(c) The adduct **15** was unstable but the bipyridyl adduct could be isolated: ^{1}H NMR (py-d_5) 3.66 (ddd, 1 H, $J_{Pt,H}$ = 93 Hz), 3.04 (m, 1 H), 3.01 (dd, 1 H, $J_{PT,H}$ = obsc), 2.76 (dd, 1 H, $J_{Pt,H}$ = obsc), 2.14 (m, 2 H), 1.5-1.0 (m, 6 H); ^{13}C NMR (py-d_5) 44.6 (d, $J_{Pt,C}$ = 98 Hz), 27.2 (t), 25.7 (t), 20.4 (t), 19.9 (t), 5.8 (d, $J_{Pt,C}$ = 369 Hz), -8.6 (t, $J_{Pt,C}$ = 354 Hz); ^{195}Pt NMR (py-d_5) 3315.

Reaction of 11 and 14 with 2,2'-Bipyridine. Three to four equivalents of bpy were added to a stirred suspension of **11** or **14** in $CHCl_3$ or pyridine, respectively. Rotoevaporation of the solution yielded a pink or yellow solid which was chromatographed on SiO_2 to give the bpy adduct (87% yield).

(a) Bpy adduct of **11**: mp >300 °C dec. Anal. Calcd for $C_{19}H_{24}N_2Cl_2Pt$: C, 41.76; H, 4.43. Found: C, 41.63; H, 4.42. ^{1}H NMR ($CDCl_3$) 3.5 (dd, 1 H, $J_{Pt,H}$ = 98 Hz), 2.9 (dddd, 1 H), 2.6 (dd, 1 H, $J_{Pt,H}$ = 91 Hz), 2.4 (dd, 1 H, $J_{Pt,H}$ = obsc), 2.2 (m, 1 H), 2.0 (m, 1 H), 1.7-1.5 (m, 9 H), 1.3 (d, 1 H); ^{13}C NMR ($CDCl_3$) 47.4 (d, $J_{Pt,C}$ = 84 Hz), 31.6 (t, $J_{Pt,C}$ ~ 38 Hz), 30.8 (t), 30.5 (t), 29.8 (t), 26.1 (t), 26.0 (t), 13.3 (d, $J_{Pt,C}$ = 341 Hz), -6.6 (t, $J_{Pt,C}$ 364 Hz); ^{195}Pt NMR ($CDCl_3$) 3076.

(b) Bpy adduct of **14**: mp 213 °C dec. Anal. Calcd for $C_{17}H_{20}N_2Cl_2Pt$: C, 39.39; H, 3.89. Found: C, 38.80; H, 3.84. ^{1}H NMR ($CDCl_3$) 3.28 (ddd, 1 H, $J_{Pt,H}$ = 92 Hz), 3.06 (m, 1 H), 2.68 (dd, 1 H, $J_{Pt,H}$ = 84 Hz), 2.46 (dd, 1 H, $J_{Pt,H}$ = 80 Hz), 2.05 (m, 1 H), 1.8-1.3 (m, 7 H); ^{13}C NMR ($CDCl_3$) 42.8 (d, $J_{Pt,C}$ = 91 Hz), 27.4 (t, $J_{Pt,C}$ = 24 Hz), 24.6 (t, $J_{Pt,C}$ = 15 Hz), 20.3 (t, $J_{Pt,C}$ = 42 Hz), 19.4 (t, $J_{Pt,C}$ = 48 Hz), 5.2 (d, $J_{Pt,C}$ = 366 Hz), -7.7 (t, $J_{Pt,C}$ = 341 Hz); ^{195}Pt NMR ($CDCl_3$) 3095.

Formation of 19 from 16. To a stirred solution of **16** (0.042 g, 0.34 mmol) in 10 mL of dry ether was added Zeise's dimer (0.100 g, 0.34 mmol). Complex **17** formed as a yellow precipitate and was isolated by filtration at the end of an hour. This precipitate was placed in 1.5 mL of chloroform, Zeise's dimer (0.100 g, 0.34 mmol) was added, and the mixture was purged with N_2 for 20-30 min. The mixture was then subjected to 3 freeze-thaw cycles and flame sealed in a thick-walled tube under vacuum. The sealed tube was heated to 95 °C for 15 min with frequent agitation. Extraction with chloroform followed by centrifugation and evaporation of the resulting solution yielded **18** as an orange solid. This solid was placed in pyridine solution and 2,2-bipyridine (0.156 g, 1.00 mmol) was added with stirring. Evaporation of the solution yielded a pink solid which was chromatographed on SiO_2 with $CHCl_3$ to give **19** as a white solid: mp 243 °C dec; ^{1}H NMR ($CDCl_3$) 5.73 (m, 2 H), 3.57 (dddd, 1 H, $J_{Pt,H}$ = 95 Hz), 3.15 (m, 1 H), 2.59 (dd, 1 H, $J_{Pt,H}$ = 91 Hz), 2.54-1.82 (m, 8 H), 1.56 (m, 1 H); ^{13}C NMR ($CDCl_3$) 131.8 (d), 130.4 (d), 47.8 (d, $J_{Pt,C}$ = 82 Hz), 34.1 (t), 32.2 (t), 25.3 (t), 24.9 (t), 12.5 (d, $J_{Pt,C}$ = 345 Hz), -6.1 (t, $J_{Pt,C}$ = 361 Hz), ^{195}Pt NMR ($CDCl_3$) 3072.

