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A NEW METHOD FOR THE SYNTHESIS OF α-D-GLUCOPYRANOSIDES

BY

© KLAUS B. HENDRIKS

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE

DEGREE OF DOCTOR OF PHILOSOPHY

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UNIVERSITY OF ALBERTA

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UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled

A NEW METHOD FOR THE SYNTHESIS OF α-D-GLUCOPYRANOSIDES

submitted by KLAUS B. HENDRIKS

in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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To Kathleen

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ABSTRACT

Reaction of tetra-O-benzyl-α-D-glucopyranosyl bromide with two mole equivalents of methanol in methylene chloride at 60°C in the presence of an aliphatic amine produced the corresponding anomeric methyl D-glucopyranosides in 80% yield with an α to β ratio of about 2.9. In the presence of an equimolar amount of tetraethylammonium bromide, the overall yield remained virtually the same, but the α to β ratio of the substituted methyl D-glucopyranosides was raised to approximately 9.3. Evidence was accumulated that the presence of bromide ion causes anomerization of the starting material, tetra-O-benzyl-α-D-glucopyranosyl bromide, to its β-anomer which is the precursor of the α-D-glucopyranosides. Thus, the presence of bromide ion in substantial concentration at the start of the reaction provides an initially rapid rate of anomerization and thereby directs the reaction to α-D-glucopyranoside formation. The kinetics of this halide-ion catalyzed alcoholysis of the D-glucopyranosyl bromides was investigated and the relative rates of formation of the anomeric D-glucopyranosides are discussed in terms of thermodynamic stability of intermediate ion-pairs. These studies were carried out

using the simple lower aliphatic alcohols methanol, ethanol and isopropanol, respectively. However, the usefulness of the method for the synthesis of more complex α-D-glucopyranosides was demonstrated by preparing two disaccharides, for example 3-O-(tetra-O-benzyl-α-D-glucopyranosyl)-1,2;5,6-di-O-isopropylidene-α-D-glucofuranose was obtained in 42% yield and 6-O-(tetra-O-benzyl-α-D-glucopyranosyl)-1,2;3,4-di-O-isopropylidene-α-D-galactopyranose was prepared in 65% yield.

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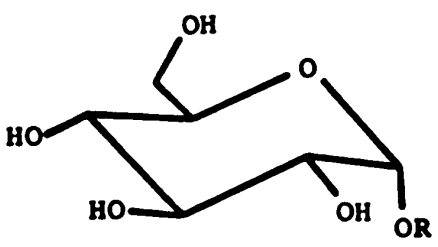
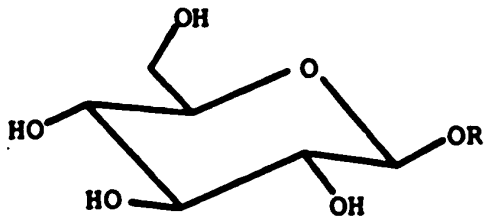
INTRODUCTION

Alkyl α- and β-D-glucopyranosides have the following configurations and conformations:



An α-D-glucopyranoside



A β-D-glucopyranoside

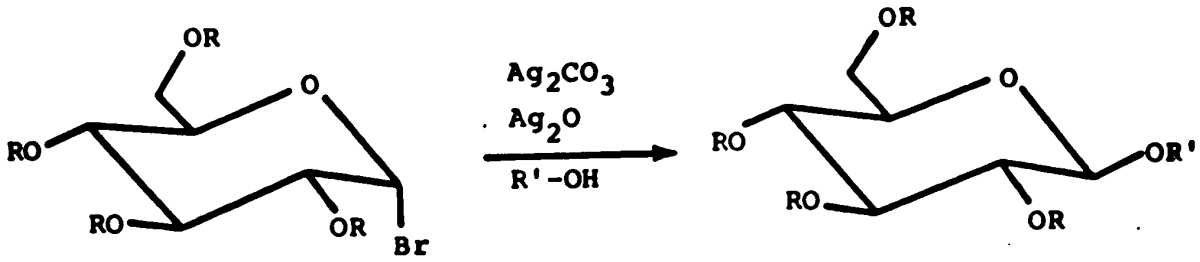
Although the formation of D-glucopyranosides from D-glucose merely involves the conversion of the cyclic hemiacetal to the corresponding cyclic acetal, the synthesis of such compounds with stereochemical control in good yield has presented a challenge which attracted the attention of chemists for nearly a hundred years, mainly because of the widespread natural occurrence of D-glucopyranosides. The chemical synthesis of all but the simplest α-D-glucopyranosides which have a 1,2-*cis*-relationship between the substituent at carbon-2 and the aglycon attached to the anomeric center has

proved to be far more difficult than that of the β-anomers. The simplest glucosides are exceptional in that the α-anomeric forms are easily prepared by the Fischer glucoside synthesis (1) in which a solution of D-glucose in the appropriate alcohol is boiled in the presence of an acid catalyst.

Several approaches have been made to solve the problem of providing a general synthetic pathway to α-D-glucopyranosides but only a few have found wide application. Most of the individual procedures are restricted to a certain aglycon type and often depend also on the configuration of the monosaccharide. Until recently, the synthesis of a given α-glycopyranoside had its optimum conditions, differing from those of the preparation of another glycoside. A review of the syntheses of the simplest representatives of aliphatic and aromatic glucosides, i.e. methyl and phenyl glucosides, has been presented by Conchie, Levvy and Marsh (2).

The alcoholysis of suitably O-protected D-hexopyranosyl halides, i.e. sugar derivatives which are activated at the anomeric center by being converted into a cyclic α-haloether, has represented the most useful method for the preparation of D-glucopyranosides. Thus, one of the oldest and by far the most widely applied synthetic method leading to β-D-glucopyranosides is the

reaction by Koenigs and Knorr (3), in which tetra-O-acetyl-α-D-glucopyranosyl bromide (the trivially called acetobromoglucose) is reacted with an excess of alcohol in the presence of a silver salt such as silver carbonate or silver oxide. The following diagram 1 shows the general scheme of a Koenigs-Knorr reaction:



R = blocking group, usually acetyl group.

Diagram 1

Of the numerous methods that have been used over the years to prepare D-glucopyranosides, only those pertinent to the present thesis will be surveyed here. They involve mainly syntheses which utilize suitably blocked D-glucopyranosyl halides as starting materials.

Concerning the reactivity of these starting materials, it is well established that the D-glucopyranosyl halides having the halogen atom in an axial

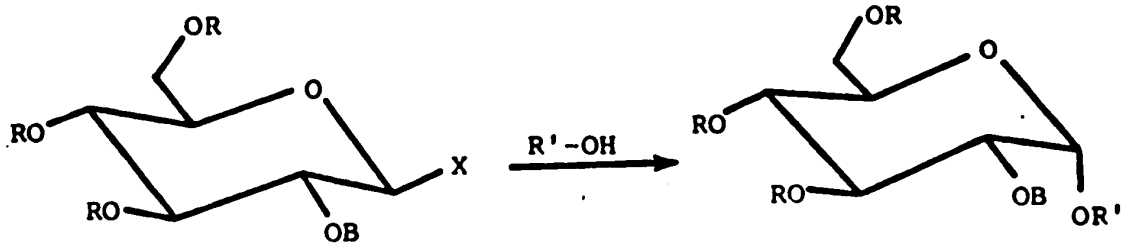
orientation are thermodynamically the more stable ones [by about 2 kcal/mole (4)] due to the "anomeric effect" (5,6). A type of bimolecular nucleophilic substitution is often observed in the reaction of the glycosyl halides with alcohols, and consequently the reaction occurs with inversion of configuration at carbon-1. This normally leads to derivatives of β-D-hexopyranosides. A brief summary of the various modifications of the Koenigs-Knorr reaction will be presented later on in this introduction.

During the past few years, derivatives of carbohydrates containing α-glycosidic linkages were discovered in various antibiotics, for example in the neomycins, the paromomycins, the kanamycins, the streptomycins including bluensomycin, the gentamycins and kasugamycin (7,8). The occurrence of α-glucopyranosides and their derivatives in living organisms, for example in starch and α-dextrins, in bacterial cell walls, glycolipids (9), and glycoproteins (10) has further stimulated interest in suitable α-glycoside syntheses and illustrates clearly the significance of this group of compounds. As mentioned earlier, the vast majority of reactions taking place under Koenigs-Knorr conditions leads to glucopyranosides in which the newly formed aglycon is *trans* to the substituent

at carbon-2 of the original glucopyranosyl halide; *cis*-1,2 α-glucosides are not obtained under normal circumstances. The reactions of acetylated 1,2-*trans*-glucopyranosyl halides are more complicated and can lead, by way of participation of the acetoxy substituent on carbon-2 in the displacement of the halogen to the formation of orthoesters as well as 1,2-*trans*-glucopyranosides with retention of configuration. A small amount of inversion to yield the *cis*-1,2-glucopyranoside may also occur and the exact composition of a given product mixture depends markedly upon the reaction conditions. Koenigs-Knorr reactions have almost invariably been carried out with acetylated glucopyranosyl halides (especially bromides) as these derivatives are normally stable, easily prepared and yet sufficiently reactive under the condensation conditions.

In the absence of neighbouring group effects of the type mentioned above, the Koenigs-Knorr reaction characteristically tends to proceed with inversion at carbon-1 of the glucopyranosyl halide. Consequently, numerous attempts have been made to obtain *cis*-1,2-glucopyranosides by Walden inversion at carbon-1 from *trans*-1,2-glucopyranosyl halides containing a neighbouring group at carbon-2 which does not participate to any appreciable extent. Such a reaction scheme is outlined

in the following diagram 2:



X = Cl (9,12,13),
F (12),
Br (11).

B = a weakly participating blocking group such as:

$-\overset{O}{\overset{\|}{C}}-CCl_3$ (11),

$-CH_2-C_6H_5$ (13),

$-NO_2$ (14,15).

R = blocking group.

Diagram 2

For example, Hickinbottom (11) in 1929 prepared tri-O-acetyl-2-trichloroacetyl-β-D-glucopyranosyl chloride and studied its reactions with alcohols. When this compound was heated in methanol or ethanol in the presence of silver carbonate or silver oxide, a mixture of the corresponding α- and β-glucopyranosides was formed, the ratio of the α- to β-glucopyranoside being approximately 7 to 3. Several attempts were made by Stacey and his associates (12) to utilize tetra-O-acetyl-β-D-glucopyranosyl fluoride as the starting material for the synthesis

of α-glucopyranosides, apparently without much success, the glucopyranosyl fluoride derivative being highly unreactive. Gorin and Perlin in 1961 (13) used 3,4,5-tri-O-acetyl-2-O-benzyl-α-D-mannopyranosyl bromide to synthesize 6-O-α-D-mannopyranosyl-D-glucopyranose from 1,2,3,4-tetra-O-acetyl-D-glucopyranose in the presence of silver oxide, iodine and drierite. In 1963, Wolfrom and coworkers (14,15) prepared the stable 3,4,6-tri-O-acetyl-2-O-nitro-β-D-glucopyranosyl chloride and reacted this with isopropanol (used simultaneously as the solvent) in the presence of silver carbonate, silver perchlorate and anhydrous calcium sulfate to give a 35% yield of the corresponding isopropyl α-D-glucopyranoside derivative. However, when only a fourfold excess of isopropanol was used in this condensation reaction in ether as solvent the yields of both the isopropyl tetra-O-acetyl-2-O-nitro-α-D-glucopyranoside and its β-anomer dropped to 15.3% and 3.2%, respectively.

In 1963, Lloyd and Roberts (16) reported the condensation of 3,4,6-tri-O-acetyl-2-deoxy-2-(2',4'-dinitroanilino)-α-D-glucopyranosyl bromide with ethanol in various solvents in the presence of a variety of condensing agents. The ratio of the α- to β-anomer for the produced ethyl glucopyranoside derivatives was found always to be greater than 1, ranging from 1.5 when

pyridine was the solvent to 9.4 when chloroform was used as solvent and pyridine as the base. A 200 molar excess of ethanol was employed in these reactions. When the condensations were carried out in chloroform in the presence of silver carbonate the α- to β-ratio was 0.12, in nitromethane this ratio was 0.02. Similarly in the presence of mercuric salts, such as mercuric acetate, mainly the ethyl tri-O-acetyl-2-deoxy-2-(2',4'-dinitroanilino)-β-D-glucopyranoside was formed. The authors discussed several possible routes by which the preferred formation of ethyl tri-O-acetyl-2-deoxy-2-(2',4'-dinitroanilino)-α-D-glucopyranoside in the presence of pyridine could be explained and finally proposed participation of the acetoxy group at carbon-6 to be responsible for the observed results as shown in the following diagram 3.

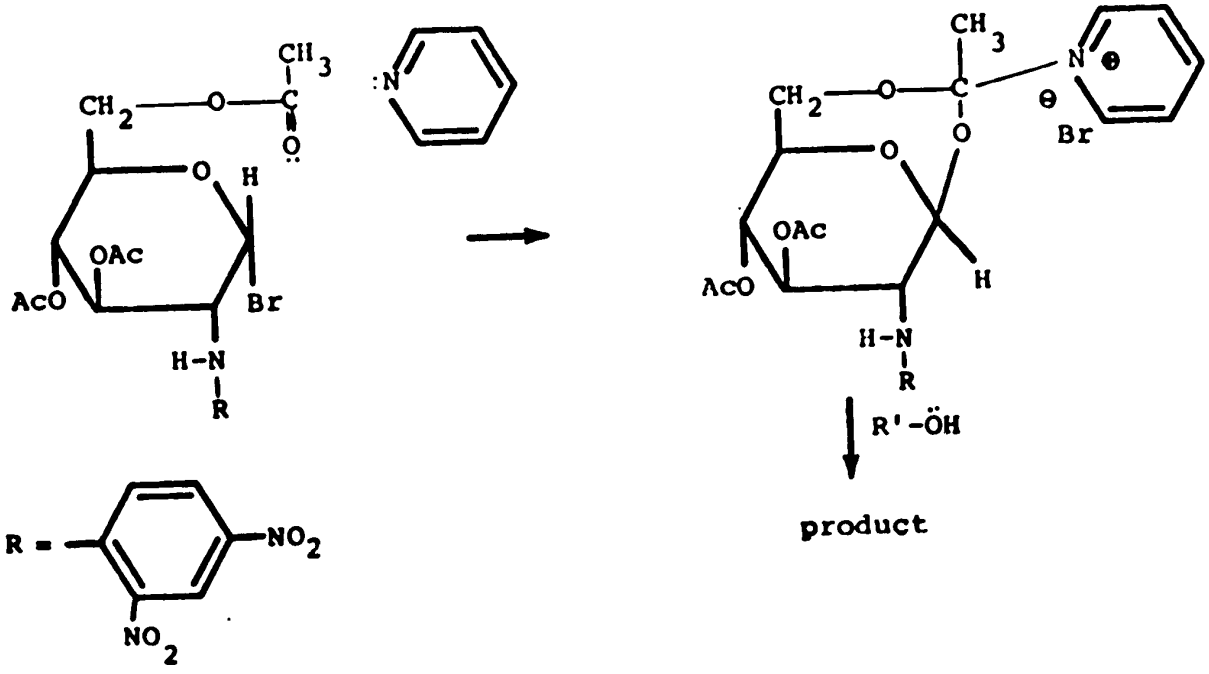


Diagram 3

A similar participation of a carbon-6 acetoxy group had been suggested earlier by Lemieux (17,18) to account for the formation of α-D-glucopyranosides from Brigl's anhydride (tri-O-acetyl-1,2-anhydro-α-D-glucopyranose), and Lloyd and Roberts quoted this proposal in support of their suggested mechanism.

An entirely new method for the synthesis of α-D-glucopyranosides and α-D-glucopyranosaminides, differing from the more classical approaches discussed above, was developed by Lemieux, Nagabhushan and Gunner (19,20). These authors showed that dimeric tri-O-acetyl-2-deoxy-2-nitroso-α-D-glucopyranosyl chloride (21,22), prepared by addition of nitrosyl chloride to tri-O-acetyl-2-deoxy-1,5-anhydro-D-*arabino*-hex-1-enitol (D-glucal triacetate), reacted with a number of alcohols either in refluxing methylene chloride or in dimethylformamide at room temperature to provide the corresponding tri-O-acetyl-2-oximino-α-D-*arabino*-hexopyranoside in yields generally greater than 80%. The reaction is believed to proceed, by an elimination-addition mechanism, by way of tri-O-acetyl-2-deoxy-2-nitroso-1,5-anhydro-D-*arabino*-hex-1-enitol (19). The intermediate 2-oximino-α-D-glucopyranosides were hydrogenated, after O-deacetylation, to 2-amino-2-deoxy-α-D-glucopyranosides (23). On the other hand, deoximation followed by reduction of the

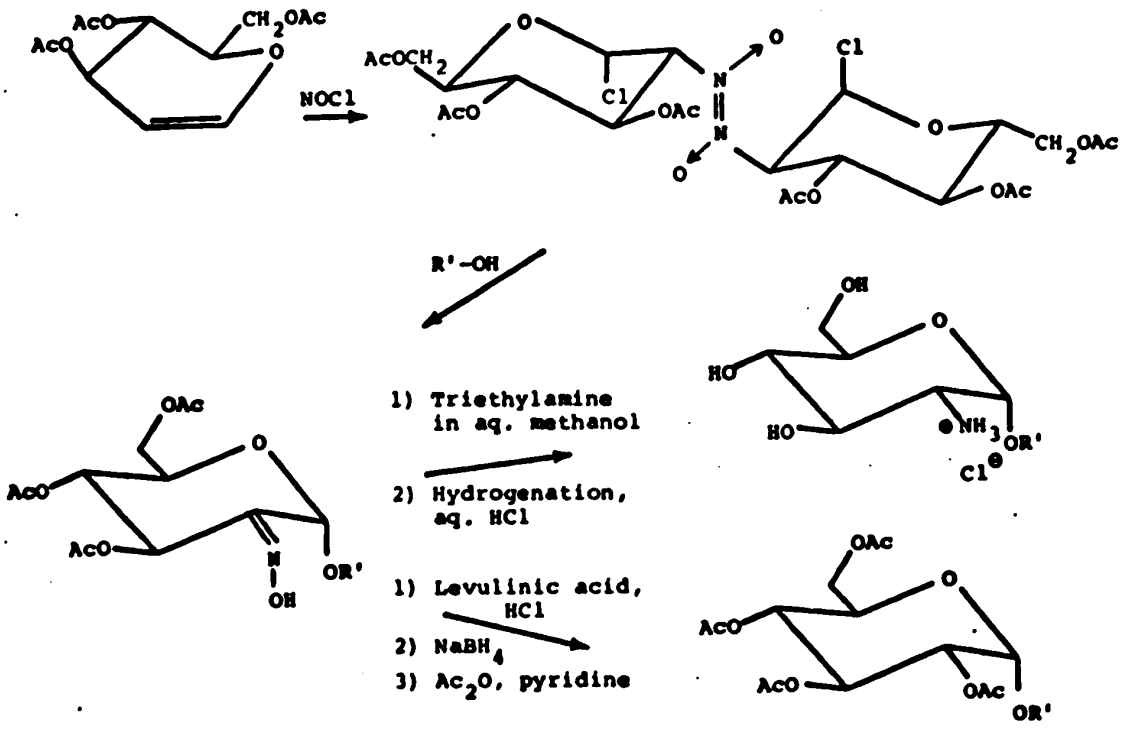


Diagram 4

resulting ketoglucopyranosides with sodium borohydride and O-acetylation, afforded the corresponding acetylated α-D-glucopyranosides (20,24). This method has since been applied to the general synthesis of 6-amino-6-deoxy-α-D-glucopyranosides (25). This new approach is outlined in diagram 4.

The major objective of the present research was to examine the possibility whether α-D-glucopyranosides could be prepared in good yield in a reaction of suitably O-protected D-glucopyranosyl halides with an alcohol in the presence of added halide ion, as outlined in diagram 5.

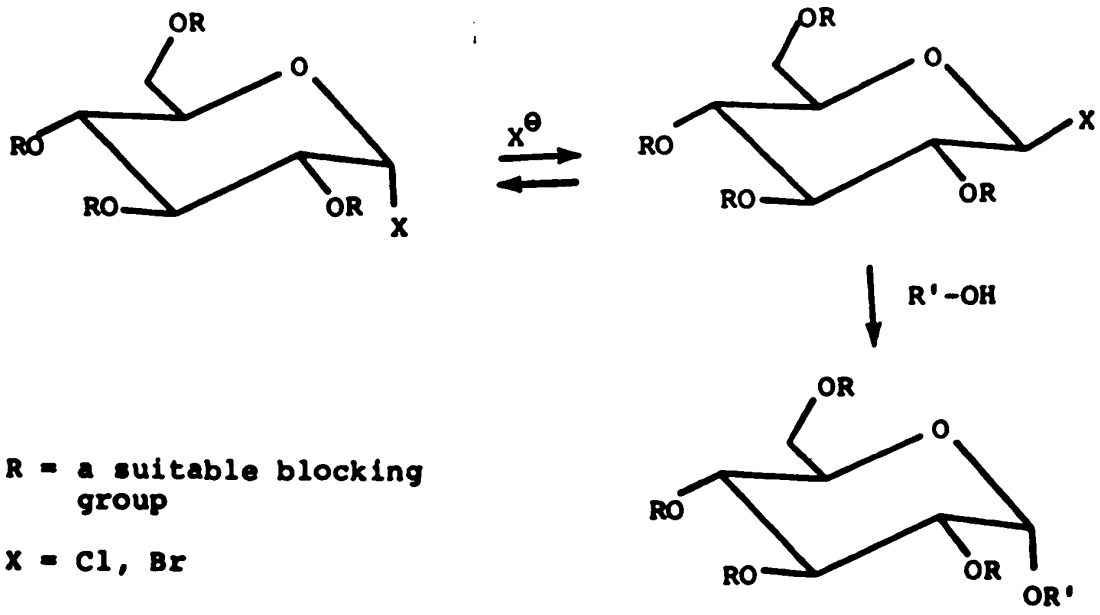


Diagram 5

Such a possibility became apparent from the investigations by Lemieux and Morgan (26). These authors reported in 1963 that if tetra-O-acetyl-α-D-glucopyranosyl bromide was

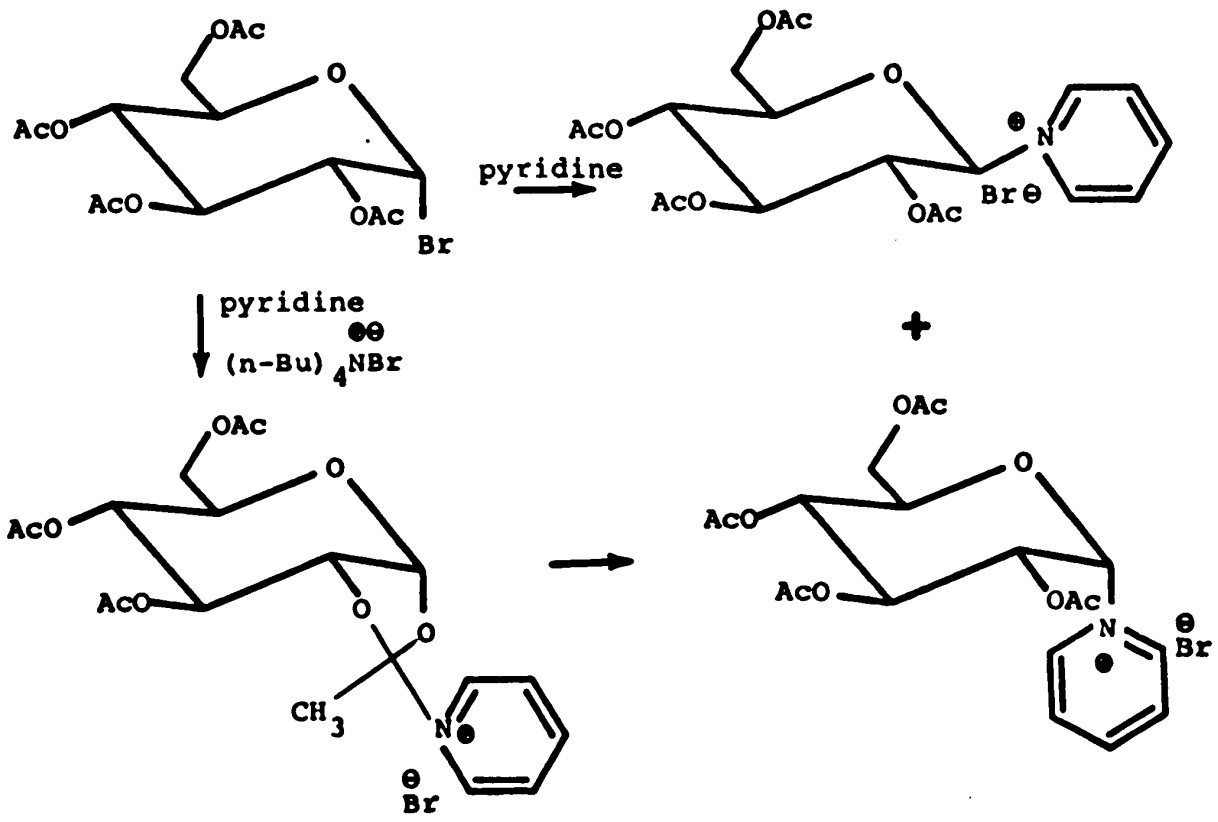


Diagram 6

reacted with pyridine, only formation of the corresponding β-N-glucoside was initially observed, but in the further course of the reaction also some of the α-anomer was

formed. If, however, the reaction was carried out in the presence of tetra-*n*-butylammonium bromide, virtually only the α-pyridinium glucoside was formed, as shown in diagram 6.

These surprising experimental results were interpreted in terms of bromide ion catalysis. If only pyridine was present in the reaction mixture, then, in forming the β-pyridinium glucopyranoside by direct nucleophilic attack of the pyridine at carbon-1, bromide ions would be liberated which would anomerize the starting material to the β-anomer. The N-α-D-glucopyranosyl pyridinium bromide derivative was then formed either by nucleophilic substitution by pyridine, or by way of a 1,2-acetoxonium ion formation followed by an intramolecular rearrangement of a transient 1,2-orthoacetyl pyridinium bromide. If, however, the reaction was carried out in the presence of added bromide ions, then fast anomerization of the tetra-O-acetyl-α-D-glucopyranosyl bromide to its β-anomer took place which yielded the α-pyridinium compound in the manner described above.

The anomerization of tetra-O-acetyl-β-D-glucopyranosyl chloride by chloride ion has been studied for the first time in great detail by Lemieux and Hayami (4). When tetra-O-acetyl-β-D-glucopyranosyl chloride was dissolved in dry acetonitrile, addition of chloride ion

in the form of tetraethylammonium chloride produced a rapid reaction as evidenced by a change in the rotation of the solution. After the optical rotation had reached a constant value the product consisted of the equilibrium mixture of the starting material and its α-anomer. The authors established that, at equilibrium, the solution contained 4.6 to 7.0% of the β-anomer. The polarimetric data provided good first-order kinetics after the reaction had been allowed to proceed for at least 10 min. Experiments related to the effect of the chloride ion concentration on the rate of anomerization showed that the reaction is first order both in chloride ion and the tetra-O-acetyl-β-D-glucopyranosyl chloride. Evidence was presented that the mechanism of the anomerization involved nucleophilic attack by chloride ion at the anomeric center with subsequent inversion of configuration. Experimental results excluded an intramolecular mechanism for the anomerization, as is known to occur for methyl tetra-O-acetyl-β-D-glucopyranoside (27). The transition state (see diagram 7) was suggested in the first step of the anomerization. That is to say that replacements at the anomeric center of sugar structures can proceed by way of bimolecular nucleophilic displacement processes which are accelerated by the participation of the ring oxygen in delocalization of the positive charge at the reacting

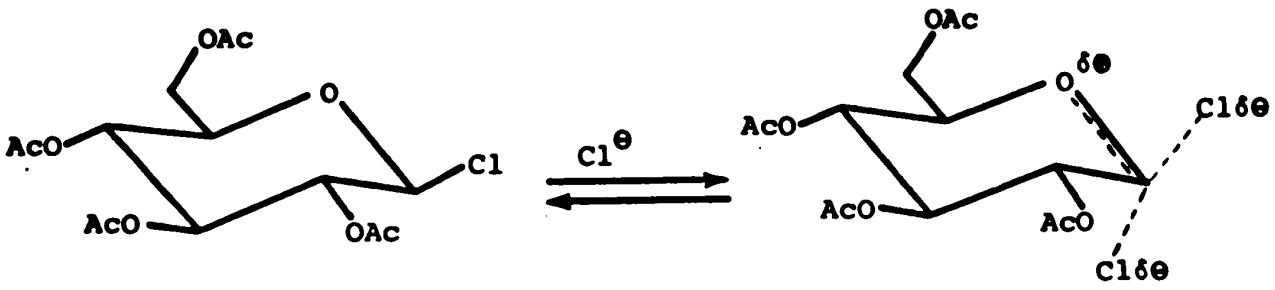


Diagram 7

center of the transition state. The nature of the bonding between the reacting center and the entering and leaving groups in the transition state may be similar to that between ions in intimate ion pairs (28). The above formulated transition state would hence represent an intimate ion triplet. It was concluded that the role of the chloride ion in the anomerization reaction may be considered to provide stabilization of the developing ion pair by leading to an ion triplet.

Numerous publications deal with the mechanism of nucleophilic substitution by an alcohol at the anomeric center of cyclic monosaccharide derivatives. In 1953, Newth and Phillips published a series of papers on the reactivity of O-acylglycosyl halides (29,30,31). They showed that solvolysis of tetra-O-acetyl-α-D-glucopyranosyl bromide in aqueous acetone or aqueous methanol

follows a unimolecular nucleophilic substitution. It was also shown that the reactivity of the halogen in the O-acyl-glycosyl halides is due to its being part of an α-halogeno-ether system and that rates of methanolysis are influenced by steric effects of substituents at carbon-2, 4 and 6. Chapman and Laird (32) pointed out that retention of configuration is not conclusive evidence against a bimolecular process since tetra-O-acetylated glucopyranosyl derivatives are well known for configurational instability at carbon-1, and the possibility of the inversion of a first-formed β-compound cannot be excluded. Lemieux and Huber (33) investigated the solvolysis of 3,4,6-tri-O-acetyl-β-D-glucosyl chloride in acetic acid and concluded that the chlorine atom is replaced by acetate by way of an intermediate carbonium ion with a strong tendency for inversion of the anomeric center. The ability of an S_N1 reaction to proceed with a high degree of inversion must be related to the conformation of the carbonium ion. Huber (34), in 1955, has reviewed the possible mechanisms for substitution reactions at carbon-1 of pyranose derivatives. Rhind-Tutt and Vernon (35) reported the results of a methanolysis study of tetra-O-methyl-α-D-glucopyranosyl chloride and its epimer of D-manno configuration. They observed that both methanolyses followed uncomplicated first-order kinetics

and concluded that the nucleophilic displacement proceeded by way of a carbonium ion intermediate. The observed nearly complete inversion of configuration at carbon-1 in the D-*gluco* compound was rationalized by a shielding effect of the departing anion. Capon, Overend and coworkers (36) concluded from measurements of the rates of solvolysis of a range of acetylated glucopyranosyl halides in aqueous acetone that the substitution of the halide at the anomeric center is unimolecular if there is a *cis* relationship between the acetoxy group at carbon-2 and the halogen at carbon-1. However, if there is a *trans* arrangement of these groups the solvolysis involves neighbour group participation. In 1966, Schroeder, Green and Johnson (37) examined the alcoholysis of tetra-O-acetyl-α-D-glucopyranosyl bromide with four primary and two secondary alcohols. They concluded that the primary alcoholyses proceeded by a unimolecular substitution mechanism, whereas a bimolecular pathway was suggested for the secondary alcoholyses. During the course of this research Ishikawa and Fletcher (38) published their work on the long-range effect of O-p-nitrobenzoyl groups as compared to O-benzyl groups on the formation and solvolysis of variously substituted D-glucopyranosyl bromides, all having an O-benzyl group at carbon-2. The rates of methanolysis of five different D-glucopyranosyl bromides

were measured and the ratio of methyl D-glucopyranosides was determined.

