Carbohydrate Research, 50 (1976) 227–238

OLIGOSACCHARIDE SYNTHESIS BY THE THIOGLYCOSIDE SCHEME ON SOLUBLE AND INSOLUBLE POLYSTYRENE SUPPORTS

SHUET-HING LEE CHIU AND LAURENS ANDERSON*

Department of Biochemistry, College of Agricultural and Life Sciences, University of Wisconsin-Madison, Madison, WI 53706 (U. S. A.)

(Received December 2nd, 1975; accepted for publication in revised form, March 1st, 1976)

ABSTRACT

The solid-phase synthesis of a disaccharide via the thioglycoside approach is described. Treatment of (chloromethyl)polystyrene, either cross-linked (**1-X**) or linear (**1-L**), with the 1-thio sugar **4** gave resins (**6-X**, **6-L**) carrying thioglycosidically bound 2,3,4-tri-*O*-benzyl-D-glucopyranosyl groups. Alternatively, the conversion of **1-X** into (mercaptomethyl)polystyrene (**3-X**) and reaction of this with the glucosyl chloride **5** gave **6-X**. By repeated treatments with 6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl bromide, a second D-glucose residue was coupled to 74–92% of the first sugar residues. The action of methyl iodide–benzyl alcohol in refluxing benzene quantitatively cleaved the sugars from the polystyrene support, forming benzyl glycosides (**13**, **7**) and, from the unreacted monosaccharide residues, the 1,6-anhydro sugar **9**. The coupling product was 92–95% α-linked. Deprotection and purification gave isomaltose.

INTRODUCTION

Syntheses of oligosaccharides on macromolecular supports after the fashion of the Merrifield peptide synthesis[1] have been described by several groups of investigators[2–6]. The approaches used by the various groups differ primarily in the way the first sugar group is attached to the support, and in the role of the support-bound sugar in coupling to the next sugar added. In most schemes, a hydroxyl group of the bound sugar serves as the glycosyl acceptor, but in one instance[5] the anomeric center is converted into a form activated for coupling. The final products of these syntheses, except that of Fréchet and Schuerch[3], have been oligosaccharides with free reducing groups, or derivatives that would give such oligosaccharides when deprotected. The synthesis of Fréchet and Schuerch yields the hydroxyethyl glycosides of the product oligosaccharides.

In a previous paper from this laboratory[7] the attachment of the first sugar to the support by a thioglycoside linkage was suggested, and the probable feasibility of

*Please address correspondence to this author.

this approach was demonstrated with model compounds. We now report the successful application of thioglycosidic linking to oligosaccharide synthesis on polystyrene. In our experiments, we have used both cross-linked, insoluble polystyrene and linear, soluble polystyrene as support materials. Cross-linked polystyrene has been the more-extensively used in syntheses-on-supports, and only with it is one working continuously with reactants in the solid phase. With linear polymers, the synthetic reactions are done in homogeneous solution. Solid phases carrying the successive products are obtained by precipitations following each step. Reports of syntheses of peptides[8], oligonucleotides[9,10], and a disaccharide[5] on linear polystyrene made a direct comparison of the types of support seem desirable.

