ELSEVIER

Carbohydrate Research 291 (1996) 43–52

CARBOHYDRATE RESEARCH

Evaluation of *C*-(β-D-galactosyl) and *C*-(2-deoxy-D-*lyxo*-hex-1-enopyranosyl) (D-galactal type) derivatives as inhibitors of β-D-galactosidase from *Escherichia coli* [1,2]

László Kiss [a], László Somsák [b,*]

[a] *Department of Biochemistry, Lajos Kossuth University, P.O. Box 50, H-4010 Debrecen, Hungary*
[b] *Department of Organic Chemistry, Lajos Kossuth University, P.O. Box 20, H-4010 Debrecen, Hungary*

Received 12 February 1996; accepted 16 May 1996

Abstract

C-(2-Deoxy-D-*lyxo*-hex-1-enopyranosyl)formamide was prepared from acetylated *C*-(β-D-galactopyranosyl)formamide by a radical-mediated bromination–zinc/*N*-methylimidazole-induced reductive elimination–Zemplén deacetylation reaction sequence. The preparation of acetylated 5-(2-deoxy-D-*lyxo*-hex-1-enopyranosyl)tetrazole was improved. Methyl *C*-(2-deoxy-D-*lyxo*-hex-1-enopyranosyl)formimidate was transformed by benzylamine into *N*-benzyl-*C*-(2-deoxy-D-*lyxo*-hex-1-enopyranosyl)formamidine and, after hydrolysis to methyl *C*-(2-deoxy-D-*lyxo*-hex-1-enopyranosyl)formate, into *N*-benzyl-*C*-(2-deoxy-D-*lyxo*-hex-1-enopyranosyl)formamide. A series of *C*-(β-D-galactopyranosyl) and *C*-(2-deoxy-D-*lyxo*-hex-1-enopyranosyl) derivatives was comparatively investigated for *E. coli* β-D-galactosidase inhibitory activity. *N*-Benzyl-*C*-(2-deoxy-D-*lyxo*-hex-1-enopyranosyl)formamidine was the best inhibitor and had $K_i = 6$ μM (on the basis of its free base concentration, $K_i = 8.3$ nM was obtained). Basicity and hydrophobicity of the aglycon proved to be more important factors for the inhibition than the conformation of the sugar ring. © 1996 Elsevier Science Ltd.

Keywords: *C*-Glycosides; D-Galactal derivatives; Radical-mediated bromination; Reductive elimination; Zinc/*N*-methylimidazole reagent; *E. coli* β-D-galactosidase; Enzyme inhibition

* Corresponding author.

[1] Dedicated to Professor István Farkas on the occasion of his 70th birthday.

[2] This work was presented in part at the *XVIth Int. Carbohydr. Symp.*, July 5–10, 1992, Paris, Book of Abstracts, p 49.

PII S0008-6215(96)00148-6

1. Introduction

Glycosidase enzymes continuously attract the attention of several disciplines [1–5] [3]. Their indirect influence on the biosynthesis of important cell-surface oligosaccharides has induced extensive synthetic activity to produce naturally occurring inhibitors and their analogues, also as tools for glycobiological manipulations [7]. On the other hand, glycosidase inhibitors may provide valuable information on the mode of action as well as the structure of the active sites of the corresponding enzymes [2,3,5].

It was shown that sugar derivatives with a planar structure around the C-1 position of the pyranose ring (e.g., lactone-, lactam-, glycal-, and C-1-exo-methylene-type compounds) efficiently inhibit several glycosidase enzymes [1,2,5]. This was attributed to the similarity of shape of these compounds and that of a glycosylium ion, the probable intermediate of the enzymatic reaction.

A more careful analysis of the inhibition of *E. coli* β-D-galactosidase by D-galactal revealed that the strong inhibition is due to the hydration of the double bond [8]. This process goes through a glycosyl-enzyme intermediate which decomposes very slowly. Thus, D-galactal itself is a rather weak inhibitor (see Table 1, Entry 1b). Two substituted D-galactal derivatives (–CHO, $-CH_2OH$ attached to C-1; Table 1, Entries 2b and 6b) not hydrated by the enzyme have a similar inhibitory effect [9].

It was also shown for β-D-galactopyranosyl derivatives that basicity and hydrophobicity of the aglycon play decisive roles in determining the strength of inhibition [10,11].

With these considerations in mind we decided to prepare [12,13] and investigate as inhibitors several derivatives of D-galactal having C-1 substituents of different basicity and hydrophobicity. It was hoped that a comparison of the inhibition of the enzyme by these compounds and by β-D-galactopyranosyl derivatives with the same (or similar) aglycons might lead to conclusions on the effect of the half-chair (i.e., 'transition-state analogue') conformation of the sugar moiety.

