Enzymatic esterification and de-esterification of carbohydrates: synthesis of a naturally occurring rhamnopyranoside of *p*-hydroxybenzaldehyde and a systematic investigation of lipase-catalysed acylation of selected arylpyranosides

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The regiocontrolled esterification and de-esterification of mono- and di-saccharides is reviewed. New results involving the enzyme-catalysed regioselective acylation of ribo-**110**, arabino-**112**, **119**, xylo-**111**, and rhamnopyranosides **116**, **118** (as well as two arylpyranosides **125** and **136**), are reported. This methodology is then applied to the total synthesis of the naturally occurring rhamnopyranoside 4-(4-*O*-acetyl-α-L-rhamnosyloxy)benzaldehyde **122**.

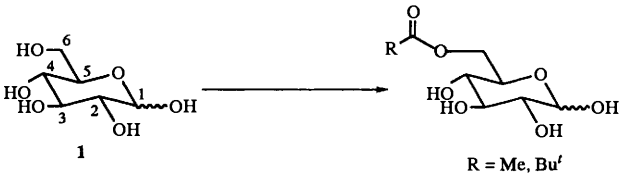
Introduction

Carbohydrates, with their multiple stereocentres and functionality, provide the synthetic chemist with an inexpensive source of chiral intermediates for use in the synthesis of natural products.[1] In order to maximize the utilization of these compounds it is often necessary to convert the peripheral hydroxy groups into other types of functionality. However, the selective manipulation of individual hydroxy groups in complex sugar moieties is often a problem. The selective protection and deprotection of carbohydrates can be achieved by various chemical techniques but this strategy often involves cumbersome multistep sequences. The possibility of using enzymatic methods to effect regioselective acylation and deacylation, is an attractive alternative proposition. Thus, while it is difficult to discriminate by chemical methods between primary and secondary hydroxy groups in acylation reactions involving carbohydrates, selective acylation of primary hydroxy groups is possible using enzymes.[2] Recent reviews have featured aspects of this topic;[3–6] this text provides a comprehensive survey of the field.

Acylation reactions

Monosaccharides – selective acylation of the primary hydroxy group

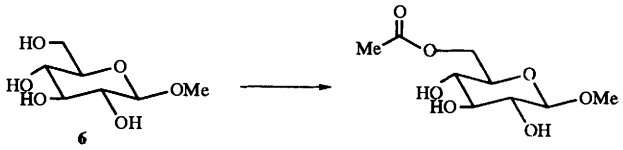
One of the initial considerations in the use of enzymes in carbohydrate chemistry was the selection of a suitable solvent. The high polarity of sugars necessitated the use of polar solvents, such as dimethyl sulfoxide, dimethylformamide, pyridine and dimethylacetamide. Unfortunately, most enzymes are inactive in these solvents and esterification reactions are, of course, impossible in water. Apart from porcine pancreatic lipase (PPL), only the lipase from *Chromobacterium viscosum* (CVL) showed any activity in pyridine, and then only to a small extent, and none exhibited appreciable activity in DMF. Moreover, PPL was found to be virtually unreactive with di- and oligo-saccharides, thereby severely restricting the scope of its potential application. Nevertheless, it was possible to achieve the selective acylation of the primary hydroxy groups of a number of unprotected monosaccharides using porcine pancreatic lipase in pyridine (Scheme 1).[2] The acylating reagents employed were a variety of 2,2,2-trichloroethyl esters. For example, D-glucopyranose **1**, D-galactopyranose **2** and D-mannopyranose **3** exhibited excellent regioselectivities, with the



Scheme 1 *Reagents and conditions:* $RCO_2CH_2CCl_3$, PPL, pyridine, 45 °C

6-*O*-acyl derivatives being obtained with 82–100% regioselectivity (see Table 1). In the case of fructose **4**, the two primary hydroxy groups displayed similar reactivities resulting in a mixture of 1-*O*-acyl and 6-*O*-acyl derivatives.

Wong and co-workers[7] found that the rate of *Candida cylindracea* lipase†-catalysed acylation of a number of monosaccharides could be enhanced by use of a co-solvent. Thus, a mixture of benzene and pyridine was used for the acetylation of D-mannopyranose **3**, *N*-acetylmannosamine (ManNAc) **5** and methyl-β-D-glucopyranoside **6** (Scheme 2)



Scheme 2 *Reagents and conditions:* Vinyl acetate, CCL, benzene–pyridine (2:1), 28 °C

with vinyl acetate as the acyl donor, giving the 6-*O*-acyl derivatives with >90% regioselectivity. The same research group also demonstrated[8] that selective acylation of various furanoses and pyranoses occurred in tetrahydrofuran, using a crude preparation of porcine pancreatic lipase and the more active acyl donor 2,2,2-trifluoroethyl acetate. For the methyl furanosides methyl α,β-D-ribofuranoside **7**, methyl α-D-arabinofuranoside **8**, methyl α,β-D-xylofuranoside **9** and methyl 2-deoxy-α,β-D-ribofuranoside **10**, good to excellent regioselectivity was achieved giving the 5-*O*-monoesters in moderate to excellent yields, 39–84% (see Table 1). Reaction times (at 37 °C) ranged from 18 to 60 h.

† *Candida cylindracea* lipase has now been renamed *Candida rugosa* lipase.

Table 1 Enzyme-catalysed acylation of the primary hydroxy group

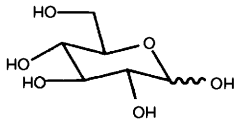
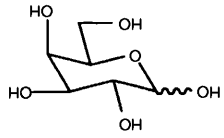
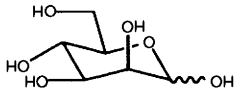
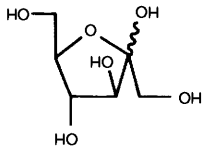
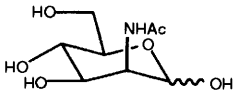
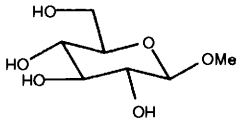
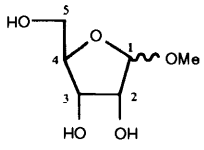
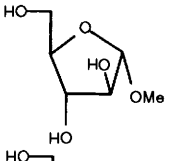
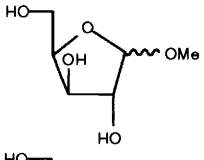
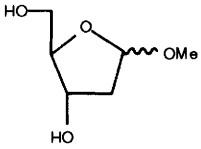
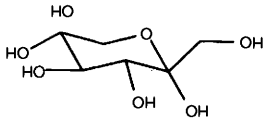
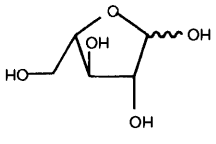
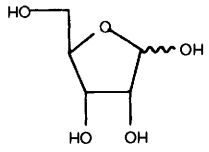
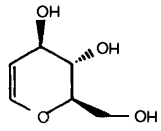
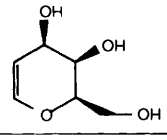
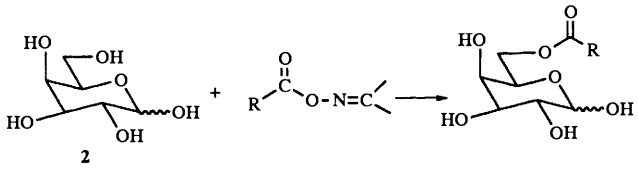
No.	Structure	Enzyme	Solvent	Acyl donor	Position	Yield (%)	Ref.
1		PPL CAL subtilisin	Pyridine Dioxane DMF	$RCO_2CH_2CCl_3$ $ROCO_2N{=}CMe_2$ $PrCO_2CH_2CCl_3$	6 6 6	19–35 15–72 60	2 10 13
2		PPL PSL CAL	Pyridine Pyridine Dioxane	$CH_3CO_2CH_2CCl_3$ $RCO_2N{=}CMe_2$ $ROCO_2N{=}CMe_2$	6 6 6	57 70–85 43–68	2 9 10
3		PPL CCL PSL CAL	Pyridine Benzene–pyridine (2:1) Pyridine Dioxane	$CH_3CO_2CH_2CCl_3$ $CH_3CO_2CH{=}CH_2$ $RCO_2N{=}CMe_2$ $ROCO_2N{=}CMe_2$	6 6 6 6	36 65–80 44–53	2 7 9 10
4		PPL	Pyridine	$CH_3CO_2CH_2CCl_3$	1 6	20 8	2
5		CCL Protease N subtilisin 8399 subtilisin BNP′	Benzene–pyridine (2:1) DMF DMF 97% DMF	$CH_3CO_2C(Me){=}CH_2$ $CH_3CO_2C(Me){=}CH_2$ $CH_3CO_2CH{=}CH_2$ $BOCHNCH_2CO_2CH_2CN$	6 6 6 6	 70 65	7 14 17 18
6		CCL	Benzene–pyridine (2:1)	$CH_3CO_2CH{=}CH_2$	6		7
7		PPL	THF	$CH_3CO_2CH_2CF_3$	5	77	8
8		PPL	THF	$CH_3CO_2CH_2CF_3$	5	77	8
9		PPL	THF	$CH_3CO_2CH_2CF_3$	5	84	8
10		PPL	THF	$CH_3CO_2CH_2CF_3$	5	39	8
11		PSL	Pyridine	$RCO_2N{=}CMe_2$	1	68–86	9
12		PSL CAL	Pyridine Dioxane	$RCO_2N{=}CMe_2$ $ROCO_2N{=}CMe_2$	5 5	45–70 	9 10

Table 1 (*continued*)

No.	Structure	Enzyme	Solvent	Acyl donor	Position	Yield (%)	Ref.
13		CAL	Pyridine	$ROCO_2N{=}CMe_2$	5	50–64	9
		CAL	Dioxane	$ROCO_2N{=}CMe_2$	5		10
16		CCL	EtOAc	$CH_3CO_2CH{=}CH_2$	6	90	19
17		CCL	EtOAc	$CH_3CO_2CH{=}CH_2$	6	93	19

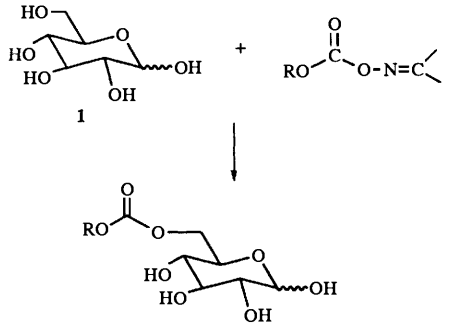
A further variation in this methodology was introduced by Gotor and co-workers,[9] who studied the selective acylation of the primary hydroxy groups of carbohydrates using oxime esters (Scheme 3). Oxime esters act as good acylating agents



Scheme 3 *Reagents and conditions:* PSL, pyridine, 25 °C

because the oxime moiety is a good leaving group. The hexoses D-galactopyranose **2**, D-mannopyranose **3** and the ketohexose L-sorbopyranose **11** were treated with an oxime ester in the presence of *Pseudomonas cepacia* lipase (PSL) for 3 days using pyridine as solvent. Acylation occurred exclusively at the primary hydroxy group giving the 6-*O*-acyl derivatives of D-galactopyranose **2** and D-mannopyranose **3**, and the 1-*O*-acyl derivative of L-sorbopyranose **11**. The pentoses L-arabinofuranose **12** and D-ribofuranose **13** were successfully acylated at the C-5 position using *Pseudomonas cepacia* lipase (PSL) and *Candida antarctica* lipase (CAL), respectively, with pyridine as solvent (see Table 1).

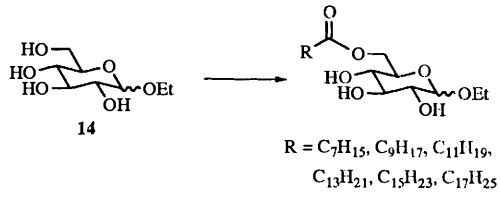
Further work by Gotor and co-workers[10] used acetone *O*-(alkoxycarbonyl)oximes as alkoxycarbonylation agents in dioxane at 60 °C (Scheme 4). In the cases of the hexoses D-



Scheme 4 *Reagents and conditions:* CAL, dioxane, 60 °C

glucopyranose **1**, D-galactopyranose **2** and D-mannopyranose **3**, *Candida antarctica* lipase (Novo SP435) gave selective esterification of the primary hydroxy group in moderate to good yields after 3 days. Despite the low polarity of the solvent, a wide variety of alkoxycarbonyl groups could be introduced. In the case of D-ribofuranose **13** and D- and L-arabinofuranose **12**, equally good results for selective C-5 alkoxycarbonylation could be achieved using different immobilized lipases from *Candida antarctica*, namely Novo SP382 and SP435 (also Novo SP435A for D-ribofuranose) in dioxane at 60 °C.

6-*O*-Monoesters of alkyl glucopyranosides have also been prepared using long chain fatty acids as the acylating agents[11,12] (Scheme 5). The reaction was carried out by simply



Scheme 5 *Reagents and conditions:* RCO_2H, CCL, 24 h, 70 °C

mixing the alkyl glucopyranoside (for example ethyl glucopyranoside **14**) with the melted fatty acid (C_8–C_{18}) at 70 °C in the presence of 5% w/w immobilized *Candida antarctica* lipase. The lipases from *Candida antarctica* are thermally stable enzymes and this convenient solvent-free process gave a series of glucoside esters, useful as nonionic surfactants and emulsifiers,[11] in 85–90% yield. Faster reactions were observed with the longer chain fatty acids (C_{12}–C_{18}) compared with the shorter chain fatty acids (C_8–C_{10}), consistent with the general preference of lipases for lipophilic substrates. The enzyme used in the reactions could be recycled several times without any noticeable loss of activity.

Considering the limited compatability between lipases and hydrophilic organic solvents, Klibanov and co-workers[13] turned their attention to the use of non-lipase hydrolases for the selective acylation of sugars. In seminal work the use of proteolytic enzymes was studied, specifically serine proteases, and it was found that *Bacillus subtilis* protease (subtilisin), a commercially available enzyme, was both stable and active in numerous anhydrous organic solvents, including pyridine and dimethylformamide. Thus, glucopyranose **1** was selectively acylated using 2,2,2-trichloroethyl butyrate in anhydrous dimethylformamide at 45 °C in a reaction catalysed by subtilisin, giving 6-*O*-butyrylglucose in 60% yield (Table 1).

