The Substrate Specificity of Amyloglucosidase (AMG). Part IV. Hydroxycyclohexyl Glucosides

Klaus Bock[a] and Susanne Refn[b]

[a] Department of Chemistry, Carlsberg Laboratory, Gl. Carlsberg Vej 10, DK-2500 Valby, Copenhagen, Denmark and [b] Department of Organic Chemistry, The Technical University of Denmark, Building 201, DK-2800 Lyngby, Denmark

Bock, K. and Refn, S., 1989. The Substrate Specificity of Amyloglucosidase (AMG). Part IV. Hydroxycyclohexyl Glucosides. – Acta Chem. Scand. 43: 373–380.

Cyclohexyl- and (1*R*, 2*R*)- and (1*S*, 2*S*)-hydroxycyclohexyl α-D-glucosides have been synthesised under halide-catalysed glycosylation reaction conditions. Furthermore, phenyl and 2-hydroxyphenyl α-D-glucopyranosides have been synthesised using fusion reactions in 60–70 % yield. Finally, methyl 4-*O*-(β-L-galactopyranosyl)-β-D-glucopyranoside has been prepared in high yield using silver triflate-promoted glycosylation conditions. All compounds have been characterised by NMR spectroscopy and their preferred solution conformations inferred from the NMR data and hard-sphere *exo*-anomeric effect calculations (HSEA). All the above-mentioned compounds have been investigated as potential substrates for the enzyme amyloglucosidase (AMG). The results show that only the compounds which have a preferred ground-state conformation similar to that of maltose can act as substrates for the enzyme.

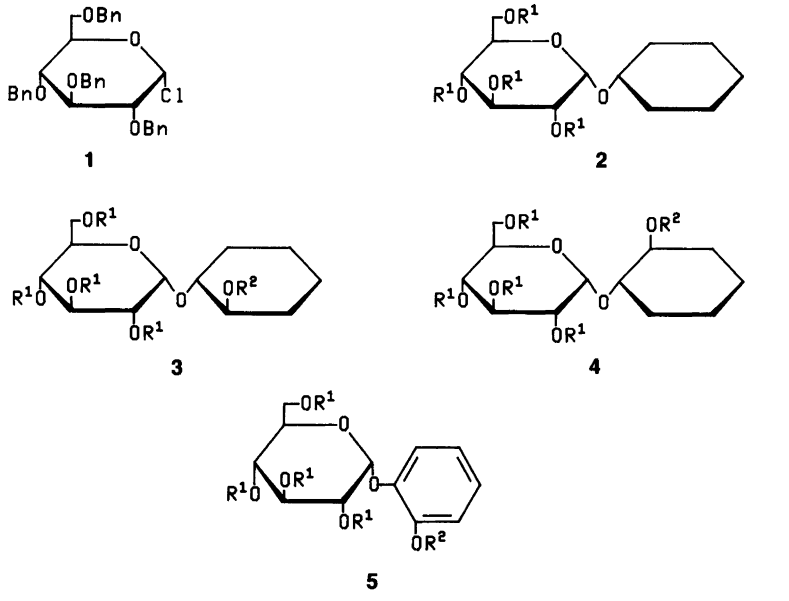
In previous papers on the substrate specificity of the enzyme amyloglucosidase (AMG),[1-6] which hydrolyses the disaccharide maltose, we have demonstrated that three hydroxy groups (3, 4′ and 6′) are essential for compounds to act as substrates for the enzyme. In this communication we elaborate on these results and demonstrate that these findings are still valid, when simple hydroxycyclohexyl α-glucosides are investigated as potential substrates for the enzyme. Furthermore, the present results substantiate that the substrates are bound to the enzyme in a conformation similar to that found in solution in the ground state, but that compounds, even though they have the key polar hydroxy groups properly oriented in space, cannot act as substrates if the pyranose rings do not adopt a conformation similar to that found in maltose.

Results and discussion

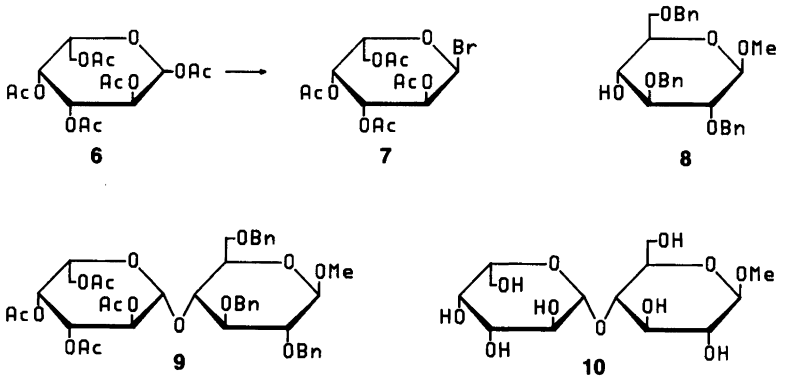
The synthesis of α-cyclohexyl glucoside (**2b**) was accomplished in 55 % yield using halide-catalysed glycosylation reaction conditions[7] with tetra-*O*-benzyl-α-D-glucopyranosyl chloride (**1**) and cyclohexanol followed by removal of the benzyl groups. Similarly, glycosylation of *trans*-cyclohexane-1,2-diol under the same reaction conditions yielded a mixture of glycosides from which the (1*R*, 2*R*)-glycoside (**3a**) could be isolated in 15 % yield. Deprotection of this in the usual way gave the glycoside **3b** in quantitative yield. The corresponding (1*S*, 2*S*)-glycoside (**4a**) could be isolated in 21.5 % yield, but was contaminated with minor amounts of β-glucosides, which were difficult to remove by preparative TLC. These impurities were therefore removed after deprotection to the free glycosides, followed by treatment with the enzyme β-glucosidase which hydrolyses the β-linked derivatives. The glucoside was isolated and characterized as the peracetate (**4c**) and finally *O*-deacetylated to the free glucoside (**4b**).

