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USE OF POSITIVELY CHARGED, LEAVING GROUPS IN THE SYNTHESIS OF α-D-LINKED GALACTOSIDES ATTEMPTED SYNTHESIS OF 3-*O*-α-D-(GALACTOPYRANOSYL)-D-GALACTOSE

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ABSTRACT

Quaternary ammonium and phosphonium salts were readily obtained by treating 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl bromide with tertiary amines and phosphines in various solvents under anhydrous conditions Optical rotations and n m r. spectra of the hygroscopic syrups indicated that they exist mainly in the β-D configuration Several dialkyl sulfides reacted very slowly with the galactosyl bromide and no conclusive evidence for sulfonium salt formation was obtained 2,3,4,6-Tetra-*O*-benzyl-α-D-galactopyranosyl chloride failed to react with any of the nucleophiles

Methanolysis reactions of the phosphonium salts were too slow to be practical and were not studied extensively Methanolyses of several quaternary ammonium salts in various solvents were not completely stereospecific, but gave good yields of methyl 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranoside Attempted reactions of benzyl 2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranoside with quaternary ammonium salts derived from 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl bromide failed to produce the corresponding derivative of 3-*O*-(α-D-galactopyranosyl)-D-galactose

INTRODUCTION

Recent reports from this laboratory[1,2] have described the use of various onium salts derived from 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl bromide as intermediates in the synthesis of α-D-glucosides These salts were shown to exist in the β-D configuration and to react with alcohols with predominant inversion at C-1 to give good yields of the α-D-glucosides The glycosidation reactions were most stereoselective when carried out in ethyl ether, or, in the case of the quaternary ammonium salts, in excess tertiary amine Since α-D-galactosides are found in many natural products, such as blood-group substances[3] and bacterial teichoic acids[4], a general method of synthesizing them would be very useful in serological and immunological studies. Herein we describe the preparation of several quaternary ammonium and phosphonium salts of 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl bromide and

their methanolysis reactions in various solvents Methanolysis was chosen as a model reaction for galactoside synthesis because the approximate reaction rates and relative amounts of anomers can be conveniently determined by n m r.

RESULTS

The preparation of 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl bromide has not been reported previously We attempted to synthesize it first from the 1-*p*-nitrobenzoate Although the reaction of 2,3,4,6-tetra-*O*-benzyl-D-galactose with *p*-nitrobenzoyl chloride gave a nearly quantitative yield of 2,3,4,6-tetra-*O*-benzyl-1-*O*-*p*-nitrobenzoyl-D-galactopyranose, the product was a mixture of the α and β anomers that could not be crystallized An alternative intermediate, 2,3,4.6-tetra-*O*-benzyl-1-*O*-(*N*-phenylcarbamoyl)-β-D-galactopyranose, was readily obtained in crystalline form and also proved to be a very useful intermediate for the preparation of the tetra-*O*-benzyl galactopyranosyl bromide and chloride The α anomer of the 1-(*N*-phenylcarbamate) was also obtained in crystalline form, although the yield was low Both 2,3,4,6-tetra-*O*-benzyl-D-galactopyranosyl bromide and chloride[5] were nearly colorless syrups Their n m r spectra and optical rotations suggested that they were the α-D-galactopyranosyl halides The bromide turned dark and developed an odor of benzyl bromide after being stored *in vacuo* for more than one day The chloride was much more stable and could be stored for several days in sealed containers without decomposition

TABLE I

APPROXIMATE RATES OF SALT FORMATION FROM 2,3,4,6-TETRA-*O*-BENZYL-α-D-GALACTOPYRANOSYL BROMIDE–NUCLEOPHILE REACTIONS

Nucleophile	*Nucleophile conc (mole/l)*	*Bromide conc (mole/l)*	*Solvent*	*Half time (h)*	*Method*
Triethylamine	4 4	0 25	$CDCl_3$	1	N m r
Triethylamine	6 5	0 03	CH_2Cl_2	2 5	Polarimetric
Tripropylamine	3 1	0 25	$CDCl_3$	1	N m r
Tris(2-methylbutyl)amine	2 0	0 09	CH_2Cl_2	2 5	Polarimetric
Tribenzylamine	1 8	0 09	CH_2Cl_2	>120	Polarimetric
Triphenylphosphine	0 3	0 25	$CHCl_3$	2	N m r
Triphenylphosphine	0 36	0 03	CH_2Cl_2	27	Polarimetric
Dimethyl sulfide	2 6	0 25	$CDCl_3$	>8	N m r.
Dimethyl sulfide	6 5	0 03	CH_2Cl_2	44	Polarimetric
Tetrahydrothiophene	2 2	0 25	$CDCl_3$	>20	N m r

