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STEREOSELECTIVITY OF GLYCOSYLATION WITH DERIVATIVES OF 2-AZIDO-2-DEOXY-D-GALACTOPYRANOSE. THE SYNTHESIS OF A DETERMINANT OLIGOSACCHARIDE RELATED TO BLOOD-GROUP A (TYPE 1)

NIKOLAI V. BOVIN, SERGEI É. ZURABYAN, AND ANATOLY YA. KHORLIN

M. M. Shemyakin Institute of Bioorganic Chemistry, U.S.S.R. Academy of Sciences, Moscow B-334 (U.S.S.R.)

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ABSTRACT

The stereoselective glycosylation of a model alcohol (cyclohexanol) by derivatives of 2-azido-2-deoxy-D-galactopyranose was studied under various conditions. 2-Azido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-galactopyranosyl chloride (**9**) was found to be the most efficient glycosylating agent for the synthesis of oligosaccharides containing 2-acetamido-2-deoxy-α-D-galactopyranose residues, and gave a tetrasaccharide, which is a determinant of the blood-group A (Type 1), *i.e.*, *O*-α-L-fucopyranosyl-(1→2)-[*O*-2-acetamido-2-deoxy-α-D-galactopyranosyl-(1→3)]-*O*-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-D-glucose, and its trisaccharide fragment, *O*-2-acetamido-2-deoxy-α-D-galactopyranosyl-(1→3)-*O*-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-D-glucose. In the course of this synthesis, the determinant trisaccharide related to the H blood-group, *i.e.*, *O*-α-L-fucopyranosyl-(1→2)-*O*-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-D-glucose, was also obtained.

INTRODUCTION

Oligosaccharides containing 2-acetamido-2-deoxy-α-D-glycopyranosyl residues are widely distributed in nature. In particular, the determinant oligosaccharides of the A blood-group Type 1 (**1** and **2**) contain 2-acetamido-2-deoxy-α-D-galactopyranosyl groups.

The formation of a 2-acetamido-2-deoxy-α-D-hexopyranosyl linkage shows the same difficulties as that of an α-D-glycoside. At present the "azide method" elaborated by Paulsen[1] shows the most promising approach to the synthesis of 2-acetamido-2-deoxy-α-D-glycopyranosides allowing the formation of various hetero-oligosaccharides. Recently, the methods of synthesis of the starting glycosylating agents (derivatives of 2-azido-2-deoxyhexoses) have been considerably simplified[2–5]. The azide group is a very suitable precursor of the acetamido group because it is nonparticipating and readily converted into the acetamido group.

Paulsen and associates used two methods for the synthesis of α-D-glycopyranosides from 2-azido-2-deoxy-derivatives. The first glycosylation was obtained with

2-azido-2-deoxy-β-D-glycopyranosyl chlorides, *e.g.*, the acetyl (**3**) and the benzyl (**4**) derivatives[6], in the presence of silver carbonate and perchlorate. This method showed a fairly high stereoselectivity and the yield[1,7-11] of α-D-glycopyranosides was as high as 85–90%. The second method, which is used less frequently, utilises 2-azido-2-deoxy-α-D-glycopyranosyl bromides, *e.g.*, the acetyl (**5**) and the benzyl (**6**) derivatives[6], under the glycosylation conditions of Helferich[12-14]. No definite indication prefering one of the two methods in a given case was provided. Model experiments[2] suggest that no stereoselectivity was obtained in the glycosylation of benzyl and *tert.*-butyl alcohols with chloride **3** and bromide **5**. Finally, the recently proposed "imidate method" for the synthesis of 1,2-*cis*-glycosides, with particular reference to azide **11**, gave poor results for the glycosylation of secondary hydroxyl groups of sugars[15].

Despite these advances in the synthesis of heterooligosaccharides having a 2-acetamido-2-deoxy-α-D-glycopyranosyl group, no systematic study of the formation of the 2-acetamido-2-deoxy-α-D-glycopyranosyl linkage depending on the structure of the glycosylating agent and reaction conditions has been performed*. In the present work, the aim was to synthesize the determinant oligosaccharide of the A blood-group Type 1 (**1**) and its fragment **2**, and also to study the stereoselectivity of glycosylation with 2-azido-2-deoxyglycosyl halides

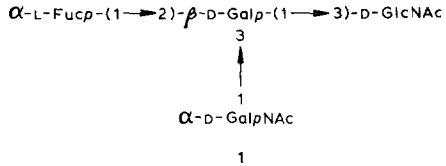
RESULTS AND DISCUSSION

Undoubtedly, extreme care should be taken to compare the results of model-alcohol glycosylation to those of the synthesis of oligosaccharides, because the change from a simple to a more complex alcohol may influence greatly the reaction stereoselectivity. Nevertheless, a study of model reactions may show a correct tendency of the stereoselectivity to depend on the nature of a glycosylating agent and reaction conditions. The hydroxyl groups of the benzyl and *tert*-butyl alcohols, which are suitable for the detection of glycosides by ^{1}H-n.m.r. data[2], differ greatly from those of carbohydrates. We selected the glycosylation of cyclohexanol because this cyclic, secondary alcohol shows a reactivity similar to that of typical carbohydrates and, at the same time, allows a quantitative analysis of mixtures of anomeric glycosides by g.l.c. (in the form of trimethylsilyl derivatives).

