Enzymes in Carbohydrate Synthesis: Lipase-Catalyzed Selective Acylation and Deacylation of Furanose and Pyranose Derivatives

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A number of furanose and pyranose derivatives were selectively acylated and deacylated on a preparative scale in lipase-catalyzed reactions. The primary hydroxyl functions of the methyl furanosides of D-ribose, D-arabinose, D-xylose, and 2-deoxy-D-ribose were selectively acetylated by crude porcine pancreatic lipase in tetrahydrofuran by using 2,2,2-trifluoroethyl acetate as the acyl donor. Selective deacetylations of the primary hydroxyl functions in the peracetylated methyl furanosides of D-ribose, D-arabinose, D-xylose, and 2-deoxy-D-ribose were best accomplished in a 9:1 solution of 0.1 N phosphate buffer (pH 7) and *N,N*-dimethylformamide using *Candida cylindracea* lipase. Selective cleavage of the 1-*O*-acetyl groups from 1,2,3,5-tetra-*O*-acetyl-D-ribose and -D-xylose were similarly accomplished with *Aspergillus niger* lipase. Similar regioselectivites were observed in the pyranose series. The *Candida* lipase was found to be the best for selective deacylation of the primary position from the peracylated methyl pyranosides, and porcine pancreatic lipase was the best for selective hydrolysis of the 1-*O*-acetyl groups from peracetylated pyranoses.

Introduction

Selective protection and deprotection of polyfunctional molecules is a critical problem in organic synthesis.[1] In carbohydrate chemistry these problems are accentuated due to the presence of multiple hydroxyl functions of very similar reactivity.[2] Reactions of carbohydrates with triphenylmethyl chloride, or one of its derivatives, has been a standard method of regioselectively incorporating acid-sensitive blocking groups into the primary hydroxyl functions.[1] Similar incorporation of base-sensitive (i.e. acyl) blocking groups usually requires a series of steps involving protections and deprotections of the other hydroxyl groups present in the molecule.[2] Regioselective deprotection of polyacylated sugars is seldom observed.[3,4]

Selective enzymatic acylation and deacylation of sugars have only been developed recently.[5-8] Acylation of the primary hydroxyl group with trichloroethyl carboxylates in some hexoses in pyridine was achieved with high regioselectivity.[5a] In this report[5a] and the subsequent report[5b] on acylation of *n*-octyl glucopyranoside, the reactions were terminated after 40–50% conversion of the starting material, presumably to preserve the initial kinetic regioselectivity. The isolated yields were thus relatively low (19–35% with only one example of 57%). In hydrolysis, *Aspergillus niger* lipase had been reported[7] to catalyze the selective cleavage of the 1-*O*-acetyl group of glucose pentaacetate after 20% conversion. When all the starting material had been consumed the product composition consisted of approximately 45% of the 2,3,4,6-tetraacetate and 55% of the 2,4,6- and 3,4,6-triacetates. In our previous work,[6] we found that certain peracylated methyl pyranosides can be selectively hydrolyzed at the primary position (C-6) with 60–100% regioselectivity.

To extend the utility of lipase-catalyzed acylation and deacylation to virtually all monosaccharides including furanose and pyranose series and their anomeric isomers, we report that the primary hydroxyl groups of the methyl furanosides of D-ribose, D-arabinose, D-xylose, and 2-deoxy-D-ribose have been selectively acetylated in tetrahydrofuran by employing a crude preparation of porcine pancreatic lipase and the more active acyl donor 2,2,2-trifluoroethyl acetate. Good to excellent regioselectivity has been achieved with these substrates even at high conversion of the starting sugars. We also report that the selective deacetylations of the primary hydroxyl functions in peracetylated methyl furanosides have been accomplished in a 10% *N,N*-dimethylformamide (DMF) solution using the lipase from *Candida cylindracea*. The 3-*O*-acetyl group, however, was selectively hydrolyzed in the 2-deoxyriboside series. In the pyranose case, we have also found that the regioselectivity can be enhanced by DMF. Procine pancreatic lipase in 10% DMF exclusively cleaved glucose pentaacetate ester at C-1 and the tetraacetate was obtained in 70% isolated yield. Similar selectivities and

Table I. Regioselective Acetylation of the Primary Hydroxyl Groups in Furanosides by Porcine Pancreatic Lipase

substrate	reactn time (h)	yield[a] (%)
methyl α,β-D-ribofuranoside (1)	60	77[b]
methyl α-D-arabinofuranoside (2)	48	77
methyl α,β-D-xylofuranoside (3)	24	84[b]
methyl 2-deoxy-α,β-D-ribofuranoside (4)	18	39[b,c]

[a] Yields reported are for anomeric mixtures. [b] The anomers were separated to facilitate spectroscopic identification of the products. [c] The product obtained was methyl 5-*O*-acetyl-2-deoxy-α,β-D-ribofuranoside. In addition 17% of methyl 3-*O*-acetyl-2-deoxy-β-D-ribofuranoside was obtained.

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(3) Haines, A. H. *Adv. Carbohydr. Chem. Biochem.* **1981**, *39*, 13–70.

(4) Wolfrom, M. L.; Szarek, W. A. In *The Carbohydrates. Chemistry and Biochemistry*, 2nd ed.; Pigman, W., Horton, D., Eds.; Academic Press: New York, 1972; Vol. IA, pp 217–51. Peracylated sugars with free anomeric hydroxyl groups are normally prepared through hydrolysis of glycosyl halides by using silver salts (Schmidt, O. T.; Herok, J. *Liebigs Ann. Chem.* **1954**, *587*, 63. Bonner, W. A. *J. Am. Chem. Soc.* **1958**, *80*, 3372) or through acid hydrolysis of protected glycosylamines (Honeyman, J. *Method. Carbohydr. Chem.* **1963**, *2*, 95). Other methods include direct deacylation with hydrazine (Excoffies, G.; Gagnaire, D.; Utille, J. P. *Carbohydr. Res.* **1975**, *39*, 368), with benzylamine (Ferrer Salat, C.; Exero Agneseti, P.; Bemporad Caniato, M. Spanish Patent 430 636 1976), with ammonia in organic solvent (Fiandor, J.; Garcia-Lopez, M. T.; de las Heras, F. G.; Mendez Castrillon, P. P. *Synthesis* **1985**, 1121), or with stannic chloride (Banaszek, A.; Bordas, C. X.; Zamojski, A. *Carbohydr. Res.* **1985**, *144*, 342).

(5) (a) Therisod, M.; Klibanov, A. M. *J. Am. Chem. Soc.* **1986**, *108*, 5638–40; (b) *Ibid.* **1987**, *109*, 3977.

(6) Sweers, H. M.; Wong, C.-H. *J. Am. Chem. Soc.* **1986**, *108*, 6421–22. The methyl glycoside derivatives of glucose, galactose, and mannose were selectively hydrolyzed at position 6.

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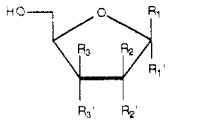
yields were obtained with six other peracylated hexoses including amino sugars. Similarly, we obtained 50–63% isolated yields in the cleavage of the 1-*O*-acetyl esters from tetra-*O*-acetyl-D-xylofuranose and tetra-*O*-acetyl-D-ribofuranose using the lipase from *A. niger*. Surprisingly, *C. cylindracea* cleaved both the 4- and 6-*O*-acetyl groups from α-D-glucose pentaacetate, giving the triacetate in 73% isolated yield.

The most interesting findings in this study are that the regioselectivity can be enhanced by DMF and that regioselective transformations can still be accomplished in the furanose series with appropriate lipases despite the fact that furanoses have more flexible conformations (mainly in the E and T forms with close energy) than pyranoses in solution. Thus both furanose and pyranose sugars can be efficiently acetylated or deacetylated by suitable lipases under proper reaction conditions.

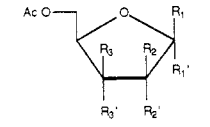
Results and Discussion

Furanose Series. Four methyl furanosides and their peracetylated derivatives were used in this study. They were methyl α,β-D-ribofuranoside, methyl α-D-arabinofuranoside, methyl α,β-D-xylofuranoside, and methyl 2-deoxy-α,β-D-ribofuranoside. Selective acetylations of these free methyl glycosides were accomplished in tetrahydrofuran solutions (16 mL/g containing 5 equiv of 2,2,2-trifluoroethyl acetate in the presence of crude porcine pancreatic lipase (3 g/g). The reactions were agitated on an orbital shaker (250 rpm) at 37 °C. The reaction times and yields are listed in Table I. No acetylations of these substrates were observed when the reactions were run in the absence of enzyme. Ethyl acetate was too slow as an acylating reagent to be of practical use. Isopropenyl acetate and trichloroethyl acetate were faster but five times slower than trifluoroethyl acetate; both reagents showed the same regioselectivity.



