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PROGRESS IN THE SYNTHESIS OF STABILIZED GLYCOCONJUGATE VACCINE CANDIDATES AGAINST *NEISSERIA MENINGITIDES* GROUP A

by

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ABBREVIATIONS

a	axial
Ac	acetyl
Bn	benzyl
BOM	benzyloxymethyl
Bz	benzoyl
CAN	ceric ammonium nitrate
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DIBALH	diisobutylaluminium hydride
DMAP	4-dimethylaminopyridine
DMF	*N,N*-dimethylformamide
DMSO	dimethyl sulfoxide
e	equatorial
Hz	hertz
J	coupling constant
KHMDS	potassium bis(trimethylsilyl)-amide or potassium hexamethyldisilylamide
Me	methyl
MEM	2-methoxyethoxymethyl
Mn	meningitis (note that in this context Mn does not refer to the element manganese)
MOM	methoxymethyl
NMR (n m r)	nuclear magnetic resonance
Ph	phenyl
PMB	*p*-methoxybenzyl
PMP	*p*-methoxyphenyl
RT	room temperature
SPh	phenylsulfonyl
TLC	thin-layer chromatography
TBS	*tert*-butyldimethylsilyl
TEAB	tetraethylammonium bromide
Tf	trifluoromethanesulfonyl
THF	tetrahydrofuran
THP	tetrahydropyran-2-yl
Tr (Trityl)	triphenylmethyl
Trisyl	2, 4, 6-triisopropylbenzenesulfonyl
Ts (Tosyl)	tosyl = *p*-toluenesulfonyl

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ABSTRACT

Meningitis is the inflammation of the lining membranes of human brains and spinal cord. It is a deadly disease that has claimed millions of lives throughout the world in particular in developing countries. *Neisseria meningitidis* serogroup A is among the leading causative agents of meningitis in Sub-Saharan Africa. Its capsular polysaccharide antigen consists of a homopolymer of α-(1→6) linked mannosamine phosphate residues.

So far, no vaccine of high efficacy to prevent the most affected age groups (infants) has been licensed against meningitis caused by serogroup A. The low efficacy of the vaccines tested is attributed to the instability of the phosphodiester glycosidic bond of the antigen towards acids. In the hope that substituents different to that of the C-2 *N*-acetyl group in the native mannosamine monomer may stabilise the glycosidic bond, we have evaluated different ways of synthesising key building blocks for preparation of novel stabilised glycoconjugates as vaccine candidates.

Three key intermediates in the preparation of desired oligosaccharide conjugates were identified and various synthetic routes to these evaluated. These intermediates were 2-azido-2-deoxy-D-mannopyranose, a suitably protected derivative of this azidomannose with selectively removable group at O-6, and an α-2-azido-D-mannopyranosyl-hydrogenphosphonate derivative as precursor to the oligomer or polymer. The first intermediate was obtained either *via* azidonitration of protected glucal or by S_N2 displacement by azide ion of suitably protected 2-*O*-triflylglucoside followed by de-*O*-acetylation. The selectively protected intermediate was synthesised making use of the formation and subsequent regioselective opening of 4,6-O-benzylidene acetals derived from the first key intermediate. Formation of the anomeric hydrophosphonate from this second intermediate was achieved with salicylchlorophosphite to give selectively the α-anomer of 2-deoxy-2-azidomannopyranosyl-hydrophosphonate.

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I declare that "**Progress in the Synthesis of Sabilized Glycoconjugate Vaccine Candidates against *Neisseria Meningitides* group A**" is my own work and that all the sources that I have used or quoted have been indicated and acknowledged by means of complete references.

HENOK H KINFE

University of Cape Town

To Kidanu and Adey (Tsegeweini)

University of Cape Town

1. INTRODUCTION

1.1 MENINGITIS

Meningitis is a disease that exclusively attacks human beings. It is an infection of the fluid that surrounds the brain and spinal cord. It is usually caused by a bacterial or viral infection.[1] The severity of the illness depends on the disease-causing agent.

1.1.1 Viral Meningitis

Viral meningitis is generally relatively less severe. It resolves in a week or two without intervention of any specific treatment. No specific kind of virus has been linked with the disease and, thus, any of a number of different viruses and mainly the entero (intestinal), varicella, the mumps and adenoviruses could be responsible for viral meningitis.[2] Viral meningitis is spread by person-to-person contact or by insects like mosquito depending on the virus involved. Diagnosis of the disease is by blood tests, however, no specific vaccine treatment is available. An outbreak of viral meningitis is rare and occurs as a single event. Taking good care of personal hygiene and avoiding mosquito bites help prevent contracting the disease.[3]

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1.1.2 Bacterial Meningitis

Bacterial meningitis, mostly caused by invasive *Haemophilus influenzae* (Hib), *Streptococcus pneumoniae* (Pn), and *Neisseria meningitides* (Mn), is one of the top ten infectious causes of death globally.[4] According to the World Health Organization (WHO) report in the year 2000, bacterial meningitis was found to be responsible for an estimated 171,000 deaths worldwide per annum. Prior to the 1990s, *Haemophilus influenzae* type b was the leading cause of bacterial meningitis, but with the introduction of glycoconjugate vaccines invasive disease due to *Haemophilus influenzae* has reduced. In the same fashion, meningitis caused by *Streptococcus pneumonia* and certain serogroups of *Neisseria meningitides* have been eliminated significantly.[4] Today, a few types of

are the leading causes of bacterial meningitis throughout the world but more pronounced in developing countries.[5] Thus, understanding the microbiologic features of *Neisseria meningitides* and their entry into the bloodstream and cerebrospinal fluid is very important in order to understand the virulence of the disease and how to combat it.

1.2 MICROBIOLOGIC FEATURES OF *NEISSERIA MENINGITIDES*

Neisseria meningitides is the leading cause of meningitis among other agents and causes endemic and epidemic diseases mostly in developing countries. *Neisseria meningitides* is an aerobic gram-negative spherical or kidney shaped diplococcus (bacilli) commonly found in pairs intracellularly (Fig. **1**). Human beings are their exclusive natural hosts. The structure of the bacterium consists of outer and inner (cytoplasmic) layers that are separated by a peptidoglycan cell wall (Fig. **2**).[6]

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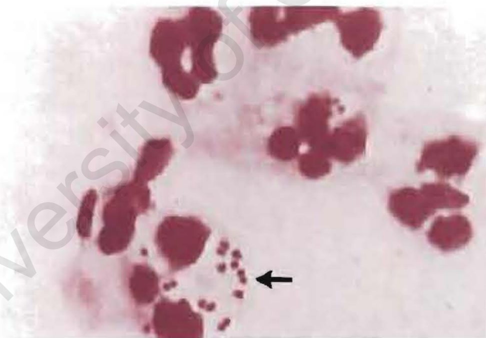

Fig. **1** *Neisseria meningitides* (Arrow) in Cerebrospinal Fluid (Gram's stain, × 1000).[7]

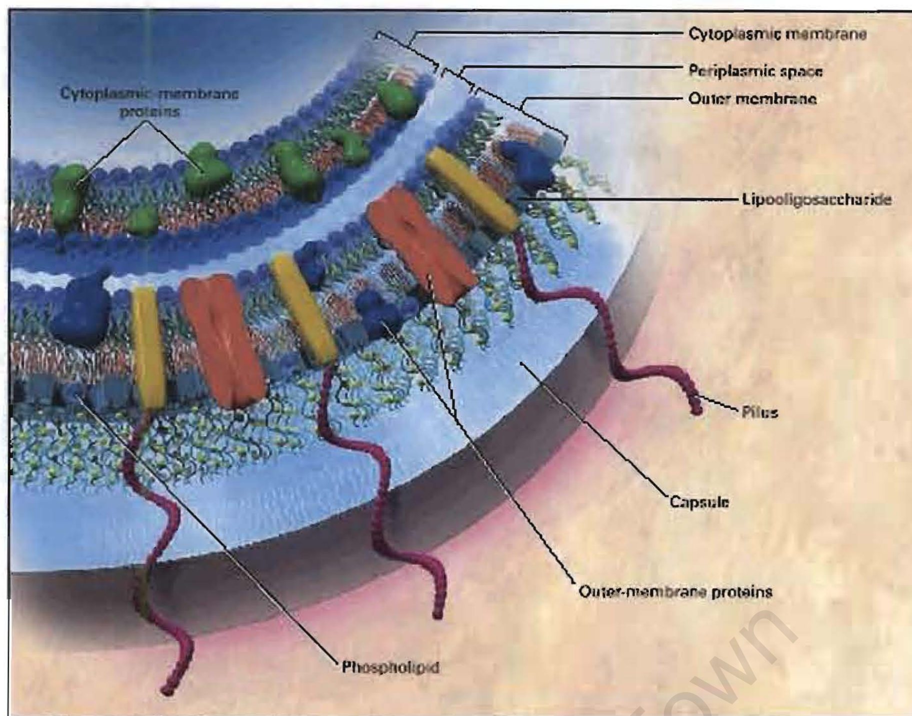

Fig. **2** Cross-Sectional View of the Meningococcal Cell Membrane.[7]

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The outer layer of the bacteria consists of:

***i.* Capsular polysaccharides**

The capsular polysaccharides are found at the outer cell membrane of the organism. Though the polysaccharides are not toxic by themselves, they are mainly engaged in the protective mechanism of the organism against dehydration and the human immune system by inhibiting phagocytosis. And hence, it enhances the survival of the organism during invasion of host cells.[5]

ii. **Pili**

Pili are organells of complex proteins that are found on the outer membrane of the meningococci.[5] On the basis of serology and structure, pili are classified into two classes namely, class I and class II pili. Both classes belong to type IV pili as they are members of the methylphenylalanine pili family.[8] Besides their assistance in adherence to host cells, pili are essential in genetic competence and twitching motility of the organism during invasion of host cells.[5] (explained more in pathogenesis).

iii. **Lipopoly(oligo)saccharide**[5]

Lipooligosaccharide (LOS or endotoxin) and lipopolysaccharide (LPS) are also parts of the outer membrane of the meningococcia bacteria. LOS and LPS are structurally related except that LOS lacks a repeating unit of O-antigen. The conserved inner core of both LOS and LPS is composed of heptose and 3-deoxy-D-manno-2-octulosonic acid (KDO) attached to a lipid A moiety embedded in the outer membrane. A more variable sequence of sugar residues composed of glucose, galactose, *N*-acetylglucosamine, and *N*-acetylneuraminic acid (NANA) is attached to the inner core heptoses. Two short oligosaccharide chains that have variable composition are attached to the two inner core heptose residues in meningococcal LOS. The variable composition of the oligosaccharides functions as a basis for immunotyping meningococcal strains. The lipopolysaccharide (endotoxin) is involved in pathogenesis as it confers serum resistance.

iv. **Outer membrane proteins (OMP)**

The outer membrane of *Neisseria meningitides* contains a number of protein structures along with the other mentioned structures. The two major porins of the bacteria are PorA and PorB. PorA is frequently recognized by the host cells and helps the meningococci to interact with the cells. PorB, on the other hand, has the ability to insert into membranes of host cells and thus facilitates adherence to and invasion of the cells by the bacteria. The outer membrane also contains other proteins that are important in pathogenesis and adherence to host cells.[5,6]

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1.3 *NEISSERIA MENINGITIDES* SEROGROUPS

In the case of bacterial meningitis, it is important to know which type of bacteria is causing the disease, as the available treatments are specific to certain types of bacteria. Meningococci are classified by serologic typing systems into different categories. On the basis of immune specificity of the capsule, *Neisseria meningitides* are classified into 13 serogroups (A, B, C, D, H, I, K, L, W135, X, Y, Z, and 29E).[9] Meningococci are also further classified depending on antigenic differences in their major outer membrane proteins PorB into serotype, other outer membrane proteins PorA into serosubtype, and the structure of the outer membrane lipopolysaccharide into immunotype.[10]

Studies have shown that most of the meningococcal diseases are caused by serogroups A, B, C, W135, and Y. Serogroups B, C, W135, and Y all contain sialic acid (*N*-acetylneuramic acid, NANA), whereas serogroup A is composed of repeating units of α-linked *N*-acetylmannosamine-1-phosphate. Reports have indicated that the average chain length and molecular weight of serogroup A are 93 residues and in excess of 100,000, respectively.[11] Although the structures of serogroups B and C are homopolymers of sialic acid, their structures differ at the glycosidic linkage of each monomer (unit). In serogroup B the units are linked α2→8 whereas in the case of serogroup C, the linkage is usually α2→9. Serogroup Y is composed of an alternating sequence of D-glucopyranose and partially *O*-acetylated NANA. The W135 serogroup differs from Y in that it contains an alternating sequence of D-galactose and NANA.[11,12,13]

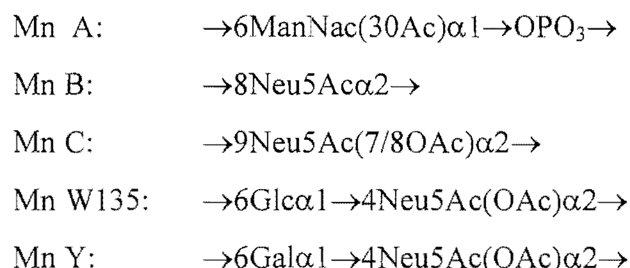

Figure 3. Structures of repeating units of *Neisseria meningitides* serogroups A, B, C, W135, and Y.[30,36]

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1.4 MENINGOCOCCAL EPIDEMIOLOGY

A, B and C are responsible for 90 % cases of the epidemics. Serogroup A accounts for most of the epidemics around the world particularly in the developing countries like the sub-Saharan Africa, Pakistan, Nepal, Bhutan and parts of India.[6,14,15] The region that extends from Senegal through Gambia from the west to Ethiopia in the east is recognized as the African meningitis belt. In the meningitis belt, serogroup A has been responsible for meningococcal epidemics for more than a century. Over 300,000 cases and 30,000 deaths were reported in the 1996-97 meningococcal epidemic outbreak in the region.[16] The epidemic occurs irregularly in 5-10 year cycles exclusively during dry seasons between November and April. The data reported by Communicable Disease Surveilance and Response (CSR) of the WHO further elaborates on the severity of meningitis in the region. As of 9 March 2003, a total of 2,056 cases including 195 deaths were reported since the Mn A outbreak began in February 2003 in Niger.[17] Besides serogroup A, serogroup W135 associated with the Hajji (Umrah) pilgrimage in Saudi Arabia resulted in 53 % of cases of the epidemics in the region in 2000. Quite recently, as of 6 March 2003 in one of the mostly affected country in the 'Meningitis Belt', Burkina Faso, suffered a total of 3,691 cases including 542 deaths since the outbreak began in January 2003. Clinical results showed that Mn A and Mn W135 were responsible for the outbreak.[18]

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Serogroup B and C are prevalent in Europe. 50 – 90 % of cases of meningococcal disease are attributed to serogroup B and the remainder to C.[18] High proportion of strain serogroup B is isolated in Norway, the Netherlands, Germany and Denmark. Whereas in Czech Republic, Slovakia, Grecee, Republic of Ireland, Spain, and the UK high proportion of strain serogroup C are isolated. In North America, most commonly isolated serogroups are then C, B, and Y.[18,19]

1.5 MENINGOCOCCAL PATHOGENESIS

Neisseria meningitides is transmitted from person to person by direct contact with respiratory secretions or *via* inhalation of aerosols. The newly arrived organisms multiply and colonise the nasopharynx by binding to the non-ciliated epithelial cells with the help of pili and opacity-associated proteins.[20,27] The epithelial cells engulf the attached organisms into the phagocytic vacuoles.[20] The IgA1 protease and PorB enhance the survival of the organisms in the epithelial cells by cleaving lysosome-associated membrane protein and crossing the cell membrane, respectively.[21] The organism can survive in the site without causing any epidemics resulting in a carrier state of the host, infact 5 to 10 % of adults are carriers of asymptomatic nasopharyngeal meningococci. As a result of the asymptomatic carriage, most carriers develop immunity to the bacterial antigens.[5,22,23,24] For unknown reasons, in certain individuals of these asymptomatic carriers, the organisms traverse the epithelial surfaces of the respiratory tract and gain access to the bloodstream to cause meningococcal septicaemia. Unless prompt medical treatment takes place, the bacteria continue to colonize and damage the endothelial cells of brain microvessels. After damaging the cells, the bacteria cross the blood-brain barrier (BBB) to gain access to the cerebrospinal fluid (CSF) as shown in Figure **4** and thereby cause meningitis.[5]

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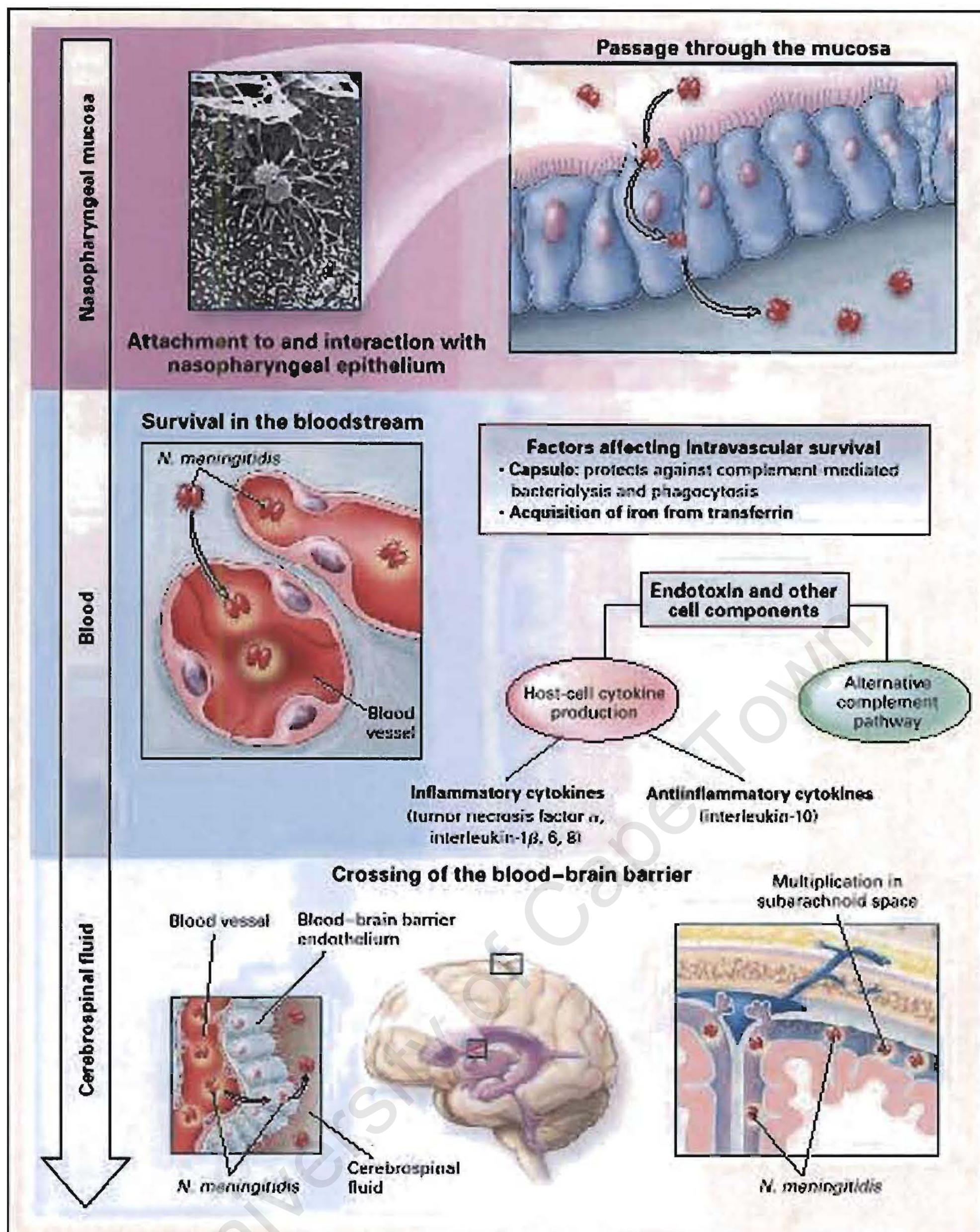

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Figure 4. Colonization of *Neisseria meningitides* in the Nasopharynx and Entry into the

Bloodstream and Cerebrospina Fluid.[7]

Meningococcal diseases are age-dependent. The incidence of the disease is highest among children between the age of 6 and 24 months and declines thereafter with age; but peaks again among people in the range of 5-24 years. Children of less than 6 months of age seem to be immunised; most likely due to the presence of maternal acquired antibodies (*via* placental or breast milk).[6,25] Some of the factors that are associated with an increase in risk of developing meningococcal disease include: overcrowding, poverty, concurrent viral infections, both active and passive smoking, chronic underlying illness, alcohol consumption, complement deficiency (C_3 and C_{5-9} deficiency), hypogammaglobulinaemia, and hyposplenism.[6,7]

1.6 MANIFESTATIONS OF MENINGITIS[7,9,26]

The similarity of meningitis symptoms to some other less serious diseases makes diagnosis of meningitis difficult. Unless prompt medical treatment with antibiotics (like penicillin) is provided, the disease can cause death in a matter of hours. Hence, knowing the symptoms is very crucial in combating the disease. The symptoms include a sudden onset of severe headache, stiffness of the neck, dislike of bright lights, fever, nausea, vomiting, drowsiness, impaired consciousness, and rash. Though stiffness of the neck and photophobia (dislike of bright lights) are not common manifestations in infants and young children, they may also suffer from a tense or bulging fontanelle. There is no specific order by which the symptoms appear and also all of them may or may not be noticed in individual patients.

Some survivors of meningitis suffer from complications of the disease. The chance of contracting the aftereffects depends on the type and severity of the disease and several other factors. The complication may be permanent or temporary, physical or emotional. The common aftereffects include: memory loss, clumsiness, residual headaches, hearing loss, learning difficulties, epilepsy, neurologic disability, speech problem, loss of sight, or loss of a limb.

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Some of the emotional aftereffects include: clinginess, temper tantrums, moodiness or aggression, disturbed sleep, nightmares, bed-wetting, changes in character, learning difficulties, and depression.

1.7 PREVENTION AND CONTROL

1.7.1 Early Treatments of Meningitis

Once the bacterium transfers from a carriage state to an invasive one, fatality of the host can result within 24 to 48 hours after the onset of symptoms if there is no immediate medical intervention. However, statistics have shown that with the introduction of antibiotics like penicillin or ampicillin with the ability to kill meningococcal bacteria in the blood and fluid around the brain, the mortality rate has decreased from 60 – 80 % to 10 %.[28]

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Although early recognition of the signs and symptoms of the disease and rapid medical treatment with antibiotics improves the chances of recovery, the similarity of the manifestations with some other non-serious illnesses can delay early appropriate treatment.

Different treatments of meningitis have been reported since the first description of meningococcal meningitis by Gaspared Vieusseux in Geneva in 1805.[29] As the etiology agent was not yet known, scientists at that time put much emphasis on stomach and bowel evacuation as the sole means of treating meningitis.[29] Identification of meningococcus from CSF by Anton Weichselbaum in 1887 enabled establishment of a link between the disease and disease-causing agent as well as a better treatment. The finding thus shifted the emphasis to drainage of CSF by repeated lumbar punctures in the late 19th century.[29] In early 1900s serogroups were identified and serum therapy was introduced by Flexner in 1913. The survival rate improved from 10 – 30 % to 69.1 % in 1,294 cases.[29] The discovery of the antibacterial activity of sulfonamides in the early 1930s opened the gate for a new way of treating meningitis. Francis Schwentker *et al.* were the first to report a cure of meningococcal meningitis with sulfanilamide therapy in

1937.[9,29] Though the sulfonamide therapy improved survival from meningitis, development of sulfonamide resistant meningococcal put an end to sulfanilamide therapy in the early 1960s.[9] The emergence of new antibiotic resistant strains and the delicacy of the cerebrospinal fluid membrane, left researchers with only one option, which is prevention to control the deadly disease. Hence, mass immunisation through vaccination was the best approach for the prevention of infection.

1.7.2 Vaccines against *Neisseria meningitides*

The history of vaccines against meningitis dates back to the early 1900s. The vaccines at that time were either heat-killed whole meningococci or crude extracts of broth cultures. However, such vaccinations resulted in adverse effects after injection and also variable efficacy. After isolation of purified capsular polysaccharides in 1935, the trial of purified serogroups A and C as vaccines by Kabat and co-workers, failed to induce antibody responses in human volunteers.[6,30] The account on the failure given by the authors was that the techniques used for the isolation of the polysaccharide were susceptible to probable enzymatic degradation of the polysaccharide to low molecular weight oligosaccharides. Gotschlich and co-workers from the Walter Reed Army Institute of Research (USA) developed a technique for the isolation of highly purified high molecular weight meningococcal polysaccharide and the polysaccharides isolated induced antibody response in adult volunteers.[6] The findings led to routine immunization with a divalent vaccine that contains serogroups A and C and a quadrivalent vaccine covering A, C, Y, and W135 since 1970s and 1982, respectively.[6,30] Although the di-and quadrivalent vaccines are administerd as a single dose, antibody responses to each of the polysaccharides are serogroup specific and independent. Antibody response to the vaccine develops within 7 to 10 days after injection and depends on several factors including: molecular size of the antigen, dose of the vaccine, number of exposures to the vaccines, and age of the recipient. The effectiveness of the polysaccharide vaccines was proved in prevention of disease due to serogroup C in US military recruits and also in controlling group A epidemics during mass campaigns in African meningitis belt. A number of reports have described the vaccines as safe with generally mild side effects consisting of pain and redness at the site of injection.[6,9,30]

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Reingold *et al.* reported that efficacy of serogroup A peaks to 90 % and 67 % for the first year of all children and 3 years in children over 4 years of age, respectively.[31] Immunogenecity studies further indicated that in infants of under 1 year of age, antibody levels decrease in less than 1 year and in less than 2 years for children in the age range of 2-3 years old though high levels persist for more than 5 years in adults after a single vaccination.[30,32,33] These results demonstrated that infants under the age of 1 year old are at high risk of contracting meningitis. It has also been reported that repeated injections resulted in decreased immunogenecity of the subject.

The limitations of most polysaccharide vaccines can be summarized as follows:

- Multiple doses of vaccine are required since the antigens fail to induce immunological memory
- Short duration and age dependency of immunity after a single dose,
- The efficacy in very young children is limited.
- They don't result in reduction of transmission of the disease in the population at risk (herd immunity).

These inhibit the usefulness of the vaccines in preventing and controlling meningitis particularly in infants who have the highest risk of sporadic disease.