The glycosylation of cyclohexanol with 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-β-D-galactopyranosyl chloride (**3**), 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D- (**5**), and -β-D-galactopyranosyl bromide (**8**) shows a strikingly wide stereoselectivity from 91% for the β-D-glycoside to 81% for the α-D-glycoside (see Table I). The ratio of glycosides obtained from the α-D-bromide **5** by the Helferich reaction (mercuric cyanide in benzene–nitromethane or in dichloromethane, Exps. 1 and 2, Table I), demon-

*After this work had been completed, a report[16] describing the effect of a catalyst on glycoside-synthesis rate has been published but without quantitative data of the stereoselectivity of the glycosylation

strates again that the stereoselectivity of this method should be evaluated with great care. Some cases show a high α stereoselectivity[12–14], some its absence[2], and the present case a high β stereoselectivity. The diphenylcyclopropenyl method[17] shows an insignificant preponderance of the α-D anomer (Exp. 4, Table I). In the halide ion-catalyzed reaction of Lemieux *et al.*[18], the ratio of α- to β-D-glycosides is fairly high (Exp. 5, Table I), but the rate of glycoside formation is very low (normally this reaction is employed for more reactive *O*-benzylglycosyl halides). An increase in the proportion of α-D-glycoside was observed with the use of β-D-glycosyl halides under the Koenigs–Knorr conditions. If the chloride **3**, obtained directly prior to glycosylation from the bromide **5**, and the chloride ion in acetonitrile[6] (as soon as the optical rotation minimum is attained) was used as the glycosylating agent, the ratio of α- to β-D-glycosides was 73:27 (Exp. 6, Table I); this ratio was obtained with crystalline[2] **3**. The bromide **7**, corresponding to chloride **3**, was obtained as follows. In the presence of silver triflate at −78°, the α bromide **5** was converted into the α-D-glycosyl triflate which, under the action of the bromide ion, gave readily, with inversion of configuration, bromide **7** containing, as indicated by ^{1}H-n.m.r.



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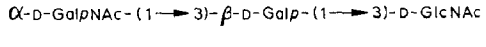

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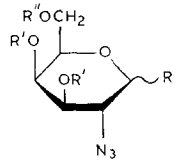
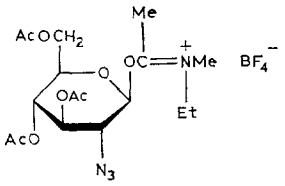


3 R = β-Cl, R′ = R″ = Ac
4 R = β-Cl, R′ = Bn, R″ = Ac
5 R = α-Br, R′ = R″ = Ac
6 R = α-Br, R′ = Bn, R″ = Ac
7 R = β-Br, R′ = R″ = Ac
8 R = α-Br, R′ = R″ = Bn
9 R = β-Cl, R′ = R″ = Bn
10 R = OAc, R′ = R″ = Bn



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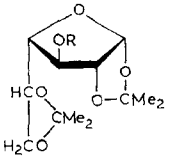


12 R = H
13 R = Ac

data, over 90% of the β-D anomer. The use of **7** in the glycosylation reaction gave the same preponderance of the α-D anomer (Exp. 8, Table 1).

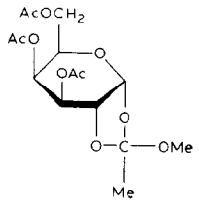
Glycosylation with glycosyl sulfonates has been studied in much detail for neutral saccharides[19]. The ratio of anomers of the resulting glycosides was shown to depend on a number of factors, such as the nature of the protecting groups of the glycosylating compound, type of leaving sulfonyl group, alcohol reactivity, alcohol-to-sulfonate ratio, and solvent. The high α stereoselectivity was favored by acyl substituents at O-6 and -4 of the glycosylating compound, and also by the use of solvents having a high donor ability and, at the same time, a low dielectric-constant (*e.g.*, ethers). Moreover, the result of the reaction depends on the ability of the leaving sulfonyl group to be replaced: thus, alkyl sulfonates, whose reactivity is higher than that of aryl sulfonates, lead to a lower stereoselectivity. The same influences were observed for the 2-azido-2-deoxy derivatives of D-galactose (see Table 1). The use of a glycosyl triflate in dichloromethane at 20° led to the preponderance of the β-glycoside (Exp. 9), a decreasing temperature giving rise to a higher β stereoselectivity (Exp. 10). Treatment with the less reactive glycosyl *p*-toluenesulfonate resulted in a drastic increase of the α anomer (Exp. 11); however, the reaction rate was not high, at least one order of magnitude lower than that of the other experiments. On the other hand, when silver *p*-toluenesulfonate was replaced by a polymeric acceptor, Dowex 50W-X4 (Ag^+) (*cf.* ref. 20), complete absence of glycosides in the reaction products was observed. Finally, replacement of dichloromethane, as solvent, by 1,2-dimethoxyethane, which has good donor-properties, resulted in a sharp increase of the α anomer (Exps. 13 and 14).

