Enzymatic β-Galactosidation of Modified Monosaccharides: Study of the Enzyme Selectivity for the Acceptor and Its Application to the Synthesis of Disaccharides

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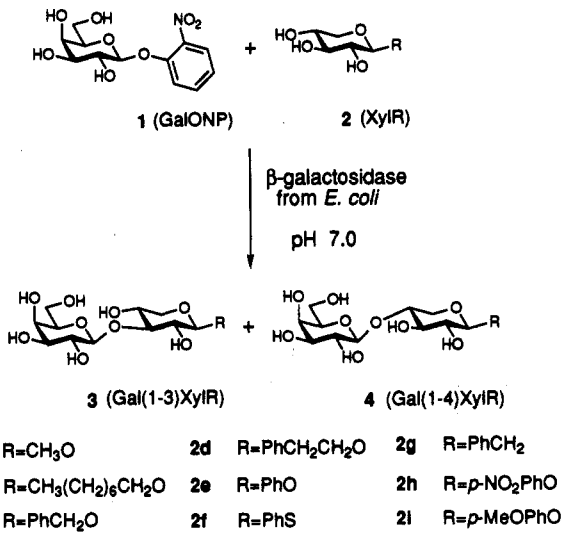
The selectivity of the *E. coli* β-galactosidase-catalyzed glycosylation of monosaccharides differently substituted at the anomeric position has been studied. Substituents bearing a phenyl ring increase the enzyme–acceptor binding; however, partial enzyme inhibition occurs. The regioselectivity of the glycosylation was dependent on small variations in the monosaccharide accceptor, such as the atom linked to the anomeric carbon and the number of methylenes between this atom and aromatic ring. A schematic model is proposed that accounts for the results. The information from this study allows the direct synthesis of disaccharides, with high regioselectivity and yields ranging from 30 to 40%.

Introduction

There is currently a great interest in the synthesis of oligosaccharides as many observations are suggesting important biological roles for these sugar chains composed of linked monosaccharides.[1] The chemical synthesis of oligosaccharides, although well-developed over the last years,[2] presents the problems of a high number of steps and the use of expensive and toxic catalysts in some glycosylation reactions. During the last years the use of enzymes in the synthesis of oligosaccharides has attracted the attention of many chemists, and in particular, the use of glycosyltransferases has strikingly shown in some cases the advantages of this approach.[3] However, these enzymes are difficult to obtain, they need expensive cofactors, and their use is confined to a limited number of substrates. A second alternative to get simple di- and trisaccharides involves the use of glycosidases in kinetically controlled reactions.[3bc,4] These hydrolytic enzymes are able to catalyze the stereospecific formation of glycosidic bonds using a sugar donor, which is specifically recognized by the enzyme, and a sugar acceptor which competes with water for the glycosyl unit to be transferred. Glycosidases do not need cofactors, and many of them are commercially available from different sources. The main drawbacks of their use for synthetic purposes are the low yields and the low regioselectivities of the disaccharide products. Several approaches have been used to control the regioselectivity of these reactions based on the configuration and the substitution on the anomeric carbon of the sugar acceptor.[5] In spite of the increasing work carried out with glycosidases, little is known about the structural requirements for the binding of the sugar acceptor to the enzyme,[6] which is essential to improve the synthetic utility of this methodology.

Scheme 1



2a	R=CH_3O	**2d**	R=$PhCH_2CH_2O$	**2g**	R=$PhCH_2$
2b	R=$CH_3(CH_2)_6CH_2O$	**2e**	R=PhO	**2h**	R=*p*-NO_2PhO
2c	R=$PhCH_2O$	**2f**	R=PhS	**2i**	R=*p*-MeOPhO

In order to get information about how substituents in the sugar acceptor can influence yield and regioselectivity we initiated a systematic study of the galactosidation of differently substituted β-xylopyranosides catalyzed by the β-galactosidase from *E. coli* (Scheme 1). The xylose does not have a higher reactive primary hydroxyl, which makes this molecule a good model for this study. Additionally, β-D-galactosyl-D-xyloses are fragments of proteoglycans and xyloglucans playing biological roles,[7] and the 4-*O*-β-D-galactopyranosyl-D-xylose has been used to evaluate the *in vivo* activity of intestinal lactase;[8] this study could therefore provide a direct access to these disaccharides. The results obtained have allowed us to predict the behavior of other sugar acceptors and to propose a model

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(2) Toshima, K.; Tatsuta, K. *Chem. Rev.* **1993**, *93*, 1503.

(3) (a) Ichikawa, Y.; Lin, Y.-C.; Dumas, D. P.; Shen, G.-J.; Garcia-Junceda, E.; Williams, M. A.; Bayer, R.; Ketcham, C.; Walker, L. E.; Paulson, J. C.; Wong, C.-H. *J. Am. Chem. Soc.* **1992**, *114*, 9283. (b) Toones, E. J.; Simon, E. S.; Bednarski, M.; Whitesides, G. M. *Tetrahedron.* **1989**, *45*, 5365. (c) David, S.; Augé, C.; Gautheron, C. *Adv. Carbohydr. Chem. Biochem.* **1991**, *49*, 175.

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(6) (a) Deschavanne, P. J.; Viratelle, O. M.; Yon, J. M. *J. Biol. Chem.* **1978**, *253*, 833. (b) Hubert, R. E.; Gaunt, M. T.; Hurlburt, K. L. *Arch. Biochem. Biophys.* **1984**, *234*, 151.

(7) Lindahl, V.; Rodén, L. In *Glycoproteins*; Elsevier: Amsterdam, 1972; pp 491–517. Albersheim, P.; Darvill, A.; Augur, C.; Cheong, J.-J.; Eberhard, S.; Hahn, M. G.; Marfa, V.; Mohhnen, D.; O'Neill, M. A.; Spiro, M. D.; York, W. S. *Acc. Chem. Res.* **1992**, *25*, 77. Chesson, A.; Gordon, A. H.; Lomax, J. *Carbohydr. Res.* **1985**, *141*, 137.

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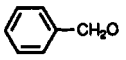
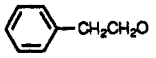
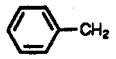
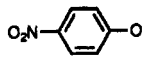
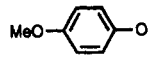
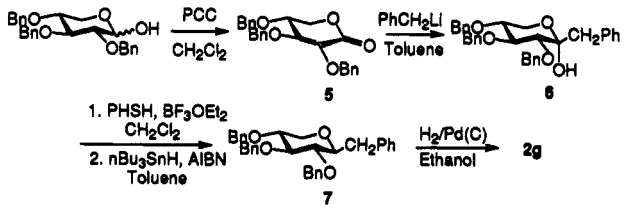
Table 1. Yield and Regioselectivity of the Disaccharide Products 3 and 4 from the β-Galactosidase-Catalyzed Reaction of 1 (100 mM) and 2 (50 mM)

compd	R in 2	yield[a] (%) 3 + 4	regioselectivity[a] ratio 3:4
2a	CH_3O	6	0:1.0
2b	$CH_3(CH_2)_6CH_2O$	8	nd[b]
2c		30	1:0.4
2d		25	1:1.2
2e		23	1:1.4
2f		19	1:2.9
2g		15	1:5.0
2h		18	1:5.5
2i		20	1:1.6

[a] Based on GLC. [b] Not determined.

Scheme 2



about how substrates can be placed in the acceptor site of the enzyme and the interactions that may play a role. In the last part of the work we use this information to get practical syntheses of disaccharides.

Results and Discussion

In a preliminary paper[9] we reported the results of the enzymatic galactosidation, using donor 1 (Scheme 1), of a variety of alkyl and aryl β-D-xylopyranosides (XylR). Two β-D-galactopyranosyl-β-D-xylopranosides were formed in all cases, the 1→3- and the 1→4-linked glycosides (compounds 3 and 4, respectively); no appreciable amount of the corresponding 1→2 was observed. The results are summarized in Table 1. We now complete this series with the C-glycoside **2g**. In the synthesis of **2g**, outlined in Scheme 2, the stereoselective reduction of the intermediate **6** turned out to be more difficult than was expected. The treatment of **6** with triethylsilane and boron trifluoride etherate[10] gave mainly elimination products, and the formation of the methyl xanthate or phenyl thiocarbonate[11] from **6** led to the cleavage of the C-glycosidic bond and other decomposition products. We obtained, however, good results through the monophenyl thioketal and subsequent radical reduction,[12] in this way, the β (equatorial) isomer **7** was exclusively obtained.