The failure to induce an immunologic memory of a polysaccharide antigen is known to be due to T cell-independent response of short duration to the antigen.[31] This failure has been solved to a certain extent in some cases of bacterial meningitis by implementing a polysaccharide-protein conjugate vaccines. Vaccines of such kind are known to be able to induce a T cell-dependent response. Conjugate vaccines against *Haemophilus influenzae* type b[27] and *Neisseria meningitides* serogroup C infections have been licensed for paediatric purposes.[34,35,36,] Recent reports indicate that, unlike polysaccharide vaccines, the vaccines induce immunological memory, elicit long-lasting protection and are immunogenic in very young children.[37,38] However, studies have shown that vaccination of animals and humans with the derived conjugate vaccine against *Neisseria meningitides* serogroup A infections resulted in poor immunogenicity.[39] As it has been mentioned earlier, the native antigen (capsular polysaccharide) of the *Neisseria meningitides*

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serogroup A is a homopolymer of $\alpha(1\rightarrow6)$ linked *N*-acetylmannosamine phosphate. The glycosidic phosphodiester bond is sensitive to both acids and bases and this lability is thought to cause instability and thus poor immunogenicity of the *Men A* conjugate vaccine.[39]

Further studies were carried on to improve the vaccines in preventing and controlling meningitis. Pozsgay *et al.* showed that a synthetic oligosaccharide – protein conjugate vaccine for *Shigella dysenteriae* type 1 to be more immunogenic than a similar one prepared from a natural polysaccharide.[28] Ali *et al.* have also stated that a synthetic polysaccharide – protein conjugate vaccine for *Haemophilus influenzae* type b elicits a higher immune response than the one made from natural polysaccharide.[28] However, no effective conjugate vaccine of any type has been licenced against *Neisseria meningitides* serogroup A infections though it is responsible for 90 % of the cases of endemic and epidemic meningitis caused by *Neisseria meningitides* bacteria worldwide.

Hence, on the basis of these findings, we set our objectives to synthesise novel stabilised synthetic capsular oligosaccharide of serogroup A, chemically conjugate it to a carrier protein and investigate the immunogenicity of the resulting glycoconjugates as vaccine candidates against *Neisseria meningitides* serogroup A infections.

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2. RESULT AND DISCUSSION

2.1 RETROSYNTHETIC ANALYSIS

The meningococcal (Mn) group A organism responsible for 90 % of the cases of endemic and epidemic meningitis has a capsular polysaccharide antigen which is a homopolymer of α-(1→6) linked *N*-acetyl-D-mannosamine phosphate **1**. The objective of this project is to develop synthetic oligosaccharide conjugates related to this structure. However, since the mannosamine phosphate **1** is known to be acid-labile, the further intention was to prepare analogues with different substituents at C-2 of the mannose unit, which may help stabilize the glycosidic linkage.

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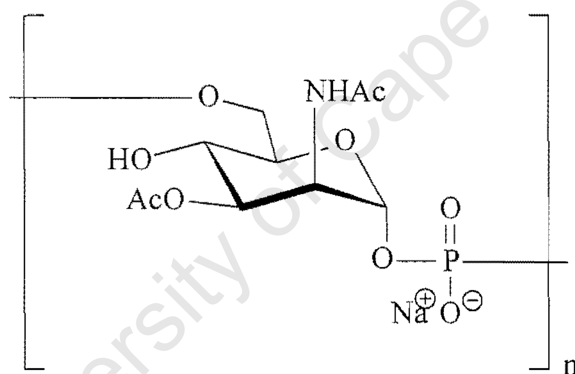
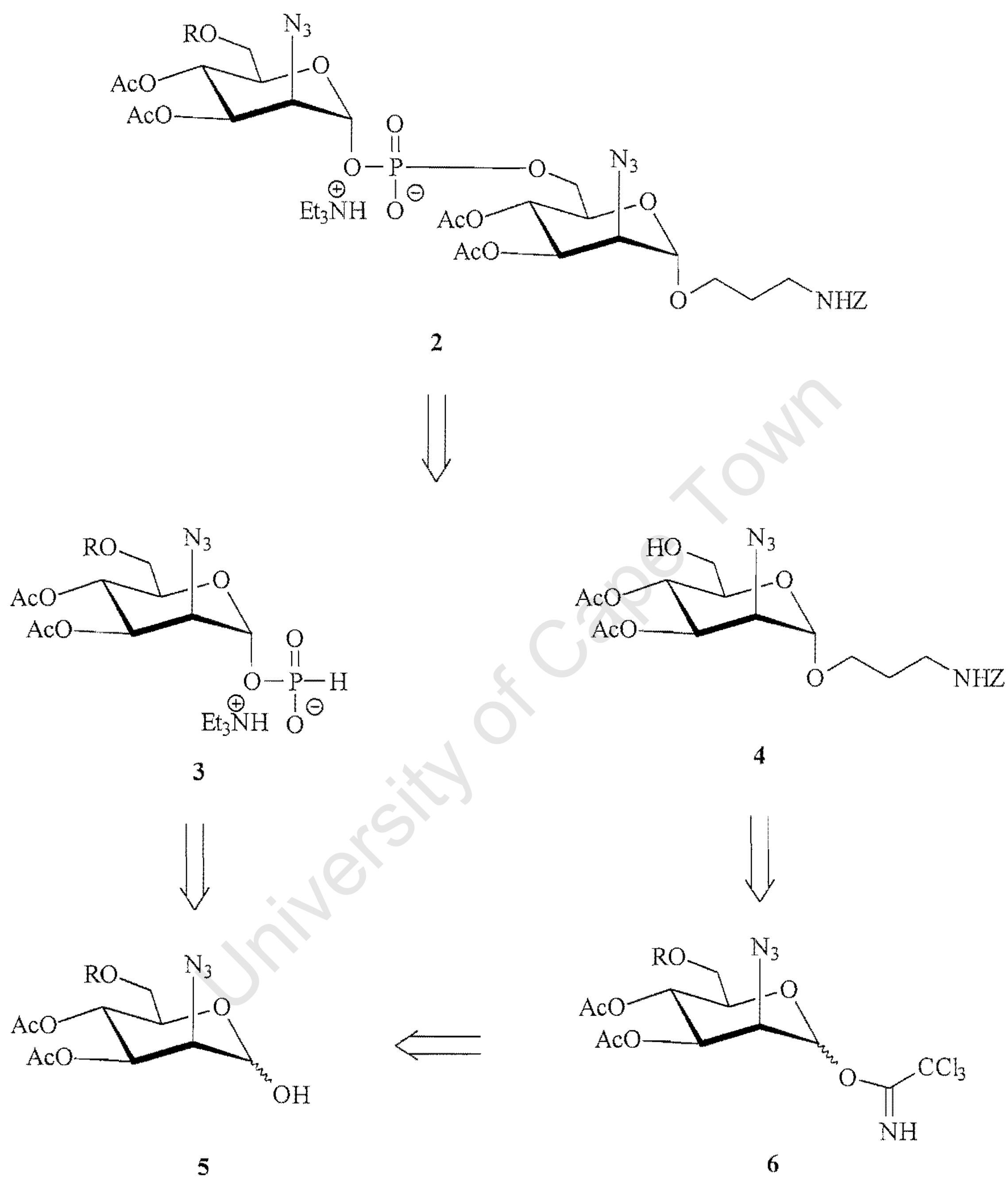


1

The initial synthetic targets were oligosaccharide conjugates depicted in structure **2**. The formation of this disaccharide glycoside requires precursor hydrophosphonate **3** and glycoside **4** which are in turn obtained from the 2-azido-2-deoxy-D-mannose **5** as common building block. 2-Azido-2-deoxymannose **5** can be readily converted either to the hydrophosphonate **3** or, after suitable activation, such as through preparation of trichloroacetimidate **6**, transformed to glycoside **4** which incorporates a functionalised "linker" for later attachment to a protein. The protecting group R in **Scheme 1** needs to be carefully chosen so that it may be selectively removed without, in particular, reducing the azide, to allow for further elongation of the oligosaccharide (**Scheme 1**).



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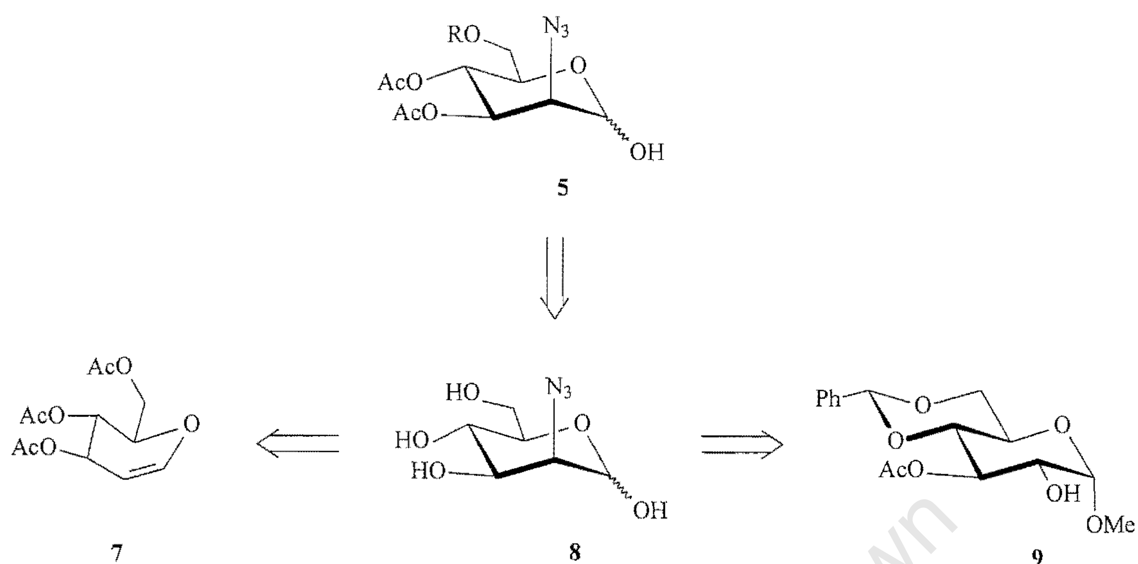
Scheme 1

The key intermediate 3,4-di-*O*-acetyl-2-azido-2-deoxy-D-mannose derivative **5** can be synthesized from 2-azido-2-deoxy-D-mannose **8** which in turn can be derived from either protected glucal **7** or selectively protected glucoside **9** (**Scheme 2**).



Scheme 2

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2.2 SYNTHESIS OF 2-AZIDO-2-DEOXYMANNOSE 8

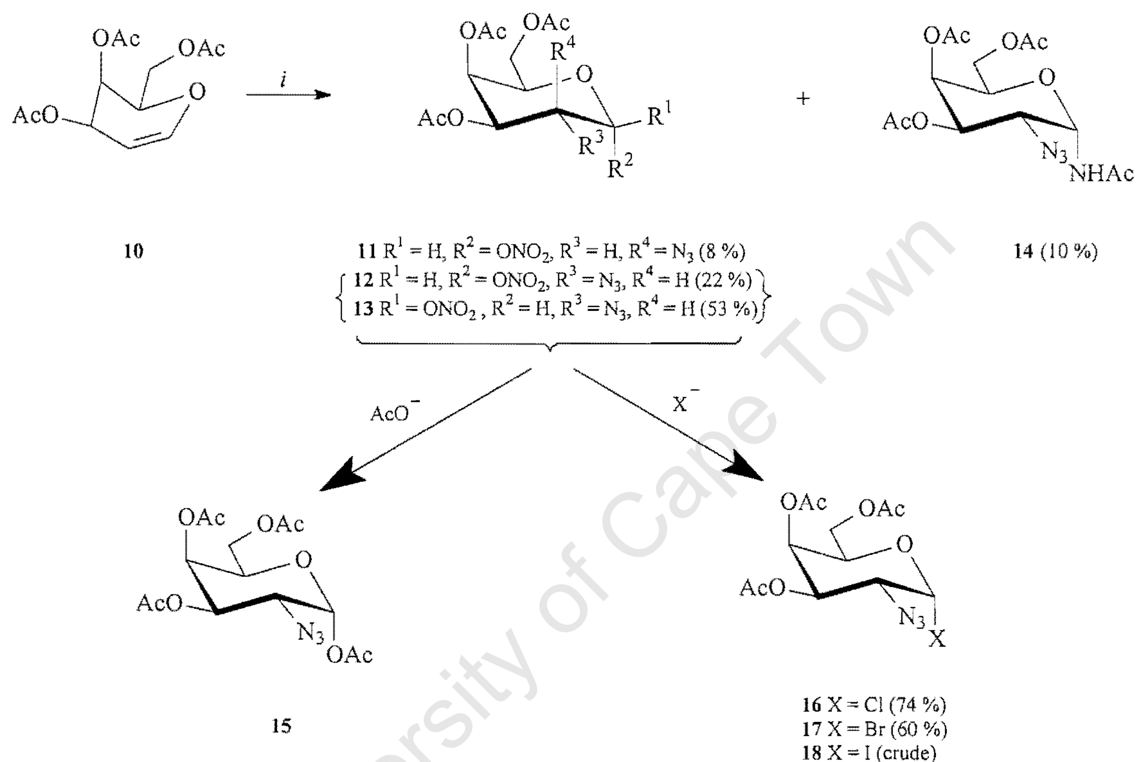
The first key synthetic intermediate required in our study was 2-azido-2-deoxymannose **8**. The azide is a non-participating group in glycosidations and can be converted to the amine *via* mild reductions, although in our case, the azide will be retained. Although 2-amino-2-deoxysugars are abundant in nature, the difficulty to separate them from natural sources limits their availability.[40] Hence, a variety of different methods of introducing the azido functionality into sugar molecules have been reported, where the desired regio- and stereoselectivity depends upon reaction conditions, reagents, and precursors used.

Widely used methods for introducing azides into sugars are:

i. Azidonitration of glycals

The use of glycals as precursors for the synthesis of 2-amino sugars was pioneered by Lemieux, who introduced the azidonitration process, where glycals are transformed to 2-aminopyranosyl derivatives.[41] In a typical example, treatment of galactal **10** with CAN and sodium azide in acetonitrile gave 2-azido-2-deoxyglycoses **11**, **12** and **13**, which

were further functionalised to provide glycosylacetate **15** and glycosylhalides **16**, **17** and **18** (**Scheme 3**). The shortcoming of this azidonitration procedure is the fact that mixtures of manno- and gluco diastereomers are obtained. When glucal is used as precursor, normally with the gluco diastereomer prepondering.



(*i*) CAN, sodium azide, CH_3CN, -15°C.

Scheme 3

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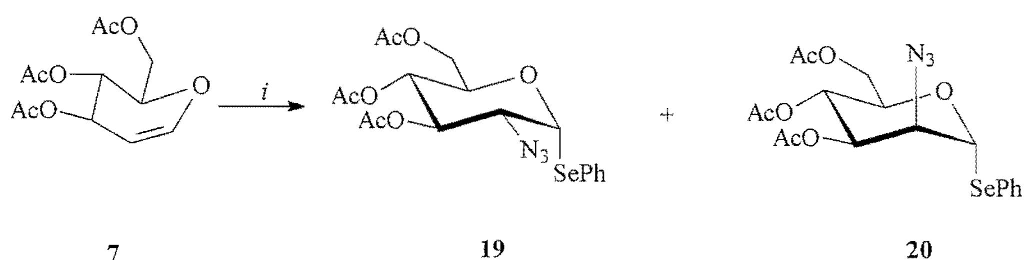
ii. Azido-phenylselenation

This method is analogous to Lemieux's azidonitration. Two methods are reported for the synthesis of phenyl 2-azido-2-deoxy-α-D-selenoglycopyranosides from protected glycals by Czernecki *et al.*.[42] The first method involves treatment of a peracetylated glycal with

sodium azide and diphenyl diselenide in the presence of (diacetoxyiodo)benzene in dichloromethane at room temperature as exemplified in **scheme 4**.



(*i*) Diphenyl diselenide, sodium azide, (diacetoxyiodo)benzene, dichloromethane, RT, 91 % (in a ratio of 3:2).

Scheme 4

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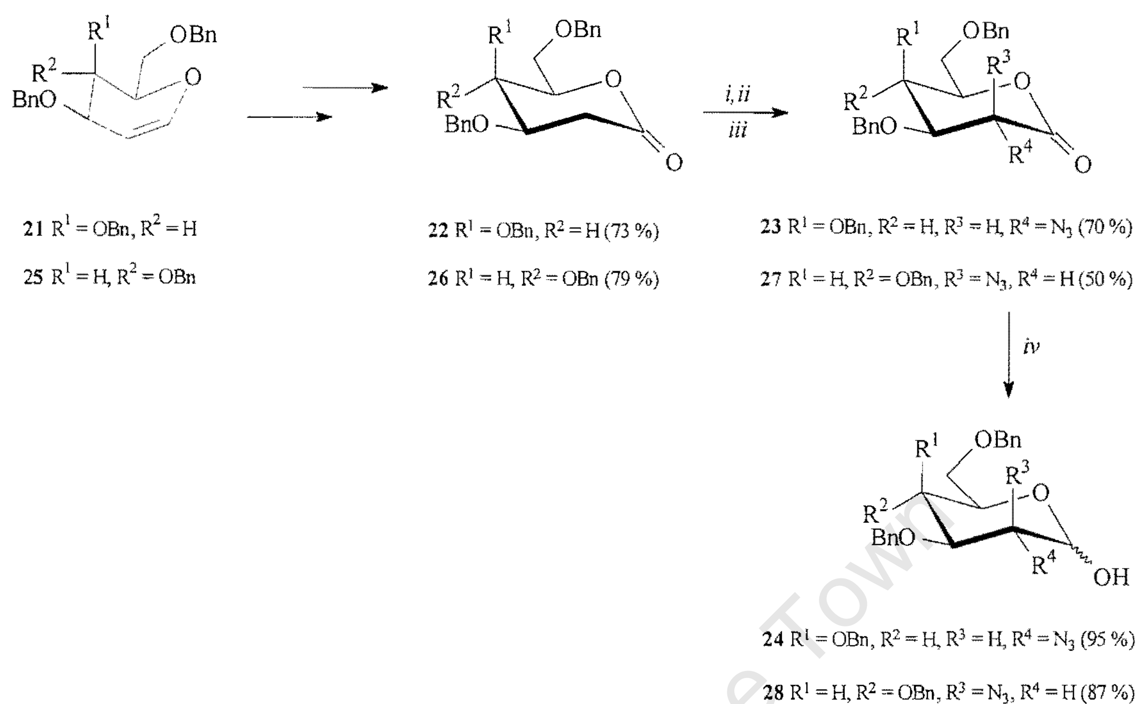
In the second method, a protected glycal is treated with trimethylsilyl azide and tetra-*n*-butylammonium fluoride in the presence of N-phenylselenophthalimide. Both methods result in mixtures of gluco and manno isomers from protected D-glucals whereas a galactal gives exclusively the galacto isomer. The former method is incompatible with benzyl ethers and acetals whereas the latter method is compatible with a variety of protecting groups including benzyl and silyl ethers as well as acetals.[42]

iii. Electrophilic azidation

Evans *et al.*[43] showed that electrophilic azide transfer from arylsulfonyl azides to enolates was a practical approach to the asymmetric synthesis of α-amino acids.[44] Later this approach was exploited for the synthesis of azidosugars. Following this protocol, the first practical synthesis of 2-azido-2-deoxy-D-mannono-1,5-lactone was achieved. Selective reduction of the lactone with DIBALH provided 2-azido-2-deoxyaldoses without epimerization at C-2 as exemplified in **Scheme 5**.[40]



(*i*) KHMDS; (*ii*) Trisyl azide; (*iii*) AcOH; (*iv*) DIBALH.

Scheme 5

***iv.* Diazo transfer for the synthesis of azides from amines**

Amines can be converted into azides by diazo transfer from TfN_3 under mild reaction conditions.[45] D-Glucosamine, D-mannosamine, and D-galactosamine were transformed to their corresponding peracetylated 2-azido-2-deoxyaldoses in 91 %, 65 %, and 70 % yield, respectively.[46] It has been reported that the diazo transfer can be also catalyzed with the help of divalent metal cations like Cu^{++}, N^{++} or Zn^{++}.[47] The general reaction conditions are as outlined in **Scheme 6:**

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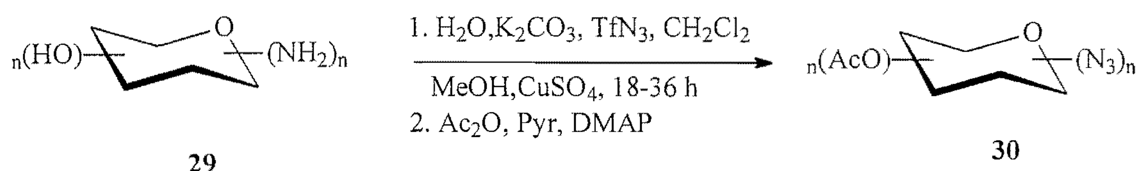
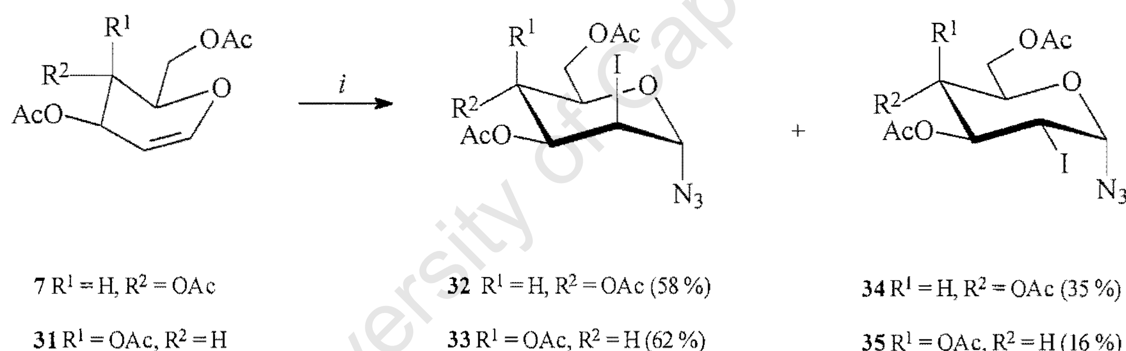


Scheme 6

v. Iodoazidation

It is possible to introduce azides into glycals other than at the C-2 position. For example, 1,2-*trans*-2-deoxy-2-iodoglycosyl azides were prepared from the reaction of glycals and iodoazides according to **Scheme 7**.[48]



(*i*) IN_3, CH_3CN, 0 °C, 2 h.

Scheme 7

vi. Nucleophilic substitution at C-2 of pyranoside derivatives

An azide is a linear triatomic anion, and because of its linear structure, is able to insert itself into almost any electrophilic site. The nucleophilicity of azides has been exploited in the synthesis of azido sugars and it has been reported that 2-*O*-triflates of carbohydrates undergo S_N2 substitution reaction with azides as outlined in **Scheme 8**.[49,50] The advantage of this method is that stereocontrol in introduction of the azide can be achieved: if a manno azide is needed, a gluco substrate is used and *vice versa.*

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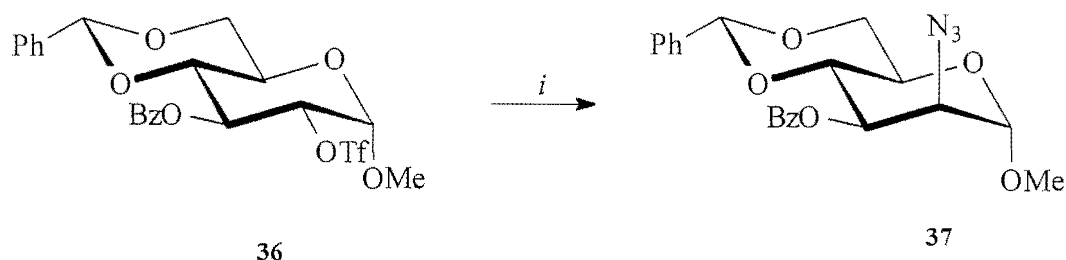

(*i*) Sodium azide, DMF, 80 °C, 2 h, 86 %.

Scheme 8

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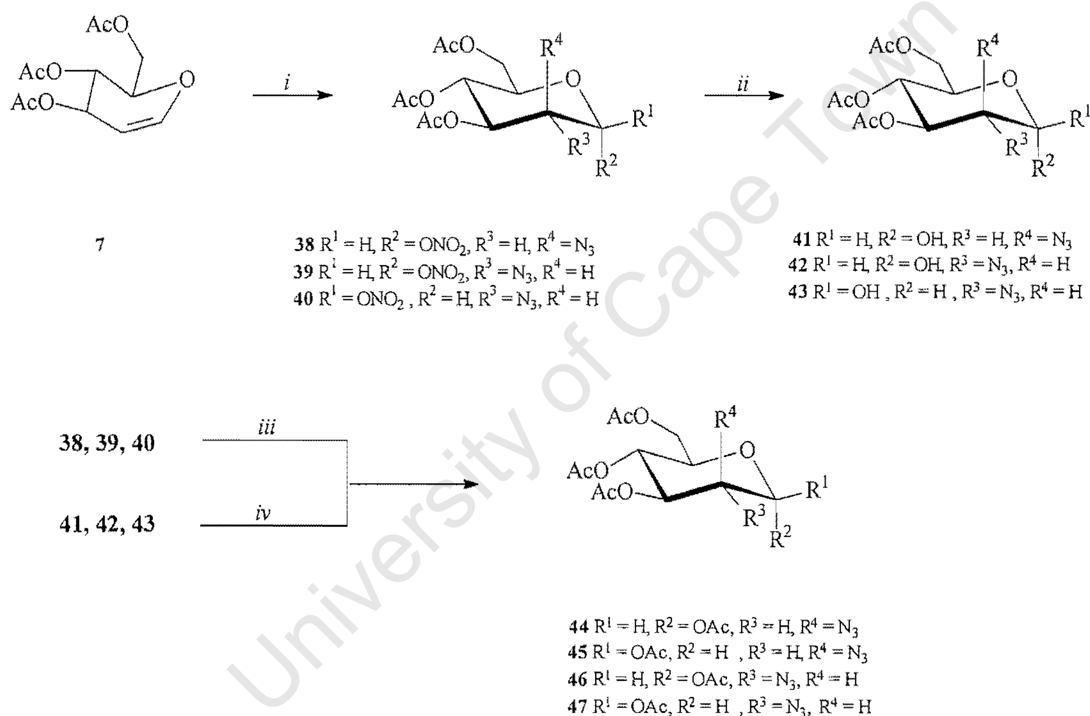
From the above mentioned methods for azide introduction, only two approaches were investigated in this project for the synthesis of the axially oriented 2-azido 2-deoxy sugar **8**. The choice was based on balancing considerations such as the practicality of the approach, availability of starting materials and compatibility in the required protecting groups. The first method investigated was the classical method of Lemieux's azidonitration of glycal followed by acetylation of the nitrate and subsequent de-*O*-acetylation.

