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NEW SYNTHETIC ROUTES TO GLYCOSIDASE INHIBITORS, BIOLOGICALLY ACTIVE DISACCHARIDES AND GLYCOSYL DONORS

presented by

Gabriela Pistia-Brueggeman

has been accepted towards fulfillment
of the requirements for

Ph.D. degree in Chemistry

Major professor

Date December 7, 2000

NEW SYNTHETIC ROUTES TO GLYCOSIDASE INHIBITORS, BIOLOGICALLY ACTIVE DISACCHARIDES AND GLYCOSYL DONORS

By

Gabriela Pistia-Brueggeman

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Submitted to
Michigan State University
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Department of Chemistry

2000

ABSTRACT

NEW SYNTHETIC ROUTES TO GLYCOSIDASE INHIBITORS, BIOLOGICALLY ACTIVE DISACCHARIDES AND GLYCOSYL DONORS

By

Gabriela Pistia-Brueggeman

Complex carbohydrates and their conjugates in biological systems are either structural or informational molecules. On the cell surface many carbohydrates of glycoconjugates are involved in various types of biochemical recognition processes, including growth, development, immune responses, infection, cell adhesion, metastasis, and numerous signal transduction events. This dissertation is focusing on the synthesis and screening of sugar analogs with high therapeutic potential, preparation of the disaccharide structural component of bacterial cell wall lipid A and finding new glycosyl donors, to facilitate oligosaccharide synthesis.

The first part of this dissertation (chapters 2 & 3) describes new synthesis and screening procedures for glycosidase inhibitors, especially 1,5-iminoalditols and aldono δ-lactones. These two classes of compounds have high inhibitory activity and they constitute viable candidates for cancer, AIDS and diabetes treatment. A new, general method for the preparation of such compounds with the D-*gluco* and D-*galacto* configuration starting

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from β-glycosides is presented. The procedure stands out among the preexisting methods due to its generality, straightforwardness, high yield and stereoselectivity. This methodology has been used in the one pot synthesis of a library of compounds belonging to 4 classes of substances: methyl glycosides, aldonic acid lactams, aldonic acid lactones and 1,5-dideoxy-1,5-iminosugars. These have been screened for activity against glucosidases. The approach provides a facile and rapid route towards the synthesis and screening of a multitude of potential glycosidase inhibitors.

Chapter 4 discusses an improvement and new application of the chromium trioxide oxidation of glycosides, while chapter 5 describes a novel protecting/activating group for N-acetylglucosamine. o-Nitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside can be activated with $ZnCl_2$ to afford, in the presence of an alcohol, high yields of N-acetamide β-D-glucosides.

The last chapter presents various attempts towards the total synthesis of the disaccharide headgroup of lipid A from *Rhizobium Trifolii.* Lipid A is a component of lipopolysaccharides (LPS), which are a unique class of glycolipids found on the surface of Gram-negative bacteria. LPS are capable of eliciting a wide array of biological responses when they interact with cellular systems of animals. Determination of their structure can help in the elucidation of their physiologic activity.

To my mother

Without her all this would not have happened

ACKNOWLEDGMENTS

Entering graduate school has been an important decision in my life, and now, at the verge of concluding this 'chapter', I can say that I cherished every moment. I want to believe that I'm emerging as a more knowledgeable, wiser, and stronger person. However, this would not have been possible without the help of some wonderful people.

Most of my gratitude goes to my advisor Rawle I. Hollingsworth. With his help I discovered the beautiful and complex world of carbohydrate chemistry and a whole new philosophy of life. He was always there for me with help, advice and encouragement from the first day I joined our lab to the last step of my graduate work. To him I owe the mastering of all techniques that I learned, the expanded vision of chemistry and the new perspective on life. Thank you Rawle, you have been a valuable mentor and at the same time a cherished friend.

I want to address my appreciation to the members of my committee, Dr. Gregory Baker, Dr. Marcos Dantus and Dr. Katharine Hunt. I want to thank Dr. Baker and Dr. Hunt for taking their time to revise this thesis and Dr. Dantus for giving me valuable advice regarding resume writing, job hunting and coping with my newborn.

I want to thank the member of Hollingsworth's lab for being there for me in good times and in stressful times, for their help and support and for being such good friends. Together with Rawle, they made coming to school not only worth wile but also a pleasure. Thank you Carol, Ben, Jim, Atima, Hussen, Lakshmi, Guangfei, Rob, Jeongrim, JJ, Ing, Guijun, Jie and Luc.

Thanks are also going to Long Lee and Kermit Johnson from the NMR facility, for their friendly help.

I am concluding my acknowledgments by thanking the most important people in my life: my parents, my son Justin and my husband Michael. There are not enough words to cover the gratitude that I have towards my parents, and especially my mother. She gave me her unconditional love and support, guided me along my life journey and was there for me whenever I needed her. I only hope that I can give back the love and help that I received. I am thanking my husband Michael for the peaceful life and support that he has offered and the cheerfulness that he has brought into our relationship. Because of this, my work did not seem as hard as it sometimes was. And at least but not at last I want to thank my precious little son Justin for the loving smiles and delightful coos and for the hope that he has brought to our lives.

To all of you THANK YOU.

TABLE OF CONTENTS

Chapt

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6

6

6

6

Table 3

Table 3.

Table 5.

LIST OF TABLES

LIST OF SCHEMES

LIST OF FIGURES

Chapter 1

Introduction for Glycosidase Inhibitors

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Abstract

Glycosidases modify glycoconjugates by hydrolyzing glycosidic linkages, a process which is essential for normal cell growth, regulation, and development. These glycoenzymes are interesting targets for inhibition, as they are involved in metabolic disorders and other diseases. Glycosidase inhibitors have the potential to produce multiple beneficial therapeutic effects including reduction of the blood glucose level, inhibition of tumor metastasis and stopping viral replication, all of which will be reviewed in this introductory chapter. Among the numerous inhibitors, three classes require special attention: iminosugars, lactams and lactones. Their mechanism of action and the advantages over the other inhibitors will be discussed.

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Figure 1.1

1.1. Classification of glycosidase inhibitors

A variety of glycosidase inhibitors are known so far, most of them being carbohydrate derivatives or their structural analogs. According to their way of action, they can be classified into reversible and irreversible inhibitors [1]. An irreversible inhibitor dissociates very slowly from its target enzyme because it becomes very tightly bound to the enzyme, either covalently or non-covalently. In contrast, reversible inhibition is characterized by a rapid dissociation of the enzyme-inhibitor complex. In competitive inhibition, the enzyme can bind the substrate (forming an ES complex) or inhibitor (EI) but not both (ESI) (Figure 1.1.1).

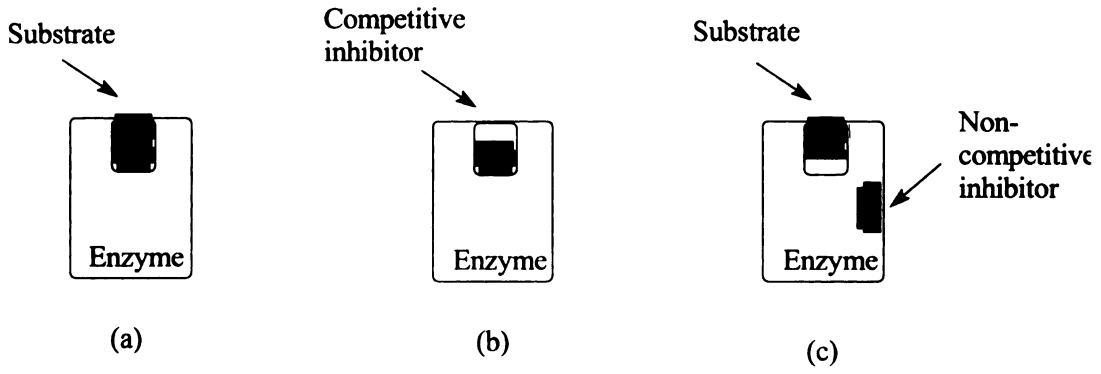

Figure 1.1.1. Distinction between competitive and noncompetitive inhibitors:

(a) enzyme-substrate complex; (b) a competitive inhibitor prevents the substrate from binding; (c) a noncompetitive inhibitor does not prevent the substrate from binding.

Many competitive inhibitors resemble the substrate and bind to the active site of the enzyme. The substrate is therefore prevented from binding to the same active site. A competitive inhibitor diminishes the rate of catalysis by reducing the proportion of enzyme molecules bound to a substrate. In noncompetitive inhibition, which is also reversible, the inhibitor and substrate can bind simultaneously to an enzyme molecule, but their binding sites do not overlap. A noncompetitive inhibitor acts by decreasing the turnover number of an enzyme rather than by diminishing the proportion of enzyme molecule that are bound to the substrate. Noncompetitive inhibition, in contrast with competitive inhibition, cannot be overcome by increasing the substrate concentration. Figure 1.1.2 presents the most common classes of glycosidase inhibitors. Reversible inhibitors are: aldonolactones, 5-amino-5-deoxyaldonolactams, glycosylamines, cyclic iminosugars like nojirimycin and deoxynojirimycin (DNJ), indolizine alkaloids like castanospermine and swainsonine, polyhydroxypyrrolidines, and aminocyclitols. There are also sugar-related compounds, called pseudosubstrates, that are chemically transformed by glycosidases, often forming long-lived intermediates and thereby acting as reversible inhibitors. Some of these enzyme-bound intermediates are cleaved so slowly that they constitute a transition towards irreversible inhibitors. Some of these pseudosubstrates are: D-glycals, heptenitols, glycosyl fluorides, and 2-deoxy-2-fluoro-glycosides. The last to be mentioned are the irreversible inhibitors, which react specifically at the active site and inactivate the enzyme, thereby providing important information about the mechanism of the enzyme-catalyzed reaction. Some examples are: conduritol epoxides (1,2-anhydroinositols), aziridines, glycosylmethyltriazenes, and glycosyl isothiocyanates [2]. This dissertation will focus on three of the most potent

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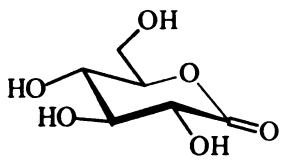
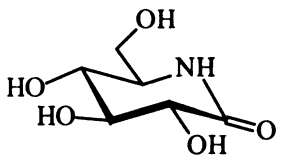
Figure 1.1

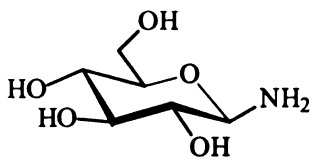
glycosidase inhibitors which are 1,5-δ-lactams, -lactones and 5-imino-1,5-dideoxysugars. Before focusing on their mechanism of action, their inhibitory activity and importance will be discussed.



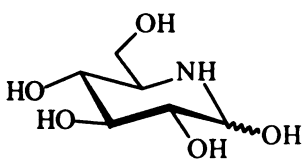
gluconolactone



5-amino-5-deoxy gluconolactam



β-D-glucosylamine



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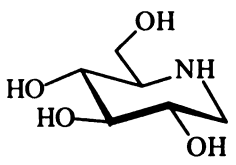
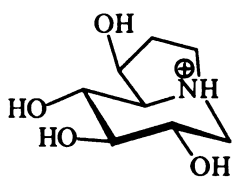
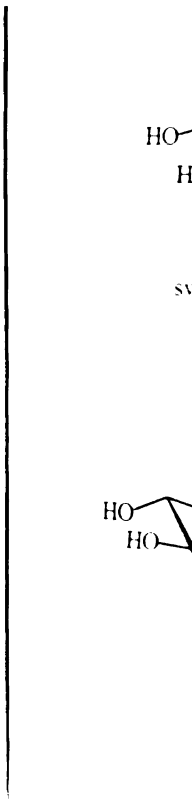


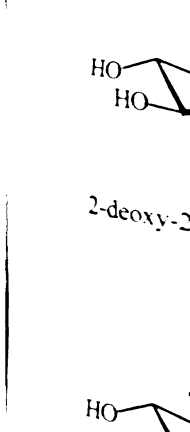
deoxynojirimycin

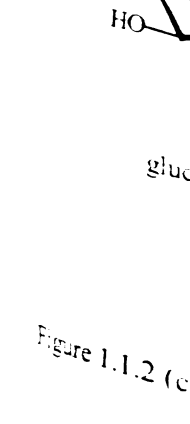


castanospermine

Figure 1.1.2 Representatives of the most common classes of glycosidase inhibitors.







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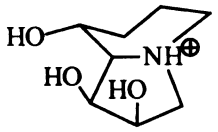

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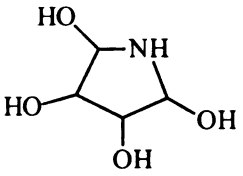
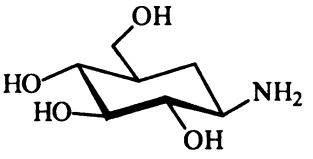
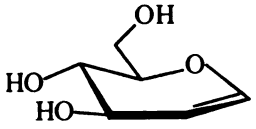


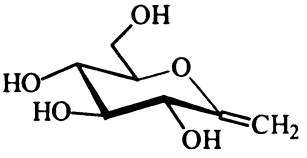
polyhydroxy-
pyrrolidine



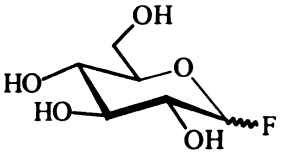
aminocyclitol



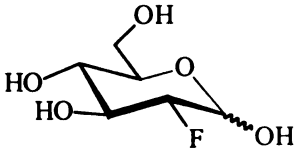
glucal



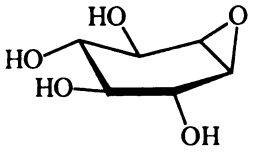
1-deoxy-D-gluco-hept-1-enitol



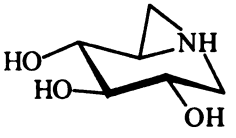
glucosyl fluoride



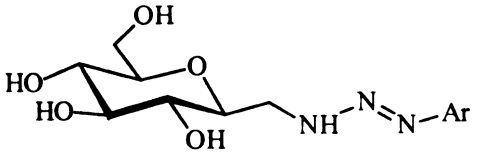
2-deoxy-2-fluoro-glucoside



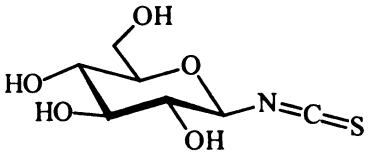
conduritol epoxide



aziridine



glucosyl methyl aryltriazene



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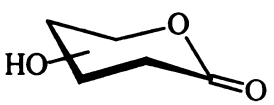
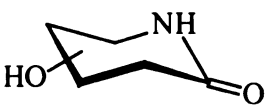
1.2. Activity and importance of glycosidase inhibitors

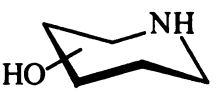
Glycosidase inhibitors have important therapeutic effects in the treatment of diabetes, cancer and AIDS. This beneficial activity derives from their interference with important biological processes that are involved in these diseases. This subsection will describe the general way glycosidases are involved in these diseases, however, it will focus only on the activity of three classes of glycosidase inhibitors, which are: 1,5-δ-lactams, -lactones and 5-imino-1,5-dideoxysugars (Figure 1.2.1).



1,5-δ-lactone



1,5-δ-lactam



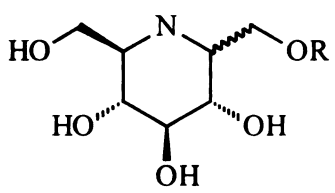
1,5-iminosugar

Figure 1.2.1 Three major classes of glycosidase inhibitors.

Diabetes

Carbohydrates are a main component of human food, 80-90% consisting of starch and sucrose. In general more than 250 g of di- and polysaccharides must be enzymatically split in the intestinal tract before they can be utilized by the organism. It has been suggested that a pharmacological interference with the intestinal carbohydrate digestion by suitable α-glucosidase inhibitors should be a feasible way to regulate and retard carbohydrate digestion, control the rate of absorption of monosaccharides and by this way influence the intermediary metabolism of the carbohydrates [3]. Glycosidases

catalyze the hydrolysis of complex saccharides and convert non-absorbable carbohydrates into absorbable sugars. The rapid action of these enzymes leads to acute undesirable elevations in blood glucose in diabetes. Potent inhibitors of these enzymes prevent harmful hyperglycemic excursions of this type. It is desirable, however, that the inhibition of these hydrolytic enzymes be limited to those present in the intestines. Otherwise, inhibition of systemic glycohydrolases or glucose transport can lead to difficulty in the utilization of intracellular carbohydrates as an energy-source and thus cause metabolic problems. It has been demonstrated that iminosugars are active as inhibitors of carbohydrate digestive enzymes and can be used in the treatment of diabetes. More specifically, they can be used to prevent the development of hyperglycemia. Rather than achieving this effect by promoting the metabolism of glucose present in the blood, some 1,5-iminosugars like homonojirimycin glycosides **1** (where R is a glycosyl radical) can act by preventing the initial formation of glucose in the body thereby holding down the quantity of glucose that could appear in the blood [4] (Figure 1.2.2). An example is the alpha-glucosidase inhibitor MDL-25637 **2** produced by Aventis Pharma and which is in phase I clinical trials [5]. N-Hydroxyethyl deoxynojirimycin **3**, commercialized by Bayer under the name of Miglitol, was launched in 1998 as a substitute for the antidiabetic Acarbose [6]. Since it has fewer gastrointestinal side effects, it is preferred. Miglitol is used to treat both type 1 and type 2 diabetes. It inhibits the action of alpha glucosidase and delays glucose absorption from the intestine, thereby preventing high blood sugar levels. Camiglibose **4** is an antidiabetic and antiobesity agent. This long-lasting alpha-glucosidase inhibitor is in preclinical trials [7]. Also in preclinical trials is the alpha-glucosidase inhibitor N-methyl deoxynojirimycin **5**, also named MOR-14 [8].



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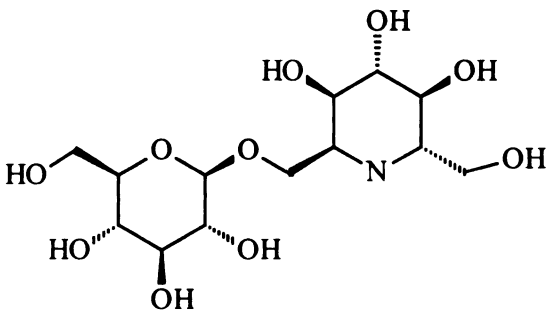

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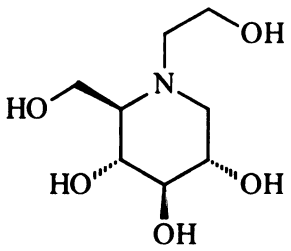

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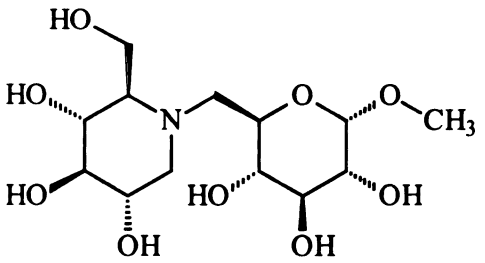

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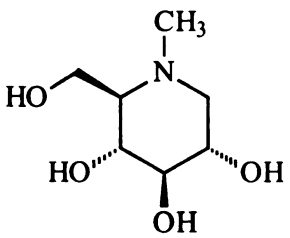

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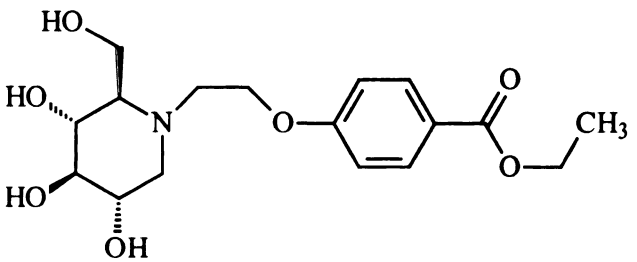

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Figure 1.2.2 Aza-sugars possessing antidiabetic activity.

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Its actions are hypoglycemic and antithrombotic. Finally, Emiglitate **6** was also considered as an antidiabetic. However, the clinical development of this compound has been discontinued due to low efficacy and poor tolerability [9].

Cancer

Cancer has been for many years one of the most fatal deseases. After its detection, the main therapeutic endeavors for its treatment were surgery, radiotherapy and chemotherapy. Surgical operation and radiotherapy may eliminate primary tumors. However, the success of these two procedures is many times hampered by the impossibility of early prognosis of the disease. The chances for an efficient therapy decrease once metastasis has started. This is why an intensified effort is applied for discovery of drugs that can stop or reverse metastasis.

Acute-transforming retroviruses carry in their genomes specific oncogenes that arose from recombination events between nontransforming viruses and normal cellular genes that control growth and/or differentiation. In most of these retroviruses a single transforming protein is synthesized from the viral oncogen, and this protein product is responsible for initiation and maintenance of the transformed, cancerous state. About 30 acute-transforming retrovirus isolates have been reported so far, and these have been found to harbor about 20 distinct oncogens. The fms, erbB, sis, and neu oncogenes are known to have glycosylated expression products and the fms and erbB glycoproteins are ultimately expressed in the plasma membrane. In general, the insertion of the cellular gene within the viral genetic framework resulted in two critical alterations that permit a

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metastasis

normal cellular gene to function in an uncontrolled manner within infected cells. First, the virus has provided a strong promoter for the cellular gene, enabling the overproduction of the acquired cellular gene, and second, in almost all cases the acquired cellular gene has been modified either through truncation or mutation. These quantitative and qualitative changes are believed to be important in neoplastic transformation by acute-transforming viruses.

It has been shown [10] that the cellular expression of acute-transforming retroviruses having glycosylated expression products is interrupted by the administration of processing glycosidase inhibitors, leading to remission of transformed cells to the normal phenotype. In particular, administration of the glucosidase I inhibitors castanospermine **7** and N-methyl-1-deoxynojirimycin **8** (Figure 1.2.3) interrupts the glycosylational processing and plasma membrane expression of the v-fms transforming glycoprotein, leading to cancer remission. These and similar processing glucosidase inhibitors should be effective in controlling cancers that are mediated by oncogenes with glycosylated products. These inhibitors are also effective in suppressing the proliferation of cells that normally express glycoprotein growth factor receptors on their surfaces. In particular, castanospermine inhibits the proliferation of monocytes and macrophages that bear glycosylated c-fms expression products on their plasma membranes, thereby affording a measure of selective immunosuppresion for therapeutic effect. Also, it is expected that the effectiveness of the existing various therapies could be further enhanced by inhibiting metastasis of cancer cells. However, there are few substances showing inhibition of metastasis as their main activity and none of them has been used in the clinical field.

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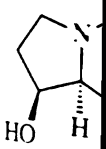

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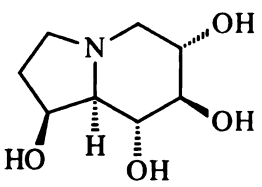
Figure 1.2

Tsuruoka has found that alkylated and arylated 5-amino-5-deoxy gluconolactams **9** posses an excellent cancer metastasis inhibiting activity [11]. D-Glucaric acid δ-lactam sodium salt **10** is an antimetastatic agent that has been found to inhibit invasion of B 16 melanoma cells. It is used in preclinical trials [12]. Its potassium analog **11** is a beta-glucuronidase inhibitor with antinephrotoxic activity [13].



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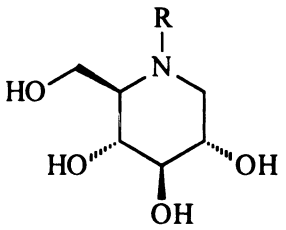

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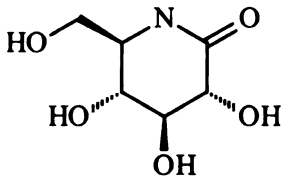

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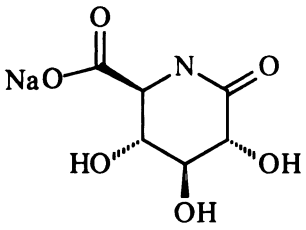

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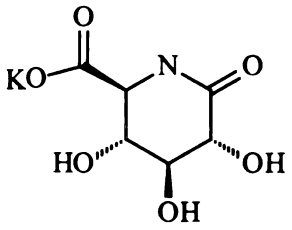

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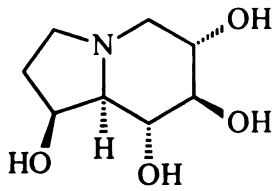
Figure 1.2.3 Compounds possessing cancer metastasis inhibitory activity.

AIDS

Acquired immunodeficiency syndrome (AIDS) is one of the most fatal disorders of our century, for which no completely successful chemotherapy has been developed so far. As a consequence, a great effort is being made to develop drugs and vaccines to combat AIDS. The AIDS virus, first identified in 1983, has the capacity to reproduce within cells of the immune system and thereby lead to a profound destruction of T2 T-cells (or CD4+ cells). The causative agent of AIDS is a retrovirus of the *Lentiviridae* family [14,15]. This enveloped single-stranded RNA virus is called human immunodeficiency virus (HIV) [16,17] and two genetically distinct subtypes, HIV-1 and HIV-2, have been characterized [18-20] of which the former has been found to be prevalent in causing the disease. The HIV-1 infection, which targets monocytes expressing surface CD4 receptors, eventually produces profound defects in cell-mediated immunity [21]. Over time, infection leads to severe depletion of CD4+ T-lymphocytes (T-cells), resulting in opportunistic infections, neurologic and neoplastic diseases, and ultimately death. Besides T-cells, other cells expressing DC4 on their surface may also harbor HIV-1 infection. These include macrophages, monocytes, and lymphoid cells [22]. Theoretically, an anti-HIV agent may exert its activity by inhibiting a variety of steps in the life cycle of the virus. However, medicinal chemists have focused their attention predominantly on the following stages: viral binding to target cells, virus cell fusion, virus uncoating, reverse transcription of genomic RNA, viral integration, gene expression, cleavage event, and virion maturation. By hitting any of these stages, the viral replication can be terminated [23]. The replicative cycle of HIV-1 presents several viable targets that could be exploited for the development of anti-HIV chemotherapy. Ideally, an

anti-HIV agent should arrest the virulence and further infection of healthy cells without displaying toxicity toward normal cellular physiology. To achieve this goal, attention has been focused on several intervention strategies and various kinds of inhibitors. Most of the compounds approved so far by the FDA in the United States for the treatment of HIV infections are reverse transcriptase inhibitors and protease inhibitors.

More recently, certain glucosidase inhibitors have been tested for activity against the AIDS virus. Because the envelope glycoproteins of HIV are heavily glycosylated, compounds that interfere with co- and posttranslational processing of glycoprotein gp120 and the transmembrane glycoprotein gp 41 may prevent viral entry into a cell. Glucosidase cleaves off glucose units from the oligosaccharide chain and thus helps the maturation of infectious virion. The inhibition of this enzyme, therefore, will lead to the inhibition of virion maturation. Polyhydroxylated compounds (Figure 1.2.4) have demonstrated inhibitory potential in preclinical evluation [24] and have been suggested as potential anti-AIDS drugs [25-28]. Castanospermine **12**, which is an alkaloid isolated from the seeds of Australian chestnut tree, has been found to interfere with normal glycosylation of HIV virions, thereby altering the envelope glycoprotein and preventing entry of HIV into target cells. It also is studied as antimetastatic, treatment for multiple sclerosis and for prevention of organ transplant rejection [29]. However, since castanospermine is in limited supply due to its natural source and its chemical synthesis is laborious, it is extremely expensive and therefore not a realistic candidate for a drug urgently required on large scale. Additionally, castanospermine has demonstrated



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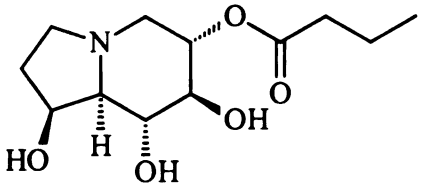

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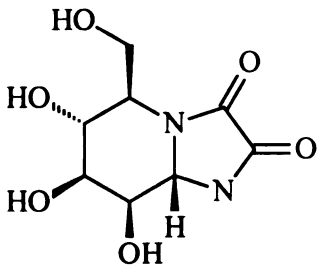

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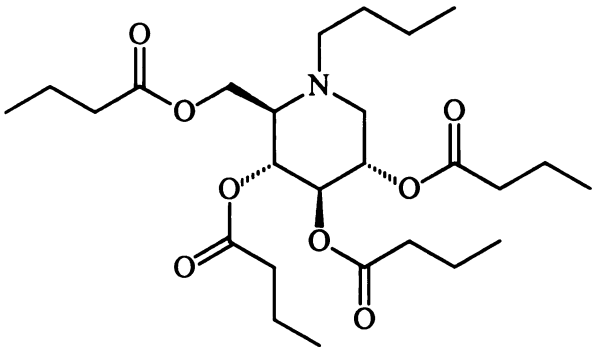

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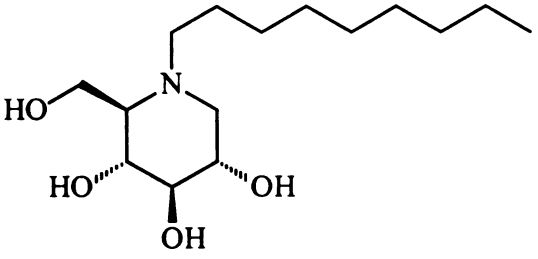

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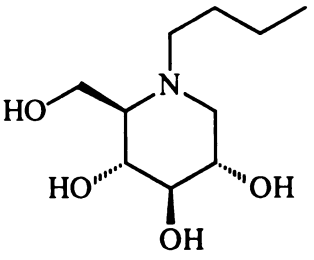

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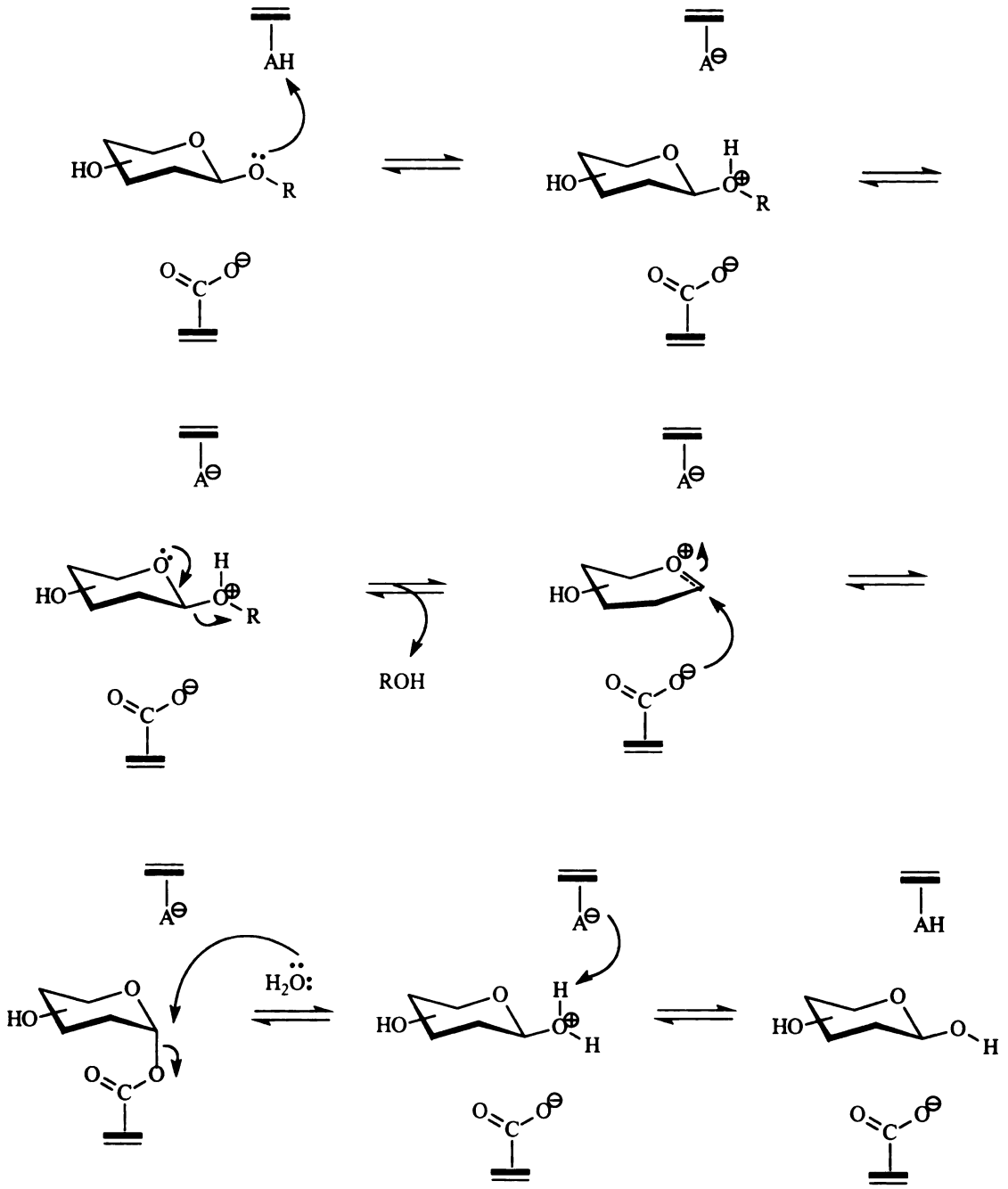
Figure 1.2.4 Compounds possessing antiviral activity.

cytotoxicity at dose levels of 0.7 mg/mL [30]. A mono-butanoyl derivative **13** of castanospermine named Celgosivir is also a potent anti-HIV agent and is tested in phase II clinical trials [31]. Alkaloid **14** called Kifunensine, produced by the actinomycete Kitasatosporia kifunense is an immunomodulator used in preclinical trials [32]. A large number of deoxynojirimycin analogs have been tested for anti-HIV activity. The pentabutanoyl derivative **15** of DNJ is a potent inhibitor in phase II preclinical trials [33]. The N-nonyl-DNJ **16**, which in 1992 was in preclinical trials, proved to be not only a potent anti-HIV agent but also active against hepatitis B and hepatitis C [34]. N-methyl deoxynojirimycin was also disclosed as having activity against HIV ostensibly based on its glucosidase I inhibitory activity. However it was subsequently shown by Fleet [35] that not all glucosidase I inhibitors are effective inhibitors of HIV. Therefore, some other mechanism may be responsible for HIV inhibitory activity. The N-butyl derivative of deoxynojirimycin has been found to have enhanced inhibitory activity against the HIV virus at not-toxic concentrations compared to that exhibited by the corresponding N-methyl and N-ethyl derivatives. N-Butyl-DNJ uniquely reduces the virus titer by over five logaritms at non-cytotoxic concentrations whereas the N-methyl- and N-ethyl-DNJ derivatives cause only a two to four log-order of reduction in the yield of infectious HIV. As such, the N-butyl derivative (BuDNJ) has significant potential use for the treatment of AIDS, and phase II clinical trials of this agent have been started. Also, long-term culture of HIV-infected cells in the presence of BuDNJ gradually decreased the number of infected cells to a point where infectious HIV was no longer detectable [36]. These data suggest that even with proviral DNA integration, HIV-infected cells probably undergo a lytic cycle and that long-term culture of infected cells in the presence of a drug such as

BuDNJ could break the cycle of HIV replication and infection. This gives hope that compounds of this type might reduce or even eliminate HIV infection *in vivo* in patients with latent infection or overt disease. Also, combinations of anti-HIV therapies which include agents that attack the HIV replicative cycle at multiple sites offer several advantages and synergistic combinations of AZT and BuDNJ have been found to give excellent results [37]. Recently it has been discovered that N-butyl DNJ is also a glycosphingolipid formation inhibitor and it is tested in the treatment for glycosphingolipid storage disorders such as Gaucher's disease and Fabry's disease [38]. Based on all this information we can conclude that there is indeed a real interest in the use of glycosidase inhibitors in the treatment of diabetes, cancer and AIDS. The discovery of simple and efficient routes for the synthesis of such compounds would increase their availability and make them viable drug candidates.

1.3. General mechanism of glycoside hydrolysis

Hen-egg lysozyme is still the only enzyme for which detailed mechanistic information is available from X-ray structure analysis with respect to active-site structure and functional groups involved in catalysis [39]. For all other glycosidases, especially the vast number of exo-hydrolases, we depend on the interpretation of kinetic and inhibition studies with reversible and irreversible inhibitors. Published data on the reversible inhibition by basic sugar analogs, and active site-directed inactivation by conduritol epoxides, show that the majority of "retaining" α- and β-glycosidases have an active site that features a proton-donating group close to the glycosidic oxygen atom, and a carboxylate group that can stabilize the partial positive charge developing on the anomeric carbon atom and that may



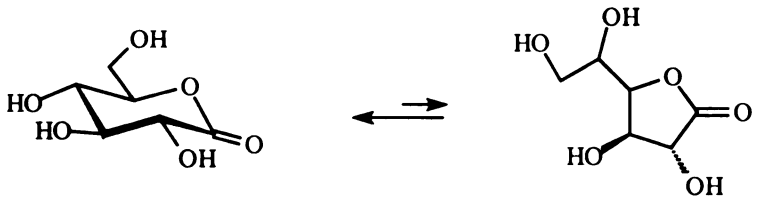
Scheme 1.3 General mechanism of glycosidase inhibitors.

possibly form a covalent glycosyl-enzyme intermediate that is subsequently hydrolyzed (Scheme 1.3). It is proposed that substrate distortion towards the transition state (change from $^4C_1$ chair towards a half-chair conformation) constitutes a major contribution to catalysis. However, the enzyme also adjusts its conformation to the requirements of the ligand, rather than forcing a drastic conformational change upon the latter. It is also assumed that access of solvent water to the active-site region of the enzyme-inhibitor complex is largely restricted. This is important for the close alignment of the substrate with respect to the catalytic groups that is required for effective catalysis.

