Chemo-Enzymatic Approaches to Morphine-6-Glucuronide and Selected Analogues

Submitted by

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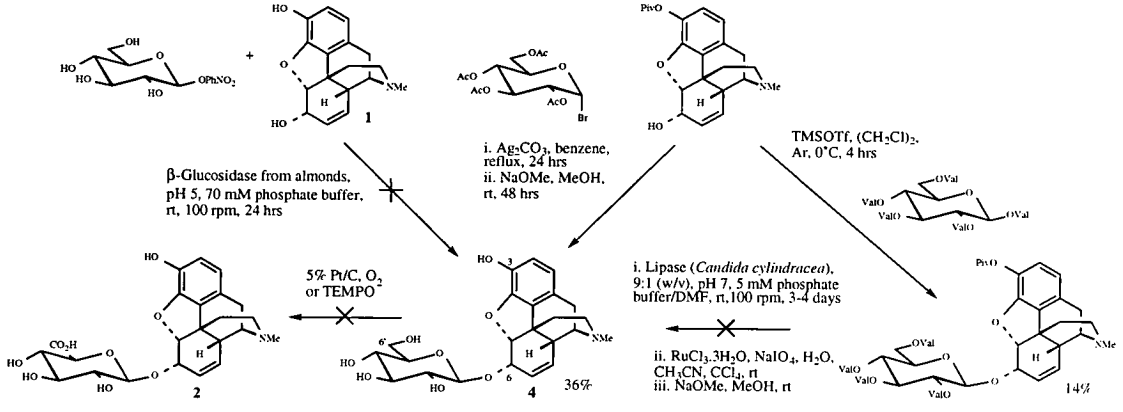
Declaration

I hereby declare that all the material included in this thesis has been composed by myself, that all material which is not my own work has been identified and no material is included that has been submitted in support of an application for a degree or qualification which has been previously conferred upon me by the University of Edinburgh or any other academic institute.

Chemo-Enzymatic Approaches to M6G and Selected Analogues

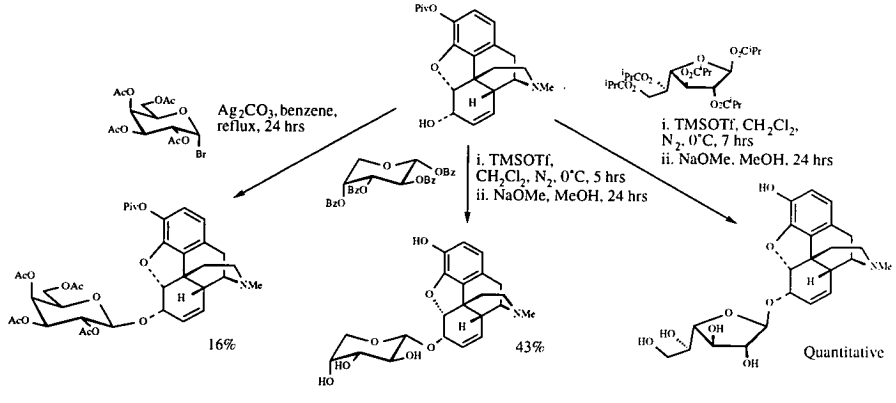
Abstract

Morphine **1** is metabolised in the liver into its active form, morphine-6-glucuronide (M6G) **2**. When M6G **2** is administered directly to patients it has greater analgesic potency than morphine **1** and this stimulated interest in the synthesis of M6G **2** as a novel analgesic. We have examined several synthetic routes to the precursor morphine-6-glucoside **4** with selective oxidation to M6G **2** as the final step (scheme 1).



Scheme 1

The coupling of a glucose residue to morphine **1** was investigated using both chemical and enzymatic approaches. However, selective oxidation at the C6' position of morphine-6-glucoside **4** did not yield the desired metabolite M6G **2**. The replacement of the glucose residue in scheme 1 by galactose and arabinose gave morphine-6-glucoside analogues which are potentially an alternative source of analgesics (scheme 2).



Scheme 2

Acknowledgements

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And there's a hand, my trusty fiere,
And gies's a hand o'thine.

Robert Burns 1759-1796

Abbreviations

AIDS	Acquired Immune Deficiency Syndrome	
Ac	Acetate	$COCH_3$
Bz	Benzoyl	COC_6H_6
Bn	Benzyl	$CH_2C_6H_6$
cm	Centimetre	10^{-2} Meters
deg	Degree	
dm	Decimetre	10^{-1} Meters
ee.	enantiomeric excess	
EtOAc	Ethyl acetate	
Et	Ethyl	CH_3CH_2
EtOH	Ethanol	
Me	Methyl	CH_3
MeOH	Methanol	
Mins	Minutes	
mM	Milli molar	10^{-3} Moles dm^{-3}
NaOMe	Sodium methoxide	
nm	Nanometer	10^{-9} Meters
^{i}pr	Isopropyl	$CH(CH_3)_2$
^{i}PrOH	Isopropanol	
Piv	Pivaloyl	$COC(CH_3)_3$
Py	Pyridine	
rt	Room temperature	
Tf	Trifluromethylsulfonate (triflate)	O_2SCF_3
TMS	Trimethylsilyl	$(CH_3)_3Si$
TEMPO	2,2,6,6-Tetramethyl-1-piperidenyloxy	
µm	Micrometer	10^{-6} Meters
µmol	Micromole	10^{-6} Moles
UTP	Uridine triphosphate	

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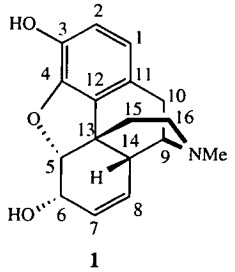
Chapter One; Introduction

Section One; Morphine and Monosaccharides

1.1. Morphine

Before the advent of modern medicine herbalists used the compounds present in plants for their healing properties. The naturally occurring substances found in the opium poppy have been used for thousands of years to relieve pain; Hippocrates mentions the use of opium in ancient Greece.[1] The word morphine **1** is derived from the Greek word Morpheus, Ovid's name for the God of dreams[2] and it was first isolated from opium in 1803 by the German pharmacist Sterner[3] who gave the alkaloid its name. Opium itself is the dried milky residue obtained from the unripe seed capsules of the opium poppy *Papaver somniferum*.[1,4]

Opium is composed of a series of alkaloids which includes morphine **1** and also codeine **17**, which itself has been in common medical use since the beginning of the century.[5] The alkaloids found in opium are called opiates;[†] the major opiate is morphine **1** (approximately 10%) and there are also appreciable amounts of codeine **17** (approximately 0.5%) and thebaine **14** (approximately 0.2%).[4] Opium alkaloids are also called isoquinoline alkaloids as they are derived by oxidation from bases of the laudanosine series.[7] Morphine **1** is derived from S-reticuline but has the R configuration at C14, this means that the amine group at C9 and the proton at C14 lie *cis* with respect to each other giving a stereoisomer of morphine **1** that is referred to as *cis* morphine (figure 1.1.).[4]



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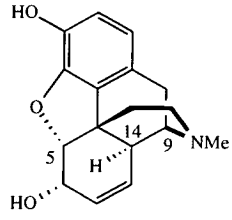


2

Stereoisomers of Morphine ***1****.*[1]

Figure 1.1.

The stereochemistry of morphine **1** is important in providing the compound with its analgesic qualities. Pharmacological studies with *trans* morphine **2**, where

[†] An opioid is any substance that produces morphine-like effects, an opiate is a morphine-like drug with a close structural similarity to morphine.[6]

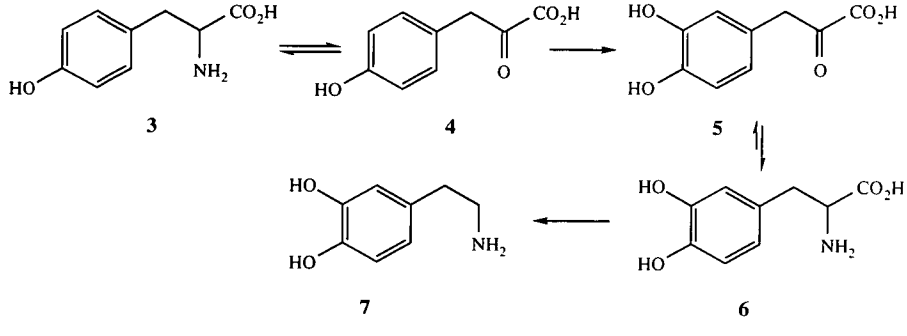
the C9 amine and C14 proton have *trans* stereochemistry have demonstrated that this compound is biologically less active than morphine **1** itself (figure 1.1.).[1] Changing the stereochemistry of morphine **1** could potentially provide compounds with potent analgesic activity.[1]

Certain structural features present in morphine **1** have been found to be important where the compound's analgesic potency is concerned. The analgesic potency of morphine **1** increases when the C6 hydroxyl group is oxidised or the *N*-methyl group is replaced by *N*-ethylphenyl;[1,7] acetylation of both C3 and C6 hydroxyl groups affords heroin **21** which is a more potent analgesic than morphine **1**.[8] The analgesic potency of morphine **1** is found to decrease on opening of the 4,5-oxygen bridge;[1,7] methylation of the phenolic hydroxyl group gives codeine **17** which is less potent than morphine **1** and is used to treat mild or moderate pain.[8]

By modifying the molecular structure of morphine **1** it was hoped alternative analgesics would be provided that did not have the side effects associated with using morphine **1**, for example, respiratory depression and dependence. However, only until the analgesic effects exerted by morphine **1** and similar compounds are understood at a molecular level can the structural features that provide these compounds with activity be identified and modified.

1.1.1. The Biosynthesis of Morphine

The biosynthetic pathway to the opium alkaloids is complex, however, the use of radiolabelled compounds in feeding studies has enabled plant biotechnologists to identify the main biosynthetic precursors of morphine **1**. The common biosynthetic intermediate of the opium alkaloids is nor-reticuline **9** which is derived ultimately from tyrosine **3** (scheme 1.1.)



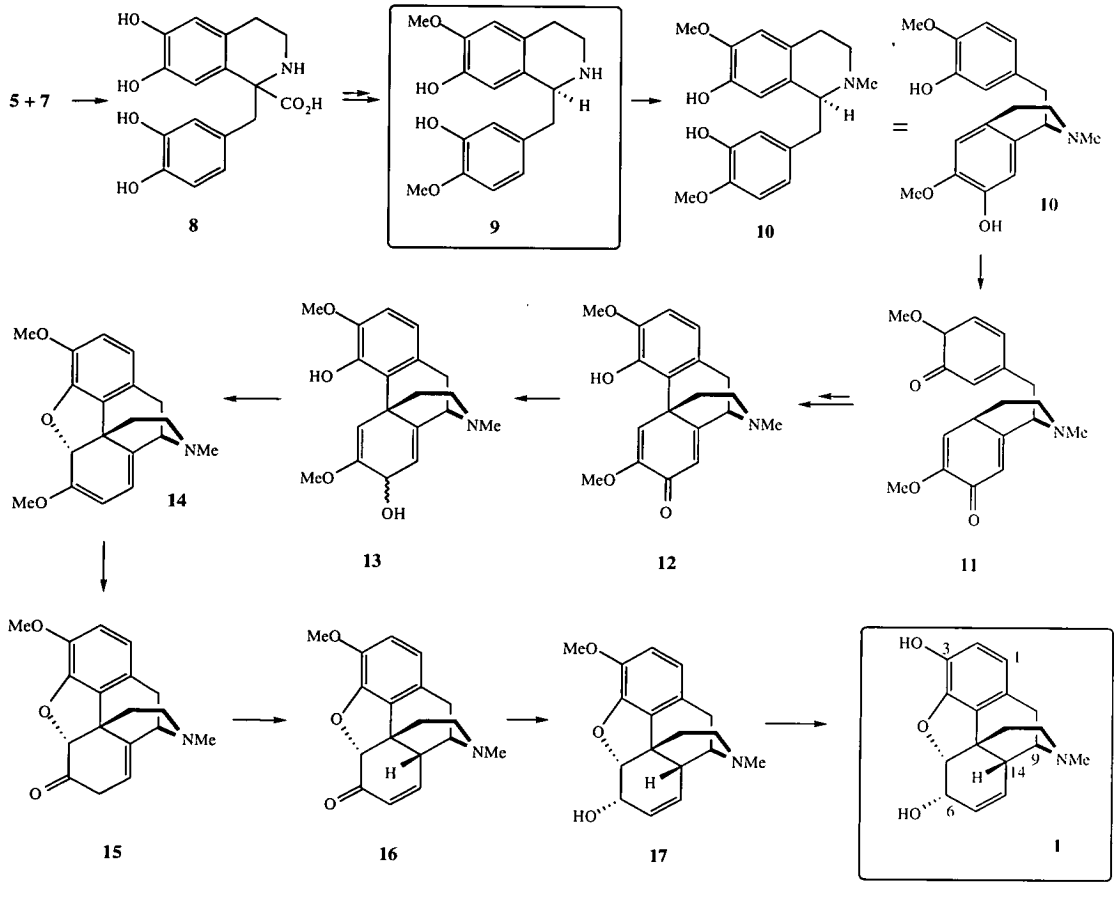
Biosynthetic Pathway of Dopamine 7.[4]

Scheme 1.1.

Tyrosine **3** is converted to the neurotransmitter dopamine **7** (scheme 1.1.),[4] whereby **3** is oxidatively deaminated to give *p*-hydroxyphenylpyruvic acid **4** which is

hydroxylated to 3,4-dihydroxyphenylpyruvic acid **5**. Reductive amination of **5** gives L-dopa **6** (the reverse reaction is very slow) which is decarboxylated to afford dopamine **7**.

The next step of the biosynthetic pathway, shown in scheme 1.2., involves coupling dopamine **7** and 3,4-dihydroxyphenylpyruvic acid **5** to give a benzylisoquinoline intermediate **8** which on decarboxylation and methylation gives nor-reticuline **9**.[1,4]



Biosynthetic Pathway of Morphine **1**[1,4]

Scheme 1.2.

N-Methylation of nor-reticuline **9** in scheme 1.2. gives reticuline **10** which is oxidised to the diketone **11**, this undergoes cyclisation followed by reduction to give salutaridine **12** which is reduced further to salutaridinol **13**. Formation of the 4,5-oxide bridge by ring closure is followed by dehydration to give thebaine **14** which is hydrolysed to noepinone **15**. Reduction of **15** gives codinone **16** which is reduced further to give codeine **17**. The final step involves demethylation of codeine **17** to give morphine **1**.[1,4]

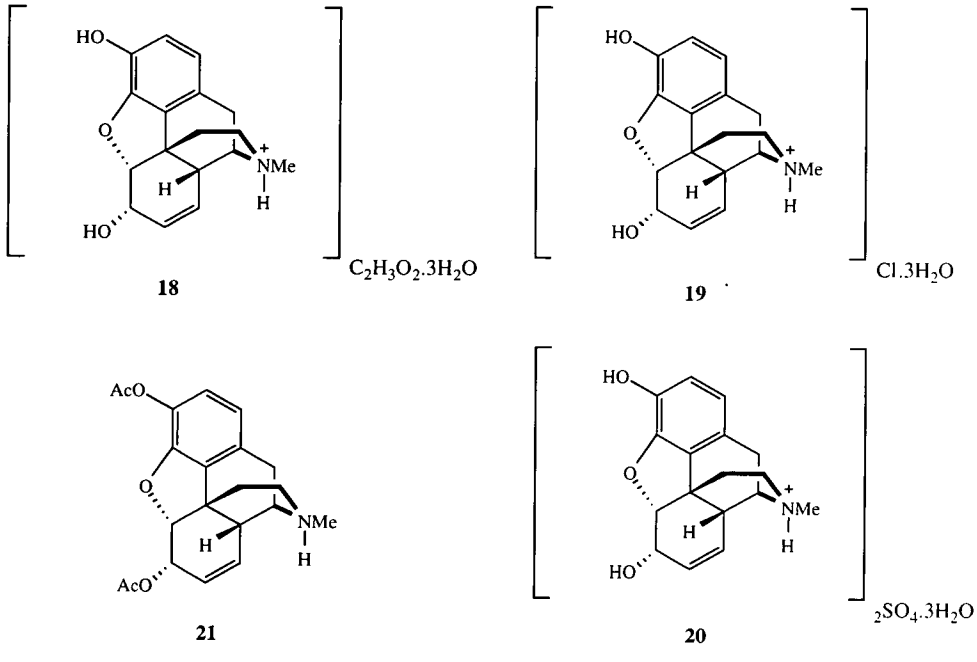
1.2. The Pharmacological Effects of Opioids on the Body

Besides analgesia opioids produce drowsiness, lethargy, apathy and euphoria or disphoria when administered to pain free volunteers. Other less pleasant effects are constipation and respiratory depression which is potentially fatal.[9,10]

1.2.1. Analgesia

Pain is seen as an infliction which affects every individual to some degree during their lives and the relief of pain, known as analgesia,[11] has been a medical problem for thousands of years. Opiates (also called narcotic analgesics[5]) have been used for hundreds of years to relieve the pain and anxiety associated with illness and surgery.[9]

Morphine **1** is generally administered orally or parenterally in the form of a salt, for example, the acetate **18**, hydrochloride **19** or sulfate **20**, salts. Heroin **21**, the diacetyl derivative of morphine has a higher analgesic potency (figure 1.2.).[12]



Some Morphine Salts and Derivatives.[12]

Figure 1.2.

Morphine **1** is one of the main drugs used to treat acute and chronic pain associated with medical illness. Opioid drugs produce analgesia over a wide range of doses and produce an analgesic effect without loss of motor and sensory functions.[8]

1.2.2. Side-Effects Associated With the Use of Opioids

The most common side-effects associated with the use of opioids are dependence, respiratory depression, sedation, nausea, constipation, and seizures.[8] Some side effects can be beneficial, for example, constipation is caused by reducing the motility and secretions of the gastrointestinal tract and so opioids can be used for the relief of diarrhoea and dysentery. In fact the first recorded use of opium was not for the treatment of pain but for the relief of diarrhoea and morphine **1** is the oldest of the opioid antidiarrhoeal drugs.[10]

One of the more adverse side effects of opioids is respiratory depression which occurs as a result of the action of opioids on the brainstem respiratory centres; death from an overdose of opioids is usually as a result of respiratory arrest. CNS depression is manifested in other side effects including mental clouding and sedation (drowsiness) both of which prevent the patient maintaining their normal working and social activities.[8]

The effect of opioids on the peripheral blood vessels and the heart can lead to a fall in blood pressure (hypotension) as a result of blood vessel dilation and a decrease in the heart rate.[9] Morphine **1** is one of the opioids associated with histamine release which can cause or exacerbate hypotension.[9] Other unpleasant side effects associated with the use of opioids includes nausea and vomiting which appears to vary with the drug, route of administration, and the patient.[8]

1.2.2.1. Dependence, Tolerance and Addiction

Morphine **1** and heroin **21** produce euphoria where the individual has feelings of well-being, relief, contentment, stimulation and freedom of imagination, and ease of mental effort.[13] Unfortunately opioids also produce a strong physical dependence when regular doses of the drug are required to prevent withdrawal symptoms.[14] Dependence is expressed as a craving for the drug and the addiction associated with the use of opioids has become a major source of crime, poverty, and health problems within modern society.[6] Heroin **21**, in particular is highly addictive and has become the recreational drug of modern society, taken by healthy individuals to experience the drug's euphoric effects.[14] However, continued use of opioids leads to tolerance, a state in which the individual needs to take increasingly larger doses of the drug to achieve the same effect.[14] Tolerance to both the narcotic and toxic effects develops rapidly and addicts need to take increasingly larger doses to achieve the desired euphoric effects.[13] Tragically addicts cannot measure their degree of tolerance as it does not increase consistently and an overdose is a frequent cause of death.[13]

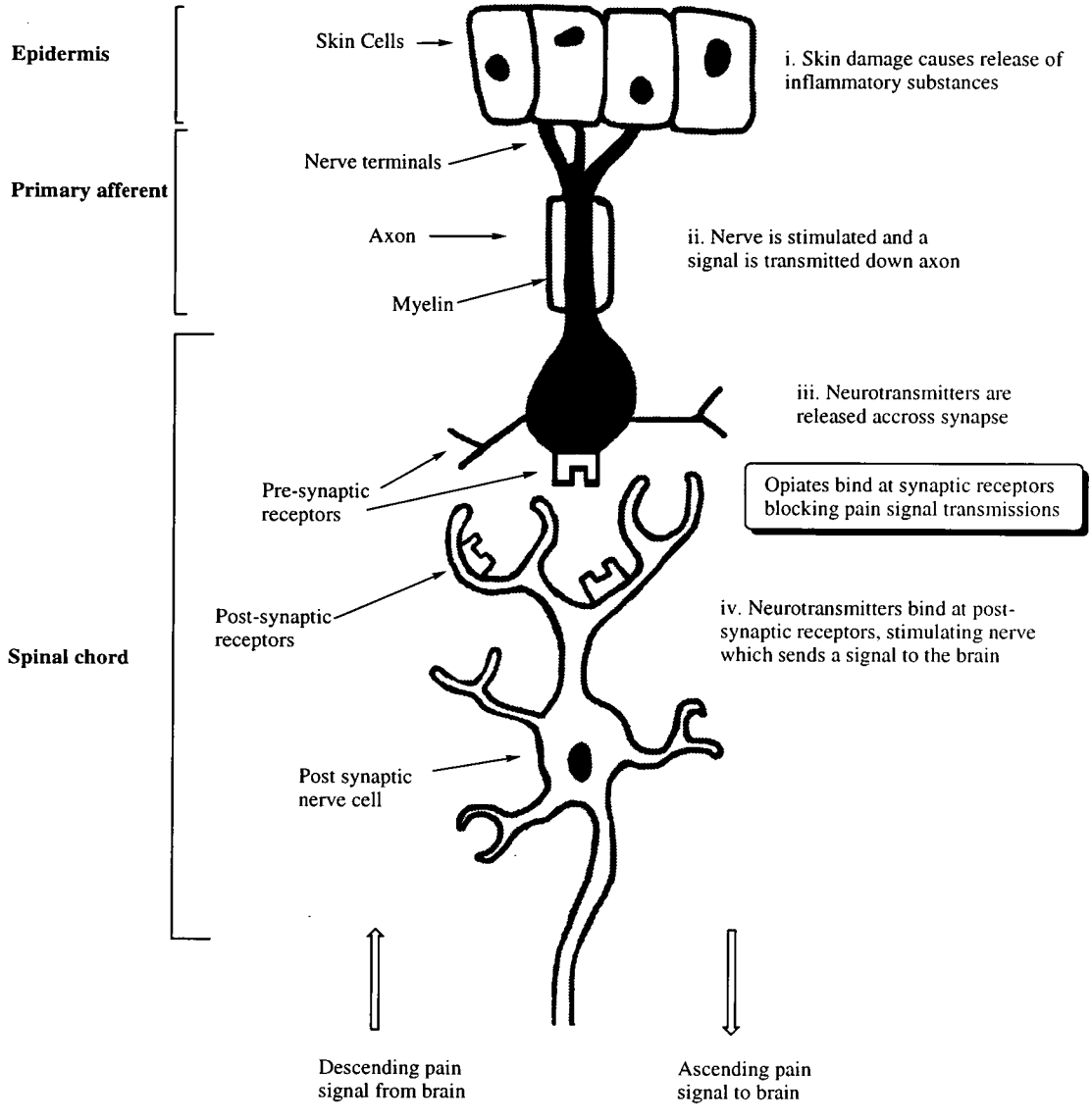
Opioid addiction hinders the effective use of opioids as clinical analgesics; alternative opioid analgesics which are more potent and have fewer side effects are of great interest and the search for alternatives to opioids and the development of other more effective analgesics may lead to some useful future therapies.

1.3. The Physiological Effects of Opioids on the Body

When the human body is physically damaged the sensation of pain is experienced. The response to pain primarily involves the nervous system which is composed of the central nervous system (CNS) (the brain and spinal chord) and the peripheral nervous system (the entire nervous system excluding the CNS).[15,16]

Both the peripheral nervous system and the CNS are composed of nerve cells or neurones which are excitatory cells that can receive and transmit electrical signals. Neurones are composed of a cell body which contains the nucleus, and an axon, the elongated conducting part of the cell which is covered in myelin, an insulating sheath. The junctions between neurones are called synapses which are either chemical or electrical. Electrical synapses pass the activity of the pre-synaptic neurone directly to the post-synaptic neurone in the form of a flow of electrical charge (*i.e.* a current). In a chemical synapse the stimulation of the pre-synaptic neurone by an electrical impulse causes the neurone to release a small chemical substance called a neurotransmitter. The neurotransmitter diffuses across the synaptic gap to the post-synaptic neurone where it binds to receptors, stimulating a change in the neurone's permeability to ions (figure 1.3.).[15,16]

When an injury to the body occurs, the sensation of pain starts at the injury site, inflammatory substances such as prostaglandins and serotonin are released which sensitise or stimulate the firing of primary afferents (pain nerve fibres) within the peripheral nervous system. The pre-synaptic end of the primary afferents are found in the spinal chord and when neurotransmitters are released from the pre-synaptic end of the primary afferents, these bind to excitatory receptors on the spinal chord. A signal is then transmitted along the spinal chord to the brain which registers a sensation of pain. The spine and brain also contain inhibitory receptors, the brain can stimulate the release of endogenous substances which can bind to these receptors reducing or inhibiting pain signals, thus reducing the sensation of pain (figure 1.3.).[3]



The Nervous System's Response to Pain.[3]

Figure 1.3.

The role of opioids in the body's response to pain has been extensively studied. Opioids bind to specialised receptors called opioid receptors which are widely distributed throughout the body, particularly within the nervous system, where opioid receptors are found in both the CNS and peripheral nervous system. In the CNS opioid receptors are found clustered in regions of the brain associated with pain regulation. The binding of opioids at these receptors means that they act directly on the part of the brain where pain is perceived providing the most effective type of analgesia. Opioids are therefore used to treat the pain arising from surgery, serious injury, and are particularly valuable for relieving the severe pain associated with terminal illness.[3,5]

In the peripheral nervous system, opioid receptors are mainly found in the pre-synaptic ends of primary afferents and the post-synaptic membranes of nerves in the spinal chord. The binding of opioids at these receptors inhibits both the pain signal to the brain and the pain signal from the brain (figure 1.3.). When the opioid morphine **1** binds to the pre-synaptic end of primary afferents, the resulting inhibitory action changes the permeability of the neurone membrane to ions, thus changing the membrane potential. The neurone can no longer release neurotransmitters and there is a decrease in the pain signals from the peripheral nervous system.[3]

1.4. Opioid Receptors

The natural endogenous ligands of opioid receptors are the peptides endorphins, enkephalins, and dynorphins. These peptides are agonists for opioid receptors and are released by the body in response to pain, binding to and stimulating opioid receptors thus reducing the perception of pain.[17] Opioids are also agonists of opioid receptors and mimic the action of the natural endogenous ligands.[3,5,17] Three types of opioid receptors have been identified on the basis of their different pharmacological and binding characteristics and different anatomical distribution, these are referred to as the mu (μ), kappa (κ), and delta (δ) receptors.[17] Receptor binding studies have shown that morphine **1** is selective for the μ opioid receptor and binds at two sites within the receptor which have been classified as μ_1 and μ_2.[18]

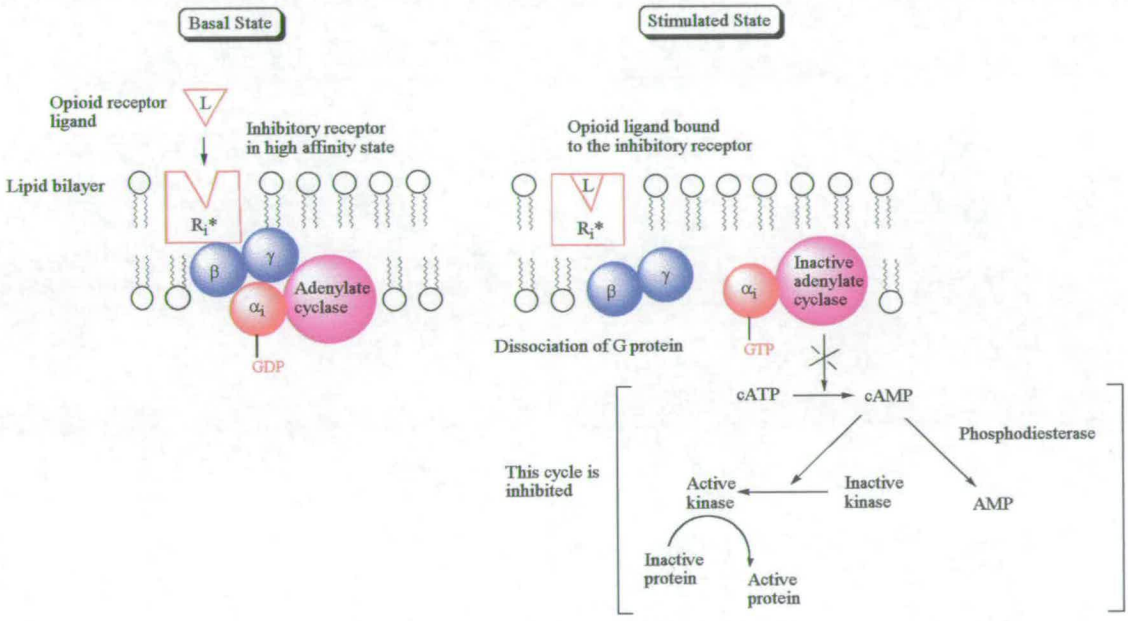
1.4.1. The Effect of Opioid Receptors on the Body

Receptors, including opioid receptors, normally consist of a recognition site to which the drug binds and are part of a transduction pathway which leads to a biological response.[19] All known opioid receptors are members of the G protein-linked receptor group which stimulate a cellular response *via* a G protein.[20,21] G proteins are a family of membrane proteins whose name refers to the protein's ability to bind guanine nucleotides and are associated with the adenylate cyclase secondary messenger systems, the phosphoinositol secondary messenger system, potassium ion channels and the operation of calcium ion channels.[20,22]

Opioid receptors have been found to associate with the adenylate cyclase secondary messenger system*via* G proteins and are inhibitory, reducing the activity of the enzyme. The adenylate cyclase secondary messenger system involves G_s and G_i proteins which stimulate and inhibit adenylate cyclase respectively. G proteins are composed of three subunits, referred to as the α, β, and γ subunits. The β and γ

subunits are identical for the G_s protein and G_i protein, however, the α subunit is different and is referred to as α_s or α_i for the stimulatory and inhibitory subunit respectively. In an unstimulated (basal) state G proteins bind guanosine diphosphate (GDP) at the guanine nucleotide binding site which is located on the larger α subunit.[20,22,23]

Binding of a natural ligand or agonist occurs at a specific ligand binding site or agonist binding site respectively, on the opioid receptor.[23] The opioid receptor can exist in either a low or high-affinity state and either stimulates or inhibits adenylate cyclase. The binding of opioids to opioid receptors inhibits adenylate cyclase and several models have been proposed for the receptor-mediated inhibition of adenylate cyclase including the direct action model shown in figure 1.4.[23]



The Direct Action Model for the Inhibition of the Adenylate Cyclase Signal Transduction Pathway by Opioid Receptor/G Protein Interactions.[23]

Figure 1.4.

In the direct action model the inhibitory receptor (R_i*) is initially bound to the G protein and the α_i subunit of the G protein is bound to guanosine diphosphate (GDP). When a ligand or agonist binds to the inhibitory receptor (R_i*) in its high affinity state it causes a conformation change and the GDP bound to the α_i-subunit is displaced by guanosine triphosphate (GTP). The G protein dissociates and the α_i subunit-GTP complex associates with adenylate cyclase inhibiting the enzyme. Active adenylate cyclase catalyses the conversion of cyclic adenosine triphosphate (cATP) to cyclic adenosine monophosphate (cAMP), this in turn activates a number

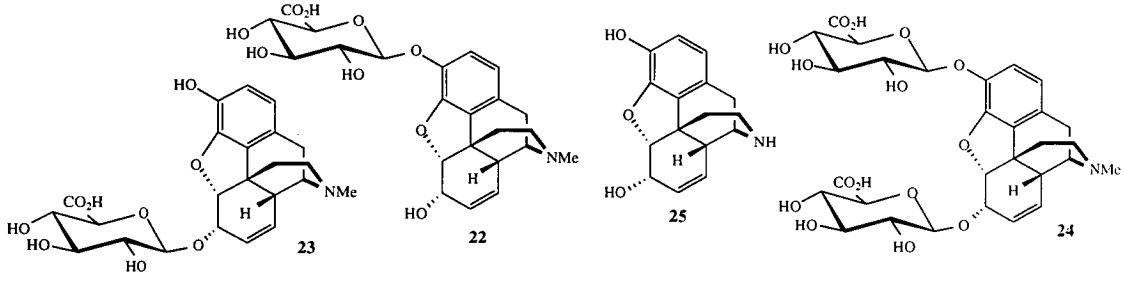
about the association of opioid receptors with cellular processes involved in the body's sensory perception and response to pain.

1.5. Morphine Metabolites

The metabolic fate of morphine **1** in both animals and humans has been studied extensively over a number of years and it has been found to undergo metabolism *via* four different pathways (figure 1.5.);[26]

1. Conjugation; gives morphine-3-glucuronide (M3G) **22**, morphine-6-glucuronide (M6G) **23** and morphine-3,6-diglucuronide **24**.
2. *N*-Demethylation; gives normorphine which is then conjugated in the liver.
3. *O*-Methylation; to form codeine **17**.
4. Oxidation; to form dihydromorphinone.

It has been known for some years that conjugation was one of the important mechanisms for the detoxification of morphine **1** in the body[27] and glucuronidation has been confirmed as the primary metabolic route for administered morphine **1**.[28]



Some Morphine Metabolites.[26]

Figure 1.5.

With the advent of sensitive modern chromatographic and spectroscopic techniques, the identity and quantitative study of morphine metabolites is achievable. Yeh[29] was one of the first to take advantage of these techniques to identify and quantify the presence of a number of morphine metabolites in the urine of subjects after the parenteral administration of morphine sulfate **20**. In a recent study Yeh and co-workers[26] reported the presence of M3G **22**, M6G **23**, morphine-3,6-diglucuronide **24**, morphine-3-ethereal sulphate, normorphine **25**, normorphine-6-glucuronide, and possibly normorphine-3-glucuronide in the urine of subjects after the parenteral administration of morphine sulfate **20**. Yeh and co-workers[26] also found that 55-64% of morphine sulfate **20** administered parenterally was conjugated in the liver where microsomal uridine diphosphate glucuronyl transferases[28] attach glucuronide residues to the morphine structure. Yeh and co-workers[26] confirmed that the major metabolite of administered morphine sulfate **20** is M3G **22** while the

amount of M6G **23** and morphine-3,6-diglucuronide **24** is found in much smaller quantities which were estimated by Yeh and co-workers[26] to be 1% of the quantity of M3G **22** found.

The identification of morphine metabolites by Yeh and co-workers[26] stimulated interest in the possible interactions these metabolites would have with μ receptors. Two *in vitro* techniques that have provided valuable information about the actions of opioid ligands and the receptors with which they interact are receptor binding studies in membrane fragments and bioassays in isolated intact tissues.[19] Pasternak and co-workers[30] examined the binding affinities of both M6G **23** and M3G **22** with μ receptors. The experiments demonstrated a high affinity of the minor metabolite M6G **23** for μ receptors, where as the major metabolite M3G **22** had a low affinity.

There was also interest in the analgesic activity of morphine metabolites with relation to the results of the binding studies. Pasternak and co-workers[30] reported that in animal studies M6G **23** was 20 times more potent than morphine, while M3G **22** lacked of any significant analgesic activity. The high potency of M6G **23** as an analgesic was confirmed by Osborne and co-workers[31] who studied the pharmacological activity of the metabolite in human subjects and suggested that most of the analgesic effect that occurred after treatment with morphine was as a result of M6G **23**. The analgesic potency of M6G **23** reflects the metabolite's high affinity for μ receptors while M3G **22** has little affinity for μ receptors and consequently lacks analgesic activity.

After the discovery of M6G's **23** analgesic potency, Osborne and co-workers[32] went on to examine the pharmacokinetics of the metabolite. After both intravenous and oral administration of morphine **1**, Osborne and co-workers[32] discovered an abundance of this metabolite in the plasma which exceeded the levels of morphine **1** itself. A recent investigation by Hanna and co-workers[33] of the pharmacokinetics and side effects associated with M6G **23** confirmed the presence of high levels of M6G **23** in plasma after intravenous administration of morphine **1**, the side effects were also of a low incidence and a shorter time was required for a full recovery.

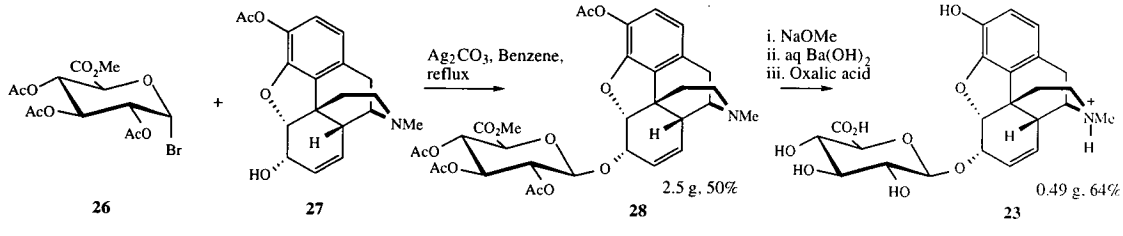
M6G **23** has also been found to penetrate the blood-brain barrier as effectively as morphine **1**. This is unusual for glucuronides and more so for M6G **23** which, as a result of the large number of hydroxyl groups and the carboxylate group, is a highly polar compound.[34] In fact studies have shown that M6G **23** is only slightly less lipophilic than morphine which explains how this metabolite is able to

penetrate the blood-brain barrier.[34] Consequently, as a result of the lipophilic nature of M6G **23** it is able to accumulate in membranes which probably accounts for the metabolite's blood-brain penetration and slow urinary excretion.[34] A publication by Carrupt and co-workers[34] suggests that M6G **23** is a "molecular chameleon" which means that the compound will fold in order to adapt to the polarity of the surrounding medium allowing what should be a highly polar hydrophilic compound to be lipophilic.

M6G **23** would appear to be a highly unusual if not a unique compound and the greater analgesic potency, longer duration of action and fewer side affects associated with this metabolite has stimulated interest in the synthesis of this novel analgesic.

1.6. The Chemical Synthesis of Morphine Glucuronides

One of the first chemical routes to M6G **23** was reported by Yoshimura and co-workers[35,36] who used a classic silver (I) carbonate promoted Koenigs-Knorr glycosidation with methyl tri-*O*-acetyl-α-D-glucosyluronate bromide **26** as the glycosyl donor and 3-*O*-acetylmorphine **27** (for synthesis see appendix A.1.) as the acceptor (scheme 1.3.). The glycosidation reaction afforded the adduct **28** in a yield of 50%, which was then deprotected to afford M6G **23** in 64% yield.

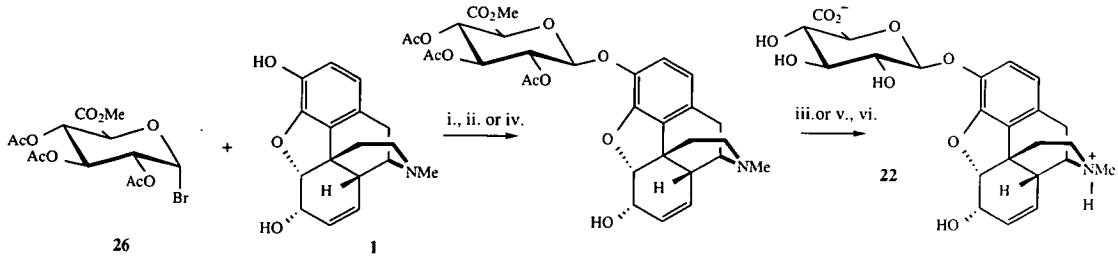


Yoshimura and Co-worker's Route to M6G 23.[35,36]

Scheme 1.3.

M3G **22** is the major metabolite of morphine and both Yoshimura[35,36] and Berrang[37] attempted its synthesis using a silver (I) carbonate promoted Koenigs-Knorr glycosidation under similar conditions used for the synthesis of M6G **23** (scheme 1.3.). However, the glycosidic bond formed at C3 was readily cleaved under the reaction conditions, therefore alternative routes were developed. Yoshimura and co-workers[35,36] reported a rather tedious reaction involving the addition of aqueous sodium hydroxide to a solution of morphine **1** and methyl tri-*O*-acetyl-α-D-glucosyluronate bromide **26** in acetone over a number of days (scheme 1.4.). A quicker synthetic route was reported by Berrang and co-workers[37] who used the

lithium salt of morphine **1** and methyl tri-*O*-acetyl-α-D-glucosyluronate bromide **26** in a reaction that afforded the desired M3G **22** in a moderate yield of 53% (scheme 1.4.).

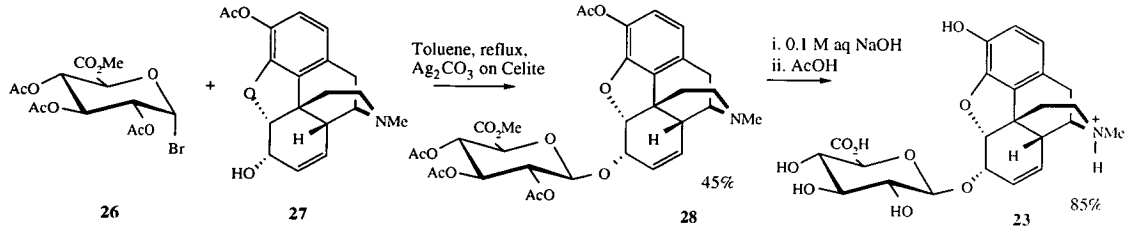


i. Acetone, aq NaOH, 7 days; ii. AcOH; iii. NH_4OH; **23** = 0.44 g, 25% (Yoshimura *et al*)[35,36]
iv. $LiOH.H_2O$, MeOH, rt, 30 mins; v. $LiOH.H_2O$, H_2O, rt, 30 mins;
vi. AcOH; **23** = 12.2 g, 53% (Berrang *et al*)[37]

Yoshimura[35,36] *and Berrang's*[37] *Synthesis of M3G 22.*

Scheme 1.4.

The discovery of M6G's **23** analgesic activity stimulated interest in the synthesis of this compound as a novel analgesic. As mentioned earlier the use of a silver (I) carbonate promoted Koenigs-Knorr reaction had been successfully reported by Yoshimura and co-workers.[35,36] A variation on this reaction was recently carried out by Lacy and Sainsbury[38] who used silver (I) carbonate supported on Celite as the promoter for a Koenigs-Knorr glycosidation with 3-*O*-acetylmorphine **27** as the acceptor and methyl tri-*O*-acetyl-α-D-glucosyluronate bromide **26** as the glycosyl donor (scheme 1.5.).



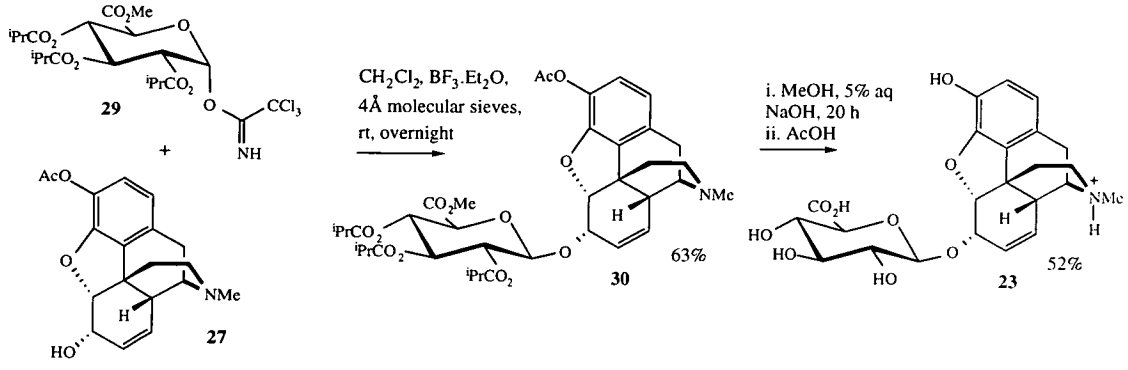
Lacy and Sainsbury's Route to M6G 23.[38]

Scheme 1.5.

Lacy and Sainsbury[38] obtained the adduct **28** in a similar yield to Yoshimura and co-workers,[35,36] but achieved a more efficient deprotection step under alkaline conditions to afford M6G **23** in a yield of 85%. However, although the use of barium salts was avoided in the deprotection step, contamination of the M6G **23** with silver would render the compound unsuitable for use in biological systems.

The publication of a route devised by Scheinmann and co-workers[39] provides a novel and efficient synthesis of M6G **23** that avoids the use of heavy metals. The

procedure involves a trimethylsilyl trifluoromethanesulfonate (trimethylsilyl triflate or TMSOTf) catalysed coupling of 3-*O*-acetylmorphine **27** and the isobutyryl protected α-D-glucosyluronate trichloroacetimidate **29** which affords the adduct **30** in a good yield of 63%. Deprotection of the adduct **30** under alkaline conditions finally gives the desired M6G **23** in 52% yield (scheme 1.6.).



Scheinmann and Co-worker's Route to M6G **23**.[39]

Scheme 1.6.

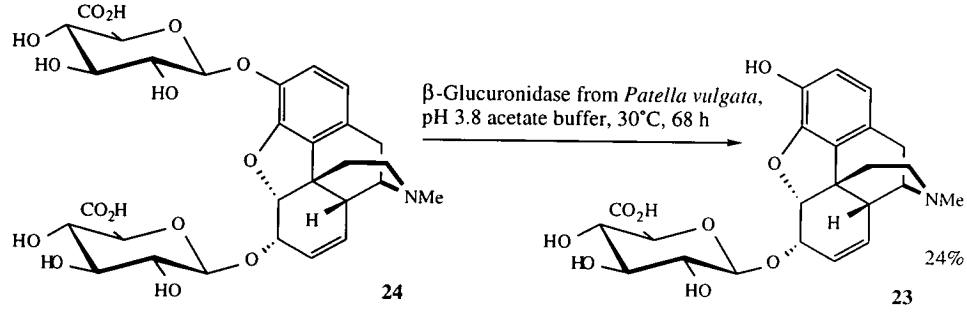
The synthesis developed by Scheinmann and co-workers[39] currently provides the best chemical synthetic route to M6G **23** and related compounds (for example, M6G hapten[40]). However, there has been recent interest in the application of biocatalysis to the synthesis of M6G **23**.

1.7. The Enzymatic Synthesis of M6G

The conjugation of morphine in the liver is catalysed by hepatic glucuronyltransferase enzymes affording a water soluble form of morphine that can be efficiently eliminated by the kidneys.[41,42] Urine samples are commonly used to measure the levels of drugs present in the body, however, conjugated forms of many drugs, including morphine, are difficult to detect.[41] The hydrolysis of conjugated forms of drugs under acidic conditions often completely destroys the conjugate, thus enzymatic hydrolysis was developed as an milder alternative.[41] Combie and co-workers[41] reported the use of β-glucuronidase from *Patella vulgata* as an efficient catalyst for the hydrolysis of morphine glucuronates in equestrian urine in the early 1980's. In a recent study by Bertholf and co-workers[42] β-glucuronidase was used to hydrolyse morphine glucuronates present in human urine. However, Bertholf and co-workers[42] demonstrated that β-glucuronidase was less able to hydrolyse the morphine glucuronates in human urine, than those in equestrian urine. Bertholf and co-workers[42] also suggested that β-glucuronidase from *Patella vulgata* is less selective for the major metabolite M3G **22** which contains a phenolic glucuronide,

than the minor metabolite M6G **23** which contains an allylic glucuronide, although this claim was not substantiated by laboratory studies.

A recent publication by Brown and co-workers[43] found that β-glucuronidase from *Patella vulgata* demonstrates selectivity for one of the glucuronide residues of morphine-3,6-diglucuronide **24** and catalyses the hydrolysis of the phenolic glucuronide at C3 with a selectivity that is 20 times greater than for the C6 glucuronide. Treatment of morphine-3,6-diglucuronide **24** with β-glucuronidase from *Patella vulgata* affords M6G **23** in a modest yield of 24%, providing a novel synthetic route to this morphine metabolite (scheme 1.7.).



24 **23**

Brown and Co-worker's Route to M6G 23 using β-glucuronidase from Patella vulgata.[43]

Scheme 1.7.

The successful synthesis of M6G **23** with β-glucuronidase from *Patella vulgata* stimulated interest in the use of other enzymes towards the synthesis of this novel analgesic. The use of enzymes in the synthesis of M6G **23** offers the advantage of using mild and selective conditions and provides attractive alternatives to chemical synthetic routes which usually require heavy metals such as silver and mercury.

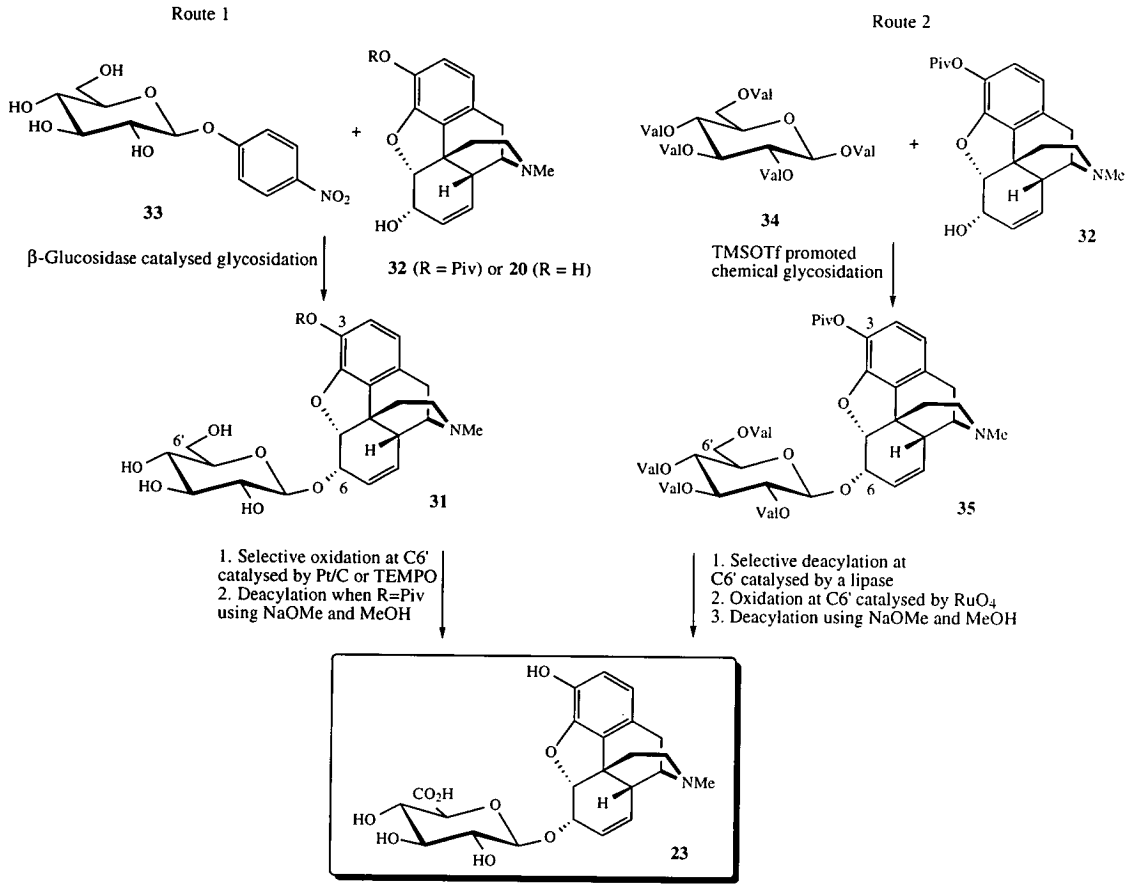
1.8. The Chemo-Enzymatic Synthesis of M6G and M6G Analogues

The project was composed of two parts; the main aim of the project was to synthesise M6G **23** using a combination of biocatalysis and organic chemistry; the second part involved an investigation of the chemical synthesis of selected M6G analogues.

1.8.1. The Chemo-Enzymatic Synthesis of M6G

Three possible routes towards the synthesis of M6G **23** were considered, whereby, two were chemo-enzymatic routes (routes 1 and 2) and one was a chemical synthetic route (route 3). The two chemo-enzymatic routes incorporated the use of either a β-glucosidase (route 1) or lipase (route 2) with a selective oxidation at C6' of

morphine-6-glucoside **31** to give M6G **23** as the final or penultimate step (scheme 1.8.).



Chemo-Enzymatic Routes to M6G 23.

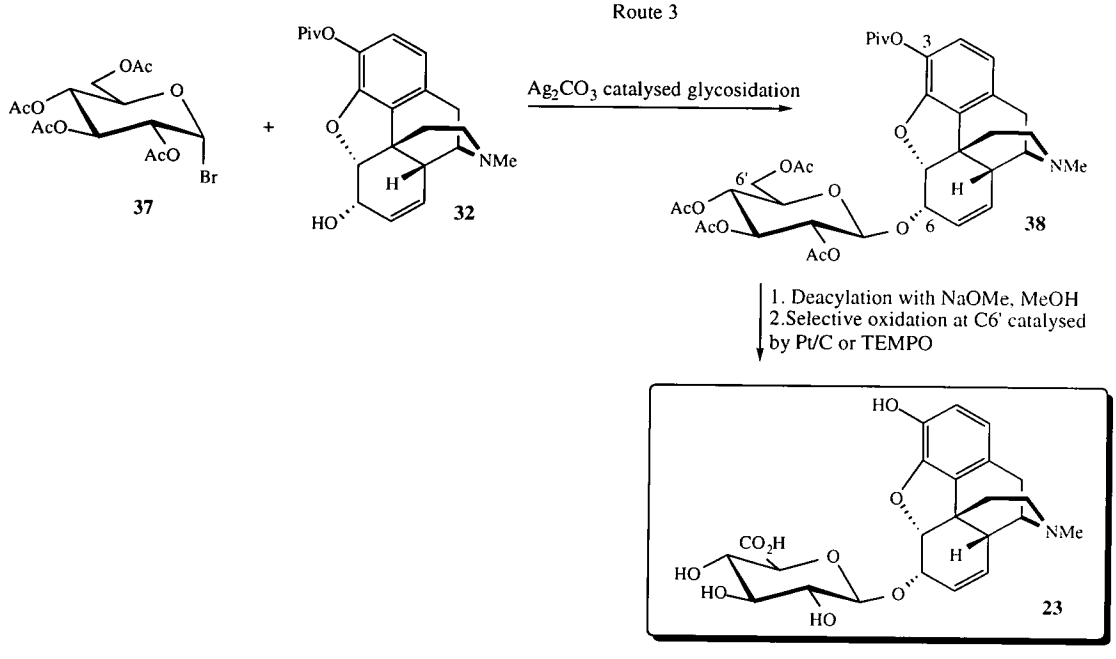
Scheme 1.8.

Route 1 in scheme 1.8. involves a transglycosidation catalysed by β-glucosidase from almonds which has been previously shown to be useful in transglycosidations with a variety of alcohols as substrate acceptors.[44-46] The acceptors for the proposed transglycosidation catalysed by β-glucosidase were a protected morphine derivative (3-*O*-pivaloylmorphine **32**) and a morphine salt (morphine sulfate **20**), with *para*-nitrophenyl-β-D-glucose **33** as the glycosyl donor. It was then proposed that a selective oxidation at C6' of morphine-6-glucoside **31** using either platinum or TEMPO as the catalyst would afford M6G **23** with a final deprotection step required when 3-*O*-pivaloylmorphine **32** is used as the acceptor to remove the pivaloyl group.

Route 2 in scheme 1.8. involves a TMSOTf promoted glycosidation of 3-*O*-pivaloylmorphine **32** with β-D-glucose pentavalerate **34** to afford 3-*O*-pivaloylmorphine-6-(2',3',4',6'-tetra-*O*-valeryl)-β-D-glucoside **35**. A selective deacylation at C6' of the morphine-β-D-glucoside **35** is then required, which can be

achieved using lipases. Lipases have proved to be versatile and selective enzymes for the acylation and deacylation of a range of compounds[47] including ester protected glycosides.[48-50] A non selective ruthenium (VIII) oxide catalysed oxidation at the C6' hydroxyl group of 3-*O*-pivaloylmorphine-6-(2',3',4'-tri-*O*-valeryl)-β-D-glucoside **36** followed by deprotection would then afford the desired M6G **23**.

Route 3 in scheme 1.9. is a chemical route to M6G **23** which involves a classic silver (I) carbonate promoted Koenigs-Knorr coupling of 3-*O*-pivaloylmorphine **32** and acetobromoglucose **37** to give the morphine-β-D-glucoside **38** which on deacylation would afford morphine-6-glucoside **31**. Thereafter a selective TEMPO or platinum catalysed oxidation at the C6' position of morphine-6-glucoside **31** would afford M6G **23** (scheme 1.9.).

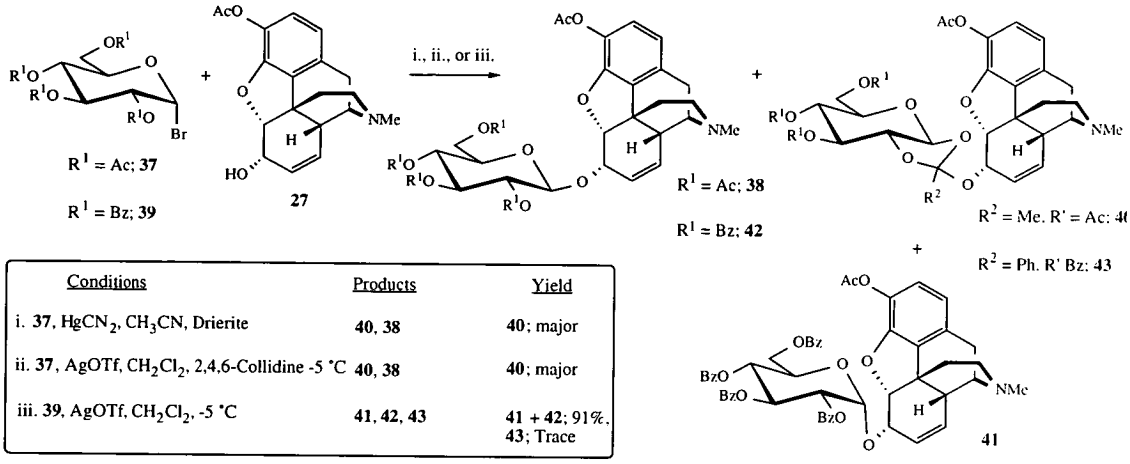


Chemical Route to M6G 23.

Scheme 1.9.

The Koenigs-Knorr glycosidation used in route 3 was established at Salford Ultrafine Chemicals. Hopkins[51] reported a silver (I) carbonate promoted Koenigs-Knorr glycosidation of 3-*O*-acetylmorphine **27** using acetobromoglucose **37** as the glycosyl donor. The reaction afforded 3-*O*-acetylmorphine-6-(2',3',4',6'-tetra-*O*-acetyl)-β-D-glucoside **38** in yields that varied between 30% and 60% with a Zemplén deprotection of **38** affording the desired morphine-6-glucoside **31** in yields in excess of 90%. Recently two new routes to morphine-6-glucoside **31** were reported by Kovác and Rice[52] who carried out either a mercury (II) cyanide or a silver (I) triflate

promoted coupling of 3-*O*-acetylmorphine **27** with either acetobromoglucose **37** or tetra-*O*-benzoyl-α-D-glucopyranosyl bromide **39** (scheme 1.10.).



Conditions	Products	Yield
i. **37**, $HgCN_2$, CH_3CN, Drierite	**40**, **38**	**40**; major
ii. **37**, AgOTf, CH_2Cl_2, 2,4,6-Collidine -5 °C	**40**, **38**	**40**; major
iii. **39**, AgOTf, CH_2Cl_2, -5 °C	**41**, **42**, **43**	**41** + **42**; 91%, **43**; Trace

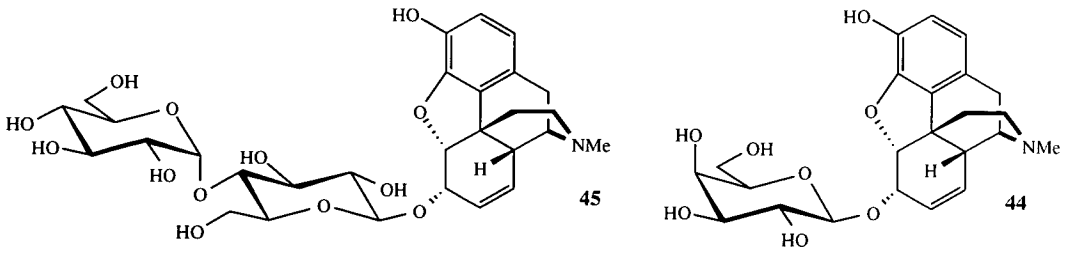
*Kovác and Rice's Synthesis of Morphine-6-glucoside **31**.*[52]

Scheme 1.10.

The mercury (II) cyanide promoted glycosidation attempted by Kovác and Rice[52] was slow and afforded the orthoester **40** as the major product and 3-*O*-acetylmorphine-6-(2',3',4',6'-tetra-*O*-acetyl)-β-D-glucoside **38** as the minor product. The silver (I) triflate promoted glycosidation although higher yielding afforded the same ratio of the orthoester **40** and the morphine-β-D-glucoside **38**. When Kovác and Rice[52] used tetra-*O*-benzoyl-α-D-glucopyranosyl bromide **39** in a silver (I) triflate promoted glycosidation, a mixture of the morphine-α-D-glucoside **41** and the morphine-β-D-glucoside **42** were found in a combined yield of 91% with only a trace of the orthoester **43**.[52] Although Kovác and Rice[52] did not attempt to explain this difference the most likely explanations are that the orthoester **43** forms more slowly as a result of steric hindrance due to the aromatic ring or if the orthoester **43** does form it rearranges to give the morphine-β-D-glucoside **42** much more rapidly than the rearrangement of orthoester **40** to the morphine-β-D-glucoside **38**. Isolation and Zemplén deprotection of the morphine-β-D-glucoside **38** afforded morphine-6-glucoside **31** in a yield of 75%.[52]

1.8.2. The Synthesis of M6G Analogues

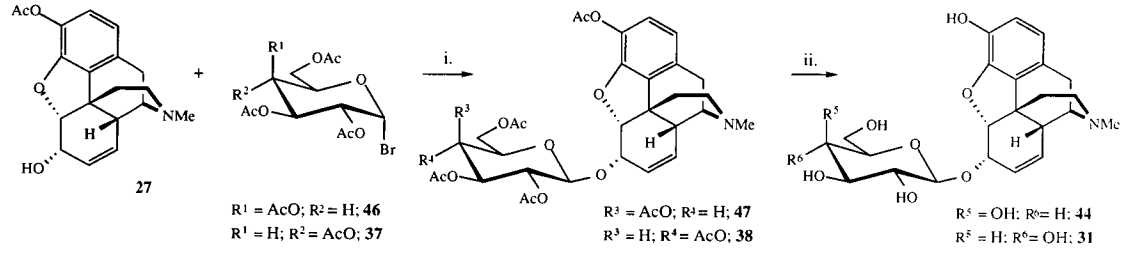
The synthesis of morphine-6-glucoside **31**, morphine-6-galactoside **44** and morphine-6-maltoside **45** has been reported by Hopkins[51] using a silver (I) carbonate promoted Koenigs-Knorr glycosidation of 3-*O*-acetylmorphine **27** with the appropriate acyl protected glycoside as the glycosyl donor (figure 1.6.).



Morphine-6-glucoside Analogues.[51]

Figure 1.6.

The best results were achieved with morphine glucosides; the Koenigs-Knorr glycosidation using acetobromoglucose **37** as the glycosyl donor gave the morphine-β-D-glucoside **38** in 32-63% yield which on Zemplén deprotection gave morphine-6-glucoside **31** in 90% yield. When acetobromogalactose **46** was used as the glycosyl donor, the morphine-β-D-galactoside **47** was obtained in much lower yields (9-28%); also the reaction was performed on a small scale, hence the isolated morphine-6-galactoside **44** could not be fully characterised. This was also the case for morphine-6-maltoside **45** (scheme 1.11.).[51]

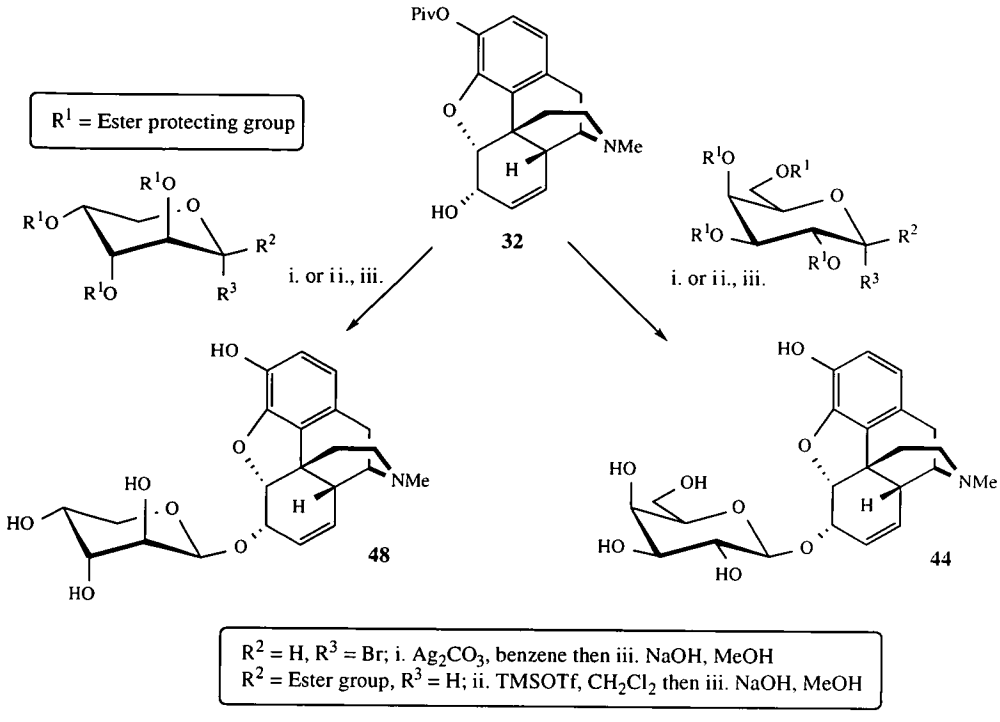


Compound	Conditions	Product	Yield
27 + 37	i. Ag_2CO_3, Benzene, reflux	38	32-63%
27 + 46	i. Ag_2CO_3, Benzene, reflux	47	9-28%
38	ii. NaOMe, MeOH	31	90%

Hopkins's Synthesis of Morphine-6-glucoside Analogues.[51]

Scheme 1.11.

There was interest in repeating the synthesis of morphine-6-galactoside **44** and attempting the synthesis of morphine-6-arabinoside **48** using both silver (I) carbonate and TMSOTf promoted couplings of 3-*O*-pivaloylmorphine **32** and the appropriate acyl protected glycosyl bromide or the peracylated sugars respectively, as the glycosyl donor (scheme 1.12.).



*Proposed Synthesis of Morphine-6-galactoside **44** and Morphine-6-arabinoside **48**.*

Scheme 1.12.

The synthesis of these morphine glycosides, if successful, would provide a series of β-glycosides, which, if biologically active, could provide alternative analgesics to M6G **23**.

Section Two; Modern Methods For Glycoside Synthesis

Interest in glycosidation reactions has grown as a result of an increased awareness of the biological significance of glycoconjugates and the biomedical potential of these compounds. Glycosidation reactions where a sugar moiety is attached to an aglycon is a common reaction in nature and natural glycosides are found throughout the plant and animal kingdoms. Glycoconjugates have a variety of functions, for example, the attachment of sugar residues to foreign compounds such as morphine **1** increases solubilization and so assists excretion *via* the kidneys. Glycoconjugates also play an important role in molecular recognition and sugars can be found attached to the proteins embedded in the surface of membranes.