Reaction of 11 with Me_2SO. Complex **11** (0.100 g, 0.26 mmol) was placed in 2 mL of cold Me_2SO. A green solution formed which was left standing for 12 **h**. After this time, the only hydrocarbon species in solution was bicyclo[6.1.0]nonane.

Thermal Decomposition of 11 in Et_2O. Complex **11** (0.100 g, 0.26 mmol) in 10 mL of dry ether was refluxed with stirring for 46 h. An aliquot was removed at 10 h. The solid products was rotoevaporated and then were added to stirred solutions of KCN (0.042 g, 0.65 mmol) in 2 mL of H_2O. Stirring was continued until the solid dissolved and then the solution was extracted with $CHCl_3$.

Reaction of 12 with CH_2N_2. Complex **12** was made from **11** with 2 equiv of pyridine in $CHCl_3$ at 0 °C. A large excess of CH_2N_2 was then added to **12** via a stream of dry N_2. Following completion of the reaction, the mixture was distilled under full vacuum to obtain any volatile products. GC/MS and NMR analysis of these products revealed 21% **28**, 23% **10**, 36% **20**, and 20% **21**, by comparison to standards.

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Regioselective Acylation of Secondary Hydroxyl Groups in Sugars Catalyzed by Lipases in Organic Solvents

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Abstract: Several unrelated, commercially available lipases (porcine pancreatic, bacterial, yeast, and fungal) can catalyze transesterification reactions between trichloroethyl butyrate and monosaccharides with blocked C-6 hydroxyl groups (enzymatically acylated or chemically alkylated) in dry organic solvents. Lipases exhibit a remarkable regioselectivity by discriminating among the four available secondary hydroxyl groups in C-6 protected glucose, galactose, and mannose. While some lipases exclusively acylate the C-3 hydroxyl group, others display an overwhelming preference toward the C-2 hydroxyl group. This positional specificity of lipases was used for the preparation of various sugar diesters and, consequently, for either fully enzymatic or chemicoenzymatic preparative synthesis of C-2 or C-3 monoesters of glucose.

Modification of only one out of several identical functional groups in a molecule is a fundamental challenge to organic chemists. An important and synthetically relevant example of this problem is the regioselective acylation of hydroxyl groups in sugars: even discrimination between primary and secondary hydroxyls usually involves multistep procedures, while there is no general basis for the positionally specific acylation of the more abundant, secondary OH groups.[1]

Although enzymes often exhibit a remarkable regioselectivity,[2] enzymatic acylation of sugars in water is thermodynamically unfavorable and hence requires expensive cofactors as a source of free energy. However, enzymes do not have to be used in aqueous solutions and can function as catalysts in organic solvents when certain straightforward rules are followed.[3] In organic media enzymes retain their inherent keen specificity; in addition, they catalyze reactions that are practically impossible in water.[3] For instance, lipases regioselectivity[4] and stereoselectivity[5] acylate alcohols in organic solvents in a quantitative fashion, whereas in

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Table I. Regioselective Acylation of Secondary Hydroxyl Groups in 6-*O*-Butyrylglucose, -galactose, and -mannose Catalyzed by Different Lipases in Organic Solvents[a]

6-*O*-butyrylmonosaccharide	product composition: ratio of 3,6-di-*O*-butyryl sugar to the 2,6-isomer[b]			
	Chromobacterium viscosum lipase[c]	porcine pancreatic lipase[c]	*Candida cylindracea* lipase[c]	*Aspergillus niger* lipase[c]
1	>50	0.1	0.4	>50
2	1.5	1.5	0.5	4.9
3	4.0	0.7	2.0	1.5

[a] A general experimental protocol was as follows: 0.6 g of **1**, **2**, or **3** and 3 mL of **4** were dissolved in 20 mL of methylene chloride (in the case of *Candida cylindracea* lipase) or 10 mL of tetrahydrofuran (in the case of all other lipases) (about 20% of acetone also had to be added to tetrahydrofuran to solubilize **2** and to CH_2Cl_2 to solubilize all sugar monoesters). Then a lipase powder (1 g, 3 g, 5 g and 3 g, respectively, in the order given in the Table) was added, and the suspension[8] was shaken at 25 °C (in the case of porcine pancreatic lipase) or 45 °C (for all other lipases) and 250 rpm for the following periods of time (the order of the enzymes is the same as in the Table) that resulted in the conversions (%) given in parentheses for **1**, **2**, and **3**, respectively: 48 h (100, 100, 100), 90 h (63, 24, 10), 7 days (19, 70, 75), and 90 h (46, 62, 25). Then the solvent was evaporated, the residue washed with 200 mL of hexane and subjected to chromatography on a silica gel column with a mixture of CH_2Cl_2 and MeOH (92:8) as a solvent. For methods, see the Experimental Section. [b] This ratio was determined by GC and/or NMR. (The actual regioselectivity of lipases may be even greater than indicated by the data in the table due to spontaneous migration of the acyl moiety.[13]) No triesters were ever formed, as evidenced by TLC and GC. [c] No appreciable reaction was detected in the absence of the enzyme.