1.4. Mechanism of action of glycosidase inhibitors

1.4.1. *Aldonolactones*

The strong inhibition of glycosidases by aldonolactones was first mentioned in 1940 by Japanese scientists who studied β-D-glucosidases from Aspergillus [40] and almonds [41]. These studies were extended to other glycosidases by Conchie and Levvy [42]. The

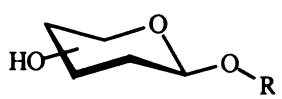


1,5-gluconolactone 1,4-gluconolactone

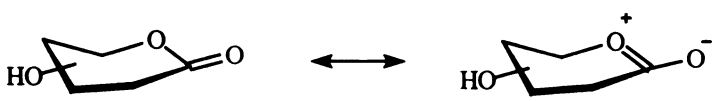
Scheme 1.4.1.1 Interconversion between 1,5-gluconolactone and 1,4-gluconolactone.

authors showed that the aldonic acids themselves are non-inhibitory, and that 1,5-lactones are better inhibitors than their 1,4-isomers. Also, the 1,4-lactones are not stable and easily convert to their 1,5-isomers (Scheme 1.4.1.1). The fact that the aldonolactones might exert their powerful inhibition by virtue of their structural similarity with a glycosyl oxocarbonium ion intermediate or a related transition state was first pointed out by Leaback [43]. Both the lactone and the oxocarbonium ion have a trigonal, planar configuration at C-1 and adopt a half-chair conformation which is in marked contrast with the tetrahedral C-1 configuration and $^{1}C_{4}$ conformation of aldopyranoside substrates and aldoses (Scheme 1.4.1.2). In addition to these geometrical factors, there have to be

Pyranosyl oxocarbenium ion
trigonal planar configuration



β-Aldopyranoside
$^{4}C_{1}$ conformation



Aldono-1,5-lactone
trigonal planar configuration

Scheme 1.4.1.2 Geometrical similarity between aldono-1,5-lactones and the pyranosyl oxocarbenium ion and the differences in the conformation of aldopyranosides.

considered electrostatic interactions arising from the large dipole moment of the lactone which would enhance lactone binding if there is a negatively charged group in close proximity to C-1 of the bound inhibitor. There is a general agreement that the aldono-1,5-lactones are better inhibitors for glycosidases than are the 1,4-isomers [44] which are probably no better inhibitors than aldoses or polyols of comparable structure. It was found that the α-specific enzymes were inhibited by D-gluconolactone at least 100-fold less potently than the β-specific ones. The latter are inhibited by aldono-1,5-lactones several hundred to many thousand-fold better than by the corresponding aldoses. It has been demonstrated that the inhibition is competitive; that is, the inhibitor competes with the substrate for the free enzyme. Generally K_i for lactones are in the micro- and even nanomolar range.

1.4.2. *5-Amino-5-deoxylactams*

The problems regarding ring size and stability that are encountered with aldonolactones disappear when the ring-oxygen atom is replaced by an NH group. The resulting 5-amino-5-deoxyglyconolactams constitute a new group of inhibitors, closely related to the lactones. Like their oxygen analogs, they have a trigonal, planar configuration at C-1; the dipole moment of the carbonyl group is expected to be even larger than that of the lactone carbonyl, due to the larger contribution of the dipolar resonance structure (Scheme 1.4.2). Differences in their interaction with glycosidases may arise if substrates and lactones are bound with the ring-oxygen atom in a closely fitting cleft of the active site. The NH group may then cause steric repulsion, or a hydrogen-bond donor for the ring-oxygen atom may fail to interact properly with the NH group because of its amide resonance. The

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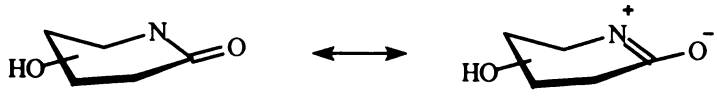
strong inhibition observed with the lactams tested so far shows that these effects are of minor importance. In cases where the inhibition constants for lactams and lactones can be compared, they have the same order of magnitude. The weak inhibition of α-specific enzymes by lactams has also been observed. The reason for this may be seen in the greater structural similarity of lactams (orientation of the C=O dipole) with the orientation of the anomeric oxygen atom of β-glycosides than of α-glycosides. If Pauling's hypothesis [45] is followed about an evolution of enzyme-active sites towards complementarity to the transition state, this would indicate a considerable resemblance of the transition-state structure with that of the substrate. 5-Amino-5-deoxyaldonolactams inhibit β-D-glycosidases 100- to > 10,000-fold better than by the parent aldoses, with K_i values from 200 μM to < 0.1 μM. The inhibition is competitive like the one for lactones. From structural consideration it may be assumed that aldonolactams are bound by the glycon binding-site of the enzyme, interacting with the same functional groups that are responsible for substrate binding and, possibly, catalysis. A molecular modeling study [46] performed by C.-H. Wong's group indicated that the 2-OH group, the 6-OH group, a



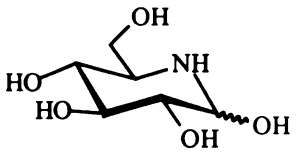
Aldono-1,5-lactam
trigonal planar configuration

Scheme 1.4.2 Configuration of aldono-1,5-lactams.

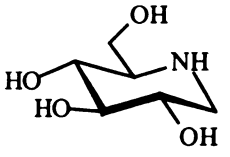
positive charge and a half-chair conformation are important for a good inhibitor binding to the enzyme. Each of these factors contributes to the overall binding.

1.4.3. *Cyclic iminosugars*

In 1970, it was reported that nojirimycin, identified as 5-amino-5-deoxy-D-glucopyranose, is a powerful inhibitor of β-D-glucosidases [47]. It had been discovered by virtue of its antibiotic activity in culture filtrates of certain strains of *Streptomyces* [48]. Its 1-deoxyderivative, a strong inhibitor of α-D-glucosidases, is produced by certain strains of *Bacillus* [49]. Subsequent studies with 5-amino-5-deoxyhexopyranoses and 1,5-dideoxy-1,5-imino-hexitols related to D-mannose [50], D-galactose [51] and 2-acetamido-2-deoxy-D-glucose [52] showed that the strong inhibition of glycosidases by



nojirimycin



deoxynojirimycin

these compounds is a general phenomenon probably based on common mechanistic features. These sugar analogs with nitrogen in the ring have much greater stability in aqueous solution. No spontaneous decomposition reactions are known for the 1,5-iminohexitols. The half-life at 25°C and pH 5 of the D-*manno* analog of nojirimycin is larger than 100 h [50]. The large inhibitory potential of these basic sugar derivatives (in the micro and nanomolar range) and their stability, have made them valuable tools not

only for mechanistic studies but also for the investigation of problems in cell biology where glycosidases are involved. Irrespective of the individual variations, it can be stated that sugar derivatives having an amino group in the ring inhibit glycosidases from several hundredfold to more than ten thousand fold better than their oxygen analogs. This means that the electrostatic interactions of protonated inhibitor and negatively charged group of the active site are not impaired by the different position of the positive center in the N-cyclic inhibitors. The formation of an ion pair consisting of the protonated inhibitor and an anionic group at the active site can take place in two ways: (i) the enzyme binds the neutral form of the inhibitor, which then gets protonated, probably by the same group which protonates the glycosidic oxygen atom during substrate hydrolysis; (ii) the enzyme binds the inhibitor cation, which then forms an ion pair with a carboxylate of the active site (Figure 1.4.3). Both modes show a similar increase of inhibitory strength with pH, because, with increasing pH, an increasing proportion of the inhibitor will be present in the unprotonated form and ionization of an active-site carboxylic group will increase the proportion of the carboxylate responsible for the tight binding of cationic glycosyl derivatives. As there is a considerable overlap of pK_a values for protonated inhibitors (pK_a 5.3 to 6.1 for glucosylamines [53], pK_a 5.1 to 5.6 for 5-amino-5-deoxyhexopyranoses [46,49,50], and pK_a 6.3 to 7.2 for 1,5-didieoxy-1,5-iminohexitols) [49,50,51] and pK_a for carboxylic groups (pK_a 4 to 6), it is not possible to discriminate between the two modes of action by pH studies with basic inhibitors alone. A straightforward discrimination between the two pathways is possible with enzymes that are inhibited by basic glycosyl derivatives as well as by permanent cationic ones. This is illustrated by β-D-glucosidases A3 form *Asp. Wentii*, where K_i values for β-D-

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glucosylpyridinium ion, β-D-glucosylamine, and nojirimycin have the same pH dependence, provided that the concentration of the last two is based on the concentration of the protonated form [54]. The most plausible explanation in this case is that the enzyme binds the inhibitor cation and the pH-dependence reflects the ionization of a carboxylic group having pK_a 5.6 which provides strong binding only when it is present as carboxylate. Binding of the basic form of the inhibitor and subsequent protonation by an acidic group of the active site probably take place with β-D-glucosidase from almonds [53] and β-D-galactosidase from E. coli [55], because here the β-glycosylpyridinium ions are bound no better than their uncharged analogs, the β-glycosylbenzenes. In cases where such isosteric pairs of cationic and neutral inhibitors as glycosylpyridinium ion and glycosylbenzene are not available, a permanently cationic derivative can be prepared by N-permethylation of a basic sugar analog. Interpretation of inhibition results present no problems if the quaternary ammonium ion is bound with affinity similar to that of the

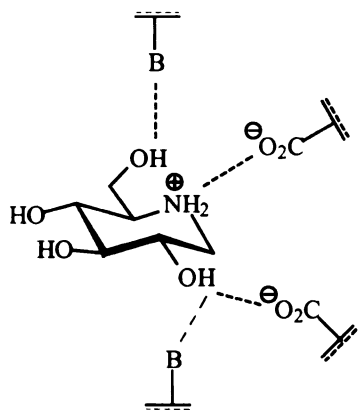

Figure 1.4.3 Assumed ground-state binding of deoxynojirimycin to β-glucosidase.

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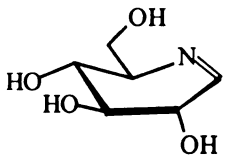
parent compound. This was observed with an α-D-glucosidase of the endoplasmic reticulum involved in glycoprotein biosynthesis (glucosidase I) which is inhibited by 1-deoxy-N,N-dimethylnojirimycin, with K_i 0.4 μM, whereas the monomethyl derivative inhibited with K_i 0.07 μM and 1-deoxynojirimycin [56] with K_i 1μM. Similar results were obtained with lysosomal β-D-glucosidase from calf spleen and human placenta, which were inhibited by 1-deoxy-N,N-dimethylnojirimycin about 4-fold better than by the unmethylated compound. These results also showed that enzyme-substrate interactions can not be very close to the ring-oxygen atom of the substrate; if they were, N-alkylation would have weakened the inhibition by steric interference, as it did [57] with glucosidase II, another α-D-glucosidase involved in glycoprotein biosynthesis and with α-D-galactosidase from coffee beans [51].

A still enigmatic feature of glycosidase inhibition by sugar analogs with a nitrogen atom in the ring is its slow onset in many cases, where K_i is in the micromolar range or below. This was first reported for β-D-glucosidase from almonds and nojirimycin by Grover and Cushley [58], and for intestinal sucrase-isomaltase and nojirimycin, 1-deoxynomirimycin, and acarbose, by Hanozet and coworkers [59]. The approach to the steady-state inhibition took place on the time-scale of minutes. Studies with other enzymes showed that this phenomenon is fairly widespread. In all cases, the enzyme-inhibitor complex is formed at a rate that is of first order with respect to the inhibitor concentration, with rate constants in the range [60-62] of 10^3 to 10^4 $M^{-1}s^{-1}$ and thus three to four orders of magnitude below those of the reactions controlled by the rate of diffusion. As the association rate showed no saturation effects with inhibitor

concentrations up to 20 K_i, any loose, pre-steady-state complex must have a dissociation constant K_i at least 50-fold larger than the steady-state K_i. Inhibition constants for intestinal sucrase measured after 2 and 10 s with nojirimycin, 1-deoxynojirimycin, and acarbose were found to be 134-, 59-, and 106-fold larger than those measured for 15 minutes. Inhibitors of this type were classified as slow, and slow, tight-binding inhibitors by Morrison and Walsh [63]. Several possible models can be discussed for the molecular basis of slow inhibition [63], but experimental evidence in support on one or the other is still lacking for glycosidases. A reversible chemical reaction at the active site, for example, formation of a cyclic imine **18** or a diffusion-controlled association with a trace of imine in equilibrium with the 5-amino-5-deoxypyranose can be precluded, because slow inhibition is also observed with 1-deoxynojirimycin and its analogs and with acarbose.



18

Another possibility could be the association of the inhibitor with a high-affinity conformer of the enzyme present in low, equilibrium concentration. Complex-formation would then shift the conformational equilibrium towards the high-affinity state. In this case, however, the rate constant should be independent of the inhibitor concentration, because the high-affinity conformer would always be saturated with the inhibitor. A third

possibility is the formation of a loose complex, having a dissociation constant K_i, which then undergoes a slow conformational change to form the tight complex. Reversibility of the inhibition requires that the dissociation of the tight enzyme-inhibitor complex must also be a slow process.

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Chapter 2

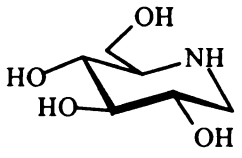
A General Approach to the Synthesis of Imino-Dideoxy and -Trideoxy Alditols from β-D-Glycosides

Abstract

Imino-sugars (also called azasugars), a class of compounds of which the 1,5-dideoxy-1,5-imino- and 1,5-imino-1,5,6-trideoxy alditols are members, are important glycosidase inhibitors with very high potential as drugs. Their potential therapeutic applications range from the treatment of diabetes to cancer and AIDS. We present here a general method for the preparation of such compounds with the D-*gluco* and D-*galacto* configurations starting from β-glycosides. The procedure is especially appealing because of its high yield, stereoselectivity and straightforwardness. The key steps are the selective oxidation of the glycosides to hexulosonic acids and reduction of the oxime derivatives to lactams which are further reduced to the target compounds. The C6 position can be deoxygenated during the reduction if it bears an acetoxy group. The trideoxy imino sugars are then produced. This saves several steps in which a protection and deprotection strategy and the installation of a halo or tosylate group at that position is avoided. Reduction of both the E and Z forms of the oxime gives the correct D-stereochemistry. Deacetylation prior to oxime reduction gives the dideoxy compounds.

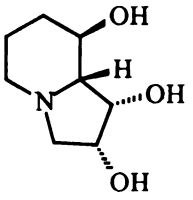
2.1. Introduction

Over the last three decades there has been a continuous interest in natural and synthetic imino-sugars because of their high potency as glycosidase inhibitors [1-18]. Glycosidases catalyze the hydrolysis of glycosidic linkages and are the key enzymes in the degradation of complex carbohydrates. One of their main metabolic roles is the conversion of complex non-absorbable carbohydrates into absorbable mono- or oligosaccharides [19]. The rapid action of these enzymes can lead, however, to undesirable elevations in blood glucose in diabetes. Iminosugars have been shown to act as glycosidase inhibitors and to retard and regulate the intestinal carbohydrate digestion. They are therefore excellent drug candidates for diabetes therapy [20]. N-Hydroxyethyl deoxynojirimycin, commercialized under the name of Miglitol, has been launched in 1996 as a very potent antidiabetic. [21]. An even more exciting potential use of iminosugars is in the treatment of cancer and viral diseases [22]. It has been shown that modification of oligosaccharide structures may alter metastatic capacity of cancer cells and 1,5-diimino-1,5-dideoxyglucitol (deoxynojirimycin) (**1**) [23] swainsonine (**2**) [24] and castanospermine (**3**) [25] (Figure 2.1.1) can markedly inhibit metastasis of cancer cells. They might, therefore, be used for the effective treatment of cancer. N-Butyl-deoxynojirimycin shows excellent activity against herpes virus [26] whilst having low cyto-toxicity and no inhibitory effect on the growth of normal cells. The greatest prospect for the use of iminosugars as drugs is probably for the treatment of AIDS. Glycosidase inhibitors prevent the processing of N-linked complex oligosaccharides. This results in the disruption of the synthesis of viral coat glycoproteins such as the critical one called gp120. This supposedly leads to the loss of recognition by the CD-4 receptor of the target



1

Deoxynojirimycin (DNJ)



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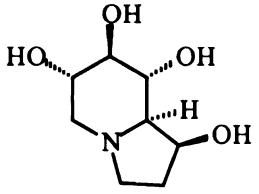
Swainsonine



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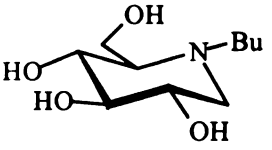
Castanospermine



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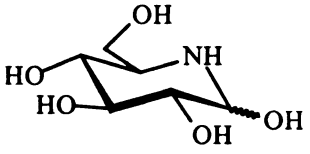
N-Butyl-DNJ



5

Nojirimycin (NJ)

6

1,6-Dideoxynojirimycin

Fig. 2.1.1 Iminosugars with therapeutic importance.

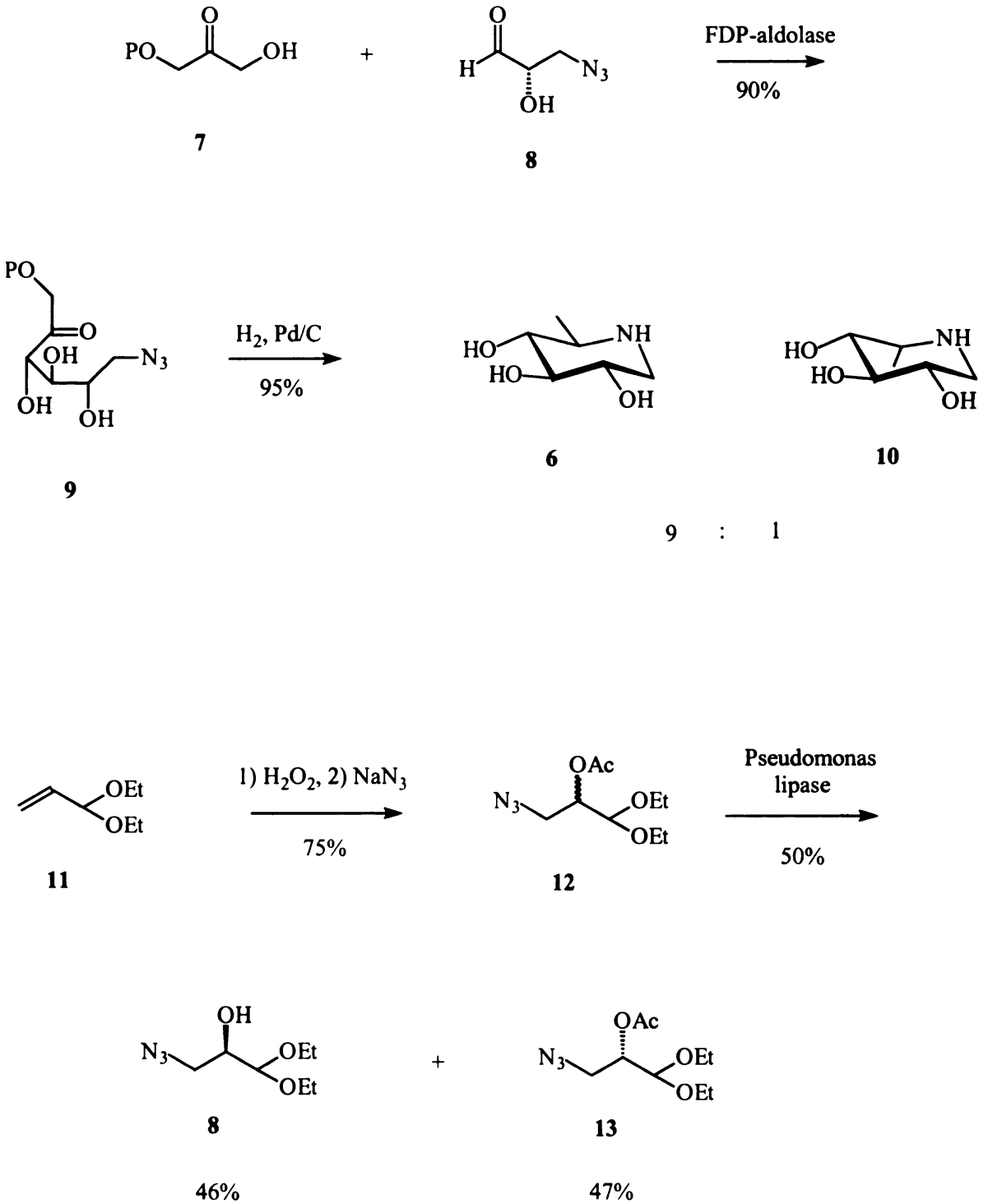
cell with concomitant reduction of syncytia formation resulting in the reduction of virus infectivity and the inhibition of viral replication [27-29]. Clinical trials have been launched for N-butyl-deoxynojirimycin (**4**) [30,31].

The iminosugars that have been the most investigated are deoxynojirimycin (DNJ) [32-46] and its N-alkyl analogues [2,32,33,47]. 1-Deoxynojirimycin was initially obtained from nojirimycin (**5**) by catalytic hydrogenation or by reduction with $NaBH_4$. Nojirimycin was first described as an antibiotic produced by *Streptomyces roseochromogenes* R-468 and *Streptomyces lavendulae*-SF-425 [48-50]. DNJ is produced by strains of the genus Bacillus [51,52]. Nojirimycin (NJ) was found to be a potent inhibitor of β–glucosidases (emulsin, fungal β-glucosidases) [53]. It also inhibits microbial α-glucosidases, but to a much lesser degree than β-glucosidases. Deoxynojirimycin is a much weaker inhibitor of emulsin than NJ but is a potent inhibitor of trehalases and of *Rhizopus niveus* glucoamylase and exo-β-1-3-glucanase. NJ and DNJ were found to be potent inhibitors of intestinal oligo- and disaccharidases of mammals [51]. Nojirimycin and deoxynojirimycin are not the only potent glycosidase inhibitors. Many other pyranoses and furanoses in which the ring oxygen has been replaced by an amino group have biological activities. For example, 1-deoxymannojirimycin inhibits α-mannosidases and α-fucosidases [54-56], 5-epi-L-deoxyrhamnojirimycin inhibits L-rhamnosidase [57], deoxyrhamnojirimycin inhibits both lysosomal α-mannosidase and α-fucosidase [58], deoxygalactonojirimycin inhibits galactosidases [59], and deoxyfuconojirimycin is a powerful and specific inhibitor of several α-L-fucosidases [60]. In addition to iminosugars, lactams have also been found to be good glycosidase

inhibitors, many times even better than the related azasugars. This may mean that the half chair conformation of the lactams resembles more closely the structure of the transition state for the enzymatic cleavage of glycosides. D-Mannolactam and D-rhamnolactam are good inhibitors of α-mannosidase [61] and of apricot β-glucosidase [62].

It has been shown that alkylation of the ring nitrogen has different effects on different glycosidases [63]. For in vivo inhibition, N-alkylation may facilitate transport of the inhibitor across the cell membrane, thereby increasing its effectiveness. This might mean that the more hydrophobic 6-deoxyiminosugars such as 1,5-imino-1,5,6-trideoxyglucitol (**6**) (1,6-dideoxynojirimycin) (Figure 2.1.1) which in vitro experiments showed to be less effective than its 1,5-dideoxy analogues [64], may be effective *in vivo* inhibitors. This is still to be investigated.

We have been able to identify three chemical syntheses for 1,6-dideoxynojirimycin in the literature. The first one involves enzymatic aldol condensation of dihydroxyacetone phosphate (**7**) (DHAP) and enantiomerically pure 3-azido-2-S-hydroxypropanal (**8**), using FDP-aldolase, followed by catalytic hydrogenation to give a 9:1 mixture of di-DNJ (**6**) and its C-5 epimer (**13**) [64] (Scheme 2.1.1). A key element used in this synthesis is the preparation of the azidoaldehyde (**8**) as an optically pure compound. This is done by peroxide oxidation of vinylaldehyde (**11**) followed by substitution with NaN_3 and acetylation, to give a racemic mixture of azides that can be resolved with lipase to give an approximatively equimolar mixture of the 2 enantiomers [65]. One disadvantage of this

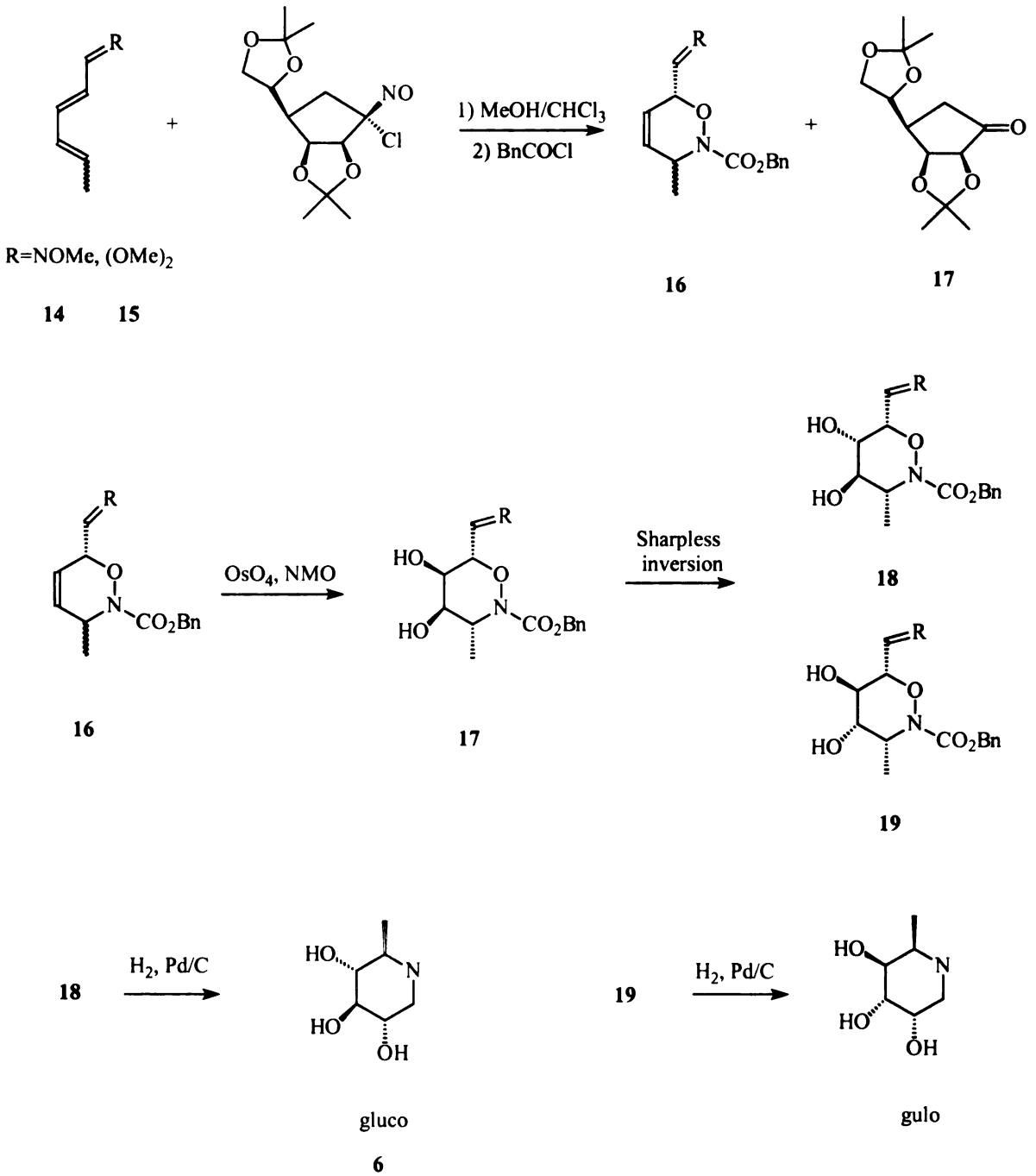


Scheme 2.1.1 Synthesis of di-DNJ by enzymatic aldol condensation.

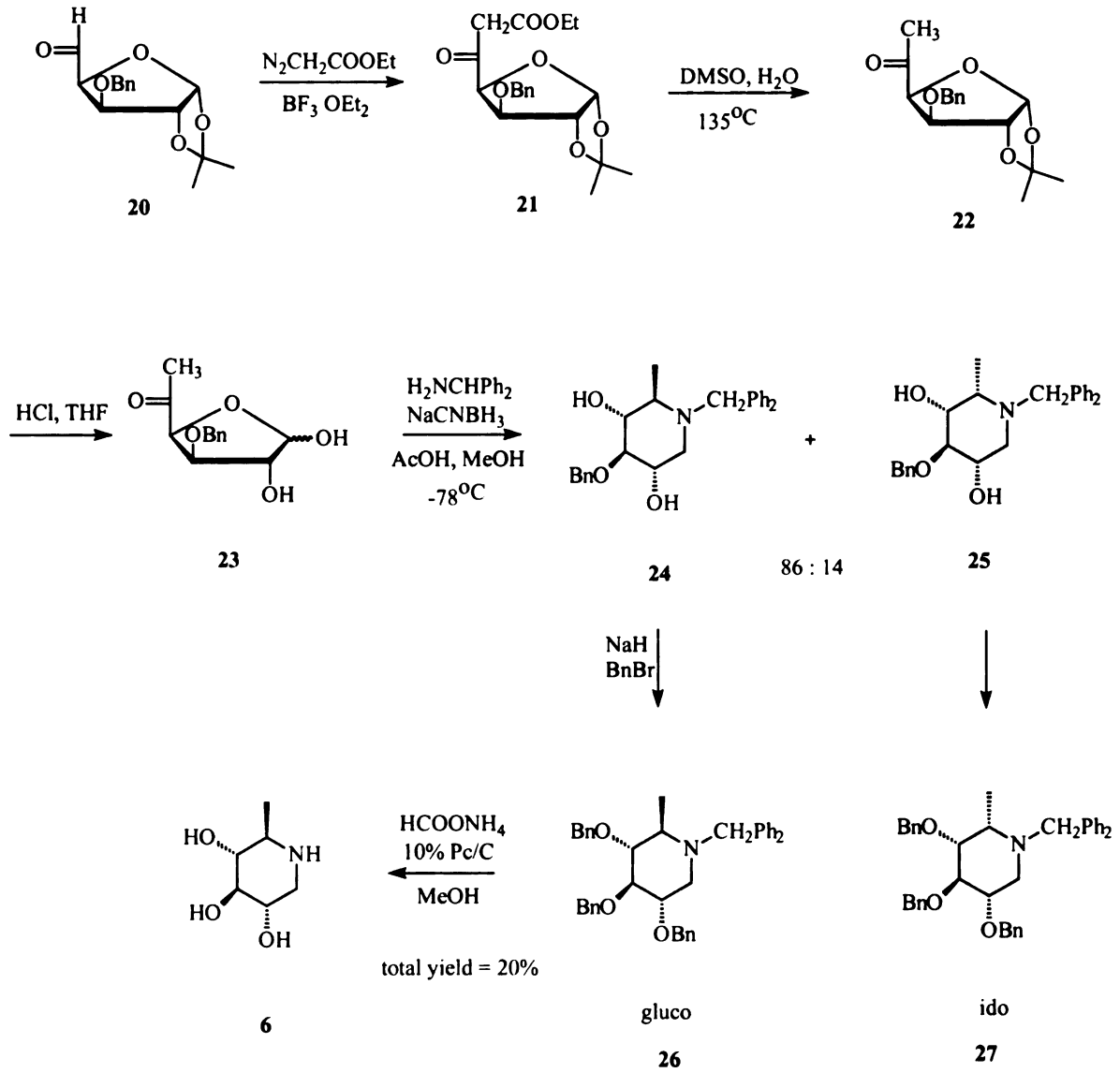
method is the necessity of using flash chromatography after each reaction step. The instability, high cost, difficulty in handling and narrow substrate specificity are other drawbacks linked to the use of enzymes as synthetic catalysts [65]. The yield of the aldol condensation reaction in the case of FDP-aldolases has been quite high, but this is not consistent and yields of only 20-40% have been reported for rhamnulose-1-phosphate- and fuculose-1-phosphate aldolases [66].

The second method is based on an asymmetric Diels-Alder reaction of sorbaldehyde O-methyloxime (**14**) or its dimethyl acetal (**15**) with a chiral chloronitroso derivative of mannose (**16**) [67,68]. N-Protection, catalytic osmylation, configurational inversion using the Sharpless method and then catalytic hydrogenolysis afford a 85:15 mixture of gluco-gulo configuration in a 6% total yield (Scheme 2.1.2). The *fuco, allo* and *tallo* configurations have also been prepared by slight modifications of this procedure. In addition to the very low yield of this method, the use of toxic OsO_4 and ruthenium and the multiple fractional separations does not make it appealing for large-scale synthesis.

The last approach uses protected 6-deoxy-5-keto-D-glucose (**23**) as substrate for a double reductive amination [69] with benzhydrylamine, $NaCNBH_3$ and acetic acid in methanol at $-78^{\circ}C$. A diastereomeric mixture of *gluco* and *ido* piperidines in an 86:14 ratio was obtained (Scheme 2.1.3). Benzylation, chromatographic separation followed by catalytic hydrogenolysis affords 1,6-dideoxynojirimycin in a 20% total yield. The *gulo* and *fuco* isomers have also been prepared. Disadvantages of this procedure are low yield, poor



Scheme 2.1.2 Synthesis of di-DNJ by asymmetric Diels-Alder reaction.



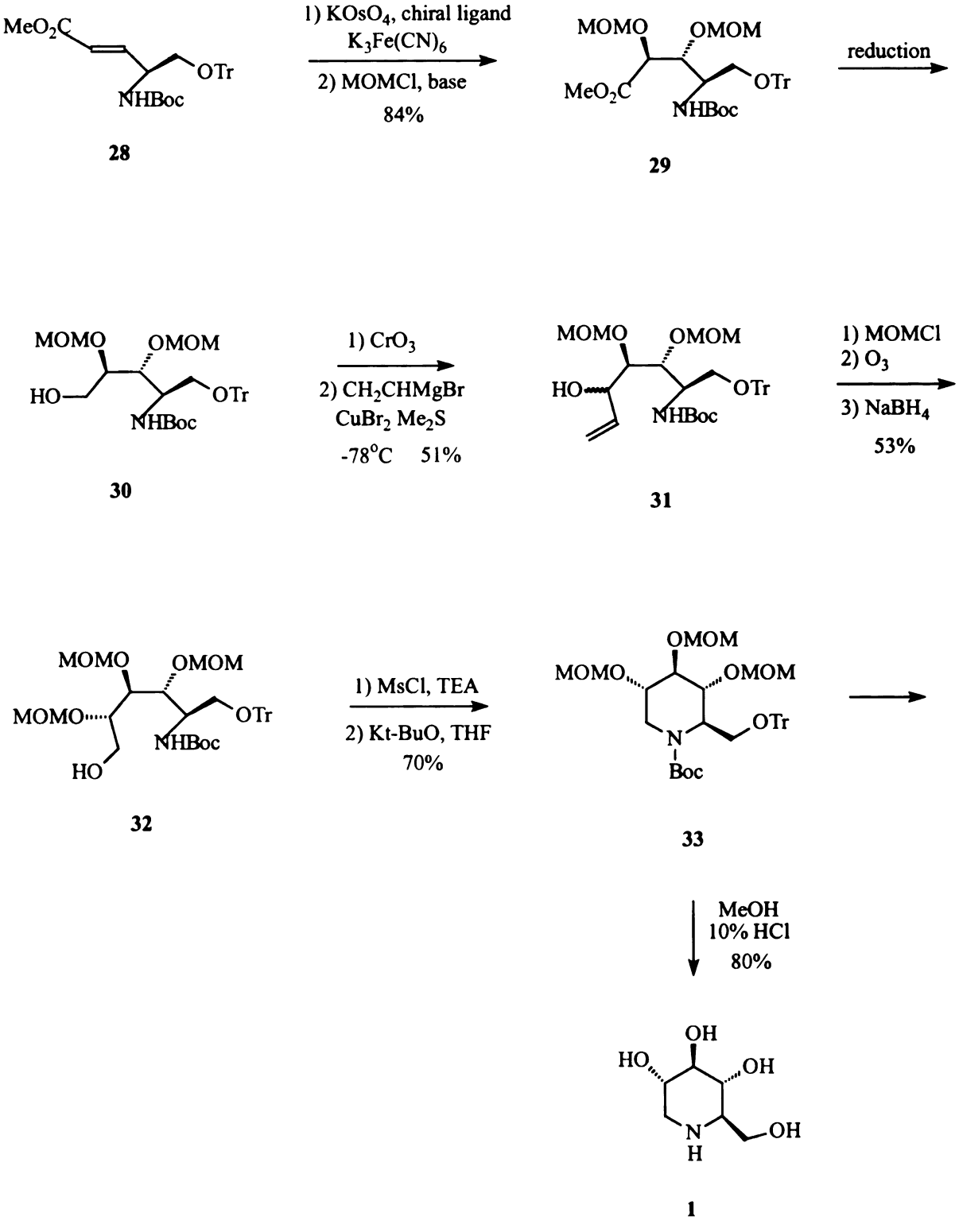
Scheme 2.1.3 Synthesis of di-DNJ by double reductive amination

stereoselectivity, harsh reaction conditions and toxic reagents, all of which is not welcomed for large-scale production. In conclusion, although these three procedures of synthesizing 1,5-imino-1,5,6-trideoxyalditols are important contributions, they generally are not stereospecific for a given sugar derivative, require drastic reagents and/or reaction conditions and the yields are below 20%.