These short introductory remarks may serve to indicate the complexity of solvolysis reactions at carbon-1 of cyclic derivatives of glycosyl halides and their application to the synthesis of α-D-glucopyranosides.

EXPERIMENTAL

A. Materials

1. Solvents

(i) Dichloromethane

Reagent grade dichloromethane was shaken with portions of concentrated sulfuric acid until the acid layer remained colorless. It was then washed successively with water and a 5% aqueous sodium hydrogen carbonate solution. After drying over anhydrous calcium chloride, the solvent was distilled from anhydrous calcium sulfate and stored in a brown bottle over Linde type 4A molecular sieves (39).

(ii) 1,2-Dichloroethane

Reagent grade 1,2-dichloroethane was shaken with concentrated sulfuric acid to remove alcohol added as an oxidation inhibitor, and then washed with aqueous sodium bicarbonate solution followed by water. It was dried over anhydrous calcium chloride at room temperature, refluxed over phosphorus pentoxide, then fractionated through a 40 cm long Vigreux column and stored over Linde type 4A molecular sieves (39).

(iii) Acetonitrile

Reagent grade acetonitrile was dried by refluxing it for 5 hr over phosphorus pentoxide and then distilling it through a 40 cm long Vigreux column (4).

(iv) Nitromethane

Reagent grade nitromethane was dried over anhydrous calcium chloride and distilled through a 40 cm long Vigreux column (39).

(v) 1,4-Dioxane

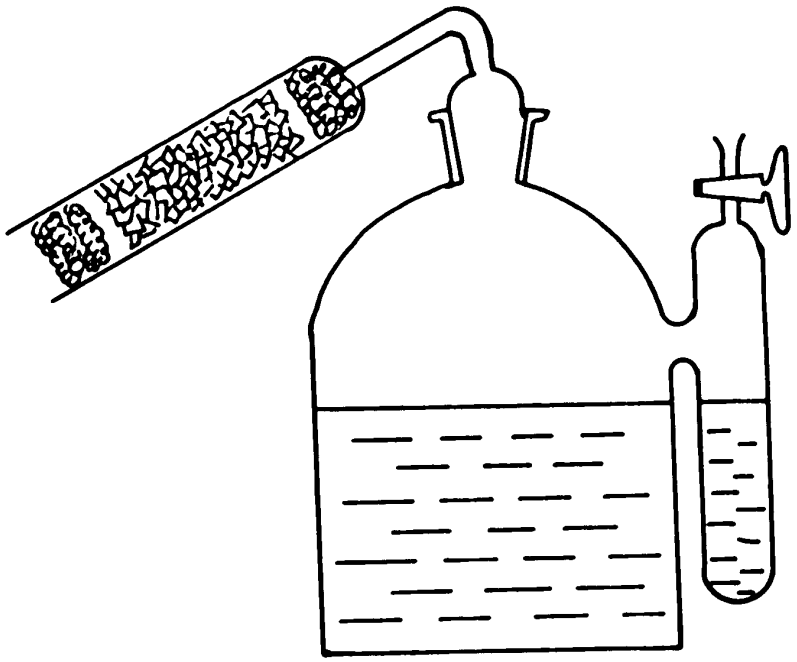
Reagent grade 1,4-dioxane was refluxed over sodium for 6 hr and fractionally distilled from fresh sodium through a 40 cm long Vigreux column (39).

(vi) Benzene

Reagent grade benzene was purified by azeotropic distillation and was then fractionated through a 40 cm long Vigreux column.

Solvents were directly distilled into a "dry solvent flask" as shown in the sketch on the following page.

The solvent was withdrawn through the serum cap with the aid of a hypodermic syringe.



2. Reagents

(i) Methanol

Reagent grade methanol was dried by heating it over magnesium metal (5 g magnesium per 500 ml methanol) in the presence of iodine (0.5 g) until all of the metal was dissolved. The solution was then refluxed for 2 hr and finally fractionated through a 40 cm long Vigreux column (39).

(ii) Ethanol

Denatured ethanol (98% ethanol, 2% benzene, approximately) was dried by reaction with magnesium

ethoxide, prepared by heating 5 g magnesium per 1 l ethanol in the presence of 0.5 g iodine. After refluxing the solution for 2 hr the ethanol was fractionated through a 40 cm long Vigreux column (39).

(iii) 2-Propanol

Reagent grade 2-propanol was refluxed with calcium oxide, distilled and the distillate further dried with calcium sulfate prior to fractional distillation using a 40 cm Vigreux column (39).

(iv) Triethylamine

Reagent grade triethylamine was dried with potassium hydroxide pellets and then distilled into a flask which was covered with black insulating tape to prevent the material from reacting under the influence of light.

(v) Diisopropylethylamine

Reagent grade diisopropylethylamine (Fluka A. G., Buchs, Switzerland) was refluxed over potassium hydroxide pellets and then distilled into a flask that was covered with black insulating tape to prevent the amine from reacting under the influence of light.

(vi) Solution of anhydrous hydrogen bromide in dry methylene chloride

This reagent was prepared by passing dry hydrogen bromide gas, with magnetic stirring, into methylene chloride. The solution was kept in the refrigerator, the container being wrapped in aluminum foil. Before each use, three 5 ml samples were taken out, suspended in a large volume of water (40 to 50 ml) and titrated with 0.1N sodium hydroxide solution against phenolphthalein as indicator, to determine the hydrogen bromide content.

(vii) Tetraethylammonium bromide

Commercial tetraethylammonium bromide (Eastman Organic Chemicals, Rochester 3, N.Y., U.S.A.) was recrystallized from absolute ethanol and thoroughly dried before use. In one case it was purified by crystallization from the solvent pair methylene chloride-diethyl ether.

(viii) 1,2;5,6-Di-O-isopropylidene-α-D-glucofuranose

This reagent was purchased from Raylo Chemicals Ltd., (Edmonton, Alberta, Canada) and was purified before use by recrystallization from Skellysolve B.

(x) 1,2;3,4-Di-O-isopropylidene-α-D-galactopyranose

This reagent was purchased from Aldrich Chemical

Co., Inc., (Milwaukee, Wis., U.S.A.) and was used without further purification.

B. Methods

1. Melting Points

All melting points were determined in capillary tubes on a Gallenkamp Melting Point Apparatus and are uncorrected.

2. Optical Rotation

Optical rotations were measured using a Perkin Elmer Model 141 automatic polarimeter. When a reaction was followed polarimetrically the temperature was kept constant by circulating thermostated water through the outer jacket of the decimeter tube.

3. Chromatography

(i) Thin Layer Chromatography

Thin layer chromatography (t.l.c.) was performed on Silica Gel G (E. Merck A. G., Darmstadt, W. Germany). Usually a solvent system containing benzene-methanol (95:5), in some cases a solvent system containing ether-Skellysolve B (7:3 to 4:6) was employed. The spots were visualized by spraying with 20% sulfuric acid and heating on a hot plate. Unsaturated products were detected by spraying the t.l.c. plates with a 1% potassium

permanganate solution (40).

(ii) Column chromatography

Wet column chromatography was carried out on columns of silicic acid (100 mesh; Mallinckrodt Chemical Works, Montreal), the fractions being collected by a mechanical fraction collector. Column size and elution solvents are given under the appropriate experiments. In some cases SilicAR CC-7 (100-200 mesh; Mallinckrodt) was used. Individual fractions were examined both by optical rotation and t.l.c.

(iii) Gas-liquid partition chromatography (G.l.p.c.)

Gas-liquid partition chromatography was performed with an F & M Programmed Temperature Gas Chromatograph (Model 500) fitted with a thermal conductivity detector. O-Trimethylsilyl derivatives of sugars were prepared according to Sweely and coworkers (41) (see also section D, paragraph 3, p. 66). These derivatives were chromatographed on a column (8' by 1/4", copper tubing) packed with 3% SE-52 which was prepared from 750 mg Silicone Gum Rubber (LP 122) and 25 g Chromosorb W, 30-60 mesh, non-acid washed. The column was purged at 225° for 24 hours. Helium was used as the carrier gas. The flow rate was approximately 100 ml/min with an inlet pressure of 30 p.s.i.

4. Spectroscopic Investigations

Nuclear magnetic resonance spectra (n.m.r.) were measured at 60 MHz with a Varian A-60 or A-56/60A spectrometer in deuterated chloroform or the solvents noted in the text. Spectra at 100 MHz were measured with a Varian HA 100 spectrometer. Chemical shifts are reported in tau (τ) values (estimated error ± 0.05 p.p.m.) and refer to resonance signals relative to the signal of tetramethyl silane (TMS) used as the internal standard. The spectra were measured by the personnel of the departmental spectroscopic services laboratories.

5. Solvent Removal

All solvents were removed from solutions on a rotary evaporator using the vacuum from a water aspirator at temperatures of 40-45°C.

C. Preparation of Starting Materials

1. Methyl tetra-O-benzyl-α-D-glucopyranoside (2)

(i) In the early stages of this work the procedure described by Tate and Bishop (42) was used to prepare the above compound.

A mixture of finely powdered methyl α-D-glucopyranoside (15 g), benzyl chloride (375 ml), dioxane (150 ml) and sodium hydride (30 g), freed from mineral oil by washing five times with Skellysolve B, was stirred mechanically under anhydrous conditions at 125 to 130°C. After 3 to 4 hr the sodium hydride was removed by filtration, washed three times with benzene and the combined filtrates were then steam-distilled until the distillate was clear. This operation required approximately one day. The oily residue was extracted with chloroform and the solution was then dried over anhydrous potassium carbonate and evaporated *in vacuo*. The product, which was formed in 75% yield, was a dark yellow syrup.

(ii) In subsequent experiments the procedure of Brown and coworkers (43) was employed.

In a three-necked flask equipped with an efficient condenser, a thermometer and a mechanical stirrer

were placed anhydrous methyl α-D-glucopyranoside (50 g, 0.258 mole) dissolved in a mixture of dry dimethylformamide (650 ml) and 1,2-dimethoxyethane (350 ml). Benzyl chloride (560 ml, 615 g, 4.85 mole) was then added and the mixture was heated to about 100°C. Sodium hydride (150 g, washed three times with Skellysolve B and partially air-dried) was added slowly in small portions to the hot solution. Immediate reaction occurred (evolution of gas, heavy reflux). After a few minutes the reaction slowed down and more of the sodium hydride was added. This procedure was repeated until all the 150 g sodium hydride was added, which required about one half hour. The mixture was then stirred for another two hours maintaining a temperature of 120°C. After cooling, the excess of sodium hydride was removed by filtration and the filtrate subjected to steam distillation under reduced pressure in a flash evaporator (cyclone type evaporator) by slowly sucking water into the system. Thus the bulk of the organic solvents and of the benzyl chloride were removed quite rapidly. The remaining oily liquid (about 200 ml) was subjected to a high vacuum distillation.

That part of compound 2 which was to be hydrolyzed as described below was not further purified.

Material which was to be used as internal standard for alcoholysis reactions was purified by chromatography through a 4 cm x 65 cm glass column packed with silicic acid as a slurry in benzene-methanol (95:5). Crude compound 2 was chromatographed in portions of 10 g using the same solvent pair as the eluant and 15 ml fractions were collected. The pure fractions were pooled, concentrated and dried under high vacuum to give 5.5 g of purified methyl tetra-O-benzyl-α-D-glucopyranoside as a light yellow syrup. It had $[\alpha]_D^{24}$ + 35° (c, 5 in chloroform, [reported for 2 (44), $[\alpha]_D^{25}$ + 32.2° (c, 5 in chloroform)]. The n.m.r. spectrum in deuteriochloroform showed a sharp singlet at τ 6.64 for the protons of the methoxy group; a band centered at about τ 2.69 for the aromatic protons; a range of peaks between τ 5.0 and τ 5.7 for the benzylic methylene protons and H-1; and a multiplet centered at around τ 6.3 for the ring protons, including methylene protons at carbon-6.

The compound was susceptible to autooxidation as indicated by an odour of benzaldehyde and a change in the n.m.r. spectrum after standing for one month. A satisfactory elementary analysis could therefore not be obtained.

2. Tetra-O-benzyl-α-D-glucopyranose (3)

Methyl tetra-O-benzyl-α-D-glucopyranoside (2;

40 g) was hydrolyzed in a mixture of glacial acetic acid (800 ml) and 2N sulfuric acid (580 ml) for 18 hr on a steam bath. However, the hydrolysate was not poured into cold water as recommended by Tate and Bishop (30), but rather left overnight at 4°C in the coolroom whereupon the product crystallized in fine long needles. After two recrystallizations from methanol the yield was ca. 30% of pure 3, m.p. 151-152°C, $[\alpha]_D^{25}$ + 19.0° (c, 2.5 in chloroform), [reported for 3 (44) $[\alpha]_D^{20}$ + 21.2 (c, 3.5 in chloroform)]. The n.m.r. spectrum at 60 MHz showed a broad peak centered around τ 4.8 for the anomeric proton; a band centered at about τ 2.79 for the aromatic protons; a range of peaks between τ 5.0 and 5.7 for the benzylic methylene protons; and a multiplet centered at around τ 6.35 for the ring protons, including the methylene protons at carbon-6.

Anal. Calcd. for $C_{34}H_{36}O_6$: M.W. 540.63.
C, 75.53; H, 6.71%.
Found: C, 75.68; H, 6.86%.

3. Tetra-O-benzyl-α-D-glucopyranosyl chloride (4)

Compound 3 (2.91 g) was added in one portion to pure thionyl chloride (10 ml) and the resulting solution was kept at 70°C for 3 hr. After concentrating *in vacuo*,

the residue was freed from the residual thionyl chloride by codistillation with toluene in the rotary evaporator. This procedure was carried out three times and the glucosyl chloride (4) was obtained as a dark brown syrup. After treatment with charcoal in chloroform, it had $[\alpha]_D^{24}$ + 92.5° (c, 3.2 in benzene), [reported for 4 (45) $[\alpha]_D$ + 95° (c, 4.0 in benzene)]. The n.m.r. spectrum showed a doublet at τ 3.95 with a spacing of 3.5 Hz for the anomeric proton; a band centered at about τ 2.74 for the aromatic protons; a range of peaks between τ 5.05 and 5.7 for the benzylic methylene protons; and a multiplet centered at around τ 6.3 for the ring protons, including the methylene protons at carbon-6.

Anal. Calcd. for $C_{34}H_{35}O_5Cl$: M.W. 559.07.
C, 73.03; H, 6.31; Cl, 6.35%.
Found: C, 73.02; H, 6.24; Cl, 6.37%.

4. Tetra-O-benzyl-1-O-(p-nitrobenzoyl)-α-D-glucopyranose (5)

To a solution of tetra-O-benzyl-α-D-glucopyranose (3; 17.5 g, 32.4 mmoles) in anhydrous pyridine (75 ml) was added p-nitrobenzoyl chloride (6.63 g, 1.1 molar excess) and the mixture was heated for 2 hr under reflux. The excess pyridine was then removed *in vacuo*. Compound 5 crystallized after treatment with ethanol in

80% yield (18.5 g), m.p. 125°C [as reported by Tate and Bishop (42)] and $[\alpha]_D^{24}$ + 72.7°, (c, 3.12 in chloroform), [reported (42) $[\alpha]_D^{26}$ + 72.3° (c, 2.13 in chloroform). The n.m.r. spectrum at 100 MHz shows: a doublet at τ 1.79 for the aromatic protons of the p-nitrobenzoyl group; a band centered at about τ 2.73 for the aromatic benzyl protons; a doublet at τ 3.42 for the anomeric proton ($J_{1,2}$ = 3.2 Hz); a range of peaks between τ 5.05 and 5.65 for the benzylic methylene protons; and a multiplet centered at about τ 6.20 for the ring protons, including the methylene protons at carbon-6.

Anal. Calcd. for $C_{41}H_{39}O_9N$: M.W. 689.73.
C, 71.39; H, 5.70; N, 2.03%.
Found: C, 71.23; H, 5.41; N, 1.83%.

5. Tetra-O-benzyl-α-D-glucopyranosyl bromide (6)

(i) In a typical experiment compound 5 (2.1 g, 3 mmole) was dissolved in methylene chloride (3 ml) and a slight excess of a standardized solution of hydrogen bromide (1.1 moles per mole of 5) in methylene chloride was added rapidly. p-Nitrobenzoic acid began to precipitate after a minute. The reaction vessel was kept in the refrigerator for 1/4 hr, then the p-nitrobenzoic acid was removed by filtration and washed with the same

solvent. The filtrate was washed twice with a concentrated aqueous solution of sodium hydrogen carbonate to remove any excess of hydrogen bromide as well as p-nitrobenzoic acid, then washed once with water, dried over anhydrous potassium carbonate and evaporated *in vacuo*. The glycosyl bromide 6, formed in approximately 95% yield, was a colourless syrup, which had $[\alpha]_D^{24}$ + 104.9° (c, 2.69 in chloroform), [reported (46), $[\alpha]_{578}^{20}$ + 151°]. The n.m.r. spectrum displayed a doublet for the anomeric proton centered at τ 3.55 with a spacing of 3.5 Hz; a band centered at about τ 2.7 for the aromatic benzyl protons; a range of peaks between τ 5.0 and 5.70 for the methylene protons of the benzyl groups; and a multiplet centered at about τ 6.25 for the ring protons, including the methylene protons at carbon-6.

The product so obtained was immediately reacted in alcoholysis experiments.

The precipitated p-nitrobenzoic acid was collected, dried and weighed for a yield determination. In the various preparations the recovered p-nitrobenzoic acid amounted to a yield of approximately 95% formation of the α-D-glucosyl bromide.

(ii) In view of the unstability and high reactivity of the tetra-O-benzylated glucosyl bromide, its purity

was checked before carrying out each glycosidation reaction in several ways:

a) The n.m.r. spectrum of the freshly prepared sample was measured, whenever this was possible. (See Fig. 5, p. 97)

b) The optical rotation of compound 6 was taken before the glycosidation reactions were carried out.

c) The glucopyranosyl halide 6 (0.5 mmole) was hydrolyzed by heating it in 5 ml 95% ethanol and 1 ml 0.75 N sodium hydroxide for 15 min. The solution was then buffered with an acetic acid - sodium acetate buffer, and the liberated bromide ion was determined by titration with a 0.05 M silver nitrate solution according to Fajan's (47) method, using dichlorofluorescein (0.1% in 70% ethanol) as indicator. As judged from the bromide ion content, the compound 6 was found to be between 90% and 95% pure. In a case where the amount of titrated bromide ion indicated a purity of less than 90% the preparation of starting material was repeated.

d) Compound 6 (0.5 mmole) was dissolved in 5 ml dichloromethane and converted into the corresponding 1-O-acetyl-tetra-O-benzyl-β-D-glucopyranose with silver acetate and a glacial acetic acid - acetic anhydride mixture, as described below. Methyl tetra-O-benzyl-α-D-

glucopyranoside (0.5 mmole) was present throughout the reaction as internal standard. After proper work-up, the ratio of the intensities of the O-methyl peak to that of the O-acetyl signal in the n.m.r. spectrum gave a fairly accurate indication of the state of purity of compound 6. In most cases, compound 6 was found to be of 90% to 95% purity and this was taken into consideration when calculating the yields of glycosidation products.

(iii) To test the stability of the glucosyl halide 6 at lower temperatures, the following experiments were carried out.

Two portions of one gram each of the freshly prepared D-glucosyl bromide were placed in small vials with a screw cap, and one was placed into a Dewar flask containing a dry ice-acetone mixture, the other into a Dewar vessel filled with liquid nitrogen. After 1, 2, 4, and 8 days, 100 mg of each sample were submitted for n.m.r. analysis. The spectra were in all cases identical with each other and with the spectrum of freshly prepared tetra-O-benzyl-α-D-glucopyranosyl bromide. Apparently this material is stable at low temperatures and can therefore, if necessary, be stored for some period of time. However, for the subsequently described alcoholysis experiments the starting material, compound 6, was freshly prepared each time.

6. Methyl tetra-O-benzyl-β-D-glucopyranoside (7)

This compound was prepared from methyl β-D-glucopyranoside in a manner similar to that described for the preparation of compound 2 (see p. 27). The product, after recrystallization from ethanol, had a m.p. of 68 to 69°C and $[\alpha]_D^{23}$ + 11° (c, 5.3 in dioxane). The n.m.r. spectrum showed a single sharp peak at τ 6.45 for the protons of the equatorially oriented methoxy group at carbon-1.

Anal. Calcd. for $C_{35}H_{38}O_6$: M.W. 554.65.

C, 75.79; H, 6.91%.

Found: C, 75.99; H, 6.88%.

7. Tetra-O-benzyl-1,5-anhydro-D-*arabino*-hex-1-enitol (8)

This compound was prepared according to the procedure outlined by Preobrazhenskaja and Suvorov (48).

A dry solution of indole (1.5 g, 12 mmole) in ether (30 ml) was added to a solution of sodium (0.1 g) in anhydrous liquid ammonia (100 ml). Twenty minutes after the blue color had disappeared from the reaction mixture, 20 ml of an ethereal solution of the glucopyranosyl bromide 6 (2 g, 3 mmole) was added. The reaction mixture was left overnight at room temperature. A further 50 ml of ether was added and the solution was washed with water, dried with anhydrous sodium sulfate

and evaporated *in vacuo* to give an oily residue which was crystallized from ethanol. The product, which was recrystallized from methanol, was formed in 53% yield (910 mg). It has a m.p. 66 - 66.5°C, $[\alpha]_D^{20}$ - 5.1° (c, 1.51 in chloroform), [reported (48) m.p. 66 - 66.5°C, $[\alpha]_D$ - 5.1° (c, 1.56 in chloroform]. The n.m.r. spectrum in deuteriochloroform showed a single sharp peak at τ 3.75 for the proton at carbon-1; a band centered at about τ 2.75 for the aromatic benzyl protons; a range of peaks between τ 5.05 and 5.65 for the methylene protons of the benzyl groups; and a multiplet centered at τ 6.1 for the ring protons, including the methylene protons at carbon-6.

Anal. Calcd. for $C_{34}H_{34}O_5$: M.W. 522.61.
C, 78.13; H, 6.56%.
Found: C, 78.14; H, 6.36%.

8. Conversion of tetra-O-benzyl-α-D-glucopyranosyl bromide into 1-O-acetyl-tetra-O-benzyl-D-glucopyranose

(i) The title compound was at first prepared according to Baddiley and coworkers (45). Tetra-O-benzyl-α-D-glucopyranose (1 g) was added to a mixture of 2.5 ml acetic anhydride and 5 ml pyridine. The mixture was left for two days at room temperature, and then the 1-O-acetate was isolated, as a syrup, in the usual manner. It was formed in 95% yield, having $[\alpha]_D^{24}$ + 50° (c, 2.2 in

benzene) [reported (45) $[\alpha]_D$ + 51° (c, 4.2 in benzene)]. The high positive rotation and also the n.m.r. spectrum indicate that mostly the α-derivative was formed. The n.m.r. spectrum showed a doublet centered at τ 3.58, $J_{1,2}$ = 3.0 Hz for the anomeric proton. There are two peaks for the acetoxy group, at τ 7.92 and τ 8.01 in the ratio of approximately 10:1, indicating an α to β ratio of 10:1. The n.m.r. spectrum of this compound is shown in Fig. 6, p. 102.

(ii) Attempts to convert the glucopyranosyl bromide 6 essentially quantitatively into the tetra-O-benzyl-1-O-acetyl-D-glucopyranose, in order to obtain a useful method of determining the amount of unreacted tetra-O-benzyl-α-D-glucopyranosyl bromide from the alcoholysis reactions, were carried out in the following manner.

Tetra-O-benzyl-α-D-glucopyranosyl bromide (302 mg, 0.5 mmole) was dissolved in dry methylene chloride (4 ml) in a 7-dram vial. Silver acetate (668 mg, 4 mmole) was added, followed by 0.210 ml of a solution containing 0.115 ml glacial acetic acid (120 mg, 2 mmole) and 0.095 ml acetic anhydride (102 mg, 1 mmole). The mixture was protected from the influence of light and was shaken for two hours at room temperature. The silver salts were then removed by filtration. The filtrate was washed three

times with water, dried over potassium carbonate and evaporated to dryness *in vacuo*. Remaining traces of acetic acid were chased out by adding 20 ml of benzene and evaporating to dryness *in vacuo*. This was done three times.

The n.m.r. spectrum of the resulting syrupy product (220 mg, 90% yield) shows a strong doublet at τ 4.37, $J^{\beta}_{1,2}$ = 7.5 Hz and a weak doublet at τ 3.63, $J^{\alpha}_{1,2}$ = 3.2 Hz. There is also a strong singlet at τ 8.03 and a smaller one at τ 7.94. The ratio of the intensities for these peaks indicate that the β-anomer was formed preferably, i.e. in a ratio of 5:1 over the α-anomer. The n.m.r. spectrum is reproduced in Fig. 7, p. 102. A 1:1 molar mixture of methyl tetra-O-benzyl-α-D-glucopyranoside and 1-O-acetyl-tetra-O-benzyl-α-D-glucopyranose was prepared and submitted for n.m.r. spectroscopy in order to check the integration response for the signal of the methoxy group as compared to the acetoxy group signal. As expected the integration ratio for the two signals was measured to be 1:1. The spectrum is shown in Fig. 8, p. 104.

The next step was to carry out this conversion under the conditions which are present during the alcoholysis reaction. The conversion of tetra-O-benzyl-α-D-glucopyranosyl bromide into the 1-O-acetate was

carried out in the presence of tetraethylammonium bromide, triethylamine and methyl tetra-O-benzyl-α-D-glucopyranoside as internal standard with: (a) only silver acetate; (b) with silver acetate and glacial acetic acid; and finally (c) with silver acetate and acetic anhydride present.

a) Tetra-O-benzyl-α-D-glucopyranosyl bromide (302 mg, 0.5 mmole) was dissolved in dry methylene chloride (4 ml) in a 5-dram vial which contained methyl tetra-O-benzyl-α-D-glucopyranoside (278 mg, 0.5 mmole). Tetraethylammonium bromide (106 mg, 0.5 mmole) was added along with triethylamine (0.12 ml, 1 mmole). Silver acetate (334 mg, 2 mmole) was then added and the mixture was shaken in the dark for two hours. After work-up as described above, the relative intensities for $-OCH_3$:$-OCOCH_3$ in the n.m.r. spectrum was 1.2:1. (Average of four measurements)

b) Exactly the same conditions were employed as under (a), except the reaction was carried out in the presence of 2 ml glacial acetic acid. The relative intensities of the $-OCH_3$:$-OCOCH_3$ signals in the n.m.r. spectrum were in a ratio of 1.1:1.

c) Exactly the same conditions were employed as under (a) except that the reaction was carried out in

the presence of 2 ml acetic anhydride. After proper work-up the relative intensities of $-OCH_3$:$-OCOCH_3$ signals in the n.m.r. spectrum had a ratio of 1.05:1.

In all three cases the β-anomers were formed predominantly as evidenced by the chemical shift for the anomeric proton and the characteristic spacing: τ 4.38, $J_{1,2}$ = 7.5 Hz. Fig. 9 on p. 104 shows the spectrum of the products obtained in the reaction described in the above paragraph.

D. Glycosidation Reactions

1. Influence of Added Halide Ion

(i) Glycosidation in the presence of halide ion (Reaction Series A)

Tetra-O-benzyl-α-D-glucopyranosyl bromide (302 mg, 0.5 mmole) of 94% purity was dissolved in 5 ml methylene chloride. Anhydrous tetraethylammonium bromide (106 mg, 0.5 mmole) was then added to the solution and the solution transferred into a Carius tube (test tube with constriction to facilitate sealing). Diisopropyl-ethylamine (0.092 ml, 0.5 mmole) and methanol (0.041 ml, 1 mmole) were introduced quickly. The tube was now sealed and placed in an oilbath maintained at 60°C. Five such experiments in five different tubes were carried out

simultaneously. Tubes were removed from the oilbath after 5, 15, 30, 60 and 120 min and the reaction quenched by immersing the tube in a dry ice/acetone bath. The tube was then opened and the content poured into a 7-dram vial containing 668 mg silver acetate (4 mmole, 7-fold excess over starting material); 0.210 ml of a solution containing 0.115 ml glacial acetic acid (120 mg, 2 mmole) and 0.095 ml acetic anhydride (102 mg, 1 mmole) were then added, the vial was protected from light and the reaction mixture shaken for 1 hr at room temperature. The silver salts were removed by filtration, the filtrate washed twice with water and the washings extracted each time with a small amount of methylene chloride. The combined methylene chloride layers were dried with potassium carbonate, filtered and evaporated *in vacuo*. Traces of acetic acid were removed by adding 20 ml of benzene and evaporating it *in vacuo*. This was done three times with each sample. The syrupy, colorless product was left connected to the high vacuum pump overnight and submitted for 100 MHz n.m.r. investigation.

This series of experiments was termed series A. Table 1 shows the relative intensities of the resonance signals for the methoxy and acetoxy protons of the products of series A. Table 2 presents the fractions of methyl tetra-O-benzyl-α- and β-D-glucopyranoside and 1-O-acetyl-

tetra-O-benzyl-D-glucopyranose of experimental series A.

TABLE 1

Relative intensities of the n.m.r. (100 MHz) signals for the methoxy and acetoxy protons of methyl tetra-O-benzyl-α- and β-D-glucopyranoside and 1-O-acetyl-tetra-O-benzyl-α- and β-D-glucopyranose from the products of reaction series A.

Time [min]	α-Methoxy intensity (c·d)*	β-Methoxy intensity (a·b)*	Acetoxy (α+β) intensity (c·d) + (a·b)*	α/β (glucoside)
5	33.6	14.5	210	2.3
15	176	34	185	5.16
30	189	22.5	74	8.4
60	260	29	45	9.0
120	223	24	27	9.3

* See pages 45 and 47.

TABLE 2

Fractions of methyl tetra-O-benzyl-α- and β-D-glucopyranoside and 1-O-acetyl-tetra-O-benzyl-D-glucopyranose in reaction series A, as calculated from the data in Table 1.

Time [min]	Appearance of α-D-glucoside	Appearance of β-D-glucoside	Disappearance of starting material
5	0.13	0.06_6	0.81
15	0.45	0.08	0.47
30	0.66	0.08	0.26
60	0.78	0.08_5	0.13
120	0.81	0.08_7	0.09_8

(ii) Glycosidation without added halide ion (Reaction Series B)

The same series of experiments as described under the previous heading was carried out, however omitting the tetraethylammonium bromide. The n.m.r. spectra of these two series of experiments are reproduced in the "Discussion" part on p. 110 and 116.

Mixtures of the two anomers of methyl tetra-O-benzyl-D-glucopyranoside with a α to β ratio of 3:1, 2:1, 1:1, 1:2 and 1:3 were prepared and 100 MHz n.m.r. spectra were taken of each of these samples in order to obtain

appropriate reference spectra. Table 3 shows the relative intensities of the resonance signals for the methoxy protons of different mixtures of methyl tetra-O-benzyl-α- and β-D-glucopyranoside.