water they would nearly completely hydrolyze the esters.[6]

Recently, we have found[7] that porcine pancreatic lipase catalyzes transesterification reactions between various unprotected monosaccharides and trichloroethyl carboxylates in anhydrous pyridine. The enzyme displays an overwhelming preference toward acylation of the sugar's primary hydroxyl group.[7] In the present study, we have employed the enzymatically formed 6-O-acylated and other 6-O-blocked monosaccharides to address the following basic questions: (i) Is it possible to enzymatically acylate secondary OH groups directly and in a regioselective manner? (ii) Will different lipases show different positional selectivity in the acylation of 6-*O*-acylsugar's secondary hydroxyls? (iii) Can enzymatic acylation in organic solvents be used for the preparation of sugars exclusively monoacylated at different positions? All three questions have been answered affirmatively.

Results and Discussion

Following our previously developed methodology,[7] we prepared multigram quantities of pure 6-*O*-butyrylglucose (**1**), 6-*O*-butyrylgalactose (**2**), and 6-*O*-butyrylmannose (**3**) via the transesterification reaction between the corresponding monosaccharides and 2,2,2-trichloroethyl butyrate (**4**) catalyzed by porcine pancreatic lipase in pyridine. The monobutyrated sugars are soluble in many more organic solvents than their unmodified precursors, including acetone, tetrahydrofuran, methylene chloride, etc. This fact is quite significant, for pyridine is a very poor reaction medium for enzymes[3]—e.g., out of a dozen commercially available lipases tested, only porcine pancreatic lipase and, to a much smaller extent, lipase from *Chromobacterium viscosum* catalyzed the reactions between the monosaccharides and **4** in pyridine. Not only does one expect more lipases to be catalytically active in acetone, tetrahydrofuran, and methylene chloride, but the lipases active in pyridine should be even more reactive in those solvents.

These predictions were verified experimentally. When 0.6 g of **1** and 3 mL of **4** were dissolved in 10 mL of anhydrous pyridine, followed by addition of 3 g of porcine pancreatic lipase, and the suspension[8] was vigorously shaken at 25 °C, only about 10% decrease in the concentration of **1** was detected by gas chromatography after 2 days. When the same experiment was repeated with dry tetrahydrofuran as the solvent, almost 40% of **1** reacted in 2 days.

Furthermore, we have found that at least three other commercially available lipases—from the bacterium *Chromobacterium viscosum*, the yeast *Candida cylindracea*, and the fungus *Aspergillus niger*—can acylate **1** with **4** in tetrahydrofuran or methylene chloride. The fact that four lipases were capable of acylating secondary hydroxyl groups in **1** afforded the opportunity to investigate their positional selectivity in that reaction. A typical protocol is exemplified by the following experiment: 2.0 g of **1** was dissolved in 30 mL of tetrahydrofuran, and then 10 mL of **4** and 3 g of *Chromobacterium viscosum* lipase were added. The suspension[8] was shaken at 45 °C for 40 h after which time virtually all **1** reacted. The enzyme was removed by filtration, the solvent was evaporated, and the residue was washed with hexane, followed by crystallization from a cyclohexane/ethyl acetate mixture. As a result, 2.0 g of white crystals was obtained, and the product was found to be a single, pure compound by TLC and GC. Its saponification confirmed that it was a diester, and a ^{13}C NMR analysis revealed its structure as 3,6-di-*O*-butyrylglucose. Hence, the enzyme exhibits a remarkable regioselectivity in the reaction with **1** by acylating only one out of four available secondary hydroxyl groups.

Similar experiments were then conducted with the three other lipases, and the data obtained are presented in the top line of Table I. One can see that *Aspergillus niger* lipase, like the enzyme from *Chromobacterium viscosum*, exclusively acylates the hydroxyl group in the C-3 position of **1**; conversely, porcine pancreatic lipase has a strong preference toward the C-2 position—90% of the product is 2,6-di-*O*-butyrylglucose. *Candida cylindracea* lipase displays comparable reactivities with respect to C-2 and C-3 hydroxyl groups (being somewhat more reactive toward the former). Thus, different lipases possess distinct, sometimes opposite, regioselectivities in the reaction with **1**, presumably due to different modes of binding of the substrate with the enzymes' active centers.

We then tested the regioselectivity of the four lipases in the reactions with 6-*O*-butyryl derivatives of two other monosaccharides, mannose and galactose (the last two lines in Table I). It is seen that although the differences in reactivities toward OH groups in C-2 and C-3 positions in the case of **2** and **3** are lower than for **1**, significant variations in enzyme positional specificities are nevertheless observed.

Recently, Sweers and Wong reported that *Candida cylindracea* lipase hydrolyzes glucose pentaacetate virtually exclusively in the C-6 position.[9] We found that the enzyme can also deacylate 2,6- and 3,6-diesters of monosaccharides with the same regioselectivity. This phenomenon was then employed for the preparation of pure 3-*O*-butyrylglucose from the enzymatically formed 3,6-di-*O*-butyrylglucose with an 85% isolated yield. In addition, we elaborated chemicoenzymatic alternatives to the foregoing fully enzymatic production of a secondary sugar monoester. To that end, the hydroxyl group in the C-6 position of α-D-glucose was

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(8) All lipases tested (as well as other enzymes) are insoluble in all organic solvents used in this work. Therefore, reaction mixtures were always suspensions of enzymes in solutions of substrates in organic solvents.