2.2.1 Azidonitration of 3,4,6-Tri-*O*-acetyl-D-glucal

Following the method of Lemieux[41], tri-*O*-acetyl glucal 7 was treated successively with sodium azide and CAN, giving an 84 % yield of a mixture of azido nitrates **38**, **39**, and **40**. Infrared bands at 2119 and 1670 cm^{-1} of the product confirmed the presence of an azide and a nitrate, respectively. The ^{1}H NMR spectrum showed three anomeric peaks at δ 5.592, 6.191, and 6.308 in a ratio of 1:2:1 that correspond to three of the possible stereoisomers. On the basis of the coupling constants (J value) and the fact that axial-axial couplings ($J_{a\text{-}a}$) are greater than axial-equatorial couplings ($J_{a\text{-}e}$), which are in turn

greater than equatorial-equatorial couplings (J_{e-e}), the signals were assigned to 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-β-D-glucopyranosyl nitrate **40**, 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-mannopyranosyl nitrate **38**, and 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-glucopyranosyl nitrate **39**, respectively. The $J_{1,2}$ value (8.8 Hz) of the β-glucose is the largest due to the *a-a* coupling between H_1-H_2 and that of the α-mannose (2.0 Hz) the smallest because of the *e-e* coupling between H_1-H_2. The presence in the ^{13}C spectrum of three signals for anomeric carbons at δ 96.30, 97.12 and 97.72, and nine for carbonyl carbons in the range 169 – 171 ppm confirmed that the product is a mixture of the above mentioned three isomers.



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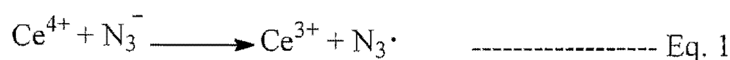
(*i*) NaN_3, CAN, CH_3CN, -20 °C, 84 %; (*ii*) PhSH, Hünig's base, CH_3CN, RT, 98 %; (*iii*) Ac_2O, Et_3N, DMAP, CH_2Cl_2, RT, 100 %; (*iv*) CH_3COONa, AcOH, 100 °C, 95%.

Scheme 9

Though the mechanism of the azidonitration is not still clear, some studies done by Trahanovsky *et al.*[51,52] led to a reasonable hypothesis for the mechanism. On studying oxidation of organic compounds with cerium(IV) to form α-azido-β-nitratoalkanes from olefins, sodium azide, and CAN, they found out that the regioselectivity of the reaction

was consistent with the initial addition of the azide. If that is the case, the reaction presumably involves addition of an azide radical to form a radical intermediate containing the azide group. The azide radical can be generated according to the reaction shown in equation (1).



$$Ce^{4+} + N_3^- \longrightarrow Ce^{3+} + N_3\cdot \quad \text{------------------ Eq. 1}$$

$$2N_3\cdot \longrightarrow 3N_2 \quad \text{----------------- Eq. 2}$$

The azide radical is trapped by the olefins in preference to forming nitrogen gas (Eq. 2). The overall reaction then proceeds as shown in **Scheme 10** where the resulting anomeric radical 2-azido-2-deoxypyranosyl **48** is oxidised by cerium(IV) to form the oxocarbenium ion **49** which can then react with the nucleophilic nitrate ion on either the α- or β- face depending probably on the orientation of the C-2 azide and the anomeric effect. Alternatively, the 2-azido-2-deoxypyranosyl radical **48** may also react directly with the ONO_2Ce^{+4} to give 3,4,6-tri-*O*-acetyl-2-azido-2-deoxypyranosyl nitrate **51**; or the radical intermediate **48** and the nitrate may form a 3,4,6-tri-*O*-acetyl-2-azido-2-deoxypyranosyl nitrate complex **50**, which is then oxidized by cerium(IV) to yield 3,4,6-tri-*O*-acetyl-2-azido-2-deoxypyranosyl nitrate **51**. All of the possible routes lead, anyway, to the same regioselectivity.

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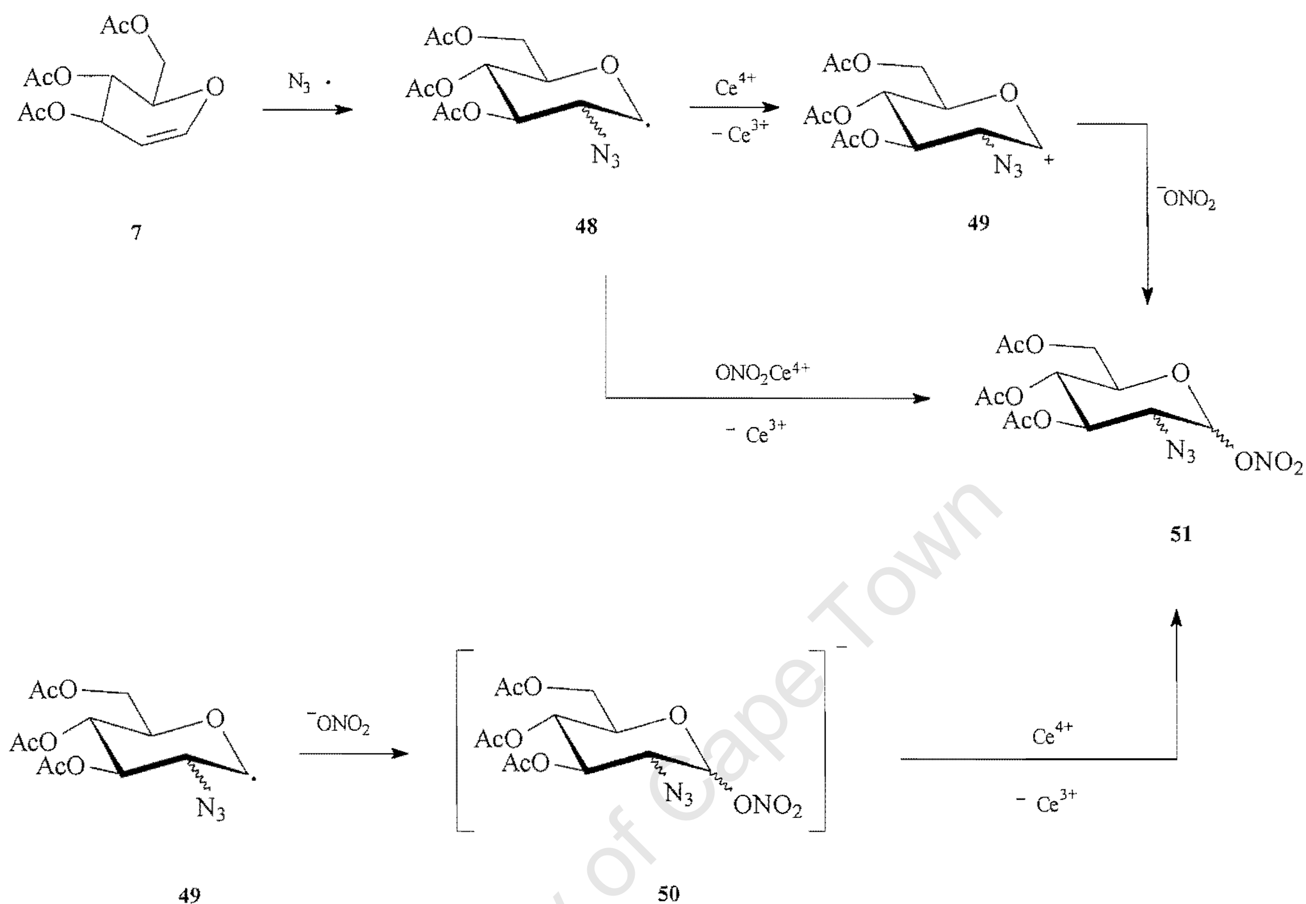

Scheme 10

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Regarding the stereoselectivity of the reaction, Seeberger *et al.*,[53] noted that it is greatly dependent on the configuration at C-4 and the protecting groups present. They found that in the case of D-galactal, the steric hindrance due to the axially oriented C-4 group resulted in good selectivity for equatorial addition of the azide radical. However, in the case of D-glucal with an equatorial C-4 group, varying selectivity was obtained. In such cases the selectivity was dependent upon the reaction temperature, ratio of reactants, and the protecting groups present.

Seeberger *et al.* found the 2-azido-2-deoxy-D-glucose in 8:1 excess over the D-mannose derivative when they carried out the azidonitration of the tri-*O*-acetyl D-glucal 7 at –15 °C to RT.[53] In contrast, we found a 1:1 ratio of the products by following a similar procedure to Seeberger's one but maintaining the temperature of the reaction at –20 °C throughout

the course of the reaction. This demonstrated that selectivity of the reaction is temperature dependent. The 1:1 selectivity at higher temperature could be attributed to the fact that neither face of the tri-*O*-acetyl D-glucal is particularly hindered in accordance with the molecular modeling results obtained by Seeberger *et al.*.[53]

In our case, the reason why a β-mannose was not formed could be attributed to the fact that the axial azide may have induced steric hindrance to the attacking nitrate ion or nitrate complex (see **Scheme 10**) or could be due to anomeric effect. However, in the case where the azide is oriented equatorially, the incoming nitrate moiety doesn't experience any steric constraint and can hence, attack equally on both faces of the sugar intermediate at the same rate resulting in a 1:1 ratio of α and β glucoses.

Separation of the isomers using column chromatography was impossible at this stage as the products had almost the same R_f. However, it is known that the anomeric nitrate group can be converted into other functionalities such as hydroxyl[54,55], acetyl[41], and halides[41]. In this case, it was converted into a hydroxyl group by treatment with thiophenol and triethylamine in acetonitrile to give 3,4,6-tri-*O*-acetyl-2-azido-2-deoxymannopyranoses **41**, **42**, and **43** (95 % yield), in the hope that these isomers were separable. Absence of a nitrate peak at around 1670 cm^{-1} and appearance of a broader –OH absorption in the 3300 – 3600 cm^{-1} region in the IR spectrum of the compound confirmed hydrolysis of the nitrate in the 2-azido-2-deoxy-D-manno and –D-gluconitrates **38**, **39**, and **40**. The upfield shift of the anomeric signals in the ^1H NMR also proved hydrolysis of the nitrate. Though a change in R_f values was observed, the differences were not enough for easy resolution of the isomeric mixture by column chromatography. However, further acetylation of the anomeric hydroxyl groups yielded separable acetylated products consisting of mixtures of α and β anomers of tetra-O-acetylated-2-azidomannose and 2-azidoglucose, respectively. As the nitrate group can be also converted into an acetyl function group, the products from the azidonitration were alternatively acetylated using acetic acid and sodium acetate at 100 °C to yield chromatographically separable products, 1,3,4,6-tetra-*O*-acetyl-2-azido-2-deoxy-α-D-mannopyranose **44**, 1,3,4,6-tetra-*O*-acetyl-2-azido-2-deoxy-β-D-mannopyranose **45**, 1,3,4,6-tetra-*O*-acetyl-2-azido-2-deoxy-α-D-glucopyranose **46**, and 1,3,4,6-tetra-*O*-acetyl-2-azido-2-deoxy-β-D-glucopyranose **47** in overall yield of 98 %. The desired 2-azido-2-

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deoxymannoses **44** and **45** had physical and spectroscopic properties consistent with the literature value.[46] The presence of four acetyl peaks at *ca* δ 2.1 in the ^{1}H NMR spectrum and four carbonyl peaks at *ca* δ 170.0 in the ^{13}C NMR spectrum confirmed the presence of four acetyl groups in each of the peracetylated 2-azido-2-deoxymannoses **44** and **45**. As summarised in **Table 1**, the anomeric doublet with the $J_{1,2}$ = 2.0 Hz and doublet of doublets at *ca* δ 5.4 with the $J_{2,3}$ = 3.7 Hz and $J_{3,4}$ = 9.8 Hz for H-3 confirmed that the azide is axially oriented. Since the coupling between H-1 and H-2 in the peracetylated 2-azido-2-deoxymannose **44** is *e-e*, the $J_{1,2}$ value was found to be in the expected range, 1 – 2 Hz. H-3 is coupled to H-4 and H-2, with a large *a-a* coupling between H-3 and H-4 and further coupling with H-2, resulting in a doublet of doublets for H-3. In the case of peracetylated 2-azido-2-deoxyglucoses **46** and **47**, the $J_{1,2}$ values were greater than that of the corresponding mannoses **44** and **45** and the signal for H-3 was an overlapping doublet of doublets due to almost identical *a-a* coupling with both H-2 and H-4 as shown in **Table 1**.

Table 1: Comparison of the multiplicity and *J* value of selected signals of peracetylated 2-azido-2-deoxypyranoses **44, 45, 46**, and **47**.

Compound	Proton	Multiplicity	J value, Hz
Mannoses **44** and **45**	H-1α	d	2.0
	H-1β	d	1.4
	H-3	dd	3.7 and 9.8
Glucoses **46** and **47**	H-1α	d	3.7
	H-1β	d	8.6
	H-3	dd	9.4 and 10.4

In order to obtain 2-azido-2-deoxymannopyranose **8**, peracetylated mannoses **44** and **45** were treated with a catalytic amount of sodium methoxide in methanol[56] (**Scheme 11**) and after 20 minutes a polar product was formed as judged by TLC. After purification through silica gel column chromatography, the 2-azido mannose **8** was obtained as an oil in 90 % yield. The spectroscopic data were consistent with the structure of the compound. ^{1}H and ^{13}C NMR spectra showed absence of any acetyl signals. The anomeric proton signals appeared at δ 5.204 and 4.972 in the ^{1}H NMR spectrum, and the integration of the

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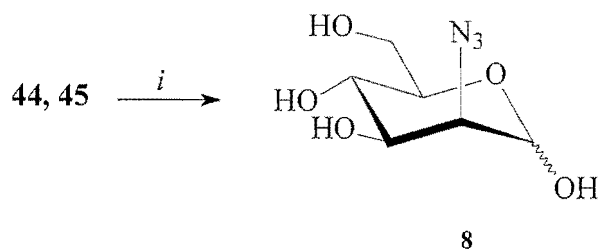
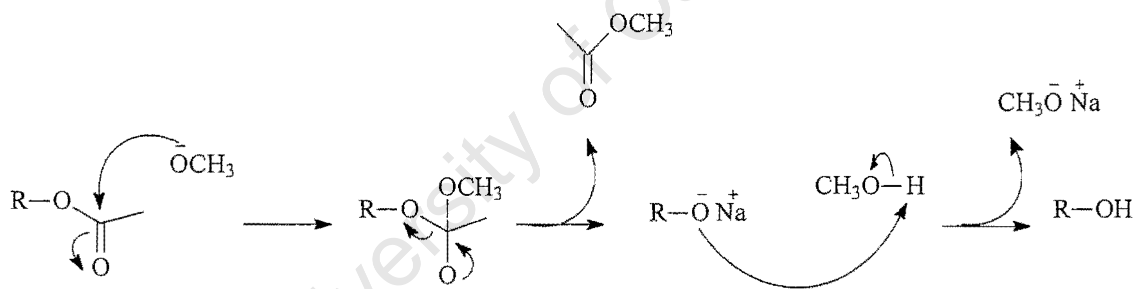
^{1}H NMR signals was consistent with the number of protons in the compound. The ^{13}C NMR spectrum showed exactly twelve signals that correspond to the carbons in the mixture of isomers that constitute azido mannose **8**. The mechanism for the de-*O*-acetylation is shown in **Scheme 12**.



(i) NaOMe, MeOH, RT, 90 %

Scheme 11



Scheme 12

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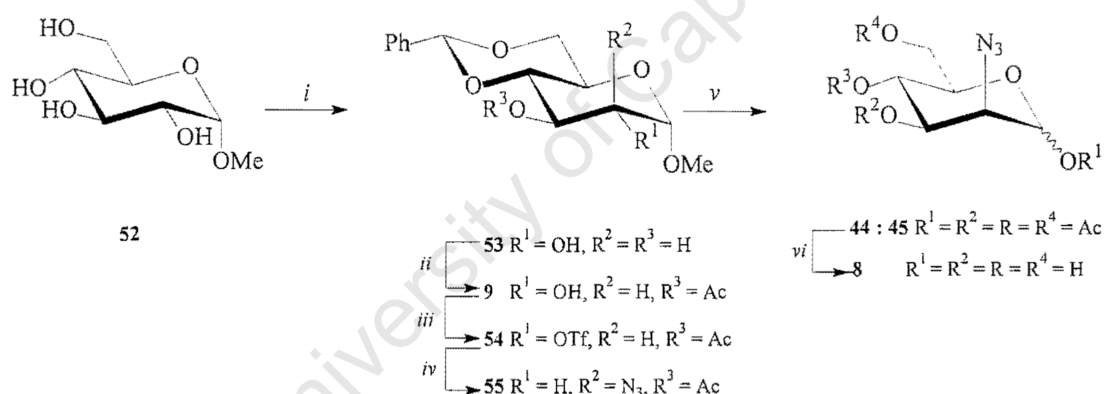
In our experience, for a small-scale (less than 1.00 gm of glucal) synthesis of the desired product, the protocol followed provided a reasonable yield (ca 50 %). However, on scaling up, significant practical difficulties were encountered. The reagents stick together at the bottom of the reaction flask, and effective stirring becomes difficult. Also, maintaining the temperature of the reaction at –20 °C for 3 h was difficult with available apparatus and in addition, a big column and a large volume of mobile solvents are needed to obtain a reasonable yield. Therefore, there was a need for us to look for a better method of obtaining the target key intermediate in order to proceed to the next stage of the project.

2.2.2 Azide S_N2-Displacement of 2-*O*-Triflate

The second method investigated for obtaining 2-azido mannose derivatives was the S_N2 displacement of a 2-*O*-triflyl glucoside. This involves selective triflation of a 2- hydroxyl group of methyl α-D-glucopyranoside. The strategy followed here was similar to that described by Oscarson *et al.* during the synthesis of an artificial antigen that corresponds to a disaccharide repeating unit of the capsular polysaccharide of *Haemophilus influenzae* type d.[49]

The first step was to protect the 6- and 4-hydroxyls of methyl α-D-glucopyranoside using Evans' method[57] followed by selective protection of the 3-O position leaving the 2-O hydroxyl for triflation.[49,58]



(*i*) benzaldehyde dimethylacetal, *p*TsOH, DMF, 70 °C, 80 %; (*ii*) a. NaH, THF, RT; b. $CuCl_2$; c. Ac_2O, 0 °C→RT, 80 %; (*iii*) Tf_2O, CH_2Cl_2-pyridine, 0°C; (*iv*) NaN_3, DMF, 70 °C, 86 %; (*v*) Ac_2O, AcOH-H_2SO_4 (1:41 v/v), RT, 90 %; (*vi*) NaOMe, MeOH, RT, 90 %.

Scheme 13

Methyl α-D-glucopyranoside **52** was treated with a molar equivalent of α,α-dimethoxytoluene (benzaldehyde dimethyl acetal) in *N,N*-dimethylformamide containing catalytic amount of *p*-toluenesulfonic acid monohydrate[57] to afford the methyl 4,6-O-

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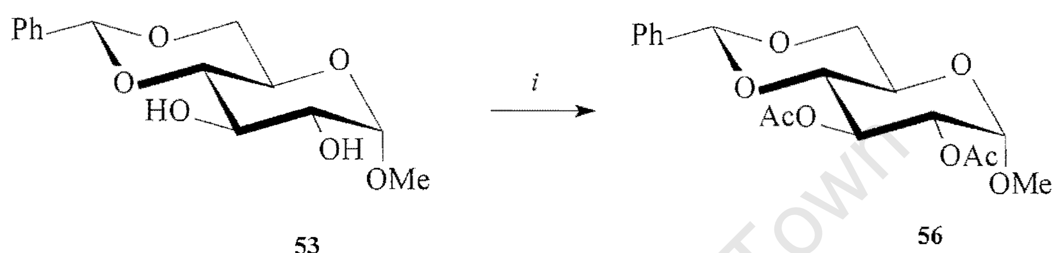
benzylidene-α-D-glucoside **53** in 80% yield. The presence of aromatic signals in the range δ = 7.504 – 7.354 and a characteristic singlet of the benzylidene (acetal) proton at δ 5.526 in the ^{1}H NMR spectrum indicated formation of a benzylidene ring in the methyl glucoside **53**. In order to fully characterize the compound; it was peracetylated using acetic anhydride, triethylamine and DMAP to give acetylated glucoside **56** in 95 %. The physical data of the product were consistent with those reported by Evans.[57]



(i) Acetic anhydride, Et_3N, DMAP, CH_2Cl_2, RT, 95 %.

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Scheme 14

Regioselective blocking of the 3-*O*- hydroxyl of the methyl glucoside **53** was achieved by the method developed by Eby *et al.*.[58] A solution of methyl glucoside **53** in THF was treated with sodium hydride and when evolution of hydrogen gas ceased, copper (II) chloride was added followed by acetic anhydride to yield the 3-*O*-acetylated ester **9** (80%). The compound was identified from its ^{1}H NMR data. The presence of a singlet integrating for three protons at δ 2.116 indicated a monoacylated product. The position of the single acetate can be established on the basis of the type and peak frequencies of H-2 and H-3 (potential positions of the acetyl). In most cases, protons H-2, H-3, and H-4 of acetoxylated carbon atoms C-2, C-3 and C-4 appear in the range of δ 3.8 – 5.7. Also, as mentioned earlier, *a-a* coupling gives larger coupling constant (9-10 Hz) compared to *a-e* (2.2-3.5 Hz) and *e-e* (1-2 Hz). An overlapping doublet of doublets (due to almost equal a_3-a_2 and a_3-a_4 J value) signal at δ 5.321 having a coupling constant of 9.8 Hz confirmed that the 3-OH was the one acetylated. If the 2-OH had been acetylated, then instead of the

overlapping doublet of doublets, a sharp doublet of doublets (*dd*) for H-2 would have appeared in the region due to unequivalent a_2-a_3 and a_2-e_1 coupling constants, and H-3 would have probably appeared further upfield than its present position. Comparison of the ^{1}H-NMR spectra of glucosides **56** and **9** confirms the structure of methyl glucoside **9** explicitly. The doublet of doublets corresponding to H-2 at δ 4.908 in the ^{1}H NMR spectrum of peracetylated **56** is absent in the ^{1}H NMR spectrum of methyl glucoside **9**, which confirms that the 2-OH in methyl glucoside **9** is not acetylated.

The regioselectivity was achieved owing to the difference in the acidity of the two hydroxyls. The sodium hydride abstracts the protons of the hydroxyl groups to form sodium alkoxides. The resulting dianion chelates with copper (II) to form a green copper complex (the color confirms the formation of the copper complex). Since the 2-hydroxyl is stronger acid than the 3-hydroxyl, the 2-alkoxide forms a stronger bond with the copper than that of the 3-alkoxide. Hence, O-2 becomes less nucleophilic than the O-3 and as a result the O-3 anion reacts faster than the O-2 to give methyl glucoside **9**.[58]

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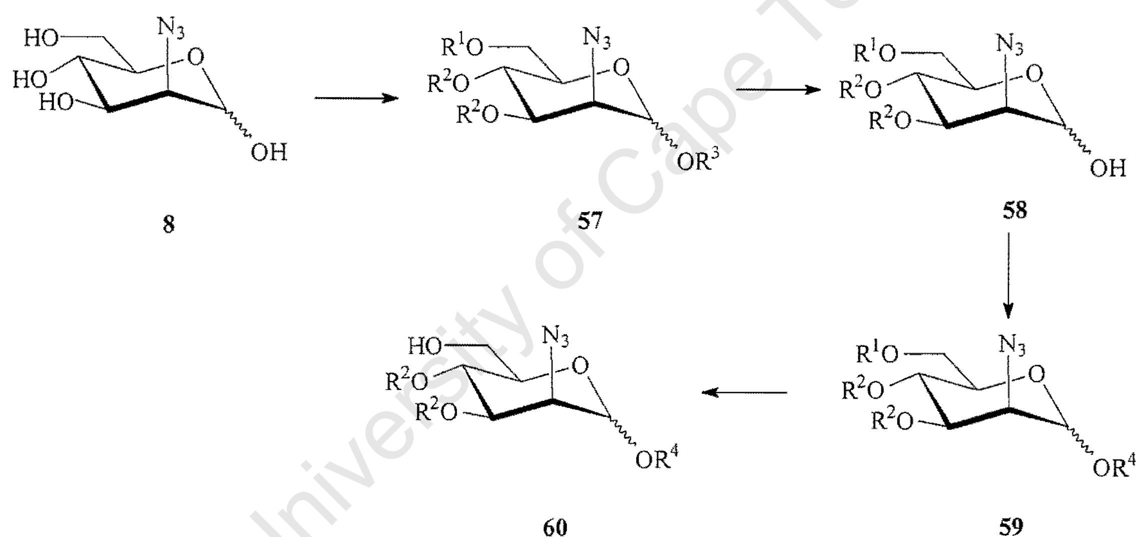
Methyl glucoside **9** was transformed to the 2-*O*-triflate after treatment of a solution of the compound in a 1:1 mixture of dichloromethane-pyridine with trifluoromethanesulfonic anhydride. A solution of the crude product in DMF was then treated with sodium azide at 70 °C to afford the 2-azido-2-deoxymannoside **55** in 86 % yield (from methyl glucoside **9**).[49] The α-D-*manno* configuration of the compound was assigned by ^{1}H NMR data. The singlets at δ 3.404 and 2.130 correspond to the OMe and OAc of the compound, respectively. The benzylidene singlet appears at δ 5.571. The anomeric signal at δ 4.696 has a $J_{1,2}$ value of 1.5 Hz, which is less than that of methyl glucoside **9** ($J_{1,2}$ = 4.0 Hz) confirming that the orientation of H-2 in 2-azido-2-deoxymannoside **55** is equatorial (in other words the 2-azide is axial) as *J* of *e-e* < *a-e*. The signal for H-3, which appeared, as an overlapping doublet of doublets in methyl glucoside **9**, is now a resolved doublet of doublets at δ 5.432 implying that the configuration at C-2 has changed and the 2-azide is axial.

2-Azido-2-deoxymannoside **55** was then subjected to acetolysis[59] in acetic anhydride and acetic acid – sulfuric acid (41:1 v/v) solution and after purification afforded the

peracetylated 2-azido-2-deoxymannoses **44** and **45** in 87 % overall yield. The physical and NMR data were in agreement with the literature values and with the same compounds synthesised as described earlier (**Scheme 9**). 2-Azido mannose **8** was obtained by de-*O*-acetylation of peracetylated 2-azido-2-deoxymannoses **44** and **45** as before (**Scheme 11**).

