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Photoinduced electron transfer and chemical α-deoxygenation of D-galactono-1,4-lactone. Synthesis of 2-deoxy-D-*lyxo*-hexofuranosides

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Dedicated to Professor Derek Horton on the occasion of his 70th birthday

Abstract

Two simple procedures for the synthesis of 2-deoxy-D-*lyxo*-hexono-1,4-lactone are described. Reductive cleavage of a 2-*O*-tosyl derivative of D-galactono-1,4-lactone in the presence of sodium iodide afforded the 2-deoxy derivative. On the other hand, α-deoxygenation of D-galactono-1,4-lactone was easily achieved by photochemical electron transfer deoxygenation of HO-2 as the 3-(trifluoromethyl)benzoate. Methyl 2-deoxy-β-D-*lyxo*-hexafuranoside ('methyl 2-deoxy-β-D-galactofuranoside') was synthesized and tested as substrate for exo β-D-galactofuranosidase from *Penicillium fellutanum*. The reaction was followed by HPAEC, showing that methyl 2-deoxy-β-D-galactofuranoside was not hydrolyzed by incubation with the enzyme. Neither the 2-deoxy lactone, nor the 2-deoxy-β-D-galactofuranoside acted as inhibitors of the reaction with the 4-nitrophenyl β-D-galactofuranoside. The present and our previous results show that the hydroxyl groups at C-2, C-3 and C-6 of the galactofuranoside are essential for interaction with the exo β-D-galactofuranosidase.

Keywords: Deoxy sugars; exo β-D-Galactofuranosidase; 2-Deoxy-D-*lyxo*-hexofuranosides; 2-Deoxy-D-*lyxo*-hexono-1,4-lactone

1. Introduction

The deoxy analogues of glycosides are useful for studies of the specificity of glycosidases. Galactofuranosidases and galactofuranosyltransferases are of great interest, because they are involved in the construction and metabolism of important glycoconjugates of pathogenic bacteria, protozoa and fungi.[1] As galactose in the furanoic configuration is not present in mammalian glycoconjugates, these enzymes are good targets for the development of antimicrobial agents. In this context, we have studied different aspects of the exo β-D-galactofuranosidase from *Penicillium fellutanum*, with the purpose of understanding the enzyme–substrate interaction. This enzyme was first studied by Gander and co-workers[2,3] We have synthesized substrates[4,5] and inhibitors,[6,7] and we recently studied the influence of the inhibitors on the culture of the microorganism.[8] An affinity chromatography system for purification of the enzyme was reported.[9]

Concerning the specificity of the exo β-D-galactofuranosidase, we have previously described simple approaches for the synthesis of 3-deoxy[10] and 6-deoxy analogues of the substrate,[11] showing that the hydroxyl groups at C-6 and C-3 of the galactofuranoside are necessary for recognition by the enzyme. The synthesis of glycosides deoxygenated at the 2-position is described in the present work in order to gain further knowledge about the glyconic specificity of this enzyme.

D-Galactono-1,4-lactone (**1**) was chosen as precursor of the furanoic sugar. The lactone was α-deoxygenated by two alternative methods. The chemical synthesis involved reductive cleavage of a 2-*O*-tosyl derivative in the presence of sodium iodide to afford the 2-deoxy

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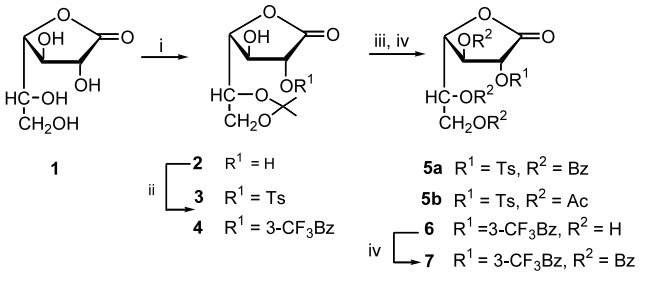
derivative. The other method is based on deoxygenation via a photoinduced electron-transfer mechanism, of HO-2 as the 3-(trifluoromethyl)benzoyl derivative. The 2-deoxylactones were selectively reduced to the corresponding sugars with diisoamyl borane[12] and glycosylated.

On the other hand, it was interesting to test the behavior of the 2-deoxy-D-*lyxo*-hexofuranosides ('2-deoxygalactofuranosides') as substrates or inhibitors of the β-D-galactofuranosidase. In this respect, methyl 2′-deoxy-β-lactoside is a potent inhibitor of β-D-galactopyranosidase.[13]

2. Results and discussion

We have previously obtained crystalline per-*O*-benzoyl-α,β-D-galactofuranose by direct benzoylation of D-galactose at high temperature.[14] The mixture of the furanosic benzoates was used for the preparation of galactofuranosyl glycosides and disaccharides.[15] The simplicity of the procedure led us to try direct benzoylation of commercially available 2-deoxy-D-*lyxo*-hexose ('2-deoxy-D-galactose') for the synthesis of the furanosic benzoates. In this case a mixture with a low proportion of α,β-furanosic forms (~ 30%), was obtained as indicated by a ^{13}C NMR spectrum.