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Table II. Lipase-Catalyzed Acylation of *n*-Octyl β-D-Glucopyranoside in Organic Solvents[a]

lipase	reaction time, h (deg of conversion,[b] %)	ratio of mono- to diester[c]	position of acylation[d] in monoester (fraction, %)	position of acylation[d] in diester (fraction, %)
Chromobacterium viscosum[e]	48 (100)	1:1	C-6 (100)	C-3, C-6 (100)
porcine pancreatic[e]	48 (100)	1:9	C-6 (100)	C-2, C-6 (100)
Aspergillus niger[e]	48 (96)	10:1	C-6 (100)	C-3, C-6 (80)
Candida cylindracea[e]	48 (29)	>50:1	C-6 (80) C-3 (10) C-2 (10)	

[a] *n*-Octyl β-D-glucopyranoside (0.6 g, purchased from Aldrich) and **1** (3 mL) were dissolved in 10 mL of methylene chloride (in the case of yeast lipase) or tetrahydrofuran (in the case of the other three lipases). Then a lipase powder was added (following the order of the enzymes given in the table from top to bottom the amounts were 0.3, 2, 3, and 3 g, respectively), and the suspension[8] was shaken at 45 °C and 250 rpm for the periods of time indicated in the table which afforded the degrees of conversion given in parentheses. After that, the enzyme was removed by filtration, the solvent evaporated, and the residue dissolved in CH_2Cl_2 and applied to a silica gel column. The loaded column was thoroughly washed with the same solvent, and then the diester was eluted with methylene chloride containing 4% (v/v) of methanol and the monoester with that containing 8% (v/v) of methanol. The products obtained were analyzed by NMR and confirmed by saponification. [b] Determined by GC on the basis of disappearance of **5**. [c] Determined by GC in the reaction mixture prior to ester separation on the basis of the ratio of the peak area for the monoesters to that for the diesters. [d] Established by NMR. [e] No appreciable reaction was detected in the absence of the enzyme.

chemically modified with triphenylmethyl chloride.[10] The resultant 6-*O*-tritylglucose was a good substrate for *Chromobacterium viscosum* lipase[11] and consequently was quantitatively acylated in the C-3 position. The resultant product was chemically detritylated[12] to yield pure crystalline 3-*O*-butyrylglucose with an 88% isolated yield. In another example, the C-6 hydroxyl group of α-D-glucose was chemically modified with a *tert*-butyldiphenylsilyl group.[13] The product was reactive not only toward the bacterial enzyme in tetrahydrofuran but also toward *Candida cylindracea* lipase in methylene chloride. Interestingly, the latter enzyme acylates 6-*O*-(*tert*-butyldiphenylsilyl)glucose virtually exclusively at the C-2 position (while with **1** as a substrate there was a mixture of C-2 and C-3 acylated compounds (Table I)); thus, increasing the size of a substituent at C-6 greatly enhances regioselectivity of the yeast lipase. The enzymatically formed 2,6-glucose derivative was chemically deblocked at C-6 to yield 2-*O*-butyrylglucose.

It was of interest to investigate the regioselectivity of lipase in the acylation of a monosaccharide in which both the primary and secondary hydroxyl groups are available and then compare it with the data reported above. To circumvent the solubility problem, instead of glucose itself we employed *n*-octyl β-D-glucopyranoside[14] (**5**), which is soluble in a number of organic solvents. The results obtained are depicted in Table II, and they afford several interesting conclusions. In the case of all four enzymes, the most reactive group in **5** is the primary hydroxyl at C-6. For the yeast lipase the acylation of **5** in the C-6 position makes the subsequent acylation impossible and hence no diester is formed. In contrast, the other three lipases form both monoesters and diesters in the transesterification reaction between **5** and **4** in organic solvents. There is a reasonable correlation between regioselectivities of different lipases in the acylation of **1** and **5**: e.g., the enzyme from *Chromobacterium viscosum* in both instances reacts only with the C-3 secondary hydroxyl group, while the porcine pancreatic enzyme has an overwhelming preference for the C-2 position (Tables I and II). As one would expect, upon a longer incubation of the biocatalyst with **5** the fraction of the diester vs. the monoester increases: doubling the reaction time yields only the diester as a product of the porcine pancreatic lipase-catalyzed acylation and reduces the monoester/diester ratio from 10:1 to 6:1 for *Aspergillus niger* lipase.

In summary, we discovered that several unrelated lipases efficiently catalyze a one-step acylation of secondary hydroxyl groups in C-6 protected monosaccharides in anhydrous organic solvents—a reaction that is impossible in water. Moreover, some lipases exhibit a striking regioselectivity in that acylation, which varies from one enzyme to another. As a result, C-2 and C-3 esters of C-6 protected sugars can be separately prepared which, following either enzymatic or chemical deprotection, afford C-2 and C-3 monoesters. This positional specificity of lipase compares favorably with classical chemical approaches: for example, when **1** was incubated with 1 mol equiv of butyric anhydride in pyridine overnight at room temperature, a mixture of an unreacted **1** and various diesters (about 40% each) and higher glucose esters was obtained; analysis of the diesters revealed the following approximate composition of the mixture—40% of 2,6- and 20% each of 3,6-, 4,6-, and 1,6-di-*O*-butyrylglucose.[15] Since all lipases used in this work are readily commercially available in large quantities (hundreds of grams) and relatively inexpensive, they appear suitable as versatile practical catalysts for preparative regioselective acylation of sugars.