The acylation reaction in DMF has been improved by the use of isopropenyl acetate to avoid the problem of reaction reversibility and product inhibition. Thus, in studies towards the synthesis of sialic acid, Protease N (subtilisin) has been used

Table 2 Enzyme-catalysed acylation of a secondary hydroxy group

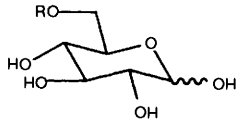
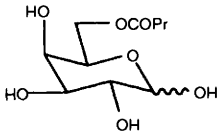
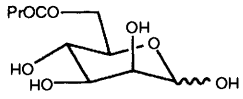
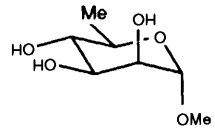
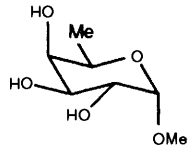
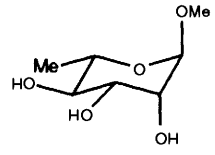
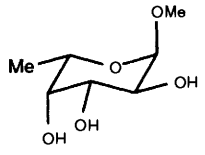
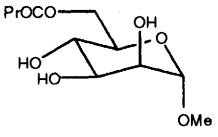
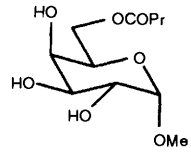
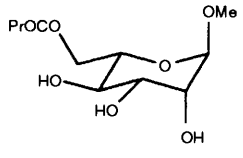
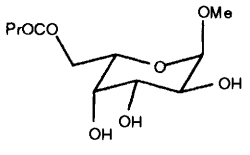
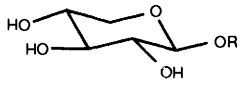
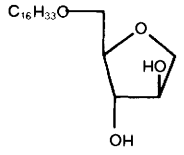
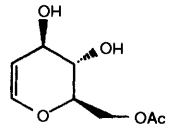
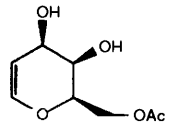
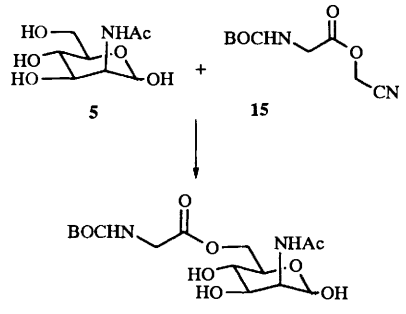
No.	Structure	Enzyme	Solvent	Acyl donor	Position	Yield (%)	Ref.
18a	 R = butyryl	ANL	THF	$PrCO_2CH_2CCl_3$	3		20
		CVL	THF	$PrCO_2CH_2CCl_3$	3	80	20
		PPL	THF	$PrCO_2CH_2CCl_3$	2	52	20
18b	R = trityl	CVL	THF	$PrCO_2CH_2CCl_3$	3	88	20
18c	R = Bu^tPh_2Si	CCL	CH_2Cl_2	$PrCO_2CH_2CCl_3$	2	45	20
19		CVL	THF	$PrCO_2CH_2CCl_3$	2	20	20
					3	31	
20		CVL	THF	$PrCO_2CH_2CCl_3$	2	13	20
					3	53	
27		PFL	THF	$PrCO_2CH_2CF_3$	2	40	22
28		PPL	THF–pyridine (4:1)	$PrCO_2CH_2CF_3$	2	78	22
		PFL	THF–pyridine (4:1)	$PrCO_2CH_2CF_3$	2	84	22
29		PPL	THF	$PrCO_2CH_2CF_3$	4	70	22
		PFL	THF	$PrCO_2CH_2CF_3$	4	60	22
30		PFL	THF–pyridine (4:1)	$PrCO_2CH_2CF_3$	4	44	22
31		CCL	CH_2Cl_2–acetone, 4:1	$PrCO_2CH_2CF_3$	2	21	23
					3	28	
					4	2	
32		PPL	THF–pyridine, 4:1	$PrCO_2CH_2CF_3$	2	85	23
		PFL	THF–pyridine, 4:1	$PrCO_2CH_2CF_3$	2	81	23
		CCL	CH_2Cl_2–acetone, 4:1	$PrCO_2CH_2CF_3$	2	80	23
33		PPL	THF	$PrCO_2CH_2CF_3$	4	65	23
		PFL	THF	$PrCO_2CH_2CF_3$	4	68	23
34		CCL	CH_2Cl_2–acetone, 4:1	$PrCO_2CH_2CF_3$	2	4	23
					3	38	
					4	10	

Table 2 (*continued*)

No.	Structure		Enzyme	Solvent	Acyl donor	Position	Yield (%)	Ref.
35a		R = Me	PSL	CH_3CN	$CH_3CO_2CH{=}CH_2$	3, 4	85	4
35b		R = octyl	PSL	Hexane	$CH_3CO_2CH{=}CH_2$	2	44	24
38			HLL RJL	Benzene Benzene	$PrCO_2CH_2CCl_3$ $PrCO_2CH_2CCl_3$	2 3	66 79	26 26
51			PFL	EtOAc	$ClCH_2CO_2CH{=}CH_2$	3	83	19
52			PFL	EtOAc	$ClCH_2CO_2CH{=}CH_2$	3	80	19

with isopropenyl acetate in DMF for the acylation of the primary hydroxy of *N*-acetyl-D-mannosamine **5**[14] (see Table 1).

The above examples[13,14] were the first reports of enzymatic reactions being carried out in DMF. However, even subtilisin is not particularly stable in this solvent and several DMF-stable subtilisin BPN′ variants have been developed, using site-directed mutagenesis to improve their stability.[15,16] The improved enzyme and acylation reaction have been used in the selective acylation of ManNAc **5**.[17] Using the engineered subtilisin variant 8399 with vinyl acetate as the acyl donor, the primary hydroxy group was acylated in 70% yield. Recently, Fitz and Wong have reported[18] a facile three-step chemo-enzymatic synthesis of fluorescent sialic acid. The first step involved transesterification of *N*-acetyl-D-mannosamine **5** with the cyanomethyl ester **15** as the acylating reagent (Scheme 6).



Scheme 6 *Reagents and conditions:* Subtilisin BNP′, 97% DMF, room temp., 3 days, 60%

The reaction was performed in 97% DMF and 3% aqueous Tris [tris(hydroxymethyl)aminomethane] buffer and was catalysed by subtilisin BNP′. Acylation occurred in 65% yield regioselectively at the primary hydroxy group of ManNAc **5** (see Table 1).

It has been demonstrated that selective acylation of the primary hydroxy group of glycals can also be achieved using lipases.[19] For example, D-glucal **16** was acylated using vinyl acetate catalysed by *Candida cylindracea* lipase in ethyl acetate to give, after 24 h, 6-*O*-acetyl-D-glucal in 90% yield. Similarly, D-galactal **17** gave, after 45 min, 6-*O*-acetyl-D-galactal in 93% yield (Table 1).

Monosaccharides – selective acylation of a secondary hydroxy group

Having established that lipases could be used to acylate selectively the primary hydroxy group of a number of unprotected monosaccharides, Therisod and Klibanov[20] then addressed the question of whether it was possible to acylate one of the secondary hydroxy groups of 6-*O*-acylated monosaccharides selectively; if so, would various lipases show different regioselectivity?

A number of 6-*O*-acylated glucopyranose **18**, galactopyranose **19** and mannopyranose **20** derivatives were found to be soluble in more organic solvents than their unmodified precursors. The significance of this finding was that whilst only porcine pancreatic lipase (PPL) showed any appreciable catalytic activity in pyridine, a range of commercially available lipases were known to be compatible with the solvents used to dissolve the monoacylated sugars, namely acetone, tetrahydrofuran and dichloromethane.[20]

The results (summarized in Table 2) show that by careful choice of lipase and solvent, it is possible to acylate selectively at the C-2 and C-3 positions of C-6 protected glucopyranose **18**, galactopyranose **19** and mannopyranose **20**. Initially, it was found that the 6-*O*-butyryl esters could be acylated with 2,2,2-trichloroethyl butyrate using several commercially available lipases, *e.g.* those from porcine pancreas, *Chromobacterium viscosum* (CVL), *Candida cylindracea* (CCL) and *Aspergillus niger* (ANL), employing tetrahydrofuran or dichloromethane as solvent. Further investigation, using 6-*O*-butyrylglucopyranoside **18a**, showed that lipases from *Aspergillus niger* and *Chromobacterium viscosum* exclusively acylated the C-3 hydroxy group. Conversely, porcine pancreatic lipase displayed a strong preference toward the C-2 position. The authors proposed that this distinct difference in regioselectivity was due to different modes of binding of the substrate within the enzymes' active centres.

In contrast, the 6-*O*-butyryl derivatives of galactopyranose **19** and mannopyranose **20**, showed only poor regioselectivities for all four lipases, with mixtures of the 2,6- and 3,6-dibutyryl esters being produced.

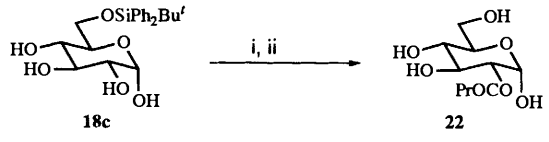
Klibanov then sought to exploit the positional specificity of the lipases with C-6 protected glucose to synthesise the C-2 or C-3 monoesters of glucose preparatively.[20] To that end, the C-6 hydroxy group of α-D-glucopyranose was chemically modified with triphenylmethyl chloride. The resultant 6-*O*-tritylglucopyranoside **18b** was a good substrate for *Chromobacterium viscosum* lipase and was quantitatively acylated in the C-3 position (Scheme 7). The resultant product was chemically

18b **21**

Scheme 7 *Reagents and conditions:* i, CVL, THF, $PrCO_2CH_2CCl_3$; ii, H^+, 88%

detritylated to yield pure 3-*O*-butyrylglucopyranoside **21** in 88% yield.

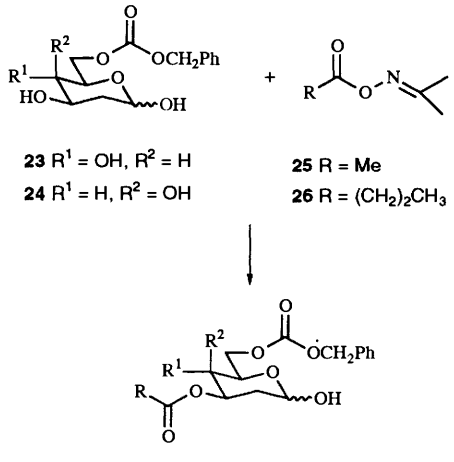
Similarly, the C-6 hydroxy group of α-D-glucopyranose was chemically modified with a *tert*-butyldiphenylsilyl group and the product **18c** was incubated with 2;2,2-trichloroethyl butyrate and *Candida cylindracea* lipase in dichloromethane. Acylation occurred exclusively at the C-2 position which, after chemical deprotection of the C-6 hydroxy group, yielded 2-*O*-butyrylglucopyranoside **22** (Scheme 8).



18c **22**

Scheme 8 *Reagents and conditions:* i, CCL, CH_2Cl_2, $PrCO_2CH_2CCl_3$, 75%; ii, Bu_4NF/AcOH, −50 °C, THF, 75%

As part of a study into how the absence of a hydroxy group at C-2 can affect the regioselectivity of lipases, Pulido and Gotor studied the enzyme-catalysed acylation of the 6-*O*-benzyloxycarbonyl derivatives of 2-deoxy-D-*arabino*-hexopyranose **23** and 2-deoxy-D-*lyxo*-hexopyranose **24**, with oxime esters.[21] The 6-*O*-benzyloxycarbonyl-2-deoxyhexoses **23** and **24**, were both selectively acylated at the 3-hydroxy position using either acetone oxime acetate **25** or acetone oxime butanoate **26** and *Pseudomonas cepacia* lipase in 1,4-dioxane at 60 °C with yields in the range 48–67% (Scheme 9).



Scheme 9 *Reagents and conditions:* PSL, 1,4-dioxane, 60 °C, 48–67%

Throughout the early work by Klibanov, no enzymatic acylation was observed at the C-4 position of the monosaccharides. Ronchetti and co-workers[22] suggested that this lack of enzymatic reactivity may be due to the presence of a polar acyloxymethyl group in the C-6 position and thus chose to study the four 6-deoxy sugars D- and L-rhamnopyranoside **27** and **28**, and D- and L-fucopyranoside **29** and **30**, with the aim of determining whether the usual enzymatic acylating system was able to esterify the previously unreactive 4-hydroxy group.

To overcome the solubility problems of 6-deoxyhexoses, the methyl α-pyranoside derivatives were used. Three commercially available crude enzyme preparations, porcine pancreatic lipase, *Candida cylindracea* lipase and *Pseudomonas fluorescens* lipase (PFL)‡ were used throughout the study. Tetrahydrofuran was used as the solvent of choice for PPL and PFL, in some cases employing pyridine as a co-solvent, while dichloromethane–acetone (4 : 1) was used for CCL-catalysed reactions. A number of acylating reagents were tested including 2,2,2-trichloroethyl butyrate, 2,2,2-trifluoroethyl butyrate and the enol esters vinyl acetate and isopropenyl acetate. Trifluoroethyl butyrate was the acylating reagent of choice since it was found to promote *relatively* fast reactions (4–12 days) with good regioselectivity.

The results are summarized in Table 2. The D-rhamnopyranoside **27** and D-fucopyranoside **28** gave the 2-butyrate esters as the main products, while the L-enantiomers **29** and **30** were preferentially acylated at the C-4 position.[22]

In order to determine the possible influence of the ester function at C-6 on the regiochemical outcome of the reaction, Ronchetti and co-workers[23] submitted the 6-*O*-butyryl derivatives of methyl α-D-mannopyranoside **31** and methyl α-D-galactopyranoside **32** plus their L enantiomers **33** and **34**, to enzymatic butyrylation using the lipase–trifluoroethyl butyrate system.

The results (summarized in Table 2) showed that the best substrate for all three lipases was methyl 6-*O*-butyryl-α-D-galactopyranoside **32** with acylation occurring at the C-2 position within 2–7 days. The efficiency and regioselectivity of this substrate closely resembled the results found for the 6-deoxy analogue, D-fucopyranoside **28**. The L-mannopyranoside **33** required longer reaction times (12 days) and furnished slighty lower yields, thus giving results comparable to the 6-deoxy analogue, L-rhamnopyranoside **29**, for which acylation occurred at the less reactive C-4 hydroxy group. In contrast, the D-mannopyranoside **31** and L-galactopyranoside **34** were found to be poor substrates, with low conversion rates and mixtures of products being observed. These reactivities are comparable to the 6-deoxy analogues, D-rhamnopyranoside **27** and L-fucopyranoside **30**.

Both the nature of the organic solvent and the aglycon moiety of the sugar can influence the regioselectivity of lipase-catalysed acylations of secondary hydroxy groups.[24] Methyl and octyl β-D-xylopyranoside **35a** and **35b** were studied in the lipase PS-catalysed acylation with vinyl acetate in acetonitrile. The results (Table 2) show that methyl β-D-xylopyranoside **35a** was acylated with vinyl acetate in acetonitrile to give the 3,4-diacetate exclusively in 85% yield. In the case of octyl β-D-xylopyranoside **35b**, a mixture of the 2,4- and 3,4-diacetates was obtained in almost equal quantities with acetonitrile as solvent; however, in hexane the 2,4-diacetate predominated. If the reaction in hexane was terminated after 3 h, the C-2 monoacetate of **35b** could be isolated in 44% yield.