Evidence for quaternary salt formation was obtained polarimetrically or by observing changes in the n m r spectra of solutions of the galactopyranosyl bromide in the presence of various nucleophiles. The n m r experiments were preferred in some cases, because the crude rate data that they provided were generally obtained at concentrations useful for synthesis (Table I) In all cases where the anomeric proton

peaks could be located, the coupling constants ($J_{1,2}$) were between 6.5 and 8 0 Hz, thus, the salts exist mainly as the β anomers (*cf.* Table II) With the exception of tribenzylamine, the amines and phosphines reacted readily with 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl bromide The sulfides reacted much more slowly and the solutions turned very dark The n m r. spectra of the sulfide solutions were very broad and complex, and there was no definite evidence for sulfonium salt formation

TABLE II

N M R DATA FOR THE QUATERNARY SALTS OF 2,3,4,6-TETRA-*O*-BENZYL-α-D-GALACTOPYRANOSYL BROMIDE

Nucleophilic reagent	*Chemical shift of the anomeric proton (p p m)*[a]	*Coupling constant (Hz)*
Triethylamine	5 80	$J_{1\,2}$ 8 0
Tripropylamine	5 63	$J_{1\,2}$ 8 0
Triphenylphosphine	5 85	J_{H-P} 2 1

[a]From the peak of tetramethylsilane used as standard

Unlike the bromide, 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl chloride did not react with triphenylphosphine, triethylamine, or dimethyl sulfide in various solvents, either at room temperature or at 50°

Methanolysis reactions of the phosphonium and ammonium salts were studied as model reactions in order to determine the best conditions for α-D-galactopyranoside formation Molar ratios of methanol to quaternary salt were kept low in order to simulate general glycoside-synthesis conditions as closely as possible The yields of the galactopyranosides and their anomer ratios were calculated from the areas of the methyl resonance-peaks, which appeared at δ 3 35 (α) and 3 52 (β) The accuracy of the analyses was limited by the fact that the methyl peak for the β-D-galactopyranoside was superimposed on a small, complex multiplet of a portion of the sugar-ring proton spectra An approximate baseline for the methyl proton peak was obtained from the spectrum of the product obtained by allowing the triethylamine salt of 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl bromide to react with methanol-d_4 The analyses are probably accurate to about ±5%

In general, the methanolysis reactions were quite slow at room temperature, especially in such solvents as ethyl ether or toluene, in which the quaternary salts are insoluble, but the reactions appeared to be substantially complete after 24 h at 50° Attempts to carry out the reactions at 70° gave a poorer yield of the galactosides and the n m r signals of the products were quite broad, indicating the occurence of side reactions

The triphenyl- and tributyl-phosphonium salts were quite unreactive toward methanol, and their reactions were not studied further

Unlike the onium salts prepared from 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl bromide, the D-galactopyranosylammonium bromide salts gave ratios of α-

to β-galactopyranoside that were nearly constant in all the solvents used (Table III) The proportion of α-D-galactopyranoside was slightly better with bulkier amines However, the most hindered amine that was tested, tribenzylamine, did not react with the D-galactopyranosyl bromide

TABLE III

METHANOLYSIS OF SOME 2,3,4 6-TETRA-*O*-BENZYL-α-D-GALACTOPYRANOSYL BROMIDE QUATERNARY SALTS

Nucleophile	*Reaction time (h)*	*Temp (°)*	*Solvent*	*Molar ratio MeOH/salt*	*Ratio α to β anomer*	*Yield (%)*
Triethylamine	19	25	Toluene	5		5
Triethylamine	24	25	Acetonitrile	5	74/26	80
Triethylamine	120	25	Ether	5	77/23	85
Triethylamine	24	25	Triethylamine	2 5	77/23	20
Triethylamine	48	50	Chloroform	3	75/25	80
Tripropylamine	48	50	Chloroform	3	82/16	80
Tripropylamine	24	70	Dichloromethane	3	86/14	60
Tributylamine	24	50	Dichloromethane	3	85/15	100
Tetramethylethylene-diamine	20	50	Dichloromethane	3	80/20	20
Triphenylphosphine	48	50	Chloroform	3		0
Tributylphosphine	48	50	Chloroform	3		0

