THE SYNTHESIS OF NOVEL MONOSACCHARIDES AS POTENTIAL ANTHELMINTICS

by

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To my mother, who gave me the confidence
and much more.

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ABSTRACT

The distribution and pathology of those filarial nematodes which affect man is reviewed, with particular emphasis on *Onchocerca volvulus*, the causative agent of onchocerciasis (River Blindness). The present control and drug treatment of onchocerciasis is outlined together with filarial carbohydrate metabolism, with a view to the rational development of selective novel chemotherapeutic agents.

The results of several studies on parasitic glucose uptake inhibition are shown and discussed and the differing structural requirements of the human erythrocyte and parasite glucose uptake systems are compared.

The identification of the primary synthetic target compound, 2-deoxy-5-thio-D-glucose is presented together with a short review of the known biochemistry of thio and amino sugars.

An overall synthetic strategy is outlined and discussed, with the a review of past syntheses of 5-thio-D-glucose and 2-acetamido-2-deoxy-5-thio-D-glucose. The use and versatility of deoxyhalogeno sugars as intermediates in the synthesis of thio, amino and deoxy sugars is reviewed and discussed.

Model studies on halogenation and displacement reactions of 3-O-acetyl-1,2-O-isopropylidene-D-glucose are presented.

The development of two novel synthetic routes to 2-acetamido-2-deoxy-5-thio-D-glucose is described; the first based upon methyl glucofuranoside intermediates and the successful second route upon acyclic dimethyl acetal derivatives. The synthesis of both 2-acetamido-2-deoxy-5-thio-D-glucose and 2-acetamido-5-azido-2,5-dideoxy-3,4-O-isopropylidene-*aldehydo*-D-glucose dimethyl acetal from a common intermediate is detailed and compared with literature syntheses.

An attempted radical-induced reductive deamination of 2-acetamido-2-deoxy-5-thio-D-glucose to the 2-deoxy analogue is described.

The development of synthetic routes towards a series of 2-acetamido-2-deoxy-4-thio and 4-amino sugars is described, including studies on the relative utility of various reagents for the selective benzoylation of N-acetylglucosamine.

A synthesis of 1,3,5,6-tetra-O-acetyl-2-acetamido-2-deoxy-4-thio-D-galactose is presented.

The synthesis of some 4-chloro and 4-bromo galactopyranose derivatives is described in conjunction with studies on the ease of halogen displacement with thiocyanate, thioacetate and azide anions. The steric and electronic factors governing such displacements are discussed and compared with literature examples.

The development of a novel synthetic route is outlined for the preparation of 4-thioglucose derivatives with overall retention of configuration.

Declaration

The work described in this thesis is the author's own work and has not been submitted in any part or form to this or any other university.

ABBREVIATIONS

The following abbreviations are used in the text and diagrams:

THF	-	tetrahydrofuran
DMF	-	N,N- dimethylformamide
^{1}H-NMR	-	proton magnetic resonance
^{13}C-NMR	-	carbon-13 magnetic resonance
Ts	-	tosyl (toluene-p-sulphonyl)
Bros	-	brosyl (bromophenyl-p-sulphonyl)
Ms	-	mesyl (methanesulphonyl)
Me	-	methyl
Ac	-	acetyl
Bz	-	benzoyl
Bzl	-	benzyl
Tr	-	trityl (triphenylmethyl)
t.l.c.	-	thin layer chromatography
TPP	-	triphenylphosphine

CONTENTS

CORRIGENDA

p13 line 24: "elephantitis" should read "elephantiasis"

p14 line 20: include "is" between "disease" and "much"

p44 line 25: "anthelmintics" should read "antiparasitics"

p54 line 11: "asymetric" should read "asymmetric"

p58 line 3: "acetylamine" should read "acetamido"

p60 line 2: "oxidation" should read "reduction"

p73 line 9: structures (16) and (135) are identical

p75 line 1: "azido" should read "azo"

p82 line 8: (47) should read (51)

p83 line 11: (47) should read (51)

p90 line 10: (64) should read (65)

line 13: "howver" should read "however"

p91 line 7: (65) should read (64)

line 20: include "an" between "gave" and "identical"

p98 line 11: "may be" should read "were"

p104 line 13: "glucofuranose" should read "galactofuranose"

p113 line 2: "tri" should read "tetra"

scheme: "DMF" should read "MeOH"

p115 line 4: "rection" should read "reaction"

p126 line 10: "occuring" should read "occurring"

line 6: include "-3-bromo-3-deoxy-" between "benzylidene" and "β"

line 9: include "-3-bromo-3-deoxy-" between "benzylidene" and "α"

p133 diagram should be labelled "scheme 19"

p134 line 14: "elctronegative" should read "electronegative"

p197 reference 81: "J. Chem. Soc." should read "J. Org. Chem."

p200 reference 144: should read "A.H. Haines, *Adv. Carbohydr. Chem. Biochem.*, (1976) **33**, 11-109.

CHAPTER ONE

1.1 Introduction

Among the many problems which beset the Third World, one of the most serious is the comparatively poor health of its population. There are many factors involved, but the prevalence of parasitic disease poses a major threat to the health of millions[1]. In order to improve this situation, multidisciplinary schemes are required, including the design, development and synthesis of new chemotherapeutic agents where existing therapy remains unsatisfactory. This is the case for onchocerciasis, a parasitic disease caused by infection with the filarial nematode, *Onchocerca volvulus*.

1.2 The Filarial Nematodes

The Filariae, so named from the long thread-like appearance of the adult worm, are classified as one of the three suborders of the Spirurida Phasmid Nematoda. They are defined as being exclusively parasites of vertebrate animals and they require an intermediate arthropod host. The mature adult female produces larvae, termed microfilariae, which do not undergo further development until ingested by the arthropod and in all cases, transmission to and from the intermediate host occurs when the arthropod takes a blood meal[2]. There are many species of filariae known in both man and animal, and many are of veterinary and medical importance.

1.2.1 Human Filarial Worms.

The taxonomy, epidemiology and distribution of those filariae which parasitise humans has been extensively reviewed[2,3,4].

1) *Wucheria bancrofti*

This is common throughout India, Indonesia, southern China and Africa, and can also be found in South and Central America. The main arthropod vectors are mosquitos, including species of *Anopheles*, *Aedes* and occasionally *Mansonia*, but especially the *Culex pipiens* "complex".

2) *Brugia Malayi*

This resembles *W. bancrofti* in lifecycle and is responsible for a similar pattern of disease. Its distribution is somewhat more restricted, being found only in India, China and Southeast Asia. *B. timori* is a related species, found only in Timor (Indonesia). Its vector is again the mosquito, the most important being *Mansonia* and a few species of *Anopheles*.

3) *Loa loa*

This is to be found in the rain forests of West and Central Africa and its vector is *Chrysops*, a species of mangrove fly.

4) *Onchocerca volvulus*

The distribution of *Onchocerca* is widespread but the majority of people at risk of disease are in Tropical Africa. However there are also foci in Guatemala, Venezuela, Columbia, Mexico and the Arabian Peninsula. The intermediate hosts are various species of blackfly of the *Simulium* genus, especially *S. damnosum*.

5) *Dipetalonema perstans*

This is non-pathogenic and occurs in both man and primates in South America and Africa. Its vector is the *Culicoides* species of midge.

6) *Dipetalonema streptocerca*

Also non-pathogenic, it affects both man and chimpanzees in Ghana, Zaire and Cameroon. The vector is mainly *Culicoides grahamii.*

7) *Mansonella ozzardi*

This occurs in the West Indies and northern parts of South America and is non-pathogenic. It affects not only man but horses, cattle and antelopes. It is nodule-forming and is transmitted by *Culicoides.*

8) *Dirofilaria immitis*

This is normally found in the right ventricle and pulmonary arteries of dogs, cats, foxes and other carnivores. However there are a few reports of human infections. The worm is widely distributed throughout the tropics, subtropics, North America and the United Kingdom with many species of fleas and mosquitos able to act as intermediate host.

1.3 The Pathology of Human filarial disease

It has been estimated that there are currently 300 million people suffering from some form of disease as a result of filarial infestation. These conditions are as follows:

1.3.1 Lymphatic Filariasis

Infection with *Wucheria banrofti*, *Brugia malayi* or *Brugia timori* may give rise to the condition termed lymphatic filariasis[2,5]. The adult worms inhabit the lymphatic tissues of man and their microfilariae migrate to both the lymph and blood vessels. Here they give rise to inflammatory reactions with recurring acute attacks of lymphangitis. Eventually due to inflammation, hyperplasia and mechanical blockage, this may lead to the fleshy deformities of the groin, breast and limbs known collectively as elephantitis.

1.3.2 Loasis

L. loa gives rise to periodic cutaneous reactions known as "calabar swellings", as well as to transient pruritus, urticaria and thickening of the skin. The tendency of the adult worms to migrate throughout the subcutaneous tissues leads to them periodically crossing the eye beneath the conjuctiva, and to their colloquial name : "eye-worm" [2,5].

1.3.3 Onchocerciasis (River Blindness)

Onchocerciasis is perhaps the most devasting of all the filarial infections with its potential to blind, as well as causing debility and a chronic skin disease. Worldwide the disease has been estimated to affect between 20 - 40 million people in developing countries[6]. It is the most difficult of the filarial diseases to adequately treat, and it is with this condition that this work has primarily been concerned.

The clinical picture of the disease is generally related to the severity of infection[7] - a light infection will typically present as pruritus, often with a reddish maculo-papular rash having an unsymmetrical distribution. There may also be a slight skin thickening and coarsening over the affected area together with a slight tenderness of the lymph nodes. If a few microfilariae are present in the eye, they may give rise to a mild punctate keratitis.

In order to confirm the diagnosis, a small shaving or "snip" of skin is taken and examined for microfilariae. If none can be detected which is not unusual in light infections, a test dose of diethylcarbamazine (DEC) is given, which in an infected individual will exacarbate the itching and rash within twenty-four hours.

In endemic areas of tropical Africa and America, the cost of the disease much greater in both human and socio-economic terms[8,9]. The vast numbers of microfilariae give rise to a much more severe skin disease termed "Onchoderma", resulting from chronic inflammation.

The skin becomes coarser, more prone to ulceration and finally atrophies. Both segments of the eye may be affected by the chronic inflammation caused by microfilarial decomposition. Lesions in the anterior segment give rise to sclerosing keratitis and iridocyclitis, and in the posterior segment cause choriodoretinitis, neuritis and atrophy of the optic nerve, any one of which can result in severe visual damage or blindness[7,8].

1.4 The Lifecycle of *O. volvulus*.

The lifecycle of *O. volvulus*, which is summarised in figure 1. overleaf, commences with the injection of the larval infective stage into the human host from the bite of the *Simulium* blackfly. *Simulium* are widespread throughout the tropical and sub-tropical world, breeding beside fast-flowing rivers in fertile land.

There then follows a prepatent period of some nine to eighteen months during which the parasites develop into adult worms and migrate to the subcutaneous tissues. The sexes mate and live coiled up in a fibrous nodule, some of which may be clearly visible or palpable - e.g. on the head or over bony prominences such as the ribs or iliac crests. Others are deeply buried, near muscles or joint capsules. The female worm may have a lifespan of fifteen years and will produce many thousands of pre-larval microfilariae daily. The microfilariae, which have a lifespan of between one and two years, migrate to the eye and to the skin surface where they are available for transmission to their intermediate blackfly host, and it is the presence of the microfilariae, rather than the relatively innocuous adults, which is responsible for the pathogenesis of onchocerciasis. Microfilarial development inside the fly is rapid and is complete within about six to nine days, whereupon they migrate to the mouthparts of the fly ready for retransmission[5,6].

THE LIFECYCLE OF ONCHOCERCA VOLVULUS.

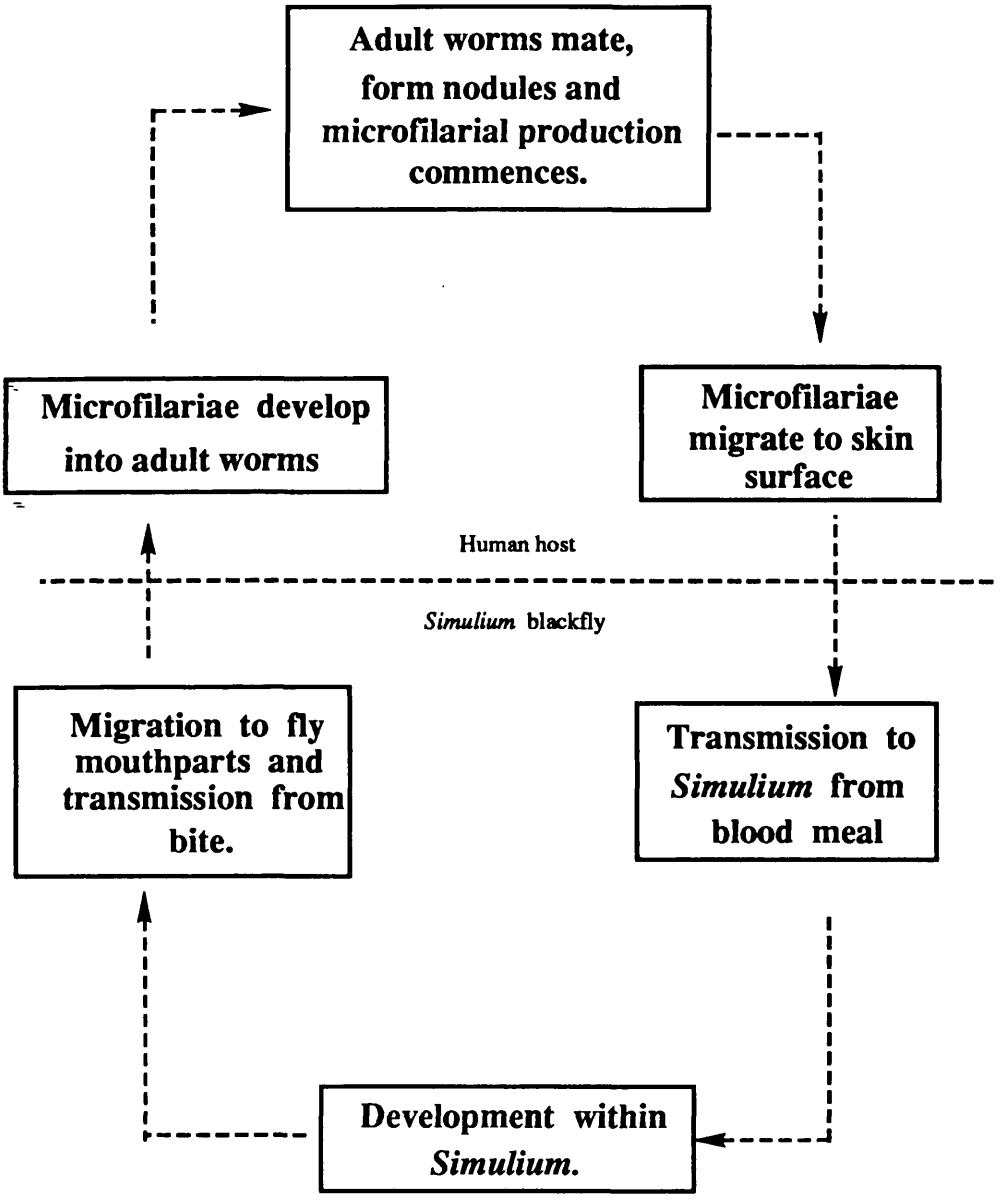


Figure 1.

1.4.1 The socioeconomic effects of Onchocerciasis

In the sub-saharan savannah belt stretching across Africa, the disease is responsible for the highest prevalence of blindness in the world. Up to 15% of the poulation may be blind, including more than 40% of the adult male workforce[9]. The chronic skin disease is debilitating and causes much distress. Remote rural communities which depend on agriculture for subsistence are forced either to accept these harsh statistics or to move from the more fertile river valleys to harsher, drier land where the crop yield may be inadequate.

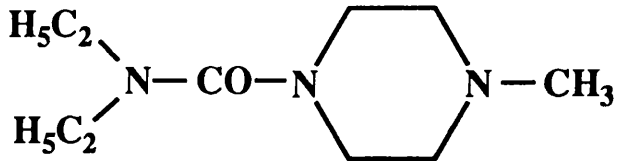
1.4.2 Control of Onchocerciasis

In 1974, the World Health Organisation (WHO) set up the Onchocerciasis Control Project (OCP) with the aim of controlling onchocerciasis over large areas in West Africa[10]. The programme has an operational budget approaching US$ 25 million, and now extends over 11 countries. In order to achieve interruption of transmission for extended periods, work has centred on *Simulium* control by treating the rivers with larvicides, mainly the biodegradable organophosphate, temephos. While this has been effective, especially in some regions, *Simulium* would quickly repopulate if spraying were to cease. It is also time-consuming and expensive, and there have been several reports of developing insecticidal resistance. Spraying has been coupled with drug treatment of the population in an attempt to decrease transmission and to alleviate the disease in those already affected. Despite major efforts in the field of immunology there is as yet no preventative vaccine.

1.4.3 Drug treatment of Onchocerciasis

Chemotherapy of onchocerciasis has centred around the following drugs, none of which are ideal. Their pharmacology and use has been extensively reviewed[3,11-14].

1.4.3.1 Diethylcarbamazine (DEC)



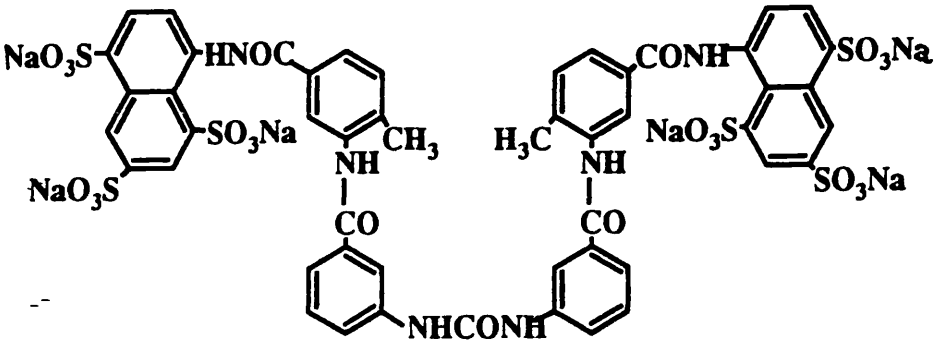
This piperazine derivative has a long history of use against filariasis and is administered orally, usually as its citrate salt. Its place in the treatment of onchocerciasis is based on its powerful microfilaricidal action. Although ineffective *in vitro*, *in vivo* it appears to act in synergism with the host immune system, sensitising the microfilariae to the host's reticuloendothelial system.

The side-effects of DEC in uninfected individuals are mild, dose-dependant and include nausea, headache, weakness, joint pain and anorexia. However in patients with onchocerciasis, DEC administration gives rise to a severe reaction, sometimes fatal, termed the Mazzotti reaction. This condition commences within hours, is normally most marked after about 16h, and is characterised by itching and oedema of the skin and eyes, photophobia, hypotension, pyrexia, lymphangitis, tachycardia and occasionally respiratory distress. Any ocular lesions will be aggravated thus precipitating blindness, and DEC treatment is generally thought inadvisable where there is significant eye involvement but some sight remains. The reaction cannot be counteracted by antiprostaglandin, antihistaminic or antiserotonergic drugs, but only by concommitant corticosteroid administration which unfortunately reduces drug efficacy. The exact mechanism of the Mazzotti reaction is not clear, but it is certainly a result of host immune reaction to antigens released by the dying microfilariae, and its severity is directly related to the microfilarial burden.

DEC has no chemoprophylactic effect and is unsuitable for mass administration programmes as patients require strict medical supervision.

The drug is normally given in combination with suramin and various drug regimens have been devised with a gradually increasing DEC dosage over four or five days. The initial dose in adults is usually 25mg (base), rising to 100mg three times daily for five or six days. Higher doses can be given to patients which an eye examination has shown to be without eye involvement. DEC is also effective against the microfilariae and adult worms of *W. bancrofti*, *B. malayi* and *L. loa*, though the mechanism of the macrofilaricidal activity remains unclear.

1.4.3.2 Suramin



Suramin is currently the only clinically available treatment which kills the adult worm as well as microfilariae. It is both trypanosomicidal and filaricidal, but its mode of action has not been fully elucidated.

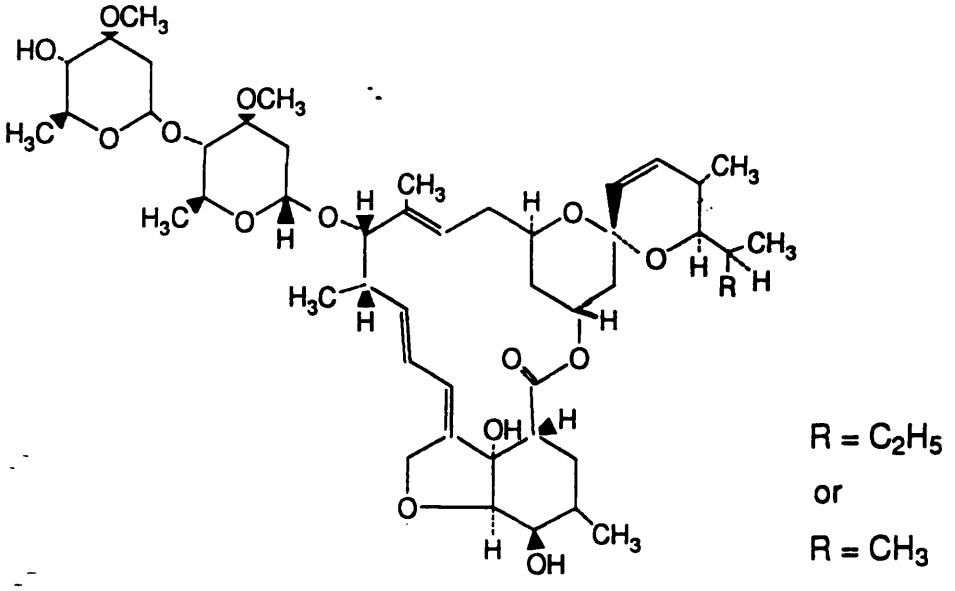
Both trypanosomes and adult filariae are killed following a latent period - in *O. volvulus* after the fourth or fifth week of treatment. Likewise, most microfilariae are killed around the sixth week of treatment. *In vitro*, suramin is an inhibitor of many glycolytic enzymes from *B. pahangi*, *O. volvulus* and *D. immitis*, notably lactate dehydrogenase and malate dehydrogenase These would be affected if *in vivo* suramin concentrations reach the relatively low $1x10^{-6}$ M. Recent interesting studies, involving a range of biochemical analysis have suggested that suramin may be an inhibitor of phosphoglycerate kinase and two separate studies have related the efficacy of suramin to glycolytic inhibition. Other studies with the model filarial worm *B. pahangi* have shown that suramin is very poorly absorbed by the parasite and have proposed that it

exerts its effect by binding to the parasite gut lining and causing degenerative changes[12]. However Suramin is also strongly bound to plasma albumin and it has also been demonstrated in *B. pahangi* that the filaricidal effect was dependent on parasite ingestion of protein-suramin complex.[6] Suramin has been noted to interfere with the DNA-RNA replication mechanism of the cell and it has been postulated that suramin causes slight errors in replication which gradually become cumulative until replication is halted. The most actively dividing cells in the adult worms are the gonads and embryos, and suramin does seem to exert major changes on these tissues. The microfilaricidal action is difficult to explain - microfilariae contain no rapidly dividing cells, but it may be the result of an immunological reaction set up by the death of the adult worms.

Suramin is normally administered by intravenous injection as its hexasodium salt. In adults, a typical dosage regimen would consist of a test dose of 0.1-0.2g, to be followed if well tolerated by 0.4g, 0.6g, 0.8g, 1.0g and 1.0g at weekly intervals, in combination with DEC. The major drawback of Suramin is its extreme cumulative toxicity, especially to the kidney. Reactions occuring immediately after injection are generally the least serious and include nausea, vomiting and slight fever. These can generally be avoided if the injection is given slowly. Later reactions up to 48 hours post-injection include fever, photophobia, constipation and hyperaesthesia of the hands and feet. Most seriously, delayed reactions include kidney damage, agranulocytosis, haemolytic anaemia, jaundice, severe diarrhoea and exfoliative dematitis and stomatitis. In addition allergic reactions to the death of adult worms and microfilariae are usual, generally occuring about the fourth week of treatment. These range from mild reactions such as tenderness and swelling around nodules, to deep abcesses or immobilisation of a joint.

Suramin has been shown to be teratogenic in rats and should not be administered to pregnant or lactating women, to those in poor general condition or those with kidney disease. Owing to its toxicity, suramin is also unsuitable for mass treatment and has no chemoprophylactic properties.

1.4.3.3 Ivermectin (IVM)



Ivermectin component B_{1a} : R = C_2H_5

Ivermectin component B_{1b} : R = CH_3

Ivermectin is a mixture of at least 83% of component B_{1a} and less than 20% of component B_{1b}

Ivermectin is a semi-synthetic macrocyclic lactone belonging to the avermectin family, and is obtained by the selective hydrogenation of avermectin B1, a fermentation product of the bacterium *Streptomyces avermitilis*. Ivermectin is a mixture of compounds, the major component being 22,23-dihydroavermectin B1. Commercial Ivermectin is active against a wide range of nematodes and arthropods and was first licensed for veterinary use in 1985. In humans, Ivermectin was found to be active against the microfilariae of *W. bancrofti* and *O. volvulus*. However it possesses no macrofilarial activity, although it does seems to temporarily halt microfilarial production.

A single dose is highly successful in clearing microfilariae from the skin. One field study showed a 95% decrease at 3 months and approximately an 80% decrease at 12 months, as assessed by microfilarial counts from skin snips, although its efficacy decreases in cases of severe infection. Microfilarial clearance from the eye is also extremely rapid and with minimal inflammatory reactions when compared with DEC. This may reflect differences in mode of action.

The drug is administered orally and one study in humans together with studies in experimental animals show that the drug is poorly metabolised with 98% excreted unchanged in the faeces. This has given rise to some environmental concern as it has been observed with treated cattle that the arthropodicidal effects of IVM prevented normal faecal decomposition[15]. In humans, the drug's plasma half-life is about 3 hours and its apparent elimination half-life is 22 hours, but nothing is known about its concentration in the nodules or skin of infected patients. Side-effects of IVM treatment are normally related to dose and severity of infection and include rash, pruritus, tenderness and enlargement of the lymph nodes, ocular reactions (rarely severe) and more seriously hypotension.

The drug's mode of action still requires further elucidation. It is known that IVM is a potent GABA agonist and that *in vivo* the drop in microfilarial count is immediate, however the adult worm appears unaffected. It has been suggested that IVM prevents uterine release of microfilariae but several experiments on model parasites would suggest this is not the case. However long-term degenerative changes in interuterine microfilariae have been observed, though the mechanism is not clear.

IVM has a high affinity for mammalian brain GABA receptors and has exhibited serious neurotoxicity in collie dogs which have a weak blood brain barrier. The drug is contraindicted if the patient is suffering from any concommitent disease which may compromise their blood-brain barrier such as meningitis or sleeping sickness. Doubts have also been raised about the wisdom of giving IVM to those suffering from malaria. It is also, at present, not licensed for use in children or pregnant women.

1.4.3.4 Other agents with antifilarial activity

There are a variety of other anthelmintics which have been found to have an effect on *O. volvulus* and other filarial worms[3]. Most of these are too toxic to have any real place in the therapy of onchocerciasis or filariasis in general. As onchocerciasis is not a life-threatening condition, even very occasional deaths attributed to drug treatment are unacceptable. Arsenicals such as Arsenamide (*p*-carbamyl-phenylarsylene-dithio diacetic acid) and Mel W (melarsenoxide potassium dimercaptosuccinate) have been shown to be macrofilaricidal to *O. volvulus*, organophosphates such as metriphonate are microfilaricidal. Benzimidazoles such as mebendazole or levamisole have some activity against the microfilariae of *W. bancrofti* and *B. malayi* but are not effective in onchocerciasis.

Most workers in the field agree that Ivermectin has proved to be a great breakthrough, and is the only drug deemed safe for mass treatment programmes. However there remains a need for a new compound which would destroy the adult worms of *O. volvulus* and which would be safe enough for mass therapy.

1.5 Carbohydrate metabolism - A potential chemotherapeutic target

Rational development of new chemotherapeutic agents requires the identification of differences between parasite and host metabolism. This should allow the design and synthesis of selective inhibitors to disrupt vital parasite processes without significant damage to the host[1]. Glucose has been generally considered to play a central role as energy source in filarial parasites, and their carbohydrate metabolism has been extensively investigated[16,17].

1.5.1 Biochemical studies - the use of model filarial species

Laboratory studies of *O. volvulus* have necessarily been limited owing to obvious difficulties in supply. These worms are extremely difficult to maintain outwith their host, and it is only recently that a successful tissue culture system was developed[18]. It is also difficult to remove these worms from their fibrous nodules without damaging them; male worms may be recovered by careful dissection and the use of collagenase, but it has proved practically impossible to isolate intact female worms. Hence the majority of biochemical studies and screens have used model species of filariae, which are readily maintained or available and whose metabolism is considered sufficiently close to *O. volvulus* to allow worthwhile comparison. The following are the most common laboratory models:

Brugia pahangi and *Acanthacheilonema viteae (Dipetalonema viteae)*[19] are filarial parasites which can conveniently be maintained in a wide range of mammals including jirds or hamsters and are readily recovered from the peritoneal cavity.

O. gutterosa, O. lienalis and *O. gibsoni*[20] are species of bovine *Onchocerca. O. gutterosa* and *O. lienalis* are widespread among herds in Britain and indeed in most parts of the world, and are transmitted by various *Simulium* species. The nodule-forming *O. gibsoni* is found mainly in Australiasia, Asia and southern Africa and its main vector is the midge *Culicoides pungens*. In comparison with human filarial species they do little harm to their host, the only clinical signs being nodular swellings under the skin. Their presence may however render the carcass unfit for sale or in need of extensive trimming. As model parasites for human filarial species they can normally be obtained from an abattoir.

Litmosoides carinii is a parasite of cotton rats, but can be maintained in laboratory rats and was much used in early work. However biochemical studies revealed that its carbohydrate metabolism had an unusually large aerobic component, and cast doubt on its use in drug screens[21,22].

Metabolic differences between species of filarial worms have become more apparent as biochemical studies have become more comprehensive. This requires that despite the convenience and availability of model filariae, care must be exercised in extrapolating results between species.

1.5.2 Catabolic pathways in *O. volvulus* and model filarial nematodes

(i) Glycolysis

The majority of filariae are regarded primarily as homolactate fermenters and do not require oxygen for life or motility. This is the case for *B. pahangi*, *A. viteae* and the cattle *Onchocerca*, *O. lienalis* and *O. gutterosa*[18,23,24].

Metabolic investigations of these parasites have made use of a variety of techniques including analysis of tissue extracts (freeze-clamping procedures), radiolabelling and metabolic studies monitored by non-invasive techniques such as ^{31}P and ^{13}C NMR spectroscopy. A study carried out by Watt *et al*[23] using NMR to monitor the use of 1-^{13}C labelled glucose found that *B. pahangi* and *A. viteae* took up glucose at a linear rate under aerobic and anaerobic conditions, with slightly higher uptake under aerobic conditions. In each case the principal metabolite was lactate which accounted for 62-71% of the labelled glucose. From analysis of the worm homogenate a further metabolite, trehalose, was detected accounting for 13-16% ^{13}C labelled glucose. Small amounts of succinate (1-5% label) were detected from subsequent analysis of the media. This work confirmed glycolysis to be the major energy-producing pathway in these model parasites, and agreed with earlier radio-labelling studies.

The glycolytic sequence in filarial nematodes is very similar to that of vertebrates, at least as far as phosphoenolpyruvate and is illustrated in figure 2. overleaf.

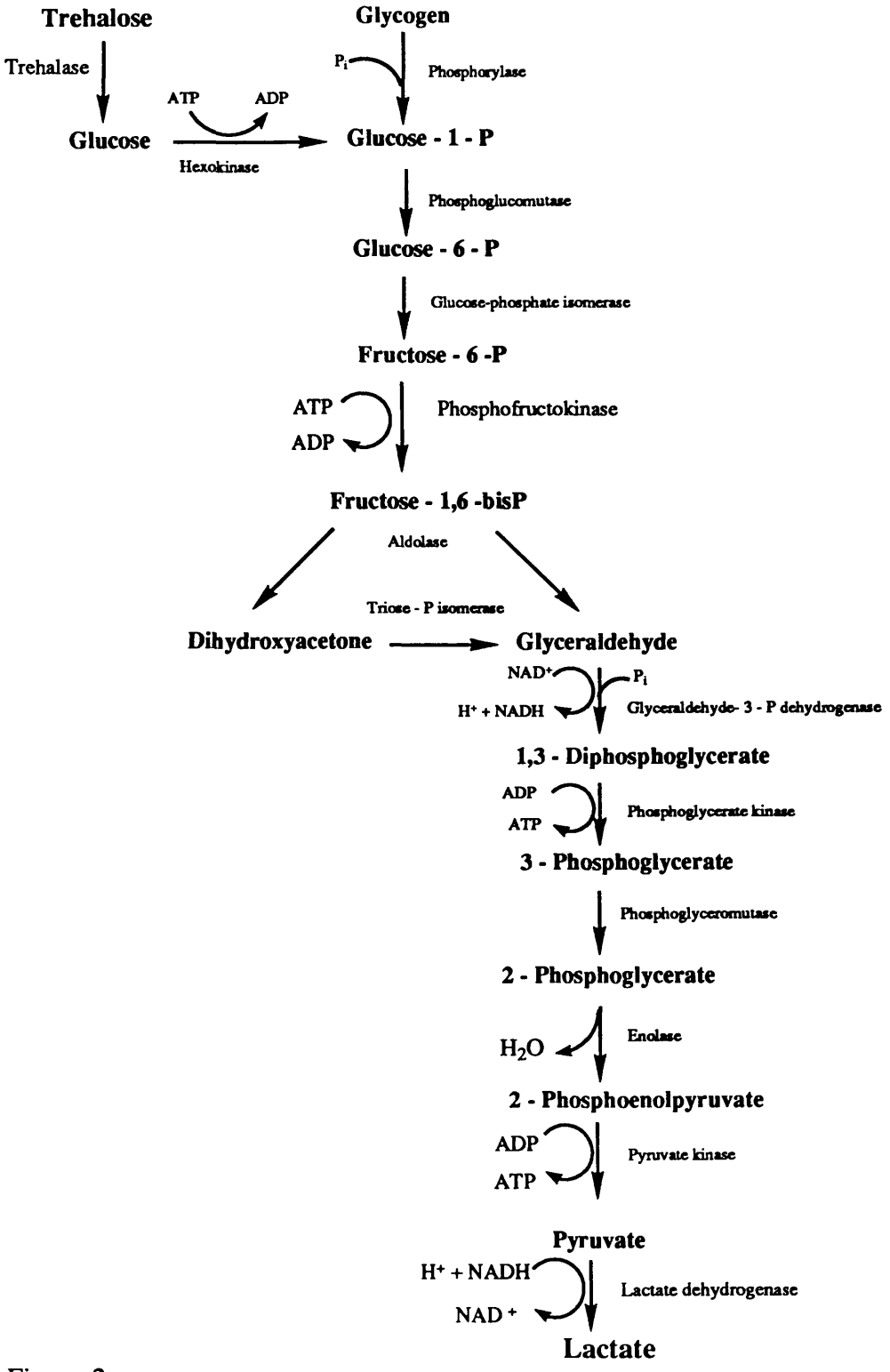

Trehalose
Trehalase
Glucose
ATP
ADP
Hexokinase
Glycogen
Pi
Phosphorylase
Glucose - 1 - P
Phosphoglucomutase
Glucose - 6 - P
Glucose-phosphate isomerase
Fructose - 6 -P
ATP
ADP
Phosphofructokinase
Fructose - 1,6 -bisP
Aldolase
Triose - P isomerase
Dihydroxyacetone
Glyceraldehyde
NAD+
H+ + NADH
Pi
Glyceraldehyde- 3 - P dehydrogenase
1,3 - Diphosphoglycerate
ADP
ATP
Phosphoglycerate kinase
3 - Phosphoglycerate
Phosphoglyceromutase
2 - Phosphoglycerate
H2O
Enolase
2 - Phosphoenolpyruvate
ADP
ATP
Pyruvate kinase
Pyruvate
H+ + NADH
NAD +
Lactate dehydrogenase
Lactate

Figure 2.

The relative activities of the glycolytic enzymes of *B. pahangi* to their mammalian counterparts have been investigated and found to be similar, however more detailed studies have shown some differences in regulatory and structural properties[27]. It has been suggested that filarial parasites, which occupy the glucose-rich lymphatics or subcutaneous tissue, can afford to rely heavily on the relatively inefficient glycolysis pathway for energy[19]. Preliminary work on the pathogen *O. volvulus* highlighted the importance of its glycolytic pathway, and seemed to indicate that breakdown of carbohydrates were the preferred energy source[27]

Carbohydrate storage forms are considered to be glycogen and trehalose, which in filarial parasites may represent the more important source. An NMR study[25] has shown that trehalose does indeed function as a carbohydrate store being quickly broken down in periods of glucose starvation. It has also been suggested that trehalose formation increases the uptake of glucose across the cuticle by helping to maintain a good concentration gradient, however in comparison with other nematodes, filarial parasites have a relatively low percentage of their wet weight in reserve as carbohydrate storage[25,26].

(ii) The partial reverse TCA cycle

In *B. pahangi*, the effects of a lowered glucose concentration had little effect on the percentage of lactate produced but did significantly increase the amount of succinate[25]. The presence of succinate had demonstrated the existence of an active partial TCA cycle in these parasites and it is unsurprising that it should become more prominent in situations of limited glucose availability, producing 4 moles ATP per mole glucose compared to the 2 moles ATP per mole glucose produced by the glycolytic sequence. It has long been known that most filarial parasites, including *O. volvulus*, contain all the enzymes necessary for a partial reverse TCA cycle and indeed for a full TCA cycle[24], although when compared with the glycolytic enzymes their activity is low and their mere presence does not guarantee an active pathway. The branchpoint in the glycolytic and partial reverse TCA cycle pathways presumably occurs at phosphoenol pyruvate and the activity of the enzyme phosphoenolpyruvate-carboxykinase is like;y to be the controlling factor in increasing the relative importance of succinate at low glucose levels. This has also been observed in other parasitic helminths such as *Schistosoma mansoni* and *A. lumbricoides*[19].

Phosphoenolpyruvate represents a branch point into a variety of other possible metabolic pathways, and the fixation of CO_2 to oxaloacetate can lead to a variety of fermentations and most importantly into the partial reverse TCA cycle illustrated overleaf in figure 3. Oxaloacetate is reduced to malate by a cytoplasmic malate dehydrogenase which reoxidises the NADH produced during glycolysis. Malate then enters the mitochondrion and undergoes decarboxylation to pyruvate. Malate is also in equilibrium with fumarate via fumarase and fumarate is reduced to succinate by a fumarase reductase complex. Hence a part of the TCA cycle is being utilised, but in the reverse direction from oxaloacetate to succinate and can proceed entirely anaerobically[17].

The partial reverse TCA cycle

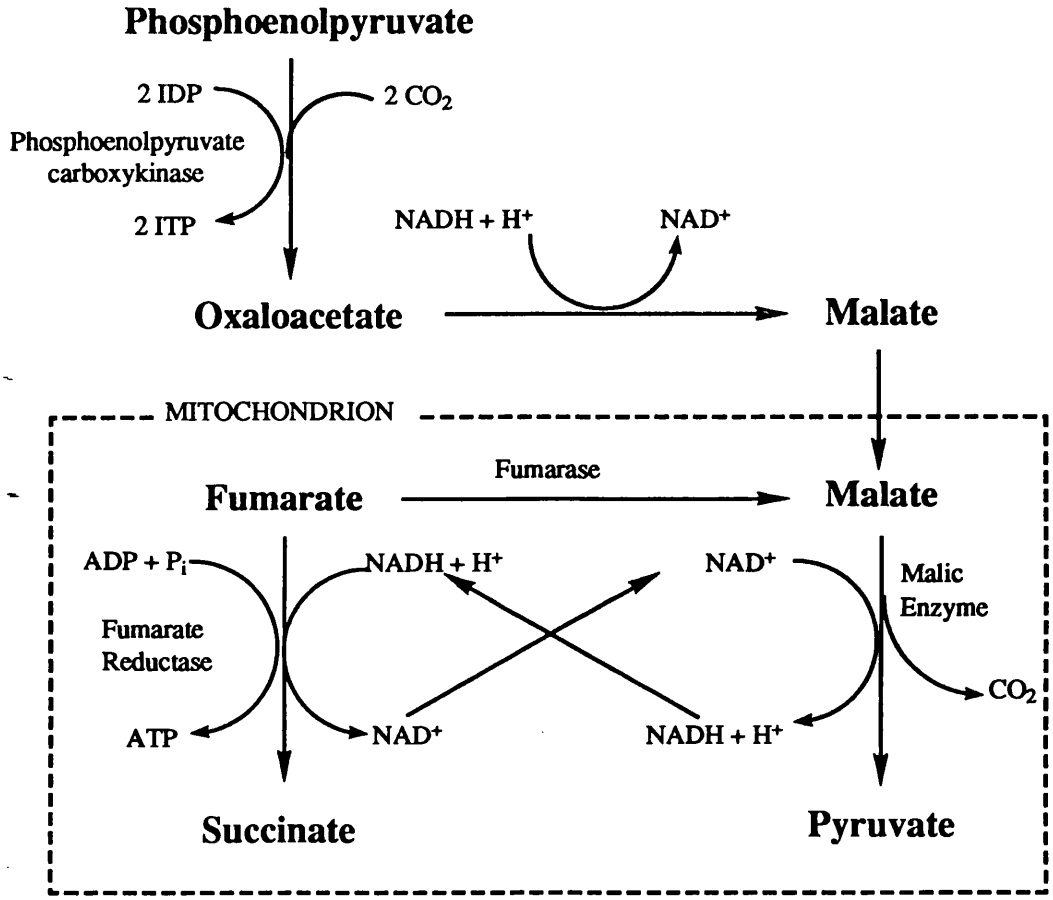
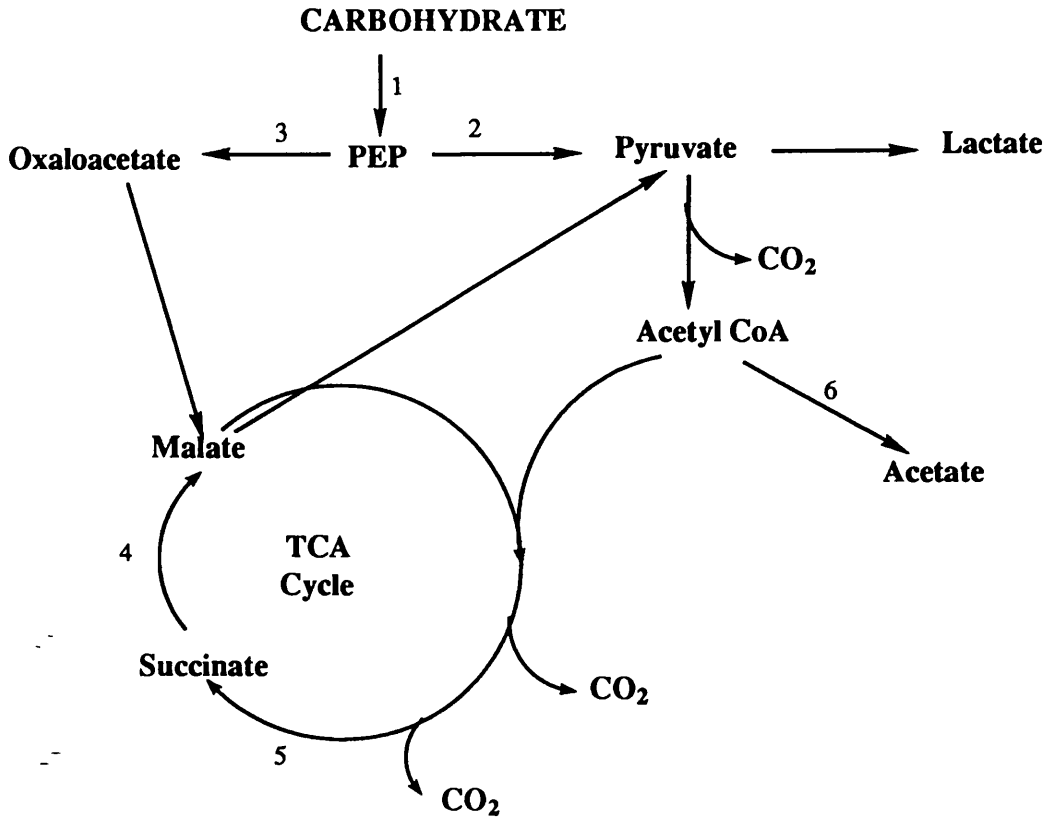


Figure 3.

(iii) Other catabolic pathways

While metabolic studies using NMR spectroscopic techniques[23-25] have shown the formation of small amounts of succinate by *B. pahangi* and *A. viteae*, they have not shown the excretion of pyruvate which must therefore be utilised in some other way. It seems likely that it is further metabolised to alanine, either by entering the TCA cycle via acetyl CoA or by re-entering glycolysis. In the cattle *Onchocerca* and *B. pahangi*, all enzymes necessary for a full TCA cycle have been found[20] although in most cases the activity of these enzymes is low. Work by Mendis and Townson[29] on mitochondrial preparations obtained from *B. pahangi* and *A. vitae* have suggested the presence of an electron transport chain similar to that of mammals and it is difficult to assess the relative importance of aerobic to anaerobic catabolism. The efficiency of the aerobic process (36 ATP per mole of glucose), when compared to glycolysis (2 ATP per mole of glucose), means that even a relatively minor aerobic contribution will be extremely significant.

In adult *B. pahangi*, measurable amounts of fumarate reductase was found and the presence of glucose-6-phosphate dehydrogenase suggests the presence of a pentose phosphate pathway[24], however the physiological significance of these routes in the filariae is questionable. However an overview of all those metabolic pathways considered to be present in filarial nematodes is shown in figure 4. overleaf.



After Kohler *et al* (1991) Parasitology Today, 7, 21-24.

Pathways of energy generation in adult filarial parasites

1. Glycolytic cascade
2. Pyruvate kinase
3. Phosphenolpyruvate carboxykinase
4. NADH-dependent fumarate reuctase
5. Succinyl CoA synthetase
6. Mechanism of possible energy generation is unclear

Figure 4.

1.5.3 Metabolic studies on *O. volvulus*

A recently published metabolic study[28] of *O. volvulus* using ^{13}C NMR spectroscopy have shown that lactate production accounts for less than half the glucose consumed from the external medium. Other products excreted by the parasite were predominantly acetate and ethanol, together with smaller amounts of succinate, formate and CO_2. The authors suggest that acetate can form from pyruvate via oxidative decarboxylation, or perhaps by the action of an enzyme analogous to the bacterial pyruvate-formate lyase.

The potential importance of the amino acid glutamine in the catabolism of *O. volvulus* has been highlighted. ^{13}C NMR spectroscopic studies have shown that glutamine does not undergo complete oxidation but is converted to acetate and CO_2.[28] Further radiolabelling studies showed that glutamine is metabolised into the TCA cycle, and stimulates the oxidative metabolism of glucose to CO_2, thus increasing the efficiency of energy production. When glutamine is the sole energy source *Onchocerca* species, *B. pahangi* and *A. viteae* all become very sensitive to known mitochondrial respiratory inhibitors[19].

1.6 Glucose uptake inhibition - an approach to novel drug design

While filarial catabolism remains a good target for rational drug design[29], many workers believe that the similarity between filarial and mammalian enzymes will not allow selective inhibition. Attempts to interrupt regulatory enzymes such as phosphoenolpyruvate carboxykinase[30,31], phosphofructokinase[32], mitochondrial respiration[33] or glutamine-dependant enzymatic reactions[34] have not been entirely successful.

The discovery that *O. volvulus* may be less dependent upon exogenous glucose than was originally thought, has proved a complication. However it is considered that while the parasite may survive short periods of glucose unavailability by switching to glutamine, this may not be sustainable for long periods. And while glutamine antagonists have been shown to kill filarial worms *in vitro*, they are inactive *in vivo*[34]. The macrofilaricidal activity of the novel antimycin A analogue BW A728C,has been attributed to a combination of glucose uptake inhibition and respiratory electron transport inhibition, and it has been shown not to directly inhibit the glycolytic sequence[35].

It may be that a combination of glucose uptake inhibition with glutamine antagonists or respiratory electron transport chain (RET) inhibitors could prove a more successful therapeutic strategy for the development of novel chemotherapy for filarial disease.

