Carbohydrate Research, 142 (1985) 47–59
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

COMPARATIVE STABILITY OF 1-ALKOXYALKYL α-D-GLUCOSIDES IN THE PRESENCE OF ACID OR α-D-GLUCOSIDASE FROM YEAST*

SIEGFRIED JEGGE AND JOCHEN LEHMANN
Institut für Organische Chemie und Biochemie der Universität Freiburg i. Br., Albertstr. 21, D-7800 Freiburg i. Br. (West Germany)

(Received August 21st, 1984; accepted for publication, December 5th, 1984)

ABSTRACT

The aminated 1-alkoxyalkyl glycosides [(*S*)-2-amino-1-methoxyethyl] 6-amino-6-deoxy-α-D-glucopyranoside (**3**) and [(*R,S*)-1-ethoxyethyl] 6-amino-6-deoxy-α-D-glucopyranoside (**4**) have been synthesised and characterised. These compounds as well as [(*R*)-2-amino-1-methoxyethyl] α-D-glucopyranoside (**1**) prepared earlier are resistant against α-D-glucosidase (maltase, α-D-glucoside glucohydrolase, E.C. 3.2.1.20) from yeast, yet undergo hydrolysis under relatively mild acidic conditions. The kinetic parameters of the interaction with α-D-glucosidase and with acid were determined. The relative rates of acid hydrolysis of aminated 1-alkoxyalkyl glycosides compared with aminated ordinary glycosides suggest essential differences in the mechanism of acid-catalysed hydrolysis.

INTRODUCTION

Electronic effects are responsible for the relative stability of aminated or halogenated glycosides in aqueous mineral acid. A general, polar shielding-effect against protonation may be responsible in the case of methyl 6-amino-6-deoxy-D-glucopyranosides[1] and methyl 6-chloro-6-deoxy-D-glucopyranosides[1], and an additional, very strong inductive effect directed against the adjacent anomeric center stabilises methyl 2-amino-2-deoxy-α-D-glucopyranosides[2] and methyl 2-chloro-2-deoxy-D-glucopyranosides[3].

As glycosylases catalyse glycoside cleavage at least partially by protonation and formation of an oxonium ion, it must be assumed that the same polar factors influence rates of enzymic hydrolyses when polar groups are present in a glycoside. In fact 4-methylumbelliferyl 2-amino-2-deoxy-β-D-glucopyranoside is practically unhydrolysable by the β-D-glucosidase from *Aspergillus wentii*, although relative affinity to the glycon binding-site is unaffected[4]. Similarly, 2,4-dinitrophenyl 2-chloro-2-deoxy-β-D-galactopyranoside is a good competitive inhibitor of reactions catalysed by the β-D-galactosidase from *Escherichia coli*, but is itself not hydrolysed by the enzyme[5].

Paradoxically, glycosylamines (which undergo hydrolysis readily in aqueous

*Dedicated to Professor Kurt Wallenfels on the occasion of his 75th birthday.

0008-6215/85/$ 03.30

acid) are equally stable against enzyme-catalysed cleavage, the affinity to the active site of the enzyme being extremely high[6]. G. Legler *et al.*[7] assumed that, with some enzymes, a catalytically active protonating group becomes deprotonated by the strongly basic glycosylamines. The stability of the ion pair then formed at a site shielded from the aqueous environment may be responsible for tight binding as well as lack of reactivity. This effect apparently becomes larger the closer the acidic group at the active site of the enzyme can be approached by the basic group in the glycoside. Such powerful glycosylase inhibitors as the naturally occurring amines, acarbose, nojirimycin, deoxynojirimycin, and swainsonine[8] confirm the aforementioned assumptions. Recently, it was shown that even an amino group attached to the aglycon of an otherwise very sensitive 1-alkoxyalkyl α-D-glucoside renders the substrate resistant to α-glucosidase-catalysed hydrolysis[9]. Analoguous derivatives lacking amino groups[9,10] are hydrolysed by the enzyme.

In order to clarify the influence of polar groups on the interaction of 1-alkoxyalkyl glycosides with enzymes and on their stability in acid solution, and to compare the two different types of reactions, a set of 1-alkoxyalkyl glucosides was synthesised and investigated kinetically.

RESULTS AND DISCUSSION

Acid- and enzyme-catalysed hydrolyses of glycosides are different in some respects. Hydrolysis in aqueous acid is a hydronium ion-catalysed reaction, whose rate depends on the hydronium-ion and substrate concentration, that is on the concentration of the conjugate acid of the substrate. The mechanism whereby the glycoside is hydrolysed depends on which bond in the conjugate acid is broken first. In ordinary alkyl glycosides, it is accepted that the pyranosyl ring remains intact and the C-1–O-1 bond is broken (Fig. 1A)[11].

With 1-alkoxyalkyl glycosides (Fig. 1B), which have not yet been studied, it may be assumed that the pyranosyl ring also remains intact, but here it could be either the C-1–O-1 (Fig. 1, B_1), the C-1′–O-1 (Fig. 1, B_2), the C-1′–O-1′ (Fig. 1, B_3), or simultaneously the C-1–O-1 and C-1′–O-1′ bonds (Fig. 1, B_4) that are broken. In enzyme-catalysed hydrolyses, the protonation actually assists the bond breaking and is specifically directed towards the most basic group in the region around the glycosidic bond (which normally[12] is the oxygen atom O-1). The protonation is strictly stereospecific[13]. With ordinary alkyl glycosides, the mechanisms of acid- or enzyme-catalysed glycoside cleavage bear a certain resemblance, both involve the breaking of the C-1–O-1 bond. With at least some 1-alkoxyalkyl glycosides, however, acid- and enzyme-catalysed hydrolysis should proceed by demonstrably different mechanisms.