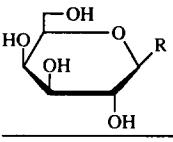
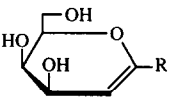
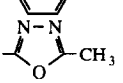
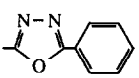
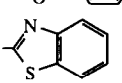
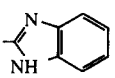
2. Results and discussion

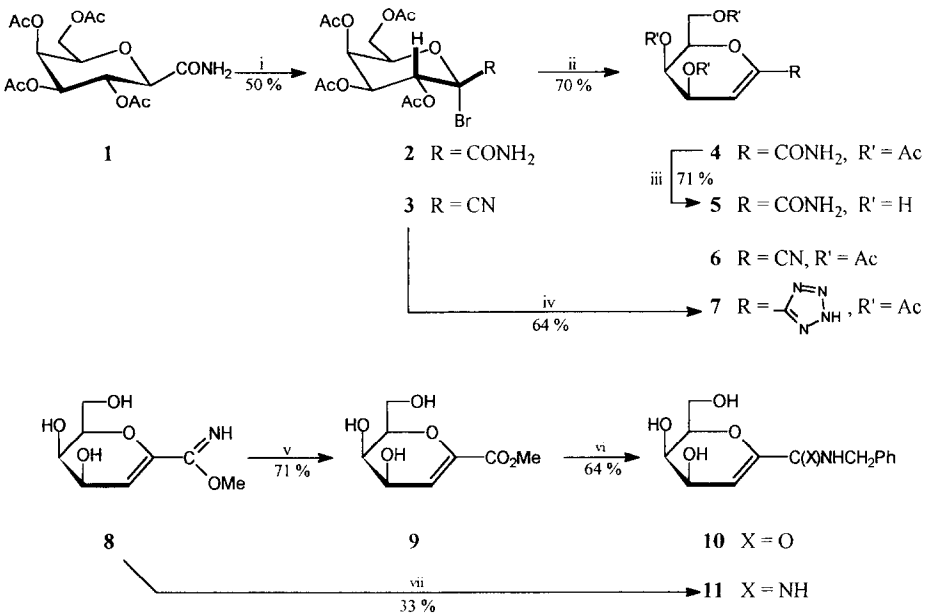
Syntheses of test-substances.—Most of the compounds of the *C*-(2-deoxy-D-*lyxo*-hex-1-enopyranosyl) (D-galactal) type have been described recently (Table 1, Entries 5b, 10b, 12b, ref. [12]; Entries 3b, 14b, 18b, 19b, 20b, ref. [13]). The *C*-(β-D-galactopyranosyl)heterocycles were prepared according to earlier methods (Table 1, Entries 5a, 12a, ref. [14]; Entries 10a, 11a, ref. [15]).

The synthesis of the unsaturated amide **5** was performed starting with **1** [16] (Scheme 1). Radical-mediated bromination [17] gave the bromide **2** showing the high radical-stabilizing capacity of the carboxamido group. The relatively low yield is similar to that obtained recently with a furanosyl derivative [18]. According to earlier considerations [19] the anomeric configuration of **2** was deduced from the downfield shift of H-3 and H-5 (0.21 and 0.54 ppm, respectively) in **2** as compared to those in **1**. This indicated that the bromine was *cis*-oriented to these protons in the $^4C_1$ conformation. The proton

[3] For a collection of papers on enzymic glycoside hydrolysis and glycosyl transfer see ref. [6].

Table 1
Inhibition constants (K_i [μM]) measured for *E. coli* β-D-galactosidase

Entry	Aglycon group (R)	 a	 b	K_{ib}/K_{ia}	pK_a of the aglycon [a]
1	–H		14 [26] (10000 [f] [26])		
2	–CHO		11000 [9]		– 10 [28]
3	–CN	4000 (2010 [11])	12400	3.1	– 10 [28]
4	$-COOCH_3$	2560 [11]	no inhibition		– 10 [28]
5		no inhibition	no inhibition		– 3 [29]
6	$-CH_2OH$	10590 [11]	22000 [9]	2.07	– 2 [28]
7	$-CONH_2$	9850 [11]	20600	2.09	– 1 [28]
8	$-CONHCH_2Ph$	821 [11]	760	0.92	
9		450 [26]			
10		6840	550	0.08	
11		15			
12		650	insoluble	23.8 [b]	1.2 [30]
13			15500		5.5 [30]
14	$-C(NH)OCH_3$		3400		5.6 [25]
15	$-CH_2NHCH_2Ph$	2.3 [11] free base: 0.2 [11]			8.3 [e] [11]
16	$-CH_2NH_2$	505 [11] free base: 7.8 [11]			9.1 [e] [11]
17	$-C(NH)NHCH_2Ph$		6 free base: 0.0083	3 [c]	10.1 [e]
18	$-C(NH)NH_2$		520 free base: 0.43	1.02 [d]	10.3 [e]
19			570		~ 10 [31]
20	$-C(NH)NHNH_2$		820		~ 10 [32]
21	$-NHCH_2Ph$	0.0095 [10]			~ 11 [28]
22	$-NH_2$	7 [10]			10–11 [28]
23	$-SCH_2Ph$	3.3 [34]			
24	–OH	21000 [10]			



