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4,6-*O*-Benzylidene-D-glucopyranose and its sodium salt: new data on their preparation and properties

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Abstract

An improved method for the preparation of 4,6-*O*-benzylidene-D-glucopyranose (**BG**), and new or corrected data on its ^{1}H and ^{13}C NMR spectra, specific rotations, and tautomeric equilibria, and on those of its anomeric sodium salt (**BGNa**), are reported. Evidence is presented in favour of the hypothesis that crystalline **BGNa** exists entirely in its β-anomeric form and that it can be useful in the access to β-glucosides in reactions with strong electrophiles under strictly heterogeneous conditions.

Keywords: 4,6-*O*-Benzylidene-D-glucopyranose; 4,6-*O*-Benzylidene-D-glucopyranose sodium salt

1. Introduction

Since the early studies by Emil Fischer [1] on the reactions of monosaccharides with aldehydes and ketones, cyclic acetal formation has become one of the most widely used techniques for the selective protection of hydroxyl groups in carbohydrate synthesis [2]. The preparation of 4,6-*O*-benzylidene-D-glucopyranose (**BG**) was first reported by Alberda van Ekenstein and Blanksma [3] in 1906, but it was only later that Zervas [4] obtained this compound in a pure state and characterized it in an adequate manner. In spite of the apparent simplicity of its preparation and of the easy removal of the 4,6-*O*-benzylidene group by hydrolytic or hydrogenolytic methods, **BG** has not been used as a protected derivative of D-glucose as extensively as one might expect, possibly because of both difficult purification and erratic yields. Recently, **BG** has received

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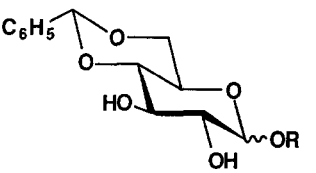
increasing attention as an anti-cancer drug, providing a water-soluble carrier of low toxicity for benzaldehyde. It has reached the clinical study phase, with significant inhibitory action on several types of advanced carcinoma [5].

In view of this recent revival of interest in **BG** and of the fact that several of its properties and its use in glucoside synthesis appear not to have been extensively investigated, we have therefore revisited this simple D-glucose derivative. The early report by Zervas [4] attracted our attention in that it gives a well-crystallized sodium salt (**BGNa**) on treatment with NaOH in aqueous ethanol: to the best of our knowledge, this is the only case of a crystalline anomeric salt of a glycopyranose to be reported in the literature. In more recent times, mention has been made of its use in a crude form as an intermediate in the preparation of 1-*O*-acyl-β-D-glucopyranoses in low to moderate yields [6]. Should this approach to anomeric nucleophilic functionalization [7] be amenable to higher yields and reaction with a reasonably large range of electrophiles, the procedure could have some practical interest as an approach to the synthesis of glucopyranosides under minimal protection conditions. Unfortunately, our preliminary results in this direction were rather disappointing. On the other hand, our work has shown that several of the data from the older literature are erroneous or incomplete, and we thought it worthwhile to present the new results on the chemistry and instrumental characterization of **BG** and its sodium salt, which are the subject of the present paper [8].



BG R = H
BGNa R = Na
1 R = Bu_4N
2 R = Me
3 R = C_6H_5CO

2. Results and discussion

Preparation and characterization of **BG**.—A reliable and reproducible method for the preparation of **BG** was highly desirable since the standard method for the benzylidenation of D-glucose, involving the use of a large excess of benzaldehyde and zinc chloride, suffers from several shortcomings, such as the formation of by-products, sensitivity to several experimental parameters, and difficulties in work-up and purification. As a consequence, reported yields have usually been low and the purity of the final

product sometimes doubtful. Early reports [4,9] gave yields as low as 10% for pure **BG**, and later improvements did not increase them above 40% [10].

We found that the transacetalation of D-glucose with α,α-dimethoxytoluene in DMF catalysed by *p*-toluenesulfonic acid provides a more satisfactory access to **BG**. This is a standard method [11] for the introduction of *O*-benzylidene groups on glycosides of mono- and oligo-saccharides, including the methyl α- and β-glycosides **2**, but has been used only occasionally on reducing sugars. This approach to **BG** was first mentioned, while the present work was already in progress, in a preliminary paper without experimental details, which reported a 71% yield [12a]. The experimental details were given in a more recent paper [12b], but a yield of only 42% was reported. No mention was made of the anomeric composition of the product in both papers. We therefore made a preliminary evaluation of the optimal ratio between D-glucose and α,α-dimethoxytoluene, which showed that a 1:1.1 molar ratio provides the best compromise between complete reaction and limitation of by-products of the bis-acetal type. A single filtration of a solution of the crude product through silica gel provided pure **BG** with a reproducible yield of 72%.

The purity of the product was ascertained by TLC, by its complete solubility in aqueous NaOH to give the sodium salt **BGNa**, and by the ^{1}H and ^{13}C NMR spectra in $(CD_3)_2SO$. The 14 non-aromatic carbon signals expected for the anomeric mixture of **BG** have chemical shift values close to those of the corresponding methyl glycosides **2** (Table 1), with the exception of C-1, in which methylation causes the expected downfield shift of ca. 7 ppm [13]. The α/β ratios were estimated on the basis of ^{1}H NMR spectra. Although the spectrum in $(CD_3)_2SO$ was rather complicated, owing to overlapping signals of CH and OH protons, the signals for the anomeric α- and β-OH protons appeared as well-separated doublets, at δ 6.34 and 6.62, respectively, well suited for analytical purposes. Alternatively, after the exchange of OH protons by addition of D_2O, the anomeric CH proton signals were simplified to non-overlapping doublets at δ 4.99 (J 3.7 Hz) and 4.46 (J 7.7 Hz), also suitable for analysis.