The 2-hydroxyphenyl α-glucoside (**5d**) was synthesized using the standard procedure for the preparation of phenyl glucosides[8] and a mixture of the tetra-*O*-acetates was isolated in 80 % yield, in an α:β ratio of 7:9. The compounds could only be separated by preparative TLC after acetylation of the phenolic hydroxy group and the α-glucoside (**5c**) was isolated in 40 % yield and fully characterized. Deprotection was accomplished by treatment with methoxide in methanol and **5b** was isolated and characterized by ^{1}H and ^{13}C NMR spectroscopy.

Conformational analysis, using the hard-sphere *exo*-anomeric effect (HSEA) method[9] of the disaccharide methyl 4-*O*-β-L-galactopyranosyl-β-D-glucopyranoside (**10**) with reference to the preferred conformation of maltose (Fig. 1) showed that the key polar hydroxy groups (3, 4′ and 6′) have relatively similar spatial orientations. It was therefore thought to be of interest to examine whether this compound would be a substrate for the enzyme AMG. The synthesis of this disaccharide was accomplished as described for the synthesis of a deuterium-labelled lactose derivative,[10] using tetra-*O*-acetyl-α-L-galactopyranosyl bromide (**7**) as the glycosylating reagent and compound **8** as the aglycone, in a silver triflate-promoted glycosylation reaction. The protected disaccharide (**9**) was isolated in 58 % yield. Deprotection by catalytic hydrogenolysis to remove the *O*-benzyl ethers followed by *O*-deacetylation



1 **2** **3** **4** **5**



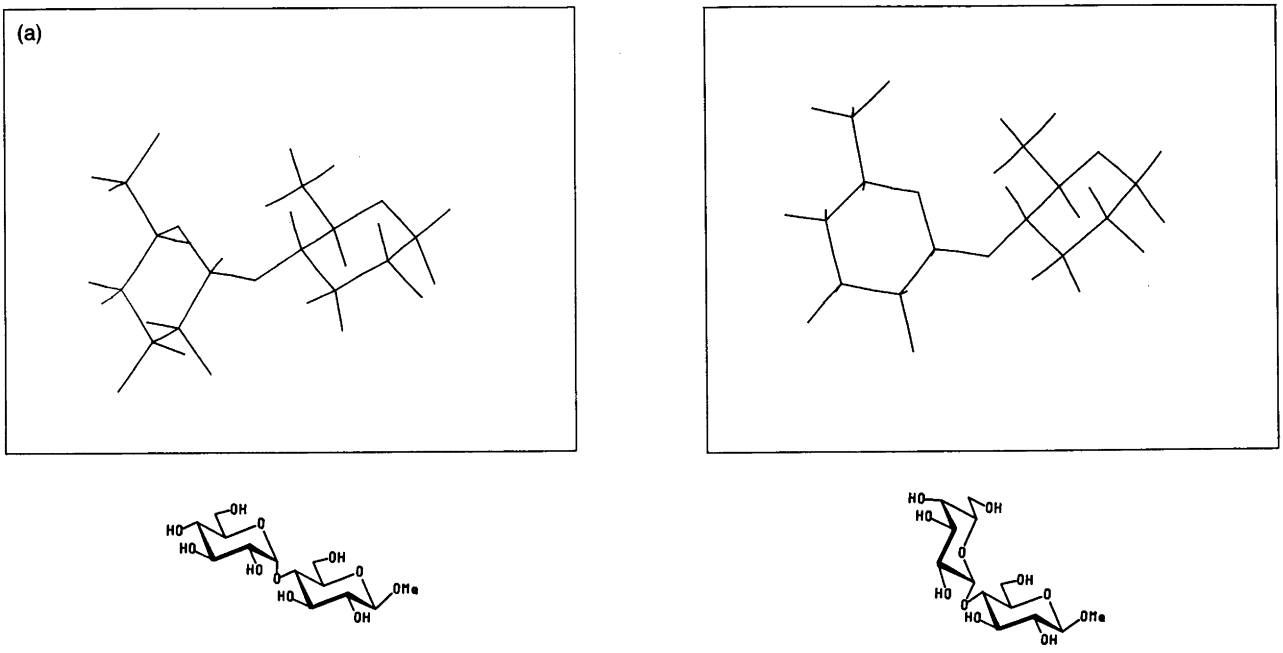
6 **7** **8** **9** **10**

a: $R^1 = Bn$, $R^2 = H$
b: $R^1 = H$, $R^2 = H$
c: $R^1 = Ac$, $R^2 = Ac$
d: $R^1 = Ac$, $R^2 = H$

gave the unprotected disaccharide (**10**) in 96 % yield. The compound could be isolated crystalline and was fully characterized.

The activities of compounds **2b**, **3b**, **4b**, **5b** and **10** as substrates for the enzyme AMG were investigated as described earlier.[1] It was found that compounds **2b**, **5b** and **10** were not substrates for the enzyme i.e. no measurable hydrolysis under standard conditions[1] occurred within 24 h. Compounds **3b** and **4b** were both substrates for the enzyme but were hydrolysed at rather different rates (Fig. 2). Compound **3b**, which has the 3-hydroxy group in the same orientation as has maltose in the ground-state conformation was hydrolysed three times faster than **4b**. Figs. 3(a) and 3(b) show the φ/ψ isoenergy contour maps from an HSEA calculation for compounds **3b** and **4b**. The observed ^{13}C NMR chemical shifts of the anomeric and aglyconic carbon atoms are in good accordance with the observed conformational preferences.[11] However, compound **4b** could be hydrolysed presumably in an unfavoured conformation **4b**,II (ca. 3 kcal mol^{-1} higher than **4b**,I) in which the hydroxy group is oriented as in maltose, but then with a much slower rate. The compound is, furthermore, not recognised by the enzyme to any significant degree because it was not possible to inhibit the hydrolysis of maltose with an equimolar amount of **10**.

