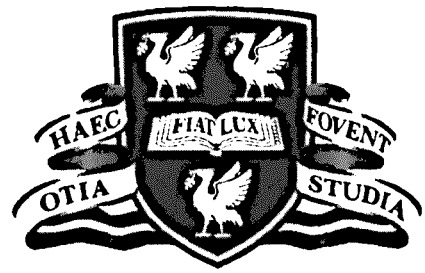

THE UNIVERSITY
of LIVERPOOL

Modification of Carbohydrates Using Enzymes as Catalysts

Thesis submitted in accordance with the requirements of
The University of Liverpool for the degree of
Doctor of Philosophy
by

Pedro Miguel Lopes Gonçalves

July 2003

To My Wife and Daughter

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Abstract

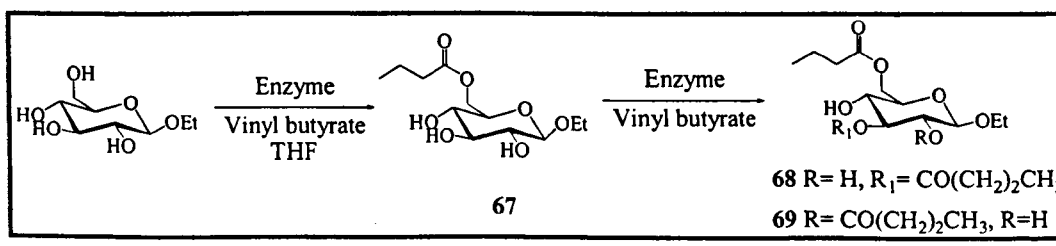
The multiple functional groups and stereocenters present in carbohydrates make them quite challenging synthetic targets for the organic chemist.

Chemical methods for the acylation of sugars generally involve protection and deprotection, often leading to low yields. Additionally, a mixture of products is also obtained in many chemical processes due to the lack of selectivity.

Therefore, enzymes have been employed for the selective (usually regioselective) transformations of readily available carbohydrates. Their ability to perform reactions in a stereospecific and regioselective manner (reducing to a minimum the use of protecting group chemistry) is one of the main attractions for the use of these biocatalysts in carbohydrate chemistry.

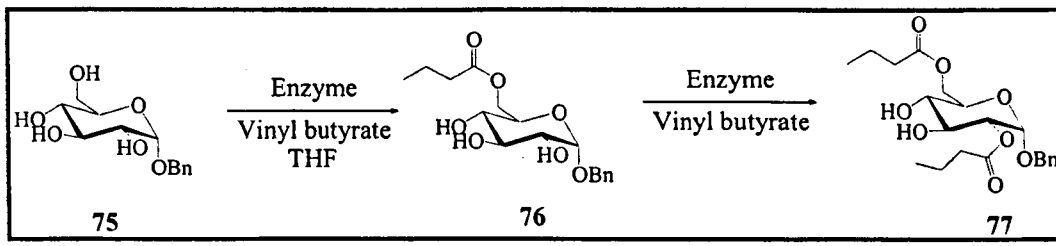
In this thesis the selective esterification of the primary and secondary hydroxyl groups of D-glucose, D-mannose and D-galactose was explored using vinyl butyrate and a variety of enzymes as catalysts.

In the first part, the α- and β-anomers of ethyl and benzyl D-glucopyranosides were selectively synthesised and then esterified. With ethyl as the protecting group, secondary esterification at the 2- or 3-positions was achieved if the protecting group was in the β-configuration (scheme **1**).



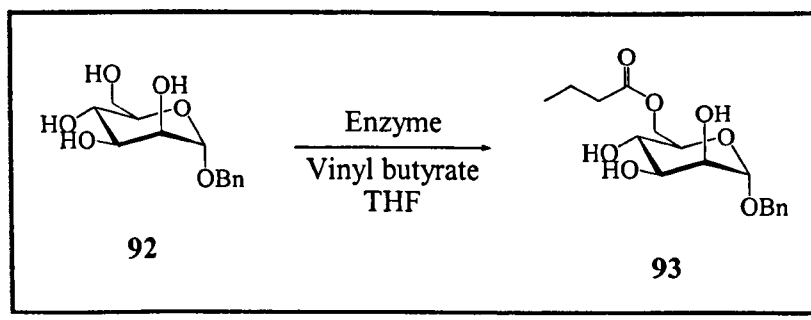
Scheme 1

With benzyl as the anomeric protecting group, only the α-anomer underwent a more selective secondary esterification, yielding a 2,6-diesterified glucopyranoside **77** (scheme **2**).



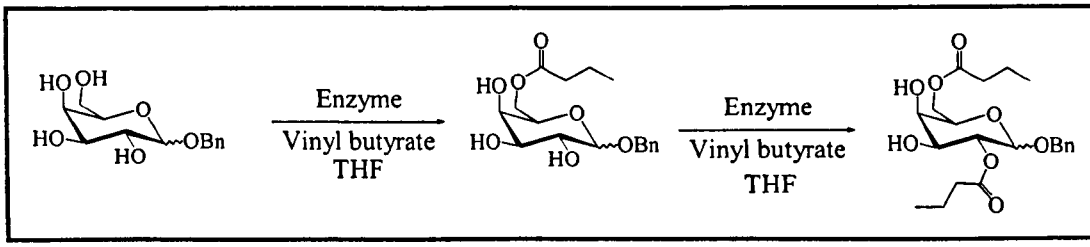
Scheme 2

Using benzyl as the anomeric protecting group, D-mannose was only mono-esterified at the primary position C-6, (scheme **3**).



Scheme 3

With benzyl-D-galactopyranoside, both α- and β-anomers have been selectively di-esterified at the 6- and 2-positions, (scheme **4**). The β-anomer has been shown to react faster then the α-anomer towards the secondary esterification.



Scheme 4

Using the difference in reactivity between the α- and β-anomers towards the esterification of the secondary hydroxyl groups, experiments aimed towards the development of a new system for the separation of anomers of D-glucose and D-galactose were performed.

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Abbreviations

Ac	Acetyl
ANL	*Aspergillus niger* lipase
Asp	Aspartic acid
Bn	Benzyl
CCL	*Candida cylindracea* lipase
CI	Chemical ionisation
COSY	Correlation Spectroscopy
CVL	*Chromobacterium viscosum* lipase
DCC	*N,N'*-Dicyclohexylcarbodiimide
DCM	Dichloromethane
DCU	*N,N'*-Dicyclohexylurea
DMAP	4-Dimethylaminopyridine
DMF	*N,N'*-Dimethylformamide
Eq.	Equivalents
Et	Ethyl
EtOAc	Ethyl acetate
EtOH	Ethanol
Gly	Glycine
HCl	Hydrochloric acid
His	Histidine
HMBC	Heteronuclear Multiple Bond Coherence
HMQC	Heteronuclear Multiple Quantum Coherence
IR	Infra-red spectroscopy

J	Coupling constant
m.p.	Melting point
Me	Methyl
MeOH	Methanol
min	Minutes
NEt_3	Triethylamine
NMR	Nuclear magnetic resonance spectroscopy
PCL	*Pseudomonas cepacia* lipase
Ph	Phenyl
PPL	Porcine pancreatic lipase
Py-GC / MS	Pyrolysis-Gas Chromatography / Mass Spectrometry
rpm	Rotations per minute
rt	Room temperature
Ser	Serine
TBDMSOTf	*tert*-Butyldimethylsilyl triflate
TFE	Trifluoroethyl
TFEB	Trifluoroethyl butanoate
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TMS	Trimethylsilyl

Chapter 1

Introduction

1.1 - Saccharides

Carbohydrates are one of the major classes of organic compounds and they are widely found in nature.[1, 2] They are, for example, structural components of the walls of plant cells.[3] They also provide most of the energy that keeps the human engine running (metabolism) and in addition, genetic information is stored and transferred by way of nucleic acids,[4] specialised derivatives of carbohydrates.[3]

Monosaccharides are the chemical units from which all members of the carbohydrates are built.[5] While the simplest are polyhydroxycarbonyl compounds, such as D-glucose, D-galactose and D-mannose, many are known which have somewhat modified formulae, notably compounds devoid of specific hydroxyl groups, such as the deoxy-sugar D-fucose **1**[6] or the so called amino sugars which possess an amino group in place of a hydroxyl group, such as D-glucosamine **2**,[7] (figure **1.1**).

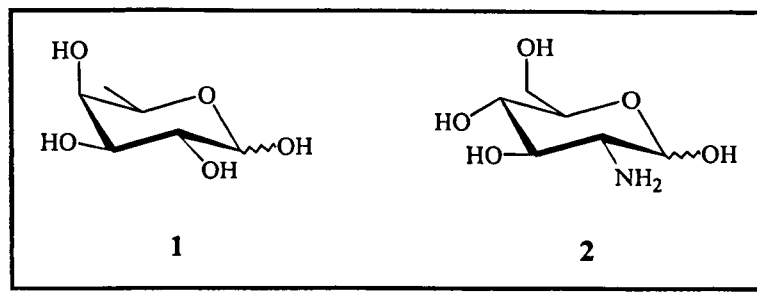


Figure 1.1

The multiple functional groups and stereocenters present in carbohydrates make them quite challenging synthetic targets for the organic chemist.[8]

1.1.1 - Structure of D-Glucose

D-Glucose is incorporated in the group of aldoses, and has two functional groups in the same molecule: a carbonyl (C=O) and a hydroxyl (OH) moiety, (figure **1.2**).

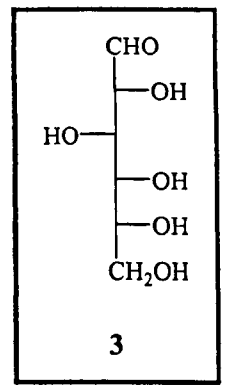


Figure 1.2: Fischer projection of D-glucose

Using neutron diffraction analysis, D-glucose **3** shows a preference for the six-membered ring **4**;[5] the numbering system is shown in figure **1.3**.

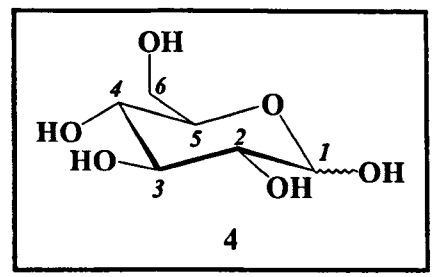
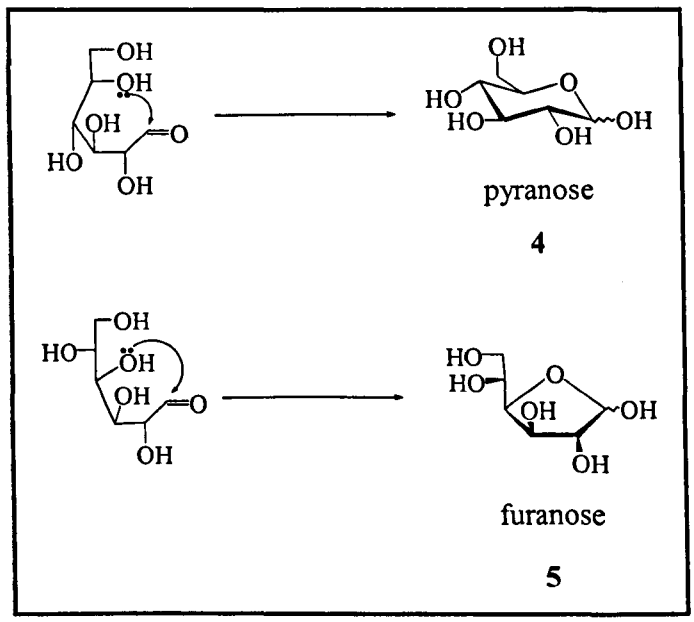


Figure 1.3: Numbering of D-glucose

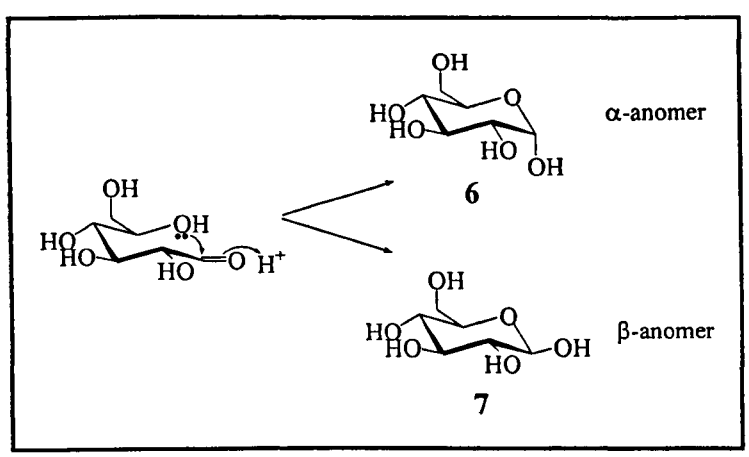
The ring closure occurs by nucleophilic attack of the hydroxyl group on the carbonyl moiety, forming a cyclic hemiacetal, where a carbon atom has two attached oxygen atoms. The resulting rings are named pyranose **4** when a six-membered cyclic

hemiacetal is formed, and a furanose **5** when a five-membered hemiacetal is obtained, (scheme **1.1**).[3, 5]



Scheme 1.1: Formation of the pyranose and furanose forms.

When the ring closure occurs by nucleophilic attack of the oxygen atom at C-5 onto the carbonyl carbon atom the resulting compound has two possible configurations (scheme **1.2**).



Scheme 1.2: Cyclisation of D-glucose

This procedure generates a new asymmetric centre at carbon C-1, called the anomeric centre. The two different structures obtained are labelled as α-anomer **6** and β-anomer **7** (scheme **1.2**).

These two anomers can be detected and differentiated by ^{1}H NMR spectroscopic analysis, by the presence in the spectrum of two doublets with a relative chemical shift between δ 4.2 ppm and δ 5.2 ppm, and by the different coupling constant *J*, (figure **1.4**). A narrow doublet at δ 5.12 ppm with a coupling constant around 3.6 Hz is characteristic of the axial proton (H-2) having an equatorial neighbouring proton (H-1) on saturated six-membered rings as is present on α-D-glucose **6**. The other doublet shown at δ 4.5 ppm with a larger coupling constant (J = 7.8 Hz) is typical for an axial H-1 with a neighbouring axial H-2, characteristic of β-D-glucose **6**.[5]

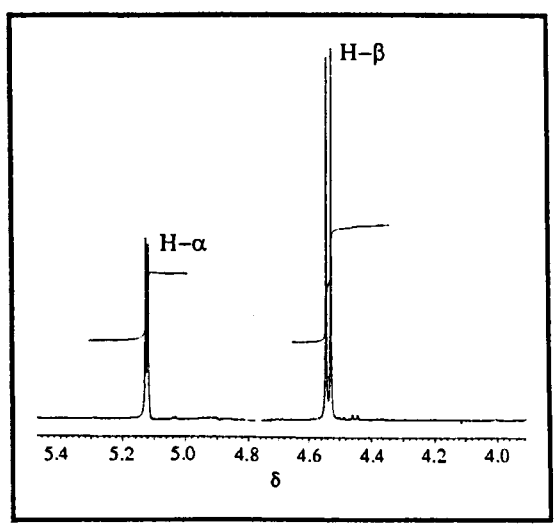


Figure 1.4: Extract of the ^{1}H NMR spectra (500 MHz) of D-glucose in D_2O[5]

Similarly, furanose **5** (scheme **1.1**) is formed by the attack of the oxygen atom at the carbon C-4 onto the carbonyl group. In D-glucose the five membered ring is

unstable compared to the six-membered ring. This observation does not apply to all sugars, as shown in table **1.1**.[5] Galactose for example, contains 6 % of the cyclic form as a five-membered ring.

Sugar	Temp. (°C)	Cyclic forms				Acyclic carbonyl form (%)
		α-pyranoside (%)	β-pyranoside (%)	α-furanoside (%)	β-furanoside (%)	
Glucose	31	38	62	-	0.14	0.02
Mannose	44	64.9	34.2	0.6	0.3	0.005
Galactose	31	30	64	2.5	3.5	0.02
Talose	22	42	29	16	13	0.03

Table 1.1: Percentage composition of various sugars in aqueous solution at equilibrium.[5]

Carbohydrates have been used as chiral synthetic intermediates in the synthesis of natural products.[8] Chemical synthesis of carbohydrate derivatives often requires tedious and extensive protecting and deprotecting steps, meaning low overall yields, and faces the problem of insufficient and often variable stereocontrol.

The broad substrate specificity exhibited by many enzymes has made the synthesis of a wide range of both natural carbohydrates and carbohydrate analogues possible.

Enzymatic acylation and deacylation has been shown to be a practical method for preparing certain carbohydrate derivatives, which are difficult to prepare chemically.[8] As the field of synthetic carbohydrate chemistry matures, enzymes are being recognised as efficient catalysts for many purposes.[8]

1.2 - Enzymes

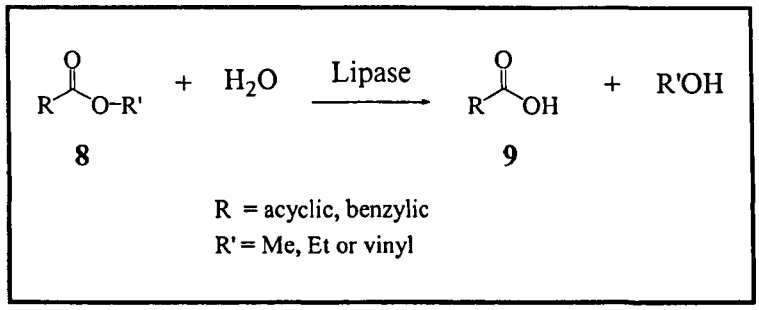
The need to design molecules with desirable properties for use in biological science and the development of economical and environmentally acceptable processes for organic synthesis have become increasingly important over the past few years. In this respect enzymes have been recognised as a useful class of catalysts. Numerous enzyme-catalysed reactions have become familiar to synthetic organic chemists, such as hydrolysis, esterification, oxidation, reduction, amide hydrolysis, Baeyer-Villiger oxidation and aldol reactions.[9]

Their ability to perform reactions in a stereospecific and regioselective manner, reducing to a minimum the use of protecting group chemistry, is one of the main attractions for the use of these biocatalysts.[2]

The majority of enzyme-catalysed reactions involve the hydrolysis of esters or the reverse process, i.e., esterification.[10] The main class of enzymes used in these reactions are lipases, which are discussed in the next section.

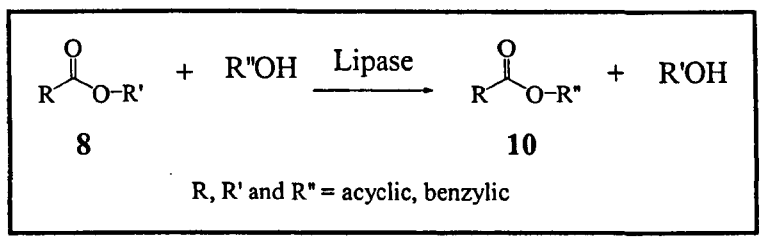
1.2.1 - Lipases

The basic schematic mechanism of hydrolysis of an ester is described in scheme **1.3**, where a lipase is used to catalyse the reaction of an ester **8**, such as a vinyl ester, with water. The reaction produces a carboxylic acid **9** and an alcohol.



Scheme 1.3: Ester hydrolysis.

Water can be substituted by alternative nucleophiles, such as alcohols or even amines. Using an alcohol as nucleophile, the resulting product will be a new ester; in this case a transesterification reaction occurs, (scheme **1.4**).



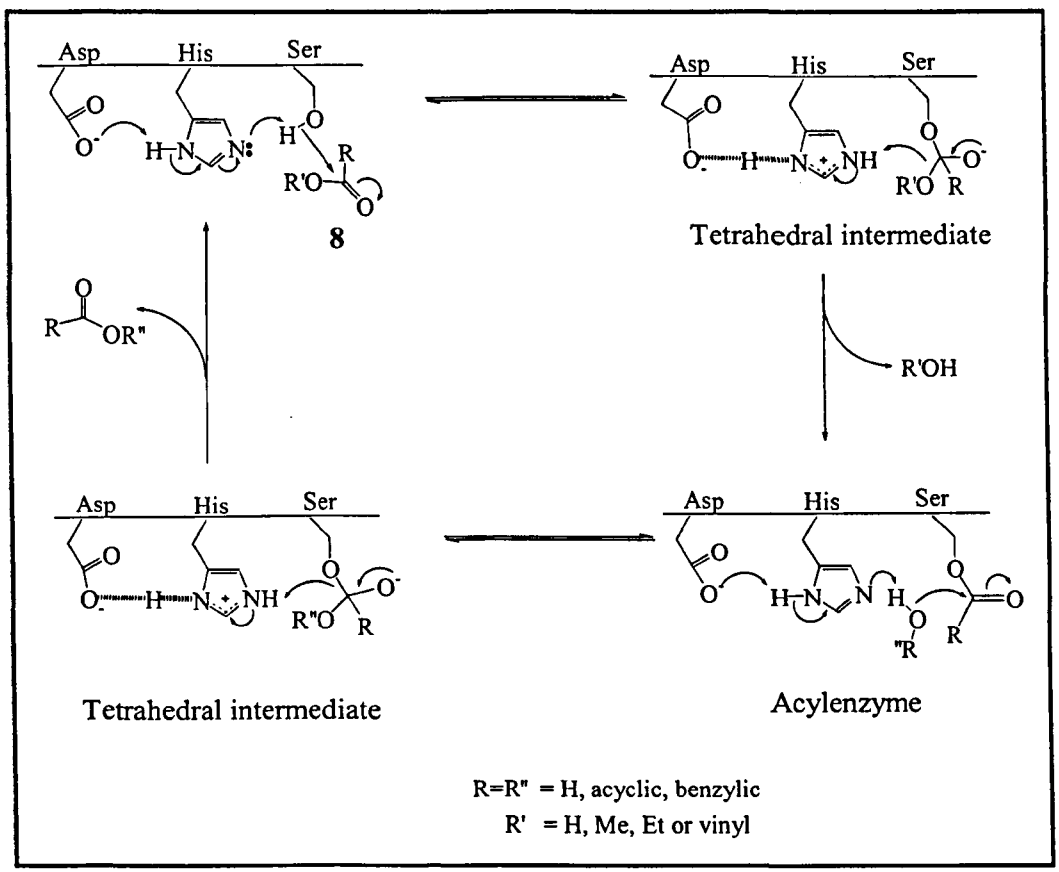
Scheme 1.4: Transesterification reaction.

The postulated mechanism at the active site of the enzyme is common to all lipases. It is well established that:

1) all lipases share primary sequence homologies including significant regions His-*X*-*Y*-Gly-*Z*-Ser-*W*-Gly or *Y*-Gly-His-Ser-*W*-Gly (where *X*, *Y*, *Z* and *W* denote generic amino acid residues)[11]

2) the serine residue at the active site is protected by a flap (or α-helical lid), which opens upon contact of the lipase with an interface and thus leads to restructuring of the lipase, which increases the affinity of the complex for lipid substrates and helps to stabilise the transition state intermediate during catalysis.[12, 13]

The general catalytic mechanism of lipases is illustrated in scheme **1.5**. Three amino acids are involved principally, Asp, His and Ser, generally known as the catalytic triad.



Scheme 1.5: General mechanism of esterification using lipases.[13]

The mechanism can be divided into two different stages, separated by an irreversible step. In the first stage, the ester (or carboxylic acid **8**) reacts with the serine residue of the active site forming the first tetrahedral intermediate. This intermediate is stabilised via hydrogen-bonding with the two other amino acids that participate in the reaction, His and Asp. The release of water (R' = H) or alcohol (R' = Me or Et) is an irreversible step forming the acylenzyme intermediate. A nucleophile, such as a molecule of water or alcohol, then reacts with the carbonyl group forming a

new tetrahedral intermediate. Subsequently, the process is followed by another step, releasing the final product, a new ester (or carboxylic acid).

Figure **1.5**, shows the X-ray structure of a *Candida rugosa* lipase, where the amino acids or the catalytic triad are presented. A hexadecylsulfonyl chloride molecule, HDS, is covalently bonded to the serine residue, and is positioned in the active site.[14]

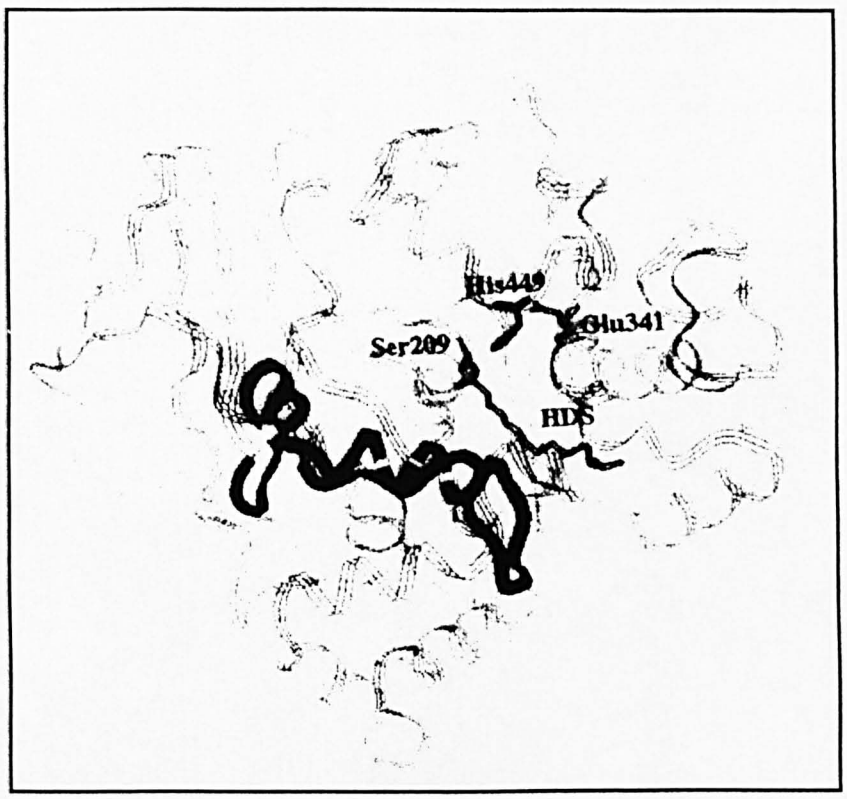


Figure 1.5: Complex formed by *Candida rugosa* lipase and HDS.[14]

Lipases are soluble in aqueous solutions (as a result of their globular protein nature), but generally their substrates, such as lipids, are not. The enzymatic acylation of sugars in aqueous solution has been reported but low yields are common, as the equilibrium for the reaction favours hydrolysis.[8] The water acts as a nucleophile on

the acylenzyme complex and hydrolyses the product of the esterification, (scheme **1.5**).

The use of organic co-solvent helps to solve the problem of lipid solubility, but monosaccharides are still only very soluble in highly polar solvents, such as DMSO, pyridine and DMF. Esterification of D-glucose in these solvents has already been reported using *Pseudomonas cepacia* lipase (PSL).[15] However enzymes have the disadvantage of not being stable in these solvents, rapidly losing their activity. Additionally these high boiling solvents can be very difficult to remove from the final product. Additionally, solvents such as methanol or *N*-methylformamide have been reported to deactivate lipases.[16]

Ionic liquids have also been applied successfully in these type of biotransformations.[16] Figure **1.6**, shows the rates of lipase-catalysed reactions as a function of solvent polarity for normal organic solvents and for ionic liquids. The acetylation of a racemic mixture of 1-phenylethanol with ethyl acetate using *Pseudomonas cepacia* lipase as catalyst was used as the model.

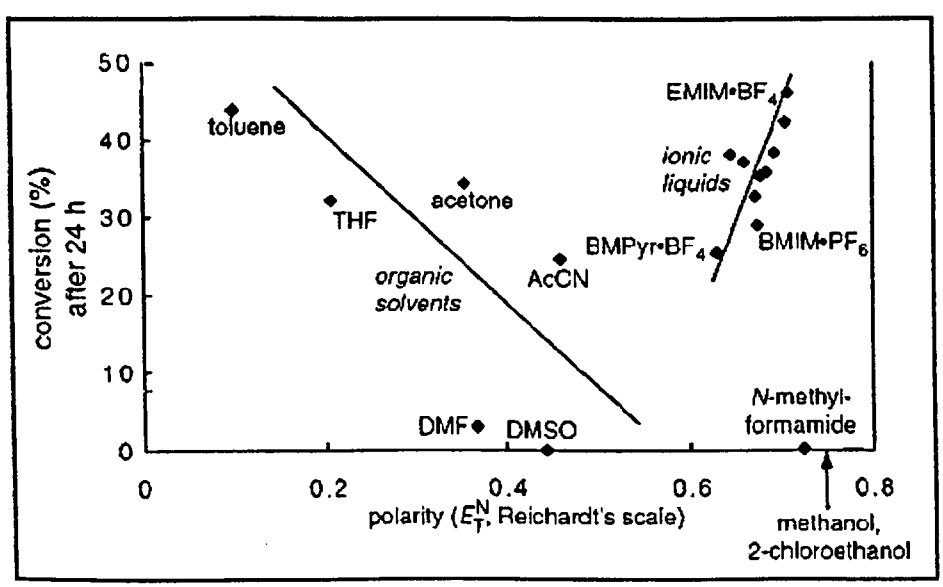
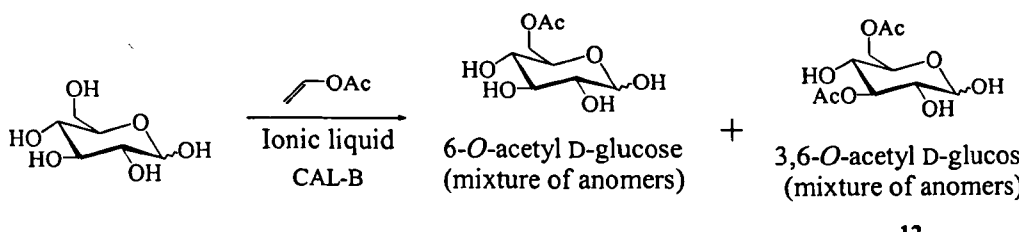


Figure 1.6: Rate of lipase-catalysed reactions as a function of solvent polarity.[16]

The acetylation proceeds well in nonpolar solvents, such as toluene or THF, almost reaching completion with toluene. With polar solvents such as DMF, DMSO or *N*-methylformamide the reaction proceeds very slowly or not at all. However, in the case of ionic liquids, whose polarity is very similar to *N*-methylformamide or methanol, the acetylation proceeds well. The use of methanol is not suitable for this acylation reaction because it would react with the acyl donor.

Interestingly, when increasing the polarity of the organic solvent the degree of conversion decreased, but when using ionic liquids the opposite effect was observed, the degree of conversion increased with the polarity.

Using ionic liquids, the acetylation of D-glucose proceeded well reaching, 99 % yield with MOEMIM·BF_4, and with 93 % formation of the 6-*O*-acetylated glucose (figure **1.7**).[16]



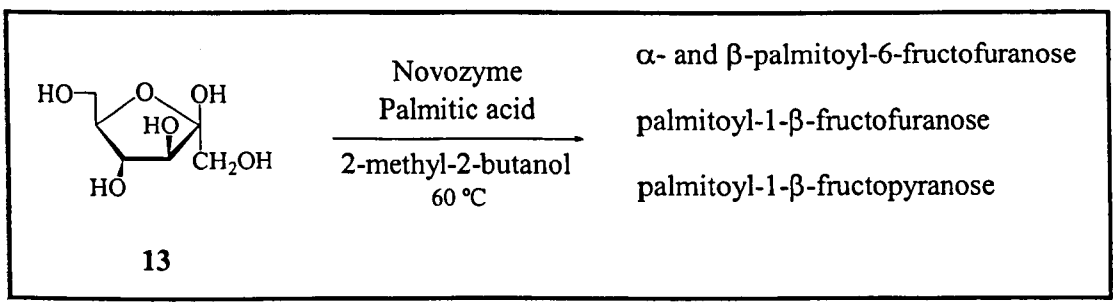
Ionic solvent	Conversion (%)	Monoacetylation (%)	6-*O*-acetyl D-glucose **11** (%) (α/β)	3,6-*O*-diacetyl D-glucose **12** (%) (α/β)
MOEMIM·BF_4	99	93	39 / 54	6.6 / 0.3
BMIM·BF_4	78	89	31 / 38	4.9 / 3.8
SBMIM·BF_4	90	88	35 / 44	6.8 / 4.0

Figure 1.7: Acetylation of D-glucose using *Candida antarctica* lipase B in ionic liquids.[16]

The high reactivity of the anomeric centre (C-1) of the monosaccharide may be used to form glycosides that are more soluble in less polar solvents. These methods will be discussed on the next section, 1.3.

Another way to increase the stability of the lipase in polar solvents is by immobilisation of the enzyme. The immobilised form of the enzyme then becomes an independent phase in the reaction and as such demonstrates increased stability at higher temperatures. For example soluble *Mucor miehei* is highly vulnerable to deactivation at 50 °C or above,[17] but its immobilised form, Lipozyme® is stable up to 60-70 °C. Moreover, several examples have been reported where immobilisation has not only improved stability but also changed the selectivity of the enzyme.[18]

Chamouleau *et al.*, using Novozyme® (a *Candida antarctica* lipase) as a catalyst in the reaction of fructose **13** with palmitic acid (scheme **1.6**), has studied the influence of the initial water activity (a_w).



13

Scheme 1.6: Reaction of fructose with palmitic acid.[19, 20]

They found that the reaction rate decreases when the initial water activity rises, (figure **1.8**).[19] This result can be explained when the role of water in the reaction is considered.

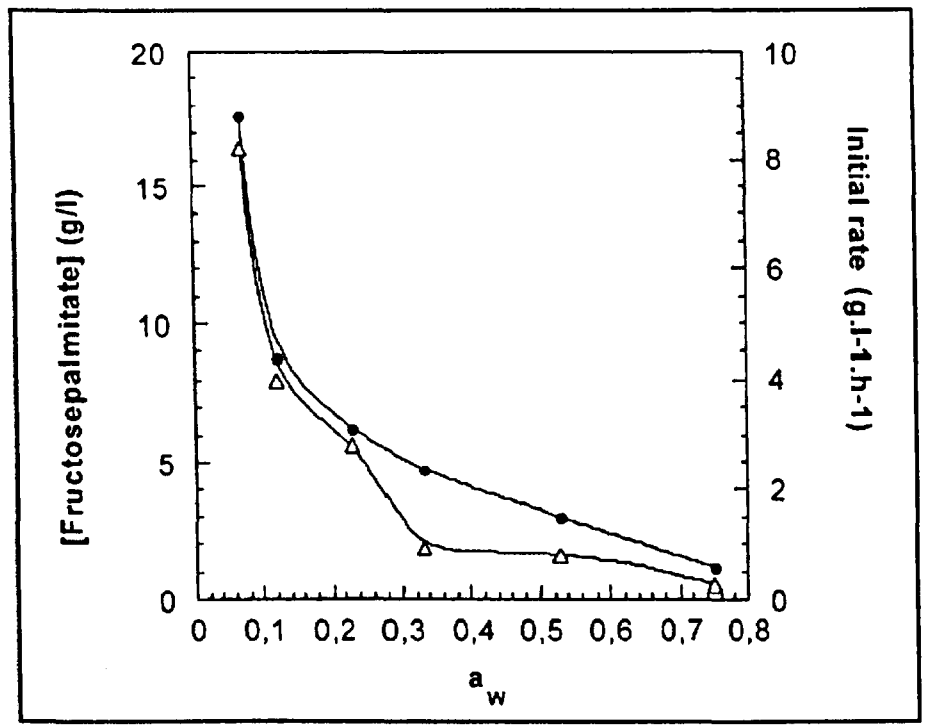


Figure 1.8: Influence of initial water activity of fructose monopalmitate steady state concentration (•) and initial rate (Δ).[19]

The water can compete with the hydroxyl group of fructose as an acyl acceptor, therefore, with the presence of a high concentration of water the equilibrium is shifted towards hydrolysis of the product. The high concentration of water can also prevent the access of the lipophilic substrate to the enzyme, by forming an aqueous layer surrounding the immobilised enzyme.[19]

This result is in accordance with the data reported by other groups, whereby addition of water provoked a decrease in the efficiency of enzymatic acylation of methyl glucopyranoside, reflected in the decrease in yield from 76.6 % to 13.3 % when 1.5 % water was added.[21]

Thus, unsurprisingly the use of molecular sieves increases the performances of the reactions, but the selectivity of the enzymes tends to decrease. This agent

removes water in the microenvironment of the enzyme and consequently the hydrophobicity increases.[19]

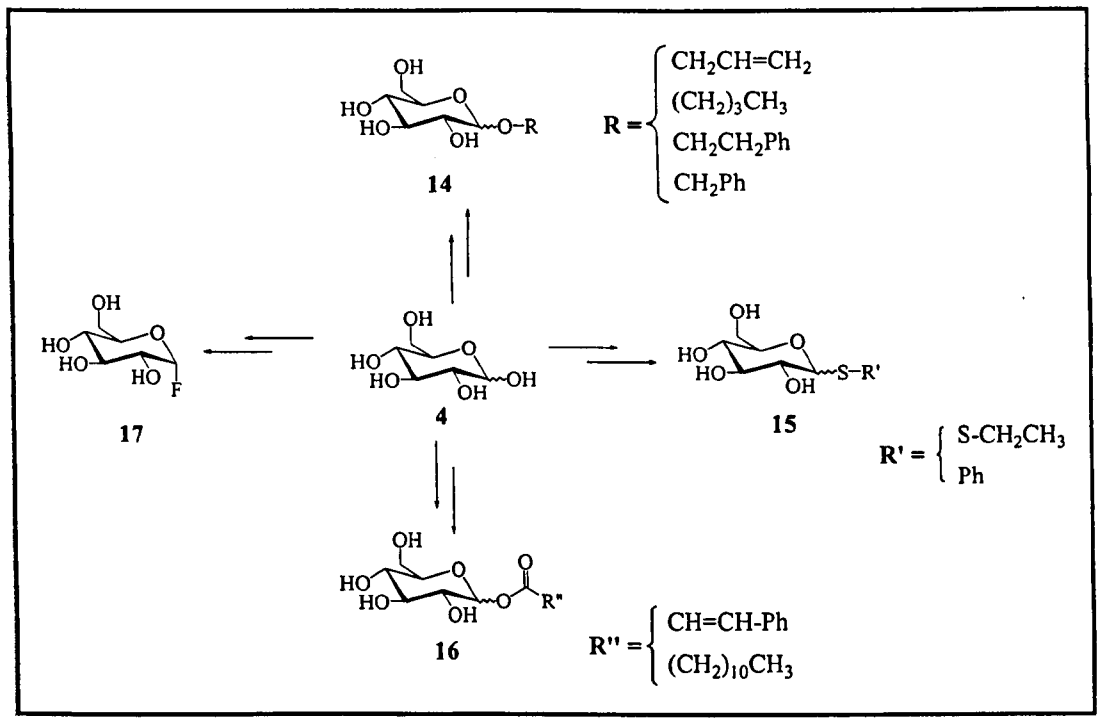
The use of reduced pressure and/or the presence of additional desiccants has been also reported to tackle the problems associated with the excess of water in the reaction mixture.[22-24]

Even after changing the reaction environment from water to an organic solvent, one of the condensation products from the reaction of the fatty acid with a sugar is water. The presence of water moved the equilibrium to the formation of the starting material when using lipases as catalysts.[22]

1.3 - Reaction at the anomeric center

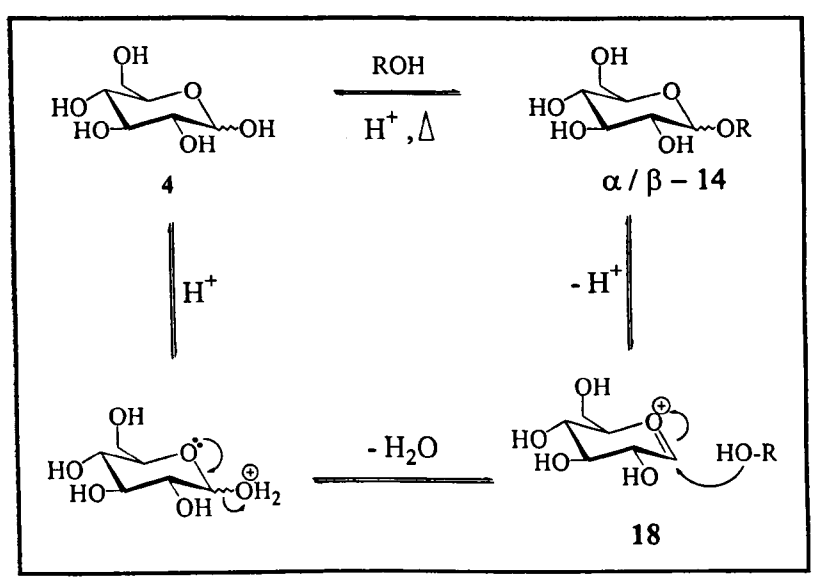
The anomeric centre is the most reactive site within the D-glucose molecule **4**, and can be used to prepare derivatives such as glycosides **14**,[22, 25-27] thioglycosides **15**,[28, 29] glycosyl esters **16**[30] and halides **17**[31] (scheme **1.7**).