After obtaining the 2-azido mannose **8**, with the required regio and stereo orientations, the next challenge was the selective protection and deprotection at O-6 and O-1 of the mannose while keeping O-3 and O-4 protected (**Scheme 15**).



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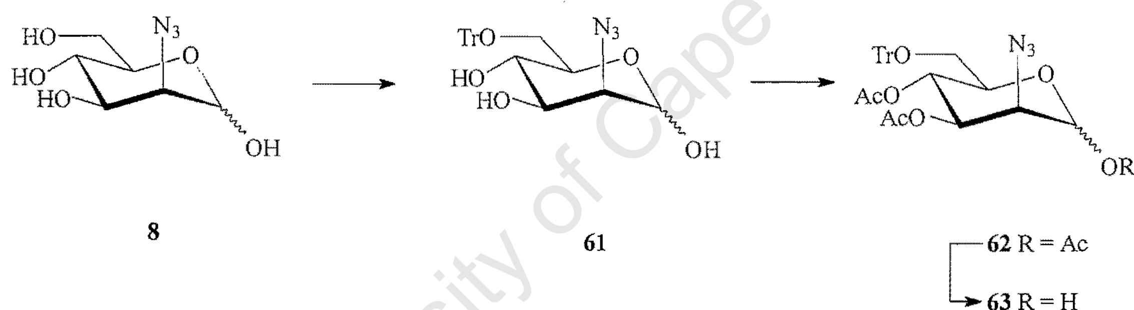
Scheme 15

2.3 SYNTHESIS OF SELECTIVELY PROTECTED 2-AZIDO MANNOSE 5

By inspection of structure of 2-azido mannose **8**, differences in the reactivity of the hydroxyls can be expected. The primary C-6 and the anomeric hydroxyls can be expected to have different reactivities from each other and the rest of hydroxyls.

2.3.1 Tritylation

As shown below in **Scheme 16,** during the course of our study towards the synthesis of compound **5** (**Scheme 1**), an attempt was made to synthesise a 6-*O*-trityl mannose from 2-azido mannose **8**.



Scheme 16

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The trityl group is widely used as a blocking group in synthesis for primary hydroxyls of carbohydrates. The classical method for the tritylation of carbohydrates is carried out in absolute pyridine and involves reaction of the substrate containing hydroxyls with a trityl chloride at a temperature ranging from room temperature to 100 °C.[60] The reaction proceeds by initial formation of a *N*-tritylpyridinium salt from the reaction between trityl chloride and the solvent (pyridine). The salt then gives the corresponding tritylated compound and pyridinium chloride on treatment with the substrate alcohol.[61]

Scheme 17

The importance of the trityl group in carbohydrate chemistry is attributed due to:[62]

i. the excellent regio-selectivity of the trityl cation towards primary hydroxyls

ii. cleavage of trityl ether under mild reaction conditions and;

iii. the advantage that many carbohydrates are soluble in pyridine.

The bulkiness of the trityl group enhances the affinity of the reagent towards the sterically less hindered hydroxyl. But very rare exceptions are reported in which a secondary hydroxyl is tritylated and a more frequent instance where there is an interaction between an anomeric hydroxyl with the tritylating agent.[62] However, under standard reaction conditions tritylation of hydroxyls other than the primary ones is relatively very slow[60] and as a result monotritylation can be achieved by using one equivalent of the reagent.[62]

Trityl ethers may be cleaved under acidic conditions or by hydrogenation depending on the substrates. Among the most common protocols are:

i. boiling of the ether with 80 % acetic acid,[62]

ii. treatment of the ether with 1 M of aq. HCl in dioxane,[63]

iv. treatment of the ether with sodium in ammonia,[64] and

v. catalytic hydrogenolysis of the ether.[62]

Although the tritylation and deprotection of trityl ether seem to be simple, treatment of a solution of 2-azido mannose **8** in pyridine with trityl chloride did not produce a product as judged by TLC even after stirring at RT for a week. Acetylation of the reaction mixture resulted in peracetylated 2-azido-2-deoxymannoses **44** and **45** implying that the tritylation process did not take place at all.

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Chaudbary *et al.* reported[61] a high yield of mono-tritylated α-methylglucoside using DMAP, triethylamine, and DMF instead of pyridine as a base and solvent, respectively. We accordingly attempted to introduce a trityl group into 2-azido mannose **8** using this method, but the reaction was unsuccessful. Though we did not conduct detailed studies as to why the reactions failed to give desired products, the failure of the reaction could probably be that since an azide ion is a linear tri-atomic species and hence the axially oriented rod-like azide might have caused steric hindrance for the incoming trityl cation;

2.3.2 Deprotection of Anomeric Oxygen

With the failure of the tritylation route, we looked for another route that employs readily available starting materials and gives a high yield of the required compounds. The route shown in **Scheme 18** seemed feasible.

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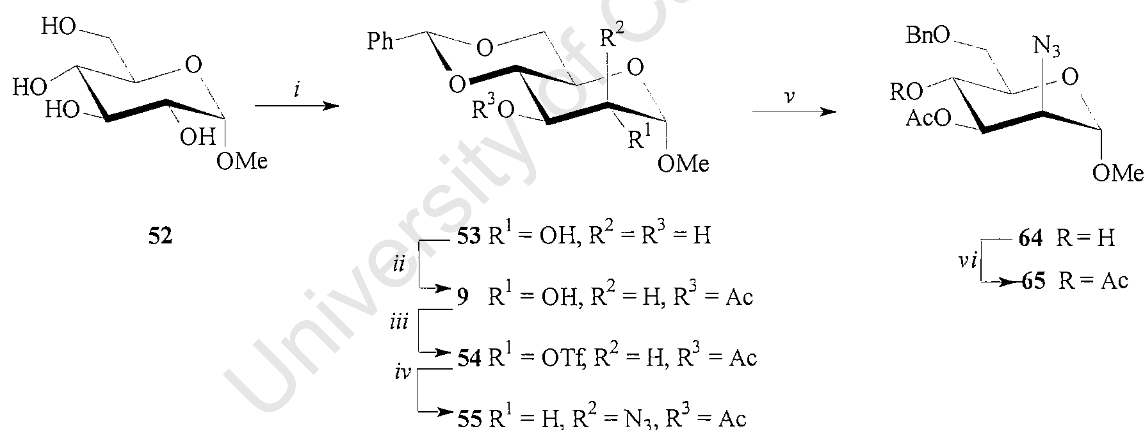
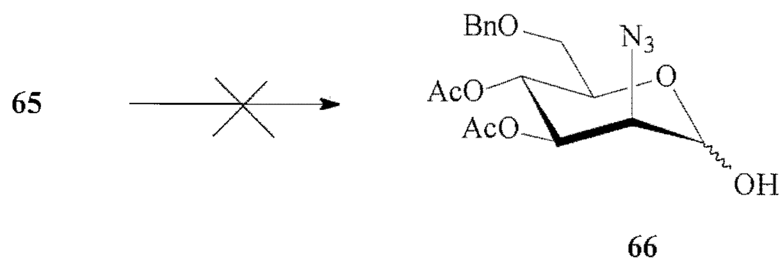


(*i*) benzaldehyde dimethylacetal, *p*TsOH, DMF, 70 °C, 80 %; (*ii*) a. NaH, THF, RT; b. $CuCl_2$; c. Ac_2O, 0 °C→RT, 80 %; (*iii*) Tf_2O, CH_2Cl_2-pyridine, 0 °C; (*iv*) NaN_3, DMF, 70 °C, 80 %; (*v*) $NaCNBH_3$, HCl in ether, THF, RT, 75 %; (*vi*) Ac_2O, DMAP, Et_3N, CH_2Cl_2, RT, 85 %.



Scheme 18

2-Azido-2-deoxymannoside **55** was obtained as explained in **Scheme 13**. When the 2-azido-2-deoxymannoside **55** was treated with sodium cyanoborohydride and hydrogen chloride in THF, the benzylidene ring was opened regioselectively towards the C-6 to give methyl 3-*O*-acetyl-2-azido-6-*O*-benzyl-2-deoxymannopyranoside **64** in 75 % yield.[65] The structure of the compound was confirmed by ^{1}H and ^{13}C NMR spectroscopy. The absence of a singlet due to the benzylidene proton peak at δ 5.571 and appearance of an AB-quartet for benzylic protons at δ 4.602, as well as a broad singlet at δ 2.814 for O*H* confirmed the opening of the benzylidene ring in the 2-azido-2-deoxymannoside **55**. After D_2O wash the ^{1}H NMR spectrum showed that the OH is attached to C-4. In addition, the appearance of the C-6 peak at *ca* δ 68.0 in the ^{13}C NMR spectrum of the compound confirmed that the 6-OH is still protected. The singlet peaks at δ 2.149 and 3.375 of the ^{1}H NMR; and characteristic carbonyl at δ 171.04 and methoxy peaks at δ 55.30 in the ^{13}C NMR spectra corresponding to acetyl and methyl groups, respectively, confirmed the 6-*O*-benzylated mannoside **64**. Acylation of 6-*O*-benzylated mannoside **64** with acetic anhydride, DMAP, and triethylamine in dichloromethane yielded 3,4-di-*O*-acetyl-2-azido-6-*O*-benzyl-2-deoxymannopyranoside **65** in 80 % yield. Two singlet acetoxy methyl peaks at *ca* δ 2.1 in the ^{1}H NMR and also two carbonyl peaks at *ca* δ 170.0 in the ^{13}C NMR confirmed presence of two acetoxy groups in the compound.

Different attempts were made to selectively cleave the methyl mannoside **65**. Jansson *et al.* synthesized 2,3,4,6-tetra-*O*-benzyl-β/α-D-glucopyranose from the corresponding methyl α-D-glucopyranose derivative.[66] Following their procedure, a solution of methyl mannoside **65** in acetic acid was treated with trifluoromethanesulfonic acid at 80 °C. After 1 h TLC showed the presence of two products, neither of which was the desired

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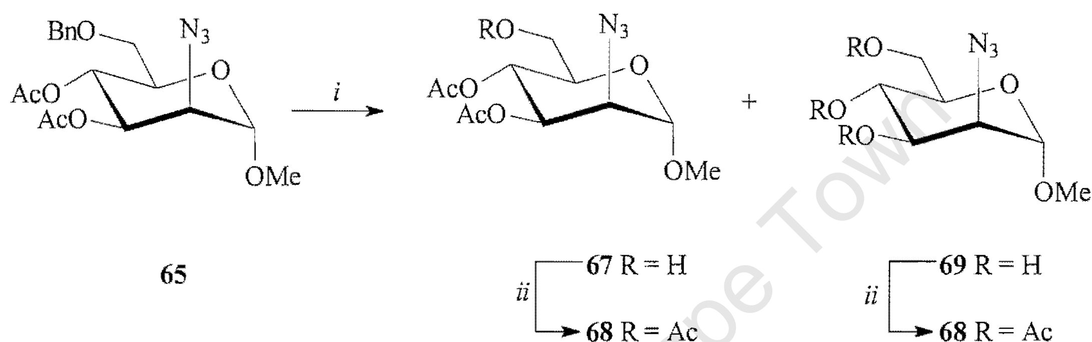
methyl 3,4-di-*O*-acetyl-2-azido-6-*O*-benzyl-2-deoxymannopyranoside **66** (**Scheme 19**). The less-polar product was identified as methyl 3,4-di-*O*-acetyl-2-azido-2-deoxymannopyranoside **67** where the benzyl had been removed and the more-polar had retained the methyl group but lost the other protecting groups (the benzyl and acetyls) *i.e* methyl 2-azido-2-deoxymannopyranoside **69**.



(*i*) AcOH, trifluoromethanesulfonic acid, 80 °C; (*ii*) Ac_2O, pyridine, RT.

Scheme 19

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The structures were deduced from ^1H NMR spectra. The ^1H NMR spectrum of methylmannoside **67** showed neither the aromatic nor the benzyl protons' characteristic signals although two singlet acetoxy methyls and a methoxy signals were identified at *ca* δ 2.1 and 3.4, respectively. In contrast, in methyl 2-azido-2-deoxymannopyranoside **69**, only a methoxy signal appeared at *ca* δ 3.4 of the ^1H NMR spectrum. Both methyl mannosides **67** and **69** gave peracetylated methyl mannoside **68** on acetylation with the structure of peracetylated methyl mannoside **68** again assigned by ^1H NMR

When selective deprotection of the *O*-methyl failed, attempts were made to convert the *O*-methyl in methyl mannoside **65** to a phenylsulfanyl by treating it with $PhSSiMe_3$ and $BF_3.Et_2O$ in dichloromethane at room temperature.[67] However, the reaction was not successful at all as starting material was recovered from the reaction mixture, and a stronger Lewis acid may be required to for this reaction.

Though the described route did not provide us with the desired intermediate **66**, it helped us to obtain 2-azido mannose **8** in a better yield than the route *via* azidonitration. In order to obtain the desired 3,4-di-*O*-acetyl-2-azido-2-deoxymannose (**5**), we therefore decided to look at alternative routes which did not utilize a methyl glycoside and incorporated a selectively removable anomeric substituent.

2.3.3 Benzylidene Acetals

The third methodological principle that we tried was to selectively protect the 6-OH with the *p*–methoxybenzyl group. It has been reported that this protecting group can be selectively introduced and cleaved in the presence of other protecting groups. It could undergo oxidative cleavage under neutral conditions using 2,3-dichloro-5,6-dicyanobenzoquinone in dichloromethane-water mixtures to give a free hydroxyl and *p*-methoxybenzaldehyde.[68] Acetyl, benzyl, benzoyl, BOM, isopropylidene acetals, MEM, MOM, TBS, THP, and tosyl protecting groups, and functional groups like epoxide, alkenes, and ketones are found to be tolerant to the reaction conditions.[69] Later, selective cleavage of this group can be optimized by using CAN in acetonitrile-water (9:1) mixtures to afford the desired alcohol without affecting acetyl, benzyl, and azide groups.[70,71,72,73] *p*-Methoxybenzyl has also been used as an *in situ*-removable protecting group in the simple one-pot synthesis of globotetraose tetrasaccharide using *N*-iodosuccinimide-trifluromethanesulfonic acid.[74]

The *p*-methoxybenzyl is introduced by etherification using *p*-methoxybenzyl chloride and sodium hydride in DMF or DMSO. But when selective protection is required in diols, regioselective reductive ring opening of *p*-methoxybenzylidene acetals results in *p*-methoxybenzyl ethers and a free hydroxyl.

Benzylidene acetals are frequently used in protection of diols. They show stability to most bases, mild oxidants, and metal hydrides, but they are readily cleaved by *N*-bromosuccinimide, ozone, and strong bases like alkyllithium reagents.[75,76] They also can be cleaved by hydrogenolysis in the presence of metal catalyst such as Pd and Pt, and acid hydrolysis. Another great virtue of benzylidene acetals is that they can undergo

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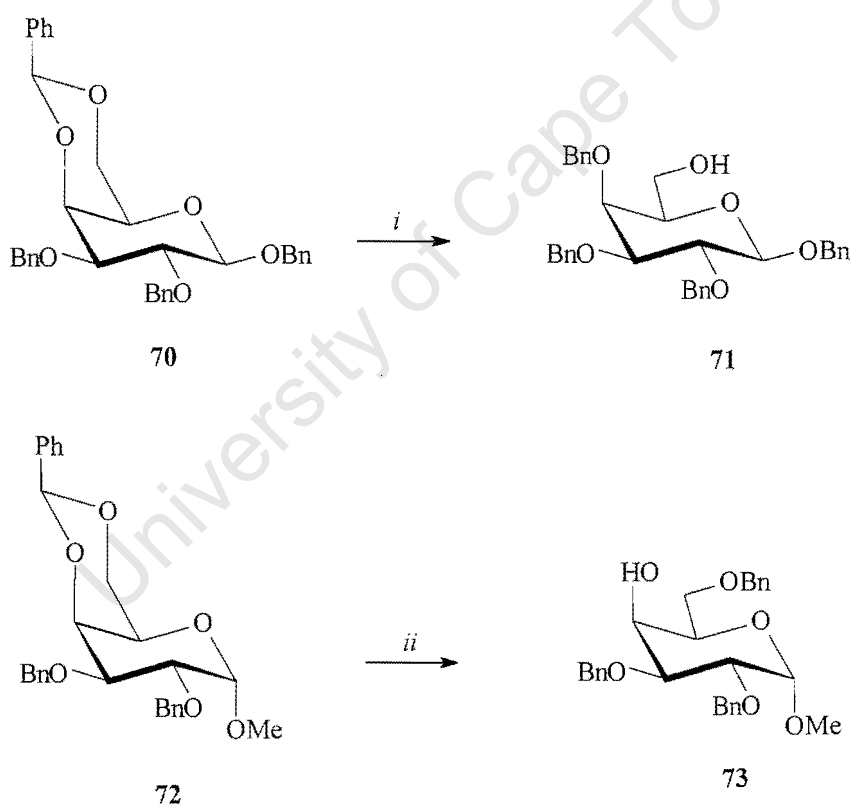
regioselective reductive ring opening to give a free OH and benzyl ethers. Because of their versatility, the reductive cleavage of benzylidene acetals of carbohydrates is well documented (**Scheme 20**).

Lipták and coworkers reported regioselective reductive cleavage of a benzylidene acetal using $LiAlH_4$-$AlCl_3$ to afford a free hydroxyl group at the less hindered position and the benzyl ether of the more hindered hydroxyl.[77] Garegg *et al* demonstrated that the benzyl ether of the less hindered hydroxyl and a free hydroxyl group at the more hindered position can be obtained with $NaCNBH_3$, and diethyl ether saturated with HCl in THF in good yield.[78]



(*i*) $AlCl_3/LiAlH_4$; (*ii*) $NaCNBH_3$, HCl, THF, 93 %.

Scheme 20

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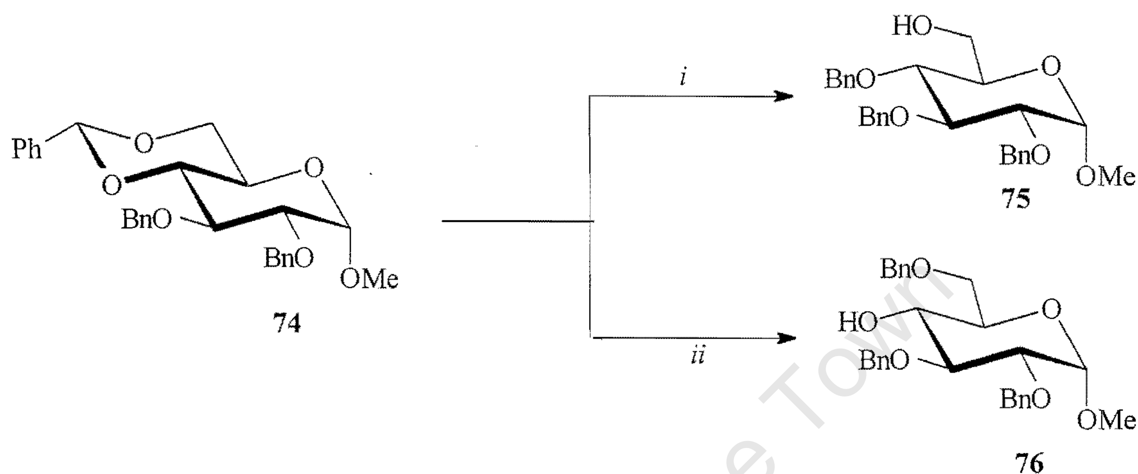
The same authors have also reported that in using $BH_3.NMe_3$ as reducing agent and $AlCl_3$ as the acid, the regioselectivity in the cleavage of the benzylidene acetal depends on the choice of the solvent though no explanation is given for this observation.[78] Using toluene as solvent, the benzyl group in the product positioned at *O*-4 and the 6-OH is free. With THF as solvent, the opposite result is obtained (**Scheme 21**).



(*i*) $Me_3NBH_3/AlCl_3$, toluene, 52 %; (*ii*) $Me_3NBH_3/AlCl_3$, THF, 72 %.

Scheme 21

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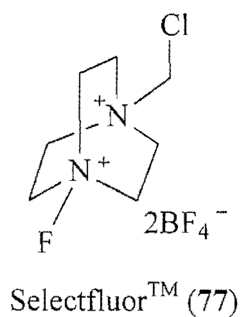
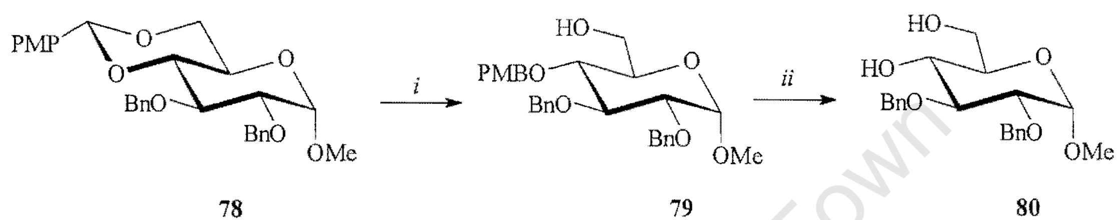
p-Methoxybenzylidene acetals, which have an electron donating group at the *p*-position, hydrolyse 10 times faster than the corresponding benzylidene acetals.[79] Deprotection of these protecting groups can be achieved using 80 % acetic acid or DDQ; however, a mild cleavage condition with Selectfluor™ **77** in CH_3CN or CH_3NO_2 having 5 % water content has been reported recently.[80] They also undergo similar kind of regioselective reductive ring opening of benzylidene acetals depending on reaction conditions, solvent, and electrophile used. Combination of reagents such as $LiAlH_4$-$AlCl_3$,[81] $BH_3.NMe_3$-$AlCl_3$,[82] BH_3 in THF at high temperature,[83] or $NaCNBH_3$-TMSCl in MeCN[70] results in the *p*-methoxybenzyl ether of the more hindered hydroxyl and a free hydroxyl at the less hindered position (**Scheme 22**).



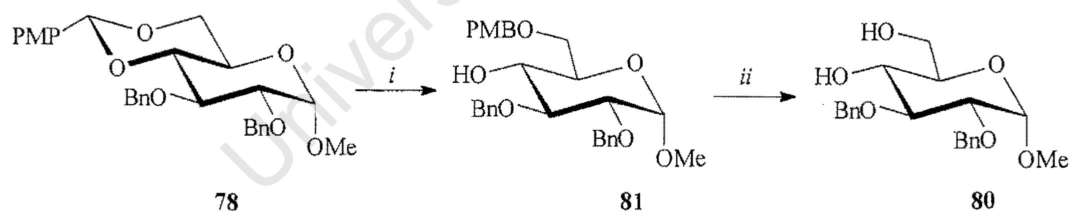
Selectfluor™ (**77**)



(*i*) $NaCNBH_3$-$(CH_3)_3SiCl$, CH_3CN, 76 %; (ii) CAN, CH_3CN-H_2O (9:1), 98 %.

Scheme 22

Nevertheless, by using $NaCNBH_3$ in a mixture of CF_3CO_2H and DMF the opposite result can be obtained, where the PMB ether is positioned at the less hindered 6-hydroxyl.[70]



(*i*) $NaCNBH_3$-CF_3CO_2H, DMF, 85 %; (*ii*) CAN, CH_3CN-H_2O (9:1), 95 %.

Scheme 23

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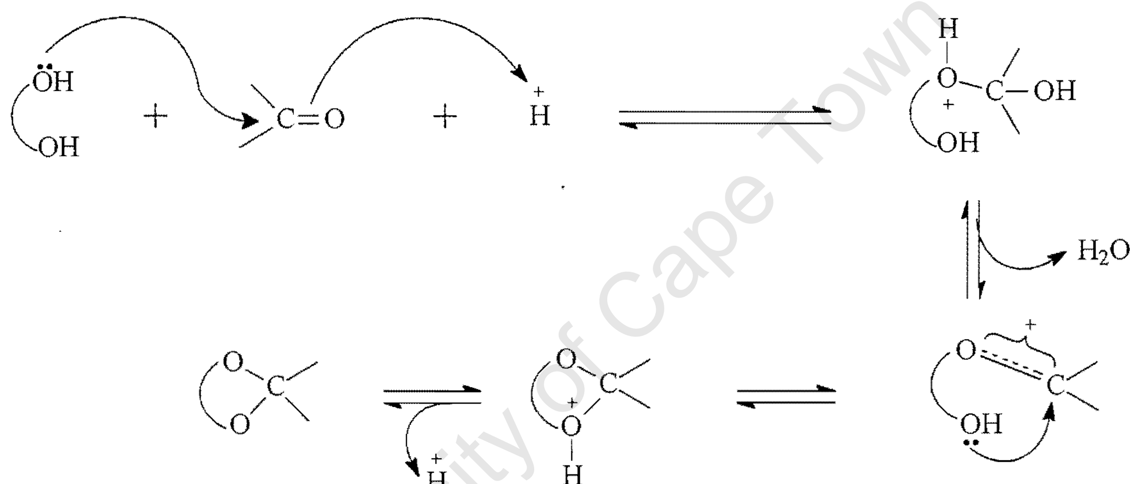
Benzylidene acetals are normally prepared by two methods:

(i) Direct condensation of a carbonyl derivative

This method involves the reaction of a diol with benzaldehyde or *p*-methoxybenzaldehyde in the presence of a protic acid or Lewis acid (usually zinc chloride is used).[84,85,86] One mole of water is formed as a by-product and has to be removed to avoid hydrolysis of the acetals. The reaction proceeds according to the following scheme.