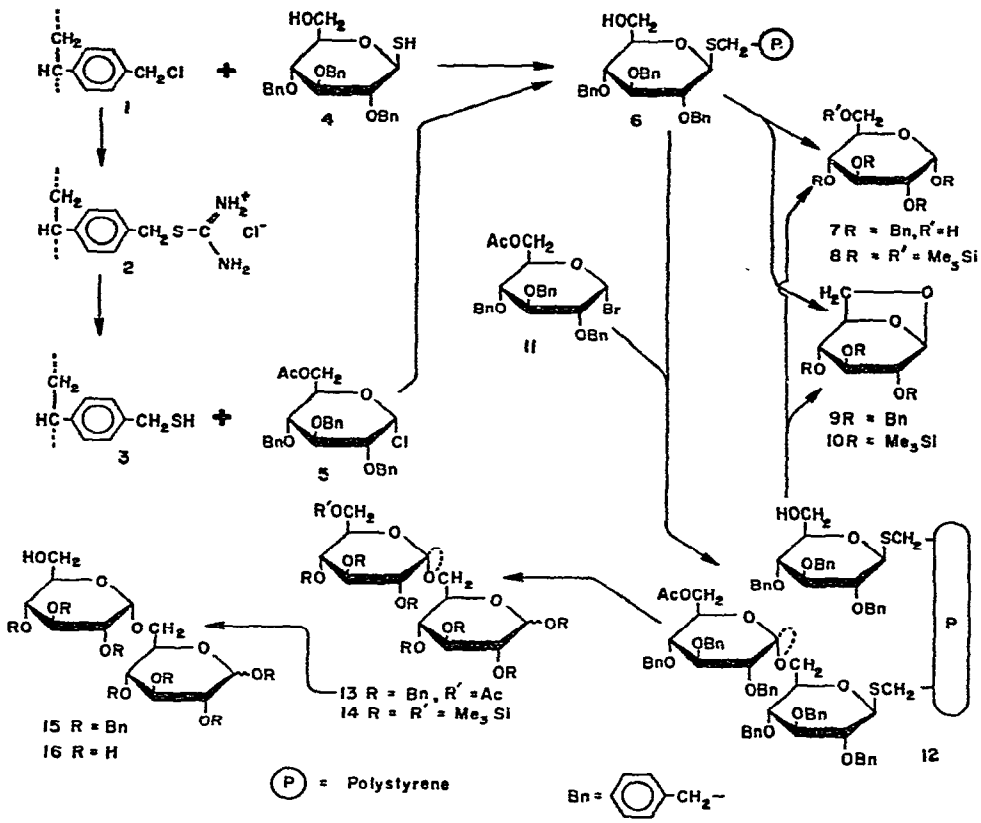
RESULTS

To generate attachment sites for the first sugar, polystyrene–1% divinylbenzene beads, or soluble, linear polystyrene, were chloromethylated in the usual way with chloromethyl methyl ether in the presence of stannic chloride[11]. Because of the large molecular weight (432) of the tri-*O*-benzylhexose monomeric units in the proposed synthesis, it was considered that the resin could accommodate fewer chains per gram than is the case in peptide synthesis. Hence, the degree of chloromethylation was kept to 1.0 mmol per g or less.

The reaction whereby the first sugar group is attached to the support may be termed the "loading" step of a solid-phase synthesis. To accomplish this, (chloromethyl)polystyrene (**1**), either cross-linked or linear, was treated with the sodium salt of the thio sugar **4** in benzene for 5 days at room temperature, to give thioglucosylated polymers (**6**). Alternatively, cross-linked **1** (**1-X**)* was first treated with an excess of thiourea in refluxing 1,4-dioxane–ethanol to give the intermediate isothiouronium salt **2-X**. This was decomposed to the thiol **3-X** by treatment with benzylamine. An overall 88% conversion of $-CH_2Cl$ to $-CH_2SH$ was achieved. Treatment of the polymer thiol **3-X** with a benzene solution of 6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl chloride (**5**), and added propanolic potassium hydroxide, again give **6** (**6-X**). (The basic conditions caused cleavage of the 6-*O*-acetyl group from the sugar.) Replacement of 71% of the resin-Cl was achieved in **6-X** prepared by the direct method, and 82% (72% overall conversion of $-CH_2Cl$ into thioglucoside) in that prepared via the polymer thiol. For product **6-L**, p.m.r. spectroscopy indicated 88% replacement of resin-Cl by thio sugar. As the thioglucose (**4**) used in the first, "direct" method is made in two steps[12] from the glucosyl chloride **5**, the number of steps in the two routes to **6** is the same.

To explore the second (polymer thiol) route with the linear polymer, the chloride of linear **1** (**1-L**) was displaced with thiolacetate ion. This reaction went smoothly, but saponification of the thiolester functions gave insoluble an product, presumably

*Formula numbers bearing the suffixes *X* and *L* designate cross-linked and linear polystyrene derivatives, respectively.



polymer cross-linked by –SS– bridges. Conceivably, the –SS– linkages could be reduced, and the product glucosylated by reaction with **5**, but in view of the availability of the "direct" reaction (**1**+**4**) this approach was not attempted.

Cleavage of the product oligosaccharide from the support is normally one of the last steps in a solid-phase synthesis. However, we elected to develop the procedure on resins loaded with single tri-*O*-benzylglucose groups, because these were more readily available. With mercuric chloride–barium carbonate, used successfully in our original solution-chemical work[7], cleavage was incomplete, even after very long reaction-times. Reductive cleavage with sodium–liquid ammonia, also previously envisaged[13], was not tried because some cleavage of glycosidic bonds was observed on application of the procedure to a model compound. Satisfactory results were obtained by refluxing the glucosylated resins in benzene containing an excess of methyl iodide and benzyl alcohol. This is a variation of a method recently described[14,15] for the regeneration of carbonyl compounds from their thioacetals. Both resins **6**-**X** and **6**-**L** gave two products, which could be separated by column chromatography. The major product was shown to be 1,6-anhydro-2,3,4-tri-*O*-benzyl-β-D-glucopyranose (**9**), and the other product was the expected benzyl 2,3,4-

tri-*O*-benzyl-D-glucopyranoside (**7**, predominantly the α anomer). Further investigation[12] showed that the anhydro product **9** is formed only from thioglycosidically bound monosaccharide groups, whence it appeared that the procedure could safely be used to remove synthesized oligosaccharides from polystyrene supports. The initial products in this case are *O*-benzylated, oligosaccharide benzyl glycosides. Removal of the benzyl groups by hydrogenolysis gives, as final products, oligosaccharides having their reducing groups free. However, it should be possible, by using a different alcohol in the cleavage reaction, to generate intermediates having a stable anomeric substituent.

