SOLID-PHASE SYNTHESIS OF OLIGOSACCHARIDES III. PREPARATION OF SOME DERIVATIVES OF DI- AND TRI-SACCHARIDES *VIA* A SIMPLE ALCOHOLYSIS REACTION

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ABSTRACT

The solid phase method has been applied to the synthesis of di- and tri-saccharides using a simple alcoholysis of 6-*O*-*p*-nitrobenzoyl- and 6-*O*-*p*-methoxybenzoyl-2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl bromides. High coupling yields were obtained with high monomer-to-resin ratios and in the presence of a small amount of 2,6-dimethylpyridine as acid acceptor. Cleavage of the completed oligomers by ozonolysis gave good yields of di- and tri-saccharides as glycosides of hydroxyethanol. All the cleaved disaccharides, irrespective of the monomer coupling sequence used in their preparation, had approximately the same optical rotation indicating that the reaction had not proceeded with the steric control characteristic of methanolysis of the same monomers.

INTRODUCTION

While several approaches are possible for the solid phase synthesis of oligosaccharides, we chose to test first a simple alcoholysis reaction in which the solid support carries the hydroxyl group and becomes linked to the anomeric center of the sugar unit. The rationale for this choice was that in the reaction of a glycosyl halide with an alcoholic hydroxyl group, side reactions are likely to occur at the anomeric center and not at the resin-bound hydroxyl group. The byproducts would therefore remain in the soluble phase and be eliminated by simple washing, while high yields of resin-bound glycosides should be obtained in the presence of a large excess of the glycosyl halide. In an alternate procedure of linking the resin to a hydroxyl group of the sugar unit, the glycosyl halide would be carried by the resin on subsequent steps and, therefore, each side reaction would cause chain termination.

The preparation[1] of a solid support with allylic alcohol functional groups and of suitable monomer units[2] carrying three types of functional groups has been described and rationalized in our previous work. Two features of a model glycoside-forming reaction have also been considered: the rate and the stereochemistry of the reaction of 6-*O*-acyl-2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl bromides (**1** and **2**). It has been shown that the proportion of anomeric glycosides produced could be varied

from over 90% α-D, in the case of the 6-*O*-*p*-nitrobenzoyl group[3] (**1**), to over 90% β-D, in the case of the 6-*O*-*p*-methoxybenzoyl group[2] (**2**). It was hoped that this type of steric control would also be applicable to the problem of solid phase oligosaccharide synthesis.

Recently, Flowers[4] prepared two α-D-glucopyranosyl disaccharides of D-galactose by the use of a glycosyl bromide having both a non-participating group at C-2 and a C-6 acyl group. By the use of a Koenigs–Knorr reaction with mercuric cyanide as condensing agent, Flowers treated 2-*O*-benzoyl-3,4,6-tri-*O*-*p*-nitrobenzoyl-β-D-glucopyranosyl bromide[3] with 1,2:3,4-di-*O*-isopropylidene-α-D-galactose or with 4,6-*O*-ethylidene-1,2-*O*-isopropylidene-α-D-galactose to produce the corresponding α-D-(1→6) or α-D-(1→3)-linked disaccharides in 63 and 47% yield, respectively. A similar treatment of the corresponding α-D-glucopyranosyl bromide was also shown to be stereospecific, although the reaction rate and yields obtained were lower. These results are consistent with those obtained by Ishikawa and Fletcher[3] in the methanolysis of these glucosyl halides, although the Koenigs–Knorr conditions are quite different from the conditions used in a methanolysis. In another recent publication, Dejter-Juszynski and Flowers[5] report that the Koenigs–Knorr condensation of 2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl bromide with benzyl 2-acetamido-3,4-di-*O*-acetyl-2-deoxy-α-D-glucopyranoside yields a disaccharide mixture containing a 7:3 ratio of α-D to β-D anomer. The lack of stereospecificity in this system and in the methanolysis of 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl bromide[3] is consistent with our suggestion that, in systems such as those used by Ishikawa and Fletcher[3] or by Flowers[4], the C-6 acyl group is responsible for the stereospecificity of the reaction.

However, the reaction conditions in a solid-phase system are very different from those used in the methanolysis or in Flowers' system. We chose, for example, to use a large excess of the glycosyl halide to ensure complete blocking of the reactive hydroxyl groups of the resin and to obtain reasonable rates. Furthermore; in a solid phase system, accessibility problems, steric hindrance, solvent differences, and the slower rate of glycosidation, may be important factors affecting steric control.

RESULTS AND DISCUSSION

Having selected the solid-support and monomers to be used in the synthesis of oligosaccharides, the problem remained to insure complete coupling of the sugar unit with the active hydroxyl groups of the resin, to find optimum reaction conditions for the cleavage of the finished product, and to test the stereospecificity of the reaction sequence.