Fig. 10 depicts a plot of the observed α to β ratio versus the actual composition. This calibration curve was used in correcting the α to β ratios for the anomeric methyl D-glucopyranosides formed in reaction series B. It was obtained, as indicated on Fig. 11, by taking the ratio c·d/a·b (height x width at half height) from the n.m.r. spectra (100 MHz) of the authentic mixtures.

TABLE 3

Relative intensities of the n.m.r. (100 MHz) signals for the methoxy protons in different mixtures of methyl tetra-O-benzyl-α- and β-D-glucopyranoside.

Composition of mixture (α to β ratio)	Ratio of intensities of α- and β-methoxy protons ($\frac{c.d}{a.b}$)	Observed α to β ratio
3:1	163:74	2.2
2:1	176:128	1.38
1:1	84:129	0.65
1:2	52:146	0.36
1:3	33:144	0.23

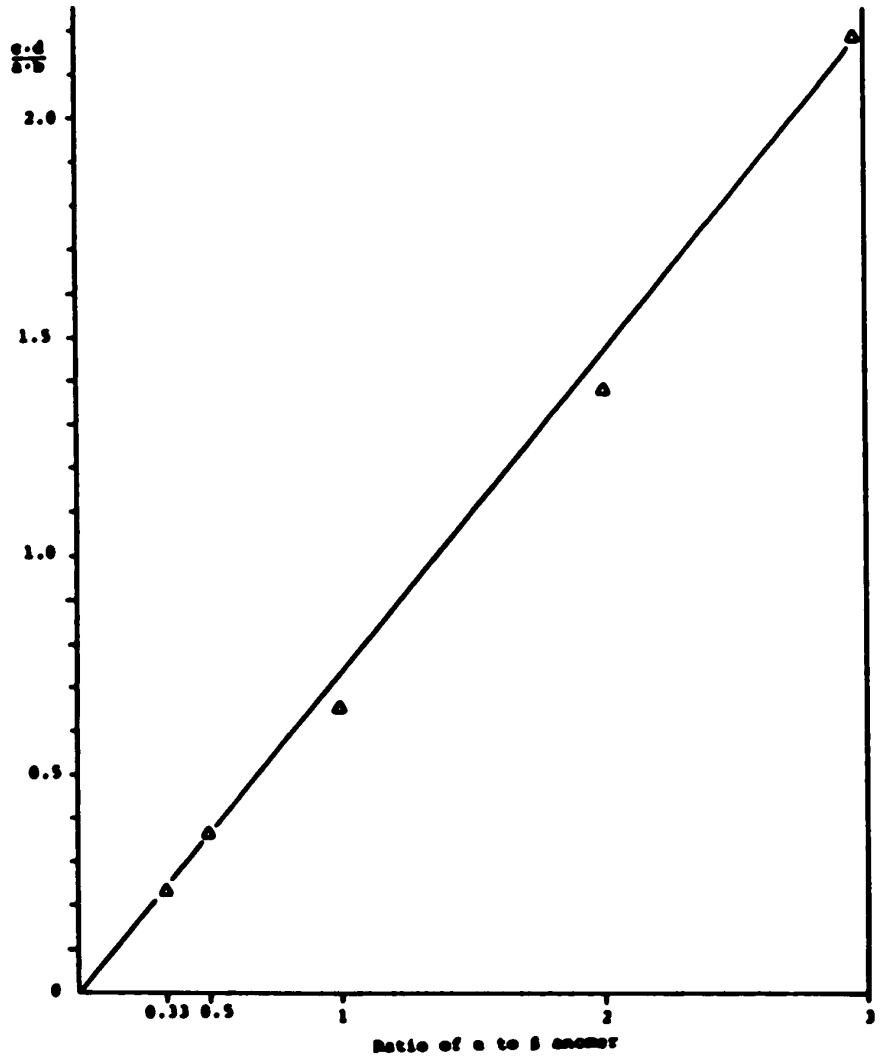


Fig. 10. Calibration curve for estimating the ratio of the α to β anomer of methyl tetra-O-benzyl-D-glucopyranoside ($\frac{c.d}{a.b}$) established as indicated in Fig. 11.

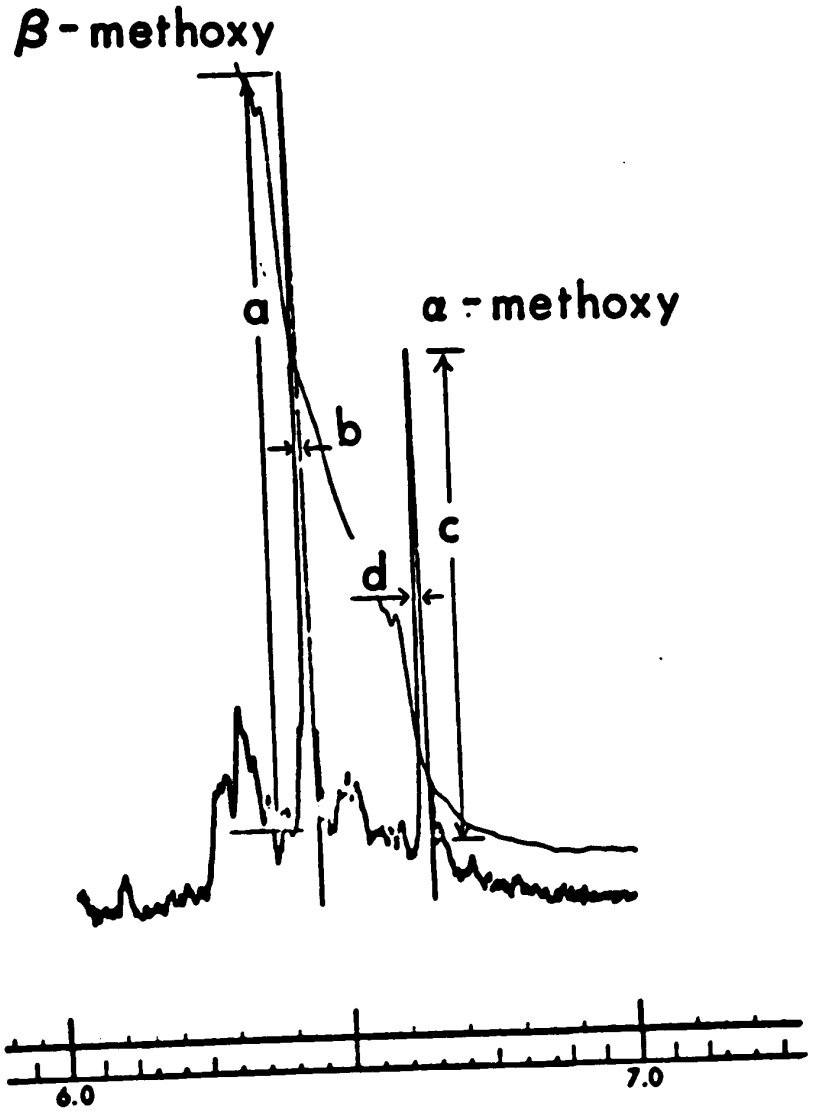


Fig. 11. Example of an n.m.r. spectrum (100MHz) and measurements made in connection with the calibration curve given in Fig. 10 to estimate the ratio of the methyl tetra-O-benzyl-α- and β-D-glucopyranosides in a product. The α to β ratio was taken as reflected by the ratio $(\frac{c \cdot d}{a \cdot b})$. The spectrum shows a 1:1 mixture of the α- and β-anomers in deuteriochloroform.

TABLE 4

Relative intensities of the n.m.r. (100 MHz) signals for the methoxy and acetoxy protons of methyl tetra-O-benzyl-α- and β-D-gluco-pyranoside and 1-O-acetyl-tetra-O-benzyl-α- and β-D-glucopyranose from the products of reaction series B.

Time [min]	α-Methoxy intensity (c.d)	β-Methoxy intensity (a.b)	Acetoxy (α+β) intensity (c·d) + (a·b)	α/β (glucoside)	α/β corrected
5	10.5	61	238	0.17	0.23
15	16.8	54	180	0.31	0.43
30	42	70	182	0.60	0.83
60	124	108	175	1.15	1.16
120	191	65	67	2.94	[2.94]

TABLE 5

Fractions of methyl tetra-O-benzyl-α- and β-D-glucopyranoside and 1-O-acetyl-tetra-O-benzyl-D-glucopyranose in reaction series B, as calculated from the data in Table 4.

Time [min]	Appearance of α-D-glucoside	Appearance of β-D-glucoside	Disappearance of starting material
5	0.03_4	0.20	0.77
15	0.06_7	0.21	0.72
30	0.14	0.24	0.62
60	0.31	0.26	0.43
120	0.59	0.20	0.21

The relative intensities of the resonance signals for the methoxy and acetoxy protons of methyl tetra-O-benzyl-α- and β-D-glucopyranoside and 1-O-acetyl-tetra-O-benzyl-α- and β-D-glucopyranose from the products of reaction series B were measured and are collected in Table 4.

Table 5 shows the appearance of the anomeric methyl D-glucopyranoside derivatives and the disappearance of starting material 6 as indicated by the disappearance of the acetoxy signal.

2. Rate of Glycoside Formation under Varying Conditions

Monitoring by n.m.r., using isopropanol as the potential aglycon and methyl tetra-O-benzyl-α-D-glucopyranoside as internal standard.

Thirteen series of experiments were carried out under various conditions. They are designated series A to M. For the first four series of experiments, reactions were stopped after 5 min, 15 min, 30 min, 1 hr, 2 hr and 4 hr. From series E to M reactions were stopped after 15 min, 30 min, 1 hr, 2 hr and 4 hr.

Experimental series A to M were conducted in the following manner.

Methyl tetra-O-benzyl-α-D-glucopyranoside, (277 mg, 0.5 mmole) was placed in a 3-dram vial. Freshly prepared tetra-O-benzyl-α-D-glucopyranosyl bromide (302 mg, 0.5 mmole) was added and this mixture was then dissolved in 4 ml dry methylene chloride. Tetraethylammonium bromide (106 mg, 0.5 mmole) was now added along with 0.14 ml triethylamine (1 mmole). After all the salt was dissolved, the solution was transferred by means of a syringe into a Carius tube (test tube with constriction to facilitate sealing). The 3-dram vial was then rinsed with 1 ml dry methylene chloride. The test tube was plugged with cotton wool and stoppered with a cork

stopper and immersed into a dry ice-acetone bath. After being cooled down sufficiently, 0.068 ml dry isopropanol (1 mmole) was added with a Hamilton syringe. The tube was then sealed quickly and put into an oil bath of 80°C.

At the end of the reaction time, the Carius tube was rapidly cooled by inserting it into a dry ice-acetone mixture, opened with a glass cutter and poured into a 7-dram vial which contained 668 mg silver acetate (4 mmole), 7-fold excess over starting material and 0.210 ml of a solution containing 0.115 ml glacial acetic acid (120 mg; 1 mmole). The vial was covered to protect it from the influence of light and the reaction mixture was shaken for at least one hour at room temperature.

The silver salts were now removed by filtration, the filtrate washed twice with water in a separatory funnel and the aqueous washings were extracted each time with a small amount of methylene chloride. The combined methylene chloride layers were dried with potassium carbonate, filtered and evaporated *in vacuo*. Traces of acetic acid were removed by adding 20 ml of benzene and evaporating it *in vacuo*. This was done three times. The syrupy, usually colorless, sometimes yellow product was left connected to the high vacuum pump overnight. The mixture (product plus

internal standard), averaging a weight of 400 mg, was then dissolved in 0.4 ml deuteriochloroform containing 1% tetramethylsilane, filtered through cotton wool into an n.m.r. tube and submitted for n.m.r. spectroscopy (60 MHz). The signals for the protons of the O-methyl, the acetoxy and of the O-isopropyl group were observed and at least five integrations were done at two different integral amplitudes. An example of such a series of n.m.r. spectra is shown and discussed in the Discussion part on p.138. Table 6, on page 53, shows the various reaction conditions.

The percentage amount of products formed was derived from the n.m.r. spectra (60 MHz) for each run in the following manner: taking into account the internal standard, the averaged height of the methoxy peak was divided by three to obtain a unit height for one proton. The averaged intensity of the signal for the isopropyl aglycon is then divided by that unit, and the per cent yield obtained by dividing the result by six. Similarly, the averaged intensity of the signal for the acetoxy group is divided by the unit for one proton, and the resulting figure is divided by 3 to obtain the per cent amount of unreacted starting material. The expression 100 - (% glucoside + % 1-O-acetate) is then considered to represent unidentified side products.

TABLE 6

Reaction Parameters for experiment series A to M.

Series	Temp [°C]	Moles per mole of D-glucopyranosyl bromide (6)		
		Tetraethyl-ammonium bromide	Triethyl-amine	Isopropanol
A	80	1	2	2
B	80	1	2	1
C	80	0.5	2	1
D	80	2	2	1
E	60	1	2	2
F	40	1	2	2
G	60	1	2*	2
H	60	-	2*	2
J	60	1	0.5*	2
L	60	1	1*	2
M	60	1**	2*	2

* diisopropylethylamine
** tetraethylammonium chloride

The following Tables show the appearance of the isopropyl D-glucopyranoside and the disappearance of starting material in the various reaction series as determined by the relative intensities for the signals of the α-methoxy, α- and β-acetoxy and of the two methyl groups of the isopropyl group, respectively.

TABLE 7

Appearance of isopropyl D-glucopyranosides (α+β) and disappearance of starting material for experimental series A.

Time [min]	% Isopropyl D-glucopyranoside	% Starting material
5	37	63
15	54.5	43
30	55.5	43
60	61	34
120	63	30
240	69	21

TABLE 8

Appearance of isopropyl D-gluco-pyranosides (α+β) and disappearance of starting material for experimental series B.

Time [min]	% Isopropyl D-glucopyranoside	% Starting material
5	34.5	65
15	39	61
30	44.5	55
60	55.5	43.5
120	53.5	46
240	59.5	40

TABLE 9

Appearance of isopropyl D-gluco-pyranosides (α+β) and disappearance of starting material for experimental series C.

Time [min]	% Isopropyl D-glucopyranoside	% Starting material
5	8.5	92
15	27.8	72
30	39	60
60	46	53
120	47.7	52
240	56.6	43

TABLE 10

Appearance of isopropyl D-gluco-pyranosides (α+β) and disappearance of starting material for experimental series D.

Time [min]	% Isopropyl D-glucopyranoside	% Starting material
5	32	68
15	40	60
30	35	65
60	39	60
120	40	60
240	47.2	49.5

TABLE 11

Appearance of isopropyl D-gluco-pyranosides (α+β) and disappearance of starting material for experimental series E.

Time [min]	% Isopropyl D-glucopyranoside	% Starting material
15	32	67
30	45	55
60	56	43
120	60.5	39
240	64	35

TABLE 12

Appearance of isopropyl D-gluco-pyranoside (α+β) and disappearance of starting material for experimental series F.

Time [min]	% Isopropyl D-glucopyranoside	% Starting material
15	14.4	85
30	16.7	83
60	20	80
120	31	69
240	44.5	55

TABLE 13

Appearance of isopropyl D-gluco-pyranoside (α+β) and disappearance of starting material for experimental series G.

Time [min]	% Isopropyl D-glucopyranoside	% Starting material
15	18.2	81
30	28.4	71
60	40.5	59
120	49	42.2
240	55.5	41

TABLE 14

Appearance of isopropyl D-gluco-pyranoside (α+β) and disappearance of starting material for experimental series H.

Time [min]	% Isopropyl D-glucopyranoside	% Starting material
15	17.6	81.6
30	20.7	74.5
60	29.8	64.5
120	39.4	43.8
240	50	18.4

TABLE 15

Appearance of isopropyl D-gluco-pyranoside (α+β) and disappearance of starting material for experimental series J.

Time [min]	% Isopropyl D-glucopyranoside	% Starting material
15	19.2	79
30	35.2	62.5
60	42	42
120	64	35
240	73.6	25

TABLE 16

Appearance of isopropyl D-gluco-pyranoside (α+β) and disappearance of starting material for experimental series L.

Time [min]	% Isopropyl D-glucopyranoside	% Starting material
15	19.8	80
30	38	62
60	48.7	48.7
120	60	35
240	61.7	26

TABLE 17

Appearance of isopropyl D-gluco-pyranoside (α+β) and disappearance of starting material for experimental series M.

Time [min]	% Isopropyl D-glucopyranoside	% Starting material
15	4.4	95
30	6.6	88
60	7.3	92
120	9.8	90
240	15.4	84

3. Investigation of Glycoside Formation by Gas-Liquid Partition Chromatography

(i) Using different solvents

In another series of experiments, seven different solvents were chosen in which glycosidation reactions were carried out, using ethanol as the potential aglycon. The reactions were performed in the manner subsequently described.

Freshly prepared tetra-O-benzyl-α-D-glucosyl bromide (1.208 g, 2 mmole) was dissolved in 10 ml solvent in a plastic-stoppered 5-dram vial. Tetraethylammonium bromide (841 mg, 4 mmole) was added and the mixture shaken until the salt was dissolved, or, if the salt appeared to be insoluble, for a total time of 5 min. Then 0.42 ml triethylamine (304 mg, 3 mmole) was added with a syringe, followed by the addition of 0.175 ml ethanol (138 mg, 3 mmole). The solution was quickly transferred into a dried Carius tube and sealed off. The test tubes were placed in an oil bath which had a temperature of 80°C. After 16 hr the tubes were cooled down, cut open, and the contents rinsed out with the aid of chloroform. The solution was washed successively with 0.1 N hydrochloric acid, saturated sodium bicarbonate solution and finally water. After each washing,

the remaining aqueous layer was washed with 10 ml chloroform. The combined organic layers were finally dried with anhydrous potassium carbonate, evaporated under diminished pressure and pumped overnight to a high vacuum.

The amount of product was then weighed and divided into two equal parts. One half was used for a n.m.r. spectrum in $CDCl_3$, t.l.c. investigation and measurement of rotation.

The second half of the reaction product was dissolved in tetrahydrofuran and debenzylated with an 8 to 10-fold excess of sodium in liquid ammonia for 6 to 8 hr (67). During the reaction period, the dry ice-acetone bath was removed from under the reaction vessel so that reflux occurred with the aid of a dry ice condenser. The reaction mixture was stirred magnetically all the time. Following this, an amount of ammonium chloride corresponding to that of the sodium present was added slowly. The blue solution turned colorless immediately. The ammonia was allowed to evaporate overnight. The solid white residue was removed from the three-necked reaction flask with the aid of methanol and transferred into a 250 ml flask. The methanol was evaporated and then 10 ml of dry pyridine was added in order to extract the debenzylated product. The mixture

was shaken for 2 hr, then the solid components were removed by filtration and the pyridine removed by evaporation. The remaining syrup was extracted with deuterium oxide, then freeze-dried. This material was then submitted for n.m.r. spectroscopy in DMSO-d_6 or D_2O.

Glycosidation reactions as described above were carried out in dioxane, benzene, methylene chloride, nitromethane, dimethylformamide, acetonitrile and dimethylsulfoxide.

Small amounts of the debenzylated glycosidation products were converted into their O-trimethylsilyl derivatives as described below and investigated by g.l.p.c. The relative amounts of α and β-anomers were determined by cutting out the respective peaks and weighing them.

The results of these experiments are summarized in Table 18. The debenzylated ethyl D-glucopyranosides were examined by n.m.r. (60 MHz) and g.l.p.c. The results of this investigation are given in Table 19.

TABLE 18

Reaction of tetra-O-benzyl-α-D-glucopyranosyl bromide (6) with 1.5 mole equivalent of ethanol at 80°C for 16 hr in the presence of 2 mole equivalents of tetraethylammonium bromide and 1.5 mole equivalent of triethylamine in different solvents.

Solvent	Solubility of tetraethylammonium bromide in solvent	Estimated yield of glucoside by t.l.c. and n.m.r. (%)
Dioxane	Mostly insoluble	72
Benzene	Mostly insoluble	91
Methylene chloride	Soluble	93
Nitromethane	Soluble	60
Dimethylformamide	Partially soluble	traces
Acetonitrile	Soluble	72
Dimethylsulfoxide	Soluble	21

TABLE 19

Reaction of tetra-O-benzyl-α-D-glucopyranosyl bromide (6) with 1.5 mole equivalent of ethanol at 80°C for 16 hr in the presence of 2 mole equivalents of tetraethylammonium bromide and 1.5 mole equivalent of triethylamine in different solvents. Debenzylated products.

Solvent	Yield of glucoside in product from n.m.r. (%)	Ratio of α to β from g.l.p.c.	Number of side products
Dioxane	91	6.6	one
Benzene	95	8.45	one
Methylene chloride	93	8.2	one (minor)
Nitromethane	98	8.1	two
Dimethyl-formamide	poor integration	2.5	four
Acetonitrile	98	6.5	one
Dimethyl-sulfoxide	poor integration	8.8	five

During work-up of these reactions, it was found that a small amount, roughly 10%, of the debenzylated material was not soluble in D_2O, but rather remained on the walls of the flask as oily droplets. Some chloroform was therefore added and the two phases were shaken and separated in a separatory funnel. The D_2O-layer was freeze-dried and weighed, the chloroform layer was

evaporated *in vacuo* to dryness, weighed and submitted for n.m.r. spectroscopy. The loss in weight caused by this procedure was in all cases approximately 10%. The n.m.r. spectra of the debenzylated D_2O-soluble glycosides purified in the described manner did not show any peaks which could be attributed to the presence of aromatic or benzylic compounds.

The chloroform layer was worked up and the resulting material submitted for n.m.r. spectroscopy. It proved identical with a product that had separated as white crystals in the neck of the flask, when the debenzylated glycosidation products were left overnight connected to the high vacuum pump. These white crystals had a melting point of 56-57°C. The n.m.r. spectrum displayed a sharp, single peak at τ 2.82 and a second sharp peak at τ 7.14. Integration showed a ratio of 9.8 to 4 for these protons. Elemental analysis and the n.m.r. spectrum of an authentic sample revealed these crystals to be 1,2-diphenylethane, $Ph\text{-}CH_2\text{-}CH_2\text{-}Ph$, which has a reported m.p. of 52.2°C. It is soluble in chloroform and is therefore the main component in the chloroform layer after partitioning the crude debenzylated product between D_2O and chloroform.

(ii) Gas-liquid partition chromatography of reference compounds

For reference purposes, the two anomeric ethyl-tetra-O-acetyl-D-glucosides were deacetylated with triethylamine in methanol (49). Each deacetylated anomer, 30 mg, was dissolved in 0.5 ml pyridine, then 0.5 ml hexamethyldisilazane and 0.3 ml trimethylchlorosilane were added (41). The mixture was shaken for 15 minutes, centrifuged and the supernatant liquid evaporated *in vacuo*. The remaining syrup was taken up in 1 ml methylene chloride and subjected to g.l.p.c. analysis. Injections of the single anomers as well as of mixtures of both showed that the α-anomer had a shorter retention time, i.e. the first peak belonged to the ethyl tetra-O-trimethylsilyl-α-D-glucopyranoside.

(iii) Using various conditions

In the following experiments, tetra-O-benzyl-α-D-glucopyranosyl bromide (6, 1.206 g, 2 mmole) was heated at 80°C in a sealed tube with 10 ml of methylene chloride in the presence of the compounds listed in Table 21, p. 135.

The products were isolated as described under section (i), p. 41, debenzylated and examined by g.l.p.c. using the trimethylsilyl derivatives as described on p. 66. The results of these analyses are given in Table 21, p. 135.

4. Preparation of 3-O-(tetra-O-benzyl-α-D-glucopyranosyl)-1,2;5,6-di-O-isopropylidene-α-D-glucofuranose (15)

(i) In ethylene chloride under reflux

Tetra-O-benzyl-α-D-glucopyranosyl bromide (12.07 g, 20 mmole) was dissolved in 60 ml ethylene chloride in a 200 ml round-bottom flask. Tetraethylammonium bromide (4.203 g, 20 mmole) was added rapidly, followed by 1,2;5,6-di-O-isopropylidene-α-D-glucofuranose (5.73 g, 22 mmole) and 3.42 ml diisopropylethylamine (2.585 g, 20 mmole). The reaction flask was fitted with

a Dimroth condenser and the mixture heated until reflux occurred. After a reaction time of 4 hours, the mixture was washed twice with water and the organic extracts were evaporated to dryness. The resulting mixture was chromatographed through a column (4 cm x 65 cm) of Silic AR CC-7 with ethyl ether-Skellysolve B (4:6) as the solvent system. Fraction I, which was collected in portions of 10 ml, of this chromatography afforded 3.29 g (20.5%) of pure 15, m.p. 90-91°C, $[\alpha]_{22}^{D}$ + 46° (c, 2.87 in chloroform). The n.m.r. spectrum in deuteriochloroform showed a doublet at τ 4.81 ($J_{1,2}$ = 3.5 Hz) for the anomeric proton of the glucoside; a doublet at τ 4.18 ($J_{1,2}$ = 3.8 Hz) for the proton at C-1 of the aglycon; a sharp signal at τ 2.78 for the aromatic benzyl protons; a multiplet centered at τ 5.35 (methylene protons of benzyl groups); a multiplet at τ 6.15 (ring protons, methylene protons at C-6); a doublet at τ 8.6 (6H) and a singlet at τ 8.79 (6H) for the isopropylidene protons of the aglycon.

Anal. Calcd. for $C_{46}H_{54}O_{11}$: M.W. 782.89.

C, 70.57; H, 6.95%.

Found: C, 70.53; H, 6.85%.

Fraction II afforded 2.182 g (21%) of the glucoseen derivative 8, m.p. 66-67°, undepressed in a mixture

with an authentic sample; $[\alpha]_{22}^{D}$ - 3.2° (c, 1.73 in chloroform); the n.m.r. spectrum was identical to that of an authentic sample.

(ii) In methylene chloride at room temperature

The above reaction was repeated using methylene chloride as the solvent. It was carried out for two days at room temperature. After chromatography on a column (4 cm x 65 cm) wet packed with silicic acid, pure compound 15 (6.268 g, 42%) was isolated; m.p. 90-91°C (undepressed with a mixture of a sample obtained in the previous experiment), $[\alpha]_{21}^{D}$ + 48°, (c, 1.53 in chloroform); n.m.r. spectrum corresponded closely to that of a sample obtained in the previous experiment.

(iii) In methylene chloride with potassium carbonate as base, at room temperature

The reaction described under the previous heading was repeated, however the amine was replaced by anhydrous potassium carbonate. After the reaction mixture was shaken for two days at room temperature, 1.017 g (13%) of the disaccharide 15 was isolated, identical in all properties with the material prepared under the above heading.

5. Preparation of 6-O-(tetra-O-benzyl-α-D-glucopyranosyl)-1,2;3,4-di-O-isopropylidene-α-D-galactopyranose (16)

(i) In methylene chloride at room temperature

Tetra-O-benzyl-α-D-glucopyranosyl bromide (6.035 g, 10 mmole) was dissolved in 30 ml methylene chloride in a 10 ml round-bottom flask. Tetraethylammonium bromide (2.1 g, 10 mmole) was added rapidly, followed by 1,2;3,4-di-O-isopropylidene-α-D-galactopyranose (2.870 g, 11 mmole) and 1.7 ml diisopropylethylamine (10 mmole). After two days at room temperature and the usual work-up, the resulting syrup was chromatographed on a column (4 cm x 65 cm) packed with silicic acid as a slurry. Fraction II, collected in portions of 10 ml, afforded 5.08 g (65%) of pure disaccharide 16 as a colorless syrup, $[\alpha]_D^{24}$ + 10.1° (c, 2.01 in chloroform). The n.m.r. spectrum showed a doublet at τ 4.47 ($J_{1,2}$ = 4.9 Hz) for the anomeric proton of the aglycon; a sharp singlet at τ 2.7 for the aromatic benzyl protons; a multiplet at τ 5.3 (anomeric proton of the glucoside, methylene protons of benzyl groups); a multiplet centered at τ 6.2 (ring protons, methylene protons at C-6); a doublet at τ 8.5 (6H) and a singlet at τ 8.68 (6H) for the protons of isopropylidene protecting groups of the aglycon.

Anal. Calcd. for $C_{46}H_{54}O_{11}$: M.W. 782.89.
C, 70.57; H, 6.95%.
Found: C, 69.47; H, 6.90%.

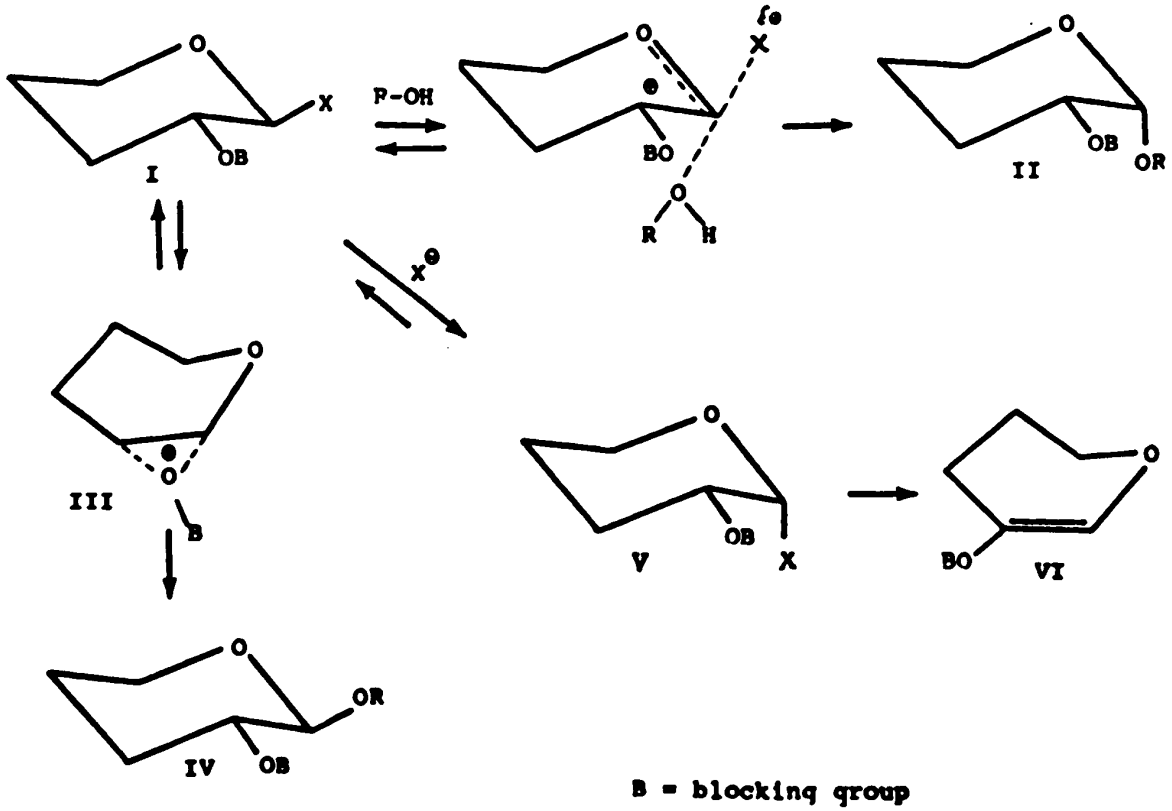
(ii) The above preparation was repeated, however substituting the amine by potassium carbonate. After the usual work-up and column chromatography, the disaccharide 16 was isolated in 28% yield (2.22 g). The physical properties of this material corresponded closely to that of the compound prepared in the previous section.