A further example of selective acylation at the C-4 hydroxy group is recorded by Rabiller and co-workers.[25] Using the conformationally rigid anhydro sugar, 1,6-anhydro-β-D-gluco-

‡ *Pseudomonas fluorescens* lipase has now been renamed as *Pseudomonas cepacia*.

pyranose **36** (glucosan), Rabiller studied the stereoselectivity as a function of the axial/equatorial situation of the hydroxy group. Using vinyl acetate as both the acyl donor and solvent, five common lipases were screened for the transesterification reaction; namely *Candida cylindracea* lipase (CCL), *Mucor miehei* lipase (MML), porcine pancreatic lipase (PPL), *Pseudomonas fluorescens* lipase (PFL) and *Rhizopus arrhizus* lipase (RAL). The results showed that while the best conversions (99%) were obtained with CCL and PFL, good regioselectivity and yield were only achieved with PFL, furnishing the 4-monoacetate **37** in 61% yield after 7 days (Scheme 10). A similar result was obtained by Pulido and Gotor

36 **37**

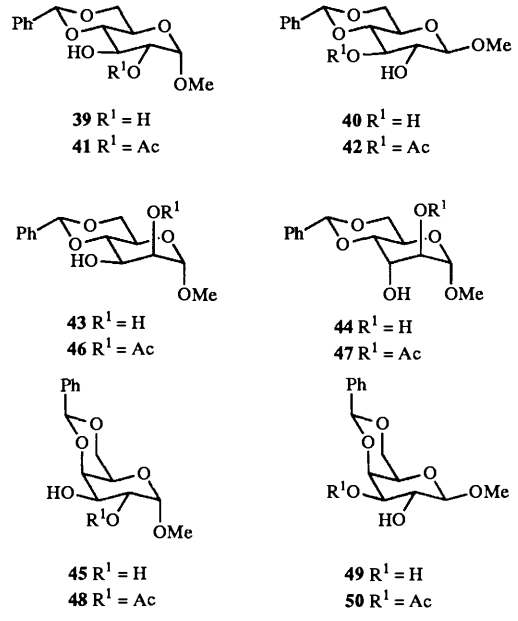
Scheme 10 *Reagents and conditions:* $MeCO_2CH{=}CH_2$, PFL, 61%, 7 days[25] or **25**, CAL, pyridine, 70%, 48 h[21]

using oxime esters as the acylating agent.[21] The best results were obtained when 1,6-anhydro-β-D-glucopyranose **36** was treated with either acetone oxime acetate **25** or acetone oxime butanoate **26** and *Candida antarctica* lipase in pyridine at room temperature, giving yields of 70 and 72%, respectively. At higher temperatures, the selectivity decreased and complex mixtures of all possible monoacylated derivatives of **36** were obtained. Experiments carried out at 30 or 60 °C in pyridine or 1,4-dioxane with *Pseudomonas cepacia* lipase as catalyst, also led to a mixture of monoacylated products.

In an attempt to differentiate between the two secondary hydroxy functions of 1,4-anhydro-5-*O*-hexadecyl-D-arabinitol **38**, Riva and co-workers[26] screened twenty different lipases using 2,2,2-trichloroethyl butyrate as the acyl donor (Table 2). Eight lipases showed an appreciable degree of conversion within 24 h and of these, six showed a good degree of regioselectivity. Four of these six enzymes were the most commonly used lipases, PFL, PPL, *Mucor miehei* lipase and CCL. PFL, PPL and *Mucor miehei* lipase showed a preference for acylation at the C-2 hydroxy group while CCL predominately acylated at the C-3 hydroxy group. However, the best results in terms of both regioselectivity and yield were obtained with *Humicula lanuginosa* lipase (HLL) and *Rhizopus japonicus* lipase (RJL). These two lipases showed complementary regioselectivity, thus, the arabinitol derivative **38** was acylated at the C-2 position using *Humicula lanuginosa* lipase giving the 2-*O*-butyryl derivative in 66% yield, while **38** was acylated at the C-3 using *Rhizopus japonicus* lipase giving the 3-*O*-butyryl derivative in 79% yield.

Protected carbohydrates, such as benzylidenes, of the *trans*-decalin type, can be regioselectively acylated using *Pseudomonas fluorescens* lipase or lipase PS (Scheme 11). In the case of methyl 4,6-*O*-benzylidene-α- and β-D-glucopyranoside **39** and **40** the regioselectivity of the lipase-catalysed acylation is dependent upon the configuration of the anomeric carbon atom.[27] Thus, using PFL and vinyl acetate, the α-anomer **39** furnished, after 3 days, the C-2 monoester **41** in 94% yield, while the β-anomer **40** gave, after 1 day, the C-3 monoester **42** in 80% yield.

Further work in this area[28] confirmed that the position of acetylation was dictated by the configuration of the anomeric centre. Using the conditions described above, methyl 4,6-*O*-benzylidene-α-D-mannopyranoside **43**, methyl 4,6-*O*-benzylidene-α-D-altropyranoside **44** and methyl 4,6-*O*-benzylidene-α-D-galactopyranoside **45**, possessing an α-configuration at the anomeric centre, were acetylated to give the C-2 monoesters



39 R^1 = H
41 R^1 = Ac

40 R^1 = H
42 R^1 = Ac

43 R^1 = H
46 R^1 = Ac

44 R^1 = H
47 R^1 = Ac

45 R^1 = H
48 R^1 = Ac

49 R^1 = H
50 R^1 = Ac

Scheme 11

46–**48**, either predominately or exclusively. Conversely, methyl 4,6-*O*-benzylidene-α-D-galactopyranoside **49**, possessing a β-configuration at the anomeric centre, gave exclusively the C-3 monoester **50**.

While the carbohydrate derivatives **39**, **40**, **43** and **44**, were found to be good substrates for the PFL-catalysed esterification giving yields of 81–94%, the α- and β-D-galactopyranosides **45** and **49** were poor substrates, each giving only a 19% yield of monoesters after 10 days. The authors proposed that the lack of reactivity of these compounds was probably due to steric factors preventing access of the sugar derivatives to the active site of the enzyme.

In a similar study, Panza and co-workers[29,30] observed the same regioselective discrimination between the α- and β-anomers of a number of 4,6-*O*-benzylidine glycosides. The reaction conditions involved using lipase PS (from *Pseudomonas cepacia*—adsorbed on Celite to improve reproducibility) in THF. A range of acylating agents were tested; the trifluoroethyl esters of chloroacetate, benzoate, pivaloate and levulinate and the vinyl esters of chloroacetate, benzoate and pivaloate. Satisfactory results were obtained with all these substrates except the benzoates and levulinates. Substrates included methyl and allyl 4,6-*O*-benzylidene-α- and β-glucopyranosides, and the corresponding derivatives of galactopyranose and mannopyranose. In agreement with the earlier results,[27,28] benzylidine glucopyranosides and benzylidine mannopyranosides were found to be much more reactive than benzylidine galactopyranosides. In general, 4,6-*O*-benzylidene-β-glycopyranosides were acylated faster, preferentially at the C-3 hydroxy group, with reaction times of 1 h and yields of 94–98%, while the corresponding α-anomers were acylated more slowly and preferentially at the C-2 hydroxy group, with average reaction times of 7 h and yields of 97–100%.

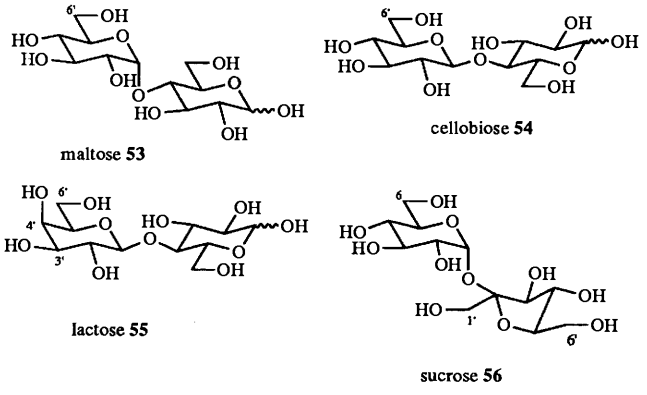
In the same way that monosaccharides possessing a masked primary hydroxy function can undergo selective acylation of the secondary hydroxy functionality, glycals have also been shown to exhibit regio-discrimination.[19] Thus, regioselective chloroacetylation of 6-*O*-acetyl-D-glucal **51** and 6-*O*-acetyl-D-galactal **52** was achieved using PFL giving the corresponding 3,6-di-*O*-acetyl derivatives in high yields (Table 2).

Di- and oligosaccharides

Compared to monosaccharides, relatively few studies have

appeared in the literature concerning the selective acylation of di- and oligo-saccharides.

As previously discussed, porcine pancreatic lipase in pyridine is virtually unreactive with di- and oligo-saccharides, thus prompting the use of the proteolytic enzyme, subtilisin. Using a method employing subtilisin in anhydrous DMF with 2,2,2-trichloroethyl butyrate as the acylating agent, it was found that the disaccharides maltose **53**, cellobiose **54**, lactose **55** and sucrose **56** all reacted readily.[13] Carrying out the reactions at 45 °C led to good substrate conversions in 2–7 days. Furthermore, the efficiency of the subtilisin-catalysed acylations for all four disaccharides was sufficient to prepare the corresponding monoesters in gram quantities with isolated yields of around 50% (Scheme 12).



Scheme 12

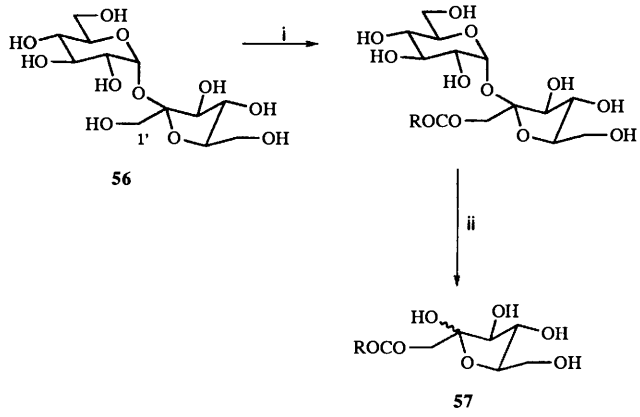
In the case of maltose **53**, acylation occurred exclusively at the C-6′ position. While chemical acylation of maltose shows the C-6′ hydroxy to be the most reactive, the regioselectivity is much poorer than that observed for the subtilisin-catalysed transesterification.[13]

In the case of cellobiose **54** (which differs from maltose **53** only in the transition from α- to β-glycosidic linkage) acylation occurred exclusively at the C-6′ hydroxy group. However, in the case of lactose **55**, the enzyme was less discriminating. Acylation occurred predominately at the C-6′ hydroxy group (74%) while reaction was also observed at the C-3′ (10%) and C-4′ hydroxy groups (10%). Unexpectedly, in the case of sucrose **56**, which has three primary hydroxy groups, subtilisin catalysed the acylation exclusively at the C-1′ hydroxy group of the fructose moiety. This is in direct contrast to chemical acylation[31] where the most reactive hydroxy group is at C-6 followed by the C-6′ hydroxy group.

A number of oligosaccharides, maltotriose to maltoheptose, were also found to be selectively acylated at the C-6 hydroxy group of the terminal glucose moiety, indicating that the enzyme is quite tolerant to the glucose chain length.

Considering the expense of highly purified subtilisin, Klibanov and co-workers[13] showed that the acylation of sucrose **56** could be carried out on a multigram scale using a crude, inexpensive preparation of subtilisin furnishing the 6′-*O*-acylated derivative in 61% yield after 6 days.

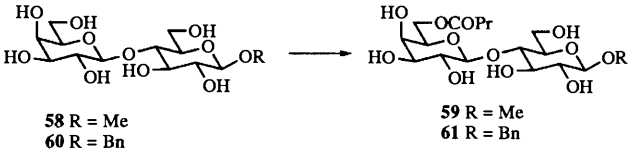
Using a similar crude preparation of subtilisin, namely protease N (PN), Riva and co-workers[32] sought to prepare a series of 1′-*O*-sucrose esters bearing acyl groups of different sizes and types. These sucrose derivatives could be hydrolysed by yeast α-glucosidase to the corresponding 1-*O*-fructose esters, which are not easily attainable by chemical methods (Scheme 13). Thus, sucrose **56** was selectively acylated at the C-1′ position using a variety of aromatic and aliphatic trifluoroethyl esters, ranging from butyl to octyl. The yields of the isolated sucrose monoesters were found to decrease dramatically with



Scheme 13 *Reagents and conditions:* i, $RCO_2CH_2CF_3$, Protease N; ii, α-glycosidase

lengthening of the acyl group. Hydrolysis using α-glucosidase gave rise to 1-*O*-acylfructoses **57**. Interestingly, direct enzymatic esterification of fructose using protease N and trichloroethyl butyrate in DMF was not selective, giving rise to a mixture of 1-*O*- and 6-*O*-butyrylfructose in a ratio of 8:2.

Using a similar approach, it has been shown[33] that it is possible to acylate the primary hydroxy group of the non-reducing sugar unit of methyl β-lactopyranoside **58** selectivity (Scheme 14). Incubation of methyl β-lactopyranoside **58** with



Scheme 14 *Reagents and conditions:* $PrCO_2CH_2CCl_3$, Subtilisin/DMF, 37 °C

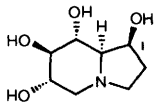
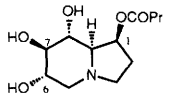
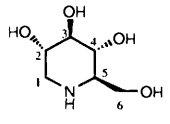
2,2,2-trichloroethyl butyrate and protease N in anhydrous DMF gave, after 5 days at 37 °C, the desired 6′-*O*-butyryllactopyranoside **59** in 73% yield. Subtilisin also catalysed monobutyration of benzyl β-lactopyranoside **60** with excellent regioselectivity, although the reaction was slow, giving rise to the 6′-*O*-butyryl derivative **61** in 71% yield after 14 days.

Aza-sugars

The enzyme-catalysed regioselective acylation of aza-sugars has, to date, attracted little attention in the literature. The most notable work involves the naturally occurring aza-sugars castanospermine **62** and 1-deoxynojirimycin **63**.[34,35] These plant alkaloids act as α-glucosidase-I inhibitors and may be of clinical value as antineoplastic agents and in the treatment of AIDS. Several *O*-acyl derivatives of castanospermine are as much as twenty times more active than castanospermine itself in inhibiting human immunodeficiency virus replication. The use of enzymatic methods, with their unparalled specificity, are an attractive alternative to the classical techniques of organic chemistry for the synthesis of these analogues. Thus, the regioselective acylation of castanospermine **62** and 1-deoxynojirimycin **63** has been accomplished using subtilisin in pyridine (see Table 3).

Castanospermine **62**, possessing four secondary hydroxy groups, is preferentially acylated at the C-1 position. Subtilisin was found to catalyse the synthesis of a wide variety of C-1 substituted castanospermine analogues incorporating a number of acylating groups such as acetyl, butyl, octanoyl or phenylacetyl or even D- and L-α-amino acyl units. In all cases, only 1-*O*-acylcastanospermine was observed. For example, castanospermine **62** was treated with vinyl acetate in pyridine at

Table 3 Enzyme-catalysed acylation of aza-sugars

No.	Structure	Enzyme	Solvent	Acyl donor	Position	Yield	Ref.
62		Subtilisin	Pyridine	$CH_3CO_2CH{=}CH_2$	1	91	34
64		CVL	THF	$PrCO_2CH_2CCl_3$	7	72	34
63		Subtilisin	Pyridine	$PrCO_2CH_2CCl_3$ (1.5 equiv.)	6	56	35
		Subtilisin	Pyridine	$PrCO_2CH_2CCl_3$ (6 equiv.)	2, 6	77	35

45 °C for 84 h, furnishing 1-*O*-acylcastanospermine in 91% yield (Table 3). Additionally, a number of 1-*O*-acyl derivatives of castanospermine, which are readily soluble in THF, were treated with 2,2,2-trichloroethyl butyrate in the presence of an enzyme, either PPL, CVL or subtilisin. The most favourable results were achieved using 1-*O*-butyrylcastanospermine **64** and lipase from *Chromobacterium viscosum*, which gave the 1,7-di-*O*-ester in 72% yield, with only a small amount (7%) of the 1,6-di-*O*-ester being produced.