The first problem was that of the reaction rate, as the relatively high rates of reaction obtained in the solvolysis of 6-*O*-acyl-2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl bromides could not be achieved under the conditions of a solid-phase synthesis. Metal ions, such as Ag^+ and Hg^{2+}, have been used in Koenigs–Knorr glycoside syntheses to enhance reaction rates; however, we chose not to use these metal ions as their presence also enhances side reactions[6]. We, therefore, used a simple alcoholysis

reaction in which the only reactants were the alcoholic hydroxyl on the resin support and the glycosyl bromide. Preliminary tests indicated that useful rates could not be obtained with a 1:1 ratio of resin to monomer, so higher concentrations of glycosyl halide were used.

TABLE I

INFLUENCE OF VARIOUS SOLVENTS ON THE EXTENT OF COUPLING

Solvent	*Yield* (%)	*Relative Ratio*[a]
2,6-Dimethylpyridine	59.2	1.67
Carbon tetrachloride	47.3	1.34
Chlorobenzene	35.7	1.01
Benzene	35.4	1.00
Nitrobenzene	28.9	0.84
Acetone	19.2	0.54
Pyridine	5.1	0.14

[a]Reaction in benzene taken as 1.00.

As can be seen in Table I, the extent of coupling increased in the series pyridine < acetone < nitrobenzene < benzene ≈ chlorobenzene < carbon tetrachloride < 2,6-dimethylpyridine. The fact that little or no reaction occurred in pyridine is probably an indication that pyridine itself acts as a nucleophile and reacts with the glycosyl bromide, as shown by Lemieux and Morgan[7]. The extent of coupling obtained in acetone, benzene or substituted benzenes, and carbon tetrachloride is presumably related to their ability to swell the resin. The reaction should proceed more readily in a good swelling agent, like carbon tetrachloride, than in acetone in which the degree of swelling is significantly lower. The higher yield of coupling obtained in 2,6-dimethylpyridine probably reflects the fact that 2,6-dimethylpyridine is a good swelling agent and also a relatively nonnucleophilic base which can accommodate the hydrogen bromide produced in the reaction. As shown in Table II, the highest coupling yields were obtained with solvents, such as benzene or carbon tetrachloride, used in combination with a small amount of base, such as 2,6-dimethylpyridine. The 2,3,4-tri-*O*-benzyl-6-*O*-*p*-nitrobenzoyl-α-D-glucopyranosyl bromide monomer (**1**) seemed to react more slowly with the solid support than the corresponding 6-*O*-*p*-methoxybenzoyl derivative (**2**), and with both monomers it was found that yields of 90% or over could only be obtained in one stage, at room temperature, if the monomer-to-resin molar ratio was ~8.1:1. In most cases where the molar ratio was of the order of 4 or 5 to 1 the reaction proceeded to an extent of 80–85%, and a second stage coupling was necessary to bring the reaction near completion. This difficulty was most often encountered in the coupling of the first unit to the resin but rarely in the coupling of subsequent units. It might have been preferable to block the less accessible hydroxyls after 80% of the reaction had been completed rather than to introduce a second coupling to complete the first reaction. A marked increase in

TABLE II

INFLUENCE OF ADDED BASE ON THE EXTENT OF COUPLING[a]

Monomer	*Monomer, mmoles*	*Monomer concentration*[b]	*Molar ratio monomer to resin*	*Solvent*[c]	*Time (days)*	*Base*[d]	*Coupling yield, %*
2	1.1	21	3.9:1	A	4	2,6-dimethylpyridine	82.5
2	1.1	21	3.9:1	A	4	1,8 BDAN[e]	72
2	1.1	21	3.9:1	B	4	2,6-dimethylpyridine	57
2	1.31	25	4.7:1	A	2	2,6-dimethylpyridine	77.8
2	1.31	25	4.7:1	A	2	pyridine	82.2
1	0.96	19.5	3.4:1	A	4.2	none	39.9
1	0.96	19.5	3.4:1	A	4.2	2,6-dimethylpyridine	65.2
1	0.96	19.5	3.4:1	A	4.2	pyridine	69.9
1	0.96	19.5	3.4:1	C	4.2	none	44.9
1	0.96	19.5	3.4:1	C	4.2	2,6-dimethylpyridine	87.9

[a]By use of a 1%-crosslinked resin (0.4 g or 0.28 mmole). [b]In g/100 ml of solution. [c](A) Benzene, (B) 2,6-dimethylpyridine, (C) carbon tetrachloride. [d]Concentration 0.43 mmole, molar ratio base to resin 1.5:1. [e]1,8-Bis(dimethylamino)naphthalene.

reaction rate was also observed when the reaction was performed at 60–65°, a 96% yield being obtained in 48 h with a monomer to resin ratio of ~6:1.