The difference in behavior between glycosylation of a model alcohol and that of sugar derivatives was clearly recognized but the results obtained with cyclohexanol give some information on the influence of the glycosylation conditions on stereoselectivity of the reaction. Thus, this model glycosylation allowed us to avoid poor conditions and rely on the more encouraging experiments based on β halides (Exps. 6–8) and glycosyl sulfonates in 1,2-dimethoxyethane (Exps. 13 and 14). These conditions were used for the glycosylation of 1,2:5,6-di-*O*-isopropylidene-α-D-galactofuranose (**12**). However, the glycosyl sulfonates in 1,2-dimethoxyethane gave no glycoside, whereas the β halides afforded exclusively, instead of the expected glycosides, the acetate **13** in high yield. Similar results were obtained for the glycosylation of more complex compounds (see later). Therefore, the acetylated glycosyl halides **3** and **5** were replaced by the completely benzylated analogs **8** and **9** in order to avoid intermolecular acetylation.

Compounds **8** and **9** were synthesized starting from acetate **10**, which was obtained by the halogenoazide method[5]. The mixture of anomers (**10**) was hydrolyzed with 0.5M hydrochloric acid in *p*-dioxane to give the free sugar, which was converted into the 1-(*p*-nitrobenzoate). Treatment with hydrogen bromide in dichloromethane gave the α-bromide **8**. For the glycosylation reaction (see later) both the crystalline α-bromide **8** and the β-chloride **9**, generated therefrom immediately before the reaction

by interaction with triethylbenzylammonium chloride in acetonitrile as soon as the optical rotation minimum had been attained, were used.

The derivatives of di- and tri-saccharides, to which the 2-acetamido-2-deoxy-α-D-galactopyranosyl group was linked at the last stage of the synthesis of oligosaccharides **1** and **2**, were synthesized from disaccharide[11] **16**. Partial chloroacetylation of **16** afforded **17** which differed in m.p. and $[\alpha]_D^{20}$ from those previously reported by Paulsen and Kolář[11]. The chloroacetate **17** was acetylated and then *O*-dechloroacetylated with thiourea[21,22] to give a disaccharide having a free OH-3′ (**18**). Its structure, and hence that of **17**, was confirmed by an alternative synthesis from 3,4,6-tri-*O*-acetyl-1,2-*O*-(1-methoxyethylidene)-α-D-galactopyranose[23] (**14**).



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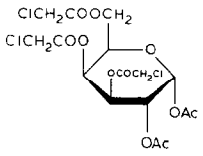

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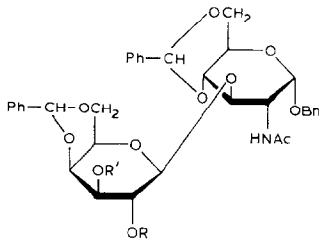
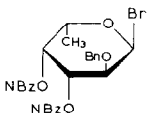


16 R = R′ = H
17 R = H, R′ = $COCH_2Cl$
18 R = Ac, R′ = H



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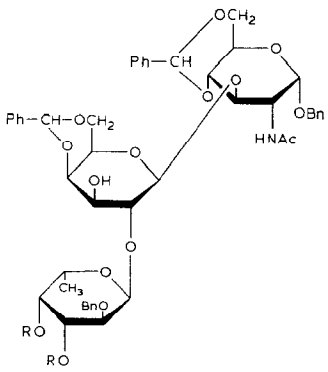


20 R = NBz
21 R = Bn

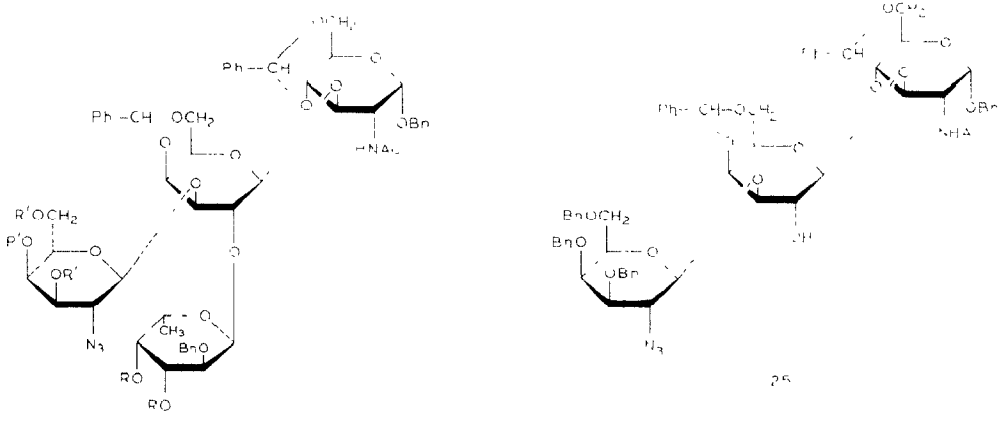
Orthoester **14** was deacetylated by the Zemplén method, the resulting compound was converted into the trichloroacetate by treatment with chloroacetic anhydride, the orthoester group was hydrolyzed with acetic acid, and the resulting compound was acetylated to give 1,2-di-*O*-acetyl-3,4,6-tri-*O*-chloroacetyl-α-D-galactopyranose (**15**). Hydrogen bromide in acetic acid converted **15** into the α-bromide, which was treated with benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside[24] under conditions similar to those described earlier[9]. The chloroacetyl groups of the resulting disaccharide were removed selectively with thiourea, with retention of the 2′-*O*-acetyl group to give a compound that was converted into the benzylidene

derivative **18** by treatment with benzaldehyde. This sequence of reactions to synthesize **18** is not convenient from a preparative viewpoint because of the many stages involved, but it allows to locate the position of the acetyl group in **18**.