The result of the enzymatic galactosidation of this new acceptor **2g** (Table 1) corroborated the previous

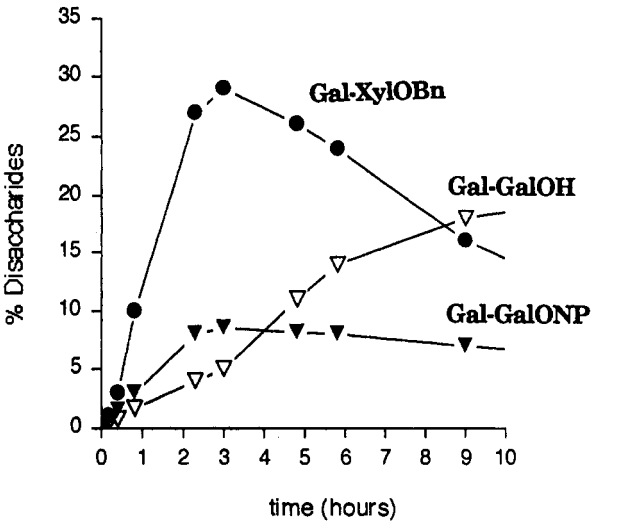
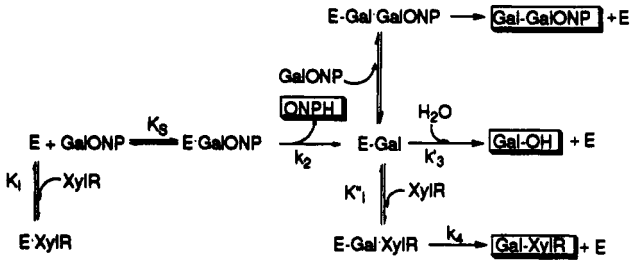


Figure 1.

Scheme 3



observations: the yield increases when the aglycon in **2** has a phenyl group, and the regioselectivity is strongly dependent on the atom linked to the anomeric carbon. Nevertheless, the reactions are more complex than one would have expected since two other monosaccharides may act as acceptors: the same donor **1** (GalONP), to give *o*-nitrophenyl β-D-galactopyranosyl-β-D-galactopyranosides (Gal-GalONP), and the galactose coming from the hydrolysis of the GalONP which, after reaching a certain concentration in the medium, gives β-D-galactopyranosyl-D-galactose (Gal-GalOH). In Figure 1 the course of the reaction in the case of the benzyl β-D-xylopyranoside (**2c**) is shown. Due to this complexity and in order to get more detailed information about the specificity of the enzyme for the substrate, we decided to measure the initial rates of formation for some selected xylose substrates.

Initial Rates Measurement. To better understand the reaction pathway for the formation of the products, the mechanism proposed for the β-galactosidase-catalyzed reactions[6] adapted for our case is represented in Scheme 3.[13] The *o*-nitrophenol (ONPH) is first released to give the intermediate E–Gal which may react either with water molecules or with added acceptor molecules which bind in the so-called acceptor site. In our case the acceptors can be GalONP or XylR, and the formed products can be galactose (GalOH) and the disaccharides Gal-GalONP and Gal-XylR:[14]

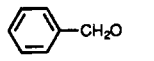
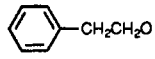
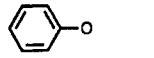
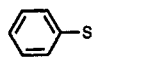
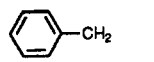
$$V_{ONPH} = V_{GalOH} + V_{GalGalONP} + V_{GalXylR}$$

Since the water and the XylR are competing substrates

(9) López, R; Fernández-Mayoralas, A. *Tetrahedron Lett.* **1992**, *33*, 5449.
(10) Czernecki, S.; Perlat, M.-C. *J. Org. Chem.* **1991**, *56*, 6289.
(11) Hartwing, W. *Tetrahedron* **1983**, *39*, 2609.
(12) Wang, Y.; Babirad, S. A.; Kishi, Y. *J. Org. Chem.* **1992**, *57*, 468.

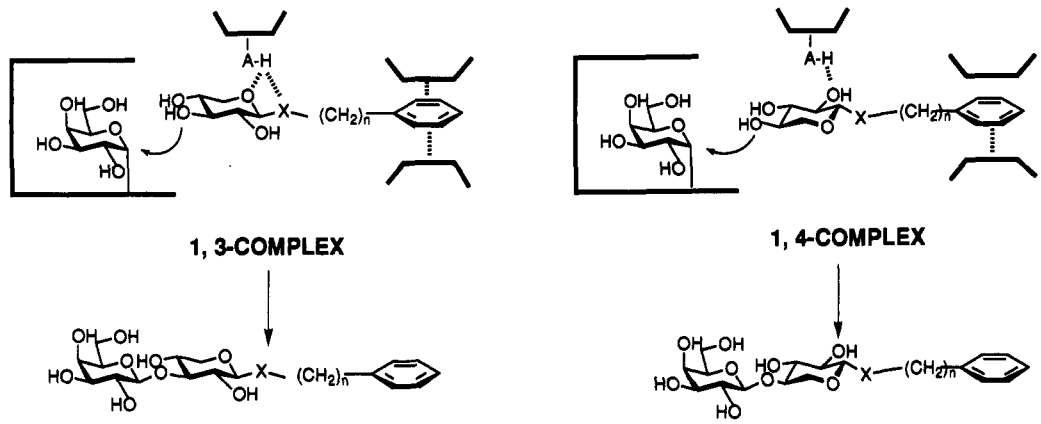
(13) In Scheme 3, E·XylR and E–Gal·XylR are, respectively, the complexes originating from the binding of the xyloside on free enzyme and on the chemical intermediate (E-Gal), and K_I and K''_I are the corresponding dissociation constants.
(14) The corresponding $V_{GalGalOH}$ is not included since in the first moments of the reaction GalGalOH disaccharides are not formed.

Table 2. Initial Rates, Relative Specificity, and Regioselectivity for the Reactions of GalONP (1, 50 mM) and Some XylR (2, 50 mM) in the Presence of β-Galactosidase from *E. coli* at pH 7.0

compd	R in 2	V_{ONPH}[a]	$V_{Gal\text{-}Gal\text{-}ONP}$[b]	$V_{Gal\text{-}Xyl}$[c]	$V_{Gal\text{-}OH}$[c]	specificity $k_4[XylR]/K''_I/k_3'$	regioselectivity $V_{1-3}:V_{1-4}$
2c		235 58	32 6	10	200 40	0.25	8.4:1.6
2d		116	13	18	90	0.20	7.5:10.5
2e		116	10	16	80	0.20	8.7:7.3
2f		118	14	10	88	0.11	2.7:7.3
2g		146	15	9	112	0.08	1.7:7.3
2a	CH_3-O	247	28	10	200	0.05	0.0:10.0

[a] Monitored by UV spectroscopy at λ = 420 nm, (μM min^{-1}). [b] Monitored by HPLC, (μM min^{-1}). [c] Monitored by GC, (μM min^{-1}).

Scheme 4



for the same intermediate E–Gal, the comparison of $V_{GalXylR}/V_{GalOH}$ ratios between the different XylR will give an idea of the relative specificity of the enzyme (in its E–Gal form) for each of the xylosides:[15]

$$V_{GalXylR}/V_{GalOH} = (k_4[XylR]/K''_I)/k'_3 \quad (1)$$

In Table 2 the results for the reactions of GalONP (50 mM) and some XylR (50mM) in the presence of the β-galactosidase (2.5 unit/mL) are summarized: the aglycon structure in the XylR, the initial rates of formation of the products, the specificity as the ratio $(k_4[XylR]/K''_I)/k'_3$ and the regioselectivity as the ratio of 1→3/1→4 Gal-XylR disaccharide rates of formation. Although an indicative value of the specificity for each xyloside can be drawn from the relative values of V_{GalOH} and $V_{GalXylR}$, more accurate information is obtained from the specificity values since these are not dependent on the nonproductive E–Gal·XylR complexes that could be formed and would affect V_{GalOH} and $V_{GalGalONP}$. It can be seen from the specificity that this is higher when the aglycon in XylR has a phenyl group as compared to the methyl derivative. For instance, from the methyloxy (**2a**) to the benzyloxy derivative (**2c**) there is a 5-fold increase in specificity.

Among the acceptors with only one atom between the aromatic ring and the anomeric carbon (compounds **2e**, **2f**, and **2g**) there are some differences in specificity depending on the atom linked to the anomeric carbon. To better understand these differences we have to look at regioselectivity. The rate of formation of 1→3 disaccharides decreases with respect to 1→4, which remains constant, in the order **2e** > **2f** > **2g**, i.e., in the same direction in which the electronegativity of the atom linked to the anomeric carbon decreases.