Work by Watt *et al* has identified glucose uptake as a potential chemotherapeutic target[36]. It was felt that this early stage of glucose metabolism would prove a more effective target than enzyme inhibition owing to the parasite's ability to switch between metabolic pathways. Several known anthelmintics are considered at least in part, to affect glucose uptake. These include the benzimidazole broad spectrum anthelminthic mebendazole which causes glycogen depletion and inhibits glucose uptake in the cestode *Avitelline lahorea*[37].

Amoscanate treatment in *Hymenoptelis diminuta* infected rats has also been shown to act by inhibition of glucose uptake and altered glycogen metabolism[38]. Amoscanate has also been shown to be effective against *B. pahangi*, *A. viteae* and *L. carinii* although over a rather lengthy timescale. A study by Nelson and Saz[39] found that glucose uptake and incorporation into glycogen, lactate accumulation and in the case of *L. carinii* acetate formation, had been inhibited 12 hours after amoscanate treatment of infected jirds. The authors considered that the most profound short-term effect had been on glucose transport.

1.6.1 Mechanisms of glucose uptake

There are a wide variety of ways in which small molecules are internalised by cellular organisms, and almost all of these have been found to apply to glucose uptake in some species. These mechanisms range from passive diffusion to active (energy requiring) transport systems and also include facilitated transport diffusion mechanisms, pore and pinocytic systems[40]. In mammalian host systems, Na^+ dependant active transport predominates in the gut and kidney, whilst a few other tissues such as the erythrocyte employ a facilitated transport mechanism[41].

Studies in other species of parasites show that cestodes such as *Hymenoptelis diminuta* possess an active Na^+ dependent uptake system,[42] however the trematode *Fasciola hepatica* shows a passive facilitated transport system[43] as does *Schistosoma mansoni*[44]. In filarial nematodes, low molecular weight nutrients like glucose may be absorbed by both the gut and the cuticle, but studies suggest that *in vitro* at least, the transcuticular route is the most important[45,46].

1.6.2 NMR studies on glucose uptake in model filarial nematodes

Studies undertaken by Watt *et al*[47,48] have examined the mechanism of filarial glucose uptake, to elucidate its mechanism, and to assess if sufficient differences exist between parasite and host systems to allow the design of a selective inhibitor.

1.6.2.1 An investigation into the mechanism of filarial glucose uptake

The first study used ^{13}C NMR spectroscopy to monitor worm metabolism of 1-^{13}C labelled glucose *in vitro*, and employed the known glucose uptake inhibitors phloridzin and phloretin. Na^+ dependant active transport is generally sensitive to competitive inhibition by phloridzin[49], a biphenolic glycoside, which usually has k_i values of the order of $5x10^{-7}M$. By contrast, the aglycone phloretin is well known as a competitive inhibitor of facilitated transport[50].

Results showed that the model filariid *B. pahangi* was little affected by phloridzin even at concentrations up to 0.5mM, with both glucose utilisation and lactate output remaining stable. However incubation with phloretin resulted in a dramatic drop in glucose utilisation and lactate output. This was associated with decreased parasite motility, and at the highest concentrations a failure to recover. The k_i value for phloretin was approximately 0.4mM. Further data from *A. viteae* confirmed these trends. This study suggests that glucose uptake by these filariae proceeds almost entirely by a facilitated transport mechanism, and that its inhibition, *in vitro* at least, results in considerable parasite morbidity.

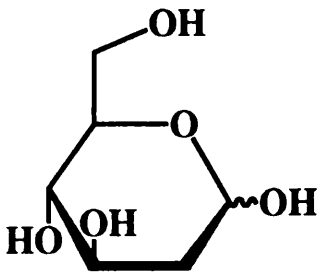
1.6.2.2 Inhibition of glucose uptake by simple glucose analogues

The second study examined the effect of simple glucose analogues on the utilisation of glucose. Again worm metabolism was monitored by ^{13}C NMR spectroscopy and the relative inhibition of uptake of 1-^{13}C labelled glucose. This has allowed a basic structure/activity relationship to be discerned and compared with those of known mammalian uptake systems.

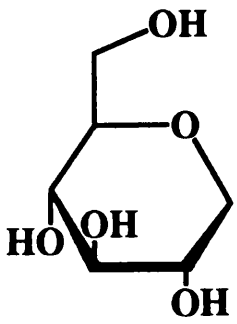
Deoxyglucose derivatives have had frequent use in a variety of studies. 2-Deoxyglucose (1) is regarded as " non-metabolisable " and it is accepted to be taken up and converted to its 6-phosphate but further metabolism is prohibited[51]. Inhibition of hexokinase has never been demonstrated for this compound. 2-Deoxyglucose is considered to share the same uptake system as glucose itself in a variety of organisms and tissues, including the mammalian brain[52], *Plasmodium* species[53] and schistosomes[44]. The kinetic parameters of glucose and 2-deoxyglucose are slightly different but within the same order of magnitude, and labelled 2-deoxyglucose has been used as a probe to investigate the relative importance and inhibition of transport processes versus late stage metabolism in *B. pahangi*[35].

Also tested were its isomers, 1-deoxyglucose (2) and 6-deoxyglucose (3), which have been commonly used in transport studies. 1-Deoxyglucose has been used as a kinetic probe for glucose uptake in *Trypanosoma brucei* and was shown to have a high affinity for its uptake system but not to undergo further metabolism[54]. In the same parasite, 6-deoxyglucose was found to inhibit 1-deoxyglucose uptake.

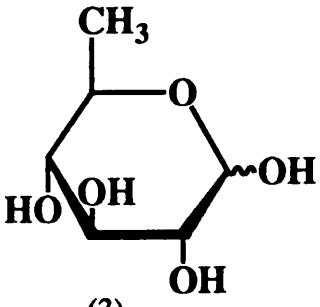
5-Thioglucose (4) is considered to be the closest structural analogue to glucose itself. As early as 1972 it was shown to inhibit cellular transport processes, both active and facilitated, in competition with D-glucose[55]. It has been demonstrated to interfere with the utilisation of glucose in the rodent intestine and can induce hyperglycaemia as a result[56,57]. It has also been shown to exert a powerful effect on *in vitro* cultures of *Schistosoma mansoni*, partly by inhibition of glucose transport but also by strong competitive inhibition of hexokinase[58]. The 1-thioglucose isomer (5) was also tested.


OH
O
OH
HO
OH

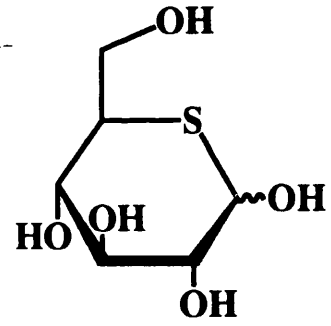
(1)


OH
O
HO
OH
OH

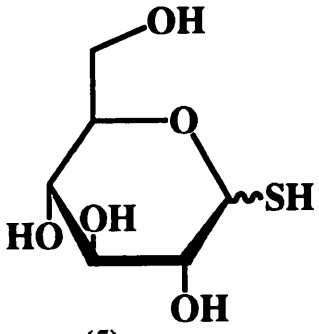
(2)


CH3
O
OH
HO
OH
OH

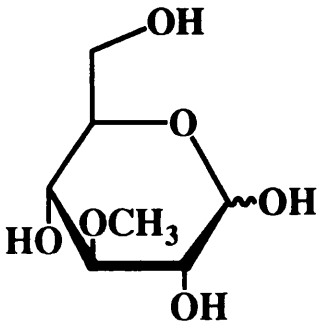
(3)


OH
S
OH
HO
OH
OH

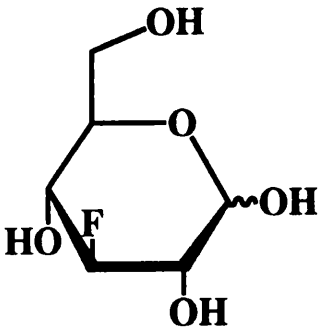
(4)


OH
O
SH
HO
OH
OH

(5)


OH
O
OH
HO
OCH3
OH

(6)


OH
O
OH
HO
F
OH

(7)

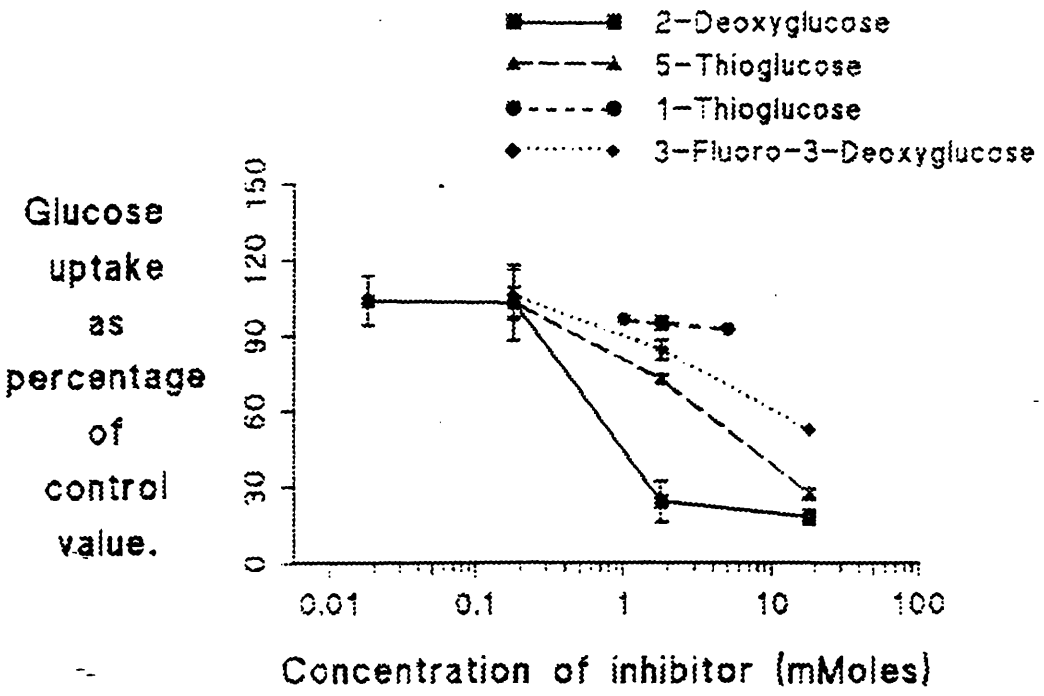
Other commonly used probes of glucose uptake include 3-O-methylglucose (6), a moderately good inhibitor of hexose uptake in the human erythrocyte[59]. Effects in parasitic organisms have been mixed. In *Fasciola hepatica* the sugar is rapidly taken by the same facilitated transport mechanism as glucose and acts as a good competitive inhibitor[43]. By contrast, in *Trypanosoma brucei*[54] and *Schistosoma mansoni*[44] 3-O-methyglucose has a much lower affinity for the carrier and is a relatively poor competitive inhibitor.

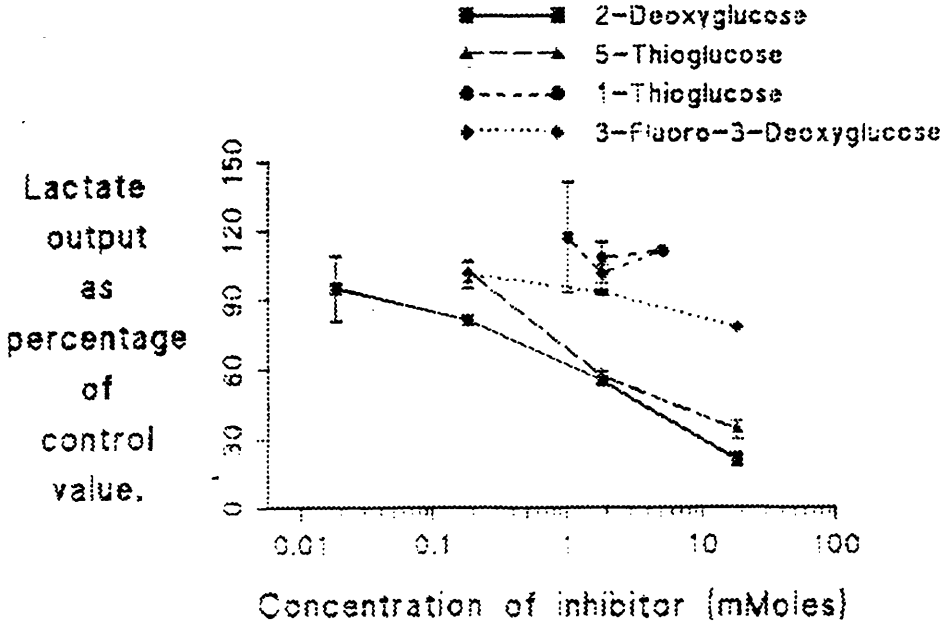
Also examined was 3-deoxy-3-fluoroglucose (7) which was of particular interest to examine by NMR methods as the sensitivity of ^{19}F, the magnetically active isotope of fluorine is almost as good as that of the proton. Rat brain metabolism of 3-deoxy-3-fluoroglucose and that of the isomeric 2-deoxy-2-fluoroglucose, in high concentration, have been followed by NMR spectroscopy[60].

Results for the inhibition of uptake of 1-^{13}C-glucose by the various glucose analogues are presented in figure 5. In *B. pahangi* macrofilariae, 2-deoxyglucose inhibited both glucose uptake and lactate output, even at the lowest concentrations (0.018mM). However a complete inhibition is not observed even at a 1:1 molar ratio. The isomeric deoxy analogues exerted no apparent effect. Those glucose analogues which lack a hydroxyl at position 3, also exert very little effect on glucose uptake. The close glucose analogue 5-thioglucose does also show a competitive inhibition closely parallel to the 2-deoxy derivative. With 3-deoxy-3-fluoroglucose a dramatic stretching effect was observed when the concentration was higher than 1.8mM inhibitor, but glucose metabolism continued, although about 50% of normal. Homogenates were prepared and assessed at the end of each metabolic run and were examined by high sensitivity NMR spectroscopy.

Trehalose was the only metabolite other than lactate which was found in fairly reproducible quantities, except in the presence of even the lowest concentrations of 5-thioglucose where its formation seems to have been suppressed.

fig 5


2-Deoxyglucose
5-Thioglucose
1-Thioglucose
3-Fluoro-3-Deoxyglucose
Glucose uptake as percentage of control value.
0
30
60
90
120
150
0.01
0.1
1
10
100
Concentration of inhibitor (mMoles)


2-Deoxyglucose
5-Thioglucose
1-Thioglucose
3-Fluoro-3-Deoxyglucose
Lactate output as percentage of control value.
0
30
60
90
120
150
0.01
0.1
1
10
100
Concentration of inhibitor (mMoles)

1.6.3 Structure/activity deductions and comparisons

The results for 2-deoxyglucose are consistent with it acting as a competitive inhibitor of glucose uptake, having an approximate K_i value of 0.2mM. This suggests that the 2-hydroxyl function is not critically involved with the parasitic glucose transporter. The K_i value is similar to that reported for *S. mansoni*[44] (mated females) and is considerably lower than the value of 3.2mM quoted for the human erythrocyte[59]. The K_i value of 6-deoxyglucose in human erythrocyte is quoted as being 6.7mM[59] whereas in *B. pahangi* the K_i value is at least one order of magnitude higher. This suggests a strong involvement for the 6-hydroxy function in the parasitic glucose uptake system which does not seem to be the case for the human erythrocyte. It also contrasts with *T. brucei* where 6-deoxyglucose was found to be a good glucose uptake inhibitor[54]. —T.b.

5-Thioglucose was also found to be a good competitive inhibitor with an estimated K_i value of 0.35mM. This is quantitatively similar to its effect on glucose uptake in *S. mansoni*[58]. However caution is required in assigning this effect to transport alone. In *S. mansoni*, 5-thioglucose was found to be a good competitive inhibitor of hexokinase by kinetic analysis on homogenates, the inhibitor having a k_i of about 1.0mM. Paranjpe and Jagannathan have reported that 5-thioglucose inhibits ox heart hexokinase[61], however it has proved a poor substrate and inhibitor for yeast hexokinase[62,63].

The apparent ineffectiveness of 1-thioglucose to inhibit glucose uptake in *B. pahangi* would tend to imply that a 1-hydroxy fuction is essential for transport. Similarly, the failure of 3-O-methylglucose as an inhibitor contrasts with its activity in the human erythrocyte where a moderate inhibition of transport is observed[59]. Its behaviour is similar to that observed in *T. brucei*[54] and *S. mansoni*[35] but differs —Tb markedly from its behaviour in *Fasciola hepatica* in which it is rapidly taken up and is a good competitive inhibitor[43]. This would imply very different susceptibility to substitution at the 3-position of monosaccharides between different parasites, and between some parasites and mammals.

The powerful physical effects exerted by 3-deoxy-3-fluoroglucose above certain concentrations would suggest that while not a powerful inhibitor of glucose uptake (estimated k_i = 0.74mM), it is recognised, taken up and toxic metabolism is taking place.The concentration at which this happens are less than have been used for quantitative NMR studies in mammalian tissues[60]. Once again, this would suggest a differential effect for substitution on this position; in fact the steric bulk at the 3-position may be particularly critical in determining the kinetics of transport.

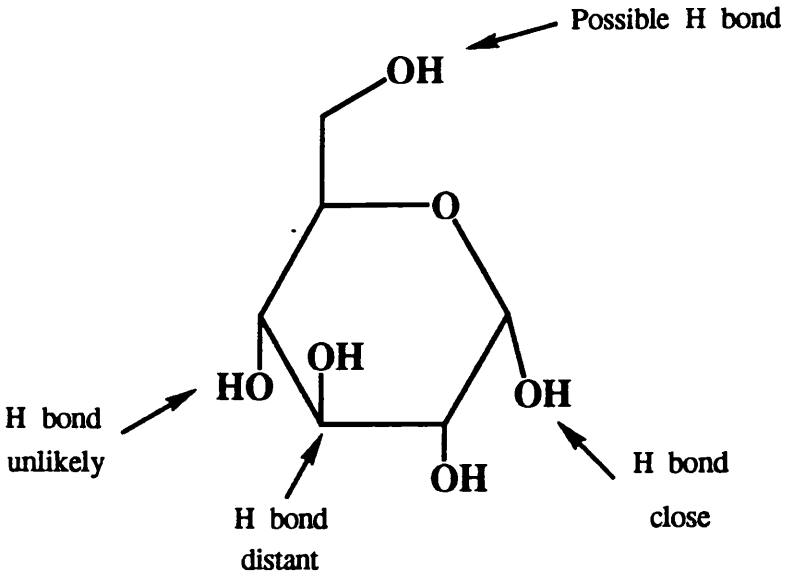
1.6.4 A comparison of the binding requirements of filarial and human erythrocyte glucose transporter

From these studies it has proved possible to develop a simple structure/activity model for the glucose transporter of *B. pahangi*. This can be compared with the known requirements of another facilitated transport mechanism, the human erythrocyte and is illustrated in figure 6 overleaf.

The glucose transport protein of the human erythrocte has been well-studied and has been isolated and purified by affinity chromatography using the powerful and specific inhibitor cytochalasin B. The study of the binding structural requirements of the human erythrocyte was undertaken by Barnett *et al*[59] who measured the inhibition constants for specifically substituted analogues of D-glucose, when L-sorbose was the penetrating sugar. L-sorbose is accepted to enter the erythrocyte by the same transport system as D-glucose although its affinity for the carrier system is low. This study showed that derivatives in which a hydroxyl group in the D-*gluco* configuration was inverted, or replaced by a hydrogen atom at C-1, C-2, C-3, C-4 and C-6, were all bound by the carrier system, showing that no single hydroxyl group is essential for binding. The binding and transport of 1-deoxy-D-glucose confirmed that the sugars bind in the pyranose form.

Erythrocyte glucose transporter

Barnett *et al* Biochem. J.



Brugia pahangi glucose transporter

Watt *et al.*

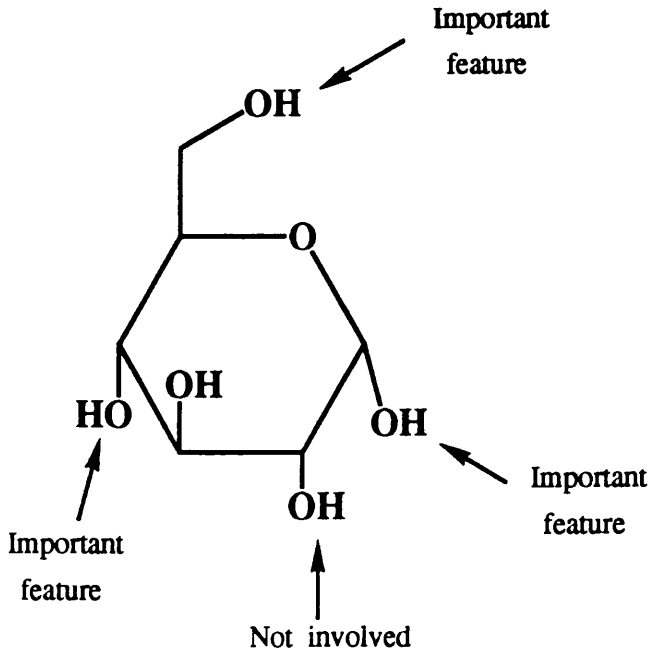
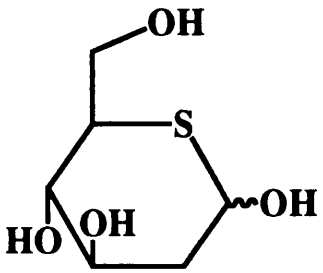


Figure 6.

Relative inhibition constants of D-glucose and various analogues were found to be consistent with the combination of β-D-glucose with the carrier by hydrogen bonds at C-1, C-3 and possibly at C-6. The authors also felt that there was probably at hydrogen bond at C-4 but work by Taylor *et al*[64] on the comparative transport parameters of D-glucose with 4-deoxy-4-fluoro-D-glucose and 4-deoxy-D-xylose in the human erythrocyte tends to indicate that O-4 in β-D-glucose is not involved in hydrogen bonding to the carrier protein. Further work by Barnett suggested that the carrier region around C-6 may contain both hydrophobic and polar binding groups. That D-glucal proved a good inhibitor shows that a strict chair form is not essential for binding.

1.7 Identification of other putative glucose uptake inhibitors

As substantial differences between structural binding requirements exist, it was decided to extend this work and to attempt the development of improved glucose uptake inhibitors for filarial nematodes. The structure/activity model has lead to the identification of a number of synthetic targets, the most obvious of which is 2-deoxy-5-thio-D-glucose (8). A literature search revealed that this compound had never been synthesised and is unknown in the natural world.



(8)

Analogues of this compound with substituents other than a hydroxyl at C-2 could also demonstrate potential selective activity.

Other compounds whose transport properties it was thought interesting to examine included other pyranose sugars containing elements other than oxygen as ring heterocycle such as nitrogen (the "aza" or piperidine sugars) with various different substitutents at C-2. An examination of binding coefficients with the corresponding furanose derivatives was also considered of some value.

1.8 Thiosugars as drugs - known pharmacological effects

Although 5-thioglucose is known to produce hyperglycaemia in rodents, it is considered to be suprisingly non-toxic, possessing a high LD_{50} value (mice) of 5.5g/Kg. Its diabetogenic properties are considered to stem from an inhibition of insulin release coupled with decreased glucose utilisation.[55,56] 5-Thioglucose has received some evaluation as an antineoplastic agent, having been shown to be selectively toxic towards and to radiosensitise hypoxic cells.[65] Overall, these effects have been attributed to interference with glucose metabolism while interaction with radiation may be due to inhibition of DNA damage repair. In conjunction with this, there is evidence that it may even protect oxygenated (i.e. normal) cells against radiation damage. Uptake of 5-thioglucose by animal tumour cells is high, and it was considered to be of greatest potential use for the treatment of certain tumours such as retinoblastoma which contain hypoxic cells.

The study[65] carried out on an ocular melanoma model in rabbits compared the local application of 5-thioglucose with *cis*-platin, a known cytotoxic with some radiosensitising activity and found a significant effect exercised by 5-thioglucose in delaying tumour growth. Good drug concentrations in the aqueous humour were achieved with no severe local reactions at relatively high doses. *Cis*-platin proved a better inhibitor of tumour growth but gave rise to more severe adverse effects.

Other novel thiosugars have recieved assessment as anthelmintics.[66] The metabolic route by which several parasitic protozoa degrade 5'-methylthioadenosine (MTA) was found to differ from its catabolism in human cells.

Two analogues of methylthioribose, an MTA catabolite, were synthesised and found *in vitro* to be selectively cytocidal to *Plasmodium falciparum* (a causative agent of malaria), *Giardia lamblia* and *Ochromonas malhamensis*. These analogues, 5-ethylthioribose and 5-isobutylthioribose, were subsequently patented as representatives of a new class of potential antiprotozoal drugs.[66]

1.9 The biological activities of piperidine and pyrollidine monosaccharides

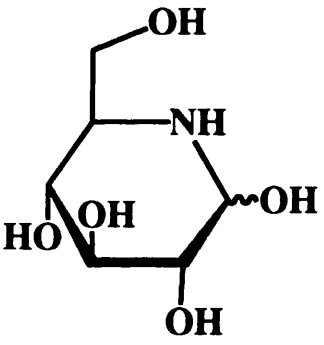
Many monosaccharide analogues in which nitrogen replaces oxygen as ring heterocycle have been identified from natural sources. The closest analogue to glucose, 5-amino-5-deoxy-D-glucose (Nojirimycin) (9) has been isolated from several strains of *Streptomyces* such as *Str. roseochromogenes* R-468, *Str. lavendulae* SF-425 and *Str.nojirensis* SF-426 and recently from *Bacillus*[67,68]. This compound has been found to possess interesting biological activity and acts as an antibiotic against drug-resistant strains of *Sarcina lutea*, *Xanthomona oryzae* and *Shigella flexneri*.

The related sugar 5-amino-1,5-dideoxy-D-glucose[69] (1-deoxynojirimycin) (10) which has been shown to possess a similar spectrum of activity was first obtained by chemical transformation of nojirimycin and subsequently isolated from plants of the species *Morus* and from *Bacillus*.

Both these compounds and many which are related to them such as galactostatin (5-amino-5-deoxy-D-galactopyranose)[70] and nojirimycin B (5-amino-5-deoxy-D-mannose)[71] act as powerful inhibitors of various α and β-glycosidases from many sources, microbial and mammalian. Structurally these are analogous to the natural products swainsonine and castanospermine which as potent glycosidase inhibitors are responsible for the disease "locoism" in cattle.[68] In this category may be considered azafuranose or pyrollidine sugars such as 1,4-dideoxy-1,4-imino-D-mannitol.[72] Their powerful inhibitory properties have been used in investigations of the mechanism of action of the glycosidic hydrolases. Information on the active site of these enzymes has come from inhibition studies with 5-amino-5-deoxyhexoses.

The impressive inhibitory power against trimming and processing glycosidases of glycoprotein synthesis has led to their examination as antiviral agents.[73] Treatment of acutely infected lymphoid cells with 2.0mM N-butyl-deoxynojirimycin was shown to reduce virus yield by more than 90% without affecting cell growth. Lower concentrations did not affect HIV-1 production but substantially reduced infectivity. They are also considered to have potential therapeutic uses in diabetes mellitus and tumour metastases.[74]

Interestingly, 1-deoxynojirimycin has also been found to inhibit insect trehalose[68], as does the related amino sugar 1,5-dideoxy-1,5-imino-D-mannitol(11). It has been postulated that the presence of such inhibitors in plants may act as a defense mechanism against phytophagous insects and has generated much interest for ecological reasons.[68]



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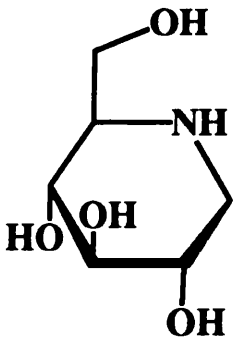

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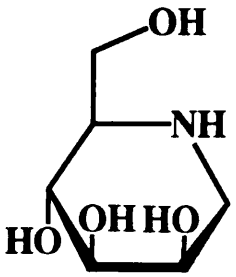
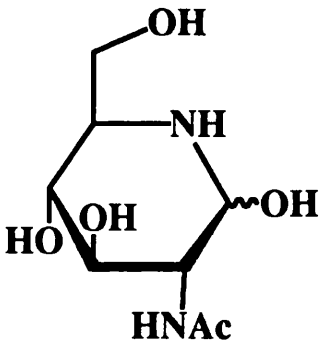


(11)

1.9.1 2-amino analogues of nojirimycin

Interest in the N-acetylglucosoaminidases, a group of enzymes analogous to the glycosidases, stemmed from their widspread occurence in both plants and animals and the need to further clarify their role. As no active-site directed inhibitors had been discovered, Kappes and Leggler[75] synthesised the amino N-acetylglucosamine (12) and 1-deoxy (13) analogues from N-acetylglucosamine. These compounds were also anticipated to find application as their N-carboxyallyl derivatives, providing ligands for enzyme isolation by affinity chromatography.

Two alternative synthetic routes have also been developed to (13) by Fleet *et al*[76] and Boshagen *et al*[77] however both are very lengthy and do not use a 2-amino sugar as starting material. There are no reports of the synthesis of any free 2-amino or 2-deoxy nojirimycin analogues in the literature.



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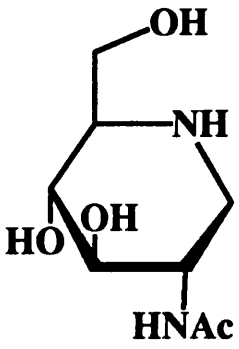


(13)

CHAPTER TWO

2.1 2-Deoxy-5-thio-D-glucose - a synthetic strategy

The synthesis of 2-deoxy-5-thio-D-glucose was considered possible by a number of routes and from various starting materials, including 2-deoxy-D-glucose and 5-thio-D-glucose. However these compounds are relatively expensive and their commercial synthesis is complex. We wished to develop routes which produced intermediates of interest in their own right for anthelmintic testing and had cheap, readily-available starting materials. Intermediates of interest included 2-amino-5-thio monosaccharides and possibly 2-amino substituted derivatives such as 2-guanidino compounds. From these considerations it was decided to use 2-amino glucose derivatives as starting materials. The preparation of the 2-deoxy sugar moiety from an intermediate amine was regarded as being relatively easily achieved by reductive deamination.

2.1.1 2-Amino monosaccharides

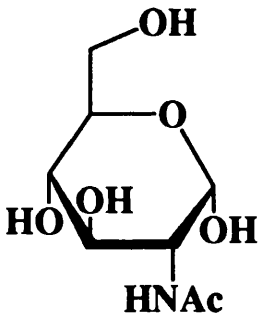
There are two readily available 2-amino-glucose derivatives, both of which are widely distributed in nature.

N-acetylglucosamine (2-acetamido-2-deoxy-D-glucose) (14) is found in both procaryotic and eucaryotic organisms; alternately β-1,4 linked with N-acetyl muramic acid, it forms part of certain bacterial cell wall polysaccharides. It is a precursor in the biosynthesis of N-acetylneuramic acid and an important constituent of the cell wall in higher animals, usually as a non-reducing end of the hetero-oligosaccharide portion of cell-surface glycoproteins and glycolipids. In eucaryotes, it is widely found as part of the carbohydrate component in various glycoproteins. In the extracellular space, various polysaccharide chain structures containing an amino sugar (either N-acetylglucosamine or N-acetylgalactosamine) as part of the disaccharide repeating unit have been identified, and are termed glycoaminoglycans.

Seven groups of glycoaminoglycans have been classified, and include hyaluronic acid which is thought to play a major role in cell-adhesion.[40]

Glucosamine (2-amino-2-deoxy-D-glucose, chitosamine) as its homopolymer chitin plays a major structural role in many organisms forming the major component of shell, which is its commercial source. Normally available as its hydrochloride salt (15), it is the least expensive of all the 2-amino sugars.

N-Galactosamine (Chondrosamine) is also commercially available but its relative rarity is reflected in its higher price.



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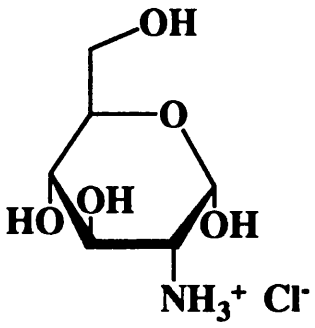
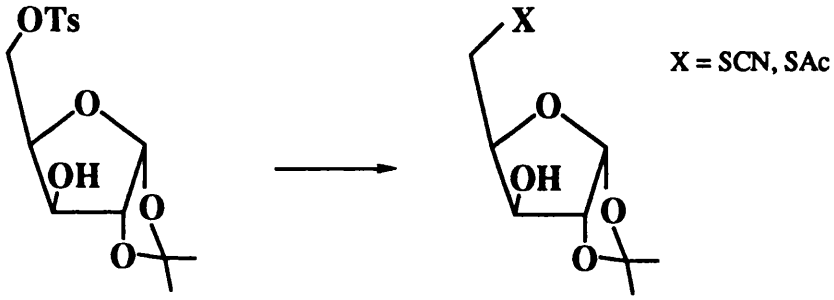


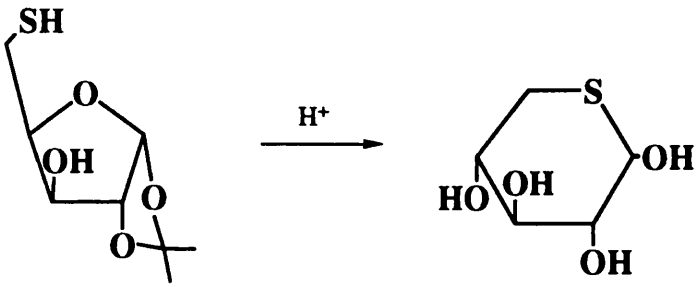
(15)

2.2 Synthesis of thio sugars

The history of the synthesis of monosaccharide derivatives in which sulphur replaces oxygen as ring heterocycle commences in Edinburgh, with the successful synthesis of 5-thioxylopyranose by Schwartz and Yule in 1961.[78] This publication was quickly followed by the synthesis of the same compound by a slightly different route from Owen and Adley at Imperial College, London.[79] Both routes commenced with 1,2-O-isopropylidene-5-O-tosyl-D-xylofuranose and involved the displacement of the tosyl group by a sulphur-containg nucleophile.



Following reduction to the free thiol, acid hydrolysis effected ring closure in each case.



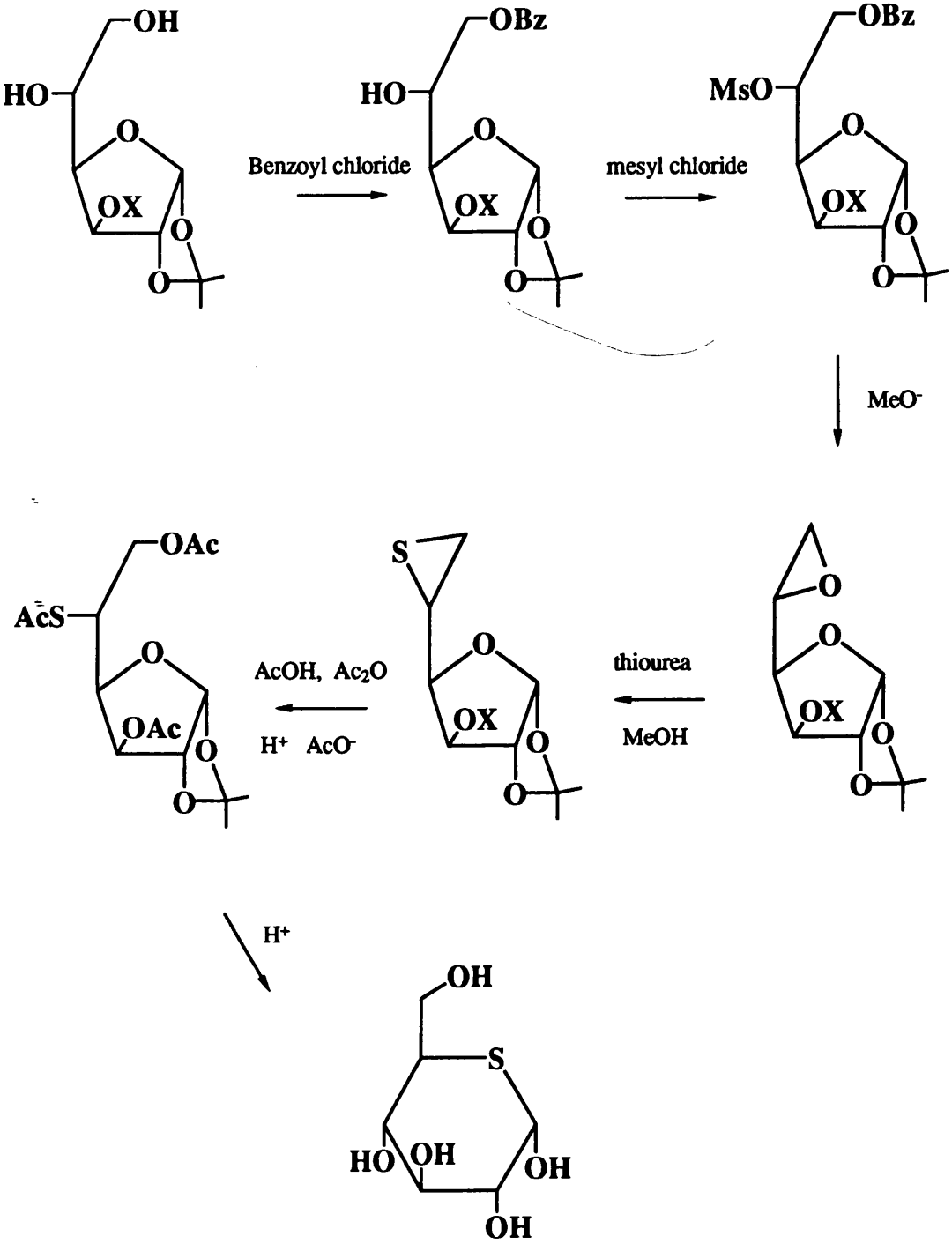
This work has lead to the synthesis of most of the 5-thio-D-hexoses and pentoses and much interest has been generated in these compounds from a biological and biochemical standpoint.

2.2.1 Literature syntheses of 5-thio-D-glucose

5-Thio-D-glucose was first synthesised in 1962 by R.L. Whistler and M.S. Feather, from the known 5,6-episulphide of 1,2-O-isopropylidene-α-D-glucofuranose by acetolysis of the episulphide ring, followed by acid-catalysed hydrolysis and ring expansion.[80] Since then, various strategies have been devised in an attempt to shorten existing synthetic routes and improve yields. All preparations of 5-thio sugars in which sulphur replaces oxygen as ring heterocycle require the substitution of a thiol precursor at C-5 and in essence this can be achieved in two ways.

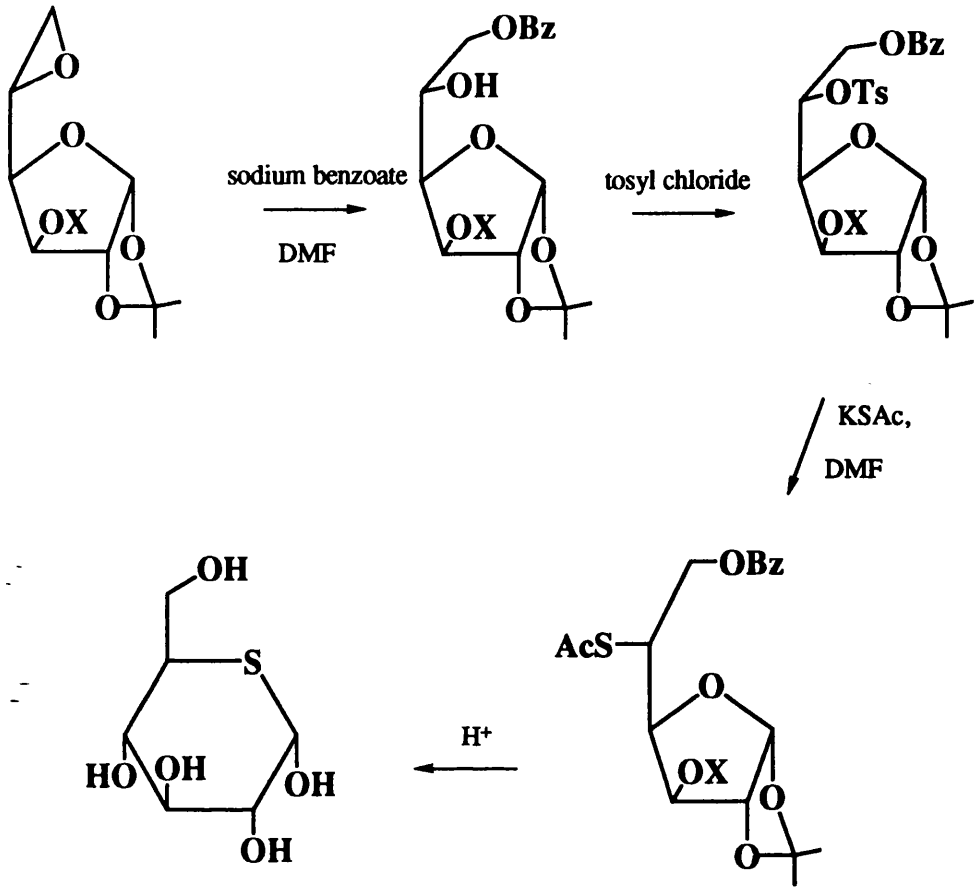
In one procedure, as in Whistler's original work, the formation of a terminal 5,6-anhydro ring is followed by its conversion to an episulphide by treatment with thiourea in methanol. This has proved a very popular means of introducing sulphur and while several transformations are required, each step normally proceeds in high yield (scheme 1.). Essentially, selective benzoylation of the primary hydroxyl group on C-6 with benzoyl chloride in pyridine at -15°C affords the 6-dibenzoate in high yield. Mesylation of the remaining free hydroxyl at C-5 with methanesulphonyl chloride gives the 5-O-mesylate. Treatment with methanolic sodium methoxide results in deacylation and anionic attack of the C-6 oxygen at C-5, leading to displacement of the sulphonate. This forms a terminal epoxide ring with concommitant inversion of stereochemistry. Treatment of this epoxide derivative with thiourea in methanol results in the formation of an episulphide ring with reinversion back to the D-*gluco* configuration. The episulphide can then be opened by a nucleophile such as acetate anion, preferential attack at the primary position results in the sulphur atom being positioned at C-5. Acid or base catalysed deacetylation will result in ring expansion with the more nucleophilic sulphur atom as ring heterocycle. This approach has proved to be most popular, with many published strategies differing in choice of starting material alone and culminating in the same final sequence of transformations.[81-83]

Scheme 1.


OH
HO
O
OX
O
O
Benzoyl chloride
OBz
HO
O
OX
O
O
mesyl chloride
OBz
MsO
O
OX
O
O
MeO⁻
OAc
AcS
O
OAc
O
O
AcOH, Ac2O
H+ AcO⁻
S
O
OX
O
O
thiourea
MeOH
O
O
OX
O
O
H+
OH
S
HO
OH
OH
OH

The second method requires the C-5 displacement of a leaving group, generally a sulphonyloxy ester, by a thiol nucleophile such as thioacetate, thiobenzoate or thiocyanate. This inevitably results in inversion of stereochemistry owing to the S_N2 nature of displacement at secondary hydroxyl functions. Therefore, either the starting sugar must be the epimer of the desired sugar at that particular carbon atom or a "double inversion" must be effected in order to retain configuration. One synthesis of 5-thio-D-glucose by Whistler[84] which uses a double inversion to maintain configuration whilst directly substituting a sulphur moiety, details the synthesis of a 5,6-anhydro-L-idofuranose derivative in an identical fashion to that described previously. However the anhydro ring then undergoes nucleophilic ring opening with sodium benzoate in dry DMF to give the 6-O-benzoyl-L-idose derivative. The free 5-hydroxyl group is tosylated and displaced with thioacetate in DMF, reinverting the stereochemistry to give a 5-S-acetyl-D-glucose derivative. Following this, conventional acid-catalysed hydrolysis and ring expansion gives 5-thio-D-glucose (scheme 2.).

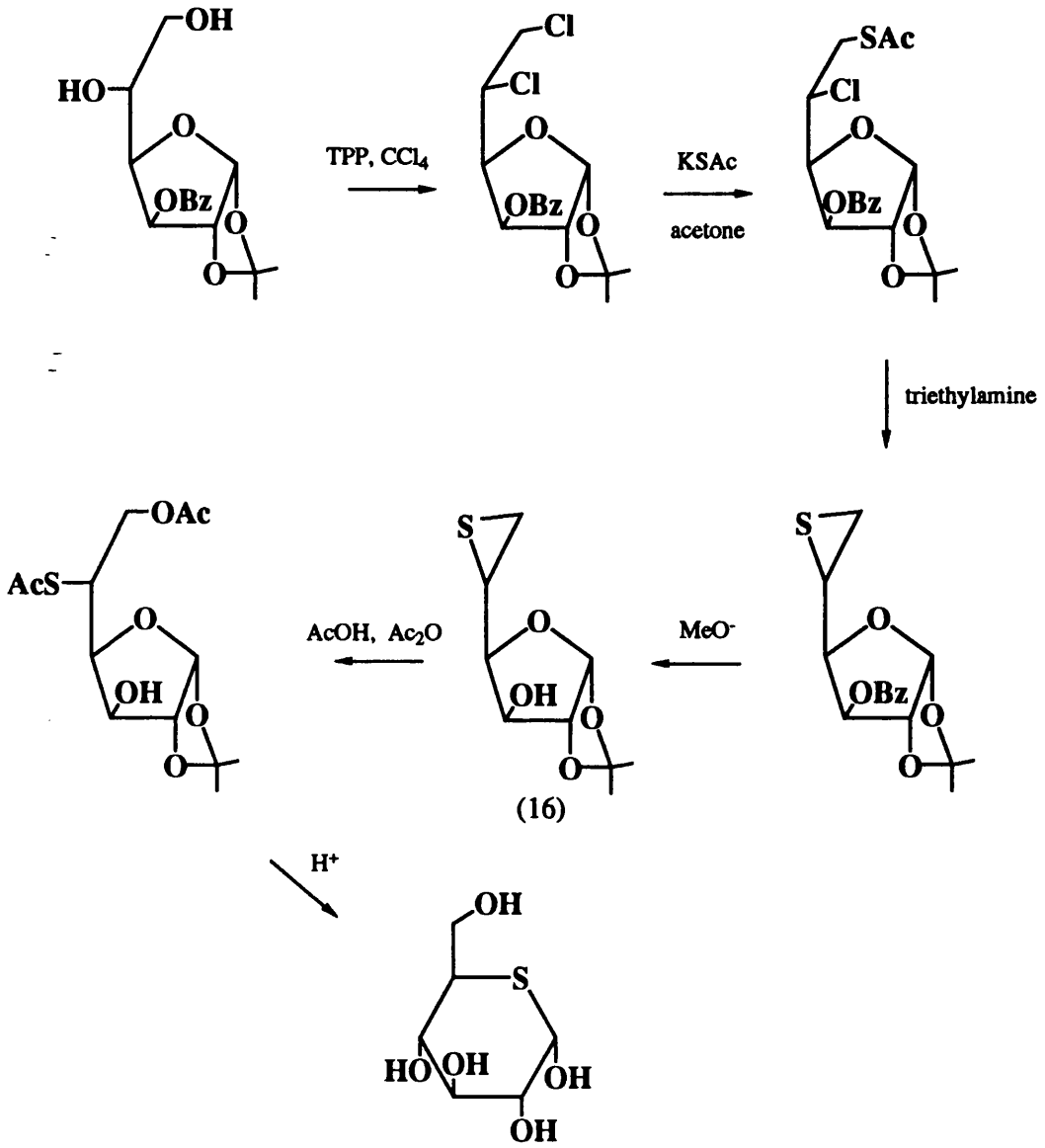
Scheme 2.



Another interesting approach also developed by Whistler[85] uses a chloro sugar intermediate, the chlorine atoms both effecting inversion and acting as leaving groups (scheme 3.). A facile chlorination of the well-characterised isopropylidene derivative 3-O-benzoyl-1,2-O-isopropylidene-α-D-glucofuranose with triphenylphosphine (TPP) in carbon tetrachloride gave 3-O-benzoyl-5,6-dichloro-5,6-dideoxy-1,2-O-isopropylidene-β-L-idose in good yield. Inversion of stereochemistry at asymetric carbon atoms is a feature of chlorination with these reagents.