Coupling of the sugar units onto the resin could be monitored conveniently by gravimetry or by i.r. spectrometry. Weight increases of the order of 40–45% of the weight of the starting resin were recorded in the coupling of the first unit. Carbonyl absorptions appeared in the i.r. spectrum at about 1715 cm^{-1} for the coupling of both monomers. In addition, several bands corresponding to the 6-*O*-acyl groups could be identified. After each coupling reaction, excess monomer was reclaimed and, after elimination of the 2,6-dimethylpyridinium hydrobromide and 2,6-dimethylpyridine, the monomer was used again in another coupling reaction.

Removal of the temporary blocking group was achieved by ester interchange with methanol in tetrahydrofuran (70%) containing a catalytic amount of sodium methoxide. Once again the reaction was monitored both by gravimetry and i.r. spectrometry. Weight reductions amounting to 23.5–25.5% (depending on the nature of the temporary blocking group) of the weight gained in the coupling of the previous unit could be followed accurately. Disappearance of the carbonyl absorption and of the bands corresponding to the C-6 acyl substituent confirmed the successful removal of the temporary blocking group.

Coupling of subsequent sugar units was effected with good yields, in a single stage in most cases. The various coupling yields reported in Table III are computed with respect to the number of sugar units attached to the resin in the previous step, since unreacted hydroxyl groups were assumed to be located in unfavorable sites of the polymeric network. The preceding assumption is ascertained by the fact that while

TABLE III

PREPARATION OF RESIN-BOUND GLYCOSIDES

Exp. No.	*Cross-linking of resin* (%)	*Monomer-coupling sequence*[a]	*Yield* (%)		
			1st Unit	*2nd Unit*	*3rd Unit*
1	1	1*	100		
2	2	1*	91		
3	1	2*	99		
4	2	2*	91		
5	1	1*, 1*	97	98	
6	1	2*, 2	99	93	
7	2	1*, 2	90	94	
8[b]	2	1*, 2	94	100	
9	2	2*, 1	92	96	
10	2	1*, 1, 1	90	91	100
11	2	1*, 2, 1	90	94	98
12	2	2*, 2, 2	92	94	99
13	2	2*, 2, 1	92	94	92

[a]Asterisk indicates 2-stage coupling of unit preceding asterisk. [b]The 2nd stage of first-unit coupling and the 2nd-unit coupling were performed at 50–55°.

all the hydroxyl groups of the starting resin can be etherified with dihydropyran (phosphorus oxychloride catalyst) under mild conditions[8], the remaining hydroxyl groups of a 2%-crosslinked resin having 93% of its hydroxyl groups blocked by sugar units can only be etherified after long refluxing periods. As can be seen in Table III, better yields were obtained for the attachment of the first sugar unit to a 1%-crosslinked resin rather than to a 2%-crosslinked resin, probably due to the higher degree of swelling of the former resin.

Cleavage of the completed glycosides was most easily accomplished by ozonolysis followed by reduction with dimethyl sulfide. To minimize side reactions which may occur at the numerous benzylic positions of both the solid support and the substituted sugar, the reaction was run at low temperature (−78°) by the use of ozone in a nitrogen carrier which gave better results than the mixture of ozone and oxygen. Partial collapse of the polymer matrix was observed when ozone–oxygen was used. The yields reported in Table IV for the ozonolysis of the various glycosides are seemingly very variable. However, the low yields obtained in some experiments (*e.g.*, products No. 10A, 11, Table IV) were most likely due to the poor design of one of the reaction vessels used, which did not allow for complete mixing of ozone–nitrogen with the polymer suspension, rather than to an intrinsic failure of the ozonolysis

TABLE IV

CLEAVAGE OF THE FINISHED GLYCOSIDES

Product No.[a]	*Coupling sequence*	*Yield of ozonolysis* (%)	*Yield of reduction at C-1 and deblocking of C-6* (%)	$[\alpha]_D^{24}$ of *product* (*degrees*)
2	1	51[b]		+41.5
4	2	82	95	+40.8
5	1, 1	87	93	+60.5
8	1, 2	61	85[c]	+61.5
6A	2, 2	87	90[c]	+60
6B	2, 2	91	95	+60.2
9A	2, 1	63	94[c]	
9B	2, 1	71	90	+59
10A	1, 1, 1	51	95[c]	
10B	1, 1, 1	83	95	+62
11	1, 2, 1	57	79[c]	+68
12A	2, 2, 2	82	93	+70
12B	2, 2, 2	74	92	+78
13A	2, 2, 1	80	94[c]	
13B	2, 2, 1	72	89	+70

[a]Refers to experiment number in Table III; where more than one experiment was performed using the same starting material, the products are designated A and B. [b]Ozonolysis performed after removal of the C-6 acyl group in the solid phase. Ozonide reduction performed with sodium borohydride in methylene chloride–ethanol. [c]Lithium aluminum hydride used to reduce the aldehyde and C-6 acyl group. All other reductions were performed with sodium borohydride and followed by saponification with potassium hydroxide.