It is interesting to note that neither the 2-hydroxyphenyl α-glucoside (**5b**) nor phenyl α-D-glucoside (or the 4-nitrophenyl derivative) are good substrates for the enzyme. This is, however, in accordance with the preferred conformation of the phenyl group where the OH-group is close to H-5 of the pyranose ring (**5b**,II). The preference for the conformer (**5b**,II) over (**5b**,I) is supported by NOE data



Methyl 4-*O*-(α-D-glucopyranosyl)-β-D-glucopyranoside

Methyl 4-*O*-(β-L-galactopyranosyl)-β-D-glucopyranoside

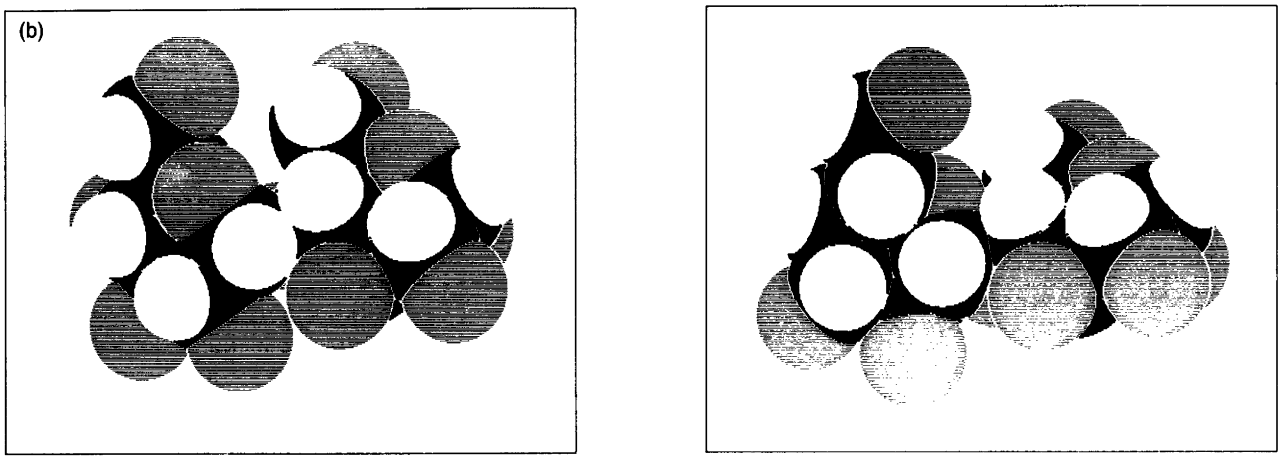


Fig. 1. (a) Stick model of minimum-energy conformation of maltose ($\varphi_H, \psi_H = -25°, -25°$) and compound **10** ($\varphi_H, \psi_H = -60°, -10°$) together with a least-squares fit of the two models using the key polar hydroxy groups and the ring and glycosidic oxygen atoms as reference points. (b) CPK models of the same two molecules to illustrate the similarity of the surfaces exposed to the enzyme.

(Table 1) and also by the observed downfield shift of H-5 (0.12–0.20 ppm) in compound **5b** compared with the corresponding phenyl (or 4-nitrophenyl) α-D-glucopyranoside, value in Table 1.

Experimental

Melting points are uncorrected. Optical rotations were measured on a Perkin Elmer 241 polarimeter. NMR spectra were obtained on Bruker WH-90 and AM-500 NMR instruments. The spectra of protected compounds were measured in $CDCl_3$. Unprotected compounds were measured in D_2O relative to the internal reference: acetone (δ 2.22) for ^1H NMR spectra and dioxane (67.4 ppm) for ^{13}C NMR spectra. Microanalyses were performed by Novo Microanalytical Laboratory, Copenhagen, Denmark. TLC was performed on silica-gel coated plates (Merck F-254). Preparative TLC was performed on 20×40 cm plates coated with a 1 mm thickness of silica gel.

Cyclohexyl α-D-glucopyranoside (**2b**). Tetra-*O*-benzyl-α-D-glucopyranosyl chloride (**1**)[12] (1.65 g, 2.96 mmol) was used as the glycosylating reagent in a halide-catalysed glycosylation reaction[7] using cyclohexanol as the aglycone followed by removal of the protecting groups by catalytic hydrogenolysis in acetic acid (5 ml) with 5 % palladium on activa-

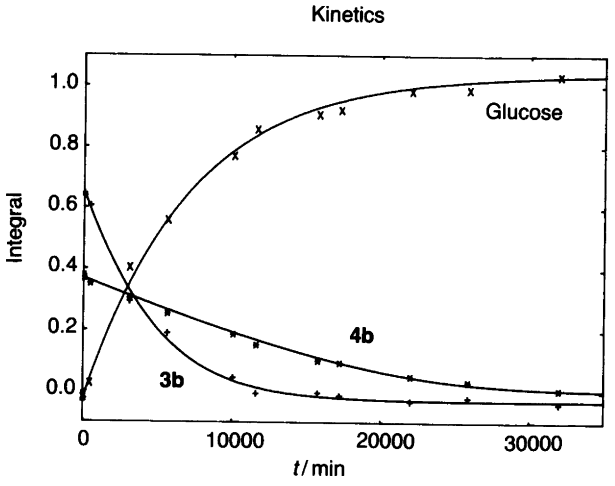
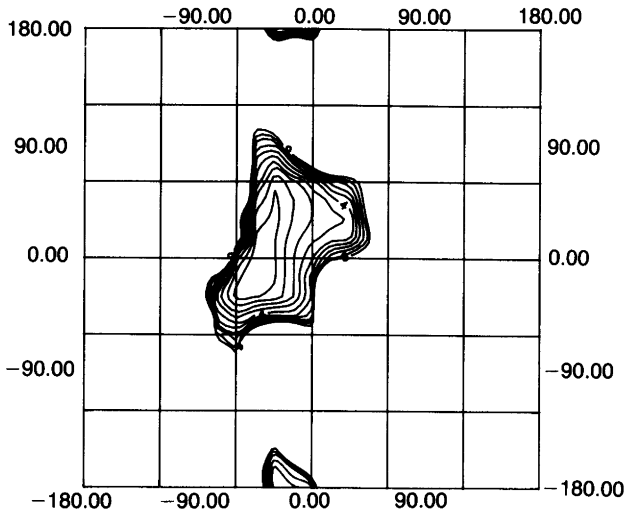


Fig. 2. Progress curves (relative concentrations, versus time) of the enzymatic hydrolysis with AMG of compounds **3b** and **4b** in single-substrate experiments under identical experimental conditions.

