Regioselective Acetylations of Alkyl β-D-Xylopyranosides by Use of Lipase PS in Organic Solvents and Application to the Chemoenzymatic Synthesis of Oligosaccharides

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Received March 28, 1994[®]

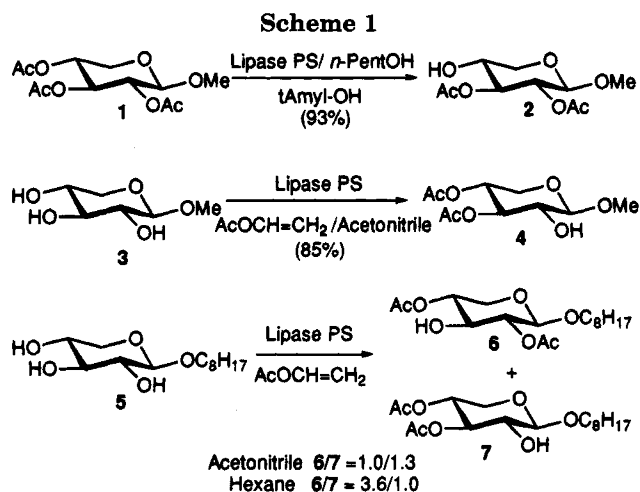
Aglycon structure and solvent can change the regioselectivity of the acetylation of alkyl β-D-xylopyranosides, catalyzed by lipase PS. The acetylation of methyl β-D-xylopyranoside (**3**) in acetonitrile gave the 3,4-diacetate **4** exclusively, whereas the reaction of octyl β-D-xylopyranoside (**5**) gave a mixture of 2,4- and 3,4-diacetates (**6** and **7**) in 1.0:1.3 and 3.6:1.0 ratios, in acetonitrile and hexane, respectively. The effect of several solvents on the selectivity in the monoacetylation of **5** was studied. The 2-monoacetate **8** was preferentially formed over the 3- and 4-monoacetates (**9** and **10**) in hydrophobic solvents. High yields of partially acetylated xylose derivatives were obtained, which were used in the syntheses of a disaccharide showing liquid crystal properties, an intermediate for the synthesis of proteoglycan fragments, and a trisaccharide potential inhibitor of plant growth.

Introduction

One of the attractive features of conducting enzyme-catalyzed reactions in organic solvents is the potential to alter the selectivity by simply changing the solvent. This effect has been reported in enantio-,[1] chemo-,[2] and regioselective[3] acylations and deacylations. A recent study[3] of solvent effect on enzyme regioselectivity in the lipase PS-catalyzed deacylation of a diester substrate containing an octyl group showed that the regioselectivity correlated with the hydrophobicity of the organic solvent. This correlation could be accounted for by an interaction of the octyl group of the substrate and a hydrophobic cleft in the enzyme. The results of this study could mitigate the limitations of conventional acylation/deacylation methodologies with carbohydrate substrates to provide partially acylated sugars—useful precursors for the synthesis of oligosaccharides and chiral intermediates for the synthesis of natural products.

We have recently reported[4] results on the lipase PS-catalyzed regioselective deacetylation of tri-*O*-acyl-β-D-xylopyranosides, which bear a methyl or octyl group as aglycon, in organic solvents. With all of the substrates and in all of the solvents used, deacylation at only the 4 position was observed. For example, the deacetylation of methyl 2,3,4-tri-*O*-acetyl-β-D-xylopyranoside (**1**) in *tert*-amyl alcohol furnishes only the HO-4 free derivative **2** in high yield (Scheme 1). This persistent regioselectivity was attributed to steric effects. Due to this observation, we planned to study the regioselectivity of the acylation, in organic solvents, of the unprotected and less sterically hindered, methyl and octyl β-D-xylopyranosides (**3** and **5**, Scheme 1). We planned to evaluate the effect of different solvents on the regioselectivity of the acetylation of **5**, owing to its solubility in a wide range of solvents and its potential for hydrophobic-type interactions. The results described herein show that hydrophobic solvents favor the acetylation at the C-2 position of **5** over the C-3 and C-4 positions. Thus, high yields of partially acetylated xylopyranosides have been obtained and their utility has been demonstrated in the synthesis of oligosaccharides.

Scheme 1



Results and Discussion

The acetylations of **3** and **5**, solubilized in organic solvent, were carried out by treatment with vinyl acetate (5 equiv) in the presence of lipase PS (25 mg/mL of organic solvent) at 30 °C. The formation of products was followed by GLC and HPLC. The course of the acetylations always followed the same pattern: monoacetates were formed first, they reached a maximum, and they subsequently gave diacetates. When nearly all monoacetates were consumed and diacetates prevailed, the reactions stopped. No appreciable amounts of triacetates were formed.

[®] Abstract published in *Advance ACS Abstracts*, October 15, 1994.

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Table 1. Monoacetylation of 5 Catalyzed by Lipase PS in Organic Solvents

5 + AcOCH=CH_2 —(Lipase PS, solvent)→ [glucopyranoside with R^3O, R^2O, R^1O, OC_8H_{17}]

8 : R^1 = Ac; R^2 = R^3 = H
9 : R^2 = Ac; R^1 = R^3 = H
10 : R^3 = Ac; R^1 = R^2 = H

solvent	time (h)	yield of monoacetates (%)	rel %		
			8	**9**	**10**
hexane	3	53	83	6	11
toluene	4	52	77	13	10
benzene	5	47	59	30	11
triethylamine	12	44	25	39	36
ethyl acetate	16	44	42	14	44
tetrahydrofurane	68	51	33	20	47
acetonitrile	22	42	35	5	60

The acetylation of **3** in acetonitrile gave the 3,4-di-*O*-acetyl derivative **4** as the only diacetylated product (Scheme 1). The reaction of the octyl derivative **5** under the same conditions gave a mixture of 2,4- and 3,4-di-*O*-acyl derivatives (**6** and **7**, respectively) in a 1.0:1.3 ratio (determined by gas chromatography). However, the latter reaction performed in hexane led to a mixture of **6** and **7** in a 3.6:1.0 ratio. These three results alone show the significant influence of both aglycon and solvent on the regioselectivity of the reaction.