	R_1	$R_{1'}$	R_2	$R_{2'}$	R_3	$R_{3'}$
1	(H, OMe)		H	OH	H	OH
2	H	OMe	OH	H	H	OH
3	(H, OMe)		H	OH	OH	H
4	(H, OMe)		H	H	H	OH

Parentheses indicate a mixture of anomers.



	R_1	$R_{1'}$	R_2	$R_{2'}$	R_3	$R_{3'}$
5	OMe	H	H	OAc	H	OAc
6	H	OMe	H	OAc	H	OAc
7	H	OMe	OAc	H	H	OAc
8	(H, OMe)		H	OAc	OAc	H
9	H	OMe	H	H	H	OAc
10	OMe	H	H	H	H	OAc
11	OAc	H	H	OAc	H	OAc
12	(H, OAc)		H	OAc	OAc	H

We also examined the above reactions using the lipase from *Psuedomonas* sp. and cholesterol esterase. *P.* sp. lipase was found to be very active with methyl D-ribofuranoside, methyl D-xylofuranoside, and methyl 2-deoxy-D-ribofuranoside as substrates but weak with methyl α-D-arabinofuranoside (50% conversion in 60 h). Both methyl D-ribofuranoside and methyl 2-deoxy-D-ribofuranoside were good substrates for cholesterol esterase but only in the case of methyl D-ribofuranoside was good selectivity observed. With both methyl D-arabinofuranoside and methyl D-xylofuranoside, poor rates and poor selectivities were observed when cholesterol esterase was employed. Neither of the above enzymes appeared to offer any great advantages over crude porcine pancreatic lipase.

Portions of each of the methyl furanosides were peracetylated in a mixture of acetic anhydride and pyridine containing a catalytic amount of 4-(dimethylamino)-pyridine. The anomeric mixtures of methyl 2,3,5-tri-*O*-acetyl-α,β-D-ribofuranoside and methyl 3,5-di-*O*-acetyl-2-deoxy-α,β-D-ribofuranosides were separated by column chromatography on silica gel to facilitate reaction monitoring and product identification. Methods for the efficient separation of the anomers of methyl 2,3,5-tri-*O*-acetyl-D-xylofuranoside were not found; therefore the 1:1 anomeric mixture was used in all reactions employing this substrate.

Table II. Enzymatic Regioselective Hydrolysis of Peracetylated Furanose Derivatives

substrate	enzyme[b]	reactn time (h)	yeild[a] (%)
methyl 2,3,5-tri-*O*-acetyl-β-D-ribofuranoside (5)	CCL	2.25	96
methyl 2,3,5-tri-*O*-acetyl-α-D-ribofuranoside (6)	CCL	3.0	85
methyl 2,3,5-tri-*O*-acetyl-α-D-arabinofuranoside (7)	CCL	2.5	98
methyl 2,3,5-tri-*O*-acetyl-α,β-D-xylofuranoside (8)	CCL	7	50[c]
methyl 3,5-di-*O*-acetyl-2-deoxy-α-D-ribofuranoside (9)	CCL	2.0	40[d]
methyl 3,5-di-*O*-acetyl-2-deoxy-β-D-ribofuranoside (10)	CCl	2.0	63
1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose (11)	ANL	0.5	63[e]
1,2,3,5-tetra-*O*-acetyl-D-xylofuranose (12)	ANL	0.5	50[e]

[a] Yields are for the 5-hydroxy products except where otherwise noted. [b] CCL = *Candida cylindraceae* lipase; ANL = *Aspergillus niger* lipase. [c] 30% of methyl 2,5-di-*O*-acetyl-β-D-xylofuranoside was also obtained. [d] 50% of methyl 5-*O*-acetyl-2-deoxy-α-D-ribofuranoside was also obtained. [e] The isolated product resulted from the hydrolysis of the 1-*O*-acetyl group.

Five enzymes were examined for use in the hydrolysis reaction. They were *Rhizopus japonics* lipase, *Mucor* sp. lipase, crude porcine pancreatic lipase, *Aspergillus niger* lipase, and *Candida cylindracea* lipase. The *M.* sp. lipase was inactive with these substrates under the reaction conditions. The lipase from *R. japonics* provided only a very slow reaction which was not selective with certain substrates. Crude pancreatic lipase, *A. niger* lipase, and *C. cylindracea* lipase accepted all the substrates examined. *A. niger* lipase and *C. cylindracea* lipase provided high reaction rates leading to monodeacetylated products. However, only *C. cylindracea* lipase was found to react quickly with high selectivity. *A. niger* lipase was found to selectively remove the anomeric group from 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose and 1,2,3,5-tetra-*O*-acetyl-D-xylofuranose. The eight substrates that were tested in this study are listed in Table II along with the calculated regioselectivities and the isolated yields of the products.

The hydrolysis reactions were best conducted by first dissolving the peracetylated substrates in *N,N*-dimethylformamide, diluting the solutions with nine volumes of 0.1 N phosphate buffer (pH 7), adding the enzyme *C. cylindracea* lipase (1 g/g), and agitating the mixture at 37 °C. In this way homogeneous reactions were obtained. The reactions were monitored by thin layer chromatography. When optimal conversions were observed the products were isolated by extracting the reaction media with three portions of ethyl acetate, drying the extract, and evaporating the solvent. The products were further purified by column chromatography on silica gel.

The hydrolysis of methyl 3,5-di-*O*-acetyl-2-deoxy-α-D-ribofuranoside was not selective. Both methyl 3-*O*-acetyl-2-deoxy-α-D-ribofuranoside and methyl 5-*O*-

Table III. Lipase-Catalyzed Selective Hydrolysis of Peracylated Sugars

substrate	product[a]	α/β ratio	regioselectivity (%)[b] (isolated yield %)	enzyme	rel activity[c]
methyl α-D-glucoside, tetraoctanoate (**13**)	6-OH		100 (78)	CCL	1.0
methyl β-D-glucoside, tetraoctanoate (**14**)	6-OH		100 (77)	CCL	0.3
methyl α-D-glucoside, tetrapentanoate (**15**)	6-OH		100 (75)	CCL	0.2
methyl α-D-galactoside, tetrapentanoate (**16**)	6-OH[d]		60 (29)[d]	CCL	0.1
methyl α-D-mannoside, tetrapentanoate (**17**)	6-OH[d]		67 (33)[d]	CCL	0.1
methyl 2-acetamido-2-deoxy-D-mannoside, tripentanoate (**18**)	6-OH[d]		70 (50)[d]	CCL	6.2
methyl 2-acetamido-2-deoxy-D-glucoside, tripentanoate (**19**)	*e*			CCL	<0.01
α-D-glucose, pentaacetate (**20**)	4,6-OH[f]		75 (73)[f]	CCL	0.3
	1-OH	70/30	100 (70)	PPL	0.04
β-D-glucose, pentaacetate (**21**)	1-OH	*g*	*g*	PPL	0.02
α-D-galactose, pentaacetate (**22**)	1-OH	78/22	100 (75)	PPL	0.02
α-D-mannose, pentaacetate (**23**)	1-OH	8/92	100 (95)	PPL	0.02
2-acetamido-2-deoxy-β-D-mannose, tetraacetate (**24**)	1-OH	66/34	100 (88)	PPL	0.02
2-acetamido-2-deoxy-β-D-glucose, tetraacetate (**25**)	1-OH	93/7	100 (96)	PPL	0.01
L-rhamnose, tetraacetate (**26**)	1-OH	85/15	100 (54)	PPL	0.04
L-fucose, tetraacetate (**27**)	1-OH	66/34	100 (71)	PPL	0.04
methyl N-acetylneuraminate, pentaacetate (**28**)	1-CO_2H		30 (30)	RJL, PPL	2.0

[a] The hydrolysis positions were determined by deuterium-induced differential isotope shift (DIS) experiments (Pfeffer, P. E.; Valentine, K. M.; Parrish, F. W. *J. Am. Chem. Soc.* **1979**, *101*, 1265–1273) and were as indicated. The reaction conditions are described in c. [b] Determined based on the ratio of the major product to other products after the substrate was completely consumed. [c] For compounds **13–18**, the rates were determined in phosphate buffer (0.1 M, pH 7) containing 0.2 M NaCl, 3 mM $CaCl_2$, and 0.1 M substrate. For compounds **19–28**, the rates were determined in 10% DMF/phosphate buffer (0.05 M, pH 7.0) containing 24 mM substrate. The specific activity for compound **13** was 0.15 U/mg enzyme. 1 U = 1 μmol based consumed/min. [d] The major byproducts isolated are the corresponding 4,6-dihydroxy derivatives (about 20%). [e] Too slow to determine. [f] In addition, the 6-hydroxy compound was isolated in 22% yield. [g] Not determined. For the preparations of products from **13–17**, see ref 6. CCL, *Candida cylindracea* lipase; PPL, porcine pancreatic lipase; RJL, *Rhizopus japonics* lipase.