As for the preparation of the 3-deoxy[10] and 6-deoxy analogues,[11] we used D-galactono-1,4-lactone (**1**) as the precursor of the 5-membered ring, with the advantage of the differential reactivity of the HO-2 due to the inductive β-carbonyl effect. Selectivity between HO-2 and -6 is not possible with the more common protective groups like benzoyl. With the aim of obtaining a derivative of **1** with HO-2 selectively substituted, we treated 5,6-*O*-isopropylidene-D-galactono-1,4-lactone (**2**)[16] with one equivalent of 4-toluenesulfonyl chloride in acetone–pyridine at 0 °C. This reaction gave regiospecifically compound **3** (Scheme 1), as evidenced from its NMR spectra (Tables 1 and 2). The signal corresponding to H-3 (4.69 ppm) was coupled ($J_{3,OH}$ 2.9 Hz) with that observed at 3.5 ppm, which disappeared after interchange with D_2O. In the ^{13}C NMR spectrum of **3** (Table 2), the signal corresponding to C-2 was shifted downfield (79.1 ppm) with respect to the C-2 signal (74.6 ppm) of **2**.



Scheme 1. (i) 2,2-Dimethoxypropane–acetone; (ii) R^1Cl, pyridine, −10 °C; (iii) 4:1 AcOH–H_2O, 50–60 °C; (iv) R^2Cl, pyridine.

Successive treatment of **3** with acetic acid and either benzoylation or acetylation led to compounds **5a**,**b**.

Paulsen and Eberstein[17] transformed α,β-epoxyuloses into α-deoxy-uloses by the action of sodium iodide in acetone. In this reaction, the epoxide is opened by the iodide, leading to an iodohydrin intermediate. The iodide of this intermediate is reduced to the deoxy derivative by the action of another iodide. We extended this reaction to the α-tosylated galactonolactone. Thus, we treated derivative **5a**,**b** with sodium iodide in the presence of TFA to produce the 2-deoxylactone **8a**,**b** in 70% yield (Scheme 2). The presence of two double doublets at high fields, with a large J_{gem} (~ 18 Hz) in the ^1H NMR spectrum (Table 1), and signals at 34.9, 34.6 ppm in the ^{13}C NMR spectrum (Table 2) confirmed the identity of the deoxy lactones **8a**,**b**. However, the acetylated derivative **5b** afforded a considerable proportion of the product of β-elimination **9**, identified on the basis of the ^{13}C NMR spectra of analogous enonolactones[18] (Table 2).

The other route relied on deoxygenation of the 3-(trifluoromethyl)benzoyl derivatives **6** or **7** (Schemes 1 and 2), accomplished via a photoinduced electron-transfer (PET) reaction using 9-methylcarbazole as photosensitizer. This reaction was first used for the synthesis of 2-deoxyribonucleosides[19,20] and later applied to the deoxygenation of a disaccharide analogue of moenomycin A.[21] Now, we extended the PET reaction to the synthesis of 2-deoxylactones. In our case, photochemical deoxygenations were carried out with a Heraeus TQ, medium-pressure Hg lamp in a Pyrex reaction vessel. We first tried deoxygenation of the perbenzoylated derivative **7** that was obtained similarly to **5a**. Monitoring the reaction by ^{13}C NMR spectroscopy (Table 2), we observed in the methylene region, not only the signal at 34.9 ppm corresponding to C-2 of compound **8a**, but also signals at δ 32.6 and 27.9 (see Section 3), indicating that the deoxygenation was not regioselective and that benzoyl groups had been also reduced. On the other hand, the 3-(trifluoromethyl)benzoyl derivative **6**, with only the 2-*O*-ester group, was deoxygenated in 1 h. The NMR spectra confirmed that 2-deoxy-D-*lyxo*-hexono-1,4-lactone (**10**) was obtained as the only product (Tables 1 and 2). Lactone **10** was previously prepared by oxidation of 2-deoxy-D-*lyxo*-hexose with bromine.[22,23]

The lactonic group of derivatives **8a**,**b** was reduced to the corresponding lactols **11a**,**b** (Scheme 3) with diisoamylborane.[12] After workup and several evaporations with methanol to eliminate boric acid, TLC analysis showed the anomeric mixture of the free lactol as the main product along with a less polar product

Table 1
^{1}H NMR (200.1 MHz, $CDCl_3$) chemical shifts and $J_{H,H}$ coupling constants values for compounds **3**–**8**, **10**, **13**, **14**