Regarding these results we postulate that two different complexes between the XylR and the enzyme produce, respectively, the 1→3 and the 1→4 Gal-XylR disaccharides as depicted in Scheme 4. We suggest that the pyranoid ring undergoes 180° flip from one to the other complex; in this way, the position of the reactive hydroxyl group (HO-3 or HO-4) is the same in each complex. In the 1,3-complex there may be an important stabilizing interaction between the lone pair electrons of the ring oxygen in the xyloside and an electrophilic center in the enzyme that would be affected by the substitution at the anomeric carbon,[16] i.e., the X atom in Scheme 4, in such a way that the higher the electron density in the X atom (oxygen, sulfur, or carbon) the higher the interaction between ring oxygen and electrophilic center. This interaction would also explain the regioselectivity obtained with the *p*-nitrophenyl derivative **2h** (Table 1). In the 1,4-complex the

(15) For Scheme 3, the specificity for each of the xylosides can be defined as the ratios k_4/K''_I (see: Fersht, A. In *Enzyme Structure and Mechanism*; Freeman: New York, 1985; p 112), which are contained in eq 1. If the concentration of XylR is fixed, k'_3 being constant, eq 1 is proportional to the specificity for each of the xylosides. This eq 1 is easily derived from Scheme 3:

$V_{GalXylR} = k_4[E\text{–}Gal\text{-}XylR]$ $V_{GalOH} = k'_3[E\text{–}Gal]$

$K''_I = [E\text{–}Gal][XylR]/[E\text{–}Gal\cdot XylR]$

(16) Lone pair interactions between oxygens in a sugar molecule determine the nucleophilicity of these oxygens: Box, V. G. S. *Heterocycles* **1982**, *19*, 1939; **1990**, *31*, 1157.

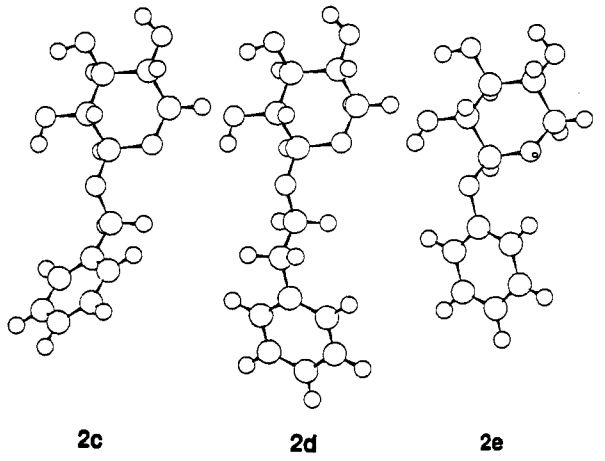


Figure 2.

HO-2 would occupy the position of the O-5 in the 1,3-complex.

In these hypothetical complexes there is an additional stabilizing interaction of the phenyl ring of the acceptor with the enzyme,[17] which must be more important in the 1,3-complex. The efficiency of this interaction in each complex would depend on the relative orientation of the phenyl ring to the hydroxyl group to react (HO-3 or HO-4 in the 1,3- or 1,4-complex, respectively). From the results of Table 1 and comparing the regioselectivity obtained with compounds **2c**, **2d**, and **2e** with respect to the nonaromatic methyl derivative **2a**, in which only the 1→4 disaccharide was obtained, this interaction for the case of the benzyl derivative **2c** seems to be optimum in the 1,3-complex and minimum in the 1,4-complex. However, for compounds **2d** and **2e** the differences in each complex are less important, and both show similar regioselectivity. As an illustrative example, Figure 2 shows the minimized gauche O.5–C.1–O.1–C conformer—the more stable conformer in O- and C-glycosides[18] —of compounds **2c**, **2d**, and **2e**. The conformations of compounds **2d** and **2e** show a similarity in the orientation of aromatic ring and HO-3 (or HO-4) (Figure 2), being in contrast to that of the benzyl derivative **2c**, which has the aromatic ring in a much different orientation in space. Therefore, the regioselectivity could be also influenced by the relative location of the aromatic ring and the reacting hydroxyls of the acceptor.

To corroborate the importance of an aromatic ring to improve the specificity, we carried out a comparative experiment using a glucose acceptor. The methyl and phenyl β-D-glucopyranosides were galactosylated under the same conditions as described above. The results are summarized in Table 3. From the specificity values a similar behavior as in the case of xylosides is observed: there is about a 3-fold increase in specificity with the phenyl with respect to the methyl derivative.

Finally, concerning the intrinsic reactivity of the hydroxyl groups in **2**, we carried out a comparative chemical glycosidation. Compound **2c** was treated with acetobromogalactose (1 equiv) in the presence of $Hg(CN)_2$ (1 equiv) and $HgBr_2$ (1 equiv) at room temperature to give a mixture of 1→2, 1→3, and 1→4 β-D-galactopyranosyl-β-D-xylopyranoside derivatives in a ratio 1.0:1.3:1.2, respectively, in 53% overall yield. Therefore, the chemical reactivities of the hydroxyl groups in **2** must be comparable, and even the HO-2 is reactive. On the other hand, all the xylopyranoside substrates used in this study seem to be in similar pyranoid ring conformations, as indicated by the values of the coupling constant of their ^1H NMR data (see Experimental Section, Table 5).

About the Disaccharide Isomerization. In comparing the regioselectivity of the Gal-XylR disaccharides at their maximum formation (Table 1) and at the initial moments of the reaction (Table 2), one can see that in some cases this varies appreciably. Two mechanisms can account for this effect: either one of the regioisomers is more rapidly hydrolyzed or there is disaccharide intramolecular isomerization catalyzed by the β-galactosidase in which the xylose portion does not become free of the enzyme during the isomerization (following the mechanism proposed for the lactose–allolactose isomerization[19]). To test the second possibility we carried out an experiment in which a 50 mM solution of Gal(1→3)XylOBn (the disaccharide that suffers more variation) was put in the presence of β-galactosidase at pH 7.0. Only the hydrolysis products (galactose and benzyl β-D-xylopyranoside) were formed, the Gal(1→4)XylOBn regioisomer was not observed at any moment of the reaction. This result shows the nonexistence of intramolecular isomerization and is consistent with our proposed complexes for the formation of Gal(1→3 and 1→4)Xyl disaccharides (Scheme 4): for the intramolecular transglycosylation the xylopyranoside has to turn 180°, a process which must have important steric barriers, thereby driving in this case diffusion into free solution.

Enzyme Inhibition by Substrate. Returning to Table 2, the following question can be asked: why do some substrates containing a phenyl group, while having a better specificity than the methyl derivative **2a**, present equal or lower Gal-Xyl disaccharide formation rates? The most plausible explanation is that these substrates can additionally bind either to the E–Gal intermediate without producing disaccharides (giving nonproductive E–Gal·XylR complexes) or to the free enzyme forming in this case an abortive E·XylR complex[20] (Scheme 3). Both cases would produce an inhibition of hydrolytic and synthetic reactions. If this is the case the actual $V_{GalXylR}$ would be higher than the observed one shown in Table 2. The first case of inhibition (by the nonproductive E–Gal·XylR complexes) is difficult to distinguish; however, the second one can be detected by determining the K_m and V_{max} of GalONP by the released of ONPH in the presence of different concentrations of xylopyranosides.[6a] We have determined these K_m and V_{max} in the presence of variable concentrations of methyl and benzyl β-D-xylopyranosides (**2a** and **2c**). The results are summarized in Table 4. It can be seen that compound **2a** has the behavior of an uncompetitive effector as deduced from the fact that the K_m/V_m ratio remains practically constant as the concentration of XylOMe is increased up to 50 mM. This means that the XylOMe at 50 mM concentration binds to the E–Gal

(17) The stacking of aromatic residues of the enzyme against the faces of sugars is a feature of protein–carbohydrate interactions which contributes to the stability and specificity of the complexes; see: N. K. Vyas. *Curr. Opin. Struct. Biol.* **1992**, *1*, 732.

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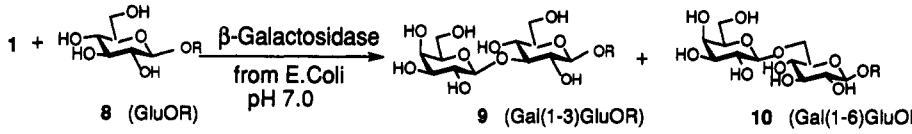
Table 3. Initial Rates, Relative Specificity, and Regioselectivity for the Reactions of GalONP (1, 50 mM) and Two Different GluOR (8, 50 mM) in the Presence of β-Galactosidase from *E. coli* at pH 7.0



8 (GluOR) 9 (Gal(1-3)GluOR) 10 (Gal(1-6)GluOR)

compd	R in 8	V_{ONPH}[a]	$V_{Gal\text{-}GalONP}$[b]	$V_{Gal\text{-}Glu}$[c]	$V_{Gal\text{-}OH}$[c]	specificity k_4[Glu-OR]/K''_I/k'_3
8a		235 124	32 10	26	200 80	0.32
8b	Me	247	22	24	200	0.12

[a] Monitored by UV spectroscopy at λ = 420 nm, (μM min^{-1}). [b] Monitored by HPLC, (μM min^{-1}). [c] Monitored by GC, (μM min^{-1}).

