ELSEVIER

Carbohydrate Research 279 (1995) 281–291

CARBOHYDRATE RESEARCH

Substituent effects on the regioselectivity of enzymatic acylation of 6-*O*-alkylglycopyranosides using *Pseudomonas cepacia* lipase

David A. MacManus, Evgeny N. Vulfson *

Biotechnology and Enzymology Department, Institute of Food Research, Earley Gate, Whiteknights Road, Reading, Berkshire, RG6 6BZ, UK

Received 20 January 1995; accepted 4 August 1995

Abstract

The regioselectivity of acylation of a range of 6-*O*-protected glycosides using *Pseudomonas cepacia* lipase in vinyl acetate was investigated. In general, α-glycosides were acylated at the O-2 position and β-glycosides were acylated at the O-3 position. The effects of varying the size/hydrophobicity of the O-6 and anomeric substituents on the regioselectivity of the reaction are discussed in terms of the binding of the substrate in the active site of the enzyme.

Keywords: Enzymes; Lipase; Regioselectivity; Acylation; Glycosides; Organic solvents

1. Introduction

The chemical synthesis of carbohydrates and their derivatives is renowned for its difficulty on account of the presence of several hydroxyl groups of similar reactivity [1,2]. In recent years, however, carbohydrate chemists have been able to benefit from the large number of transformations carried out by enzymes which, in many cases, exhibit a high degree of regioselectivity [3]. In particular, glycosidases and glycosyltransferases have facilitated the syntheses of oligosaccharides which would otherwise have required complex synthetic methodology [4–7].

Another class of enzymes which is valuable in carbohydrate modification is that of lipases. The ability of lipases to introduce ester groups into carbohydrates selectively is

* Corresponding author.

Elsevier Science Ltd.
SSDI 0008-6215(95)00292-8

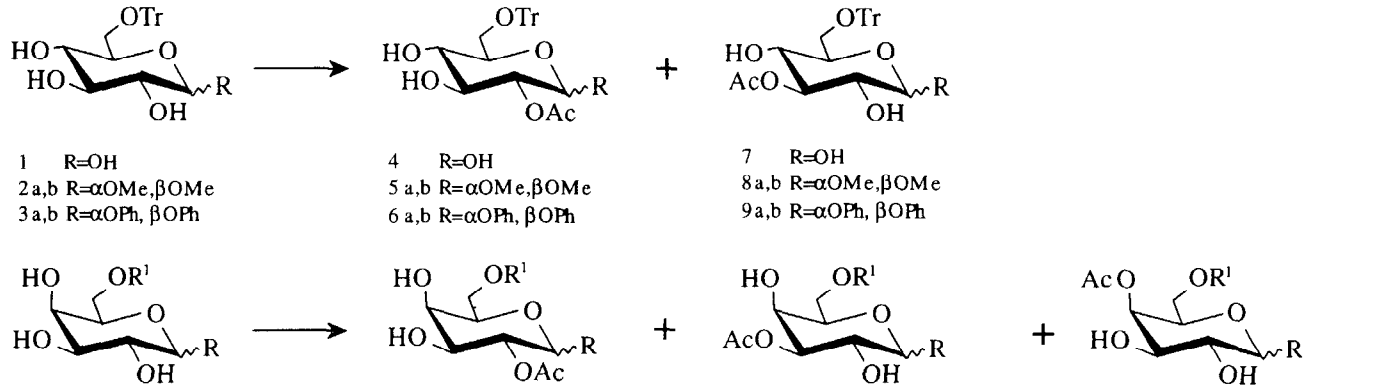
particularly useful on account of their utility as protecting groups and the difficulties associated with regioselective hydroxyl group protection using standard chemical methods [8–13].

In general, lipases exhibit a preference for primary hydroxyl functions, and regioselective acylation at O-6 may be achieved easily [3,14–18]. More recently, however, the specificity of lipases in regard to secondary hydroxyl groups has also been examined and high degrees of regioselectivity were achieved using 6-deoxy sugars and monosaccharides protected at their primary positions [19–25]. In other cases, however, the degree of discrimination between hydroxyl groups was found to be much lower. Recently Chinn et al. [23], Panza et al. [24], and Iacazio and Roberts [25] have shown that considerable control over the regioselectivity of acylation of several methyl 4,6-*O*-benzylidene monosaccharides may be exerted by changing the configuration of the anomeric centre. In order to extend these results and gain a better understanding of the binding of these and similar substrates in the active site of the enzyme, a study of the various factors likely to influence the regioselectivity of the transformation was initiated. In particular a range of glycosides was investigated to probe the effects of (*i*) varying the anomeric substituent, (*ii*) varying the bulkiness of the O-6 substituent, and (*iii*) including the 4-position as a possible site for acylation.