It may be assumed that enzyme catalysis, after all, involves breaking of the C-1–O-1 bond, whereas acid catalysis should favour breaking of the C-1′–O-1 or the C-1′–O-1′ bond (Fig. 1, B_2 or B_3), because this should give the most-stable cation if R′, like an alkyl group, does not exert any electronic influence (Fig. 1).

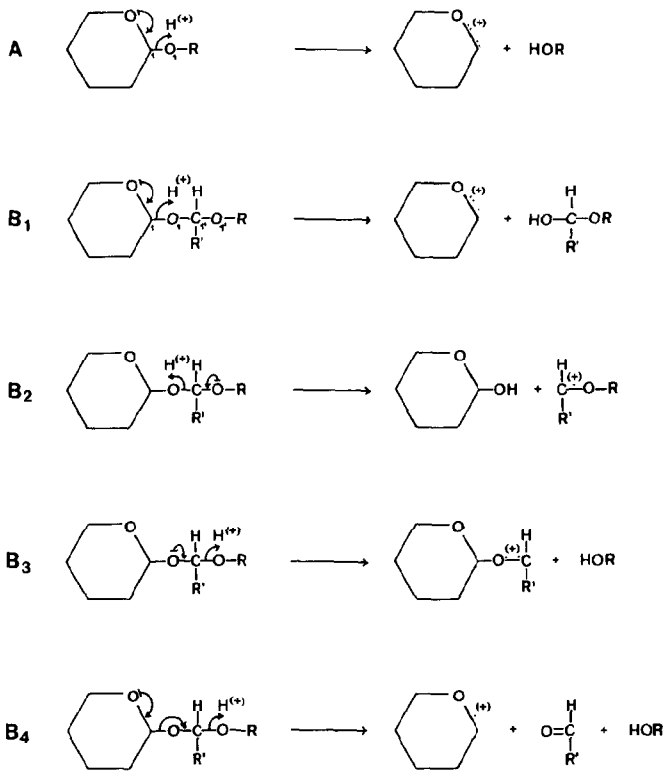
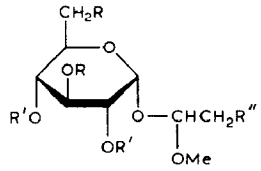


Fig. 1.

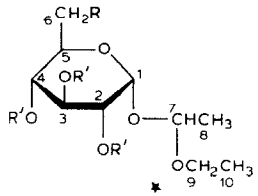
These assumptions were tested with the 1-alkoxyalkyl glycosides [(*R*)-2-amino-1-methoxyethyl] α-D-glucopyranoside[14] (**1**) [(*R*)-1-ethoxyethyl] α-D-glucopyranoside[10] (**2**), [(*S*)-2-amino-1-methoxyethyl] 6-amino-6-deoxy-α-D-glucopyranoside (**3**), [(*R,S*)-1-ethoxyethyl] 6-amino-6-deoxy-α-D-glucopyranoside (**4**), and [(*R*)-2-bromo-1-methoxyethyl] α-D-glucopyranoside[14] (**5**).

A. Hydrolysis of compounds **1–5** *in* M *HCl.* — All of the compounds, when treated with M HCl at different temperatures, are hydrolysed, as expected, according to a first-order rate law (Table I). The neutral 1-alkoxyalkyl glycoside **2** resembles an alkyl 2-deoxy-α-D-glucopyranoside in steric as well as in polar properties, and it is to be expected that **2** should be at least as labile in acid solution as methyl 2-deoxy-α-D-glucopyranoside[15] (see Table I).

In fact, as comparison of the relative rates show, compound **2** is hydrolysed ~40 times faster than methyl 2-deoxy-α-D-glucopyranoside. Introduction of an amino group at C-6 decreases the relative rate of hydrolysis in ordinary methyl glycosides by a factor of ~10. In the corresponding 1-alkoxyalkyl derivative **4**, this general shielding effect is less pronounced as compared with the standard **2**. If the cleavage followed mechanism B_2 or B_3, compounds **1** and **3** ought to show both general shielding effects as well as strong inductive effects, comparable to those

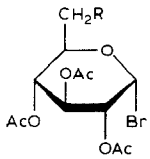


Compound	R	R′	R″
1	OH	H	NH_2
3	NH_2	H	NH_2
5	OH	H	Br
6	$OSiMe_3$	$SiMe_3$	Br
7	N_3	Ac	N_3
12	OH	$SiMe_3$	Br
13	OSO_2Me	$SiMe_3$	Br
14	OSO_2Me	H	Br
15	OSO_2Me	Ac	Br
16	N_3	H	N_3

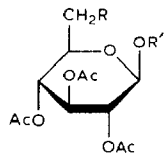


Compound	R	R′
2	OH	H
4	NH_2	H
10	I	Ac
17	N_3	Ac
19	N_3	H

* numbers relevant for ^{1}H-n m r data



8 R = I



Compound	R	R′
9	I	H
11	I	—CHMe | OEt
18	N_3	—CHMe | OEt

responsible for the extreme stability of methyl 2-amino-2-deoxy-α-D-gluco-pyranoside in acid solution (Table I, compared with methyl 2-deoxy-α-D-*arabino*-hexopyranoside). No such extreme stabilisation is, however, observed (Table I, compare compounds **1** and **2**).

These results indicate that, in the 1-alkoxyalkyl glycosides **1, 3, 4,** and **5**, containing polar substituents, a general shielding effect against protonation indeed exists, which is more pronounced when the polar grouping is attached to the aglycon moiety as in compounds **1, 3,** and **5**. However, an inductive effect, destabilising a potential carboxonium-ion intermediate (Fig. 1, B_2 or B_3), must be ruled out. The experimental results gained with the 1-alkoxyalkyl glycosides **1, 3,** and **5** agree best with a mechanism whereby protonation of O-1′ leads to the formation of free aldehyde, the alcohol R-OH, and a pyranosyl cation (Fig. 1, B_4). The generally increased acid lability also of the 1-alkoxyalkyl glycosides **2** and **4**, as compared with ordinary glycosides, may equally be explained by this essentially different mechanism of acid-catalysed hydrolysis.