Selective displacement of the primary chlorine with a single molar equivalent of potassium thioacetate in acetone at 40°C gave the 6-thioacetate. In dry triethylamine this underwent deacetylation, followed by attack of the thiol on C-5 and displacement of 5-Cl to give a 5,6-episulphide.

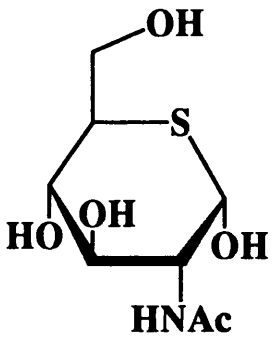
Base-catalysed deacetylation with methanolic potassium hydroxide gave 5,6-dideoxy-5,6-epithio-1,2-O-isopropylidene-α-D-glucofuranose (16). Conventional nucleophilic ring opening with acetate anion was then accomplished according to previous procedure.



Scheme 3.

2.2.2 Literature syntheses of thio analogues of amino sugars

The first report of the preparation of an amino sugar with sulphur in the ring was that of 6-amino-6-deoxy-5-thio-D-glucopyranose.[86] This was followed by a published synthesis[87] of 2-acetamido-2-deoxy-5-thio-D-glucopyranose (17). Perhaps owing to the widespread abundance of N-acetylglucosamine in many biological systems and as a natural product, the 5-thio analogue has since been the subject of much synthetic interest.



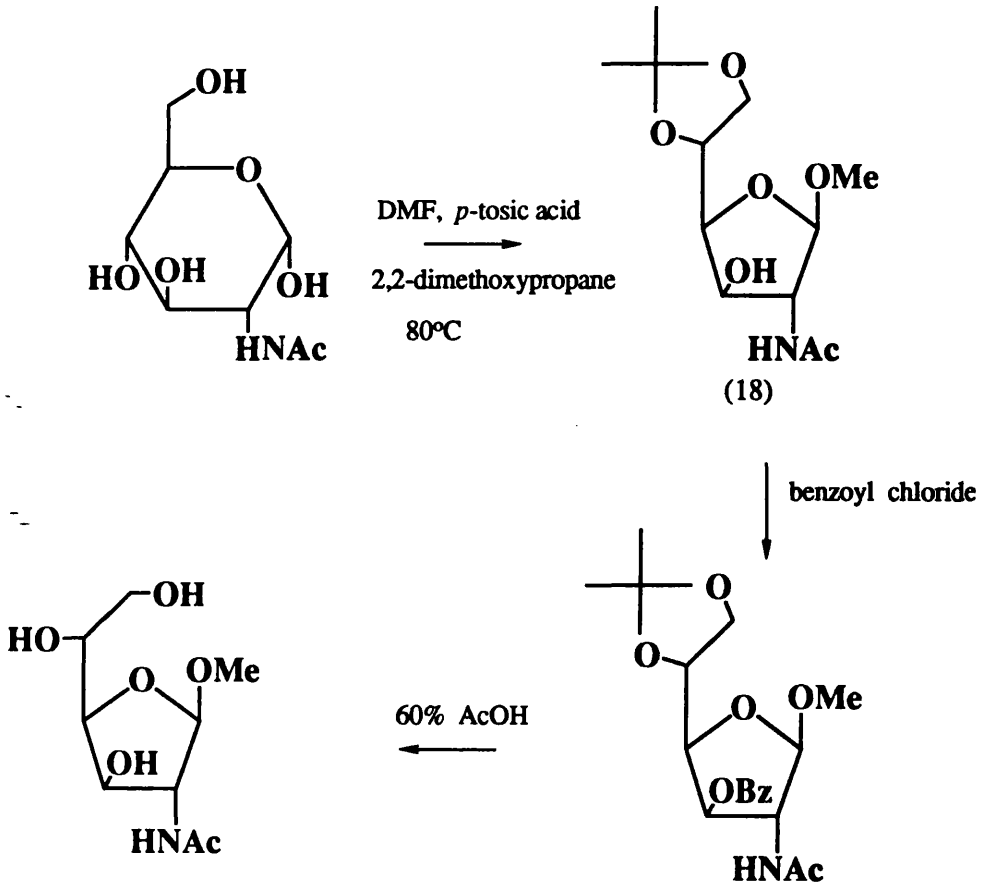
(17)

As with all syntheses involving replacement of the ring heterocycle, access to C-5 requires that the sugar is prepared in either a suitably protected acyclic or furanose form. This poses a particular problem with 2-amino sugars which show a marked preference for the pyranose configuration.

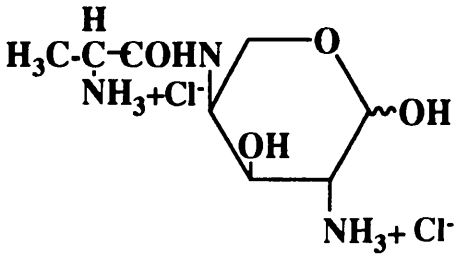
The first reported synthesis of 2-acetamido-2-deoxy-5-thio-D-glucose in 1978 by Hasegawa *et al*[87] used as starting material the glucose derivative, methyl 2-acetamido-2-deoxy-5,6-O-isopropylidene-β-D-glucofuranoside (18), which was readily prepared from 2-acetamido-2-deoxy-D-glucopyranose. Earlier work by Hasegawa[88,89] had shown that several N-substituted 2-amino-2-deoxy-D-aldohexoses react with 2,2-dialkoxypropane-N,N-dimethylformamide-*p*-toluenesulphonic acid at 80-90°C to give 5,6-O-isopropylidene furanosides. The treatment of 2-acetamido-2-deoxy-D-glucose in dry DMF with *p*-toluenesulphonic acid and 2,2-dimethoxypropane at 90°C for 1 hour gives methyl 2-acetamido-2-deoxy-5,6-O-isopropylidene-β-D-glucopyranoside

(18) in 34% yield. (scheme 4.) Likewise the same conditions have produced 2-acetamido-2-deoxy-5,6-O-isopropylidene-α-D-mannofuranoside from 2-acetamido-2-deoxy-α-D-mannopyranose.[88]

Scheme 4.



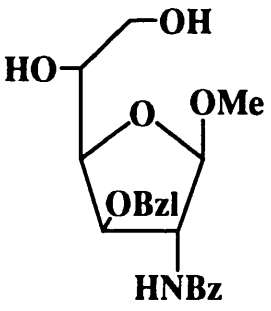
These derivatives have proved to be versatile aminofuranose intermediates and (18) has also been used in the preparation of the amino sugar derivative Prumycin (19).[90,91]



(19)

Hasegawa's synthesis of 2-acetamido-2-deoxy-5-thio-D-glucopyranose (17) involves the benzoylation of (18) at C-3 following which, removal of the 5,6-O-isopropylidene group under mildly acidic conditions gave methyl 2-acetylamine-3-O-benzoyl-2-deoxy-β-D-glucopyranoside. Conversion to the 5,6-epoxide followed by formation of the 5,6-episulphide was identical to the series of transformations discussed earlier in the synthesis of 5-thio-D-glucose. Final deacetylation and ring closure was achieved with sodium methoxide, giving the required product (17) as a crystalline compound. A similar synthesis of 2-acetamido-2-deoxy-5-thio-α-D-mannopyranose by Hasegawa[92] uses the same reaction sequence, but commencing with the mannofuranoside analogue.

This work was quickly followed by Guthrie and O'Shea[93] in 1981, who used the well characterised glucose 1,2-phenyloxazoline derivative as intermediate to the preparation of a methyl glucofuranoside. The development and synthesis of oxazoline derivatives will be discussed later. Following the isolation of the key intermediate methyl 2-benzamido-3-O-benzyl-2-deoxy-β-D-glucofuranoside (20), the pattern of introduction of the sulphur nucleus is identical. The use of a benzyl protecting group at C-3 gave the possibility of its selective removal by hydrogenolysis, allowing the preparation of selectively substituted derivatives at this position if required.



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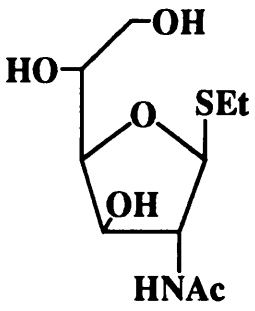
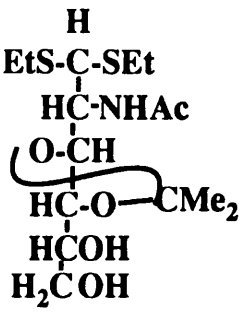


(21)

A further two starting materials were introduced in 1981 by Whistler *et al* in alternative approaches[94], which however retained the traditional sequence of a 5,6-anhydro-episulphide conversion, the first being the cyclic derivative ethyl 2-acetamido-2-deoxy-1-thio-α-D-glucofuranoside (21). The second synthesis, which was reported to be more convenient and to give higher yields, used an acyclic diethyl dithioacetal glucose derivative. This obviated the requirement to lock the sugar in a furanose configuration in order to gain access to C-5. The diethyl dithioacetals are perhaps the most well-known of the acyclic monosaccharide derivatives and have been the subject of extensive review.[95] They are readily prepared by treating the free monosaccharide with ethanethiol in the presence of catalytic amounts of acid (commonly concentrated hydrochloric acid). Synthesis of the 3,4-O-isopropylidene derivative (22) allowed selective access to C-5 and C-6. Again the pattern of thiol introduction was unchanged, going through an anhydro-episulphide conversion.

In 1983, a further approach by Hasegawa[96] utilised an acyclic 2-acetylamine dimethyl acetal derivative, which is conveniently synthesised in high yield from 2-acetamido-2-deoxy-D-glucopyranose itself. In an earlier paper[97] by the same author it was shown that the treatment of 2-acetamido-2-deoxy-α-D-glucopyranose and certain other glucopyranose derivatives, with a large excess of 2,2-dimethoxypropane or 2,2-dibenzyloxypropane and catalytic amounts of *p*-toluenesulphonic acid in 1,4-dioxane solution at 60-70°C yields the corresponding 3,4:5,6-di-O-isopropylidene-*aldehydo*-D-glucose dimethyl acetal in good yield (@ 65%).

This derivative will undergo selective deacetalation with weak acid to the 3,4-O-monoisopropylidene derivative (23), thus allowing access to C-5 and C-6. Once more the conventional sequence of of thiol introduction leads to the synthesis of 2-acetamido-2-deoxy-5-thio-D-glucose in good yield.



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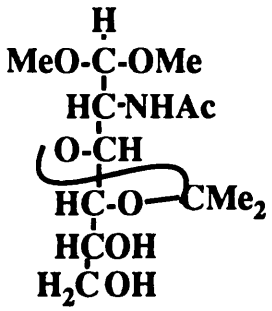
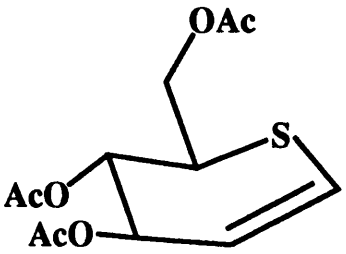


(23)

Only one other synthesis of 2-acetamido-2-deoxy-5-thio-D-glucose is detailed in the literature and this uses 5-thio-D-glucal (24), obtained by oxidation of 5-thio-D-glucose, as starting material.[98] Although the double bond of the 5-thio derivative has been reported to be less reactive than that of the corresponding D-glucal, it underwent ready azidonitration to give 2-azido-1-nitrate addition products. Further treatment with sodium acetate in glacial acetic acid at 100°C gave a mixture of 2-azido-2-deoxy-*manno* and *gluco* tetraacetates in a 1:1.7 ratio. Chromatographic separation and hydrogenation in the presence of acetic anhydride gave the corresponding 2-acetamido monosaccharides.

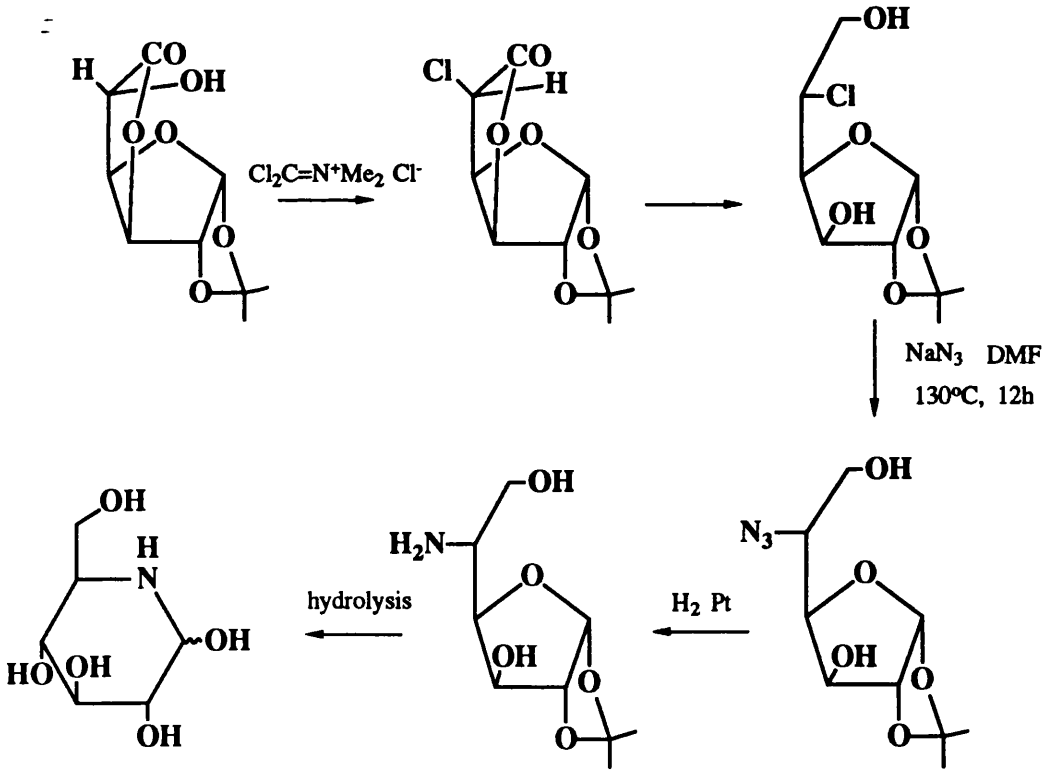


(24)

2.3 An overview of synthetic approaches to nojirimycin

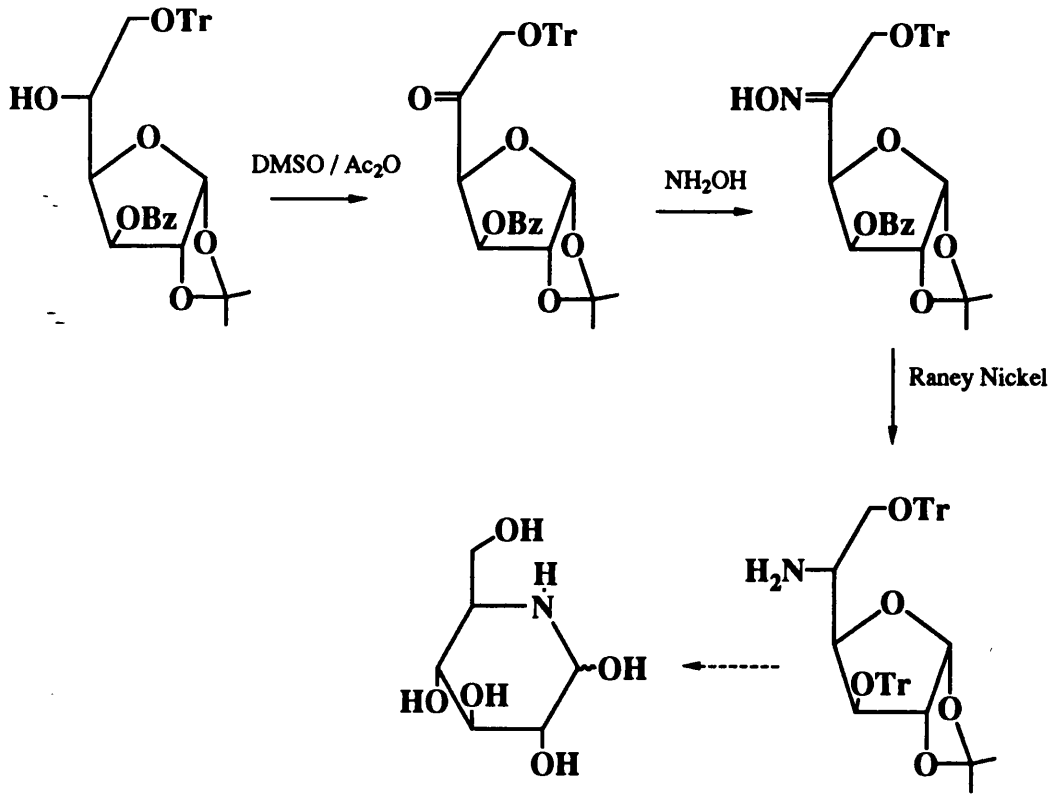
Since the isolation and identification of nojirimycin and its analogues, many different preparations of these compounds have appeared in the literature.[67, 99-103] Most differ in choice of starting material alone, with the placing of an amine precursor at C-5 performed in one of two ways.

The first method uses a direct displacement of a leaving group, generally a sulphonate ester by the nitrogen nucleophile, azide. There is also one preparation of nojirimycin[101] commencing from a glucofuranose derivative where a C-5 chlorine acts as the leaving group and this is shown below (scheme 5.). Again the S_N2 nature of displacement results in inversion of stereochemistry.



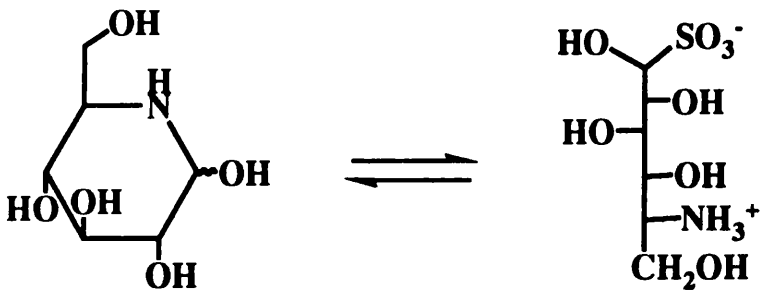
Scheme 5.

The second method involves the formation of an oxime derivative by the treatment of a 5-keto function with hydroxylamine. Normally this requires a C-5 oxidation step followed by oxime formation and reduction. The presence of a bulky substituent at C-6 normally directs the reduction to give a preponderance of the D-*gluco* isomer. This approach was used in the original synthesis of nojirimycin by Inouye *et al*[67] and is outlined below (scheme 6.).



Scheme 6.

Nojirimycin and its analogues are unstable in neutral or acidic conditions and these compounds are normally prepared as acyclic bisulphite adducts which crystallise from aqueous solution saturated with SO_2. The bisulphite adduct can be cleaved with concommitant ring closure to the active compound by alkaline hydrolysis followed by column chromatography on anionic ion exchange resin.



2.3.1 The synthesis of 2-acetamido-2-deoxy-nojirimycin and 2-acetamido-1,2-dideoxynojirimycin

There is only one report in the literature of the synthesis of 2-acetamido-2-deoxynojirimycin which appears together with a synthesis of 2-acetamido-1,2-dideoxynojirimycin. This publication[75] details a 10 step synthetic route commencing with 2-acetamido-2-deoxy-D-glucose which was converted to its methyl 1,2-oxazoline derivative according to the method of Mack.[104] From the corresponding methyl furanoside, the 5,6-O-isopropylidene ring was opened and the primary hydroxyl group underwent tritylation. The amine precursor was introduced by oxidation at C-5 followed by treatment with hydroxylamine. Stereoselective reduction of the resulting oxime with Raney nickel was reported as giving 95% of the D-*gluco* isomer.

Final hydrolysis and formation of the bisulphite adduct was found to be problematic owing to the stabilising effects of the amide on the glycosidic bond. The group's final successful method was the use of trifluoromethanesulphonic acid to form the 1-O-trifluoromethanesulphonate, followed by treatment with SO_2 and eventual recovery of the bisulphite by lyophilisation.

2.4 The use of deoxyhalogeno intermediates in the synthesis of deoxy, thio and amino sugars

Deoxyhalogeno sugars have found use as versatile precursors to a number of biologically important sugars, many of which are vital constituents of antibiotics[105]. In addition, halogenated antibiotics and especially nucleosides have in many cases shown enhanced biological or chemotherapeutic effects.[106] In the field of nucleotide synthesis, the utility of the glycosyl halides in coupling reactions is well-documented.[107] The substitution of chlorine into sucrose and several other sugar derivatives has sometimes conferred enhanced sweetening properties and resulted in considerable commercial interest.[108]

The carbon-halogen bond is susceptible to nucleophilic displacement by a variety of nucleophiles, the products of which may undergo further chemical modification to useful compounds.[109] This bond is also readily reduced with lithium aluminium hydride to the corresponding deoxy sugar.[110] Therefore, the development of new halogenating reagents and conditions has been the subject of some research.

2.4.1 Direct halogenation reagents for carbohydrates

The literature on carbohydrate halogenation has been comprehensively reviewed[111] on carbohydrate halogenation, but the main reagent categories which involve the direct replacement of hydroxyl groups by halogens will be briefly outlined.

Sulphuryl chloride-pyridine[112,113] was one of the earliest direct halogenating reagents to be developed and is considered to act by the primary formation of a sulphonyl chloride ester which then undergoes displacement by chloride anion. The reagent exhibits little selectivity between primary and secondary hydroxyl groups and often gives rise to by-products such as cyclic sulphates, anhydrides and elimination products. In order to avoid side-reactions, temperature must be very strictly controlled.

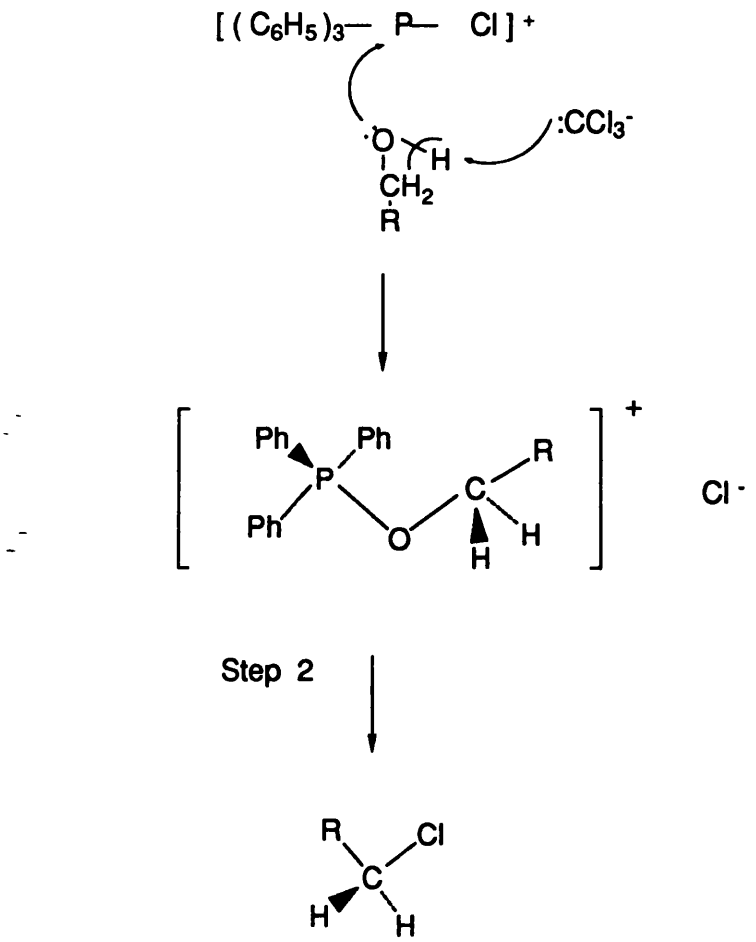
Methanesulphonyl chloride - DMF[114] as a chlorinating agent generally gives only moderate yields of chlorinated derivatives at best. It does display some selectivity for primary hydroxyl groups[115], but formylation of secondary and hindered hydroxyl groups is a common side reaction.

Triphenylphosphine (TPP) - N-halosuccinimide - DMF has been shown to give good to moderate yields of halogenated derivatives at primary hydroxyl groups in various carbohydrates[116,117]. However acetal migration has been noted with this reagent mixture[118] and purification is often difficult.

TPP-carbon tetrahalide has been used for the successful replacement of both primary and secondary hydroxyl groups by halogen, proceeding in a variety of solvents. It is a simple, mild method proceeding under essentially neutral conditions over a relatively wide temperature range (5-70°C).[119,120] The mechanism of this halogenating reagent has recieved considerable attention. Studies[120] have suggested that the reaction proceeds in two steps, shown in scheme 7. The first step involves the formation of a complex between triphenylphosphine and carbon tetrahalide. This then reacts with an alcohol to give the ion-pair intermediate with phosphorus tetrahedrally co-ordinated. Dissociation of the ion-pair gives the alkyl halide with inversion of configuration, but its mechanism is dependent upon solvent polarity. The ion-pair formed between TPP, neopentyl alcohol and carbon tetrachloride has been shown to decompose bimolecularly in acetonitrile and unimolecularly in deuteriochloroform. However, the use of TPP in CCl_4 has in some instances been associated with acetal migration: the attempted chlorination of 1,2:5,6-di-O-isopropylidene-α-D-glucofuranose resulted in the rearrangement of the 5,6-acetal to the 3,5-acetal with chlorination at C-6.[119]

Step 1

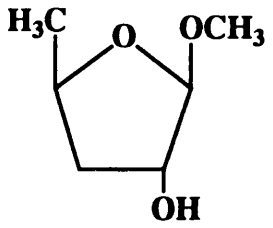
$$(C_6H_5)_3P + CCl_4 \longrightarrow [(C_6H_5)_3P\text{-}Cl]^+ : CCl_3^-$$



Step 2

Scheme 7.

There is one report[121] of oligomerisation taking place on treatment with this reagent mixture. The attempted chlorination of C-2 in methyl 3,5-dideoxy-β-D-erythropentofuranoside (25) with TPP in CCl_4 gave none of the expected 2-chloro derivative, but a mixture of 1-2 linked oligosaccharides, terminated by a 2-chloro-2,3,5-trideoxy-α-D-threo-pento-furanosyl unit. At least two mechanisms, each involving isomerisation of a phosphonium group between O-1 and O-2 have been proposed to explain this oligomerisation.



(25)

The use of pyridine as a solvent with TPP and a carbon tetrahalide, usually tetrachloride or tetrabromide, has markedly improved the utility of this reagent in terms of speed, selectivity and yield[122]. Both primary and secondary hydroxyl groups may be halogenated and improved yields have been achieved by optimising the molar ratio of TPP and carbon tetrahalide. In most cases temperature control provides a means of selectively halogenating primary hydroxyl groups. This improved selectivity has been associated with a bulky halogenating complex formed from triphenylphosphinedihalide and pyridine.

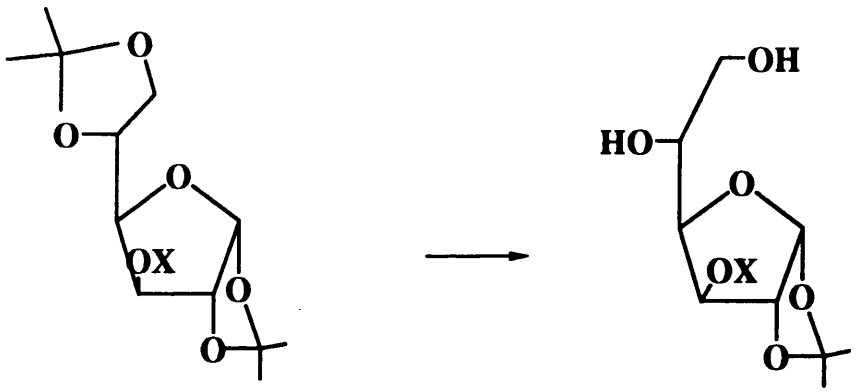
It is noteworthy that these chlorination conditions have not been associated with acetal migration and attempted chlorination at C-3 in 1,2:5,6-di-O-isopropylidene-α-D-glucofuranose simply gave no reaction.[122]

2.5 The development of novel routes to 2-acetamido-2-deoxy-5-thio-D-glucose

Despite the interest in 2-acetamido-2-deoxy-5-thio-D-glucose, there are no reports in the literature of the N-deacetylation of this compound to the free amine, or of any other amino derivatives of this sugar. Preparation of this particular compound was of great interest and it was decided to attempt its synthesis if possible by an alternate route, one which would perhaps allow the synthesis of 2-acetamido-5-amino-2,5-dideoxy-D-glucopyranose from a common intermediate. The known versatility of the deoxyhalogeno compounds in this regard made them intermediates of choice. In glucofuranose derivatives, the displacement of a C-5 chlorine has been demonstrated with both azide[101] and thioacetate[85] anions.

2.5.1 - Model studies with 1,2-O-isopropylidene derivatives

To test the viability of this approach, a model series of compounds were prepared in order to attempt some trial displacements. It was decided to use 1,2:5,6-di-O-isopropylidene-α-D-glucose (26) as starting material which was conveniently prepared from D-glucose according to the method of Schmidt.[123] At this stage, acylation will give the corresponding 3-O-substituted derivative and a quantity of the 3-O-acetate (27) was prepared by conventional acetylation with acetic anhydride in pyridine. The two isopropylidene groups of this derivative are known to display different hydrolysis velocities and selective removal of the 5,6-O-isopropylidene group was readily accomplished[123] in 60% aqueous acetic acid at room temperature. Thus, both 1,2-O-isopropylidene-α-D-glucofuranose (28) and its 3-O-acetate (29) were prepared as model compounds for chlorination investigations.

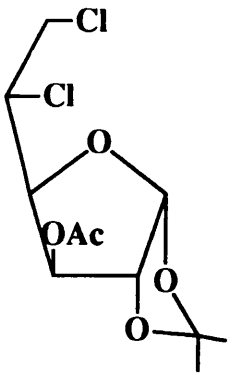


(26) X = H
(27) X = Ac

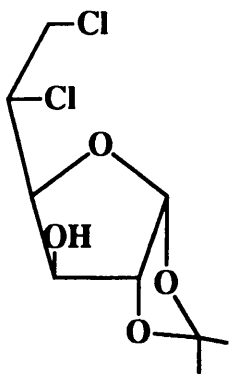
(28) X = H
(29) X = Ac

TPP and CCl_4 in anhydrous pyridine was judged to be the most suitable reagent to effect chlorination of both primary and secondary hydroxyl groups, with a minimum of side-reactions. Earlier work by Khan *et al* has established that a favourable reagent ratio for a proposed dichlorination was 6 molar equivalents of TPP and 3 molar equivalents of CCl_4.[108]

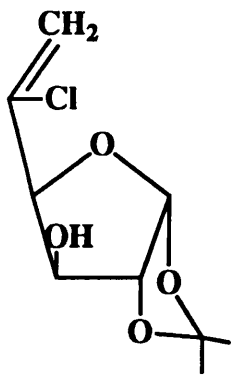
Established reaction conditions were followed and the 3-O-acetate derivative (29) reacted smoothly to give 3-O-acetyl-5,6-dichloro-5,6-dideoxy-1,2-O-isopropylidene-β-L-idofuranose (30) in 81% yield. In (28) the well-documented unreactivity of C-3 to these chlorination conditions was confirmed,[122] giving 5,6-dichloro-5,6-dideoxy-1,2-O-isopropylidene-β-L-idofuranose (31) as expected in 65% yield. Spectral and microanalytical data were in full agreement with the structures assigned to (30) and (31).



(30)



(31)



(32)

It is difficult with the more flexible furanose derivatives to definitively establish inversion of configuration at C-5 by ^{1}H NMR. However proof that (30) possesses the L-*ido* configuration can best be achieved by its subsequent conversion via a 6-thioacetate to the known D-*gluco* episulphide. As the formation of the episulphide must also take place with inversion of configuration, the overall retention of stereochemistry establishes that the corresponding 5-chloro sugar must have been an L-idose derivative.

From each reaction mixture, a small amount (<10%) of a second less polar product was isolated by chromatography. Mass spectroscopy revealed a pseudomolecular ion as a 3:1 doublet, 36 mass units below that expected for the dichloro compound. This was identified in each case as the elimination product. It was noticed that yields for the dichlorination of the 3-hydroxy derivative are significantly less than those of the 3-acetate derivative, this was attributed to a greater amount of the elimination product (32) as well as to significantly more breakdown. With this in mind it was decided to continue the study with the 3-acetate derivative alone.

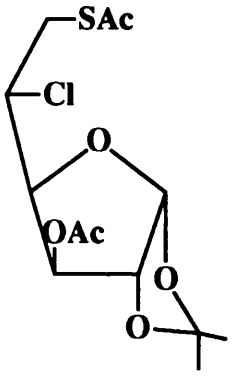
2.5.2 The reactions of 3-O-acetyl-5,6-dichloro-1,3-O-isopropylidene-β-L-furanose with sulphur-containing nucleophiles

In Whistler and Chiu's synthesis of 5-thio-D-glucose,[85] the selective displacement of the primary chloro group with thioacetate was reported to occur from treatment in dry acetone at 40°C with 1 molar equivalent of potassium thioacetate. These conditions were repeated with (30) and gave 3-O-acetyl-6-S-acetyl-5-chloro-5,6-dideoxy-1,2-O-isopropylidene-β-L-idofuranose (33) in 65% yield following chromatography, which matches closely the yields obtained by Whistler for the 3-benzoate analogue. ^{1}H-NMR showed the presence of a single thioacetate peak at δ 2.21, whilst mass spectrometry revealed a pseudomolecular ion at 356 and 358 $(M+NH_4)^+$ as a 3:1 doublet.

It seemed of interest at this stage to examine the reaction of the 5,6-dichloro derivative with another commonly-used sulphur-containing nucleophile, the thiocyanate anion. To a solution of the monosaccharide (30) in dry DMF was added potassium thiocyanate (10 molar equivalents) and the temperature gradually increased until t.l.c. indicated the formation of another more polar compound. Heating was maintained at 90°C for 48 hours, at this point no starting material remained. Following workup, a crystalline compound was isolated, the i.r. spectrum of which showed a strong absorption at 2160cm^{-1}, indicative of substitution by thiocyanate. In the identification of thiocyanates and isothiocyanates, the i.r. spectrum is frequently more informative than ^{1}H-NMR, thiocyanates being characterised by a sharp, medium-strong band in the range 2175-2100cm^{-1}. In contrast, isothiocyanates show a strong double band in the range 2020-1990cm^{-1}. Thermal rearrangements of thiocyanates to isothiocyanates are well-documented,[124] but this was not observed under these conditions.

C.I. mass spectrometry revealed strong pseudomolecular ions of m/z 339 and 341 as a 3:1 doublet, indicating the presence of one chlorine atom. As for the 6-thioacetate derivative (31), the ^{1}H-NMR spectrum of the thiocyanate showed an upfield shift of signals corresponding to H-6 and H-6', (0.67ppm for H-6', 0.3ppm for H-6) but with negligable change for H-5. It was therefore concluded that displacement of the primary chlorine had occurred as expected and the compound was identified as

3-O-acetyl-5-chloro-5,6-dideoxy-1,2-O-isopropylidene-6-thiocyano-β-L-idofuranose (34). Microanalytical results were consistent with this assignment. The yield of the monothiocyanate was in excess of 90% and being crystalline was easily purified. This compared very favourably with the preparation of the 6-thioacetate which was oily and contaminated with both starting material (10%) and dithioacetate (14%).



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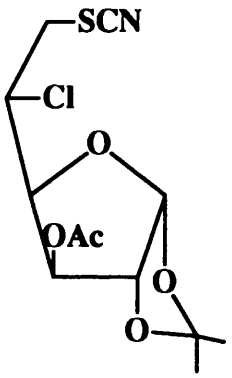
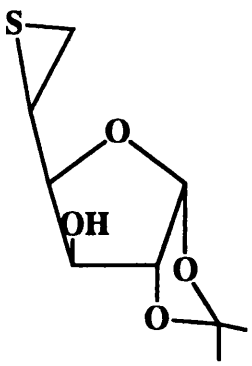


(34)



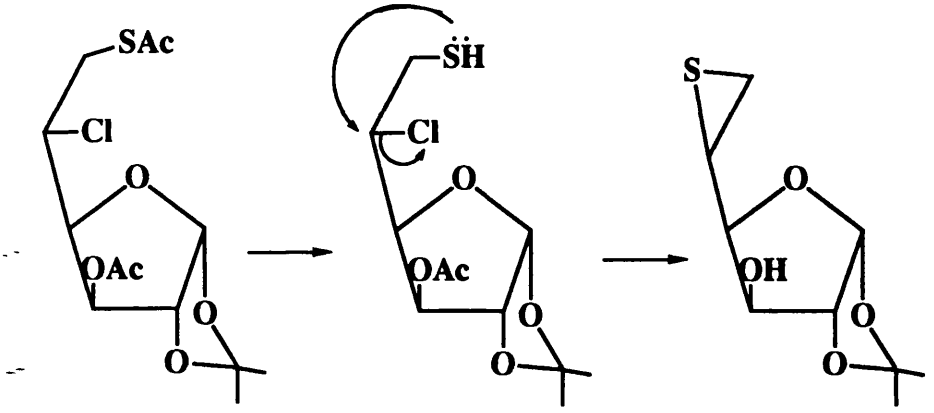
(35)

The reactivity of the 5-chloro substituent to thiocyanate was assessed by changing the reaction conditions. The compound in dry DMF was again treated with 10 molar equivalents of potassium thiocyanate, and was heated at 140°C for 48 hours but even under these drastic conditions the only product isolated was the 6-thiocyanate. At temperatures above 140°C, a steady discolouration and decomposition was observed.

Since nucleophilic displacements with certain leaving groups such as sulphonates have been reported to take place more efficiently in the presence of crown ethers,[125] reaction of the 5,6-dichloro derivative with a 10 molar excess of potassium thiocyanate at 140°C was repeated with the inclusion of 18-crown-6. The reaction time was noticeably shortened but nothing corresponding to a 5,6-dithiocyano derivative could be found. Substitution with thiocyanate, at least in DMF, would therefore seem to be completely selective for the primary position. As Whistler has shown the C-5 chlorine to be readily replaceable with thioacetate anion[85] this was unexpected but perhaps reflects the greater nucleophilicity of thioacetate anion over thiocyanate anion.

2.5.3 The formation of an episulphide derivative

Formation of the 5,6-episulphide from 3-O-acetyl-6-S-acetyl-5-chloro-5,6-dideoxy-1,2-O-isopropylidene was carried out according to the method of Whistler.[85] As described earlier, de-S-acetylation with strong base liberates the free thiol which under these conditions attacks at C-5 with the displacement of the 5-chloro function and reinversion to give a D-*gluco* episulphide.



Treatment of a methanolic solution of the 6-S-acetyl derivative with dry triethylamine at 40°C, overnight, as with the literature synthesis, resulted in a good yield (75%) of an episulphide, 5,6-dideoxy-5,6-epithio-1,2-O-isopropylidene-α-D-glucofuranose (16), but with the concommitant loss of the 3-O-acetate function. This key intermediate was identical to that described by Whistler, with a satisfactory ^{1}H-NMR, melting point and microanalysis.

Formation of the same intermediate from the 6-thiocyanate was considered. There are various methods documented in the literature for the reduction of monosaccharide thiocyanates to thiols.[124,126] It was anticipated that any attempted isolation of the free thiol could prove disadvantageous as many monosaccharide thiols are readily oxidised to their disulphide derivative even by exposure to the atmosphere.[126] Reduction with sodium sulphide[124] was attempted but following treatment of the thiocyanate with an aqueous sodium sulphide solution over 24 hours, t.l.c still indicated the presence of some starting material, together with the formation

of two distinct products which were difficult to separate. The mixture was extracted with chloroform and evaporated to a syrup, but i.r. spectroscopy did not reveal any thiol absorbance at 2550cm^{-1} and it was concluded oxidation and perhaps some breakown had taken place.

Zinc-acetic anhydride in acetic acid[126] was then considered as a means of reducing the thiocyanate coupled with *in situ* conversion to the stable thioacetate. However this was considered to offer few advantages over the direct preparation of the thioacetate. Sodium methoxide in methanol[124] is known to be effective in the reduction of thiocyanates to thiols and it was anticipated that such strongly basic conditions could also bring about episulphide formation. The addition of sodium methoxide in dry methanol at 0°C was monitored by t.l.c and first gave rise to deacetylation at C-3; this was followed by the slow formation of a second product isolated in 75% yield. ^{1}H-NMR and mass spectrometry showed this to be 5,6-dideoxy-5,6-epithio-1,2-O-isopropylidene-α-D-glucofuranose (16), identical to that prepared earlier.

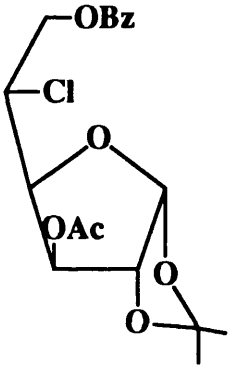
From this intermediate, Whistler's publication[85] details the synthesis of 5-thio-D-glucose in a further two steps: reaction with potassium acetate in acetic acid-acetic anhydride (1:10) to give the 5-S-acetyl derivative followed by acetolysis to give 1,2,3,4,6-penta-O-acetyl-5-thio-α-D-glucopyranose. The synthesis of the 6-thiocyanate (32), followed by its methoxide reduction with concommitant episulphide formation would appear to represent a shorter and more convenient route to the preparation of this key episulphide (16) with improved yields.

2.5.4 Studies towards the development of a common intermediate for both azido and thio sugar synthesis

As many syntheses of 5-amino sugars employ a displacement at C-5, the chloro sugar was interest, but in order to achieve a single substitution at C-5, the production of a 5-monochloro derivative becomes necessary. It was therefore decided to prepare 3-O-acetyl-6-O-benzoyl-5-chloro-5-deoxy-1,2-O-isopropylidene-β-L-idofuranose (36). This could be achieved by two routes, either:

(i) The synthesis of the dichloro compound followed by selective displacement of the primary chloro group with benzoate anion.
or
(ii) The selective benzoylation of the diol, followed by chlorination at C-5.

Initially route (i) seemed most favourable, however both routes were attempted so that yields could be compared.



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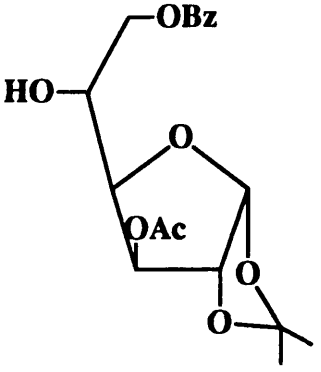


(37)

Displacement of 6-Cl of (30) in DMF at 120°C with benzoate anion proceeded unexpectedly poorly, requiring long periods of time and resulting in a significant degree of breakdown. By contrast, the selective benzoylation of 3-O-acetyl-1,2-O-isopropylidene-α-D-glucofuranose (29) in dry pyridine at -40°C afforded the monobenzoate (37) in 85% yield, displaying a high degree of selectivity. Halogenation with TPP (6 molar equivs) and CCl_4 (3 molar equivs) gave after chromatography 64%

overall yield of the desired derivative (36). ^{1}H-NMR, mass spectroscopy and microanalysis were in agreement with the assigned structure.

This derivative (36) was heated at 90°C with potassium thioacetate (3 molar equivalents) in dry DMF for 24 hours. Following workup and chromatography, the bulk of the starting material was recovered unchanged. This was confirmed by mass spectroscopy where no molecular or pseudomeolecular ions corresponding to 3-O-acetyl-5-S-acetyl-6-O-benzoyl-5-deoxy-1,2-O-isopropylidene-α-D-glucofuranose were seen. A similar reaction was attempted with azide anion, and again heating at 130°C for 48 hours in DMF did not result in any discernable substitution.

These conditions are considerably more severe than was required for the displacement of the C-5 chlorine on the dichloro derivative (30), and are at least parallel to the conditions employed for displacement of a C-5 chlorine by azide, in Klemer's synthesis of nojirimycin.[101] As a result it could be speculated that the presence of the bulky benzoate group at C-6 gives rise to considerable steric hindrance, thus preventing reaction. It was decided not reattempt these displacements following cleavage of the 6-benzoate as there was sufficient literature precedent to continue however the possible inhibitory effects of a bulky substituent at C-6 were noted. It was considered that a 5-S-acetate should easily undergo acetolysis and ring-expansion as in Whistler's synthesis[85] to afford in one step, 1,2,3,4,6-penta-O-acetyl-5-thio-D-glucopyranose while a corresponding azide derivative is analogous to 5-azo intermediates in published syntheses of nojirimycin and 1-deoxynojirimycin. Catalytic hydrogenation or lithium aluminium hydride reduction to afford the amine, followed by the formation of the bisulphite should then proceed as previously documented.[100,101,103]

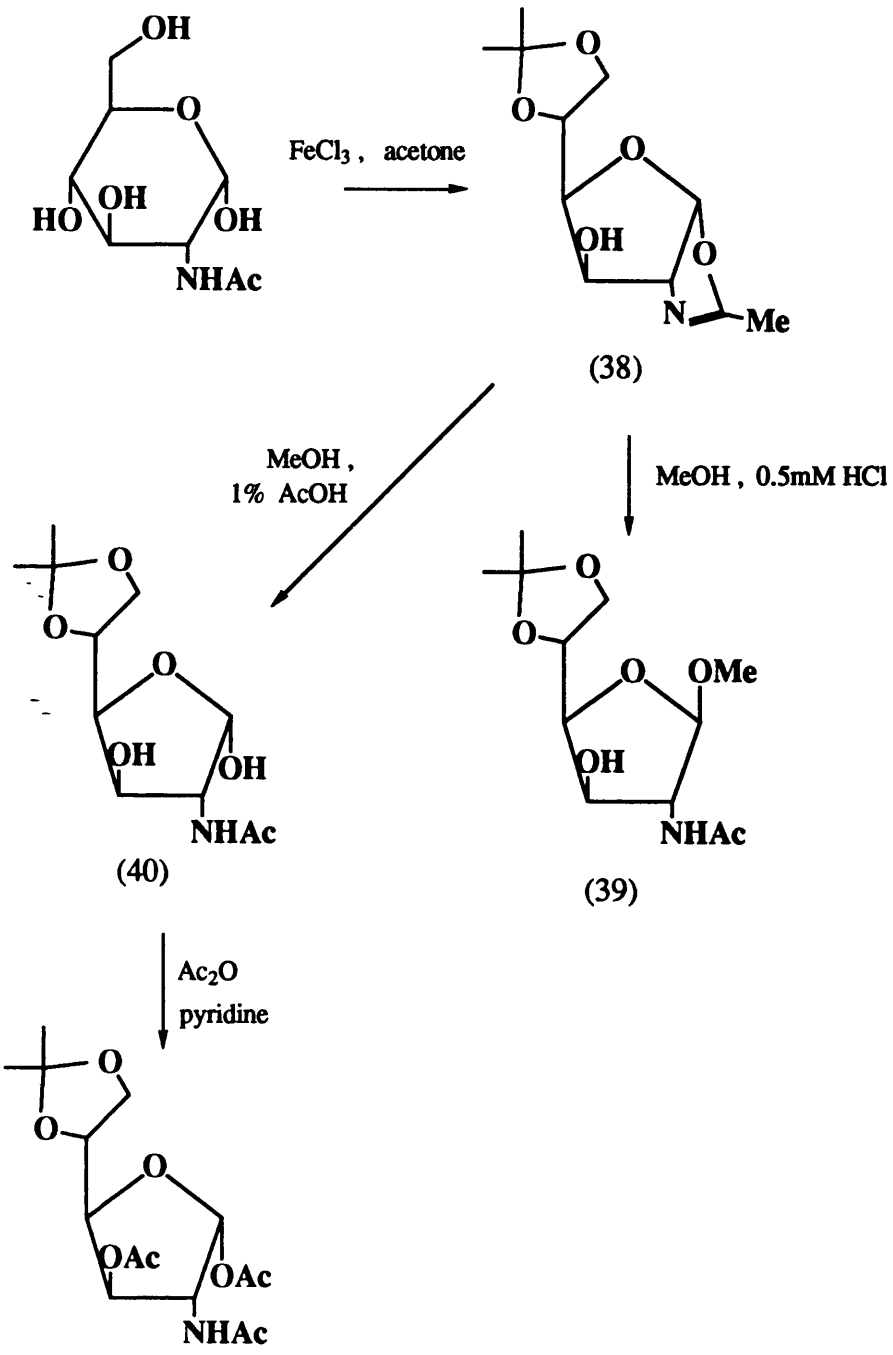
The use of chlorine intermediates both at the 5 and 6 positions of a furanose derivative was considered to be a viable approach to the incorporation of both sulphur and nitrogen heteroatoms at C-5 with overall retention of configuration. It was therefore decided to select a suitable 2-amino precursor and attempt a similar series of transformations.