2. Results and discussion

Our studies of lipase-mediated acylation were initially focused on the 6-trityl ethers of D-glucose, D-galactose, D-mannose, and the corresponding methyl α- and β-glycosides which, to our knowledge, have received little attention as acceptors in transesterification reactions (Scheme 1). These substrates were attractive, however, on account of the ubiquity of the trityl group in carbohydrate chemistry and the fact that all secondary hydroxyl groups are available for derivatisation. For the purpose of this study *Pseudomonas cepacia* lipase (PAL) from Amano was selected as it has previously been shown to exhibit high degrees of regioselectivity in the acylation of 4,6-*O*-benzylidene monosaccharides. All reactions were carried out in vinyl acetate which acted as both solvent and acyl donor for the transesterification and rendered the reaction completely irreversible [26] (Table 1). In all cases the reaction stopped at the monoester stage and product ratios were independent of reaction time.

Despite the bulkiness of the trityl group, PAL was found to be an effective catalyst for the acylation of a wide range of *O*-trityl sugars, but high degrees of regioselectivity could only be obtained with *O*-tritylglycosides. Thus in the cases of 6-*O*-trityl-D-glucose (**1**), 6-*O*-trityl-D-galactose (**10**), and 6-*O*-trityl-D-mannose (**30**), the best results were achieved with **1** which gave two products, namely, 2-*O*-acetyl-6-*O*-trityl-D-glucose (**4**) and 3-*O*-acetyl-6-*O*-trityl-D-glucose (**7**) in the ratio 85:15 as determined from the ^{1}H-NMR spectrum (Table 1). Interestingly, this regioselectivity observed with PAL was found to be opposite to that of *Chromobacterium viscosum* lipase which has been shown to acylate **1** exclusively at the O-3 position using trichloroethyl butyrate in anhydrous pyridine [19]. In the cases of **10** and **30**, a mixture of products, which was unresolvable by column chromatography, was obtained. Although the identification of individual



1 R=OH
2a,b R=αOMe,βOMe
3a,b R=αOPh, βOPh

4 R=OH
5a,b R=αOMe,βOMe
6a,b R=αOPh, βOPh

7 R=OH
8a,b R=αOMe,βOMe
9a,b R=αOPh, βOPh

10 R=OH; R^1=Tr
11a,b R=αOMe,βOMe; R^1=Tr
12a,b R=αOPh,βOPh; R^1=Tr
13a,b R=αOMe,βOMe; R^1=^{i}Pr
14a,b R=αOMe,βOMe; R^1=Bn

15 R=OH; R^1=Tr
16a,b R=αOMe,βOMe; R^1=Tr
17a,b R=αOPh,βOPh; R^1=Tr
18a,b R=αOMe,βOMe; R^1=^{i}Pr
19a,b R=αOMe,βOMe; R^1=Bn

20 R=OH; R^1=Tr
21a,b R=αOMe,βOMe; R^1=Tr
22a,b R=αOPh,βOPh; R^1=Tr
23a,b R=αOMe,βOMe; R^1=^{i}Pr
24a,b R=αOMe,βOMe; R^1=Bn

25 R=OH; R^1=Tr
26a,b R=αOMe,βOMe; R^1=Tr
27a,b R=αOPh,βOPh; R^1=Tr
28a,b R=αOMe,βOMe; R^1=^{i}Pr
29a,b R=αOMe,βOMe; R^1=Bn

30 R=OH
31a,b R=αOMe,βOMe

32 R=OH
33a,b R=αOMe,βOMe

34 R=OH
35a,b R=αOMe,βOMe

Scheme 1.

Table 1
Enzymatic acetylation of partially protected monosaccharide derivatives using *Pseudomonas cepacia* lipase from Amano in vinyl acetate (VA)

Substrate	% Conversion [a]		% Acylation in position 2 [a]	% Acylation in position 3 [a]	% Acylation in position 4 [a]
	10 h	24 h			
TrGlc (**1**)	90	100	85 [b]	15 [b]	–
TrGlc αOMe (**2a**)	32	75	100	–	–
TrGlc βOMe (**2b**)	95	100	–	100	–
TrGlc αOPh (**3a**)	50	65	100	–	–
TrGlc βOPh (**3b**)	75	> 95	28	72 [c]	–
in 9:1 acetone–VA	22	55	14	86	–
in 9:1 hexane–VA	67	> 95	64	36	–
TrGal (**10**)	95	100	– [d]	– [d]	– [d]
TrGal αOMe (**11a**)	78	100	85 [e]	–	15
TrGal βOMe (**11b**)	100	100	< 5	> 95 [f]	–
TrGal αOPh (**12a**)	76	100	91	–	9
TrGal βOPh (**12b**)	84	100	35 [g]	65	–
^{i}PrGal αOMe (**13a**)	100	–	> 95 [b]	< 5 [b]	–
^{i}PrGal βOMe (**13b**)	100	–	25 [b]	60 [b]	15 [b]
BnGal αOMe (**14a**)	100	–	> 95 [b]	–	< 5 [b]
BnGal βOMe (**14b**)	100	–	30 [b]	70 [b]	–
TrMan (**30**)	47	83	– [d]	– [d]	– [d]
TrMan αOMe (**31a**)	0	0	–	–	–
TrMan βOMe (**31b**)	56	> 95	–	100	–