Scheme 1. i, NBS/Bz_2O_2, CCl_4/$CBrCl_3$, reflux, 2 h; ii, Zn/*N*-methylimidazole, EtOAc, reflux, 1 h; iii, NaOMe/MeOH; iv, Zn/*N*-methylimidazole, PhH, reflux, 1 h, then NaN_3, NH_4Cl, DMF, room temp., 6 days; v, H^+ (Amberlyst 15)/H_2O, room temp., 2 h; vi, $PhCH_2NH_2$/MeOH, reflux, 1 h; vii, $PhCH_2NH_2$/EtOH, reflux, 5 h.

coupled ^{13}C spectrum contained a broad singlet for $CONH_2$, suggesting a $^3J_{H\text{-}2,CONH_2}$ < 1 Hz heteronuclear coupling from which the *gauche* orientation of the nuclei involved follows in the given conformation. Reductive elimination with zinc–*N*-methylimidazole [20,21] gave the amide **4** which was deacetylated to **5** by the Zemplén method. Attempted preparations of **4** by elimination of acetic acid with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) from **1** or by partial hydrolysis of the cyano group in the 1-cyano-galactal [20] **6** with HBr–AcOH [22] or $TiCl_4$–H_2O–AcOH [16] resulted in complex mixtures.

The preparation of the unsaturated tetrazole **7** [12] has been improved by using [21] activated zinc and *N*-methylimidazole for the reductive elimination step from **3**. The ring closure of the raw 1-cyano-galactal **6** by azide ions [12] was performed at room temperature and gave **7** in 64% yield for the two steps.

Notes to Table 1:

[a] Values for the given functional groups/heterocycles attached to simple alkyl groups or hydrogen from the indicated references [9–11,25,26,28–32,34].

[b] K_{i13b}/K_{i12a}.

[c] K_{i17b}/K_{i15a}.

[d] K_{i18b}/K_{i16a}.

[e] Exactly measured value for the given sugar derivative.

[f] Inhibition constant from pre-steady state rates.

The *N*-benzyl-amide **10** was prepared with benzylamine from the ester **9** (for similar compounds see refs. [23,24]) which was obtained from imidate **8** [13] by acid-catalyzed hydrolysis. The reaction of **8** with benzylamine gave directly the *N*-benzyl-amidine **11**.

*p*K$_a$ *Determinations.*—The dissociation constants of **11** and its unsubstituted analogue (Table 1, Entries 17b and 18b) were determined by pH-metric titrations. The pK_a values (10.1 and 10.3, respectively) proved to be lower as compared to those of simple amidines [25] (acetamidine, 12.4; benzamidine, 11.6).

Enzymatic investigations.—Inhibition constants (K_i) were measured with *E. coli* β-D-galactosidase (EC 3.2.1.23), using 4-nitrophenyl β-D-galactopyranoside as substrate. All the investigated compounds inhibited the enzyme in a competitive fashion. The results together with literature values are collected in Table 1, and are arranged in the approximate order of basicity of the aglycon.

The trend of increasing inhibition with stronger basicity and higher hydrophobicity of the aglycon established for *C*-(β-D-galactopyranosyl) derivatives (for an illustration by literature examples, see Table 1: Entries 3a, 4a, 6a–8a, 15a, 16a; for other derivatives, Entries 21a–24a) can also be observed for the unsaturated analogues (see pairs of Entries 7b, 18b; 8b, 17b; 14b, 18b–20b for increasing basicity; pairs of Entries 7b, 8b; 18b, 17b for increasing hydrophobicity).

It is interesting to point out that 2-(β-D-galactopyranosyl)benzothiazole is a similar inhibitor as β-D-galactopyranosylbenzene [26] (Entries 12a and 9a), while the 5-methyl-1,3,4-oxadiazole derivative (Entry 10a) is one order of magnitude weaker. This may be due to the less hydrophobic character of the oxadiazole ring. However, changing the 5-methyl to a 5-phenyl group (Entry 11a) strengthens the inhibition significantly (more than two orders of magnitude), indicating that the position of the hydrophobic group (compare Entry 9a) is also of great importance.

Comparison of the inhibition by 2-deoxy-D-*lyxo*-hex-1-enopyranosyl derivatives with that of the corresponding β-D-galactopyranosyl compounds (Table 1, see K_{ib}/K_{ia} values) shows that the unsaturated substances are generally weaker inhibitors. This is in keeping with the fact that glycals, because of lack of the 2-OH substituent, typically bind worse to glycosidases than do the corresponding glycosides [5]. A further point could be that the C-1 substituent in the D-galactal derivatives cannot fit well into the active site of the enzyme. The best example for this may be the benzimidazole/benzothiazole pair (Entries 12a and 13b). (Because of insufficient solubility the unsaturated benzothiazole (Entry 12b) could not be investigated.) Obviously, the benzene part of the benzimidazole cannot be correctly placed into the hydrophobic cleft because of the rigidity of structure around C-1. Therefore, in spite of its higher basicity, this is a much less efficient inhibitor than the 2-(β-D-galactopyranosyl)benzothiazole.