Notably these processes may often be performed directly without prior protection of the additional hydroxyl groups.



Scheme 1.7: Reactions at the anomeric centre.

The synthesis of these derivatives can be achieved chemically. The simplest way is the well known Fischer glycosidation, where D-glucose and an alcohol are reacted in the presence of an acid catalyst, (scheme **1.8**).[32]

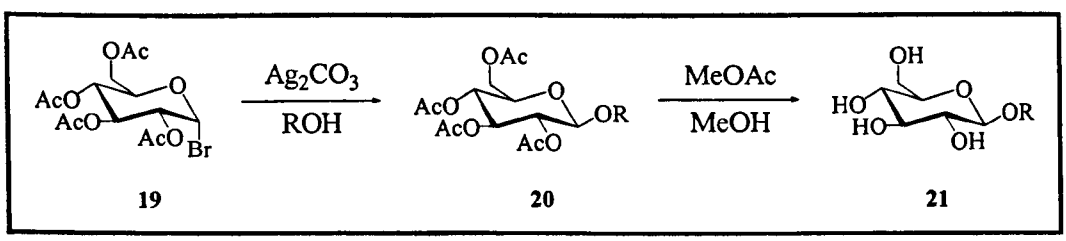


Scheme 1.8: Fischer glycosidation.

The reaction proceeds via an intermediate, the oxocarbonium ion **18**. The nucleophilic attack of an alcohol on **18** can occur on the top face of the molecule, forming the β-anomer or on the bottom face forming the α-anomer.

The resulting product formed in such a way is generally a mixture of glucopyranosides without selectivity in terms of α- or β-anomers.

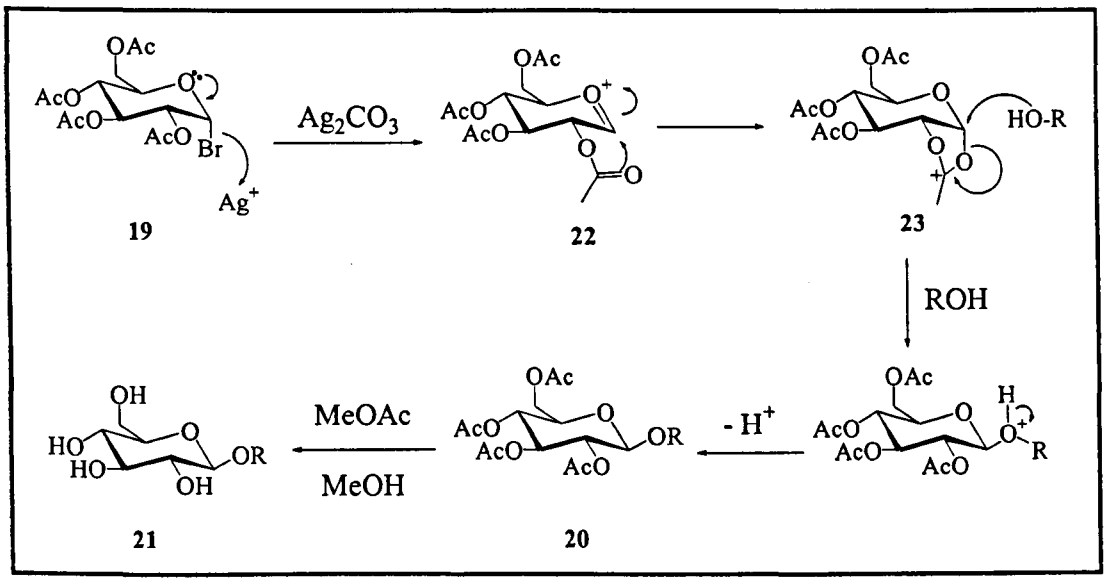
For the selective chemical synthesis of β-glucopyranosides, the Koenigs-Knorr synthesis can be used (scheme **1.9**).[33-37] Starting from the commercially available acetobromo-α-D-glucopyranoside **19**, the process of interest is followed by the removal of the acetate protecting groups, yielding the β-glucopyranoside **21** (scheme **1.9**).



Scheme **1.9**: Koenigs-Knorr synthesis.

The reaction uses silver carbonate as a Lewis acid promoting the removal of the bromine atom from the anomeric position of the acetobromo α-D-glucopyranoside **19** (scheme **1.10**). The resulting oxocarbonium ion **22** is then selectively attacked by the carbonyl of the acetate group from carbon C-2, on the bottom face, forming a new carbocation, **23**. This intramolecular reaction involves neighbouring group participation, this is the acetate group at carbon C-2. Due to this intramolecular neighbouring group participation, the α-position of the intermediate **23** is blocked and can only be attacked by a nucleophile, in this case an alcohol, at the β-position leading

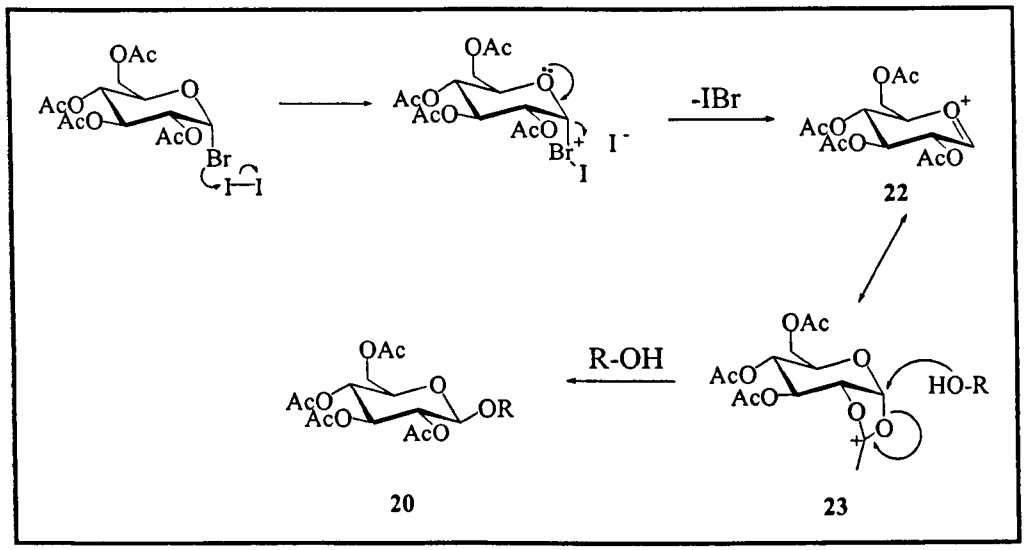
to the selective formation of the β-anomer, **20**. Removal of the acetate groups leads to the final compound, **21**.



Scheme 1.10: Mechanism of the Koenigs-Knorr synthesis

Acetobromo aldopyranoses used in conjunction with iodine are also a good alternative to the traditional Koenigs-Knorr glycosidation of a range of alcohols.[38, 39]

The mechanism is not fully understood but it is conceivable that iodine acts as a halophile resulting in iodobromonium ion formation, followed by fragmentation to give I-Br and a carbohydrate-derived oxocarbonium ion (scheme **1.11**).



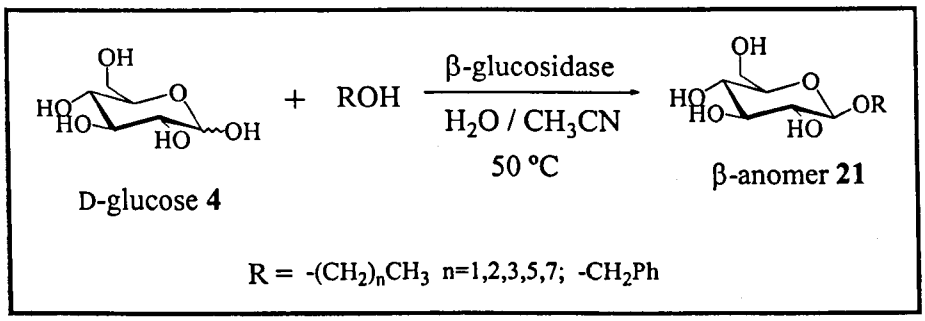
Scheme 1.11: Variation of the Koenigs-Knorr reaction.[39]

These representative examples demonstrate that the selective formation of α- and β-anomers can be achieved chemically. However, these methods typically involve multistep process where the use of protecting and deprotecting steps become necessary. Enzymes can be used in this reaction, affording moderate to good yields of the desired anomer.

Methods such as glycosidase-catalysed glycosidation reactions are stereoselective processes and are good alternatives to chemical syntheses.[2]

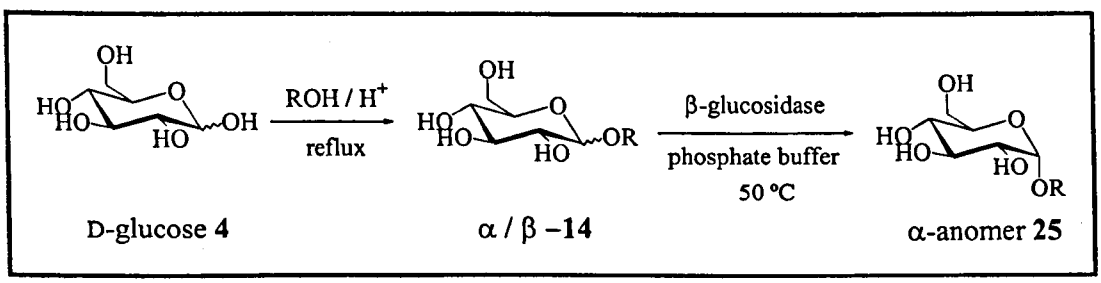
The formation of the β-glucopyranoside derivatives of a variety of primary alcohols can be achieved in one step from a carbohydrate precursor using the enzyme β-glucosidase, isolated from almonds,[25, 26] in a reversed hydrolysis or alcoholysis mode.[40]

By tuning the reaction conditions, for example by working at a high substrate concentration, β-glucosidase can also glucosylate alcohols (scheme **1.12**).[41, 42]



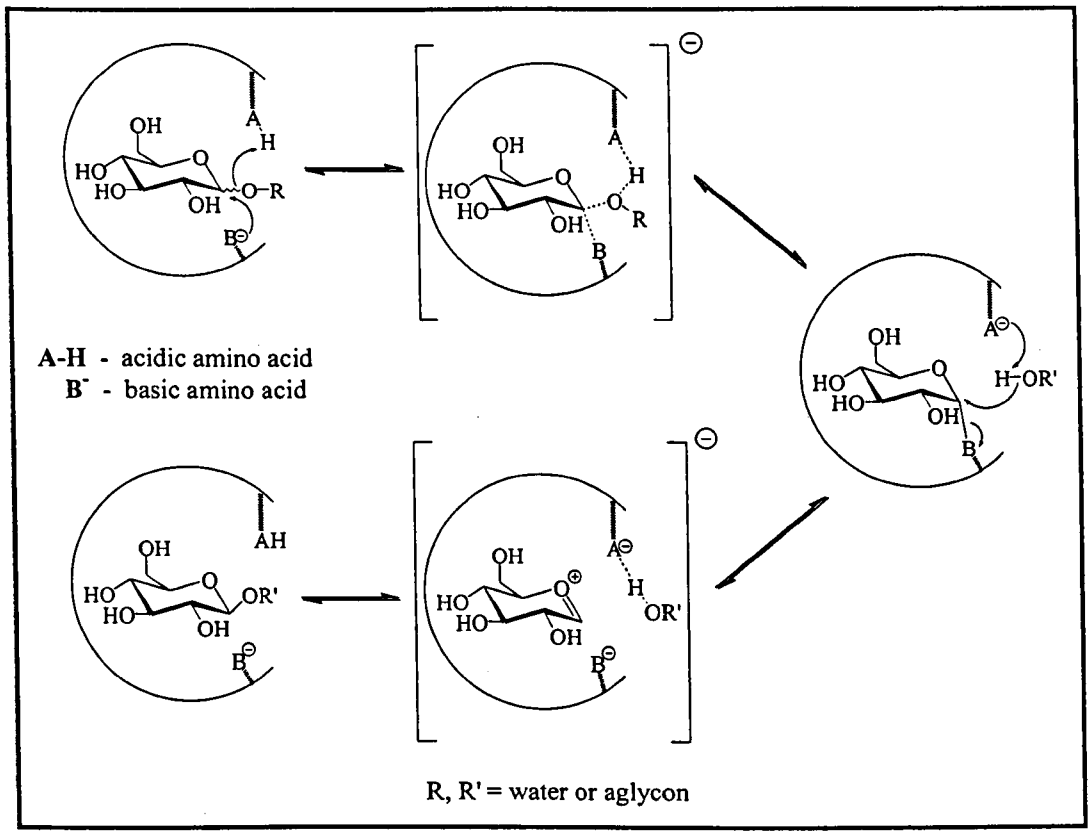
Scheme 1.12: Synthesis of different β-glucosidases.[26]

The synthesis of the α-anomer can be achieved in a two step procedure. The first step is the chemical synthesis of the mixture of anomers followed by the selective hydrolysis of the β-anomer with β-glucosidase from almonds in a buffer (scheme **1.13**).[22]



Scheme 1.13: Synthesis of the α-glucopyranosides.[22]

The mechanism of the glucosidation is shown in scheme **1.14**.



Scheme 1.14: Mechanism of the β-glucosidase.[42]

In the first step, the capacity of the aglycon (**O-R**) to act as a leaving group is enhanced by its protonation by an acidic amino acid (**A-H**). The anomeric centre is also attacked by a basic amino acid ($\mathbf{B^-}$) of the enzyme at the alpha position, forming a covalent enzyme-α-glucose complex. The complex enzyme-α-glucose then collapses forming an oxocarbonium ion. This ion suffers nucleophilic attack by water or another aglycon forming the β-glucopyranoside.

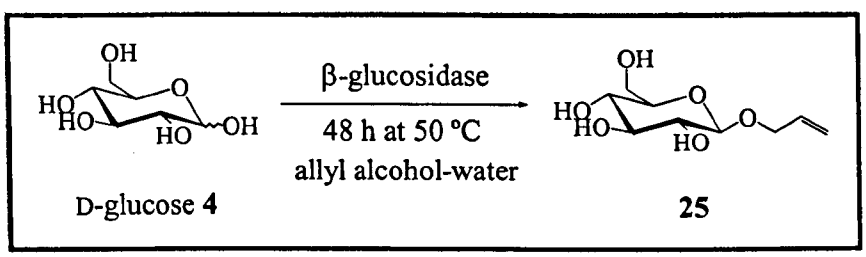
It has been proposed that the formation of the covalent enzyme-α-glucose complex, leads to the selective formation of the β-anomer by blocking the α-position.[43]

A drawback of the glucosydation reaction catalysed by β-glucosidases is the unfavourable equilibrium position that often results in low product yield.[42] However,

improvement of the product yield is possible by "tuning" the reaction conditions, such as working at high substrate concentrations,[44, 45] working at low water activities[46] or selectively removing the product.[45]

It was found that a minimum amount of water was necessary to maintain enzymatic activity when reactions were carried out in an alcohol as solvent.

Vic. *et al.*, studied the effect of water concentration in the reaction medium using the synthesis of allyl β-D-glucopyranoside **25** catalysed by β-glucosidase (Scheme **1.15**).[25]



Scheme 1.15: Synthesis of allyl β-D-glucopyranoside **25**.

Figure **1.9** shows the time course for the synthesis of allyl β-D-glucopyranoside catalysed by β-glucosidase in different allyl alcohol-water mixtures.

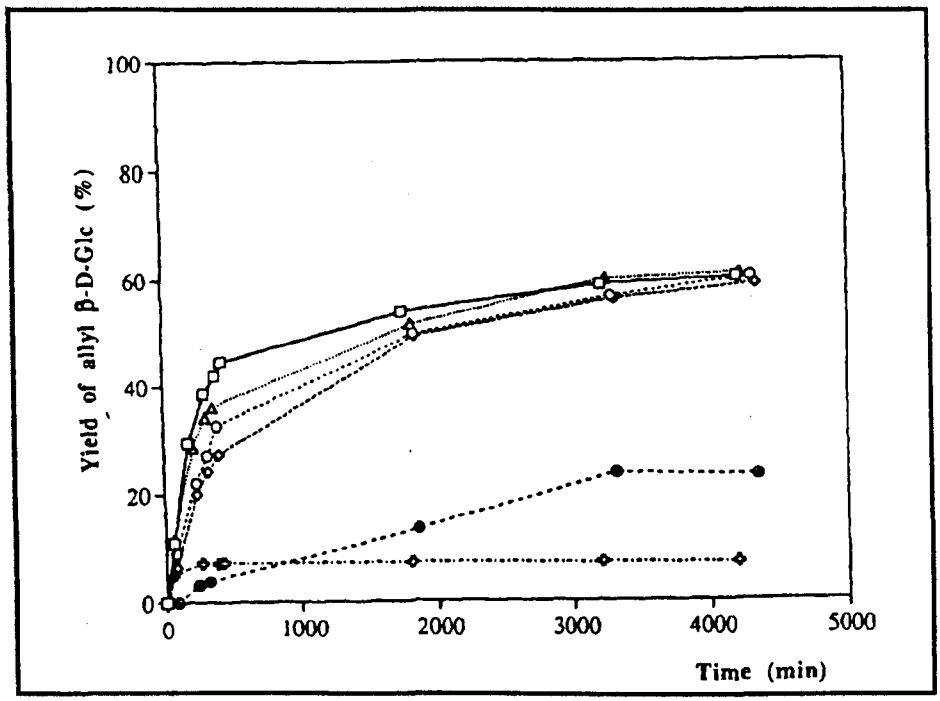


Figure 1.9: Time course of the synthesis of allyl β-D-glucopyranoside catalysed by almond β-D-glucosidase in different allyl alcohyl-water mixtures: (- · - · + · - · -) 80:20 (v / v); (—|—) 90:10 (v / v); (· · · Δ · · ·) 92:8 (v / v); (---O---) 94:6 (v / v); (---◊---) 96:4 (v / v); (---⊕---) 98:2 (v / v). D-Glucose concentration is fixed at 36 g/L. The yields, estimated by HPLC, were based on D-glucose added.[25]

The optimum yield was obtained with concentration of water between 4 and 10 % (v/v). Futher increase of the water concentration only increases the rate of synthesis.[25]

1.4 - Synthesis of sugar esters

Long fatty acid chains, containing 8 to 18 carbons, constitute an interesting group of non-ionic surfactants whose physical properties are due principally to their surface-active properties. They have important applications in detergents, food,

cosmetics and pharmaceuticals.[22, 47] They are an integral part of many industrial, agricultural and food processes.[48, 49]

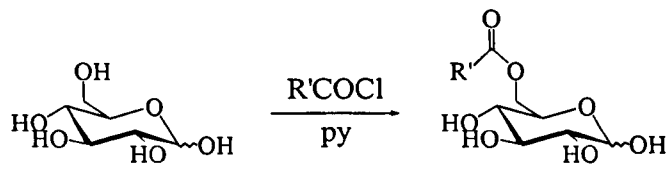
Shorter fatty acid chains, 2 to 6 carbons, are used to inhibit the growth of some viruses like Herpes virus or Epstein-Barr virus as well as tumour cells.[50, 51] However, their method of delivery (as the sodium or silver salt) and short lifetime, limit their use. The acylation of these acids with sugars such as D-glucose and D-galactose has proven to be an efficient method to deliver the acid *in vivo* (in rats), where it is then liberated by enzymatic hydrolysis.[50, 51]

In the field of synthetic organic chemistry, the efficient synthesis of oligosaccharides is still under active investigation. Most research projects have focused on the control of the stereochemistry at the anomeric position. The control of the regiochemistry at the other hydroxyl groups is usually achieved by multiple protection-deprotection procedures.[52]

1.4.1 - Acylation

In 1979 Yoshimoto *et al.*, synthesised, by a multistep procedure, a series of mono-acyl-D-glucopyranosides, such as 6-*O*-acyl, 4-*O*-acyl, 3-*O*-acyl and 2-*O*-acyl-D-glucopyranosides.[53]

The 6-*O*-acyl-D-glucopyranosides (**26** a-e) were obtained as major products by direct acylation of D-glucose **4** with acyl chlorides in pyridine, (table **1.2**). Together with the 6-*O*-acyl-D-glucopyranoside a mixture of di-*O*-acyl and tri-*O*-acyl derivatives were obtained as by-products and these were separated by chromatography.



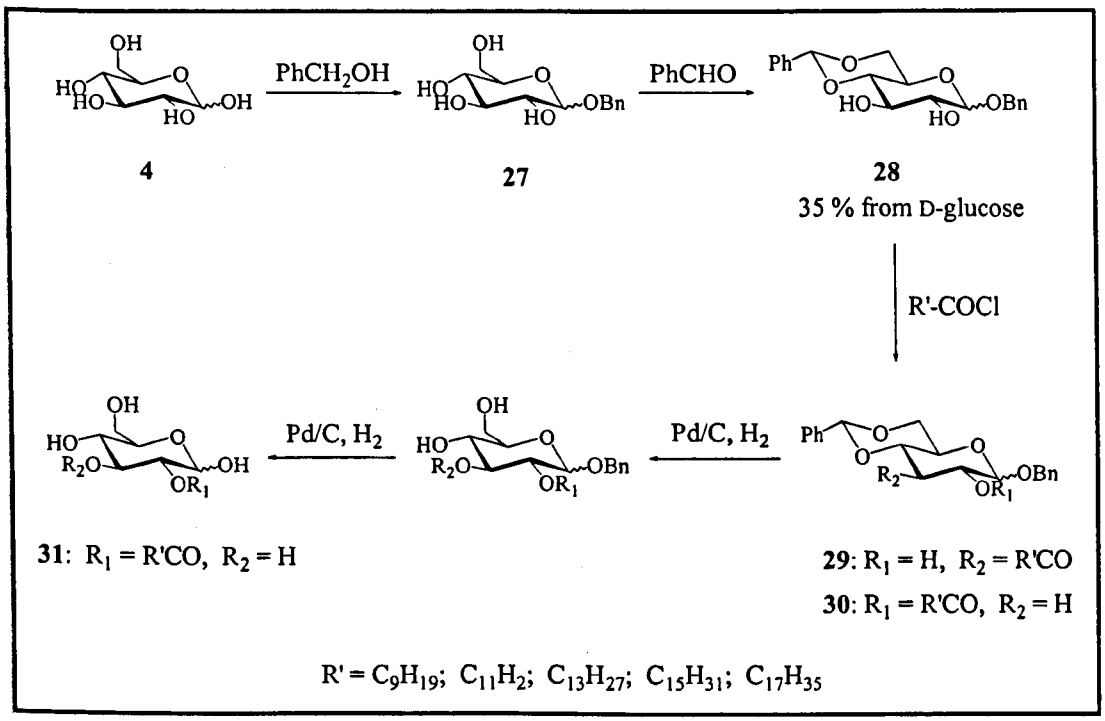
D-glucose **4** **26**

26	**R'**	**Isolated yield (%)**
a	C_9H_{19}	23
b	$C_{11}H_{23}$	25
c	$C_{13}H_{27}$	15
d	$C_{15}H_{31}$	15
e	$C_{17}H_{35}$	13

Table 1.2: Synthesis of 6-*O*-acyl-glucopyranosides.

The 2-*O*-acyl-glucopyranoside was synthesised by using a 4 step procedure. The key intermediate, benzyl 4,6-*O*-benzylidene-α-D-glucopyranoside **28**, was prepared in 35 % yield from D-glucose **4** via benzyl α-D-glucopyranoside **27**. This intermediate was then partially acylated with the acyl chlorides affording the 2-*O*-acyl-D-glucopyranosides **29** in 40 to 50 % yield. 3-*O*-Acyl-D-glucopyranosides **30** were also obtained in very small quantities. The benzyl and benzylidene protecting groups were selectively removed under mild conditions using Pd/C and H_2, affording the unprotected 2-*O*-acyl-D-glucopyranosides **31** in 80 to 85 % yield (scheme **1.16**).

The overall yield of the 2-*O*-acyl-D-glucopyranosides **31** was *ca.* 30 % from D-glucose.

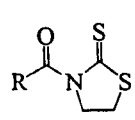


Scheme 1.16: Synthesis of 2,6-*O*-acyl and 3,6-*O*-acyl-glucopyranosides.[53]

Due to the high reactivity of an acid chloride, useful levels of selectivity can only be achieved by protection and deprotection of the hydroxyl groups.

Thus, Plusquellec *et al.*, regioselectively esterified non-protected glucose and protected glucose, mannose and galactose derivatives at the 6-position, using activated esters **32, 33** and **34** (table **1.3**).[54, 55]

The reactions were performed in anhydrous pyridine in the presence of NaH:DMAP or in DMF using triethylamine or DBU as the base or catalyst. The reaction time varied from 0.5 h to 2 h, depending on the nature of the sugar. Methyl-α-D-glucopyranoside was reacted with **32** at room temperature in pyridine without additional initiator or catalyst, but required 16 h to reach completion.



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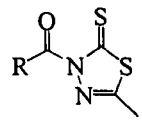

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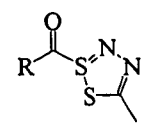


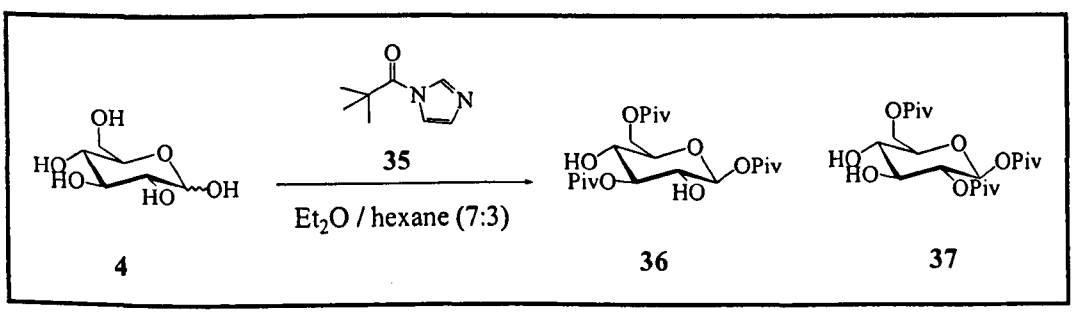
34

R = CH_3-$(CH_2)_n$; n = 6, 8, 10, 12, 14, 16

Glycopyranoside	Yield (%)
Methyl α-D-glucopyranoside	55 – 88
Methyl α-D-mannopyranoside	45 - 55
Methyl β-D-galactopyranoside	75
α-D-glucose	60 - 67
α-D-galactose	66

Table 1.3: Synthesis of 6-*O*-monoesters using activated esters.[54, 55]

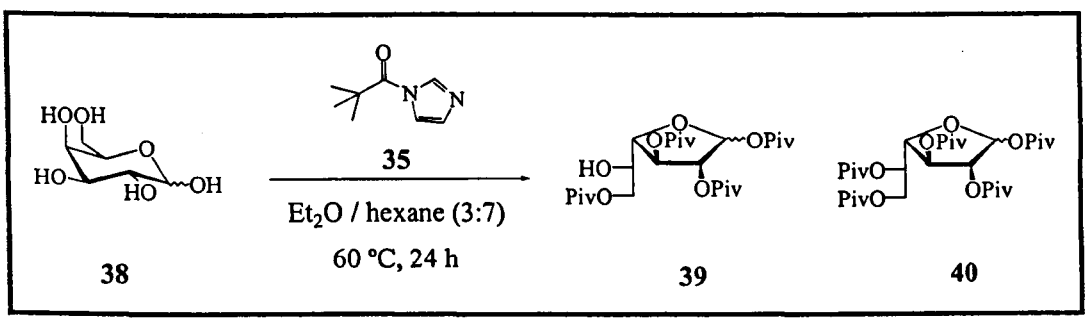
Selective acylation of carbohydrates has also been achieved by combining the different reactivity of the hydroxyl groups with the use of hindered carboxylic acid chlorides, such as pivaloyl chloride (^{t}BuCOCl).[56] The reaction of D-glucose **4** with a less reactive pivaloyl species, such as *N*-pivaloyl imidazole **35** (6 eq.), at room temperature for 8 h, led to a mixture of tripivaloylated derivatives, 1,3,6-tri-*O*-pivaloyl-β-D-glucopyranoside **36** in 33 % yield, and 1,2,6-tri-*O*-pivaloyl-β-D-glucopyranoside **37** in 48 % yield (scheme **1.17**).[57]



4 36 37

Scheme 1.17: Selective acylation of D-glucose using pivaloyl derivatives.

However, by increasing the temperature to 60 °C and changing the solvent to Et_2O / hexane (1:1), the only product obtained was the tripivaloylated compound **36** in 50 % yield. When D-mannose was similarly pivaloylated at 60 °C, the resulting product was a complex mixture of tripivaloylated and tetrapivaloylated mannopyranoside.[57] D-Galactose **38** showed a different behaviour and the corresponding acylation reaction led to protected furanoside derivatives. Thus, acylation with *N*-pivaloyl imidazole **35** (5 eq.) for 24 hours at 60 °C, gave a mixture of 1,2,3,6-tetra-*O*-pivaloyl-D-galactofuranoside **39** in 55 % yield and 1,2,3,5,6-penta-*O*-pivaloyl-D-galactofuranoside **40** in 19 % yield, (scheme **1.18**).[57]



38 **39** **40**

Scheme 1.18: Acylation of D-galactose with *N*-pivaloyl imidazole.[57]

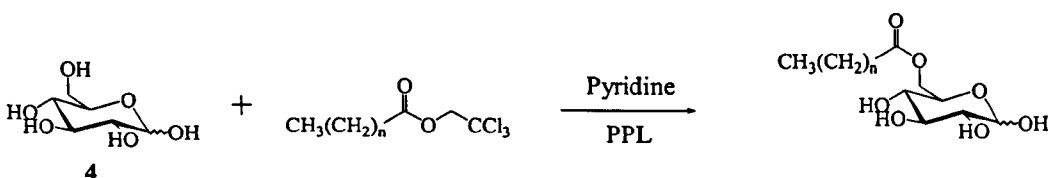
1.4.2 - Enzymatic method

As discussed above, the use of chemical methods for the acylation of sugars generally involves protection and deprotection, often leading to low yields. Additionally, a mixture of products is also obtained in many chemical processes due to the lack of selectivity.

Therefore, enzymes have been employed for the selective (usually regioselective) transformations of readily available carbohydrates, including selective acylations[8, 18, 58-64] and deacylations.[8, 18, 65-67]

Monoacylated sugars have been obtained by lipase,[67-69] or protease-catalysed[70] esterification or transesterification in pyridine or DMF. In these reactions, yields are often low considering the amount of enzyme used. The efficiency of the acylation has shown to be dramatically dependent on both the substrate and on the acylating reagent.[55]

Selective esterification of the primary hydroxyl group of unprotected D-glucose **4** in pyridine using 2,2,2-trichloroethyl butyrate, (n=2 in table **1.4**), and porcine pancreatic lipase (PPL) as catalyst was reported in 1986.[68]



N	Regioselectivity (%)	Isolated yield (%)[a]
0	85	76
2	82	50
6	84	57
9	95	91

a) Calculated taking into account the degree of conversion and the regioselectivity achieved

Table 1.4: Acylation of free D-glucose with 2,2,2-trichloroethyl esters.[68]

The reaction was performed using 6 grams of D-glucose and 18 grams of crude enzyme. The reaction mixture was shaken at 250 rpm and 45 °C for 2 days, yielding 50 % of the 6-*O*-butyryl-D-glucose.[68] Subsequently, this procedure was applied to

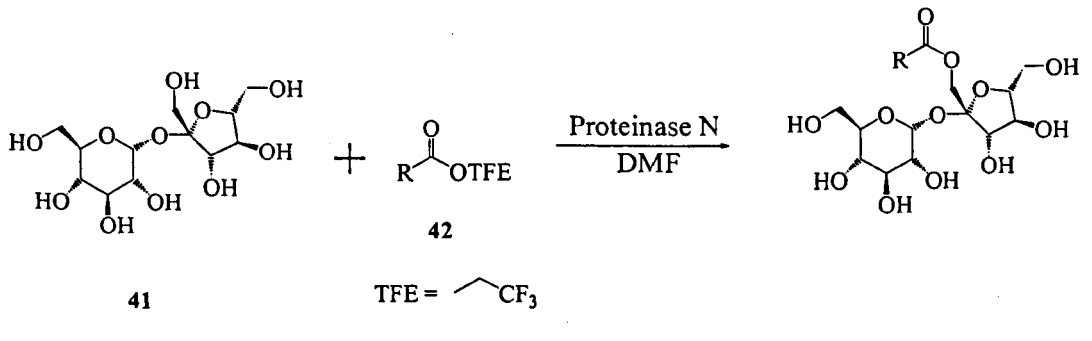
other sugars, such as D-mannose and D-galactose, and similar high levels of regioselectivity were obtained.

The main problems of this reaction were the use of dry pyridine and a large excess of enzyme compared to the amount of substrates.

Usually lipases have proven to be inactive in DMF, although the protease subtilisin has been employed for the regioselective acylation of the primary hydroxyl group of D-glucose in anhydrous DMF.[71] In this reaction, 77 % conversion was achieved after 5 days, yielding 66 % of the isolated 6-*O*-butyryl-D-glucose.[71] Disaccharides, such as maltose, cellobiose, lactose and sucrose were also acylated with an average yield of 50 %, with (in the case of maltose and cellobiose) a regioselectivity greater than 95 % for the primary hydroxyl group. With lactose the enzyme was less selective, furnishing the 6-*O*-mono-butyryl-lactose with 75 % regioselectivity as the main product and 10 % each of the 3-*O*- and 4-*O*-butyryl-lactose. Concerning the two saccharides present in lactose (D-galactose and D-glucose) the enzyme only reacts with the glucose moiety.

Surprisingly, with sucrose, the hydroxyl group acylated was the anomeric position of the fructose moiety with 90 % regioselectivity, whereas in the chemical acylation the most reactive OH is the primary hydroxyl group.[72]

The same result in the esterification of sucrose **41** were obtained by Potier *et al.* using a different enzyme (proteinase N) and using activated trifluoroethyl (TFE) esters **42** in anhydrous DMF (figure **1.10**).[73]

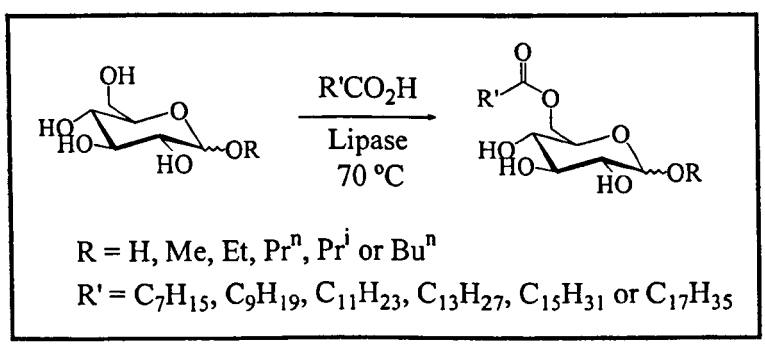


R	n-C_3H_7	i-C_3H_7	(*E*)-CH_3CH=CH-	H_2C=$C(CH_3)$-
Analytical yield (%)	76	43	6	15

Figure 1.10: Acylation of sucrose using proteinase N as catalyst in dry DMF.[73]

The presence of up to 7 % (v/v) of water in the reaction medium increases the rate of the reaction. This result can be explained by the formation of an optimised conformation of the proteinase N enzyme. Further increasing the amount of water in the reaction mixture resulted in lower yields since, it was argued, water becomes a competitive nucleophile.[73]

Lipase catalised regioselective esterification of the primary hydroxyl group of D-glucopyranosides with long fatty acid chains (C_8-C_{18}) has been successful.[47] The reaction was performed by mixing D-glucose with the fatty acid in the presence of an immobilised enzyme, *Candida antarctica* lipase, at 70 °C under reduced pressure to remove the water formed during the reaction (scheme **1.19**).



Scheme 1.19: Esterification of glucopyranosides with long fatty acid chains.[47]

Generally, this type of D-glucose esterification has proved to be very slow.[65, 74] The reaction of D-glucose with dodecanoic acid at 70 °C and *Candida antarctica* lipase shows a conversion lower than 5% after 24 hours (table **1.5**).[47] Even with methyl α-D-glucopyranoside the reaction only reached 53.3 % conversion after 24 hours.

Carbohydrate	Conversion after 24 h (%)	Diester (%)
α-D-glucose	< 5	-
Methyl α-D-glucopyranoside	53.3	3.5
Ethyl D-glucopyranoside	92.5	4.9
Isopropyl D-glucopyranoside	93.2	4.2
n-Propyl D-glucopyranoside	95.6	17.3
n-Butyl D-glucopyranoside	94.4	21.8

Table 1.5: Conversion of different glucopyranosides.[47]

Ethyl D-glucopyranoside has shown a dramatic change in reactivity compared with glucose or methyl α-D-glucopyranoside. This higher reactivity could be due to higher solubility of the glucopyranoside in the reaction medium.

The selective acetylation of D-glucose in an organic solvent such as acetone or THF has been also tried but resulted in low selectivity.[16] The poor selectivity is likely to be due to solubility problems of D-glucose in these organic solvents.

The results indicate that by increasing the carbon chain length at the anomeric position, greater reactivity has been achieved resulting in a greater quantity of diester being formed.