The proton spectra in $(CD_3)_2SO$ and in C_5D_5N were completely interpreted and the spectroscopic parameters for the two anomers are collected in Table 2, together with relevant diagnostic signals of incompletely resolved spectra recorded in other solvents.

The sample of **BG**, obtained as above after filtration through silica gel and analysed in $(CD_3)_2SO$ by this method, contained a slight excess of the α anomer (52–60%), which slowly decreased with time to reach equilibrium at an $\alpha:\beta$ ratio of 36:64 after 24 h. The equilibrium values in other solvents, determined by ^{1}H NMR, are collected in Table 3; as expected [14], the amount of the α anomer is smallest in water, owing to the greater operation of the anomeric effect in non-aqueous solvents. The corresponding reported values relative to D-glucopyranose in $(CD_3)_2SO$, CD_3CN, and C_5D_5N (also shown in Table 3) are very close to those found for **BG**, a fact that proves that bridging between HO-4 and HO-6 has little influence on the relative stabilities of anomers.

When the chromatographically pure **BG** was subjected to repeated crystallizations from water containing NH_3, as reported by Zervas [4] and Fletcher [6b], we obtained a product, mp 178–179°C, with an equilibrium $[\alpha]_D$ (MeOH) value of $-5.0°$, corresponding closely to those reported by these authors ($-4°$ and $-4.9°$). NMR analysis showed that it is a 1:1 mixture of α- and β-**BG**, possibly a eutectic of the type described by

Table 1
^{13}C NMR data (δ, ppm) for **BG**, **BGNa**, and **2** [a] in various solvents

Compound	Solvent	C-1	C-2	C-3	C-4	C-5	C-6	PhCH	Aromatic				OMe
α-BG	Me_2SO	93.0	72.8	69.6	81.6	61.8	68.3	100.6	137.8	128.5	127.7	126.1	—
	1:1 Me_2SO–D_2O	94.3	73.7	71.1	82.1	63.4	69.6	102.9	138.0	129.0	128.2	126.6	—
	8:92 Me_2SO–D_2O	94.6	73.7	71.4	82.2	63.9	69.9	103.4	137.8	131.6	130.4	127.9	—
	CD_3OD	94.7	74.4	71.8	83.0	63.5	70.2	103.0	139.2	129.9	129.0	127.5	—
	C_5D_5N	94.5	74.5	71.6	83.2	63.0	69.7	101.9	138.8	128.8	128.1	126.9	—
	THF–D_2O	95.0	74.7	72.0	83.5	63.9	70.6	103.1	140.0	130.1	129.4	128.0	—
β-BG	Me_2SO	97.5	75.7	72.9	80.8	65.7	67.9	100.5	137.7	128.5	127.7	126.1	—
	1:1 Me_2SO–D_2O	98.4	76.7	74.0	81.5	67.2	69.2	102.5	138.0	129.0	128.2	126.6	—
	8:92 Me_2SO–D_2O	98.3	76.6	74.2	81.7	67.6	69.5	103.5	137.8	131.6	130.4	127.9	—
	CD_3OD	98.9	77.1	74.6	82.3	67.7	69.7	102.8	139.1	129.9	129.0	127.5	—
	C_5D_5N	99.2	77.5	74.6	82.5	67.1	69.3	101.9	138.7	128.8	128.1	126.9	—
	THF–D_2O	99.3	77.4	74.9	82.7	68.1	70.2	102.9	139.9	130.1	129.4	128.0	—
α-BGNa	D_2O–NaOD (4°C)	100.1	76.6	72.1	83.1	62.2	70.4	103.1	137.7	131.4	130.2	127.8	—
β-BGNa	D_2O–NaOD (4°C)	104.5	79.8	74.5	82.7	66.8	70.0	103.1	137.7	131.4	130.2	127.8	—
BGNa	D_2O–NaOD (17°C)	104.2	79.7	74.3	82.7	66.5	69.9	102.8	137.6	131.1	130.0	127.5	—
α-2	Me_2SO	100.7	72.6	70.1	81.5	62.6	68.4	101.1	138.0	129.0	128.2	126.6	54.9
	4:1 $CDCl_3$–CD_3OD [b]	99.9	72.4	70.5	80.8	62.0	68.5	101.5					54.9
β-2	Me_2SO	104.7	74.4	73.0	80.8	65.9	68.1	100.8	137.9	129.0	128.2	126.5	56.5
	4:1 $CDCl_3$–CD_3OD [b]	104.2	74.2	72.9	80.3	65.9	68.3	101.5					56.8

[a] Internal standards: Me_2SO δ 39.5, dioxane δ 67.8 (for spectra in D_2O), CD_3OD δ 49.9, C_5D_5N δ 123.2, THF δ 26.0.
[b] From ref. [24].