Table 4. V_{max} and K_m values for the Release of ONPH (Scheme 3) at Different Concentrations of XylOMe (2a) and XylOBn (2c)

[2a] (mM)	$V_{max} \times 10^{-4}$ (mM s^{-1})	K_m (mM)	K_m/V_{max} (s)
0	24.5 ± 0.1	0.103 ± 0.001	42.0
10	22.5 ± 0.3	0.105 ± 0.005	46.7
20	21.9 ± 0.4	0.094 ± 0.006	42.9
50	18.5 ± 0.2	0.087 ± 0.003	47.0
100	19.8 ± 0.3	0.081 ± 0.005	40.9
200	17.0 ± 0.4	0.100 ± 0.007	58.5

[2c] (mM)	$V_{max} \times 10^{-4}$ (mM s^{-1})	K_m (mM)	K_m/V_{max} (s)
0	24.5 ± 0.1	0.103 ± 0.001	42.0
10	12.1 ± 0.1	0.069 ± 0.002	56.2
12	11.3 ± 0.2	0.067 ± 0.005	59.2
20	8.3 ± 0.1	0.064 ± 0.003	77.1
50	5.5 ± 0.2	0.060 ± 0.009	109.1
100	2.8 ± 0.1	0.041 ± 0.009	146.4

intermediate preferently. However, the XylOBn behaves as a mixed effector, and the K_m and V_{max} vary differently at small concentrations of XylOBn; this means that the XylOBn is able to bind both free enzyme and the E–Gal intermediate with comparable binding constants.

The following equation[6a] gives a linear relationship as a function of XylOMe or XylOBn concentrations allowing us to determine K_I of Scheme 3 (where I = XylOMe or XylOBn):

$$K_{m,I}/k_{cat,I} = K_s/k_2\,(1 + [I]/K_I)$$

From this equation and the values from Table 4 we calculated K_I for the reactions in the presence of XylOMe and XylOBn being 560 and 33 mM, respectively. Thus, at the concentration of XylOBn in which the results of Table 2 were obtained (i.e., 50 mM) a certain percentage of hydrolytic and synthetic reactions are inhibited, and therefore, the actual $V_{GalXylOBn}$ must be higher than the observed value. In the case of the XylOMe this inhibition is negligible.

Synthetic Applications. At this moment we have collected enough information to synthesize disaccharides controlling yield and regioselectivity by using appropriate concentrations of substrates and choosing the convenient substituted acceptor. From the results of Table 2 and looking at the regioselectivity values, we can choose the benzyl β-D-xylopyranoside (**2c**) to prepare the Gal(1→3)-Xyl disaccharide and the methyl β-D-xylopyranoside (**2a**) for the corresponding Gal(1→4)Xyl disaccharide. In order to get a good yield the obvious thing to do is to work at high concentration of xylose acceptor; in this manner, we will favor the reaction in the direction of Gal-Xyl products (Scheme 3). In the case of using the XylOMe this is feasible since the K_I of the reaction in the presence of this substrate is very high. However, with the XylOBn, which has a lower K_I value, we have to take into account that enzyme inhibition will occur. Fortunately, the XylOBn shows a better specificity than the XylOMe.

After these considerations we carried out the synthesis of Gal-XylOBn using GalONP (50 mM) and XylOBn (100 mM) in the presence of β-galactosidase at pH 7.0 to get, in 40% isolated yield, the Gal(1→3)XylOBn disaccharide and the 1→4 regioisomer in a 4:1 ratio. Under similar conditions using GalONP (0.1 M) and XylOMe (1.0 M) only the Gal(1→4)XylOMe disaccharide was obtained in 33% isolated yield. Therefore, different galactosyl-xylose disaccharides were obtained in good yields and regioselectivities, making the isolation procedure easier, which has been claimed to be one of the main drawbacks in glycosidase-catalyzed synthesis.

Conclusions

The present work gives a schematic picture of how variations of substituents in the acceptor substrate can alter the selectivity in the enzymatic galactosidation of monosaccharides, allowing the controlled synthesis of disaccharides. A similar approach can be applied to the study of other glycosidases and monosaccharides. This work also promotes a field of research aimed at the design of new acceptors by introducing certain functional groups well-disposed with respect to the hydroxyl to be glycosylated. In this respect, enzymatic glycosylations could also be applicable to other noncarbohydrate acceptors with pharmacological properties in which the attachment of a sugar residue could improve their activity (e.g., enkephalin analogues when glycosylated give glycopeptides with high antinociceptive activity as compared to morphine[21]) and in substrates which cannot be chemically glycosylated due to their sensitivity to acid medium (e.g., the synthesis of unstable cardiac glycosides was succesfully accomplished using β-galactosidase enzymes[22]).

Experimental Section

Materials and Methods. β-Galactosidase from *E. coli* was purchased from a commercial source and used without further purification. *o*-Nitrophenyl β-D-galactopyranoside, methyl and *p*-nitrophenyl β-D-xylopyranosides, and methyl and phenyl β-D-glucopyranosides were from commercial sources. Other substrates were chemically synthesized.

Melting points are uncorrected. TLC was performed on silica gel GF_{254} with detection by charring with H_2SO_4. Column chromatography was performed on silica gel (70–230 mesh) and

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Table 5. Chemical Shifts (ppm) and Coupling Constants (Hz) of Xylose Derivatives 2

compd	H-1	H-2	H-3	H-4	H-5a	H-5e	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5a}$	$J_{4,5e}$	$J_{5a,5e}$
2a	4.22	3.15	3.33	3.52	3.22	3.87	7.8	9.3	9.1	10.5	5.4	11.5
2b	4.24	3.09	3.28	3.45	3.17	3.79	7.9	9.2	9.2	9.9	5.2	11.4
2c	4.41	3.23	3.33	3.54	3.23	3.89	7.7	9.2	9.2	10.3	5.4	11.6
2d	4.40	3.21	3.40	3.58	3.27	3.91	7.8	9.1	9.1	10.5	5.4	11.5
2e	5.00	3.46	3.46	3.58	3.38	3.92	7.7			10.4	5.4	11.5
2f	4.71	3.33	3.44	3.58	3.30	3.95	9.7	8.5	9.1	10.2	5.3	11.3
2g	3.56	3.26	3.42	3.54	3.13	3.82	9.0	9.0	9.2	10.4	5.4	11.3
2h	5.22	3.60	3.60	3.75	3.60	4.05	7.2				5.2	11.4
2i	4.99	3.59	3.59	3.73	3.44	4.04	7.63		9.0	10.5	5.5	11.3

on Sephadex G-10. ^{1}H-NMR spectra were measured at 300 or 200 MHz. ^{13}C-NMR spectra were obtained at 50 MHz. Gas–liquid chromatographic analysis was carried out on a chromatograph with FID detector using a fused SE-54 capillary column (10 m, 0.3-mm i.d., and 0.15 μm film). A flow rate of 1 mL/min of nitrogen was utilized. HPLC chromatographic analysis was carried out using a Carbohydrate Analysis column (85% acetonitrile/water isocratic, 1 mL/min). *o*-Nitrophenol released was recorded using a UV spectrophotometer.

The geometries of compounds **2c**, **2d**, and **2e** (Figure 2) describing minima were calculated in vacuo using the CVFF[23] and the Discover 2.8 program.[24]

Analysis of Reaction Products. *o*-Nitrophenol released was recorded by UV: to 20 μL aliquots was added 1 mL of Na_2CO_3 (0.5 M), and the amount of *o*-nitrophenolate was determined by absorbance at 420 nm. *o*-Nitrophenyl β-D-galactopyranosyl-β-D-galactopyranosides (Gal-GalONP) were monitored by HPLC: 20 μL aliquots were heated at 100 °C for 10 min, diluted with water (140 μL), and applied to the HPLC system. The compounds were detected by their absorbance at 254 nm.

Galactopyranosyl-β-D-xylo(gluco)pyranosides and galactose were determined by GC: 20-μL aliquots were removed and added to 1.5-mL plastic centrifuge tubes with the caps open. These aliquots were immediately heated at 100 °C for 20 min. Internal standard (20 μL of a solution of 10.0 mM 1-benzyl-DL-inositol in pyridine) and trimethylsilyl imidazole (20 μL) were added and the mixture heated at 60 °C for 30 min. The amounts of disaccharides were determined from previous calibration curves obtained for each sugar.

Preparation of Xylose Substrates. Octyl β-D-Xylopyranoside (2b). To a solution of tetra-*O*-acetyl-β-D-xylopyranose (7.1 g, 22.3 mmol) in dry CH_2Cl_2 (185 mL) were added 1-octanol (4.2 mL, 26.8 mmol, 1.2 equiv) and BF_3·OEt_2 (2.6 mL, 22.3 mmol, 1.0 equiv) at room temperature under argon atmosphere. The solution was stirred for 1 h and then diluted with CH_2Cl_2 (70 mL) and washed successively with saturated hydrogen carbonate (2 × 40 mL) and water. The residue (7.8 g) was purified by column chromatography (hexane/ethyl acetate (8:1)) to give octyl 2,3,4-tri-*O*-acetyl-β-D-xylopyranoside (4.6 g, 53%) as a solid. This compound was dissolved in methanol and treated with sodium methoxide in methanol (0.1 M) at room temperature. After 15 min the mixture was neutralized with Amberlite IR-120 (H^+) resin, filtered, and concentrated. After recrystallization from ethyl acetate **2b** (2.9 g, 95%) was obtained: mp 89–91 °C; $[\alpha]_D$ −42.3 (MeOH, *c* 1) [lit.[25] mp 91–92 °C; $[\alpha]_D$ −45.1 (MeOH, *c* 2)]; ^{1}H NMR, see Table 5.