The esterification was found to be catalised by a variety of lipases, such as *Candida antarctica*, *Mucor miehei*, *Humicola sp.*, *Candida cylindracea* and

Pseudomonas sp.[47] However, the best results were obtained with *Candida antarctica* lipase with its high conversion at 24 hours and limited formation of diesters, (table **1.6**). *Candida antarctica* lipase is active even at low water activities.[75]

Lipase	After 24 h		After 48 h	
	Conversion	Diester	Conversion	Diester
Candida antarctica	96.1	3.1	96.3	5.2
Mucor miehei	97.9	19.3	97.9	28.5
Humicola sp.	99.0	45.8	99.7	64.3
Candida cylindracea	21.2	1.2	39.6	6.1
Pseudomonas sp.	1.5	--	14.3	21.6

Table 1.6: Rates of conversion of ethyl D-glucopyranoside with dodecanoic acid using different lipases.[47]

The length of the acyl chain at the anomeric position is not the only factor to influence the rate of conversion, the α- and β-anomers have also shown a difference in reactivity.

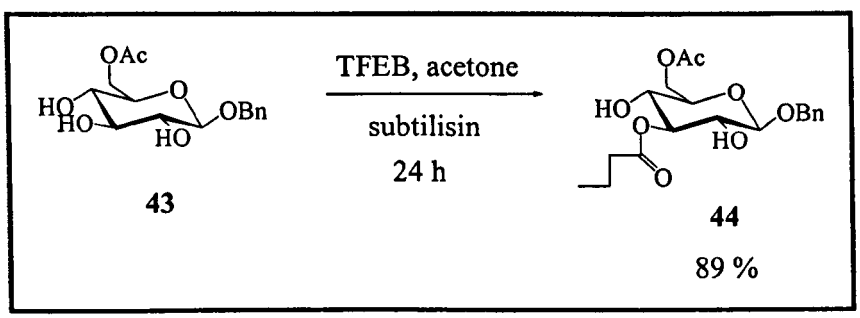
Adelhorst *et al.*, have shown that, typically, the α- and β-ethyl D-glucopyranosides show different reactivities in the enzyme-catalysed esterification reaction at the 6-group. Ethyl β-D-glucopyranoside exhibits about twice the reactivity of the ethyl α-D-glucopyranoside.[22] This may be explained by the different polarities of the anomers and thereby their solubilities in fatty acids or, alternatively, the enzyme's preference for the β-anomer.

The primary hydroxyl group has been proven to be the most reactive site and this observation can be explained by the fact that is the least sterically hindered position in the molecule.

Kurahashi *et al.*, has constructed enzyme-like catalysts for regioselective functionalisation of unprotected sugars, by introducing substituents regioselectively to a desired OH group through non-covalent interactions. These catalysts are chiral cavities constructed around 4-dimethylaminopyridine (DMAP), 4-pyrrolidinopyridine or imidazole.[52] Unexpectedly, in the DMAP-catalised acetylation reaction, secondary OH groups of glucose were preferentially acetylated in the presence of the primary OH group at position 6.[52]

Fabre *et al.*, has reported the synthesis of a 2,6-di-*O*-stearoyl butyl-α-D-glucopyranoside in 96 % yield after 5 days of reaction, catalised by Lipozyme in hexane and starting from the monoacylated 6-*O*-stearoyl derivative.[27]

The acylation of benzyl 6-*O*-acetyl β-D-glucopyranoside **43** with trifluoroethyl butanoate in the presence of subtilisin, gave the benzyl 3-*O*-butanoyl 6-*O*-acetyl β-D-glucopyranoside **44** in 89 % yield (scheme **1.20**).[76]

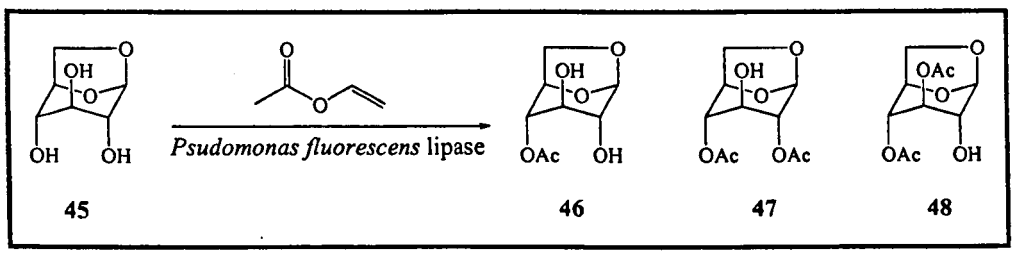


Scheme 1.20: Selective acylation of benzyl 6-*O*-acetyl β-D-glucopyranoside.[76]

The acetyl group introduced at the 3-position considerably reduced the reactivity of the neighbouring 4-OH group.[52] This observation can be explained by the increased steric hindrance at this position.

The use of anhydro sugars was also reported. In these cases the anomeric position and the primary hydroxyl group are "protected". The regioselective

transesterification of 1,6-anhydro-β-D-glucopyranose **45** was achieved using *Pseudomonas fluorescens* lipase. After 7 days, the 4-monoacetate **46** 81 %, the 2,4-diacetate **47** 9 % and the 3,4-diacetate **48** 6 % were formed (scheme **1.21**).[77]

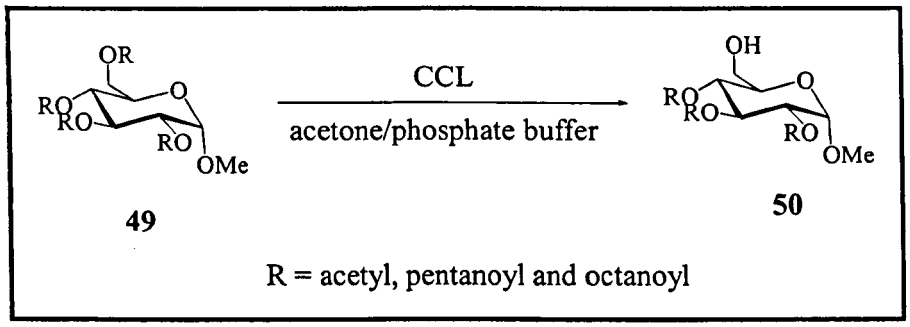


Scheme 1.21: Acylation of anhydrous glucose.[77]

1.4.3 - Deacylation

Regioselective deacylation is another way to synthesise sugar esters. For this reaction the main issue is the solubility of the pentaacylated sugar in the solvent, generally an aqueous buffer. This problem is generally overcome with the use of organic co-solvents, generally mixtures of DMF / buffer[78] or acetone / buffer.[67]

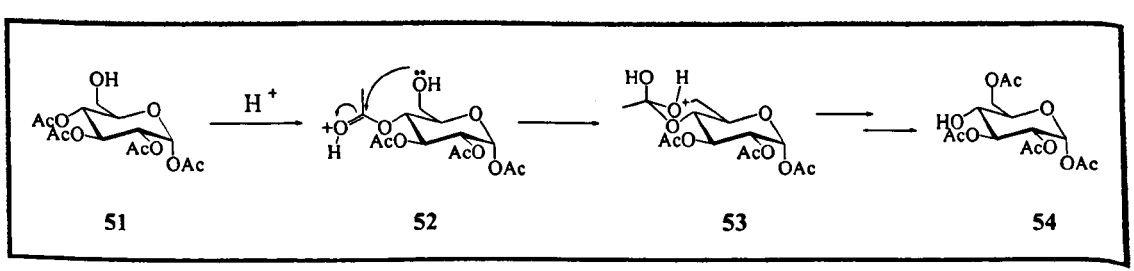
The most reactive position in the deacylation reaction has been shown to be the primary position. Thus, regioselective deacylation of the primary hydroxyl group of methyl 2,3,4,6-tetra-*O*-acyl-D-hexopyranosides **49** has been achieved with good yields (80 – 90 %) using *Candida cylindracea* lipase (CCL) as the catalyst (scheme **1.22**).[67]



Scheme 1.22: Regioselective deacylation of methyl tetra-*O*-acyl-glucopyranosides.[67]

Peracylated methyl glycosides have also been hydrolysed using CCL as catalyst in a solution of 10 % (v/v) DMF/phosphate buffer.[78] *Aspergillus niger* lipase has been used for the hydrolysis of 1-*O*-acetyl groups of di-, tri- and higher oligosaccharides.[79] Hydrolysis of peracylated sugars with the esterase from rabbit serum demonstrates a preference for the anomeric centre, followed by the primary position.[80] After initial deacylation of the primary position, intramolecular migration of acetyl groups towards the 6-OH has been shown to lead to complete deprotection of the sugar.[80] The mechanism of the acyl migration is explained in scheme **1.23**, where, in the presence of an acid, the 4 → 6 acyl migration can be accomplished. The oxonium ion intermediate **53** formed can adopt a six-membered ring chair-like conformation (scheme **1.23**).[66]

The use of apolar solvents such as hexane have been shown to limit this acyl-group migration.[27]



Scheme 1.23[66]

Chapter 2

Synthesis of sugar esters

2.1 - Project aims

The selective esterification of the primary and secondary hydroxyl groups of D-glucose, D-mannose and D-galactose was explored using vinyl butyrate and a variety of enzymes as catalysts. The thermal stability of the resulted sugar esters and the release pattern of butyric acid moieties under pyrolysis conditions were investigated.

Prior to the esterification, the anomeric position, C-1, was protected to mask its high reactivity; simultaneously this protection helps to increase the solubility of the glycosides in solvents such as THF and chloroform.

Both ethyl and benzyl groups were used to protect the anomeric position. The ethyl protecting group was already shown by other workers to increase the reaction rate as compared to the methyl protecting group or free glycosides;[47] descriptions of the syntheses of the required ethyl D-glycopyranosides were available in the literature.[26, 81-83]

The benzyl group was also tested, due to the selective conditions necessary for its removal, generally with H_2 in the presence of Pd/C as catalyst.[84]

After the protection at the anomeric position, the glycopyranosides were screened against several commercially available enzymes (table **2.1**). At the same time, a small scale reaction where the glycopyranosides were reacted with vinyl butyrate in the absence of enzyme was performed to verify if the esterification was chemical or indeed catalysed, as expected, by the enzymes.

Novozyme 435 an immobilised *Candida antarctica* lipase B on acrylic resin.
Esterase from hog liver immobilised on Eupergit C.
Pancreatin® from hog pancreas.
Lipozyme® an immobilised lipase from *Mucor miehei.*
Candida antarctica lipase immobilised in a sol-gel-AK support.
Lipase PS-C II from Amano, an immobilised lipase from *Pseudomonas cepacia.*
Pseudomonas cepacia lipase, as a free enzyme.
Lipase AY® from Amano, a *Candida rugosa* lipase.
Lipase AK® from Amano, a lipase from *Pseudomonas fluorescens.*
Acylase I, from *Aspergillus melleus*.

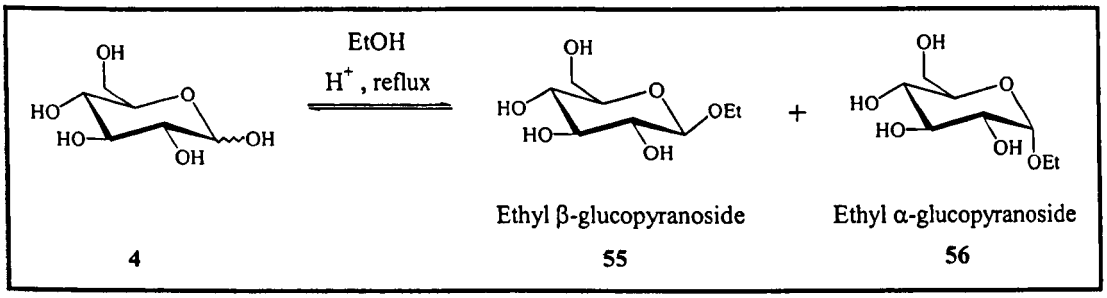
Table 2.1: Enzymes screened for the esterification of glucopyranosides.

2.2 - D-Glucose

2.2.1 - Ethyl-D-glucopyranoside

When D-glucose reacts with ethanol in the presence of catalytic amount of HCl or an ion exchange resin (H^+ form) at reflux for 12 h, the resulting product after column chromatography is a syrup of ethyl D-glucopyranoside.

The resultant ethyl D-glucopyranoside is a mixture of anomers, the ethyl β-glucopyranoside **55** and ethyl α-glucopyranoside **56** (scheme **2.1**).



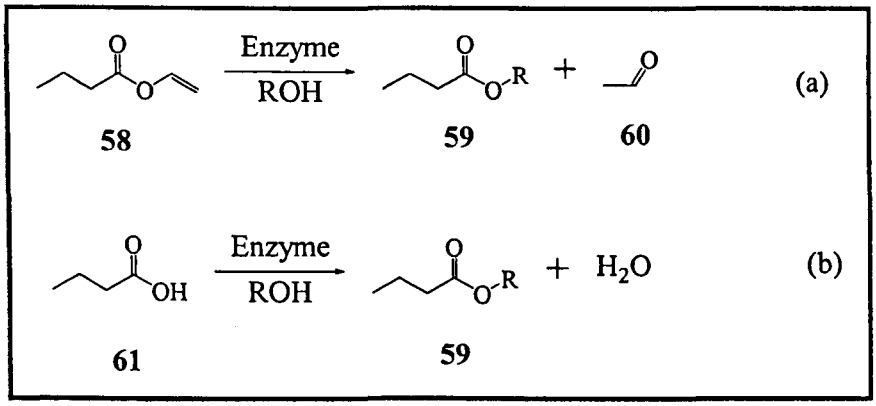
4 55 56

Scheme 2.1: Synthesis of ethyl D-glucopyranoside.

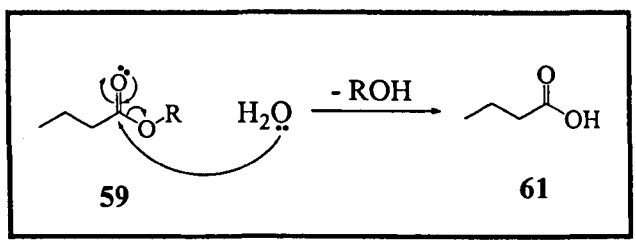
This reaction is an example of the Fischer glycosidation (for the mechanism, see chapter 1, scheme **1.8**).

The ethyl D-glucopyranoside **57** was screened for selective esterification with the enzymes described above (table **2.1**). The reactions were performed on a small scale with 10 % (w/w) of enzyme and using vinyl butyrate as both the acyl transfer agent and the solvent at 35 °C.

The choice between vinyl butyrate **58** and butyric acid **61**, both commercially available, was driven mainly by consideration of the side product formed in the reaction. With vinyl butyrate **58** the side product is acetaldehyde **60**, which is volatile (scheme **2.2**, (a)). Direct esterification of a carboxylic acid with an alcohol leads to the production of water (scheme **2.2** (b)). The presence of water in the esterification reaction is thermodynamically unfavorable to ester synthesis,[19] and moves the equilibrium toward the hydrolysis of the products (scheme **2.3**).



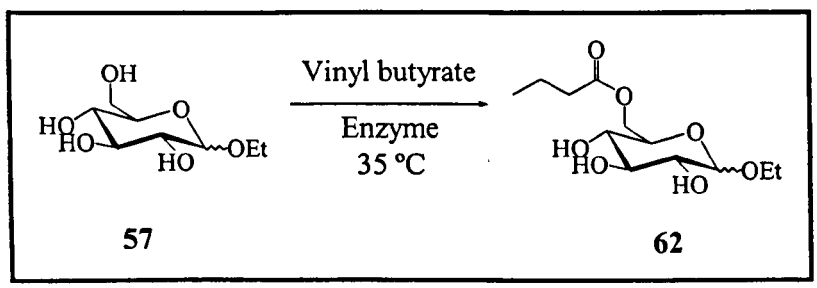
Scheme 2.2: Reaction of butyric acid and vinyl butyrate with an alcohol.



Scheme 2.3: Hydrolysis of an ester.

Several methods have been used to remove water, such as the use of high vacuum,[22, 47] low boiling-point azeotrope,[85] semipermeable silicone tubing,[86] saturated salt solution,[86] sparging dried air through the reaction medium,[87 88] and using molecular sieves in a reflux trap.[89] For instance, the use of high vacuum has been shown to be very effective in solvent-free systems, but cannot be applied to solvent-phase reactions.[87] The use of molecular sieves has been shown to have a detrimental effect on the enzyme.[90] Reflux of the solvent through a trap is limited by the number of solvents which have suitable boiling points.[86]

After two hours of reaction, only two enzymes had shown reaction by thin layer chromatography (TLC), namely Lipozyme and Novozyme 435. The reaction with Novozyme 435 was cleaner than with Lipozyme, presenting only a major spot and very small impurity. Both ^{1}H and ^{13}C NMR spectroscopic analysis of the major product of the reaction confirmed this compound to be ethyl 6-*O*-butyryl D-glucopyranoside **62**, as a mixture of α- and β-anomers (scheme **2.4**).



Scheme 2.4: Esterification of ethyl D-glucopyranose.

The yields vary from 72 % for Lipozyme after 3 hours of reaction to 83 % for Novozyme 435 after only 2 hours of reaction.

The reaction at the primary hydroxyl group was expected, because from all the hydroxyl groups present in ethyl D-glucopyranoside, the primary hydroxyl group is the most reactive, mainly due to its being less hindered.[53, 81, 91-94]

Most enzymes are only stable to temperatures below 40 °C, but Lipozyme and Novozyme 435 have already been used at higher temperatures, e.g. 70 °C.[22, 47, 93]

Increasing the temperature from 35 °C to 60 °C gave a decrease in the reaction time, especially when Novozyme 435 was used. With Novozyme 435 the reaction time decreased to 1 hour with ethyl 6-*O*-butyryl D-glucopyranoside **62** being observed by TLC.

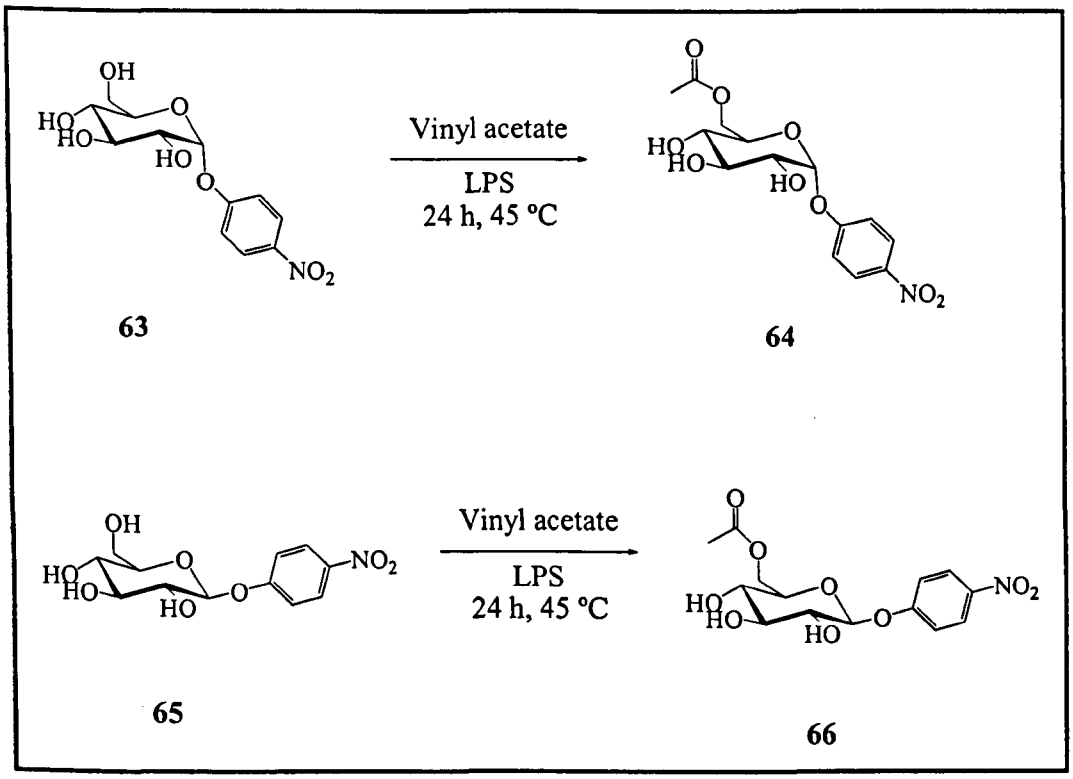
The solubility of ethyl D-glucopyranoside in neat vinyl butyrate proved to be problematic, with some starting material remaining insoluble after 10-20 minutes of

reaction. The addition of 5 % (v/v) of THF helped to solve the solubility problem, however the enzymes showed a different reactivity in the presence of THF. With two equivalents of vinyl butyrate and THF as solvent, the time for the reaction catalysed by Lipozyme increased from 3 to 4 hours; the yield remained the same. However, using Novozyme 435 as catalyst the reaction time and the yield was unchanged.

After the esterification of the primary position, only secondary hydroxyl groups remain in the glucopyranoside.

Studies aimed at the selective esterification of the secondary alcohols were then attempted using the enzymes already described. However, due to the presence of the α- and β-anomers, the reaction led to the formation of a complex mixture. Consequently, the isolation and identification of the different products proved to be difficult.

Watanabe *et al.*, have reported a difference in reactivity of the acylation of the α- and β-anomer of *p*-nitrophenyl D-glucopyranoside with vinyl acetate and lipase *Pseudomonas cepacia* (LPS) as catalyst in pyridine. When *p*-nitrophenyl α-D-glucopyranoside **63** was used, a 76 % yield of the *p*-nitrophenyl 6-*O*-acetyl α-D-glucopyranoside **64** was obtained, while the acylation of the *p*-nitrophenyl β-D-glucopyranoside **65** gave only a 19 % yield of the corresponding β-anomer **66** (scheme **2.5**).[95]

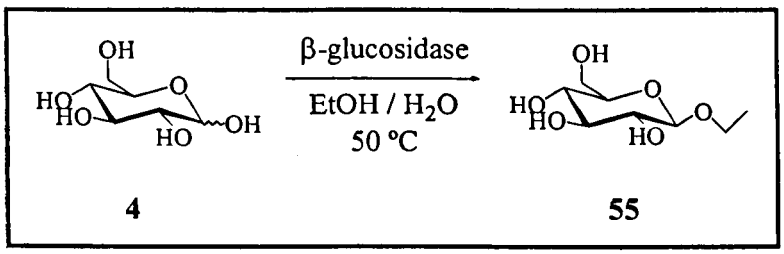


Scheme 2.5: Esterification of *p*-nitrophenyl D-glucopyranoside

This might be explained by differences in the steric hindrance and stereoelectronic effects of the anomeric substituents.[95] Therefore, it was concluded that in order to simplify the identification of the second esterification, the selective synthesis of the α- and β-anomers must be achieved.

2.2.2 - Ethyl β-D-glucopyranoside

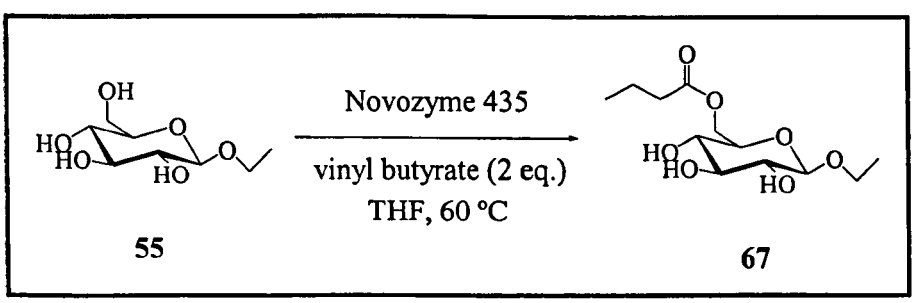
Ethyl β-D-glucopyranoside was synthesised from D-glucose in 85 % yield, using the enzyme β-glucosidase from almonds (scheme **2.6**).[25 26]



Scheme 2.6: Enzymatic synthesis of ethyl β-glucopyranoside.

Several enzymes, (table **2.1**) were then screened for the selective esterification of the primary alcohol of ethyl β-glucopyranoside in the presence of vinyl butyrate at 35 °C. As expected, based on the result discussed previously, only two enzymes gave a positive reaction, Lipozyme and Novozyme 435. Both enzymes were tested in different concentrations of the acylating agent, but no major differences were detected between either the reactivity or the selectivity of the two enzymes.

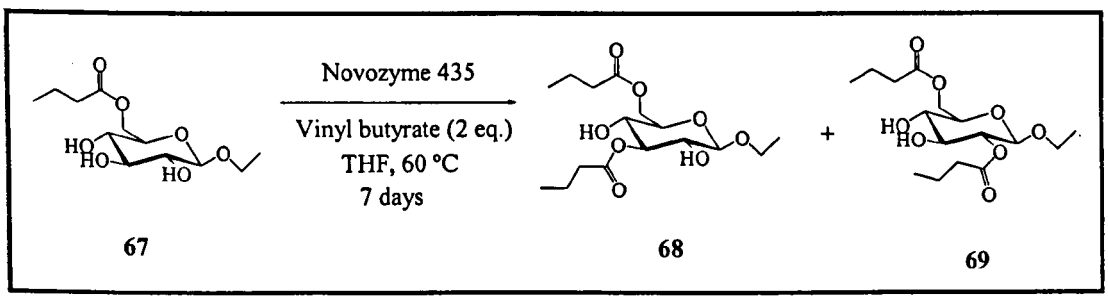
Ethyl 6-*O*-butyryl β-glucopyranoside **67** was obtained in all the reactions, however Novozyme 435 gave a better yield and a cleaner reaction (scheme **2.7**).



Scheme 2.7: Synthesis of ethyl 6-*O*-butyryl β-D-glucopyranoside.

Several enzymes were then screened for the selective esterification of the remaining secondary hydroxyl groups in **67**, but Novozyme 435 was the only enzyme to produce new compounds (by TLC), even after two weeks.

The products of this esterification were ethyl 3,6-*O*-butyryl β-D-glucopyranoside **68** (50 % yield) and ethyl 2,6-*O*-butyryl β-D-glucopyranoside **69** (30 %) (scheme **2.8**). In the absence of enzyme, no esterification was observed. The identification of the position of esterification was assigned by ^{13}C, ^{1}H and correlation NMR (COSY, HMQC and HMBC).



Scheme 2.8: Acylation of the secondary alcohols of D-glucose.

The esterification of the second hydroxyl group was found to be very slow, taking 7 days to reach completion. Even with long reaction times, the enzyme was stable and was reused 10 times without loss of reactivity or selectivity.

The formation of the 2,6-diester could be due to a migration of the ester from the 3- to the 2- position (or vice-versa). It is known that an acyl group can easily migrate to a neighbouring OH group especially under basic conditions.[52, 66]

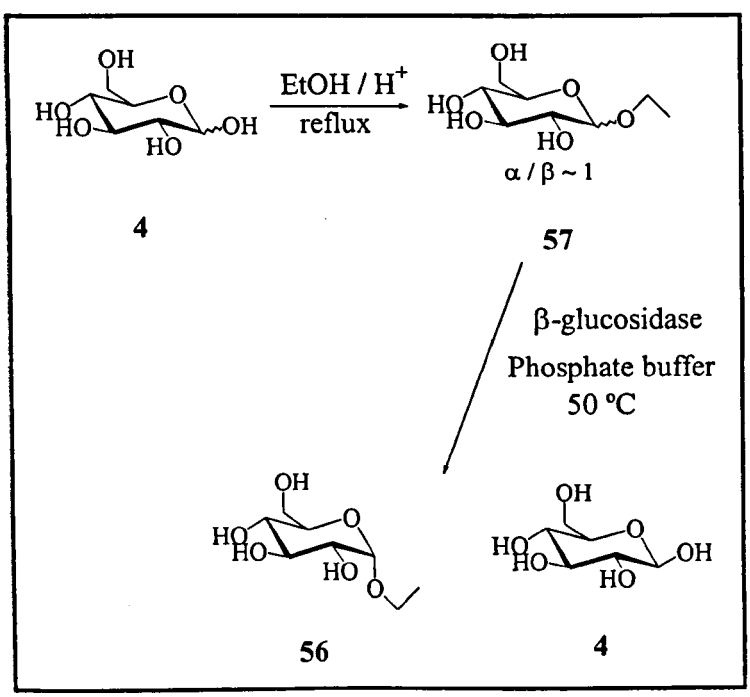
To verify if the acyl group migrates, the 3,6-esterified sugar **68** was heated to 60 °C in THF. The same reaction was applied to the 2,6-ester. No new products were observed, and consequently no migration of the acyl group took place, these experiments indicate that the second acylation reaction is unselective.

Several groups have also reported similar 3,6- selectivity for the enzymatic or chemical esterification of secondary hydroxyl group of glycosides. [28 74, 96, 97]

Attempts at the further esterification of one of the remaining hydroxyl groups in ethyl 3,6-*O*-butyryl β-D-glucopyranoside **68** and ethyl 2,6-*O*-butyryl β-D-glucopyranoside **69**, were unsuccessful. No reaction was detected with any of the enzymes tested. It is interesting to note that Kurahashi *et al.* has shown that an acetyl group introduced in the 3-position considerably reduced the chemical reactivity of the neighbouring 4-OH group.[52]

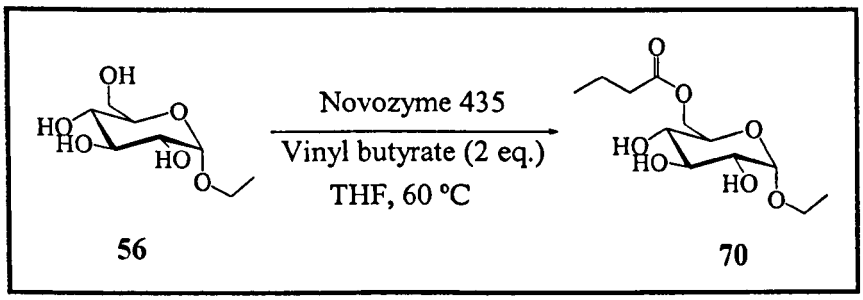
2.2.3 - Ethyl α-D-glucopyranoside

Ethyl α-D-glucopyranoside was synthesised from D-glucose following a two-step process. The first step was the synthesis of ethyl-D-glucopyranoside, as a mixture of anomers, and the second step was the selective hydrolysis of the β-anomer in a buffered solution of β-glucosidase, yielding the pure α-anomer in 30 % yield over two steps (scheme **2.9**).[22]



Scheme 2.9: Synthesis of ethyl α-glucopyranoside

The resulting α-glycopyranoside was esterified with vinyl butyrate using Novozyme 435, yielding a single product, ethyl 6-*O*-butyryl α-D-glucopyranoside **70** in 60 % yield (scheme **2.10**).



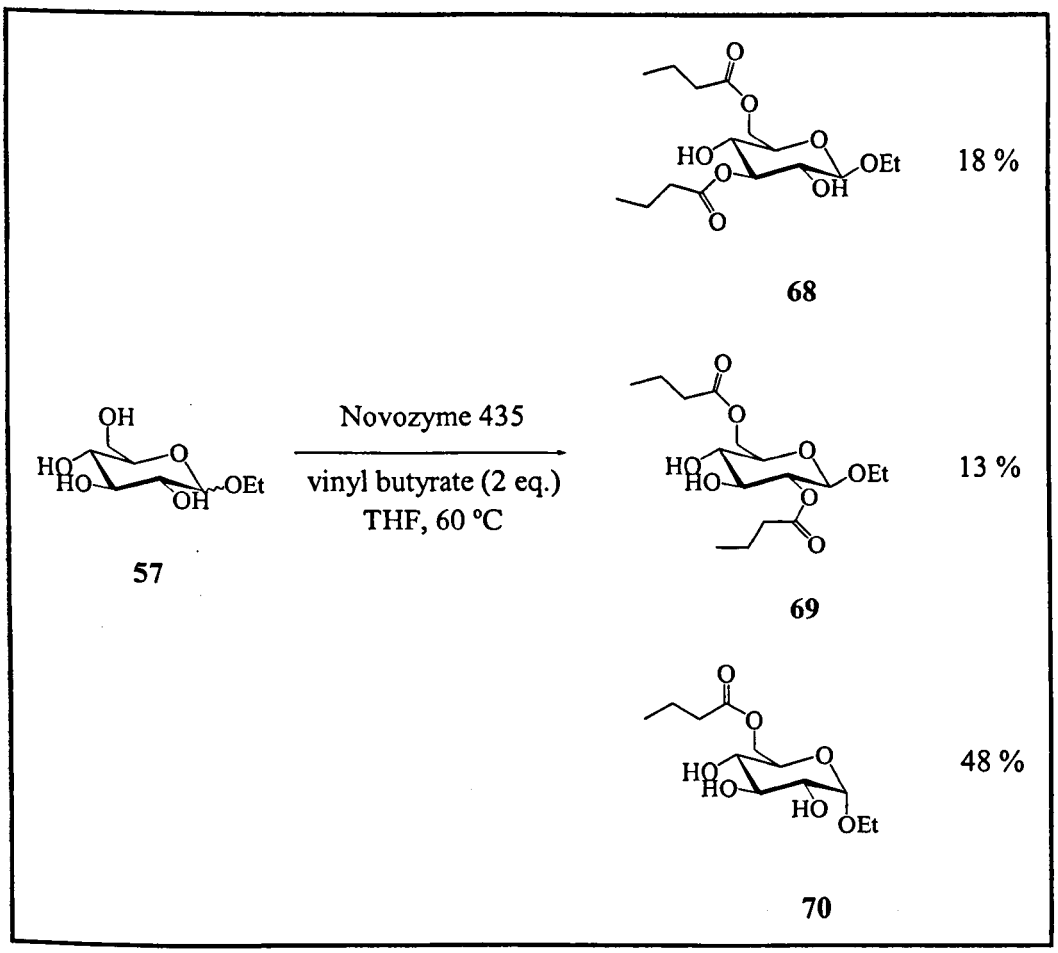
Scheme 2.10: Esterification of ethyl α-D-glucopyranoside.

It was apparent that the α-anomer **56** reacted slower than the corresponding β-anomer **57** for this 6-OH esterification: i.e., 1 hour for the β-anomer **57**, against 6 hours for the α-anomer **56**. Previous work performed by Adelhorst *et al.*, involving the esterification of the same glycopyranosides with fatty acids (C_8-C_{18}) and using Lipozyme (a lipase from *Mucor miehei*) as catalyst indicated that the β-anomer **57** displayed about twice the reactivity of the α-anomer **56**.[22]

When the 6-*O* ester **70** was tested with different enzymes for the esterification of the secondary alcohols, no reaction was detected even after two weeks. In this case the presence of the ethyl group in α-position may prohibit the enzyme-glucopyranoside complex being formed, and consequently the remaining hydroxyl groups cannot be acylated.

Using this difference in reactivity between the α- and β-anomers towards esterification of the secondary hydroxyl groups, experiments aimed towards the development of a new system for the separation of anomers were performed. Thus, when ethyl D-glucopyranoside **57**, as a mixture of α- and β-anomers, was reacted with

vinyl butyrate in the presence of Novozyme 435 for 4 days the resulting product was a mixture of three products. The diesterified β-anomers, ethyl 3,6-*O*-butyryl β-D-glucopyranoside **68** (18 % yield), ethyl 2,6-*O*-butyryl β-D-glucopyranoside **69** (13 % yield) and the monoesterified α-anomer ethyl 6-*O*-butyryl α-D-glucopyranoside **70** (48 % yield) (scheme **2.11**).

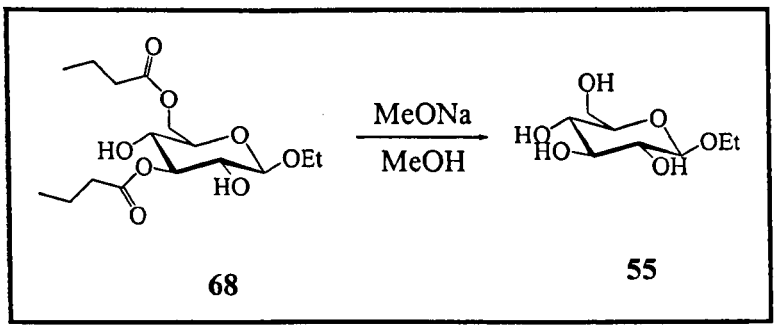


57 **68** **69** **70**

Scheme 2.11

The selective cleavage of the esters from ethyl 3,6-*O*-butyryl β-D-glucopyranoside **68** with sodium methoxide gave ethyl-β-D-glucopyranoside **55**, as the pure β-anomer **55** in quantitative yield (scheme **2.12**).

The same method was applied for the ethyl 2,6-*O*-butyryl β-D-glucopyranoside **69** and ethyl 6-*O*-butyryl α-D-glucopyranoside **70**, giving pure ethyl β-D-glucopyranoside **55** and α-D-glucopyranoside **56**, respectively.



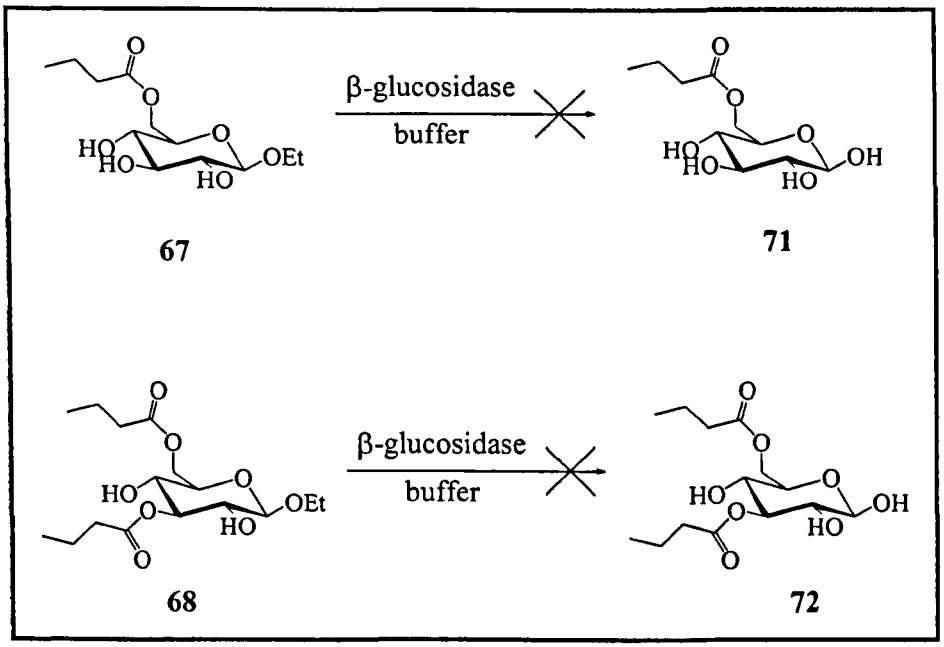
Scheme 2.12

2.2.4 - Selective removal of the ethoxy group

After the selective introduction of the butyric moieties into D-glucose **4**, the following step was the removal of the ethoxy group, which was employed to increase the solubility of D-glucose in more suitable solvents for the enzymatic reactions. The most direct method to remove this group would be with an acid, but the presence of acid sensitive butyric esters does not permit the use of acids.

Previously, the enzyme β-glucosidase, from almonds, was used to insert the ethyl group at the anomeric centre in the β-position. By changing the solvent from an alcohol to water or buffer, the same enzyme could be used to cleave selectively the ether bond at the anomeric position.

β-Glucosidase was used in different buffer solutions, from pH 6.5 to pH 8. In almost all cases no reaction was observed, and when a reaction was observed the product was D-glucose (scheme **2.13**).



Scheme 2.13: Attempt for the selective removal of the ethoxy group.

The difficulty for the glucopyranoside to "fit" in the active site could explain the lack of reactivity of the enzyme; an alternative explanation is that the other hydroxyl groups present on the molecules are necessary for the enzyme to recognise the glucopyranoside. The presence of a large excess of water could also catalyse the loss of the butyric moieties leading to the formation of D-glucose. The hydrolysis with β-glucosidase in THF with 10 % water was also tried, but in this case no reaction was detected.