Table 2
^{1}H NMR data (δ, ppm) for **BG** [a] in various solvents

Anomer	Solvent	H-1	H-2	H-3	H-4	H-5	H-6ax	H-6eq	PhCH	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6ax}$	$J_{5,6eq}$	$J_{6ax,6eq}$
α-**BG**	Me_2SO–D_2O	4.99	4.29	3.63	3.33	3.80	3.65	4.08	5.55	3.7	8.7	9.5	9.8	9.1	4.4	9.4
	CD_3CN	5.09	3.43	3.74	3.40	3.87	3.67	4.15	5.54	3.8	9.2	9.3	9.4	10.0	4.7	9.8
	CD_3OD	5.14	3.48	3.87	3.43	3.97	3.72	4.18	5.57	3.8	9.3	9.3	9.4	9.7	4.8	9.8
	D_2O	5.14				3.89		4.13	5.55	3.8			9.6	10.0	4.7	10.0
	C_5D_5N	5.78	4.17	4.64	3.98	4.60	3.95	4.48	5.79	3.8	9.2	9.3	9.6	9.0	4.8	10.0
β-**BG**	Me_2SO–D_2O	4.46	3.06	3.40	3.36	3.33	3.66	4.15	5.56	7.7	8.6	9.4	9.8	9.1	4.4	9.4
	CD_3CN	4.56	3.19					4.22	5.54	7.7	8.7				4.5	10.3
	CD_3OD	4.61	3.26	3.65	3.48	3.46	3.75	4.26	5.56	7.7	8.8			9.8	4.5	10.2
	D_2O	4.60	3.22					4.18	5.55	7.9	8.7				4.5	10.3
	C_5D_5N	5.28	4.10	4.29	3.96	3.75	3.98	4.51	5.83	7.6	8.8	9.1	10.0	9.2	4.9	9.8

[a] Signals of aromatic protons resonate always between δ 7.70 and 7.30.

Table 3
Anomeric equilibria for **BG** [a,b]

Solvent	α Anomer (%)	β Anomer (%)
Me_2SO	36 (38)	64 (62)
CD_3CN	50	50
CD_3CD	46	54
D_2O	34 (36)	66 (64)
C_5D_5N	48 (48)	52 (52)

[a] Estimated error ± 3%.
[b] Values in parentheses are those reported for D-glucopyranose [14].

Pfeffer et al. [6c] for 2,3,4,6-tetra-*O*-benzyl-D-glucopyranose, in contrast to the suggestion [4] that it is pure **α-BG**.

In an attempt to obtain pure **α-BG**, the 1:1 mixture was recrystallized from ethyl acetate to give a product in which the α/β ratio was 4:1. When this product was dissolved in acetonitrile at room temperature and cooled to 5°C, needles with mp 179–180°C were obtained, NMR analysis of which showed that it was pure **α-BG**, with an initial $[\alpha]_D$ of +49° in Me_2SO. From this value and from the anomeric ratio at equilibrium it was further possible to deduce an $[\alpha]_D$ value of approximately −42° for **β-BG**. These specific rotations agree well with the Hudson isorotation rules, their difference (91°), reflecting the contribution of the anomeric centre, being very close to that between the anomers of D-glucose (94°).

The mutarotation of **BG** was followed by polarimetry (see Experimental section). The rates of equilibration in different solvents had the following order: H_2O > 1:1 Me_2SO–H_2O > MeOH > DMF > Me_2SO. The very low rate of equilibration of **BG** in Me_2SO makes this solvent highly suitable for the determination of anomer ratios by polarimetric and NMR methods.

Preparation and characterization of **BGNa**.—The preparation of the sodium salt of **BG** (**BGNa**), according to Zervas [4], gave the reported 'prismatische, sternförmig angeordnete Krystalle' only when the addition of ethanol to the aqueous alkaline solution of **BG** was carried out very carefully. It was more easily obtained by the method of Fletcher [6b], involving addition of MeONa in MeOH to a solution of **BG** in absolute ethanol. This product was precipitated as a powder, but, when observed under a microscope, was found to consist of microcrystals with, at most, small amounts of amorphous matter. Since the products obtained by the two methods had identical NMR spectra, the Fletcher salt was used subsequently for the preparation of **BGNa**.

The salt can be stored in vacuo over P_4O_{10} for several days without change. Its aqueous solution has an initial $[\alpha]_D^{20}$ of −17.4°, which slowly decreases with time. This observed mutarotation is not due to an anomeric equilibration, as indicated by the appearance of a yellowish colour.

The negative value of the specific rotation of **BGNa** points to a prevalence of the β form in the anomeric equilibrium. If one assumes that the specific rotations of α- and **β-BGNa** are not too different from those of α- and **β-BG**, an initial value of −17.4° would correspond to a β/α ratio of ca. 7:3 for **BGNa**. This was confirmed by NMR studies.

Table 4
^{13}C NMR chemical shift differences between anomeric salts and their conjugate acids

	C-1	C-2	C-3	C-4	C-5	C-6
BGNa–BG						
α	5.5	2.9	0.7	0.9	−1.7	0.5
β	6.2	3.2	0.3	1.0	−0.8	0.5
GlcNa–Glc [a]						
α	4.8	3.0	1.0	1.0	−1.4	0.4
β	5.5	3.0	0.7	1.2	−0.4	0.6

[a] From ref. [15].

The changes induced by salt formation involving the more acidic anomeric hydroxyl group of monosaccharides have been studied mainly by ^{13}C NMR spectroscopy [15], since ^{1}H NMR spectra of these salts are not usually susceptible to detailed analysis owing to extensive overlap of signals. However, in the proton spectrum of **BGNa** in D_2O solution, measured at 0°C, we observed two signals at δ 4.9 and 5.5, a doublet (J 7.2 Hz) and a broad singlet, in a ratio of 76:24, which, also on the basis of their chemical shifts, were likely to correspond, respectively, to the anomeric protons of the β and α forms.

The ^{13}C NMR spectrum of **BGNa** was more informative. Although the stability of this salt in water is limited, it was possible to record fairly good spectra at room temperature and better ones at lower temperature. At 4°C two sets of six signals were clearly visible for the pyranose moiety of the salt which were assigned, respectively, to the β and α forms, by comparison with those reported [15] for the sodium salts of β- and α-D-glucopyranose (Tables 1 and 4). A rough evaluation of the β/α ratio gave a value of 4:1, in fairly good agreement with the ratio estimated from the proton spectrum. The benzylidene carbons gave a single set of five signals showing that they are isochronous in the two anomeric forms.