acetyl-2-deoxy-α-D-ribofuranoside were isolated in a 4:5 ratio from this reaction. The hydrolysis of methyl 2,3,5-tri-*O*-acetyl-α,β-D-xylofuranoside provided two products in a 5:3 ratio. One product was the expected methyl 2,3-di-*O*-acetyl-α-D-xylofuranoside. The other product was identified by 400-MHz proton NMR spectroscopy as methyl 2,5-di-*O*-acetyl-β-D-xylofuranoside. Further confirmation of the above structure was obtained when the relative downfield shifts of the various proton resonances were measured in the presence of an europium shift reagent. The downfield shifts of H2, H4, H5, and H5′ were approximately twice as large as the downfield shift experienced by H1. The europium-induced shifts in the spectrum of methyl 3,5-di-*O*-acetyl-β-D-xylofuranoside, on the other hand, should have been large for H1 and H3 and quite small for H5. These results established that the α-isomer was hydrolyzed at position 5 with 100% regioselectivity. The β-isomer, however, was not hydrolyzed at position 5 but at position 3, again with 100% regioselectivity. All other furanose derivatives tested showed 100% regioselectivity at C-1 for peracetylated furanoses and at C-5 for peracetylated methyl furanosides.

Pyranose Series. The hydrolyses of pyranose derivatives are summarized in Table III. The regioselectivity in the hydrolysis of peracylated methyl glycosides observed in this study was essentially the same as that observed before[6] in reactions with other sugars, all being preferentially hydrolyzed at the primary positions. Of the substrates with different lengths of the acyl group tested, the one with the longer acyl group was hydrolyzed faster. The reactions with methyl galactoside and methyl mannoside derivatives showed less regioselectivity (60–70%) than that with methyl glucoside derivatives (100%). The derivative of *N*-acetylglucosamine (**19**) was a very poor substrate. When the C-1 functionality was protected as acetate instead of methyl ether, all of the sugar acetates tested as substrates for pancreatic lipase were hydrolyzed (in 0.05 M phosphate/10% DMF) at the C-1 positions giving 1-OH sugars. In the absence of DMF the selectivity of these reactions was much poorer. The reaction of glucose pentaacetate with *Candida* lipase, however, showed a different selectivity, both positions 4 and 6 being cleaved.

In conclusion, acylated furanose and pyranose derivatives can be selectively hydrolyzed at the anomeric or the primary positions in reactions catalyzed by lipases. Acylation of methyl furanosides or pyranosides at the primary position can be achieved with lipase catalysis using trifluoroethyl acetate as acylating reagent. These regioselective transformations should find general use in synthetic organic chemistry using sugar-related substances as starting materials.

Experimental Section

General Methods. *Candida cylindracea* lipase (Type VII) and porcine pancreas lipase (Type II) were from Sigma. Cholesterol esterase and the other lipases were from Amano. All enzymes were used as received. Tetrahydrofuran (THF) was distilled from sodium–benzophenone just prior to use. The 2,2,2-trifluoroethyl acetate (bp 77–78 °C) used in these reactions was prepared from acetyl chloride and 2,2,2-trifluoroethanol. All other solvents and chemicals were reagent grade and were used as received. Optical rotations were measured on a Perkin-Elmer Model 240 polarimeter. Proton NMR spectra were obtained at 200 and 400 MHz, respectively. Carbon NMR spectra were obtained at 50 MHz. Assignments of anomeric configurations in the furanose series were based on the comparison of the obtained ^1H NMR spectra and the reported spectra of the parent methyl glycosides.[10,11] The sites of acetylation were determined from the resultant shifts in the product ^1H NMR spectra[12,13] as compared to the spectra of the starting materials. Low resolution mass spectra (MS) were obtained on a GC/MS quadrapole operating at 70 eV. High-resolution mass spectra (HRMS) were obtained at 70 eV. Thin layer chromatography was done with 0.25-mm layers of silica gel (60A) on glass plates as supplied by J. T. Baker Chemical Company. The compounds were visualized

(10) Serianni, A. S., Barker, R. J. *Org. Chem.* **1984**, *49*, 3292–3300.
(11) Gerlt, J. A., Youngblood, A. V. *J. Am. Chem. Soc.* **1980**, *102*, 7433–7438.
(12) Mitsunobu, O.; Kimura, J.; Iiizumi, K.; Yanagida, N. *Bull. Chem. Soc. Jpn.* **1976**, *49*, 510–3.
(13) See footnote 10 in ref 6.

by spraying the plates with a 10% sulfuric acid in ethanol solution and heating. Column chromatography was done on Baker Flash-chromatography silica gel (230–400 mesh). Trimethylsilyl ethers were prepared by treating 1–5 mg samples with a mixture of chlorotrimethylsilane, hexamethyldisilazane, and pyridine (1:3:9) for 30 min at 60 °C. The TMS ethers were analyzed by GC on a methylsilicone column (0.53 mm × 5 m): initial temperature, 170 °C; final temperature, 210 °C; rate, 2 deg/min; flow rate, 15 mL/min. HPLC was performed on a RESOLVEX-Sil column (Fisher) and a refractive index detector; flow rate, 1.5 mL/min; mobile phase, hexane/ethyl acetate, 2/1 v/v. The isolated yields of all reported products were for chromatographically and spectrally clean materials, thus indicating a purity of >97% in all cases. Elemental analysis samples were obtained from syrups by rechromatographing the purified products and removing a center cut from the elution band followed by evaporation of the solvent in vacuo.

Methyl 5-*O*-Acetyl-D-ribofuranoside. To a solution of 1.64 g (10 mmol) of methyl D-ribofuranoside[9] in 24 mL of THF were added 5.75 mL (50 mmol) of 2,2,2-trifluoroethyl acetate and 5.0 g of crude porcine pancreatic lipase. The mixture was placed on an orbital shaker at 250 rpm and 37 °C. After 60 h the reaction was stopped by filtering off the enzyme and washing the solids with fresh THF. The combined filtrate and washings were evaporated to dryness and loaded into a 21 mm × 500 mm silica gel flash chromatography column. The column was developed with 5% methanol in chloroform. After elution of a minor amount of less polar byproducts, the fractions containing the pure anomers were pooled separately for spectroscopic identification. The total yield of the desired syrupy anomeric products was 1.35 g (77%). β anomer: ^{1}H NMR (DMSO-d_6) δ 2.01 (s, 3 H, acetyl), 3,17 (s, 3 H, OCH_3), 3.68–3.74 (m, 1 H), 3.82–3.96 (m, 3 H), 4.16–4.30 (m, 1 H), 4.61 (s, 1 H, H1), 4.98 (d, 1 H, OH, J = 2.0 Hz), 5.11 (d, 1 H, OH, J = 1.8 Hz); ^{13}C NMR (DMSO-d_6) 20.55, 54.04, 64.76, 70.94, 73.90, 79.72, 108.02, 170.16 ppm; MS, m/z (relative intensity) 175 (6.2), 146 (2.8), 133 (21.6), 115 (21.0), 103 (31.0), 86 (86.8), 73 (88.4), 57 (100); $[\alpha]^{24}_D$ −52.8° (c 1, EtOAc); HRMS calcd for $C_7H_{11}O_5$ ($M^+ - OCH_3$) 175.0606, obsd 175.0601. α anomer: ^{1}H NMR (DMSO-d_6) δ 2.02 (s, 3 H, acetyl), 3.29 (s, 3 H, OCH_3), 3.68–3.79 (m, 1 H), 3.80–4.20 (m, 4 H), 4.35 (d, 1 H, OH, J = 8.6 Hz), 4.76 (d, 1 H, H1, J = 4.4 Hz), 4.82 (d, 1 H, OH, J = 5.8 Hz); ^{13}C NMR (DMSO-d_6) 20.63, 54.86, 64.30, 69.59, 71.12, 81.35, 103.22, 170.72 ppm; MS, m/z (relative intensity) 175 (21.5), 146 (1.8), 133 (8.5), 115 (49.6), 103 (29.6), 86 (80.0), 73 (75.1), 57 (100); $[\alpha]^{24}_D$ +150.8° (c 1, EtOAc); HRMS calcd for $C_7H_{11}O_5$ ($M^+ - OCH_3$) 175.0606, obsd 175.0591.