Experimental Section

Materials. Lipases (EC 3.1.1.3) were obtained as follows: porcine pancreatic and *Candida cylindracea* from Sigma Chemical Co. (St. Louis, MO), *Aspergillus niger* (lipase K) from Amano International Enzyme Co. (Troy, VA), and *Chromobacterium viscosum* (lipase CV) from FinnSugar Biochemicals (Elk Grove Village, IL). Their specific activities were 11, 700, 30, and 120 units/mg of solid. Yeast and fungal lipases were used "straight from the bottle". Porcine pancreatic lipase was kept under vacuum for 3 days prior to use which lowered its water content[16] from 3.6 to 0.5%. Lipase from *Chromobacterium viscosum* was dissolved in a minimal amount of water, pH was adjusted to 7.0, and the solution was freeze-dried. This "pH-adjustment"[3,6] increases catalytic activity of the bacterial lipase in organic media approximately by a factor of 15 (while not significantly affecting that of the other three lipases). All four lipases used in this work were crude preparations; this was viewed as an advantage from the practical standpoint, as it resulted in low cost of catalysts.

Trichloroethyl butyrate was synthesized from butyryl chloride and 2,2,2-trichloroethanol following the general methodology[17] and had the same characteristics as previously described.[5a] We prepared **1**, **2**, and

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3 as reported in ref 7. The properties of the last two, not provided in ref 7, were mp 95–98 °C for **2** (**3** was an oil) and $[\alpha]_D^{30}$ +56.8° (*c* 2, H_2O, eq.) and +13.4° (*c* 8, H_2O, eq.), respectively. **2**: ^{13}C NMR (67.9 MHz, D_2O) δ 96.5, 92.4, 72.6, 72.5, 71.8, 69.3, 69.0, 68.8, 68.2, 68.1, 64.2, 64.0 (a mixture of α and β anomers) (C1, β) (C1, α) (C3, β) (C5, β) (C2, β) (C4, α) (C3, α) (C4, β) (C2, α) (C5, α) (C6, α) (C6, β); these NMR data are consistent with those reported for 6-*O*-acetylgalactose.[18] Anal. Calcd for $C_{10}H_{18}O_7$: C, 48.04; H, 7.21; O, 44.83. Found: C, 48.21; H, 7.32; O, 44.74. Chemical synthesis of **2** was mentioned by Hori[19] but no physical properties of the compound, obtained as a syrup, were reported. **3**: ^{13}C NMR (67.9 MHz, D_2O) δ 94.2, 93.8, 73.6, 72.9, 71.1, 70.6, 70.1, 70.0, 66.8, 66.6, 63.8 (a mixture of α and β anomers) (C1, α) (C1, β) (C5, β) (C3, β) (C2, β) (C2, α) (C3, α) (C5, α) (C4, α) (C4, β) (C6, α,β). Anal. Calcd for $C_{10}H_{18}O_7$: C, 48.01; H, 7.24; O, 44.81. Found: C, 47.84; H, 7.41; O, 44.56. 6-*O*-Tritylglucose and (*tert*-butyldiphenylsilyl)glucose were prepared as described in the literature (ref 10 and 13, respectively). All other chemicals used in this work were obtained commercially and were of reagent grade. Pyridine was purified and dried as outlined before;[7] all other solvents (analytical grade) employed as reaction media for lipase-catalyzed reactions were dried by shaking overnight with 3 A molecular sieves (Linde).

Assays. All sugar derivatives in this work were determined by gas chromatography with a 5-m HP1 capillary fused silica gel column coated with methylsilicon gum (Hewlett-Packard) (N_2 carrier gas, 30 mL/min, detector and injector port temperature 250 °C). All reaction mixtures were subjected to precolumn derivatization with 1,1,1,3,3,3-hexamethyldisilazane.[20]

In addition to GC, the purity of all products was confirmed by TLC with use of precoated silica gel IB-F sheets (J.T. Baker) and ethyl acetate/methanol/water (100:10:1) or methylene chloride containing 5–10% (v/v) methanol as solvents for sugar monoesters and other derivatives, respectively. The spots were developed by spraying with concentrated H_2SO_4, followed by heating.

Optical rotations were measured at 589 nm (sodium line) and 30 °C in a Perkin-Elmer 234 B polarimeter.