To study the origin of the regioselectivity change in the acetylation of **5**, the effect of several solvents on the formation of the initial products, i.e., the monoacetates, was determined. The acetylations of **5** in the various solvents were carried out on a preparative scale and were interrupted when the maximum formation of monoacetates was reached. In Table 1, the solvents used (from more to less hydrophobic), the reaction times, the yields, and the relative percentages of isolated monoacetates are shown. From the relative percentage of monoacetates, a clear tendency of the 2-monoacetate **8** to be formed in the more hydrophobic solvents could be seen, whereas the 4-monoacetate **10** seems to be formed in the most polar solvents. Behavior leading to the 3-monoacetate **9** is not so clear. In the structure of **5**, HO-3 lies between the other two hydroxyls. The values in Table 1 must be considered in a qualitative manner since two factors can affect the regioselectivity observed at the time of maximum monoacetates: as the monoacetates are produced, each regioisomer can be further acetylated at a different rate and intramolecular migration could occur during the reaction and purification.[5]

In order to circumvent these problems, the rates of formation of the monoacetates **8**–**10** were measured at the initial stages of the reaction, before diacetates formed. Samples were analyzed by HPLC after making sure that no intramolecular migrations of acetates took place during the analysis time. In Table 2, the results are summarized. In general, the catalytic efficiency of the lipase increased as the solvent hydrophobicity increased. Examination of the individual rate values corroborates the observation at the time of maximum formation of monoacetates: acetylation at HO-2 is preferred in hydrophobic solvents while acetylation at HO-4 is favored as the solvent becomes more hydrophilic. In the last column of Table 2, the ratios of V_8/V_{10} clearly show this behavior. In changing from acetonitrile to hexane, the ratio increases 11-fold. In Figure 1A–B, the V_8/V_{10} ratios are plotted versus log P[6] (solvent hydrophobicity) and the polarity index[7] of the solvents. Except for the basic triethylamine, a correlation was found with these solvent parameters.

To determine if monoacetates disappear at different velocities under the reaction conditions, we measured the initial rates of formation of the diacetates from the three monoacetyl regioisomers separately, in two distinct solvents, acetonitrile and toluene.[8] From the results shown in Table 3, it is noteworthy that the enzyme in toluene

Table 2. Initial Rates[a] of Formation of 8 (V_8), 9 (V_9), and 10 (V_{10}) in the Monoacetylation of 5 (10 mM) Catalyzed by Lipase PS in Organic Solvents

solvent	V_8	V_9	V_{10}	V_8/V_{10}
hexane	262.9	113.8	50.9	5.16
toluene	174.8	88.0	57.1	3.07
benzene	137.0	145.3	61.8	2.22
triethylamine	22.8	42.0	43.8	0.52
ethyl acetate	19.8	12.9	18.9	1.05
tetrahydrofurane	5.8	3.9	6.1	0.95
acetonitrile	10.7	5.8	23.8	0.45

[a] $\mu M \cdot h^{-1} \cdot (mg \cdot mL^{-1}$ enzyme$)^{-1}$.

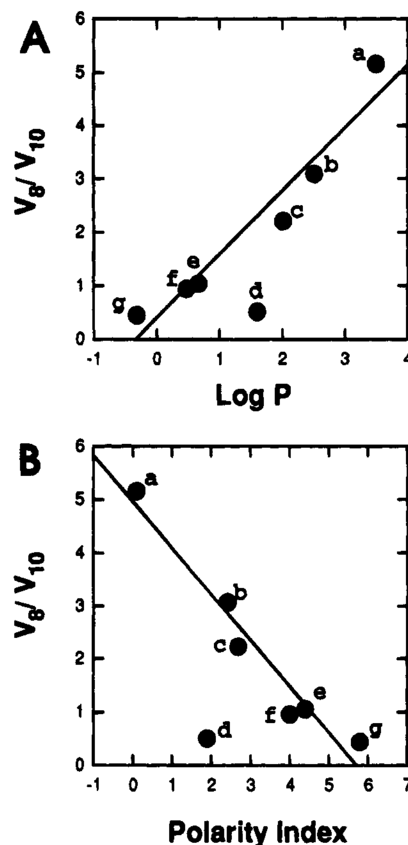

Figure 1. Regioselectivity 2-/4-acetate (V_8/V_{10}) in the monoacetylation of **5** catalyzed by lipase PS in anhydrous solvents as function of solvent log P[6] (A) and solvent polarity index[7] (B): a, hexane; b, toluene; c, benzene; d, triethylamine; e, ethyl acetate; f, tetrahydrofuran; g, acetonitrile.

(5) The monoacetates are stable in the selected solvents for the time of the reaction in the absence or in the presence of enzyme; however, intramolecular migrations during the separation by silica gel column cannot be precluded.

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(8) We chose toluene as the hydrophobic solvent since monoacetate **9** was not completely soluble in hexane at 20 mM.

Table 3. Initial Rates[a] for the Acetylation of Monoacetates 8, 9, and 10 (20 mM) Catalysed by Lipase PS, in Toluene and Acetonitrile at 30 °C

monoacetates	solvent	rate[b]	diacetate products (ratio)
8	toluene	1.66	**6**
9	toluene	5.57	**7**
10	toluene	18.02	**7**
8	acetonitrile	0.99	**6**
9	acetonitrile	1.44	**7**
10	acetonitrile	1.30	**6** + **7** (1:3)

[a] Determined by GC. [b] $mM \cdot h^{-1} \cdot (mg \cdot mL^{-1}\ enzyme)^{-1}$.