Methyl 5-*O*-Acetyl-α-D-arabinofuranoside. To a solution of 1.40 g (8.5 mmol) of methyl α-D-arabinofuranoside[14] in 22 mL of THF were added 9.8 mL (42.5 mmol) of 2,2,2-trifluoroethyl acetate and 4.2 g of crude porcine pancreatic lipase. The mixture was shaken as above. After 16 h an additional 2.1 g of crude porcine pancreatic lipase was added. After a total of 48 h the reaction was stopped and the product recovered as above. The residue was loaded into a 21 × 470 mm silica gel column and the product was eluted from the column with a 25–50% gradient of ethyl acetate in n-hexane. The product fractions were combined and evaporated to yield 1.35 g (77%) of a viscous oil which was crystallized from EtOAc: mp 83–84 °C; ^{1}H NMR (DMSO-d_6) δ 2.05 (s, 3 H, acetyl), 3.27 (s, 3 H, OCH_3), 3.58–3.70 (m, 1 H), 3.73–3.93 (m, 2 H), 3.94–4.08 (m, 1 H), 4.24 (dd, 1 H, J_1 = 11.8 Hz, J_2 = 3.0 Hz), 4.69 (d, 1 H, H1, J = 2.0 Hz), 5.34 (d, 1 H, OH, J = 4.0 Hz), 5.45 (d, 1 H, OH, J = 4.0 Hz); ^{13}C NMR (DMSO-d_6) 20.66, 54.65, 64.05, 77.48, 80.42, 81.82, 109.36, 170.72 ppm; MS, m/z (relative intensity) 175 (7.4), 146 (1.2), 133 (8.7), 115 (20.8), 103 (29.2), 86 (84.7), 73 (81.1), 57 (100.0); $[\alpha]^{24}_d$ +126.2° (c 0.6, EtOAc); HRMS calcd for $C_7H_{11}O_5$ ($M^+ - OCH_3$) 175.0606; obsd 175.0615. Anal. Calcd for $C_8H_{14}O_6$: C, 46.60; H, 6.84. Found: C, 46.3; H, 6.72.

Methyl 5-*O*-Acetyl-D-xylofuranoside. To a solution of 1.64 g (10 mmol) of methyl D-xylofuranoside[15] in 24 mL of THF were added 5.75 mL (50 mmol) of 2,2,2-trifluoroethyl acetate and 5.0 g of crude porcine pancreatic lipase. The mixture was shaken as before at 37 °C for 24 h. The product was then isolated and then loaded into a 35 mm × 480 mm silica gel flash chromatography column. The column was developed with a gradient of 50–100% ethyl acetate in hexane. After elution of a nonpolar byproduct, the fractions containing the pure anomers were pooled separately for spectroscopic identification. The total yield of syrupy products was 1.72 g (84%). β anomer: ^{1}H NMR (DMSO-d_6) δ 3.80–3.86 (m, 1 H, H2), 3.91–4.07 (2 m, 2 H, H3 and H5), 4.15–4.27 (2 m, 2 H, H4 and H5′), 4.62 (d, 1 H, H1, J = 1.2 Hz), 5.04 (d, 1 H, 3-OH, J = 5.0 Hz), 5.37 (d, 1 H, 2-OH, J = 4.2 Hz); ^{13}C NMR (DMSO-d_6) 20.70, 54.60, 64.45, 75.88, 79.32, 80.89, 109.82, 170.81 ppm; MS m/z (relative intensity) 175 (6.4), 146 (2.8), 133 (13.0), 115 (30.3), 103 (24.3), 86 (82.1), 73 (77.2), 57 (100); $[\alpha]^{24}_D$ −61.8° (c 1, EtOAc); HRMS calcd for $C_7H_{11}O_5$ ($M^+ - OCH_3$) 175.0606, obsd 175.0603. α anomer: ^{1}H NMR (DMSO-d_6) δ 2.02 (s, 3 H, acetyl), 3.31 (s, 3 H, OCH_3), 3.77–3.87 (m, 1 H), 3.89–4.22 (m, 4 H), 4.75 (d, 1 H, H1, J = 4.2 Hz), 4.89 (d, 1 H, OH, J = 6.4 Hz), 5.29 (d, 1 H, OH, J = 4.8 Hz); ^{13}C NMR (DMSO-d_6) 20.67, 54.88, 63.84, 74.48, 74.41, 76.96, 102.21, 170.28 ppm; MS, m/z (relative intensity) 175 (5.0), 146 (2.2), 133 (7.2), 115 (17.3), 103 (31.0), 86 (79.2), 73 (69.2), 57 (100); $[\alpha]^{24}_D$ +128.5° (c 0.4, EtOAc); HRMS calcd for $C_7H_{11}O_5$ ($M^+ - OCH_3$) 175.0606, obsd 175.0603. The proton assignments for the β anomer determined from a proton COSY experiment.

Methyl 5-*O*-Acetyl-2-deoxy-D-ribofuranoside and Methyl 3-*O*-Acetyl-2-deoxy-β-ribofuranoside. To a solution of 1.0 g (6.8 mmol) of methyl 2-deoxy-D-ribofuranoside[16] in 18 mL THF were added 4.0 mL (34 mmol) of 2,2,2-trifluoroethyl acetate and 3.0 g of crude porcine pancreatic lipase. The mixture was shaken as usual for 18 h and the product recovered was loaded into a 21 mm × 415 mm silica gel column. The column was developed with a gradient of 25–50% ethyl acetate in hexane. After elution of diester byproducts, a clean separation of two monoester products was obtained. The fractions containing the pure components were pooled separately to yield viscous oils. The faster moving component was shown by NMR spectroscopy to be methyl 3-*O*-acetyl-2-deoxy-β-D-ribofuranoside, 0.22 g (17%): ^{1}H NMR ($CDCl_3$) δ 2.06 (s, 3 H, acetyl), 2.23 (ddd, ^{1}H, H2, J_1 = 14.7 Hz, J_2 = 5.6 Hz, J_3 = 4.1 Hz), 2.38 (ddd, 1 H, H2′, J_1 = 14.7 Hz, J_2 = 7.2 Hz, J_3 = 2.6 Hz), 3.01 (br, 1 H, OH), 3.41 (s, 3 H, OMe), 3.71 (d, 2 H, H5, H5′, J = 3.4 Hz), 4.21 (dd, 1 H, H4, J_1 = 3.4 Hz, J_2 = 2.6 Hz), 5.18 (dd, 1 H, H1, J_1 = 5.6 Hz, J_2 = 2.6 Hz); ^{13}C NMR ($CDCl_3$) 21.21, 40.04, 55.82, 64.12, 75.77, 86.44, 106.02, 171.51 ppm MS, m/z (relative intensity) 159 (33.5), 130 (5.0), 100 (9.8), 99 (100); HRMS calcd for $C_7H_{11}O_4$ (M^+ − OMe) 159.0657, obsd 159.0663. The slower moving component was identified as methyl 5-*O*-acetyl-2-deoxy-D-ribofuranoside, 0.50 g (39%): ^{1}H NMR ($CDCl_3$) δ 2.02 and 2.03 (2 s, 3 H, acetyl), 2.03–2.26 (m, 2 H, H2 and H2′), 3.89 (br s, 1 H, OH), 3.27 and 3.33 (2 s, 3 H, OCH_3), 3.96–4.11 (m, 2 H), 4.11–4.24 and 4.28–4.39 (2 m, 1 H), 4.98–5.07 (m, 1 H, H1); ^{13}C NMR ($CDCl_3$) 20.91, 21.16, 39.23, 41.54, 55.02, 55.09, 62.57, 65.39, 72.52, 74.27, 83.51, 83.60, 104.94, 105.15, 171.12, 171.48 ppm.

Methyl 2,3-Di-*O*-acetyl-β-D-ribofuranoside. A solution of 580 mg (2 mmol) of methyl 2,3,5-tri-*O*-acetyl-β-D-ribofuranoside in 6 mL of *N*,*N*-dimethylformamide was diluted with 54 mL of 0.1 M phosphate buffer (pH 7) and 580 mg of *C. cylindracea* lipase was added. The mixture was heated to 37 °C and shaken at 250 rpm on an orbital shaker. After 2.25 h the reaction was completed and the reaction mixture was extracted with 3 × 125 mL of ethyl acetate. The combined extracts were dried ($MgSO_4$) and evaporated to dryness to yield 480 mg of 96% chromatographically pure product as a clear oil: ^{1}H NMR ($CDCl_3$) δ 2.07 (s, 3 H, acetyl), 2.12 (s, 3 H, acetyl), 3.44 (s, 3 H, OCH_3), 3.58–3.73 (m, 1 H, H5), 3.75–3.88 (m, 1 H, H5′), 4.19–4.28 (m, 1 H, H4), 4.92 (s, 1 H, H1), 5.24 (d, 1 H, H2, J = 5.4 Hz), 5.37 (dd, 1 H, H3, J_1 = J_2 = 5.4 Hz); ^{13}C NMR ($CDCl_3$) 20.61 (2 C), 55.78, 62.87, 71.23, 75.24, 82.43, 106.49, 169.68, 170.08; MS, m/z (relative intensity) 217 (42.0), 158 (4.0), 157 (21.5), 128 (8.0), 115 (100.0), 103 (6.2), 99 (4.4) ppm; $[\alpha]^{24}_D$ −26.9° (c 0.55, EtOAc); HRMS calcd for $C_9H_{13}O_6$ (M^+ − OMe) 217.0712, obsd 217.0684. Anal. Calcd for $C_{10}H_{16}O_7$: C, 48.38; H, 6.50. Found: C, 48.46; H, 6.32.