2.6 The development of novel synthetic routes for 2-acetamido-2-deoxy-5-thio-D-glucose

Previous literature syntheses of 2-acetamido-2-deoxy-5-thio-D-hexoses reviewed earlier, had identified a number of suitable starting materials, both cyclic furanosides and acyclic derivatives. The synthetic sequences developed with the model series while potentially applicable to all these intermediates, had been investigated with a cyclic furanose derivative, so it was decided in the first instance to choose one of the known 2-acylamino furanosides as starting material. In terms of yield and versatility, methyl furanosides obtained from the well-characterised phenyl oxazoline derivatives seemed a good choice.

2.6.1 Oxazoline derivatives of 2-acylamino monosaccharides

The two most common oxazoline derivatives described in the literature are the phenyl 1,2-oxazolines and the methyl 1,2-oxazolines. The preparation of methyl 1,2-oxazolines was first described by Mack *et al*[104] who showed that the treatment of 2-acetamido-2-deoxy-D-glucopyranose with strong Lewis acids such as $FeCl_3$ in acetone at 80°C gave methyl-(2-deoxy-5,6-O-isopropylidene-α-D-glucofurano)-Δ^2-oxazoline (38) in 62% yield. Treatment of this derivative with 0.5mM methanolic HCl gives the corresponding β-methyl furanoside (39); this derivative was used as starting material by Kappes and Legler for their preparation of 2-acetamido-2-deoxy-nojirimycin. Interestingly Mack has also demonstrated that treating the methyl oxazoline (38) with cold methanol containing 1% acetic acid yields 2-acetamido-2-deoxy-5,6-O-isopropylidene-D-glucofuranose (40) which will undergo conventional acetylation to give largely the α-1,3-diacetate (41). (scheme 8)

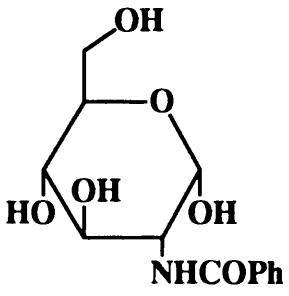

OH
O
HO
OH
OH
NHAc
FeCl3 , acetone
O
O
O
OH
O
N
Me
(38)
MeOH ,
1% AcOH
MeOH , 0.5mM HCl
O
O
O
OH
OH
NHAc
(40)
O
O
O
OMe
OH
NHAc
(39)
Ac2O
pyridine
O
O
O
OAc
OAc
NHAc
(41)

Scheme 8.

The synthesis of glucofuranose 1,2-phenyloxazolines from 2-benzamido-2-deoxy-D-glucopyranose (42) was first reported in 1959 by Zervas and co-workers.[127] He also showed that these oxazolines could be converted to β-methyl furanosides by the action of catalytic amounts of acid in methanol. The versatility of these oxazoline derivatives has been demonstrated by Gigg and Warren[128] who have prepared 2-amino-2-deoxy-D-allose, 2-amino-2-deoxy-D-ribose and 2-amino-2-deoxy-D-xylose from 1,2 and 2,3-phenyl oxazolines. The formation of a 2,3-phenyl oxazoline with concommitant inversion of configuration at C-3 was achieved by warming a solution of a 3-mesyl 2-benzamido derivative and forming a 2,3-oxazoline, the partial hydrolysis of which gave a readily separable mixture of allose derivatives.

2.6.2 The preparation of some phenyl 1,2-oxazoline derivatives

2-Benzamido-2-deoxy-D-glucopyranose (42) was prepared in high yield from D-glucosamine HCl (15) according to the method of Gigg and Warren[128]. Following this a suspension of dry, powdered 2-benzamido-2-deoxy-D-glucopyranose (42) was stirred for approximately 12 hours at room temperature in a 0.7M solution of HCl in dry acetone. Following neutralisation with gaseous ammonia and filtration, the highly crystalline 1,2-phenyloxazoline was easily isolated by fractional crystallisation from ethyl acetate-petrol. Yields are generally only moderate (@ 40%), but the availability and low cost of glucosamine hydrochloride allows the preparation of large quantities and without the need for chromatography.



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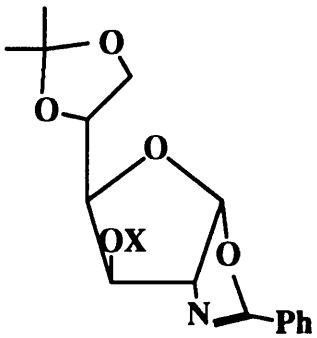


(43) X = H

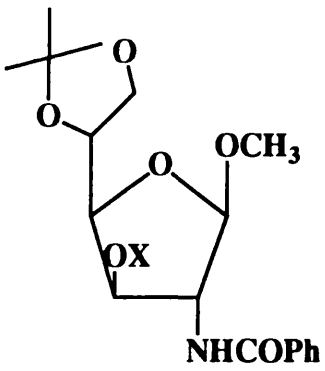
(44) X = Ac

(45) X = Bz

2.6.3 Preparation of the methyl furanoside

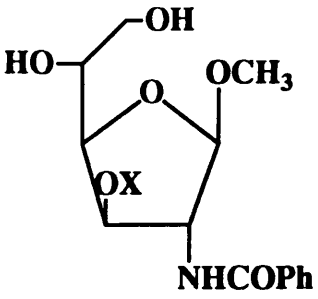
Owing to the lower yields observed with the model series from the chlorination of compounds with a free hydroxyl group at position 3, it was decided to protect this position by esterification. Both the acetate (44) and the benzoate (45) were prepared and were subjected to the glycosidation conditions set out by Guthrie and O'Shea. However although on standing at room temperature in a solution of 0.5mM HCl in methanol, t.l.c. indicated the slow formation of the glycoside, many days were required to achieve complete reaction. This was especially true for the 3-benzoyl oxazoline (45) for which the complete reaction of a 1g quantity took 5 days, whereas Guthrie and O'Shea[93] report only a 24 hour timecourse for large quantities of an analagous 3-benzyl oxazoline.

In an attempt to remedy this, the concentration of HCl solution was increased to 1.0mM with only slight improvements in reaction time. Further increases in acid concentration resulted in a substantial amount of breakdown. It was thought that perhaps the poorer solubility in methanol of these derivatives, especially the benzoate, was in part to blame. However considerable improvements were found when the reaction mixture (0.5mM HCl) was allowed to stir at 40°C which brought the reaction time to within 48 hours for 1g quantities of both esters. It is of course difficult to rule out the possibility of contamination with some alkaline substance, neutralising the trace quantities of acid. However the substantial improvements in reactivity brought about by warming would tend to imply that this is not the case.



(46) X = Ac

(47) X = Bz



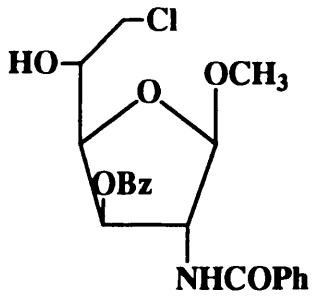
(48) X = Ac

(49) X = Bz

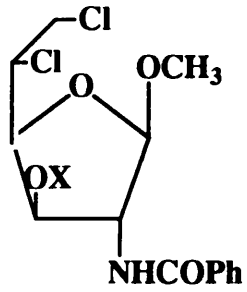
The synthesis of both methyl furanosides (46) and (47) were followed by a selective deacetalation with cold 60% aqueous acetic acid to give the 5,6-diol. Yields of methyl 3-O-acetyl-2-benzamido-β-D-glucofuranoside (48) were only moderate owing to an acid-catalysed de-esterification, however the more acid-stable benzoate derivative gave an almost quantitative yield of the diol (49).

2.6.4 Chlorination reactions of oxazoline-derived β-methyl furanosides

The chlorination of methyl 2-benzamido-3-O-benzoyl-2-deoxy-α-D-glucofuranoside (49) was attempted in an identical fashion to the chlorination of the isopropylidene derivative 3-O-acetyl-1,2-O-isopropylidene-α-D-glucofuranose (29) described earlier. Following recommended reagent quantities, 6 molar equivalents of TPP and 3 molar equivalents of carbon tetrachloride in dry pyridine were used. After 30 minutes at 0°C, the reaction mixture was gradually warmed and at 40-50°C t.l.c indicated a complete loss of starting material and the formation of a single less polar spot, corresponding to the formation of the 6-monochloro derivative. A quantity of the reaction mixture was removed, quenched with methanol and workup gave methyl 2-benzamido-3-O-benzoyl-6-chloro-2,6-dideoxy-β-D-glucofuranoside (50) as a syrup.



(50)



(51) X = Bz

(52) X = Ac

Further heating at 65-70°C resulted in the loss of this material and the simultaneous formation of a further fast-moving spot, presumed to be the dichloro compound and two polar spots with a lower Rf than the starting material. These were far stronger and upon quenching with methanol and the usual workup, the dichloro derivative methyl 2-benzamido-3-O-benzoyl-5,6-dichloro-2,5,6-trideoxy-α-L-idofuranoside (51) was isolated in only 8% yield contaminated with a slightly less polar product which from the mass spectrum was tentatively considered to be the elimination product. The empirical structure assigned to (51) agreed with mass spectral and microanalytical data. A similar pattern of reaction was observed with the 3-O-acetate derivative (48), which gave only small amounts of methyl 2-benzamido-3-O-acetyl-5,6-dichloro-2,5,6-trideoxy-α-D-idofuranoside (52). It was decided to attempt chlorination with only 3 molar equivalents of TPP, in the hope that smaller quantities of reagent would bring about a more gentle reaction but as the temperature was increased, extensive decomposition was observed as before and after 45 minutes, there remained a mixture of mono and dichloro sugars.

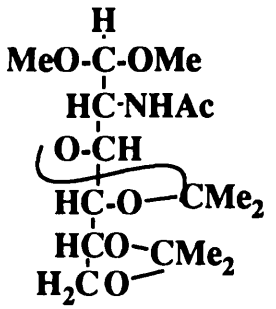
From the aqueous extract a large amount of yellow syrup was obtained, which was only sparingly soluble in organic solvents. From t.l.c (ethanol-ammonia 9:1) there appeared to be one major component with a mixture of minor spots. FAB mass spectrometry showed a number of peaks in the range 800-1000 amu and the possibility of oligomerisation was considered. This was considered unlikely however as the possibility of an isomerisation between C-5 and C-1 seemed remote.

Further investigations into this anomalous reaction were instituted at a later date. Treatment of the polar extract with cold 1M HCl for several days which would be expected to cleave any intermolecular bonds did not bring about any noticeable change in the t.l.c. An attempted acetylation brought about a suprising shift in polarity with the production of one fast-moving spot. The crude acetylation mixture was examined by mass spectrometry however no firm conclusions could be drawn and the precise nature of the polar product produced by the attempted dichlorination remains a mystery, however a possible attack at C-1 by TPP to produce some form of phosphonium intermediate which may then undergo further reaction, cannot be ruled out.

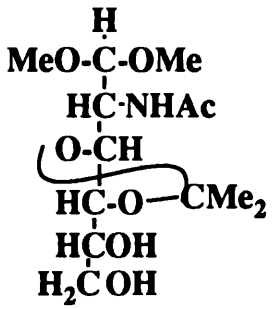
The poor yield of (47) cast doubt on these particular derivatives as intermediates in this route and it was considered that any attempted chlorination of C-5 alone would prove equally unfavourable, if concurrent attack at the β-methyl group indeed takes place. It could be speculated that the preparation of the 1,3-diacetate (41) derived from a methyl 1,2-oxazoline following deacetalation would perhaps prove more stable to these chlorination conditions, though time did not allow this to be further investigated. In the light of these findings it was decided to change to an acyclic series of intermediates and attempt the same series of transformations.

2.6.5 Acyclic dimethylacetal derivatives of 2-acylamino monosaccharides

We therefore turned our attention to the two acyclic derivatives used in previous syntheses of 2-acetamido-2-deoxy-5-thio-D-glucose, which were a dimethyl acetal[96] (23) and a diethyl dithioacetal[94] (22). The ease of preparation of a suitably protected acyclic derivative was examined and Hasegawa's preparation of various di-isopropylidene dialkyl acetals directly from 2-aminoglucose derivatives was judged most favourable. Hasegawa has shown that several 2-aminoglucoses upon treatment with 2,2-dialkoxypropane and p-toluenesulphonic acid in 1,4-dioxane solution at 60-70°C give mainly their 3,4:5,6-di-O-isopropylidene dimethyl or dibenzyl acetals.[97] This quick one-pot preparation of 2-acetamido-2-deoxy-3,4:5,6-di-O-isopropylidene-*aldehydo*-D-glucose dimethyl acetal (53) was accordingly carried out and purified to give a yellow syrup. This, although chromatographically pure, proved refractory to all attempts to induce crystallisation. Following receipt of satisfactory ^{1}H-NMR and mass spectral data, selective deacetalation with 80% acetic acid and chromatography gave 2-acetamido-2-deoxy-3,4-O-isopropylidene-*aldehydo*-D-glucose dimethyl acetal (23) in 62% overall yield.



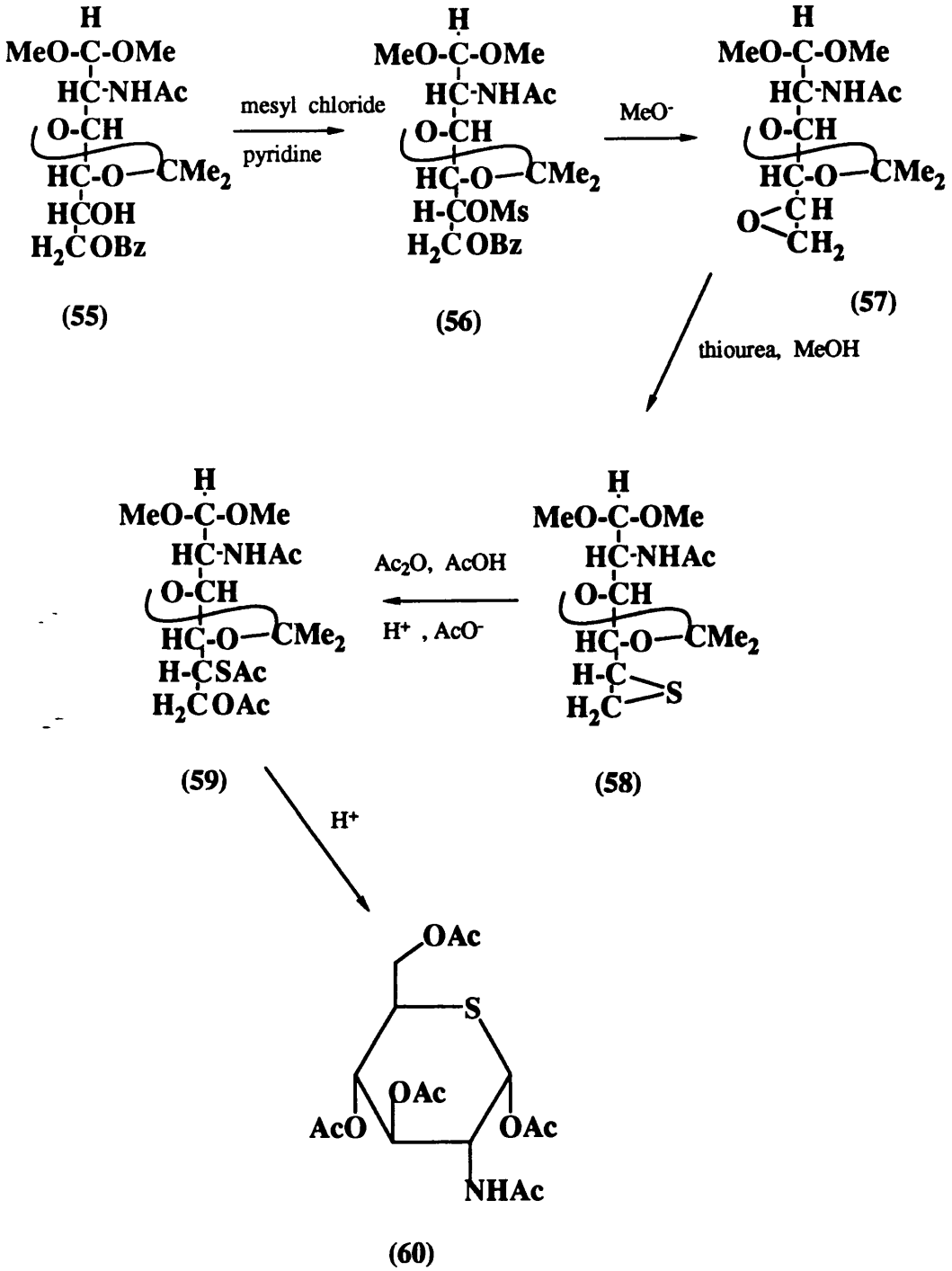
(53)



(23)

Hasegawa has detailed a synthesis of 2-acetamido-2-deoxy-5-thio-D-glucose from this derivative, which follows a conventional terminal epoxide-episulphide conversion.[94] In order to more readily compare yields and for easy characterisation of the final product by a novel route, it was decided to carry out this synthesis in parallel to our own. The methods and reaction conditions set out in this publication were followed closely and are detailed in scheme 9. As the reaction intermediates are already fully characterised, microanalytical data was not routinely obtained if mass spectral and ^{1}H-NMR data was satisfactory.

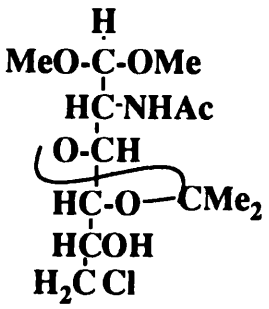
The final product 2-acetamido-2-deoxy-5-thio-D-glucose was obtained as its crystalline pentaacetate in 12% overall yield, which it should be noted is slightly poorer than the literature yield of 17%. Melting point, microanalysis and ^{1}H-NMR all match reported data for this compound and ammonia C.I. mass spectroscopy gave a pseudomolecular ion of 346 mass units corresponding to $(M+H)^+$. This material was used for final comparative purposes and for subsequent de-N-acetylation and deamination reactions.


mesyl chloride
pyridine
MeO-
thiourea, MeOH
Ac2O, AcOH
H+ , AcO-
H+
(55)
(56)
(57)
(58)
(59)
(60)

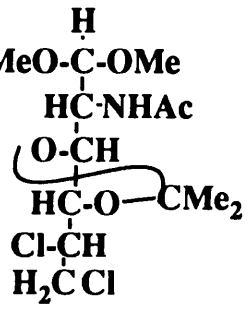
Scheme 9.

2.6.6 Chlorination of the acyclic diol

Selective primary chlorination at C-6 of the acyclic diol (23) was first attempted to assess the ease of halogenation with the same reagent mixture. As with the oxazoline derivative, at low temperatures (40-50°C), with 2 molar equivalents of TPP and 1 molar equivalent of CCl_4, an almost quantitative yield of crystalline 2-acetamido-6-chloro-2,6-dideoxy-3,4-O-isopropylidene-*aldehydo*-D-glucose dimethyl acetal (61A) was obtained. Mass spectroscopic, ^1H NMR and microanalytical data were all in agreement with the assigned structure.



(61A)

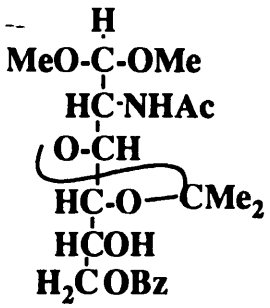


(61B)

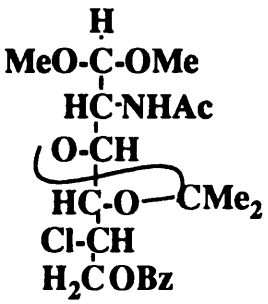
An attempted dichlorination reaction was then performed with the same reagent ratio as used for the oxazoline derivtives; 6 molar equivalents TPP and 3 molar equivalents CCl_4. The temperature was slowly raised and the reaction followed by t.l.c. Quantitative formation of the monochloro derivative (60) was observed as expected at 40-50°C, but no further reaction had taken place after 30 minutes at 50°C. The temperature was cautiously raised and at 70°C the formation of a second less polar spot was observed which was assumed to be 2-acetamido-5,6-dichloro-2,5,6-trideoxy-3,4-O-isopropylidene-*aldehydo*-L-idose dimethyl acetal (61B). Heating was continued between 70-75°C for a further 15 minutes at which point t.l.c. indicated an absence of starting material or (61A). This was accompanied by the appearance of a small amount of base-line material, indicating a certain degree of decomposition. The usual workup procedure of ether extraction followed by column chromatography gave (61B) as a clear syrup in 61% yield.

Mass spectrometry gave a pseudomolecular ion $(M+Na)^+$ of 366 as a 9:6:1 doublet indicating the presence of two chlorine atoms. As with furanose derivatives, the flexibility of the acyclic ring can make it difficult to identify which positions have undergone substitution but ^{1}H-NMR was satisfactory for the disubstitution of the two hydroxyl groups by chlorine.

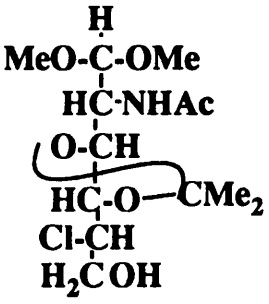
The relative molar quantities of TPP were altered in an attempt to improve the yield. At 70°C, reaction of the diol (23) with 3 molar equivalents of TPP and 3 molar equivalents of CCl_4, resulted in the formation of (61B) with very little detectable breakdown. No detectable traces of the monochloro derivative (61A) were found, indicating that the reaction had proceeded to completion. The number of molar equivalents of TPP required may therefore be dependant upon the type of derivative and ease of halogenation as well as the desired level of substitution.



(55)



(62)



(63)

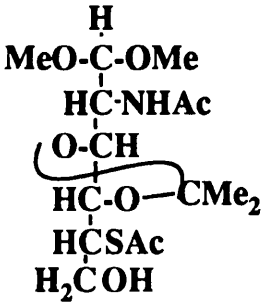
The first step of Hasegawa's synthesis[94] involved the selective benzoylation of the diol (23) to produce 2-acetamido-6-O-benzoyl-2-deoxy-3,4-O-isopropylidene-aldehydo-D-glucose dimethyl acetal (55). The halogenation of this derivative was also attempted, using 3 molar equivalents TPP and 3 molar equivalents CCl_4. Again no reaction was observed until the temperature was raised to 70-75°C, at which point t.l.c. showed a smooth conversion to a single fast-moving spot, with no detectable breakdown. Following workup and chromatography, this was isolated in 90% yield as a yellow syrup which proved refractory to crystallisation. Mass spectrometry gave a pseudomolecular ion $(M+Na)^+$ of 430 as expected as a 3:1 doublet. The material was therefore confirmed as being 2-acetamido-6-O-benzoyl-5-chloro-2,5-dideoxy-3,4-O-isopropylidene-*aldehydo*-L-idose dimethyl acetal (62) and in subsequent reactions was used as a foam. This derivative corresponded closely to the isopropylidene derivative 3-O-acetyl-6-O-benzoyl-5-chloro-5-deoxy-1,2-O-isopropylidene-β-L-idofuranose (36) prepared earlier and as the presence of a bulky benzoate had been shown in this derivative to inhibit reactivity, (62) was treated with methoxide in order to debenzoylate C-6. This resulted in the preparation of 2-acetamido-5-chloro-2,5-dideoxy-3,4-O-isopropylidene-*aldehydo*-L-idose dimethyl acetal (58) in almost quantitative yield. Displacement reactions with azide and thiol nucleophiles were then attempted with both (62) and (63).

2.6.7 Halide displacement reactions in some dimethyl acetal derivatives

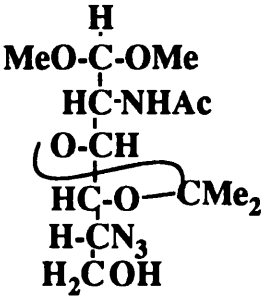
Primarily, the preparation of a common intermediate for the introduction of both amino and thiol functions was envisaged by displacement of the C-5 chlorine by azide and thioacetate. This was first attempted with 2-acetamido-6-O-benzoyl-5-chloro-2,5-dideoxy-3,4-O-isopropylidene-*aldehydo*-L-idose dimethyl acetal (62). This compound was heated to 120°C in dry DMF with a molar excess of both azide and thioacetate, however no reaction could be detected by t.l.c. Further examination of the $CHCl_3$ extract by mass spectrometry revealed only unreacted starting material, and i.r. failed to show the presence of organic azide or thioacetate. It was therefore concluded that the chlorine atom in this derivative was very resistant to displacement in a similar fashion to the isopropylidene derivative (36).

Two portions of the debenzoylated derivative, 2-acetamido-2-deoxy-5-chloro-3,4-O-isopropylidene-*aldehydo*-L-idose dimethyl acetal (63) was also treated with azide and thioacetate in the same way. Following workup in each case, two spots could be seen by t.l.c., one corresponding to unreacted starting material and the other to slightly more polar product.

In the case of the azide-treated compound, i.r. spectroscopy revealed the presence of organic azide and chromatography gave the purified material in 62% yield together with unreacted starting material. Mass spectroscopy gave a pseudomolecular ion $(M+H)^+$ of 437 and ^1H NMR was consistent with the preparation of 2-acetamido-5-azido-2,5-dideoxy-3,4-O-isopropylidene-*aldehydo*-D-glucose dimethyl diacetal (65). Time did not allow the further reaction of this material, and reversion to the D-gluco configuration can only be tentatively assigned upon the basis of the S_N2 nature of displacement at a secondary carbon atom. It is to be hoped howver that this material could undergo reduction and cyclisation to form 2-acetamido-2-deoxy-D-nojirimycin (12) and thus would represent an alternative route to the preparation of this derivative.



(64)



(65)

In a similar fashion, the reaction of (63) with potassium thioacetate in hot DMF, gave rise to one major polar product. Following chromatography this was isolated as an orange syrup in 40% yield together with an almost equal proportion of unreacted starting material. Examination by ^{1}H NMR revealed the presence of a thioacetate protons at δ 2.23 and mass spectroscopy revealed a pseudomolecular ion $(M+H)^+$ of 458, corresponding to the formation of 2-acetamido-5-S-acetyl-2,5-dideoxy-3,4-O-isopropylidene-*aldehydo*-D-glucose dimethyl acetal (65). Again the acyclic nature of this derivative makes it difficult to draw firm configurational conclusions but reinversion to the D-*gluco* configuration was assumed to have taken place and this was subsequently confirmed by its conversion to 2-acetamido-2-deoxy-5-thio-α-D-glucopyranose (17) on acid hydrolysis.

2.6.8 The preparation of 2-acetamido-1,3,4,6-tetra-O-acetyl-2-deoxy-5-thio-α-D-glucopyranose

From 2-acetamido-5-S-acetyl-2,5-dideoxy-3,4-O-isopropylidene-*aldehydo*-D-glucose dimethyl acetal (65), the preparation of 1,3,4,6-tetra-O-acetyl-2-acetamido-2-deoxy-5-thio-D-glucopyranose (17) was easily accomplished using the conditions outlined by Hasegawa. The material was warmed to 40°C in 10:1 acetic acid-2M hydrochloric acid for 6 hours, then cooled and neutralised. Following evaporation, this residue underwent conventional acetylation with acetic anhydride in pyridine and on workup gave a white solid. This gave identical ^{1}H NMR spectrum and mass spectrum to the sample of 1,3,4,6-tetra-O-acetyl-2-acetamido-2-deoxy-5-thio-α-D-glucopyranose (60) prepared according to the method of Hasegawa. A mixed melting point with this authentic material showed no depression and thus confirmed the identity of the material prepared by our novel route.

In terms of final yield Hasegawa's route appears superior and possesses the added advantage that the majority of his synthetic intermediates are crystalline. The majority of intermediates in our novel route resisted all attempts to induce solidification making purifiction difficult and chromatography was required at almost every stage. However the versatility of the halo compounds as synthetic intermediates perhaps compensates for difficulty of handling and other intermediates may be worth further investigation.

No attempt was made to repeat work accomplished with the isopropylidene model compounds by attempting a displacement of the primary chlorine of the dichloro derivative, 2-acetamido-5,6-dichloro-2,5,6-trideoxy-3,4-O-isopropylidene-*aldehydo*-L-idose dimethyl acetal (61B). Such a displacement is likely to be more readily achieved and it may be supposed that formation of the episulphide upon methoxide treatment followed by acetolysis and acid-catalysed ring closure may provide a second novel route to (60) perhaps in higher yield.

CHAPTER THREE

3.1 The regeneration of the amino function from acetamidodeoxy sugars

The preparation of both 2-amino-2-deoxy-5-thio-D-glucose and 2-deoxy-5-thio-D-glucose had been envisaged from the synthetic intermediate 2-acetamido-1,3,4,6-tetra-O-acetyl-2-deoxy-5-thio-D-glucose. In order to prepare both compounds it was first necessary to deacetylate the amine function at C-2. There are several well-documented methods of affecting this transformation and these are listed below.

The N-acetyl group may be cleaved under both acidic and basic conditions to regenerate the amino function. However the method of hydrolysis must be carefully chosen if other acid or base sensitive functions are to be preserved. The following hydrolytic reagents have been shown to effect de-N-acetylation of sugar derivatives and possess a degree of selectivity.

Glacial acetic acid saturated with hydrogen bromide has been used to effect selective de-N-acetylation of α-D-glucosamine pentaacetate. It is presumed to act through an oxazolinium intermediate to give 1,3,4,6-tetra-O-acetyl-2-amino-2-deoxy-α-D-glucopyranose hydrobromide.

Triethyloxonium fluoroborate in dichloromethane also reacts selectively with the acetamido group in the presence of O-acetyl functions and has been used in a variety of acetylated sugar derivatives. Initial reaction gives an O-ethyl acetamidium fluoroborate which are often crystalline and can easily be isolated. Upon treatment with water or aqueous sodium hydrogen carbonate, these salts are hydrolysed to give the corresponding O-acetyl amines. This reagent has also been employed in the presence of glycosidic linkages.

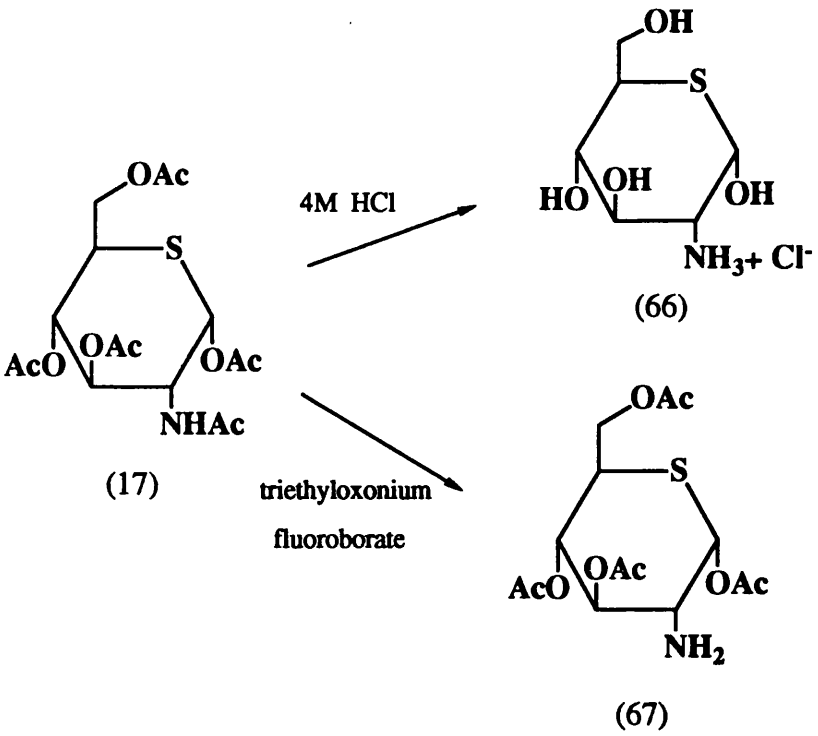
Boron trichloride in dichloromethane has also been used to form 2-amino-2-deoxy-D-glucose hydrochloride from 2-acetamido-2-deoxy-D-glucose, under conditions in which O-acetyl groups should be stable.

Unselective hydrolysis reagents include hot aqueous hydrochloric acid (2-4M) which normally gives the amino sugar as the hydrochloride salt. In some cases e.g. that of D-allose, yields by this method are high, but in the presence of other substituents, yields appear to suffer. Some basic reagents such as aqueous sodium or barium hydroxide or hydrazine hydrate have also found use.

3.2 The De-N-acetylation of 2-acetamido-2-deoxy-5-thio-D-glucopyranose

It was decided to attempt the de-N-acetylation of 2-acetamido-1,3,4,6-tetra-O-acetyl-2-deoxy-5-thio-D-glucopyranose (17), with both a selective and an unselective reagent. (Scheme 10) Literature reports concerning triethyloxonium fluoroborate indicated the possibility of superior yields and a readily purified intermediate. It was therefore decided to employ this reagent in the first instance. On stirring at room temperature over several hours with a molar excess of triethyloxonium fluoroborate in dry dichloromethane, t.l.c. showed little change and nothing corresponding to a solid intermediate was isolated. Following treatment with dilute acid however, a small amount of material was isolated by chromatography. This was analysed by mass spectroscopy and contained a peak of 363 corresponding to the expected pseudomolecular ion $(M+H)^+$ for 1,3,4,6-tetra-O-acetyl-2-amino-2-deoxy-5-thio-D-glucopyranose (67). Despite several attempts, this route was never optimised and yields remained poor.

For comparison, de-N-acetylation was then attempted with aqueous hydrochloric acid. A small sample of (17) in 4M HCl was heated in a boiling water bath for several minutes. T.l.c. revealed rapid deacetylation followed by slower conversion to a single polar spot which was assumed to be 2-amino-2-deoxy-5-thio-D-glucopyranose (66). This reaction proved to be both rapid and readily controllable although no crystalline hydrochloride salt was obtained, possibly due to lack of material. Again support for the assigned structure was obtained by mass spectroscopy which showed a pseudomolecular ion of 196 corresponding to $(M+H)^+$.

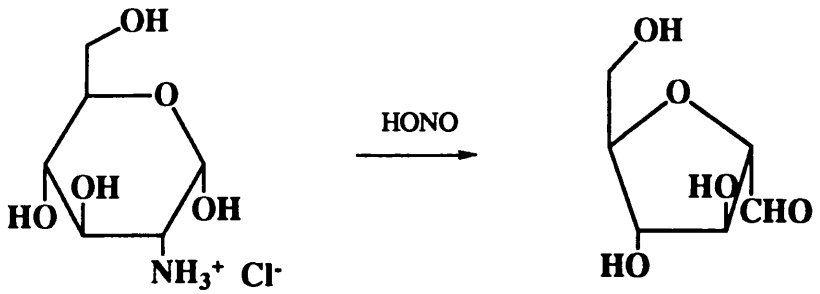


(17) (66) (67)

Scheme 10.

3.3 Deamination Reactions of aminosugars

The selective replacement of an amino function by a hydrogen atom is a reaction of particular utility in natural product chemistry and especially in the modification of aminoglycoside antibiotics. Difficulties in the reduction of primary amine fuctions have largely been overcome, but selective reduction of secondary amines in carbohydrates have until recently been problematic. Most deaminations involve ionic intermediates which readily undergo side reactions and in many cases give products which are configurationally different from the parent sugar. Nitrous acid has been a widely used reagent, but in the case of 2-amino-2-deoxy-D-glucopyranose also results in rearrangement and ring-contracture to form 2,5-anhydro-D-mannose by an accompanying attack of O-5 at C-2 (Scheme 11).



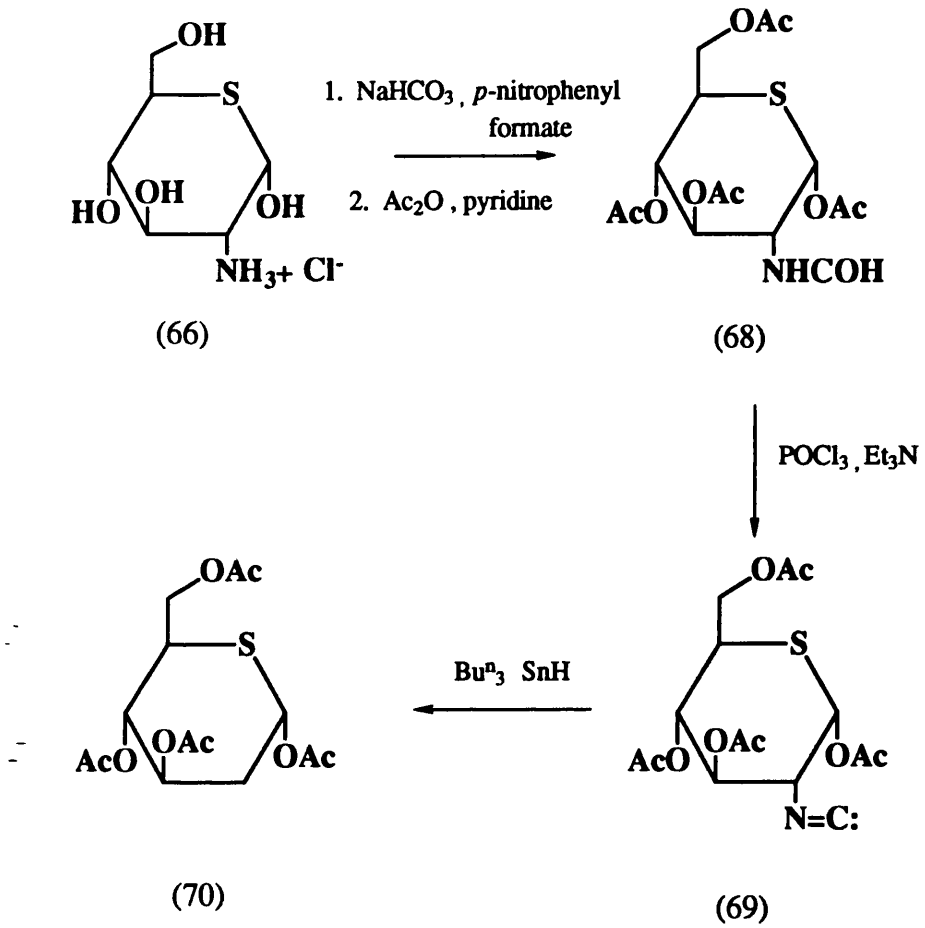
Scheme 11.

3.3.1 A radical-induced deamination reaction

Work by Barton *et al* has developed a short and efficient radical reaction carried out under neutral conditions, without the possibilty of rearrangement. This radical-induced reductive deamination has been employed with success on various natural products and carbohydrates and including the preparation of either anomer of 2-deoxy-D-glucopyranose from 2-amino-2-deoxy-D-glucopyranose. The method has also been shown to be compatible with common carbohydrate functional groups such as sulphonate esters.

The reaction sequence is as follows. Formylation of the free amine with either *p*-nitrophenyl formate or acetic-formic anhydride is then followed by its dehydration with phosphorus oxychloride and triethylamine to an isocyanide moiety. Reflux of this with tri-n-butylstannane in dry toluene in the presence of azobisisobutyronitrile (AIBN) as radical initator leads to carbon-nitrogen bond cleavage and formation of the deoxy sugar. Further work has shown the unique role of the isocyanide moiety as leaving group; carbon-nitrogen cleavge was not obtained in the reactions of n-octadecyl isocyanate or DCC with tri-n-butylstannane.

It was considered that this reaction sequence offered the best chance of preparing 2-deoxy-5-thio-D-glucopyranose by deamination and the proposed reaction sequence is shown overleaf (Scheme 12).



(66) (68)

(70) (69)

Scheme 12.

The intermediate 1,3,4,6-tetra-O-acetyl-2-amino-2-deoxy-D-glucopyranose hydrochloride produced from de-N-acetylation would have been ideal for the purposes of this reaction, however only a small quantity had been purified. It was therefore decided to prepare crude 2-amino-2-deoxy-5-thio-D-glucopyranose hydrochloride by treatment with hydrochloric acid. This then underwent a sequence of N-formylation followed by O-acetylation in a similar manner to Barton's preparation of 2-deoxy-D-glucose from D-glucosamine hydrochloride. A facile formylation was achieved in quantitative yield with p-nitrophenyl formate in aqueous 1,4-dioxan. The aqueous extract was dried and treated with acetic anhydride in pyridine to afford 1,3,4,6-tetra-O-acetyl-2-deoxy-2-formamido-5-thio-D-glucopyranose.

A small quantity of this N-formyl material (20mg) was then treated with phosphorus oxychloride and triethylamine at -70°C. Control of reaction temperature and time has been found to be critical at this stage,and any excess of phosphorus oxychloride must be quenched with gaseous ammonia at low temperature to minimise overreaction during workup. The reaction was monitored by t.l.c. and following chromatography a clear oil was isolated. This was examined by i.r. and showed a strong absorbance at 2140 cm^{-1} indicating the presence of the isocyanide moiety.

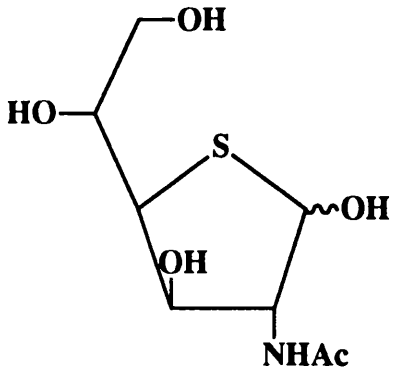
Owing to lack of time and material only a single attempt to induce deamination was carried out. Treatment with tri-n-butylstannane and AIBN in refluxing toluene followed by extraction into ether gave a small amount of material which did not show an isocyanide absorbtion in its i.r. spectrum. However mass spectral results may be ambiguous and though a peak corresponding to the product could be found, further experiments on a larger scale are necessary in order to definitively characterise the main product of this reaction.

CHAPTER 4

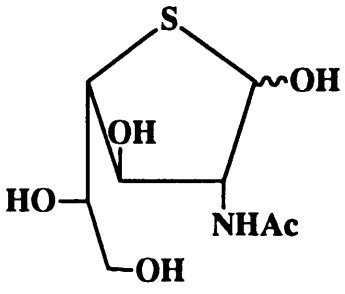
4.1 4-Thiofuranose sugars - configurational isomers

Following the successful development of a route allowing the incorporation of heteroatoms at C-5 of 2-acetamido-2-deoxy-D-glucopyranose with overall retention of configuration, we turned our attention to the preparation of the furanose analogues.

Many 4-thiohexofuranose sugars have been prepared, including those with the D-*gluco*,[132] D-*galacto*,[133] D-*manno*,[134] 6-deoxy-D-*gluco*,[135] 6-deoxy-D-*ido*,[136] 6-deoxy-D-*altro*,[136] D-*talo*[137] and 6-deoxy-D-*gulo*[138] configurations but it is interesting that no 2-acetamido or 2-deoxy-4-thiofuranose sugars are reported in the literature or in the natural world. As these compounds are merely the configurational isomers of our original target compounds, their preparation was also of interest. While it is known that the human erythrocyte glucose uptake system primarily interacts with the pyranose form of D-glucose, this has not been investigated for filarial parasites and we therefore became interested in the synthesis of 2-acetamido-2-deoxy-4-thio-D-glucofuranose (71) and also its 4-epimer, 2-acetamido-2-deoxy-4-thio-D-galactose (72).



(71)

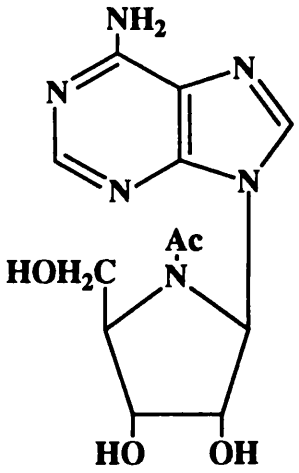


(72)

4.1.1 Biological properties of some 4-thio and 4-amino-furanose sugars

The powerful biological properties which have been demonstrated by thio sugars has resulted in considerable work towards the preparation of thiofuranose analogues of the common hexoses and pentoses. In particular, interest was generated in the 4-thio analogues of nucleosides as antimetabolites for potential chemotherapeutic and pharmacological use.[139,140] Hexofuranose sugars are relatively uncommon but have been found in several biological systems, including parasites. In particular, D-galactofuranose has been discovered as a constituent of a glycoconjugate from membranes of the parasitic flagellated protozoan, *Trypanosoma* , responsible for trypanosomiasis (sleeping sickness) and Chagas disease, a chronic ailment affecting several millions in South and Central America. The presence of galactose in the furanoid configuration markedly distinguishes the protozoan from the mammalian cell, in which apparently, no D-galactofuranose has been found. This has directly led to the synthesis of 4-thiogalactofuranose for the investigation of its potential biological properties in this regard.[135]

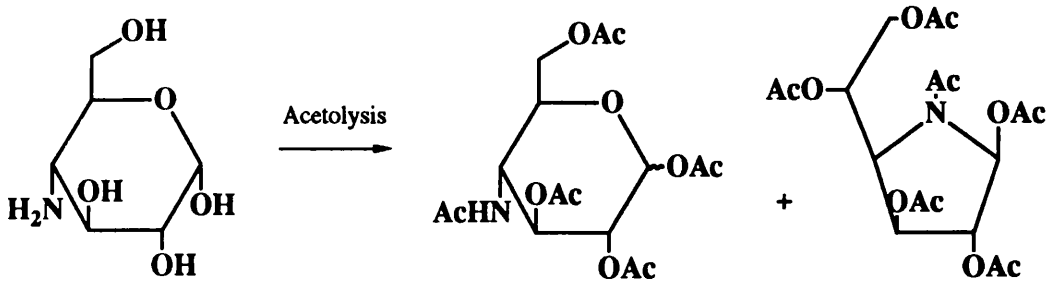
The potential properties of furanose sugars in which nitrogen replaces oxygen as ring heterocycle has also generated much interest and synthetic endevour. Several 4,6-dideoxy-4-amino-D-hexoses have been found in the natural world, as components of some antibiotics and within the lipopolysaccharide chains of certain bacteria.[141] Several of these pyrollidine sugars have been successfully prepared from methyl 4-acetamido-4-deoxypentopyranosides, which on sulphuric acid-catalysed acetolysis exclusively yield 4-acetamido-4-deoxyfuranoses. In this way derivatives of the pentose sugar 4-amino-4-deoxy-D-ribose has been prepared together with its nucleoside, 4'-acetamidoadenosine (73).[142]



(73)

In contrast, methyl 4-amino-4-deoxyhexopyranosides possessing the 6-deoxy-D-*gluco* and 6-deoxy-D-*galacto* configurations have been shown to preferentially, if not exclusively, maintain the pyranose ring on acetolysis. Although other products have been isolated from acetolysis mixtures, nothing corresponding to a pyrollidine sugar has been isolated.

However a paper[143] details the isolation of significant quantities of acetyliminofuranose derivatives, as well as pyranose derivatives from the acetolysis of both methyl 4-acetamido-4-deoxy-D-glucopyranoside and methyl 4-acetamido-4-deoxy-D-galactopyranoside, the ease of hydrolysis and the relative ratios of which can be linked with configuration at C-4. From the acetolysis of the D-glucohexoside over several days, the ratio of furanose to pyranose product was 2:3, whereas for the D-galactohexoside reaction was essentially complete within 24 hours and gave the furanose derivative as the major component in a ratio with the pyranose derivative of 5:2, and was isolated in an overall yield of 51%. A comprehensive mechanistic study revealed the importance of the methyl pyranoside as intermediate and showed that the pentaacetate did not undergo any conversion to the furanose derivative under acetolysis conditions. (Scheme 13)

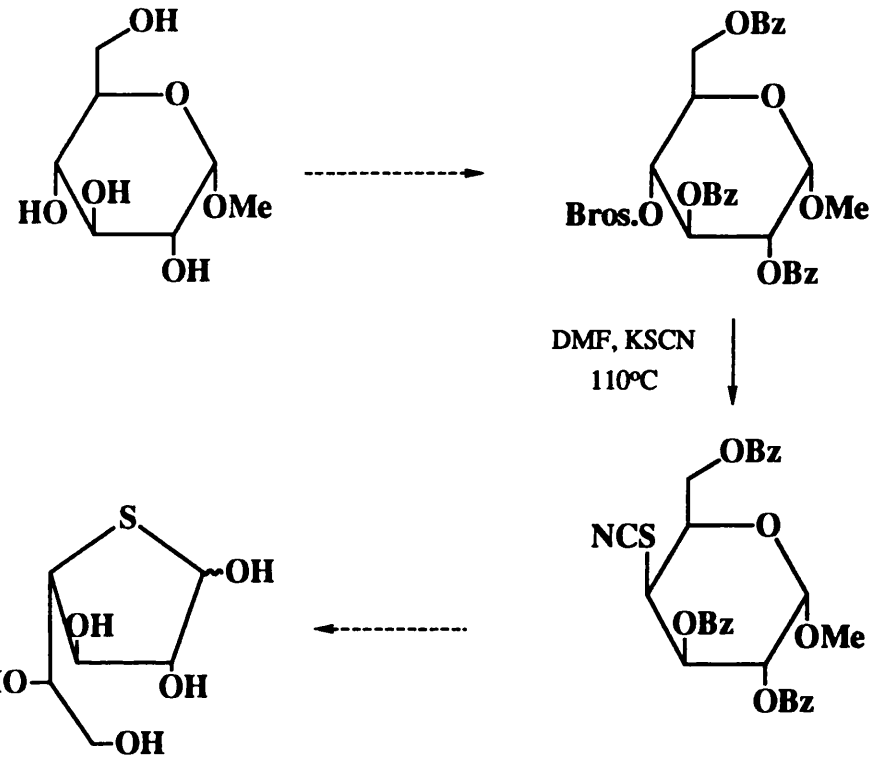


Scheme 13.