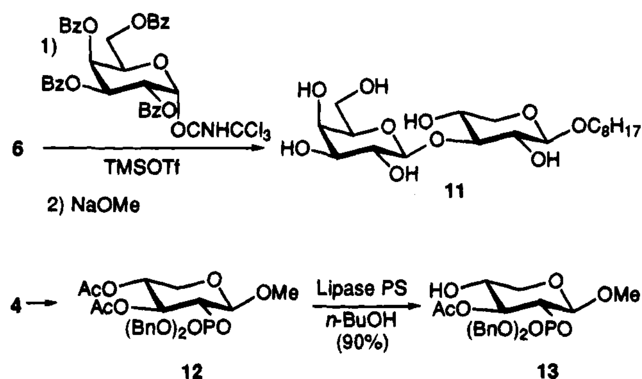
catalyzes the acetylation of the 4-acetate (**10**) 11 and 3 times faster than the acetylations of the 2- and 3-acetates (**8** and **9**, respectively). In acetonitrile, the three monoacetates react at similar rates. The rates in toluene might explain the difference between the ratio of V_8/V_{10} at the initial moments (Table 2) and the ratio of **8**:**10** at maximum formation of monoacetates (Table 1), in toluene and hexane. Although monoacetates disappear at different rates depending on the solvent used, the ratio of diacetates formed is not significantly changed by the nature of the solvent (Table 2).

Examination of the regioselectivity values from Tables 1–3 suggests that the regioselectivity in the formation of **6** and **7** from **5** in acetonitrile and hexane (Scheme 1) is mainly dictated in the first acetylation of **5**. In hexane, acetylation at HO-2 is more rapid than at HO-3 and HO-4. The 2-acetate **8** slowly produces 2,4-diacetate **6**, while the 3- and 4-acetates **9** and **10** give 3,4-diacetate **7**. Since the final ratio of **6**:**7** is higher than the ratio of initial rates $V_8/(V_9 + V_{10})$ in hexane, it is possible that 3,4-diacetate **7**, formed at the beginning of the reaction, is partially isomerized to **6** during the reaction course.[9] In acetonitrile **8**–**10** are initially formed in a 1.9:1.0:4.2 ratio and are subsequently acetylated at comparable rates to produce diacetates: **8** gives **6**, **9** gives **7**, and **10** gives **6** and **7** in a 1:3 ratio. The final ratio of **6**:**7** (1:1.3) in this solvent is thus accounted for. A rationalization of the solvent effect on this type of enzymatic reactions must be tentative, since many factors could be involved.[1,3] Recently-determined structures of some lipases reveal that important conformational changes take place at an oil–water interface,[10] which could be a general feature in this family of enzymes.

For comparative purposes, we carried out a chemical acetylation of **5**. In acetonitrile or hexane, **5** was treated with Ac-Cl (5 equiv) and pyridine (10% v/v) at room temperature. When diacetate formation reached its maximum, the ratio of **6** to **7** was similar in both solvents and even the 2,3-diacetyl derivative[4] was observed. Ratios of 2,3-, 2,4-, and 3,4-diacetates were 3.8:2.1:4.1 and 3.2:2.3:4.5 in acetonitrile and hexane, respectively. Furthermore, some amount of triacetate was always formed.

Overall, the present and previous[4] studies illustrate the versatility of lipase-catalyzed reactions in organic solvents. All possible diacetyl xylopyranosides can be obtained in high yield by simple acylations or deacylations, with variance of the solvent and the substitution

Scheme 2



on the substrate. Diacetate **2** can be prepared from the deacetylation of the triacetate **1** in *tert*-amyl alcohol, **4** from the acetylation of **3** in acetonitrile, and **6** from the acetylation of **5** in hexane, in 93, 85, and 70% isolated yield, respectively (Scheme 1). These acetates can be used as intermediates for the synthesis of xylose-containing oligosaccharides such as the oligosaccharide fragments of glycoproteins,[11] xyloglucans and arabinoxylans from cell walls,[12] and of steroid glycosides.[13] We present below some applications. Since compound **5** has an amphiphilic character, its partially acetylated derivatives could be of use in the synthesis of surfactants, membrane transports, and liquid crystals.[14] By way of example, the 2,4-diacetate **6** was galactosylated and subsequently deacylated to give octyl 3-*O*-(β-D-galactopyranosyl)-β-D-xylopyranoside (**11**, Scheme 2). Compound **11** showed all mesophases expected for a liquid crystal.

One advantage of enzymatic reactions is the mild conditions used. Given the high selectivity in the deacetylation of **1** (Scheme 1), we carried out the enzymatic deacetylation on a similar substrate containing a phosphate group at position 2 (compound **12**), prepared by phosphorylation of the alcohol **4** (Scheme 2). This reaction showed high regioselectivity for deacetylation at the 4 position. The alcohol **13** was isolated in 90% yield. The phosphate group, which is sensitive to basic media, remained intact. Compound **13** has recently been used in the synthesis of oligosaccharide fragments of proteoglycans.[15] It can be prepared from **3** in a three-step sequence, two of them catalyzed by the lipase, in 67% overall yield.

Trisaccharide α-Fuc-1,2-β-Gal-1,2-Xyl (compound **19** in Scheme 3 as the methyl glycoside) is a fragment of a nonasaccharide repeating unit of plant cell wall xyloglucan, which acts as an endogenous hormone to regulate cell growth.[16] This trisaccharide is postulated to be the minimum structural feature responsible for the inhibition of 2,4-dichlorophenoxyacetic acid-stimulated growth of pea stem.[17] Two reports[18,19] dealing with the synthesis of the nonasaccharide and some fragments make use of

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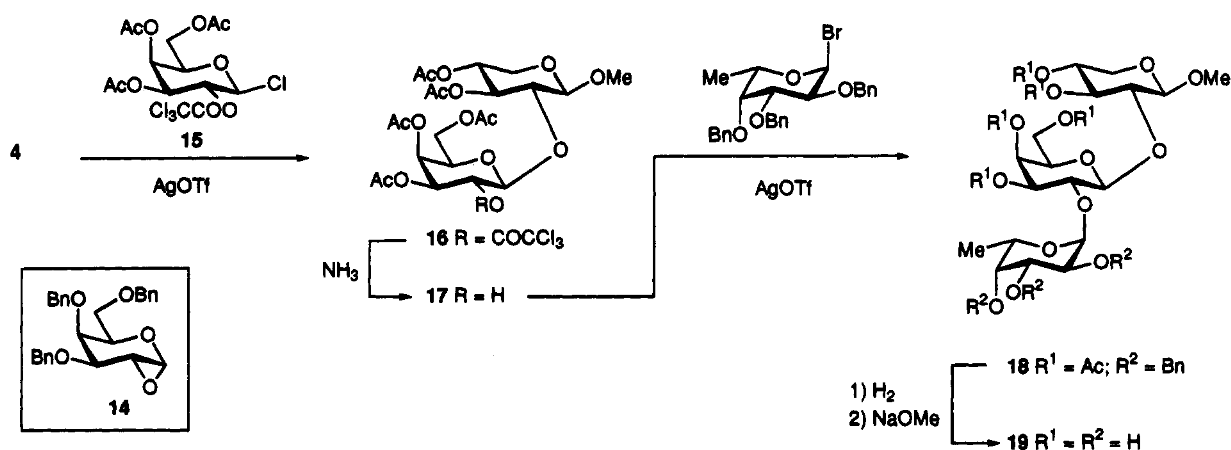
Scheme 3