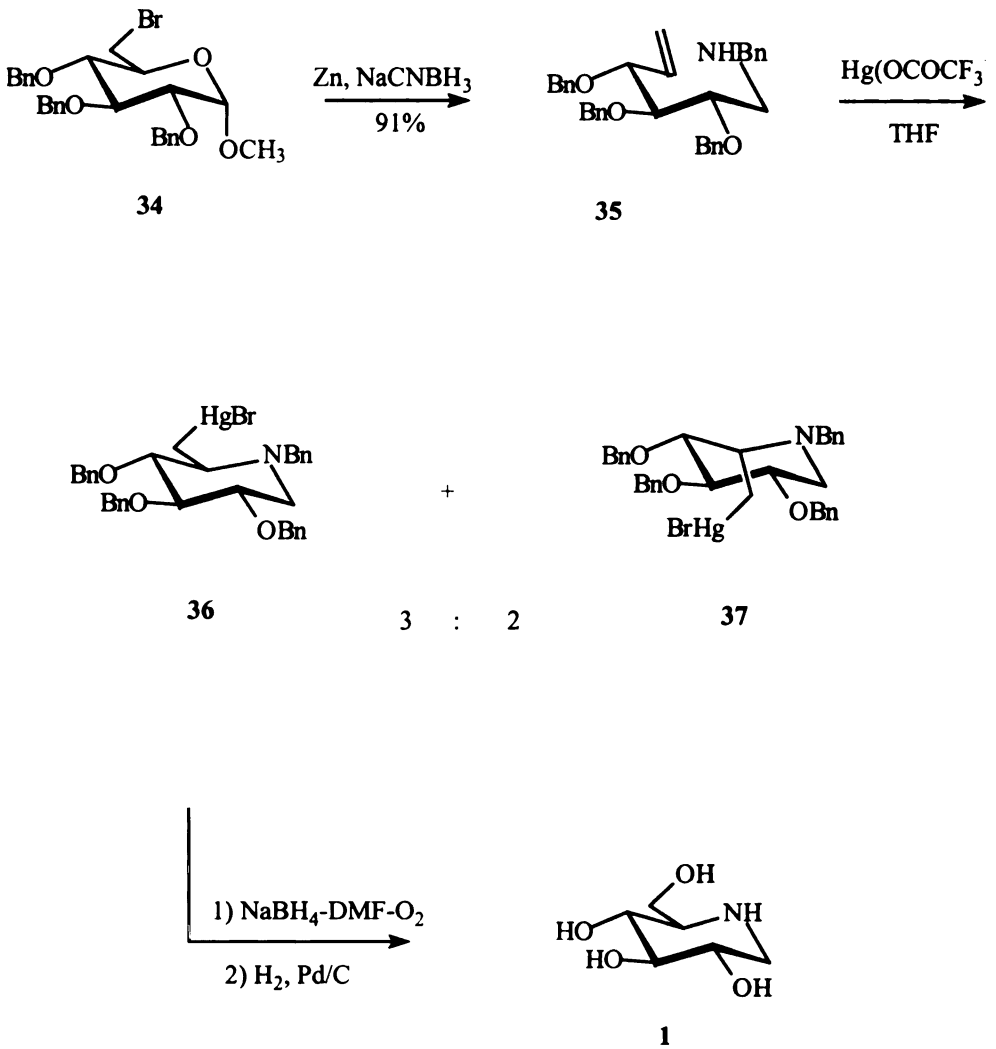
The literature contains many syntheses for deoxynojirimycin and its analogues. According to the methods that are involved they can be classified into: chemical, chemical-microbiological and chemo-enzymatic syntheses.

Chemical synthesis

Many of the purely chemical methods that have been published involve many steps with multiple protections and deprotections. They often utilize carbohydrates, lactones or other chiral pool sources containing one or more chiral centers as starting materials. Yields are generally low and multiple chromatographic separations are needed. An example of a quite elaborate synthesis is the one published by Ikota in 1997 [39]. He used (S)-pyroglutamic acid (**28**) as starting material. This is dihydroxylated using potassium osmate and hydroquinidine-1,4-phthalazinediyl diether as a chiral ligand in the presence of $K_3Fe(CN)_6$, K_2CO_3 and $MeSO_2NH_2$ (Scheme 2.1.4). Protection of the alcohol groups was followed by CrO_3 oxidation and Grignard reaction to give a diastereomeric mixture where the desired product was predominant. The major diastereomer was converted into the corresponding MOM-ether and was treated with ozone followed by reductive work-



Scheme 2.1.4 Synthesis of DNJ from S-pyroglutamic acid.

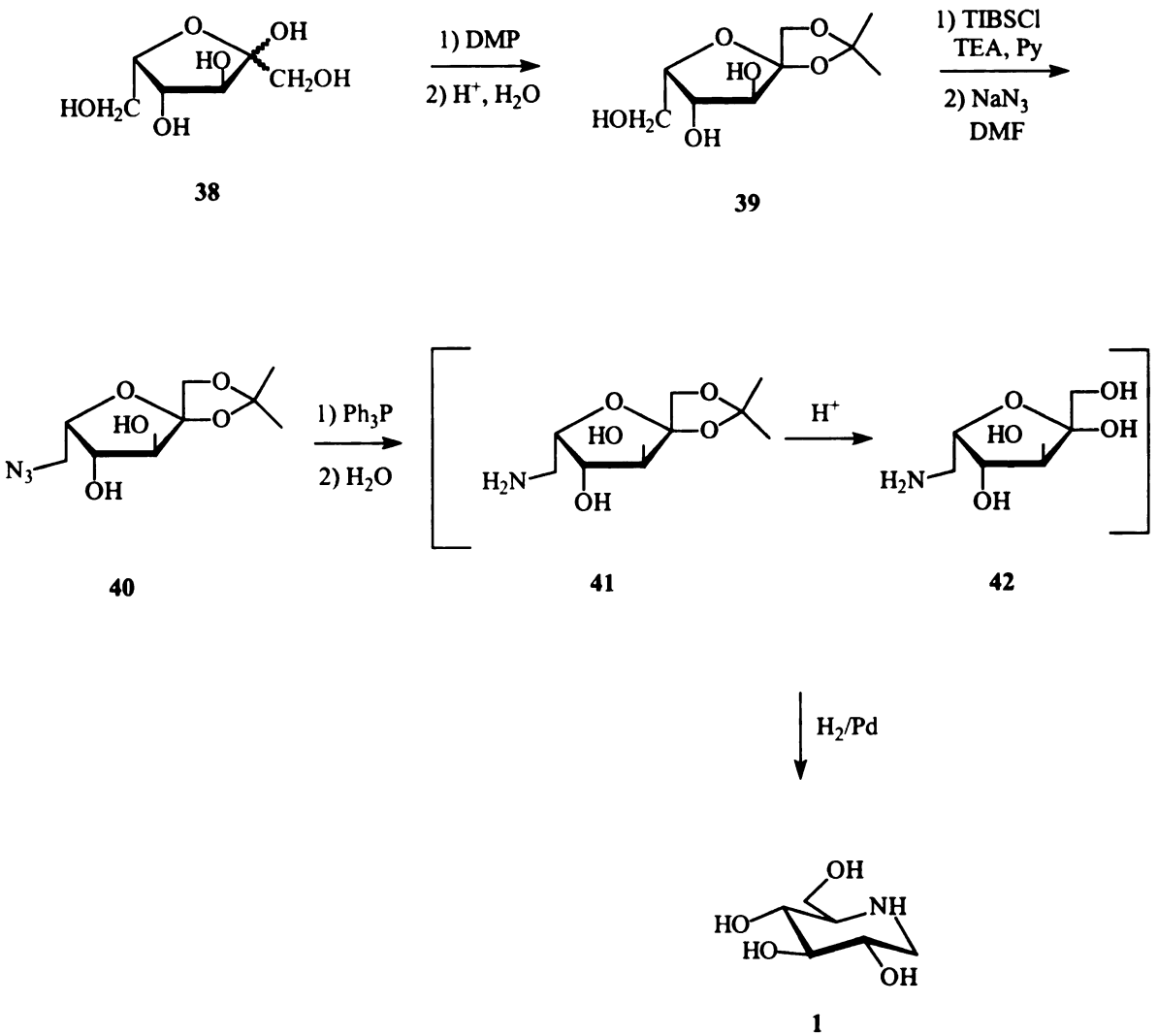


Scheme 2.1.5 Synthesis of DNJ by intramolecular aminomercuration.

up to give the alcohol in 53% yield. Mesylation followed by cyclization and deprotection gave 1-deoxynojirimycin in an 11% total yield. The disadvantages of this procedure are the high number of reaction steps, low total yield, multiple separations, and the use of toxic reagents. Other 1-deoxyazasugars have also been synthesized by this procedure.

A different approach towards DNJ has been attempted by Ganem [46] who devised an enantioslective synthetic route using intramolecular aminomercuration (Scheme 2.1.5). A one-pot reductive ring opening and reductive amination of the pyranose were achieved by heating tri-O-benzyl-6-bromopyranoside (**34**) with acid-washed zinc dust in 19:1 propanol-water containing benzylamine and $NaBH_3CN$ to afford an aminoalkene (**35**) in 91% overall yield. Reaction with mercuric trifluoroacetate in anhydrous THF gave a 3:2 mixture of bromomercurials after ligand exchange with LiBr-THF. Following chromatographic separation, the major cyclization product could be transformed to DNJ by reductive oxygenation and hydrogenolytic deprotection. The total yield starting with the bromobenzylglycoside was 35%, however the use of toxic cyanide and mercury compounds excludes the procedure from large-scale synthetic considerations.

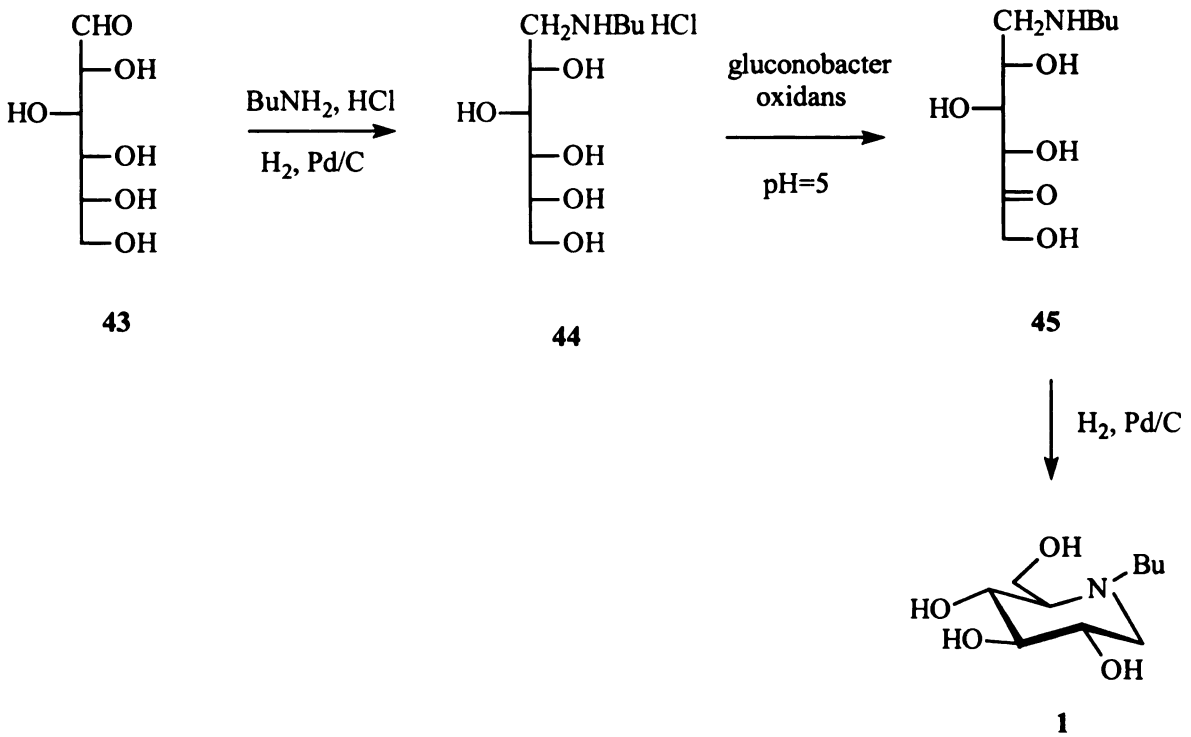
One method worthy of special mention is Behling's short and enantiospecific synthesis of deoxynojirimycin starting from L-sorbose (**38**) [42]. This has been transformed to the 1,2-isopropyllidene sorbofuranose (**39**) and then selectively derivatized at the primary position. Reduction, deprotection and catalytic hydrogenation leads to DNJ which has been crystallized in a 17% total yield. The only drawback of this procedure is that it cannot be extended to other sugars (Scheme 2.1.6).



Scheme 2.1.6 Synthesis of DNJ from L-sorbose.

Chemo-microbiological synthesis

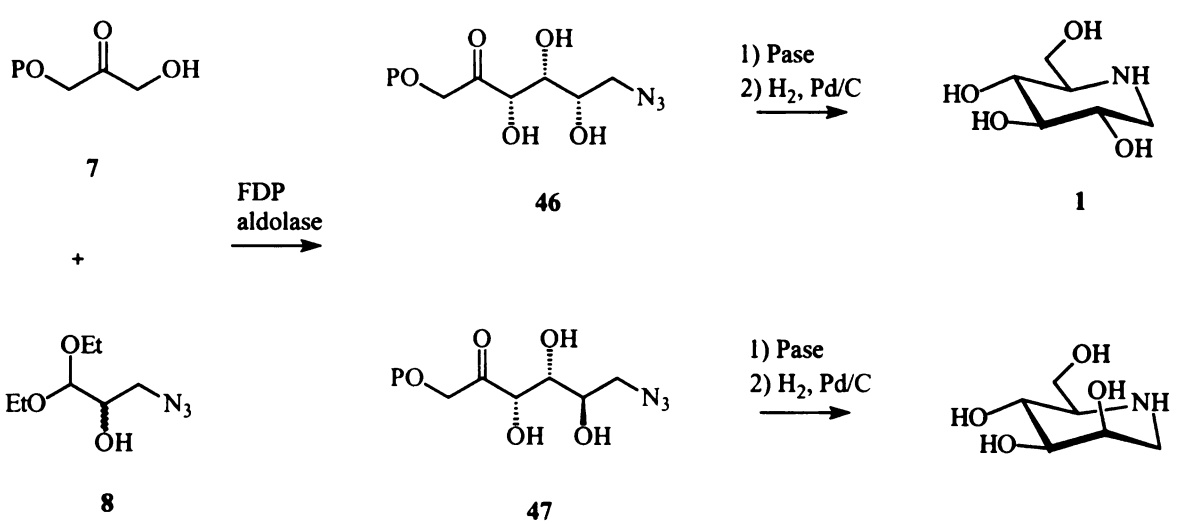
The chemo-microbiological method patented by Grabner [1,47] provides an elegant way of transforming glucose into 1-amino-1-deoxy-sorbitol (**44**), which is subsequently transformed by bacteria into an amino hydroxyketone (**45**), that undergoes intramolecular reductive amination to give DNJ in a good total yield of 45% (Scheme 2.1.7). The extension of this method to deoxy sugars and various substituted sugars is limited by the specificity of the organisms. So far, only mannose, allose, altrose, ribose and arabinose could be used as starting materials, since gluconobacter selectively oxidizes the central of three hydroxyl groups in D-erythro compounds. Since bacteria are being used, a requirement of this procedure is avoidance of contamination of the reaction media.



Scheme 2.1.7 Chemo-microbiological synthesis of DNJ.

Chemo-enzymatic synthesis

The chemo-enzymatic approach has been extensively developed by C.-H. Wong and involves aldolase promoted condensation reactions between DHAP (**7**) and a chiral N-containing aldehyde (**8**) (Scheme 2.1.8). To date, three aldolases have been used to make iminosugars and these are FDP (fructose-di-phosphate), Fuc-1-P (fuculose-1-phosphate) and Rha-1-P (rhamnulose-1-phosphate), the products being: D,L-*gluco* DNJ, D-*galacto*-DNJ, D,L-*manno*-DNJ and D-*talo*-DNJ. In the case of FDP-aldolase and using a racemic azide, a 1:4 mixture of *gluco*- and *manno*-DNJ has been obtained in a 59% yield based on FDP. So far it is not clear whether the enzyme is available in sufficient quantity to allow large-scale synthesis.



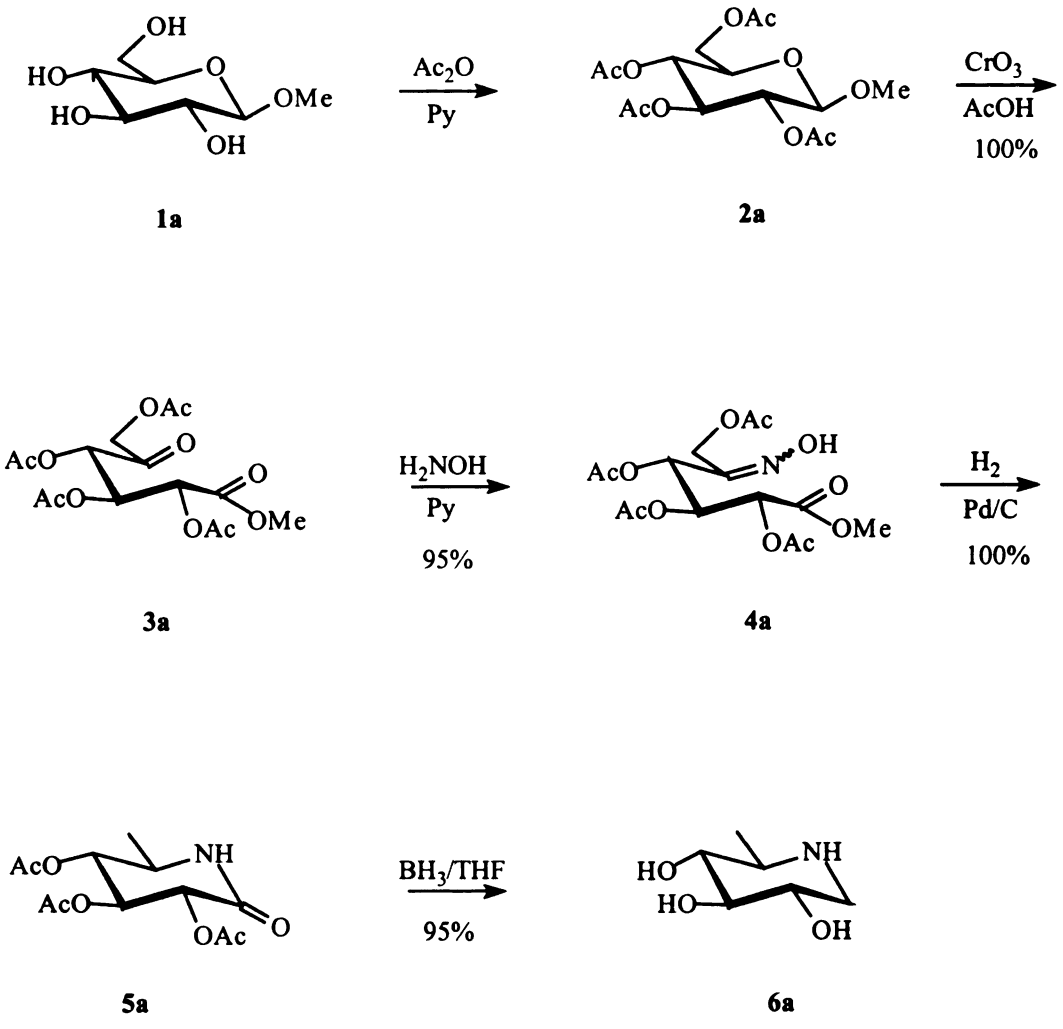
Scheme 2.1.8 Chemo-enzymatic synthesis of DNJ.

2.2. Results and discussions

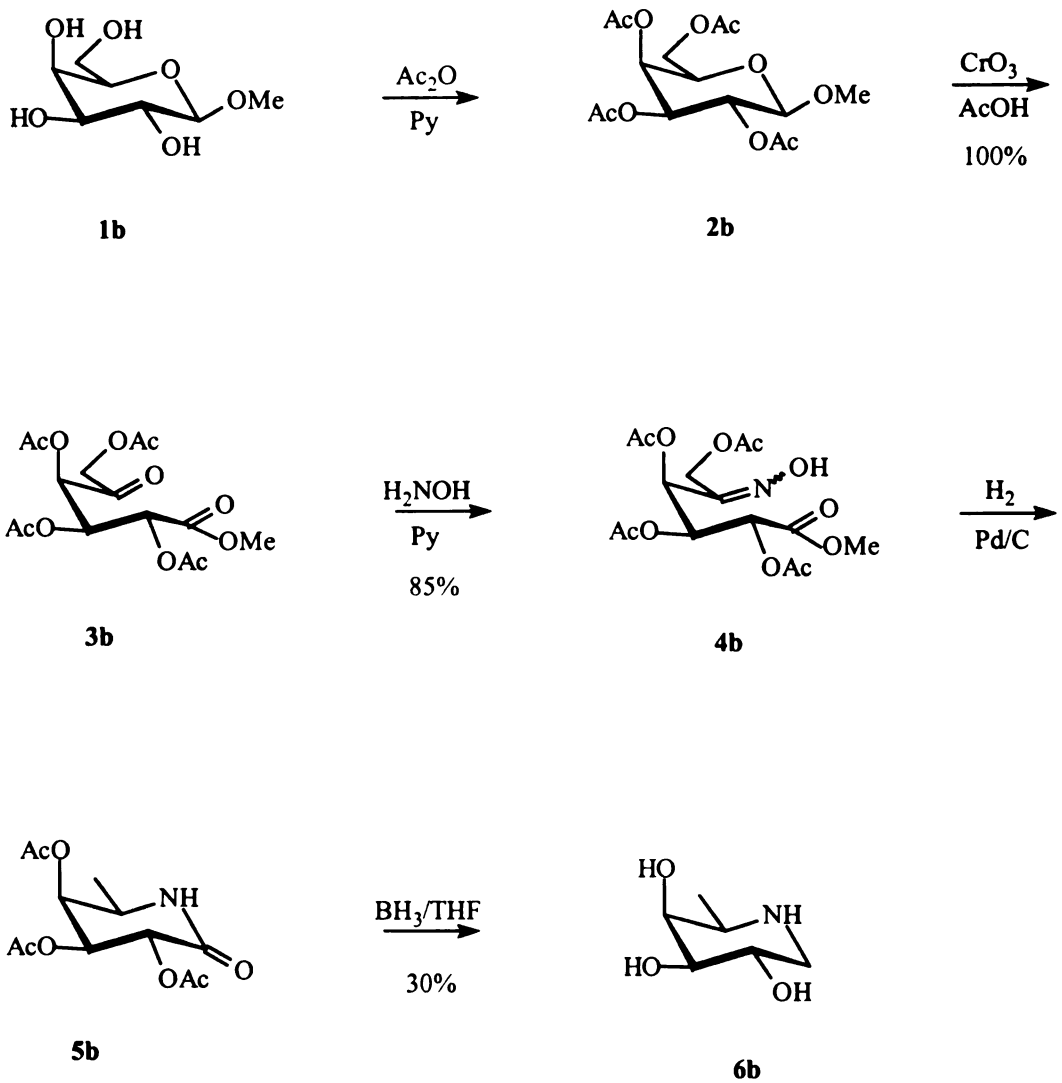
As indicated in the previous chapter, most of the chemical methods for the preparation of deoxynojirimycin, dideoxynojirimycin and their analogs are quite elaborate, require multiple protection and deprotection steps, fractional separation, involve toxic reagents or expensive chiral catalysts and provide low total yields. On the other hand, the alternative microbiological and enzymatic methods need special care with respect to reaction conditions, work-up and disposal of media. Finally, a common shortcoming to all these procedures is their lack of generality. They are restricted to specific azasugars and there is no single synthesis that can be applied for the preparation of all naturally occurring imine carbohydrate analogs. Finding a general synthetic procedure would be extremely appealing since the majority of N-sugar-analogs have glycosidase inhibitory action. Here we present a general, simple and high yield new synthesis for iminosugars especially for the 1,5,6-trideoxy analogs. The routes start from readily available β–glycosides and require just a few steps with straightforward reaction conditions. They allow access to both 1,5-dideoxy- and 1,5,6-trideoxy-1,5-iminoalditols. The enantiochemistry does not have to be induced but is derived from the starting sugar and is therefore always close to 100%. Hence the *galacto*- isomers can be obtained simply by using a galactoside. The method also allows access to the 5-amino-5-deoxy-aldonic acid δ–lactams. These are excellent glycosidase inhibitors at concentrations 100 times lower than most of the other inhibitors tested [64]. So far, the *glucono*-lactam has been made by the oxidation of nojirimycin [64], a procedure that is quite expensive considering the high cost of nojirimycin ($ 25 for 1 mg). The approach we use here is based on the chromium trioxide oxidation of glycosides to yield ketoaldonic acid esters that are then converted to cyclic

iminosugars by reductive ring closure with a nitrogen species. Our research has focused only on compounds with the D-gluco and D-galacto configurations.

The oxidation of the anomeric carbon of glycosides is well-documented in patents and papers. Examples are the microbiological oxidation with *gluconobacter oxidans* [32, 47] or the use of oxidants such as $(Bu_3Sn)_2O$ [36] and RuO_4 with or without partial protection of the hydroxyl groups. The chromium trioxide oxidation we use here typically employs a large excess of CrO_3 [70] but we obtained excellent results using only 2 equivalents. The peracetate of methyl-β-D-glucopyranoside **2a**, on oxidation with chromium trioxide gave the keto-ester **3a** in quantitative yield (Scheme 2.2.1). Our first attempts at reductive amination using $NaCNBH_3$ and different amino-group sources such as NH_4OH, NH_4Cl, NH_4OAc were unsuccessful. The use of the more nucleophilic hydroxylamine yielded the oxime **4a**, which crystallized as a mixture of cis- and trans-isomers in 95% yield. Reduction of this oxime with hydrogen in the presence of palladium on carbon led to formation of the 5-amino-5-deoxy derivative which spontaneously cyclized to form the δ–lactam. The hydrogenation conditions also led to reductive cleavage of the acetoxy group to form a 6-deoxy function. This preceded the reduction of the oxime and allowed access to the 1,5,6-trideoxyderivatives. Despite the presence of both the cis- and trans oximes, no L-derivatives were formed. The desired isomer was formed exclusively. Lactam **5a** was formed quantitatively and was then reduced and deacetylated with borane-THF, to afford the 6-dideoxynojirimycin **6a** in 84.8% total yield, better than any previously published synthesis.

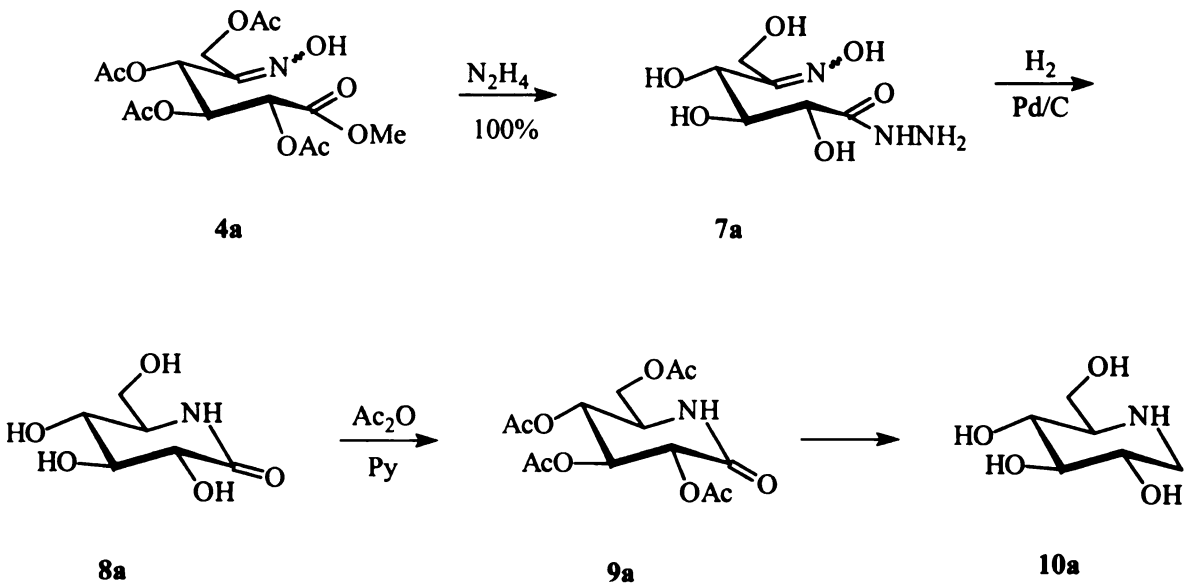


Scheme 2.2.1 Synthesis of 1,5,6-trideoxy-1,5-iminoglucitol.



Scheme 2.2.2 Synthesis of 1,5,6-trideoxy-1,5-iminogalactitol.

The same reaction sequence was applied to methyl-β-D-galactopyranoside **1b**. While the first three reactions occurred with the same high yields, the catalytic reduction was accompanied by partial deacetylation, making chromatographic separation necessary and therefore decreasing the yield. Even with this inconvenience, this method affords a convenient and high-yield route for preparing 1,6-dideoxy-D-galactonojirimycin **6b** (Scheme 2.2.2). Access to the 6-hydroxy derivatives (deoxy-D-*galacto* and *gluco*-nojirimycins) was readily achieved by deacetylating the oxime with hydrazine prior to reduction. The deacylation yielded the acyl hydrazide **7a** in quantitative yield (Scheme 2.2.3). Catalytic hydrogenation of the latter with Pd/C provided the δ–lactam **8a.** To facilitate isolation, **8a** was re-acetylated to **9a** which was recovered in 35% total yield by chromatography. This can be reduced with BH_3/THF, using the previously indicated procedure to yield DNJ.



Scheme 2.2.3 Synthesis of gluconolactam and DNJ.

2.3. Conclusion

In summary, we have developed a facile and effective synthesis of 1,6-dideoxynojirimycin, 1,6-dideoxygalactonojirimycin and deoxynojirimycins as well as the corresponding 5-amino-5-deoxy-aldonic acid δ-lactams using β-glycosides as starting materials. β-Glycosides, in addition to the fact that they are relatively cheap and readily available, have the advantage of containing the desired stereochemistry, making chiral induction unnecessary. The synthesis is short and requires minimal or no chromatographic separation, which accounts for the high yield and simplicity of the procedure. The discovery that 6-acetoxy oximes can be deoxygenated is especially important because the 6-deoxy-D-hexoses are extremely rare and alternative routes would involve several protection and activation steps. This approach should be general to any β-glycoside, especially deoxy sugars and those with substituents such as ether groups, to give general access to substituted or functionalized iminosugars. This is of great importance since to date, there is no general procedure for synthesizing N-analogs of all naturally occurring monosaccharides. The use of alkylamines instead of hydroxylamine should give access to N-alkylated iminosugars.

2.4. Experimental section

General methods. Melting points were measured on a Fisher-Johns melting point apparatus. Optical rotations were measured (λ = 589 nm) at room temperature using a Perkin-Elmer 341 polarimeter in a 1 mL cell. The ^{1}H and ^{13}C NMR spectra were recorded at 300 MHz on a Varian VXR spectrometer. The HRMS FAB mass spectra were

obtained using a JEOL HX-110 double focusing mass spectrometer operating in positive ion mode.

Methyl 2,3,4,6-tetra-O-acetyl-β-D-*glucopyranoside* **(2a)**

The tetraacetate was prepared from methyl-β–D-glucopyranoside **1a**, pyridine and acetic anhydride according to standard procedures [71].

Methyl-2,3,4,6-*tetra*-O-*acetyl*-D-xylo-*hex-5-ulosonate* **(3a)**

To a solution of **2a** (7.0 g, 19.3 mmol) in acetic acid (125 mL) and acetic anhydride (10 mL), CrO_3 (3.8 g, 38.0 mmol) was added and the suspension was stirred at 50°C for 2 hours. The mixture was then poured slowly, with stirring into cold water (500 mL). The water was extracted three times with chloroform. The combined chloroform layers were decolorized with activated charcoal, filtered and washed with saturated $NaHCO_3$ solution and then with water. After drying with Na_2SO_4 and rotary evaporation of the solvent the ketone **3a** (7.2 g, 100%) was obtained as a colorless syrup: $[\alpha]^{23}D$ -2.4° (c 1.67,CH_3Cl) lit.-5.8°, (c 1.65, CH_3Cl) [21]; ^1H NMR ($CDCl_3$) δ 2.08 (s, 3 H, OAc), 2.08 (s, 3 H, OAc), 2.10 (s, 3 H, OAc), 2.13 (s, 3 H, OAc), 3.70 (s, 3H, OCH_3), 4.73 (d, 1 H, $J_{6a,6b}$ 17.3 Hz, H-6a), 4.84 (d, 1 H, H-6b), 5.26 (d, 1 H, $J_{2,3}$ 4.4 Hz, H-2), 5.43 (d, 1 H, $J_{3,4}$ 4.4 Hz, H-4) 5.65 (t, 1 H, H-3); ^{13}C NMR ($CDCl_3$) δ 20.1, 20.2, 20.3, 52.8, 66.4, 69.3, 69.6, 73.6, 167.0, 169.1, 169.3, 169.7, 197.0.

Methyl 2,3,4,6-*tetra*-O-*acetyl*-D-xylo-*hex*-5-*ulosonate oxime* **(4a)**

The ketone **3a** (7.0 g, 18.6 mmol) was dissolved in pyridine (16 mL) and the solution cooled to 0°C. Hydroxylamine hydrochloride (2.0 g, 28.7 mmol) was then added and the solution stirred at 0°C for 15 minutes and then for another 2 hours at room temperature. The mixture was poured onto ice and water and then extracted three times with chloroform. The combined chloroform layers were subsequently washed with water, dried with Na_2SO_4 and evaporated. Crystallization from hot ethanol afforded white crystals of the oxime **4a** (6.9 g, 95%) as a 3:2 mixture of cis-trans isomers: Isomer 1: ^{1}H NMR ($CDCl_3$) δ 1.93 (s, 3 H, OAc), 1.94 (s, 3 H, OAc), 2.00 (s, 3 H, OAc), 2.01 (s, 3 H, OAc), 3.56 (s, 3H, OCH_3), 4.36 (d, 1H, $J_{6a,6b}$ 12.4 Hz, H6-a), 4.72 (d, 1H, H6-b), 4.99 (d, 1H, $J_{3,4}$ 2.6 Hz, H-4), 5.72 (dd, 1H, $J_{3,2}$ 7.8 Hz, H-3), 6.28 (d, 1H, H-2); ^{13}C NMR($CDCl_3$) δ 20.5, 20.4, 52.8, 61.3, 66.1, 69.5, 69.8, 149.9, 167.3, 169.4, 169.5, 170.1; HRMS ($M+H^+$) calcd. 392.1193, found 392.1198. Isomer 2: mp 121-122°C; ^{1}H NMR($CDCl_3$) δ 1.88 (s, 3 H, OAc), 1.89 (s, 3 H, OAc), 1.98 (s, 3 H, OAc), 2.00 (s, 3 H, OAc), 3.56 (s, 3H, OCH_3), 4.82 (s, 2H, H-6), 5.16 (d, 1H, $J_{3,4}$ 2.6 Hz, H-4), 5.62 (d, 1H, $J_{3,2}$ 8.5, H-2), 5.78 (dd, 1H, H-3); ^{13}C NMR ($CDCl_3$) δ 20.5, 20.4, 52.8, 61.3, 66.1, 69.5, 69.8, 149.9, 167.3, 169.4, 169.5, 170.1.

2,3,4-Tri-O-*acetyl*-5-*amino*-5,6-*dideoxy*-D-*glucono*-*1,5-lactam* **(5a)**

A solution of **4a** (6.9 g, 17.6 mmol) in glacial acetic acid (275 mL), containing 10% Pd/C (2.7 g) was hydrogenated in a Parr reactor under a H_2 pressure of 300-400 psi for 40 hours at 55°C. The reaction mixture was filtered through Celite and washed with ethanol. The solvent was rotary-evaporated and the lactam **5a** (5 g, 100%) was obtained as a light

yellow syrup: $[\alpha]^{23}D$ +70.0° (c 1.56, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.11 (d, 3H, $J_{5,6}$ 6.3 Hz, H-6), 1.94 (s, 3 H, OAc), 1.98 (s, 3 H, OAc), 2.00 (s, 3 H, OAc), 3.51 (m, 1H, $J_{4,5}$ 9.7, Hz, H-5), 4.94 (t, 1H, $J_{2,3}$=$J_{3,4}$ 9.7 Hz, H-3), 4.96 (d, 1H, H-2), 5.40 (t, 1H, H-4); ^{13}C NMR ($CDCl_3$) δ 18.0, 20.3, 20.3, 48.7, 70.6, 70.9, 71.4, 166.7, 169.4, 169.6, 169.8; HRMS ($M+H^+$) calcd. 288.1083, found 288.1089.