When the ^{13}C NMR spectrum was recorded at a higher temperature (17°C) the signals for the minor α anomer merged into the background noise, while the C-1, C-2, C-3, and C-5 signals of the β anomer decreased their intensities with respect to those of the benzylidene carbons and also of C-4 and C-6, the chemical shifts of which, as shown in Table 1, differ very little in the two anomers. This clearly pointed to a situation of dynamic equilibrium between the two anomeric forms, approaching coalescence of signals. A further increase in temperature led to the disappearance of all signals into the increased background noise, except for the isochronous ones, at around 35°C, which should therefore approximately correspond to the coalescence temperature. A more precise determination of this temperature was not possible owing to the degradation of the solute becoming too fast. It may be noted that these data show that for **BGNa** the coalescence temperature is significantly lower than for the sodium salt of D-glucopyranose, since on recording the ^{13}C NMR spectrum of the latter up to 20°C the signals of the α and β forms did not undergo the changes in relative intensity observed for **BGNa**. This is in accordance with the observation [16] that 4,6-*O*-ethylidene-D-glucose undergoes base-catalysed mutarotation much faster than D-glucose, showing that bridg-

ing of the 4,6-positions facilitates opening and closing of the pyranose ring of the anomeric anions.

The fact that the β/α anomer ratio increases on going from **BG** to its salt agrees with previous results concerning D-glucopyranose [15], and its tetra-*O*-methyl [15] and tetra-*O*-benzyl [6c] derivatives. Although no rigorous experimental proof, such as solid-state NMR, is yet available, it is very likely that the crystalline salt prepared according to Zervas [4] (and probably also that obtained according to Fletcher [6b]) is the pure β anomer.

Use of **BGNa** *as a nucleophile in glycosylation reactions.*—Glycosylations involving direct nucleophilic displacements by anomeric anions on activated glycosyl acceptors, although less versatile than the classical electrophilic glycosylations (Fischer, Koenigs–Knorr, etc.), have in some cases provided a simple approach to glycoside synthesis [7a], including those in which O-unprotected aldose anions were converted in fair yields into mixtures of glycoside anomers through reactions with reactive electrophiles [7b]. As mentioned at the beginning of this paper, very little was known of the possible use in reactions of this type of **BGNa** as an easily prepared nucleophilic D-glucose derivative.

Preliminary solubility tests on **BGNa** showed that it is practically insoluble in all solvents, except water. For instance, addition of one drop of 2 M aqueous sodium hydroxide to a solution of **BG** in Me_2SO produces immediate precipitation of microcrystals of **BGNa**. Although the low solubility of **BGNa** in organic solvents poses a severe limit to its use under homogeneous conditions, it may turn out to be advantageous in heterogeneous reactions, if, as is likely, crystalline **BGNa** is the pure β anomer, and provided that conditions are found in which it reacts as a solid.

It was further found that the solubility of the **BG** anion can be greatly increased when it is converted into the tetrabutylammonium salt **1**, prepared in situ from **BG**. This salt is easily soluble in several aprotic solvents and can therefore be used for homogeneous reactions.

Anomeric methylation.—Zervas [4] isolated 30% of the methyl β-glycoside (β-**2**) from the reaction of **BGNa** with Me_2SO_4 in water, no mention being made of the concurrent formation of any α anomer or other products. More recently Szeja [17] reported that **BG**, when treated with Me_2SO_4 and tetrabutylammonium chloride (1:1.5:0.05 molar ratio) under phase transfer conditions in a benzene–aq 50% NaOH system, gave β-**2** and α-**2** in a ratio of 78:22. We obtained similar results (Table 5) in reactions conducted on **BGNa** under homogeneous (Me_2SO_4, THF–H_2O) or heterogeneous conditions (MeI–Me_2SO), and on the soluble tetrabutylammonium salt **1** (MeI–Me_2SO and Me_2SO_4–THF). The fact that, under a fairly wide range of conditions, the methyl β-glycoside is constantly the main product is in accordance with previous results [7a,b] on alkylations of anomeric anions and with the fact that, owing to fast equilibration, the more nucleophilic β-anion reacts preferentially [18,19]. One may assume also that in the case of the apparently heterogeneous reaction conducted in Me_2SO a certain, even if very limited, solubility of the salt and the highly favourable conditions for a nucleophilic displacement may allow a homogeneous reaction.

In order to check this hypothesis the methylation of **BGNa** was carried out with a highly reactive electrophile (methyl triflate) in a poor solvent (CH_2Cl_2), conditions

Table 5
Methylation of **BG** salts [a]

Salt	Reagent	Molar ratio	Solvent (mL/mmol salt)	Reaction time (h)	Yield (%) $2\alpha + 2\beta$	Ratio [b] $2\alpha - 2\beta$
BGNa	MeI	1:1.5	Me_2SO (10)	12	70	32:68
BGNa	Me_2SO_4	1:1.05	2:1 THF–H_2O (6)	5	75	20:80
BGNa	MeOTf	1:1	CH_2Cl_2 (6)	7	60	< 2: > 98
1	MeI	1:1.5	3:1 Me_2SO–MeOH (6)	5	40	38:62
1	Me_2SO_4	1:1.05	3:1 THF–MeOH (6)	5	65	30:70

[a] All reactions carried out at room temperature.
[b] Determined by HPLC (see Experimental section).

under which a heterogeneous reaction was expected to be particularly favoured. In this case, only β-**2** was formed. Weaker electrophiles, such as MeI and Me_2SO_4, did not react with **BGNa** in CH_2Cl_2, whereas $Me_3O^+BF_4^-$ gave degradation products only.

The exclusive formation of the methyl β-glycoside in the methyl triflate reaction could be taken as confirmation of the hypothesis that crystalline **BGNa** exists entirely in its β-tautomeric form.