Whilst such hexofuranoses are stable as their penatacetates, it is difficult to judge their stability following deacylation and it is probable that such a compound would quickly revert to an acyclic or pyranose form in an aqueous environment, perhaps even at neutral pH. However these compounds retain interest from the proven biological activity of their counterparts.

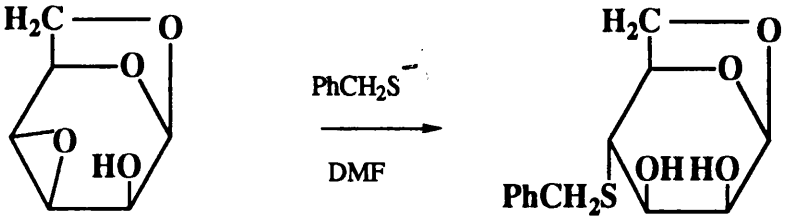
4.2 Literature syntheses of 4-thiofuranose monosaccharides

The preparation of 4-thiopentoses and hexoses is well-documented, with several published synthetic strategies.[132-138] Again the substitution of a thiol precursor at C-4 is essential, however since most free monosaccharides and many of their derivatives are stable in the pyranose configuration access to C-4 does not present difficulties. Following the preparation of a partially protected derivative suitable for substition, the most common method of thiol introduction has involved the displacement of a sulphonyloxy ester leaving group at C-4. This approach has been used for the majority of these syntheses, although it has been noticed that ease of displacement at C-4 by nucleophiles is markedly dependant on configurational, steric and electronic factors. As before, such a displacement takes place with inversion of stereochemistry, so that the starting material must be the 4-epimer of the desired sugar. This approach was used by Varela[133] in his successful synthesis of 4-thiogalactofuranose from methyl α-D-glucopyranoside via the 4-O-bromophenylsulphonyl derivative. (Scheme 14)



Scheme 14.

An entirely different method by which sulphur may be introduced at C-4 is by fission of a 3,4-epoxide with a thiol anion, provided that the compound has a conformation such that the rule of diaxial opening leads to attack at the required position. Therefore such epoxide derivatives require a "locked" conformation which can normally be provided by an anhydro bridge. Such an approach can be illustrated from Owen's reaction of 1,6:3,4-dianhydro-β-D-talose with benzyl sodium sulphide to give 1,6-anhydro-4-benzylthio-4-deoxy-β-D-mannose in excellent yield.[135]



Unfortunately in his attempt to prepare 4-thiomannofuranose from this derivative, the author found that the vigorous conditions required to hydrolyse the 1,6-anhydro bridge resulted in extensive decomposition with no identifiable product finally being obtained.

Following the substitution of a thiol moiety at C-4, sulphuric acid-catalysed acetolysis usually yields the thiofuranose as its pentaacetate. This ring-contracture occurs relatively easily with the more powerfully nucleophilic sulphur atom providing the driving force of the reaction. The preferred conformation of the thiofuranose over the pyranose configuration is comparable to the enhanced stability of thioacetals to acetals. Following the isolation of the 4-thiofuranose, deacetylation with methoxide affords the free monosaccharide.

4.3 2-acetamido-4-thiofuranose sugars - a synthetic overview

Whereas the synthesis of 2-acetamido-2,4-dideoxy-4-thio-D-glucofuranose was regarded as relatively easily achieved from 2-acetamido-2-deoxy-α-D-glucopyranose, by displacement of a 4-sulphonate, the synthesis of its D-*gluco* epimer appeared more problematic. However it was hoped that a similar strategy to that developed earlier for the 5-heterosugars could be successfully employed, whereby a halogen atom allowed the direct introduction of a thiol precuror with overall retention of configuration.

4.4 The preparation of a partially protected intermediate

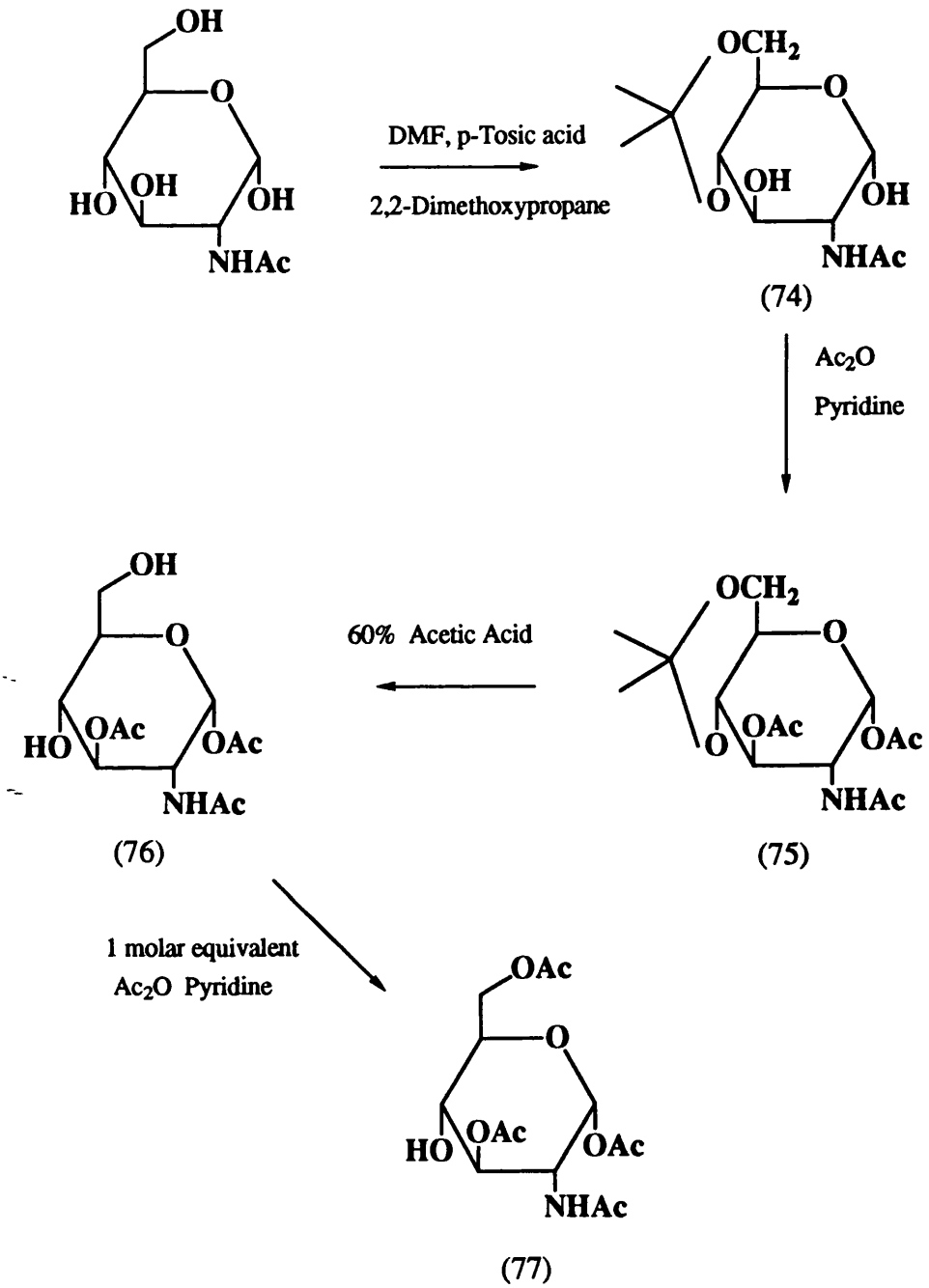
In order to successfully prepare 4-thiofuranose sugars (71) and (72) by the direct introduction of a thiol nucleophile, the synthesis of a partially protected intermediate was required. The protecting groups employed had to be stable under the relatively mild conditions used for halogenation or nucleophilic displacement and be readily removed under mild conditions to afford either the free monohalo or thio sugar. It was felt that such requirements were best met by a partially acylated derivative which was readily prepared by several routes.

4.4.1 A multi-step synthesis

The first method employed involved a protection-deprotection sequence using the known 4,6-O-isopropylidene derivative, a facile synthesis of which was developed by Hasegawa.[89] (Scheme 15) In this sequence, 2-acetamido-2-deoxy-D-glucopyranose was reacted with 2,2-dimethoxypropane in dry DMF with a catalytic quantity of *p*-toluenesulphonic acid to form 2-acetylamine-2-deoxy-4,6-O-isopropylidene-α-D-glucopyranose (74) in high yield. This was then acetylated at O-1 and O-3 with acetic anhydride-pyridine to give 1,3-di-O-acetyl-2-acetamido-2-deoxy-4,6-O-isopropylidene-α-D-glucopyranose (75) which was isolated in almost quantitative yield. The acetal was then cleaved to the free 4,6-diol, 1,3-di-O-acetyl-2-acetamido-2-deoxy-α-D-glucopyranose (76) using cold 60% aqueous acetic acid over several hours. This underwent selective acetylation at the primary 6-OH with 1 molar equivalent of acetic anhydride in pyridine at -40°C to form a mixture of products from which the 1,3,6-triacetate was isolated by column chromatography in 79% yield. This three-step sequence gave 1,3,6-tri-O-acetyl-2-acetamido-2-deoxy-α-D-glucopyranose (77) in an overall yield of approximately 60%.

A final selective benzoylation may well have resulted in an improved yield as compared with a selective acetylation, the more bulky benzoate group being associated with greater selectivity between primary and secondary hydroxyl groups.[144] However the triacetate was initially considered to be of greater value than a mixed ester derivative in the later testing of the 4-halo compounds with selected nucleophiles.

This route was also adapted for the later preparation of methyl 2-acetamido-6-O-benzoyl-4-chloro-2,4-dideoxy-3-O-methyl-α-D-galactopyranoside, by methylation of (74) followed by acetal cleavage and selective benzoylation.


OH
O
HO
OH
OH
NHAc
DMF, p-Tosic acid
2,2-Dimethoxypropane
OCH2
O
O
OH
OH
NHAc
(74)
Ac2O
Pyridine
OH
O
HO
OAc
OAc
NHAc
(76)
60% Acetic Acid
OCH2
O
O
OAc
OAc
NHAc
(75)
1 molar equivalent
Ac2O Pyridine
OAc
O
HO
OAc
OAc
NHAc
(77)

Scheme 15.

This multistep approach was also considered to be potentially useful for the preparation of 6-deoxy-4-thio derivatives owing to the ready preparation of a 4,6-disulponate ester. It is well-documented that lithium aluminium hydride differs in its reaction with primary and secondary sulphonates, with the primary groups giving rise to the deoxy compound, while secondary sulphonates, if they react at all, are cleaved to the free hydroxyl group which may then be further derivatised depending upon the method of thiol introduction[135,145] e.g. resulphonation or halogenation.

But although largely satisfactory, this route remained cumbersome and time-consuming and we became interested in the development of a facile, one-pot synthesis of the partially protected derivative in comparable yield.

4.4.2 Selective Acylation

The relative reactivities of hydroxyl groups in carbohydrates and selective protction or deprotection has been the subject of extensive review,[144] with almost every type of reagent. By far the greatest body of information concerns acylation, which has been widely studied with the polar and steric factors which influence reactivity noted. The usefulness especially of partially benzoylated derivatives has been proven, and in some cases selective benzoylation can be accomplished with little or no anomerisation.

Differences in reactivity between primary and secondary hydroxyl groups to many acylation conditions have long been known, but differential reactivity between secondary hydroxyl groups has become increasingly apparent with some reagents. These differences together with differences in substitution between reagents and conditions are often explained from the standpoint of intramolecular hydrogen bonding and steric factors. NMR spectroscopy has proved of great value in the examination of acylation reactions and the assessment of order of reactivity between secondary hydroxyl functions. There are several reports in the literature of the lower reactivity of OH-4 in many hexopyranose derivatives including those with the D-*gluco* configuration with a variety of acylating agents and these are discussed overleaf, with the most widely used reagents briefly reviewed.

4.4.2.1 Acyl chloride or anhydride/pyridine

Selective tribenzoylation of methyl α-D-glucopyranoside, methyl α-D-mannopyranoside and α-D-galactopyranoside with benzoyl chloride (4.2 molar equivalents) in pyridine at -40°C each gave the 2,3,6-tri-O-benzoate in yields of greater than 50%.[145] Further dibenzoylation studies[147] of these glycosides have suggested that for each, OH-4 has the lowest reactivity among the secondary hydroxyl groups, though reactivity differs between glycosides for OH-2 and OH-3. This same reagent combination has been used to effect the preparation of the 2,3-dibenzoate of both methyl 6-deoxy-α and β D-glucopyranosides as the major product in 61% and 29% yields respectively, similarly treatment of α-D-glucopyranose with 4.2 molar equivalents of benzoyl chloride in dry pyridine at -35°C gave a mixture of penta and tetrabenzoates from which 1,2,3,6-tetrabenzoate was isolated in 50% overall yield.[148] Reaction of 2-deoxy-D-*arabino*-hexose with this combination of reagents produced the 1,3,6-tribenzoate in 79% yield[149] while treatment of 2-benzamido-2-deoxy-D-glucopyranose with benzoyl chloride and the selective pivaloylation of 2-acetamido-2-deoxy-D-glucose with pivaloyl chloride have both indicated the lowest reactivity for OH-4.[144]

The low reactivity of OH-4 in α-D-galactose derivatives can be readily explained[144] by its axial configuration, but in α-D-*gluco* and α-D-*manno* derivatives it has been attributed to steric hind rance through *gauche* interactions with OH-3 and a benzoyloxymethyl or methyl group at C-5 (It is presumed that the primary hydroxyl is acylated first). The 3-OH is presumed to suffer less steric interaction, possessing two adjacent *gauche* orientated hydroxyl or substituted groups.

4.4.2.2 N-Acylimidazoles

The N-acylimidazoles as selective acylating agents are finding increasing use in carbohydrate chemistry and were developed from work by Staab.[150] In contrast to other benzoylation procedures a high degree of discrimination between the hydroxyl groups at C-2 and C-3 has been observed with some *gluco* derivatives as well as with *galacto* derivatives; methyl 4,6-O-benzylidene-α-D-glucopyranoside reacts with one molar equivalent of N-benzoylimidazole in refluxing chloroform to give the 2-O-benzoyl derivative in 78% yield.[151] However on similar treatment the corresponding *manno* derivative gave its 2- and 3-monobenzoates in almost equal amounts.[144] Upon treatment with N-acylimidazoles, imidazole-catalysed acyl migration ensures that the esterification of such vicinal *cis*-diols is subject to thermodynamic control and the reflux of methyl 3-O-benzoyl-4,6-O-benzylidene-α-D-mannop yranoside in chloroform containg imidazole led to a 1:1 equilibrium of 2- and 3- benzoates in less than 1 hour.[144] In contrast, similar treatment of 3-O-benzoyl-4,6-O-benzylidene-α-D-glucopyranoside, a 2,3-*trans* diol, isomerised to the extent of only 10% after 12 hours.[152] A similar effect has been noted with the attempted esterification of a ribofuranose derivative with N-acetylimidazole which showed very little selectivity between 2 and 3-OH, and this has again been attributed to a facile base-catalysed ester migration.[144] Variations in time of reaction, solvent polarity or temperature did not significantly affect the stereoselectivity of this reaction.

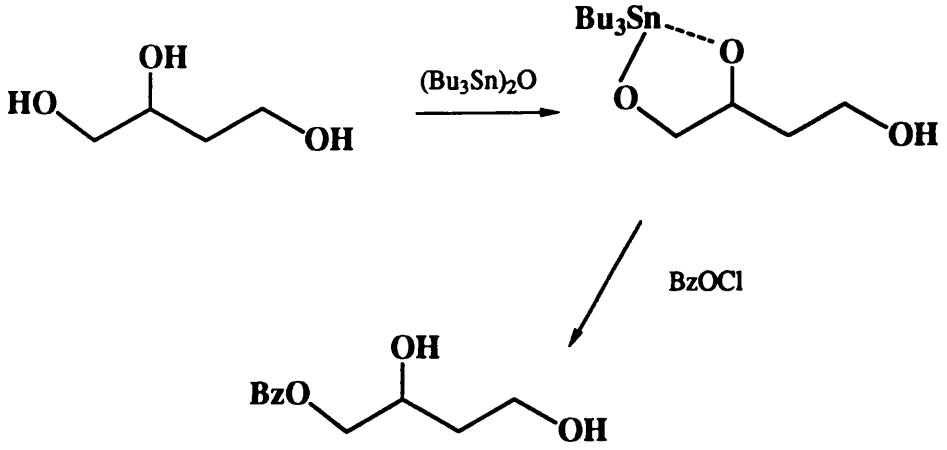
It has been noted that N-acetylimidazole shows no appreciable selectivity towards certain glucopyranose derivatives.[144] Treatment of methyl α-D-glucopyranoside with unimolecular quantities of this reagent showed no significant selectivity and lead to partial substitution at all four hydroxyl groups.[153] However the benzoylation of the same compound with 3.6 molar equivalents of N-benzoylimidazole in refluxing acetonitrile resulted in the isolation of the 2,3,6-tribenzoate in 71% yield, with the yield of only 2.7% of the isomeric 2,4,6-tribenzoate.[133] The superior selectivity often demonstrated by N-benzoylimidazole when compared with the acyl halides or anhydrides in pyridine has been attributed in part to their lower reactivity; they often require many hours at 60-80°C to achieve complete reaction.[144]

4.4.2.3 Acyl cyanides

This class of acylating agent has only recently found use in carbohydrate chemistry, but has shown great promise, matching the selectivity of N-benzoylimidazole in several cases.[144] The unimolecular benzoylation of methyl 4,6-O-benzylidene-α-D-glucopyranoside, methyl 4,6-O-benzylidene-α-D-altropyranoside and benzyl 4,6-O-benzylidene-β-D-altropyranoside with benzoyl cyanide gave good yields of the corresponding 2-,2- and 3- benzoates respectively.[154] It has also been used for the succesful selective monobenzoylation of methyl 4,6-O-benzylidene-α-D-mannopyranoside as the product of the benzoylating reagent being a weak acid does not catalyse a transesterification and the final product ratio reflects the original formation under kinetic control. Treatment of this diol in acetonitrile at room temperature with benzoyl cyanide resulted in the isolation of the 2- and 3- benzoates in the ratio 2.3:1.[154]

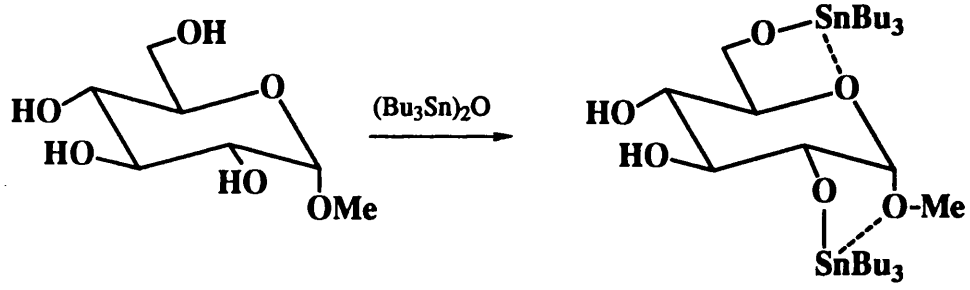
4.4.2.4 Trialkyltin alkoxides

The use of tributylstannane derivatives for the selective acylation and alkylation of vicinal diol systems in nucleosides was first introduced by Moffatt *et al*,[155] and was developed by Ogawa for the stannylation of methyl α-D-glucopyranoside giving a synthesis of methyl 2,3,6-tri-O-benzoyl-α-D-glucopyranoside in 63% yield.[156] Regioselective stannylation with tributylstannane oxide occurs from its reaction with hydroxyl group to form a trialkyltinalkoxide. This alkoxide being more nucleophilic than the original hydroxyl group forms a coordination bond with a neighbouring oxygen atom across the tin atom. Just as *cis* 1,2-diols form isopropylidene derivatives more readily than *trans* 1,2-diols, the formation of coordination complexes with *cis* vicinal diols is favoured. Subsequent reaction of the complex with an electrophile leads to a regioselectively modified derivative. (Scheme 16)



Scheme 16.

In the case of methyl α-D-glucopyranoside, the following nucleophile activated complex has been proposed,[156] leading to the formation of 2,3,6-tribenzoate upon treatment with an excess of benzoyl chloride.



Stannylation of 1,6-anhydro-D-glucose has allowed selective monoacylation and etherification at 4-OH. The most commonly used reagent for effecting stannylation is tributyltin oxide, which is however extremely toxic and requires very careful handling.[157]

4.4.3 The action of some benzoylating reagents with 2-acetamido-2-deoxy-α-D-glucose

In view of the general low reactivity of OH-4 to acylation demonstrated by many glucopyranose derivatives, it was decided to attempt the selective preparation of 1,3,6-tribenzoate. Yields and reagents are displayed in the following table, the solvent used is indicated in the text, comparable molar ratios of reagents to starting material were used. The yield percentages are averaged from at least two experiments of the selective benzoylation of approximately 1g of 2-acetamido-2-deoxy-α-D-glucopyranose and showed an average variation in yield of not more than 10%.

Reagent	Tetra-benzoate	1,3,6-tri-benzoate	1,4,6-tri-benzoate
N-Benzoyl Imidazole	10 %	61 %	12 %
Benzoyl chloride	14 %	43 %	32 %
Benzoyl Cyanide	9%	63 %	10 %

With all of the reagents shown above, a mixture of benzoylated products were obtained. T.l.c. (dichloromethane-ethyl acetate 3:2) revealed a complex mixture of compounds which could be distinguished into three fast-moving major products, and small amounts of a complex mixture of polar products presumed to be starting material, mono and dibenzoates. The three major components were recovered and purified by silica gel chromatography.

The fastest migrating component ($R_f = 0.63$) was presumed to be 2-acetamido-1,3,4,6-tri-O-benzoyl-2-deoxy-D-glucopyranose (78). It crystallised readily on standing and mass spectrometry revealed a pseudomolecular ion of 661 $(M+Na)^+$ which was correct for the tetrabenzoate. ^1H NMR gave an aromatic integral correct for 20 aromatic protons and the coupling constant of the anomeric proton J=4Hz is in agreement with an α configuration. There was no spectroscopic evidence of the coexistence of the β isomer. It was noticeable that the dibenzoylation of OH-3 and OH-4 shifted both H-3 and H-4 signals downfield by more than 1.0ppm.

The next fraction (R_f = 0.56) 2-acetamido-1,4,6-tri-O-benzoyl-2-deoxy-α-D-glucopyranose (79) was isolated as a colourless syrup. Mass spectrometry revealed a pseudomolecular ion of 556 $(M+Na)^+$, correct for a tribenzoate. From work by Varela with a similar solvent system, it was expected that the isomeric 1,4,6-tribenzoate would migrate faster than the 1,3,6-tribenzoate, in line with the methyl pyranoside tribenzoates.[133] However the structures assigned on this basis were confirmed by ^1H NMR spectroscopy. The coupling constant of the anomeric proton revealed only the α configuration (J=3.5Hz). When compared with the ^1H NMR spectrum for the 1,3,6-tribenzoate (80), the signals corresponding to H-4 are shifted downfield by 1.5ppm, obviously by benzoylation of OH-4. However the signals of the vicinal protons, H-3 and H-5 were shifted downfield less than 0.5ppm.

The main product 2-acetamido-1,3,6-tri-O-benzoyl-2-deoxy-α-D-glucopyranose (80) was isolated from the next chromatographic fraction (R_f = 0.40). Mass spectrometry revealed a pseudomolecular ion of 556 $(M+Na)^+$ again correct for a tribenzoate. It was noticeable however that the relative ion intensities, together with fragmentation patterns were quite different for the two tribenzoates which could be relatively easily distinguished. ^1H NMR again gave the anomeric configuration as α. In comparison with the 1,4,6-tribenzoate (79) the signals for H-3 were shifted downfield by 1.7ppm by O-3 benzoylation while the signals of the vicinal proton H-2 shows a slight downfield shift (0.3ppm). In line with previous observations, H-4 shifts upfield and is indistinguishable from H-5.

Selective benzoylation of 2-acetamido-2-deoxy-D-glucopyranose with benzoyl chloride in pyridine at -40°C proved to be disappointing, producing a large amount of the isomeric 1,4,6-tribenzoate and making complete chromatographic separation difficult, although the overall amount of 1,3,6-tribenzoate produced is comparable with yields for other D-glucopyranose derivatives. More stringent temperature control (-70°C, rising to -15°C for 12 hours) did not result in any significant improvements, the reagent still displaying a general lack of selectivity between secondary hydroxyl groups in this derivative. This is in agreement with the reagent's behaviour with methyl α-D-glucopyranoside where selective benzoylation with 3 molar equivalents of benzoyl chloride in pyridine at -40°C gave the 2,3,6-tribenzoate in 67% yield, but also gave the isomeric 2,4,6-tribenzoate in 28% yield.[145]

Use of N-benzoylimidazole in refluxing dichloromethane, the conditions used for most monobenzoylations described in the literature,[144,154] resulted in no reaction. This was attributed to the poor solubility of 2-acetamido-2-deoxy-D-glucopyranose in dichloromethane and the solvent was changed to acetonitrile, which proved successful. A relatively high percentage of the 1,3,6-tribenzoate was finally isolated, and with much lower quantities of the isomeric 1,4,6-tribenzoate which allowed complete, facile chromatographic separation. However, it was noted that the required reaction time was double that required for similar treatment of methyl glucopyranoside together with a slightly lower overall yield. It was considered that this could perhaps also be due to the poorer solubility of the 2-acetamido sugar in acetonitrile. A further benzoylation with this reagent was performed in DMF, however, suprisingly, this did not result in any significant improvements in yield or reaction time.

The possibility of a trans-esterification reaction between 3-OH and 4-OH was considered, but this was considered unlikely in view of their diequatorial relationship. However, this was examined further by refluxing a solution of the pure 1,3,6-tribenzoate in dry acetonitrile in the presence of 3 molar equivalents of imidazole over 24 hours. The formation of 1,4,6-tribenzoate as assessed by t.l.c was very slight, and only barely detectable at the end of 24 hours, but a certain amount of baseline degradation products were also observed. So the rather poorer ratio of 1,3,6-

tribenzoate to 1,4,6-tribenzoate observed for 2-acetamido-2-deoxy-D-glucopyranose cannot be explained in terms of a simple trans-esterification over time.

It was decided to attempt a similar benzoylation with benzoyl cyanide, first employing similar conditions to those used in literature reactions. No reaction was observed on stirring with 3 molar equivalents of benzoyl cyanide in DMF or acetonitrile at room temperature for 48 hours. When benzoyl cyanide was heated at 90°C in DMF for 24 hours, a smooth reaction took place, resulting in the isolation of comparable quantities of the 3 major products. With 2-acetamido-2-deoxy-α-D-glucopyranose, this reagent displays very similar specificity to N-benzoylimidazole. The absence of reaction at room temperature is interesting, but again may reflect the poor solubility of the free monosaccharide even in very polar organic solvents rather than inherent unreactivity. It would be of interest to examine the benzoylation of other more substituted derivatives of this sugar at room temperature and at raised temperatures.

One attempt was made to use TBTO to effect a stannylation. 2-Acetamido-2-deoxy-D-glucose was refluxed with 3 molar equivalents of TBTO in dry toluene for 5 hours hours, upon cooling, 3.0 molar equivalents of benzoyl chloride were added and the mixture left to stir for 48 hours at 50°C. However following this, t.l.c. revealed the presence of some tetrabenzoate and both tribenzoates in an approximately 1:1 mixture. It is not certain whether the formation of the tin coordination complex with the monosaccharide was successful, if not, this would account for the absence of differential benzoylation. More work is necessary to assess the usefulness of this reagent in the selective benzoylation of this monosaccharide.

4.5 A Synthesis of 2-acetamido-1,3,5,6-tetra-O-acetyl-2-deoxy-4-thio-D-galactose

The synthesis of a 4-thio-D-galactose derivative from a D-glucose derivative requires an inversion of stereochemistry at C-4 which is readily accomplished by the formation of a 4-sulphonate and its displacement with a thiol nucleophile. Varela's synthesis of 4-thio-D-galactose[133] was achieved in this fashion, with the formation of 4-brosyl and 4-tosyl derivatives from a crude mixture of partially benzoylated derivatives of methyl glucopyranoside. By the use of these very crystalline sulphonates they were able to circumvent the requirement for column chromatography in order to isolate pure methyl 2,3,6-tri-O-benzoyl-α-D-glucopyranoside, instead sulphonylation of the crude benzoylation mixture allowed the isolation of both the 4-brosyl and 4-tosyl derivatives directly, by crystallisation from ethanol.

In an attempt to repeat this elegant separation, a crude extract of partially benzoylated 2-acetamido-2-deoxy-α-D-glucopyranose, obtained by reaction of N-benzoylimidazole in acetonitrile was tosylated with an excess of tosyl chloride in dry pyridine. However, despite repeated attempts with various solvent systems, no crystallisation could be induced. Tosylation of some pure material gave crystals identified as 2-acetamido-1,3,6-tri-O-benzoyl-2-deoxy-4-tosyl-α-D-glucopyranose (81), but seeding experiments with several different solvent systems did not result in significant further crystallisation. It was therefore concluded that the larger amount of contaminating benzoylated derivatives, perhaps coupled with inherently less crystalline material would not allow this approach and column chromatography would be necessary in order to prepare the pure 4-sulphonyltribenzoate.

The methanesulphonate (mesyl) group is generally considered to act as the most efficient leaving group of all the commonly-used sulphonyl esters, and while its derivatives are rarely readily crystallisable, it is associated with higher yields in displacement reactions.[135] 2-acetamido-1,3,6-tri-O-benzoyl-2-deoxy-4-O-mesyl-α-D-glucopyranose (82) was accordingly prepared from column purified 2-acetamido-1,3,6-tri-O-benzoyl-2-deoxy-α-D-glucopyranose and was obtained in quantitative yield as a pale yellow syrup which although refractory to crystallisation was readily dried and

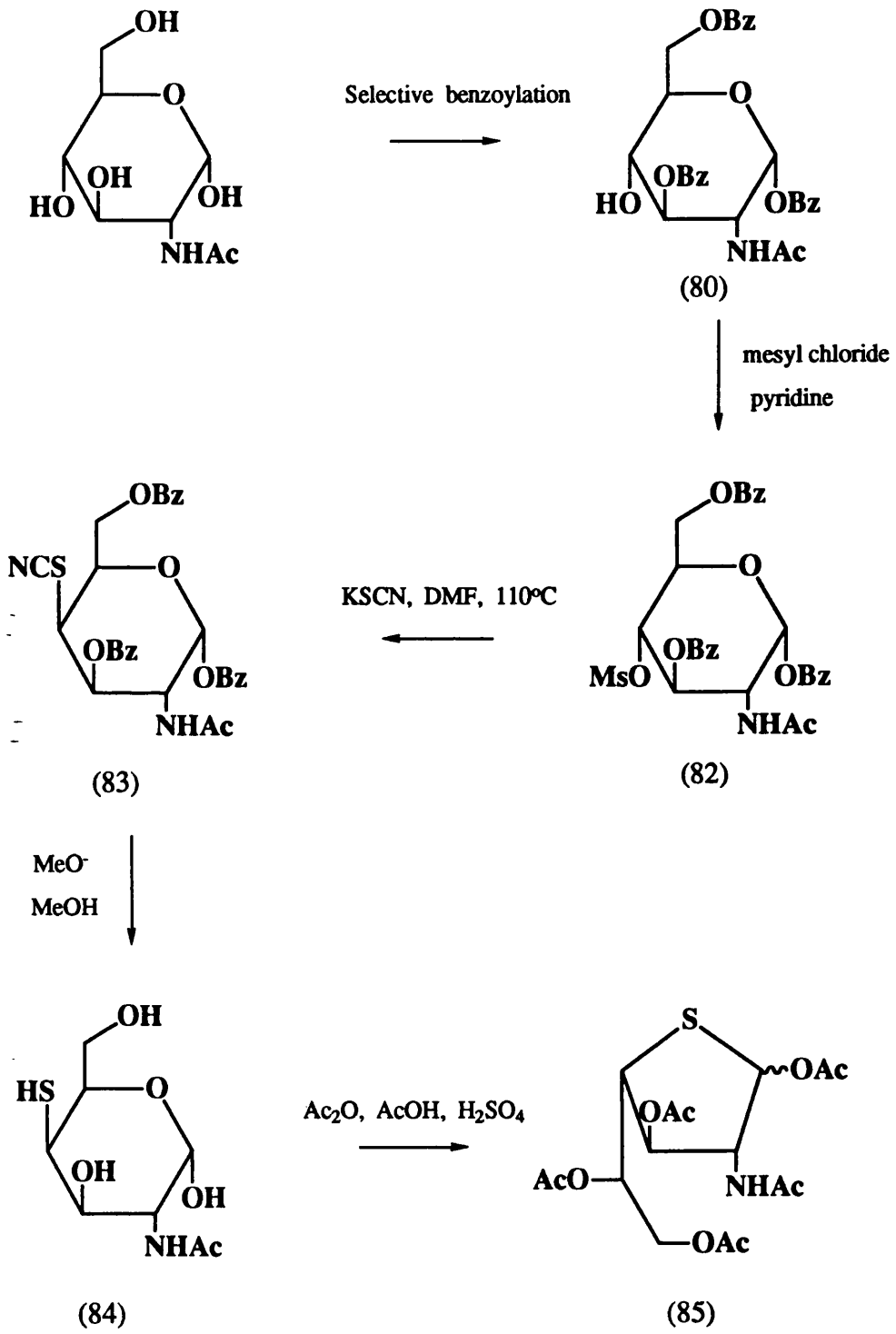
used as a foam.

This was heated with 10 molar equivalents of sodium thiocyanate in DMF at 110°C for 48 hours which resulted in a smooth conversion of the mesyl derivative to the thiocyanate derivative, 2-acetamido-1,3,6-tri-O-benzoyl-2,4-dideoxy-4-thiocyano-α-D-galactopyranose (83) in 74% yield. This compares well with Varela's conversion of methyl 2,3,6-tri-O-benzoyl-4-O-brosyl-α-D-glucopyranoside to methyl 2,3,6-tri-O-benzoyl-4-deoxy-4-thiocyano-α-D-galactopyranoside in 70% yield. (83) proved to be a crystalline compound, which sublimed readily and the i.r. spectrum as expected showed a strong SCN absorbance at 2180 cm^{-1} and the ^1H NMR spectrum showed features consistent with the change of configuration from D-*gluco* to D-*galacto*.

Reduction of the thiocyanate to the thiol was considered by two methods, either by reflux with zinc powder in acetic acid or with sodium methoxide in methanol. It was considered that for our purposes sodium methoxide would prove the superior reagent, giving rise to debenzoylation in addition to thiocyanate reduction. Although in his synthesis of 4-thio-D-galactofuranose,[133] Varela reports a slightly superior overall yield from the sequential reduction of the analagous thiocyanate with zinc, followed by debenzoylation with sodium methoxide (63%) as compared with direct use of methoxide (50%), this was barely significant and must be balanced against the introduction of a further step.

Treatment of (83) with a solution of sodium (100mg) in methanol (5ml) at room temperature for 12 hours gave a syrup which following column chromatography gave the thiol (84) in 54% yield, as a solid which could be recrystallised from ethanol. I.r. spectroscopy showed an absence of SCN, but the presence of the characteristic SH absorbance at 2550 cm^{-1}.

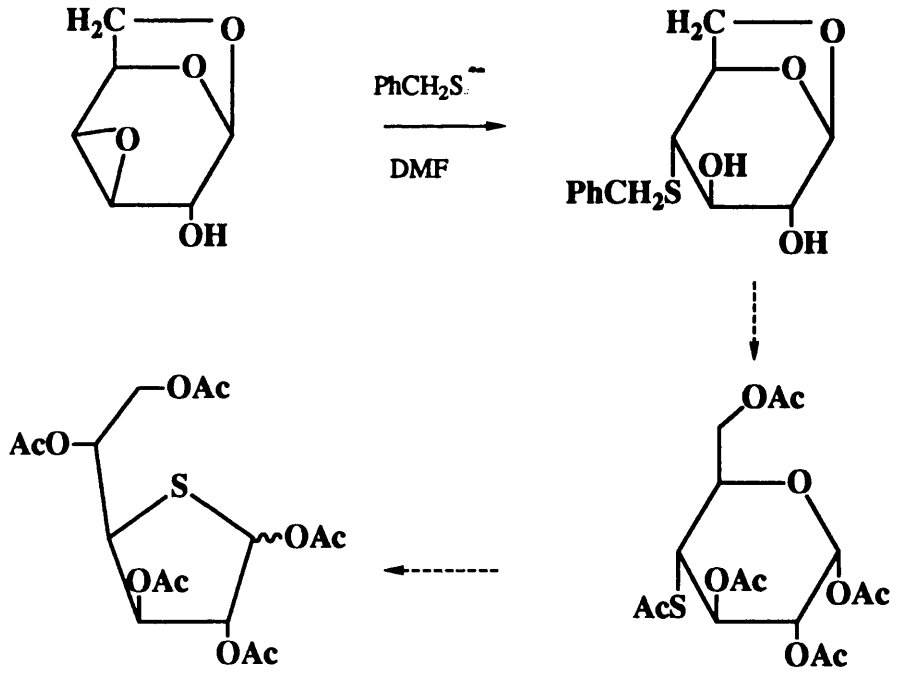
This compound was acetolysed with acetic acid-acetic anhydride-sulphuric acid 17:17:1 at 4°C to give a mixture of two main products which were separated by column chromatography. The fraction containing the lowest migrating product were pooled. Only a small amount of this material was obtained however and complete confirmtion of the thiogalactose ring was not possible. However mass spectroscopy showed a pseudomolecular ion of 406 corresponding to $(M+H)^+$. No SH absorbance was observed from its i.r. spectrum and on this basis the compound has been tentitively identified as 2-acetamido-1,3,5,6-tetra-O-acetyl-2-deoxy-5-thio-D-galactofuranose (85). A complete scheme of this reaction is detailed in scheme 17 overleaf.


OH
O
HO
OH
OH
NHAc
Selective benzoylation
OBz
O
HO
OBz
OBz
NHAc
(80)
mesyl chloride
pyridine
OBz
NCS
O
OBz
OBz
NHAc
KSCN, DMF, 110°C
OBz
O
MsO
OBz
OBz
NHAc
(83)
(82)
MeO⁻
MeOH
OH
HS
O
OH
OH
NHAc
Ac2O, AcOH, H2SO4
S
OAc
OAc
AcO
NHAc
OAc
(84)
(85)

Scheme 17.

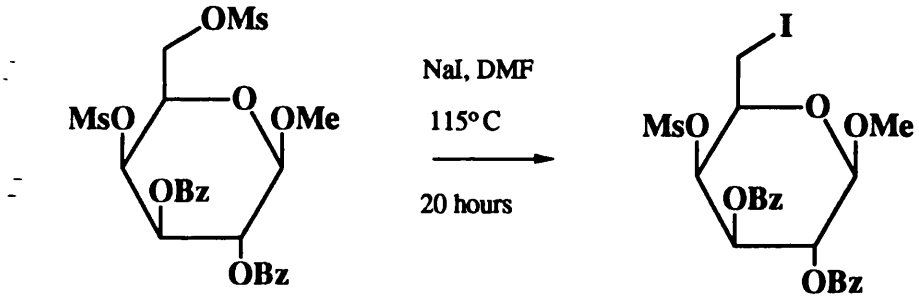
4.6 2-acetamido-2-deoxy-4-thioglucofuranose - a synthetic strategy

2-acetamido-2-deoxy-4-thio-D-glucofuranose (71) remained the principal target thiofuranose monosaccharide, but its synthesis posed several problems. The published prepartion of 4-thio-D-glucofuranose by Vegh and Hardegger[132] utilised 1,6:3,4-dianhydro-galactopyranose. Treatment of this with sodium benzyl sulphide and sodium methoxide in methanol gave 1,6-anhydro-4-S-benzyl-4-deoxy-D-glucopyranose in 23% yield. Following this, cleavage of the 1,6-anhydro ring, acetylation to give 1,3,4,6-tetra-O-acetyl-4-S-acetyl-4-deoxy-β-D-glucopyranose and acetolysis gave the ring-contracted product as its pentaacetate in 40% overall yield.

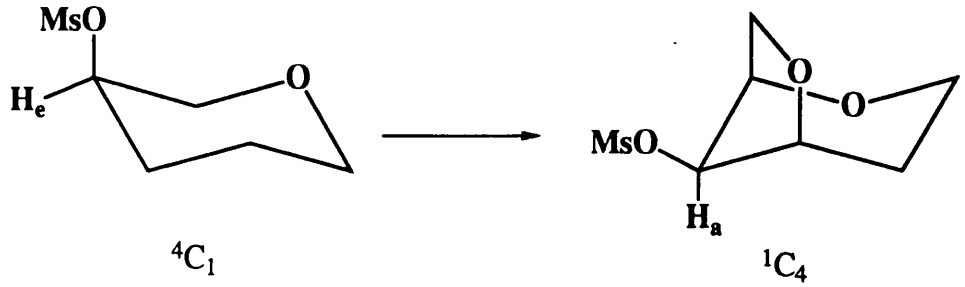


However, in our case thiol introduction by the nucleophilic opening of an epoxide was not favoured. This approach would not allow overall retention of configuration and in order to prepare the direct epoxide opening, would require the preparation of a conformationally restricted 1,6-anhydro derivative. This not only requires extra steps for preparation but the harsh conditions required for the final cleavage of anhydro linkages has been associated with decomposition and poor yields.[135]

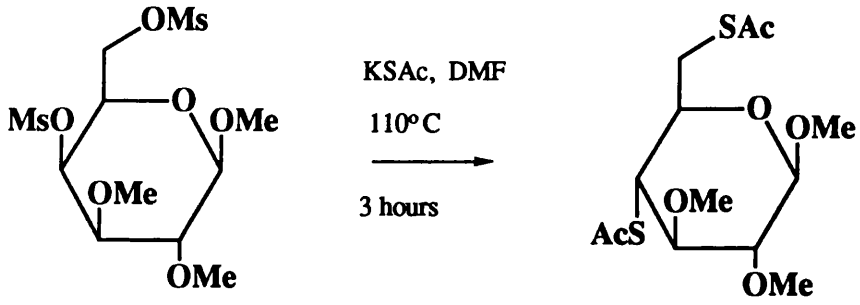
The preparation of this derivative from 2-acetamido-2-deoxy-D-galactopyranose by S_N2 displacement of a 4-sulphonate was considered, although this starting material is almost 40 times more expensive than its D-*gluco* analogue.[158] In addition it has been observed by other workers that even the primary sulphonyloxy group in a galactose derivative is of relatively low reactivity to nucleophiles.[159,160] In the 4,6-dimesyl galactose derivative, the primary group was not completely replaced by treatment with sodium iodide for 20 hours at 115°C, starting material was still present and only a 40% yield of methyl 2,3-di-O-benzoyl-6-deoxy-6-iodo-4-O-mesyl-β-D-galactoside was obtained. For analagous reactions on glucose derivatives, 6 hours at 100°C normally effects a complete primary displacement.



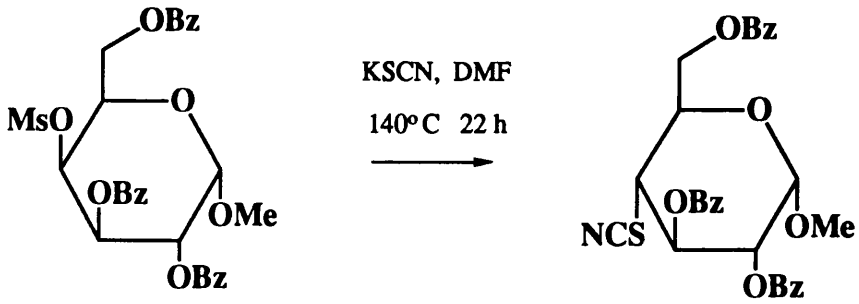
Similarly, the mesyl group in methyl 2,3,6-tri-O-benzoyl-4-O-mesyl-β-D-galactopyranoside has been shown to be completely unreactive to displacement with a variety of thiol nucleophiles in harsh conditions.[135] This effect has been attributed largely to the axial orientation of the C-4 group. Accordingly, a 3,6-anhydro bridge was formed, the presence of which changes the most stable conformation from $^4C_1$ to $^1C_4$; the sulphonyloxy group was now equatorial. However this derivative was still shown to be completely unreactive to any thiol nucleophile.[135]



It has been noted that in methyl 4,6-di-O-mesyl-2,3-di-O-methyl-β-D-galactopyranoside both mesyl groups undergo ready displacement with thioacetate in DMF to give after only 3 hours at 110°C, a good yield of methyl 4,6-di-S-acetyl-4,6-dideoxy-2,3-di-O-methyl-β-D-glucopyranoside.[135]



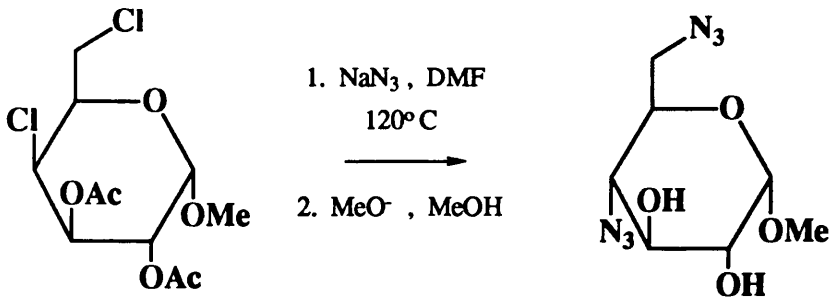
This contrasting reactivity tends to implicate steric crowding of the bulky benzoate groups as perhaps an important reason for the low reactivity of this particular derivative. It is however interesting to note that in a later publication,[161] Gero reports the reaction of an analagous α-galactopyranoside, methyl 2,3,6-tri-O-benzoyl-4-O-methanesulphonyl-α-D-galactopyranoside with potassium thiocyanate in DMF at 140°C for 22 hours gave methyl 2,3,6-tri-O-benzoyl-4-deoxy-4-thiocyano-α-D-glucopyranoside in 47% yield.



This difference in reactivity at position 4 which is apparently the result of anomeric configuration alone, is hard to rationalise, but is presumably the result of an overall steric effect. In addition, differences in the configuration of the final product have also been observed in some halogenation reactions described later are also apparently dependent upon configuration at the anomeric carbon atom.[108]
It can perhaps be speculated that differences in solvation or in configuration are responsible for this and a comprehensive study on the factors affecting sulphonate displacement has been complied by Richardson[162] and these will be discussed later.

A 2-acetamido-2-deoxy-4-O-mesyl-galactopyranose derivative was therefore thought to hold little advantages over the possible preparation of a 2-acetamido-2,4-dideoxy-4-halo-galactopyranose derivative, where the halogen atom acts as leaving group. In addition the halo derivative should be readily prepared from an appropriately protected 2-acetamido-2-deoxy-D-gluco monosaccharide. A further advantage of a halo derivative is a more ready selective deprotection if an 1,3,6-triacyl derivative proved unreactive, allowing assessment of the reactivity of the trihydroxy derivative.

Thus it was hoped that a strategy could be developed along similar lines to that adopted in the synthesis of the 5-thiopyranose derivatives, in which a halogen leaving group was used both to retain overall configuration and to develop common intermediates for the preparation of both thio and amino sugars. However unlike the synthesis of 5-thio monosaccharides, a literature survey revealed no precedent for halogen displacement by a thiol nucleophile at C-4 of either a galactopyranose or glucopyranose derivative. Only a single published synthesis of some azido sugars by Szarek[163] details a double displacement reaction of methyl 2,3-di-O-acetyl-4,6-dichloro-4,6-dideoxy-α-D-galactopyranoside with a twofold excess of sodium azide in DMF at 120-130°C. After 12 hours this gave a syrupy product which following deacetylation gave methyl 4,6-diazido-4,6-dideoxy-α-D-glucopyranoside in 90% yield from the original dichloro derivative.(Scheme 18) A similar displacement of a C-3 chlorine in an allopyranoside derivative is reported in the same publication.