Compd	H-1 ($J_{1,2}$) ($J_{1,2'}$)	H-2 ($J_{2,3}$)	H-2′ ($J_{2',3}$) ($J_{2,2'}$)	H-3 ($J_{3,4}$)	H-4 ($J_{4,5}$)	H-5 ($J_{5,6}$)	H-6	H-6′ ($J_{5,6'}$) ($J_{6,6'}$)
3		4.99		4.69	4.17	4.36	4.13	4.00
		(7.9)		(8.2)	(3.3)	(6.8)		(7.0) (8.8)
4		5.56		4.67	4.34	4.43	4.15	4.11
		(7.6)		(7.3)	(3.3)	(7.0)		(6.6) (8.8)
5a		5.63*		5.63*	4.91	5.91		4.66*
				(5.12)	(2.19)			
5b		5.23		5.42	4.54	5.32	4.28	4.20
		(5.84)		(5.48)	(2.2)	(5.20)		(6.2) (11.7)
6[a]		5.94		4.65	4.40	3.76		4.47*
		(9.8)		(8.4)	(1.8)			
7		6.05		5.93	5.06	6.00	4.79	4.70
		(6.2)		(5.8)	(2.7)	(6.3)		(6.2) (12.0)
8a		3.01	2.75	5.55	5.04	5.98	4.72	4.64
		(7.7)	(1.8) (17.8)		(2.2)	(5.1)		(7.0) (11.7)
8b		2.91	2.54	5.19	4.67	5.36	4.33	4.21
		(7.3)	(2.2) (18.6)			(5.5)		(6.6) (11.7)
10[b]		3.01	2.51	4.57	4.52	3.89	3.64	3.60
		(7.2)	(2.8) (18.7)	(5.5)	(2.6)	(5.5)		(7.2) (11.5)
13bα[c]	5.09	2.37	2.07	5.21	4.17*	5.25	4.34	4.17*
	(2.2) (5.4)	(7.2)	(–) (13.5)		(3.2)	(4.1)		(6.4) (11.8)
13bβ[c]	5.09	2.37	1.95	4.97	4.23	5.29	4.32	4.21
	(1.1) (5.5)	(8.4)	(2.5) (14.5)	(4.5)	(3.9)	(4.4)		(7.1) (11.6)
14α[c]	5.15	2.19	2.10	4.40	3.84		3.66*	3.56
	(2.4) (5.4)	(6.8)	(6.5) (13.8)		(5.0)			
14β[c]	5.12	2.29	1.85	4.30	3.97	3.75	3.65	3.57
	(5.5) (1.2)	(7.5)	(2.5) (14.4)		(3.7)	(4.6)	(7.8)	(7.8) (11.7)

[a] DMSO-d_6.
[b] D_2O.
[c] 500 MHz.
* Center of a complex multiplet.

that was isolated by column chromatography and characterized as the anomeric methyl glycosides **13a**,**b** (15%). In the case of the acetylated lactone **8b**, rearrangement to the pyranosic lactol was also observed. This result, and the fact that β-elimination of **5b** could not be avoided during the reduction with NaI, led us to choose the benzoylated derivatives for the synthetic sequence.

Lactol **11a** was acetylated to give **12** (Scheme 3) with the purpose to activate the anomeric HO for glycosylation reactions. However, many efforts for glycosylation of **12** were unsuccessful. Treatment of **12** with $SnCl_4$, and addition of either methanol[24] or 4-nitrophenol[11] (or the tetrabutylammonium salt of 4-nitrophenol[5]), did not afford the corresponding glycosides, indicating that in comparison with the galactofuranose derivative, the absence of HO-2 dramatically changes the reactivity of the anomeric center.

The fact that lactols **11a**,**b** had been easily glycosylated during the workup of the diisoamylborane reduction suggested that boron trifluoride could be a good promoter of the glycosylation reaction. Thus, compounds **11a**,**b** or **12** were treated with BF_3·MeOH, and methyl glycosides **13a**,**b** were obtained in both cases in ~90% yield as a 1:1 anomeric mixture. The lack of stereoselectivity in this reaction was predictable, due to the absence of a participating group at C-2. The anomeric mixture **13a** was chromatographically homogeneous, but **13b** could be resolved by column chromatography. However, the ^{1}H NMR spectra of both anomers of **13b** were almost identical because of the absence of a substituent at C-2. Nevertheless, the anomeric configuration could be assigned on the basis of the coupling constants observed in the ^{1}H NMR spectrum (Table 1).

The anomeric mixture **13a** was debenzoylated, and the free glycosides **14α** and **14β** were separated by fast silicagel column chromatography. The hydrolytic instability of the 2-deoxyglycofuranosides was shown by the formation of 2-deoxy-D-galactose during the purification. The more polar product (48%) gave $[\alpha]_D$ −65°, suggesting a β configuration, in agreement with the ^{1}H

