Carbohydrate Research, 50 (1976) 203–214

THE STEPWISE SYNTHESIS OF METHYL α-ISOMALTOOLIGOSIDE DERIVATIVES AND METHYL α-ISOMALTOPENTAOSIDE

RONALD EBY AND CONRAD SCHUERCH

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

(Received February 24th, 1976; accepted for publication, April 5th, 1976)

ABSTRACT

Methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside was treated with 2,3,4-tri-*O*-benzyl-6-*O*-(*N*-phenylcarbamoyl)-1-*O*-tosyl-D-glucopyranose in diethyl ether to give methyl 2,3,4,2′,3′,4′-hexa-*O*-benzyl-6′-*O*-(*N*-phenylcarbamoyl)-α-isomaltoside. The disaccharide was decarbanilated in ethanol with sodium ethoxide to give methyl 2,3,4,2′,3′,4′-hexa-*O*-benzyl-α-isomaltoside. The sequence of coupling with the same 1-*O*-tosyl-D-glucose derivative followed by removal of the *N*-phenylcarbamate group was repeated until the hexasaccharide derivative, methyl octadeca-*O*-benzyl-α-isomaltohexaoside, was formed. Methyl α-isomaltopentaoside was prepared by debenzylation of the corresponding benzylated oligosaccharide. The structures of the oligosaccharides were determined with the aid of both ^{1}H- and ^{13}C-n.m.r. spectroscopy. From spectral data, we estimate the coupling reaction to be 95% stereoselective.

INTRODUCTION

Practical preparative syntheses of oligosaccharides are almost unknown in carbohydrate chemistry. The major obstacle to their synthesis has been the lack of glycoside-forming reactions that are completely stereoselective and give high yields of product. Until recently, the synthesis even of di- and tri-saccharides usually has resulted in low yields (20–50%) due to the formation of mixtures of anomers and by-products. The synthesis of higher oligosaccharides has rarely been attempted since the yields usually diminish with increasing molecular weight.

Recently, new glycoside-forming reactions have been developed in which glycosyl derivatives having nonparticipating blocking groups—notably benzyl ethers—at C-2 have been used. These reactions often give products enriched in α linkages and are more stereoselective or lead to higher yields than do previous methods[1–7]. Glycosyl bromides having a *p*-nitrobenzoyl blocking group at O-6 or O-4 have been used to synthesize α-glycosides quite extensively[5,8–11] since their introduction in 1969, by Ishikawa and Fletcher[1]. The reaction is, however, not completely stereoselective and rates of reaction are low unless enhanced by metal salts.

Lemieux and associates[12–14] have shown that the glycosidation reaction catalyzed by a halide-ion is stereoselective and gives α-glycosides in good yields. In these reactions, a glycosyl halide, chloride or bromide, and a sugar alcohol are coupled in the presence of a quaternary ammonium halide. Under these conditions the glycosyl halide equilibrates rapidly to both the α and β anomer, and the more reactive β-halide couples with the alcohol to give the α-glycoside preferentially. The yields of coupling with favorable conditions are good but the reactions are slow and require about four days for completion. Nevertheless, a most important series of di- and branched tri-saccharides have been synthesized using this reaction scheme.

Work in our laboratory has comprised a systematic investigation of glycoside-forming reactions of sugars with various leaving groups at C-1, and temporary blocking groups at C-6, in a number of solvents, and under a variety of reaction conditions. We have attempted to develop glycoside-forming reactions that are rapid, completely stereoselective, and suitable for oligosaccharide synthesis. Of the systems studied, we have reported[6] one that includes 2,3,4-tri-*O*-benzyl-6-*O*-(*N*-phenylcarbamoyl)-1-*O*-tosyl-D-glucopyranose (**1**) in diethyl ether for the rapid stereoselective synthesis of α-linked D-glucosides in high yields. We have now applied this system to the stereoselective synthesis of a hexasaccharide derivative, methyl octadeca-*O*-benzyl-α-isomaltohexaoside, and to the synthesis of methyl α-isomaltopentaoside.

RESULTS AND DISCUSSION

In previous articles[6,15], **1** was synthesized from the corresponding α-D-glucopyranosyl bromide. It has since been reported[12,16], and results in our experiments also show, that many D-glucosyl bromides having a benzyl group at C-2 are relatively unstable and lose the benzyl group at C-2 readily. To avoid this problem, the corresponding α-D-glucosyl chloride was synthesized and this compound proved to be much more satisfactory. In the past, the synthesis of α-D-glucosyl chlorides from compounds having an ester function at C-1 involved a long reaction time of usually 2 to 4 days at 0°. In this work, the derivative having an *N*-phenylcarbamoyl group at O-1 was used and the reaction time at room temperature was thus reduced to one hour. The by-product of the reaction, aniline hydrochloride, is easily removed by filtration. Thus, the use of the α-D-glucosyl chloride gave a better coupling reaction without the presence of degradation products.

Methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (**2**) was coupled with **1** to give the disaccharide, methyl 2,3,4,2′,3′,4′-hexa-*O*-benzyl-6′-*O*-(*N*-phenylcarbamoyl)-α-isomaltoside (**3**). The yield of this syrupy product was almost 92% and the losses were caused by the manipulation, as t.l.c. of the crude reaction mixture did not show the presence of any unreacted **2**. The specific rotation of the syrup was the same as that of the crystalline product, suggesting that only one anomer, namely α, was present. The optical rotation of +61.6° was a good indication of an α-linked product since a similar disaccharide, methyl 2,3,4,2′,3′,4′,6′-hepta-*O*-benzyl-α-isomaltoside[17], in the same solvent and at the same temperature, showed a rotation of +59°.

The structure of the disaccharide was confirmed by removal of the blocking groups (the *N*-phenylcarbamoyl group by sodium ethoxide in ethanol and the benzyl groups by hydrogenolysis on palladium black). The resulting glassy product had $[\alpha]_D^{25}$ +166° (*c* 1, water), which agrees with the literature values of +177.4° (*c* 2, water)[18] and +164° (*c* 0.4, water)[17] for methyl α-isomaltoside. The product was benzoylated in pyridine to give crystalline methyl hepta-*O*-benzoyl-α-isomaltoside in a 75% yield, m.p. 186–188°, $[\alpha]_D^{25}$ +99.5° (*c* 1, chloroform). The corresponding literature values are: m.p. 193–194°, $[\alpha]_D^{25}$ +100° (*c* 1, chloroform)[17] and m.p. 188–189°, $[\alpha]_D^{20}$ +108° (*c* 2, chloroform)[19].

Removal of the *N*-phenylcarbamoyl group of **3** gave methyl 2,3,4,2′,3′,4′-hexa-*O*-benzyl-α-isomaltoside (**4**) in good yield. The ethyl *N*-phenylcarbamate that was formed during the reaction was removed by passing the solution through a small column of silicic acid and eluting with benzene. The decarbanilated disaccharide was eluted with dichloromethane as a colorless syrup that was crystallized from ether–petroleum ether in an 85% yield. Under some conditions the syrup, which had the same specific rotation as the crystalline material, failed to crystallize and was used to prepare the higher oligosaccharides without any loss of purity, as shown by the identical specific rotations of the products.

The trisaccharide was synthesized by coupling **1** with **4** under the same conditions as in the case of the disaccharide synthesis. An excess of **1** (1.1 equiv.) was used to insure the complete conversion of **4** into the trisaccharide **5**. 2,3,4-Tri-*O*-benzyl-6-*O*-(*N*-phenylcarbamoyl)-D-glucopyranose that was formed by the hydrolysis of the excess of **1** could be removed from the crude reaction product by passage through a small column of silicic acid. Thin-layer chromatography of the crude reaction mixture showed only the presence of the trisaccharide and of the free sugar resulting from the hydrolysis of **1**. The trisaccharide was isolated by chromatography and decarbanilated with sodium ethoxide to give an oligosaccharide with a free hydroxyl group at C-6.

The sequence of coupling an oligosaccharide having a free hydroxyl group at C-6 with an excess of **1**, followed by decarbanilation with sodium ethoxide was repeated until the hexamer, methyl octadeca-*O*-benzyl-α-isomaltohexaoside, was obtained. The physical constants of the oligosaccharides (see Table I) are based on several experiments, each of which gave the same product with the same physical constants. For example, the synthesis of methyl nona-*O*-benzyl-α-isomaltotrioside was performed four times, with weights of **4** that ranged from 0.25 g to 2.5 g, and in each case the yield and the specific rotation of the trisaccharide obtained was the same. Increasing the weights from milligram to gram quantities had no effect on the yield or stereoselectivity of the reactions.