ted carbon. The reaction was worked up in the usual way and crystallization from water gave crude crystalline **2b** (420 mg). Recrystallization from ether gave pure crystals (400 mg) with m.p. 118–121 °C. Lit.,[13] m.p. 125 °C. ^{1}H NMR (500 MHz, D_2O) of (**2a**): δ 5.08 (H-1, d, $J_{1,2}$ 3.9 Hz), 3.53 (H-2, dd, $J_{2,3}$ 9.8 Hz), 3.71 (H-3, t, $J_{3,4}$ 9.2 Hz), 3.41 (H-4, t, $J_{4,5}$ 9.3 Hz), 3.77 (H-5), 3.85 (H-6_a), 3.77 (H-6_b), 3.64 (cyclohexane H-1) 1.95–1.2 (5×CH_2). ^{13}C NMR (125.7 MHz, D_2O): δ 97.07 (C-1), 73.99, 72.56, 72.12, 70.53 (C-2,-3,-4,-5), 61.40 (C-6), 77.52 (cyclohexane C-1), 33.82, 31.87, 25.91, 24.83, 24.60 (5×CH_2).

*(1*R,2R*)- and (1*S,2S*)-2-Hydroxycyclohexyl 2,3,4,6-tetra-O-benzyl-α-*D*-glucopyranoside* (**3a**), (**4a**). A solution of *trans*-cyclohexane-1,2-diol (464 mg, 4.0 mmol) and tetraethylammonium bromide (890 mg, 4.0 mmol) in dichloromethane (2 ml) and *N,N*-dimethylformamide (0.4 ml) was stirred with 4 Å molecular sieves under nitrogen for 1 h. A solution of 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl chloride (**1**) (2.23 g, 4.0 mmol) in dichloromethane (2 ml) was added and the mixture was stirred for 16 h. The mixture was diluted with dichloromethane (25 ml) and stirred for 1 h and filtered. The organic phase was successively washed with 0.1 M hydrochloric acid (12 ml), saturated sodium hydrogen carbonate solution and water. Drying ($MgSO_4$) and concentration of the organic layer gave a syrup (2.26 g) which was purified by preparative TLC using ethyl acetate–hexane (1:3) as the eluant to give two main fractions. Work-up of the faster-moving fraction yielded **3a** (374 mg, 0.59 mmol, 15 %). The product was pure according to the ^{1}H NMR spectrum. ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.94 (H-1, d, $J_{1,2}$ 3.8 Hz), 3.51 (H-2, dd, $J_{2,3}$ 9.8 Hz), 3.98 (H-3, t, $J_{3,4}$ 10.5 Hz), 3.59 (H-4, t, $J_{4,5}$ 10.1 Hz), 3.98 (H-5, ddd, $J_{5,6a}$ 3.9, $J_{5,6b}$ 2.0 Hz), 3.69 (H-6_a, dd, $J_{6a,6b}$ 10.6 Hz), 3.57 (H-6_b, dd), 3.18 (cyclohexane H-1, ddd), 3.42 (cyclohexane H-2, ddd), 2.00–1.22 (4×CH_2). Work-up

(a) (1*R*, 2*R*)-2-Hydroxycyclohexyl α-D-glucopyranoside (**3b**)



(b) (1*S*, 2*S*)-2-Hydroxycyclohexyl α-D-glucopyranoside (**4b**)

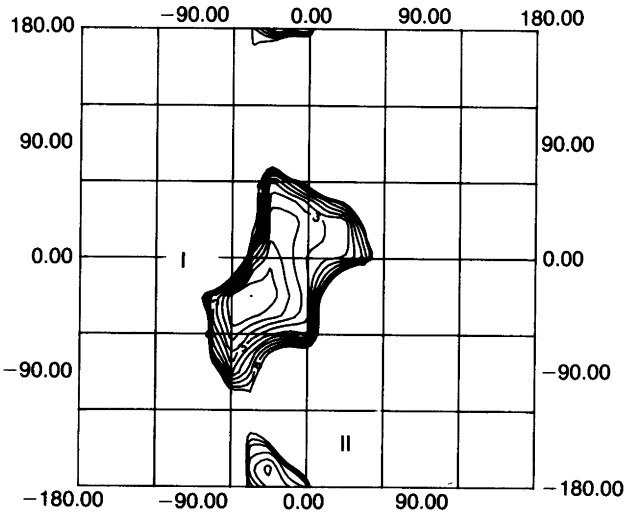
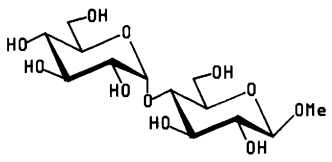


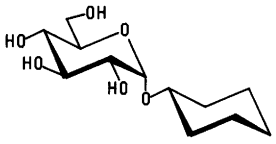
Fig. 3. Isoenergy contour maps from the HSEA calculations of compounds **3b** and **4b**, the spacing between the contours being 1 kcal mol^{-1}. (a) Compound **3b** (minimum $\varphi_H, \psi_H = -50°, -10°$). (b) Compound **4b** (minimum $\varphi_H, \psi_H = -50°, -30°$).

of the slower-moving fraction yielded a syrup, which, according to the ^{13}C NMR spectrum, consisted mainly of **4a** (551 mg, 0.86 mmol, 21.5 %), and small amounts of the two β-D-glucopyranosides corresponding to **3a** and **4a** as seen from a ^{13}C NMR spectrum (δ 103.5, C-1). ^{1}H NMR (500 MHz, $CDCl_3$) of (**4a**): δ 4.97 (H-1, d, $J_{1,2}$ 3.7 Hz), 3.59 (H-2, dd, $J_{2,3}$ 9.7 Hz), 3.99 (H-3, t, $J_{3,4}$ 9.7 Hz), 3.63 (H-4, dd, $J_{4,5}$ 9.6 Hz), 4.02 (H-5, ddd, $J_{5,6a}$ 4.1, $J_{5,6b}$ 2.2 Hz), 3.70 (H-6_a, dd, $J_{6a,6b}$ 10.7 Hz), 3.65 (H-6_b, dd), 3.29 (cyclohexane H-1, ddd), 3.48 (cyclohexane H-2), 2.00–1.2 (4×CH_2). ^{13}C NMR (125.7 MHz, $CDCl_3$): δ 95.01 (C-1).