The *N*-benzyl-amide derivatives (Entry 8) are almost equally efficient inhibitors. Molecular modelling has revealed that the orientation of the phenyl group is superimposable in the two derivatives (Fig. 1). Thus, the hydrophobic affinity of the enzyme towards the phenyl group can be the same in both cases.

Similar efficiencies can also be seen for the strongly basic derivatives (Entries 15a, 17b; 16a, 18b). (Unfortunately, our attempts to prepare the unsaturated analogues of the *C*-(β-D-galactopyranosyl)methylamines by reduction of the unsaturated amides **4**, **5**, and **10**, as well as the 1-cyano-galactal **6**, by several methods have failed; therefore direct

Fig. 1. View of conformations adopted in aqueous medium by *N*-benzyl-*C*-(β-D-galactopyranosyl)formamide (Entry 8a; top) and *N*-benzyl-*C*-(2-deoxy-D-*lyxo*-hex-1-enopyranosyl)formamide (Entry 8b; bottom) generated by Macromodel software.

comparison is impossible.) Although the amidines are somewhat stronger bases than the methylamines they are not more powerful inhibitors on the basis of their total concentration. However, taking into account the concentration of the free bases, that is the actual inhibitors [3,10,11,27], we found a significantly stronger inhibition by the amidines (Entries 17b, 18b) than by the methylamines [11] (Entries 15a, 16a). This finding may reveal that aglycon basicity and hydrophobic interactions with the enzyme are equally important, and that this is independent of the presence of a 1,2-double bond in the galactosyl moiety.

The only example in our study showing clearly higher inhibitory activity for the derivative of the D-galactal type is the pair of 5-methyl-1,3,4-oxadiazoles (Entry 10). We cannot provide a clearcut explanation for this behaviour because the neutral [33] and weakly hydrophobic character of this aglycon means that the stronger inhibition may also be attributed to the half-chair conformation of the ring.

In conclusion, we have shown for the majority of a series of *C*-(β-D-galactopyranosyl) and *C*-(2-deoxy-D-*lyxo*-hex-1-enopyranosyl) compounds with the same (or similar) groups attached to C-1 that the introduction of 1,2-unsaturation into the molecule generally does not increase (or even lowers) the enzyme inhibitory activity. The basicity and/or the hydrophobicity of the aglycon are much more important factors in binding the inhibitor to the enzyme than the conformation of the sugar moiety. The change in the conformation (i.e., the 'transition-state analogy') of the sugar ring seems to be effective only in that case when the electrostatic and/or hydrophobic interactions do not override its influence.

3. Experimental

Materials.—*E. coli* β-D-galactosidase (EC 3.2.1.23) and 4-nitrophenyl β-D-galactopyranoside were purchased from Sigma.

General methods.—Melting points were measured in open capillary tubes or on a Kofler hot-stage and are uncorrected. Optical rotations were determined with a Perkin–Elmer 241 polarimeter at room temperature. NMR spectra were recorded with a Bruker WP 200 SY spectrometer (^{1}H, 200 MHz; ^{13}C, 50.3 MHz). TLC was performed on DC-Alurolle, Kieselgel 60 F_{254} (Merck), and the plates were visualised by gentle heating. Organic solutions were dried over anhyd $MgSO_4$ and concentrated in vacuo at 40–50 °C (water bath).

C-*[(1S)-2,3,4,6-Tetra*-O-*acetyl-1-bromo*-D-*galactopyranosyl]formamide (3,4,5,7-tetra*-O-*acetyl-α*-D-*galacto-hept-2-ulopyranosylonamide bromide)* (**2**).—A mixture of *C*-(2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranosyl)formamide [16] (**1**; 0.94 g, 2.5 mmol), *N*-bromosuccinimide (1.8 g, 10 mmol), and benzoyl peroxide (0.06 g, 0.25 mmol) was refluxed in CCl_4 (40 mL) and $CBrCl_3$ (10 mL) for 1 h. After cooling, CH_2Cl_2 was added to dissolve all the solids and the solution was washed with aq 5% $NaHSO_3$ and water (3 ×). After drying the solvents were removed and the residue was crystallised from abs EtOH to give **2** (0.57 g, 50%); mp 164–167 °C; $[\alpha]_D$ +138° (*c* 2.30, $CHCl_3$); ^{1}H NMR ($CDCl_3$): *δ* 6.49, 6.03 (2 br s, 2 H, $CONH_2$), 5.54 (dd, 1 H, $J_{4,5}$ 1 Hz, H-4), 5.43 (d, 1 H, $J_{2,3}$ 10.3 Hz, H-2), 5.31 (dd, 1 H, $J_{3,4}$ 3 Hz, H-3), 4.53 (ddd, 1 H, $J_{5,6a}$ 6.9 Hz, H-5), 4.32 (dd, 1 H, $J_{6a,6b}$ 11.5 Hz, H-6a), 4.16 (dd, 1 H, $J_{5,6b}$ 5.5 Hz, H-6b), 2.18, 2.12, 2.09, 1.99 (4 s, 12 H, 4 Ac); ^{13}C NMR ($CDCl_3$): *δ* 170.41, 169.80, 169.26 (C=O), 167.27 ($CONH_2$, $^3J_{H\text{-}2,CONH_2}$ < 1 Hz), 94.13 (C-1), 73.54, 69.68, 66.61, 66.45 (C-2,3,4,5), 60.82 (C-6), 20.77, 20.48, 20.39 (Me). Anal. Calcd for $C_{15}H_{20}BrNO_{10}$ (454.2): C, 39.66; H, 4.44; Br, 17.59; N, 3.08. Found: C, 39.74; H, 4.32; N, 3.08; Br, 17.62; N, 3.08.