The isolation and synthesis of natural glycosides such as glycoproteins, for example, transmembrane protein anchors found in rat brains[53,54] and glycosphingolipids such as the tumour associated antigen, Lewis X[55] has lead to the development of a number of new and novel glycosidation procedures. Glycosidation reactions were an essential part of the synthetic routes examined for the synthesis of M6G **23** and M6G analogues. Both classic and relatively modern methods of glycoside synthesis have been studied, for example, the Koenigs-Knorr glycosidation and the trichloroacetimidate method respectively. However, these are just two of the variety of methods currently available for the synthesis of glycosides.

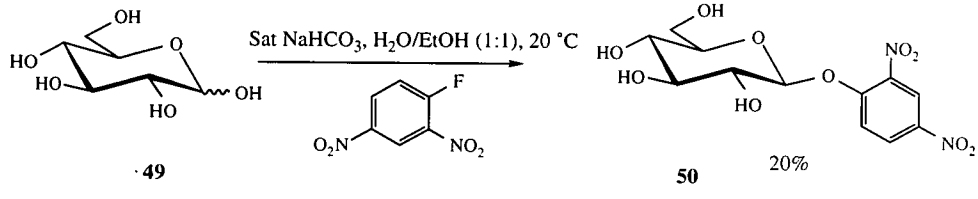
2.1. Classic Glycosidation Reactions

The classic glycosidation reactions for the synthesis of *O*-glycosides include; the Fischer glycosidation reaction,[56,57] which is the acid catalysed substitution of an alcohol with the anomeric hydroxyl group of a free sugar, the reaction normally produces a mixture of furanosyl and pyranosyl glycosides as a mixture of α and β-anomers. The Purdie method[58] is a classic synthesis of methyl glycosides by direct alkylation of a suitably protected hemiacetal using methyl iodide and silver oxide. The Koenigs-Knorr glycosidation[59] involves the displacement of a halide ion from a glycosyl halide in the presence of heavy metal salts, either silver or mercury, which is followed by glycosyl transfer to a free hydroxyl group on the glycosyl acceptor.[57]

Classic glycosidation reactions have been well documented over the years and some recent reviews have been published by Schmidt[60] and Paulsen[61,62]. However, classic glycosidation reactions have their limitations, for example, the mixture of compounds produced in the Fischer glycosidation reaction can be inconvenient.[56] The glycosyl halides used in the Koenigs-Knorr glycosidation have

low thermal stability, are sensitive to hydrolysis and are generated under harsh conditions, while the heavy metal salts are expensive and can be toxic (mercury) or explosive (silver perchlorate).[60]

The limitations of these classic glycosidation reactions led to searches for improved glycosidation procedures. A number of alkylating agents are now available for the direct alkylation of protected hemiacetals, for example, primary triflates such as methyl triflate have been found to give the methyl glycoside in high yields; by varying the reaction conditions, e.g., reaction temperature, stereoselectivity can be introduced.[60] The development of some reagents has even enabled the alkylation of unprotected sugars, for example, 1-*O*-arylation of D-glucose **49** occurs in the presence of sodium hydrogen carbonate using 1-fluoro-2,4-dinitrobenzene to give the 2,4-dinitrophenyl glucoside **50** (scheme 2.1.).[63]



Anomeric O-*Arylation of* D-*Glucose* ***49***.[63]

Scheme 2.1.

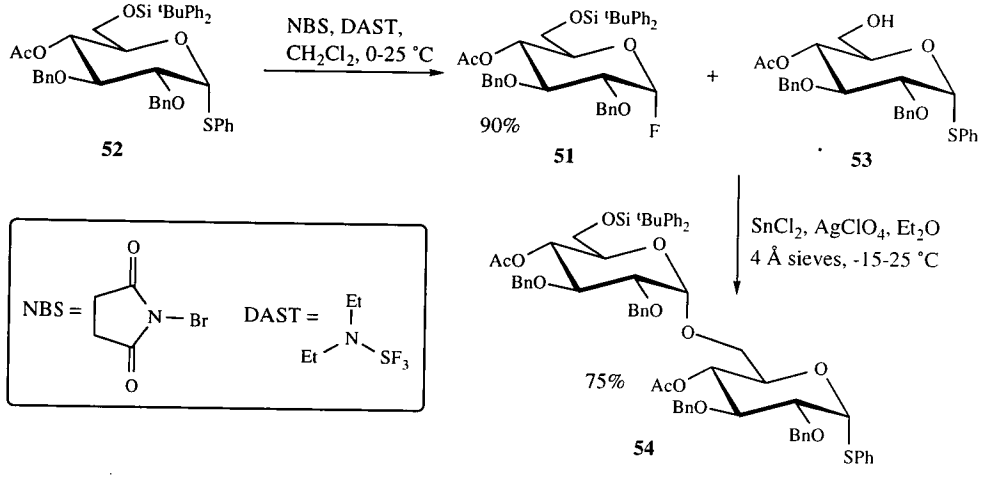
The original Koenigs-Knorr glycosidation is based on the displacement of a good leaving group from the anomeric centre of the glycosyl donor. However the limitations of glycosyl halides as glycosyl donors has lead to the development of alternative anomeric leaving groups.

2.2. Anomeric Leaving Groups

The nucleophilic displacement of an electrophilically activated leaving group at the anomeric carbon of sugars is one of the most synthetically useful reactions in carbohydrate chemistry and many glycosidation reactions, including the Koenigs-Knorr glycosidation, are based on this principle.[60] The anomeric leaving group of the Koenigs-Knorr glycosidation is generally a chloride or bromide (the glycosyl iodide is very reactive and is not commonly used[56]). However, as a result of the limitations of glycosyl bromides and chlorides attention was turned to the use of glycosyl fluorides as donors for glycosidation reactions.

2.2.1. Glycosyl Fluorides

Glycosyl fluorides have high thermal stability and are unreactive towards many reagents as a result of the short C-F bond. Until recently there were few good synthetic routes towards the preparation of glycosyl fluorides,[56,57] but it is now possible to synthesise glycosyl fluorides using a number of procedures, for example, from thioglycosides[56,64] in scheme 2.2.



Scheme 2.2.

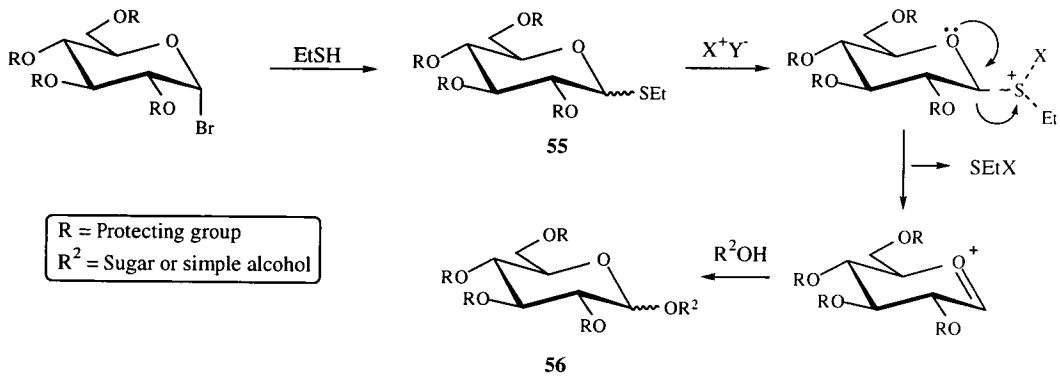
Synthesis of Glycosyl Fluorides and their use as Glycosyl Donors.[64]

In the mid 1980's specific fluorophilic promoters were developed enabling the use of glycosyl fluorides as glycosyl donors in glycosidation reactions.[57,60] Early fluorophilic promoters for glycosidation reactions with glycosyl fluorides were strong Lewis acids such as boron trifluoride diethyl etherate ($BF_3.Et_2O$)[65], other fluorophilic promoters include tin (II) chloride (scheme 2.2.) and lithium perchlorate ($LiClO_4$),[66] a mild fluorophilic promoter. Nicolaou and co-workers[64] synthesised the silyl protected glucosyl fluoride **51** from the silyl protected thioglucoside **52** using diethylaminosulfur trifluoride (DAST) (scheme 2.2.). A glycosidation reaction promoted by tin (II) chloride and silver perchlorate using the thioglycoside **53** as the glycosyl acceptor gave the α-disaccharide **54** in good yield.[64]

2.2.2. Glycosyl Amines, Phosphonium and Nitrilium Compounds

The replacement of the anomeric oxygen of glycosides with other elements such as sulfur, nitrogen or phosphorus has allowed the introduction of a range of new glycosyl donors. Sulfur, nitrogen or phosphorus based leaving groups allow the formation of stabilised carbonium ions which will undergo a glycosidation reaction with a suitable glycosyl acceptor.[60] The halide leaving group of glycosyl halides is readily replaced by an alkyl-thio or aryl-thio group to afford thioglycosides, such as

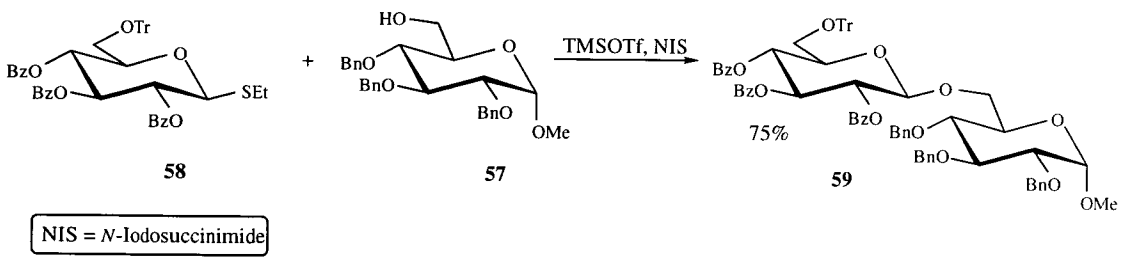
thioglycoside **55** in scheme 2.3., which on treatment with a suitable promoter (X^+Y^-) react with a glycosyl acceptor (R^2OH) to give the desired glycoside **56**.[56]



Thioglycosides as Glycosyl Donors in Glycosidation Reactions.[56,67]

Scheme 2.3.

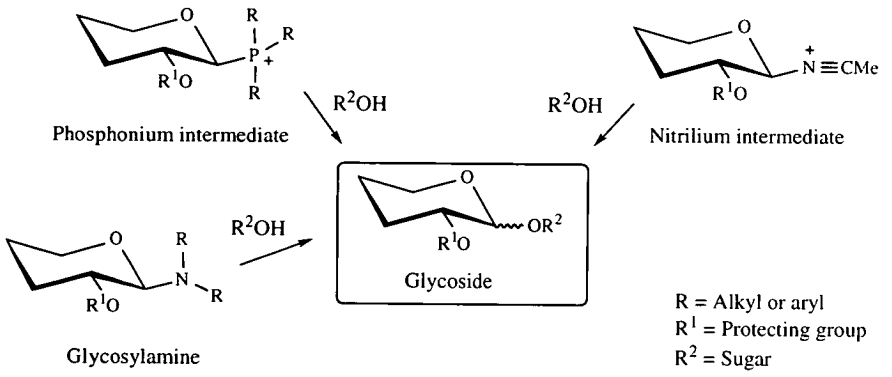
Early glycosidation promoters of thioglycosides were electrophilic reagents such as mercury (II), lead (II), copper (II) and silver (I) salts (X= Hg, Pb, Cu or Ag in scheme 2.3.).[56] Thioalkyl groups in particular are good leaving groups and in the presence of metal salts are readily converted into sulfonium cations which are easily displaced by a nucleophile.[56] A number of non-metalic glycosidation promoters are now available for thioglycosides, such as TMSOTf with *N*-iodosuccinimide (NIS)[68] or *N*-bromosuccinimide (NBS),[57,60] bis(trifluoroacetoxy)iodobenzene (BTIB).[67] For example, Boons and co-workers[68] used TMSOTf-NIS to promote the glycosidation of the thioglucoside **57** with the benzyl protected glucoside **58** affording the β-D-disaccharide **59** in 75% yield (scheme 2.4.).



Synthesis of β-D-Glucopyranosyl Disaccharides from Thioglycoside Donors.[68]

Scheme 2.4.

Sulfonium leaving groups are more reactive than ammonium leaving groups which are in turn more reactive than phosphonium leaving groups and so interest has mainly concentrated on the use of thioglycosides in glycosidation reactions. However, ammonium, phosphonium, and acetonitrile groups at the anomeric centre of sugars potentially provide useful glycosyl donors (scheme 2.5.).[57,60]



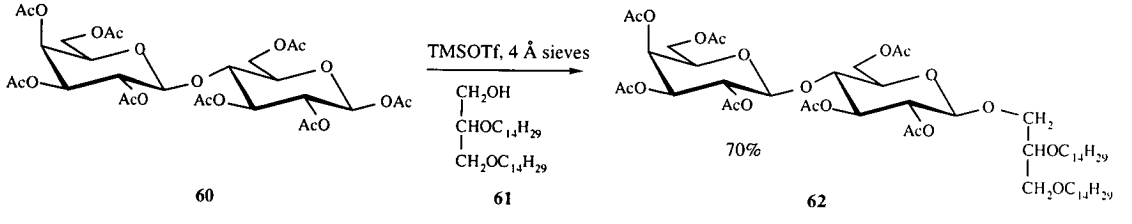
Potential Glycoside Donors for Glycosidation Reactions.[56]

Scheme 2.5.

Of the investigations with ammonium, phosphonium and nitrile glycosides, glycosidation reactions predominately give α-glycosides as the reaction products.[57,60]

2.2.3. Glycosyl Acyl Esters

Acyl esters are now commonly used in glycosidation reactions, early promoters for these reactions were Lewis acids such as zinc (II) chloride and tin (IV) chloride. These promoters were then followed by triflates such as TMSOTf which is a powerful glycosidation promoter. The use of TMSOTf as a promoter of glycoside synthesis was first established in the early 1980's by Ogwa and co-workers[69] who used the promoter in the glycosidation of β-lactose octaacetate **60** using the alcohol **61** to give the β-D-disaccharide **62** (scheme 2.6.).

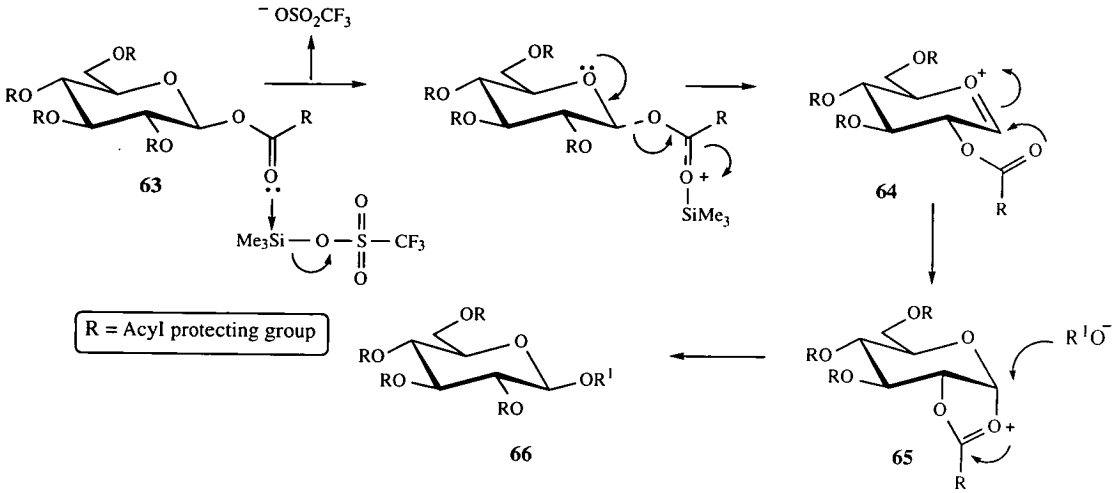


First Example of the Use of Trimethylsilyl Triflate as a Glycosidation Promoter.[69]

Scheme 2.6.

A possible mechanism of TMSOTf promoted glycosidations with glycosyl donors which have an acyl ester group at the anomeric centre is shown in scheme 2.7. whereby co-ordination of the electropositive silyl group to the carbonyl oxygen of the anomeric acyl group weakens the ester bond. Loss of the 1-*O*-acyl group in compound **63** gives the oxonium ion **64** and neighbouring group participation from the 2-*O*-acyl group gives the acetoxonium ion **65**. As the α-face is blocked the

acetoxonium ion **65** undergoes equatorial attack by the alcohol acceptor to give the β-D-glycoside **66**.[70]



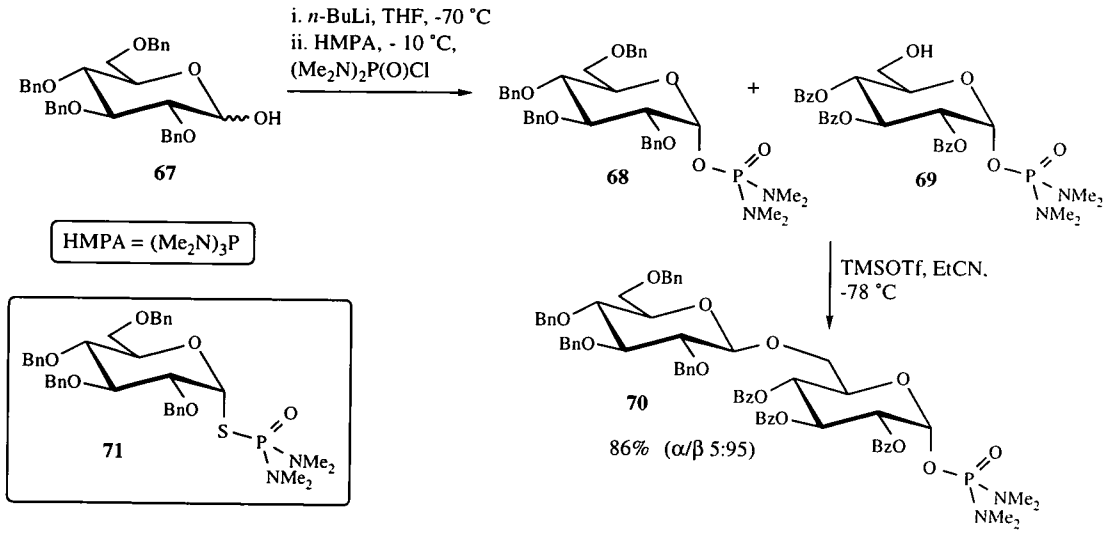
Mechanism of The Trimethylsilyl Triflate Promoted Glycosidation.[70]

Scheme 2.7.

TMSOTf promoted glycosidations using acylated sugars are most successful when the glycosyl donor has a 1,2-*trans* configuration; the acylated β-anomer is more reactive than the anomerically more favoured and more stable acylated α-anomer.[62]

2.2.4. Glycosyl Phosphates

In nature glycosyl transfer generally occurs with a sugar nucleotide containing a pyrophosphate linkage or with glycosyl phosphates as glycosyl donors; this observation stimulated interest for the use of glycosyl phosphates as glycosyl donors for chemical synthesis.[56] Glycopyranosyl phosphoramidates in particular are becoming increasingly popular as glycosyl donors, being stable, readily prepared from acylated glucopyranoses[71] or glycosyl phosphites[72] and they will couple with alcohol acceptors under a range of reaction conditions. For example, the glucosyl tetramethylphosphoramidate **68**, readily prepared from the tetra-*O*-benzylglucopyranose **67**, will react with the partially protected sugar acceptor tri-*O*-benzoyl glucoside **69** in the presence of TMSOTf to give predominately the β-D-disaccharide **70** in scheme 2.8.[71]



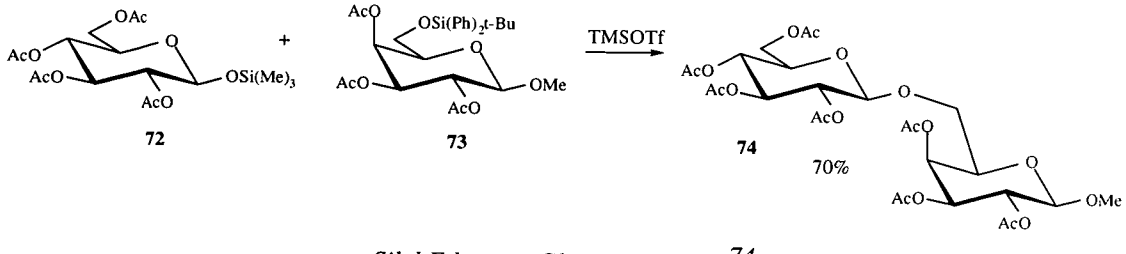
Glycopyranosyl Tetramethylphosphoramidates as Glycosyl Donors.[71]

Scheme 2.8.

Hashimoto and co-workers have investigated the use of glycopyranosyl tetramethylphosphoramidates to synthesise a variety of compounds including the anticancer agents podophyllum lignan glycosides[73] and oligosaccharides.[71] Recently Hashimoto and co-workers[71] have extended their investigation into the use of more reactive glycosyl donors, such as the glycosyl phosphordiamidothioate **71** in scheme 2.8. which give predominately α-D-glucosides.

2.2.5. Trialkylsilyl Glycosides

Silyl ethers provide another source of glycosyl donor, for example, Nashed and co-workers[74] coupled the trimethylsilyl-β-D-glucoside **72** to the silyl protected galactoside **73** in the presence of TMSOTf to give the β-disaccharide **74** in a highly stereoselective reaction (scheme 2.9.).

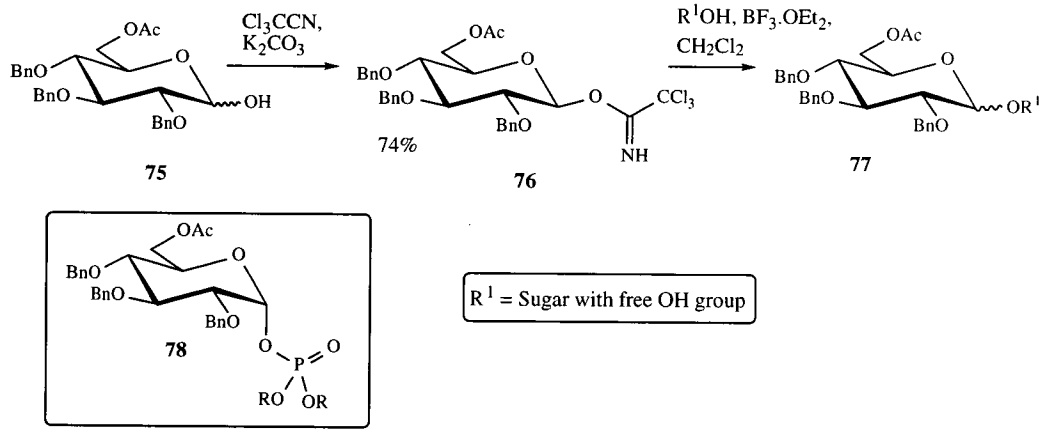


Silyl Ethers as Glycosyl Donors.[74]

Scheme 2.9.

2.2.6. Glycosyl Trichloroacetimidates

Imidates have proved to be very versatile glycosyl donors and are extensively used in glycosidation reactions. Glycosyl imidates were first introduced as glycosyl donors by Pougny and Sinaÿ[75] who examined the use of glycosyl acetimidates and benzylimidates in glycosidation reactions with silver (I) oxide as the promoter. Trichloroacetimidates are among the most commonly used glycosyl imidates, they are formed from a nucleophilic reaction between the unprotected anomeric hydroxyl group (the remaining hydroxyl groups are protected) and trichloroacetonitrile in the presence of base (scheme 2.10.). Potassium carbonate is generally used to obtain β-glycosyl trichloroacetimidates while a stronger base such as sodium hydride is required for α-glycosyl trichloroacetimidates (scheme 2.10.).[56] Glycosyl trichloroacetimidates will react with a suitable alcohol acceptor in the presence of Lewis acid catalysts such as TMSOTf or $BF_3.Et_2O$ to give the desired glycoside.[57] For example, the trichloroacetimidate glucoside **76** is readily prepared from the tri-*O*-benzyl glucose **75** and will react with an alcohol acceptor (R^1OH) in the presence of $BF_3.Et_2O$ to give the 1-*O*-glucoside **77** (scheme 2.10.).[57]

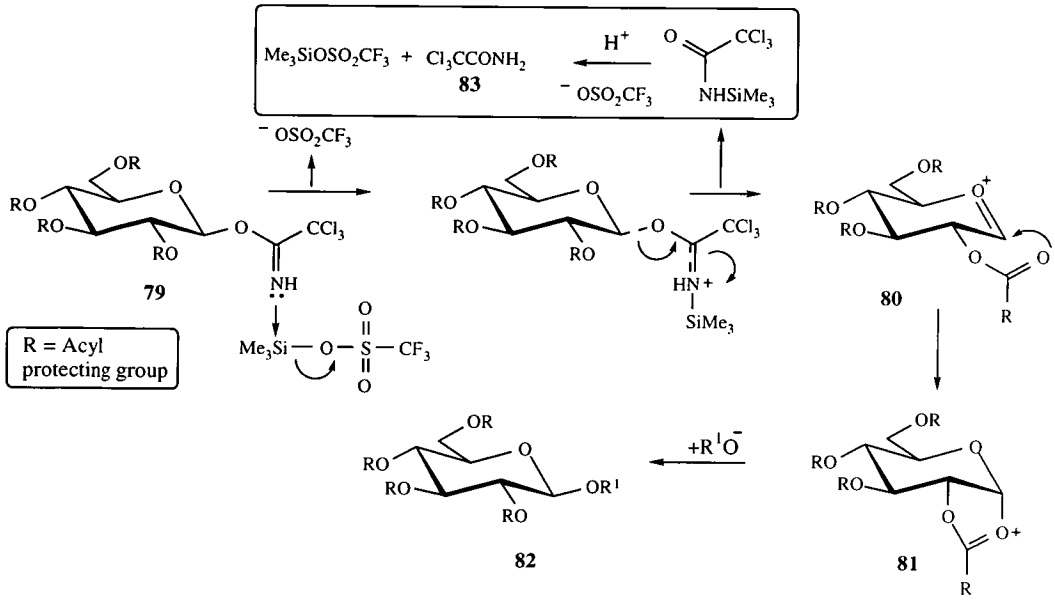


Synthesis of 1-O-Glycosides Using 1-O-Glycosyl Trichloroacetimidates.[56,57]

Scheme 2.10.

When phosphoric acids are used as the acceptor, TMSOTf promoted glycosidations with glycosyl trichloroacetimidates afford glycosyl phosphates e.g. **78** in scheme 2.10. These glycoconjugates are components of biological membranes and so their synthesis is of significant interest.[60] The mechanism of glycosidation reactions with trichloroacetimidate donors such as **79** in scheme 2.11. involves initial co-ordination of a Lewis acid, such as TMSOTf with the nitrogen lone pair of the trichloroacetimidate group. Generation of the oxonium ion **80** is brought about by the loss of the trichloroacetamide-Lewis acid complex, which will pick up a proton to

form trichloroacetamide **83** and regenerate the Lewis acid. Neighbouring group participation from the 2-*O*-acyl group gives the acetoxonium ion **81** which undergoes nucleophilic attack by the acceptor to form the β-glycosidic bond of the β-glycoside **82**.[70]

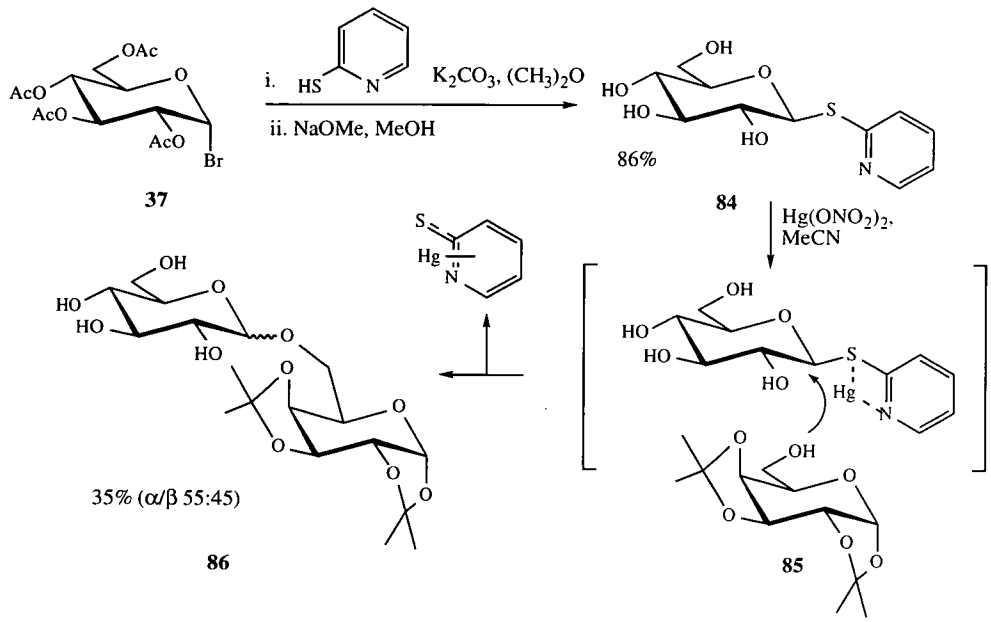


Mechanism of the Trimethylsilyl Triflate Promoted Glycosidation with Trichloroacetimidate Donors.[70]

Scheme 2.11.

2.2.7. Glycosyl 2-Pyridine Carboxylates

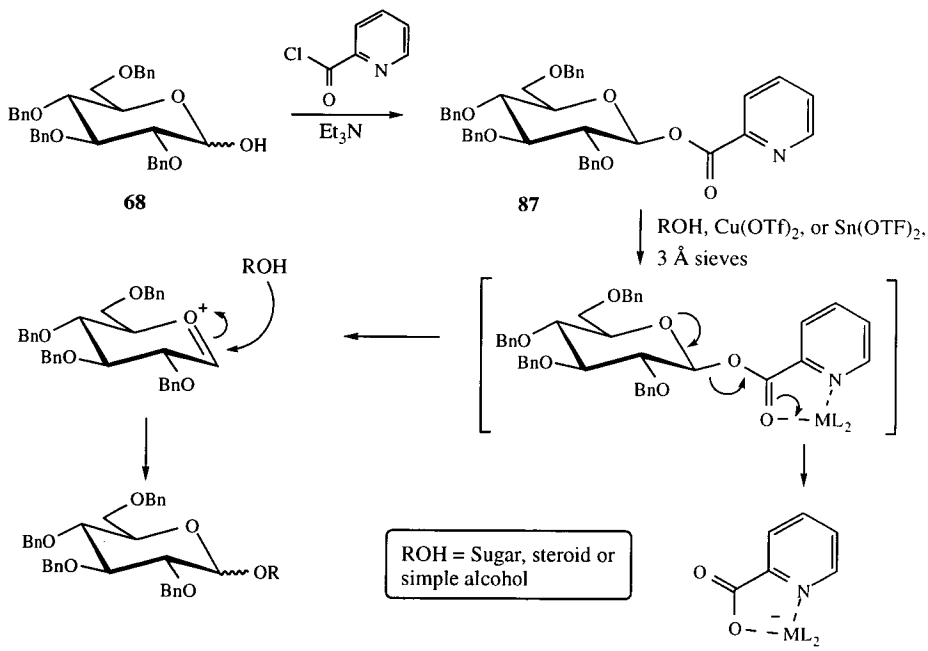
Novel glycoside donors are being reported on a regular basis, amongst the more unusual glycosyl donors being glycosyl 2-pyridine carboxylates, reported by Koide and co-workers[76] which were developed on the basis of the Remote Activation Concept. Remote Activation, a term originally introduced by Hanessian and co-workers,[77] involves the activation of a suitable group on the anomeric carbon of an unprotected sugar producing a leaving group followed by nucleophilic attack to give the desired glycoside. For example, the isopropylidine protected galactoside **85** was coupled with pyridin-2-yl thio-β-glucoside **84** in the presence of mercury (II) nitrate to afford a mixture of α and β-disaccharides **86** in a moderate yield (scheme 2.12.).[77]



Formation of Glycosides by Remote Activation.[77]

Scheme 2.12.

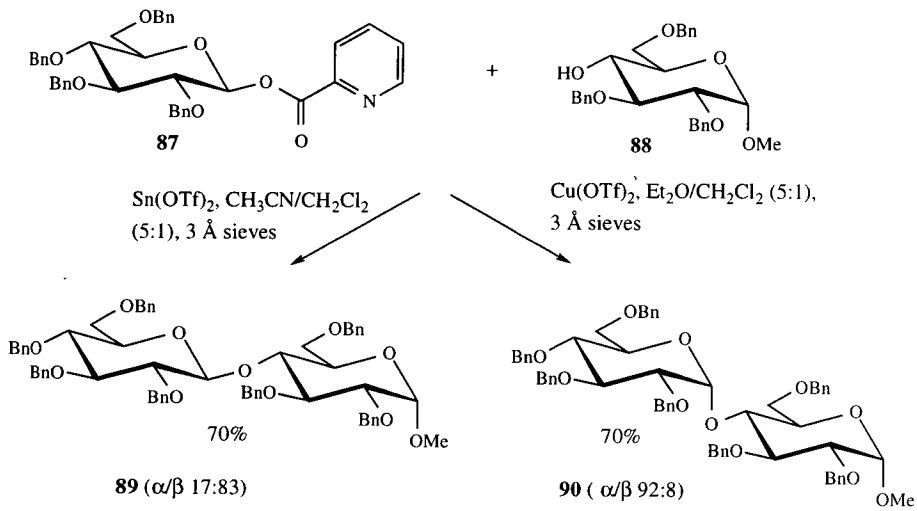
Koide and co-workers[76] applied the principle of Remote Activation to glycosidation reactions using glycosyl pyridine carboxylates in the presence of a range of activators including mild Lewis acids such as copper (II) triflate and tin (II) triflate to give either α or β-glycosides with good stereoselectivity (scheme 2.13.).[76]



Glycosyl Pyridine Carboxylates as Glycosyl Donors.[76]

Scheme 2.13.

The glycosyl pyridine carboxylates, such **87** in scheme 2.13, were readily prepared from the tetra-*O*-benzyl glucose **68** and picolinyl chloride in the presence of triethylamine. Koide and co-workers[76] found that copper (II) triflate promoted glycosidations of glycosyl pyridine carboxylates afforded predominately α-glycosides while tin (II) triflate promoted glycosidations gave predominately β-glycosides. For example, in scheme 2.14., the tin (II) triflate promoted glycosidation of the 1-*O*-methyl tri-*O*-benzyl glucoside **88** with benzylglucopyranosyl 2-pyridine carboxylate **87** as the glycosyl donor afforded predominantly the β-D-glucoside **89** while copper (II) triflate promoted the synthesis of the α-D-glucoside **90** (scheme 2.14.).[76]

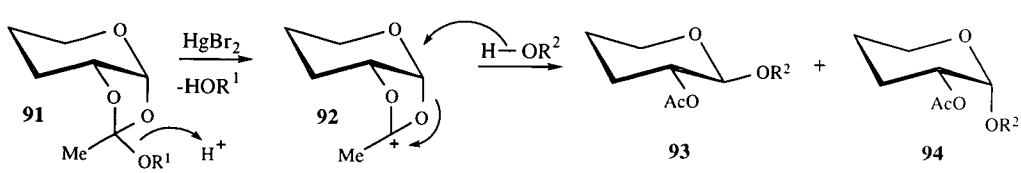


Influence of Promoter on Remote Activation Based Glycosidations.[76]

Scheme 2.14.

2.2.8. Orthoester Derivatives as Glycosides

Other novel glycosyl donors recently reported include orthoesters such as compound **91** (scheme 2.15.) In the presence of a Lewis acid such as mercury (II) bromide, loss of the alkoxy ion ($^-OR^1$) from compound **91** gives carbocation **92** which will react with an alcohol acceptor (R^2OH) to give a mixture of α and β-glycosides such as the 1,2-*trans*-glycoside **93** and the 1,2-*cis*-glycosides **94** (scheme 2.15.).[56,61]



Synthesis of 1-O-Glycosides Using 1,2-Orthoesters.

Scheme 2.15.

With simple orthoesters stereoselectivity is difficult to achieve and the alcohol released (R^1OH) may compete with the intended glycosyl acceptor (R^2OH) in the glycosidation reaction. To overcome this problem a sterically hindered orthoester is used such as *tert*-butyl orthoester, which will provide steric bulk, and the released *tert*-butyl alcohol will be less reactive than the glycosyl acceptor.[56,61]

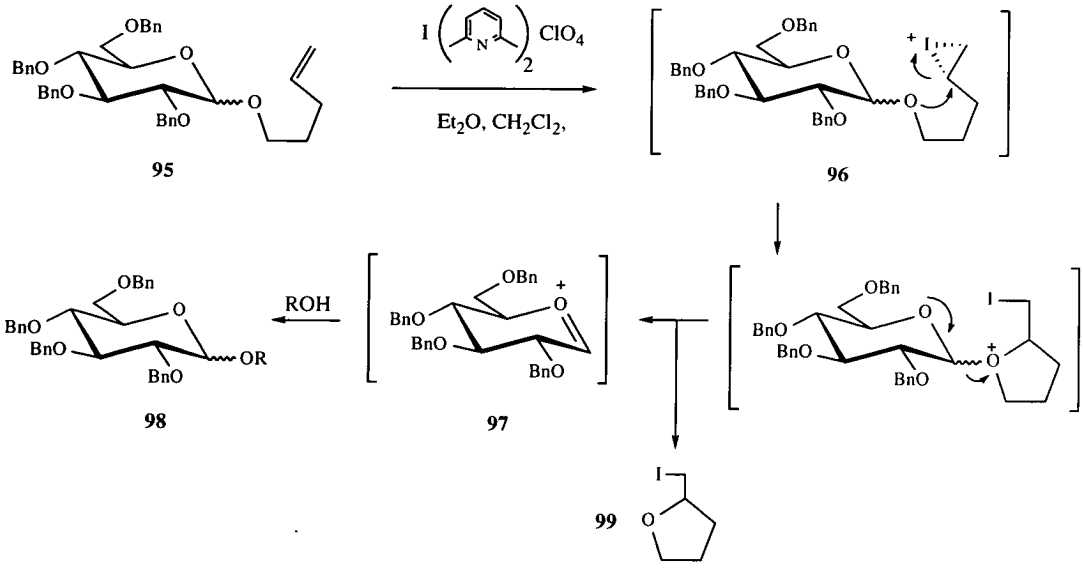
An alternative route to glycosides using orthoesters has been recently examined by Iimori and co-workers[78] who examined the reduction of cyclic orthoesters with diisobutylaluminium hydride (DIBAH) or lithium aluminium hydride ($LiAlH_4$) to obtain disaccharides in excellent yields and selectivity.

Currently glycosyl fluorides, trichloroacetimidates, and sulfides provide the most widely used glycosyl donors for glycosidation reactions. New and novel glycosyl donors are constantly appearing in the literature and a more comprehensive review on glycosyl donors has been published by Toshima and Tatsuta.[79] In the search for new glycosidation procedures one area in particular, namely oligosaccharide synthesis, has provided some innovative glycosidation techniques including the armed-disarmed glycosylation strategy.

2.3. The Armed-Disarmed Glycosylation Concept

The armed-disarmed approach to glycoside synthesis was first introduced by Fraser-Reid and co-workers[80] who used the term to describe glycosidation reactions incorporating the use of pentenyl glycosides. During glycosidation reactions Fraser-Reid and co-workers[80] found that the reactivity of glycosyl donors was decreased when an ester group was present at C2 (disarmed) and increased when an ether group was present at C2 (armed). The pentenyl group provides protection for the anomeric centre during protection-deprotection steps involving non-anomeric hydroxyl groups, and allows activation of the anomeric centre towards glycosidation reactions.

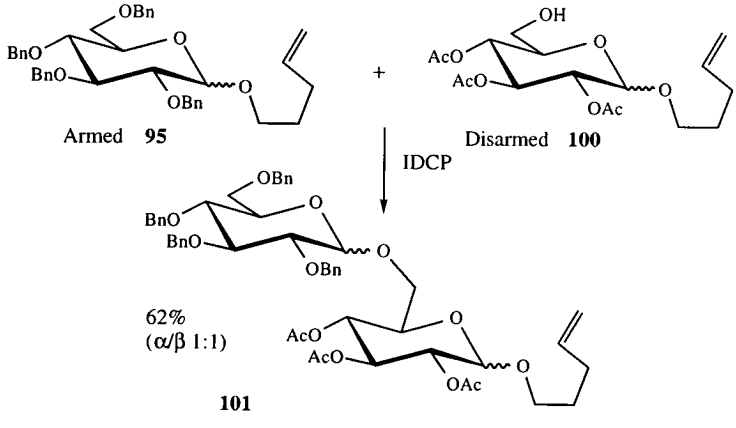
The pentenyl group is typically formed by a silver (I) triflate or tin (IV) chloride promoted glycosidation using penten-1-ol as the acceptor. Activation of the pentenyl group is achieved by the addition of a halogen to the double bond using iodonium dicollidine perchlorate (IDCP) or NBS. The armed pentenyl tetra-*O*-benzylglucoside **95** in scheme 2.16. is initially activated with IDCP to give the activated glucosyl donor **96**, the pentenyl group then rearranges to give 2-halomethylfuran **99** and the oxonium ion **97**, nucleophilic attack of the oxonium ion **97** by the desired alcohol acceptor gives the 1-*O*-glucoside **98**.[56]



Synthesis of 1-O-Glycosides Using Pentenyl Glycosides.[56]

Scheme 2.16.

Fraser-Reid and co-workers[80] have used the armed-disarmed approach to synthesise a variety of compounds from simple disaccharides such as the α/β-D-disaccharide **101** which was synthesised from the armed pentenyl glucoside **95** and disarmed pentenyl glucoside **100** in scheme 2.17.[80] to more complex heptasaccharides such as that found in the glycosylphosphatidylinositol (GPI) anchor.[81]



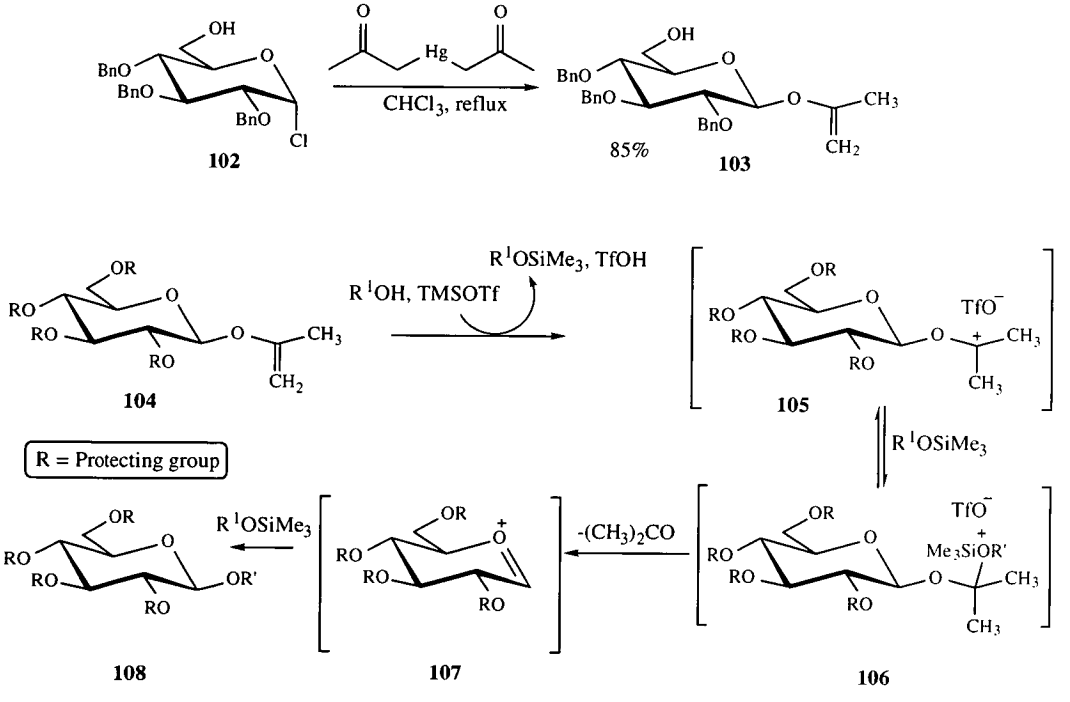
The Armed-Disarmed Approach to Glycoside Synthesis.[80]

Scheme 2.17.

Pentenyl glycosides provide the opportunity to perform glycosidation reactions under mild conditions and provide a valuable tool for the synthesis of oligosaccharides.[56] The armed-disarmed strategy provides both versatility and selectivity for glycoside synthesis[80] and can be applied to other novel glycosidation procedures such as those involving glycals (section 2.5.).

2.4. Isopropenyl Glycosyl Donors

The isopropenyl glycosides are similar to the pentenyl glycosides whereby, the propenyl group provides protection at the anomeric centre during hydroxyl group manipulations. Isopropenyl glycosides such as the isopropenyl glucoside **103** in scheme 2.18. are prepared from glycosyl halides such as the tri-*O*-benzylglucopyranosyl chloride **102** with bis(acetonyl)mercury. In the presence of an electrophile, such as a proton, the isopropenyl group is activated, the hydroxyl group of the acceptor adds to the double bond of the isopropenyl glycosyl donor to give a mixed sugar acetal which rearranges to give acetone and the β-glycoside. For example, when a Lewis acid catalyst such as TMSOTf is used, reaction with the glycosyl acceptor (R^1OH) gives triflic acid which protonates the enol ether **104** to produce the carbocation **105** (scheme 2.18.). Nucleophilic attack by the silylated glycosyl acceptor (R^1OSiMe_3) gives a mixed acetal **106** which rearranges with loss of acetone to give the oxonium ion **107**. Nucleophilic attack of **107** by a second molecule of the silylated glycosyl acceptor (R^1OSiMe_3) finally gives the desired β-D-glucoside **108**.[56,82,83]



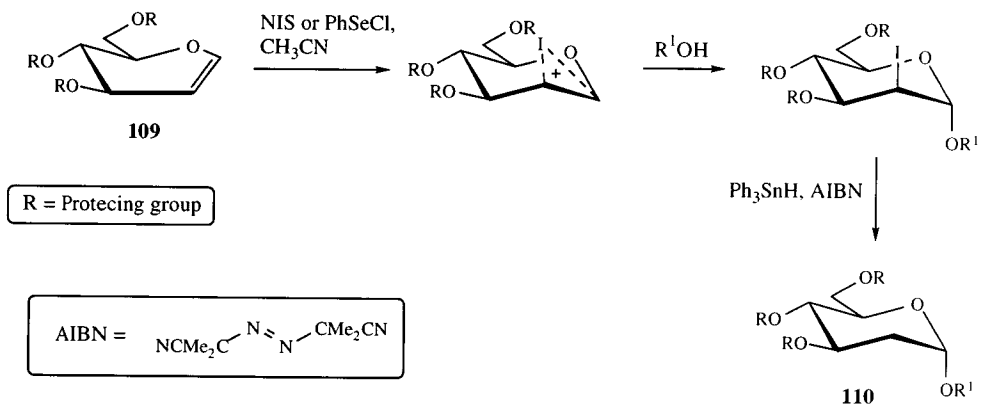
Synthesis of 1-O-Glycosides Using Isopropenyl Glycosides.[83]

Scheme 2.18.

As observed with pentenyl glycosides, isopropenyl glycosides are a useful tool, providing the versatility and selectivity required for oligosaccharide synthesis.[83]

2.5. Glycals

Another important glycosidation procedure involves the use of glycals which have recently been demonstrated by Danishefsky and co-workers[84] to provide a useful route to α and β-glycosides. Glycals are readily prepared from acylated glycosyl halides using zinc and acetic acid and when treated with electrophilic species such as $PhSe^+$ or I^+ from NIS, the electrophile adds across the double bond between C1 and C2.[56] If the 1,2-iodonium ion intermediate that forms is then treated with a nucleophile such as an alcohol, the nucleophile adds to C1 to give the α or β-glycoside while the electrophilic species adds to C2. The iodide ion influences the stereochemistry of the glycoside and is easily removed from the glycoside with, for example, nickel (II) chloride and sodium borohydride to give the 2-deoxy sugar. For example, the D-glucal **109** forms the iodonium ion on the β-face of the double bond, addition of an alcohol acceptor, this is followed by radical displacement of iodine initiated by 2, 2-azobisisobutyronitrile (AIBN) gives the 2-deoxy-α-D-glucoside **110** (scheme 2.19.).[56,57]

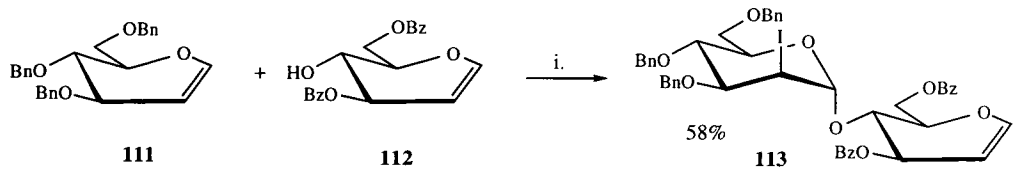


109 **110**

Synthesis of 2-deoxy Glycosides From Glycals.[57,84]

Scheme 2.19.

Glycals provide useful starting materials for 2-deoxy sugars and are extensively used for the synthesis of oligosaccharides.[56] The reaction between two glycals is possible using the armed-disarmed approach which allows one of the glycals to provide a donor function. This is achieved by protecting the free hydroxyl groups with ether functions while the acceptor is protected by ester groups (scheme 2.20.).[84]



111 **112** **113**

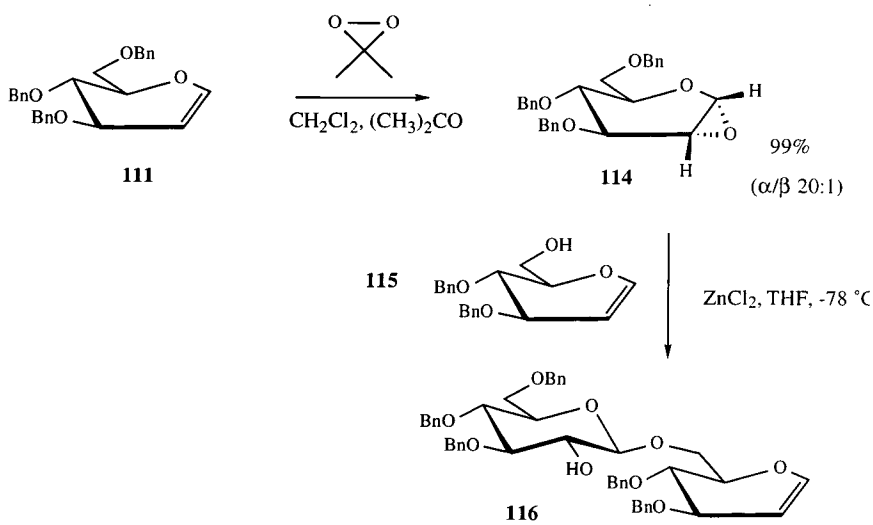
i. (sym-collidine)$_2$I$^+$ClO$_4^-$, CH_2Cl_2, 4Å sieves

Disarmed-Armed Approach to The Coupling Reaction Between Two Glycals.[84]

Scheme 2.20.

In scheme 2.20 the armed tri-*O*-benzyl-D-glucal **111** and the disarmed tri-*O*-benzoyl-D-glucal **112** couple to afford the α-linked disaccharide **113** in good yield and stereoselectivity.[84]

Recently glucals have been shown by Halcomb and Danishefsky[85] to be useful precursors of 1,2-anhydro sugars which are potentially good glycosyl donors as a result of the strain in the alkoxy epoxide moiety. Glucals are readily epoxidised with dimethyldioxirane to afford 1,2-anhydro sugars, for example, when the tri-*O*-benzyl-D-glucal **111** (scheme 2.20.) is treated with dimethyldioxirane the reaction gives the 1,2-anhydroglucoside **114** as predominately the α-anomer in high yield and stereoselectivity. When the 1,2-anhydroglucoside **114** is treated with a suitable alcohol, such as the di-*O*-benzyl-D-glucal **115** in the presence of zinc (II) chloride the epoxide ring undergoes *trans*-diaxial opening to afford selectively the β-linked disaccharide **116** (scheme 2.21.).[85]

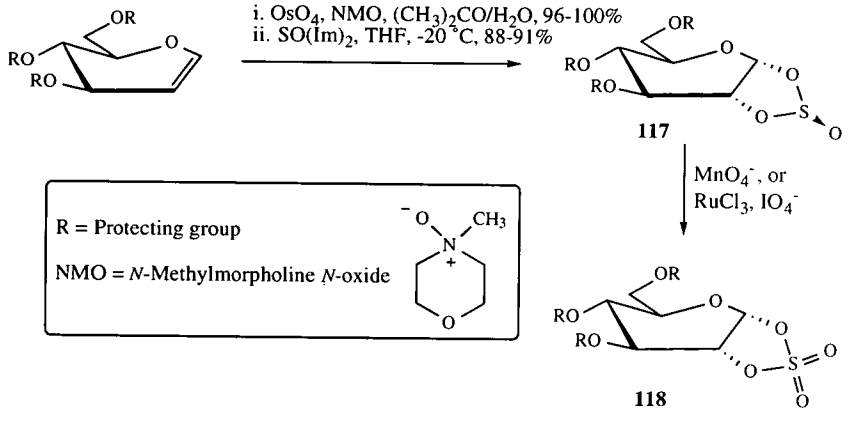


Synthesis of β-D-Glycosides from Glucals.[85]

Scheme 2.21.

The high selectivity of the glycosidation reaction is achieved as a result of nucleophilic attack on C1 equatorially, Waldern inversion at C1 then gives the 1,2-*trans* configuration expected in an S_N2 reaction.[56]

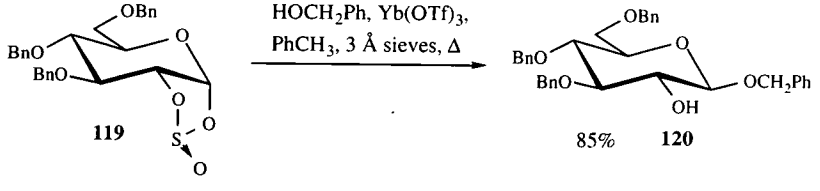
Unfortunately anhydro sugars are not stable and dimethyldioxirane is difficult to use on a large scale and so interest has turned to cyclic sulfites and sulfates as epoxide equivalents. Cyclic sulfites such as **117** are readily prepared from glucals *via* the 1,2-diol using thionyl chloride or thionyl diimidazole. Oxidation of cyclic sulfites with permanganate, ruthenium (III) chloride or periodate affords cyclic sulfates such as **118** (scheme 2.22.).[56,86]



Synthesis of Cyclic Sulfites and Sulfates.[56,86]

Scheme 2.22.

The treatment of cyclic sulfites with lanthanide (III) triflates as glycosidation promoters has recently been reported by Sanders and co-workers,[86] who observed the stereoselective glycosidation of glucose 1,2-cyclic sulfites, for example, **119** which coupled to benzyl alcohol to give the β-D-glucoside **120** in a good yield (scheme 2.23.).



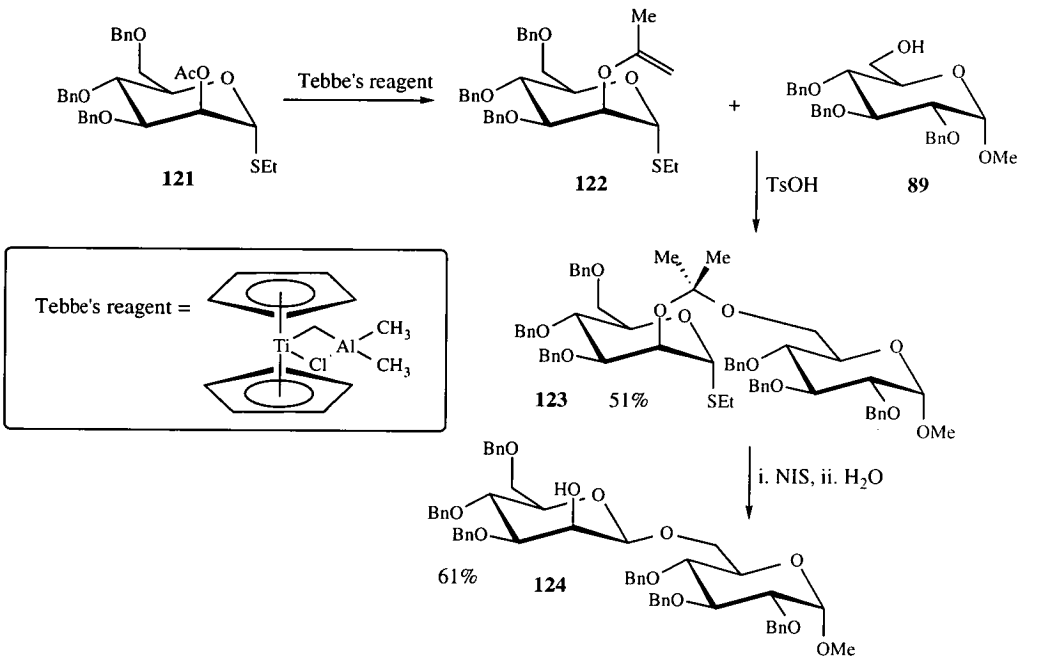
*Synthesis of 1,2-*Trans*-β-D-glucosides From Cyclic Sulfites.*[86]

Scheme 2.23.

2.6. Intramolecular Aglycon Delivery

The potential of a functional moiety present on the sugar that provides both activation and stereo-control during glycoside formation offers a novel glycosidation

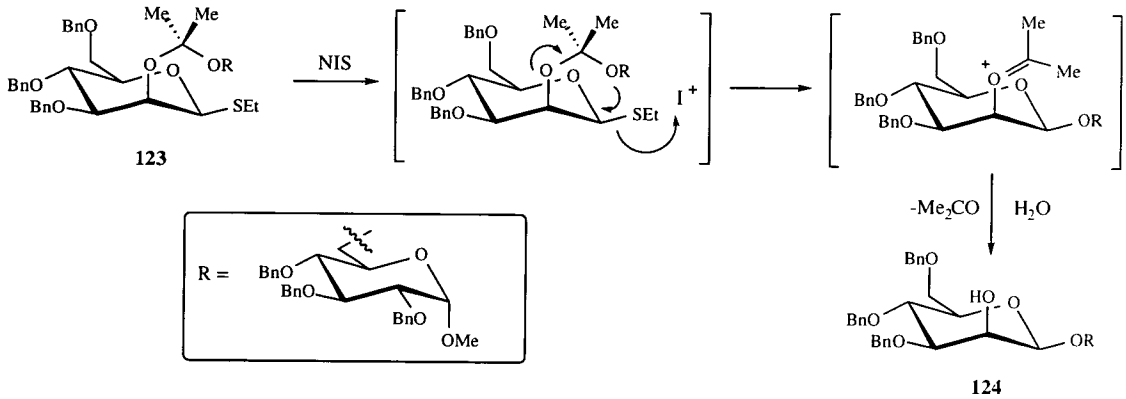
procedure that potentially could be of great significance in oligosaccharide synthesis. Intramolecular aglycon delivery provides a useful strategy for the synthesis of 1,2-*cis*-β-D-mannopyranoside linkages which are difficult to construct using traditional glycosidation reactions such as the Koenigs-Knorr procedure.[56,87] For glycosidation to occur a good leaving group, for example, a thiol group, is required at the anomeric position, while either a carbon bridge or a silicon bridge is present at C2.[56] Both approaches have been successfully used to synthesise β-D-mannosides, for example, the synthesis of the β-D-mannoside **124** using a carbon bridge by Barresi and Hindsgaul[87] (scheme 2.24.).



The Intramolecular Synthesis of a β-D-Mannodisaccharide.[87]

Scheme 2.24.

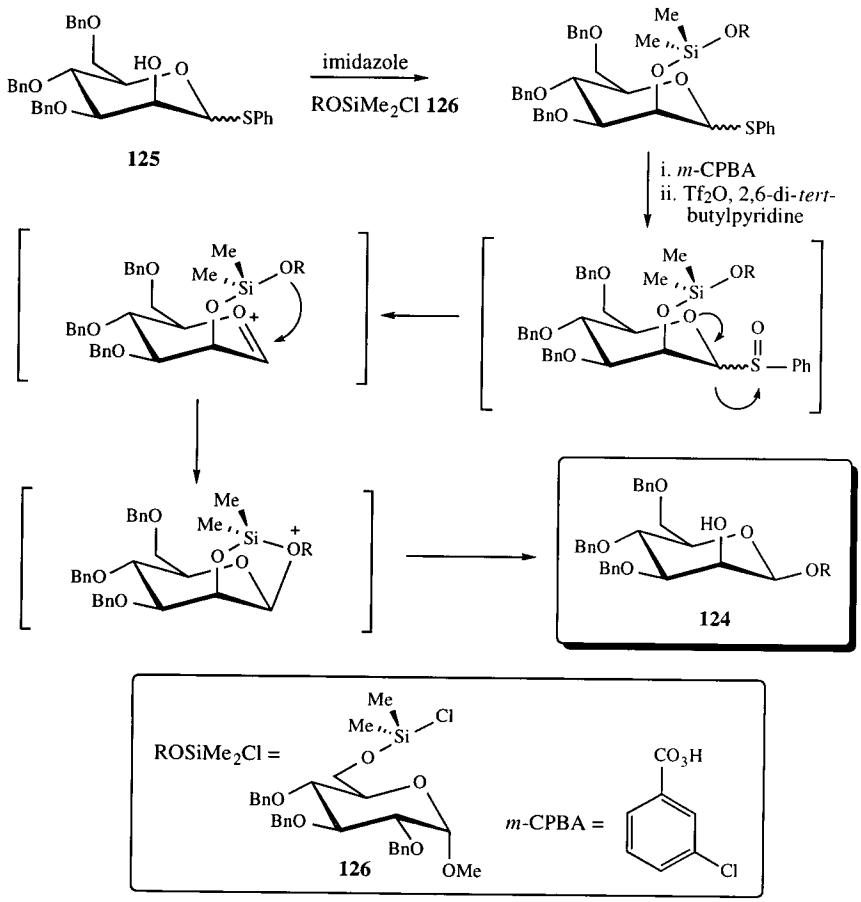
The carbon bridge is readily prepared by the methylene-de-oxo-bisubstitution at the 2-*O*-acetate group of the 2-*O*-acetylbenzyl 1-thiomannoside **121** using Tebbe's Reagent (a titanium cyclopentadiene complex, $Cp_2Ti(CH_2)(Cl)AlMe_2$)[88] to give the 2-*O*-isopropyl derivative **122** (scheme 2.24.). Coupling of compound **122** and the 1-*O*-methyl tri-*O*-benzyl glucoside **89** at the isopropenyl group occurs in the presence of tosic acid to give the bridged disaccharide **123** (scheme 2.24.). The thio group of the bridged disaccharide **123** is activated by NIS (for mechanism see scheme 2.25.). Equatorial attack of the anomeric centre from the alkoxy group is followed by loss of acetone to afford the β-mannoside **124** exclusively.[56]



Mechanism of Intramolecular Aglycon Delivery.[56,87]

Scheme 2.25.

The silicon bridge is also readily prepared, for example, by condensation of a free alcohol group on the thiomannoside **125** with the chlorosilylether group on C2 of the thiomannoside **126**, activation of the thiol group and equatorial attack again affords the β-D-mannoside **124** exclusively (scheme 2.26.).[56,89]



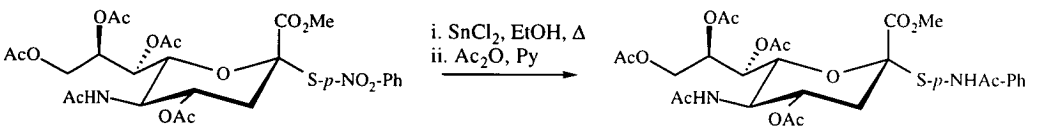
Intramolecular Aglycon Delivery Incorporating a Silicon Bridge.[56]

Scheme 2.26.

The temporary carbon and silicon connections provide a useful and stereoselective synthesis of glycosides particularly the difficult 1,2-*cis*-β-mannoside linkages.[89]

2.7. Latent-Active Glycosidations

Influencing the reactivity of the anomeric centre towards glycosidation is a concept that has been examined with the armed-disarmed approach demonstrated with pentenyl glycosides.[80] Latent-active glycosidations also affect the level of reactivity of the anomeric centre to glycosidation and provide further novel routes to glycosides. Roy and co-workers[90] have recently reported the use of a thioglycoside which has an electron-withdrawing group on the thioaryl substiuent, rendering the thioglycosyl acceptor latent and unreactive towards glycosidation. However, on replacing the electron-withdrawing group with an electron-donating group the thioglycoside acceptor becomes active, for example, the active thioglucoside donor **128** was synthesised from acetochloroneuraminic acid **127** using tin (II) chloride (scheme 2.27.).[90]

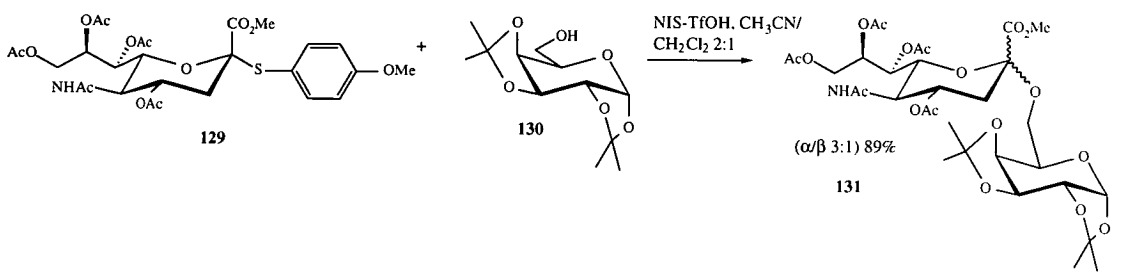


127 **128** 98%

Conversion of Latent Thioglycosyl Donor into the Active Thioglycosyl Donor.[90]

Scheme 2.27.

Roy and co-workers[90] coupled active thioglycosyl donors such as the 4-methoxyphenylthio-α-sialyl donor **129** to glucosyl acceptors such as the galactosyl acceptor **130** to give predominately the α-D-glucoside **131** in high yield (scheme 2.28.).[90]

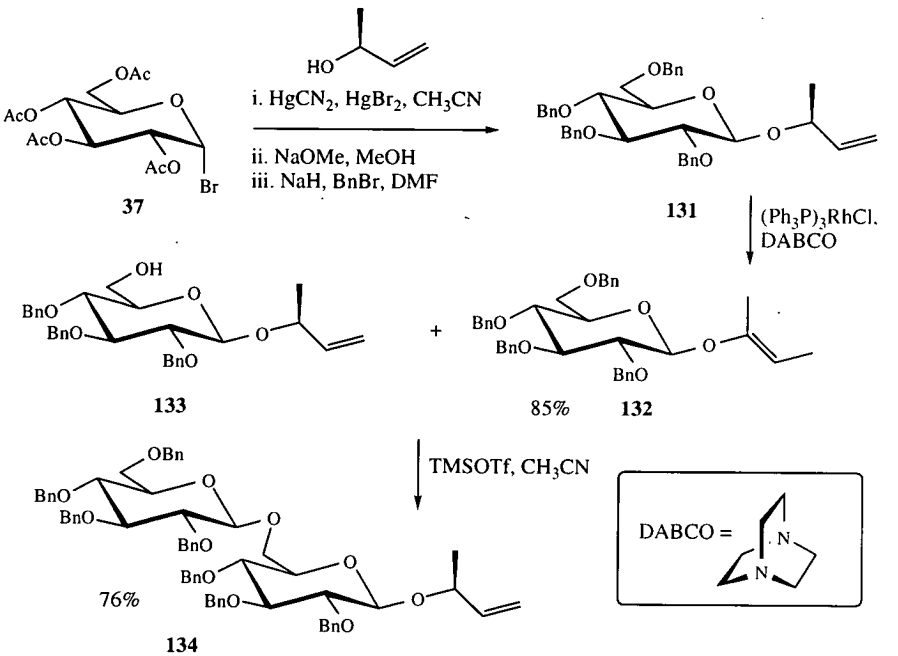


129 **130** (α/β 3:1) 89% **131**

Latent-Active Approach to Glycosidation Reactions.[90]

Scheme 2.28.