a similar strategy. With an enzymatically useful synthesis of 4 in hand, we decided to explore an original straightforward route to **19** (Scheme 3). We first examined the β-galactosidation of 4 with galactal epoxide **14** and $ZnCl_2$ catalyst, following Danishefsky's methodology.[20] Unfortunately, no glycosidation was observed, probably due to the low reactivity of the glycosyl acceptor **4**. We then turned our attention to the 2-*O*-trichloroacetyl derivative **15**, a glycosylating agent with a conveniently differentiated 2-position, that has been recently used[21] in the synthesis of sarsasapogenin glycosides. Compound **15** is prepared directly from commercially available galactose pentacetate. Glycosidation of **4** with **15**, using silver triflate as promoter, gave the desired β-disaccharide **16** together with the α-anomer ($\alpha/\beta \sim$ 1:1) in 76% overall yield. Although no stereoselectivity was achieved, we continued the synthesis with **16** to prove the validity of this approach. Removal of the trichloroacetyl group of **16** with ammonia took place smoothly to give **17** (90%), which was subsequently α-fucosylated to afford protected trisaccharide **18** (70%). Hydrogenolysis of **18** followed by deacetylation gave the target trisaccharide **19**. The activity of **19** in vegetal cells is currently being evaluated.

Conclusions

Lipase-catalyzed reactions are gaining importance in carbohydrate transformations, since they provide selectivity in some cases in which it is difficult to achieve by conventional reactions. Previous work has shown that the regioselectivity is dependent upon the lipase[22] and the acyl donor[23] used, the relative orientation of the hydroxyls,[24] and the anomeric configuration[25] of the sugar. In this paper, the dependence of regioselectivity on the nature of the organic solvent and upon the aglycon of the sugar has been defined.

Experimental Section

Materials and Methods. Lipase PS was purchased from a commercial source and used without further purification. 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). ^{1}H NMR spectra were measured at 300 or 200 MHz. ^{13}C NMR spectra were obtained at 50 MHz. HPLC chromatographic analysis was carried out on a chromatograph equipped with a normal-phase column (Licrospher Si 60.5 μm). Each sample was injected through a 200 μL loop and eluted at 1.5 mL/min. The elution of monoacetates was followed with a refraction index detector. 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 id, and 0.15 μm film). Temperature program: initial temperature 200 °C; rate 5 °C/min; final temperature 250 °C. A flow rate of 1 mL/min of nitrogen was utilized. The samples were prepared from an aliquot (20 μL) of the reaction mixture, treated with pyridine (20 μL) and hexanoic anhydride (40 μL), and kept for 1 h at 35 °C.

Monoacetylation of 5 in Different Organic Solvents. To a solution of octyl β-D-xylopyranoside[26] (**5**, 0.2 g, 0.8 mmol) in the organic solvent (38 mL) were added vinyl acetate (0.35 mL, 4 mmol, 5 equiv), 4-Å molecular sieves (1.9 g), and Lipase PS (1.1 g). The mixture was stirred at 30 °C and 250 rpm in an orbital shaker and the reaction was monitored by TLC (hexane–ethyl acetate) and GC. When the maximum of monoacetates was reached and reaction was stopped by filtration of the enzyme. The solvent was eliminated, and the residue was fractionated by column chromatography (ethyl acetate–hexane (3:1)) to give the three monoacetates **8–10** in different ratios depending on the solvent used (see Table 1).

Octyl 2-*O*-acetyl-β-D-xylopyranoside (8): white solid; mp 47–50 °C; $[\alpha]_D$ −46.3° ($CHCl_3$, c 1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.74 (d, 1H, J = 4.9, 5.1 Hz), 4.53 (d, 1H, J = 4.9 Hz), 4.07 (dd, 1H, J = 3.4, 12.0 Hz), 3.78 (dt, 1 H, J = 6.5, 9.6 Hz), 3.65 (m, 2H), 3.45 (dt, 1H, J = 6.5, 9.6 Hz), 3.41 (dd, 1H, J = 7.2, 11.9 Hz), 3.03 (s, 1H), 2.63 (s, 1H), 2.12 (s, 3H), 1.59 (m, 2H), 1.28 (m, 10H), 0.85 (m, 3H). Anal. Calcd for $C_{15}H_{28}O_6$: C, 59.10; H, 9.27. Found: C, 59.33, H, 9.60.

Octyl 3-*O*-acetyl-β-D-xylopyranoside (9): white solid; mp 66–70 °C; $[\alpha]_D$ = 33.3° ($CHCl_3$, *c* 0.9); ^{1}H NMR (200 MHz, $CDCl_3$) δ 4.80 (t, 1H, J = 8.6 Hz), 4.28 (d, 1H, J = 7.0 Hz), 4.05 (dd, 1H, J = 5.1, 11.6 Hz), 3.84 (dt, 1H, J = 6.7, 9.4 Hz), 3.75 (m, 1H), 3.50 (dd, 1H, J = 6.7, 9.4 Hz), 3.49 (m, 1H), 3.30

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(dd, 1H, J = 9.4, 11.7 Hz), 2.64 (d, 1H, J = 5.1 Hz), 2.39 (d, 1H, J = 3.0 Hz), 2.15 (s, 3H), 1.60 (m, 2H), 1.23 (m, 10H), 0.83 (m, 3H). Anal. Calcd for $C_{15}H_{28}O_6$: C, 59.10; H, 9.27. Found: C, 58.87; H, 9.45.