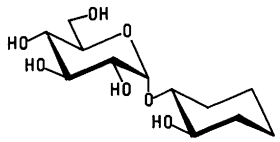
*(1*R,2R*)-2-Hydroxycyclohexyl α-D-glucopyranoside* (**3b**). To a solution of **3a** (374 mg, 0.59 mmol) in methanol (25 ml) and acetic acid (5 ml) was added 5 % palladium on



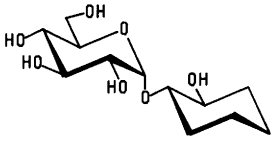
Methyl β-maltoside



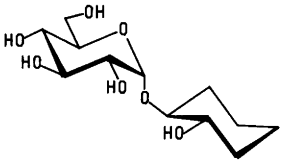
2b



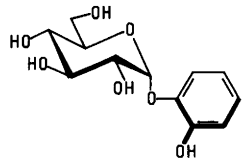
3b



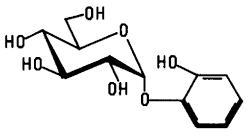
4b, I



4b, II



5b, I



5b, II

activated carbon (150 mg), and the mixture was stirred for 16 h under 100 kPa hydrogen pressure. The catalyst was filtered off and washed well with methanol. The combined filtrates were concentrated, and evaporated twice more with water to yield pure **3b** (170 mg, ca. 100 %) as a syrup which was characterized by its ^{1}H and ^{13}C NMR spectra: ^{1}H NMR (500 MHz, D_2O): δ 5.14 (H-1, d, $J_{1,2}$ 4.0 Hz), 3.54 (H-2, dd, $J_{2,3}$ 10.0 Hz), 3.72 (H-3, dd, $J_{3,4}$ 9.6 Hz), 3.41 (H-4, t, $J_{4,5}$ 9.6 Hz), 3.79 (H-5, ddd, $J_{5,6a}$ 2.1, $J_{5,6b}$ 5.2 Hz), 3.85 (H-6_a, dd, $J_{6a,6b}$ 11.8 Hz), 3.75 (H-6_b, dd), 3.44 (cyclohexane H-1, ddd), 3.59 (cyclohexane H-2, ddd), 1.63–1.47 (4×CH_2). ^{13}C NMR (125.7 MHz, D_2O): δ 100.7 (C-1), 72.6 (C-2), 73.8 (C-3), 70.4 (C-4), 72.7 (C-5), 61.3 (C-6), 85.4 (cyclohexane C-1), 74.5 (cyclohexane C-2), 33.1, 32.3, 24.5, 24.2 (4×CH_2). The compound was also fully characterized as its pentaacetate (**3c**), which had m.p. 142–144 °C, $[\alpha]_D^{20}$ 87.1° (*c.* 3.9, $CHCl_3$). Anal. for $C_{22}H_{32}O_{12}$: C, H. ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.32 (H-1, d, $J_{1,2}$ 3.8 Hz), 4.79 (H-2, dd, $J_{2,3}$ 9.6 Hz), 5.42 (H-3, dd, $J_{3,4}$ 9.6 Hz), 5.00 (H-4, t, $J_{4,5}$ 9.6 Hz), 4.08 (H-5, ddd, $J_{5,6a}$ 2.1, $J_{5,6b}$ 5.2 Hz), 4.20 (H-6_a, dd, $J_{6a,6b}$ 11.8 Hz), 4.09 (H-6_b, dd), 3.64 (cyclohexane H-1, ddd), 4.73 (cyclohexane H-2, ddd), 1.80–1.20 (4×CH_2). ^{13}C NMR (125.7 MHz, $CDCl_3$): δ 95.7 (C-1), 70.7 (C-2), 69.9 (C-3), 68.6 (C-4), 67.3 (C-5), 61.9 (C-6), 76.2, 75.9 (cyclohexane C-1, C-2), 31.5, 30.0, 23.6, 22.1 (4×CH_2).

(1S,2S)-2-Hydroxycyclohexyl α-D-glucopyranoside (**4b**). To a solution of **4a** (281 mg, 0.44 mmol) in methanol (20 ml) and acetic acid (4 ml) was added 5 % palladium on activated carbon (120 mg), and the mixture was stirred for 16 h under 100 kPa hydrogen pressure. The catalyst was filtered off and washed well with methanol. The combined filtrates were concentrated, and evaporated twice more with water to yield an impure syrup (145 mg). According to the ^{1}H NMR and the ^{13}C NMR spectra, the product was mainly **4b** (ca. 71 %) and the two β-D-glucopyranosides corresponding to **3b** and **4b** (ca. 29 %). ^{1}H NMR: δ 4.6 and 4.5 for H-1, respectively, and ^{13}C NMR: δ 101.0 and 104.0 (C-1, respectively). Part of the crude mixture (57 mg, 0.21 mmol) was treated with β-glucosidase from *Aspergillus Niger* and concentrated. As seen from the ^{13}C NMR spectrum only the two β-D-glucosides were hydrolysed. The product was treated with pyridine (4 ml) and acetic anhydride (4 ml) for 3.5 h. The reaction mixture was concentrated, and evaporated twice with toluene. The resulting compound was dissolved in dichloromethane (25 ml) and filtered. Evaporation of the organic phase yielded the crude product which was purified by preparative TLC using ethyl acetate–hexane (1:2) as the eluant. The main fraction gave (1*S*,2*S*)-2-acetoxycyclohexyl 2,3,4,6-*O*-acetyl-α-D-glucopyranoside (**4c**) (74 mg) with m.p. 144–146 °C. Recrystallization from ethyl acetate–hexane gave **4c** (51 mg, 0.10 mmol) with m.p. 153–155 °C. $[\alpha]_D^{20}$ 146.0° (*c.* 0.9, $CHCl_3$). Anal. for $C_{22}H_{32}O_{12}$: C, H. ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.22 (H-1, d, $J_{1,2}$ 3.6 Hz), 4.82 (H-2, dd, $J_{2,3}$ 10.2 Hz), 5.42 (H-3, t, $J_{3,4}$ 9.5 Hz), 5.06 (H-4, t, $J_{4,5}$ 9.5 Hz), 4.19 (H-5, ddd, $J_{5,6a}$ 4.1, $J_{5,6b}$ 2.1 Hz), 4.24 (H-6_a, ddd, $J_{6a,6b}$ 12.0 Hz), 4.15 (H-6_b, dd), 3.59 (cyclohexane H-1, ddd), 4.76 (cyclohexane H-2, ddd), 1.02–2.01 (4×CH_2). ^{13}C NMR (125.7 MHz, $CDCl_3$): δ 92.9 (C-1), 70.9 (C-2), 70.1 (C-3), 68.4 (C-4), 67.3 (C-5), 61.6 (C-6), 76.2, 74.5 (cyclohexane C-1, C-2), 29.7, 28.7, 23.0, 22.9 (4×CH_2).