C-*(3,4,6-Tri*-O-*acetyl-2-deoxy*-D-lyxo-*hex-1-enopyranosyl)formamide (4,5,7-tri*-O-*acetyl-2,6-anhydro-3-deoxy*-D-lyxo-*hept-2-enonamide)* (**4**).—A mixture of **2** (0.82 g, 1.8 mmol) and Zn dust (0.9 g; activated by successive washing with 2 M HCl, water, acetone, and Et_2O followed by air-drying on a glass filter) was heated to reflux, and then *N*-methylimidazole (0.285 mL, 3.6 mmol) was added in one portion. Boiling with intensive stirring was continued for 1 h. The mixture was then diluted with EtOAc and filtered, and the filtrate was washed with 2 M HCl, satd aq $NaHCO_3$, and water. Removal of the solvent after drying gave chromatographically pure crystalline **4** (0.4 g, 70%); mp 130–132 °C. An analytical sample was recrystallised from EtOH (0.26 g, 65% recovery); mp 135–136 °C; $[\alpha]_D$ −47° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): *δ* 6.42, 6.11 (2 br s, 2 H, $CONH_2$), 5.91 (dd, 1 H, $J_{2,3}$ 2, $J_{2,4}$ 2 Hz, H-2), 5.68 (ddd, 1 H, $J_{3,4}$ 4, $J_{3,5}$ 1.5 Hz, H-3), 5.48 (ddd, 1 H, $J_{4,5}$ 1.5 Hz, H-4), 4.45 (dddd, 1 H, $J_{5,6a}$ 5.5 Hz, H-5), 4.36 (dd, 1 H, $J_{6a,6b}$ 11.4 Hz, H-6a), 4.25 (dd, 1 H, $J_{5,6b}$ 5 Hz, H-6b), 2.12, 2.10, 2.04 (3 s, 9 H, 3 Ac); ^{13}C NMR ($CDCl_3$): *δ* 170.26, 169.89 (C=O), 162.67 ($CONH_2$), 146.41 (C-1), 103.70 (C-2), 74.22, 64.48, 62.64 (C-3,4,5), 61.54 (C-6). Anal. Calcd for $C_{13}H_{17}NO_8$ (315.3): C, 49.52; H, 5.43; N, 4.44. Found: C, 50.19; H, 5.48; N, 4.43.

C-*(2-Deoxy*-D-lyxo-*hex-1-enopyranosyl)formamide (2,6-anhydro-3-deoxy*-D-lyxo-*hept-2-enonamide)* (**5**).—To a solution of **4** (200 mg, 0.63 mmol) in MeOH (2 mL) was added one drop of 1 M methanolic NaOMe. Crystallisation began shortly and the mixture was stirred at room temperature for 1 h. After keeping it in a refrigerator for several hours filtration gave **5** (85 mg, 71%); mp 228–232 °C; $[\alpha]_D$ −25° (*c* 1.0, H_2O);

^{1}H NMR (D_2O): δ 5.90 (dd, 1 H, $J_{2,3}$ 2.3, $J_{2,4}$ 1.7 Hz, H-2), 4.74 (ddd, 1 H, $J_{3,4}$ 4.5, $J_{3,5}$ ~ 1 Hz, H-3), 4.34 (dddd, 1 H, $J_{5,6a}$ 7.9 Hz, H-5), 4.13 (ddd, 1 H, $J_{4,5}$ 0.8 Hz, H-4), 4.08 (dd, 1 H, $J_{6a,6b}$ 11.9 Hz, H-6a), 3.99 (dd, 1 H, $J_{5,6b}$ 4.5 Hz, H-6b); ^{13}C NMR (Me_2SO-d_6): δ 163.16 ($CONH_2$), 145.36 (C-1), 107.48 (C-2), 79.14, 64.49, 63.83 (C-3,4,5), 61.19 (C-6). Anal. Calcd for $C_7H_{11}NO_5$ (189.2): C, 44.44; H, 5.86; N, 7.40. Found: C, 44.45; H, 6.05; N, 7.19.