1-Deoxynojirimycin **63** has one primary and three secondary hydroxy groups plus a potentially reactive amino function. When a small excess of the acylating agent, 2,2,2-trichloroethyl butyrate was used, the 6-*O*-ester was formed in 56% yield. However, use of a large excess of the acylating reagent resulted in the 2,6-di-*O*-ester in 77% yield. No enzymatic acylation of the amino group was observed.

Deacylation reactions

Monosaccharides – selective deacylation of the primary hydroxy group

Since all the hydroxy groups of carbohydrates can be readily acylated by chemical methods (for example, the treatment of glucose with acetic anhydride results in the production of glucose pentaacetate[36]) then the regioselective enzymatic deacylation should provide access to sugars with varying degrees of acylation.

In an early study, Sweers and Wong[37] reported that, for several tetra-acylated monosaccharides in which the hydroxy function at the anomeric carbon was protected, selective deacylation of the primary hydroxy site at C-6 was observed (see Table 4). For example, methyl 2,3,4,6-tetra-*O*-pentanoyl-β-D-glucopyranoside **65** was incubated with *Candida cylindracea* lipase in an acetone–0.1 mol dm^{-3} phosphate buffer solution (1 : 10) at room temperature furnishing the 6-hydroxy product in 90% yield after 3 days. Using these reaction conditions, a number of different acyl pyranoses (**65–69**) were tested.[8] Although the octanoyl derivatives were found to be the best substrates, for various reasons the pentanoyl derivatives were found to be the best compounds with which to work on a large scale. While good yields were reported for the methyl α- and β-D-glucopyranosides **65** and **66**, CCL was found to be less discriminating in the cases of methyl α-D-galacto- and manno-pyranosides **67** and **68** and the methyl 2-acetamido-2-deoxy-D-mannopyranoside **69**, where the major by-products (approx. 20%) were found to be the corresponding 4,6-dihydroxy derivatives.

Similarly, for a series of methyl furanosides, selective deprotection of the primary hydroxy site at C-5, was possible in most cases. Five enzymes were examined for use in these hydrolysis reactions: *Rhizopus japonicus* lipase, *Mucor* sp. lipase, crude porcine pancreatic lipase, *Aspergillus niger* lipase and *Candida cylindracea* lipase. The *Mucor* sp. lipase was inactive with the substrates under the reaction conditions and *Rhizopus japonicus* lipase reacted only slowly and was not selective. Crude porcine pancreatic lipase, *Aspergillus niger* lipase and *Candida cylindracea* lipase all accepted the substrates but only CCL was found to react quickly with a high degree of regioselectivity. The hydrolysis reactions were conducted by dissolving the peracylated substrates in DMF, diluting with 0.1 mol dm^{-3} phosphate buffer then adding the enzyme and stirring at 37 °C. In this way homogeneous reaction conditions were obtained.

The results, summarized in Table 4, show that in the cases of methyl α- and β-D-ribofuranoside **70** and **71**, methyl α-D-arabinofuranoside **72**, methyl α-D-xylofuranoside **73** and methyl 2-deoxy-β-D-ribofuranoside **74**, deprotection occurred exclusively at the primary hydroxy site to give the corresponding 5-hydroxy products in high yields. Cleavage of the C-3 secondary hydroxy group was observed for methyl β-D-xylofuranoside **75** while in the case of methyl 3,5-di-*O*-acetyl-2-deoxy-α-D-ribofuranoside **76** the deprotection was not selective giving rise to a mixture of the 5-hydroxy and 3-hydroxy derivatives in the ratio 4 : 5, respectively.

Monosaccharides – selective deacylation of a secondary hydroxy group

The enzymatic hydrolysis of β-D-glucose pentaacetate **77** has been investigated using a number of lipases and esterases (see Table 5).[38] The substrate was hydrolysed by four enzymes namely lipases from *Aspergillus niger*, *Rhizopus oryzae* and wheat germ, and porcine liver carboxyl esterase. The most favourable results were obtained with *Aspergillus niger* lipase in 0.5 mol dm^{-3} phosphate buffer at 30 °C. By terminating the reaction at certain time intervals, it was possible to obtain varying degrees of the tetra-, tri- and di-acetates. For example, after 30 min, glucose tetraacetate constituted 74% of the total material (33% isolated yield), the two major contaminants being glucose penta- and tri-acetate. NMR evidence showed that the initial deacylation had occurred at C-1, the anomeric carbon atom. With increased reaction times, greater amounts of glucose tri- and di-acetate were observed; at all times mixtures of products were observed.

More favourable results are summarized in Table 5.[8] All the sugar acetates (**78–84**) were hydrolysed by porcine pancreatic lipase in 10% DMF–phosphate buffer, to give 1-hydroxy

Table 4 Enzyme-catalysed deacylation of the primary hydroxy group

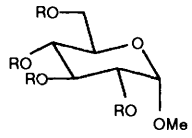
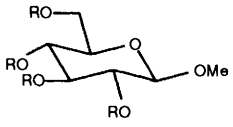
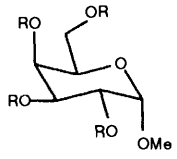
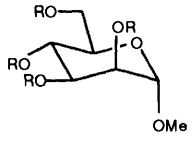
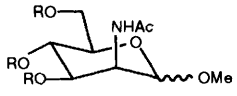
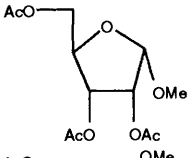
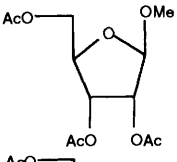
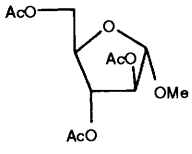
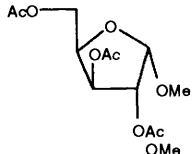
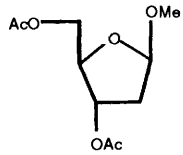
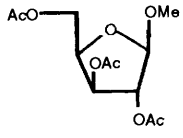
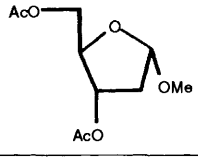
No.	Structure	R group	Enzyme	Solvent	Position	Yield (%)	Ref.
65		octanoyl	CCL	0.1 mol dm^{-3} phosphate buffer	6-OH	78	8
		pentanoyl	CCL	0.1 mol dm^{-3} phosphate buffer	6-OH	75	8
66		octanoyl	CCL	0.1 mol dm^{-3} phosphate buffer	6-OH	77	8
		pentanoyl	CCL	acetone–0.1 mol dm^{-3} phosphate buffer (1 : 10)	6-OH	90	37
67		pentanoyl	CCL	0.1 mol dm^{-3} phosphate buffer	6-OH	29	8
68		pentanoyl	CCL	0.1 mol dm^{-3} phosphate buffer	6-OH	33	8
69		pentanoyl	CCL	0.1 mol dm^{-3} phosphate buffer	6-OH	50	8
70			CCL	DMF–0.1 mol dm^{-3} phosphate buffer (1 : 10)	5-OH	96	8
71			CCL	DMF–0.1 mol dm^{-3} phosphate buffer (1 : 10)	5-OH	85	8
72			CCL	DMF–0.1 mol dm^{-3} phosphate buffer (1 : 10)	5-OH	98	8
73			CCL	DMF–0.1 mol dm^{-3} phosphate buffer (1 : 10)	5-OH	98	8
74			CCL	DMF–0.1 mol dm^{-3} phosphate buffer (1 : 10)	5-OH	63	8
75			CCL	DMF–0.1 mol dm^{-3} phosphate buffer (1 : 10)	3-OH	60	8
76			CCL	DMF–0.1 mol dm^{-3} phosphate buffer (1 : 10)	5-OH 3-OH	40 50	8

Table 5 Enzyme-catalysed deacylation of a secondary hydroxy group

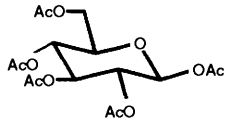
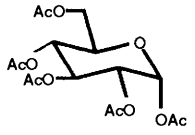
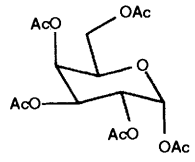
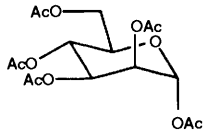
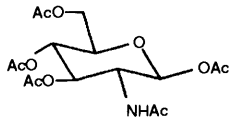
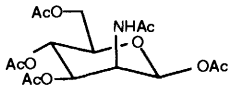
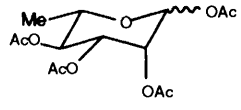
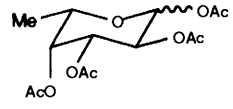
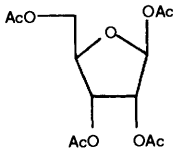
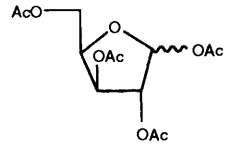
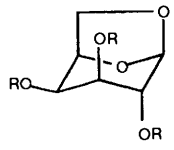
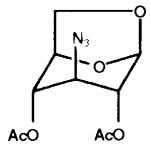
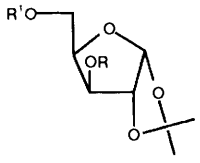
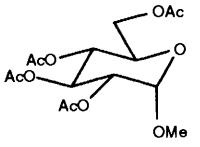
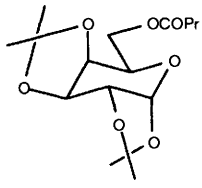
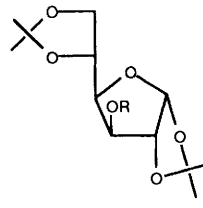
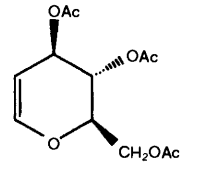
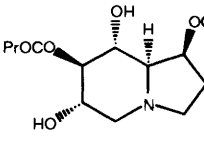
No.	Structure	R group	Enzyme	Solvent	Position	Yield (%)	Ref.
77			PPL	DMF–0.1 mol dm^{-3} phosphate buffer (1 : 10)	1-OH		8
			ANL	0.5 mol dm^{-3} phosphate buffer	1-OH	33	38
78			CCL	DMF–0.1 mol dm^{-3} phosphate buffer (1 : 10)	4,6-OH		8
			PPL	DMF–0.1 mol dm^{-3} phosphate buffer (1 : 10)	1-OH		8
79			PPL	DMF–0.1 mol dm^{-3} phosphate buffer (1 : 10)	1-OH	75	8
80			PPL	DMF–0.1 mol dm^{-3} phosphate buffer (1 : 10)	1-OH	95	8
81			PPL	DMF–0.1 mol dm^{-3} phosphate buffer (1 : 10)	1-OH	88	8
82			PPL	DMF–0.1 mol dm^{-3} phosphate buffer (1 : 10)	1-OH	96	8
83			PPL	DMF–0.1 mol dm^{-3} phosphate buffer (1 : 10)	1-OH	54	8
84			PPL	DMF–0.1 mol dm^{-3} phosphate buffer (1 : 10)	1-OH	71	8
85			ANL	DMF–0.1 mol dm^{-3} phosphate buffer (1 : 10)	1-OH	63	8
86			ANL	DMF–0.1 mol dm^{-3} phosphate buffer (1 : 10)	1-OH	50	8
93		butyryl	CCL (immob.)		2-OH	90	40
			PPL		2,4-OH	65	40
96			CCL	0.1 mol dm^{-3} phosphate buffer	4-OH	85–90	41
			alcalase	0.1 mol dm^{-3} phosphate buffer	2-OH	82	41

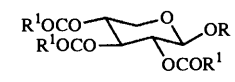
Table 5 (*continued*)

No.	Structure	R group	Enzyme	Solvent	Position	Yield (%)	Ref.
97		R = butyryl R^1 = pivaloyl	CCL or PPL		3-OH		42
98		R = acetyl R^1 = butyryl	CCL	0.1 mol dm^{-3} phosphate buffer	6-OH		42
99			CCL	0.1 mol dm^{-3} phosphate buffer	6-OH		42
100			PPL	0.1 mol dm^{-3} phosphate buffer	6-OH		42
101		R = acetyl	PPL	0.1 mol dm^{-3} phosphate buffer	3-OH		42
102		R = butyryl	CCL or PPL	0.1 mol dm^{-3} phosphate buffer	3-OH		42
103			PPL	0.01 mol dm^{-3} phosphate buffer	3-OH	90	19
104			Subtilisin PLE	0.01 mol dm^{-3} phosphate buffer 0.01 mol dm^{-3} phosphate buffer	1-OH 7-OH	64 75	34 34

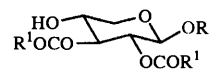
sugars. A difference in selectivity was observed in the reaction of glucose pentaacetate **78** with CCL, which showed deacylation at both the C-4 and C-6 positions.

The two furanose tetraacetates **85** and **86**, showed complete regioselectivity for hydrolysis at the C-1 position using *Aspergillus niger* lipase (Table 5). López and co-workers.[39] have recently shown that Lipase PS-catalysed regioselective deacylation of tri-*O*-acyl-β-D-xylopyranosides bearing a methyl or octyl as aglycon, can occur in organic solvents. This is in contrast to the usual hydrolytic conditions which employ either buffer or a biphasic system. In this study, six different solvents with varying degrees of hydrophobicity were used and the authors found that deacylation of the C-4 position was observed, regardless of the solvent used. The highest rates were found with *tert*-pentyl alcohol, 2-methylheptane and pentan-1-ol, after 4 days incubation. For example, methyl 2,3,4-tri-*O*-acetyl-β-D-xylopyranoside **87** in *tert*-pentyl alcohol furnished exclusively methyl 2,3-di-*O*-acetyl-β-D-xylopyranoside **88** in 93% yield. The octyl derivatives **89** and **90** also gave the desired 2,3-di-*O*-acyl-β-D-xylopyranosides **91** and **92** in 42 and 45% yields, respectively (Scheme 15). From a synthetic point of view, this high regioselectivity has practical advantages, such that oligosaccharides can be synthesized using these partially protected xylose derivatives.