Scheme 24

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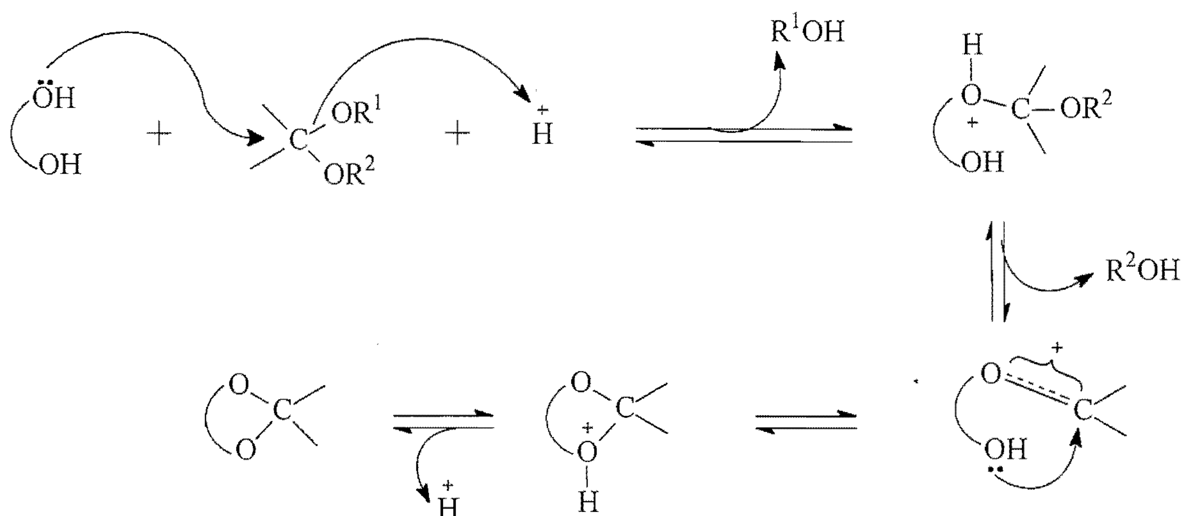
(ii) Transacetalation

This method is based on acetal exchange in the presence of catalytic amount of an acid. The reaction takes place using *p*-methoxybenzaldehyde dimethyl acetal or benzaldehyde dimethyl acetal in the presence of *p*-toluenesulfonic acid in DMF to prepare their respective benzylidenes as shown in **Scheme 25**.[57] The reaction can be carried out at high temperatures and reduced pressure in order to remove the two moles of methanol produced as by-product during the course of the reaction.



Scheme 25

The second method is more advantageous than the first method. In this transacetalation, strictly anhydrous reaction conditions can be followed whereas in the first continuous removal of water is required.

2.3.3.1 *p*-Methoxy benzyl ether

By virtue of its easy introduction, removal and compatibility with the protecting groups that are in our intermediate compounds, selective protection of the 6-OH with *p*-methoxybenzyl was taken as an option. Accordingly, a solution of 2-azido-2-deoxymannopyranose **8** and catalytic amount of *p*-toluenesulfonic acid in DMF was treated with *p*-methoxybenzaldehyde dimethyl acetal at 70 °C to afford 2-azido-4,6-(4-methoxybenzylidene)-2-deoxymannopyranose **82** in 75% yield. The compound and ring size of the compound were ascertained by ^{1}H NMR data. The doublets at *ca* δ 7.8 and 6.8 correspond to the two sets of protons on the aromatic ring of the *p*-methoxybenzylidene acetal. The acetal proton appeared at *ca* δ 5.5 confirming that the acetal is a six membered ring (dioxane ring), as does the acetal carbon at *ca* δ 102.1 in the ^{13}C NMR of the compound.[87] The methoxy carbon gave a signal at *ca* δ 3.8. The reaction was carried out under reduced pressure in order to remove the MeOH produced (see **Scheme 25**) so

that hydrolysis of the product wouldn't take place. The transacetalation takes place according to the mechanism outlined above (**Scheme 25**).

p-Methoxybenzylidene mannose **82** was taken up in dichloromethane for acetylation using acetic anhydride, catalytic amount of DMAP, and triethylamine to give 1,3-di-*O*-acetyl-2-azido-4,6-*O*-(*p*-methoxybenzylidene)-2-deoxymannopyranose **83** in 80 % yield. In the ^{1}H NMR spectrum of the compound, the characteristic proton signals of the aromatic ring appeared at *ca* δ 7.2 and 6.9. The acetal and methoxy proton signals showed up at *ca* δ 5.5 and 3.8, respectively. The presence of two acetyl groups in the compound was confirmed by the presence of two singlet acetoxy methyl signals at *ca* δ 2.1 in the ^{1}H NMR spectrum and two carbonyl signals *ca* δ 160 in the ^{13}C NMR spectrum of the compound. 1,3-Di-*O*-acetyl-2-azido-2-deoxy-6-*O*-(*p*-methoxybenzyl)mannopyranose **84** was obtained in 70 % yield by treating a solution of peracetylated ***p***-methoxybenzylidene mannose **83** in DMF with $NaCNBH_3$ and CF_3CO_2H.[70] Absence of an acetal proton signal at around δ 5.5, appearance of a AB-quartet at δ 4.513 and a broad singlet peak at *ca* δ 2.7 of an OH proved the opening of the acetal and formation of a *p*-methoxybenzyl ether in which the methoxy showed up at δ 3.802. D_2O-washed ^{1}H NMR spectrum showed that the OH is attached to C-4 and this was also supported by the appearance of C-6 signal at *ca* δ 68.1 in the ^{13}C NMR because protected C-6 of an ether appears at ca. δ 68.0.

The regioselectivity of this reaction could be mainly due to steric factors. Since the acetyl at O-3 is not bulky enough to exert steric hindrance to an incoming electrophile and also the H^+ is too small to experience such hindrance, protonation can either take place at O-4 or O-6 depending on their relative acidity. But primary alcohols are more acidic than secondary ones. And hence, O-4 is protonated in preference to O-6 and a *p*-methoxybenzylic cation intermediate is formed, which, is then stabilized by the slightly basic polar solvent, DMF.[70] Then reduction with the reducing agent is followed to give the corresponding 6-*O*-(*p*-methoxybenzyl) ether **84** as outlined in **Scheme 26**.

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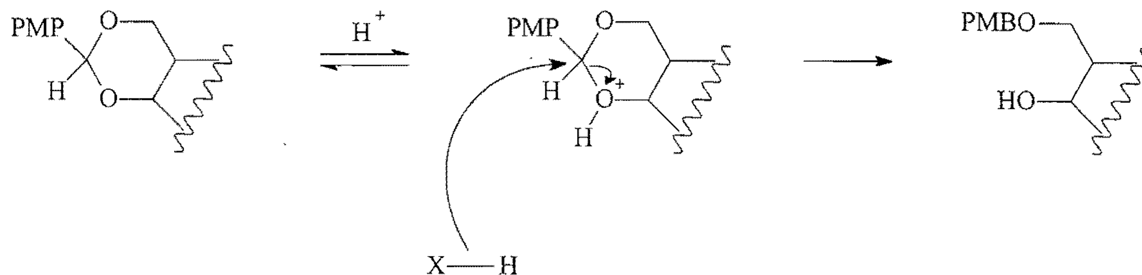

Scheme 26

Acetylation of 6-*O*-(*p*-methoxybenzyl) mannose **84** afforded 1,3,4-tri-*O*-acetyl-2-azido-2-deoxy-6-*O*-(*p*-methoxybenzyl)mannopyranose **85** in 85 % yield. The downfield shift of H-4 to δ 5.389 in peracetylated 6-*O*-(*p*-methoxybenzyl) mannose **85** from 4.102 in 6-*O*-(*p*-methoxybenzyl) mannose **84** (δ = 3.987 in the case of the β isomer) shows that the *O*-4 in 6-*O*-(*p*-methoxybenzyl) mannose **84** was not protected. And hence, moved downfield upon acetylation. Three acetoxy peaks corresponding to the three acetyls in peracetylated 6-*O*-(*p*-methoxybenzyl) mannose **85** appeared at *ca* δ 2.1 in the ^{1}H NMR spectrum. Carbonyl signals at δ 170.01, 169.34, and 168.24 in the ^{13}C NMR spectrum of peracetylated 6-*O*-(*p*-methoxybenzyl) mannose **85** indicated presence of three acetyl groups. The acetyl at the anomeric center was selectively removed after treatment of a solution of peracetylated 6-*O*-(*p*-methoxybenzyl) mannose **85** in DMF at 50 °C with 1.2 eq. of hydrazine acetate to afford 3,4-di-*O*-acetyl-2-azido-2-deoxy-6-*O*-(*p*-methoxybenzyl)mannopyranose **86** in 82 % yield.[88] ^{1}H NMR showed two signals at *ca* δ 2.0 indicating the presence of only two acetyls. Upfield shift of the anomeric peak from δ 6.090 to δ 5.159 in the ^{1}H NMR is also an indication of anomeric de-*O*-acetylation.

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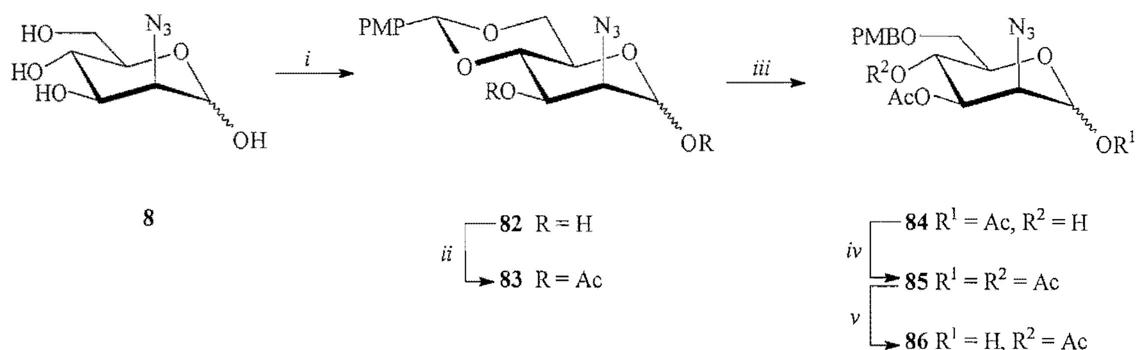

(*i*) *p*-methoxybenzaldehyde dimethylacetal, *p*-TsOH, DMF, 70 °C, 75 %; (*ii*) Ac_2O, DMAP, Et_3N, dichloromethane, RT, 80 %; (*iii*) $NaCNBH_3$, CF_3CO_2H, DMF, RT, 70 %; (*iv*) Ac_2O, DMAP, Et_3N, dichloromethane, RT, 85 %; (*v*) hydrazine acetate, DMF, 50 °C, 82 %.

Scheme 27

Synthesis of 6-*O*-(*p*-methoxybenzyl) mannopyranose **86** was a major achievement for our project. The next step was to couple 6-*O*-(*p*-methoxybenzyl) mannopyranose **86** with the linker benzyl *N*-(3-hydroxypropyl)carbamate **88** to obtain carbobenzoxyamino 3,4-di-O-acetyl-2-azido-2-deoxy-6-*O*-(*p*-methoxybenzyl)mannopyranoside **89** (**Scheme 28**). As the anomeric hydroxyl has to be transformed into a good leaving group in order for glycosidation to take place, 6-*O*-(*p*-methoxybenzyl) mannose **86** had to be transformed into an imidate.

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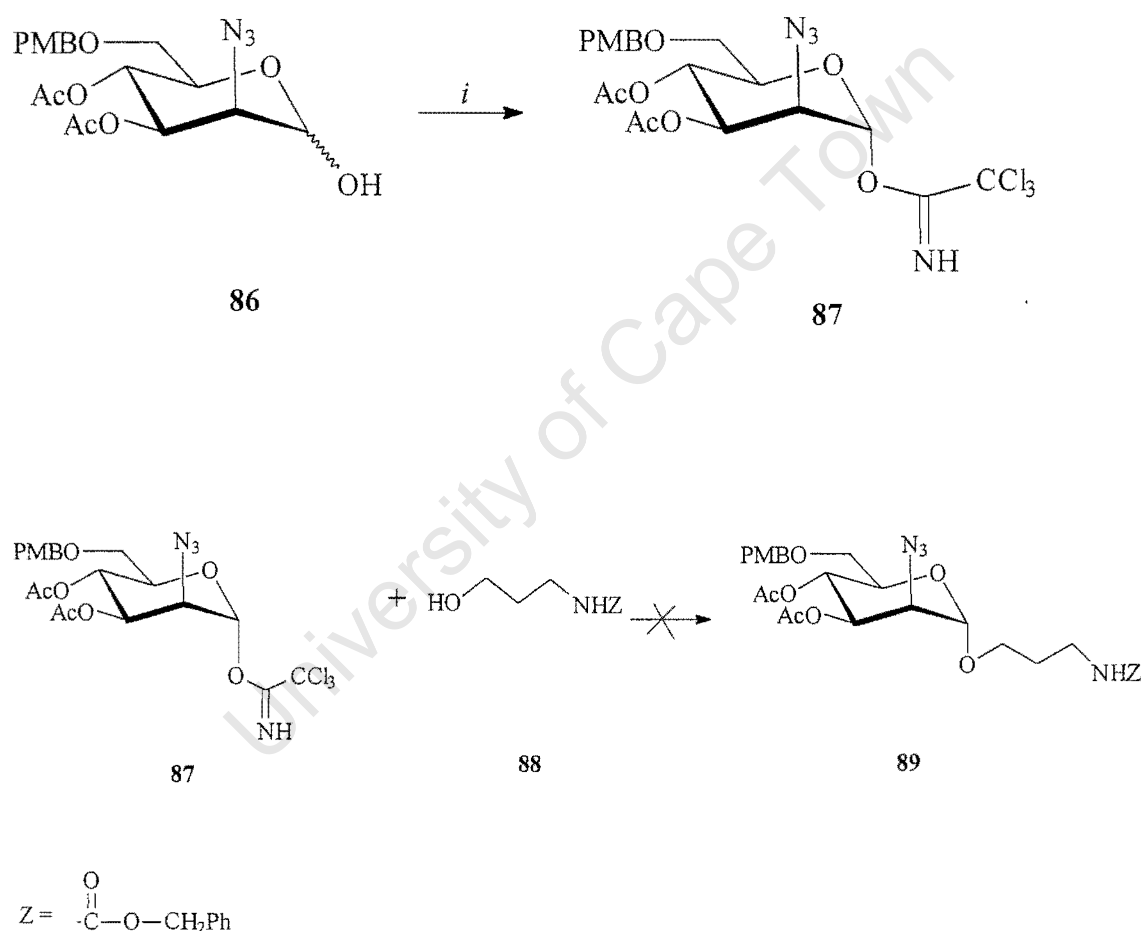
A solution of 6-*O*-(*p*-methoxybenzyl) mannopyranose **86** and trichloroacetonitrile in dry dichloromethane was treated with finely powdered K_2CO_3 to give 3,4-di-*O*-acetyl-2-azido-2-deoxy-6-*O*-(*p*-methoxybenzyl)-D-mannopyranosyl trichloroacetimidate **87** in 65 % yield.[89] Since imidate formation is reversible, the thermodynamically more stable α-imidate was the major product. The structure of the compound was ascertained by ^{1}H and ^{13}C NMR data. Downfield shift of the anomeric signal to δ 6.266 signifies *O*-alkylation of the OH. Appearance of characteristic imidate peak at δ 8.741 was also an indication of formation of the imidate. Besides shift of anomeric peak in the ^{13}C NMR, the presence of an additional peak at δ 160.09 that corresponds to the imidate carbon is evidence of the existence of a trichloroacetimidate.[89] The C-1 orientation was confirmed

by the J value of H-1 and *dd* signal of H-2 in the ^{1}H NMR of the compound. The small J value (1.8 Hz) of H-1 corresponds to *e-e* coupling. The signal corresponding to H-2 showed up as a doublet of doublet as a result of unequal coupling between H-1 – H-2 and H-2 – H-3. Coupling of H-2 with H-3 afforded a doublet with a J value of 9.7 Hz, which was then split into doublets (J = 3.6 Hz) by coupling with H-1 to give an overall doublet of doublet signal for H-2. If the C-1 orientation had been β, $J_{1,2}$ and $J_{2,3}$ would have closely similar values, resulting in an overlapping doublet of doublets for H-2.



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(*i*) Trichloroacetonitrile, K_2CO_3, CH_2Cl_2, RT, 65 %.

Scheme 28

However, glycosidic bond formation between imidate **87** and the carbamate **88** employing Lewis acids such as BF_3-Et_2O was not successful. The position (being at the

primary) might have added to the susceptibility of the PMB group to the acids, which were removed under the reaction conditions prior to glycosidation and probably led to formation of an anhydro compound.

Therefore, we had to look for another protecting group with greater stability to acids than the PMB. As mentioned earlier PMB groups are 10 times more labile to acids than their corresponding benzyl ethers. Hence, we decided to try benzyl ether as a protecting group for the O-6 in the hope that this could be later selectively removed without reducing the azide with reagents such as BBr_3. The benzyl group was introduced in the same fashion as the PMB was introduced.

2.3.3.2 Benzyl ether

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A solution of 2-azido mannose **8** and *p*-toluenesulfonic acid monohydrate in DMF was treated with benzaldehyde dimethyl acetal (α, α -dimethoxytoluene) at room temperature to afford 2-azido-4,6-*O*-benzylidene-2-deoxy-D-mannopyranose **90** in 75 % yield.[57] The appearance of an acetal signal at *ca* δ 5.553 and *ca* 102 in the ^{1}H and ^{13}C NMR of the product, respectively, indicated formation of the benzylidene ring. The compound was fully characterized after being peracetylated. The 4,6-*O*-benzylidene mannose **90** was taken up in dichloromethane and acetic anhydride, triethylamine, and DMAP were then added to give 1,3-di-*O*-acetyl-2-azido-4,6-*O*-benzylidene-2-deoxy-D-mannopyranose **91** after stirring for 0.5 h at room temperature in an excellent yield. The acetal signals both in ^{1}H and ^{13}C NMR spectra were intact ascertaining that the benzylidene ring was unaffected. Two singlets at δ 2.175 and 2.159 (δ 2.184 and 2.151 in the case of the β anomer) in the ^{1}H NMR spectrum, the two carbonyl signals at δ 170.31 and 168.62 (δ 170.34 and 168.42 for the β anomer) and two methyl signals at δ 21.10 and 20.80 (δ 20.88 and 20.81 in the case of the β anomer) in the ^{13}C NMR spectrum witnessed the presence of two acetyl groups. H-3 appeared as a doublet of doublet at δ 5.470 whereas H-4 showed a signal far upfield at *ca* δ 4.0 indicating that 3-*O* was acetylated. H-1α appeared at δ 6.080 as a doublet with a J = 1.6 Hz. 1,3-Di-*O*-acetyl-2-azido-6-*O*-benzyl-2-deoxy-D-mannopyranose **92** was obtained in 65 % yield after the benzylidene ring in peracetylated 4,6-*O*-benzylidene mannose **91** was regioselectively opened upon treatment

with sodium cyanoborohydride and ether saturated with HCl using THF as solvent. The product was assigned by NMR. The disappearance of the acetal peaks in both ^{1}H and ^{13}C NMR spectra and on the other hand appearance of CH_2Ph signals confirmed opening of the ring. The CH_2 gave a distorted quartet at δ 4.578 in the ^{1}H NMR spectrum of the product. Appearance of a broad singlet peak at δ 2.896 of the ^{1}H NMR of the product corresponding to O*H* was additional evidence of the opening of the benzylidene ring. Opening of the ring towards O-6 was ascertained by the fact that H-4 appeared upfield *ca* δ 4.0 in the ^{1}H NMR and C-6 appeared at δ 69.905. D_2O wash also confirmed that the O*H* had a long range coupling with H-4 not with H-6 *i.e* the OH is attached to C-4.

A solution of 6-*O*-benzyl mannose **92** in dichloromethane was treated with acetic anhydride, triethylamine, and DMAP to afford 1,3,4-tri-*O*-acetyl-2-azido-6-*O*-benzyl-2-deoxy-D-mannopyranose **93** in 85 % yield. The number of acetyl groups present in the compound was consistent with ^{1}H and ^{13}C NMR spectra. The O*H* peak is absent in the ^{1}H NMR spectrum. H-4 shifted downfield to δ 5.233 while the other protons didn't show significant change. The downfield shift of H-4 indicates that O-4 is acetylated and thus the OH in 6-*O*-benzyl mannose **92** was positioned at C-4. Anomeric de-*O*-acetylation was achieved in 80 % yield to obtain 3,4-di-*O*-acetyl-2-azido-6-*O*-benzyl-2-deoxy-D-mannopyranose **66** by treatment of peracetylated 6-*O*-benzyl mannose **93** with hydrazine acetate at 50 °C in DMF. The anomeric proton (α) shifted upfield to δ 5.134 while the rest of the protons were intact and an additional peak of O*H* appeared at δ 4.247. These pieces of information prove that anomeric de-*O*-acetylation took place. A D_2O shake ^{1}H NMR also gave sharper signals for both α and β anomeric protons signifying that the OH is attached to C-1. The number of acetyls in the compound was in agreement with the number of acetoxy and carbonyl peaks in the ^{1}H and ^{13}C NMR spectra.

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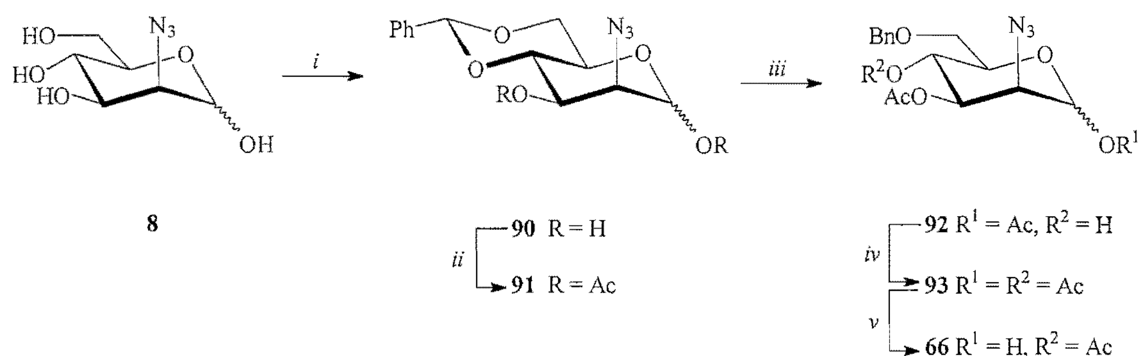

(*i*) α,α-Dimethoxytoluene, *p*-TsOH, DMF, RT, 75 %; (*ii*) Ac_2O, DMAP, Et_3N, dichloromethane, RT, 90 %; (*iii*) $NaCNBH_3$, HCl-ether, THF, RT, 65 %; (*iv*) Ac_2O, DMAP, Et_3N, dichloromethane, RT, 85 %; (*v*) hydrazine acetate, DMF, 50 °C, 80 %.

Scheme 29

2.4 PHOSPHORYLATION

Satisfied with the result achieved, we wanted to attempt synthesis of the α-hydrophosphonate monomer **3** from 6-*O*-benzyl mannose **66**. Synthesis of this monomer having the right orientation at the anomeric center was crucial for synthesis of the desired target **2**

Different techniques for the preparation of hydrophosphonates have been reported so far. Marugg *et al* demonstrated a general and simple method for the preparation of d-nucleoside-3'-hydrophosphonates using monofunctional phosphitylating reagents **97** and **95** according to **Scheme 30**.[90] Phosphitylation of **94** with reagent **95** yielded intermediate **96**. Treatment of a solution of **96** in dioxane with acetic acid followed by excess pyridine-water gave homogeneous **99** in 87 % yield. Alternatively, treatment of a solution of **94** in dioxane and pyridine with a slight excess of 1.25 molar solution of salicylchlorophosphite **97** in dioxane followed by treatment of the reaction mixture with water gave them 88 % of hydrophosphonate **99** (**Scheme 30**).