2.2.5 - Benzyl D-glucopyranoside

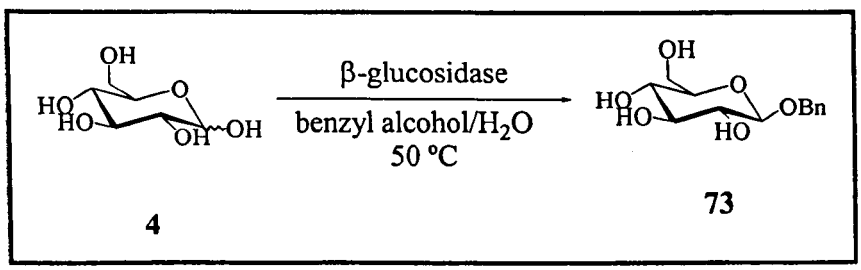
Due to the difficulty found on the selective removal of the ethoxy group, the benzyl alcohol was tested as a protecting/solubilising group for the anomeric position.

This group can be removed under mild, selective conditions; for example H_2 and catalytic Pd/C at room temperature is often used.

Due to the different reactivity found in both anomers of the ethyl glucopyranoside, the selective synthesis of the α- and β-anomers of benzyl-D-glucopyranoside was attempted.

2.2.6 - Benzyl β-D-glucopyranoside

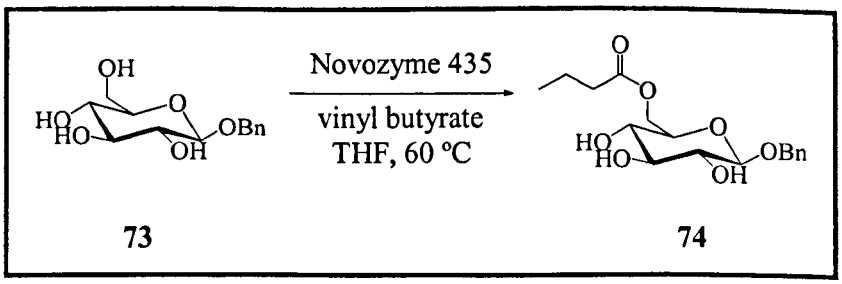
The addition of D-glucose **4** to a solution of benzyl alcohol and water (9/1, v/v) followed by the addition of the enzyme β-glucosidase from almonds, and stirring for 2 days at 50 °C, afforded benzyl β-D-glucopyranoside **73** in a 43 % yield (scheme **2.14**).



Scheme 2.14: Synthesis of benzyl β-glucopyranoside.

Several enzymes, described in table **2.1**, were then screened with benzyl β-D-glucopyranoside **73** as substrate. Using vinyl butyrate as solvent, the only enzyme that gave a positive reaction was Novozyme 435; the product of the reaction was the benzyl 6-*O*-butyryl β-D-glucopyranoside **74** in 98 % yield (scheme **2.15**). No reaction was detected in the absence of enzyme.

The amount of vinyl butyrate was decreased to two equivalents by using THF as solvent; no change in the reactivity was observed, but the amount of impurities decreased.

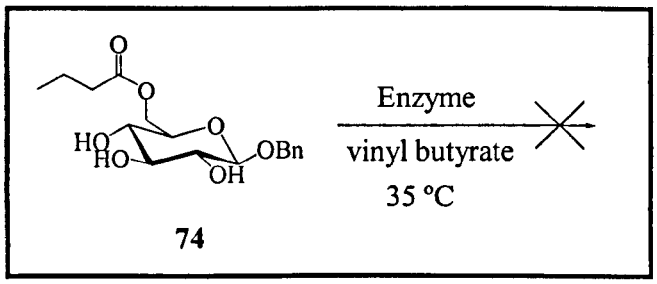


73 **74**

Scheme 2.15: Esterification of the primary position

The reaction was slower than observed using ethyl-β-D-glucopyranoside **55**, probably due to increased solubility of the ethyl β-D-glucoside **55** in THF.

When benzyl 6-*O*-butyryl β-glucopyranoside **74** was tested for esterification of a secondary alcohol group none of the tested enzymes gave a positive reaction after several days, even with different concentrations of vinyl butyrate (scheme **2.16**).



74

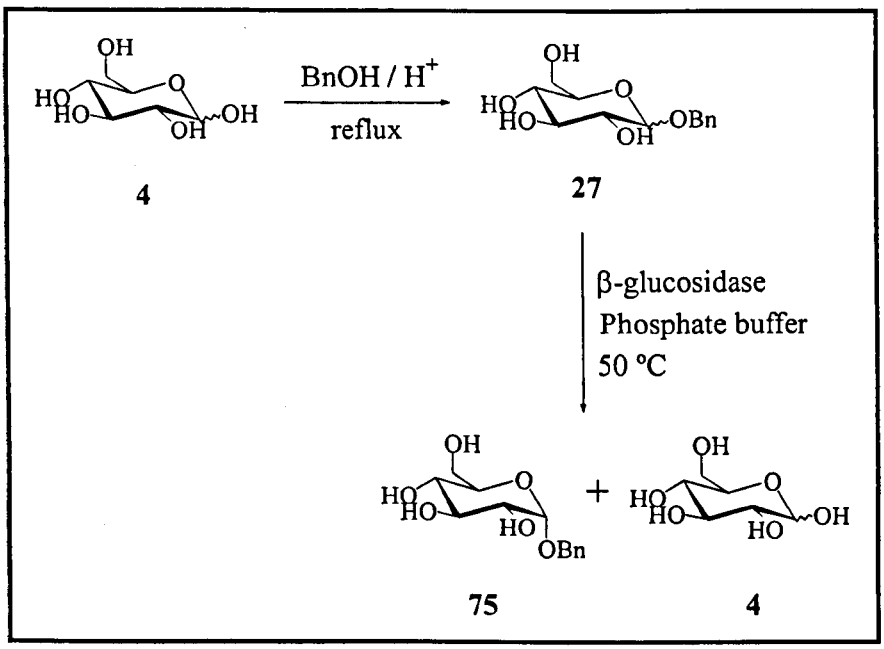
Scheme 2.16: Attemted esterification of monoesterified benzyl-β-glucopyranoside.

This result was surprising, highlighting the difference in reactivity with the ethyl 6-*O*-butyryl β-glucopyranoside **67** (scheme **2.8**), which reacted at the hydroxyl groups C-2 and C-3. The inclusion of a large benzyl ring on the molecule can limit the movement of the molecule inside the active site of the enzyme, having a "negative" interaction with the amino acids surrounding the active site. This interaction only allowed reaction at the primary hydroxyl group, perhaps because this hydroxyl group is the least hindered of all the hydroxyl groups present on the molecule.

2.2.7 - Benzyl α-D-glucopyranoside

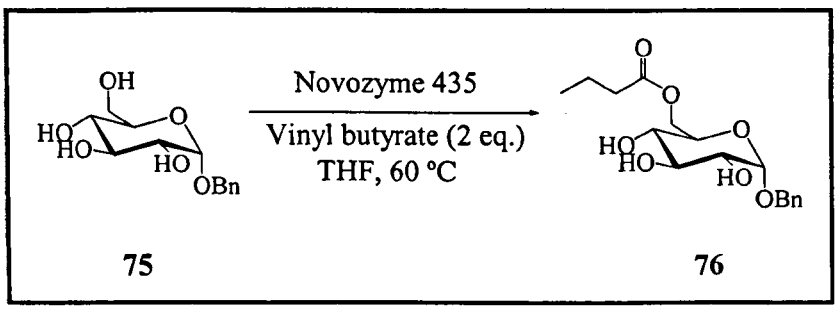
The synthesis of the benzyl α-D-glucopyranoside **75** involves two steps, a chemical process for the synthesis of the mixture of benzyl glucopyranoside **27** as mixture of α- and β-anomers, and then the selective hydrolysis of the β-anomer with β-glucosidase in a buffer solution (scheme **2.17**).

This two step method gave a good yield (67 %) when compared to the previously reported preparation of the benzyl α-glucopyranoside **75** by a Fischer reaction of D-glucose with benzyl alcohol in the presence of *p*-toluenesulfonic acid, yielding a 36 % yield of the desired compound.[98]



Scheme 2.17: Synthesis of benzyl α-glucopyranoside.

The esterification of the primary hydroxyl group was carried out in 97 % yield after 30 minutes, using the same enzyme as for the β-anomer, Novozyme 435 (scheme **2.18**).

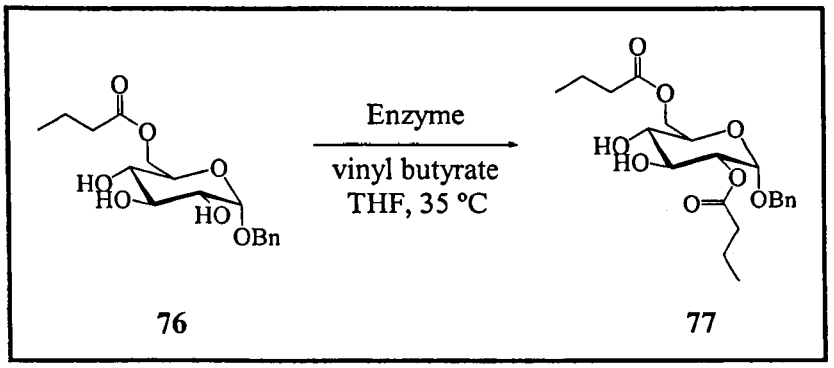


Scheme 2.18: Synthesis of the monoesterified benzyl α-glucopyranoside.

The α-anomer reacted faster than the β-anomer, showing a different reactivity when compared with the ethyl-D-glucopyranoside.

Again several enzymes were tested for the esterification of a secondary alcohol group. Three enzymes catalysed this reaction, as observed by TLC, namely lipase PS-C II, from *Pseudomonas cepacia*, lipase AK from *Pseudomonas fluorescens* and Pancreatin from hog pancreas.

Using THF as solvent and with 2 eq. of vinyl butyrate at 35 °C, all three enzymes gave moderate yields (50 - 66 %) over long reaction times (18 days was the shortest, for lipase PS-C II). The product of the reaction for the three enzymes was the same, benzyl 2,6-*O*-butyryl α-D-glucopyranoside **77** (scheme **2.19**).



76 **77**

Scheme 2.19: Selective esterification of the secondary hydroxyl groups.

All the enzymes presented a high selectivity for the hydroxyl group at position 2, in contrast with the esterification of ethyl 6-*O*-butyryl β-D-glucopyranoside **67** (scheme **2.8**) where the 2- and 3-hydroxyl groups were esterified.

To improve the yield and the reaction time of the process catalysed by the lipase PS-C II two variables were tested, temperature and the ratio THF / vinyl butyrate. This enzyme has been shown to be sensitive to increases of temperature, the optimum balance between yield and reaction time was obtained at a temperature of 40 °C. Above this temperature no reaction was detected, probably due to denaturation of the enzyme.

When vinyl butyrate was used as the solvent at 40 °C, the rate and the yield of the reaction increased. For lipase PS-C II the yield increased from 66 to 86 % and the time of the reaction decreased from 18 to 3 days; for the other two enzymes, no significant change was noticeable.

The nature of the organic solvent has been shown to affect the enzymatic catalysis by causing deactivation/inhibition by directly interacting with the enzyme or with the diffusible substrate/product, and by direct interaction of the water layer in the vicinity of the enzyme.[99] More hydrophilic solvents such as dioxane and THF remove the essential hydration layer present on the enzyme surface and this causes enzyme deactivation.[100] On the other hand hydrophobic solvents such as *n*-hexane and chloroform preserve the microaqueous layer around the enzyme thereby retaining its activity.[100] The water layer acts as lubricant, providing the enzyme with the flexibility necessary for enzymatic catalysis.

The reactivity of the benzyl α-glucopyranoside **75** was different to that of ethyl α-glucopyranoside **56**. With ethyl α-glucopyranoside **56** no reaction was detected for the esterification of one or more of the secondary hydroxyl groups. With the benzyl α-glucopyranoside **75** three enzymes have shown reactivity, all of them at the same hydroxyl group, namely at C-2.

The lipase from *Pseudomonas cepacia* has been extensively used for the resolution of secondary alcohols and its enantiopreference has been widely studied.[101, 102] Previous mapping of the specificity of lipase *Pseudomonas cepacia* has established a simple rule that predicts its enantiopreference toward secondary alcohols (figure **2.1**).[103]

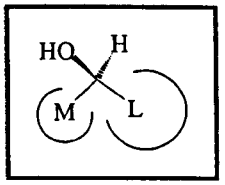


Figure 2.1[103]

This rule predicts which enantiomer reacts faster based on the sizes of the substituents at the stereocenters, where **M** represents a medium sized substituent such as CH_3 while **L** represents a large substituent, such as a benzyl group.[103] If **M** and **L** is substituted by components of benzyl 6-*O*-butyryl α-glucopyranoside **76**, **M** will become carbon atom C-3 and **L** will be the anomeric position with the benzyl group attached to it (figure **2.2**).

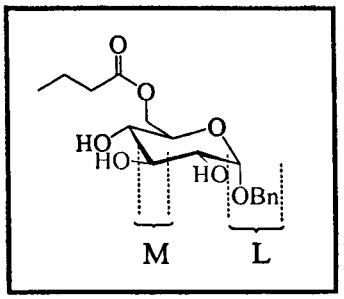
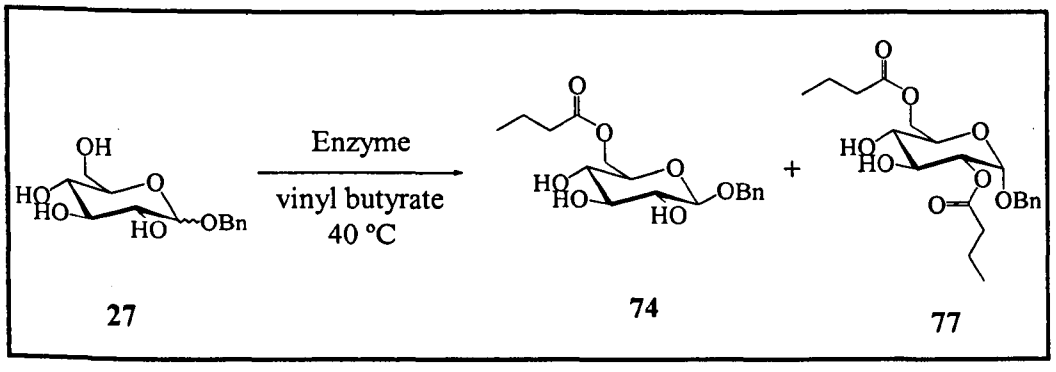


Figure 2.2

With this configuration it was felt that the sugar could be accommodated into the active site of the enzyme and the hydroxyl group at the carbon atom C-2 is in the preferred position to be esterified.

As with ethyl D-glucopyranoside **57** the separation of the α- and β-anomers of benzyl D-glucopyranoside **27** was performed in one-pot using the difference in reactivity between the anomers. The use of two enzymes was necessary, Novozyme

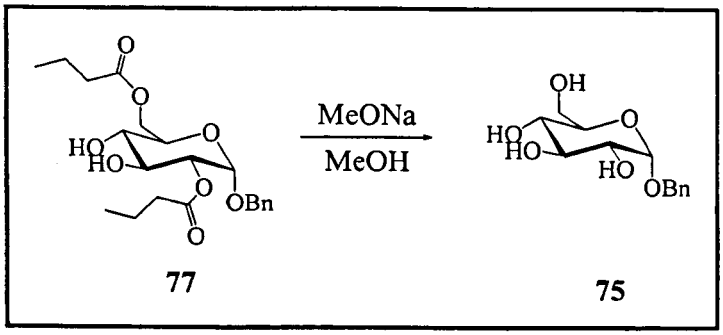
435 for the esterification of the primary hydroxyl group, followed by the use of lipase PS-C II for the esterification of the hydroxyl group at C-2 of the α-anomer. A mixture of the two enzymes was employed and THF was eliminated as solvent due to the poor yield registered for the esterification with lipase PS-C II. The rate limiting step in this reaction is the esterification of the hydroxyl group at carbon atom C-2, requiring 3 days to reach completion. After two hours all the benzyl D-glucopyranoside **27** was found to be monoesterified (by TLC). The reaction was then left for 4 days allowing all the α-anomer to react. The resulting product of the reaction was benzyl 6-*O*-butyryl β-D-glucopyranoside **74** in 36 % yield and benzyl 2,6-*O*-butyryl α-D-glucopyranoside **77** in 28 % yield (scheme **2.20**).



Scheme 2.20

The butyric units in benzyl 2,6-*O*-butyryl α-D-glucopyranoside **77** were then cleaved with sodium methoxide giving benzyl α-D-glucopyranoside **75**, as the pure α-anomer in quantitative yield (scheme **2.21**).

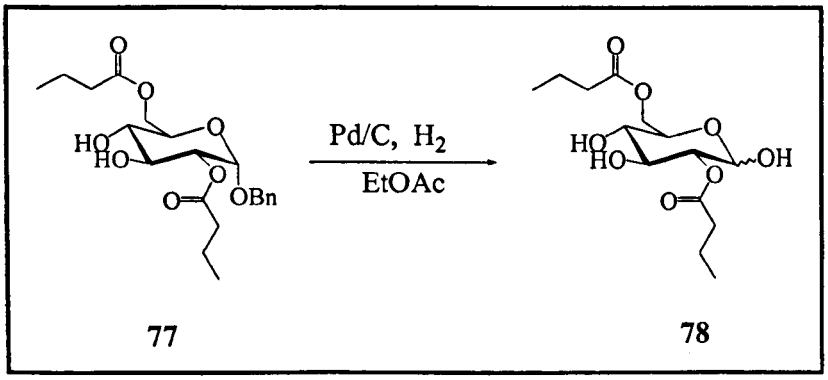
The same protocol was used for the selective cleavage of the butyric side chains in benzyl 6-*O*-butyryl β-D-glucopyranoside **74**, yielding pure benzyl β-D-glucopyranoside **73**.



Scheme 2.21

2.2.8 - Selective hydrogenation of the benzyl group

The selective removal of the benzyl protecting group using H_2 in EtOAc and Pd/C as catalyst, was successfully tested on the benzyl 2,6-*O*-butyryl α-D-glucopyranoside **77**, yielding the desired unprotected sugar 2,6-O-butyryl D-glucopyranoside **78** in 75 % yield (scheme **2.22**).



Scheme 2.22

As expected under these selective conditions, no loss of the butyric acid moieties were detected.

2.2.9 - Ester as the anomeric protecting group

At this stage all the glucopyranosides that had been used as substrates were protected at the anomeric position with an ether unit comprising an ethyl and benzyl group.

The synthesis of monoesters at the anomeric position has traditionally used two methods. One method uses the Koenigs-Knorr type reaction (see scheme **1.9**) with the use of tetraacetylated bromo sugar in the presence of silver carboxylates.[104, 105] However, the final deprotection of the acetate group has proved to be difficult with the migration and hydrolysis of the remaining acyl groups. The second method uses direct condensation of an acylating agent, such as free carboxylic acid or acid chloride, with the anomeric hydroxyl group of tetrabenzylated sugars.

The direct esterification using the free carboxylic acid needs the employment of *N,N'*-dicyclohexylcarbodiimide (DCC) as condensation agent and imidazole as catalyst.[106] The use of acid chlorides has been also tried on tetra 2,3,4,6-benzyl-D-glucopyranosides.[107]

The major disadvantage of these two methods is the use of protecting and deprotecting procedures. Side reactions such as acyl migration and hydrolysis are a major problem, giving a low overall yield of the desired product.

Plusquellec *et al.*, have acylated the anomeric position of unprotected lactose, maltose and glucose using activated long chain fatty acid esters (figure **2.3**).[30] These activated esters are less reactive and more selective than the acid chlorides.

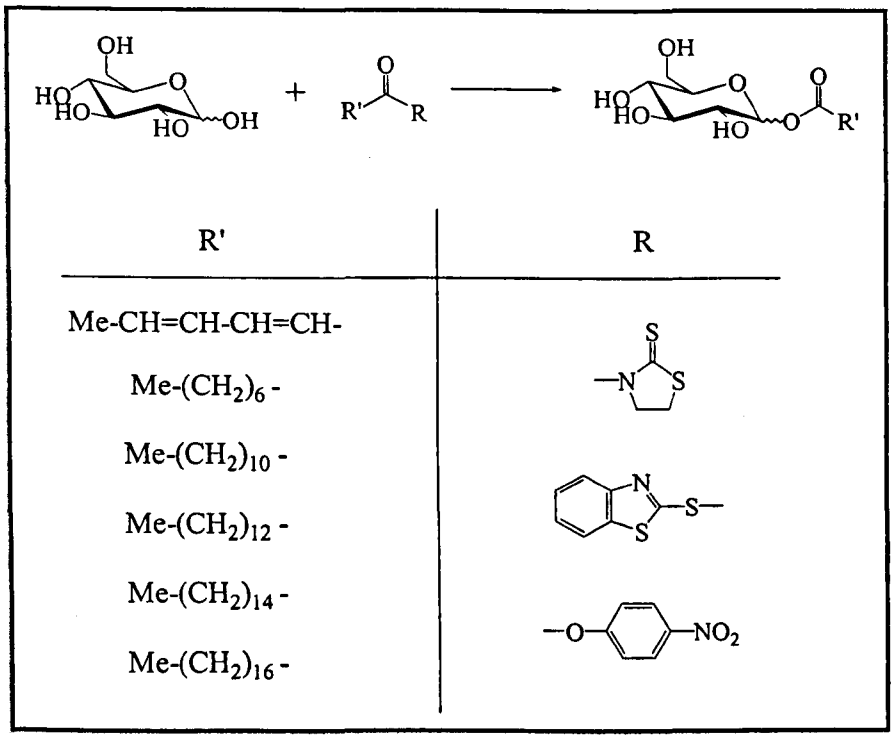
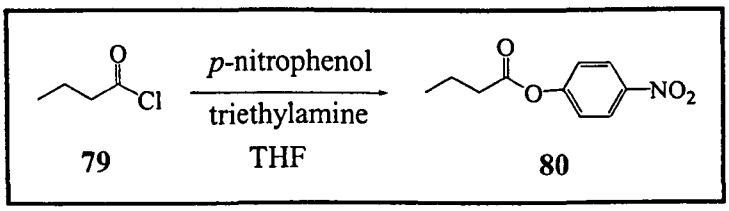


Figure 2.3

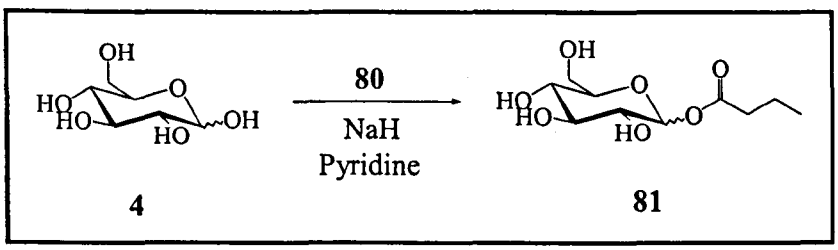
The method used by Plusquellec *et al.*, was applied for the acylation of the anomeric position of D-glucose with butyric acid, using *p*-nitrophenol as an activating agent. Reacting butyryl chloride **79** with *p*-nitrophenol in the presence of triethylamine yields the activated ester, butyric acid 4-nitro-phenyl ester **80**, in a quantitative yield (scheme **2.23**)



79 **80**

Scheme 2.23

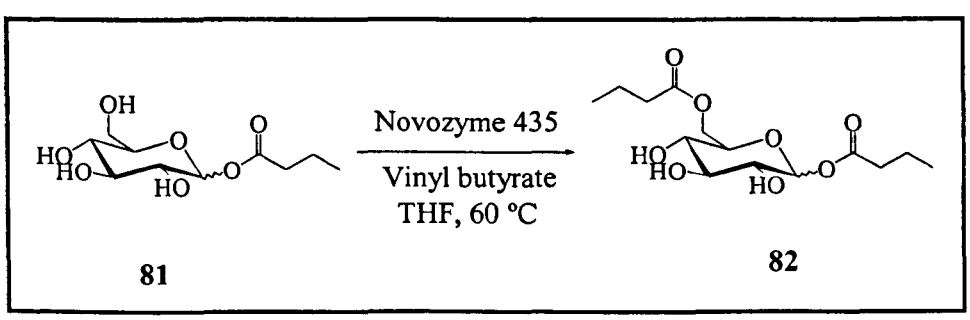
The reaction of D-glucose with 1 equivalent of the activated ester **80** in the presence of sodium hydride in pyridine gave the desired monoacylated glucopyranoside **81** in 75 % yield, as a mixture of α- and β-anomers (scheme **2.24**).



4 **81**

Scheme 2.24

With a different protecting group at the anomeric centre, the next step was the selective esterification of the remaining hydroxyl groups present on the glucopyranoside. Novozyme 435 was used to catalyse the reaction of the protected sugar with vinyl butyrate in THF affording 1,6-*O*-butyryl D-glucopyranoside **82** in 56 % yield (scheme **2.25**).



81 **82**

Scheme 2.25

The diester was then used to screen several enzymes for the selective acylation of the remaining hydroxyl groups. THF was used as solvent due to the poor solubility

of the glucopyranoside in vinyl butyrate. After seven days no esterification was detected; only degradation of the starting material was detected by TLC

In summary, the presence of an ester at the anomeric centre does not affect the esterification of the primary hydroxyl group, but the yield was lower than the esterification of the ether protected glucopyranosides, mainly due to a higher level of impurities formed during the reaction. The esterification of the secondary hydroxyl group shows a dependence on the protecting group at the anomeric centre. When the protecting group is an ether, such as benzyl or ethyl, esterification of one or more of the secondary hydroxyl groups was observed. With an ester as the protecting group no further esterification was observed.

2.2.10 - Deacylation

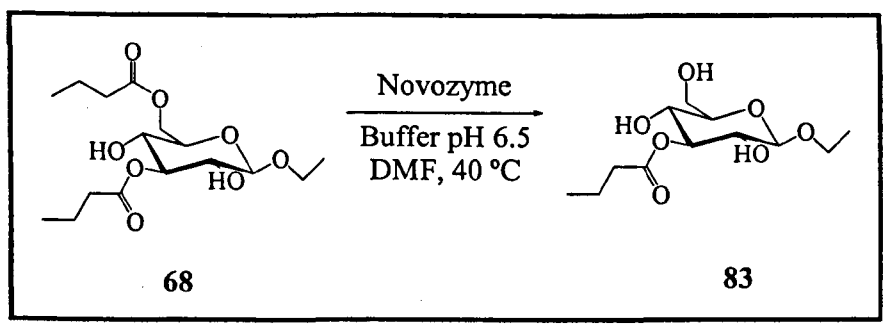
Partial deacylation of peracylated sugars using chemical methods is restricted by the position of deacylation and problems of poor selectivity.[66] In contrast, enzymic techniques have been used successfully in the selective deacylation of one or more acyl groups in peracylated sugars.[49, 65, 80, 108-110]

The regioselectivity observed in the esterification reactions of the D-glucopyranosides indicates that the reverse reaction, i.e., hydrolysis of esterified glucopyranosides, may also be regioselective.

To verify the regioselectivity of the hydrolysis reaction, a diester previously prepared by enzymatic methods, ethyl 3,6-*O*-butyryl β-D-glucopyranoside **68**, was used. Novozyme 435 was the catalyst employed, regioselectively to acylate the

primary hydroxyl group (C-6) followed by the secondary hydroxyl group (C-3) (see section **2.12**).

The butyric acid moiety at the primary position should be also the first to be cleaved. Thus, the ethyl 3,6-*O*-butyryl β-D-glucopyranoside **68** was reacted in the presence of Novozyme 435 in a buffer solution at pH 6.5 with 5 % DMF, yielding ethyl 3-*O*-butyryl β-D-glucopyranoside **83** in 95 % yield (scheme **2.26**).

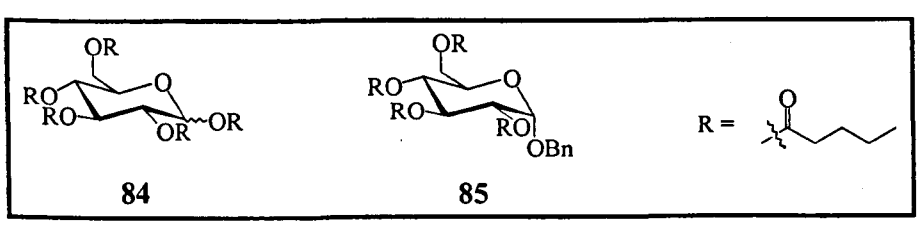


68 **83**

Scheme 2.26

DMF was used to increase the solubility of the glycopyranoside in the buffer solution and has been shown to enhance the regioselectivity of the hydrolysis.[78] As expected the butyric moiety at the primary position (C-6) was hydrolysed selectively.

Two different compounds were tried, the 1,2,3,4,6-penta-*O*-butyryl-D-glucopyranose **84** and benzyl 2,3,4,6-tetra-*O*-butyryl α-D-glucopyranoside **85** (figure **2.4**).

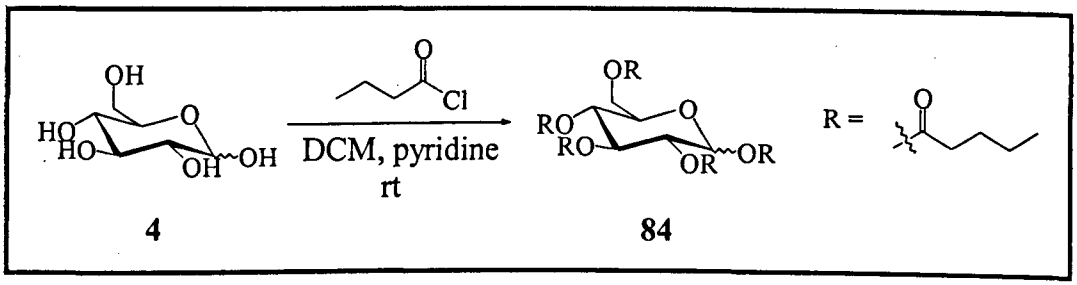


84 **85**

Figure 2.4

1,2,3,4,6-Penta-*O*-butyryl-D-glucopyranose 84

The pentaesterified glucopyranoside **84** was synthesised in an 85 % yield from D-glucose **4** with excess butyryl chloride **79** in pyridine at room temperature for 15 hours (scheme **2.27**).

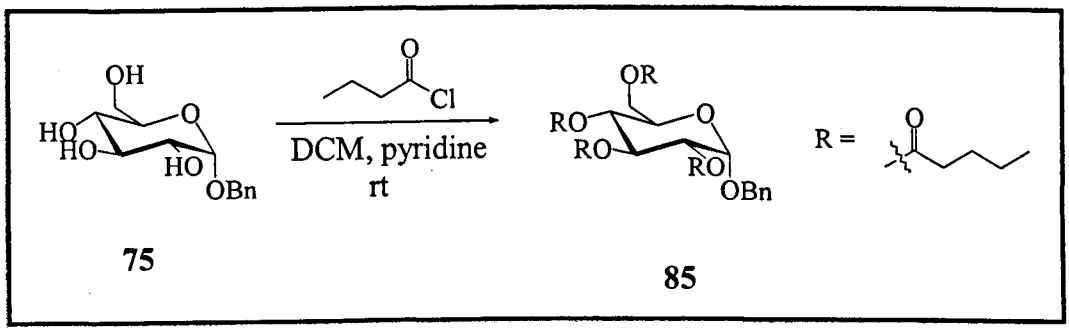


Scheme 2.27

Several lipases and esterases were then tested in pH 7.0 buffer employing 10 % DMF due to the poor solubility of the glucopyranoside in buffer. However, after two weeks no reaction was detected with any of the enzymes. The lack of reactivity could be due to the large size of the pentaesterified sugar, making the access of the sugar to the active site of the enzyme impossible.

Benzyl 2,3,4,6-tetra-*O*-butyryl α-D-glucopyranoside 85

Due to the differences in reactivity of D-glucose derivatives under the acylation conditions benzyl 2,3,4,6-tetra-*O*-butyryl α-D-glucopyranoside **85** was prepared and tested. The synthesis of the tetra-esterified glucopyranoside followed the same procedure described for the pentaesterified sugar **84**, in 77 % yield (scheme **2.28**).



Scheme 2.28

Again, several enzymes were screened for the selective hydrolysis of the butyric group.

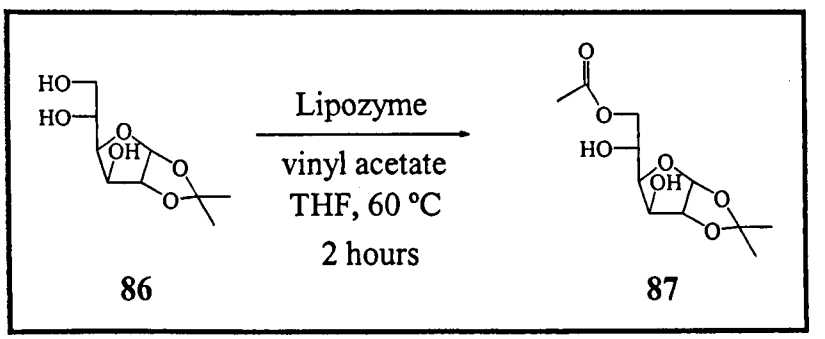
Lipozyme and Novozyme 435 did not give any reaction. Lipase PS-C II, Pancreatin and lipase AY showed some reaction by TLC. However, the NMR spectrum of the product of the reaction showed several impurities and D-glucose. No ester or benzyl group was detected by NMR or IR spectroscopy. The same enzymes catalysed selective esterification of benzyl 6-*O*-butyryl α-D-glucopyranoside **76** (section **2.1.7**).

2.2.11 - 1,2-O-Isopropylidene-α-D-glucofuranose

The use of 1,2-*O*-isopropylidene-α-D-glucofuranose **86** has been used to transport and then to release, *in vivo*, a butyric acid moiety by enzymatic hydrolysis.[111]

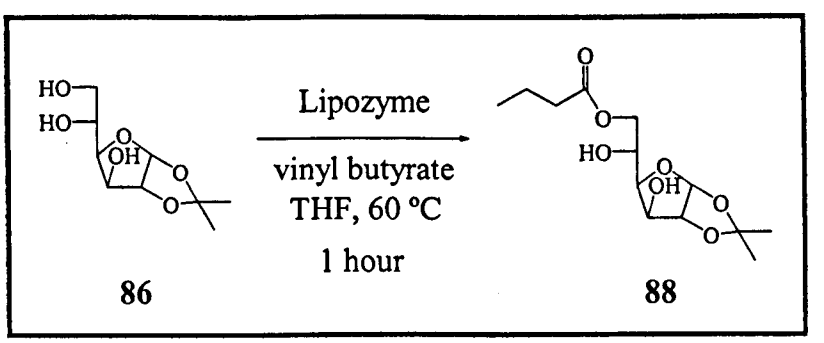
The regioselective synthesis of monoesters of 1,2-*O*-isopropylidene-α-D-glucofuranose **86**, however, has presented some challenges due to the lack of selectivity and the need of protection and deprotection steps.

1,2-*O*-Isopropylidene-α-D-glucofuranose **86** was tested as a substrate for the selective esterification of the primary hydroxyl group. Using Lipozyme as catalyst and vinyl acetate as acylating agent, 1,2-*O*-isopropylidene-α-D-glucofuranose **86** was acylated at the primary hydroxyl group yielding 6-*O*-acetyl 1,2-*O*-isopropylidene α-D-glucofuranose **87** in 92 % yield (scheme **2.29**).



Scheme 2.29

Using the same enzyme and vinyl butyrate as acylating agent, 1,2-*O*-isopropylidene-α-D-glucofuranose **86** was also acylated at the primary hydroxyl group. After 1 hour 6-*O*-butyryl 1,2-*O*-isopropylidene α-D-glucofuranose **88** was isolated in 99 % yield (scheme **2.30**)

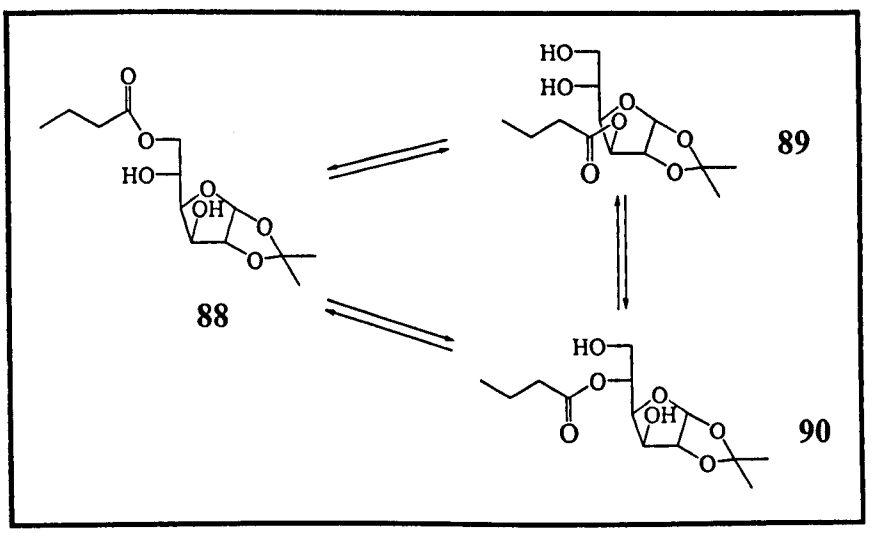


Scheme 2.30

With the increase in the chain length of the acylating agent from acetate to butyrate, the reaction was found to be faster and cleaner. This seems to be consistent with previously published results. Adelhorst *et al.* observed a faster reaction time with fatty acids having 12 to 18 carbons compared with shorter ones containing 8 to 10 carbons.[22]

The ester **88** was shown to undergo internal transesterification in water solution, leading to mixtures of 3-*O*-butanoyl and 5-*O*-butanoyl 1,2-*O*-isopropylidene α-D-glucofuranose, **89** and **90** respectively (scheme **2.31**).[50] This phenomenon is due to the favourable orientation of the 6-*O*-acyl group toward the nucleophilic attack of the neighbour OH groups at the C-5 and C-3 positions.[50]

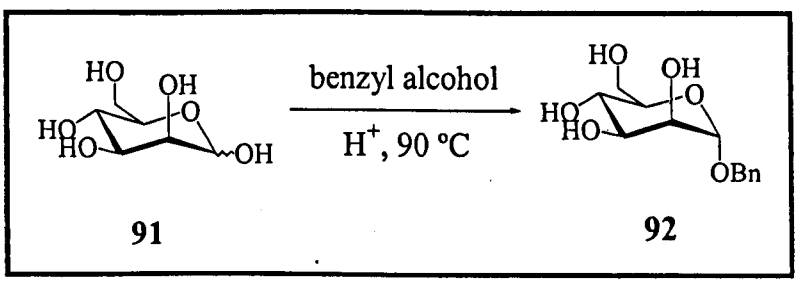
In solvents such as THF and chloroform no transesterification has been observed.



Scheme 2.31

2.3 - D-Mannose

D-Mannose **91** was also tested for the selective esterification of the hydroxyl groups. Again the main problem of D-mannose **91** is the solubility in suitable solvents for enzymatic esterification. The reaction of D-mannose **91** with benzyl alcohol in the presence of catalytic H^+, yielded benzyl α-D-mannopyranoside **92** in 63 % yield (scheme **2.32**); notably, the only product formed in this reaction was the α-anomer.