TABLE V

OPTICAL ROTATIONS OF GLYCOSIDES PREPARED BY SOLID-PHASE SYNTHESIS AND OF MODEL COMPOUNDS

Compound	*Method of preparation*[a]	$[\alpha]_D^b$ (*degrees*)	$[\phi]$ (*degrees*)
2-Hydroxyethyl 2,3,4-tri-*O*-benzyl-D-glucopyranoside	Prod. 4	+40.8	+202
2-Hydroxyethyl 2,3,4-tri-*O*-benzyl-D-glucopyranoside	Prod. 2	+41.5	+205
2-Hydroxyethyl 2,3,4-tri-*O*-benzyl-D-glucopyranoside	8[c]	+17.5	+119
Methyl 2,3,4-tri-*O*-benzyl-β-D-glucopyranoside	Ref. 10	+10	+46
Methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside	Ref. 9	+26.3	+122
Methyl 2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranoside	Ref. 11	+12.6	+70
Methyl 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranoside	Ref. 12	+32.2	+179
2-Hydroxyethyl 2,3,4-tri-*O*-benzyl-6-*O*-(2,3,4-tri-*O*-benzyl-D-glucopyranosyl)-D-glucopyranoside	Prod. 5, 6, 8, 9	+59 to +61.5	+547 to +569
Methyl 2,3,4-tri-*O*-benzyl-6-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)-α-D-glucopyranoside	Ref. 9	+17.4	+172
Methyl 2,3,4-tri-*O*-benzyl-6-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-α-D-glucopyranoside	Ref. 9	+59	+582

[a]Refers to product number in Table IV or to the literature. [b]In chloroform. [c]Model compound 8 is expected to contain a large proportion of the β-D-glucoside.

reaction. After reduction of the ozonides with dimethyl sulfide, the products, isolated as glycosides of hydroxyethanal, had n.m.r. spectra consistent with those expected for the mono-, di-, or tri-saccharides. Specifically, protons characteristic of the terminal C-6 blocking group were in proper proportion to other protons, within experimental error. In most cases, a small amount of impurity (usually amounting to 2% or less of the total number of protons) was observed at δ 1–1.4 p.p.m.

Although removal of the C-6 acyl group was not desired since it provided a convenient standard for n.m.r. analysis, it was usually removed at the same time as the aldehyde group was reduced to the corresponding alcohol group. Cleavage of the C-6 ester group was complete with lithium aluminum hydride as the reducing agent, and only partial with sodium borohydride. Sodium borohydride reduction, followed by saponification with potassium hydroxide, was preferred to lithium aluminum hydride reduction, as the separation of the final product was easier in the borohydride reaction (separation of the product from an acid salt *vs.* separation from an alcohol) and as lithium aluminum hydride is known to react with nitro groups.

As can be seen in Table IV, the optical rotations of the disaccharides prepared by the solid-phase method are in the range 59–61.5°, irrespective of the monomer-coupling sequence, thus indicating that the method, as applied, did not produce the expected stereochemical results. Since no anomeric protons can be observed in the n.m.r. spectra, it is difficult to draw any conclusion on the optical purity of the products from the data available. Conceivably, these products could be made up of a mixture of all possible isomers or of one major component with smaller amounts of the other isomers. Although no comparable glycol glycosides are described in the literature, a comparative study of the optical rotations of the products obtained in this work and of the optical rotations of various methyl glycosides can be attempted. The results of this comparative study, shown in Table V, suggest that the products obtained by the solid-phase method contain a high proportion of α-D linkages. This indication is supported further by the high positive optical rotation of compound **14** which was obtained by debenzylation of disaccharides prepared by the solid-phase method, followed by acetylation of the resulting product. A comparison of the optical rotation of **14** with those of a few disaccharide methyl glycosides found in the literature is presented in Table VI.

The solid-phase synthesis of oligosaccharides is probably a feasible proposition, since the present work is only a first approach to this new method of glycoside synthesis. In this system, however, reaction rates are too low and stereochemical results observed in related solvolyses are not obtained. Obviously, steric control by C-6-participating substituents[2–5] is markedly sensitive to reaction conditions. Until new glycoside-forming reactions are developed, efforts should be directed toward an increase in reaction rates, possibly by addition of metal ions in a system comparable to that used by Flowers[4,5]. This change may make lower monomer concentrations practical and may also tend to produce loose ion-pair intermediates. The latter have been postulated as the intermediates which favor steric control by C-6 acyl groups[2].