Boons and Isles[91] also examined latent-active glycosidations with 3-buten-2-yl glycosides such as the latent 3-buten-2-yl glycoside **131** which can be isomerised with Wilkinson catalyst (chlorotris(triphenylphosphine)rhodium, $RhCl(Ph_3P)_3$)[88] into the active 2-buten-2-yl glycoside **132** which will undergo glycosidation reactions with suitable glycosyl acceptors such as the latent tri-*O*-benzyl glucoside **133** (scheme 2.29.).[91]



Latent-Active Glycosidation of 3-Buten-2-yl glycosides.[91]

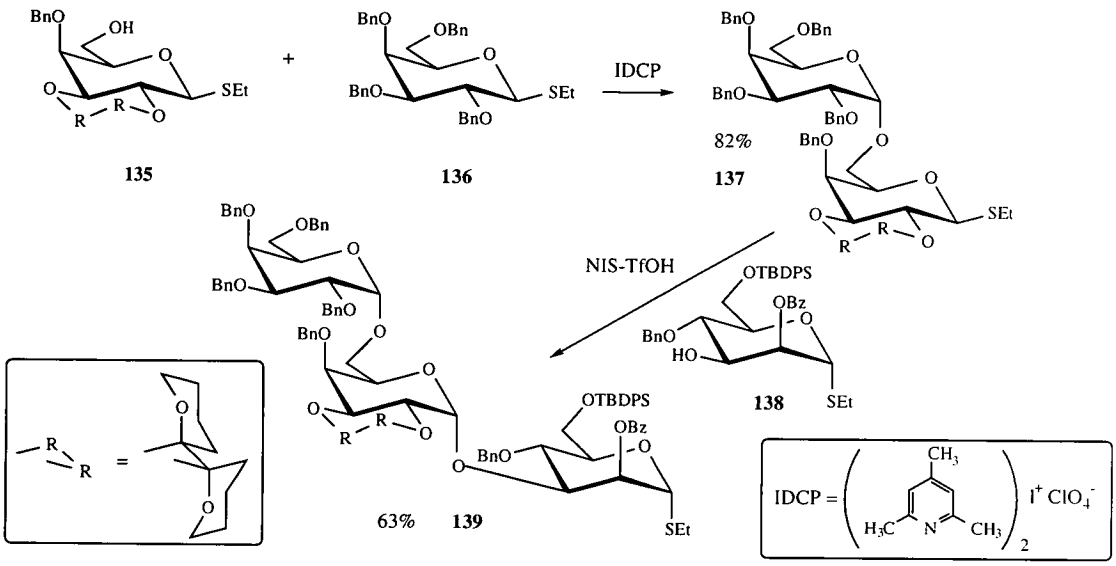
Scheme 2.29.

The latent 3-buten-2-yl glycosides are readily prepared from the corresponding bromide and are stable during many functional and protecting group manipulations, for example, deprotection under Zemplén conditions and benzylation. In the presence of TMSOTf the active 2-buten-2-yl glycoside **132** couples with the latent 3-buten-2-yl glycoside **133** and predominately forms the β-D-glucoside **134** in excellent yield providing a highly stereoselective alternative to the armed-disarmed glycosidation strategy.[91]

2.8. Differentially Activated Glycosyl Donors and Acceptors

Ley and co-workers[92] have recently reported a novel approach to the activation of the anomeric centre involving the use of dispiroketal (dispoke) protected glycosides. Dispoke protection, for example in the thio galactoside **135** (scheme 2.30.), increases the rigidity of the sugar ring and inhibits the formation of the flattened oxonium ion intermediate. The thio galactoside **135** may act as an

acceptor in the presence of mild activating conditions such as iodonium di-*sym*-collidine perchlorate (IDCP),[93] which activates the armed benzyl protected thio galactoside **136** to afford the α-D-galactoside **137** in good yield. Coupling of compound **137** and the disarmed thiomannoside **138** in the presence of strong activating conditions (NIS-TfOH) affords the trisaccharide **139** in good yield as almost exclusively the α-anomer (scheme 2.30.).[92]



Glycoside Synthesis With Differentially Activated Glycoside Donors And Acceptors.[92]

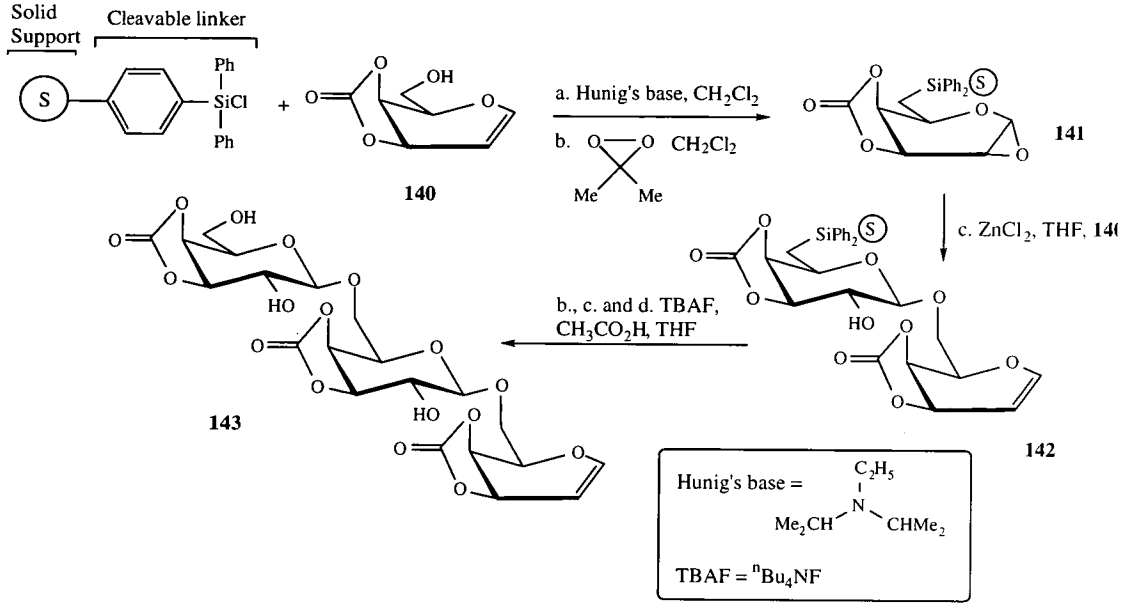
Scheme 2.30.

The use of differentially activated glycosyl donors and acceptors provides a useful preparation of complex oligosaccharides avoiding the numerous functional group manipulations that are usually required.

2.9. Glycosidations on Solid Supports

A relatively new but rapidly expanding area of glycoside synthesis can be found in solid-phase chemistry where oligosaccharides or glycopeptides can be synthesised rapidly without the numerous isolation steps required for solution based oligosaccharide synthesis. Danishefsky and co-workers[94] have examined the use of polymer bound glycals for oligosaccharide synthesis, which have the versatile feature of behaving as glycosyl donors or acceptors depending on the protecting group present (see the armed-disarmed concept discussed in section 2.3.). The solid support used is a copolymer of polystyrene cross-linked with 1% divinylbenzene, and functionalised with triphenylsilyl chloride, which requires a simple silylation using Hunig's base (ethyldiisopropylamine), a good proton acceptor,[95] to afford glycal **140**

which on epoxidation gives compound **141**. Treatment of the immobilised epoxide **141** with acceptor **140** and zinc chloride affords disaccharide **142** (scheme 2.31.).[94]



Solid-Phase Oligosaccharide Synthesis.[94]

Scheme 2.31.

The synthetic steps epoxidation and glycosidation in scheme 2.31. can be repeated a number of times to obtain the oligosaccharide of the desired size, for example, the trisaccharide **143** in scheme 2.31., which can be cleaved from the solid support with tetrabutylammoniumfluoride (TBAF) and acetic acid. The average yield of the coupling cycle (epoxidation and glycosidation) was 70% making solid-phase synthesis with glycals an attractive synthetic route for oligosaccharides and potentially a useful glycosidation procedure.[94]

Section Three; The Use of Enzymes in Glycosidation Reactions

Most of the chemical glycosidations discussed in section 2 rely on the use of protected glycosyl donors with a selectively protected acceptor which will couple in the presence of a highly reactive and often expensive catalyst or promoter. An attractive alternative to the chemical glycosidations currently used are enzyme catalysed glycosidations which enable the selective synthesis of glycosides under mild reaction conditions and without the need for protection.

3.1. General Features of Enzymes

Enzymes are biological catalysts which have three distinguishing characteristics;[96]

1. Enzymes increase the rate of a reaction
2. Enzyme activity can be regulated
3. Enzymes display substrate selectivity

All three characteristics are important when considering the use of enzymes in synthesis. The ability of enzymes to increase the rate of a reaction has been studied in great detail, the kinetics and the mechanisms of enzyme action are major fields in their own right and can provide a useful insight into how and why enzymes catalyse synthetically useful reactions.[96] The catalytic activity of enzymes can be influenced by a number of factors; the concentrations of substrates and products in the reaction mixture; the requirement for co-factors such as the reduced co-enzyme nicotinamide adenine dinucleotide (NADH); extreme heat, pH and mechanical damage which can denature enzymes as they are protein in nature. From a synthetic point of view some of the factors that regulate and so control enzyme activity can be problematic, for example, if accumulation of a product in the reaction mixture inhibits enzyme activity, or if expensive co-factors such as adenosine triphosphate (ATP) are required. However, some features that affect enzyme activity can be useful, for example, enzyme reactions are often reversible; forcing the reaction in one direction can afford the desired product in maximum yield with few side-products.[96]

Enzymes in their natural role display high selectivity for a specific substrate, however, when used for synthetic purposes, many enzymes have been found to exhibit broad substrate specificity enabling the synthesis of a large range of natural products and analogues.[48]

As the field of biocatalysis has grown, enzymes have become common tools for the synthetic chemist. From a synthetic point of view enzymes provide a useful alternative to traditional organic chemistry, performing chemically difficult reactions under very mild reaction conditions of pH, temperature, and pressure. Enzymes are also chiral catalysts and are able to produce optically active molecules that can be used as the building blocks or synthons for homochiral compounds.[48,97] An increasing number of enzymes are now commercially available and have also become more economical to use with the development of techniques such as enzyme immobilisation which increases enzyme stability and allows the catalysts to be recovered and reused. Enzymes that require co-factors are now cheaper to use as a result of the development of regenerating systems which allows catalytic amounts of co-factors such as ATP to be used in enzyme catalysed reactions.[98]

Enzymes are used in a wide range of reactions including C-C bond formation, oxidations, and glycosidations. The enzymes used in glycosidation reactions are from two classes; the glycosyltransferases and glycosidases.

3.2. Glycosidation Reactions With Glycosyltransferases

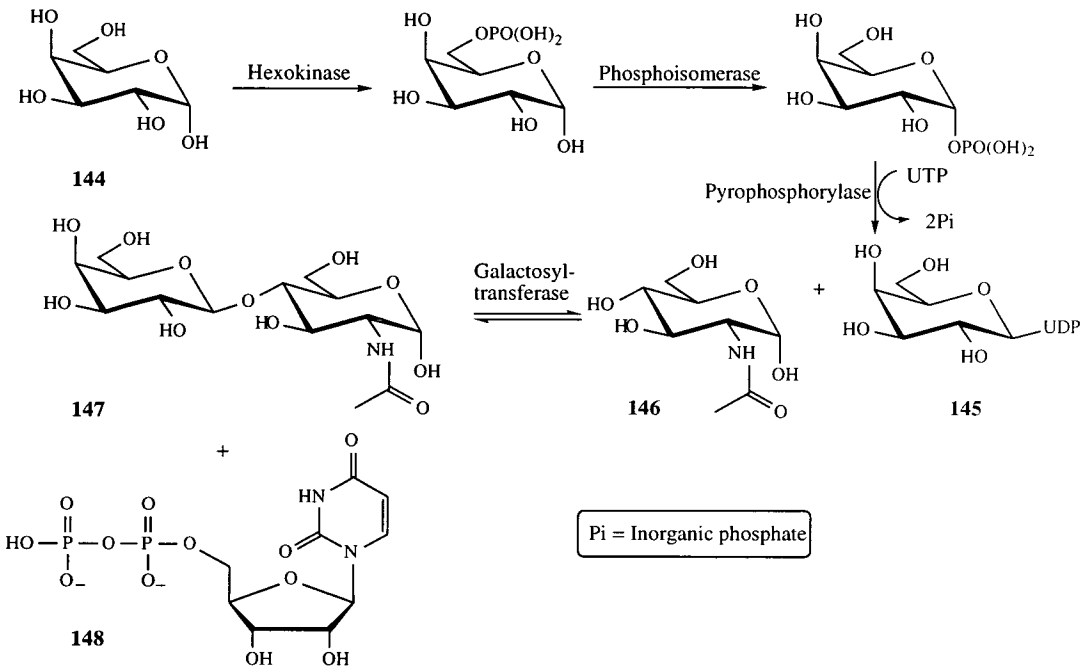
There are two classes of glycosyltransferase dependent on the type of glycosyl donor they accept, these are called Leloir-type glycosyltransferases and non-Leloir glycosyltransferases.[99] Leloir-type glycosyltransferases are the enzymes involved in the Leloir pathway, a biosynthetic route to sugar nucleotides discovered by Luis Leloir who examined the role of UDP-glucose in the biosynthesis of glycogen.[100] Non-Leloir glycosyltransferases are not involved in the Leloir pathway and include some phosphorylase enzymes such as trehalose phosphorylase and sucrose phosphorylase.[98]

3.2.1. Glycosidation Reactions With Leloir-Type Glycosyltransferases

The Leloir pathway[101] involves four stages with four different types of enzymes.[48] The first part of the Leloir pathway is the phosphorylation of sugars, this is catalysed by kinases such as hexokinase. Phosphoisomerases then remove a phosphate residue from the C6 hydroxyl group and attach a phosphate residue to the anomeric hydroxyl group. Pyrophosphorylases, which are also called nucleotide transferases, then attach nucleoside 5'-monophosphates (also called nucleotides) to the phosphorylated sugar producing a sugar nucleotide.[†] In the final step,

[†] Nucleotides are phosphate esters of glycosides of all heterocyclic bases, nucleosides are glycosides of heterocyclic bases and in particular purines and pyrimidines.[22]

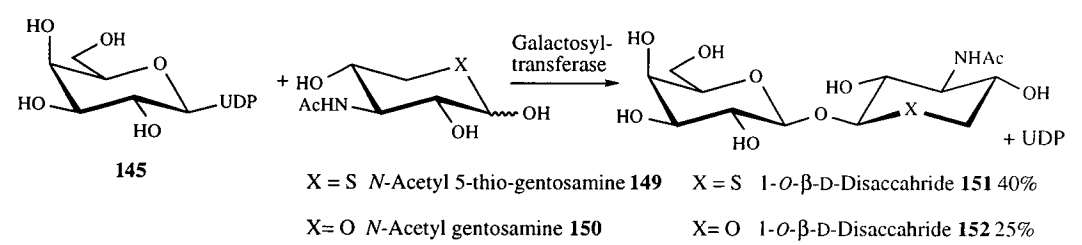
glycosyltransferases transfer a monosaccharide unit from a sugar nucleotide to a protein, lipid or non-reducing end of a growing oligosaccharide.[98] For example, the Leloir pathway enzymes convert D-galactose **144** into UDP-galactose **145** which is used by galactosyl transferase to transfer a galactose residue to *N*-acetylglucosamine **146** affording *N*-acetyllactosamine **147** and uridine diphosphate (UDP) **148** (scheme 3.1.).[22]



Synthesis of N-*Acetyllactosamine* ***147*** *Catalysed by Galactosyltransferase.*[22]

Scheme 3.1.

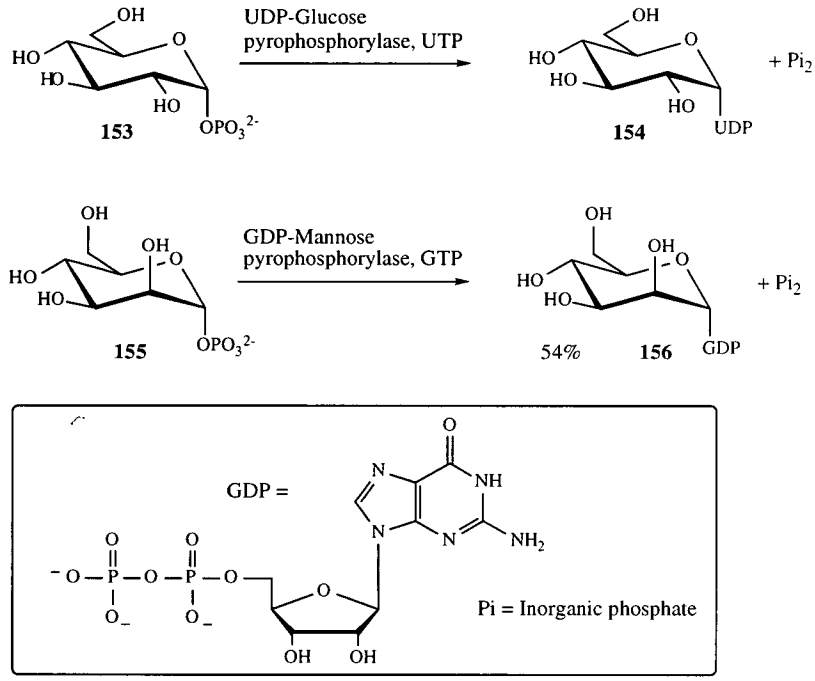
A number of compounds have been synthesised using Leloir-type glycosyltransferases, for example, the synthesis of disaccharides using galactosyl transferase from bovine milk has been reported by Nishida and co-workers[102,103] who used the enzyme to catalyse the transfer of galactose from UDP-galactose **145** to the anomeric centre of the thio-gentosamine **149** and the gentosamine **150** to give β-D-galactosides **151** and **152** respectively (scheme 3.2.).



Synthesis of Disaccharides Using Galactosyltransferase.[102,103]

Scheme 3.2.

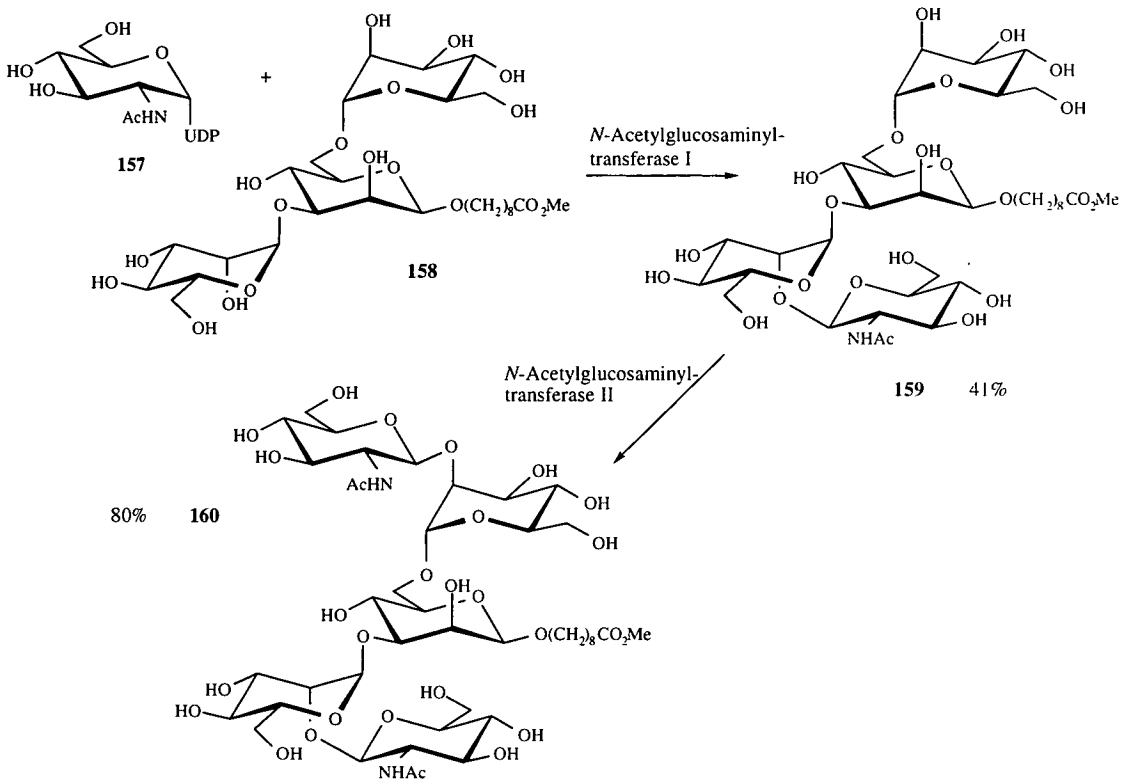
Leloir-type glycosyltransferases have also been used to synthesise nucleotide sugars, for example, UDP-glucose pyrophosphorylase and GDP-mannose pyrophosphorylase from brewers yeast were used by Simon and co-workers[104] to synthesise nucleoside phosphate sugars. The enzymes were used to synthesise UDP-glucose **154** and GDP-mannose **156** from α-D-glucose-1-phosphate **153** and α-D-mannose-1-phosphate **155** respectively (scheme 3.3.).[104]



Synthesis of Nucleotide Sugars with Glycosyltransferases.[104]

Scheme 3.3.

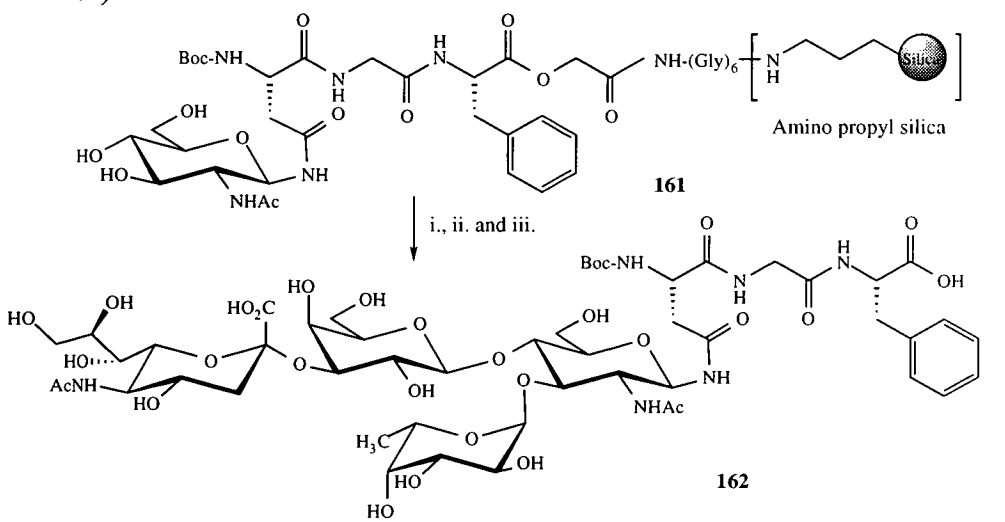
Many Leloir glycosyltransferase enzymes have been used in oligosaccharide synthesis, galactosyl and sialyltransferases are frequently used, others include fucosyltransferases, glucosyltransferases and *N*-acetylglucosaminyltransferases which Kaur and co-workers[105] used to synthesise the tetrasaccharide **159** and the pentasaccharide **160** from the trimannoside **158** by transferring *N*-acetylglycosyl residues from UDP-*N*-acetylglucosamine **157** (scheme 3.4.).[98]



The Use of Transferase Enzymes in Oligosacchride Synthesis.[105]

Scheme 3.4.

Recently the solid-phase synthesis of compounds such as oligosaccharides and glycopeptides has been achieved with Leloir glycosyltransferase enzymes (scheme 3.5.).



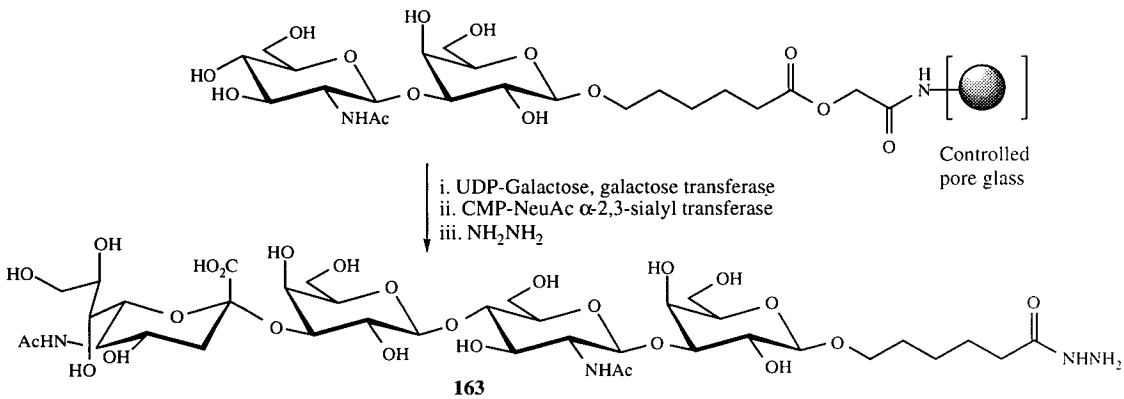
i. β-1,4-Galactosyltransferase, UDP-galactose, 0.1 M HEPES (pH 7.0), 10 mM $MnCl_2$, 55%
ii. α-2,3-Sialyltransferase, CMP-NeuAc, 0.1 M HEPES (pH 7.0), 5 mM $MnCl_2$, 65% iii. a. α-Chemotrypsin, H_2O (pH 7.0); b. ultrafiltration; c. α-1,3-fucosyltransferase, GDP-Fuc, 0.1 M HEPES (pH 7.0), 95%

Solid-Phase Synthesis of the Sialyl Lewis X Glycopeptide ***162***.[106]

Scheme 3.5.

The synthesis of the sialyl Lewis X glycopeptide **162** in scheme 3.5. has been examined by Schuster and co-workers[106] using a silica based solid support to which *N*-acetylglucosamine was linked through a peptide spacer **161** and monosaccharide units were added in a stepwise fashion.

The synthesis of an oligosaccharide, antigen **163** which is used against *Helicobacter pylori* was achieved by Halcomb and co-workers[107] using controlled pore glass as the solid support (scheme 3.6.).



Use of Transferase Enzymes in the Solid-Phase Synthesis of Oligosaccharides.[107]

Scheme 3.6.

3.2.2. Glycosidation Reactions With Non Leloir-Type Glycosyltransferases

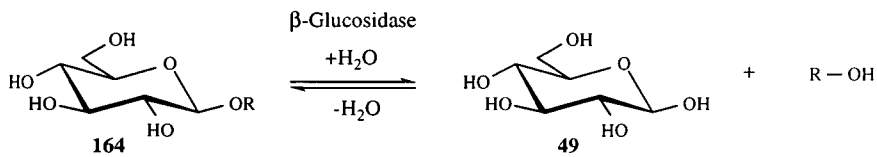
Non-Leloir glycosyltransferases include trehalose phosphorylase and sucrose phosphorylase which have been used to synthesise trehalose and sucrose respectively. Nucleoside phosphorylases are non-Leloir glycosyltransferases which cleave the glycosidic bond of nucleosides to give the corresponding base and sugar phosphate and are of particular interest for the synthesis of unnatural nucleosides.[98,99]

As a result of the high regio and stereoselectivity of glycosyltransferases, they are potentially a valuable tool in both solution and solid-phase synthesis of oligosaccharides.[99] Glycosyltransferases provide a useful alternative to the current complex, multistep chemical synthesis of oligosaccharides, which involves selective protection of the acceptor hydroxyl groups, and for each glycosidation step that follows exposure of the hydroxyl group is required for glycosidation, and a final deprotection step gives the desired product.[56] In addition to oligosaccharides glycosyltransferases have also been demonstrated to be highly useful in the synthesis of a wide variety of natural and un-natural products including small saccharides and nucleosides.

Unfortunately, many glycosyltransferases are unstable and difficult to isolate, (especially from mammalian sources as the proteins are found in low concentrations and are membrane bound) hence only a small number are available from commercial sources.[48,98] Little is also known about the specificity of many glycosyltransferases, however, as more enzymes become available, substrate specificity and their potential use in glycosidation reactions can be more thoroughly evaluated.[98]

3.3. Glycosidation Reactions With Glycosidases

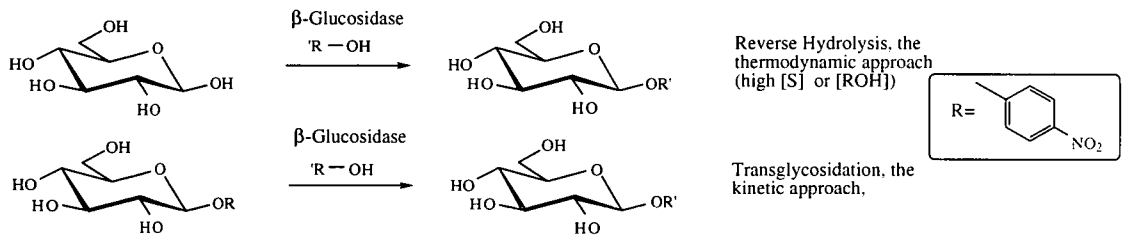
Glycosidases are hydrolases and in their natural role will hydrolyse glycoside bonds, for example, β-glucosidase will hydrolyse the β-glycosidic bond of the β-D-glucoside **164** to give D-glucose **49** and the alcohol R-OH (scheme 3.7.).[22]



The Reaction Catalysed by β-Glucosidase.[22]

Scheme 3.7.

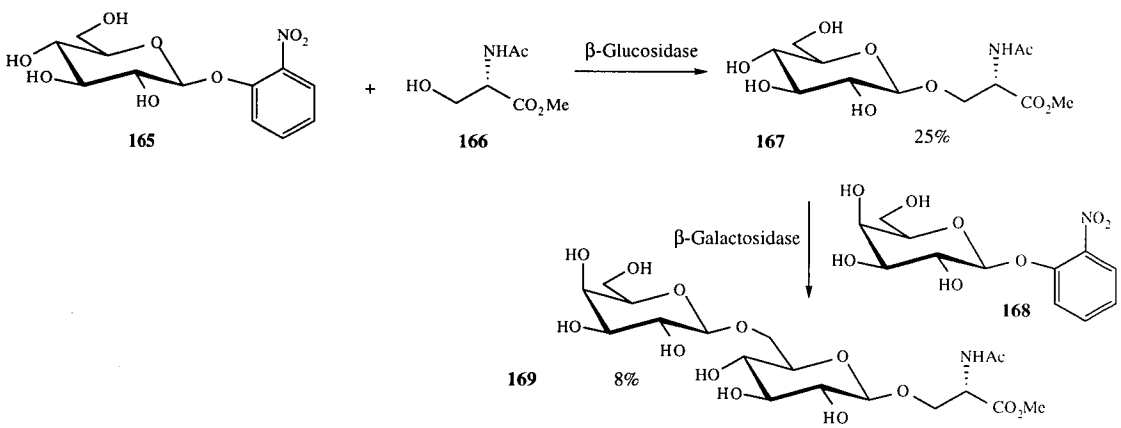
The reaction catalysed by β-glucosidases exists as an equilibrium and so changing the reaction conditions will push the equilibrium in favour of the reactants or products. Reverse hydrolysis occurs if the β-glucoside is removed from the reaction, an excess of the starting material is present or the reaction is performed with the alcohol acceptor as solvent; this is the thermodynamic approach to glycoside synthesis using glycosidases (scheme 3.8.). A transglycosidation reaction occurs if an efficient leaving group is present on the anomeric carbon of the β-glucoside glycosyl donor, whereby the enzyme catalyses the transfer of the glycosyl moiety to a suitable alcohol acceptor; this is the kinetic approach to glycoside synthesis using glycosidases.[108]



Synthesis of Glucosides by Reverse Hydrolysis and Transglycosidation Reactions.[108]

Scheme 3.8.

Transglycosidation reactions have been employed for the synthesis of a variety of compounds including simple glycosides and oligosaccharides.[108] The glycosyl donor requires a good leaving group at the anomeric centre, usually an aryl group such as *para*-nitrophenoxide[98] which can concentrate in the reaction medium as *para*-nitrophenol without poisoning the catalyst.[108] The transglycosidation product tends to accumulate rapidly during the reaction and can concentrate in the reaction medium without affecting the glycosidation reaction.[108] Glycosidases are substrate specific and so β-galactosidases and β-glucosidases are required for the synthesis of β-galactosides and β-glucosides respectively. These enzymes are readily available and have been used to attach glucose and galactose residues to a variety of alcohol acceptors. The synthesis of glycopeptide analogues and disaccharides was recently reported by Baker and co-workers[44] using β-galactosidases and β-glucosidases (scheme 3.9.).



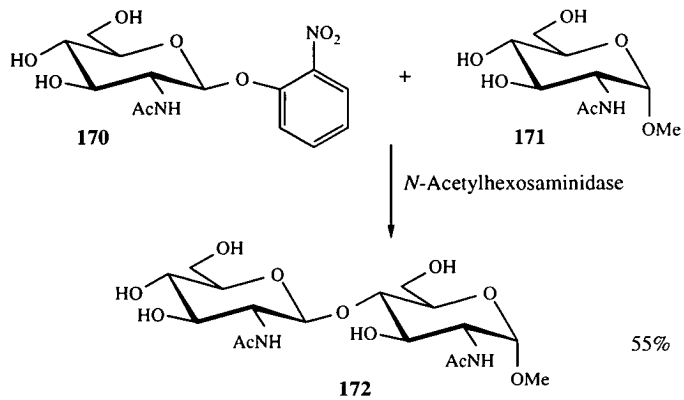
Transglycosidation Reactions With β-Galactosidase and β-Glucosidase.[44]

Scheme 3.9.

The transglycosidation reaction catalysed by β-glucosidase with the amino acid derivative *N*-acetylamine ester **166** as the alcohol acceptor and *O*-nitrophenyl-β-D-glucose **165** as the donor afforded the glycoside **167** and suggests the potential application of transglycosidation reactions to the synthesis of glycopeptides.[44,45] The second transglycosidation reaction catalysed by β-galactosidase between glycoside **167** and *O*-nitrophenyl-β-D-galactose **168** to afford the disaccharide **169** demonstrates the usefulness of glycosidases for the synthesis of small oligosaccharides.

The enzyme *N*-acetylhexosaminidase from *Aspergillus oryzae* has recently been shown by Sing and co-workers[109] to be a useful catalyst for transglycosidation reactions involving the synthesis of disaccharides. The enzyme *N*-acetylhexosaminidase was used in a transglycosidation reaction between *N*-

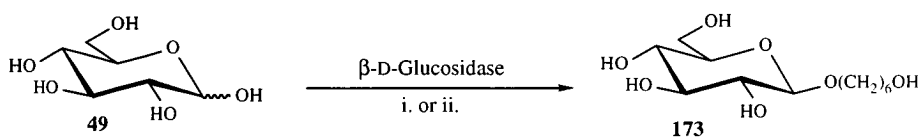
acetylamine nitrophenyl-β-D-glucose **170** and 1-*O*-methyl *N*-acetyl-α-D-glucosamide **171** to afford the disaccharide **172** in a good yield (scheme 3.10.).[109]



Transglycosidation Reaction Catalysed by N-*Acetylhexosaminidase.*[109]

Scheme 3.10.

The activated glycosyl donor required for transglycosidation reactions can be expensive and side products formed during the transglycosidation are often difficult to separate from the intended product.[110] The use of reverse hydrolysis has recently been considered by Vic and co-workers[46,108,110,111] as a more cost effective alternative to transglycosidation reactions. Reverse hydrolysis catalysed by β-D-glucosidase was used to transfer glucose residues onto simple alcohol acceptors in a reaction medium that contained *tert*-butanol as a co-solvent to increase the solubility of the alcohol acceptor (scheme 3.11.).[111]



i. $HO(CH_2)_6OH$, *tert*-$BuOH/H_2O$ 90:10 (v/v) 22%

ii. $HO(CH_2)_6OH/H_2O$ 90:10 (v/v) 61%

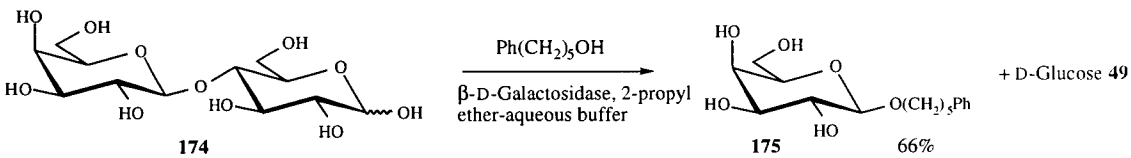
Glycosidation Reactions Using Reverse Hydrolysis.[111]

Scheme 3.11.

Water is a necessary requirement for enzyme activity to be maintained although many enzymes can tolerate high concentrations of organic solvent.[111] Vic and co-workers[111] found that in 80-90% *tert*-butanol, β-D-glucosidase from almonds was stable and afforded glucoside **173** in 22% yield. However by using the alcohol as the solvent 61% yield of **173** was achieved (scheme 3.12.).[111]

The yields of reverse hydrolysis reactions are often low due to the competing hydrolysis reaction. To avoid this problem Mori and co-workers[112] have used a two

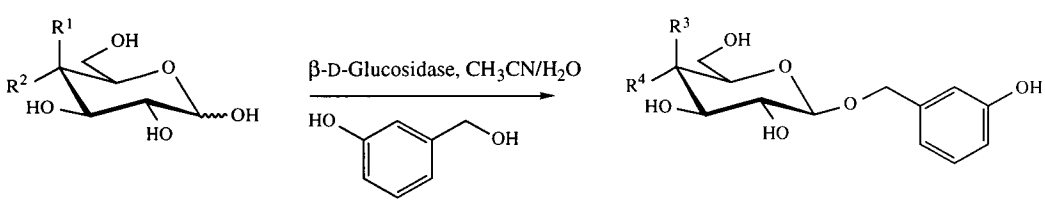
phase aqueous-organic reaction system with lipid coated β-galactosidase. This allows the enzyme to exist in organic solvents, which would normally cause denaturation of the native enzyme. The β-D-galactopyranoside **175** was prepared in 66% yield by reacting lactose **174** and 5-phenyl-1-pentanol in the presence of the lipid coated β-galactosidase using 2-propylether and an aqueous buffer as the biphasic solvent system. The reaction product **175** remains in the organic layer, therefore preventing its hydrolysis by the galactosidase (scheme 3.12.).[112]



Reverse Hydrolysis with Lipid Coated β-Galactosidase.[112]

Scheme 3.12.

An interesting feature of the use of β-glucosidase in reverse hydrolysis reactions is the broad substrate specificity that this enzyme has been found to exhibit. For example, when 2-hydroxybenzyl alcohol is used as the acceptor β-glucosidase catalyses the coupling of both D-glucose **49** and D-galactose **144** to 2-hydroxybenzyl alcohol affording 2-hydroxybenzyl-β-D-galactopyranoside **176** and 2-hydroxybenzyl-β-D-glucopyranoside **177,** respectively, both in 9% yield (scheme 3.13).[108]



R^1	R^2	Glycosyl donor
H	OH	D-Glucose
OH	H	D-Galactose

R^3	R^4	Reverse hydrolysis product (yield)
H	OH	2-Hydroxybenzyl β-D-glucopyranoside **176** (9%)
OH	H	2-Hydroxybenzyl β-D-galactopyranoside **177** (9%)

Example of the Broad Specificity of β-Glucosidase in Reverse Hydrolysis Glycosidations.[108]

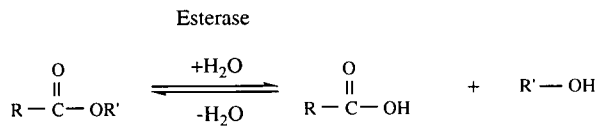
Scheme 3.13.

Other enzymes, for example, lipases and proteases are useful for the synthesis of chiral starting materials or to catalyse specific functional group transformations (enzyme catalysed acylation and deacylation reactions are discussed in section four) further demonstrating the extensive applications of enzyme catalysed reactions in carbohydrate chemistry.[99]

Section Four; Enzyme Catalysed Acylation and Deacylation Reactions

Glycosidation reactions are only one of a wide range of chemical reactions that enzymes can catalyse. Since the mid 1980's there has been an enormous amount of research into the use of enzymes and whole cells in organic synthesis; biocatalysis is now a well established area within this field. Enzyme catalysed acylation and deacylation reactions were used in the synthetic routes examined towards M6G **23**, and provided a useful alternative to the chemical manipulations currently available. In general, regioselective acylations and deacylations under acid or base catalysed conditions can only be achieved with the careful choice of protecting group and by using reaction conditions which exploit the different labilities and cleavage conditions required for protecting groups. The use of enzymes provides the opportunity to achieve regioselective acylations and deacylations under mild conditions and has become a common feature in the synthesis and manipulation of carbohydrates; several reviews on the use of enzymes for selective acylation and deacylation reactions in carbohydrate chemistry have been published by Drueckhammer *et al*[48] and Bashir and co-workers.[113]

The use of enzymes in synthesis exploits their natural catalytic abilities, esterases in particular are attractive for synthetic work being readily available, inexpensive, and stable.[96] Esterases are a class of hydrolase enzymes which catalyse the hydrolysis of ester bonds to give the alcohol and carboxylic acid (scheme 4.1.).[22]



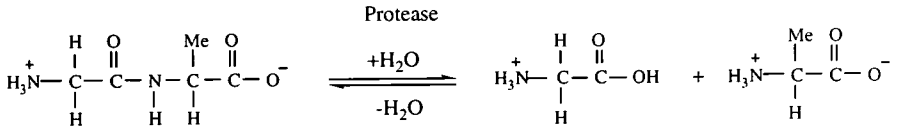
The Hydrolysis of Esters Catalysed by Esterases.

Scheme 4.1.

The hydrolysis reaction is reversible and so the removal of water or the presence of excess alcohol will force the equilibrium in favour of the acylation reaction (reverse hydrolysis), esterases can therefore catalyse the acylation of alcohols and the deacylation of esters. A frequently used and versatile class of esterase are lipases which are inexpensive, have high stability, and are used in a variety of acylation and deacylation reactions.[50,113] The natural substrates of lipases are glycolipids, whereby the enzyme hydrolyses ester bonds to release the component fatty acids.[22] However, lipases are found to exhibit a lack of substrate

specificity and will accept a variety of compounds making these enzymes particularly useful for synthetic work.[96]

Proteolytic enzymes or proteases are another class of enzymes that can participate in acylation and deacylation reactions. Proteases catalyse the hydrolysis of peptide bonds exposing the carboxylic acid group of one peptide and the amine group of another (scheme 4.2.).[22]



The Hydrolysis of Peptides Catalysed by Proteases.[22]

Scheme 4.2.

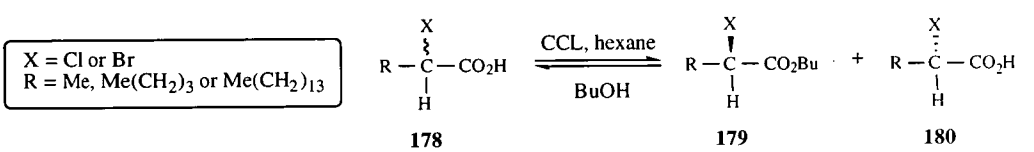
The hydrolysis reaction catalysed by proteases is reversible and an amine group can be esterified if water is removed or excess acid is present.

4.1. Enzyme Catalysed Acylation Reactions

There are two potential routes towards the synthesis of esters with esterases and proteases; direct esterification and transesterification.[114]

4.1.1. Enzyme Catalysed Direct Esterifications

The direct esterification of compounds with esterases and proteases offers some practical difficulties; the presence of excess acid is required, which lowers the pH of the reaction medium and may inactivate the enzyme, and the ester produced in the reaction may be susceptible to hydrolysis. These problems can be overcome by conducting the reaction in organic media, for example, *Candida cylindracea* lipase (CCL) will catalyse the esterification of R-acid **178** with butanol in hexane (scheme 4.3.).[114]



178 **179** **180**

CCL Catalysed Direct Esterification of Acids.[114]

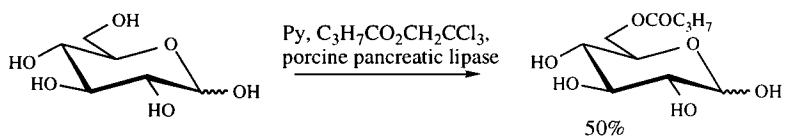
Scheme 4.3.

The use of excess alcohol forces the reaction towards esterification with the R-acid **178** providing the substrate to give the R-ester **179**. The S-acid **180** is not used as a substrate by the enzyme and remains in the reaction medium. Unfortunately, with direct esterification reactions the product will be hydrolysed by

the enzyme back to the acid and the alcohol which explains the low yields observed for these reactions.[114]

4.1.2. Enzyme Catalysed Transesterifications

Transesterifications provide a useful alternative to direct esterifications and involve the use of high concentration of the ester (the acyl donor) which is transferred to a free hydroxyl group on the acyl acceptor.[48] The use of activated esters such as trihaloethyl esters[115] and anhydrides[116] as acyl donors facilitates the reaction and makes it kinetically irreversible.[114] Lipases in particular are very useful in transesterification reactions demonstrating broad substrate specificity, regioselectivity and enantioselectivity. The regioselectivity of lipases has been demonstrated by Therisod and Klibanov[115] who found that D-glucose **49** can be acylated selectively at C6 using 2,2,2-trichloroethyl butyrate in a reaction catalysed by porcine pancreatic lipase to afford the 6-*O*-butyrylglucose **181** in 50% yield (scheme 4.4.).[115]

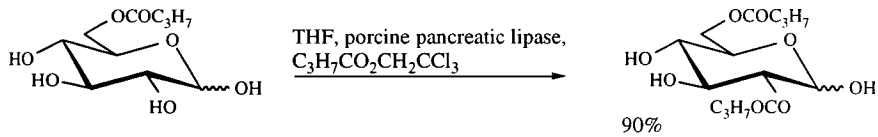


49 **181**

*Acylation of D-Glucose **49** Catalysed by Porcine Pancreatic Lipase.*[115]

Scheme 4.4.

Transesterifications catalysed by lipases will generally occur at the primary hydroxyl group as it is the least sterically hindered, however, if the primary hydroxyl group is protected selective acylation at the secondary hydroxyl groups C2 and C3 is possible.[48] This has been demonstrated by Therisod and Klibanov[117] who found that lipases in particular could discriminate between the four remaining hydroxyl groups of a C6 protected sugar (scheme 4.5.).[117]



181 **182**

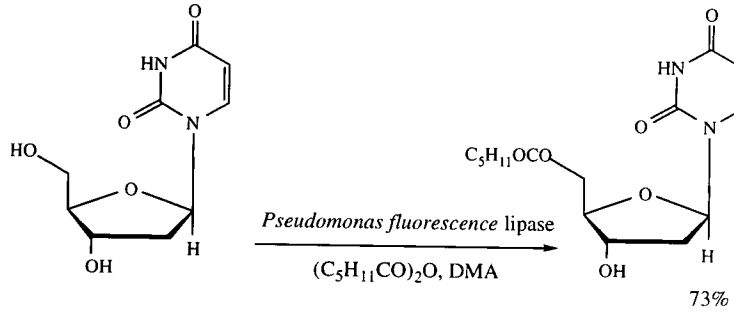
The Selectivity of Porcine Pancreatic Lipase with C6 Protected Glucose.[117]

Scheme 4.5.

When 6-*O*-butyrylglucose **181** was treated with porcine pancreatic lipase the enzyme displayed a strong preference for the C2 position resulting in the formation

of 2,6-di-*O*-butyrylglucose **182** (scheme 4.4.). Of the other enzymes examined, *Aspergillus niger* lipase was found to be selective for the C3 position and catalysed the acylation of **181** to give 3,6-di-*O*-butyrylglucose.[117]

The regioselectivity of lipase catalysed transesterifications has been particularly useful for the selective acylation of compounds with multiple hydroxyl groups. For example, the acylation of nucleosides was recently reported by Uemura and co-workers[116] who used *Pseudomonas fluorescence* lipase to acylate 2'-deoxyuridine **183** with hexanoic anhydride in dimethylacetamide (DMA) to give 2'-deoxy-5-*O*-hexanoyluridine **184** in 73% yield (scheme 4.6.).

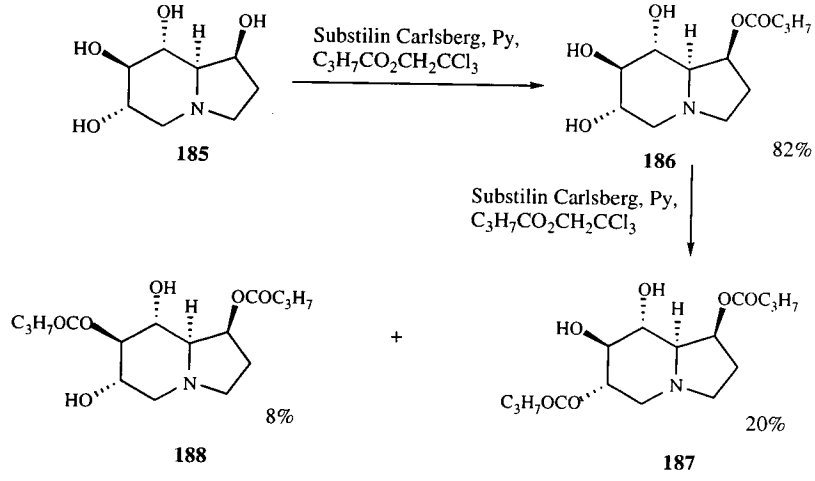


183 **184**

The Selective Acylation of Nucleosides with Lipases.[116]

Scheme 4.6.

Proteases also have broad substrate specificity, regioselectivity, and enantioselectivity; this has been demonstrated by Margolin and co-workers[118] who recently reported the use of the protease Subtilisin Carlsberg to acylate the plant alkaloid castanospermine **185**, a potential anti AIDS drug (scheme 4.7.).



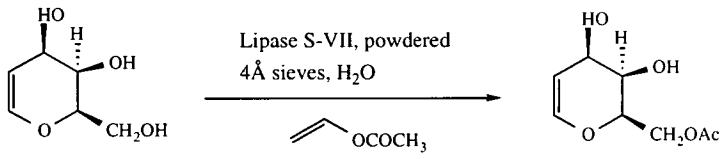
185 **186** **188** **187**

Regioselective Acylation of Castanospermine with Substilin Carlsberg.[118]

Scheme 4.7.

When castanospermine **185** is treated with 2,2,2-trichloroethyl butyrate and Subtilisin Carlsberg, the C1 hydroxyl group is selectively acylated to give 1-*O*-butyrylcastanospermine **186** in 82% yield (scheme 4.7.). Subtilisin Carlsberg was also found to display selectivity and when the C1 position was protected would acylate primarily at C6 to give 1,6-*O*-butyrylcastanospermine **187** in 20% yield and the 1,7-*O*-butyrylcastanospermine **188** in 8% yield. The enzyme accepted a variety of acyl donors as substrates and no multiple acylations of castanospermine **185** were observed. The broad substrate specificity and enantioselectivity of Subtilisin Carlsberg make this enzyme a potentially useful tool in synthetic organic chemistry.

One of the main problems with esterase catalysed transesterifications is a tendency for the enzyme to hydrolyse the newly formed ester bond which can give low yields. A recent development in transesterifications has been the use of unstable alcohols as acyl donors, for example, enol esters which allow the reversible acylation reaction to become irreversible. When enol esters are used the enol released during the transesterification spontaneously toutomerises into an aldehyde or ketone causing the reaction to become irreversible.[119] For example, the enol ester vinyl acetate was used by Holla[120] as the acyl donor for the irreversible acylation of glycals (scheme 4.8.).



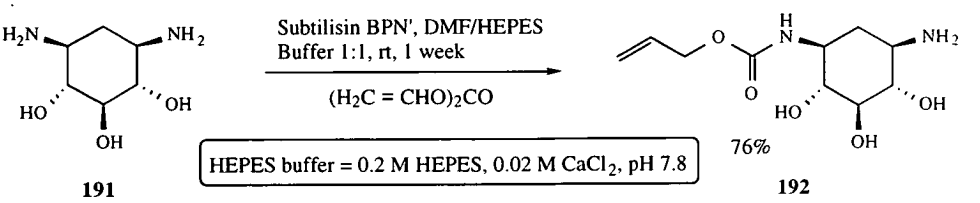
189 **190**

Lipase Catalysed Acylation of Glycals.[120]

Scheme 4.8.

A number of lipases were examined by Holla[120] and lipase S-VII in particular was found to catalyse the acylation of D-galactal **189** at the primary hydroxyl group to give 6-*O*-acetyl-D-galactal **190** in excellent yield (scheme 4.8.). Glycals are useful synthons in carbohydrate chemistry and enzyme catalysed acylations of these compounds are highly useful reactions.[120]

Esterases and proteases are usually used to acylate free hydroxyl groups and will accept a variety of substrates. Recently it has been shown by Orsat and co-workers[119] that these enzymes are also capable of protecting amines when vinyl carbonates are used as the donor (scheme 4.9.).[119]



191 **192**

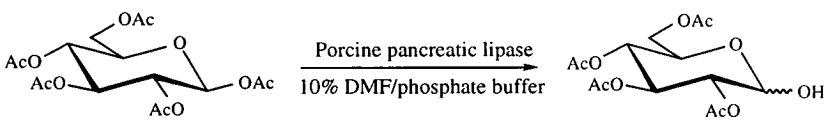
The Protection of Amines as Carbamates Catalysed by the Protease Subtilisin BPN'.[119]

Scheme 4.9.

The protection of 2-deoxystreptamine **191** with diallyl carbonate was catalysed by subtilisin BPN' to afford the carbamate **192** in good yield and high enantioselectivity (99% ee.) (scheme 4.8.).[119] The reactions showed high chemo- and enantioselectivity and the low cost of homocarbonates makes the protection of amines as carbamates an attractive proposition.

4.2. Enzyme Catalysed Deacylation Reactions

The hydrolysis reaction catalysed by lipases and esterases provides an effective means for the selective cleavage of one or more ester groups in polyacylated compounds. The selective deacylation of fully acylated sugars has been well studied and lipases in particular have provided a useful alternative to the tedious chemical steps required to expose a desired hydroxyl group. In a fully acylated sugar the anomeric hydroxyl group is generally the most easily cleaved,[48] this has been demonstrated by Hennen and co-workers[50] who have found that porcine pancreatic lipase and *Aspergillus niger* lipase are the most effective enzymes for this reaction. For example, porcine pancreatic lipase in 10% dimethylformamide (DMF) cleaved the C1 acetate of β-D-glucose pentaacetate **193** to afford glucose tetraacetate **194** in good yield (scheme 4.10.).[50]



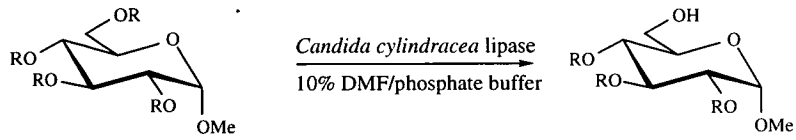
193 **194**

Lipase Catalysed Selective Deacylation at C1 of a Polyacylated Glycosides.[50]

Scheme 4.10.

Hennen and co-workers[50] also found that in acetylated methyl glycosides, the primary hydroxyl group was selectively cleaved; the lipases catalysing this reaction were found to favour ester groups with longer chain lengths. For example, *Candida cylindracea* lipase catalysed the deacylation of the C6 ester in 1-*O*-methyl α-D-

glucose tetraoctanoate **196** and 1-*O*-methyl α-D-glucose tetrapentanoate **195** in good yields (scheme 4.11.).[50]



R	Deacylation product	% Yield
C_5H_9	**195**	75
C_8H_{15}	**196**	78

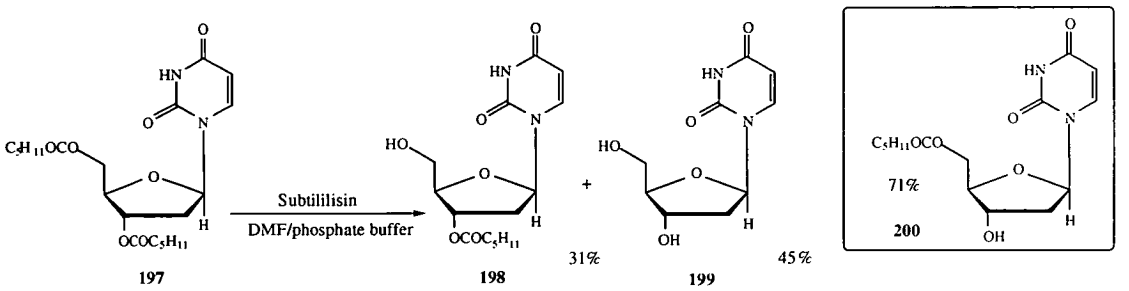
Lipase Catalysed Deacylation at C6 of Acyl Protected Glycosides.[50]

Scheme 4.11.

Hennen and co-workers[50] observed that the use of organic solvents in lipase catalysed deacylation reactions enhanced enzyme selectivity and improved the solubility of hydrophobic compounds.[48] In general only a thin layer of water is required to retain a catalytically active conformation[121] and lipases have been found to operate best at water-organic interfaces.[96] The solvent systems generally used for lipase catalysed reactions are monophasic organic solvents, however, the use of co-solvents, such as *t*-butanol, acetone,[122] and DMF,[50] in a biphasic (water-organic) solvent system have been found to enhance the yields, selectivity, and improve the solubility of hydrophobic substrates.[48,50]

Although lipases exhibit a good degree of tolerance to organic media, denaturation is still a problem and some recent publications have approached this difficulty in various ways, for example, by using lipid coated lipases; reported by Okahata and co-workers,[123] dialysis membrane enclosed lipases; reported by Bednarski and co-workers,[124] and lipases immobilised on anion exchange resin by Ballesteros and co-workers.[49] Immobilisation of enzymes onto an insoluble support (e.g. beads or resins) allows easier separation of the enzymes from the reaction products and also offers the opportunity to recycle the enzyme which is attractive for large scale reactions.[124]

Esterases have also proved useful in selective deacylation reactions of polyacylated compounds. For example, Uemura and co-workers[125] have found that the protease substilisin from *Bacillus subtilis* cleaves the primary and secondary acyl group of pyrimidine nucleosides such as 3',5'-di-*O*-hexanoyluridine **197** to give the 2'-deoxy-3'-*O*-hexanoyluridine **198** in 31% yield and 2'-deoxyuridine **199** in 45% yield (scheme 4.12.).[125]



Subtilisin Catalysed Deacylation of Pyrimidine Nucleosides.[125]

Scheme 4.12.

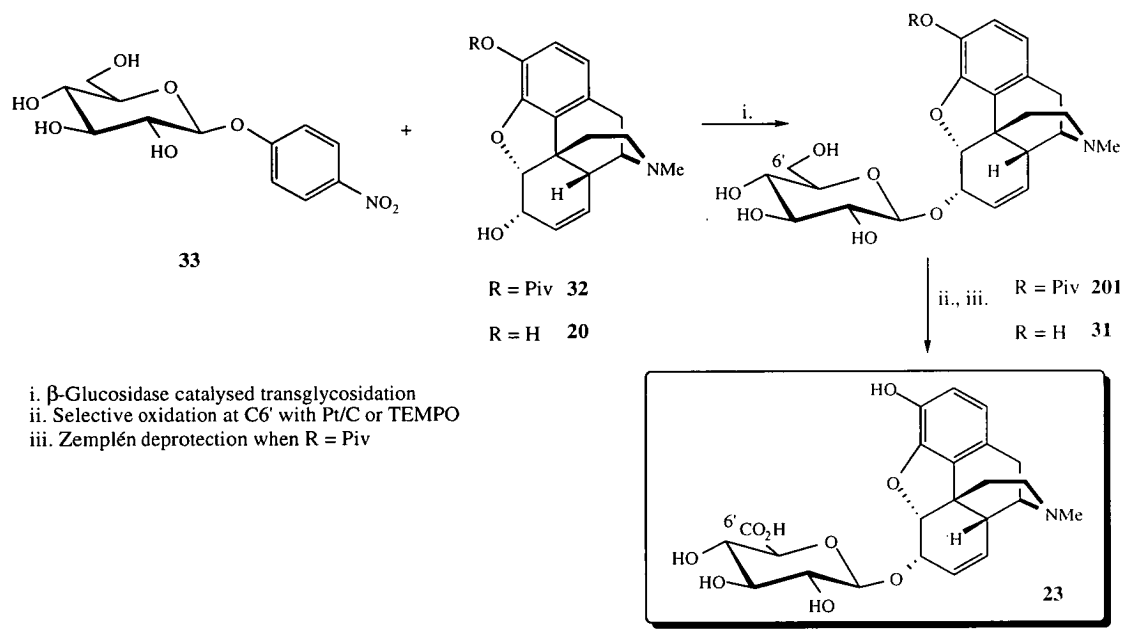
The proteases substilisin in common with lipases is able to tolerate dry organic solvents[126] and the use of co-solvents such as DMF enhances selectivity and substrate solubility. Unfortunately in the conditions used by Uemura and co-workers[125] substilisin could not distinguish between the primary and secondary acyl group and better selectivity was achieved with *Pseudomonas fluorescens* lipase which cleaved the C3 acyl group affording the 2'-deoxy-5'-*O*-hexanoyluridine **200** in a yield of 71% and 2'-deoxyuridine **199** as the minor product in a yield of 23%.[125]

Chapter Two; Results and Discussion

Section One; Chemo-Enzymatic Approaches to M6G

1. Synthesis of M6G; Synthetic Route One

Synthetic route 1 incorporates the use of β-glucosidase to catalyse a transglycosidation reaction between a glycosyl donor **33** which has a good leaving group, and a morphine derivative (the alcohol acceptor **32** or **20**). Thereafter, selective oxidation of the C6' hydroxyl group of the resulting 3-*O*-pivaloylmorphine glucoside **201** or morphine-6-glucoside **31**, followed by removal of the protecting groups would afford M6G **23** (scheme 1.1.).



Proposed Synthesis of M6G 23; Synthetic Route One.

Scheme 1.1.

The availability of some starting materials, particularly the morphine derivatives, was limited and model studies were used to establish suitable conditions for each step of the synthetic routes examined. Some of the model studies gave interesting results and are discussed with the reactions involving the morphine compounds.

The first step of route one is a transglycosidation catalysed by β-glucosidase from almonds. The enzyme β-glucosidase is a hydrolase which can be used for glycosidation reactions under certain conditions, catalysing either reverse hydrolysis or a transglycosidation reaction.[108] A transglycosidation reaction was used as the

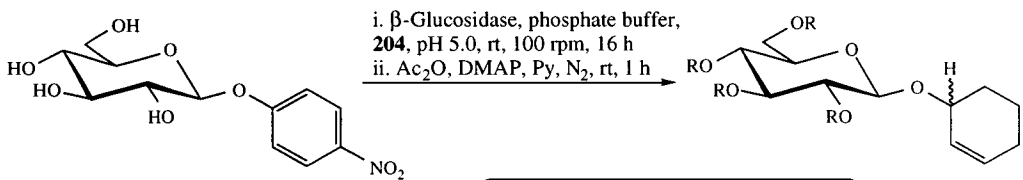
yields are generally higher than those of reverse hydrolysis, and β-glucosidase from almonds had already been shown to catalyse transglycosidation reactions with a number of alcohol acceptors.[44,45]

1.1. β-Glucosidase Catalysed Transglycosidation Reactions

To establish suitable conditions for a transglycosidation reaction with the morphine derivatives available, namely 3-*O*-pivaloylmorphine **32** (for synthesis see appendix A.1.) and morphine sulphate **20**, transglycosidations with simple alcohol acceptors were initially examined in a model study.

1.1.1. Model Study

The acceptor 2-cyclohexen-1-ol **204** provides a good model for the morphine **1** structure since it contains an allylic alcohol. A transglycosidation catalysed by β-glucosidase from almonds with excess 2-cyclohexen-1-ol **204** and *p*-nitrophenyl-β-D-glucose **33** as the glycosyl donor afforded cyclohex-2-enyl β-D-glucoside **202** in 7% crude yield (scheme 1.2.).



33

R	Compound	% Yield
H	**202**	7
Ac	**203**	1

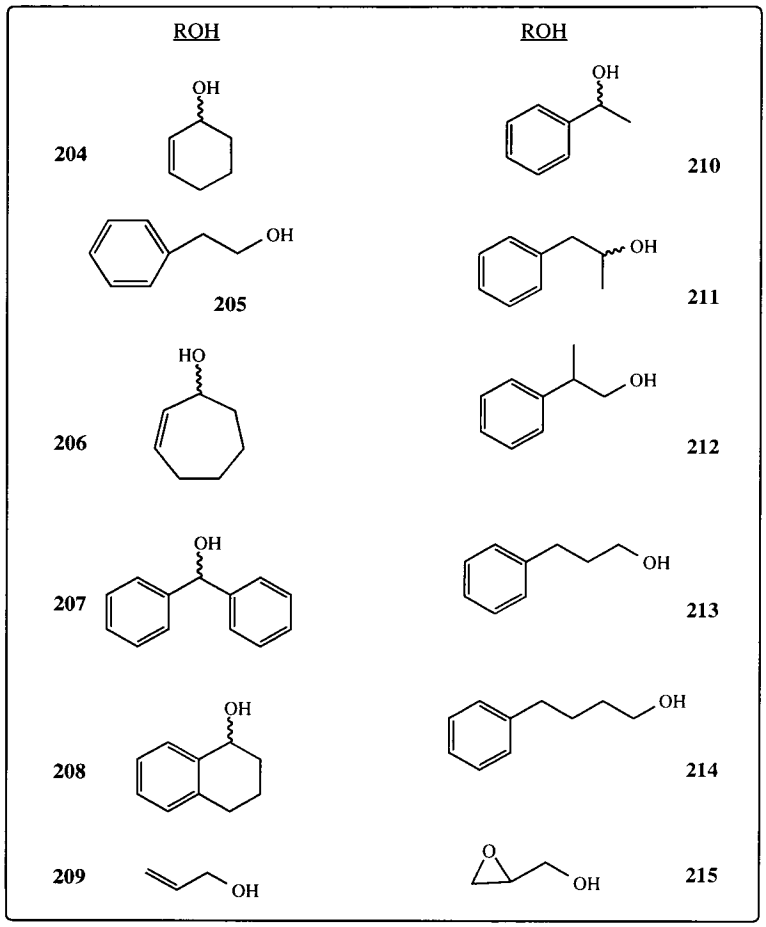
*Synthesis of the Cyclohexenyl Tetraacetyl-β-D-glucoside **203*** via *a β-glucosidase Catalysed Transglycosidation.*

Scheme 1.2.

After isolation and purification, the transglycosidation product was acetylated to afford the cyclohexenyl tetraacetyl-β-D-glucoside **203** in 1% overall yield. The ^{1}H NMR spectrum of the acetylated transglycosidation product **203** revealed two anomeric protons as expected for a mixture of two diastereoisomers, however, the relative heights of the two peaks were in a ratio of 3:2. The β-glucosidase was, therefore, displaying stereoselectivity, and this observation stimulated interest in examining related alcohols as potential acceptors for this β-glucosidase.

A number of allylic alcohols were either purchased or synthesised and used as acceptors in a series of β-glucosidase catalysed transglycosidations (figure 1.1.).

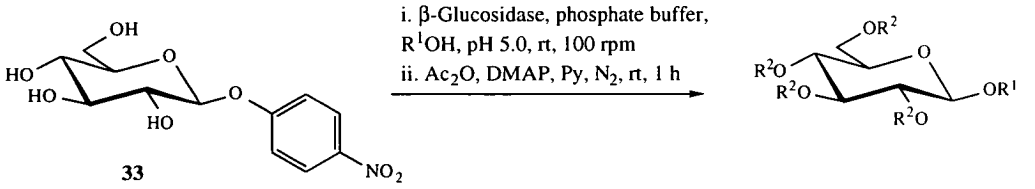
The alcohol 2-cycloheptan-1-ol **206** was synthesised in 80% yield using a procedure reported by Gemal and Luche[127] which involved the reduction of 2-cycloheptanone with sodium borohydride.

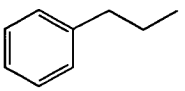
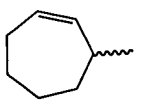


Alcohol Acceptors for β-Glucosidase from Almonds Catalysed Glycosidations.