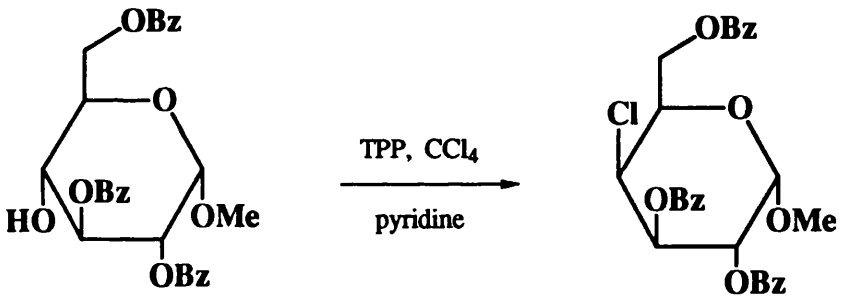
Scheme 18.

On consideration of this reported favourable substitution, it was decided to prepare several 2-acetamido-2,4-dideoxy-4-halo-*galacto* derivatives and to study displacement reactions with a variety of nucleophiles, in an attempt to effect a direct displacement and to add to the literature understanding of the factors controlling such displacements.

Owing to the paucity of literature information dealing with the substitution of the axially orientated 4-halogen atoms in galactose derivatives by thiol nucleophiles, it was decided to assess the reactivity of a series of derivatives, in the hope that it would prove possible by these means to devise a preparation of 2-acetamido-2-deoxy-4-thio-D-glucofuranose from 2-acetamido-2-deoxy-D-glucopyranose. So a series of halogenated intermediates were prepared either by selective acylation or by modifications to the multistep route outlined earlier. Certain of the halogenated intermediates developed for testing were prepared from methyl 2,3,6-tri-O-benzoyl-α-D-glucopyranoside (86). This was synthesised from methyl α-D-glucopyranoside in 70% yield by selective benzoylation with N-benzoylimidazole in acetonitrile according to the method of Varela.[133] This derivative which was used as a foam, gave an ^{1}H NMR spectrum which matched closely with that described in the literature, and gave a satisfactory mass spectrum and microanalysis.

4.6.1 The halogenation of 4-OH in suitably protected α-D-glucopyranose derivatives

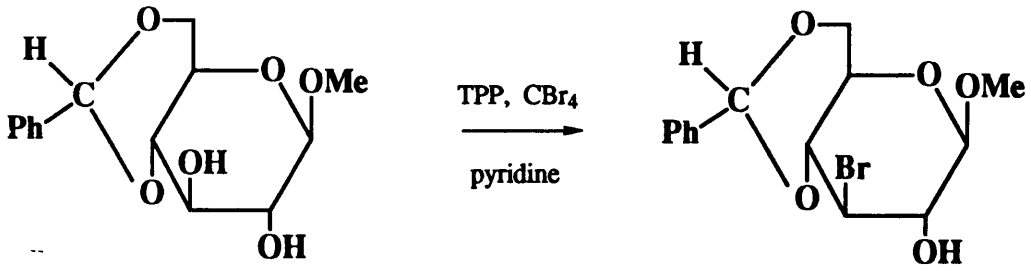
Chlorination of methyl 2,3,6-tri-O-benzoyl-α-D-glucopyranoside (86) was attempted with 2 molar equivalents of TPP and 1 molar equivalent of CCl_4[108] and proceeded smoothly and at noticeably lower temperatures than were previously required for the chlorinate secondary hydroxyl groups in furanose derivatives. T.l.c. showed complete reaction after 1 hour at 50-60°C and the formation of a single, fast-moving product. Following column chromatography, this was isolated as a crystalline substance, whose mass spectrum gave a pseudomolecular ion and ion isotope pattern correct for the substitution of one chlorine atom. Inversion of stereochemistry at C-4 was confirmed by examination of the ^1H NMR spectrum, the coupling constant $J_{3,4}$=4Hz were indicative of a galactose derivative. The compound was therefore identified as methyl 2,3,6-tribenzoyl-4-chloro-4-deoxy-α-D-galactopyranoside (87).



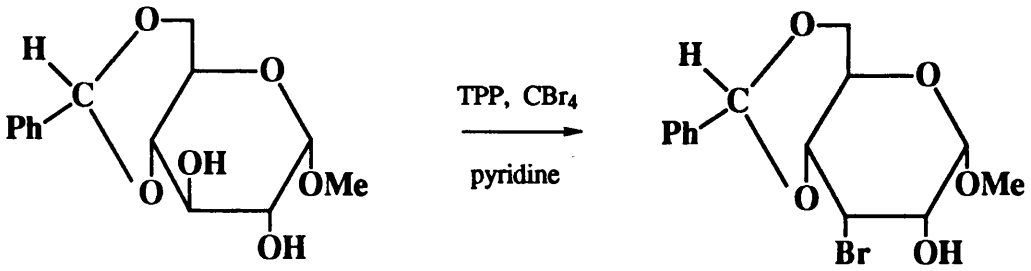
(86) (87)

The leaving group ability of the halogens increases with increasing molecular weight, thus bromine and iodine are both known to be better leaving groups than chlorine. It was therefore decided to prepare the 4-bromo galactoside and to attempt the preparation of an iodo galactoside. However it was considered that these improved properties could give rise to problems of configuration on halogenation.

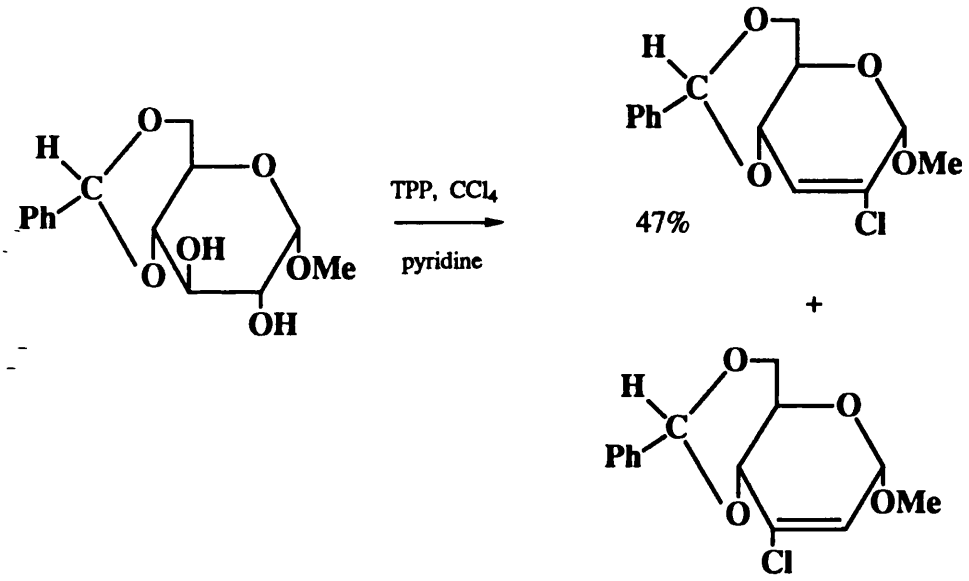
Some previous brominations at secondary hydroxyl groups with a TPP/ CBr_4 reagent mixture have sometimes given rise to bromination with retention of configuration, owing to an initial bromination then undergoing a second nucleophilic displacement by excess brominating reagent. This has been shown to occur in the bromination of methyl 4,6-benzylidene-β-D-glucopyranoside which gave methyl 4,6-benzylidene-β-D-glucopyranoside in 66% yield.[108]



It is however interesting to note that treatment of methyl 4,6-benzylidene-α-D-glucopyranoside with the same molar ratio of the brominating reagent gave methyl 4,6-benzylidene-α-D-mannopyranoside in 47% yield, with inversion of stereochemistry apparently occuring as expected.[108]



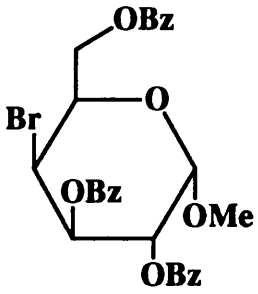
There is also the risk of an increasing amount of elimination side-product especially in the presence of excess reagent. Treatment of methyl 4,6-benzylidene-α-D-glucopyranoside with excess TPP and CCl_4 has been shown to give predominantly methyl 4,6-O-benzylidene-2-chloro-2,3-dideoxy-α-D-*erythro*-hex-2-enopyranoside and methyl 4,6-O-benzylidene-3-chloro-2,3-dideoxy-α-D-*erythro*-hex-2-enopyranoside as a minor product. These elimination reactions were considered to also be potentially troublesome in basic conditions such as those used for deacylation.[108]



The bromination of methyl 2,3,6-tri-O-benzoyl-α-D-glucopyranoside (80) was attempted with 2 molar equivalents of TPP and 1 molar equivalent of CBr_4. Temperature was strictly controlled and the reaction followed by t.l.c. and heating to 50-60°C for 1 hour proved sufficient to give a smooth transformation of the starting material to a single fast-moving spot. Following conventional workup, this was isolated as a white crystalline powder in 80% yield. The mass spectrum revealed a pseudomolecular ion of 591 corresponding to $(M+Na)^+$ with the corrct isotope pattern for one bromine atom. This was identified as being correct for the replacement of the 4-OH by bromine, but the configuration remained unknown.

Examination of the ^1H NMR spectrum revealed a similar pattern to that of the chlorine derivative (87), with $J_{3,4}$= 4.2Hz, correct for the D-*galacto* configuration, however an 0.5ppm upfield shift of H-1 was noticed with the shielding effect probably arising due to the greater bulk of the bromine atom.

On the basis of its ^1H NMR spectrum, this compound was identified as methyl 2,3,6-tri-O-benzoyl-4-bromo-4-deoxy-α-D-galactopyranoside (88).



(88)

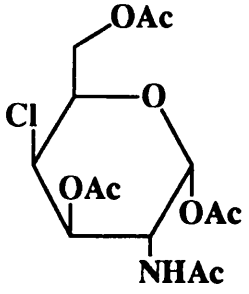
Attempts to iodinate (70) by a similar method failed. Prolonged heating at 90°C with 3 molar equivalents of TPP and 3 molar equivalents of CI_4 resulted in no discernable reaction and following workup unchanged starting material was recovered. On another occasion, following prolonged heating under these conditions, 1 molar equivalent of CBr_4 was added which resulted in the rapid formation of a fast-moving product, discovered to be methyl 2,3,6-tri-O-benzoyl-4-bromo-4-deoxy-α-D-galactopyranose (88). This rapid incorporation of bromine after a long period of inactivity in the presence of CI_4 rules out the accidental presence of water, which may have provided an alternative explanation for reaction failure.

Although the use of TPP and CI_4 in pyridine has proved an efficient direct reagent for iodinating primary hydroxyl groups,[164] no reports exist for its use with secondary hydroxyl groups in monosaccharide derivatives and it was considered that perhaps the greater bulk of the iodine complex prevents its reaction with the more hindered secondary hydroxyl functions. However, another reagent mixture which has been successfully employed in the iodination of secondary hydroxyl functions is imidazole, iodine and triphenylphosphine in toluene. Originally employed to convert *trans* 1,2-diol system in hexopyranosides to form the olefin, it was found that treating methyl 3,4,6-tri-O-benzyl-α-D-glucopyranoside with the same reagent mixture gave methyl 3,4,6-tri-O-benzyl-2-deoxy-2-iodo-α-D-mannopyranoside in high yield.[165] A similar treatment of of 1,2:5,6-di-O-isopropylidene-α-D-glucofuranose has been claimed to give 3-deoxy-3-iodo-1,2:5,6-O-isopropylidene-α-D-allofuranose, without acetal migration.[108]

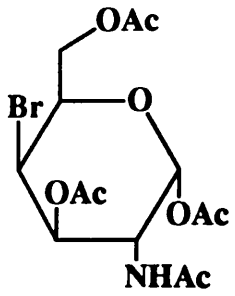
Time did not allow the use of this reagent with our derivatives but it probably represents the reagent of choice for the attempted preparation of methyl 2,3,6-tri-O-benzoyl-4-deoxy-4-iodo-α-D-galactopyranose and it would be interesting to compare its properties with those of the corresponding chloro and bromo derivatives.

4.6.2 2-acetamido-4-halo-galactopyranose derivatives

It was decided to also prepare some acetylated derivatives, analagous to those used by Szarek[163] in his azide displacement reactions, but possessing the 2-acetamido function. These were conveniently prepared from 2-acetamido-2-deoxy-α-D-glucose by the multistep pathway outlined earlier. Chlorination of the 1,3,6-triacetate (76) in a similar fashion to that employed earlier gave 1,3,6-tri-O-acetyl-2-acetamido-4-chloro-2,4-dideoxy-α-D-galactopyranose (88) in 87% yield. In addition the bromine derivative 1,3,6-tri-O-acetyl-2-acetamido-4-bromo-2,4-dideoxy-α-D-galactopyranose (89) was also prepared. The ^1H NMR spectra of both derivatives bore similarities to those of the tribenzoate derivatives (87) and (88) in terms of coupling constants.



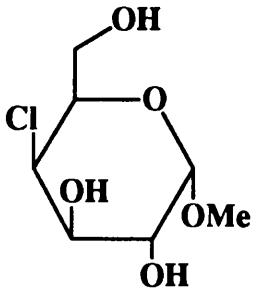
(89)



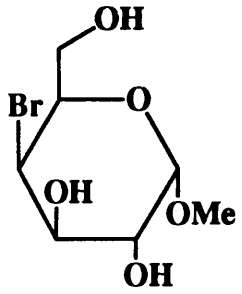
(90)

4.6.3 Deacylation with sodium methoxide

From Owen's work with the displacement of an analogous 4-mesyl group with thiol nucleophiles,[135] it was anticipated that the steric bulk of the benzoyl groups may inhibit reactivity. It was therefore decided to attempt to debenzoylate (87) and (88) with sodium methoxide despite the possibility of base-catalysed elimination. In each case, treatment with a solution of sodium in methanol at room temperature effected a smooth debenzoylation to give methyl 4-chloro-4-deoxy-α-D-galactopyranoside (91) and methyl 4-bromo-4-deoxy-α-D-galactopyranoside (92) respectively in high yield.



(91)



(92)

In each case, examination of the ^{1}H NMR spectrum revealed similar coupling to that observed previously, confirming the D-*galacto* configuration.

4.6.4 The reaction of the 4-halogalactopyranose derivatives with various nucleophiles

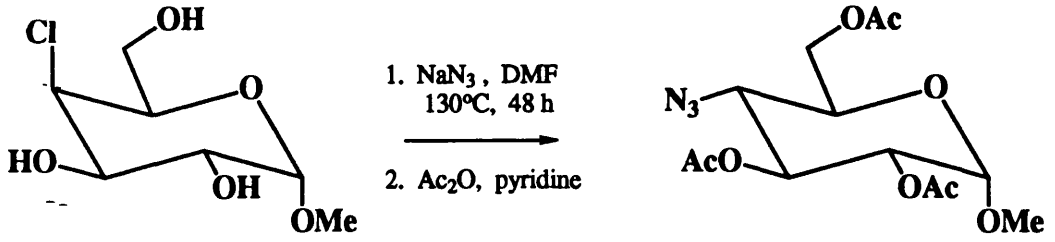
Following the preparation of these halogenated derivates, their reaction with selected nucleophiles was assessed, although on a purely qualitative basis. In each case the compound was heated to 130°C in dry DMF under an inert atmosphere for 48 hours, with a molar excess of the nucleophile in question. Following this, it was judged that any reaction which had taken place should be readily detectable.

The reactions were monitored by t.l.c and also in a more definitive manner, by i.r. spectroscopy. This proved a convenient yet powerful technique of judging on a qualitative basis whether there was any reaction with azide or thiocyanate. After 24 hours, a small quantity of the reaction mixture was removed and evaporated. The residue was extracted in $CHCl_3$ and washed with several portions of water, dried and evaporated under reduced pressure to a syrup, a thin film of which was examined by i.r. The presence of organic thiocyanate can readily be distingiushed from inorganic thiocyanate by its shift to a higher frequency (typically 2140-2180 cm^{-1})[166] and the same was found to be true of organic azide with shifts from 2130cm^{-1} (inorganic) to 2150cm^{-1} (organic). After 48 hours of reaction, the bulk of the material underwent similar workup and examination by i.r., together with mass spectroscopic examination of the crude material.

Methyl 2,3,6-tetra-O-benzoyl-4-chloro-4-deoxy-α-D-galactopyranoside (87) was treated with potassium thioacetate, under the forcing conditions outlined above but no reaction was observed and starting material was recovered. As thiocyanate anion is more stable in hot DMF it was hoped that this would prove more successful, however following heating at 130°C for 48 hours, only starting material was recovered and i.r. spectroscopy revealed no organic thiocyanate. Suprisingly, treatment with 3 molar equivalents of sodium azide at 120°C for 48 hours also failed, no organic azide could be detected and the bulk of starting material was recovered.

As this lack of reactivity could be attributable to the presence of the three bulky benzoyl groups, the 2-acetamido triacetate derivative (89) was also examined with thiocyanate, but again even under forcing conditions, no reaction took place.

The deprotected 4-chloro derivative, methyl 4-chloro-4-deoxy-α-D-galactopyranoside (91) was next examined, with both thiocyanate and azide. In agreement with Szarek's findings,[163] it reacted readily with azide in DMF at 120-130°C to give following acetylation, methyl 2,3,6-tri-O-acetyl-4-azido-4-deoxy-α-D-glucopyranoside (93) in 62% yield under the conditions outlined earlier (scheme 18).



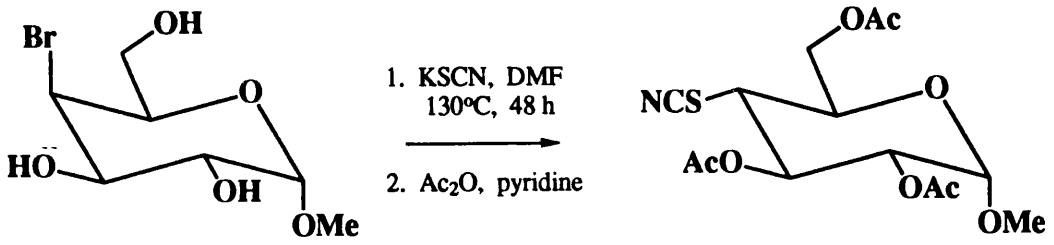
(91) (93)

Scheme 18.

However no reaction was observed with thiocyanate and following acetylation, methyl 2,3,6-tri-O-acetyl-4-chloro-4-deoxy-α-D-galactopyranoside was isolated in almost quantitative yield from (91).

Next, the series of brominated derivatives were examined as it was hoped that the improved leaving-group ability of bromine over chlorine would lead to a more reactive molecule. First was methyl 2,3,6-tri-O-benzoyl-4-bromo-4-deoxy-α-D-galactopyranoside (88) but unsuprisingly, this derivative displayed no reactivity with either thiocyanate or azide anions. Again, this perhaps should not be attributed solely to an inherently unreactive molecule but also to the possible steric inhibition of the surrounding benzoate groups.

1,3,6-tri-O-Acetyl-2-acetamido-4-bromo-2,4-dideoxy-α-D-galactopyranose (90) was only tested with thiocyanate anion but no reaction was observed even under forcing conditions. However when methyl 4-bromo-4-deoxy-α-D-galactopyranoside (92) was treated with thiocyanate anion in DMF at 120-130°C for 48 hours then, following acetylation and workup, an i.r. spectrum of the crude syrup showed the presence of organic thiocyanate. The material was purified by column chromatography to give methyl 2,3,6-tri-O-acetyl-4-deoxy-4-thiocyano-α-D-glucopyranoside (94) in 64% yield together with some unreacted starting material identified as methyl 2,3,6-tri-O-acetyl-4-bromo-4-deoxy-α-D-galactopyranoside. (Scheme 19.)



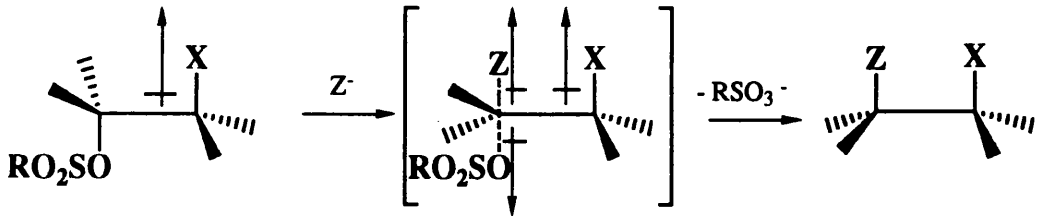
(92) (94)

Although the yield of the thiocyanate was only moderate, it was considered that a synthesis of a 4-thioglucofuranose was feasible by this means. Reduction of (94) with methoxide should yield a thiol analogous to that described by Vegh and ring-contraction should take place under the similar conditions. It is proposed that this sequence may represent a shorter and more convenient route for the preparation of 4-thioglucofuranose, with overall retention of configuration.

4.7 Factors affecting nucleophilic displacement at secondary hydroxyl groups

A review by Richardson[162] has compliled the factors thought to be important in the displacement of sulphonates by anionic nucleophiles and it is of interest to examine these. Displacement reactions at secondary positions by various nucleophiles provides a versatile method of introducing desired funtional groups such as thiols, amines and deoxy derivatives. The great enhancement of reactivity in polar aprotic solvents such as DMF is generally considered to occur by greater solvation of the transition state thus lowering the activation enthalpy of the reaction. The position of the leaving group still plays a critical role in its reactivity as do other steric and polar factors which may influence the formation of the S_N2 transition state.

The geometry of the S_N2 transition state is well-documented and its formation involves the simultaneous generation of two highly polarised bonds, one in the process of formation and one in the process of degeneration (scheme 20). The formation of these bonds will be affected by the nature of neighbouring substituents and if these are elctronegative in character such as halogens or hydroxyl groups then the permanent dipole moment of the substituent-to-carbon bond may inhibit the development of the transition state when an anionic nucleophile approaches. This hinderance is described as being greatest when the dipoles are antiparallel. On the other hand the use of neutral nucleophiles will reverse the polarity of the forming bond in the transition state, giving rise to favourable polar interactions with neighbouring electronegative substituents.

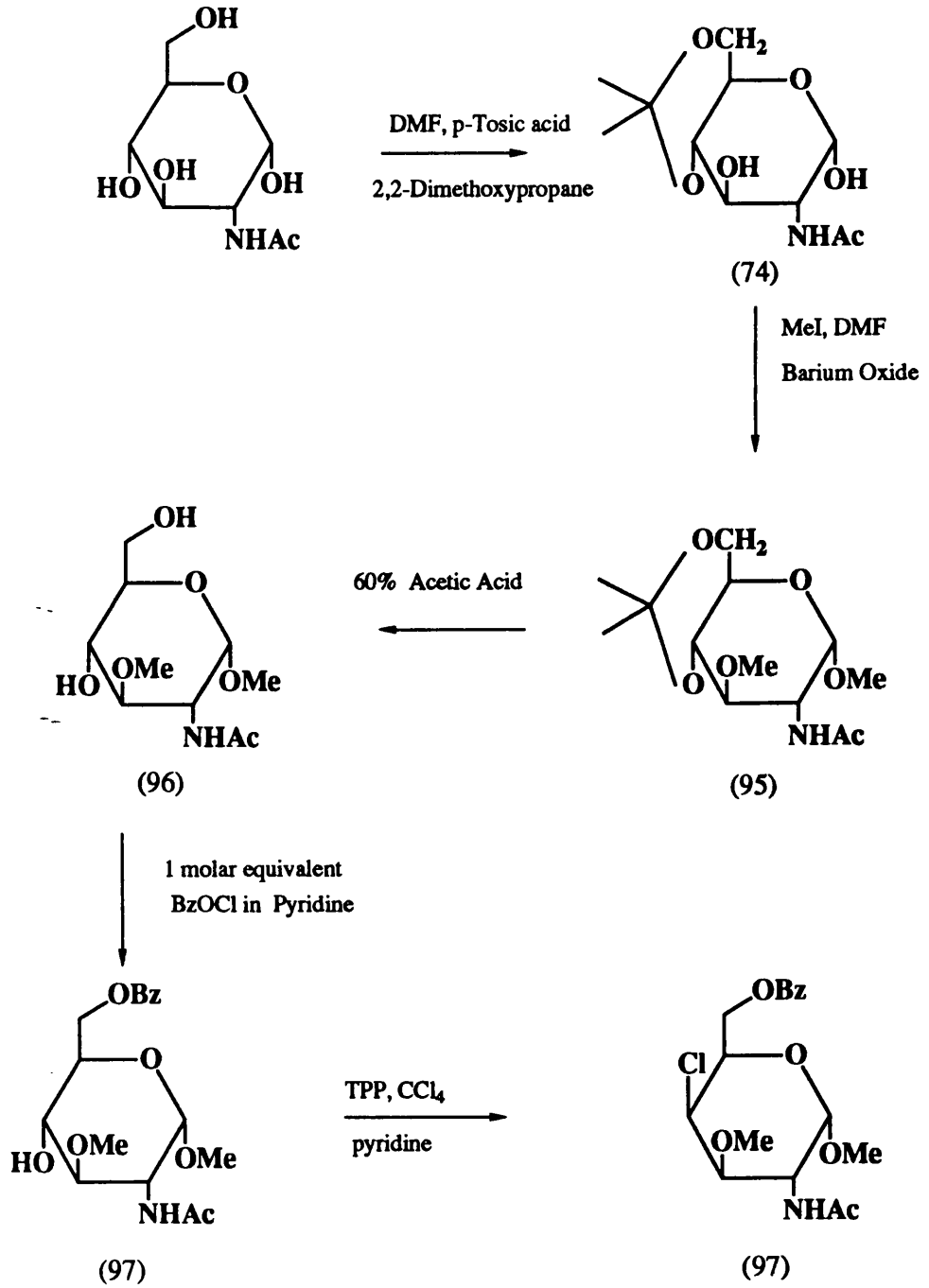


Scheme 20.

This approach has been used to predict reactivity in a qualitative manner, but other factors which must also be taken into careful account include the size of neighbouring substituents and the possibility of the molecule reacting in an alternative conformation to its ground state.

The relative strength of the nucleophile must also be considered. Although it is difficult to directly compare nucleophiles, relative base strengths may provide a rough guide. Although the pka values quoted were determined in aqueous solution and by different techniques, HN_3 was valued at approximately 4.5, whereas estimated values for HSCN are approximately 0.5 with no dissociation being detected in water.[167] Therefore when we consider the strengths of the conjugate bases, N_3^- is a more powerful base than SCN^- and although in DMF this may differ, on a rough basis it would imply that N_3^- was the more powerful nucleophile. This would match our experimental findings.

Following the lack of success in the displacement of chlorine derivatives, it was decided to prepare a 3-O-methyl derivative in an attempt to assess whether chlorine in this axial position was too poor a leaving group to undergo displacement by thiocyanate, or whether a polar dipole effect set up by the adjacent 3-OH was proving inhibitory to development of the transition state. Methyl 2-acetamido-6-O-benzoyl-4-chloro-2,4-dideoxy-3-O-methyl-α-D-galactopyranoside (97) was accordingly prepared by the multistep route (scheme 21) used to prepare the triacetate derivative (89) earlier. It would perhaps have been better to prepare a trimethyl ether derivative by permethylation of (91) however it was considered that C-3 substituents were likely to influence reactivity to the greatest extent and that the presence of a 6-benzoate should not inhibit reactivity in this case.



Scheme 21.

Following reaction, the starting material was recovered in almost quantitative yield, and it must be concluded that 4-Cl does not seem amenable to displacement by SCN^-, at least under these conditions.

CHAPTER FIVE

CONCLUSIONS

The development of novel therapies to combat parasitic disease continues to be of vital importance for the welfare of entire nations. Scarce resources demand a rational approach towards drug design and in the case of filarial parasites several possible biochemical targets have been identified. In this regard, selective glucose uptake inhibition is attractive with an effective agent perhaps being most useful in conjunction with other treatments. Work has been presented on the identification of putative inhibitors in model filarial worms and the development of further synthetic targets with possible improved activity - the 2-deoxy-5-thio and 5-amino hexoses perhaps via their corresponding 2-amino derivatives which are of interest in their own right .

Novel routes for the preparation of 5-thio and 5-amino hexopyranoses were developed using deoxyhalo intermediates. Initial studies with a model glucofuranose compound pointed to several interesting synthetic alternatives. The displacement of a primary chlorine atom by thiocyanate followed by a one-step reduction and cyclisation shortened an already proven method of thiol introduction. The ease of halogen displacement at C-5 led to further work which highlighted the potential inhibitory properties of bulky groups at C-6.

In the design of these synthetic pathways the choice of a suitably protected 2-amino derivative was essential. The use of methyl 2-benzamido-glucofuranoses derived from the well-known phenyl oxazolines seemed at first to be ideal. However difficulties were encountered in the halogenation of C-5, this reaction gave only a low yield of the desired material. The identity of the major product remains unknown although further investigation would be interesting and perhaps a non-glycosidic derivative would have more closely matched the model compound.

However the use of an acyclic dimethyl acetal proved successful, with a novel route involving direct displacement of C-5 halogen with thioacetate being compared with the traditional anhydro-episulphide conversion. It was interesting that C-6 benzoylation precluded C-5 halogen displacement as earlier demonstrated in the model series. The novel route gave a lower overall yield with intermediates which were not crystalline and difficult to purify, however it is proposed that this novel route could be developed perhaps by investigation of C-6 halogen displacement and is inherently more versatile.

In order to prepare the desired 2-deoxy derivatives, de-N-acetylation followed by reductive deamination was necessary. Application of a method of radical-induced deamination has produced 2-deoxy-D-glucose from 2-amino-2-deoxy-D-glucose and this method was employed with 2-amino-2-deoxy-5-thio-D-glucose. Although insufficient quantities were prepared to fully characterise the final product, mass spectral evidence seems to support the successful preparation of 2-deoxy-5-thio-D-glucose.

The 4-thio and amino hexoses - configurational isomers of the previous compounds were then examined. The requirement for partially protected intermediates led to a study of the selective benzoylation of 2-acetamido-2-deoxy-D-glucose. A one-pot preparation of 2-acetamido-1,3,6-tri-O-benzoyl-2-deoxy-α-D-glucopyranose was investigated with high yields and selectivity being achieved with both N-benzoylimidazole and benzoyl cyanide. A preparation of 1,3,5,6-tetra-O-acetyl-2-acetamido-2-deoxy-4-thio-D-galactofuranose was performed via thiocyanate displacement of a 4-sulphonate.

The preparation of 4-thio and amino glucofuranose derivatives with overall retention of configuration remained a major goal. Model studies with methyl glycoside derivatives were carried out and confirmed the utility of the deoxyhalo derivatives in this regard. The introduction of azide as an amino precusor was succesfully achieved by chlorine displacement in methyl 4-chloro-4-deoxy-D-galactopyranoside. In a similar fashion it proved possible to displace bromine with thiocyanate in methyl 4-bromo-4-deoxy-D-galactopyranoside. It is anticipated that these reactions will represent the key step in more convenient preparations of the 4-thio and amino hexoses.

The potential diverse and powerful biological properties of monosaccharide derivatives are under investigation in many areas besides parasitology, therefore the continued development of monosaccharide chemistry is increasingly relevant. Many candidate drug substances are or contain monosaccharides and the search for new, improved synthetic pathways has been the inspiration behind this work.

CHAPTER SIX

General Experimental Details

Instrumentation

Melting points were measured on an electrothermal Gallenkamp melting point apparatus and are uncorrected. Melting point ranges are quoted in ^{o}C and the abbreviation "d" refers to decomposition at the melting point.

Infrared spectra were recorded from potassium bromide discs (unless otherwise stated) on a Perkin-Elmer 841 Infrared Spectrophotometer, and values are quoted in cm^{-1}.

^{1}H-Nuclear magnetic resonance spectra were recorded on a Bruker WP80SY FT-NMR spectrometer, a 300MHz Varian XL300 FT-NMR spectrometer or a Bruker AM500 500MHz FT-NMR spectrometer, in deuteriochloroform solution (unless otherwise stated) using TMS as internal standard. The following descriptions of signals have been used.

s = singlet, d = doublet, dd = doublet of doublets,
t = triplet, q = quartet, m = multiplet, b = broad,
dist. d = distorted doublet.

^{13}C-Nuclear magnetic resonance spectra were recorded on a 300MHz Varian FT-NMR spectrometer or on a Bruker 80-FT NMR spectrometer in deuteriochloroform solution (unless otherwise stated) with a TMS internal standard. Chemical shifts (δ) are quoted in parts per million and coupling constants (J) in Hertz (Hz).

Elemental analyses were determined by a Carlo Erba elemental analyser model 1160.

FAB mass spectra were recorded on a VG Analytical ZAB-SE double focussing magnetic sector mass spectrometer and low resolution EI and CI mass spectra on a VG Masslab 12-250 Quadrupole mass spectrometer.

Chromatography

Analytical chromatographic separations (t.l.c.) were performed on 0.2mm x 2cm x 5cm strips of Keiselgel 60F 254 and preparative layer chromatographic (p.l.c.) separations were performed on 0.75mm x 20cm x 20cm layers of Keiselgel GF 254; these separations were visualised either by viewing under ultraviolet light or by spraying with an acidic solution of ceric ammonium sulphate (2%w/v ceric ammonium sulphate in 50%v/v aqueous sulphuric acid). Large scale separations were accomplished by flash chromatography using the method outlined by Still.[168]

Silica gel for t.l.c.: Keiselgel 60 254 (Merck) contains approximately 13% w/w added calcium sulphate and an inorganic fluorescent dye. Silica gel for flash chromatography: Sorbsil C 60-H (40-60μm) (Rhone-Poulenc).

General Solvents, reagents and reaction conditions

Reactions involving dry solvents were carried out under an inert atmosphere (argon or nitrogen) with all glassware predried in an oven (110°C) and cooled in an inert atmosphere prior to use.

Commercial reagent grade solvents were used, unless otherwise stated and were dried as follows:-

Methanol and Ethanol were dried by boiling under reflux over "activated" magnesium turnings, followed by distillation onto activated 4A molecular sieves.

Pyridine was dried by reflux over potassium hydroxide pellets, followed by distillation and stored under N_2 over fresh potassium hydroxide pellets.

Dry DMF was obtained by boiling the commercial reagent under reflux over calcium hydride for 30 minutes, followed by distillation under reduced pressure onto activated molecular sieves.

Acetone was dried by standing over anhydrous potassium carbonate overnight, followed by distillation onto 4A molecular sieves.

Chloroform, dichloromethane and carbon tetrachloride were dried and/or freed from any ethanol stabiliser by distillation from phosphorus pentoxide.

Acetonitrile was boiled under reflux over calcium hydride, distilled and stored over 4A molecular sieves.

1,4-Dioxan was continously distilled over sodium metal in an inert atmosphere using benzophenone as indicator, then distilled into the reaction vessel.

Toluene and tetrahydrofuran were similarly dried over potassium.

Certain reagents required purification before use. Commercial benzoyl choride was purified by fractional distillation and stored in ampoules under N_2. Commercial triethylamine and diethylamine were also distilled before use.

In the following section, mass spectral data for chlorine or bromine-containing compounds may be superscripted as follows:- Ions (a) show an isotope pattern corresponding to 1 chlorine atom - a 3:1 doublet, 2 mass units apart. Ions (b) show isotope pattern corresponding to 2 chlorine atoms - a 9:6:1 triplet, 2 mass units apart. Ions (c) show an isotope pattern corresponding to 1 bromine atom - a 1:1 doublet, 2 mass units apart.

1,2:5,6-Di-O-isopropylidene-α-D-glucofuranose (26)

Sulphuric acid (96%, 1ml) was added dropwise to an ice-cooled suspension of anhydrous D-glucose (10g) in acetone (200ml) and the mixture left to stir at room temperature for 12 hours. The resulting solution was then cooled to 0°C and neutralised by the addition of small amounts of 50% NaOH solution. A small amount of solid $NaHCO_3$ was added to maintain the solution at near neutrality. After standing for 3 hours, the salts were removed by filtration and the acetone solution was concentrated under reduced pressure to a thick syrup which solidified on standing. Recrystallisation from ethyl acetate-petrol gave 7.90g (60%) of white needles, m.p. 110-112°C (lit[123]: 110-111°C)

Mass spectral data (Ammonia C.I.)	$(M+NH_4)^+$ m/z 278 (97%), $(M+H)^+$ 261 (100%), 245 (13%), 220 (63%), 203 (19%), 180 (7%), 162 (8%), 144 (6%), 101 (20%).
^{1}H-NMR data (500MHz)	δ 5.92 (d, 1H, $J_{1,2}$=3.6, H-1); 4.51 (d, 1H, $J_{1,2}$=3.6, H-2); 4.31 (m, 2H, H-3, H-5); 4.14 (dd, 1H, $J_{6,6'}$=8.6, $J_{5,6}$=6.2, H-6); 4.04 (dd, 1H, $J_{4,5}$=7.8, $J_{3,4}$=2.7, H-4); 3.97 (dd, 1H, $J_{6,6'}$=8.6, $J_{5,6'}$=5.3, H-6'); 2.78 (bs, 1H, 3-OH); 1.48, 1.42, 1.37, 1.30 (4s, 12H, 1,2:5,6-O-isopropylidene).
Analysis	$C_{12}H_{20}O_6$ requires C 55.37 H 7.74 found C 55.58 H 7.74

3-O-Acetyl-1,2:5,6-di-O-isopropylidene-α-D-glucofuranose (27)

A solution of (26) (7g) in dry pyridine (20ml) underwent conventional acetylation, with the addition of acetic anhydride (2.80ml, 1.1 molar equiv.) and storage at 4°C for 24 hours. The solution was then poured onto ice and extracted with $CHCl_3$ (2x75ml). The combined $CHCl_3$ solution was then washed with 2M H_2SO_4 (2x25ml), dilute $NaHCO_3$ solution (2x10ml) and water (1x10ml). The solution was dried over $MgSO_4$, filtered and evaporated under reduced pressure to a syrup which crystallised from ether-hexane giving 7.8g (96%) of white prisms, mp 57-59°C.

Mass spectral data (Ammonia C.I.)	$(M+H)^+$ m/z 303 (13%), 287 (19%), 262 (8%), 245 (100%), 187 (9%), 113 (8%), 101 (40%), 58 (12%).
^{1}H-NMR (300MHz)	δ 6.02 (d, 1H, $J_{1,2}$=3.8, H-1); 4.60 (dd, 1H $J_{1,2}$=3.8, H-2); 4.52 (m, 3H, H-3,H-4,H-5); 3.91 (m, 2H, H-6, H-6'); 2.12 (s, 3H, 3-Ac); 1.57, 1.52, 1.33, 1.25 (4s, 12H, 1,2:5,6-O-isopropylidene).
Analysis	$C_{14}H_{22}O_7$ requires C 55.62 H 7.33 found C 55.73 H 7.29

1,2-O-Isopropylidene-α-D-glucofuranose (28)

A solution of (26) (0.65g) in aqueous 60% acetic acid was kept at room temperature for 24 hours, then evaporated under reduced pressure. Three portions of water (5ml) were added and evaporated to remove any traces of acetic acid. The resulting syrup slowly crystallised on standing and was recrystallised from methanol-ether to give 0.51g (93%) of white needles, mp 159-161°C (lit[123]: 160-161°C).

Mass spectral data: (Ammonia C.I.)	$(M+NH_4)^+$ m/z 238 (33%), 220 (6%), 205 (22%), 180 (100%), 162 (16%), 145 (10%), 127 (11%), 113 (16%), 100 (21%), 85 (16%), 76 (8%), 71 (8%), 59 (15%), 43 (9%).

^{1}H-NMR (500MHz) (D_2O)	δ 6.05 (d, 1H, $J_{1,2}$=5.0, H-1); 4.60 (d, 1H, $J_{1,2}$=5.0, H-2); 4.35 (d, 1H, $J_{3,4}$=2.7, H-4); 4.11 (dd, 1H, $J_{4,5}$=9.0, $J_{3,4}$=2.7, H-3); 3.94 (m, 1H, H-5); 3.83 (dd, 1H, $J_{6,6'}$=12.1, $J_{5,6}$=2.9, H-6); 3.67 (m, 1H, $J_{6,6'}$=12.1, H-6'); 1.54, 1.39 (2s, 6H, 1,2-isopropylidene).	
Analysis	$C_9H_{16}O_6$ requires	C 49.09 H 7.32
	found	C 49.18 H 7.26

3-O-Acetyl-1,2-O-isopropylidene-α-D-glucofuranose (29)

A solution of (27) (4g) in aqueous 60% acetic acid (20ml) was kept at room temperature for 24 hours, then evaporated under reduced pressure. Three portions of water (5ml) were added and evaporated to remove any remaining traces of acetic acid. The resulting syrup was chromatographed on an SiO_2 column with $CHCl_3$: MeOH 9:1 to give 3.12g (90%) of white needles, mp 122-124°C.

Mass spectral data (Ammonia C.I.)	$(M+NH_4)^+$ m/z 280 (100%), 245 (23%), 222 (36%), 205 (50%).
^{1}H NMR data (500MHz)	δ 5.97 (d, 1H, $J_{1,2}$=3.7, H-1); 5.28 (d, 1H, $J_{3,4}$=2.5, H-3); 4.56 (d, 1H, $J_{1,2}$=3.7, H-2); 4.18 (dd, 1H, $J_{3,4}$=2.5, $J_{4,5}$=9.0, H-4); 3.85 (bd, 1H, $J_{5,OH}$=11.1, OH-5); 3.73 (bm, 1H, H-5); 3.65 (bm, 1H, H-6); 3.04 (bm, 1H, H-6') 2.22 (bs, 1H, 6-OH); 2.11 (s, 3H, 3-Ac); 1.59, 1.53 (2s, 6H, 1,2-O-isopropylidene).

Analysis	$C_{11}H_{18}O_7$ requires	C 50.38 H 6.92
	found	C 50.62 H 7.05

5,6-Dichloro-5,6-dideoxy-1,2-O-isopropylidene-β-L-idofuranose (31)

To an ice-cooled solution of 1,2-O-isopropylidene-α-D-glucofuranose (28) (0.1g, 0.45mmol) in dry pyridine (5ml) under argon, was added TPP (0.72g, 6 molar equivs) and CCl_4 (0.135ml, 3 molar equivs). The solution was stirred at 0°C for 30 minutes and then allowed to warm to room temperature. The solution was then heated at 70°C for a further 30 minutes at which point t.l.c. ($CHCl_3$:MeOH 9:1) showed an absence of starting material and two fast-moving spots. The reaction was cooled, quenched by the addition of methanol (1ml) and evaporated under reduced pressure to a syrup. This was extracted with ether (3x30ml), concentrated to a small volume and crystalline TPP oxide was removed by filtration. The remaining syrup was chromatographed on an SiO_2 column with ethyl acetate-petrol 4:1 to give 0.076g (65%) of the major product (31) as a clear syrup.

Mass spectral data (E.I.): $M^{+\cdot}$ m/z 256 not seen, $(M-15)^{+\cdot}$ 241[b] (17%), 231 (8%), 219 (100%), 201 (69%), 181 (37%), 163 (39%), 145 (43%), 127 (35%).

^{1}H-NMR (300MHz): δ 5.96 (d, 1H, $J_{1,2}$=4.6, H-1); 4.54 (d, 1H, $J_{1,2}$=4.6, H-2); 4.38 (d, 1H, $J_{4,5}$=2.3, H-4); 4.13 (m, 2H, H-3, H-5); 3.87 (dist. dd, 1H, $J_{6,6'}$=11.4, $J_{5,6}$=2.3, H-6); 3.74 (dd, 1H, $J_{6,6'}$=11.4, $J_{5,6'}$=5.7, H-6'); 3.16 (bs, 3-OH); 2.03 (s, 3H, 3-Ac); 1.50, 1.30 (2s, 6H, 1,2-isopropylidene).

3-O-Acetyl-5,6-dichloro-5,6-dideoxy-1,2-O-isopropylidene-β-L-idofuranose (30).

To an ice-cooled solution of 3-O-acetyl-1,2-O-isopropylidene-α-D-glucofuranose (27) (2.49g, 9.5mmol) in dry pyridine (20ml) under argon, was added TPP (14.96g, 6 molar equivs) and CCl_4 (2.76ml, 3 molar equivs). The solution was stirred at 0°C for 30 minutes and then allowed to warm to room temperature. The solution was then heated at 70°C for a further 30 minutes at which point t.l.c. (petrol-ethyl acetate 7:3)

indicated an absence of starting material and two fast-moving spots. The reaction was cooled, quenched by the addition of methanol (5ml) and evaporated under reduced pressure to a syrup. This was extracted with ether (3x50ml), concentrated to a small volume and the crystalline TPP oxide was removed by filtration. The remaining syrup was chromatographed on an SiO_2 column with ethyl acetate-petrol 3:7 to give 2.30g (81%) of (30) as white needles, mp 91-93°C.

Mass spectral data (Ammonia C.I.)	$(M+NH_4)^+$ m/z 316[b] (42%), 283[b] (19%), 258[b] (58%), 241[b] (100%), 205[a] (10%), 198[b] (13%), 143[a] (9%), 85 (10%), 76 (10%), 58 (9%).
^{1}H-NMR data (300MHz)	δ 5.96 (d, 1H, $J_{1,2}$=3.7, H-1); 5.29 (d, 1H, $J_{1,2}$=3.7, H-2); 4.56 (m, 2H, H-3,H-4); 4.28 (q, 1H, $J_{4,5}=J_{5,6}=J_{5,6'}$=6.7, H-5); 3.78 (m, 2H, H-6, H-6'); 2.11 (s, 3H, 3-Ac); 1.53, 1.32 (2s, 6H, 1,2-O-isopropylidene).

Analysis	$C_{11}H_{16}Cl_2O_5$	requires	C 44.16 H 5.39
		found	C 44.43 H 5.35

3-O-Acetyl-6-S-acetyl-5-chloro-5,6-dideoxy-1,2-O-isopropylidene-β-L-idofuranose (33)

Potassium thioacetate (0.76g, 1.3 molar equivs.) was added to a solution of (30) (0.153g, 0.51mmol) in dry acetone (10ml) under argon. The solution was warmed at 40°C for 14 hours then evaporated under reduced pressure to a syrup which was chromatographed on an SiO_2 column with ether-hexane 3:2 to give 0.12g (65%) of (33) as a straw-coloured syrup. Also isolated were the 5,6-dithioacetate (0.03g, 14%) and unchanged starting material (0.03g, 10%).

Mass spectral data (Ammonia C.I.)	$(M+NH_4)^+$ m/z 356[a] (100%), 298[a] (29%), 221[a] (23%).

^{1}H-NMR data (300MHz)	δ 5.94 (d, 1H, $J_{1,2}$=3.5, H-1); 5.30 (d, 1H, $J_{3,4}$=3.3, H-3); 4.51 (d, 1H, $J_{1,2}$=3.5, H-2); 4.36-4.15 (m, 1H, H-4); 3.77 (m, 1H, H-5); 3.40 (dd, 1H, $J_{6,6'}$=14.2, $J_{5,6}$=3.8 H-6); 2.98 (dd, 1H, $J_{6,6'}$=14.2, $J_{5,6'}$=9.3, H-6'); 2.34 (s, 3H, SAc); 1.52, 1.31 (2s, 6H, 1,2-isopropylidene).

3-O-Acetyl-5-chloro-5,6-dideoxy-1,2-O-isopropylidene-6-thiocyano-β-D-idofuranose (34).

To a solution of (30) (0.1g, 0.34mmol) in dry DMF (10ml) under argon was added potassium thiocyanate (0.326g, 10 molar equivs.) and the solution was stirred at 100-110°C for 48 hours. T.l.c. (petrol-ethyl acetate 7:3) indicated an absence of starting material and the formation of a single polar product. The reaction mixture was filtered and concentrated to a syrup which was taken up in $CHCl_3$ (2x25ml). The combined $CHCl_3$ portions were washed with water (2x10ml), dried over $MgSO_4$, filtered and concentrated under reduced pressure to a syrup which crystallised on standing to give 0.106g (92%) of crude solid. This could be recrystallised from ethyl acetate-petrol to give white plates mp 94-95°C.