Glycosylation of the disaccharide derivative **15** with 2-*O*-benzyl-3,4-di-*O*-*p*-nitrobenzoyl-α-L-fucopyranosyl bromide **19**[22,25] gave, after removal of the chloroacetyl group with thiourea, a partially protected trisaccharide **20**. Attempt to link a 2-azido-2-deoxy-α-D-galactopyranosyl group to **20**, by the method used by Paulsen and Kolář[11] for the similar trisaccharide **21**, by treating **20** with bromide **5** in the presence of mercuric cyanide and bromide, or mercuric bromide with 2,4,6-trimethylpyridine for over a month was unsuccessful (some product, probably tetrasaccharide **22**, was identified by i.r. and u.v. spectra only). Treatment of **20** with chloride **3** led, as that of **12**, to the corresponding aglycon acetate rather than to the glycoside. We were unable to repeat the glycosylation of the known trisaccharide **21**, which was obtained by the method of Paulsen and Kolář[11], and also from **20**, by substituting the *p*-nitrobenzoyl by benzyl groups[22,26]. Bromide **5** did not afford a condensation product, whereas chloride **3** afforded, as described by Paulsen and Kolář[11], a mixture of the desired glycoside with aglycon acetate, but in a considerably lower yield. Similar results were also obtained for the glycosylation of the disaccharide derivative **18** with halides **3** and **5**.

Further glycosylations were attempted with completely benzylated glycosyl halides. 2-Azido-3,4,6-tri-*O*-benzyl-2-deoxy-α-D-galactopyranosyl bromide (**8**) was inactive in the halide ion-catalyzed reaction described by Lemieux *et al.*[18], but the β-chloride **9** (obtained from **8**) reacted, in the presence of silver carbonate and perchlorate, with the trisaccharide derivatives **20** and **21** to give the 2-azido-2-deoxy-γ-D-galactosides **23** and **24**, in 82 and 60% yield, respectively.

Trisaccharide **25** was synthesized by two methods. Glycosylation of **18** with



22 R = NBz, R' = Ac
23 R = NBz, R = Bn
24 R = P = Bn

25

Bn = $C_6H_5CH_2$
NBz = $OC(C_6H_4NO_2)$

α-L-Fucp-(1→2)-β-D-Galp-(1→3)-2-azido-NAc

chloride **9**, followed by deacetylation, gave **25** in 74% yield, while glycosylation of diol **16** with the same chloride **9** gave **25** in 33% yield, in one step.

Deprotection of tetrasaccharides **23** and **24** gave **1**, and that of trisaccharides **25** and **20**, gave **2** and **26**, respectively. The last-named compound is the H blood-group determinant. Methanolysis, followed by g.l.c. analysis indicated a correct monosaccharide composition for **1**, **2**, and **26**, and the α configuration of the L-fucopyranosyl and 2-acetamido-2-deoxy-D-galactopyranosyl groups was ascertained by ^{1}H-n.m.r. spectrometry. The homogeneity of **1**, **2**, and **26** was confirmed by chromatography on anion-exchange resin.

In conclusion, use of the glycosylating chloride **9** excluded the formation of by-products due to intermolecular acetylation, and permits the synthesis of complex heterooligosaccharides containing the 2-acetamido-2-deoxy-α-D-galactopyranosyl group.

EXPERIMENTAL

General methods. — Melting points were determined with a Boetius apparatus. T.l.c. was performed on Silica gel 60 F-254 plates (E. Merck), and column chromatography on Silica gel L 40–100 μm (Chemapol, Czechoslovakia). All proportions of the solvents were v/v. G.l.c. was performed with a Hewlett–Packard 5710A chromatograph, equipped with a flame-ionization detector, in a capillary column (50 m × 0.24 mm) containing SE-30 phase, at 140–220° (2°/min), with helium as carrier gas (1.2 atm). Analytical ion-exchange chromatography was performed with a carbohydrate analyzer (Biotronic). The solvents were evaporated *in vacuo* at 30–40°. ^{1}H-N.m.r. spectra were recorded with a Varian-XL-100 spectrometer (at 100 MHz) with tetramethylsilane as the internal standard. Optical rotations were determined with a Perkin–Elmer 141 polarimeter.

*3,4,6-Tri-*O*-acetyl-2-azido-2-deoxy-β-D-galactopyranosyl chloride* (**3**). — To a solution of 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl bromide[6] (**5**, 5 mmol) in acetonitrile (40 mL) was added tetrabutylammonium chloride (10 mmol). After 30 min, the solution was diluted with benzene (200 mL), washed three times with water, dried (magnesium sulfate), and evaporated, and the residue was crystallized from ether to give **3** in 72% yield, m.p. 98–99°, $[\alpha]_D^{20}$ −16° (*c* 1, chloroform); lit.[2] m.p. 102–104°, $[\alpha]_D^{20}$ −16.5° (*c* 1, chloroform).