Figure 1.1.

Among the transglycosidation reactions attempted, the most successful alcohol acceptors were phenethyl alcohol **205** and 2-cycloheptan-1-ol **206**, where the transglycosidation products **216** and **218** were afforded in crude yields of 15% and 2% respectively. The compounds were isolated as the acylated glycosides, phenethyl tetraacetyl-β-D-glucoside **217** and cycloheptenyl tetraacetyl-β-D-glucoside **219** in 13% and 1% yield respectively (scheme 1.3.).



R^1	R^2	Compound	% Yield
	H, Ac	**216, 217**	15, 13
	H, Ac	**218, 219**	2, 1

*Synthesis of the Phenethyl Tetraacetyl-β-D-glucoside **217** and the Cycloheptenyl Tetraacetyl-β-D-glucoside **219**.*

Scheme 1.3.

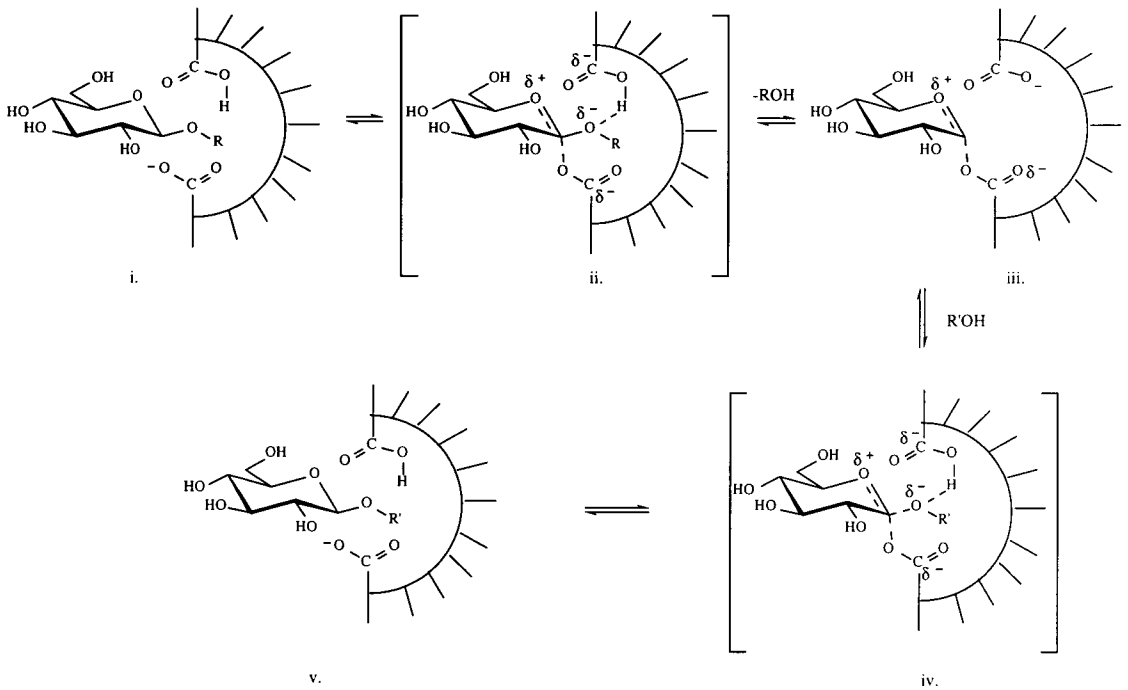
Comparison of the relative peak heights for the anomeric protons in the ^{1}H NMR spectrum of the cycloheptenyl tetraacetyl-β-D-glucoside **219** revealed a 3:2 ratio of diastereoisomers. This was also observed with the synthesis of the cyclohexenyl tetraacetyl-β-D-glucoside **203** confirming the enzyme has a preference for either the R or S isomer of cyclic allylic alcohols.

The success of alcohol acceptors as substrates for β-glucosidase depends on the ability of the alcohol to enter the active site of the enzyme and associate with the glycosyl donor. However, the exact mechanism of the hydrolysis reaction catalysed by β-glucosidases from almonds is still not fully understood.[128]

The glucosidases obtained from plants are often present in multimolecular forms, β-glucosidase from almonds is a mixture of at least two isoenzymic forms which have different kinetic properties and so mechanistic studies of this plant enzyme are difficult, and there is only a limited amount of basic structural and mechanistic information available.[128]

Other β-glucosidases isolated from bacteria and fungi have been studied more thoroughly and mechanisms of reverse hydrolysis and transglycosidation reactions have been proposed.[48,129] The reactions are thought to occur with initial loss of the anomeric group (as water or alcohol respectively) from the glycosyl donor within the active site of the enzyme. The resulting intermediate is stabilised by carboxyl groups attached to amino acid residues (aspartic acid or glutamic acid), if this stabilisation is concerted then retention of configuration occurs and nucleophilic attack by the

alcohol will afford the transglycosidation product as the β-anomer (scheme 1.4.).[48,129]



Proposed Mechanism of Reverse Hydrolysis and Transglycosidation Reactions.

Scheme 1.4.

The failure of the transglycosidations with the alcohol acceptors **207** to **215** was probably as a result of the alcohol acceptors either being too sterically hindered to enter the active site, for example, benzhydrol **207**, or if small enough to enter could either not associate with the glycosyl donor within the active site or if glycosidation did occur, the rate of cleavage of the newly formed glycosidic bond exceeded the rate of glycosidation.

The partial success of the model studies with β-glucosidase from almonds was followed by an attempt to use the enzyme to catalyse the synthesis of morphine-6-glucoside **31**.

1.1.2. Attempted Synthesis of Morphine-6-glucoside

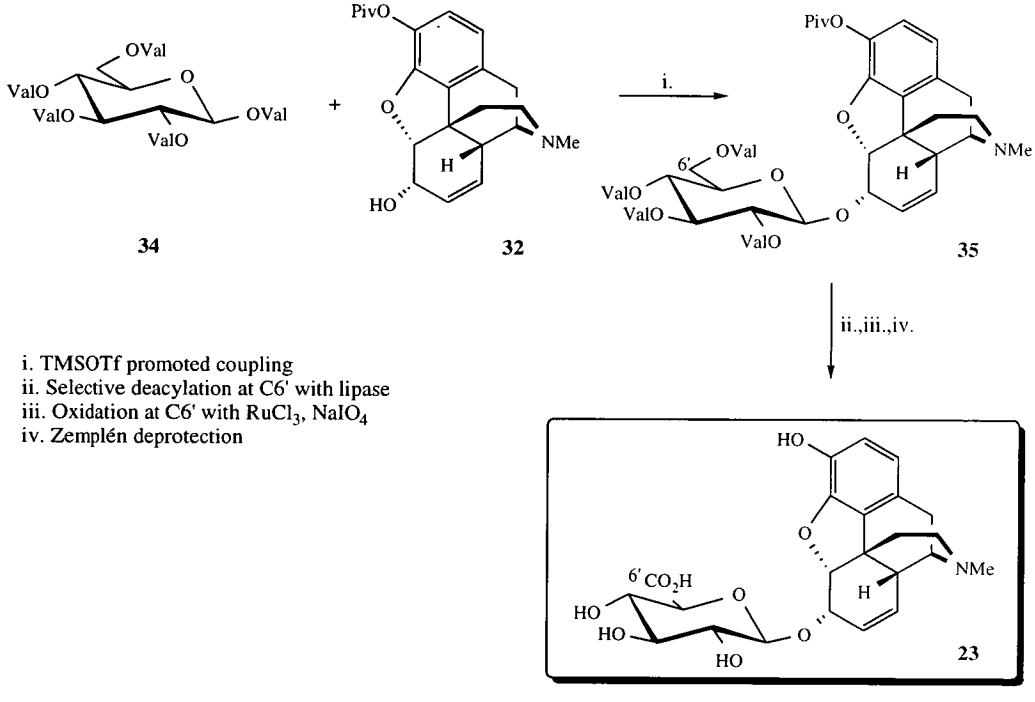
A β-glucosidase catalysed transglycosidation with 3-*O*-pivaloylmorphine **32** was examined first; the glycosyl donor **33** was consumed after 15 hours and 3-*O*-pivaloylmorphine-6-β-D-glucoside **220** was not detected. It was thought the poor solubility of 3-*O*-pivaloylmorphine **32** in the aqueous reaction medium could be a factor in the failure of the reaction so a second transglycosidation reaction was examined using morphine sulphate **20** as the acceptor. The glycosyl donor **33** was

consumed in 4 hours but despite the addition of further glycosyl donor the presence of morphine-6-glucoside **31** was not detected and the reaction was terminated after 36 hours. As morphine sulphate **20** was freely soluble in the reaction medium, solubility was not a factor in the failure of the reaction and it was more probable that 3-*O*-pivaloylmorphine **32** and morphine sulphate **20** were too bulky to participate as substrates for the enzyme.

As a result of the failure of the β-glucosidase catalysed transglycosidation reactions attempted using morphine compounds as acceptors, the synthesis of M6G **23** was examined using synthetic route two.

2. Synthesis of M6G; Synthetic Route Two

Synthetic route 2 incorporates a TMSOTf promoted glycosidation between an acyl protected glycosyl donor such as β-D-glucose pentavalerate **34** and 3-*O*-pivaloylmorphine **32** as the acceptor to afford 3-*O*-pivaloylmorphine-6-(tetravaleryl)-β-D-glucoside **35**. Selective deprotection of the primary valeryl group of the glucoside **35** *via* an enzyme catalysed reaction is followed by oxidation of the C6' hydroxyl group and deprotection to afford M6G **23** (scheme 1.5.).



Proposed Synthesis of M6G; Synthetic Route Two.

Scheme 1.5.

The use of traditional Koenigs-Knorr catalysts in the synthesis of M6G **23** is not suitable as any contamination with heavy metal ions means that M6G **23** could

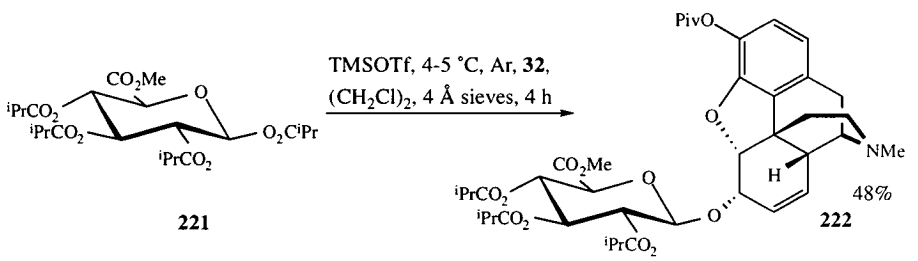
not be used as a medical drug. The use of TMSOTf provides an attractive alternative as heavy metals are avoided, hence, a TMSOTf promoted coupling was examined as the first step of route two.

2.1. TMSOTf Promoted Couplings

TMSOTf promoted glycosidations of morphine derivatives with acylated glycosyl donors have been examined at Salford Ultrafine Chemicals.[130] The conditions developed there were examined in a model study before attempting the glycosidation reaction on the morphine compounds of interest.

2.1.1. Model Study

A TMSOTf promoted coupling between methyl tetraisobutyryl-β-D-glucuronate **221** and 3-*O*-pivaloylmorphine **32** afforded 3-*O*-pivaloylmorphine triisobutyryl-β-D-glucuronate **222** in 48% yield (scheme 1.6.).



Synthesis of 3-O-Pivaloylmorphine Triisobutyryl-β-D-glucuronate 222.

Scheme 1.6.

The yield was comparable to that reported previously (55%)[130] and although the melting point was slightly lower than the reported value of 199-200 °C[130] (uncorrected), elemental analysis and spectroscopic data suggested a high degree of purity for the isolated compound.

The ^{1}H NMR spectra of 3-*O*-pivaloylmorphine triisobutyryl-β-D-glucuronate **222** showed the anomeric proton signal of the sugar residue as a doublet with a coupling constant of 8 Hz, typical for β-glycosides, at a chemical shift of δ 4.95 ppm. Of the morphine protons, the C10 equatorial proton appears as a doublet at δ 3.04 ppm with a large geminal coupling constant of 19 Hz. The unusually large geminal coupling for the protons at C10 occurs as a result of the close proximity of the aromatic ring allowing an overlap between the C-H bond and the π bond of the ring. The C10 equatorial proton also lies at a dihedral angle of about 90° to the C9 proton and so according to the Karplus equation, no coupling exists between these two protons and only geminal coupling is observed. The axial proton at C10 is found as a

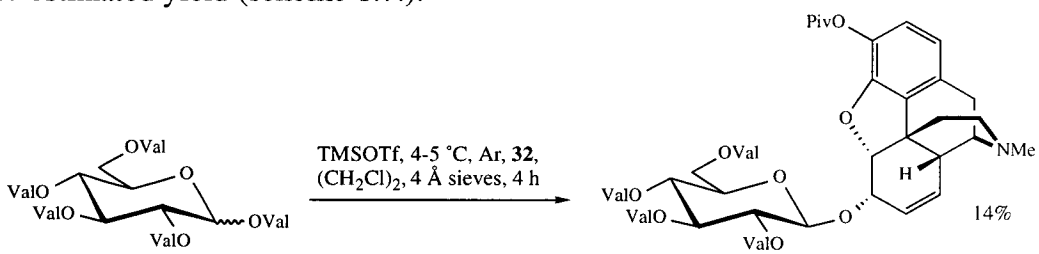
doublet of doublets at δ 2.30 ppm and has a dihedral angle of approximately 30° to the C9 proton enabling coupling between these protons to occur.

The conditions examined for the TMSOTf promoted coupling did not include the presence of an acid scavenger such as 2,4,6-collidine (2,4,6-trimethylpyridine) which is sometimes required in order to neutralise the triflic acid that forms during the reaction, preventing the acid attacking the newly formed glycosidic bond (for mechanism see introduction scheme 2.7.).[61,131] Recent investigations have shown that the presence of 2,4,6-collidine can result in the formation of orthoester side products,[52,132] thus, it was not used in any of the TMSOTf promoted glycosidations of the morphine derivatives examined.

Having established the conditions for TMSOTf promoted couplings with morphine derivatives the synthesis of 3-*O*-pivaloylmorphine tetravaleryl-β-D-glucoside **35** was examined.

2.1.2. Synthesis of 3-*O*-Pivaloylmorphine Tetravaleryl-β-D-glucoside

The TMSOTf promoted coupling between 3-*O*-pivaloylmorphine **32** and an inseparable mixture of the β-D-glucose pentavalerate **34** and the α-D-glucose pentavalerate **225** afforded 3-*O*-pivaloylmorphine tetravaleryl-β-D-glucoside **35** in 14% estimated yield (scheme 1.7.).



225, 34 **35**

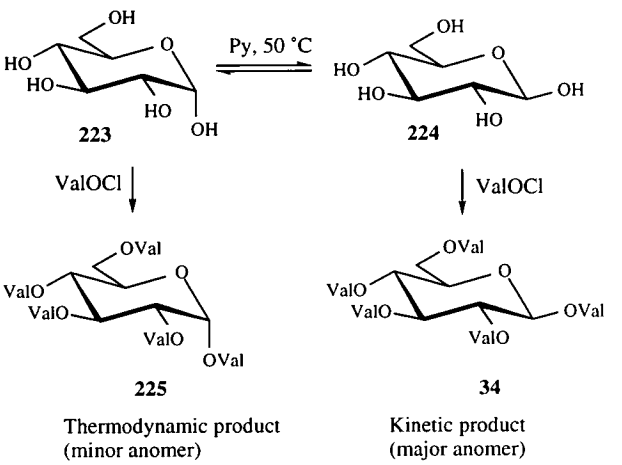
Synthesis of 3-O-Pivaloylmorphine Tetravaleryl-β-D-glucoside 35.

Scheme 1.7.

The yield of the coupling reaction was lower than expected, yields in excess of 50% are common for TMSOTf catalysed glycosidations of morphine derivatives and glycosyl trichloroacetimidates.[39,40,130] However, as the quantity of β-D-glucose pentavalerate **34** used in the glycosidation reaction was estimated, the yield of 3-*O*-pivaloylmorphine tetravaleryl-β-D-glucoside **35** could only be approximated.

The mixture of the β-D-glucose pentavalerate **34** and α-D-glucose pentavalerate **225** (in a 3:1 ratio respectively) used as the glycosyl donor in the coupling reaction was obtained from the base catalysed acylation of D-glucose **49**

with valeryl chloride and pyridine (which supplied both solvent and base catalyst) in quantitative yield (scheme 1.8.).



*Synthesis of β-D-Glucose Pentavalerate **34** and α-D-Glucose Pentavalerate**225**.*

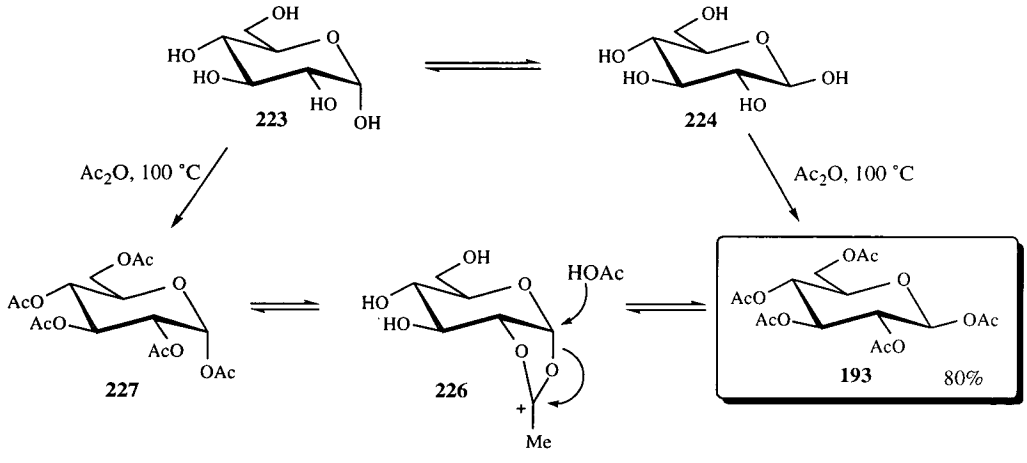
Scheme 1.8.

The mixture of β-D-glucose pentavalerate **34** and α-D-glucose pentavalerate **225** was isolated in a ratio of 3:1 respectively. The anomeric ratio was estimated from the relative peak heights of the anomeric protons in the ^{1}H NMR spectrum and is strongly influenced by the anomeric effect.[†] When D-glucose **49** is dissolved in pyridine an equilibrium develops between the α and β anomers. As a result of the repulsion between the lone electron pair of the ring oxygen and the lone electron pair of the C1 oxygen (the kinetic anomeric effect), the anomeric hydroxyl group of β-D-glucose **224** is more reactive than that of the α-D-glucose **223**. This means that the acylated β-anomer, the kinetic product of esterification, forms faster than the acetylated α-anomer. However, the anomeric effect also favours an axial configuration for electron-withdrawing groups such as esters at C1 which means that in the presence of acid, anomerisation of the acylated product will occur to give the α-anomer exclusively.[56]

Unfortunately the base catalysed acylation of D-glucose **49** did not produce the β-anomer exclusively and the temperature of the acylation reaction needed to be increased so that the rate of equilibrium between α-D-glucose **223** and β-D-glucose **224** was greater than the rate of acylation of α-D-glucose **223** so that only β-D-glucose **224** is acylated affording β-D-glucose pentavalerate **34** exclusively.

[†] The anomeric effect is the preference for substituents on the anomeric carbon of sugars to adopt the sterically more hindered axial position.[56]

Alternatively a different base, for example, sodium valerate could be used for the acylation reaction. This reaction is a modification of the synthesis of β-D-glucose pentaacetate **193** using D-glucose **49** in hot acetic anhydride which is treated with sodium acetate to afford the acylated β-anomer **193** in high yield (scheme 1.9.).[56]



Synthesis of the β-D-Glucose Pentaacetate 193.[56]

Scheme 1.9.

A high yield of β-D-glucose pentaacetate **193** is obtained as a result of anomerisation of the acetylated products *via* the acetoxonium ion **226**, therefore, the acylated β-anomer, the kinetic product of acylation, is obtained in excess. However, this reaction still gives some α-D-glucose pentaacetate **227** and this would probably occur if the reaction was adapted for the synthesis of β-D-glucose pentavalerate **34**.

Improving the yields of Koenigs-Knorr glycosidations can be achieved with the presence of excess alcohol acceptor, although the presence of excess 3-*O*-pivaloylmorphine **32** did not appear to be significantly advantageous in improving the yield of the TMSOTf promoted glycosidation reaction examined.

A sufficient quantity of 3-*O*-pivaloylmorphine tetravaleryl-β-D-glucoside **35** was available for the second step of synthetic route two, a selective deacylation of the C6' valeryl group catalysed by a lipase.

2.2. Lipase Catalysed Deacylations

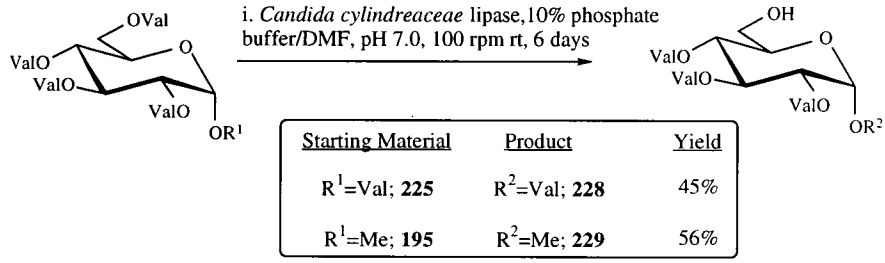
Lipases are now widely used in deacylation reactions and provide the most effective means for the selective cleavage of ester groups in polyacylated sugars.[48] The selective cleavage of ester groups within glycosides has been demonstrated by Hennen and co-workers[50] who reported that acylated furanose and pyranose derivatives can be selectively deacylated at C1 and C6 with the lipase favouring an optimal ester group chain length of five carbons. This stimulated interest in the use of

lipases in the synthesis of M6G **23** and a selective deacylation catalysed by a lipase was incorporated into synthetic route two.

In order to establish suitable conditions for the lipase catalysed deacylation of the valeryl group at C6' of 3-*O*-pivaloylmorphine tetravaleryl-β-D-glucoside **35**, enzyme catalysed deacylations of 1-*O*-methyl α-D-glucoside tetravalerate **195** and α-D-glucose pentavalerate **225** were examined using the conditions reported by Hennen and co-workers.[50]

2.2.1. Model Study

The selective removal of the C6 valeryl group of 1-*O*-methyl tetravaleryl-α-D-glucoside **195** and α-D-glucose pentavalerate **225** with *Candida cylindraceae* lipase afforded 1-*O*-methyl trivaleryl-α-D-glucoside **229** in 56% yield and α-D-glucose tetraavalerate **228** in 45% yield (scheme 1.10.).



Starting Material	Product	Yield
R^1=Val; **225**	R^2=Val; **228**	45%
R^1=Me; **195**	R^2=Me; **229**	56%

*Synthesis of 1-O-Methyl 2,3,4-Tri-O-valeryl-α-D-glucoside **229** and 2,3,4,6-TetraO-valeryl-α-D-Glucose **228**.*

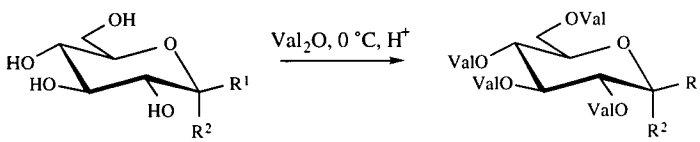
Scheme 1.10.

Examination of the ^{1}H NMR spectra for the isolated products revealed the loss of one valeryl group and a reduction in the chemical shift value of the C6 protons. The chemical shift values for the C6 protons of α-D-glucose pentavalerate **225** and 1-*O*-methyl tetravaleryl-α-D-glucoside **195** were 4.06 and 4.17 ppm, and 4.14 ppm respectively, the chemical shift values for compounds **228** and **229** were 3.64 and, 3.60 and 3.80 ppm respectively. This reduction in the chemical shift value was expected for the removal of the ester group from the C6 position and confirmed the identity of compounds **229** and **228** as 1-*O*-methyl trivaleryl-α-D-glucoside and the α-D-glucose tetraavalerate respectively.

In agreement with Hennen and co-workers[50] 100% regioselectivity was observed for the lipase catalysed deacylation although the yield was lower than the 75%-90% previously reported.[50,122] The reaction time of 6 days was twice as long as that reported by Sweers and Wong[122] and the optical rotation of $[\alpha]_D^{26}$ +91.50 (*c* 0.41, $CHCl_3$) was lower than the value of $[\alpha]_D^{22}$ +98.2 (*c* 0.55, $CHCl_3$) reported by

Sweers and Wong.[122] The *Candida cylindraceae* lipase used in the deacylation reaction was purchased from the same supplier as Hennen and co-workers,[50] however, the slow reaction time and lower yield of the 1-*O*-methyl trivaleryl-α-D-glucoside **229** isolated in scheme 1.10. would suggest the enzyme had lower unit activity than the enzyme used by Hennen and co-workers[50] or Sweers and Wong.[122]

Synthesis of 1-*O*-methyl tetravaleryl-α-D-glucoside **195** and the α-D-glucose pentavalerate **225** was achieved by treating methyl α-D-glucoside **230** and D-glucose **49** respectively, with valeric anhydride and a catalytic amount of perchloric acid (scheme 1.11.). The acid catalysed acylation reactions afforded 1-*O*-methyl tetravaleryl-α-D-glucoside **195** in 51% yield and α-D-glucose pentavalerate **225** in 76% yield.



Starting Material	Product	Yield
R^1 = OH/H; R^2 = H/OH; **49**	R^1 = H; R^2 = OVal; **225**	76%
R^1 = H; R^2 = OMe; **230**	R^1 = H; R^2 = OMe; **195**	51%

*Synthesis of 1-O-Methyl Tetravaleryl-α-D-glucoside **195** and α-D-Glucose Pentavalerate 225.*

Scheme 1.11.

Under the reaction conditions of low temperature (below 10 °C) the esterification of D-glucose **49** would be expected to occur faster than anomerisation. The product of the acylation reaction will, therefore, be predominantly the β-anomer, however, the presence of perchloric acid causes the acetylated product to anomerise and under the influence of the anomeric effect the acylated α-anomer (the thermodynamic product of acetylation) is obtained exclusively.

Acetylations of simple sugars are generally in excess of 80-90% yield and so the 51% yield obtained for 1-*O*-methyl tetravaleryl-α-D-glucoside **195** was lower than expected. The compound should be stable under the acylation conditions and so the low yield could be a result of terminating the reaction before all the starting material had been consumed. Increasing the reaction time for the acylation of methyl α-D-glucoside **230** could, therefore, give an improved yield of 1-*O*-methyl tetravaleryl-α-D-glucoside **195**.

The model studies with *Candida cylindraceae* lipase had proved successful so an attempt was made to selectively deacylate the C6' valeryl group of 3-*O*-pivaloylmorphine tetravaleryl-β-D-glucoside **35**.

2.2.2. Lipase Catalysed Selective Deacylation of 3-*O*-Pivaloylmorphine Tetravaleryl-β-D-glucoside

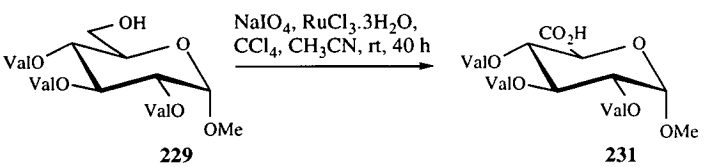
Teatment of 3-*O*-pivaloylmorphine tetravaleryl-β-D-glucoside **35** with *Candida cylindraceae* lipase as described above did not result in any deacylation reaction. It would appear that the enzyme could not accept 3-*O*-pivaloylmorphine tetravaleryl-β-D-glucoside **35** as a substrate, which is probably as result of the molecule being too large for the enzyme's active site.

2.3. Oxidations

Despite the failure of the lipase catalysed deacylation reactions, it was decided to investigate the C6 oxidation reactions. The third step of route two was a ruthenium (VIII) oxide catalysed oxidation of the C6' hydroxyl group of 3-*O*-pivaloylmorphine trivaleryl-β-D-glucoside **36**. Ruthenium (VIII) oxide is a strong oxidising reagent first introduced in the 1960's by Beynon and co-workers.[133] Ruthenium (VIII) oxide provides an efficient oxidant for a number of compounds including nucleosides[134,135] and carbohydrates,[136] oxidising isolated secondary alcohols and primary alcohols to give the dialdose and uronic acid, respectively. Carlsen and co-workers[137] have recently developed new rapid and mild conditions for ruthenium (VIII) oxide catalysed reactions based on a biphasic reaction mixture, avoiding the sluggish reactions previously encountered. These new conditions developed by Carlsen and co-workers[137] were applied to some simple sugars before attempting the oxidation on the morphine glycosides.

2.3.1. Model Study

The ruthenium (VIII) oxide catalysed oxidation of the 1-*O*-methyl trivaleryl-α-D-glucoside **229** afforded 1-*O*-methyl trivaleryl-α-D-glucuronide **231** in quantitative yield (scheme 1.12.).



229 **231**

*Synthesis of the 1-O-Methyl Trivaleryl-α-D-glucuronide **231**.*

Scheme 1.12.

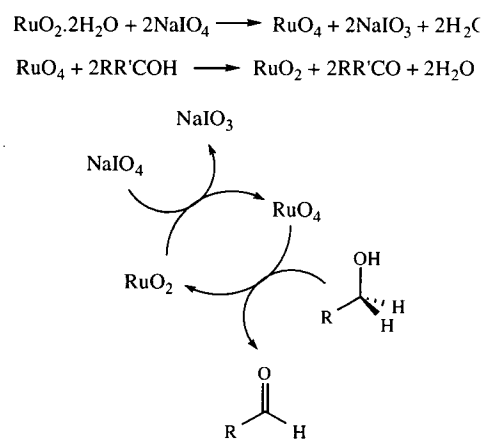
Examining the ^{1}H NMR spectra of the product confirmed the presence of 1-*O*-methyl trivaleryl-α-D-glucuronide **231** isolated as an impure red solid. The C6 protons observed in the ^{1}H NMR spectra of the 1-*O*-methyl trivaleryl-α-D-glucoside

231 were absent and the C5 multiplet at a chemical shift of δ 3.78 ppm had become a doublet and moved downfield to δ 4.16 ppm in agreement with the formation of the carboxylic acid at C6.

Several attempts were made to remove the red colour of 1-*O*-methyl trivaleryl-α-D-glucuronide **231** with decolourising charcoal, however the colour remained. This suggested contamination with ruthenium which would explain the failure to record the $[\alpha]_D$ value, and potentially could pose a problem in the use of ruthenium catalysed oxidations for the synthesis of compounds which are required for use in biological systems.

The ruthenium (VIII) oxide catalysed oxidation was complete after 40 hours, which is slow compared to the reaction times of up to 6 hours reported by Carlsen and co-workers[137] for some simple olefins. A quicker ruthenium (VIII) oxide catalysed oxidation (30 mins to 3 hours) for some acyl protected sugars has been reported by Smejkal and Kalvoda,[136] however, the catalyst used is much harsher than that used by Carlsen and co-workers[137] and involves fusing a mixture of powdered ruthenium, sodium hydroxide and sodium nitrate with heat and adding the melt to sodium periodate in water.

The mechanism of ruthenium (VIII) oxide catalysed oxidations is not well understood and could be similar to that reported for chromium (VI) oxide (scheme 1.13.).[138] The reaction probably involves initial oxidation of either ruthenium (III) chloride or ruthenium (IV) oxide with periodate to ruthenium (VIII) oxide, which on oxidising the alcohol to the carboxylic acid is reduced to ruthenium (IV).[133]



Ruthenium (VIII) Oxide Catalysed Oxidation of Alcohols.

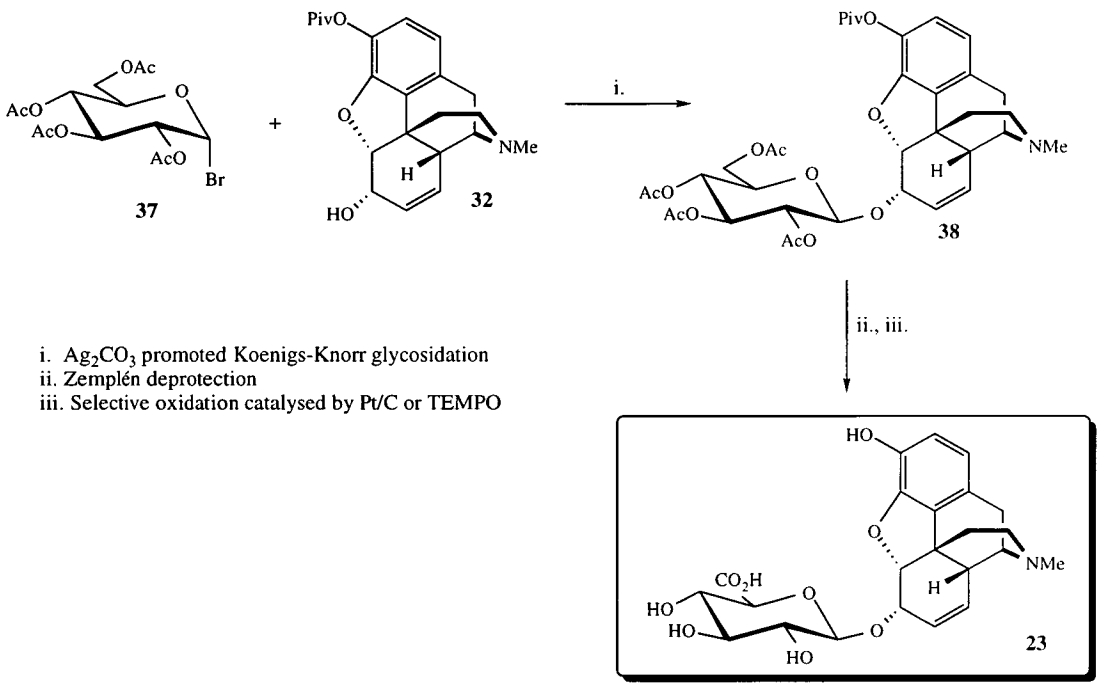
Scheme 1.13.

Unfortunately the failure to synthesise 3-*O*-pivaloylmorphine trivaleryl-β-D-glucoside **36** did not allow the opportunity to attempt the ruthenium (VIII) oxide

catalysed oxidation on this compound which was demonstrated in the model study to be a useful reagent for the oxidation of simple sugars.

3. Synthesis of M6G; Synthetic Route Three

Synthetic route 3 is a chemical synthesis of M6G **23** incorporating three steps; firstly, a silver (I) carbonate promoted Koenigs-Knorr glycosidation between acetobromoglucose **37** and 3-*O*-pivaloylmorphine **32**; secondly, a Zemplén deprotection of 3-*O*-pivaloylmorphine-6-(tetraacetyl)-β-D-glucoside **38**; thirdly, a selective oxidation of the C6' hydroxyl group of morphine-6-glucoside **31** using either platinum or TEMPO as catalysts to afford the desired M6G **23** (scheme 1.14.).



Proposed Synthesis of M6G 23; Synthetic Route Three.

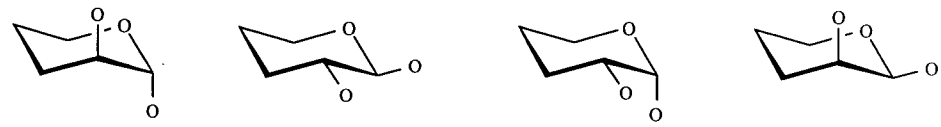
Scheme 1.14.

The silver (I) promoted Koenigs-Knorr glycosidation of 3-*O*-pivaloylmorphine **32** with acetobromoglucose **37** was initially examined.

3.1. Koenigs-Knorr Glycosidations

The original promoters employed by Koenigs and Knorr[59] for glycosidation reactions were silver (I) oxide and silver (I) carbonate. These promoters still prove valuable in the synthesis of the four basic structural types of glycosides, α-mannose, β-glucose/galactose, α-glucose/galactose, and β-mannose which are progressively

more difficult to synthesise as a result of the anomeric effect and potential participation of the C2 neighbouring group (figure 1.2.).[56]



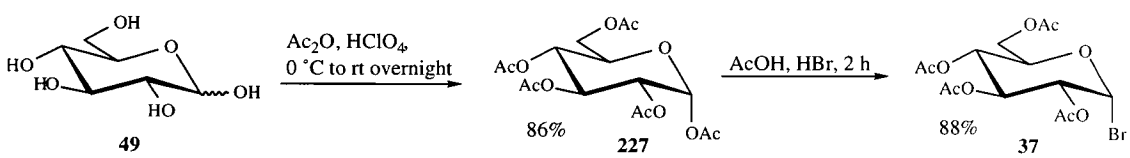
The Four Structural Types of 1-O-Glycopyranosides.

Figure 1.2.

The silver (I) carbonate promoted Koenigs-Knorr synthesis of morphine glucosides still provides a useful route to these compounds. However, interest has turned to the use of silver (I) triflate as a highly reactive glycosidation promoter for the synthesis of a variety of β-glycosides including morphine glucosides,[52] β-disaccharides,[131,139] and the more difficult β-mannodisaccharides.[140]

3.1.1. Silver (I) Triflate Promoted Glycosidations

The Koenigs-Knorr glycosidation of a variety of simple alcohol acceptors using acetobromoglucose **37** as the donor was examined. The glycosyl donor **37** is commercially available although it is readily synthesised from D-glucose **49** using an acid catalysed acetylation with perchloric acid and acetic anhydride to give α-D-glucose pentaacetate **227** followed by bromination with a 45% solution of hydrogen bromide in glacial acetic acid to afford acetobromoglucose **37** in 88% yield (scheme 1.15.).



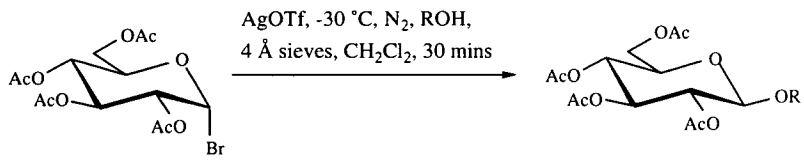
Synthesis of Acetobromoglucose 37.

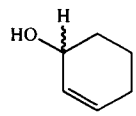
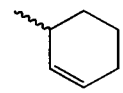
Scheme 1.15.

Glycosyl bromides were the preferred glycosyl halide to use for the glycosidation reactions as the bromides generally have greater reactivity towards glycosidation than the chlorides or fluorides.[61]

The silver (I) triflate promoted glycosidation of two primary alcohols, *n*-butanol, *n*-octanol and the secondary alcohol, 2-cyclohexen-1-ol **204** with acetobromoglucose **37** was examined using the conditions described by Hanessian and Banoub[131] who used equimolar quantities of the promoter (in common with most Koenigs-Knorr promoters[57]) with respect to the glycosyl donor. The highly

reactive nature of the promoter requires the use of anhydrous conditions and low temperatures (a lower temperature of -30 °C[141] was used in preference to the reported temperature of 0 °C) (scheme 1.16.).



ROH	R	Product	Yield
^{n}BuOH	^{n}Bu	**232**	40%
$C_8H_{17}OH$	^{n}Octyl	**233**	63%
		**203**	28%

Synthesis of the n-*Butyl Tetraacetyl-β-D-glucoside* ***232****, the* n-*Octyl Tetraacetyl-β-D-glucoside* ***233*** *and the Cyclohexenyl Tetraacetyl-β-D-glucoside* ***203****.*

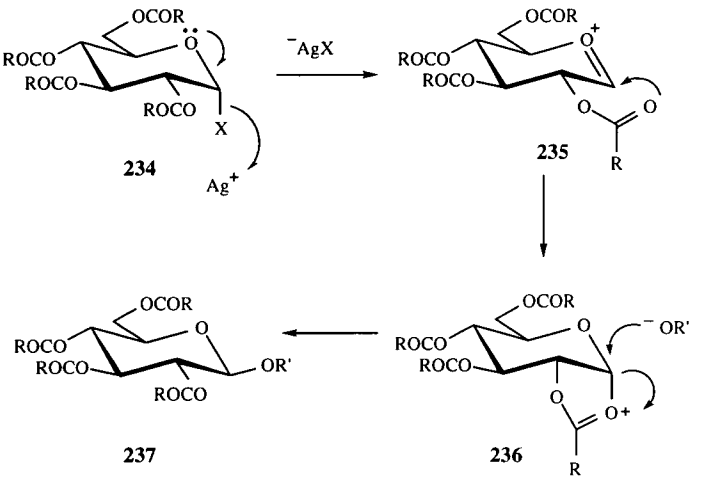
Scheme 1.16.

The initial reactions carried out used equimolar amounts of the promoter, glycosyl donor, and alcohol acceptor, however, the transglycosidation products were isolated in low yield (less than 20%). The quantity of the alcohol acceptor was increased to 10 molar equivalents and a significant increase in yield was observed with the glycosidation reactions affording the *n*-butyl tetraacetyl-β-D-glucoside **232** in 40% yield, the *n*-octyl tetraacetyl-β-D-glucoside **233** in 63% yield, and the cyclohexenyl tetraacetyl-β-D-glucoside **203** as a 1:1 ratio of diastereoisomers in 28% yield.

The use of 2-cyclohexen-1-ol **204** as an acceptor was of interest as it contains an allylic alcohol group which is also present in the morphine structure. 2-Cyclohexen-1-ol **204** is a secondary alcohol and when used as the acceptor, the transglycosidation product is isolated in a lower yield compared to the primary alcohols, *n*-butanol and *n*-octanol. This was expected as secondary alcohols are more sterically hindered.

Two diastereoisomers of cyclohexenyl tetraacetyl-β-D-glucoside **203** were observed in the ^{1}H NMR spectrum. The anomeric protons were easily identified as two doublets with chemical shifts of δ 4.60 and 4.64 ppm, as expected the coupling constant of the anomeric proton had increased from 4 Hz to 8 Hz indicating the anomeric proton has changed from an axial position in the acetobromoglucose **37** to a equatorial position in the cyclohexenyl tetraacetyl-β-D-glucoside **203**.

The mechanism of silver (I) triflate promoted Koenigs-Knorr glycosidations involves initial halogen abstraction from the glycosyl donor **234** by the silver ion affording the oxonium ion **235** (scheme 1.17.). Neighbouring group participation gives the acetoxonium ion **236** which is attacked by the alcohol equatorially, affording the β-D-glucoside **237**.



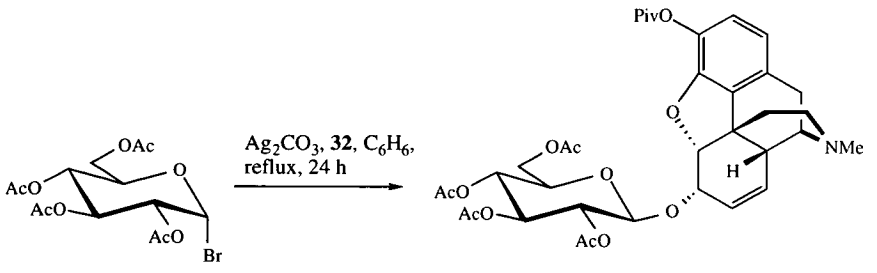
Mechanism of the Silver Triflate Catalysed Koenigs-Knorr Glycosidation.

Scheme 1.17.

Triflic acid is formed as a by-product of the reaction, this can be removed by the inclusion of an acid scavenger which neutralises the acid and prevents attack of the newly formed glycosidic bond.[61,131]

3.1.2. Silver (I) Carbonate Promoted Glycosidations

Conditions for this reaction have been reported by Hopkins and co-workers[51] who used of a large excess of the glycosyl donor **37** and catalyst which were heated under reflux in benzene for approximately 24 hours. The exact quantities of donor, acceptor and catalyst were not given and so the initial reaction examined used equimolar quantities of silver (I) carbonate, 3-*O*-pivaloylmorphine **32** and acetobromoglucose **37** to afford 3-*O*-pivaloylmorphine tetraacetyl-β-D-glucoside **38** in 10% yield. Despite being one of the more thermally stable glycosyl bromides, decomposition of acetobromoglucose **37** under the reaction conditions occurred and only by increasing the quantities of both the glycosyl donor **37** and catalyst to 5 molar equivalents was 3-*O*-pivaloylmorphine tetraacetyl-β-D-glucoside **38** isolated in an improved yield of 36% (scheme 1.18.).



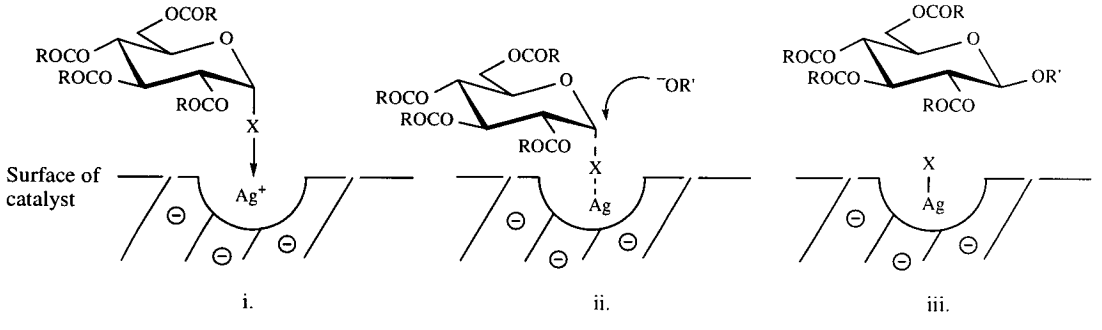
37 36% **38**

Synthesis of 3-O-Pivaloylmorphine Tetraacetyl-β-D-glucoside 38.

Scheme 1.18.

Hopkins and co-workers[51] obtained 3-*O*-pivaloylmorphine tetraacetyl-β-D-glucoside **38** in 32-63% yield, demonstrating how difficult it is to achieve consistently high yields for this reaction.

Silver (I) carbonate promoted glycosidations are thought to occur at the surface of the catalyst where the halide of the glycosyl halide associates with a silver ion (scheme 1.19.).[142] The alcohol approaches the surface bound glycosyl halide equatorially and an S_N2 reaction allows cleavage of the carbon-halide bond and formation of the glycosidic bond to take place simultaneously with inversion of configuration.



Proposed Mechanism of the Koenigs-Knorr Glycosidation with the Promoters Silver (I) Oxide or Silver (I) Carbonate.

Scheme 1.19.

The reaction between protons, released from the alcohol, and carbonate ions released from the catalyst, produces small amounts of water during silver (I) carbonate promoted Koenigs-Knorr glycosidations.[142] Although the use of Dean-Stark conditions removes any water present in the reaction medium, this might not have been done efficiently enough during the coupling reaction to prevent hydrolysis of the glycosyl halide and the new glycosidic bond. Improved yields for the synthesis of 3-*O*-pivaloylmorphine tetraacetyl-β-D-glucoside **38** by silver (I) carbonate

promoted Koenigs-Knorr glycosidations might be achieved by the use of a drying agent such as Drierite and dry benzene.

Alternative Koenigs-Knorr catalysts which avoid the creation of water during the glycosidation reactions have been used for the synthesis of morphine glycosides. For example, silver (I) triflate is a highly reactive, soluble Koenigs-Knorr catalyst which has been used by Kovác and Rice.[52] The soluble nature of the promoter means that the anomeric effect and neighbouring group participation have more influence over the reaction mechanism than occurs with the insoluble heterogenous catalysts. However, the formation of orthoesters has been associated with the use of silver (I) triflate,[52] one advantage of the silver (I) carbonate promoted Koenigs-Knorr glycosidations is the absence of side-products such as transacylation products and orthoesters.

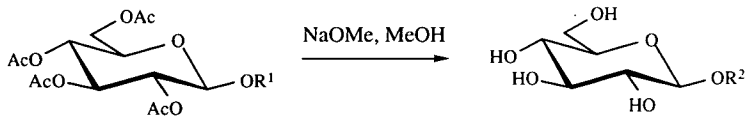
3.2. Zemplén Deprotections

The cleavage of ester protecting groups of monosaccharides can be achieved under basic or acidic conditions or with lipases which provide mild and selective deprotection conditions. A common basic deacylation procedure for ester protecting groups, particularly acetates, is the Zemplén deprotection which involves the use of catalytic amounts of sodium methoxide in methanol.[56]

Esters can be cleaved with a variety of other reagents, for example, barium hydroxide and amines such as dimethylamine and triethylamine which are relatively mild reagents. If base labile groups are present, hydrochloric acid in methanol can be used.[56,143] The Zemplén procedure is rapid and has been used for the deacylation of morphine glycosides and glucuronides, however, contamination of the morphine glycosides or glucuronide with sodium acetate has been a problem.[51]

3.2.1. Deacylations of Some Simple Transglycosidation Products

A Zemplén deprotection was attempted with the *n*-butyl tetraacetyl-β-D-glucoside **232**, the *n*-octyl tetraacetyl-β-D-glucoside **233**, and the cyclohexenyl tetraacetyl-β-D-glucoside **203** to afford *n*-butyl β-D-glucoside **238**, *n*-octyl β-D-glucoside **239**, and cyclohexenyl β-D-glucoside **202** respectively, in quantitative yields (scheme 1.20.).

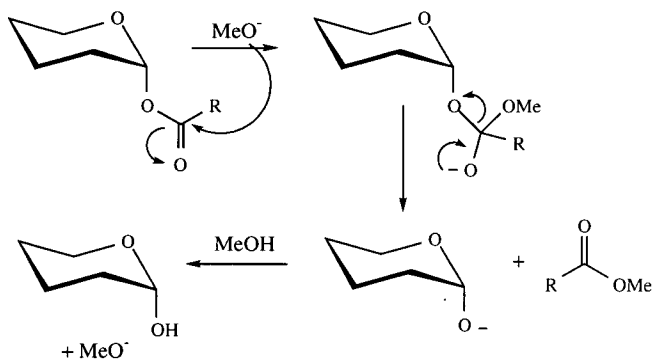


R^1	Starting Material	R^2	Product	% Yield
C_4H_9	**232**	C_4H_9	**238**	Quantitative
C_8H_{17}	**233**	C_8H_{17}	**239**	Quantitative
	**203**		**202**	Quantitative

*Synthesis of n-Butyl β-D-glucoside **238**, n-Octyl β-D-glucoside **239** and Cyclohexenyl β-D-glucoside **202**.*

Scheme 1.20.

The mechanism of Zemplén deprotections involves nucleophilic attack of the methoxide ion on the carbonyl group generating the methyl ester and regenerating the methoxide ion from methanol to give the alcohol (scheme 1.21.).[56]



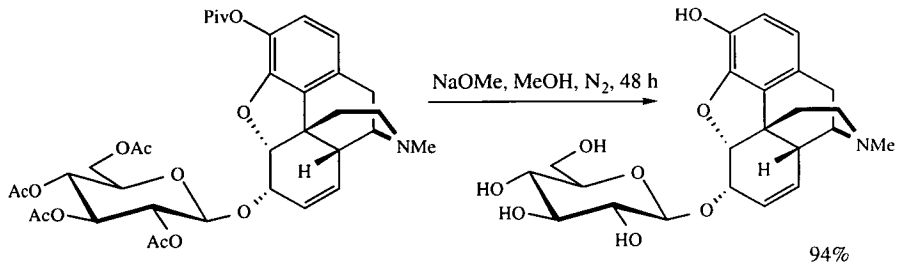
The Zemplén Deprotection.

Scheme 1.21.

Sodium acetate is sometimes produced as a side product of the Zemplén deprotection, however, *n*-butyl β-D-glucoside **238**, *n*-octyl β-D-glucoside **239** and cyclohexenyl β-D-glucoside **202** were formed rapidly in excellent yield without the formation any side products. A Zemplén deprotection was therefore used for the synthesis of morphine-6-glucoside **31**.

3.2.2. Synthesis of Morphine-6-glucoside

The Zemplén deprotection of 3-*O*-pivaloylmorphine tetraacetyl-β-D-glucoside **38** afforded morphine-6-glucoside **31** in 94% yield (scheme 1.22.).



38 **31**

*Synthesis of Morphine-6-glucoside **31**.*

Scheme 1.22.

The formation of morphine-6-glucoside **31** was confirmed by ^{1}H NMR spectroscopy (for NMR spectra see appendix A.3.), however, the removal of the acetate groups created a highly polar compound which was difficult to assign as a result of extensive overlapping of the spectral peaks. The deacylation reactions are typically slow for acylated morphine glucosides and glucuronides (20-72 hours)[39,51] particularly with the presence of the pivaloyl group which is more difficult to cleave than acetate protecting groups.

Both the ^{1}H NMR spectra and HPLC trace of morphine-6-glucoside **31** (figure 1.2.) indicated a good degree of purity, unfortunately, this was not confirmed by elemental analysis. Attempts were made to purify morphine-6-glucoside **31** by crystallisation using a range of solvents including isopropanol-water and methanol-ether, however, they were unsuccessful. Purification of morphine-6-glucoside **31** using size exclusion chromatography was also unsuccessful as the eluent contained sodium chloride which could not be removed. Alternatively morphine-6-glucoside **31** could be purified with preparative HPLC, however the eluent used contained phosphate salts which would have also contaminated morphine-6-glucoside **31** and this procedure was not attempted.

The contamination of morphine-6-glucoside **31** was only slight and the contaminants, possibly sodium acetate and sodium pivaloate from the deprotection reaction, were unlikely to affect further reactions and so an attempt was made to oxidise morphine-6-glucoside **31** to M6G **23**.

3.3. Oxidations

The selective oxidation of morphine-6-glucoside **31** to M6G **23** has not been previously attempted although a number of mild reagents have been developed for the selective oxidation of unprotected and partially protected carbohydrates, these include platinum, TEMPO, and more recently enzymes and whole cells.

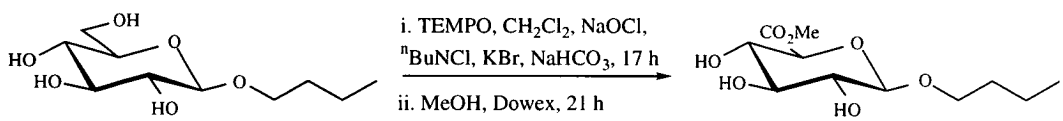
3.3.1. TEMPO Catalysed Oxidations

The use of nitroxyl radicals (a recent review has been published by de Nooy *et al*[144]) as mild oxidising agents has been known since the latter part of the last century. TEMPO in particular has received much attention and the development of a useful procedure for the selective oxidation of primary alcohols in the presence of secondary alcohols has been of particular significance.[145,146] Recently the use of TEMPO for the synthesis of uronic acids was reported by Davis and Flitsch[147] and this has led to the establishment of TEMPO as a useful oxidising agent in carbohydrate chemistry.[144,148]

Before attempting a TEMPO catalysed oxidation on morphine-6-glucoside **31** the reaction was examined with a simple glycoside using the conditions reported by Davis and Flitsch.[147]

3.3.1.1. Model Studies

The oxidation of *n*-butyl β-D-glucoside **238** under biphasic alkaline conditions afforded the crude uronic acid in 52% yield which on methylation gave the desired methyl (*n*-butyl β-D-glucopyranosid)uronate **240** in 20% yield (scheme 1.23.).



238 **240** 20%

*Synthesis of Methyl (n-Butyl β-D-glucopyranosid)uronate **240**.*

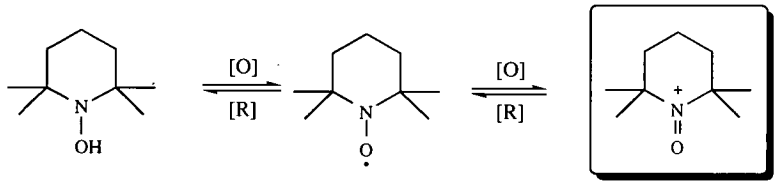
Scheme 1.23.

Examination of the isolated compound by ^{1}H NMR spectroscopy revealed a loss of the C6 protons and the presence of a methyl ester group thereby confirming the formation of methyl (*n*-butyl β-D-glucopyranosid)uronate **240**. Yields for TEMPO catalysed oxidations of simple sugars in the presence of hypochlorite-bromide are generally good. A related glycoside *n*-octyl β-D-glucoside **238** was readily oxidised and methylated by Davis and Flitsch[147] to afford the methyl uronate **240** in 67% yield. The yield of the methyl (*n*-butyl β-D-glucopyranosid)uronate **240** was lower than expected, the crude yield of the isolated uronate was moderate and so the methylation procedure could be the yield limiting step.

The TEMPO catalysed oxidation of carbohydrates commonly requires the glycoside to be stirred at low temperature with a catalytic amount of TEMPO and sodium bromide while a solution of sodium hypochlorite is added. The reaction

mixture can be monophasic (aqueous) or biphasic (aqueous-organic) with a phase transfer catalyst such as tetrabutylammonium chloride (Bu_4NCl) required for biphasic reaction mixtures. The conditions can be alkaline or acidic using sodium hydrogen carbonate or aqueous hydrochloric acid respectively, with the uronic acid isolated as the sodium salt.[149] TEMPO oxidations can be carried out with or without the presence of a secondary oxidant depending on the presence of a catalytic or stoichiometric amount of TEMPO respectively.

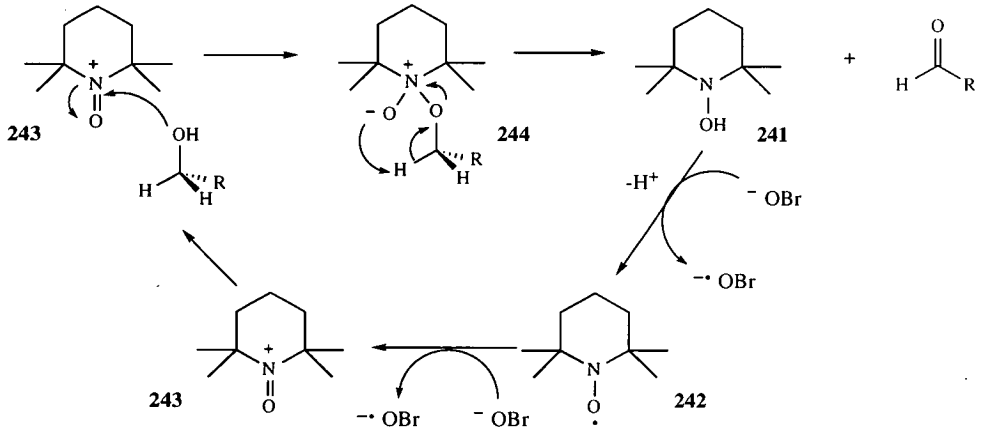
The primary oxidant of TEMPO catalysed oxidations has been identified as the oxoammonium salt **243** which is obtained by oxidation of the nitroxyl radical **242** which can also be reduced to the hydroxylamine **241** (scheme 1.24.).[149]



Oxidation/Reduction Cycle of TEMPO.

Scheme 1.24.

The exact mechanism of the TEMPO catalysed oxidation of alcohols is still unclear and a number of possible routes have been proposed.[150,151] The pH of the reaction has been found to be important and different mechanisms are observed in acidic and alkaline conditions.[149] The TEMPO oxidation of glycosides often occurs under alkaline conditions, under these conditions a sterically confined reaction mechanism is possible (scheme 1.25.).[149]



Proposed Mechanism for the TEMPO Catalysed Oxidation of Alcohols in Alkaline Conditions.

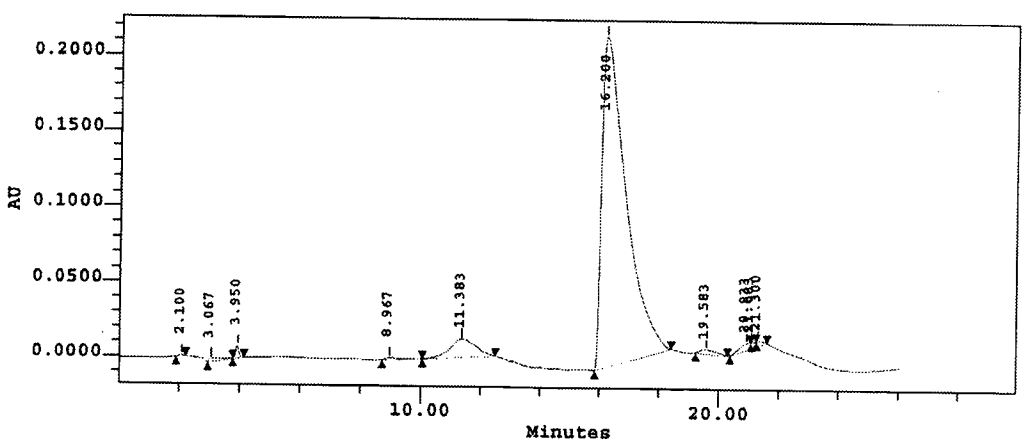
Scheme 1.25.

The sterically confining reaction mechanism involves initial abstraction of an electron from the nitroxyl radical **242** by the secondary oxidant hypobromite, which is a more active oxidant than hypochlorite, to generate the oxoammonium salt **243**. Compound **243** is then attacked by the alcohol to give the adduct **244** which rearranges to release the aldehyde and the hydroxylamine **241**. The aldehyde is thought to be oxidised in its hydrated form to the carboxylic acid using the same mechanism as the oxidation of the alcohol.[149] TEMPO catalysed reactions with hypochlorite-bromide are usually very rapid with the rate limiting step being regeneration of the oxoammonium salt **243**.[149]

As a result of the successful TEMPO catalysed oxidation of *n*-butyl β-D-glucoside **238** to methyl (*n*-butyl β-D-glucopyranosid)uronate **240**, the TEMPO catalysed oxidation of morphine-6-glucoside **31** was examined.

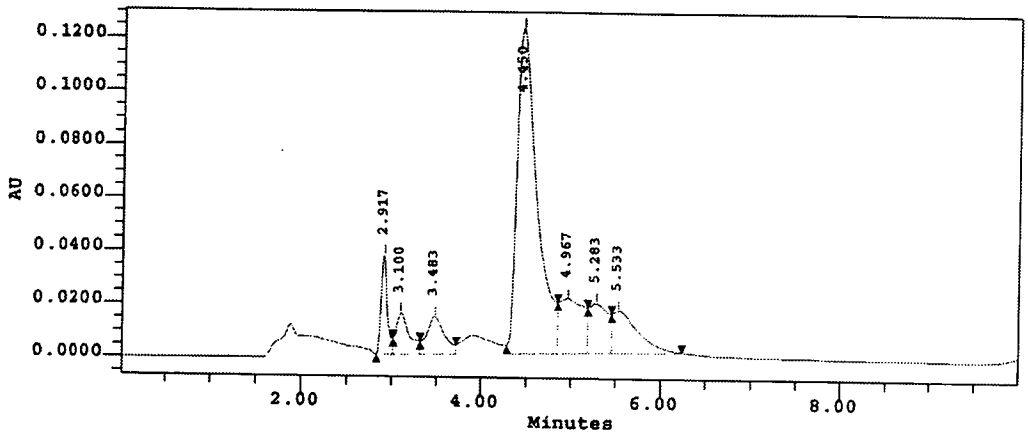
3.3.1.2. TEMPO Catalysed Oxidation of Morphine-6-glucoside

Before the oxidation of morphine-6-glucoside **31** could be attempted a suitable assay had to be established which would allow the reaction to be monitored accurately. The progress of the oxidation was followed by HPLC using a C18 reverse phase column with acetonitrile-pH 6.5, 5 mM phosphate buffer as the eluent, which was set up as a gradient from 10% to 90% acetonitrile (see general experimental table 1.1.). Using this eluent the more polar M6G **23** had a considerably shorter retention time (4.45 mins) than morphine-6-glucoside **31** (16.20 mins) as demonstrated in the HPLC traces (figures 1.3. and 1.4.)



*HPLC Trace of Morphine-6-glucoside **31** (retention time 16.20 mins).*

Figure 1.3.



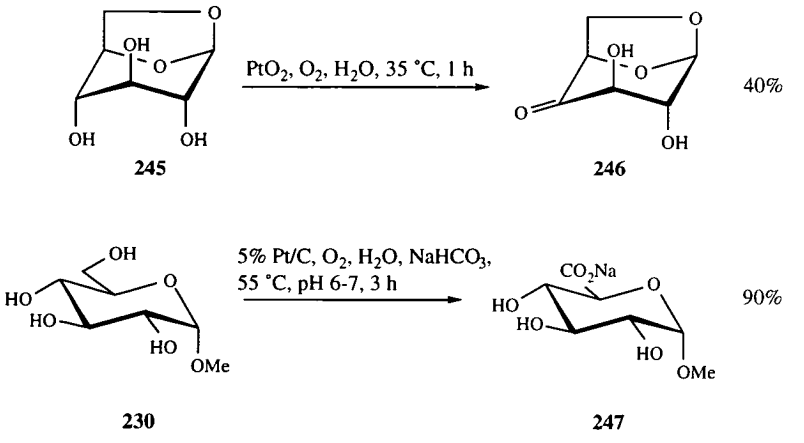
HPLC Trace of M6G 23 (retention time of 4.45 mins).

Figure 1.4.

Unfortunately, when the TEMPO oxidation was attempted with the morphine-6-glucoside **31** the reaction conditions appeared to completely destroy the morphine skeleton as none of the relevant peaks on the HPLC trace were observed. The destruction of the starting material was confirmed by ^{1}H NMR spectroscopy, no trace of morphine-6-glucoside **31** or desired the M6G **23** was observed. Although the reaction medium contains a number of components, the morphine-6-glucoside **31** was probably destroyed by the sodium hypochlorite which is a powerful oxidising agent in its own right. Milder oxidising conditions were required and a platinum catalysed oxidation was examined.

3.3.2. Platinum Catalysed Oxidations

The use of platinum as a catalyst for the oxidation of primary and secondary alcohols was established in the last century and can be used as a mild and selective catalyst for the oxidation of carbohydrates.[152] The oxidation of a number of sugar derivatives has been achieved using platinum catalysts, for example, the synthesis of the aldonic acid 1,6-anhydro-β-D-xylo-hexopyranos-3-ulose **246** from 1,6-anhydro-β-D-galactopyranoside **245**[152] and sodium methyl α-D-glucopyranosiduronate **247** from methyl α-D-glucoside **230**[153] (scheme 1.26.).[56]



*Platinum Oxidation of 1,6-Anhydro-β-D-galactopyranose **245** and Methyl α-D-Glucoside **230**.*

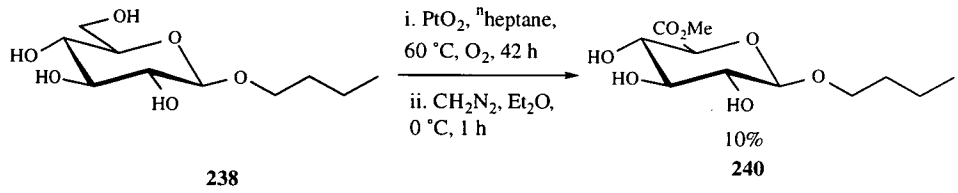
Scheme 1.26.

A variety of conditions have been developed for platinum catalysed oxidations. Adams catalyst (platinum (IV) oxide) and 5-10% Pt/C are frequently used and oxygen or air is usually bubbled through the reaction mixture which is maintained at a temperature of between 20-100 °C depending on the substrate reactivity.[152] The solvent is usually water, although a variety of other solvents are also suitable including acetone[154] and *n*-heptane.[155] The acid that is produced during the oxidation reaction lowers the pH of the reaction medium, and the addition of sodium hydrogen carbonate is required to maintain neutrality; the reaction affords the sodium salt of the acid, in often excellent yields.[153] Platinum catalysts readily oxidise the anomeric carbon of aldoses to give aldonic acids, thus, anomeric protection is required for oxidations at non-anomeric carbons.[56] As the oxidation of morphine glycosides was of interest the sensitivity of the anomeric carbon to oxidation did not have to be considered.

The conditions used by Fabre and co-workers[153] and Heyns and Blazejewicz[155] were of particular interest. Fabre and co-workers[153] prepared uronic acids in high yields with Pt/C catalysed oxidations while Heyns and Blazejewicz[155] successfully oxidised some simple alcohols with platinum (IV) oxide in *n*-heptane, this reaction was examined first.

3.3.2.1. Model Study

The oxidation of *n*-butyl β-D-glucoside **238** under the conditions reported by Heyns and Blazejewicz[155] afforded the crude uronic acid in 37% yield, which was isolated as the methyl (*n*-butyl β-D-glucopyranosid)uronate **240** in 10% yield (scheme 1.27.).