TABLE VI

OPTICAL ROTATION OF 14 AND OF MODEL COMPOUNDS

Derivatives of 6-O-(D-glucopyranosyl)-D-glucopyranoside	*Method of Preparation*[a]	$[\alpha]_D^b$ (*degrees*)	$[\phi]$ (*degrees*)
2-Acetoxyethyl (2,3,4,6-tetra-*O*-acetyl)-2,3,4-tri-*O*-acetyl-	**14**	+122.6	+886
2-Acetoxyethyl (2,3,4,6-tetra-*O*-acetyl-β)-2,3,4-tri-*O*-acetyl-α-	**11**	+49.5	+358
Methyl (2,3,4,6-tetra-*O*-acetyl-β)-2,3,4-tri-*O*-acetyl-β-	Ref. 13	−17	−111
Methyl (2,3,4,6-tetra-*O*-benzoyl-β)-2,3,4-tri-*O*-benzoyl-β-	Ref. 14	+8.3	+90
Methyl (2,3,4,6-tetra-*O*-benzoyl-α)-2,3,4-tri-*O*-benzoyl-β-	Ref. 14	+56.4	+611
Methyl (2,3,4,6-tetra-*O*-benzoyl-α)-2,3,4-tri-*O*-benzoyl-α-	Ref. 14	+108	+1170

[a]Refers to products described in Experimental or to the literature. [b]In chloroform.

EXPERIMENTAL

General methods. — I.r. spectra were measured with a Perkin–Elmer 137 infrared spectrophotometer on samples at 4–7% concentration in potassium bromide pellets. Since the pellets were usually opaque, a Perkin–Elmer reference beam attenuator was used during the recording of the spectra. N.m.r. spectra were measured with a Varian A-60 spectrometer in chloroform-*d* with tetramethylsilane as internal reference. Optical rotations were determined in a Perkin–Elmer model 141 polarimeter with jacketed 1-dm cells kept at 24° by circulation of water from a thermostatted bath. Microanalyses were performed by Galbraith Laboratories.

Preparation of the solid-support resin. — The resin was prepared as described previously[1]. A 1%-crosslinked resin having a capacity of 0.70 mmole/g and 2%-crosslinked resins having capacities of 0.78 and 0.70 mmole/g were prepared and used in this work.

Preparation of the monomers. — Crystalline 2,3,4-tri-*O*-benzyl-1,6-di-*O*-*p*-nitrobenzoyl-β-D-glucopyranose and 2,3,4-tri-*O*-benzyl-1,6-di-*O*-*p*-methoxybenzoyl-β-D-glucopyranose were prepared as described previously[2,3]. The corresponding α-D anomers were isolated from the mother liquor by column chromatography on Silica Gel with 200:1 benzene–ether for the 1,6-di-*O*-*p*-nitrobenzoyl derivative and 100:1 benzene–ether for the 1,6-di-*O*-*p*-methoxybenzoyl derivative. Both anomers were used in the preparation of the corresponding α-D glucopyranosyl bromides (**1**,**2**).

Glycoside-forming reaction: determination of optimum experimental conditions. — The influence of the nature of the solvent was studied as follows: to 0.195 mmole of 2%-crosslinked resin (capacity 0.78 mmole/g) was added 1.9 ml of a solution containing 0.453 g (0.7 mmole) of 2,3,4-tri-*O*-benzyl-6-*O*-*p*-methoxybenzoyl-α-D-glucopyranosyl bromide (**2**) in each of the following solvents: acetone, benzene, carbon tetrachloride, chlorobenzene, 2,6-dimethylpyridine, nitrobenzene, and pyridine. The reaction was allowed to proceed with stirring for 4.5 days at room temperature. The resin was then collected on a glass filter and extensively rinsed with various solvents. The i.r. spectra of the resin after reaction indicated that the reaction had proceeded to

various extents in the different solvents, and the yields of the coupling step could be estimated from the gain in weight of the resin after reaction. The results of this study are shown in Table I.

A procedure similar to the one just described was used to determine the influence of an added base to the extent of the coupling reaction. The experimental conditions and results are summarized in Table II.

The influence of the monomer concentration was studied in a similar way: to 0.4 g (0.28 mmole) of 1%-crosslinked resin was added 3.5 ml of a benzene solution containing either 1.6 g (2.41 mmole, 5 samples) or 0.4 g (0.6 mmole, 4 samples) of monomer **1**. After addition of 2,6-dimethylpyridine (0.05 ml, 0.43 mmole) each sample was stirred at room temperature. The samples were processed at appropriate intervals and the coupling yields estimated from the weight increase of each sample. The results of this study are shown in Fig. 1.