*Improved procedure for 5-(3,4,6-tri-*O*-acetyl-2-deoxy-*D*-lyxo-hex-1-enopyranosyl)tetrazole* (**7**).—Zinc dust (13 g; activated by washing with 2 M HCl, water, acetone, and Et_2O, then air-dried on a glass filter) was suspended in dry benzene (80 mL), and *N*-methylimidazole (3.2 mL, 0.04 mol) was added. The mixture was vigorously stirred with an efficient mechanical stirrer and heated to reflux. A solution of (1*R*)-2,3,4,6-tetra-*O*-acetyl-1-bromo-D-galactopyranosyl cyanide [19] (**3**; 8.74 g, 0.02 mol) in dry benzene (20 mL) was added dropwise to the above mixture during 20 min. Stirring and reflux were continued until TLC indicated the disappearance of the starting material (~ 30 min). Charcoal was added and the warm reaction mixture was filtered on a Celite bed. The filtrate was washed successively with 2 M HCl, satd aq $NaHCO_3$, and aq NaCl. After drying, evaporation of the solvent left 5.02 g (0.0169 mol, 84%) of the per-*O*-acetylated 1-cyano-D-galactal **6** as white crystals; mp 111–113 °C, lit [20] mp 113–115 °C for the purified material. This crude product was dissolved in dry *N*,*N*-dimethylformamide (25 mL), NaN_3 (2.18 g, 0.0335 mol) and NH_4Cl (1.80 g, 0.0335 mol) were added, and the mixture was stirred at room temperature for 6 days. It was then diluted with water, acidified with 2 M HCl, and extracted by EtOAc (3 ×). The organic phase was washed with water (3 ×) and satd aq NaCl, dried, and concentrated to a crystalline mass. This was dissolved in warm acetone (~ 30 mL), hexane (~ 100 mL) was added, and cooling then gave **7** (4.37 g, 76%; 64% from **3**); mp with decomposition from 182 °C, lit [12] mp 174–177 °C; $[\alpha]_D$ −59° (*c* 1.08, pyridine), lit [12] −53°.

Methyl C-*(2-deoxy-*D*-lyxo-hex-1-enopyranosyl)formate (methyl 2,6-anhydro-3-deoxy-*D*-lyxo-hept-2-enonate)* (**9**).—Methyl 2,6-anhydro-3-deoxy-D-*lyxo*-hept-2-enonimidate [13] (**8**; 1 g, 4.92 mmol) was dissolved in warm water (50 mL) and the solution then cooled down to room temperature. Amberlyst 15 (H^+ form) [2 g, rinsed by water (20 mL)] was added and the mixture was stirred at room temperature for 1 h. The resin was removed and the water evaporated to leave a crystalline solid which was dried in a vacuum desiccator over P_4O_{10} to give **9** (0.71 g, 71%); mp 140–145 °C. An analytical sample was obtained by recrystallisation from MeOH; mp 143–144 °C; $[\alpha]_D$ −42° (*c* 1.03, H_2O); ^{1}H NMR (D_2O): δ 5.96 (dd, 1 H, $J_{2,3}$ 2.5, $J_{2,4}$ 1.5 Hz, H-2), 4.62 (ddd, 1 H, $J_{3,4}$ 4.3, $J_{3,5}$ ~ 1 Hz, H-3), 4.18 (dddd, 1 H, $J_{5,6a}$ 7.6 Hz, H-5), 4.01 (ddd, 1 H, $J_{4,5}$ ≤ 1 Hz, H-4), 3.93 (dd, 1 H, $J_{6a,6b}$ 12 Hz, H-6a), 3.83 (dd, 1 H, $J_{5,6b}$ 4.8 Hz, H-6b), 3.82 (s, 3 H, CH_3); ^{13}C NMR (Me_2SO-d_6): δ 164.88 (CO_2CH_3), 143.65 (C-1), 113.26 (C-2), 79.19, 65.36, 64.46 (C-3,4,5), 61.79 (C-6), 53.56 (CO_2CH_3). Anal. Calcd for $C_8H_{12}O_6$ (204.2): C, 47.05; H, 5.92. Found: C, 46.78; H, 5.80.