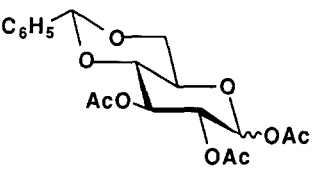
Anomeric acylation.—The only type of reaction in which **BGNa** has so far found some practical use is the preparation of β-1-*O*-acyl derivatives of D-glucose. Zervas [4] was the first to report the formation of the β-1-*O*-benzoyl derivative β-**3** in 75% yield in the reaction of **BGNa** with benzoyl chloride in chloroform; on the other hand, later papers on the same reaction [6b,g], or on similar reactions with substituted benzoyl chlorides [6a,f], gave yields in the range of 40–50%, or did not mention yields. None of these reports contained an accurate analysis of reaction products, nor was it ascertained if the acylation was β-stereospecific, or if some α anomer was also formed.

We therefore repeated the benzoylation of **BGNa** under Fletcher's conditions [6b] (PhCOCl–CH_2Cl_2) and obtained the pure β-1-*O*-benzoyl derivative β-**3** in 90% yield. NMR analysis of the crude product proved α-**3** to be completely absent. Again, the reaction of **BGNa** with a highly reactive electrophile seems to occur entirely under heterogeneous conditions to produce an ester having the same anomeric configuration as the salt.

The picture is much less clear with other types of acyl chlorides, which have been reported to give, with **BGNa**, mixtures of α- and β-anomeric esters in ratios strongly depending on the structure of the acyl chloride and on the reaction conditions. For instance, in the acylation of **BGNa** with long-chain acyl chlorides, α/β product ratios were found to depend strongly on the presence and number of double bonds [6c,e], and on small changes in the **BGNa**/acyl chloride ratios [6d,e]; it may be observed that, in most cases, crude products were not fully analysed, isolated product yields often being minimal (7–20%). The easy acyl shift from the anomeric to the free HO-2 and HO-3 [6c,e] may have contributed in complicating product analysis, but an accurate re-examination of older work would certainly be desirable.

Acetylation of **BG** *and* **BGNa**.—Zervas [4] described the formation of the β-triacetate β-**4** in the acetylation of **BGNa** with acetic anhydride–sodium acetate in 73%

yield, α-**4** also being formed in an unspecified yield. He also reported that the reaction of **BG** with acetic anhydride–pyridine gave only a 30% yield of β-**4**, and that α-**4** was also present in substantial amounts, although isolated in an impure form. When we repeated the latter reaction on a sample of **BG** in which the α and β anomer were present in a 92:8 ratio, the triacetate **4** was formed in a nearly quantitative yield with an α/β ratio of 87:13. Pure α-**4** was obtained by recrystallization. Both anomers of **4** can thus be prepared by starting from either **BGNa**, or α-enriched **BG**. Whereas in the former reaction the β-selectivity should be determined by the low solubility of the β salt, in the latter reaction a much lower rate of the anomeric equilibration of **BG** with respect to the rate of acetylation is likely to cause the α/β ratio of the product to reflect closely the anomeric ratio of the starting **BG**.



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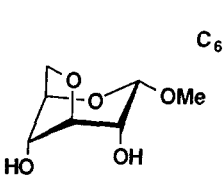
Attempts to prepare a disaccharide.—With the goal of achieving glycosylation reactions under minimal protection conditions, **BGNa** was treated with methyl 6-*O*-tosyl-α-D-glucopyranoside in DMF or Me_2SO. Careful analysis of the peracetylated product showed that the gentiobiose derivative **6** was formed only in traces (< 2%), recovered tosylate, **BG**, and the 3,6-anhydro derivative **5** [20] being the main components of the reaction mixture. Evidently, **BGNa** acts mainly as a base, favouring an intramolecular displacement of the tosyloxy group by the free HO-3 over an intermolecular glycosylation reaction. A reference sample of **6** was obtained by benzylidenation of methyl α-gentiobioside [19].



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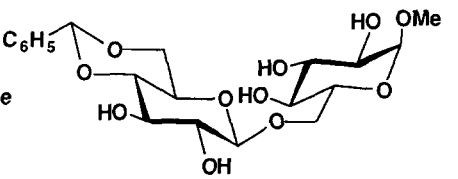

6

Also, when the 6-*O*-triflyl and 6-*O*-tosyl derivatives of methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside were used as glycosyl acceptors, the results were totally negative: no reaction of the 6-*O*-triflyl derivative with **BGNa** occurred in dioxane, CH_2Cl_2, or toluene, even in the presence of a crown ether, whereas in Me_2SO only detriflation took place. The tosyl derivative was unreactive under all of these conditions, even when the reaction was conducted in Me_2SO.

One can conclude that **BGNa** offers only very limited possibilities as a precursor for stereospecific synthesis of glucosides. Its reactions under heterogeneous conditions with simple strong electrophiles (alkyl triflates, aroyl chlorides, etc.) may be competitive with other methods for the preparation of β-glucosides, but an extension of its use for the synthesis of di- and oligo-saccharides appears to be hopeless. A rigorous re-investigation of acylations with non-aromatic acyl halides could, however, give some results of practical interest.