Selective deacylation at the 2- and 4-positions has been demonstrated for the 1,6-anhydro-β-D-galactopyranose derivative **93**.[40] In the case of the 2,3,4-tri-*O*-butyryl derivative, good selectivity was observed using CCL immobilized on agarose. Selective cleavage of the C-2 ester gave rise to the 3,4-di-*O*-butyryl derivative in 90% yield, the reaction practically stopping after 29 h. The only by-product observed was a small amount of the 3-*O*-butyryl derivative (8%) resulting from cleavage of the C-4 ester. A similar result was obtained using lipase from pig pancreas, *i.e.* a 90% yield of the 3,4-di-*O*-butyryl derivative was obtained after 24 h, but in this case the reaction proceeded further to cleave the C-4 ester group giving, after 52



87 R = CH_3, R^1 = CH_3
89 R = $CH_3(CH_2)_7$, R^1 = CH_3
90 R = $CH_3(CH_2)_7$, R^1 = $CH_3(CH_2)_4$

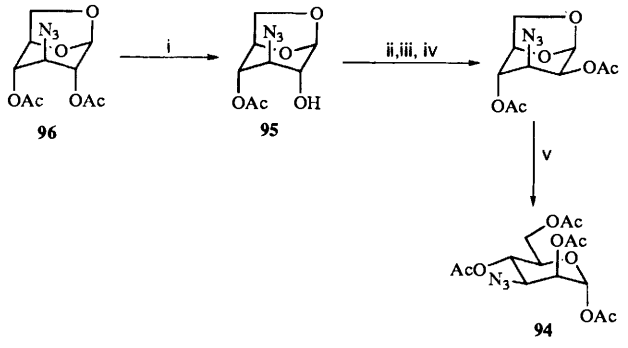


88 R = CH_3, R^1 = CH_3
91 R = $CH_3(CH_2)_7$, R^1 = CH_3
92 R = $CH_3(CH_2)_7$, R^1 = $CH_3(CH_2)_4$

Scheme 15 *Reagents and conditions:* Lipase PS, pentanol or *tert*-pentyl alcohol

h, the 3-*O*-butyryl derivative in 65% yield. Hydrolysis of the 2,3,4-tri-*O*-acetyl derivative under the same reaction conditions resulted in low yields.

Selective enzymatic deacylation has been employed in the chemo-enzymatic preparation of 1,2,4,6-tetra-*O*-acetyl-3-azido-3-deoxy-α-D-mannopyranoside **94**[41] (Scheme 16). The



Scheme 16 *Reagents and conditions:* i, Alcalase; ii, Tf_2O, py; iii, NaOAc, DMF; iv, Ac_2O, py; v, Ac_2O, $HClO_4$

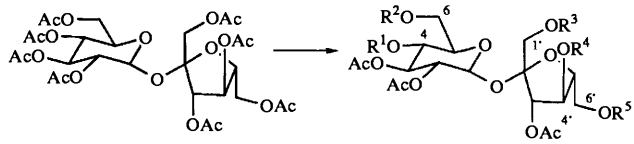
strategy involved an inversion at C-2 of a 3-azido-3-deoxy-β-D-glucopyranoside **95** and, subsequently, reaction to give 1,6-anhydro-2,4-di-*O*-acetyl-3-azido-3-deoxy-β-D-glucopyranose **96**, allowing selective manipulation of the C-2 and C-4 functionalities. Lipase from *Candida cylindracea* resulted in hydrolysis of the anhydro sugar **96** at the C-4 position to give the C-2 monoacetate in 85–90% yield (Table 5). Conversely, hydrolysis at the C-2 position was achieved using alcalase, giving the C-4 monoacetate in 82% yield. With the ability to differentiate between the C-2 and C-4 hydroxy groups using enzymatic methodology, it was then possible, by subsequent chemical steps, to manipulate the anhydro sugar to provide 1,2,4,6-tetra-*O*-acetyl-3-azido-3-deoxy-D-mannopyranoside **94**.

For a number of furanoses and pyranoses, the chain length and steric bulk of various protecting groups can influence the regioselectivity of deacylation.[42] Hence, using either CCL or PPL it was shown that deprotection of a secondary hydroxy group was possible in the presence of a primary hydroxy protected as the pivaloyl ester **97** (Table 5). When the primary hydroxy group was protected as the less bulky butyryl derivative **98**, deprotection of the C-6 primary hydroxy occurred as expected. Other results reported include the deprotection of the primary hydroxy group of methyl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside **99** using CCL and the diacetonide **100** with PPL. Protection of the primary hydroxy and anomeric carbon as the diisopropylidene derivatives **101** and **102**, allowed deprotection of the secondary hydroxy group at C-3.

Deacylation of tri-*O*-acetyl-D-glucal[19] **103** was carried out using PFL in a weakly buffered solution, resulting in cleavage of the secondary hydroxy group at C-3, giving 4,6-di-*O*-acetyl-D-glucal in 90% yield (see Table 5). Complementary regioselectivities were observed in the hydrolysis of 1,7-di-*O*-butyrylcastanospermine **104** with porcine liver esterase (PLE) and subtilisin.[34] PLE preferentially cleaved the butyryl group from the C-7 position [with regioselectivity (C-1 : C-7) > 1 : 25] giving rise to 1-*O*-butyrylcastanospermine in 75% yield (see Table 5). Conversely, subtilisin hydrolysed the ester group at the C-1 position with regioselectivity > 25 : 1, giving rise to 7-*O*-castanospermine in 64% yield. The significance of these results is that it allows the preparation of 7-*O*-acyl analogues of castanospermine in three enzymatic steps from castanospermine using subtilisin in the final deprotection step.

Disaccharides

Palmer and Terradas[43] studied the hydrolysis of sucrose octaacetate **105** with a number of lipases and proteases in organic solvent containing small amounts of water. The aim of the study was to prepare 2,3,4,3′,4′-pentaacetylsucrose **106** and 2,3,6,3′,4′-pentaacetylsucrose **107** which are both intermediates in the synthesis of high intensity, non-nutritive sweeteners. The synthesis of pentaacetate **106** requires deprotection of all three primary hydroxy sites, (C-6, C-1′ and C-6′) while the preparation of **107** requires deprotection of the two primary hydroxy sites of the fructose moiety (C-1′ and C-6′) plus the C-4 secondary hydroxy group of the glucose ring (Scheme 17).



105

106 R^1 = R^4 = Ac; R^2 = R^3 = R^5 = H
107 R^2 = R^4 = Ac; R^1 = R^3 = R^5 = H
108 R^1 = R^2 = R^3 = R^5 = Ac; R^4 = H
109 R^1 = R^2 = R^4 = Ac; R^3 = R^5 = H

Scheme 17 *Reagents and conditions:* Protease, diisopropyl ether

Sucrose octaacetate **105** was treated with a number of lipases in diisopropyl ether. Lipases from *Candida cylindracea*, porcine pancreas, *Candida antarctica* and *Aspergillus niger* yielded either mixtures of products or, in the case of PPL, deacylation of the 4′-position to give the sucrose heptaacetate **108**. Since none of the lipases exhibited the selectivity required for the synthesis of **106** and **107**, attention was turned to the use of proteases.

Virtually no regioselectivity was observed using the serine protease N from *Bacillus subtilis*. In contrast, both proleather, a serine protease from *Bacillus subtilis* and alcalase, a form of subtilisin Carlsberg from *Bacillus licheniformis*, predominately deacylated the C-1′ position. Prolonged treatment of the octaacetate with either proleather or alcalase (4 to 5 days), resulted in a mixture of products. The mixture comprised of two heptaacetates, resulting from deprotection of either the C-1′ or C-6′ positions, the hexaacetate **109**, resulting from deprotection of *both* the C-1′ and C-6′ positions, plus two pentaacetates. Isolation and characterization of these two pentaacetates confirmed them to be the desired products **106** and **107**. However, silica gel chromatography catalysed the rearrangement of the acetate at the C-4 position of the glucose moiety to the C-6 position resulting in the isolation of only one pentaacetate **107**. Alternatively, the pentaacetate **107** was also

synthesised by isolation of the hexaacetate **109** from this reaction mixture and subsequent treatment with lipase AP 12. This resulted in the same pentaacetate mixture, **106** and **107**, which, after chromatography, gave only the pentaacetate **107**.

Treatment of sucrose octaacetate with CCL resulted in selective cleavage of the 6-*O*-acetyl group of the glucose moiety while alcalase or protease N was selective for the 1-*O*-acetyl group of the fructose moiety.[44] This latter result agrees with the findings of Palmer and Terradas.[43]

Circumspection

In the acylation of monosaccharides, disaccharides, oligosaccharides and aza-sugars, the primary hydroxy group shows the greatest reactivity and can be selectively acylated with good regioselectivity and in high yields. When the primary hydroxy group is protected or absent, selective acylation of the C-2, C-3 or C-4 hydroxy group can be achieved with varying success depending on the choice of enzyme.

In the deacylation of monosaccharides, an ester group at the anomeric carbon centre shows the greatest reactivity and can be deacylated with good regioselectivity and high yields. If the hydroxy group of the anomeric carbon atom is protected, usually as the methyl ether, then selective deacylation of the primary hydroxy group can be achieved in high yields. Selective deacylation of the C-2, C-3 or C-4 hydroxy groups requires protection of the hydroxy group at the anomeric carbon atom and the primary hydroxy group either as the 1,6-anhydro sugar or by blocking with, for example, isopropylidene groups. However, a few examples of selective C-2, C-3 or C-4 deacylation have been reported.

The most common lipases which have been used to catalyse the acylation and deacylation of carbohydrates are *Pseudomonas cepacia* lipase, porcine pancreatic lipase, *Candida cylindracea* lipase, *Candida antarctica* lipase, *Aspergillus niger* lipase and *Chromobacterium viscosum* lipase, while the only protease to have been used is *Bacillus subtilis* protease (subtilisin).

The most common solvents which have been used in the acylation reactions include pyridine, dioxane, THF, DMF and benzene. For the deacylation reactions, 0.1 mol dm^{-3} phosphate buffer has been the most frequently used solvent and in many cases DMF has been used as a co-solvent to improve solubility. The acylating agents of choice have included trichloroethyl butyrate, trifluoroethyl butyrate, oxime esters, and the enol esters, vinyl acetate and isopropenyl acetate. Most of the acylation and deacylation reactions were carried out at 25–30 °C with reaction times averaging 2–12 days.

In conclusion, it has been shown that enzymes possess the ability to discriminate between the individual hydroxy groups in the regioselective acylation and deacylation of carbohydrates. As shown below, this methodology can now be exploited for regioselective transformation of selected hydroxy groups.

Results and discussion

Acetylation of some ribo-, arabino-, xylo- and rhamno-pyranosides

Methyl β-D-ribopyranoside **110**, methyl β-D-arabinopyranoside **111** and methyl β-D-xylopyranoside **112** were prepared according to literature procedures.[45] The methyl pyranosides **110**–**112** were then subjected to lipase-catalysed acylation, using vinyl acetate as both solvent and acyl donor. Three lipases were screened for their suitability in this reaction, namely, *Pseudomonas fluorescens* lipase, *Candida cylindracea* lipase and porcine pancreatic lipase. The reactions were carried out at 37 °C and the only products isolated were the corresponding 4-acetates **113**, **114** and **115**, respectively, with yields almost always in the range 50–65% after reaction times of 5–10 days. From the results presented in Table 6, it can be deduced that lipases from *Pseudomonas fluorescens*, *Candida cylindracea* and porcine pancreas have, in general, achieved good regioselectivity and conversion. It is evident that *Pseudomonas fluorescens* lipase is the preferred enzyme for acetylation under the specified conditions, giving higher isolated yields of the acetylated products. Although *Candida cylindracea* lipase and porcine pancreatic lipase gave similar results, the isolated yields were slightly inferior.

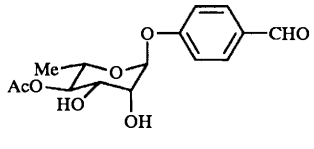
The position of acetylation was established by ^{1}H NMR spectroscopy. Initial analysis of the acetate **113**, identified the anomeric proton as a doublet appearing at 4.48 ppm with a *J* value of 3.7 Hz. Having identified the anomeric proton, the techniques of homonuclear decoupling and nuclear Overhauser enhancement (NOE) were then used to assign the remaining protons. In this way, the full coupling relationships and the assignments of the protons were made. The site of acetylation was then determined to be the C-4 position by identification of the 4-H proton, which appeared as a doublet of doublets at 4.80 ppm with *J* values of 5.0 and 3.5 Hz. Using these techniques, the site of acetylation for acetates **114** and **115**, was also determined to be the C-4 position.

The results described here, form a pattern which is consistent with the results discussed earlier (Table 2).

Interestingly, methyl α-L-rhamnopyranoside **116** behaved similarly, furnishing the 4-acetate **117**. However, the epimers of the compounds **111** and **116**, namely **118** and **119**, gave completely different results, in a similar fashion to other pyranosides that we have studied previously.[27,28] Thus, methyl β-L-rhamnopyranoside **118** gave only the diacetate **120** under the usual reaction conditions. No monoesters were observed even after short reaction times. Methyl α-D-arabinopyranoside **119** was difficult to obtain in a pure state in that it was contaminated by the isomer **111**. However, enzyme-catalysed acetylation of a mixture of **111** and **119** gave the ester **121** as the only product derived from the α-anomer. The reluctance of the 4-hydroxy group in compounds **119** and **121** to undergo reaction may reflect its predominant existence as an axial substituent.

In summary, the β-anomers of the D-pyranosides **110**, **111** and **112** and the α-anomer of the L-pyranoside **116** were cleanly acetylated on the 4-hydroxy group. The regioselectivity of the reaction of two of the corresponding epimers, namely **118** and **119** was less easy to predict.

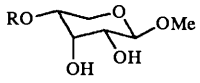
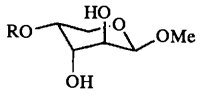
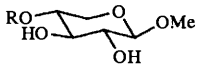
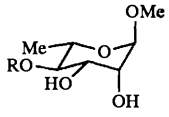
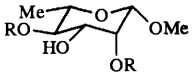
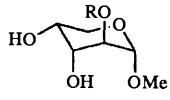
The selective acylation of arylglycosides became an issue of interest to us when it was announced[46] that some naturally occurring 4-acetylphenylglycosides, similar to compound **122**, possessed noteworthy biological activity. Having successfully applied the aforementioned methodology to some simple methyl pyranosides, it was envisaged that targets such as compound **122**, could also be synthesised utilising this methodology.