Since the quaternary ammonium salts appeared to be useful intermediates in the synthesis of α-D-galactopyranosides, attempts were made to synthesize 3-*O*-α-D-galactopyranosyl-D-galactose The synthesis of this disaccharide has not been reported, although it is part of a trisaccharide end-group that provides B specificity to human blood-group substances[3] *O*-α-L-fucopyranosyl-(1→2)-*O*-[α-D-galactopyranosyl(1→3)]-D-galactose Benzyl 2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranoside was chosen as the monomeric unit for the attempted synthesis because it contains an easily removable blocking group at C-2, a prerequisite for subsequent synthesis of the aforementioned trisaccharide Unfortunately, attempts to condense benzyl 2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranoside with several of the quaternary ammonium salts of 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl bromide yielded the unreacted alcohol along with 2,3,4,6-tetra-*O*-benzyl-D-galactose and other products, including 1,5-anhydro-2,3,4,6-tetra-*O*-benzyl-D-*lyxo*-1-enitol Even extended reaction times at 50° were unsuccessful In some experiments, the reactions were quenched with methanol and interpretation of the n m r spectra of purified product fractions suggested that appreciable yields of methyl α- and β-2,3,4,6-tetra-*O*-benzyl-D-galactopyranosides had been obtained Thus, the major problem appeared to be the low reactivity of the quaternary salts, and further study of the synthesis of the disaccharide was not continued

DISCUSSION

The nucleophilic attack of nitrogen, phosphorus, and sulfur compounds on 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl bromide and the reactions of the resulting onium salts with alcohols differ in several respects from the reactions occurring with the isomeric compounds in the D-glucose series

First, the galactopyranosyl bromide derivative reacted very slowly with dialkyl sulfides If sulfonium salts were formed in the reactions, they were apparently unstable because the solutions turned very dark and the peaks of the n m r spectra became very broad Hydrolysis could not occur in these experiments, because the reactions were performed in sealed n m r tubes, which were prepared with high vacuum techniques Under the conditions reported here, the comparatively low reactivity of the phosphonium salts of 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl bromide is, at least, in part due to the much lower concentrations of methanol used than those in the D-*gluco* series[1,2] We chose these particular conditions for the model reactions in order to simulate the conditions that would be used in the synthesis of complex galactopyranosides Presumably, the phosphonium salts would have undergone solvolysis much more readily if a large excess of methanol had been used

The third major difference, in comparing the present reactions to similar reactions performed in the D-*gluco* series, was the nearly complete lack of solvent dependence in the stereoselectivity of the methanolysis reactions The observed pattern of behavior is in line with our experience that, with less reactive glycosyl derivatives, the stereochemical course of methanolysis generally appears to be relatively independent of the solvent

The failure of the quaternary ammonium salts to react with benzyl 2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranoside is not unexpected in retrospect, since both reactants are quite hindered at their reaction sites Perhaps future work will lead to the synthesis of more reactive quaternary salts, which will be more generally useful in the synthesis of α-galactosides

EXPERIMENTAL

General — N m r spectra were obtained with a Varian A-60-A spectrometer, chloroform-*d* containing tetramethylsilane as an internal reference was used as the solvent Optical rotations were determined with a Perkin–Elmer model 141 polarimeter with jacketed 1-dm cells kept at 25° by circulating water from a constant-temperature bath

*Methyl 2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranoside* — Methyl 2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranoside was prepared by conventional procedures from methyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside (44 4 g) in toluene (150 ml), with powdered potassium hydroxide (200 g) and benzyl chloride (70 ml and 140 ml) Addition of a small amount of ethanol to the isolated product induced crystallization (56 2 g, 74%), m.p 84–85°, $[\alpha]_D^{25}$ +17° (*c* 3 6, *p*-dioxane), lit [5] m p 80–81°, $[\alpha]_D$ +18° (*c* 3 6, *p*-dioxane)

2,3,4,6-Tetra-O-benzyl-1-O-(N-phenylcarbamoyl)-α- and β-D-galactopyranose — Methyl 2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranoside (45 g) was heated on a steam bath with a mixture of 80% acetic acid (720 ml) and M hydrochloric acid (225 ml) for 4 h The mixture was cooled and extracted with chloroform After drying and distilling off the solvent, the remaining syrup was homogeneous on t l c with dichloromethane as eluent and its n m r spectrum was consistent with the assigned structure of 2,3,4,6-tetra-*O*-benzyl-D-galactose No attempt was made to crystallize the product, which appeared to be a mixture of anomers