TABLE I

RATES FOR THE ACID HYDROLYSIS OF SUBSTITUTED METHYL D-GLUCOPYRANOSIDES AND 1-ALKOXYALKYL α-D-GLUCOPYRANOSIDES **1–5**

Glucoside	*Relative rates* k_{rel}	*First-order rate constants (M HCl)* $10^5 \cdot k\ (sec^{-1})$	*Lit.*
Methyl α-D-glucopyranoside	1.0		1
Methyl 6-deoxy-α-D-glucopyranoside	5.05		1
Methyl 6-amino-6-deoxy-α-D-glucopyranoside	0.102		1
Methyl 6-chloro-6-deoxy-α-D-glucopyranoside	0.144		1
Methyl 2-deoxy-α-D-*arabino*-hexopyranoside	2090		15
Methyl 2-amino-2-deoxy-α-D-glucopyranoside	0.011		2[a]
Methyl 2-chloro-2-deoxy-β-D-glucopyranoside	0.080[b]		15
1	225	5.63 (55°)	—
2	80200	96.27 (−10°)	—
3	42	1.04 (55°)	—
4	18130	88.87 (−5°)	—
5	2176	54.40 (55°)	—

[a]The values given in ref. 16 are based on false citation. [b]The corresponding α anomer could not be found in the literature. Its expected relative rate of hydrolysis ought, however, to be comparable to that for the β anomer. Compare also the relative rates for methyl α- and β-D-glucopyranoside.

TABLE II

KINETIC PARAMETERS FOR INTERACTIONS OF **1–5** WITH YEAST α-GLUCOSIDASE

Compound	K_M[mM]	K_i[mM]	*Lit.*
1		3.62	9
		3.95	9
2	22.9		10
3		6.7	
4		37	
5	2.35		9
	2.44		9

B. Interactions of compounds **1–5** *with the α-D-glucosidase from yeast.* — Consistent with the proposal by Legler[7] that basic glycosides tend to deprotonate the catalytically active proton-donating group in the active site of glycosylases, thereby forming stable ion-pairs, all of the aminated mixed-acetal glucosides in buffer solution at pH 6.8, were inert in the presence of α-D-glucosidase, even with enzyme concentrations exceeding those in an ordinary test by a factor of 1500. The neutral glycosides **2** and **5** were rapidly hydrolysed (Table II) under conditions the same as those described by Dettinger *et al.*[10] and Blanc-Muesser *et al.*[9]. The fact that a polar group, such as bromine in compound **5**, does not lower the rate of enzymic hydrolysis of that compound proves that only the formation of stable ionic species can account for the observed exceptional resistance of the aminated glucosides to hydrolysis catalysed by a yeast α-D-glucosidase. The K_M value for the

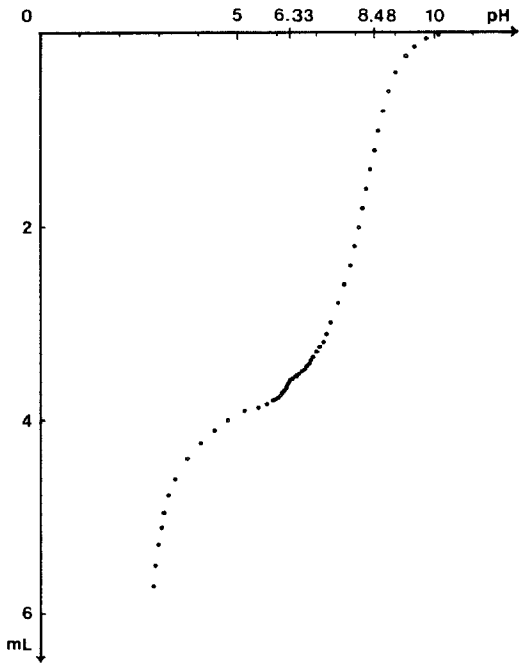


Fig. 2. Titration of [(*S*)-2-amino-1-methoxyethyl] 6-amino-6-deoxy-α-D-glucopyranoside (**3**) with 10mM HCl. The pK values were found to be 8.48 and 6.33.

glycosides **2** and **5** and the K_i values of the aminated compounds **1, 3,** and **4** are comparable. It is interesting that the K_i of the diamine **3** (see Fig. 3) is almost equal to that of monoamine **1** but only 20% of the K_i of monoamine **4** (see Fig. 4). If, according to Legler[7] the glyconic moieties may only enter the active site in unprotonated form, then diamine **3** must have a greater chance, in fact almost equal to that of monoamine **1**, to enter the active site than monoamine **4**, as at pH 6.8 it is less than 75% protonated (see titration curve, Fig. 2), whereas the monoamine **4** is almost completely protonated.

Work is in progress to investigate the general applicability of aminated 1-alkoxyalkyl glycosides of other monosaccharides and of oligosaccharides as hydrolysis-resistant, competitive inhibitors for saccharide-degrading enzymes. The preparation of such compounds may be performed by simple procedures.

C. Chemical syntheses of compounds **3** *and* **4**. — Syntheses of **3** and **4**, prepared for the first time, are described in the Experimental section.