*2-Azido-3,4,6-tri-*O*-benzyl-2-deoxy-α-D-galactopyranosyl bromide* (**8**). — The mixture of anomer acetates (**10**, 10 g), obtained as described earlier[5], was boiled for 4 h in a mixture of 1,4-dioxane (400 mL) and 0.5M hydrochloric acid (50 mL), and then evaporated, and the residue chromatographed in 9:1 toluene–acetone. Alternatively, similar hydrolysis was carried out on a crude mixture resulting from the addition of chloroazide to 1,5-anhydro-3,4,6-tri-*O*-benzyl-2-deoxy-D-*lyxo*-hex-1-enitol and subsequent treatment with mercuric acetate[5]. The resulting mixture of reducing sugars was dissolved in a mixture of dichloromethane (150 mL) and pyridine (20 mL), and *p*-nitrobenzoyl chloride (6 g) was added portion-wise at 20°. After

TABLE I

GLYCOSYLATION OF CYCLOHEXANOL WITH GLYCOSYL HALIDES **3**, **5**, AND **7**

Experiment	*Glycosyl halide*	*Catalyst and (or) acceptor*	*Molar ratio of halide[a] to catalyst and (or) acceptor*	*Time of reaction (h)*	*Solvent*	*Ratio of α- to β-D-glycoside*
1	**5**	$Hg(CN)_2$ – $HgBr_2$	1:2:0.5	24	1:1 C_6H_6 – $MeNO_2$	9:91
2	**5**	$Hg(CN)_2$ – $HgBr_2$	1:2:0.5	24	CH_2Cl_2	11:89
3	**5**	Ag_2CO_3	1:2	24	CH_2Cl_2	43:57
4	**5**	$AgClO_4$[a]	1:2	1	C_6H_6	62:38
5	**5**	Bu_4NBr + $(Me_2CH)_2NEt$	1:3:2	72	1:1 C_6H_6 – $HCONMe_2$	77:23[b]
6	**3**[c]	Ag_2CO_3 – $AgClO_4$	1:2:0.2	4	CH_2Cl_2	73:27
7	**3**[d]	Ag_2CO_3 – $AgClO_4$	1:2:0.2	4	CH_2Cl_2	77:23
8	**7**[e]	Ag_2CO_3 – $AgClO_4$	1:2:0.2	4	CH_2Cl_2	81:19
9	**5**	$AgOSO_2CF_3$ 2,4,6-trimethylpyridine	1:2:2	4	CH_2Cl_2	36:64
10	**5**	$AgOSO_2CF_3$ 2,4,6-trimethylpyridine	1:2:2	4[f]	CH_2Cl_2	16:84
11	**5**	AgOTs 2,4,6-trimethylpyridine	1:2:2	72	CH_2Cl_2	61:39
12	**5**	Dowex 50W-X4 (Ag^+) 2,4,6-trimethylpyridine	1:10:2	120	CH_2Cl_2 or 1,4-dioxane	[g]
13	**5**	$AgOSO_2CF_3$ 2,4,6-trimethylpyridine	1:2:2	24	$(CH_2OMe)_2$	73:27
14	**5**	$AgOSO_2CH_3$ 2,4,6-trimethylpyridine	1:2:2	24	$(CH_2OMe)_2$	79:21

[a] Glycosylation by the diphenylcyclopropenyl method[17]. [b] The yield of glycosides is ~20%; in the remaining cases (except for Exp. 12) this yield is over 90%. [c] Obtained from **5** by action of Bu_4NCl. [d] Crystalline compound. [e] Obtained from **5** by action of $AgOSO_2CF_3$ and then Bu_4NBr. [f] At −30°; in the remaining cases at 20°. [g] No reaction.

15 min, the mixture was washed successively with water, 0.5M hydrochloric acid (500 mL), sodium hydrogencarbonate, and water, dried (calcium chloride), and evaporated. The resulting 1-(*p*-nitrobenzoate) was kept, without purification, for 5 h at 0°, in a solution of hydrogen bromide in dichloromethane (100 mL). The solution was evaporated at 20°, and the remaining solvent was coevaporated with benzene. To the residue was added benzene, the precipitate was filtered off, the filtrate evaporated, the residue dissolved in dichloromethane (15 mL), and heptane added until the solution became cloudy. After 10 min, silica gel (2 g) was added, the mixture was vigorously shaken and quickly filtered. Compound **8** crystallized from the decolorized solution in 60% yield (based on acetates **10**), m.p. 92–93°, $[\alpha]_D^{20}$ +180° (*c* 1, acetonitrile), +146° (*c* 1, chloroform); ^{1}H-n.m.r. (CCl_4): δ 6.35 (d, 1 H, $J_{1,2}$ 2.7 Hz, H-1) and 7.3 (m, 15 H, 3 Ph).

Anal. Calc. for $C_{27}H_{28}BrN_3O_4$: C, 60.22; H, 5.25; Br, 14.84; N, 7.81. Found: C, 60.30; H, 5.19; Br, 14.72; N, 7.80.

Glycosylation of cyclohexanol. — In all cases, a two-fold amount of cyclohexanol (or cyclohexyl 2,3-diphenyl-2-cyclopropen-1-yl ether) to glycosyl halide was used. The end of the reaction was detected by t.l.c. The pure anomers of cyclohexyl glycosides, used as the standards for g.l.c., were isolated by column chromatography of the reaction mixture with 7:3 ether–hexane. The anomeric configuration of the glycosides was assigned by n.m.r. spectrometry. After glycosylation, the reaction mixture was diluted with chloroform and filtered, and the filtrate was washed successively with 0.5M hydrochloric acid and sodium hydrogencarbonate, dried, evaporated, and deacetylated by the Zemplén method. The ratio of anomers was determined by g.l.c. after silylation. The results are shown in Table I.