Saponification experiments to ascertain the molar ratios of the butyryl groups to the monosaccharide moieties in sugar esters were conducted according to the literature procedure.[21]

Structure Determinations. The positions of acylation in all sugar derivatives enzymatically prepared in this work were established by ^{13}C NMR (Brucker WM 270 spectrometer). The general strategy was the same as previously developed by Yoshimoto et al.[22] As established by these authors, acylation of a hydroxyl group at C-3, C-4, or C-6 positions of glucose results in a downfield shift (1–3 ppm) of the peak corresponding to the O-acylated carbon and an upfield shift (1–3 ppm) of the peak(s) corresponding to the neighboring carbon(s). O-Acylation at C-1 results in a downfield shift of the peaks of both C-1 and C-2. O-Acylation at C-2 results in a downfield shift of the peaks corresponding to C-2 and C-1 and in a normal upfield shift of the C-3 peak. The shift values are virtually independent of the nature of the acyl moiety and the solvent. The rules established for enzymatically formed glucose derivatives were subsequently applied to those of galactose and mannose. The NMR spectra of the monoesters were compared to those of the unmodified, authentic monosaccharides, and the spectra of the diesters were compared to those of the previously identified monoesters (all in acetone or D_2O, tetramethylsilane as an internal or external reference). While the 6-O-monoesters **1**, **2**, and **3** were enzymatically prepared from the monosaccharides as described above, their 2-O and 3-O counterparts were produced from the corresponding 2,6-O- and 3,6-O-diesters via the C-6 selective hydrolysis catalyzed by *Candida cylindracea* lipase (see the text and below).

Enzymatic Synthesis of 3,6- and 2,6-Di-*O*-butyrylglucose. Preparation of 3,6-di-*O*-butyrylglucose from **1** catalyzed by *Chromobacterium viscosum* lipase is described in the Results and Discussion section. The product was obtained in an 80% isolated yield and had mp 85–89 °C and $[\alpha]_D^{30}$ +56.0° (*c* 1, H_2O, eq.). ^{13}C NMR (67.9 MHz, acetone) δ 97.4, 93.1, 78.3, 77.1, 76.3, 73.8, 72.5, 71.5, 69.2, 61.9 (a mixture of α and β anomers) (C1, β) (C1, α) (C3, β) (C5, β) (C3, α) (C2, β) (C5, α) (C2, α) (C4, α,β) (C6, α,β). Anal. Calcd for $C_{14}H_{24}O_8$: C, 52.53; H, 7.54; O, 39.99. Found: C, 52.62; H, 7.61; O, 39.88.

2,6-Di-*O*-butyrylglucose was synthesized as follows: 2 g of **1** and 10 mL of **4** were dissolved in 30 mL of tetrahydrofuran, and then 10 g of porcine pancreatic lipase was added. The suspension[8] was shaken at 25 °C and 250 rpm for 48 h to reach a 56% conversion; then the enzyme was replaced with a new batch and the suspension shaken for another 48 h to reach an 89% conversion of **1**. The enzyme was removed by filtration, the solvent evaporated, and the residue washed with hexane and then chromatographed on a silica gel column with a CH_2Cl_2–MeOH mixture (92:8) as a solvent. The resultant light-yellow oil was crystallized from isopropyl ether to form 1.3 g of white crystals (51% overall isolated yield). The product was pure by GC, TLC, and NMR and had mp 82–85 °C and $[\alpha]_D^{30}$ +50.8° (*c* 4, H_2O, eq.). ^{13}C NMR (67.9 MHz, acetone) δ 95.7, 90.5, 75.7, 75.2, 74.6, 74.5, 71.4, 71.3, 70.0, 64.3, 64.1 (a mixture of α and β anomers) (C1, β) (C1, α) (C2, β) (C3, β), (C5, β) (C2, α) (C3, α) (C4, α,β) (C5, α) (C6, β) (C6, α). These NMR data are consistent with those reported for 2,6-di-*O*-myristoylglucose.[22] Anal. Calcd for $C_{14}H_{24}O_8$: C, 52.46; H, 7.54; O, 40.02. Found: C, 52.24; H, 7.48; O, 39.84.

Enzymatic Synthesis of 2,6- and 3,6-Di-*O*-butyrylgalactose. Two grams of **2** and 10 mL of **4** were dissolved in 30 mL of tetrahydrofuran, followed by addition of 3 g of *Chromobacterium viscosum* lipase. The suspension[8] was shaken at 45 °C and 250 rpm for 40 h, after which time virtually all **2** has reacted. Then the enzyme was filtered out and the solvent evaporated. The residue was washed with hexane, dissolved in water, and washed with hexane again, and then water was evaporated. The residue was subjected to a separation by preparative HPLC (silica gel column, a mixture of ethyl acetate and hexane (6:4) as a solvent, flow rate of 60 mL/min). As a result, pure (by GC, TLC, and NMR) 2,6-di-*O*-butyrylgalactose (0.5 g of white crystals) and 3,6-di-*O*-butyrylgalactose (0.8 g of an oil which slowly crystallized upon storage) were separately obtained. The products (combined isolated yield of 51%) had mp 98–103 and 70–74 °C and $[\alpha]_D^{30}$ +64.5° and +81.5° (*c* 1, H_2O, eq.), respectively. 2,6-Di-*O*-butyrylgalactose: ^{13}C NMR (67.9 MHz, acetone) δ 96.1, 90.8, 74.6, 73.3, 72.3, 70.3, 69.8, 68.5, 67.6, 64.3, 64.1 (a mixture of α and β anomers) (C1, β) (C1, α) (C2, β) (C5, β) (C2, α; C3, β) (C4, α) (C4, β) (C5, α) (C3, α) (C6, α) (C6, β). Anal. Calcd for $C_{14}H_{24}O_8$: C_7 52.47; H, 7.52; O, 40.03. Found: C, 52.53; H, 7.58; O, 39.92. 3,6-Di-*O*-butyrylgalactose: ^{13}C NMR (67.9 MHz, acetone) δ 98.2, 93.6, 76.4, 73.6, 73.0, 70.4, 68.5, 68.0, 67.4, 67.2, 64.0, 63.9 (a mixture of α and β anomers) (C1, β) (C1, α) (C3, β) (C3, α) (C5, β) (C2, β) (C4, α) (C5, α) (C4, β) (C2, α) (C6, α) (C6, β). Anal. Calcd for $C_{14}H_{24}O_8$: C, 52.51; H, 7.51; O, 39.99. Found: C, 52.54; H, 7.44; O, 39.96.

