacted hydroxyl group bound to silicon or zirconium.

A solution of the monosodium salts of either of the diols in 1:1 (v/v) dimethyl sulfoxide–tetrahydrofuran was passed through a column of the treated glass in tetrahydrofuran, displacing the solvent. The use of a column prevented the breaking of the glass particles and also resulted in a more complete reaction. Analysis of the isolated glass showed that the reaction reached 98% of completion after 30 min, and that the sum of the unreacted bromide derivative and of the olefin present equalled the starting capacity of the glass. Therefore, no loss of functionality had occurred.

The coupling of a saccharide unit to the glass was attempted with several D-glucosyl derivatives of 2,3,4-tri-*O*-benzyl-6-*O*-(*N*-phenylcarbamoyl)-D-glucopyranose, namely the D-glucosyl quaternary ammonium salt[13,14], the D-glucosyl bromide[15], and the D-glucosyl 1-*p*-toluenesulfonate[16]. The yield of coupling was determined by comparing the weight of the glass before and after the reactions. The best coupling (about 20%) was that of the D-glucosyl 1-*p*-toluenesulfonate derivative, the D-glucosyl bromide gave between 0 and 10% coupling, and the ammonium salt showed no coupling. Analysis of the glass for olefin content showed that the functional group was still present and analysis of the D-glucosyl derivatives showed that they were still active after the reaction.

The causes for the incomplete coupling are not known. Impeded diffusion was not the cause, since several glasses of various pore diameters, including a 2500-Å pore diameter, were used. Coating of the glass with hexamethyldisilazane should have removed any surface hydroxyl groups that might have interferred with the coupling reaction. Finally, the coupling of diols of different chain-lengths and of 3-hexene-1,6-diol demonstrated that the distance of the reactive hydroxyl groups from the glass surface and the allylic alcohol function, respectively, were not the cause. Unless the reason for the low coupling-yields can be identified and corrected, it seems that porous glass of this type cannot be used as a solid support in the solid-phase synthesis of oligosaccharides.

EXPERIMENTAL

Materials. — Porous glasses (Bio-glas from Bio-rad Lab, 96% silica) with pore sizes of 500, 1000, and 2500 Å diameter was dried at 100° *in vacuo* before use. (*p*-Bromomethylphenyl)trichlorosilane was prepared by the method of Parr and Grohmann[9]. 2-Butene-1,4-diol was distilled and dried over molecular sieves 4A. 3-Hexene-1,6-diol was prepared by the reduction of diethyl *β*-hexenedioate with lithium aluminum hydride by the method of Lukes[17].

Preparation of zirconium oxide-coated glass. — The dried, porous glass (14.7 g) was mixed with a solution of zirconium tetrachloride (4.0 g) in dry 1,2-dimethoxyethane (30 ml). The solvent was slowly distilled off on a rotary evaporator at low pressure. The granular mixture was dried in a vacuum oven for 3 h at 150°, and then heated to 350°. The mixture changed from light yellow to black and then back to white in the course of 16 h. The glass was cooled, washed with water, dilute sulfuric acid, water, and acetone, and dried at 100° in a vacuum oven. The weight of the dried glass was 17.1 g, corresponding to an increase of ~16% in weight.

Preparation of the benzyl bromide-bound glass. — Zirconium-coated glass (17.1 g) was mixed with *p*-(bromomethylphenyl)trichlorosilane (5.0 ml) in toluene (50 ml). The mixture was heated at reflux for 3 h and any entrapped air removed by vacuum. The glass was filtered off, washed with benzene, benzene–ethanol, ethanol, and ethanol–water (1:1), heated to 100° for 24 h *in vacuo*, and then washed with benzene, ethanol, and acetone, and dried at 60° *in vacuo*. The capacity of the glass was determined by weighing a sample (100–300 mg) into a flask and adding a 5M sodium hydroxide solution (5–10 ml). After being heated for 1 h, the mixture was cooled and acidified with M nitric acid. A 10mM silver nitrate solution (10.0 ml) was added with a small amount of ferric nitrate. The excess silver nitrate was titrated with 10mM potassium thiosulfate. The capacity of the glass varied from 0.04 to 0.08 mmol of bromine derivative per g of glass. The glass was heated at reflux with hexamethyldisilazane (5.0 ml) in dry hexane (30 ml) for 2 h, filtered off, washed with hexane, and dried at 60° *in vacuo*. It showed no loss of bromine after this treatment.

Binding of 4-benzyloxy-3-butene-1-ol and 6-benzyloxy-3-hexene-1-ol to porous glass. — The benzyl bromide-bound glass (8.0 g having a capacity of 0.04 mmol of bromine/g) was mixed with dry tetrahydrofuran (20 ml), the entrapped air was removed by suction, and the suspension was transferred onto a chromatographic column. The solvent was allowed to drain until it reached the top of the porous glass. A solution of 2-butene-1,4-diol or 3-hexene-1,6-diol (2 ml) 1:1 (v/v) in tetrahydrofuran–dimethyl sulfoxide (10 ml) was treated with sodium (0.5 g), and then added to the column. The solution was allowed to drain through until the effluent was basic, and then the flow was stopped. After 30 min, the column was washed with 1:1 tetrahydrofuran–dimethyl sulfoxide, tetrahydrofuran, acetone, methanol, 1:1 methanol–water, and acetone. The glass was dried at 60° *in vacuo*.

The glass was analyzed for unreacted bromide derivative as just described. Olefinic bond content was determined by placing the glass (200–300 mg) in a flask, and adding 68mM potassium bromate solution (5.0 ml) and potassium bromide (100 mg). The mixture was slightly acidified with dilute nitric acid, the entrapped air was removed by suction, and the flask was stoppered and kept for 30–60 min. The excess of bromine was replaced by iodine by the addition of a potassium iodide solution, and the iodine was titrated to a starch end-point with 10mM sodium thiosulfate solution. The results showed that the reaction was completed to 98% (0.039 mmol/g) and that the sum of the unreacted bromide derivative and of the olefin present was equal to the total starting capacity of the glass (0.04 mmol/g).

Attempted coupling of a saccharide unit. — A weighed sample of the glass (5.3858 g, capacity 0.039 mmol/g) was dried under high vacuum for 3 h. A solution of 2,3,4-tri-*O*-benzyl-6-*O*-(*N*-phenylcarbamoyl)-D-glucopyranosyl or pyranose derivative [bromide[15], 1-*p*-toluenesulfonate[16], or quaternary ammonium salt[14] (0.02 mol, 10-fold excess)] in an appropriate solvent (8.0 ml) [diethyl ether for the bromide and 1-*p*-toluenesulfonate derivatives and 5:2 (v/v) triethylamine–dichloromethane for the glucosyl quaternary ammonium salt] was added. The entrapped nitrogen was removed by vacuum and the mixture was kept for 2 days. The glass was filtered off, washed with acetone and ether, dried at 60° *in vacuo*, and weighed. The glass showed little or no gain of weight. The maximum yield of coupling was 20%. The treated glass was analyzed for the presence of olefin and was found to have the starting capacity.

ACKNOWLEDGMENT

This work was completed with financial support from the Division of General Medical Sciences, National Institutes of Health, U.S. Public Health Service (grant GM 06168). The authors thank Dr. W. Côté and Mr. A. Day for preparing the scanning electron micrographs.

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