Table 2
^{13}C NMR chemical shifts (50.3 MHz, $CDCl_3$) for compounds **2–14**

Compd	C-1	C-2	C-3	C-4	C-5	C-6
2	174.5	74.6	74.4*	79.9	74.1*	65.0
3	166.7	79.1	73.6	79.6	73.0	64.9
4	169.4	77.6	73.8	79.7	73.3	65.0
5a	166.7	76.0	73.6	79.5	69.5	62.1
5b	166.5	75.8	72.6	78.7	68.3	61.4
6[a]	170.1	76.0	69.9	79.9	67.9	61.6
7	171.6	73.6	73.0	78.8	69.8	62.3
8a	173.7	34.9	72.1	83.2	70.8	62.3
8b	173.7	34.6	71.0	82.4	70.5	61.5
9*Z*		138.6	124.5	146.5	110.7	58.6
9*E*		140.3	121.0	146.9	109.8	58.3
10[b]	180.4	38.4	71.7	88.9	69.8	62.9
11aα,β	104.4	39.4	75.1	82.3	71.8	63.9
	103.7	39.3	74.6	82.3	71.1	63.4
12α,β	98.5	38.4	74.1	83.2	70.8	63.1
	97.9	38.5	74.6	84.3	71.0	63.1
13bα,β	105.2	39.0	73.9	81.6	70.9	62.8
	104.9	39.1	73.7	80.6	70.2	62.8
14α[b]	105.9	41.0	73.0	86.3	71.7	63.4
14β[b]	106.3	41.2	72.2*	86.1	72.4*	63.6

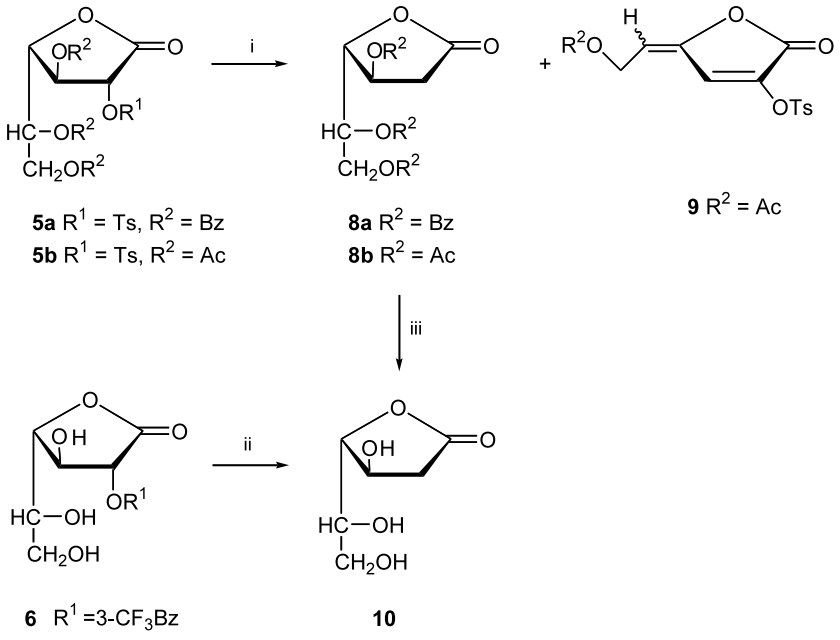
[a] DMSO-d_6.
[b] D_2O.
* Interchangeable.

NMR spectrum (Table 1). The H-2′, in a trans relationship with both H-3 and H-1, indicated that these hydrogens were oriented to the same face, so the anomeric configuration was β. The less polar methyl 2-deoxy-α-D-*lyxo*-hexofuranoside (**14α**) was isolated with 48% yield and gave $[\alpha]_D$ 40°. The methyl α,β glycosides **14**, were prepared by Fisher glycosylation, but they were not fully characterized.[23]

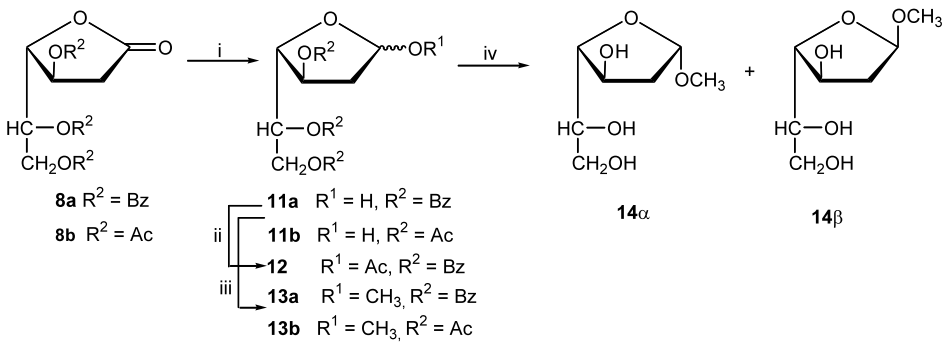
We also considered the advantage of performing the deoxygenation step after the reduction of the lactone group and with further glycosylation so the anchimeric effect of the substituent at HO-2 would be effective for stereoselective β-glycosylation. However, neither chemical deoxygenating of the 2-*O*-tosyl glycosides with NaI, nor PET deoxygenation of β-D-galactofuranosides derivatized with 3-(trifluoromethyl)benzoyl at HO-2, were successful.