Compound **4c** (51 mg, 0.10 mmol) was de-*O*-acetylated in 0.1% sodium methoxide in methanol (10 ml) for 16 h. Neutralization with carbon dioxide and concentration yielded **4b** (64 mg) as a mixture with inorganic salts. The mixture was purified by chromatography on a Sephadex G-15 column using methanol–water (1:1) as the eluant and pure **4b** (37 mg, ca. 0.13 mmol) was obtained. ^{1}H NMR (500 MHz, D_2O): δ 5.10 (H-1, d, $J_{1,2}$ 4.1 Hz), 3.56 (H-2, dd, $J_{2,3}$ 9.8 Hz), 3.78 (H-3, t, $J_{3,4}$ 9.3 Hz), 3.43 (H-4, t, $J_{4,5}$ 10.0 Hz), 3.89 (H-5, ddd, $J_{5,6a}$ 2.5, $J_{5,6b}$ 5.5 Hz), 3.86 (H-6_a, dd, $J_{6a,6b}$ 12.3 Hz), 3.76 (H-6_b, dd), 3.45 (cyclohexane H-1), 3.56 (cyclohexane H-2), 1.64–1.47 (4×CH_2). ^{13}C NMR (125.7 MHz, D_2O): δ 95.3 (C-1), 72.0 (C-2), 73.7 (C-3), 70.4 (C-4), 72.4 (C-5), 61.3 (C-6), 80.2 (cyclohexane C-1), 73.5 (cyclohexane C-2), 33.3, 29.2, 24.3, 24.1 (4×CH_2).

Table 1. NMR data for aromatic glucosides.

(a) ^{1}H NMR data

Chemical shift relative to $\delta[(CH_3)_2CO] = 2.22$ (J/Hz)

	4-Nitrophenyl α-glucoside	Phenyl α-glucoside	2-Hydroxyphenyl glucoside (**5b**)
H-1	5.85 (3.8)	5.66 (3.5)	5.60 (3.8)
H-2	3.82 (10.0)	3.74 (10.0)	3.78 (10.0)
H-3	4.00 (9.5)	3.96 (10.0)	4.00 (9.5)
H-4	3.58 (9.5)	3.54 (10.0)	3.58 (9.5)
H-5	3.69	3.79	3.88
H-6	3.78 (2.4,12.2)	3.77	3.82 (2.4,12.2)
H-6′	3.74 (4.9)	3.77	3.78 (6.1)
H-2′	7.35 (9.0,0.6)	7.22 (7.5)	
H-3′	8.28 (9.0,0.6)	7.44 (7.5)	7.05 (8.0,1.5)
H-4′		7.16	7.00 (8.0,8.0,1.5)
H-5′	8.28	7.44	6.96 (8.0,8.0,1.5)
H-6′	7.35	7.22	7.30 (8.0,1.5)

(b) Nuclear Overhauser enhancements

Saturated	Proton observed	Observed	Proton saturated	Observed
H-3′	12.0 (H-2′)	8.0 (H-2′)	H-3′, H-5′	3.0 (H-6′)
	−0.6 (H-1)	−0.6 (H-1)		−0.3 (H-1)
H-2′	16.9 (H-3′)	23.0 (H-3′)	H-3′, H-5′	5.6 (H-6′)
	7.3 (H-1)	17.5 (H-1)		−0.5 (H-1)
	−0.3 (H-2)	−0.3 (H-2)		
	0.5 (H-5)	0.7 (H-5)	H-6′	10.5 (H-5′)
				10.6 (H-1)
				1.6 (H-5)
				−0.8 (H-2)
H-1	−1.4 (H-3′)	7.5 (H-2′)	H-1	9.3 (H-6′)
	18.3 (H-6′)			10.2 (H-2)
	12.2 (H-2)	14.0 (H-2)		
	−0.7 (H-4)			

*2-Hydroxyphenyl 2,3,4,6-tetra-*O*-acetyl-α-*D*-glucopyranoside* (**5d**) *and 2-hydroxyphenyl 2,3,4,6-tetra-*O*-acetyl-β-*D*-glucopyranoside.* A mixture of benzene-1,2-diol (5 g, 45.5 mmol) and penta-*O*-acetyl-β-D-glucopyranose (5 g, 12.8 mmol) were fused[8] on an oil bath at 130–135 °C. Dry $ZnCl_2$ (0.2 g) was added in one portion. The reaction mixture was kept at 135 °C under diminished pressure for 20 min (a spontaneous reaction set in after ca. 2 min). Ethanol (20 ml) was then added to give a dark solution. No crystals were obtained after the solution had been cooled overnight. The solution was concentrated and dichloromethane (20 ml) and water (50 ml) were added to the residue. The water phase was washed with dichloromethane (20 ml), and the combined dichloromethane solutions were washed with water (3×50 ml), dried ($MgSO_4$) and concentrated to a syrup (5.5 g). Part of the residue (1 g) was purified by flash chromatography using ethyl acetate–hexane, (1:1) as the eluant. This gave a syrup (866 mg, 80 %) which was characterized by its NMR spectra (^{1}H and ^{13}C) and shown to be a mixture of anomers, α:β = 7:9.