*Benzyl 2-acetamido-4,6-benzylidene-3-*O*-(4,6-*O*-benzylidene-3-*O*-chloroacetyl-β-*D*-galactopyranosyl)-2-deoxy-α-*D*-glucopyranoside* (**17**). — To a solution of disaccharide[11] **16** (30 mmol) in abs. 1,4-dioxane (1.5 L) was added 2,4,6-trimethylpyridine (60 mmol) and, during 24 h at 20°, chloroacetyl chloride (~60 mmol); the reaction was continuously monitored by t.l.c. After the reaction was complete, the mixture was evaporated, the residue dissolved in chloroform, and the solution washed successively with water, 0.5M hydrochloric acid, sodium hydrogencarbonate, and water, dried (calcium chloride), and evaporated. The residue was crystallized from nitromethane to give **17** in 60% yield, m.p. 297°, $[\alpha]_D^{20}$ +115° (*c* 0.5, chloroform); lit.[11] m.p. 271°, $[\alpha]_D^{22}$ +103° (*c* 0.5, chloroform); the n.m.r. data are consistent with those reported in the literature[11].

*Benzyl 2-acetamido-3-*O*-(2-*O*-acetyl-4,6-*O*-benzylidene-β-*D*-galactopyranosyl)-4,6-*O*-benzylidene-2-deoxy-α-*D*-glucopyranoside* (**18**). — *Method A.* Chloroacetate **17** was treated with a mixture of pyridine and acetic anhydride at 20°. After 2 h, the mixture was diluted with chloroform, washed successively with water, 0.5M hydrochloric acid and sodium hydrogencarbonate, and dried (calcium chloride). After evaporation, the residue was dissolved in 3:1 pyridine–ethanol and boiled for 30 min with thiourea (1.2 equiv.). The mixture was evaporated, and chromatography of the

residue in 3:1 chloroform–acetone afforded **18** in 75–80% yield, m.p. 258° (from nitromethane–ether), $[\alpha]_D^{20}$ +56 (*c* 0.5, chloroform).

Anal. Calc. for $C_{37}H_{41}NO_{12}$: C, 64.23; H, 5.99; N, 2.03. Found: C, 64.29; H, 6.02; N, 1.96.

Method B. 3,4,6-Tri-*O*-acetyl-1,2-*O*-(1-methoxyethylidene)-α-D-galactopyranose (**14**, 30 mmol), obtained as previously reported[23], was deacetylated by the Zemplén method, the solution was evaporated to dryness, and the residue dissolved in a mixture of pyridine (50 mL) and dichloromethane (100 mL). To this solution was added at 0° chloroacetic anhydride (105 mmol). After 2 h, the mixture was diluted with chloroform (500 mL), washed with water, and, then, 0.5M hydrochloric acid, and evaporated. The residue was dissolved in 90% acetic acid (100 mL) and, after 10 min, the solution was evaporated. The residue was acetylated with acetic anhydride and pyridine, the mixture was diluted with chloroform, washed as usual, and chromatographed in 9:1 toluene–acetone to give 1,2-di-*O*-acetyl-3,4,6-tri-*O*-chloroacetyl-α-D-galactopyranose (**15**) in 32% yield, as a syrup; ^{1}H-n.m.r (chloroform-*d*): δ 6.43 (d, 1 H, $J_{1,2}$ 2.9 Hz, H-1), 4.18 (s, 2 H, $ClCH_2$), 4.06 (s, 2 H, $ClCH_2$), 4.01 (s, 2 H, $ClCH_2$), 2.17 (s, 3 H, Ac), and 2.02 (s, 3 H, Ac). The resulting compound **15** was dissolved in 30% hydrogen bromide in acetic acid (100 mL), and kept for 15 h at 0°. The solution was diluted with cold chloroform, washed successively with cold water, sodium hydrogencarbonate, and water, and dried (calcium chloride). The glycosyl halide obtained by evaporation was treated with benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside[24], as described by Paulsen *et al.*[9] for 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide. The protected disaccharide was isolated by chromatography in 55:9:1 chloroform–ether–methanol, and treated with thiourea (3.6 equiv.) according to method A. After evaporation of the reaction mixture, the residue was treated, without further purification, for 15 h with benzaldehyde in the presence of zinc chloride. Chromatographic purification in 3:1 chloroform–acetone gave **18**, identical with that synthesized by method A. The yield of **18** (based on orthoester **14**) was 9%.

Benzyl O-[2-O-*benzyl*-3,4-*di*-O-(p-*nitrobenzoyl*)-α-L-*fucopyranosyl*]-(1→2)-O-(4,6-O-*benzylidene*-β-D-*galactopyranosyl*)-(1→3)-2-*acetamido*-2-*deoxy*-α-D-*glucopyranoside* (**20**). — A solution **17** (1.0 mmol), mercuric cyanide (2.0 mmol), and mercuric bromide (0.3 mmol) in a mixture of nitromethane (35 mL) and benzene (35 mL) was evaporated to three-quarters of its initial volume. 2-*O*-Benzyl-3,4-di-*O*-(*p*-nitrobenzoyl)-α-L-fucopyranosyl bromide[22,25] (**19**, 0.6 mmol) was added within 1 h at 50°, and then an additional amount of **19** (0.6 mmol) within 1 h without heating. The mixture was kept for 24 h at 20°, diluted with dichloroform, washed successively with water and sodium hydrogencarbonate, and dried (calcium chloride). After evaporation, the residue was boiled for 1 h with thiourea (1.2 mmol) in a mixture of pyridine (45 mL) and ethanol (15 mL). Evaporation and chromatography in 17:3 toluene–acetone gave **20** in 65% yield, m.p. 166–169°, $[\alpha]_D^{20}$ −43 (*c* 1, chloroform); ^{1}H-n.m.r. (chloroform-*d*): δ 7.50–6.90 (2AA′BB′, 8 H, 2 C_6H_4), 6.40