[a] Degrees of conversion were determined by HPLC or GLC analysis and no variations in regioisomer ratios with time were observed.
[b] As determined by ^{1}H-NMR.
[c] 100% of the 3-acetate may be obtained using *Chromobacterium viscosum* lipase immobilised on EP100.
[d] Unresolvable mixture of acetates obtained.
[e] 100% of the 2-acetate may be obtained using Lipozyme or *Pseudomonas* lipase from Boehringer.
[f] 100% of the 2-acetate may be obtained using Lipozyme.
[g] 100% of the 2-acetate may be obtained using *Candida antarctica* lipase immobilised on EP100.

regioisomers and their anomers was not successful, it was evident that acetylation occurred at a number of hydroxyl groups; in the case of **30** acylation was even found to occur at the anomeric hydroxyl group as shown by the low-field doublet at δ 6.07 ($J_{1,2}$ 1.7 Hz) in the ^{1}H-NMR spectrum.

When methyl α- and β-glycosides (**2**, **11**, **31**) were used as substrates, exclusive regioselectivity was observed; α-glycosides gave the 2-acetate and β-glycosides gave the 3-acetate. However, notable exceptions to this occurred in the cases of TrGal αOMe (**11a**) and TrMan αOMe (**31a**). With **11a** an 85:15 mixture of monoacetates was formed. The products, which could be easily separated by column chromatography, were identified as the 2-acetate (**16a**) (major) and, interestingly, the 4-acetate (**26a**). The regioselectivity of this reaction was significantly improved, however, by using a *Pseudomonas* lipase from another supplier (Boehringer Mannheim) or *Mucor miehei* lipase (Lipozyme IM); both gave **16a** as the sole product. It was also noted that by using *Mucor miehei* lipase it was possible to acylate TrGal βOMe (**11b**) exclusively in the 2-position. TrMan αOMe (**31a**) was the only substrate among the glycosides examined which was not acylated even after lengthy periods of incubation.

The general pattern of acylation for methyl 6-*O*-trityl-D-glycopyranosides compares well with those determined by Chinn et al. [23], Panza et al. [24], and Iacazio and Roberts [25] who used the corresponding 4,6-*O*-benzylidene substrates and *P. cepacia* lipase from Amano and *P. fluorescens* lipase from Biocatalysts, respectively. Some notable differences in the rates of reaction were, however, noted. In particular, whereas methyl 4,6-*O*-benzylidenegalactosides were found to be acetylated very slowly or not at all, the 6-*O*-substituted galactosyl derivatives which we examined were all acetylated easily. Also a significant difference in reaction rate occurs between methyl 4,6-*O*-benzylidene-α-D-mannopyranoside and TrManαOMe (**31a**); the former was readily acylated, whereas **31a** was completely unreactive. These results show that high degrees of regioselectivity can be achieved in the synthesis of any 2-*O*- and 3-*O*-acetyl monosaccharide glycoside using *Pseudomonas* lipases and an appropriate choice of protecting group.

The marked difference between the reactivities of methyl 6-*O*-trityl-α- and -β-D-mannopyranosides is a reflection of the consistent way in which trityl monosaccharide glycosides reside in the active site. Thus in the case of TrManβOMe it is likely that a situation analogous to that with TrGlcβOMe and TrGalβOMe exists, allowing acetylation at O-3. With TrManαOMe, however, the orientation in the active site corresponds to those of TrGlcαOMe and TrGalαOMe but acetylation at O-2 is precluded on account of its axial orientation. In order to gain further insight into the factors affecting the regioselectivity of these reactions, the study was widened to encompass several derivatives featuring changes in the O-6 substituent (*galacto*-series) and the anomeric substituent (*galacto*- and *gluco*-series). The results obtained with 6-*O*-benzyl and 6-*O*-isopropyl derivatives of methyl α- and β-D-galactopyranoside and phenyl 6-*O*-tritylglycosides are summarised in Table 1.

From these results it is possible to draw some tentative conclusions regarding the effects of size/hydrophobicity on product formation. In the α-*galacto*-series it is clear that the 2-acetate is the strongly preferred product and that the size of the O-6 substituent is less critical than in the β-series. In the α-series it was also clear that the size of the anomeric substituent has little or no bearing on the regioselectivity (cf. **11a** and **12a**) whereas its configuration is critical. The formation of small amounts of the 4-acetate in the cases of **11a** and **12a** was unexpected on account of steric hindrance by the trityl group. Since, however, the 4-position is not acylated when the substituent at O-6 is isopropyl (i.e., with **13a**) it is evident that large hydrophobic groups at this position impose a certain degree of conformational restraint. This effect is also seen, though to a lesser degree, in the case of **14b** where a small amount of the 4-acetate is formed to the total exclusion of the 3-acetate. The result obtained in the case of **13a** is interesting for the information it provides about the 4-position; clearly this is not a 'natural' site for acylation using this enzyme and there is no requirement for protection of this position, e.g., using benzylidene, to achieve selectivity. Finally, the difference between the regioselectivities observed in the cases of TrGlcαOMe (**2a**) and TrGalαOMe (**11a**) [and, to a lesser extent, TrGlcβOMe (**2b**) and TrGalβOMe (**11b**)] is assumed to result from conformational differences brought about by the repulsion between the axial C-4 hydroxyl group and the trityl group.