This is the first report on the PET reaction applied to an aldonolactone, and the effectiveness of this strategy could be attributed to the combination of two factors: the selectivity of the substitution because of the lactonic carbonyl group, and the activation of the photochemical reaction, due to the formation of a radical intermediate stabilized by conjugation with the carbonyl group. However, the stability of the resulting radical would not account for deoxygenation at other ring carbons when benzoyl groups are present. Further work is in progress to identify the products and to study the kinetics of this reaction.

The biological interactions of the exo β-D-galactofuranosidase from *P. fellutanum* with the 2-deoxylactone **10** and the 2-deoxyglycosides **14α** and **14β** were investigated. We have previously reported[6] that D-galactono-1,4-lactone (**1**) is a good inhibitor of β-D-galactofuranosidase. Thus, we studied the inhibitory properties of the 2-deoxylactone **10** by incubating the enzyme in the presence of both the substrate 4-nitro-



Scheme 2. (i) NaI, TFA, acetone; (ii) 9-methylcarbazole, 9:1 *n*-PrOH–H_2O, *hv*; (iii) HCl, aq MeOH, reflux.



Scheme 3. (i) DSB, THF; (ii) Ac_2O, pyridine; (iii) BF_3, MeOH; (iv) MeONa, MeOH.

phenyl β-D-galactofuranoside[4,6] and compound **10**. We also examined the hydrolytic activities on the 2-deoxygalactofuranosides **14α** and **14β** and their inhibitory properties.

After incubation, the enzymatic mixtures were analyzed by high-pH anion-exchange chromatography with pulse amperometric detection (HPAEC–PAD), which proved to be an excellent method for the resolution of 2-deoxygalactose and the methyl glycosides **14α,β** (Fig. 1). The absence of 2-deoxygalactose indicated that no hydrolysis of **14β** occurred (not shown). On the other hand, the activity of the enzyme towards 4-nitrophenyl β-D-galactofuranoside as substrate, was not affected by the presence of **10**, **14α** or **14β**, indicating that these compounds do not act as inhibitors, and thus no interaction with the enzyme takes place in the absence of HO-2. The methyl β-D-galactofuranoside had been used as substrate for the determination of the exo β-D-galactofuranosidase of *P. fellutanum*.[2] Many enzymes show broad substrate specificity, and deoxygenated analogues of the substrates can be hydrolyzed with a characteristic kinetics,[25] or act as inhibitors of the glycosidase.[12] However, the present and our previous results[9,10] show that the exo β-D-galactofuranosidase from *P. fellutanum* has a strict selectivity against the glycon structure, and that the hydroxyl groups at C-2,3 and 6 of the substrate are essential for interaction with the enzyme.

3. Experimental

General methods.—Thin-layer chromatography (TLC) was carried out on 0.2 mm Silica Gel 60 F_{254} (E. Merck) aluminum supported plates, using the following solvents: (a) 9:1 toluene–EtOAc, (b) 20:1 toluene–EtOAc, (c) 8:1 EtOAc–MeOH, (d) 19:1 EtOAc–MeOH. Detection was effected by exposure to UV light and by spraying with 10% (v/v) H_2SO_4 in EtOH and charring. Column chromatography was performed on Silica Gel 60 (200–400 mesh, E. Merck). Optical rotations were measured with a Perkin–Elmer 343 polarimeter. NMR spectra were recorded with a Bruker AC 200 spectrometer at 200 MHz (^{1}H) and 50 MHz (^{13}C) or with a Bruker AM 500 spectrometer at 500 MHz (^{1}H) and 125 MHz (^{13}C).

Benzoylation of 2-deoxy-D-lyxo-*hexose at high temperature.*—The procedure previously described for the preparation of penta-*O*-benzoyl-α,β-D-galactofuranose[14] was adapted for 2-deoxy-D-*lyxo*-hexose. A solution of 2-deoxy-D-*lyxo*-hexose (0.5 g, 2.77 mmol) in anhyd Py (7.0 mL) was stirred for 2 h in a water bath at 100 °C. The temperature was lowered to 60 °C, and BzCl was added dropwise. After 1.5 h at 60 °C the mixture was poured into ice-water, and the syrup that separated was dissolved in CH_2Cl_2 and sequentially washed with 5% HCl, satd aq $NaHCO_3$, and then dried (Na_2SO_4). The syrup obtained after evaporation of the solvent was a mixture of α-pyranosic and α,β-furanosic forms. ^{13}C NMR ($CDCl_3$) inter alia, δ: 99.3, 99.2 (C-1 α,β-*f*), 92.4 (C-1 α-*p*), 84.9, 83.6 (C-4 α,β-*f*), 63.4 (C-6 α,β-*f*), 62.4 (C-6 α-*p*), 38.9 (C-2 α,β-*f*), 31.5 (C-2 α-*p*). From the C-2 signals a 30% content of furanosic forms was estimated.