The reaction was repeated with the inclusion of 18-crown-6 (100mg) and the same quantities of reactants at 110°C. This resulted in complete reaction within 36 hours but with no significant difference in yield. Nothing corresponding to a dithiocyanate could be detected. The reaction was repeated again with 10 molar equivalents of potassium thiocyanate and 18-crown-6 at 140°C, but again only monothiocyanate could be found.

Mass spectral data (Ammonia C.I.)	$(M+NH_4)^+$ m/z 339[a] (100%), 281[a] (15%), 264 (13%).
I.R.	strong peak 2160cm^{-1} (SCN)

^{1}H-NMR data (300MHz)	δ 5.96 (d, 1H, $J_{1,2}$=3.7, H-1); 5.30 (d, 1H, $J_{3,4}$=3.0, H-3); 4.56 (d, 1H, $J_{1,2}$=3.7, H-2); 4.45 (dd, 1H, $J_{4,5}$=9.9, $J_{3,4}$=3.0, H-4); 4.33 (m, 1H, H-5); 3.48 (dd, 1H, $J_{6,6'}$=14.0, $J_{5,6}$=3.4, H-6); 3.11 (dd, 1H, $J_{6,6'}$=14.0, $J_{5,6'}$=9.2, H-6); 2.15 (s, 3H, 3-Ac); 1.59, 1.53 (2s, 6H, 1,2-isopropylidene).	
^{13}C-NMR data (75MHz)	δ 169.7 (carbonyl C); 112.8 (C-1); 111.1 (SCN); 104.6 (C-4) ; 83.6 (C-3); 79.8 (C-5); 76.0 (C-2); 57.2 (C-6); 38.5 (3-CH3); 26.8; 26.3 (Me_2) 20.9 (C-Me_2).	
Analysis	$C_{12}H_{16}ClNO_5S$ requires	C 44.79 H 5.01 N 4.35
	found	C 45.01 H 4.99 N 4.57

5,6-dideoxy-5,6-epithio-1,2-O-isopropylidene-α-D-glucofuranose (35)

Method 1.

To an ice-cooled solution of 3-O-acetyl-6-S-acetyl-5-chloro-5,6-dideoxy-1,2-O-isopropylidene-β-L-idofuranose (33) (0.05g) in dry methanol (5ml) was added triethylamine (2ml) and the mixture allowed to stand at room temperature for 24 hours. The solution was then evaporated under reduced pressure and the residue extracted with $CHCl_3$ (2x20ml). The combined $CHCl_3$ extract was washed with water (2x10ml), dried over $MgSO_4$, filtered and evaporated under reduced pressure to give 0.025g (75%) of (35) as a white solid, mp 138-139°C (lit: 138-140°C)

Method 2.

Sodium metal (100mg) was added to an ice-cooled solution of 3-O-acetyl-5-chloro-5,6-dideoxy-1,2-O-isopropylidene-6-thiocyano-β-L-idofuranose (34) (0.1g) in dry methanol. The solution was kept at 4°C for 12 hours then allowed to stand at room temperature for a further 4 hours. T.l.c ($CHCl_3$-MeOH 9:1) revealed an initial fast conversion to a more polar product (corresponding to deacetylation), followed by the

slow formation of a further product. The cloudy solution was neutralised with Amberlite IR-120 cation exchange resin, filtered and evaporated under reduced pressure to give a syrup which was chromatographed on an SiO_2 column with $CHCl_3$-MeOH 19:1 to give 0.057g (84%) of (35) as white crystals, mp 138-139°C (lit[85]: 138-140°C).

Mass spectral data (Ammonia C.I.)	$(M+NH4)^+$ m/z 236 (37%), 215 (59%), 200 (55%), 175 (62%), 146 (35%), 126 (100%), 99 (17%), 76 (17%), 58 (30%).
^{1}H-NMR (300MHz)	δ 5.97 (d, 1H, $J_{1,2}$=3.7, H-1); 4.53 (d, 1H, $J_{1,2}$=3.7, H-2); 4.23 (d, 1H, $J_{3,4}$=2.6, H-3); 3.61 (dd, 1H, $J_{4,5}$=8.6, $J_{3,4}$=2.6, H-4); 3.14-3.07 (m, 4H, H-5, H-6, H-6', 3-OH); 1.46, 1.31 (2s, 6H, 1,2-isopropylidene).

Analysis	$C_9H_{14}O_4S$ requires	C 49.52	H 6.46
	found	C 49.61	H 6.53

3-O-Acetyl-6-O-benzoyl-1,2-O-isopropylidene-α-D-glucofuranose (37)

To a solution of 3-O-acetyl-1,2-O-isopropylidene-α-D-glucofuranose (29) (0.257g, 0.98mmol) in dry pyridine (4ml) held at -40°C, was added benzoyl chloride (0.11ml, 1 molar equiv.). The solution was kept below 0°C for 1 hour, stored at 4°C for 12 hours, then poured onto ice and extracted with $CHCl_3$ (2x30ml). The combined $CHCl_3$ extracts were washed with 2M H_2SO_4 (2x10ml), dilute $NaHCO_3$ solution (1x10ml) and water (1x10ml), dried over $MgSO_4$, filtered and concentrated under reduced pressure to a syrup. This was immediately chromatographed on an SiO_2 column with $CHCl_3$-MeOH 19:1 to give 0.27g (85%) of a clear syrup which proved refractory to crystallisation.

Mass spectral data: (Ammonia C.I.)	$(M+NH_4)^+$ m/z 384 (76%), $(M+H)^+$ 367 (98%), 349 (28%), 326 (16%), 309 (100%), 266 (23%), 245 (77%), 231 (47%), 201 (12%), 177 (15%), 143 (26%), 105 (62%).
^{1}H-NMR (500MHz)	δ 8.06-7.41 (m, 5H, aromatic H's); 5.92 (d, 1H, $J_{1,2}$=3.7, H-1); 5.33 (d, 1H, $J_{3,4}$=2.7, H-3); 4.72 (dd, 1H, $J_{6,6'}$=11.9, $J_{5,6}$=2.3, H-6); 4.56 (d, 1H, $J_{1,2}$=3.7, H-2); 4.42 (dd, 1H, $J_{6,6'}$=11.9, $J_{5,6}$=6.1, H-6'); 4.30 (dd, 1H, $J_{4,5}$=9.2, $J_{3,4}$=2.7, H-4); 3.99 (m, 1H, H-5); 3.07 (bs, 1H, 5-OH); 2.14 (s, 3H, 3-Ac); 1.49, 1.31 (2s, 6H, 1,2-isopropylidene).

3-O-Acetyl-6-O-benzoyl-5-chloro-5-deoxy-1,2-O-isopropylidene-β-L-idofuranose (36)

Method 1.

To a solution of 3-O-acetyl-5,6-dichloro-5,6-dideoxy-1,2-O-isopropylidene-β-L-idofuranose (30) (0.16g) in dry DMF under argon was added sodium benzoate (0.2g) and the mixture stirred at 120°C for 24 hours. The mixture was then evaporated under reduced pressure and extracted with $CHCl_3$ (2x40ml). The combined $CHCl_3$ extracts were washed with water (2x20ml), dried over $MgSO_4$, filtered and evaporated under reduced pressure to a clear syrup. This was chromatographed on an SiO_2 column with ethyl acetate-toluene 1:1 to give 0.06g (36%) of (36) as white needles, mp 138-140°C together with unreacted starting material (0.06g).

Method 2.

To an ice-cooled solution of 3-O-acetyl-6-O-benzoyl-1,2-O-isopropylidene-α-D-glucofuranose (37) (0.5g, 1.37mmol) in dry pyridine (5ml) was added TPP (1.07g, 3 molar equiv.) and CCl_4 (0.4ml, 3 molar equivs.) and the solution maintained at 0°C for 30 minutes. The solution was then allowed to warm to room temperature and finally heated at 70°C for 30 minutes, at which point t.l.c. ($CHCl_3$-MeOH 9:1) indicated complete reaction. The reaction was then quenched by the addition of methanol (1ml) and evaporated under reduced pressure to a syrup. This was extracted

into ether and crystalline TPP oxide was removed by filtration. The remaining syrup was chromatographed on an SiO_2 column with ethyl acetate-toluene 1:1 to give 0.44g (85%) of (36) as a clear syrup which crystallised from ethyl acetate-petrol as white needles, mp 138-140°C. A mixed melting point with material obtained by method 1. showed no depression.

Mass spectral data (Ammonia C.I.)	$(M+NH_4)^+$ m/z 402[a] (100%), 369[a] (12%), 344[a] (42%), 327[a] (97%), 258 (5%), 241 (11%), 227 (10%), 201 (5%), 188 (7%), 171 (19%), 143 (12%), 123 (12%), 105 (85%).
^1H NMR (500MHz)	δ 8.04-7.44 (m, 5H, aromatic H's); 5.96 (d, 1H, $J_{1,2}$=3.8, H-1); 5.28 (d, 1H, $J_{3,4}$=3.0, H-3); 4.61-4.43 (m, 5H, H-2, H-4, H-5, H-6, H-6'); 2.07 (s, 3H, 3-Ac); 1.53, 1.32 (2s, 6H, 1,2-isopropylidene).

Analysis	$C_{18}H_{21}ClO_6$ requires	C 56.18	H 5.50
	found	C 55.98	H 5.49

2-Benzamido-2-deoxy-α-D-glucopyranose (42)

To a solution of glucosamine hydrochloride (50g) in water (600ml) was added sodium hydrogen carbonate (68g) and the solution stirred until evolution of CO_2 had ceased. Benzoyl chloride (30ml, 1.1 molar equivs.) was then added dropwise and the mixture allowed to stir at room temperature for 12 hours. Following storage at 4^0C for 4h, the resulting crystals were filtered under suction, washed with small portions of ice-cold water and dried under reduced pressure over P_2O_5. A further crop of crystals were obtained from the evaporation of the filtrate to half-volume and storage at 4^oC. This gave 51g (71%) of 2-benzamido-2-deoxy-α-D-glucopyranose (42), m.p 198-200°C (lit[128]: 196-200°C).

2-Phenyl-4,5-(5,6-isopropylidene-D-glucofurano)-Δ^2-oxazoline.(43)

Dry, powdered 2-benzamido-2-deoxy-α-D-glucopyranose (42) (54g) was added to a solution of dry hydrogen chloride (38g) in dry acetone (1.5 litre) and the mixture was stirred at room temperature for 24 hours. The stirred solution was cooled in ice and neutralised with gaseous ammonia. Following filtration, the acetone solution was concentrated under reduced pressure and the residue extracted with petroleum ether (b.p. 60-80°C) to remove condensation products of acetone. The crude product was crystallised from ethyl acetate - light petroleum (1:2) to give 20g (37%) of oxazoline m.p. 160-161°C (lit[128]: 159-161°C)

Mass spectral data: (Ammonia C.I.) $(M+H)^+$ m/z 306 (100%), 164 (6%), 139 (9%), 122 (5%).

^{1}H-NMR data (500MHz) δ 8.00-7.41 (m, 5H, aromatic H's); 6.37 (d, 1H, $J_{1,2}$=5.0, H-1); 4.71 (d, 1H, $J_{1,2}$=5.0, H-2); 4.55 (d, 1H, $J_{3,4}$=2.8, H-3); 4.35 (m, 1H, H-5); 4.05 (dd, 1H, $J_{6,6'}$=8.6, $J_{5,6}$=5.5, H-6); 3.98 (dd, 1H, $J_{6,6'}$=8.6, $J_{5,6}$=4.9, H-6'); 3.79 (dd, 1H, $J_{3,4}$=2.8, $J=_{4,5}$=7.9, H-4); 2.85 (bs, 1H, 3-OH) 1.39, 1.35 (2s, 6H, 5,6-O-isopropylidene).

Analysis	$C_{16}H_{19}NO_5$ requires	C 62.95	H 6.27 N 4.60
	found	C 62.86	H 6.29 N 4.71

2-Phenyl-4,5-(3-O-benzoyl-5,6-O-isopropylidene-D-glucofurano)-Δ^2-oxazoline. (45)

Benzoyl chloride (7.75ml, 1.1 molar equivs) was added dropwise to an ice-cooled solution of the oxazoline (43) (18.5g) in dry pyridine (20ml). The solution was kept at room temperature for 12 hours then poured onto ice. After standing for 1 hour, the mixture was extracted twice with $CHCl_3$ (2x75ml) and the combined extracts washed sequentially with 2M H_2SO_4 (2x25ml), dilute $NaHCO_3$ solution (2x25ml) and water (1x30ml). The extract was dried over $MgSO_4$, filtered and concentrated under reduced pressure to a syrup which crystallised on standing. Recrystallisation from ethyl acetate gave 23.5g (95%) of (45) as white needles, mp 155-156°C.

Mass spectral data (Ammonia C.I.)	$(M+H)^+$ m/z 410 (100%), 105 (8%).
^{1}H-NMR data (500MHz)	δ 8.09-7.43 (m, 10H, aromatic H's); 6.37 (d, 1H, $J_{1,2}$=5.0, H-1); 5.74 (d, 1H, $J_{3,4}$=3.0, H-3); 4.82 (d, 1H, $J_{1,2}$=5.0, H-2); 4.39 (m, 1H, H-5); 4.10 (dd, 1H, $J_{6,6'}$=8.6, $J_{5,6}$=3.0, H-6); 4.07 (m, 2H,H-6, H-4); 1.36, 1.25 (2s, 6H, 5,6-O-isopropylidene).

Analysis	$C_{23}H_{23}NO_6$ requires	C 67.47	H 5.66 N 3.42
	found	C 67.58	H 5.66 N 3.29.

2-Phenyl-4,5-(3-O-acetyl-5,6-O-isopropylidene-D-glucofurano)-Δ^2-oxazoline. (44)

Acetic anhydride (1.7ml, 1.1 molar equivs) was added dropwise to an ice-cooled solution of the oxazoline (43) (5g, 16.4mmol) in dry pyridine (8ml). This was kept at room temperature for 12 hours, then poured onto ice. The solution was extracted twice with $CHCl_3$ (2x50ml) and the combined extracts washed with 2M H_2SO_4 (2x25ml),

dilute $NaHCO_3$ solution (2x10ml) and water (1x20ml). The extract was dried over $MgSO_4$, filtered and concentrated under reduced pressure to a crystalline solid which was recrystallised from ethyl acetate - light petroleum (1:1) to give 5.43g (97%) of (44) as white needles, mp 150-153°C (d).

Mass spectral data: (Ammonia C.I.) $(M+H)^+$ m/z 348 (100%), 332 (7%), 101 (12%).

^{1}H-NMR (500MHz) δ 8.00-7.41 (m, 5H, aromatic H's); 6.30 (d, 1H, $J_{1,2}$=5.0, H-1); 5.49 (d, 1H, $J_{3,4}$=3.0, H-3); 4.68 (d, 1H, $J_{1,2}$=5.0, H-2); 4.25 (m, 1H, H-5); 4.07 (dd, 1H, $J_{6,6'}$=8.6, $J_{5,6}$=6.1, H-6); 4.00 (dd, 1H, $J_{6,6'}$=8.6, $J_{5,6'}$=5.0, H-6'); 3.94 (dd, 1H, $J_{4,5}$=7.5, $J_{3,4}$=3.0, H-4); 2.13 (s, 3H, 3-Ac); 1.35, 1.30 (2s, 6H, 5,6-isopropylidene).

Analysis			
$C_{18}H_{21}NO_6$ requires	C 62.24	H 6.09	N 4.03
found	C 62.25	H 6.24	N 4.27

Methyl 2-benzamido-3-O-acetyl-2-deoxy-5,6-O-isopropylidene-β-D-glucofuranoside (46)

The oxazoline derivative (44) (1g) was dissolved in a 0.5mM hydrogen chloride in dry methanol solution (10ml) and left to stir at 40-50°C for 24 hours at which point t.l.c. (ether-hexane 4:1) indicated an absence of starting material and the formation of a single more polar product. Methanolic potassium hydroxide (0.1M, 0.5ml) was added, followed by solid CO_2 (1g). Evaporation under reduced pressure gave 0.93g (85%) of (46) as a white solid.

Mass spectral data: (Ammonia C.I.) $(M+H)^+$ m/z 380 (60%), 348 (100%), 158 (8%), 122 (13%), 105 (21%).

^{1}H-NMR data (500MHz)	δ 7.77-7.42 (5H, N-benzoyl); 6.39 (bd, 1H, NH); 5.44 (dd, 1H, $J_{1,2}$=6.0, H-1); 4.96 (d, 1H, J=1.8, H-3); 4.38 (m, 3H, H-2, H-4, H-5); 4.05 (m, 2H, H-6, H-6'); 3.40 (s, 3H, 1-OMe); 2.08 (s,3H, 3-Ac); 1.38,1.31 (2s, 6H, 5,6-isopropylidene).			
Analysis	$C_{19}H_{31}NO_7$ requires	C 59.20	H 8.11	N 3.63
	found	C 59.15	H 8.07	N 3.79

Methyl 3-O-acetyl-2-benzamido-β-D-glucofuranoside (48)

A suspension of (46) (0.8g) in 60% aqueous acetic acid was warmed at 40°C until complete solution was achieved, then stirred at room temperature for 24 hours at which point t.l.c. ($CHCl_3$-MeOH 9:1) indicated an absence of starting material and the formation of more polar products. The mixture was extracted with $CHCl_3$ (2x25ml) and the combined extracts washed with dilute $NaHCO_3$ solution (2x15ml). The $CHCl_3$ solution was then dried over $MgSO_4$, filtered and evaporated under reduced pressure to a yellow solid which crystallised from ethyl acetate giving 0.59g (82%) of (48) as white needles.

Mass spectral data (Ammonia C.I.)	$(M+H)^+$ m/z 340 (56%), 308 (100%).
^{1}H-NMR (500MHz)	δ 7.72-7.42 (5H, N-benzoyl); 6.40 (bd, 1H, NH); 5.41 (dd, 1H, $J_{1,2}$=5.8, H-1); 4.94 (d, 1H, J=2.0, H-3); 4.38 (m, 3H, H-2, H-4, H-5); 4.00 (m, 2H, H-6, H-6'); 3.40 (s, 3H, 1-OMe); 2.10 (s, 3H, 3-OAc).

Methyl 3-O-acetyl-2-benzamido-5,6-dichloro-2,5,6-trideoxy-α-L-idofuranoside (52)

To an ice-cooled solution of (48) (1.89g, 5.74mmol) in dry pyridine (20ml) under argon was added TPP (9.18g, 6 molar equivs) and CCl_4 (1.7ml, 3 molar equivs). The solution was stirred at 0°C for 30 minutes and then allowed to warm to room temperature. Finally the solution was heated at 70-80°C for 30 minutes at which point t.l.c. ($CHCl_3$-MeOH 9:1) showed an an absence of starting material, one fast-moving spot and two more polar spots. The reaction was quenched by the addition of methanol (5ml) and evaporated under reduced pressure. The resulting syrup was chromatographed on an SiO_2 column with petrol-ethyl acetate 7:3 to give 0.24g (11%) of the fast-migrating compound (52) as white needles.

Mass spectral data (Ammonia C.I.)	$(M+H)^+$ m/z 376[b] (17%), 348 (45%), 340[a] (100%), 308[a] (84%), 219 (11%), 105 (85%), 80 (16%).
^{1}H-NMR (500MHz)	δ 7.75-7.42 (5H, N-benzoyl); 6.45 (bd, 1H, NH); 5.52 (dd, 1H, $J_{1,2}$=6.0, H-1); 5.10-4.20 (m, 6H, H-2, H-3, H-4, H-5, H-6, H-6'); 3.40 (s, 3H, 1-OMe); 2.10 (s, 3H, 3-OAc).

Methyl 2-benzamido-3-O-benzoyl-2-deoxy-5,6-O-isopropylidene-β-D-glucofuranoside (47)

The oxazoline derivative (45) (17g) was dissolved in 0.5mM hydrogen chloride in dry methanol (100ml) and left to stir at 40-50°C for three days at which point t.l.c. (ether-hexane 4:1) indicated an absence of starting material and the formation of a single polar product. The solution was neutralised by the addition of methanolic potassiun hydroxide (0.1M, 3ml), followed by solid CO_2. Evaporation under reduced pressure gave 17.6g (96%) of (47) as a yellow syrup which proved refractory to crystallisation.

Mass spectral data (Ammonia C.I.)	$(M+H)^+$ m/z 442 (50%), 410 (48%), 220 (29%), 91 (100%), 74 (32%).

^{1}H-NMR data (500MHz)	δ 8.00-7.30 (m, 11H, aromatic H's & NH); 5.76 (dd, 1H, $J_{1,2}$=6.0, H-1); 5.03 (s, 1H, H-3) 4.54 (m, 2H, H-2, H-4); 4.41 (m, 1H, H-5); 4.05 (m, 2H, H-6, H-6'); 3.39 (s, 3H, OCH_3); 1.30, 1.16 (2s, 6H, 5,6-O-isopropylidene).

Methyl 2-benzamido-3-O-benzoyl-2-deoxy-β-D-glucofuranoside. (49)

A suspension of (47) (5.0g) in 60% aqueous acetic acid was warmed at 40^{0}C until complete solution was achieved, then stirred at room temperature for 24 hours at which point t.l.c. ($CHCl_3$-MeOH 9:1) indicated an absence of starting material and the formation of a single more polar product. The mixture was extracted with $CHCl_3$ (2x25ml) and the combined extracts washed with dilute $NaHCO_3$ solution (2x15ml). The $CHCl_3$ solution was then dried over $MgSO_4$, filtered and evaporated under reduced pressure to a yellow solid which crystallised from ethyl acetate giving 0.41g (90%) of (49) as white needles, mp 164-166°C.

Mass spectral data (Ammonia C.I.)	$(M+H)^+$ m/z 402 (100%), 370 (87%), 286 (5%), 257 (5%), 105 (16%).
^{1}H-NMR data (500MHz)	δ 8.07-7.41 (m, 10H, aromatic H's); 6.61 (d, 1H, $J_{2,NH}$=6.7,NH); 5.68 (d, 1H, $J_{1,2}$=4.8, H-1); 5.09 (d, 1H, $J_{3,4}$=1.5, H-3); 4.59 (m, 1H, $J_{2,NH}$=6.7, H-2); 4.47 (m, 1H, H-4); 3.91 (m, 1H, H-5); 3.87 (bs, 1H, OH); 3.82 (m, 2H, H-6, H-6'); 3.47 (s, 3H, OCH_3);3.21 (d, 1H, $J_{5,6}$=4.3, H-6), 2.32 (bs, 1H, OH).

Analysis	$C_{21}H_{23}NO_7$ requires	C 62.84	H 5.78 N 3.49
	found	C 62.77	H 5.74 N 3.56

Methyl 2-benzamido-3-O-benzoyl-6-chloro-2,6-dideoxy-β-D-glucofuranoside. (50)

To a solution of (49) (4.5g, 0.112 mol) in dry pyridine (10ml) under argon was added TPP (5.875g, 2 molar equivs) and CCl_4 (1.085ml, 1 molar equiv). The solution was stirred at 0°C for 30 minutes and then allowed to rise to room temperature. Finally the solution was warmed at 40°C for 30 minutes at which point t.l.c. (ethyl acetate - petrol 1:1) showed an absence of starting material and one fast-moving spot. The reaction mixture was then quenched with methanol (5ml) and evaporated under reduced pressure. The resulting yellow syrup was chromatographed on an SiO_2 column with ethyl acetate - petrol 3:2 to yield 4.32g (92%) of (50) as a syrup which slowly crystallised on standing.

Mass spectral data (Ammonia C.I.)	$(M+H)^+$ m/z 420[a] (100%), 388[a] (84%), 352 (13%), 298 (5%), 279 (5%), 158 (13%), 105 (40%), 74 (5%).
^{1}H-NMR data (500MHz)	δ 8.00-7.28 (m, 10H, aromatic H's); 5.87 (d, 1H, $J_{1,2}$=5.2, H-1); 5.07 (m, 1H, H-3); 4.55 (d, 1H, J=6.7, H-4); 4.50 (dd, 1H, J=9.0, J=5.2, H-2); 4.13 (bs, OH); 4.06 (q, 1H, J=7.1, H-5); 3.84 (dist.d, 1H, $J_{6,6'}$=11.3, H-6); 3.74 (dd, 1H, $J_{6,6'}$=11.3, $J_{5,6'}$=5.2, H-6'); 3.41 (s, 3H, OCH_3).

Analysis	$C_{21}H_{22}ClNO_6$	requires	C 60.07	H 5.28 N 3.34
		found	C 59.73	H 5.39 N 3.42

Methyl 2-benzamido-3-O-benzoyl-5,6-dichloro-1,5,6-trideoxy-α-L-idofuranoside (51).

To an ice-cooled solution of (49) (1.03g, 2.58mmol) in dry pyridine (20ml) under argon was added TPP (4.06g, 6 molar equivs) and CCl_4 (0.75ml, 3 molar equivs). The solution was stirred at 0°C for 30 minutes and then allowed to warm to room temperature. Finally the solution was heated at 70-80°C for 30 minutes at which point t.l.c. ($CHCl_3$-MeOH 9:1) showed an an absence of starting material, one fast-moving

spot and two more polar spots. The reaction was quenched by the addition of methanol (5ml) and evaporated under reduced pressure. The resulting syrup was chromatographed on an SiO_2 column with petrol-ethyl acetate 7:3 to yield 0.09g (8%) of (51) as white needles.

Mass spectral data (Ammonia C.I.)	$(M+H)^+$ m/z 438[b] (9%), 406[b] (17%), 370[a] (13%), 105 (100%), 94 (7%), 74 (8%).
^{1}H-NMR data (500MHz)	δ 8.06-7.42 (m, 10H, aromatic H's); 6.54 (bd, 1H, $J_{2,NH}$=7.1, NH); 5.82 (d, 1H, $J_{1,2}$=6.1, H-1); 5.21 (d, 1H, $J_{3,4}$=3.0, H-3); 4.92 (t, 1H, $J_{4,5}$=$J_{5,6}$=5.8, H-5); 4.55 (m, 1H, H-5); 4.43 (m, 1H, J=5.4, H-4); 3.90 (dd, 1H, $J_{6,6'}$=11.8, $J_{5,6}$=5.8, H-6); 3.79 (dd, 1H, $J_{6,6'}$=11.8, $J_{5,6'}$=5.8, H-6'); 3.54 (s, 3H, OCH_3).

2-Acetamido-2-deoxy-3,4:5,6-di-O-isopropylidene-*aldehydo*-D-glucose dimethyl acetal (53)

To a stirred suspension of 2-acetamido-2-deoxy-α-D-glucopyranose (15.0g) in 1,4-dioxane was added *p*-toluenesulphonic acid (0.5g) and 2,2-dimethoxypropane (30ml). The mixture was stirred at 60-70^{0}C for 5 hours until complete solution had been achieved and t.l.c. ($CHCl_3$-MeOH 9:1) showed the formation of one major fast-moving component. The solution was neutralised with Amberlite IR-45 (OH^-) ion exchange resin, filtered and evaporated under reduced pressure to a syrup which was chromatographed on SiO_2 gel with $CHCl_3$-MeOH 19:1 which gave 15.3g (65%) of the title compound as a yellow syrup.

Mass spectral data (Ammonia C.I.) $(M+H)^+$ m/z 348 (22%), 332 (9%), 316 (100%), 75 (21%).

^{1}H-NMR data (500MHz) δ 5.93 (d, 1H, $J_{NH,2}$=9.9, NH); 4.34 (m, 1H, H-1); 4.26 (d, 1H, $J_{3,4}$=6.1, H-3); 4.14 (d, 1H, $J_{5,6}$=7.9, H-5); 3.98 (m, 2H, H-2,H-4); 3.86 (m, 1H, H-6); 3.53 (m, 1H, H-6'); 3.30, 3.25 (2s, 6H, di-OCH_3); 1.93 (s, 3H, NAc); 1.40, 1.29, 1.27, 1.23 (4s, 12H, 1,2-isopropylidene, 3,4-isopropylidene).

2-Acetamido-2-deoxy-3,4-O-isopropylidene-*aldehydo*-D-glucose dimethyl acetal. (23)

A solution of (53) (10g) in 80% aqueous acetic acid was warmed at 40°C for 5 hours until t.l.c. ($CHCl_3$-MeOH 9:1) indicated an absence of starting material and the formation of a single more polar spot. The solution was then evaporated under reduced pressure and three further portions of water (10ml) were added and evaporated in order to remove any residual traces of acetic acid. The syrup was then chromatographed on an SiO_2 column with $CHCl_3$-MeOH 9:1 to give 8.2g (93%) of a syrup which solidified on standing and was recrystallised from ethyl acetate to give white prisms, mp 108-110°C (lit[97]:110-111°C)

Mass spectral data (Ammonia C.I.)	$(M+H)^+$ m/z 308 (36%), 276 (100%), 75 (29%).
^{1}H-NMR data (500MHz)	δ 6.29 (d, 1H, $J_{NH,2}$=9.0, NH); 4.38 (d, 1H, $J_{1,2}$=7.0, H-1); 4.30 (t, 1H, $J_{6,OH}$=$J_{6',OH}$=6-OH); 4.19 (d, 1H, $J_{4,5}$=8.0, H-4);3.99 (d, 1H, $J_{4,5}$=8.0, H-5); 3.70-3.53 (bm, 5H, 5-OH, H-2, H-3, H-6, H-6') 3.32, 3.23 (2s, 6H, di-OCH_3); 1.98 (s, 3H, NAc); 1.29 (s,6H, 3,4-O-isopropylidene).
Analysis	$C_{13}H_{25}NO_7$ requires C 50.80 H 8.20 N 4.56 found C 50.63 H 8.34 N 4.22

Synthesis of 2-acetylamine-5-thio-D-glucose

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2-Acetamido-6-O-benzoyl-2-deoxy-3,4-O-isopropylidene-*aldehydo*-D-glucose dimethyl acetal.(55)

To a stirred solution of (23) (3.5g) in dry pyridine (20ml) was added benzoyl chloride (1.32ml 1.1 molar equivs.) at -20^{0}C. The mixture was held below 0^{0}C for 6h and then poured onto ice. The mixture was then extracted twice with $CHCl_3$ (2x50ml) and the combined extracts washed successively with 2M H_2SO_4 (2x25ml), dilute $NaHCO_3$ solution (2x25ml) and water (1x25ml). The $CHCl_3$ extract was dried over $MgSO_4$, filtered and evaporated under reduced pressure to a syrup which was chromatographed on an SiO_2 column to give 4.03g (86%) of (55) as white needles, mp 125-127°C (lit[96]: 125°C)

Mass spectral data (Ammonia C.I.)	$(M+H)^+$ m/z 412 (2%), 394 (3%), $(M-OCH_3)^+$ 380 (100%), 340 (3%), 322 (4%), 308 (1%), 75 (5%).

^{1}H NMR (500MHz)	δ 8.20-7.28 (m, 5H, 6-OBz); 6.62 (d, 1H, $J_{2,NH}$=8.0, NH); 4.35 (d, 1H, $J_{1,2}$=6.6, H-1); 4.20 (d, 1H, $J_{4,5}$=7.5, H-4); 4.10 (d, 1H, $J_{4,5}$=7.5, H-5); 3.95-3.60 (bm, 5H, 5OH, H-2, H-3, H-6, H-6'); 3.36, 3.26 (2s, 6H, di-OMe); 2.10 (s, 3H, NAc); 1.34 (s, 6H, 3,4-isopropylidene)

Analysis	$C_{20}H_{29}NO_8$ requires	C 58.38	H 7.10	N 3.40
	found	C 58.09	H 7.22	N 3.46

2-Acetamido-6-O-benzoyl-2-deoxy-3,4-O-isopropylidene-5-O-mesyl-*aldehydo*-D-glucose dimethyl acetal.(56)

To an ice-cooled solution of (55) (3.0g) in dry pyridine (10ml) was added, with stirring, methanesulphonyl chloride (1.1 molar equivs) and the mixture was kept for 24 hours at room temperature. The solution was then poured onto ice, then extracted twice with $CHCl_3$ (2x50ml). The combined $CHCl_3$ extract was then washed sequentially with 2M H_2SO_4 (2x20ml), dilute $NaHCO_3$ solution (2x10ml) and water (1x10ml) then dried over $MgSO_4$, filtered and evaporated under reduced pressure to a syrup. The product was chromatographed on an SiO_2 column with $CHCl_3$-MeOH 19:1 to give 3.2g (96%) of (56) as a pale yellow syrup.

Mass spectral data (Ammonia C.I.)	$(M+H)^+$ m/z 490 (1%), $(M-OCH_3)^+$ 458 (100%), 400 (2%), 380 (1%), 362 (23%), 285 (2%), 105 (5%), 75 (14%).
^{1}H NMR (80MHz)	δ 8.2-7.3 (m, 5H, 6-O-Bz); 5.95 (bd, 1H, NH); 5.2-3.5 (m, 6H, H-1-H-6); 3.35, 3.25 (2s, 6H, di-OCH_3); 3.1 (s, 3H, SO_2CH_3); 2.0 (s, 3H, NAc); 2.3 (s, 6H, 3,4-isopropylidene).

2-Acetamido-5,6-anhydro-2-deoxy-3,4-O-isopropylidene-*aldehydo*-L-idose dimethyl acetal.(57)

To a solution of (56) (2.8g) in dry $CHCl_3$ (20ml) at 0^oC was added a freshly prepared solution of sodium (200mg) in dry methanol (10ml). The resulting solution was kept at 4^0C for 12 hours and then neutralised by the addition of Amberlite IR-120 cation exchange resin, followed by extraction into $CHCl_3$ (50ml). The $CHCl_3$ extract was washed with dilute $NaHCO_3$ solution (2x20ml), dried over $MgSO_4$, filtered and evaporated under reduced pressure to give 1.44g (87%) of a syrup which crystallised on standing. Recrystallisation from ether-hexane gave white prisms, mp 93-95°C (lit[96]: 95°C)

Mass spectral data (Ammonia C.I.)	$(M+H)^+$ m/z 290 (33%), $(M-OCH_3)^+$ 258 (100%), 232 (8%), 75 (34%).
^1H NMR (80MHz)	δ 5.95 (bd, 1H, NH); 4.5-3.5 (m, 4H, H-1, H-2, H-3, H-4); 3.35, 3.25 (2s, 6H, di-OCH_3); 2.6-3.1 (m, 3H, H-5, H-6, H-6'); 1.95 (s, 3H, NAc); 1.3 (s, 6H, 3,4-isopropylidene).

2-Acetamido-2,5,6-trideoxy-5,6-epithio-3,4-O-isopropylidene-*aldehydo*-D-glucose dimethyl acetal (58).

To a solution of (57) (2.3g) in methanol (20ml) was added thiourea (1.5g) and the mixture was heated at 60^0C with stirring for 1.5 hours, cooled and evaporated under reduced pressure. The residue was taken up in $CHCl_3$ (70ml) and washed with 2M H_2SO_4 (2x25ml) and water (1x10ml). The $CHCl_3$ extract was then dried over $MgSO_4$, filtered and evaporated under reduced pressure to yield 2.33g (96%) of a syrup which solidified on standing. Recrystallisation from ethyl acetate-hexane gave white needles, mp 96-98°C (lit[96]: 97°c)

Mass spectral data (Ammonia C.I.)	$(M+H)^+$ m/z 306 (6%), $(M-OCH_3)^+$ 274 (100%), 75 (9%).

^{1}H NMR (80MHz)	δ 5.9 (bd, 1H, NH); 3.7-4.8 (m, 4H, H-1, H-2, H-3, H-4); 3.35, 3.25 (2s, 6H, di-OCH_3); 2.2-3.2 (m, 3H, H-5, H-6, H-6'); 2.0(s, 3H, NAc); 1.25, 1.35 (2s, 6H, 3,4-isopropylidene).

2-Acetamido-6-O-acetyl-5-S-acetyl-2,5-dideoxy-3,4-O-isopropylidene-5-thio-*aldehydo*-D-glucose dimethyl acetal (59).

To a solution of (58) (1g) in acetic anhydride (10ml) was added potassium acetate (0.2g) and acetic acid (1ml). The solution was boiled under reflux for 15 hours until t.l.c. ($CHCl_3$: MeOH 19:1) indicated an absence of starting material and the formation of one fast-moving spot. The mixture was then poured onto ice and stirred for 1 hour, then extracted with $CHCl_3$ (2x50ml). The combined extracts were washed with dilute $NaHCO_3$ solution (3x25ml), dried over $MgSO_4$, filtered and evaporated under reduced pressure to a syrup which was chromatographed on an SiO_2 column with $CHCl_3$ - MeOH 29:1. This gave 0.8g (82%) of (59) as a clear syrup which solidified on standing.

Mass spectral data (Ammonia C.I.)	$(M+H)^+$ m/z 408 not seen, $(M-OCH_3)^+$ 376 (23%), 366 (8%), 350 (13%), 334 (19%), 292 (15%), 274 (84%), 242 (44%), 216 (37%), 183 (27%), 152 (23%), 75 (100%).
^{1}H NMR (80MHz)	δ 5.9 (bd, 1H, NH); 3.5-4.5 (m, 4H, H-1, H-2, H-3, H-4); 3.35, 3.25 (2s, 6H, di-OCH_3); 3.2-2.4 (m, 3H, H-5, H-6, H-6');2.2 (s, 3H, SAc); 2.0, 2.05 (2s, 6H, 6-O-Ac, NAc); 1.3, 1.35 (2s, 6H, 3,4-isopropylidene).

2-Acetamido-1,3,4,6-tetra-O-acetyl-2-deoxy-5-thio-α-D-glucopyranose (60).

To a solution of (59) (0.6g) in 10:1 acetic acid - 2M hydrochloric acid (10ml) was heated for 6 hours at 40^0C, cooled and treated with Amberlite IR-45 (OH^-) ion exchange resin until neutral. The resin was filtered off and washed with methanol and the solutions and washings combined and evaporated under reduced pressure. The residue was acetylated with acetic anhydride (2ml) and pyridine (6ml) for 12 hours at room temperature. The mixture was then poured onto ice and extracted twice with $CHCl_3$ (2x40ml). The combined extract was washed with 2M H_2SO_4 (2x10ml), dilute $NaHCO_3$ solution (2x10ml) and water (1x10ml), dried over $MgSO_4$, filtered and evaporated under reduced pressure to give a yellow syrup. This was chromatographed on an SiO_2 column with $CHCl_3$-MeOH 9:1 to give 0.4g (72%) of a clear syrup which crystallised from ethyl acetate-petrol as white needles, mp 165-167°C (lit[96]: 166-167°C)

Mass spectral data (FAB) $(M+H)^+$ m/z 405 (100%), 330 (20%), 286 (22%), 226 (8%).

^{1}H-NMR (500MHz) δ 5.95 (d, 1H, $J_{1,2}$=3.1, H-1); 5.71 (bd, 1H, $J_{2,NH}$=8.3, NH); 5.38 (t, 1H, $J_{3,4}$=$J_{4,5}$=10.8, H-4); 5.18 (t, 1H, $J_{2,3}$=$J_{3,4}$=10.8, H-3); 4.64 (m, 1H, $J_{2,NH}$=8.4, $J_{2,3}$=10.8, $J_{1,2}$=3.1, H-2); 4.34 (dd, 1H, $J_{6,6'}$=12.1, $J_{5,6}$=5.0, H-6); 4.04 (dd, 1H, $J_{6,6'}$=12.1, $J_{5,6}$=5.0, H-6'); 3.48 (m, 1H, H-5); 2.20, 2.07, 2.05, 2.04 (4s, 12H, 4 OAc); 1.91 (s, 3H, NAc).

Analysis			
	$C_{16}H_{23}NO_9S$ requires	C 47.40	H 5.72 N 3.46
	found	C 47.11	H 5.78 N 3.32

2-Acetamido-6-chloro-2,6-dideoxy-3,4-O-isopropylidene-*aldehydo*-D-glucose dimethyl acetal (61A)

To an ice-cooled solution of (23) (1.0g) in dry pyridine under argon was added TPP (5.13g, 6 molar equivs.) and CCl_4 (0.33ml, 3 molar equivs.). The solution was stirred at 0°C for 30 minutes then allowed to warm to room temperature and finally heated at 40°C for 1 hour. The mixture was quenceched by the addition of MeOH (2ml) and evaporated under reduced pressure to a syrup which was chromatographed on an SiO_2 column with $CHCl_3$-MeOH 19:1 to give 1.0g (94%) of (61A) as white needles.

Mass spectral data (FAB+NaI)	$(M+Na)^+$ m/z 348[a] (78%), $(M+H)^+$ 326[a] (15%), 294[a] (86%), 236 (9%), 204 (7%), 106 (8%), 116 (25%), 75 (100%).
^1H NMR (500MHz)	δ 5.97 (bd, 1H, NH); 4.50 (d, 1H, $J_{1,2}$=6.0, H-1); 4.40 (m, 1H, H-2); 4.20 (dd, 1H, J=9.0, J=2.0, H-3); 3.8-3.53 (m, 5H, H-4, H-5, H-6, H-6', 5-OH); 3.39, 3.30 (2s, 6H, di-OCH_3); 2.05 (s, 3H, NAc); 1.34, 1.31 (2s, 6H, 3,4-isopropylidene).

Analysis	$C_{13}H_{24}ClNO_6$ requires	C 47.93	H 7.42 N 4.30
	found	C 47.80	H 7.63 N 4.57

2-Acetamido-5,6-dichloro-2,5,6-trideoxy-3,4-O-isopropylidene-*aldehydo*-L-idose dimethyl acetal (61B)

To an ice-cooled solution of (23) (0.204g, 0.364mmol) in dry pyridine (8ml) under argon was added TPP (1.05g, 6 molar equivs) and CCl_4 (0.195ml, 3 molar equivs). The solution was stirred at 0°C for 30 minutes, then allowed to warm to room temperature and finally heated at 70-75°C for 30 minutes at which point t.l.c. ($CHCl_3$-MeOH 9:1) showed an absence of starting material and one fast-moving spot. The reaction was quenched by the addition of MeOH (3ml) and the mixture evaporated under reduced pressure to a syrup which was chromatographed on an SiO_2 column with ethyl acetate-petrol 7:3 to give 0.14g (61%) of (61B) as a clear syrup.

Mass spectral data (FAB + NaI)	$(M+Na)^+$ m/z 366[b] (19%), 330[b] (9%), 301 (70%), 279 (100%), 201 (13%), 116 (5%), 75 (44%).
^{1}H-NMR data (500MHz)	δ 6.20 (1H, bd, NH); 4.35 (dd, $J_{1,2}$=6.0, $J_{1,NH}$=1.5, H-1); 4.27-4.15 (m, 3H, H-2, H-3, H-4); 3.73 (m, 1H, H-5); 3.70 (m, 2H, H-6, H-6'); 3.31, 3.26 (2s, 6H, di-O-CH_3); 1.96 (s, 3H, NAc); 1.34, 1.31 (2s, 6H, CMe_2).

2-Acetamido-6-O-benzoyl-5-chloro-2,5-dideoxy-3,4-O-isopropylidene-*aldehydo*-L-idose dimethyl acetal (62)

To an ice-cooled solution of (55) (2.0g, 4.66mM) in dry pyridine (10ml) under argon was added TPP (7.34g, 6 molar equivs.) and CCl_4 (1.35ml, 3 molar equivs.). The solution was stirred at 0°C for 30 minutes then allowed to warm to room temperature and finally heated at 70°C for 30 minutes at which point t.l.c. (ethyl acetate-petrol 1:1) showed an absence of starting material and one fast-moving spot. The reaction was quenched by the addition of MeOH (3ml) and the mixture evaporated under reduced pressure to give a syrup which was chromatographed on an SiO_2 column with ethyl acetate -petrol 1:1 to give 1.88g (90%) of (55) as a clear syrup.

Mass spectral data (FAB)	$(M+H)^+$ m/z 430[a] (8%), 398[a] (100%), 279 (8%), 105 (11%), 75 (84%).
^{1}H-NMR (500MHz)	δ 8.05-7.36 (m, 5H, 6-OBz); 6.42 (bd, 1H, NH); 5.06 (d, 1H, $J_{1,2}$=5.4, H-1); 4.20 (m, 3H, H-2, H-3, H-4); 3.95 (m, 3H, H-5, H-6, H-6') 3.41 (s, 6H, di-OCH_3); 1.34, 1.31 (2s, 6H, 3,4-isopropylidene).

2-Acetamido-5-chloro-2,5-dideoxy-3,4-O-isopropylidene-*aldehydo*-D-glucose dimethyl acetal (63)

To an ice-cooled solution of (62) (0.8g) in dry methanol (10ml) was added sodium metal (200mg) and the solution allowed to stir at room temperature for 2 hours, at which point t.l.c. ($CHCl_3$-MeOH 9:1) indicated an absence of starting material and the formation of one more polar spot. The solution was neutralised with Amberlite IR-120 cation exchange resin, filtered and evaporated under reduced pressure to give 0.57g (94%) of (63) as a yellow syrup.

Mass spectral data (FAB)	$(M+H)^+$ m/z 322[a] (2%), 294[a] (9%), 279 (100%), 201 (9%).
^1H NMR (500MHz)	δ 6.35 (bd, 1H, NH); 4.73 (d, 1H, $J_{1,2}$=6.0, H-1); 4.0 (m, 3H, H-2, H-3, H-4); 3.60 (bm, 4H, H-5, H-6, H-6', 6-OH); 3.39, 3.30 (2s, 6H, di-OCH_3); 2.00 (s, 3H, NAc); 1.30, 1.34 (2s, 6H, 3,4-isopropylidene)

2-Acetamido-5-azido-2,5-dideoxy-3,4-O-isopropylidene-*aldehydo*-D-glucose dimethyl acetal (65)

To a solution of (63) (0.5g) in dry DMF under argon was added sodium azide (0.3g) and the solution heated at 120°C for 12 hours. The solution was then cooled and evaporated under reduced pressure to a solid which was extracted twice with $CHCl_3$ (2x25ml). The combined $CHCl_3$ extract was then washed with water (2x30ml), dried over $MgSO_4$, filtered and evaporated to a straw-coloured syrup. This was chromatographed on an SiO_2 column with $CHCl_3$-MeOH 9:1 to give 0.32g (62%) of (65) as a clear syrup.

Mass spectral data (FAB+NaI)	$(M+H)^+$ 329 (2%), 279 (100%), 246 (19%), 214 (8%), 182 (6%), 75 (17%).

I.r. data	strong peak 2150cm-1 (N_3)
^{1}H NMR (500MHz)	δ 6.32 (bd, 1H, NH); 4.65 (d, 1H, $J_{1,2}$=6.0, H-1); 3.9 (m, 4H, H-2, H-3, H-4, H-5); 3.55 (bm, 2H, H-6, H-6'); 3.39 (2s, 6H, di-OCH_3); 2.00 (s, 3H, NAc); 1.30, 1.34 (2s, 6H, 3,4-isopropylidene).

2-Acetamido-5-S-acetyl-2,5-dideoxy-3,4-O-isopropylidene-5-thio-*aldehydo*-D-glucose dimethyl acetal (64)

To a solution of (63) (0.5g) in dry DMF (10ml) under argon was added potassium thioacetate (0.5g) and the solution heated at 80°C for 24 hours. The mixture was then cooled and evaporated under reduced pressure to a sticky brown solid. This was extracted with $CHCl_3$ (2x25ml) and the combined $CHCl_3$ fraction was washed with water (2x25ml), dried over $MgSO_4$, filtered and evaporated. This left a sticky orange syrup which was purified by column chromatography to give 0.27g (40%) of (64) as an orange syrup.

Mass spectral data (FAB)	$(M+H)^+$ m/z 366 not seen, 336 (9%), 279 (100%), 201 (8%), 93 (12%), 75 (24%).
^{1}H NMR (500MHz)	δ 6.25 (bd, 1H, NH); 4.60 (d, 1H, $J_{1,2}$=5.0, H-1); 4.0 (m, 4H, H-2, H-3, H-4, H-5); 3.51 (bm, 2H, H-6, H-6'); 3.40 (2s, 6H, di-OCH_3); 2.23 (s, 3H, SAc); 2.00 (s, 3H, NAc); 1.32, 1.28 (2s, 6H, 3,4-isopropylidene).

2-Acetamido-1,3,4,6-tetra-O-acetyl-2-deoxy-5-thio-α-D-glucopyranose (60)

To a solution of (64) (0.25g) in 10:1 acetic acid - 2M hydrochloric acid (10ml) was heated for 6 hours at 40°C, cooled and neutralised with Amberlite IR-45 anion exchange resin. The resin was removed by filtration and washed with methanol and the solution and washings combined and evaporated under reduced pressure. The residue was acetylated with acetic anhydride (2ml) and pyridine (6ml) for 12 hours at room temperature. The mixture was then poured onto ice and extracted twice with $CHCl_3$ (2x40ml). The combined extract was washed with 2M H_2SO_4 (2x10ml), dilute $NaHCO_3$ solution (2x10ml) and water (1x10ml), dried over $MgSO_4$, filtered and evaporated under reduced pressure to give a yellow syrup. This was chromatographed on an SiO_2 column with $CHCl_3$-MeOH 9:1 to give 0.27g (80%) of a clear syrup which crystallised from ethyl acetate-petrol as white needles, mp 165-167°C (lit[96]: 166-167°C)

A mixed melting point with a sample of previously prepared authentic material showed no depression.