EXPERIMENTAL

Methods. — All reactions were monitored by t.l.c. on silica gel 60 F_{254} (Merck), using the solvents indicated. G.l.c. was performed with a Pye-Unicam GCD chromatograph, with glass columns and SE-52 (3%) on Chromosorb G, AW-DMCS. Flash chromatography[17] was performed on silica gel (230–400 mesh,

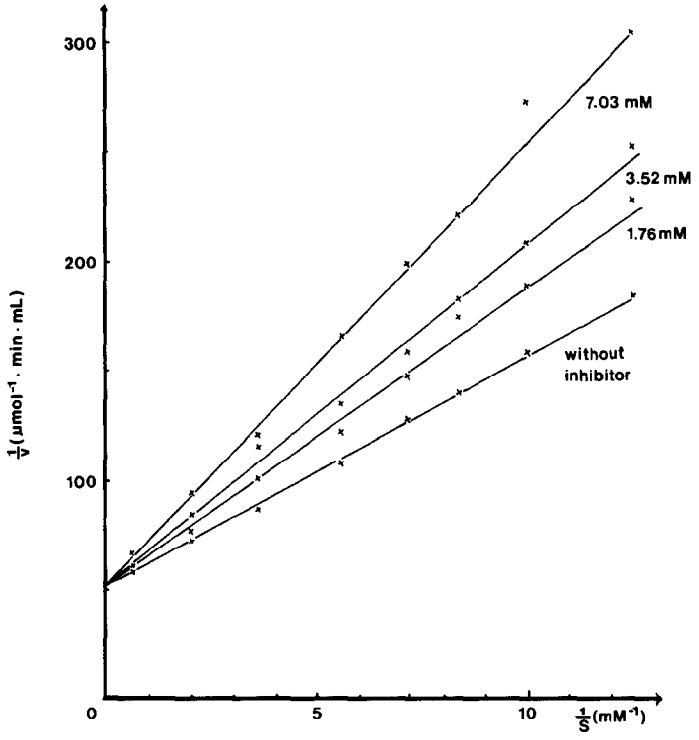


Fig. 3. Determination of the inhibition constant (K_i) for [(*S*)-2-amino-1-methoxyethyl] 6-amino-6-deoxy-α-D-glucopyranoside (**3**) in sodium phosphate buffer (0.05M, pH 6.8, containing calcium chloride (mM) at 30°. The concentrations of substrate (*p*-nitrophenyl α-D-glucopyranoside) and inhibitor were varied as indicated.

Merck). Optical rotations were measured with a Perkin–Elmer 141 polarimeter. ^{1}H-N.m.r. spectra were recorded with a Bruker WM 250 (250 MHz) spectrometer for solutions in $CDCl_3$ (internal Me_4Si) or D_2O (internal 2,2,3,3-tetradeuterio-4,4-dimethyl-4-silapentanoate). Light petroleum refers to the fraction b.p. 60–70°.

Enzymic reactions. — α-D-Glucosidase (maltase, α-D-glucoside glucohydrolase, EC 3.2.1.20) from yeast was purchased from Boehringer, Mannheim. The inhibition constants were determined spectrometrically at 405 nm using *p*-nitrophenyl α-D-glucopyranoside. Tests were performed in sodium phosphate buffer (0.05M, pH 6.8), containing calcium chloride (mM), with various concentrations of substrate (0.08–1.59mM). The solutions for all tests also contained inhibitor (0–19.2 and 0–7.03mM). Lineweaver–Burk plots of the results are shown in Figs. 3 and 4. Calculation of the competitive inhibition was based on the extinction coefficient of 9.6 $cm^2/\mu mol$.

Acid hydrolysis. — The compound to be hydrolysed (2 mmol) was dissolved in M hydrochloric acid (100 μL) at various temperatures as indicated in Table I. Aliquots (10 μL) were taken at intervals and treated with pyridine (150 μL) and acetic anhydride (100 μL). After 6 h at room temperature, the acetylation was

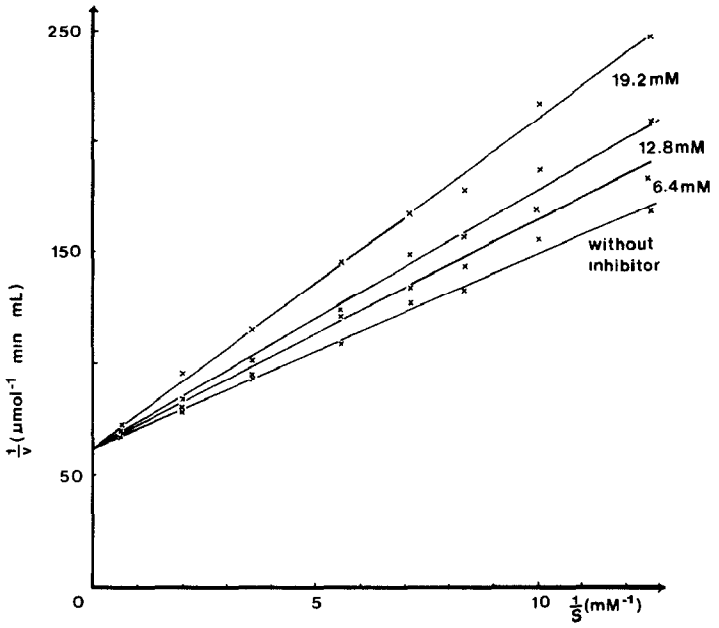


Fig. 4. Determination of the inhibition constant (K_i) for [(*R,S*)-1-ethoxyethyl] 6-amino-6-deoxy-α-D-glucopyranoside (**4**) in sodium phosphate buffer (0.05M, pH 6.8, containing calcium chloride (mM) at 30°. The concentrations of substrate (*p*-nitrophenyl α-D-glucopyranoside) and inhibitor were varied as indicated.

complete and the excess of reagent was decomposed with water (50 μL). The solution was extracted with dichloromethane (20 μL) and the organic layer analysed by g.l.c.