3. Experimental

General methods and products.—Melting points were determined with a Kofler apparatus and are uncorrected. Optical rotations were measured with a Perkin–Elmer 241 polarimeter at 20°C. NMR spectra were recorded with a Varian CFT-20 and with a Bruker 200 AC instrument in the stated solvent (Me_4Si was used as the internal standard, unless stated otherwise). Analytical TLC was carried out on silica gel plates (Merck, PSC Fertigplatten, Kieselgel 60 F_{254}) with detection by charring with methanolic 50% H_2SO_4 or ethanolic 10% phosphomolybdic acid. Kieselgel 60 (Merck, 70–230 and 230–400 mesh, respectively) was used without pre-treatment for column and flash chromatography. HPLC analyses were performed with a Pye Unicam 4895 liquid-chromatograph equipped with a LiChrosorb 10RP18 (250 × 2 mm) column; UV detector at 210 nm; eluent, 80:20 H_2O–MeCN; flow, 1.5 mL/min; relative retention times for **BG** (two peaks), β-**2**, and α-**2**: 1.00, 1.16, 2.27, 3.00. The previously described procedures were utilized for the preparation of methyl 6-*O*-tosyl-α-D-glucopyranoside [21] and its 2,3,4-triacetate [22], methyl 3,6-anhydro-α-D-glucopyranoside (**5**) and its 2,4-diacetate [23], and methyl α-gentiobioside [19]; in all cases good agreement was obtained between the physico-chemical properties of the samples prepared by us and the reported ones.

4,6-O-Benzylidene-D-glucopyranose (**BG**).—A mixture of pre-dried (3 h at 60°C and 0.1 Torr) D-glucose (10.00 g, 55.5 mol), benzaldehyde dimethyl acetal (9.3 g, 61.0 mol), and *p*-toluenesulfonic acid (12 mg) in dry DMF (40 mL) was heated at 60°C with vigorous stirring in a flask connected to a water pump that ensured an internal pressure of 250 Torr, N_2 being bubbled through the reaction mixture in order to ensure a rapid removal of MeOH. The glucose was completely dissolved after 30 min. The reaction was stopped by cooling and adding an excess of Et_3N (0.3 mL). Complete evaporation of the solvent under reduced pressure (0.1 Torr) left 19.8 g of a dark-yellow, very viscous syrup consisting (TLC, EtOAc) mainly of **BG** (R_f 0.34), with very small amounts of by-products with R_f 0.0 (glucose), 0.39 (α-**2**), and 0.54, 0.63, 0.71, and 0.82 (unidentified). The crude product was dissolved in dioxane (20 mL) and adsorbed on silica gel (20 g), the solvent was evaporated from the slurry under reduced pressure, and the dry solid obtained was placed on top of a short flash-chromatographic column of silica gel (4 × 30 cm) and eluted with EtOAc + 0.1% Et_3N (linear flow of 5 cm/min). The fractions containing only the compound with R_f 0.34 were collected and evaporated to give 10.7 g (72% yield) of pure **BG** as an amorphous anomeric mixture, $[\alpha]_D$

$+11.3° \rightarrow -3.5°$ (*c* 3.9, MeOH); lit. [4] $[\alpha]_D$ (equilibrium) $-4.0°$ (MeOH). NMR data for both α and β anomers of **BG** are collected in Tables 1 and 2.

The anomeric composition of different samples obtained as above, ascertained by NMR measurements in $(CD_3)_2SO$, showed a slight excess of the α anomer in the range 52–60%.

The solubilites of **BG** in various solvents were as follows: (mg/mL) 0.08 (CH_2Cl_2); 0.15 ($CHCl_3$); 8.0 (EtOAc); 16.8 (MeCN); > 100 (dioxane, DMF, Me_2SO). In hydroxylic solvents some decomposition with formation of benzaldehyde was observed on warming and standing; a rough estimation of the solubility (mg/mL) is ca. 0.5 (H_2O) and ca. 50 (MeOH).

Chromatographically pure **BG** was recrystallized from H_2O according to Fletcher [6b]: 1.0 g of **BG** was dissolved in H_2O (10 mL) containing concentrated NH_3 (2 drops) under stirring at 90°C for 3 min. The hot solution was rapidly filtered through a short layer of activated charcoal and immediately cooled at 0°C; the recovered solid {0.6 g, mp 178–179°C; $[\alpha]_D$ $+17.8° \rightarrow -5.0°$ (*c* 4.0, MeOH); lit. [12b] mp 186–187°C; $[\alpha]_D$ $+14° \rightarrow +4.4°$ (EtOH)} was found by ^1H NMR analysis in $(CD_3)_2SO$ to be a ca. 1:1 mixture of α- and β-**BG**. Crystallization from EtOAc gave a product, mp 165–167°C; $[\alpha]_D$ (equilibrium) $-3.4°$ (*c* 4, MeOH); as a 78:22 mixture of α- and β-**BG**.

Dissolving the above sample in MeCN at room temperature followed by cooling at +5°C gave colourless needles with mp 179–180°C; $[\alpha]_D$ $+49.0° \rightarrow -8.7°$ (*c* 3, Me_2SO); which ^1H NMR analysis showed to be pure α-**BG**. On the basis of initial and final specific rotation values in Me_2SO and of the anomeric equilibrium value obtained by ^1H NMR spectroscopy in $(CD_3)_2SO$, the specific rotation value of β-**BG** should be close to $[\alpha]_D$ $-42°$.

The mutarotation of **BG** was followed by polarimetry, starting from α-**BG**/β-**BG** mixtures. The experimental values were in all cases in accordance with the kinetic law (A).

$$k_1 + k_2 = 1/t \cdot \log(r_t - r_\infty)(r_0 - r_\infty) \quad \text{(A)}$$

The mutarotation parameters $(k_1 + k_2)$ and $[\alpha]_D$ (equilibrium) in various solvents at 25°C were as follows: Me_2SO (*c* 3.0), $2.57 \pm 0.18 \times 10^{-3}$, $-8.7°$; DMF (*c* 3.0), $3.69 \pm 0.03 \times 10^{-2}$, $+2.3°$; MeOH (*c* 3.9), $3.41 \pm 0.27 \times 10^{-2}$, $-4.4°$; 1:1 Me_2SO–H_2O (*c* 3.0), $1.33 \pm 1.33 \times 10^{-2}$, $-13.6°$; H_2O (*c* 0.5), $1.17 \pm 0.20 \times 10^{-1}$, $-18.4°$.