2,3,4,6-Tetra-*O*-benzyl-D-galactose (18 3 g) was dissolved in dry pyridine (50 ml) Phenyl isocyanate (5 0 ml) was added at 0° The solution was stirred for 3 days at room temperature and poured into water The syrup was extracted with chloroform, and the organic layer was dried (sodium sulfate) and evaporated to a syrup Crystallization from benzene gave a small amount of diphenylurea After filtration, crystallization of the mother liquor from ethanol gave 7 4 g (33 2%) of the β-anomeric *N*-phenylcarbamate, m p 133–134° (after recrystallization from ethanol), $[\alpha]_D^{25}$ −22° (*c* 1 7, chloroform), n m r data (chloroform-*d*) doublet centered at δ 5 70, $J_{1,2}$ 7 7 Hz Crystallization of the mother liquor from ethanol gave 3 6 g (15 6%) of the α anomer, m p 145–147° (after recrystallization from ethanol), $[\alpha]_D^{25}$ +68° (*c* 1 7, chloroform), n m r spectrum doublet centered at δ 6 40, $J_{1,2}$ 3 8 Hz

Anal Calc for $C_{41}H_{41}NO_7$ C, 74 65, H, 6 27; N, 2 12 Found for α-D anomer C, 74 56, H, 6 32, N, 2 08 Found for β-D-anomer C, 74 68, H, 6 33; N, 2 10

Under similar conditions, 2,3,4,6-tetra-*O*-benzyl-α,β-1-*O*-*p*-nitrobenzoyl-D-galactopyranose was prepared from 2,3,4,6-tetra-*O*-benzyl-D-galactose (10 0 g), dry pyridine (30 ml), and *p*-nitrobenzoyl chloride (4 0 g) The product was homogeneous on t l c with 1 9 acetone–benzene as eluent and, according to its n m r spectrum, was a mixture of 60% of the β and 40% of the α anomer However, the product did not crystallize from a number of solvents and was not fully characterized

2,3,4,6-Tetra-O-benzyl-α-D-galactopyranosyl bromide — 2,3,4,6-Tetra-*O*-benzyl-1-*O*-(*N*-phenylcarbamoyl)-β-D-galactopyranose (650 mg, 0 986 mmole), was dissolved in dry dichloromethane (20 ml), and hydrogen bromide was bubbled through the solution for 5 min. A precipitate of anilinium hydrobromide appeared after about 0 5 min The solvent was evaporated at room temperature and replaced by 5 ml of fresh dichloromethane The anilinium salt was filtered off, dried, and weighed (169 mg, 0 97 mmole, 98 4%) The remaining solution was decolorized by filtration through silica gel, the solvent was evaporated off, and the syrup was dried *in vacuo* overnight, $[\alpha]_D^{25}$ +117° (*c* 1 7, chloroform), n m r spectrum (chloroform-*d*) broad, poorly resolved doublet centered at δ 6 55 that was, however, too narrow to be typical of a β anomeric proton, the ratio of aromatic to aliphatic proton peaks was consistent with the assumed structure

2,3,4,6-Tetra-O-benzyl-α-D-galactopyranosyl chloride — Hydrogen chloride was bubbled through a solution of 2,3,4,6-tetra-*O*-benzyl-1-*O*-(*N*-phenylcarbamoyl)-

β-D-galactopyranose (1 0 g) in 4 1 ether–dichloromethane (10 ml) for 60 min A precipitate of anilinium hydrochloride began to form after about 5 min The mixture was evaporated to dryness, treated with 5 ml of fresh dichloromethane, and filtered to give 194 0 mg (98 7%) of anilinium hydrochloride The solution was passed through a layer of silica gel and evaporated to a colorless syrup, $[\alpha]_D^{25}$ +151° (*c* 2 0, benzene), n m.r spectrum doublet centered at δ 6 18, $J_{1,2}$ 3 7 Hz, lit.[5] $[\alpha]_D$ +147° (*c* 2 0, benzene)