Synthesis of [(S)-*2-amino-1-methoxyethyl*]*6-amino-6-deoxy-α-D-glucopyranoside* (**3**). — [(S)-*2-Bromo-1-methoxyethyl*]*2,3,4,6-tetra*-O-*trimethylsilyl-α-D-glucopyranoside* (**6**). Compound **5** (ref. 14) (6.15 g, 19.39 mmol) in pyridine (90 mL) was treated with hexamethyldisilazane (7.81 g) and chlorotrimethylsilane (4.77 g) for 2 h at 0°. The mixture was evaporated under diminished pressure. The semisolid residue was extracted with dichloromethane (3 × 200 mL), and the combined extracts were washed with ice–water (200 mL), dried ($MgSO_4$), and evaporated to yield **6** as a colourless syrup (11.27 g, 96%); R_F 0.77 (1:2 ethyl acetate–light petroleum); $[\alpha]_{589}^{25}$ +189° (*c* 1.9, chloroform); ^{1}H-n.m.r. data: see Tables III and IV.

[(S)-*2-Bromo-1-methoxyethyl*] *2,3,4-tri*-O-*acetyl-6*-O-*mesyl-α-D-glucopyranoside* (**15**). — Compound **6** (6.68 g, 11.02 mmol) in pyridine (60 mL) was treated with water (1 mL) and acetic acid (0.7 mL) for 148 h. The reaction was monitored by g.l.c. Dichloromethane (150 mL) was added, the mixture was washed with ice–water (3 × 150 mL), and the extract was dried ($MgSO_4$) and evaporated *in vacuo*. To the colourless, syrupy [(*S*)-2-bromo-1-methoxyethyl] 2,3,4-tri-*O*-trimethylsilyl-α-D-glucopyranoside (**12**) (5.0 g, 9.37 mmol, R_F 0.40 in 1:1 ether–light petroleum)

TABLE III

^{1}H-N M R CHEMICAL SHIFT DATA (250 MHz) FOR COMPOUNDS **3, 4, 6, 7, 8, 9** AND **15–19** ($CDCl_3$, INTERNAL Me_4Si) OR (D_2O, INTERNAL DSS)

Proton	*Compound*										
	3	**4**	**6**	**7**	**8**	**9**	**15**	**16**	**17**	**18**	**19**
H-1	5 03 d	5.14 d 5 19 d	4.89 d	5.40 d	6 61 d	4.79 d	5 38 d	5 08 d	4.87 d 4 91 d	4.79 d 4 84 d	5.13 d 5 19 d
H-2	3.42 dd	3 22–3.96 m	3.53 dd	4 93 dd	4.83 dd	4.90 dd	4.92 dd	3.65 dd	5 38 dd	5.04 dd	3 42–3 96 m
H-3	3.51 dd	3.22–3 96 m	3 30–3 78 m	5 51 dd	5 56 dd	5.26 dd	5.52 dd	3.18–3.90 m	5 51 dd	5 21 d 5 22 d	3 42–3.96 m
H-4	3.13 dd	3.22–3.96 m	3.30–3 78 m	5.03 dd	5 04 dd	4.94 dd	5.07 dd	3 18–3.90 m	4.98 dd	4.97 dd	3 42–3 96 m
H-5	3 10 ddd	2 83 ddd	3.30–3.78 m	4 09 ddd	4.02 ddd	3.51 ddd	4.38 ddd	3.18–3 90 m	4 05 ddd 4 15 ddd	3 18–3 83 m	3 42–3.96 m
H-6	2.83 dd	3 35 dd	3 30–3.78 m	3 34 m	3 36 dd	3 33 dd	4.29 m	3.18–3.90 m	3 32 dd	3 18–3 83 m	3.42–3 96 m
H-6′	2 69 dd	3.07 dd	3.30–3 78 m	3.34 m	3.22 dd	3.19 dd	4 29 m	3.18–3.90 m	3.29 dd	3 18–3.83 m	3 42–3 96 m
H-7	4 58 t	5 02 q 5 06 q	4 72 dd	4 78 t	—	—	4.87 t	4.74 dd	4.88 t 4.94 t	4 88 q 5 02 q	4 90 q 4 93 q
H-8	2.64 d	1 38 d 1 39 d	3.30–3 78 m	3.39 d	—	—	3 44 d	3 30 d	1 38 d 1 41 d	1 36 d 1 39 d	1.40 d 1 41 d
H-8′	2.64 d	—	3 30–3.78 m	3.43 d	—	—	3.48 d	3.31 d	—	—	—
H-9	3.28 s	3 22–3 96 m	3 45 s	3 44 s	—	—	3.39 s	3 51 s	3 47 dq 3 51 dq	3 18–3 83 m	3 42–3.96 m
H-9′	—	3 22–3 96 m	—	—	—	—	—	—	3 65 dq 3 80 dq	3 18–3 83 m	3 42–3.96 m
H-10	—	1 21 t 1.22 t	—	—	—	—	—	—	1.19 dd 1 20 dd	1.22 t, 1 23 t	1 22 t 1.23 t
-OAc	—	—	—	2.03 s; 2 05 s	2 04 s, 2 08 s	2 03 s, 2 07 s	2 03 s; 2 06	—	2 01 s, 2 04s	2 01 s; 2 03 s	—
				2.08 s	2.10 s	2 10 s	2 06 s	—	2 06 s	2 04 s	—
$-SO_2CH_3$	—	—	—	—	—	—	3.05 s	—	—	—	—
$-Si(CH_3)_3$	—	—	0 04–0 19 m	—	—	—	—	—	—	—	—

TABLE IV

^{1}H-N.M R SPIN-COUPLING DATA FOR COMPOUNDS **3, 4, 6, 7, 8, 9,** AND **15–19**[a]