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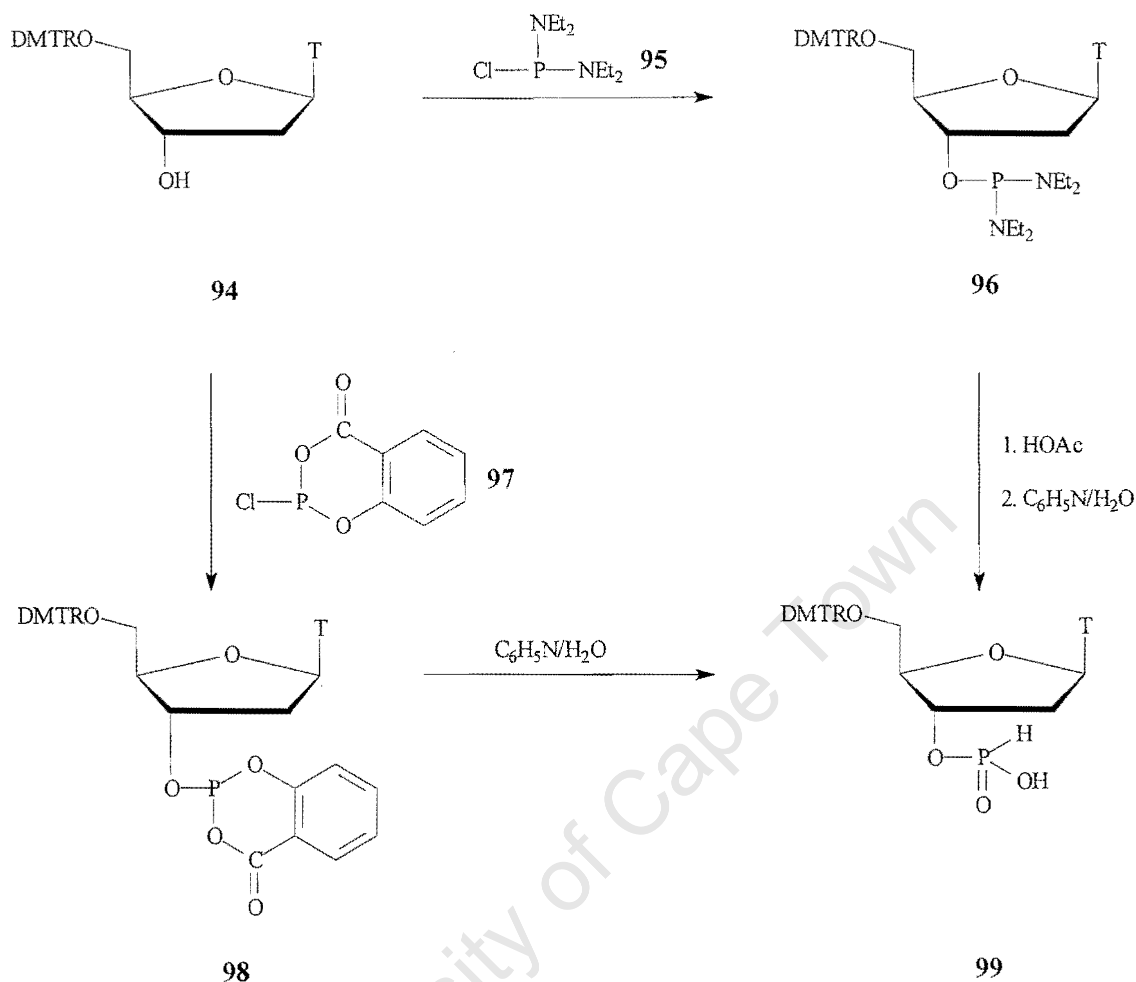

Scheme 30

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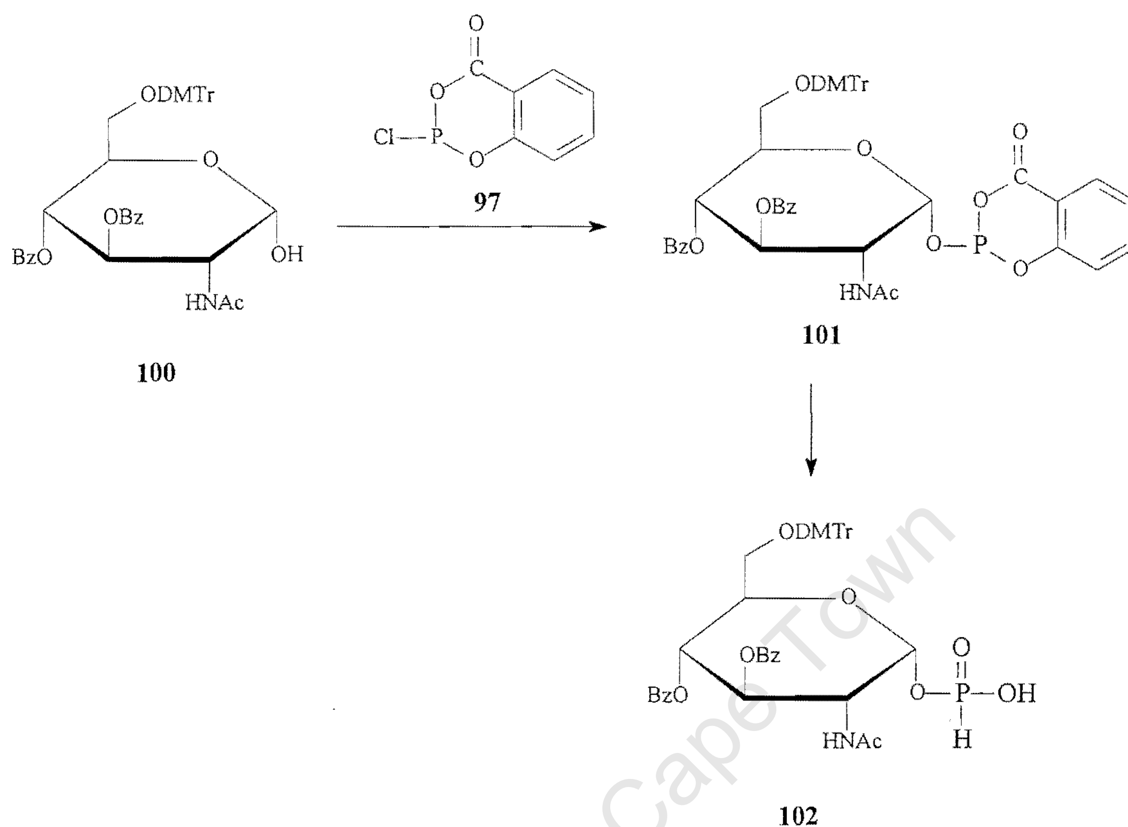
The same authors slightly modified the second phosphitylation method when they were synthesizing a fragment of GlcNAc-α(1→P→6)-GlcNAc of the cell wall polymer of *Staphylococcus Lactis* having repeating *N*-acetyl-D-glucosamine phosphate units.[91] They treated a solution of *N*-acetylglucosamine **100** in dioxane containing triethylamine with salicylchlorophosphite **97** followed by hydrolysis to obtain hydrophosphonate **102** in a yield of 86 % (**Scheme 31**).



Scheme 31

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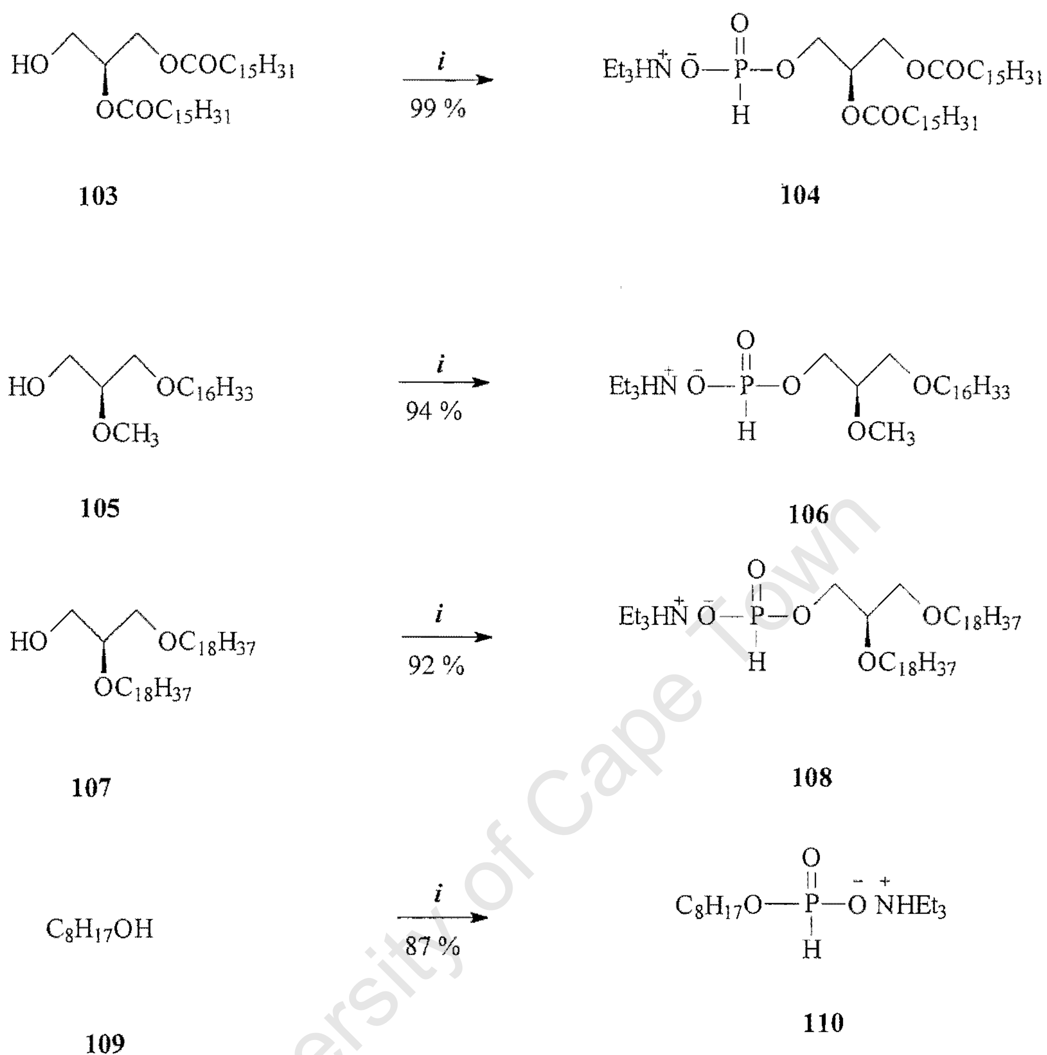
It has been described that none sugar hydrophosphonates can also be synthesized using salicyl chlorophosphite phosphitylating reagent. Brimacombe *et al.* synthesized a series of hydrophosphonates during the synthesis of further substrate analogues of early intermediates in the biosynthetic pathway of glycosylphosphatidylinositol membrane anchors.[92] They showed that treatment of a solution of salicyl chlorophosphite in THF with a solution of the corresponding alcohol in THF-pyridine followed by hydrolysis with 1M aq. triethylammonium hydrogen carbonate buffer solution yielded the respective hydrophosphonates[90] (**Scheme 32**).



(*i*) a. Salicylchlorophosphite in THF, pyridine-THF; b. 2.1 M (aq) triethylammonium hydrogen carbonate.

Scheme 32

The other method of preparing hydrophosphonates is using *in situ* prepared tri-imidazolylphosphine phosphitylating reagent. The reagent is prepared from PCl_3, imidazole, and Et_3N in acetonitrile. Nikolaev *et al* elucidated use of this reagent for the synthesis of hydrophosphonates **113** and **114** from the corresponding alcohols (**Scheme 33**).[93]

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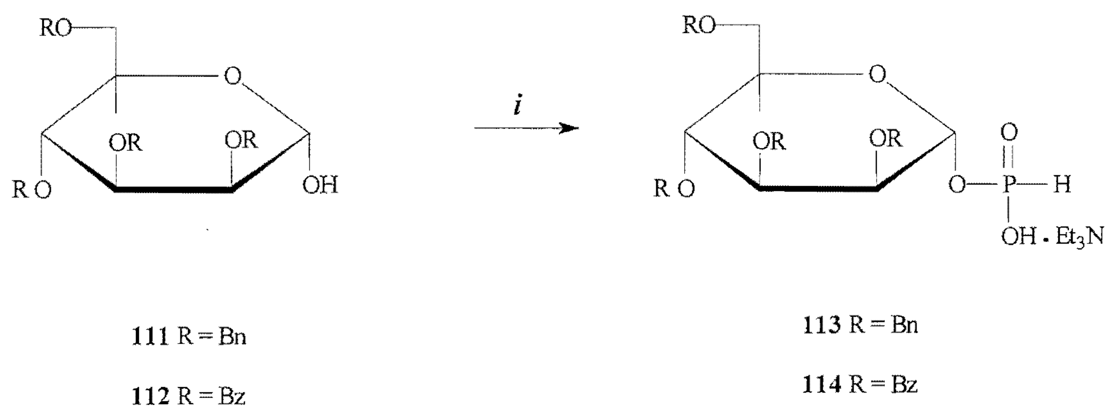
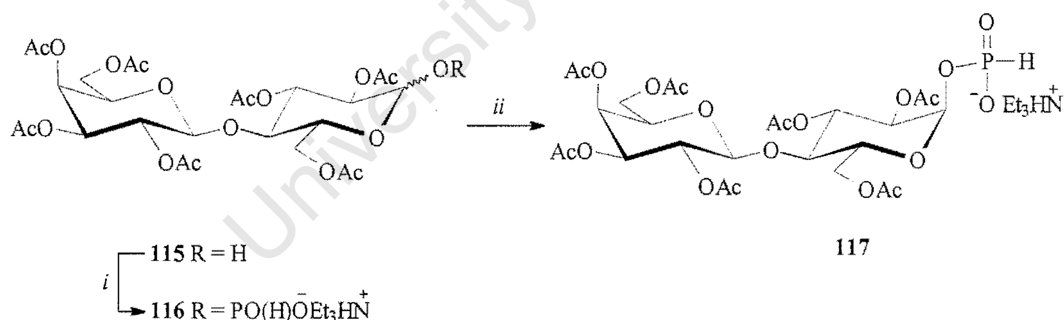


(*i*) Tri-imidazolylphosphine, CH_3CN, 100 %.

Scheme 33

The same authors further elaborated the scope of the reagent when they were synthesizing phosphodisaccharide synthetic probes and substrate analogues for the elongating α-D-mannopyranosylphosphate transferase in the *Leishmania* (**Scheme 34**).[94]



(*i*) a. Triimidazolylphosphine, CH_3CN; b. $Et_3\overset{\oplus}{N}H\ HCO_3$, H_2O (pH 7); (*ii*) H_3PO_3, CH_3CN, 48 %.

Scheme 34

Bearing these H-phosphonating techniques in mind, we tried to adopt them to our own substrate 3,4-di-*O*-acetyl-2-azido-6-*O*-benzyl-2-deoxy-D-mannopyranose **66**. Experiments employing triimidazolylphosphine and salicyl chlorophosphite with

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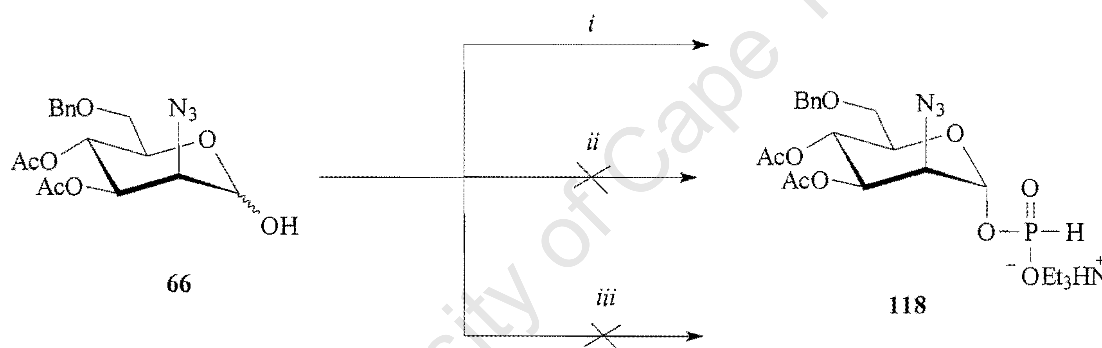
triethylamine as a base were unsuccessful. However, reaction was moderately successful when a solution of 6-*O*-benzyl **66** in dioxane-pyridine (in 3:1 ratio) was treated with 1.25M solution of salicyl chlorophosphite in dioxane followed by quenching with TEAB to afford hydrophosphonate **118**. The reaction failed to go to completion and only *ca* 50 % conversion was achieved, with starting material being recovered after work-up. ^{1}H as well as ^{31}P NMR data of hydrophosphonate **118** were in complete accordance with its proposed structure. The characteristic NMR data for hydrophosphonate **118** were as follows: a singlet ^{31}P signal at δ -0.816; a doublet signal at δ 6.949 with a J value of 647.1 Hz that corresponds to the P-H in the ^{1}H NMR spectrum were typical for α-hydrophosphonate derivatives.[93] The anomeric proton appeared at δ 5.622 as an unresolved doublet of doublets with $J_{1,P}$ = 8.4 Hz and $J_{1,2}$ = 1.2 Hz.



(*i*) a. Salicyl chlorophosphite in dioxane, dioxane-pyridine; b. aq. TEAB, RT, 40 %; (*ii*) a. Salicyl chlorophosphite, Et_3N, dioxane; b. aq. TEAB; (*iii*) a. Tri-imidazolylphosphine, CH_3CN; b. aq. TEAB.

Scheme 35

An investigation was carried out on the model compounds 2,3,4,6-tetra-*O*-acetyl-D-glucopyranose **119** and 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-D-manno and-D-glucopyranose **121** to find out if the reaction goes to completion. Model 2,3,4,6-tetra-*O*-acetyl-D-glucopyranose **119** has a participating group at C-2 while the 2-azido-2-deoxypyranoside derivative **121** bears a non-participating group at the same position

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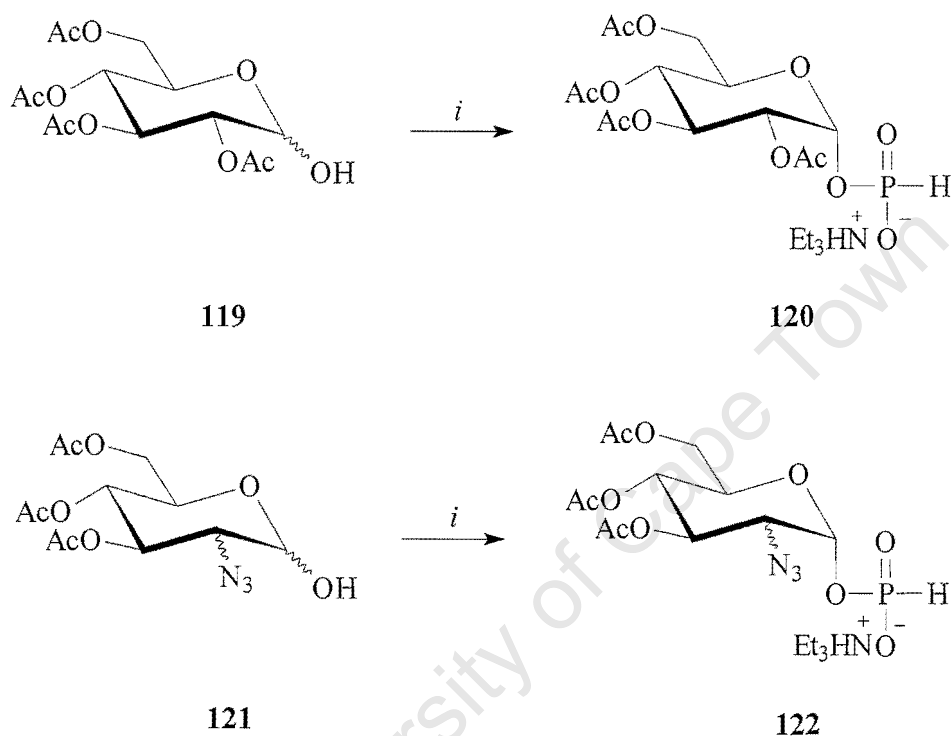
(**Scheme 36**). The reaction took place on both models but without going to completion. These findings proved that the type (participating or non-participating) and orientation of the C-2 constituent does not play any role in preventing the reaction from going to completion and on the stereochemistry of the product.



(i) a. Salicyl chlorophosphite in dioxane, dioxane-pyridine, RT; b. aq. TEAB

Scheme 36

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3. CONCLUSION

This study has succeeded in developing methods for the synthesis of the selectively protected α-1-hydrophosphonate **118**, which is the cornerstone for synthesis of potential glycoconjugate vaccine candidates. It has also been demonstrated that the most efficient route to the desired precursor 2-azido-2-deoxymannopyranose derivative **8** is *via* selective protection of methyl4,6-O-benzylidene-α-D-glucoside followed by azide displacement of a 2-O-triflate derivative rather than via azidonitration of protected glucal. The former route gave higher yields and avoided the difficult separations of diastereoisomers in the latter route. The impracticability of 6-O-tritylation of 2-azidomannose **8** was also demonstrated, establishing that this was not a feasible route to 3,4-di-*O*-acetyl-2-azido-2-deoxymannose derivative **5**.

In the future, mannose unit **66** will also be converted to a glucoside containing a linker suitable for subsequent conjugation to a protein. Selective removal of the 6-*O*-benzyl group by non-hydrogenolytic methods will allow for linking to the hydrophosphonate **118**. It is envisaged that a range of oligosaccharide conjugates can be prepared in this way and evaluated to determine the optimum size of the epitope required for activity.

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4. EXPERIMENTAL

Melting points were determined using a Reichert-Jung Thermovar hot-stage microscope and are uncorrected. Optical rotations were determined on a Perkin-Elmer 141 polarimeter in chloroform solutions and are recorded in units of $10^{-1}deg.Cm^2.g^{-1}$. Infrared spectra were recorded using a Perkin-Elmer Paragon 1000 FT-IR spectrometer.

All proton nuclear magnetic resonance (^{1}H-n.m.r) spectra were recorded unless otherwise specified, as deuteriochloroform solutions using tetramethylsilane as an internal standard on a Varian VXR-200 (200 MHz) or a Varian Mercury Spectrometer 300 MHz or a Varian Unity Spectrometer 400 MHz. Carbon 13 nuclear magnetic resonance (^{13}C-n.m.r) spectra were recorded on the same instruments at 75 or 100 MHz using tetramethylsilane as an internal standard. ^{31}P n.m.r were also recorded in the same way using the same instruments. High-resolution mass spectrometry was performed at the mass-spectrometry unit of the Cape Technikon using a VG70-SEQ Micromass spectrometer.

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All reactions were monitored by TLC on aluminum-backed silica gel 60 F_{254} plates using an ascending technique. The plates were visualised by spraying with ceric ammonium sulfate in 8 M sulfuric acid or a 1:1 solution of 5 % *p*-anisaldehyde in ethanol and 10 % sulfuric acid in ethanol baking at 200 °C. Gravity column chromatography was done on Merck silicagel 60 (70 – 230 mesh).

All the solvents used were dried by appropriate techniques[95,96] and all reactions were carried out under nitrogen or argon. All reagents were purchased from the chemical suppliers except for α, α-dimethoxytoluene,[57] 4-methoxybenzaldehyde dimethyl acetal,[70] and TEAB.[97]

BENZALDEHYDE DIMETHYL ACETAL

A solution of benzaldehyde (10.0mL, 98.4 mmol) and trimethyl orthoformate (12.4 mL, 112.9 mmol) in dry methanol (50 mL) containing Amberlite IR – 120 (H^+) ion exchange resin (500 mg; freshly washed with dry methanol) was refluxed for 3 h under nitrogen atmosphere. After TLC showed reaction was complete, the reaction mixture was cooled to room temperature, the resin was filtered off, and the filtrate evaporated at 30 °C on a rotary evaporator. The residue was distilled under vacuum to afford the title compound in 80 % yield; ^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.44 – 7.34 (m, 5 H, ArH), 5.40 (s, 1 H, H-7), 3.34(s, 6 H, OCH_3).

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4-METHOXYBENZALDEHYDE DIMETHYL ACETAL

A solution of 4-methoxybenzaldehyde (10.0 mL, 82.2 mmol), trimethyl orthoformate (9.9 mL, 90.6 mmol) and *p*-toluenesulphonic acid monohydrate (780 mg, 0.042 mmol) was stirred at room temperature for 21 h. After TLC (ethyl acetate/petroleum ether 3:7) showed completion of reaction, sodium carbonate (90 mg, 0.001 mmol) was added and stirred for an additional hour. The reaction mixture was then filtered and the formate distilled off with a rotary evaporator. The crude product was purified by distillation under reduced pressure to afford the title compound in 85% yield; ^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.386 (d, 2 H, J = 8.5 Hz, ArH_a), 6.911 (d. 2 H, J = 8.5 Hz, ArH_b), 5.369 (s, 1 H, H-7), 3.821 (s, 3 H, OMe), 3.327 (s, 6 H, 2 OMe).

TETRAETHYLAMMONIUM BROMIDE (TEAB)

A 1:1 mixture of triethylamine and ethyl bromide in acetone was refluxed overnight. The mixture was cooled to room temperature and the crystals were collected by filtration. Recrystalisation was carried out by dropwise addition of ethyl ether to a solution of TEAB in methanol; m.p 284 – 286 °C (*lit.* 285 °C).

3,4,6-TRI-O-ACETYL-2-AZIDO-2-DEOXY-D-GLUCO- AND D-MANNOPYRANOSYL NITRATES (38, 39, 40)

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A solution of tri-*O*-acetylglucal 7 (695 mg, 2.55 mmol) in dry acetonitrile (13 mL) was cooled to –20 °C. To the solution at –20 °C was added sodium azide (250 mg, 3.85 mmol) followed by cerium(IV) ammonium nitrate (4.20 g, 7.66 mmol). The mixture was stirred vigorously while maintaining the temperature until TLC showed complete reaction (3 h). The mixture was diluted with ethyl acetate and washed with water until the organic layer was colorless, then with brine, dried with anhydrous magnesium sulphate, filtered and concentrated to syrup. The residue was purified by column chromatography to afford a mixture of the title compounds in 84 % total yield; R_f 0.64 (ethyl acetate/petroleum ether 1:1); IR (NaCl) ν_{max}: 2119 (N_3) cm^{-1}, 1670 (ONO_2) cm^{-1},, 1752 (C=O) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.308 (d, 1 H, $J_{1,2}$ = 4.4 Hz, H-1 of compound **39**), 6.191 (d, 1 H, $J_{1,2}$ = 2.0 Hz, H-1 of compound **38**), 5.592 (d, 1 H, $J_{1,2}$ = 8.8 Hz, H-1 of compound **40**), 5.003 – 5.405 (m, 4 H), 4.047 – 4.318 (m, 6H), 3.668 (dd, 1 H, J = 9.2 and 10.0 Hz), 3.826 – 3.869 (m, 2 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 170.45, 170.35, 170.29, 169.70, 169.61, 169.59, 169.44, 169.41, 169.23 (OAcs of compounds **38**, **39**, and **40**), 97.72, 97.12, 96.30 (C-1s of compounds **39**, **38**, and **40**, respectively), 77.42, 77.00, 76.56, 72.88, 72.84, 71.21, 70.50, 70.36, 67.51, 64.88, 61.42, 61.17, 61.06, 60.54, 59.41, 58.79 (C-2, C-3, C-4, C-5, and C-6) 20.55, 20.52, 20.48, 20.41, 20.33 (OAc).

***3,4,6-TRI-O-ACETYL-2-AZIDO-2-DEOXY-D-GLUCO- AND D-MANNOPYRANOSES** (41, 42, and 43)*

To a solution of the nitrates obtained above (**38**, **39** and **40**) in acetonitrile (26 mL), thiophenol (0.8 mL, 7.79 mmol) and Hunig's base (0.4 mL, 2.30 mmol) were added. The reaction mixture was stirred under nitrogen. After 30 minutes TLC showed completion of reaction. The solvent was evaporated and the crude product subjected to column chromatography using ethyl acetate/petroluem ether mixture as eluant to give a mixture of 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-D-gluco- and D- mannopyranoses **41**, **42**, and **43** in 84 % yield; R_f = 0.74 (ethyl acetate/petroleum ether 1:1); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.000 (d, 0.25 H, J = 3.9 Hz, H-1), 4.901 (d, 0.50 H, J = 1.2 Hz, H-1), 4.727 (d, 0.25 H, J = 8.1 Hz, H-1); ^{13}C NMR (75 MHz, $CDCl_3$) selected data δ: 170.93, 170.75, 170.07, 170.05, 170.03, 169.77, 169.67, 169.63, 169.52 (OAc), 92.87 (C-1), 92.74 (C-1), 92.09 (C-1), 20.74, 20.69, 20.66, 20.63, 20.57, 20.52 (OAc).

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***1,3,4,6-TETRA-O-ACETYL-2-AZIDO-2-DEOXY-D-GLUCO- AND D-MANNOPYRANOSES** (44, 45, 46, and 47)*

a. To a solution of 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-manno and glucopyranoses **41**, **42**, and **43** (500 mg, 0.54 mmol) in pyridine (5 mL) was added acetic anhydride (0.1 mL, 1.08 mmol) and DMAP (3.0 mg, 0.027 mmol). After stirring the mixture for 45 minutes at room temperature, TLC (ethyl acetate/petroleum ether 3:7) showed two new spots. The reaction mixture was then poured into 50 mL of ice and water. The products were extracted with ethyl acetate and washed with brine, dried (magnesium sulphate), filtered, and concentrated. The residue was purified and resolved into the respective stereoisomers by column chromatography (ethyl acetate/petroleum ether 3:7) to afford peracetylated 2-azido-2-deoxymannoses **44**, **45** (combined yield *ca* 50 %), and peracetylated 2-azido-2-deoxyglucoses **46**, and **47** (*ca* 50 %).

b. Alternatively, a suspension of the syrup (**38**, **39**,and **40**) (2.13 g, 5.59 mmol) and sodium acetate (920 mg, 11.20 mmol) in acetic acid (14 mL) was heated to 100 °C and the temperature maintained for 3 h. The reaction mixture was then cooled down to room temperature and diluted with dichloromethane. The mixture was successively washed with ice-cold water, saturated sodium bicarbonate, and water. The organic layer was then concentrated and purified by column chromatography to afford peracetylated 2-azido-2-deoxypyranoses **44**, **45**, **46** and **47** in 98 %.