The specific rotations of the oligosaccharides increased steadily as the degree of polymerization (D.P.) increased for both the carbanilated and decarbanilated oligosaccharides. A plot of molecular rotation *vs.* the D.P. of both oligosaccharide series was linear (see Fig. 1). The linear relationship has been interpreted by Turvey and Whelan[20], among others, to indicate that the products belong to a homologous series

TABLE I

PHYSICAL CONSTANTS OF METHYL α-ISOMALTOSIDES

Compound	*M.p.* (°)	$[\alpha]_D^{25}$ (°)[a]	$[M]_D^{25} \times 10^{-2}$ (°)	Anal[b]			*Yield* (%)
				C	*H*	*N*	
6′-*O*-(*N*-Phenylcarbamoyl)-hexa-*O*-benzyl- (3)	123–124	+61.6	625	73.19	6.40	1.35	85
				73.28	6.45	1.38	
Hexa-*O*-benzyl- (4)	104–106	+62.9	564	73.46	6.79		90
				73.64	6.74		
6′-*O*-(*N*-Phenylcarbamoyl)-nona-*O*-benzyl- (5)		+74.5	1079	73.70	6.73	1.03	85
				73.79	6.47	0.97	
Nona-*O*-benzyl- (6)		+77.7	1033	73.88	6.58		89
				74.08	6.67		
6′-*O*-(*N*-Phenylcarbamoyl)-dodeca-*O*-benzyl-		+77.1	1450	74.30	6.50	0.81	85
				74.06	6.48	0.74	
Dodeca-*O*-benzyl-[c]	189–191	+80.3	1415	74.25	6.58		90
				74.30	6.64		
6′-*O*-(*N*-Phenylcarbamoyl)-pentadeca-*O*-benzyl-		+81.0	1874	74.40	6.15	0.62	84
				74.23	6.49	0.61	
Pentadeca-*O*-benzyl-		+85.0	1865	74.58	6.57		87
				74.43	6.61		
6′-*O*-(*N*-Phenylcarbamoyl)-octadeca-*O*-benzyl-		+85.5	2348	74.68	6.52	0.54	83
				74.35	6.50	0.51	
Octadeca-*O*-benzyl-		+87.3	2293	74.24	6.52		84
				74.52	6.60		

[a](*c* 1, Chloroform). [b]Upper line, experimental value; lower line, calculated value. [c]Only a small portion of the compound crystallized from the syrup in ether–petroleum ether. The mother liquors had the same specific rotation as the crystalline material.

in which the glycosyl bonds have the same structure. In such a synthetic sequence, however, the linear relationship merely demonstrates the same degree of stereoselectivity at each step.

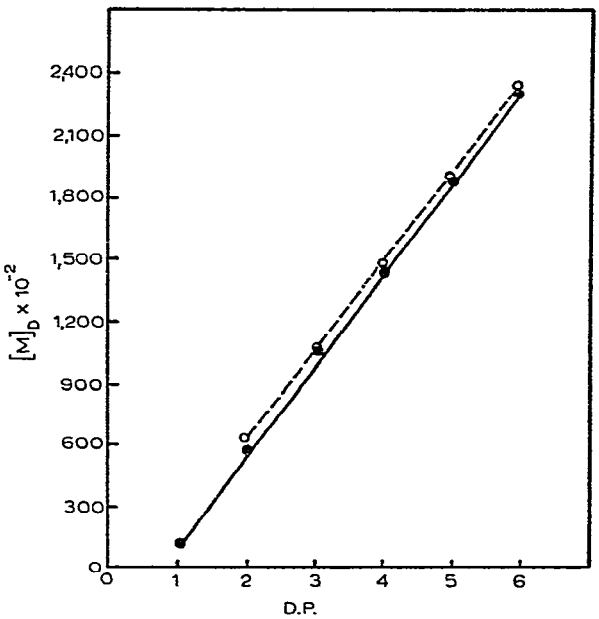

Fig. 1. Relation between D.P. and molecular rotation $[M]_D$ for carbanilated oligosaccharides (○) and decarbanilated oligosaccharides (●).

The ^{1}H-n.m.r. spectrum of each oligosaccharide was used to determine the D.P. of the oligosaccharide and the presence of the *N*-phenylcarbamoyl group. The D.P. was determined by the ratio of the aromatic (δ 7.45) to the benzyl and ring (δ 3.7–5.1) protons or to the N–H proton of the carbamoyl group (δ 6.45). The spectra also showed the presence of an α-methoxyl group at δ 3.37. The signal of anomeric protons coincided with the signals of the benzyl and other ring protons and could not be used to identify the configuration of the D-glucosyl linkages.

The α-linked structure of each of the oligosaccharides was determined from the ^{13}C-n.m.r. spectra. Resonances were observed at 97–98.5 p.p.m. from the signal of tetramethylsilane and were assigned to the C-1 atom bearing the α-methoxyl group and to the C-1 atoms of the α-D-glucosyl interunit linkages. No other resonances were observed between 95 and 110 p.p.m., which is the region where C-1 atoms of D-glucosides absorb. In particular, only signals due to noise were observed at the 105.5-p.p.m. signal that indicates a C-1 atom bearing a β-D linkage. Thus, it was concluded that only α-D-glucosyl bonds were present.