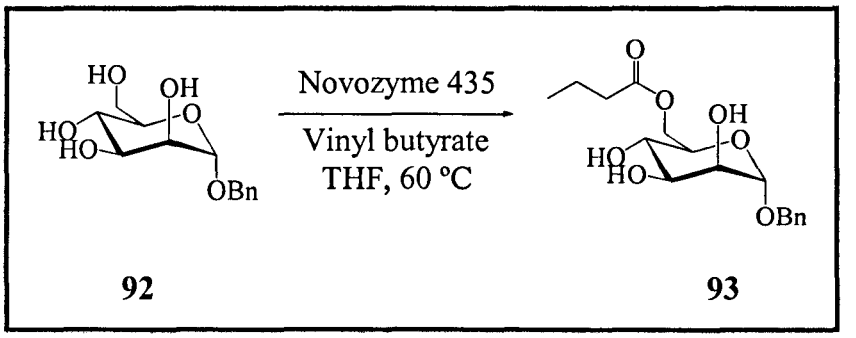


91 **92**

Scheme 2.32

In this process a combination of steric factors and the anomeric effect control the α/β-pyranose ratio. When the hydroxyl group at carbon C-2 is equatorial, such as in D-glucose and D-galactose, the anomeric effect is overcome by the tendency of the anomeric hydroxyl group to assume the equatorial orientation for steric reasons and the equatorial anomers are favoured.[5] When the hydroxyl group at carbon C-2 is axial, such as mannose, the anomeric effect increases in significance. Additionally, axial anomers have one destabilising *gauche* interaction.[5]

The selective acylation of the primary hydroxyl group with butyric acid was then tested using Novozyme 435 as catalyst. After one hour of reaction, benzyl 6-*O*-butyryl α-D-mannopyranoside **93** was obtained in good yield (76 %) (scheme **3.33**).



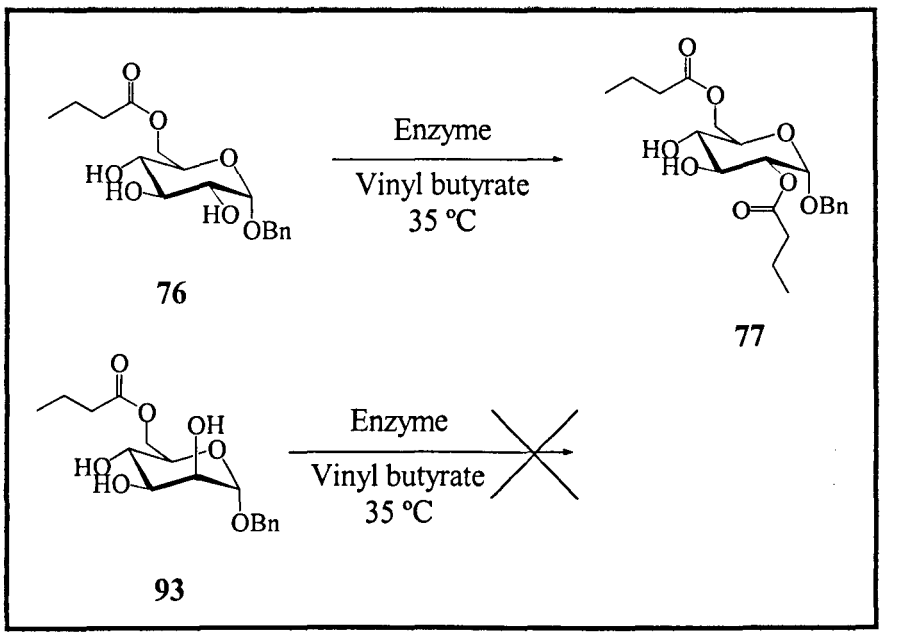
Scheme 2.33

The reactivity of benzyl α-mannopyranoside **92** was similar to benzyl α-glucopyranoside **75**.

The monoesterified mannopyranoside **93** was then used to screen several enzymes for the selective esterification of the secondary hydroxyl groups. After seven days, no reaction was detected with any of the enzymes. Even using vinyl butyrate as the solvent, no reaction was detected.

It is important to compare this result to that obtained with the esterification of benzyl 6-*O*-butyryl α-D-glucopyranoside **76** (section **2.1.7**). With the glucopyranoside, lipase PS-C II, Pancreatin and lipase AK, catalysed the acylation of the hydroxyl group at carbon C-2. The change in stereochemistry of the hydroxyl group at C-2 from equatorial (glucose) to axial (mannose), shows its influence on the reactivity of the secondary hydroxyl groups (scheme **2.34**).

Based on this experimental observation it was proposed that the change from equatorial to axial can orient the hydroxyl group at C-2 away from the active site of the enzyme, and consequently, its esterification is not possible



Scheme 2.34

Again, applying the rule that predicts the enantiopreference toward secondary alcohols by lipase (see figure **2.1**, page 58), it can bee seen that the hydroxyl group at C-2 from D-glucose(figure **2.2**, page 58) has the opposite conformation to that verified in D-mannose (figure **2.5**).

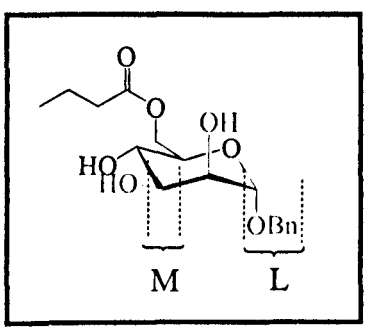
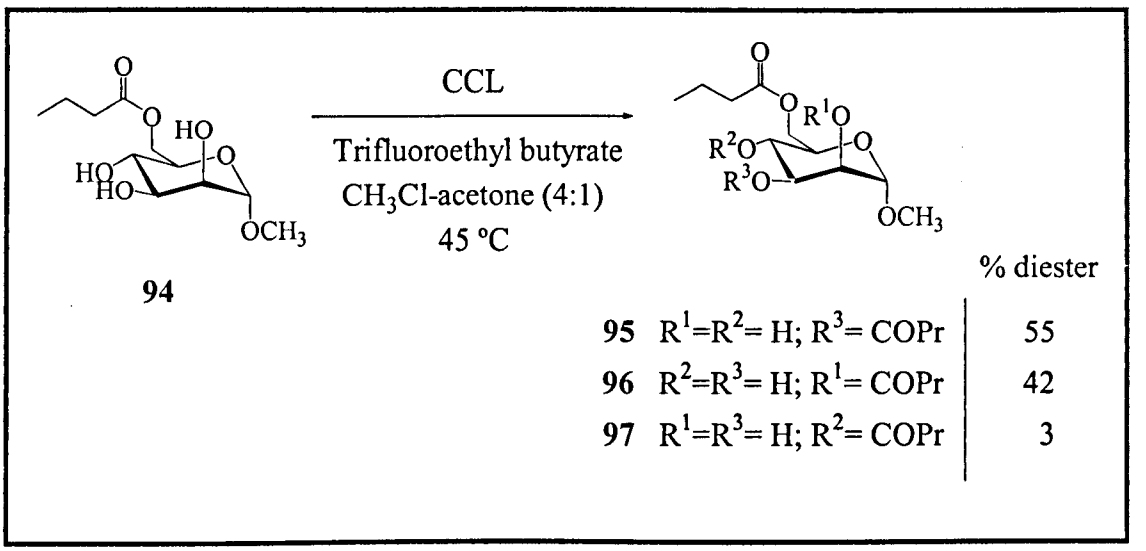


Figure 2.5

However it is interesting to note that acylation of methyl 6-*O*-butyryl α-D-mannopyranoside **94** has been reported by Colombo *et al.* Using *Candida cylindracea* lipase (CCL) as catalyst and a trifluoroethyl ester as acylating agent, they

obtained a mixture of diesters in moderate yield (50 %) after 12 days of reaction. The major diester obtained was the methyl 3,6-*O*-butyryl α-D-mannopyranoside **95** in 55 % yield (scheme **2.35**).[112]



Scheme 2.35

2.4 - D-Galactose

After discovering the difference in reactivity between D-glucose **4** and D-mannose **92**, it was interesting to investigate how D-galactose **38** would behave (figure **2.6**).

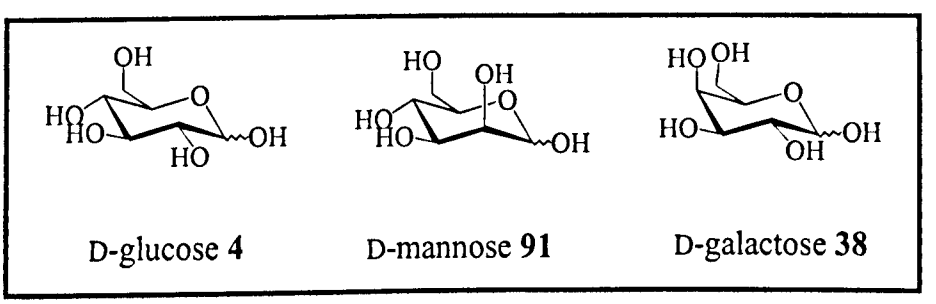
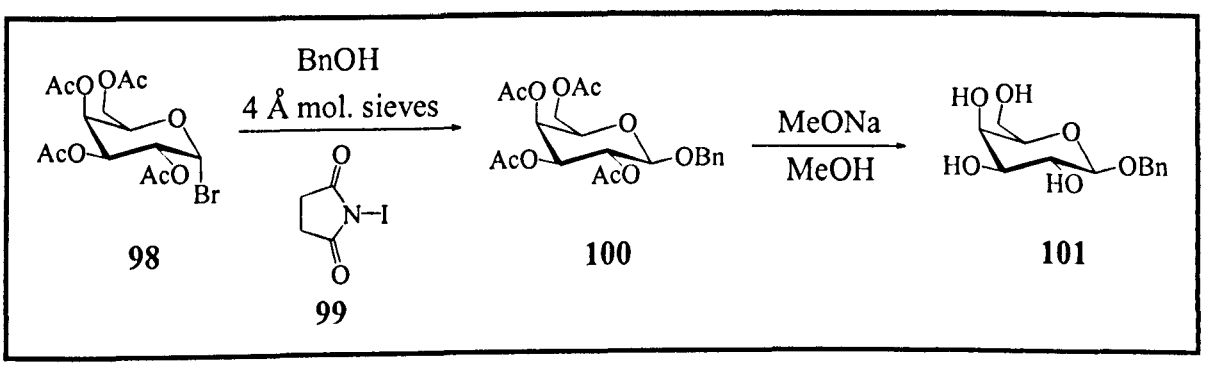


Figure 2.6

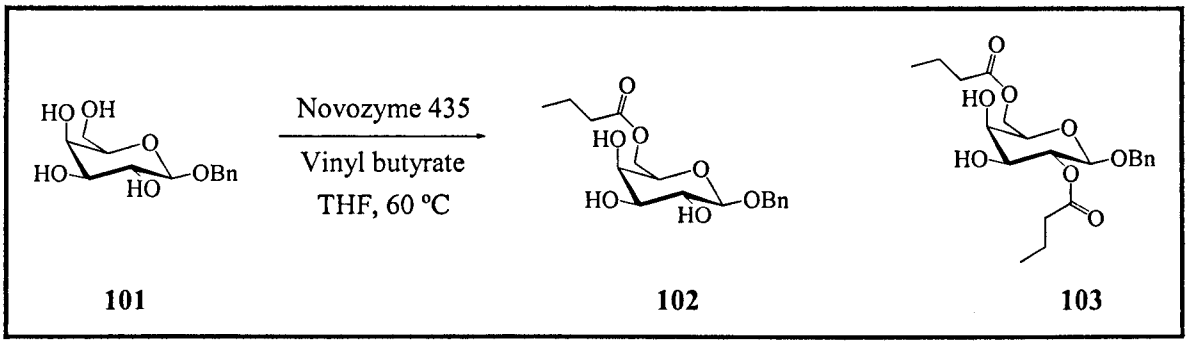
The first step was the protection of the anomeric hydroxyl group with benzyl alcohol. Again the synthesis of both anomers was explored.

Benzyl β-D-galactopyranoside **101** was synthesized using a modified Koenigs-Knorr glycosidation, in which N-iodosuccinimide was used as a promoter. Acetobromo α-D-glactose **98** was reacted with benzyl alcohol in the presence of *N*-iodosuccinimide **99** and 4 Å molecular sieves. The resulting product, benzyl β-D-galactopyranoside tetraacetate **100**, was then reacted with sodium methoxide selectively to remove the protecting acetate groups, yielding the desired benzyl β-D-galactopyranoside **101** in 59 % yield over the two steps (scheme **2.36**).



Scheme 2.36: Synthesis of benzyl β-D-galactopyranoside.

Benzyl β-D-galactopyranoside **101** was then esterified with vinyl butyrate using Novozyme 435 as catalyst. After three hours, all the starting material was consumed and two products were detected by TLC. After purification the two products were assigned as benzyl 6-*O*-butyryl β-D-galactopyranoside **102** (64 % yield) and benzyl 2,6-*O*-butyryl β-D-galactopyranoside **103** (14 % yield) (scheme **2.37**) by NMR spectroscopy.



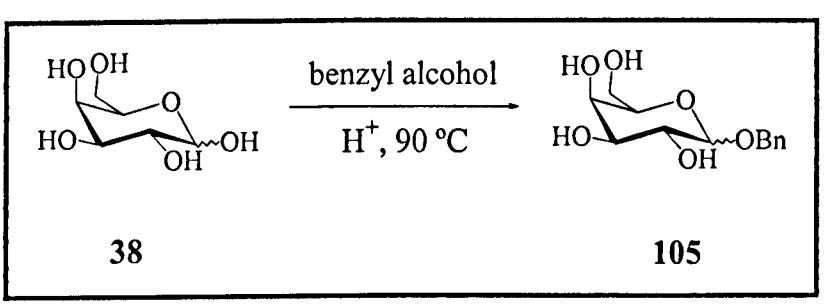
Scheme 2.37

Benzyl β–D-galactopyranoside **101** was less reactive than the corresponding benzyl β-D-glucopyranoside **73**, taking two more hours to reach completion. Additionally Novozyme 435 was shown not be totally selective for the esterification of the primary hydroxyl group, as observed with benzyl β-D-glucopyranoside **74** (see scheme **2.15**).

After the selective esterification of the secondary hydroxyl group of benzyl β-D-galactopyranoside **101**, it was necessary to verify the reactivity of benzyl α-D-galactopyranoside **104** towards the secondary acylation.

Due to the difficulty found for the synthesis of the benzyl α-galactopyranoside **104**, the use of a mixture of α- and β-galactopyranosides was the only option.

The Fischer glycosidation reaction was used for the synthesis of benzyl D-galactopyranoside **105** (47 % yield) as a mixture of α- and β-anomers, (scheme **2.38**)

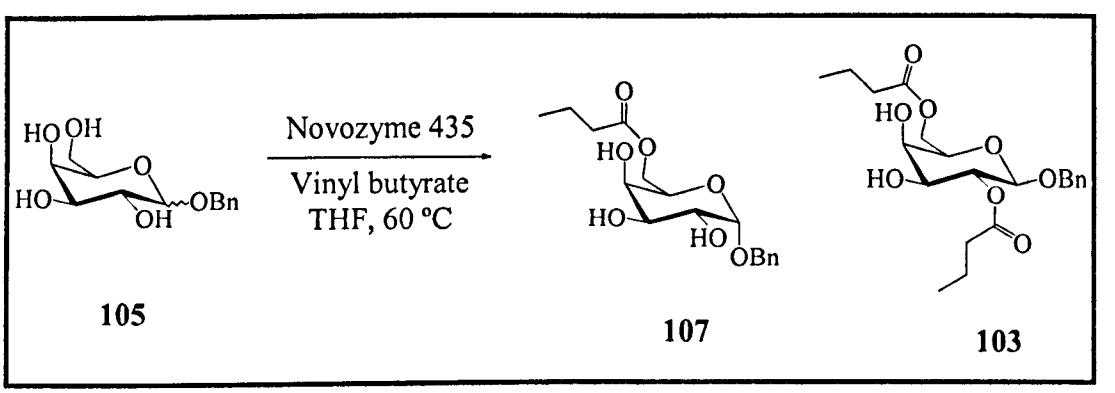


Scheme 2.38

Using benzyl D-galactopyranoside **105**, several enzymes were screened for the selective acylation of the primary hydroxyl group. Three enzymes showed a positive reaction by TLC, Novozyme 435, Lipozyme and lipase AK.

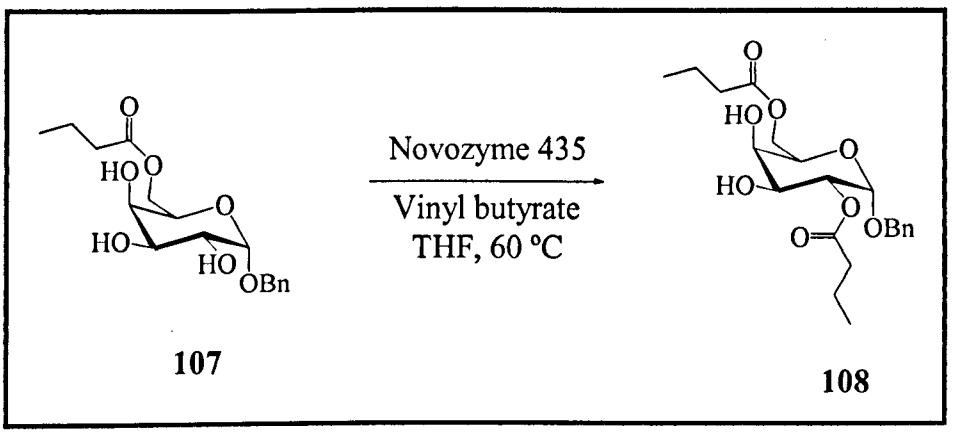
With Novozyme 435 and lipase AK, all the starting material was consumed between 5 and 9 hours of reaction, yielding two products by TLC However, with Lipozyme, starting material was still present even after 2 weeks of reaction, and the reaction mixture gave numerous spots by TLC analysis.

Benzyl 6-*O*-butyryl D-galactopyranoside **106** (α- and β-anomers) and a mixture of polyesterified galactopyranosides were isolated from the reactions when Novozyme 435 and lipase AK were used. After fine-tuning the reaction conditions by varying the reaction time it was possible to establish a difference in reactivity of the enzymes toward the acylation of benzyl D-galactopyranoside **105**. Lipase AK always gave the C-6 monoesterified galactopyranoside and a mixture of polyesterified galactopyranosides, independent from the time of the reaction. With Novozyme 435, benzyl 6-*O*-butyryl α-D-galactopyranoside **107** and benzyl 2,6-*O*-butyryl β-D-galactopyranoside **103** were obtained within 7.5 hours of reaction (scheme **2.39**).



Scheme 2.39

If the reaction was left for a longer period, the monoesterified α-galactopyranoside **107** was also acylated, yielding a mixture of diesters. With a shorter reaction time, ca. 5 h, mixtures of α- and β-monoesterified galactopyranosides and pure benzyl 2,6-*O*-butyryl β-D-galactopyranoside **103** were obtained. Using Novozyme 435 as catalyst, benzyl 6-*O*-butyryl α-D-galactopyranoside **107** was reacted in the presence of vinyl butyrate, yielding after 24 h, benzyl 2,6-*O*-butyryl α-D-galactopyranoside **108** in 81 % yield (scheme **2.40**).



Scheme 2.40

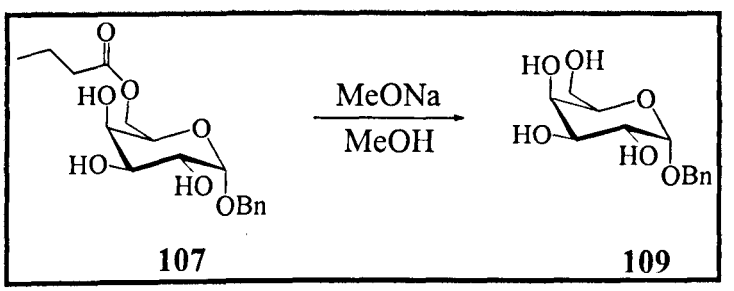
The difference in reactivity between the α- and β-galactopyranoside toward the secondary acylation can explain why it was possible to obtain pure benzyl 6-*O*-butyryl α-D-galactopyranoside **107** and pure benzyl 2,6-*O*-butyryl β-D-galactopyranoside **103** when starting with a mixture of anomers (scheme **2.39**).

The change of the hydroxyl group at carbon C-4 from equatorial to axial, has caused a large change in reactivity toward esterification. With benzyl D-glucopyranoside **27**, where all the hydroxyl groups are equatorial, no secondary esterification was detected. With benzyl D-galactopyranoside **105** no selectivity is

observed in terms of α- and β-anomers was detected; both compounds are acylated at carbon C-2, if the reaction time is longer than 9 hours.

Again the difference in reactivity of both anomers towards secondary esterification can be used to prepare pure α- and β-glycopyranoside.

The saponification of benzyl 6-*O*-butyryl α-D-galactopyranoside **107** gave pure benzyl α-D-galactopyranoside **109** (scheme **2.41**) in 91 % yield.



107 **109**

Scheme 2.41

2.5 - Summary and conclusions

In conclusion, the regioselective synthesis of different sugar esters was achieved. The reactivity of the secondary hydroxyl group shows dependence on the anomeric protecting group. When ethyl is the protecting group, esterification of 2- and 3-positions of D-glucose was obtained, but on changing the protecting group from ethyl to benzyl, only the 2-position is esterified.

The α- or β-configuration of the anomeric protecting group also influences the esterification of the secondary hydroxyl group. With ethyl as the protecting group, the β-anomer was selectively esterified at 2- and 3-position, however, none of the secondary hydroxyl groups of the α-anomer was esterified. With benzyl as the

anomeric protecting group, only the α-anomer was selectively esterified at the 2-position. The glycoside also presented a different reactivity toward secondary esterification. No secondary esterification of D-mannose was observed. With D-galactose both the α- and β-anomers were esterified at the 2-position, but with different reaction rates.

Using the difference in reactivity of the anomers, an alternative synthesis of pure α- and β-D-glucopyranosides and D-galactopyranosides was developed.

Chapter 3

Pyrolysis

3.1 - Introduction

Pyrolysis-Gas Chromatography/Mass Spectrometry (Py-GC/MS) is being increasingly used both in the field of structural characterisation,[113] and as a quantitative method of assessing thermal stability.[114] Table **3.1** summarises some of the representative applications of Py-GC/MS.[113]

Polymer chemistry	Clinical science
Process control of polymeric materials	Forensic science
Biochemistry	Food science
Organic chemistry	Wood science
Soil chemistry	Toxicology
Microbiology	Environmental science
Numerical taxonomy	Energy conservation
Pathology	Extraterrestrial studies

Table 3.1: Areas of application for analytical pyrolysis.[113]

A typical Py-GC/MS measuring system is composed of a pyrolyser connected to the injector of a gas-chromatograph, which is in turn connected to a quadrupole mass spectrometer (figure **3.1**).[113]

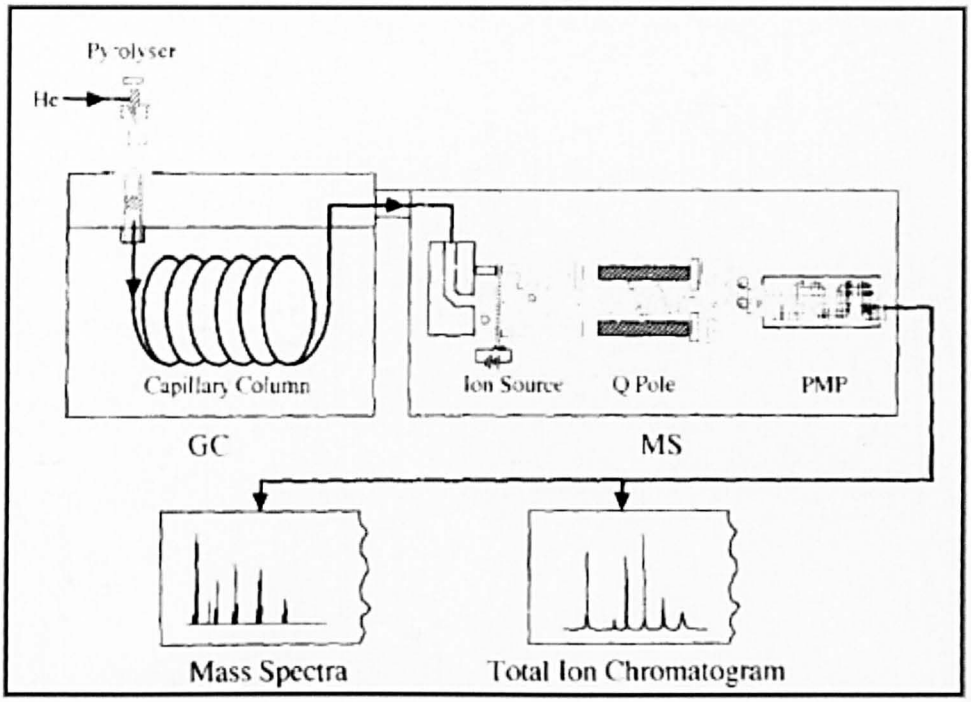


Figure 3.1: Py-GC/MS measuring system using a micro-furnace pyrolyser.[113]

The pyrolyser is usually an interface equipped with a pyroprobe, where the samples are pyrolysed, and N_2/O_2 gas inlets. The products of the pyrolysis are trapped with liquid nitrogen in the injector of the GC. The gas chromatograph is equipped with a chemically inert capillary column where the different compounds are separated, followed by a quadrupole mass spectrometer with an EI/CI ionisation source for analysis of the different products from pyrolysis.

3.2 – Aims, materials and methods

The thermal stability of the sugar esters (prepared as described in chapter 2) and the thermal release pattern of the resultant butyric acid moieties were investigated. Using Py-GC/MS, the obtained information might partially reflect the controlled release properties of the sugar esters of their corresponding butyric acid moiety.

Consequently, the outcome may be beneficial for the food, flavour and fragrance industries.

The different sugar esters pyrolysed in this manner is shown in figure **3.2.**

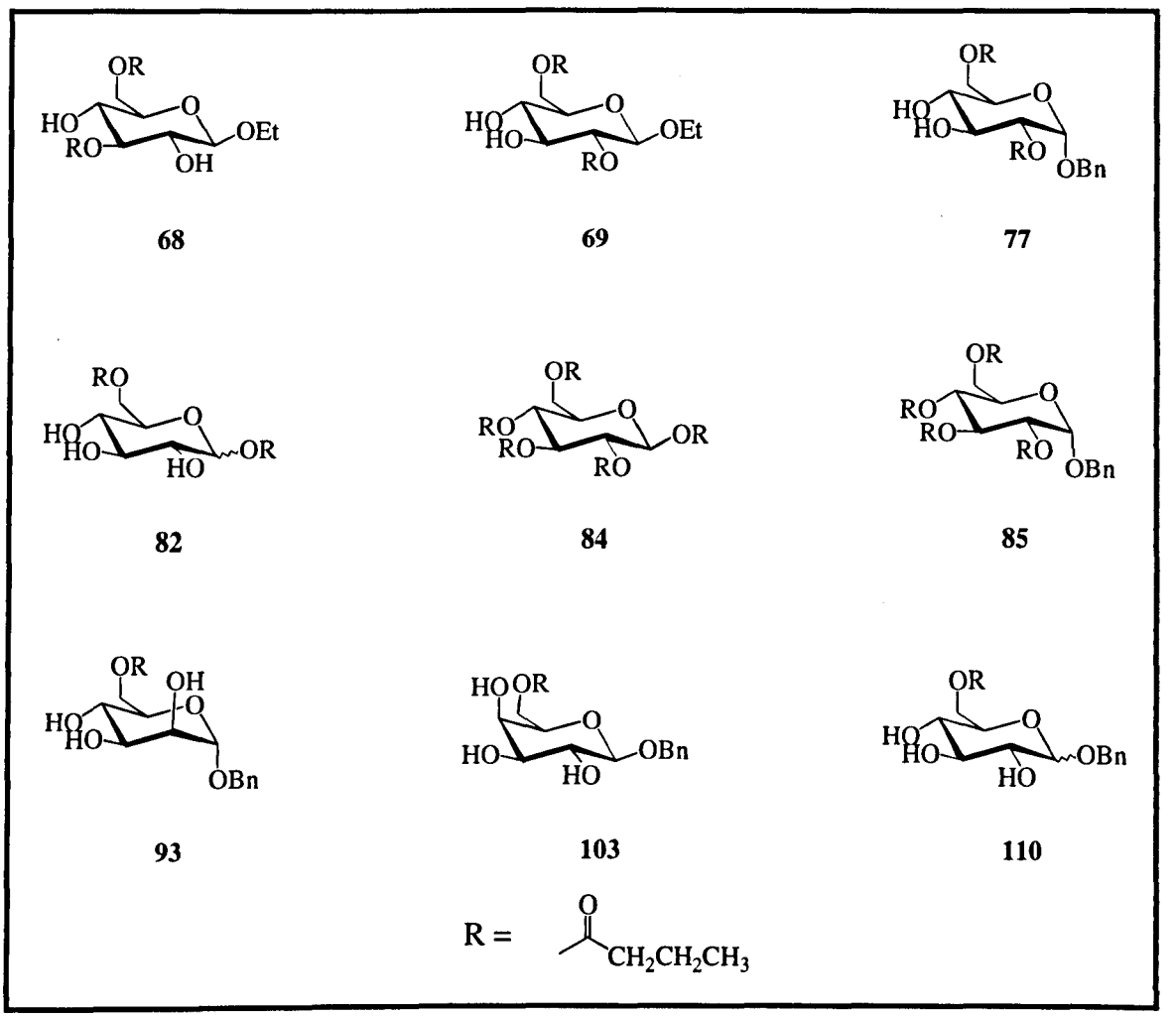


Figure 3.2: Structure of the pyrolysed compounds.

Solutions of the sample tested were prepared with a concentration of 10 mg/cm^3. A small piece of quartz wool was inserted in a quartz tube and 2 µL of the solution previously prepared was injected onto the quartz wool. The quartz tube was then inserted into the pyroprobe, which was then inserted in the interface, previously stabilised at 250 or 25 °C. The samples were then pyrolysed under a flow of nitrogen with 10 % of oxygen, at a flow rate of 25 cm^3/min.

Two different pyrolysis tests were performed, one with a dynamic system and another with a static system. In the dynamic system, an initial temperature of 300 °C, was held for 5 seconds followed by a rapid increase of the temperature (30 °C/s) to 900 °C. The final temperature was maintained for another 5 seconds (figure **3.3**).

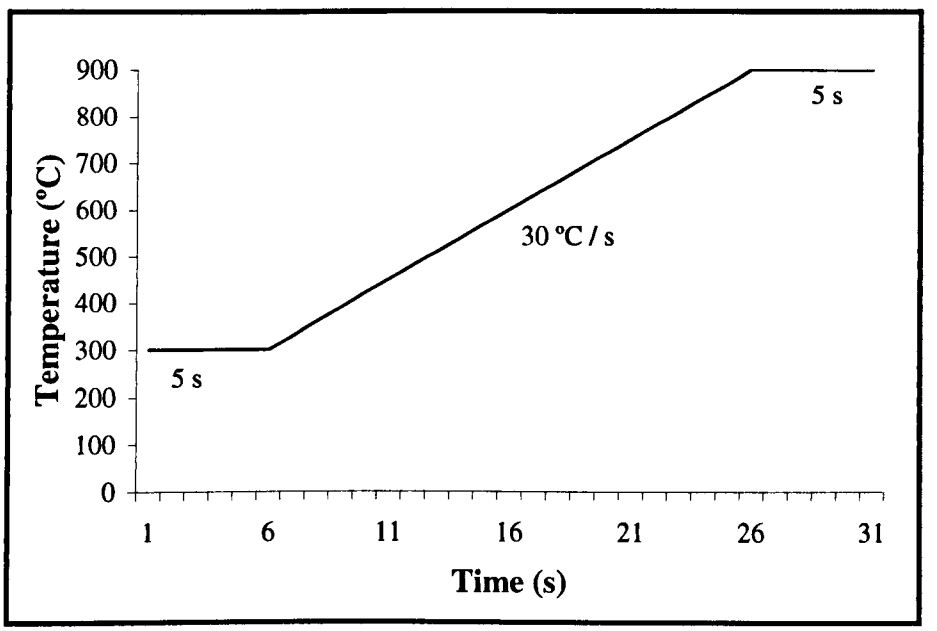


Figure 3.3: Temperature profile of the dynamic system.

In the static system, the different compounds were pyrolysed at a constant temperature for 30 seconds. The different temperatures used were 25, 200, 400, 600 and 800 °C.

After pyrolysis the products were trapped with liquid nitrogen in the injector of the GC. The products of pyrolysis were then blast heat at injector to transfer the products to the GC column using helium as carrier gas at constant flow of $1cm^3/min$. To eliminate carry-over, a blank sample was run between samples.

3.3 - Results and discussion

Pyrolysis of the sugars esters was expected to release butyric acid molecules and products from the degradation of the sugar moiety.

The pyrolysis of ethyl 3,6-butyryl β-glucopyranoside **68** and ethyl 2,6-butyryl β-glucopyranoside **69** was used to verify if the butyric acid moieties exhibit similar reactivity, under the same pyrolysis conditions, in both molecules. These two compounds have two butyric acid units, each differing only in the position of esterification of the secondary hydroxyl group.

In order to verify the influence of the anomeric protecting group, benzyl 2,6-butyryl α-glucopyranoside **77** was pyrolysed. 1,6-Butyryl glucopyranoside **82** was also tested. In this compound one of the butyric acid units is attached to the anomeric position. Chemically, the anomeric position of D-glucose is the most reactive and it was also expected to be the most reactive under pyrolysis conditions, showing a good release of butyric acid.

Three monoesterified glycopyranosides, benzyl 6-butyryl α-mannopyranoside **93**, benzyl 6-butyryl β-galactopyranoside **103** and benzyl 6-butyryl glucopyranoside **110**, were pyrolysed so as to test the difference in reactivity of the different carbohydrates.

Two compounds without free hydroxyl groups, a pentaesterified glucopyranoside, 1,2,3,4,6-butyryl-glucopyranoside **84** and a tetraesterified glycopyranoside, benzyl 2,3,4,6-butyryl α-glucopyranoside **85** where also pyrolysed. These compounds have the highest loading of butyric acid and were expected to give the greatest release of butyric acid of all the compounds tested.

The choice of solvent influenced the pyrolysis of glycopyranosides. Three different solvents were tested for the pyrolysis, namely ethanol, methanol and

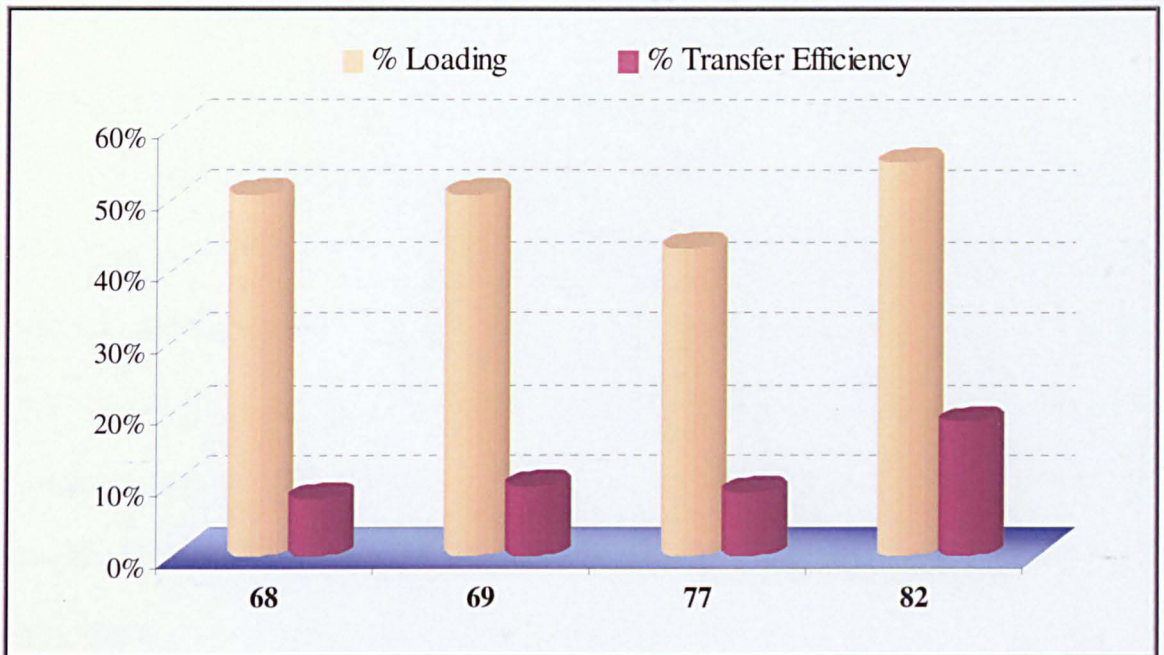
chloroform. It was found that ethanol and methanol interfere with the pyrolysis process of the glycopyranosides generating a large peak corresponding to butyric ethyl ester or butyric methyl ester respectively. This result can be explained by the reaction of the alcohol with the esters present on the glucopyranoside.

When chloroform was used as solvent, no interfering peaks were detected.

3.3.1 - Pyrolysis using a dynamic system

The results of the pyrolysis are expressed in terms of % loading and % transfer efficiency. The % loading is the percentage of butyric acid on the sugar derivative, as demonstrated in the following example: each mole of ethyl 2,6-butyryl β-glucopyranoside **69** has 2 moles of butyric acid attached, corresponding to 51 % in weight of the total molecule of the glycopyranoside. The % transfer efficiency is the % of butyric acid released on pyrolysis.

The pyrolysis of the diesters **68**, **69**, **77** and **82** using the dynamic system previously described, shows the different release of butyric acid units (figure **3.4**).



% Loading	51	51	43	55
% Transfer efficiency	8	10	9	19

Figure 3.4: Loading and transfer efficiency for the pyrolysis of diesters **68**, **69**, **77** and **82** (structures in fig **3.2**).