*2-Acetoxyphenyl 2,3,4,6-tetra-*O*-acetyl-α-*D*-glucopyranoside* (**5c**). The product prepared above (**5d** and its β-anomer) (580 mg, 1.32 mmol) was acetylated with acetic anhydride (8 ml) and pyridine (8 ml) for 2 h. The mixture was concentrated, and the residue was evaporated three times (with toluene, 5 ml). This gave a mixture of the acetylated α- and β-glucosides (550 mg, 1.14 mmol, 86 %), which was purified by preparative TLC using ethyl acetate–hexane (1:2) as the eluant. The faster-running fraction gave **5c** (223 mg, 0.46 mmol). Crystallization of this from ethanol gave **5c** m.p. 112–113 °C. $[\alpha]_D^{20}$ + 113.7° (*c*. 1.95, $CHCl_3$). Anal. for $C_{22}H_{26}O_{12}$: C, H. ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.70 (H-1, d), 4.96 (H-2, dd), 5.64 (H-3, t), 5.16 (H-4, t), 4.08 (H-5, ddd), 4.23 (H-6a, dd), 4.05 (H-6b, dd), $J_{1,2}$ 3.5, $J_{2,3}$ 10.3, $J_{3,4}$ 9.8, $J_{4,5}$ 10.2, $J_{5,6a}$ 4.4, $J_{5,6b}$ 2.2, $J_{6a,6b}$ 12.4 Hz. ^{13}C NMR (125.77 MHz, $CDCl_3$): δ 93.4 (C-1), 146.4 (aryl C-1′), 139.2 (aryl C-2).

The slower-moving fraction gave β-**5c** (191 mg, 0.40 mmol). Crystallization of this from ethanol gave β-**5c** m.p. 130–131 °C. $[\alpha]_D^{20}$ −6.4° (*c*. 1.96, $CHCl_3$). Anal. for

$C_{22}H_{26}O_{12}$: C, H. ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.08 (H-1, d), 5.32–5.26 (H-2, H-3), 5.13 (H-4, t), 3.87 (H-5, ddd), 4.28 (H-6a, dd), 4.15 (H-6b, dd), $J_{1,2}$ 7.5, $J_{3,4}$ 9.6, $J_{4,5}$ 10.0, $J_{5,6a}$ 5.4, $J_{5,6b}$ 2.4, $J_{6a,6b}$ 12.3 Hz. ^{13}C NMR (125.77 MHz, $CDCl_3$): δ 97.3 (C-1), 147.0 (aryl C-1), 138.7 (aryl C-2). The corresponding tetraacetate β-**5d** has been described previously by Bretschneider and Beran.[14]

2-Hydroxyphenyl α-D-glucopyranoside (**5b**). Compound **5c** (110 mg, 0.23 mmol) was de-*O*-acetylated in 0.1% sodium methoxide in methanol (10 ml). After the reaction mixture had been concentrated, a solution of the product in D_2O (1.5 ml) was neutralized with a few drops of trichloroacetic acid in D_2O. A ^{1}H NMR spectrum of the product showed it to be **5b**, the NMR data of which are given in Table 1.

1,2,3,4,6-Penta-O-acetyl-α,β-L-galactopyranose (**6**). A solution of α,β-L-galactopyranose[15] (500 mg, 2.78 mmol) in acetic anhydride (10 ml) and a few drops of perchloric acid, was cooled in ice–water, and stirred at 20 °C for 3 h. The mixture was poured onto ice–water and stirred, and sodium acetate (3 g) in water was added. The solution was extracted three times with dichloromethane (25 ml), and the organic phase was washed with saturated sodium chloride solution (25 ml) and twice with saturated sodium hydrogen carbonate solution (25 ml) and finally with water (25 ml). Drying ($MgSO_4$) and concentration of the organic extract yielded a syrup which consisted of penta-*O*-acetyl-α,(β)-L-galactopyranose (**6**) (1.07 g, ca. 99 %), which was characterized by ^{1}H NMR spectroscopy.

2,3,4,6-Tetra-O-acetyl-α-L-galactopyranosyl bromide (**7**). To a solution of **6** (720 mg, 1.85 mmol) in dichloromethane (3 ml) was added acetic acid saturated with hydrogen bromide (7 ml), and the mixture was stirred for 1 h. To the mixture was added dichloromethane (25 ml) and ice, and the organic phase was washed five times with water (25 ml), dried ($MgSO_4$) and concentrated to yield **7** (740 mg, 1.79 mmol, ca. 97 %) as a syrup which was used without further purification.

Methyl 2,3,6-tri-O-benzyl-4-O-(2,3,4,6-tetra-O-acetyl-β-L-galactopyranosyl)-β-D-glucopyranoside (**9**). A solution of methyl 2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**8**)[16] (419 mg, 0.90 mmol), silver trifluoromethanesulfonate (470 mg, 1.83 mmol) and *N,N,N′,N′*-tetramethylurea (0.217 ml, 1.92 mmol) in dichloromethane (5 ml) was stirred for 1 h with 4 Å molecular sieves under nitrogen. The mixture was cooled to −20 °C and a solution of **7** in dichloromethane (5 ml) was added and the mixture was stirred for 3 h under nitrogen. The mixture was diluted with dichloromethane (25 ml) and filtered into saturated sodium hydrogen carbonate solution containing ice. The residue was washed with dichloromethane (25 ml) and the combined organic phases were washed once with water (25 ml). Drying ($MgSO_4$) and concentration of the organic layer gave a syrup (1.08 g), which was purified by preparative TLC using ethyl acetate–hexane (1:1) as the eluant. Work-up of the fastest running fraction gave **9** (421 mg, 58 %): ^{1}H NMR (500 MHz, $CDCl_3$): Glc: δ 4.31 (H-1, d, $J_{1,2}$ 7.8 Hz), 3.41 (H-2, dd, $J_{2,3}$ 8.7 Hz), 3.58 (H-3, t, $J_{3,4}$ 8.5 Hz), 3.67 (H-4, t, $J_{4,5}$ 8.7 Hz), 3.52 (H-5), 3.53 (H-6_a), 3.98 (H-6_b, dd, $J_{5,6b}$ 5.3, $J_{6a,6b}$ 13.5 Hz); Gal: δ 4.97 (H-1, d, $J_{1,2}$ 8.0 Hz), 5.16 (H-2, dd, $J_{2,3}$ 10.5 Hz), 4.93 (H-3, dd, $J_{3,4}$ 3.5 Hz), 5.32 (H-4, dd, $J_{4,5}$ ca. 1 Hz), 3.71 (H-5, dd, $J_{5,6a}$ and $J_{5,6b}$ 6.3 Hz), 3.98 (H-6, dd, $J_{6a,6b}$ 11.3 Hz), 4.06 (H-6_b, dd), 3.57 (OCH_3). ^{13}C NMR (125.7 MHz, $CDCl_3$): Glc: δ 104.36 (C-1), 82.27 (C-2), 84.50 (C-3), 74.93 (C-4), 74.18 (C-5), 69.80 (C-6); Gal: δ 100.35 (C-1), 69.17 (C-2), 70.93 (C-3), 67.20 (C-4), 70.57 (C-5), 61.22 (C-6), 57.05 (OCH_3).