The synthesis of methyl α-isomaltopentaoside showed, however, that the coupling reactions were not completely stereoselective. Methyl pentadeca-*O*-benzyl-α-

isomaltopentaoside was debenzylated reductively with sodium in liquid ammonia. The crude oligosaccharide was separated from the inorganic salts by chromatography on Sephadex G-10. The ^{13}C-n.m.r. spectrum of the product indicated that the product was completely α-D-(1→6) linked and crystallization of the pentasaccharide was attempted by the use of the method reported by Jones *et al.*[18]. The product would not crystallize until the solution was seeded with a sample of pure methyl α-isomaltopentaoside.

The crystalline material had the same melting point as previously reported[18] and it did not depress the melting point of an authentic sample of methyl α-isomaltopentaoside. The optical rotation was about 3% lower than the reported value. The synthetic material showed a single spot on paper chromatography in 2:1:1 (v/v) butanol–water–2-methoxyethanol and cochromatographed with the authentic material. The ^{13}C-n.m.r. spectrum showed the compound to have only α-D linkages, and the positions and number of carbon peaks agreed with the structure of a methyl α-isomaltooligosaccharide (see Fig. 3.)

The ^{13}C-n.m.r. spectrum of the mother liquors, however, showed the presence of β-D linkages to the extent of 12% based on the relative peak heights of the α-D-glucosyl (87.3 p.p.m.) and β-D-glucosyl (102.2 p.p.m.) absorbances. Since pure methyl α-isomaltopentaoside was obtained in a 61% yield and showed no β-D linkages, the total amount of β-D linkages in the starting crude oligosaccharide was estimated at 4.6%. This indicates that the coupling reactions are not completely stereoselective and that about 5% of β-D linkages are formed at each coupling.

Assuming a 95% stereoselectivity at each of the four coupling steps, the yield of methyl α-isomaltopentaoside is calculated to be 81% of the final, crude product. The presence of β-D-linked oligosaccharide in the crude product undoubtedly inhibited crystallization of the pentasaccharide and accounted for the 61% yield of crystalline product obtained from the crude syrup.

The ^{13}C-n.m.r. spectra of methyl α-isomaltopentaoside and its mother liquors are a reliable measure of the amount of α- and β-D linkages in these products due to the increase in the signal-to-noise ratio. The increase in sensitivity is due mainly to an increase in the molar concentration of a sample of the debenzylated oligosaccharide, as compared to the same weight of a fully blocked (benzylated) oligosaccharide. This can be seen by comparing the ^{13}C-n.m.r. spectra reproduced in Figs. 2 and 3, both of which were run at the same concentration (200 mg/ml) (5 mm tube) for the same time. The C-1 glucosyl atoms of the debenzylated oligosaccharide also resonated at the same frequency, which also caused an increase in the sensitivity. The benzylated oligosaccharide (Fig. 2) shows a broad absorption for the C-1 glucosyl atoms with a resulting decrease in sensitivity.

The success of the synthesis depends on several factors: The 1-*O*-*p*-toluenesulfonate is preformed and the reaction is, therefore, between two species in homogeneous solution. The *p*-toluenesulfonate group is a good-leaving and a poor-nucleophilic group. The reaction mechanism is, therefore, comparatively simple[6], and a systematic investigation of the conditions was possible. The stereoselectivity