238 **240**

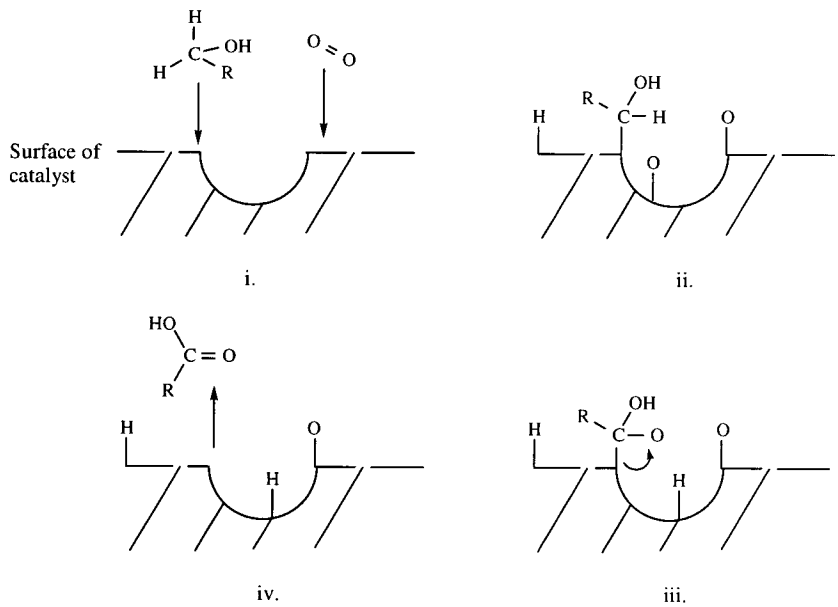
*Synthesis of Methyl (*n*-Butyl β-D-glucopyranosid)uronate **240**.*

Scheme 1.27.

^{1}H NMR spectroscopy of the isolated material revealed the loss of the C6 protons and the presence of a methyl ester as expected for the oxidation of a glycoside to its uronic acid confirming the formation of methyl (*n*-butyl β-D-glucopyranosid)uronate **240**.

Although both primary and secondary alcohol groups are present in *n*-butyl β-D-glucoside **238**, primary alcohols in pyranoid rings are generally oxidised more readily than the secondary alcohols,[56] essentially none of the secondary alcohol groups present in compound **238** were oxidised.

A proposed mechanism for platinum catalysed oxidations is shown in scheme 1.28. and possibly involves an exchange of hydrogen atoms for oxygen atoms at surface of the catalyst.[138]

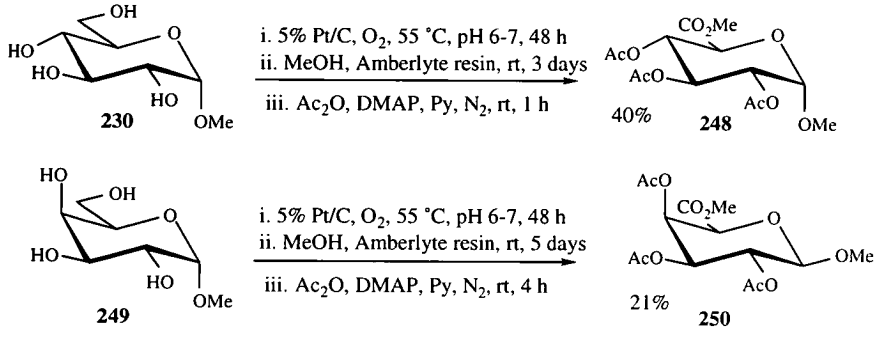


Proposed Mechanism of Platinum Catalysed Oxidations.

Scheme 1.28.

The poor yield of methyl (*n*-butyl β-D-glucopyranosid)uronate **240** was discouraging, therefore, the platinum (IV) oxide catalysed oxidation of morphine-6-glucoside **31** was not attempted as the conditions were considered unfavourable and the reaction not likely to be successful.

Attention turned to the platinum catalysed oxidation reported by Fabre and co-workers.[153] The oxidation of methyl α-D-glucoside **230** and methyl β-D-galactoside **249** afforded the crude sodium glucuronate and galacturonate respectively, in quantitative yields. Methylation followed by acetylation gave methyl (methyl 2,3,4-tri-*O*-acetyl-α-D-glucopyranosid)uronate **248** in 40% yield and methyl (methyl 2,3,4-tri-*O*-acetyl-β-D-galactopyranosid)uronate **250** in 21% yield (scheme 1.29.).



230 **248** **249** **250**

*Synthesis of Methyl (Methyl 2,3,4-tri-O-acetyl-α-D-glucopyranosid)uronate **248** and Methyl (Methyl 2,3,4-tri-O-acetyl-β-D-galactopyranosid)uronate **250**.*

Scheme 1.29.

Examination of the ^{1}H NMR spectra for methyl (methyl 2,3,4-tri-*O*-acetyl-α-D-glucopyranosid)uronate **248** and methyl (methyl 2,3,4-tri-*O*-acetyl-β-D-galactopyranosid)uronate **250** revealed the loss of the C6 proton signals as expected and the presence of two strong singlets for the OMe group and the CO_2Me group.

The formation of the crude glucuronate and galacturonate in quantitative yields were comparable to the 80-90% yields of the sodium glucuronate **247** isolated by Fabre and co-workers.[153] However, derivatisation of the crude glucuronate **248** and galacturonate **250** to give compounds that were easier to handle and charaterise significantly reduced the overall yield of the isolated uronates. Although the use of methanol and Amberlyte resin provided the mild methylation conditions desired, the reactions were slow and afforded the methylated uronic acids in crude yields of less than 50%. The low yields of the methylation were probably as a result of the equilibrium that develops between the free uronic acid and the methyl ester. This equilibrium occurs as the water produced in the methylation reaction can attack the methyl ester to give the uronic acid. The removal of water from the reaction medium by a drying agent such as Drierite would have pushed the equilibrium in favour of the methylated uronate, possibly reducing the reaction time and increasing the yield of the methylated uronic acids.

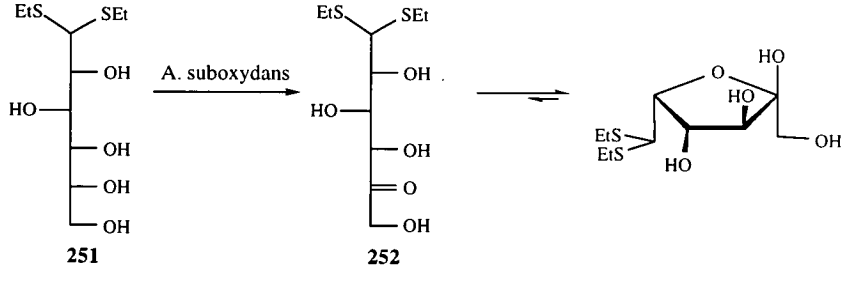
Other methylation procedures for uronic acids include the use diazomethane and acidified methanol,[156] attempts to use the former procedure resulted in disappointingly low yields of the uronic acid methyl ester, and the latter conditions were considered too harsh to be examined.

3.3.2.2. Platinum Catalysed Oxidation of Morphine-6-glucoside

After the successful oxidation of methyl α-D-glucoside **230** and methyl β-D-galactoside **249** with 5% Pt/C the reaction was attempted with the morphine-6-glucoside **31** using the conditions reported by Fabre and co-workers.[153] The progress of the reaction was followed by reverse phase tlc using 20% methanol in 0.5 M aq sodium chloride and HPLC. However, the reaction conditions again appeared to harsh for morphine-6-glucoside **31** which was completely destroyed.

3.3.3. Enzyme Catalysed Oxidations

The failure of the TEMPO and platinum catalysed oxidations of morphine-6-glucoside **31** was disappointing. Other possible routes to M6G **23** exist, and one of these may be the use of enzyme catalysed oxidations. A number of isolated enzymes and whole cells are currently available for the mild and selective oxidation of a variety of compounds including carbohydrates. One such organism is the bacterium *Acetobacter suboxydans* which is isolated from stale beer and is well established as an oxidant for a variety of alcohols[157] and carbohydrates.[56] The organism has dehydrogenase activity, these are enzymes that catalyse the oxidation of a substrate by removing hydrogen, usually with the presence of the co-enzyme nicotinamide adenine dinucleotide (NAD) which transfers hydrogen during the oxidation reaction.[16] The bacterium *Acetobacter suboxydans* readily oxidises free aldoses to aldonic acids, for example, D-glucose diethyl dithioacetal **251** is oxidised to D-*xylo*-hexos-5-ulose diethyl dithioacetal **252** when treated with the bacterium in neutral aqueous solutions (scheme 1.30.).[56]

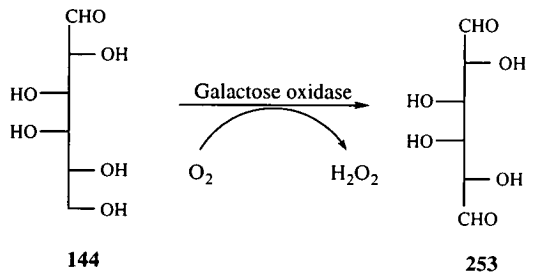


251 **252**

*Oxidation of D-Glucose Diethyl Dithioacetal **251** with* Acetobacter suboxydans.

Scheme 1.30.

Enzymes that have recently been used for the mild and selective oxidation of carbohydrates include oxidase enzymes and oxidoreductases, for example, the metallo-oxidase galactose oxidase (*Dactylium deudroides*) which contains copper (II) as a co-factor, oxidises D-galactose **144** at the C6 position in the presence of oxygen to give D-galactohexodialdose **253** (scheme 1.31.).[158]



144 **253**

*Oxidation of D-Galactose **144** with Galactose Oxidase from* Dactylium deudroides.

Scheme 1.31.

Enzymatic oxidoreductions are not common reactions in organic synthesis, although they are potentially very useful tools in many areas of chemistry including carbohydrate chemistry; a recent review published by Fang *et al*[158] explores the potential applications of these enzymes in detail.

Enzymes and whole cells could provide the mild and selective oxidation required to synthesise the elusive M6G **23** and succeed where chemical methods have so far failed.

Section Two; Synthesis of M6G Analogues

2.1. Synthesis of Morphine-6-Galactoside

In a previous unpublished study by Hopkins and co-workers,[51] a silver (I) carbonate promoted coupling of the acetobromogalactose **46** and 3-*O*-acetylmorphine **27** followed by a Zemplén deprotection afforded morphine-6-galactose **44** as an impure solid in low yield (9-28%). In order to synthesise morphine-6-galactose **44** in an improved yield and higher degree of purity, the silver (I) carbonate promoted couplings were re-examined using the glycosyl donors acetobromogalactose **46** and tetra-*O*-benzoyl-α-D-galactopyranosyl bromide **256**, with 3-*O*-pivaloylmorphine **32** as the glycosyl acceptor.

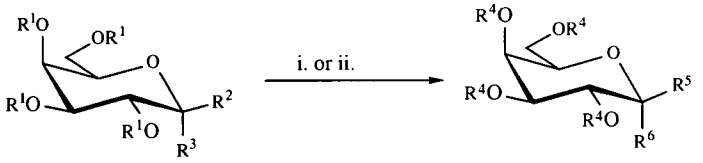
2.1.1. Koenigs-Knorr Couplings

Of the four structural types of 1-*O*-glycosides that exist, 1,2-*trans*-β-D-linkages of glucosides and galactosides are among the most straight forward to synthesise with a Koenigs-Knorr coupling, where glycosyl bromides and chlorides are typically used as glycosyl donors.[56]

2.1.1.1. Synthesis of Tetra-*O*-benzoyl-α-D-galactopyranosyl Bromide and Tetra-*O*-acetyl-α-D-galactopyranosyl Bromide

Glycosyl bromides and chlorides are synthesised by treating peracylated sugars with hydrogen halides in acetic acid.[56] The halide displaces the C1 acyl group to give the β-glycosyl halide (the kinetic product) which undergoes anomerisation to the α-glycosyl halide (the thermodynamic product) exclusively.[56] In this manner the glycosyl bromides **46** and **256** were prepared from the peracylated galactose **254** and perbenzoylated galactose **255**, respectively, with hydrogen bromide-glacial acetic acid (scheme 2.1.).

Peracetylated α-D-galactose **254** is commercially available but can be readily prepared with a base catalysed esterification of the free sugar with pyridine and acetic anhydride. The base catalysed esterification of free sugars with the appropriate anhydride or acyl chloride is a well known procedure for the synthesis of peracylated sugars. Varying the acylation conditions will determine the ratio of the α/β-anomers and pyranose/furanose ring sizes.[56] At low temperatures in pyridine the esterification reaction will proceed faster than anomerisation of the free sugar and so formation of the acylated α-anomer which retains a pyranose conformation is generally favoured.[56]



R^1	R^2	R^3		Conditions	R^4	R^5	R^6	Product	Yield
Ac	H	OAc	**254**	i. AcOH, HBr, **254**	Ac	H	Br	**46**	77%
H	H/OH	H/OH	**144**	ii. BzOCl, Py, **144**, 0 °C, 3 h	Bz	H	OBz	**255**	25%
Bz	H	OBz	**255**	i. AcOH, HBr, **255**	Bz	H	Br	**256**	25%

*Synthesis of Glycosyl Donors **46** and **256**.*

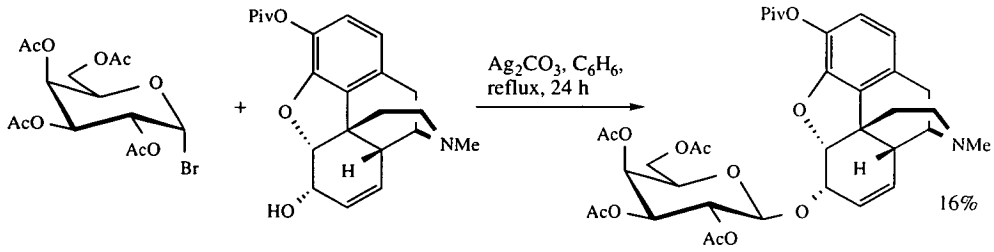
Scheme 2.1.

As shown in scheme 2.1., the benzoylation of D-galactose **144** afforded the perbenzoylated α-D-galactopyranoside **255** exclusively in 25% yield after chromatography and crystallisation. The presence of compound **255** was confirmed by ^{1}H NMR spectroscopy which showed the presence of 5 aromatic rings at a chemical shift of δ 7.25-8.40 ppm with the anomeric proton clearly visible as a doublet with a coupling constant of 4 Hz typical for α-glycosides at δ 6.95 ppm. An analogous reaction reported by Ness and co-workers[159] with D-glucose **49** under the same conditions gives the penta-*O*-benzoyl-α-D-glucopyranoside in a moderate yield of 50%.

The peracetylated α-D-galactose **254** and the perbenzoylated α-D-galactose **255** were readily brominated with hydrogen bromide in glacial acetic acid to afford acetobromogalactose **46** in 77% yield and the tetra-*O*-benzoyl galactopyranosyl bromide **256** in a lower yield of 25% (scheme 2.1.). ^{1}H NMR and mass spectroscopy readily confirmed the identity of the compounds **46** and **256** indicating that 4 acyl groups and a bromine group were present (for experimental see sections 1.5.1. and 1.5.8. respectively).

2.1.1.2. Synthesis of Morphine Tetraacetyl-β-D-galactoside

The initial silver (I) carbonate promoted coupling with the acetobromogalactose **46** and 3-*O*-pivaloylmorphine **32** afforded the acyl protected morphine-6-galactoside **257** in 16% yield (scheme 2.2.).



46 32 257

Synthesis of The Acyl Protected Morphine-6-galactoside 257 via *a Koenigs-Knorr Coupling.*

Scheme 2.2.

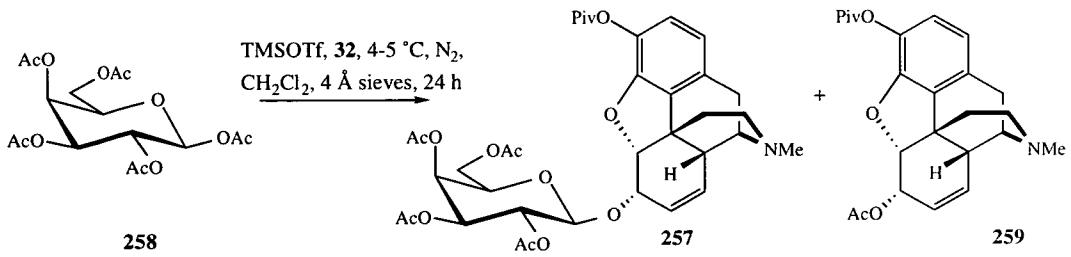
The presence of the acyl protected morphine-6-galactoside **257** was confirmed by the ^{1}H and ^{13}C NMR spectra (for ^{1}H NMR spectra see appendix A.6.). The anomeric proton was observed as a doublet with a coupling constant of 8 Hz at a chemical shift of δ 4.80 ppm typical of a β-glycosidic linkage and the pivaloyl and four acetyl protecting groups were observed at chemical shift of δ 1.35 and 1.97-2.14 ppm, respectively. There was a slight overlap between the morphine and galactose protons, however, the morphine protons chemical shift changes very little after glycosidation and so by using the ^{1}H NMR spectra of 3-*O*-pivaloylmorphine **32** (see appendix A.2.) the morphine protons were easily separated from the galactosyl protons.

Although no side products such as the orthoester or transesterification products were observed, the yield of the acyl protected morphine-6-galactoside **257** was lower than those previously reported.[51] This was probably the result of rapid decomposition of the glycosyl donor **46** under the reaction conditions. The coupling reaction with the tetra-*O*-benzoyl-α-D-galactopyranosyl bromide **256** was not attempted as benzoylated glycosyl halides are often less reactive than the acetylated analogues, probably due to the bulky aromatic rings providing steric hindrance and conformational inertia, and the reaction yield would have probably been poorer.[56]

The thermal instability of glycosyl halides is a common problem with Koenigs-Knorr glycosidations, thus an alternative method was sought. The synthesis of 3-*O*-pivaloylmorphine-6-(2',3',4',6'-tetra-*O*-valeryl)-β-D-glucoside **35** with a mixture of the glucose pentavalerate donors **225** and **34** and TMSOTf as the glycosidation promoter was successful, therefore these conditions were applied to the synthesis of morphine-6-galactose **44**.

2.1.2. TMSOTf Promoted Couplings

The first coupling reaction attempted was between the β-D-galactose pentaacetate **258** and 3-*O*-pivaloylmorphine **32** (scheme 2.3.).



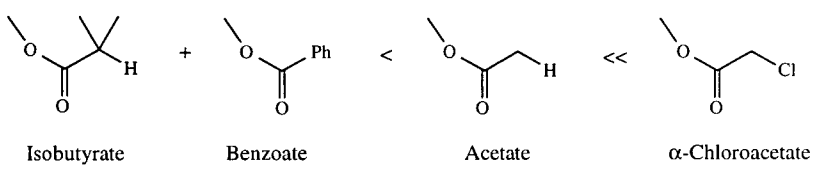
Formation of the Morphine-β-D-Galactoside ***257*** *and the Side-Product Pivaloyl-6-O-acetyl-morphine* ***259****.*

Scheme 2.3.

The reaction product was isolated and examined by ^{1}H NMR spectroscopy which revealed the presence of two pivaloyl groups and five acetyl groups. On comparing the ^{1}H NMR spectra of the isolated compound with that for the acetyl protected morphine-6-galactoside **257**, the presence of compound **257** could be confirmed, however, the extra peaks present suggested a side product had been formed and this was identified as 3-*O*-pivaloyl-6-*O*-acetyl-morphine **259**.

Unfortunately the presence of the side product **259** showed that the reaction conditions were favourable for acyl migration. The acetyl group released from the anomeric carbon of the β-D-galactose pentaacetate **258** migrated to the free hydroxyl group of the alcohol acceptor affording the side product **259**.

Acetyl protecting groups are generally the most labile of the ester protecting groups, although the presence of an electron-withdrawing group such as chlorine at the α position enhances lability (figure 2.1.).[160]



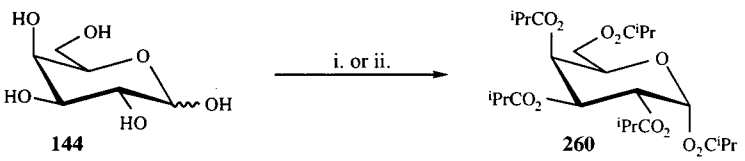
The Relative Order of Susceptibility Towards Acyl Migration.

Figure 2.1.

Benzoates, pivaloates and isobutyrates are less susceptible to migration as they are large, bulky groups. Lability is reduced if the ester carbonyl group has a reduced electrophilic character; the delocalization of electrons over the benzene ring in benzoates reduces the electrophilic nature of the ester carbonyl group.[160] Interest therefore moved to the use of benzoates and isobutyrates as alternative acyl protecting groups for the galactosyl donor.

2.1.2.1. Synthesis of Pentaisobutyryl-β-D-Galactose and Pentabenzoyl-β-D-Galactose

As a result of the anomeric effect the acylated β-glycosyl donor is more reactive than the acylated α-anomer for TMSOTf promoted couplings. The synthesis of pentaisobutyryl-β-D-galactose **261** was examined first using a base catalysed acylation reaction. The initial reactions were performed under low temperature conditions, however, only pentaisobutyryl-α-D-galactose **260** was isolated in 92% crude yield (scheme 2.4.).

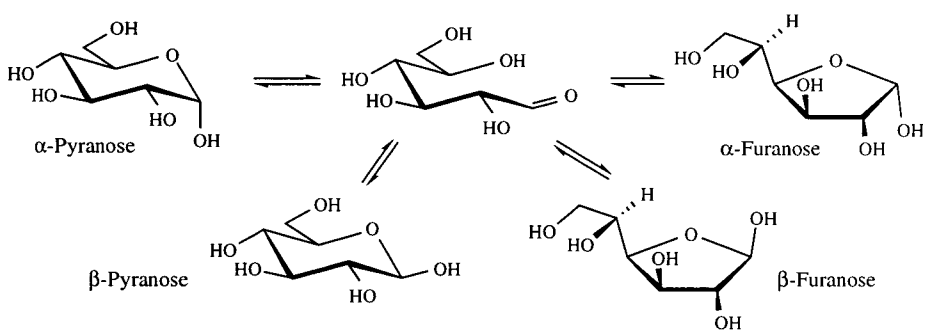


Conditions	Crude Yield
i. $^{i}PrCO_2Cl$, DMAP, Py, -10 °C, 3 h	92%
ii. $^{i}PrCO_2Cl$, DMAP, Py, 50 °C, 2 h	84%

*Base Catalysed Acylation of D-Galactose **144** with* Isobutyryl Chloride.

Scheme 2.4.

When D-galactose **144** is dissolved in a solvent such as pyridine an equilibrium develops between the different anomers and ring sizes. Studies have shown that when sugars are dissolved in more polar solvents such as water a complex equilibrium develops between the α and β-anomers of the furanose and pyranose rings *via* the acyclic sugar such as that shown for D-glucose **49** (scheme 2.5.).[56]



*The Equilibrium of D-Glucose **49** in Water.*[56]

Scheme 2.5.

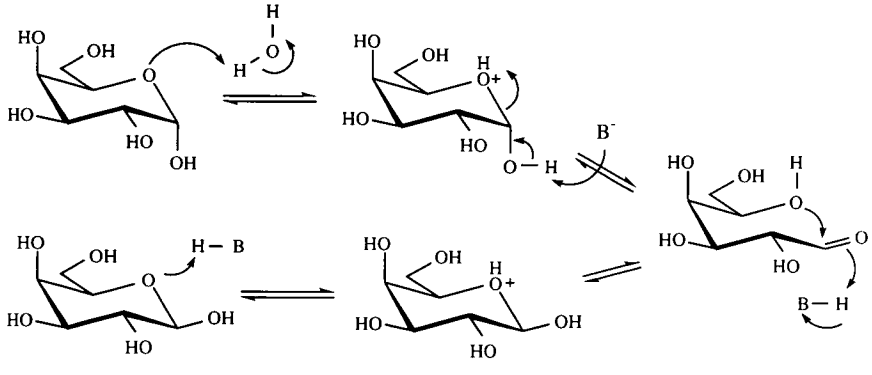
The proportions of the different anomers of D-glucose **49** and D-galactose **144** in water are shown in table 2.1. with the equilibrium favouring the β-pyranose for both D-glucose **49** and D-galactose **144**.[56]

Sugar	The % composition at 31 °C in aqueous solution at equilibrium.			
	% α-Pyranose	% β-Pyranose	% α-Furanose	% β-Furanose
Glucose	38	62	0	0.14
Galactose	30	64	2.5	3.5

*Proportions of Anomers and Isomers Present at Equilibrium for D-Glucose **49** and D-Galactose **144** in Water.*[56]

Table 2.1.

The equilibrium that develops when D-glucose **49** or D-galactose **144** is dissolved in water can be acid or base catalysed and favours cyclic forms over acyclic forms and pyranose rings over furanose rings (scheme 2.6.).[56]



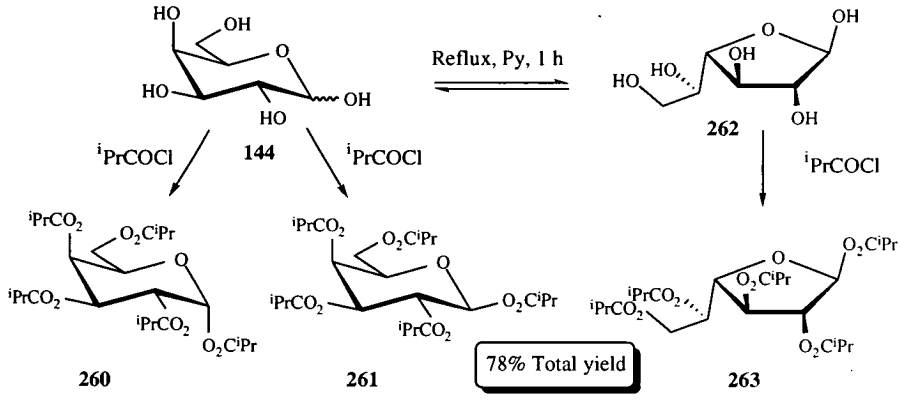
*Acid Catalysed Equilibration of D-Galactose **144** in Water.*

Scheme 2.6.

In cold (0-4 °C) pyridine D-galactose **144** dissolves reluctantly and any equilibrium that does develop must heavily favour the α-D-galactopyranose. This would explain why isobutyrylation of D-galactose **144** in cold pyridine afforded the α-D-galactoside **260** exclusively. By increasing the temperature of the acylation reaction to 50 °C it was hoped to increase the rate of anomerisation between β-D-galactose and α-D-galactose to a point where it exceeds the rate of acylation. As the β-anomer of D-galactose is more reactive than α-D-galactose the formation of the β-acylated galactose, the kinetic product of acylation, will be favoured at higher temperatures. Unfortunately when the acylation reaction was repeated at 50 °C only pentaisobutyryl-α-D-galactoside **260** was obtained in a crude yield of 84% (scheme 2.6.).

The temperature of the acylation reaction was still not high enough to encourage formation of the perisobutyryl-β-D-galactoside **261**, thus the reaction was

repeated, this time heating a suspension of D-galactose **144** under reflux for 1 hour before the addition of the isobutyryl chloride (scheme 2.7.).



*Base Catalysed Acylation of D-Galactose **144** with* Isobutyryl Chloride *Under Refluxing Conditions.*

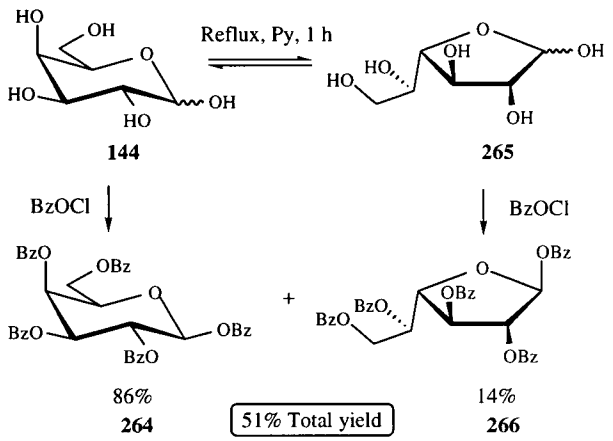
Scheme 2.7.

Examination of the ^{1}H NMR spectrum of the crude product revealed three signals at δ 5.71 ppm (d), 6.12 ppm (s), and 6.32 ppm (d) in a ratio of 1:2:7. These signals corresponded respectively, to an inseparable mixture of pentaisobutyryl-α-D-galactopyranose **260**, pentaisobutyryl-β-D-galactofuranose **263**, and pentaisobutyryl-β-D-galactopyranose **261** isolated in 78% yield after chromatography (scheme 2.7.). The acylated α and β-pyranose isomers were readily distinguished in the ^{1}H NMR spectrum by the coupling constants of the anomeric protons of 4 and 8 Hz respectively. However, as a result of the numerous signals in the ^{1}H NMR spectrum, the presence of pentaisobutyryl-β-D-galactofuranose **261** could only be confirmed by examining the chemical shifts of the anomeric carbons in the ^{13}C NMR spectrum. The anomeric carbons of the peracylated sugars typically have chemical shifts values of 90-100 ppm and so the signals observed at δ 91.88 ppm, δ 93.02 ppm, and δ 98.81 ppm were identified as the anomeric carbons for pentaisobutyryl-β-D-galactopyranose **261**, pentaisobutyryl-α-D-galactopyranose **260**, and pentaisobutyryl-β-D-galactofuranose **263**, respectively.

Unfortunately the furanose forms of D-galactose **144**, for example, the β-D-galactofuranose **262** in scheme 2.7., are stable and the mixture of pyranose and furanose forms found in acylation reactions performed at high temperatures in pyridine is common.[161] There are other potential acylation procedures that could possibly give the pentaisobutyryl-β-D-galactopyranose **261** exclusively, for example, using a different base, such as sodium isobutyrate or using the anhydride.[56]

The use of benzoate groups as protecting groups for D-galactose **144** was also examined and synthesis of pentabenzoyl-β-D-galactopyranose **264** was attempted

using the base catalysed acylation procedure established for the synthesis of pentaisobutyryl-β-D-galactopyranose **261**. A suspension of D-galactose **144** in pyridine was heated under reflux for 1 hour before the addition of benzoyl chloride. An inseparable mixture of β-D-galactofuranose pentabenzoate **266** and β-D-galactopyranose pentabenzoate **264** was obtained in a 1:6 ratio in 51% yield (scheme 2.8.).



*Synthesis of the Pentabenzoyl-β-D-Galactofuranose **266** and the Pentabenzoyl-β-D-Galactopyranose **264**.*

Scheme 2.8.

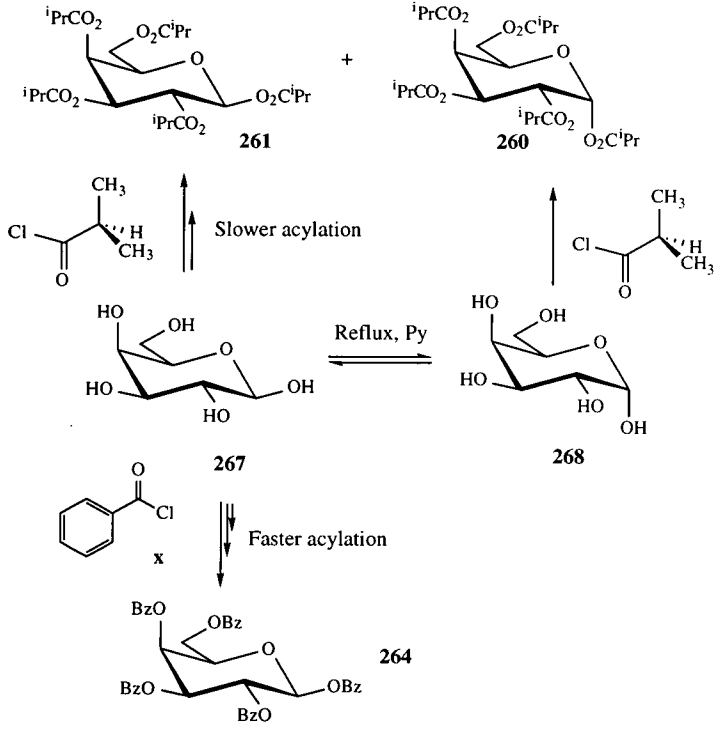
As observed with the isobutyrylation of D-galactose **144**, conditions of refluxing pyridine were again required to increase the rate of anomerisation between β-D-galactose **267** and α-D-galactose **268** sufficiently above the rate of acylation to ensure that β-D-galactopyranose pentabenzoate **264** was the major product.

The anomeric protons of pentabenzoyl-β-D-galactopyranose **264** and the pentabenzoyl-β-D-galactofuranose **266** were clearly visible in the ^{1}H NMR spectrum as a doublet with a coupling constant of 8 Hz at δ 6.29 ppm and broad singlet at δ 6.80 ppm respectively. The anomeric proton of galactofuranoses characteristically appears as a singlet in the ^{1}H NMR spectra as the dihedral angle between the anomeric proton and the C2 proton is 90° which means the protons do not couple.[56,162]

The chemical shift of the anomeric carbons in the ^{13}C NMR spectrum could be found at δ 92.91 ppm for the pentabenzoyl-β-D-galactopyranose **264** and δ 99.75 ppm for the pentabenzoyl-β-D-galactofuranose **266**. These results were in agreement with the results of the ^{13}C NMR studies of benzoylated D-galactose **144** by D'Accorso and Thiel[161] who reported the signal of the anomeric carbon of penta-*O*-benzoyl-α-D-galactopyranose at δ 90.65 ppm, pentabenzoyl-β-D-galactopyranose

264 at δ 92.78 ppm, penta-*O*-benzoyl-α-D-galactofuranose at δ 94.25 ppm, and pentabenzoyl-β-D-galactofuranose **266** at δ 99.77 ppm.

The benzoylated galactofuranose was the minor product of the benzoylation of D-galactose **144** as a result of the formation of α/β-D-galactofuranose **265**, but unlike the isobutyrylation of D-galactose **144**, the acylated α-galactopyranose (the thermodynamic product of acylation for the galactopyranose) was not observed and this could reflect the relative rates of isobutyrylation and benzoylation for D-galactose **144**. At equilibrium in boiling pyridine the β-anomer of D-galactose **267** could be benzoylated rapidly enough to pull the equilibrium entirely in favour of the acylated β-pyranose. If isobutyrylation of the β-anomer of D-galactose **267** occurs at a significantly slower rate than a small amount of the thermodynamic product (the acylated α-pyranose) will be observed (scheme 2.9.).



Comparison of the Base Catalysed Benzoylation and Isobutyrylation *of D-Galactose **144**.*

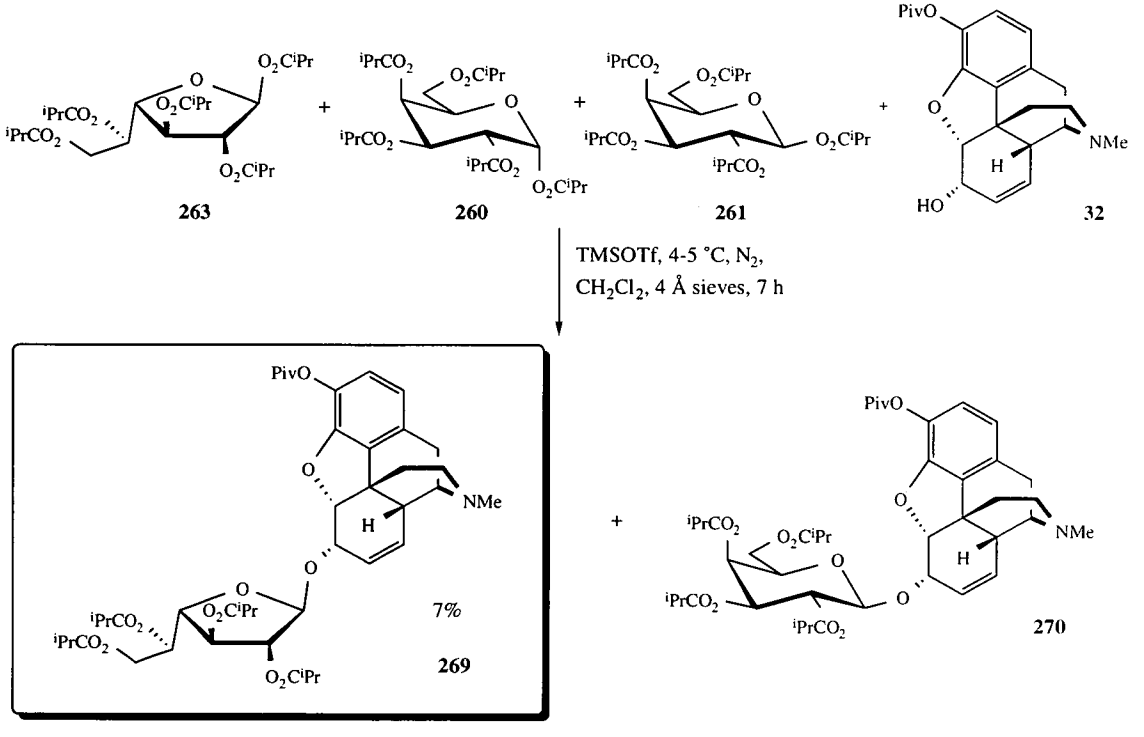
Scheme 2.9.

The acylation reactions produced inseparable mixtures of pentabenzoyl-β-D-galactopyranose **264** and pentabenzoyl-β-D-galactofuranose **266**, and pentaisobutyryl-β-D-galactopyranose **261**, pentaisobutyryl-α-D-galactopyranose **260**, and pentaisobutyryl-β-D-galactofuranose **263**. These mixtures were used without further purification in TMSOTf promoted couplings with 3-*O*-pivaloylmorphine **32** in an attempt to synthesise 3-*O*-pivaloylmorphine tetraisobutyryl-β-D-

galactopyranoside **270** and 3-*O*-pivaloylmorphine tetrabenzoyl-β-D-galactopyranoside **272**.

2.1.2.2. Synthesis of 3-*O*-Pivaloylmorphine Tetraisobutyryl-β-D-galactopyranoside and 3-*O*-Pivaloylmorphine Tetrabenzoyl-β-D-galactopyranoside

The synthesis of 3-*O*-pivaloylmorphine tetraisobutyryl-β-D-galactopyranoside **270** was attempted using the inseparable mixture of pentaisobutyryl-β-D-galactopyranose **261**, pentaisobutyryl-α-D-galactopyranose **260**, and pentaisobutyryl-β-D-galactofuranose **263** with 3-*O*-pivaloylmorphine **32** in a TMSOTf promoted coupling (scheme 2.10.).



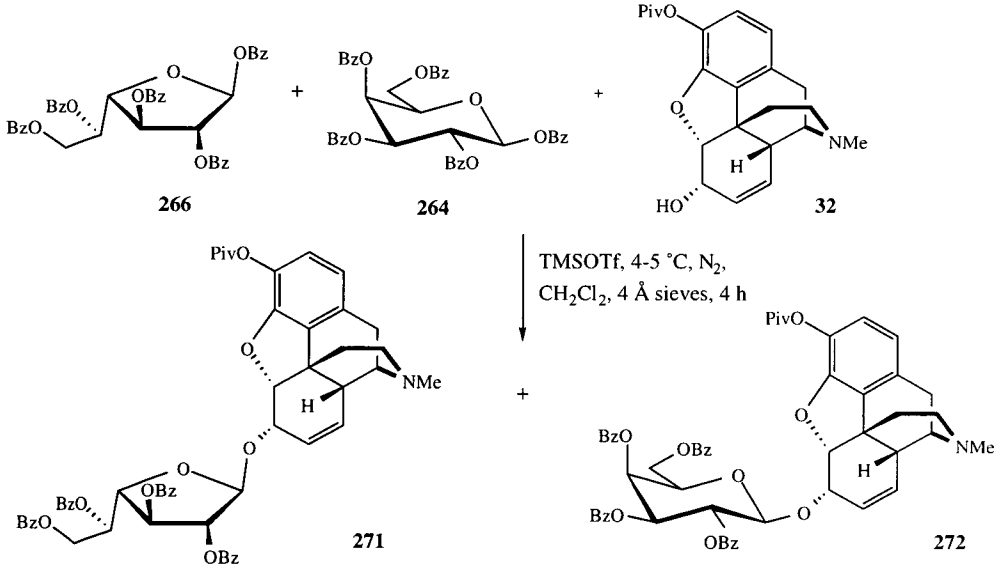
*Synthesis of 3-O-Pivaloylmorphine Tetraisobutyryl-β-D-galactofuranoside **269** and 3-O-Pivaloylmorphine Tetraisobutyryl–β-D-galactopyranoside **270**.*

Scheme 2.10.

Using an excess of the glycosyl donor mixture, the coupling reaction afforded a mixture of glycosides which, after examination by ^{1}H NMR spectroscopy, were found to be 3-*O*-pivaloylmorphine tetraisobutyryl β-D-galactofuranoside **269** and 3-*O*-pivaloylmorphine tetraisobutyryl β-D-galactopyranoside **270**. Several attempts were made to separate this mixture by crystallisation but only a small quantity of one of the adducts was isolated in 7% yield. This was identified by the ^{1}H NMR spectrum as 3-*O*-pivaloylmorphine tetraisobutyryl β-D-galactofuranoside **269** by the presence

of a singlet at δ 5.30 ppm corresponding to the anomeric proton (for NMR spectra see appendix A.7.) (scheme 2.10.). The replacement of the acetyl ester protecting group with the less labile isobutyryl protecting group ensured there was no acyl migration during the glycosidation reaction. However, the mixture of morphine glycosides afforded by the glycosidation reaction demonstrates the need for pure glycosyl donors.

The synthesis of 3-*O*-pivaloylmorphine tetrabenzoyl-β-D-galactopyranoside **272** was attempted using the mixture of β-D-galactofuranose pentabenzoate **266** and β-D-galactopyranose pentabenzoate **264** with 3-*O*-pivaloylmorphine **32** in a TMSOTf promoted coupling (scheme 2.11.), which afforded a mixture of two products. These products were identified from the ^{1}H NMR spectrum as 3-*O*-pivaloylmorphine tetrabenzoyl-β-D-galactopyranoside **272** and 3-*O*-pivaloylmorphine tetrabenzoyl-β-D-galactofuranoside **271**. However, repeated attempts to isolate the two morphine galactosides using chromatography and crystallisation failed.



266 264 32 271 272

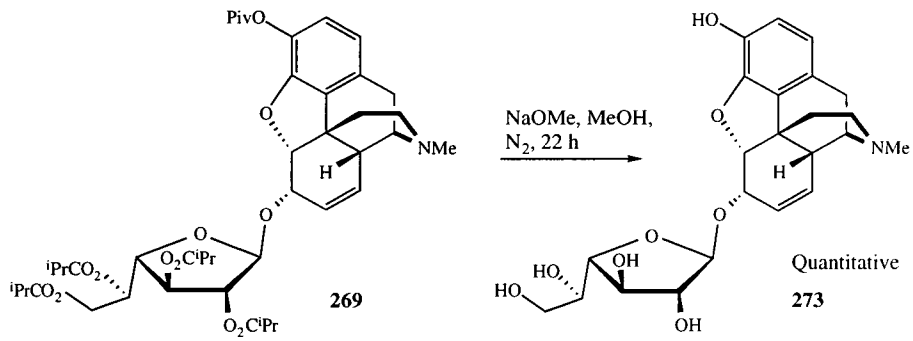
*Synthesis of 3-O-Pivaloylmorphine Tetrabenzoyl-β-D-galactopyranoside **272** and 3-O-Pivaloylmorphine Tetrabenzoyl-β-D-galactofuranoside **271**.*

Scheme 2.11.

The formation of both 3-*O*-pivaloylmorphine tetrabenzoyl-β-D-galactopyranoside **272** and 3-*O*-pivaloylmorphine tetrabenzoyl-β-D-galactofuranoside **271** indicates that perbenzoylated β-D-galactofuranoses are highly reactive glycosyl donors. Unfortunately there was not enough time to investigate further acylation reactions which would possibly afford pentabenzoyl-β-D-galactofuranose **266** and pentabenzoyl-β-D-galactopyranose **264** exclusively.

2.1.2.3. Synthesis of Morphine-6-galactofuranoside

The potential biological activity of morphine-6-galactofuranoside **273** was of interest and a Zemplén deprotection of 3-*O*-pivaloylmorphine tetraisobutyryl-β-D-galactofuranoside **269** was attempted in order to obtain this novel morphine glycoside. 3-*O*-Pivaloylmorphine tetraisobutyryl-β-D-galactofuranoside **269** was treated with 10 equivalents of sodium methoxide in methanol for 22 hours affording morphine-6-galactofuranoside **273** in quantitative yield (scheme 2.12.).



Synthesis of The Morphine Galactofuranoside 273.

Scheme 2.12.

Examination of the crude product by ^{1}H NMR spectroscopy revealed the absence of the isobutyryl groups and pivaloyl group as expected. There was some overlapping of the morphine and galactosyl protons which were closer together on the spectra as a result of the high number of hydroxyl groups present. However, examination of the COSY spectra allowed all the signals to be assigned and the product identified as morphine-6-galactofuranoside **273**.

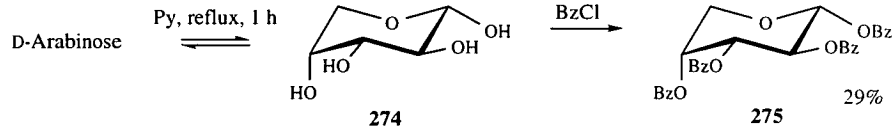
The large quantity of sodium methoxide required for the deprotection demonstrated the reduced lability of the isobutyryl groups compared to the acetyl protecting groups under Zemplén conditions. An attempt was made to purify the crude morphine galactofuranoside **273** by crystallisation from isopropanol-methanol, however, examination of the crystallised material revealed a small amount of impurity (for NMR spectra see appendix A.4.). Despite repeated attempts to recrystallise the material, elemental analysis did not indicate a high state of purity and as was the case with morphine-6-glucoside **31**, contamination from by-products such as sodium isobutyrate and sodium pivaloate from the deprotection reaction had probably occurred. This was disappointing as the morphine galactofuranoside **273** was not pure enough to undergo tests for biological activity and further attempts to recrystallise it resulted in a gradual decomposition.

2.2. Synthesis of Morphine-6-arabinopyranoside

Among the morphine glycosides synthesised there have been no attempts to prepare morphine-6-β-D-arabinopyranoside **48**.[51] Synthesis of morphine-6-β-D-arabinopyranoside **48** would, with morphine-6-β-D-galactopyranoside, morphine-6-β-D-galactofuranoside **273** and morphine-6-β-D-glucopyranoside **31**, complete a series of morphine β-glycosides which if biologically active could provide some novel analgesics.

2.2.1. Attempted Synthesis of Tetrabenzoyl-β-D-Arabinopyranose

The synthesis of tetrabenzoyl-β-D-arabinopyranose was attempted with a base catalysed acylation reaction, in which a suspension of D-arabinose was heated under reflux in pyridine for 1 hour before the addition of benzoyl chloride (scheme 2.13.).



Synthesis of Tetrabenzoyl-α-D-Arabinopyranose ***275****.*

Scheme 2.13.

The isolated material (in 29% yield) was examined by ^{1}H NMR spectroscopy and the signal corresponding to the anomeric proton was identified as a doublet at δ 6.25 ppm with a coupling constant of 5 Hz. The ^{1}H NMR data was in agreement with the data reported by Durette and Horton[163] for the tetrabenzoyl-α-D-arabinopyranose **275** and there was no evidence of the tetrabenzoyl-β-D-arabinose or the furanose isomers present.

	The % composition at 31 °C in aqueous solution at equilibrium.			
Sugar	% α-Pyranose	% β-Pyranose	% α-Furanose	% β-Furanose
Arabinose	60	35.5	2.5	2

Proportions of Anomers and Isomers Present at Equilibrium for D-Arabinose in Water.[56]

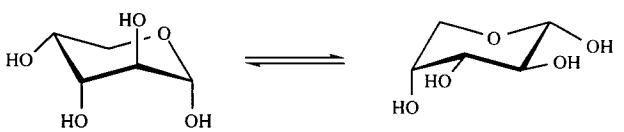
Table 2.2.

Arabinose is a sugar which readily adopts different conformations and in polar solvents such as water (table 2.2.)[56] the α-pyranose isomer is favoured as a result of the presence of an axial C2 hydroxyl group which increases the anomeric effect. The furanose conformation of D-arabinose is unfavourable as a result of steric

interactions, these are destabilising interactions between the substituents on C1 and C3 in the α-anomer and C1 and C2 in the β-anomer.[56]

The isomers adopted by D-arabinose after acylation have been studied by Durette and Horton[163] who found that the peracylated sugar prefers to adopt the α-pyranose isomer. It has been suggested by Durette and Horton[163] that the preference for the α-pyranose conformation is a result of an increase in the anomeric effect when an electronegative substituent, such as a benzoyl group, is placed on C1, C2, C3, and C4. As a result of this effect, the peracylated arabinopyranose will adopt a chair conformation where the anomeric substituent is axial. Durette and Horton[163] have also shown that the type of chair isomer peracylated D-arabinopyranose prefers depends on the substituent present. Durette and Horton[163] suggested that perbenzoylated arabinopyranose is found as the $^1C_4$ isomer possibly as a result of the attractive interactions between the *syn*-diaxial benzoxy groups at C2 and C4, however further investigation is required.

These findings would suggest that in refluxing pyridine a complex equilibrium exists between the α and β-anomers of D-arabinopyranose with the pyranose ring equilibrating between the $^1C_4$ and $^4C_1$ conformers, for example, the equilibrium between the $^1C_4$ conformer **276** and the $^4C_1$ conformer **274** of α-D-arabinopyranose (scheme 2.14.).



276 **274**

$^4C_1$ $^1C_4$

The Equilibrium Between the $^1C_4$ and $^4C_1$ Conformers of α-D-Arabinopyranose.

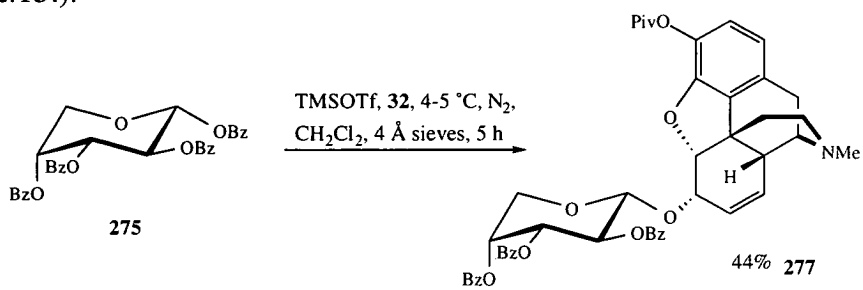
Scheme 2.14.

Unfortunately the use of a benzoyl protecting group for the acylation of D-arabinose encouraged the formation of the α-D-arabinopyranose and not the desired β-anomer, however, there was interest in observing whether the perbenzoylated α-D-arabinopyranose **275** would participate as a glycosyl donor in a TMSOTf promoted coupling reaction with 3-*O*-pivaloylmorphine **32**.

2.2.2. Synthesis of 3-*O*-Pivaloylmorphine Tribenzoyl-α-D-arabinopyranoside

A TMSOTf promoted glycosidation with tetrabenzoyl-α-D-arabinopyranose **275** and 3-*O*-pivaloylmorphine **32** afforded 3-*O*-pivaloylmorphine tribenzoyl-α-D-

arabinopyranoside **277** in 44% yield after chromatography and crystallisation (scheme 2.15.).

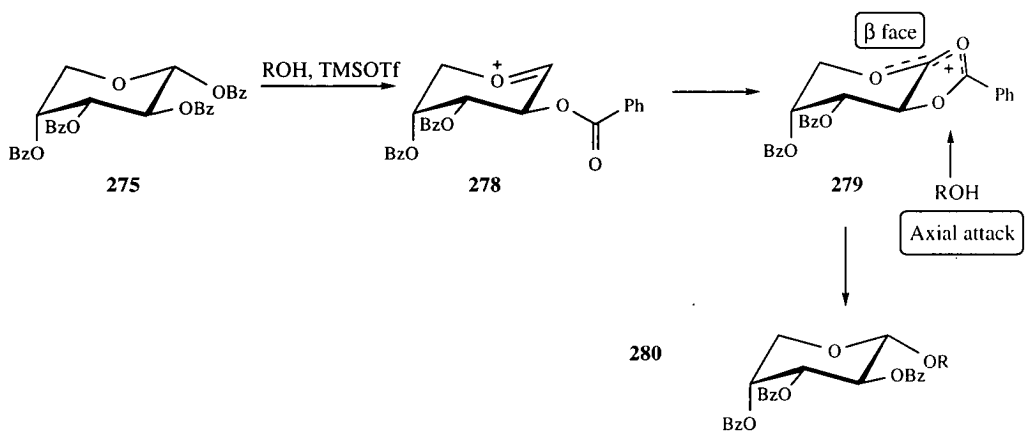


Synthesis of 3-O-Pivaloylmorphine Tribenzoyl-α-D-arabinopyranoside 277.

Scheme 2.15.

The formation of 3-*O*-pivaloylmorphine tribenzoyl-α-D-arabinopyranoside **277** was confirmed by examination of the ^{1}H NMR spectra. Three benzoyl groups were present at δ 7.23-8.13 ppm, however, the anomeric proton was less clearly defined as its signal appeared under the signal for the C5 morphine proton at δ 5.08 ppm. The signal for the C2 proton was more clearly visible at δ 5.52 ppm as a doublet of doublets with a coupling constant of 5 Hz which is the same value as the signal for the C2 proton in the ^{1}H NMR spectra of tetrabenzoyl-α-D-arabinopyranose **275**. As a result of the presence of the benzoyl protecting groups it is possible that the sugar moiety of 3-*O*-pivaloylmorphine tribenzoyl-α-D-arabinopyranoside **277** will retain the $^1C_4$ chair conformation observed in the perbenzoylated α-D-arabinopyranose **275**.

The mechanism for the TMSOTf promoted glycosidation of 3-*O*-pivaloylmorphine **32** with tetrabenzoyl-α-D-arabinopyranose **275** is possibly similar to the TMSOTf promoted glycosidation reactions discussed earlier (scheme 2.16.).



Proposed Mechanism for TMSOTf Promoted Glycosidation of 3-O-Pivaloylmorphine 32 with Tetrabenzoyl-α-D-arabinopyranose 275.[70]

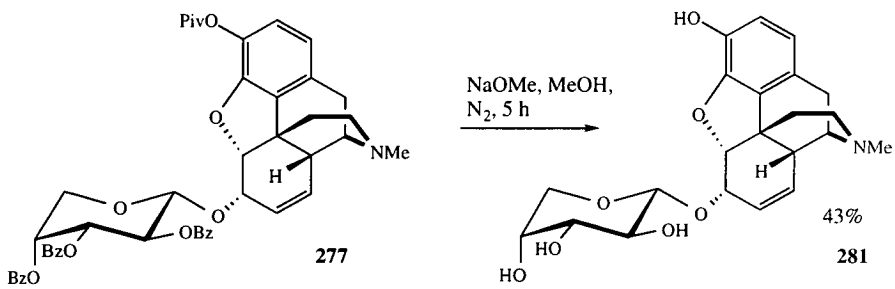
Scheme 2.16

Initial loss of the anomeric acyl group in scheme 2.16. gives the oxonium ion **278**, this is followed by neighbouring group participation from the C2 acyl group to afford intermediate **279** which will be attacked by the alcohol (ROH) from the α-face only affording the α-glycoside **280**. The β-face of intermediate **279** is inaccessible to the incoming nucleophile (ROH) and so the α-glycosidic linkage is formed exclusively.[70]

The use of pure acylated α-pyranose anomer as the glycosyl donor in the glycosidation reaction avoided the mixture of products seen with the synthesis of the previous morphine galactosides. 3-*O*-Pivaloylmorphine tribenzoyl-α-D-arabinopyranoside **277** is a novel morphine glucoside and its potential biological activity was of interest. An attempt was made to obtain the pure morphine-6-α-D-arabinopyranoside **278** using a Zemplén deprotection.

2.2.3. Synthesis of Morphine-6-α-D-arabinopyranoside

A Zemplén deprotection of 3-*O*-pivaloylmorphine tribenzoyl-α-D-arabinopyranoside **277** afforded morphine-6-α-D-arabinopyranoside **281** in 43% yield (scheme 2.17.).



*Synthesis of Morphine-6-α-D-arabinopyranoside **281**.*

Scheme 2.17.

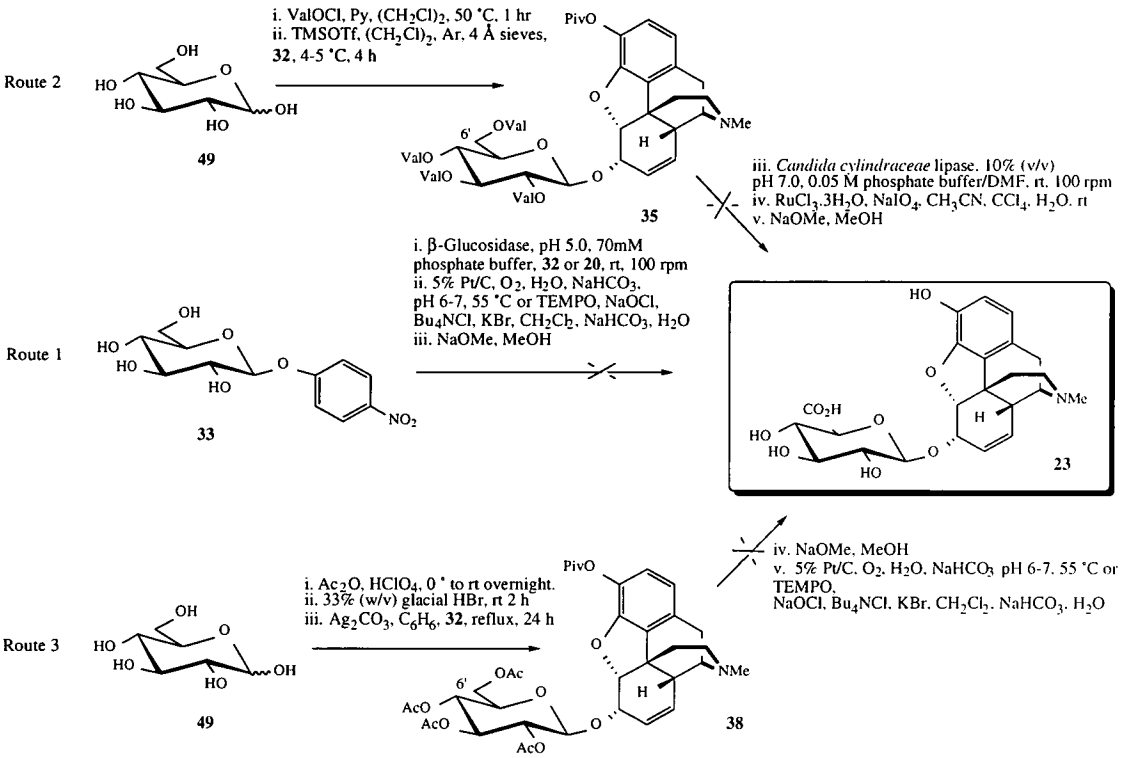
Examination of the crude product by ^{1}H NMR spectroscopy confirmed the formation of morphine-6-α-D-arabinopyranoside **281**. The ^{1}H NMR spectra revealed the loss of the benzoyl groups and pivaloyl group as expected while the signal at 4.48 ppm corresponded to the anomeric proton which appeared as a doublet with a coupling constant of 7 Hz. This high coupling constant would suggest that an α-glycosidic linkage is present, this is expected as the presence of an axial C2 on the sugar creates a strong anomeric effect which favours the α-anomer. It is also possible that the arabinose ring has retained the $^1C_4$ chair conformation and that this could be as a result of the attractive interactions between the *syn*-diaxial benzoxy groups at C2 and C4 suggested by Durette and Horton.[163] Another possibility is that the

introduction of a large substituent at the anomeric position of D-arabinose could result in the $^4C_1$ isomer being a higher energy or less accessible conformation than the $^1C_4$ chair conformation, thus, making this the preferred conformation for the sugar moiety of morphine-6-α-D-arabinopyranoside **278**.

The deprotection reaction went to completion much more rapidly than the Zemplén deprotection of 3-*O*-pivaloylmorphine tetraisobutyryl-β-D-galactofuranoside **269** demonstrating the increased lability of benzoyl groups compared to isobutyryl protecting groups under these conditions. Unfortunately, as was the case with morphine-6-galactofuranoside **273**, attempts to crystallise the crude morphine-6-α-D-arabinopyranoside **281** from isopropanol-methanol failed to produce a compound which was pure enough to test for biological activity (for NMR spectra see appendix A.5.).

Section Three; Summary and Conclusions

Attempts to synthesise M6G **23** using synthetic routes 1, 2 and 3 involved the use of a wide variety of chemical techniques and some interesting results were obtained. However, all three routes failed to produce the desired target compound **23** (scheme 1.1.).



Summary of the Three Synthetic Routes Towards M6G 23.

Scheme 1.1.

The transglycosidation reactions examined in route 1 confirmed the versatility that β-glucosidase from almonds has for a variety of alcohol acceptors. The model studies examining the glycosidations of alcohols containing an α-allyl group were very encouraging, but disappointingly, the attempts to synthesise morphine-6-glucoside **31** using morphine derivatives **32** and **20** as acceptors were unsuccessful.

The model studies for the glycosidation, selective deacylation, oxidation and deacylation steps of route two suggested this could provide a useful synthesis of M6G **23**. However, there was a potential problem with contamination of the product with ruthenium as this was observed in the product of the ruthenium catalysed oxidation of 1-*O*-methyl 2,3,4-tri-*O*-valeryl-α-D-glucoside **229**.

Unfortunately, the selective deacylation of 3-*O*-pivaloylmorphine tetravaleryl-β-D-glucoside **35** was unsuccessful. Although the particular lipase used (*Candida cylindracea* lipase) did not cleave the valerate ester at C6' of 3-*O*-pivaloylmorphine tetravaleryl-β-D-glucoside **35** there are currently many other sources of lipase commercially available, for example, *Aspergillus niger* lipase and porcine pancreatic lipase which might have accepted 3-*O*-pivaloylmorphine tetravaleryl-β-D-glucoside **35** as a substrate. The use of other ester groups such as acetates could also have been influential in increasing the acceptability of 3-*O*-pivaloylmorphine tetravaleryl-β-D-glucoside **35** as a substrate for these enzymes.

Route 3 resulted in the successful synthesis of morphine-6-glucoside **31** using a Koenigs-Knorr glycosidation promoted by silver (I) carbonate. Investigating the glycosidation reaction with some simple alcohols demonstrated the usefulness of the soluble Koenigs-Knorr promoter silver (I) triflate. This promoter could be useful for the synthesis of morphine-6-glucoside **31** if the reaction conditions are carefully controlled to avoid the formation of orthoester side-products.

The model studies investigating the oxidation of simple sugars to uronic acids demonstrated the suitability of TEMPO and platinum catalysts for this reaction. Pt/C was found to be a mild and selective oxidant affording high yields of the uronic acids. However, the reactions were slow, taking several hours to complete and contamination with platinum could render the oxidation product unsuitable for use in biological analysis. TEMPO was, therefore, considered a more suitable oxidant. Attempts to oxidise morphine-6-glucoside **31** using both Pt/C and TEMPO were unsuccessful and it would appear that milder reaction conditions will be required. Degradation of morphine-6-glucoside **31** could have been avoided by adjusting the oxidation reaction conditions. For example, during the TEMPO catalysed oxidation, lower temperatures, smaller quantities of sodium hypochlorite or even attempting the reaction in acidic or monophasic (aqueous) conditions might have proved effective, while attempting the platinum catalysed oxidations at lower temperatures could have been examined.

After the successful synthesis of morphine-6-glucoside **31**, attempts to synthesise analogues of this compound resulted in the production of two novel morphine glycosides, morphine-6-α-D-arabinopyranoside **281** and morphine-6-galactofuranoside **273**. The use of TMSOTf demonstrated that this is an efficient promoter for the glycosidation of morphine. The reaction conditions did not degrade the glycosyl donors unlike the conditions required for silver (I) carbonate promoted glycosidations. However, some problems were encountered. Firstly, suitable

protecting groups on the glycosyl donor were required to avoid migration of the acyl group onto the free hydroxyl group of the alcohol acceptor and secondly, the glycoside donor cannot be used as a mixture of β-furanose and β-pyranose isomers as the formation of an inseparable mixture of 3-*O*-pivaloylmorphine tetraisobutyrate-β-D-galactofuranoside **269**, and 3-*O*-pivaloylmorphine tetraisobutyrate)-galactopyranoside **270** demonstrated.

The problems encountered with the use of acetate protecting groups for the glycosyl donor in TMSOTf promoted glycosidation reactions led to attempts to synthesise alternative ester derivatives of D-galactose **144** and D-arabinose. In general, the acid catalysed esterifications to afford the α-anomer of acylated D-glucose **49** and D-galactose **144** were very straight forward reactions. Preparing the anomerically less favourable acylated β-anomers required elevated temperatures which created some problems particularly with D-galactose **144** as the stability of the furanose isomer resulted in a mixture of isomers forming during base catalysed acylations.

The base catalysed acylation of D-arabinose afforded the acylated α-pyranose isomer exclusively and unfortunately there was not time to attempt a synthesis of the acyl protected β-arabinose. The conformation of this sugar is strongly influenced by the type of protecting group present and in order to obtain the β-anomer the increased anomeric effect caused by an axial substituent on C2 would have to be overcome. It is possible that a less electron withdrawing group such as a benzyl group could influence the anomeric effect enough to obtain some of the β-anomer although it is likely that to achieve the β-anomer exclusively will be difficult.

The investigations with the monosaccharides D-glucose **49**, D-galactose **144**, and D-arabinose demonstrated how differently these sugars can behave under the same reaction conditions and provided a fascinating insight into the vast field of carbohydrate chemistry.

Chapter Three; Experimental

1.1. General Experimental

All solvents and reagents used were of analytical grade, obtained from commercial suppliers and used as supplied unless stated. Diethyl ether and tetrahydrofuran were dried over sodium benzophenone ketyl and dichloromethane was dried over calcium hydride prior to use. Petroleum ether with a boiling point (b.p.) range of 40-60 °C is referred to as "light petroleum". High purity water (18.2 MΩcm) was obtained from a Milli-Q Ultra Pure Water System.

Reagent quantities are reported in units of grams (g), milligrams (mg), millimoles (mmol) and millilitres (ml), units of time are reported as minutes (mins) and hours (h). Ambient or room temperature is referred to as "rt" and revolutions per minute as "rpm".

Solution concentrations are expressed in the number of moles (M) per dm^3, or as weight/volume (w/v) or volume/volume (v/v) ratios. Saturated (sat) solutions were prepared by dissolving the maximum quantity of solid in the required volume of solvent, aqueous solutions are referred to as "aq". Solutions that have a concentration greater than 2 M dm^3 are referred to as concentrated (conc) and saturated aqueous solutions of sodium chloride are referred to as "brine".

Analytical thin layer chromatography (tlc) was conducted on Merck (Darmstadt, Germany) Kieselgel 60 F_{254} 0.25 nm pre-coated, glass backed plates and Merck HPTLC RP-18 F_{254} S pre-coated glass backed plates, retention factor (R_f) values are reported. The plates were visualised using a low frequency ultraviolet (UV) lamp or the following dips[164] as stated;

Naphthoresorcinol; an acidic solution for free or protected carbohydrates, preparation; add conc hydrochloric acid (4 ml) to a solution of naphthoresorcinol (1,3-dihydroxynaphthalene) (0.2 g) and diphenylamine (0.4 g) in 95% (v/v) ethanol (100 ml), store in an amber vessel, heating of the tlc plate is required.

Bromocresol green; a basic solution for carboxylic acids, preparation; dissolve bromocresol green (0.04 g) in 96% (v/v) ethanol (100 ml), add 0.1 M sodium hydroxide until a blue coloration appears, store in an amber vessel, heating of the tlc plate is not required.