N-*Benzyl*-C-*(2-deoxy-*D*-lyxo-hex-1-enopyranosyl)formamide (2,6-anhydro-*N*-benzyl-3-deoxy-*D*-lyxo-hept-2-enonamide)* (**10**).—A mixture of **9** (1.85 g, 9 mmol) and benzylamine (2.3 mL, 21 mmol) was refluxed in MeOH (8 mL) for 3 h. The solvent was evaporated and the crystalline residue was triturated with Et_2O. The solid was filtered

off and recrystallised from THF. After filtration the crystals were thoroughly washed with Et_2O to give **10** (1.61 g, 64%); mp 119–120 °C. An analytical sample was obtained by a further recrystallisation from THF; mp 123–124 °C; $[\alpha]_D$ −16° (*c* 1.12, H_2O); ^1H NMR (D_2O): δ 7.42–7.29 (m, 5 H, aromatics), 5.75 (dd, 1 H, $J_{2,3}$ ~ 3, $J_{2,4}$ ~ 2 Hz, H-2), 4.59 (ddd, 1 H, $J_{3,4}$ ~ 5, $J_{3,5}$ ~ 1 Hz, H-3), 4.47 (s, 2 H, CH_2), 4.18 (dddd, 1 H, $J_{5,6a}$ ~ 7 Hz, H-5), 3.98 (ddd, 1 H, $J_{4,5}$ ~ 1 Hz, H-4), 3.88 (dd, 1 H, $J_{6a,6b}$ 12 Hz, H-6a), 3.84 (dd, 1 H, $J_{5,6b}$ ~ 5 Hz, H-6b); ^{13}C NMR (Me_2SO-d_6): δ 162.24 (CONH), 145.55 (C-1), 139.61, 129.07, 128.06, 127.72 (aromatics), 108.45 (C-2), 79.61, 64.88, 64.28 (C-3,4,5), 61.72 (C-6), 42.59 (CH_2). Anal. Calcd for $C_{14}H_{17}NO_5$ (279.3): C, 60.20; H, 6.13; N, 5.01. Found: C, 59.87; H, 6.33; N, 5.06.

N-*Benzyl*-C-*(2-deoxy*-D-lyxo-*hex-1-enopyranosyl)formamidine (2,6-anhydro*-N-*benzyl-3-deoxy*-D-lyxo-*hept-2-enonamidine)* (**11**).—A mixture of methyl 2,6-anhydro-3-deoxy-D-*lyxo*-hept-2-enonimidate [13] (**8**; 0.203 g, 1 mmol) and benzylamine (0.33 mL, 3 mmol) was refluxed in abs EtOH (5 mL) for 5 h. The solvent was evaporated and the residue was triturated with Et_2O. The insoluble material was applied to a short column of silica gel, and was eluted by MeOH. Unreacted **8** contaminated with an unidentified byproduct was collected first, then the last fractions gave **11** as a solid foam (0.093 g, 33%); R_f 0.11 (7:3 $CHCl_3$–MeOH); $[\alpha]_D$ −13° (*c* 1.16, H_2O); ^1H NMR (D_2O): δ 7.45–7.28 (m, 5 H, aromatics), 5.57 (dd, 1 H, $J_{2,3}$ ~ 2.5, $J_{2,4}$ ~ 1 Hz, H-2), 4.61 (ddd, 1 H, $J_{3,4}$ ~ 4.5, $J_{3,5}$ ~ 1 Hz, H-3), 4.45 (s, 2 H, CH_2), 4.21 (dddd, 1 H, $J_{5,6a}$ ~ 8 Hz, H-5), 4.01 (ddd, 1 H, $J_{4,5}$ ~ 1 Hz, H-4), 3.96 (dd, 1 H, $J_{6a,6b}$ 12 Hz, H-6a), 3.85 (dd, 1 H, $J_{5,6b}$ 4.5 Hz, H-6b); ^{13}C NMR (D_2O): δ 158.35 [C(NH)NH], 146.51 (C-1), 138.73, 129.41, 128.01 (aromatics), 105.01 (C-2), 79.28, 65.31, 64.72 (C-3,4,5), 62.03 (C-6), 46.29 (CH_2). Anal. Calcd for $C_{14}H_{18}N_2O_4$ (278.3): C, 60.42; H, 6.51; N, 10.06. Found: C, 60.11; H, 6.62; N, 10.01.

pK_a *Determinations.*—Aqueous solutions of the totally protonated samples (2–4 mM) were titrated by a KOH solution (0.1722 M) from an automatic buret (Radiometer ABU 13) using pH-metric detection (Radiometer pHM 93, K401 reference-electrode, G202C measuring-electrode) at 25 ± 0.1 °C under argon (I = 0.2 M KCl). Experimental data were evaluated by 'SUPERQUAD' computer software.

Enzyme assays.—β-D-Galactosidase activities were measured in 0.1 M phosphate buffer (pH 7.3) containing 0.1 M $MgCl_2$ at 37 °C. The total volume was 1 mL. The reaction was initiated by addition of the substrate. It was allowed to proceed for 10 min then stopped by the addition of 0.5 M borate buffer (2 mL, pH 10). The concentration of *p*-nitrophenolate was measured spectrophotometrically at 400 nm. The inhibitors were dissolved in the same buffer as above. Data were determined from Dixon plots using a Grafit computer programme.