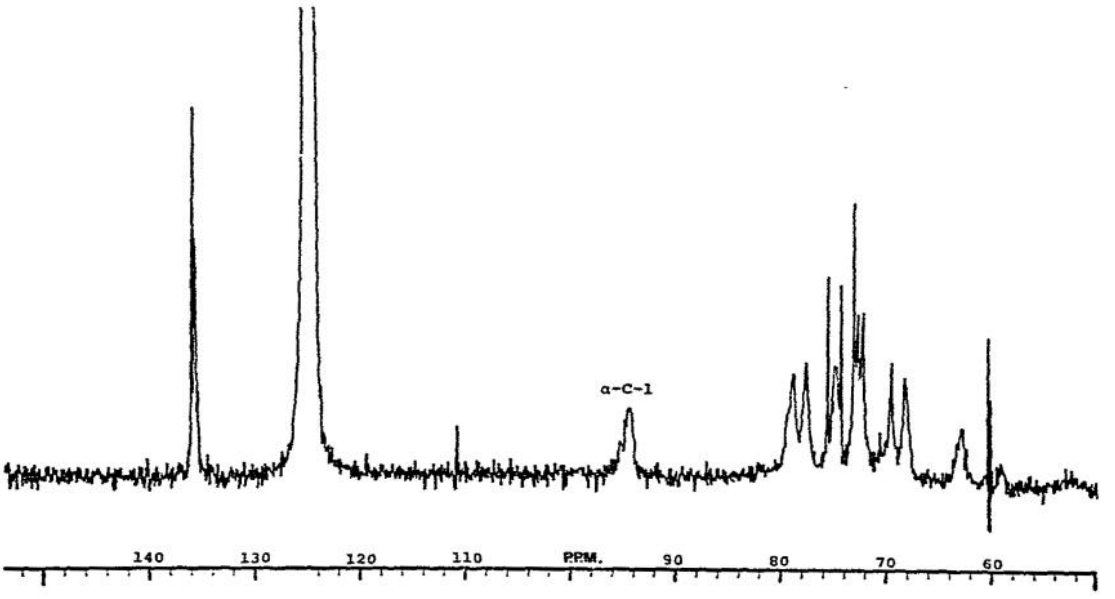

Fig. 2. ^{13}C-n.m.r. spectrum of methyl octadeca-*O*-benzyl-α-isomaltohexaoside.

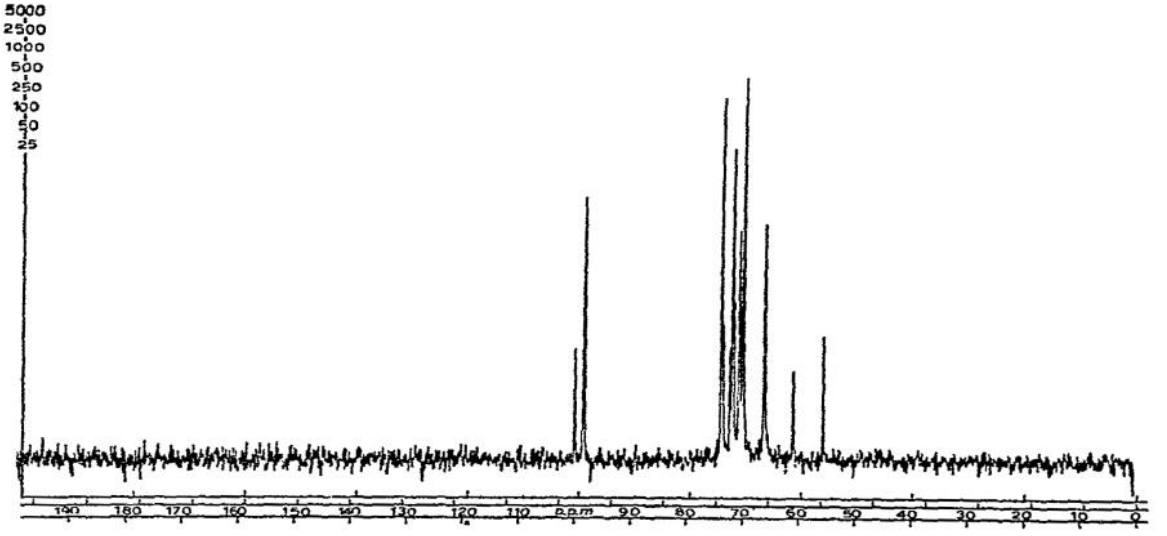

Fig. 3. ^{13}C-n.m.r. spectrum of methyl α-isomaltopentaoside.

of glycoside-forming reactions are profoundly influenced by interactions between substituent blocking-groups, the leaving group, the configuration of the sugar, the solvent, and the concentration of the reacting alcohol, and these influences are not completely understood at this time. However, in the present case the optimal conditions were carefully established[6] before the reaction was undertaken.

Recently Koto *et al.*[11] have reported a synthesis of isomaltooctaose. In the first step of this synthesis, 2,3,4-tri-*O*-benzyl-6-*O*-*p*-nitrobenzoyl-α-D-glucopyranosyl bromide[1] was treated with ethyl 2,3,4-tri-*O*-benzyl-1-thio-α-D-glucopyranoside in nitromethane solution in the presence of dimethylpyridine. A homogeneous oil was isolated in a yield of 92%, by chromatography of the product mixture, and was indicated to be the expected isomaltose derivative. The tetrasaccharide was synthesized by conversion of half of the disaccharide into the glycosyl bromide with bromine and half into a disaccharide alcohol by ester interchange. These two compounds were condensed to form a product mixture from which the desired tetrasaccharide was obtained in a 49% yield. A repetition of this sequence of reactions produced an octasaccharide in 11% yield. It is not clear whether the low yields are due to very slow rates of reaction or to by-product formation. It is clear, in any case, that any influence that the 6-*O*-*p*-nitrobenzoyl group may have had on the stereoselectivity of the first coupling must not have been present in the second and third couplings. Fortunately, it appears that 2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl bromides, irrespective of the substituent group at C-6, tend to give α-D-glucosides with an α stereoselectivity better than 90% when equivalent amounts of glycosyl halide and alcohol are used[4]. There is a marked difference in stereoselectivity[5] only when a very large excess of alcohol is present. Nevertheless, very important improvements for oligosaccharide synthesis can be achieved by careful selection of the solvent, and leaving and substituent groups. Unfortunately, the influence of the factors is not general and must be determined for each case.