DISCUSSION OF RESULTS

As mentioned in the Introduction, the main purpose of this investigation was to examine the possibility whether α-D-glucopyranosides (II) could be synthesized from a suitably protected glucopyranosyl halide under conditions which would promote displacement of the halogen by the alcohol with the glucopyranosyl halide being in the β-form (I). Thus, the reaction may be evidenced to proceed by way of an inversion at the anomeric center to provide the desired α-glucoside in acceptable yield. Successful use of this approach would presumably require the following controls:

(1) Use of the β-halide (I) as reactant with the 2-position blocked by groups (B) not strongly prone to participate in the solvolysis of I. Such a participation would most likely arise from the 2-substituent to lead to a cationic intermediate (III) in the α-configuration. Reaction of III at the anomeric center with the alcohol would lead to the β-glucopyranoside (IV) rather than the desired α-glucopyranoside (II).

(2) Use of conditions which would strongly depress the development of halide ion since it is well established that halide ion catalyzes the



anomerization of glucosyl halides (4) and primarily because of the anomeric effect (5,6), the equilibrium is probably shifted by more than 90% toward the α-anomer V (4).

(3) The use of reaction conditions and choice of reactants which would not be conducive to dehydrohalogenation of V to form the "1,2-glucoseen" type

product (VI) and thus lead to loss of the substrate (I or V).

These considerations for the development of a synthetic route to α-glucopyranosides are not novel and several attempts have been recorded as pointed out earlier in the Introduction (11,12,14,15).

The present research was based on the premise (26) that it may be possible to establish conditions where the formation of the α-glucopyranoside (II) would proceed in acceptable yield from the equilibrium mixture of the anomeric glucopyranosyl halides (I and V), should the following conditions exist:

1) The reaction conditions must be such that the rate of α $\rightleftharpoons$ β anomerization of the glucopyranosyl halide is much greater than the rate of glucopyranoside (either α or β) formation.

2) The rate of glucopyranoside formation must be reasonably high in order to provide a convenient reaction time - preferably one day.

3) The glucopyranoside formation must be the predominant route of reaction and not dehydrohalogenation.

4) Most importantly, the rate of formation of α-glucopyranoside must be substantially greater than that of the formation of the β-anomer to overcome the fact

that, at equilibrium, the concentration of the α-halide (V) will be about ten times greater than that of the β-halide (I) (4) which in all likelihood is the immediate precursor of the desired α-glucopyranoside (II).

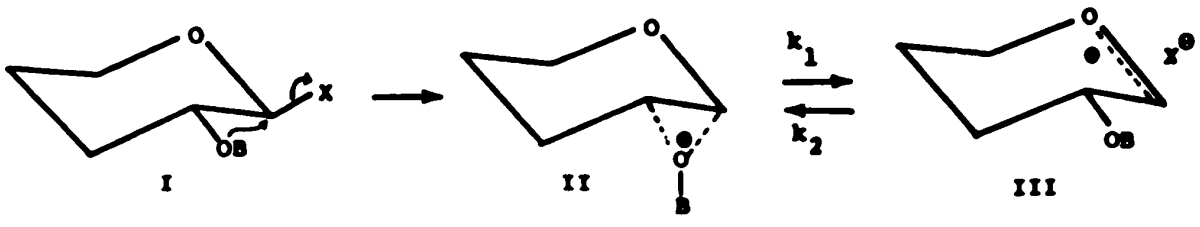
Some control of the above first three conditions seemed possible but the last condition would largely rest on the assumption, mainly based on the work by Lemieux and Morgan (26), that β-glucopyranosyl halides react much more rapidly with alcohols than do the α-anomers. For example, Lemieux and Huber (33) showed many years ago that 3,4,6-tri-O-acetyl-β-D-glucopyranosyl chloride undergoes acetolyses about 100 times faster than its α-anomer.

The above considerations called for the preparation of a suitably O-protected glucopyranosyl halide and examination of its reaction with an alcohol in the presence of halide ion under a variety of conditions with the hope that a general method could be developed for the synthesis of α-D-glucopyranosides. A further objective was to investigate, once a satisfactory method for the synthesis of simple α-D-glucopyranosides had been established, the applicability of the method for the synthesis of more complex α-D-glucopyranosides in which the aglycon comprised another sugar molecule.

The trivially called acetohalogeno sugars, tetra-O-acetyl-α-D-glucopyranosyl bromide (acetobromoglucose) in particular, represent a common example of O-protected glucopyranosyl halides which have in general played a major role as starting materials in reactions leading to the formation of O-glucopyranosides. It is well established that alcoholysis of this acetobromoglucose in the presence of a base such as silver carbonate or silver oxide leads generally to inversion of configuration at the anomeric center to produce β-glucopyranosides. Attempts to synthesize α-glycopyranosides by way of intermediate tetra-O-acetyl-β-D-glucopyranosyl bromide have led to the formation of orthoesters arising from participation of the carbon-2 acetoxy group (50). Baddiley and coworkers (45), in trying to circumvent this undesired course of reaction, used O-benzylated glycopyranosyl halides instead of the corresponding acetylated sugars. These authors prepared in 1964 the tetra-O-benzyl-α-D-glucopyranosyl chloride (4) by reacting 1-O-acetyl-tetra-O-benzyl-D-glucopyranose with a 4% solution of hydrogen chloride in dioxane. The resulting glucopyranosyl halide derivative, believed to be a mixture of the α- and β-anomers, was reacted with 1,3-di-O-benzylglycerol in the presence of silver perchlorate

and silver carbonate in benzene as solvent. The formation of a mixture of the corresponding α- and β-D-glucopyranosides was observed, the ratio of α:β anomers being about 4:1. When the tetra-O-benzyl-D-glucopyranosyl chloride (4), which was prepared from reaction of tetra-O-benzyl-α-D-glucopyranose with thionyl chloride, was reacted with 1,3-di-O-benzyl glycerol under essentially the same conditions as above, the ratio of the α:β anomers in the product was 2:1.

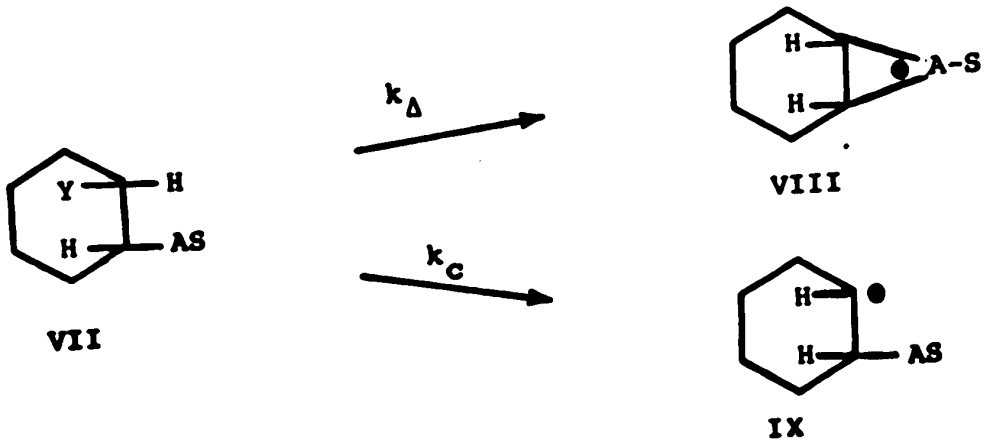
Compound 4 was thought to be a suitable starting material for our preliminary studies of alcoholysis reactions in the presence of added halide ion. In this type of reaction of glycopyranosyl halides which have a *trans* relationship of the halogen atom at carbon-1 with the substituent at carbon-2, one considers the product as a result of a competition between the blocking group at carbon-2 and the solvent molecules in making a rear-ward attack at carbon-1 for the expulsion of the halogen atom:



If $k_1 << k_2$, neighboring group participation would lead to predominant formation of the β-glucoside. Winstein and coworkers (51) have made an assessment of the nature of participating groups by introducing a variable L termed the driving force due to the participation of the neighboring group in the rate-determining step. The variable L is defined by the equation:

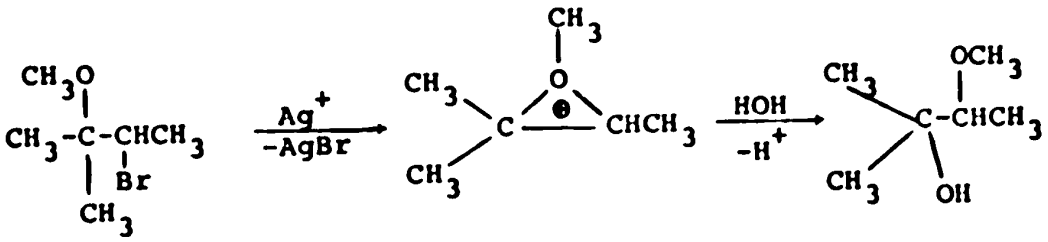
$$L = R \cdot T \ln (k_\Delta / k_c)$$

where k_Δ is the specific rate constant for the ionization to the cyclic ion VIII, and k_c is the specific rate constant for the ionization to the open carbonium ion



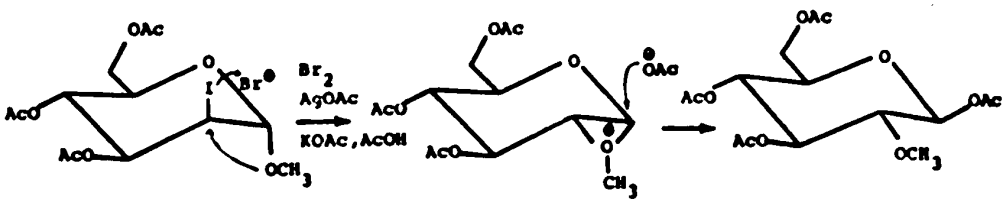
IX. The driving force is therefore a measure of the decrease in free energy accompanying the conversion of

an open carbonium ion IX to the cyclic carbonium ion VIII. The value for L for an acetoxy group was found to be 4.60 kcal/mole, for a methoxy group, however, 0.86 kcal/mole at 25°C. (k_Δ/k_c for methoxy : 4.3; for acetoxy: 2.33×10^3). Although the authors claim no accuracy for the fraction of ionization leading to the cyclic carbonium ion VIII in case of a methoxy group, it is seen that the tendency for a neighboring acetoxy group to participate in the formation of a cyclic carbonium ion is about 500 times larger than for a neighboring methoxy group. Consequently there are only few reports of participation by a methoxy group. Winstein and Ingraham (52) observed a methoxy group migration in the silver ion assisted solvolysis of 2-methyl-2-methoxy-3-bromobutane:



Also, for example, for $CH_3\text{-}O(CH_2)_nOBs$, neighboring group participation was important for n = 4 or 5 (corresponding to a five- or six-membered cyclic intermediate) but not

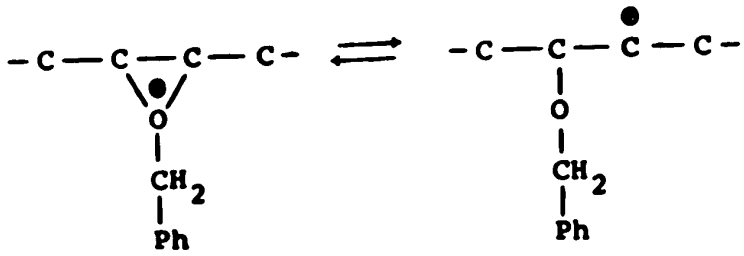
for n = 2,3 or 6 (53). In 1964, Lemieux and Fraser-Reid (54) reported a 1,2-methoxy group migration in carbohydrate chemistry when tetra-O-acetyl-2-O-methyl-β-D-glucopyranose was obtained in small yield from methyl 3,4,6-tri-O-acetyl-2-deoxy-2-iodo-α-D-mannopyranoside:



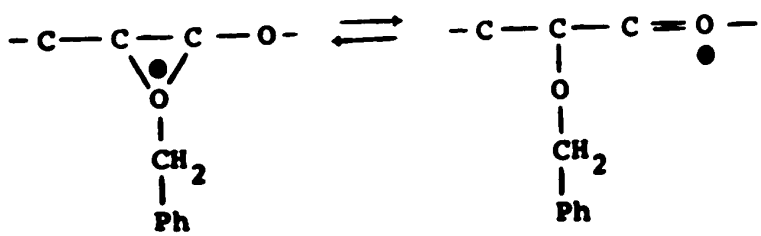
In 1965, Hughes and Speakman (55) reported a 1,4-migration of a methoxy group during a benzoate displacement reaction and postulated a five-membered cyclic oxonium ion intermediate as a result of favourable participation by the methoxy group.

The minor tendency of a methoxy group to participate in solvolysis reactions at the neighbouring carbon atom as compared to that of an acetoxy group is due to the necessity of forming a three-membered cylic intermediate and thus having to introduce considerable amount of strain into the ring. The tendency of a benzyloxy group to form a participating benzyl oxonium ion should not be too different from that for a methoxy

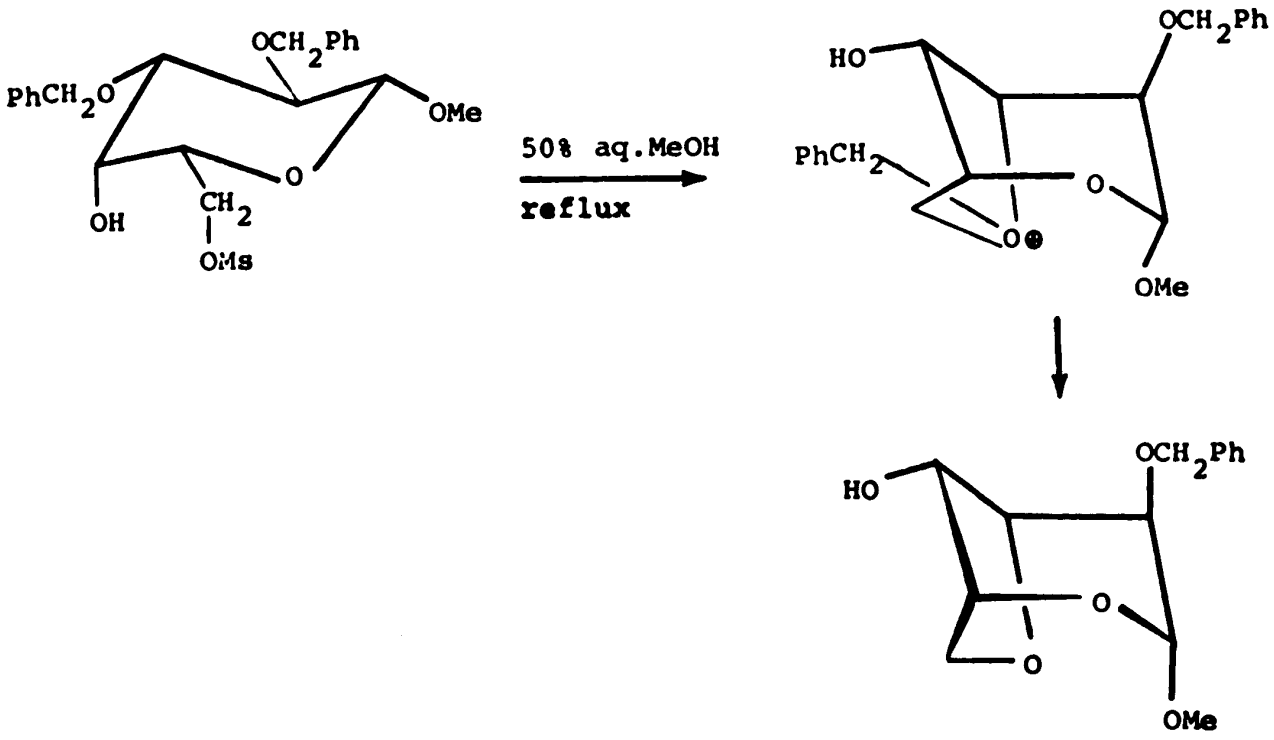
group. Here we would also have formation of a three-membered cyclic intermediate, however steric factors, such as the size of the benzyloxy group may decrease its tendency to participate even further. The possibility of participation by a benzyloxy group at carbon-2 of a cyclic monosaccharide in nucleophilic displacement reactions at the anomeric center may be further decreased through the assistance by the ring oxygen in delocalizing the positive charge of the developing carbonium ion. Therefore, in a simplified scheme, the following equilibrium:



may be expected to be more in favor of the cyclic benzyloxonium intermediate than in a case where the possibility of forming an oxocarbonium ion exists:



However, participation of the benzyloxy group by way of a five-membered cyclic intermediate has been reported in the literature. Barker and coworkers (56) noted the rapid solvolysis of 4-O-benzyl-1-O-p-tolylsulfonyl-1,4-pentanediol in ethanol to give tetrahydro-2-methylfuran, benzyl ethyl ether and p-toluenesulfonic acid, a reaction where the rate and products clearly indicate anchimeric assistance from the benzyloxy group by forming a five-membered, cyclic, oxonium-ion intermediate. Brimacombe and Ching (57,58) have presented evidence for the formation and participation of a benzyloxonium ion across the monosaccharide ring in the solvolysis of methyl 2,3-di-O-benzyl-6-O-methanesulfonyl-β-D-galactopyranoside with a 50% aqueous methanol solution to form a 3,6-anhydrosugar.



Ryan and coworkers (59) have pointed out that the benzyloxy group provides little or no assistance in the reaction of methyl 2-O-benzyl-5-deoxy-3-O-methanesulfonyl-α-D-xylofuranoside with sodium benzoate in boiling dimethyl formamide, whereas a benzoyloxy group at C-2 is highly effective. The topic of neighboring group participation has been extensively reviewed by Goodman (60) and more recently by Brimacombe (61).

Besides its little tendency of participating in displacement reactions at the adjacent carbon atom thus avoiding the formation of undesired side products, the

benzyloxy group is weakly electronegative as compared to an acyloxy group. This important characteristic is a necessary condition for the proposed anomerization of a perbenzylated glucosyl halide by added halide ion. With a more strongly electronegative substituent at carbon-2, highly stable β-D-glucosyl halides are obtained for example, the tri-O-acetyl-2-O-trichloroacetyl-β-D-glucopyranosyl chloride (62), prepared by Brigl in 1921, or the tri-O-acetyl-2-O-nitro-β-D-glucopyranosyl chloride described by Wolfrom and Gillam (14). Due to the strong electronegativity of the C-2 blocking group, these halides are quite inert and unreactive which renders them not too useful for the synthesis of disaccharides in the absence of some assistance as provided, for example, by silver salts. The anomerization of a perbenzylated α-D-glucopyranosyl halide to its β-anomer by added halide ion and subsequent dissociation is expected to be facile, but must become much slower if the substituent at carbon-2 is highly electronegative (63). Thus, halide ion catalysis would become impossible in the case where the presence of silver ions is required to assist the reaction because of the unreactivity of the β-D-glucopyranosyl halide without such an assistance by silver ions.

Benzyl ethers of sugars in general have played an important role in carbohydrate chemistry, because these generally can be prepared in high yield and

because the benzyl groups can normally be readily removed under mild conditions by various methods, such as hydrogenolysis (64), by sodium in liquid ammonia (65,66,67), or through free-radical bromination in either chloroform, carbon tetrachloride, benzene or Sulfolane at 0-25°C, followed by hydrolysis in saturated sodium carbonate or calcium hydroxide solutions (68). The subject of benzyl ethers of sugars has been extensively reviewed by McCloskey (69).

The two different methods described by Baddiley (45) to prepare tetra-O-benzyl-D-glucopyranosyl chloride (4) start out from 2,3,4,6-tetra-O-benzyl-D-glucose (5) which in turn is prepared by acid hydrolysis of methyl tetra-O-benzyl-α-D-glucypyranoside (2). This latter compound was synthesized by Schmidt and coworkers (44) in 1960 who used the "classical" method of benzylating methyl α-D-glucopyranoside, i.e. powdered potassium hydroxide as the base with benzyl chloride as the alkylating agent. Upon hydrolysis of the amorphous methyl tetra-O-benzyl-α-D-glucopyranoside with glacial acetic acid and 2N HCl at 80°C for 24 hr, they obtained for the first time crystalline tetra-O-benzyl-α-D-glucopyranose (3). Baddiley and coworkers (45) reported that use of dioxane as a solvent in the benzylation reaction increased

the yield of the required product.

Tate and Bishop (42) improved the method by using sodium hydride as the base. This procedure was used in the initial stage of the present work. During the course of this research Brown and coworkers (43) published the details for a convenient methylation procedure, utilizing sodium hydride and a solvent system that consisted roughly of a 2:1 (by volume) mixture of dimethylformamide and 1,2-dimethoxyethane This method was adopted by us for benzylation, especially in large scale preparations of compound 2. Care must be taken so as not to add the required excess of sodium hydride at once to the reaction mixture. The process is strongly exothermic at 100°C and to keep control, sodium hydride must be added slowly and in small portions. After filtering off the excess sodium hydride at the end of the reaction, the bulk of the organic solvents and of the benzyl chloride can be removed quite rapidly by steam distillation in a cyclone-type evaporator. Fig. 1 shows the n.m.r. spectrum (60 MHz) of the compound in deuteriochloroform.

For the hydrolysis of the methyl tetra-O-benzyl-α-D-glucopyranoside (2), 2N sulfuric acid was recommended by Tate and Bishop (42) over 2N hydrochloric acid, and

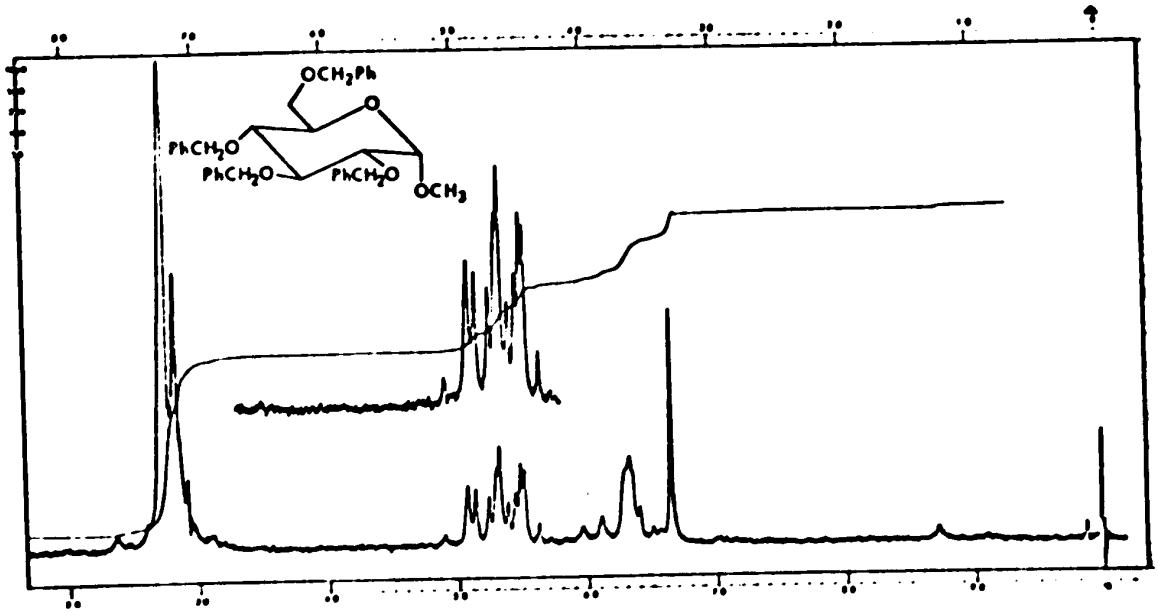


Fig. 1. N.m.r. spectrum (60 MHz) of methyl tetra-O-benzyl-α-D-glucopyranoside (2) in deuteriochloroform.

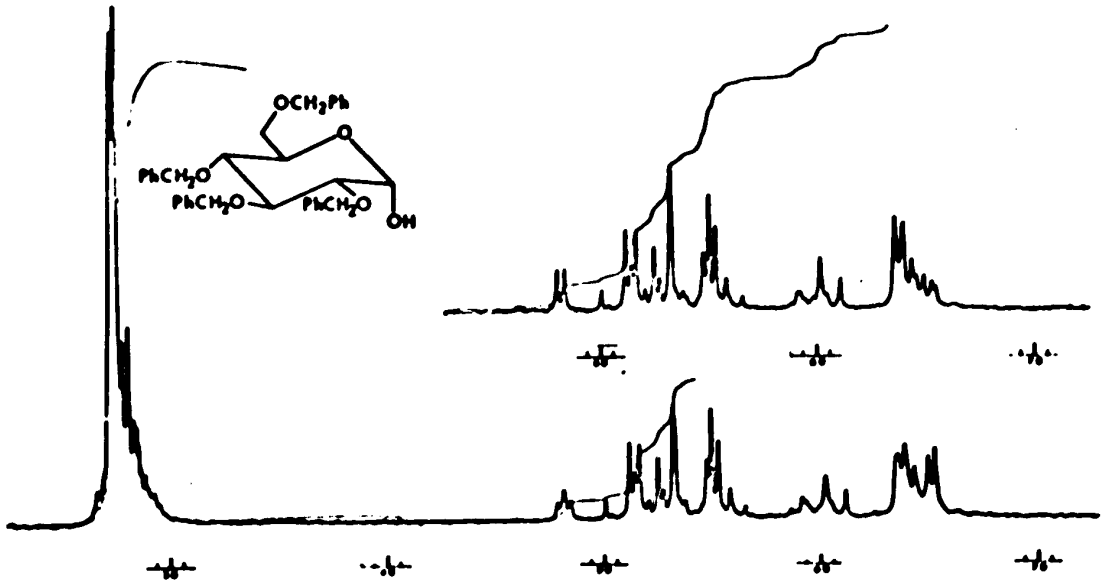


Fig. 2. N.m.r. spectrum (100 MHz) of tetra-O-benzyl-α-D-glucopyranose (3) in deuteriochloroform. Inset, after exchange with D_2O.

in this study sulfuric acid was used along with glacial acetic acid with good results.

The n.m.r. spectrum (100 MHz) of tetra-O-benzyl-α-D-glucopyranose (3) in deuteriochloroform is shown in Fig. 2. The signal for the anomeric proton appears as a triplet at τ 4.82, $J_{1,2}$ = 3.1 Hz, since it is split not only by coupling with hydrogen-2, but also by coupling with the proton of the hydroxyl group at carbon-1. After addition of a few drops of deuterium oxide to the sample, the triplet collapsed, as expected, to a sharp doublet at τ 4.82, $J_{1,2}$ = 3.3 Hz.

Besides using a substrate with a blocking group at carbon-2 which can readily be removed, is only weakly electronegative and is virtually non-participating in displacement reactions at the anomeric carbon, the basic idea leading to the present research was to provide a rapidly equilibrating mixture of the anomeric glucopyranosyl halides as the glycosylating agent for the synthesis of α-D-glucopyranosides. Rapid reversible anomerization of glucopyranosyl halides by added halide ion, for example a tetraalkylammonium halide was expected to provide this condition. The necessary reagents in such a synthesis are a dry and non-hydroxylic solvent, since for the method of producing O-glycosides

to be useful for the synthesis of complex glycosides, the alcohol cannot be the solvent at the same time. The ultimate goal was to synthesize α-D-glucopyranosides in which the aglycon is a sugar molecule itself, that is the production of di- and oligosaccharides. To avoid the development of strong acid conditions arising out of the liberation of the hydrogen halide, the synthesis would be best conducted in the presence of a base such as a tertiary amine which would, for steric reasons, not form a stable N-glucopyranoside from the glucopyranosyl halide. For a number of preliminary experiments methanol was chosen as the alcohol and diisopropylethylamine ("Hünig's base") (71) as the base, for the reasons outlined below.

The two anomeric forms of methyl tetra-O-benzyl-D-glucopyranoside can clearly be distinguished by their respective n.m.r. spectra due to a difference in chemical shift for the protons of the two methoxy groups (70). In chloroform the signal for the protons of the α-methoxy group appears as a sharp singlet at τ 6.64 whereas the corresponding signal for the β-isomer appears as a singlet further downfield at τ 6.45. Diisopropylethylamine, introduced by Hünig in 1958 (71) is known to be a strongly hindered base and, since it forms only weak complexes with silver salts, likely

would not lead to N-glucopyranoside formation.

For a number of preliminary experiments, tetra-O-benzyl-D-glucopyranosyl chloride (4) was prepared according to the procedure given by Baddiley (45) from tetra-O-benzyl-α-D-glucopyranose and thionyl chloride.

The n.m.r. spectrum (60 MHz) of this glucopyranosyl halide is shown in Fig. 3. The doublet at τ 3.96 is assigned to the anomeric proton, and from its relative intensity and the spacing $J_{1,2}$ = 3.4 c.p.s. the major component is considered to be the α-anomer.

When compound 4 was reacted with two molar equivalents of methanol in the presence of one mole of tetraethylammonium chloride and one mole of diisopropylethyl amine for 2½ hrs at 100°C in acetonitrile as solvent, the n.m.r. spectrum of the worked-up reaction mixture displayed a sharp single peak at τ 6.64. From the ratio of the relative intensities of the signal for the aromatic protons to that of the α-methoxy group, an amount of 80% of α-D-glucopyranoside was estimated to be present in the reaction mixture. When, however, compound 4 was reacted with methanol in the presence of silver perchlorate under otherwise equal conditions, but omitting the tetraethylammonium chloride, the n.m.r. spectrum of the product mixture, after proper work-up,

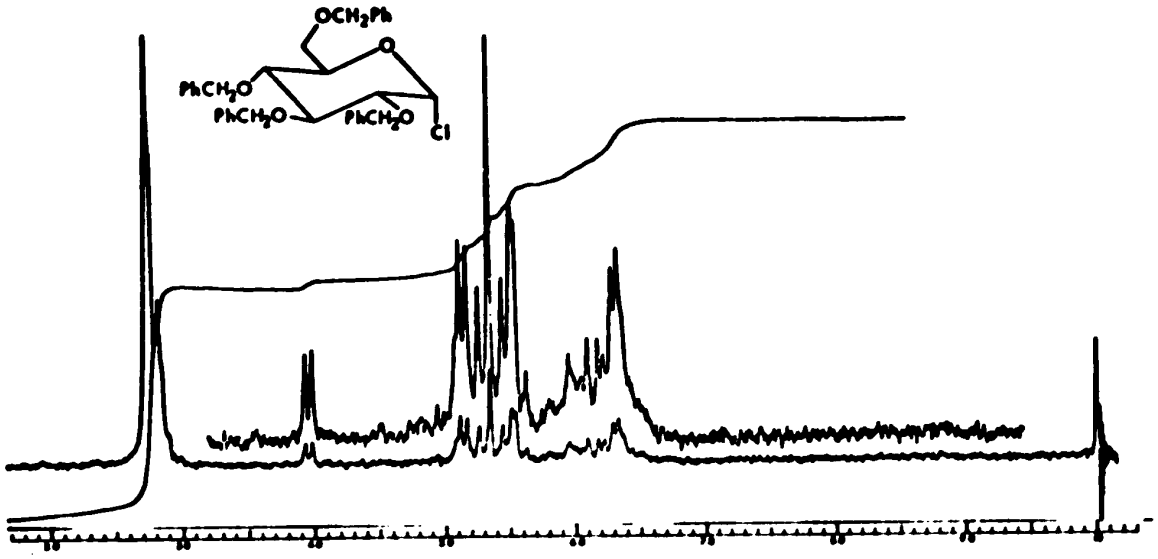


Fig. 3. N.m.r. spectrum (60 MHz) of tetra-O-benzyl-α-D-glucopyranosyl chloride in deuteriochloroform.

showed a sharp singlet at τ 6.45, corresponding to an 82% yield of β-glucoside formation. There was also a much weaker signal at τ 6.46 indicating an estimated α-glucoside formation of 15%.