1,5,6-*trideoxy*-1,5-*imino*-D-*glucitol* **(6a)**

1M BH_3/THF (50 mL, 50.0 mmol) was added under N_2 to a solution of **5a** (5.0 g, 17.4 mmol) in THF (33 mL). The mixture was stirred at room temperature for 1.5 hours and then refluxed for another 1.5 hour. After cooling to room temperature 9% methanolic HCl (40 mL) was carefully added and the resulting solution was refluxed for 30 minutes. The THF was removed by rotary evaporation and the reaction mixture was dissolved repeatedly in methanol, followed by evaporation to remove borates. Water was added to the dry crude product and the solution was passed through an anion exchange resin (Amberlite IR-45 OH-form) and then dried on the rotary evaporator. To remove the last traces of borates, a solution of 1M NaOH (15 mL) and methanol (6 mL) were added to the crude product and the mixture was stirred overnight at room temperature. The methanol was evaporated and the aqueous solution was lyophylized. A methanolic HCl solution was added, which precipitated NaCl while the methanolic solution was dried, to give product **6a** (2.4 g, 95%): $[\alpha]^{23}D$ +15.5° (c 1.88, H_2O), lit. +13° (c 1.0, H_2O) [18]; ^1H NMR (D_2O) δ 1.25 (d, 3H, $J_{5,6}$ 6.3 Hz, H-6), 2.77 (dd, 1H, $J_{1a,1e}$ 12.4 Hz, $J_{1a,2}$ 11.7 Hz, H-1a), 3.02 (dd, 1H, $J_{4,5}$ 10.0 Hz, H-5), 3.23 (dd, 1H, $J_{3,4}$ 9.0 Hz, H-4), 3.33 (dd, 1H, $J_{1e,2}$ 5.1

Hz, H-1e), 3.31 (dd, 1H, $J_{2,3}$ 9.2 Hz, H-3), 3.63 (ddd, 1H, H-2); ^{13}C NMR (D_2O) δ 17.5, 49.5, 55.2, 71.4, 76.7, 79.0.

Methyl 2,3,4,6-tetra-O-acetyl-β-D-galactopyranoside **(2b)**

This was prepared from methyl-β-D-galactopyranoside hydrate **1b** (5.0 g, 24.6 mmol) using the same procedure described for the gluco-compound.

Methyl 2,3,4,6-*tetra*-O-*acetyl-L*-arabino-*hex*-5-*ulosonate* **(3b)**

This was prepared from **2a** using the same procedure as described for **3a** except that the reaction time was 3 hours. Yield is 100%: $[\alpha]^{23}$D -24.3° (c 1.4, $CHCl_3$), lit.-12.0°, (c 2.8, $CHCl_3$) [21]; ^{1}H NMR ($CDCl_3$) δ 2.06 (s, 3 H, OAc), 2.10 (s, 3 H, OAc), 2.11 (s, 3 H, OAc), 2.16 (s, 3 H, OAc), 3.71 (s, 3H, OCH_3), 4.80 (d, 1H, $J_{6a,6b}$ 17.5 Hz, H-6a), 4.92 (d, 1H, H-6b), 5.20 (d, 1H, $J_{2,3}$ 8.7 Hz, H-2), 5.27 (d, 1 H, $J_{3,4}$ 2.4 Hz, H-4), 5.66 (dd, 1 H, H-3); ^{13}C NMR ($CDCl_3$) δ 20.1, 20.2, 20.3, 20.4, 52.8, 67.1, 69.3, 69.5, 71.0, 167.0, 168.9, 169.4, 169.8, 198.1.

Methyl-2,3,4,6-*tetra*-O-*acetyl*-L-arabino-*hex*-5-*ulosonate oxime* **(4b)**

This was prepared from the ketone **3b** as described for the corresponding D-xylo compound. White crystals of **4a** (85%) were obtained as a mixture of cis-trans isomers: ^{1}H NMR ($CDCl_3$) δ isomer 1: 1.98 (s, 3 H, OAc), 2.01 (s, 3 H, OAc), 2.08 (s, 3 H, OAc), 2.15 (s, 3 H, OAc), 3.70 (s, 3H, OCH_3), 4.82 (d, 1H, $J_{6a,6b}$ 14.6 Hz, H6-a), 5.11 (d, 1H, H6-b), 5.35 (d, 1H, $J_{3,4}$ 1.9 Hz, H-4), 5.68 (d, 1H, $J_{3,2}$ 9.0, Hz, H-2), 5.84 (dd, 1H, H-3);

^{13}C NMR ($CDCl_3$) δ 20.2, 20.3, 20.4, 20.5, 52.6, 56.4, 68.7, 69.2, 69.6, 149.9, 167.5, 168.9, 169.3, 170.0, 170.3.

1,5,6-*trideoxy*-1,5-*imino*-D-*galactitol* **(6b)**

This was prepared from **4b** (7.4 g, 18.9 mmol) as described for the corresponding *gluco* compound **5a**. 7.4 g of a crude product was obtained which was subjected to borane reduction. The product was isolated as described for the *gluco* isomer. Flash column chromatography using a chloroform-methanol (6:1) mixture gave the product **6b** (1.5 g, 30%): $[\alpha]^{23}D$ +27.0° (c 1.3, $CHCl_3$) , lit. +49.0° (c 1, $CHCl_3$) [20]; ^1H NMR (D_2O) δ 1.21 (d, 3H, $J_{5,6}$ 6.6 Hz, H-6), 2.73 (t, 1H, $J_{1a,1e}$, $J_{1a,2}$ 11.9 Hz, H-1a), 3.30 (dd, 1H, $J_{1e,2}$ 5.4 Hz, H-1e), 3.37 (m, 1H, H-5), 3.50 (dd, 1H, $J_{2,3}$ 9.6 Hz, $J_{3,4}$ 3.1 Hz, H-3), 3.90 (d, 1H, $J_{4,5}$ 3.1 Hz, H-4), 3.91 (ddd, 1H, H-2); ^{13}C NMR (D_2O) δ 14.2, 46.1, 55.0, 64.4, 69.9, 73.1.

2,3,4,6-Tetra-O-*acetyl*-5-*amino*-5-*deoxy*-*D*-*glucono*-*1,5*-*lactam* **(9)**

The acetylated oxime **4a** (1.5 g, 3.8 mmol) was deacetylated with concomitant conversion to the acyl hydrazide by treatment with anhydrous hydrazine (0.75 mL, 23.8 mmol) in methanol (15 mL) at room temperature for 2 hours. Evaporation of the solvent gave crude **7a:** ^1H NMR (D_2O) δ 4.18 (1H, dd, *J* 4.6 Hz, *J* 7.0 Hz) 4.51 (1H, d, *J* 6.5 Hz), 4.43 (1H, d, *J* 14.9 Hz), 4.53 (1H, d, *J* 14.8 Hz), 5.18 (1H, d, *J* 4.6 Hz); ^{13}C NMR (D_2O) δ 61.1, 69.1, 73.4, 73.5, 160.7, 173.4.

7a was hydrogenated in glacial acetic acid with 10%, Pd/C (0.4 g) at 50°C and 300 psi pressure of H_2 for 2 days. After filtration through Celite, the solution was dried on the rotary evaporator and the crude product acetylated with acetic anhydride (15 mL) and

pyridine (15 mL) for 5 hours at room temperature. The mixture was poured into cold water and extracted with chloroform. The chloroform layer was dried with Na_2SO_4. Evaporation of the solvent gave crude product (1.4 g), which was subjected to flash chromatography on silica (eluent hexane-acetone = 2:1) to give the peracetylated lactam **9a** (0.5 g, 34% total yield from **4a**) and its C-5 epimer: **9a**: mp=177-178°C; $[\alpha]^{23}_D$ +88.6° (c 1.11, $CHCl_3$), lit. +104° (c 1.73, $CHCl_3$) [17]; ^{1}H NMR ($CDCl_3$) δ 2.03 (s, 3 H, OAc), 2.06 (s, 3 H, OAc), 2.08 (s, 3 H, OAc), 2.10 (s, 3 H, OAc), 3.75 (ddd, 1H, $J_{4,5}$ 9.7 Hz, $J_{5,6a}$ 2.9 Hz, $J_{5,6b}$ 6.5 Hz, H-5), 3.96 (dd, 1H, $J_{6a,6b}$ 11.7 Hz, H6-b), 4.22 (dd, 1H, H-6a), 5.06 (d, 1H, $J_{3,2}$ 9.5 Hz, H-2), 5.20 (t, 1H, $J_{3,4}$ 9.5 Hz, H-3), 5.53 (dd, 1H, H-4), 6.48 (s, 1H, s, NH) ;^{13}C NMR($CDCl_3$) δ 20.5, 20.5, 20.5, 20.6, 52.4, 62.7, 67.2, 70.4, 70.5, 166.2, 169.4, 169.6, 170.0, 170.4. HRMS ($M+H^+$) calcd. 346.1060, found 346.1143. **9a**-epimer: $[\alpha]^{23}_D$ +3.1° (c 1.81, $CHCl_3$); ^{1}H NMR ($CDCl_3$) 1.98 (s, 3 H, OAc), 1.99 (s, 3 H, OAc), 2.00 (s, 3 H, OAc), 2.02 (s, 3 H, OAc), 3.88 (1H, m, H-5), 4.04 (dd, 1H, $J_{6a,6b}$ 11.4 Hz, $J_{5,6b}$ 6.3 Hz, H6-b), 4.18 (dd, 1H, $J_{5,6a}$ 3.9 Hz, H-6a), 5.15 (dd, 1H, $J_{4,5}$ 9.5 Hz, $J_{3,4}$ 7.5 Hz, H-4), 5.15 (d, 1H, $J_{2,3}$ 7.5 Hz, H-2), 5.39 (t, 1H, H-3), 7.27 (1H, s, broad, NH); ^{13}C NMR ($CDCl_3$) δ 20.2, 20.3, 20.4, 50.0, 62.0, 68.0, 69.8, 70.0, 166.7, 169.3, 169.7, 170.3, 170.6.

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Chapter 3

A Preparation and Screening Strategy for Glycosidase Inhibitors

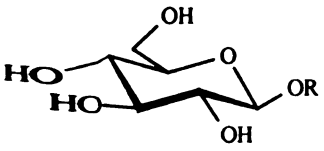
Abstract

Glycosidases play important roles in numerous biological processes. Inhibitors of glycosidases have the potential of producing several beneficial therapeutic effects. However, their synthesis is not straightforward and their inhibitory activity can be estimated only by testing them one by one, which proved to be laborious and time-consuming. Finding a method of synthesis by which a large spectrum of compounds can be produced and tested would be extremely advantageous.

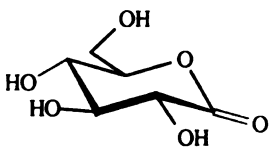
Here we present a one-pot procedure that we have employed in the synthesis of a library of compounds belonging to 4 classes of substances: methyl glycosides, aldonic acid lactams, aldonic acid lactones and 1,5-dideoxy-1,5-iminosugars. The complex mixture has been separated into individual components that have been screened for activity against α- and β-glucosidases. The active molecules have been identified by nuclear magnetic resonance spectroscopy and high-resolution mass spectrometry. This methodology provides a straightforward, general and time-efficient way to synthesize, test and analyze an extensive number of glucosidase inhibitors. It can also be extended to many other compounds.

3.1. Introduction

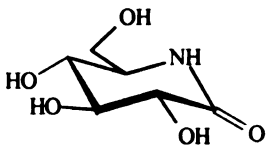
Glycosidase inhibitors have the potential to produce several beneficial therapeutic effects including reduction of the blood glucose level [1], inhibition of tumor metastasis [2] and inhibition of viral replication [3-5]. Four of the most common and potent classes of glycosidase inhibitors are glycosides, aldono-1,5-lactones, amino-deoxyaldonic acid lactams, and 1,5-dideoxy-1,5-imino alditols (aza-sugars). There are several authoratative reviews on this area [6-9]. Table 3.1.1 summarizes their inhibition of α- and β-glucosidases.



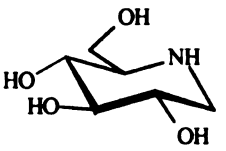
glucosides



aldono-1,5-lactone

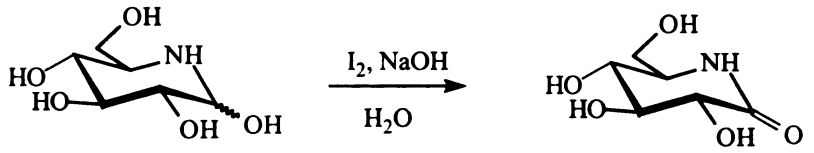


amino deoxyaldonic acid lactams



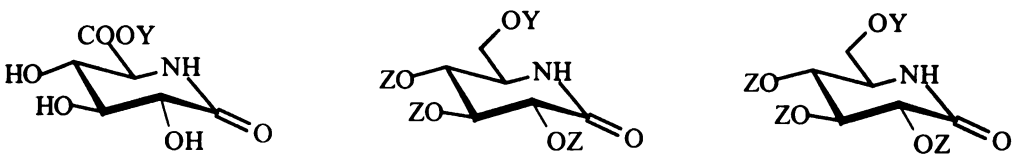
1,5-dideoxy-1,5 -iminoglucitols

The classes of inhibitory molecules mentioned above are usually prepared using unrelated synthetic routes although one way of preparing δ-lactams has been to oxidize 1,5-dideoxy-1,5-iminosugars such as nojirimycin (Scheme 3.1.1) [18]. However, considering the high cost and instability of nojirimycin, this way of preparing lactams is not at all appealing.



Scheme 3.1.1 Preparation of 1,5-gluconolactam from nojirimycin.

The inhibitory activity of these classes of compounds can be modulated by substitution. For instance, some O-alkylated and -arylated δ-lactams have been found to markedly inhibit metastasis of cancer cells [19] (Figure 3.1.1). Substitution therefore affords a way of influencing the specificity, efficacy, potency and bio-availability of drug targets based on these molecules. Routes for the syntheses of carbohydrate glycosidase inhibitors, especially with O-substituents, are very involved. Because of this, it is not possible to routinely prepare and screen a significant number of candidates. An integrated combinatorial approach that gives access to candidates from all three classes mentioned above and with variations in substitution would be an ideal and invaluable strategy.



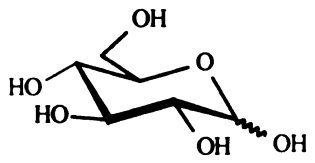
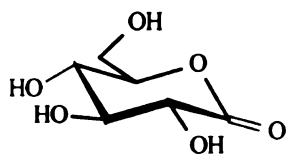
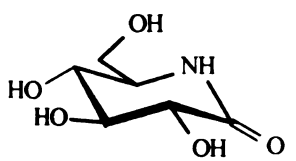
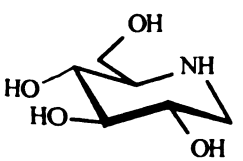
Y = H, R

Y = H, RCO
Z = H, RCO, ArCO

Y = H, Ar
Z = H, RCO

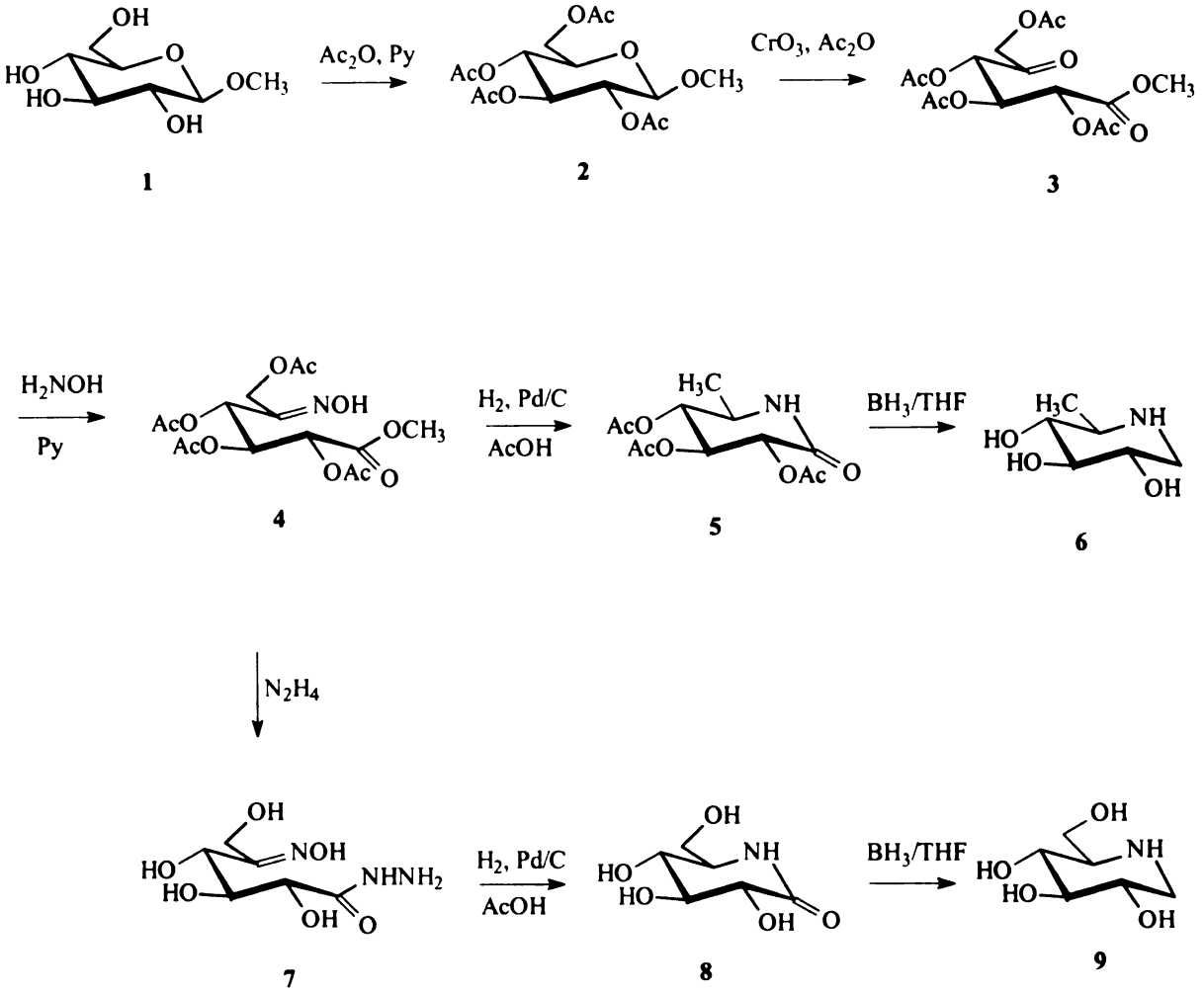
Figure 3.1.1 Biologically active δ-lactams.

Table 3.1.1. Inhibitory action of some glucosidases

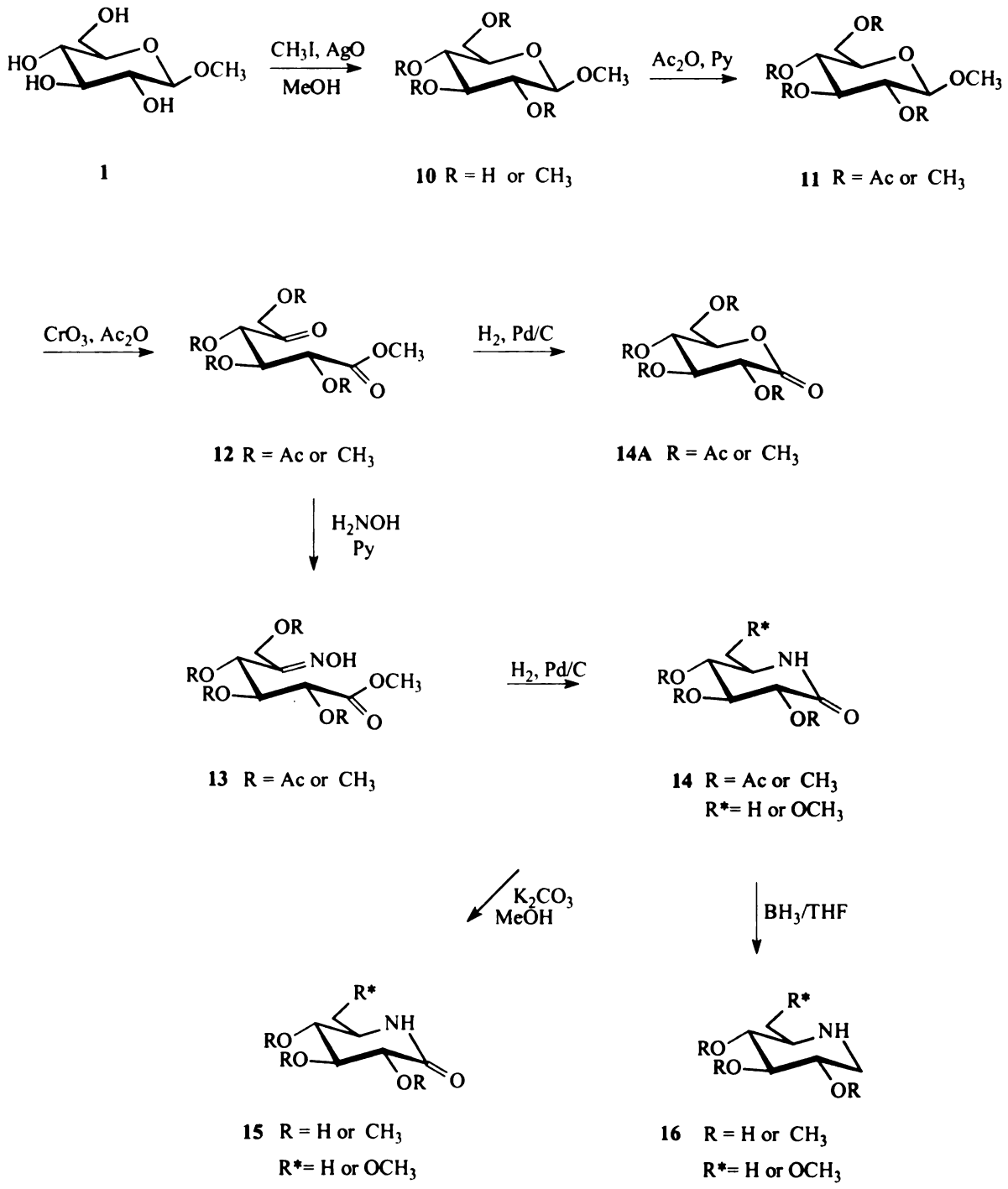
Inhibitor	Glucosidase (Source)	K_i (μM)	Reference
	α-D-Glucosidase		
	Rabbit,intestinal sucrase	19000	10
	β-D-Glucosidase		
	Alcaligenes faecalis	6400	11
	Aspergillus wentii	2800	12
	Sweet almonds, A	189000	13
	B	80000	14
	Human liver, cytosolic	55000	15
	α-D-Glucosidase		
	Rabbit, intestinal sucrase	10000	10
	β-D-Glucosidase		
	Alcaligenes faecalis	1.7	11
	Aspergillus wentii	9.5	12
	Sweet almonds, A	200	13
	B	36	14
	Human liver, cytosolic	15	15
	α-D-Glucosidase		
	Rabbit, intestinal sucrase	23000	16
	β-D-Glucosidase		
	Aspergillus wentii	36	12
	Sweet almonds, B	37	13
	α-D-Glucosidase		
	Rabbit, intestinal sucrase	0.032	16
	Yeast	12.6	16
	Rice	0.01	17
	β-D-Glucosidase		
	Aspergillus wentii	2.7	12
	Sweet almonds, B	47	13
	Calf liver	210	15
	α-D-Glucosidase		
	Brewers yeast	1560	18
	β-D-Glucosidase		
	Sweet almonds	780	18

3.2. Results and discussions

In chapter 2 of this dissertation we have described in detail the procedure for the transformation of methyl-β-D-glucopyranoside **1** into 5-amino-D-gluconic acid lactams **5**, **8** and then to deoxynojirimycin **9** and dideoxynojirimycin **6** (Scheme 3.2.1) [20]. This involves the chromium trioxide oxidation of methyl-β-D-glucopyranoside to form 5-keto aldonic acid methyl ester, which is then converted to a cyclic aminosugar by reductive ring closure with a nitrogen species. One important feature of this method is that it can potentially afford access to all three of these classes of molecules. We used this procedure as the basis for the design of a combinatorial approach to partially methylated β-methyl-D-glucopyranosides, 5-amino-5-deoxy-D-gluconic acid lactams and 1,5-dideoxy-1,5-imino glucitols. This method has the advantage of producing structural diversity and a comprehensive spectrum of molecules through just a few simple and high yield reactions. The strategy is illustrated in Scheme 3.2.2. Methyl-β-D-glucoside **1** was partially methylated and then acetylated, giving an array of differently substituted glucosides **11**. This mixture of partially methylated, partially acetylated glucosides was subjected to chromium trioxide oxidation to give 5-keto-gluconic acid methyl ester derivatives **12.** These were reacted with hydroxylamine to produce oximes **13.** The oximes were then converted to δ-lactams **14** by catalytic hydrogenation. Part of this reaction mixture was then deacetylated to give **15**, while another portion was reduced to the 1,5-dideoxy-1,5-imino-D-glucitols **16** [21]. Both **15** and **16** were subjected to HPLC separation. All of the individual components from HPLC separation of mixtures **15** and **16** were assayed for their inhibitory activity against α- and β-glucosidases. Six compounds proved to be active. Structure determination using NMR spectroscopy and



Scheme 3.2.1 Synthesis of δ-gluconolactam, dideoxynojirimycin and deoxynojirimycin.



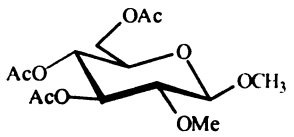
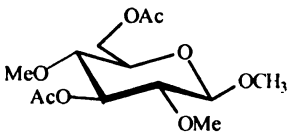
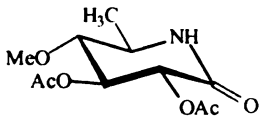
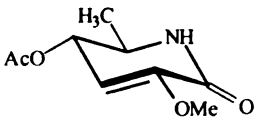
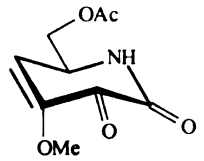
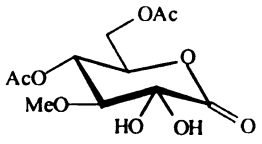
Scheme 3.2.2 Synthesis of partially methylated 5-amino-5-deoxy-gluconic acid lactams, 1,5-dideoxy-1,5-imino-glucitols and gluconic acid lactones.

high resolution mass spectrometry (HRMS) on the acetylated active substances led to the structures in Table 3.2.1. A variety of positive candidate molecules were obtained from the screen. Methyl-β-D-glucosides **17, 18** with the hydroxyl at C-2 methylated were quite common, indicating that the presence of the methoxy group retarded chromium trioxide oxidation at the adjacent anomeric site. Three different lactams, the 5,6-dideoxy-lactam **19**, the 2,3-dehydro-3,5,6-trideoxy lactam **20** and the enone **21**, all displayed good to excellent inhibitory activity despite their difference in functionality and the extent of their structural planarity. The 2-oxo-lactone present as its hydrate **22** also shows good inhibitory activity. The presence of this lactone indicates that some proportion of the 5-keto-gluconic acid methyl ester was not oximated, and the carbonyl group was reduced back to a mixture of D-*gluco* and L-*ido* aldonic esters. These subsequently cyclized to produce the derivatized lactones. In the scheme described here, the proportion of lactam species can be controlled by varying the amount of hydroxylamine used. For instance, the use of half the required amount of hydroxylamine would increase the proportion of lactone species produced.

3.3. Conclusions

Using a combinatorial library approach towards synthesis and screening, we have identified methylated methyl-β-D-glucosides, lactams and lactones with inhibitory activity towards α- and β-glucosidases. This method, which is primarily a discovery and screening routine, is very convenient and practical. It circumvents the complexity and tremendous effort needed to access these classes of molecules when treated separately

Table 3.2.1. Structure, characterization and inhibitory activity of some synthesized glucosidase inhibitors.

Compound	Structure	^{1}H-NMR (500 MHz)	HRMS[a]	Inhibitory activity[b]
17		1.99 (s), 2.04 (s), 2.06 (s), 3.15 (dd, J=7.5, 9.3), 3.48 (s), 3.54 (s), 3.63 (m), 4.09 (dd, J=12.2, 1.9), 4.26 (dd, J=12.2, 4.6), 4.98 (t, J=9.3), 5.07 (t, J=9.2).	calcd. 335.1264 found 335.1353	85% (β)
18		2.10 (s), 2.13 (s), 3.05 (dd, J=9.5), 3.26 (t, J=9.5), 3.40 (s), 3.48 (s), 3.51 (m), 3.54 (s), 4.24 (dd, J= 11.9, 5.1), 4.27 (d, J=7.7), 4.36 (dd, J=11.9, 2.2), 5.09 (t, J=9.5).	calcd. 307.1393 found 307.1407	20% (β) 25% (α)
19		1.21 (d, J=6.6), 2.12 (s), 2.17 (s), 3.45 (s), 3.52 (m), 3.72 (t, J=8.6), 4.96 (t ,J=8.6), 5.16 (d, J=8.6), 5.45 (s).	calcd. 260.1134 found 260.1136	25% (β)
20		1.25 (d, J=6.8), 2.05 (s), 3.66 (s), 3.88 (m), 5.19 (m), 5.36 (s), 5.58 (d, J=6.3).	calcd. 200.0923 found 200.0923	15% (α) 25% (β)
21		2.07 (s), 3.80 (s), 4.19 (dd, J=12.1, 5.7), 4.30 (dd, J=12.1, 3.5), 5.10 (ddd, J=2.0, 5.7, 3.5), 5.98 (d, J=2.0).		90% (α)
22		2.10 (s), 2.18 (s), 3.74 (s), 4.46 (dd, J=9.1, 6.6), 4.50 (dd, J=9.1, 7.9), 4.89 (ddd, J=7.9, 5.3, 6.6), 5.20 (d, J=2.8), 5.62 (dd, J=2.8, 5.3).	calcd. 291.0954 found 291.0726 M^+	84% (α)

a. Fast atom bombardment high resolution mass spectrometry (FABHRMS), $M+H^+$ given.

b. % inhibition per 1mM compound, measured for 0.5 mM α- and β-p-nitrophenylglucoside respectively and 0.2 mg/mL of enzyme at 37°C. In brackets, the type of glucosidase is given.

and gives a wide cross section of candidates. This procedure is general and can be used to prepare, isolate and screen other classes of alkylated and arylated carbohydrate derivatives

3.4. Experimental section

3.4.1. Synthesis

In this procedure, the product of each reaction is used, after workup, without further purification or separation. In a 200 mL round-bottom flask, equipped with a condenser and drying tube, methyl-β-D-glucopyranoside **1** (10.0 g, 49.2 mmol) was dissolved in methyl iodide (30.0 mL, 492.0 mmol) and methanol (40 mL) and heated under reflux for 6 h. AgO (25.0 g, 107.8 mmol) is added to the solution in 10 equal portions at 0.5 h intervals. After the final addition, heating is continued for 4 h, followed by stirring at room temperature for another 12 h. The suspension was then filtered, extracted with chloroform and dried on the rotary evaporator to give **10**.

Acetylation of the partially methylated methyl-β-D-glucopyranoside was performed using standard procedures to give **11**.

To a solution of **11** (14.0 g) in acetic acid (280 mL) and acetic anhydride (24 mL), CrO_3 (9.0 g) was added and the suspension was stirred at 50°C for 2 h. The mixture was then poured slowly, with stirring into cold water (1000 mL). The water was extracted three times with chloroform. The combined chloroform layers were decolorized with activated

charcoal, filtered and washed with saturated $NaHCO_3$ solution and then with water. After drying with Na_2SO_4 and rotary evaporation of the solvent the ketone **12** was obtained.

This reaction mixture (4.0 g) was dissolved in pyridine (40 mL) and the solution cooled to 0°C. Hydroxylamine hydrochloride (5.0 g) was then added and the solution stirred at 0°C for 15 minutes and then for another 2 h at room temperature. The mixture was poured onto ice and water and then extracted three times with chloroform. The combined chloroform layers were subsequently washed with water, dried with Na_2SO_4 and then evaporated to give oxime **13**.

A solution of **13** (12.5 g) in glacial acetic acid (300 mL), containing 10% Pd/C (5 g) was hydrogenated in a Parr reactor under a H_2 pressure of 300-400 psi for 40 hours at 55°C. The reaction mixture was filtered through Celite and washed with ethanol. The solvent was rotary-evaporated and the lactam **14** (15.0 g) was obtained.

Deacetylation was performed by dissolving **14** (1.0 g) into methanol (20 mL) and water (3 mL) to which K_2CO_3 (1.0 g) was added and stirred overnight at room temperature. The suspension was evaporated, partially redissolved in ethanol and filtered. The filtrate was then dried by rotary evaporation, after which the crude reaction mixture **15** was subjected to HPLC separation.

A solution of the lactam **14** (1.2 g) in THF (10 mL) was reduced by adding 1M BH_3/THF (13 mL) and stirring at room temperature for 1.5 h and then refluxing for another 1.5 h. After cooling to room temperature 9% methanolic HCl (10 mL) was carefully added and the resulting solution was refluxed for 30 minutes. The THF was removed by rotary evaporation and the reaction mixture was dissolved repeatedly in methanol, followed by evaporation to remove borates. Thus, **16** was synthesized which was then separated by HPLC.

3.4.2. Separation of the reaction mixtures

The crude reaction mixtures **15** and **16** were separated via reverse-phase high performance liquid chromatography (HPLC) using a Waters 600 multisolvent delivery system with an ODS Ultrasphere 4.6 x 250 mm column, a Spectoflow 783 programmable absorbance detector and a Waters 740 Data Module. The reaction mixture was dissolved in a 5% solution of acetonitrile in water and 70μL (20 mg) loaded on the column. Running time was set to 75 minutes, with a flow rate of 1.5 mL/min. The starting solvent was 5% acetonitrile in water while the second solvent was water. Gradient curve shape 6 from the Waters gradient program was chosen to mix the two solvents. The absorbance detector was set to 219 nm while the detection range was programmed to 0.2 AUFS. 28 fractions were collected from reaction mixture **15**, and 52 fractions from reaction mixture **16**. These were subjected to inhibition studies against α- and β-glucosidase.

3.4.3. Inhibition studies

Materials

The enzyme, buffers and substrates were purchased from Sigma. These include: α–D-glucosidase (maltase, 45U/mg), phosphate buffer, p-nitrophenyl α-D-glucoside, β-D-glucosidase (from Almonds, 30U/mg), citrate buffer, p-nitrophenyl β-D-glucoside.

Preparation of solutions

(a) α-D-Glucosidase: The stock enzyme solution was prepared by dissolving 0.2 mg of solid protein in 1 mL of buffer solution. This enzyme solution was diluted 10-fold for the enzymatic assay. (b) β-D-Glucosidase: The stock enzyme solution was prepared by dissolving 0.4 mg of solid protein in 1 mL of buffer solution. This enzyme solution was diluted 10-fold for the enzymatic assay. (c) Phosphate buffer (0.5 M with 0.244 M K_2HPO_4 and 0.256 M KH_2PO_4, pH 6.5): 24.4 mL of 1 M K_2HPO_4 and 25.6 mL of 1 M KH_2PO_4 were mixed and diluted to 100 mL. (d) Citrate buffer (0.3 M, pH 5) (e) p-nitrophenyl α-D-glucoside (0.5 mM) and p-nitrophenyl β-D-glucoside (0.5 M) were dissolved in the corresponding buffer solutions. (f) 1 mM solutions of inhibitors were used.

General procedure for enzyme assay

To a 96-well disposable microtiter plate with a maximum volume per well being 300 μL, the assay solutions and the potential inhibitors collected from reaction mixtures **15** and **16** were added as follows: 20 μL of buffer, 20 μL inhibitor, 10 μL enzyme and 40 μL of water. The solutions were mixed and then incubated at 37°C for 15 minutes. Ten μL of

substrate were then added and the plate was incubated at 37°C for another 35 minutes. After this, the molar extinction coefficient was read in a spectrophotometer with a microtiter plate reader set at 420 nm. This procedure has been used for both α- and β-glucosidase assays, with the only difference being basification of the β-glucosidase solutions with Na_2CO_3 prior to readings.

3.4.4. Structure identification

The 1H-NMR spectra were recorded at 500 MHz on a Varian VXR spectrometer.

The fast atom bombardment high resolution mass spectra (FABHRMS) were obtained using a JEOL HX 110 double focusing mass spectrometer operating in positive ion mode.