It could be shown that the cleavage by methyl iodide was quantitative after 4–5 days of reaction. To accomplish this, samples of the crude cleavage-product were debenzylated, trimethylsilylated, and analyzed by g.l.c. From the measured proportions of Me_3Si-derivatives **8** (and its β-anomer) and **10**, and the weight of the material, the amount of monosaccharide cleaved from the resin could be calculated.

To couple a second glucosyl group by an α-D-(1→6) linkage to the glucose residue attached to the support, the "alcoholysis" procedure of Fréchet and Schuerch[3] was employed. These authors treated loaded resin with a 4- to 5-fold excess of 6-*O*-(substituted benzoyl)-2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl bromide in benzene solution, in the presence of a hindered pyridine base to bind protons, but with no other catalyst. With reaction times of 5 days at room temperature, coupling yields of over 90% (that is, glucosylation of >90% of the resin-bound, acceptor-sugar residues) were obtained.

In the absence of evidence that an aromatic 6-*O*-acyl group is needed, we used the more readily available 6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl bromide **11** as the activated sugar in our coupling experiments. We used essentially the conditions of Fréchet and Schuerch[3], except for elevation of the temperature, a modification known to be feasible from their observations, and from our own with a model system[12]. In spite of this, we found a low degree of coupling after 3.5 days of reaction at 60–65° with **6-X** as acceptor, and less than 50% coupling with **6-L**. Acceptable degrees of coupling were achieved by repeating the procedure two or three times with fresh solvent and reagents. This repetition was done to avoid interference from accumulated side-products, even though t.l.c. showed that the glucosyl bromide was still present at the end of each reaction period. The results of an experiment with **6-X** are given in Table I, and those of an experiment with **6-L** in Table II.

A number of methods for determining coupling yield were tested. These were: (**1**) conventional gravimetry, (**2**) measurement of the weight increase following dinitrobenzoylation of the free hydroxyl groups of the unreacted monosaccharide residues, (**3**) measurement of the acetyl content by the hydroxamate method, and (**4**) analysis of the products cleaved from a sample of the resin. With the cross-linked resin, methods **1**, **2**, and **4** gave good agreement, but the hydroxamate analysis (**3**) gave high results. Methods **1** and **2** are not applicable to the linear polymer, but, in this instance, methods **3** and **4** agreed well. On balance, we concluded that analysis of the cleavage products is the most accurate method. The products from a partially

TABLE I

COUPLING OF THE SECOND SUGAR TO LOADED, CROSS-LINKED POLYSTYRENE

Stage	*Weight of resin (g)*	*Volume of reaction mixture (ml)*	*Glucosyl bromide (mmol)*	*2,6-Lutidine (mmol)*	*Cumulative degree of coupling*[a] (%)
1st	1.008[b]	10	1.44	4.32	26
2nd	0.816	10	1.70	2.59	54
3rd	0.629	5	1.32	1.73	74

[a]By gravimetry. [b]Resin 6'X carrying 0.55 mmol of acceptor groups (monosaccharide) per g.

TABLE II

COUPLING OF THE SECOND SUGAR TO LOADED, LINEAR POLYSTYRENE

Stage	*Weight of resin (g)*	*Volume of reaction mixture (ml)*	*Glucosyl bromide (mmol)*	*2,6-Lutidine (mmol)*	*Cumulative degree of coupling* (%)
1st	0.833[a]	15	2.39	0.86	40[b]
2nd	0.718	12	1.13	0.86	54[c]
3rd	0.500	9	1.92	1.73	70[c]
4th	0.195	5	0.55	1.73	92[c]

[a]Resin 6'L carrying 1.02 mmol of acceptor groups (monosaccharide) per g. [b]By column chromatography. [c]By g.l.c.

coupled resin (**12**), consisting of **7** and **9** from the unreacted monosaccharide derivatives and a mixture of disaccharide benzyl glycosides (**13**), were deblocked and trimethylsilylated. The trimethylsilyl derivatives were separated, by temperature programming, on the g.l.c. column used for the monomer derivatives **8** and **10**. The octakis(trimethylsilyl) disaccharides (**14**) came out as a single peak. The mole percentages of monomer and dimer were calculated from the peak areas, after correction by the appropriate response-factors.

Determination of the stereoselectivity of the coupling reaction was also accomplished by g.l.c. analysis. For this purpose, the mixtures to be analyzed were debenzylated and reduced to alditols with sodium borohydride. The Me_3Si-derivatives of the α- and β-linked disaccharide alditols separated cleanly on the column*. Measurement of the peak areas showed that 92–95% of the disaccharide formed was α-linked (isomaltose). These results confirm and extend those of Fréchet and Schuerch[3], whose mixtures of hydroxyethyl glycosides, on the basis of their optical rotations, contained a high but undetermined proportion of α-linkages.