$J_{H,H}$ (Hz)	Compound										
	3	**4**	**6**	**7**	**8**	**9**	**15**	**16**	**17**	**18**	**19**
1,2	3.8	3.9	3.6	3.9	4.2	8.3	3.8	4.1	3.8	7.8	3.8
2,3	10.5	—	11.1	10.5	10.2	9 6	10.4	9.6	8.3	9.6	—
3,4	9.7	—	—	9.8	9.3	9.3	9.9	—	10.2	9.8	—
4,5	9.9	13.5	—	10.6	10.4	9.8	10.6	—	9.8	9.6	—
5,6	3.3	3.5	—	4.5	3.0	3.0	3.4	—	3.2	—	—
5,6′	7.2	7.2	—	4.5	5.7	6.8	6.9	—	6.0	—	—
6,6′	13.4	14.3	—	—	11.7	11.1	—	—	13.5	—	—
7,8	5.4	5.1	3.7	5.3	—	—	5.4	3.9	5.3	5.1	5.4
		5.1							5.3	5 1	5.4
7,8′	5.4	—	7.2	5.3	—	—	5.4	5.4	—	—	—
9,10	—	7.2	—	—	—	—	—	—	6.8	7.4	7.2
		7.2							6.8	7.4	7.2
9′,10	—	7.2	—	—	—	—	—	—	6.8	7 4	7.2
		7.2							6.8	7 4	7.2
9,9′	—	—	—	—	—	—	—	—	9.3	—	—

[a]Conditions described in Table III.

in pyridine (40 mL), methanesulfonyl chloride (1.55 mL) was added at 0°. After 8 h the mixture was poured into ice–water (100 mL) and extracted with dichloromethane (3 × 100 mL). The extract was dried ($MgSO_4$) and concentrated *in vacuo* to dryness. To the residue, [(*S*)-2-bromo-1-methoxyethyl] 6-*O*-mesyl-2,3,4-tri-*O*-trimethylsilyl-α-D-glucopyranoside (**13**) (4.76 g, 7.78 mmol, R_F 0.33, 1:1 ether–light petroleum), in methanol (50 mL) water (5 mL) was added and the solution boiled for 3 h under reflux. The mixture was concentrated to dryness *in vacuo*. The product [(*S*)-2-bromo-1-methyoxyethyl] 6-*O*-mesyl-α-D-glucopyranoside (**14**) (R_F 0.42, 7:2:1 ethyl acetate–methanol–water) was treated with pyridine (12 mL) and acetic anhydride (9 mL) for 6 h. The mixture was then processed conventionally to yield **15** (3.77 g, 7.23 mmol), m.p. 128° (ethyl acetate–light petroleum); R_F 0.20 (1:1 ethyl acetate–light petroleum); $[\alpha]_{589}^{25}$ +114° (*c* 1.2, chloroform); ν_{max}^{KBr} 1190, 1380 cm^{-1} (-O-SO_2); ^{1}H-n.m.r. data: see Tables III and IV.

Anal. Calc. for $C_{16}H_{25}BrO_{12}S$: C, 36.86; H, 4.83. Found: C, 36.74; H, 4.92.

[(S)-*2-Azido-1-methoxyethyl*]*2,3,4-tri*-O-*acetyl-6-azido-6-deoxy*-α-D-*glucopyranoside* (**7**). Compound **15** (3.2 g, 6.13 mmol) was kept for 20 h at 80° with dried sodium azide (2.10 g) in dry dimethyl sulfoxide (52 mL). The mixture was cooled, acetone was added (500 mL) with stirring, the inorganic precipitate filtered off, and the filtrate concentrated *in vacuo*. The residue was taken up in water (300 mL), the suspension was extracted with ether (3 × 300 mL), and the combined extracts were washed with water (300 mL), dried ($MgSO_4$) and evaporated to yield **7**. Recrystallisation from ethanol gave 2.06 g, 78%; m.p. 76–77°, R_F 0.38 (1:1 ethyl acetate–light petroleum); $[\alpha]_{589}^{25}$ +123° (*c* 1.5, chloroform); ν_{max}^{KBr} 2060 cm^{-1} (N_3); ^{1}H-n.m.r. data: see Tables III and IV.

Anal. Calc. for $C_{15}H_{22}N_6O_9$: C, 41.86; H, 5.15; N, 19.53. Found: C, 41.92; H, 5.12; N, 20.16.

[(S)-*2-Azido-1-methoxyethyl*]*6-azido-6-deoxy-α*-D-*glucopyranoside* (**16**). Compound **7** (1.9 g, 4.41 mmol) was deacetylated (Zemplén) to give **16** (1.30 g, 97%); R_F 0.63 (7:2:1 ethyl acetate–methanol–water); m.p. 67–68° (ethanol–light petroleum); $[\alpha]_{589}^{25}$ +69° (*c* 1.5, chloroform); ν_{max}^{KBr} 2095 cm^{-1} (N_3); ^{1}H-n.m.r. data: see Tables III and IV.

Anal. Calc. for $C_9H_{16}N_6O_6$: C, 35.53; H, 5.30; N, 27.62. Found: C, 35.46; H, 5.37; N, 27.93.

[(S)-*2-Amino-1-methoxyethyl*]*6-amino-6-deoxy-α*-D-*glucopyranoside* (**3**). Compound **16** (1.2 g, 3.94 mmol) in ethanol (50 mL) was hydrogenated in the presence of Adams' catalyst (35 mg PtO_2). The reaction was complete after 3 h. After filtration, the strongly basic filtrate was evaporated *in vacuo* to yield amorphous **3** (975 mg, 98%); R_F 0.41 (7:3:3:2:3:2 1-propanol–ethanol–ethyl acetate–pyridine–water–acetic acid); $[\alpha]_{589}^{25}$ +106° (*c* 0.95, water); ν_{max}^{film} 1620 cm^{-1} (-NH_2); ^{1}H-n.m.r. data: see Tables III and IV.

Anal. Calc. for $C_9H_{20}N_2O_6$: C, 42.85; H, 7.99; N, 11.10. Found: C, 42.73; H, 8.09; N, 11.43.