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Methyl 2,3-Di-*O*-acetyl-α-D-ribofuranoside. A solution of 580 mg (2 mmol) of methyl 2,3,5-tri-*O*-acetyl-α-D-ribofuranoside in 6 mL of DMF was processed as described for the preparation of the β anomer. After 3 h the reaction was complete and extractive workup provided 420 mg (85%) of pure oil: ^{1}H NMR ($CDCl_3$) δ 2.14 (S, 6 H, acetyls), 2.52 (br, 1 H, OH) 3.45 (s, 3 H, OCH_3), 3.74–3.94 (m, 2 H, H5, H5′), 4.15 (ddd, 1 H, H4, $J_1 = J_2 = J_3 = 3.4$ Hz), 4.97 (dd, 1 H, H2, $J_1 = 17.4$ Hz, $J_2 = 3.4$ Hz), 5.15 (d, 1 H, H1, $J = 4.4$ Hz), 5.22 (dd, 1 H, H3, $J_1 = 7.4$ Hz, $J_2 = 3.4$ Hz); ^{13}C NMR ($CDCl_3$) 20.60, 20.92, 55.52, 62.16, 70.00, 71.20, 82.50, 101.56, 170.07, 170.86 ppm; MS, *m/z* (relative intensity) 217 (42.8), 157 (23.5), 128 (19.5), 115 (100.0), 103 (12.9), 99 (8.6); $[\alpha]^{24}_D$ +162° (*c* 0.54, EtOAc); HRMS calcd for $C_9H_{13}O_6$ (M^+ – OMe) 217.0712, obsd 217.0690.

Methyl 2,3-Di-*O*-acetyl-D-arabinofuranoside.[17] A solution of 580 mg (2 mmol) of methyl 2,3,5-tri-*O*-acetyl-α-D-arabinofuranoside in 6 mL of *N,N*-dimethylformamide was processed as described for the preparation of the ribose derivatives. The reaction time was 2.5 h. After extractive workup 490 mg (98%) of syrupy diacetate was obtained: ^{1}H NMR ($CDCl_3$) δ 2.11 (s, 3 H, acetyl), 2.12 (s, 3 H, acetyl), 2.38 (br, 1 H, OH), 3.41 (s, 3 H, OCH_3), 3.81 (dd, 1 H, H5, $J_1 = 12.0$ Hz, $J_2 = 4.4$ Hz), 3.91 (dd, 1 H, H5′, $J_1 = 12.0$ Hz, $J_2 = 3.4$ Hz), 4.11 (ddd, 1 H, H4, $J_1 = 5.4$ Hz, $J_2 = 4.4$ Hz, $J_3 = 3.4$ Hz), 4.93 (s, 1 H, H1), 5.01–5.06 (m, 1 H, H3), 5.09–5.12 (m, 1 H, H2); ^{13}C NMR ($CDCl_3$) 20.75, 20.82, 54.85, 61.94, 77.14, 81.60, 82.91, 106.55, 169.80, 170.67 ppm.

Methyl 2,3-Di-*O*-acetyl-α-D-xylofuranoside and Methyl 2,5-Di-*O*-acetyl-β-D-xylofuranoside from Methyl 2,3,5-Tri-*O*-acetyl-D-xylofuranoside. A solution of 580 mg (2 mmol) of methyl 2,3,5-tri-*O*-acetyl-α,β-D-xylofuranoside in 6 mL of DMF was processed as described for the ribose derivatives. After 7 h the reaction was complete and worked up in the normal manner to give 470 mg of crude oil containing two major components. This mixture was loaded into a 21 × 390 mm silica gel flash column. The products were eluted with a 25–100% gradient of ethyl acetate in hexane. The fractions containing the two components were pooled separately to give 150 mg (30%) of methyl 2,5-di-*O*-acetyl-β-D-xylofuranoside as a syrup: ^{1}H NMR ($CDCl_3$) δ 2.10 (s, 3 H, acetyl), 2.12 (s, 3 H, acetyl), 3.00 (d, 1 H, OH, $J = 10.2$ Hz), 3.43 (s, 3 H, OCH_3), 4.20 (ddd, 1 H, H3, $J_1 = 10.2$ Hz, $J_2 = 4.3$ Hz, $J_3 = 1.0$ Hz), 4.25 (dd, 1 H, H5, $J_1 = 11.0$ Hz, $J_2 = 6.6$ Hz), 4.41–4.50 (m, 2 H, H4, H5′), 4.94 (s, 1 H, H1), 5.02 (s, 1 H, H2, $J = 1.0$ Hz); ^{13}C NMR ($CDCl_3$) 20.77, 20.96, 55.48, 63.70, 74.19, 80.94, 81.00, 106.41, 169.90, 170.90 ppm; MS, *m/z* (relative intensity) 217 (7.5), 175 (29.1), 157 (8.9), 145 (9.2), 128 (22.2), 115 (100.0), 103 (28.2), 99 (14.0); $[\alpha]^{24}_D$ –17.7° (*c* 0.13, EtOAc); HRMS calcd for $C_9H_{13}O_6$ (M^+ – OMe) 217.0712, obsd 217.0706. The second component consisted of 250 mg (50%) of an oil identified as methyl 2,3-di-*O*-acetyl-α-D-xylofuranoside: ^{1}H NMR δ 2.12 (s, 3 H, acetyl), 2.13 (s, 3 H, acetyl), 2.47 (br, 1 H, OH), 3.45 (s, 3 H, OCH_3), 3.64–3.76 (m, 2 H, H5, H5′), 4.44–4.54 (m, 1 H, H4), 4.89 (d, 1 H, H1, $J = 1.0$ Hz) 5.19 (dd, 1 H, H2, $J_1 = 2.8$ Hz, $J_2 = 1.2$ Hz), 5.30 (dd, 1 H, H3, $J_1 = 6.3$ Hz, $J_2 = 2.8$ Hz); ^{13}C NMR ($CDCl_3$) 20.75, 20.80, 55.93, 61.45, 75.62, 81.05, 81.09, 107.05, 169.67, 170.55; MS, *m/z* (relative intensity) 217 (24.8), 157 (14.2), 128 (12.2), 114 (100.0), 103 (11.4) ppm; $[\alpha]^{24}_D$ –24.3° (*c* 1, EtOAc) 99 (7.2); HRMS calcd for $C_9H_{13}O_6$ (M^+ – OMe) 217.0712, obsd 217.0705.

Methyl 3- and 5-*O*-Acetyl-2-deoxy-α-D-ribofuranosides from Methyl 3,5-Di-*O*-acetyl-2-deoxy-α-D-ribofuranoside. A solution of 240 mg (1.03 mmol) of methyl 3,5-di-*O*-acetyl-2-deoxy-α-D-ribofuranoside in 3.0 mL of DMF was diluted with 27 mL of 0.1 M phosphate buffer (pH 7). The mixture was processed as above for 2 h and then extracted with three 50-mL portions of ethyl acetate. The combined extracts were dried over anhydrous magnesium sulfate and evaporated to dryness. The residue (290 mg) was loaded into a 21 × 400 mm silica gel flash column and the products were eluted from the column with a 0–1% gradient of methanol in chloroform. The first component, 100 mg (50%), was an oil that was identified as methyl 5-*O*-acetyl-2-deoxy-α-D-ribofuranoside: ^{1}H NMR ($CDCl_3$) δ 1.97–2.22 (m, 2 H, H2, H2′), 2.09 (s, 3 H, acetyl), 3.02 (dd, 1 H, OH, $J_1 = 10.1$ Hz, $J_2 = 2.1$ Hz), 3.40 (s, 3 H, OMe), 4.01–4.19 (m, 3 H, H4, H5, H5′), 4.22–4.30 (m, 1 H, H3), 5.11 (dd, 1 H, H1, $J_1 = 4.4$ Hz, $J_2 < 1.0$ Hz); ^{13}C NMR ($CDCl_3$) 20.81, 40.83, 54.96, 64.26, 72.88, 84.67, 105.43, 170.80 ppm; MS, *m/z* (relative intensity) 159 (20.1), 130 (4.2), 117 (49.1), 103 (47.9), 99 (86.3), 88 (100.0); $[\alpha]^{24}_D$ +105.5° (*c* 0.36, EtOAc); HRMS calcd for $C_7H_{11}O_4$ (M^+ – OMe) 159.0657, obsd 159.0665. The second component, 80 mg (40%), was also an oil and was identified as methyl 3-*O*-acetyl-2-deoxy-α-D-ribofuranoside: ^{1}H NMR ($CDCl_3$) δ 2.03 (ddd, 1 H, H2, $J_1 = 14.7$ Hz, $J_2 = 2.1$ Hz, $J_3 = 1$ Hz), 2.10 (s, 3 H, acetyl), 2.35 (br, 1 H, OH), 2.38 (1 H, H2′, $J_1 = 14.7$ Hz, $J_2 = 8.4$ Hz, $J_3 = 5.0$ Hz, 3.40 (s, 3 H, OCH_3), 3.70–3.80 (m, 2 H, H5, H5′; collapses to a 2-H triplet in D_2O: 3.78, $J = 3.9$ Hz), 4.11 (ddd, 1 H, H4, $J_1 = J_2 = J_3 = 3.9$ Hz), 5.08 (ddd, 1 H, H3, $J_1 = 4.3$ Hz, $J_2 = 4.0$ Hz, $J_3 = 2.1$ Hz), 5.10 (d, 1 H, H1, $J = 5.0$ Hz); ^{13}C NMR ($CDCl_3$) 21.15, 39.23, 55.08, 62.55, 74.25, 83.54, 104.94, 171.41 ppm; MS, *m/z* (relative intensity) 159 (30.8), 130 (8.5), 99 (100), 87 (39.3); HRMS calcd for $C_7H_{11}O_4$ (M^+ – OMe) 159.0657, obsd 159.0665.