122

Retrosynthetic analysis of arylglycoside **122** (Scheme 18) required the consideration of two key factors: firstly, the final step should involve lipase-catalysed regioselective acylation and secondly, the synthetic route should afford only the desired α-anomer. With these considerations in mind, the arylglycoside

Table 6 Regioselective acylation of some pyranosides

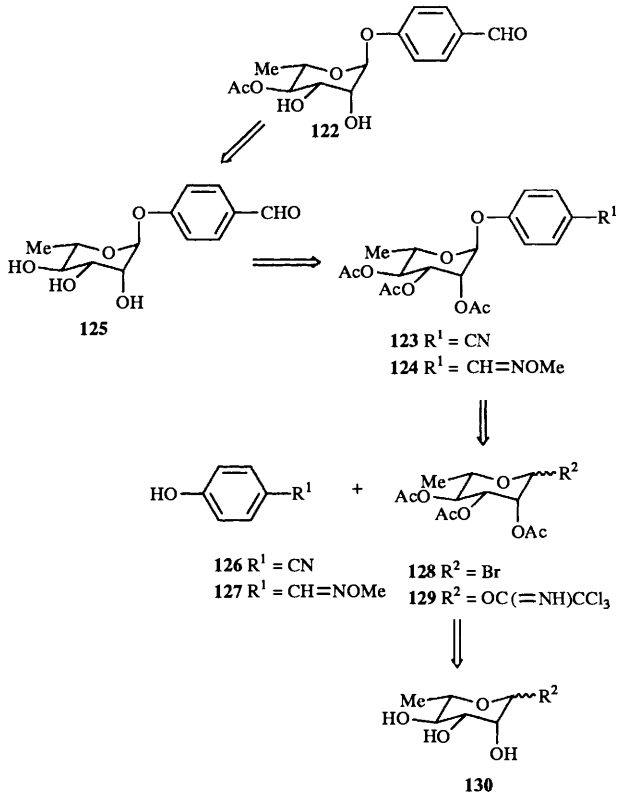
	Compound no.		Enzyme	Reaction time (days)	Yield (%)
	**110**	R = H	PFL	5–7	55
	113	R = COMe	PPL		40
			CCL		50
	**111**	R = H	PFL	5–7	60
	114	R = COMe	PPL		—
			CCL		50
	**112**	R = H	PFL	5–7	65
	115	R = COMe	PPL		—
			CCL		59
	**116**	R = H	PFL	5–7	65
	117	R = COMe	PPL		40
			CCL		50
	**118**	R = H	PFL	5–7	50
	120	R = COMe	PPL		40
			CCL		40
	**119**	R = H	PFL	7–10	30
	121	R = COMe	PPL		20
			CCL		—

122 was envisaged as being prepared from the masked aldehydes **123** or **124** *via* the triol **125**. Compounds **123** and **124** could be prepared by coupling of the phenol components **126** and **127**, respectively, with either the activated glycoside **128** using Koenigs–Knorr conditions,[47] or *via* the trichloroacetamide **129**.[48] Finally, the glycoside components **128** and **129** could be prepared from L-rhamnose **130** by literature methods.[48]

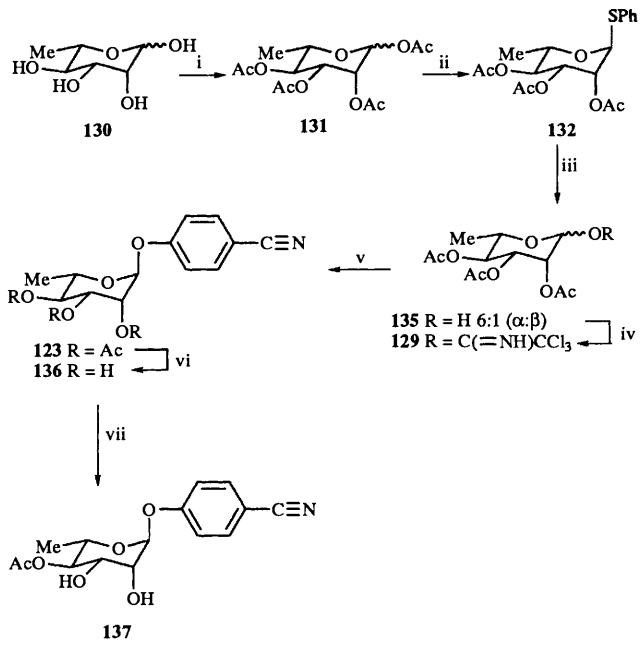
Initial attempts to prepare arylglycoside **122** focused on the route involving Koenigs–Knorr coupling, with the hope that the mixture of α- and β-anomers could be separated efficiently. Consequently, bromide **128** was prepared by literature methods from L-rhamnose.[47] Attempts to couple bromide **128** and phenol **126** using 1 mol dm^{-3} sodium hydroxide in acetone, resulted in a complex mixture of products, which was found to be problematic during purification. As a result, attention was then focused on a more lengthy route in which the *p*-benzonitrile rhamnopyranoside **123** was prepared from L-rhamnose **130** *via* the trichloroacetimidate[48] **129**, as described in Scheme 19: L-rhamnose **130** was peracetylated, using standard chemical methods, to give the tetraacylated rhamnopyranoside **131**. Treatment of the peracetate **131** with benzenethiol in the presence of tin(IV) chloride gave the α-phenylglycoside **132** as a single anomer in 95% yield.[48]

At this point, a model study was carried out to investigate whether phenyl glycosides are substrates for hydrolytic enzymes (Scheme 20). Hence, phenylsulfanylglycoside **132** was deacylated to give the triol **133** which was then screened for lipase activity. The 4-acetoxy compound **134** was obtained in 90% yield using *Pseudomonas fluorescens* lipase and vinyl acetate at 37 °C over 120 h. The position of acetylation was deduced by the sequence of events described earlier using NMR spectroscopy methods.

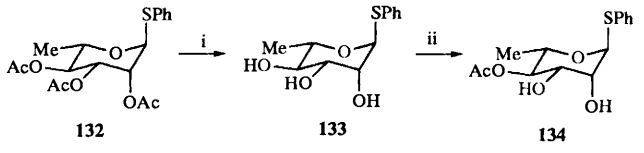
Having established that phenyl glycosides are indeed substrates for *Pseudomonas fluorescens* lipase, furnishing the desired 4-acetoxy glycoside **134**, the synthesis of the required triol **125** was then completed.



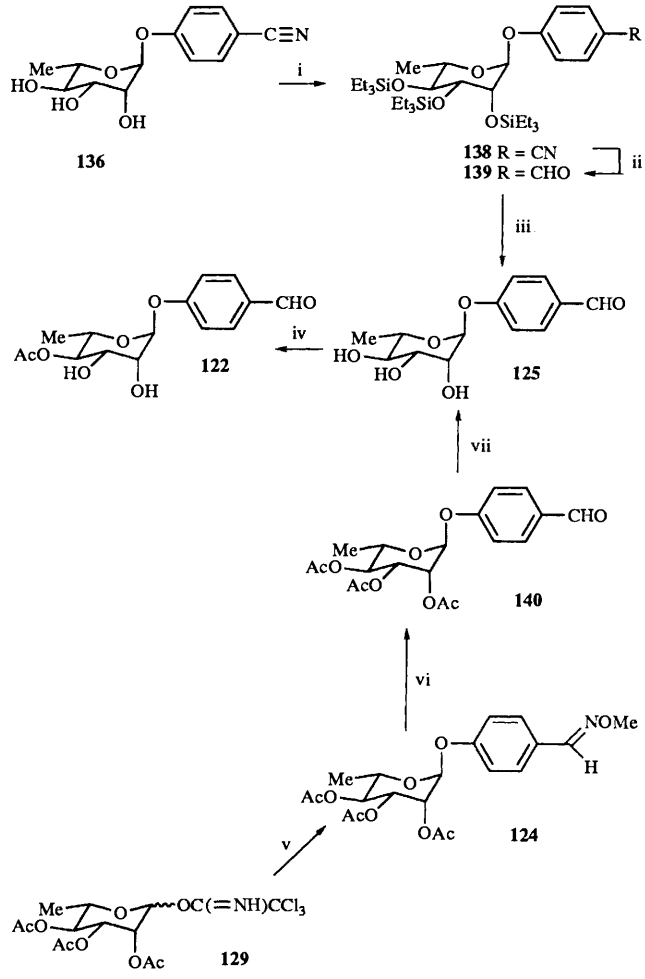
Scheme 18 Retrosynthetic analysis



Scheme 19 *Reagents and conditions:* i, $(CH_3CO)_2O$, Et_3N, DMAP (cat.), CH_2Cl_2, 0–25 °C, 12 h; ii, PhSH, $SnCl_4$, CH_2Cl_2, 0 °C, 8 h, 90%; iii, NBS, Me_2CO–H_2O (20:1), 25 °C, 4 h, 60%; iv, excess Cl_3CCN, NaH, CH_2Cl_2, 0–25 °C, 1.5 h; v, *p*-hydroxybenzonitrile **126**, BF_3·Et_2O, 4 Å sieves, CH_2Cl_2, −78 to −20 °C, 70% over two steps; vi, K_2CO_3 (cat.), THF–MeOH (1:1), 2 h, 90%; vii, *Pseudomonas fluorescens* lipase, vinyl acetate, 37 °C, 60 h, 85%



Scheme 20 *Reagents and conditions:* i, K_2CO_3 (cat.), THF–MeOH (1:1), 1 h; ii, *Pseudomonas fluorescens* lipase, vinyl acetate, 37 °C, 120 h, 90% over two steps



Scheme 21 *Reagents and conditions:* i, Et_3SiOTf, 2,6-lutidine, CH_2Cl_2, −20–0 °C, 2 h, 90%; ii, DIBAL-H, toluene, −78 °C to room temp., 12 h, 40%; iii, KF·$2H_2O$, THF, 18-crown-6, 25 °C, 7 h; iv, *Pseudomonas fluorescens* lipase, vinyl acetate, 37 °C, 90 h, 70% over two steps; v, *p*-hydroxybenzaldehyde *O*-methyloxime **127**, BF_3·Et_2O, −78 to −20 °C, 80%; vi, $NaHSO_3$, EtOH–H_2O (1:1), reflux, 60 h, 86%; vii, K_2CO_3 (cat.), THF–MeOH (1:1), 25 °C, 1 h

Oxidative removal of the phenylsulfanyl moiety from compound **132** using *N*-bromosuccinimide gave the lactol **135** as a 6:1 mixture of α- and β-anomers. The anomeric position was activated as the trichloroacetimidate **129** and was subsequently coupled with *p*-hydroxybenzonitrile **126** in the presence of boron trifluoride to give the α-benzonitrile glycoside **123** in good yield. Deprotection of compound **123** gave the required intermediate **136** in 90% yield. This triol **136** was then subjected to selective acylation using vinyl acetate and PFL at 37 °C over 60 h giving the 4-acetoxy glycoside analogue **137** in 85% yield.

Triol **136** was then protected as the silyl ether **138** and subsequently reduced using DIBAL to give the aldehyde **139** in 40% yield (Scheme 21). Removal of the silyl ether groups was achieved using potassium fluoride dihydrate[49] to give the crude triol **125** which was subjected, without purification, to regioselective acylation using vinyl acetate and PFL at 37 °C over 90 h, to give the naturally occurring 4-acetoxy glycoside **122** in 70% yield over two steps. The structure of this compound was elucidated by ^1H NMR spectroscopy and by comparing these data to those obtained from the natural product.[46]

The low yield of the aldehyde **139** prompted us to investigate a second synthetic route. This route started from commercially available *p*-hydroxybenzaldehyde which was protected as its *O*-methyl oxime derivative **127**, and coupled to the trichloroacetimidate **129** to give exclusively the α-oxime glycoside **124**.

Cleavage of the oxime was achieved using sodium bisulfite in aqueous ethanol which gave the free aldehyde **140** in excellent yield. Deprotection and regioselective acetylation of the α-rhamnopyranoside **140** was accomplished in 70% yield using PFL in vinyl acetate at 37 °C over 90 h, furnishing the naturally occurring rhamnopyranoside of *p*-hydroxybenzaldehyde **122**.

The latter results should serve to demonstrate that, with the accumulated knowledge of the regioselectivity of enzyme-catalysed esterification/de-esterification reactions on sugars and sugar-derivatives, it is possible to incorporate a key lipase-promoted reaction at a late stage in a multi-step synthesis of a carbohydrate derivative.

Experimental

IR spectra were recorded on a Perkin-Elmer 881 spectrometer. ^1H and ^{13}C NMR spectra were recorded on a Bruker AM 300 spectrometer at 300 and 75.0 MHz, respectively, using $CDCl_3$ as the solvent with $CHCl_3$ as the internal standard. All chemical shifts (δ) are reported in parts per million (ppm) and the coupling constants (J) are quoted in Hz. The mps were determined on a capillary apparatus. $[\alpha]_D$ Values, in units of 10^{-1} deg cm^2 g^{-1}, were recorded on an Optical Activity Ltd AA

1000 polarimeter. Lipases from *Pseudomonas fluorescens* (PFL), *Candida cylindracea* (CCL) and porcine pancreas (PPL) were purchased from Biocatalyst and Sigma. Vinyl acetate was purchased from Aldrich and used without further purification. Solvents were distilled prior to use. Tetrahydrofuran (THF) and diethyl ether were distilled from sodium metal/benzophenone under nitrogen. Dichloromethane was distilled from calcium hydride under nitrogen. Acetone was dried over 4 Å molecular sieves and distilled under nitrogen. Light petroleum refers to the fraction with bp 40–60 °C. All reactions were carried out under an argon atmosphere with freshly distilled anhydrous solvents, unless otherwise stated. Flash column chromatography was carried out using silica gel 60 H (Merck 7385). Thin-layer chromatography (TLC) was performed on Merck 60F-254 (Merck 5715) glass-backed silica gel plates with visualization by UV light (254 nm), *p*-anisaldehyde solution and 5% ammonium molybdate/cerium sulfate (solution in sulfuric acid). All compounds were homogenous by TLC analysis using the solvent systems employed in the purification process.

Enzymatic reactions

Esterification of the substrates was carried out according to the general procedure (using the specified amounts, unless otherwise stated). A mixture of substrate (150 mg), vinyl acetate (30 cm^3) and lipase (150 mg) was stirred at 37 °C until TLC analysis showed complete consumption of the starting material. The reaction was terminated by removal of the lipase by filtration through a pad of Celite. The residue was washed with ethyl acetate (50 cm^3), the filtrate dried ($NaSO_4$) and the solvent removed under reduced pressure. The resulting residue was purified by flash chromatography, eluting with dichloromethane–methanol (9:1), to afford the acetylated products.

Methyl 4-*O*-acetyl-β-D-ribopyranoside 113. The title compound **113** was isolated as an oil, $[\alpha]_D$ −98.6 (*c* 0.8, $CHCl_3$); ν_{max}(thin film)/cm^{-1} 3465, 2947, 1738, 1385, 1240, 1137 and 1071; δ_H 4.80 (1 H, dd, *J* 5.0 and 3.5, 4-H), 4.48 (1 H, d, *J* 3.7, 1-H), 3.90 (1 H, m, OH), 3.86 (1 H, dd, *J* 3.7 and 3.5, 3-H), 3.64 (1 H, m, 5-H_{eq}), 3.81 (1 H, m, OH), 3.60 (1 H, m, 5-H_{ax}), 3.45 (1 H, dd, *J* 3.7 and 3.5, 2-H), 3.23 (3 H, s, OMe) and 2.01 (3 H, s, $COCH_3$); δ_C 170.4, 101.5, 70.5, 69.9, 65.5, 60.2, 55.6 and 20.8 (Found: M^+, 206.195. $C_8H_{14}O_6$ requires *M*, 206.195).