Potassium permanganate; a basic solution and universal reagent, preparation; dissolve potassium permanganate (10.0 g), potassium carbonate (50.0 g) and sodium hydroxide (40 pellets) into water (1 L), heating of the tlc plate is not required.

Iodoplatinate; a neutral solution for alkaloids and various nitrogen containing heterocyclic compounds, preparation; dissolve hydrogen hexachloroplatinate (IV) hydrate (chloric acid) (0.3 g) in water (100 ml), add a solution of potassium iodide (6.0 g) in water (100 ml), store in an amber vessel, heating of the tlc plate is not required.

Flash column-chromatography was conducted on Merck Kieselgel 60H silica gel, particle size 0.04-0.063 nm, 230-400 mesh ASTM under pressure and LiChroprep RP-18 was used for reverse-phase chromatography, particle size 40-63 μm under pressure. Size exclusion chromatography was conducted on Sigma Sephadex G-15 and a Pharmacia Biotech PD-10 column containing Sephadex G-25 M under pressure. Eluent systems are given in volume/volume (v/v) ratios.

Melting point (m.p.) and decomposition (decomp.) values, which are given in units of degrees centigrade (°C), were determined using a Gallenkamp melting point apparatus and are uncorrected.

Elemental analysis[†] was conducted in the Department of Chemistry, University of Edinburgh at the Elemental Analysis Laboratory using a Perkin Elmer 2400 CHN Elemental Analyser.

Infra-red (IR) measurements were made on a Perkin Elmer 881 Infra-red Grating Spectrometer, Bio-Rad FTS-7 Infra-red Spectrometer or a Perkin Elmer FT-IR Paragon 1000 Spectrometer as solutions, nujol mulls, thin films on sodium chloride plates or as a potassium bromide (KBr) disc as stated. The frequencies are measured in wavenumbers (cm^{-1}) and are reported as strong (s), medium (m) or weak (w), frequencies associated with aryl groups are referred to as "Ar".

Optical rotations were performed on an Optical Activity Ltd., AA-1000 polarimeter (serial number 84-16-08/A1000 DW) using a sodium D-line (589.5 nm) light source. Solvents were of spectroscopic grade, the cell path length was 0.5 or 1.0 dm and optical rotation ($[\alpha]_D$) values are given in units of 10^{-1} deg cm^2 g^{-1}.

Proton (^1H) and carbon (^{13}C) nuclear magnetic resonance (NMR) spectra were recorded on either a Brüker AM 250 instrument at 250 megahertz (MHz) and 63 MHz respectively, a Brüker AC 300 instrument at 300 and 75 MHz respectively, or a Variable Gemini 2000 instrument at 200 and 50 MHz respectively. Correlated spectroscopy (COSY) was recorded on a Brüker AM 250 instrument at 250 MHz. The chemical shifts (δ_H) of ^1H NMR spectra are reported to the nearest 0.01 ppm relative to the following residual protic solvents; deuterated chloroform ($CHCl_3$) (δ_H 7.25, singlet), deuterated methanol (CD_2HOD) (δ_H 3.35, quintet), deuterated dimethyl

[†] Elemental analysis is reported, where possible, for novel compounds obtained as solids or syrups.

sulfoxide (DMSO) (δ_H 2.50, quintet) and deuterated water (D*H*O) (δ_H 4.70, singlet). The spectral peaks are described using the following abbreviations; singlet (s), doublet (d), triplet (t), quartet (q), doublet of doublets (dd), triplet of doublets (td), quartet of doublets (qd), multiplet (m), and broad (b). Coupling constants (*J*) are quoted to the nearest 0.5 Hz and are further qualified as $^3J_{1,2}$ which denotes the coupling of the protons at carbons C1 and C2 which are separated by 3 bonds, geminal (gem) coupling is reported as $^2J_{gem}$. The protons are reported as ^{x}CH and are further qualified as axial (ax), equatorial (eq) and where a mixture of isomers is present[†] as alpha pyranose (αP), beta pyranose (βP), beta furanose (βF), furanose (F), and pyranose (P).

For ^{13}C NMR spectra chemical shifts (δ_C) are reported to the nearest 0.01 ppm relative to the following residual solvents; *C*DCl$_3$ (δ_C 77.00, t), *C*D$_3$OD (δ_C 49.00, septet), and DMSO (δ_C 39.70, septet) and the carbon-substituted patterns were assigned using Distortionless Enhancement by Polarisation Transfer (DEPT). The spectral peaks are described using the following abbreviations; quaternary carbon (Q), aryl (Ar), anomeric carbon (An) and where a mixture of isomers is present are further qualified as described for ^{1}H NMR spectral peaks.

Mass spectra (MS) were recorded at the Chemistry Department, University of Exeter using a Kratos Profile HV-3 high-resolution instrument, at the Chemistry Department, University of Edinburgh using a MS-50 high-resolution instrument or at the EPSRC Mass Spectrometry Centre, Swansea using a VG ZAB-E high-resolution instrument under Electron Impact (E.I.), Chemical Ionisation (C.I.) or Fast Atom Bombardment (F.A.B.) conditions as stated. The mass to charge ratios (*m/z*) of the molecular ion (M^+) and daughter fragments are reported with the most abundant ion arbitrarily assigned a value of 100%.

Enzymes were obtained from Sigma-Aldrich Company Ltd. and were used as supplied. β-Glucosidase was used as a crude lyophilised powder from almonds (14 U per mg). Enzyme activity is defined in units where one unit hydrolyses 1.0 μmol of glucose from salicin per min at pH 5.0 at 37 °C. *Candida cylindracea* lipase type VII was used as a crude lyophilised powder (700 - 1, 500 U per mg), enzyme activity is defined in units where one unit hydrolyses 1 μmol of fatty acid from a triglyceride per h at pH 7.2 at 37 °C.

Analytical high pressure liquid chromatography (HPLC) was carried out using a Waters™ 600 pump fitted with a Rheodyne 7725i manual injector and a

[†] Mixtures of anomers are regarded as different isomers of the same compound, mixtures of furanose and pyranose forms of the same monosaccharide are considered as separate compounds.

Waters™ 486 detector set at 254 nm. A reverse phase analytical column (25 x 4.6 mm internal diameter) packed with Spherisorb 5 μ octadecylsilyl (ODS) 2 was used. Solvents were of HPLC grade, filtered through Phenomenex (No. AFO-0504) nylon filter membranes, 0.45 μm thickness, 47 mm diameter prior to use and then purged at a rate of 40 ml per min with helium. Samples were dissolved in high purity water and injected onto the column through a 20 μl injection loop. The solvent system of acetonitrile and pH 6.5, 5 mM phosphate buffer (solutions of 5 mM dipotassium hydrogen phosphate and 5 mM potassium dihydrogen phosphate in high purity water were used in the appropriate proportions to give the desired pH) was applied as a gradient at a rate of 1 ml per min (figure 1.1).

Time (mins)	Acetonitrile %	Phosphate buffer %
0-4.3	10	90
4.3-4.6	20	80
4.6-5.0	30	70
5.0-6.0	40	60
6.0-7.0	45	55
7.0-15.0	50	50

HPLC Gradient.

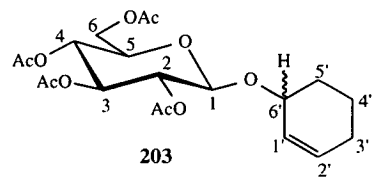
Figure 1.1

Note:

For compounds obtained as an inseparable mixture or synthesised using a procedure previously reported in the literature, the recorded data is limited to the m.p. (if a solid), R_f, IR, ^{1}H NMR, ^{13}C NMR and nominal MS values.

1.2. Synthesis of Morphine-6-glucuronide; Synthetic Route One

1.2.1. Cyclohex-6'-enyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside 203

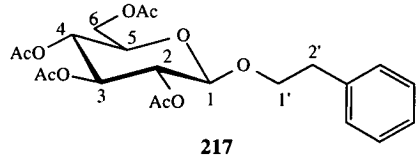


203

A solution of β-glucosidase from almonds (4 mg, 50 U) in pH 5.0, 70 mM phosphate buffer (1 ml) was added to a suspension of *para*nitrophenyl-β-D-glucopyranoside **33** (0.100 g, 0.33 mmol) in 2-cyclohexen-1-ol **204** (1.630 g, 16.60 mmol). The reaction mixture was stirred at rt at 100 rpm for 16 h; tlc at that time revealed that the reaction had gone to completion. The reaction was quenched by the addition of MeOH (5 ml) and the solvent removed by rotary evaporation to give a yellow solid (2.099 g) which was loaded onto silica (1 g). Flash column-chromatography (9:2:1, EtOAc-CH_2Cl_2-MeOH) afforded a yellow oil (6 mg) which was suspended in dry Py (0.5 ml) and treated with Ac_2O (0.541 g, 5.30 mmol) and DMAP (3 mg, 0.02 mmol). The reaction mixture was stirred under an atmosphere of N_2 at rt for 1 h; tlc at that time revealed that the reaction had gone to completion. The reaction was poured onto a mixture of ice (5 g) and 2 M aq HCl (10 ml), extracted with EtOAc (3x 10 ml) and the combined organic extracts washed with 1 M aq HCl (2x 50 ml), sat aq $NaHCO_3$ (2x 50 ml), water (50 ml), brine (50 ml), dried over $MgSO_4$ and the solvent removed by rotary evaporation to give a yellow oil (0.072 g). Flash column-chromatography (1:1, light petroleum-EtOAc) of the crude acetylated product followed by crystallisation (light petroleum-EtOAc) afforded *the title compound* in a 3:2 ratio of diastereoisomers[†] as a white solid (2 mg, 1%); R_f 0.56 (1:1, light petroleum-EtOAc); the spectroscopic data was in agreement with that reported for cyclohex-6'-enyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside **196** (section 1.4.5.).

[†] Diastereoisomer ratio is calculated from the relative peak heights of the anomeric protons in the ^1H NMR spectrum.

1.2.2. 2'-Phenethyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside 217

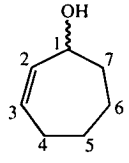


217

A solution of β-glucosidase from almonds (0.023 g, 322 U) in pH 5.0, 70 mM phosphate buffer (0.8 ml) was added to a suspension of *para*nitrophenyl-β-D-glucopyranoside **33** (1.903 g, 6.32 mmol) and phenethyl alcohol **198** (4.637 g, 37.96 mmol) in pH 5.0, 70 mM phosphate buffer (30 ml). The reaction mixture was stirred at 100 rpm for 15 h at rt when further β-glucosidase (0.023 g, 322 U) in pH 5.0, 70 mM phosphate buffer (0.8 ml) was added. Tlc revealed that the reaction had gone to completion after 24 h, the reaction was quenched by the addition of MeOH (50 ml) and the solvent removed by rotary evaporation to give a yellow solid (6.534 g) which was loaded onto silica (5 g). Flash column-chromatography (9:1, EtOAc-MeOH) afforded a yellow syrup (0.498 g) which was suspended in dry Py (5 ml) and treated with Ac_2O (1.08 g, 10.60 mmol) and DMAP (11 mg, 0.09 mmol) and stirred under an atmosphere of N_2 at rt. Tlc revealed that the reaction had gone to completion after 7 h and the reaction was poured onto a mixture of ice (20 g) and 2 M aq HCl (25 ml), extracted with EtOAc (3x 10 ml) and the combined organic extracts washed with 2 M aq HCl (2x 100 ml), sat aq $NaHCO_3$ (2x 100 ml), water (100 ml), brine (100 ml), dried over $MgSO_4$ and the solvent removed by rotary evaporation to give a yellow oil (0.659 g). Flash column-chromatography (1:1, light petroleum-EtOAc) of the crude acetylated product followed by crystallisation (light petroleum-ether) afforded *the title compound* as a white crystalline solid (0.358 g, 13%); R_f 0.59 (6:4, light petroleum-EtOAc); m.p. 64-65 °C (from light petroleum-EtOAc); $[\alpha]_D^{21}$ -20.94 (*c* 1.02, $CHCl_3$); ν_{max} ($CHCl_3$)/cm^{-1} 3028s (CH), 2948s (CH), 2884s (CH), 1750s (CO_2), 1604m (Ar CH), 1432s (CH), 1374s (CH_3), 1051s (C-O-C); δ_H (250 MHz; $CDCl_3$) 1.87 (3H, s, OAc), 1.97 (3H, s, OAc), 2.00 (3H, s, OAc), 2.06 (3H, s, OAc), 2.87 (2H, t, $^3J_{1',2'}$ 7, $^{2'}CH_2$), 3.65 (2H, m, $^2J_{gem}$ 10, $^{1'}CH$, ^5CH), 4.11 (2H, m, $^2J_{gem}$ 10, $^{1'}CH$, ^6CH), 4.24 (1H, dd, $^2J_{gem}$ 12, ^6CH), 4.46 (1H, d, $^3J_{1,2}$ 8, ^1CH), 4.97 (1H, dd, $^3J_{2,1}$ 8, ^2CH), 5.60 (1H, dd, $^3J_{4,3}$ 9, ^4CH), 5.16 (1H, t, $^3J_{3,4}$ 9, ^3CH), 7.13-7.30 (5H, m, C_6H_5); δ_C (63 MHz; $CDCl_3$) 20.42 (CH_3), 20.47 (2x CH_3), 20.62 (CH_3), 35.75 (CH_2), 61.77 (CH_2), 68.23 (CH), 70.52 (CH_2), 70.94 (CH), 71.63 (CH), 72.62 (Ar CH), 100.61 (An CH), 126.16 (Ar CH), 128.19 (2x Ar CH), 128.80 (2x Ar CH), 138.27 (Ar Q), 169.14 (Q), 169.27 (Q), 170.16 (Q), 170.57 (Q); *m/z* 452 (M^+, 1%), 451 (M^+ -H, 1%),

393 (M^+ -$C_2H_3O_2$, 3%), 331 (M^+ -C_8H_9O, 74%), 169 (M^+ -$C_{14}H_{19}O_6$, 94%), 105 (M^+ -$C_{16}H_{27}O_8$, 69%), 43 (M^+ -$C_{20}H_{25}O_9$, 100%); [Found MH^+ 453.1733. $C_{22}H_{28}O_{10}$ requires *M*H 453.1761] (F.A.B.).

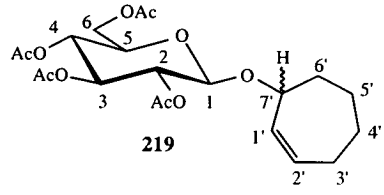
1.2.3. 2-Cyclohepten-1-ol 206



206

A solution of $CeCl_3.7H_2O$ (8.454 g, 22.69 mmol) in MeOH (25 ml) was added dropwise at rt to a stirring solution of cyclopentenone (2.495 g, 22.56 mmol) in MeOH (25 ml). Finally $NaBH_4$ (0.852 g, 22.53 mmol) was added over 15 mins, the temperature of the reaction mixture increased to 35 °C and when the effervescence had subsided, the reaction mixture was cooled to rt with an ice bath; the formation of a white precipitate was observed. Tlc at that time revealed that the reaction had gone to completion, the reaction mixture was poured onto ice, neutral pH was achieved with the addition of 1 M aq HCl and the solution extracted with ether (4x 50 ml), dried over $MgSO_4$ and the solvent removed by rotary evaporation to give a yellow oil (13.521 g). Flash column-chromatography (ether) of the crude residue afforded *the title compound* as a colourless oil (2.060 g, 81%); R_f 0.35 (1:1, light petroleum-EtOAc); ν_{max} (nujol)/cm^{-1} 3336s (OH), 3022m (C=*C-H*), 2924s (CH), 2851s (CH), 1652m (C=C); δ_H (250 MHz; $CDCl_3$) 1.17-2.21 (8H, m, $^4CH_2$, $^5CH_2$, $^6CH_2$, $^7CH_2$), 2.32 (1H, m, OH), 4.35 (1H, d, $^3J_{1,OH}$ 7, ^1CH), 5.69 (2H, m, $^3J_{2,3}$ 2, ^2CH, ^3CH); δ_C (50 MHz; $CDCl_3$) 26.41 (CH_2), 26.56 (CH_2), 28.29 (CH_2), 36.37 (CH_2), 71.78 (CH), 129.72 (C=*C*H), 137.71 (C=*C*H); *m/z* 113 (MH^+, 21%), 112 (M^+, 66%), 111 (M^+ -H, 41%), 97 (M^+ -CH_3, 68%), 95 (M^+ -OH, 68%), 55 (M^+ -C_3H_5O, 100%) (E.I.).

1.2.4. Cyclohept-7'-enyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside 219



219

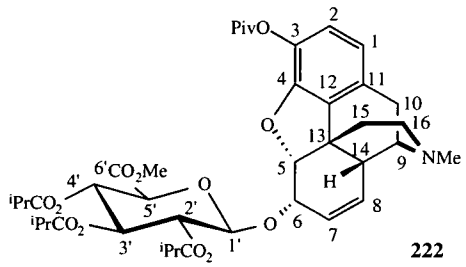
A solution of β-glucosidase from almonds (1 mg, 140 U) in pH 5.0, 70 mM phosphate buffer (1 ml) was added to a suspension of *para*nitrophenyl-β-D-glucopyranoside **33** (0.107 g, 0.36 mmol) and 2-cyclohepten-1-ol **197** (0.251 g, 2.24 mmol) in pH 5.0, 70 mM phosphate buffer (5 ml) and stirred at rt at 100 rpm. Tlc revealed that the reaction had gone to completion after 2 h, the reaction was quenched by the addition of MeOH (25 ml), the solvent removed by rotary evaporation to give a yellow solid (0.199 g) which was loaded onto silica (1 g). Flash column-chromatography (9:1, EtOAc-MeOH) afforded a colourless syrup (2 mg) which was suspended in dry Py (1 ml) and treated with Ac_2O (0.541 g, 5.30 mmol) and DMAP (1 mg, 0.008 mmol) with stirring at rt under an atmosphere of N_2. Tlc revealed that the reaction had gone to completion after 1 h and the reaction was poured onto a mixture of ice (5 g) and 1 M aq HCl (10 ml), extracted with EtOAc (2x 25 ml) and the combined organic extracts washed with 1 M aq HCl (2x 50 ml), sat aq $NaHCO_3$ (2x 50 ml), water (50 ml), brine (50 ml), dried over $MgSO_4$ and the solvent removed by rotary evaporation to give a colourless oil (0.011 g). Flash column-chromatography (3:2, light petroleum-EtOAc) of the crude acetylated product afforded *the title compound* in a 3:2 ratio of diastereoisomers[†] as a colourless syrup (5 mg, 1%); R_f 0.55 (1:1, light petroleum-EtOAc); $[\alpha]_D^{22}$ -42.33 (*c* 0.11, $CHCl_3$); ν_{max} ($CHCl_3$)/cm^{-1} 2927m (CH), 2854m (CH), 1753s (CO_2), 1373m (CH_3), 1063m (C-O-C), 1039s (C-O-C); δ_H (250 MHz; $CDCl_3$) 1.20-1.67 (8H, m, $^{3'}CH_2$, $^{4'}CH_2$, $^{5'}CH_2$, $^{6'}CH_2$), 1.99 (3H, s, OAc), 2.01 (3H, s, OAc), 2.02 (1.2H, s, OAc), 2.03 (1.8H, s, OAc), 2.06 (1.2H, s, OAc), 2.07 (1.8H, s, OAc), 3.67 (1H, m, ^5CH), 4.11 (1H, dt, $^2J_{gem}$ 12, ^6CH), 4.26 (1H, dd, $^2J_{gem}$ 12, ^6CH), 4.34 (1H, bdd, $^3J_{7',1'}$ 8, $^{7'}CH$), 4.57 (0.4H, d, $^3J_{1,2}$ 8, ^1CH), 4.60 (0.6H, d, $^3J_{1,2}$ 8, ^1CH), 4.97 (1H, d, $^3J_{2,1}$ 8, ^2CH), 5.07 (1H, d, $^3J_{4,3}$ 10, ^4CH), 5.19 (1H, d, $^3J_{3,4}$ 10, ^3CH), 5.67-5.87 (2H, m, $^{1'}CH$, $^{2'}CH$); δ_C (63 MHz; $CDCl_3$) 20.49 (CH_3), 20.52 (CH_3), 20.59 (CH_3), 20.63 (CH_3), 26.36 (0.6x CH_2), 26.41 (0.4x CH_2), 26.84 (0.6x CH_2), 28.30 (0.4x CH_2), 28.46 (0.6x CH_2), 29.58 (0.4x CH_2),

[†] Diastereoisomer ratio is calculated from the relative peak heights of the anomeric protons in the ^1H NMR spectrum.

32.63 (0.4x CH_2), 33.50 (0.6x CH_2), 61.99 (CH_2), 68.47 (CH), 71.38 (0.6x CH), 71.44 (0.4x CH), 71.60 (1.4x CH), 72.85 (0.6x CH), 78.46 (0.6x CH), 80.09 (0.4x CH), 99.07 (0.6x An CH), 99.67 (0.4x An CH), 130.66 (0.4x CH), 132.56 (0.6x CH), 134.12 (0.6x CH), 135.14 (0.4x CH), 169.09 (0.4x Q), 169.16 (0.6x Q), 169.29 (Q), 170.23 (Q), 170.58 (Q); *m/z* 443 (MH^+, 1%), 331 (M^+ -$C_7H_{11}O$, 31%), 271 (M^+ -$C_9H_{15}O_3$, 1%), 169 (M^+ -$C_{13}H_{21}O_6$, 66%), 111 (M^+ -$C_{14}H_{19}O_9$, 6%); [Found MH^+ 443.1926. $C_{21}H_{30}O_{10}$ requires *M*H 443.1917] (F.A.B.).

1.3. Synthesis of Morphine-6-glucuronide; Synthetic Route Two

1.3.1. Morphine-3-*O*-pivaloyl-6-(methyl (2',3',4'-tri-*O*-isobutyryl)-β-D-glucopyranosid)uronate 222

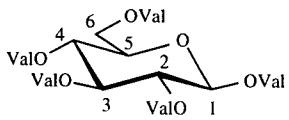


222

A solution of methyl (1,2,3,4-tetra-*O*-isobutyryl-β-D-glucopyranosid)uronate **221** (2.480 g, 5.18 mmol) in dry $(CH_2Cl)_2$ (5 ml) was added to 3-*O*-pivaloylmorphine **32** (0.740 g, 2.00 mmol) and 4 Å molecular sieves under an atmosphere of Ar. The reaction mixture was stirred at rt for 1 h and then at 4-5 °C for 30 mins, whereafter TMSOTf (2.013 g, 9.06 mmol) was added, the reaction mixture stirred for a further 4 h at 4-5 °C when tlc revealed that the reaction had gone to completion. The reaction mixture was poured onto a mixture of ice (10 g) and sat aq $NaHCO_3$ (30 ml), extracted with EtOAc (2x 25 ml) and the combined organic extracts washed with 5% (w/v) aq citric acid (3x 20 ml), sat aq $NaHCO_3$ (2x 20 ml), water (2x 20 ml), brine (2x 20 ml), dried over Na_2SO_4 and the solvent removed by rotary evaporation to give a white solid (3.110 g). Flash column-chromatography (1:9, MeOH-CH_2Cl_2) of the crude residue followed by crystallisation (^{i}PrOH-water) afforded *the title compound* as a white crystalline solid (0.740 g, 48%); R_f0.46 (1:9, MeOH-CH_3Cl); m.p. 191-192 °C (from ^{i}PrOH-water); [Found: C, 63.83%; H, 7.08%; N, 1.58%. $C_{41}H_{55}NO_{13}$ requires C, 63.95%; H, 7.21%; N, 1.82%]; $[\alpha]_D^{30}$ -110.38 (*c* 0.99, $CHCl_3$); ν_{max} ($CHCl_3$)/cm^{-1} 2940s (CH), 2900s (CH), 2840m ($CO\mathit{CH}_3$), 2760m (NMe), 1720s (CO_2), 1020s (C-O-C); δ_H (250 MHz; $CDCl_3$) (COSY) 1.11-1.35 (18H, m, 6x $CHC\mathit{H}_3$), 1.38 (9H, s, $C(C\mathit{H}_3)_3$), 1.90-2.08 (3H, m, $C\mathit{H}CH_3$, $^{15}CH_{eq}$, $^{15}CH_{ax}$), 2.30 (1H, bdd, $^2J_{gem}$ 19, $^{10}CH_{ax}$), 2.40 (3H, s, NMe), 2.42-2.58 (4H, m, 2x $C\mathit{H}CH_3$, $^{16}CH_{eq}$, $^{16}CH_{ax}$), 2.62 (1H, bt, $^3J_{14,9}$ 3, ^{14}CH), 3.04 (1H, d, $^2J_{gem}$ 19, $^{10}CH_{eq}$), 3.35 (1H, m, $^3J_{9,14}$ 3, ^{9}CH), 3.70 (3H, s, OMe), 4.11 (1H, m, $^3J_{6,7}$ 10, ^{6}CH), 4.28 (1H, m, $^{4'}$CH), 4.92 (1H, dd, $^3J_{5',4'}$ 7, $^{5'}$CH), 4.95 (1H, d, $^3J_{1',2'}$ 8, $^{1'}$CH), 5.07 (1H, m, $^3J_{2',1'}$ 8, $^{2'}$CH), 5.24-5.32 (3H, m, $^3J_{7,8}$ 10, ^{7}CH, ^{5}CH, $^{3'}$CH), 5.65 (1H, bd, $^3J_{8,7}$ 10, ^{8}CH), 6.52 (1H, d, $^3J_{2,1}$ 9, ^{1}CH), 6.60 (1H, d, $^3J_{2,1}$ 9, ^{2}CH); δ_C (63 MHz; $CDCl_3$) 18.57 (CH_3), 18.63 (CH_3), 18.93 (CH_3), 20.66 (CH_2), 27.00 (6x CH_3), 33.62 (3x CH), 35.56 (CH_2), 38.85 (Q),

40.91 (CH), 42.92 (NMe), 43.70 (Q), 46.07 (CH_2), 52.65 (OMe), 58.46 (CH), 68.95 (CH), 70.67 (CH), 71.29 (CH), 72.61 (CH), 73.18 (CH), 89.64 (CH), 98.75 (An CH), 118.77 (CH), 121.49 (CH), 128.52 (CH), 130.32 (CH), 131.13 (Q), 131.64 (Q), 131.90 (Q), 150.11 (Q), 167.30 (Q), 175.10 (Q), 175.16 (Q), 175.73 (Q), 176.28 (Q); *m/z* 770 (MH^+, 6%), 769 (M^+, 31%), 685 (M^+ -$C_4H_4O_2$, 7%), 354 (M^+ -$C_{19}H_{27}O_{10}$, 19%); [Found MH^+ 770.3784. $C_{41}H_{55}NO_{13}$ requires *M*H 770.3752] (F.A.B.).

1.3.2. β-D-Glucopyranose Pentavalerate 34

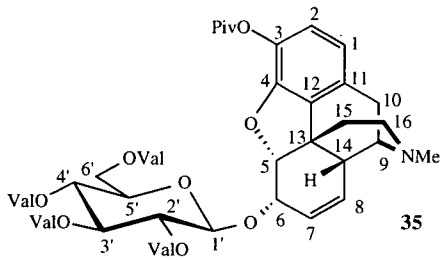


34

A solution of valeryl chloride (5.486 g, 45.50 mmol) in dry $(CH_2Cl)_2$ (5 ml) was added dropwise to a suspension of D-(+)-glucose **49** (1.020 g, 5.66 mmol) in dry Py (10 ml) and dry $(CH_2Cl)_2$ (5 ml). The resulting mixture was stirred at 50 °C with the exclusion of water provided by a drying tube containing self indicating silica gel, tlc revealed that the reaction had gone to completion after 1 h. The reaction mixture was allowed to cool to rt, extracted with CH_2Cl_2 (6x 20 ml) and the combined organic extracts washed with 1 N aq HCl (2x 50 ml), sat aq $NaHCO_3$ (2x 50 ml), water (2x 50 ml), brine (2x 50 ml), dried over $MgSO_4$ and the solvent removed by rotary evaporation to give a dark orange oil (4.570 g). Flash column-chromatography (100% hexane then a gradient to 100% EtOAc) of the crude residue afforded an inseparable mixture in a 3:1 ratio of *the title compound* and α-D-glucopyranose pentavalerate **225** as an orange syrup (4.410 g, quantitative yield; approximately 2.205 g, 3.67 mmol of *the title compound*); R_f 0.61 (4:1, hexane-EtOAc); $[\alpha]_D^{24}$ +22.33 (*c* 1.17, $CHCl_3$); ν_{max} (neat)/cm^{-1} 2962s (CH), 2936s (CH), 2875s (CH), 1755s (CO_2), 1381m (CH_3), 1078s (C-O-C); δ_H (300 MHz; $CDCl_3$) 0.85-0.98 (15H, m, 5x βP-CH_3, 5x αP-CH_3), 1.24-1.42 (10H, m, 5x βP-CH_2CH_3, 5x αP-CH_2CH_3), 1.42-1.69 (10H, m, 5x βP-OCH_2CH_2 , 5x βP-OCH_2CH_2), 2.18-2.30 (10H, m, 5x βP-OCH_2, 5x αP-OCH_2), 3.83 (0.75H, m, $^3J_{5,4}$ 10, βP-^{5}CH), 4.06-4.26 (2.25H, m, $^2J_{gem}$ 12, βP-$^6CH_2$, αP-^{5}CH, αP-$^6CH_2$), 5.06-5.20 (2H, m, $^3J_{2,1}$ 4, βP-^{2}CH, βP-^{4}CH, αP-^{2}CH, αP-^{4}CH), 5.29 (0.75H, t, $^3J_{3,4}$ 10, βP-^{3}CH), 5.51 (0.25H, t, $^3J_{3,4}$ 10, αP-^{3}CH), 5.73 (0.75H, d, $^3J_{1,2}$ 8, βP-^{1}CH), 6.35 (0.25H, d, $^3J_{1,2}$ 4, αP-^{1}CH); δ_C (75 MHz; $CDCl_3$) 13.55 (2.25x βP-CH_3, 0.75x αP-CH_3), 13.62 (1.5x βP-CH_3, 0.5x αP-CH_3), 22.16 (3.75x βP-CH_2, 1.25x αP-CH_2), 26.53 (1.5x βP-CH_2, 0.5x αP-CH_2), 26.74 (1.5x βP-CH_2, 0.5x

αP-CH_2), 26.85 (0.75x βP-CH_2, 0.25x αP-CH_2), 33.66 (1.5x βP-CH_2, 0.5x αP-CH_2), 33.80 (1.5x βP-CH_2, 0.5x αP-CH_2), 34.49 (0.75x βP-CH_2, 0.25x αP-CH_2), 61.38 (0.75x βP-CH_2, 0.25x αP-CH_2), 67.67 (0.75x βP-CH, 0.25x αP-CH), 70.02 (0.75x βP-CH, 0.25x αP-CH), 72.41 (0.75x βP-CH, 0.25x αP-CH), 73.23 (0.75x βP-CH, 0.25x αP-CH), 91.65 (0.75x An βP-CH, 0.25x An αP-CH), 172.04 (3.75x βP-Q, 1.25x αP-Q); *m/z* 600 (M^+, 6%), 584 (M^+ -CH_4, 33%), 553 (M^+ -CH_3O_2, 10%), 500 (M^+ -$C_5H_8O_2$, 29%), 499 (M^+ -$C_5H_9O_2$, 100%), 498 (M^+ -$C_5H_{10}O_2$, 16%); [Found M^+ 600.3535. $C_{31}H_{52}O_{11}$ requires *M* 600.3510] (E.I.).

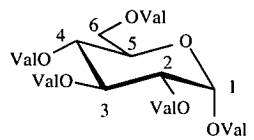
1.3.3. 3-*O*-Pivaloylmorphine-6-(2',3',4',6'-tetra-*O*-valeryl)-β-D-glucopyranoside 35



To a stirred solution of 3-*O*-pivaloylmorphine **32** (0.670 g, 1.82 mmol) in dry $(CH_2Cl)_2$ (2.5 ml) in the presence of 4 Å molecular sieves under an atmosphere of Ar was added a mixture of α-D-glucopyranose pentavalerate **225** and β-D-glucopyranose pentavalerate **34** (2.000 g; approximately 1.021 g, 1.70 mmol of β-D-glucopyranose pentavalerate **34**) in dry $(CH_2Cl)_2$ (2.5 ml). The reaction mixture was stirred for a further 1 h, whereafter, the temperature was lowered to 0 °C and TMSOTf (90.805 g, 3.62 mmol) was added dropwise while maintaining the temperature below 5 °C. The reaction mixture was stirred for a further 4 h at 0 °C until tlc revealed that the reaction had gone to completion. The reaction mixture was poured onto a mixture of ice (20 g) and sat aq $NaHCO_3$ (20 ml), the aqueous layer was isolated and extracted with EtOAc (2x 25 ml). The combined organic extracts were washed with 5% aq citric acid (5x 25 ml), sat aq $NaHCO_3$ (2x 25 ml), water (2x 25 ml), brine (2x 25 ml), dried over $MgSO_4$ and the solvent removed by rotary evaporation to give a dark yellow oil (1.700 g). Flash column-chromatography (1:9, MeOH-CH_2Cl_2) of the crude residue followed by crystallisation (^{i}PrOH-water) afforded *the title compound* as a white crystalline solid (0.016 g, approximately 14%); R_f 0.43 (1:9, MeOH-CH_2Cl_2); m.p. 82-84 °C (from ^{i}PrOH-water); $[\alpha]_D^{26}$ -53.75 (*c* 2.00, $CHCl_3$); [Found: C, 66.29%; H, 7.94%; N, 1.69%. $C_{48}H_{69}NO_{13}$ requires C, 66.40%; H, 8.02%; N, 1.61%]; ν_{max} ($CHCl_3$)/cm^{-1} 2920s (CH), 2880s (CH), 2820s (CH), 1720s (CO_2),

1010m (C-O-C); δ_H (250 MHz; $CDCl_3$) (COSY) 0.78-0.92 (12H, m, 4x CH_2CH_3), 1.22-1.47 (8H, m, 4x CH_2CH_3), 1.35 (9H, s, $C(CH_3)_3$), 1.49-1.64 (8H, m, 4x OCH_2CH_2), 1.93 (1H, bs, $^{15}CH_{eq}$), 1.98 (1H, bs, $^{15}CH_{ax}$), 2.18-2.34 (8H, m, 4x OCH_2), 2.43-2.52 (2H, m, $^{10}CH_{ax}$, $^{16}CH_{ax}$), 2.56 (3H, s, NMe), 2.77 (1H, bd, $^{16}CH_{eq}$), 2.94 (1H, bs, ^{14}CH), 3.06 (1H, d, $^2J_{gem}$ 19, $^{10}CH_{eq}$), 3.54 (1H, bs, ^9CH), 3.74 (1H, m, $^3J_{5',4'}$ 10, $^{5'}CH$), 4.16 (2H, d, $^3J_{6',5'}$ 4, $^{6'}CH_2$), 4.26 (1H, m, $^3J_{6,5}$ 3, ^6CH), 4.83-5.00 (2H, m, $^3J_{2',1'}$ 8, $^{2'}CH$, ^5CH), 5.04 (1H, d, $^3J_{1',2'}$ 8, $^{1'}CH$), 5.05-5.20 (2H, m, $^3J_{4',5'}$ 10, $^{4'}CH$, $^{3'}CH$), 5.24 (1H, dt, $^3J_{7,8}$ 10, ^7CH), 5.73 (1H, bd, $^3J_{8,7}$ 10, ^8CH), 6.55 (1H, d, $^3J_{1,2}$ 8, ^1CH), 6.70 (1H, d, $^3J_{2,1}$ 8, ^2CH); δ_C (63 MHz; $CDCl_3$) 13.50 (2x CH_3), 13.57 (2x CH_3), 21.14 (CH_2), 21.98 (CH_2), 22.05 (2x CH_2), 26.63 (CH_2), 26.66 (2x CH_2), 26.90 (CH_2), 27.03 (3x CH_3), 29.57 (Q), 33.56 (OCH_2), 33.61 (2x OCH_2), 33.75 (OCH_2), 34.58 (CH_2), 38.90 (Q), 39.74 (CH_2), 42.41 (NMe), 43.11 (Q), 46.38 (CH_2), 59.08 (CH), 61.69 (CH_2), 68.02 (CH), 71.00 (CH), 72.11 (CH), 72.22 (CH), 73.15 (CH), 77.10 (CH), 89.72 (CH), 99.36 (An CH), 118.90 (CH), 122.07 (CH), 127.00 (CH), 130.49 (Q), 131.45 (CH), 132.09 (Q), 150.31 (Q), 171.90 (Q), 172.00 (Q), 172.72 (Q), 173.28 (Q), 176.25 (Q); *m/z* 868 (MH^+, 81%), 867 (M^+, 32%), 784 (M^+ -C_5H_7O, 34%), 499 (M^+ -$C_{22}H_{26}NO_4$, 15%), 370 (M^+ -$C_{26}H_{41}O_9$, 39%), 368 (M^+ -$C_{26}H_{43}O_9$, 47%); [Found MH^+ 868.4847. $C_{48}H_{69}NO_{13}$ requires *M*H 868.4717] (F.A.B.).

1.3.4. α-D-Glucopyranose Pentavalerate 225

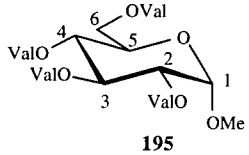


225

Valeric anhydride (4.710 g, 25.29 mmol) was stirred at 0 °C and 70% (w/v) aq perchloric acid (0.832 g; 0.350 g, 3.48 mmol $HClO_4$) added dropwise while maintaining the temperature below 10 °C. D-(+)-Glucose **49** (1.000 g, 5.56 mmol) was added over 30 mins and stirring was continued for a further 30 mins at 0 °C and then at rt for 90 mins. The reaction mixture was poured onto ice (22 g), partitioned with CH_2Cl_2 (20 ml) and the organic extract washed with water (5x 10 ml), sat aq $NaHCO_3$ (4x 10 ml), water (8x 10 ml) until the aqueous washings reached neutral pH, washed with brine (10 ml), dried over $MgSO_4$ and the solvent removed by rotary evaporation to give an orange oil (2.680 g). Flash column-chromatography (13:1, hexane-^{i}PrOH) of the crude residue afforded *the title compound* as a yellow syrup (2.549 g, 76%); R_f 0.50 (light petroleum); $[\alpha]_D^{27}$ +63.10 (*c* 1.04, $CHCl_3$); ν_{max}

(neat)/cm^{-1} 2963s (CH), 2936s (CH), 2875s (CH), 1752s (CO_2), 1467s (CH), 1381s (CH_3), 1107s (C-O-C), 1037s (C-O-C); δ_H (250 MHz, $CDCl_3$) 0.80-0.96 (15H, m, 5x CH_3), 1.08-1.42 (10H, m, 5x CH_2CH_3), 1.43-1.70 (10H, m, 5x OCH_2CH_2), 2.28 (10H, m, 5x OCH_2), 4.06 (1H, dd, $^2J_{gem}$ 12, ^{6}CH), 4.10 (1H, m, $^3J_{5,6}$ 5, ^{5}CH), 4.17 (1H, dd, $^2J_{gem}$ 12, ^{6}CH), 5.06 (1H, dd, $^3J_{2,1}$ 4, ^{2}CH), 5.12 (1H, t, $^3J_{4,3}$ 10, ^{4}CH), 5.46 (1H, t, $^3J_{3,4}$ 10, ^{3}CH), 6.31 (1H, d, $^3J_{1,2}$ 4, ^{1}CH); δ_C (63 MHz, $CDCl_3$) 13.47 (3x CH_3), 13.51 (2x CH_3), 22.04 (2x CH_2), 22.12 (3x CH_2), 26.68 (CH_2), 26.71 (CH_2), 26.77 (CH_2), 26.83 (2x CH_2), 33.47 (CH_2), 33.63 (CH_2), 33.75 (CH_2), 33.79 (2x CH_2), 61.41 (CH_2), 67.73 (CH), 69.26 (CH), 69.57 (CH), 70.05 (CH), 88.86 (An CH), 171.38 (Q), 171.97 (Q), 172.21 (Q), 172.68 (Q), 173.22 (Q); *m/z* 601 (MH^+, 2%), 600 (M^+, 35%), 585 (M^+ -CH_3, 100%), 499 (M^+ -$C_5H_9O_2$, 46%), 368 (M^+ -$C_{11}H_{20}O_5$, 27%), 284 (M^+ -$C_{16}H_{28}O_6$, 50%); [Found M^+ 600.3536. $C_{31}H_{52}O_{11}$ requires *M* 600.3501] (E.I.).

1.3.5. 1-*O*-Methyl 2,3,4,6-tetra-*O*-valeryl-α-D-glucopyranoside 195

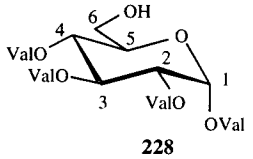


195

Valeric anhydride (4.710 g, 25.29 mmol) was stirred at 0 °C and 70% (w/v) aq perchloric acid (0.832 g; 0.350 g, 3.48 mmol $HClO_4$) added dropwise while maintaining the temperature below 10 °C. Methyl α-D-glucopyranoside **230** (1.006 g, 5.18 mmol) was added over 5 mins and stirring continued for a further 45 mins at 0 °C. The reaction mixture was then allowed to warm to rt and poured onto ice (22 g), partitioned with CH_2Cl_2 (20 ml) and the organic extract washed with water (5x 10 ml), sat aq $NaHCO_3$ (3x 20 ml), water (2x 20 ml) until the aqueous washings reached neutral pH, washed with brine (20 ml), dried over Na_2SO_4 and the solvent removed by rotary evaporation to give a yellow oil (2.749 g). Flash column-chromatography (9:1, light petroleum-EtOAc) of the crude residue afforded *the title compound* as a yellow syrup (1.401 g, 51%); R_f 0.57 (8:2, light petroleum-EtOAc); $[\alpha]_D^{27}$ +83.09 (*c* 0.47, $CHCl_3$); ν_{max} (neat)/cm^{-1} 2962s (CH), 2960s (CH), 2874s (CH), 1754s (CO_2), 1462s (CH), 1418m (CH), 1339m (CH_3), 1170s (C-O-C), 1107s (C-O-C), 1047s (C-O-C); δ_H (250 MHz, $CDCl_3$) 0.79-0.97 (12H, m, 4x CH_3), 1.18-1.41 (8H, m, 4x CH_2CH_3), 1.45-1.68 (8H, m, 4x OCH_2CH_2), 2.16-2.39 (8H, m, 4x OCH_2), 3.25 (3H, s, OMe), 3.95 (1H, m, $^3J_{5,6}$ 5, ^{5}CH), 4.14 (2H, qd, $^2J_{gem}$ 12, $^6CH_2$), 4.87 (1H, dd, $^3J_{2,1}$ 4, ^{2}CH), 4.92 (1H, d, $^3J_{1,2}$ 4, ^{1}CH), 5.07 (1H, t, $^3J_{4,3}$ 10, ^{4}CH), 5.47 (1H, t, $^3J_{3,4}$ 10, ^{3}CH);

δ_C (63 MHz, $CDCl_3$) 13.56 (2x CH_3), 13.63 (2x CH_3), 22.89 (2x CH_2), 22.16 (2x CH_2), 26.74 (CH_2), 26.82 (CH_2), 26.89 (2x CH_2), 33.69 (CH_2), 33.73 (CH_2), 33.83 (2x CH_2), 55.38 (OMe), 61.82 (CH_2), 67.36 (CH), 68.30 (CH), 69.68 (CH), 70.78 (CH), 96.83 (An CH), 172.23 (Q), 172.61 (Q), 172.94 (Q), 173.38 (Q); *m/z* 531 (MH^+, 1%), 530 (M^+, 1%), 499 (M^+ -CH_3O, 31%), 428 (M^+ -$C_5H_{10}O_2$, 11%), 369 (M^+ -$C_8H_{17}O_3$, 82%), 271 (M^+ -$C_{13}H_{23}O_5$, 100%); [Found M^+ 530.3069. $C_{27}H_{46}O_{10}$ requires *M* 530.3091] (E.I.).

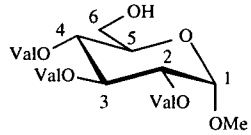
1.3.6. 1,2,3,4-Tetra-*O*-valeryl-α-D-glucopyranose 228



228

A suspension of α-D-glucopyranose pentavalerate **225** (0.212 g, 0.35 mmol) in 10% (v/v) pH 7.0, 0.05 M phosphate buffer-DMF (20 ml) was treated with *Candida cylindraceae* lipase (0.305 g, 213,500-457,500 U) and the reaction mixture stirred at rt at 100 rpm. The neutral pH was maintained by the addition of 1 M aq NaOH for 6 days; tlc at that time revealed that the reaction had gone to completion. The reaction mixture was extracted with EtOAc (4x 20 ml), the combined organic extracts dried over Na_2SO_4 and the solvent removed by rotary evaporation to give a yellow oil (0.850 g). Flash column-chromatography (24:1, hexane-^{i}PrOH) of the crude residue afforded *the title compound* as a colourless syrup (0.083 g, 45%); R_f 0.20 (9:1, hexane-^{i}PrOH); $[\alpha]_D^{27}$ +81.42 (*c* 0.60, $CHCl_3$); ν_{max} (neat)/cm^{-1} 2962s (CH), 2936s CH), 2874s (CH), 1759s (CO_2), 1463m (CH), 1418m (CH), 1380m (CH_3), 1165s (C-O(H)), 1105s (C-O-C), 1048s (C-O-C); δ_H (300 MHz; $CDCl_3$) 0.84-0.97 (12H, m, 4x CH_3), 1.16-1.39 (8H, m, 4x CH_2CH_3), 1.41-1.58 (8H, m, 4x OCH_2CH_2), 2.18-2.34 (8H, m, 4x OCH_2), 3.64 (2H, bdd, $^2J_{gem}$ 12, $^6CH_2$), 3.90 (1H, m, $^3J_{5,6}$ 4, ^{5}CH), 5.03-5.15 (2H, m, $^3J_{4,3}$ 10, ^{4}CH, ^{2}CH), 5.56 (1H, t, $^3J_{3,4}$ 10, ^{3}CH), 6.37 (1H, d, $^3J_{1,2}$ 4, ^{1}CH); δ_C (75 MHz; $CDCl_3$) 13.57 (2x CH_3), 13.59 (2x CH_3), 22.09 (2x CH_2), 22.15 (CH_2), 22.18 (CH_2), 26.70 (CH_2), 26.83 (CH_2), 26.91 (2x CH_2), 33.50 (CH_2), 33.73 (CH_2), 33.82 (CH_2), 33.84 (CH_2), 60.92 (CH_2), 68.10 (CH), 69.21 (CH), 69.37 (CH), 72.18 (CH), 88.92 (An CH), 171.66 (Q), 172.33 (Q), 172.75 (Q), 173.06 (Q); *m/z* 415 (M^+ -$C_5H_9O_2$, 6%), 284 (M^+ -$C_{11}H_{20}O_5$, 11%), 279 (M^+ -$C_{13}H_{17}O_4$, 20%), 149 (M^+ -$C_{19}H_{27}O_7$, 36%), 85 (M^+ -$C_{21}H_{35}O_9$, 100%); [Found M^+ -$C_5H_9O_2$ 415.2328. $C_{26}H_{44}O_{10}$ requires *M*-$C_5H_9O_2$ 415.2332] (E.I.).

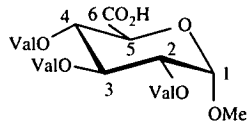
1.3.7. 1-*O*-Methyl 2,3,4-tri-*O*-valeryl-β-D-glucopyranoside 229



229

A suspension of 1-*O*-methyl 2,3,4,6-tetra-*O*-valeryl-α-D-glucopyranoside **195** (0.061 g, 0.12 mmol) in 10% (v/v) pH 7.0, 0.05 M phosphate buffer-DMF (20 ml) was treated with *Candida cylindraceae* lipase (0.091 g, 63,700-136,500 U) and the reaction mixture stirred at rt at 100 rpm. The neutral pH was maintained by the addition of 1 M aq NaOH for 6 days; tlc at that time revealed that the reaction had gone to completion. The reaction mixture was extracted with EtOAc (5x 20 ml), the combined organic extracts dried over Na_2SO_4 and the solvent removed by rotary evaporation to give a yellow oil (0.592 g). Flash column-chromatography (24:1, hexane-^{i}PrOH) of the crude residue afforded *the title compound* as a colourless syrup (0.029 g, 56%); R_f 0.65 (9:1, CH_2Cl_2-MeOH); $[\alpha]_D^{26}$ +91.50 (*c* 0.41, $CHCl_3$), (Lit.[122] $[\alpha]_D^{22}$ +98.2 (*c* 0.55, $CHCl_3$); ν_{max} ($CHCl_3$)/cm^{-1} 2920s (CH), 1740s (CO_2), 1140s (C-O(H)), 1070s (C-O-C), 1030s (C-O-C); δ_H (300 MHz; $CDCl_3$) 0.80-1.03 (9H, m, 3x CH_3), 1.21-1.43 (6H, m, 3x CH_2 CH_3), 1.49-1.67 (6H, m, 3x OCH_2CH_2), 2.18-2.39 (6H, m, 3x OCH_2), 3.43 (3H, s, OMe), 3.60 (1H, dd, $^2J_{gem}$ 12, ^{6}CH), 3.78 (1H, m, ^{5}CH), 3.80 (1H, dd, $^2J_{gem}$ 12, ^{6}CH), 4.88 (1H, dd, $^3J_{2,1}$ 4, ^{2}CH), 4.98 (1H, d, $^3J_{1,2}$ 4, ^{1}CH), 5.03 (1H, t, $^3J_{4,3}$ 10, ^{4}CH), 5.60 (1H, t, $^3J_{3,4}$ 10, ^{3}CH); δ_C (75 MHz; $CDCl_3$) 13.63 (3x CH_3), 22.20 (OMe), 26.88 (2x CH_2), 27.00 (CH_2), 33.76 (CH_2), 33.80 (CH_2), 33.90 (CH_2), 55.42 (3x CH_2), 61.04 (CH_2), 68.73 (CH), 69.26 (CH), 69.38 (CH), 70.96 (CH), 96.86 (An CH), 172.62 (Q), 173.01 (Q), 173.51 (Q); *m/z* 446 (M^+, 1%), 415 (M^+ -CH_3O, 100%), 344 (M^+ -$C_5H_{10}O_2$, 2%), 103 (M^+ -$C_{17}H_{27}O_7$, 13%), 85 (M^+ -$C_{17}H_{29}O_8$, 100%); [Found M^+ 446.2518. $C_{22}H_{38}O_9$ requires *M* 446.2516] (E.I.).

1.3.8. 1-*O*-Methyl 2,3,4-tri-*O*-valeryl-α-D-glucopyranosiduronic acid 231

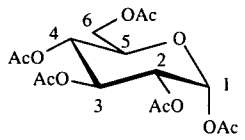


231

To a solution of $NaIO_4$ (0.047 g, 0.22 mmol) in water (3 ml) was added a solution of 1-*O*-methyl 2,3,4-tri-*O*-valeryl-α-D-glucopyranoside **229** (0.021 g, 0.05 mmol) in CH_3CN (2 ml) and CCl_4 (2 ml) and a solution of $RuCl_3.3H_2O$ (5 mg, 0.02 mmol) in CH_3CN (1 ml). The reaction mixture was stirred vigorously at rt for 40 h; tlc at that time revealed that the reaction had gone to completion. The aqueous layer was isolated, extracted with CH_2Cl_2 (4x 10 ml) and the combined organic extracts were dried over $MgSO_4$, filtered through a pad of Celite and the solvent removed by rotary evaporation to give *the title compound* as a red solid (0.029 g, quantitative yield); R_f 0.76 (45:15:10:30, *n*-BuOH-acetone-acetic acid-1.6% (v/v) aq NH_3); ν_{max} (neat)/cm^{-1} 2963m, (CH), 2933m (CH), 2872m (CH), 1743s (CO_2), 1375w (CH_3), 1166s (C-O(H)), 1105s (C-O-C), 1048s (C-O-C); δ_H (300 MHz; $CHCl_3$) 0.76-0.96 (9H, m, 3x CH_3), 1.13-1.39 (6H, m, 3x CH_2CH_3), 1.40-1.62 (6H, m, 3x OCH_2CH_2), 2.13-2.40 (6H, m, 3x OCH_2), 3.41 (3H, s, OMe), 4.16 (1H, bd, $^3J_{5,4}$ 10, ^{5}CH), 4.89 (1H, dd, $^3J_{2,1}$ 4, $^3J_{2,3}$ 10, ^{2}CH), 5.05-5.28 (2H, m, $^3J_{4,3}$ 10, ^{4}CH, ^{1}CH), 5.53 (1H, t, $^3J_{3,4}$ 10, ^{3}CH); δ_C (75 MHz; $CHCl_3$) 13.57 (3x CH_3), 22.07 (CH_2), 22.13 (2x CH_2), 26.64 (CH_2), 26.81 (CH_2), 26.90 (CH_2), 29.64 (OMe), 33.70 (CH_2), 33.75 (CH_2), 33.82 (CH_2), 69.33 (CH), 69.57 (CH), 70.53 (2x CH), 96.90 (An CH), 171.61 (Q), 172.45 (2x Q), 173.03 (Q); *m/z* 460 (M^+, 1%), 429 (M^+ -CH_3O, 1%), 301 (M^+ -$C_7H_{11}O_4$, 7%), 241 (M^+ -$C_{10}H_{19}O_5$, 17%), 85 (M^+ -$C_{17}H_{27}O_9$, 97%); [Found M^+ 460.2313. $C_{22}H_{36}O_{10}$ requires *M* 460.2309] (E.I.).

1.4. Synthesis of Morphine-6-glucuronide; Synthetic Route Three

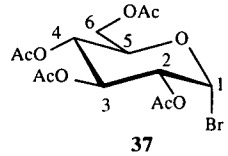
1.4.1. α-D-Glucopyranose Pentaacetate 227



227

Acetic anhydride (270.5 g, 2.65 mol) was stirred at 0 °C and 70% (w/v) aq perchloric acid (3.9 g; 1.6 g, 0.02 mol, $HClO_4$) added dropwise while maintaining the temperature below 10 °C. D-(+)-Glucose **49** (50.0 g, 0.28 mol) was added over 30 mins and stirring continued for a further 30 mins at 0 °C, then allowed to warm to rt overnight. The reaction mixture was poured onto ice (500 g), partitioned with CH_2Cl_2 (4x 250 ml) and the combined organic extracts washed with water (10x 250 ml), sat aq $NaHCO_3$ (8x 250 ml), water (2x 250 ml) until the aqueous washings reached neutral pH, washed with brine (250 ml), dried over $MgSO_4$ and the solvent removed by rotary evaporation to give a white solid (136.5 g). Crystallisation (EtOH-water) of the crude residue afforded *the title compound* as a white crystalline solid (93.6 g, 86%); R_f 0.53 (1:1, light petroleum-EtOAc); m.p. 98-99 °C (from EtOH-water), (Lit.[165] 109-111 °C); $[\alpha]_D^{23}$ +93.95 (*c* 1.00, $CHCl_3$), (Lit.[165] $[\alpha]_D^{20}$ +102.00 (*c* 1.00, $CHCl_3$); ν_{max} (KBr)/cm^{-1} 1754s (CO_2), 1430w (CH), 1371m (CH_3), 1043s (C-O-C); δ_H (250 MHz, $CDCl_3$) 1.97 (3H, s, OAc), 1.99 (3H, s, OAc), 2.00 (3H, s, OAc), 2.05 (3H, s, OAc), 2.14 (3H, s, OAc), 3.97-4.13 (2H, m, $^2J_{gem}$ 12, ^{6}CH, ^{5}CH), 4.23 (1H, dd, $^2J_{gem}$ 12, ^{6}CH), 5.05 (1H, dd, $^3J_{2,1}$ 4, ^{2}CH), 5.08 (1H, t, $^3J_{4,3}$ 10, ^{4}CH), 5.43 (1H, t, $^3J_{3,4}$ 10, ^{3}CH), 6.29 (1H, d, $^3J_{1,2}$ 4, ^{1}CH); δ_C (75 MHz, $CDCl_3$) 20.35 (CH_3), 20.48 (CH_3), 20.57 (CH_3), 20.60 (CH_3), 20.78 (CH_3), 61.45 (CH_2), 67.91 (CH), 69.18 (CH), 69.81 (2x CH), 89.04 (An CH), 168.65 (Q), 169.31 (Q), 169.56 (Q), 170.12 (Q), 170.51 (Q); *m/z* 390 (M^+, 1%), 347 (M^+ -C_2H_3O, 6%), 332 (M^+ -$C_2H_2O_2$, 16%), 331 (M^+ -CH_3O, 100%); [Found M^+ 390.1153. $C_{16}H_{22}O_{11}$ requires *M* 390.1162] (E.I.).

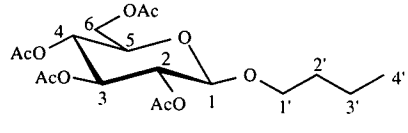
1.4.2. Tetra-*O*-acetyl-α-D-glucopyranosyl Bromide 37



37

A solution of 33% (w/v) HBr in glacial acetic acid (36.0 g; 11.9 g, 0.15 mol HBr) was stirred at rt while α-D-glucopyranose pentaacetate **227** (20.0 g, 0.05 mol) was added over 5 mins. Stirring was continued for 2 h after which time tlc revealed that the reaction had gone to completion. The reaction mixture was poured onto ice (500 g), extracted with $CHCl_3$ (250 ml) and the organic extract washed with ice cold water (2x 250 ml), sat aq $NaHCO_3$ (250 ml), water (2x 250 ml), brine (250 ml), dried over $MgSO_4$ and the solvent removed by rotary evaporation to give a white solid (21.0 g). Crystallisation (light petroleum-ether) of the crude residue afforded *the title compound* as a white crystalline solid (18.5 g, 88%); R_f 0.67 (1:1, light petroleum-EtOAc); m.p. 87-89 °C (from light petroleum-ether), (Lit.[165] 88-89 °C); $[\alpha]_D^{21}$ +195.80 (*c* 1.00, $CHCl_3$), (Lit.[165] $[\alpha]_D^{28}$ +196.00 (*c* 2.00, $CHCl_3$); ν_{max} (KBr)/cm^{-1} 2962w (CH), 2925w (CH), 2854w (CH), 1745s (CO_2), 1433m (CH), 1370s (CH_3), 1042s (C-O-C); δ_H (300 MHz, $CDCl_3$) 1.99 (3H, s, OAc), 2.01 (3H, s, OAc), 2.05 (3H, s, OAc), 2.06 (3H, s, OAc), 4.09 (1H, dt, $^2J_{gem}$ 11, ^{6}CH), 4.25-4.37 (2H, m, $^2J_{gem}$ 11, ^{6}CH, ^{5}CH), 4.80 (1H, dd, $^3J_{2,1}$ 4, ^{2}CH), 5.12 (1H, t, $^3J_{4,3}$ 10, ^{4}CH), 5.52 (1H, t, $^3J_{3,4}$ 10, ^{3}CH), 6.58 (1H, d, $^3J_{1,2}$ 4, ^{1}CH); δ_C (75 MHz, $CDCl_3$) 20.46 (CH_3), 20.53 (CH_3), 20.55 (CH_3), 20.57 (CH_3), 60.93 (CH_2), 67.17 (CH), 70.15 (CH), 70.56 (CH), 72.14 (CH), 86.58 (An CH), 169.36 (Q), 169.67 (Q), 169.72 (Q), 170.37 (Q); *m/z* 411 (MH^+, 3%), 331 (M^+ -Br, 54%), 289 (M^+ -C_2H_2OBr, 22%), 70 (M^+ -$C_{11}H_{17}O_7Br$, 100%); [Found MH^+ 411.0270. $C_{14}H_{19}O_9Br$ requires *M*H 411.0291] (E.I.).

1.4.3. *n*-Butyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside 232

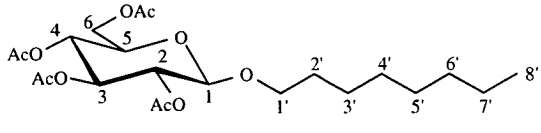


232

A suspension of AgOTf (0.311 g, 1.21 mmol) and *n*-butanol (0.810 g, 10.93 mmol) in dry CH_2Cl_2 (5 ml) was stirred at -30 °C, in the presence of 4Å molecular sieves, under an atmosphere of N_2 and in the absence of light. A solution of tetra-*O*-acetyl-α-D-glucopyranosyl bromide **37** (0.518 g, 1.26 mmol) in dry CH_2Cl_2 (10 ml)

was added over 10 mins while maintaining the temperature below -20 °C. The reaction mixture was stirred at this temperature for a further 30 mins; tlc at that time revealed that the reaction had gone to completion. The reaction mixture was allowed to warm to rt, filtered through a pad of Celite and the filtrate washed with water (25 ml), sat aq $NaHCO_3$ (25 ml), dried over Na_2SO_4 and the solvent removed by rotary evaporation to give a clear colourless oil (0.736 g). Flash column-chromatography (8:2, light petroleum-EtOAc) of the crude residue followed by crystallisation (ether-light petroleum) afforded *the title compound* as a white crystalline solid (0.204 g, 40%); R_f 0.81 (1:1, light petroleum-EtOAc); m.p. 58-59 °C (from ether-light petroleum), (Lit.[166] 61-62 °C); $[\alpha]_D^{23}$ -12.55 (*c* 1.00, $CHCl_3$), (Lit.[166] $[\alpha]_D$ -20.6 (*c* 2, $CHCl_3$); [Found: C, 52.88%; H, 6.98%; N, 0%. $C_{18}H_{28}O_{10}$ requires C, 53.44%; H, 6.56%; N, 0%]; ν_{max} (KBr)/cm^{-1} 2963m (CH), 2874m (CH), 1758s (CO_2), 1432m (CH), 1376s (CH_3), 1041s (C-O-C); δ_H (250 MHz; $CDCl_3$) 0.88 (3H, t, $^3J_{4',3'}$ 7, $^{4'}CH_3$), 1.32 (2H, m, $^3J_{3',4'}$ 7, $^{3'}CH_2$), 1.52 (2H, m, $^3J_{2',3'}$ 7, $^{2'}CH_2$), 1.98 (3H, s, OAc), 1.99 (3H, s, OAc), 2.01 (3H, s, OAc), 2.06 (3H, s, OAc), 3.46 (1H, td, $^2J_{gem}$ 10, $^{1'}CH$), 3.66 (1H, m, $^3J_{5,4}$ 10, ^5CH), 3.83 (1H, td, $^2J_{gem}$ 10, $^{1'}CH$), 4.07 (1H, dd, $^2J_{gem}$ 12, ^6CH), 4.21 (1H, dd, $^2J_{gem}$ 12, ^6CH), 4.47 (1H, d, $^3J_{1,2}$ 8, ^1CH), 4.95 (1H, dd, $^3J_{2,1}$ 8, ^2CH), 5.05 (1H, t, $^3J_{4,3}$ 10, ^4CH), 5.18 (1H, t, $^3J_{3,4}$ 10, ^3CH); δ_C (75 MHz; $CDCl_3$) 13.66 (CH_3), 18.93 (CH_2), 20.55 (CH_3), 20.58 (2x CH_3), 20.68 (CH_3), 31.38 (CH_2), 62.04 (CH_2), 68.56 (CH), 69.86 (OCH_2), 71.39 (CH), 71.76 (CH), 72.90 (CH), 100.85 (An CH), 169.24 (Q), 169.36 (Q), 170.27 (Q), 170.63 (Q); *m/z* 405 (MH^+, 2%), 404 (M^+, 2%), 331 (M^+ -C_4H_9O, 100%), 243 (M^+ -$C_6H_9O_5$, 15%), 103 (M^+ -$C_{13}H_{17}O_8$, 25%), 73 (M^+ -$C_{14}H_{19}O_9$, 22%); [Found MH^+ 405.1737. $C_{18}H_{28}O_{10}$ requires *M*H 405.1761] (F.A.B.).

1.4.4. *n*-Octyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside 233

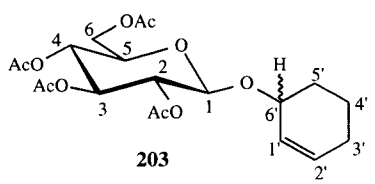


233

A suspension of AgOTf (0.311 g, 1.21 mmol) and *n*-octanol (1.675 g, 12.86 mmol) in dry CH_2Cl_2 (2 ml) was stirred at -30 °C, in the presence of 4Å molecular sieves, under an atmosphere of N_2 and in the absence of light. A solution of tetra-*O*-acetyl-α-D-glucopyranosyl bromide **37** (0.497 g, 1.21 mmol) in dry CH_2Cl_2 (2 ml) was added over 15 mins while maintaining the temperature below -20 °C. The reaction mixture was stirred at this temperature for a further 30 mins, whereafter, tlc

revealed that the reaction had gone to completion. The reaction mixture was allowed to warm to rt, filtered through a pad of Celite and the filtrate washed with water (2x 25 ml), 1% (w/v) aq $NaHCO_3$ (2x 25 ml), dried over Na_2SO_4 and the solvent removed by rotary evaporation to give a yellow oil (1.863 g). Flash column-chromatography (1:1, light petroleum-EtOAc) of the crude residue followed by a Kugelrohr distillation (to remove excess *n*-octanol) and finally crystallisation (EtOAc-EtOH) afforded *the title compound* as a white crystalline solid (0.352 g, 63%); R_f 0.62 (1:1, light petroleum-ethyl acetate); m.p. 57-58 °C (from EtOAc-EtOH), (Lit.[167] 54 °C); $[\alpha]_D^{24}$ -20.71 (*c* 1.00, $CHCl_3$), (Lit.[167] $[\alpha]_D$ -23.8); [Found: C, 57.49%; H, 8.07%; N, 0%. $C_{22}H_{36}O_{10}$ requires C, 57.36%; H, 7.88%; N, 0%]; ν_{max} (KBr)/cm^{-1} 2930m (CH), 2862w (CH), 1750s (CO_2), 1373m (CH_3), 1044s (C-O-C); δ_H (300 MHz; $CDCl_3$) 0.88 (3H, t, $^3J_{8',7'}$ 7, $^{8'}CH_3$), 1.32 (12H, bs, $^{7'}CH_2$, $^{6'}CH_2$, $^{5'}CH_2$, $^{4'}CH_2$, $^{3'}CH_2$, $^{2'}CH_2$), 2.00 (3H, s, OAc), 2.02 (3H, s, OAc), 2.03 (3H, s, OAc), 2.08 (3H, s, OAc), 3.45 (1H, td, $^3J_{1',2'}$ 10, $^{1'}CH$), 3.68 (1H, m, ^5CH), 3.86 (1H, td, $^3J_{1',2'}$ 10, $^{1'}CH$), 4.13 (1H, dd, $^2J_{gem}$ 12, ^6CH), 4.26 (1H, dd, $^2J_{gem}$ 12, ^6CH), 4.49 (1H, d, $^3J_{1,2}$ 8, ^1CH), 4.95 (1H, dd, $^3J_{2,1}$ 8, ^2CH), 5.08 (1H, t, $^3J_{4,3}$ 9, ^4CH), 5.20 (1H, t, $^3J_{3,4}$ 9, ^3CH); δ_C (75 MHz; $CDCl_3$) 14.03 (CH_3), 20.56 (2x CH_3), 20.59 (CH_3), 20.69 (CH_3), 22.61 (CH_2), 25.78 (CH_2), 29.21 (CH_2), 29.34 (CH_2), 29.38 (CH_2), 31.77 (CH_2), 62.04 (CH_2), 68.56 (CH), 70.21 (OCH_2), 71.40 (CH), 71.76 (CH), 72.91 (CH), 100.84 (An CH), 169.22 (Q), 169.36 (Q), 170.28 (Q), 170.64 (Q); *m/z* 460 (M^+, 1%), 459 (M^+ - H, 4%), 401 (M^+ -$C_2H_3O_2$, 21%), 387 (M^+ -$C_3H_5O_2$, 100%), 331 (M^+ -$C_8H_{17}O$, 10%), 157 (M^+ -$C_{13}H_{19}O_8$, 94%); [Found M^+ 460.2325. $C_{22}H_{36}O_{10}$ requires *M* 460.2309] (E.I.).