These observations gave a clear indication that added halide ions may catalyze the formation of glycosides in such a way as to give preferentially the product with the α-configuration, whereas in the presence of a halide ion scavenger, such as silver perchlorate, the β-glucoside is formed as the major product.

It seemed worthwhile at this point to investigate the preparation and properties of other tetra-O-benzylated D-glucopyranosyl halides and their use in glycosidation reactions of the above kind. Weygand and Ziemann (46) in 1962 reported, presumably for the first time, the preparation of the tetra-O-benzyl-α-D-glucopyranosyl bromide (6) by reacting ethyl tetra-O-benzyl-D-thioglucopyranoside (either anomer) with bromine in ether. If the β-thioglucoside is used as the starting material, inversion of configuration is observed, whereas in the reaction of the α-thioglucoside the configuration is retained. By following the change of the optical rotation during the latter reaction, the authors concluded that the β-(1,2-*trans*)-bromocompound is formed first under inversion, which rearranges slowly into the more

stable α-anomer. It is interesting to note in this connection that Bonner in 1948 has obtained α-acetobromoglucose in a similar reaction from phenyl tetra-O-acetyl-β-D-thioglucopyranoside and bromine (72). Weygand et al (73) have also reacted the tetra-O-acetylated anomers of ethyl thioglucopyranoside and succeeded to prepare that way both the α- and β-acetobromoglucopyranoses.

The compound important to the present study, tetra-O-benzyl-α-D-glucopyranosyl bromide (6), is described by Weygand (46) only as a light yellow syrup which has $[\alpha]_{578}^{20}$ + 151°. No rotation at the sodium D-line is given, neither a solvent nor the concentration. Ishikawa and Fletcher (38) have reported the preparation of compound 6 by reacting tetra-O-benzyl-1-O-(p-nitrobenzoyl)-α-D-glucopyranose (5) with a saturated solution of hydrogen bromide in methylene chloride. This convenient method, utilized in different laboratories to prepare halides of several per-O-benzylated and benzoylated pentoses (74,75,76,77), takes advantage of the very low solubility of p-nitrobenzoic acid in methylene chloride. No physical constants whatsoever are given by Fletcher and Ishikawa (38) to characterize compound 6. The same is true for a publication by Preobrazhenskaja and Suvorov (48) who also report the preparation of compound

6 by treating 5 with hydrogen bromide in methylene chloride, but do not characterize it beyond stating that it is a yellow syrup.

Compound 6 is expected to be considerably more reactive than the corresponding D-glucopyranosyl chloride due to the greater bond length of the carbon-bromine bond and thus lower bond energy, which should be especially useful in glycosidation reactions in which the potential aglycon is another sugar molecule. Thus, compound 5, tetra-O-benzyl-1-O-(p-nitrobenzoyl)-α-D-glucopyranose, was prepared by reacting tetra-O-benzyl-α-D-glucopyranose with p-nitrobenzoyl chloride in pyridine at elevated temperatures. It crystallizes readily from ethanol and is, because of its stability, the preferred starting material which can safely be stored in large amounts, whereas the glucopyranosyl bromide 6 should be used soon after its preparation. Fig. 4 shows the n.m.r. spectrum (100 MHz) of compound 5 in deuteriochloroform. The doublet at τ 1.79 arises from the four aromatic protons of the p-nitrobenzoyl group at the anomeric center. The anomeric proton itself appears as a doublet centered at τ 3.42, $J_{1,2}$ = 3.3 Hz. There is no evidence that some of the material is present in the β-form. The aromatic protons of the protecting O-benzyl groups appear as a triplet at τ 2.73. A broad multiplet between τ 5.85

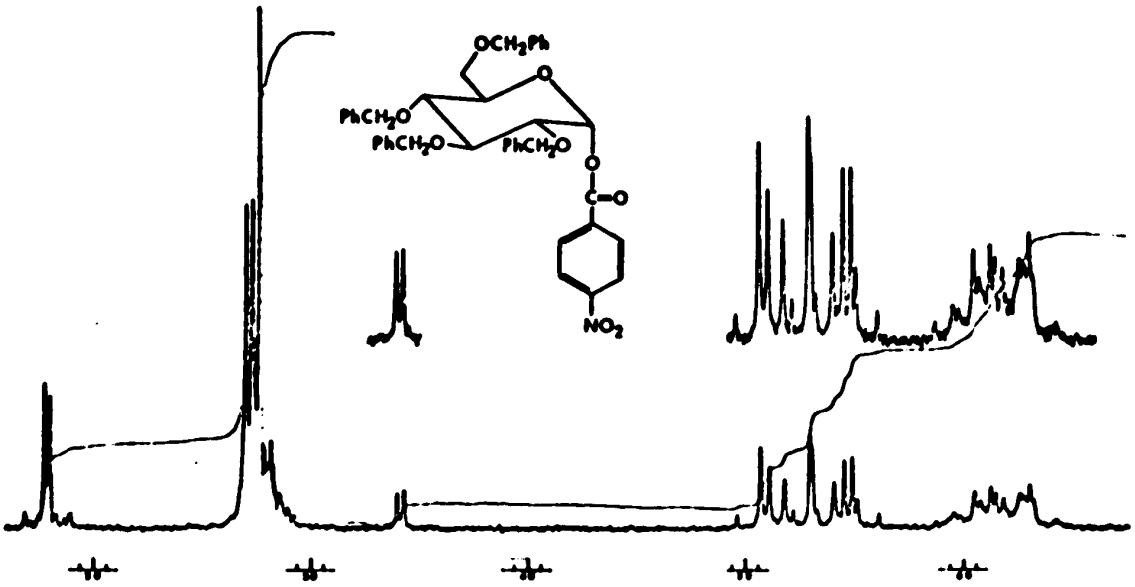


Fig. 4. N.m.r. spectrum (100 MHz) of tetra-O-benzyl-1-O-(p-nitrobenzoyl)-α-D-glucopyranose (5) in deuteriochloroform.

and τ 6.5 includes the ring protons and the methylene protons at carbon-6 of the sugar moiety. The signals for the remaining four methylene groups from the benzyloxy part of the molecule appear between τ 4.95 and τ 5.65.

The tetra-O-benzyl-α-D-glucopyranosyl bromide was prepared next following Fletcher's (38) procedure. A 10% molar excess of a solution of hydrogen bromide in methylene chloride is added to a solution of compound 5 in methylene chloride. p-Nitrobenzoic acid precipitates after a minute. It has been described in the literature to be sufficient to evaporate the excess solvent after removal of the p-nitrobenzoic acid by filtration. However, in the present research it was found to be of advantage to wash the filtrate a couple of times with sodium bicarbonate and once with water in order to remove the bulk of the unreacted hydrogen bromide and residues of p-nitrobenzoic acid. The product so obtained has to be reacted within ½ hr of its preparation, since it decomposes rapidly, as indicated by a change in color from virtually colorless to orange, dark red and brown after about 4 hr.

Fig. 5 shows the n.m.r. spectrum (60 MHz) of freshly prepared tetra-O-benzyl-α-D-glucopyranosyl bromide (6) in deuteriochloroform. The anomeric signal appears

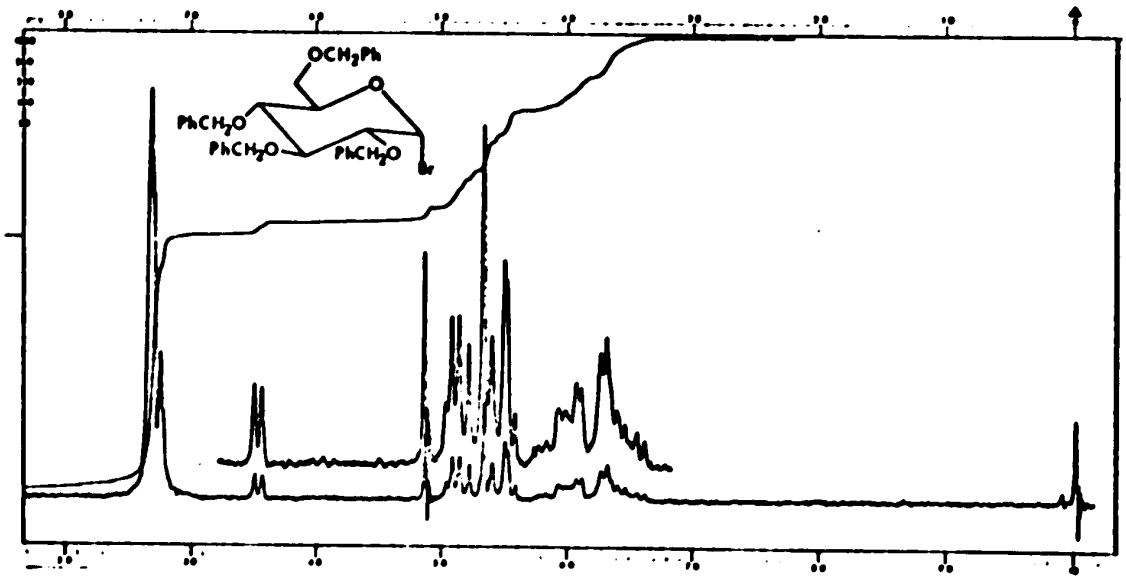


Fig. 5. N.m.r. spectrum (60 MHz) of tetra-O-benzyl-α-D-glucopyranosyl bromide in deuteriochloroform.

at τ 3.55 with a spacing of 3.5 Hz with sufficient intensity to indicate that compound 6 is predominantly present in the α-configuration.

Protons for the four methylene groups of the benzyloxy blocking groups in the molecule appear at τ 5.0 to τ 5.7, and the signals upfield from τ 5.7 are due to the ring protons including the two protons at carbon-6. The single peak at τ 4.86 is due to the presence of methylene chloride in the product. This remaining solvent which is trapped in the syrup is the reason for inaccurate values of the elemental analysis.

Fletcher and Ishikawa (38) have recently commented on the formation of tetra-O-benzyl-α-D-glucopyranosyl bromide from compound 5. They observed a dextromutarotation after the precipitate of p-nitrobenzoic acid had settled and the supernatant liquid was transferred into a polarimeter tube. This is consistent with the view that the product formed first when tetra-O-benzyl-1-O-(p-nitrobenzoyl)-α-D-glucopyranose is treated with hydrogen bromide in dichloromethane solution is, at least predominantly, the corresponding β-D-glucopyranosyl bromide. As the reaction time is extended, the β-D-glucopyranosyl bromide tends to equilibrate with its α-anomer. Weygand (46) discussed the mechanism of anomerization and assumed, in accordance with suggestions

made earlier by Lemieux (18), that cleavage occurs of the carbon to halogen bond which is similar to a dissociation, but that the halogen does not leave the region of the carbonium ion.

Recently, Lemieux and Hayami (4) have shown that the anomerization of D-glucopyranosyl halides is caused by halide ions and that already traces of halide ion are sufficient to cause anomerization. The reaction time of Weygand's method for preparing the tetra-O-benzylated glucopyranosyl bromide is 2 hr which is sufficient for the anomerization of the β-glucopyranosyl bromide to its α-anomer.

The preparation of compound 6 and its further reaction with alcohols is outlined in diagram 8.

As previously mentioned the main purpose of the present research was to assess the alcoholysis of suitably O-protected D-glucopyranosyl halides in the presence of added halide ions as a possible route for the preparation of α-D-glucopyranosides.

For precise kinetic investigations, it is essential to have a pure substrate or to know exactly the degree of purity of the substrate. The starting material for the present investigation, compound 6, has the disadvantage of being a syrup and was shown to

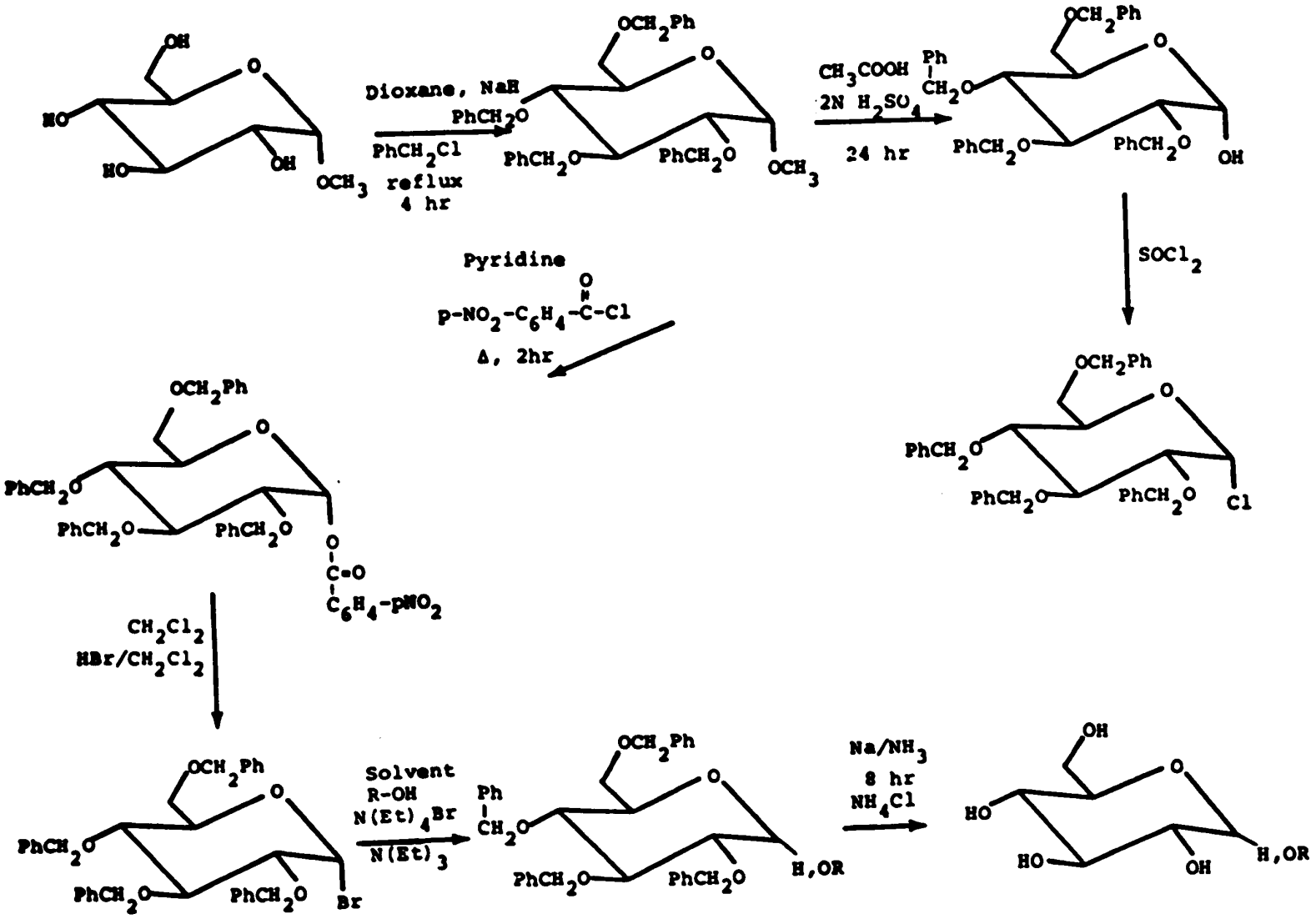

Dioxane, NaH
PhCH2Cl
reflux
4 hr
CH3COOH
2N H2SO4
24 hr
SOCl2
Pyridine
p-NO2-C6H4-C(=O)-Cl
Δ, 2hr
CH2Cl2
HBr/CH2Cl2
Solvent
R-OH
N(Et)4Br
N(Et)3
Na/NH3
8 hr
NH4Cl

Diagram 8

contain varying amounts of methylene chloride, the solvent in which it is prepared. Before carrying out reactions to determine the rate of formation of glycosides, the freshly prepared compound 6 was analyzed for purity by n.m.r. spectroscopy and optical rotation measurements. Another sample from the same batch was hydrolyzed with a 95% ethanol solution containing a two-fold molar excess of sodium hydroxide. The liberated bromide ions were titrated according to the procedure by Fajans (47) with silver nitrate using a 0.1% dichlorofluorescein solution as indicator. Finally, attempts were made to convert as quantitatively as possible the D-glucopyranosyl bromide 6 into the corresponding 1-O-acetyl-tetra-O-benzyl-D-glucopyranose. The α-anomer was first prepared by following the procedure described by Tate and Bishop (42), in which tetra-O-benzyl-α-D-glucopyranose is treated with acetic anhydride in pyridine at room temperature. The n.m.r. spectrum (60 MHz) of the 1-O-acetyl-tetra-O-benzyl-D-glucopyranose in deuteriochloroform is shown in Fig. 6. The chemical shift and spacing for the anomeric proton signal (τ 3.59, $J_{1,2}$ = 3.0 Hz) and the chemical shift for the protons of the acetoxy group (τ 7.92) are evidence for the α-anomer. A small peak at τ 8.01 for the equatorially oriented acetyl group at carbon-1

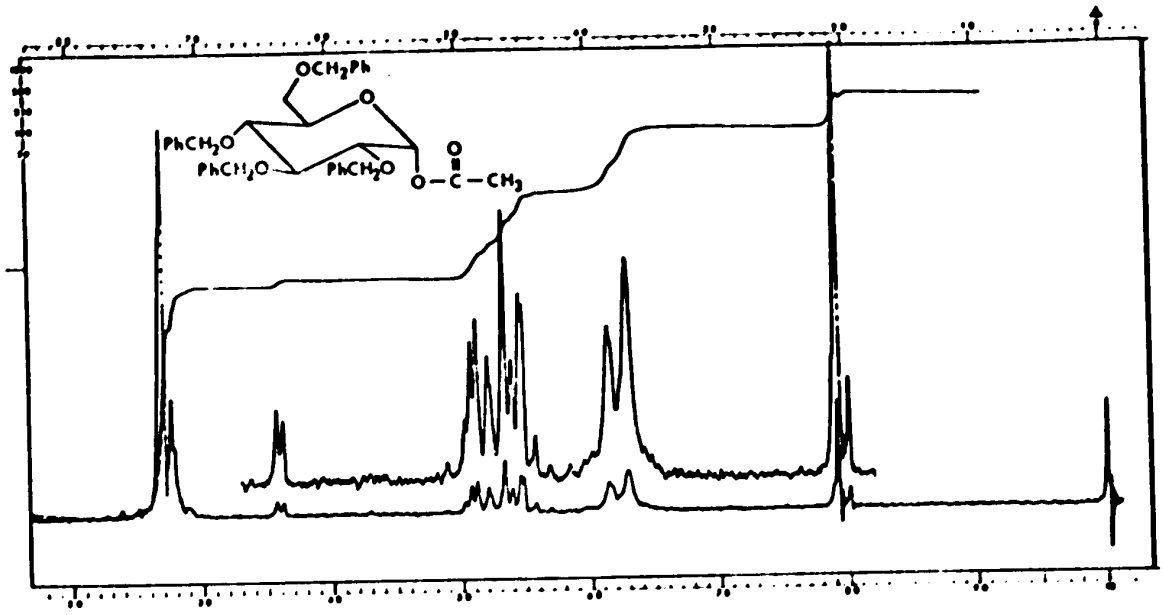


Fig. 6. N.m.r. spectrum (60 MHz) of 1-O-acetyl-tetra-O-benzyl-α-D-glucopyranose in deuteriochloroform.

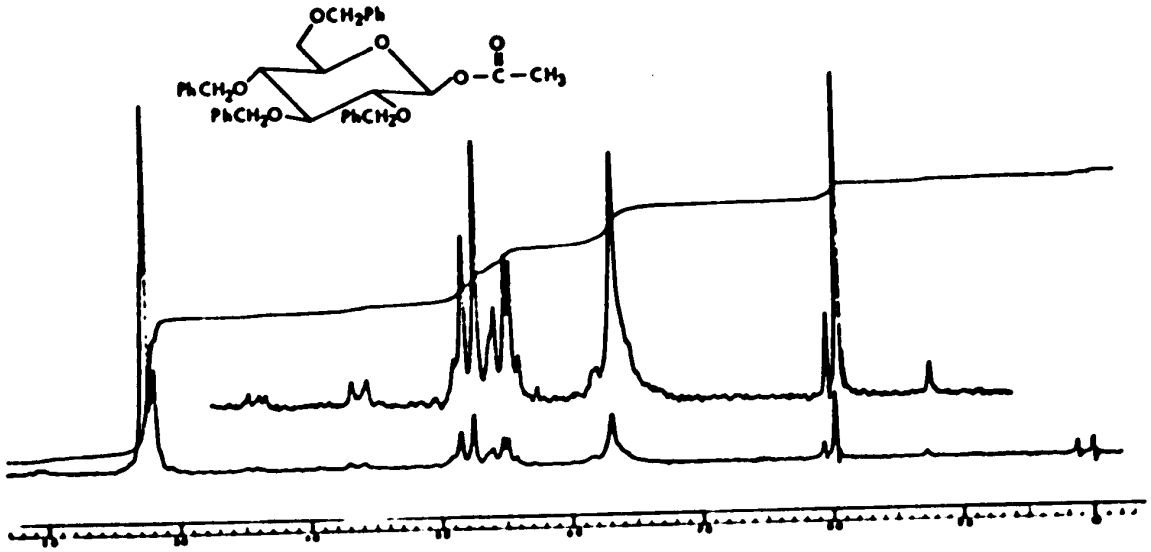


Fig. 7. N.m.r. spectrum (60 MHz) of 1-O-acetyl-tetra-O-benzyl-β-D-glucopyranose in deuteriochloroform.

indicated the presence of the β-anomer. Comparison of the relative intensities of the O-acetyl signals indicated that the α-anomer was the major product and that the β-anomer was present to an extent of less than 10%.

Compound 6 was then reacted in methylene chloride with silver acetate in a mixture of glacial acetic acid and acetic anhydride. The n.m.r. spectrum (60 MHz) of the syrupy product in deuteriochloroform, shown in Fig. 7, confirmed the expectation that mainly the β-anomer would be formed in this reaction. The signal for the anomeric proton appears at τ 4.37 with a spacing of 7.2 Hz. There is also a weak doublet at τ 3.62, $J_{1,2}$ = 3.2 Hz. Two signals for the acetoxy group in the ratio of approximately 5:1 at τ 8.03 (for the β-anomer) and τ 7.93 (for the α-anomer), respectively, gave an indication of the relative amounts of the two anomers present (approximately 83% β-anomer and 17% α-anomer).

In order to check for the integration response for the methoxy protons as compared to the acetoxy proton signal at carbon-1, a 1 to 1 molar mixture of methyl tetra-O-benzyl-α-D-glucopyranoside (2) and 1-O-acetyl-tetra-O-benzyl-α-D-glucopyranose (9) was prepared and submitted for n.m.r. spectroscopy. The spectrum (60 MHz) in deuteriochloroform is shown in Fig. 8. Three

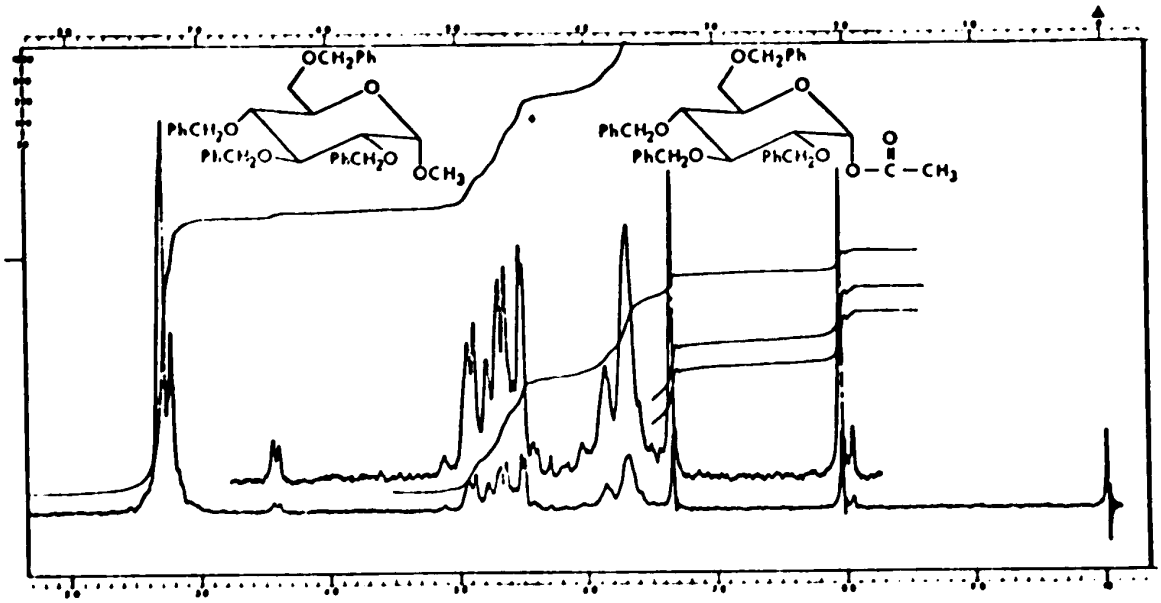


Fig. 8. N.m.r. spectrum (60 MHz) of a 1 to 1 molar mixture of methyl tetra-O-benzyl-α-D-glucopyranoside and 1-O-acetyl-tetra-O-benzyl-α-D-glucopyranose in deuteriochloroform.

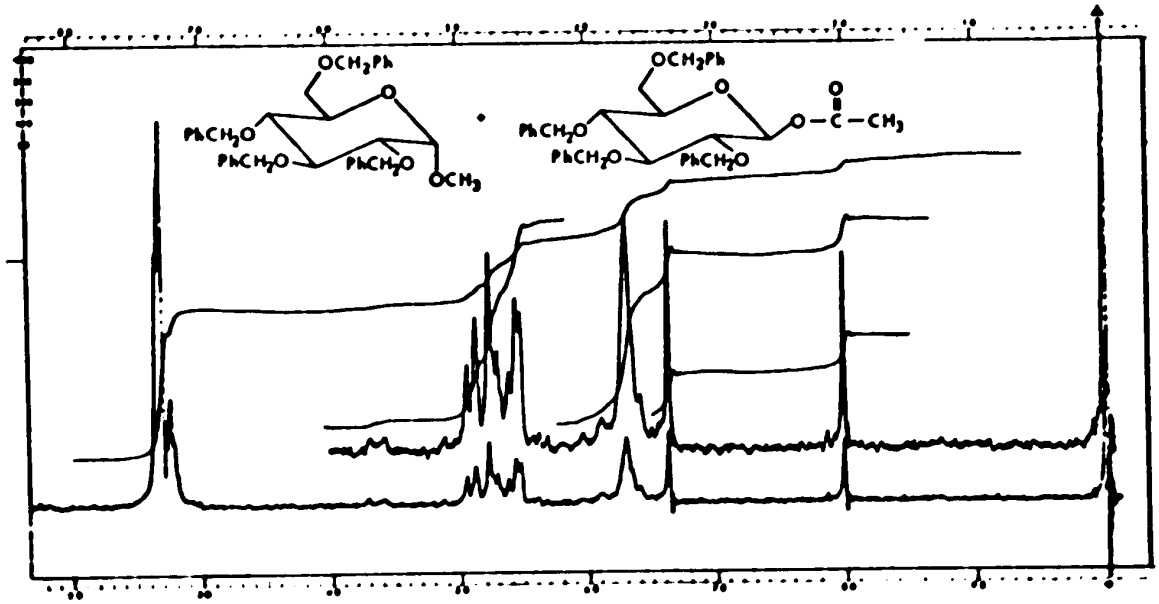


Fig. 9. N.m.r. spectrum (60 MHz) of the product of the reaction of tetra-O-benzyl-α-D-glucopyranosyl bromide with silver acetate in glacial acetic acid - acetic anhydride in the presence of 1 mole equivalent of methyl tetra-O-benzyl-α-D-glucopyranoside as internal standard.

integrations at two different integral amplitudes show the ratio of the relative intensities of the methoxy signal to the acetoxy signal to be 1:1. Therefore, n.m.r. spectroscopy could be utilized to establish the purity of the D-glucopyranosyl bromide 6. If one millimole of compound 6 was reacted with an excess of silver acetate and a mixture of glacial acetic acid and acetic anhydride under the conditions of the glycosidation reaction in the presence of one millimole of methyl tetra-O-benzyl-α-D-glucopyranoside (2) as internal standard, the n.m.r. spectrum of the worked-up reaction mixture gave a good indication of the purity of compound 6. The n.m.r. spectrum (60 MHz) of such a product mixture is shown in Fig. 9. If compound 6 were completely free of impurities, the ratio of the relative intensities for the proton signal of the methoxy group to that of the acetoxy group would be 1:1. In the example shown, the integration (methoxy:acetoxy = 15:13.9 = 1.08) indicates a purity for compound 6 of approximately 92%. The combined results of these test experiments showed that the degree of purity for the D-glucopyranosyl bromide 6 was generally between 90% and 95%.

Having established the quality of the starting material 6, attempts were undertaken to find conditions

under which large amounts of it could be stored for longer periods of time. Samples of freshly prepared glucopyranosyl bromide 6 were placed in small test tubes which were subsequently sealed off. Some of the tubes were placed in a dry ice-acetone bath and the rest in liquid nitrogen. After 4 hr, 16 hr, 24 hr, 48 hr and 5 days, respectively, the material was submitted for n.m.r. spectroscopy and was found virtually unchanged in all cases. However, for all glycosidation reactions described subsequently, the substrate 6 was prepared freshly each time.

The problem now was to establish a method for product analysis which would avoid work-up of the reaction mixture with subsequent separation of sugar components by column chromatography. In order to obtain some insight into the course and mechanism of halide ion catalyzed glycosidation reactions it was decided to carry out five or six reactions under equal conditions for 5 min, 15 min, 30 min, 1 hr, and 2 hr, respectively. Of interest to us was not only the rate of formation of glucopyranosides but also the stereoselectivity of the reaction in the presence of added halide ion as compared to that without added halide ion. The significant difference in chemical shift for the methoxy signal of methyl α- and β-D-glucopyranosides mentioned earlier was

expected to prove useful in analyzing the composition of the reaction mixture. Fig. 12 and 13 show the n.m.r. spectra (100 MHz) of the two anomers of methyl tetra-O-benzyl-D-glucopyranoside (2 and 7) in deuteriochloroform. The chemical shift difference of 0.16 τ-units between the two methoxy signals is clearly apparent from the spectra.

Formation of side products was expected and, therefore, in order to obtain some knowledge about the rate of disappearance of the starting material, it was desired to convert it into a stable derivative, the presence of which could be detected by n.m.r. spectroscopy. We have seen that the glucopyranosyl bromide 6 is converted quantitatively into the 1-O-acetyl-tetra-O-benzyl-D-glucopyranose (predominantly the β-anomer) in the reaction with an excess of silver acetate in a mixture of glacial acetic acid and acetic anhydride. As mentioned earlier, the resonance signal for the equatorially oriented acetoxy group at carbon-1 appears at τ 8.03 whereas the axial acetoxy group at the same position appears farther downfield at τ 7.93. Both signals are well separated from all other signals in the molecule of the O-benzylated methyl α- and β-D-glucopyranosides, and therefore well suited for the determination of the amount of unreacted glucopyranosyl bromide 6 after converting it into the stable 1-O-acetoxy derivative.