Methyl 4-*O*-acetyl-β-D-arabinopyranoside 114. The title compound **114** was isolated as an oil, $[\alpha]_D$ −20.4 (*c* 0.9, $CHCl_3$); ν_{max}(thin film)/cm^{-1} 3440, 2940, 1742, 1374, 1249 and 1084; δ_H 5.40 (1 H, m, 4-H), 4.70 (1 H, d, *J* 3.3, 1-H), 4.00 (1 H, br s, OH), 3.85 (1 H, d, *J* 9.7, 3-H), 3.75 (1 H, m, 2-H), 3.70 (1 H, m, 5-H_{ax}), 3.60 (1 H, m, 5-H_{eq}), 3.58 (1 H, br s, OH), 3.34 (3 H, s, OCH_3) and 2.05 (3 H, s, $COCH_3$); δ_C 171.2, 99.9, 71.7, 69.5, 68.2, 60.6, 55.5 and 21.0 (Found: M^+, 206.193 16. $C_8H_{14}O_6$ requires *M*, 206.193 16).

Methyl 4-*O*-acetyl-β-D-xylopyranoside 115. The title compound **115** was isolated as an oil, $[\alpha]_D$ −70.6 (*c* 0.8, $CHCl_3$); ν_{max}(thin film)/cm^{-1} 3466, 2940, 1746, 1380, 1242, 1137 and 1071; δ_H 4.75 (1 H, m, 4-H), 4.60 (1 H, d, *J* 3.6, 1-H), 4.05 (2 H, br s, 2 × OH), 3.76 (1 H, m, 3-H), 3.65 (1 H, m, 2-H), 3.60 (2 H, m, 5-H_{ax}, 5-H_{eq}), 3.31 (3 H, s, OCH_3) and 2.10 (3 H, s, $COCH_3$); δ_C 172.4, 97.2, 73.3, 71.8, 70.4, 61.1, 55.1 and 20.9 (Found: M^+, 206.198. $C_8H_{14}O_6$ requires *M*, 206.198).

Methyl 4-*O*-acetyl-α-L-rhamnopyranoside 117. The title compound **117** was isolated as a white solid, mp 109–110 °C; $[\alpha]_D$ −67.2 (*c* 1.0, $CHCl_3$); ν_{max}(KBr)/cm^{-1} 3332, 2950, 1740, 1379, 1247, 1133 and 1078; δ_H 4.88 (1 H, dd, *J* 9.9 and 9.9, 4-H), 4.64 (1 H, d, *J* 1.8, 1-H), 3.89 (1 H, m, 2-H), 3.78 (2 H, br s, OH and 3-H), 3.68 (1 H, dd, *J* 9.7 and 6.2, 5-H), 3.50 (1 H, br s, OH), 3.30 (3 H, s, OCH_3), 2.06 (3 H, s, $COCH_3$) and 1.15 (3 H, d, *J* 6.2, CH_3); δ_C 171.7, 100.7, 74.8, 70.8, 69.9, 65.7, 54.85, 21.0 and 17.3 [Found: $(M + H)^+$, 221.102 17. $C_9H_{16}O_6$ requires $(M + H)$, 221.094 68].

Methyl 2,4-di-*O*-acetyl-α-L-rhamnopyranoside 120. The title compound **120** was isolated as an oil, $[\alpha]_D$ −40.6 (*c* 0.1, $CHCl_3$); ν_{max}(thin film)/cm^{-1} 3340, 2980, 1740, 1738, 1379, 1132 and 1079; δ_H 5.20 (1 H, dd, *J* 3.6 and 1.6, 2-H), 4.82 (1 H, d, *J* 9.9, 4-H), 4.65 (1 H, d, *J* 1.6, 1-H), 4.00 (1 H, m, 3-H), 3.78 (1 H, dd, *J* 9.9 and 6.2, 5-H), 3.35 (3 H, s, OCH_3), 2.38 (1 H, br s, OH), 2.13 (3 H, s, $COCH_3$), 2.11 (3 H, s, $COCH_3$) and 1.20 (3 H, d, *J* 6.2, CH_3); δ_C 171.4, 170.6, 98.2, 74.65, 72.6, 68.4, 65.8, 55.1, 20.9, 20.9 (2 × C, overlapping) and 17.3 [Found: $(M + H)^+$, 263.251 82. $C_{11}H_{18}O_7$ requires $(M + H)$, 263.243 92].

Methyl 2-*O*-acetyl-α-D-arabinopyranoside 121. The mixture of anomers was subjected to the enzyme protocol using the general procedure and purification by flash chromatography gave the desired product (30%) as a yellow oil, $[\alpha]_D$ +25.6 (*c* 0.1, $CHCl_3$); ν_{max}(thin film)/cm^{-1} 3440, 2945, 1749, 1650, 1325, 1249 and 1084; δ_H 5.05 (1 H, dd, *J* 5.9 and 3.2, 2-H), 4.70 (1 H, d, *J* 3.3, 1-H), 4.00 (1 H, br s, OH), 3.85 (1 H, d, *J* 9.1, 3-H), 3.80 (1 H, m, 4-H), 3.70 (1 H, m, 5-H_{ax}), 3.60 (1 H, m, 5-H_{eq}), 3.58 (1 H, br s, OH), 3.34 (3 H, s, OCH_3) and 2.12 (3 H, s, $COCH_3$); δ_C 170.2, 99.8, 72.1, 68.8, 67.9, 60.6, 55.5 and 21.0 (Found: M^+, 206.2091. $C_8H_{14}O_6$ requires *M*, 206.1932).

Acetyl 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranoside 131

L-Rhamnose **130** monohydrate (10.0 g, 54.9 mmol) suspended in dichloromethane (50 cm^3) was treated at 0 °C with triethylamine (30 cm^3), DMAP (4-dimethylaminopyridine; 0.7 g, 0.55 mmol) and acetic anhydride (30 cm^3). The reaction mixture was allowed to warm to ambient temperature, stirred for 12 h then treated with methanol (1 cm^3). After concentration, the residue was dissolved in ethyl acetate (100 cm^3), and washed with brine (2 × 50 cm^3). The aqueous phase was extracted with ethyl acetate (3 × 30 cm^3) and the combined organic extracts were washed with 0.5 mol dm^{-3} HCl (15 cm^3), saturated aqueous sodium hydrogen carbonate and then brine (30 cm^3). This solution was dried (Na_2SO_4) and concentrated to afford the crude tetraacetate **131**.

Phenyl 2,3,4-tri-*O*-acetyl-1-thio-α-L-rhamnopyranoside 132

Benzenethiol (6 cm^3, 52.6 mmol) and tin(IV) chloride (20 cm^3, 20 mmol, 1 mol dm^{-3} solution in dichloromethane) was added to a solution of L-rhamnose tetraacetate **131** (17.5 g, 52.6 mmol) in dichloromethane (150 cm^3) at 0 °C. The mixture was stirred at 0 °C for 8 h, diluted with diethyl ether (1 dm^3) and washed with 1 mol dm^{-3} HCl (120 cm^3), water (2 × 20 cm^3), saturated aqueous sodium hydrogen carbonate and then brine (100 cm^3). Subsequently the organic layer was dried (Na_2SO_4), concentrated and purified by flash column chromatography to give only the α-anomer of thioglycoside **132** (19 g, 95%) as a white solid, mp 120 °C; $[\alpha]_D$ −96.7 (*c* 1.0, $CHCl_3$); ν_{max}(KBr)/cm^{-1} 3064, 2985, 1740, 1570, 1449, 1377, 1245, 1101 and 1050; δ_H 7.33 (2 H, m, Ar-H), 7.27 (3 H, m, Ar-H), 5.50 (1 H, m, 2-H), 5.40 (1 H, d, *J* 1.3, 1-H), 5.35 (1 H, m, 3-H), 5.20 (1 H, dd, *J* 9.9 and 9.9, 4-H), 4.35 (1 H, m, 5-H), 2.13 (3 H, s, $COCH_3$), 2.09 (3 H, s, $COCH_3$), 2.00 (3 H, s, $COCH_3$) and 1.20 (3 H, d, *J* 6.2, CH_3) (Found: M^+, 382.107. $C_{18}H_{22}O_7S$ requires *M*, 382.107).

Phenyl 1-thio-α-L-rhamnopyranoside 133

Potassium carbonate (200 mg) was added to a solution of the phenylsulfanyl glycoside **132** (400 mg, 1.046 mmol) in THF–MeOH (1:1; 10 cm^3). The reaction was stirred at room temperature for 2 h. The reaction mixture was filtered through a pad of Celite, concentrated and purified by flash column chromatography, eluting with dichloromethane–methanol (95:5), to give the triol **133** (200 mg, 67%) as a clear oil, ν_{max}(thin film)/cm^{-1} 3395, 3063, 2977, 1650, 1559, 1538, 1527, 1476, 1440, 1382, 1252, 1134 and 1054; δ_H(250 MHz) 7.38 (2 H, m, Ar-H), 7.13 (3 H, m, Ar-H), 5.50 (1 H, m, 1-H), 4.93 (1 H, br s, 3-H), 4.63 (2 H, br s, OH and 2-H), 4.25 (1 H, br s, OH),

4.12 (1 H, m, 4-H), 3.88 (1 H, m, OH), 3.52 (1 H, m, 5-H), 1.25 (3 H, d, *J* 6.2, CH_3) (Found: M^+, 256.076 89. $C_{12}H_{16}O_4S$ requires *M*, 256.076 93).

Phenyl 4-*O*-acetyl-1-thio-α-L-rhamnopyranoside 134

The triol **133** (200 mg, 0.78 mmol) was subjected to the general esterification procedure over 120 h and purified by flash column chromatography, eluting with light petroleum–diethyl ether (7:3), to give the desired product **134** as a clear oil (180 mg, 90%), $[\alpha]_D$ −148.1 (*c* 1.0, $CHCl_3$); ν_{max}(thin film)/cm^{-1} 3752, 3353, 2985, 2318, 1734, 1584, 1540, 1476, 1449, 1377, 1245, 1101 and 1050 cm^{-1}; δ_H 7.43 (2 H, m, Ar-H), 7.25 (2 H, m, Ar-H), 5.52 (1 H, d, *J* 1.5, 1-H), 4.92 (1 H, dd, *J* 9.7 and 9.7, 4-H), 4.28 (1 H, dd, *J* 9.7 and 6.4, 5-H), 4.20 (1 H, dd, *J* 3.5 and 1.5, 2-H), 3.90 (1 H, dd, *J* 9.7 and 3.5, 3-H), 3.50 (2 H, br s, 2 × OH), 2.13 (3 H, s, $COCH_3$) and 1.20 (3 H, d, *J* 6.2, CH_3); δ_C 171.9, 133.9, 131.3, 129.1, 127.4, 87.6, 75.2, 72.5, 70.6, 67.2, 21.1 and 17.3 (Found: M^+, 298.087 16. $C_{14}H_{18}O_5S$ requires *M*, 298.087 49).

2,3,4-Tri-*O*-acetyl-α-L-rhamnopyranoside 135

A solution of thioglycoside **132** (7.63 g, 19.9 mmol) in dry acetone (50 cm^3) was treated at 0 °C with *N*-bromosuccinimide (7.1 g) and water (2.5 cm^3). The reaction mixture was allowed to warm to ambient temperature, and stirring was continued for 8 h. Acetone was removed under reduced pressure and the residue was taken up in ethyl acetate (200 cm^3), washed with saturated aqueous sodium hydrogen carbonate (2 × 50 cm^3) and brine (50 cm^3). The organic layer was dried (Na_2SO_4), concentrated and purified by flash column chromatography, eluting with light petroleum–diethyl ether (3:2), to give the lactol **135** (3.97 g, 60%) as a 6:1 anomeric mixture, ν_{max}(thin film)/cm^{-1} 3444, 2910, 1745, 1725, 1443, 1377, 1259, 1228, 1177 and 1056; δ_H 5.34 (1 H, dd, *J* 10.1 and 3.5, 3-H), 5.23 (1 H, dd, *J* 7.35 and 1.9, 2-H), 5.15 (1 H, d, *J* 1.9, 1-H), 5.04 (1 H, dd, *J* 10.1 and 10.1, 4-H), 4.12 (1 H, m, 5-H), 3.86 (1 H, br s, OH), 2.12 (3 H, s, $COCH_3$), 2.02 (3 H, s, $COCH_3$), 1.97 (3 H, s, $COCH_3$) and 1.18 (3 H, d, *J* 6.2, CH_3); δ_C 170.3, 170.2, 170.1, 92.0, 71.2, 70.4, 68.9, 66.3, 20.8, 20.7, 20.6 and 17.4 (Found: M^+, 290.2543. $C_{12}H_{18}O_8$ requires *M*, 290.2543).

4-(2,3,4-Tri-*O*-acetyl-α-L-rhamnosyloxy)benzonitrile 123

A solution of the lactol **135** (1.94 g, 6.68 mmol) in dichloromethane (40 cm^3) and trichloroacetonitrile (5.35 cm^3, 53.45 mmol) was treated at 0 °C with sodium hydride (0.139 g, 6.0 mmol) and the mixture was stirred for 90 min at room temperature. The reaction mixture was filtered through a pad of Celite and concentrated to give the crude trichloroacetimidate **129** (2.56 g).

A mixture of the crude trichloroacetimidate **129** (0.776 g) and 4-hydroxybenzonitrile (0.164 g, 1.37 mmol) was taken up in dichloromethane (20 cm^3). Dried 4 Å molecular sieves were added and the mixture was cooled to −78 °C and treated with boron trifluoride–diethyl ether (0.25 cm^3, 2.0 mmol). The reaction mixture was allowed to warm to −20 °C and solid sodium hydrogen carbonate (0.4 g) was added. After 45 min, water (5 cm^3) was added and the mixture was diluted with dichloromethane (20 cm^3), and filtered through a pad of Celite. The filtrate was dried (Na_2SO_4), concentrated and purified by flash column chromatography, eluting with light petroleum–diethyl ether (7:3), to give the desired glycoside **123** as a white solid (255 mg, 60%), mp 62 °C; $[\alpha]_D$ −16.9 (*c* 0.2, $CHCl_3$); ν_{max}(KBr)/cm^{-1} 3481, 2988, 2229, 1750, 1605, 1579, 1430, 1373, 1222, 1177, 1135, 1055 and 1030; δ_H 7.62 (2 H, m, Ar-H), 7.12 (2 H, m, Ar-H), 5.52 (1 H, d, *J* 1.9, 1-H), 5.48 (1 H, dd, *J* 10 and 3.5, 3-H), 5.42 (1 H, dd, *J* 3.5 and 1.8, 2-H), 5.16 (1 H, dd, *J* 9.9 and 9.9, 4-H), 3.90 (1 H, m, 5-H), 2.19 (3 H, s, $COCH_3$), 2.05 (3 H, s, $COCH_3$), 2.03 (3 H, s, $COCH_3$) and 1.2 (3 H, d, *J* 6.2, CH_3); δ_C 169.9 (2 × C overlapping), 169.8, 134.1, 118.6, 116.9, 106.3, 95.5, 70.5, 69.3, 68.6, 67.7, 20.8, 20.7, 20.6 and 17.4 [Found: $(M + H)^+$, 392.135 47. $C_{19}H_{21}NO_8$ requires (*M* + H), 392.135 47].