Pure white crystal of peracetylated 1,3,4,6-tetra-*O*-acetyl-2-azido-2-deoxy-D-mannopyranoside **44** was obtained from the mixture by crystallization using ethanol and petroleum ether; m.p of **44** = 130 °C; $[\alpha]_D^{20}$ of **44** = + 81.5; R_f 0.16 for **44 and 45** and 0.26 for **46 and 47**; ^{1}H NMR (400 MHz, $CDCl_3$) **44**: δ = 6.103 (d, 1 H, $J_{1,2}$ = 2.0 Hz, H-1), 5.408 – 5.339 (m, 2 H, H-3 and H-4), 4.232 (dd, 1 H, J = 4.8 Hz and 12.4 Hz, H-6a), 4.086 (dd, 1 H, J = 2.4 Hz, and 12.4 Hz, H-6b), 4.018 (dd, 1 H, $J_{1,2}$ = 2.0 Hz, $J_{2,3}$ = 3.2 Hz, H-2), 4.012 – 3.880 (m, 1 H, H-5), 2.150 (s, 3 H, OAc), 2.102 (s, 3 H, OAc), 2.080 (s, 3 H, OAc), 2.042 (s, 3 H, OAc); ^{13} C NMR (100 MHz, $CDCl_3$) **44**: δ = 170.625, 169.988, 169.301, 168.114 (4 C=O), 91.380 (C-1), 70.770, 70.542, 65.352, 61.785, 60.518 (C-2, C-3, C-4, C-5, and C-6), 20.808, 20.637, 20.539, 20.444 (OAc); ^{1}H NMR (400 MHz, $CDCl_3$) of **44** and **45** (70:30): δ = 6.102 (d, 0.7 H, J = 2.0 Hz, H-1), 5.811 (d, 0.3 H, J = 1.5 Hz, H-1), 5.408 – 5.339 (m, 1.4 H, H-3, H-4), 5.268 (t, 0.3 H, J = 9.8 Hz, H-4), 5.043 (dd, 0.3 H, J = 3.7 Hz, 9.8 Hz, H-3), 4.232 (dd, 0.7 H, J = 4.8 Hz, 12.4 Hz, H-6a), 4.214 (dd, 0.3 H, 4.7 Hz, 12.4 Hz, H-6a), 4.086 (dd, 0.7 H, J = 2.4 Hz, 12.4 Hz, H-6b), 4.067 (dd, 0.3 H, J = 2.2 Hz, 12.4 Hz, H-6b), 4.025 – 3.998 (m, 1 H, H-2, H-5), 3.997 (dd, 0.7 H, J = 2.0 Hz, 3.3 Hz, H-2), 3.704 (ddd, 0.3 H, J = 2.4 Hz, 4.8 Hz, 9.8 Hz, H-5), 2.161 (s, 0.9 H, OAc), 2.150 (s, 2.1 H, OAc), 2.102 (s, 3 H, OAc), 2.080 (s, 2.1 H, OAc), 2.065 (s, 0.9 H, OAc), 2.042 (s, 2.1 H, OAc), 2.024 (s, 0.9 H, OAc); ^{1}H NMR (400 MHz, $CDCl_3$) **46** and **47** (40:60): δ = 6.252 (d, 0.4 H, $J_{1,2}$ = 3.7 Hz, H-1), 5.513 (d, 0.6 H, $J_{1,2}$ = 8.6 Hz, H-1), 5.420 (dd, 0.4 H, J = 9.4, 10.4 Hz, H-3), 5.101 – 4.904 (m, 1.6 H, H-3, 2H-4), 4.244 (dd, 0.6 H, J = 4.6, 12.4 Hz, H-6a), 4.252 (dd, 0.4 H, J = 4.2, 12.5 Hz, H-6a), 4.040 (dd, 0.6 H, J = 2.2, 12.4 Hz, H-6b), 4.121 – 3.978 (m, 0.8 H, H-5 and H-6b), 3.761 (ddd, 0.6 H, J = 2.2, 4.6, 9.8 Hz, H-5), 3.619 (dd, 0.4 H, J = 3.7, 10.4 Hz, H-2), 3.616 (dd, 0.6 H, J = 8.6, 9.8 Hz, H-2), 2.153 (s, 1.2 H, OAc), 2.139 (s, 1.8 H,OAc),

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2.057 (s, 1.2 H, OAc), 2.040 (s, 1.8 H, OAc), 2.029 (s, 3 H, 2 OAc), 2.001 (s, 1.2 H, OAc), 1.980 (s, 1.8 H, OAc).

2-AZIDO-2-DEOXY-D-MANNOPYRANOSE (8)

To a solution of peracetylated 2-azido-2-deoxymannoses **44** and **45** (1.30 g, 3.49 mmol) in dry methanol (60 mL) was added 2 mL of 0.3 M of freshly prepared sodium methoxide (in methanol) while stirring. The reaction mixture was stirred for 30 min at room temperature. After completion of reaction, a test drop from the mixture gave alkaline reaction to litmus. The sodium was removed by stirring with Amberlite IR – 120 (H^+) until the solution became neutral to litmus. The resin was then filtered and the solution concentrated to a syrup. The residue was purified by column chromatography (MeOH/ethyl acetate 1:9) to afford 2-azido-2-deoxy-D-mannopyranose **8** in 90% yield; (*m/z* = 204.9) (*lit. m/z* = 205.2)[99]; R_f 0.51 (MeOH/ethyl acetate 1:4); ^{1}H NMR (300 MHz, D_2O): δ = 5.204 (s, 0.5 H, H-1α), 4.972 (d, 0.5 H, $J_{1,2}$ = 1.5 Hz, H-1β), 4.030 – 3.280 (m, 6 H, H-2, H-3, H-4, H-5, H-6a, and H-6b) {*lit.* 5.05 (d, 0.64 H, $J_{1,2}$ = 1.7 Hz, H-1α), 4.81 (d, 0.36 H, $J_{1,2}$ = 1.5 Hz, H-1β)}[99]; ^{13}C NMR (75 MHz, D_2O): δ = 92.97, 92.12, 76.32, 72.50, 72.35, 70.11, 66.81, 66.55, 65.92, 64.38, 60.80, 60.68.

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METHYL 4, 6-O-BENZYLIDENE-α-D-GLUCOPYRANOSIDE (53)

A solution of methyl α-D-glucopyranoside **52** (10 g, 51.49 mmol), benzaldehyde dimethyl acetal (7.73 mL, 51.60 mmol), in dry DMF (40 mL) containing *p*-toluenesulphonic acid monohydrate (26 mg, 0.14 mmol) was boiled under reflux for 2.5 hrs. After 2 h, TLC (ethyl acetate/petroleum ether 2:3) showed the formation of a new spot, and the mixture was cooled down to room temperature, ice-cold saturated sodium hydrogen carbonate was added while stirring, extracted with ethyl acetate (3 x). The organic layer was washed with water, dried (anhydrous magnesium sulfate), filtered, and concentrated. The residue was purified by column chromatography (ethyl acetate/petroleum ether 2:3) to obtain 4,6-*O*-benzylidene acetal **53** in 80 % yield; m.p = 164-166°C (*lit.* 163-164 °C)[98]; R_f 0.28 (ethyl acetate/petroleum ether 1:1); $[\alpha]_D^{20}$ = 109. (*lit.* +110 in chlorform)[98]; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.504 – 7.354 (m, 5 H, Ar*H*), 5.526 (s, 1 H, H-7), 4.784 (d, 1 H, $J_{1,2}$ = 4.0 Hz, H-1), 4.288 (dd, 1 H, $J_{5,6a}$ = 4.4 Hz, $J_{6a,6b}$ = 9.6 Hz, H-6a), 3.920 (t, 1 H, J = 9.2 Hz, H-3), 3.834 – 3.775 (m, 1 H, H-5), 3.734 (t, 1 H, J = 10.4 Hz, H-6b), 3.631 (bs, 1 H, H-2), 3.482 (t, 1 H, J = 9.6 Hz, H-4), 3.455 (s, 3 H, OMe), 2.835 and 2.358 (2s, 2 H, 2 O*H*); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 153.54, 137.30, 129.46, 128.53, 126.53 (Ar), 102.18 (C-7), 100.03 (C-1), 81.18 (C-4), 73.13 (C-2), 72.01 (C-3), 69.16 (C-6), 62.61 (C-5), 55.78 (OMe).

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METHYL 2, 3-DI-O-ACETYL-4, 6-O-BENZYLIDENE-α-D-GLUCOPYRANOSIDE (56)

To a solution of 4,6-*O*-benzylidene acetal **53** (100 mg, 0.29 mmol) and triethylamine (0.16 mL, 1.16 mmol) in dry dichloromethane (5 mL) were added acetic anhydride (0.27 mL, 2.90 mmol) and DMAP (2 mg, 0.02 mmol). After stirring for 40 min, the mixture was diluted with dichloromethane, washed with 10 % (w/v) aqueous sodium chloride, and the organic layer was dried (anhydrous magnesium sulfate), filtered, and concentrated. The residue was purified by column chromatography (ethyl acetate/petroleum ether 3:7) to give **56** (95 %); m.p = 110 °C (*lit.* 108-109 °C)[98]; $[\alpha]_D^{20}$ = +66.0 (*lit.* +75.5 in chloroform)[98]; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.504 – 7.338 (m, 5 H, Ar*H*), 5.582 (t, 1 H, $J_{2,3} = J_{3,4}$ = 9.6 Hz, H-3), 5.504 (s, 1 H, H-7), 4.942 (d, 1 H, $J_{1,2}$ = 3.6 Hz, H-1), 4.908 (dd, 1 H, $J_{1,2}$ = 3.6 Hz, $J_{2,3}$ = 9.6 Hz, H-2), 4.303 (dd, 1 H, $J_{5,6a}$ = 4.8 Hz, $J_{6a,6b}$ = 10.4 Hz, H-6a), 3.929 (sextet, 1 H, $J_{4,5}$ = 9.6 Hz, $J_{5,6a}$ = 4.8 Hz, $J_{5,6b}$ = 10.4 Hz, H-5), 3.768 (t, 1 H, $J_{5,6b} = J_{6a,6b}$ = 10.4 Hz, H-6b), 3.647 (t, 1 H, $J_{3,4} = J_{4,5}$ = 9.6 Hz, H-4), 3.415 (s, 3 H, OMe), 2.090 and 2.050 (2s, 6 H, OAc); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.60, 169.97 (OAc), 153.54, 137.19, 129.27, 128.42, 126,38 (Ar), 101.82 (C-7), 97.89 (C-1), 79.48 (C-4), 71.87 (C-2), 69.24 (C-3), 69.09 (C-6), 62.57 (C-5), 55.58 (OMe), 20.00 and 20.94 (OAc).

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METHYL 3-O-ACETYL-4, 6-O-BENZYLIDENE-α-D-GLUCOPYRANOSIDE (9)

A mixture of **53** (1.00 g, 3.58 mmol) and sodium hydride (170 mg, 7.08 mmol) in dry THF (50 mL) was stirred at RT. After 2 h evolution of hydrogen ceased and anhydrous cupric chloride (477 mg, 3.54 mmol) was then added. After 10 min, a green solution that indicated formation of the copper chelate resulted. The reaction mixture was cooled down to 0 °C, acetic anhydride (0.50 mL, 540 mg, 5.31 mmol) was added dropwise, and the temperature was maintained at 0 °C for 30 min and then for 1.5 h at room temperature. After adding water (2 mL) and acetic acid (1 mL), the reaction mixture was concentrated. The residue was diluted with dichloromethane, washed successively with water, aqueous sodium hydrogen carbonate, and saturated aqueous sodium chloride, dried (anhydrous magnesium sulfate), and concentrated. The solid residue was purified by column chromatography to yield monoacetylated glucoside **9** (80 %); R_f 0.85 (ethyl acetate/petroleum ether 2:3); m.p. 170-172 °C (*lit.* 174-175.5 °C)[98]; $[\alpha]_D^{20}$ = +74.0 (*lit.* +110 in chloroform)[98]; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.458 – 7.340 (m, 5 H, ArH), 5.488 (s, 1 H, H-7), 5.321 (t, 1 H, J = 10.0 and 9.6 Hz, H-3), 4.800 (d, 1 H, $J_{1,2}$ = 4.0 Hz, H-1), 4.299 (dd, 1 H, $J_{6a,6b}$ = 4.6 Hz, $J_{5,6a}$ = 10.2 Hz, H-6a), 3.865 (m, 1 H, H-2), 3.745 (t, 1 H, J = 10.2 Hz, H-6b), 3.659 (m, 1 H, H-5), 3.578 (t, 1 H, J = 9.6 Hz, H-4), 3.467 (s, 3 H, OMe), 2.241 (d, 1 H, J = 11.2 Hz, OH), 2.116 (s, 3 H, OAc); ^{13}CNMR (75 MHz, $CDCl_3$): δ = 171.26 (OAc), 137.28, 129.27, 128.42, 126.41 (Ar), 101.77 (C-7), 100.38 (C-1), 78.91 (C-4), 72.56 (C-3), 72.12 (C-5), 69.14 (C-6), 62.99 (C-2), 55.78 (OMe), 21.23 (OAc).

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METHYL 3-*O*-ACETYL-2-AZIDO-4,6-*O*-BENZYLIDENE-2-DEOXY-α-D-MANNOPYRANOSIDE (55)

To a cooled (0 °C) solution of monoacetylated glucoside **9** (380 mg, 1.18 mmol) in dry dichloromethane-pyridine (2:1, 12 mL) was added trifluoromethanesulphonic anhydride (0.50 mL, 3.07 mmol) at 0 °C and the mixture was stirred at 0 °C for 30 min. The solution was then diluted with dichloromethane, washed with saturated aqueous sodium hydrogen carbonate, dried (anhydrous magnesium sulfate), and concentrated to dryness. To a stirred solution of the crude product in DMF (13.00 mL) was added sodium azide (374 mg, 5.75 mmol) and the mixture was kept at 70 °C for 3 h, cooled down to room temperature, filtered through Celite, and concentrated. The residue was purified by column chromatography (ethyl acetate/petroleum ether 1:9) to give azido mannoside **55** in an over all yield of 86 %; R_f 0.83 (ethyl acetate: petroleum ether 3:7); $[\alpha]_D^{20}$ = 20.6; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.477 – 7.341 (m, 5 H, ArH), 5.571 (s, 1 H, H-7), 5.432 (dd, 1 H, $J_{3,2}$ = 3.9 Hz, $J_{3,4}$ = 10.2 Hz, H-3), 4.696 (d, 1 H, $J_{1,2}$ = 1.5 Hz, H-1), 4.319 (dd, 1 H, $J_{5,6a}$ = 3.2 Hz, $J_{6a,6b}$ = 8.7 Hz, H-6a), 4.157 (dd, 1 H, $J_{1,2}$ = 1.5 Hz, $J_{2,3}$ = 3.9 Hz, H-2), 4.068 (t, 1 H, J = 9.0 and 10.2 Hz, H-4), 3.935 – 3.794 (m, 2 H, H-5, H-6b), 3.404 (s, 3 H, OMe), 2.130 (s, 3 H, OAc); ^{13}CNMR (75 MHz, $CDCl_3$): δ = 170.00 (OAc), 137.09 (C-Ph_q), 129.12, 128.24, 126.19 (C-Ph), 101.99 (C-7), 99.91 (C-1), 76.03 (C-4), 70.22 (C-3), 68.68 (C-6), 63.75 (C-5), 62.02 (C-2), 55.14 (OMe), 20.63 (OAc).

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1,3,4,6-TETRA-*O*-ACETYL-2-AZIDO-2-DEOXY-D-MANNOPYRANOSES (44 and 45)

A solution of **55** (1.50 g, 4.33 mmol) in acetic anhydride (303 mL, 328 g, 3.22 mol) and acetic acid-sulfuric acid (41:1 v/v, 31 mL) was stirred at room temperature. After 3 h, sodium hydrogen carbonate (powder) was added to the reaction mixture and cooled down to 0 °C. Methanol was added dropwise to the cooled reaction mixture and stirred for another 30 min, diluted with water and the product extracted with dichloromethane. The organic layer was dried (anhydrous magnesium sulfate), filtered, and concentrated. The crude solid product was purified by column chromatography (ethyl acetate/petroleum ether 3:7) to afford **44** and **45** in a total yield of 87 %.

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METHYL 3-*O*-ACETYL-2-AZIDO-6-*O*-BENZYL-2-DEOXY-α-D-MANNOPYRANOSIDE (64)

A solution of **55** (364 mg, 1.05 mmol) in dry freshly distilled THF (15 mL) containing 3Å molecular sieves was stirred at room temperature for 30 min. Sodiumcyanoborohydride (793 mg, 12.62 mmol) was added to the solution and the mixture stirred for an additional 10 min. Diethyl ether saturated with hydrogen chloride was then added to the reaction mixture with a syringe until evolution of gas ceased. After 10 min TLC showed completion of reaction. The mixture was diluted with dichloromethane and water, filtered with aid of Celite. The organic layer was washed with water followed by saturated sodium hydrogencarbonate, dried (anhydrous magnesium sulfate), and concentrated to a syrup. The crude residue was purified by column chromatography (ethyl acetate/petroleum ether 1:4) to afford a 75 % yield of the title compound **64**; (*m/z* = 350.1); R_f 0.50 (ethyl acetate: petroleum ether 3:7); $[\alpha]_D^{20}$ = 31.7; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.350 – 7.258 (m, 5 H, ArH), 5.211 (dd, 1 H, *J* =

4.0 Hz, 9.6 Hz, H-3), 4.684 (d, 1 H, J = 1.6 Hz, H-1), 4.602 (ABq, 2 H, J = 11.6 Hz, 26.4 Hz, PhCH_2), 3.999 (dd, 1 H, J = 1.6 Hz, J = 4.0 Hz, H-2), 3.999 (m, 1 H, H-4), 3.753 (m, 3 H, H-5, H-6a, H-6b), 3.375 (s, 3 H, OMe), 2.814 (bs, 1 H, OH), 2.149 (s, 3 H, OAc); ^{13}CNMR (100 MHz, $CDCl_3$): δ = 171.04, (OAc), 138.00, 128.67, 128.02, 127.92 (Ar), 99.25 (C-1), 73.96 (PhCH_2, C-3), 71.12 (C-5), 70.39 (C-6), 67.36 (C-4), 61.54 (C-2), 55.30 (OMe), 20.06 (OAc).

METHYL 3, 4-DI-*O*-ACETYL-2-AZIDO-6-*O*-BENZYL-2-DEOXY--α-D-MANNOPYRANOSIDE (65)

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To a solution of **64** (50 mg, 0.14 mmol) and triethyl amine (0.077 mL, 0.56 mmol) in dry dichloromethane (2 mL) were added acetic anhydride (0.13 mL, 1.40 mmol) and DMAP (0.86 mg, 0.007 mmol). After stirring for 40 min, the mixture was diluted with dichloromethane, washed with 10 % (w/v) aqueous sodium chloride, and the organic layer was dried (anhydrous magnesium sulfate), filtered, and concentrated. The residue was purified by column chromatography (ethyl acetate/petroleum ether 3:7) to give the title compound (90 %); (m/z = 392.6); R_f 0.67 (ethyl acetate/petroleum ether 3:7); $[\alpha]_D^{20}$ = 72.9; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.323 – 7.310 (m, 5 H, ArH), 5.356 (dd, 1 H, $J_{2,3}$ = 3.6 Hz, $J_{3,4}$ = 9.6 Hz, H-3), 5.307 (t, 1 H, J = 9.6 Hz, H-4), 4.724 (d, 1 H, $J_{1,2}$ = 1.6 Hz, H-1), 4.541 (ABq, 2 H, J = 12.0 Hz, 24.8 Hz, PhCH_2), 4.002 (dd, 1 H, $J_{1,2}$ = 1.6 Hz, $J_{2,3}$ = 3.6 Hz, H-2), 3.870 (ddd, 1 H, J = 4.0 Hz, 4.8 Hz, 4.4 Hz, H-5), 3.551 – 3.534 (m, 2 H, H-6a, H-6b), 3.396 (s, 3 H, OMe), 2.074 (s, 3 H, OAc), 1.908 (s, 3 H, OAc); ^{13}CNMR (100 MHz, $CDCl_3$): δ = 169.93, 169.52 (OAc), 137.80, 128.25, 127.72, 127.56 (Ar), 98.80 (C-1), 73.50 (PhCH_2), 71.12 (C-3), 69.62 (C-5), 68.93 (C-6), 66.76 (C-4), 61.52 (C-2), 55.08 (OMe), 20.45 (OAc), 20.44 (OAc).

2-AZIDO-2-DEOXY-4, 6-*O*-(4-METHOXYBENZYLIDENE)-D-MANNOPYRANOSE (82)

A solution of **8** (74 mg, 0.36 mmol) and 4-methoxybenzaldehyde dimethyl acetal (0.010 mL, 0.54 mmol) in dry DMF (4 mL) containing catalytic amount of *p*-toluenesulphonic acid monohydrate was boiled under reflux for 2.5 h. TLC showed the formation of a new spot, and the mixture was cooled down to room temperature, ice-cold saturated sodium hydrogen carbonate was added while stirring, extracted with ethyl acetate (3 x). The organic layer was washed with water, dried (anhydrous magnesium sulfate), filtered, and concentrated. The residue was purified by column chromatography (ethyl acetate/petroleum ether 2:3) to obtain **82** in 70 % yield; (*m/z* = 322.9); R_f 0.19 (ethyl acetate/petroleum ether 2:3); ^{1}H NMR (300 MHz, $CDCl_3$) (α:β, 2:1): δ = 7.422 – 7.382 (m, 2 H, ArH_a), 6.896 (d, 2 H, J = 8.1 Hz, ArH_b), 5.530 (s, 0.67 H, H-7), 5.503 (s, 0.33 H, H-7), 5.484 (d, 0.67 H, J = 2.7 Hz, H-1), 5.289 (d, 0.33 H, J = 1.2 Hz, H-1), 4.343 – 3.720 (m, 9 H, H-2, H-3, H-4, H-5, H-6a, H-6b, OMe); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 160.61, 160.56, 132.26, 129.78, 129.57, 127.82, 127.79, 114.57, 114.00 (Ar), 102.46, 102.38 (C-7), 94.11, 93.73 (C-1), 79.26, 78.43, 71.69, 68.88, 68.76, 68.61, 68.53, 67.12, 65.82, 64.14, 63.76 (C-3, C-5, C-6, C-4, C-2), 55.79, 55.55 (OMe).

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1,3-DI-*O*-ACETYL-2-AZIDO-2-DEOXY-4, 6-*O*-(4-METHOXYBENZYLIDENE)-D-MANNOPYRANOSE (83)

To a solution of **82** (385 mg, 1.20 mmol) in dry dichloromethane (5 mL) and triethylamine (0.7 mL, 5.0 mmol) were added acetic anhydride (1.2 mL, 12.72 mmol) and DMAP (8 mg, 0.065 mmol). After stirring for 40 min, the mixture was diluted with dichloromethane, washed with 10 % (w/v) aqueous sodium chloride, and the organic layer was dried (anhydrous magnesium sulfate), filtered, and concentrated. The residue was purified by column chromatography (ethyl acetate/petroleum ether 3:7) to give **83** in 80 % yield; (m/z = 406.7); R_f 0.56 (ethyl acetate/petroleum ether 3:7); ^{1}H NMR (300 MHz, $CDCl_3$) (α:β, 43:57) δ = 7.391 – 7.349 (m, 2 H, ArH_a), 6.896 – 6.863 (m, 2 H, ArH_b), 6.061 (d, 0.43 H, J = 1.8 Hz, H-1), 5.896 (d, 0.57 H, J = 1.5 H, H-1), 5.531 (s, 0.43 H, H-7), 5.496 (s, 0.57 H, H-7), 5.437 (dd, 0.43 H, J = 3.6 Hz, J = 10.2 Hz, H-3), 5.133 (dd, 0.57 H, J = 3.8 Hz, 10.4 Hz, H-3), 4.340 – 3.525 (m, 8 H, H-2, H-4, H-5, H-6a, H-6b, OMe), 2.176 (s, 1.71 H, OAc), 2.164 (s, 1.29 H, OAc), 2.140 (s, 3 H, OAc); ^{13}CNMR (100 MHz, $CDCl_3$): δ = 170.36, 168.63, 168.43 (OAc), 160.51, 129.58, 129.49, 127.74, 113.90 (Ar), 102.23 (C-7), 92.25 (C-1β), 91.86 (C-1α), 75.63, 75.17, 71.30, 70.08, 68.52, 68.35, 68.28, 66.31, 62.00, 61.33 (C-2, C-3, C-4, C-5, C-6), 55.49 (OMe), 21.07, 20.87, 20.79 (OAc).