Methyl 2-O-methyl-3,4,6-tri-O-acetyl-β-D-glucopppyranoside (**17**)

^{1}H-NMR (500 MHz, $CDCl_3$); δ 1.99 (s, 3H, OAc), 2.04 (s, 3H, OAc), 2.06 (s, 3H, OAc), 3.15 (dd, 1H, $J_{1,2}$ 7.57 Hz, $J_{2,3}$ 9.28 Hz, H_2), 3.48 (s, 3H, OCH_3), 3.54 (s, 3H, OCH_3), 3.63 (m, 1H, H_5), 4.09 (dd, 1H, $J_{6a,6b}$ 12.2 Hz, $J_{6a,5}$ 1.95 Hz, H_{6a}), 4.26 (dd, 1H, $J_{6a,6b}$ 12.2 Hz, $J_{6b,5}$ 4.64 Hz, H_{6b}), 4.98 (t, 1H, $J_{3,2} = J_{3,4}$ 9.28Hz, H_3), 5.076 (t, 1H, $J_{3,4} = J_{4,5}$ 9.28 Hz, H_4); HRFABMS ($M+H^+$) calcd. 335.1264, found 335.1353.

Methyl 3,6-di-O-acetyl-2,4-di-O-methyl -β-D-glucopyranoside (**18**)

^{1}H-NMR (500 MHz, $CDCl_3$); δ 2.10 (s, 3H, OAc), 2.13 (s, 3H, OAc), 3.05 (dd, 1H, $J_{1,2} = J_{2,3}$ = 9.5 Hz, H_2), 3.26 (t, 1H, $J_{3,4} = J_{4,5}$ 9.5 Hz, H_4), 3.40 (s, 3H, OCH_3), 3.48 (s, 3H, OCH_3), 3.51 (m, 1H, H_5), 3.54(s, 3H, OCH_3), 4.24 (dd, 1H, $J_{6a,6b}$ 11.93 Hz, $J_{6a,5}$ 5.08 Hz,

H_{6a}), 4.27 (d, 1H, $J_{1,2}$ 7.73 Hz, H_1), 4.36 (dd, 1H, $J_{6a,b}$ 11.93 Hz, $J_{6b,5}$ 2.21 Hz, H_{6b}), 5.09 (t, 1H, $J_{2,3} = J_{3,4}$ 9.5 Hz, H_3); HRFABMS ($M+H^+$) calcd. 307.1393, found 307.1407.

2,3-Di-O-acetyl-4-O-methyl-5-amino-5,6-dideoxy-D-glucono-1,5-lactam **(19)**

^{1}H-NMR (500 MHz, $CDCl_3$); δ 1.21 (d, 3H, $J_{5,6}$ 6.62 Hz, CH_3), 2.12 (s, 3H, OAc), 2.17 (s, 3H, OAc), 3.45 (s, 3H, OCH_3), 3.52 (m, 1H, H_5), 3.72 (t, 1H, $J_{4,5} = J_{3,4}$ 8.61 Hz, H_4), 4.96 (t, 1H, $J_{2,3} = J_{3,4}$ 8.61 Hz, H_3), 5.16 (d, 1H, $J_{2,3}$ 8.61 Hz, H_2), 5.45 (br s, 1H, NH); HRFABMS ($M+H^+$) calcd. 260.1134, found 260.1136.

4-O-Acetyl-2-O-methyl-2,3-dehydro -3,5,6-trideoxy-5-amino-1,5-lactam **(20)**

^{1}H-NMR (500 MHz, $CDCl_3$); δ 1.25 (d, 3H, $J_{5,6}$ 6.84 Hz, CH_3), 2.054 (s, 3H, OAc), 3.66 (s, 3H, OCH_3), 3.88 (m, 1H, H_5), 5.19 (m, 1H, H_4), 5.36 (br s, 1H, NH), 5.58 (d, 1H, $J_{3,4}$ 6.35 Hz, H3); HRFABMS ($M+H^+$) calcd. 200.0923, found 200.0923.

6-O-Acetyl-3-O-methyl-5-amino-4,5-dideoxy-3,4-dehydro-D-gluco-2-ulosonic acid lactam **(21)**

^{1}H-NMR (500 MHz, $CDCl_3$); δ 2.07 (s, 3H, OAc), 3.80 (dd, 1H, $J6_{a,b}$ 12.15 Hz, $J_{6a,5}$ 5.74 Hz, H_{6a}), 4.30 (dd, 1H, $J6_{a,b}$ 12.15 Hz, $J_{6b,5}$ 3.53 Hz, H_{6b}), 5.10 (ddd, 1H, $J_{5,4}$ 1.99 Hz, $J_{6a,5}$ 5.74 Hz, $J_{6b,5}$ 3.54 Hz, H_5), 5.98 (d, 1H, $J_{4,5}$ 1.99 Hz, H_4).

4,6-Di-O-acetyl-3-O-methyl-D-gluco-2-ulosonic acid lactone hydrate (**22**)

^{1}H-NMR (500 MHz, $CDCl_3$); δ 2.10 (s, 3H, OAc), 2.18 (s, 3H, OAc), 3.74 (s, 3H, OCH_3), 4.46 (dd, 1H, $J_{6a,b}$ 9.06 Hz, $J_{6a,5}$ 6.63 Hz, H_{6a}), 4.50 (dd, 1H, $J_{6a,b}$ 9.06 Hz, $J6_{b,5}$ 7.95 Hz, H_{6b}), 4.89 (ddd, 1H, $J_{6a,5}$ 6.63 Hz, $J_{5,6b}$ 7.95 Hz, $J_{4,5}$ 5.3, H_5), 5.20 (d, 1H, $J_{3,4}$ 2.87 Hz, H_3), 5.62 (dd, 1H, $J_{3,4}$ 2.87 Hz, $J_{4,5}$ 5.3 Hz, H_4).

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Chapter 4

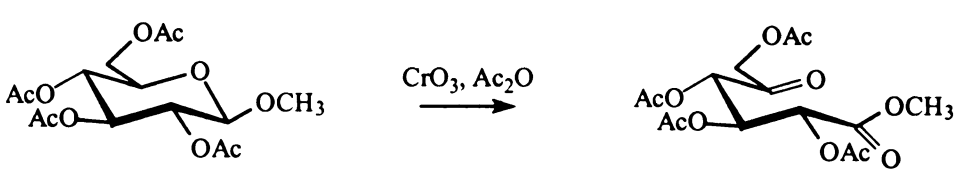
Chromium Trioxide Oxidation of Glycosides. A Comparative Study

Abstract

The chromium trioxide oxidation of glycosides has been known for a long time [1,2]. It required large excesses of oxidant, which was a deterrent, considering the high toxicity of Cr^{6+}. This chapter presents a synthetic improvement over the old method, reducing the amount of oxidant used by more than 10-fold and improving the yield of the reaction to 100%. We also describe the discovery that α-benzyl glycosides are converted to α-benzoates in quantitative yield. This reaction loses its specificity in the case of β-benzyl glycosides where anomeric cleavage competes with benzyl oxidation. A detailed study of this behavior is presented.

4.1. Introduction

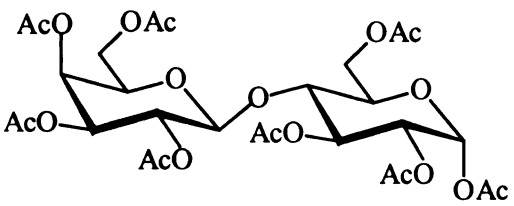
In 1970 Angyal published his results on the chromium trioxide oxidation of carbohydrates [1,2]. Chromium trioxide in acetic acid has been shown to oxidize acetals of aldehydes, $R^1CH(OR^2)OR^3$, to esters, R^1COOR^2, in which one of the alcohols constituting the acetal has been retained while the other is lost during oxidation of the alkylidene fragment to a ketone. Glycosides, being acetals, are oxidized according to this general scheme, the aglycon being retained and the ring being ruptured. The products are therefore esters of 5-hexulosonic acids from pyranosides and 4-hexulosonic acids from furanosides [3]. Scheme 4.1.1 exemplifies this chemistry for methyl-β-D-glucoside **1**. A remarkable feature of the reaction is that it is specific to β-pyranosides but occurs with both α- and β–furanosides. Since primary and secondary hydroxyl groups are rapidly oxidized by the reagent, they must be protected, for example by esterification, since esters are stable under these conditions. Acetals, being attacked, are not suitable for



1 **2**

Scheme 4.1.1 Chromium trioxide oxidation of methyl 2,3,4,6-tetra-O-acetyl-glucoside.

protecting the hydroxyl groups. Methyl and benzyl ethers are also attacked by chromium trioxide in acetic acid [4], and hence these are also unsuitable as protecting groups. Methyl ethers are converted into formic esters, while benzyl groups are transformed to benzoates. Disaccharides, being glycosides, are similarly oxidized. For example, the octaacetate of lactose **3**, which is a β-3-O-glucosyl-galactoside, is smoothly converted into a keto ester, while maltose **4**, being a α–glycoside does not react.



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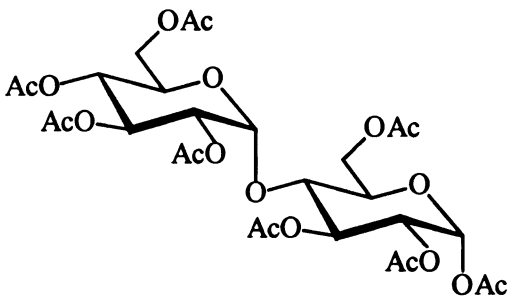


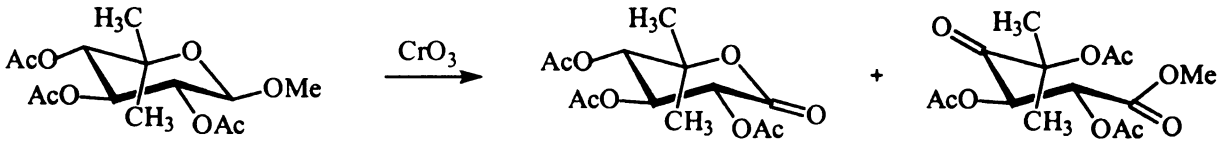
4

The chromium trioxide oxidation has also been used to determine the anomeric natures of sugar residues in oligo- and polysaccharides [5]. The materials are fully acetylated, oligosaccharides having first been reduced to their alditols, and the products are then treated with chromium trioxide in acetic acid in the presence of an internal standard. A comparison of sugar analyses, performed before and after oxidation, reveals what sugar residues have been oxidized. A prerequisite for obtaining reliable results is that the carbohydrate does not contain free hydroxyl groups but is fully acetylated and that the conformational stability of the chain forms having axially attached aglycons is large enough to ensure that the proportion of the alternate form is negligible. This supposition is valid for the α-pyranosides of the common hexoses, 6-deoxyhexoses, 2-acetamido-2-

deoxyhexoses and xylose. This technique has been applied for the elucidation of the anomeric configuration of bacterial lipopolysaccharides from *Salmonella typhi* and *Salmonella Strasbourg* [6]. The method may also, in favorable circumstances, be used for sequence analysis, like in the case of the *Pneumococcus* type 2 capsular polysaccharide [7,8].

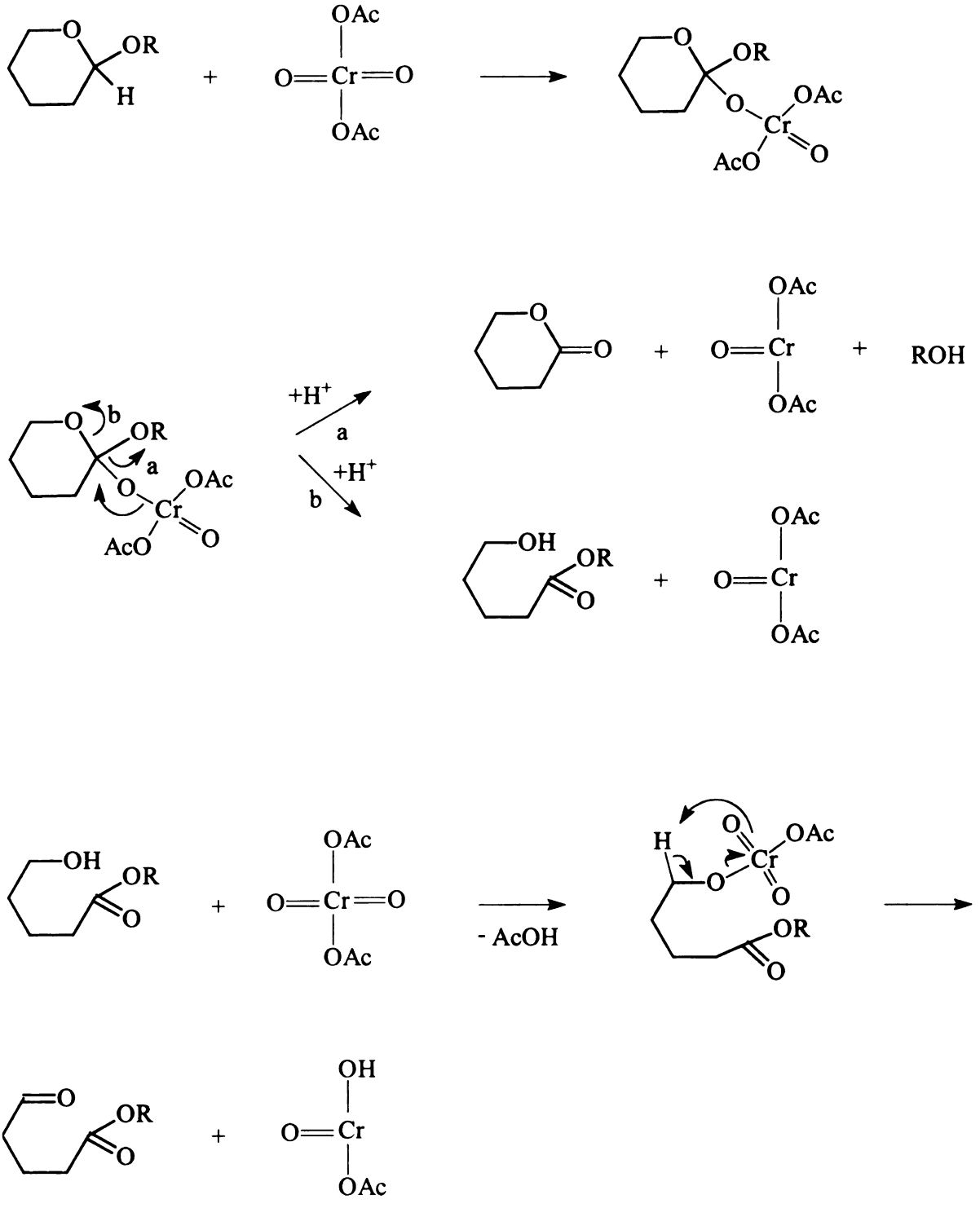
The mechanism of the oxidation of acetals by chromium trioxide in acetic acid is not yet known. Presumably the actual oxidizing agent is chromyl acetate, $CrO_2(OAc)_2$, since chromium trioxide in water or pyridine will not give keto esters. It is not clear whether the oxidation of the acetal to ester and that of one of the alkoxyl groups to ketone are simultaneous or consecutive reactions. In order to decide this point, the oxidation of the β-glucoside was carried out in acetic anhydride rather than in acetic acid according to the reasoning that if the acetal were first oxidized to an ester, liberating a hydroxyl group, the latter might be acetylated faster than it is oxidized. However, the same keto ester was obtained and the experiment was inconclusive. It was then argued that if the two reactions are concerted, oxidation of the glycopyranoside could not occur if there were no hydrogen atom on C5. A suitable model compound that is a branched-chain glycoside is methyl 6-deoxy-5-C-methyl-β-D-*xylo*-hexopyranoisde. The acetylated glycoside **5** was treated with CrO_3. Oxidation occurred at a slower rate than that of the unbranched β-glycosides and two products were obtained: the minor one was the lactone **6** and the major one was methyl 2,3,5-tri-O-acetyl-6-deoxy-5-C-methyl-L-*threo*-4-hexulosonate **7** (Scheme 4.1.2). It appears that acetyl migration occurred, presumably after oxidation of the glycoside to ester, from O4 to O5, followed by oxidation of the free hydroxyl group

on C4. The experiment would suggest that removal of the two hydrogen atoms - from the acetal and from the alkoxyl group - is not a concerted process: oxidation of the acetal will occur even if there is no hydrogen atom on C5 (Scheme 4.1.3). However, this does not preclude the possibility of the oxidation proceeding by two different mechanisms, a concerted one operating with unbranched β-glycopyranosides. The ring-opening of the branched-chain pyranoside (b) is, however, slower, allowing the competing reaction - breaking of the bond between the anomeric carbon and the aglycon (a) to give the lactone - to achieve some degree of prominence. It is possible that the first step in the oxidation is the formation of a complex between the acetal and chromyl acetate; this complex then breaks down at a rate, and in a direction, dependent on the nature of the two alkoxyl groups of the acetal.



5 **6** **7**

Scheme 4.1.2 Oxidation of methyl 6-deoxy-5-C-methyl-β-D-*xylo*-hexopyranoisde.

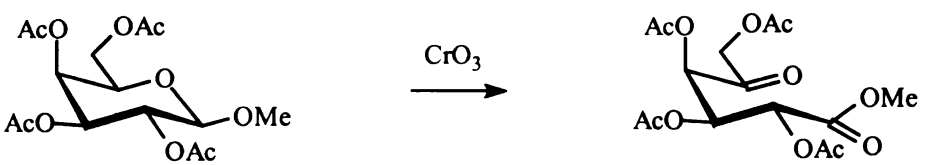


Scheme 4.1.3 Proposed mechanism for the oxidation of glucopyranosides by CrO_3.

4.2. Results and discussions

4.2.1. Improvements in the oxidation of methyl β-D-glycosides

An important previous disadvantage of this method has been the employment of large excesses of CrO_3. In his experiments, Angyal was using 28 equivalents of oxidant obtaining a maximum of 70% conversion. In our efforts to minimize the quantity of CrO_3, we obtained complete conversion of β-methyl glucoside **1** (Scheme 4.1.1) and β-methyl galactoside **9** (Scheme 4.2.1) to products using only 2 equivalents of oxidant. This represents a significant improvement from the initial 28 equivalents used, considering the toxicity of the oxidant and the difficulty in removing traces of CrO_3. In our procedure we used a 12.5:1 (vol) mixture of glacial acetic acid and acetic anhydride. It is possible that acetic anhydride removes the traces of water and improves the yield of the reaction. It also seems that the 100% conversion is general and does not depend on the nature of the β-glycoside substrate.

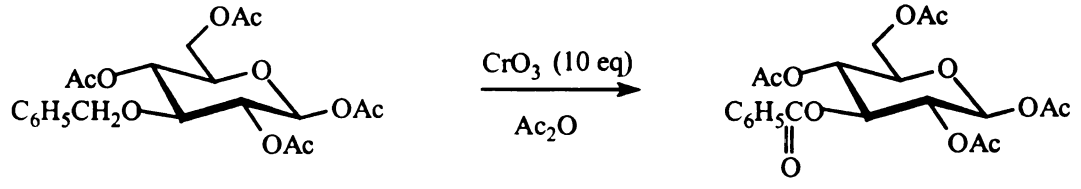


8 **9**

Scheme 4.2.1 Chromium trioxide oxidation of methyl 2,3,4,6-tetra-O-acetyl-galactoside.

4.2.2. Oxidation of benzyl glycosides to glycosyl benzoates

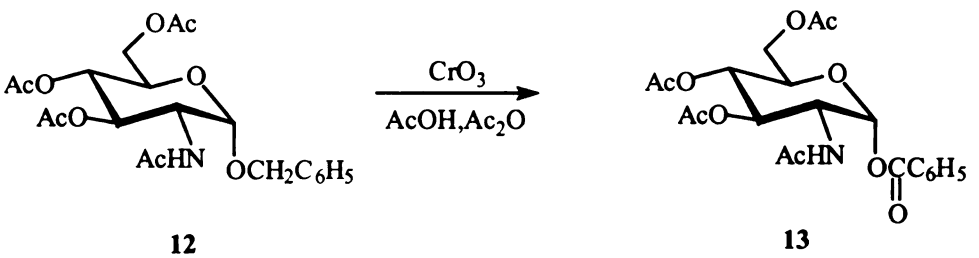
Angyal reported [4] that O-benzyl ethers are converted into benzoates. The substrate that he analyzed was 1,2,4,6-tetra-O-acetyl-3-O-benzyl-β-D-glucopyranose **10** which he transformed into 1,2,4,6-tetra-O-acetyl-3-O-benzoyl-β-D-glucopyranose **11** with a 50% total yield and using 10 equivalents of chromium trioxide (Scheme 4.2.2). Benzyl groups are important protecting groups that are orthogonal to esters. They are inert to acids and bases and can be removed only by catalytic hydrogenolysis. A benzyl group in the anomeric position constitutes a good protecting group that can be easily introduced. Once the anomeric site needs to be activated, the protecting group has to be removed. This is traditionally done by hydrogenolysis. However, if the benzyl group is transformed into a benzoate, the latter can be either hydrolyzed under basic conditions or directly transformed into a glycoside, following activation.



10 **11**

Scheme 4.2.2 Oxdation of 1,2,4,6-tetra-O-acetyl-3-O-benzyl-β-D-glucopyranose.

By using the same reaction conditions as earlier described for the oxidation of methyl glycosides and using again the same low amount of chromium trioxide, we succeeded in transforming benzyl α-D-glucoside **12** into α-glucosyl benzoate **13** in 100% yield (Scheme 4.2.3). The ester group can easily be removed by hydrolysis if needed.



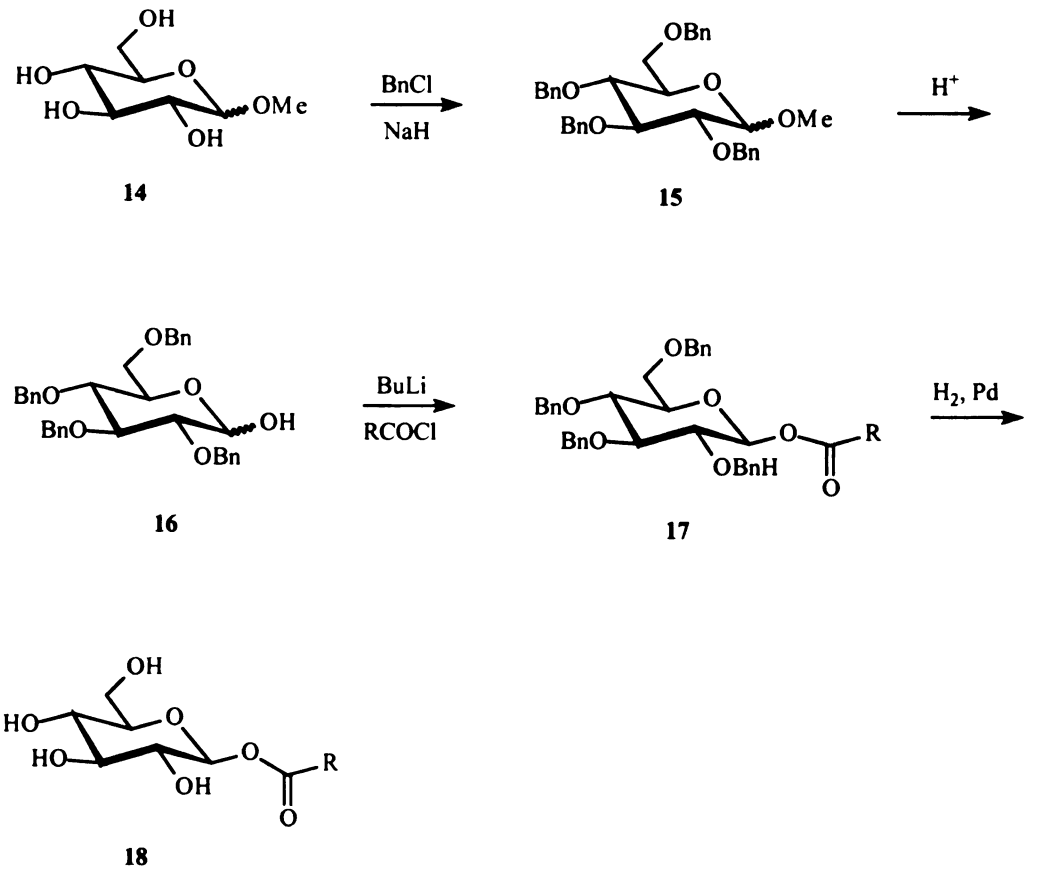
12 **13**

Scheme 4.2.3 Oxidation of benzyl 2-acetamido-2-deoxy-3,4,6-tri-O-acetyl-α-D-glucopyranoside.

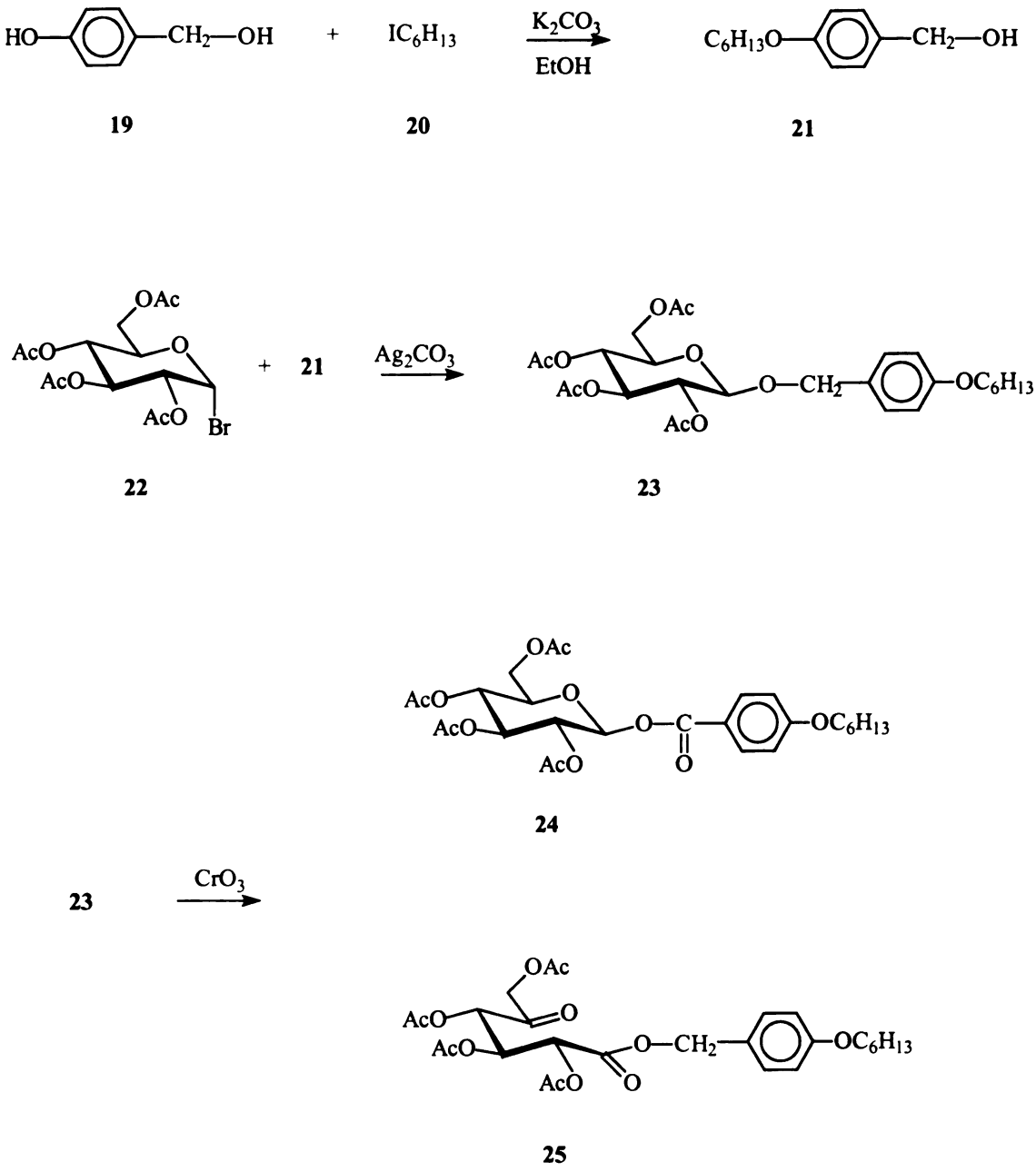
4.2.3. Chromium trioxide oxidation of benzyl β-glycosides. Competition between ring cleavage and benzyl oxidation.

The synthesis of acyl glycosides is not very straightforward. The classical route for making them involves benzyl protection of a glycoside, deprotection of the anomeric center, acylation followed by catalytic hydrogenolysis (Scheme 4.2.4). We already demonstrated the quantitative conversion of α-benzyl glycosides into benzoates and we also wanted to analyze the behavior of β-benzyl glycosides or analogs thereof. Since glycosides with long chain aglycons are of interest because of their tendency to

aggregate, we considered synthesizing long-chain *p*-alkoxy-β-benzoyl glycosides. For this purpose we chose a six-carbon alkyl chain. Scheme 4.3.5 outlines the synthesis. 4-Hexyloxy benzylalcohol **21** was prepared from 4-hydroxy benzylalcohol **19** and 1-iodohexane **20**. This was then reacted with 2,3,4,6-tetra-O-acetyl glucosylbromide **22**, in the presence of silver carbonate, to give *p*-hexyloxybenzyl-2,3,4,6-tetra-O-acetyl-β-D-glucoside **23**. On oxidation with chromium trioxide, we obtained, however, not only the



Scheme 4.2.4 Classical route for the synthesis of benzoyl glycosides.



Scheme 4.2.5 Competition between benzyl oxidation and ring opening in the oxidation of benzyl β-D-glucopyranosides.

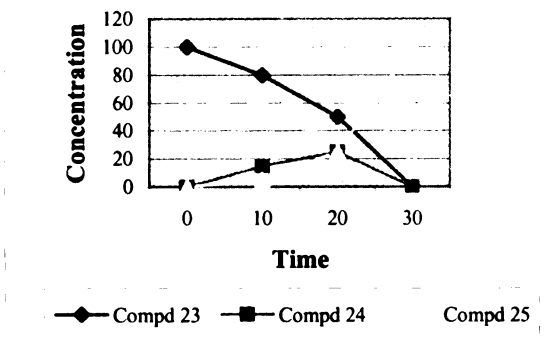
desired *p*-hexyloxybenzoyl-2,3,4,6-tetra-O-acetyl-β-D-glucoside **24** but also compound **25** with the ruptured ring. The two compounds are easily distinguished by their phenyl proton signals **24** has two doublets at 6.96 and 7.89 ppm, while the same proton signals in **25** are shifted to 6.89 and 7.98 ppm respectively. Our next goal was to create conditions in which formation of **25** is avoided or diminished. Since the first oxidation attempt was performed using 2 equivalents of CrO_3 and 50°C, we thought that reducing the amount of oxidant might improve the yield of **24** but this did not happen. Next, the reaction was run at different temperatures: 20 and 1°C. The course of the reaction was determined by taking samples at different times and estimating the conversions from NMR spectra. The values are indicated in the tables associated with the graphs in Figure 4.2.1. It can be concluded that modification in the concentration of oxidant does not affect conversion much, while the temperature has quite a large effect. The best conversions are obtained at the lowest temperature. However, none of the conditions accomplishes total transformation of the benzyl into a benzoate and the ring cleavage cannot be totally annulled. Therefore oxidation of β-benzyl glycosides proves to be less appealing than that of α-glycosides.

4.3. Conclusions

The oxidation of β-D-glycosides to keto esters has been known since 1970. The disadvantage of the method is the high excess of chromium trioxide used. By using small volumes of acetic anhydride, we succesfully lowered the amounts of CrO_3 used to two equivalents and also obtained quantitative yields. This is a considerable improvement over the original procedure, considering the high toxicity of the oxidant. Oxidation of

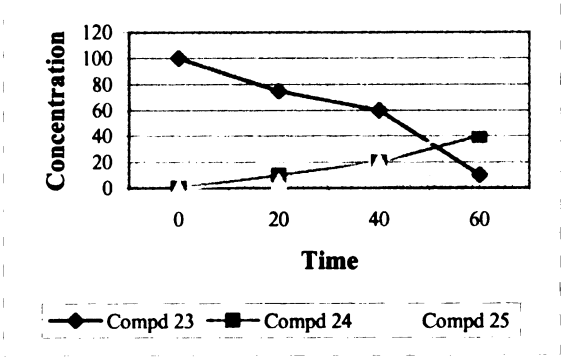
50° C

	Time			
Compound	0	10	20	30
23	100	80	50	0
24	0	15	25	0
25	0	5	25	100



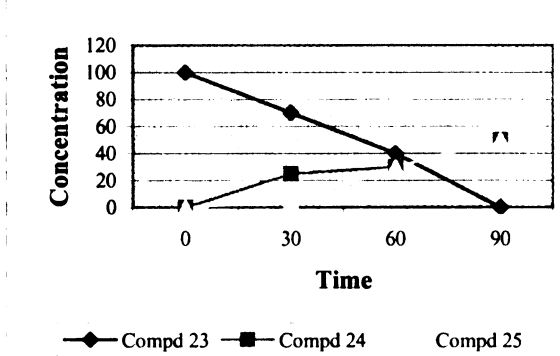
20° C

	Time			
Compound	0	20	40	60
23	100	75	60	10
24	0	10	20	40
25	0	5	20	50



1° C

	Time			
Compound	0	30	60	90
23	100	70	40	0
24	0	25	30	50
25	0	5	30	50



50° C (2eq)

	Time			
Compound	0	10	20	30
23	100	66	40	24
24	0	22	25	20
25	0	12	35	56

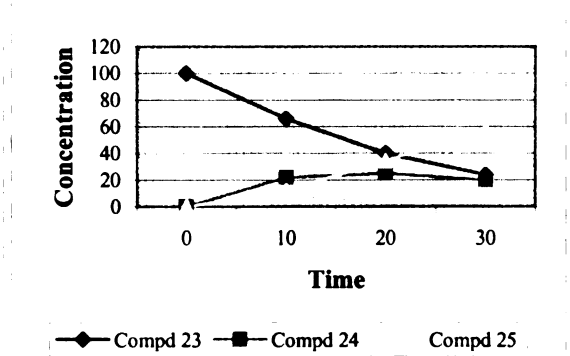


Figure 4.2.1. Oxidation of benzyl-β-D-glucopyranoside.

benzyl α-D-glycosides to α-glycosyl benzoates can be achieved in quantitative yield by using the same procedure. Acid or base catalyzed hydrogenolysis provides an alternative to the traditional debenzylation via catalytic hydrogenolysis. Chromium trioxide oxidation of β-benzyl glycosides, however, targets both the benzyl group and the sugar ring, making the reaction less appealing from the standpoint of conversion.

4.4. Experimental section

Methyl-2,3,4,6-*tetra*-O-*acetyl*-D-xylo-5-*hexulosonate* **(2)** and *Methyl*-2,3,4,6-*tetra*-O-*acetyl*-L-*arabino*-5-*hexulosonate* **(9)**

The procedure and spectral characterizations are described in the experimental section of chapter 2.

Benzoyl-2-acetamido-2-deoxy-3,4,6-tri-O-acetyl-α-D-glucose **(13)**

To a solution of benzyl-2-acetamido-2-deoxy-3,4,6-tri-O-acetyl-α-D-glucose **12** (140.00 mg, 0.34 mmol) in acetic acid (1.5 mL) and acetic anhydride (0.1 mL), CrO_3 (180.00 mg, 1.80 mmol) was added and the suspension was stirred at 50°C for 30 minutes. The mixture was then poured slowly, with stirring into cold water (10 mL). The water was extracted three times with chloroform. The combined chloroform layers were decolorized with activated charcoal, filtered and washed with saturated $NaHCO_3$ solution and then with water. After drying with Na_2SO_4 and rotary evaporation of the solvent the ester **13** (143.00 mg, 100%) was obtained as a colorless syrup: ^1H NMR ($CDCl_3$) δ 1.87 (3 H, s, NHAc), 2.03 (6 H, s, OAc), 2.04 (3H, s, OAc), 4.04 (1H, dd, $J_{6a,6b}$ 12.2 Hz, $J_{6a,5}$ 12.45 Hz, H-6a), 4.04 (1H, m, H-5), 4.21 (1H,

dd, H-6b, $J_{6b,5}$ 3.17 Hz, H-6b), 4.57 (1H, ddd, $J_{1,2}$ 8.79 Hz, $J_{2,3}$ 9.77 Hz, $J_{2,NH}$ 2.93, H-2), 5.25 (1H, t, $J_{4,5}$ =$J_{3,4}$ 9.77 Hz, H-4), 5.37 (1H, t, H-3), 5.68 (1H, d, H_1), 6.41 (1H, d, NH) 7.50 (2H, dd, $J_{Ara,b}$ 7.57 Hz, $J_{Arb,c}$ 7.08 Hz, H-Ar_b), 7.63 (1H, t, H-Ar_c), 8.06(2H, d, H-Ar_c); ^{13}C NMR ($CDCl_3$) δ 20.5, 20.6, 20.7, 22.9, 57.2, 61.3, 67.4, 70.0, 70.7, 91.1, 128.6, 128.8, 129.8, 134.0, 164.1, 169.0, 170.0, 170.6, 171.6.