Octyl 4-*O*-acetyl-β-D-xylopyranoside (10): white solid; mp 107–109 °C; $[\alpha]_D$ −67.5° (CHCl3, *c* 1.1); ^{1}H NMR (200 MHz, $CDCl_3$) δ 4.84 (m, 1H), 4.44 (d, 1H, J = 5.5 Hz), 4.10 (dd, 1H, J = 4.2, 12.2 Hz), 3.81 (t, 1H, J = 7.6 Hz), 3.79 (dt, 1H, J = 9.5, 6.8 Hz), 3.51 (m, 1H), 3.40 (dd, 1H, J = 7.0, 12.3 Hz), 3.04 (d, 1H, J = 5.6 Hz), 2.62 (d, 1H, J = 5.7 Hz), 2.12 (s, 3H), 1.63 (m, 2H), 1.29 (m, 10H), 0.85 (m, 3H). Anal. Calcd for $C_{15}H_{28}O_6$: C, 59.19; H, 9.27. Found: C, 58.90; H 9.45.

Initial Rates Experiments. A solution of compound **5** (10 mM) and vinyl acetate (50 mM) in the corresponding organic solvent (Table 2), with 25 mg/mL of 4-Å molecular sieves, was incubated in the presence of the lipase (14.5 mg/mL) at 30 °C. Aliquots (500 μL) of the reaction mixture were withdrawn at different times starting just after the addition of the enzyme. The reaction was stopped in each aliquot separating the enzyme by filtration (Durapore filter, 0.45 μm). The filtered solution (375 μL) was dried with argon stream and redissolved in 250 μL of HPLC elution mixture (hexane–ethyl acetate (43: 57)). The amount of each monoacetate was calculated from the integration of the chromatogram peaks. Previously, a calibration curve for each monoacetate was obtained under the same chromatographic conditions. Initial velocities were calculated from the slope of the plots of monoacetate production against time when the transformation of the substrate was below 10% of its initial concentration.

Octyl 2,4- and 3,4-Di-*O*-acetyl-β-D-xylopyranosides (6 and 7). A mixture of octyl β-D-xylopyranoside (**5**, 0.5 g, 1.4 mmol), vinyl acetate (0.9 mL, 9.5 mmol, 5 equiv), 4-Å molecular sieves (4.8 g), and Lipase PS (2.86 g) in hexane (95 mL) was stirred at 30 °C and 250 rpm in an orbital shaker. The reaction mixture was monitored by TLC and GC and after 74 h (when the diacetates were the main products) was stopped by filtration of the enzyme. The residue was fractionated by column chromatography (hexane–ethyl acetate (3:1)) to give first **7** (0.11 g, 28%) as a white solid: mp 35–36 °C; $[\alpha]_D$ −28° ($CHCl_3$, *c* 1.1); ^{1}H NMR (200 MHz, $CDCl_3$) δ 5.1 (t, 1H, J = 8.9 Hz), 4.92 (m, 1H), 4.32 (d, 1H, J = 7.1 Hz), 4.07 (dd, 1H, J = 5.2, 11.6 Hz), 3.85 (dt, 1H, J = 6.7, 9.4 Hz), 3.52 (m, 2H), 3.32 (dd, 1H, J = 9.4, 11.6 Hz), 2.42 (d, 1H, J = 3.7 Hz), 2.09 (s, 3H), 2.04 (s, 3H), 1.61 (m, 2H), 1.27 (m, 10H), 0.87 (m, 3H). Anal. Calcd for $C_{17}H_{30}O_7$: C, 58.94; H, 8.73. Found: C, 59.21; H, 9.01. Next eluted was **6** (0.43 g, 70%) as a white solid: mp 49–51 °C; $[\alpha]_D$ −56.5° ($CHCl_3$, *c* 1); ^{1}H NMR (200 MHz, $CDCl_3$) δ 4.79 (m, 2H), 4.57 (d, 1H, J = 4.6 Hz), 4.09 (dd, 1H, J = 3.9, 12.6 Hz), 3.76 (m, 2H), 3.40 (m, 2H), 3.03 (d, 1H, J = 7.9 Hz), 2.09 (s, 3H), 2.08 (s, 3H), 1.56 (m, 2H), 1.27 (m, 10H), 0.85 (m, 3H). Anal. Calcd for $C_{17}H_{30}O_7$: C, 58.94; H, 8.73. Found: C, 59.10; H, 9.00.

Methyl 3,4-Di-*O*-acetyl-β-D-xylopyranoside (4). To a solution of methyl β-D-xylopyranoside (**3**, 1 g, 6.1 mmol) in acetonitrile (300 mL) were added vinyl acetate (2.8 mL, 30.5 mmol, 5 equiv), 4-Å molecular sieves (15.2 g), and Lipase PS (9.1 g). The reaction mixture was shaken at 30 °C and 250 rpm for 55 h, and then the reaction was stopped by filtration of the enzyme. The solvent was eliminated, and the residue (1.7 g) was purified by column chromatography (ethyl acetate–hexane (1:1)) to give **4** (1.2 g, 85%): mp 110 °C (transition temperature) → 120 °C; $[\alpha]_D$ −32.4° ($CHCl_3$, *c* 1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.05 (t, 1H, J = 8.9 Hz), 4.90 (m, 1H), 4.22 (d, 1H, J = 7.1 Hz), 4.05 (dd, 1H, J = 5.2, 11.7 Hz), 3.50 (s, 3H), 3.48 (ddd, 1H, J = 9.0, 7.1, 3.4 Hz), 3.30 (dd, 1H, J = 9.4, 11.7 Hz), 2.56 (d, 1H, J = 3.7 Hz), 2.06 (s, 3H), 2.01 (s, 3H). Anal. Calcd for $C_{10}H_{16}O_7$: C, 48.38; H, 6.50. Found: C, 48.38; H, 6.80.