Methyl 5-*O*-Acetyl-2-deoxy-β-D-ribofuranoside. A solution of 230 mg (1 mmol) of methyl 3,5-di-*O*-acetyl-2-deoxy-β-D-ribofuranoside was processed as described for the corresponding α anomer. After silica gel chromatography using a 0–2% gradient of methanol in chloroform, 120 mg (63%) of methyl 5-*O*-acetyl-2-deoxy-β-D-ribofuranoside was obtained as an oil: ^{1}H NMR ($CDCl_3$) δ 2.09 (ddd, 1 H, H2, $J_1 = 13.4$ Hz, $J_2 = 6.8$ Hz, $J_3 = 5.1$ Hz), 2.11 (s, 3 H, acetyl), 2.25 (ddd, 1 H, H2′, $J_1 = 13.4$ Hz, $J_2 = 6.8$ Hz, $J_3 = 1.8$ Hz) 3.12 (br, 1 H, OH), 3.33 (s, 3 H, OCH_3), 3.90–4.14 (m, 2 H, H5, H5′), 4.20–4.31 (m, 1 H, H4), 4.32–4.44 (m, 1 H, H3), 5.07 (dd, 1 H, H1, $J_1 = 5.1$ Hz, $J_2 = 1.8$ Hz); ^{13}C ($CDCl_3$) 20.93, 41.44, 55.01, 65.47, 72.26, 83.57, 105.17, 171.34 ppm; MS, *m/z* (relative intensity) 159 (11.9), 130 (3.5), 117 (67.5), 103 (29.3), 99 (100.0), 88 (60.1); HRMS calcd for $C_7H_{11}O_4$ (M^+ – OMe) 159.0657, obsd 159.0664.

2,3,5-Tri-*O*-acetyl-D-ribofuranose. A solution of 634 mg (2 mmol) of 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose in 6.4 mL of *N,N*-dimethylformamide was diluted with 57.6 mL of 0.1 N phosphate buffer (pH 7), and 634 mg of *A. niger* lipase was added with vigorous agitation. The reaction mixture was shaken at 250 rpm and 26 °C for 30 min after which it was extracted with two 125-mL portions of ethyl acetate. The combined extracts were dried over anhydrous magnesium sulfate and evaporated to dryness to yield 550 mg of crude product. The residue (550 mg) was loaded into a 21 × 450 mm flash chromatography column using a gradient of 25–50% ethyl acetate in hexanes. The product fractions were pooled and evaporated to dryness to yield 350 mg (63%) of pure oil: ^{1}H NMR ($CDCl_3$) δ 2.04–2.20 (6 s, 9 H, acetyls), 3.28–3.37 (br d, 0.4 H, OH) 3.44–3.52 (br, 0.6 H, OH), 4.12–4.56 (m, 3 H, H4, H5, H5′), 5.06–5.13 and 5.22–5.32 and 5.35–5.43 and 5.51–5.58 (4 m, 3 H, H1, H2, H3); ^{13}C ($CDCl_3$) 20.49, 20.51, 20.59, 20.68, 20.77, 20.81, 63.51, 64.54, 70.69, 70.92, 71.23, 75.36, 78.61, 79.17, 95.37, 100.10, 169.76, 169.82 (2 C), 169.93, 170.63, 170.98 ppm; MS, *m/z* (relative intensity) 259 (1.2), 217 (3.0), 203 (12.1), 187 (8.3), 157 (10.8), 156 (18.9), 143 (100), 86 (56.4); $[\alpha]^{24}_D$ –30.2° (*c* 0.56, EtOAc); HRMS calcd for $C_{11}H_{15}O_7$ (M^+ – OH) 259.0818, obsd 259.0819. Anal. Calcd for $C_{11}H_{16}O_8$: C, 47.82; H, 5.84. Found: C, 48.01, H, 5.97.

2,3,5-Tri-*O*-acetyl-D-xylofuranose. A 634 mg (2 mmol) sample of 1,2,3,5-tetra-*O*-acetyl-D-xylofuranose was processed under the same conditions as described above to yield 280 mg (50%) of syrup after chromatography: ^{1}H NMR ($CDCl_3$) δ 2.08–2.19 (6 s, 9 H, acetyls) 3.53 (d, 0.5 H, OH, $J = 6.0$ Hz), 3.60 (d, 0.5 H, OH, $J = 4.0$ Hz), 4.16 (d, 0.5 H, $J = 2.0$ Hz), 4.18 (d, 0.5 H, $J = 3.0$ Hz), 4.22–4.41 (m, 1 H), 4.50–4.69 (m, 1 H), 5.02–5.10 (m, 1 H), 5.32 (d, 0.5 H, $J = 6.0$ Hz), 5.38 (dd, 0.5 H, $J_1 = 5.4$ Hz, $J_2 = 1.6$ Hz), 5.53 (dd, 0.5 H, $J_1 = 6.1$ Hz, $J_2 = 5.1$ Hz), 5.66 (t, 0.5 H, $J = 4.8$ Hz); ^{13}C NMR ($CDCl_3$) 20.71 (2 C), 20.80 (2 C), 20.90 (2 C), 62.14, 63.17, 74.17, 75.05 (2 C), 77.17, 78.55, 81.40, 94.38, 101.17, 170.16 (2 C), 170.66, 170.69, 171.17, 171.35 ppm; *m/z* (relative intensity) 259 (2.3), 217 (5.7), 203 (22.4), 187 (16.5), 157 (22.2), 156 (31.6), 143 (99.7), 86 (100); $[\alpha]^{24}_D$ –62.8° (*c* 1, EtOAc); HRMS calcd for $C_{11}H_{15}O_7$ (M^+ – OH) 259.0818, obsd 259.0819.

General Procedure for Porcine Pancreatic Lipase Catalyzed Hydrolyses of Pyranose Derivatives. The sugar (10 mg/mL buffer) was suspended in 10% (v/v) DMF/phosphate buffer (0.05 M, pH 7). Lipase was added (0.75 g/mmol sugar),

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and the suspension was stirred at room temperature. The pH was periodically adjusted with 1.0 N NaOH. The reaction progress was monitored by TLC ($CHCl_3$/MeOH 5:1) and the suspension extracted with ethyl acetate (three times reaction volume) when the substrate could no longer be detected. The combined organic extracts were dried over anhydrous sodium sulfate, filtered, and evaporated in vacuo. The resulting syrup was purified on silica gel column chromatography. The following 1-OH sugars were prepared from the peracetylated precursors.

2,3,4,6-Tetra-*O*-acetyl-D-glucopyranose: yield 0.60 g, 70% (syrup); ^{1}H NMR ($CDCl_3$) δ 5.51 (t, 1 H, H2, J = 9.8 Hz), 5.43 (d, 1 H, H1α, J = 3.5 Hz), 5.22 (t, 1 H, H3β, J = 9.4 Hz), 5.05 (dt, 2 H, H4$\alpha\beta$), 4.92–4.82 (m, 2 H, 4.72 (d, 1 H, H1β, J = 4.7 Hz), 4.28–3.98 (m, 5 H), 3.77–3.47 (m, 1 H, H5β), 2.06 (s, 3 H, acetyl), 2.05 (s, 3 H, acetyl), 2.01 (s, 3 H, acetyl), 2.00 (s, 3 H, acetyl), 1.99 (s, 3 H, acetyl); ^{13}C NMR ($CDCl_3$) α anomer 90.12, 71.43, 70.21, 68.84, 67.22, 62.29, β-anomer 95.48, 73.21, 72.74, 72.18, 68.74, 62.29 ppm; GLC (Me_3Si ether) t_R 4.10 min. The partial NMR data reported[18] are in agreement with the indicated values.