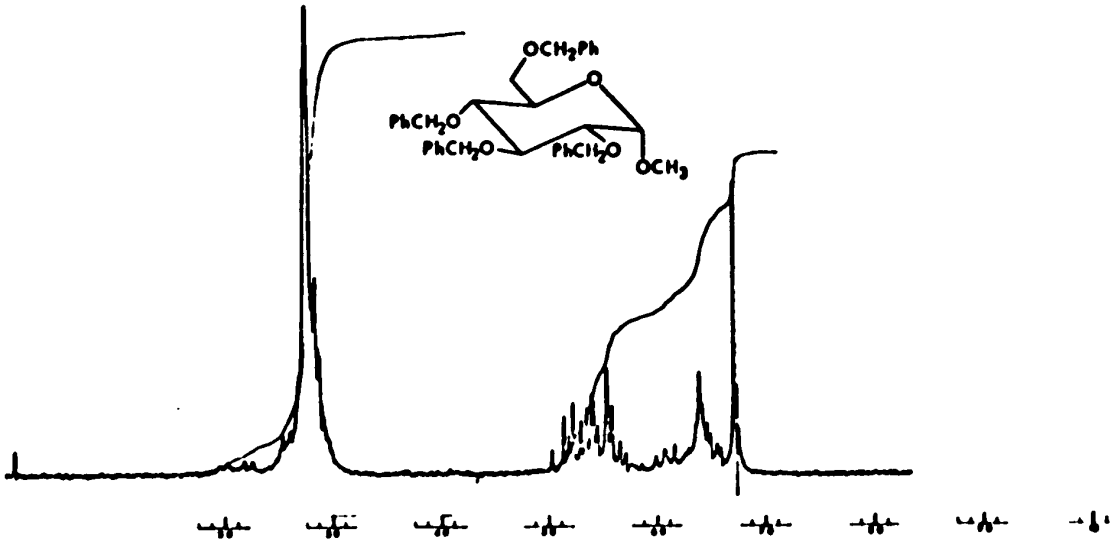


Fig. 12. N.m.r. spectrum (100 MHz) of methyl tetra-O-benzyl-α-D-glucopyranoside in deuteriochloroform.

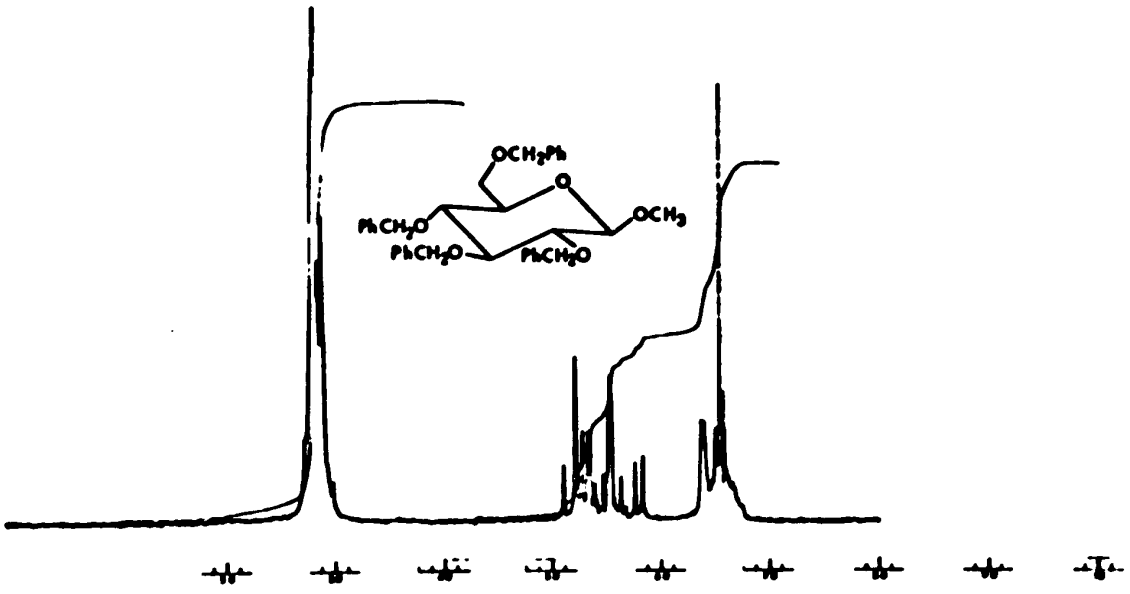


Fig. 13. N.m.r. spectrum (100 MHz) of methyl tetra-O-benzyl-β-D-glucopyranoside in deuteriochloroform.

Two series of reactions were carried out, termed series A and B. In reaction series A, one mole of tetra-O-benzyl-α-D-glucopyranosyl bromide (6) was reacted with 2 moles of methanol in methylene chloride at 60°C in the presence of one mole of diisopropylethylamine and one mole of tetraethylammonium bromide. The glycosidation reactions were quenched after 5 min, 15 min, 30 min, 1 hr and 2 hr periods by adding silver acetate to the reaction mixture along with glacial acetic acid and acetic anhydride. The products were then worked up as described in the Experimental and submitted for n.m.r. spectroscopy (100 MHz). Fig. 14 shows reproductions of these spectra for the products of experiment series A. The chemical shifts for the methoxy and acetoxy signals, respectively, are shown in Table 20.

The relative intensities of the α- and β-methoxy and acetoxy signals were measured as described in the Experimental Part. Fig. 15 shows a plot of the rate of disappearance of starting material 6 as well as of the rate of formation of methyl tetra-O-benzyl-α-D-glucopyranoside and its β-anomer. Fig. 15 shows the formation of methyl tetra-O-benzyl-D-glucopyranoside to proceed at a fairly fast rate; moreover the rate of formation of the α-anomer is much faster than that of

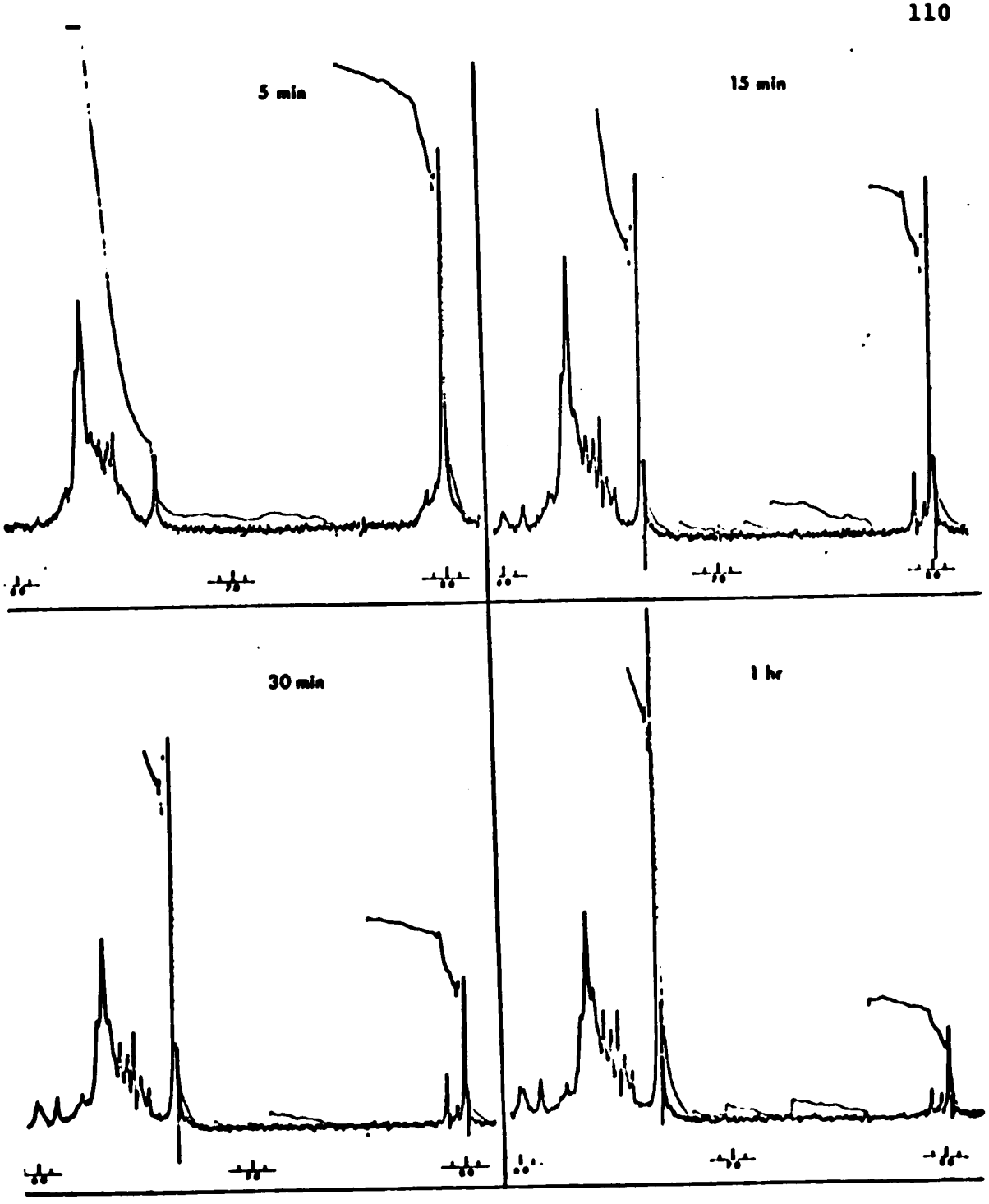


Fig. 14. N.m.r. spectra (100 MHz) of the products of the reaction of tetra-O-benzyl-α-D-glucopyranosyl bromide (6) with 2 moles of methanol in methylene chloride at 60°C in the presence of 1 mole of diisopropylethylamine and 1 mole of tetraethylammonium bromide (series A). Reaction times are indicated on the spectra.

TABLE 20

Chemical shifts (τ-values) for the methoxy and acetoxy groups, respectively, of methyl tetra-O-benzyl-D-glucopyranoside and 1-O-acetyl-tetra-O-benzyl-D-glucopyranose in deuteriochloroform at 100 MHz (TMS internal standard).

Configuration at carbon-1	Methoxy signal	Acetoxy signal
α	6.65	
β	6.47	
α		7.99
β		7.92

the β-anomer. The latter is formed only during the early stages of the reaction, its amount remaining virtually constant after a reaction time of 15 minutes.

We shall consider the course of the reaction with the aid of the formula scheme in diagram 9. This scheme will be somewhat simplified since it does not account for the possible formation of side products, the two most important of which would be anticipated to be the 1,2-glucoseen type derivative arising from dehydrohalogenation of compound 6, (48,81) and the product of hydrolysis of compound 6, tetra-O-benzyl-D-glucopyranose.

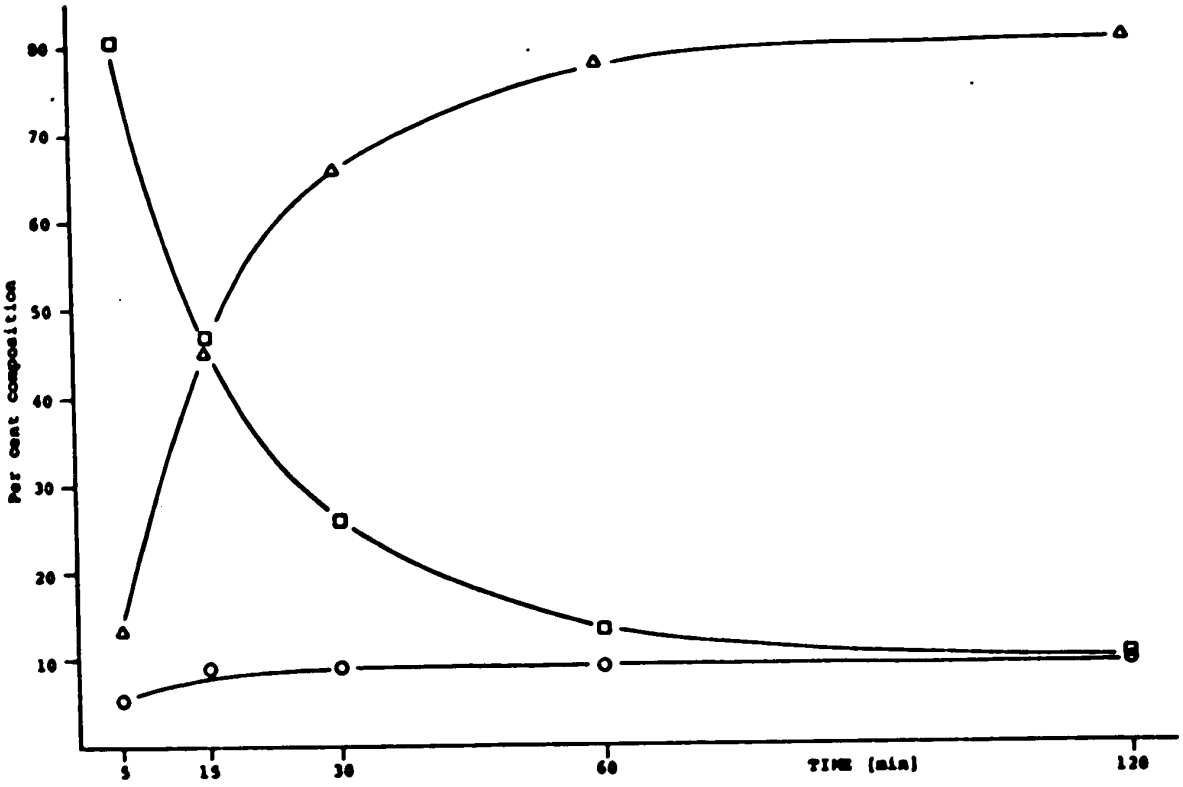
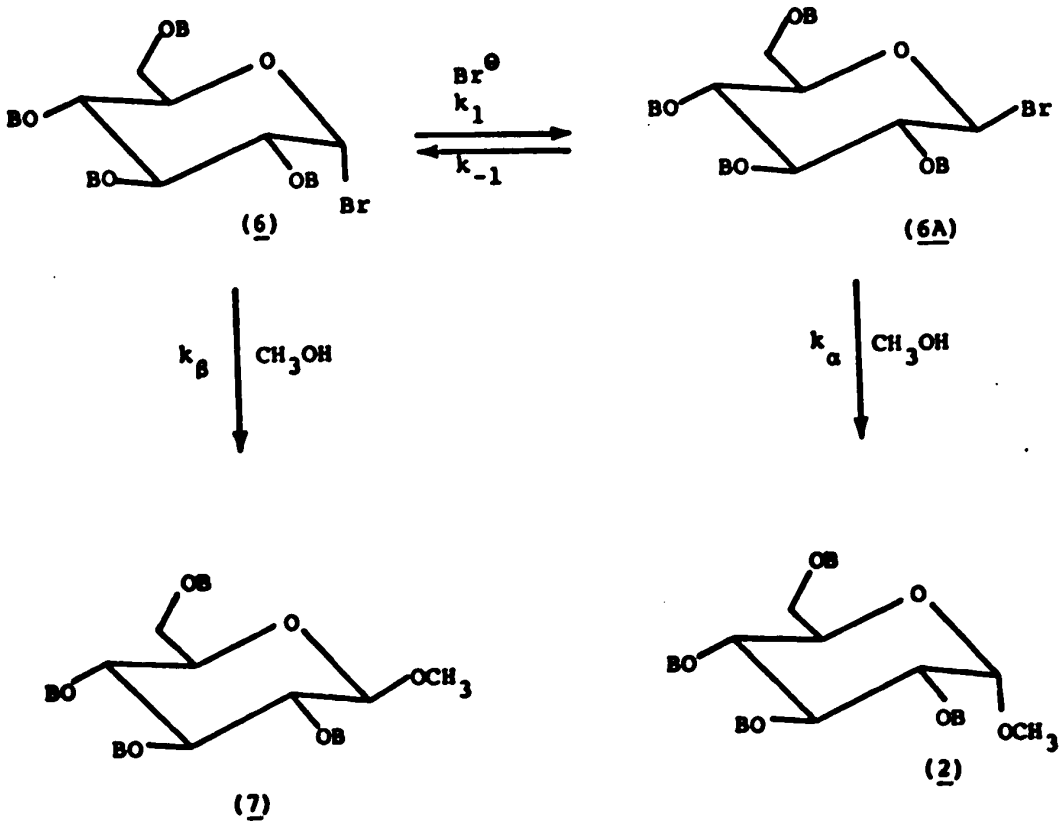


Fig. 15. Rates of formation of tetra-O-benzylated methyl α- and β-D-glucopyranosides on reacting tetra-O-benzyl-α-D-glucopyranosyl bromide (6), 0.1 M in methylene chloride, at 60°C with two mole equivalents of methanol and in the presence of one mole equivalent diisopropylethylamine and one mole equivalent of tetraethylammonium bromide.

□, tetra-O-benzyl-α-D-glucopyranosyl bromide (6)

△, methyl tetra-O-benzyl-α-D-glucopyranoside (2)

○, methyl tetra-O-benzyl-β-D-glucopyranoside (7)



B = blocking groups

Diagram 9.

If it is assumed that the α-D-glucopyranoside (2) arises from nucleophilic attack by methanol at the anomeric center of the β-D-glucopyranosyl bromide (6A) and the β-D-glucopyranoside (7) from nucleophilic attack by the alcohol at the anomeric center of the

α-D-glucopyranosyl bromide 6, then $k_\alpha > k_\beta$ and $k_1 > k_\alpha$ in order that the concentration of 6Λ not be depleted and thus maintain the high rate of formation of 2 as compared to 7. Lemieux and Hayami (4) have demonstrated that the anomerization of tetra-O-acetyl-β-D-glucopyranosyl chloride to its α-anomer is first order in chloride ion. One may therefore envisage rapid interconversion of the tetra-O-benzyl-D-glucopyranosyl bromides at rates which are considerably faster than the reaction of either of the anomeric D-glucopyranosyl bromides with alcohol, especially in the presence of a high concentration of bromide ion provided by the tetraethylammonium bromide.

The equilibrium constant for the α ⟷ β equilibration found for the tetra-O-acetyl-D-glucopyranosyl chlorides (4) may be expected of the same order of magnitude as that for the equilibration of the tetra-O-benzyl-D-glucopyranosyl bromides, that is 16. A further indication for the magnitude of the equilibrium constant for the equilibration of the tetra-O-benzyl-D-glucopyranosyl bromides is given by the fact that reaction of 6 with silver acetate in glacial acetic acid - acetic anhydride gave the β-O-acetate in about 83% yield, with 17% being the α-O-acetate. That the methyl α-D-glucopyranoside (2) is formed predominantly from compound 6 in the presence of added bromide ion must then be taken as

strong evidence for rapid equilibration of 6 to its β-anomer on the one hand, and for the greater reactivity of the β-D-glucopyranosyl bromide with methanol than the α-anomer 6 on the other hand, i.e. $k_\alpha > k_\beta$. In other words, the predominant formation of the α-D-glucoside in this reaction is the result of kinetic control.

In reaction series B, one mole of tetra-O-benzyl-α-D-glucopyranosyl bromide (6) was reacted with 2 moles of methanol in methylene chloride at 60°C in the presence of one mole of diisopropylethylamine. The glycosidation reactions were quenched after 5 min, 15 min, 30 min, 1 hr and 2 hr periods by adding silver acetate to the reaction mixture along with glacial acetic acid and acetic anhydride. The products were then worked up as described in the Experimental Part and submitted for n.m.r. spectroscopy (100 MHz). Fig. 16 shows reproductions of these spectra for the products of experiment series B. The chemical shifts for the methoxy and acetoxy signals, respectively, are given in Table 20, p. 111.

The relative intensities of the α- and β-methoxy and acetoxy signals were measured as described in the Experimental Part. Fig. 17 shows a plot of the rate of disappearance of starting material 6 as well as of the rate of formation of methyl tetra-O-benzyl-α-D-gluco-

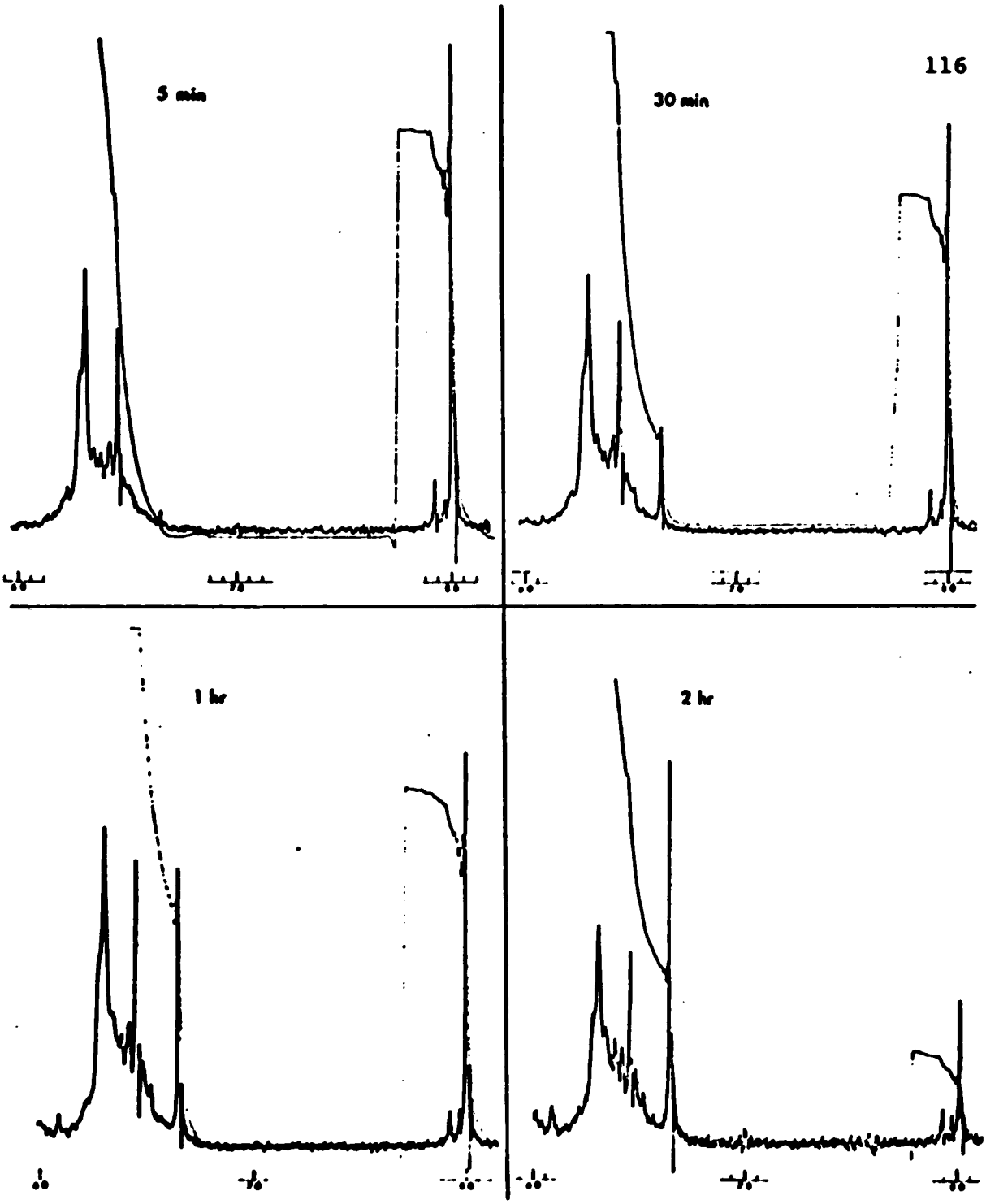


Fig. 16. N.m.r. spectra (100 MHz) of the products of the reaction of tetra-O-benzyl-α-D-glucopyranosyl bromide (6) with 2 moles of methanol in methylene chloride at 60°C in the presence of 1 mole of diisopropylamine (series B). Reaction times are indicated on the spectra.

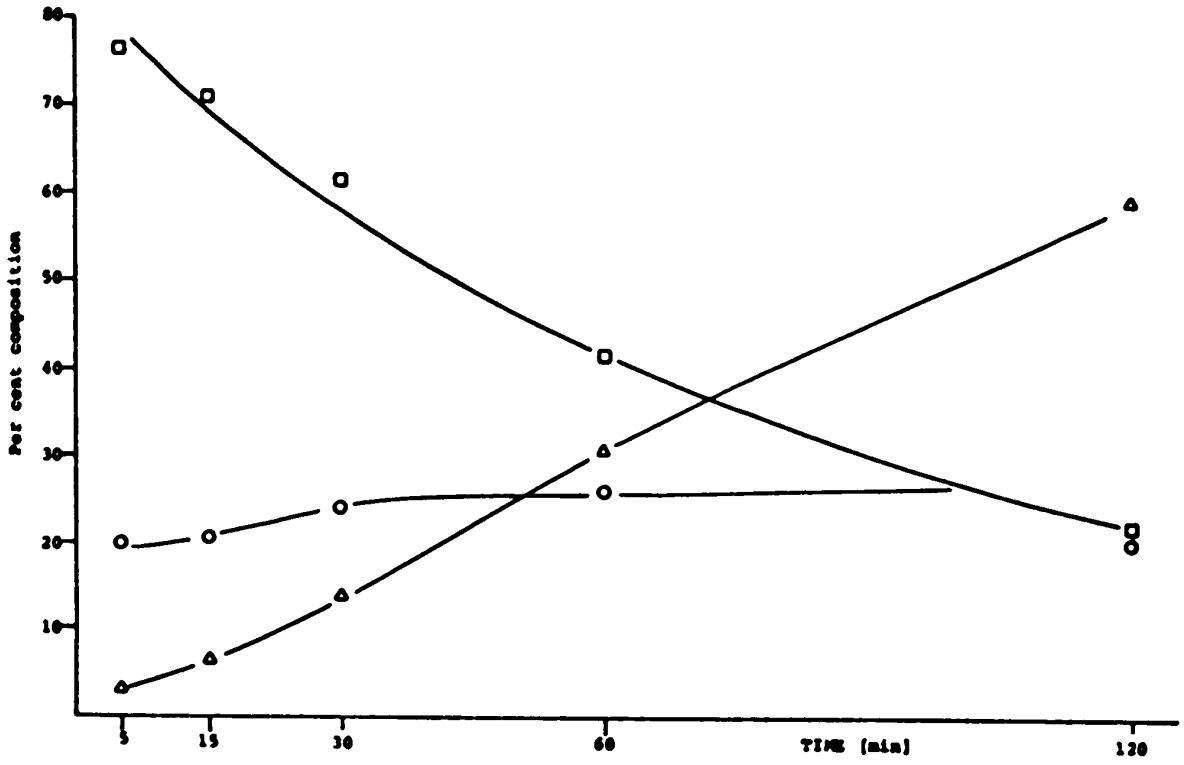


Fig. 17. Rates of formation of tetra-O-benzylated methyl α- and β-D-glucopyranosides in the absence of added halide ion on reacting tetra-O-benzyl-α-D-glucopyranosyl bromide (6), 0.1 M in methylene chloride, at 60°C with two mole equivalents of methanol and in the presence of one mole equivalent diisopropylethylamine.

□, tetra-O-benzyl-α-D-glucopyranosyl bromide (6)

△, methyl tetra-O-benzyl-α-D-glucopyranoside (2)

○, methyl tetra-O-benzyl-β-D-glucopyranoside (7)

pyranoside and its β-anomer. The plot shows the rate of glycosidation generally to be slowed down somewhat after the first five minutes. A considerable amount (approximately 20%) of β-glucoside has been formed during that initial period of reaction. The rate of formation of β-glucoside slows down dramatically after 5 minutes reaction time and becomes virtually constant, whereas that of formation of α-glucoside increases steadily. The experimental observations may be interpreted in the following way.

The α-bromide 6 first undergoes nucleophilic attack by alcohol with inversion of the configuration at carbon-1 to form β-D-glucopyranoside (7) and thus releasing bromide ion. The liberated bromide ions then lead to equilibration of the α-bromide 6 with its β-anomer (6A). That the bromide ions catalyze the overall reaction is evident from a comparison of the two plots for the rate of disappearance of starting material in Fig. 15 and 17.

The very rapid formation of the methyl β-D-glucopyranoside during the initial stages of the reaction is subject to some speculation. One reason for this phenomenon lies probably in the high alcohol concentration at the beginning of the reaction which may cause

some strong solvation of the initially released bromide ions. As the reaction proceeds the alcohol concentration decreases while more bromide ions are liberated. The anomerization of the α-bromide 6 to its β-anomer soon becomes the dominating process which seems to control the rate of the reaction. After about 15 minutes the amount of β-D-glucoside formed remains virtually constant at about 25%. The anomerization governs the further course of the reaction and subsequently the almost exclusive formation of the α-D-glucoside is observed.

Fig. 18 shows a plot of the ratio of the α- to β-anomer of the methyl D-glucopyranosides formed in the previous two reaction series with and without added halide ion. The figure clearly demonstrates the effect of bromide ions on the stereochemical result of the reaction. The α to β values for the experimental series B which was carried out in the absence of added bromide ions were determined with the aid of a calibration curve. This curve was obtained by preparing five different mixtures of anomeric methyl tetra-O-benzyl-D-glucopyranosides with an α to β ratio of 3:1, 2:1, 1:1, 1:2 and 1:3. The n.m.r. spectra (100 MHz) of these mixtures were taken and the relative intensities of the signals for the α- and β-methoxy protons were obtained by measuring the peak height and multiplying it with the width at

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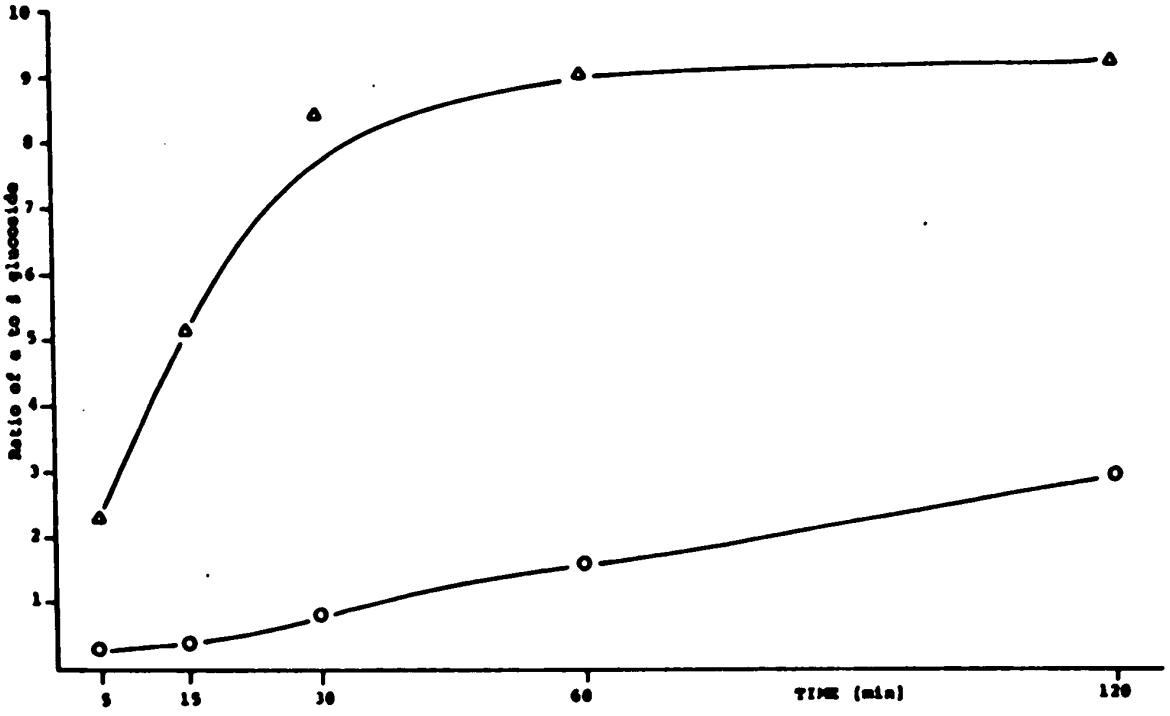

Fig. 18. Effect of bromide ions on the rates of formation of tetra-O-benzylated methyl α- and β-D-glucopyranosides on reacting tetra-O-benzyl-α-D-glucopyranosyl bromide (6), 0.1 M in methylene chloride, at 60°C with two mole equivalents of methanol and in the presence of one mole equivalent diisopropylethylamine.