In the case of the β-series, the 3-acetate is the preferred product but the size of the

O-6 substituent is more critical than in the α-series, with any reduction resulting in a marked decrease in regioselectivity (cf. **11b**, **13b**, **14b**). The size of the anomeric substituent is also more important in the β-series with a change from OMe to OPh allowing the formation of considerable amounts of the 2-acetate. The decrease in regioselectivity through the β-galactoside series **11b, 13b, 14b** may be explained in terms of conformational restraints imposed by large hydrophobic substituents. Thus substituents such as trityl cannot be accommodated in the active site of the enzyme and remain largely exposed to the solvent, thus 'anchoring' the monosaccharide in a particular position. On the other hand, when the 6-*O*-substituent is smaller, e.g., isopropyl, the binding is more flexible, resulting in a relatively poor regioselectivity. If this is the case, a certain balance between the hydrophobicity of the 6-*O*- and the anomeric substituents would be important to provide the regioselectivity required. Although it is not possible to verify such a model on the basis of the results obtained so far, it would suggest that the regioselectivity of the enzymatic esterification would be markedly dependent on the nature of organic solvent used. Indeed, we have observed that the ratio of TrGlcβOPh(2-OAc) (**9a**) to TrGlcβOPh(3-OAc) (**9b**) could be changed from 28:72 to 14:86 or 64:36 simply by changing the solvent from vinyl acetate to acetone–vinyl acetate (90:10 v/v) or hexane–vinyl acetate (90:10 v/v), respectively (Table 1). To the best of our knowledge, this is the first demonstration of the effect of organic solvents on regioselectivity of enzymatic acylation of sugars. Experiments are currently being performed to investigate this phenomenon in detail and to assess its usefulness in terms of preparative synthesis. With the availability of three-dimensional structures of lipases and recent advances in molecular modelling software it is anticipated that the effects of substituents on the regioselectivity of these reactions will soon be understood and that, with the aid of protein engineering, it will be possible to tailor enzymes to specific functions. Indeed, the structural basis for chiral preference of lipases [27,28] and the change in enantioselectivity and substrate specificity of these enzymes achieved by mutagenesis have recently been reported [29].

3. Experimental

General methods.—Amano 'PS' lipase was a gift from the Amano Pharmaceutical Corporation. *Pseudomonas* species lipase was a lipoprotein lipase obtained from Boehringer Mannheim GmbH. *Chromobacterium viscosum* lipase was obtained from Genzyme and immobilised on EP100 by Unilever Research (Colworth House, Sharnbrook, Beds.). *Mucor miehei* lipase (Lipozyme) and *Candida antarctica* lipase (Novozyme 435) were obtained from Novo Nordisk. Melting points are uncorrected. Optical rotations were measured using a Thorn-NPL Automatic Polarimeter Type 243 at ~ 20°C using $CHCl_3$ as solvent unless otherwise stated. NMR spectra were recorded with a Jeol EX270 spectrometer for solutions in $CDCl_3$ (internal Me_4Si) unless otherwise stated. Elemental analyses were carried out using a Perkin–Elmer 2400 Series 2 analyzer. TLC was performed on Silica Gel 60 (Merck) precoated on aluminium sheets using $CHCl_3$–MeOH mixtures of appropriate polarity as eluent. Products were visualised using the orcinol–$FeCl_3$ spray reagent (Sigma).

Preparative chromatography was performed by gradient elution (also $CHCl_3$–MeOH) from columns of silica gel (70–230 mesh, 60 Å, Aldrich). HPLC analysis was performed with a Spherisorb 5ODS2 column (25 cm × 4.6 mm i.d.) using a Gilson pump system with UV detection at 254 nm. Products were eluted with a water–MeCN gradient (1:9 to 9:1) over a period of 10 min. GLC analysis was performed on a 25 m SGE BPX70 column (i.d. 0.33 mm, 0.25 mm film thickness) in a Hewlett–Packard 5890 GC programmed from 260 to 290°C at 5°C min^{-1}. Samples were derivatised with bis(trimethylsilyl)trifluoroacetamide.

Phenyl α-D-galactopyranoside [30], methyl 6-*O*-benzyl-α- and -β-D-galactopyranosides [31], and methyl β-D-mannopyranoside [32] were prepared according to published procedures.