Benzyl β-D-Xylopyranoside (2c). This compound was synthesized as previously described:[8] ^{1}H NMR, see Table 5.

2-Phenylethyl β-D-Xylopyranoside (2d). To a solution of tetra-*O*-acetyl-β-D-xylopyranose (0.5 g, 1.6 mmol) in dry CH_2Cl_2 (13 mL) were added 2-phenylethanol (0.23 mL, 1.92 mmol, 1.2 equiv) and BF_3·OEt_2 (1.0 mL, 8 mmol, 5 equiv) at room temperature under argon atmosphere. The solution was stirred for 4 h and then diluted with CH_2Cl_2 (10 mL), washed successively with saturated hydrogen carbonate (2 × 20 mL) and water (2 × 20 mL), dried over sodium sulfate, and concentrated in vacuo. The residue (0.68 g) was purified by column chromatography (hexane–ethyl acetate (3:1)) to give 2-phenylethyl 2,3,4-tri-*O*-acetyl-β-D-xylopyranoside (0.26 g g, 42%) as a solid. This product was dissolved in methanol (5 mL) and treated with a solution of sodium methoxide in methanol (0.1 M) at room tempereture. After 20 min the reaction mixture was neutralized with Amberlite IR-120(H^+) resin, filtered, and concentrated. Column chromatography (ethyl acetate–methanol (10:1)) of the residue gave pure **2d** (0.15 g, 90%) as a solid: mp 98–100 °C; $[\alpha]$ −44.4 ($CHCl_3$, *c* 0.28); ^{1}H NMR, see Table 5; ^{13}C NMR (50 MHz, D_2O) δ 130.07, 128.83 (2C), 128.44 (2C), 126.38, 103.03, 75.70, 72.78, 70.49, 69.49, 65.12, 36.03.

Anal. Calcd for $C_{13}H_{18}O_5$: C, 61.40; H, 7.13. Found: C, 61.18; H, 7.26.

Phenyl β-D-Xylopyranoside (2e). A mixture of tetra-*O*-acetyl-β-D-xylopyranose (1.0 g, 7 mmol), phenol (2.0 g, 21 mmol, 3 equiv), and *p*-toluensulfonic acid (12 mg) was stirred under vacuum in the rotavapor heating at 80 °C for 1 h 30 min. After this time the mixture was diluted with CH_2Cl_2 (25 mL) and then washed successively with a 0.1 N sodium hydroxide (2 × 25 mL) and water (2 × 25 mL). The organic phase was dried over sodium sulfate and concentrated in vacuo. The residue was purified by column chromatography (hexane–ethyl acetate (4:1)) to give pure phenyl 2,3,4-tetra-*O*-acetyl-β-D-xylopyranoside (0.27 g, 47%). This compound was dissolved in methanol (5 mL) and treated with a solution of sodium methoxide in methanol (0.1 M) as described above. Column chromatography (ethyl acetate) of the residue gave **2e** (0.17 g, 95%) as a solid: mp 145–147 °C; $[\alpha]$ −44.2 (H_2O, *c* 0.40); ^{1}H NMR, see Table 5; ^{13}C NMR (50 MHz, D_2O) δ 157.59, 131.22 (2C), 124.70, 117.96 (2C), 102.07, 76.79, 74.10, 70.32, 66.43.

Anal. Calcd for $C_{11}H_{14}O_5$: C, 58.40; H, 6.23. Found: C, 58.25; H, 5.98.

Phenyl 1-Thio-β-D-xylopyranoside (2f). To a solution of tetra-*O*-acetyl-β-D-xylopyranose (2.0 g, 6.3 mmol) in dry CH_2Cl_2 (8 mL) were added thiophenol (0.77 mL, 7.6 mmol, 1.2 equiv) and BF_3·OEt_2 (2.32 mL, 18.9 mmol, 3 equiv) at 0 °C under argon atmosphere. The solution was stirred for 2 h at room temperature and then diluted with CH_2Cl_2 (8 mL). The resulting solution was washed successively with saturated sodium hydrogen carbonate (2 × 25 mL) and water (2 × 15 mL), dried over sodium sulfate, and concentrated in vacuo. The residue was dissolved in methanol (6 mL) and a 0.1 M solution of sodium methoxide in methanol was added. After 15 min the mixture was neutralized with Amberlite IR-120(H^+) resin, filtered, and concentrated. After recrystallization of the residue from acetone–hexane, **2f** was obtained (0.96 g, 64%) as a solid: mp 143–145 °C; $[\alpha]$ −70.0 (MeOH, *c* 0.9); ^{1}H NMR, see Table 5.

Anal. Calcd for C_{11} $H_{14}O_4S$: C, 54.35; H, 5.88; S, 13.21. Found: C, 54.53; H, 5.82; S, 12.98.

Benzyl 1-*C*-β-D-Xylopyranoside (2g). To a stirred solution of pyridinium chlorochromate (2.4 g, 11.4 mmol) in dry CH_2Cl_2 (50 mL) was added a solution of 2,3,4-tri-O-benzyl-D-xylopyranose[26] (4.0 g, 9.5 mmol) in dry CH_2Cl_2 (10 mL) with stirring at room temperature for 4 h. The reaction mixture was diluted with ethylic ether (150 mL) and filtered through silica gel. The filtrate was washed successively with water and saturated aqueous sodium hydrogen carbonate, dried (Na_2SO_4), and concentrated. Column chromatography (hexane–ethyl acetate (3:1)) of the residue afforded 2,3,4-tri-*O*-benzyl-D-xylonolactone (**5**, 3.4 g, 85%) as a solid: mp 112–114 °C; $[\alpha]$ 5.2° ($CDCl_3$, *c* 0.53); ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.38 (m, 15 H), 5.04 and 4.67 (AB syst, 2H, J = 11.5 Hz), 4.67 and 4.58 (AB syst, 2H, J = 11.6 Hz), 4.58 and 4.52

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(AB syst, 2H, J = 12.0 Hz), 4.40 (ddd, 1H, J = 12.3, 3.5, 1.5 Hz), 4.29 (dd, 1H, J = 12.1, 2.2 Hz), 4.15 (d, 1H, J = 6.6 Hz), 3.90 (ddd, 1H, J = 6.5, 3.7, 2.0 Hz), 3.77 (m, 1H).

Lactone **5** (1.0 g, 2.4 mmol) was dissolved in 20 mL of dry toluene, and the solution was cooled to −78 °C under argon atmosphere. Benzyllithium[27] (8 mL of 0.33 M, 2.64 mmol, 1.1 equiv) was then added, and the mixture was stirred for 45 min at −78 °C. The reaction was quenched with a saturated aqueous solution of ammonium chloride (15 mL), and the temperature was then allowed to rise to room temperature. After being stirred for 30 min, the mixture was extracted with ethyl acetate (3 × 30 mL), and the organic layer was dried (Na_2SO_4) and concentrated. Column chromatography (hexane–ethyl acetate (5:1)) of the residue (1.6 g) gave pure **6** (0.9 g, 75%) as a solid: mp 92–95 °C; [α] 7.6° ($CHCl_3$, c 0.86); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.24 (m, 15H), 4.96 and 4.66 (AB syst, 2H, J = 8.5 Hz), 4.93 and 4.80 (AB syst, 2H, J = 10.9 Hz), 4.67 and 4.59 (AB syst, 2H, J = 11.6 Hz), 3.93 (t, 1H, J = 8.9 Hz), 3.58 (m, 3H), 3.30 (dd, 1H, J = 9.2, 0.9 Hz), 3.0 and 2.72 (AB syst, 2H, J = 13.5 Hz), 2.39 (d, 1H, J = 1.0 Hz).

Anal. Calcd for $C_{33}H_{34}O_5$: C, 77.62; H, 6.71. Found: C, 77.86; H, 7.01.