Confirmatory characterization of the coupling product was accomplished by cleaving the sugars from a batch of the soluble resin (**12-L**) that was coupled to a

*Petitou and Sinaÿ (ref. 17) have used this method to test the purity of an isolated, synthetic disaccharide. They observed a single peak.

degree of 68%. The disaccharide fraction obtained by chromatography of the crude cleavage-product was deacetylated and further fractionated. The major component, on debenzylation, yielded pure isomaltose **(16)**.

DISCUSSION

In the application of the thioglycoside scheme to the synthesis of a disaccharide on polystyrene supports, some attractive features are evident. Functionalization of the resins is readily accomplished in one step by a standard method, and the loading and final cleavage steps of the synthesis proceed with excellent yields. Rather long reaction-times were used for these steps, but these could no doubt be shortened by judicious changes in conditions. The method appears to have potential for synthesizing oligosaccharide glycosides as well as oligosaccharides having free reducing end-groups.

The aspect of the synthesis requiring the most further exploration is the coupling step. The "alcoholysis" coupling reaction used in the present work required more time and activated sugar than is desirable, and stereoselectivity, while good, was less than ideal. For the incorporation of α-linked D-glucose residues into oligosaccharides, the solution to these problems may be the use of the recently described, improved glucosylating agent 2,3,4-tri-*O*-benzyl-6-*O*-(*N*-phenylcarbamoyl)-D-glucopyranose 1-*p*-toluenesulfonate[18,19].

Contrary to what might have been expected, the reactions of soluble polystyrene derivatives were not significantly faster at any stage of the synthesis than those of cross-linked polystyrene derivatives. As the precipitation and washing procedures required to recover the soluble resin after each step are more troublesome than the simple washing used with such materials as cross-linked resin or porous glass, these latter appear more promising as supports for oligosaccharide synthesis. A forthcoming paper will describe our results with porous glass as a support.

EXPERIMENTAL

General. — Instrumental and chromatographic procedures have been described in the earlier papers in this series. Gas–liquid chromatography was performed with a Varian–Aerograph Hy-Fi instrument, model 600-D, equipped with a linear temperature-programmer and a flame-ionization detector.

Collection and washing of the cross-linked resin, at the conclusion of a reaction, was done on a sintered-glass funnel. After removal of the reaction liquor, generous portions of washing solvents, as listed for each procedure, were added sequentially. Each solvent was allowed to remain in contact for 1–2 min, and was then drawn off by gentle suction. The washed resin was dried, first in air and then overnight in a vacuum oven, at a temperature of 45° or higher.

Mixtures containing soluble resin were usually evaporated under diminished pressure. The syrupy residue was dissolved in 6–10 volumes of benzene or chloroform and the solution was added dropwise, with stirring, to 100 volumes of methanol. The

precipitated resin was collected on a sintered filter, washed with methanol, and dried in the same way as the cross-linked resin. Samples for analysis were subjected to a second precipitation cycle before oven-drying.

(Chloromethyl)polystyrene. — A. Cross-linked resin (**1-X**). The starting material was Bio-Beads S-X1 (polystyrene–1% divinylbenzene), 200–400 mesh, from Bio-Rad Laboratories. The (chloromethyl)ation procedure given by Stewart and Young[11] was followed closely, but it was necessary to determine by experiment the proportions of stannic chloride and chloromethyl methyl ether required to give degrees of (chloromethyl)ation of 1 mmol/g or less. CAUTION: *Stringent precautions must be taken to avoid inhaling the vapor of chloromethyl methyl ether. It is carcinogenic.*

B. Linear resin (**1-L**). A portion (21.5 g) of the polystyrene (sample No. S104, $\overline{M}_w$ 164,000, $\overline{M}_w/\overline{M}_n$ 1.17, from Dr. J. F. Rudd of the Dow Chemical Co.) was dissolved in 300 ml of chloroform (dried over calcium chloride and distilled). The solution, in a two-necked flask equipped with a thermometer and dropping funnel, was stirred and cooled to 0°. Chloromethyl methyl ether (50 ml) containing 3.0 ml of stannic chloride was slowly added (6 min), and stirring was continued for 10 min at 0–1°. The solution was then diluted with 250 ml of chloroform and poured into 400 ml of ice–water. The resulting emulsion was extracted with 400 ml of chloroform, the chloroform layer was washed with sodium carbonate (3 × 200 ml) and water (5 × 200 ml), and dried with sodium sulfate. Concentration of the filtrate gave a thick paste, which was taken up in 120 ml of benzene and precipitated and dried as described in the General Methods section. Analysis for chloromethyl groups gave (mmol/g): Volhard[11], 1.01; combustion (3.62% Cl), 1.02; integration of p.m.r. signals at τ 2.63–3.90 (Ph-*H*), 5.34–5.67 (CH_2Cl), and 7.67–9.07 (C*H*, CH_2), 1.00.