2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-D-glucopyranose: yield 0.82 g, 96% (syrup); ^{1}H NMR ($CDCl_3$) δ 6.22 (d, 1 H, NHα, J = 10 Hz), 5.77 (d, 1 H, NHβ, J = 8.6 Hz), 5.32 (t, 1 H, H3α, J = 9.4 Hz), 5.22 (d, 1 H, H1α, J = 4 Hz), 5.13 (dt, 2 H, J = 9 Hz), 4.74 (d, 1 H, H1β, J = 8.4 Hz), 4.38–3.91 (m, 4 H), 3.77–3.68 (m, 1 H), 2.10 (s, 3 H, acetyl), 2.03 (s, 3 H acetyl), 2.02 (s, 3 H, acetyl), 1.97 (s, 3 H, acetyl); ^{13}C NMR ($CDCl_3$) 91.60, 71.30, 68.70, 67.45, 62.49, 52.58 ppm; $[\alpha]^{23}_D$ +49.1° (c 1, $CHCl_3$) [lit.[19] $[\alpha]_D$ +49.4°].

2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-D-mannopyranose: yield 0.75 g, 88% (solid). This solid was recrystallized from absolute ethanol and n-hexane (55%): ^{1}H NMR ($CDCl_3$) δ 6.06 (d, 1 H, NHβ, J = 7.7 Hz), 5.89 (d, 1 H, NHα, J = 7.7 Hz), 5.41 (t, 1 H, H3, J = 4.4 Hz, J = 10.2 Hz), 5.17 (d, 1 H, H1α, J = 1.3 Hz), 5.12–5.06 (m, 1 H), 5.02 (d, 1 H, H1β, J = 1.6 Hz), 4.68–4.64 (m, 1 H, Hβ), 4.58 (dd, 1 H, H2α, J = 1.4 Hz, J = 4.2 Hz), br s, 1 H, OH), 4.33–4.22 (m, 2 H), 4.14–4.03 (m, 1 H), 3.77–3.64 (m, 1 H, H5β), 2.12 (s, 3 H, acetyl), 2.11 (s, 3 H, acetyl), 2.10 (s, 3 H, acetyl), 2.06 (s, 3 H, acetyl), 2.05 (s, 3 H, acetyl), 2.02 (s, 3 H, acetyl), 2.00 (s, 3 H, acetyl); ^{13}C NMR ($CDCl_3$) 93.51, 69.56, 67.90, 66.53, 63.09, 51.44 ppm; $[\alpha]^{23}_D$ +30.5° (c 0.97, $CHCl_3$); mp 149–151 °C [lit.[20] $[\alpha]^{19}_D$ +30.4° ($CHCl_3$); mp 134–137 °C].

2,3,4,6-Tetra-*O*-acetyl-D-galactopyranose: yield 0.64 g, 75%, syrup; ^{1}H NMR ($CDCl_3$) δ 5.55–5.42 (m, 2 H, H4α, H2α, J = 3.4 Hz, J = 3.4 Hz, J = 1.6 Hz), 5.42–5.37 (m, 1 H, H1α, J = 3.4 Hz), 5.32 (s, 1 H), 5.18–5.05 (m, 2 H, J = 12.3 Hz, J = 3.6 Hz), 4.74 (d, 1 H, H1β, J = 6.2 Hz), 4.49 (br t, 1 H, J = 7 Hz), 4.43–4.30 (br s, 1 H, OH), 4.19–3.93 (m, 2 H), 2.09 (s, 3 H, acetyl), 2.04 (s, 3 H, acetyl), 1.99 (s, 3 H, acetyl), 1.93 (s, 3 H, acetyl); ^{13}C NMR ($CDCl_3$) α anomer 90.72, 68.70, 68.48, 67.55, 66.14, 60.66, β anomer 96.02, 71.07, 70.98, 70.85, 67.46, 60.66 ppm; mp 135–137 °C [lit.[18] mp 136–138 °C].

2,3,4,6-Tetra-*O*-acetyl-D-mannopyranose: yield 0.77 g, 95%, syrup; ^{1}H NMR ($CDCl_3$) δ 5.42 (dd, 1 H, H4, J = 9 Hz, J = 3 Hz), 5.37–5.20 (m, 2 H, H1,2, J = 1.3 Hz), 4.70–4.40 (br s, 1 H, OH), 4.32–4.05 (m, 3 H, H5,6,6′), 2.17 (s, 3 H, acetyl), 2.11 (s, 3 H, acetyl), 2.06 (s, 3 H, acetyl), 2.00 (s, 3 H, acetyl) ^{13}C NMR ($CDCl_3$) 92.23, 70.52, 69.20, 68.42, 66.46, 62.87 ppm; mp 106–108 °C; $[\alpha]^{23}_D$ −12° (c 1, $CHCl_3$) [lit.[21] mp 105–110 °C; $[\alpha]_D$ −12° (c 1, $CHCl_3$)].

2,3,4-Tri-*O*-acetyl-L-rhamnose: yield 124 mg, 54% (syrup); ^{1}H NMR ($CDCl_3$) δ 5.29 (dd, 1 H, H3α, J = 10 Hz, J = 3.5 Hz), 5.18 (dd, 1 H, H2α, J = 1.2 Hz, J = 3.5 Hz), 5.08 (d, 1 H, H1α, J = 1.2 Hz), 4.98 (t, 1 H, H4α, J = 10 Hz), 4.22–3.97 (m, 2 H, H5α, OH), 2.07 (s, 3 H, acetyl), 1.98 (s, 3 H, acetyl), 1.93 (s, 3 H, acetyl), 1.19 (d, 3 H, $CH_3\beta$), 1.14 (d, 3 H, $CH_3\alpha$). The ^{13}C data are in agreement with reported values.[22]

2,3,4-Tri-*O*-acetyl-L-fucose: yield 163 mg (71%, syrup); ^{1}H NMR ($CDCl_3$) δ 5.40 (d, 1 H, H1α, J = 3 Hz), 5.38 (dd, 1 H, H2α, J = 10 Hz, J = 3 Hz), 5.27 (t, 1 H, H4α, J = 3 Hz), 5.20 (t, 1 H, H4β, J = 2 Hz), 5.11 (dd, 1 H, H3α, J = 10 Hz, J = 3 Hz), 5.02 (m, 2 H, H3β, H2β), 4.62 (d, 1 H, H1β, J = 8 Hz), 4.38 (q, 1 H, H5α), 3.82 (9, 1 H, H5β), 3.25 (brs, 2 H, OH), 2.14 (s, 3 H, acetyl), 2.12 (s, 3 H, acetyl), 2.05 (s, 3 H, acetyl), 1.94 (s, 3 H, acetyl), 1.17 (d, 3 H, $CH_3\beta$, J = 6 Hz), 1.03 (d, 3 H, $CH_3\alpha$, J = 6 Hz), 1.03 (d, 3 H, $CH_3\alpha$, J = 6 Hz); the ^{13}C data are in agreement with the reported values.[23]

Methyl 2-Acetamido-2-deoxy-D-glucopyranoside. *N*-Acetyl-D-glucosamine (0.5 g, 2.1 mmol) was suspended in dry methanol (10 mL). Dry Dowex 50 (H^+) (0.3 g) was added and the suspension stirred under reflux for 1–1.5 h. The resin was filtered off and rinsed with MeOH and the solvents were evaporated to yield the title compound as a hygroscopic white solid: mp 60–69 °C; yield 0.41 g (81%); ^{1}H NMR (D_2O) δ 5.07 (d, 1 H, H1α, J = 3 Hz), 4.64 (d, 1 H, H1β, J = 4 Hz), 4.32 (d, 1 H, NH, J = 8 Hz), 3.38 (s, 3 H, OMe), 3.26 (s, 3 H, OMe), 1.91 (s, 3 H, acetyl).

Methyl 2-Acetamido-2-deoxy-D-mannoside. This compound was prepared as described above to give a mixture of pyranose and furanose rings: yield 3.27 g, 61.5% (white foam); ^{1}H NMR (D_2O) δ 5.08 (d, 1 H, J = 1.4 Hz), 4.98 (br t, 1 H, J = 2 Hz), 4.95 (d, 1 H, J = 1.7 Hz), 4.66 (d, 1 H, J = 1.4 Hz), 3.39 (s, 3 H, OMe), 3.36 (s, 3 H, OMe), 3.33 (s, 3 H, OMe), 3.31 (s, 3 H, OMe), 2.05 (s, 3 H, acetyl), 2.04 (s, 3 H, acetyl), 2.03 (s, 3 H, acetyl), 2.01 (s, 3 H, acetyl).