To a solution of **6** (0.9 g, 1.8 mmol) in dry CH_2Cl_2 (20 mL) were added thiophenol (4 mL) and BF_3·OEt_2 (0.3 mL, 2.9 mmol, 1.6 equiv). The reaction was stirred at 0 °C for 1 h and quenched with saturated sodium hydrogen carbonate. Aqueous workup (CH_2Cl_2) gave 0.93 g of crude which was dissolved without further purification in in dry toluene (60 mL), and then n-Bu_3SnH (8.3 mL, 30 mmol) and AIBN (5 mg) were added. The reaction was heated at 110 °C for 3 h, cooled to room temperature, and then concentrated in vacuo. Purification by silica gel chromatography (hexane–ethyl acetate (30:1)) afforded **7** (0.5 g, 56%) as a single product: mp 72–74 °C; [α] 2.1° ($CDCl_3$, c 0.70); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.20 (m, 15H), 4.92 and 4.77 (AB syst, 2H, J = 11.0 Hz), 4.89 and 4.58 (AB syst, 2H, J = 11.0 Hz), 4.63 and 4.53 (AB syst, 2H, J = 11.6 Hz), 3.86 (dd, 1H, J = 11.3, 4.8 Hz), 3.55 (m, 2H), 3.37 (dt, 1H, J = 9.2, 2.3 Hz), 3.18 (t, 1H, J = 8.7 Hz), 3.0 (dd, 1H, J = 14.3, 2.3 Hz), 2.51 (dd, J = 14.3, 9.0 Hz).

Anal. Calcd for $C_{33}H_{34}O_4$: C, 80.13; H, 6.92. Found: C, 79.85; H, 7.03.

Hydrogenation of **7** (0.5 g) with 10% Pd/C (0.5 g) in methanol (20 mL) for 4 days at atmospheric pressure afforded **2g** (0.176 g, 80%) as a syrup: [α] −38.7 (MeOH, c 1.5); ^{1}H NMR, see Table 5; ^{13}C NMR (50 MHz, CD_3OD) δ 140.20, 130.56 (2C), 128.99 (2C), 82.71, 79.94, 75.04, 71.53, 70.81, 39.01.

Anal. Calcd for $C_{12}H_{16}O_4$: C, 64.27; H, 7.19. Found: C, 63.80; H, 7.32.

***p*-Methoxyphenyl β-D-Xylopyranoside (2i).** A mixture of tetra-*O*-acetyl-β-D-xylopyranose (0.5 g, 1.6 mmol), *p*-methoxyphenol (0.40 g, 3.2 mmol, 2 equiv) and *p*-toluenesulfonic acid (6 mg) was stirred at 100 °C under vacuum in the rotavapor. After 2 h the mixture was diluted with CH_2Cl_2 (15 mL) and then was washed successively with 0.1 N sodium hydroxide (2 × 10 mL) and water (2 × 10 mL). The organic phase was dried (Na_2SO_4) and concentrated. The residue (0.8 g) was purified by column chromatography (hexane–ethyl acetate (4:1)) to give *p*-methoxyphenyl 2,3,4-tetra-*O*-acetyl-β-D-xylopyranoside (0.21 g, 34%). This compound in methanol (3 mL) was treated with a solution of sodium methoxide (0.1 M) for 20 min. The reaction mixture was neutralized with Amberlite IR-120 (H^+) resin, filtered, and concentrated. The residue was purified by column chromatography (ethyl acetate–methanol (10:1)) to give **2i** (0.13 g, 90%) as a solid: mp 146–148 °C; [α] −19.7 (H_2O, c 0.31); ^{1}H NMR, see Table 5; ^{13}C NMR (50 MHz, D_2O) δ 156.11, 151.93, 119.67 (2C), 116.36 (2C), 103.18, 76.81, 74.13, 70.34, 66.44, 57.09.

Anal. Calcd for $C_{12}H_{16}O_6$: C, 56.24; H, 6.29. Found: C, 56.10; H, 6.06.

Characterization of Disaccharides. In order to prepare and characterize the disaccharides formed from the different acceptors in the enzymatic reactions, the following general procedure was used: To a solution of **1** (100 mM) and aryl/alquil-β-D-xylo(gluco)pyranoside (compound **2** or **8**, 50 mM) in buffer (0.05 M Na phosphate, 1 mM $MgCl_2$, 5 mM mercaptoethanol, pH 7.0) was added β-galactosidase from *E. coli* (12 u/mL). The mixture was left at room temperature, and the course of the reaction was monitored by TLC and GC. The reaction was stopped by heating for 10 min at 100 °C when the maximun formation of galactosylxylose(glucose) disaccharides was reached. The yellow solution was concentrated, and the residue was fractionated by column chromatography on a Sephadex G-10 (distilled water as eluent). The fractions containing galactosylxylose(glucose) disaccharides were collected and concentrated. Conventional acetylation and column chromatography on silica gel (hexane–ethyl acetate) gave the separated acetylated disaccharides, which were subsequently deacetylated and used to assign peaks in the gas chromatogram. The structure of the disaccharides was deduced from the ^{1}H NMR (300 MHz, $CDCl_3$) of their acetates:

Peracetylated 4a: δ 5.37 (dd, 1H, J = 3.4, 1.1 Hz), 5.14 (t, 1H, J = 8.3 Hz), 5.12 (dd, 1H, J = 10.4, 7.6 Hz), 4.98 (dd, 1H, J = 10.4, 3.4 Hz), 4.84 (dd, 1H, J = 8.6, 6.9 Hz), 4.51 (d, 1H, J = 7.7 Hz), 4.36 (d, 1H, J = 6.8 Hz), 4.11 (m, 2H), 3.98 (dd, 1H, J = 11.6, 5.0 Hz), 3.90 (td, 1H, J = 6.2, 1.2 Hz), 3.81 (m, 1H), 3.47 (s, 3H), 3.34 (dd, 1H, J = 11.6, 8.9 Hz).

Peracetylated 3c: δ 5.36 (dd, 1H, J = 3.4, 1.1 Hz), 5.14 (dd, 1H, J = 10.4, 7.9 Hz), 4.96 (dd, 1H, J = 10.4, 3.4 Hz), 4.92 (dd, 1H, J = 7.1, 5.8 Hz), 4.91 (m, 1H), 4.59 (d, 1H, J = 7.9 Hz), 4.50 (d, 1H, J = 5.8 Hz), 4.17 (dd, 1H, J = 11.1, 6.4 Hz), 4.13 (dd, 1H, J = 12.1, 4.4 Hz), 4.06 (dd, 1H, J = 11.1, 7.2 Hz), 3.88 (m, 1H), 3.85 (t, 1H, J = 7.1 Hz), 3.39 (dd, 1H, J = 12.1, 7.0 Hz).

Peracetylated 4c: δ 5.32 (dd, 1H, J = 3.4, 1.1 Hz), 5.07 (t, 1H, J = 8.4 Hz), 5.05 (dd, 1H, J = 10.4, 7.7 Hz), 4.95 (dd, 1H, J = 10.4, 3.4 Hz), 4.87 (dd, 1H, J = 8.4, 6.6 Hz), 4.49 (d, 1H, J = 6.6 Hz), 4.48 (d, 1H, J = 7.7 Hz), 4.07 (d, 2H, J = 6.7 Hz), 3.97 (dd, 1H, J = 11.8, 4.9 Hz), 3.86 (dt, 1H, J = 6.7, 1.1 Hz), 3.80 (m, 1H), 3.30 (dd, 1H, J = 11.8, 8.8 Hz).

Peracetylated 3d: δ 5.37 (dd, 1H, J = 3.4, 1.2 Hz), 5.15 (dd, 1H, J = 10.5, 7.9 Hz), 4.96 (dd, 1H, J = 10.5, 3.4 Hz), 4.88 (dd, 1H, J = 7.6, 6.0 Hz), 4.90 (m, 1H), 4.57 (d, 1H, J = 7.9 Hz), 4.41 (d, 1H, J = 6.0 Hz), 4.19 (dd, 1H, J = 11.1, 6.4 Hz), 4.06 (m, 3H), 3.89 (td, 1H, J = 7.3, 1.2 Hz), 3.83 (t, 1H, J = 7.6 Hz), 3.64 (dt, 1H, J = 9,6, 7.1 Hz), 3.32 (dd, 1H, J = 12.1, 7.4 Hz), 2.88 (t, 2H, J = 6.8 Hz).

Peracetylated 4d: δ 5.32 (dd, 1H, J = 3.4, 1.1 Hz), 5.07 (dd, 1H, J = 10.8, 7.4 Hz), 5.06 (t, 1H, J = 8.3 Hz), 4.95 (dd, 1H, J = 10.8, 3.4 Hz), 4.80 (dd, 1H, J = 8.5, 6.7 Hz), 4.46 (d, 1H, J = 7.4 Hz), 4.40 (d, 1H, J = 6.7 Hz), 4.09 (d, 2H, J = 6.7 Hz), 4.01 (dt, 1H, J = 6.9, 4.3 Hz), 3.87 (m, 2H), 3.76 (m, 1H), 3.63 (dt, 1H, J = 9.6, 6.9 Hz), 3.26 (dd, 1H, J = 11.7, 8.8 Hz), 2.84 (t, 2H, J = 6.7 Hz).