Methyl 4-O-(β-L-galactopyranosyl)-β-D-glucopyranoside (**10**). To a solution of **9** (184 mg, 0.23 mmol) in methanol (10 ml) and acetic acid (1 ml) was added 5 % palladium-on-carbon (80 mg). The mixture was stirred under 100 kPa hydrogen pressure for 16 h. The catalyst was filtered off and washed with methanol. The filtrate and washings were combined and concentrated to yield a syrup of methyl 4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-L-galactopyranosyl)-β-D-glucopyranoside (117 mg, 96 %). The syrup was de-*O*-acetylated in a mixture of methanol (10 ml) and 1 M sodium methoxide in methanol (1.6 ml) overnight. Neutralization and removal of the sodium ions with Amberlite IRC-50 (H^+) ion-exchange resin followed by filtration and concentration of the eluate yielded **10** (92 mg ca. 100 %) as a crystalline compound with m.p. 184–190 °C. Recrystallization from 95 % ethanol gave crystals of **10** (40 mg) which decomposed at 228–232 °C. $[\alpha]_D^{20}$ −16.3° (*c*. 1.53, H_2O). Anal. for $C_{13}H_{24}O_{11}$: C, H. ^{1}H NMR (500 MHz, D_2O): Glc: δ 4.40 (H-1, d, $J_{1,2}$ 7.8 Hz), 3.30 (H-2, dd, $J_{2,3}$ 9.4 Hz), 3.73 (H-3, H-4), 3.53 (H-5), 3.95 (H-6_a), 3.93 (H-6_b). Gal: δ 4.68 (H-1, d, $J_{1,2}$ 7.9 Hz), 3.55 (H-2, dd, $J_{2,3}$ 10.2 Hz), 3.67 (H-3, dd, $J_{3,4}$ 3.9 Hz), 3.92 (H-4), 3.71 (H-5), 3.75 (H-6_a, dd, $J_{5,6a}$ 4.2, $J_{6a,6b}$ 11.6 Hz), 3.80 (H-6_b, dd, $J_{5,6b}$ 8.3 Hz), 3.58 (OCH_3). ^{13}C NMR (125.7 MHz, D_2O): Glc: δ 104.0 (C-1), 73.84 (C-2), 76.37 (C-3), 77.55 (C-4), 75.35 (C-5), 61.17 (C-6), 57.99 (OCH_3). Gal: δ 104.5 (C-1), 72.09 (C-2), 73.51 (C-3), 69.36 (C-4), 75.90 (C-5), 61.82 (C-6).

Enzyme reactions were carried out as described in Ref. 1.

HSEA calculations were performed on an IBM PS2 model 80 computer using the program described.[9] The coordinates for the α-D-glucopyranose and the β-L-galactopyranose units were taken from neutron-diffraction data.[17,18] The coordinates for the hydroxycyclohexyl units were generated by the program Alchemy.[19]

Acknowledgements. The 500 MHz NMR spectrometer was provided by the Danish Natural Science Research Council and The Carlsberg Foundation.

References

1. Bock, K. and Pedersen, H. *Acta Chem. Scand., Ser. B 41* (1987) 617.
2. Bock, K. *Molecular Recognition of Oligosaccharides Related to Starch Studied by NMR Spectroscopy and HSEA Calculations.* In: Jarozewski, J., Schaumburg, K. and Kofod, H., Eds., Alfred Benzon Symposium 26. *NMR Spectroscopy and Drug Design,* Munksgaard, Copenhagen 1988.
3. Bock, K., Bolanos Guzman, J. F. and Refn, S. *Glycoconjugate J. 4* (1987) 283.
4. Bock, K. and Pedersen, H. *Acta Chem. Scand., Ser. B 42* (1988) 75.
5. Adelhorst, K., Bock, K., Pedersen, H. and Refn, S. *Acta Chem. Scand., Ser. B 42* (1988) 196.
6. Bock, K. *Pure Appl. Chem. 59* (1987) 1447.
7. Lemieux, R. U., Hendriks, K. B., Stick, R. V. and James, K. *J. Am. Chem. Soc. 97* (1975) 4056.
8. Jermyn, M. A. *Aust. J. Chem. 7* (1954) 202.
9. Bock, K. *Pure Appl. Chem. 55* (1983) 605.
10. Bock, K. and Refn, S. *Acta Chem. Scand., Ser. B 41* (1987) 469.
11. Bock, K., Brignole, A. and Sigurskjold, B. W. *J. Chem. Soc., Perkin Trans. 2* (1986) 1711.
12. Ishikawa, T. and Fletcher, H. G., Jr. *J. Org. Chem.* 34 (1969) 563.
13. Wing, R. E. and BeMiller, J. N. *Carbohydr. Res. 10* (1969) 441.
14. Bretschneider, H. and Beran, K. *Monatsh. Chem. 80* (1949) 262.
15. Whispler, R. L., Wolfrom, M. L., BeMiller, J. N. and Shafizadeh, F., Eds., *Methods Carbohydr. Chem. 1,* Academic Press, New York 1962, p. 122.
16. Garegg, P. J., Hultberg, H. and Wallin, S. *Carbohydr. Res. 108* (1982) 97.
17. Brown, G. A. and Levy, H. A. *Acta Crystallogr., Sect. B 35* (1979) 656.
18. Takagi, S. and Jeffrey, G. A. *Acta Crystallogr., Sect. B 35* (1979) 902.
19. *Alchemy.* Molecular Modeling Software, Tripos Associates, Inc. St. Louis, USA 1987.

Received November 15, 1988.