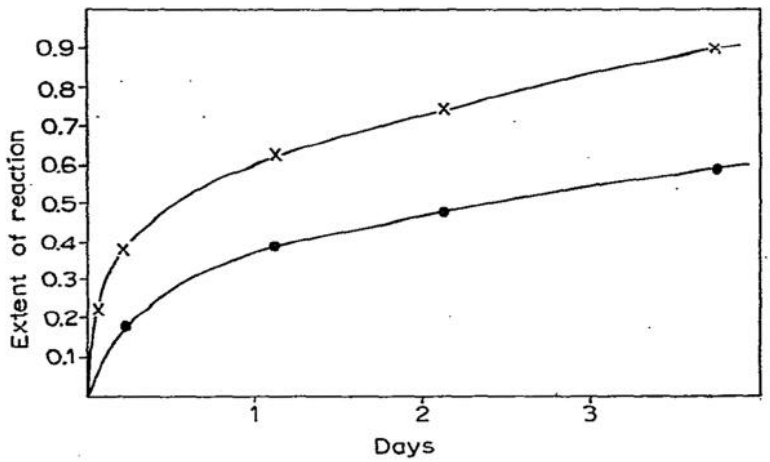

Fig. 1. Reaction of monomer **1** with resin. The molar ratio of monomer to resin was 2.1:1 (●) and 8.5:1 (×).

Preparation of a disaccharide. — A typical procedure for the preparation of a disaccharide from monomer **2** is given in the next paragraph. The same procedure was used for the coupling of monomer **1** with minor adjustments to take into account the difference in the molecular weights of the two monomers. Detailed information on the various yields obtained in the preparation of each oligomer can be found in Tables III and IV.

Preparation of **3** *by coupling of the first sugar unit to the resin.* — To 1.8276 g (1.28 mmole) of 1%-crosslinked resin was added a solution (15.5 ml) containing the monomer **2** (3.9 g, 6.02 mmoles) and 2,6-dimethylpyridine (0.20 ml, 1.72 mmole) in dry benzene. The molar ratio of monomer to 2,6-dimethylpyridine to resin was 4.7:1.34:1 and the monomer concentration 25 g/100 ml. The air in the flask containing the mixture was evacuated while the mixture was stirred to remove any air which might

have remained trapped in the resin. After reaction for 72 h with stirring at room temperature, the resin was collected on a glass filter and rinsed with dry benzene, and the excess of monomer reclaimed. Removal of any remaining monomer or side product was effected by washing the resin successively with acetone, 4:1 pyridine–water, 1:1 pyridine–water, water, tetrahydrofuran, 4:1 tetrahydrofuran–water, 1:1 tetrahydrofuran–water, water, tetrahydrofuran, acetone, ethanol, acetone, benzene, and methylene chloride. After being dried overnight in a vacuum oven at 70°, the product (**3**) weighed 2.4249 g. The gain in weight of 597.3 mg indicated a coupling yield of 82% (calc.: 725.6 mg for 100% coupling). All the resin collected was then allowed to react again, as just described, for 4 additional days. After the usual processing, 2.5460 g of product **3** was obtained for a total weight gain of 718.4 mg (99%) in the two-stage coupling reaction.

Preparation of **4** *by removal of the temporary blocking group of* **3**. — To a stirred suspension of resin **3** (2.4551 g) in tetrahydrofuran–methanol (20 ml) was added a catalytic amount of sodium methoxide. The mixture was heated for 90 min at reflux. After being cooled to room temperature, the resin was collected on a fritted-glass filter and rinsed with 7:3 tetrahydrofuran–methanol, 4:1 tetrahydrofuran–water, 1:1 tetrahydrofuran–water, water, ethanol, acetone, tetrahydrofuran, acetone, benzene, and methylene chloride. After being dried overnight in a vacuum oven, the resin **4** weighed 2.2893 g indicating that 165.8 mg had been lost in this step, and that removal of the C-6 acyl group was essentially complete (calc.: 24% of weight gained in first step or 166.2 mg). The absence of absorption in the carbonyl group region of the i.r. spectrum confirmed that the temporary blocking group had been cleaved.

Preparation of **5** *by coupling of a second sugar unit to the resin.* **4**. — To 2.1486 g (~1.16 mmole) of the resin-bound monosaccharide **4** was added a solution (15 ml) containing 3.6 g (5.56 mmoles) of monomer **2** and 0.25 ml (2.15 mmoles) of 2,6-dimethylpyridine in dry benzene. The mixture was stirred for 5 days at room temperature. The resin was then collected on a fritted-glass filter and rinsed as described for the preparation of **3**. After being dried overnight, 2.7508 g of resin-bound disaccharide **5** was obtained. The gain in weight of the resin was 0.6022 g indicating a coupling yield of 93% with respect to the resin-bound monosaccharide **3**.

Cleavage of the disaccharide **6** *from the resin* **5** *by ozonolysis.* — In most cases the resin used in the decoupling step still contained the C-6 acyl group. The ozone used in this experiment was prepared in advance and stored under nitrogen in a glass coil cooled to −78° and containing 30 g of previously ozonized and activated Silica Gel Davison grade 05 (6–16 mesh). Only a fraction of the coil was saturated with ozone. Nitrogen was used as carrier gas for the desorption of ozone and was passed through the cooled (−78°) coil at a rate of 150 ml/min.