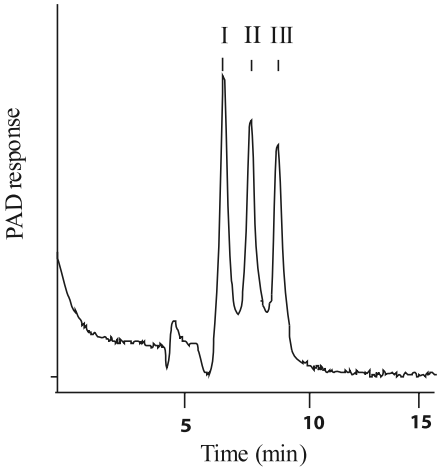


Fig. 1. HPAEC–PAD separation of 2-deoxy-D-*lyxo*-hexose (**I**), methyl 2-deoxy-β-D-*lyxo*-hexofuranoside (**II**) and methyl 2-deoxy-α-D-*lyxo*-hexofuranoside (**III**). A CarboPac MA-10 anion-exchange column under conditions described in Section 3 was used.

*5,6-Di-*O*-isopropylidene-2-*O*-tosyl-*D*-galactono-1,4-lactone* (**3**).—To a solution of 5,6-di-*O*-isopropylidene-D-galactono-1,4-lactone[16] (**2**, 5.8 g, 26 mmol) in dry Py (25 mL), cooled at 0 °C, 4-toluenesulfonyl chloride (5.6 g, 29 mmol) diluted in acetone (12 mL) was added during 1 h. After stirring for 4 h at 0 °C, the mixture was kept at 4 °C overnight. The mixture was then poured into ice-water and the syrup that separated was dissolved in CH_2Cl_2, and washed with HCl (5%), water, satd aq $NaHCO_3$, water, and then dried ($MgSO_4$). After evaporation of the solvent, the syrup that was obtained was recrystallized from toluene to afford compound **3** (6.8 g, 70%), mp 140–141 °C, $[\alpha]_D$ −94° (*c* 1, $CHCl_3$). Anal. Calcd for $C_{16}H_{20}O_8S$: C, 51.61; H, 5.41. Found: C, 51.85; H, 5.23.

*5,6-Di-*O*-isopropylidene-2-*O*-[3-(trifluromethyl)benzoyl]-*D*-galactono-1,4-lactone* (**4**).—To a solution of 5,6-di-*O*-isopropylidene-D-galactono-1,4-lactone[16] (**2**, 1.0 g, 4.5 mmol) in CH_2Cl_2 (15 mL), cooled at −15 °C, dry Py (1.5 mL) was added. 3-(Trifluromethyl)benzoyl chloride (0.81 mL, 5.4 mmol) diluted in CH_2Cl_2 (5.0 mL) was added during 2 h. After stirring for 4 h at −15 °C, the mixture was poured over ice-water and the resulting syrup was dissolved in CH_2Cl_2, and washed with HCl (5%), water, satd aq $NaHCO_3$, water, and then dried ($MgSO_4$). Evaporation of the solvent and recrystallization from toluene afforded compound **4** (2.23 g, 68%), mp 166–168 °C, $[\alpha]_D$ 21° (*c* 1.3, $CHCl_3$). Anal. Calcd for $C_{17}H_{17}F_3O_7$: C, 52.31; H, 4.39. Found: C, 52.26; H, 4.62.

*2-*O*-[3-(Trifluromethyl]benzoyl-*D*-galactono-1,4-lactone* (**6**).—Compound **4** (2.0 g, 5.73 mmol) was treated with 4:1 AcOH–water (40 mL) for 1.5 h at 60 °C. The solvent was evaporated under reduced pressure, and the remaining acid was removed by several evaporations with water. A white solid was obtained (1.68 g, 90%) that upon recrystallization from water gave R_f 0.65 (solvent c), mp 176–177 °C, $[\alpha]_D$ −7° (*c* 1, $CHCl_3$). Anal. Calcd for $C_{15}H_{16}F_3O_7$: C, 49.32; H, 4.41. Found: C, 49.27; H, 4.18.

*3,5,6-Tri-*O*-benzoyl-2-*O*-tosyl-*D*-galactono-1,4-lactone* (**5a**).—Compound **2** (1.5 g, 4.5 mmol) was treated with 4:1 AcOH–water (30 mL) at 60 °C during 1.5 h. After evaporation of the solvent and several co-evaporations with water, the syrup obtained was dried and treated with a solution of Py–BzCl (1:1, 10 mL) at 0 °C. After 2 h of stirring the mixture was poured over ice-water. The resulting syrup was extracted with CH_2Cl_2, and the extract was washed as usual and dried. Benzoic acid was eliminated by dissolution of the syrup in Et_2O and precipitation with hexane. Compound **5a** (2.30 g, 90%) was characterized as a syrup that gave $[\alpha]_D$ + 38° (*c* 1, $CHCl_3$). Anal. Calcd for $C_{34}H_{28}O_{11}S$: C, 63.35; H, 4.38. Found: C, 63.10; H, 4.50.