EXPERIMENTAL

General. — ^{1}H-n.m.r. spectra were determined with a Varian A-60-A spectrometer on solution in chloroform-*d* and with Me_4Si as internal standard. ^{13}C-n.m.r. spectra were determined with a Varian XL-100-15 instrument in pulsed, Fourier-transform, proton-noise, decoupled mode with chloroform-*d* as the solvent and Me_4Si as internal standard. All chemical shifts of the ^{13}C-spectra are reported in p.p.m. from the signal of Me_4Si. Optical rotations were determined with a Perkin–Elmer model 141 polarimeter with jacketed 1-dm cells kept at 25° by circulating water from a constant-temperature bath.

Materials. — Spectrograde dichloromethane and acetonitrile were dried with CaH_2. Diethyl ether was stored over Na wire. Ag *p*-toluenesulfonate (Eastman Organic Chemicals, Rochester, N.Y. 14650) was dried under high vacuum before use. Methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside and 2,3,4-tri-*O*-benzyl-1,6-di-*O*-(*N*-phenylcarbamoyl)-α,β-D-glucopyranose were prepared as described in our previous report[6].

*2,3,4-Tri-*O*-benzyl-6-*O*-(*N*-phenylcarbamoyl)-*D*-glucopyranosyl chloride.* — A weighed amount of 2,3,4-tri-*O*-benzyl-1,6-di-*O*-(*N*-phenylcarbamoyl)-(α,β)-D-glucopyranose was dissolved in dichloromethane. Dry HCl gas was bubbled into the solution for 1 h. During this time, a precipitate of anilinium hydrochloride formed. Dry N_2 was then bubbled into the solution to remove the excess HCl and the suspension was filtered. The anilinium salt was washed with a small quantity of dichloromethane, dried, and weighed. The filtrate and washings were evaporated to give a clear, colorless syrup under reduced pressure at a temperature below 20°. The weight of anilinium hydrochloride gave a measure of the extent of the reaction, which in most cases was found to be at least 99% complete after 1 h. The glucosyl chloride was used in syrupy form and no further purification was required. After being dried in a high vacuum, the chloride had $[\alpha]_D^{25}$ +56° (*c* 1, chloroform); the n.m.r., spectrum showed two doublets centered at δ 6.1, ($J_{1,2}$ 3.7 Hz) and 5.3 ($J_{1,2}$ 8.0 Hz) corresponding to the α and β anomers, respectively. On being kept, the β anomer slowly isomerized into the α anomer. The compound was quite stable at room temperature and no decomposition could be detected after 1 day when kept dry.

*Methyl 2,3,4,2',3',4'-hexa-*O*-benzyl-6'-*O*-(*N*-phenylcarbamoyl)-α-isomaltoside* (**3**). — The reaction was performed on a high-vacuum rack in a reaction vessel that had two chambers separated by a fritted-glass filter. In one of the chambers was added methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (**2**, 0.32 g, 0.50 mmol) and in the other chamber Ag *p*-toluenesulfonate (0.19 g, 0.68 mmol). The reaction vessel was evacuated for several hours to dry the reagents. A solution of 2,3,4-tri-*O*-benzyl-6-*O*-(*N*-phenylcarbamoyl)-α-D-glucopyranosyl chloride (0.38 g, 0.60 mmol) in diethyl ether (3.0 ml) was added to the chamber containing the Ag salt. All additions were carried out under an atmosphere of dry N_2. The diethyl ether was removed by high vacuum distillation and dry acetonitrile was added by distillation (3.0 ml). The formation of the 1-*O*-tosyl-D-glucopyranose derivative (**1**) took place immediately as shown by the rapid precipitation of AgCl. After 10 min, the acetonitrile was distilled off under vacuum and dry diethyl ether (4.0 ml) was added by distillation. After mixing, the ether solution of **1** was introduced through a filter into the chamber containing the methyl glucoside. Suction was provided by cooling the receiver. The reactants were mixed and then placed in the dark for 16 h at room temperature. Dichloromethane (30 ml) was added and the solution washed with saturated $NaHCO_3$, water, and saturated NaCl. The organic phase was dried ($MgSO_4$) and evaporated to a syrup. The syrup was chromatographed on a column of SiO_2 (1.5 × 5 cm) with dichloromethane as the eluting solvent (50–100 ml). The disaccharide fraction was evaporated to give 639 mg (91.8%) of a syrup, $[\alpha]_D^{25}$ +61.5° (*c* 1, chloroform), that was homogeneous by t.l.c. on SiO_2 developed with 19:1 (v/v) benzene–methanol. The product was crystallized from either ethanol or diethyl ether–petroleum ether to give 590 mg (85%) of methyl 2,3,4,2',3',4'-hexa-*O*-benzyl-6'-*O*-(*N*-phenylcarbamoyl)-α-isomaltoside, m.p. 123–124°, $[\alpha]_D^{25}$ +61.6° (*c* 1, chloroform).