Synthesis of [(R,S)-*1-ethoxyethyl*] *6-amino-6-deoxy-α*-D-*glucopyranoside* (**4**). — *2,3,4-Tri*-O-*acetyl-6-deoxy-6-iodo-β*-D-*glucopyranosyl bromide* (**8**). A solution of 1,2,3,4-tetra-*O*-acetyl-6-deoxy-6-iodo-β-D-glucopyranoside[18] (21 g, 45.83 mmol) in dichloromethane (50 mL) was treated at 0° with hydrogen bromide in glacial acetic acid (33%, 85 mL), stirred for 10 h, and then poured into ice–water (250 mL) while stirring. The mixture was extracted with dichloromethane (3 × 250 mL). The combined extracts were washed with saturated aqueous hydrogencarbonate (250 mL) and water (250 mL), dried ($MgSO_4$), and evaporated *in vacuo* to yield crystalline **8** (20.5 g, 93.4%); m.p. 178° (dec.), $[\alpha]_{589}^{25}$ +157° (*c* 1.3, chloroform); R_F 0.52 (1:1 ethyl acetate–light petroleum); ^{1}H-n.m.r. data: see Tables III and IV.

Anal. Calc. for $C_{12}H_{16}BrIO_7$: C, 30.09; H, 3.37. Found: C, 29.98; H, 3.29.

2,3,4-Tri-O-*acetyl-6-deoxy-6-iodo-β*-D-*glucopyranose* (**9**). Compound **8** (20.0 g, 41.75 mmol) was added to a suspension of Ag_2CO_3 (15 g) in acetone (250 mL) and water (10 mL). The mixture was stirred for 15 min in darkness, the inorganic precipitate was filtered off, and the filtrate was evaporated under diminished pressure. The residue was recrystallised from ethanol to yield **9** (16.8 g, 96.7%); m.p. 154–155°, $[\alpha]_{589}^{25}$ +31.5° (*c* 0.4, chloroform); R_F 0.35 (1:1 ethyl acetate–light petroleum); ^{1}H-n.m.r. data: see Tables III and IV.

Anal. Calc. for $C_{12}H_{17}IO_8$: C, 34.63; H, 4.12. Found: C, 34.75; H, 4.19.

[(R,S)-*1-Ethoxyethyl*] *2,3,4-tri*-O-*acetyl-6-deoxy-6-iodo-α*-D-*glucopyranoside* (**10**) *and* [(R,S)-*1-ethoxyethyl*] *2,3,4-tri*-O-*acetyl-6-deoxy-6-iodo-β*-D-*glucopyranoside* (**11**). A mixture of **9** (16.6 g, 39.89 mmol) and acetaldehyde diethyl acetal (100 mL) containing acetic acid (0.1 mL) was boiled under reflux for 20 h. Approximately 60 mL of the solvent was slowly distilled off together with the liberated ethanol, and was continuously replaced by fresh acetaldehyde diethyl acetal during a period of

12 h. The mixture was evaporated *in vacuo*. Flash chromatography (1:3 ethyl acetate–light petroleum) yielded a colourless syrup (13.8 g, 71%) R_F 0.57 (1:1 ethyl acetate–light petroleum). As determined by ^{1}H-n.m.r. the syrup consisted of four stereoisomers, which were subsequently submitted to the azide-exchange reaction.

[(R,S)-*1-Ethoxyethyl*] *2,3,4-tri*-O-*acetyl-6-azido-6-deoxy-α*-D-*glucopyranoside* (**17**) *and* [(R,S)-*1-ethoxyethyl*] *2,3,4-tri*-O-*acetyl-6-azido-6-deoxy-β*-D-*glucopyranoside* (**18**). The mixture of **10** and **11** (13.8 g, 28.26 mmol) in dry dimethyl sulfoxide (200 mL) was heated for 8 h with dried sodium azide (14.2 g) at 70°. The mixture was processed as described for **7**. The resulting, colourless syrup consisted of two products which were separated by flash chromatography (1:4 ethyl acetate–light petroleum) to yield crystalline **17** (5.46 g, 57%); m.p. 102° (ethanol); $[\alpha]_{589}^{25}$ +130° (*c* 1.4, chloroform); R_F 0.58 (1: ethyl acetate–light petroleum); ν_{max}^{KBr} 2100 cm^{-1} (N_3); ^{1}H-n.m.r. data: see Tables III and IV.

Anal. Calc. for $C_{16}H_{25}N_3O_9$: C, 47.64; H, 6.25; N, 10.42. Found: C, 47.53; H, 6.36; N, 10.56.

The isomer **18** (3.65 g, 38%) had m.p. 99° (ethanol); R_F 0.53 (1:1 ethyl acetate–light petroleum); $[\alpha]_{589}^{25}$ −58° (*c* 1.1, chloroform); ν_{max}^{KBr} 2140 cm^{-1} (N_3); ^{1}H-n.m.r. data: see Tables III and IV.

Anal. Calc. for $C_{16}H_{25}N_3O_9$: C, 47.64; H, 6.25; N, 10.42. Found: C , 47.56; H, 6.18; N, 10.64.

[(R,S)-*1-Ethoxyethyl*] *6-azido-6-deoxy-α*-D-*glucopyranoside* (**19**). Zemplén deacetylation of **18** (5.3 g, 13.14 mmol) gave **19** (3.39 g, 93%); m.p. 72° (ether–light petroleum); R_F 0.67 (7:2:1 ethyl acetate–methanol–water); $[\alpha]_{589}^{25}$ +107° (*c* 1.5, chloroform); ν_{max}^{KBr} 2110 cm^{-1} (N_3); ^{1}H-n.m.r. data: see Tables III and IV.

Anal. Calc. for $C_{10}H_{19}N_3O_6$: C, 43.32; H, 6.91; N, 15.15. Found: C, 43.17; H, 7.04; N, 15.49.