Displacement by thiourea. — Chloromethylated, cross-linked resin **1-X** (19.879 g, 0.39 mmol $-CH_2Cl$ per g) was allowed to swell in 45 ml of 1,4-dioxane for 0.5 h. A solution of thiourea (0.683 g, 1.16 molar equivalents) in 220 ml of 95% ethanol was added, and the suspension was boiled for 26 h under reflux. The resin isothiouronium chloride **2-X** was collected and washed with ethanol, 1:1 ethanol–1,4-dioxane, 1,4-dioxane, 2:1 1,4-dioxane–water, 1:1 1,4-dioxane–water, water, 1:1 methanol–water, 2:1 methanol–water, and methanol. The gain in weight of the dried (16 h, 45°) resin was 0.541 g. This gain corresponds to a displacement of 92% of the Cl of the starting **1-X**, and a degree of substitution of 0.35 mmol of isothiouronium chloride groups per g*.

(Mercaptomethyl)polystyrene. — A. Cross-linked resin (**3-X**). The isothiouronium chloride **2-X** (19.515 g) was allowed to swell for 0.5 h in 120 ml of 2-methoxyethanol in a two-necked flask equipped with a condenser and gas inlet-tube. Benzylamine (2.4 g, 3.3 molar equivalents) was added and the suspension was refluxed for 4 h with slow bubbling of nitrogen. After collection (see foregoing) the wash sequence was: 2-methoxyethanol, 1:1 2-methoxymethanol–1,4-dioxane, 1:1 1,4-dioxane–methanol, methanol, 3:1 methanol–water, 1:1 methanol–water, water, methanol, 2:1 methanol–

*This value is less than 92% of 0.39 because of the increase in the weight of the resin.

chloroform, 1:1 methanol–chloroform, and chloroform. Drying was for 12 h at 50°. The product weighed 19.061 g.

A portion (1.3005 g) of the resin and 3,5-dinitrobenzoyl chloride (0.332 g) were refluxed for 10 h in dry pyridine. The reddish-brown product was collected, washed (pyridine, pyridine–benzene, benzene, benzene–acetone, acetone–1,4-dioxane, 1,4-dioxane, 1,4-dioxane–methanol, methanol, chloroform), and dried. The weight increase was 0.087 g, corresponding to 0.345 mmol of –SH per g of resin, or 96% conversion of the isothiouronium chloride groups. This composition would require a sulfur content of 1.15%. Found: S, 1.22.

B. Attempted preparation of mercaptomethyl linear polystyrene. Chloromethyl resin **1-L** (3.2 g, 3.23 mmol $-CH_2Cl$) in 155 ml of 29:21 chloroform–1,4-dioxane was treated with potassium thiolacetate (0.656 g, 5.74 mmol) for 21 h at room temperature. The solution was washed twice with water, and the organic layer was dried (sodium sulfate) and evaporated to a thick syrup. Precipitation and collection as described for **1-L** gave 2.2 g of acetylthiomethyl resin; p.m.r. ($CDCl_3$) τ 6.00 ($-CH_2S-$) and 7.70 ($COCH_3$), no signal for $-CH_2Cl$ at ~5.50. Calc. for complete replacement of Cl (0.97 mmol –SAc per g), S, 3.11%. Found: S, 3.61.

The acetylthiomethyl resin was treated with sodium methoxide in tetrahydrofuran–methanol, under nitrogen, for 72 h. Sodium ions were removed with a cation exchanger, and the resin recovered in the usual way. The product showed no C=O absorption in the infrared, but could not be redissolved in benzene or chloroform.

The loading step. — A. Cross linked resin, Ⓟ–CH_2Cl+thio sugar. (Chloromethyl)polystyrene (**1-X**, 2.000 g, 2.04 mmol $-CH_2Cl$) and dry benzene (13 ml) were added to a solution of 0.990 g (2.12 mmol) of 2,3,4-tri-*O*-benzyl-1-thio-β-D-glucopyranose[12] (**4**) in 7 ml of 0.4M methanolic sodium methoxide. The mixture was stirred for 5 days at room temperature. After collection, the wash sequence was: chloroform, 4:1 chloroform–methanol, 1:1 chloroform–methanol, methanol, 1:1 methanol–1,4-dioxane, 1,4-dioxane, 4:1 1,4-dioxane–water, 1:1 1,4-dioxane–water, 1,4-dioxane, 1:1 1,4-dioxane–methanol, 1:4 methanol–chloroform, chloroform. Drying was for 16 h at 60°.

The weight increase of 0.621 g indicates 71% reaction (71% of the resin-Cl replaced by thioglucose groups), and thus a degree of loading of 0.55 mmol of sugar per g of the product **6-X**. This composition would require a sulfur content of 1.77%. Found: S, 1.75.