Salts of **BG**.—*Method A: according to Zervas* [4]. **BG** (528 mg, 1.97 mmol) was dissolved in 1 M NaOH (2 mL) and water (0.8 mL), and the solution and EtOH (25 mL), both cooled at 0°C, were mixed, with immediate precipitation of a microcrystalline solid, which was collected, washed with EtOH, then with Et_2O, and dried in vacuo, to give **BGNa** (466 mg, 81% yield). Slow addition of EtOH to the aqueous solution of **BGNa** gave a lower yield of larger crystals, $[\alpha]_D^{20}$ $-17.5°$ (*c* 1.0, H_2O, after 5 min), $-15.9°$ (10 min), $-13.5°$ (1 h), $-4.9°$ (48 h). These rotation changes are due to degradation of the product, rather than to mutarotation, as shown by NMR analysis. For the ^{13}C NMR spectrum, see Table 1.

Method B: according to Fletcher [6b]. A solution of **BG** (547 mg, 2.04 mmol) in absolute EtOH (10 mL), cooled at $-10°$C, was treated under Ar with 1 M methanolic NaOMe (2 mL). The precipitated powder was separated by centrifugation, washed with

absolute EtOH and twice with Et_2O, and dried in vacuo. The product (519 mg, 88% yield), when observed under the microscope, was mostly microcrystalline, with only a small amorphous part. The ^{13}C NMR spectrum and $[\alpha]_D^{20}$ were identical with those of the product obtained by method A.

BGNa is practically insoluble in all common solvents except water. Homogeneous solutions were, however, obtained when **BG** in THF or MeOH was treated with an equimolar amount of 1 M aqueous NaOH. Their ^{13}C NMR spectra were identical with those of solutions prepared starting from pre-formed **BGNa**.

Tetrabutylammonium salt (**1**).—Homogeneous solutions of this salt were obtained when 0.25 M solutions of **BG** in THF, MeOH, or Me_2SO were treated with 1.3 equivalents of commercial 0.8 M tetrabutylammonium hydroxide in MeOH. Their ^{13}C NMR spectra were identical in their glucidic part to those of **BGNa** in D_2O (Table 1).

Methylation of **BG** *salts*.—The salt, either pre-formed or prepared in situ, suspended or dissolved in the appropriate solvent, was treated with the methylating agent under Ar. When TLC showed completion of the reaction, the product methyl glycosides were extracted into CH_2Cl_2, the washed and dried extracts were evaporated, and the crude residues were directly analysed by HPLC under the conditions given in the 'General methods and products' section. The results are summarized in Table 5.

*1-O-Benzoyl-4,6-O-benzylidene-β-*D*-glucopyranose* (**β-3**).—Benzoyl chloride (80 μL, 0.69 mmol) was added to **BGNa** (200 mg, 0.69 mmol) suspended in CH_2Cl_2 (2.5 mL). After shaking for 15 h at room temperature the solvent was evaporated, the residue taken up in saturated aq $NaHCO_3$, left 4 h at 5°C, collected, and dried over P_4O_{10} to yield practically pure **β-3** (234 mg, 91% yield). No **α-3** or other by-products were detected by ^1H NMR spectroscopy. **β-3** had R_f 0.37 (1:1 hexane–EtOAc), mp 212–214°C; $[\alpha]_D^{20}$ −31.8° (*c* 0.9, Me_2CO). Lit. [6b]: mp 208–211°C, $[\alpha]_D^{20}$ −34°. ^1H NMR (Me_2SO): δ 3.46–3.78 (m, 5 H, H-2, H-3, H-4, H-5, H-6ax), 4.23 (dd, 1 H, $J_{6eq,6ax}$ 9.0, $J_{6eq,5}$ 4.0 Hz, H-6eq), 5.61 (s, 1 H, C*H*C_6H_5), 5.79 (d, 1 H, $J_{1,2}$ 7.84 Hz, H-1), 7.74–7.35 (m, 8 H), and 8.03 (m, 2 H) (aromatic); ^{13}C NMR: δ 66.44 (C-5), 67.47 (C-6), 72.49 (C-3), 73.26 (C-2), 80.07 (C-4), 94.98 (C-1), 100.51 (*C*HC_6H_5), 126.03, 127.66, 128.84, 128.46, 128.46, 129.25, 133.48, and 137.46 (aromatic), 164.25 (C=O).

*1,2,3-Tri-O-acetyl-4,6-O-benzylidene-α-*D*-glucopyranose* (**α-4**).—A sample of **BG** (134 mg, 0.50 mmol, α/β ratio 92:8) was acetylated with Ac_2O (2.0 mL, 21.2 mmol) in pyridine (4.0 mL) for 24 h at room temperature, to give, after evaporation of excess of reagent and solvent followed by repeated coevaporation with toluene, a quantitative yield of **4** (α/β ratio 87:13). Crystallization from EtOAc–hexane gave pure **α-4** (53% yield), mp 158–159°C; $[\alpha]_D^{20}$ +51.8° (*c* 0.9, $CHCl_3$). ^1H NMR (CD_3CN): δ 1.99, 2.02, and 2.15 (3 s, each 3 H, 3 × OAc), 3.75 (dd, 1 H, $J_{6ax,6eq}$ 9.95, $J_{6ax,5}$ 10.01 Hz, H-6ax), 3.85 (dd, 1 H, $J_{3,4}$ 9.84, $J_{4,5}$ 9.60 Hz, H-4), 4.04 (ddd, 1 H, $J_{5,6eq}$ 4.71 Hz, H-5), 4.24 (dd, 1 H, H-6eq), 5.06 (dd, 1 H, $J_{2,3}$ 9.98 Hz, H-2), 5.48 (dd, 1 H, H-3), 5.58 (s, 1 H, C*H*C_6H_5), 6.22 (d, 1 H, $J_{1,2}$ 3.86 Hz, H-1), 7.42–7.36 (m, 5 H, aromatic); ^{13}C NMR: δ 20.70, 20.90, and 21.03 (3 × OAc), 65.67 (C-5), 68.92 (C-6), 69.61 (C-3), 70.67 (C-2), 78.89 (C-4), 90.21 (C-1), 102.27 (*C*HC_6H_5), 127.13, 129.18, 130.06, and 138.29 (aromatic), 170.28, 170.86, and 170.92 (3 C=O). Anal. Calcd for $C_{19}H_{22}O_9$: C, 57.9; H, 5.6. Found: C, 58.1; H, 5.8.