Δ, in the presence of one mole equivalent of tetraethylammonium bromide

O, without added bromide ion

half height. From the plot of the observed α to β ratio vs the actual composition of the mixture, it is seen that the intensity of the β-methoxy signal was too high in all five cases. The β-methoxy signal appears on top of a broad multiplet which is due to the ring protons and the two methylene protons at carbon-6. Only the α to β ratios of the series B were corrected since the observed anomeric ratios for the reaction done in the absence of added halide ion were within the range of the calibration curve. For the reactions which were performed in the presence of added halide ion, the anomeric ratio of the produced methyl glucosides was so strongly in favor of the α-anomer, that a correction seemed not necessary.

That the α-D-glucopyranoside is formed preferentially over its β-anomer in the absence of a halide ion scavenger after a prolonged reaction time may be attributed to the effect of the released bromide ion on the stereochemical route of the reaction. Thus the amount of β-glucoside formed in the reaction remains virtually constant after a relatively large yield in the early stages of the reaction. The α to β ratio, as shown in Fig. 18, increases constantly after about 15 minutes reaction time, after it remained constant and smaller than 1 for the first 15 minutes of the reaction.

Of particular interest is therefore the nature of the very first reaction step, i.e. the anomerization of the α-D-glucopyranosyl bromide 6 to its β-anomer by bromide ion. As mentioned earlier, one must consider here a transition state from which the α-D-glucopyranoside is formed almost exclusively. In other words, it seems possible to assume that the nature of a reactive intermediate governs the stereochemical result of the product. One may speculate at this point as to the likely structure of that transition state and how it is possibly formed. A mechanism which is analogous to that discussed by Lemieux and Hayami (4) for the anomerization of tetra-O-acetyl-β-D-glucopyranosyl chloride by chloride ion may be considered here. Thus the mechanism of the anomerization of tetra-O-benzyl-α-D-glucopyranosyl bromide likely involves nucleophilic attack by bromide ion at the anomeric center to form an intimate ion-triplet, see diagram 10 p. 123.

Under alcoholysis conditions one should expect formation of two different ion pairs of different thermodynamic stability from the ion triplet. The main driving force for such formation of ion-pairs is the participation of the ring oxygen in delocalization of the positive charge of the developing carbonium ion.

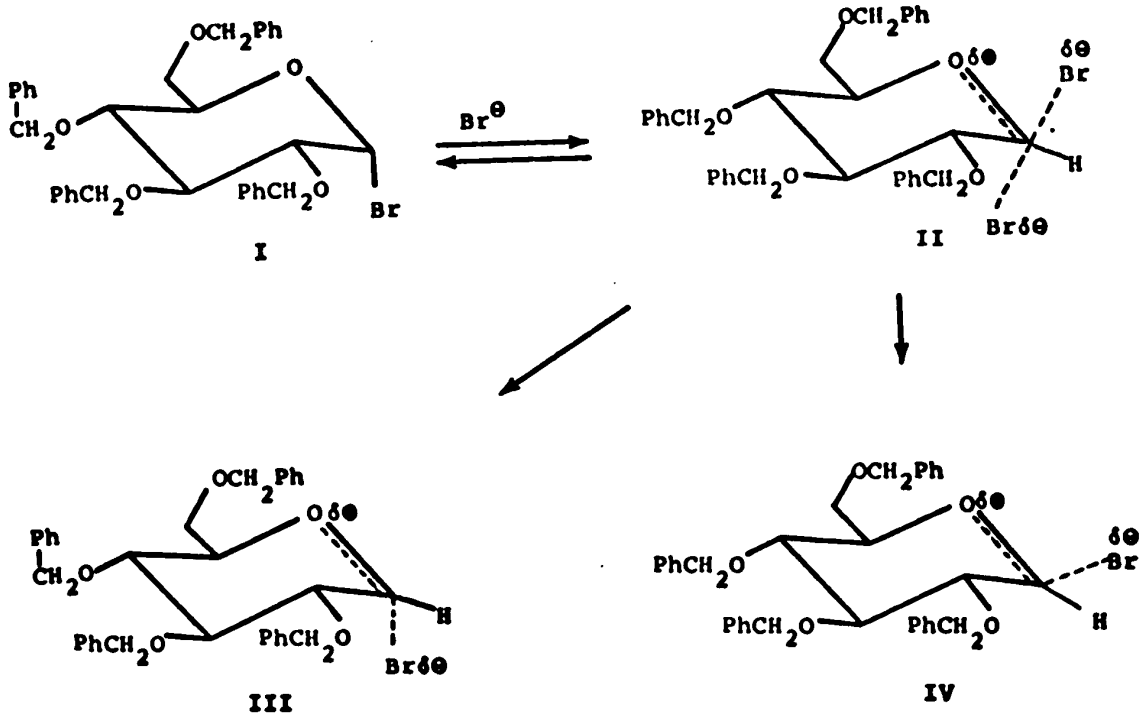

Diagram 10

One would expect the ion-pair with the halide in an equatorial position at the reacting center (structure IV) energetically favored, since the halide is oriented *trans* to the bulky non-participating substituent at C-2. Ion-pair III, however, having the halide ion at C-1 in an axial orientation, is therefore expected to be less stable because of the 1,2-*cis* relationship between the halide ion and the substituent at C-2. Formation of ion-pair IV will therefore require less energy and can be expected to be a much faster process than the formation

of an ion-pair like structure III. Nucleophilic attack by alcohol upon the anomeric centre of the two outlined probable ion-pairs leads to the formation of glycosides, ion-pair III presumably furnishing the β-D-glucopyranoside whereas ion-pair IV is expected to yield the α-D-glucopyranoside. The two processes should be energetically of similar kind, that is equally favored (or unfavored). This means that the observed preference of α-D-glucopyranoside formation is caused entirely by the presence of bromide ion, and is dependant upon the rate of ion-pair formation to give the thermodynamically more stable ion-pair which has the halide ion in an equatorial position at the reacting center. The nature and the thermodynamic stability of the intermediate ion pairs would on this basis govern the stereochemical route of the reaction in such a way as to make it highly stereoselective.

The reaction of O-protected glucosyl halides with alcohols, often in the presence of silver salts and under solvolysis conditions has been widely described in the literature for the past 75 years. The effect of halide ion on the stereochemical outcome of reactions of these glucosyl halides with alcohols is apparent in these reports. In some cases, the authors either did not

try to give an explanation, or, in our opinion, came to erroneous interpretations owing to a lack of an appreciation of the phenomenon we have termed halide-ion catalysis.

In 1929, Hickinbottom (11) reported the results of a study of some alcoholysis reactions of tri-O-acetyl-2-trichloroacetyl-β-D-glucopyranosyl chloride and 3,4,6-tri-O-acetyl-β-D-glucopyranosyl chloride. Both compounds have only weakly participating groups at the 2-position. When either compound was dissolved in dry methanol and the change in optical rotation of the solution followed, a rise in rotation to a maximum was observed. The rotation then gradually fell to an equilibrium value. In the presence of silver carbonate or silver oxide the α-D-glucopyranoside was formed in 70% yield. This yield was raised to 90% by adding pyridine and using silver nitrate. The course of the reaction of the substrate with methanol in the absence of silver salts appeared to be somewhat complex. The upward trend of the rotation suggested that the β-D-glucosyl chloride was converted into compounds belonging to the α-series, either α-D-glucopyranoside or the α-D-glucopyranosyl chloride. Hickinbottom was able, after deacetylation with "alcoholic ammonia", to isolate by fractional crystallization, pure specimens of the methyl α- and

β-D-glucopyranosides. If ethanol was used in the deacetylation reaction, then the observed formation of the anomeric methyl D-glucopyranosides occurred as a result of the alcoholysis reaction. He concluded that the β-D-glucopyranoside had formed from the α-D-glucosyl chloride present in the solution, which in turn had formed from the β-D-glucosyl chloride by anomerization. He showed that the isomerization did not occur in dry benzene in the presence of either silver oxide or silver chloride. The conclusion was reached that the anomerization occurred through an action of the alcohol. Whereas some methyl β-D-glucopyranoside may have formed by anomerization of the corresponding α-isomer by methanolysis in the presence of eliminated hydrogen chloride (84), we suggest that the chloride ion produced in the initial stage of the reaction was the agent responsible for the anomerizatin of the starting material to the α-halide which subsequently formed the methyl β-D-glucopyranoside.

In 1953, Helferich, Doppstadt and Gottschlich (79) reported the formation of methyl tetra-O-acetyl-β-D-glucopyranoside along with "acetylated free sugars" in the reaction of tetra-O-acetyl-α-D-glucopyranosyl bromide ("acetobromoglucose") with an excess of methanol in the presence of lutidine. While the formation of

the β-D-glucopyranoside can be attributed to a direct nucleophilic attack of the alcohol on the anomeric center, the formation of the "acetylated free sugars" which after acetylation yielded the α- and β-pentaacetates remained obscure. The possibility that these compounds were formed by direct hydrolysis of the acetobromoglucose through the presence of water in the system can be discounted. We consider that they were produced by the following more complex mechanism. Likely the initial formation of methyl β-D-glucopyranoside liberated bromide ions which anomerized the starting substrate, α-acetobromoglucose. Thus, β-acetobromoglucose was available for orthoester formation (50). Hydrolysis of the highly acid-sensitive orthoesters on work-up would then have been the source of the "acetylated free sugars" which gave the α- and β-pentaacetates upon acetylation. The formation of the isopropyl orthoester from α-acetobromoglucose and isopropanol was demonstrated in the same paper.

In 1956, Helferich and Weis (80) reported the formation of methyl and benzyl orthoesters in the reaction of tetra-O-benzoyl-α-D-glucopyranosyl bromide ("benzobromoglucose") with methanol and benzyl alcohol, respectively, in nitromethane and in the presence of collidine. Again the formation of the orthoesters

likely did not arise from the glucosyl halide which has the halogen atom attached to the anomeric center in an axial orientation. Anomerization to the β-halide through catalysis by liberated halide ion must be considered (50) to be the first step in the formation of the orthoester.

A striking example of catalysis by halide ion appears to be present in the work published by Lloyd and Roberts (16) who demonstrated the formation of substituted ethyl 2-amino-2-deoxy-α- and β-D-glucopyranosides in condensation reactions of 3,4,6-tri-O-acetyl-2-deoxy-2-(2',4'-dinitroanilino)-α-D-glucopyranosyl bromide with ethanol in various solvents in the presence of a variety of condensating agents (see Introduction, p. 7 and 8). Their experimental results seem best explained by assuming that the initial formation of substituted 2-amino-2-deoxy-β-D-glucopyranoside liberated bromide ions which effectively catalyzed the anomerization of the starting glucosyl bromide to its β-anomer which in turn yielded predominantly the substituted ethyl 2-amino-2-deoxy-α-D-glucopyranoside.

A recent paper by Ishikawa and Fletcher (38) reports the formation of the two anomers of methyl D-glucopyranoside from variously substituted D-glucosyl bromides under alcoholysis conditions, both in the

presence and in the absence of added bromide ion (tetra-n-butylammonium bromide). The reaction of tetra-O-benzyl-α-D-glucopyranosyl bromide (6) with a 75-fold molar excess of methanol in dichloromethane at room temperature yielded the α- and β-anomers of the corresponding methyl D-glucopyranosides in the ratio 0.9:1.1, respectively. No absolute yields were reported. When the reaction was performed in the presence of a 4 molar excess of tetra-n-butylammonium bromide, the α to β ratio in the product mixture was 2.6:1 as expected from the phenomenon of halide-ion catalysis.

It appears then that the reaction of suitably O-protected α-D-glucopyranosyl halides with alcohols can be classified as follows: (a) in the presence of silver carbonate, silver oxide or any other effective halide-ion scavenger; (b) in the absence of a halide-ion scavenger; and (c) in the presence of added halide ion. In the first case, the reaction leads to a predominant formation of derivatives of β-D-glucopyranosides as is documented throughout the chemical literature, most recently by Brennan and Finan (82), as well as by the present research. In the absence of silver salts, or other halide-ion scavengers, the reaction yields initially the glucosidic product with inversion of configuration at the anomeric center. However, as a consequence halide

ion is produced which leads to an increasing rate of anomerization of the glucosyl halides, and reaction by way of the more reactive β-glucosyl halide gradually predominates. The presence of an initially high concentration of halide ion in the reaction mixture provides rapid anomerization of the glucosyl halide from the beginning of the reaction.

In order to further verify these conclusions and to gain more insight into the course of the reaction, a number of glucosidations were carried out under varying conditions. In the experiments discussed so far, nuclear magnetic resonance spectroscopy was employed as the analytical method. The different chemical shifts for the methoxy protons of the tetra-O-benzyl derivatives of the anomeric methyl D-glucopyranosides allowed a fairly accurate assessment of the amounts of the glucoside formed in a given reaction. Another method of product analysis employed was based on gas-liquid partition chromatography (g.l.p.c.). In 1963, Sweely and coworkers (41) published a detailed study of g.l.p. chromatography of sugars which had been converted into the trimethylsilyl derivatives. They reported the successful separation of the two anomers of methyl D-glucopyranoside. However, difficulties in reproducing their reported

separation prompted the preparation of the trimethylsilyl derivatives of ethyl α- and β-D-glucopyranosides and to subject these to g.l.p.c. analysis. Injections of the single anomers as well as of mixtures of both showed that the α-anomer had the shorter retention time. The ethyl D-glucopyranosides were obtained from the ethyl tetra-O-benzyl-D-glucopyranoside mixtures by de-O-benzylation using sodium in liquid ammonia (67). In a typical experiment, the products from the reactions of the syrupy tetra-O-benzyl-α-D-glucopyranosyl bromide with ethanol in various solvents (see Table 18) were examined by n.m.r. and thin-layer chromatography using tetra-O-benzyl-α-D-glucopyranose and ethyl tetra-O-benzyl-β-D-glucopyranoside as reference compounds. This allowed a semiquantitative estimate of the total amount of the glucosides formed. A more accurate determination of the total yield of the glucosides formed was made by comparing the intensities of the resonance signals for the aromatic protons of the benzyl groups (about τ 2.65, 20 H) with the intensity of the signal for the methyl protons of the aglycon which appears as a triplet centered at τ 8.73 (3 H). The reaction product was then debenzylated with an 8 to 10 fold excess of sodium in liquid ammonia (67). The debenzylated ethyl D-glucopyranosides

were extracted with pyridine from the solid residue. The bibenzyl (1,2-diphenylethane, identified by n.m.r.) was also extracted. Therefore, after removing the bulk of the pyridine by vacuum evaporation, the resulting syrup was extracted into D_2O and the resulting solution washed with chloroform. The material left in the D_2O layer was then submitted for n.m.r. analysis. That the material represented a fairly pure mixture of the ethyl α- and β-D-glucopyranosides was evident from the spectra, for example, the spectrum reproduced in Fig. 19. In order to establish the relative amounts of the α- and β-anomers formed, the D_2O solution was reduced to dryness and the residue was trimethylsilylated. The trimethylsilyl derivatives thus obtained were analyzed by gas-liquid partition chromatography. The detector response of the particular Gas Chromatograph used in these experiments was found to be the same for both the α- and β-anomers of ethyl tetra-O-trimethylsilyl-D-glucopyranoside. This was established by trimethylsilylations of authentic samples of a 1:1 mixture of these D-glucopyranosides. Table 19 on p. 64 shows the results of these investigations. As seen in Table 19, dimethylsulfoxide and dimethylformamide are the least suitable solvents, since either the α to β ratio of the produced ethyl D-glucopyranosides is

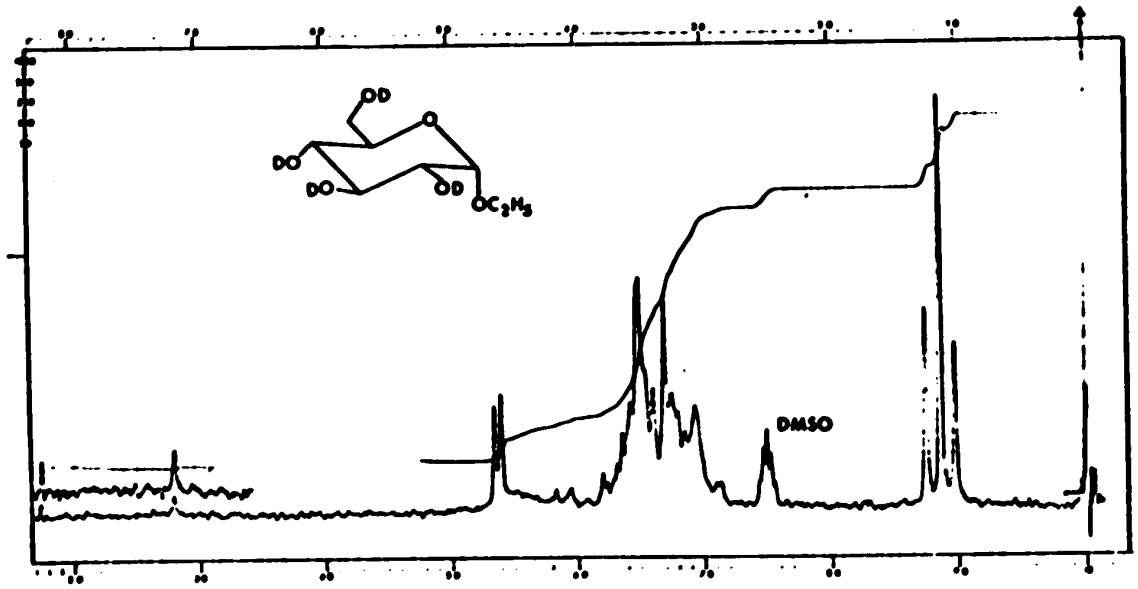

Fig. 19. N.m.r. spectrum (60 MHz) of a typical ethyl α- and β-D-glucopyranoside mixture in DMSO-d_6, obtained by de-O-benzylation prior to trimethylsilylation for g.l.p.c. analysis.

unfavorable or the number of side products is too high. The glycosidation proceeded best in benzene, methylene chloride and acetonitrile. The results obtained in benzene were somewhat surprising since tetraethylammonium bromide is only slightly soluble in this solvent. Methylene chloride was chosen to examine the reaction in further detail, because the tetra-O-benzyl-α-D-glucopyranosyl bromide was prepared in this solvent. It was mentioned earlier that this reagent could not completely be freed from methylene chloride.

A series of five reactions were carried out in methylene chloride using ethanol to form the aglycon. The results are summarized in Table 21. The reaction performed in the absence of both a base and tetraethylammonium bromide produced the anomeric ethyl tetra-O-benzyl-D-glucopyranosides with an α to β ratio of approximately 2:1. The α to β ratios were established by gas-liquid partition chromatography as described above. The n.m.r. method is of limited accuracy. Nevertheless, the reasonably good agreement of the two values for the α to β ratios in this and the following experiments may be noted. The dramatic effect of added bromide ions on the stereochemical route of the reaction was clearly demonstrated by the results of experiment No. 2. It is seen that the formation of the α-D-

TABLE 21

Reaction parameters, yields and observed α to β ratios of D-glucosides formed on reacting tetra-O-benzyl-α-D-glucopyranosyl bromide (6), 0.2 M in methylene chloride, at 80°C with two mole equivalents of ethanol under varying conditions.

Experiment No.	Moles per mole of 6		Total yield of glucoside (%)	Ratio of α to β glucoside	
	Tetraethyl-ammonium bromide	Triethyl-amine		by g.l.p.c.	by n.m.r.
1	-	-	64	2.5	1.6
2	2	-	75	4.6	4.8
3	-	1.5	96	5.2	5.3
4*	-	-	72	2.4	2.8
5	2	1.5	93	8.2	-

* Dioxane was the solvent

glucopyranoside was nearly doubled. The presence of a tertiary amine also increased the yield of α-D-glucopyranoside formed and appeared to accelerate the reaction (see experiment No. 3). The experiment carried out using dioxane as solvent and in the absence of both the triethylamine and tetraethylammonium bromide gave results similar to those using methylene chloride and, therefore, dioxane was not further explored as a solvent (compare experiment Nos. 1 and 4 of Table 21). The experiment using methylene chloride as solvent and adding both triethylamine and tetraethylammonium bromide produced an α to β ratio for the ethyl tetra-O-benzyl-D-glucopyranosides of 8.2:1 (Experiment No. 5). This result is in agreement with that found for the glycosidation reactions with methanol which were discussed earlier (p. 110). The results of these experiments clearly confirm the effect of bromide ions on the stereochemical route of the reaction in that the presence of bromide ions in the reaction mixture at initially high concentrations leads to predominant formation of α-D-glucopyranosides.

In order to gain a proper insight into the kinetic course of the glycosidation reaction using tetra-O-benzyl-α-D-glucopyranosyl bromide as the substrate

it was necessary to develop a reasonably rapid analytical method to determine the amounts of products formed after various reaction times. The previously employed method of debenzylation and subsequent analysis by g.l.p.c. was considered too time-consuming for this purpose. Therefore, the following method based on n.m.r. was established. As for the experiments described on p. 50 (Experimental Part), reactions of tetra-O-benzyl-α-D-glucopyranosyl bromide (6) with isopropanol under varying conditions were stopped by mixing with silver acetate in acetic acid-acetic anhydride. However, methyl tetra-O-benzyl-α-D-glucopyranoside was present in known concentrations in the reaction mixture. Thus, the intensity of the n.m.r. signal for the methyl group could serve as internal standard to establish the amount of substituted isopropyl D-glucopyranoside formed. As before, the amount of unreacted starting material (6) was estimated from the intensity of the signal for the acetyl groups introduced into the product in the reaction with silver acetate. Typical spectra are presented in Fig. 20, showing the products of reaction series G. The spectra were taken in such a way as to show only the high field part of the spectrum containing the signals for the methoxy, acetoxy and isopropyl group. It can be seen that the ratio of the intensity

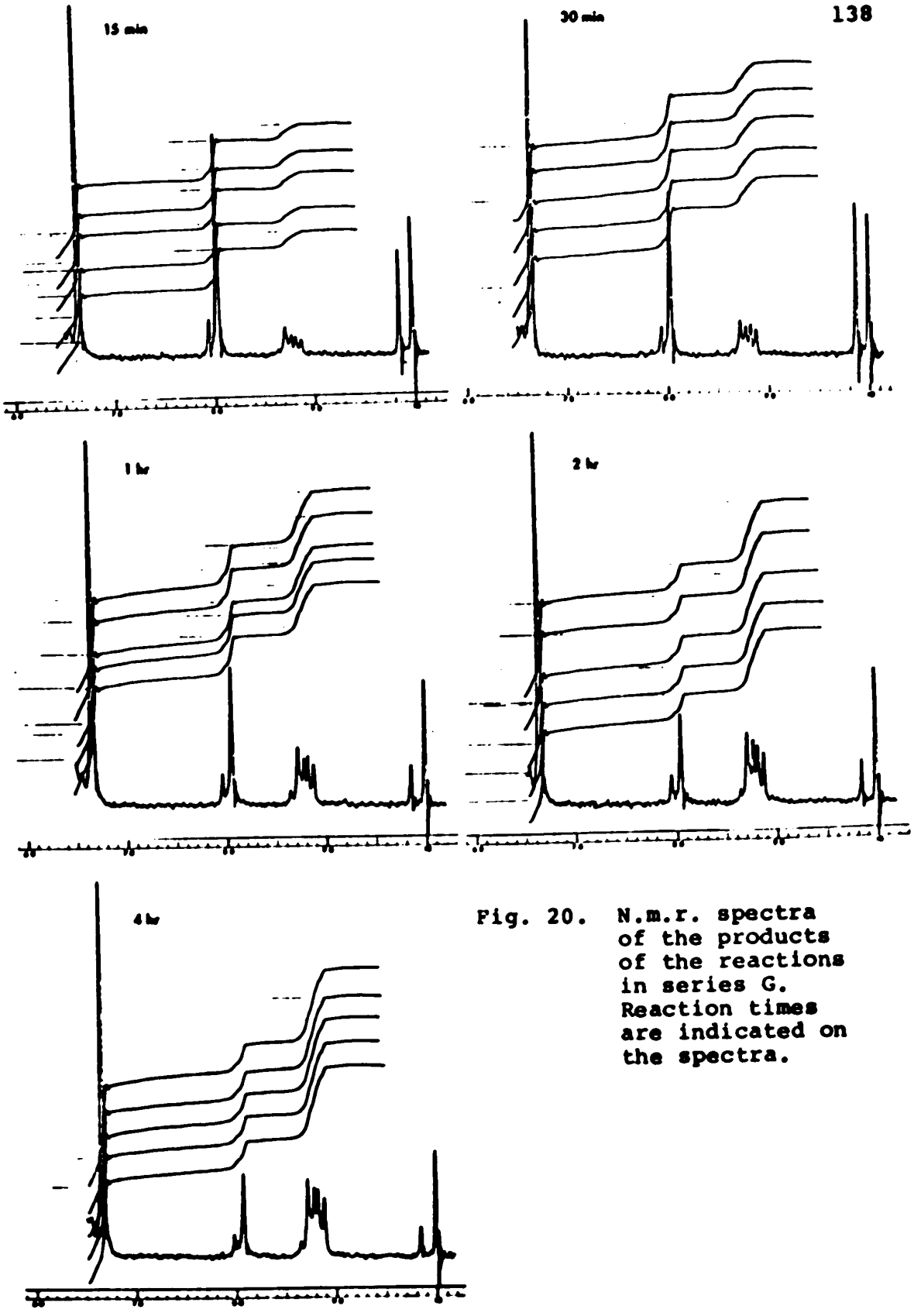

Fig. 20. N.m.r. spectra of the products of the reactions in series G. Reaction times are indicated on the spectra.

for the α-methoxy signal (at τ 6.67) to that of the acetoxy signal (at τ 8.01) increases as the reaction proceeds. At the same time the ratio of the methoxy signal intensity to that of the signals for the methyl protons of the isopropyl group (at τ 8.74) decreases with increasing reaction time. The effect of doubling the concentration of isopropanol on the rate and extent of total glucoside formation under the conditions given in Fig. 21 indicates that the alcohol is probably involved in the transition states of the rate-determining stages. The effect of bromide ion concentration can be assessed from the data plotted in Fig. 22. It is seen that doubling the bromide ion concentration substantially increased the rate of glucoside formation as expected for bromide ion catalysis. These studies would have been more profitable if made at a lower temperature in order to better define the initial rate of reaction. However, the expenditure in time required did not allow such studies. The effect of temperature on the rate of glucoside formation is demonstrated by the plots in Fig. 23. The effect of temperature on yield seems to indicate that glucoside formation is greater at 80° than at 60° and, therefore, the temperature gradient for glucoside formation is greater than those for the reactions leading to by-products. The effect of

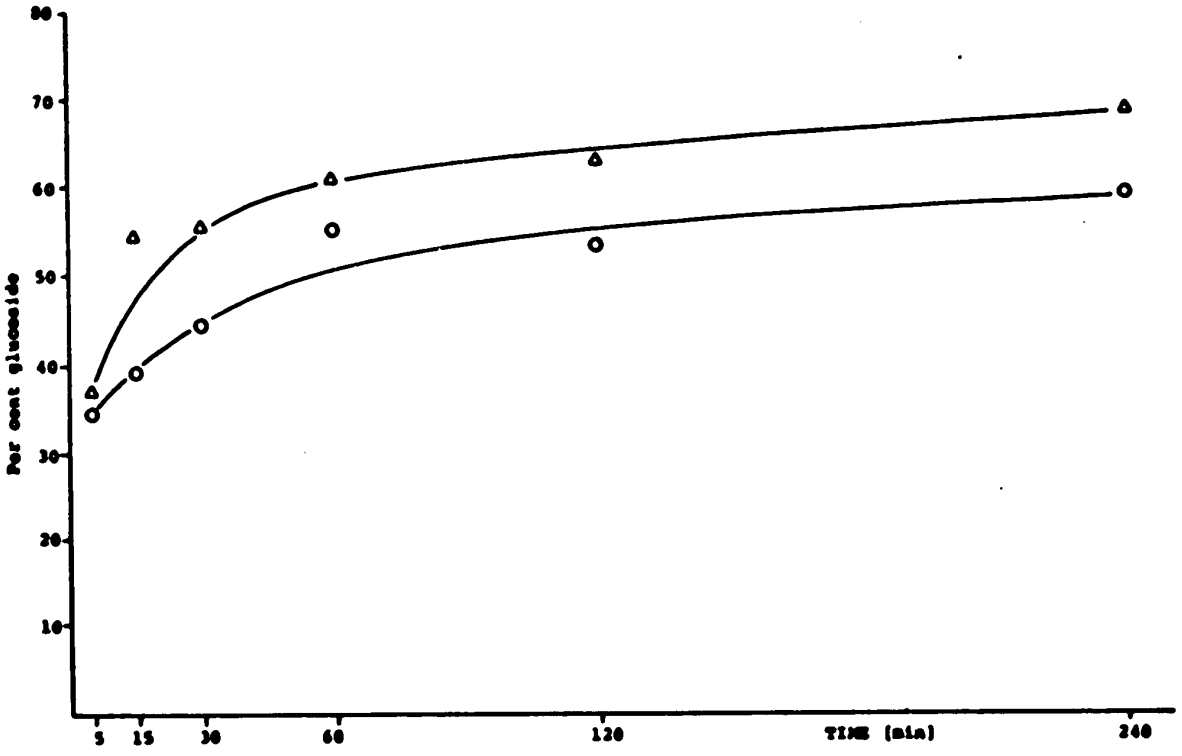

Fig. 21. Effect of alcohol concentration on rate of total glucoside formation on reacting tetra-O-benzyl-α-D-glucopyranosyl bromide (6), 0.1 M in methylene chloride at 80°C with Isopropanol and in the presence of two mole equivalents of triethylamine and one mole equivalent of tetraethylammonium bromide.

○, one mole equivalent of isopropanol

△, two mole equivalents of isopropanol.