B. Cross-linked resin, Ⓟ–CH_2SH+glucosyl chloride. The mercaptomethyl resin **3-X** (1.002 g, 0.346 mmol of –SH) was stirred for 0.5 h in a solution of 0.522 g (1.02 mmol) of 6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl chloride[20] (**5**) in 40 ml of dry benzene. Then 80 mg (1.4 mmol) of potassium hydroxide in 0.2 ml of 1-propanol was added, and stirring was continued, at room temperature, for 5 days. After collection of the resin, the wash sequence was: benzene, 2:1 benzene–ethyl acetate, ethyl acetate, 1:1 ethyl acetate–methanol, methanol, 3:1 methanol–water, 3:1:1 methanol–1,4-dioxane–water, methanol, 1:1 methanol–chloroform, chloroform, dichloromethane. Drying was for 15 h at 50°. The weight increase of 0.123 g indicates

82% reaction of the resin –SH groups, and a degree of loading of 0.25 mmol of sugar per g of the product **6-X**.

C. Linear resin, Ⓟ–CH_2Cl+thio sugar. A solution of 1.30 g (2.8 mmol) of thio sugar **4** in 11 ml of 0.27M methanolic sodium methoxide was added with stirring to 2.77 g of chloromethyl resin **1-L** (2.8 mmol of –CH_2Cl) in 30 ml of dry benzene. Turbidity appeared immediately. More benzene (5 ml) was added, and stirring was continued for 5 days at room temperature. The resulting suspension was filtered, and 3.24 g of **6-L** recovered in the usual way. Evaporation of the filtrate from the precipitation of the polymer and recycling of the residue yielded an additional 0.12 g of product. Integration of the p.m.r. spectrum indicated a degree of loading of 0.64 mmol of sugar per g, corresponding to 88% replacement of resin-Cl and requiring a sulfur content of 2.06%. Found: S, 2.10.

Cleavage of sugars from the support. — In analytical experiments with cross-linked polystyrene, samples (0.3–0.5 g) of the loaded (**6-X**) or loaded and coupled (**12-X**) resin were refluxed for 4–5 days in 50 volumes of 75:5:1 benzene–methyl iodide–benzyl alcohol (v/v/v). For preparative work, convenience would require smaller relative volumes of benzene, with adjustments in the amounts of methyl iodide and benzyl alcohol to maintain these reagents in substantial molar excess with respect to the resin-bound sugar. After collection, the resin was washed with benzene, 1:1 benzene–chloroform, chloroform, 4:1 chloroform–methanol, 1:1 chloroform–methanol, methanol, chloroform, and dichloromethane. The combined filtrate and washings were evaporated and the residue was dissolved in 10 ml of chloroform. The chloroform solution was washed twice with 0.2M sodium thiosulfate, twice with water, dried (sodium sulfate), and evaporated (water pump). Residual benzyl alcohol was removed by a final evaporation under an oil-pump vacuum.

Linear-polystyrene samples were dissolved in the benzene–methyl iodide–benzyl alcohol reagent. The solution was refluxed for 4–5 days, and then evaporated. The residual syrup was taken up in a minimal volume of chloroform, and the resin precipitated and reprecipitated. The combined filtrates and washings were processed as described for the cross-linked resin.

Identification of the cleavage products from monosaccharide–resin. — The cleavage reaction performed with 1.04 g of **6-L** gave 250 mg of residue. This was chromatographed on silica gel (25 g, 1.5 cm diameter), with 23:2 chloroform–ethyl acetate as eluant. The material in the first peak (200 mg) crystallized on storage, and was recrystallized from ethyl acetate–hexane. It was identified as 1,6-anhydro-2,3,4-tri-*O*-benzyl-β-D-glucopyranose (**9**) by its m.p. of 93–94° (lit.[21] m.p. 90°), $[\alpha]_D^{25}$ −32.3° in chloroform (lit.[21] $[\alpha]_D^{20}$ −29.5°), and p.m.r. and i.r. spectra, which were identical with those of an authentic sample. T.l.c. of the material of the second peak (35 mg) showed a major and a minor component having closely similar R_F values in a variety of solvent systems. Recrystallization gave the pure major component as silky needles having m.p. 83–84°, $[\alpha]_D^{25}$ +63.4°, $[\alpha]_{436}^{25}$ +123.5° (*c* 1, chloroform). The p.m.r. spectrum was that expected for a tetra-*O*-benzylhexose. Found: C, 75.64; H, 6.54. $C_{34}H_{36}O_6$ (540.63) requires C, 75.53; H, 6.71. These properties, and its

origin, characterize the product as *benzyl 2,3,4-tri-O-benzyl-α-D-glucopyranoside* (**7**). (The known β-anomer[22] has $[\alpha]_D^{20}$ −9.2°.) As required by the proposed structure, **7** on acetylation gave a monoacetate; syrup; p.m.r. ($CDCl_3$): τ 2.38–2.82 (m, 20, Ph-*H*) and 7.97 (CH_3CO); relative to the spectrum of **7** there was a downfield shift of intensity from the upfield portion (τ 6.4–6.6, H-6 and H-6') of the sugar-proton envelope.