A portion (1.3703 g containing 0.5463 g of bound sugar) of the resin-bound sugar **5** was suspended in dry dichloromethane (10 ml). After the suspension had been cooled to −78°, a stream of ozone in nitrogen carrier was bubbled through the mixture for 2 h. During the course of the reaction, the color of the resin changed from brown to light yellow. After flushing the excess of ozone with nitrogen, approx-

imately 3–4 ml of the dichloromethane was evaporated at a temperature not exceeding −10°, and methanol (3 ml) pre-cooled to −78° was added and the mixture shaken. Dimethyl sulfide (2 ml) was added, and the mixture was kept for 1 h at −78° with intermittent shaking. The temperature was then allowed to rise to ambient, and the resin was filtered off on a fritted-glass filter and thoroughly rinsed with dichloromethane and chloroform. The resin was then extracted repeatedly with chloroform, 7:3 chloroform–acetone, and 7:3 chloroform–ethanol, heated at reflux. The combined filtrates were evaporated in a flash evaporator, leaving a yellow oil which was dissolved in chloroform and then washed with distilled water to remove the dimethyl sulfoxide produced in the reduction of the ozonide. After drying and evaporation of the solvent, the residual oil was dried under high vacuum to yield 0.4961 g of the desired disaccharide **6** obtained as a glycoside of hydroxyethanal. The n.m.r. spectrum of the product corresponded to that expected and showed the presence of a small amount (~2% of the total number of protons) of impurity with high field resonance in the region δ 1–1.4 p.p.m.

Preparation of **7** *by reduction of the hydroxyethanal glucoside* **6** *and removal of the C-6 blocking group.* — The disaccharide **6** (0.496 g, 0.461 mmole) was dissolved in dry tetrahydrofuran (3 ml), and the solution was added dropwise through a dropping funnel to sodium borohydride (50 mg, 1.3 mmole) in ethanol (8 ml) at 0°. The solution was stirred for 90 min at room temperature, 0.5M potassium hydroxide (2 ml) was added, and the mixture was heated for 3 h at reflux. After evaporation of approximately half the solvent, the mixture was extracted with chloroform, the chloroform extract was rinsed with water, dried, and evaporated to yield 414 mg of the disaccharide glycol glycoside **7**.

*2-Hydroxyethyl 2,3,4-tri-*O*-benzyl-*D*-glucopyranosides* (**8** *and* **9**). — To a stirred solution of 2,3,4-tri-*O*-benzyl-6-*O*-*p*-methoxybenzoyl-α-D-glucopyranosyl bromide (**2**, 1 g, 1.54 mmole) in acetone (4 ml) was added purified 1,2-ethanediol (10 ml, 145.3 mmoles) and 2,6-dimethylpyridine (0.2 ml, 1.72 mmole). After 4 days at room temperature, the acetone was evaporated in a flash evaporator and the mixture extracted with chloroform and water. The chloroform extract was washed with water, dried, and evaporated to a light yellow oil which was saponified in ethanol–water–potassium hydroxide. The resulting product was chromatographed on a column of silica gel using 100:1 benzene–methanol for elution. After evaporation of the solvent and drying under high vacuum, the product **8** (412 mg, 54%), isolated as a slightly yellow oil, had $[\alpha]_D^{24}$ +17.5° (*c* 1.5, chloroform).

A similar reaction was carried out on a 1-g sample of 2,3,4-tri-*O*-benzyl-6-*O*-*p*-nitrobenzoyl-α-D-glucopyranosyl bromide (**1**) and yielded product **9** with $[\alpha]_D^{24}$ +25.4° (*c* 3, chloroform). The two products prepared by this method had similar n.m.r. spectra which could not be assigned to either anomer, because of the lack of identifiable anomeric proton.

*2-Hydroxyethyl 2,3,4-tri-*O*-acetyl-6-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-α-D-glucopyranoside* (**10**). — β-Gentiobiose octaacetate (1 g, 1.47 mmole) was dissolved in dry dichloromethane (10 ml). A current of dry hydrogen bromide was

bubbled through the solution for 10 min at room temperature. The solvent was evaporated in a flash evaporator, and successive portions of dichloromethane were evaporated *in vacuo* from the residual syrup at room temperature. Acetic acid produced in the reaction was eliminated by azeotropic distillation with dry benzene. The resulting hepta-*O*-acetylgentiobiosyl bromide was dissolved in acetone (3 ml) and treated with 1,2-ethanediol (10 ml, 145.3 mmoles) and 2,6-dimethylpyridine (0.17 ml, 1.47 mmole), and the mixture was stirred for 4.5 days at room temperature. After evaporation of the acetone, the mixture was extracted with chloroform and washed repeatedly with water. Evaporation of the chloroform yielded a clear syrup which was crystallized from ether (0.793 g, 79% yield). After recrystallization from ether–pentane, **10** had m.p. 144–45°, $[\alpha]_D^{24}$ +51° (*c* 1.3, chloroform), Its n.m.r. exhibited the expected proton ratio and included a low-field doublet (δ 6.21 p.p.m., $J_{1,2}$ 4 Hz) suggesting that the α-D anomer had been obtained.