4-(α-L-Rhamnopyranosyloxy)benzonitrile 136

Potassium carbonate (100 mg) was added to a solution of the triacetate **123** (250 mg, 0.64 mmol) in THF–MeOH (1:1; 10 cm^3). The reaction mixture was stirred at room temperature for 45 min and then filtered through a pad of Celite, and concentrated to give the triol **136** (148 mg, 90%) as a clear oil. Due to instability, this product was used further without any purification; ν_{max}(thin film)/cm^{-1} 3360, 2934, 2229, 1667, 1605, 1559, 1509, 1435, 1314, 1248, 1175, 1077 and 1030; δ_H(250 MHz) 7.45 (2 H, m, Ar-H), 7.12 (2 H, m, Ar-H), 5.51 (1 H, d, *J* 1.8, 1-H), 5.00 (1 H, br s, 3-H), 4.65 (1 H, br s, 2-H), 4.30 (2 H, br s, OH, 4-H), 4.24 (1 H, br s, OH), 3.85 (1 H, br s, OH), 3.53 (1 H, m, 5-H) and 1.3 (3 H, d, *J* 6.2, CH_3) (Found: M^+, 265.1059. $C_{13}H_{15}NO_5$ requires *M*, 265.1059).

4-(4-*O*-Acetyl-α-L-rhamnosyloxy)benzonitrile 137

The triol **136** (100 mg, 0.37 mmol) was subjected to the general esterification procedure over 60 h and the product was purified by column chromatography, eluting with dichloromethane–methanol (95:5), to give the desired product **137** as a clear oil (80 mg, 85%), $[\alpha]_D$ −29.7 (*c* 0.1, $CHCl_3$); ν_{max}(thin film)/cm^{-1} 3824, 3463, 2938, 2228, 1734, 1672, 1605, 1579, 1420, 1373, 1236, 1175 and 1054; δ_H 7.55 (2 H, d, *J* 8.6, 3,5-H), 7.20 (2 H, d, *J* 8.6, 2- and 6-H), 5.60 (1 H, d, *J* 1.8, 1-H), 4.92 (1 H, dd, *J* 9.9 and 9.9, 4-H), 4.14 (1 H, dd, *J* 3.5 and 1.8, 1-H), 4.06 (1 H, dd, *J* 9.9 and 3.5, 3-H), 3.78 (1 H, m, 5-H), 3.50 (2 H, br s, 2 × OH), 2.15 (3 H, s, $COCH_3$) and 1.16 (3 H, d, *J* 6.2, CH_3); δ_C 172.0, 159.2, 134.1, 118.8, 116.9, 105.7, 97.4, 74.6, 70.4, 69.8, 67.1, 21.0 and 17.4 (Found: M^+, 307.106 64. $C_{15}H_{17}NO_6$ requires *M*, 307.106 64).

4-(2,3,4-Tris-*O*-triethylsilyl-α-L-rhamnosyloxy)benzonitrile 138

Triethylsilyl trifluoromethanesulfonate (0.84 g, 756 mmol) was added to a solution of the triol **136** (150 mg, 0.189 mmol) in dichloromethane (5 cm^3) containing 2,6-lutidine (2,6-dimethylpyridine; 0.2 cm^3, 1.4 mmol) at −20 °C. The mixture was allowed to warm to 0 °C over a period of 2 h and was then diluted with diethyl ether (25 cm^3), washed with water (2 × 15 cm^3), saturated aqueous sodium hydrogen carbonate and then brine (2 × 25 cm^3), dried (Na_2SO_4), concentrated and purified by flash column chromatography, eluting with light petroleum–diethyl ether (8:2) to give the title compound **138** as a clear oil (149.5 mg, 90%), $[\alpha]_D$ −38.5 (*c* 0.1, $CHCl_3$); ν_{max}(thin film)/cm^{-1} 2957, 2913, 2879, 2229, 1672, 1667, 1582, 1495, 1462, 1377, 1260, 1238 and 1073; δ_H(250 MHz) 7.55 (2 H, m, Ar-H), 7.12 (2 H, m, Ar-H), 5.52 (1 H, m, 1-H), 4.30 (1 H, m, 3-H), 4.25 (1 H, m, 2-H), 3.90 (2 H, m, 4- and 5-H), 1.30 (3 H, d, *J* 6.2, CH_3), 1.20 [27 H, m, 3 × $Si(CH_2CH_3)_3$] and 1.1 [18 H, m, 3 × $Si(CH_2CH_3)_3$] (Found: M^+, 608.0573. $C_{31}H_{57}NO_5Si_3$ requires *M*, 608.0573).

4-(2,3,4-Tris-*O*-triethylsilyl-α-L-rhamnosyloxy)benzaldehyde 139

DIBAL-H (0.2 cm^3; 1.5 equiv.) was added dropwise to a solution of the benzonitrile glycoside **138** (139 mg, 0.23 mmol) in freshly distilled toluene (5 cm^3) at −78 °C. The mixture was stirred for 3 h at −78 °C and allowed to warm to ambient temperature over a period of 2 h. It was then stirred for a further 10 h, and diluted with ethyl acetate (30 cm^3) and stirring was continued for a further 30 min. Saturated aqueous ammonium chloride (20 cm^3) was added and after 20 min 1 mol dm^{-3} H_2SO_4 (5 cm^3) was added dropwise and the product was extracted with ethyl acetate and diethyl ether. The combined

organic layers were washed with brine, dried (Na_2SO_4), concentrated and purified by flash column chromatography, eluting with light petroleum–diethyl ether (3:2) to give the desired product **139** as a pale yellow oil (50 mg, 40%), $[\alpha]_D$ −36.4 (*c* 0.1, $CHCl_3$); ν_{max}(thin film)/cm^{-1} 3000, 2958, 2913, 2879, 1772, 1734, 1672, 1667, 1601, 1559, 1495, 1467, 1265, 1238 and 1073; δ_H 9.87 (1 H, s, 7-H), 7.80 (2 H, d, *J* 8.8, 3- and 5-H), 7.15 (2 H, d, *J* 8.8, 2, 6-H), 5.37 (1 H, m, 1-H), 4.07 (1 H, m, 3-H), 3.87 (1 H, m, 2-H), 3.62 (1 H, m, 4-H), 3.50 (1 H, m, 5-H), 1.30 (3 H, d, *J* 6.2, CH_3), 1.20 [27 H, m, 3 × $Si(CH_2CH_3)_3$] and 1.10 [18 H, m, 3 × $Si(CH_2CH_3)_3$]; δ_C 190.8, 131.8, 116.5, 98.7, 70.6, 69.3, 67.3, 65.8, 15.2, 7.0, 6.8, 6.7, 5.2 and 5.1 (Found: M^+, 610.353 37. $C_{31}H_{58}O_6Si_3$ requires *M*, 610.354 12).

4-(α-L-Rhamnopyranosyloxy)benzaldehyde 125

To a solution of glycoside **139** (40 mg, 0.057 mmol) in dry THF (5 cm^3) potassium fluoride dihydrate (130 mg, 0.1 mmol; 18 equiv.) was added. The suspension was stirred for 5 min at room temperature, 18-crown-6 was added and the suspension was stirred for 7 h under an argon atmosphere, when TLC analysis showed complete conversion of the starting material to the triol. The suspension was filtered through a pad of Celite, concentrated to dryness, redissolved in ethyl acetate (25 cm^3) and then washed with brine (2 × 10 cm^3). The organic layer was dried (Na_2SO_4), concentrated and purified by flash column chromatography, eluting with dichloromethane–methanol (95:5) to give the desired product **125** as a semi-solid (25 mg, 60%); ν_{max}(thin film)/1 cm^{-1} 3330, 3200, 2967, 2879, 1768, 1698, 1660, 1601, 1559, 1495, 1350, 1265, 1238 and 1070; δ_H(250 MHz) 9.87 (1 H, s, 7-H), 7.80 (2 H, m, Ar-H), 7.15 (2 H, m, Ar-H), 5.45 (1 H, m, 1-H), 4.50 (1 H, m, 3-H), 4.30 (1 H, m, 2-H), 3.62 (2 H, br s, 2 × OH), 3.55 (2 H, br s, 4-H and OH), 3.50 (1 H, m, 5-H) and 1.20 (3 H, d, *J* 6.2, CH_3).

4-(4-*O*-Acetyl-α-L-rhamnosyloxy)benzaldehyde 122

The triol **125** (20 mg, 0.074 mmol) was subjected to the general esterification procedure over 90 h and purified by flash column chromatography, eluting with dichloromethane–methanol (95:5), to give the desired product **122** as a clear oil (14 mg, 70%), $[\alpha]_D$ −13.7 (*c* 0.1, $CHCl_3$); ν_{max}(thin film)/cm^{-1} 3830, 3000, 1765, 1698, 1605, 1595, 1495, 1375, 1235, 1138 and 1029; δ_H 9.92 (1 H, s, 7-H), 7.86 (2 H, d, *J* 8.7, 3- and 5-H), 7.20 (2 H, d, *J* 8.7, 2- and 6-H), 5.65 (1 H, d, *J* 1.8, 1-H), 4.88 (1 H, dd, *J* 9.9 and 9.9, 4-H), 4.18 (1 H, dd, *J* 3.4 and 1.6, 1-H), 4.10 (1 H, dd, *J* 9.9 and 3.5, 3-H), 3.85 (1 H, m, 5-H), 3.10 (1 H, br s, OH), 2.65 (1 H, br s, OH), 2.17 (3 H, s, $COCH_3$) and 1.20 (3 H, d, *J* 6.2, CH_3); δ_C 190.7, 172.2, 160.8, 147.8, 131.9, 131.2, 128.5, 116.4, 97.2, 70.5, 70.05, 66.85, 66.53, 20.9 and 17.5 (Found: M^+, 310.1044. $C_{15}H_{18}O_7$ requires *M*, 310.1050).

4-Hydroxybenzaldehyde *O*-methyloxime 127

O-Methylhydroxylamine hydrochloride (1.4 g, 18 mmol) and 4-hydroxybenzaldehyde (1.4 g, 12 mmol) was stirred for 24 h in dry pyridine (15 cm^3) at room temperature. The pyridine was removed under reduced pressure and the residue azeotroped with toluene (25 cm^3). The residue was re-dissolved in ethyl acetate (30 cm^3), washed with brine (2 × 15 cm^3), dried (Na_2SO_4), concentrated and purified by flash column chromatography, eluting with light petroleum–diethyl ether (1:1), to give the oxime **127** as an oil (1.5 g, 93%), ν_{max}(thin film)/cm^{-1} 3300, 2815, 1709, 1617, 1518, 1439, 1347, 1104 and 1051; δ_H 8.00 (1 H, s, 7-H), 7.42 (2 H, m, Ar-H), 6.75 (2 H, m, Ar-H), 6.42 (1 H, br s, OH) and 3.95 (3 H, s, CH_3).

4-(2,3,4-Tri-*O*-acetyl-α-L-rhamnosyloxyl)benzaldehyde *O*-methyloxime 124

A mixture of the crude trichloroacetimidate **129** and 4-hydroxybenzaldehyde *O*-methyloxime **127** (1.0 g, 6.6 mmol) was taken up in dichloromethane (30 cm^3) under an argon atmosphere. Dried 4 Å molecular sieves were added and the mixture was cooled to −78 °C and treated with boron trifluoride–diethyl ether (0.7 cm^3, 5.6 mmol). The solution was allowed to warm to −30 °C over a period of 90 min and then solid sodium hydrogen carbonate (1.0 g) was added. After 60 min, water (5 cm^3) was added and the mixture was diluted with dichloromethane (50 cm^3). The suspension was filtered through a pad of Celite, dried (Na_2SO_4), concentrated and purified by flash column chromatography, eluting with light petroleum–diethyl ether (7:3), to give the desired oxime glycoside **124** (650 mg, 60%), $[\alpha]_D$ −43.6 (*c* 0.2, $CHCl_3$); ν_{max}(thin film)/cm^{-1} 3378, 3310, 2936, 1761, 1608, 1566, 1387 and 1055; δ_H 8.00 (1 H, s, 7-H), 7.50 (2 H, d, *J* 7.5, 3- and 5-H), 7.04 (2 H, d, *J* 7.5, 2- and 6-H), 5.50 (1 H, m, 1-H), 5.48 (1 H, m, 3-H) 5.20 (1 H, m, 2-H), 5.12 (1 H, dd, *J* 9.9 and 9.9, 4-H), 3.96 (1 H, m, 5-H), 3.92 (3 H, s, CH_3), 2.17 (3 H, s, $COCH_3$), 2.04 (3 H, s, $COCH_3$), 2.01 (3 H, s, $COCH_3$) and 1.19 (3 H, d, *J* 6.2, CH_3); δ_C 170.05, 170.0, 169.9, 156.9, 147.7, 128.5, 126.8, 116.5, 95.5, 70.9, 69.6, 68.9, 67.3, 61.9, 20.8, 20.7, 20.6 and 17.4 (Found: M^+, 423.1540. $C_{20}H_{25}NO_9$ requires *M*, 423.1540).

4-(2,3,4-Tri-*O*-acetyl-α-L-rhamnosyloxyl)benzaldehyde 140

The oxime glycoside **124** (150 mg, 0.3 mmol) and sodium bisulfite (150 mg, 1.2 mmol) were stirred in aqueous ethanol (15 cm^3) at room temperature for 10 min. The suspension was then refluxed for 60 h, cooled to room temperature and evaporated to dryness. The residue was re-dissolved in dichloromethane (50 cm^3), washed with 0.5 mol dm^{-3} HCl (2 × 10 cm^3) and brine (15 cm^3). The organic layer was subsequently dried (Na_2SO_4), evaporated and purified by flash column chromatography, eluting with light petroleum–diethyl ether (7:3), to give the free benzaldehyde glycoside **140** (134 mg, 90%) as a semi-solid, $[\alpha]_D$ −37.8 (*c* 0.1, $CHCl_3$); ν_{max}(thin film)/cm^{-1} 3367, 2987, 2350, 1760, 1691, 1602, 1582, 1432, 1375, 1220, 1166, 1135 and 1029; δ_H 9.92 (1 H, s, 7-H), 7.86 (2 H, d, *J* 8.8, 3- and 5-H), 7.20 (2 H, d, *J* 8.8, 2- and 6-H), 5.56 (1 H, d, *J* 1.8, 1-H), 5.50 (1 H, dd, *J* 9.9 and 3.5, 3-H), 4.45 (1 H, dd, *J* 3.6 and 1.9, 2-H), 4.18 (1 H, dd, *J* 9.9 and 9.9, 4-H), 3.94 (1 H, m, 5-H), 2.20 (3 H, s, $COCH_3$), 2.05 (3 H, s, $COCH_3$), 2.03 (3 H, s, $COCH_3$) and 1.20 (3 H, d, *J* 6.2, CH_3); δ_C 190.6, 170.0 (2 × C overlapping), 169.9, 160.4, 131.9, 131.5, 116.5, 95.4, 70.7, 69.4, 68.7, 67.6, 20.8, 20.7, 20.6 and 17.4 (Found: M^+, 394.3739. $C_{19}H_{22}O_9$ requires *M*, 394.3739).

4-(4-*O*-Acetyl-α-L-rhamnosyloxy)benzaldehyde 122

The oxime glycoside was deprotected using potassium carbonate in THF–methanol (1:1) to give the triol **125** in quantitative yield. Triol **125** was then subjected to the standard enzyme methodology over 90 h, to give the title compound **122** in 70% yield. Data for this compound **122** were in agreement with those given previously.

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