De-N-acetylation, radical deamination and amine derivatives

De-N-Acetylation with triethyloxonium fluoroborate

An ice-cooled solution of 2-acetamido-1,3,4,6-tetra-O-acetyl-2-deoxy-5-thio-α-D-glucopyranose (60) (27mg, 0.067mmol) in dry dichloromethane (2ml) under nitrogen was treated with a 1M solution of triethyloxonium tetrafluoroborate in dry dichloromethane (0.08ml, 1.3 molar equivs). The solution was held at 0°C for 10 minutes and then kept at room temperature for 1 hour. During this time no crystallisation was observed. The straw-coloured solution was then evaporated under reduced pressure to a syrup which was dissolved in 0.005 M hydrochloric acid (1ml), then neutalised with cold dilute sodium hydrogen carbonate solution after 15 minutes. This solution diluted with water and extracted with $CHCl_3$ (2x10ml). The combined extracts were dried over $MgSO_4$, filtered and evaporated under reduced pressure to give 22mg of a clear syrup which t.l.c. ($CHCl_3$-MeOH 9:1) showed to consist largely of unreacted starting material together with one slightly more polar substance. Column chromatography failed to completely separate the mixture.

Mass spectral data (Ammonia C.I.)	Together with peaks corresponding to unreacted (60) a peak was observed at m/z 381 possibly corresponding to $(M+NH_4)^+$

2-Amino-2-deoxy-5-thio-D-glucopyranose hydrochloride (66)

A solution of (60) (0.04g) in 4M HCl (3ml) was heated at 100°C for 45 minutes, at which point t.l.c. ($CHCl_3$-MeOH 4:1) showed an absenec of starting material and a single polar spot. The solution was evaporated under reduced pressure to give 0.02g of (66) as an orange syrup. Attempts to induce crystallisation from MeOH-acetone proved unsuccessful and the syrup was used in its crude form.

Mass spectral data (FAB)	$(M+H)^+$ m/z 196 (free base) (26%), 178 (6%), 167 (14%), 131 (8%), 115 (34%), 85 (9%).

1,3,4,6-Tetra-O-acetyl-2-formamido-2-deoxy-5-thio-D-glucopyranose (68)

To a solution of crude (66) (0.04g) in 50% aqueous 1,4-dioxane (10ml) was added sodium hydrogen carbonate (0.3g) and *p*-nitrophenyl formate (0.4g) and the mixture was stirred for 24 hours at room temperature. The dioxane was removed under reduced pressure and the residual aqueous phase was extracted with ether (2x20ml) and then concentrated to dryness. Acetic anhydride (1ml) and pyridine (5ml) were added and the solution was kept at room temperature for 12 hours, then poured onto ice. The mixture was extracted with $CHCl_3$ (2x30ml) and the combined extracts washed with 2M H_2SO_4 (2x10ml), dilute $NaHCO_3$ solution (1x10ml) and water (1x10ml), dried over $MgSO_4$, filtered and evaporated under reduced pressure to give 0.08g of (68) as a syrup which was dried and used as a foam.

Mass spectral data (FAB) $(M+H)^+$ m/z 391 (5%),

^1H NMR (500MHz) δ 7.80 (bs, 1H, aldehyde H); 6.05 (d, 1H, $J_{1,2}$=4.0, H-1); 5.73 (bd, 1H, $J_{2,NH}$=8.0, NH); 5.30 (m, 1H, H-4); 5.10 (m, 1H, H-3); 4.65 (m, 1H, H-2); 4.32 (dd, 1H, $J_{6,6'}$=12.0, $J_{5,6}$=4.8, H-6); 4.05 (dd, 1H, $J_{6,6'}$=12.0, $J_{5,6}$=4.7) 3.50 (m, 1H, H-5); 2.20-2.02 (4s, 12H, 4 OAc); 1.95 (s, 3H, NAc).

1,3,4,6-Tetra-O-acetyl-2-deoxy-2-isocyano-D-glucopyranose (69)

A stirred solution of (68) (0.06g) in dry dichloromethane (20ml) was cooled to -40°C and triethylamine (2ml) was added. Phosphorus oxychloride (1ml) was added dropwise over 10 minutes and the mixture allowed to warm to room temperature. After 2 hours t.l.c. (toluene-tetrahydrofuran 22:3) indicated complete reaction. The reaction mixture was concentrated to 3ml and loaded onto a silica column packed in toluene. Elution with toluene-tetrahydrofuran 0.054g of the isocyanide (69) as a clear syrup.

Mass spectral data (FAB)	$(M+H)^+$ m/z 374 (8%), 331 (100%), 309 (31%), 278 (12%), 214 (6%), 149 (34%).
I.r. data	strong peak 2160cm^{-1} (N=C)

1,3,4,6-tetra-O-acetyl-2-deoxy-5-thio-D-glucopyranose (70)

To a stirred solution of tri-n-butylstannane (0.05g) in dry toluene (10ml) at 80°C was added dropwise a solution of the crude isocyanide (0.04g) and azobisisobutyronitrile (AIBN) (0.01g) in dry toluene (5ml). After 4 hours the solution was cooled, reduced in volume and treated with several drops of a solution of iodine in toluene until the iodine colour persisted. The solution was filtered through a short SiO_2 column and evaporated under reduced pressure. The colourless product was dissolved in acetonitrile and washed with pentane (2x10ml) and evaporated under reduced pressure. The residual syrup was taken up in $CHCl_3$ and washed with aqueous KF (2x10ml), dried over $MgSO_4$, filtered and evaporated under reduced pressure to give 0.02g of a pale yellow syrup.

Mass spectral data (FAB + NaI)	Together with several peaks which corresponded to unreacted (69) a strong peak corresponding to $(M+Na)^+$ m/z 370

2-Acetamido-2-deoxy-4,6-O-isopropylidene-α-D-glucopyranose (74).

To a suspension of 2-acetamido-2-deoxy-D-glucopyranose (10.0g) in dry DMF (250ml) was added *p*-toluenesulphonic acid (0.3g) and 2,2-dimethoxypropane (30ml). The mixture was stirred at room temperature for 24 hours at which point complete solution was achieved. The solution was treated with Amberlite IR-45 anion exchange resin to neutralise, then filtered and evaporated under reduced pressure to a white powder which was washed with portions of ice-cold ether. The compound could be recrystallised from methanol-ether to give white needles, mp 187-189 (lit[88]: 189-190°C)

Mass spectral data (Ammonia C.I.)	$(M+H)^+$ m/z 262 (92%), 244 (46%), 226 (9%), 204 (8%), 186 (16%), 160 (10%), 132 (6%), 126 (6%), 120 (16%), 16%), 102 (100%), 60 (6%).
^{1}H-NMR data (500MHz)	δ 5.22 (d, 1H, $J_{1,2}$=3.6, H-1); 4.02-3.83 (m, 4H, H-2, H-3, H-4, NH); 3.76 (m, 1H, H-5); 3.67 (m, 1H, H-6); 3.47 (m, 1H, H-6'); 2.07 (s, 3H, NAc); 1.60, 1.47 (2s, 6H, 4,6-isopropylidene).

Analysis	$C_{11}H_{19}NO_6$ requires	C 50.57	H 7.33	N 5.36
	found	C 50.42	H 7.39	N 5.28

1,3-Di-O-acetyl-2-acetamido-2-deoxy-4,6-O-isopropylidene-α-D-glucopyranose (75)

To an ice-cooled solution of (74)(5g, 19.0mmol) in dry pyridine was added acetic anhydride (4.0ml, 2.2 molar equivs.) and the mixture kept for 12 hours at room temperature, then poured onto ice and extracted with $CHCl_3$ (2x50ml). The combined $CHCl_3$ extracts were then washed with 2M H_2SO_4 (2x20ml), dilute $NaHCO_3$ solution (2x10ml) and water (1x10ml), dried over $MgSO_4$, filtered and evaporated under reduced pressure to a syrup which crystallised fron methanol-ether, to give 6.2g (94%) of a white solid, mp 137-139°C (lit[88]: 137-138°C)

Mass spectral data (Ammonia C.I.)	$(M+H)^+$ m/z 346 (1%), 330 (5%), 286 (100%), 226 (6%), 114 (6%), 96 (6%).
^1H NMR (300MHz)	δ 6.09 (d, 1H, $J_{1,2}$=4, H-1); 5.75 (bd, 1H, $J_{NH,2}$=10, NH); 5.15 (dd, 1H, $J_{3,4}$=9, $J_{2,3}$=2, H-3); 4.43 (m, 1H, H-2); 3.93-3.69 (m, 4H, H-4, H-5, H-6, H-6'); 2.20 (s, 3H, NAc); 2.10, 2.07 (2s, 6H, 1-OAc, 3-OAc); 1.50, 1.38 (2s, 6H, 4,6-isopropylidene).

Analysis	$C_{15}H_{23}NO_8$ requires	C 52.17	H 6.71	N 4.06
	found	C 52.38	H 6.65	N 4.36

1,3-Di-O-acetyl-2-acetamido-2-deoxy-α-D-glucopyranose (76)

A solution of (75) (3.0g) was dissolved in 60% acetic acid and kept at room temperature for 24 hours, at which point t.l.c. ($CHCl_3$-MeOH 9:1) indicated reaction to be almost complete. The mixture was evaporated under reduced pressure and three potions of water (5ml) were added and evaporated to remove any traces of acetic acid. The resulting syrup was triturated with $CHCl_3$ to give 2.25g (85%) of (76) as a white solid, which could be recrystallised from MeOH-ether to give white needles, mp 169-171°C (lit[88]: 172-173°C)

Mass spectral data (Ammonia C.I.)	$(M+H)^+$ m/z 306 (2%), 288 (6%), 246 (100%), 186 (12%), 168 (20%), 126 (13%), 114 (7%), 102 (9%), 72 (6%).
^1H NMR (500MHz) (D_2O)	δ 5.22 (d, 1H, $J_{1,2}$=3.6, H-1); 4.02-3.47 (m, 6H, H-2, H-3, H-4, H-5, H-6, H-6'); 2.07 (s, 3H, NAc); 1.60, 1.42 (2s, 6H, 4,6-isopropylidene).

Analysis	$C_{12}H_{19}NO_8$ requires	C 47.21	H 6.27	N 4.59
	found	C 47.58	H 6.23	N 4.59

1,3,6-Tri-O-acetyl-2-acetamido-2-deoxy-α-D-glucopyranose (76).

To a solution of (76) (4.52g,14.8mmol) in dry pyridine/$CHCl_3$ (20ml) at -70°C was added acetic anhydride (1.54ml, 1.1 molar equivs.) and the solution kept at -20°C for 12 hours. The solution was slowly allowed to warm to room temperature and kept for a further 12 hours, then poured onto ice and extracted with $CHCl_3$ (2x40ml). The combined $CHCl_3$ extract was washed with 2M H_2SO_4 (2x20ml), dilute $NaHCO_3$ solution (2x10ml) and water (1x10ml), dried over $MgSO_4$, filtered and evaporated under reduced pressure to a syrup which was chromatographed on an SiO_2 column with $CHCl_3$-MeOH 9:1 to give 4.67g (79%) of a clear syrup, which was subsequently dried and used as a foam.

Mass spectral data (FAB + NaI)	$(M+Na)^+$ m/z 370 (31%), $(M+H)^+$ 348 (10%), 330 (2%), 310 (3%), 288 (100%), 246 (7%), 228 (26%), 186 (8%), 168 (14%), 137 (8%), 126 (18%), 108 (7%).
^1H NMR (300MHz)	δ 5.92 (d, 1H, $J_{1,2}$=3.6, H-1); 5.06 (dd, 1H, $J_{3,4}$=10.9, $J_{2,3}$=2.2, H-3); 4.20-4.14 (m, 4H, NH, H-2, H-4, H-5); 3.98 (m, 2H, H-6, H-6'); 3.54 (dd, 1H, $J_{3,4}$=10.9, $J_{4,5}$=1, H-4); 2.07 (s, 3H, NAc); 1.96, 1.95, 1.80 (3s, 9H, 1-OAc, 3-OAc, 6-OAc).

Preparation of N-benzoylimidazole

An ice-cooled solution of imidazole (2 molar equivalents) in dry CH_3CN (20ml/g imidazole) was treated with benzoyl chloride (1 molar equiv) slowly with stirring. After 15 minutes, the solution was filtered under suction to remove precipitated imidazole hydrochloride which was subsequently washed with CH_3CN (20ml) and the solution and washings combined.

2-Acetamido-1,3,6-tri-O-benzoyl-2-deoxy-α-D-glucopyranose (80).

A: Benzoyl chloride / pyridine

A suspension of N-acetylglucosamine (1.0g, 4.5mmol) in dry pyridine (20ml) was treated with a solution of benzoyl chloride (1.73ml, 3.3 molar equivs.) in dry $CHCl_3$ (10ml) at -20°C for 30 minutes. The solution was then kept at 4°C overnight. The mixture was then poured onto ice and extracted with $CHCl_3$ (2x40ml). The combined $CHCl_3$ fractions were then washed with 2M H_2SO_4 (2x20ml), dilute $NaHCO_3$ solution (2x20ml) and water (1x20ml), dried over $MgSO_4$, filtered and evaporated under reduced pressure to yield a yellow syrup. This was chromatographed on an SiO_2 column with dichloromethane-ethyl acetate 3:2 to give the tetrabenzoate (78) and the two isomeric tribenzoates (79) and (80).

B: N-Benzoylimidazole / CH_3CN

To a suspension of N-acetylglucosamine (1.5g, 6.77mmol) in dry CH_3CN (70ml) was boiled under reflux with the addition of portions (10ml) over 5 hours of a freshly prepared solution of N-benzoylimidazole (23.3mmol) in CH_3CN (70ml). Following complete addition, the mixture was heated for a further 48 hours until a clear yellow solution was achieved. Water (3ml) was added and the mixture was stirred at room temperature for 30 minutes. The CH_3CN solution was evaporated under reduced pressure and the residue was taken up in $CHCl_3$ (2x70ml). The combined $CHCl_3$ fractions were extracted with 2M H_2SO_4 (2x30ml), dilute $NaHCO_3$ solution (2x30ml)

and water (1x30ml), dried over $MgSO_4$, filtered and concentrated under reduced pressure to a brown syrup which was chromatographed on an SiO_2 column with dichloromethane-ethyl acetate 3:2.

C: Benzoyl cyanide / DMF

To a suspension of N-acetylglucosamine (1.0g, 4.5mmol) in dry DMF (40ml) was added benzoyl cyanide (0.195g, 3.3 molar equivs.) and the mixture stirred and heated at 70-80°C for 24 hours. The resulting yellow solution was then evaporated under reduced pressure and the residue taken up in $CHCl_3$ (60ml). This was then washed with water (3x20ml), dried over $MgSO_4$, filtered and evaporated to syrup which was chromatographed on an SiO_2 column with dichloromethane-ethyl acetate 3:2

2-Acetamido-1,3,4,6-tetrabenzoyl-2-deoxy-α-D-glucopyranose (78)

Rf = 0.63 (dichloromethane-ethyl acetate 3:2)

Mass Spectral data (FAB + NaI)	$(M+Na)^+$ m/z 661 (19%), $(M+H)^+$ 639 (2%), 601 (5%), 579 (16%), 539 (5%), 517 (6%), 334 (15%), 301 (5%), 252 (23%), 230 (100%), 212 (30%).
^{1}H-NMR: (500MHz)	δ 8.12-7.27 (m, 20H, aromatic H's); 6.57 (d, 1H, $J_{1,2}$=4.0, H-1); 6.44 (d, 1H, $J_{2,NH}$=9.0, NH); 5.69 (dd, 1H, $J_{3,4}$=11.0, $J_{2,3}$=1.7, H-3); 4.74 (m, 2H, H-6, H-4); 4.49 (dd, 1H, $J_{6,6'}$=12.4, $J_{5,6}$= 2.1, H-6); 4.09 (m, 1H, H-2); 1.78 (s, 3H, NAc).

Analysis	$C_{36}H_{31}NO_{10}$ requires	C 67.81	H 4.90 N 2.20
	found	C 67.95	H 4.95 N 2.01

B: 2-Acetamido-1,4,6-tri-O-benzoyl-2-deoxy-α-D-glucopyranose (79)

Rf = 0.56 (dichloromethane-ethyl acetate 3:2)

Mass spectral data (FAB + NaI): $(M+Na)^+$ m/z 556 (26%), 538 (13%), 434 (100%), 412 (10%), 312 (5%), 290 (4%), 105 (46%), 77 (5%).

^{1}H-NMR data (500MHz): δ 6.25 (d, 1H, $J_{1,2}$=3.5, H-1); 5.86 (d, 1H, $J_{2,NH}$=8.4, NH); 5.60 (dd, 1H, $J_{3,4}$=11.0, $J_{4,5}$=1.8, H-4); 4.89 (dd, 1H, $J_{6,6'}$=12.6, $J_{5,6}$=3.1, H-6); 4.68 (m, 1H, H-2); 4.43 (dd, 1H, $J_{6,6'}$=12.6, $J_{5,6'}$=2.3, H-6'); 4.16 (m, 1H, H-5); 3.97 (m, 1H, H-3); 3.53 (bd, 1H, $J_{4,OH}$=4.5, 4-OH); 1.80 (s, 3H, NAc).

Analysis: $C_{29}H_{27}NO_9$ requires C 65.29 H 5.10 N 2.62; found C 65.36 H 5.13 N 2.86

C: 2-Acetamido-1,3,6-tri-O-benzoyl-2-deoxy-α-D-glucopyranose (79)

Rf = 0.40 (dichloromethane-ethyl acetate 3:2)

mp 134-137°C (d)

Mass spectral data (FAB + NaI): $(M+Na)^+$ m/z 556 (34%), 494 (2%), 452 (5%), 434 (100%), 413 (5%), 360 (5%), 330 (4%), 312 (5%), 289 (14%),176 (11%), 133 (6%), 105 (24%), 77 (3%).

^{1}H-NMR data (500MHz): δ 8.08-7.39 (m, 15H, aromatic H's); 6.25 (d, 1H, $J_{NH,2}$=9.6, NH); 6.06 (d, 1H, $J_{1,2}$=5.6, H-1); 5.51 (m, 1H, H-3); 4.76 (dd, 1H, $J_{6,6'}$=12.2, $J_{5,6}$=2.8, H-6); 4.62 (m, 2H, H-6', H-2); 3.96 (m, 2H, H-4, H-5); 3.80 (bs, 1H, OH); 1.75 (s, 3H, NAc).

Analysis	$C_{29}H_{27}NO_9$ requires	C 65.29 H 5.10 N 2.62
	found	C 65.15 H 5.27 N 2.76

2-Acetamido-1,3,6-tri-O-benzoyl-2-deoxy-4-tosyl-α-D-glucopyranose (81)

A crude quantity (1.00g) of benzoylated 2-acetamido-2-deoxy-D-glucose prepared by method B. was taken up in dry pyridine and excess tosyl chloride (0.50g) was added. The resulting solution was kept at room temperature for 48 hours then poured onto ice and extracted with $CHCl_3$ (2x40ml). The combined $CHCl_3$ extracts were washed with 2M H_2SO_4 (2x10ml), dilute $NaHCO_3$ solution (2x10ml) and water (1x10ml), dried over $MgSO_4$, filtered and evaporated under reduced pressure to a syrup. T.l.c. (CH_2Cl_2-ethyl acetate 3:2) revealed one major spot with significant amounts of contaminants. Attempts to crystallise the 4-tosyl derivative (81) from cold ethanol, methanol-ether or ethyl acetate-petrol failed. A sample of crystalline material was prepared by the reaction of pure (80) (0.2g, 0.37mmol) and tosyl chloride (0.08g, 1.1 molar equivs) in dry pyridine (5ml) by the same method. Attempts to crystallise (81) from the crude tosylated mixture in various solvents by "seeding" with authentic material also failed.

Mass spectral data (FAB + NaI)	$(M+Na)^+$ m/z 710 (4%), 566 (5%), 413 (4%), 394 (3%), 272 (3%), 245 (5%), 200 (4%), 105 (100%), 77 (24%).
^{1}H-NMR (500MHz)	δ 8.11-7.32 (m, 19H, aromatic H's); 6.01 (m, 2H, NH, H-1); 5.63 (dd, 1H, $J_{3,4}$=11, $J_{2,3}$=1, H-3); 5.23 (dd, 1H, $J_{3,4}$=11, $J_{4,5}$=1, H-4); 4.65 (m, 2H, H-2, H-6); 4.31 (dd, 1H, $J_{6,6'}$=13, $J_{5,6'}$=4, H-6'); 4.10 (m, 1H, H-5); 1.72 (s, 3H, NAc).

Analysis	$C_{36}H_{33}NO_{11}S$ requires	C 62.87 H 4.84 N 2.04
	found	C 62.29 H 5.12 N 1.95

2-Acetamido-1,3,6-tri-O-benzoyl-2-deoxy-4-O-mesyl-α-D-glucopyranose (82)

To an ice-cooled solution of (80) (0.2g, 0.375mmol) in dry pyridine (5ml) was added mesyl chloride (0.035ml, 1.2 molar equivs) and the solution was kept at 4°C for 48h. Water (1ml) was then added and the mixture was extracted with $CHCl_3$ (1x50ml). The $CHCl_3$ solution was then washed with 2M H_2SO_4 solution (2x10ml), dilute $NaHCO_3$ solution (1x10ml) and water (1x10ml), dried over $MgSO_4$, filtered and evaporated under reduced pressure to a yellow syrup which was chromatographed on an SiO_2 column with CH_2Cl_2-ethyl acetate 3:2 to give (82) in quantitative yield as a clear syrup which proved refractory to crystallisation and in further reactions was used as a foam.

Mass spectral data (FAB + NaI)	$(M+Na)^+$ m/z 634 (53%), 612 $(M+H)^+$ (1%), 538 (17%), 512 (6%), 490 (20%), 413 (11%), 394 (10%), 329 (6%), 301 (4%), 272 (7%), 230 (4%), 200 (6%), 176 (35%), 154 (15%), 136 (16%), 105 (100%), 89 (16%).
^1H NMR (500MHz)	δ 8.11-7.43 (m, 15H, aromatic H's); 6.08 (m, 2H, NH, H-1); 5.68 (dd, 1H, $J_{3,4}$=10.1, $J_{2,3}$=1.1, H-3); 5.22 (dd, 1H, $J_{3,4}$=10.1, $J_{4,5}$=1.0, H-4); 4.73 (m, 2H, H-2, H-6); 4.59 (dd, 1H, $J_{6,6'}$=12.7, $J_{5,6'}$=4.1, H-6'); 4.17 (m, 1H, H-5); 2.91 (s, 3H, SO_2Me); 2.16 (s, 3H, NAc).

2-acetamido-1,3,6-tri-O-benzoyl-2,4-dideoxy-4-thiocyano-α-D-glucopyranose (83)

To a solution of (82) (0.275g, 0.45mmol) in dry DMF (10ml) under argon was added potassium thiocyanate (0.48g, 10 molar equivs). The mixture was heated with stirring to 110°C for 48 hours, then evaporated under reduced pressure to a solid residue which was taken up in $CHCl_3$ (50ml). The $CHCl_3$ extract was washed with water (2x20ml), dried over $MgSO_4$, filtered and evaporated under reduced pressure to a syrup which was chromatographed on an SiO_2 column with ethyl acetate-toluene 7:3 to give 0.185g (70%) of (83) as white needles.

I.R. data	strong peak 2180cm^{-1} (SCN)
Mass spectral data (FAB + NaI)	M^+ m/z 586 not seen, 529 (M-SCN) (32%), 479 (7%), 441 (14%), 413 (62%), 393 (12%), 352 (5%), 329 (70%), 307 (10%), 289 (8%), 245 (10%), 223 (7%), 199 (15%), 176 (100%), 154 (67%), 136 (55%), 105 (41%), 89 (22%).
^{1}H NMR (500MHz)	δ 8.15-7.35 (m, 15H, aromatic H's); 5.82 (dd, 1H, $J_{NH,2}$=9.7, NH); 5.28 (dd, 1H, $J_{3,4}$=10.3, $J_{2,3}$=3.7, H-3); 5.16 (d, 1H, $J_{1,2}$=3.7, H-1); 4.80 (dd, 1H, $J_{6,6'}$=12.2, $J_{5,6}$=4.7, H-6); 4.66 (dd, 1H, $J_{6,6'}$=12.2, $J_{5,6'}$=2.3, H-6'); 4.13 (m, 2H, H-2, H-4); 3.91 (m, 1H, H-5); 2.18 (s, 3H, NAc).

2-Acetamido-2,4-dideoxy-4-thio-α-D-galactopyranose (84).

To an ice-cooled solution of (83) (0.04g) in dry methanol (5ml) was added sodium metal (100mg) and the resulting solution was stirred at room temperature for 4 hours, at which point no starting material could be detected by t.l.c. The solution was neutralised with Amberlite IR-120 cation exchange resin, filtered and evaporated under reduced pressure to give 0.014g (87%) of (84) as a clear syrup.

Mass spectral data (Ammonia C.I.)	M^+ m/z 237 not seen, 204 (M-SH) (6%), 202 (24%), 186 (7%), 168 (9%), 152 (10%), 144 (7%), 135 (6%), 126 (37%), 102 (33%), 102 (33%), 77 (100%), 60 (61%), 44 (18%).
I.r. data	2550cm^{-1} (SH)

1,3,5,6-tetra-O-acetyl-2-acetamido-2-deoxy-4-thio-D-galactofuranose (85)

Solid (84) (0.1g) was dissolved in an ice-cooled solution of glacial acetic acid (2.5ml), acetic anhydride (2.5ml) and sulphuric acid (0.2ml). The solution was kept at $4^{o}C$ for 48 hours then sodium acetate (1.7g) was added. After 30 minutes of stirring at room temperature, the solution was poured onto ice and extracted with $CHCl_3$ (2x20ml). The combined $CHCl_3$ extract was washed with dilute $NaHCO_3$ solution (2x5ml), dried over $MgSO_4$, filtered and evaporated under reduced pressure to a syrup. This was chromatographed on an SiO_2 column with $CHCl_3$-MeOH 9:1 to give a few milligrams of material with Rf 0.42.

Mass spectral data (FAB)	$(M+H)^+$ m/z 406 (12%), 395 (21%), 371 (45%), 334 (15%), 235 (50%), 231 (45%).

Methyl 2,3,6-tri-O-benzoyl-α-D-glucopyranoside (86).

A suspension of methyl α-D-glucopyranoside (3.0g, 0.0155 mol) in dry CH_3CN was boiled under reflux and 10ml portions of a freshly prepared solution of N-benzoylimidazole (0.046 mol) in CH_3CN (70ml) were added over 5 hours. Following complete addition, the mixture was heated for a further 24 hours. Water (3ml) was added to the resulting clear solution and the mixture stirred at room temperature for 30 minutes. The CH_3CN solution was evaporated under reduced pressure and the residue was poured into ice-water to give a sticky precipitate which was taken up in $CHCl_3$ (2x70ml). The combined $CHCl_3$ solution was extracted with 2M H_2SO_4 (2x30ml), dilute $NaHCO_3$ solution (2x30ml) and water (1x30ml), dried over $MgSO_4$, filtered and concentrated under reduced pressure to a clear syrup which was chromatographed on an SiO_2 column with toluene-ethyl acetate 1:1 to give 5.48g (70%) of (86). The compound could be crystallised from di-isopropyl ether-hexane to give white plates mp 126-128°C (lit[133]: mp 127-129°C) but was normally used as a foam.

Mass spectral data (Ammonia C.I.)	$(M+NH_4)^+$ m/z 524 (17%), 508 (12%), 475 (89%), 402 (18%), 385 (88%), 371 (13%), 353 (52%), 337 (33%), 298 (5%), 280 (8%), 266 (10%), 248 (40%), 231 (30%), 215 (15%), 165 (9%), 139 (42%), 122 (100%), 105 (82%).
^{1}H-NMR data (500MHz)	δ 8.10-7.31 (m, 15H, aromatic H's); 5.84 (m, 1H, H-3); 5.28 (dd, 1H, $J_{2,3}$=10.2, $J_{1,2}$=3.7, H-2); 5.16 (d, 1H, $J_{1,2}$=3.7, H-1); 4.77 (dd, 1H, $J_{6,6'}$=11.9, $J_{5,6}$=4.2, H-6); 4.67 (d, 1H, $J_{6,6'}$=11.9, H-6'); 4.15 (dd, 1H, $J_{4,5}$=9.8, $J_{5,6'}$=2.6, H-5); 3.92 (m, 1H, $J_{4,5}$=9.8, H-4); 3.65 (bs, 1H, 4-OH); 3.45 (s, 3H, OCH_3).
^{13}C-NMR data (75MHz)	δ 167.3, 166.9, 166.0 (carbonyl C's); 133.6-128.4 (aromatic C's); 97.1 (C-1); 73.9 (C-3); 71.4 (C-2); 70.1 (C-5); 69.7 (C-4); 63.5 (C-6); 55.4 (OCH_3).

Analysis	$C_{28}H_{26}O_9$ requires	C 66.40 H 5.17
	found	C 66.64 H 5.26

Methyl 2,3,6-tri-O-benzoyl-4-chloro-4-deoxy-α-D-galactopyranose (87).

To an ice-cooled solution of (86) (1.951g, 8.86mmol) in dry pyridine (20ml) under argon was added TPP (6.07g, 6 molar equivs.) and CCl_4 (1.123ml, 3 molar equivs). The solution was stirred at 0°C for 30 minutes, then allowed to warm to room temperature. Finally the solution was heated at 40-50°C for 1 hour at which point t.l.c. ($CHCl_3$-MeOH 19:1) showed an absence of starting material and one fast-moving spot. The reaction was quenched by the addition of MeOH (5ml) and evaporated under reduced pressure to a yellow syrup which was chromatographed on an SiO_2 column with petrol-ethyl acetate 3:2 to give (87) in quantitative yield as white needles mp 136-138°C

Mass spectral data (Ammonia C.I.)	$(M+NH_4)^+$ m/z 542[a] (11%), 509[a] (4%), 493[a] (16%), 419[a] (12%), 391 (100%), 371[a] (29%), 337[a] (71%), 279[a] (41%), 223[a] (96%), 177 (38%), 122 (37%), 105 (56%).
^{1}H-NMR data (500MHz)	δ 8.07-7.37 (m, 15H, aromatic H's); 5.87 (dd, 1H, $J_{2,3}$=10.5, H-3); 5.69 (dd, 1H, $J_{2,3}$=10.5, $J_{1,2}$=3.7, H-2); 5.24 (d, 1H, $J_{3,4}$=3.7, H-1); 4.82 (d, 1H, $J_{3,4}$=3.7, H-4); 4.65-4.54 (m, 3H, H-5, H-6, H-6'); 3.46 (s, 3H, OCH_3).
^{13}C-NMR data (75MHz)	δ 166.1, 165.9, 165.7 (carbonyl C's); 133.5-128.4 (aromatic C's); 97.5 (C-1); 69.0 (C-3); 68.6 (C-2); 66.7 (C-5); 64.0 (C-6); 59.6 (C-4); 55.6 (OCH_3).

Analysis	$C_{28}H_{27}ClO_8$ requires	C 64.04 H 4.79
	found	C 63.99 H 5.01

Methyl 2,3,6-tri-O-benzoyl-4-bromo-4-deoxy-α-D-galactopyranoside (88)

To an ice-cooled solution of (86) (0.64g, 1.265mmol) in dry pyridine (20ml) under argon was added TPP (1.99g, 6 molar equivs) and CBr_4 (1.26g, 3 molar equivs). The solution was stirred for 30 minutes and then allowed to warm to room temperature and finally heated at 40-50°C for 1 hour at which point t.l.c. (petrol-ethyl acetate 1:1) indicated an absence of starting material and one fast-moving spot. The reaction was quenched by the addition of methanol (3ml) and the mixture evaporated under reduced pressure to a brown syrup which was chromatographed on an SiO_2 column with petrol-ethyl acetate 3:2 to give 0.67g (93%) of (88) as white needles, mp 120-121°C.

Mass spectral data (FAB + NaI)	$(M+Na)^+$ m/z 591[c]
^{1}H-NMR data (500MHz)	δ 8.06-7.37 (m, 15H, aromatic H's); 5.70 (m, 2H, H-1, H-2); 5.23 (d, 1H, $J_{2,3}$=3.0, H-3); 4.87 (m, 1H, H-4); 4.63 (dd, 1H, $J_{6,6'}$=11.3, $J_{5,6}$= H-6); 4.49 (m, 2H, H-6); 3.45 (s, 3H, OCH_3).
Analysis	$C_{28}H_{25}BrO_8$ requires C 59.06 H 4.42
	found C 59.34 H 4.55

1,3,6-Tri-O-acetyl-2-acetamido-4-chloro-2,4-dideoxy-α-D-galactopyranose (89)

To an ice-cooled solution of (77) (0.50g, 1.44mmol) under argon was added TPP (0.756g, 2 molar equivs.) and CCl_4 (0.14ml, 1 molar equiv.) and was stirred at 0°C for 30 minutes. The solution was then allowed to warm to room temperature and was finally heated at 60-65°C for a further 45 minutes at which point t.l.c. showed an absence of starting material and the frormation of a single less polar product. The reaction was quenched by the addition of methanol (3ml) and evaporated under reduced pressure to a syrup which chromatographed on an SiO_2 column with ethyl acetate-petrol 7:3 to give 0.43g (82%) of (89) as white needles.

Mass spectral data (FAB + NaI)	$(M+Na)^+$ m/z 388[a] (100%), 364 (6%), 328 (10%), 306[a] (14%), 149 (20%).
^1H NMR (500MHz)	δ 6.18 (d, 1H, $J_{1,2}$=3.6, H-1); 5.45 (bd, 1H, $J_{NH,2}$=9.3, NH); 5.25 (dd, 1H, $J_{2,3}$=11.2, $J_{1,2}$=3.6, H-2); 4.85 (m, 1H, H-3); 4.44 (d, 1H, $J_{3,4}$=3.4, H-4); 4.31-4.18 (m, 3H, H-5, H-6, H-6'); 2.16 (s, 3H, NAc); 2.14, 2.06, 1.95 (3s, 9H, OAc).

1,3,6-Tri-O-acetyl-2-acetamido-4-bromo-2,4-dideoxy-α-D-galactopyranose (90)

To an ice-cooled solution of (77) (0.572g, 0.65mmol) under argon was added TPP (0.86g, 2 molar equivs) and CBr_4 (0.55g, 1 molar equiv). The solution was stirred at 0°C for 30 minutes then allowed to warm to room temperature and finally heated at 60-65°C for a further 45 minutes at which point t.l.c. showed the absence of starting material and the formation of a single fast-moving product. The reaction was quenched by the addition of methanol (3ml) and evaporated under reduced pressure to a syrup which was chromatographed on an SiO_2 column with ethyl acetate - petrol 4:1 to give 0.61g (90%) of (90) as a white solid.

Mass spectral data (FAB + NaI)	$(M+Na)^+$ m/z 433[c] (41%), 413 (100%), 391 (12%), 370 (11%), 352 (15%), 329 (5%), 301 (12%), 279 (14%), 176 (42%), 149 (84%).
^1H NMR (500MHz)	δ 6.20 (d, 1H, $J_{1,2}$=3.6, H-1); 5.45 (bd, 1H, NH); 5.25 (dd, 1H, $J_{2,3}$=11.4, $J_{1,2}$=3.6, H-2); 4.90 (m, 1H, H-3); 4.46 (d, 1H, $J_{3,4}$=3.4, H-4); 4.31-4.18 (m, 3H, H-5, H-6, H-6'); 2.20 (s, 3H, NAc); 2.17, 2.09, 1.98 (3s, 9H, OAc).

Methyl 4-chloro-4-deoxy-α-D-galactopyranoside (91)

To an ice-cooled suspension of (87) (0.2g) under argon in dry methanol was added sodium metal (100mg). The resulting mixture was stirred at room temperature for 6 hours at which point t.l.c ($CHCl_3$-MeOH 9:1) indicated an absence of starting material and a single polar spot. The solution was neutralised with Amberlite IR-120 cation exchange resin, filtered and evaporated under reduced pressure to a pale syrup. This was chromatographed on an SiO_2 column with $CHCl_3$-MeOH 4:1 to give 0.09g (91%) of (91) as a white powder which could be recrystallised from ethyl acetate.

Mass spectral data (Ammonia C.I.)	$(M+NH_4)^+$ m/z 230[a] (42%), 163 (7%), 140 (8%), 122 (8%), 94 (15%), 78 (60%).
^1H NMR (500MHz)	δ 4.95 (d, 1H, $J_{1,2}$=2.0, H-1); 4.60 (d, 1H, J=1, H-3); 4.04 (m, 1H, H-2); 3.95 (m, 2H, H-4, H-5); 3.84 (dd, 1H, $J_{6,6'}$=11.7, $J_{5,6}$=7.9, H-6); 3.75 (dd, 1H, $J_{6,6'}$=11.7, $J_{5,6'}$=4.7, H-6'); 3.40 (s, 3H, OCH_3).
Analysis	$C_7H_{13}ClO_5$ requires C 39.54 H 6.16 found C 39.72 H 6.20

Methyl 4-bromo-4-deoxy-α-D-galactopyranoside (92)

To an ice-cooled suspension of (88) (0.3g) in dry methanol was added sodium metal (100mg) and the mixture stirred at room temperature for 6 hours at which point t.l.c showed an absence of starting material and a single polar spot. The solution was neutralised with Amberlite IR-120 cation exchange resin, filtered and evaporated under reduced pressure to a a syrup which was chromatographed on an SiO_2 column with $CHCl_3$-MeOH 4:1 to give 0.11g (85%) of (92) as a white powder which could be recrystallised from ethyl acetate, mp 114-116°C.

Mass spectral data (FAB + NaI)	$(M+Na)^+$ m/z 279[c] (24%), 242 (5%), 220 (6%), 199[c] (10%), 176 (47%), 154 (50%), 136 (45%), 91 (26%), 76 (35%), 68 (100%).
^1H NMR (500MHz)	δ 4.88 (d, 1H, $J_{1,2}$=2.0, H-1); 4.60 (d, 1H, J=1, H-3); 4.04 (m, 1H, H-2); 3.94 (m, 2H, H-4, H-5); 3.82 (dd, 1H, $J_{6,6'}$=11.7, $J_{5,6}$=7.4, H-6); 3.74 (dd, 1H, $J_{6,6'}$=11.7, $J_{5,6'}$=4.7, H-6'); 3.46 (s, 3H, OCH_3).
Analysis	$C_7H_{13}BrO_5$ requires C 32.70 H 5.10 found C 32.87 H 4.91

Methyl 2,3,6-tri-O-acetyl-4-azido-4-deoxy-α-D-glucopyranoside (93)

To a solution of methyl 4-chloro-4-deoxy-α-D-galactopyranoside (91) (0.03g, 0.12mmol) in dry DMF (5ml) under argon was added sodium azide (0.02g, 2 molar equivs) and the solution heated to 120°C for 48 hours. The solution was then cooled and evaporated under reduced pressure to a solid to which was added dry pyridine (5ml) and acetic anhydride (2ml) and the mixture kept at room temperature for 12 hours. The mixture was then poured onto ice and extracted with $CHCl_3$ (2x30ml) and the combined $CHCl_3$ extract was washed with 2M H_2SO_4 (2x10ml), dilute $NaHCO_3$ solution (2x10ml) and water (1x10ml), dried over $MgSO_4$, filtered and evaporated under reduced pressure to a clear syrup. This was chromatographed on an SiO_2column with $CHCl_3$-MeOH 9:1 to give 0.03g (62%) of (93) as a clear syrup assuming quantitative acetylation.

Mass spectral data (Ammonia C.I.)	$(M+NH_4)^+$ m/z 363 (3%), 279 (8%), 183 (100%), 125 (6%), 98 (40%), 81 (32%).
i.r. data	strong peak 2050cm-1 (N_3)

Methyl 2,3,6-tri-O-acetyl-4-deoxy-4-thiocyano-α-D-glucopyranoside (94)

To a solution of methyl 4-bromo-4-deoxy-α-D-galactopyranoside (92) (0.03g, 0.12mmol) in dry DMF (5ml) under argon was added potassium thiocyanate (0.115g, 10 molar equivs) and the solution heated to 120°C for 48 hours. The solution was then cooled and evaporated under reduced pressure to a solid to which was added dry pyridine (5ml) and acetic anhydride (2ml) and the mixture kept at room temperature for 12 hours. The mixture was then poured onto ice and extracted with $CHCl_3$ (2x30ml) and the combined $CHCl_3$ extract was washed with 2M H_2SO_4 (2x10ml), dilute $NaHCO_3$ solution (2x10ml) and water (1x10ml), dried over $MgSO_4$, filtered and evaporated under reduced pressure to a brown syrup. This was chromatographed on an SiO_2 column with $CHCl_3$-MeOH 19:1 to give 0.025g (54%) of (94) as a clear syrup assuming quantitative acetylation. 0.01g of a less polar material was also recovered which mass spectral data proved to be methyl 2,3,6-tri-O-acetyl-4-bromo-4-deoxy-α-D-galactopyranoside.

Mass spectral data (Ammonia C.I.)	$(M+NH_4)^+$ m/z 379 (100%), 213 (18%), 183 (7%), 141 (10%), 125 (6%), 82 (26%).
I.r. data	2180 cm^{-1} SCN
^1H NMR (500MHz)	δ 4.95 (d, 1H, $J_{1,2}$=5.6, H-1); 4.57 (dd, 1H, $J_{3,4}$=10.7, $J_{2,3}$=9.9, H-3); 4.89 (dd, 1H, $J_{2,3}$=9.9, $J_{1,2}$=5.6, H-2); 4.47 (m, 2H, H-4, H-6); 4.13 (m, 1H, H-6'); 3.43 (s, 3H, OCH_3); 3.22 (t, 1H, J=10.8, H-5); 2.20-2.12 (3s, 9H, 2-Ac, 3-Ac, 6-Ac).

Methyl 2,3,6-tri-O-acetyl-4-bromo-4-deoxy-α-D-galactopyranoside

Mass spectral data (Ammonia C.I.)	$(M+NH_4)^+$ m/z 400[c] (100%), 351[c] (71%), 307 (7%), 293[c] (11%), 79 (9%), 262 (10%), 243 (9%), 230 (6%), 168 (10%), 153 (14%), 125 (15%), 111 (8%), 98 (19%), 82 (59%), 60 (14%).

Methyl2-Acetamido-2-deoxy-4,6-O-isopropylidene-3-O-methyl-α-D-glucopyranoside (95)

To an ice-cooled solution of (74) (3.0g, 11.5mmol) in dry DMF (20ml) was added barium oxide (5g) and dropwise, methyl iodide (5ml) and the mixture was stirred at room temperature for 12 hours. Dichloromethane (50ml) was added and the mixture was filtered under suction through a bed of celite. The filtrate was evaporated under reduced pressure to give 3.0g (90%) of (95) as a syrup which solidified on standing, and could be recrystallised from ethyl acetate to give white needles, mp 199-202°C.

Mass spectral data (FAB)	$(M+H)^+$ m/z 290 (40%), 274 (5%), 258 (43%), 232 (13%), 200 (7%), 168 (8%), 140 (8%), 128 (26%), 115 (70%), 101 (20%), 86 (26%), 73 (100%), 42 (70%).
^{1}H NMR (500MHz)	δ 5.89 (bd, 1H, $J_{2,NH}$=7.2, NH); 4.82 (d, 1H, $J_{1,2}$=8.2, H-1); 3.88 (m, 2H, H-2, H-4); 3.76 (m, 1H, H-3); 3.60 (m, 1H, H-5); 3.49, 3.47 (2s, 6H, 1-OMe, 3-OMe); 3.33 (m, 1H, H-6); 3.20 (m, 1H, H-6'); 2.00 (s, 3H, NAc); 1.49, 1.40 (2s, 6H, 4,6-isopropylidene).

Analysis	$C_{13}H_{23}NO_6$ requires	C 53.97	H 8.01 N 4.84
	found	C 54.03	H 7.97 N 5.02

Methyl 2-Acetamido-2-deoxy-3-O-methyl-α-D-glucopyranoside (96)

A solution of (2) (0.95g) in 60% aqueous acetic acid (5ml) was kept for 24 hours wherupon t.l.c. ($CHCl_3$-MeOH 9:1) showed an absence of starting material and the formation of a single polar spot. The solution was evaporated under reduced pressure to a syrup which was chromatographed on an SiO_2 column with $CHCl_3$-MeOH 19:1 to give 0.75g (92%) of (96) as a white solid, mp 107-108°C.

Mass spectral data: (FAB)	$(M+H)^+$ m/z 250 (79%), 218 (100%), 200 (5%), 184 (6%), 170 (6%), 152 (5%).	
^{1}H-NMR (500MHz)	δ 6.01 (bd, 1H, NH); 4.73-3.82 (m, 5H, H-1, H-2, H-3, H-4, H-5); 3.48, 3.43 (2s, 6H, 1-OMe, 3-OMe); 3.32 (m, 1H, H-6); 3.21 (m, 1H, H-6'); 2.01 (s, 3H, NAc).	
Analysis	$C_{10}H_{19}NO_6$ requires	C 48.10 H 7.68 N 5.62
	found	C 47.97 H 7.64 N 5.55

Methyl 2-Acetamido-6-O-benzoyl-2-deoxy-3-O-methyl-α-D-glucopyranoside (97)

A solution of (96) (0.5g, 2.01mmol) in a mixture of dichloromethane (5ml) and dry pyridine (5ml), was cooled to -20°C and benzoyl chloride (0.26ml, 1.1 molar equivs) was added dropwise with stirring. The mixture was kept at 4°C for 12 hours then poured onto ice and extracted with $CHCl_3$ (2x40ml). The combined $CHCl_3$ extracts were washed with 2M H_2SO_4 (2x10ml), dilute $NaHCO_3$ solution (2x10ml) and water (1x10ml), dried over $MgSO_4$, filtered and evaporated under reduced pressure to a solid which was purified on an SiO_2 column with $CHCl_3$-MeOH 19:1 to give 0.64g (90%) of (97) as a white solid, mp 124-125°C.

Mass spectral data (Ammonia C.I.)	$(M+H)^+$ m/z 354 (22%), 322 (84%), 290 (6%), 218 (4%), 200 (4%), 168 (8%), 115 (8%), 105 (14%), 74 (77%).	
^{1}H-NMR (500MHz)	δ 8.12- 7.20 (m, 5H, 6-O-benzoyl); 6.02 (bd, 1H, NH); 4.50-4.39 (m, 5H, H-1, H-2, H-3, H-6, H-6'); 3.75-3.65 (m, 2H, H-4, H-5); 3.45, 3.42 (2s, 6H, 1-OMe, 3-OMe); 2.02 (s, 3H, NAc).	
Analysis	$C_{17}H_{23}NO_7$ requires	C 57.78 H 6.56 N 3.96
	found	C 57.95 H 6.81 N 3.75

Methyl 2-Acetamido-6-O-benzoyl-4-chloro-2,4-dideoxy-3-O-methyl-α-D-galactopyranose (98)

To an ice-cooled solution of (97) (0.245g, 0.694mmol) in dry pyridine (10ml) under argon was added TPP (1.092, 6 molar equivs) and CCl_4 (0.201ml, 3 molar equivs). The solution was stirred at 0°C for 30 minutes then allowed to warm to room temperature and finally heated at 40-50°C for 1 hour. T.l.c. ($CHCl_3$-MeOH 9:1) showed an absence of starting material and one fast-moving spot. The reaction was quenched by the addition of methanol (2ml) and the solution was evaporated under reduced pressure to a syrup which was chromatographed on an SiO_2 column with ethyl acetate-petrol 4:1 to give 0.22g (82%) of (98) as a clear syrup.

Mass spectral data (Ammonia C.I.) $(M+NH_4)^+$ m/z 398[a] (12%), 105 (19%), 75 (100%).

^1H NMR (500MHz) δ 8.14-7.32 (m, 5H, 6-O-benzoyl); 6.02 (bd, 1H, NH); 4.52-4.41 (m, 5H, H-1, H-2, H-3, H-6, H-6'); 4.25 (dd, 1H, $J_{4,5}$=8.5, $J_{3,4}$=1.6, H-4); 3.71 (dd, 1H, $J_{4,5}$=8.5, $J_{5,6}$=3.4, H-5); 3.40, 3.30 (2s, 6H, 1-OMe, 3-OMe); 2.05 (s, 3H, NAc).

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