Acknowledgements

This work was supported by the Hungarian National Science Foundation (Grant No. OTKA I/3 1721.). The authors are grateful to Professor E. Farkas for the pK_a determinations, Dr. Gy. Batta for the molecular modelling, and Mrs. L. Kóder and Miss R. Somosi for technical assistance.

References

[1] G.A. Levvy and S.M. Snaith, *Adv. Enzymol.*, 36 (1972) 151–181.
[2] P. Lalegerie, G. Legler, and J.M. Yon, *Biochimie*, 64 (1982) 977–1000.
[3] M.L. Sinnott, in M. Page (Ed.), *Enzyme Mechanisms*, The Royal Society of Chemistry, London, 1987, pp 259–297.
[4] M.L. Sinnott, *Chem. Rev.*, 90 (1990) 1171–1202.
[5] G. Legler, *Adv. Carbohydr. Chem. Biochem.*, 48 (1990) 319–384.
[6] *Carbohydr. Res.*, 250 (1993).
[7] B. Winchester and G.W.J. Fleet, *Glycobiology*, 2 (1992) 199–200.
[8] D.F. Wentworth and R. Wolfenden, *Biochemistry*, 13 (1974) 4715–4720.
[9] H.-M. Dettinger, G. Kurz, and J. Lehmann, *Carbohydr. Res.*, 74 (1979) 301–307.
[10] G. Legler and M. Herrchen, *Carbohydr. Res.*, 116 (1983) 95–103.
[11] J.N. BeMiller, R.J. Gilson, R.W. Myers, M.M. Santoro, and M.P. Yadav, *Carbohydr. Res.*, 250 (1993) 93–100.
[12] S.H. Mahmoud, L. Somsák, and I. Farkas, *Carbohydr. Res.*, 254 (1994) 91–104.
[13] L. Somsák, *Carbohydr. Res.*, 286 (1996) 167–171.
[14] I. Farkas, I.F. Szabó, and R. Bognár, *Carbohydr. Res.*, 56 (1977) 404–406.
[15] I. Farkas, I.F. Szabó, L. Szilágyi, and R. Bognár, *Khim. Geterotsikl. Soedin.*, (1978) 893–896.
[16] J.N. BeMiller, M.P. Yadav, V.N. Kalabokis, and R.W. Myers, *Carbohydr. Res.*, 200 (1990) 111–126.
[17] L. Somsák and R.J. Ferrier, *Adv. Carbohydr. Chem. Biochem.*, 49 (1991) 37–92.
[18] P.M. Harrington and M.E. Jung, *Tetrahedron Lett.*, 35 (1994) 5145–5148.
[19] L. Somsák, Gy. Batta, and I. Farkas, *Carbohydr. Res.*, 124 (1983) 43–51.
[20] L. Somsák, I. Bajza, and Gy. Batta, *Liebigs Ann. Chem.*, (1990) 1265–1268.
[21] L. Somsák and I. Németh, *J. Carbohydr. Chem.*, 12 (1993) 679–684.
[22] R.W. Myers and Y.C. Lee, *Carbohydr. Res.*, 152 (1986) 143–158.
[23] P.A. Driguez, B. Barrere, G. Quash, and A. Doutheau, *Carbohydr. Res.*, 262 (1994) 297–310.
[24] A. Banaszek, *Tetrahedron*, 51 (1995) 4231–4238.
[25] G. Häfelfinger, in S. Patai (Ed.), *The Chemistry of Amidines and Imidates*, Wiley, 1975, pp 14–15.
[26] R.S.T. Loeffler, M.L. Sinnott, B.D. Sykes, and S.G. Withers, *Biochem. J.*, 177 (1979) 145–152.
[27] M.P. Dale, H.E. Ensley, K. Kern, K.A.R. Sastry, and L.D. Byers, *Biochemistry*, 24 (1985) 3530–3539.
[28] J. March, *Advanced Organic Chemistry*, 4th ed., Wiley, 1992, pp 250–252.
[29] R.N. Butler, in A.R. Katritzky (Ed.), *Comprehensive Heterocyclic Chemistry*, Vol. 5, Pergamon, 1984, p 813.
[30] A.R. Katritzky and J.M. Lagowski, in A.R. Katritzky (Ed.), *Comprehensive Heterocyclic Chemistry*, Vol. 5, Pergamon, 1984, p 50.
[31] M.R. Grimmett, in A.R. Katritzky (Ed.), *Comprehensive Heterocyclic Chemistry*, Vol. 5, Pergamon, 1984, p 425.
[32] D.G. Neilson, R. Roger, J.W.M. Heatlie, and L.R. Newlands, *Chem. Rev.*, 70 (1970) 151–170.
[33] J. Hill, in A.R. Katritzky (Ed.), *Comprehensive Heterocyclic Chemistry*, Vol. 6, Pergamon, 1984, p 431.
[34] C.K. De Bruyne and M. Yde, *Carbohydr. Res.*, 56 (1977) 153–164.