Octyl 3-*O*-(β-D-Galactopyranosyl)-β-D-xylopyranoside (11). To a mixture of **6** (0.24 g, 0.7 mmol) and 4-Å molecular sieves (0.5 g) in dry CH_2Cl_2 (25 mL) was added a solution of 2 M trimethylsilyltriflate (1.4 mL, 0.28 mmol, 0.4 equiv) at −20 °C under argon atmosphere. Then 2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl trichloroacetimidate[27] (1.1 g, 1.4 mmol, 2 equiv) was added in four equal portions during 2h. After 3 h the reaction was allowed to reach room temperature and triethylamine (2 mL) was added. The reaction mixture was diluted with CH_2Cl_2, filtered, and concentrated. Column chromatography (toluene–ethyl acetate (12:1)) of the residue gave first octyl 2,4-di-*O*-acetyl-3-*O*-(2,3,4,6-tetra-*O*-benzoyl-α-D-galactopyranosyl)-β-D-xylopyranoside. Next eluted was the desired octyl 2,4-di-*O*-acetyl-3-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-β-D-xylopyranoside (0.55 g) accompanied with some decomposed benzoyl donor (<10%): ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.54 (m, 20H), 5.97 (d, 1H, J = 3.3 Hz), 5.71 (dd, 1H, J = 7.6, 10.6 Hz), 5.58 (dd, 1H, J = 10.3, 3.3 Hz), 5.01 (d, 1H, J = 7.5 Hz), 5.00 (m, 1H), 4.83 (dd, 1H, J = 5.2, 6.8 Hz), 4.67 (m, 1H), 4.38 (d, 1H, J = 5.0 Hz), 4.36 (m, 2H), 4.11 (dd, 1H, J = 4.2, 12.2 Hz), 3.97 (t, 1H, J = 6.7 Hz), 3.53 (dt, 1H, J = 6.7, 9.8 Hz), 3.35 (dd, 1H, J = 6.6, 12.2 Hz), 3.22 (dt, 1H, J = 6.8, 9.8 Hz), 1.95 (s, 6H), 1.60 (m, 2H), 1,18 (m, 10H), 0.86 (m, 3H). This mixture dissolved in methanol was treated with sodium methoxide (0.1 M) at room temperature, neutralized with Amberlite IR-120 (H^+), filtered, and concentrated. Column chromatography (ethyl acetate–methanol (4: 1)) of the residue gave a crystalline compound **11** (0.16 g, 53% from **6**): mp 98 °C (crystal → ordered mesophase); transition point 155 °C (ordered mesophase → smectic A); clearing point 220 °C (smectic A → isotropic liquid); $[\alpha]_D$ −34.1° (MeOH, *c* 1); ^{1}H NMR (200 MHz, CD_3OD) δ 4.53 (d, 1H, J = 7.6 Hz), 4.29 (d, 1H, J = 7.5 Hz), 3.95 (dd, 1H, J = 11.5, 5.3 Hz), 3.27 (dd, 1H, J = 11.6, 9.8 Hz), 1.63 (m, 2H), 1.37 (m, 10H), 0.95 (m, 3H); ^{13}C NMR (50 MHz, CD_3OD) δ 105.9, 104.9, 87.7, 77.4, 75.0, 74.4, 73.3, 71.1, 70.6, 70.3, 66.9, 62.9, 33.3, 31.1, 30.8, 30.7, 27.4, 24.0, 14.7. Anal. Calcd for $C_{19}H_{36}O_{10}$: C, 53.76; H, 8.55. Found: C, 53.53; H, 8.22.

A sample of **11** was conventionally acetylated to confirm its structure: ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.36 (dd, 1H, J = 3.5, 1.1 Hz, H-4′), 5.13 (dd, 1H, J = 10.5, 7.9 Hz, H-2′), 4.96 (dd, 1H, J = 10.4, 3.4 Hz, H-3′), 4.90 (m, 1H, H-4), 4.87 (dd, H, J = 7.9, 6.1 Hz, H-2), 4.59 (d, 1H, J = 7.9 Hz), 4.40 (d, 1H, J = 6.2 Hz), 4.19 (dd, 1H, J = 11.0, 7.3 Hz), 4.09 (dd, 1H, J = 12.1, 4.8 Hz), 4.05 (dd, 1H, J = 11.0, 7.2 Hz), 3.90 (td, J = 7.3, 1.1 Hz), 3.85 (t, 1H, J = 7.7 Hz, H-3), 3.75 (dt, 1H, J = 9.7, 6.5 Hz), 3.41 (dt, 1H, J = 9.7, 6.7 Hz), 3.35 (dd, 1H, J = 12.0, 7.7 Hz), 2.15 (s, 3H), 2.10 (s, 3H), 2.05 (s, 6H), 2.03 (s, 3H), 1.97 (s, 3H), 1.56 (m, 2H), 1.26 (m, 10H), 0.86 (m, 3H).

Methyl 3,4-Di-*O*-acetyl-2-*O*-[bis(benzyloxy)phosphoryl]-β-D-xylopyranoside (12). To a solution of **4** (0.6 g, 2.4 mmol) in a mixture dichloromethane-acetonitrile (1:1, 90 mL) were added 1*H*-tetrazole (0.5 g, 7.3 mmol, 1.5 equiv) and dibenzyl *N,N*-diisopropylphosphoramidite[28] (1.6 mL, 3.6 mmol, 1.5 equiv) at room temperature. The solution was stirred for 2 h and then $RuCl_3 \cdot 3H_2O$ (catalytic amount), sodium periodate (1.0 g, 4.8 mmol, 2 equiv), and water were added. After 1 h the reaction mixture was diluted with CH_2Cl_2 and washed succesively with aqueous sodium bisulfate (3 × 30 mL) and water (3 × 30 mL), dried over sodium sulfate, and concentrated. Column chromatography of the residue (ethyl acetate–hexane (1:2)) gave compound **12** (1.1 g, 90%): mp 74–76 °C; $[\alpha]_D$ 4.5° ($CHCl_3$, *c* 1); ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.34 (s, 5H), 7.33 (s, 5H), 5.27 (t, 1H, J = 8.9 Hz), 5.06 (d, 2H, J = 7.32 Hz), 5.00 (d, 2H, J = 6.6 Hz), 4.92 (m, 1H), 4.38 (d, 1H, J = 6.5 Hz), 4.34 (m, 1H), 4.11 (dd, 1H, J = 5.4, 11.7 Hz), 3.45 (s, 3H), 3.34 (dd, 1H, J = 9.4, 11.7 Hz), 2.02 (s, 3H), 1.87 (s, 3H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 170.1, 169.8, 135.7, 128.4, 102.2 (d, J = 4.8), 76.1, 72.0, 69.4 (dd, J = 5.7, 6.7 Hz), 69.1, 62.3, 56.7, 20.6. Anal. Calcd for $C_{24}H_{29}O_{10}P$: C, 56.69; H, 5.75. Found: C, 56.91; H, 5.95.