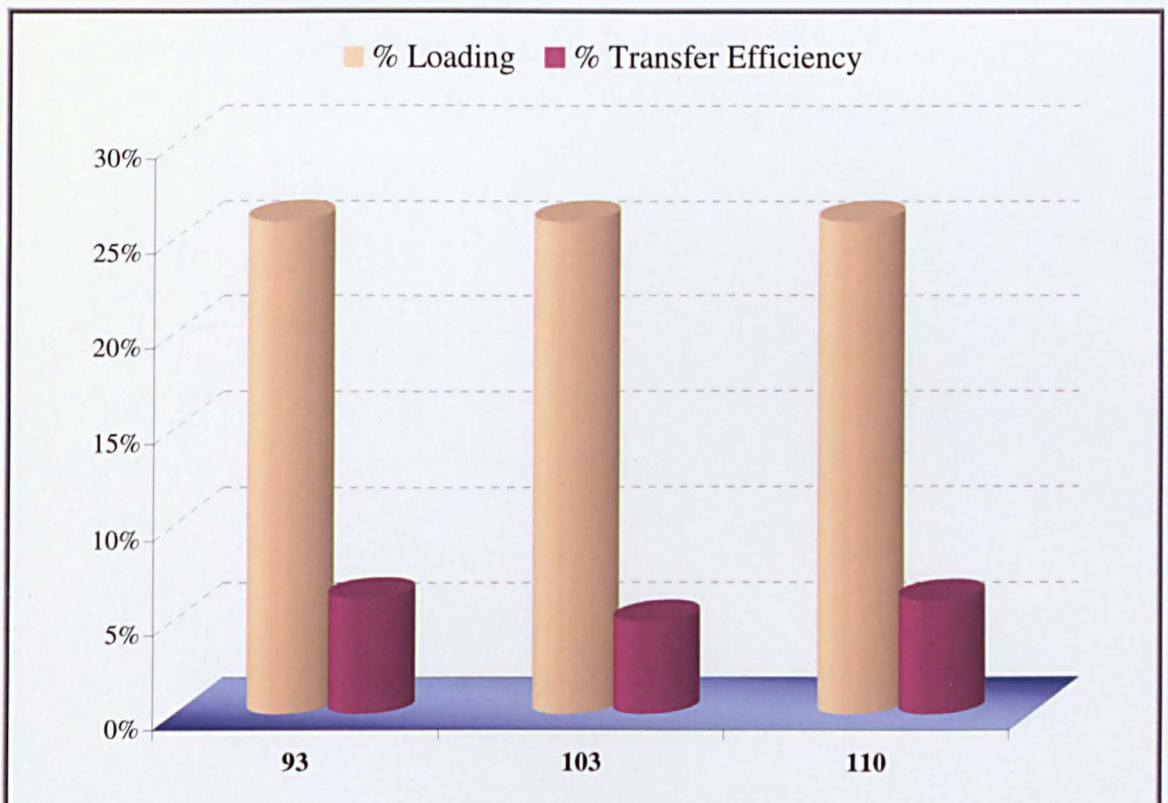
The ethyl 3,6- and 2,6-butyryl glycopyranosides, **68** and **69**, have the same anomeric protecting group, differing only in the position of esterification of the secondary hydroxyl group, C-3 and C-2 respectively. The ability to deliver butyric acid was very similar (10 and 8 % transfer efficiency respectively) the maximum transfer efficiency is 51 % (figure **3.4**). Irrespective of the position of esterification (at a secondary hydroxyl group) the percentage efficiency of butyric acid release is very similar under the pyrolysis conditions.

With the change of the anomeric protecting group from ethyl to benzyl, in 2,6-butyryl benzyl α-glucopyranoside **77**, the efficiency increased only slightly (figure **3.4**).

1,6-Butyryl-glucopyranoside **82** bearing one butyric acid molecule linked at the anomeric position, gave the best result for transfer efficiency (19 %) when compared

with the previous three diesters, (figure **3.4**). This result can be explained by a higher reactivity when the acyl unit is attached at the anomeric position.

Different glycopyranosides could show a difference in efficiency for the release of butyric acid. To test this hypothesis monoesterified glycopyranosides, benzyl 6-butyryl glucopyranoside **110**, benzyl 6-butyryl galactopyranoside **103** and benzyl 6-butyryl mannopynoside **93**, were pyrolysed. All the three glycopyranosides were esterified at the primary hydroxyl group and have the same anomeric protecting group, namely benzyl. Results indicated that the efficiency of butyric acid release was very similar, irrespective of the sugar used (figure **3.5**).

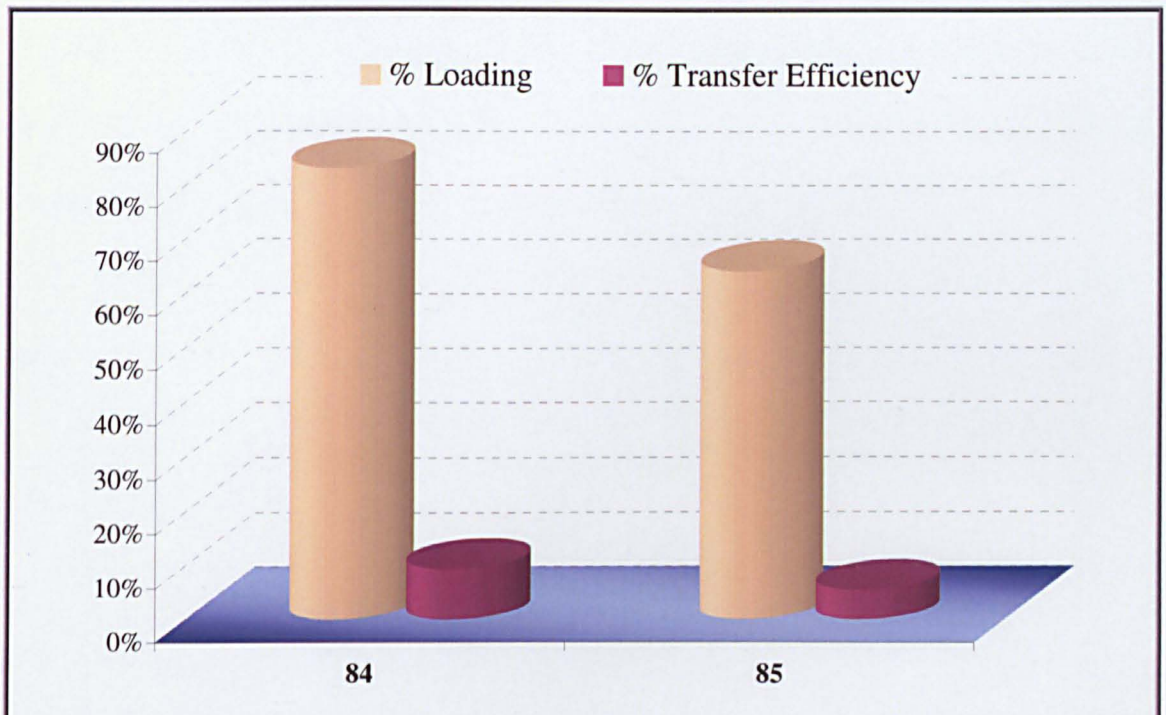


% Loading	26	26	26
% Transfer efficiency	6	5	6

Figure 3.5: Loading and transfer efficiency for the pyrolysis of pyranosydes **93**, **103**, and **110**.

The pentaesterified glucopyranoside **84** and the tetraesterified glucopyranoside **85**, have the highest percentage loading of butyric acid of all the compounds tested and were therefore expected to show the highest percentage of release, especially the pentaesterified glucopyranoside **84**, due to the presence of a butyric acid unit at the anomeric position.

However, in practice, the efficiency of release was poor compared to the 1,6-butyryl glucopyranoside **82** (figure **3.6**). The pentaesterified sugar has a better percentage release than the tetraesterified sugar. This efficiency is due to the presence of a butyric acid molecule at the anomeric position, which, as observed previously, is the best position from which to release butyric acid.



% Loading	83	64
% Transfer efficiency	9	5

Figure 3.6: Loading and transfer efficiency for the pyrolysis of the penta and tetraesterified compounds, **84** and **85**.

The low efficiency of these two compounds could be explained if the glycopyranoside had a relatively low boiling point, leading to a rapid distillation instead of pyrolysis, however, no peak of the pentaesterified compound was detected by the MS. The absence of free hydroxyl groups, which could aid the release of butyric acid, can also explain this low efficiency.

3.3.2 - Pyrolysis using a static system

Four different glycopyranosides, 3,6-butyryl ethyl-β-glucopyranoside **68**, 2,6-butyryl ethyl-β-glucopyranoside **69**, 2,6-butyryl benzyl-α-glucopyranoside **77** and 1,2,3,4,6-butyryl-glucopyranoside **84**, were pyrolysed at constant temperature, to determine the optimum temperature for the release of butyric acid.

Starting with the pyrolysis at room temperature (25 °C) no release of butyric acid was detected (figure **3.7**).

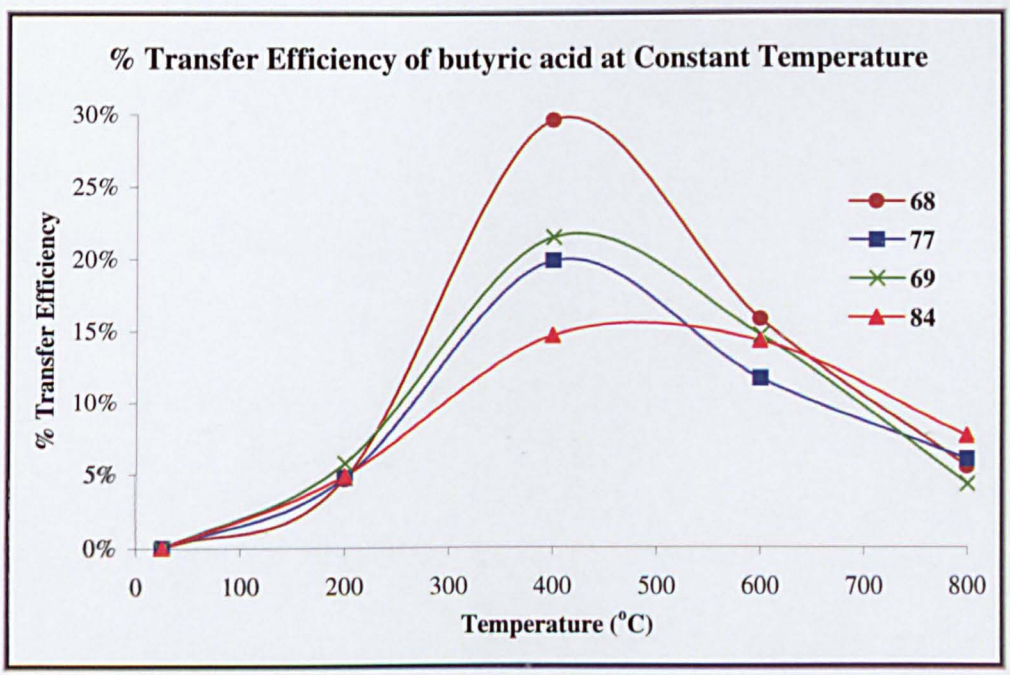


Figure 3.7: Results from the pyrolysis using a static system.

With the increase of the pyrolysis temperature to 200 °C, all the glycopyronisides had a similar increase on the release of butyric acid with a percentage of transfer efficiency of 5 %. At 400 °C all the glycopyranosides obtained a maximum transfer efficiency, but with different values for each compound. The best transfer efficiency was observed for 3,6-butyryl ethyl β-glucopyranoside **68** (—●—), (30 %) followed by 2,6-butyryl ethyl β-glucopyranoside **69** (—x—) (21 %), showing a distinct difference between the two secondary esters. This higher efficiency could be due to less steric hindrance of the ester at carbon C-3.

The change of the anomeric protecting group from ethyl to benzyl did show a significant variation on the yield obtained, 20 % for the glycopyranoside **77** (—■—). Again the poorest result was obtained with the pentaesterified glycopyranoside **84** (—▲—) with 15 % transfer efficiency, confirming the result obtained with the dynamic system.

With the increase of the temperature to 600 °C the % transfer efficiency decreased in all the diesters, dropping to values between 12 and 16 %. Only the pentaesterified glycopyranoside compound, with 14 %, maintained close to the value obtained at 400 °C. At 800 °C all the glycopyranosides show low % transfer efficiency with values around 5 %.

The decrease in transfer efficiency at 600 and 800 °C can be explained by the degradation of the butyric acid released at high temperatures.

3.4 - Summary and conclusions

Under dynamic conditions, the diester compounds **68** and **69** afforded a similar yield of butyric acid, showing no dependence on the site of esterification. Due to similar reactivity, these two compounds could be used as a mixture, avoiding an extra separation step [see section (**2.1.2**)].

The anomeric protecting group does not interfere with the release of the butyric acid molecules on the other positions of the glycopyranosides, as shown by the results obtained for ethyl and benzyl protected glycopyranosides (**69** and **77**).

The best transfer efficiency was observed when a butyrate was attached at the anomeric position, due to its more rapid loss from this position.

Release of butyric acid was not affected by the class of glycopyranoside used, as shown by the results obtained for the pyrolysis of the monoesterified glucopyranoside, mannopyranoside and galactopyranoside.

The pentaesterified glycopyranoside **84** or the tetraesterified glycopyranoside **85** did not afford the highest transfer efficiency, contrary to what was expected, showing that a high loading does not necessary result in an enhanced release of butyric acid. The presence of a free hydroxyl group on the glycopyranoside could be important in assisting the mechanism of pyrolysis.

With the static system, a maximum transfer efficiency of butyric acid, under pyrolysis, was observed around 400 °C. Below this temperature the transfer efficiency is low and at 25 °C no release was detected. Above 400 °C the degradation of the butyric acid released starts to be important, and at 900 °C most of the butyric acid is destroyed.

Chapter 4

Experimental

4.1 - General procedures

All reactions were monitored by thin layer chromatography, which was performed on 200-250 μm thickness Merck silica gel plates (60_{F254}). Compounds were detected by ultraviolet light or by staining with ceric ammonium molybdate solution followed by heating as a developing agent. Column chromatography was performed on Merck-60 silica gel (230-240 mesh). Melting points were recorded on a Reichert microscope and are uncorrected. Elemental analysis was performed on a Carlo Erba Elemental Analyser model 1106. IR spectra were recorded on a Perkin Elmer 883 spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were recorded in deuteriated solutions (Aldrich, Fluorochem) on AVANCE 400 MHz instrument or Varian Gemini300 instruments. ^{1}H- and ^{13}C- spectra were referenced using TMS or residual solvent peaks as internal standards. Chemical shifts (δ) are quoted in ppm and the absolute value of the coupling constants (J) in Hertz (Hz). The following abbreviations are used to describe the multiplicity: s, singlet; d, doublet; t, triplet; tt, triplet of triplets; q, quartet; dd, doublet of doublets; ddd, doublet of doublets of doublets; dt, doublet of triplets; m, multiplet; br, broad. Optical rotations $[\alpha]_D^T$ (concentration in g / 100 cm^3, solvent) were recorded on an Optical Activity A1000 polarimeter at 589 nm, where T is the temperature in °C. Low resolution CI mass spectra were measured on a Fisons TRIO 1000 spectrometer. Accurate mass spectra were obtained on a VG Analytical 7070E double focussing magnetic mass spectrometer. β-Glucosidase from almonds (EC) number 3.2.1.21, Novozyme® 435 (EC) number 3.1.1.3, Lipozyme® (EC) number 3.1.1.1, Pancreatin® and *Candida antarctica* lipase immobilised in a sol-gel-AK (EC) number EINECS were purchased

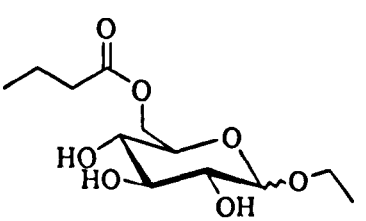
from Sigma-Aldrich. Lipase PS-C II was purchased from Amano Pharmaceutical. Lipase AY, lipase AK and Acylase I were a gift from Amano Pharmaceutical.

All solvents and reagents were purchased and used directly from commercial suppliers without purification.

4.2 - D-Glucose series

4.2.1 - Ethyl as protecting group

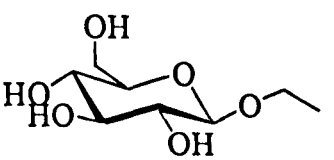
Ethyl 6-*O*-butyryl D-glucopyranoside (62)



A solution of ethyl-D-glucopyranoside **40** (1.93 g, 9.28 mmol) and vinyl butyrate (2.5 cm^3) in THF (20 cm^3) was prepared and was heated to 60 °C with stirring. When all the glucopyranoside was dissolved, Novozyme 435 (1.01 g) was added, and the stirring was maintained for 1 h. The enzyme was then filtered and washed with THF (10 cm^3). The reaction mixture was concentrated under reduced pressure giving a syrup which was chromatographed on silica gel with ethyl acetate /

ethanol (95:5) yielding a mixture of anomers as a white solid *ethyl 6-O-butyryl* D-*glucopyranoside* **62** (2.13 g, 83 %); (Found C, 51.58; H, 8.05. $C_{12}H_{22}O_7$ required C, 51.79; H, 7.97 %); m.p. 80 - 82 °C; ν_{max} ($CHCl_3$) / cm^{-1}: 1687 (C=O); δ_H (400 MHz, $CDCl_3$): 0.95 (3H, t, *J* 7.6 Hz, $^{\alpha}CH_3$), 0.96 (3H, t, *J* 7.3 Hz, $^{\beta}CH_3$), 1.25 (3H, t, *J* 7.1 Hz, $^{\alpha}CH_3$), 1.26 (3H, t, *J* 7.1 Hz, $^{\beta}CH_3$), 1.6-1.72 (4H, m, $^{\alpha+\beta}CH_2CH_3$), 2.33 (2H, t. *J* 7.5 Hz, $^{\alpha}CH_2CO$), 2.34 (2H, t. *J* 7.3 Hz, $^{\beta}CH_2CO$), 3.30-3.40 (2H, m, $^{2}CH+^{\beta}CH_2+$ ^{5}CH), 3.45-3.65 (4H, m, $^{\beta}CH_2+^{2'}CH+^{\alpha}CH_2+^{5}CH$), 3.70-3.80 (4H, m, $^{\alpha}CH_2+^{4\alpha+\beta}CH$), 3.90-4.0 (2H, m, $+^{3\alpha+\beta}CH$), 4.29 (1H, d, *J* 7.7 Hz, $^{1\beta}CH$), 4.30-4.4 (4H, m, $^{6\alpha+6\beta}CH_2$), 4.87 (1H, d, *J* 3.9 Hz, $^{1\alpha}CH$); δ_C (100 MHz, $CDCl_3$): 13.66 ($^{\alpha+\beta}CH_3$), 15.03 ($^{\alpha}CH_3$), 15.15 ($^{\beta}CH_3$), 18.42 ($^{\alpha+\beta}CH_2CH_3$), 36.10 (CH_2CO), 63.36 ($^{\alpha}CH_2$), 63.49 ($^{\beta}CH_2$), 63.98 ($^{\alpha}CH_2$), 65.58 ($^{\beta}CH_2$), 69.92, 70.37, 72.13, 73.57, 73.97, 74.47, 76.32, 98.17 ($^{\alpha}CH$), 102.48 ($^{\beta}CH$), 174.15 ($^{\alpha}C$=O), 174.17 ($^{\beta}C$=O); *m/z* (CI) $[M + NH_4]^+$ 296 (81 %).

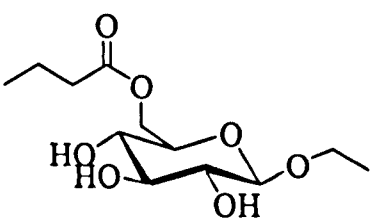
Ethyl β-D-glucopyranoside (55)



D-Glucose **4** (0.81 g, 4.48 mmol) was added to a solution of water (2 cm^3) in ethanol (18 cm^3), and heated to 50 °C, with stirring. After 10 minutes β-glucosidase from almonds (0.11 g) was added to the reaction mixture and stirred for 3 days. The enzyme was filtered through Celite® and washed with ethanol (20 cm^3). The excess of solvent was removed under reduced pressure yielding a yellow crude product, which was chromatographed on silica gel with chloroform-methanol (9:1) yielding a

white solid, *ethyl β-D-glucopyranoside* **55** (0.79 g, 85 %); m.p. 82-84 °C (lit.,[115] 98-100 °C); (Found: C, 45.88; H, 7.80. $C_8H_{16}O_6$ requires C, 46.15; H, 7.75 %); $[\alpha]_D^{23}$ – 28.57 (*c* 1.0 in MeOH); δ_H (400 MHz, D_2O): 1.11 (3H, t, *J* 7.1 Hz, CH_3), 3.12 (1H, t, *J* 9.1 Hz, ^3CH), 3.25 (1H, t, *J* 9.1 Hz, ^4CH), 3.31-3.38 (2H, m, CH_2), 3.56-3.64 (2H, m, $^{5+6}CH_2$), 3.79 (1H, d, *J* 12.1 Hz, ^6CH), 3.84 (1H, t, *J* 8.1 Hz, ^2CH), 4.34 (1H, d, *J* 8.1 Hz, $^\beta CH$); δ_C (100 MHz, D_2O) 14.71 (CH_3), 61.28, 66.62, 70.15, 73.60, 76.30, 76.37, 102.33 ($^\beta CH$); *m/z* (CI) $[M + NH_4]^+$ 226 (100 %).

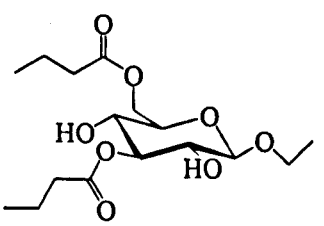
Ethyl 6-*O*-butyryl β-D-glucopyranoside (67)



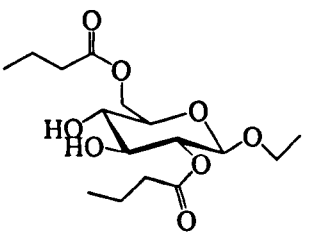
Novozyme 435 (0.42 g) was added to a solution of ethyl β-D-glucopyranoside **55** (0.42 g, 2.01 mmol) in vinyl butyrate (0.52 cm^3, 4.1 mmol) and THF (10 cm^3). The reaction mixture was stirred for 1 hour at 60 °C, after which the enzyme was filtered and washed with THF (10 cm^3). The excess of THF was evaporated under reduced pressure to afford a crude residue. The residue was purified by flash chromatography on silica gel with ethyl acetate-ethanol (95:5) yielding *ethyl 6-O-butyryl β-D-glucopyranoside* **67** (0.45 g, 81 %) as a white solid; m.p. 80 °C; (Found: C, 51.92; H, 8.06. $C_{12}H_{22}O_7$ requires C, 51.79; H, 7.97 %); $[\alpha]_D^{26}$ – 51 (*c* 1.0 in $CHCl_3$); ν_{max} ($CHCl_3$) / cm^{-1} 1730 (C=O); δ_H (400 MHz, $CDCl_3$) 0.96 (3H, t, *J* 8.1 Hz, CH_3), 1.26

(3H, t, *J* 7.1 Hz, C*H*$_3$), 1.63-1.71 (2H, m, C*H*$_2$CH$_3$), 2.36 (2H, t, *J* 8.1 Hz, C*H*$_2$CO), 3.35-3.41 (2H, m, ^{4}C*H*+^{2}C*H*), 3.46 (1H, ddd, *J* 2.0, 5.1 and 9.1 Hz, ^{5}C*H*), 3.55-3.65 (2H, m, ^{3}C*H* + C*H*$_2$), 3.96 (1H, dq, *J*, 3.0 and 14.2 Hz, C*H*$_2$) 4.29 (1H, d, *J* 7.1 Hz, $^{\beta}$C*H*), 4.30 (1H, dd, *J* 12.1 and 2.0 Hz, ^{6}C*H*), 4.49 (1H, dd, *J* 12.1 and 5.1 Hz, $^{6'}$C*H*); δ_C (100 MHz, $CDCl_3$) 13.98 (C*H*$_3$), 15.48 (C*H*$_3$), 18.78 (C*H*$_2$CH$_3$), 36.41 (C*H*$_2$CO), 63.45 (^{6}*C*H), 66.02 (*C*H$_2$), 70.42 (^{2}*C*H), 74.02, 74.45, 76.34, 102.88 ($^{\beta}$C*H*), 174.90 (CO); *m/z* (CI) $[M + NH_4]^+$ 296 (100 %).

Ethyl 3,6-*O*-butyryl *β*-D-glucopyranoside (68) and ethyl 2,6-*O*-butyryl *β*-D-glucopyranoside (69)



68



69

Novozyme (0.21 g) was added to a solution of ethyl 6-*O*-butyryl *β*-D-glucopyranoside **67** (0.44, 1.59 mmol) and vinyl butyrate (0.4 cm^3, 3.16 mmol) in THF (10 cm^3). The reaction mixture was heated to 60 °C and stirred for 7 days, after which time the enzyme was filtered off and washed with THF (10 cm^3). The solvent was evaporated under reduced pressure to afford a crude residue. The crude was chromatographed on silica gel with ethyl acetate-ethanol (95:5) yielding a mixture of compounds. The mixture was re-chromatographed on silica gel with ethyl acetate-

petroleum ether (2:1) affording *ethyl 3,6-O-butyryl β-D-glucopyranoside* **68** (0.28 g, 50 %) and *ethyl 2,6-O-butyryl β-D-glucopyranoside* **69** (0.17 g, 30 %).

Ethyl 3,6-O-butyryl β-D-glucopyranoside **68**

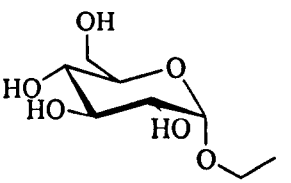
(Found: C, 54.97; H, 8.17. $C_{16}H_{28}O_8$ requires C, 55.16; H, 8.10 %); $[\alpha]_D^{27}$ – 17.59 (*c* 1.1 in $CHCl_3$); m.p. 36-38 °C; ν_{max} ($CHCl_3$) / cm^{-1} 1730 (C=O); δ_H (400 MHz, $CDCl_3$) 0.94-0.99 (6H, m, C*H*$_3$), 1.26 (3H, t, *J* 7.1 Hz, C*H*$_3$), 1.62-1.74 (4H, m, 2xC*H*$_2$CH$_3$), 2.35 (2H, t, *J* 8.1 Hz, C*H*$_2$CO), 2.40 (2H, t, *J* 7.1 Hz, 'C*H*$_2$CO), 3.46-3.55 (3H, m $^{2+4+5}$C*H*), 3.63 (1H, dq, *J* 2.0 and 16.2 Hz, 'C*H*$_2$), 3.96 (1H, dq, *J* 3.0 and 14.2 Hz, C*H*$_2$), 4.34 (1H, d, *J* 8.1 Hz, $^{\beta}$C*H*), 4.35-4.44 (2H, m, ^{6}C*H*$_2$), 4.93 (1H, t, *J* 9.1 Hz, ^{3}C*H*); δ_C (100 MHz, $CDCl_3$) 13.53 ('*C*H$_3$), 13.63 (*C*H$_3$), 15.12 (*C*H$_3$), 18.43 ('*C*H$_2$CH$_3$), 18.48 (*C*H$_2$CH$_3$), 36.06 ('*C*H$_2$CO), 36.24 (*C*H$_2$CO), 63.04 (^{6}*C*H$_2$), 65.76 (*C*H$_2$), 69.46, 72.13, 74.40, 77.62, 102.67 ($^{\beta}$*C*H), 174.09 ('C=O), 175.19 (C=O); *m/z* (CI) $[M + NH_4]^+$ 366 (88 %).

Ethyl 2,6-O-butyryl β-D-glucopyranoside **69**

(Found: C, 54.96; H, 8.15. $C_{16}H_{28}O_8$ requires C, 55.16; H, 8.10 %); $[\alpha]_D^{26}$ – 48.8 (*c* 1.1 in $CHCl_3$); m.p. 48 °C; ν_{max} $CHCl_3$ / cm^{-1} 1760 (C=O); δ_H (400 MHz, $CDCl_3$) 0.94-0.99 (6H, m, 2xC*H*$_3$), 1.19 (3H, t, *J* 7.1 Hz, C*H*$_3$), 1.63-1.73 (4H, m, 2xC*H*$_2$CH$_3$), 2.36 (4H, t, *J* 7.1 Hz, 2xC*H*$_2$CO), 3.41-3.48 (2H, m, $^{4+5}$C*H*), 3.51-3.62 (2H, m, ^{3}C*H*+C*H*$_2$), 3.89 (1H, dq, *J* 2.0 and 16.2 Hz, 'C*H*$_2$), 4.32 (1H dd, *J* 2.0 and 12.1 Hz $^{6'}$C*H*$_2$), 4.43 (1H, d, *J* 8.1 Hz, $^{\beta}$C*H*), 4.44 (1H dd, *J* 2.0 and 12.1 Hz ^{6}C*H*$_2$), 4.76 (1H, dd, *J* 9.1 and 8.1 Hz, ^{2}C*H*); δ_C (100 MHz, $CDCl_3$) 13.86 ('*C*H$_3$), 13.98 (*C*H$_3$), 15.41 (*C*H$_3$), 18.78 ('*C*H$_2$CH$_3$), 18.87 (*C*H$_2$CH$_3$), 36.40 ('*C*H$_2$CO), 36.60

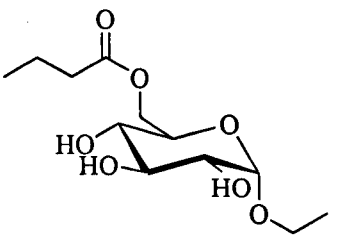
(CH_2CO), 63.31 ($^6CH_2$), 65.75 (CH_2), 71.18, 74.14, 74.19 (^2CH), 75.72 (^3CH), 101.04 ($^\beta CH$), 174.08 and 174.89 (C=O); *m/z* (CI) $[M + NH_4]^+$ 366 (37 %).

Ethyl α-D-glucopyranoside (56)



A reaction mixture consisting of Amberlite IR-120 (H^+) (3.5 g) and anhydrous D-glucose **4** (3.02 g, 16.76 mmol) in ethanol (25 cm^3) was refluxed for 20 hours followed by cooling to room temperature. After filtration of the resin, the excess of ethanol was evaporated under reduced pressure affording a yellow syrup. This syrup was dissolved in buffer pH 5.0 (25 cm^3) and heated to 35 °C. β-Glucosidase from almonds (0.047 g) was added to the reaction mixture and allowed to react for 3 days, after which time the enzyme was filtered off through Celite® and the buffer evaporated under reduced pressure. The crude product was chromatographed with chloroform-methanol (9:1) affording *ethyl α-D-glucopyranoside* **56** as a syrup (1.02 g, 30 %); (Found C, 45.98; H, 7.88. $C_8H_{16}O_6$ required C, 46.15; H, 7.75 %); $[\alpha]_D^{22}$ + 147.90 (*c* 0.57 in MeOH) [lit.,[116] + 150 (*c* 1.0 in MeOH)]; δ_H (400 MHz, DMSO): 1.12 (3H, t, *J* 7.2 Hz, CH_3), 3.01-3.09 (1H, m, ^2CH), 3.14-3.21 (1H, m), 3.32-3.48 (5H, m, $^{3+5+6}CH+CH_2$), 3.56-3.65 (2H, m, $^4CH+^1CH_2$), 4.63 (1H, d, *J* 3.9 Hz, $^\alpha CH$); δ_C (100 MHz, DMSO) 15.24 (CH_3), 61.18 ($^6CH_2$), 62.77, 70.45, 70.58, 72.02, 72.09, 98.45 ($^\alpha CH$); *m/z* (CI) $[M + NH_4]^+$ 226 (100 %).

Ethyl 6-*O*-butyryl α-D-glucopyranoside (70)



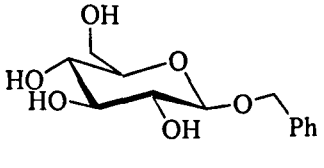
A solution of ethyl α-D-glucopyranoside **56** (0.22 g, 1.04 mmol) and vinyl butyrate (0.2 cm^3, 1.58 mmol) in THF (10 cm^3) was prepared and immersed in a oil bath at 60 °C. After 10 minutes Novozyme (0.13 g) was added to the reaction mixture, and the mixture was stirred for 6 hours, after which time the enzyme was filtered off and washed with THF (5 cm^3). Excess solvent was then evaporated under reduced pressure and the crude product was chromatographed over silica with ethyl acetate-ethanol (95:5) yielding *ethyl 6-O-butyryl α-D-glucopyranoside* **70** as a white solid (0.18 g, 60 %); (Found C, 51.80; H, 7.99. $C_{12}H_{22}O_7$ required C, 51.79; H, 7.97 %); m.p. 70-72 °C; $[\alpha]_D^{21}$ + 81.9 (*c* 1.05 in $CHCl_3$); ν_{max} ($CHCl_3$) / cm^{-1} 1730 (C=O); δ_H (400 MHz, $CDCl_3$): 0.96 (3H, t, *J* 7.1 Hz, C*H*$_3$), 1.25 (3H, t, *J* 7.1 Hz, C*H*$_3$), 1.61-1.73 (2H, m, C*H*$_2$CH$_3$), 2.36 (2H, t, *J* 8.1 Hz, C*H*$_2$CO), 3.45 (1H, t, *J* 9.1 Hz, ^{4}C*H*), 3.47-3.60 (2H, m, C*H*$_2$ + ^{2}C*H*), 3.67 (1H, br s, O*H*), 3.72-3.85 (3H, m $^{3+5}$C*H*+'C*H*$_2$), 4.26 (1H, dd, *J* 12.1 and 2.0 Hz, ^{6}C*H*$_2$), 4.47 (1H, dd, *J* 12.1 and 4.0 Hz, $^{6'}$C*H*$_2$), 4.89 (1H, d, *J* 4.0 Hz, $^{\alpha}$C*H*); δ_C (100 MHz, $CDCl_3$): 13.66 (*C*H$_3$), 15.02 (*C*H$_3$), 18.40 (*C*H$_2$CH$_3$), 36.02 (*C*H$_2$CO), 63.10 (*C*H$_2$), 63.93 (^{6}*C*H$_2$), 69.85, 70.02 (^{4}*C*H), 72.09 (^{2}*C*H), 74.40, 98.06 ($^{\alpha}$*C*H), 174.44 (*C*=O); *m/z* (CI) $[M + NH_4]^+$ 296 (100 %).

Separation of anomers of ethyl D-glucopyranoside

Novozyme 435 (0.53 g) was added to a solution of ethyl D-glucopyranoside **40** (0.88 g, 4.24 mmol) and vinyl butyrate (1.1 cm^3, 8.66 mmol) in THF (20 cm^3). The reaction mixture was immersed in a oil bath at 60 °C and stirred for 4 days after which time the enzyme was filtered off and washed with THF (10 cm^3). The excess of solvent was then evaporated under reduced pressure and the crude product was chromatographed over silica gel using a gradient of ethyl acetate-petroleum ether (2:1) to ethyl acetate (100 %), affording *ethyl 3,6-O-butyryl β-D-glucopyranoside* **68** (0.27 g, 18 %), *ethyl 2,6-O-butyryl β-D-glucopyranoside* **69** (0.19 g, 13 %), and *ethyl 6-O-butyryl α-D-glucopyranoside* **70** (0.57 g, 48 %).

4.2.2 - Benzyl as protecting group

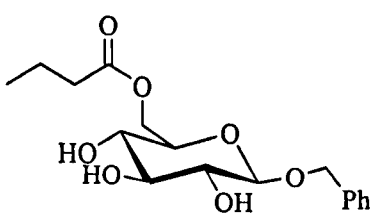
Benzyl-β-D-glucopyranoside (73)



β-Glucosidase from almonds (120 mg) was added to a solution of D-glucose **4** (0.81 g, 4.48 mmol) in distilled water (2 cm^3) and benzyl alcohol (18 cm^3). The solution was stirred for 30 h at 50 °C, after which time the enzyme was filtered off and washed with distilled water (5 cm^3). The excess of benzyl alcohol was removed

under reduced pressure at 90 °C and 1 mbar. Flash chromatography of the residue on silica gel with chloroform-methanol (9:1) as the eluent afforded the *benzyl-β-D-glucopyranoside* **73** (0.52 g, 43 %) as a white solid, (Found C, 57.73; H, 6.77. $C_{13}H_{18}O_6$ required C, 57.77; H, 6.71 %), m.p. 104 °C (lit.,[117] 120-121 °C); $[\alpha]_D^{27}$ – 52 (*c* 0.5 in MeOH) [lit.,[25] –55.1 (*c* 1.0 in MeOH)]; $\nu_{max}(CHCl_3)/cm^{-1}$ 1140 (C=O); δ_H (400 MHz, DMSO): 3.02-3.19 (3H, m), 3.44-3.52 (2H, m), 3.71 (1H, dd, *J* 11.6 and 4.0 Hz), 4.24 (1H, d, *J* 7.8 Hz, $^{\beta}CH$), 4.49 (1H, t, *J* 6.1 Hz), 4.59 (1H, d, *J* 12.4 Hz, CH_2), 4.83 (1H, d, *J* 12.4 Hz, $'CH_2$), 4.88 (2H, dd, *J* 11.6 and 4.8 Hz), 7.26-7.41 (5H, m, ^{Ph}CH); δ_C (100 MHz, DMSO): 48.95, 61.46, 69.79, 70.45, 73.84, 77.07, 77.31, 102.41 ($^{\beta}C$), 127.69 (^{Ph}CH), 127.95 (^{Ph}CH), 128.45 (^{Ph}CH), 138.40 (^{Ph}C); *m/z* (CI) $[M + NH_4]^+$ 288 (100%).

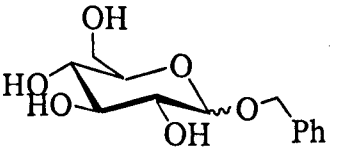
Benzyl 6-*O*-butyryl β-D-glucopyranoside (74)



Novozyme 435 (0.10 g) was added to a solution of β-benzyl D-glucopyranoside **73** (0.31 g, 1.14 mmol) and vinyl butyrate (0.3 cm^3, 2.36 mmol) in dry THF (10 cm^3) and immersed in a oil bath at 60 °C with stirring. After 3 hour the reaction was complete by TLC, and was cooled to room temperature; the excess of solvent was evaporated under reduced pressure. Flash chromatography of the residue over silica

gel with EtOAc / EtOH (95:5) as eluent gave *benzyl 6-O-butyryl β-D-glucopyranoside* **74** as a white solid (0.38 g, 98 %); (Found C, 59.77; H, 7.17. $C_{17}H_{24}O_7$ required C, 59.99; H, 7.11 %); m.p. 65 °C; $[\alpha]_D^{23}$ - 55.76 (*c* 1.1 in $CHCl_3$); ν_{max} ($CHCl_3$) / cm^{-1} 1720 (C=O); δ_H (400 MHz, $CDCl_3$): 0.96 (3H, t, *J* 8.1 Hz, CH_3), 1.62-1.71 (2H, m, CH_2CH_3), 2.36 (2H, t, *J* 7.1 Hz, CH_2CO), 3.35-3.43 (2H, m, ^{2+5}CH), 3.51 (1H, t, *J* 8.1 Hz, ^{3}CH), 4.30-4.32 (1H, m ^{4}CH), 4.33 (1H, d, *J* 7.1 Hz, $^{\beta}CH$), 4.40 (1H, d, *J* 4.0 Hz, ^{6}CH), 4.43 (1H, d, *J* 4.0 Hz, $^{6'}CH$), 4.59 (1H, d, *J* 12.1 Hz, CH_2Ph) 4.89 (1H, d, *J* 12.1 Hz, CH_2Ph), 7.27-7.36 (5H, m, ^{Ph}CH); δ_C (100 MHz, $CDCl_3$): 13.68 (CH_3), 18.42 (CH_2CH_3), 36.04 (CH_2CO), 63.15 ($^{6}CH_2$), 70.03, 71.09 (CH_2Ph), 73.52, 73.96, 75.92 (^{3}CH), 101.41 ($^{\beta}CH$), 128.12 (^{Ph}CH), 128.27 (^{Ph}CH), 128.53 (^{Ph}CH), 136.80 (^{Ph}C), 174.44 (*C*=O); *m/z* (CI) $[M + NH_4]^+$ 358 (100%).