Methyl 6-O-isopropyl-α-D-galactopyranoside (**13a**).—Methyl α-D-galactopyranoside (3.88 g, 0.02 mol) was dissolved in dry *N,N*-dimethylformamide and the solution cooled in an ice–salt bath. Sodium hydride (0.528 g, 0.022 mol) was added and the mixture stirred for 1 h. 2-Iodopropane (2.2 mL, 0.022 mol) was then added and the mixture allowed to warm to room temperature. After a further 2 h the mixture was warmed to 70°C for 1 h, MeOH (1 mL) was added, and the solvent was removed under reduced pressure. The resultant solid was triturated with 30:1 $CHCl_3$–MeOH (5 mL), filtered, and the filtrate applied to a column of silica gel. Gradient elution ($CHCl_3$–MeOH, 30:1 to 10:1) yielded **13a** (0.90 g, 19%); mp 153–155°C (from EtOAc); R_f 0.30 (6:1 $CHCl_3$–MeOH); $[\alpha]_D$ +120.4° (*c* 0.7, MeOH); NMR data (CD_3OD): ^1H, δ 1.15 (d, 6 H, *J* 6.3 Hz, 2 × Me), 3.39 (s, 3 H, OMe), 3.54–3.68 (m, 4 H, H-3, C*H*Me_2, H-6a,b), 3.73 (dd, 1 H, $J_{2,3}$ 9.3 Hz, H-2), 3.77–3.86 (m, 2 H, H-4, H-5), 4.68 (d, 1 H, $J_{1,2}$ 3.3 Hz, H-1); ^{13}C, δ 22.4 (Me), 55.6 (OMe), 68.7 (C-6), 70.2 (C-2), 70.8 (C-5), 71.1 (C-4),

Table 2
Physical data for monoacetylated monosaccharide derivatives

Compound	Mp (°C)	$[\alpha]_D$ (deg)	Anal.
TrGlcαOMe(2-OAc) (**5a**)	78–80 (ether–hexane)	+68 (*c* 1.0)	C, 70.30; H, 6.30
TrGlcβOMe(3-OAc) (**8b**)	–	−6.0 (*c* 0.7)	C, 70.25; H, 6.30
TrGlcαOPh(2-OAc) (**6a**)	96–98 (ether)	+127 (*c* 0.3)	C, 73.30; H, 6.01
TrGlcβOPh(2-OAc) (**6b**)	–	−24 (*c* 0.5, MeOH)	C, 73.35; H, 5.89
TrGlcβOPh(3-OAc) (**9b**)	–	−17 (*c* 0.9, MeOH)	C, 73.24; H, 5.92
^{i}PrGalαOMe(2-OAc) (**18a**)	–	+128 (*c* 1.1, MeOH)	C, 51.83; H, 7.81
BnGalαOMe(2-OAc) (**19a**)	–	+133 (*c* 0.7)	C, 58.69; H, 6.87
TrGalαOMe(2-OAc) (**16a**)	77–78 (MeOH)	+86 (*c* 0.4)	C, 70.34; H, 6.37
TrGalαOMe(4-OAc) (**26a**)	86–88 (MeOH)	+39 (*c* 0.6)	C, 70.30; H, 6.31
TrGalβOMe(2-OAc) (**16b**)	78–80 (MeOH)	−22 (*c* 0.6)	C, 70.36; H, 6.28
TrGalβOMe(3-OAc) (**21b**)	89–91 (ether–hexane)	+15 (*c* 1.2)	C, 70.36; H, 6.34
TrGalαOPh(2-OAc) (**17a**)	–	+36 (*c* 0.3)	C, 73.36; H, 5.90
TrGalαOPh(4-OAc) (**27a**)	–	+59 (*c* 0.8)	C, 73.40; H, 5.96
TrGalβOPh(2-OAc) (**17b**)	89–91 (MeOH)	+7.6 (*c* 0.9)	C, 73.42; H, 6.01
TrGalβOPh(3-OAc) (**22b**)	–	+7.9 (*c* 0.5)	C, 73.39; H, 5.92
TrManαOMe(3-OAc) (**35a**)	78–81 ($CHCl_3$)	+41 (*c* 0.7)	C, 70.24; H, 6.39
TrManβOMe(3-OAc) (**35b**)	156–159 (MeOH)	−73 (*c* 1.0)	C, 70.32; H, 6.40