(m, 20 H, 4 Ph), 2.94 (s, 1 H, OH), 1.95 (s, 3 H, Ac), and 1.14 (d, 3 H, $J_{5'',6''}$ 6.6 Hz, CH_3 of L-fucose).

Anal. Calc. for $C_{62}H_{61}N_3O_{21}$: C, 62.88; H, 5.20; N, 3.55. Found: C, 62.83; H, 5.02; N, 3.42.

Benzyl O-(*2,3,4-tri*-O-*benzyl*-α-L-*fucopyranosyl*)-(*1→2*)-O-(*4,6*-O-*benzylidene*-β-D-*galactopyranosyl*)-(*1→3*)-*2-acetamido-2-deoxy*-α-D-*glucopyranoside* (**21**). — To a solution of **20** (2 mmol) in oxolan (20 mL) was added 2,4,6-trimethylpyridine (20 mmol) and, then, at 0°, portionwise during 1 h, a reagent[26] made of oxolan, sulfuryl chloride (20 mmol), and triethylamine (20 mmol). After 2 h, the precipitate was filtered off, the solution evaporated, and the residue deacetylated by the Zemplén method. It is to be noted that the required amount of sodium methoxide may be more than usual, as a certain amount of amine hydrochloride remains in the mixture. After evaporation, the residue was dissolved in *N,N*-dimethylformamide (30 mL), and treated with sodium hydride (6 mmol) and benzyl chloride (6 mmol) at 20°. After 15 h, the mixture was diluted with chloroform, washed with water, and evaporated. The residue was kept for 15 h at 20° in 3 : 1 : 1 acetic acid–oxolan–water. Evaporation and chromatography in 5:2 hexane–acetone gave amorphous **21** in 80% yield, $[\alpha]_D^{20}$ +20° (*c* 1, chloroform); lit.[11] m.p. 117°, $[\alpha]_D^{20}$ +16.1° (*c* 1, chloroform); the ^{1}H-n.m.r. data are consistent with those reported in the literature[11]. The same compound **21** was obtained by the method described by Paulsen and Kolář[11].

Benzyl O-[*2*-O-*benzyl-3,4-di*-O-(p-*nitrobenzoyl*)-α-L-*fucopyranosyl*]-(*1→2*)-[O-(*2-azido-3,4,6-tri*-O-*benzyl-2-deoxy*-α-D-*galactopyranosyl*)-(*1→3*)]-O-(*4,6*-O-*benzylidene*-β-D-*galactopyranosyl*)-(*1→3*)-*2-acetamido-4,6*-O-*benzylidene-2-deoxy*-α-D-*glucopyranoside* (**23**). — To a solution of bromide **8** (1 mmol) in acetonitrile (45 mL) was added a solution of triethylbenzylammonium chloride (2 mmol) in acetonitrile (4 mL). After 9 min (minimum optical rotation), the mixture was diluted with dichloromethane (100 mL), washed three times with cold water, dried (magnesium sulfate), and evaporated. The resulting β-chloride **9** (a syrup containing, as indicated by t.l.c., a negligible amount of impurity of the starting compound **8**), was added within 30 min to a mixture of trisaccharide **20** (0.5 mmol), silver carbonate (2.5 g), silver perchlorate (70 mg), and molecular sieves 4 A (5 g) in dichloromethane (40 mL). After 30 min, the mixture was filtered, and the filtrate washed successively with water and sodium hydrogencarbonate, dried (calcium chloride), and evaporated. Chromatography of the residue in 17:3 benzene–ethyl acetate gave **23** in 82% yield, m.p. 151–153°, $[\alpha]_D^{20}$ +9° (*c* 1, chloroform); ^{1}H-n.m.r. (chloroform-*d*): δ 8.4–7.6 (2 AA′BB′, 8 H, 2 C_6H_4), 5.56 (s, 1 H, PhC*H*), 5.52 (s, 1 H, PhC*H*), 1.78 (s, 3 H, Ac), and 0.66 (d, 3 H, $J_{5'',6''}$ 6.0 Hz, CH_3 of L-fucose).

Anal. Calc. for $C_{89}H_{88}N_6O_{25}$: C, 65.10; H, 5.41; N, 5.12. Found: C, 65.00; H, 5.52; N, 5.09.