1.4.5. Cyclohex-6'-enyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside 203

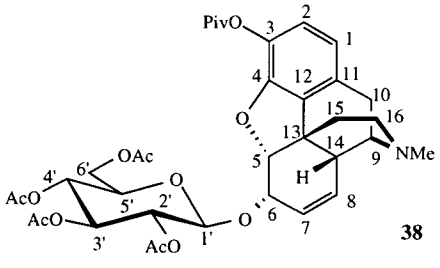


203

A suspension of AgOTf (0.312 g, 1.21 mmol) and 2-cyclohexen-1-ol **204** (1.189 g, 12.21 mmol) in dry CH_2Cl_2 (2 ml) was stirred at -30 °C, in the presence of 4Å molecular sieves, under an atmosphere of N_2 and in the absence of light. A solution of tetra-*O*-acetyl-α-D-glucopyranosyl bromide **37** (0.509 g, 1.24 mmol) in dry CH_2Cl_2 (2 ml) was added over 10 mins while maintaining the temperature below -20 °C. The reaction mixture was stirred at this temperature for a further 30 mins, whereafter, tlc revealed that the reaction had gone to completion. The reaction

mixture was allowed to warm to rt, filtered through a pad of Celite and the filtrate washed with water (2x 25 ml), 1% (w/v) aq $NaHCO_3$ (2x 25 ml), dried over Na_2SO_4 and the solvent removed by rotary evaporation to give a yellow oil (1.001 g). Flash column-chromatography (1:1, light petroleum-EtOAc) of the crude residue followed by crystallisation (EtOAc-light petroleum) afforded *the title compound* in a 1:1 ratio of diastereoisomers[†] as a colourless crystalline solid (0.150 g, 28%); R_f 0.64 (1:1, light petroleum-ethyl acetate); m.p. 156-158 °C (from EtOAc-light petroleum); $[\alpha]_D^{24}$ -22.90 (*c* 1.00, $CHCl_3$); ν_{max} (KBr)/cm^{-1} 2938w (CH), 1754s (CO_2), 1633w (CH), 1039s (C-O-C); δ_H (250 MHz; $CDCl_3$) 1.24 (2H, t, $^3J_{4',5'}$ 7, $^{4'}CH_2$), 1.44-1.90 (4H, m, $^{3'}CH_2$, $^{5'}CH_2$), 2.00 (3H, s, OAc), 2.01 (3H, s, OAc), 2.02 (1.5H, s, OAc), 2.03 (1.5H, s, OAc), 2.08 (3H, s, OAc), 3.68 (1H, m, ^5CH), 4.05-4.29 (3H, m, $^6CH_2$, $^{6'}CH$), 4.60 (0.5H, d, $^3J_{1,2}$ 8, ^1CH), 4.64 (0.5H, d, $^3J_{1,2}$ 8, ^1CH), 4.95 (1H, dd, $^3J_{2,1}$ 8, ^2CH), 5.07 (1H, dt, $^3J_{4,3}$ 9, ^4CH), 5.19 (1H, t, $^3J_{3,4}$ 9, ^3CH), 5.70 (1H, m, $^3J_{1',2'}$ 11, $^{1'}CH$), 5.86 (1H, m, $^{2'}CH$); δ_C (75 MHz; $CDCl_3$) 18.68 (0.5x CH_2), 18.89 (0.5x CH_2), 20.57 (2x CH_3), 20.60 (CH_3), 20.70 (CH_3), 24.92 (0.5x CH_2), 25.03 (0.5x CH_2), 28.52 (0.5x CH_2), 29.72 (0.5x CH_2), 62.18 (0.5x CH_2), 68.64 (CH), 68.68 (CH), 71.57 (0.5x CH), 71.73 (0.5x CH), 71.76 (0.5x CH), 72.92 (0.5x CH), 72.98 (0.5x CH), 73.40 (0.5x CH), 73.52 (0.5x CH), 99.47 (0.5x An CH), 99.56 (0.5x An CH), 126.12 (0.5x CH), 127.49 (0.5x CH), 131.64 (0.5x CH), 132.33 (0.5x CH), 169.14 (Q), 169.23 (0.5x Q), 169.36 (Q), 170.32 (Q), 170.63 (0.5x Q); *m/z* 429 (MH^+, 10%), 428 (M^+, 3%), 331 (M^+ -C_6H_9O, 35%), 271 (M^+ -$C_8H_{13}O_3$, 14%), 97 (M^+ -$C_{14}H_{19}O_9$, 59%); [Found MH^+ 429.1913. $C_{20}H_{28}O_{10}$ requires *M*H 429.1761] (E.I.).

1.4.6. 3-*O*-Pivaloylmorphine-6-(2',3',4',6'-tetra-*O*-acetyl)-β-D-glucopyranoside **38**



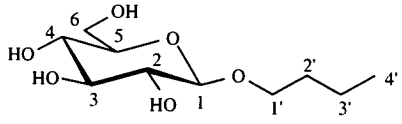
38

To a solution of 3-*O*-pivaloylmorphine **32** (1.238 g, 3.35 mmol) and Ag_2CO_3 (4.472 g, 16.22 mmol) in dry benzene (20 ml) stirring at rt was added a solution of tetra-*O*-acetyl-α-D-glucopyranosyl bromide **37** (6.873 g, 16.71 mmol) in dry benzene

[†] Diastereoisomer ratio is calculated from the relative peak heights of the anomeric protons in the ^1H NMR spectrum.

(20 ml). The reaction mixture was heated under reflux using Dean-Stark apparatus, at 18 h tlc showed that some starting material remained, thus, further Ag_2CO_3 (2.035 g, 7.38 mmol) and dry benzene (10 ml) were added. Six hours later, tlc revealed that the reaction had gone to completion. The reaction mixture was allowed to cool to rt, filtered through a pad of Celite and the filtrate concentrated by rotary evaporation to give a dark red oil (6.193 g). The crude residue was dissolved in CH_2Cl_2 (150 ml) and the solution washed with 5% (w/v) aq citric acid (2x 250 ml), sat aq $NaHCO_3$ (2x 250 ml), water (250 ml), brine (250 ml), dried over $MgSO_4$ and the solvent removed by rotary evaporation to give a brown foam (5.919 g). Flash column-chromatography (1:4, MeOH-$CHCl_3$) of the crude residue followed by crystallisation (EtOH-water) afforded *the title compound* as a white crystalline solid (0.852 g, 36%); R_f 0.22 (1:9, MeOH-$CHCl_3$); m.p. 195 °C (from EtOH-water); $[\alpha]_D^{23}$ -105.88 (*c* 1.01, $CHCl_3$); [Found: C, 62.08%; H, 6.59%; N, 2.00%. $C_{36}H_{45}NO_{13}$ requires C, 61.78%; H, 6.49%; N, 2.00%]; ν_{max} (KBr)/cm^{-1} 2969m (CH), 1756s (CO_2), 1640m (Ar CH), 1449m (Ar CH), 1117s (C-O-C); δ_H (250 MHz; $CDCl_3$) (COSY) 1.34 (9H, s, $C(CH_3)_3$), 1.91 (1H, m, $^3J_{15eq,16ax}$ 5, $^{15}CH_{eq}$), 1.94 (1H, m, $^{15}CH_{ax}$), 1.99 (3H, s, OAc), 2.01 (3H, s, OAc), 2.03 (3H, s, OAc), 2.05 (3H, s, OAc), 2.29 (1H, dd, $^2J_{gem}$ 19, $^{10}CH_{ax}$), 2.37 (1H, d, $^3J_{16ax,15eq}$ 5, $^{16}CH_{ax}$), 2.41 (3H, s, NMe), 2.51 (1H, d, $^3J_{16eq,15ax}$ 3, $^{16}CH_{eq}$), 2.61 (1H, m, $^3J_{14,9}$ 3, ^{14}CH), 3.04 (1H, d, $^2J_{gem}$ 19, $^{10}CH_{eq}$), 3.34 (1H, q, $^3J_{9,14}$ 3, ^9CH), 3.74 (1H, m, $^3J_{5',6'}$ 2, $^{5'}CH$), 4.18 (2H, dd, $^3J_{6',5'}$ 2, $^{6'}CH_2$), 4.20 (1H, m, ^6CH), 4.84 (2H, d, $^3J_{2',1'}$ 8, $^{2'}CH$, ^5CH), 4.97-5.21 (3H, m, $^{1'}CH$, $^{3'}CH$, $^{4'}CH$), 5.28 (1H, dt, $^3J_{7,8}$ 10, ^7CH), 5.70 (1H, bd, $^3J_{8,7}$ 10, ^8CH), 6.52 (1H, d, $^3J_{1,2}$ 8, ^1CH), 6.67 (1H, d, $^3J_{2,1}$ 8, ^2CH); δ_C (63 MHz; $CDCl_3$) 20.48 (CH_2), 20.61 (3x CH_3), 27.02 (4x CH_3), 35.63 (CH_2), 38.85 (Q), 40.97 (CH), 42.94 (NMe), 43.59 (Q), 46.08 (CH_2), 58.46 (CH), 61.80 (CH_2), 68.26 (CH), 71.23 (CH), 71.79 (CH), 72.61 (CH), 73.53 (CH), 89.87 (CH), 99.18 (An CH), 118.82 (CH), 121.51 (CH), 128.44 (CH), 130.53 (CH), 131.12 (Q), 131.63 (Q), 131.95 (Q), 150.16 (Q), 169.16 (Q), 169.28 (Q), 170.09 (Q), 170.46 (Q), 176.25 (Q); *m/z* 700 (MH^+, 56%), 699 (M^+, 11%), 656 (M^+ -C_2H_3O, 3%), 462 (M^+ -$C_8H_{13}O_3$, 3%), 368 (M^+ -$C_{16}H_{29}NO_6$, 31%); [Found MH^+ 700.2969. $C_{36}H_{45}NO_{13}$ requires *M*H 700.2969] (F.A.B.).

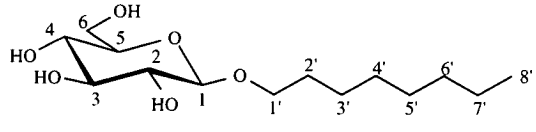
1.4.7. *n*-Butyl β-D-glucopyranoside 238



238

To a solution of *n*-butyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside **232** (0.071 g, 0.18 mmol) in dry MeOH (1 ml) under an atmosphere of N_2 was added a solution of NaOMe (0.013 g, 0.24 mmol) in dry MeOH (1 ml). The reaction was stirred for 1 h; tlc at that time revealed that the reaction had gone to completion. The solvent was removed by rotary evaporation to give a yellow oil (0.082 g), flash column-chromatography (9:1, CH_2Cl_2-MeOH) of the crude residue afforded *the title compound* as a yellow syrup (0.043 g, quantitative yield); R_f 0.63 (9:4:2, EtOAc-^{i}PrOH-water); Lit.[168] m.p. 68-69 °C; $[\alpha]_D^{23}$ -23.08 (*c* 1.06, MeOH), (Lit.[168] $[\alpha]_D^{20}$ -36.9); ν_{max} (neat)/cm^{-1} 2961s (CH), 2935s (CH), 2876s (CH), 1377m (CH_3), 1268m (C-O(H)), 1078s (C-O-C), 1032s (C-O-C); δ_H (300 MHz; CD_3OD) 0.95 (3H, t, $^3J_{4',3'}$ 7, $^{4'}CH_3$), 1.42 (2H, m, $^3J_{3',4'}$ 7, $^{3'}CH_2$), 1.63 (2H, m, $^3J_{2',3'}$ 7, $^{2'}CH_2$), 3.16 (1H, dd, $^3J_{2,3}$ 8, ^{2}CH), 3.21-3.28 (3H, m, $^3J_{1',2'}$ 7, $^{1'}$CH, ^{6}CH, ^{3}CH), 3.55 (1H, td, $^2J_{gem}$ 13, ^{6}CH), 3.68 (1H, dd, $^3J_{4,5}$ 5, ^{4}CH), 3.79-3.99 (2H, m, ^{5}CH, $^{1'}$CH), 4.26 (1H, d, $^3J_{1,2}$ 8, ^{1}CH); δ_C (75 MHz; CD_3OD) 14.12 (CH_3), 20.09 (CH_2), 32.79 (CH_2), 62.70 (CH_2), 70.49 (OCH_2), 71.60 (CH), 75.03 (CH), 77.78 (CH), 78.04 (CH), 104.26 (An CH); *m/z* 237 (MH^+, 1%), 163 (M^+ -C_4H_9O, 3%), 116 (M^+ -$C_6H_{12}O_2$, 7%), 103 (M^+ -$C_5H_{11}O_4$, 21%), 57 (M^+ -$C_6H_{11}O_6$, 100%); [Found MH^+ 237.1333. $C_{10}H_{20}O_6$ requires *M*H 237.1416] (E.I.).

1.4.8. *n*-Octyl β-D-glucopyranoside 239

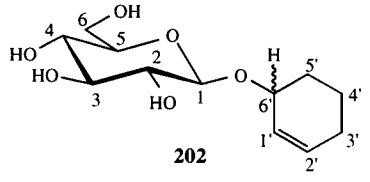


239

To a solution of *n*-octyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside **233** (0.101 g, 0.22 mmol) in dry MeOH (1 ml) under an atmosphere of N_2 was added a solution of NaOMe (0.017 g, 0.32 mmol) in dry MeOH (1.5 ml). The reaction was stirred for 10 mins; tlc at that time revealed that the reaction had gone to completion. The solvent was removed by rotary evaporation to give a yellow oil (0.104 g), flash column-chromatography (9:1, CH_2Cl_2-MeOH) of the crude residue afforded *the title*

compound as a yellow syrup (0.067 g, quantitative yield); R_f 0.78 (9:4:2, EtOAc-^{i}PrOH-water); Lit.[169] m.p. 110 °C; $[\alpha]_D^{24}$ -20.50 (*c* 1.34, MeOH), (Lit.[170] $[\alpha]_D^{25}$ -28.3); ν_{max} (neat)/cm^{-1} 2961s (CH), 1376m (CH_3), 1030s (C-O-C); δ_H (250 MHz; CD_3OD) 0.89 (3H, t, $^3J_{8',7'}$ 7, $^{8'}CH_3$), 1.30 (12H, bs, $^{7'}CH_2$, $^{6'}CH_2$, $^{5'}CH_2$, $^{4'}CH_2$, $^{3'}CH_2$, $^{2'}CH_2$), 3.17 (1H, dd, $^3J_{2,1}$ 8, ^{2}CH), 3.22-3.41 (3H, m, $^6CH_2$, ^{3}CH), 3.53 (1H, td, $^3J_{1',2'}$ 10, $^{1'}$CH), 3.67 (1H, dd, $^3J_{4,5}$ 5, ^{4}CH), 3.83 (1H, m, ^{5}CH), 3.90 (1H, td, $^3J_{1',2'}$ 10, $^{1'}$CH), 4.25 (1H, d, $^3J_{1,2}$ 8, ^{1}CH); δ_C (75 MHz; CD_3OD) 14.40 (CH_3), 23.66 (CH_2), 27.07 (CH_2), 30.35 (CH_2), 30.54 (CH_2), 30.79 (CH_2), 32.96 (CH_2), 82.64 (CH_2), 70.98 (OCH_2), 71.74 (CH), 75.14 (CH), 77.85 (CH), 78.14 (CH), 104.36 (An CH); *m/z* 293 (MH^+, 100%), 292 (M^+, 1%), 261 (M^+ -CH_3O, 82%), 163 (M^+ -$C_8H_{17}O$, 21%), 144 (M^+ -$C_5H_8O_5$, 26%), 133 (M^+ -$C_9H_{19}O_2$, 2%); [Found MH^+ 293.1955. $C_{14}H_{28}O_6$ requires *M*H 293.1964] (E.I.).

1.4.9. Cyclohex-6'-enyl β-D-glucopyranoside 202



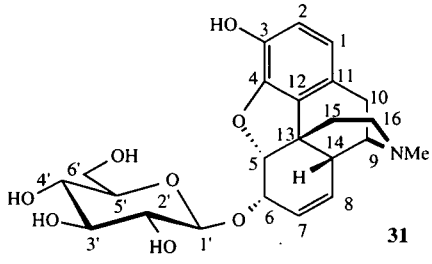
202

To a solution of cyclohex-6'-enyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside **203** (0.071 g, 0.18 mmol) in dry MeOH (1 ml) under an atmosphere of N_2 was added a solution of NaOMe (0.022 g, 0.41 mmol) in dry MeOH (1.5 ml). The reaction was stirred for 30 mins; tlc at that time revealed that the reaction had gone to completion. The solvent was removed by rotary evaporation to give a yellow oil (0.148 g), flash column-chromatography (9:1, CH_2Cl_2-MeOH) of the crude residue afforded *the title compound* in a 1:1 ratio of diastereoisomers[†] as a colourless oil (0.097 g, quantitative yield); R_f 0.66 (9:4:2, EtOAc-^{i}PrOH-water); $[\alpha]_D^{24}$ -22.03 (*c* 0.45, MeOH); ν_{max} (neat)/cm^{-1} 2980m (CH), 2935s (CH), 1682m (C=C-H), 1169s (C-O(H)), 1047s (C-O-C); δ_H (300 MHz; CD_3OD) 1.47-2.16 (6H, m, $^{3'}CH_2$, $^{4'}CH_2$, $^{5'}CH_2$), 3.16 (1H, dd, $^3J_{2,1}$ 8, ^{2}CH), 3.21-3.42 (3H, m, $^{6'}$CH, ^{3}CH, ^{4}CH), 3.68 (1H, dd, $^2J_{gem}$ 12, ^{6}CH), 3.87 (1H, bd, $^2J_{gem}$ 12, ^{6}CH), 4.29 (1H, m, ^{5}CH), 4.39 (0.5H, d, $^3J_{1,2}$ 8, ^{1}CH), 4.42 (0.5H, d, $^3J_{1,2}$ 8, ^{1}CH), 5.47-5.95 (2H, m, $^{1'}$CH, $^{2'}$CH); δ_C (75 MHz; CD_3OD) 20.03 (0.5x CH_2), 20.36 (0.5x CH_2), 25.94 (0.5x CH_2), 25.99 (0.5x CH_2), 29.27 (0.5x CH_2), 31.15 (0.5x CH_2), 62.69 (CH_2), 71.59 (CH), 73.60 (0.5x CH), 74.51 (0.5x CH), 74.97 (0.5x CH), 75.09 (0.5x CH), 77.75 (0.5x CH), 77.78 (0.5x CH), 78.00 (CH), 102.53 (0.5x An

[†] Diastereoisomer ratio is calculated from the relative peak heights of the anomeric protons in the ^{1}H NMR spectrum.

CH), 103.23 (0.5x An CH), 128.51 (0.5x CH), 129.79 (0.5x CH), 131.41 (0.5x CH), 131.70 (0.5x CH); *m/z* 261 (MH^+, 2%), 260 (M^+, 1%), 163 (M^+ -C_6H_9O, 19%), 143 (M^+ -$C_4H_5O_4$, 25%), 97 (M^+ -$C_6H_{11}O_5$, 20%), 81 (M^+ -$C_6H_{11}O_6$, 100%), 73 (M^+ -$C_9H_{15}O_4$, 34%); [Found M^+ 260.1274. $C_{12}H_{20}O_6$ requires *M* 260.1260] (E.I.).

1.4.10. Morphine-6-β-D-glucopyranoside 31

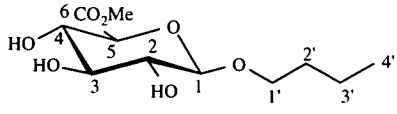


31

A suspension of 3-*O*-pivaloylmorphine-6-(2,3,4,6-tetra-*O*-acetyl)-β-D-glucopyranoside **38** (0.252 g, 0.36 mmol) in dry MeOH (10 ml) was stirred at rt under an atmosphere of N_2. A 0.23 M solution of NaOMe (0.012 g, 0.23 mmol) in dry MeOH (1 ml) was added with the addition of further NaOMe (0.012 g, 0.23 mmol) in dry MeOH (1 ml) at 17 h, 21 h and 24 h. Tlc revealed that the reaction had gone to completion after 48 h, the pH of the reaction mixture was neutralised using 2 M aq HCl and the solvent removed by rotary evaporation to give an off white solid (0.380 g). Crystallisation (MeOH-ether) of the crude residue afforded *the title compound* as an off white solid (0.151 g, 94%); R_f 0.30 (1:1:1, butanol-acetone-water); m.p./decomp. 234-235 °C (from MeOH-ether); $[\alpha]_D^{25}$ -140.22 (*c* 0.37, water), (Lit.[52] $[\alpha]_D^{25}$ -132 (*c* 1, water); [Found: C, 53.84%; H, 6.43%; N, 1.79%. $C_{23}H_{29}NO_8$ requires C, 61.72%; H, 6.55%; N, 3.10%]; ν_{max} (KBr)/cm^{-1} 1655m (CH), 1616m (CH), 1459s (CH), 1040s (C-O-C); δ_H (250 MHz; D_2O) (COSY) 2.06 (1H, bd, $^2J_{gem}$ 12, $^{15}CH_{eq}$), 2.27 (1H, bt, $^2J_{gem}$ 12, $^{15}CH_{ax}$), 2.83-3.14 (2H, m, ^{14}CH, $^{10}CH_{ax}$), 2.94 (3H, s, NMe), 3.21 (3H, bs, $^{16}CH_{ax}$), 3.27-3.55 (6H, m, $^2J_{gem}$ 19, $^{10}CH_{eq}$, $^{2'}CH$, $^{3'}CH$, $^{4'}CH$, $^{5'}CH$, $^{16}CH_{eq}$), 3.68 (1H, dd, $^2J_{gem}$ 12, $^{6'}CH$), 3.86 (1H, dd, $^2J_{gem}$ 12, $^{6'}CH$), 4.18 (1H, m, ^9CH), 4.55 (1H, m, ^6CH), 4.70 (1H, d, $^3J_{1',2'}$ 8, $^{1'}CH$), 5.21 (1H, d, $^3J_{5,6}$ 8, ^5CH), 5.38 (1H, bd, $^3J_{7,8}$ 11, ^7CH), 5.82 (1H, bd, $^3J_{8,7}$ 11, ^8CH), 6.62 (1H, d, $^3J_{1,2}$ 8, ^1CH), 6.73 (1H, d, $^3J_{2,1}$ 8, ^2CH); δ_C (63 MHz; D_2O) 20.93 (CH_2), 34.47 (CH_2), 38.55 (CH), 40.94 (NMe), 41.59 (Q), 47.20 (CH_2), 60.61 (CH_3, CH_2), 69.51 (CH), 72.78 (CH), 73.24 (CH), 75.66 (CH), 76.06 (CH), 88.14 (CH), 101.52 (An CH), 117.73 (CH), 120.45 (CH), 123.28 (Q), 126.04 (CH), 129.05 (Q), 131.40 (CH), 137.97 (Q), 145.58 (Q); *m/z* 448 (MH^+, 17%), 269 (M^+ -$C_6H_{11}O_6$, 7%), 237 (M^+ -$C_7H_{16}NO_6$, 31%), 214

(M^+ -$C_{16}H_9O_2$, 28%); [Found MH^+ 448.1961. $C_{23}H_{29}NO_8$ requires *M*H 448.1971] (F.A.B.).

1.4.11. Methyl (*n*-Butyl β-D-glucopyranosid)uronate 240



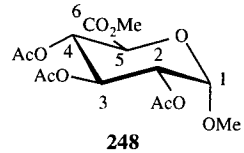
240

TEMPO Oxidation; A solution of *n*-butyl β-D-glucopyranoside **238** (0.054 g, 0.23 mmol) and TEMPO (1 mg, 0.01 mmol) in CH_2Cl_2 (1 ml) stirring at rt was treated with a solution of KBr (0.028 g, 0.23 mmol) and Bu_4NCl (3 g, 0.23 mmol) in sat aq $NaHCO_3$ (1 ml), whereafter, the temperature was lowered to 0 °C and a solution of 0.6 M NaOCl[†] (0.0828 g, 0.05 mmol) in sat aq $NaHCO_3$ (0.5 ml) and brine (1 ml) was added over 45 mins. Further 0.6 M NaOCl (0.120 g, 0.072 mmol) was added at 3 h. Tlc revealed that the reaction had gone to completion after 17 h, the organic layer was isolated, washed with water (3x 5 ml) and the pH of the combined aqueous washings was adjusted to pH 3 with 2 M HCl, extracted with EtOAc (5x 25 ml) and the volume of the combined organic extracts reduced by rotary evaporation to give a white solid (0.018 g). Flash column-chromatography (9:1, CH_2Cl_2-MeOH) of the crude residue afforded a yellow syrup (0.030 g) which was dissolved in MeOH (4 ml) and stirred at rt with Dowex 50W x8 H^+ (0.114 g), for 21 h. The reaction mixture was filtered and the volume of the filtrate reduced by rotary evaporation to give a yellow syrup (0.011 g). Flash column-chromatography (9:1, CH_2Cl_2-MeOH) of the crude residue afforded *the title compound* as a yellow syrup (1 mg, 20%); R_f 0.72 (9:1, CH_2Cl_2-MeOH); $[\alpha]_D^{24}$ -27.22 (*c* 0.19, MeOH); ν_{max} (nujol)/cm^{-1} 1744s (CO_2), 1068s (C-O-C), 1026s (C-O(H)); δ_H (300 MHz; CD_3OD) 0.95 (3H, t, $^3J_{4',3'}$ 7, $^4'CH_3$), 1.41 (2H, sextet, $^3J_{3',4'}$ 7, $^3'CH_2$), 1.61 (2H, quintet, $^3J_{2',3'}$ 7, $^2'CH_2$), 3.21 (1H, dd, $^3J_{2,3}$ 8, ^{2}CH), 3.39 (1H, t, $^3J_{3,4}$ 9, ^{3}CH), 3.47-3.61 (2H, m, $^3J_{1',2'}$ 7, $^1'$CH, ^{4}CH), 3.76 (3H, s, CO_2CH_3), 3.79-3.87 (2H, m, $^2J_{gem}$ 13, $^1'$CH, ^{5}CH), 4.29 (1H, d, $^3J_{1,2}$ 8, ^{1}CH); δ_C (63 MHz; CD_3OD) 14.12 (CH_3), 20.10 (CH_2), 32.62 (CH_2), 57.79 (CO_2CH_3), 70.93 (OCH_2), 73.15 (CH), 74.80 (CH), 76.63 (CH), 77.41 (CH), 104.80 (An CH), 171.30 (Q); *m/z* 282 (MNH_4^+, 86%), 265 (MH^+, 7%), 208 (M^+ -C_4H_8, 100%), 191 (M^+ -C_4H_9O, 30%); [Found MNH_4^+ 282.1552. $C_{11}H_{20}O_7$ requires *M*NH_4^+ 282.1553] (C.I.).

[†] Each batch of sodium hypochlorite solution obtained from the commercial supplier contained varying quantities of sodium hypochlorite. Initial titration against potassium iodide and standardised sodium thiosulfate gave a molarity of 0.6 M for the sodium hypochlorite solution.

Platinum Oxidation; A solution of PtO_2 (Adams Catalyst) (0.031 g, 0.11 mmol) in high purity water (2 ml) was stirred for 5 h at rt under an atmosphere of H_2. A solution of *n*-butyl β-D-glucopyranoside **238** (0.031 g, 0.13 mmol) in *n*-heptane (2 ml) was added and the reaction mixture heated to 60 °C with a continuous stream of O_2 supplied by a sintered bleed. After 42 h at this temperature, tlc revealed that the reaction had gone to completion. The reaction mixture was allowed to cool to rt, filtered through a pad of Celite and the volume of the filtrate concentrated by rotary evaporation to give a yellow syrup (0.012 g). The crude residue was dissolved in MeOH (2 ml) and the temperature of the solution lowered to 0 °C, a solution of diazomethane (0.424 g, 10.08 mmol) in ether (10 ml) was added dropwise. After 1 h at this temperature, tlc revealed the reaction had gone to completion, the reaction mixture was allowed to warm to rt and the solvent removed by rotary evaporation to give a yellow syrup (9 mg). Flash column-chromatography (9:1, CH_2Cl_2-MeOH) of the crude residue afforded *the title compound* as a white solid (3 mg, 10%).

1.4.12. Methyl (methyl 2,3,4-tri-*O*-acetyl-α-D-glucopyranosid)uronate 248

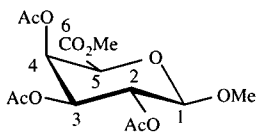


248

A solution of methyl α-D-glucopyranoside **230** (0.307 g, 1.6 mmol) in high purity water (50 ml) was stirred for 15 mins at rt while a continuous stream of O_2 supplied by a sintered bleed was applied at a vigorous rate. The rate of O_2 delivery was reduced to a gentle stream and 5% Pt/C (0.500 g) was added. The reaction mixture heated to 55 °C and the pH maintained between pH 6-7 by the addition of 0.1 M aq $NaHCO_3$, after 48 h tlc revealed that the reaction had gone to completion. The reaction mixture was allowed to cool to rt, filtered through a pad of Celite and the solvent removed by rotary evaporation to give an off white solid (1.870 g). A suspension of the crude residue was stirred at rt in MeOH (60 ml) with Amberlyte Resin 1R 120(H) (15 g), tlc revealed that the reaction had gone to completion after 3 days. The reaction mixture was filtered and the solvent removed by rotary evaporation to give a glassy solid (1.369 g), flash column-chromatography (9:1, EtOAc-MeOH) of the crude residue afforded the methyl (methyl glucopyranosid)uronate as a colourless glass (0.824 g). The methyl (methyl glucopyranosid)uronate was stirred in dry Py (5 ml) under an atmosphere of N_2 at rt and treated with with Ac_2O (4.328 g, 42.39 mmol) and DMAP (4 g, 0.03 mmol).

After 1 h tlc revealed that the reaction had gone to completion, the reaction mixture was poured onto a mixture of ice (40 g) and 2 M aq HCl (25 ml), extracted with EtOAc (4x 50 ml) and the combined organic extracts washed with 2 M aq HCl (2x 100 ml), sat aq $NaHCO_3$ (2x 100 ml), water (100 ml), brine (100 ml) and dried over $MgSO_4$ and the solvent removed by rotary evaporation to give a yellow syrup (1.172 g). Flash column-chromatography (3:2, light petroleum-EtOAc) of the crude acetylated product afforded *the title compound* as a clear, glassy solid (1.088 g, 40%); R_f 0.61 (1:1, light petroleum-EtOAc), Lit.[171] m.p. 150-151 °C; $[\alpha]_D^{22}$ +121.77 (*c* 0.98, $CHCl_3$), (Lit.[172] $[\alpha]_D^{22}$ +106 (*c* 0.65, $CHCl_3$); [Found: C, 48.13%; H, 6.07%; N, 0%. $C_{14}H_{20}O_{10}$ requires C, 48.26%; H, 5.79%; N, 0%]; ν_{max} (nujol)/cm^{-1} 1755s (CO_2), 1133s (C-O-C), 1051s (C-O-C); δ_H (250 MHz; $CDCl_3$) 1.98 (3H, s, OAc), 1.99 (3H, s, OAc), 2.04 (3H, s, OAc), 3.42 (3H, s, OMe), 3.74 (3H, s, CO_2CH_3), 4.26 (1H, d, $^3J_{5,4}$ 10, ^{5}CH), 4.86 (1H, dd, $^3J_{2,1}$ 4, ^{2}CH), 4.99 (1H, d, $^3J_{1,2}$ 4, ^{1}CH), 5.13 (1H, t, $^3J_{4,3}$ 10, ^{4}CH), 5.48 (1H, t, $^3J_{3,4}$ 10, ^{3}CH); δ_C (63 MHz; $CDCl_3$) 20.33 (CH_3), 20.49 (2x CH_3), 52.71 (OMe), 55.83 (CO_2CH_3), 67.95 (CH), 69.09 (CH), 69.44 (CH), 70.30 (CH), 96.92 (An CH), 167.92 (Q), 169.36 (Q), 169.72 (Q), 169.84 (Q); *m/z* 349 (MH^+, 2%), 348 (M^+, 1%), 257 (M^+ -$C_3H_7O_3$, 5%), 197 (M^+ -$C_5H_{11}O_5$, 19%), 155 (M^+ -$C_7H_{13}O_6$, 62%), 43 (M^+ -$C_{12}H_{17}O_9$, 100%); [Found MH^+ 349.1136. $C_{14}H_{20}O_{10}$ requires *M*H 349.1135] (F.A.B.).

1.4.13. Methyl (methyl 2,3,4-tri-*O*-acetyl-β-D-galactopyranosid)uronate 250



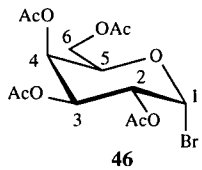
250

A solution of 1-*O*-methyl β-D-galactopyranoside **249** (1.535 g, 7.90 mmol) in high purity water (50 ml) was stirred for 5 mins at rt while a continuous stream of O_2 supplied by a sintered bleed was applied at a vigorous rate. The rate of O_2 delivery was reduced to a gentle stream and 5% Pt/C (0.494 g) was added. The reaction mixture heated to 55 °C and the pH was maintained between pH 6-7 by the addition of 0.1 M aq $NaHCO_3$, after 48 h tlc revealed that the reaction had gone to completion. The reaction mixture was allowed to cool to rt, filtered through a pad of Celite and the solvent removed by rotary evaporation to give an off white solid (1.873 g). A suspension of the crude residue was stirred at rt in MeOH (60 ml) with Amberlyte Resin 1R 120(H) (5 g), tlc revealed that the reaction had gone to completion after 5 days, the reaction mixture was filtered and the solvent removed by

rotary evaporation to give an off white syrup (1.412 g), flash column-chromatography (17:3, EtOAc-MeOH) of the crude residue afforded the methyl (methyl galctopyranosid)uronate as a white solid (0.665 g). A suspension of the methyl (methyl galctopyranosid)uronate was stirred in dry Py (5 ml) and treated with Ac_2O (3.246 g, 31.80 mmol) and DMAP (5 g, 0.04 mmol). After 4 h tlc revealed that the reaction had gone to completion. The reaction mixture was poured onto a mixture of ice (20 g) and 1 M aq HCl (50 ml), extracted with EtOAc (4x 50 ml) and the combined organic extracts washed with 1 M aq HCl (2x 100 ml), sat aq $NaHCO_3$ (2x 100 ml), water (100 ml), brine (100 ml), dried over $MgSO_4$ and the solvent removed by rotary evaporation to give a white foam (0.875 g). Flash column-chromatography (1:1, light petroleum-EtOAc) of the crude acetylated product followed by crystallisation (ether-EtOAc-light petroleum) afforded *the title compound* as a white crystalline solid (0.571 g, 21%); R_f 0.34 (1:1, light petroleum-EtOAc); m.p. 106-107 °C (from ether-EtOAc-light petroleum), (Lit.[173] 95-96 °C); $[\alpha]_D^{24}$ +15.52 (*c* 1.07, $CHCl_3$), (Lit.[173] $[\alpha]_D^{22}$ +160 ($CHCl_3$); [Found: C, 48.33%; H, 5.85%; N, 0%. $C_{14}H_{20}O_{10}$ requires C, 48.26%; H, 5.79%; N, 0%]; ν_{max} (KBr)/cm^{-1} 3014w (CH), 2968w (CH), 1754s (CO_2), 1455m (CH_3), 1374s ($COCH_3$), 1146s (C-O-C), 1081s (C-O-C), 1054s (C-O-C); δ_H (250 MHz; $CDCl_3$) 1.96 (3H, s, OAc), 2.04 (3H, s, OAc), 2.10 (3H, s, OAc), 3.55 (3H, s, OMe), 3.74 (3H, s, CO_2CH_3), 4.30 (1H, d, $^3J_{5,4}$ 2, ^{5}CH), 4.41 (1H, d, $^3J_{1,2}$ 8, ^{1}CH), 5.05 (1H, dd, $^3J_{3,2}$ 10, ^{3}CH), 5.23 (1H, dd, $^3J_{2,1}$ 8, ^{2}CH), 5.68 (1H, dd, $^3J_{4,5}$ 2, ^{4}CH); δ_C (63 MHz; $CDCl_3$) 20.43 (CH_3), 20.45 (CH_3), 20.64 (CH_3), 52.67 (OMe), 57.22 (CO_2CH_3), 68.20 (CH), 68.26 (CH), 70.42 (CH), 72.21 (CH), 101.82 (An CH), 166.37 (Q), 169.26 (Q), 169.75 (Q), 169.96 (Q); *m/z* 349 (MH^+, 7%), 348 (M^+, 1%), 317 (M^+ -CH_3O, 100%), 197 (M^+ -$C_5H_{11}O_5$, 31%), 155 (M^+ -$C_7H_{13}O_6$, 62%), 43 (M^+ -$C_{12}H_{17}O_9$, 76%); [Found MH^+ 349.1148. $C_{14}H_{20}O_{10}$ requires *M*H 349.1135] (F.A.B.).

1.5. Synthesis of Morphine-6-β-D-glucoside Analogues

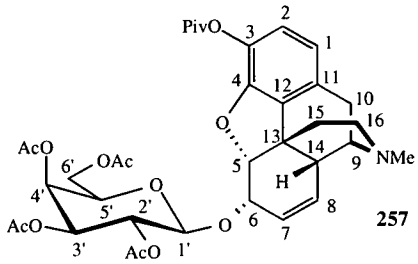
1.5.1. Tetra-*O*-acetyl-α-D-galactopyranosyl Bromide 46



46

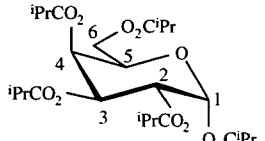
A solution of 45% (w/v) HBr in glacial acetic acid (1 ml; 0.450 g, 5.60 mmol HBr) was stirred at rt while α-D-galactopyranose pentaacetate (0.715 g, 1.80 mmol) was added over 5 mins, whereafter stirring was continued for 30 mins; tlc revealed at that time that the reaction had gone to completion. The reaction mixture was poured onto ice (20 g), partitioned with $CHCl_3$ (15 ml) and the organic extract was washed with ice cold water (4x 15 ml), sat aq $NaHCO_3$ (2x 15 ml), water (15 ml), brine (15 ml) and dried over $MgSO_4$. The solvent was removed by rotary evaporation to give a clear colourless oil (0.764 g), crystallisation (from ether-light petroleum) of the crude residue afforded *the title compound* as a white crystalline solid (0.579 g, 77%); R_f 0.67 (1:1, light petroleum-EtOAc); m.p. 79-80 °C (from ether-light petroleum), (Lit.[174] 83-85 °C); $[\alpha]_D^{24}$ +215.23 (*c* 1.01, $CHCl_3$), (Lit.[174] $[\alpha]_D^{20}$ +215 ($CHCl_3$); [Found: C, 40.47%; H, 4.71%; N, 0%. $C_{14}H_{19}O_9Br$ requires C, 40.97%; H, 4.67%; N, 0%]; ν_{max} ($CHCl_3$)/cm^{-1} 3000s (CH_3), 1750s (CO_2), 1078m (C-O-C); δ_H (250 MHz; $CDCl_3$) 2.00 (3H, s, OAc), 2.05 (3H, s, OAc), 2.10 (3H, s, OAc), 2.14 (3H, s, OAc), 4.14 (2H, qd, $^3J_{6,5}$ 7, $^6CH_2$), 4.48 (1H, t, $^3J_{5,6}$ 7, ^5CH), 5.04 (1H, dd, $^3J_{2,1}$ 4, ^2CH), 5.40 (1H, dd, $^3J_{3,4}$ 3, ^3CH), 5.50 (1H, d, $^3J_{4,3}$ 3, ^4CH), 6.68 (1H, d, $^3J_{1,2}$ 4, ^1CH); δ_C (63 MHz; $CDCl_3$) 20.39 (CH_3), 20.42 (CH_3), 20.47 (CH_3), 20.58 (CH_3), 60.66 (CH_2), 66.80 (CH), 67.59 (CH), 67.81 (CH), 70.88 (CH), 87.94 (An CH), 169.59 (Q), 169.73 (Q), 169.90 (Q), 170.16 (Q); *m/z* 411 (MH^+, 1%), 331 (M^+ -Br, 55%), 353 (M^+ -$C_2H_2O_2$, 28%), 351 (M^+ -$C_2H_4O_2$, 28%), 169 (M^+ -$C_6H_{10}O_5Br$, 100%); [Found M^+ 410.0343. $C_{14}H_{19}O_9Br$ requires *M* 410.0212] (F.A.B.).

1.5.2. 3-*O*-Pivaloylmorphine-6-(2',3',4',6'-tetra-*O*-acetyl)-β-D-galactopyranoside 257



A solution of 3-*O*-pivaloylmorphine **32** (0.105 g, 0.29 mmol) and Ag_2CO_3 (0.480 g, 1.74 mmol) in dry benzene (10 ml) was stirred at rt, a solution of tetra-*O*-acetyl-α-D-galactopyranosyl bromide **46** (0.195 g, 0.48 mmol) in dry benzene (10 ml) added and the reaction mixture heated under reflux using Dean-Stark apparatus. After 24 h tlc revealed that the reaction had gone to completion. The reaction mixture was allowed to cool to rt, filtered through a pad of Celite and the filtrate concentrated by rotary evaporation to give a dark red oil (0.362 g). Flash column-chromatography (13:1, CH_2Cl_2-MeOH) of the crude residue afforded *the title compound* as a white solid (0.032 g, 16%); R_f 0.62 (9:1, $CHCl_3$-MeOH); δ_H (250 MHz; $CDCl_3$) 1.35 (9H, s, $C(CH_3)_3$), 1.90 (1H, m, $^{15}CH_{eq}$), 1.97 (3H, s, OAc), 2.01 (1H, m, $^{15}CH_{ax}$) 2.03 (3H, s, OAc), 2.06 (3H, s, OAc), 2.14 (3H, s, OAc), 2.38 (1H, dd, $^2J_{gem}$ 19, $^{10}CH_{ax}$), 2.42 (3H, s, NMe), 2.44 (1H, m, $^{16}CH_{ax}$), 2.56 (1H, bd, $^3J_{16,15}$ 5, $^{16}CH_{eq}$), 2.64 (1H, m, ^{14}CH), 3.05 (1H, d, $^2J_{gem}$ 19, $^{10}CH_{eq}$), 3.36 (1H, q, $^3J_{9,14}$ 4, ^9CH), 3.94 (1H, t, $^3J_{5',6'}$ 7, $^{5'}CH$), 4.02-4.20 (2H, m, $^3J_{6',5'}$ 7, $^{6'}CH_2$, ^6CH), 4.80 (1H, d, $^3J_{1,2}$ 8, $^{1'}CH$), 4.88 (1H, dd, $^3J_{5,6}$ 6, ^5CH), 5.00 (1H, dd, $^3J_{3',4'}$ 4, $^{3'}CH$), 5.25 (1H, dd, $^3J_{2,1}$ 8, $^{2'}CH$), 5.30 (1H, m, ^7CH), 5.38 (1H, dd, $^3J_{4',3'}$ 4, $^{4'}CH$), 5.72 (1H, bd, $^3J_{8,7}$ 10, ^8CH), 6.53 (1H, d, $^3J_{1,2}$ 8, ^1CH), 6.67 (1H, d, $^3J_{2,1}$ 8, ^2CH); δ_C (63 MHz; $CDCl_3$) 20.50 (CH_3), 20.56 (2x CH_3), 20.74 (CH_3), 27.05 (3x CH_3), 29.55 (CH_2), 35.61 (CH_2), 38.87 (Q), 40.95 (CH), 42.92 (NMe), 43.59 (Q), 46.11 (CH_2), 58.51 (CH), 61.22 (CH_2), 66.97 (CH), 68.68 (CH), 70.70 (CH), 70.80 (CH), 73.94 (CH), 90.00 (CH), 100.11 (An CH), 118.81 (CH), 121.60 (CH), 128.30 (CH), 130.71 (CH), 131.11 (Q), 131.65 (Q), 131.82 (Q), 150.25 (Q), 169.29 (Q), 170.05 (Q), 170.17 (Q), 170.24 (Q), 176.29 (Q); a full characterisation was not possible as result of the limited quantity of material available.

1.5.3. α-D-Galactopyranose Pentaisobutyrate 260



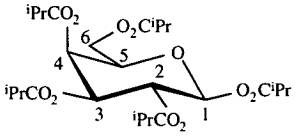
260

Base catalysed synthesis at low temperature; A suspension of D-(+)-galactose **144** (0.107 g, 0.59 mmol) and DMAP (5 g, 0.04 mmol) was stirred in dry Py (5 ml) at -10 °C with the exclusion of water provided by a drying tube containing $CaCl_2$. Isobutyryl chloride (0.712 g, 6.68 mmol) was added dropwise over 45 mins while maintaining the temperature below -5 °C. The reaction mixture was stirred for a further 2 h at this temperature and then allowed to warm to rt overnight. The reaction mixture was poured onto ice (20 g), extracted with CH_2Cl_2 (4x 20 ml) and the combined organic extracts were washed with 2 M aq HCl (2x 100 ml), sat aq $NaHCO_3$ (2x 100 ml), water (100 ml), brine (100 ml), dried over $MgSO_4$ and the solvent removed by rotary evaporation to give a yellow oil (0.289 g). Flash column-chromatography (8:2, light petroleum-EtOAc) afforded *the title compound* as a colourless syrup (0.534 g, 87%); R_f 0.50 (8:2, light petroleum-EtOAc); $[\alpha]_D^{22}$ +84.62 (*c* 1.06, $CHCl_3$); [Found: C, 59.07%; H, 7.89%; N, 0%. $C_{26}H_{42}O_{11}$ requires C, 58.84%; H, 8.98%; N, 0%]; ν_{max} (neat)/cm^{-1} 2974s (CH), 2936s (CH), 2877s (CH), 1754s (CO_2), 1099s (C-O-C), 1037s (C-O-C); δ_H (250 MHz; $CDCl_3$) 1.04-1.26 (30H, m, 10x CHCH_3), 2.31-2.74 (5H, m, 5x CHCH$_3$), 4.02 (2H, d, $^3J_{6,5}$ 7, $^6CH_2$), 4.35 (1H, t, $^3J_{5,6}$ 7, ^{5}CH), 5.36 (2H, dd, $^3J_{2,1}$ 2, ^{2}CH, ^{4}CH), 5.52 (1H, t, $^3J_{3,4}$ 2, ^{3}CH), 6.35 (1H, d, $^3J_{1,2}$ 2, ^{1}CH); δ_C (63 MHz; $CDCl_3$) 18.36 (CH_3), 18.49 (CH_3), 18.61 (4x CH_3), 18.69 (CH_3), 18.77 (CH_3), 18.81 (CH_3), 18.95 (CH_3), 33.61 (CH), 33.67 (CH), 33.75 (CH), 33.83 (CH), 33.89 (CH), 60.96 (CH_2), 66.30 (CH), 66.93 (CH), 67.35 (CH), 68.81 (CH), 89.29 (An CH), 174.75 (Q), 175.55 (Q), 175.71 (Q), 175.79 (Q), 176.29 (Q); *m/z* 531 (MH^+, 2%), 530 (M^+, 3%), 445 (M^+ -$C_4H_5O_2$, 100%), 375 (M^+ -$C_8H_{13}O_3$, 52%), 375 (M^+ -$C_8H_{13}O_4$, 17%); [Found 530.2734. $C_{26}H_{42}O_{11}$ requires 530.2727] (F.A.B.).

Base catalysed synthesis at an elevated temperature; A suspension of D-(+)-galactose **144** (0.105 g, 0.58 mmol) and DMAP (0.022 g, 0.18 mmol) was stirred in dry Py (5 ml) with the exclusion of water provided by a drying tube containing $CaCl_2$. Isobutyryl chloride (0.712 g, 6.68 mmol) was added dropwise and the reaction mixture heated to 50 °C, and stirring was continued at this temperature for 2 h; tlc

revealed that the reaction had gone to completion. The reaction mixture was poured onto ice (20 g) and extracted with CH_2Cl_2 (4x 20 ml). The combined organic extracts were washed with 2 M aq HCl (2x 100 ml), sat aq $NaHCO_3$ (2x 100 ml), water (100 ml), brine (100 ml), dried over $MgSO_4$ and the solvent removed by rotary evaporation to give a yellow oil (0.261 g, 84% crude yield); the spectroscopic data was in agreement with that reported for the title compound of the previous synthesis.

1.5.4. β-D-Galactopyranose Pentaisobutyrate 261

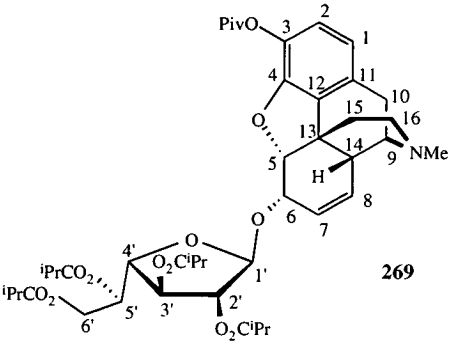


261

A suspension of D-(+)-galactose **144** (5.070 g, 28.14 mmol) in dry Py (48.900 g, 618.20 mmol) was heated under reflux with the exclusion of water provided by a drying tube containing $CaCl_2$, for 1 h, thereafter isobutyryl chloride (30.510 g, 286.34 mmol) was added dropwise and the reaction mixture heated under reflux for a further 1 h, then allowed to cool to rt. The reaction mixture was poured onto a mixture of ice (100 g) and 2 M aq HCl (100 ml), extracted with EtOAc (3x 100 ml) and the combined organic extracts washed with sat aq $NaHCO_3$ (4x 250 ml), water (2x 250 ml), brine (2x 250 ml), dried over $MgSO_4$ and the solvent removed by rotary evaporation to give a brown oil (16.698 g). Flash column-chromatography (1:99, EtOAc-light petroleum) of the crude residue afforded an inseparable mixture in a ratio 7:1:2 of β-D-galactopyranose pentaisobutyrate **261**, α-D-galactopyranose pentaisobutyrate **260** and β-D-galactofuranose pentaisobutyrate **263** respectively, as a yellow syrup (11.711 g, 78%; approximately 9.452 g, 2.17 mmol of *the title compound*); R_f0.56 (8:2, light petroleum-EtOAc); ν_{max} (neat)/cm^{-1} 2981s (CH), 2940s (CH), 2876s (CH), 1749s (CO_2), 1386s (CH_3), 1112s (C-O-C); δ_H (250 MHz; $CDCl_3$) 1.00-1.24 (60H, m, 10x βF-CHCH_3, 10x P-CHCH_3), 2.39-2.70 (10H, m, 5x βF-CHCH$_3$, 5x P-CHCH$_3$), 4.00-4.40 (5H, m, βF-^{5}CH, βF-^{6}CH$_2$, βP-^{5}CH, βP-^{6}CH$_2$, αP-^{5}CH, αP-^{6}CH$_2$), 5.05-5.15 (2.6H, m, βP-^{4}CH, βP-^{3}CH, βF-^{2}CH), 5.19-5.55 (4.4H, m, $^3J_{2,1}$ 8, βP-^{2}CH, αP-^{3}CH, βF-^{2}CH, αP-^{4}CH, βF-^{3}CH, βF-^{4}CH, αP-^{2}CH), 5.71 (0.8H, d, $^3J_{1,2}$ 8, βP-^{1}CH), 6.12 (1H, s, βF-^{1}CH), 6.32 (0.2H, d, $^3J_{1,2}$ 4, αP-^{1}CH); δ_C (63 MHz, $CDCl_3$) 18.17-19.00 [18.17, 18.27, 18.32, 18.40, 18.52, 18.54, 18.59, 18.65, 18.69, 18.78, 19.00] (10x βF-CH_3, 10x P-CH_3), 33.56-33.87 [33.56, 33.59, 33.66, 33.70, 33.76, 33.82, 33.87] (5x βF-CH, 5x P-CH), 60.51 (0.8x βP-CH_2), 61.70 (0.2x αP-CH_2), 62.26 (βF-CH_2), 66.30 (0.8x βP-CH), 67.51 (0.8x βP-CH), 68.79 (βF-CH),

70.09 (0.2x αP-CH), 70.64 (0.8x βP-CH), 71.68 (0.8x βP-CH), 73.11 (0.2x αP-CH), 75.16 (0.2x αP-CH), 75.70 (βF-CH), 78.90 (0.2x αP-CH), 80.22 (βF-CH), 82.27 (βF-CH), 91.88 (0.8x An βP-CH), 93.02 (0.2x An αP-CH), 98.81 (An βF-CH), 174.77-176.21 [174.77, 174.89, 175.16, 175.51, 175.55, 175.78, 175.85, 176.21] (5x βF-Q, 5x P-Q); *m/z* 529 (MH^+, 2%), 457 (M^+-C_4H_9O, 6%), 443 (M^+ -$C_4H_7O_2$, 88%), 442 (M^+-$C_4H_8O_2$, 42%), 197 (M^+-$C_{15}H_{25}O_8$, 100%) (F.A.B.).

1.5.5. 3-*O*-Pivaloylmorphine-6-(2',3',4',6'-tetra-*O*-isobutyryl)-β-D-galactofuranoside 269

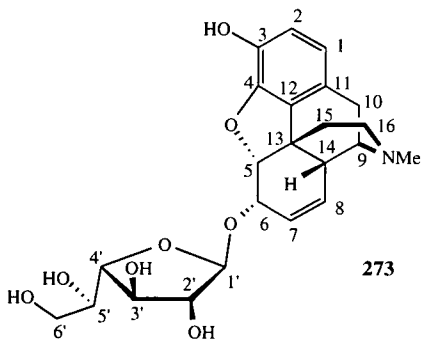


269

A solution of the inseperable β-D-galactopyranose **261**, α-D-galactopyranose **260** and β-D-galactofuranose pentaisobutyrate **263** (7.988 g, approximately 1.598 g, 3.01 mmol of β-D-galactofuranose pentaisobutyrate **263**) in dry CH_2Cl_2 (20 ml) was added to a solution of 3-*O*-pivaloylmorphine **32** (0.829 g, 2.24 mmol) in dry CH_2Cl_2 (11 ml) under an atmosphere of N_2. The resulting solution was stirred at rt for 10 mins, whereafter the temperature was lowered to 0 °C and TMSOTf (2.415 g, 10.87 mmol) was added dropwise while maintaining the temperature below 5 °C. Tlc at 3 h showed the reaction was incomplete, thus, further 3-*O*-pivaloylmorphine **32** (0.687 g, 1.84 mmol) in dry CH_2Cl_2 (9 ml) and TMSOTf (1.150 g, 5.17 mmol) was added and more TMSOTf (1.150 g, 5.17 mmol) added again at 5 h. Tlc revealed the reaction had gone to completion after 7 h; the reaction mixture was poured onto a mixture of ice (100 g) and sat aq $NaHCO_3$ (100 ml), extracted with CH_2Cl_2 (4x 100 ml) and the combined organic extracts washed with 5% (w/v) aq citric acid (3x 250 ml), sat aq $NaHCO_3$ (2x 250 ml), water (2x 250 ml), brine (250 ml), dried over $MgSO_4$ and the solvent removed by rotary evaporation to give a yellow oil (8.147 g). Flash column-chromatography (1:29, MeOH-$CHCl_3$) of the crude residue followed by crystallisation (EtOH-water) afforded an inseparable mixture of *the title compound* and 3-*O*-pivaloylmorphine-6-(2',3',4',6'-tetra-*O*-isobutyryl)-β-D-galactopyranoside **270** as a white foam (0.778 g) and *the title compound* as a white solid (0.238 g, 7%);

R_f 0.54 (9:4:2, EtOAc-^{i}PrOH-water); m.p. 124-125 °C (from EtOH-water); [Found: C, 65.18%; H, 7.73%; N, 1.52%. $C_{44}H_{61}NO_{11}$ requires C, 65.07%; H, 7.58%; N, 1.73%]; $[\alpha]_D^{23}$ -130.96 (*c* 1.00, $CHCl_3$); ν_{max} (KBr)/cm^{-1} 2976m (CH), 2932m (CH), 2871m (CH), 2792w (NMe), 1741s (CO_2), 1388m (CH_3), 1153s (C-O-C); δ_H (250 MHz; $CDCl_3$) (COSY) 1.10-1.19 (24H, m, 8x CHCH_3), 1.30 (9H, s, C(CH_3)$_3$), 1.95-1.97 (2H, m, $^{15}CH_{eq}$, $^{15}CH_{ax}$), 2.34 (1H, dd, $^2J_{gem}$ 19, $^{10}CH_{ax}$), 2.42 (3H, s, NMe), 2.44-2.64 (7H, m, 4x CHCH$_3$), $^{16}CH_{eq}$, $^{16}CH_{ax}$, ^{14}CH), 3.05 (1H, d, $^2J_{gem}$ 19, $^{10}CH_{eq}$), 3.35 (1H, q, $^3J_{9,14}$ 3, ^{9}CH), 4.12-4.22 (3H, m, ^{6}CH, $^{6'}CH_2$), 4.36 (1H, dd, $^3J_{5',6'}$ 12, $^{5'}$CH), 4.99 (2H, m, $^{3'}$CH, ^{5}CH), 5.08 (1H, d, $^{2'}$CH), 5.28-5.40 (3H, m, $^3J_{7,8}$ 10, ^{7}CH, $^{1'}$CH, $^{4'}$CH), 5.63 (1H, bd, $^3J_{8,7}$ 10, ^{8}CH), 6.53 (1H, d, $^3J_{2,1}$ 8, ^{1}CH), 6.70 (1H, d, $^3J_{2,1}$ 8, ^{2}CH); δ_C (63 MHz; $CDCl_3$) 18.59 (CH_3), 18.63 (CH_3), 18.66 (2x CH_3), 18.73 (2x CH_3), 18.78 (CH_3), 18.83 (CH_3), 20.75 (CH_2), 27.01 (3x CH_3), 33.53 (CH), 33.57 (CH), 33.74 (CH), 33.89 (CH), 35.56 (CH_2), 38.87 (Q), 40.97 (CH), 42.99 (NMe), 43.48 (Q), 46.22 (CH_2), 58.65 (CH), 62.19 (CH_2), 68.93 (CH), 69.82 (CH), 76.26 (CH), 80.48 (CH), 81.08 (CH), 88.73 (CH), 102.90 (An CH), 118.86 (CH), 121.50 (CH), 128.82 (CH), 130.29 (CH), 131.38 (Q), 131.97 (Q), 131.99 (Q), 150.26 (Q), 175.00 (Q), 175.69 (Q), 175.82 (Q), 176.04 (Q), 176.27 (Q); *m/z* 812 (MH^+, 2%), 811 (M^+, 14%), 724 (M^+ -$C_4H_7O_2$, 5%), 638 (M^+ -$C_8H_{13}O_4$, 5%), 443 (M^+ -$C_{22}H_{26}NO_4$, 14%), 268 (M^+ -$C_{27}H_{43}O_{11}$, 15%); [Found MH^+ 812.4216. $C_{44}H_{61}NO_{13}$ requires *M*H 812.4221] (F.A.B.).

1.5.6. Morphine-6-β-D-galactofuranoside 273

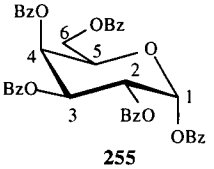


273

A suspension of 3-*O*-pivaloylmorphine-6-(2',3',4',6'-tetra-*O*-isobutyryl)-β-D-galactofuranoside **269** (0.094 g, 0.12 mmol) in dry MeOH (5 ml) was stirred at rt for 1 h under an atmosphere of N_2. A 0.52 M solution of NaOMe (0.056 g, 1.04 mmol) in dry MeOH (2 ml) was added and stirring was continued for 2 h. Tlc indicated that the reaction was proceding slowly, thus a further addition of NaOMe (0.056 g, 1.04

mmol) in dry MeOH (2 ml) was carried out. Tlc revealed that the reaction had gone to completion after 22 h, the reaction mixture was neutralised with 2 M aq HCl and the solvent removed by rotary evaporation to give an off white solid (0.181 g). The crude residue was dissolved in warm MeOH and filtered through a sintered funnel. Rotary evaporation of the filtrate gave a white solid (0.151 g) which on crystallisation (MeOH-^{i}PrOH) afforded *the title compound* as a white solid (0.061 g, quantitative yield); Rf 0.36 (9:4:2, EtOAc-^{i}PrOH-water); m.p./decomp. 158 °C (from MeOH-EtOAc); $[\alpha]_D^{24}$ -89.35 (*c* 0.47, MeOH); [Found: C, 29.60%; H, 3.42%; N, 1.35%. $C_{23}H_{29}NO_7$ requires C, 61.72%; H, 6.54%; N, 3.13%]; ν_{max} (KBr)/cm^{-1} 2921s (CH), 2852m (CH), 1612m (Ar CH), 1558m (Ar CH), 1499m (Ar CH), 1387m (CH_3), 1121s (C-O(H)), 1097s (C-O-C), 1028s (C-O-C); δ_H (250 MHz; CD_3OD) 1.96 (1H, bd, $^2J_{gem}$ 11, $^{15}CH_{eq}$), 2.23 (1H, td, $^2J_{gem}$ 11, $^{15}CH_{ax}$), 2.57 (1H, dd, $^2J_{gem}$ 19, $^{10}CH_{ax}$), 2.60 (1H, m, $^{16}CH_{ax}$), 2.61 (3H, s, NMe), 2.77 (1H, bd, $^2J_{gem}$ 11, $^{16}CH_{eq}$), 2.82 (1H, m, ^{14}CH), 3.16 (1H, d, $^2J_{gem}$ 19, $^{10}CH_{eq}$), 3.58 (1H, m, ^9CH), 3.71 (1H, d, $^3J_{2',3}$ 6, $^{2'}CH$), 3.83 (1H, m, $^{3'}CH$), 3.99-4.20 (4H, m, $^{5'}CH$, $^{4'}CH$, $^{6'}CH_2$), 4.42 (1H, m, ^6CH), 5.12 (1H, dd, $^3J_{5,6}$ 6, ^5CH), 5.33 (1H, d, $^3J_{1',OH}$ 2, $^{1'}CH$), 5.46 (1H, dt, $^3J_{7,8}$ 11, ^7CH), 5.81 (1H, bd, $^3J_{8,7}$ 11, ^8CH), 6.54 (1H, d, $^3J_{1,2}$ 8, ^1CH), 6.64 (1H, d, $^3J_{2,1}$ 8, ^2CH); δ_C (63 MHz; CD_3OD) 19.92 (CH_2), 34.09 (CH_2), 39.28 (CH), 40.97 (NMe), 42.34 (Q), 45.78 (CH_2), 58.65 (CH), 62.54 (CH_2), 70.18 (CH), 70.48 (CH), 76.80 (CH), 81.48 (CH), 82.84 (CH), 88.08 (CH), 105.93 (An CH), 115.83 (CH), 118.49 (CH), 124.52 (CH), 127.08 (CH), 129.60 (Q), 130.54 (CH), 138.07 (Q), 145.48 (Q); *m/z* 448 (MH^+, 1%), 385 (M^+ -$C_2H_6O_2$, 19%), 357 (M^+ -$C_3H_6O_3$, 25%), 338 (M^+ -$C_6H_5O_2$, 1%), 268 (M^+ -$C_6H_{11}O_6$, 6%), 43 (M^+ -$C_{21}H_{26}NO_7$, 100%); [Found MH^+ 448.1958. $C_{23}H_{29}NO_8$ requires *M*H 448.1971] (F.A.B.).

1.5.7. α-D-Galactopyranose Pentabenzoate 255

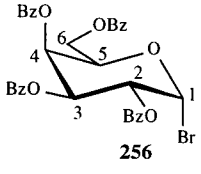


255

A cooled (0 °C) and stirred solution of D-(+)-galactose **144** (1.018 g, 5.65 mmol) in Py (9.780 g, 123.64 mmol) was treated with benzoyl chloride (7.872 g, 56.00 mmol), added dropwise, while the temperature was maintained below 4 °C. After 3 h a white precipitate had formed which dissolved on addition of $CHCl_3$ (20 ml). The reaction mixture was poured onto ice (20 g), the aqueous layer isolated and extracted with $CHCl_3$ (3x 30 ml). The combined organic extracts were washed with 1

M aq HCl (2x 100 ml), water (100 ml), sat aq $NaHCO_3$ (2x 100 ml), water (100 ml), brine (100 ml), dried over $MgSO_4$ and the solvent removed by rotary evaporation to give a yellow oil (5.588 g). Flash column-chromatography (8:2, light petroleum-EtOAc) of the crude residue followed by crystallisation (ether-light petroleum) afforded *the title compound* as a white powder (1.002 g, 25%); R_f0.59 (7:3, light petroleum-EtOAc); m.p. 149-150 °C (from ether-light petroleum); $[\alpha]_D^{23}$ +182.18 (*c* 1.00, $CHCl_3$), (Lit.[175] $[\alpha]_D$ +187.1); [Found: C, 69.86%; H, 4.50%; N, 0%. $C_{41}H_{32}O_{11}$ requires C, 70.27%; H, 4.61%; N, 0%]; ν_{max} (KBr)/cm^{-1} 2975w (CH), 1730s (CO_2), 1602m (Ar CH), 1454m (Ar CH), 1108s (C-O-C), 1026s (C-O-C); δ_H (250 MHz; $CDCl_3$) 4.42 (1H, dd, $^3J_{6,5}$ 7, ^{6}CH), 4.64 (1H, dd, $^3J_{6,5}$ 7, ^{6}CH), 4.84 (1H, t, $^3J_{5,6}$ 7, ^{5}CH), 6.03 (1H, dd, $^3J_{2,3}$ 7, ^{2}CH), 6.13 (1H, dd, $^3J_{3,2}$ 4, ^{3}CH), 6.19 (1H, dd, $^3J_{4,3}$ 4, ^{4}CH), 6.95 (1H, d, $^3J_{1,2}$ 4, ^{1}CH), 7.25-8.40 (25H, m, 5x C_6H_5); δ_C (63 MHz; $CDCl_3$) 61.69 (CH_2), 67.54 (CH), 68.31 (CH), 68.39 (CH), 69.29 (CH), 90.53 (An CH), 128.23 (2x Ar CH), 128.28 (5x Ar CH), 128.55 (Ar Q), 128.61 (Ar Q), 128.66 (3x Ar CH), 128.80 (Ar Q), 128.83 (Ar Q), 129.13 (Ar Q), 129.63 (7x Ar CH), 129.82 (3x Ar CH), 133.12 (Ar CH), 133.28 (Ar CH), 133.36 (Ar CH), 133.61 (Ar CH), 133.79 (Ar CH), 164.41 (Q), 165.35 (Q), 165.43 (Q), 165.57 (Q), 165.79 (Q); *m/z* 699 (M^+ - H, 1%), 580 (M^+ -$C_7H_4O_2$, 30%), 579 (M^+ -$C_7H_5O_2$, 80%), 459 (M^+ -$C_{14}H_9O_4$, 1%), 335 (M^+ -$C_{21}H_{17}O_6$, 4%); [Found 699.1885. $C_{41}H_{32}O_{11}$ requires 699.1866] (F.A.B.).