Methyl 3-*O*-Acetyl-2-*O*-[bis(benzyloxy)phosphoryl]-β-D-xylopyranoside (13). To a solution of **12** (0.68 g, 1.3 mmol) in toluene (44 mL) were added *n*-butanol (0.4 mL, 4.4 mmol, 3.4 equiv) and Lipase PS (4.4 g). The reaction mixture was

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shaken at 35 °C and 250 rpm until complete disappearance of **12**. The reaction was stopped by filtration of the enzyme. The solvent was eliminated, and column chromatography of the residue (ethyl acetate–hexane (1:1)) afforded compound **13** (0.55 g, 90%): syrup; $[\alpha]_D$ −8.2° ($CHCl_3$, *c* 0.8) (lit.[15] $[\alpha]_D$ −7.5° ($CHCl_3$, *c* 0.6)); ^1H NMR (200 MHz, $CDCl_3$) δ 7.34 (s, 5H), 7.33 (s, 5H), 5.01 (m, 5H), 4.37 (d, 1H, *J* = 7.1 Hz), 4.29 (m, 1H), 4.04 (dd, 1H, *J* = 5.1, 11.6 Hz), 3.79 (m, 1H), 3.44 (s, 3H), 3.32 (dd, 1H, *J* = 9.5, 11.6 Hz), 2.99 (d, 1H, *J* = 5.6 Hz), 1.92 (s, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 171.3, 135.7, 128.0, 102.2 (d, *J* = 4.7 Hz) 76.4, 75.7, 69.4 (d, *J* = 5.8 Hz), 69.1 (d, *J* = 5.4 Hz), 68.4, 65.4, 56.5, 20.7. Anal. Calcd for $C_{22}H_{27}O_9P$: C, 56.60; H, 5.83. Found: C, 56.61; H, 5.97.

Methyl 3,4-Di-*O*-acetyl-2-*O*-[3,4,6-tri-*O*-acetyl-2-*O*-(trichloroacetyl)-β-D-galactopyranosyl]-β-D-xylopyranoside (16). A mixture of **4** (1.2 g, 4.8 mmol), silver triflate (2.5 g, 9.6 mmol, 2 equiv), 2,6-di-*tert*-butyl-4-methylpyridine (0.99 g, 4.8 mmol, 1 equiv), and 4-Å molecular sieves (3 g) in dry CH_2Cl_2 (30 mL) was stirred at room temperature under argon atmosphere. After 30 min the 2,3,6-tri-*O*-acetyl-2-*O*-(trichloroacetyl)-β-D-galactopyranosyl chloride[21] (**15**, 4.5 g, 14.4 mmol, 3 equiv) was added in six equal portions during 48 h. One equivalent more of silver triflate and 2 equiv more of base were added during the course of the reaction. After 4 days the reaction mixture was filtered, and the filtrate was poured into ice–water. The organic phase was washed successively with sodium hydrogen carbonate (2 × 50 mL) and water (2 × 50 mL), dried over sodium sulfate, and concentrated. The residue was fractionated by column chromatography (hexane–ethyl acetate (3:1)) to give first methyl 3,4-di-*O*-acetyl-2-*O*-[3,4,6–tri-*O*-acetyl-2-*O*-(trichloroacetyl)-α-D-galactopyranosyl]-β-D-xylopyranoside (1.24 g, 39%) as a syrup: ^1H NMR (200 MHz, $CDCl_3$) δ 5.72 (d, 1H, *J* = 3.8 Hz), 5.44 (d, 1H, *J* = 2.5 Hz), 5.33 (dd, 1H, *J* = 10.7, 3.3 Hz), 5.14 (m, 2H), 4.89 (m, 1H), 4.27–3.96 (m, 5H), 3.66 (dd, 1H, *J* = 9.3, 7.27 Hz), 3.41 (s, 3H), 3.30 (dd, 1H, *J* = 9.9, 11.6 Hz), 2.13 (s, 3H), 2.07 (s, 3H), 2.05 (s, 3 H), 2.02 (s, 3H), 1.95 (s, 3H).

The second product to elute was **16** (1.18 g, 37%) as a syrup: $[\alpha]_D$ + 7.6° ($CDCl_3$, *c* 0.8); ^1H NMR (300 MHz, $CDCl_3$) δ 5.38 (dd, 1H, *J* = 4.1, 1.1 Hz), 5.21 (dd, *J* = 10.4, 7.7 Hz), 5.10 (dd, 1H, *J* = 10.3, 3.3 Hz), 5.05 (t, 1H, *J* = 7.2 Hz), 4.92 (d, 1H, *J* = 7.7 Hz), 4.78 (td, 1H, *J* = 6.8, 4.3 Hz), 4.57 (d, 1H, *J* = 5.1 Hz), 4.10 (m, 2H), 4.02 (dd, 1H, *J* = 12.1, 5.5 Hz), 3.93 (td, 1H, *J* = 6.5, 1.1 Hz), 3.60 (dd, 1H, *J* = 7.1, 5.1 Hz), 3.46 (s, 3H), 3.41 (dd, 1H, *J* = 7.1, 5.1 Hz), 2.03 (s, 6H), 2.02 (s, 3H), 2.01 (s, 3H), 1.9 (s, 3H). Anal. Calcd for $C_{24}H_{31}O_{16}Cl_3$: C, 42.35; H, 4.59. Found: C, 42.59; H, 4.51.