Hexyloxy benzylalcohol **(21)**

To a solution of 4-hydroxy benzylalcohol **19** (6.0 g, 48.3 mmol) and 1-iodohexane **20** (8.6 mL, 58.0 mmol) in ethanol (40 mL), potassium carbonate (26.8 g, 193.9 mmol) was added and the suspension was stirred at 55°C for 15 h. A second amount of iodohexane (1.5 mL, 10.0 mmol) was added and stirring was continued for another 5 h at 55°C and then15 h at room temperature. The suspension was filtered, the precipitate washed with ethanol and the mother liquor rotary evaporated. The potassium iodide that is soluble in ethanol, was removed from the product **21** by precipitation from chloroform/hexane solution. After filtration, the solution was evaporated and the hydroxyether **21** (9.9 g) was obtained in 100% yield. ^{1}H NMR ($CDCl_3$) δ 1.04 (3 H, t, $J_{i,j}$ 6.84 Hz, H-j) 1.46 (2H, hex, $J_{i,h}$ 3.66 Hz, H-i), 1.48 (2H, p, H-h), 1.89 (2H, p, $J_{e,f}$ 6.59 Hz, , H-f), 2.98 (1H, s, OH), 4.04 (2H, t, H-e), 4.46 (2H, d, H-d), 6.97 (2H, d, J 8.56 Hz, H-Ar_a), 7.33 (2H, d, H-Ar_b); ^{13}C NMR ($CDCl_3$) δ 13.9, 22.4, 25.5, 29.0, 31.4, 64.5, 67.8, 114.2, 128.4, 132.8, 158.4.

p-Hexyloxybenzyl-2,3,4,6-tetra-O-acetyl-β-D-glucoside **(23)**

Hexyloxy benzylalcohol **21** (5.0 g, 24.0 mmol) was dried for 1 h in the vacuum oven and then dissolved in a 1:1 mixture of dry benzene and nitromethane. Calcium sulphate was added and the suspension was stirred at room temperature for 1 h. To this, silver carbonate (1.4 g, 5.0 mmol) and 2,3,4,6-tetra-O-acetyl glucosylbromide **22** (2.0 g, 4.8 mmol) were added and stirred for 20 h. Some more **22** (1.2 g, 3.0 mmol) was added and the temperature was increased slowly to 55°C and stirring was continued for another 24 h. The suspension was filtered over Celite, washed with chloroform and the solution then rotary evaporated. The crude product was purified by column chromatography using a 1:6 acetone-hexane mixture as the eluent. Pure *p*-hexyloxybenzyl-2,3,4,6-tetra-O-acetyl-β-D-glucoside **(23)** (2.4 g) was obtained in a 57% yield. ^{1}H NMR ($CDCl_3$) δ 0.88 (3H, t, $J_{i,j}$ 7.08 Hz, H-j), 1.23 (2H, m, H-i), 1.31 (2H, s, J 3.42 Hz, H-h), 1.43 (2H, m, H-g), 1.76 (2H, m, J 6.59 Hz, J 7.3 Hz, H-f,), 1.97 (6H, s, OAc), 1.99 (3H, s, OAc), 2.08 (3H, s, OAc), 3.6 (1H, ddd, $J_{5,4}$ 9.28 Hz, $J_{5,6a}$ 2.44 Hz, $J_{5,6b}$ 4.64 Hz, H-5), 3.92 (2H, t, $J_{e,f}$ 6.59 Hz, H-e), 4.13 (1H, dd, $J_{6a,b}$ 12.21 Hz, $J_{6b,5}$ 2.44 Hz, H-6b), 4.75 (1H, dd, H-6a), 4.48 (1H, d, $J_{1,2}$ 7.81 Hz, H-1), 4.53 (1H, d, H-ad), 4.79 (1H, d, H-db), 5.01 (1H, dd, H-2), 5.07 (1H, t, $J_{4,5}$ 9.28 Hz, H-4), 5.13 (1H, t, $J_{3,4}$=$J_{2,3}$ 9.28 Hz, H-3), 6.84 (2H, d, J_{ab} 8.3 Hz, H-a), 7.16 (2H, d, H-b); ^{13}C NMR ($CDCl_3$) δ 14.0, 20.6, 20.7, 20.8, 22.5, 25.6, 29.1, 31.5, 61.9, 68.0. 68.3, 70.4, 71.2, 71,7, 72.8, 98.7, 114.3, 128.2, 129.5, 159.0, 163.4, 170.2, 170.7.

p-hexyloxybenzoyl-2,3,4,6-*tetra-O-acetyl-β-D-glucoside* **(24)** and *p-hexylbenzyloxo*-2,3,4,6-*tetra*-O-*acetyl*-D-xylo-5-*hexulosonate* **(25)**

p-Hexyloxybenzyl-2,3,4,6-tetra-O-acetyl-β-D-glucoside **(23)** (0.15 g, 0.27 mmol) was dissolved in a mixture of glacial acetic acid (1.5 mL) and acetic anhydride (0.2 mL) and chromium trioxide (0.14 g, 1.40 mmol) was added. The suspension was stirred at 50°C for 30 minutes and samples were taken at 10 minute intervals, and were worked up using the following procedure. The suspension was poured over cold water (15 mL) and extracted three times with chloroform (10 mL). The solution was rotary evaporated and the crude solid was analyzed by NMR. **24**: ^{13}C NMR ($CDCl_3$) δ14.0, 20.5, 20.7, 22.5, 25.5, 28.9, 31.4, 61.2, 66.3, 67.1, 69.7, 70.9, 90.1, 114.1, 114.7, 137.2, 161.9, 169.3, 170.1, 170.7, 176.7.

4.5. References

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Chapter 5

Development of a New Glycosylation Procedure for 2-Aamino-2-Deoxy Sugars

Abstract

Amino sugars occur in a host of different compounds mainly as components of poysaccharides and sometimes in low molecular weight metabolic products, but rarely in the free form. The finding of aminosugars in antibiotics, in microorganisms, higher plants, invertebrates and human body has deepened the interest in this class of biologically important compounds. It appears that in these natural products the amino groups of the sugars are always protected, most often by acetyl, and occasionally by formyl or methyl groups. N-Acetylglucosamine is the most commonly occurring amino sugar. Therefore, glycosylation reactions involving them are of high interest. There are only two procedures that involve N-acetylated glucosamine as starting material: the Koenigs-Knorr and the oxazoline methods. Here we present a different approach that consists of activation of a nitro-phenyl leaving group with $ZnCl_2$ to afford high yields of N-acetamide β-D-glucosides.

5.1. Introduction

Amino sugars represent a class of compounds that in diversity and ubiquity has few parallels in the biological field. The number of amino sugars identified in living organisms is greater than that of all other sugars. Amino sugars have also been found in all tissues and fluids of pluricellular organisms. Their structure, which blends those of both amino acid and common sugar, gives them a marked lyophilic character that may explain their association with most proteins and with many lipids. Glycoconjugates such as the bacterial lipopolysaccharides, cell surface glycoproteins and glycolipids, as well as other complex proteoglycans of immunological significance are involved in a host of biological processes. Their oligosaccharide parts have received much attention and have acquired an importance as great as that of proteins and nucleic acids. Their functions are less well understood, but it is clear that the oligosaccharide moieties of biological membranes play a decisive role for specific immune reactions [1]. Moreover, they fulfill important functions in intercellular recognition and interaction, in the control of cell growth and thus tumor formation, and in the interaction with biologically active factors such as enzymes, hormones, bacteriotoxin, and viruses. This may be due to the fact that strongly hydrophilic glycan chains will normally be located at the outer surface of molecules in aqueous environments, which render them available for interactions with other molecules. The wide structural variety makes sugars and in particular oligosaccharides ideal as carriers of biological information. In contrast to peptides and nucleotides, in which the informational content is determined solely by the number and sequence of different monomer units, the informational content of oligosaccharides is fixed additionally by the site of coupling, by the configuration of the glycosidic linkage,

and by the occurrence of branching. Thus, polymers made up of carbohydrates can carry considerably more information per building block then proteins and nucleic acids [2]. N-Acetylglucosamine is an important component of oligosaccharides with biological importance. Glycosides of N-acetylglucosamine are widely distributed in living organisms where they constitute building blocks of glycoconjugates such as peptidoglycans, mucopolysaccharides (hyaluronic acids, keratan sulfates and inner and outer core regions of glycoproteins). They are also encountered in human milk, in blood group substances, in bacterial lipopolysaccharide antigens where they constitute part of the epitopes, and in plants [3]. A sulfated and acetylated glucosamine oligosaccharide is recognized by root cells of leguminous plants and acts as a signal for symbiotic host-specificity of *Rhizobium* bacteria. This shows that receptors for amino sugars are also present in plant cells. Of special interest is the occurrence of aminosugars in a variety of useful antibiotics, such as kanamycin C, paromomycin, hydroxymycin, trehalosamine and zygomycin A [4].

The construction of each individual oligosaccharide poses a new challenge, requiring knowledge of methods, experience, and experimental dexterity, especially because there are no universal methods for their synthesis. This chapter will focus on 1,2-trans-glycosylations of N-acetylglucosamine. Glycosylation reactions are not restricted only to the actual process of coupling of the donor D with the acceptor A in the presence of different promoters P, but are constituted from a series of reaction steps: protection of both the acceptor and the donor, introduction of a participating or nonparticipating group at C-2 of the donor, activation of the anomeric position of the donor, glycosylation in the

presence of a suitable protecting group P and finally deprotection of C-2 and its retransformation in an amido group. Protecting groups are used to block certain hydroxyl groups in a carbohydrate, so that these will not interfere in reactions. In the case of the donor sugar, all hydroxyl groups have to be protected, to avoid its self-condensation. On the other hand, in the case of the acceptor sugar, it has to be ensured that only the oxygen atom in the desired position forms the glycosidic bond. This can be achieved if that particular hydroxyl group is the most reactive in the molecule, or it is the only one available for reaction. If the synthesis of a reducing di- or oligosaccharide is desired, the anomeric site not involved in glycosylation has to be protected, since it is the most reactive position in the molecule. There are three major protecting agents: esters, ethers and cyclic acetals, which can be used alternatively to achieve the desired protection pattern. The suitable protection of the OH groups determines the regioselectivity of the reaction. The diastereoselectivity of the glycosylation, that is the α or β configuration of the newly formed glycosidic bond, is determined to a large extent by the blocking groups of the donor sugar, but also by the type of promoter and solvent used. In order to achieve 1,2-trans glycosylation, the most often encountered linkage for NAG, two approaches are of general interest (Figure 5.1.1). The most widely used method involves a glycosylation donor containing a C-2 participating group as the amino protective function. The formation of a cyclic intermediate (**D**) by anchimeric assistance results in a shielding of the α-face of the donor, allowing the reaction of the acceptor alcohol on the β-face only, thus affording the 1,2-trans glycoside (**F**) with a high degree of stereoselectivity. This approach has been used in reactions in which the assisting group is N-acetyl or phthalimido and the leaving group is a halogen. A second approach involves 1,2-cis-2-

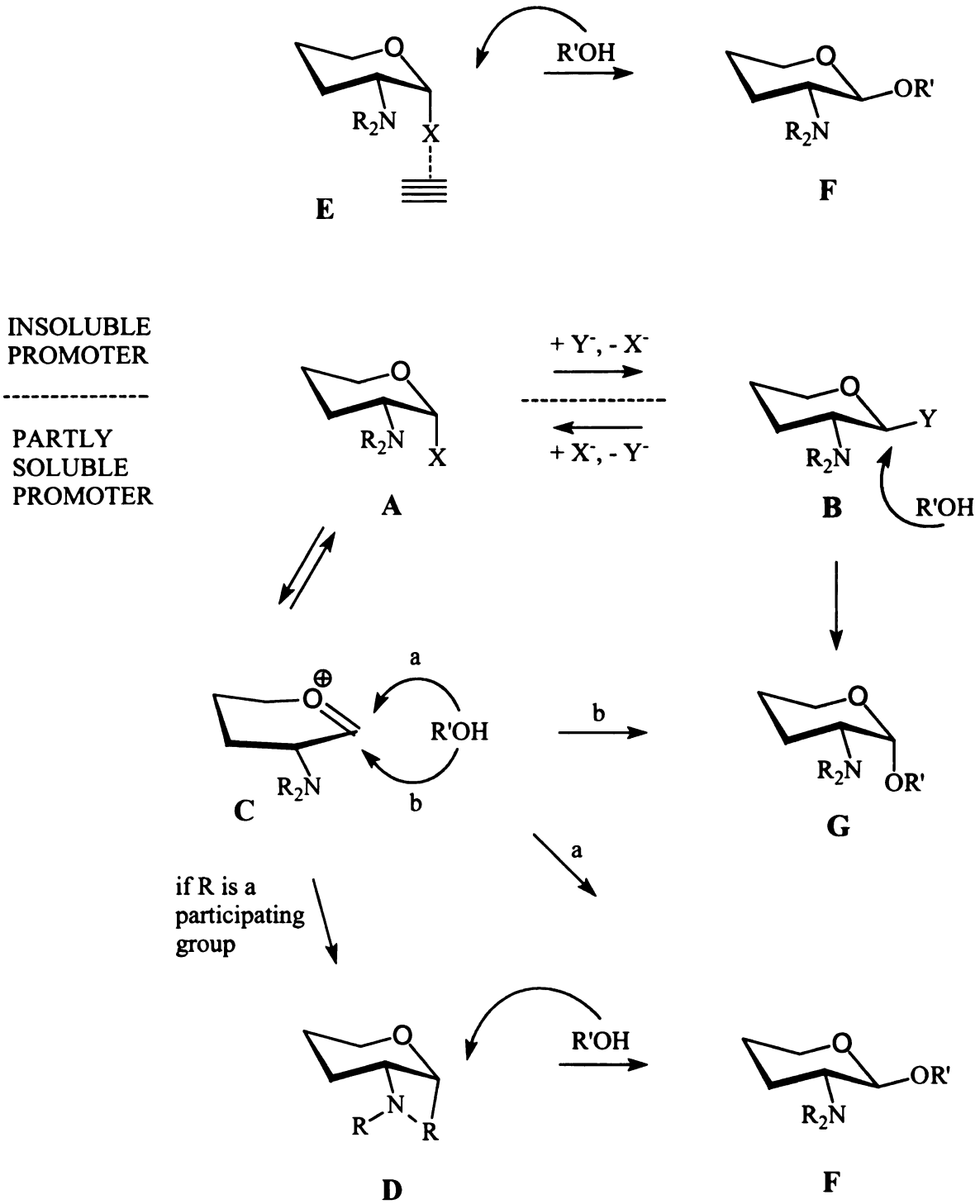
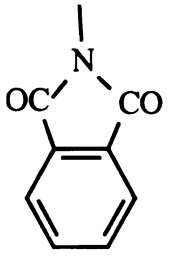


Figure 5.1.1 General scheme of glycosylations.

amino-2-deoxy-α-D-glycopyranosyl halides (**A**) (having an amino nonparticipating protecting group) and an insoluble promoter able to shield the α-face of the donor (**E**) during the substitution with the acceptor alcohol. Selectivities are generally lower than those observed with the donors containing C-2 participating groups. An important issue is the survival of the N-protecting group under the conditions necessary for activation of the anomeric position. The most often employed leaving-groups are: halogens, acetyls, thiols, trichloroacetimide and 4-pentenyl. The promoters can be used either in catalytic amounts or in stoechiometric proportions. For oxygenated leaving groups, Lewis acids have proven adequate ($ZnCl_2$, $SnCl_4$, $AlCl_3$, $FeCl_3$, BF_3) and for the rest, silver and mercury compounds have been used (Ag_2O, Ag_2CO_3, $Hg(CN)_2$, $HgBr_2$, $F_3C\text{-}SO_3\text{-}Ag$, $AgClO_4$, the activity increasing in the order given). The role of the promoter is both as and activator as well as an acid scavanger. The promoter should assist the departure of the anomeric leaving group (X) in a way which avoids the formation of the oxocarbonium ion, which results, most of the time, in a lack of stereoselectivity and affords mixtures of α- and β-glycosides (**G**, **F**). Thus, after choosing the proper N-protecting group, the promoter and solvent, the glycosylation reaction is performed to give exclusively or predominantly the desired anomeric glycosidic linkage. The final step of this procedure is deprotection. Table 5.1.1 gives a summary of the main leaving groups, N-protecting groups, and the corresponding promoters. Also, the advantages and limitations of the particular methods are indicated [32-34]. There are many glycosylation methods but each of them has disadvantages or limitations. One major disadvantage common to all glycosyl halides is their low thermal stability and high sensitivity towards hydrolysis.

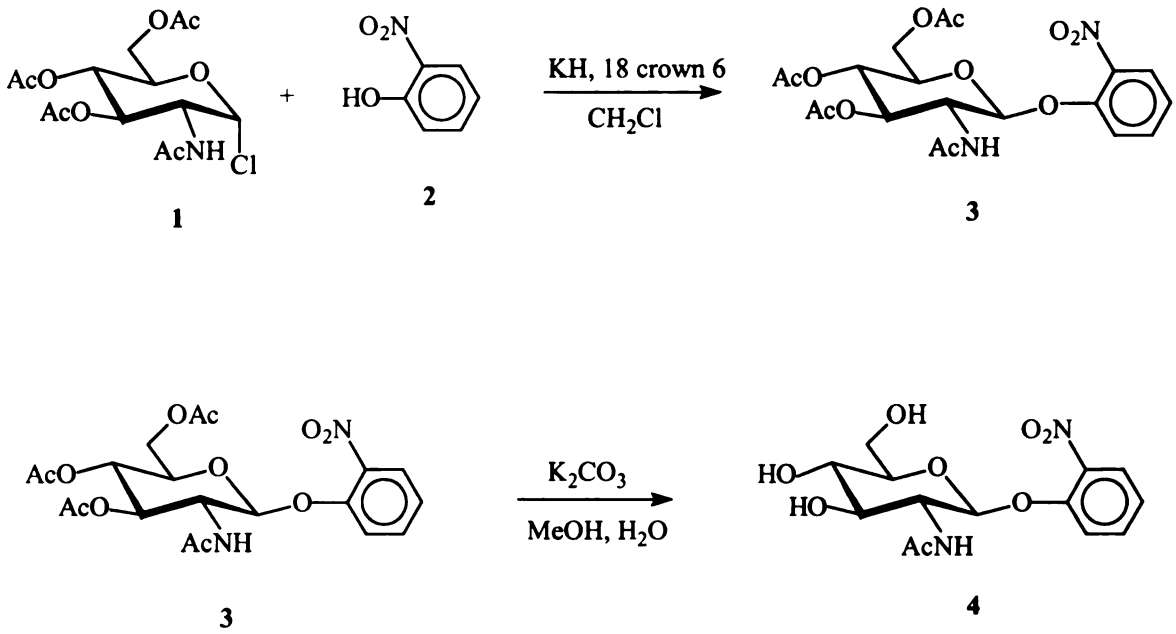
Table 5.1.1 Conditions, advantages and limitations for the most common glycosylation procedures.

Leaving group	N-protecting gr.	Promoters	Advantages, limitations & ref
Koenigs -Cl Knorr	-N-CO-CH_3	Ag salt //Hg salts	only active alcohols//low yields [5-8]
Oxazoline -Cl -Br	-N-CO-CH_3	$ZnCl_2$//$SnCl_4$//$AlCl_3$// $FeCl_2$//$FeCl_3$	harsh cond, not for sec OH.// migration of acid sensitive gr.// partial anomerization [9-11]
-Cl		AgTfl-collidine // Ag Zeolite // Ag salicilate // $AgClO_4$-$AgCO_2$//$HgBr_2$	good for both primary and secondary OH; deprotection: H_2N-NH_2, EtOH // $NaBH_4$ [12,13]
-OAc		$SnCl_4$ // $BF_3.Et_2O$ // Me_3Si-OSO_2-CF_3	good yields [14,15]
-S-R		MeTfl // NIS+CF_3SO_3H AgTfl+Br_2	good yields // MeTfl is toxic [16,17]
-O-CNH-CCl_3		BF_3·Et_2O//Me_3SiTfl	good yields and selectivity; low temp [18-20]
-O$(CH_2)_3CHCH_2$		$[(coll)_2I]ClO_4$	good yields [21,22]
-Cl	$-N_3$	insol. Ag promoters	deprotection: reduction [23-26]
-O-CNH-CCl_3			BF_3 [27,28]
-S-R		MeTfl	α-selectivity only // low yields for oligosaccharides [29,30]
-O-Ac	-NH-CO-OR	Me_3Si-OSO_2CF_3	good yields for primary & secondary OH [31]

Usually, Cl is favored over Br, which is more reactive but less stable. The Koenig-Knorr and oxazoline methods do not use N-protecting groups different from acetyl, but this advantage is counteracted by the fact that only reactive acceptors in excess can react. The phthalimido method [7] is the method of choice so far, giving 1,2-cis NAG disaccharide in good yields and high stereoselectivity, but it has the inconvenience of requiring removal of the phthalimido group, once the reaction is completed. Fraser-Reid discovered not long ago that 4-pentenyl glycosides can be activated by NBS to give a bromonium ion, which will be attacked by the alcohol nucleophile to give the trans 1,2-glycoside, but this procedure also requires the presence of protecting groups. Even though a multitude of procedures are employed in the synthesis of N-acetylglucosamine glycosides, none of them is a 'perfect choice'. This is why the search for better activating groups and promoters is still going on.

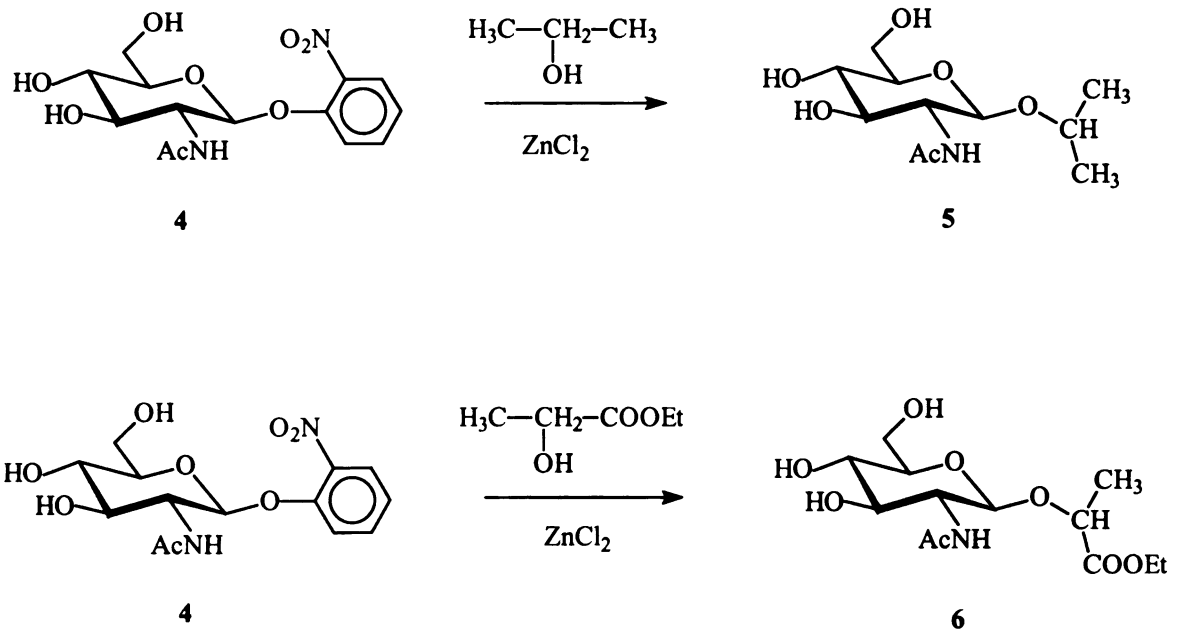
5.2. Results and Discussions

We have demonstrated that the *o*-nitrophenyl can act both as a protecting group of the anomeric center and as a leaving group, upon activation with a Lewis acid. The promoter used is $ZnCl_2$, which is a mild Lewis acid. This constitutes an advantage over many other highly toxic or highly unstable reagents. The *o*-nitrophenyl glycoside of NAG can easily be synthesized by the reaction of 2-acetamido-2-deoxy-3,4,6-tri-O-acetyl-α-D-glucopyranosylchloride **1** with *o*-nitrophenol **2** in the presence of a base like KH and 18-crown-6-ether (Scheme 5.2.1). The role of the crown ether is to complex the potassium cation so that the hydride is free and available to abstract the phenolic proton. The *o*-nitrophenylglycoside **3** is stable at room temperature and also in the presence of bases.



Scheme 5.2.1 Synthesis of nitrophenyl-2-amino-2-deoxy-glucoside.

This is a big advantage, considering the lability of the very often-used sugar halide donors and how easily they decompose in the presence of moisture or even in an improper solvent system. Under standard deacetylation conditions, **3** is converted to *o*-nitrophenyl-2-amino-2-deoxy-glucoside **4**, that has a long shelf stability. The suitability of the o-nitrophenyl group as a leaving group was evaluated by reacting glycoside **4** with two secondary alcohols: isopropanol and ethyl lactate (Scheme 5.2.2). Heating the solution of glycoside in excess alcohol at 70°C in the presence of $ZnCl_2$ afforded high yields of isopropyl-2-amino-2-deoxy-β-D-glucoside **5** and ethyl lactyl-2-amino-2-deoxy-β-D-glucoside **6** respectively. The β-isomer is formed exclusively. It can be concluded that *o*-nitrophenyl is a good protecting/activating group. It can easily be activated by

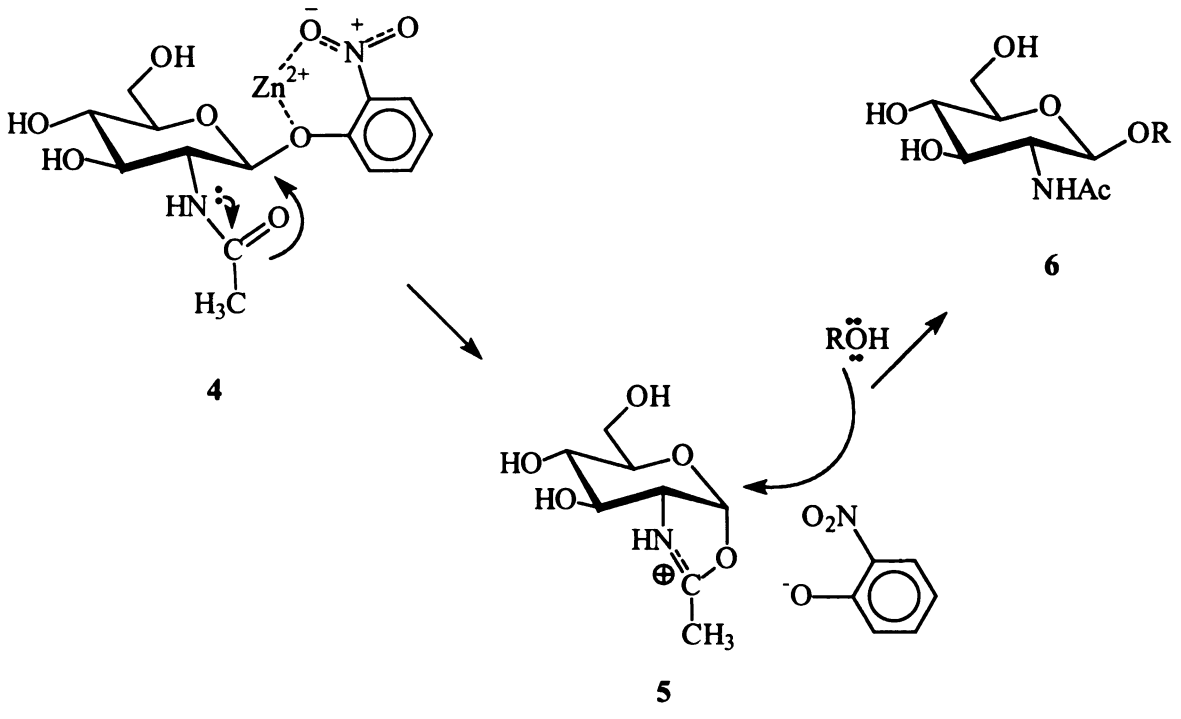


Scheme 5.2.2 Transglycosylation between o-nitrophenylglycosides and alcohols.

$ZnCl_2$ and substituted by primary and secondary alcohols but is not removed under alkaline conditions. The glycosylation reaction occurs in high yields, with total β-diastereospecificity. If the synthesis of the N-acetyl glycoside is desired, this procedure is advantageous since it employs acetyl as a starting protecting group. Thus, it circumvents the introduction of other miscellaneous protecting groups that need further deprotection, followed by N-acetylation, in order to obtain the desired product.

The mechanism that we propose for the transglycosylation reaction is depicted in Scheme 5.2.3. The acetamido functionality is a very good participating group, and after zinc chloride mediated removal of the *o*-nitrophenyl leaving group, the oxazolinium ion **7** is formed. Since the conditions of the reaction are acidic and Zn coordinates to both the

nitro- and phenyl hydroxyl groups, the oxazoline is not formed by abstraction of the proton from the nitrogen. This explains the absence of oxazoline byproducts. This cyclic intermediate formed has the α-face shielded, allowing the attack of the acceptor alcohol on the β-face only and thus affording the trans-glycoside **8** with total stereoselectivity.



Scheme 5.2.3 Mechanism of transglycosylation reaction.

5.3. Conclusions

Glycosylations are important reactions in carbohydrate chemistry. They are the key step in the synthesis of complex oligosaccharides and glycoconjugates. Since the outcome of the reaction is determined by the structure of the substrates, the nature of the substituents, and the desired stereochemistry in the products, it is not possible to use one generally applicable technique. 2-Amino-2-deoxy sugars do not form glycosides when treated with the conventional acid-alcohol mixtures, owing to the electrostatic shielding effect of the $-NH_3^+$ group, which prevents the approach of cationic groups to the glycosidic center. Glycoside formation takes place normally when the amino group is suitably blocked, as with the N-acetyl, N-(benzyloxycarbonyl) or phthalimido derivatives. If an N-acetyl-glucosamine glycoside is desired, the last two N-protecting groups mentioned need to be removed so that the amino group can be transformed into an acetamide. Only two procedures use acetyl protected glucosamine as starting material. The Koenigs-Knorr method has the disadvantage of using toxic silver or mercury salts and giving only low yields. The oxazoline method requires harsh reaction conditions, leading to migration and partial anomerization and affords satisfactory results only for primary alcohols. We developed a new glycosylation procedure where the leaving group is *o*-nitrophenyl. This provides good protection of the anomeric center against bases and can be activated with $ZnCl_2$ to render β-glucosamine glycosides in high overall yield.

5.4. Experimental section

For the subsequent reactions, all solvents were distilled in order to remove traces of water. The solid reactants were dried in a vacuum oven at room temperature. Potassium hydride was washed with hexane before weighing.

o-Nitrophenyl-2-acetamido-2-deoxy-3,4,6-tri-O-acetyl-β-D-glucoside (**3**)

In a 2 neck round bottom flask equipped with a dropping funnel and a nitrogen tube was added potassium hydride (12.8 g, 0.2 mol) as a 60% suspension in mineral oil, over dichloromethane (80 mL), keeping the suspension in an ice-water bath. To this, a solution of 18-crown-6 (26.5 g, 0.1 mol) and *o*-nitrophenol (28.0 g, 0.2 mol) in dichloromethane (100 mL) was and added through the dropping funnel over 1 h. The suspension was kept stirring for another 2 h, after which chloroglucosamine (36.6 g, 0.1 mol) was added and stirring was continued at room temperature for another 2 h. The reaction was quenched with glacial acetic acid (12 mL) during 0.5 h of mixing. For workup, 5% $NaHCO_3$ solution (1 L) was added and the product was extracted into the organic layer and dried on the rotary evaporator to give crude **3** (45.0 g), which was used without further purification in the deacetylation reaction.

o-Nitrophenyl-2-acetamido-2-deoxy-β-D-glucoside (**4**)

To a solution of **3** in methanol (1050 mL) and water (70 mL) potassium carbonate (55.0 g, 0.4 mol) was added and the suspension was stirred overnight at room temperature. After removal of the salts by filtration, the solution was rotary evaporated. More methanol was added to precipitate the excess salt which was then filtered off. By dissolving the crude reaction mixture in water and rotary evaporating it at 40°C, the *o*-

nitrophenol was removed. Recristallization from hot ethanol gave **4** (18.4 g) in a 54% overall yield starting from **1**. ^{1}H-NMR ($CDCl_3$) δ 1.85 (3H, s, NHAc), 3.48 (1H, t, J 8.31 Hz, H-4), 3.55 (1H, t, $J_{2,3}$=$J_{3,4}$ 8.61 Hz, H-3), 3.57 (1H, m, H-5), 3.72 (1H, dd, $J_{6a, 6b}$ 12.37 Hz, $J_{6a,5}$ 5.3 Hz, H-6a), 3.88 (1H, dd, $J_{6b,5}$ 2.43 Hz, H6-b), 3.92 (1H, dd, H-2), 5.11 (1H, d, $J_{1,2}$ 10.16 Hz, H-1), 7.17 (1H, t, J=8.62 Hz, H-c), 7.36 (1H, d, H-a), 7.57 (1H, t, J=8.63, H-b), 7.77 (1H, d, H-d).

Isopropyl-2-acetamido-2-deoxy-β-D-glucoside (**5**)

A suspension of *o*-nitrophenyl-2-acetamido-2-deoxy-β-D-glucoside (**4**) (0.10 g, 0.29 mmol) and $ZnCl_2$ (0.10 g, 0.70 mmol) in dry *i*-propanol (5 mL) was heated at 70°C, when the solution became homogeneous and stirring was continued for 22 h. The solvent was removed and the crude reaction mixture was dissolved in water and rotary evaporated to distill off the *o*-nitrophenol. Column chromatography with a chloroform-methanol (6:1 vol) eluent was used to remove the traces of $ZnCl_2$. The product **5** (0.068 g) was recovered in 95% yield. ^{1}H-NMR ($CDCl_3$); δ 1.10 (3H, d, J=6.1 Hz,CH_3), 1.16 (3H, d, CH_3), 2.01 (3H, s, $NHCH_3$), 3.40 (2H, m, H-6a, H-6b), 3.46 (1H, m, H-5), 3.56 (1H, dd, J 8.05 Hz, J 11.97 Hz, H-2), 3.62 (1H, dd, J 12.65 Hz, J 7.2 Hz, H-4), 3.71 (1H, dd, H3), 3.98 (1H, m, H-5), 4.56 (1H, d, H-1).

Ethyl lactyl-2-acetamido-2-deoxy-β-D-glucoside (**6**)

A suspension of *o*-nitrophenyl-2-acetamido-2-deoxy-β-D-glucoside (**4**) (0.10 g, 0.29 mmol) and $ZnCl_2$ (0.10 g, 0.70 mmol) in dry ethyl lactate (2 mL) was heated at 70°C, when the solution became homogeneous and stirring was continued for 20 h. The solvent was removed and the crude reaction mixture was dissolved in water and

rotary evaporated to distill off the *o*-nitrophenyl. Column chromatography with a chloroform-methanol (7:1 vol) eluent was used to remove the traces of $ZnCl_2$ and separate the product from the ethyl lactate dimmer. The product **6** (0.06 g) was recovered in 63.78 % yield. ^{1}H-NMR (D_2O); δ 1.05 (3H, t, J 7.08 Hz, CH_3), 1.14 (3H, d, J 6.84 Hz, CH_3), 1.78 (3H, s, NHAc), 3.03 (1H, m, H-5), 3.08, (1H, m, $J_{2,1}$ 8.06 Hz, $J_{NH,2}$ 1.71 Hz, H-2), 3.26 (1H, dd, $J_{2,3}$ 8.06 Hz, $J_{3,4}$ 10.25 Hz, H-3), 3.37 (1H, dd, H-4), 3.43 (1H, dd, $J_{6a,b}$ 11.97 Hz, $J_{6a,5}$ 5.61 Hz, H-6a), 3.65 (1H, dd, H-6b), 3.96 (2H, 2, J 7.08 Hz, H-d), 4.26 (1H, d, H-a), 4.28 (1H, 2, J 6.84 Hz, H-b); ^{13}C-NMR (D_2O) δ 8.7, 13.3, 17.7, 50.9, 56.0, 57.8, 65.2, 68.8, 69.0, 71.3, 95.6, 169.9, 170.0.

5.5. References

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Chapter 6

Approaches Towards the Total Synthesis of the Disaccharide Head-Group of Lipid A of *Rhizobium Trifolii*

Abstract

Lipopolysaccharides (LPS) are a unique class of glycolipids found on the surface of Gram-negative bacteria. LPS are capable of eliciting a wide array of biological responses when they interact with cellular systems of animals. Recently, the LPS from *Rhizobium Trifolii* has been isolated from bacterial extracts and its structure elucidated. The total synthesis of the disaccharide head-group of lipid A has been attempted in order to prove the originally assigned structure. The most successful method involves coupling of a trichloroacetimidate donor sugar with an acceptor benzyl glucosamine having an unprotected primary hydroxyl, followed by deprotection and radical oxidation. Three different approaches are described.