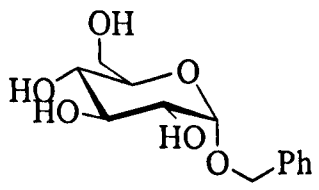
Benzyl-D-glucopyranoside (27)



37 % Hydrochloric acid (1 cm^3) was added dropwise to benzyl alcohol (25 cm^3) and the solution was then immersed in an oil bath at 80 °C and stirred for five minutes. Anhydrous D-glucose **4** (1.18 g, 6.55 mmol) was added portionwise over 15 minutes. The reaction mixture was allowed to react for 6 h at 80 °C. After that time the reaction was cooled to room temperature and $NaHCO_3$ (0.5 g) was added to the reaction mixture and stirred for 10 min (liberation of gas was observed). The reaction

mixture was filtered through Celite® and the excess of benzyl alcohol was distilled under high vacuum (1 mbar) at 90 °C. The resulting yellow crude was chromatographed on silica gel with chloroform-methanol (9:1) yielding a yiellow syrup *benzyl-D-glucopyranoside* **27** (0.78 g, 44%), (Found C, 57.33; H, 6.82. $C_{13}H_{18}O_6$ required C, 57.77; H, 6.71 %); δ_H (400 MHz, D_2O): 3.49-3.69 (1H, m), 3.77 (1H, dd, *J* 9.1 and 3.0 Hz), 3.84-4.00 (1H, m), 4.13 (1H, d, *J* 12.1 Hz), 4.73 (1H, d, *J* 8.1 Hz, $^{\beta}CH$), 4.91-5.02 (2H, m), 4.83 (1H, d, *J* 12.1 Hz), 5.15 (1H, d, *J* 11.1 Hz), 5.23 (1H, d, *J* 3.0 Hz, $^{\alpha}CH$), 7.57-7.73 (10H, m, ^{Ph}CH); δ_C (100 MHz, D_2O) 60.91, 70.05, 71.79, 72.42, 73.59, 76.30, 76.42, 98.15 ($^{\alpha}C$), 101.71 ($^{\beta}C$), 128.75 (^{Ph}CH), 128.93 (^{Ph}CH), 128.97(^{Ph}CH), 129.17(^{Ph}CH), 137.56 (^{Ph}C); *m/z* (CI) $[M + NH_4]^+$ 288 (100 %).

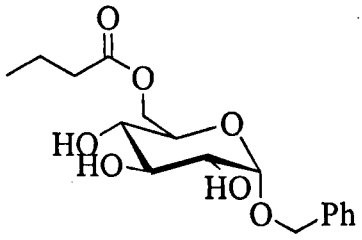
Benzyl-α-D-glucopyranoside (75)



β-Glucosidase from almonds (0.06 g) was added to a solution of benzyl-D-glucopyranoside **27** (0.42 g, 1.56 mmol) in a citric acid buffer pH 5.0 (20 cm^3). The reaction mixture was stirred for 3 days at 50 °C. The enzyme was then filtered and the excess of buffer was evaporated under reduced pressure. The crude product was chromatogrphed over silica gel with chloroform-methanol (9:1) yielding *benzyl-α-D-glucopyranoside* **75** (0.28 g, 67 %), (Found C, 57.49; H, 6.77. $C_{13}H_{18}O_6$ required

C, 57.77; H, 6.71 %); m.p. 102 °C (lit.,[118] 122 °C); $[\alpha]_D^{22}$ + 134.7 (*c* 1.01 in MeOH) [lit.,[118] + 133.5 (*c* 2.5 in H_2O)];; δ_H (400 MHz, DMSO) 3.04-3.10 (1H, m), 3.20-3.25 (1H, m), 3.41-3.50 (3H, m), 3.63 (1H, dd, *J* 6.1 and 9.1 Hz), 4.41-4.48 (2H, m), 4.66 (1H, s, O*H*), 4.70 (2H, d, *J* 6.1 Hz), 4.73 (1H, d, *J* 3.0 Hz, $^{\alpha}$C*H*), 4.83 (1H, d, *J* 5.1 Hz), 7.25-7.38 (5H, m); δ_C (100 MHz, DMSO) 61.37, 68.24, 70.77, 72.34, 73.40, 73.71, 98.28 ($^{\alpha}$*C*), 127.66 (^{Ph}*C*H), 127.88 (^{Ph}*C*H), 128.50 (^{Ph}*C*H), 138.50 (^{Ph}*C*); *m/z* (CI) $[M + NH_4]^+$, 288 (100 %).

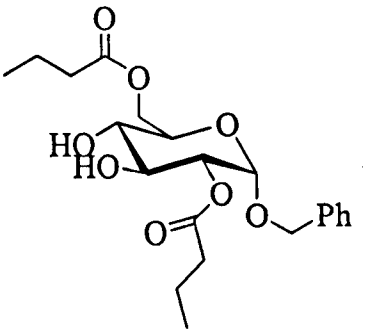
Benzyl 6-*O*-butyryl α-D-glucopyranoside (76)



Vinyl butyrate (0.2 cm^3, 1.576 mmol) was added to a solution of benzyl-α-D-glucopyranoside **75** (0.25 g, 0.906 mmol) in THF (20 cm^3) and stirred for 5 min at room temperature. Novozyme 435 (0.10 g) was added and the reaction mixture was heated to 60 °C and stirred for 1 h. The enzyme was filtered and washed with THF (10 cm^3). The excess of solvent was evaporated under reduced pressure and the crude product chromatographed on silica gel with ethyl acetate-ethanol (95:5) yielding *benzyl 6-O-butyryl α-D-glucopyranoside* **76** (0.30 g, 97 %); (Found C, 60.20; H, 7.15. $C_{17}H_{24}O_7$ required C, 59.99; H, 7.11 %); m.p. 72 °C; $[\alpha]_D^{21}$ + 77.8 (*c* 1.0 in $CHCl_3$); ν_{max} ($CHCl_3$) / cm^{-1} 1729 (C=O); δ_H (400 MHz, $CDCl_3$) 0.95 (3H, t, *J* 7.1 Hz,

CH_3), 1.62-1.71 (2H, m, CH_2CH_3), 2.35 (2H, t, *J* 7.1Hz, CH_2CO), 3.33 (1H, t, *J* 9.1 Hz, ^4CH), 3.5 (1H, dd, *J* 3.0 and 9.1 Hz, ^2CH), 3.74-3.78 (2H, m, ^{3+5}CH), 4.19 (1H, dd, *J* 2.0 and 12.1 Hz, ^6CH), 4.42 (1H, dd, *J* 4.0 and 12.1 Hz, $^{6'}CH$), 4.53 (1H, d, *J* 11.1 Hz, CH_2), 4.72 (1H, d, *J* 11.1 Hz, $'CH_2$), 4.95 (1H, d, *J* 4.0 Hz, $^{\alpha}CH$), 7.28-7.37 (5H, m); δ_C (100 MHz, $CDCl_3$) 14.01 (CH_3), 14.55 (CH_2CH_3), 18.79 (CH_2CO), 36.42, 60.76, 63.38, 70.34, 70.42, 70.54, 72.57, 74.79, 98.07 ($^{\alpha}CH$), 128.55 (^{Ph}CH), 128.96 (^{Ph}CH), 137.20 (^{Ph}C), 174.70 (C=O); (Found: $[M + NH_4]^+$, 358.186 $C_{17}H_{28}O_7N$ requires $[M + NH_4]^+$, 358.187); *m/z* (CI) $[M + NH_4]^+$ 358 (100 %).

Benzyl 2,6-*O*-butyryl α-D-glucopyranoside (77)



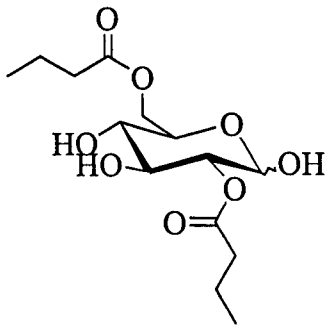
Lipase PS-C II (0.054 g) was added to a slurry of benzyl 6-*O*-butyryl α-D-glucopyranoside **76** (0.03 g, 0.09 mmol) in vinyl butyrate (5 cm^3) and immersed in a oil bath at 30 °C and stirred for 3 days. The enzyme was filtered and washed with THF (5 cm^3), the excess of solvent was evaporated under reduced pressure to afford a crude residue. Flash chromatography of the crude residue over silica gel with ethyl acetate-petroleum ether (2:1) afforded *benzyl 2,6-O-butyryl α-D-glucopyranoside* **77** as a yellow syrup (0.032 g, 86 %); $[\alpha]_D^{24}$ + 46 (*c* 0.5 in $CHCl_3$); ν_{max} ($CHCl_3$) / cm^{-1}

1720 (C=O); δ_H (400 MHz, $CDCl_3$) 0.94 (3H, t, *J* 8.1 Hz, CH_3), 0.97 (3H, t, *J* 7.1 Hz, $'CH_3$), 1.59-1.73 (4H, m, $2xCH_2CH_3$), 2.39-2.39 (4H, m, $2xCH_2CO$), 3.41 (1H, t, *J* 10.1 Hz, ^4CH), 3.82 (1 H, dd, *J* 10.1, 4.0 and 2.0 Hz, ^5CH), 4.04 (1H, t, *J* 10.1 Hz, ^3CH), 4.17 (1H, dd, *J* 12.1 and 2.0 Hz, ^6CH), 4.48-4.53 (2H, m, $^{6'}CH+CH_2$), 4.67-4.71 (2H, m, $^2CH + 'CH_2$), 5.11 (1H, d, *J* 4.0 Hz, $^{\alpha}CH$), 7.27-7.36 (5H, m, ^{Ph}CH); δ_C (100 MHz, $CDCl_3$) 13.94, 14.01, 18.58, 18.73, 36.36, 36.42, 63.18, 70.20, 70.22, 70.96, 71.70, 73.38, 95.92, 128.27, 128.36, 128.81, 137.42, 173.84, 174.94; (Found: $[M]^+$ 411.203 $C_{21}H_{31}O_8$ requires $[M]^+$, 411.202); *m/z* (CI) $[M]^+$ 411 (6 %).

Separation of anomers from benzyl D-glucopyranoside

Novozyme 435 (0.054 g) and lipase PS-C II (0.22 g) was added to a reaction mixture of benzyl D-glucopyranoside **27** (0.31 g, 1.15 mmol) in vinyl butyrate (5 cm^3). The reaction was stirred for 4 days at 40 °C, after which time the enzymes were filtered off and washed with THF (10 cm^3). Excess solvent was evaporated under reduced pressure and the crude product chromatographed over silica gel with ethyl acetate-petroleum ether (2:1) affording *benzyl 6-O-butyryl β-D-glucopyranoside* **74** (0.141 g, 36 %) and *benzyl 2,6-O-butyryl α-D-glucopyranoside* **77** (0.13 g, 28 %).

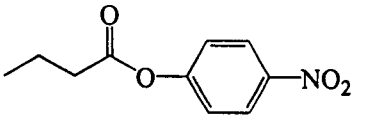
2,6-*O*-Butyryl D-glucopyranoside (78)



Benzyl 2,6-butyryl α-D-glucopyranoside **77** (0.35 g, 0.86 mmol) was dissolved in ethyl acetate, followed by the addition of Pd/C (10 %) (0.11 g). The reaction mixture was purged with hydrogen and then placed under pressure (0.2 bar) with stirring for 7 days, at room temperature. After completion of the reaction, the catalyst was filtered and washed with ethyl acetate. The excess of solvent was evaporated under reduced pressure and the crude product was chromatographed on silica gel using ethyl acetate-petroleum ether (2:1) as eluent affording *2,6-O-butyryl D-glucopyranoside* **78** (0.22 g, 75 %), as an α and β mixture; (Found C, 52.63; H, 7.62. $C_{14}H_{24}O_8$ required C, 52.49; H, 7.55 %); $[\alpha]_D^{22}$ + 49.6 (*c* 0.6 in $CHCl_3$); m.p. 70 °C; ν_{max} ($CHCl_3$) / cm^{-1} 1740 (C=O); δ_H (400 MHz, $CDCl_3$): 0.93-0.99 (12H, m, 4x $^{\alpha+\beta}CH_3$), 1.62-1.72 (8 H, m, 2x $^{\alpha+\beta}CH_2CH_3$) 2.33-2.41 (8 H, m, 2 x $^{\alpha+\beta}CH_2CO$), 3.23 (1H, bs), 3.43 (2H, t, *J* 9.2 Hz), 3.52 (1H, ddd, *J* 9.9, 4.8 and 2.2 Hz, ^5CH), 3.63-3.71 (3H, m), 3.78 (1H, bs) 4.00-4.05 (3H, m), 4.33 (2H, td, *J* 12.4 and 2.2 Hz), 4.43-4.49 (2H, m), 4.64 (1H, t, *J* 7.3 Hz), 4.67-4.72 (2H, m), 5.41 (1H, bs); δ_C (100 MHz, $CDCl_3$) 13.91, 13.95 and 14.01 ($^{\alpha+\beta}CH_3$), 18.74, 18.78 (CH_2CH_3), 36.35, 36.39, 60.88, 63.31, 63.43, 69.90, 70.90, 71.22 73.52, 74.53, 74.74, 75.84, 90.90 ($^{\alpha}CH$), 95.97 ($^{\beta}CH$), 175.20 and 175.26 (*C*=O); *m/z* (CI) $[M + NH_4]^+$ 338 (64%).

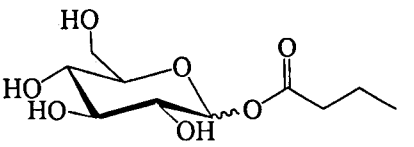
4.2.3 - Ester as protecting group

Butyric acid 4-nitro-phenyl ester (80)



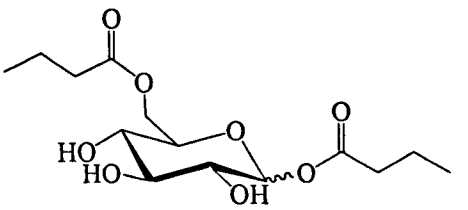
Under argon, triethylamine (3 cm^3) was added to a solution of *para*-nitrophenol (3.01 g, 21.64 mmol) in dry THF (20 cm^3) with stirring. After 20 minutes a solution of butyryl chloride **79** (3 cm^3, 39.74 mmol) in dry THF (10 cm^3) was added to the reaction mixture, forming a yellow precipitate. After 1.5 h the reaction was stopped, the THF was evaporated under reduced pressure and the crude product was dissolved in dichloromethane (20 cm^3). The solution was washed with HCl (0.5 N) (2 x 50 cm^3) followed by concentrated $NaHCO_3$ (2 x 50 cm^3) and then distilled water (2 x 50 cm^3). The organic layer was dried with magnesium sulphate, filtered and the excess of solvent evaporated under reduced pressure yielding *butyric acid 4-nitro-phenyl ester* **80** as a yellow liquid (4.18 g, 93 %); ν_{max} ($CHCl_3$) / cm^{-1} 1760 (C=O), 1352 and 1531 (-NO_2); δ_H (400 MHz, $CDCl_3$): 1.05 (3H, t, *J* 7.3 Hz, CH_3), 1.75-1.85 (2H, m, CH_3CH_2), 2.59 (2H, t, *J* 7.3 Hz, CH_2CO), 7.28 (2H, d, *J* 9.2 Hz, ^{Ph}CH), 8.25 (2H, d, *J* 9.2 Hz, ^{Ph}CH); δ_C (100 MHz, $CDCl_3$) 13.90 (CH_3), 18.63 (CH_2), 36.50 (CH_2), 122.79 (^{Ph}CH), 123.03 (^{Ph}CH), 125.14 (^{Ph}CH), 125.50 (^{Ph}CH), 145.64 (^{Ph}C), 155.92 (^{Ph}C), 171.45 (C=O); (Found: [M], 209.07 $C_{10}H_{11}O_4N$ requires [M], 209.07); *m/z* (EI) [M] 209 (4 %), 71 (100), 43 (86).

1-*O*-Butyryl-D-glucopyranoside (81)



Under argon a mixture of D-glucose **4** (5.03 g, 27.92 mmol) in pyridine (30 cm^3) was immersed in an oil bath at 100 °C for 30 minutes with stirring. When D-glucose **4** was completely dissolved the reaction was cooled to 30 °C. Sodium hydride, 60% suspension in mineral oil, was washed with n-hexane, and 0.150 g (6.25 mmol) was added to the reaction mixture, followed by the addition of butyric acid 4-nitro-phenyl ester **80**. The formation of a precipitate was observed, but after 20 minutes the reaction mixture was clear. One hour after the addition of the ester, the reaction was stopped and the mixture cooled to room temperature, followed by the evaporation of pyridine under reduced pressure. The crude product was chromatographed over silica with chloroform-methanol (9:1) yielding a syrup of *1-O-butyryl D-glucopyranoside* **81** as a mixture of α- and β-anomers, (0.94 g, 75 %); ν_{max} ($CHCl_3$) / cm^{-1} 1721 (C=O); δ_H (400 MHz, DMSO): 0.88 (3H, t, *J* 7.3 Hz, $^{\beta}CH_3$), 0.89 (3H, t, *J* 7.3 Hz, $^{\alpha}CH_3$) 1.48-1.60 (4H, m, $^{\alpha+\beta}CH_2CO$), 2.24-2.29 (4H, m, $^{\alpha+\beta}CH_2$), 3.55-3.77 (1H, m), 3.73-3.79 (1H, m), 3.96-4.02 (2H, m), 4.28 (3H, dt, *J* 1.9 and 11.8 Hz), 4.47 (1H, d, *J* 7.6 Hz, $^{\beta}CH$), 4.95-5.04 (2H, m), 6.29 (1H, s); δ_C (100 MHz, DMSO) 13.74, 18.30, 35.64, 64.20, 69.52, 70.95, 76.79, 92.63, 97.25, 173.12; (Found: $[M + NH_4]^+$, 268.140 $C_{10}H_{22}O_7N$ requires $[M + NH_4]^+$, 268.140); *m/z* (CI) $[M + NH_4]^+$ 268 (70 %).

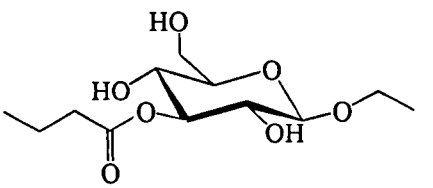
1,6-*O*-Butyryl-D-glucopyranoside (82)



Novozyme 435 (0.21 g) was added to a solution of 1-*O*-butyryl-D-glucopyranoside **81** (0.74 g, 2.94 mmol) and vinyl butyrate (1 cm^3, 7.888 mmol) in dry THF (20 cm^3). The reaction mixture was stirred for 1 hour at 60 °C, after which time the enzyme was filtered and washed with THF (10 cm^3). The excess of solvent was then evaporated under reduced pressure and the crude product was chromatographed with ethyl acetate-ethanol (95:5) yielding a syrup of *1,6-O-butyryl-D-glucopyranoside* **82** as a mixture of α- and β-anomers (0.53 g, 56 %); ν_{max} ($CHCl_3$) / cm^{-1} 1720 (C=O); δ_H (400 MHz, $CDCl_3$): 0.94-0.99 (12H, m, C*H*$_3$), 1.62-1.73 (8H, m, C*H*$_2$), 2.34-2.43 (8H, m, C*H*$_2$), 3.41-3.53 (4H, m), 3.60 (1H, dd, *J* 9.1 and 3.0 Hz), 3.67 (1H, t, *J* 9.1 Hz), 4.01-4.10 (3H, m), 4.28 (1H, d, *J* 2.0 Hz), 4.31-4.36 (2H, m), 4.39 (1H, d, *J* 3.0 Hz), 4.43 (1H, d, *J* 4.0 Hz), 4.45-4.51 (2H, m), 4.68-4.72 (2H, m), 5.29 (1H, d, *J* 4.0 Hz, $^{\beta}$C*H*), 5.42 (1H, d, *J* 3.0 Hz, $^{\alpha}$C*H*); δ_C (100 MHz, $CDCl_3$) 13.55, 13.58, 13.64, 18.37, 18.41, 35.98, 36.01, 36.11, 36.27, 62.86, 62.88, 68.70, 69.59, 70.05, 70.48, 70.82, 70.96, 73.14, 74.23, 75.74, 76.71, 90.58, 92.63, 95.70, 174.50, 174.85, 174.92, 175.62; (Found: $[M + NH_4]^+$, 338.181 $C_{14}H_{28}O_8N$ requires $[M + NH_4]^+$, 338.182); *m/z* (CI) $[M + NH_4]^+$ 338 (51 %).

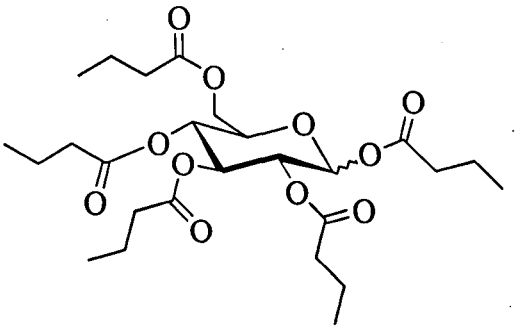
4.2.4 - Deacylation

Ethyl 3-*O*-butyryl β-D-glucopyranoside (83)



Novozyme 435 (0.086 g) was added to a solution of ethyl 3,6-butyryl β-D-glucopyranoside **68** (0.15g, 0.422 mmol) in buffer tris(hydroxymethyl) aminomethane at pH 6.5 (10 cm^3) and DMF (0.5 cm^3) and stirred for 7 h at 40 °C. The enzyme was filtered and washed with THF (10 cm^3) and the excess of solvent was then removed under reduced pressure. The resulting crude product was chromatographed over silica gel with ethyl acetate-ethanol (95:5) affording *ethyl 3-O-butyryl β-D-glucopyranoside* **83** as an syrup (0.11 g, 95 %); $[\alpha]_D^{25}$ - 16.58 (*c* 0.97 in MeOH); ν_{max} ($CHCl_3$) / cm^{-1} 1735 (C=O); δ_H (400 MHz, $CDCl_3$): 0.98 (3H, t, *J* 8.1 Hz, CH_3), 1.27 (3H, t, *J* 7.1 Hz, CH_3), 1.65-1.75 (2H, m, CH_2), 2.41 (2H, t, *J* 8.1 Hz, CH_2), 3.40-3.44 (1H, m, ^5CH), 3.48 (1H, t, *J* 9.1 Hz, ^2CH), 3.61-3.69 (2H, m, ^3CH+'CH_2), 3.83 (1H, d, *J* 12.1Hz, ^6CH), 3.91-4.01 (2H, m, $^{4+6'}CH$), 4.39 (1H, d, *J* 7.1 Hz, $^{\beta}CH$), 4.91 (1H, t, *J* 9.1 Hz, ^3CH); δ_C (100 MHz, $CDCl_3$): 13.54, 15.13, 18.44, 36.21, 62.18, 65.87, 69.49, 72.08, 75.69, 77.75, 102.56 ($^{\beta}CH$), 175.42 (C=O); (Found: $[M]^+$, 279.145 $C_{12}H_{23}O_7$ requires $[M]^+$, 279.144); *m/z* (CI) $[M + NH_4]^+$ 296 (76 %).

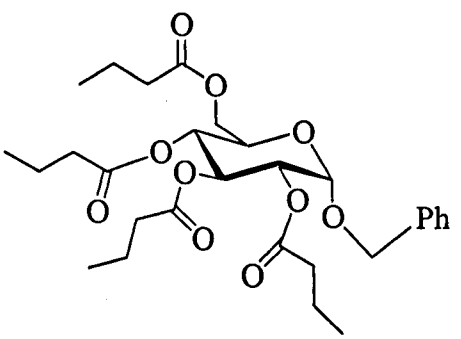
1,2,3,4,6-Penta-*O*-butyryl-D-glucopyranose (84)



A suspension of D-glucose **4** (2.07 g, 11.49 mmol) in pyridine (15 cm^3) was heated to 100 °C with stirring for 30 minutes and then cooled to 0 °C. A solution of butyryl chloride **79** (7 cm^3, 66.88 mmol) in DCM (20 cm^3) previously prepared was added dropwise during 1 h, keeping the temperature of the reaction below 10 °C. The reaction mixture was then cooled to room temperature and stirred for 15 h, the reaction mixture was then diluted with DCM (50 cm^3) and washed with HCl (0.1 M) (3 x 50 cm^3) followed by $NaHCO_3$ (conc., 3 x 50 cm^3). The organic layer was added to an aqueous amonia 25% (50 cm^3) and stirred for 10 min. followed by washing with brine (3 x 50 cm^3), the organic layer was dried with anhydrous sodium sulphate, filtered and the DCM evaporated under reduced pressure giving a yellow syrup which was chromatographed over silica gel with n-hexane-ethyl acetate (3:1) yielding a mixture of anomers of *1,2,3,4,6-penta-O-butyryl-D-glucopyranose* **84** as a syrup (5.18 g, 85 %); (Found C, 59.01; H, 8.06. $C_{26}H_{42}O_{11}$ required C 58.85; H, 7.98 %); ν_{max} ($CHCl_3$) / cm^{-1} 1726 (C=O); δ_H (400 MHz, $CDCl_3$): 0.87-1.02 (30H, m, CH_3), 1.51-1.76 (20H, m, CH_2CH_3), 2.18-2.37 (20H, m, CH_2CO), 3.85 (1H, ddd, *J* 10.1, 4.6 and 2.5 Hz, ^5CH), 4.08-4.16 (3H, m, C*H*), 4.23 (2H, td, *J* 12.6 and 5.0 Hz, C*H*), 5.08-5.20 (4H, m, C*H*+CH_2), 5.29 (1H, t, *J* 10.1 Hz, C*H*), 5.51 (1H, t, *J* 10.11 Hz, C*H*) 5.74

(1H, d, *J* 8.1 Hz, $^{\beta}CH$), 6.35 (1H, d, *J* 3.5 Hz, $^{\alpha}CH$); δ_C (100 MHz, $CDCl_3$): 13.48, 13.52, 13.58, 13.60 and 13.62 (5x CH_3), 18.04, 18.17, 18.20, 18.23 and 18.28 (5x CH_2CH_3), 35.83, 35.86, 35.89, 35.97 and 36.02 (5x CH_2CO), 61.30, 67.57, 69.20, 69.97, 70.05, 72.39, 72.88, 88.85 ($^{\alpha}C$), 91.63 ($^{\beta}C$), 171.40, 171.62, 171.79, 171.94 and 172.22 (5x C=O); (Found: $[M + Na]^+$, 553.262 $C_{26}H_{42}O_{11}Na$ requires $[M + Na]^+$, 553.263).

Benzyl 2,3,4,6-tetra-*O*-butyryl α-D-glucopyranoside (85)



Benzyl-α-D-glucopyranose **75** (0.401 g, 1.48 mmol) was dissolved in pyridine (5 cm^3) at room temperature for 10 minutes, and then cooled to - 5 °C. A solution of butyryl chloride **79** (0.9 cm^3, 7.09 mmol) in DCM (5 cm^3) was prepared and added dropwise, keeping the temperature below 10 °C, when the addition was complete, the reaction mixture was allowed to room temperature, and stirred for 15 h. The reaction mixture was diluted with DCM (5 cm^3) and washed with HCl 0.1 M (3x 50 cm^3) followed by $NaHCO_3$ (conc.) (3x 50 cm^3), the organic layer was added to a solution of aqueous amonia (50 cm^3) and stirred for 20 minutes. After this time the reaction mixture was washed with brine (3x 50 cm^3), the organic layer was dried with

anhydrous sodium sulphate and filtered. The excess of solvent was evaporated and the resultant crude product was chromatographed on silica gel with n-hexane-ethyl acetate (3:1) as eluent, yielding a syrup of *benzyl 2,3,4,6-tetra-O-butyryl α-D-glucopyranoside* **85** (0.63 g, 77 %); (Found C, 63.29; H, 7.68. $C_{29}H_{42}O_{10}$ required C, 63.26; H, 7.69 %); $[\alpha]_D^{21}$ + 90.6 (*c* 1.25 in $CHCl_3$); ν_{max} ($CHCl_3$) / cm^{-1} 1698 (C=O); δ_H (400 MHz, $CDCl_3$): 0.8-1.0 (12H, m, CH_3), 1.5-1.7 (8H, m, CH_2CH_3), 2.2-2.25 (8H, m, CH_2CO), 2.34 (2H, td, *J* 7.1 and 2.5 Hz CH_2CO), 4.0-4.1 (2H, m, ^4CH + $^6CH_2$), 4.22 (1H, dd, *J* 12.6 and 5.0 Hz, $^{6'}CH_2$), 4.5 (1H, d, *J* 12.1 Hz, O'CH_2), 4.7 (1H, d, *J* 12.1 Hz, OCH_2), 4.9 (1H, dd, *J* 10.6 and 4.0 Hz), 5.10 (1H, d, *J* 9.6 Hz), 5.14 (1H, d, *J* 3.5 Hz, $^{\alpha}CH$), 5.57 (1H, t, *J* 9.6 Hz), 7.34 (5H, m, ^{Ph}CH); δ_C (100 MHz, $CDCl_3$): 13.57, 13.60, 13.67 and 14.21 (CH_3), 18.23, 18.30, 18.34 and 21.06 (CH_2CH_3), 35.86, 35.92 and 36.02 (CH_2CO), 61.62 ($^6CH_2$), 67.68, 68.18, 69.76, 69.90, 70.66, 95.03 ($^{\alpha}C$), 127.7 (^{Ph}CH), 128.1 (^{Ph}CH), 128.4 (^{Ph}CH), 136.66 (^{Ph}C), 172.12, 172.56, 172.71 and 173.27 (*C*=O); *m/z* (CI) $[M + NH_4]^+$ 568 (100 %).

4.2.5 - Saponification

General procedure

A solution of NaOMe (0.11 g, 2 mmol) in MeOH (25 cm^3) was prepared. The glucopyranoside was added to 10 cm^3 of the previous solution and stirred for 2 to 4 minutes at room temperature, followed by the evaporation of the solvent under reduced pressure. The resulted crude product was filtered off through silica using

chloroform-methanol (9:1) as eluent. The excess of solvent was then evaporated under reduced pressure, yielding the pure anomer.

Saponification of ethyl 3,6-*O*-butyryl β-D-glucopyranoside (68)

From ethyl 3,6-*O*-butyryl β-D-glucopyranoside **68** (0.045 g, 0.129 mmol) was obtained *ethyl β-D-glucopyranoside* **55** (0.027 g, 99 %).

Saponification of ethyl 2,6-*O*-butyryl β-D-glucopyranoside (69)

From ethyl 2,6-*O*-butyryl β-D-glucopyranoside **69** (0.050 g, 0.144 mmol) was obtained *ethyl β-D-glucopyranoside* **55** (0.029 g, 97 %).

Saponification of ethyl 6-*O*-butyryl α-D-glucopyranoside (70)

From ethyl 6-*O*-butyryl α-D-glucopyranoside **70** (0.046 g, 0.165 mmol) was obtained *ethyl α-D-glucopyranoside* **56** (0.034 g, 99 %).

Saponification of benzyl 2,6-*O*-butyryl α-D-glucopyranoside (77)

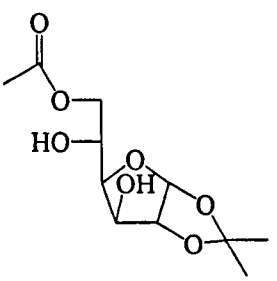
From benzyl 2,6-*O*-butyryl α-D-glucopyranoside **77** (0.053 g, 0.129 mmol) was obtained *benzyl α-D-glucopyranoside* **75** (0.034 g, 97 %).

Saponification of benzyl 6-*O*-butyryl β-D-glucopyranoside (74)

From benzyl 6-*O*-butyryl β-D-glucopyranoside **74** (0.057 g, 0.167 mmol) was obtained *benzyl β-D-glucopyranoside* **73** (0.043 g, 95 %).

4.2.6 - 1,2-O-isopropylidene-α-D-glucofuranose

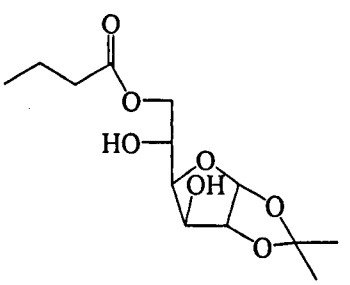
6-*O*-Acetyl 1,2-*O*-isopropylidene-α-D-glucofuranose (87)



1,2-*O*-Isopropylidene-α-D-glucofuranose **86** (0.51 g, 2.31 mmol) was added to a solution of vinyl acetate (20 cm^3, 0.21 mol) and THF (0.5 cm^3), the reaction mixture was stirred at 60 °C for 10 minutes, followed by the addition of Lipozyme (0.25 g). The reaction was stirred at 60 °C for another 2 hours after which time the enzyme was filtered and washed with vinyl acetate (5 cm^3), the excess of solvent was removed under reduced pressure to afford a crude residue. Flash chromatography of the crude residue over silica with chloroform-methanol (9:1) afforded a white solid, *6-O-acetyl 1,2-O-isopropylidene-α-D-glucofuranose* **87** (0.56 g, 92 %); (Found: C, 50.49; H,

6.91. $C_{11}H_{18}O_7$ requires C, 50.38; H, 6.92 %); $[\alpha]_D^{26}$ + 13 (*c* 1.0 in $CHCl_3$); m.p. 122 °C; ν_{max} ($CHCl_3$) / cm^{-1} 1741 (C=O); δ_H (400 MHz, $CDCl_3$) 1.32 (3H, s, C*H*$_3$), 1.49 (3H, s, C*H*$_3$), 2.12 (3H, s, C*H*$_3$), 2.95 (1H, d, *J* 3.6 Hz, O*H*), 3.08 (1H, d, *J* 3.6 Hz, O*H*), 4.08 (1H, dd, *J* 5.7 and 2.7 Hz, ^{5}C*H*), 4.21-4.26 (2H, m, ^{4}C*H* + ^{6}C*H*$_2$), 4.37 (1H, t, *J* 2.7 Hz, ^{3}C*H*), 4.43 (1H, dd, *J* 8.7 and 5.7 Hz, $^{6'}$C*H*$_2$), 4.53 (1H, d, *J* 3.9 Hz, ^{2}C*H*), 5.96 (1H, d, *J* 3.6 Hz, ^{1}C*H*); δ_C (100 MHz, $CDCl_3$) 21.15, 26.55, 27.19, 66.51, 69.79, 76.07, 79.66, 85.58, 105.36, 112.25, 171.95 (*C*=O); *m/z* (CI) $[M + NH_4]^+$ 280 (100 %).

6-*O*-Butanoyl 1,2-*O*-isopropylidene-α-D-glucofuranose (88)

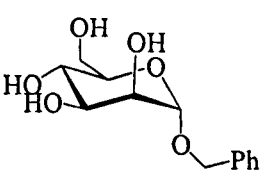


1,2-*O*-Isopropylidene-α-D-glucofuranose **86** (0.21 g, 0.96 mmol) was added to a solution of vinyl butyrate (10 cm^3) and THF (0.5 cm^3). The reaction mixture was stirred at 60 °C for 10 minutes, followed by the addition of Lipozyme (0.20 g). The reaction was stirred at 60 °C for 1 hour, after which time the enzyme was filtered off and washed with THF (10 cm^3). The excess of solvent was removed under reduced pressure to afford a crude residue. Flash chromatography of the crude residue over silica gel with chloroform-methanol (9:1) afforded a white solid, *6-O-butanoyl 1,2-O-isopropylidene-α-D-glucofuranose* **88** (0.28 g, 99 %); (Found C, 54.00; H, 7.71.

$C_{13}H_{22}O_7$ required C, 53.78; H, 7.64 %); $[\alpha]_D^{21}$ - 1.33 (*c* 1.0 in $CHCl_3$); m.p. 80 °C (lit: [119] 85 °C); ν_{max} ($CHCl_3$) / cm^{-1}: 1738 (C=O); δ_H (400 MHz, $CDCl_3$): 0.97 (3H, t, *J* 7.1 Hz, CH_3), 1.32 (3H, s, CH_3), 1.49 (3H, s, CH_3), 1.63-1.73 (2H, m, CH_2CH_3), 2.36 (2H, t, *J* 7.1 Hz, CH_2CO), 2.9 (1H, d, *J* 3.0 Hz, O*H*), 3.0 (1H, d, *J* 3.0 Hz, O*H*), 4.09 (1H, dd, *J* 6.1 and 3.0 Hz, ^5CH), 4.23 (1H, d, *J* 2.0 Hz, ^4CH), 4.27 (1H, d, *J* 6.1 Hz, $^6CH_2$), 4.37 (1H, m, ^3CH), 4.44 (1H, dd, *J* 14.2 and 5.1 Hz, $^{6'}CH_2$), 4.54 (1H, d, *J* 3.0 Hz, ^2CH), 5.97 (1H, d, *J* 3.0 Hz, $^{\alpha}CH$); δ_C (100 MHz, $CDCl_3$) 13.64 (CH_3), 18.40 (CH_2), 26.22 (CH_3), 26.85 (CH_3), 36.04 (CH_2), 65.96, 69.64, 75.79, 79.36, 85.25, 105.04, 111.89, 174.31 (*C*=O); *m/z* (CI) $[M + NH_4]^+$ 308 (64 %).

4.3 - D-Mannose series

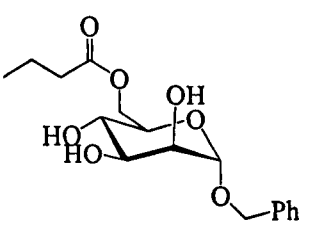
Benzyl α-D-mannopyranoside (92)



D-Mannose **91** (1.63 g, 9.07 mmol) was added to a solution of HCl 37% (2 cm^3) in benzyl alcohol (15 cm^3). The reaction mixture was heated to 90 °C and stirred for 7 h, after which time it was cooled to room temperature. $NaHCO_3$ (0.2 g) was added to the reaction slowly with vigorous stirring due to the liberation of gas. The reaction was filtered through Celite® and washed with benzyl alcohol (10 cm^3). The excess of

solvent was evaporated under high vacuum at 90 °C affording a yellow crude product. Column chromatography of the crude product in silica with chloroform-methanol (9:1) gave *benzyl α-mannopyranoside* **92** as a yellow syrup (1.54 g, 63 %); (Found C, 57.55; H, 6.73. $C_{13}H_{18}O_6$ required C, 57.77; H, 6.71 %); $[\alpha]_D^{26}$ + 80 (*c* 1.1 in MeOH) [lit.,[120] + 75.5 (*c* 1.35 in H_2O)]; δ_H (400 MHz, DMSO): 3.36-3.42 (2H, m, CH_2+^4CH), 3.44-3.53, (2H, m, ^3CH+CH_2), 3.64 (1H, br s, O*H*), 3.69 (1H, dd, *J* 11.4 and 5.7 Hz, ^2CH), 4.44-4.47 (2H, m, O*H*+$^6CH_2$), 4.52 (1H, d, *J* 6.0 Hz, $^{\alpha}CH$) 4.65 (1H, s, O*H*), 4.68-4.71 (3H, m, O*H*+^5CH+$^{6'}CH_2$), 7.28-7.38 (5H, m, ^{Ph}CH); δ_C (100 MHz, DMSO) 61.63 ($^6CH_2$), 67.36, 67.78 (CH_2), 70.64, 71.37, 74.63, 99.28 ($^{\alpha}CH$), 127.86 (^{Ph}CH), 128.12 (^{Ph}CH), 128.15 (^{Ph}CH), 128.54 (^{Ph}CH), 128.64 (^{Ph}CH), 138.22 (^{Ph}C); *m/z* (CI) 288 $[M + NH_4]^+$.