Enzymatic Synthesis of 2,6- and 3,6-Di-*O*-butyrylmannose. The two diesters were prepared from **3** the same way as described above for the galactose diesters, except that a mixture of CH_2Cl_2 and MeOH (96:4) was used as a solvent in preparative HPLC. As a result, pure (by GC, TLC and NMR) 3,6-di-*O*-butyrylmannose (oil, 1.3 g) and 2,6-di-*O*-butyrylmannose (oil, 0.33 g) were obtained. The products (combined isolated yield of 65%) had $[\alpha]_D^{30}$ +9.8° and −1.5° (*c* 1.5, H_2O, eq.), respectively. 2,6-Di-*O*-butyrylmannose: ^{13}C NMR (67.9 MHz, acetone) δ 92.3 (C1), 74.0 (C2), 71.0 (C5), 69.5 (C3), 68.2 (C4), 64.1 (C6). Anal. Calcd for $C_{14}H_{24}O_8$: C, 52.52; H, 7.48; O, 40.04. Found: C, 52.62; H, 7.41; O, 40.17. 3,6-Di-*O*-butyrylmannose: ^{13}C NMR (67.9 MHz, acetone) δ 95.2 (C1), 75.0 (C3), 71.5 (C5), 70.1 (C2), 65.6 (C4), 64.4 (C6) (all for the pedominant α anomer). Anal. Calcd for $C_{14}H_{24}O_8$: C, 52.46; H, 7.52; O, 40.03. Found: C, 52.24; H, 7.59; O, 39.92.

Enzymatic Synthesis of 3-*O*-Butyrylglucose. Two independent, alternative methods were employed. (i) 3,6-Di-*O*-butyrylglucose (0.32 g), enzymatically prepared as described above, was dissolved in 10 mL of aqueous buffer (0.1 M Tris·HCl, pH 7.6), and then 0.16 g of *Candida cylindracea* lipase was added. The solution was stirred for 8 h, while pH was maintained between 7 and 8 by adding 1 N NaOH, after which time the C-6 butyryl moiety was almost completely cleaved off. Then three volumes of acetone were added, and the mixture was placed in a freezer for several hours to precipitate the enzyme which was subsequently removed by filtration. The resultant aqueous solution was evaporated to dryness and the residue subjected to column silica gel chromatography (a mixture of ethyl acetate, methanol, and H_2O (100:10:1) as a solvent). As a result, 0.21 g (85% isolated yield) of crystalline 3-*O*-butyrylglucose (pure by TLC and GC) with $[\alpha]_D^{30}$ +56.7° (*c* 2.5, H_2O, eq.) was obtained (the product was too hygroscopic to measure melting point).[23] (ii) We dissolved 1.4 g of 6-*O*-tritylglucose (prepared chemically through a standard procedure[10]) and 3 mL of **4** in 10 mL of tetrahydrofuran. Then 0.3 g of *Chromobacterium viscosum* lipase was added, and the suspen-

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(24) This work was financially supported by W. R. Grace & Co.

sion[8] was shaken at 45 °C and 250 rpm. After 48 h, when virtually all sugar substrate had reacted, the enzyme was filtered out, and the remaining solution was added to a mixture of 25 mL of *n*-butanol and 8 mL of trifluoroacetic acid to remove the trityl moiety.[12] After a 10-min incubation at room temperature the mixture was evaporated to dryness under vacuum, and the residue was washed with 100 mL of toluene, dissolved in ethyl acetate, and applied to a silica gel column equilibrated with the same solvent. The column was thoroughly washed with ethyl acetate, and then the product was eluted with a mixture of ethyl acetate, methanol, and water (100:10:1). As a result, 0.75 g (88% isolated yield) of pure (by TLC, GC, and NMR) crystalline 3-*O*-butyrylglucose (identical to that prepared in (i)) was obtained. ^{13}C NMR (67.9 MHz, acetone) δ 97.4, 93.1, 78.3, 77.1, 76.3, 73.8, 72.5, 71.4, 69.2, 61.9 (a mixture of α and β anomers) (C1, β) (C1, α) (C3, β) (C5, β) (C3, α) (C2, β) (C5, α) (C2, α) (C4, α,β) (C6, α,β). These data are in agreement with those reported for 3-*O*-acetylglucose.[22] Anal. Calcd for $C_{10}H_{18}O_7$·EtOAc: C, 49.68; H, 7.69; O, 42.51. Found: C, 49.53; H, 7.62; O, 42.43.