6.1. Introduction

Lipopolysaccharides (LPS) were first discovered by Richard Pfeiffer in 1892. He found that Gram-negative bacteria produce toxins that are distinct from the more commonly known exotoxins in that they were heat-stable and remained associated with the bacteria. Due to these unique properties, he called them endotoxins [1]. Since this first discovery, a tremendous amount of effort went into elucidating the chemical structure of endotoxins. It was found that they consisted of three regions. The O-antigen region is involved in the host-to-parasite interactions [2]. The distal core region serves as the linker arm connecting the O-antigen to the rest of the LPS molecule. The inner core region (rich in charged groups) appears to maintain the barrier properties of the outer membrane, and, finally, the lipid A is the hydrophobic moiety that anchors the LPS to the outer membrane.

Lipid A has a highly conserved structure. When the structure of this glycolipid from many bacterial sources is examined, certain common features are revealed. They appear to have the following: (1) β–(1-6)-linked disaccharide backbone containing amino sugars, (2) five to seven fatty acyl groups of which the sugar-linked acids are 3-hydroxy fatty acids, (3) acyloxyacyl fatty acids, and (4) one to two phosphate groups at the 1- and 4'-positions. The structural role of lipid A as a hydrophobic anchor is provided by the abundant fatty acyl groups attached to the sugar. The presence of the acyloxyacyl group further enhances the hydrophobicity of the lipid A. It is not known why a disaccharide would be needed instead of a monosaccharide. Perhaps a higher degree of hydrophobicity is required and can be achieved by a disaccharide, due to the greater capacity for acyl

groups. Amino sugars could provide the more stable amide bond with the fatty acids rather than the labile ester bond provided by neutral sugars. This might be an advantage for survival of bacteria. The reason for the presence of hydroxy fatty acids is not clear. They might be involved in intermolecular hydrogen bonding (i.e. with outer membrane proteins) to provide further stability to the outer membrane by cross-linking adjacent lipid A molecules via divalent cations. It appears that the entire lipid A molecule is important for biological activities, since alteration of the previously mentioned characteristics alter biological activity [3].

The pathophysiological effects of lipid A on the mammalian system have created great interest [4]. LPS is known to elicit a variety of pathological effects as a result of an adverse host inflammatory response [5] and lipid A is responsible for triggering these events [6]. Recently, nontoxic lipid A molecules were isolated from bacterial extracts and shown to exhibit LPS antagonistic properties [7, 8]. These properties are useful for investigations into the mechanisms of LPS action and have served as the basis for speculation about strategies for the possible therapeutic intervention in LPS-related disease states. It is tempting to develop structure-relationships among lipid A analogs as an aid toward understanding LPS action at the molecular level. However, such an approach critically depends upon firm structural information and supplies of homogeneous materials. Hollingsworth and Wang [9] have recently solved the structure of *Rhizobium Trifolii* lipid A. On the basis of degradation and spectral studies, they have suggested the structure depicted in Figure 6.1.1. Our attempt was the total synthesis of the head group of lipid A, in order to prove the initially assigned structure.

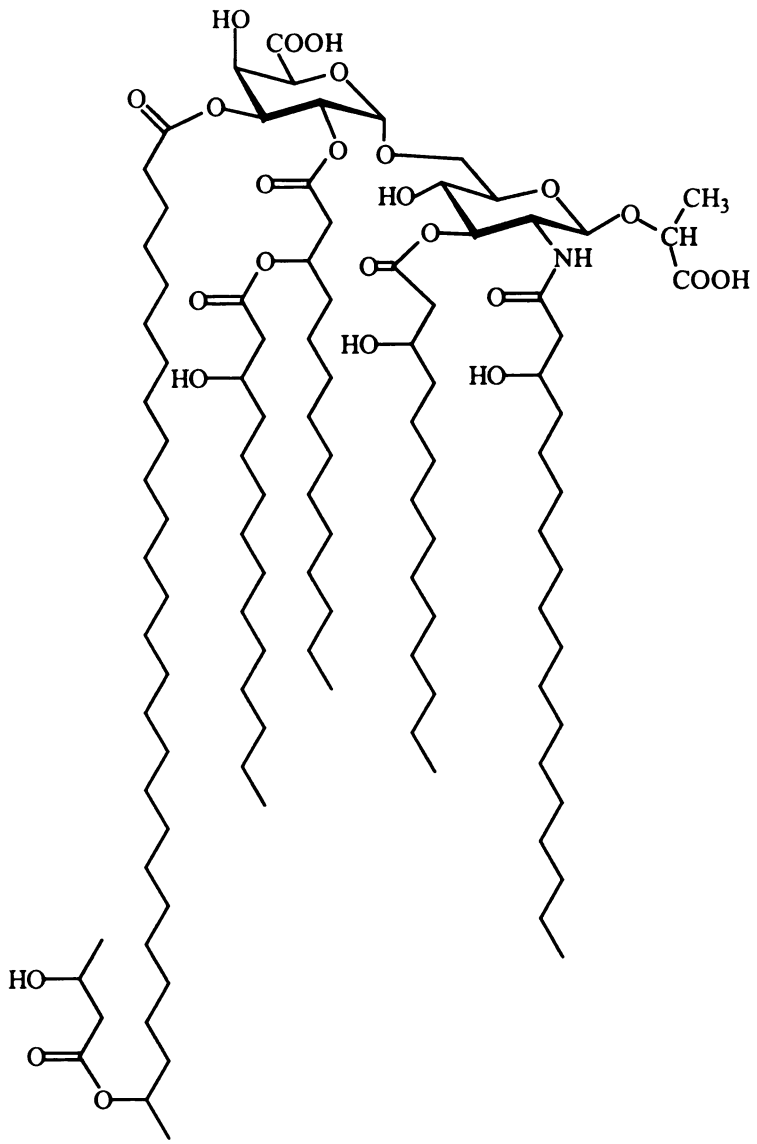


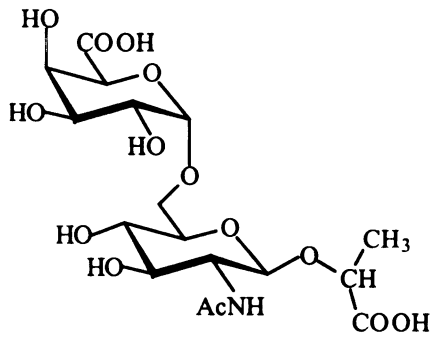
Figure 6.1.1 Structure of *Rhizobium Trifolii* lipid A.

6.2. Results and Discussions

Three attempts were made to prepare the lipid A head group **1** (Figure 6.2.1) and each will be described with their successes and failures.

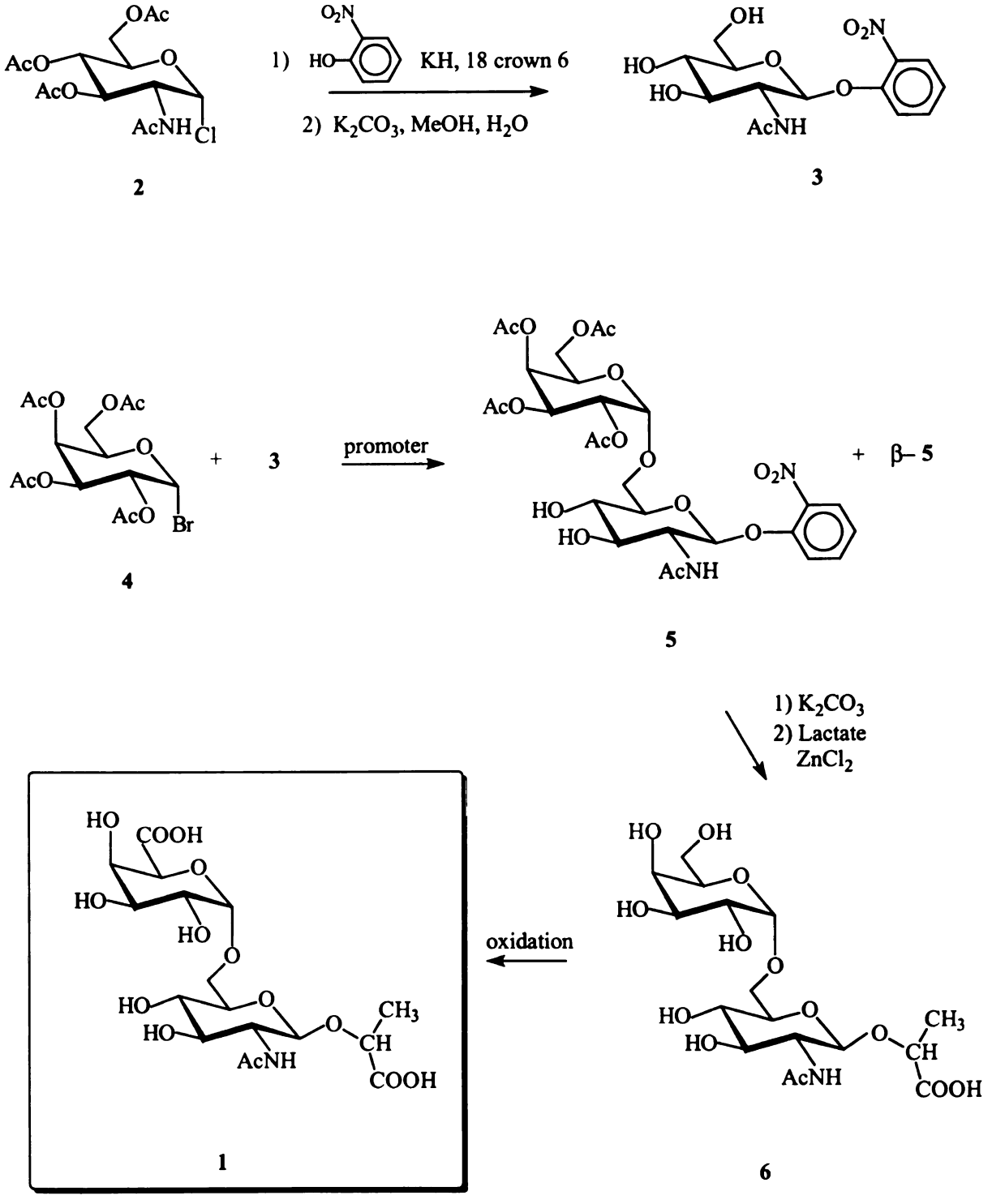
6.2.1. Method I

The first procedure was designed to take advantage of the new anomeric nitrophenyl activating group described in chapter 5. The idea was to use *o*-nitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside **3**, and react it with 2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl bromide **4** in the presence of a promoter, to obtain an α/β mixture of



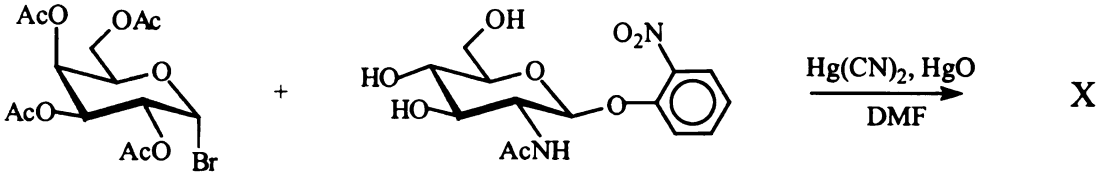
1

Figure 6.2.1 Assigned structure for the lipid A head-group of *Rhizobium Trifolii.*



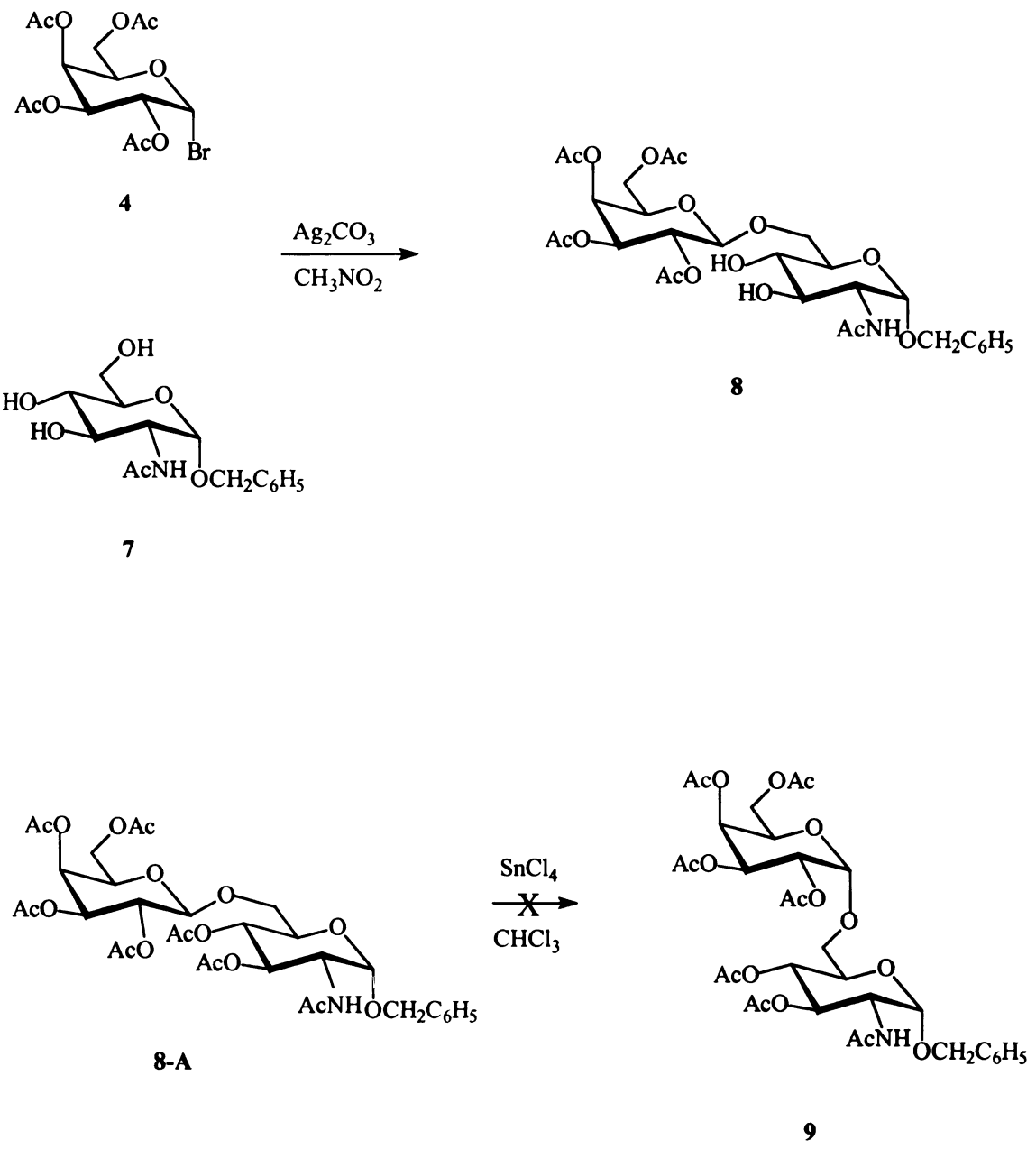
Scheme 6.2.1 First attempt at the synthesis of the disaccharide head-group of lipid A.

disaccharides **5**. The α–isomer would then be deacetylated and transglycosylated with lactic ethyl ester, to give disaccharide **6**, which would be oxidized to the final product **1**, according to Scheme 6.2.1. The synthesis of compound **3** occured in 65% overall yield and has been described in detail in chapter 5. It is known that the reactivity of the pyranose hydroxyl groups decreases in the order 6 OH >> 3 OH > 2 OH > 4 OH. Since primary hydroxyl groups are much more reactive than secondary ones, we expect the reaction of the unprotected nitrophenyl glycoside **3** with acetobromogalactose **4** to afford only the 1-6 disaccharide. Because the sugar acceptor is very polar, the only acceptable solvent for the reaction proved to be DMF. Unfortunately, acetobromogalactose decomposes under these conditions before it gets a chance to react with **3**. HgO and $Hg(CN)_2$ have been used as promoters, but only unreacted nitrophenyl glycoside and 2,3,4,6-tetra-O-acetyl galactose have been isolated from the product mixture (Scheme 6.2.2).



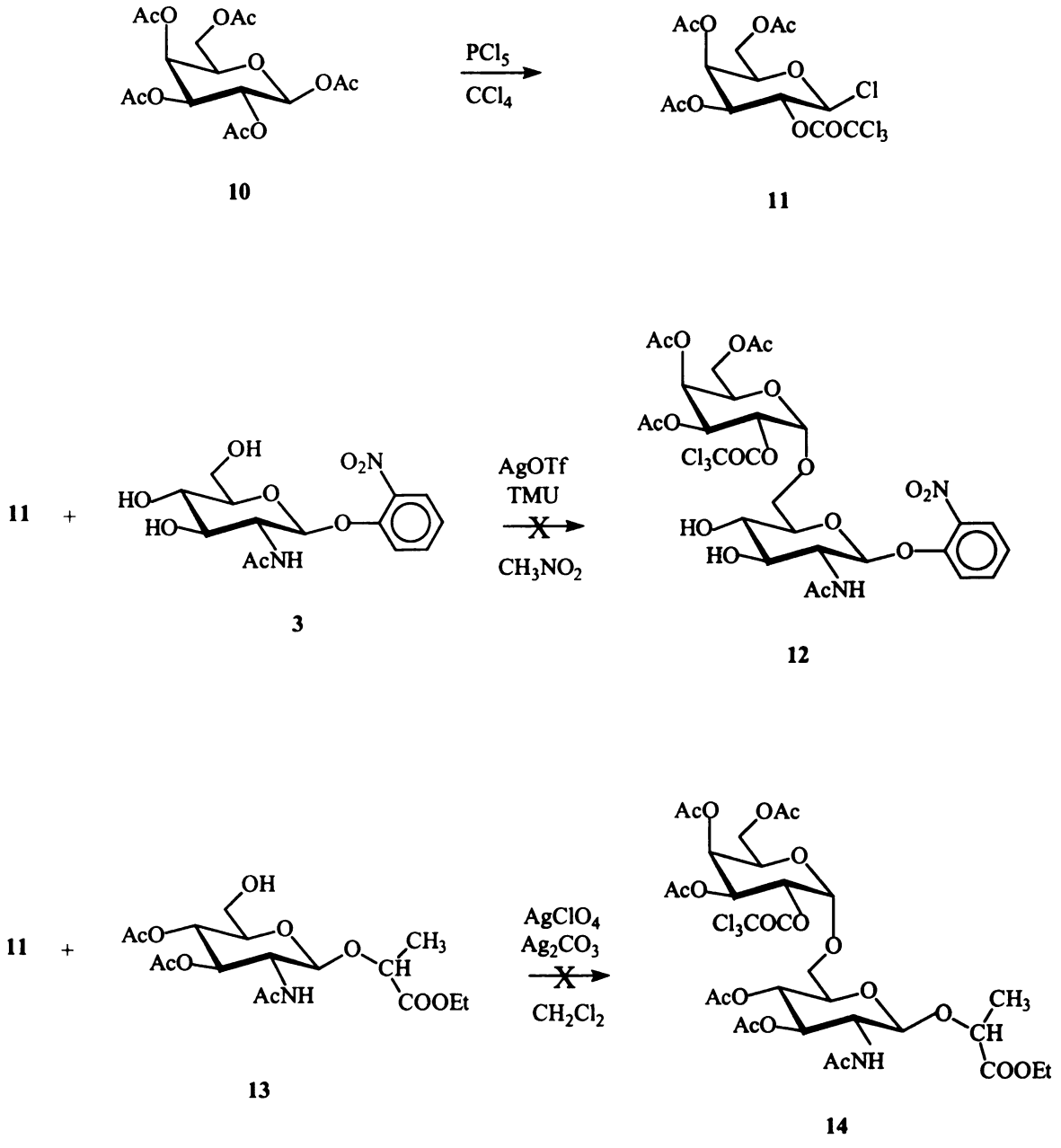
4 **3**

Scheme 6.2.2 Unsuccessful glycosylation between acetobromogalactose and o-nitrophenyl glucosamine.

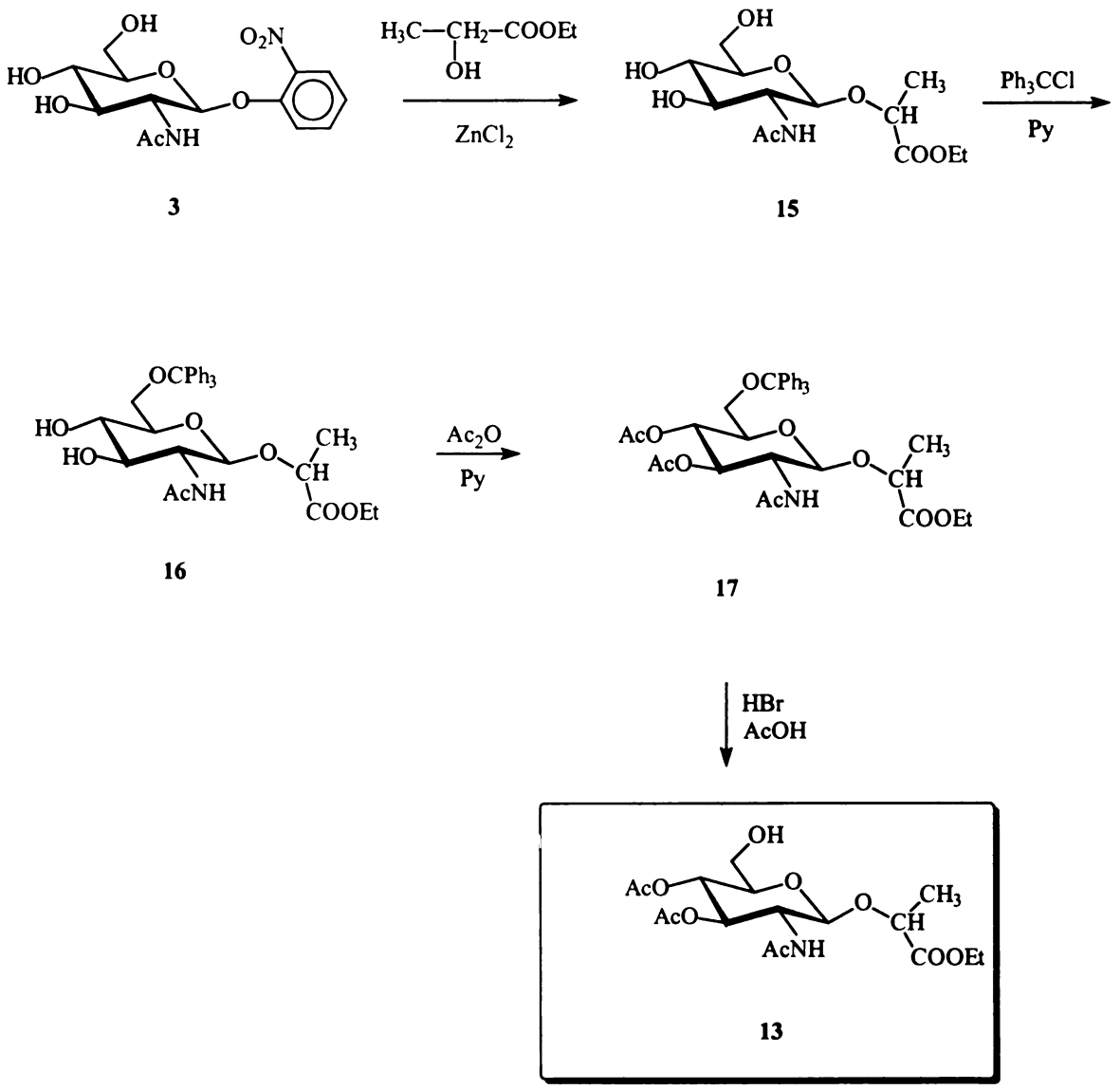


Scheme 6.2.3 Synthesis of β-disaccharide **8** and unsuccessful anomerization towards **9**.

Because of the difficulties described above, the use of a different acceptor molecule was considered. Benzyl 2-acetamido-2-deoxy-α-D-glucopyranoside **7** was reacted with the same sugar donor **4** in nitromethane and in the presence of silver carbonate [10], to give solely the β–disaccharide **8** in a 33% yield (Scheme 6.2.3). Other promoters such as $Hg(CN)_2$-HgO or $Hg(CN)_2$-Ag_2CO_3 were considered but no improvement in yield was obtained. In the first case, using a 3:1:1:2 ratio between **4**, **7**, $Hg(CN)_2$ and HgO the yield was 20%, while in the second case, a 24% yield was obtained using a 2.5:1:2:2 ratio of **4**, **7**, $Hg(CN)_2$ and Ag_2CO_3. We tried to invert the stereochemistry of the disaccharide linkage by refluxing the peracetylated disaccharide **8-A** in chloroform or THF in the presence of Lewis acids like $TiCl_4$, $ZnCl_2$, BF_3, $SnCl_2$, $SnCl_4$. However, unlike the anomerization of methyl 2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside [11], these reactions proved unsuccessful (Scheme 6.2.3). Since the β-stereoselectivity of the reaction is due to the activating effect of the C-2 acetyl group, we considered using a different donor molecule that has a less participating C-2 substituent, in order to favor α–stereoselectivity. The compound chosen was 3,4,6-tri-O-acetyl-2-O-trichloroacetyl-β-D-glucopyranosyl chloride **11**, obtained from pentaacetyl-β-D-galactoside **10** by treatment with PCl_5 in CCl_4 [12-14] (Scheme 6.2.4). In this case, the assisting effect of the trichloroacetyl group at C-2 is minimized by the inductive effect of the three chlorine groups. This new glycosyl donor was reacted first with the unprotected nitrophenyl glycoside **3** in the presence of AgOTf, and TMU [15] and also with the ethyl lactyl 3,4-tri-O-acetyl-2-acetamido-2-deoxy-β-D-glucoside **13**, in the presence of $AgClO_4$ and silver carbonate [16]. Unfortunately no product has been isolated, since **11** decomposed.



Scheme 6.2.4 Synthesis of glycosyl donor **11** and attempted glycosylation reactions.

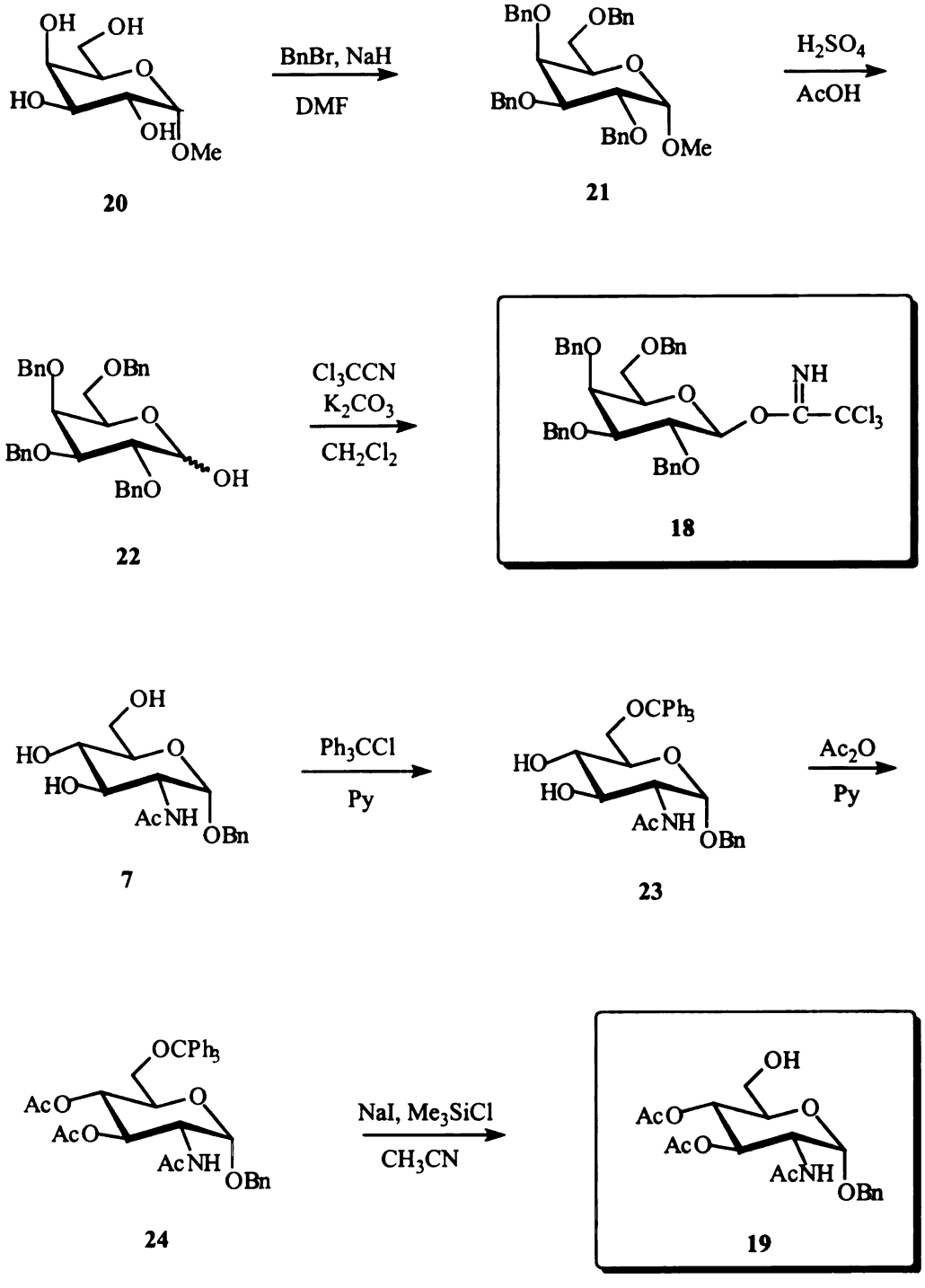


Scheme 6.2.5 Synthesis of lactyl 2-acetamido-2-deoxy-3,4-di-O-acetyl-β-D-gluco-pyranoside ethyl ester **13**.

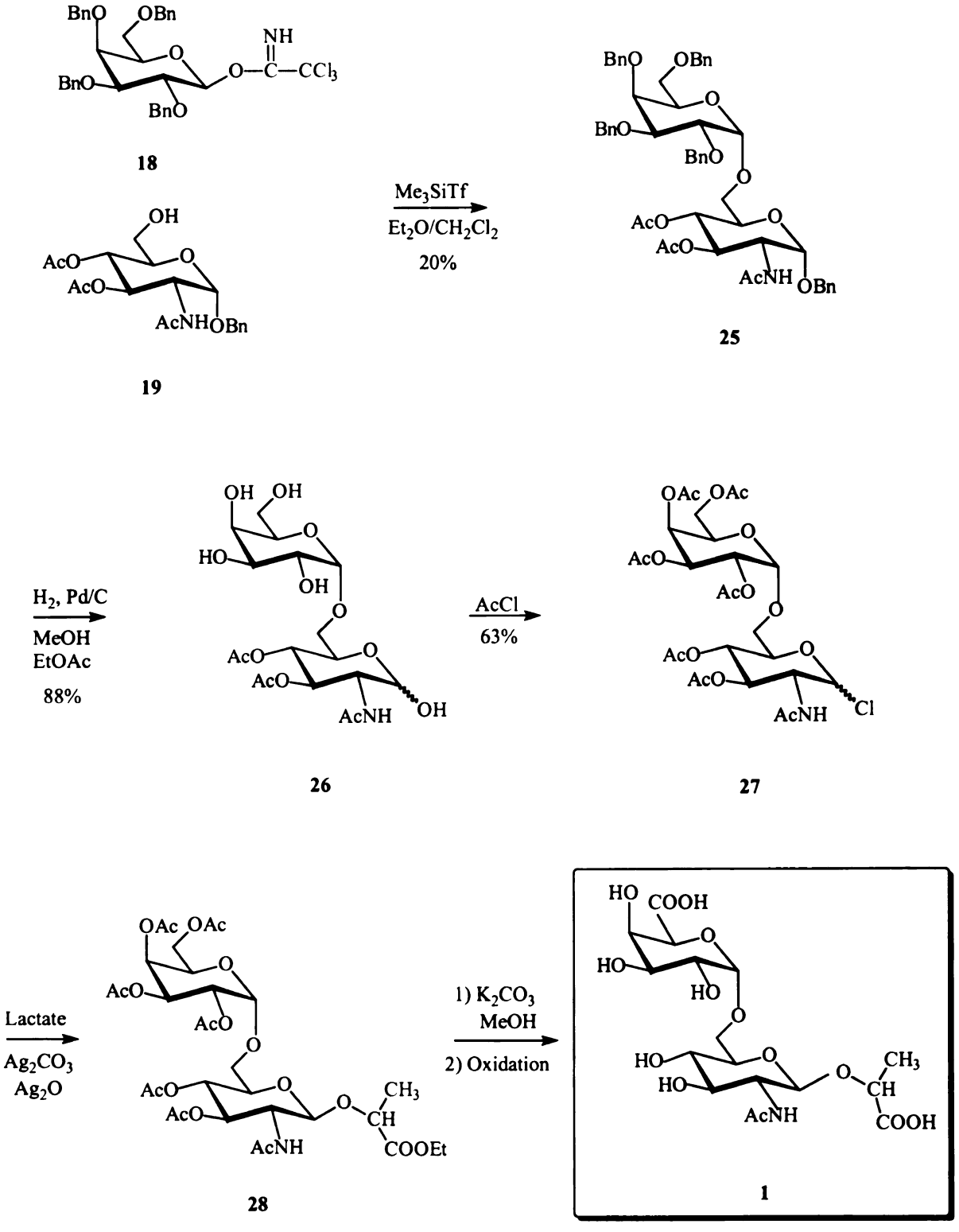
Compound **13** was synthesized (Scheme 6.2.5) from nitrophenyl glycoside **3**, which in ethyl lactate and in the presence of $ZnCl_2$ gives the β-lactyl glycoside **15**. Its primary hydroxyl group was protected by tritylation [17], followed by acetylation of the secondary hydroxyls. The intermediate **16** was not isolated because the one pot reaction gives a higher yield of **17**. Deprotection in the presence of HBr and acetic acid gave **13**. It is very important to conduct the detritylation very rapidly, otherwise the peracetylated side-product will predominate.

6.2.2. Method II

Because the former approach proved unsuccessful, a totally different strategy was used. 2,3,4,6-Tetra-O-benzyl-β-D-glycopyranosyl trichloroacetimidate **18** was chosen as donor, while benzyl-2-acetamido-2-deoxy-3,4-di-O-acetyl-β-D-glucopyranoside **19** served as acceptor. Scheme 6.2.6 summarizes their synthesis. Methyl α-D-galactopyranoside **20** was perbenzylated with benzyl chloride in the presence of sodium hydride and then the anomeric hydroxyl group deprotected [18] under acidic conditions. Reaction of benzyl hemiacetal **22** with trichloroacetonitrile in the presence of potassium carbonate gave trichloroacetimidate **18** in very good yield. The acceptor **19** was obtained from benzylglucosamine **7** after tritylation, acetylation and detritylation using NaI and trimethylsilyl chloride [19]. This new detritylation method proved to be much better than the one that involves HBr and AcOH, since peracetylation is avoided. It seems that a (trimethylsilyl)trityloxonium ion intermediate is formed, that is displaced by iodide. The trichloroacetimidate **18** was reacted with the benzyl glucosamine **19** in the presence of trimethylsilyl triflate [20] to give the α-disaccharide **25** in 20.3% yield after

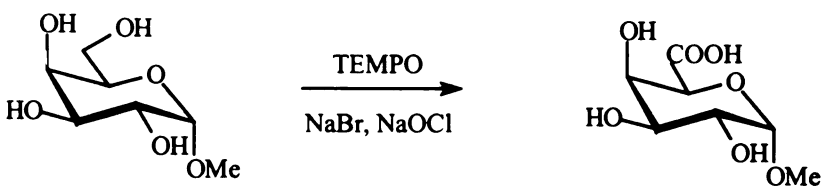


Scheme 6.2.6 Synthesis of donor **18** and acceptor sugar **19**.



Scheme 6.2.7 Second attempt at the synthesis of the disaccharide head-group of lipid A.

chromatographic separation (Scheme 6.2.7). Catalytic hydrogenolysis removed all the benzyl groups and also deprotected the anomeric center. Peracetylation with acetyl chloride was accompanied by activation of the reducing end of the disaccharide, affording **27.** This has been glycosylated with ethyl lactate in the presence of Ag_2O and Ag_2CO_3 to afford β-lactyl galactosyl glucoside **28**. Unfortunately, the total synthesis could not be completed because compound **28** was not isolated in sufficient amount to undergo deacetylation and oxidation. However, oxidation can easily be accomplished by using TEMPO (2,2,6,6-tetramethyl-1-piperidinyl-1-oxy) in the presence of NaBr and NaOCl [21]. TEMPO is a stable radical, which can be oxidized by several agents to give a nitosonium ion. The latter is a strong oxidant and shows selectivity towards primary hydroxyl functions over secondary ones. The reaction has been tested on methyl-α-D-galactopyranoside **20** and produced **29** in quantitative yield (Scheme 6.2.8).