Coupling of the second sugar. — *A. Cross-linked resin.* The monosaccharide–resin (**6-X**) was stirred for 0.5 h at room temperature in a solution of 6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl bromide[16] (**11**) in dry benzene. The proportions are shown in Table I. 2,6-Lutidine was then added, and the suspension was heated for 3 days at 65°. The resin was collected and washed with benzene, chloroform, 1:1 chloroform–methanol, methanol, 2:1 methanol–1,4-dioxane, 1:1 methanol–1,4-dioxane, 1,4-dioxane, 2:1 1,4-dioxane–methanol, 3:1:1 methanol–1,4-dioxane–water, methanol, 3:1 methanol–chloroform, and chloroform. Drying was for 18 h at 60°. Samples of the product (**12-X**) were taken for analysis, and the coupling procedure was repeated on the remainder, as indicated in Table I.

B. Linear resin. The monosaccharide resin (**6-L**) was added to the freshly prepared, syrupy glucosyl bromide **11**, these were dissolved in dry benzene, and 2,6-lutidine was added. The proportions are given in Table II. The solution was heated for 3 days at 65°, diluted with benzene, and filtered to remove lutidinium bromide. The filtrate was concentrated to low volume, and then added dropwise to Skellysolve B to precipitate the resin. The resin was dissolved (chloroform) and reprecipitated (methanol) until t.l.c. of a chloroform solution showed no fast-moving spots. The sample was dried for 12 h at 35°; higher temperatures gave difficultly soluble material. The product (**12-L**), after removal of samples for analysis, was used for repetition of the coupling procedure, as shown in Table II.

Determination of the degree and stereoselectivity of the coupling. — The mixture of products cleaved from the resin (**12-X** or **12-L**) was deacetylated by ester exchange (sodium methoxide in 1:1 methanol–dichloromethane). The residue obtained after deionization (Amberlite IR-120, H^+) and evaporation was taken up in 25 ml of 90% ethanol and stirred with 5% Pd/C catalyst under hydrogen at atmospheric pressure and room temperature. The hydrogenolysis, monitored by t.l.c. in 26:23:1 chloroform–methanol–acetic acid, was complete in 2–3 days. Evaporation, after removal of the catalyst, gave a glassy solid.

Samples of this solid (~5 mg) were treated for 0.5 h at room temperature with 0.5 ml of a 7:2:1 (v/v/v) mixture of pyridine, hexamethyldisilazane, and chlorotrimethylsilane. G.l.c. was performed on a column (3.7 m × 3.2 mm) of 5% SE-52 on Anakrom A. The carrier gas was nitrogen, flow rate 60 ml/min; initial temperature 180°, with linear programming at 5°/min to 260°. Under these conditions, **10** and the anomers of **8** gave separate peaks, and the disaccharide mixture **14** gave a single peak. Peak areas were determined by cutting and weighing, and the molar ratio of disaccharide to monosaccharide determined with the aid of calibration curves.

To determine the ratio of α-linked to β-linked disaccharide, the sample of

mixed, debenzylated sugars (~12 mg) in 7 ml of 96% aqueous methanol was stirred with sodium borohydride (~30 mg) for 2 h at 0°. T.l.c. showed complete conversion into alditols. The solution was deionized, boric acid was removed by repeated evaporation with methanol, and the residue was trimethylsilylated. On the column just described, operated isothermally at 265°, Me_3Si-isomaltitol (retention time 19.5 min) was cleanly separated from Me_3Si-gentiobiitol (retention time 22.3 min). Measurement of the peak areas gave the α:β ratio. The values found were: for a sample from **12-X**, 92:8; for a sample from **12-L**, 95:5.

Characterization of the major coupling-product as isomaltose. — The syrupy cleavage-product (468 mg) from a batch of linear resin (**12-L** from **6-L** subjected twice to the coupling procedure) was chromatographed on silica gel (80 g, 2-cm diameter, 5-ml fractions). Elution with 23:2 chloroform–ethyl acetate gave first a component (A) of high R_F value on t.l.c., and then mixtures of A and the anhydro sugar **9**. The latter were resolved by thick-layer chromatography (1:1 ether–hexane). The fractions of component A were combined (211 mg), deacetylated (see foregoing), and concentrated to a thin syrup which, on dropwise addition to hexane, yielded 133 mg of solid precipitate (**15**), m.p. 120–125°, $[\alpha]_D^{25}$ +73.5°, having the p.m.r. spectrum and elemental analysis expected of a hepta-*O*-benzyl disaccharide. Found: C, 75.50; H, 6.70. $C_{61}H_{64}O_{11}$ (973.12) requires C, 75.28; H, 6.63.