Rate studies — A N m r. method An n m r tube fused to a high vacuum apparatus was evacuated and filled with nitrogen The apparatus was opened up under a flow of nitrogen and 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl bromide (130 mg) in dry ether (1 ml) was added The ether solution was frozen with liquid air and the apparatus was sealed After evacuation and degassing of the frozen solution, the ether was distilled off *in vacuo* and the remaining syrup was dried *in vacuo* for several h Chloroform-*d* (0 5 ml) and the nucleophile (0 3 ml) were distilled into the n m r tube from evacuated containers containing calcium hydride The n m r tube was melted off and stored in liquid air until the rate studies were initiated Spectra of the solutions were obtained at regular intervals until the reactions were complete The approximate half time of the reaction was taken as the time at which half of the galactopyranosyl bromide was consumed

In cases where the nucleophile was nonvolatile, it was added directly to the n m r tube under a stream of nitrogen

B Polarimetric method The galactopyranosyl bromide, solvent, and nucleophile were mixed in a small volumetric flask, and the solution was transferred to a 1-dm tube The optical rotation was recorded as a function of time until the reaction was complete The approximate half time was taken to be the time at which $\alpha_0-\alpha/\alpha_0-\alpha_\infty$ was equal to 0 5 Solvents and liquid nucleophiles used in the polarimetric studies were dried over calcium hydride

Methanolysis experiments (example) — 2,3,4,6-Tetra-*O*-benzyl-α-D-galactopyranosyl bromide (245 mg) in ether (2 ml) was introduced into a high-vacuum reaction tube under a stream of nitrogen The ether solution was frozen with liquid air and the tube was sealed The tube was evacuated, the ether solution was frozen with liquid air, and the tube was sealed The tube was evacuated, the ether was distilled off, and the syrup was dried for several h *in vacuo* Triethylamine (5 0 ml) was distilled into the tube, and the mixture was stirred to dissolve the bromide The solution was allowed to stand overnight, the triethylamine was distilled off, and acetonitrile (5 0 ml) and methanol (60 mg) were distilled in The solution was stirred to mix the reactant compounds and allowed to stand for 24 h The tube was opened, the solution poured into water (50 ml), and the product extracted with dichloromethane The organic solution was washed with water, dried (magnesium sulfate), and evaporated to dryness The remaining syrup was dried *in vacuo* and dissolved in chloroform-*d* for n m r analysis A very similar procedure was used for studies with the other tertiary amines in various solvents All solvents and nucleophiles for the methanolysis studies were dried over calcium hydride Methanol was distilled from a

solution of sodium methoxide into small, calibrated pyrex tubes and transferred into the reaction medium by breaking the tubes with a magnetic hammer and distilling *in vacuo*

Disaccharide preparation (example) — An ether solution of 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl bromide (250 mg, 0 415 mmole) was evaporated to dryness *in vacuo* in a high-vacuum reaction vessel, tripropylamine (0 6 ml) was added, and the solution was mixed well and kept overnight Benzyl 2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranoside, was prepared according to the method of Chittenden and Buchanan[6], $[\alpha]_D^{25}$ −23° (*c* 1 0, chloroform), all physical constants of this compound and its precursors were in agreement with the published data[6] except $[\alpha]_D^{21}$ +24° (*c* 1 0, chloroform) This compound (230 mg, 0 498 mmole) was added under a stream of nitrogen The mixture was frozen in liquid air, evacuated, degassed, and the apparatus was melted off the vacuum rack The tube was heated in an oil bath (50°) and shaken occasionally for 4 days Methanol (~5 ml) was added and the mixture was allowed to stand for 4 h The solvent was distilled from the mixture, and an excess of dichloromethane was added The mixture was washed twice with 1 5M sulfuric acid and once with saturated sodium hydrogen carbonate solution, and dried (magnesium sulfate) After removal of the solvent, the residual gel was chromatographed on silica gel A mixture of 2,3,4,6-tetra-*O*-benzyl-α- and β-D-galactopyranoside (138 mg, 60%) was eluted with benzene Elution with chloroform gave a small, impure fraction (2 spots on t l c) and a fraction of crystalline benzyl 2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranoside (220 mg, 95%)

Very similar results were obtained when *N*-methylmorpholine and tetramethylethylenediamine were used instead of tripropylamine Reactions with these two amines were performed for 3 weeks at 50° The reaction mixture in tetramethylethylenediamine gave, in 89% yield, the starting benzylidene compound, an estimated 20% yield of 2,3,4,6-tetra-*O*-benzyl-D-*lyxo*-hex-1-enose, and an impure fraction which, from its n m r spectrum, appeared to contain both methyl 2,3,4,6-tetra-*O*-benzyl-α- and β-D-galactopyranosides, the starting benzylidene compound, and 2,3,4,6-tetra-*O*-benzyl-D-galactose

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