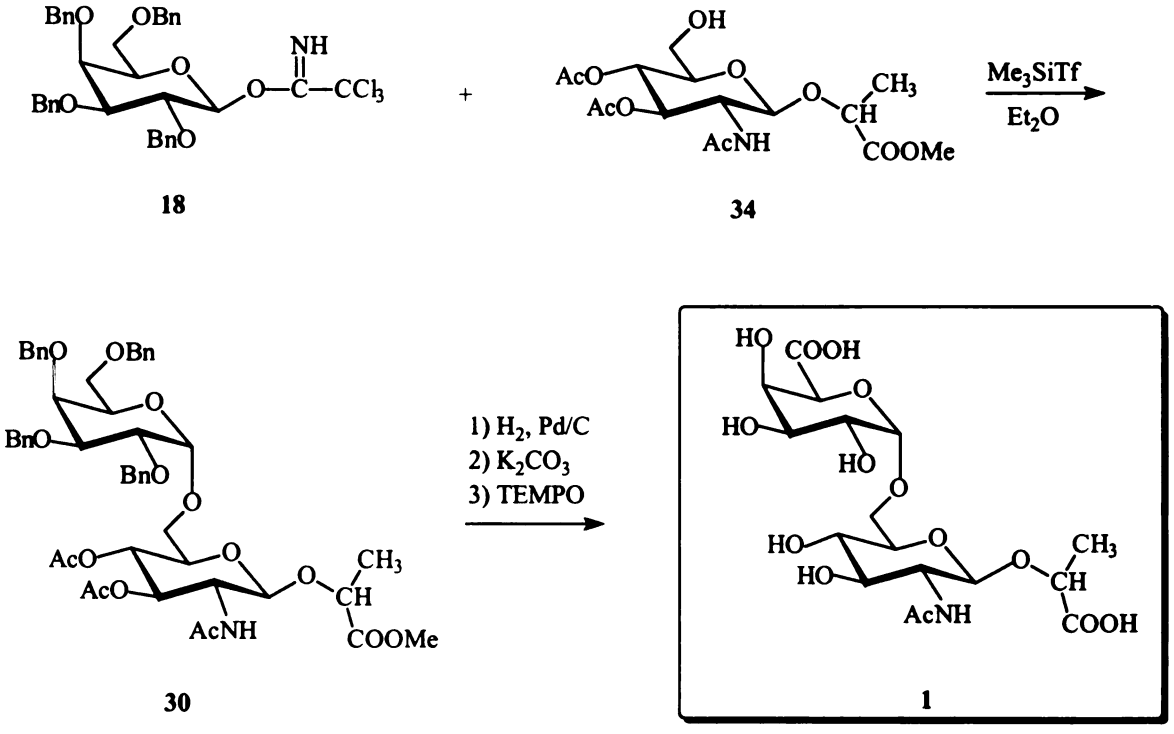


20 **29**

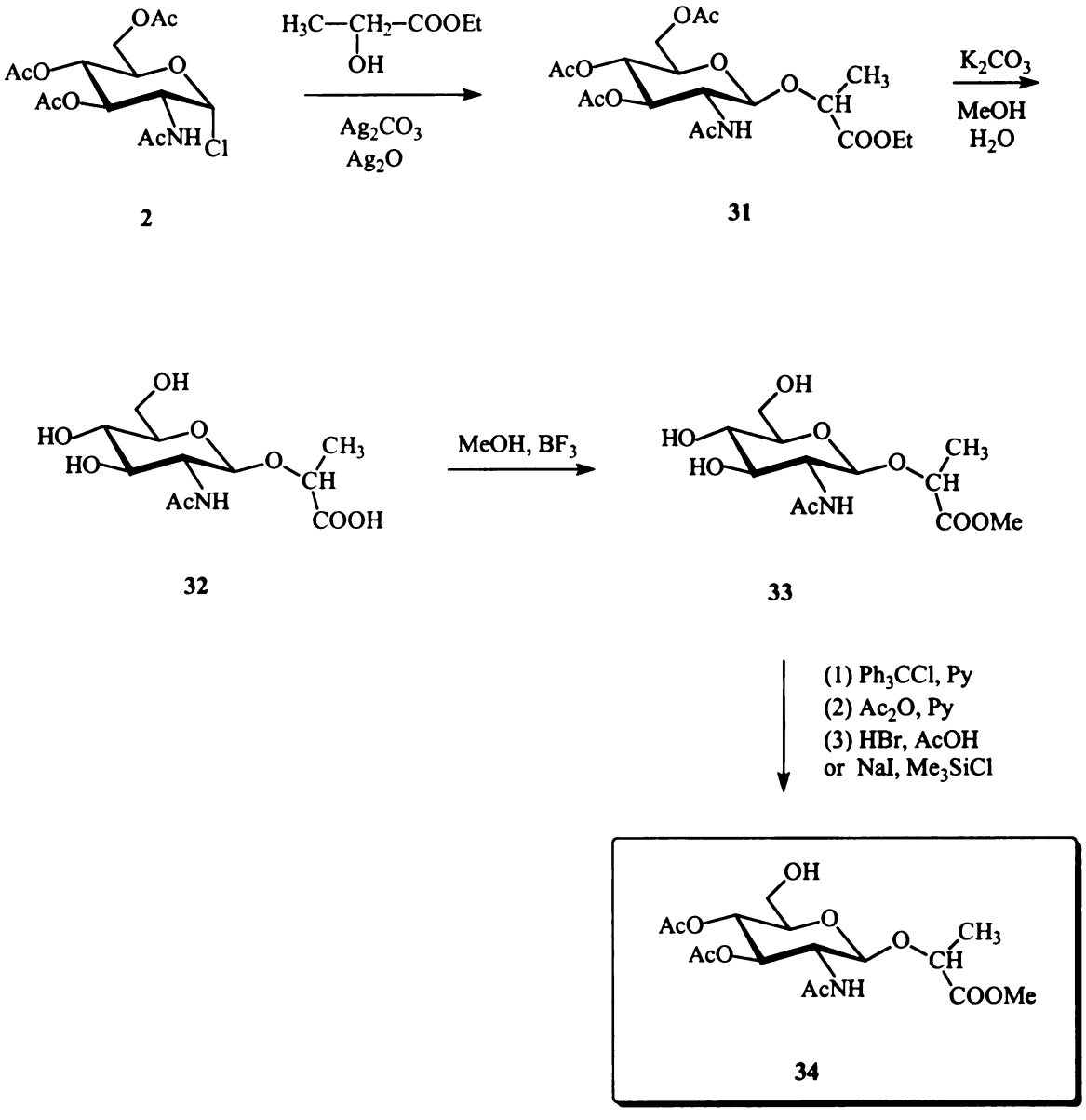
Scheme 6.2.8 Oxidation of methyl α-D-galactoside with TEMPO.

6.2.3. Method III

Since the glycosylation with ethyl lactate gives only low yields, it would be better if it would occur early in the synthesis, so that after multiple reaction steps, there will be enough material for charachterization at the end of the synthesis. The idea was to couple the trichloroacetimidate **18** with the lactyl glycoside **34** to give the α-disaccharide, followed by catalytic hydrogenation, deacetylation and oxidation (Scheme 6.2.9). A different approach for the synthesis of the sugar acceptor started from 2-acetamido-2-deoxy-3,4,6-tri-O-acetyl-α-D-glucopyranosyl chloride **2** which is transformed into the β-lactyl glycoside **31** in the presence of Ag_2CO_3 and Ag_2O. After deacetylation, the lactic acid is reesterified with MeOH and BF_3 to give **33**. Tritylation, peracetylation followed by detritylation with NaI affords **34**. The rest of the synthesis is still to be completed.



Scheme 6.2.9 Third attempt at the synthesis of the disaccharide head-group of lipid A.



Scheme 6.2.10 Synthesis of lactyl 2-acetamido-2-deoxy-3,4-di-O-acetyl-β-D-gluco-pyranoside methyl ester **33**.

6.3. Conclusions

Lipopolysaccharides are a unique class of glycolipids found on the surface of Gram-negative bacteria. LPS are capable of eliciting a wide array of biological responses when they interact with cellular systems of animals. Recently, the structure of lipid A of *Rhizobium Trifolii* has been determined by degradation of bacterial extracts and spectroscopic studies. Its headgroup consists of a galacturonic acid 1(α)-6 linked to glucosamine. The reducing end of the glucosamine is coupled to lactic acid. We attempted to chemically synthesize this disaccharide. In one method we tried to couple tetraacetobromogalactose to an *o*-nitrophenyl glucosamine acceptor. The high polarity of the acceptor molecule and the β-orienting effect of the donor made this synthesis unsuccessful. A different approach used tetrabenzyl β-D-galactopyranosyl trichloro-acetimidate as a nonparticipating donor while benzyl 2-acetamido-2-deoxy-3,4-di-O-acetyl-α-D-glucopyranoside with only the 6-hydroxyl group unprotected was the acceptor. The α-disaccharide was produced, but glycosylation with lactic ethyl ester proved to be a very low yield reaction, preventing us from reaching the final target.

6.4. Experimental section

o-Benzy,-6-O-(2,3,4,6-tetra-O-acetyl-α–D-galactopyranosyl)-2-acetamido-2-deoxy-β-D-glucopyranoside (**8**)

In a 25 mL stoppered round bottom flask, dried benzyl 2-acetamido-2-deoxy-α–D-glucopyranoside **7** (0.10 g, 0.32 mmol) was dissolved in dry nitromethane (6 mL). Silver carbonate (0.22 g, 0.79 mmol) and calcium sulphate (0.50 g) were added and the suspension was stirred in the dark at room temperature for 5 h. A solution of bromo

2,3,4,6-tetra-O-acetyl-α-D-galactopyranoside **4** (0.39 g, 0.95 mmol) dissolved in nitromethane (0.5 mL) was added at 60°C over a period of 10 min., after which the temperature returned to room temperature. Stirring was continued for 48 h. The suspension was filtered through Celite and washed several times with chloroform and acetone. The solution was rotary evaporated and the crude product stirred with chloroform. The disaccharide and unreacted or decomposed bromoacetogalactose dissolved in chloroform while the benzylglucosamine precipitated and was filtered off. The solution was rotary evaporated and the product was isolated by flash column chromatography, using 5:1 (vol) eluent of acetone-hexane as the eluent. After evaporating the fractions, **8** (0.07 g) was obtained in 33.35% yield. ^{1}H-NMR ($CDCl_3$) δ 1.95 (3H, s, OAc), 2.01 (3H, s, NHAc), 2.02 (3H, s, OAc), 2.09 (3H, s, OAc), 2.14 (3H, s, OAc), 3.45 (1H, m, H-5), 3.65 (1H, m, H-5'), 3.70 (1H, dd, H-6a), 3.75 (1H, dd, H-6b), 3.76 (1H, m, H-2), 3.90 (1H, t, $J_{2,3}$ 6.7 Hz, H-3), 4.03 (1H, t, $J_{3,4}$ 6.7 Hz, H-4), 4.10 (1H, dd, $J_{6a,5}$ 6.98 Hz, $J_{6a,6b}$ 11.16 Hz, H-6a'), 4.16 (1H, dd, $J_{6b,5}$ 7.26 Hz, H-6b'), 4.41 (1H, d, J 11.72 Hz, H-bn), 4.54 (1H, d, J 7.82 Hz, H-1'), 4.71 (1H, d, J 11.72 Hz, H-bn), 4.83 (1H, d, J 3.63 Hz, H-1), 5.00 (1H, dd, $J_{3,2}$ 10.3 Hz, $J_{3,4}$ 3.35 Hz, H-3'), 5.21 (1H, dd, H-2'), 5.35 (1H, dd, H-4'); ^{13}C-NMR ($CDCl_3$) δ 20.4, 20.4, 20.5, 20.6, 23.1, 53.4, 61.1, 66.9, 68.6, 68.9, 69.2, 69.4, 70.4, 71.6, 74.0, 96.0, 101.6, 128.0, 128.2, 128.4, 137.0, 170.0, 170.3, 170.4, 170.9, 172.0.

3,4,6-tri-O-acetyl-2-O-trichloroacetyl-β-D-glucopyranosyl chloride (**11**)

A suspension of β-pentaacetylgalactose (3.0 g, 7.6 mmol), phosphorus pentachloride (6.0 g, 28.0 mmol) and carbon tetrachloride (1.5 mL, 15.5 mmol) was refluxed for 5 h. The

mixture was poured into ice and water (30 mL) and extracted three times with dichloromethane (10 mL). The combined organic extracts were successively washed with water, saturated aqueous $NaHCO_3$ solution and then again with water. After drying over Na_2SO_4, the solution was filtered and the filtrate was evaporated under reduced pressure at 40°C to give a residue that was subjected to column chromatography. The eluent was a 1:11 solution of EtOAc-$CHCl_3$. After recrystallization from ether, **11** (0.36 g) was obtained in 10.33% yield. ^{1}H-NMR ($CDCl_3$) δ 1.99 (3H, s, OAc), 2.07 (3H, s, OAc), 2.20 (3H, s, OAc), 4.07 (1H, dd, J 6.59 Hz, H 7.08 Hz, H-6a), 4.19 (1H, d, H-6b), 4.19 (1H, d, J 6.59 Hz, H-1), 5.18 (1H, m H-5), 5.40 (1H, t, H-2), 5.41 (1H, dd, H-3), 5.48 (1H, dd, J 3.17 Hz, H-4); ^{13}C-NMR ($CDCl_3$) δ 20.1, 20.4, 20.5, 60.9, 66.7, 70.2, 74.6, 75.1, 86.7, 160.3, 169.3, 169.8, 170.1.

(D-ethyl propionyl) 2-acetamido-2-deoxy-3,4-di-O-acetyl-6-triphenylmethyl-β-D-glucopyranoside (**17**)

In a round bottom flask, lactyl glucosamine **15** (1.34 g, 4.17 mmol) was dissolved in pyridine (7.5 mL). Triphenyl methylchloride (1.22 g, 4.30 mmol) and dimethyl aminopyridine (DMAP) (0.046 g, 0.370 mmol) were added, and the solution was stirred at 40°C for 14 h. **16** was obtained, and used without isolation in the following reaction. After cooling the reaction mixture to room temperature, acetic anhydride (1.9 mL) was added and stirring was continued for another 2 h. The solution was poured slowly and with stirring over ice and water (100 mL), extracted with chloroform, washed several times with ice/water and dried with Na_2SO_4. The chloroform layer was rotary evaporated and the product was recrystallized from pure ethanol to give **17** (2.27 g) in an 84.34%

total yield. ^{1}H-NMR ($CDCl_3$) δ 1.30 (3H, t, J 7.33 Hz, OEt), 1.52 (3H, d, J 7.08 Hz, $OCHCH_3$), 1.72 (3H, s, NHAc), 2.00 (3H, s, OAc), 2.02 (3H, s, OAc), 3.08 (1H, dd, $J_{6a,b}$ 10.74 Hz, $J_{6a,5}$ 4.88 Hz, H-6a), 3.23 (1H, dd, $J_{6b,5}$ 2.24 Hz, H-6b), 3.49 (1H, m, H-5), 4.06 (2H, q, OCH_2), 4.21 (1H, dd, J 8.31 Hz, J 8.06 Hz, H-2), 4.48 (1H, q, $OCHCH_3$), 4.57 (1H, d, H-1), 5.06 (1H, t, J 9.77 Hz, H-3), 5.17 (1H, t, H-4), 5.94 (1H, d, NH), 7.26 (3H, t, H-ph), 7.44 (1H, d, H-ph).

(2-Ethyl propionyl) 2-acetamido-2-deoxy-3,4-di-O-acetyl-β-D-glucopyranoside (**13**)

The tritylated lactylglucosamine **17** (2.27 g, 3.50 mmol) was dissolved on a steam bath in glacial acetic acid (13.6 mL). The clear solution was cooled to 10°C and HBr (1.5 mL) was added, shaking the flask for 40 seconds. The trityl alcohol fell out of solution and the solution was filtered immediately through Celite and washed with acetic acid into a filter flask that contained ice and water (100 mL). The detritylated product was extracted with chloroform, washed with water several times, dried with Na_2SO_4 and rotary evaporated at 35°C. Chromatographic separation using a 1:1 eluent of chloroform-ethyl acetate gave **13** (0.3 g) in a 21.4% yield. ^{1}H-NMR ($CDCl_3$) δ 1.26 (3H, t, J 7.08 Hz, OCH_2CH_3), 1.40 (3H, d, J 6.84 Hz, $OCHCH_3$), 1.94 (3H, s, NHAc), 2.00 (3H, s, OAc), 2.01 (3H, s, OAc), 32.14 (3H, s, OAc), 3.46 (1H, m, H-5), 3.67 (1H, dd, H-6a), 3.9 (1H, dd, J 1.95 Hz, J 12.69 Hz, H-6b), 3.95 (2H, q, OCH_2CH_3), 4.16 (1H, m, H-2), 4.39 (1H, q, $OCHCH_3$), 4.58 (1H, d, J 7.57 Hz, H-1), 4.99 (1H, t, J 9.77 Hz, H-3), 5.15 (1H, t, J 10.50 Hz, H-4), 6.29 (1H, d, J 7.81 Hz, NH); ^{13}C-NMR ($CDCl_3$) δ 14.1, 18.5, 20.6, 70.7, 23.3, 54.2, 61.4, 62.0, 68.2, 72.2, 72.4, 73.3, 99.8, 127.2, 127.9, 143.1, 170.8, 170.9, 173.1.

Methyl 2,3,4,6-tetra-O-benzyl-α–D-galactopyranoside (**21**)

NaH in mineral oil (6.72 g, 0.28 mol) was added to a 1000 mL round bottom flask. After washing 5 times with hexane to remove the mineral oil, anhydrous DMF (200 mL) was added and the suspension was stirred at 60°C for 45 min. The methyl galactoside **20** (7.76 g, 0.04 mol) was added and the reaction mixture stirred for 3 h, lowering the temperature gradually to 15°C. Benzyl bromide (23.75 mL, 0.2 mol) was slowly added from a dropping funnel with the flask cooled in an ice-water bath. The temperature of the reaction mixture was increased to 40°C and stirring continued overnight. The solvent was rotary evaporated and the oily mixture separated by flash chromatography using 10:1 chloroform-hexane as the eluent. **21** (19.9 g) was obtained in 90.5% yield as a yellowish syrup. ^{1}H-NMR ($CDCl_3$) δ 3.37 (3H, s, OCH3), 3.52 (1H, d, J 6.50 Hz, H-2), 3.91 (1H, dd, H-6a), 3.92 (1H, m H-5), 3.95 (1H, m, H-3), 4.04 (1H, dd, J 11.03 Hz, J 3.42 Hz, H-6b), 4.39 (1H, d, J 11.72 Hz, H-bn), 4.48 (1H, d, H-bn), 4.56 (1H, d, J 3.42 Hz, H-4), 4.58 (1H, d, J 11.47 Hz, H-bn), 4.69 (1H, d, H-1), 4.69 (1H, d, J 12.2 Hz, H-bn), 4.74 (1H, d, H-bn), 4.84 (1H, d, J H-bn), 4.85 (1H, d, H-bn), 4.95 (1H, d, H-bn), 7.30 (1H, d, H-bn); ^{13}C-NMR ($CDCl_3$) δ 55.0, 69.2, 73.2, 73.4, 74.9, 75.2, 78.8, 78.9, 79.0, 79.2, 98.9, 127.6, 127.8, 128.1, 137.9, 138.4, 138.5, 138.7.

2,3,4,6-Tetra-O-benzyl-α-D-gacactopyranoside (**22**)

Methyl 2,3,4,6-tetra-O-benzyl-α–D-galactopyranoside (**21**) (17 g, 0.03 mol) was dissolved in glacial acetic acid (315 mL) and heated at 100°C [22]. A solution of 2N H_2SO_4 (68 mL) was added and refluxed for 1 h. A second portion of sulfuric acid was added and the solution was left at 100°C with stirring, overnight. The reaction mixture

was cooled and poured into water (2.5 L) and left at RT for 2 days. After extraction with chloroform and drying, **22** (16.2 g) was obtained in 100% yield. ^{13}C-NMR ($CDCl_3$) δ 55.7, 69.4, 69.5, 73.5, 73.7, 73.8, 75.1, 75.4, 78.0, 79.3, 99.1, 127.8, 127.9, 128.0, 128.1, 128.4, 128.5, 128.6, 128.6, 128.7, 130.4, 138.2, 138.8, 138.9, 139.1.

2,3,4,6-Tetra-O-benzyl-α-D-galactopyranosyl trichloroacetimimdate **(18)**

To a solution of **22** (9.0 g, 0.016 mol) in dry CH_2Cl_2, K_2CO_3 (8.65 g) and Cl_3CCN (8.65 mL) were added [23]. The suspension was vigorously stirred at room temperature for 17 h. The reaction mixture was filtered through Celite and washed with chloroform. The mother liquors were rotary evaporated and **18** (11.4 g) was obtained as a yellow syrup in quantitative yield. ^{13}C-NMR ($CDCl_3$) δ 66.2, 68.0, 72.9, 73.2, 73.3, 74.2, 74.6, 75.1, 77.9, 82.0, 98.6, 127.4, 127.6, 127.7, 127.7, 127.9, 128.0, 128.1, 128.3, 128.4, 137.7, 138.1, 138.3, 161.4.

Benzyl 2-acetamido-2-deoxy-3,4-di-O-acetyl-6-O-trityl-α-D-glucopyranoside **(24)**

Benzyl 2-acetamido-2-deoxy-α-D-glucopyranoside **7** (10.0 g, 32.0 mmol), DMAP (0.35 g, 2.80 mmol) and Ph_3CCl (10.0 g, 35.9 mol) were dissolved in pyridine (50 mL) and the resulting solution was stirred at 40°C for 48 h. The reaction mixture was cooled and after addition of acetic anhydride (30 mL), stirring was continued at room temperature for 4 h. The solution was poured over ice and water (800 mL) with strong agitation. The crude product was extracted into chloroform and the chloroform layer dried over Na_2SO_4. The mixture of **24** and peracetylated benzylgalactoside was not purified any more, but used in the following reaction without purification. ^{1}H-NMR ($CDCl_3$) δ 1.93 (6H, s, OAc), 2.06

(3H, s, NHAc), 3.32 (1H, dd, $J_{6a,b}$ 10.01 Hz, $J_{6a,5}$ 5.86 Hz, H-6a), 3.40 (1H, dd, $J_{6b,5}$ 2.93, H-6b), 3.55 (1H, t, $J_{3,4}$ 8.79 Hz, $J_{4,5}$ 9.77 Hz, H-4), 3.72 (1H, dd, $J_{3,2}$ 10.5 Hz, H-3), 3.86 (1H, m, H-5), 4.15 (1H, m, $J_{2,NH}$ 8.78 Hz, $J_{2,1}$ 3.91 Hz, H-2), 4.50 (1H, d, J 11.72 Hz, H-bn), 4.83 (1H, d, H-bn), 4.95 (1H, d, J 3.91, H-1), 6.23 (1H, d, NH), 7.28 (20H, m, H-bn); ^{13}C-NMR ($CDCl_3$) δ 23.1, 53.4, 63.5, 69.0, 71.0, 71.8, 73.3, 81.6, 86.4, 96.3, 124.0, 126.0, 172.0.

Benzyl 2-acetamido-2-deoxy-3,4-di-O-acetyl-α-D-glucopyranoside (**19**)

Crude **24** (12.4 g, 19.4 mmol) was dissolved in acetonitrile (145 mL). After addition of NaI (5.1 g, 34.0 mmol) and Me_3SiCl (4.37 mL, 34 mmol), the solution was stirred at room temperature for 15 min. Water (115 mL) was added and the mixture stirred for another 15 minutes at 0°C. The precipitated trityl alcohol was removed by filtration and the filtrate extracted with CH_2Cl_2, after which the organic phase was washed with aqueous 10% sodium thiophosphate. The aqueous phase was washed 2 times with dichloromethane, and then the combined CH_2Cl_2 layers were rotary evaporated. Chromatographic separation with 4:1 hexane-acetone gave pure **19** (3.7 g) in 50.4% total yield. ^{1}H-NMR ($CDCl_3$) δ 1.87 (3H, s, OAc), 1.99 (3H, s, OAc), 2.02 (3H, s, NHAc), 3.56 (1H, dd, $J_{6a,b}$ 12.69 Hz, $J_{6a,5}$ 4.15, H-6a), 3.61 (1H, dd, $J_{6b,5}$ 2.2 Hz, H-6b), 3.77 (1H, m, H-5), 4.31 (1H, ddd, H-2), 4.49 (d, 1H, J 11.72 Hz, H-bn), 4.70 (1H, d, H-bn), 4.92 (1H, d, $J_{1,2}$ 3.67, H-1), 5.04 (1H, t, J 10.01 Hz, H-4), 5.26 (1H, t, H-3), 5.78 (1H, d, J 9.52 Hz, NH), 7.3 (5H, m, H-bn); ^{13}C-NMR ($CDCl_3$) δ 20.5, 20.6, 23.0, 51.8, 60.8, 68.4, 69.9, 70.0, 70.9, 96.4, 128.0, 128.2, 128.5, 136.5, 169.9, 170.2, 171.2.

Benzyl-6-O-(2,3,4,6-tetra-O-benzyl-α-D-galactopyranosyl)-2-aceatamido-2-deoxy-3,4-di-O-acetyl-α-D-glucopyranoside (**25**)

The trichloroacetimidate donor **18** (1.4 g, 3.5 mmol) and the acceptor sugar **19** (5.0 g, 7.3 mmol) were dissolved in a mixure of diethylether (30 mL) and dichloromethane (5 mL). Me_3SiTf (0.3 mL, 1.6 mmol) was added and the solution stirred at RT for 10 h. The reaction mixture was treated with excess solid $NaHCO_3$ and then with ether/$NaHCO_3$ solution in water. The ether extract was washed with water, dried with Na_2SO_4 and then concentrated. The crude product was separated by chromatography with a 3:1 hexane-acetone eluent. Pure **25** (0.65 g) was obtained in 20.21 % yield. ^{1}H-NMR ($CDCl_3$) δ 1.89 (3H, s, OAc), 1.96 (3H, s, OAc), 2.00 (3H, s, NHHc), 3.46 (1H, dd, J6a,b 10.7 Hz, J6a,5 2.0 Hz, H-6a), 3.50 (1H, d, J 6.35 Hz, H-4'), 3.74 (1H, dd, J 10.98 Hz, H-3'), 3.80 (1H, m, H-5), 3.95 (1H, dd, J 2.69 Hz, H-6a'), 3.97 (1H, m, H-5'), 4.07 (1H, dd, J 4.2 Hz, J 10.6 Hz, H-6b'), 4.30 (1H, dd, J 3.66, H-2), 4.37 (1H, d, J 11.72 Hz, H-bn), 4.47 (1H, d, J 11.96 Hz, H-bn), 4.57 (1H, d, J 11.47 Hz, H-bn), 4.67 (1H, d, J 11.48 Hz, H-bn), 4.71 (1H, d, J 9.52 Hz, H-bn), 4.79 (1H, d, H-2'), 4.81 (1H, d, J 3.42 Hz, H-1'), 4.85 (1H, d, J 3.66 Hz, H-1), 4.93 (1H, d, J 11.48 Hz, H-bn), 5.09 (1H, t, J 9.77 Hz, H-4), 5.22 (1H, t, H-3), 5.64 (1H, d J 9.53 Hz, NH); ^{13}C-NMR ($CDCl_3$) δ 20.5, 20.6, 23.0, 51.6, 66.3, 68.6, 68.7, 68.8, 69.4, 69.5, 71.5, 73.0, 73.3, 74.6, 75.0, 76.2, 78.6, 95.9, 97.6, 127.3, 127.4, 127.6, 127.6, 127.7, 128.1, 128.2, 128.3, 124.4, 128.5, 136.4, 137.8, 138.5, 138.6, 169.5, 169.4, 171.3.

6-O-(α-D-galactopyranosyl)-2-aceatamido-2-deoxy-3,4-di-O-acetyl-α-D-glucopyranoside (**26**)

In a Parr reactor, benzyl disaccharide **25** (0.65 g, 0.7 mmol) was dissolved in a mixture of ethyl acetate (50 mL) and methanol (50 mL), to which Pd/C (0.15 g) was added. The suspension was stirred at room temperature and 200 psi H_2 for 18 h. The reaction mixture was filtered through Celite and the filtrate rotary evaporated to give crude product. Recristallization from ethanol afforded pure **26** (0.35 g) in 88.40 % yield. ^{13}C-NMR ($CDCl_3$) δ 20.6, 20.7, 53.0, 62.5, 66.5, 68.7, 70.1, 70.9, 72.2, 72.5, 78.8, 79.1, 79.6, 92.3, 100.0, 171.7, 179.9.

6-O-(2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl)-2-aceatamido-2-deoxy-3,4-di-O-acetyl-α-D-glucopyranosyl chloride (**27**)

In a 25 mL round bottom flask equipped with a condenser and drying tube, the dry disaccharide hemiacetal **26** (0.35 g, 0.62 mmol) was dissolved in acetyl chloride (2 mL) and the solution was stirred at room temperature for 40 h. After adding dichloromethane (5 mL), the solution was poured over ice and water (5 mL) in a separatory funnel and shaken. The organic layer was poured rapidly into another separatory funnel containing ice and saturated sodium bicarbonate solution, and extracted without delay. The organic layer was then poured into a flask that contained $MgSO_4$ for drying. After decanting, the solution was dried on the rotary evaporator at 50°C. Over the still warm solution, ether was added and crystals of **27** formed (0.3 g) in 62.9 % yield in a 1:1 mixture with peracetylated disaccharide.

(2-Ethyl propionyl) 6-O-(2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl)-2-acetamido-2-deoxy-3,4-di-O-acetyl-α-D-glucopyranoside (**28**)

To a 25 mL round bottom flask equipped with a drying tube, glycosyl chloride **27** (0.30 g, 0.39 mmol) was dissolved in lactic acid ethyl ester (7 mL) and $CaSO_4$ (0.1 g) was added to remove any residual moisture. After stirring for 30 minutes, Ag_2O (0.15 g, 0.64 mmol) and Ag_2CO_3 (0.3 g, 1.1 mmol) were added and stirring was continued at room temperature for 48 h. The suspension was filtered through Celite, washed with dichloromethane and then evaporated. The crude product was purified using column chromatography with a 2:1 mixture of hexane-acetone as the eluent. Pure lactyl glycoside (0.15 g) was obtained. ^{13}C-NMR ($CDCl_3$) δ 14.0, 20.6, 22.5, 23.0, 29.6, 50.9, 51.7, 61.3, 61.7, 66.3, 67.4, 67.9, 68.2, 68.7, 69.6, 71.2, 72.4, 96.0, 99.7, 169.2, 169.9, 171.3.

1-O-Methyl-α-D-galacturonic acid (**29**)

Methyl α-D-galactopyranoside **20** (0.1 g, 0.5 mmol) was dissolved in water (10 mL). To this was added NaBr (0.025 g, 0.25 mmol), NaClO (0.525 mL, 1.1 mmol) and TEMPO (0.0008 g, 0.005 mmol) and the solution was strongly stirred at 0°C and pH 11. The pH was checked every 5 minutes and NaClO was added to maintain pH 11. After 20 minutes, the pH was stabilized and the reaction completed. The reaction was quenched with ethanol (0.5 mL) and then neutralized to pH 7 by adding 4 N HCl. The solution was concentrated at 35°C to about 1 mL and then ethanol was added to precipitate the product, which was isolated by centrifugation. After drying pure 1-O-methyl galacturonic acid (0.1 g) was obtained in quantitative yield. ^{1}H-NMR ($CDCl_3$) δ 3.4 (3H, s, OMe), 3.6 (1H, dd, H-6), 3.7 (1H, dd, H-2), 3.72 (1H, m, H-5), 3.75 (1H, m, H-3), 3.8 (1H, dd, H-

6), 4.24 (1H, dd, H-4), 4.9 (1H, d, H-1); ^{13}C-NMR ($CDCl_3$) δ 51.0, 63.5, 64.3, 66.0, 66.3, 95.0, 172.0.

2-acetamido-2-deoxy-3,4,6-tri-O-α-D-glucopyranosyl chloride (**2**)

2-Acetamido-2-deoxy glucose (50.0 g, 226.0 mmol) and acetyl chloride (100 mL) were added to a 500 mL round bottom flask equipped with a magnetic stirrer, reflux condenser and drying tube. The mixture was stirred at room temperature for 16 h. Dichloromethane (400 mL) was added through the top of the condenser and the resulting solution was poured with vigorous stirring on ice (400 g) and water (100 mL) in a 3 L beaker. The organic solution was drawn off without delay into a 3 L beaker containing ice and saturated sodium bicarbonate solution (400 mL). After neutralization wass complete, the organic layer was run directly into a flask containing anhydrous $MgSO_4$ (25 g) and stirred for 10 minutes. The suspension was filtered and washed with dry dichloromethane directly into a 1 L round bottom flask, and then concentrated on a rotary evaporator at 50°C to about 75 mL. Dry ether (500 mL) was added to the warm solution, rapidly and with swirling, and the product crystallized out (63 g) in a 76 % yield. ^{1}H-NMR ($CDCl_3$) δ 1.96 (3H, s, OAc), 2.02 (3H, s, OAc), 2.03 (3H, s, OAc), 2.08 (3H, s, OAc), 4.12 (1H, dd, H-6a), 4.24 (1H, m, H-5), 4.25 (1H, dd, H-6b), 4.51 (1H, ddd, J 9.77 Hz, J 8.3 Hz, J 3.9 Hz, H-2), 5.19 (1H, J 9.77 Hz, H-4), 5.29 (1H, t, H-3), 5.8 (1H, d, NH), 6.15 (1H, d, J 3.41 Hz, H-1); ^{13}C-NMR ($CDCl_3$) δ 20.4, 20.6, 23.0, 53.3, 61.0, 66.8, 70.0, 70.8, 93.5, 169.1, 170.1, 170.5, 171.4.

(2-Ethyl propionyl) 2-acetamido-2-deoxy-3,4,6-tri-O-acetyl-β-D-glucopyranoside (**31**)

In a round bottom flask covered with aluminum foil, Ag_2O (7 g, 30 mmol), Ag_2CO_3 (15.0 g, 54.4 mmol) and $CaSO_4$ (15 g), all dried in the vacuum oven for 1 h, were added to lactic acid ethyl ester (200 mL) and the suspension was stirred for 1.5 h at room temperature. N-acetylglucosamine **2** (15.0 g, 41 mmol) was added and stirring is continued for 24 h. The reaction mixture was filtered through Celite and washed with chloroform. To eliminate all Ag salts, the residue was dissolved in chloroform and extracted 2 times with the same volume of aqueous 2 % NH_4OH and then with water. After drying over Na_2SO_4, the solution was rotary evaporated. After one day, pure **31** crystallized out (3.3 g) in 17 % yield. ^{1}H-NMR ($CDCl_3$) δ 1.29 (3H, t, OCH2CH3), 1.44 (3H, d, OCH), 1.98 (3H, s, OAc), 2.00 (3H, s, OAc), 2.02 (3H, s, OAc), 2.08 (3H, s, OAc), 3.64 (1H, m, J 2.14 Hz, J 4.39 Hz, J 9.77 Hz, H-5), 4.01 (1H, q, J 8.30 Hz, H-lac), 4.10 (1H, dd, J 12.45 Hz, H-6a), 4.21 (1H, ddd, H-2a), 4.24 (1H, dd, H-6b), 4.40 (2H, q, J 7.08 Hz, H-lac), 4.56 (1H, d, J 8.55 Hz, H-1), 5.09 (1H, t, J 9.52 Hz, H-4), 5.13 (1H, t, J 9.79 Hz, H-3), 6.10 (1H, t, J 7.82 Hz, NH); ^{13}C-NMR ($CDCl_3$) δ 14.1, 18.5, 20.5, 20.7, 23.3, 54.1, 61.3, 61.9, 68.2, 72.1, 72.4, 73.2, 99.7, 169.2, 170.8, 173.0.

Lactyl 2-acetamido-2-deoxy-β-D-glucopyranoside (**32**)

To a solution of **31** (3.34 g, 7.7 mmol) in methanol (67 mL) was added a solution of K_2CO_3 (3.34 g) in water (5 mL) and the suspension was stirred at room temperature for 24 h. After drying the solvent on a rotary evaporator at 30°C, water was added and the solution was passed over a cation exchange strongly acidic resin. Lyophilization afforded pure product (2.25 g) in quantitative yield. ^{1}H-NMR (D_2O) δ 1.21 (3H, d, J 7.08 Hz, H-

lac), 1.83 (3H, s, OAc), 3.22 (1H, m, H-3), 3.23 (1H, m, H-5), 3.34 (1H, m, H-5), 3.47 (1H dd, J 1.46 Hz, J 6.60 Hz, H-6a), 3.49 (1H, dd, J 2.93 Hz, H-6b), 3.70 (1H, d, H-4), 4.32 (1H, q, J 7.08 Hz, H-lac), 4.38 (1H, d, J 8.31 Hz, H-1); ^{13}C-NMR (D_2O) δ 13.4, 17.6, 50.8, 56.0, 65.1, 68.8, 69.0, 71.2, 95.7, 170.1, 171.8.

(2-Methyl propionyl) 2-acetamido-2-deoxy-β-D-glucopyranoside (**33**)

32 (2.26 g, 7.7 mmol) was dissolved in methanol (70 mL) and BF_3 etherate (7 mL) was added. The mixture was stirred at room temperature for 2 h. The solution was dried on a rotary evaporator several times by adding larger amounts of methanol. After lyophylization, crude product (2.2 g) was obtained. Recrystallization from 1:1 ethanol/ether gave pure **33** (1.2 g) in a 50 % yield. ^{13}C-NMR (D_2O) δ 13.4, 17.6, 48.16, 50.8, 56.0, 65.1, 68.8, 69.0, 71.2, 95.7, 170.1, 171.8.

6.5. References

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