Table 3
^{1}H-NMR data for monoacetylated monosaccharide derivatives

Compound	H-1	$J_{1,2}$	H-2	$J_{2,3}$	H-3	$J_{3,4}$	H-4	$J_{4,5}$	H-5	H-6	AcO-2	AcO-3	AcO-4	OMe	CH_2Ph ($CHMe_2$)
TrGlc α OMe (2-OAc) (**5a**)	4.90	3.6	4.70	9.8	3.91	8.9	3.54	9.8	3.67	3.38	2.11	–	–	3.38	–
TrGlc β OMe (3-OAc) (**8b**)	4.26	7.9	3.46	[a]	4.93	4.51	3.67	4.2	3.46	3.46	–	2.15	–	3.57	–
TrGlc α OPh (2-OAc) (**6a**)	5.72	3.6	4.84	9.9	4.17	9.9	3.66	9.6	3.83	3.36	2.13	–	–	–	–
TrGlc β OPh (2-OAc) (**6b**)	4.97	7.9	5.03	8.9	3.55	8.3	3.4	[a]	3.5	3.3/3.4	2.02	–	–	–	–
TrGlc β OPh (3-OAc) (**9b**)	4.94	7.9	3.79	9.2	4.98	9.2	3.66	8.9	3.47	3.47	–	2.16	–	–	–
BnGal α OMe (2-OAc) (**19a**)	4.91	3.6	5.07	10.2	3.93	[a]	4.08	[a]	3.93	3.76	2.14	–	–	3.38	4.59
BnGal β OMe (2-OAc) (**19b**)	4.29	7.9	4.98	9.9	3.6	3.3	4.13	[a]	3.6	3.75	2.12	–	–	3.49	4.57
BnGal β OMe (3-OAc) (**24b**)	4.24	7.9	3.87	10.2	4.81	3.3	4.13	[a]	3.65	3.75	–	2.15	–	3.56	4.57
^{i}PrGal α OMe (2-OAc) (**18a**)	4.93	3.6	5.10	10.2	3.94	3.3	4.13	[a]	3.87	3.75	2.15	–	–	3.39	(3.73)
^{i}PrGal β OMe (2-OAc) (**18b**)	4.29	7.9	4.99	9.6	3.63	3.3	4.00	[a]	3.65	3.65	2.13	–	–	3.49	(3.74)
^{i}PrGal β OMe (3-OAc) (**23b**)	4.25	7.9	3.88	10.2	4.80	3.0	4.15	[a]	3.65	3.65	–	2.17	–	3.57	(3.74)
TrGal α OMe(2-OAc) (**16a**)	4.90	3.6	5.02	10.2	3.92	3.3	4.00	[a]	3.83	3.41	2.12	–	–	3.38	–
TrGal α OMe(4-OAc) (**26a**)	4.79	4.0	3.73	9.6	3.95	3.3	5.46	[a]	3.95	3.06/3.31	–	–	1.91	3.43	–
TrGal β OMe (2-OAc) (**16b**)	4.22	7.9	4.89	9.7	3.51	3.3	3.95	[a]	3.46	3.48	2.06	–	–	3.44	–
TrGal β OMe (3-OAc) (**21b**)	4.22	7.6	3.82	10.2	4.81	3.0	4.11	[a]	3.60	3.37	–	2.14	–	3.56	–
TrGal α OPh (2-OAc) (**17a**)	5.73	3.6	5.16	10.2	4.14	3.3	4.00	[a]	4.00	3.29/3.44	2.10	–	–	–	–
TrGal α OPh(4-OAc) (**27a**)	5.58	3.6	3.91	10.2	4.17	3.0	5.45	[a]	4.11	3.04/3.29	–	–	1.94	–	–
TrGal β OPh (2-OAc) (**17b**)	4.95	7.9	5.24	9.6	3.64	3.3	3.94	[a]	3.64	3.40/3.58	2.12	–	–	–	–
TrGal β OPh (3-OAc) (**22b**)	4.94	7.6	4.18	10.2	4.89	3.0	4.10	[a]	3.71	3.42	–	2.17	–	–	–
TrMan α OMe (3-OAc) (**35a**)	4.70	1.3	4.00	3.0	5.07	9.6	3.93	[a]	3.73	3.45	–	2.13	–	3.39	–
TrMan β OMe (3-OAc) (**35b**)	4.47	1.0	4.10	3.0	4.81	9.9	4.05	[a]	3.42	3.50	–	2.17	–	3.55	–

[a] Not determined.

Table 4
^{13}C-NMR data for monoacetylated monosaccharide derivatives

Compound	C-1	C-2	C-3	C-4	C-5	C-6	CH_3CO	OMe	CPh_3	CH_2Ph	$CHMe_2$
TrGlc α OMe(2-OAc) **(5a)**	96.8	73.2	71.5	72.3	69.5	63.9	21.0	55.1	87.0	–	–
TrGlc β OMe(3-OAc) **(8b)**	103.6	72.2	77.7	70.9	74.2	64.2	21.0	57.1	87.2	–	–
TrGlc α OPh(2-OAc) **(6a)**	94.5	72.9	71.4	72.0	70.4	63.5	20.9	–	87.0	–	–
TrGlc β OPh(2-OAc) **(6b)**	98.7	73.4	75.3	71.2	74.9	63.3	20.9	–	86.6	–	–
TrGlc β OPh(3-OAc) **(9b)**	100.8	72.0	77.8	70.5	74.7	63.8	21.0	–	87.1	–	–
BnGal α OMe(2-OAc) **(19a)**	97.4	71.4	68.2 [a]	69.9	68.3 [a]	69.5	21.0	55.3	–	73.7	–
BnGal β OMe(2-OAc) **(19b)**	101.7	72.6	73.2 [a]	69.4	73.6 [a]	69.2	20.7	56.6	–	73.6	–
BnGal β OMe(3-OAc) **(24b)**	104.2	69.0	75.1	67.9	72.9	69.2	21.0	57.2	–	73.6	–
^{i}PrGal α OMe(2-OAc) **(18a)**	97.5	71.5	68.0	70.8	68.5	68.4	21.0	55.3	–	–	72.9
^{i}PrGal β OMe(2-OAc) **(18b)**	101.7	72.5 [a]	73.3	69.3	72.6 [a]	67.1	21.0	56.5	–	–	72.8
^{i}PrGal β OMe(3-OAc) **(23b)**	104.3	68.8	75.2	67.9	72.6	67.4	21.0	57.1	–	–	72.8
TrGal α OMe(2-OAc) **(16a)**	97.2	71.6	68.3	70.2	68.5	63.3	21.0	55.1	87.0	–	–
TrGal α OMe(4-OAc) **(26a)**	99.4	68.0	69.8	70.4	69.6	61.5	20.7	55.6	86.8	–	–
TrGal β OMe(2-OAc) **(16b)**	101.6	73.0	72.7	69.5	73.3	62.6	21.1	56.5	87.0	–	–
TrGal β OMe(3-OAc) **(21b)**	104.2	69.2	75.1	67.9	73.1	62.9	21.0	56.9	87.1	–	–
TrGal α OPh(2-OAc) **(17a)**	95.0	71.2	68.2	69.6	70.3	63.5	20.9	–	87.0	–	–
TrGal α OPh(4-OAc) **(27a)**	97.6	69.5	69.8	70.4	69.1	61.7	20.7	–	86.7	–	–
TrGal β OPh(2-OAc) **(17b)**	99.1	72.8	72.7	69.6	73.9	63.1	21.0	–	87.1	–	–
TrGal β OPh(3-OAc) **(22b)**	101.5	69.1	75.0	68.2	73.6	63.4	21.0	–	87.2	–	–
TrMan α OMe(3-OAc) **(35a)**	100.4	69.1	74.2	67.7	70.5	64.6	21.1	55.0	87.3	–	–
TrMan β OMe(3-OAc) **(35b)**	100.2	68.9	75.5	67.3	74.0	64.5	21.1	56.8	87.3	–	–