Methyl 2-Acetamido-2-deoxy-3,4,6-tri-*O*-pentanoyl-D-glucopyranoside. The methyl glycoside prepared above 0.41 g, 1.7 mmol) was taken up in anhydrous pyridine (4 mL) and valeric anhydride (Aldrich) (2.4 mL, 11.9 mmol) was added. The solution was allowed to stand overnight at room temperature. Ice was added and the emulsion stirred for 2 h. It was extracted into $CHCl_3$ (40 mL) and washed subsequently with 2 N HCl (2 × 40 mL), 5% $NaHCO_3$ (2 × 40 mL), and water (1 × 40 mL). The organic phase was dried over anhydrous $MgSO_4$ and filtered and the solvent removed under reduced pressure: yield 0.82 g (100%, wax); ^{1}H NMR ($CDCl_3$) δ 5.75 (d, NH, J = 10 Hz), 5.62 (d, NH, J = 9 Hz), 4.75 (d, 1 H, H1α, J = 3.6 Hz), 4.55 (d, 1 H, H1β, J = 8.3 Hz), 3.53 (s, 3 H, OMe), 3.43 (s, 3 H, OMe), 1.97 (s, 3 H, *N*-acetyl); MS, m/z (relative intensity) 185 (23), 140 (34), 139 (39), 138 (13), 114 (22), 101 (38), 98 (18), 97 (18), 96 (17), 86 (14), 85 (base peak), 84 (11), 83 (12), 80 (11), 41 (38); HRMS calcd for $C_{24}H_{41}NO_9$ (M^+) 487.2782, found 487.2781.

Methyl 2-Acetamido-2-deoxy-3,4,6-tri-*O*-pentanoyl-D-mannoside. This compound was prepared via acylation as described above. Full acylation was confirmed by IR spectroscopy since the NMR spectra are complicated by the presence of five- and six-membered rings: yield 5.10 g, 82% (syrup); MS m/z (relative intensity) 241 (17), 240 (13), 223 (18), 168 (10), 167 (12), 139 (16), 114 (24), 96 (15), 85 (base peak), 84 (12), 60 (11), 57 (95), 41 (30), 32 (17), 29 (32), 27 (11); HRMS calcd for $C_{23}H_{38}NO_8$ (M^+ −OMe) 456.2652, found 456.2597.

Methyl 2-Acetamido-2-deoxy-3,4-di-*O*-pentanoyl-D-mannopyranoside. Methyl 2-acetamido-2-deoxy-3,4,6-tri-*O*-pentanoyl-D-mannoside (2 g, 4.1 mmol) was added to phosphate buffer (25 mL pH 7, 0.5 M) containing 0.2 M NaCl and 3 mM $CaCl_2$. CCL (1 g) was added and the suspension stirred at room temperature. The reaction process was monitored on TLC ($CHCl_3$MeOH 10:1). After 3 days the suspension was extracted with ethyl acetate (3 × 50 mL), the extracts were dried over anhydrous Na_2SO_4 and filtered, and the solvent was evaporated. The residue was purified by flash chromatography (ethyl acetate/petroleum ether, 1:1) to give the title compound: yield 0.83 g, 50% (syrup); ^{1}H NMR ($CDCl_3$) δ 6.75 (d, 1 H, NH, J = 9.2 Hz), 5.42 (dd, 1 H, J = 10.8 Hz, J = 4 Hz, 5.18 (t, 1 H, J = 10 Hz), 4.91 (br s, 1 H, OH), 4.67 (s, 1 H, H1), 4.72–4.61 (m, 1 H, H2), 3.81–3.54 (m, 3 H, H5,6,6′), 3.39 (s, 3 H, OMe); ^{13}C NMR ($CDCl_3$) 174.10, 172.68 and 170.80 (carbonyl), 100.37 (C1), 70.11, 68.88, 66.05, 60.89 (C6), 55.25 (C2), 50.22 ppm (OMe); MS, m/z (relative intensity) 114 (16), 103 (15), 101 (26), 98 (12), 85 (base peak), 84 (10), 41 (24), 32 (14); HRMS calcd for $C_{18}H_{30}NO_7$ (M^+ − OMe) 372.2065, found 372.2024.

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1,2,3,4-Tetra-*O*-acetyl-α-D-glucopyranose and 1,2,3-Tri-*O*-acetyl-α-D-glucopyranose. To a suspension of α-D-glucose pentaacetate (0.1 g/100 mL) in phosphate buffer (0.05 M, pH 7) containing 10% (v/v) DMF was added CCL (1 g/g substrate), and the reaction was stirred. The reaction progress was monitored by TLC (EtOAc/petroleum ether 2:1). When all starting material had disappeared (~36 h) the reaction was worked up as described in the general procedure above. Yield (1,2,3,4-tetraacetate): 0.23 g, 27% (syrup); ^{1}H NMR ($CDCl_3$) δ 6.32 (d, 1 H, H1, J = 4 Hz), 5.50 (t, 1 H, H3, J = 10 Hz), 5.08 (t, 1 H, H4, J = 10 Hz), 5.04 (dd, 1 H, H2, J = 10 Hz, J = 4 Hz), 3.9 (m, 1 H, H5), 3.71 (dd, 1 H, H6, J = 11 Hz, J = 2 Hz), 3.52 (dd, 1 H, H6′, J = 11 Hz, J = 4 Hz), 2.15 (s, 3 H, acetyl), 2.05 (s, 3 H, acetyl), 2.01 (s, 3 H, acetyl), 1.99 (s, 3 H, acetyl); ^{13}C NMR ($CDCl_3$) 88.67, 71.71, 69.28, 68.95, 67.82, 60.23 ppm; GLC (Me_3Si ether) t_R 3.86 min. Yield (1,2,3-triacetate): 0.51 g, 73% (syrup); ^{1}H NMR ($CDCl_3$) δ 6.25 (d, 1 H, H1, J = 4 Hz), 5.38–5.25 (m, 1 H, H3), 4.96 (d, 1 H, H2, J = 10 Hz, J = 4 Hz), 3.83–3.70 (m, 4 H, H4,5,6,6′), 2.14 (s, 3 H, acetyl), 2.08 (s, 3 H, acetyl), 1.99 (s, 3 H, acetyl); ^{13}C NMR ($CDCl_3$) 89.03, 73.67, 71.85, 69.19, 67.88, 60.83 ppm; GLC (Me_3Si ether) t_R 4.23 min; $[\alpha]^{26}_D$ +104.0° (*c* 2.77, $CHCl_3$).

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Synthesis, X-ray Crystal Structure, and Antimitotic Properties of 6-Chloro-2-methoxy-5-(2′,3′,4′-trimethoxyphenyl)cyclohepta-2,4,6-trien-1-one, a Bicyclic Analogue of Colchicine

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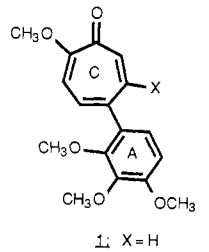
A regiocontrolled synthesis of the bicyclic colchicine analogue **2** has been achieved. Thus, the readily available cyclohexenone **10** was elaborated to the tricyclic compounds **15a**, **16b**, and **17b**, which were converted into the α-methoxy enone **6**. Reaction of **6** with 1,8-diazabicyclo[5.4.0]undec-7-ene gave the title compound (**2**). Subjection of diol **17b** to a Swern reaction, using excess oxidant, afforded the free tropolone **22**, which, on O-methylation, gave a 1:1 mixture of **2** and isomer **23**. An X-ray crystal structure of **2** reveals an angle of 77.5° between the planes of the two rings. Like colchicine, compound **2** is a potent antimitotic agent. Isomer **23** is much less active.

Introduction

In 1976, Fitzgerald reported[1] that the 5-(trimethoxyphenyl)tropolone methyl ether **1** (MTPT) retains the potent antimitotic activity of the alkaloid colchicine (**3**).[2] Subsequent and extensive studies[3] on **1** have helped to provide a better understanding of the mode of biological action of **3**. Interestingly, in spite of several reported attempts,[4] only two successful routes to 5-aryltroponoids have been published[5] since this time. As one of us has recently developed new syntheses of troponoid compounds,[6,7] we sought to prepare systems related to **1** for the purposes of structure–activity studies. We now described a fully regiocontrolled synthesis of the chloro analogue **2**, report its X-ray crystal structure, and detail some of the compound's biological properties. Interest in compound **2** derives not only from its demonstrated antimitotic activity but also from the fact that it is suitably constituted for elaboration to the natural product **3**.



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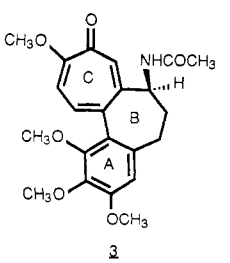
2: X = Cl



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