[(R,S)-*1-Ethoxyethyl*] *6-amino-6-deoxy-α*-D-*glucopyranoside* (**4**). Compound **19** (2.1 g, 7.57 mmol) in ethanol (200 mL) was hydrogenated in the presence of Adams' catalyst (70 mg PtO_2). After 1 h the mixture was filtered and the filtrate concentrated *in vacuo*. The product was purified by flash chromatography (17:2:1 ethyl acetate–methanol–water) and by elution from Sephadex LH20 (1:3 methanol–water) to yield amorphous **4** (1.82 g, 96%). According to g.l.c. of the *O*-trimethylsilylated derivative, **4** was homogenous. R_F 0.59 (7:3:3:2:3:2 1-propanol–ethanol–ethyl acetate–pyridine–water–acetic acid); $[\alpha]_{589}^{25}$ +110° (*c* 0.5, water); ν_{max}^{film} 1585 cm^{-1} (-NH_2); ^{1}H-n.m.r. data: see Tables III and IV.

Anal. Calc. for $C_{10}H_{21}NO_6$: C, 47.80; H, 8.42; N, 5.57. Found: C, 47.62; H, 8.29; N, 5.83.

ACKNOWLEDGMENT

We thank the Deutsche Forschungsgemeinschaft for financial support.

REFERENCES

1 T. E. TIMELL, W. ENTERMANN, F. SPENCER, AND E. J. SOLTES, *Can. J. Chem.*, 43 (1965) 2296–2305; T. E. TIMELL, *Chem. Ind. (London)*, (1964) 503–504.
2 R. C. G. MOGGRIDGE AND A. NEUBERGER, *J. Chem. Soc.*, (1938) 745–750; K. ONODERA AND T. KOMANO, *Agr. Biol. Chem. (Tokyo)*, 25 (1961) 932–936.
3 T. ISHII, A. ISHIZU AND J. NAKANO, *Carbohydr. Res.*, 48 (1976) 33–40.
4 K. R. ROESER AND G. LEGLER, *Biochim. Biophys. Acta*, 657 (1981) 321–333.
5 W. HENGSTENBERG AND K. WALLENFELS, *Carbohydr. Res.*, 11 (1969) 85–91.
6 H.-Y. L. LAI AND B. AXELROD, *Biochem. Biophys. Res. Commun.*, 54 (1973) 463–468.
7 P. LALÉGERIE, G. LEGLER, AND J. M. YON, *Biochimie*, 64 (1982) 977–1000; G. LEGLER AND M. HERRCHEN, *Carbohydr. Res.*, 116 (1983) 95–103.
8 E. T. REESE, F. W. PARRISH, AND M. ETTLINGER, *Carbohydr. Res.*, 18 (1971) 381–388; G. SEMENZA AND A.-K. VON BALTHAZAR, *Eur. J. Biochem.*, 41 (1974) 149–162; A. COGOLI AND G. SEMENZA, *J. Biol. Chem.*, 250 (1975) 7802–7809; D. D. SCHMIDT, W. FROMMER, B. JUNGE, L. MULLER, W. WINGENDER, E. TRUSCHEIT, AND D. SCHAFER, *Naturwissenschaften*, 64 (1977) 535–536; W. F. CASPARY AND S. GRAF, *Res. Exp. Med.*, 175 (1979)) 1–6; S. MURAO AND K. OHYAMA, *Agr. Biol. Chem.*, 43 (1979) 679–681; P. R. DORLING, C. R. HUXTABLE, AND S. M. COLGATE, *Biochem. J.*, 191 (1980) 649–651; G. HANOZET, H.-P. PICHLER, P. VANNI, B. OESCH, AND G. SEMENZA, *J. Biol. Chem.*, 256 (1981) 3703–3711.
9 M. BLANC-MUESSER, J. DEFAYE, S. HUTER-PAULUS, AND J. LEHMANN, *Liebigs Ann. Chem.*, (1983) 147–149.
10 H.-M. DETTINGER, J. LEHMANN, AND K. WALLENFELS, *Carbohydr. Res.*, 87 (1980) 63–70.
11 A. B. FOSTER AND W. G. OVEREND, *Chem. Ind. (London)*, (1955) 566–567; J. T. EDWARD, *Chem. Ind. (London)*, (1955) 1102–1104; J. LEHMANN, E. PFEIFFER, AND H. REINSHAGEN, *Chem. Ber.*, 102 (1969) 2745–2751.
12 C. C. F. BLAKE, L. N. JOHNSON, G. A. MAIR, A. C. T. NORTH, D. C. PHILLIPS, AND V. R. SARMA, *Proc. Roy. Soc. (London)* B, 167 (1967) 378–388; C. A. VERNON, *Proc. Roy Soc. (London)* B, 167 (1967) 389–401; P. J. MARHALL, M. L. SINNOTT, P. J. SMITH, AND D. WIDDOWS, *J. Chem. Soc., Perkin Trans. 1*, (1981) 366–376.
13 H. FRITZ, J. LEHMANN, AND P. SCHLESSELMANN, *Carbohydr. Res.*, 113 (1983) 71–92; C. F. BREWER, E. J. HEHRE, J. LEHMANN, AND W. WEISER, *Liebigs Ann. Chem.*, (1984) 1078–1087.
14 M. BLANC-MUESSER, J. DEFAYE, AND J. LEHMANN, *Carbohydr. Res.*, 108 (1982) 103–110.
15 W. G. OVEREND, C. W. REES, AND J. S. SEQUEIRA, *J. Chem. Soc.*, (1962) 3429–3440.
16 J. N BEMILLER, *Adv. Carbohydr. Chem.*, 22 (1967) 25–108; A. F. BOCHKOV, *Chemistry of the O-Glycosidic Bond: Formation and Cleavage*, Pergamon Press, 1979.
17 W. C. STILL, M. KAHN, AND A. MITRA, *J. Org. Chem.*, 43 (1978) 2923–2925.
18 E. HARDEGGER AND R. M. MONTAVON, *Helv. Chim. Acta*, 29 (1946) 1199–1203.