1.5.8. Tetra-*O*-benzoyl-α-D-galactopyranosyl Bromide 256

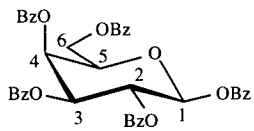


256

A solution of 45% (w/v) HBr in glacial acetic acid (15 ml; 6.750 g, 83.33 mmol HBr) was stirred at rt while α-D-galactopyranose pentabenzoate **255** (3.151 g, 4.50 mmol) was added over 5 mins. Stiring was continued for 4 h; tlc at that time revealed that the reaction had gone to completion. The reaction mixture was poured onto ice (20 g), extracted with CH_2Cl_2 (4x 30 ml) and the combined organic extracts washed with water (2x 100 ml), sat aq $NaHCO_3$ (2x 100 ml), water (100 ml), brine (100 ml), dried over $MgSO_4$ and the solvent removed by rotary evaporation to give a white foam (2.591 g). Flash column-chromatography (8:2, light petroleum-EtOAc) of the crude residue followed by crystallisation (light petroleum) afforded *the title compound* as a fine white powder (0.750 g, 25%); R_f 0.54 (7:3, light petroleum-EtOAc); m.p. 61-62 °C (from light petroleum); $[\alpha]_D^{23}$ +193.88 (*c* 1.04, $CHCl_3$),

(Lit.[176] $[\alpha]_D^{20}$ +219.7 (*c* 1, $CHCl_3$); [Found: C, 62.13%; H, 4.20%; N, 0%. $C_{34}H_{27}O_9Br$ requires C, 61.81%; H, 4.06%; N, 0%); ν_{max} (KBr)/cm^{-1} 3071s (CH), 2968s (CH), 1730s (CO_2), 1602m (Ar CH), 1451m (Ar CH), 1095s (C-O-C); δ_H (250 MHz; $CDCl_3$) 4.47 (1H, dd, $^3J_{6,5}$ 7, ^{6}CH), 4.65 (1H, dd, $^3J_{6,5}$ 7, ^{6}CH), 4.93 (1H, t, $^3J_{5,6}$ 7, ^{5}CH), 5.68 (1H, dd, $^3J_{2,1}$ 4, ^{2}CH), 6.07 (1H, dd, $^3J_{3,4}$ 4, ^{3}CH), 6.13 (1H, dd, $^3J_{4,3}$ 4, ^{4}CH), 6.99 (1H, d, $^3J_{1,2}$ 4, ^{1}CH), 7.20-8.10 (20H, m, 4x C_6H_5); δ_C (63 MHz; $CDCl_3$) 61.52 (CH_2), 67.91 (CH), 68.43 (CH), 68.73 (CH), 71.67 (CH), 88.13 (An CH), 128.20 (2x Ar CH), 128.32 (2x Ar CH), 128.36 (Ar Q), 128.42 (2x Ar CH), 128.60 (2x Ar CH), 129.07 (Ar Q), 129.60 (Ar Q), 129.66 (3x Ar CH), 129.80 (2x Ar CH), 129.87 (3x Ar CH), 133.20 (Ar Q), 133.25 (2x Ar CH), 133.66 (2x Ar CH), 165.17 (Q), 165.21 (Q), 165.41 (Q), 165.77 (Q); *m/z* 661 (MH^+, 1%), 660 (M^+, 2%), 579 (M^+ -Br, 43%), 539 (M^+ -$C_7H_5O_2$, 7%), 459 (M^+ -$C_7H_5O_2Br$, 4%); [Found M^+ 660.0831. $C_{34}H_{27}O_9Br$ requires *M* 660.0818] (F.A.B.).

1.5.9. β-D-Galactopyranose Pentabenzoate 264

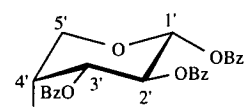


264

A suspension of D-(+)-galactose **144** (0.500 g, 2.77 mmol) in Py (10 ml) was heated under reflux for 1 h with the exclusion of water provided by a drying tube containing $CaCl_2$. Benzoyl chloride (3.633 g, 25.85 mmol) was added dropwise and the reaction mixture was heated under reflux for a further 1 h, then allowed to cool to rt. The reaction mixture was poured onto a mixture of ice (20 g) and sat aq $NaHCO_3$ (20 ml), extracted with CH_2Cl_2 (3x 20 ml) and the combined organic extracts washed with 1 M aq HCl (4x 100 ml), sat aq $NaHCO_3$ (2x 100 ml), water (100 ml), brine (100 ml), dried over $MgSO_4$ and the solvent removed by rotary evaporation to give a brown oil (3.840 g). Flash column-chromatography (8:2, light petroleum-EtOAc) of the crude residue followed by crystallisation (light petroleum-ether) afforded an inseparable mixture in a 6:1 ratio of *the title compound* and β-D-galactofuranose pentabenzoate **266** respectively, as an off white foam (0.991 g, 51%; approximately 0.849 g, 1.21 mmol of *the title compound*); R_f 0.46 (7:3, light petroleum-EtOAc); Lit.[175] m.p. 169-170 °C; Lit.[175] $[\alpha]_D^{20}$ +53.5 (*c* 0.8, $CHCl_3$); ν_{max} (KBr)/cm^{-1} 3067m (CH), 2974 (CH), 2361m (Ar CH), 2341m (Ar CH), 1731s (CO_2), 1107s (C-O-C), 1068s (C-O-C), 1026s (C-O-C); δ_H (250 MHz; $CDCl_3$) 4.45 (1H, dd, $^3J_{6,5}$ 6, βP-^{6}CH),

4.59 (1H, t, $^3J_{5,6}$ 6, βP-^{5}CH), 4.68 (1H, dd, $^2J_{gem}$ 10, βP-^{6}CH), 4.78 (2H, m, $^3J_{6,5}$ 5, βF-$^6CH_2$), 4.87 (1H, t, $^3J_{5,6}$ 5, βF-^{5}CH), 5.78 (2H, m, βF-^{3}CH, βF-^{4}CH), 5.79 (1H, dd, $^3J_{3,4}$ 9, βP-^{3}CH), 6.06-6.16 (3H, m, $^3J_{2,1}$ 8, βP-^{2}CH, βP-^{4}CH, βF-^{2}CH), 6.29 (1H, d, $^3J_{1,2}$ 8, βP-^{1}CH), 6.80 (1H, bs, βF-^{1}CH), 7.20-8.15 (50H, m, 5x βP-C_6H_5, 5x βF-C_6H_5); δ_C (63 MHz; $CDCl_3$) 61.63 (βP-CH_2), 63.50 (βF-CH_2), 67.79 (βP-CH), 68.58 (βP-CH), 70.25 (βF-CH), 71.41 (βP-CH), 72.28 (βP-CH), 76.90 (βF-CH), 81.00 (βF-CH), 84.40 (βF-CH), 92.91 (An βP-CH), 99.75 (An βF-CH), 128.20 (2x Ar βP-CH, 2x βF-CH), 128.30 (4x Ar βP-CH, 4x βF-CH), 128.40 (2x Ar βP-CH, 2x βF-CH), 128.51 (Ar βP-Q), 128.55 (2x Ar βP-CH, 2x βF-CH), 128.64 (Ar βP-Q, 2x Ar βF-Q), 128.80 (Ar βP-Q), 129.09 (Ar βF-Q), 129.15 (Ar βP-Q), 129.32 (Ar βF-Q), 129.57 (Ar βP-Q, Ar βF-Q), 129.62 (2x Ar βP-CH, 2x βF-CH), 129.67 (Ar βP-CH, Ar βF-CH), 129.71 (Ar βP-CH, Ar βF-CH), 129.80 (Ar βP-CH, Ar βF-CH), 129.91 (2x Ar βP-CH, 2x βF-CH), 130.05 (Ar βP-CH, Ar βF-CH), 130.12 (2x Ar βP-CH, 2x βF-CH), 132.89 (Ar βF-CH), 133.14 (Ar βP-CH), 133.26 (Ar βP-CH, Ar βF-CH), 133.32 (Ar βP-CH), 133.41 (Ar βF-CH), 133.56 (Ar βP-CH), 133.59 (Ar βP-CH), 133.73 (2x Ar βF-CH), 164.50 (βF-Q), 164.56 (βP-Q, βF-Q), 165.17 (βP-Q, βF-Q), 165.34 (2x βP-Q, βF-Q), 165.80 (βF Q), 165.86 (βP-Q); *m/z* 700 (M^+, 1%), 699 (M^+ -H, 1%), 579 (M^+ -$C_7H_5O_2$, 82%), 459 (M^+ -$C_{14}H_9O_2$, 1%), 105 (M^+ -$C_{34}H_{27}O_{10}$, 100%) (F.A.B.).

1.5.10. α-D-Arabinopyranose Tetrabenzoate 275

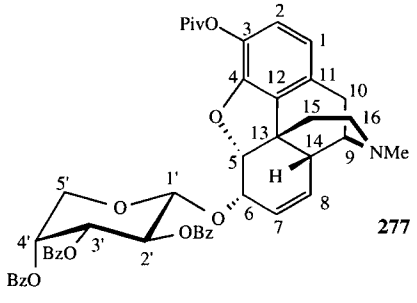


275

A suspension of D-(-)-arabinose (5.024 g, 33.47 mmol) in dry Py (48.900 g, 618.20 mmol) was heated under reflux for 1 h with the exclusion of water provided by a drying tube containing $CaCl_2$. Benzoyl chloride (48.440 g, 344.60 mmol) was added dropwise and the reaction mixture heated under reflux for a further 1 h, then allowed to cool to rt. The reaction mixture was poured onto a mixture of ice (100 g) and 2 M aq HCl (100 ml), extracted with EtOAc (3x 100 ml) and the combined organic extracts washed with sat aq $NaHCO_3$ (4x 250 ml), water (4x 250 ml), brine (2x 250 ml), dried over $MgSO_4$ and the solvent removed by rotary evaporation to give a brown oil (33.480 g). Flash column-chromatography (1:9, EtOAc-light petroleum) of the crude residue followed by crystallisation (EtOAc-light petroleum) afforded *the title compound* as a white crystalline solid (0.821 g, 29%); R_f 0.53 (8:2,

light petroleum-EtOAc); m.p. 148-149 °C (from EtOAc-light petroleum), (Lit.[177] 162-163 °C); $[\alpha]_D^{21}$ -112.61 (*c* 1.09, $CHCl_3$), (Lit.[177] $[\alpha]_D^{21}$ -112 (*c* 0.55, $CHCl_3$); [Found: C, 69.74%; H, 4.66%; N, 0%. $C_{33}H_{26}O_9$ requires C, 69.95%; H, 4.63%; N, 0%]; ν_{max} (KBr)/cm^{-1} 1729s (CO_2), 1636m (Ar CH), 1602m (Ar CH), 1492m (Ar CH), 1087s (C-O-C); δ_H (250 MHz; $CDCl_3$) 4.14 (1H, dd, $^2J_{gem}$ 12, ^{5}CH), 4.43 (1H, dd, $^2J_{gem}$ 12, ^{5}CH), 5.79 (2H, m, ^{3}CH, ^{4}CH), 5.94 (1H, dd, $^3J_{2,1}$ 5, ^{2}CH), 6.25 (1H, d, $^3J_{1,2}$ 5, ^{1}CH), 7.28-7.62 (12H, m, Ar CH), 7.93-8.00 (8H, m, Ar CH); δ_C (63 MHz; $CDCl_3$) 62.61 (CH_2), 67.43 (CH), 68.78 (CH), 69.78 (CH), 92.30 (An CH), 128.37 (4x Ar CH), 128.41 (4x Ar CH), 128.67 (Ar Q), 128.73 (Ar Q), 128.97 (Ar Q), 129.16 (Ar Q), 129.79 (3x Ar CH), 130.03 (5x Ar CH), 133.38 (2x Ar CH), 133.48 (Ar CH), 133.59 (Ar CH), 164.62 (Q), 165.00 (Q), 165.35 (Q), 165.44 (Q); *m/z* 567 (MH^+, 1%), 566 (M^+, 1%), 461 (M^+-C_7H_5O, 1%), 446 (M^+-$C_7H_4O_2$, 10%), 445 (M^+-$C_7H_5O_2$, 50%), 443 (M^+-$C_7H_7O_2$, 29%), 105 (M^+-$C_{27}H_{21}O_8$, 59%); [Found MH^+ 565.1489. $C_{33}H_{26}O_9$ requires *M*H 565.1499] (F.A.B.).

1.5.11. 3-*O*-Pivaloylmorphine-6-(2',3',4'-tri-*O*-benzoyl)-α-D-arabinopyranoside 277

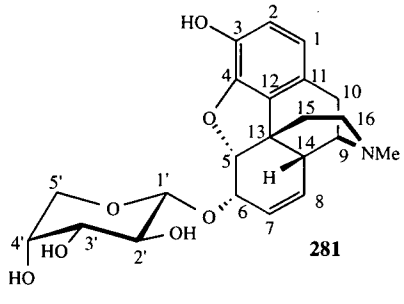


277

A solution of α-D-arabinopyranose tetrabenzoate **275** (2.033 g, 3.59 mmol) in dry CH_2Cl_2 (15 ml) was added to a solution of 3-*O*-pivaloylmorphine **32** (0.277 g, 0.75 mmol) in dry CH_2Cl_2 (2.8 ml) under an atmosphere of N_2. The resulting mixture was stirred at rt for 10 mins, whereafter, the temperature was lowered to 0 °C and TMSOTf (0.805 g, 3.62 mmol) was added dropwise while maintaining the temperature below 5 °C. The reaction was stirred at this temperature for a further 2 h; tlc at that time showed that the reaction was not complete so further 3-*O*-pivaloylmorphine **32** (0.297 g, 0.80 mmol) in dry CH_2Cl_2 (3 ml) and TMSOTf (0.805 g, 3.62 mmol) was added. TMSOTf (0.345 g, 1.55 mmol) was added again at 4 h, and after 5 h tlc revealed that the reaction had gone to completion. The reaction mixture was poured onto a mixture of ice (50 g) and sat aq $NaHCO_3$ (50 ml), extracted with CH_2Cl_2 (4x 100 ml) and the combined organic extracts washed with

5% (w/v) aq citric acid (3x 100 ml), sat aq $NaHCO_3$ (2x 100 ml), water (2x 100 ml), brine (100 ml), dried over $MgSO_4$ and the solvent removed by rotary evaporation to give an off white foam (0.724 g). Flash column-chromatography (1:19, MeOH-$CHCl_3$) of the crude residue followed by crystallisation (EtOH-water) afforded *the title compound* as a white solid (0.724 g, 44%); R_f 0.55 (1:9, MeOH-CH_3Cl); m.p. 138-140 °C (from EtOH-water); [Found: C, 70.41%; H, 5.91%; N, 1.54%. $C_{48}H_{47}NO_{11}$ requires C, 70.82%; H, 5.82%; N, 1.72%]; $[\alpha]_D^{23}$ -92.63 (*c* 1.06, $CHCl_3$); ν_{max} (KBr)/cm^{-1} 2971m (CH), 2924m (CH), 2857m (CH), 2780m (NMe), 1731s (CO_2), 1121s (C-O-C); δ_H (250 MHz; $CDCl_3$) (COSY) 1.00 (9H, s, $C(CH_3)_3$), 1.95 (1H, bd, $^2J_{gem}$ 12, $^{15}CH_{eq}$), 2.06 (1H, td, $^2J_{gem}$ 12, $^{15}CH_{ax}$), 2.33 (1H, dd, $^2J_{gem}$ 19, $^{10}CH_{ax}$), 2.42 (3H, s, NMe), 2.46 (1H, d, $^3J_{16ax,15eq}$ 4, $^{16}CH_{ax}$), 2.55 (1H, d, $^3J_{16eq.15ax}$ 3, $^{16}CH_{eq}$), 2.62 (1H, m, $^3J_{14,9}$ 3, ^{14}CH), 3.08 (1H, d, $^2J_{gem}$ 19, $^{10}CH_{eq}$), 3.37 (1H, q, $^3J_{9.14}$ 3, ^9CH), 4.00 (1H, dd, $^3J_{5',4'}$ 4, $^{5'}CH$), 4.14 (1H, m, $^3J_{6,5}$ 3, ^6CH), 4.74 (1H, dd, $^2J_{gem.}$ 11, $^{5'}CH$), 5.08 (2H, m, $^3J_{5,6}$ 3, ^5CH, $^{1'}CH$), 5.29 (1H, dt, $^3J_{7,8}$ 10, ^7CH), 5.52 (1H, dd, $^3J_{2'.1'}$ 5, $^{2'}CH$), 5.60 (1H, d, $^3J_{8,7}$ 10, ^8CH), 5.71 (2H, m, $^3J_{3',2'}$ 3, $^{3'}CH$, $^{4'}CH$), 6.57 (1H, d, $^3J_{1,2}$ 8, ^1CH), 6.74 (1H, d, $^3J_{2,1}$ 8, ^2CH), 7.23-7.67 (9H, m, Ar), 7.81-8.13 (6H, m, Ar); δ_C (63 MHz; $CDCl_3$) 20.88 (CH_2), 26.85 (3x CH_3), 35.72 (CH_2), 38.55 (Q), 41.48 (CH), 43.02 (NMe), 44.08 (Q), 46.04 (CH_2), 58.42 (CH), 58.88 (CH_2), 67.02 (CH), 68.51 (CH), 69.98 (2x CH), 92.54 (CH), 99.75 (An CH), 118.82 (CH), 121.66 (CH), 128.21 (3x CH), 128.33 (2x CH), 128.42 (2x CH), 129.07 (Q), 129.34 (Q), 129.38 (Q), 129.63 (CH), 129.83 (4x CH), 130.14 (2x CH), 131.57 (Q), 131.82 (Q), 132.20 (Q), 133.07 (CH), 133.20 (CH), 133.41 (CH), 150.71 (Q), 165.00 (Q), 165.47 (Q), 165.60 (Q), 176.16 (Q); *m/z* 814 (MH^+, 40%), 813 (M^+, 46%), 712 (M^+ -$C_5H_9O_2$, 1%), 693 (M^+ -$C_7H_4O_2$, 2%), 445 (M^+ -$C_{22}H_{26}NO_4$, 65%); [Found MH^+ 814.3218. $C_{48}H_{47}NO_{11}$ requires *M*H 814.3227] (F.A.B.).

1.5.12. Morphine-6-α-D-arabinopyranoside 281



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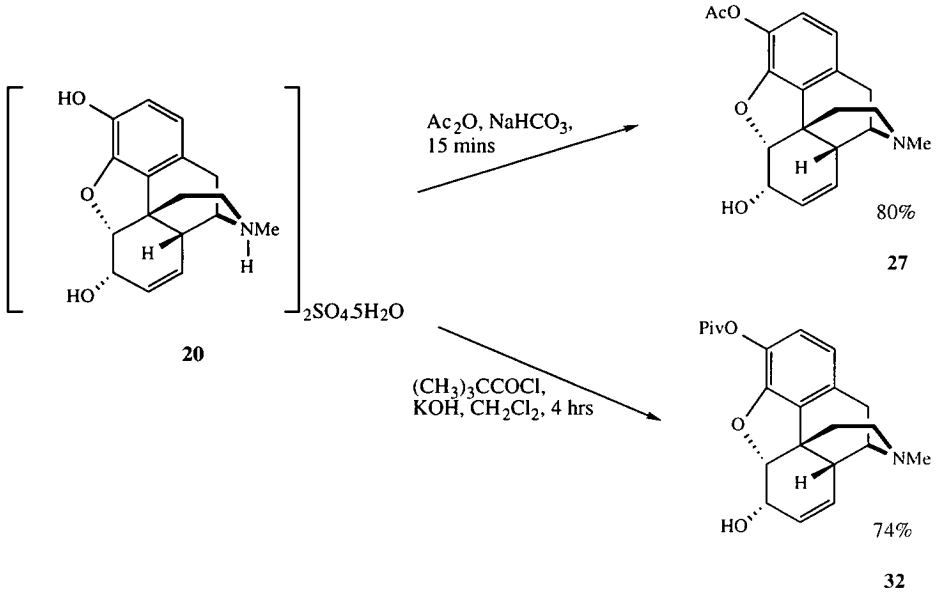
A suspension of 3-*O*-pivaloylmorphine-6-(2',3',4'-tri-*O*-benzoyl)-α-D-arabinopyranoside **277** (0.270 g, 0.25 mmol) in dry MeOH (10 ml) was stirred at rt for 1 h under an atmosphere of N_2. A 0.52 M solution of NaOMe (0.140 g, 2.60 mmol) in dry MeOH (5 ml) was added and stirring was continued for 2 h. Tlc indicated that the reaction was proceding slowly, thus a further addition of NaOMe (0.084 g, 1.56 mmol) in dry MeOH (3 ml) was carried out. Tlc revealed that the reaction had gone to completion after 5 h, the reaction mixture was neutralised with 2 M aq HCl and the solvent removed by rotary evaporation to give an off white solid (0.351 g). The crude residue was dissolved in MeOH and filtered through a sintered funnel. Rotary evaporation of the filtrate gave a white solid (0.250 g) which on crystallisation (MeOH-EtOAc) afforded *the title compound* as a white solid (0.043 g, 43%); R_f 0.29 (9:4:2, EtOAc-^{i}PrOH-water); m.p./decomp. 159 °C (from MeOH-EtOAc); $[\alpha]_D^{24}$ -129.78 (*c* 0.39, MeOH); [Found: C, 51.74%; H, 5.95%; N, 2.56%. $C_{22}H_{27}NO_7$ requires C, 63.28%; H, 6.52%; N, 3.36%]; ν_{max} (KBr)/cm^{-1} 2921m (CH), 2852w (CH), 1386m (CH_3), 1087s (C-O(H)), 1067s (C-O-C); δ_H (250 MHz; CD_3OD) (COSY) 1.94 (1H, dd, $^2J_{gem}$ 13, $^{15}CH_{eq}$), 2.25 (1H, td, $^2J_{gem}$ 13, $^{15}CH_{ax}$), 2.59 (1H, dd, $^2J_{gem}$ 19, $^{10}CH_{ax}$), 2.63 (3H, s, NMe), 2.69 (1H, dd, $^2J_{gem}$ 13, $^{16}CH_{ax}$), 2.82 (2H, m, ^{14}CH, $^{16}CH_{eq}$), 3.17 (1H, d, $^2J_{gem}$ 19, $^{10}CH_{eq}$), 3.61-3.72 (3H, m, ^9CH, $^{2'}CH$, $^{3'}CH$), 3.77 (1H, dd, $^2J_{gem}$ 12, $^{5'}CH$), 3.94 (1H, m, $^{4'}CH$), 4.05 (1H, dd, $^2J_{gem}$ 12, $^{5'}CH$), 4.42 (1H, m, ^6CH), 4.48 (1H, d, $^3J_{1',2'}$ 7, $^{1'}CH$), 5.05 (1H, dd, $^3J_{5,6}$ 6, ^5CH), 5.46 (1H, dt, $^3J_{7,8}$ 10, ^7CH), 5.90 (1H, dq, $^3J_{8,7}$ 10, ^8CH), 6.55 (1H, d, $^3J_{1,2}$ 8, ^1CH), 6.63 (1H, d, $^3J_{2,1}$ 8, ^2CH); δ_C (63 MHz; CD_3OD) 20.05 (CH_2), 33.96 (CH_2), 39.40 (CH), 40.91 (NMe), 42.70 (Q), 45.65 (CH_2), 58.61 (CH), 65.07 (CH_2), 67.60 (CH), 70.36 (CH), 72.21 (CH), 73.84 (CH), 90.84 (CH), 103.12 (CH), 115.87 (CH), 118.61 (CH), 124.33 (Q), 127.91 (CH), 129.38 (CH), 129.56 (Q), 138.15 (Q), 145.50 (Q); *m/z* 418 (MH^+, 55%), 268 (M^+ -$C_5H_9O_5$, 16%), 239 (M^+ -$C_6H_{12}NO_5$, 100%), 237 (M^+ -$C_6H_{14}NO_5$, 51%), 131 (M^+ -$C_{17}H_{20}NO_3$, 96%); [Found MH^+ 418.1864. $C_{22}H_{27}NO_7$ requires *M*H 418.1866] (F.A.B.).

Appendix

A.1. Synthesis of 3-*O*-Pivaloylmorphine and 3-*O*-Acetylmorphine

The synthesis of 3-*O*-pivaloylmorphine **32** is achieved in 74% yield by treating morphine sulphate **20** with pivaloyl chloride and potassium hydroxide (scheme A.1.).[178]



Synthesis of 3-O-Pivaloylmorphine 32 and 3-O-Acetylmorphine 27.

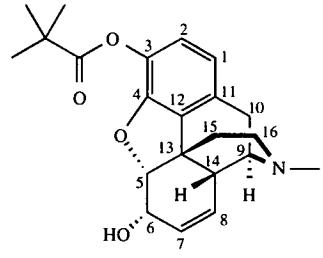
Scheme A.1.

The synthesis of 3-*O*-acyl morphine derivatives under basic conditions has been established for many years and a classic example is the synthesis of 3-*O*-acetylmorphine **27** which was reported by Welsh[179] in the 1950's. This acylation reaction is still commonly used and involves treating morphine with acetic anhydride and sodium bicarbonate to afford 3-*O*-acetylmorphine **27** in good yields (scheme A.1.).[39]

A.2. ^{1}H and COSY NMR Spectra of 3-*O*-Pivaloyl Morphine

Assigning the peaks in the ^{1}H NMR spectra of the synthesised morphine glycosides was made more straight forward when the peaks corresponding to the morphine protons could be identified. The ^{1}H NMR (figure A.2.) and COSY (figure A.3.) spectra of 3-*O*-pivaloylmorphine **32** were therefore recorded and proved to be of great value in understanding the ^{1}H NMR spectra of the morphine glycosides synthesised.

3-*O*-Pivaloylmorphine **32** contains 5 chiral centres at C5, C6, C9, C13 and C14 with the protons at C9 and C14 lying *trans* to each other. There are three CH_2 groups at C10, C15 and C16, two benzylic protons on C1 and C2 and two allylic protons on C7 and C8 (figure A.1.).



Structure of 3-O-Pivaloylmorphine 32.

Figure A.1.

By examining the three dimensional structure of 3-*O*-pivaloylmorphine **32** (figure A.4.) and applying the Karplus equation the splitting patterns observed in the ^1H NMR spectrum (figure A.2.) can be explained;

The signals of the C15 and C16 protons are split as a result of two types of coupling, geminal and vicinal, however, the spatial orientation of the vicinal protons greatly affects the splitting patterns observed. The equatorial C15 proton is initially coupled to the axial C15 proton with an observed geminal coupling constant of 13 Hz. The signal for the equatorial C15 proton is then split as a result of coupling to the C16 protons, both of which are at a dihedral angle of 50-60° to the equatorial C15 proton which according to the Karplus equation should give a coupling constant of approximately 4 Hz. However, multiple splitting of the peaks and extensive overlapping results in the signal for the equatorial C15 proton appearing as a broad doublet at δ 1.91 ppm in the ^1H NMR spectrum. Coupling of the axial C15 proton to the C16 protons, which are at a dihedral angle of approximately 50° and 180°, gives observed coupling constants of 5 and 12 Hz respectively, and signal appears as a triplet of doublets at δ 2.1 ppm.

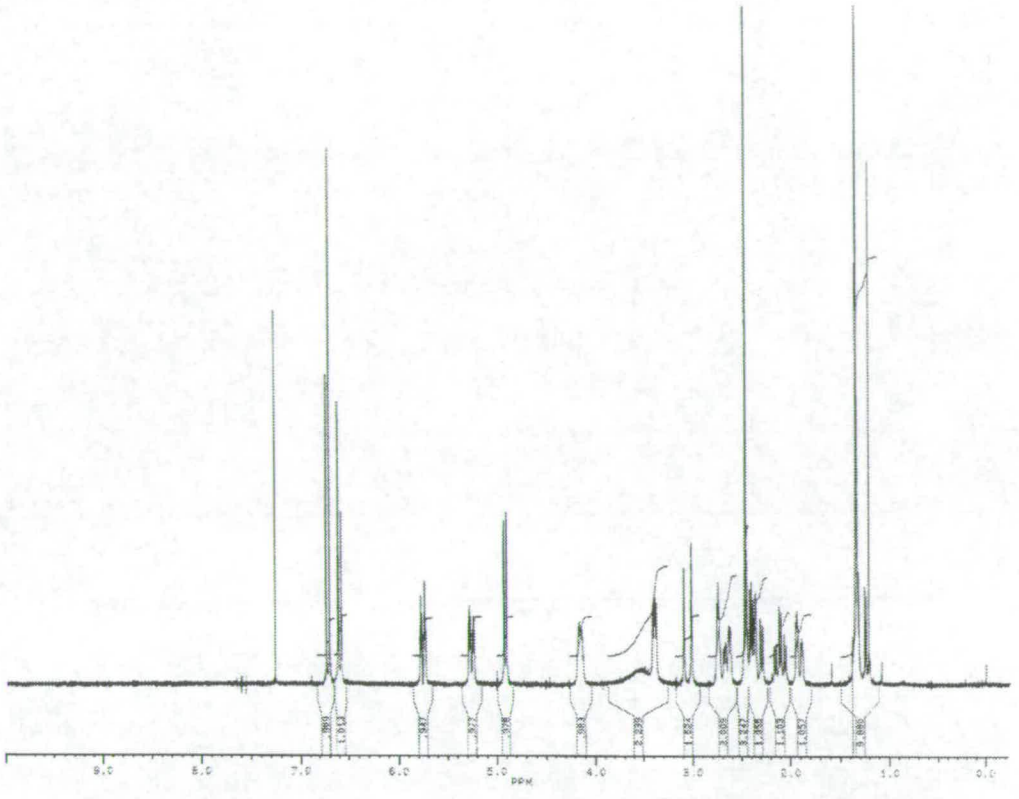
A similar splitting pattern is observed for the two C16 protons, the signal for the axial C16 proton lies under the peaks of the axial C10 proton at δ 2.38 ppm and only one coupling constant of 4 Hz can be measured, which corresponds to the coupling of the equatorial C15 proton. The signal of the equatorial C16 proton appears at δ 2.65 ppm, the equatorial C16 proton is initially coupled to the axial C16 proton with an observed coupling constant of 13 Hz. The signal is then split again by the two C15 protons which both lie at a dihedral angle of 50-60° to the equatorial

C16 proton and the spectral lines extensively overlap to give the broad doublet of doublets observed in the ^{1}H NMR spectrum.

The geminal coupling for the protons at C10 has an unusually large coupling constant of 19 Hz as a result of the close proximity of the aromatic ring. The axial and equatorial protons at C10 have different signal splitting patterns in the ^{1}H NMR spectrum, the equatorial C10 proton at δ 3.04 ppm appears as a doublet while the axial C10 proton appears at δ 2.32 ppm as a doublet of doublets. The equatorial C10 proton lies at a dihedral angle of about 90° to the C9 proton and so according to the Karplus equation, no coupling exists between these two protons and this is what is observed in the ^{1}H NMR spectrum. However, the axial C10 proton lies at a dihedral angle of approximately 30° to the C9 proton and there is an observed coupling constant of 6 Hz between these two protons.

The peak corresponding to the C6 hydroxyl proton lies between δ 3.4-3.7 ppm, its close proximity to the C6 proton distorts its signal so that it is not possible to measure any coupling constants. However, the value of the coupling constant for the C6 proton can be obtained from the C5 proton signal which is observed to be 7 Hz corresponding to a vicinal coupling between the C5 and C6 protons which have *cis* stereochemistry.

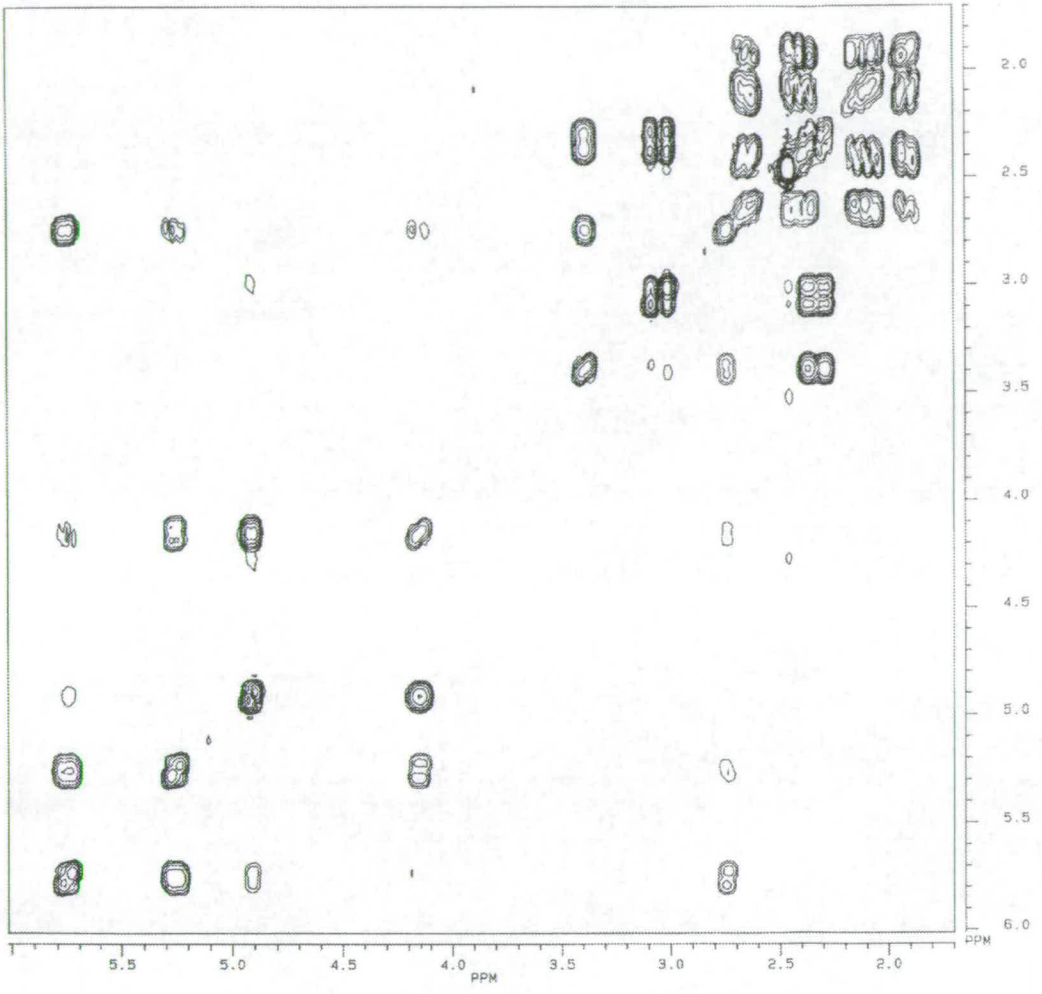
The coupling constant of the C9 and C14 protons is observed to be 3 Hz and so the dihedral angle of these vicinal protons would be approximately 60°. The coupling constant of the C7 and C8 protons is observed to be 10 Hz and this confirms that the protons are in a *cis* conformation. The C8 proton is observed to have a higher chemical shift than C7 as a result of the electron withdrawing effect of the C6 hydroxyl group through the double bond (figure A.2.).



^{1}H NMR Spectrum of 3-O-Pivaloylmorphine ***32***.

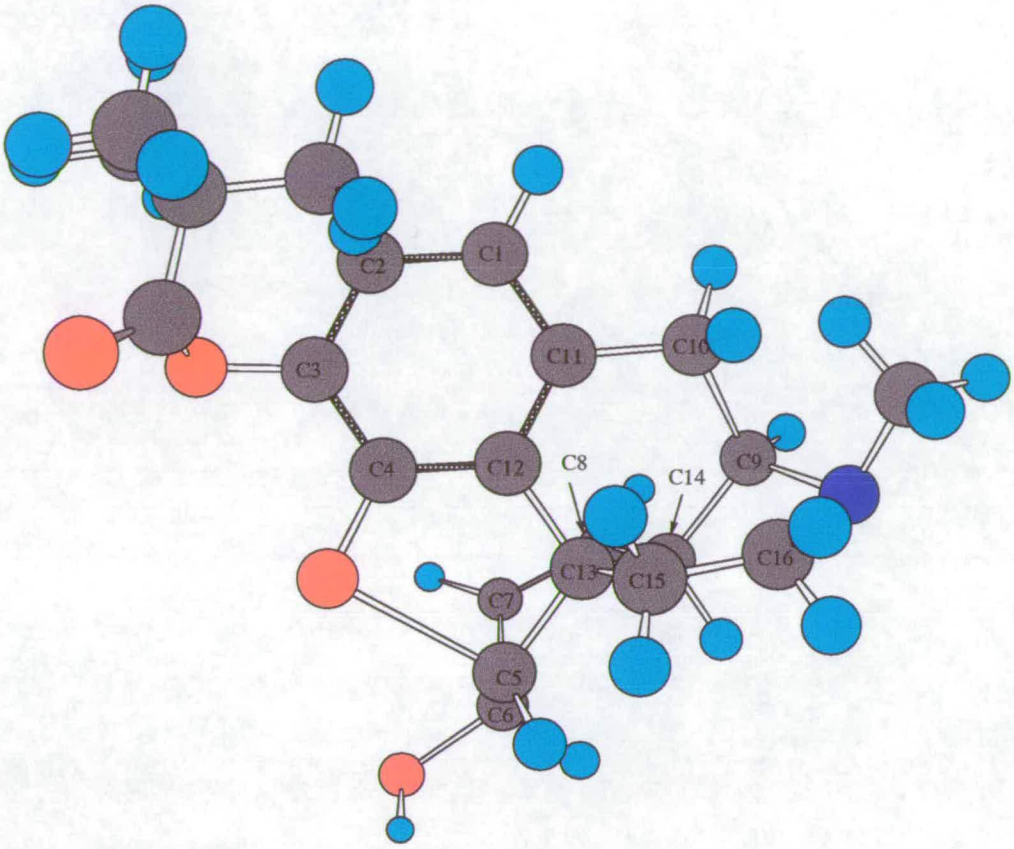
Figure A.2.

δ_H (250 MHz; $CDCl_3$) 1.33 (9H, s, $(CH_3)_3$), 1.91 (1H, bd, $^{2}J_{gem}$ 13, $^{15}CH_{eq}$), 2.10 (1H, td, $^{2}J_{gem}$ 13 , $^{15}CH_{ax}$), 2.32 (1H, dd, $^{2}J_{gem}$ 19 , $^{10}CH_{ax}$), 2.38 (1H, m, $^{3}J_{16ax,15eq}$, 4, $^{16}CH_{ax}$), 2.45 (3H, s, NCH_3), 2.65 (1H, bdd, $^{2}J_{gem}$ 13, $^{16}CH_{eq}$), 2.74 (1H, t, $^{3}J_{14,9}$ 3, ^{14}CH), 3.04 (1H, d, $^{2}J_{gem}$ 19, $^{10}CH_{eq}$), 3.38 (1H, q, $J_{9,14}$ 3, ^{9}CH), 4.13 (1H, m, ^{6}CH), 4.91 (1H, dd, $^{3}J_{5,6}$ 7, ^{5}CH), 5.25 (1H, bd, $^{3}J_{7,8}$ 10, ^{7}CH), 5.75 (1H, bd, $^{3}J_{8,7}$ 10, ^{8}CH), 6.59 (1H, d, $^{3}J_{1,2}$ 8, ^{1}CH), 6.71 (1H, d, $^{3}J_{2,1}$ 8, ^{2}CH).



^{1}H-^{1}H Correlated (COSY) NMR Spectrum of 3-O-Pivaloylmorphine ***32***.

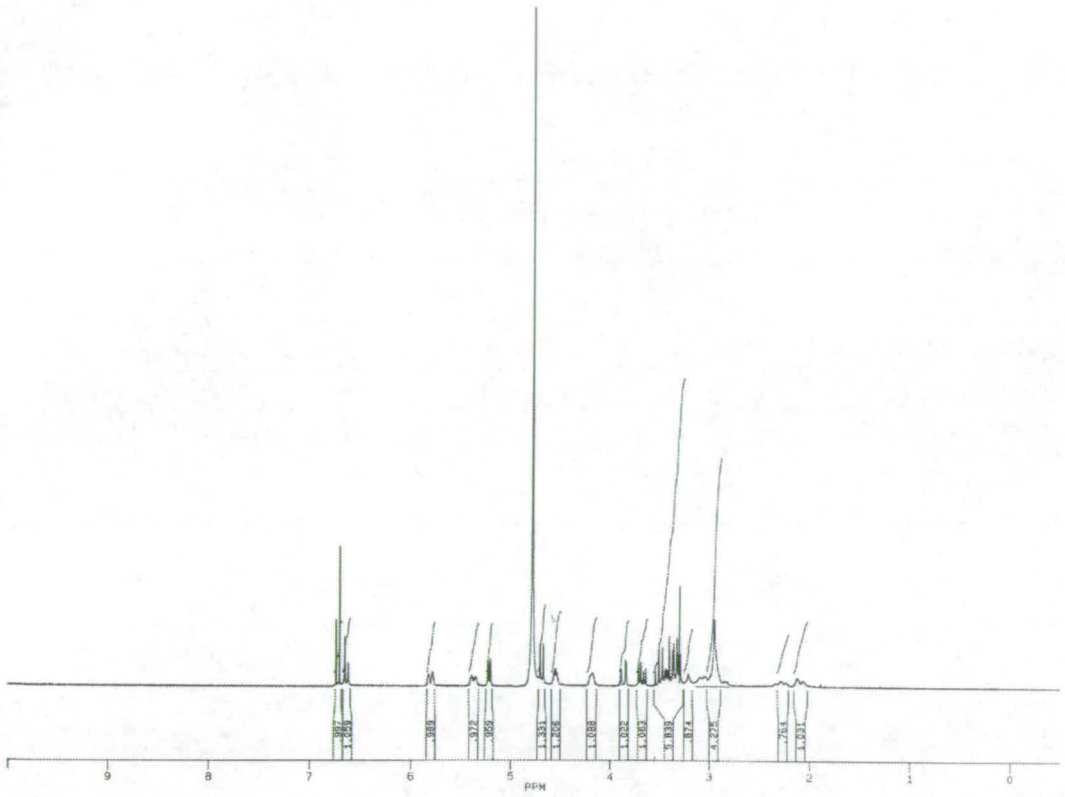
Figure A.3.



The Three Dimensional Structure of 3-O-Pivaloylmorphine ***32****.*

Figure A.4.

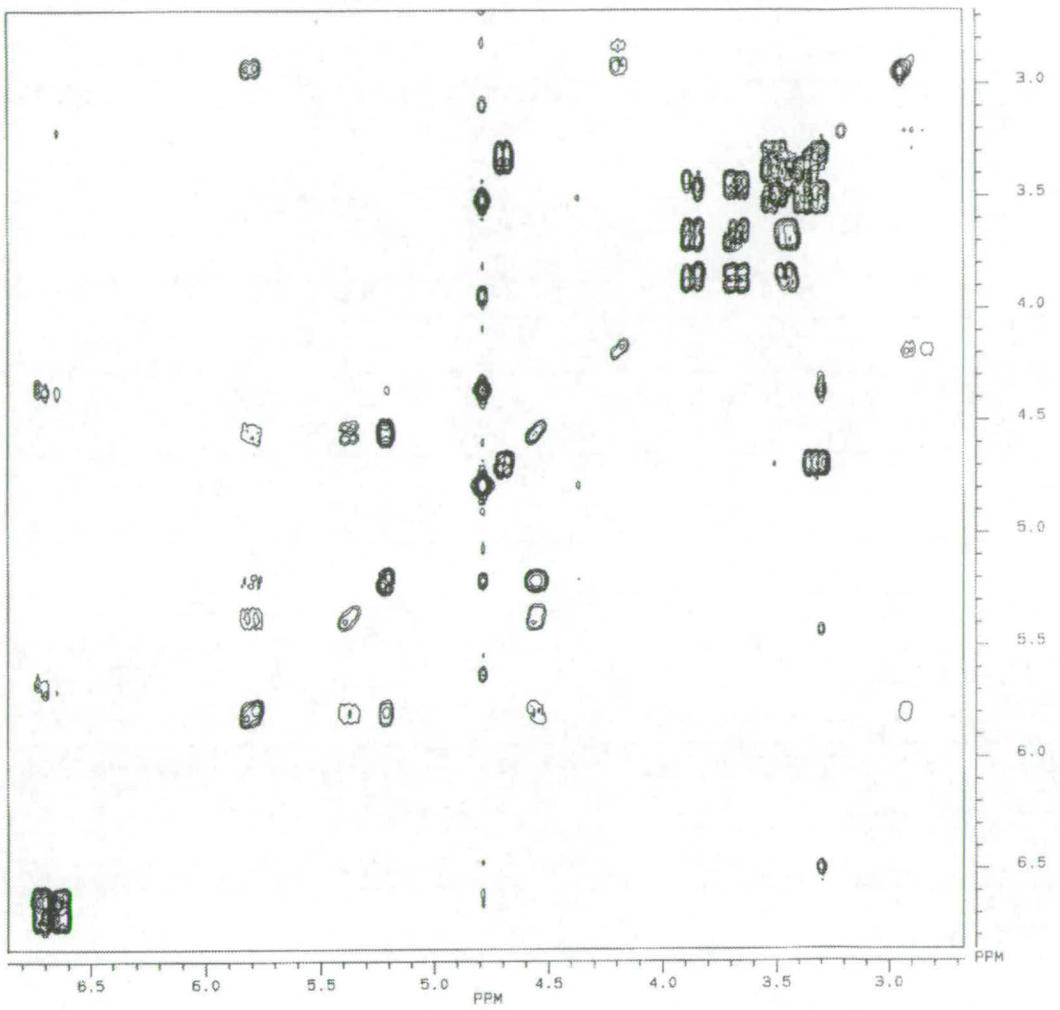
A.3. ^{1}H and COSY NMR Spectra of Morphine-6-β-D-glucoside 31



*^{1}H NMR Spectrum of Morphine-6-β-D-glucoside **31**.*

Figure A.5.

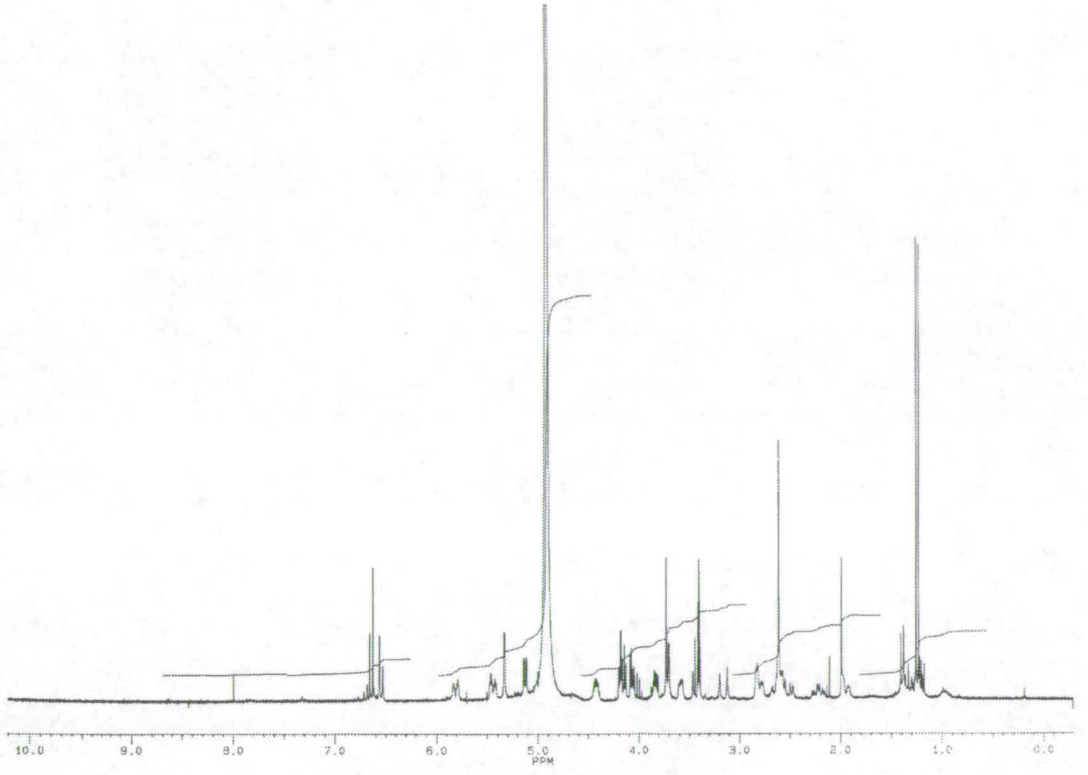
δ_H (250 MHz; D_2O) (COSY) 2.06 (1H, bd, $^{2}J_{gem}$ 12, $^{15}CH_{eq}$), 2.27 (1H, bt, $^{2}J_{gem}$ 12, $^{15}CH_{ax}$), 2.83-3.14 (2H, m, ^{14}CH, $^{10}CH_{ax}$), 2.94 (3H, s, NMe), 3.21 (3H, bs, $^{16}CH_{ax}$), 3.27-3.55 (6H, m, $^{2}J_{gem}$ 19, $^{10}CH_{eq}$, $^{2'}CH$, $^{3'}CH$, $^{4'}CH$, $^{5'}CH$, $^{16}CH_{eq}$), 3.68 (1H, dd, $^{2}J_{gem}$ 12, $^{6'}CH$), 3.86 (1H, dd, $^{2}J_{gem}$ 12, $^{6'}CH$), 4.18 (1H, m, ^{9}CH), 4.55 (1H, m, ^{6}CH), 4.70 (1H, d, $^{3}J_{1',2'}$ 8, $^{1'}CH$), 5.21 (1H, d, $^{3}J_{5,6}$ 8, ^{5}CH), 5.38 (1H, bd, $^{3}J_{7,8}$ 11, ^{7}CH), 5.82 (1H, bd, $^{3}J_{8,7}$ 11, ^{8}CH), 6.62 (1H, d, $^{3}J_{1,2}$ 8, ^{1}CH), 6.73 (1H, d, $^{3}J_{2,1}$ 8, ^{2}CH).



^{1}H-^{1}H Correlated (COSY) NMR Spectrum of Morphine-6-β-D-glucoside ***31***.

Figure A.6.

A.4. ^{1}H NMR Spectrum of Morphine-6-β-D-galactofuranoside 273

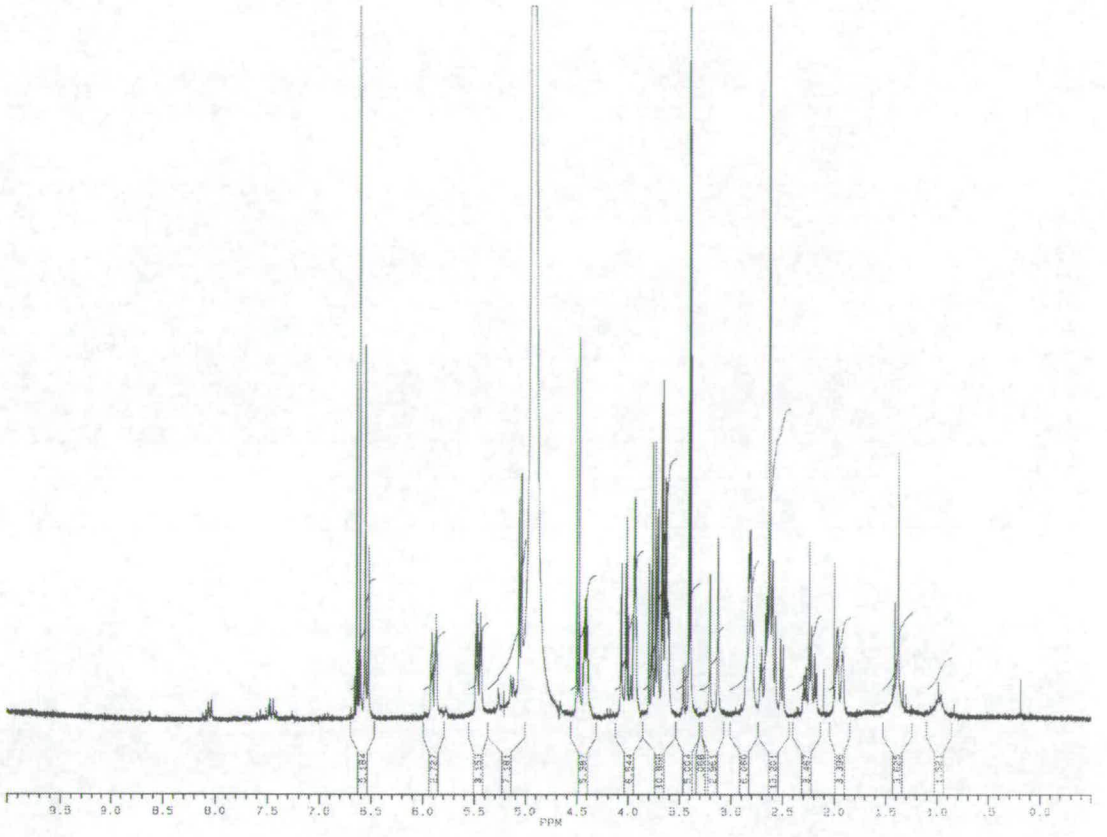


*^{1}H NMR Spectrum of Morphine-6-β-D-galactofuranoside **273**.*

Figure A.7.

δ_H (250 MHz; CD_3OD) 1.96 (1H, bd, $^{2}J_{gem}$ 11, $^{15}CH_{eq}$), 2.23 (1H, td, $^{2}J_{gem}$ 11, $^{15}CH_{ax}$), 2.57 (1H, dd, $^{2}J_{gem}$ 19, $^{10}CH_{ax}$), 2.60 (1H, m, $^{16}CH_{ax}$), 2.61 (3H, s, NMe), 2.77 (1H, bd, $^{2}J_{gem}$ 11, $^{16}CH_{eq}$), 2.82 (1H, m, ^{14}CH), 3.16 (1H, d, $^{2}J_{gem}$ 19, $^{10}CH_{eq}$), 3.58 (1H, m, ^{9}CH), 3.71 (1H, d, $^{3}J_{2',3}$ 6, $^{2'}CH$), 3.83 (1H, m, $^{3'}CH$), 3.99-4.20 (4H, m, $^{5'}CH$, $^{4'}CH$, $^{6'}CH_2$), 4.42 (1H, m, ^{6}CH), 5.12 (1H, dd, $^{3}J_{5,6}$ 6, ^{5}CH), 5.33 (1H, d, $^{3}J_{1',OH}$ 2, $^{1'}CH$), 5.46 (1H, dt, $^{3}J_{7,8}$ 11, ^{7}CH), 5.81 (1H, bd, $^{3}J_{8,7}$ 11, ^{8}CH), 6.54 (1H, d, $^{3}J_{1,2}$ 8, ^{1}CH), 6.64 (1H, d, $^{3}J_{2,1}$ 8, ^{2}CH).

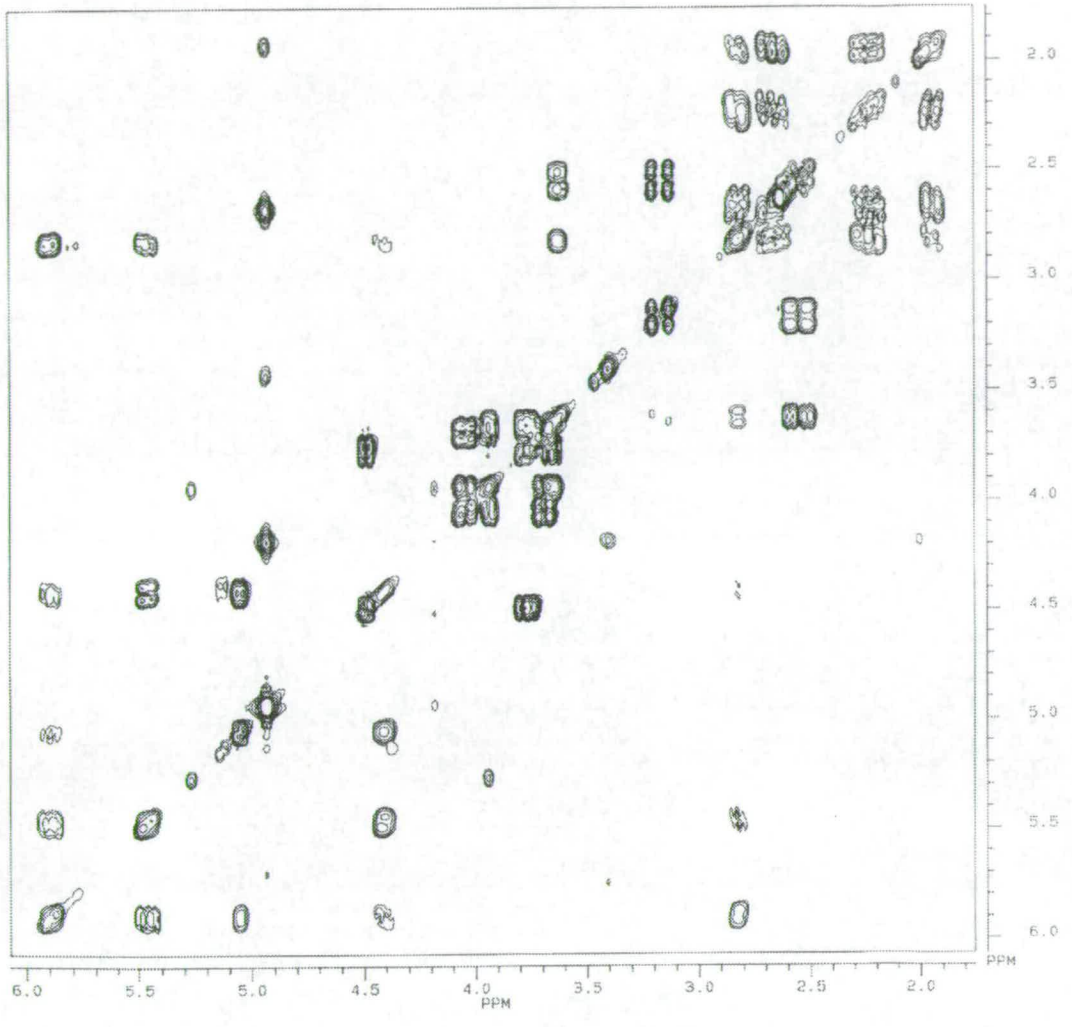
A.5. ^{1}H and COSY NMR Spectra of Morphine-6-α–D-arabinopyranoside 278



^{1}H NMR Spectrum of Morphine-6-α-D-arabinopyranoside 278.

Figure A.8.

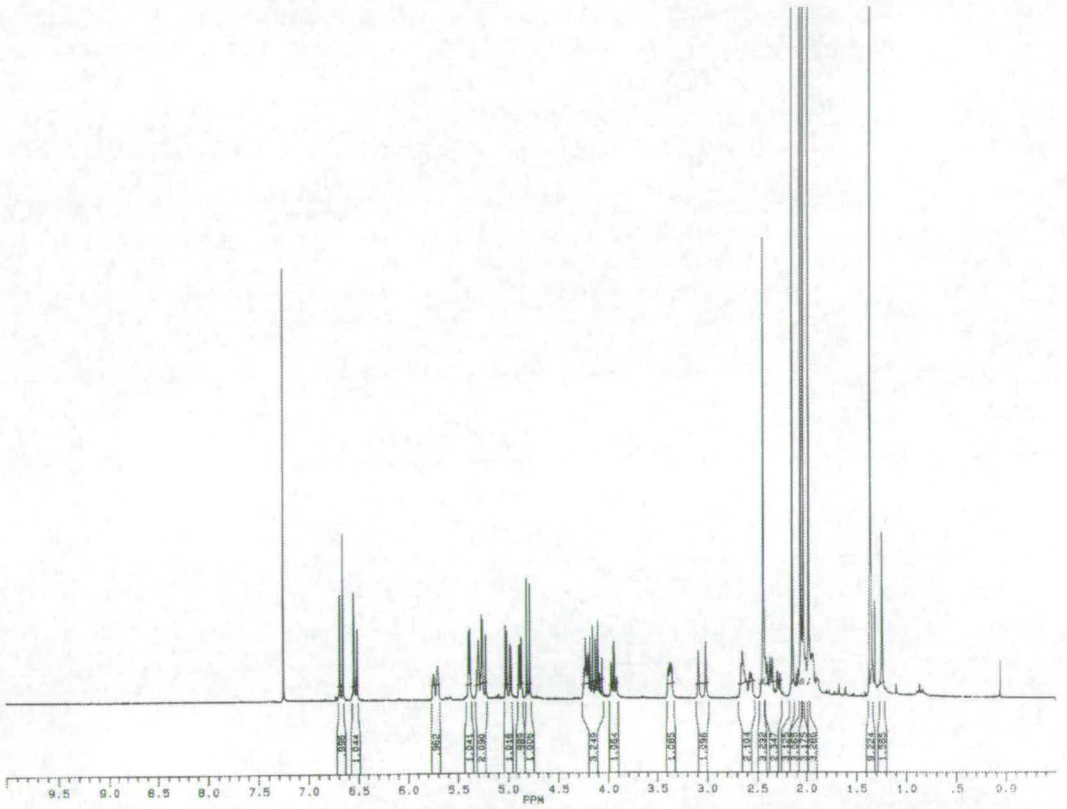
δ_H (250 MHz; CD_3OD) (COSY) 1.94 (1H, dd, $^{2}J_{gem}$ 13, $^{15}CH_{eq}$), 2.25 (1H, td, $^{2}J_{gem}$ 13, $^{15}CH_{ax}$), 2.59 (1H, dd, $^{2}J_{gem}$ 19, $^{10}CH_{ax}$), 2.63 (3H, s, NMe), 2.69 (1H, dd, $^{2}J_{gem}$ 13, $^{16}CH_{ax}$), 2.82 (2H, m, ^{14}CH, $^{16}CH_{eq}$), 3.17 (1H, d, $^{2}J_{gem}$ 19, $^{10}CH_{eq}$), 3.61-3.72 (3H, m, ^{9}CH, $^{2'}CH$, $^{3'}CH$), 3.77 (1H, dd, $^{2}J_{gem}$ 12, $^{5'}CH$), 3.94 (1H, m, $^{4'}CH$), 4.05 (1H, dd, $^{2}J_{gem}$ 12, $^{5'}CH$), 4.42 (1H, m, ^{6}CH), 4.48 (1H, d, $^{3}J_{1',2'}$ 7, $^{1'}CH$), 5.05 (1H, dd, $^{3}J_{5,6}$ 6, ^{5}CH), 5.46 (1H, dt, $^{3}J_{7,8}$ 10, ^{7}CH), 5.90 (1H, dq, $^{3}J_{8,7}$ 10, ^{8}CH), 6.55 (1H, d, $^{3}J_{1,2}$ 8, ^{1}CH), 6.63 (1H, d, $^{3}J_{2,1}$ 8, ^{2}CH).



^{1}H-^{1}H Correlated (COSY) NMR Spectrum of Morphine-6-α-D-arabinopyranoside ***278****.*

Figure A.9.

A.6. ^{1}H NMR Spectrum of 3-*O*-Pivaloylmorphine-6-(2',3',4',6'-tetra-*O*-acetyl)-β-D-galactopyranoside 257

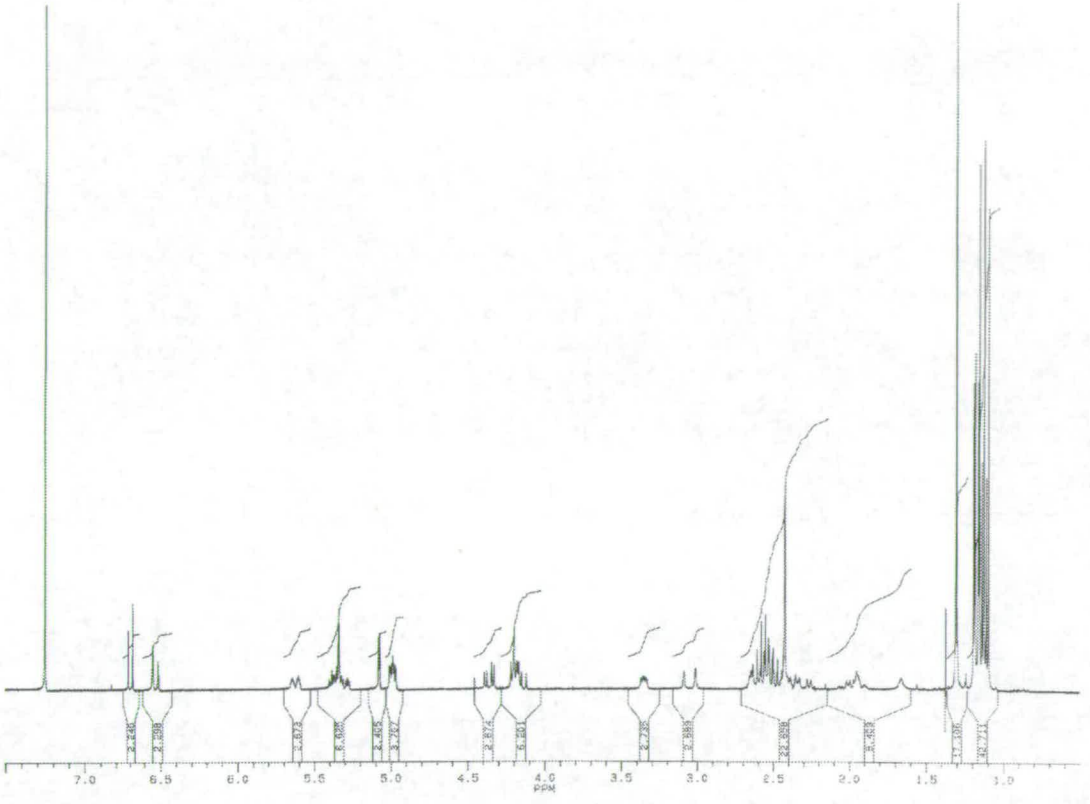


^{1}H Proton NMR Spectrum of 3-O-Pivaloylmorphine-6-(2',3',4',6'-tetra-O-acetyl)-β-D-galactopyranoside ***257***.

Figure A.10.

δ_H (250 MHz; $CDCl_3$) 1.35 (9H, s, $C(CH_3)_3$), 1.90 (1H, m, $^{15}CH_{eq}$), 1.97 (3H, s, OAc), 2.01 (1H, m, $^{15}CH_{ax}$) 2.03 (3H, s, OAc), 2.06 (3H, s, OAc), 2.14 (3H, s, OAc), 2.38 (1H, dd, $^{2}J_{gem}$ 19, $^{10}CH_{ax}$), 2.42 (3H, s, NMe), 2.44 (1H, m, $^{16}CH_{ax}$), 2.56 (1H, bd, $^{3}J_{16,15}$ 5, $^{16}CH_{eq}$), 2.64 (1H, m, ^{14}CH), 3.05 (1H, d, $^{2}J_{gem}$ 19, $^{10}CH_{eq}$), 3.36 (1H, q, $^{3}J_{9,14}$ 4, ^{9}CH), 3.94 (1H, t, $^{3}J_{5',6'}$ 7, $^{5'}CH$), 4.02-4.20 (2H, m, $^{3}J_{6',5'}$ 7, $^{6'}CH_2$, ^{6}CH), 4.80 (1H, d, $^{3}J_{1,2}$ 8, $^{1'}CH$), 4.88 (1H, dd, $^{3}J_{5,6}$ 6, ^{5}CH), 5.00 (1H, dd, $^{3}J_{3',4'}$ 4, $^{3'}CH$), 5.25 (1H, dd, $^{3}J_{2',1'}$ 8, $^{3}J_{2',3'}$ 10, 2'CH), 5.30 (1H, m, ^{7}CH), 5.38 (1H, dd, $^{3}J_{4',3'}$ 4, $^{4'}CH$), 5.72 (1H, bd, $^{3}J_{8,7}$ 10, ^{8}CH), 6.53 (1H, d, $^{3}J_{1,2}$ 8, ^{1}CH), 6.67 (1H, d, $^{3}J_{2,1}$ 8, ^{2}CH).

A.7. ^{1}H NMR Spectrum of 3-*O*-Pivaloylmorphine-6-(2',3',4',6'-tetra-*O*-isobutyryl)-β-D-galactofuranoside 269



*^{1}H Proton NMR Spectrum of 3-*O*-Pivaloylmorphine-6-(2',3',4',6'-tetra-*O*-isobutyryl)-β-D-galactofuranoside **269**.*

Figure A.11.

δ_H (250 MHz; $CDCl_3$) (COSY) 1.10-1.19 (24H, m, 8x CHCH_3), 1.30 (9H, s, C(CH_3)$_3$), 1.95-1.97 (2H, m, $^{15}CH_{eq}$, $^{15}CH_{ax}$), 2.34 (1H, dd, $^{2}J_{gem}$ 19, $^{10}CH_{ax}$), 2.42 (3H, s, NMe), 2.44-2.64 (7H, m, 4x C*H*CH$_3$), $^{16}CH_{eq}$, $^{16}CH_{ax}$, ^{14}CH), 3.05 (1H, d, $^{2}J_{gem}$ 19, $^{10}CH_{eq}$), 3.35 (1H, q, $^{3}J_{9,14}$ 3, ^{9}CH), 4.12-4.22 (3H, m, ^{6}CH, $^{6'}CH_2$), 4.36 (1H, dd, $^{3}J_{5',6'}$ 12, $^{5'}CH$), 4.99 (2H, m, $^{3'}CH$, ^{5}CH), 5.08 (1H, d, $^{2'}CH$), 5.28-5.40 (3H, m, $^{3}J_{7,8}$ 10, ^{7}CH, $^{1'}CH$, $^{4'}CH$), 5.63 (1H, bd, $^{3}J_{8,7}$ 10, ^{8}CH), 6.53 (1H, d, $^{3}J_{2,1}$ 8, ^{1}CH), 6.70 (1H, d, $^{3}J_{2,1}$ 8, ^{2}CH).

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