Peracetylated 3e: δ 5.40 (dd, 1H, J = 3.4, 1.0 Hz), 5.26 (d, 1H, J = 4.3 Hz), 5.23 (dd, 1H, J = 10.5, 7.9 Hz), 5.07 (dd, 1H, J = 5.9, 4.3 Hz), 5.02 (dd, 1H, J = 10.5, 3.5 Hz), 5.0 (m, 1H), 4.68 (d, 1H, J = 8.0 Hz), 4.23 (dd, 1H, J = 12.6, 3.8 Hz), 4.22 (dd, 1H, J = 11.1, 6.6 Hz), 4.10 (dd, 1H, J = 11.1, 7.0 Hz), 3.98 (t, 1H, J = 5.9 Hz), 3.94 (td, 1H, J = 6.0, 1.0 Hz), 3.56 (dd, 1H, J = 12.5, 5.2 Hz).

Peracetylated 4e: δ 5.37 (dd, 1H, J = 3.4, 1.1 Hz), 5.24 (t, 1H, J = 7.4 Hz), 5.13 (dd, 1H, J = 10.5, 7.6 Hz), 5.14 (d, 1H, J = 5.7 Hz), 5.10 (dd, 1H, J = 7.4, 6.1 Hz), 5.0 (dd, 1H, J = 10.4, 3.4 Hz), 4.55 (d, 1H, J = 7.9 Hz), 4.07 (m, 2H), 4.08 (dd, 1H, J = 11.5, 4.6 Hz), 3.93 (td, 1H, J = 6.2, 1.1 Hz), 3.89 (m, 1H,), 3.49 (dd, 1H, J = 11.5, 8.1 Hz).

Peracetylated 3f: δ 5.39 (dd, 1H, J = 3.4, 1.1 Hz), 5.21 (dd, 1H, J = 10.5, 7.9 Hz), 5.0 (dd, 1H, J = 10.9, 3.4 Hz), 4.99 (m, 2H), 4.93 (m, 1H), 4.65 (d, 1H, J = 7.9), 4.41 (dd, 1H, J = 12.2, 3.7 Hz), 4.21 (dd, 1H, J = 11.1, 6.3 Hz), 4.13 (dd, 1H, J = 7.6, 1.9 Hz), 4.09 (dd, 1H, J = 11.1, 6.7 Hz), 3.94 (m, 1H), 3.53 (dd, 1H, J = 12.5, 6.2 Hz).

Peracetylated 4f: δ 5.35 (dd, 1H, J = 3.4, 1.1 Hz), 5.16 (t, 1H, J = 7.8 Hz), 5.10 (dd, 1H, J = 10.5, 7.8 Hz), 4.97 (dd, 1H, J = 10.5, 3.4 Hz), 4.90 (t, 1H, J = 7.9 Hz), 4.80 (d, 1H, J = 8.1 Hz), 4.50 (d, 1H, J = 7.0 Hz), 4.15 (dd, 1H, J = 11.9, 4.8 Hz), 4.10 (d, 2H, J = 6.6 Hz), 3.89 (td, 1H, J = 6.6, 1.1 Hz), 3.80 (m, 1H), 3.40 (dd, 1H, J = 11.9, 8.7 Hz).

Peracetylated 3g: δ 5.33 (dd, 1H, J = 4.2, 1.1 Hz), 5.10 (dd, 1H, J = 10.1, 8.2 Hz), 5.92 (m, 3H), 4.54 (d, 1H, J = 7.8 Hz), 4.18 (dd, 1H, J = 11.4, 6.5 Hz), 3.73 (dd, 1H, J = 11.4, 8.2Hz), 4.00

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(dd, 1H, J = 6.8, 5.4 Hz), 3.85 (td, 1H, J = 7.4, 1.1 Hz), 4.60 (t, 1H, J = 9.2 Hz), 3.45 (m, 1H), 3.12 (dd, 1H, J = 11.6, 11.1 Hz).

Peracetylated 4g: δ 5.34 (d, 1H, J = 2.4 Hz), 5.08 (t, 1H, J = 9.2 Hz), 5.07 (dd, 1H, J = 10.4, 7.7 Hz), 4.95 (dd, 1H, J = 10.2, 3.4 Hz), 4.84 (t, 1H, J = 9.5 Hz), 4.44 (d, 1H, 7.8 Hz), 4.10 (d, 2H, J = 6.8 Hz), 3.38 (m, 3H), 3.60 (m, 1H), 3.16 (t, 1H, J = 10.7 Hz).

Peracetylated 3h: δ 5.42 (d, 1H, J = 3.2 Hz), 5.38 (dd, 1H, J = 3.4, 0.9 Hz), 5.23 (dd, 1H, J = 10.5, 8.0 Hz), 4.99 (dd, 1H, J = 10.5, 3.4 Hz), 4.98 (m, 2H), 4.64 (d, 1H, J = 7.9 Hz), 4.17 (dd, 1H, J = 12.4, 2.9 Hz), 4.13 (m, 2H), 3.96 (t, 1H, J = 4.8 Hz), 3.93 (td, 1H, J = 6.8, 1.0 Hz), 3.62 (dd, 1H, J = 12.5, 1.0 Hz).

Peracetylated 4h: δ 5.38 (dd, 1H, J = 3.4, 1.1 Hz), 5.32 (d, 1H, J = 5.1 Hz), 5.27 (t, 1H, J = 6.7 Hz), 5.17 (dd, 1H, J = 10.4, 7.8 Hz), 5.09 (dd, 1H, J = 5.1, 6.8 Hz), 5.01 (dd, 1H, J = 10.5, 3.4 Hz), 4.58 (d, 1H, 7.9 Hz), 4.11 (m, 3H), 3.94 (td, 1H, J = 7.1, 1.1 Hz), 3.89 (m, 1H), 3.58 (dd, 1H, J = 12.2, 6.6 Hz).

Peracetylated 3i: δ 5.39 (dd, 1H, J = 3.4, 1.0 Hz), 5.23 (dd, 1H, J = 10.3, 7.8 Hz), 5.13 (d, 1H, J = 4.2 Hz), 5.06 (dd, 1H, J = 4.7, 6.4 Hz), 5.01 (dd, 1H, J = 10.4, 3.3 Hz), 4.90 (m, 1H), 4.68 (d, 1H, J = 7.9 Hz), 4.22 (m, 2H), 4.13 (m, 2H), 3.94 (td, J = 7.7, 1.0 Hz), 3.78 (s, 3H), 3.53 (dd, 1H, J = 12.2, 5.1 Hz).

Peracetylated 4i: 5.37 (dd, 1H, J = 3.4, 0.7 Hz), 5.21 (t, 1H, J = 7.7 Hz), 5.14 (dd, 1H, J = 10.5, 7.8 Hz), 5.07 (t, 1H, J = 6.4 Hz), 5.00 (d, 1H, J = 6.5 Hz), 4.54 (d, 1H, J = 7.8 Hz), 4.13 (d, 2H,), 4.07 (dd, 1H, J = 11.9, 4.7 Hz), 3.85 (m, 2H), 3.78 (s, 3H), 3.45 (dd, 1H, J = 11.9, 8.3 Hz).

Peracetylated 10a: δ 5.32 (dd, 1H, J = 3.4, 1.1 Hz), 5.26 (t, 1H, J = 9.5 Hz), 5.20 (t, 1H, J = 9.6 Hz), 5.18 (dd, 1H, J = 10.6, 8.0 Hz), 5.04 (d, 1H, J = 7.6 Hz), 4.93 (t, 1H, J = 10.0 Hz), 4.92 (dd, 1H, J = 10.4, 3.3 Hz), 4.50 (d, 1H, J = 8.1 Hz), 4.10 (m, 2H), 3.85 (m, 2H), 3.78 (td, 1H, J = 6.5, 1.1 Hz), 3.65 (dd, 1H, J = 11.5, 8.1 Hz).

Peracetylated 9a: δ 5.33 (dd, 1H, J = 3.4, 1.0 Hz), 5.05 (dd, 1H, J = 10.4, 7.8 Hz), 4.96 (t, 1H, J = 8.2 Hz), 4.93 (t, 1H, J = 10.1 Hz), 4.91 (dd, 1H, J = 10.5, 3.4 Hz), 4.53 (d, 1H, J = 7.8 Hz), 4.28 (d, 1H, J = 7.9 Hz), 4.16 (m, 3H), 4.03 (dd, 1H, J = 10.9, 7.3 Hz), 3.88 (t, 1H, J = 9.3 Hz), 3.86 (m, 1H), 3.65 (m, 1H), 3.45 (s, 3H).