*3,5,6-Tri-*O*-acetyl-2-*O*-tosyl-*D*-galactono-1,4-lactone* (**5b**).—Compound **5b** was obtained in 96% yield by the procedure described for **5a**, but using Ac_2O instead of BzCl; mp 155–156 °C, $[\alpha]_D$ + 15° (*c* 1, $CHCl_3$).

*3,5,6-Tri-*O*-benzoyl-2-*O*-[3-(trifluromethyl)benzoyl]-*D*-galactono-1,4-lactone* (**7**).—Compound **6** (1.50 g, 4.10 mmol) was treated with 1:1 BzCl–Py (10.0 mL) at 0 °C for 3 h. After workup, compound **7** was obtained as a syrup (2.65 g, 93%), R_f 0.63 (solvent a), $[\alpha]_D$ + 2° (*c* 1, $CHCl_3$). Anal. Calcd for $C_{37}H_{32}F_3O_{10}$: C, 64.07; H, 4.65. Found: C, 64.32; H, 4.50.

*Deoxygenation step—(a) Chemical α-deoxygenation. 3,5,6-Tri-*O*-benzoyl-2-deoxy-*D*-lyxo-hexono-1,4-lactone* (**8a**). To a solution of compound **5a** (1.0 g, 1.6 mmol) in acetone (18 mL), trifluoroacetic acid (2 mL) and NaI (5.0 g, 33.0 mmol) were added. After 16 h of stirring the dark, mixture was diluted with CH_2Cl_2, washed sequentially with satd aq solutions of $NaHSO_3$, $NaHCO_3$, NaCl, and water and dried ($MgSO_4$). The syrup obtained after evaporation of the solvent was purified by column chromatography (20:1 toluene–EtOAc), recrystallized from EtOH (0.53 g, 70%), to give a solid with mp 137–138 °C, $[\alpha]_D$ −35° (*c* 1, $CHCl_3$). Anal. Calcd for $C_{27}H_{22}O_8$: C, 68.35; H, 4.67. Found: C, 68.50; H, 4.50.

*3,5,6-Tri-*O*-acetyl-2-deoxy-*D*-lyxo-hexono-1,4-lactone* (**8b**).—Compound **8b** was prepared from **5b** (1.0 g, 2.2 mmol) by the procedure described for **8a**. After workup TLC examination of the syrup obtained showed a main product (R_f 0.25, solvent b) and minor amount of a faster moving product (R_f 0.40), which were separated by column chromatography (20:1 toluene–EtOAc). The product of R_f 0.40 was spectroscopically identified as 6-*O*-acetyl-2-*O*-tosyl-hexa-2,4-dien-4-olide (**9**) by comparison with the spectra for 2,6-dibenzoyloxy-2,4-hexadien-4-olide.[18] Fractions containing the product of R_f 0.25 were evaporated, and the syrup obtained (**8b**, 0.38 g, 60%) gave the same spectroscopic properties as those reported.[22]

(b) Photochemical deoxygenation. General procedure.—In a custom-made Pyrex reaction vessel equipped with a cold finger, a solution containing 0.15 mmol of the substrate, $Mg(ClO_4)_2$ (66 mg, 0.3 mM), and methylcarbazole (3 mg, ~0.015 mmol) in 100 mL of 10% deionized water–2-propanol was degassed by bubbling UHP Ar through the solution for 30 min. The reaction was photolyzed with a Heraeus TQ medium-pressure Hg lamp, while the temperature was maintained at 25 °C with a circulating water bath. The following compounds were photolyzed:

(i) 3,5,6-Tri-*O*-benzoyl-2-*O*-[3-(trifluromethyl)benzoyl]-D-galactono-1,4-lactone (**7**, 0.10 g, 0.15 mmol). TLC analysis after 3 h of reaction showed a product with the same R_f as **8a** (R_f 0.25, solvent a). The reaction mixture was evaporated to remove the 2-PrOH, and the aq residue was extracted with CH_2Cl_2. The organic phase was washed with water, dried ($MgSO_4$) and evaporated. ^{13}C NMR ($CDCl_3$) methylene region, δ 34.9, 32.6 and 27.9.

(ii) 2-*O*-[3-(Trifluromethyl]benzoyl-D-galactono-1,4-lactone, (**6**, 0.06 g, 0.16 mmol). After 1 h TLC analysis showed a single spot (R_f 0.64, solvent c). The solvent was evaporated, and the residue was dissolved in MeOH and filtered, and the filtrate was evaporated to give 2-deoxy-D-*lyxo*-hexono-1,4-lactone (**10**, 0.02 g, 88%), $[\alpha]_D$ −10° (*c* 1, water), in agreement with literature data.[23]

3,5,6-Tri-O-*benzoyl-2-deoxy*-D-lyxo-*hexose* (**11a**).—To a solution of freshly prepared bis(3-methyl-2-butyl)borane (5.6 mmol) in anhyd THF (5.0 mL),[12] compound **8a** (0.66 g, 1.4 mmol) was added. The solution was stirred for 16 h at rt and then processed as already described.[11] After column chromatography purification (10:1 toluene–EtOAc), compound **11a** (0.63 g, 95%) was obtained as an amorphous solid, $[\alpha]_D$ −19° (*c* 1, $CHCl_3$). Anal. Calcd for $C_{27}H_{24}O_8$: C, 68.06; H, 5.08. Found: C, 68.30; H, 4.90.