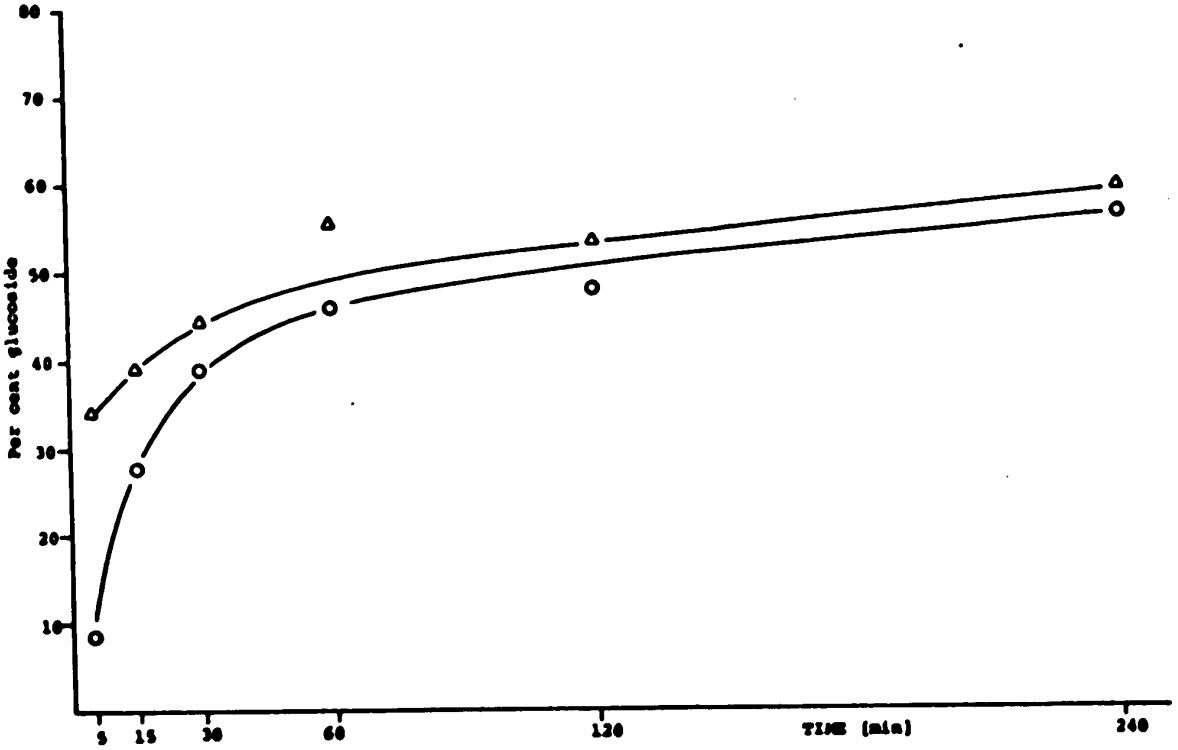

Fig. 22. Effect of bromide ion concentration on rate of total glucoside formation on reacting tetra-O-benzyl-α-D-glucopyranosyl bromide (6), 0.1 M in methylene chloride at 80°C, with one mole equivalent of isopropanol in the presence of two mole equivalents of triethylamine and tetraethylammonium bromide.

O, 0.5 mole equivalents of tetraethylammonium bromide

Δ, 1 mole equivalent of tetraethylammonium bromide

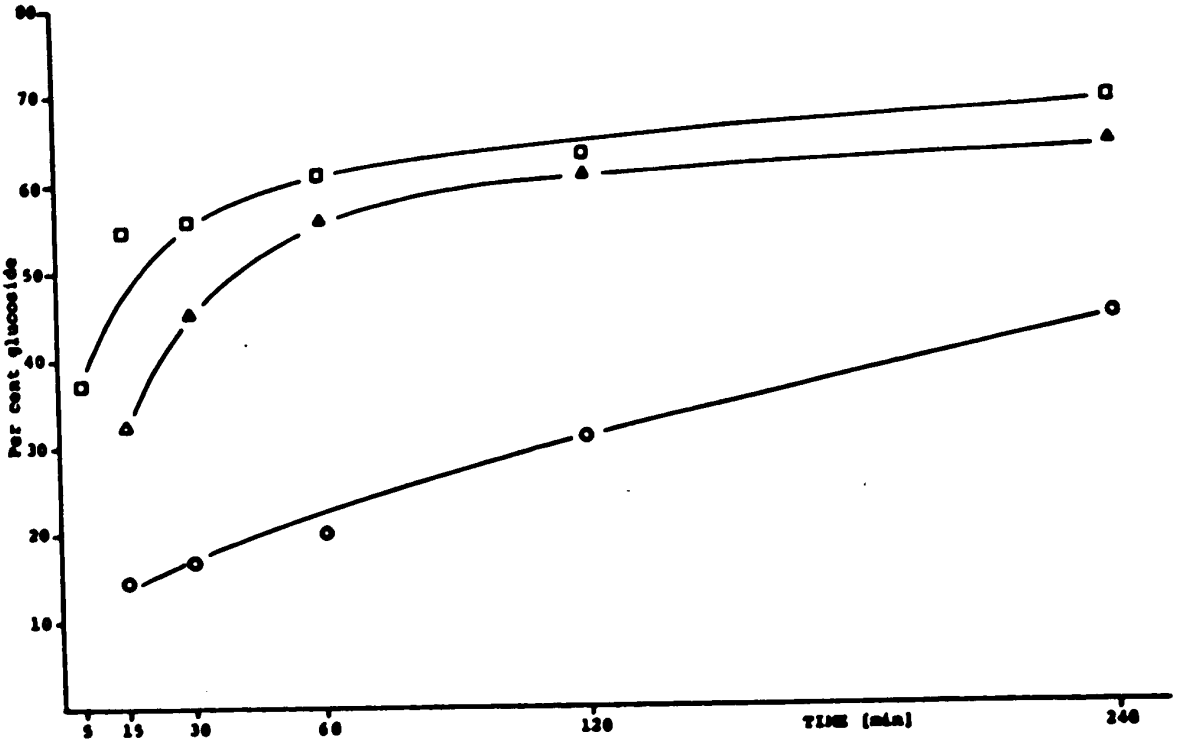

Fig. 23. Effect of temperature on rate of total glucoside formation on reacting tetra-O-benzyl-α-D-glucopyranosyl bromide (6), 0.1 M in methylene chloride with two mole equivalents of isopropanol and in the presence of two mole equivalents of triethylamine and one mole equivalent of tetraethylammonium bromide.

○, at 40°C

△, at 60°C

□, at 80°C

substituting diisopropylethylamine (Hünig's base) for triethylamine is demonstrated by the plots in Fig. 24. Apparently, the use of a more hindered amine caused a faster glucosidation reaction and higher yield. Judging from the data plotted in Fig. 25, the higher the concentration of amine present, the lower the yield of glucoside. Of course, the tertiary amine was added to the reaction medium to avoid the development of strongly acidic conditions as a result of the liberation of hydrogen bromide. On the other hand, the presence of the amine can be expected to lead to side reactions. Lemieux and Lineback (81) have recently discussed the role of amines in the formation of glucoseen derivatives by dehydrohalogenation of D-glucopyranosyl halides. They reported that the dehydrobromination of tetra-O-acetyl-α-D-glucopyranosyl bromide to tetra-O-acetyl-1-deoxy-D-*arabino*-hex-1-enopyranose is catalyzed by secondary amines containing n-alkyl groups. It was concluded that catalysis is highly dependent on the structure of the amine used. When the amine used was triethylamine, the glucoseen derivative was formed only in 19% yield, the rest being the N-glucosides. These N-glucosides were not isolated but their existence inferred from the n.m.r. spectra of the reaction mixture. It can be anticipated that the highly labile tetra-O-benzyl-α-D-glucopyranosyl

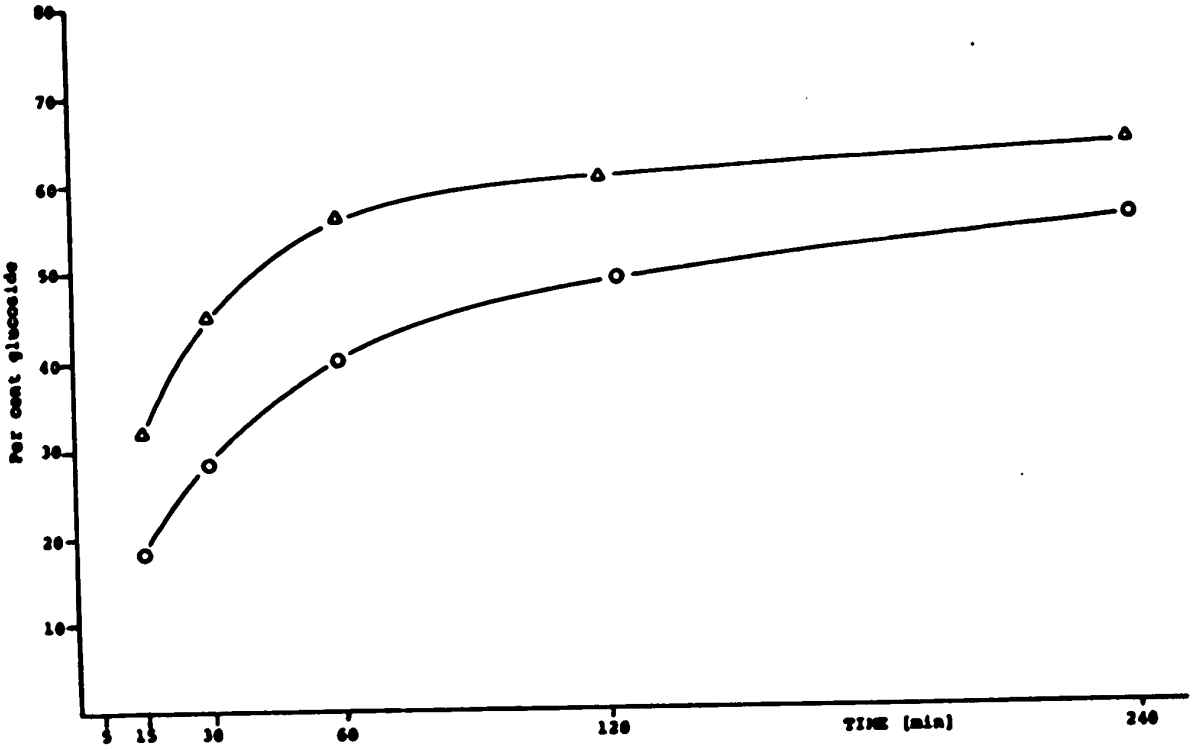

Fig. 24. Effect of base on rate of total glucoside formation on reacting tetra-O-benzyl-α-D-glucopyranosyl bromide (6), 0.1 M in methylene chloride at 60°C, with two mole equivalents of isopropanol and in the presence of a tertiary amine and one mole equivalent of tetraethylammonium bromide.

○, two mole equivalents of triethylamine

△, two mole equivalents of diisopropylethylamine

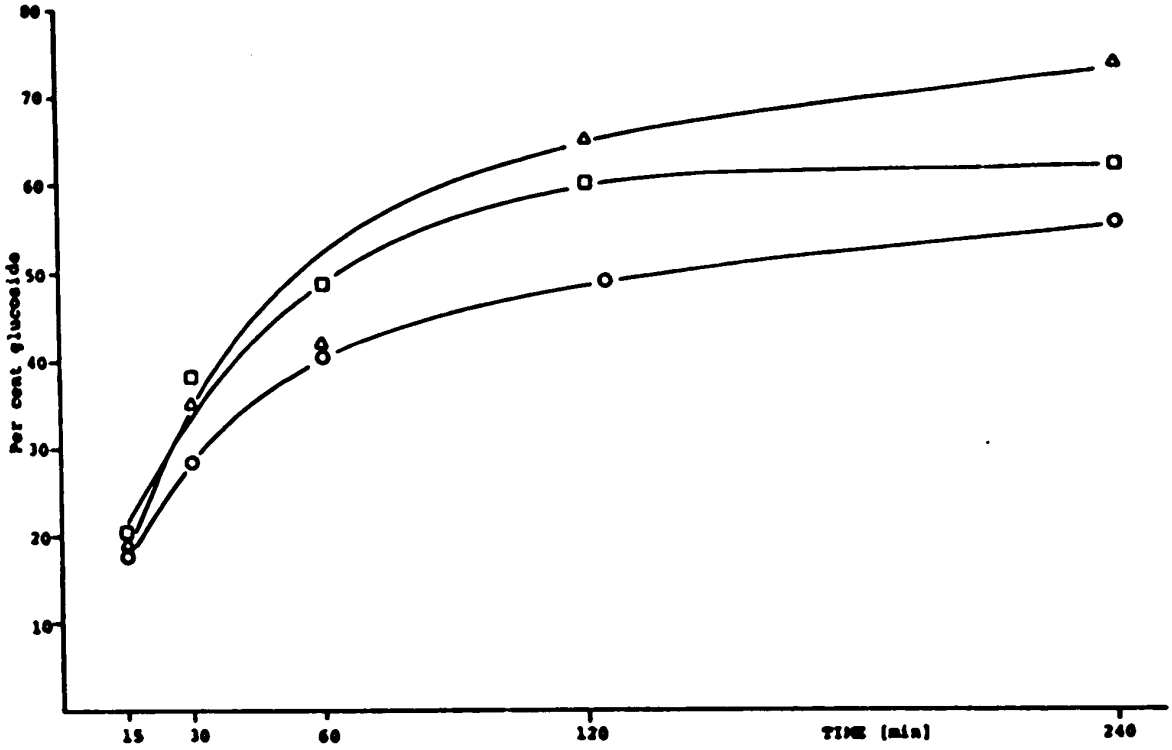

Fig. 25. Effect of amine concentration on rate of total glucoside formation on reacting tetra-O-benzyl-α-D-glucopyranosyl bromide (6), 0.1 M in methylene chloride, at 60°C with two mole equivalents of isopropanol and in the presence of one mole equivalent of tetraethylammonium bromide and diisopropylethylamine.

△ , 0.5 mole equivalent of diisopropylethylamine

□ , 1 mole equivalent of diisopropylethylamine

○ , 2 mole equivalents of diisopropylethylamine

bromide (6) used in this work can undergo dehydrobromination in the presence of an amine. Furthermore, glucosyl halides are known to form quaternary ammonium halides with trimethylamine (83). However, such N-glucosyl derivatives appear to be extremely labile if the amine is a more highly hindered amine. It is for this reason that triethylamine and diisopropylethylamine were used as buffers in the present research. Likely, the higher yield of glucoside obtained with the more hindered base (Fig. 24) results from the more hindered base being less effective in stabilizing the transition state for the dehydrobromination. Evidence for the formation of the glucoseen derivative 8 was obtained by the resonance signal for the anomeric proton, which appears as a singlet at τ 3.74 in deuteriochloroform. The n.m.r. spectrum (60 MHz) of the glucoseen 8 which was prepared following a procedure by Preobrazhenskaja and Suvorov (48) is shown in Fig. 26. The characteristic signal for the anomeric proton was often observed in the products of the various glycosidation reactions and its amount was estimated to be approximately 5 to 10%. The effect of different halide ions on the rate of total glucoside formation is demonstrated by the plots in Fig. 27. That the rate of glucoside formation is slowed

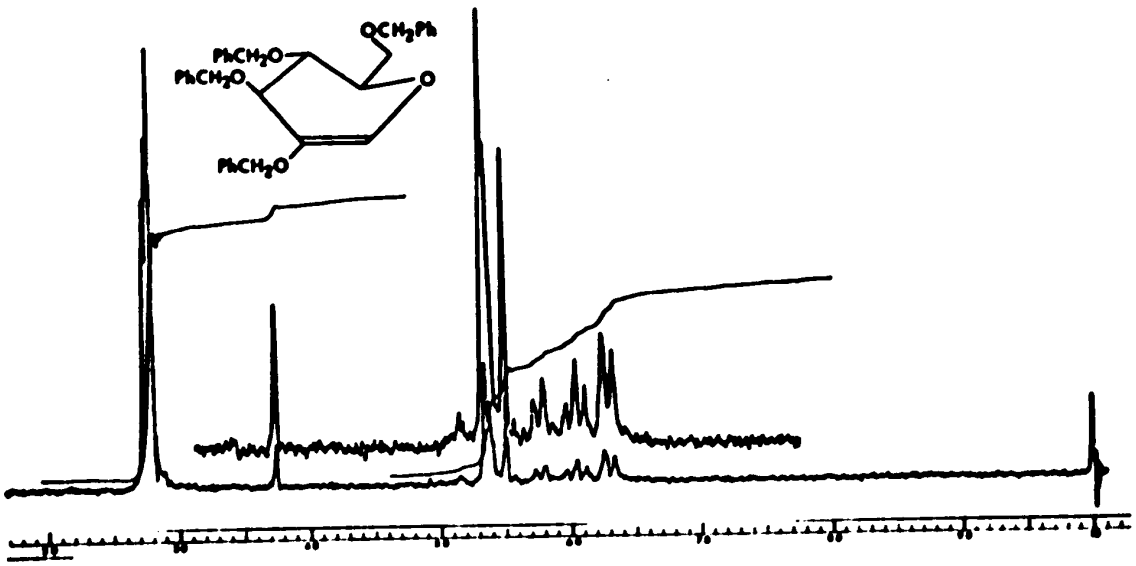

Fig. 26. N.m.r. spectrum (60 MHz) of tetra-O-benzyl-1-deoxy-D-*arabino*-hex-1-enopyranose in deuteriochloroform.

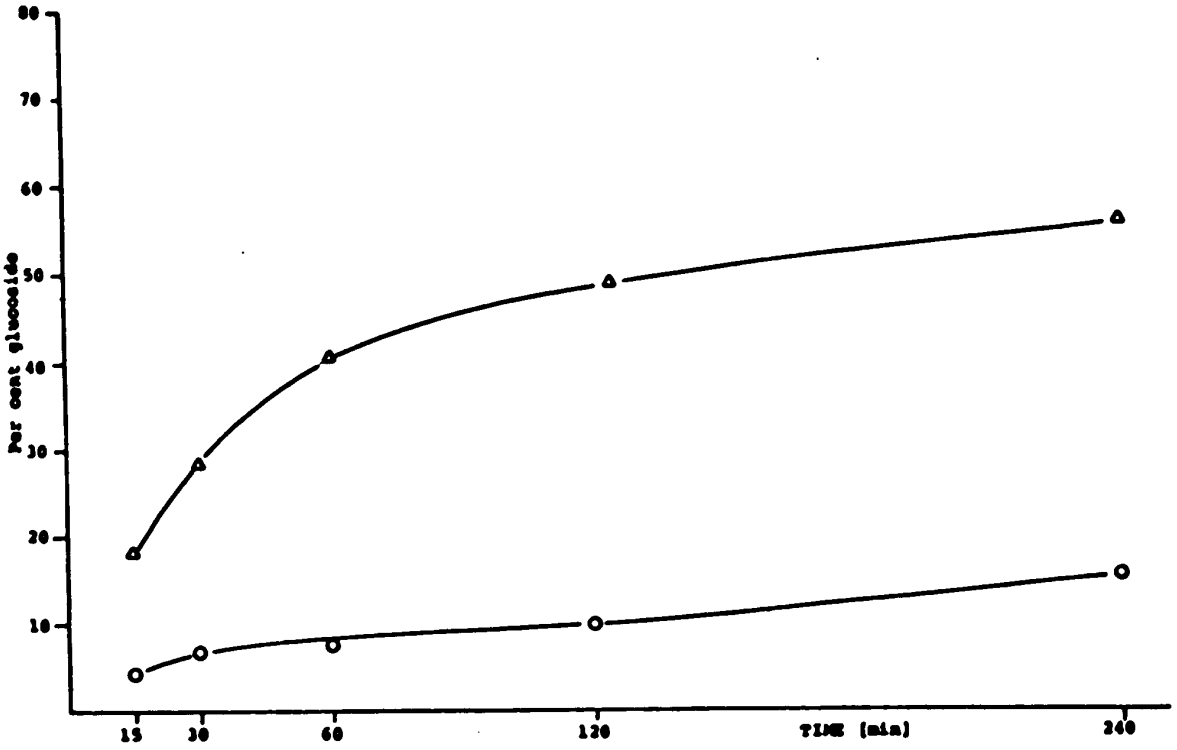

Fig. 27. Effect of halide ion on rate of total glucoside formation on reacting tetra-O-benzyl-α-D-glucopyranosyl bromide (6), 0.1 M in methylene chloride at 60°C, with two mole equivalents of isopropanol and in the presence of two mole equivalent of tetraethylammonium halide.

○, tetraethylammonium chloride

△, tetraethylammonium bromide

down considerably when chloride ion was used is taken as evidence for the suggestion that attack of halide ion upon the anomeric center is the initial step in the halide-ion catalyzed glucoside formation. The resulting tetra-O-benzylated D-glucosyl chloride is expected to be much less reactive, thus causing the slow rate of glucoside formation.

The isopropyl tetra-O-benzyl-α-D-glucopyranoside and its β-anomer were prepared for reference purposes to compare their n.m.r. spectra with those obtained from the products of the various glucosidation reactions. The α-anomer was prepared under the conditions of reaction series G (see p. 53), the β-glucoside was synthesized in the absence of tetraethylammonium bromide but with two moles of silver perchlorate per mole of substrate present in the reaction mixture. The n.m.r. spectra of these two anomeric glucosides are shown in Fig. 28 and Fig. 29, respectively.

Reaction of the tetra-O-benzyl-α-D-glucopyranosyl bromide (6) with the hydroxyl group of a suitably blocked sugar molecule should provide a good test for the usefulness of the reaction towards the synthesis of disaccharides. Compound 6 was reacted for 4 hours with a 10% molar excess of 1,2;5,6-di-O-isopropylidene-α-D-glucofuranose in ethylene chloride under reflux in the presence of one

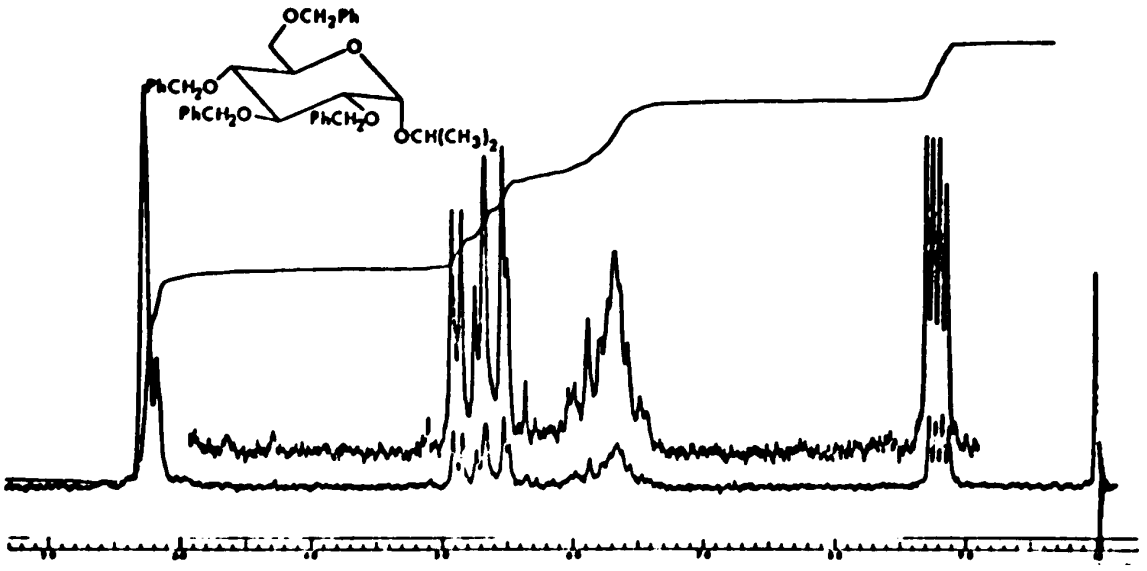

Fig. 28. N.m.r. spectrum (60 MHz) of isopropyl tetra-O-benzyl-α-D-glucopyranoside in deuteriochloroform.

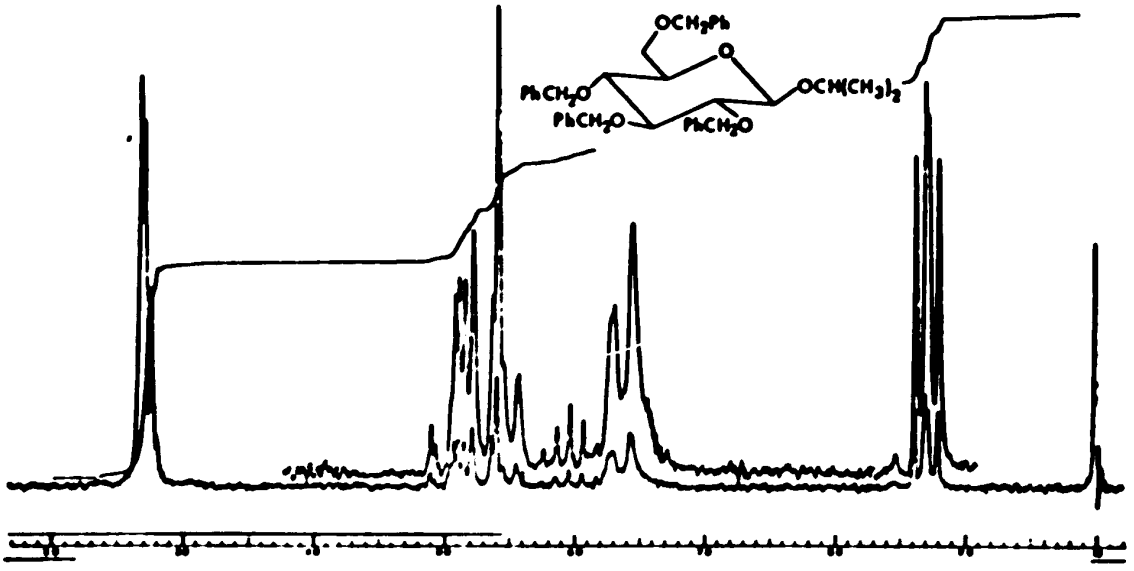

Fig. 29. N.m.r. spectrum (60 MHz) of isopropyl tetra-O-benzyl-β-D-glucopyranoside in deuteriochloroform.

mole equivalent of tetraethylammonium bromide and one mole equivalent of diisopropylethylamine. Ethylene chloride was chosen as the solvent because it has similar properties as methylene chloride, but can be refluxed at about 80°C. After work-up of the reaction mixture and separation of the products by column chromatography, the disaccharide 3-O-(tetra-O-benzyl-α-D-glucopyranosyl)-1,2;5,6-di-O-isopropylidene-α-D-glucofuranose (15) was isolated in nearly the same yield (20.5%) as the product of the dehydrohalogenation, the tetra-O-benzyl-1,5-anhydro-D-*arabino*-hex-1-enitol (8; 21%). Fig. 30 shows the n.m.r. spectrum (100 MHz) of the disaccharide 15, Fig. 26 presents the n.m.r. spectrum (60 MHz) of the glucoseen 8. The glycosidation reaction was then repeated at room temperature using methylene chloride as the solvent. The disaccharide 15 could be isolated from the product mixture in 42% yield. In a further experiment in methylene chloride at room temperature the amine was substituted for anhydrous potassium carbonate. The yield of isolated disaccharide 15 had dropped to 13%. The results show that reaction of 6 with a highly hindered alcohol at room temperature in the presence of added bromide ions produces the corresponding α-D-glucopyranoside in an acceptable yield. At elevated temperatures however, dehydrobromination

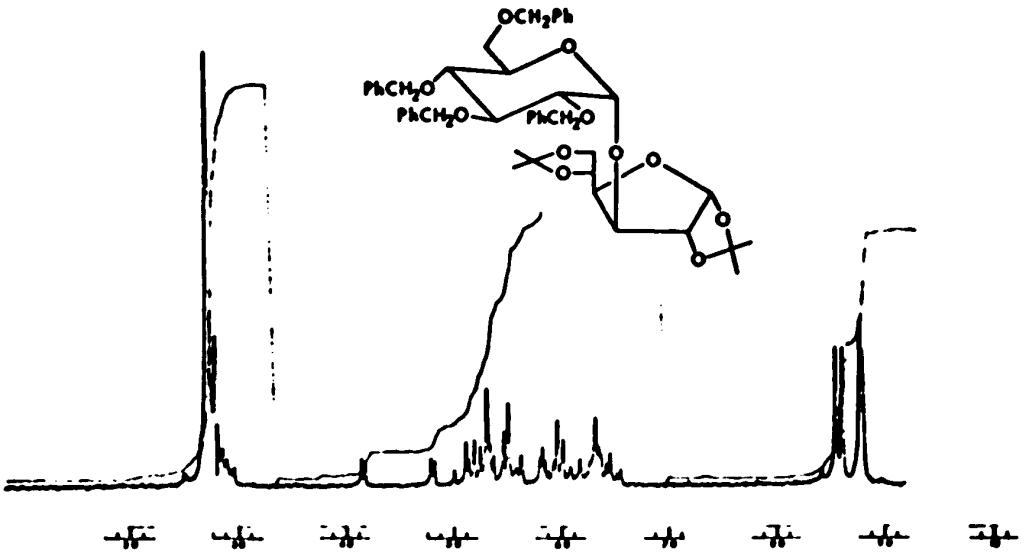

Fig. 30. N.m.r. spectrum (100 MHz) of 3-O-(tetra-O-benzyl-α-D-glucopyranosyl)-1,2;5,6-di-O-isopropylidene-α-D-glucofuranose (15) in deuteriochloroform.

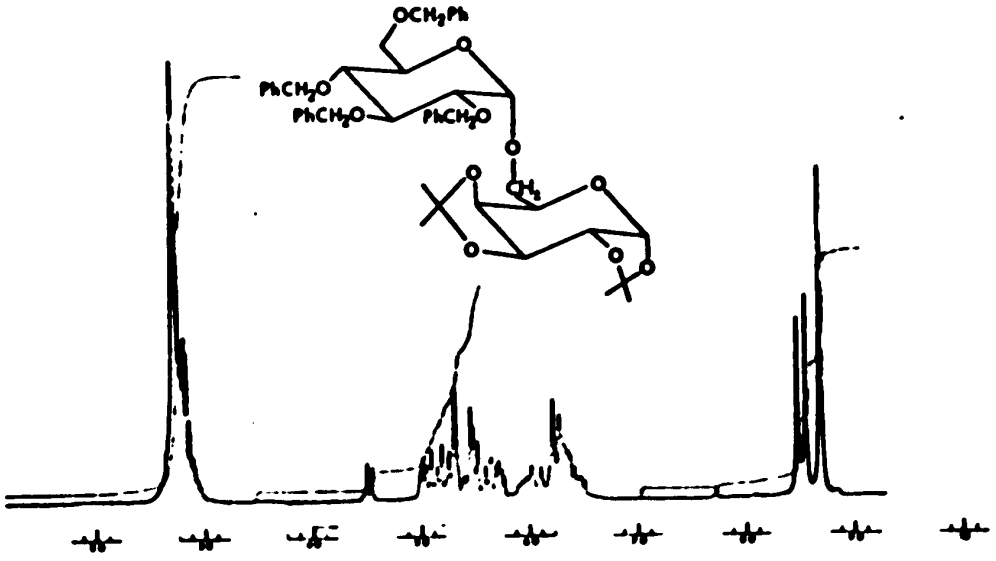

Fig. 31. N.m.r. spectrum (100 MHz) of 6-O-(tetra-O-benzyl-α-D-glucopyranosyl)-1,2;3,4-di-O-isopropylidene-α-D-galactopyranose (16) in deuteriochloroform.

becomes an important side-reaction. The tertiary amine was therefore substituted by anhydrous potassium carbonate which was thought to suppress formation of the glucoseen 8 derivative, yet was considered to be strong enough a base to catalyze the formation of the disaccharide 15. The low yield of 13% formation of disaccharide however indicates that potassium carbonate is not as suited as a tertiary amine to catalyze the glucosidation reaction. Tetra-O-benzyl-α-D-glucopyranosyl bromide (6) was reacted further with a 10% molar excess of 1,2;3,4-di-O-isopropylidene-α-D-galactopyranose in methylene chloride at room temperature in the presence of one mole equivalent of tetraethylammonium bromide and one mole equivalent of diisopropylethylamine. The disaccharide 6-O-(tetra-O-benzyl-α-D-glucopyranosyl)-1,2;3,4-di-O-isopropylidene-α-D-galactopyranose (16) was isolated in 65% yield. The n.m.r. spectrum of compound 16 is shown in Fig. 31. When anhydrous potassium carbonate was used as the base, the yield of disaccharide had dropped to 28%. These results allow the conclusion that the reactions of tetra-O-benzyl-α-D-glucopyranosyl bromide (6) with alcohols under the conditions established in this study may prove useful in the synthesis of derivatives of α-D-glucopyranosides.

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