Anal. Calc. for $C_{28}H_{40}O_{19}$: C, 49.41; H, 5.92. Found: C, 49.26; H, 5.88.

2-Acetoxyethyl 2,3,4-tri-O-acetyl-6-O-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-α-D-glucopyranoside (**11**). — Compound **10** (0.618 g, 0.91 mmole) was acetylated in the usual manner with dry pyridine (3 ml) and acetic anhydride (2 ml) to yield 0.592 g (90%) of **11** crystallized from ether. After recrystallization from chloroform–ether the product had m.p. 182–83°, $[\alpha]_D^{24}$ +49.5°; its n.m.r. spectrum included a doublet ($J_{1,2}$ 3.4 Hz) corresponding to 1 proton and centered at δ 6.39 p.p.m. assigned to the α-D anomer.

Anal. Calc. for $C_{30}H_{42}O_{20}$: C, 49.86; H, 5.86. Found: C, 49.49; H, 5.79.

2-Hydroxyethyl 2,3,4-tri-O-acetyl-6-O-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-D-glucopyranoside (**12**). — Hepta-*O*-acetylgentiobiosyl bromide, prepared as just described from octa-*O*-acetyl-β-gentiobiose (1 g, 1.47 mmole) and hydrogen bromide in dichloromethane, was dissolved in acetone (10 ml) and added dropwise to a mixture of 1,2-ethanediol (15 ml, 218 mmoles), silver carbonate (0.3 g), and powdered Drierite (2 g). The mixture was stirred for 24 h at room temperature, acetone was evaporated *in vacuo*, and the remaining syrup was extracted with chloroform and water. The chloroform extract was washed with water, dried, and evaporated *in vacuo*. Attempts to crystallize the resulting oil (0.81 g, 80%) failed, and the product was freeze-dried from benzene to a white solid having $[\alpha]_D^{24}$ +13.9° (*c* 1.9, chloroform).

2-Acetoxyethyl 2,3,4-tri-O-acetyl-6-O-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-D-glucopyranoside (**13**). — Acetylation of **12** (0.61 g, 0.9 mmole) in pyridine (2 ml) and acetic anhydride (1.5 ml) followed by the usual work-up of the product yielded 0.589 g (90%) of a slightly yellow oil. Its n.m.r. spectrum included two low-field doublets accounting for a total of one proton and centered at δ 5.79 p.p.m. ($J_{1,2}$ 5.9 Hz) and at δ 6.39 p.p.m. ($J_{1,2}$ 3.4 Hz) in a 7:3 ratio indicating that the product contained a mixture of **13** and **11** in 7:3 ratio. The product had $[\alpha]_D^{24}$ +22.5° (*c* 2.2, chloroform).

Anal. Calc. for $C_{30}H_{42}O_{20}$: C, 49.86; H, 5.86. Found: C, 49.69; H, 5.88.

Preparation of **14** *by debenzylation of* 7 *and acetylation.* — To a stirred solution of 7 (0.472 g, 0.5 mmole) in liquid ammonia (75 ml) and dry tetrahydrofuran

(10 ml) was added, portionwise, a total of 140 mg (6.1 mmoles) of sodium. After addition of each piece of sodium, a transient blue color developed around the metal and subsided rapidly. After addition of the last piece of sodium, a blue coloration developed throughout the solution but disappeared within 30 sec. Upon addition of another 40 mg (1.7 mmole) of sodium, the intense blue coloration reappeared and persisted until a small excess of ammonium chloride was added after 30 min of stirring. The solvents were evaporated under a stream of nitrogen, and the solid residue was extracted with chloroform, and then water. The water phase was washed several times with benzene, chloroform, and ether, and then evaporated to dryness. After being dried for 24 h in a vacuum oven at 85° the solid residue, which contained both the debenzylated product and appreciable quantities of inorganic byproducts, was acetylated with acetic anhydride (2 ml) in pyridine (3 ml) for 2 days at room temperature. The acetylation mixture was worked-up in the usual manner, and the desired product was isolated as an oil (**14**) which failed to crystallize. After elimination of a small amount of colored impurities on a small Silica Gel column, the product was freeze-dried to a slightly colored solid (191 mg, 52%) which had $[\alpha]_D^{24}$ +122.6° (*c* 1.3, chloroform). The n.m.r. spectrum of **14** was not identical with either that of **11** nor that of **13** but the proton amount of 18 in the region δ 3.5–3.9 and 24 in the acetyl region of the spectrum was similar to that of **11** and **13**.

Anal. Calc. for $C_{30}H_{42}O_{20}$: C, 49.86; H, 5.86. Found: C, 49.74; H, 5.95.

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