Methyl 3,4-Di-*O*-acetyl-2-*O*-(3,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-β-D-xylopyranoside (17). **16** (220 mg, 0.33 mmol) was dissolved in ammonia-saturated ether (15 mL), cooled at 0 °C, and shaken vigorously. After 3 h the reaction mixture was concentrated to give **17** (160 mg, 90%) as a syrup: $[\alpha]_D$ +1.1° ($CHCl_3$, *c* 0.2); ^1H NMR (300 MHz, $CDCl_3$) δ 5.36 (dd, 1H, *J* = 3.4, 1.1 Hz), 5.15 (t, 1H, *J* = 7.6 Hz), 4.90 (dd, 1H, *J* = 8.2, 3.3 Hz), 4.89 (m, 1H), 4.51 (d, 1H, *J* = 5.5 Hz), 4.48 (d, 1H, *J* = 7.9 Hz), 4.13 (d, 2H, *J* = 6.6 Hz), 4.09 (dd, 1H, *J* = 12.3, 4.9 Hz), 3.89 (td, 1H, *J* = 6.5, 1.2 Hz), 3.79 (ddd, *J* = 10.3, 8.0, 2.0 Hz), 3.70 (dd, 1H, *J* = 5.5, 7.6 Hz), 3.49 (s, 3H), 3.43 (dd, 1H, *J* = 12.1, 7.4 Hz), 2.65 (d, 1H, *J* = 2.0 Hz), 2.12 (s, 3H), 2.07 (s, 3H), 2.05 (s, 3H), 2.04 (s, 3H), 2.03 (s, 3H). Anal. Calcd for $C_{22}H_{32}O_{15}$: C, 49.24; C, 6.01. Found: C, 49.50; H, 5.98.

Methyl 3,4-Di-*O*-acetyl-2-*O*-[3,4,6-tri-*O*-acetyl-2-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-β-D-galactopyranosyl]-β-D-xylopyranoside (18). A suspension of **17** (160 mg, 0.3 mmol), silver triflate (77 mg, 0.3 mmol), 2.6-di-*tert*-butyl-4-methylpyridine (62 mg, 0.3 mmol), and 4-Å molecular sieves (1 g) in dry CH_2Cl_2 (15 mL) at −78 °C was stirred under argon atmosphere for 45 min. After this time 2,3,4-tri-*O*-benzylfucopyranosyl bromide[29] (270 mg, 1.8 equiv) was added in six equal portions during 5 h. A half equivalent more of silver triflate was added during the course of the reaction. After 7 h the reaction mixture was filtered and poured into ice–water. The organic phase was washed successively with sodium hydrogen carbonate (2 × 20 mL) and water (2 × 30 mL), dried over sodium sulfate, and concentrated. Column chromatography of the residue (hexane–ethyl acetate (2:1)) gave pure **18** (202 mg, 70%) as a syrup: $[\alpha]_D$ −26.1° ($CHCl_3$, *c* 0.4); ^1H-NMR (200 MHz, $CDCl_3$) δ 5.34 (dd, 1H, *J* = 3.5, 1.1 Hz), 5.21 (d, 1H, *J* = 3.9 Hz), 5.16 (t, 1H, *J* = 8.0 Hz), 5.05 (dd, *J* = 10.0, 3.4 Hz), 4.96 (d, 1H, *J* = 11.6 Hz), 4.9 (m, 1H), 4.76 (s, 2H), 4.73 (d, 1H, *J* = 11.6 Hz), 4.64 (d, 1H, *J* = 6.4 Hz), 4.63 (d, 1H, *J* = 12.8 Hz), 4.61 (d, 1H, *J* = 11.6 Hz), 4.54 (d, 1H, *J* = 4.7 Hz), 4.20–3.76 (m, 10H), 3,45 (dd, *J* = 12.0, 6.8 Hz), 3.44 (s, 3H), 2.12 (s, 3H), 2.04 (s, 3H), 2.04 (s, 3H), 2.02 (s, 3H), 1.76 (s, 3H), 1.19 (d, 3H, *J* = 6.5 Hz). Anal. Calcd for $C_{49}H_{60}O_{19}$: C, 61.74; H, 6.35. Found: C, 61.90; H, 6.61.

Methyl 2-*O*-[2-*O*-(α-L-Fucopyranosyl)-β-D-galactopyranosyl]-β-D-xylopyranoside (19). Hydrogenation of **18** (130 mg, 0.14 mmol) with 10% Pd/C (0.5 g) in ethyl acetate (10 mL) for 18 h at atmospheric pressure afforded methyl 3,4-di-*O*-acetyl-2-*O*-[3,4,6-tri-*O*-acetyl-2-*O*-(α-L-fucopyranosyl)-β-D-galactorpyanosyl]-β-D-xylopyranoside (90 mg) as a syrup. This product was dissolved in methanol (10 mL), and a 0.1 M solution of sodium methoxide in methanol was added. After 1 h the mixture was neutralized with Amberlite IR-120(H^+) resin, filtered, and concentrated. After purification by column chromatography (ethyl acetate–methanol (1:1)) **19** was obtained (58 mg, 90% from **18**): mp 122–125 °C; $[\alpha]_D$ −150° (H_2O, *c* 0.4); ^1H NMR (200 MHz, D_2O) δ 5.15 (d, 1H, *J* = 3.9 Hz), 4.81 (d, 1H, *J* = 7.9 Hz), 4.3 (d, 1H, *J* = 6.5 Hz), 3.22 (s, 3H), 1.12 (d, 3H, *J* = 6.6 Hz); ^{13}C NMR (50 MHz, D_2O) δ 105.17, 103.48, 102.32, 79.91, 79.82, 79.50, 77.87, 76.45, 74.70, 72.44, 72.30, 71.95, 71.26, 69.71, 67.93, 63.91, 59.58, 18.41. Anal. Calcd for $C_{18}H_{32}O_{14}$: C, 45.74; H, 6.83. Found: C, 45.32; H:, 7.21.

Acknowledgment. Financial support by DGICYT (Grant PB90-0076), Comunidad de Madrid (Grant C258/91), and European Community (Bridge Programme, Grant BIOT-CT90-0176) are gratefully acknowledged. We thank Professor Martín-Lomas and Dr. H. Driguez for helpful discussions and Dr. Vokmar Vill for suggesting the synthesis of compound **11** and determining its liquid crystal properties.

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