[a] May be interchanged.

71.5 (C-3), 73.5 (CMe_2), 101.5 (C-1). Anal. Calcd for $C_{10}H_{20}O_6$: C, 50.84; H, 8.53. Found: C, 51.12; H, 8.96.

Methyl 6-O-isopropyl-β-D-galactopyranoside (**13b**).—Methyl β-D-galactopyranoside was treated in an analogous method to that described above for **13a**. Column chromatography gave **13b** as a white solid (0.95 g, 21%); R_f 0.28 (6:1 $CHCl_3$–MeOH); $[\alpha]_D$ −15.2° (*c* 0.7, MeOH); NMR data (CD_3OD): ^1H, δ 1.17 (d, 6 H, *J* 6.3 Hz, 2 × Me), 3.48 (dd, 1 H, $J_{2,3}$ 10.2 Hz, H-2), 3.55 (s, 3 H, OMe), 3.62 (dd, 1 H, $J_{3,4}$ 3.3 Hz, H-3), 3.65–3.73 (m, 3 H, H-5, H-6a,b), 3.76 (m, 1 H, C*H*Me_2), 3.88 (d, 1 H, H-4), 4.28 (d, 1 H, $J_{1,2}$ 7.6 Hz, H-1); ^{13}C, δ 22.0/22.1 (Me), 58.2 (OMe), 68.1 (C-6), 69.9 (C-4), 71.6 (C-2), 73.7 (C-3), 74.0 (CMe_2), 74.6 (C-5), 101.4 (C-1). Anal. Calcd for $C_{10}H_{20}O_6$: C, 50.84; H, 8.53. Found: C, 51.02; H, 8.89.

6-O-Trityl monosaccharides and monosaccharide glycosides.—A typical procedure for the preparation of these compounds is outlined as follows. Trityl chloride (1.87 g, 6.7 mmol) was added in portions to a solution of methyl β-D-mannopyranoside (1.0 g, 5.15 mmol) in dry *N,N*-dimethylformamide containing triethylamine (3 mL). The mixture was stirred overnight at room temperature and then poured onto ice. The aqueous phase was decanted and the residue dissolved in $CHCl_3$. After drying over $MgSO_4$ the solution was chromatographed on silica gel. Elution with $CHCl_3$ followed by acetone yielded methyl 6-*O*-trityl-β-D-mannopyranoside (**31b**) (1.7 g, 76%) which was recrystallised from MeOH–water; mp 74–76°C; $[\alpha]_D$ −66° (*c* 0.6); lit. [33] $[\alpha]_D$ −63.6°; NMR data ($CDCl_3$): ^1H, δ 3.21 (m, 1 H, H-5), 3.40 (m, 3 H, H-3, H-6), 3.55 (s, 3 H, OMe), 3.68 (m, 1 H, H-4), 3.95 (m, 1 H, H-2), 4.37 (s, 1 H, H-1); ^{13}C, δ 56.8 (OMe), 64.3 (C-6), 69.7 (C-4), 70.3 (C-2), 74.1 (C-3, C-5), 87.0 (CPh_3), 100.7 (C-1), 127.1, 127.9, 128.6, 143.6 (Ph). Anal. Calcd for $C_{26}H_{28}O_6$: C, 71.54; H, 6.47. Found: C, 71.36; H, 6.56.