Compound **15** (100 mg) was debenzylated (see foregoing), and the product was dissolved in ethanol. Addition of ethyl acetate precipitated 31 mg of *isomaltose* (**16**, 88% from **15**), identified by its $[\alpha]_D^{25}$ of +117° (*c* 0.48, water) (lit.[23,24] $[\alpha]_D^{25}$ +122°, +120°), its paper-chromatographic mobility (18:3:1:4 ethyl acetate–acetic acid–formic acid–water), and g.l.c. of the Me_3Si ether of the derived alditol.

Thick-layer chromatography (1:1 ether–hexane) of the concentrated mother liquor from the precipitation of **15** gave additional **15**, and a component having $[\alpha]_D^{25}$ +20°, presumably the gentiobioside.

ACKNOWLEDGMENTS

This work was supported by the College of Agricultural and Life Sciences, University of Wisconsin-Madison, and by grant No. AM-10588 from the National Institute of Arthritis, Metabolism, and Digestive Diseases, NIH.

We thank Dr. J. F. Rudd of the Dow Chemical Company for his generosity in supplying the linear polystyrene. Ed Blake of this Laboratory gave valuable help by preparing the 1,6-di-*O*-acetyl-2,3,4-tri-*O*-benzyl-D-glucopyranose used to make the glucosyl halides.

REFERENCES

1 R. B. Merrifield, *J. Am. Chem. Soc.*, 85 (1963) 2149–2154; 86 (1964) 304–305.
2 J. M. Fréchet and C. Schuerch, *J. Am. Chem. Soc.*, 93 (1971) 492–496.
3 J. M. Fréchet and C. Schuerch, *Carbohydr. Res.*, 22 (1972) 399–412.
4 G. Excoffier, D. Gagnaire, J.-P. Utille, and M. Vignon, *Tetrahedron Lett.*, (1972) 5065–5068; *Tetrahedron*, 31 (1975) 549–553.

5 R. D. GUTHRIE, A. D. JENKINS, AND G. A. F. ROBERTS, *J. Chem. Soc. Perkin Trans I*, (1973) 2414–2417.
6 U. ZEHAVI AND A. PATCHORNIK, *J. Am. Chem. Soc.*, 95 (1973) 5673–5677.
7 P. J. PFÄFFLI, S. H. HIXSON, AND L. ANDERSON, *Carbohydr. Res.*, 23 (1972) 195–206.
8 M. M. SHEMYAKIN, YU. A. OVCHINNIKOV, A. A. KINYUSKHIN, AND I. V. KOZHEVNIKOVA, *Tetrahedron Lett.*, (1965) 2323–2327.
9 F. CRAMER, R. HELBIG, H. HETTLER, K. H. SCHEIT, AND H. SELIGER, *Angew. Chem. Int. Ed. Engl.*, 5 (1966) 601.
10 H. HAYATSU AND H. G. KHORANA, *J. Am. Chem. Soc.*, 88 (1966) 3182–3183; 89 (1967) 3880–3887.
11 J. M. STEWART AND J. D. YOUNG, *Solid Phase Peptide Synthesis*, W. H. Freeman and Co., San Francisco, 1969, p. 27 and pp. 55–56.
12 S. A. HOLICK, S.-H. L. CHIU, AND L. ANDERSON, *Carbohydr. Res.*, 50 (1976) 215–225 (immediately preceeding paper).
13 S. A. HOLICK AND L. ANDERSON, *Carbohydr. Res.*, 34 (1974) 208–213.
14 H.-L. W. CHANG, *Tetrahedron Lett.*, (1972) 1989–1990.
15 M. FÉTIZON AND M. JURION, *Chem. Commun.*, (1972) 382–383.
16 J. M. FRÉCHET AND C. SCHUERCH, *J. Am. Chem. Soc.*, 94 (1972) 604–609.
17 M. PETITOU AND P. SINAŸ, *Carbohydr. Res.*, 40 (1975) 13–22.
18 R. EBY AND C. SCHUERCH, *Carbohydr. Res.*, 34 (1974) 79–90.
19 R. EBY AND C. SCHUERCH, *Macromolecules*, 7 (1974) 397–398.
20 N. PRAVDIĆ AND D. KEGLEVIĆ, *Tetrahedron*, 21 (1965) 1897–1901.
21 G. ZEMPLÉN, Z. CSŰRÖS, AND S. ANGYAL, *Ber.*, 70 (1937) 1848–1856.
22 E. ZISSIS AND H. G. FLETCHER, JR., *Carbohydr. Res.*, 12 (1970) 361–368.
23 E. M. MONTGOMERY, F. B. WEAKLEY, AND G. E. HILBERT, *J. Am. Chem. Soc.*, 71 (1949) 1682–1687.
24 A. JEANES, C. A. WILHAM, R. W. JONES, H. M. TSUCHIYA, AND C. E. RIST, *J. Am. Chem. Soc.*, 75 (1953) 5911–5915.