Anal. Calc. for $C_{62}H_{65}NO_{12}$: C, 73.28; H, 6.45; N, 1.38. Found. C, 73.19, H, 6.51; N, 1.35.

Methyl 2,3,4,2′,3′,4′-hexa-O-benzyl-α-isomaltoside. — To a solution of methyl 2,3,4,2′,3′,4′-hexa-*O*-benzyl-6′-*O*-(*N*-phenylcarbamoyl)-α-isomaltoside (400 mg) in ethanol (50 ml) was added a solution of sodium ethoxide (0.2 g) in ethanol (5 ml). The solution was boiled at reflux for 3 h and then neutralized with glacial acetic acid. The solvent was distilled off and the product extracted with dichloromethane. The solution was washed with dilute acetic acid, water, and saturated NaCl, dried ($MgSO_4$), and evaporated to a syrup. Chromatography on a column of SiO_2 (1.5 × 5 cm), with benzene (50–100 ml) as the eluting solvent, separated the ethyl *N*-phenylcarbamate from the disaccharide. The disaccharide fraction was eluted with dichloromethane (50–100 ml) and crystallized from diethyl ether–petroleum ether to give 300 mg of methyl 2,3,4,2′,3′,4′-hexa-*O*-benzyl-α-isomaltoside, m.p. 104–106°, $[\alpha]_D^{25}$ +62.9° (*c* 1, chloroform).

Anal. Calc. for $C_{55}H_{60}O_{11}$: C, 73.64; H, 6.74. Found: C, 73.46; H, 6.79.

Preparation of higher oligosaccharides. — The higher oligosaccharides, trimer through hexamer, were synthesized in the same manner as the disaccharide. An oligosaccharide having a free hydroxyl at C-6 was coupled with an excess of **1** in diethyl ether as just described. The processing of the reaction was essentially the same as that described and the product was isolated by chromatography on a column of SiO_2 (1.5 × 5 cm) to remove the monomeric impurities [mostly 2,3,4-tri-*O*-benzyl-6-*O*-(*N*-phenylcarbamoyl)-D-glucopyranose and some colored material]. None of the higher oligosaccharides were crystalline and the yields and optical rotations were obtained from the syrup isolated by column chromatography. The products were homogeneous by t.l.c. on SiO_2 with 19:1 (v/v) benzene–diethyl ether as eluting solvent, and no trace of the starting oligosaccharide was observed.

The *N*-phenylcarbamate group was removed with sodium ethoxide in refluxing ethanol. The higher oligosaccharides having a D.P. of 4–6 were only partially soluble in absolute ethanol, and benzene was added to obtain a homogeneous reaction mixture. The decarbanilated product was isolated by column chromatography on SiO_2 (1.5 × 5 cm), with benzene (50–100 ml) eluting ethyl *N*-phenylcarbamate and dichloromethane (50–100 ml) eluting the oligosaccharides. Except for minor quantities, the products were not crystalline and the yields and optical rotations were obtained on the isolated syrups.

The steps of coupling with **1**, followed by decarbanilation, were repeated until a hexamer was synthesized. The physical constants of the oligosaccharides are reported in Table I.

The D.P. of each of the oligosaccharides was determined from the ratio of the N–H proton (δ 6.85) of the *N*-phenylcarbamate group to the aromatic, or benzyl and ring-proton absorptions in the ^{1}H-n.m.r. spectrum.

The ^{13}C-n.m.r. spectra were determined on samples (~250–300 mg) in chloroform-*d* (3 ml) containing 1% (v/v) Me_4Si. Figure 1 shows the spectrum of methyl octadeca-*O*-benzyl-α-isomaltohexaoside.