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1,3-DI-*O*-ACETYL-2-AZIDO-2-DEOXY-6-*O*-(4-METHOXYBENZYL)-D-MANNOPYRANOSE (84)

A mixture of **83** (405 mg, 99.51 mmol), sodium cyanoborohydride (314 mg, 5.00 mmol), and 3Å molecular sieves in DMF (8 mL) was stirred for 30 min at room temperature. A solution of trifluoroacetic acid (0.77 mL, 10 mmol) in DMF (6 mL) was added dropwise at 0°C to the stirring reaction mixture. When TLC (ethyl acetate/petroleum ether 2:3) after 2 h showed completion of reaction, the mixture was diluted with dichloromethane and water, and filtered with the aid of Celite. The organic layer was washed successively with water, saturated aqueous sodium hydrogen carbonate, dried (anhydrous magnesium sulfate), and concentrated to a syrup. The crude residue was purified by column chromatography to give **84** in 70 % yield; (*m/z* = 409.2); R_f 0.22 (ethyl acetate/petroleum ether 2:3); ^{1}H NMR (300 MHz, $CDCl_3$) (α:β, 45:55): δ = 7.244 (d, 2 H, J = 7.5 Hz, ArH), 6.877 (d, 2 H, J = 8.7 Hz, ArH), 6.058 (d, 0.45 H, $J_{1,2}$ = 2.1 Hz, H-1), 5.793 (d, 0.55 H, $J_{1,2}$ = 1.2 Hz, H-1), 5.236 (dd, 0.45 H, $J_{2,3}$ = 3.6 Hz, $J_{3,4}$ = 9.6 Hz, H-3), 4.914 (dd, 0.55 H, $J_{2,3}$ = 3.6 Hz, $J_{3,4}$ = 9.6 Hz, H-3), 4.513 (ABq, 2 H, J = 11.4 and 24.3 Hz, CH_2Ph), 4.102 (dt, 0.45 H, $J_{4,OH}$ = 3.0 Hz, $J_{3,4}$ = $J_{4,5}$ = 9.6 Hz, H-4), 4.012 (dd, 0.45 H, $J_{1,2}$ = 2.1 Hz, $J_{2,3}$ = 3.6 Hz, H-2), 3.987 (dt, 0.55 H, $J_{4,OH}$ = 3.0 Hz, $J_{3,4}$ = $J_{4,5}$ = 9.6 Hz, H-4), 3.881 – 3.622 (m, 2.45 H, H-5, H-6a, H-6b), 3.802 (s, 3 H, OMe), 3.565 (m, 0.55 H, H-5), 2.856 (d, 1 H, $J_{4,OH}$ = 3.3 Hz, OH), 2.176 (s, 1.6 H, OAc), 2.159 (s, 3 H, OAc), 2.135 (s, 1.35 H, OAc); ^{13}CNMR (100 MHz, $CDCl_3$): δ = 170.07, 169.45, 168.43 (OAc), 159.32, 129.79, 129.72, 129.57, 113.77 (Ar), 91.35, 91.25 (C-1s), 75.01, 73.19, 72.06, 71.64, 70.41, 68.34, 68.19, 66.26, 65.85, 61.17, 60.89, 55.26 (OMe), 20.84, 20.77, 20.63 (OAc).

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1,3,4-TRI-*O*-ACETYL-2-AZIDO-2-DEOXY-6-*O*-(4-METHOXYBENZYL)-D-MANNOPYRANOSE (85)

To a solution of **84** (134 mg, 0.33 mmol) in dry dichloromethane (5 ml) and triethylamine (0.18 ml, 1.31 mmol) were added acetic anhydride (0.31 ml, 3.28 mmol) and DMAP (2 mg, 0.02 mmol). After stirring for 40 min, the mixture was diluted with dichloromethane, washed with 10 % (w/v) aqueous sodium chloride, and the organic layer was dried (anhydrous magnesium sulfate), filtered, and concentrated. The residue was purified by column chromatography (ethyl acetate/petroleum ether 3:7) to give **85** in 85 % yield; (*m/z* = 450.6); R_f 0.48 (ethyl acetate/petroleum ether, 2:3); ^{1}H NMR (300 MHz, $CDCl_3$) (α:β, 67:33): δ = 7.210 (d, 2 H, J = 8.6 Hz, ArH_a), 6.843 (d, 2 H, J = 8.6 Hz, ArH_b), 6.090 (d, 0.67 H, $J_{1,2}$ = 2.1 Hz, H-1), 5.806 (d, 0.33 H, $J_{1,2}$ = 1.5 Hz, H-1), 5.389 (t, 1 H, J = 9.6 Hz, H-4), 5.296 (dd, 0.67 H, $J_{2,3}$ = 3.6 Hz, $J_{3,4}$ = 9.6 Hz, H-3), 5.041 (dd, 0.33 H, $J_{2,3}$ = 3.6 Hz, $J_{3,4}$ = 9.6 Hz, H-3), 4.433 (ABq, 2 H, J = 11.5 Hz, 12.0 Hz, $PhCH_2$), 4.119 (dd, 0.33 H, $J_{1,2}$ = 1.5 Hz, $J_{2,3}$ = 3.8 Hz, H-2), 4.009 (dd, 0.67 H, $J_{1,2}$ = 2.1 Hz, $J_{2,3}$ = 3.4 Hz, H-2), 3.973 – 3.916 (m, 0.67 H, H-5), 3.773 (s, 3 H, OMe), 3.693 – 3.634 (m, 0.33 H, H-5) 3.505 (dd, 2 H, J = 4.1 Hz, J = 7.9 Hz, H-6_a and H-6_b), 2.155 (s, 0.99 H, OAc), 2.124 (s, 2.01 H, OAc), 2.085 (s, 3 H, OAc), 1.908 (s, 2.01 H, OAc), 1.890 (s, 0.99 H, OAc); ^{13}CNMR (75 MHz, $CDCl_3$): δ = 170.01, 169.34, 168.24 (OAc), 159.24, 129.66, 129.54, 129.50, 113.68 (C-Ar), 91.36 (C-1α), 91.26 (C-1β), 74.55 (C-5β), 73.17 (CH_2Ph), 72.07 (C-5α), 71.94 (C-3β), 70.61 (C-3α), 68.35 (C-6α), 68.20 (C-6β), 66.34 (C-4α), 65.94 (C-4β), 61.21 (C-2β), 60.54 (C-2α), 55.17 (OMe), 20.79, 20.62, 20.53, 20.46, 20.42 (OAc).

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3,4-DI-*O*-ACETYL-2-AZIDO-2-DEOXY-6-*O*-(4-METHOXYBENZYL)-D-MANNOPYRANOSE (86)

To a stirred solution of **85** (140 mg, 0.31 mmol) in DMF (1 mL) at 50 °C was added hydrazine acetate (34 mg, 0.37 mmol). After 10 min stirring at 50 °C of the reaction mixture, TLC showed completion of reaction. The reaction mixture was then cooled down to room temperature and diluted with ethyl acetate. The organic layer was washed with 5% aqueous NaCl solution, dried (anhydrous magnesium sulfate), filtered, and concentrated. The crude product was purified by column chromatography (ethyl acetate/petroleum ether 3:7) to afford **86** in 82 % yield; (*m/z* = 408.8); R_f 0.34 (ethyl acetate: petroleum ether 2:3); ^{1}H NMR (300 MHz, $CDCl_3$) α/β = 0.67:0.33 δ = 7.212 (d, 2 H, J = 8.6 Hz, ArH_a), 6.844 (d, 2 H, J = 8.6 Hz, ArH_b), 5.416 (dd, 0.67 H, $J_{2,3}$ = 3.9 Hz, $J_{3,4}$ = 9.6 Hz, H-3), 5.246 (t, 1 H, J = 9.8 Hz, H-4), 5.159 (d, 0.67 H, $J_{1,2}$ = 1.6 Hz, H-1), 5.008 (dd, 0.33 H, $J_{2,3}$ = 3.9 Hz, $J_{3,4}$ = 9.6 Hz, H-3), 4.815 (s, 0.33 H, H-1), 4.503 – 4.436 (m, 3 H, CH_2Ph and O*H*), 4.059 – 4.169 (m, 1 H, H-5), 3.954 (dd, 0.67 H, $J_{1,2}$ = 1.6 Hz, $J_{2,3}$ = 3.8 Hz, H-2), 3.795 (s, 3 H, OCH_3), 3.510 – 3.444 (m, 2 H, H-6a and H-6b), 2.102 (s, 0.99 H, OAc), 2.085 (s, 2.01 H, OAc), 1.952 (s, 2.01 H, OAc), 1.921 (s, 0.99 H, OAc); ^{13}CNMR (100 MHz, $CDCl_3$): δ = 159.40, 129.86, 129.75, 129.72, 129.64, 113.86, 93.54, 92.79, 73.20, 72.16, 71.02, 70.89, 68.78, 68.46, 67.06, 66.82, 61.20, 60.67, 55.25, 20.86, 20.77, 20.64.

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3,4-DI-*O*-ACETYL-2-AZIDO-2-DEOXY-6-*O*-(4-METHOXYBENZYL)-D-MANNOPYRANOSYL TRICHLOROACETIMIDATE (87)

To a solution of **86** (75 mg, 0.183 mmol) in dry dichloromethane (2 mL) at room temperature were added trichloroacetonitrile (0.070 mL, 0.71 mmol) and dry finely powdered potassium carbonate (112 mg, 0.81 mmol). After 4 h, the reaction mixture was diluted with dichloromethane, filtered through Celite and concentrated to a syrup. The residue was purified by column chromatography (ethyl acetate/petroleum ether 1:9) to obtain imidate **87** (65%); (m/z = 551.6); R_f 0.59 (ethyl acetate: petroleum, 2:3); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.741 (s, 1 H, N*H*), 7.206 (d, 2 H, J = 8.4 Hz, ArH_a), 6.837 (d, 2 H, J = 8.8 Hz, ArH_b), 6.266 (d, 1 H, $J_{1,2}$ = 1.8 Hz, H-1), 5.457 (t, 1 H, J = 9.7 Hz, H-4), 5.395 (dd, 1 H, $J_{2,3}$ = 3.5 Hz, $J_{3,4}$ = 9.7 Hz, H-3), 4.444 (ABq, 2 H, J = 11.3 Hz, J = 37.4 Hz, $PhCH_2$), 4.254 (dd, 1 H, $J_{1,2}$ = 1.8 Hz, $J_{2,3}$ = 3.7 Hz, H-2), 4.111 – 4.068 (m, 1 H, H-5), 3.775 (s, 3 H, OMe), 3.524 (d, 2 H, J = 4.0 Hz, H-6a and H-6b), 2.087 (s, 3 H, OAc), 1.917 (s, 3 H, OAc); ^{13}CNMR (100 MHz, $CDCl_3$): δ = 170.08, 169.53 (OAc), 160.09 ($CNHCCl_3$), 159.41, 129.91, 129.65, 113.87 (Ar), 95.63 (C-1), 73.28 (CH_2Ph), 72.71 (C-5), 70.95 (C-3), 68.30 (C-6), 66.24 (C-4), 60.10 (C-2), 55.37 (OMe), 20.74, 20.60 (OAc).

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2-AZIDO-4,6-*O*-BENZYLIDENE-2-DEOXY-D-MANNOPYRANOSE (90)

A solution of **8** (103 mg, 0.50 mmol) and benzaldehyde dimethyl acetal (0.38 mL, 385 mg, 2.50 mmol) in dry DMF (13 mL) containing *p*-toluenesulphonic acid monohydrate (250 mg, 0.1 mmol) was stirred at room temperature for 3 h. TLC showed the formation of a new spot, and ice-cold saturated sodium hydrogen carbonate was then added while stirring, extracted with ethyl acetate (3 x). The organic layer was washed with water, dried (anhydrous magnesium sulfate), filtered, and concentrated. The residue was purified by column chromatography (ethyl acetate/petroleum ether 2:3) to obtain **90** (75 %); (m/z = 293.1); R_f 0.63 (ethyl acetate: petroleum ether 2:3); ^{1}H NMR (400 MHz, $CDCl_3$) (α:β,

0.67:0.33): δ = 7.487 – 7.374 (m, 5 H, 2ArH), 5.580 (s, 0.67 H, H-7), 5.553 (s, 0.33 H, H-7), 5.178 (d, 0.67 H, J = 1.6 Hz, H-1), 4.823 (d, 0.37 H, J = 1.6 Hz, H-1), 4.359 – 3.313 (m, 6 H, H-2, H-3, H-4, H-5, H-6a, H-6b), 2.865 (bs, 2 H, 2OH); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 137.03, 129.42, 129.35, 128.41, 128.38, 126.25, 126.21 (Ar), 102.30, 102.21 (C-7), 93.86 (C-1β), 93.49 (C-1α), 79.08, 78.26, 71.44, 68.67, 68.51, 68.31, 66.85, 65.57, 63.89, 63.52 (C-3, C-5, C-6, C-4, and C-2, respectively).

1,3-DI-*O*-ACETYL-2-AZIDO-4,6-*O*-BENZYLIDENE-2-DEOXY-D MANNOPYRANOSE (91)

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To a solution of **90** (100 mg, 0.34 mmol) in dry dichloromethane (3 mL) and triethyl amine (0.2 mL, 0146 mg, 1.36 mmol) were added acetic anhydride (0.32 mL, 346 mg, 3.40 mmol) and DMAP (2 mg, 0.017 mmol). After stirring for 40 min, the mixture was diluted with DCM, washed with 10 % (w/v) aqueous sodium chloride, and the organic layer was dried (anhydrous magnesium sulfate), filtered, and concentrated. The residue was purified by column chromatography (ethyl acetate/petroleum ether 3:7) to give **91** (90%); (*m/z* = 376.95); R_f 0.71 (ethyl acetate: petroleum ether 2:3); ^{1}H NMR (400 MHz, $CDCl_3$) (α:β, 50:50): δ = 7.479 – 7.157 (m, 5 H, ArH), 6.080 (d, 0.5 H, $J_{1,2}$ = 1.6 Hz, H-1), 5.906 (d, 0.5 H, $J_{1,2}$ = 1.6 Hz, H-1),5.586 (s, 1 H, H-7), 5.470 (dd, 0.5 H, $J_{2,3}$ = 3.6 Hz, $J_{3,4}$ = 10.4 Hz, H-3), 5.151 (dd, 0.5 H, $J_{2,3}$ = 3.8 Hz, $J_{3,4}$ = 10.2 Hz, H-3), 4.342 (dd, 0.5 H, $J_{5,6a}$= 4.8 Hz, $J_{6a,6b}$ = 10.4 Hz, H-6a), 4.280 (dd, 0.5 H, $J_{5,6a}$= 4.8 Hz, $J_{6a,6b}$ = 10.4 Hz, H-6a), 4.275 (dd, 0.5 H, $J_{1,2}$ = 1.6 Hz, $J_{2,3}$ = 4.0 Hz, H-2), 4.169 – 4.119 (m, 1 H, H-2, H-4), 4.012 (t, 0.5 H, J = 10.0 Hz, H-4), 3.980 (ddd, 0.5 H, $J_{4,5}$ = 9.6 Hz, $J_{6a,5}$ = 4.8 Hz, $J_{5,6b}$ = 10.4 Hz, H-5), 3.831 (t, 0.5 H, J = 10.2 Hz, H-6b), 3.807 (t, 0.5 H, J = 10.4 Hz, H-6b), 3.586 (ddd, 0.5 H, $J_{5,6a}$ = 4.8 Hz, $J_{4,5}$ = 10.4 Hz, $J_{5,6b}$ = 14.4 Hz, H-5), 2.184 (s, 1.5 H, OAc), 2.175 (s, 1.5 H, OAc), 2.159 (s, 1.5 H, OAc), 2.151 (s, 1.5 H, OAc); ^{13}CNMR (100 MHz, $CDCl_3$): α δ = 170.31, 168.62 (OAc), 137.10, 129.44, 129.40,129.25, 128.51, 128.48 (Ar), 102.25 (C-7), 92.26 (C-1), 75.73 (C-4), 70.05 (C-3), 68.59 (C-6), 66.31 (C-5), 61.36 (C-2), 21.10, 20.80 (OAc); ^{13}CNMR (100 MHz, $CDCl_3$): β δ = 170.34, 168.42

(OAc), 136.99, 129.46, 128.51, 126.40 (Ar), 102.26 (C-7), 91.86 (C-1), 75.24 (C-4), 71.27 (C-3), 68.35 (C-6), 68.33 (C-5), 62.01 (C-2), 20.88, 20.81 (C-OAc).

1,3-DI-*O*-ACETYL-2-AZIDO-6-*O*-BENZYL-2-DEOXY-D-MANNOPYRANOSE (92)

A solution of **91** (400 mg, 1.06 mmol) in dry freshly distilled THF (11 mL) containing 3Å molecular sieves was stirred at room temperature for 30 min. Sodium cyanoborohydride (0.73 g, 11.66 mmol) was added to the solution and the mixture stirred for an additional 10 min. Diethyl ether saturated with hydrogen chloride was then added to the reaction mixture with a syringe until evolution of gas ceased. After 10 min, TLC (ethyl acetate/petroleum ether 2:3) showed completion of reaction. The mixture was diluted with dichloromethane and water, filtered with aid of Celite. The organic layer was washed with water followed by saturated sodium bicarbonate, dried (anhydrous magnesium sulfate), and concentrated to a syrup. The crude residue was purified by column chromatography (ethyl acetate/petroleum ether 3:7) to afford **92** (65 %); (*m/z* = 378.9); R_f 0.66 (ethyl acetate: petroleum, 2:3); ^{1}H NMR (300 MHz, $CDCl_3$): (α:β, 60:40) δ = 7.350 - 7.298 (m, 5 H, ArH), 6.068 (d, 0.6 H, $J_{1,2}$ = 1.8 Hz, H-1), 5.802 (d, 0.4 H, $J_{1,2}$ = 1.2 Hz, H-1), 5.239 (dd, 0.6 H, $J_{2,3}$ = 3.6 Hz, $J_{3,4}$ = 9.6 Hz, H-3), 4.919 (dd, 0.4 H, $J_{2,3}$ = 3.8 Hz, $J_{3,4}$ = 9.6 Hz, H-3), 4.578 (ABq, 2 H, J = 12.0 and 11.7 Hz, PhCH_2), 4.131 (dd, 0.4 H, $J_{1,2}$ = 1.2 Hz, $J_{2,3}$ = 3.8 Hz, H-2), 4.026 – 3.545 (m, 4 H, H-2, H-4, H-6a, H-6b), 3.414 (ddd, 0.6 H, $J_{4,5}$ = 9.6 Hz, $J_{5,6}$ = 4.8 Hz, H-5), 2.896 (bs, 1 H, OH), 2.174 (s, 1.2 H, OAc), 2.169 (s, 1.2 H, OAc), 2.159 (s, 1.2 H, OAc), 2.134 (s, 1.8 H, OAc); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 170.88, 170.53, 168.43 (OAc), 137.32 (Ar_q), 128.50, 128.47, 127.97, 127.93, 127.86, 127.82 (Ar), 91.58 (C-1α), 91.41 (C-1β), 75.07, 74.29, 73.85, 70.00, 69.90, 66.87, 66.69, 61.04, 59.37 (C-3, CH$_2$Ph, C-5, C-6, C-4, C-2), 20.88, 20.71, 20.67 (OAc).

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1,3,4-TRI-*O*-ACETYL-2-AZIDO-6-*O*-BENZYL-2-DEOXY-D-MANNOPYRANOSE (93)

To a solution of **92** (60 mg, 0.16 mmol) in dry dichloromethane (0.5 mL) and triethyl amine (0.09 mL, 0.65 mmol) were added acetic anhydride (0.15 mL, 1.58 mmol) and DMAP (0.001 gm, 0.008 mmol). After stirring for 40 min, the mixture was diluted with dichloromethane, washed with 10 % (w/v) aqueous sodium chloride, dried (anhydrous magnesium sulfate), filtered, and concentrated. The residue was purified by column chromatography (ethyl acetate/petroleum ether 3:7) to give **93** (85 %); (*m/z* = 420.8); R_f 0.75 (ethyl acetate/petroleum ether 2:3); ^{1}H NMR (400 MHz, $CDCl_3$) (α:β, 80:20): δ = 6.070 (d, 0.8 H, $J_{1,2}$ = 2.0 Hz, H-1), 5.802 (d, 0.2 H, $J_{1,2}$ = 1.2 Hz, H-1), 5.381 (t, 0.2 H, $J_{3,4}$ = $J_{4,5}$ = 9.6 Hz, H-4), 5.049 (dd, 0.2 H, $J_{2,3}$ = 3.8 Hz, $J_{3,4}$ = 9.6 Hz, H-3), 5.233 (t, 0.8 H, $J_{3,4}$ = $J_{4,5}$ = 9.6 Hz, H-4), 5.329 (dd, 0.8 H, $J_{2,3}$ = 3.4 Hz, $J_{3,4}$ = 9.6 Hz, H-3), 4.529 – 4.417 (m, 2 H, C*H*$_2$Ph), 4.101 (dd, 0.2 H, $J_{1,2}$ = 1.6 Hz, $J_{2,3}$ = 3.6 Hz, H-2), 3.995 (dd, 0.8 H, $J_{1,2}$ = 2.0 Hz, $J_{2,3}$ = 3.6 Hz, H-2), 4.005 – 3.920 (m, 1 H, H-6a), 3.665 (ddd, 1 H, $J_{4,5}$ = 9.6 Hz, $J_{5,6a}$ = 4.4 Hz, $J_{5,6b}$ = 3.6, Hz, H-5), 3.60 – 3.454 (m, 1 H, H-6b), 2.120 (s, 0.6 H, OAc), 2.091 (s, 2.4 H, OAc), 2.050 (s, 0.6 H, OAc), 2.046 (s, 2.4 H, OAc), 1.88 (s, 2.4 H, OA), 1.862 (s, 0.6 H, OAc); ^{13}CNMR (100 MHz, $CDCl_3$): δ = 170.09, 170.04, 169.37, 168.41, 168.31, (OAc), 137.66,137.60 (Ar), 128.34, 128.32, 127.98, 127.94, 127.72 (Ar), 91.45 (C-1α), 91.33 (C-1β), 74.64, 73.66, 73.61, 72.03, 70.68, 68.71, 68.57, 66.38, 65.98 (C-3, C-4, C-5, C-6), 61.27 (C-2α), 60.61 (C-2β), 20.88, 20.70, 20.61, 20.57, 20.54, 20.49 (OAc).

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3,4-DI-*O*-ACETYL-2-AZIDO-6-*O*-BENZYL-2-DEOXY-D-MANNOPYRANOSE (66)

To a solution of **93** (100 mg, 0.24 mmol) in DMF (3 mL) at 50 °C was added hydrazine acetate (26 mg, 0.28 mmol). After 10 minutes stirring at 50 °C of the reaction mixture, TLC showed completion of reaction. The mixture was then cooled to room temperature and diluted with ethyl acetate. The organic layer was washed with 5 % aqueous NaCl solution, dried (anhydrous magnesium sulfate), filtered, and concentrated. The crude product was purified by column chromatography (ethyl acetate/petroleum ether 3:7) to afford 80 % of **66**; (*m/z* = 379.1); R_f 0.63 (ethyl acetate: petroleum ether 2:3); ^{1}H NMR (300 MHz, $CDCl_3$) (α:β, 60:40) δ: 7.370 – 7.243 (m, 5 H, ArH), 5.412 (dd, 0.6 H, $J_{2,3}$ = 3.9 Hz, $J_{3,4}$ = 9.6 Hz, H-3), 5.182 (t, 1 H, $J_{3,4}$ = 9.9 Hz, $J_{4,5}$ = 10.2 Hz, H-4), 5.134 (d, 0.6 H, $J_{1,2}$ = 1.5 Hz, H-1), 5.006 (dd, 0.4 H, $J_{2,3}$ = 3.9 Hz, $J_{3,4}$ = 9.9 Hz, H-3), 4.757 (bs, 0.4 H, H-1), 4.503 (ABq, 2 H, J = 11.7 and 14.7 Hz, CH_2Ph), 4.247 (bs, 1 H, OH), 4.140 (ddd, 1 H, $J_{4,5}$ = 9.9 Hz, $J_{5,6a}$ = 6.8 Hz, $J_{5.6b}$ = 2.4 Hz, H-5), 3.910 (dd, 1 H, $J_{1,2}$ = 1.8 Hz, $J_{2,3}$ = 3.6 Hz, H-2), 3.452 (dd, 1 H, $J_{6a,6b}$ = 10.4 Hz, $J_{5,6b}$ = 2.7 Hz, H-6b), 3.543 (dd, 1 H, $J_{6a,6b}$ = 10.4 Hz, $J_{5,6a}$ = 6.8 Hz, H-6a), 2.090 (s, 1.20 H, OAc) 2.074 (s, 1.80 H, OAc), 1.937 (s, 1.80 H, OAc), 1.912 (s, 1.20 H, OAc); ^{13}C NMR (75 MHz, $CDCl_3$) for α δ: 170.23, 170.04 (OAc), 128.66, 128.42, 128.33, 128.17, 128.11 (Ar), 92.68, 70.99, 69.58, 69.50, 67.18, 62.29 (C-1, 3, 5, 6, 4, and 2, respectively), 20.85 (OAc), 20.75 (OAc); ^{13}C NMR (75 MHz, $CDCl_3$) for β δ: 93.14, 72.87, 69.15, 66.62, 63.61 (C-1, 3, 6, 4, and 2, respectively), 20.69 (OAc).

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TRIETHYLAMMONIUM 3,4-DI-*O*-ACETYL-2-AZIDO-6-*O*-BENZYL-2-DEOXY-α-D-MANNOPYRANOSYL HYDROPHOSPHONATE (118)

A solution of **66** (150 mg, 0.4 mmol) in dry dioxane (1.5 mL) and pyridine (0.5 mL) was treated with a solution of salicylchlorophosphite (1 mL, 1.25M stock solution salicylchlorophosphite in dioxane) and stirred at room temperature for 20 minutes. The reaction was quenched with cold 0.5 M TEAB (aq) solution and stirred for additional 30 minutes. The reaction mixture was evaporated to remove the dioxane. The residue was diluted with chloroform, washed successively with cold water and TEAB solution. The organic layer was concentrated and purified by column chromatography using 10 % MeOH/$CHCl_3$/Et_3N solvent system to yield 50 % of hydrophosphonate **118**; R_f 0.44 (MeOH/chloroform and a drop of triethyl amine 1:4); ^1H NMR (300 MHz, $CDCl_3$): δ = 7.258 – 7.237 (m, 5 H, ArH), 6.949 (d, 1 H, $J_{P,H}$ = 647.1 hz, PH), 5.622 (dd, 1 H, $J_{1,P}$ = 8.4 Hz, $J_{1,2}$ = 1.2 Hz, H-1), 5.426 (dd, 1 H, $J_{2,3}$ = 3.6 Hz, $J_{3,4}$ = 9.9 Hz, H-3), 5.313 (t, 1 H, J = 9.9 Hz, H-4), 4.446 (ABq, 2 H, J = 12.0 Hz, 24.6 Hz, PhCH_2), 4.158 – 4.087 (m, 3 H, H-5, H-6a, H-6b), 3.470 (d, 1 H, $J_{2,3}$ = 3.6 Hz, H-2), 3.052 (q, 2 H, J = 7.2 Hz, 14.4 Hz, CH_3CH_2), 2.012 (s, 3 H, OAc), 1.849 (s, 3 H, OAc), 1.309 (t, 3 H, J = 7.2 Hz, CH_3CH_2); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 169.79, 169.46 (OAc), 137.70, 128.11, 127.74, 127.43 (Ar), 92.99 (C-1), 73.29 (PhCH_2), 70.67, 70.56, 68.64, 66.52, 62.26 (C-2, C-3, C-4, C-5, C-6), 45.76 (Et_3N), 20.47 (OAc), 20.37 (OAc), 8.45 (Et_3N); ^{31}P (300 MHz, $CDCl_3$): δ = - 0.82.

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