Enzymatic Synthesis of 2-*O*-Butyrylglucose. We dissolved 2 g of 6-*O*-(*tert*-butyldiphenylsilyl)glucose (prepared chemically[13]) and 5 mL of **4** in 20 mL of methylene chloride. Then 5 g of *Candida cylindracea* lipase was added, and the suspension[8] was shaken at 45 °C and 250 rpm. After 60 h, when 60% of the glucose substrate had reacted, the enzyme was removed by filtration and the solvent and unreacted **4** were evaporated at 100 °C under vacuum. The residue was dissolved in CH_2Cl_2, applied on a silica gel column equilibrated with the same solvent, and thoroughly washed with it. Then the product was eluted with methylene chloride containing 4% (v/v) of methanol, and consequently 1.1 g (75% isolated yield) of light yellow oil was obtained which was determined to be pure (GC, NMR, and TLC) 2-*O*-butyryl-6-*O*-(*tert*-butyldiphenylsilyl)glucose. To remove the protective group in the C-6 position with a minimal migration of the butyryl moiety we followed the procedure kindly suggested to us by Professor Stephen Hanessian of Universite de Montreal. The enzymatically formed product (2 mmol) was dissolved in 60 mL of dry tetrahydrofuran, and the solution was cooled to −50 °C. A solution of 2 mmol of tetrabutylammonium fluoride and 10 mmol of acetic acid in 25 mL of tetrahydrofuran was then added dropwise, and the temperature of the mixture was allowed to reach 25 °C and remain at that temperature overnight. Following evaporation of the solvent under vacuum at room temperature, the residue was purified by a silica gel column chromatography with use of a mixture of ethyl acetate, methanol, and water (100:10:1) as a solvent. As a result, we obtained 0.41 g (75% isolated yield) of 2-*O*-butyrylglucose which contained about 10% of the 3-O-isomer (as determined by GC and NMR). This contamination was presumably due to migration of the acyl moiety, also observed in other systems.[13] The 2-O-isomer: ^{13}C NMR (67.9 MHz, acetone) δ 95.7, 90.5, 77.3, 75.9, 75.4, 74.7, 72.3, 71.5, 71.4, 71.2, 62.2 (a mixture of α and β anomers) (C1, β) (C1, α) (C5, β) (C2, β) (C3, β) (C2, α) (C5, α) (C3, α) (C4, α) (C4, β) (C6, α,β). These data are in agreement with those reported for 2-*O*-myristoylglucose.[22] Anal. Calcd for $C_{10}H_{18}O_7$: C, 49.74; H, 7.70; O, 42.49. Found: C, 49.82; H, 7.61; O, 42.33.

Enzymatic Hydrolysis of Di-*O*-butyryl Sugars. The procedure described above for 3,6-di-*O*-butyrylglucose did not afford a satisfactory selectivity when applied to the other diesters. Therefore, the following protocol was employed instead. A diester (0.1 g) was dissolved in 5 mL of a CH_2Cl_2–acetone–water (8:5:0.4) mixture, and then 0.1 g of *Candida cylindracea* lipase was added. The suspension was shaken at 25 °C and 250 rpm for 24–48 h, after which time the enzyme was removed by filtration and the solvent evaporated. The residue was subjected to silica gel column chromatography with an ethyl acetate–methanol–water mixture (100:10:1) as solvent.

A General Method for the Synthesis of Chiral Multifunctional Chain Compounds Incorporating Pentitol Fragments[1]

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Abstract: A new method for the synthesis of pentitol derivatives **21**–**32** of all possible configurations is described. The method is based on the use of 2,3-*O*-isopropylidene-D-glyceraldehyde (**3**) as a chiral starting material. Compound *anti*-**6** is obtained by the zinc bromide mediated addition of furyllithium to **3**, while *syn*-**8** is prepared by L-Selectride reductrion of ketone **7**; in both cases the diastereoselectivity is at least 20:1. It is shown that the furan ring is a very convenient precursor of the enedione system. The regioselective protection of one of the carbonyl groups of enedione allows for stereoselective reduction of ketones **15**, **16**, **19**, and **20**. Syn selectivity greater than 14:1 is observed for **15** and **19** when diisobutylaluminum hydride is used as reducing agent. In contrast, the reduction of **16** and **20** with zinc borohydride is 20:1 antiselective. Stereochemical assignments for **23**, **26**, and **29** were established by unambigous chemical correlations with compounds synthesized from natural D-arabinose, D-xylose, and D-ribose, respectively.

In recent years there has been a growing interest in the synthesis of natural products possessing a dense array of chiral centers bearing hydroxy groups. These structure types are characteristic not only of carbohydrates but also of other highly important, biologically active compounds such as macrolides[4] and antitumor antibiotics[5] as well as some marine toxins, e.g., palitoxin.[6] Significant advances in syntheses of such compounds have recently occurred.[7] Among many methodologies developed, those leading in an acyclic manner and with higher selectivity to the 1,3-dimethyl-2-hydroxy unit (A) have attracted great attention.[7,8] On the other hand, methodologies for the steroselective construction

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