The enzymatic acylation of all substrates was carried out in vinyl acetate obtained from Aldrich and used without purification. All products were purified by column chromatography which was effective, where necessary, in separating mixtures of monoacetates. A typical procedure is given as follows. The substrate (100 mg) was dissolved in vinyl acetate (3 mL) and incubated with shaking at 37°C with Amano lipase 'PS' (250 mg). The reaction was monitored by TLC (25:1 $CHCl_3$–MeOH) and HPLC. When the reaction was complete, the enzyme was removed by filtration and the vinyl acetate was removed in vacuo. The resultant syrup was chromatographed on silica gel using a gradient elution system of $CHCl_3$ followed by $CHCl_3$–MeOH (up to 20:1). Product acetates, obtained in almost quantitative yield, were then, where possible, recrystallised. Physical data for all characterised monoester products are given in Tables 2–4.

References

[1] H. Paulsen, *Chem. Soc. Rev.*, 13 (1984) 15–45.
[2] R.R. Schmidt, *Angew. Chem., Int. Ed. Engl.*, 25 (1986) 212–235.
[3] C.H. Wong and G.M. Whitesides, *Enzymes in Synthetic Organic Chemistry*, Elsevier, Oxford, 1994.
[4] D.H.G. Crout, D.A. MacManus, J.-M. Ricca, S. Singh, P. Critchley, and W.T. Gibson, *Pure Appl. Chem.*, 64 (1992) 1079–1084.

[5] K.G.I. Nilsson, *ACS Symp. Ser.*, 466 (1991) 51–62.
[6] K.G.I. Nilsson, *TIBTECH*, 6 (1988) 256–264.
[7] Y. Ichikawa, G.C. Look, and C.H. Wong, *Anal. Biochem.*, 202 (1992) 215–238.
[8] J.M. Williams and A.C. Richardson, *Tetrahedron*, 23 (1967) 1369–1378.
[9] A.H. Haines, *Adv. Carbohydr. Chem. Biochem.*, 33 (1976) 11–109.
[10] D. Plusquellec and K. Baczko, *Tetrahedron Lett.*, 28 (1987) 3809–3812.
[11] K. Ishihara, H. Kurihara, and H. Yamamoto, *J. Org. Chem.*, 58 (1993) 3791–3793.
[12] A. Liguori, A. Procopio, G. Romeo, G. Sindona, and N. Uccella, *J. Chem. Soc., Perkin Trans. 1*, (1993) 1783–1786.
[13] H. Cho, J. Yu, and J.R. Falck, *J. Am. Chem. Soc.*, 116 (1994) 8354–8355.
[14] A.M. Klibanov, *Acc. Chem. Res.*, 23 (1990) 114–120.
[15] S. Riva and F. Secundo, *Chimicaoggi*, June 1990, pp 9–16.
[16] V. Gotor and R. Pulido, *J. Chem. Soc., Perkin Trans. 1*, (1991) 491–492.
[17] A. Sharma and S. Chattopadhyay, *Biotechnol. Lett.*, 15 (1993) 1145–1146.
[18] S. Riva, J. Chopineau, A.P.G. Kieboom, and A.M. Klibanov, *J. Am. Chem. Soc.*, 110 (1988) 584–589.
[19] M. Therisod and A.M. Klibanov, *J. Am. Chem. Soc.*, 109 (1987) 3977–3981.
[20] P. Ciuffreda, D. Colombo, F. Ronchetti, and L. Toma, *J. Org. Chem.*, 55 (1990) 4187–4190.
[21] D. Colombo, F. Ronchetti, and L. Toma, *Tetrahedron*, 47 (1991) 103–110.
[22] F. Nicotra, S. Riva, F. Secundo, and L. Zucchelli, *Tetrahedron Lett.*, 30 (1989) 1703–1704.
[23] M.J. Chinn, G. Iacazio, D.G. Spackmann, N.J. Turner, and S.M. Roberts, *J. Chem. Soc., Perkin Trans 1*, (1992) 661–662.
[24] L. Panza, M. Luisetti, E. Crociati, and S. Riva, *J. Carbohydr. Chem.*, 12 (1993) 125–130.
[25] G. Iacazio and S.M. Roberts, *J. Chem. Soc., Perkin Trans. 1*, (1993) 1099–1101.
[26] M. Degueil-Castaing, B. DeJesco, S. Drouillard, and B. Maillard, *Tetrahedron Lett.*, 28 (1987) 953–954.
[27] M. Cygler, P. Grochulski, R.J. Kazlauskas, J.D. Schrag, F. Bouthillier, B. Rubin, A.N. Serreqi, and A.K. Gupta, *J. Am. Chem. Soc.*, 16 (1994) 3180–3186.
[28] R.J. Kazlauskas, *TIBTECH*, 12 (1994) 464–472.
[29] M. Holmquist, M. Martinelle, P. Berglund, I.G. Clausen, S. Patkar, A. Svendsen, and K. Hult, *J. Protein Chem.*, 12 (1993) 749–757.
[30] M.J. Clancy, *J. Chem. Soc.*, (1960) 3421–3423.
[31] H.M. Flowers, *Carbohydr. Res.*, 100 (1982) 418–423.
[32] P.J. Garegg and T. Iversen, *Carbohydr. Res.*, 70 (1979) c13–c14.
[33] B. Bernet and A. Vasella, *Helv. Chim. Acta*, 62 (1979) 2400–2410.