Benzyl 6-*O*-butyryl α-mannopyranoside (93)

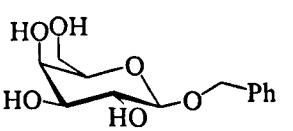


Novozyme 435 (0.10 g) was added to a solution of benzyl α-mannopyranoside **92** (0.31 g, 1.14 mmol) and vinyl butyrate (0.3 cm^3, 2.36 mmol) in THF (20 cm^3). The reaction mixture was stirred at 60 °C for 1 h after which time the enzyme was filtered off and washed with THF (20 cm^3). The excess of solvent was evaporated under reduced pressure affording a crude mixture that was chromatographed over silica with ethyl acetate-ethanol (95:5) as eluent affording *benzyl 6-O-butyryl α-*

mannopyranoside **93** as a syrup (0.29 g, 76 %); (Found C, 59.66; H, 7.15. $C_{17}H_{24}O_7$ required C, 59.99; H, 7.11 %); $[\alpha]_D^{22}$ +31.26 (*c* 1.03 in $CHCl_3$); ν_{max} ($CHCl_3$) / cm^{-1} 1720 (C=O); δ_H (400 MHz, $CDCl_3$): 0.94 (3H, t, *J* 7.1 Hz, CH_3), 1.60-1.70 (2H, m, CH_2CH_3), 2.35 (2H, t, *J* 7.6 Hz, CH_2CO), 3.63 (1H, t, *J* 9.6 Hz, ^4CH), 3.72-3.79 (4H, m, ^4CH+3xO*H*), 3.86 (1H, dd, *J* 9.6 and 3.5 Hz, ^3CH), 3.96 (1H, dd, *J* 3.0 and 1.5 Hz, ^2CH), 4.26 (1H, dd, *J* 12.1 and 2.6 Hz, $^6CH_2$), 4.47 (1H, dd, *J* 12.1 and 5.6 Hz, $^{6'}CH_2$), 4.49 (1H, d, *J* 11.6 Hz, CH_2Ph), 4.69 (1H, d, *J* 12.1 Hz, 'CH_2Ph), 4.90 (1H, d, *J* 1.5 Hz, $^{\alpha}CH$), 7.28-7.36 (5H, m, ^{Ph}CH); δ_C (100 MHz, $CDCl_3$) 13.66 (CH_3), 18.26 (CH_2CH_3), 38.11 (CH_2CO), 63.53 ($^6CH_2$), 67.84, 69.31, 67.99, 70.76, 71.55, 99.07 ($^{\alpha}CH$), 128.02 (^{Ph}CH), 128.05(^{Ph}CH), 128.53(^{Ph}CH), 137.02 (^{Ph}C), 174.70 (*C*=O); *m/z* (CI) 358 $[M + NH_4]^+$.

4.4 - D-Galactose series

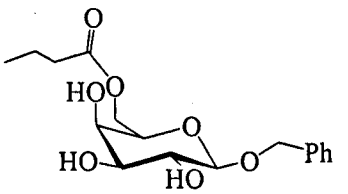
Benzyl β-D-galactopyranoside (101)



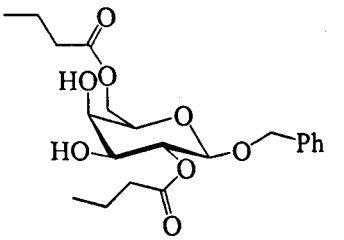
In a flask protected from light with aluminum foil, benzyl alcohol (0.4 cm^3) and acetobromo-α-D-glucose **98** (1.02 g, 2.47 mmol) were dissolved in DCM (20 cm^3). 4 Å Molecular sieves (0.10 g) were added to the reaction mixture, followed by

cooling to 0 °C with stirring. *N*-Iodosuccinimide **99** (0.75 g, 3.33 mmol) was added to the reaction mixture. The reaction was stirred for 16 hours at 0 °C in the dark, after which time the mixture was diluted with EtOAc (100 cm^3) and washed with a solution of $Na_2S_2O_3$ (3x 100 cm^3), followed by $NaHCO_3$ (aq.) (3x 100 cm^3), distilled water (3x 100 cm^3) and finally by brine (3x 100 cm^3). The organic phase was then dried with Na_2SO_4, filtered and the excess of solvent was evaporated under reduced pressure, yielding a white syrup. The white syrup was then dissolved in 20 cm^3 of a previous prepared solution of NaOMe (0.26 g, 4.89 mmol) in MeOH (100 cm^3). The reaction mixture was stirred at room temperature for 2 h after which Amberlyst® 15 resin (H^+ form) (1 g) was added and the reaction mixture was stirred for another 10 minutes. The resin was filtered off and washed with MeOH (20 cm^3), followed by the evaporation of excess solvent under reduced pressure. The resulting crude material was chromatographed over silica with chloroform-methanol (6:1) as eluent, affording *benzyl β-D-galactopyranoside* **101** (0.39 g, 59 %); (Found C, 57.20; H, 7.67. $C_{13}H_{18}O_6$ required C, 57.77; H, 6.71 %); $[\alpha]_D^{22}$ - 23.6 (*c* 1.01 in MeOH) [lit.,[121] -24.7 (*c* 1 in $CHCl_3$)]; m.p. 112-114 °C (lit.,[121] 116-118 °C); δ_H (400 MHz, D_2O): 3.54 (1H, dd, *J* 7.6 and 1.3 Hz, ^2CH), 3.59 (1H, dd, *J* 9.9 and 2.5 Hz, ^3CH), 3.64-3.66 (1H, m, ^4CH), 3.72-3.81 (3H, m, $^5CH+^6CH_2$), 3.90 (1H, br s, O*H*), 4.45 (1H, d, *J* 7.6, $^{1\beta}CH$), 4.74, (1H, d, *J* 11.8 Hz, CH_2Ph), 4.93 (1H, d, *J* 11.8 Hz, 'CH_2Ph), 7.38-7.47 (5H, m, Ph); δ_C (100 MHz, D_2O) 61.41 ($^6CH_2$), 69.10, 71.23, 71.82, 73.29, 75.62, 102.25 ($^{\beta}C$), 128.52 (^{Ph}CH), 128.87 (^{Ph}CH), 129.16 (^{Ph}CH), 137.18 (^{Ph}C).

Benzyl 6-*O*-butyryl β-D-galactopyranoside (102) and benzyl 2,6-*O*-butyryl β-D-galactopyranoside (103)



102



103

Novozyme 435 (0.10 g) was added to a solution of benzyl β-D-galactopyranoside **101** (0.13 g, 0.47 mmol) and vinyl butyrate (0.160 cm^3, 1.26 mmol) in THF 10 cm^3. After 3 h of stirring at 60 °C, the enzyme was filtered off and washed with THF (10 cm^3). The solvent was evaporated under reduced pressure and the resulting crude product was chromatographed over silica with ethyl acetate-ethanol (95:5) afforded *benzyl 6-O-butyryl β-D-galactopyranoside* **102** (0.10 g, 64 %) and *benzyl 2,6-O-butyryl β-D-galactopyranoside* **103** (0.024 g, 14 %).

Benzyl 6-O-butyryl β-D-galactopyranoside **102**

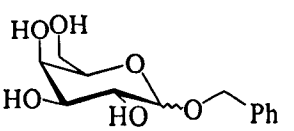
(Found C, 60.41; H, 7.39. $C_{17}H_{24}O_7$ required C, 59.99; H, 7.11 %); $[\alpha]_D^{22}$ - 15.86 (*c* 1.03 in $CHCl_3$); m.p. 82 - 83 °C; ν_{max} ($CHCl_3$) / cm^{-1} 1750 (C=O); δ_H (400 MHz, $CDCl_3$): 0.97 (3H, t, *J* 7.6 Hz, CH_3), 1.63-1.72 (2H, m, CH_2CH_3), 2.35 (2H, t, *J* 7.3 Hz, CH_2CO), 3.56-3.61 (1H, m, ^3CH), 3.65-3.70 (2H, m, ^{2+4}CH), 3.9 (1H, t, *J* 3.2 Hz, ^5CH), 4.31 (1H, dd, *J* 11.4 and 6.7 Hz, $^6CH_2$), 4.33 (1H, d, *J* 7.6, $^\beta CH$), 4.4 (1H, dd, *J* 6.4 and 6 Hz, $^{6'}CH_2$), 4.61 (1H, d, *J* 11.4 Hz, CH_2Ph), 4.93 (1H, d, *J* 11.8 Hz, $'CH_2Ph$), 7.29-7.38 (5H, m, ^{Ph}CH); δ_C (100 MHz, $CDCl_3$) 13.68 (CH_3), 18.45

(CH_2CH_3), 36.09 (CH_2CO), 62.42 ($^6CH_2$), 68.29 (CH_2Ph), 71.04, 72.00, 72.43, 73.22, 101.69 ($^{\beta}CH$), 128.14 (^{Ph}CH), 128.30 (^{Ph}CH), 128.55 (^{Ph}CH), 136.67 (^{Ph}C), 173.68 (C=O); *m/z* (CI) 358 $[M + NH_4]^+$.

Benzyl 2,6-O-butyryl β-D-galactopyranoside **103**

(Found C, 61.33; H, 7.37. $C_{21}H_{30}O_8$ required C, 61.45; H, 7.37 %); $[\alpha]_D^{22}$ - 24.17 (*c* 0.8 in $CHCl_3$); ν_{max} ($CHCl_3$) / cm^{-1} 1738 (C=O); δ_H (400 MHz, $CDCl_3$): 0.94 (3H, t, *J* 7.6 Hz, CH_3), 0.97 (3H, t, *J* 7.6 Hz, 'CH_3), 1.60-1.72 (4H, m, CH_2CH_3), 2.29-2.36 (4H, m, CH_2CO), 3.60-3.67 (2H, m, ^{3+5}CH), 3.87 (1H, d, *J* 2.9 Hz, ^4CH), 4.32 (1H, dd, *J* 11.4 and 6.7 Hz, $^6CH_2$), 4.43 (1H, dd, *J* 11.4 and 6.4 Hz, $^{6'}CH_2$), 4.45 (1H, d, *J* 7.9 Hz, $^{\beta}CH$), 4.62 (1H, d, *J* 12.1 Hz, CH_2Ph), 4.88 (1H, d, *J* 12.1 Hz, 'CH_2Ph), 5.01 (1H, dd, *J* 7.9 and 9.5 Hz, ^2CH), 7.26-7.36 (5H, m, ^{Ph}CH); δ_C (100 MHz, $CDCl_3$) 13.93 and 14.03 (CH_3), 18.74 and 18.79 (CH_2CH_3), 36.42 and 36.56 (CH_2CO), 62.78 (^6CH), 69.10 (CH_2Ph), 70.79, 72.53, 73.15, 73.66, 99.81 ($^{\beta}CH$), 128.20 (^{Ph}CH), 128.23 (^{Ph}CH), 128.73 (^{Ph}CH), 137.38 (^{Ph}C), 174.03 and 174.68 (C=O); *m/z* (CI) 428 $[M + NH_4]^+$.

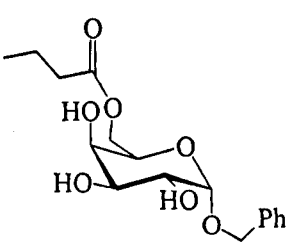
Benzyl D-galactopyranoside (105)



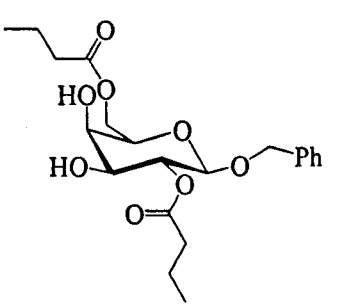
37 % Hydrochloric acid (2 ml) was added to benzyl alcohol (40 ml) and then heated to 100 °C. D-Galactose **38** (4.16 g, 23.06 mmol) was added portionwise over

30 minutes. The reaction was allowed to react during 6 hours at 100°C and then was cooled to room temperature. $NaHCO_3$ (0.1 g) was added to the reaction mixture slowly with vigorously stirring. When the liberation of gas was finished, the reaction was filtered through Celite® and washed with benzyl alcohol (10 ml), the excess of benzyl alcohol was distilled under high vacuum at 100 °C, yielding a yellow syrup. The syrup was chromatographed on silica with chloroform-methanol (9:1) yielding *benzyl D-glucopyranoside* **105** (α:β 1.6:1) as a syrup (2.84 g, 47 %); (Found C, 57.48; H, 6.80. $C_{13}H_{18}O_6$ required C, 57.77; H, 6.71 %); δ_H (400 MHz, DMSO): 3.27-3.41 (3H, m), 3.46-3.57 (4H, m), 3.61-3.70 (5H, m), 3.75 (2H, t, *J* 3.0 Hz), 4.20 (1H, d, *J* 7.6 Hz, $^{\beta}CH$), 4.34 (1H, d, *J* 3.5 Hz), 4.35 (2H, d, *J* 4.0 Hz), 4.44 (1H, d, *J* 12.1 Hz, $^{\alpha}CH_2Ph$), 4.50-4.57 (5H, m), 4.69 (1H, d, *J* 12.1 Hz, $^{\alpha}CH_2Ph$), 4.77 (1H, d, *J* 3.5 Hz, $^{\alpha}CH$), 4.81 (1H, d, *J* 12.6 Hz $^{\beta}CH_2Ph$), 4.91 (1H, d, *J* 5.1 Hz), 7.26-7.41 (10H, m, $^{\alpha+\beta Ph}CH$); δ_C (100 MHz, DMSO) 60.88, 61.06, 67.37, 68.30, 68.57, 68.78, 69.29, 69.78, 70.02, 71.02, 71.87, 73.83, 75.67, 98.65 ($^{\alpha}CH$), 103.10 ($^{\beta}CH$), 127.62 (^{Ph}CH), 127.82 (^{Ph}CH), 127.94 (^{Ph}CH), 128.44 (^{Ph}CH), 128.49 (^{Ph}CH), 138.56 (^{Ph}C), 138.59 (^{Ph}C); *m/z* (CI) 288 $[M + NH_4]^+$.

Benzyl 6-*O*-butyryl α-D-galactopyranoside (107) benzyl 2,6-*O*-butyryl β-D-galactopyranoside (103)



107



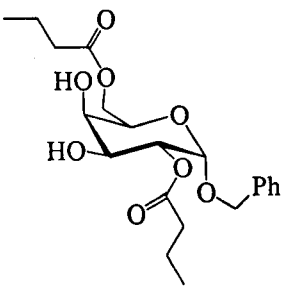
103

Novozyme 435 (0.12 g) was added to a solution of benzyl D-galactopyranoside **105** (0.12 g, 0.43 mmol) and vinyl butyrate (0.1 cm^3, 0.79 mmol) in THF (10 cm^3). The reaction mixture was heated to 60 °C and stirred for 7 ½ h; the enzyme was filtered off and washed with THF (10 cm^3). Excess solvent was evaporated under reduced pressure, and the crude product was chromatographed over silica with ethyl acetate-ethanol (95:5) as eluent yielding *benzyl 6-O-butyryl α-D-galactopyranoside* **107** (0.067 g, 46 %) and *benzyl 2,6-O-butyryl β-D-galactopyranoside* **103** (0.041 g, 23 %).

Benzyl 6-O-butyryl α-D-galactopyranoside **107**

(Found C, 59.94; H, 7.12. $C_{17}H_{24}O_7$ required C, 59.99; H, 7.11 %); $[\alpha]_D^{21}$ + 92.5 (*c* 0.56 in $CHCl_3$); m.p. 106-108 °C; ν_{max} ($CHCl_3$) / cm^{-1} 1735 (C=O); δ_H (400 MHz, $CDCl_3$): 0.96 (3H, t, *J* 7.6 Hz, CH_3), 1.62-1.72 (2H, m, CH_2CH_3), 2.34 (2H, t, *J* 7.6 Hz, CH_2CO), 3.79-3.87 (2H, m, ^{2+4}CH), 3.97 (1H, d, *J* 2.0 Hz, ^{3}CH), 4.01 (1H, t, *J* 6.6 Hz, ^{5}CH), 4.23 (1H, dd, *J* 11.6 and 6.57 Hz, $^{6}CH_2$), 4.41 (1H, dd, *J* 11.6 and 6.1 Hz, $^{6'}CH_2$), 4.54 (1H, d, *J* 11.6 Hz CH_2Ph), 4.76 (1H, d, *J* 11.6 Hz 'CH_2Ph), 5.05 (1H, d, *J* 3.5 Hz $^{\alpha}CH$), 7.31-7.40 (5H, m, ^{Ph}CH); δ_C (100 MHz, $CDCl_3$) 13.70 (CH_3), 18.39 (CH_2), 36.05 (CH_2), 63.02 ($^{6}CH_2$), 68.35, 68.88, 69.48, 69.81, 70.88 (CH_2Ph), 97.61 ($^{\alpha}CH$), 128.18 (^{Ph}CH), 128.62 (^{Ph}CH), 136.77 (^{Ph}C), 173.73(*C*=O; *m/z* (CI) 358 [M + NH_4]$^+$.

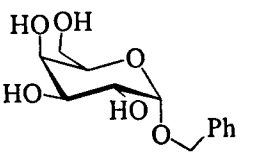
Benzyl 2,6-*O*-butyryl α-D-galactopyranoside (108)



Benzyl 6-*O*-butyryl α-D-galactopyranoside **107** (0.025 g, 0.075 mmol) was added to a solution of vinyl butyrate (5 cm^3) and THF (0.5 cm^3). The reaction mixture was stirred for 5 minutes to dissolve all the galactopyranoside, after which time Novozyme 435 (0.026 g) was added, and the reaction immersed in an oil bath at 60 °C and stirred for 24 h. The enzyme was filtered off and washed with THF (10 cm^3), the excess of solvent was evaporated under reduced pressure and the crude product was chromatographed over silica gel with ethyl acetate-n-hexane (2:1) affording *benzyl 2,6-O-butyryl α-D-galactopyranoside* **108** as a syrup (0.025 g, 81 %); (Found C, 61.33; H, 7.37. $C_{21}H_{30}O_8$ required C, 61.45; H, 7.37 %); $[\alpha]_D^{22}$ +159.09 (*c* 1.1 in $CHCl_3$); ν_{max} ($CHCl_3$) / cm^{-1} 1736 (C=O); δ_H (400 MHz, $CDCl_3$): 0.942 (3H, t, *J* 7.3 Hz, C*H*$_3$), 0.961 (3H, t, *J* 7.3 Hz, 'C*H*$_3$), 1.62-1.72 (4H, m, C*H*$_2$CH$_3$), 2.30-2.39 (4H, m, C*H*$_2$CO), 3.96 (1H, m, ^{4}C*H*), 4.03-4.11 (2H, m, $^{3+5}$C*H*), 4.19 (1H, dd, *J* 11.4 and 6.7 Hz, ^{6}C*H*$_2$), 4.44 (1H, dd, *J* 11.4 and 6.7 Hz, $^{6'}$C*H*$_2$), 4.52 (1H, d, *J* 12.1 Hz, C*H*$_2$Ph), 4.71 (1H, d, *J* 12.1 Hz, 'C*H*$_2$Ph), 5.01 (1H, dd, *J* 10.2 and 3.82 Hz, ^{2}C*H*), 5.10 (1H, d, *J* 3.8 Hz, $^\alpha$C*H*), 7.30-7.37 (5H, m, ^{Ph}C*H*); δ_C (100 MHz, $CDCl_3$) 13.60 and 13.68 (*C*H$_3$), 18.41 and 21.19 (*C*H$_2$CH$_3$), 36.09 and 36.10 (*C*H$_2$CO), 62.76 (^{6}*C*H$_2$), 67.90, 68.18, 69.34, 69.67, 71.51, 95.68 ($^\alpha$*C*H), 127.86 (^{Ph}*C*H), 128.29

(^{Ph}CH), 128.48 (^{Ph}CH), 137.09 (^{Ph}C), 173.77 and 174.09 (C=O); *m/z* (CI) 428 [M + NH_4]$^+$.

Benzyl α-D-galactopyranoside (109)



Benzyl 6-*O*-butyryl α-D-galactopyranoside **107** (0.065 g, 0.19 mmol) was added to 10 cm^3 of a solution of sodium methoxide in methanol (0.08 M). The reaction mixture was stirred for 3 minutes after which time the solvent was evaporated under reduced pressure affording a crude product, which was chromatographed over silica with chloroform-methanol (9:1) as eluent, yielding a syrup of *benzyl–α-D-galactopyranoside* **109** (0.047 g, 91 %); $[\alpha]_D^{24}$ + 96.13 (*c* 1.55 in MeOH); δ_H (400 MHz, DMSO): 3.41-3.56 (2H, m, ^{2+3}CH), 3.59-3.69 (3H, m, ^4CH + O*H*) 3.73 (1H, br s, O*H*), 4.31-4.34 (1H, br s, O*H*), 4.23 (1H, d, *J* 12.4, CH_2Ph), 4.49-4.55 (3H, m, ^5CH + $^6CH_2$), 4.68 (1H, d, *J* 12.1, 'CH_2Ph), 4.76 (1H, d, *J* 3.2, $^{\alpha}CH$), 7.26-7.69 (5H, m, ^{Ph}CH); δ_C (100 MHz, DMSO) 61.04, 68.29, 68.76, 69.28, 70.00, 71.86, 98.64 ($^{\alpha}CH$), 127.61 (^{Ph}CH), 127.81 (^{Ph}CH), 128.48(^{Ph}CH), 138.58 (^{Ph}C); (Found: $[M+NH_4]^+$, 288.145 $C_{13}H_{18}O_6N$ requires $[M+NH_4]^+$, 288.145); *m/z* (CI) $[M+NH_4]^+$ 288 (100 %).

4.5 - Pyrolysis-GC / MS parameters

Pyrolysis operating conditions

Instrument	CDS Pyroprobe 2000 / 1500 Valved Interface
Pyrolysis interface	250 °C
Pyroprobe initial temperature	300 °C
Initial hold time	5 seconds
Pyroprobe final temperature	900 °C
Final hold time	5 seconds
Ramp rate	30 °C / S
Pyrolysis gas	10% Oxygen / 90% Nitrogen
Nitrogen flow rate	250 cm^3/min
Oxygen flow rate	25 cm^3/min

GC operating conditions

Instrument	HP 6890 GC / 5973 MSD with Gerstel CIS 4 cooled injector
Column	HP-5
Column dimensions	30.0m x 0.25mm id x 0.25μm film thickness
Injection mode	Split
Split flow	100 cm^3/min
Carrier gas	Helium at constant flow of 1 cm^3/min
Initial injector temperature	- 60 °C
Initial hold time	1 minute
Ramp rate 1	12 ° C/s
Temperature 1	260 °C
Hold time 1	10 minutes
Ramp rate 2	12 ° C/s
Final temperature	350 °C
Final hold time	10 minutes
Initial oven temperature	40 °C
Initial hold time	3 minutes

Ramp rate 1	10 °C/min
Final temperature	240 °C
Final hold time	7 minutes

MS operating conditions

Operating mode	Scan
Mass range	29-400 amu
Threshold	50
Solvent delay	3.5 minutes
Transfer line temperature	280 °C
Source temperature	230 °C
Quad temperature	150 °C
Tune file	ATUNE

Bibliography

1. C.-H. Wong, R. L. Halcomb, Y. Ichikawa and T. Kajimoto, *Angew. Chem., Int. Ed.*, 1995, **34**, 412.

2. H. J. M. Gijsen, L. Qiao, W. Fitz and C.-H. Wong, *Chem. Rev.*, 1996, 443

3. F. A. Carey, in *Organic Chemistry*, McGraw-Hill, 1996, p. 1151.

4. K. Show and S. Danishefsky, *J. Org. Chem.*, 1990, **55**, 4211.

5. P. M. Collins and R. J. Ferrier, in *Monosaccharides - Their Chemistry and Their Roles in Natural Products*, John Wiley & Sons, 1995, p. 594.

6. L. M. Lerner, *Carbohydr. Res.*, 1993, **241**, 291.

7. I. Carvalho and A. H. Haines, *J. Chem. Soc., Perkin Trans. 1*, 1999, 1795.

8. D. G. Drueckhammer, W. J. Hennen, R. L. Pederson, C. F. B. III, C. M.-. Gautheron, T. Krach and C.-H. Wong, *Synthesis*, 1991, 499

9. S. M. Roberts, *J. Chem. Soc., Perkin Trans. 1*, 1999, 1.

10. S. M. Roberts, *J. Chem. Soc., Perkin Trans. 1*, 1998, 157.

11. E. Antonian, *Lipids*, 1988, **23**, 1101.

12. V. M. Balcão, A. L. Paiva and F. X. Malcata, *Enzyme Microb. Technol.*, 1996, **18**, 392.

13. D. M. Quinn and S. R. Feaster, in *Esterases and Lipases*, ed. M. Sinnott, Academic Press Limited, 1998, I.

14. P. Grochulski, F. Bouthillier, R. J. Kazlauskas, A. N. Serrequi, J. D. Schrag, E. Ziomek and M. Cygler, *Biochem.*, 1994, **33**, 3494.

15. R. Pulido, F. L. Ortiz and V. Gotor, *J. Chem. Soc., Perkin Trans. 1*, 1992, **21**, 2891.

16. S. Park and R. J. Kazlauskas, *J. Org. Chem.*, 2001, **66**, 8395.

17. N. N. Gandhi, S. B. Sawant, J. B. Joshi and D. Mukesh, *Enzyme Microb. Technol.*, 1995, **17**, 373.

18. A. Ballesteros, M. Bernabé, C. Cruzado, M. Martín-Lomas and C. Otero, *Tetrahedron*, 1989, **45**, 7077.

19. F. Chamouleau, D. Coulon, M. Girardin and M. Ghoul, *J. Mol. Catal. B: Enzymatic*, 2001, **11**, 949.

20. J. A. Arcos, M. Bernabé and C. Otero, *Enzyme Microb. Technol.*, 1998, **22**, 27.

21. L. N. Mutua and C. C. Akoh, *J. Am. Oil Chem. Soc.*, 1993, **70**, 43.

22. K. Adelhorst, F. Björkling, S. E. Godtfredsen and O. Kirk, *Synthesis*, 1990, 112.

23. Y. Watanabe, Y. Miyawaki, S. Adachi, K. Nakanishi and R. Matsuno, *J. Mol. Catal. B: Enzymatic*, 2000, **10**, 241.

24. P. Degn, L. H. Pedersen, J. O. Duus and W. Zimmermann, *Biotechnol. Lett.*, 1999, **21**, 275.

25. G. Vic and D. H. G. Crout, *Carbohydr. Res.*, 1995, **279**, 315.

26. G. Vic and D. Thomas, *Tetrahedron Lett.*, 1992, **33**, 4567

27. J. Fabre, D. Betbeder, F. Paul, P. Monsan and J. Perie, *Carbohydr. Res.*, 1993, **243**, 407

28. Y. Ikeda, K. Furukawa and H. Yamada, *Carbohydr. Res.*, 2002, **337**, 1499.

29. B. G. Davis, S. J. Ward and P. M. Rendle, *J. Chem. Soc., Chem. Commun.*, 2001, **2**, 189.

30. D. Plusquellec, F. Roulleau, F. Bertho, M. Lefeuvre and E. Brown, *Tetrahedron*, 1986, **42**, 2457

31. J. Defaye and A. Gadelle, *Carbohydr. Res.*, 1982, **110**, 217.

32. J. M. J. Ruiz, G. Oβwald, M. Petersen and W.-D. Fessner, *J. Mol. Catal. B: Enzymatic*, 2001, **11**, 189.

33. J. Greiner, A. Milius and J. G. Riess, *Tetrahedron Lett.*, 1988, **29**, 2193.

34. D. A. Leigh, J. P. Smart and A. M. Truscello, *Carbohydr. Res.*, 1995, **276**, 417.

35. A. Temeriusz, B. Piekarska, J. Radomski and J. Stepinski, *Carbohydr. Res.*, 1982, **108**, 298.

36. K. Takeo, *Carbohydr. Res.*, 1979, **77**, 131.

37. K. Takeo, *Carbohydr. Res.*, 1980, **87**, 147.

38. A. V. Stachulski, *Tetrahedron Lett.*, 2001, **42**, 6611.

39. K. P. R. Kartha, M. Aloui and R. A. Field, *Tetrahedron Lett.*, 1996, **37**, 8807.

40. M. W. Oosterom, H. J. A. v. Belle, F. v. Rantwijk and R. A. Sheldon, *J. Mol. Catal. A: Chem.*, 1998, **134**, 267.

41. S. Takayama, G. J. McGarvey and C.-H. Wong, *Chem. Soc. Rev.*, 1997, **26**, 407.

42. B. M. d. Roode, H. Zuilhof, M. C. R. Franssen, A. v. d. Padt and A. d. Groot, *J. Chem. Soc., Perkin Trans. 2*, 2000, 2217.

43. D. L. Zechel and S. G. Withers, *Acc. Chem. Res.*, 2000, **33**, 11.

44. A. Millqvist-Fureby, I. S. Gill and E. N. Vulfson, *Biotechnol. Bioeng.*, 1998, **60**, 190.

45. B. M. d. Roode, L. Oliehoek, A. v. d. Padt and M. C. R. F. M. Boom, *Biotechnol. Prog.*, 2001, **17**, 881.

46. M. Gelo-Pujic, E. Guibé-Jampel, A. Loupy and A. Trincone, *J. Chem. Soc., Perkin Trans. 1*, 1997, 1001.

47. F. Björkling, S. E. Godtfredsen and O. Kirk, *J. Chem. Soc., Chem. Commun.*, 1989, 934

48. H. Seino, T. Uchibori, T. Nishitani and S. Inamasu, *J. Am. Oil Chem. Soc.*, 1984, **61**, 1761.

49. J. F. Shaw and A. M. Klibanov, *Biotechnol. Bioeng.*, 1987, **29**, 648.

50. P. Pouillart, O. Douillet, B. Scappini, A. Gozzinni, V. Santini, A. Grossi, G. Pagliai, P. Strippoli, L. Rigacci, G. Ronco and P. Villa, *Eur. J. Pharmaceutical Sceinces*, 1998, **7**, 93.

51. P. Pouillart, G. Ronco, F. Pieri, M. Brazier and P. Villa, *Eur. J. Med. Chem.*, 1989, **24**, 471.

52. T. Kurahashi, T. Mizutani and J.-i. Yoshida, *J. Chem. Soc., Perkin Trans. 1*, 1999, 465.

53. K. Yoshimoto, K. Tahara, S. Suzuki, K. Sasaki, Y. Nishikawa and Y. Tsuda, *Chemical Pharmacology Bulletin Tokyo*, 1979, **27**, 2661

54. D. Plusquellec and K. Baczko, *Tetrahedron Lett.*, 1987, **28**, 3809.

55. K. Baczko and D. Plusquellec, *Tetrahedron*, 1991, **47**, 3817

56. F. Santoyo-González, F. García-Calvo-Flores, J. Isac-García, R. Robles-Díaz and A. Vargas-Berenguel, *Synthesis*, 1994, 97.

57. F. Santoyo-González, C. Uriel and J. A. Calvo-Asín, *Synthesis*, 1998, 1787.

58. F. Nicotra, S. Riva, F. Secundo and L. Zucchelli, *Tetrahedron Lett.*, 1989, **30**, 1703.

59. R. Eby, K. T. Webster and C. Schuerch, *Carbohydr. Res.*, 1984, **129**, 111.

60. P. López-Serrano, J. A. Jongejan, F. v. Rantwijk and R. A. Sheldon, *Tetrahedron: Asymmetry*, 2001, **12**, 219.

61. J. J. Gridley, H. M. I. Osborn and W. G. Suthers, *Tetrahedron Lett.*, 1999, **40**, 6991

62. M. P. Bousquet, R. M. Willemot, P. Monsan and E. Boures, *Biotechnol. Bioeng.*, 1999, **63**, 730.

63. C. Tsitsimpikou, H. Stamatis, V. Sereti, H. Daflos and F. N. Kolisis, *J. Chem. Technol. Biotechnol.*, 1998, **71**, 309.

64. D. B. Sarney, M. J. Barnard, D. A. MacManus and E. N. Vulfson, *J. Am. Oil Chem. Soc.*, 1996, **73**, 1481.

65. M. Kloosterman, E. W. J. Mosmaller, H. E. Shoemaker and E. M. Meijer, *Tetrahedron Lett.*, 1987, **28**, 2989.

66. T. Horrobin, C. H. Tran and D. Crout, *J. Chem. Soc., Perkin Trans. 1*, 1998, 1069

67. H. M. Sweers and C.-H. Wong, *J. Am. Chem. Soc.*, 1986, **108**, 6421.

68. M. Therisod and A. M. Klibanov, *J. Am. Chem. Soc.*, 1986, **108**, 5638.

69. Y. Wang, J. J. Lálonde, M. Momongan, D. E. Bergbreiter and C. J. Wong, *J. Am. Chem. Soc.*, 1988, **110**, 7200.

70. G. Carrea, S. Riva, F. Secundo and B. Danieli, *J. Chem. Soc., Perkin Trans. 1*, 1989, **5**, 1057.

71. S. Riva, J. Chopineau, A. P. G. Kieboom and A. M. Klibanov, *J. Am. Chem. Soc.*, 1988, **110**, 584.

72. R. Khan, *Pure Appl. Chem.*, 1984, **56**, 833.

73. P. Potier, A. Bouchu, G. Descotes and Y. Queneau, *Tetrahedron Lett.*, 2000, **41**, 3597

74. M. Therisod and A. M. Klibanov, *J. Am. Chem. Soc.*, 1987, **109**, 3977.

75. C. Tsitsimpikou, H. Daflos and F. N. Kolisis, *J. Mol. Catal. B: Enzymatic*, 1997, **3**, 189.

76. B. Danieli, F. Peri, G. Roda, G. Carrea and S. Riva, *Tetrahedron*, 1999, **55**, 2045

77. C. Chon, A. Heisler, N. Junot, F. Levayer and C. Rabiller, *Tetrahedron: Asymmetry*, 1993, **4**, 2441.

78. W. J. Hennen, H. M. Sweers, Y. F. Wang and C. H. Wong, *J. Org. Chem.*, 1988, **53**, 4939.

79. A. Giordano and A. Trincone, *Tetrahedron Lett.*, 2002, **43**, 4939.

80. S. Tomié, D. Ljevakovié and J. Tomasié, *Tetrahedron*, 1993, **49**, 10977.

81. F. Theil and H. Schick, *Synthesis*, 1991, **7**, 533

82. M. Nuchter, B. Ondruschka and W. Lautenschlager, *Synth. Commun.*, 2001, **31**, 1277.

83. W. Green and E. Pacsu, *J. Am. Chem. Soc.*, 1937, **59**, 1205.

84. C. Fernández, O. Nieto, E. Rivas, G. Montenegro, J. A. Fontenla and A. Fernández-Mayoralas, *Carbohydr. Res.*, 2000, **327**, 353.

85. Y. Yan, U. T. Bornscheuer and R. D. Schmid, *Biotechnol. Bioeng.*, 2002, **78**, 31.

86. E. Wehtje, J. Kaur, P. Adlercreutz, S. Chand and B. Mattiasson, *Enzyme Microb. Technol.*, 1997, **21**, 502.

87. J. C. Jeong and S. B. Lee, *Biotechnol. Tech.*, 1997, **11**, 853.

88. P. E. Napier, H. M. Lacerda, C. M. Rosell, R. H. Valivety, A. M. Vaidya and P. J. Halling, *Biotechnol. Prog.*, 1996, **12**, 47.

89. S. Bloomer, P. Adlercreutz and B. Mattiasson, *Enzyme Microb. Technol.*, 1992, **14**, 546.

90. F. Monot, F. Borzeix, M. Bardin and J. P. Vandecasteele, *Appl. Microbiol. Biotechnol.*, 1991, **35**, 759.

91. M. Gelo-Pujic, E. Guibé-Jampel, A. Loupy, S. A. Galema and D. Mathé, *J. Chem. Soc., Perkin Trans. 1*, 1996, 2777

92. Y. Watanabe, Y. Miyawaki, S. Adachi, K. Nakanishi and R. Matsuno, *Biochem. Eng. J.*, 2001, **8**, 213.

93. K. S. Bisht, F. Deng, R. A. Gross, D. L. Kaplan and G. Swift, *J. Am. Chem. Soc.*, 1998, **120**, 1363.

94. K. Yoshimoto, Y. Itatani, K. Shibata and Y. Tsuda, *Chem. Pharm. Bull.*, 1980, **28**, 208

95. T. Watanabe, R. Matsue, Y. Honda and M. Kuwahara, *Carbohydr. Res.*, 1995, **275**, 215

96. D. A. MacManus and E. N. Vulfson, *Carbohydr. Res.*, 1995, **279**, 281.

97. D. Kadereit and H. Waldmann, *Chem. Rev.*, 2001, **101**, 3367.

98. S. Koto, S. Inada, T. Yoshida, M. Toyama and S. Zen, *Can. J. Chem.*, 1981, **59**, 255.

99. G. V. Chowdary, M. N. Ramesh and S. G. Prapulla, *Proc. Biochem.*, 2000, **36**, 331.

100. A. Zaks and A. M. Klibanov, *J. Biol. Chem.*, 1988, **263**, 8017.

101. R. J. Kazlauskas, A. N. E. Weissfloch, A. T. Rappaport and L. A. Cuccia, *J. Org. Chem.*, 1991, **56**, 2656.

102. Z.-F. Xie, *Tetrahedron: Asymmetry*, 1991, **2**, 733.

103. A. N. E. Weissfloch and R. J. Kazlauskas, *J. Org. Chem.*, 1995, **60**, 6959

104. Y. Nishikawa, K. Yoshimoto, G. Kurono and K. Michishta, *Chem. Pharm. Bull. Tokyo*, 1975, **23**, 597.

105. K. Yoshimoto, M. Taru and Y. Tsuda, *Tetrahedron Lett.*, 1983, **24**, 2779.

106. H. Tsutsumi and Y. Ishido, *Carbohydr. Res.*, 1982, **111**, 75.

107. P. E. Pfeffer, E. S. Rothman and G. G. Moore, *J. Org. Chem.*, 1976, **41**, 2925.

108. G.-T. Ong, S.-H. Wu and K.-T. Wang, *Bioorg. Med. Chem. Lett.*, 1992, **2**, 161.

109. K.-Y. Chang, S.-H. Wu and K.-T. Wang, *Carbohydr. Res.*, 1991, **222**, 121.

110. K. Kefurt, Z. Kefurtová, J. Jary, I. Horáková and M. Marek, *Carbohydr. Res.*, 1992, **223**, 137.

111. K. N. Prasad, *Life Sci.*, 1980, **27**, 1351.

112. D. Colombo, F. Ronchetti and L. Toma, *Tetrahedron*, 1991, **47**, 103.

113. S. Tsuge and H. Ohtani, *Polym. Degrad. Stab.*, 1997, **58**, 109.

114. R. S. Lehrle, R. Duncan, Y. Liu, I. W. Parsons, M. Rollinson, G. Lamb and D. Barr, *J. Anal. Appl. Pyrolysis*, 2002, **64**, 207.

115. R. Butler, *J. Chem. Soc.*, 1950, 1433.

116. R. J. Ferrier, R. W. Hay and N. Vethaviyasar, *Carbohydr. Res.*, 1973, **27**, 55.

117. H. Arita, K. Sugita, A. Nomura, K. Sato and J. Kawanami, *Carbohydr. Res.*, 1978, **62**, 143.

118. E. V. Piel and C. B. Purves, *J. Am. Chem. Soc.*, 1939, **61**, 2978.

119. P. Y. Goueth, P. Gogalis, R. Bikanga, P. Gode, D. Postel, G. Ronco and P. Villa, *J. Carbohydr. Chem.*, 1994, **13**, 249.

120. L. J. J. Hronowski, W. A. Szarek, G. W. Hay, E. R. Ison and A. K. T. Depew, *Carbohydr. Res.*, 1991, **219**, 51.

121. R. Csuk and P. Dorr, *Tetrahedron*, 1994, **50**, 9983.