Benzyl O-(*2,3,4-tri*-O-*benzyl*-α-L-*fucopyranosyl*)-(*1→2*)-[O-(*2-azido-3,4,6-tri*-O-*benzyl-2-deoxy*-α-D-*galactopyranosyl*)-(*1→3*)]-O-(*4,6*-O-*benzylidene*-β-D-*galactopyranosyl*)-(*1→3*)-*2-acetamido-4,6*-O-*benzylidene-2-deoxy*-α-D-*glucopyranoside* (**24**). — This compound was obtained, as described for the preparation of trisaccharide **21**,

in 60% yield as a syrup, $[\alpha]_D^{20}$ +26° (*c* 1, chloroform); ^{1}H-n.m.r. (chloroform-*d*): δ 7.3 (m, 45 H, 9 Ph), 5.51 (s, 1 H, PhC*H*), 5.39 (s, 1 H, PhC*H*), 1.46 (s, 3 H, Ac), and 1.04 (d, 3 H, $J_{5',6'}$ 6.0 Hz, CH_3 of L-fucose).

Anal. Calc. for $C_{89}H_{94}N_4O_{16}$: C, 70.14; H, 6.23; N, 3.68. Found: C, 70.09; H, 6.23; N, 3.60.

Benzyl O-(*2-azido-3,4,6-tri-*O*-benzyl-2-deoxy-*α-D-*galactopyranosyl*)-(*1→3*)-O-(*4,6-O*-benzylidene β-D-galactopyranosyl*)-(*1→3*)-2-acetamido-4,6-O-benzylidene-2-deoxy-*α-D-*glucopyranoside* (**25**). — *Method 1.* Glycosylation of disaccharide **18** was performed similarly to that of trisaccharide **20**. The reaction mixture was *O*-deacetylated by the Zemplén method, and then chromatographed in 3:2 chloroform–ethyl acetate to give **25** in 74% yield, m.p. 265° (dec., from methanol–nitromethane), $[\alpha]_D^{20}$ +91° (*c* 1, chloroform); ^{1}H-n.m.r. (dichloromethane-d_2) δ 7.35 (m, 30 H, 6 Ph), 6.44 (d, 1 H, $J_{NH,2}$ 8.5 Hz, NH), 5.59 (s, 2 H, 2 PhC*H*), and 1.90 (s, 3 H, Ac).

Anal. Calc. for $C_{62}H_{60}N_4O_{15}$: C, 67.25; H, 6.02; N, 5.06. Found: C, 67.31; H, 6.11; N, 4.98.

Method B. To a mixture of **16** (1 mmol), silver carbonate (2.5 g), silver perchlorate (70 mg), and molecular sieves 4 A (5 g) in dichloromethane (200 mL) was added, within 3 h, a solution of chloride **9**, obtained from bromide **8** (1 mmol) as just described. After 30 min, the mixture was filtered, and the filtrate washed successively with water and sodium hydrogencarbonate to give, after chromatography, **25** in 33% yield.

O-α-L-*Fucopyranosyl*-(*1→2*)-[O-*2-acetamido-2-deoxy*-α-D-*galactopyranosyl*-(*1→3*)]-O-β-D-*galactopyranosyl*-(*1→3*)-*2-acetamido-2-deoxy*-D-*glucose* (**1**). — *Method A.* Tetrasaccharide **23** was *O*-deacetylated by the Zemplén method in 3:1 methanol–benzene, and the solution was made neutral with acetic acid and evaporated. The product was hydrogenated in methanol solution for 2 days in the presence of palladium-on-charcoal. The mixture was cooled, filtered, and a ten-fold excess of acetic anhydride was added to the filtrate. After 24 h, the mixture was evaporated at 20°, and a solution of residue in water passed through a column of Bio-Gel P-2; evaporation of the eluate gave, in a nearly quantitative yield, amorphous **1**, $[\alpha]_D^{20}$ +49° (*c* 1, methanol); lit.[11] $[\alpha]_D^{20}$ +53.8° (*c* 1, methanol); the ^{1}H-n.m.r. spectrum was consistent with that reported in the literature[11].

Method B. Similarly, except for the *O*-deacetylation step, tetrasaccharide **1** was obtained from the protected derivative **24**.

O-*2-Acetamido-2-deoxy*-α-D-*galactopyranosyl*-(*1→3*)-O-β-D-*galactopyranosyl*-(*1→3*)-*2-acetamido-2-deoxy*-D-*glucose* (**2**). — Trisaccharide **25** gave, under conditions similar to those described under method B, a nearly quantitative yield of amorphous trisaccharide **2**, $[\alpha]_D^{20}$ +130° (*c* 1, water); ^{1}H-n.m.r. (methanol-d_4): δ 5.18 (d, 1 H, $J_{1,2}$ 3.4 Hz, H-1), 5.08 (d, 1 H, $J_{1',2'}$ 3.2 Hz, H-1'), and 2.00 (s, 6 H, 2 Ac); lit.[9] $[\alpha]_D^{20}$ +135.1° (*c* 1, water).

O-α-L-*Fucopyranosyl*-(*1→2*)-O-β-D-*galactopyranosyl*-(*1→3*)-*2-acetamido-2-deoxy*-D-*glucose* (**26**). — Trisaccharide **20** was *O*-deacylated and hydrogenated, as described for the preparation of **1** (method A). The product was dissolved in water,

washed with benzene, and treated with Amberlite IR-120 (H^+), and the solution was evaporated to dryness. The amorphous trisaccharide **26** was obtained in a nearly quantitative yield, $[\alpha]_D^{20}$ $-18°$ (*c* 1, methanol); lit.[11] $[\alpha]_D^{20}$ $-19.4°$ (*c* 1, methanol); the n.m.r. spectrum is consistent with that reported in the literature[11].

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