1-O-*Acetyl-3,5,6-tri*-O-*benzoyl-2-deoxy*-α,β-D-lyxo-*hexofuranose* (**12**).—Compound **11a** (0.67 g, 1.4 mmol) was conventionally acetylated (1:1, Ac_2O–Py, 10.0 mL) to afford compound **12** (0.70 g, 96%), as a 1:1 anomeric mixture that gave $[\alpha]_D$ −26° (*c* 1, $CHCl_3$). Anal. Calcd for $C_{29}H_{26}O_9$: C, 67.18; H, 5.05. Found: C, 67.04; H, 5.16.

Methyl 3,5,6-tri-O-*benzoyl-2-deoxy*-D-lyxo-*hexofuranoside* (**13a**).—A solution of either **11a** (0.27 g, 0.57 mmol) or **12** (0.29 g, 0.57 mmol) in 14% BF_3–MeOH (10.0 mL) was stirred for 3 h at rt. The solvent was evaporated, and the syrup obtained was dissolved in CH_2Cl_2, washed with $NaHCO_3$, water and dried ($MgSO_4$). After evaporation of the solvent, the syrup was purified by column chromatography (20:1 toluene–EtOAc). Compound **13a** (0.25 g, 90%) was obtained as an anomeric mixture. Anal. Calcd for $C_{28}O_{26}O_8$. C, 68.56; H, 5.34. Found: C, 68.44; H, 5.16.

Methyl 2-deoxy-α-D-lyxo-*hexofuranoside* (**14α**) *and methyl 2-deoxy*-β-D-lyxo-*hexofuranoside* (**14β**).—The anomeric mixture of **13a** (0.40 g, 0.80 mmol) was suspended in a 0.5 M solution of NaOMe in MeOH (5.0 mL) and stirred until complete dissolution occurred (2 h). The solution was neutralized with Dowex 50W (H^+) and concentrated. Methyl benzoate was eliminated by repeated evaporation with water. TLC examination showed the presence of two products of R_f 0.40 and 0.30 (solvent d). After column chromatography purification (19:1 EtOAc–MeOH) compound **14α** (0.08 g, 55%) was obtained as a syrup that gave $[\alpha]_D$ 40° (*c* 0.3, water). Fractions containing the product of R_f 0.3 (**14β**), were evaporated (0.06 g, 41%) and gave $[\alpha]_D$ −65° (*c* 0.3, water). Anal. Calcd for $C_7H_{14}O_5$: C, 47.19; H, 7.92. Found: C, 46.41; H, 8.09.

Assays for specificity of exo β-D-*galactofuranosidase from* P. fellutanum.—Enzymatic assays were performed using the filtered medium of a stationary culture of *P. fellutanum*[2] as the enzyme source. The enzyme was incubated with 4-nitrophenyl β-D-galactofuranoside[4] as a control reaction, or with the deoxylactone **10** or the methyl glycosides **14α**,**β**, for studying the specificity of the enzyme, or with each of these compounds together with 4-nitrophenyl β-D-galactofuranoside, for the inhibition tests. The assays were carried out by incubating 100 μL of the culture medium containing the enzyme (20 μg of protein) with 60 μL of a 5 mM solution of the substrate, 100 μL of 66 mM AcONa buffer (pH 4.0), and the inhibitors (1 mM) in a final volume of 500 μL. The enzymatic reaction was stopped after 1.5 h incubation at 37 °C by adding 1 mL of 0.1 M Na_2CO_3 buffer (pH 9.0). The released 4-nitrophenol was measured spectrophotometrically at 410 nm.

For the specificity studies, incubation at 37 °C was performed overnight. The sample was centrifuged for 25 min at 10000 × *g* through an Ultrafree-MC centrifugal filter (MW 5000). The filtrate was analyzed by TLC and HPAEC–PAD.

HPAEC–PAD analysis.—Analysis by HPAEC–PAD was performed using a Dionex DX 300 high performance liquid chromatography (HPLC) system with pulse amperometric detection (PAD), set at 30 nA and $E_1 = +0.05$ V, $E_2 = +0.60$ V, and $E_3 = -0.60$ V. The column used was a CarboPac MA-10 anion-exchange analytical column (4 × 250 mm) equipped with a guard column MA-10 (4 × 50 mm). The separations were performed isocratically in 70 mM NaOH at a flow rate of 0.4 mL min^{-1}.

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