Peracetylated 10b: δ 5.37 (dd, 1H, J = 3.3, 0.8 Hz), 5.19 (dd, 1H, J = 10.5, 7.9 Hz), 5.18 (t, 1H, J = 9.5 Hz), 4.98 (dd, 1H, J = 10.5, 3.4 Hz), 4.92 (dd, 1H, J = 11.0, 8.0 Hz), 4.88 (t, 1H, J = 9.7 Hz), 4.54 (d, 1H, J = 8.0 Hz), 4.39 (d, 1H, J = 7.9 Hz), 4.13 (t, 1H, J = 6.5 Hz), 4.09 (t, 1H, J = 6.4 Hz), 7.0 (m, 2H), 3.68 (dd, 1H, J = 10.0, 1.7 Hz), 3.60 (dd, 1H, J = 10.7, 7.3 Hz), 3.50 (s, 3H).

Peracetylated 9b: δ 5.33 (dd, 1H, J = 3.4, 1.0 Hz), 5.05 (dd, 1H, J = 10.4, 7.8 Hz), 4.96 (t, 1H, J = 8.2 Hz), 4.93 (t, 1H, J = 10.1 Hz), 4.91 (dd, 1H, J = 10.5, 3.4 Hz), 4.53 (d, 1H, J = 7.8 Hz), 4.28 (d, 1H, J = 7.9 Hz), 4.16 (m, 3H), 4.03 (dd, 1H, J = 10.9, 7.3 H), 3.88 (t, 1H, J = 9.3 Hz), 3.86 (m, 1H), 3.65 (m, 1H), 3.45 (s, 3H).

Conventional deacetylation of peracetylated compounds **3** and **4** and **9** and **10** gave the corresponding unprotected disaccharides. GC retention times for compounds **3** and **4** (temperature program: initial temp 200 °C; rate 5 °C/min; final temp 250 °C): **4a**, 8.00 min; **3c**, 12.60 min; **4c**, 15.55 min; **3d**, 16.70 min; **4d**, 22.60 min; **3e**, 12.20 min; **4e**, 14.75 min; **3f**, 13.40 min; **4f**, 15.55 min; **3g**, 12.40 min; **4g**, 18.50 min; **3h**, 30.00 min; **4h**, 40.50; **3i**, 15.90 min; **4i**, 19.90 min. GC retention times for compounds **9** and **10** (temperature program: initial temp 180 °C; rate 5 °C/min; final temp 250 °C): **9a**, 20.35 min; **10a**, 21.85 min; **9b**, 13.13 min; **10b**, 15.50 min.

Chemical Glycosidation of 2c. To a mixture of **2c** (0.57 g, 2.4 mmol), $Hg(CN)_2$ (0.6 g, 2.4 mmol, 1 equiv), $HgBr_2$ (0.86 g, 2.4 mmol, 1 equiv) and 4-Å molecular sieves (5 g) in 1:1 toluene–nitrometane (40 mL) was added 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide[28] (0.98 g, 2.4 mmol, 1 equiv) at room temperature under argon atmosphere. After 5 h the mixture was diluted with CH_2Cl_2 (20 mL) and filtered. The filtrate was washed successively with a 10% solution of sodium iodide (2 × 60 mL) and sodium hydrogencarbonate (2 × 60 mL), dried (Na_2SO_4), and concentrated. Column chromatography (hexane–ethyl acetate (1:1)) of the residue (0.96 g) afforded benzyl 2-*O*-(β-D-galactopyranosyl)-β-D-xylopyranoside (0.14 g) and compounds **3c** (0.19 g) and **4c** (0.17 g) in 53% overall yield. The first product had: mp 179–182 °C; [α] 21.2° (MeOH, *c* 0.34); ^{13}C NMR (50 MHz, D_2O) δ 136.60, 129.99 (2C), 129.78, 129.67 (2C), 104.68, 102.10, 81.52, 76.40, 76.33, 73.88, 72.85, 72.63, 70.27, 69.72, 65.94 and 61.90 (CH_2).

Anal. Calcd for $C_{18}H_{26}O_{10}$: C, 51.41; H, 6.66. Found: C, 51.22: H, 6.80.

Compound **3c**: mp 143–146 °C; [α] −46.7 (*c* 0.1, MeOH); ^{13}C NMR (50 MHz, D_2O) δ 137.62, 130.03 (2C), 129.92 (2C), 129.77, 104.52 and 103.13, 85.53, 76.57, 73.84, 73.78, 72.88, 72.48, 69.86, 69.27, 65.90 and 62.35.

Anal. Calcd for $C_{18}H_{26}O_{10}\cdot H_2O$: C, 51.41; H, 6.72. Found: C, 51.12; H, 6.86.

Compound **4c**: mp 141–143 °C; [α] −42.7 (MeOH, *c* 0.5); ^{13}C NMR (50 MHz, D_2O) δ 139.34, 131.58 (2C), 131.52 (2C), 131.35, 104.77 and 104.70, 79.34, 78.15, 76.83, 75.69, 75.43, 74.51, 73.47, 71.45, 65.83, and 83.96.

Anal. Calcd for $C_{18}H_{26}O_{10}\cdot H_2O$: C, 51.41; H, 6.72. Found: C, 51.36; H, 6.66.

Conventional acetylation of the first product gave benzyl 3,4-di-*O*-acetyl-2-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-xylopyranoside: ^1H NMR (300 MHz, $CDCl_3$) δ 7.35 (m, 5H), 5.34 (dd, 1H, J = 3.2, 0.7 Hz), 5.19 (dd, 1H, J = 10.5, 7.9 Hz), 5.11 (t, 1H, J = 7.8 Hz), 4.94 (dd, 1H, J = 10.4, 3.4 Hz), 4.88 and 4.59 (AB syst, 2H, J = 11.9 Hz), 4.85 (m, 1H), 4.71 (d, 1H, J = 5.6 Hz), 4.69 (d, 1H, J = 7.9 Hz), 4.80 (m, 3H), 3.78 (td, 1H, J = 7.2, 0.9 Hz), 3.74 (dd, 1H, J = 7.9, 5.7 Hz), 3.44 (dd, 1H, J = 12.1, 7.4 Hz).

Acetylation of **3c** and **4c** gave the corresponding peracetylated derivatives (^1H NMR, see above).

Initial Rate Experiments. Enzyme assays were carried out at 25 °C adding β-galactosidase from *E. coli* (2.5 u/mL) to a solution of *o*-nitrophenyl β-D-galactopyranoside (50 mM) and aryl/alquil-β-D-xylo(gluco)pyranoside (50 mM) in buffer (0.05 M Na phosphate, 1 mM $MgCl_2$, 5 mM mercaptoethanol, pH 7.0). At various times after the enzyme was added to the reaction mixture, aliquots were removed to determine the amounts of products formed per unit of time (see results in Table 2).

Inhibition Experiments: K_m and V_{max} Measurements. Enzyme assays were carried out at 25 °C in buffer (pH 7.0) adding β-galactosidase from *E. coli* (0.005 u/mL) to solutions with differents concentrations of *o*-nitrophenyl β-D-galactopyranoside (1) (0.05, 0.1, 0.2, 0.4, and 0.8 mM) in the presence of variable amounts of inhibitors (**2a** and **2c**, see Table 4). The incubation was stopped after 20 min (by adding Na_2CO_3 (0.5 M)), and the *o*-nitrophenyl released was measured as mentioned above. All enzymic assays were performed at least in duplicate. The kinetic calculations and curve fits were carried out with the help of the Enzfiter program.[29]

Enzymatic Synthesis of 3c and 4c. To a solution of 1 (65 mg, 50 mM) and **2c** (103 mg, 100 mM) in buffer (4.3 mL, pH 7.0) was added β-galactosidase from *E. coli* (50 u/mL), and the mixture was left at 25 °C. After 4 h 30 min the reaction was stopped by heating for 10 min at 100 °C. The solution was concentrated, and the residue (180 mg) was fractionated by column chromatography on a Sephadex G-10 (water as eluent). The fractions containing **3c** and **4c** were collected and concentrated (60 mg). Column chromatography on silica gel (ethyl acetate–methanol (20:1)) of the residue gave pure **3c** (28 mg, 32%) as a white solid and **4c** (7 mg, 8%). Compounds **3c** and **4c** showed melting points and optical rotations identical with the chemically obtained samples.

Enzymatic Synthesis of 4a. To a solution of 1 (70 mg, 100 mM) and **2a** (377 mg, 1 M) in buffer (2.3 mL, pH 7.0) was added β-galactosidase from *E. coli* (50 u/mL) and the mixture was left at 25 °C. After 1 h the reaction was stopped by heating for 10 min at 100 °C. The solution was concentrated, and the residue was fractionated by column chromatography on a Sephadex G-10. The fractions containing **4a** were collected and concentrated. Further column chromatography on silica gel (ethyl acetate–methanol (20:1)) gave pure **4a** (25 mg, 33%) as a solid: mp 246–

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248 °C; [α] −8.2 (c 0.5, water) [lit.[30] mp 248–250 °C; $[\alpha]_D$ −8.7 (c 0.1, water)].

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Supplementary Material Available: ^1H NMR spectra of peracetylated **3d-i**, **4d-i**, **9a,b**, and **10a,b** (16 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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