Methyl 6-O-(4,6-O-benzylidene-β-D-glucopyranosyl)-α-D-glucopyranoside (**6**).—A solution of methyl α-gentiobioside [19] (164 mg, 0.46 mmol), α,α-dimethoxytoluene (74 mg, 0.49 mmol), and *p*-toluenesulfonic acid (5 mg) in DMF (3 mL) was stirred at 60°C for 90 min under a pressure of 250 Torr. Triethylamine (3 drops) was added and the solvent was evaporated at 1 Torr. Crystallization of the residue from MeOH gave **6** (152 mg, 74% yield), mp 243–246°C; $[\alpha]_D^{20}$ +24.8° (*c* 1.0, H_2O); R_f 0.28 (8:2 EtOAc–MeOH). ^{1}H NMR (80 MHz, Me_2SO–H_2O): δ 3.29 (s, 3 H, OMe), 4.41 (d, 1 H, $J_{1',2'}$ 7.5 Hz, H-1'), 4.54 (d, 1 H, $J_{1,2}$ 3.2 Hz, H-1), 5.56 (s, 1 H, PhC*H*). Anal. Calcd for $C_{20}H_{28}O_{11}$: C, 54.0; H, 6.3. Found: C, 53.7; H, 6.3.

Compound **6** was acetylated with Ac_2O in pyridine for 24 h at room temperature to give, after usual work-up and preparative TLC (6:4 hexane–EtOAc, R_f 0.23), the pentaacetate, mp 163–166°C; $[\alpha]_D^{20}$ +27.4° (*c* 1.0, $CHCl_3$). ^{1}H NMR (CD_3CN): δ 3.57 (m, 1 H, H-5'), 3.60 (m, 1 H, H-6a), 3.78 (m, 2 H, H-6'ax and H-4'), 3.87 (m, 1 H, H-6b), 3.88 (m, 1 H, H-5), 4.31 (dd, 1 H, $J_{6'ax,6'eq}$ 10.47, $J_{5,6'eq}$ 4.75 Hz, H-6'eq), 4.76 (dd, 1 H, $J_{2,3}$ 10.35, $J_{1,2}$ 3.52 Hz, H-2), 4.76 (d, 1 H, $J_{1',2'}$ 7.93 Hz, H-1'), 4.87 (d, 1 H, H-1), 4.91 (dd, 1 H, $J_{2',3'}$ 9.47 Hz, H-2'), 4.93 (dd, 1 H, $J_{4,5}$ 10.04, $J_{3,4}$ 9.36 Hz, H-4), 5.27 (dd, 1 H, $J_{3',4'}$ 9.47 Hz, H-3'), 5.33 (dd, 1 H, H-3), 5.54 (s, 1 H, C*H*C_6H_5), 7.41–7.34 (m, 5 H, aromatic); ^{13}C NMR: δ 20.85 (5 OAc), 67.03 (C-5'), 69.18 (C-5), 68.71 (C-6), 68.94 (C-6'), 69.18 (C-4), 70.73 (C-3), 71.31 (C-2), 72.38 (C-3'), 72.64 (C-2'), 79.05 (C-4'), 97.46 (C-1), 102.23 (C-1'), 102.08 (*C*HC_6H_5), 127.11, 129.14, 130.00, and 138.38 (aromatic), 170.48, 170.53, 170.85, 170.85, and 171.00 (5 C=O). Anal. Calcd for $C_{30}H_{38}O_{16}$: C, 55.0; H, 5.8. Found: C, 55.3; H, 6.0.

Reaction between **BGNa** *and methyl 6-O-tosyl-α-D-glucopyranoside.*—A suspension of **BGNa** (367 mg, 1.26 mmol) and methyl 6-*O*-tosyl-α-D-glucopyranoside (330 mg, 0.95 mmol) in DMF (3 mL) was shaken under Ar at room temperature for 24 h, to obtain an orange solution. TLC revealed the presence of only a trace amount of a product having the R_f of the desired disaccharide **6**. The solution was evaporated to dryness in vacuo, the residue acetylated with Ac_2O (2 mL) and pyridine (5 mL) for 2 h at room temperature, and the mixture diluted with CH_2Cl_2, washed with 2 M HCl, then with saturated aqueous NaCl, and dried. After evaporation, the brown residue was subjected to chromatography on a column of silica gel (6:4 EtOAc–hexane). The main products were the anomeric mixture of the triacetates **4**, methyl 2,3,4-tri-*O*-acetyl-6-*O*-tosyl-α-D-glucopyranoside [22], and the diacetate of **5** [23], identified by comparison with independently prepared samples. A very small amount of a product (16 mg, 2% yield) having R_f 0.23 (6:4 hexane–EtOAc) and spectral parameters corresponding to those of the pentaacetate of **6** was also isolated.

The same reaction, when conducted at 60°C, or with Me_2SO in place of DMF, gave similar disappointing results.

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