Methyl α-isomaltopentaoside. — Methyl pentadeca-*O*-benzyl-α-isomaltopentaoside (**10**, 975 mg) was dissolved in 1:1 (v/v) anhydrous toluene–dimethoxyethane

(50 ml). The solution was added dropwise into liquid NH_3 (100 ml) containing Na (0.3 g). After 3 h, the solution was still blue and NH_4Cl was added to react with the excess Na. The mixture was evaporated to dryness under a stream of dry N_2 and the resulting white solid was dissolved in water (50 ml). The solution was extracted twice with dichloromethane (20 ml). The aqueous layer was concentrated to 5 ml and placed on a Sephadex G-10 column (2 × 40 cm). The column was eluted with distilled water and 1-ml fractions were collected. The fractions were analyzed for the presence of halide ions with $AgNO_3$ and those fractions containing no halide ion were combined and evaporated to a glassy syrup. The yield of crude methyl α-isomaltopentaoside was 300 mg (80%). The remaining 20% was found in the dichloromethane washings as partially debenzylated material. The crude material was dissolved in hot water and ethanol added until turbidity, and the solution allowed to cool. Repeated attempts failed to produce crystals. The solution was finally seeded with authentic methyl α-isomaltopentaoside and crystals formed. The white solid was filtered off, washed with ethanol, and dried in a vacuum oven at 80°. The yield was 182 mg (61%), based on the weight of the crude methyl α-isomaltopentaoside, m.p. 161–163°, $[\alpha]_D^{25}$ +182° (*c* 1, water); lit.[18]: m.p. 161–164°, $[\alpha]_D^{25}$ +188.7° (*c* 2, water).

The oligosaccharide was chromatographed on paper with standards of methyl α-D-glucopyranoside and methyl α-isomaltopentaoside in 2:1:1 (v/v) butanol–water–2-methoxyethanol as the developing solvent, as described by Jones *et al.*[18]. The compounds were detected with the ammoniacal $AgNO_3$ spray. The synthetic compound had the same R_F values as the authentic sample and gave a single spot. The ^{13}C-n.m.r. spectrum is shown in Fig. 3.

ACKNOWLEDGMENTS

The present work was supported by a research grant (1-RO1-AI-12509-01) of the National Institute of Allergy and Infectious Diseases and is based on earlier work supported by research grant GM-06169 of the Division of General Medical Sciences, National Institutes of Health. The authors wish to express their appreciation to Dr. Allene Jeanes for kindly supplying a sample of methyl α-isomaltopentaoside.

REFERENCES

1 T. Ishikawa and H. G. Fletcher, Jr., *J. Org. Chem.*, 34 (1969) 563–571.
2 A. C. West and C. Schuerch, *J. Am. Chem. Soc.*, 95 (1973) 1333–1335.
3 A. C. West and C. Schuerch, unpublished results.
4 F. J. Kronzer and C. Schuerch, *Carbohydr. Res.*, 27 (1973) 379–390.
5 J. M. Frechet and C. Schuerch, *J. Am. Chem. Soc.*, 94 (1971) 604–609.
6 R. Eby and C. Schuerch, *Carbohydr. Res.*, 34 (1974) 79–90.
7 F. J. Kronzer and C. Schuerch, *Carbohydr. Res.*, 34 (1974) 71–78.
8 H. M. Flowers, *Carbohydr. Res.*, 18 (1971) 211–218.
9 M. Dejter-Juszynski and H. M. Flowers, *Carbohydr. Res.*, 18 (1971) 219–226.
10 M. Dejter-Juszynski and H. M. Flowers, *Carbohydr. Res.*, 30 (1973) 287–292.
11 S. Koto, T. Uchida, and S. Zen, *Bull. Chem. Soc. Jpn.*, 46 (1973) 2520–2523.
12 R. U. Lemieux, K. B. Hendriks, R. V. Stick, and K. James, *J. Am. Chem. Soc.*, 97 (1975) 4056–4062.

13 R. U. LEMIEUX AND H. DRIGUEZ, *J. Am. Chem. Soc.*, 97 (1975) 4063–4068.
14 R. U. LEMIEUX AND H. DRIGUEZ, *J. Am. Chem. Soc.*, 97 (1975) 4069–4075.
15 R. EBY AND C. SCHUERCH, *Macromolecules*, 7 (1974) 397–398.
16 R. U. LEMIEUX AND T. KONDO, *Carbohydr. Res.*, 35 (1974) C4–C5.
17 S. HIXSON, Ph.D. Thesis, Univ. of Wisconsin, Madison, 1970.
18 R. W. JONES, A. JEANES, C. S. STRINGER, AND H. M. TSUCHIYA, *J. Am. Chem. Soc.*, 78 (1956) 2499–2502.
19 H. BREDERECK, A. WAGNER, D. GEISSEL, AND H. OTT, *Chem. Ber.*, 95 (1962) 3064–3069.
20 J. R. TURVEY AND W. J. WHELAN, *Biochem. J.*, 67 (1957) 49–52.